THE MYCOTA

A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research

Edited by
K. Esser

Growth, Differentiation and Sexuality

I

2nd Edition

U. Kües · R. Fischer
(Volume Editors)

Springer

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Volume Editors:
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Karl Esser

(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

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(born 1958) finished her graduate studies in biology at the Ruhr-Universität Bochum (Germany) in 1983, with a Master's thesis in yeast genetics under the supervision of Ulf Stahl and Karl Esser. After completing her Ph.D. in microbiology at the Technical University of Berlin (1988), and a Post-Doc at the Free University of Berlin, in 1990 she joined Lorna Casselton in London and Oxford to study fungal mating types. In 1993, she was awarded a Glasstone Research Fellowship of the University of Oxford for research in fungal development. As a group leader at the ETH Zurich in Switzerland (1994–2001), in 1999 she completed her post-doctoral thesis ("Habilitation") and obtained a Venia Legendi in Molecular Microbiology and Genetics. Since 2001, she is full professor in Molecular Wood Biotechnology at the Georg-August-University Göttingen (Germany), in a post funded by the Deutsche Bundesstiftung Umwelt. Her research focuses on basidiomycete development and wood decay.

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(born 1962) studied biology at the Philipps University of Marburg, Germany and obtained his Ph.D. in 1990 under the supervision of Rolf Thauer, on the topic of acetate metabolism in the archaeon *Methanosarcina barkeri*. In 1992, he switched his attention to *Aspergillus nidulans* fungal development when he joined William E. Timberlake's group as a Post-Doc at the University of Georgia, GA, USA, returning 1994 to Marburg to the department led by Rolf Thauer at the Max-Planck-Institute for Terrestrial Microbiology. In 1998 he completed his "Habilitation" and was awarded a Venia Legendi in Microbiology and Cell Biology. He was appointed as professor for Applied Microbiology in 2004 at the University of Karlsruhe, Germany. His work is focused on the analysis of *A. nidulans* development, with a current interest in light regulation. In a second line of research, he is studying polarized growth and the involvement of molecular motors.

Series Preface

Mycology, the study of fungi, originated as a subdiscipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for selfincompatibility, termed "heterothallism", and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with *Neurospora crassa*, led to the one gene: one enzyme hypothesis and to a second Nobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to *Saccharomyces cerevisiae*, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics and molecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology, medical mycology, and systematics. Today, there is much interest in the genetic manipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title *The Mycota*. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this Series of Volumes the names adopted for major groups of fungi (representative genera in parentheses) are as follows:

Pseudomycota

Division:	Oomycota (*Achlya*, *Phytophthora*, *Pythium*)
Division:	Hyphochytriomycota

Eumycota

Division:	Chytridiomycota (*Allomyces*)
Division:	Zygomycota (*Mucor*, *Phycomyces*, *Blakeslea*)
Division:	Dikaryomycota
Subdivision:	Ascomycotina

Class:	Saccharomycetes (*Saccharomyces*, *Schizosaccharomyces*)
Class:	Ascomycetes (*Neurospora*, *Podospora*, *Aspergillus*)
Subdivision:	Basidiomycotina
Class:	Heterobasidiomycetes (*Ustilago*, *Tremella*)
Class:	Homobasidiomycetes (*Schizophyllum*, *Coprinus*)

We have made the decision to exclude from *The Mycota* the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.

The Series throughout will address three basic questions: what are the fungi, what do they do, and what is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this Series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature of fungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather than ingest, nutrients and they have rigid cell walls. The vast majority of fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on their method of production have been used to delineate most fungal taxa. Although there is a multitude of spore forms, fungal spores are basically only of two types: (i) asexual spores are formed following mitosis (mitospores) and culminate vegetative growth, and (ii) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondary metabolites, is also noteworthy. The metabolic capacities of fungi have attracted much interest in natural products chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the microfungi that grow in the anaerobic environment of the rumen, the many insect-associated fungi and the medically important pathogens afflicting humans. Yes, fungi are ubiquitous and important.

There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contribute to this Series. We are especially indebted and grateful for the initiative and leadership shown by the Volume Editors in selecting topics and assembling the experts. We have all been a bit ambitious in producing these Volumes on a timely basis and therein lies the possibility of mistakes and oversights in this first edition. We encourage the readership to draw our attention to any error, omission or inconsistency in this Series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine Volumes.

Bochum, Germany
Auburn, AL, USA
April 1994

Karl Esser
Paul A. Lemke
Series Editors

Addendum to the Series Preface

In early 1989, encouraged by Dieter Czeschlik, Springer-Verlag, Paul A. Lemke and I began to plan *The Mycota*. The first volume was released in 1994, 12 volumes followed in the subsequent years. Unfortunately, after a long and serious illness, Paul A. Lemke died in November 1995. Thus, it was my responsibility to proceed with the continuation of this series, which was supported by Joan W. Bennett for Volumes X–XII.

The series was evidently accepted by the scientific community, because first volumes are out of print. Therefore, Springer-Verlag has decided to publish some of the completely revised and updated new editions of Volumes I, II, III, IV, VI, and VIII. I am glad that most of the volume editors and authors have agreed to join our project again. I would like to take this opportunity to thank Dieter Czeschlik, his colleague, Andrea Schlitzberger, and Springer-Verlag for their help in realizing this enterprise and for their excellent cooperation for many years.

Bochum, Germany
July 2005

Karl Esser

Volume Preface to the First Edition

Hyphal growth, leading to the formation of a mycelium, is the most characteristic feature of fungi. Many fungi, however, propagate as yeasts or alternate between the yeast and mycelial form. Both growth forms have in common that they are manifestations of polarized cytoplasmic activities. A large part of this Volume of The Mycota therefore deals with growth and differentiation in both yeast-like and mycelial fungi and also the transition between them. Sexuality is the other main topic of this Volume.

Yeasts offer many methodological advantages, particularly with respect to applying microbiological and molecular techniques, and therefore have become major experimental objects for advanced research. Among processes studied in depth are the regulation of mitosis and the cell division cycle, the synthesis of wall components, the secretion of proteins, the regulation of meiosis and sporulation by the mating-type genes, the mating-type switch, and the nature and action of mating hormones. Apart from their relevance to general eukaryotic cell biology, these studies form a basis for exploring the nature of similar processes in mycelial fungi.

The rigid wall ultimately determines the shape of fungal structures and therefore attention is given to wall structure and biogenesis. The mechanisms by which the cells locate wall synthesis and exocytosis of proteins at denned sites are still problematic. Attention therefore focuses on the role of the cytoskeleton and plasma membrane proteins in the polarized activities of the cell, the role of turgor as a driving force for growth and the role of electrical currents that often accompany polarized growth.

Hyphae regularly branch according to a distinct pattern and form a mycelium that enables transport of water and nutrients. With the apically growing hyphae that release digestive enzymes, the mycelium is thus ideally adapted to colonize dead or living substrata. Vegetatively growing mycelia may eventually grow old and die, necessitating a sexual cycle for rejuvenescence.

Anastomosis of fungal mycelia occurs frequently in nature but heterogenic incompatibility systems often prevent the coexistence of genetically different nuclei in a common cytoplasm, whereas homogenic incompatibility often governs sexual interactions which lead to meiosis and the formation of meiospores.

The role of mating-type genes in homogenic incompatibility is best understood in some yeast species, but rapid progress is being made in elucidating the complex interactions between mating-type genes that govern sexual processes in mycelial fungi. Some of these mycelial fungi also offer excellent opportunities to study meiosis.

The mycelial growth habit allows for long-range intercellular communication and transport, permitting the emergence of aerial structures for dispersal of asexual and sexual spores. Extensive molecular-genetic studies are being carried out on conidiation and fruit body formation in a few selected species.

Differentiation in fungi also often involves signaling substances, indicated as hormones in the Chapter titles, although the authors may prefer different designations for these substances. Again, the yeast systems have yielded most to molecular studies while the mycelial systems offer a wealth of cases to be studied in depth.

Where possible we have selected authors who are actively engaged in advanced research on the topics mentioned. This means that the emphasis is on molecular research

probably with some neglect of important research that has not yet entered this arena. A comprehensive treatment of the topics would require more than one Volume of The Mycota. However, the contributors were asked to consider all relevant aspects and to stress future developments in their specific and related areas of research. We therefore hope that this Volume will not only provide a source of information for what has already been achieved but that it may also influence future research and developments in this field.

Groningen, The Netherlands
Münster, Germany
April 1994

Joseph G.H. Wessels
Friedhelm Meinhardt
Volume Editors

Volume Preface to the Second Edition

This volume is an update of Volume I of the series The Mycota, which appeared more than ten years ago. To us, the first edition of the book has been and, in some areas, still is a major source of ideas and knowledge concerning fungal development and reproduction. Since then, however, three major breakthroughs in molecular biology have caused fast progress in our understanding of fungal development and have also opened up new research fields. First, the improvement of PCR-based methods has facilitated faster access to genes and, in combination with reverse transcriptase, to transcripts. In addition, these methods have made it possible to investigate fungi which do not grow well on agar plates, such as obligate biotrophic fungi, and they have offered us the opportunity to analyse fungal populations in the field. Second, the advent of green and other fluorescent proteins in fungi in 1995 has caused a "green revolution", just like in many other fields of biology. Third, the publication of the full *Saccharomyces cerevisiae* genome in 1996, and of many more fungal genomes since then, has opened the way to genome-wide assessments and the widespread use of reverse genetics approaches.

We are very much indebted to Jos Wessels for his initial input in structuring this new Volume I, and his wealth of scientific and editorial experience has greatly helped to get the final design of the volume. Several of the authors of chapters in the first edition agreed to update their contributions, in many instances with a totally new focus. Others, however, had retired from science and, above all, we mourn the loss of Graham Gooday. New authors were asked to join the project, particularly in fast-progressing sectors and also in newly emerged fields dealing with novel developments in studies of growth, differentiation and sexuality. Due to space limitations, some subject matter, formerly treated in several chapters, has now been condensed, with the inevitable cost of loosing out on some knowledge and detailed information. An example is the field of meiosis, which formerly was presented in three chapters, now only in one. Likewise, in the first edition four chapters dealt with aspects of mating type genes, but in this second edition we have only two chapters specifically assigned to these genes. We therefore apologize to authors and readers if excellent parts of the former book have been left out of the current version, and we would like to draw attention to the older Volume I in The Mycota series, which in many cases still adds to the text presented here. Furthermore, we refer the reader to other books in The Mycota series, in particular to Volume 8, dealing with the related field of cell biology. Cross-references within the chapters of the present book will help in learning more about related topics in this and other books of the series.

Issues of vegetative processes and growth are nowadays not easily fully separated from those dealing with reproduction, and vice versa. As will be repeatedly found in this book, often the same cellular players are involved in growth and in reproduction. Grouping of chapters into general themes is thus not always that clear cut. This second edition of Volume 1 has been divided into three sections, according to the main foci of the chapters. The first ten chapters deal with vegetative processes and growth. There is one chapter on fungal cell types, and two chapters on organelle inheritance and mitosis. The following two contributions summarize our current knowledge about the fungal cell wall and its biogenesis. How fungal compartments are separated is described in Chapter 6. Chapters 7 and 8 deal with mechanisms of fungal cell fusion and incompatibility

reactions. These are followed by two chapters on programmed cell death and senescence. The second section comprises three chapters on signals in growth and development, with one chapter on autoregulatory signals, another on pheromones, and yet another on photomorphogenesis and gravitropism. The last section describes, in seven chapters, asexual and sexual reproductive processes in asco- and basidiomycetes, and ends with one chapter on meiosis. Reproductive processes in other groups of fungi are less well understood, and available information has been included mainly in the second section of chapters.

We hope that the book will give its readers the same joy as we had when learning all about new information and ideas in these rapidly evolving fields. We would like to express our gratitude to all the authors for their excellent input into this book, which hopefully will enjoy the same success as its predecessor.

Göttingen, Germany
Karlsruhe, Germany
July 2005

Ursula Kües
Reinhard Fischer
Volume Editors

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Reproductive Processes

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Vegetative Processes and Growth

1 Morphogenesis: Control of Cell Types and Shape

K.J. Boyce[1,2], A. Andrianopoulos[1]

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I. Introduction Cell Types and Cell Shapes: a Diverse Array of Form

Fungi generate a variety of cellular morphologies in order to colonize and adapt to new environments. The most commonly utilized cell shapes include spherical, ellipsoidal or cylindrical yeast cells or chains of highly polarised cylindrical cells which form pseudohyphae or hyphae (Fig. 1.1). These common cell shapes encompass a large number of cell types, which may or may not be terminally differentiated, and which differ in their physiologies. Some fungi are capable of growing vegetatively in two or more of these morphologies, and are termed dimorphic. Fungi are also capable of producing a large variety of specialized cell types with unique cellular morphologies during developmental processes such as asexual and sexual reproduction, pathogenesis for host penetration or host association during symbiosis. This chapter will attempt to cover the most common of fungal cell types and highlight the current research into the molecular components which govern cell type and shape establishment through the control of actin polarisation.

II. Polarity

The establishment and maintenance of polarity is central to the generation of the wide variety of cell morphologies found in organisms. It relies on the ability to mark specific regions of the cell by protein localisation and results in distinct cellular morphologies or function. These marked regions are used for many cellular processes involving the asymmetric distribution of cellular components such as receptors and transporters or in establishing growth polarity by directing growth to specific areas of the cell. Controlling polarisation during growth is required for both the maintenance of cell morphology during vegetative growth and cell division, as well as the alteration of cell morphology which is required for the differentiation of distinct cell types during development (Fig. 1.2; see Chaps. 2–6, this volume, and various chapters in The Mycota, Vol. VIII). The ability of cells to polarise growth is also crucial for rapid morphological responses to the environment. Control of polarisation is directly dependent on the cytoskeleton and its dynamics.

A. Cytoskeleton

The cytoskeleton is composed of three types of protein filaments – microtubules, microfilaments

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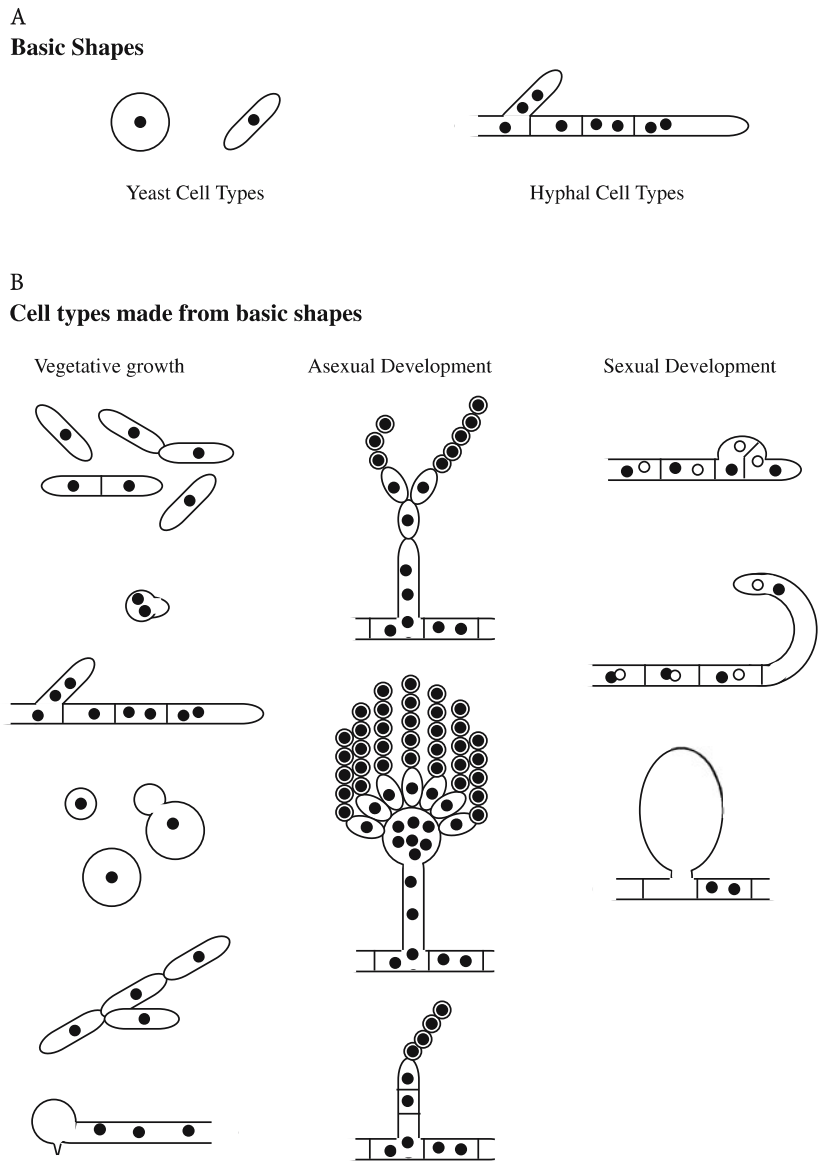

Fig. 1.1. Cell types in fungi. **A** The basic shapes of fungal cells associated with the two major growth forms: yeast cells and hyphal cells. **B** Diagrammatic representations of some of the cell types which can be generated using the basic fungal cell shapes. These can be involved in vegetative growth (fission yeast, germinating spores, hyphae, budding yeast, pseudohyphae, appressoria; *top to bottom*), asexual development (biverticilliate conidiophore, vesicular conidiophore, arthroconidiating conidiophore; *top to bottom*) and sexual development (dikaryotic clamp cell, ascogenous hypha, Hülle cell; *top to bottom*)

and intermediate filaments. These filaments provide structure and organisation to the cytoplasm and give shape to the cell. Intermediate filaments, which appear to be higher eukaryote specific and for which there is no firm evidence in fungi, are thought to provide internal mechanical support for cells whereas microtubules are required for transport between intracellular compartments, during formation of the mitotic spindle (division) and during cell motility (beating of cilia and flagella; Glotzer 2005; Palmer et al. 2005). Microfilaments are also necessary for the transport of intracellular components (see below) but differ from microtubular-based movement which often functions over long distances, such as for the transport of vesicles from the endoplasmic reticulum to the expanding growth region of hyphal cells (Steinberg et al. 2000). Details of the specific roles of intermediate filaments and microtubules are beyond the scope of this chapter. Microfilaments are comprised of actin polymers and, when associated with a variety of interacting proteins, comprise the actin cytoskeleton (Schmidt and Hall 1998). The actin cytoskeleton is required for numerous cellular functions, including polarised growth. In mammals and many other organisms, actin is required for cell motility, changes in cell shape, muscle contraction, cytokinesis, cell–substrate

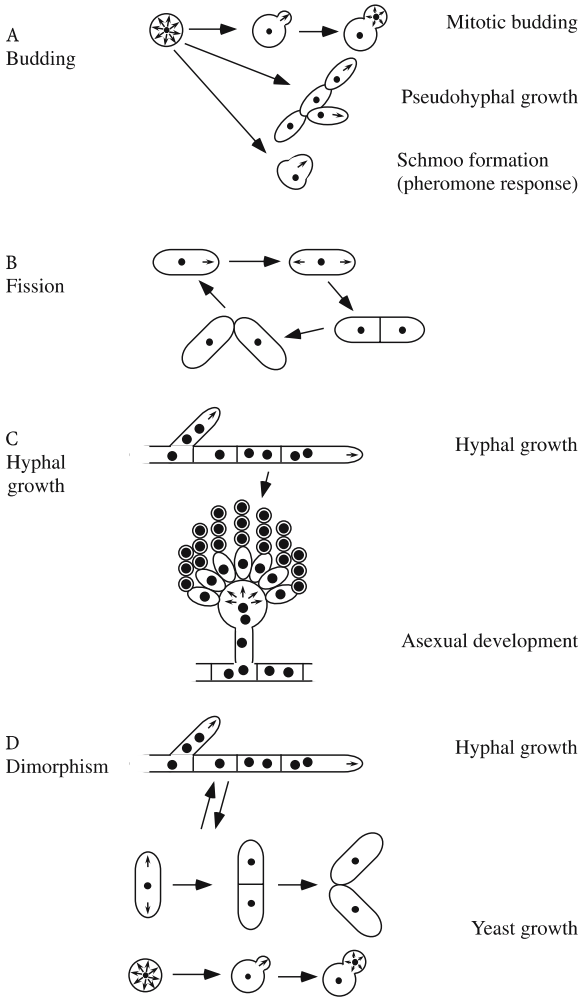

Fig. 1.2. Polarisation and fungal cell types. **A** Budding yeast are uninucleate and unicellular, growing by isotropic expansion. The uninucleate cells undergo mitotic budding to reproduce, which requires polarised growth towards the emerging bud. Budding yeasts can also grow as chains of elongated cells termed pseudohyphae. This growth form also requires polarised growth towards the pseudohyphal apex. Upon exposure to pheromone, budding yeasts like *S. cerevisiae* can polarise growth in the direction of the external pheromone source (schmoo formation). **B** Fission yeast are uninucleate and unicellular, growing initially by polarising growth to one end of the cell, followed by polarised growth at both ends. Cell elongation is followed by cell division and cell separation. **C** Mycelial fungi grow as multinucleate, branched hyphae which are divided at regular intervals by septa. Hyphal growth is polarised towards the hyphal and branch apices. Fungi such as *A. nidulans* can also undergo asexual development, a process with similarities to budding in yeast and which requires multiple rounds of initiation of polarised growth. **D** Some fungi can grow both as unicellular yeast (budding or fission) and as multicellular hyphae, and these are termed dimorphic. The switch between these growth states requires regulated changes in the modes of polarised growth. *Arrows* indicate direction of polarisation

interactions, endocytosis and secretion (Schmidt and Hall 1998). Likewise, in *Saccharomyces cerevisiae*, the actin cytoskeleton is required for a range of cellular processes including bud formation, movement of vesicles, localisation of chitin, cytokinesis, endocytosis, organelle movement and shape changes in response to environmental stimuli (Schmidt and Hall 1998).

Cells are reliant on the actin cytoskeleton in order to direct growth in a polarised manner. So, in order to regulate or initiate polarised growth during development, cells must be able to polarise the actin cytoskeleton towards the imminent growth site. However, as the actin cytoskeleton influences a wide variety of other cellular processes, the organisation must be very tightly regulated. This is achieved by regulating actin nucleation and polymerisation, and by regulating the cellular sites where nucleation and polymerisation occur (Schmidt and Hall 1998). Actin exists as either a monomeric form (G-actin) or a polymeric form (F-actin). Actin monomers bind and hydrolyse ATP in order to be incorporated into the polymer. The rate-limiting step in actin polymerisation is nucleation, the assembly of new monomers to form a filament (Schmidt and Hall 1998). This can be enhanced by scaffold proteins at the cell membrane.

B. Isotropic to Polarised Growth

1. The Establishment of Polarised Growth in Budding Yeast

The mechanisms regulating polarised growth establishment and the switch from isotropic to polarised growth in fungal cells has been best characterised in *S. cerevisiae* (reviewed in Johnson 1999). During the mitotic cell cycle, *S. cerevisiae* divides by a process termed budding. This involves the selection of a non-random budding site, the organisation of proteins at this site, and the rearrangement of the actin cytoskeleton (Fig. 1.3). The bud emerges and growth is directed exclusively to the expanding bud. Bud expansion is followed by cytokinesis, septum formation and cell separation.

2. Bud Site Selection

During bud initiation in *S. cerevisiae*, the site of bud emergence is selected by the location of cortical markers and the recruitment of the GTP-bound, small Ras-like GTPase, Rsr1p (Bud1p;

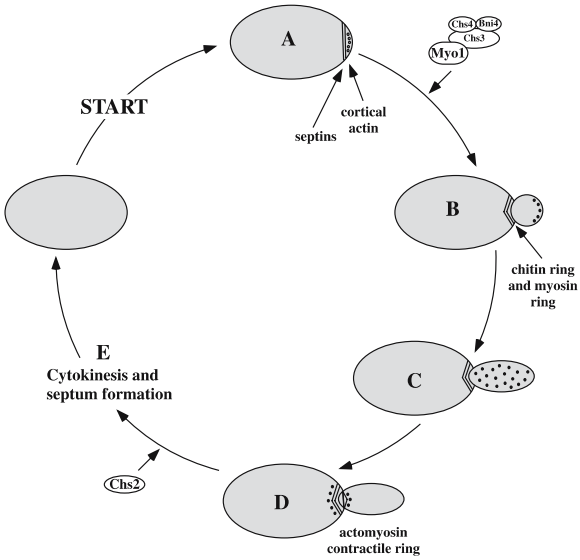

Fig. 1.3. Mitotic budding in *S. cerevisiae*. **A** A non-random budding site is selected, and proteins required for polarised growth are localised to this site. **B** Through the action of the type II myosin (Myo1p), chitin synthase (Chs3p and Chs4p) and the bud neck-involved (Bni4p) proteins, the bud emerges and growth is directed specifically to this site via a polarised actin cytoskeleton. **C** The bud begins to grow isotropically, expanding growth in all directions. **D** The actomyosin contractile ring forms in the mother–bud neck region, and a third chitin synthase (Chs2p) is recruited for sepum formation. **E** Cytokinesis occurs, followed by septum formation and cell separation

Bender and Pringle 1989; Fig. 1.4). The Cdc24p guanine exchange factor (GEF) for the small Rho-type GTPase Cdc42p associates with the Rsr1p GTPase and the Bem1p protein located at the incipient bud site. Bound Cdc24p and Bem1p interact with GDP-bound Cdc42p, which is bound to the guanine dissociation inhibitor (GDI) for Cdc42p, Rdi1p (Fig. 1.4). This interaction results in the loss of GDP-Cdc42p-Rdi1p binding, and the GEF Cdc24p catalyses the Cdc42p-bound GDP to GTP exchange (Ziman and Johnson 1994; Zheng et al. 1994, 1995; Richman et al. 2004; Fig. 1.4). These events now establish the site of bud emergence. The mechanisms which select new sites of growth in other fungi are poorly understood. It is clear that bud site selection in the dimorphic, opportunistic human pathogen *Candida albicans* is regulated by some of the same proteins as those identified in *S. cerevisiae* (Table 1.1). The *C. albicans* Rsr1p Ras-like GTPase regulates bud site selection, and the Cdc42p Rho GTPase is required for the establishment of polarity during budding

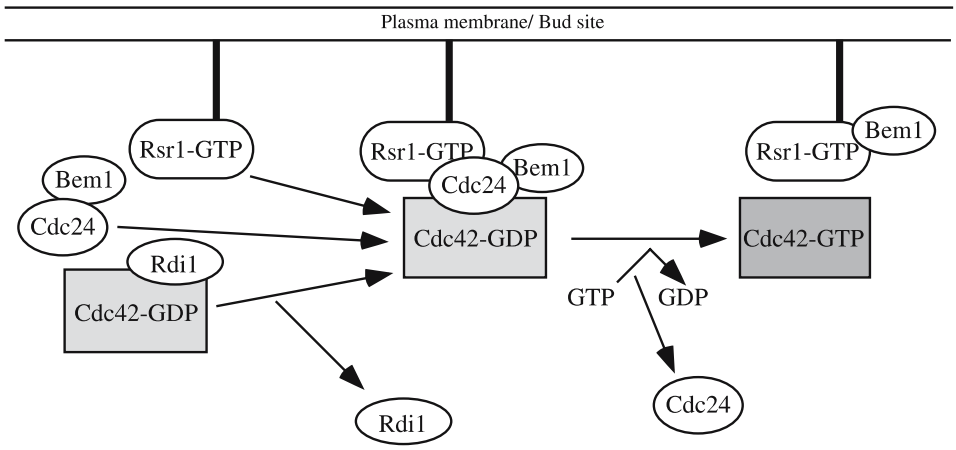

Fig. 1.4. Selection of a bud or polarisation site in *S. cerevisiae*. The organisation of protein complexes required for bud site selection and polarised growth during mitotic budding is shown (*left to right*). The process begins with localisation of GTP-Rsr1p to the membrane at the imminent bud site. GTP-bound Rsr1p interacts with Cdc24p, GDP-bound Cdc42p, Rdi1p and Bem1p at the plasma membrane. This interaction results in the loss of GDP-Cdc42p-Rdi1p binding, and the GEF Cdc24p catalyses the Cdc42p-bound GDP to GTP exchange. (Adapted from Johnson 1999)

Table. 1.1. Major protein families required for polarity establishment in fungi

Protein type	Protein	Organism	Role	Reference
Guanine exchange factor (GEF)	Cdc24p	*S. cerevisiae*	Catalyses GDP to GTP exchange of Cdc42p	Adams et al. (1990)
	Cdc24p	*U. maydis*	Cell separation during budding	Weinzierl et al. (2002)
	Cdc24p	*A. gossypii*	Establishment of actin polymerisation and polarised hyphal growth	Wendland and Philippsen (2001)
	Cdc24p	*C. albicans*	Hyphal germ tube emergence	Bassilana et al. (2003)
Guanine dissociation inhibitor (GDI)	Rdi1p	*S. cerevisiae*	Prevents Cdc42p-GDP associating with the membrane	Johnson (1999)
Bud initiation	Bem1p	*S. cerevisiae*	Associates with Cdc24p and Rsr1p during the activation of Cdc42p	Peterson et al. (1994)
GTPase activating protein (GAP)	Bem2p	*S. cerevisiae*	Activates Rho1p and Cdc42p during bud initiation	Marquitz et al. (2002)
	Bem2p	*A. gossypii*	Polarised hyphal growth and actin polarisation	Wendland and Philippsen (2000)
Ras-like GTPase	Rsr1p/Bud1p	*S. cerevisiae*	Bud site selection	Bender and Pringle (1989)
	Rsr1p	*C. albicans*	Bud site selection	Yaar et al. (1997)
	Rsr1p	*A. gossypii*	Hyphal growth guidance and morphology. Required for the localisation of the polarisome component Spa2	Bauer et al. (2004)
Rho GTPase	Cdc42p	*S. cerevisiae*	Regulates budding frequency. Organises proteins required for polarised growth at bud site. Activates PAK kinases, resulting in the formation of septin, chitin and myosin ring at the presumptive bud site. Localises actin. Assembles cytokinesis proteins and actin complex in mother–bud region. Leads to activation of MAPK cascade during pseudohyphal growth	Hartwell (1974); reviewed in Johnson (1999)
	Cdc42p	*C. albicans*	Required for bud formation and polarised growth of hyphae	Ushinsky et al. (2002)
	Cdc42p	*A. gossypii*	Establishment of actin polymerisation and polarised hyphal growth	Wendland and Philippsen (2001)
	CflA	*P. marneffei*	Initiation of germination (establishment of polarised hyphal growth), actin polarisation during hyphal growth (maintenance of polarised growth) and the polarised growth of yeast cells	Boyce et al. (2001)
	CflB	*P. marneffei*	Actin-dependent polarisation of hyphae (maintenance of polarised hyphal growth) and conidiophores	Boyce et al. (2003)
	Rac1	*C. trifolii*	Required for polarised hyphal growth	Chen and Dickman (2004)
	Rho1p	*S. cerevisiae*	Establishment of cell polarity. Regulates protein kinase C (Pkc1p) and cell wall-synthesizing enzyme 1,3-beta-glucan synthase (Fks1p and Gsc2p)	Drgonova et al. (1996)
	Rho2p	*S. cerevisiae*	Establishment of cell polarity and microtubule assembly	Madaule et al. (1987)
	Rho3p	*S. cerevisiae*	Establishment of cell polarity	Matsui and Toh-E (1992)
	Rho4p	*S. cerevisiae*	Establishment of cell polarity	Matsui and Toh-E (1992)
	Rho5p	*S. cerevisiae*	Involved in protein kinase C-dependent signal pathway which controls cell integrity	Schmitz et al. (2002)
	Rho1p	*A. gossypii*	Maintenance of polarised hyphal growth	Wendland and Philippsen (2001)

Table. 1.1. (continued)

Protein type	Protein	Organism	Role	Reference
	Rho3p	*A. gossypii*	Maintenance of hyphal growth and polarity at the hyphal tip	Wendland and Philippsen (2001)
PAK kinase	Cla4p	*S. cerevisiae*	Required for cytokinesis. Phosphorylates myosin and septin ring in mother–bud neck region	Benton et al. (1997)
	Cla4p	*C. albicans*	Required for cytokinesis in the yeast phase and polarised growth of hyphae	Leberer et al. (1997)
	Ste20p	*S. cerevisiae*	Phosphorylates septins and myosins during the establishment of polarised growth during budding	Leberer et al. (1992)
	Cst20p	*C. albicans*	Required for filamentous growth	Köhler and Fink (1996); Leberer et al. (1996)
	Don3p	*U. maydis*	Required for cytokinesis and cell separation	Weinzierl et al. (2002)
	Skm1p	*S. cerevisiae*	Phosphorylates septins and myosins during the establishment of polarised growth during budding	Johnson (1999)
Formin	Bni1p	*S. cerevisiae*	Scaffold protein for protein complex at presumptive bud site	Evangelista et al. (1997)
	SepA	*A. nidulans*	Polarised growth and septation of hyphae and conidiophores	Harris et al. (1997)
	MesA	*A. nidulans*	Promotes the assembly of actin cables by recruitment of SepA formin and sterol-rich membrane domains	Pearson et al. (2004)
Myosins	Myo3p, Myo5p, Myo2p, Myo4p	*S. cerevisiae*	Form a ring at the mother–bud neck. When phosphorylated, contracts and cytokinesis occurs	Wu et al. (1996)
	Myo5 3/5 homologue	*C. albicans*	Required for polarised growth and actin localisation of yeast cells during budding and for polarised growth of hyphae	Oberholzer et al. (2002)
Septins	Cdc3p, Cdc10p, Cdc11p, Cdc12p, Sep7p	*S. cerevisiae*	Form a ring at the mother–bud neck. When phosphorylated, contracts and cytokinesis occurs	Trimble (1999)
	Cdc10p, Cdc11p	*C. albicans*	Required for cytokinesis of yeast cells and polarised growth and chitin deposition during hyphal growth	Warenda and Konopka (2002)
	AspB	*A. nidulans*	Required for correct septation and branching patterns and maturation of conidiophores	Westfall and Momany (2002)
14-3-3 protein	Bmh1p, Bmh2p	*S. cerevisiae*	Regulates cell elongation	Roberts et al. (1997)
	Bmh1p	*C. albicans*	Required for germ tube formation and polarised growth of hyphae	Cognetti et al. (2002)
	ArtA	*A. nidulans*	Germ tube emergence	Kraus et al. (2002)
Polarisome component	Spa2p	*S. cerevisiae*	Part of the polarisome complex which polarises actin	Sheu et al. (1998)
	Spa2p	*C. albicans*	Polarised growth establishment and maintenance during budding and filamentation. Component of polarisome which polarises actin assembly	Zheng et al. (2003)
	Spa2p	*A. gossypii*	Determination of the area of growth at hyphal tips. Component of polarisome	Knechtle et al. (2003)
WASP	Bee1p	*S. cerevisiae*	Required during budding and cytokinesis to interact with Arp2/3 to nucleate actin filament assembly	Li (1997)
	Wal1p	*A. gossypii*	Maintenance of polarised growth of hyphae, actin polarisation, traffic of endosomes and vacuoles to the hyphal tip	Walther and Wendland (2004a)

Table. 1.1. (continued)

Protein type	Protein	Organism	Role	Reference
	Wal1p	*C. albicans*	Bud site selection, actin polarisation, endocytosis and hyphal formation	Walther and Wendland (2004b)
Ras GTPase	Ras2p	*S. cerevisiae*	Required for activation of both the cAMP and MAPK cascades which initiate pseudohyphal growth	Toda et al. (1985); Gimeno et al. (1992)
	Ras1p	*C. albicans*	Required for activation of both the cAMP and MAPK cascades which initiate filamentous growth	Leberer et al. (2001)
	RasA	*A. nidulans*	Initiation of polarised growth during conidial germination. Onset of asexual development	Som and Kolparthi (1994); Osherov and May (2000)
	RasA	*P. marneffei*	Initiation of polarised growth during conidial germination. Maintenance of polarised growth of hyphae. Onset of asexual development. Polarised growth of yeast cells	Boyce et al. (2005)
	Ras1	*C. trifolii*	Required for polarisation of hyphae, sporulation and appressoria formation	Memmot et al. (2002)
MAPKKK, MAPKK, MAPK	Ste11p, Ste7p, Kss1p	*S. cerevisiae*	Required for pseudohyphal differentiation	Reviewed in Lengeler et al. (2000)
	Hst11, Hst7p, Cek1p	*C. albicans*	Required for filamentous growth	Leberer et al. (1996)
	Ubc4p, Ubc5p, Ubc3p/Fuz7p	*U. maydis*	Required for filamentous growth, mating and pheromone production	Mayorga and Gold (1999)
Protein kinase A components	Bcy1p, Tpk1p, Tpk3p, Tpk2p	*S. cerevisiae*	Required for pseudohyphal differentiation	Reviewed in Lengeler et al. (2000)
	Tpk2	*C. albicans*	Regulates filamentous growth	Lengeler et al. (2000)
	Uac1p, Ubc1p	*U. maydis*	Required to activate filamentous growth	Gold et al. (1994); Mayorga and Gold (1999)

(Yaar et al. 1997; Ushinsky et al. 2002). *Ashbya gossypii* is a morphologically simple mycelial fungus, and recent studies of *AgRSR1* have shown that it is not required for the establishment of hyphal polarisation during spore germination. It is, however, important for maintenance of polarisation during hyphal growth and is localised at the tips of growing hyphae (Bauer et al. 2004). In more complex mycelial fungi, even less is known but the *A. gossypii* data suggest that selection of polarisation sites may not be solely due to *RSR1* orthologues.

3. Bud Emergence

S. cerevisiae GTP-bound Cdc42p regulates the establishment of polarised growth by recruiting additional proteins to the bud site (reviewed in Johnson 1999). After bud site selection, GTP-bound Cdc42p dissociates from both Cdc24p and Bem1p proteins and interacts with Gic1p, Gic2p and members of the family of p21-activated kinases (PAKs; reviewed in Brown et al. 1997; Johnson 1999; Richman et al. 1999). This complex binds to the Bni1p formin, which acts as a scaffold protein and binds a number of additional proteins including phosphorylated myosins and actin-associated proteins (Evangelista et al. 1997; Lechler et al. 2000; Fig. 1.5). This complex at the incipient bud site promotes the consequent localisation of the septin, chitin and myosin rings, and actin polymerisation at the bud tip (Lechler et al. 2000; Gladfelter et al. 2004; Kadota et al. 2004).

Different cell types can exhibit different cell cycle characteristics, and there is a close interplay between regulatory mechanisms which control cell growth and polarisation and cell cycle progression. Cell cycle control is imposed during budding by

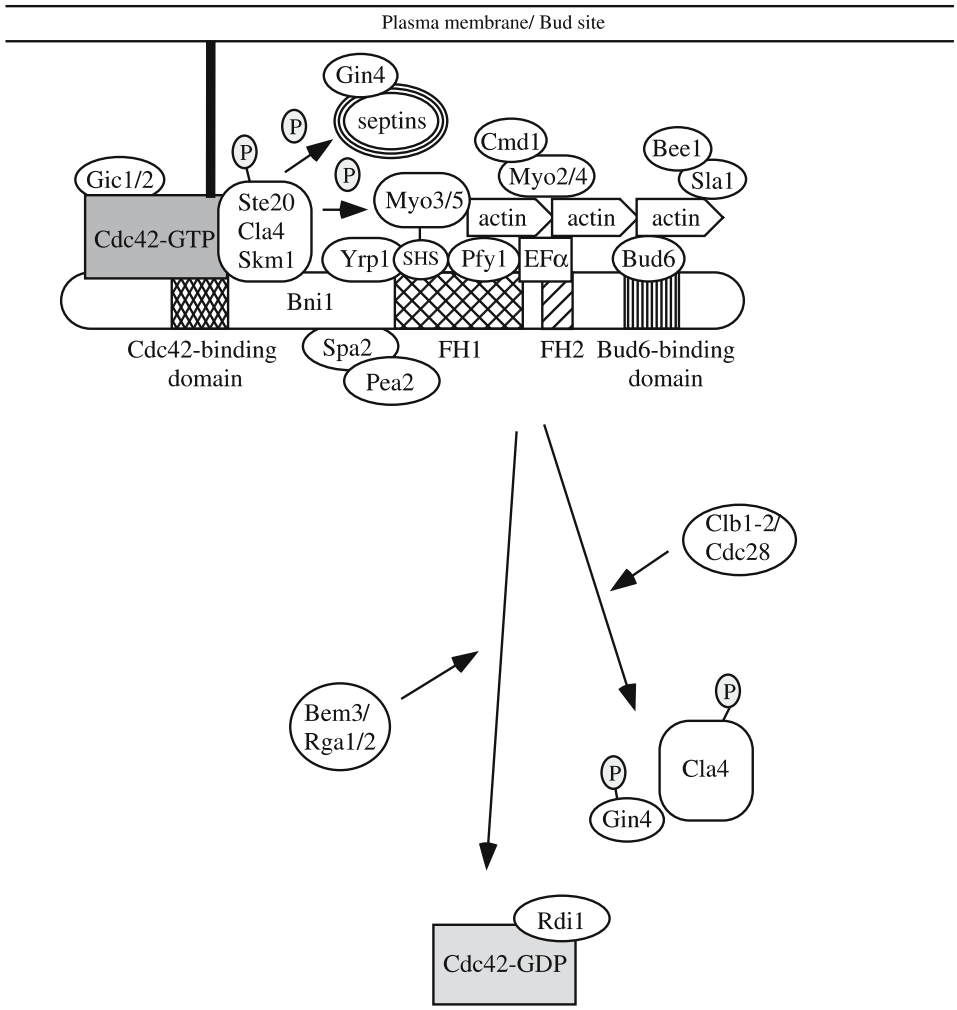

Fig. 1.5. Initiation of polarised growth in *S. cerevisiae*. The complex required for bud emergence through the initiation of polarised growth is shown. GTP-Cdc42p dissociates from Cdc24p and Bem1p, and interacts with Gic1p, Gic2p, Cla4p, Ste20p, Skm1p, and Bni1p. Bni1p acts as a scaffold protein and binds myosins (Myo3p and/or Myo5p, Myo2p and Myo4p), actin-associated proteins (Pfy1p, EFα, Bee1p, Bud6p and Sla1p), actin, additional Bni1p-binding proteins (Spa2p, Pea2p, and Yrp1p), myosin-associated protein Cmd1p, septin-associated protein Gin4p, the septin ring (Cdc3p, Cdc10p, Cdc11p, Cdc12p and Sep7p), chitin ring and myosin ring (Myo3p and Myo5p). Cdc42p causes the phosphorylation of PAK kinase Cla4p and septin-associated protein Gin4p, which leads to Cdc42p GTP to GDP exchange, catalysed by GAP proteins Bem3p, Rga1p or Rga2p. The growth switch also depends on the activation of the Clb1p-2p/Cdc28p kinase complex. Rdi1p removes GDP-bound Cdc42p from the membrane. Myo1p, and Chs3p (chitin synthase III) and associated proteins Bni4p and Chs4p act to expand the bud. (Adapted from Johnson 1999)

the cyclin-dependent kinase Cdc28p, which maintains the order of DNA replication, chromosome segregation and actin localisation (Lew and Reed 1993). Cdc28p is required to phosphorylate various proteins during each step of the budding process, and this activity can determine if a cell grows in a polarised manner or isotropically. To promote polarised growth by the restriction of actin polymerisation to the cell apex, Cdc28p is activated by the G1 cyclins Cln1p and Cln2p, and the Cdc28p-Cln2p complex can phosphorylate the PAK Ste20p (Lew and Reed 1995; Wu et al. 1998). Conversely, to promote isotropic growth, the cyclins Clb1p and Clb2p activate Cdc28p to direct not only chromosome segregation during mitosis but also redistribution of actin throughout the bud (Lew and Reed 1995; McMillan et al. 1998). The apical to isotropic switch also requires the activation of the Clb1p,

Clb2p and Cdc28p complex which phosphorylates the Nim-1-related kinase Gin4p and the PAK Cla4p (Longtine et al. 2000). Gin4p and Cla4p are required for the formation of the septin ring (Longtine et al. 2000; Mortensen et al. 2002; Fig. 1.5).

4. Pseudohyphal Growth

Under conditions of nitrogen starvation, diploid *S. cerevisiae* cells undergo a dimorphic transition involving changes in cell shape and division, termed pseudohyphal growth (Fig. 1.2A). Unlike the ellipsoidal cells, pseudohyphal cells are elongate and do not separate completely. The initiation of pseudohyphal growth requires polarity reestablishment and changes to actin polymerisation at the terminal ends of pseudohyphal cells. Although the mechanisms of polarised growth establishment during pseudohyphal growth is not as well characterised as budding, it is clear that this process uses some of the same proteins and mechanism (Table 1.1). However, unlike budding which operates on intrinsic cues, pseudohyphal growth is regulated by signal transduction pathways which relay extrinsic signals and lead to the up-regulation of genes required for changes to the cell's shape, adhesion properties and budding pattern.

The signal(s) inducing pseudohyphal growth remains unclear, but two signalling pathways have been implicated in pseudohyphal growth, a cAMP signalling pathway and a MAPK signalling pathway. The cAMP pathway signals through a heterotrimeric G protein (Gpa2p) which is associated with the Gpr1p glucose-sensing G protein coupled receptor (Lengeler et al. 2000). This signal and/or a signal from GTP-bound active Ras2p leads to activation of adenylate cyclase and results in the production of cAMP (Lengeler et al. 2000). The second pathway regulating pseudohyphal growth is also mediated by the Ras2p GTPase. GTP-bound Ras2p activates the GEF Cdc24p, which catalyses the GDP to GTP exchange of Cdc42p. Cdc42p-GTP consequently interacts with, and phosphorylates the PAK Ste20p, which results in the sequential phosphorylation and activation of components of the mitogen-activated protein kinase (MAPK) cascade (Madhani and Fink 1997).

Similarly to *S. cerevisiae*, there are two signalling pathways in *C. albicans* which regulate the transition from budding yeast to a filamentous form in response to environmental stimuli (such as pH, temperature and serum) – a cAMP-PKA pathway and a MAPK pathway, both of which are activated by the *C. albicans RAS1/2* homologue, *RAS1* (Lo et al. 1997; Lengeler et al. 2000). Ras1p regulates the activity of adenylate cyclase, which in turn regulates the cAMP pathway, and which is also regulated by a G-protein α subunit (Toda et al. 1985). Ras1p is considered to control Cdc42p activity, which leads to activation of the MAPK cascade (Leberer et al. 1996, 2001). Similarly to *S. cerevisiae* and *C. albicans*, a cAMP and a Ras signalling pathway also lead to the activation of a MAPK cascade regulating yeast-hyphal morphogenesis in the plant pathogen *Ustilago maydis* (Lee and Kronstad 2002; see Chap. 18, this volume).

In addition to the conservation of signalling pathways regulating morphogenesis, *C. albicans* requires some of the same factors for the establishment of polarisation during filamentous growth as does *S. cerevisiae*. Similarly to *S. cerevisiae*, the guanine exchange factor Cdc24p, the Rho GTPase Cdc42p and, to a lesser extent, the Ras-like GTPase Rsr1p are required for the establishment of polarisation during filamentous growth in *C. albicans* (Yaar et al. 1997; Ushinsky et al. 2002; Bassilana et al. 2003; Table 1.1). Polarity is also thought to be established by phosphorylation of myosins by PAKs activated by Cdc42p, as mutations in both the PAKs, Ste20p and Cla4p, and myosin-encoding Myo5p (a homologue of *S. cerevisiae* Myo3/5p) result in a loss of filamentous growth and incorrect localisation of cortical actin at cell tips (Oberholzer et al. 2002; Table 1.1). The Cdc24p, Cdc42p and Rsr1p proteins are also required for the initiation of polarised hyphal growth in the simple mycelial fungus *A. gossypii* (Wendland and Philippsen 2001; Bauer et al. 2004).

5. The Establishment of Polarised Growth in Mycelial Fungi – Conidial Germination

The formation of dormant spores is common in fungi, and these spores can be either sexually or asexually derived. Under appropriate conditions,

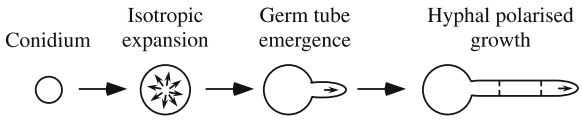

Fig. 1.6. Conidial germination. The dormant conidium begins germination after activation by environmental signals, and begins isotropic expansion (swelling). After mitosis, a site of polarisation is established and the conidium extends a germ tube. As the growth tip extends, nuclear division continues and septation is triggered to partition cells

spores will initiate growth, leading to the formation of a new colony, and the mechanisms by which this occurs involve significant cell shape or polarisation changes. Spherical asexual spores of the mycelial fungus *Aspergillus nidulans* germinate in the presence of water and a carbon source, by initiating isotropic growth until the first nuclear division, followed by the establishment of polarised growth to allow a germ tube to emerge (Harris et al. 1999; Momany et al. 1999; Osherov and May 2000; Fig. 1.6). Like *S. cerevisiae* and *C. albicans*, the signal to grow filamentously in *A. nidulans* is controlled by two signalling pathways, a cAMP pathway and a Ras pathway (Som and Kolaparthi 1994; Osherov and May 2000; Fillinger et al. 2002). However, unlike *S. cerevisiae* and *C. albicans*, the signalling pathways act independently in *A. nidulans*, as RasA activity does not regulate adenylate cyclase activity (Fillinger et al. 2002). It is possible that there is also a MAPK cascade regulating germination in *A. nidulans*, as the 14-3-3 protein, ArtA, is required for the establishment of polarised growth during germination. The homologues of this 14-3-3 protein, Bmh1/2p, in *S. cerevisiae* are required for MAPK cascade activation during pseudohyphal growth (Roberts et al. 1997; Kraus et al. 2002).

cAMP and Ras pathways also appear to regulate conidial germination in the dimorphic fungus *Penicillium marneffei*. The *P. marneffei* heterotrimeric G-protein α subunit encoded by *gasC* is required during conidial germination, and this most likely operates through a cAMP signalling pathway. Deletion of *gasC* results in severely delayed germination, whereas expression of a dominant activating allele shows a significantly accelerated germination rate (Zuber et al. 2003; Fig. 1.7). The *A. nidulans* orthologue of *gasC*, *ganB*, operates in a very similar manner, except that *P. marneffei gasC* mutants are still dependent on a carbon source for germination whereas *A. nidulans ganB* mutants are not (Zuber et al. 2003; Chang et al. 2004). Likewise, the *P. marneffei* Ras homologue, RasA, is required for conidial germination, with expression of a dominant negative or an activated *rasA* allele both resulting in a delay in germination and in conidia with abnormal isotropic growth (Boyce et al. 2005). Genes required for polarity establishment during budding and pseudohyphal growth in yeasts are also required during spore germination in mycelial fungi. Expression of a dominant negative or a constitutively active allele of the *CDC42* homologue in *P. marneffei*, *cflA*, results in a decrease or increase in the rate of germination respectively

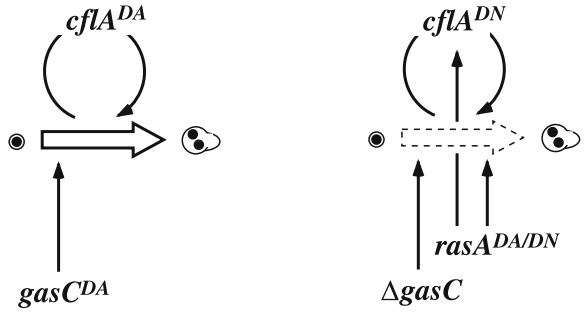

Fig. 1.7. Control of conidial germination in *P. marneffei*. Conidial germination in *P. marneffei* is precocious when either a dominant activated allele of the G α subunit-encoding *gasC* (*gasC*^{DA}) gene or a dominant activated allele of the small Rho-type (*CDC42*) GTPase orthologue *cflA* (*cflA*^{DA}) are expressed. Conversely, conidial germination is delayed when either *gasC* is deleted, a dominant negative allele of *cflA* (*cflA*^{DN}) is expressed, or a dominant negative or an activated allele of the *rasA* (*rasA*$^{DA/DN}$) GTPases are expressed

(Boyce et al. 2001). The dominant activated *cflA* allele (which results in an accelerated rate of germination) suppresses the germination delay of the dominant negative *rasA* mutant, suggesting that RasA acts upstream of CflA during germination in *P. marneffei* (Boyce et al. 2005).

6. Polarised Growth Maintenance

Once polarity is initiated in mycelial fungi, it must be maintained in order for linear hyphal growth to proceed, and be re-established when hyphal branching occurs. Polarisation of hyphae requires cell growth to be continually directed to the hyphal apex. Actin and microtubules facilitate the transport of vesicles containing cell wall material and enzymes to the apical region. Actin is concentrated at the germ tube tip, and it has been shown that the actin inhibitor cytochalasin A disrupts this localisation and thus inhibits hyphal growth (d'Enfert 1997).

The core components regulating the maintenance of actin polarisation to the site of growth appear to be conserved in both budding yeast and mycelial fungi. In *S. cerevisiae*, actin polarisation is maintained at the site of growth by the Rho GTPase Cdc42, involving a number of different mechanisms. Cdc42p interacts with a protein complex, called the polarisome, at the site of polarised growth (Evangelista et al. 1997; Fujiwara et al. 1998). In *S. cerevisiae*, proteins in the polarisome include the scaffold protein Spa2p, the WASP (Wiskott Aldrich Syndrome protein) protein Bee1p

and the formin Bni1p, and polarisome localisation to growth sites occurs in a cell cycle-dependent manner (Evangelista et al. 1997; Fujiwara et al. 1998). *C. albicans SPA2* homologue mutants exhibit wide, elongated hyphae with nuclear positioning defects and reduced actin localisation, suggesting that the polarisome is essential for polarity maintenance in mycelial fungi (Zheng et al. 2003). The second mechanism of Cdc42-dependent maintenance of polarised growth is via the activation of the PAKs Ste20p and Cla4p, which regulate myosins, WASP proteins and Arp2/3-dependent actin nucleation to form actin patches. Intrinsic to these processes is the role of *S. cerevisiae* Cdc42 in endocytosis and exocytosis (reviewed in Johnson 1999). The Cdc42p protein is also required for polarity maintenance in mycelial fungi, although the details of how this is achieved have yet to be uncovered. Expression of a dominant negative or a constitutively active allele of the Cdc42p homologue in *P. marneffei*, *cflA*, results in swollen, aberrantly shaped hyphae with a depolarised actin cytoskeleton (Boyce et al. 2001). Homologues of proteins which are present in the polarisome of *S. cerevisiae* are also required for the maintenance of polarised growth in mycelial fungi, suggesting that the Cdc42p/polarisome actin regulation pathway is conserved. The homologue of the Bni1p formin in *A. nidulans*, *sepA*, is required for actin-mediated polarised growth and septation of both hyphae and conidiophores (acting as a scaffold), and is recruited to the hyphal tip by the transmembrane protein MesA (Harris et al. 1997; Pearson et al. 2004). Similarly, the Spa2p homologue in *A. gossypii* determines the site of growth at hyphal tips (Knechtle et al. 2003).

Similarly to *S. cerevisiae*, Cdc42-dependent maintenance of polarised growth in mycelial fungi also leads to activation of WASP proteins, possibly via the activation of the PAK Cla4p, which regulates myosins, WASP proteins and Arp2/3-dependent actin nucleation to form actin patches in *S. cerevisiae*. In *S. cerevisiae*, the WASP protein Bee1p (which is also a polarisome protein) interacts with Arp2/3p to nucleate actin during budding and cytokinesis (Li 1997). In both *C. albicans* and *A. gossypii*, the WASP homologue (Wal1p) regulates the maintenance of polarised growth by regulating actin polymerisation and trafficking of endosomes and vacuoles (Walther and Wendland 2004a,b).

Despite some of the core components regulating the maintenance of polarised growth being conserved between budding yeast and mycelial fungi, the requirement for additional cell shapes and developmental programs in mycelial fungi makes the situation more complex. In higher eukaryotes, a second Cdc42-like Rho GTPase, named Rac, also exists. Rac orthologues are not found in less morphologically complex eukaryotes such as *S. cerevisiae* and *A. gossypii*, suggesting that *RAC* genes may have evolved with increasing cellular complexity. The *RAC* orthologue in the dimorphic fungus *P. marneffei*, *cflB*, is also required during polarised growth maintenance (Fig. 1.8). Deletion of *cflB* results in depolarised, swollen hyphae with a delocalised actin cytoskeleton, and CflB colocalises with actin at the tips of vegetative hyphal cells (Boyce et al. 2003). A *P. marneffei* double mutant with the *cflB* deletion and the dominant activated *cflA* allele has a depolarised phenotype more severe than either of the single mutants alone, suggesting that CflA and CflB may have overlapping roles during actin-mediated polarised growth maintenance (Boyce et al. 2005). Despite some overlapping

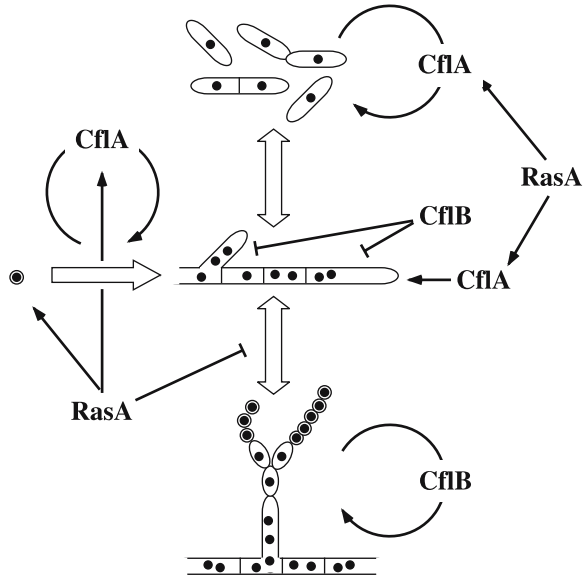

Fig. 1.8. Small GTPases differentially regulate different cell types in *P. marneffei*. Representation of the various cell types of *P. marneffei*; conidia (*centre*, *left*) can germinate to produce hyphal cells (*centre*) which consist of actively growing apical cells, branch cells and subapical cells, which in turn can undergo the asexual development program at 25 °C to produce foot, stalk, sterigmata and conidial cell types (*bottom*), or the arthroconidiation program at 37 °C to produce the yeast cell type (*top*). The various Ras- and Rho-type GTPases which control morphogenesis of these cell types are shown, as is their proposed action

roles, CflA and CflB clearly have additional, unique roles in *P. marneffei*. The phenotypes of *cflA* (*CDC42* homologue) and *cflB* mutants are distinguishable. Deletion of *cflB* affects not only polarisation of hyphae but also subapical and apical branching and the formation of asexual development structures, but not the polarity of yeast cells (Boyce et al. 2003). By contrast, mutation of *cflA* affects polarity of yeast cells but not hyphal branching and asexual development structures (Boyce et al. 2001). The *RAC* homologue in the alfalfa pathogen *Colletotrichum trifolii* is also required for polarised hyphal growth (Chen and Dickman 2004).

Polarity mutants have been isolated in *A. nidulans* and *Neurospora crassa*, but the genes mutated in these mutants are only just beginning to be cloned and analysed (Kaminskyj and Hamer 1998; Harris et al. 1999; Momany et al. 1999; Seiler and Plamann 2003). Recently, a novel regulator of hyphal polarity, *hbrB*, was identified in *A. nidulans* by complementation of a hyperbranching and hyperseptated mutant named *hbrB3* (Gatherar et al. 2004). The gene is essential for hyphal growth and polarity, and appears specific for mycelial fungi, with no obvious homologues in non-mycelial fungi (Gatherar et al. 2004).

7. Vesicle Transport

Polarised tip growth relies on the fusion of vesicles at the hyphal apex. SNARE (SNAP (*s*oluble *N*SF *a*ttachment *p*rotein) *re*ceptor) proteins are present on the surface of both vesicles and target membranes, and these interact to regulate vesicle fusion (Ungar and Hughson 2003). In *N. crassa*, expression of an antisense construct for *nssyn1*, which encodes a SNARE protein homologous to *S. cerevisiae* Sso1p, results in smaller hyphal diameter, reduced hyphal polarity and abnormal branching (Gupta et al. 2003). In addition, RIP (*r*epeat *i*nduced *p*oint mutation)-generated mutations in the SNARE-encoding gene *nssyn2* (homologous to *S. cerevisiae* Sso2p) produced hyphae with swollen apices and abnormal apical branching (Gupta et al. 2003).

Vesicles transported to the apical region contain enzymes required to expand the apex, such as chitin synthetases and glucan synthetases (Gow 1994). In *S. cerevisiae*, the Rho GTPase Rho1p regulates cell wall deposition, in addition to playing a role during the establishment of cell polarity and reorganisation of the actin cytoskeleton. How Rho1p affects the establishment of polarity is unclear, but mutations in *RHO1* have been shown to result in loss of Cdc42p and Spa2p to the presumptive bud site (Drgonova et al. 1999). In addition, Rho1p and Cdc42p both have a direct role in mediating the docking stage of vacuole fusion, which may be important for cell polarity (Eitzen et al. 2001). During cell wall deposition, Rho1p regulates protein kinase C (encoded by *PKC1*) and the cell wall-synthesizing enzyme 1,3-beta-glucan synthase (encoded by *FKS1* and *GSC2*; Drgonova et al. 1996; Qadota et al. 1996). Likewise, in *A. nidulans*, *rhoA* is required for the regulation of cell wall deposition (Guest et al. 2004). Expression of a constitutively active *rhoAG14V* or a dominant negative *rhoAE40I* allele in *A. nidulans* results in abnormal branch emergence and cell wall deposition defects or accelerated germ tube and branch emergence, cell wall defects and cell lysis respectively (Guest et al. 2004). Rho1p and Rho3p in *A. gossypii* are also required for the maintenance of polarised hyphal growth (Wendland and Philippsen 2001).

Chitin is a major structural component of fungal cell walls, and the rapid synthesis and assembly of chitin at the hyphal apex is crucial for hyphal extension. Growth at the hyphal apex also requires the rapid degradation of chitin, as existing cell wall must be removed before the hypha can be extended. Chitin synthases catalyse chitin polymerisation, and disruption of the chitin synthase-encoding gene *csmA* results in hyphal cells with depolarised, swollen apical cells and the production of intrahyphal hypha (hyphae within hyphae; Horiuchi et al. 1999). The deletion of other biosynthetic enzymes required for polarised growth at the hyphal apex also results in polarisation defects. Sphingolipids are major components of the plasma membrane. The inactivation of serine palmitoyltransferase, the first enzyme in the sphingolipid biosynthesis pathway, abolishes cell polarity, resulting in apical branching. This branching is a result of abnormal actin localisation in this strain (Cheng et al. 2001).

Vacuolar functions are also required for organelle positioning and polarised growth. Deletion of *A. nidulans digA*, a homologue of the *S. cerevisiae PEP3* gene which is required for intracellular vesicle sorting, results in dichotomous branching, clustered mitochondria and nuclei, and a defect in the polarisation of the actin cytoskeleton (Geissenhoner et al. 2001). In *A. nidulans*, the *hypA*, also known as *podA* and *swoE*, has been shown to be required for the secretory pathway (Kaminskji

and Hamer 1998; Harris et al. 1999; Momany et al. 1999; Shi et al. 2004). Deletion of *hypA* results in swollen hyphae which lyse, abnormal conidia and nuclear defects. In addition, the *ΔhypA* and *hypA1* mutant strains showed decreased secretion of amylose, disorganisation of endomembranes and loss of the Spitzenkörper (an apical cluster of vesicles and cytoskeletal elements visible at the cell apex), which suggests a role in the secretory pathway (Shi et al. 2004).

C. Polarised to Isotropic Growth

1. Budding

After bud emergence in *S. cerevisiae*, a switch from polarised growth to isotropic growth occurs, allowing the bud to grow in all directions. This transition involves the hydrolysis of GTP bound to Cdc42p, catalysed by one or more GTPase activating proteins, and results in the phosphorylation of Gic2p, the PAK Cla4p and the cell cycle-associated protein Gin4p, thereby effecting the switch from apical growth of the bud to isotropic growth. This growth switch also depends on the activation of the complex containing the cyclins Clb1p and Clb2p, and the cyclin-dependent kinase (CDK) Cdc28p, as bud morphogenesis is coupled to cell division control. Bud emergence is followed by the formation of an actomyosin contractile ring at the mother–bud junction, cytokinesis and cell separation (reviewed in Johnson 1999).

2. Appressorium Peg

During infection, hyphae of plant pathogens differentiate specialized infection structures called appressoria. Appressoria are formed by the swelling of the germ tube tip (analogous to a switch from polarised growth to isotropic growth) when the appropriate signal(s) are received from the plant surface. Subsequently, formation of the penetration peg which enters the plant requires an isotropic to polarised switch. Unfortunately, little is known about the role of cell polarity determinants in appressorium formation. It has been shown in the rice pathogen *Magnarporthe grisea* that cAMP signalling regulates appressorium formation (Xu and Hamer 1996). In addition, a number of MAPKs have been identified in *M. grisea* which are required for appressorium formation and cell wall integrity, including Pmk1 (homologue of *S. cerevisiae* Fus3p), Mps1 (homologue of *S. cerevisiae* Slt2p), Mst7 and Mst11 (homologues of *S. cerevisiae* Ste7p MEK and Ste11p MEKK) (Xu et al. 1993; Xu and Hamer 1996; Xu et al. 1998; Zhao et al. 2005). The *RAS* homologue *C. trifolii* is also required for polarised growth of hyphae and appressoria formation (Memmott et al. 2002).

3. Conidiophore Production

Many mycelial fungi produce asexual spores on specialized cellular structures termed conidiophores (see Chap. 14, this volume). The development of conidiophores requires the differentiation of morphologically distinct cell types from the vegetative mycelium, and thus changes in polarised growth. In *A. nidulans*, the conidiation process begins with the growth of a conidiophore stalk by apical extension from a thick-walled vegetative cell termed a foot cell (Fig. 1.9). The tip of the stalk then undergoes an apical to isotropic switch to produce the conidiophore vesicle. The foot cell, stalk and vesicle are not separated by septa. A layer of uninucleate cells termed metulae bud from the vesicle, and these metulae then bud from their distal end to produce uninucleate cells termed phialides, in a process analogous to polar budding in *S. cerevisiae*. Repeated asymmetric divisions by phialides produce chains of uninucleate conidia (Adams et al. 1998). The central regulatory pathway controlling asexual development includes three genes specifically required for conidiation, *brlA*, *abaA* and *wetA* (Adams et al. 1988; Mirabito et al. 1989). These gene products control conidiation-specific gene expression and the order of gene expression during conidiophore development. Two other developmental genes, *stuA* and *medA*, are necessary for the spatial pattern of the conidiophore (Adams et al. 1998). The central regulatory pathway is inhibited by a G-protein-mediated signalling pathway (Adams et al. 1998). The small GTPase RasA also regulates the onset of asexual development in *A. nidulans*, independently of the G-protein signalling pathway (Som and Kolaparthi 1994). Although the morphology of *P. marneffei* conidiophores differs from that of *A. nidulans*, the molecular mechanisms which control this developmental program are conserved (Zuber et al. 2002; Boyce et al. 2005).

How changes in polarity are mediated during asexual development in mycelial fungi remains largely unknown. Similarly to other developmental pathways, some of the key components required for polarity establishment and maintenance have

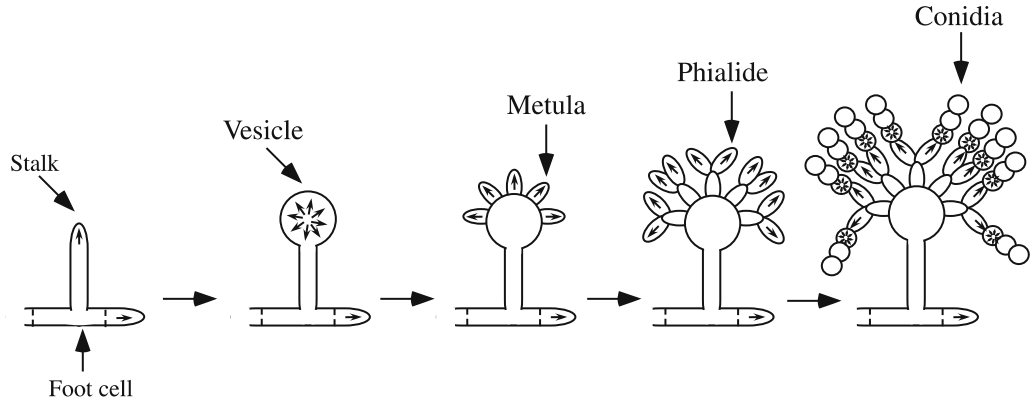

Fig. 1.9. Polarisation events during asexual development in *A. nidulans*. Hyphal cells grow by directing growth exclusively to the hyphal apex, followed by septation behind the growing front at regular intervals. Branching requires the re-initiation of polarised growth in subapical cells. During asexual development, an aerial stalk cell is produced from a thick-walled cell of the basal mycelium (foot cell) by apical extension. The stalk grows to a predetermined height, and then growth becomes depolarised at the tip to produce a globose vesicle. Uninucleate cells termed metulae bud from the vesicle, in a process analogous to budding in yeast. Phialides bud from metulae which, in turn, bud off conidia by basipetal division to produce long chains of uninucleate conidia. *Arrowheads* in cells indicate directions of growth

been shown to play a role during conidiophore development. In *A. nidulans*, deletion of the MAPKK-encoding gene *steC* (which shows homology to *S cerevisiae STE11*) results in a slower growth rate, curled and branched hyphae, and altered conidiophore development (Wei et al. 2003). Conidiophores have altered stalk height, large conidia, some metulae fail to develop further, and secondary conidiophores are produced from the vesicle (Wei et al. 2003). Genes encoding proteins required for downstream polarity events have also been identified in *A. nidulans*. One such family is the septin proteins, which act as organisational scaffolds in areas of new growth. The *A. nidulans* septin *aspB* mutant strain, in addition to possessing septation defects in hyphae, also has defects in conidiation. In contrast to the wild type which shows mature conidiophores by 24 h the *aspB-318* strain produced only immature reproductive structures, the majority of conidiophores arresting at the vesicle stage and only a small proportion going on to form metulae (Westfall and Momany 2002). AspB localizes transiently to the vesicle/metula and metula/phialide interfaces in an actin-dependent manner and consistently at the phialide/conidia interface, suggesting that it is crucial for these cell division events (Westfall and Momany 2002).

CDC42 homologues regulate the establishment of polarised growth in *S. cerevisiae* and many other fungi (reviewed in Johnson 1999). It would therefore be expected that *CDC42* homologues are required during the switch from isotropic to polarised growth during metula, phialide or conidial production. However, the *CDC42* homologue from *P. marneffei*, *cflA*, does not appear to have a role during conidiophore development. Expression of dominant negative or dominant activated *cflA* alleles does not affect conidiophore development, despite having major effects on the underlying vegetative hyphal cells from which conidiophores derive (Boyce et al. 2001). Interestingly, when the mutant alleles of *P. marneffei cflA* were expressed in *A. nidulans*, a complete lack of conidiation was observed, suggesting that the *CDC*42 homologue in *A. nidulans* may be required during conidiophore formation (Boyce et al. 2001). In contrast to *cflA*, the *RAC* homologue in *P. marneffei*, *cflB*, is required for conidiophore development. Deletion of *cflB* results in swollen, aberrantly shaped cells in the conidiophores which lack actin at the site of polarised growth (Boyce et al. 2003). Despite the loss in shape and polarisation, these cells still produce conidia and these conidia are viable, indicating that although cell shape is lost, the integrity of the differentiated cell types is maintained. In addition, when dominant negative and dominant constitutively activated *cflB* alleles were expressed in *A. nidulans*, conidiophores with abnormally extended metulae or phialides were produced, suggesting that Rac regulates the switch from isotropic to polarised growth, and vice versa in *A. nidulans* (Boyce et al. 2003). The disparity in phenotypes of *cflA* mutants

in *P. marneffei* and *A. nidulans* suggests that a specific Rho GTPase can regulate different developmental programs in different fungal species. In addition, the difference in phenotypes produced by different *cflB* mutant alleles in *P. marneffei* and *A. nidulans* suggests that a specific Rho GTPase can also regulate different aspects within a developmental program. These differences are most likely attributed to varied regulation of the GTPases and differential activation of downstream effectors.

III. Septation

Cellular division, called septation in fungi, is an important aspect of cell type generation. In *S. cerevisiae* and *C. albicans*, cellular division requires the formation of a septin/actomyosin contractile ring at the mother–bud junction, cytokinesis and cell separation (Leberer et al. 1997; Ushinsky et al. 2002; Warenda and Konopoka 2002). In mycelial fungi, cytokinesis is completed by the formation of crosswalls called septa. Septa are laid down at uniform intervals along the hyphae, behind the growing apical tip, and have septal pores which facilitate the transport of cytoplasm and organelles between cells; this cellular transport promotes a rapid rate of apical cell growth (Jedd and Chua 2000). Microtubules and actin are essential for the septation process (Momany and Hamer 1997; Westfall and Momany 2002).

Interestingly, many components of the machinery required for the establishment of polarised growth in fungi are also utilized during septation. The *RAS*, *CDC42* and *RAC* homologues in *P. marneffei* are required for polarised growth of hyphae but also play a role during septation. Both the CflA and CflB GTPases are localised to nascent septation sites, and mutations in either *rasA*, *cflA* or *cflB* result in hyphae with aberrantly positioned septa (Boyce et al. 2001, 2003). Likewise, the PAKs Cla4p and Ste20p, myosins and septins in *C. albicans*, and septins and formins in *A. nidulans* are required for both septation and polarised growth of hyphae (Leberer et al. 1992, 1996, 1997; Köhler and Fink 1996; Harris et al. 1997; Oberholzer et al. 2002; Warenda and Konopka 2002; Westfall and Momany 2002). Clearly, many of the polarisation components required for cellular expansion during growth are also involved in cell separation. Although this is an obvious link for coupled cell division and cytokinetic events in yeast such as *S. cerevisiae* and *C. albicans*, the link in mycelial fungi where polarised growth is distant from sites of septation is intriguing.

IV. Conclusions

Many of the genes required during polarised growth establishment and maintenance comprise a conserved core which is used to orchestrate global changes in morphology in a broad range of fungi, and therefore generate the various cell types produced by these organisms. The ability to utilize these conserved core components to elicit a variety of cell shapes in different organisms is most likely a result of differences in the specific modes of regulation of these proteins and their upstream activators, in addition to a diverse set of downstream effectors. The field is at an exciting stage, with the major players identified and characterised in a number of systems, and it will be interesting to see how these players are regulated and their activates recruited differently in different contexts to effect the various cell types and shapes which exist.

References

Adams TH, Boylan MT, Timberlake WE (1988) *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell 54:353–362

Adams AE, Johnson DI, Longnecker RM, Sloat BF, Pringle JR (1990) *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J Cell Biol 111:131–142

Adams TH, Wieser JK, Yu J (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Bassilana M, Blyth J, Arkowitz RA (2003) Cdc24, the GDP-GTP exchange factor for Cdc42, is required for invasive hyphal growth of *Candida albicans*. Eukaryot Cell 2:9–18

Bauer Y, Knechtle P, Wendland J, Helfer H, Philippsen P (2004) A Ras-like GTPase is involved in hyphal growth guidance in the filamentous fungus *Ashbya gossypii*. Mol Biol Cell 15:4622–4632

Bender A, Pringle JR (1989) Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including the *ras*-related gene *RSR1*. Proc Natl Acad Sci USA 86:9976–9980

Benton BK, Tinkelenberg A, Gonzalez I, Cross FR (1997) Cla4p, a *Saccharomyces cerevisiae* Cdc42p-activated kinase involved in cytokinesis, is activated at mitosis. Mol Cell Biol 17:5067–5076

Boyce KJ, Hynes MJ, Andrianopoulos A (2001) The *CDC42* homolog of the dimorphic fungus *Penicillium marneffei* is required for correct cell polarization during growth but not development. J Bacteriol 183:3447–3457

Boyce KJ, Hynes MJ, Andrianopoulos A (2003) Control of morphogenesis and actin localization by the *Penicillium marneffei RAC* homolog. J Cell Sci 116:1249–1260

Boyce KJ, Hynes MJ, Andrianopoulos A (2005) The Ras and Rho GTPases genetically interact to co-ordinately regulate cell polarity during development in *Penicillium marneffei*. Mol Microbiol 55:1487–1501

Brown JL, Jaquenoud M, Gulli MP, Chant J, Peter M (1997) Novel Cdc42-binding proteins Gic1 and Gic2 control cell polarity in yeast. Genes Dev 11:2972–2982

Chang MH, Chae KS, Han DM, Jahng KY (2004) The GanB Gα-protein negatively regulates asexual sporulation and plays a positive role in conidial germination in *Aspergillus nidulans*. Genetics 167:1305–1315

Chen C, Dickman MB (2004) Dominant active Rac and dominant negative Rac revert the dominant active Ras phenotype in *Colletotrichum trifolii* by distinct signalling pathways. Mol Microbiol 51:1493–1507

Cheng J, Park T, Fischl AS, Ye XS (2001) Cell cycle progression and cell polarity require sphingolipid biosynthesis in *Aspergillus nidulans*. Mol Cell Biol 21:6198–6209

Cognetti D, Davis D, Sturtevant J (2002) The *Candida albicans* 14-3-3 gene, *BMH1*, is essential for growth. Yeast 19:55–67

d'Enfert C (1997) Fungal spore germination: insights from the molecular genetics of *Aspergillus nidulans* and *Neurospora crassa*. Fungal Genet Biol 21:163–172

Drgonova J, Drgon T, Tanaka K, Kollar R, Chen GC, Ford RA, Chan CS, Takai Y, Cabib E (1996) Rho1p, a yeast protein at the interface between cell polarization and morphogenesis. Science 272:277–279

Drgonova J, Drgon T, Roh DH, Cabib E (1999) The GTP-binding protein Rho1p is required for cell cycle progression and polarization of the yeast cell. J Cell Biol 146:373–387

Eitzen G, Thorngren N, Wickner W (2001) Rho1p and Cdc42p act after Ypt7p to regulate vacuole docking. EMBO J 20:5650–5656

Evangelista M, Blundell K, Longtine MS, Chow CJ, Adames N, Pringle JR, Peter M, Boone C (1997) Bni1p, a yeast formin linking Cdc42p and the actin cytoskeleton during polarized morphogenesis. Science 276:118–122

Fillinger S, Chaveroche M, Shimizu K, Keller N, d'Enfert C (2002) cAMP and Ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 44:1001–1016

Fujiwara T, Tanaka K, Mino A, Kikyo M, Takahashi K, Shimizu K, Takai Y (1998) Rho1p-Bni1p-Spa2p interactions: implication in localization of Bni1p at the bud site and regulation of the actin cytoskeleton in *Saccharomyces cerevisiae*. Mol Biol Cell 9:1221–1233

Gatherar IM, Pollerman S, Dunn-Coleman N, Turner G (2004) Identification of a novel gene *hbrB* required for polarised growth in *Aspergillus nidulans*. Fungal Genet Biol 41:463–471

Geissenhoner A, Sievers N, Brock M, Fischer R (2001) *Aspergillus nidulans* DigA, a potential homolog of *Saccharomyces cerevisiae* Pep3 (Vps18), is required for nuclear migration, mitochondrial morphology and polarized growth. Mol Gen Genomics 266:672–685

Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. Cell 68:1077–1090

Gladfelter AS, Zyla TR, Lew DJ (2004) Genetic interactions among regulators of septin organization. Eukaryot Cell 3:847–854

Glotzer M (2005) The molecular requirements for cytokinesis. Science 307:1735–1739

Gold S, Duncan G, Barrett K, Kronstad J (1994) cAMP regulates morphogenesis in the fungal pathogen *Ustilago maydis*. Genes Dev 8:2805–2816

Gow NAR (1994) Growth and guidance of the fungal hypha. Microbiology 140:3193–3205

Guest GM, Lin X, Momany M (2004) *Aspergillus nidulans* RhoA is involved in polar growth, branching, and cell wall synthesis. Fungal Genet Biol 41:13–22

Gupta GD, Free SJ, Levina NN, Keranen S, Heath IB (2003) Two divergent plasma membrane syntaxin-like SNAREs, *nsyn1* and *nsyn2*, contribute to hyphal tip growth and other developmental processes in *Neurospora crassa*. Fungal Genet Biol 40:271–286

Harris SD, Hamer L, Sharpless KE, Hamer JE (1997) The *Aspergillus nidulans sepA* gene encodes an FH1/2 protein involved in cytokinesis and the maintenance of cellular polarity. EMBO J 16:3474–3483

Harris SD, Hofman AF, Tadford HW, Lee MP (1999) Identification and characterisation of genes required for hyphal morphogenesis in the filamentous fungus *Aspergillus nidulans*. Genetics 151:1015–1035

Hartwell LH (1974) *Saccharomyces cerevisiae* cell cycle. Bacteriol Rev 38:164–198

Horiuchi H, Fujiwara M, Yamashita S, Outa A, Takagi M (1999) Proliferation of intrahyphae caused by disruption of *csmA*, which encodes a class V chitin synthase with a myosin motor-like domain in *Aspergillus nidulans*. J Bacteriol 181:3721–3729

Jedd G, Chua N (2000) A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat Cell Biol 2:226–232

Johnson DI (1999) Cdc42: An essential Rho-type GTPase controlling eukaryotic cell polarity. Microbiol Mol Biol Rev 63:54–105

Kadota J, Yamamoto T, Yoshiuchi S, Bi E, Tanaka K (2004) Septin ring assembly requires concerted action of polarisome components, a PAK kinase Cla4p, and the actin cytoskeleton in *Saccharomyces cerevisiae*. Mol Biol Cell 15:5329–5345

Kaminskyj SG.W, Hamer JE (1998) *hyp* loci control cell pattern formation in the vegetative mycelium of *Aspergillus nidulans*. Genetics 148:669–680

Knechtle P, Dietrich F, Philippsen P (2003) Maximal polar growth potential depends on the polarisome component AgSpa2 in the filamentous fungus *Ashbya gossypii*. Mol Biol Cell 14:4140–4154

Köhler J, Fink GR (1996) *Candida albicans* strains heterozygous and homozygous for mutations in mitogen-activated protein kinase signalling components have defects in hyphal development. Proc Natl Acad Sci USA 93:13223–13228

Kraus PR, Hofmann A, Harris SD (2002) Characterisation of the *Aspergillus nidulans* 14-3-3-homologue, ArtA. FEMS Microbiol Lett 210:61–66

Leberer E, Dignard D, Harcus D, Thomas DY, Whiteway M (1992) The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein βγ subunits to downstream signalling components. EMBO J 11:4815–4824

Leberer E, Harcus D, Broadbent ID, Clark KL, Dignard D, Ziegelbauer Z, Schmidt A, Gow NAR, Brown AJP, Thomas D (1996) Signal transduction through homologs of the Ste20p and Ste7p protein kinases can trigger hyphal formation in the pathogenic fungus *Candida albicans*. Proc Natl Acad Sci USA 93:13217–13222

Leberer E, Ziegelbauer K, Schmidt A, Harcus D, Dignard D, Ash J, Johnson L, Thomas DY (1997) Virulence and hyphal formation of *Candida albicans* require the Ste20p-like protein kinase CaCla4p. Curr Biol 7:539–546

Leberer E, Harcus D, Dignard D, Johnson L, Ushinsky SC, Thomas DY, Schroppel K (2001) Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus *Candida albicans*. Mol Microbiol 42:673–687

Lechler T, Shevchenko A, Li R (2000) Direct involvement of yeast type I myosins in Cdc42-dependent actin polymerization. J Cell Biol 148:363–73

Lee N, Kronstad JW (2002) *ras2* controls morphogenesis, pheromone response, and pathogenicity in the fungal pathogen *Ustilago maydis*. Eukaryot Cell 1:954–966

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen W, Wang P, Ran X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Lew DJ, Reed SI (1993) Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. J Cell Biol 120:1305–1320

Lew DJ, Reed SI (1995) Cell cycle control of morphogenesis in budding yeast. Curr Opin Genet Dev 5:17–23

Li R (1997) Bee1, a yeast protein with homology to Wiscott-Aldrich syndrome protein, is critical for the assembly of cortical actin cytoskeleton. J Cell Biol 136:649–658

Lo HJ, Köhler B, DiDomenico B, Loebenberg D, Cacciapuoti A, Fink GR (1997) Nonfilamentous *C. albicans* mutants are avirulent. Cell 90:939–949

Longtine MS, Theesfeld CL, McMillan JN, Weaver E, Pringle JR, Lew DJ (2000) Septin-dependent assembly of a cell cycle-regulatory module in *Saccharomyces cerevisiae*. Mol Cell Biol 20:4049–4061

Madaule P, Furuyashiki T, Eda M, Bito H, Ishizaki T, Narumiya S (1987) Characterization of two members of the *rho* gene family from the yeast *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 84:779–783

Madhani HD, Fink GR (1997) Combinatorial control required for the specificity during yeast MAPK signalling. Science 275:1314–1317

Marquitz AR, Harrison JC, Bose I, Zyla TR, McMillan JN, Lew DJ (2002) The Rho-GAP Bem2p plays a GAP-independent role in the morphogenesis checkpoint. EMBO J 21:4012–4025

Matsui Y, Toh-E A (1992) Yeast *RHO3* and *RHO4* ras superfamily genes are necessary for bud growth, and their defect is suppressed by a high dose of bud formation genes *CDC42* and *BEM1*. Mol Cell Biol 12:5690–5699

Mayorga ME, Gold SE (1999) A MAP kinase encoded by the *ubc3* gene of *Ustilago maydis* is required for filamentous growth and full virulence. Mol Microbiol 34:485–497

McMillan JN, Sia RA, Lew DJ (1998) A morphogenesis checkpoint monitors the actin cytoskeleton in yeast. J Cell Biol 142:1487–1499

Memmott SD, Ha YS, Dickman MB (2002) Proline reverses the abnormal phenotypes of *Colletotrichum trifolii* associated with expression of endogenous constitutively active Ras. Appl Environ Microbiol 68:1647–1651

Mirabito PJ, Adams TH, Timberlake WE (1989) Interactions of three sequentially expressed genes control temporal and spatial specificity in *Aspergillus* development. Cell 57:859–868

Momany M, Hamer JE (1997) Relationship of actin, microtubules and crosswall synthesis during septation in *Aspergillus nidulans*. Cell Motil Cytoskeleton 38:373–384

Momany M, Westfall PJ, Abramowsky G (1999) *Aspergillus nidulans swo* mutants show defects in polarity establishment, polarity maintenance and hyphal morphogenesis. Genetics 151:557–567

Mortensen EM, McDonald H, Yates J III, Kellogg DR (2002) Cell cycle-dependent assembly of a Gin4-septin complex. Mol Biol Cell 13:2091–2105

Oberholzer U, Marcil A, Leberer E, Thomas DY, Whiteway M (2002) Myosin I is required for hypha formation in *Candida albicans*. Eukaryot Cell 1:213–228

Osherov N, May G (2000) Conidial germination in *Aspergillus nidulans* requires RAS signalling and protein synthesis. Genetics 155:647–656

Palmer KJ, Watson P, Stephens DJ (2005) The role of microtubules in transport between the endoplasmic reticulum and Golgi apparatus in mammalian cells. Biochem Soc Symp 72:1–13

Pearson CL, Xu K, Sharpless KE, Harris SD (2004) MesA, a novel fungal protein required for the stabilization of polarity axes in *Aspergillus nidulans*. Mol Biol Cell 15:3658–3672

Peterson J, Zheng Y, Bender L, Myers A, Cerione R, Bender A (1994) Interactions between the bud emergence proteins Bem1p and Bem2p and Rho-type GTPases in yeast. J Cell Biol 127:1395–1406

Qadota H, Python CP, Inoue SB, Arisawa M, Anraku Y, Zheng Y, Watanabe T, Levin DE, Ohya Y (1996) Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-β-glucan synthase. Science 272:279–281

Richman TJ, Sawyer MM, Johnson DI (1999) The Cdc42p GTPase is involved in a G2/M morphogenetic checkpoint regulating the apical-isotropic switch and nuclear division in yeast. J Biol Chem 274:16861–16870

Richman TJ, Toenjes KA, Morales SE, Cole KC, Wasserman BT, Taylor CM, Koster JA, Whelihan MF, Johnson DI (2004) Analysis of cell-cycle specific localization of the Rdi1p RhoGDI and the structural determinants required for Cdc42p membrane localization and clustering at sites of polarized growth. Curr Genet 45:339–349

Roberts RL, Mösch HU, Fink GR (1997) 14-3-3 proteins are essential for Ras/MAPK cascade signalling during pseudohyphal development in *S. cerevisiae*. Cell 89:1055–1065

Schmidt A, Hall MN (1998) Signaling to the actin cytoskeleton. Annu Rev Cell Dev Biol 14:305–338

Schmitz HP, Huppert S, Lorberg A, Heinisch JJ (2002) Rho5p downregulates the yeast cell integrity pathway. J Cell Sci 115:3139–3148

Seiler S, Plamann M (2003) The genetic basis of cellular morphogenesis in the filamentous fungus *Neurospora crassa*. Mol Biol Cell 14:4352–4364

Sheu YJ, Santos B, Fortin N, Costigan C, Snyder M (1998) Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. Mol Cell Biol 18:4053–4069

Shi X, Sha Y, Kaminskyj S (2004) *Aspergillus nidulans hypA* regulates morphogenesis through the secretion pathway. Fungal Genet Biol 41:75–88

Som T, Kolaparthi VSR (1994) Developmental decisions in *Aspergillus nidulans* are modulated by Ras activity. Mol Cell Biol 14:5333–5348

Steinberg G, Wedlich-Söldner R, Brill M, Schulz I (2000) Microtubules in the fungal pathogen *Ustilago maydis* are highly dynamic and determine cell polarity. J Cell Sci 114:609–622

Toda T, Uno I, Ishikawa T, Powers S, Kataoka T, Broek D, Cameron S, Broach J, Matsumoto K, Wigler M (1985) In yeast, RAS proteins are controlling elements of adenylate cyclase. Cell 40:27–36

Trimble WS (1999) Septins: a highly conserved family of membrane-associated GTPases with functions in cell division and beyond. J Membrane Biol 169:75–81

Ungar D, Hughson FM (2003) SNARE protein structure and function. Annu Rev Cell Dev Biol 19:493–517

Ushinsky SC, Harcus D, Ash J, Dignard D, Marcil A, Morchhauser J, Thomas DY, Whiteway M, Leberer E (2002) Cdc42 is required for polarized growth in the human pathogen *Candida albicans*. Eukaryot Cell 1:95–104

Walther A, Wendland J (2004a) Apical localization of actin patches and vacuolar dynamics in *Ashbya gossypii* depend on the WASP homolog Wal1p. J Cell Sci 117:4947–4958

Walther A, Wendland J (2004b) Polarized hyphal growth in *Candida albicans* requires the Wiskott-Aldrich Syndrome protein homolog Wal1p. Eukaryot Cell 3:471–482

Warenda AJ, Konopka JB (2002) Septin function in *Candida albicans* morphogenesis. Mol Biol Cell 13:2732–2746

Wei H, Requena N, Fischer R (2003) The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol Microbiol 47:1577–1588

Weinzierl G, Leveleki L, Hassel A, Kost G, Wanner G, Bolker M (2002) Regulation of cell separation in the dimorphic fungus *Ustilago maydis*. Mol Microbiol 45:219–231

Wendland J, Philippsen P (2000) Determination of cell polarity in germinated spores and hyphal tips of the filamentous ascomycete *Ashbya gossypii* requires a rhoGAP homolog. J Cell Sci 113:1611–1621

Wendland J, Philippsen P (2001) Cell polarity and hyphal morphogenesis are controlled by multiple Rho-protein modules in the filamentous ascomycete *Ashbya gossypii*. Genetics 157:601–610

Westfall PJ, Momany M (2002) *Aspergillus nidulans* septin AspB plays pre- and postmitotic roles in septum, branch and conidiophore development. Mol Biol Cell 13:110–118

Wu CL, Lee SF, Furmaniak-Kazmierczak E, Cote GP, Thomas DY, Leberer E (1996) Activation of myosin-I by members of the Ste20p protein kinase family. J Biol Chem 271:31787–31790

Wu C, Leeuw T, Leberer E, Thomas DY, Whiteway M (1998) Cell cycle- and Cln2p-Cdc28p-dependent phosphorylation of the yeast Ste20p protein kinase. J Biol Chem 273:28107–28115

Xu JR, Hamer JE (1996) MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. Genes Dev 10:2696–2706

Xu JR, Staiger CJ, Hamer JE (1993) Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA 95:12713–12718

Xu JR, Staiger CJ, Hamer JE (1998) Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA 95:12713–12718

Yaar L, Mevarech M, Koltin Y (1997) A *Candida albicans RAS*-related gene (*CaRSR1*) is involved in budding, cell morphogenesis and hyphal development. Microbiology 143:3033–3044

Zhao X, Kim Y, Park G, Xu JR (2005) A mitogen-activated protein kinase cascade regulating infection-related morphogenesis in *Magnaporthe grisea*. Plant Cell 17:1317–1329

Zheng Y, Cerione R, Bender A (1994) Control of the yeast bud-site assembly GTPase Cdc42. Catalysis of guanine nucleotide exchange by Cdc24 and stimulation of GTPase activity by Bem3. J Biol Chem 269:2369–2372

Zheng Y, Bender A, Cerione RA (1995) Interactions among proteins involved in bud-site selection and bud-site assembly in *Saccharomyces cerevisiae*. J Biol Chem 270:626–630

Zheng XD, Wang YM, Wang Y (2003) *CaSPA2* is important for polarity establishment and maintenance in *Candida albicans*. Mol Microbiol 49:1391–1405

Ziman M, Johnson DI (1994) Genetic evidence for a functional interaction between *Saccharomyces cerevisiae CDC24* and *CDC42*. Yeast 10:463–474

Zuber S, Hynes MJ, Andrianopoulos A (2002) G-protein signalling mediates asexual development at 25 °C but has no effect on yeast-like growth at 37 °C in the dimorphic fungus *Penicillium marneffei*. Eukaryot Cell 1:440–447

Zuber S, Hynes MJ, Andrianopoulos A (2003) The G-protein α subunit GasC plays a major role in germination in the dimorphic fungus *Penicillium marneffei*. Genetics 164:487–499

2 Organelle Inheritance in Yeasts and Other Fungi

L.J. García-Rodríguez[1], A.C. Gay[1], L.A. Pon[1]

CONTENTS

I. Introduction

Internal compartmentalization is a hallmark of the eukaryotic cell. The evolution from prokaryote to eukaryote resulted in the physical separation of the genome from the cytoplasm, and the development of dynamic subcellular compartments that coordinate their functions and abundance according to the needs of the cell. Organelles create order within a cell, prevent undesirable interactions between the various cellular components, and perform specialized functions. While each organelle has a defined cellular role, their localization, copy number, size, and morphology can vary. For instance, variations in the carbon source for growth of budding yeast can impact peroxisomal biosynthesis and result in a tenfold change in the abundance of mitochondria (Pon and Schatz 1991; Einerhand et al. 1992).

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Studies of organelle inheritance have focused primarily on the nucleus. Nuclear inheritance, which uses the mitotic spindle to segregate nuclei and chromosomes, occurs by a conserved and tightly regulated mechanism. The inheritance of other organelles, on the other hand, occurs by more diverse and less well-understood mechanisms. Organelles like mitochondria and chloroplasts cannot be produced de novo. This is true, in part, because they contain DNA, which must undergo template-dependent replication. As a result, these organelles must be transferred from mother to daughter cells to ensure normal daughter cell viability and/or function, and more than one mechanism for these essential inheritance events has been identified.

On the other hand, since the endoplasmic reticulum is continuous with the outer nuclear envelope, Golgi and lysosomes (vacuoles) can, in principle, be produced de novo from ER that is inherited with the nucleus. Interestingly, even in cases where cellular control of organelle inheritance is not obligatory, mechanisms for inheritance have been identified. Thus, the high cost of de novo organelle synthesis may have driven the development of mechanisms for organelle inheritance even when they are not required.

The use of modern microscopy techniques, such as time-lapse fluorescence imaging, in combination with genetic and molecular biology approaches, has led to a rapid expansion of our understanding of organelle distribution and inheritance. Fungi, and yeasts in particular, have been used as model systems for these combined-approach studies of organelle inheritance. Thus, this chapter describes organelle distribution and inheritance in yeasts and other fungi. Specifically, we focus on the mechanisms underlying organelle abundance in dividing cells, in addition to a brief consideration of nuclear migration in non-dividing cells.

The Mycota I
Growth, Differentation and Sexuality
Kües/Fischer (Eds.)

II. Cytoskeletal Organization and Function in Organelle Movement and Inheritance

In eukaryotic organisms, the cytoskeleton network is required for essential biological processes including the maintenance of growth polarity and cellular shape, cytokinesis, chromosome segregation, cell motility, and organelle transport. Both cytoskeleton-dependent and -independent mechanisms have been proposed as models for organelle inheritance and distribution. However, recent studies suggest that the cytoskeleton plays a role in all known strategies for organelle inheritance.

Two mechanisms have been identified that control organelle localization during asymmetric division in budding yeast (Fig. 2.1). One mechanism employs cytoskeletal tracks for the active transport of organelles from mother to daughter cell. Motor molecules provide the force for many of these track-dependent movements. Recent evidence suggests that forces generated by cytoskeletal polymerization can also drive linear, cytoskeleton-dependent organelle movement and inheritance. The second mechanism is based on the capture of organelles and their subsequent anchorage in mother and/or daughter cells. These regional capture events are cytoskeleton-dependent and ensure that the organelle is present at specific sites within a cell during cytokinesis. Neither of the proposed mechanisms is mutually exclusive. Indeed, budding yeast utilize both mechanisms for the process of mitochondrial inheritance.

A. Organization of the Actin Cytoskeleton in Fungi

In fungi, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus nidulans* and *Ustilago maydis*, F-actin assembles into two structures that persist throughout the cell division cycle: actin patches and actin cables (Botstein et al. 1997; Harris 2001; Banuett and Herskowitz 2002). Actin patches are cortical, F-actin-containing "spots". Actin cables are bundles of actin filaments that align along the axis of cell growth.

Polarization of actin patches and cables is essential for cell division in the budding yeast, *S. cerevisiae* (Fig. 2.2). During G_1, yeast cells commit to a round of cell division and select a bud site. These events stimulate a reorganization of the actin cytoskeleton: actin patches become concentrated at the selected bud site and within the bud as it develops, and actin cables are deposited along the mother–bud axis. These rearrangements occur before START, require activation of Cdc28p protein kinase by G_1 cyclins, and are mediated by the Rho-type GTPase, Cdc42p. As the cell division cycle progresses, activation of Cdc28p protein kinase by G_2 cyclins stimulates depolarization of the actin cytoskeleton. Finally, degradation of G_2 cyclins at M phase triggers an accumulation of actin patches at, and orientation of actin cables to, the bud neck where cytokinesis occurs (Schmidt and Hall 1998).

Actin patches are required for septation and polarity events including the maintenance of hyphal tips in mycelial fungi. In budding yeast, actin patches are linked to endocytosis (Engqvist-

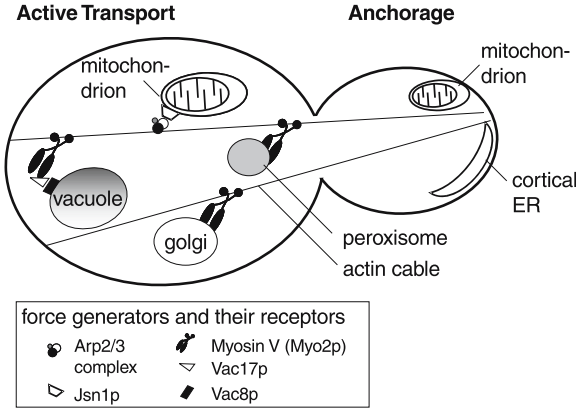

Fig. 2.1. Strategies for organelle inheritance in budding yeast. Please refer to text for description

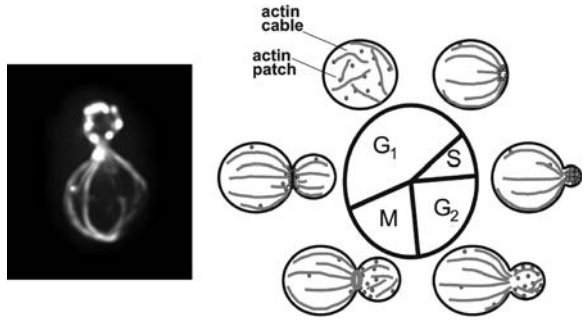

Fig. 2.2. Organization of the actin cytoskeleton in budding yeast. The image shown is a fluorescence micrograph of a yeast cell in G_2 phase of the cell division cycle that is fixed and stained for the actin cytoskeleton using fluorophore-coupled phalloidin. Not visible. Please refer to text for description of the schematic. Images and schematic provided by T. Huckaba (Columbia University)

Goldstein and Drubin 2003). Proteins that are required for endocytosis, including actin, Arp2/3 complex and its activators, as well as endocytic adapters and scaffolds, localize to actin patches. Elegant studies have revealed a pathway for the association of receptors, adaptors, and actin patches during endocytic internalization (Kaksonen et al. 2003). Consistent with this, recent studies showed that the lipophilic endosomal marker, FM4-64, colocalizes with actin patches during their assembly, internalization, and movement from the bud into the mother cell (Huckaba et al. 2004). Thus, there is direct evidence that actin patches of budding yeast are early endosomes.

Actin cables exist in many fungi, and are best characterized in budding yeast. These structures have been implicated as tracks for the movement of mitochondria, secretory vesicles, mRNA, and spindle alignment elements from mother cells to developing daughter cells during yeast cell division (Simon et al. 1997; Takizawa et al. 1997, 2000; Pruyne et al. 1998; Schott et al. 1999, 2002; Beach et al. 2000; Yin et al. 2000; Fehrenbacher et al. 2003). Moreover, actin cables are required for normal inheritance of Golgi and vacuoles (Hill et al. 1996; Rossanese et al. 2001).

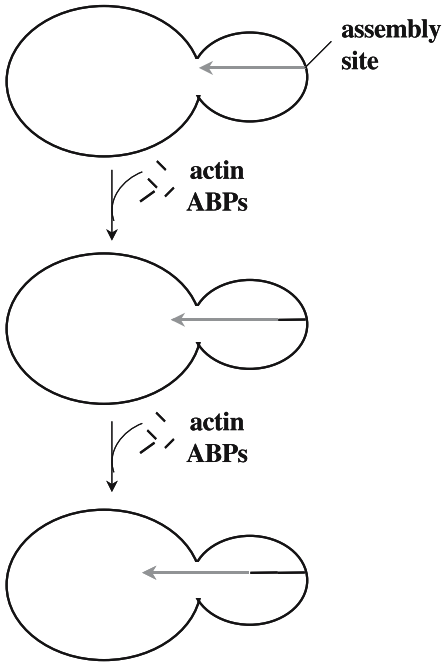

Fig. 2.3. Model for retrograde flow of actin cables during their assembly and elongation in budding yeast. *ABPs*: Actin-binding proteins. Please refer to text for description

Recent studies indicate that actin cables undergo retrograde movement, i.e., movement in the direction opposite that of cargo movement, as they assemble and elongate (Yang and Pon 2002). This process is mediated by the formins (Bni1p and Bnr1p), proteins that stimulate actin polymerization at sites of actin cable assembly, and resident actin cable proteins including the actin bundling proteins, fimbrin (Sac6p) and Abp140p, and tropomyosin proteins (Tpm1p and Tpm2p; Adams et al. 1991; Drees et al. 1995; Asakura et al. 1998; Pruyne et al. 1998, 2002; Evangelista et al. 2002; Sagot et al. 2002a,b). Actin cable assembly and elongation occurs at two sites in budding yeast: the bud tip and bud neck (Yang and Pon 2002). During elongation of these structures, new material is incorporated into an existing actin cable at its assembly site, and the growing actin cable moves toward the distal tip of the mother cell with an average velocity of $\sim 0.29 \pm 0.11$ µm/s (Fig. 2.3). This "retrograde flow" of actin cables has an impact on both the velocity and direction of organelle and vesicle movement in budding yeast (Fehrenbacher et al. 2004; Huckaba et al. 2004).

B. Organization of the Microtubule Cytoskeleton in Fungi

The microtubule cytoskeleton of fungi, like that of other cell types, is highly dynamic and undergoes ordered changes throughout the cell cycle (Hagan 1998; Sato and Toda 2004). Fungi typically differ regarding how their microtubules are nucleated. For example, the spindle pole body (SPB), a structure that is embedded in the nuclear envelope, nucleates both cytoplasmic and nuclear microtubules in non-dividing yeast, and nucleates microtubules in the spindle apparatus in dividing yeast (Fig. 2.4). By contrast, microtubules in *U. maydis* and *S. pombe* are nucleated by other, cytoplasmically located nucleation centers (Straube et al. 2003).

In the fission yeast, *S. pombe*, bundles of microtubules extend along the major axis of the cell during interphase. The microtubules in these bundles are arranged in an antiparallel manner, with the plus ends oriented toward the cell tips (Tran et al. 2001). Microtubule nucleation during interphase occurs from the SPB and from the interphase microtubule organizing center (iMTOC; Tran et al. 2001). In mitotic yeast, the SPB is duplicated during late G_2 phase. During prophase, microtubules nucleated from the SPB form a short, bipolar spin-

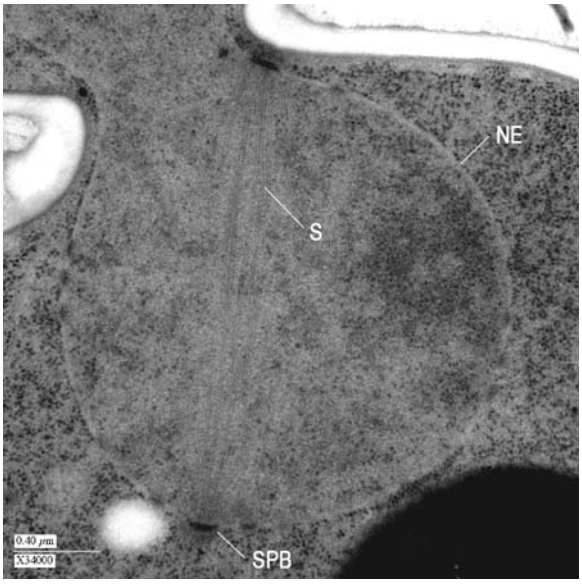

Fig. 2.4. The spindle pole body and spindle apparatus in budding yeast. Transmission electron micrographs of the nucleus in a dividing yeast cell. *NE* Nuclear envelope, *S* spindle, *SPB* spindle pole body. Image generously provided by M. Winey (University of Colorado)

dle. The spindle elongates during metaphase, spanning from the nucleus to the ends of the cell. At this point, another microtubule nucleation center, the equatorial microtubule organization center (eMTOC), appears and nucleates microtubules from the cell equator that contribute to sustaining the contractile ring and determining the site for cell division during cytokinesis. As cells progress to interphase, microtubule nucleation occurs from the iMTOC and SPB again to reestablish the interphase microtubules organizing center (Sato and Toda 2004).

Microtubule dynamics throughout the cell cycle have also been studied in mycelial fungi. Bundles of microtubules are present in haploid *U. maydis* cells during interphase (Steinberg et al. 2001). As the bud grows during S and G_1 phases, bipolar microtubule bundles traverse the length of the cell. These microtubule bundles are not associated with the spindle pole body. Rather, they are organized by polar MTOCs that assemble at the bud neck (Straube et al. 2003). Later, in mitosis, the SPB is activated, forming the mitotic spindle and nucleating astral microtubules that contact the cell periphery for the segregation of chromosomes. After cytokinesis, the nucleus is relocated to the center of the cell and the SPB is inactivated again (Steinberg and Fuchs 2004).

III. Nuclear Migration in Fungi

Nuclei are dynamic organelles that undergo directed movement during cell division, development, and establishment of cell polarity. Fungi have been used extensively for studies on nuclear positioning, in part because they can be genetically manipulated, and in part because the defined shape of fungi provides landmarks for the analysis of nuclear migration. The widely studied function of the cytoskeleton in spindle formation and function in fungi is the topic of numerous reviews (Xiang and Fischer 2004). Here, we describe recent findings on nuclear position in non-dividing cells, that is, during interphase in *S. pombe* and filamentous fungi.

A. Control of Nuclear Position During Interphase in *S. pombe*

In *S. pombe*, maintenance of the nucleus at the center of the cell is critical for determining the plane of cell division (Chang and Nurse 1996). A role for microtubules and microtubule dynamics in this process emerged from phenotypic analysis of fission yeast mutants, and visualization of microtubules and nuclei in living cells. Tran et al. (2000) observed that mutation of tubulin results in defects in positioning of the nucleus during interphase and of the division plane during mitosis. Subsequent imaging studies revealed that interphase microtubules are organized from MTOCs that are embedded in the nuclear envelope, and that microtubules that emerge from these MTOCs exert forces that affect nuclear position when they polymerize to, and make contact with, the tips of the cell (Tran et al. 2001). These findings support the model that positioning of the nucleus in the center of fission yeast during interphase occurs as a result of the balanced pushing forces generated by microtubules emerging from medial MTOCs at opposite ends of the nucleus.

B. Nuclear Migration in Hyphae

Formation of polarized hyphae is essential for growth and invasion by filamentous fungi. In multinucleated filamentous fungi including *Aspergillus* and *Neurospora*, nuclei are evenly distributed along the hyphae (Fig. 2.5). Several studies

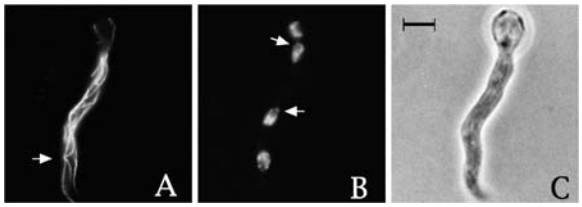

Fig. 2.5. A–C Disposition of microtubules and nuclei in an interphase germling of *Aspergillus nidulans*. **A** Immunofluorescence micrographs of microtubules. The *arrow* points to a site where several microtubules converge, known from other studies to correspond to the spindle pole body. **B** Nuclei stained with the DNA-binding dye DAPI. The unstained regions correspond to nucleoli (*arrows*). **C** Phase contrast image of the same field. Preprinted from Jung et al. (1998) and Oakley (2004), with permission. Bar = 5 μm

have implicated microtubules and microtubule-dependent motors in nuclear positioning in hyphae. Specifically, fungi bearing mutations in dynein subunits, dynactin, the num1p-related protein apsA, kinesin, or in three proteins required for dynein function, NUDF/LIS1, NUDE/RO11 and NUDC, display defects in nuclear position (Graia et al. 2000; Xiang et al. 2000; Alberti-Segui et al. 2001; Han et al. 2001; Requena et al. 2001).

Currently, the mechanism underlying dynein function in nuclear migration is not well understood. Since dynein, dynactin, NUDF/LIS1, and NUDE/RO11 all form comet-like structures on the plus ends of microtubules, and microtubule plus ends are less dynamic in dynein mutants (Han et al. 2001; Zhang et al. 2002, 2003; Efimov 2003), it is possible that dynein-mediated microtubule dynamics control nuclear position during interphase in filamentous fungi. This may occur by a pushing mechanism similar to that described in fission yeast (Tran et al. 2000). Alternatively, dynein may pull microtubules and their associated nuclei toward the cell cortex by a mechanism similar to that which contributes to nuclear migration during anaphase in budding yeast. During this process, dynein is found on the plus ends of astral microtubules emerging from the spindle pole body. Moreover, evidence from two groups supports a model whereby dynein that is associated with microtubule plus ends is delivered to, and activated at, the cell cortex. Once activated, the cortical dynein can exert a pulling force on nuclear-associated microtubules, effectively pulling them toward the cell cortex (Lee et al. 2003; Sheeman et al. 2003).

IV. Organelle-Specific Inheritance in Fungi

A. Mitochondria

Mitochondria are essential for their role in aerobic energy mobilization and the biosynthesis of amino acids, fatty acids, pyrimidines, heme, and steroid hormones. Although mitochondria contain DNA (mtDNA) and the machinery to express mtDNA, over 95% of the proteins in mitochondria are encoded in the nucleus and synthesized in the cytoplasm prior to import into the organelle. Since the machinery for the transport of proteins into mitochondria is encoded in the nucleus and must be imported into mitochondria, mitochondrial membranes can be produced only from preexisting, import-competent mitochondrial membranes. Thus, mitochondrial membranes and mtDNA cannot be produced de novo, and both must be transferred from mother to daughter cells to ensure that the cells contain fully functional mitochondria.

Many yeasts are not obligate aerobes and can survive under conditions which produce lethality in other eukaryotic cells. As a result, mitochondrial biogenesis and inheritance have been studied extensively in fungal systems. Visualization of mitochondrial motility in budding yeast has revealed the "mitochondrial inheritance cycle", a series of cell cycle-linked motility events that result in an equal distribution of the organelle during cell division (Fig. 2.6). Mitochondria are tubular structures that form an extended cytoplasmic network and align along the axis of growth in budding yeast and other fungi (Stevens 1977; Lazzarino et al. 1994; Prokisch et al. 2000; Suelmann and Fischer

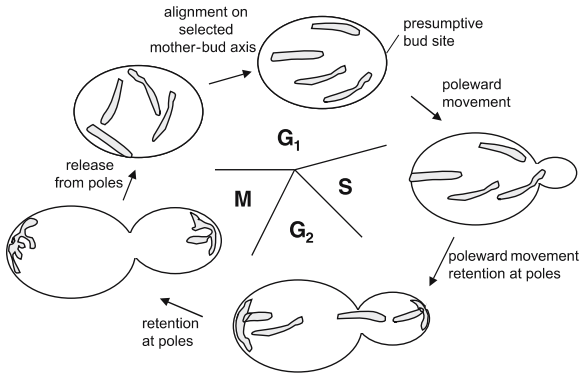

Fig. 2.6. The mitochondrial inheritance cycle. Please refer to text for description

2000). In budding yeast, mitochondria align along the mother–bud axis and orient toward the site of bud emergence at G_1 phase, after the selection of a bud site. During S, G_2, and M phases, mitochondria display a pattern of movement that resembles chromosome movement during cell division. Mitochondria undergo poleward movement; i.e., linear movements toward the bud tip (anterograde movement) or toward the distal tip of the mother cell (retrograde movement). After mitochondria have reached the poles, they are immobilized at "retention zones" in the bud tip or mother cell tip. Finally, at the end of the cell division cycle, mitochondria are released from the retention zones, and are redistributed in the dividing cells (Simon et al. 1997; Boldogh et al. 2001b; Fehrenbacher et al. 2004).

These studies indicate that cell cycle-linked mitochondrial motility events are the basis for the physical transfer and equal distribution of the organelles among mother and daughter cells. In addition, it is now clear that (1) mitochondrial position, shape, and movement are controlled by their interaction with the cytoskeleton; (2) the inheritance of mtDNA and mitochondrial membranes is coordinated; (3) multiple pathways, including those controlling mitochondrial fission, fusion, protein import, and respiratory activity, can impact on mitochondrial inheritance; and (4) strategies and mechanisms for mitochondrial inheritance vary among different cell types.

In *Fusarium acuminatum*, *Uromyces phaseoli*, *Nectria haematococca*, *N. crassa*, *S. pombe*, and *Allomyces macrogymus*, mitochondrial distribution is microtubule-dependent (Howard and Aist 1980; Heath et al. 1982; Kanbe et al. 1989; Aist and Bayles 1991; Steinberg and Schliwa 1993; Yaffe et al. 1996; McDaniel and Roberson 2000). Current evidence supports the existence of both motor-dependent and -independent mechanisms for microtubule control of mitochondrial distribution. For example, in *N. crassa*, mitochondrial morphology and distribution are abnormal in "*ropy*" dynein mutants (Minke et al. 1999). Similarly, in *N. haemotococca*, deletion of the conventional kinesin gene NhKIN1 results in defects in mitochondrial distribution during hyphal growth (Wu et al. 1998). However, deletion of the genes that encode kinesin or dynein has no obvious effect on mitochondrial distribution in *S. pombe*, suggesting that mitochondrial motility may be controlled by microtubule polymerization in fission yeast (Yaffe et al. 2003).

By contrast, the actin cytoskeleton is required for proper mitochondrial morphology and motility in *S. cerevisiae* and *A. nidulans* (Drubin et al. 1993; Lazzarino et al. 1994; Hermann and Shaw 1998; Suelmann and Fischer 2000). Several lines of evidence support a role for actin cables as tracks for mitochondrial movement during inheritance in budding yeast. First, mitochondrial movement is linear and polarized, like other known track-dependent movements (Simon et al. 1995). Second, mitochondria co-localize with actin cables in fixed yeast cells and display ATP-sensitive, reversible, protein-mediated binding to F-actin in cell-free assays (Lazzarino et al. 1994). Third, mutations that destabilize actin cables but have no effect on actin patch polarity block mitochondrial movements (Simon et al. 1997; Fehrenbacher et al. 2004).

Consistent with this, recent studies have documented a direct interaction between mitochondria and actin cables (Fehrenbacher et al. 2004). Simultaneous two-color, time-lapse imaging revealed movement of mitochondria along actin cables in living yeast cells. Since actin cables have been implicated as the tracks for bud-directed movement of particles including mitochondria, secretory vesicles, mRNA, and spindle alignment elements, this is the first direct evidence that one of the proposed cargos uses actin cables as tracks for anterograde transfer from mother cells to buds. These imaging studies also revealed that the retrograde flow of actin cables affects both the velocity and direction of mitochondrial movement – that is, the transfer of mitochondria from mother cells to buds requires forces for anterograde movement that must overcome the opposing force of retrograde actin cable flow. By contrast, during retrograde movement mitochondria associate with actin cables undergoing retrograde flow, and exhibit a velocity of retrograde movement that is similar to that of actin cable movement during retrograde flow.

Type V myosins have been implicated as motors for the anterograde movement of many cargos along actin cables in budding yeast. Moreover, many of the known adaptors that link type V myosins to their cargos are Rab-like proteins. Itoh et al. (2002) provided evidence that Myo2p, a type V myosin, and Ypt11p, a Rab-like protein, participate in mitochondrial inheritance in budding yeast. They showed that (1) the Myo2p tail can bind to Ypt11p, (2) deletion of *YPT11* produced a partial delay of mitochondrial transmission to the bud, (3) overexpression of *YPT11* resulted in excess ac-

cumulation of mitochondria in the bud, and (4) two *MYO2* alleles that showed genetic interactions with *YPT11* also affected mitochondrial inheritance.

These findings raise the possibility that Myo2p and Ypt11p may drive mitochondrial movement during inheritance. However, recent studies indicate that neither protein is required for mitochondrial motility in budding yeast (Boldogh et al. 2004). That is, deletion of *YPT11*, reduction of the length of the Myo2p lever arm (*myo2–Δ6IQ*), or deletion of *MYO4*, the other type V myosin of yeast, have no effect on mitochondrial morphology, co-localization of mitochondria with actin cables, or the velocity of bud-directed movement of mitochondria. By contrast, retention of mitochondria in the bud, a process that results in the immobilization and accumulation of mitochondria in the bud tip, was compromised in *YPT11* and *MYO2* mutants. Since Myo2p and Ypt11p localize to the bud tip, they may serve as capture devices that contribute to the retention of mitochondria in the bud tip. Alternatively, Myo2p and/or Ypt11p may mediate the transport of retention factors or mRNA that encode retention factors to the bud tip.

An alternative, motor-independent mechanism for mitochondrial movement in budding yeast has been identified (Boldogh et al. 2001a). This mechanism is similar to that used by bacterial and viral pathogens for their movement through the cytoplasm of infected cells. Propulsion of these pathogens occurs by Arp2/3 complex-mediated nucleation of F-actin. This evolutionarily conserved, seven-subunit complex is regulated by a variety of nucleation-promoting factors such as WASp. In its activated state, Arp2p and Arp3p subunits of the complex serve as platforms for the nucleation of F-actin. In addition, the Arp2/3 complex can bind to the pointed, slow-growing end of F-actin, allowing for the addition of actin monomers at the barbed, fast-growing end. Finally, Arp2/3 complex can bind to the lateral surface of F-actin, creating and stabilizing filament branches (reviewed by Pollard and Beltzner 2002). These activities of the Arp2/3 complex lead to the assembly of a higher-order dendritic array at the membrane surface, eventually leading to a branched network of actin filaments in vivo and in vitro (Svitkina and Borisy 1999; Volkmann et al. 2001).

In budding yeast, the Arp2/3 complex is associated with endosomes (actin patches) and vacuoles (Moreau et al. 1996; Winter et al. 1997; Insall et al. 2001; Eitzen et al. 2002; Chang et al. 2003). Several findings also support a role for the Arp2/3 complex in driving mitochondrial movement during inheritance in budding yeast (Boldogh et al. 2001a). First, Arp2/3 complex subunits co-localize with mitochondria in intact cells and are recovered with mitochondria after subcellular fractionation. Second, Arp2/3 complex activity is detected on mitochondria in living yeast and in isolated yeast mitochondria. Third, mitochondrial movement requires constant actin assembly and disassembly, and is impaired by an agent that perturbs actin dynamics. Fourth, mutations in Arp2/3 complex subunits inhibit mitochondrial movements but have no effect on the co-localization of mitochondria with actin cables. These findings indicate that the Arp2/3 complex is associated with yeast mitochondria, and that mitochondria use Arp2/3-complex-driven actin assembly to drive their movement during inheritance.

One feature that distinguishes mitochondrial movement from other Arp2/3 complex-driven movements is track dependence. In contrast to endosomes and bacterial pathogens that undergo non-linear movement, yeast mitochondria use actin cables as tracks for linear anterograde and retrograde movement during cell division. Molecules that contribute to the association of mitochondria with actin cables were revealed by visual screens for mutations that interfere with normal *m*itochondrial *d*istribution and *m*orphology (MDM) and *m*itochondrial *m*orphology *m*aintenance (MMM; McConnell et al. 1990; Burgess et al. 1994; Sogo and Yaffe 1994; Hermann et al. 1997). Three of the genes identified (*MMM1*, *MDM10*, and *MDM12*) encode integral membrane proteins that form a complex in the mitochondrial outer membrane (Boldogh et al. 2003).

Two lines of evidence support a role for the Mmm1p/Mdm10p/Mdm12p complex in the association of mitochondria to actin cables during movement and inheritance. First, deletion of any subunit in the complex results in the loss of all anterograde and retrograde mitochondrial movement, and produces a pattern of mitochondrial movement similar to that observed upon destabilization of actin cables (Boldogh et al. 2001a, 2003; Fehrenbacher et al. 2004). Second, subunits of this complex are required for reversible, ATP-sensitive binding of mitochondria to F-actin in vitro (Boldogh et al. 2001a). These observations support a role for Mmm1p/Mdm10p/Mdm12p in mediating interactions between mitochondria and actin cables through cyclic, reversible, ATP-sensitive binding of mitochondria to F-actin within actin cables.

MMM1, *MDM10*, and *MDM12* homologues have been found in other species of fungus. Deletion of *MMM1* produces female sterility in *N. crassa*, of *MDM10* results in mitochondrial morphology and a shortened lifespan in *Podospora anserina*, and of *MDM12* results in mitochondrial morphology in *S. pombe* (Berger et al. 1997; Jamet-Vierny et al. 1997; Prokisch et al. 2000). Interestingly, there is also evidence for a role of the Mmm1p/Mdm10p/Mdm12p complex in the inheritance of mtDNA in budding yeast (Hobbs et al. 2001; Boldogh et al. 2003). First, deletion of any subunit in the complex results in a high rate of mtDNA loss and a corresponding loss of mitochondrial respiratory activity. Second, the complex may contribute to mtDNA maintenance through effects on the assembly and/or maintenance of mtDNA nucleoids. Third, the complex localizes to punctate structures in close proximity to mtDNA nucleoids. These findings support the notion that the Mmm1p/Mdm10p/Mdm12p complex may be functionally similar to the kinetochore. Thus, it links the minimum heritable mitochondrial unit – mitochondrial membranes and mtDNA – to the cytoskeleton-based force-generating machinery that drives movement of that unit from mother cells to developing daughter cells during cell division. Accordingly, the complex is referred to as the "mitochore".

Together, these observations support models for anterograde and retrograde mitochondrial movement and inheritance in budding yeast. For retrograde movement, the mitochore mediates binding of mitochondria to actin cables undergoing retrograde flow. Once bound, mitochondria use actin cables as conveyor belts for transport toward the distal tip of the mother cell. Interestingly, mitochondria are not the only organelles that use this mechanism for retrograde movement. Endosomes (actin patches) of budding yeast also associate with actin cables and use the force of retrograde actin cable flow to drive their movement from the bud toward the mother cell (Huckaba et al. 2004).

For anterograde, bud-directed movement, the mitochore mediates cyclic binding of mitochondrial membranes and mtDNA to actin cables for movement in the presence of an applied force. The force for movement is produced by (1) Arp2/3 complex-stimulated actin polymerization and assembly at the interface between mitochondria and actin cables, and (2) cross-linking of mitochondria-associated actin polymers in parallel to F-actin within actin cables. When the forces for anterograde mitochondrial movement are sufficient to overcome the forces for retrograde actin cable flow, mitochondria undergo anterograde movement on actin cables, i.e., movement from mother to daughter cells.

Finally, the regulatory mechanisms that coordinate mitochondrial distribution and movement in budding yeast during cell cycle progression are not well understood. *MDM28*, a gene that when mutated results in a delay in mitochondrial inheritance, is allelic to *PTC1*, a gene that encodes a serine/threonine phosphatase (Roeder et al. 1998). Ptc1p is a negative regulator of the high osmolarity glycerol (HOG) response pathway and catalyzes dephosphorylation of the HOG pathway components Pbs2p, a MAPKKinase, and Hog1p, a MAPKinase. As a result, both Pbs2p and Hog1p activities are increased in *ptc1* mutants (Warmka et al. 2001). The mechanism for Ptc1p control of mitochondrial inheritance appears to be HOG-independent, since *hog1 ptc1* and *pbs2 ptc1* mutants show the same delay in mitochondrial inheritance as a *ptc1* single mutant. Future studies may reveal the precise role of Ptc1p and other cell cycle-regulatory elements on mitochondrial inheritance.

B. Endoplasmic Reticulum

The endoplasmic reticulum (ER) is a continuous, membrane-bound organelle, the major site of lipid biosynthesis, and essential for protein secretion. In addition, it stores Ca^{++}, an ion that functions in many signal transduction cascades (Meldolesi and Pozzan 1998). Proteins that follow the secretory pathway are glycosilated in the ER lumen before they are exported from the organelle (Hedge and Lingappa 1999). Lastly, the ER is the final destination for retrograde vesicles (Pelham 1996).

In *S. cerevisiae*, the ER consists of (1) a reticulum underlying the plasma membrane (cortical ER), (2) ER associated with the nuclear envelope (nuclear ER), and (3) finger-like projections connecting cortical and nuclear ER (Preuss et al. 1991). In contrast to mammalian cells, ribosomes are associated with both cortical and nuclear ER in budding yeast. To date, there is no evidence for the existence of smooth ER in budding yeast (Baba and Osumi 1987). Time-lapse microscopy experiments revealed that tubular ER structures undergo sliding, branching, and fusion (Prinz et al. 2000). Since treatment of cells with the actin-destabilizing drug

Latrunculin A (Lat-A) decreases ER motility, it is clear that the actin cytoskeleton plays a role in cortical ER dynamics and morphology in budding yeast (Prinz et al. 2000; Fehrenbacher et al. 2002). However, the precise function of the actin cytoskeleton in ER dynamics is not well understood.

The dimorphic heterobasidiomycete *U. maydis* serves as an example of how the ER is organized in mycelial fungi. In *U. maydis* the ER forms a polygonal network of tubules that is associated with the cell cortex and makes contact with the nuclear envelope. As in *S. cerevisiae* (Prinz et al. 2000), the ER in *U. maydis* is a highly dynamic structure, with ER tubules undergoing continuous extension, sliding, and fusion. Analysis of the effect of the destabilization of microtubules or mutations in microtubule-dependent motors revealed that ER motility in *U. maydis* requires microtubules and cytoplasmic dynein (Wedlich-Söldner et al. 2002a,b). Although microtubules support ER movement in *U. maydis*, as in vertebrate cells (Dabora and Sheetz 1988), they are not required for peripheral organization of the network. However, in contrast to animal systems, motility events are not essential for ER inheritance in *U. maydis*; ER tubules are present in the growing bud in cells with conditional mutations in tubulin at permissive and restrictive conditions (Wedlich-Söldner et al. 2002a).

In *S. cerevisiae*, nuclear ER undergoes spindle-driven inheritance in conjunction with the nucleus. By contrast, inheritance of cortical ER occurs by a fundamentally different, actin-dependent mechanism. Cortical ER is the first organelle inherited during the cell cycle (Preuss et al. 1991; Koning et al. 1996; Du et al. 2001). Moreover, cortical ER morphology is sensitive to treatment with *Lat-A* and to mutations in the actin-encoding *ACT1* gene (Fehrenbacher et al. 2002). Using Sec63p-GFP to visualize cortical ER, Fehrenbacher et al. (2002) found that cortical ER is anchored to sites of bud emergence and apical bud growth during the S and G_2 phases of the cell cycle, and that this anchorage allows cortical ER to be drawn into and maintained in the bud as it develops. These observations support a mechanism for cortical ER inheritance that is actin cytoskeleton-dependent but relies on anchorage, not directed, organelle movement.

In support of this model, a protein that localizes to the site of ER anchorage has been implicated in cortical ER inheritance. The exocyst component Sec3p localizes to the bud tip where it mediates post-Golgi membrane traffic (Walch-Solimena et al. 1997; Grote et al. 2000). Since *sec3Δ* cells exhibit a defect in cortical ER inheritance but not in Golgi and mitochondrial inheritance, it has been proposed that Sec3p contributes to anchoring cortical ER at the bud tip (Wiederkehr et al. 2003). In addition deletion of Aux1p/Swa2p, a protein that localizes to ER membranes but has no obvious role in membrane traffic, produces a delay in the transfer of cortical ER tubules to daughter cells (Gall et al. 2000; Pishvaee et al. 2000; Du et al. 2001). Therefore, it is possible that this protein also contributes to anchorage of ER at the bud tip.

Other studies support a role for a type V myosin in cortical ER inheritance. Deletion of the *MYO4* gene results in defects in actin cable-dependent movement of mRNAs from mother to daughter cells during cell division (Bertrand et al. 1998; Bohl et al. 2000). The She2p/She3p protein complex serves as an adaptor to bind mRNA to a region in the Myo4p C-terminal tail (Bohl et al. 2000). Recent studies indicate that a point mutation in the ATP-binding region of the motor domain of Myo4p or a mutation of She3p inhibit ER inheritance (Estrada et al. 2003). Moreover, both She3p and Myo4p are recovered in fractions enriched in ER-derived membranes after subcellular fractionation. These findings raise the possibility that myosin may drive transport of cortical ER from mother to daughter cells in budding yeast. Thus, it is possible that two distinct processes, anchorage of ER in the bud tip and active transport of ER into the bud, may contribute to the inheritance of cortical ER. Alternatively, Myo4p and She3p may mediate the transport of ER anchoring proteins or mRNAs that encode ER anchoring proteins from mother to the bud tip.

C. Vacuoles, the Lysosomes of Yeast

Vacuoles are evenly distributed among mother and daughter cells in *S. cerevisiae*. Early studies indicated that the yeast vacuole fragments into small vesicles that are then distributed between the mother cell and the bud (Wiemken et al. 1970; Severs et al. 1976). However, more recent work indicates that vacuoles remain relatively constant in size during cell division, and that the primary event during vacuole inheritance is the formation of a tubular, vacuole-derived "segregation structure" (Weisman et al. 1987; Weisman and Wickner 1988). The segregation structure forms near the bud and rapidly extends from the mother cell to the bud before the nucleus enters into the neck. Thereafter, the segregation structure disappears,

and fusion of vacuolar vesicles produces the large vacuoles typical of daughter cells (Conradt et al. 1992; Jones et al. 1993).

Vacuole inheritance has been studied also during hyphal growth in *Candida albicans*. Time-lapse microscopy and three-dimensional imaging studies indicate that vacuoles are inherited asymmetrically during germ tube formation. After the first division, the subapical cell in the incipient hypha inherits most of the vacuolar compartment and arrests in G_1. Subapical cells are released from their arrest when the vacuoles decrease in size. These observations indicate that vacuole segregation is coordinated with the cell cycle, possibly by cell cycle-regulatory machinery that monitors vacuole size (Barelle et al. 2003).

Genetic screens have revealed genes required for vacuole inheritance in budding yeast. These "*vac*" mutants are classified according to the morphology of their vacuoles (for review, see Weisman 2003). The first class of *vac* mutants display defects in inheritance, but not in vacuolar morphology. Many class I mutations affect the actin cytoskeleton. Specifically, mutations in genes encoding actin (*ACT1*), profilin (*PFY1*), and a type V myosin (*MYO2*) were identified in class I mutations (Hill et al. 1996). Some *MYO2* mutations that interfere with vacuolar inheritance are in the Myo2p motor, and therefore affect many Myo2p-dependent processes. However, other mutations in the Myo2p tail (e.g., *myo2-2*) appear to be vacuole-specific (Catlett and Weisman 1998; Catlett et al. 2000).

Other studies support a role for the peripheral and integral vacuolar membrane proteins, Vac17p and Vac8p, in recruiting Myo2p to vacuoles. *VAC17* was isolated as a multi-copy suppressor of the vacuole inheritance defect of the *myo2-2* allele. Vac17p is required for normal vacuolar inheritance, and is not required for the inheritance of peroxisomes, late Golgi, or secretory vesicles (Ishikawa et al. 2003). Vac17p immunoprecipitates with Myo2p, can bind to the Myo2p tail in two-hybrid tests, and is required to recruit Myo2p to vacuolar membranes. Vac8p, an integral vacuolar membrane protein, interacts with Vac17p in co-immunoprecipitation experiments and two-hybrid tests, and is required for vacuole inheritance and recruitment of Vac17p to the organelle (Tang et al. 2003). Finally, recent studies indicate that Vac17p protein and mRNA levels are high when segregation structures are formed, and implicate a PEST sequence in Vac17p in its turnover (Tang et al. 2003). Thus, there is evidence for cell cycle-dependent regulation of Vac17p levels.

According to one model for vacuolar inheritance, binding of Vac17p to the vacuole-specific region of the globular tail of Myo2p and to the integral vacuolar membrane protein Vac8p results in recruitment of Myo2p to the vacuolar surface. This, in turn, allows for Myo2p-driven movement of the vacuolar segregation structure from mother cell to developing bud, using actin cables as tracks. Finally, PEST-sequence-mediated degradation of Vac17p in the bud tip releases the motor from its cargo, contributing to the retention of a newly inherited vacuole in the bud (Tang et al. 2003; Weisman 2003).

This model for Myo2p-driven vacuolar inheritance is based, in part, on the established role of type V myosins in mediating the movement of secretory vesicles using actin cables as tracks in budding yeast (Pruyne et al. 1998; Schott et al. 1999). Studies of vacuolar motility with better temporal resolution, including analyses of the direct interaction between vacuoles and actin cables and the effect of *MYO2* mutations on vacuolar motility and association with actin cables, may provide additional support for this model of organelle inheritance. However, since Myo2p is enriched in the bud tip (Lillie and Brown 1994), it is possible that it serves as a capture device to retain vacuoles at that site.

D. The Golgi Apparatus

Two models for Golgi function address mechanisms for Golgi biogenesis and inheritance. According to the "stable compartments model", the Golgi consists of stable cisternae, and vesicles carry proteins across these cisternae (Dunphy and Rothman 1985). In this model, the Golgi is a distinct entity that may be produced in either a template-dependent or -independent process. By contrast, according to the "cisternal progression model", the Golgi apparatus is a dynamic membrane system that can be produced de novo by fusion of ER-derived membranes, and mediates protein trafficking by continuous movement of membranes from its cis to trans face (Beams and Kessel 1968; Morré 1987). Studies in the yeast *Pichia pastoris*, which support the cisternal progression model, indicate that late Golgi membranes appear to form subsequent to the formation of translational ER (tER), ER subdomains where the coat protein (COP) II

transport vesicles are formed and that are morphologically and functionally distinct from the rest of the ER (Soderholm et al. 2004). In these studies, apparent formation of tER and late Golgi were monitored using Sec7p-DsRed and Sec13p-GFP, respectively. Consistent with this, tER localizes to regions in close proximity to Golgi stacks in *P. pastoris*. These observations lend further support to a refined model of Golgi inheritance in which fusion of the COPII vesicles results in the de novo synthesis of Golgi cisternae (Bevis et al. 2002).

There is no detectable tER in *S. cerevisiae*. Indeed, the Golgi of budding yeast does not appear to be organized in stacks. Rather, they are dispersed throughout the cytoplasm (Rossanese et al. 1999). Nonetheless, Golgi inheritance is believed to be a cell-cycle-dependent, non-random process (Rossanese et al. 2001). Using Sec7p-GFP as a marker, late Golgi membranes are detected near the incipient budding site and dispersed throughout the bud. Moreover, movement of Sec7p-GFP from the bud neck to bud tip has been documented. Since mutation of the Myo2p motor domain (*myo2-66*) results in defects in late Golgi localization, it is possible that late Golgi movement during inheritance is mediated by Myo2p-driven processes (Rossanese et al. 2001).

A capture mechanism, similar to that first observed during mitochondrial inheritance, has been detected during late Golgi inheritance in budding yeast – that is, late Golgi elements accumulate in the bud tip and are released from their retention site in the bud tip prior to cytokinesis. Finally, during cytokinesis, Golgi cisternae appear grouped together with secretory vesicles at sites of cell wall synthesis in order to deposit cell surface material (Preuss et al. 1992). Since destabilization of F-actin reduced the amount of Sec7p-GFP that accumulates in the bud tip, retention of late Golgi in the bud tip, like retention of mitochondria at that site, appears to be actin-dependent. Consistent with this, a mutation in Cdc1p (*cdc1-304*), which results in depolarization of the actin cytoskeleton in budding yeast, also results in defects in retention of late Golgi elements in the bud tip, but has no effect on the inheritance of early Golgi (Rossanese et al. 2001).

E. Peroxisomes

Peroxisomes are small, lipid bilayer-bound organelles that perform diverse functions including fatty acid β-oxidation or H_2O_2 metabolism (van den Bosch et al. 1992). Peroxisome abundance depends on a balance between biogenesis, division, and degradation. In budding yeast, peroxisome biogenesis is induced by growth conditions (e.g., fatty acid- or methanol-based growth media) that require peroxisome activity (see review by Veenhuis and Harder 1988). Under inducing conditions, peroxisomes can occupy up to 80% of the cytoplasmic volume in *Hansenula polymorpha* (Veenhuis et al. 1979). Conversely, peroxiphagy (rapid autophagy of peroxisomes) occurs after removal of peroxisome inducers. The protein Pex14p contributes to peroxiphagy in *H. polymorpha* (Bellu et al. 2001).

According to the classical model of peroxisome biogenesis, peroxisomes arise by fission from preexisting peroxisomes (Lazarow and Fujiki 1985). This view has been challenged recently by evidence from several groups supporting the de novo synthesis of early and immature peroxisomes (Eckert and Erdmann 2003; Lazarow 2003). The proteins that contribute to peroxisome fission and movement during inheritance are described below.

Several "peroxins", proteins required for normal peroxisome development and function, have been identified and characterized in budding yeast (Distel et al. 1996). Peroxins contribute to the maintenance of the peroxisomal membrane, import of proteins into the peroxisome matrix, and the control of peroxisome abundance or morphology. Pex11p is important for peroxisome proliferation in budding yeast. *PEX11* overexpression produces proliferation of very small peroxisomes. On the other hand, *pex11* deletion strains contain a small number of abnormally large peroxisomes (Erdmann and Bolbel 1995). These results suggest that Pex11p functions mainly in dividing the peroxisomal compartment. An additional role of Pex11p in fatty acid oxidation has also been proposed (van Roermund et al. 2000). Another protein required for peroxisome division in *S. cerevisiae* is Vps1p (one of the three dynamin-like proteins in budding yeast). Mutants lacking *VPS1* show single, big peroxisomes or clusters of small peroxisomes that failed to separate during the fission process (Hoepfner et al. 2001).

In *Y. lipolytica*, coordination of peroxisome maturation and division occurs by redistribution of the peroxisomal protein acyl-CoA oxidase (Aox) from the matrix to the membrane, and its interaction with the membrane-associated protein Pex16p (Guo et al. 2003). Peroxisome maturation in this cell type requires budding of a COP-vesicle from

the ER (Titorenko et al. 2000). This suggests that ER-derived vesicles can fuse with preexisting peroxisomes or mature into peroxisomes themselves (Erdmann et al. 1997; Titorenko and Rachubinski 1998; Tabak et al. 1999).

Finally, in *S. cerevisiae*, peroxisomes segregate in a cell cycle-dependent manner. Time-lapse microscopy experiments revealed that they move along the cell cortex before bud formation, and from the incipient bud site into the bud during bud growth. In small buds, peroxisomes are present at the bud tip, and as the bud grows they move to the bud cortex (Hoepfner et al. 2001). Peroxisomes colocalize with actin cables, and depolymerization of the actin cytoskeleton after Lat-A treatment results in a loss of peroxisome-directed movement. Moreover, the type V myosin Myo2p is involved in peroxisome movement and inheritance in *S. cerevisiae*, as peroxisome localization in the buds is delayed upon incubation of a conditional *MYO2* mutant at restrictive conditions.

V. Conclusions

Organelle inheritance has emerged as an active and fundamentally important branch of cell biology. As a result of advances in our understanding of organelle biogenesis, cell cycle progression, cytoskeletal dynamics, establishment of cell polarity, and membrane–cytoskeletal interactions, we are now in a position to uncover the molecular basis for organelle inheritance. Diversity appears to be a theme in organelle inheritance, from the use of immobilization versus selective mobilization for control of organelle position, in the type of force-generating machinery that is used to drive organelle motility, and in the type of cytoskeletal network used for all forms of positional control. Fungi will continue to play a central role as model systems to understand the mechanisms that ensure proper organelle segregation during cell division in dividing cells.

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References

Adams AEM, Botstein D, Drubin DG (1991) Requirement of yeast fimbrin for actin organization and morphogenesis in vivo. Nature 354:404–408

Aist JR, Bayles CJ (1991) Organelle motility within mitotic asters of the fungus *Nectria haematococca*. Eur J Cell Biol 56:358–363

Alberti-Segui C, Dietrich F, Altmann-Johl R, Hoepfner D, Philippsen P (2001) Cytoplasmic dynein moves nuclei towards the hyphal tip in the filamentous ascomycete *Ashbya gossypii*. J Cell Sci 114:975–986

Asakura T, Sasaki T, Nagano F, Satoh A, Obaishi H, Nishioka H, Imamura H, Hotta K, Tanaka K, Nakanishi H et al. (1998) Isolation and characterization of a novel actin filament-binding protein from *Saccharomyces cerevisiae*. Oncogene 16:121–130

Baba M, Osumi M (1987) Transmission and scanning electron microscopy examination of intracellular organelles in freeze-substituted *Kloeckera* and *Saccharomyces cerevisiae* yeast cells. J Electron Microsc 5:249–261

Banuett F, Herskowitz I (2002) Bud morphogenesis and the actin and microtubule cytoskeletons during budding in the corn smut fungus *Ustilago maydis*. Fungal Genet Biol 37:149–170

Barelle CJ, Bohula EA, Kron SJ, Wessels D, Soll DR, Schafer A, Brown AJ, Gow NA (2003) Asynchronous cell cycle and asymmetric vacuolar inheritance in true hyphae of *Candida albicans*. Eukaryot Cell 2:398–410

Beach DL, Thibodeaux J, Maddox P, Yeh E, Bloom K (2000) The role of the proteins Kar9 and Myo2 in orienting the mitotic spindle of budding yeast. Curr Biol 10:1497–1506

Beams HW, Kessel RG (1968) The Golgi apparatus: structure and function. Int Rev Cytol 23:209–276

Bellu AR, Komori M, van der Klei IJ, Kiel JA, Veenhuis M (2001) Peroxisome biogenesis and selective degradation converge at Pex14p. J Biol Chem 276:44570–44574

Berger KH, Sogo LF, Yaffe MP (1997) Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. J Cell Biol 136:545–553

Bertrand E, Chartrand P, Schaefer M, Shenoy SM, Singer RH, Long RM (1998) Localization of *ASH1* mRNA particles in living yeast. Mol Cell 2:437–445

Bevis BJ, Hammond AT, Reinke CA, Glick BS (2002) De novo formation of transitional ER sites and Golgi structures in *Pichia pastoris*. Nat Cell Biol 4:750–756

Bohl F, Kruse C, Frank A, Ferring D, Jansen RP (2000) She2p, a novel RNA-binding protein tethers *ASH1* mRNA to the Myo4p myosin motor via She3p. EMBO J 19:5514–5524

Boldogh IR, Yang HC, Nowakowski WD, Karmon SL, Hays LG, Yates JR III, Pon LA (2001a) Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast. Proc Natl Acad Sci USA 98:3162–3167

Boldogh IR, Yang HC, Pon LA (2001b) Mitochondrial inheritance in budding yeast. Traffic 2:368–374

Boldogh IR, Nowakowski DW, Yang HC, Chung H, Karmon S, Royes P, Pon LA (2003) A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. Mol Biol Cell 14:4618–4627

Boldogh IR, Ramcharan SL, Yang HC, Pon LA (2004) A type V myosin (Myo2p) and a Rab-like G-protein (Ypt11p) are required for retention of newly inherited mitochondria in yeast cells during cell division. Mol Biol Cell 15:3994–4002

Botstein D, Amberg D, Mulholland J, Huffaker T, Adams A, Drubin D, Stearns T (1997) The yeast cytoskeleton. In: Pringle JR, Broach JR, Jones EW (eds) The molecular and cellular biology of the yeast *Saccharomyces*: cell cycle and cell biology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 1–90

Burgess SM, Delannoy M, Jensen RE (1994) *MMM1* encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. J Cell Biol 126:1375–1391

Catlett NL, Weisman LS (1998) The terminal tail region of a yeast myosin-V mediates its attachment to vacuole membranes and sites of polarized growth. Proc Natl Acad Sci USA 95:14799–14804

Catlett N, Duex J, Tang F, Weisman L (2000) Two distinct regions in a yeast myosin-V tail domain are required for the movement of different cargoes. J Cell Biol 150:513–526

Chang F, Nurse P (1996) How fission yeast fission in the middle. Cell 84:191–194

Chang FS, Stefan CJ, Blumer KJ (2003) A WASp homolog powers actin polymerization-dependent motility of endosomes in vivo. Curr Biol 13:455–463

Conradt D, Shaw J, Vida T, Emr S, Wickner W (1992) In vitro reactions of vacuole inheritance in *Saccharomyces cerevisiae*. J Cell Biol 119:1469–1479

Dabora SL, Sheetz MP (1988) The microtubule-dependent formation of a tubulovesicular network with characteristics of the ER from cultured cell extracts. Cell 54:27–35

Distel B, Erdmann R, Gould SJ, Blobel G, Crane DI, Cregg JM, Dodt G, Fujiki Y, Goodman JM, Just WW et al. (1996) A unified nomenclature for peroxisome biogenesis factors. J Cell Biol 135:1–3

Drees B, Brown C, Barrell BG, Bretscher A (1995) Tropomyosin is essential in yeast, yet the *TPM1* and *TPM2* proteins perform distinct functions. J Cell Biol 128:383–392

Drubin DG, Jones HD, Wertman KF (1993) Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. Mol Biol Cell 4:1277–1294

Du Y, Pypaert M, Novick P, Ferro-Novick S (2001) Aux1p/Swa2p is required for cortical endoplasmic reticulum inheritance in *Saccharomyces cerevisiae*. Mol Biol Cell 12:2614–2628

Dunphy WG, Rothman JE (1985) Compartmental organization of the Golgi stack. Cell 42:13–21

Eckert JH, Erdmann R (2003) Peroxisome biogenesis. Rev Physiol Biochem Pharmacol 147:75–121

Efimov VP (2003) Roles of NUDE and NUDF proteins of *Aspergillus nidulans*: insights from intracellular localization and overexpression effects. Mol Biol Chem 14:871–888

Einerhand AW, van der Leij I, Kos WT, Distel B, Tabak HF (1992) Transcriptional regulation of genes encoding proteins involved in biogenesis of peroxisomes in *Saccharomyces cerevisiae*. Cell Biochem Funct 10:185–191

Eitzen G, Wang L, Thorngren N, Wickner W (2002) Remodeling of organelle-bound actin is required for yeast vacuole fusion. J Cell Biol 158:669–679

Engqvist-Goldstein AE, Drubin DG (2003) Actin assembly and endocytosis: from yeast to mammals. Annu Rev Cell Dev Biol 19:287–332

Erdmann R, Blobel G (1995) Giant peroxisomes in oleic acid-induced *Saccharomycs cerevisiae* lacking the peroxisomal membrane protein Pmp27p. J Cell Biol 128:509–523

Erdmann R, Veenhuis M, Kunau WH (1997) Peroxisomes: organelles at the crossroads. Trends Cell Biol 7:400–407

Estrada P, Kim J, Coleman J, Walker L, Dunn B, Takizawa P, Novick P, Ferro-Novick S (2003) Myo4p and She3p are required for cortical ER inheritance in *Saccharomyces cerevisiae*. J Cell Biol 163:1255–1266

Evangelista M, Pruyne D, Amberg DC, Boone C, Bretscher A (2002) Formins direct Arp2/3-independent actin filament assembly to polarize cell growth in yeast. Nat Cell Biol 4:260–269

Fehrenbacher KL, Davis D, Wu M, Boldogh I, Pon LA (2002) Endoplasmic reticulum dynamics, inheritance, and cytoskeletal interactions in budding yeast. Mol Cell Biol 13:854–865

Fehrenbacher KL, Bodogh IR, Pon LA (2003) Taking the A-train: actin-based force generators and organelle targeting. Trends Cell Biol 13:472–477

Fehrenbacher KL, Yang HC, Gay AC, Huckaba TM, Pon LA (2004) Live cell imaging of mitochondrial movement along actin cables in budding yeast. Curr Biol 14:1996–2004

Gall WE, Higginbotham MA, Chen C, Ingram MF, Cyr DM, Graham TR (2000) The auxilin-like phosphoprotein Swa2p is required for clathrin function in yeast. Curr Biol 10:1349–1358

Graia F, Berteaux-Lecellier V, Zickler D, Picard M (2000) ami1, an orthologue of the *Aspergillus nidulans* apsA gene, is involved in nuclear migration throughout the life cycle of *Podospora anserine*. Genetics 155:633–646

Grote E, Carr CM, Novick PJ (2000) Ordering the final events in yeast exocytosis. J Cell Biol 151:439–452

Guo T, Kit YY, Nicaud JM, Le Dall MT, Sears SK, Vali H, Chan H, Rachubinski RA, Titorenko VI (2003) Peroxisome division in the yeast *Yarrowia lipolytica* is regulated by a signal from inside the peroxisome. J Cell Biol 162:1255–1266

Hagan IM (1998) The fission yeast microtubule cytoskeleton. J Cell Sci 111:1603–1612

Han G, Liu B, Zhang J, Zuo W, Morris NR, Xiang X (2001) The *Aspergillus* cytoplasmic dynein heavy chain and NUDF localize to microtubule plus ends and affect microtubule dynamics. Curr Biol 11:719–724

Harris SD (2001) Septum formation in *Aspergillus nidulans*. Curr Opin Microbiol 4:736–739

Heath IB, Heath MC, Herr F (1982) Motile systems in fungi. Symp Soc Exp Biol 35:563–587

Hedge RS, Lingappa VR (1999) Regulation of protein biogenesis at the endoplasmic reticulum membrane. Trends Cell Biol 9:132–137

Hermann GJ, Shaw JM (1998) Mitochondrial dynamics in yeast. Annu Rev Cell Dev Biol 14:265–303

Hermann GJ, King EJ, Shaw JM (1997) The yeast gene, *MDM20*, is necessary for mitochondrial inheritance and organization of the actin cytoskeleton. J Cell Biol 137:141–153

Hill KL, Catlett NL, Weisman LS (1996) Actin and myosin function in directed vacuole movement during cell division in *Saccharomyces cerevisiae*. J Cell Biol 135:1535–1549

Hobbs AE, Srinivasan M, McCaffery JM, Jensen RE (2001) Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. J Cell Biol 152:401–410

Hoepfner D, van den Berg M, Philippsen P, Tabak HF, Hettema EH (2001) A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae*. J Cell Biol 155:979–990

Howard RJ, Aist JR (1980) Cytoplasmic microtubules and fungal morphogenesis: ultrastructural effects of methyl benzimidazole-2-ylcarbamate determined by freeze-substitution of hyphal tip cells. J Cell Biol 87:55–64

Huckaba TM, Gay AC, Pantalena LF, Yang H-C, Pon LA (2004) Live cell imaging of assembly, disassembly, and actin cable-dependent movement of endosomes and actin patches in the budding yeast, *Saccharomyces cerevisiae*. J Cell Biol 167:519–530

Insall R, Muller-Taubenberger A, Machesky L, Kohler J, Simmeth E, Atkinson SJ, Weber I, Gerisch G (2001) Dynamics of the *Dictyostelium* Arp2/3 complex in endocytosis, cytokinesis, and chemotaxis. Cell Motil Cytoskeleton 50:115–128

Ishikawa K, Catlett NL, Novak JL, Tang F, Nau JJ, Weisman LS (2003) Identification of an organelle-specific myosin V receptor. J Cell Biol 160:887–897

Itoh T, Watabe A, Toh-e A, Matsui Y (2002) Complex formation with Ypt11p, a rab-type small GTPase, is essential to facilitate the function of Myo2p, a class V myosin, in mitochondrial distribution in *Saccharomyces cerevisiae*. Mol Cell Biol 22:7744–7757

Jamet-Vierny C, Contamine V, Boulay J, Zickler D, Picard M (1997) Mutations in genes encoding the mitochondrial membrane proteins Tom70 and Mdm10 of *Podospora anserina* modify the spectrum of mitochondrial DNA rearrangements associated with cellular death. Mol Cell Biol 17:6359–6366

Jones HD, Schliwa M, Drubin DG (1993) Video microscopy of organelle inheritance and motility in budding yeast. Cell Motil Cytoskeleton 25:129–142

Jung MK, May GS, Oakley BR (1998) Mitosis in wild-type and beta-tubulin mutant strains of *Aspergillus nidulans*. Fungal Genet Biol 24:146–160

Kaksonen M, Sun Y, Drubin DG (2003) A pathway for association of receptors, adaptors, and actin during endocytic internalization. Cell 115:475–487

Kanbe T, Kobayashi I, Tanaka K (1989) Dynamics of cytoplasmic organelles in the cell cycle of the fission yeast *Schizosaccharomyces pombe*: three-dimensional reconstruction from serial sections. J Cell Sci 94:647–656

Koning AJ, Roberts CJ, Wright RL (1996) Different subcellular localization of *Saccharomyces cerevisiae* HMG-CoA reductase isoenzymes at elevated levels corresponds to distinct endoplasmic reticulum proliferations. Mol Biol Cell 7:769–789

Lazarow PB (2003) Peroxisome biogenesis: advances and conundrums. Curr Opin Cell Biol 15:489–497

Lazarow PB, Fujiki Y (1985) Biogenesis of peroxisomes. Annu Rev Cell Biol 1:489–530

Lazzarino DA, Boldogh I, Smith MG, Rosand J, Pon LA (1994) Yeast mitochondria contain ATP-sensitive, reversible actin-binding activity. Mol Biol Cell 5:807–818

Lee WL, Oberle, JR, Cooper JA (2003) The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. J Cell Biol 160:355–364

Lillie SH, Brown SS (1994) Immunofluorescence localization of the unconventional myosin, Myo2p, and the putative kinesin-related protein, Smylp, to the same regions of polarized growth in *Saccharomyces cerevisiae*. J Cell Biol 125:825–842

McConnell SJ, Stewart LC, Talin A, Yaffe MP (1990) Temperature-sensitive mutants defective in mitochondrial inheritance. J Cell Biol 111:967–976

McDaniel DB, Roberson RW (2000) Microtubules are required for motility and positioning of vesicles and mitochondria in hyphal tip cells of *Allomyces macrogynus*. Fungal Genet Biol 31:233–244

Meldolesi J, Pozzan T (1998) The endoplasmic reticulum Ca^{2+} store: a view from the lumen. Trends Biochem Sci 23:10–14

Minke PF, Lee IH, Plamann M (1999) Microscopic analysis of *Neurospora* ropy mutants defective in nuclear distribution. Fungal Genet Biol 28:55–67

Moreau V, Madania A, Martin RP, Winson B (1996) The *Saccharomyces cerevisiae* actin-related protein Arp2 is involved in the actin cytoskeleton. J Cell Biol 134:117–132

Morré DJ (1987) The Golgi apparatus. Int Rev Cytol Suppl 17:211–253

Oakley (2004) Tubulins in *Aspergillus nidulans*. Fungal Genet Biol 41:420–427

Pelham HR (1996) The dynamic organization of the secretory pathway. Cell Struct Funct 21:413–419

Pishvaee B, Costaguta G, Yeung BG, Ryazantsev S, Greener T, Greene LE, Eisenberg E, McCaffery JM, Payne GS (2000) A yeast DNA J protein required for uncoating of clathrin-coated vesicles *in vivo*. Nat Cell Biol 2:958–963

Pollard TD, Beltzner CC (2002) Structure and function of the Arp2/3 complex. Curr Opin Struct Biol 12:768–774

Pon LA, Schatz G (1991) Biogenesis of yeast mitochondria. In: Pringle JR, Broach JR, Jones EW (eds) The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis and energetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 334–406

Preuss D, Mulholland J, Kaiser CA, Orlean P, Allbright C, Rose MD, Robbins PW, Botstein D (1991) Structure of the yeast endoplasmic reticulum: localization of ER proteins using immunofluorescence and immunoelectron microscopy. Yeast 7:891–911

Preuss D, Mulholland J, Franzusoff A, Segev N, Botstein D (1992) Characterization of the *Saccharomyces* Golgi complex through the cell cycle by immunoelectron microscopy. Mol Biol Cell 3:89–803

Prinz W, Grzyb L, Veenhuis M, Kahana JA, Silver PA, Rapoport TA (2000) Mutants affecting the structure of

the cortical endoplasmic reticulum in *Saccharomyces cerevisiae*. J Cell Biol 150:461–474

Prokisch H, Neupert W, Westermannn B (2000) Role of MMM1 in maintaining mitochondrial morphology in *Neurospora crassa*. Mol Biol Cell 11:2961–2971

Pruyne DW, Schott DH, Bretscher A (1998) Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J Cell Biol 143:1931–1945

Pruyne D, Evangelista M, Yang C, Bi E, Zigmond S, Bretscher A, Boone C (2002) Role of formins in actin assembly: nucleation and barbed-end association. Science 297:612–615

Requena N, Alberti-Segui C, Wizenburg E, Horn C, Schiliwa M, Philippsen P, Liese R, Fischer R (2001) Genetic evidence for a microtubule-destabilizing effect of convectional kinesin and analysis of its consequences for the control of nuclear distribution in *Aspergillus nidulans*. Mol Microbiol 42:121–132

Roeder AD, Hermann GJ, Keegan BR, Thatcher SA, Shaw JM (1998) Mitochondrial inheritance is delayed in *Saccharomyces cerevisiae* cells lacking the serine/threonine phosphatase *PTC1*. Mol Biol Cell 9:917–930

Rossanese OW, Soderholm J, Bevis BJ, Sears IB, O'Connor J, Williamson EK, Glick BS (1999) Golgi structure correlates with transitional endoplasmic reticulum organization in *Pichia pastoris* and *Saccharomyces cerevisiae*. J Cell Biol 145:69–81

Rossanese OW, Reinke CA, Bevis BJ, Hammond AT, Sears IB, O'Connor J, Glick BS (2001) A role for actin, Cdc1p and Myo2p in the inheritance of late Golgi element in *Saccharomyces cerevisiae*. J Cell Biol 153:47–62

Sagot I, Klee SK, Pellman D (2002a) Yeast formins regulate cell polarity by controlling the assembly of actin cables. Nat Cell Biol 4:42–50

Sagot I, Rodal AA, Moseley J, Goode BL, Pellman D (2002b) An actin nucleation mechanism mediated by Bni1 and profilin. Nat Cell Biol 4:626–631

Sato M, Toda T (2004) Reconstruction of microtubules; entry into interphase. Dev Cell 6:456–458

Schmidt A, Hall MN (1998) Signalling to the actin cytoskeleton. Annu Rev Cell Dev 14:305–338

Schott D, Ho J, Pruyne D, Bretscher A (1999) The COOH-terminal domain of Myo2p, a yeast myosin V, has a direct role in secretory vesicle targeting. J Cell Biol 147:791–808

Schott DH, Collins RN, Bretscher A (2002) Secretory vesicle transport velocity in living cells depends on the myosin-V lever arm length. J Cell Biol 156:35–39

Severs NJ, Jordan EG, Willamson DH (1976) Nuclear pore absence form areas of close association between nucleus and vacuole in synchronous yeast cultures. J Ultrastruct Res 54:374–387

Sheeman B, Carvalho P, Sagot I, Geiser J, Kho D, Hoyt MA, Pellman D (2003) Determinants of *S. cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. Curr Biol 139:985–994

Simon VR, Swayne TC, Pon LA (1995) Actin-dependent mitochondrial motility in mitotic yeast and cell-free systems: identification of a motor activity on the mitochondrial surface. J Cell Biol 130:345–354

Simon VR, Karmon SL, Pon LA (1997) Mitochondrial inheritance: cell cycle and actin cable dependence of polarized mitochondrial movements in *Saccharomyces cerevisiae*. Cell Motil Cytoskeleton 37:199–210

Soderholm J, Bhattacharyya D, Strongin D, Markovitz V, Connerly PL, Reinke CA, Glick BS (2004) The transitional ER localization mechanism of *Pichia pastoris* Sec12. Dev Cell 6:649–659

Sogo LF, Yaffe MP (1994) Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. J Cell Biol 126:1361–1373

Steinberg G, Fuchs U (2004) The role of microtubules in cellular organization and endocytosis in the plant pathogen *Ustilago maydis*. J Microsc 214:114–123

Steinberg G, Schliwa M (1993) Organelle movements in the wild type and wall-less fz;sg;os-1 mutants of *Neurospora crassa* are mediated by cytoplasmic microtubules. J Cell Sci 106:555–564

Steinberg G, Wedlich-Söldner R, Brill M, Schulz I (2001) Microtubules in the fungal pathogen *Ustilago maydis* are highly dynamic and determine cell polarity. J Cell Sci 114:609–622

Stevens BJ (1977) Variation in number and volume of the mitochondria in yeast according to growth conditions. A study based on serial sectioning and computer graphics reconstruction. Biol Cell 29:37–56

Straube A, Brill M, Oakley BR, Horio T, Steinberg G (2003) Microtubule organization requires cell cycle-dependent nucleation at dispersed cytoplasmic sites: polar and perinuclear microtubule organizing centers in the plant pathogen *Ustilago maydis*. Mol Biol Cell 14:642–657

Suelmann R, Fischer R (2000) Mitochondrial movement and morphology depend on an intact actin cytoskeleton in *Aspergillus nidulans*. Cell Motil Cytoskeleton 45:42–50

Svitkina TM, Borisy GG (1999) Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. J Cell Biol 145:1009–1026

Tabak HF, Braakman I, Distel B (1999) Peroxisomes: simple in function but complex in maintenance. Trends Cell Biol 9:447–453

Takizawa PA, Vale RD (2000) The myosin motor, Myo4p, binds Ash1 mRNA via the adapter protein, She3p. Proc Natl Acad Sci USA 97:5273–5278

Takizawa PA, Sil A, Swedlow JR, Herskowitz I, Vale RD (1997) Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. Nature 389:90–93

Takizawa PA, DeRisi JL, Wilhelm JE, Vale RD (2000) Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. Science 290:341–344

Tang F, Kauffman EJ, Novak JL, Nau JJ, Catlett NL, Weisman LS (2003) Regulated degradation of class V myosin receptor directs movement of the yeast vacuole. Nature 422:87–92

Titorenko VI, Rachubinski RA (1998) The endoplasmic reticulum plays an essential role in peroxisome biogenesis. Trends Biochem Sci 23:231–233

Titorenko VI, Chan H, Rachubinski RA (2000) Fusion of small peroxisomal vesicles in vitro reconstructs an early step in the in vivo multistep peroxisome assembly pathway of *Yarrowia lipolytica*. J Cell Biol 148:29–44

Tran PT, Doye V, Chang F, Inoue S (2000) Microtubule-dependent nuclear positioning and nuclear-dependent septum positioning in the fission yeast *Schizosaccharomyces pombe*. Biol Bull 199:205–206

Tran PT, Harsh L, Doye V, Inoue S, Chang F (2001) A mechanism for nuclear positioning in fission yeast based on microtubule pushing. J Cell Biol 153:397–412

Van den Bosch H, Schutgens RB, Wanders RJ, Tager JM (1992) Biochemistry of peroxisomes. Annu Rev Biochem 61:157–197

Van Roermund CW, Tabak HF, van Den Berg M, Wanders RJ, Hettema EH (2000) Pex11p plays a primary role in medium-chain fatty acid oxidation, a process that affects peroxisome number and size in *Saccharomyces cerevisiae*. J Cell Biol 150:489–498

Veenhuis M, Harder W (1988) Microbodies in yeasts: structure, function and biogenesis. Microbiol Sci 5:347–351

Veenhuis M, Keizer I, Harder W (1979) Characterization of peroxisomes in glucose-grown *Hansenula polymorpha* and their development after the transfer of cells into methanol-containing media. Arch Microbiol 120:167–175

Volkmann N, Amann KJ, Stoilova-McPhie S, Egile C, Winter DC, Hazelwood L, Heuser JE, Li R, Pollard TD, Hanein D (2001) Structure of Arp2/3 complex in its activated state and in actin filament branch junctions. Science 293:2456–2459

Walch-Solimena C, Collins RN, Novick PJ (1997) Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. J Cell Biol 137:1495–1509

Warmka J, Hanneman J, Lee J, Amin D, Ota I (2001) Ptc1, a type 2C Ser/Thr phosphatase, inactivates the HOG pathway by dephosphorylating the mitogen-activated protein kinase Hog1. Mol Cell Biol 21:51–60

Wedlich-Söldner R, Schulz I, Straube A, Steinberg G (2002a) Dynein supports motility of endoplasmic reticulum in the fungus *Ustilago maydis*. Mol Biol Cell 13:965–977

Wedlich-Söldner R, Straube A, Friedrich MW, Steinberg G (2002b) A balance of KIF1A-like kinesin and dynein organizes early endosomes in the fungus *Ustilago maydis*. EMBO J 21:2946–2957

Weisman LS (2003) Yeast vacuole inheritance and dynamics. Annu Rev Genet 37:435–460

Weisman LS, Wickner W (1988) Intervacuole exchange in the yeast zygote: a new pathway in organelle communication. Science 241:589–591

Weisman LS, Bacallao R, Wickner W (1987) Multiple methods of visualizing the yeast vacuole permit evaluation of its morphology and inheritance during the cell cycle. J Cell Biol 105:1539–1547

Wiederkehr A, Du Y, Pypaert M, Ferro-Novick S, Novick P (2003) Sec3p is needed for the spatial regulation of secretion and for the inheritance of the cortical endoplasmic reticulum. Mol Biol Cell 14:4770–4782

Wiemken A, Matile P, Moor H (1970) Vacuolar dynamics in synchronously budding yeast. Arch Mikrobiol 70:89–103

Winter D, Podtelejnikov AV, Mann M, Li R (1997) The complex containing actin-related proteins Arp2 and Arp3 is required for the motility and integrity of yeast actin patches. Curr Biol 7:519–529

Wu Q, Sandrock TM, Turgeon BG, Yoder OC, Wirsel SG, Aist JR (1998) A fungal kinesin required for organelle motility, hyphal growth, and morphogenesis. Mol Biol Cell 9:89–101

Xiang X, Fischer R (2004) Nuclear migration and positioning in filamentous fungi. Fungal Genet Biol 41:411–419

Xiang S, Han G, Winkelmann DA, Zuo W, Morris NR (2000) Dynamics of cytoplasmic dynein in living cells and the effect of mutation in the dynactin complex actin-related protein Arp1. Curr Biol 9:1211–1220

Yaffe MP, Harata D, Verde F, Eddison M, Toda T, Nurse P (1996) Microtubules mediate mitochondrial distribution in fission yeast. Proc Natl Acad Sci USA 93:11664–11668

Yaffe MP, Stuurman N, Vale RD (2003) Mitochondrial positioning in fission yeast is driven by association with dynamic microtubules and mitotic spindle poles. Proc Natl Acad Sci USA 100:11424–11428

Yang HC, Pon LA (2002) Actin cable dynamics in budding yeast. Proc Natl Acad Sci USA 99:751–756

Yin H, Pruyne D, Huffaker TC, Bretscher A (2000) Myosin V orientates the mitotic spindle in yeast. Nature 406:1013–1015

Zhang J, Han G, Xiang X (2002) Cytoplasmic dynein intermediate chain and heavy chain are dependent upon each other for microtubule end localization in *Aspergillus nidulans*. Mol Microbiol 44:381–391

Zhang J, Li S, Fischer R, Xiang X (2003) The accumulation of cytoplasmic dynein and dynactin at microtubule plus ends is kinesin dependent in *Aspergillus nidulans*. Mol Biol Chem 14:1479–1488

3 Mitosis in Filamentous Fungi

S.D. Harris[1]

CONTENTS

I. Introduction

The cell cycle is a fundamental feature of all dividing cells (Murray and Hunt 1993). It comprises the orderly series of events that lead to the duplication (DNA replication) and segregation (mitosis) of a cell's chromosomes. Through the ability to coordinate the cell cycle with growth and cytokinesis, somatic cells insure the formation of identical duplicate progeny. The dramatic changes in nuclear morphology that accompany the cell cycle attracted the interest of early microscopists (cf. Nurse 2000), which led to a detailed morphological description of these events. This was particularly true for the fungi, where, by the early 20th century, descriptive analyses had already shown that fungal mitoses were largely identical to those of higher animals (reviewed by Aist and Morris 1999). However, it was not until the late 1970s that an understanding of the molecular mechanisms underlying the fungal cell cycle began to emerge. This progress was achieved primarily through genetic and molecular study of the cell cycle in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Lew et al. 1997; Su and Yanagida 1997). Most importantly, these studies confirmed what was previously hinted at by the earlier microscopic analyses, namely, that the core biochemistry of the cell cycle is conserved between fungi and animals. The advances made using yeast were superbly summarized in the previous edition of this volume (MacNeill 1994). In this edition, emphasis is placed on describing progress toward understanding mitosis in the filamentous fungi. Much has been learned over the past decade, including important similarities and key differences with yeast. Notable distinctions from yeast include insights into the regulation of mitosis within the context of multinucleate cells and elaborate morphogenetic programs. The new results are summarized, along with recent findings that provide a novel perspective on the regulation of fungal mitosis.

II. Fungal Mitosis

A. Chromosome and Spindle Organization

As recently summarized by Aist and Morris (1999), the patterns of chromosome movement and spin-

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dle organization that accompany mitosis were accurately described by the mid-20th century using conventional light and electron microscopy. Key pre-mitotic events include duplication and separation of the spindle pole bodies (SPBs), the fungal equivalent of microtubule-organizing centers (MTOCs). These structures are embedded within the nuclear envelope, and uniquely to fungi, appear to be tethered to interphase chromosomes via chromatin (Heath 1994). As they migrate, each SPB generates a half-spindle composed of microtubules. Once the SPBs are aligned opposite to each other, the half-spindles come into register to form a full, bipolar spindle. During the process of spindle assembly, interphase chromosomes undergo marked condensation near the developing spindle. Eventually, each chromosome is captured by spindle microtubules that bind to the kinetochore. Notably, in contrast to animals and plants, the nuclear envelope does not breakdown in preparation for mitosis (Aist and Morris 1999).

Unlike animals and plants, filamentous fungi do not display the classic metaphase alignment of chromosomes at the mid-point of the mitotic spindle (Fig. 3.1). Instead, metaphase chromosomes appear to occupy the middle one-third to one-half of the spindle. Chromosome segregation then occurs in two steps – anaphase A and anaphase B (Fig. 3.1). During anaphase A, sister chromatids disjoin and move toward the opposite spindle poles. Remarkably, microscopic examination of this process revealed that chromosome disjunction is not synchronous (Aist 1969). Accordingly, during anaphase A, separating chromatids are often aligned along the entire length of the spindle, rather than moving in a synchronous wave toward the pole. Anaphase B begins when the chromatids reach the poles, and is characterized by rapid elongation of the spindle and marked development of spindle asters. Another distinct feature of fungal mitosis is the absence, during anaphase B, of a well-defined spindle mid-zone consisting of antiparallel arrays of overlapping polar microtubules (Aist and Bayles 1991). This may have important implication for the subsequent regulation of cytokinesis. Finally, midway during anaphase B, the nuclear envelope collapses and re-forms around the segregated chromosomes. Following the completion of mitosis in fungal hyphae, nuclei undergo a period of rapid oscillatory movement within the hyphal cell. As a result, they achieve proper alignment throughout the cell prior to cytokinesis. At this point, it should be emphasized that not all mitoses are necessarily coupled to cytokinesis in hyphal cells (Clutterbuck 1970).

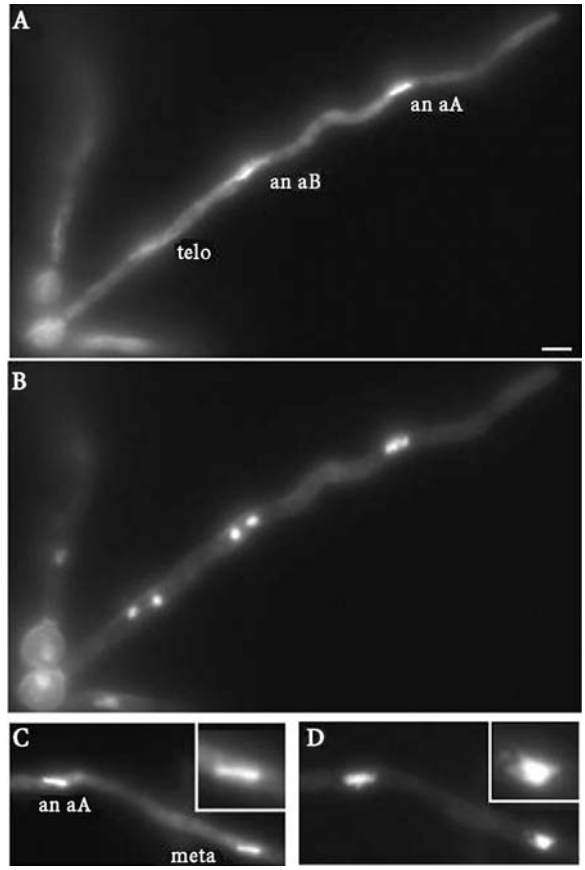

Fig. 3.1. A–D Progression of mitosis in *Aspergillus nidulans* hyphae. Mitotic spindles (**A**,**C**) are visualized using a functional GFP-TubA (α-tubulin) fusion provided by X. Xiang. Nuclei (**B**,**D**) are stained using Hoechst 33258. **A**,**B** Adjacent nuclei engaged in anaphase A (*anaA*), anaphase B (*anaB*), and telophase (*telo*). **C**,**D** Adjacent nuclei found in metaphase (*meta*) and anaphase A. *Insets* Higher magnification of the metaphase nucleus, showing that the nuclear material occupies approximately one-half of the spindle. *Bar* 4 μm (8 μm for *insets*)

B. The Duplication Cycle

Because hyphal cells do not separate following cytokinesis, the fungal cell cycle has been termed the duplication cycle (Fiddy and Trinci 1976; Trinci 1978). Like the cell cycle, the duplication cycle is defined by the period of time required to duplicate and segregate cellular constituents. For example, the *Aspergillus nidulans* cycle typically requires 120 min for completion, of which only about 5 min is devoted to mitosis (Bergen and Morris 1983).

As in yeast and animals, cell growth appears to be coordinated with the duplication cycle during the G1 phase. However, it is not clear if, like for both *S. cerevisiae* and *S. pombe*, filamentous fungi also possess a distinct cell size control that governs mitotic entry at the G2/M boundary. A unique feature of the duplication cycle in *A. nidulans* germlings is the existence of a novel post-mitotic cell size control that temporally regulates the formation of the first septum (Wolkow et al. 1996). This has led to the notion that mitotic entry may be subject to at least two different modes of regulation in hyphal cells (Harris 1997). Prior to formation of the first septum (i.e., in pre-divisional hyphae), mitotic exit is not coupled to cytokinesis and there may be no G2/M size control. By contrast, after the first septation event (i.e., in post-divisional hyphae), mitotic exit is linked to cytokinesis and normal G2/M size controls presumably exist. Additional modification of the controls linking mitosis with growth may occur during development. For example, the ability of *A. nidulans* phialides to produce large numbers of uninucleate conidiospores over a relatively short period of time (Timberlake 1990) may imply that controls normally operating during G1 and G2 are dispensed with altogether.

III. Regulation of Mitotic Entry

A. The CDK Module

Cyclin-dependent protein kinases (CDKs) are the central regulators of the eukaryotic cell cycle (Murray and Hunt 1993). They function as a module with an associated cyclin partner that targets the kinase to the appropriate substrates. Like yeast, filamentous fungi appear to possess a single Cdk2 homologue that regulates cell cycle progression. Of these homologues, the best characterized is *A. nidulans* NimX, which is required for both the G1/S and G2/M transitions (Osmani et al. 1994). Furthermore, based on the recent annotation of genome sequences (Borkovich et al. 2004), the fungal CDK family also includes homologues of yeast Pho85 and other CDKs potentially involved in transcriptional regulation. Notably, the *A. nidulans* Pho85 homologues PhoA and PhoB have no apparent role in phosphate metabolism, but do act in tandem to control an essential function required for polarized morphogenesis (Dou et al. 2003). Finally, a somewhat divergent CDK that is conserved in filamentous fungi, but not yeast, is involved in some aspect of the fungal DNA damage response (Fagundes et al. 2004).

CDK function is regulated by associated cyclins (Murray and Hunt 1993). For example, B-type cyclins universally regulate mitotic entry by targeting CDKs to specific phosphorylation substrates. Several mechanisms ensure proper regulation of mitotic cyclin activity (Fig. 3.2; Murray 2004), including stringent control of transcript accumulation, association with CDK inhibitors, and regulated proteolysis mediated by the anaphase-promoting complex (APC). Unlike *S. cerevisiae* and the closely related filamentous fungus *Ashbya gossypii*, which possess multiple distinct B-type cyclins, filamentous fungi generally appear to possess only two (Borkovich et al. 2004). Amongst those that have been characterized are *A. nidulans* NimE, which associates with NimX to control mitotic entry (O'Connell et al. 1992), and *Ustilago maydis* Clb1 and Clb2, which appear to regulate distinct aspects of mitotic progression (Garcia-Muse et al. 2004). Notably, *U. maydis* Clb2 also regulates morphogenesis and pathogenic development, perhaps as the target of a G2/M size control that responds to developmental signals. Filamentous fungi also possess homologues of the Cln and the Pcl cyclins that regulate the G1/S transition in *S. cerevisiae* (Schier et al.

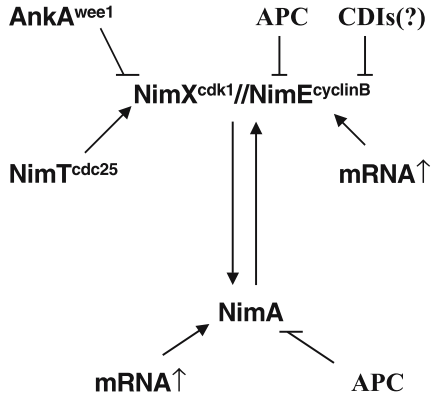

Fig. 3.2. Regulation of the NimX CDK module and the NimA kinase. The transcription of both *nimA* and *nimE* is tightly regulated, such that their expression is largely confined to the G2 phase. Once expressed, NimE is presumably held inactive by CDK inhibitors (i.e., CDIs). The balance of AnkA versus NimT activity maintains the NimX CDK module in an inactive state until mitotic entry, where NimT activity predominates. Activation of NimX subsequently promotes NimA activity, which in turn facilitates the localization of the NimX CDK module. The APC-mediated degradation of both NimE and NimA is required for mitotic exit. See text for additional details

2001; Borkovich et al. 2004). However, with the exception of *A. nidulans* PclA, which associates with NimX and likely regulates its activity during asexual development (Schier and Fischer 2002), the function of these cyclins remains unknown. Nevertheless, the paucity of fungal cyclins predicted to regulate Cdk2 (i.e., three vs. nine in *S. cerevisiae*) is somewhat surprising. Perhaps filamentous fungi resort to a similar mechanism as has been proposed for *S. pombe* (Stern and Nurse 1996), whereby threshold levels of a single B-type cyclin are sufficient to regulate all cell cycle transitions.

It has been firmly established that mitotic entry is regulated by reversible phosphorylation of the conserved CDK tyrosine-15 residue (Murray and Hunt 1993). Phosphorylation of this site by the Wee1 kinase inhibits CDK activity and blocks mitotic entry, whereas de-phosphorylation by the Cdc25 phosphatase stimulates CDK activity and promotes mitotic entry (Fig. 3.2). Accordingly, the relative balance of Wee1 and Cdc25 activities determines the timing of mitotic entry in response to growth and checkpoint signals. *A. nidulans* is the only filamentous fungus in which the roles of Wee1 and Cdc25 have been extensively characterized. As predicted, mutational inactivation of the Wee1 homologue, AnkA, largely abolishes NimX tyrosine-15 phosphorylation and triggers premature mitotic entry (Ye et al. 1996; Kraus and Harris 2001), whereas mutations in the Cdc25 homologue, NimT, cause a mitotic block with elevated NimX tyrosine-15 phosphorylation (Osmani et al. 1991). Unexpectedly, AnkA- and NimT-mediated regulation of NimX also exerts a post-mitotic effect on the timing of septum formation in pre-divisional hyphae (Harris and Kraus 1998; Kraus and Harris 2001), and is also modulated by developmental signals to control cell patterning during conidiation (Ye et al. 1999). These observations place NimX tyrosine-15 phosphorylation at the interface between mitotic regulation and morphogenesis in *A. nidulans*, though the precise mechanisms by which this occurs await investigation.

CDK inhibitors play a crucial role in the regulation of mitotic entry by interfering with the phosphorylation of substrates. In yeast, the CDK inhibitor Sic1 binds to mitotic cyclins and must be degraded to permit their accumulation (Cross 2003). The lack of obvious homology has precluded the straightforward identification of fungal CDK inhibitors by sequence annotation (Borkovich et al. 2004). However, in light of the finding that filamentous fungi possess fewer cyclins despite their increased morphological complexity, CDK inhibitors may provide one mechanism for imposing developmental constraints upon mitotic entry.

B. NimA Kinase

In *A. nidulans*, mitotic entry requires the parallel activity of both the CDK module and the NimA kinase (Fig. 3.3; Ye et al. 1995). NimA was first identified via temperature sensitive (Ts) mutations that caused a reversible arrest in late G2 (Morris 1976). Notably, at the arrest point, the CDK NimX is in an active, tyrosine-15 de-phosphorylated state (Osmani et al. 1991), such that mitosis rapidly ensues when the block is released. NimA is a conserved protein kinase, with functional homologues present in other filamentous fungi (Pu et al. 1995). Additional homologues with mitotic functions have been characterized in *S. pombe* and animals (O'Connell et al. 2003; Grallert et al. 2004). Indeed, NimA is the founding member of a family of protein kinases involved in diverse aspects of mitosis.

NimA is subject to multiple modes of regulation that share some features in common with cyclins (Fig. 3.2). For example, *nimA* expression

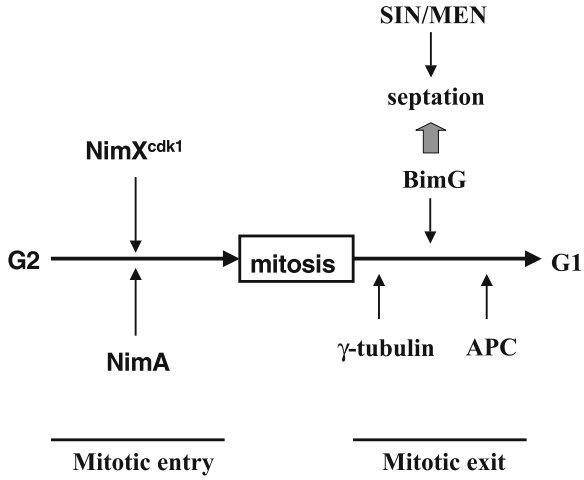

Fig. 3.3. Overview of mitotic regulatory circuits in *Aspergillus nidulans*. Mitotic entry (G2→M) and mitotic exit (M→G1) are depicted. The NimX CDK module and the NimA kinase act in a coordinate manner to regulate mitotic entry. Mitotic exit requires the APC, the BimG phosphatase, and γ-tubulin. BimG is also involved in septum formation. Unlike yeast, the SIN/MEN has no apparent role in mitotic exit, and is required only for septation. See text for additional details

is tightly regulated and confined to S phase and G2 (Osmani et al. 1987). In addition, like cyclins, NimA is degraded by proteolysis during mitosis, and expression of a non-degradable version prevents mitotic exit (Pu and Osmani 1995). Moreover, NimA and the NimX-NimE CDK complex co-regulate each other within an apparent feedback loop (Fig. 3.2). As part of this loop, NimA is hyper-phosphorylated by activated NimX, which boosts NimA activity at the point of mitotic entry (Ye et al. 1995). In parallel, NimA promotes the localization of the NimX-NimE CDK module to chromatin, the nucleolus, and SPBs during G2 (Wu et al. 1998). This mutual regulation presumably coordinates the activity of the two kinases and ensures timely entry into mitosis (Osmani and Ye 1996).

What is the mitosis promoting function of NimA? In *A. nidulans*, as well as *S. pombe*, NimA can promote chromosome condensation (Osmani et al. 1988a; O'Connell et al. 1994). NimA possesses the ability to phosphorylate histone H3 at the conserved serine-10 residue, and localizes to chromatin at the point of mitotic entry when this phosphorylation event occurs (de Souza et al. 2000). Notably, histone H3 kinase activity and nuclear localization appear to occur after the NimX-NimE CDK module hyper-phosphorylates NimA. Although this activity may account for the effect of NimA on chromatin condensation, it does not fully explain how NimA promotes mitotic entry. For example, NimA also localizes to mitotic spindles and SPBs after mitotic entry (de Souza et al. 2000). How NimA may regulate spindle organization and/or SPB function during mitosis remains to be determined. However, one mechanism may be via interaction with the NimA-interacting protein TinA, which localizes to SPBs during mitosis and regulates microtubule-nucleating capacity (Osmani et al. 2003).

Recent observations have provided further insight into the role of NimA in promoting mitotic entry in *A. nidulans*. In particular, mitotic entry is coupled to the rapid influx of tubulin from the cytoplasm into the nucleus, thereby enabling assembly of the mitotic spindle (Ovechkina et al. 2003). This influx occurs downstream of NimX activation, and presumably depends upon a sudden increase in the permeability of the nuclear envelope. Strikingly, concomitant genetic analyses suggest that NimA may regulate nuclear transport during mitotic entry. Mutations affecting two different components of the nuclear pore complex, SonA and SonB, can suppress the partially active *nimA1* allele (Wu et al. 1998; de Souza et al. 2003), apparently by restoring transport of both NimA1 and the NimX-NimE CDK module. Although it remains to be tested, bioinformatic analyses suggest that both SonA and SonB are potential phosphorylation substrates of NimA. Taken together, these results set up an attractive model whereby NimA activity directly modifies nuclear pore complexes to permit import of mitotic regulators, tubulin, and other factors required for mitosis.

IV. Regulation of Mitotic Exit

A. Anaphase-Promoting Complex

The anaphase-promoting complex (APC) is a multi-protein complex that functions as an E3 ubiquitin ligase that targets specific proteins for proteolytic degradation (Murray 2004). Key targets defined in yeast include securin, which blocks the dissolution of sister chromatid cohesion, and B-type cyclins (Thornton and Toczyski 2003). Accordingly, by eliminating sister chromatid cohesion, the APC promotes mitotic progression, and by destroying mitotic cyclins, also triggers exit from mitosis (Fig. 3.3). Components of the APC have been characterized in *A. nidulans*, where Ts mutations in *bimA* and *bimE* arrest cells in mitosis (Morris 1976). BimA (=Apc3) and BimE (=Apc1) associate within a complex that is slightly larger than the typical APC (Lies et al. 1998), and phenotypic characterization of mutations affecting either gene shows that mitotic exit requires APC function (Osmani et al. 1988b; O'Donnell et al. 1991). How does the APC trigger mitotic exit in *A. nidulans*? Likely targets include NimA and the cyclin NimE (Fig. 3.2; Lies et al. 1998; Ye et al. 1998), both of which must be degraded to permit exit from mitosis. Notably, genetic and biochemical evidence suggests that BimA may have a specific role in targeting the APC to NimA (Ye et al. 1998). The fungal APC has also been implicated in a potentially novel checkpoint function that may prohibit mitotic entry in response to specific interphase perturbations (Ye et al. 1996; Lies et al. 1998). Although it remains unclear how this checkpoint may operate, it presumably involves APC-mediated destruction of a key mitotic regulator such as NimE.

The mechanisms underlying the temporal regulation of APC function have been partially charac-

terized in *A. nidulans*. Phenotypic analysis of *bimA* mutants suggests that the accumulation of mitotic regulators such as NimA and NimE to a threshold level triggers activation of the APC (Ye et al. 1998). This may reflect the operation of negative feedback loops required for timely mitotic exit, whereby NimA and NimE promote their own destruction by activating the APC. The APC then appears to remain active until the initiation of DNA replication at the G1/S boundary. At this point, the regulatory controls that prevent inappropriate mitotic entry are turned over to inhibitory tyrosine-15 phosphorylation of NimX (Ye et al. 1997a).

B. The SIN/MEN Pathway

The SIN (septation initiation network)/MEN (mitotic exit network) is a GTPase-regulated protein kinase cascade that promotes cytokinesis and septum formation in both *S. cerevisiae* and *S. pombe* (reviewed by Simanis 2003). In addition, this signaling network triggers mitotic exit in *S. cerevisiae* by mediating the inactivation of mitotic CDKs. In both yeasts, the activating GTPase (Tem1p in *S. cerevisiae*, Spg1 in *S. pombe*) displays asymmetric localization to one of the two duplicated SPBs, where it facilitates the recruitment of the downstream protein kinases (including *S. cerevisiae* Cdc15p and *S. pombe* Cdc7). Ultimately, the terminal kinase (Dbf2p in *S. cerevisiae*, Sid2 in *S. pombe*) re-localizes to the septation site, where it promotes septum formation. In *S. cerevisiae*, MEN activity also regulates the Cdc14p phosphatase, which triggers mitotic exit by activating the APC and promoting the accumulation of CDK inhibitors.

Annotation of genome sequences reveals that the SIN/MEN pathway is conserved in filamentous fungi (S. Harris, unpublished data). However, functional characterization of *A. nidulans* SepH, which is a homologue of the Cdc15p/Cdc7 kinase, reveals two important differences from yeast (Bruno et al. 2001; Harris 2001). First, because a null mutation in *sepH* has no discernable effect on mitotic progression (Bruno et al. 2001), the SIN/MEN appears to be completely dispensable for mitotic exit in filamentous fungi (Fig. 3.3). Second, SIN/MEN signaling is required for assembly of the contractile actin ring at septation sites (Bruno et al. 2001; Sharpless and Harris 2002), whereas in yeast it acts after ring assembly to promote deposition of the division septum. Despite these initial insights, many questions remain regarding the role of the SIN/MEN in promoting mitotic exit, if any, and in the regulation of cytokinesis. For example, is SIN/MEN activity required for mitotic exit during development, where mitosis in typically coupled to cytokinesis? In addition, how is SIN/MEN activity spatially regulated in multinucleate hyphal cells, where each mitotic event is not necessarily coupled to cytokinesis?

C. γ-Tubulin

γ-tubulin was originally identified in *A. nidulans* (Oakley and Oakley 1989), and was subsequently shown to be universally required for microtubule nucleation at the SPB (or centrosome in animal cells; Oakley and Akkari 1999). Original observations highlighted the essential role of γ-tubulin in assembly of the mitotic spindle (Oakley et al. 1990). However, recent genetic studies have uncovered novel spindle-independent phenotypes caused by specific mutations in *A. nidulans mipA*, which encodes γ-tubulin (Jung et al. 2001). Notably, one mutation, *mipAD159*, disrupts the organization of late mitotic events (Prigozhina et al. 2004). As a result, nuclei that have not completed anaphase or chromosome segregation exit mitosis prematurely. These studies suggest that γ-tubulin may have an additional function that promotes the formation of SPB signaling complexes that regulate mitotic progression (Fig. 3.3). The role of the SPB as a signaling center has been established in both *S. cerevisiae* (Pereira and Schiebel 2001) and *S. pombe* (Grallert et al. 2004), and it has been shown that failure to properly recruit γ-tubulin to the *S. pombe* SPB causes premature activation of the SIN (Vardy et al. 2002). Accordingly, in *A. nidulans* and other filamentous fungi, proteins required for orderly mitotic exit might associate with the SPB in a γ-tubulin-dependent manner.

D. BimG

Because protein phosphorylation plays such a critical role in the regulation of mitosis, it is somewhat intuitive that de-phosphorylation would also be important. In support of this argument, BimG is a type I protein phosphatase required for mitotic exit in *A. nidulans* (Fig. 3.3; Doonan and Morris 1989). Mutations affecting BimG lead to mitotic arrest in anaphase, with increased phosphorylation of a mitosis-specific marker. BimG localizes to SPBs throughout mitosis, except for a brief pe-

riod in early mitosis when CDK and NimA activity are presumably at their highest (Fox et al. 2002). The potential targets of BimG remain unknown, but its association with duplicated SPBs suggests that it might regulate the activity and/or assembly of a mitotic exit complex. BimG also localizes to nucleoli, which appear to divide surprisingly late in fungal mitosis (Fox et al. 2002). In yeast, another protein phosphatase, Cdc14, is required for mitotic exit (Seshan and Amon 2004), and is normally retained in the nucleolus prior to anaphase. However, it is possible that Cdc14 may not regulate mitotic exit in filamentous fungi. Although it is conserved in several sequenced fungal genomes, the network of proteins that link its function to anaphase progression appears to be absent (S. Harris, unpublished data). In this context, it is tempting to speculate that BimG may in part fulfill the Cdc14 mitotic exit function.

V. Mitotic and Post-Mitotic Functions of Motor Proteins

A. Motor Proteins and Mitosis

The microtubule-based motor proteins kinesin and dynein are required for hyphal morphogenesis, which has been attributed to their ability to transport vesicles and organelles in a directed manner to and from hyphal tips (Seiler et al. 1999). However, they have also been implicated in multiple aspects of mitosis, including the assembly of a bipolar spindle, chromosome segregation during anaphase A, and spindle elongation during anaphase B (reviewed by Aist 2002). Kinesins are plus-end directed motor proteins, whereas dyneins are minus-end directed motors. Both proteins are ATPases that convert chemical energy into mechanical force that permits progressive movement along microtubules. Kinesins and dyneins are conserved in all sequenced filamentous fungal genomes (Schoch et al. 2003; Xiang and Plamann 2003). In fact, the diverse roles of microtubules in mitosis and morphogenesis are reflected by the presence of up to 11 kinesins in fungi such as *A. nidulans* (Rischitor et al. 2004).

BimC is the prototype of a family of kinesins involved in mitosis. In *A. nidulans*, *bimC* mutants undergo SPB duplication, but the SPBs fail to separate (Enos and Morris 1990). As a result, the mutant arrests with short monopolar spindles. BimC acts as a cross-bridge between anti-parallel microtubules and, because it is a plus-end directed motor, it effectively pushes the SPBs apart to generate a bipolar spindle. Another kinesin, KlpA, possesses an opposing force that appears to resist BimC and modulate the kinetics of SPB separation (O'Connell et al. 1993). Kinesins are also involved in anaphase B, where an inward-directed force within the mitotic spindle counters the astral microtubule-mediated pulling apart of the spindle (Aist 2002). In *Fusarium solani*, the kinesin NhKRP1 is apparently responsible for the inward-directed force (Aist 2002). In *A. nidulans*, another kinesin, KipB, contributes to mitosis, where it may promote the disassembly of the spindle (Rischitor et al. 2004).

In filamentous fungi, the primary function of cytoplasmic dynein is to control nuclear distribution (Xiang and Fischer 2004). However, in *Nectria haematococca*, it has been shown that dynein also plays a role in spindle elongation during anaphase B (Inoue et al. 1998a). In particular, the astral microtubule-based pulling force is eliminated in *Nhdhc1* mutants. Similarly, in *A. nidulans*, a genetic screen for synthetic lethal mutants uncovered a previously unexpected role for dynein in mitosis (Efimov and Morris 1998). As in *N. haematococca*, the rate of spindle elongation during anaphase B was dramatically reduced in *nudA* mutants (Efimov and Morris 1998). It has been proposed that dynein may generate force through the destabilization of astral microtubules, which may in turn allow the cortical attachment needed for proper spindle elongation and nuclear migration (Efimov and Morris 1998).

B. Post-Mitotic Nuclear Movement

Post-mitotic nuclear movement occurs during the period between completion of anaphase B and septum formation (Aist and Morris 1999). During this time, the movement of daughter nuclei relative to each other establishes the normal pattern of nuclear distribution in hyphal cells. In basidiomycete hyphae, this involves nuclear migration through the clamp connection (Iwasa et al. 1998). Less is known about this process in filamentous ascomycetes, although the non-random distribution of labeled nuclei observed in *A. nidulans* (Rosenberger and Kessel 1967) suggests that nuclei may slide past one another. The mechanisms underlying post-mitotic nuclear movement remain unclear, although SPBs and

astral microtubules seem to play a key role in this process (Aist and Morris 1999). In particular, astral microtubules emanating from the SPB make cortical contacts that appear to be necessary for post-mitotic nuclear movement (Inoue et al. 1998b). The motor proteins that mediate the process remain unknown, although dynein is an obvious candidate, based on its likely role in assembling astral microtubules (Inoue et al. 1998b). In addition, as characterized in *A. nidulans*, ApsA represents a putative cortical anchor for astral microtubules (Xiang and Fischer 2004).

VI. Coordination of Mitotic Events in a Multinucleate Hyphal Cell

A characteristic feature of filamentous fungi is the presence of multinucleate hyphal cells. Nuclear numbers per multinucleate cell range from two in dikaryotic basidiomycetes to as many as one hundred in the primary hyphae of *Neurospora crassa*. Fungi appear to have evolved two distinct strategies for coordinating mitosis within multinucleate hyphal cells (Fig. 3.4). One approach, observed in fungi such as *A. nidulans*, *Alternaria solani*, and *Fusarium oxysporum* (Rosenberger and Kessel 1967; Aist 1969; King and Alexander 1969; Clutterbuck 1970; Fiddy and Trinci 1976), is characterized by a parasynchronous wave of mitosis that progresses through the hyphal cell, such that neighboring nuclei are simultaneously engaged in mitosis. By contrast, fungi such as *N. crassa* and *A. gossypii* (Serna and Stadler 1978; A. Gladfelter, personal communication) employ an alternative approach characterized by asynchronous mitoses, where individual nuclei undergo mitosis in an autonomous manner. The mechanisms underlying either mode of mitotic behavior remain unknown. However, parasynchronous mitoses are presumably triggered by a wave of mitosis-inducing factors such as activated CDKs or NimA. Coupling this wave to regulated nuclear transport might allow nuclei to sequentially enter mitosis as the wave propagates through the hyphal cell. Moreover, asynchronous mitoses could conceivably be established by merely eliminating the wave, such that mitotic regulators are uniformly distributed throughout the hyphal cell. In this scenario, tightly regulated nuclear transport and/or localized nuclear autonomous translation might permit individual nuclei to enter

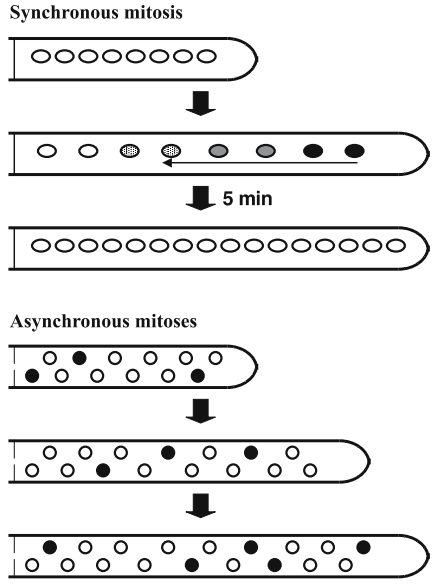

Fig. 3.4. Comparison of synchronous and asynchronous mitoses. During a synchronous mitosis, a mitotic wave progresses through the hyphal tip cell (*arrow*). The degree of shading correlates with extent of progression through mitosis (i.e., *darkly shaded nuclei* adjacent to the tip are in anaphase, whereas *non-shaded nuclei* near the septum are still in G2). No wave is observed during asynchronous mitoses. Instead, nuclei appear to enter mitosis (*darkly shaded nuclei*) in a completely autonomous manner

mitosis regardless of the mitotic status of their neighbor. Preliminary evidence supporting this model for asynchronous mitoses was recently obtained in *A. gossypii*, where the entire cell cycle can be completed despite the presence of uniform levels of mitotic B-type cyclins (A. Gladfelter, personal communication).

At this point, it is not clear why mitosis occurs in a synchronous manner in some filamentous fungi, whereas it is asynchronous in others. An additional complication is that mitotic synchrony can break down under sub-optimal growth conditions, as observed in *A. nidulans* (Rosenberger and Kessel 1967). To some extent, the explanation may lie in the relative advantages of mitotic asynchrony. For example, in fungi that possess a high ratio of nuclei per unit of cytoplasm, synchronous mitoses may not be sufficient to ensure that all nuclei divide within one round of the duplication cycle. Accordingly, asynchrony may be the only way to maintain the appropriate ratio. Alternatively, mitotic asynchrony may allow hyphal cells to better cope with DNA damage by limiting the number of actively dividing nuclei at any given time. This may not be important for fungi such as *A. nidulans*, where

sub-apical hyphal cells likely provide a reservoir of mitotically quiescent nuclei that are somewhat resistant to the effects of DNA damage (Harris 1997). Because similar compartments of mitotically inactive nuclei do not appear to exist in *N. crassa* and *A. gossypii*, these fungi may be more reliant upon mitotic asynchrony to maintain viability after exposure to DNA damaging agents.

VII. Coordination of Mitosis with Branch Formation

The formation of lateral branches is a morphogenetic process unique to filamentous fungi (Momany 2002). Several studies have shown that branch formation is tightly coordinated with growth (Trinci 1978). For example, new tips typically form to accommodate the increased volume of cytoplasm generated under optimal growth conditions. More recently, it was also demonstrated that branch formation correlates with mitosis (Dynesen and Nielsen 2003). In particular, although the inhibition of mitosis in *A. nidulans* using either *nimX* or *nimA* mutations does not affect growth, it clearly prevents the initiation of new branches. The coordination of branch formation with mitosis may ensure the appropriate ratio of cytoplasmic volume per nucleus is maintained in growing hyphae.

The mechanisms underlying the relationship between mitosis and branch formation remain an interesting puzzle. Although mitosis is required for branching, a recent study suggested that early events in branch formation might precede mitosis (Westfall and Momany 2002). Specifically, in *A. nidulans*, localization of the septin AspB to the incipient branch site occurs before the previously quiescent nuclei reenter mitosis. Moreover, septin localization persists during the first mitosis, before finally disappearing as the new branch elongates. Because septins have a conserved role in cellular morphogenesis as organizational scaffolds capable of linking cytoskeletal dynamics with the cell cycle (Gladfelter et al. 2001), this observation suggests a potential model for the reciprocal coordination of mitosis and branch formation (Fig. 3.5). In particular, septins may provide an anchor for the localized recruitment of regulatory proteins that promote mitotic reentry, such as the Cdc25 phosphatase. At the same time, other mitotic regulators may influence septin organization, thereby modulating cytoskeletal function to prevent commitment to branch formation if mitosis does not proceed properly. It should be possible to test this speculative model using the tools available for *A. nidulans*. More importantly, it will be necessary to determine if the coordination of branching with mitosis is a general feature of filamentous fungi, or if it is limited to only those fungi that, like *A. nidulans*, form obviously differentiated hyphal compartments (Harris 1997).

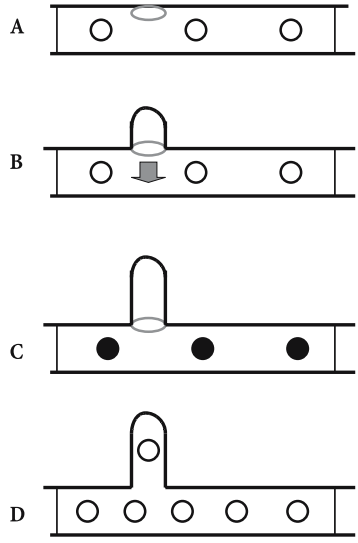

Fig. 3.5. A–D Model for the coordinated regulation of branch formation and mitosis. **A** A septin ring forms at the presumptive branch site (*shaded circle*). Nuclei remain in G2. **B** A signal generated by regulators associated with the septin ring promotes mitotic entry as the new branch emerges. **C** Nuclei undergo mitosis (*closed circles*) as the new branch continues to extend. **D** The septin ring disassembles, and post-mitotic nuclear migration into the new branch occurs

VIII. Regulation of Mitosis in Response to DNA Damage and Replication Stress

A characteristic feature of the DNA damage response is the activation of checkpoints that block mitosis when DNA is damaged (Zhou and Elledge 2000). Typically, DNA damage inhibits CDK activation and causes arrest prior to mitotic entry. Because *A. nidulans* possesses two mitosis-promoting protein kinases, it was of interest to determine how their respective activities were affected by DNA damage. As demonstrated by Ye et al. (1997b), exposure of hyphal cells to DNA damage blocks mitotic

entry, and the CDK NimX accumulates in an inactive, tyrosine-15 phosphorylated form (Fig. 3.6). By contrast, NimA activity is not affected. This mechanism is sufficient to block entry into mitosis, as the arrest is abrogated by mutations that eliminate the AnkA kinase or change tyrosine-15 to a residue that cannot be phosphorylated (Ye et al. 1997b; Kraus and Harris 2001). Accordingly, as established in animal cells and *S. pombe*, DNA damage presumably alters the balance of AnkA (Wee1) and NimT (Cdc25) activity (Elledge 1996), leading to increased tyrosine-15 phosphorylation of NimX and inhibition of mitotic entry.

The CDK tyrosine-15 regulatory module also prevents inappropriate mitosis when DNA replication is slowed in *A. nidulans* (i.e., using 5 mM hydroxyurea; Ye et al. 1996). However, when replication is completely blocked (i.e., using 100 mM hydroxyurea), a functional APC is also required to inhibit mitotic entry. Presumably, this additional level of regulation ensures that NimA kinase remains inactive and cannot initiate premature mitosis (Fig. 3.6; Ye et al. 1996).

The DNA damage signaling pathway that leads to mitotic checkpoint activation has been extensively characterized in yeast (Zhou and Elledge 2000). Components of this pathway, including UvsB (=Mec1), UvsD (=Rad26), ScaA (=Nbs1), and MreA (=Mre11), have also been identified in *A. nidulans*, where they appear to perform functions similar to the yeast homologues (Fig. 3.6; de Souza et al. 1999; Hofmann and Harris 2000; Bruschi et al. 2001; Semighini et al. 2003). Nevertheless, it is not clear how this pathway regulates CDK tyrosine-15 phosphorylation or NimA activity to arrest mitosis. For example, does this step require homologues of the Chk1 and/or Chk2 protein kinases, as it does in yeast and animal cells? Moreover, genetic studies clearly show that the fungal DNA damage signaling pathway regulates additional functions that contribute to viability when genome integrity has been compromised (Ye et al. 1997b; Hofmann and Harris 2000). These functions likely include the activation of DNA repair pathways (Hofmann and Harris 2000; Semighini and Harris, unpublished data) and the inhibition of DNA re-replication (de Souza et al. 1999). Further studies also revealed a novel post-mitotic function for the DNA damage signaling pathway. In particular, low levels of damage that are not sufficient to block mitosis prevent septum formation in pre-divisional hyphae (Harris and Kraus 1998). This response is mediated by the DNA damage signaling pathway and NimX tyrosine-15 phosphorylation (Harris and Kraus 1998; Kraus and Harris 2001), though the underlying mechanism remains unknown.

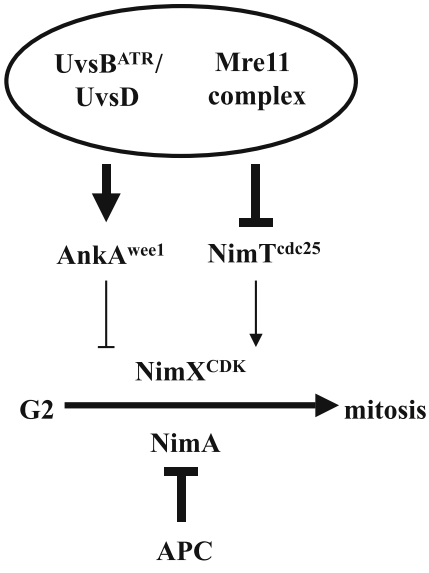

Fig. 3.6. Model for checkpoint regulation of mitosis in *Aspergillus nidulans*. The UvsB/UvsD and Mre11 complexes are activated in response to DNA damage or replication stress. In analogy to yeast and animal cells, these complexes presumably regulate the activity of both AnkA (up-regulated) and NimT (down-regulated), thereby preventing activation of the NimX CDK. Under severe replication stress, NimA activity is also inhibited, presumably via the APC. See text for additional details

The observation that DNA damage triggers the checkpoint-mediated arrest of mitotic entry raises two important questions in the context of fungal biology. First, how does a single nucleus respond to DNA damage within a multinucleate hyphal cell? In yeast and animal cells, the DNA damage checkpoint signal is nuclear autonomous (Sluder et al. 1995; Demeter et al. 2000). It is not clear if this is also true for filamentous fungi, particularly those that display a "parasynchronous" mitotic wave. If so, damaged nuclei must possess a mechanism that makes them refractory to a passing wave. Alternatively, the presence of a threshold level of damaged nuclei may abolish the wave and revert the cell to an asynchronous pattern of mitosis. Second, how is the mitotic checkpoint modulated during development? In some filamentous fungi (i.e., *A. nidulans*; Timberlake 1990), the cell cycle appears to accelerate during asexual development to permit the rapid production of spores. This may imply that mitotic checkpoints are eliminated, such that, like the syn-

cytial divisions during *Drosophila* embryogenesis, damaged nuclei are simply discarded and do not contribute to subsequent generations.

IX. Regulation of Mitosis in Response to Spindle Damage

The spindle checkpoint arrests mitosis if a properly assembled bipolar spindle is not present. The checkpoint also becomes operative if chromosomes do not properly attach to an assembled spindle. A complex of proteins, including Bub1-Bub3 and Mad1-Mad3, detect spindle abnormalities and trigger checkpoint activation, which ultimately results in inhibition of APC activation (Lew and Burke 2003). As a result, mitosis cannot proceed past the metaphase-to-anaphase transition because sister chromatids remain attached.

Limited genetic analyses in *A. nidulans* highlight the existence of the spindle checkpoint in filamentous fungi, and suggest that it is organized in a manner similar to yeast and animal cells. The *A. nidulans* homologues of Bub1 and Bub3, SldA and SldB, respectively, were identified in a screen for mutants that could not tolerate loss of dynein function (Efimov and Morris 1998). Characterization of *sldA* and *sldB* mutants revealed phenotypes consistent with a defective spindle checkpoint, including loss of viability when spindle assembly is compromised. In addition, a homologue of Mad2 has been implicated in the *A. nidulans* spindle checkpoint response (Prigozhina et al. 2004). Notably, the latter study uncovered a possible role for γ-tubulin in the spindle checkpoint, where it may facilitate the formation of an SPB-associated complex that detects spindle abnormalities and halts mitosis. Another recent study suggested that telomeres could also mediate spindle checkpoint function (Pitt et al. 2004). In particular, mutations affecting NimU, the *A. nidulans* homologue of the telomere-binding protein Pot1, permit mitotic progression in the presence of spindle defects. Despite this progress, a coherent picture of the spindle checkpoint in filamentous fungi has yet to emerge. One interesting issue that warrants investigation is whether a single detached chromosome in one nucleus can arrest the propagation of a "parasynchronous" mitotic wave. Presumably, the affected nucleus would become refractory to the wave and would not divide. However, segregation of detached chromosomes may be tolerated in a multinucleate cell, as it could provide a mechanism for chromosome exchange during the parasexual cycle.

X. Future Challenges

Considerable progress has been made toward understanding the regulation of mitosis in filamentous fungi. Important insights into the mechanisms that regulate mitotic entry have been obtained. In addition, the mode by which DNA damage checkpoint signals impinge upon the mitotic regulatory network has been described. Nevertheless, key questions remain unanswered. In many cases, these questions reflect unique features of fungal biology that must be taken into account to understand mitosis and its coordination with other components of the duplication cycle. Several of the more significant questions are discussed below.

A. What Is the Critical Function of NimA in the Regulation of Mitotic Entry?

NimA is clearly required for multiple functions that promote mitotic entry in *A. nidulans*, and presumably other filamentous fungi. Although these functions include chromosome condensation and spindle organization, recent results suggest that regulation of nuclear transport may be the most important downstream effect (De Souza et al. 2004). If so, this would provide a potential explanation for why NimA is essential for mitotic entry in filamentous fungi, but not animal cells. Because the nuclear envelope does not break down, regulated nuclear transport may allow the rapid accumulation of CDKs and other factors at the G2/M transition. This may be particularly important for propagation of the parasynchronous mitotic wave in multinucleate hypha cells.

B. How Is Mitotic Exit Coordinated with Cytokinesis?

The mechanisms underlying mitotic exit in filamentous fungi remain to be determined. Furthermore, it is not known how cytokinesis is coordinated with the completion of mitosis (Wendland and Walther, Chap. 6, this volume). In animal cells, the spindle mid-zone appears to play a key role in the spatial and temporal regulation of cytokinesis. However, although the mitotic spindle regulates septum formation in *A. nidulans* (Momany

and Hamer 1997), there is no evidence for a well-defined spindle mid-zone. Either the spindle mid-zone exists, but has an unusual appearance in filamentous fungi, or an alternative structure, such as astral microtubules, regulates septation. Regardless of the specific structure involved, it presumably triggers septation by controlling the timing and location of SIN/MEN pathway activation, an aspect that also needs to be tested.

C. How Is Nuclear Autonomy Established in a Multinucleate Hyphal Cell?

In those filamentous fungi that display asynchronous mitosis (i.e., *N. crassa*, *A. gossypii*, *A. nidulans* incubated under poor growth conditions), it remains to be determined how individual nuclei can regulate mitotic entry and exit regardless of the mitotic status of their neighbor. Presumably, the action of mitosis-promoting factors is limited to specific nuclear domains. Moreover, since mitotic cyclin levels do not appear to fluctuate (A. Gladfelter, personal communication), autonomous expression and/or degradation of CDK inhibitors may play a crucial role in permitting nuclear-autonomous mitotic entry. It is also interesting to speculate on how mitotic synchrony could be imposed on a population of asynchronously dividing nuclei. Could this be accomplished by merely incorporating the regulated expression of NimA into the mitotic regulatory network? Finally, it is important to understand how individual nuclei within a synchronously dividing cell respond to DNA damage or spindle perturbations. Do they become insulated from the parasynchronous mitotic wave, or is a single damaged nucleus sufficient to arrest propagation of the wave?

D. How Is Mitotic Regulation Altered by Developmental Signals?

The rapid development of spores allows fungi to efficiently exploit available resources. This may come with a cost, however, as the marked acceleration in the cell cycle that appears to accompany spore formation may compromise regulatory mechanisms that maintain genome integrity. For example, does the shortened cell cycle of *A. nidulans* phialides come at the expense of the DNA damage and spindle checkpoints that block mitotic entry when the cell cycle is perturbed? Is the potential loss of genome integrity an acceptable risk that is tolerated to permit the rapid generation of a clonal population of spores? In many filamentous fungi, spore formation also necessitates dramatic changes in the spatial pattern of mitosis. In particular, cells often switch from a multinucleate to a uninucleate mode of division. In *A. nidulans*, this switch appears to require developmental regulation of CDK expression (Ye et al. 1999). Whether this is a general feature of fungal development remains to be determined. Nevertheless, given the multitude of cell types formed even by a relatively simple fungus such as *N. crassa* (Bistis et al. 2003), it is likely that filamentous fungi will provide an excellent opportunity to identify conserved mechanisms for the developmental regulation of mitosis.

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References

Aist JR (1969) The mitotic apparatus in fungi, *Ceratocystis fagacearum* and *Fusarium oxysporum*. J Cell Biol 40:120–135

Aist JR (2002) Mitosis and motor proteins in the filamentous ascomycete, *Nectria haematococca*, and some related fungi. Int Rev Cytol 212:239–263

Aist JR, Bayles CJ (1991) Ultrastructural basis of mitosis in the fungus *Nectria haematococca* (sexual stage of *Fusarium solani*) II. Spindles. Protoplasma 161:123–136

Aist JR, Morris NR (1999) Mitosis in filamentous fungi: how we got where we are. Fungal Genet Biol 27:1–25

Bergen LG, Morris NR (1983) Kinetics of the nuclear division cycle of *Aspergillus nidulans*. J Bacteriol 156:155–160

Bistis GN, Perkins DD, Read ND (2003) Different cell types in *Neurospora crassa*. Fungal Genet Newslett 50:17–19

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Bruno KS, Morrell JL, Hamer JE, Staiger CJ (2001) SEPH, a Cdc7p orthologue from *Aspergillus nidulans*, functions upstream of actin ring formation during cytokinesis. Mol Microbiol 42:3–12

Bruschi GC, de Souza CC, Fagundes MR, Dani MA, Goldman, MH, Goldman GH (2001) Sensitivity to camptothecin in *Aspergillus nidulans* identifies a novel gene, *scaA*$^+$, related to the cellular DNA damage response. Mol Genet Genomics 265:264–275

Clutterbuck AJ (1970) Synchronous nuclear division and septation in *Aspergillus nidulans*. J Gen Microbiol 60:133–135

Cross FR (2003) Two redundant oscillatory mechanisms in the yeast cell cycle. Dev Cell 4:741–752

Demeter J, Lee SE, Haber JE, Stearns T (2000) The DNA damage checkpoint signal in budding yeast is nuclear limited. Mol Cell 6:487–492

De Souza CP, Ye XS, Osmani SA (1999) Checkpoint defects leading to premature mitosis also cause endoreduplication of DNA in *Aspergillus nidulans*. Mol Biol Cell 10:3661–3674

De Souza CP, Osmani AH, Wu LP, Spotts JL, Osmani SA (2000) Mitotic histone H3 phosphorylation by the NIMA kinase in *Aspergillus nidulans*. Cell 102:293–302

De Souza CP, Horn KP, Masker K, Osmani SA (2003) The SONB (Nup98) nucleoporin interacts with the NIMA kinase in *Aspergillus nidulans*. Genetics 165:1071–1081

De Souza CP, Osmani AH, Hashmi SB, Osmani SA (2004) Partial nuclear pore complex disassembly during closed mitosis in *Aspergillus nidulans*. Curr Biol 14:1973–1984

Doonan JH, Morris NR (1989) The *bimG* gene of *Aspergillus nidulans*, required for completion of anaphase, encodes a homolog of mammalian phosphoprotein phosphatase I. Cell 57:987–996

Dou X, Wu D, An W, Davies J, Hashmi SB, Ukil L, Osmani SA (2003) The PHOA and PHOB cyclin-dependent kinases perform an essential function in *Aspergillus nidulans*. Genetics 165:1105–1115

Dynesen J, Nielsen J (2003) Branching is coordinated with mitosis in growing hyphae of *Aspergillus nidulans*. Fungal Genet Biol 40:15–24

Efimov V, Morris NR (1998) A screen for dynein synthetic lethals in *Aspergillus nidulans* identifies spindle assembly checkpoint genes and other genes involved in mitosis. Genetics 149:101–116

Elledge SJ (1996) Cell cycle checkpoints: preventing an identity crisis. Science 274:1664–1672

Enos AP, Morris NR (1990) Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. Cell 23:1019–1027

Fagundes MR, Lima JF, Savoldi M, Malavazi I, Larson RE, Goldman MH, Goldman GH (2004) The *Aspergillus nidulans npkA* gene encodes a Cdc2-related kinase that genetically interacts with the $UvsB^{ATR}$ kinase. Genetics 167:1629–1641

Fiddy C, Trinci APJ (1976) Mitosis, septation, branching and the duplication cycle in *Aspergillus nidulans*. J Gen Microbiol 97:169–184

Fox H, Hickey PC, Fernandez-Abalos JM, Lunness P, Read ND, Doonan JH (2002) Dynamic distribution of $BIMG^{PP1}$ in living hyphae of *Aspergillus* indicates a novel role in septum formation. Mol Microbiol 45:1219–1230

Garcia-Muse T, Steinberg G, Perez-Martin J (2004) Characterization of B-type cyclins in the smut fungus *Ustilago maydis*: roles in morphogenesis and pathogenicity. J Cell Sci 117:487–506

Gladfelter AS, Pringle JR, Lew DJ (2001) The septin cortex at the yeast mother-bud neck. Curr Opin Microbiol 4:681–689

Grallert A, Krapp A, Bagley S, Simanis V, Hagan IM (2004) Recruitment of NIMA kinase shows that maturation of the *S. pombe* spindle-pole body occurs over consecutive cell cycles and reveals a role for NIMA in modulating SIN activity. Genes Dev 18:1007–1021

Harris SD (1997) The duplication cycle in *Aspergillus nidulans*. Fungal Genet Biol 22:1–12

Harris SD (2001) Septum formation in *Aspergillus nidulans*. Curr Opin Microbiol 4:736–739

Harris SD, Kraus PR (1998) Regulation of septum formation in *Aspergillus nidulans* by a DNA damage checkpoint pathway. Genetics 148:1055–1067

Heath IB (1994) The cytoskeleton in hyphal growth, organelle movement, and mitosis. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol I. Growth, differentiation, and sexuality. Springer, Berlin Heideberg New York, pp 43–65

Hofmann AF, Harris SD (2000) The *Aspergillus nidulans uvsB* gene encodes an ATM-related kinase required for multiple facets of the DNA damage response. Genetics 154:1577–1586

Inoue S, Yoder OC, Turgeon BG, Aist JR (1998a) A cytoplasmic dynein required for mitotic aster formation in vivo. J Cell Sci 111:2607–2614

Inoue S, Turgeon BG, Yoder OC, Aist JR (1998b) Role of fungal dynein in hyphal growth, microtubule organization, spindle pole body motility and nuclear migration. J Cell Sci 111:1555–1566

Iwasa M, Tanabe S, Kamada T (1998) The two nuclei in the dikaryon of the homobasidiomycete *Coprinus cinereus* change position after each conjugate division. Fungal Genet Biol 23:110–116

Jung MK, Prigozhina N, Oakley CE, Nogales E, Oakley BR (2001) Alanine-scanning mutagenesis of *Aspergillus* gamma-tubulin yields diverse and novel phenotypes. Mol Biol Cell 12:119–136

King SB, Alexander LJ (1969) Nuclear behavior, septation, and hyphal growth of *Alternaria solani*. Am J Bot 56:249–253

Kraus PR, Harris SD (2001) The *Aspergillus nidulans snt* genes are required for the regulation of septum formation and cell cycle checkpoints. Genetics 159:557–569

Lew DJ, Burke DJ (2003) The spindle assembly and spindle position checkpoints. Annu Rev Genet 37:251–282

Lew DJ, Weinert T, Pringle JR (1997) Cell cycle control in *Saccharomyces cerevisiae*. In: Pringle JR, Broach JR, Jones EW (eds) The molecular and cellular biology of the yeast *Saccharomyces*. Cell cycle and cell biology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 607–695

Lies CM, Cheng J, James SW, Morris NR, O'Connell MJ, Mirabito PM (1998) $BIMA^{APC}$, a component of the *Aspergillus* anaphase promoting complex/cyclosome, is required for a G2 checkpoint blocking entry into mitosis in the absence of NIMA function. J Cell Sci 111:1453–1465

MacNeill SA (1994) Cell cycle control in yeast. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol I. Growth, differentiation, and sexuality. Springer, Berlin Heidelberg New York, pp 3–23

Momany M (2002) Polarity in filamentous fungi: establishment, maintenance and new axes. Curr Opin Microbiol 5:580–585

Momany M, Hamer JE (1997) Relationship of actin, microtubules, and crosswall synthesis during septation in *Aspergillus nidulans*. Cell Motil Cytoskeleton 38:373–384

Morris NR (1976) Mitotic mutants of *Aspergillus nidulans*. Genet Res Camb 26:237–254

Murray AW (2004) Recycling the cell cycle: cyclins revisited. Cell 116:221–234
Murray AW, Hunt T (1993) The cell cycle: an introduction. Freeman, New York
Nurse P (2000) A long twentieth century of the cell cycle and beyond. Cell 100:71–78
Oakley BR, Akkari YN (1999) Gamma-tubulin at ten: progress and prospects. Cell Struct Funct 24:365–372
Oakley CE, Oakley BR (1989) Identification of gamma-tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*. Nature 338:662–664
Oakley BR, Oakley CE, Yoon Y, Jung MK (1990) Gamma-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. Cell 61:1289–1301
O'Connell MJ, Osmani AH, Morris NR, Osmani SA (1992) An extra copy of $nimE^{cyclinB}$ elevates pre-MPF levels and partially suppresses mutation of $nimT^{cdc25}$ in *Aspergillus nidulans*. EMBO J 11:2139–2149
O'Connell MJ, Meluh PB, Rose MD, Morris NR (1993) Suppression of the *bimC4* mitotic spindle defect by deletion of *klpA*, a gene encoding a KAR3-related kinesin-like protein in *Aspergillus nidulans*. J Cell Biol 120:153–162
O'Connell MJ, Norbury C, Nurse P (1994) Premature chromatin condensation upon accumulation of NIMA. EMBO J 13:4926–4937
O'Connell MJ, Krien MJ, Hunter T (2003) Never say never. The NIMA-related protein kinases in mitotic control. Trends Cell Biol 13:221–228
O'Donnell KL, Osmani AH, Osmani SA, Morris SA (1991) *bimA* encodes a member of the tetratricopeptide repeat family of proteins and is required for the completion of mitosis in *Aspergillus nidulans*. J Cell Sci 99:711–719
Osmani SA, Ye X (1996) Cell cycle regulation in *Aspergillus* by two protein kinases. Biochem J 317:633–641
Osmani SA, May GS, Morris NR (1987) Regulation of the mRNA levels of *nimA*, a gene required for the G2-M transition in *Aspergillus nidulans*. J Cell Biol 104:1495–1504
Osmani SA, Pu RT, Morris NR (1988a) Mitotic induction and maintenance by over-expression of a G2-specific gene that encodes a potential protein kinase. Cell 53:237–244
Osmani SA, Engle DB, Doonan JH, Morris NR (1988b) Spindle formation and chromatin condensation in cells blocked at interphase by mutation of a negative cell cycle control gene. Cell 52:241–251
Osmani AH, McGuire SL, Osmani SA (1991) Parallel activation of the NIMA and $p34^{cdc2}$ cell cycle-regulated protein kinases is required to initiate mitosis in *A. nidulans*. Cell 67:283–291
Osmani AH, van Peij N, Mischke M, O'Connell MJ, Osmani SA (1994) A single $p34^{cdc2}$ protein kinase ($nimX^{cdc2}$) is required at G1 and G2 in *Aspergillus nidulans*. J Cell Sci 107:1519–1528
Osmani AH, Davies J, Oakley CE, Oakley BR, Osmani SA (2003) TINA interacts with the NIMA kinase in *Aspergillus nidulans* and negatively regulates astral microtubules during metaphase arrest. Mol Biol Cell 14:3169–3179
Ovechkina Y, Maddox P, Oakley CE, Xiang X, Osmani SA, Salmon ED, Oakley BR (2003) Spindle formation in *Aspergillus* is coupled to tubulin movement into the nucleus. Mol Biol Cell 14:2192–2200
Pereira G, Schiebel E (2001) The role of the yeast spindle pole body and the mammalian centrosome in regulating late mitotic events. Curr Opin Cell Biol 13:762–769
Pitt CW, Moreau E, Lunness PA, Doonan JH (2004) The $pot1^{+}$ homologue in *Aspergillus nidulans* is required for ordering late mitotic events. J Cell Sci 117:199–209
Prigozhina NL, Oakley CE, Lewis AM, Nayak T, Osmani SA, Oakley BR (2004) gamma-tubulin plays an essential role in the coordination of mitotic events. Mol Biol Cell 15:1374–1386
Pu RT, Osmani SA (1995) Mitotic destruction of the cell cycle regulated NIMA protein kinase of *Aspergillus nidulans* is required for mitotic exit. EMBO J 14:995–1003
Pu RT, Xu G, Wu L, Vierula J, O'Donnell K, Ye XS, Osmani SA (1995) Isolation of a functional homolog of the cell cycle-specific NIMA protein kinase of *Aspergillus nidulans* and functional analysis of conserved residues. J Biol Chem 270:18110–18116
Rischitor PE, Konzack S, Fischer R (2004) The Kip3-like linesin KipB moves along microtubules and determines spindle position during synchronized mitoses in *Aspergillus nidulans* hyphae. Eukaryot Cell 3:632–645
Rosenberger RF, Kessel M (1967) Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans*. J Bacteriol 94:1464–1469
Schier N, Fischer R (2002) The *Aspergillus nidulans* cyclin PclA accumulates in the nucleus and interacts with the central cell cycle regulator NimX(Cdc2). FEBS Lett 523:143–146
Schier N, Liese R, Fischer R (2001) A Pcl-like cyclin of *Aspergillus nidulans* is transcriptionally activated by developmental regulators and is involved in sporulation. Mol Cell Biol 21:4075–4088
Schoch CL, Aist JR, Yoder OC, Turgeon BG (2003) A complete inventory of fungal kinesins in representative filamentous ascomycetes. Fungal Genet Biol 39:1–15
Seiler S, Plamann M, Schliwa M (1999) Kinesin and dynein mutants provide novel insights into the roles of vesicle traffic during cell morphogenesis in *Neurospora*. Curr Biol 12:779–785
Semighini CP, Fagundes MR, Ferreira JC, Pascon RC, Goldman MH, Goldman GH (2003) Different roles of the Mre11 complex in the DNA damage response in *Aspergillus nidulans*. Mol Microbiol 48:1693–1709
Serna L, Stadler D (1978) Nuclear division cycle in germinating conidia of *Neurospora*. J Bacteriol 136:341–351
Seshan A, Amon A (2004) Linked for life: temporal and spatial coordination of late mitotic events. Curr Opin Cell Biol 16:41–48
Sharpless KE, Harris SD (2002) Functional characterization and localization of the *Aspergillus nidulans* formin SEPA. Mol Biol Cell 13:469–479
Simanis V (2003) Events at the end of mitosis in the budding and fission yeasts. J Cell Sci 116:4263–4275
Sluder G, Thompson EA, Rieder CL, Miller FJ (1995) Nuclear envelope breakdown is under nuclear not cytoplasmic control in sea urchin zygotes. J Cell Biol 129:1447–1458
Stern B, Nurse P (1996) A quantitative model for the cdc2 control of S phase and mitosis in fission yeast. Trends Genet 12:345–350
Su S, Yanagida M (1997) Mitosis and cytokinesis in the fission yeast, *Schizosaccharomyces pombe*. In: Pringle JR,

Broach JR, Jones EW (eds) The molecular and cellular biology of the yeast *Saccharomyces*. Cell cycle and cell biology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 765–825

Thornton BR, Toczyski DP (2003) Securin and B-cyclin/CDK are the only essential targets of the APC. Nat Cell Biol 5:1090–1094

Timberlake WE (1990) Molecular genetics of *Aspergillus* development. Annu Rev Genet 24:5–36

Trinci APJ (1978) The duplication cycle and vegetative development in molds. In: Smith JE, Berry DR (eds) The Filamentous Fungi, vol 3. Developmental mycology. Arnold, London, pp 132–163

Vardy L, Fujita A, Toda T (2002) The gamma-tubulin complex protein Apl4 provides a link between the metaphase checkpoint and cytokinesis in fission yeast. Genes Cells 7:365–373

Westfall PJ, Momany M (2002) *Aspergillus nidulans* septin AspB plays pre- and postmitotic roles in septum, branch, and conidiophore development. Mol Biol Cell 13:110–118

Wolkow TD, Harris, SD, Hamer JE (1996) Cytokinesis in *Aspergillus nidulans* is controlled by cell size, nuclear positioning and mitosis. J Cell Sci 109:2179–2188

Wu L, Osmani SA, Mirabito PM (1998) A role for NIMA in the nuclear localization of cyclin B in *Aspergillus nidulans*. J Cell Biol 141:1575–1587

Xiang X, Fischer R (2004) Nuclear migration and positioning in filamentous fungi. Fungal Genet Biol 41:411–419

Xiang X, Plamann M (2003) Cytoskeleton and motor proteins in filamentous fungi. Curr Opin Microbiol 6:628–633

Ye XS, Xu G, Pu RT, Fincher RR, McGuire SL, Osmani AH, Osmani SA (1995) The NIMA protein kinase is hyperphosphorylated and activated downstream of $p34^{cdc2}$/cyclin B: coordination of two mitosis promoting kinases. EMBO J 14:986–994

Ye XS, Fincher RR, Tang A, O'Donnell K, Osmani SA (1996) Two S-phase checkpoint systems, one involving the function of both BIME and Tyr15 phosphorylation of $p34^{cdc2}$, inhibit NIMA and prevent premature mitosis. EMBO J 15:3599–3610

Ye XS, Fincher RR, Tang A, McNeal KK, Gygax SE, Wexler AN, Ryan KB, James SW, Osmani SA (1997a) Proteolysis and tyrosine phosphorylation of $p34^{cdc2}$/cyclin B. The role of MCM2 and initiation of DNA replication to allow tyrosine phosphorylation of $p34^{cdc2}$. J Biol Chem 272:33384–33393

Ye XS, Fincher RR, Tang A, Osmani SA (1997b) The G2/M DNA damage checkpoint inhibits mitosis through Tyr15 phosphorylation of $p34^{cdc2}$ in *Aspergillus nidulans*. EMBO J 16:182–192

Ye XS, Fincher RR, Tang A, Osmani AH, Osmani SA (1998) Regulation of the anaphase-promoting complex/cyclosome by $bimA^{APC3}$ and proteolysis of NIMA. Mol Biol Cell 9:3019–3030

Ye XS, Lee SL, Wolkow TD, McGuire SL, Hamer JE, Wood GC, Osmani SA (1999) Interaction between developmental and cell cycle regulators is required for morphogenesis in *Aspergillus*. EMBO J 18:6994–7001

Zhou BB, Elledge SJ (2000) The DNA damage response: putting checkpoints in perspective. Nature 408:433–439

4 Apical Wall Biogenesis

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CONTENTS

I. Introduction

Polarised growth is a distinctive feature of the fungal kingdom. Although this process also occurs in some plant cells, notably pollen tubes and root hairs, plant cells generally grow by diffuse or intercalary extension of their walls, a process only exceptionally seen in fungi, such as in expansion of mushrooms (see The Mycota, Vol. I, 1st edn., Chap. 22). Even growth by budding, as in yeasts, can be viewed as a form of apical growth, only different from hyphal growth with respect to the degree of polarisation of the wall synthetic activities (Wessels 1990).

The essential features of hyphal tip growth are polarised synthesis and extensibility of the wall, and a suitable gradient of rigidification of the wall to produce the hyphal tube. When the synthetic activities of the organism exceed the rate at which individual hyphae can extend, branching occurs, producing new volume for the increasing cytoplasm (see The Mycota, Vol. I, 1st edn., Chap. 10). The branches extend and proliferate, giving rise to the expanding fungal colony. The adaptive value of this mode of growth can hardly be doubted: like mobility in animals, apical growth allows the fungus to exploit a vast area for nutrients, particularly because translocation within the hyphal system allows the fungus to grow for long distances in nonnutritive areas (see The Mycota, Vol. I, 1st edn., Chap. 9, and Vol. VIII, Chap. 6).

Apical extension also allows hyphae to penetrate solid substrates such as wood and living tissues of plants and animals. In relation to this invasive growth, turgor plays an important role (see The Mycota, Vol. I, 1st edn., Chap. 4, and Vol. VIII, Chap. 1), as does the excretion of large amounts of proteins. Such proteins appear excreted primarily at the growing tips and consist largely of lytic enzymes which can digest the substrate polymers, providing nutrients for the fungus and clearing the path for further penetration. The hyphal wall is an apparent diffusion barrier for the passage of these proteins into the milieu. Therefore, specialised regions in the hyphae or specialised mechanisms have to be envisaged to let these molecules pass.

This review deals with polarised wall biosynthesis and with the excretion of proteins through the nascent wall.

II. General Overview of Apical Growth

Many observations on apical wall growth were already made in the 19th century and various hypotheses then put forward to explain apical growth still survive today. Most notable is a publication

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by Reinhardt (1892) in which he described studies on the wide hyphae of *Peziza* species showing that symptoms of growth are first manifested at the apices of hyphae. Many of these observations were later repeated and extended by Robertson (1958, 1965). With regard to the mechanism involved in apical wall extension, the conclusions put forward by Reinhardt are still controversial. He discussed the then prevailing theory of leading botanists who regarded enlargement of wall area as a process in which a plastic wall expands due to turgor pressure while new wall material is being added by apposition or intussusception. However, he considered such a theory inadequate because it would require an increase in mechanical strength of the wall, going from the apex to the base of the extension zone. As he puts it, such an increase in strength could be achieved by a proportional increase in wall thickness or a change in the quality of the molecules making up the wall. He found no evidence for a change in wall thickness. Later investigators (Girbardt 1969; Grove and Bracker 1970; Trinci and Collinge 1975) also found that wall thickness remains uniform in the extension zone. A change in quality of the wall molecules, which is precisely the kind of change suggested by work from our laboratory (Wessels 1986, 1988, 1990, 1993; see also Sect. IV), was considered unlikely by Reinhardt. In that case, the weakest spot of the wall would be at the extreme tip, and flooding with water should result in swelling or bursting at the very tip and not, as he observed, at the base of the apex where the cylindrical form is attained. Swelling and bursting subapically have also been observed by later investigators (Robertson 1958, 1965; Bartnicki-Garcia and Lippman 1972; Wessels 1988). Reinhardt concluded that the wall must have uniform strength over the whole apex, and does not grow by plastic expansion but by intussusception of wall material maximally at the extreme tip and declining to zero at the base of the extension zone. Consequently, turgor was not considered to play a role in apical wall expansion. However, the orthogonal trajectories of markers at the extending tip, also surmised by Reinhardt, have been taken as evidence by Bartnicki-Garcia et al. (2000) that the wall expands under uniform pressure which can be exerted only by turgor.

The development of the technique of microautoradiography has made it possible to prove that wall material is indeed maximally deposited at the extreme tip and declines to low values at the base of the extension zone (Bartnicki-Garcia and Lippman 1969; Gooday 1971; Katz and Rosenberger 1971a). However, these observations do not clarify the mechanism of wall growth at the apex.

Assuming that turgor is the driving force of apical wall expansion, and probably inspired by considerations of D'Arcy Thompson (1917, see Bonner 1961) on the origin of cellular form, mathematical models have been put forward to describe apical morphogenesis. They all rely on the concept that extension at the hyphal apex is due to the presence of a gradient in the plasticity of the wall, such that there is a decrease in the tendency of the wall to yield to turgor pressure from the extreme tip downwards (da Riva Ricci and Kendrick 1972; Green 1974; Trinci and Saunders 1977; Saunders and Trinci 1979; Koch 1982). In all these models, expansion at any point on the apical dome is determined by its position, according to a mathematical function which depends on the shape of the apex.

To put these models through a test, it would seem necessary to determine the plastic and elastic properties of the wall at various points over the growing apex but obviously such measurements are difficult to make. It has, however, been recognised that in order to maintain uniform thickness of the expanding wall, a gradient in wall plasticity must be paralleled by a gradient in wall deposition. Gooday and Trinci (1980) have indeed shown that the deposition of chitin, measured by autoradiography, closely parallels the derived mathematical equation for wall plasticity.

If apical wall expansion is driven by turgor, then why do hyphal tips, when subjected to high turgor pressure, swell and burst at the base of the apical dome, rather than at the weakest spot, the extreme apex? Wessels (1988), inspired by observations of Picton and Steer (1982) on the growth of pollen tubes, suggested that cytoskeletal elements in the hyphal apex could protect the delicate, newly formed wall from becoming subject to high hydrostatic pressure. Indeed, evidence was obtained in both oomycetes and true fungi that actin reinforces the hyphal apex (see The Mycota, Vol. I, 1st edn., Chap. 3, and Heath 2000), and at least the oomycete *Saprolegnia ferax* appears to be able to generate normal hyphal growth in the absence of measurable turgor pressure (Money and Harold 1993). Although such observations have still to be reported for true fungi, this indicates that Reinhardt may have been correct in believing that the shape of the apical wall is primarily determined by the cytoplasm, turgor playing only a secondary role. These observations are also consistent with his view that

fungi can be regarded as "tube-dwelling amoebae", more recently advocated by Gregory (1984) and Heath and Steinberg (1999).

However, if the structured apical cytoplasm prevents turgor pressure from rupturing the most apical part of the wall (Wessels 1988), then Reinhardt's conclusion that the wall must have uniform strength over the apex, growing by intussusception, can be dismissed. New models taking into account the presence of cytoskeletal elements might assign a fundamental role to the cytoplasm in shaping the hyphal apex while retaining the concept of a deformable wall expanding under the influence of pressure exerted by the cytoplasm (Heath and Steinberg 1999; Heath 2000). If there is a gradient in the plasticity of the wall, maximal at the very apex and declining towards the base of the extension zone, then how is this gradient generated? Two possibilities have been considered: either the wall is originally plastic and expands until it becomes rigid or the wall is synthesised as a rigid entity and cannot expand unless it is plastified by wall-modifying enzymes. Robertson (1958, 1965) implicitly based his explanation of the behaviour of living hyphae on a concept of "setting" of the wall after its formation. Work on wall biogenesis in *Schizophyllum commune* has given this concept a molecular basis and has been named the "steady-state model of apical wall growth" (Wessels 1986). The concept that a wall must be continuously loosened by lytic enzymes in order to expand is well established for intercalary wall growth in bacteria (Koch 1988) and plants (Cleland 1981), and has also been suggested to occur during growth of some mushrooms (see The Mycota, Vol. I, 1st edn., Chap. 22). Johnson (1968), Bartnicki-Garcia (1973) and Gooday (1978) have advocated that wall-loosening enzymes also play a role in apical extension of mycelial fungi. However, direct evidence for this concept, which presumes a "delicate balance between wall synthesis and wall lysis" (Bartnicki-Garcia 1973), has not been obtained to date. In fact, inhibition of chitinases does not affect apical growth (Gooday et al. 1992). However, the possibility that other hydrolases or transferases, some with as yet unknown specificities, play a role in modifying the apical wall has not been ruled out, and it may be difficult to do so. For the moment, the strength of the evidence supports the alternative concept of an inherently plastic wall which undergoes hardening with time (Sect. V).

Although the present review concentrates on the biogenesis of the wall over the apex, it is clear that hyphal morphogenesis by apical growth involves activities of the whole hypha (Harold 1990, 1999), with an emphasis on activities involving the cytoskeleton (see The Mycota, Vol. I, 1st edn., Chap. 3; Heath 2000; Steinberg 2000). Molecular genetic studies are currently identifying the participating components (McGoldrick et al. 1995; Harris et al. 1999; Weber et al. 2003). Molecular genetic studies pertaining to signalling cascades are discussed below (Sect. VI.B). The advance provided by these studies is that they guide us to proteins which contribute to the intricate network of reactions occurring at the growing tip. Indications were obtained that microtubules and microfilaments are involved in the apical organization of the Spitzenkörper, which is a specialised accumulation of vesicles implicated in the polar exocytosis of fungal hyphae. Both kinesins and dynesins motor molecules are thought to participate in organizing the Spitzenkörper (Seiler et al. 1997; Riquelme et al. 2000; Weber et al. 2003).

III. Chemical Composition of Fungal Walls

For detailed information on the chemistry of fungal walls and its relation to taxonomy, the reader is referred to a number of reviews (Bartnicki-Garcia 1968; Wessels and Sietsma 1981; Bartnicki-Garcia and Lippman 1982; Farkas 1985; Ruiz-Herrera 1992; see The Mycota, Vol. I, 1st edn., Chap. 6, Vol. VIII, Chap. 9, and Latgé and Calderone, Chap. 5, this volume). Only general aspects will be mentioned here, with an emphasis on the cross-linking between different polymers.

The gross monomeric composition of an average wall of a species belonging to the Dikaryomycota (Ascomycetes or Basidiomycetes) shows a predominance of glucose (about 60%–70%) and aminosugars (15%–20%), predominantly *N*-acetyl-glucosamine. In budding yeasts, mannose is prominently present as a constituent of mannoproteins (see The Mycota, Vol. I, 1st edn., Chap. 6, and Vol. VIII, Chap. 9). In members of the Zygomycetes, glucose is usually replaced by glucuronic acid and the predominant aminosugar is glucosamine, rather than *N*-acetylglucosamine (Datema et al. 1977b).

Roughly half the amount of glucan in hyphal walls of the Dikaryomycota is alkali-soluble and represents in most cases a (1-3)-α-linked glucan

partly present as crystalline material at the outer wall surface (Wessels et al. 1972). This type of glucan usually contains minor amounts of (1-4)-α-linkages, which have a function in the initiation of synthesis, similar to that found for bacterial glycogen synthesis (Grün et al. 2005). Also, part of the (1-3)-β-glucan is usually found to be soluble in alkali (Sietsma and Wessels 1981).

The alkali-insoluble fraction of the wall contains glucan and aminosugar polymers. The glucan consists of (1-3)-β-/(1-6)-β-linked glucose residues and is apparently highly branched. The aminosugars in the alkali-insoluble fraction are generally considered to derive from chitin, (1-4)-β-linked poly-*N*-acetyl-glucosamine. However, it is difficult to ascertain whether these aminosugars are present as *N*-acetyl-glucosamine or glucosamine, because an accurate estimation of the aminosugar content is usually done after complete acid hydrolysis, a procedure removing any acetyl groups. Digestion of samples with chitinase usually yields chitobiose, indicating the presence of extensive stretches of poly-*N*-acetyl-glucosamine chains. However, this enzymic treatment usually fails to hydrolyse the aminosugar-containing polymers completely, probably because of the close association with the (1-3)-β-glucan and the possible presence of non-acetylated glucosamine residues. Successive treatments with nitrous acid, which degrades glucosaminoglycans at non-acetylated glucosamine residues, and chitinase not only degrades the glucosaminoglycan but also solubilises all the glucan (Sietsma and Wessels 1979, 1981, 1990; Kamada and Takemaru 1983; Suarit et al. 1988; Mol and Wessels 1987; Mol et al. 1988; van Pelt-Heerschap and Sietsma 1990), suggesting the occurrence of covalent linkages between the β-glucan and the glucosaminoglycan.

In members of the Zygomycetes the hyphal wall contains, in addition to chitin, long stretches of (1-4)-β-linked glucosamine residues (chitosan), which can be isolated from these walls as an alkali-insoluble but acid-soluble polymer (Kreger 1954; Bartnicki-Garcia and Nickerson 1962). At least in *Mucor mucedo*, glucosaminoglycans occur containing both acetylated and non-acetylated glucosamine residues (Datema et al. 1977b). Destruction of these cationic glucosaminoglycans by nitrous acid released a heteropolymer containing glucuronic acid, fucose, mannose and some galactose (Datema et al. 1977a). This acidic heteropolymer was apparently held insoluble in the wall by ionic linkage to the polycationic glucosaminoglycan.

IV. Biosynthesis of Fungal Walls

The tubular form of the hypha must be the consequence of the way the wall is synthesised and assembled at the tip. At the base of the extension zone, the wall must have enough strength to withstand the turgor pressure.

Not much is known about the role of (1-3)-α-glucan, the prominent alkali-soluble component of the wall of many fungi. In *Schizosaccharomyces pombe* walls, this glucan has an essential structural role and is not covalently linked to other wall components (Hochstenbach et al. 1998; Grün et al. 2005). However, this organism is an exception because chitin, as a structural component, is here present at very low levels, if at all (Sietsma and Wessels 1990). Mutants of *Aspergillus nidulans* unable to synthesise α-glucan have been described (Zonneveld 1974; Polacheck and Rosenberger 1977), and these were affected in cleistothecia formation but no effects on osmotic sensitivity or hyphal morphogenesis were reported. Also, inhibition of synthesis of this glucan by 2-deoxyglucose during cell wall regeneration by protoplasts of *S. commune* had no effect on the regeneration of hyphae in osmotically stabilised medium (Sietsma and Wessels 1988). These findings suggest that this glucan does not play a significant role in hyphal morphogenesis.

On the other hand, when during protoplast regeneration in *S. commune* chitin synthesis was inhibited by polyoxin-D, no hyphal tubes were formed (Sonnenberg et al. 1982). Under these conditions, the protoplasts became osmotically stable but the wall contained (1-3)-α-glucan only. The β-glucan was normally synthesised but as a water/alkali-soluble component, whereas no alkali-insoluble glucan was formed, apparently because the aforementioned linkage to chitin could not be established. Similar observations have been made during regeneration of the wall in *Candida albicans* protoplasts (Elorza et al. 1987). Pulse-chase experiments with regenerating protoplasts (Sonnenberg et al. 1982) and growing hyphae (Wessels et al. 1983) of *S. commune* have shown that the water- and alkali-soluble (1-3)-β-glucan is indeed a precursor of the alkali-insoluble β-glucan.

In agreement with an essential role of the chitin–glucan complex in hyphal morphogenesis, mutants of *A. nidulans* and *Neurospora crassa* affected either in chitin or β-glucan synthesis show osmotic sensitivity and abnormal morphology (Leal-Morales and Ruiz-Herrera 1985; Martinez et al. 1989; Borgia and Dodge 1992). Because chitin and (1-3)-β-glucan are synthesised by different enzymes at the plasma membrane (see below), linkage of these two polymers to each other can occur only outside the plasma membrane within the wall domain.

A. Chitin Synthesis

1. Regulation of Chitin Synthase Activity

It is now generally accepted that chitin is synthesised by a trans-membrane protein, accepting its substrate, uridine-diphospho-*N*-acetyl-glucosamine (UDP-Glc*N*ac) at the cytoplasmic site while the (1-4)-β-linked *N*-acetyl-glucosamine polymer is extruded to the outside (Duran et al. 1975; Vermeulen et al. 1979; Cabib et al. 1983). This topic has been reviewed by Roncero (2002, in The Mycota, Vol. III, 2nd edn., Chap. 14), and by Latgé and Calderone (Chap. 5, this volume).

In fungi, chitin synthases are encoded by multigene families containing from three members in *Saccharomyces cerevisiae* to eight (e.g. for *Benjaminiella poitrasii*) or even ten members (e.g. *Phycomyces blakesleeanus*; Bulawa 1993; Miyazaki and Ootaki 1997; Chitnis et al. 2002). Based on sequence homology, chitin synthase genes are divided into five classes. Studies on disruptions of these genes provide more data for the function of these genes, indicating that different functions can be assigned to members of different classes. When disrupted, class I genes show hardly any phenotypical effect, a repair function during cytokinesis having been assigned only to the yeast member of this class (Cabib et al. 1992). Disruption of class II genes had an effect on septum synthesis and conidiogenesis (Fujiwara et al. 2000; Munro et al. 2001). Class IV contains chitin synthase genes coding for enzymes responsible for the synthesis of the bulk of chitin present in yeast or hyphal cell wall. Although disruption of these genes causes a considerable reduction in the amount of chitin in the wall, it does not produce aberrant hyphal morphologies (Din et al. 1996; Specht et al. 1996). Genes belonging to classes III and V are present only in mycelial fungi and absent in yeasts (Weber et al. 2003). Disruption of these genes shows in several cases abnormal hyphal growth (Yarden and Yanofsky 1991; Mellado et al. 1996). Significantly, genes belonging to class V code for fusion proteins between myosin and chitin synthase, the myosin part possibly playing a major role in directing chitin synthase to the site of action, the hyphal tip (Aufauvre-Brown et al. 1997; Roncero 2002). In *S. cerevisiae*, evidence exists that a myosin motor molecule (Myo2) transports chitin synthase to its site of action (Santos and Snyder 1997). However, Myo2 is not involved in chitin synthase transport in mycelial fungi (Weber et al. 2003).

The aforementioned autoradiographic studies indeed show chitin synthase to be particularly active at growing hyphal apices and at developing septa. This suggests precise localisation of chitin synthase and/or its precise local activation.

Chitin synthase, like other membrane proteins, may arise at the ER far behind the hyphal tip and then be transported by vesicles to the apex where it is inserted into the plasma membrane by vesicle fusion. Docking SNARE and vesicle SNARE molecules are found to be present at hyphal tips and are thought to play a role in the precise localisation of vesicle fusion to the cytoplasmic membrane (Gupta et al. 2003). Vesicle-like particles called chitosomes, containing inactive chitin synthase, have been isolated from a variety of fungi (Bartnicki-Garcia et al. 1978). They show a variety of proteins and lipids, which seem to be essential for the integrity and functioning of the chitosomes (Flores-Martinez et al. 1990); upon activation with proteolytic enzymes, they produce crystalline chitin in vitro. However, these chitosomes are much smaller than the usual secretory vesicles present at the hyphal apex, and do not seem to be delineated by a unit membrane (Bracker et al. 1976). Therefore, it is questionable whether these structures can be called true vesicles. Chitosomes may be unique assemblages of lipids and proteins, the latter possibly synthesised on free ribosomes. Significantly, the chitin synthase genes cloned thus far do not indicate the presence of canonical signal sequences for secretion (Silverman 1989).

Proteolytic activation in vitro of chitin synthases has been generally observed (Bartnicki-Garcia et al. 1978; Cabib et al. 1982). Cabib et al. (1982) have implied local proteolytic activation of chitin synthase at the site of septum formation in *S. cerevisiae*, assuming a zymogenic form of the enzyme uniformly present in the plasma membrane. However, there is no direct evidence that proteolytic activation does occur in vivo. In

fact, chitin synthase 3, thought to be responsible in this yeast for most chitin synthesis in vivo, appears non-zymogenic (Orlean 1987; Shaw et al. 1991; Bulawa 1992). Another model for the regulation of chitin synthase activity, based on the study of secretory mutants in *S. cerevisiae*, has been proposed, involving a specialised mechanism of vesicle sorting (Ziman et al. 1996). In this model, chitin synthase is maintained inside specialised vesicles (chitosomes) and shuttled between a store of these vesicles, where it is in an inactive form, and the site of function, where it becomes activated by insertion into the cytoplasmic membrane. Inactivation occurs by endocytosis. The enzyme is initially not degraded but stored inside chitosomes until needed, and eventually shuttled to the vacuole and degraded by proteolysis (Chuang and Schekman 1996; Valdivia et al. 2002).

This is in agreement with the finding in earlier studies that isolated protoplasts from different parts of hyphae start chitin synthesis without delay when transferred to regenerating conditions, even in the presence of antibiotics inhibiting protein synthesis (Sonnenberg et al. 1982). During the formation of protoplasts, insertion of preformed cytoplasmic chitin synthase into the plasma membrane may have occurred. Inhibitors of protein synthesis have also little influence on the rate of chitin synthesis in growing hyphae; they only induce a shift of chitin synthesis from apical to subapical regions of the hyphae (Katz and Rosenberger 1971a; own unpublished data), indicating a slow turnover of chitin synthase molecules.

Being an integral membrane protein, the lipid environment can be surmised as important for regulating the activity of chitin synthase. Arrhenius plots show clear transition points in this activity, and delipidification inactivates the enzyme whereas addition of phospholipids restores the activity of partially delipidified chitin synthase (Duran and Cabib 1978; Vermeulen and Wessels 1983; Montgomery and Gooday 1985). Fungicides which interfere with sterol synthesis (imidazole derivatives) cause irregular deposition of chitin (Kerkenaar and Barug 1984), while polyene antibiotics, known to interact with sterols, inhibit chitin synthesis by chitosomes in vitro (Rast and Bartnicki-Garcia 1981). These data agree with the notion that the insertion of chitin synthase into the lipid environment of the plasma membrane in itself could result in activation of the enzyme.

An unexpected mode of chitin synthesis regulation was recently proposed in yeast (Lagorce et al. 2002). This mode of regulation is based on the availability of the substrate for chitin synthase, *N*-acetylglucosamine. The expression of the *GFA1* gene, which is directly involved in glucosamine metabolism, is increased when more chitin is required, in a sort of salvage mechanism when the cell wall is weakened by the inhibition of the synthesis of wall components other than chitin. In earlier studies with mutants of *A. nidulans* in which cell wall rigidity was affected, chitin content of the wall was directly correlated to mutations in genes involved in the synthesis of *N*-acetylglucosamine (Katz and Rosenberger 1971b; Borgia and Dodge 1992). It has recently been found that also in the mycelial fungi *Aspergillus niger*, *Penicillium chrysogenum* and *Fusarium oxysporum*, expression of the homologue of *GFA1* was increased during stress conditions caused by cell wall weakening (R. Damsveld and A. Ram, unpublished data), indicating that this mechanism of regulating chitin synthesis could be a more general phenomenon.

2. Chitin Modifications

The product of chitin synthase is a homopolymer of (1-4)-β-linked *N*-acetylglucosamine. In vitro, the polymer chains spontaneously crystallise by forming inter- and intra-molecular hydrogen bonds. X-ray diffraction then shows a crystalline configuration known as α-chitin, in which chains are presumably antiparallel (Minke and Blackwell 1978). Because newly formed chitin is very sensitive to modifying enzymes, e.g. chitinase and chitin deacetylase (Davis and Bartnicki-Garcia 1984; Vermeulen and Wessels 1984, 1986), it was concluded that there is a time lapse between enzymic synthesis and crystallisation. On the other hand, modification of chitin, e.g. by deacetylation or linkage to other polysaccharides, would likely interfere with the crystallisation process. In growing regions, therefore, where chitin synthesis occurs, competition may exist between crystallisation and modification. In most fungal species, the modification of chitin after synthesis probably accounts for the fact that, in mature hyphal walls, crystalline chitin can rarely be detected by X-ray diffraction. Crystallisation of chitin can be prevented experimentally by addition of compounds which strongly adhere to the native chains, e.g. the optical brightener calcofluor white and congo red. These compounds maintain chitin in a reactive form which is susceptible to the action of dilute acids and chitinase (Vermeulen and Wessels 1984;

Kopecka and Gabriel 1992). They also induce the formation of aberrant growth forms (Pancaldi et al. 1984; C.A. Vermeulen and J.G.H. Wessels, unpublished data on *S. commune*). During the reactive state immediately following synthesis, chitin may be (partially) deacetylated by a deacetylase (Ariko and Ito 1975; Davis and Bartnicki-Garcia 1984) and/or covalently linked to β-glucan chains. Fragments have been isolated from *S. cerevisiae* (Kollar et al. 1995), *C. albicans* (Suarit et al. 1988) and *A. fumigatus* (Fontaine et al. 2000), indicating a linkage between the reducing end of chitin and either (1-3)-β- and (1-6)-β-glucan chains. However, the alkali-insoluble glucan is resistant to β-elimination, indicating that the reducing end of the (1-3)-β-glucan must also be protected. Whether this involves (1-6)-β-linkages or whether the reducing end of the (1-3)-β-glucan is protected by another type of linkage is not known yet.

In *A. fumigatus*, instead of (1-6)-β-linkages which are generally found in the alkali-insoluble glucan of hyphal walls, (1-4)-β-linkages were supposedly involved (Fontaine et al. 2000). Because (1-4)-β-linkages are thought to be absent in true fungi, this needs confirmation, for instance, by periodate oxidation and/or sensitivity to hydrolysis by different types of cellulases.

B. β-Glucan Synthesis

1. Synthesis of (1-3)-β-Glucan

Autoradiographic studies with *S. cerevisiae* (Shematek et al. 1980) and *N. crassa* (Jabri et al. 1989) suggest that, like chitin synthase, glucan synthase is a trans-membrane protein, accepting its substrate UDP-glucose at the cytoplasmic site and extruding its product outside the plasma membrane. By incubating isolated membrane preparations with UDP-glucose in the presence of amylase, a glucan with exclusively (1-3)-β-linkages was obtained, i.e. no (1-6)-β-linkages could be found. With enzyme preparations contaminated with wall material, a low amount of (1-6) linkages could be detected (Balint et al. 1976; Lopez-Romero and Ruiz-Herrera 1977). Studies with membrane preparations from several fungi (Szaniszlo et al. 1985) have shown that (1-3)-β-glucan synthase does not occur in a zymogenic form but its activity is stimulated by nucleoside triphosphates, GTP being the most effective. Further assessments of the regulatory system show that the fungal (1-3)-β-D-glucan synthase can be dissociated into two components, one soluble and the other particulate. Both components, as well as GTP, are required for activity; the soluble fraction appears to be a GTP-binding protein belonging to the *ras* homologue family (Kang and Cabib 1986; Mol et al. 1994; Beauvais et al. 2001), linking (1-3)-β-D-glucan synthesis with events occurring during the cell cycle.

2. Modifications of (1-3)-β-Glucan

The in vitro product of (1-3)-β-glucan synthase, a homopolymer of (1-3)-β-linked glucose residues, forms triple helices, stabilised by hydrogen bonds (Jelsma and Kreger 1975; Marchessault and Deslandes 1979). Crystallites of pure (1-3)-β-glucan take the form of ribbon-like fibrils when the glucan is synthesised on the surface of *S. cerevisiae* protoplasts (Kreger and Kopecka 1975) or with isolated (1-3)-β-glucan synthase preparations (Wang and Bartnicki-Garcia 1976). Similarly to chitin, the formation of these crystallites can be hampered by the addition of congo red during protoplast regeneration (Kopecka and Gabriel 1992).

Crystalline (1-3)-β-glucan has not been detected in normal fungal walls, probably because the chains are heavily modified after their extrusion into the wall. Among these modifications is the aforementioned linkage to glucosaminoglycan (chitin). In mature fungal walls, the (1-3)-β-glucan is heavily branched, often with (1-6)-β-linked glucose branches (Sietsma and Wessels 1977). The properties in solution of (1-3)-β-glucan with short (1-6)-β-linked glucose branches indicate a triple helix conformation, as in pure (1-3)-β-glucan (Sato et al. 1981).

Little is known of the mechanism by which branching occurs or how (1-6)-β-linked glucan is formed. However, there is clear evidence suggesting that the β-glucan at the very tip of growing hyphae in *S. commune* is unbranched, and that the introduction of (1-6) linkages occurs with maturation of the wall in the subapical region (Sonnenberg et al. 1985; Sietsma et al. 1985). Recently, enzymes have been isolated from *C. albicans* and *A. fumigatus* which specifically cleave laminaribiose from the reducing end of a linear (1-3)-β-glucan and transfer the remainder to another laminarioligosaccharide, creating a (1-6)-β-linked branching point (Hartland et al. 1991; Fontaine et al. 1996). With respect to (1-6)-β-linkages in linear chains, the work on the killer-resistant (*KRE*) mutants of *S. cerevisiae* is relevant. In this organism, a (1-6)-β-glucan is the receptor for the yeast killer-

toxin (Hutchings and Bussey 1983). Bussey and coworkers have used killer-toxin-resistant (*KRE*) mutants to identify several genes required for the synthesis of (1-6)-β-glucan. The analyses of numerous mutants suggest that the polymer is synthesised in a sequential manner involving several gene products, some of which are in the secretory pathway, others being cytoplasmic or membrane proteins (Brown et al. 1993). It appears that both in yeast and mycelial fungi, glyco-proteins, synthesised on a GPI-anchor, are involved in linking a (1-6)-β-glucan protein complex to the cell wall matrix. The protein part often plays an active role in fungal wall synthesis and modification outside the plasma membrane (Mouyna et al. 2000; Klis et al. 2002).

V. Wall Modifications During Wall Expansion

A. Steady-State Model for Apical Wall Growth

The simplest interpretation of the events occurring at the hyphal tip, based on what is known of the structure of the mature wall and the synthesis of individual wall components described in the preceding section, is that the major polysaccharides, (1-3)-β-glucan and chitin, are extruded into the apical wall as individual chains. Together with water, they probably constitute a plastic, hydrated, gel-like wall, easily deformable by pressure exerted by the cytoplasm. However, as soon as the two primary components are extruded into the wall domain, they become subject to enzymic modifications, become covalently linked to each other, and intra-molecular hydrogen bonds are formed. It is plausible that the total of these changes results in a gradual change in the mechanical properties of the wall, going from plastic to more rigid. Because cross-linking of the primary wall polymers is a time-dependent process and the newly formed wall continuously falls behind the expanding apical wall, this would imply the presence of a steady-state amount of plastic wall material at the growing hyphal tip, and the presence of hardened wall at the base of the extension zone. This mechanism has therefore been called the "steady-state" model of apical growth (Wessels 1986). In accordance with this model, it was found that the structure of the growing apical wall is indeed unique. Early studies (reviewed by Wessels 1986) and a recent study (Momany et al. 2004) have shown that the hyphal apex differentially binds the dyes calcofluor white and congo red, the lectin wheat agglutinin and some antibodies raised against cell walls. More specifically it was found that, in contrast to the subapical wall, the wall at the growing apex contains non-fibrillar chitin and no alkali-insoluble glucan, and that cessation of growth transforms the apical wall into a structure indistinguishable from that of the mature subapical wall (Wessels et al. 1983; Vermeulen and Wessels 1984; Sietsma et al. 1985).

Direct experimental evidence, obtained with spore germlings of *S. commune* (Sietsma et al. 1985) supporting the steady-state growth theory of apical wall growth, is summarised in Fig. 4.1.

– Pulse-labelling with [^{3}H]acetylglucosamine resulted in the labelling of chitin, nearly all alkali-insoluble, maximally at the extreme apex and rapidly decreasing subapically. Labelling with [^{3}H]glucose resulted in the labelling of total glucan in the wall in a similar pattern; after the short labelling period, no label was detectable in chitin. However, at the apex very little label appeared in alkali-insoluble glucan;

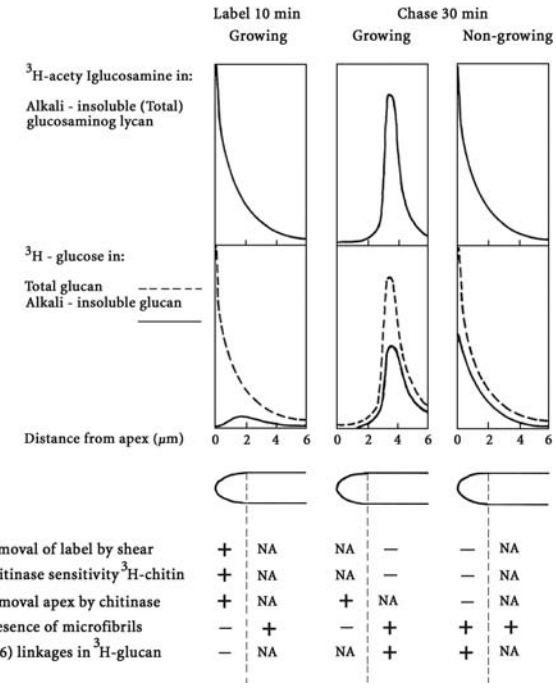

Fig. 4.1. Experimental evidence supporting the steady-state growth theory of apical wall expansion. Patterns of labelling and tabulated results are from Wessels et al. (1983), Vermeulen and Wessels (1984), and Sietsma et al. (1985). *NA* Not applicable. See text for more explanation (from Wessels et al. 1990, with permission)

nearly all the label was present in water- and alkali-soluble glucans. After a chase of radioactivity and continued incubation in the presence of unlabelled precursor, two patterns of labelling were observed. Some of the hyphae show displacement of the label to subapical regions, indicating that these hyphae had grown during the chase period. Other hyphae had stopped growing, due to mechanical disturbance by the chase procedure, and retained all label in their apices. In both cases, however, during the incubation in unlabelled medium a considerable part of the label appeared in the alkali-insoluble fraction, at the expense of label in the water/alkali-soluble glucan fraction. It was found that the glucan transferred from the water- and alkali-soluble fractions into the alkali-insoluble fraction almost exclusively contained (1-3)-β-linkages.

- After pulse-labelling with [^{3}H]glucosamine or [^{3}H]glucose, labelling patterns shown in Fig. 4.1 were observed only after removal of cytoplasm by extraction with ethanolic KOH. Removal of the cytoplasm by mechanical breakage of the hyphae resulted in disappearance of labelled apices; labelled glucan was solubilised, and labelled chitin was dispersed. After a chase, however, all label, now present subapically in growing hyphae and still apically in non-growing ones, was resistant to the shearing forces produced by mechanical breakage. This indicates transfer of label during the chase period from mechanically fragile to rigid wall structures.
- Immediately after pulse-labelling with [^{3}H]glucosamine, incubation of the alkali-insoluble wall residue with chitinase or hot dilute mineral acid affected solubilisation of most of the label incorporated into chitin. After a chase of 60 min, the chitin became resistant to such treatment. This may reflect the gap between polymerisation and crystallisation of chitin but also the protection of chitin chains against chitinase by the attachment of glucan chains.
- The growing hyphae, when extracted with alkali, did not show microfibrils over their apices, and apical chitin was rapidly disintegrated by treatment with chitinase, again indicating the absence of crystallinity. Chitin in non-growing apices became resistant to chitinase, and did reveal microfibrils after alkali extraction and extraction of β-glucan with hot dilute acid.
- By using glucose labelled with [^{3}H] at either the C_3 or C_2 position, and localising the label by autoradiography before and after treatment with periodate, it was found that the most apical region of growing apices contained few (1-6) linkages in the alkali-insoluble glucan. Subapically, the number of (1-6) linkages in this glucan rose rapidly. An abundance of (1-6) linkages was also recorded in the alkali-insoluble glucan which covered non-growing apices, so that also in this respect the wall over these apices became very similar to the subapical wall.

Importantly, in growing hyphae all the abovementioned wall modifications continue beyond the extension zone. We therefore surmise that, at the base of the extension zone, these wall modifications have only progressed sufficiently to produce a wall resisting turgor but that the wall is not maximally hardened. Although the most apical wall may be protected by the structured cytoplasm, this may explain why the wall bulges and eventually ruptures just under the apex when turgor is suddenly increased (see Sect. I).

B. Determinate Wall-Growth Model for Budding Yeasts

Whereas in mycelial fungi the wall continuously expands during growth, wall expansion in budding and fission yeasts is discontinuous. Considering only budding yeasts, after the bud has attained a certain size it stops growing and then the wall is apparently loosened again at predetermined sites to allow for evaginations which grow into new buds. There is a growing body of evidence indicating that this process is similar to branching and apical growth in mycelial fungi, with the important difference that the gradient in wall synthesis is less steep than that in hyphae, or becomes so during bud growth (Staebell and Soll 1985; Klis et al. 2002).

Dimorphic fungi, such as *C. albicans*, are able to modulate the pattern of wall deposition and are thus able to switch between yeast and hyphal growth, depending on environmental conditions (see The Mycota, Vol. I, 1st edn., Chap. 8, and Vol. VIII, Chap. 3). Careful measurements of wall expansion in *C. albicans* (Soll et al. 1985; Staebell and Soll 1985) have shown that the first phase of bud growth is dominated by polarised wall expansion whereas in the second phase, expansion of the bud wall is more general. Only during the first phase

can inductive conditions transform the bud into a hypha. In the cytoplasm this change in the pattern of wall deposition is mirrored by a change in actin distribution from apically concentrated to more dispersed.

Notwithstanding its advanced genetics, knowledge of the chemistry of the wall of *S. cerevisiae* is not more advanced than in other fungi. For some time it was generally assumed that the small amount of chitin present (1%–2% of wall preparations) was only important for construction of the septum (Cabib et al. 1988). However, the demonstration that the degradation of chitin (including stretches with deacetylated residues) renders all the β-glucan in the wall soluble in alkali (Mol and Wessels 1987) indicates that also in this fungus chitin plays an important role in maintaining the integrity of the whole wall. We then showed that throughout the cell cycle, (1-3)-β-glucan is extruded into the wall in an alkali-soluble form and is then slowly sequestered in an insoluble form, apparently by linkage of the alkali-insoluble chitin (Hartland et al. 1994). As proposed for mycelial fungi (Sect. V.A), this time-dependent process of insolubilisation, together with hydrogen bonding among the glucan chains, may lead to a gradual change in the mechanical properties of the wall, going from a plastic to a more rigid composite.

If the above reasoning is accepted, then it is clear that deposition of plastic wall material over the whole surface of the bud must eventually result in hardening of the whole wall and cessation of growth (Fig. 4.2B). The presence of a polarised component in wall deposition during early bud growth would lead to an oval, rather than a spherical cell. Growth of the wall is therefore determinate, and the proliferation of cells can occur only by reiteration of the process by local wall softening and emergence of new evaginations. Persistence of polarised wall growth for an extended period would lead to very elongated buds and the formation of pseudohyphae. Only when the gradient of wall deposition becomes very steep and persists would continuously growing hyphae arise (Fig. 4.2A). In these hyphae, the growing wall continuously escapes the zone of rigidification which spreads into the apical wall only when the forces which drive elongation subside.

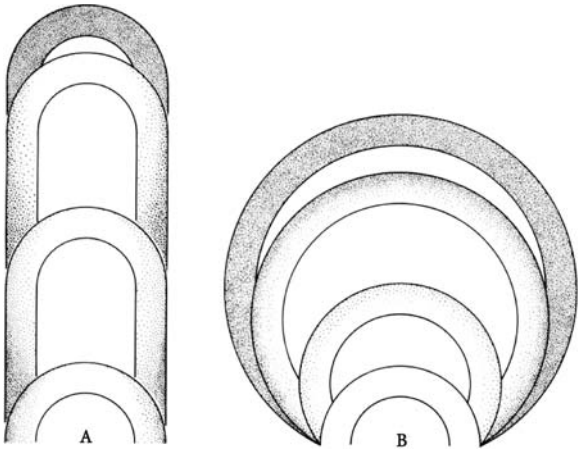

Fig. 4.2. Comparison of **A** the steady-state growth model of apical wall extension, which is based on a steep gradient in wall apposition, with **B** determinate growth resulting from general wall deposition. In both cases, cross-linking and rigidification of the wall occurs (*increased shading*), but the steep gradient of wall deposition in **A** permits the maintenance of a steady-state amount of viscoelastic wall material which can continuously expand. Cessation of the steady state and rigidification of the wall over the apex occur only when expansion is interrupted. In **B**, a steady state is never attained and expansion ceases because rigidification spreads over the whole growing area of the wall. In both cases, after expansion comes to an end, further proliferation can occur only by formation of a new evagination by wall loosening (formation of a branch or a bud; from Wessels 1990, with permission)

VI. Apical Gradient in Wall Synthesis

According to the steady-state model of apical wall growth, the crucial aspect for generation of a hypha is the generation of an appropriate gradient in wall biosynthetic activity. Little is know about the mechanism(s) by which this gradient is generated, and only hypotheses exist which are awaiting experimental consideration.

A. The Vesicle Supply Centre Model

Bartnicki-Garcia et al. (1989, 1990) developed a computer program for fungal cell growth based on the incorporation of vesicles carrying all the components for wall growth. The following assumptions were made: (1) the cell is a two-dimensional object, (2) the vesicles are released from a point source called the vesicle supply centre (VSC), (3) from the VSC, vesicles migrate randomly in all directions and (4) upon hitting the cell surface, a vesicle inserts its mass into the wall, supplying all ingredients to expanding the surface by one unit. Note that it is implicit that the wall vesicles carry not only biosynthetic

ingredients such as wall components and synthesising enzymes but also lytic enzymes presumed necessary to plastisise the wall and allow insertion of new wall material, as originally conjectured by Bartnicki-Garcia (1973). By assuming the VSC to be stationary, a spherical cell was obtained, growing only in diameter. By assuming the VSC to move in one direction, a gradient in wall expansion was simulated, resembling the outline of a growing hypha. Qualitatively this can be easily appreciated by realising that a maximum of vesicles reach the surface in the direction of movement of the VSC. Mathematically, the relationship was expressed as $y = x \cot x\mathrm{V/N}$, where x and y are the axes of the two dimensions, V is the rate of linear displacement of the VSC, and N is the rate of increase in area, equivalent to the number of vesicles released by the VSC per unit of time. The ratio V/N is the distance, d, between the VSC and the wall at the extreme tip. When plotting y versus x, a curve is obtained, called a hyphoid, which faithfully outlines the shape of median sections through growing hyphal tips of many fungi, the emplacement of the VSC closely approximating the position of the Spitzenkörper. The Spitzenkörper (apical body) was so named by Brunswick (1924) who observed it as an iron-haematoxylin positive area in the cytoplasm of the hyphal tip. A recent review on the nature and possible roles of the Spitzenkörper is given by Harris et al. (2005). Girbardt (1955), using phase contrast microscopy, observed a Spitzenkörper in growing hyphae of several fungi. He found that when growth was arrested, the Spitzenkörper vanished and reappeared again just before growth resumed. Electron microscopical observations subsequently revealed the accumulation of numerous vesicles at the site where the Spitzenkörper was observed (Girbardt 1969; Grove and Bracker 1970). A role in hyphal growth was also evident from Girbardt's finding that an off-centre displacement of the Spitzenkörper preceded a change in growth direction of the hypha (Girbardt 1957). This observation was corroborated and extended by observing the growth direction and shape of fungal hyphae after experimental displacement of the Spitzenkörper (Bartnicki-Garcia et al. 1995) or following normal trajectories of the Spitzenkörper (Riquelme et al. 1998). All these observations were taken as strong evidence that the Spitzenkörper is the VSC which collects secretory vesicles from the subapical cytoplasm and then radiates these vesicles in all directions. While moving forwards, being pushed or pulled (Bartnicki-Garcia et al. 1990), it would create the necessary gradient in vesicles, fusing with the apical plasma membrane. The VSC model has been criticised on both cytological grounds (Heath and Janse van Rensburg 1996) and on the basis that it is a two-dimensional model which does not apply to the three-dimensional shape of the hypha (Koch 1994). The latter critique was recently addressed by Bartnicki-Garcia and Gierz (2001), showing that a mathematical treatment of three dimensions, incorporating an orthogonal wall expansion (Bartnicki-Garcia et al. 2000), essentially leads to the same model as devised for a two-dimensional projection of the hypha.

The VSC model envisioned by Bartnicki-Garcia (1990, 2002) incorporates the concept that the nascent wall is a basically rigid structure and that the wall vesicles contain plastisising enzymes. In the steady-state model of apical wall growth referred to above, lytic enzymes or other plastisising agents are not deemed necessary, except for initiation of a new apical growth point. Johnson et al. (1996) and Bartnicki-Garcia (2002), who all have advanced the idea of a balance between lysis and synthesis in apical wall growth, have argued that the steady-state model of wall growth would benefit from incorporating the concept of lysins. This would lead to a reconciliation of the VSC and steady-state models. On the other hand, Wessels (1999) has argued that there is no contradiction between the models if the need for lytic action is removed from the VSC model. The VSC model would then address only the mechanism by which a gradient in wall synthesis can become established, the essential feature of the model. As noted by its inventor (Bartnicki-Garcia 2002), the ultimate validity of the VSC hypothesis depends on the demonstration that the flow of wall-building vesicles passes through a Spitzenkörper control gate. Such traffic of vesicles in/out of the Spitzenkörper is yet to be demonstrated and measured.

B. The Self-Sustained Gradient Model

As noted above, the wall retains uniform thickness during apical extension, meaning that the thinning of the older wall due to expansion must be exactly compensated by addition of new wall material. Gooday and Trinci (1980) have indeed shown that the deposition of chitin at the apex closely parallels

the derived mathematical equation for wall plasticity. This implies that the cytoplasm must obtain information about the degree of plasticity of the wall at each point on the growing apex, and accordingly regulates the wall synthetic activity at this site. To explain this tight coupling between wall plasticity and wall synthesis, Wessels (1990, 1993) suggested that mechano-sensitive proteins in the plasma membrane might sense stretch of the membrane at sites where the wall can yield to turgor pressure. Sensing the plasticity of the wall could regulate the recruitment and/or activity of wall-synthesising enzymes, either directly or indirectly via ion channels. Ion current could also direct vesicle fusion. Such processes could balance wall expansion and wall addition, ensuring constant wall thickness. The discovery of stretch-activated Ca^{2+} channels in *Uromyces appendiculatus* (Zhou et al. 1991) and *Saprolegnia ferax* (Garill et al. 1993) provides some support for this contention. In the latter case, these channels were suggested to be particularly abundant in the hyphal tips. Bovine brain calmodulin has been shown to activate chitin synthase (Martinez-Cadena and Ruiz-Herrera 1987), suggesting that chitin synthase at the hyphal apex may be regulated by calcium. In general, it would seem worthwhile to consider the possibility that effects of mutations in the signal transduction pathways which influence hyphal growth are due to defects in reporting wall extensibility to the cytoplasm. This would apply, for instance, to mutations in *ras* genes (Truesdell et al. 1999) and genes encoding protein kinases (Yarden et al. 1992; Dickman and Yarden 1999). It would also be interesting to investigate a possible role of small GTP-ases, encoded by *rho* and *rac* genes which interact with the cytoskeleton (Raudaskoski et al. 2001).

Whatever the mechanism by which wall plasticity is sensed and by which the information is relayed to the cytoplasm, a tight coupling between the degree of wall plasticity (as envisaged by the steady-state model) and wall synthesis would mean that, once a wall-building gradient is established, this gradient would be self-sustained. It can be assumed that at branch initiation local wall softening occurs. It can be speculated that such a local increase of plasticity of the wall would generate a signal in the cytoplasm which leads to activation at this site of wall synthases and the acquisition of exocytotic vesicles. Probably the establishment of the gradient in wall synthesis would heavily depend on the polarised structure of the cytoplasm at the nascent apex.

VII. Passage of Proteins Through the Hyphal Wall

A. The Secretory Pathway

In accordance with their habit of colonizing polymeric substrates, mycelial fungi are able to secrete large amounts of lytic enzymes into the milieu. They are therefore also regarded as attractive hosts for the production of heterologous proteins by genetic engineering (van den Hondel et al. 1991; MacKenzie et al. 1993; Conesa et al. 2001; see The Mycota, Vol. X, Chap. 21).

In essence, the secretory pathway for proteins in eukaryotes appears universal, and entails an initial translocation of proteins across the membrane of the endoplasmic reticulum (ER), followed by movement from the ER to the Golgi apparatus and on through secretory vesicles to the cell surface or the vacuole. Specific sorting apparently operates mainly through a process of selective retention so that, in the absence of specific signals, protein transport occurs by default route, which is through secretory vesicles to the cell surface (Barinaga 1993). These insights have emerged mainly from studying mammalian cells and the yeast *S. cerevisiae*, the latter on account of the many temperature-sensitive *sec* mutants with specific defects in protein excretion (Schekman 1985, and see The Mycota, Vol. I, 1st edn., Chap. 2). These *sec* mutants also reveal the tight coupling between membrane biogenesis, cell surface growth and excretion in these fungal cells. This warrants the suggestion that in mycelial fungi similar mechanisms could operate, although the secretory process via the endomembrane system in these organisms has barely been explored (MacKenzie et al. 1993; see The Mycota, Vol. X, Chap. 21).

A concentration of vesicles at the site of growth seems to be a general feature of apical extension, both in fungal and plant kingdoms. Gooday and Trinci (1980) found that in *N. crassa* the number of vesicles does not change much along the hyphal axis but that the vesicle concentration increased at the apex, due to the decrease in cytoplasmic volume in the tapering apical dome. As referred to above, Bartnicki-Garcia et al. (1989) have suggested the Spitzenkörper to be a vesicle supply centre (VCS), collecting the vesicles from subapical regions and radiating them uniformly in all directions until they encounter the cytoplasmic membrane, resulting in exocytosis. Because in their opinion the VCS moves forwards at a constant speed, a gradient

in membrane enlargement and exocytosis would occur.

The apical vesicles are thought to arise in the endoplasmic reticulum far behind the tip, pass through Golgi equivalents, and be then transported vectorially to the apex where they fuse with the plasma membrane (see The Mycota, Vol. I, 1st edn., Chap. 3). In their membranes, these vesicles may carry proteins destined for the plasma membrane, such as polysaccharide synthases, ion channels and ATP-ase. In their lumina, they may carry some wall components, wall-modifying enzymes, as well as lytic enzymes destined for excretion to digest polymeric substrates. The cytological and enzymological evidence attesting to this view has been reviewed by Gooday and Gow (1990). As mentioned above, evidence is accumulating that chitin synthesis in yeast and at the hyphal tip is regulated by exo- and endocytosis of chitin synthase-containing particles (see The Mycota, Vol. III, 2nd edn., Chap. 14).

B. The Hyphal Wall as a Barrier for Passage of Proteins

The cell wall is the last barrier proteins secreted into the medium have to take. Slime mutants of *N. crassa* which are defective in wall synthesis over-excrete enzymes, including invertase (Bigger et al. 1972; Casanova et al. 1987; Pietro et al. 1989), alkaline phosphatase (Burton and Metzenberg 1974), alkaline protease and aryl-β-glucosidase (Pietro et al. 1989). This suggests that the wall acts as a barrier in protein excretion. Moreover, the problem of passage through the wall is compounded by the fact that excreted proteins are sometimes much larger than the estimated average pore size of the walls of yeasts (Scherrer et al. 1974; Cope 1980) and mycelial fungi (Trevithnick and Metzenberg 1966). These pore sizes were measured with isolated cell wall fractions, or determined by the solute exclusion method, using living hyphae (Money 1990).

On the basis of the existence of a correlation between the sizes of excreted proteins and numbers of hyphal tips present, Chang and Trevithick (1974) have proposed that the proteins are excreted at the hyphal apices and that the nascent wall at the apex contains larger pores than the mature subapical wall. Because of the small proportion of apical wall material in wall preparations used for pore-size measurements, such large pores would go undetected. The description of wall deposition at the extreme apex of the hypha, given in Sect. V., indeed indicates that the nature of the wall in that region differs completely from that in subapical regions; it can easily be envisaged that the assumed gel-like, highly hydrated condition of the wall at the extreme apex facilitates the diffusion of large proteins over this barrier. However, the measurements of Money (1990) on solute exclusion in living hyphae do not support this contention.

An alternative is offered by the bulk-flow hypothesis proposed by Wessels (1990, 1993, 1999), which assumes that proteins secreted at the very tip are pushed through the wall from the inside to the outside by the accretion of plastic wall polymers during wall growth. A theoretical consideration on how a wall volume travels through an expanding wall at the apex is given by Green (1974). Considering the model for apical wall growth discussed in Sect. V., it is plausible that proteins extruded into the wall will be carried by the flow of plastic wall material (Fig. 4.3). In general, if the gradient of extrusion of a particular protein were similar to the gradient in wall synthesis, then this protein would be expected to become evenly distributed throughout the wall. However, if its gradient of extrusion were steeper, then it would be preferentially located in the outer wall region (Fig. 4.3). Across the wall, this region is oldest, most rigidified and most stretched. Consequently, the wall may have larger pores in this region than at the inside, and

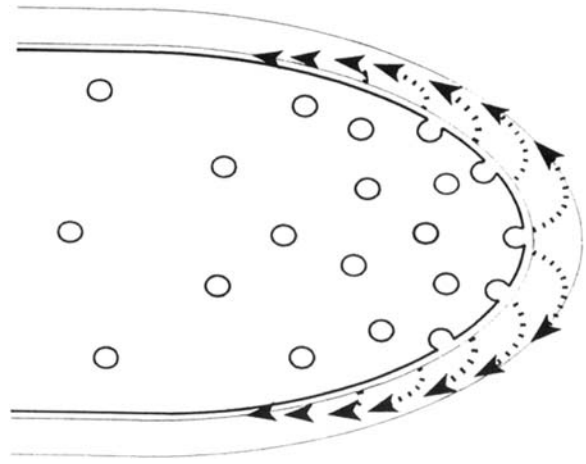

Fig. 4.3. Schematic presentation of the "bulk-flow" hypothesis for protein translocation through the wall in a growing fungal hypha. Proteins contained in secretion vesicles which fuse with the plasma membrane at the most apical site are transported by the flow of nascent wall polymers to the outside of the subapical wall and, if not anchored to the wall, can then easily diffuse into the medium. Proteins from vesicles which fuse more subapically with the plasma membrane become trapped in the inner portion of the wall

would permit larger proteins to diffuse readily from this region into the medium. Within this context, differences in the gradients by which wall components are extruded into the apical wall could also account for some of the layering seen in the walls of fungi, without inferring temporal differences in the synthesis of these wall components (Wessels 1990).

A similar mechanism of passage of proteins through the wall could apply for yeasts during bud growth, particularly during the first polarised growth phase. Even during the phase of more general wall expansion, proteins should be pushed into the wall by the accretion of wall material at the inside. A coupling between wall expansion and excretion has been documented in yeasts (Schekman 1985). However, protein translocation over the wall by the proposed mechanism is expected to be less efficient than in mycelial fungi where the most apically excreted proteins would flow continuously to the outside regions of the wall. In addition, proteins in yeasts would not be released into the medium if they became cross-linked to the polysaccharides of the wall, as seems to be the case for the high-mannan protein fraction of the yeast cell wall (see The Mycota, Vol. I, 1st edn., Chap. 6, and Vol. XIII, Chap. 9) and the sexual adhesion protein α-agglutinin (Schreuder et al. 1993). Moreover, the presence of these structural mannoproteins seems to impede the release of other proteins into the medium (Zlotnik et al. 1984; de Nobel and Barnett 1991).

What then is the evidence for protein excretion occurring mainly at the growing apex? Direct evidence was obtained by growing colonies of *A. niger* and *Phanerochaete chrysosporium* in a very thin layer between two porous polycarbonate membranes (Wösten et al. 1991; Moukha et al. 1993a,b). By this method it was possible to localise, by autoradiography, both apical wall growth and the excretion of proteins. Apical growth was monitored by the incorporation of radioactive *N*-acetyl-glucosamine into chitin, protein synthesis and excretion by the incorporation of radioactive sulphate and catching excreted proteins on a protein-binding membrane underneath the sandwiched colony. In young, actively growing colonies, chitin labelling occurred most intensely at apices at the periphery of the colony, as expected. However, whereas cytoplasmic proteins were labelled throughout the colony, protein excretion occurred almost exclusively in the peripheral growth zone. In *A. niger*, the excretion of glucoamylase could be immunologically detected in this zone, and at the hyphal level it could be shown that a considerable portion is actually excreted at hyphal apices. Another portion of the glucoamylase, however, is retained for some time in the hyphal wall and appears to diffuse into the medium only slowly (Wösten et al. 1991). In *A. niger*, apical secretion and transient retaining of protein in the subapical wall was also demonstrated for a glucoamylase:green fluorescent protein fusion (Gordon et al. 2000). Importantly, apical excretion was also suggested for idiophase enzymes which are excreted only after growth of the mycelium as a whole has ceased. In *Ph. chrysosporum*, Moukha et al. (1993a,b) found that after cessation of radial growth of the colony a new growth zone arises in the centre of the colony, characterized by branching of the resident hyphae and accompanied by the formation of mRNAs for lignin peroxidase and Mn^{2+}-dependent peroxidase and the excretion of these enzymes into the medium, apparently by the newly formed branches. This reflects the ability of colonies of these mycelial fungi to redirect growth even in the absence of external nutrients, by redistribution of previously assimilated nutrients (see The Mycota, Vol I, 1st edn., Chap. 9).

The results of these experiments clearly show that in mycelial fungi there is a tight coupling between apical wall growth and excretion of proteins into the medium, both during primary growth and during idiophase when net growth has come to a halt. The wall over the growing apex is apparently the major site for the passage of proteins. If the very polarised excretion of wall polymers is instrumental in the passage of proteins over the wall, then this may explain the tremendous capacity of mycelial fungi, in contrast to yeasts, to export sometimes about half of the proteins they make into the external milieu (Wessels 1993).

VIII. Conclusion

The cylindrical hyphal form is generated by cell wall synthesis at the apex. The cell wall components, chitin, (1-3)-β-glucan and (1-3)-α-glucan, are synthesised separately at the apex, and cross-linked and modified in subapical regions. In this way, a cell wall is generated which is plastic at the very apex, expandable by turgor pressure and rigid at subapical parts of the hypha, resistant to turgor pressure. At the apex a gradient of wall synthe-

sis occurs, coinciding with a gradient of plasticity. The rigidification process occurs in the wall area, outside the cytoplasmic membrane. In this process, chitin and (1-3)-β-glucan become covalently linked and the glucan part becomes modified, branched and (1-6)-β-linkages are inserted. This model explains not only the generation of the hyphal form but also the fact that the secretion of large molecules occurs exclusively at growing hyphal apices. The looser and highly hydrated construction of the wall at the very apex allows proteins and other large molecules to diffuse across the wall, whereas in subapical regions the wall is rigid and forms a barrier for large molecules. Components of the wall-synthesising apparatus and proteins which need to be secreted are probably all synthesised at the ER, packed in vesicles and guided by the cytoskeleton to the apex. Therefore, at the apex all vesicles are collected in a microscopically visible structure which has been referred to as the Spitzenkörper or vesicle supply centre. The function and role of this structure is not well understood yet.

References

Ariko Y, Ito E (1975) A pathway of chitosan formation in *Mucor rouxii*. Enzymatic deacetylation of chitin. Eur J Biochem 55:71–78

Aufauvre-Brown A, Mellado E, Gow NAR, Holden DW (1997) *Aspergillus fumigatus chsE*: a gene related to *CHS3* of *Saccharomyces cerevisiae* and important for hyphal growth and conidiophore development but not pathogenicity. Fungal Genet Biol 21:141–152

Balint S, Farkas V, Bauer S (1976) Biosynthesis of β-glucans catalyzed by a particulate enzyme preparation from yeast. FEBS Lett 64:44–47

Barinaga M (1993) Secrets of secretion revealed. Science 260:487–489

Bartnicki-Garcia S (1968) Cell wall chemistry, morphogenesis and taxonomy of fungi. Annu Rev Microbiol 22:87–108

Bartnicki-Garcia S (1973) Fundamental aspects of hyphal morphogenesis. In: Ashworth JM, Smith E (eds) Microbial differentiation. Symp Soc Gen Microbiol, Cambridge University Press, Cambridge, pp 245–267

Bartnick-Garcia S (1990) Role of vesicles in apical growth and a new mathematical model of hyphal morphogenesis. In: Heath IB (ed) Tip growth in plants and fungal cells. Academic Press, San Diego, pp 211–232

Bartnicki-Garcia S (2002) Hyphal tip growth: outstanding questions. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 29–58

Bartnicki-Garcia S, Gierz G (2001) A three-dimensional model of fungal morphogenesis based on the vesicle supply center concept. J Theor Biol 208:151–164

Bartnicki-Garcia S, Lippman E (1969) Fungal morphogenesis: cell wall construction in *Mucor rouxii*. Science 165:302–304

Bartnicki-Garcia S, Lippman E (1972) The bursting tendency of hyphal tips of fungi: presumptive evidence for a delicate balance between wall synthesis and wall lysis in apical growth. J Gen Microbiol 73:487–500

Bartnicki-Garcia S, Lippman E (1982) Fungal cell wall composition. Handb Microbiol 4:229–252

Bartnicki-Garcia S, Nickerson WJ (1962) Isolation, composition and structure of cell walls of filamentous and yeast-like forms of *Mucor rouxii*. Biochim Biophys Acta 58:102–119

Bartnicki-Garcia S, Bracker CE, Reyes E, Ruiz-Herrera J (1978) Isolation of chitosomes from taxonomically diverse fungi and synthesis of chitin microfibrils in vitro. Exp Mycol 2:173–192

Bartnicki-Garcia S, Hergert F, Gierz G (1989) Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. Protoplasma 153:46–57

Bartnicki-Garcia S, Hergert F, Gierz G (1990) A novel computer model for generating cell shape: application to fungal morphogenesis. In: Kuhn PJ, Trinci APJ, Jung MJ, Goosey MW, Copping LG (eds) Biochemistry of cell walls and membranes in fungi. Springer, Berlin Heidelberg New York, pp 43–60

Bartnicki-Garcia S, Bartnicki DD, Gierz G, López-Franco R, Bracker CE (1995) Evidence that Spitzenkörper behavior determines the shape of a fungal hypha: a test of the hyphoid model. Exp Mycol 19:153–159

Bartnicki-Garcia S, Bracker CE, Gierz G, López-Franco R, Lu H (2000) Mapping the growth of fungal hyphae: orthogonal cell wall expansion during tip growth and the role of turgor. Biophys J 79:2382–2390

Beauvais A, Bruneau JM, Mole PC, Buitrago MJ, Legrand R, Latge JP (2001) Glucan synthase complex of *Aspergillus fumigatus*. J Bacteriol 183:2273–2279

Bigger CH, White MR, Braymer, HD (1972) Ultrastructure and invertase secretion of the slime mutant of *Neurospora crassa*. J Gen Microbiol 71:159–166

Bonner JT (ed) (1961) D'Arcy Thompson, on growth and form. Cambridge University Press, London

Borgia P, Dodge C (1992) Characterization of *Aspergillus nidulans* mutants deficient in cell wall chitin or glucan. J Bacteriol 174:377–383

Bracker CE, Ruiz-Herrera J, Bartnicki-Garcia S (1976) Structure and transformation of chitin synthase particles (chitosomes) during microfibril synthesis in vitro. Proc Natl Acad Sci USA 73:4570–4574

Brown JL, Kossaczka Z, Jiang B, Bussey H (1993) A mutational analysis of killer toxin resistance in *Saccharomyces cerevisiae* identifies new genes involved in cell wall (1-6)-β-glucan synthesis. Genetics 133:837–849

Brunswick H (1924) Untersuchungen über Geschlechts- and Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*. In: Goebel K (ed) Botanische Abhandlungen, vol 5. Fischer, Jena, pp 243–305

Bulawa CE (1992) CSD2, CSD3 and CSD4 genes required for chitin synthesis in *Saccharomyces cerevisiae*; the CSD2 gene product is related to chitin synthases and to developmental regulated proteins in *Rhizobium* and *Xenopus laevis*. Mol Cell Biol 12:1764–1776

Bulawa CE (1993) Genetics and molecular biology of chitin synthesis in fungi. Annu Rev Microbiol 47:505–534

Burton EG, Metzenberg RL (1974) Properties of repressible alkaline phosphatase from wild type and a wall-less mutant of *Neurospora crassa*. J Biol Chem 249:4679–4688

Cabib E, Roberts R, Bowers B (1982) Synthesis of the yeast wall and its regulation. Annu Rev Biochem 51:763–793

Cabib E, Bowers B, Roberts RL (1983) Vectorial synthesis of a polysaccharide by isolated plasma membranes. Proc Natl Acad Sci USA 80:3318–3321

Cabib E, Bowers B, Sburlati A, Silverman SJ (1988) Fungal cell wall synthesis: the construction of a biological structure. Microbiol Sci 5:370–375

Cabib E, Silverman SJ, Shaw JA (1992) Chitinase and chitin synthase 1: counterbalancing activities in cell separation of *Saccharomyces cerevisiae*. J Gen Microbiol 138:97–102

Casanova A, Martinez JP, Gil ML, Sentandreu R, Ruiz-Herrera J (1987) Different molecular forms of invertase in the slime variant of *Neurospora crassa*: comparison with the wild-type strain. J Gen Microbiol 133:2447–2456

Chang PLY, Trevithick JR (1974) How important is secretion of exoenzymes through apical cell walls of fungi? Arch Mikrobiol 101:281–293

Chitnis MV, Munro CA, Brown AJ, Gooday GW, Gow NA, Deshpande MV (2002) The zygomycetous fungus *Benjaminiella poitrasii* contains a large family of differentially regulated chitin synthase genes. Fungal Genet Biol 36:215–223

Chuang JS, Schekman RW (1996) Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p. J Cell Biol 135:597–610

Cleland RE (1981) Wall extensibility: hormones and wall extension. In: Tanner W, Loewus FA (eds) Encyclopedia of Plant Physiology, vol 13B. Springer, Berlin Heidelberg New York, pp 255–276

Conesa A, Punt PJ, van Luyk N, van den Hondel CAMJJ (2001) The secretory pathway in filamentous fungi: a biotechnical view. Fungal Genet Biol 33:155–171

Cope J (1980) The porosity of the cell wall of *Candida albicans*. J Gen Microbiol 119:253–255

Da Riva Ricci D, Kendrick B (1972) Computer modelling of hyphal tip growth in fungi. Can J Bot 50:2455–2462

Datema R, van den Ende H, Wessels JGH (1977a) The hyphal wall of *Mucor mucedo* 1. Polyanionic polymers. Eur J Biochem 80:611–619

Datema R, Wessels JGH, van den Ende H (1977b) The hyphal wall of *Mucor mucedo* 2. Hexosamine-containing polymers. Eur J Biochem 80:621–626

Davis LL, Bartnicki-Garcia S (1984) Chitosan synthesis by the tandem action of chitin synthetase and chitin deacetylase from *Mucor rouxii*. Biochem J 23:1065–1068

De Nobel JG, Barnett JA (1991) Passage of molecules through yeast cell walls: a brief assay-review. Yeast 7:313–323

Dickman MB, Yarden O (1999) Serine/threonine protein kinases and phosphatases in filamentous fungi. Fungal Genet Biol 26:99–117

Din AB, Specht CA, Robbins PW, Yarden O (1996) *chs-4*, a class IV chitin synthase gene from *Neurospora crassa*. Mol Gen Genet 250:214–222

Duran A, Cabib E (1978) Solubilization and partial purification of yeast chitin synthetase. J Biol Chem 253:4419–4425

Duran A, Bowers B, Cabib E (1975) Chitin synthetase zymogen is attached to the yeast plasma membrane. Proc Natl Acad Sci USA 72:3952–3955

Elorza MV, Murgui A, Rico H, Miragall F, Sentandreu R (1987) Formation of a new cell wall by protoplasts of *Candida albicans*: effect of papulacandin B, tunicamycin and nikkomycin. J Gen Microbiol 133:2315–2325

Farkas V (1985) The fungal cell wall. In: Peberdy JF, Ferenczy L (eds) Fungal protoplasts. Dekker, New York, pp 3–29

Florez-Martinez A, Lopez-Romero E, Martinez JP, Bracker CE, Ruiz-Herrera J, Bartnicki-Garcia S (1990) Protein composition of purified chitosomes of *Mucor rouxii*. Exp Mycol 14:160–168

Fontaine T, Mouyna I, Hartland RP, Paris S, Latge JP (1996) From the surface to the inner layer of the fungal cell wall. Biochem Soc Trans 25:194–199

Fontaine T, Simenel C, Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M, Latge JP (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. J Biol Chem 275:27594–27607

Fujiwara M, Ichinomiya M, Motoyama T, Horiuchi H, Ohta A, Takagi M (2000) Evidence that the *Aspergillus nidulans* class I and class II chitin synthase genes, *chsC* and *chsA*, share critical roles in hyphal wall integrity and conidiophore development. J Biochem 127:359–366

Garill A, Jackson SL, Lew RR, Heath IB (1993) Ion channel activity and tip growth: tip localized stretch activated channels generate an essential Ca^{2+} gradient in the oomycete *Saprolegnia ferax*. Eur J Cell Biol 60:358–365

Girbardt M (1955) Lebendbeobachtungen an *Polystictus versicolor*. Flora 50:47–59

Girbardt M (1957) Der Spitzenkörper von *Polystictus versicolor*. Planta 50:47–59

Girbardt M (1969) Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma 67:413–441

Gooday GW (1971) An autoradiographic study of hyphal growth of some fungi. J Gen Microbiol 76:125–133

Gooday GW (1978) The enzymology of hyphal growth. In: Smith JE, Berry DR (eds) Filamentous Fungi, vol 3. Arnold, London, pp 51–77

Gooday GW, Gow NAR (1990) Enzymology of tip growth in fungi. In: Heath IB (ed) Tip growth in plants and fungal cells. Academic Press, San Diego, pp 31–58

Gooday GW, Trinci AJP (1980) Wall structure and biosynthesis in fungi. Symp Soc Gen Microbiol 30:207–251

Gooday GW, Zhu W, O'Donell RW (1992) What are the roles of chitinases in the growing fungus? FEMS Microbiol Lett 100:387–392

Gordon CL, Khalaj V, Ram AFJ, Archer DB, Brookman JL, Trinci APJ, Jeenes DJ, Doonan JH, Wells B, Punt PJ et al. (2000) Glucoamylase:green fluorescent protein fusions to monitor protein secretion in *Aspergillus niger*. Microbiology 146:415–426

Green PB (1974) Morphogenesis of the cell and organ axis. Biophysical models. Brookhaven Symp Biol 25:166–190

Gregory PH (1984) The fungal mycelium: an historical perspective. Trans Br Mycol Soc 82:1–11

Grove SN, Bracker CE (1970) Protoplasmic organization of hyphal tips among fungi. J Bacteriol 104:989–1009

Grün CH, Hochstenbach F, Humbel BM, Verkley AJ, Sietsma JH, Klis FM, Kamerling JP, Vliegenthart FG (2005) Synthesis of cell wall alpha-glucan requires cou-

pling of two (1-3)-alpha-glucan segments and affects fission-yeast morphogenesis. Glycobiology 15:245–257
Gupta GD, Free SJ, Levina NN, Keränen S, Heath IB (2003) Two divergent plasma membrane syntaxin-like SNAREs, *nsyn1* and *nsyn2*, contribute to hyphal tip growth and other developmental processes in *Neurospora crassa*. Fungal Genet Biol 40:271–286
Harold FM (1990) The shape of a cell: an inquiry into the causes of morphogenesis of microorganisms. Microbiol Rev 54:381–431
Harold FM (1999) In pursuit of the whole hypha. Fungal Genet Biol 27:128–133
Harris SD, Hofmann AF, Tedford HW, Lee MP (1999) Identification and characterization of genes required for hyphal morphogenesis in the filamentous fungus *Aspergillus nidulans*. Genetics 151:1015–1025
Harris SD, Read ND, Roberson RW, Shaw B, Seiler S, Plamann M, Momany M (2005) Polarisome meets Spitzenkörper: microscopy, genetics, and genomics converge. Eukaryot Cell 4:225–229
Hartland RP, Emerson GW, Sullivan PA (1991) A secreted β-glucan-branching enzyme from *Candida albicans*. Proc R Soc Lond B 246:155–160
Hartland RP, Vermeulen CA, Klis FM, Sietsma JH, Wessels JGH (1994) The linkage of (1-3)-β-glucan to chitin during cell wall assembly in *Saccharomyces cerevisiae*. Yeast 10:1591–1599
Heath IB (2000) Organization and functions of actin in hyphal tip growth. In: Staiger CJ (ed) Actin: a dynamic framework for multiple plant cell functions. Kluwer, Dordrecht, pp 275–300
Heath IB, Janse van Rensburg EJ (1996) Critical evaluation of the VSC model for tip growth. Mycoscience 37:1–10
Heath IB, Steinberg G (1999) Mechanisms of hyphal tip growth: tube-dwelling amebae revisited. Exp Mycol 28:79–93
Hochstenbach F, Klis FM, van den Ende H, van Donselaar E, Peters PJ, Klausner RD (1998) Identification of a putative α-glucan synthase essential for cell wall construction and morphogenesis in fission yeast. Proc Natl Acad Sci USA 95:9161–9166
Hutchins K, Bussey H (1983) Cell wall receptor for yeast killer toxin: involvement of (1-6)-β-D-glucan. J Bacteriol 154:161–169
Jabri E, Quigley DR, Alders M, Hrmova M, Taft CS, Phelps P, Selitrennikoff CP (1989) (1-3)-β-glucan synthesis of *Neurospora crassa*. Curr Microbiol 19:153–161
Jelsma J, Kreger DR (1975) Ultrastructural observations on (1-3)-β-glucan from fungal cell walls. Carbohydr Res 43:200–203
Johnson BF (1968) Lysis of yeast cell walls induced by 2-deoxyglucose at their sites of glucan synthesis. J Bacteriol 95:1169–1172
Johnson BF, Calleja GB, Yoo BY (1996) A new model for hyphal tip extension and its application to differential fungal morphogenesis. In: Chiu S-W, Moore D (eds) Patterns in fungal development. Cambridge University Press, London, pp 37–69
Kamada T, Takemaru T (1983) Modification of cell wall polysaccharides during stipe elongation in the basidiomycete *Coprinus cinereus*. J Gen Microbiol 76:319–330
Kang MS, Cabib E (1986) Regulation of fungal cell wall growth: a guanine nucleotide-binding, proteinaceous component required for activity of (1-3)-β-D-glucan synthase. Proc Natl Acad Sci USA 83:5808–5812
Katz D, Rosenberger RF (1971a) Hyphal wall synthesis in *Aspergillus nidulans*: effect of protein synthesis inhibition and osmotic shock on chitin insertion and morphogenesis. J Bacteriol 108:184–192
Katz D, Rosenberger RF (1971b) Lysis of an *Aspergillus nidulans* mutant blocked in chitin synthesis and its relation to wall assembly and wall metabolism. Arch Mikrobiol 80:286–292
Kerkenaar A, Barug D (1984) Fluorescence microscope studies of *Ustilago maydis* and *Penicillium italicum* after treatment with imazalilor fenpropimorph. Pest Sci 15:199–205
Klis FM, Mol P, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 26:239–256
Koch AL (1982) The shape of the hyphal tips of fungi. J Gen Microbiol 128:947–951
Koch AL (1988) Biophysics of bacterial walls viewed as stress-bearing fabric. Microbiol Rev 52:337–353
Koch AL (1994) The problem of hyphal growth in streptomycetes and fungi. J Theor Biol 171:137–150
Kollar R, Petrakova E, Ashwell G, Robbins PW, Cabib E (1995) Architecture of the yeast cell wall: the linkage between chitin and β-1,3-glucan. J Biol Chem 270:1170–1178
Kopecka M, Gabriel M (1992) The influence of congo red on the cell wall and (1-3)-β-D-glucan microfibril biogenesis in *Saccharomyces cerevisiae*. Arch Microbiol 158:115–126
Kreger DR (1954) Observations on cell walls of yeasts and some other fungi by X-ray diffraction and solubility tests. Biochim Biophys Acta 13:1–9
Kreger DR, Kopecka M (1975) On the nature and formation of the fibrillar nets produced by protoplasts of *Saccharomyces cerevisiae* in liquid media: an electronmicroscopic, X-ray diffraction and chemical study. J Gen Microbiol 92:207–212
Lagorce A, Le Berre-Anton V, Aguilar-Uscanga B, Martin-Yken H, Dagkessamanskaia A, Francois J (2002) Involvement of *GFA1*, which encodes glutamine-fructose-6-phosphate amido-transferase, in the activation of the chitin synthesis pathway in response to cell wall defects in *Saccharomyces cerevisiae*. Eur J Biochem 269:1697–1707
Leal-Morales CA, Ruiz-Herrera J (1985) Alterations in the biosynthesis of chitin and glucan in the slime mutant of *Neurospora crassa*. Exp Mycol 9:28–32
Lopez-Romero E, Ruiz-Herrera J (1977) Biosynthesis of β-glucan by cell free extract from *Saccharomyces cerevisiae*. Biochim Biophys Acta 500:372–384
MacKenzie DA, Jeenes DJ, Belshaw NJ, Archer DB (1993) Regulation of secreted protein production by filamentous fungi: recent developments and perspectives. J Gen Microbiol 139:2295–2307
Marchessault RH, Deslandes Y (1979) Fine structure of (1-3)-β-D-glucans: curdlan and paramylon. Carbohydr Res 75:231–242
Martinez JP, Gimenez G, Bartnicki-Garcia S (1989) Sedimentation properties of chitosomal chitin synthetase from the wild type strain and the slime variant of *Neurospora crassa*. Biochim Biophys Acta 990:45–52

Martinez-Cadena G, Ruiz-Herrera J (1987) Activation of chitin synthase from *Phycomyces blakesleeanus* by calcium and calmodulin. Arch Microbiol 148:280–285

McGoldrick CA, Gruver C, May GS (1995) Myo A of *Aspergillus nidulans* encodes an essential myosin I required for secretion and polarized growth. J Cell Biol 128:577–587

Mellado E, Aufauvre BA, Gow NAR, Holden DW (1996) The *Aspergillus fumigatus* chsC and chsG genes encode class III chitin synthases with different functions. Mol Microbiol 20:667–679

Minke R, Blackwell J (1978) The structure of α-chitin. J Mol Biol 120:167–181

Miyazaki A, Ootaki T (1997) Multiple genes for chitin synthase in the zygomycete fungus *Phycomyces blakesleeanus*. J Gen Appl Microbiol 43:333–340

Mol PC, Wessels JGH (1987) Linkages between glucosaminoglycan and glucan determine alkali-insolubility of the glucan in walls of *Saccharomyces cerevisiae*. FEMS Microbiol Lett 41:95–99

Mol PC, Vermeulen CA, Wessels JGH (1988) Glucan-glucosaminoglycan linkages in fungal walls. Acta Bot Neerl 37:17–21

Mol PC, Park HM, Mullins JT, Cabib E (1994) A GTP-binding protein regulates the activity of (1-3)-β-glucan synthase, an enzyme directly involved in yeast cell wall morphogenesis. J Biol Chem 269:31267–31274

Momany M, Lindsey R, Hill TW, Richardson EA, Momany C, Peseira M, Guest GM, Fisher J, Hessler RB, Roberts KA (2004) The *Aspergillus fumigatus* fungal cell wall is organized in domains that are remodelled during polarity establishment. Microbiology 150:3261–3268

Money NP (1990) Measurement of pore size in the hyphal cell wall of *Achlya bisexualis*. Exp Mycol 14:234–242

Money NP, Harold FM (1993) Two water molds can grow without measurable turgor pressure. Planta 190:426–430

Montgomery GWG, Gooday GW (1985) Phospholipid-enzyme interactions of chitin synthase of *Coprinus cinereus*. FEMS Microbiol Lett 27:29–33

Moukha SM, Wösten HAB, Asther M, Wessels JGH (1993a) *In situ* localization of lignin peroxidase excretion in colonies of *Phanerochaete chrysosporium* using sandwiched mode of culture. J Gen Microbiol 139:969–978

Moukha SM, Wösten HAB, Mylius EJ, Asther M, Wessels JGH (1993b) Spatial and temporal accumulation of mRNAs encoding two common lignin peroxidases in *Phanerochaete chrysosporium*. J Bacteriol 175:3672–3678

Mouyna I, Fontaine T, Vai M, Monod M, Fonzi WA, Diaquin M, Popolo L, Hartland RP, Latge JP (2000) Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. J Biol Chem 275:14882–14889

Munro CA, Winter K, Buchan A, Henry K, Becker JM, Brown AJ, Bulawa CE, Gow NAR (2001) Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis at the septum and for cell integrity. Mol Microbiol 39:1414–1426

Orlean P (1987) Two chitin synthases in *Saccharomyces cerevisiae*. J Biol Chem 262:5732–5739

Pancaldi S, Poli F, Dall'Olio G, Vannini GA (1984) Morphological abnormalities induced by Congo red in *Aspergillus niger*. Arch Microbiol 137:185–187

Picton JM, Steer MW (1982) A model for the mechanism of tip extension in pollen tubes. J Theor Biol 98:15–20

Pietro RCLR, Jorge JA, Terenzi HF (1989) Pleiotropic deficiency in the control of carbon-regulated catabolic enzymes in the 'slime' variant of *Neurospora crassa*. J Gen Microbiol 135:1375–1382

Polacheck I, Rosenberger RF (1977) *Aspergillus nidulans* mutants lacking (1-3)-β-D-glucan, melanin and cleistothecia. J Bacteriol 132:650–656

Rast D, Bartnicki-Garcia S (1981) Effects of amphotericin B, nystatin and other polyene antibiotics on chitin synthase. Proc Natl Acad Sci USA 78:1233–1236

Raudaskoski M, Pardo AG, Tarkka MT, Gorfer M, Hanif M, Laitainen E (2001) Small GTPases. Cytoskeleton and signal transduction in filamentous homobasidiomycetes. In: Geitman A, Cresti M, Heath IB (eds) Cell biology of plant and fungal tip growth. NATO Science Series, vol 328. IOS Press, Amsterdam, pp 123–136

Reinhardt MO (1892) Das Wachstum der Pilzhyphen. Jahrb Wissen Bot 23:479–565

Riquelme M, Reynaga-Peña CG, Gierz G, Bartnicki-Garcia S (1998) What determines growth direction in fungal hyphae? Fungal Genet Biol 24:101–109

Riquelme M, Gierz G, Bartnicki-Garcia S (2000) Dynein and dynactin deficiencies affect the formation and function of the Spitzenkörper and distort hyphal morphogenesis of *Neurospora crassa*. Microbiology 146:1743–1752

Robertson NF (1958) Observations of the effect of water on the hyphal apices of *Fusarium oxysporium*. Ann Bot 22:159–173

Robertson NF (1965) The fungal hypha. Trans Br Mycol Soc 48:1–8

Roncero C (2002) The genetic complexity of chitin synthesis in fungi. Curr Genet 41:367–378

Ruiz-Herrera J (1992) Fungal cell wall: structure, synthesis, and assembly. CRC Press, Boca Raton

Santos B, Snyder M (1997) Targeting of chitin synthase 3 to polarized growth sites in yeast requires Chs5p and Myo2p. J Cell Biol 136:95–110

Sato T, Novisuye T, Fujita H (1981) Melting behavior of *Schizophyllum commune* polysaccharides in mixture of water and dimethyl sulfoxide. Carbohydr Res 95:195–204

Saunders PT, Trinci AJP (1979) Determining the tip shape in fungal hyphae. J Gen Microbiol 110:469–473

Schekman R (1985) Protein localization and membrane traffic in yeast. Annu Rev Cell Biol 1:115–143

Scherrer R, Louden L, Gerhardt P (1974) Porosity of the yeast cell wall and membrane. J Bacteriol 118:534–540

Schreuder MP, Brekelmans S, van den Ende H, Klis FM (1993) Targeting of a heterologous protein to the cell wall of *Saccharomyces cerevisiae*. Yeast 9:399–409

Seiler S, Nargang FE, Steinberg G, Schliwa M (1997) Kinesin is essential for cell morphogenesis and polarized secretion in *Neurospora crassa*. EMBO J 16:3025–3034

Shaw JA, Mol P, Bowers B, Silverman SJ, Valdivieso MH, Duran A, Cabib E (1991) The function of chitin synthase 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J Cell Biol 114:111–123

Shematek EM, Braatz JA, Cabib E (1980) Biosynthesis of the yeast cell wall. I. Preparation and properties of β-(1-3)-glucan synthetase. J Biol Chem 255:888–894

Sietsma JH, Wessels JGH (1977) Chemical analysis of the hyphal wall of *Schizophyllum commune*. Biochim Biophys Acta 496:225–239

Sietsma JH, Wessels JGH (1979) Evidence for covalent linkages between chitin and β-glucan in a fungal wall. J Gen Microbiol 114:99–108

Sietsma JH, Wessels JGH (1981) Solubility of (1-3)-β-D/(1-6)-β-D-glucan in fungal walls: importance of presumed linkages between glucan and chitin. J Gen Microbiol 125:209–212

Sietsma JH, Wessels JGH (1988) Total inhibition of wall synthesis by 2-deoxy-glucose and polyoxin D in protoplasts of *Schizophyllum commune*. Acta Bot Neerl 37:23–29

Sietsma JH, Wessels JGH (1990) The occurrence of glucosaminoglycan in the wall of *Schizosaccharomyces pombe*. J Gen Microbiol 136:2261–2265

Sietsma JH, Sonnenberg ASM, Wessels JGH (1985) Localization by autoradiography of synthesis of (1-3)-β and (1-6)-β linkages in a wall glucan during hyphal growth of *Schizophyllum commune*. J Gen Microbiol 131:1331–1337

Silverman SJ (1989) Similar and different domains of chitin synthase 1 and 2 of *S. cerevisiae*: two isozymes with distinct functions. Yeast 5:459–467

Silverman SJ, Sburlati A, Slater ML, Cabib E (1988) Chitin synthase 2 is essential for septum formation and cell division in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 85:4735–4739

Soll DR, Herman MA, Staebell M (1985) The involvement of cell wall expansion in the two modes of mycelium formation of *Candida albicans*. J Gen Microbiol 128:2667–2674

Sonnenberg ASM, Sietsma JH, Wessels JGH (1982) Biosynthesis of alkali-insoluble cell wall glucan in *Schizophyllum commune* protoplasts. J Gen Microbiol 128:2667–2674

Sonnenberg ASM, Sietsma JH, Wessels JGH (1985) Spatial and temporal differences in the synthesis of (1-3)-β and (1-6)-β linkages in a wall glucan of *Schizophyllum commune*. Exp Mycol 9:141–148

Specht CA, Liu Y, Robbins PW, Bulawa CE, Iartchouk N, Winter KR, Riggle PJ, Rhodes JC, Dodge CL, Culp DW et al. (1996) The *chsD* and *chsE* genes of *Aspergillus nidulans* and their roles in chitin synthesis. Fungal Genet Biol 20:153–167

Staebell M, Soll DR (1985) Temporal and spatial differences in cell wall expansion during bud and mycelium formation in *Candida albicans*. J Gen Microbiol 131:1467–1480

Steinberg (2000) The cellular roles of molecular motors in fungi. Trends Microbiol 8:162–168

Suarit R, Gopal PK, Shepherd MG (1988) Evidence for a glycosidic linkage between chitin and glucan in the cell wall of *Candida albicans*. J Gen Microbiol 134:1723–1730

Szaniszlo PJ, Kang MS, Cabib E (1985) Stimulation of β-(1-3)-glucan synthetase of various fungi by nucleoside triphosphates: generalized regulatory mechanism for cell wall biosynthesis. J Bacteriol 161:1188–1194

Trevithick JR, Metzenberg RL (1966) Genetic alteration of pore size and other properties of the *Neurospora* cell wall. J Bacteriol 92:1016–1020

Trinci APJ, Collinge A (1975) Hyphal wall growth in *Neurospora crassa* and *Geotrichum candida*. J Gen Microbiol 91:355–361

Trinci APJ, Saunders PT (1977) Tip growth of fungal hyphae. J Gen Microbiol 91:355–361

Truesdell GM, Jones C, Holt T, Henderson G, Dickman MB (1999) A Ras protein from a phytopathogenic fungus causes defects in hyphal growth polarity, and induces tumors in mice. Mol Gen Genet 262:46–54

Valdiovieso MH, Duran A, Roncero C (2004) Chitin biosynthesis and morphogenetic processes. In: Brambl R, Marzluf GA (eds) The Mycota, vol III, 2nd edn. Biochemistry and molecular biology. Springer, Berlin Heidelberg New York, pp 275–290

Valdivia RH, Baggot D, Chuang JS, Schekman R (2002) The yeast clathrin adaptor protein complex 1 is required for the efficient retention of a subset of late Golgi membrane proteins. Dev Cell 2:283–294

Van den Hondel CAMJJ, Punt PJ, van Gorcum RFM (1991) Heterologous gene expression in filamentous fungi. In: Bennett JW, Lasure LL (eds) More gene manipulations in fungi. Academic Press, London, pp 396–428

Van Pelt-Heerschap H, Sietsma JH (1990) Analysis of the hyphal wall of *Didymella bryoniae*: composition and sensitivity to hydrolytic enzymes. Mycol Res 94:979–984

Vermeulen CA, Wessels JGH (1983) Phospholipid requirement of chitin synthase in *Schizophyllum commune*. Curr Microbiol 8:67–71

Vermeulen CA, Wessels JGH (1984) Ultrastructural differences between wall apices of growing and non-growing hyphae of *Schizophyllum commune*. Protoplasma 120:123–131

Vermeulen CA, Wessels JGH (1986) Chitin biosynthesis by a fungal membrane preparation. Evidence for a transient non-crystalline state of chitin. Eur J Biochem 158:411–415

Vermeulen CA, Raeven MBJM, Wessels JGH (1979) Localization of chitin synthase in subcellular fractions of *Schizophyllum commune* protoplasts. J Gen Microbiol 114:87–97

Wang MC, Bartnicki-Garcia S (1976) Synthesis of β-1,3-glucan microfibrils by a cell-free extract from *Phytophthora cinnamomi*. Arch Biochem Biophys 175:351–356

Weber I, Gruber C, Steinberg G (2003) A class-V myosin required for mating, hyphal growth and pathogenicity in the dimorphic plant pathogen *Ustilago maydis*. Plant Cell 15:2826–2842

Wessels JGH (1986) Cell wall synthesis in apical hyphal growth. Int Rev Cytol 104:37–79

Wessels JGH (1988) A steady-state model for apical wall growth in fungi. Acta Bot Neerl 37:3–16

Wessels JGH (1990) Role of wall architecture in fungal tip growth generation. In: Heath IB (ed) Tip growth in plant and fungal cells. Academic Press, San Diego, pp 1–29

Wessels JGH (1993) Wall growth, protein excretion and morphogenesis in fungi. New Phytol 123:397–413

Wessels JGH (1999) Fungi in their own right. Fungal Genet Biol 27:134–145

Wessels JGH, Sietsma JH (1981) Fungal cell walls: a survey. In: Tanner W, Loewus FA (eds) Encyclopedia of Plant Physiology, vol 13B. Springer, Berlin Heidelberg New York, pp 352–394

Wessels JGH, Kreger DR, Marchant R, Regensburg BA, de Vries OMH (1972) Chemical and morphological characterization of the hyphal wall surface of the basidiomycete *Schizophyllum commune*. Biochim Biophys Acta 273:346–358

Wessels JGH, Sietsma JH, Sonnenberg ASM (1983) Wall synthesis and assembly during morphogenesis in *Schizophyllum commune*. J Gen Microbiol 129:1599–1605

Wessels JGH, Mol PC, Sietsma JH, Vermeulen CA (1990) Wall structure, wall growth and fungal cell morphogenesis. In: Kuhn PJ, Trinci APJ, Jung MJ, Goosey MW, Copping LG (eds) Biochemistry of cell walls and membranes in fungi. Springer, Berlin Heidelberg New York, pp 81–95

Wösten HAB, Moukha SM, Sietsma JH, Wessels JGH (1991) Localization of growth and secretion of proteins in *Aspergillus niger*. J Gen Microbiol 137:2017–2023

Yarden O, Yanofsky C (1991) Chitin synthase 1 plays a major role in cell wall biogenesis in *Neurospora crassa*. Genes Dev 5:2420–2430

Yarden O, Plamann MD, Ebbole DJ, Yanofsky C (1992) *cot-1*, a gene of *Neurospora crassa* required for hyphal elongation encodes a protein kinase. EMBO J 11:2159–2166

Zhou XL, Stumpf MA, Hoch HC, Kung C (1991) A mechanosensitive channel in whole cells and in membrane patches of the fungus *Uromyces*. Science 253:1415–1417

Ziman M, Chuang JS, Schekman RW (1996) Chs1p and Chs3p, two proteins involved in chitin synthesis, populate a compartment of the *Saccharomyces cerevisiae* endocytotic pathway. Mol Biol Cell 7:1909–1919

Zlotnik H, Fernandes MP, Bowers B, Cabib E (1984) *Saccharomyces cerevisiae* mannoproteins from a external cell wall layer that determines wall porosity. J Bacteriol 159:1018–1026

Zonneveld BJM (1974) α-1,3-glucan synthesis correlated with α-1,3-glucanase synthesis, conidiation and fructification in morphogenetic mutants of *Aspergillus nidulans*. J Gen Microbiol 81:445–451

5 The Fungal Cell Wall

J.P. Latgé[1], R. Calderone[2]

CONTENTS

I. Introduction

A thorough analysis of the biochemical organisation and biogenesis of the fungal cell wall is essential to obtain a good understanding of the growth of fungi in vitro and in their natural environment. All exchanges between the fungal cell and its environment rely upon a functional and permeable cell wall. In plant and mammalian pathogens, the cell wall is continuously in contact with the host, and acts as a sieve and a reservoir for molecules such as enzymes, antigens and elicitors or toxins which play an active role during infection (Mouyna and Latgé 2001). Moreover, it is essential to the resistance of fungi to host defence reactions (Philippe et al. 2003). The cell wall plays an essential role in sensing adverse or favourable environments and, particularly, it provides the fungus with adaptive responses to variable osmotic pressures and other stress factors. Fungi have developed cell wall repair mechanisms which are rapidly activated following damage to this structure (Klis et al. 2002).

Cell wall remodelling continuously occurs during morphogenesis (Fig. 5.1). For example, in moulds like *Aspergillus fumigatus*, the conidium has a double-layered cell wall, the outer layer

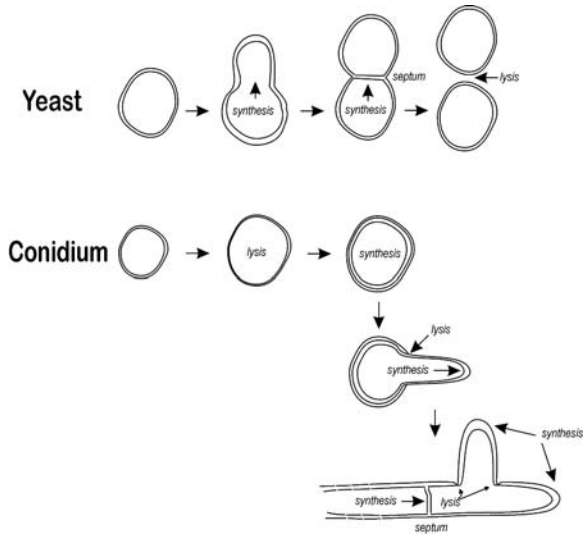

Fig. 5.1. Cell wall modifications (synthesis and lysis) during morphogenesis of yeasts and conidia

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being composed of melanin. During the first stages of germination (swelling), polysaccharide hydrolysis occurs to soften the cell wall, along with the de novo synthesis of a new electron lucent inner layer. Hyphae which emerge have a single mono-layered cell wall. A similar situation exists in yeast, although biosynthetic and lytic sites are differently located (Cabib et al. 2001). Cell wall biosynthesis appears then as a dynamic, essential and timely process which is correlated with growth (Latgé et al. 2005). Polysaccharide components of the cell wall are unique to fungi and, consequently, putative inhibitors of the biosynthetic pathways responsible for cell wall construction are therefore unlikely to have secondary toxic effects, as is the case for some existing anti-fungal drugs such as amphotericin B. The recent initiatives by the pharmaceutical industry with the echinocandins, drugs inhibiting β1,3 glucan synthase, has validated the enormous potential of the cell wall as a source of targets for the development of lead anti-fungal targets (Denning 2003; Walsh et al. 2004). Inhibitors of cell wall polysaccharide synthesis have not been developed in plant pathogenic fungi. However, in these fungi, inhibitors of the synthesis of cell wall pigment are powerful fungicides (Yamaguchi and Kubo 1992).

These reasons are sufficient to investigate the polymer organisation of the fungal cell wall and to characterize proteins/genes involved in the biosynthesis of the constitutive polymers of the cell wall. In this chapter, we will review our current understanding of the structural organisation of the cell wall and of the enzymes responsible for the biosynthesis of cell wall components. This analysis takes into account recent genomic data from the comparison of yeast and mould genome sequences as well as chemical data on the cell wall of these fungi to identify general core pathways common to all fungi.

II. Cell Wall: Composition and Organisation

A. Techniques to Study Cell Wall

The cell wall is a highly insoluble material. Chemical analysis of cell wall components requires first their solubilization. Different techniques, using mostly chemicals, have been used to solubilize cell wall fractions (Fleet 1985, 1991; Perez and Ribas 2004). Numerous protocols are given in the literature, without any comparative efficiency in the qualitative or quantitative recovery of the different constitutive polymers. Although some of the chemical methods are recommended for specific polymer extractions (for example, long *N*-mannans in yeast are easily recovered by Fehling's solution or cetavlon precipitation; Fleet 1985, 1991), no consensus exists today on the best methods to use and their most appropriate sequence to analyse cell walls. In spite of this, the protocol given in Fig. 5.2 has gained the most acceptance for cell wall extraction of most fungi. Sodium hydroxide treatment (sometimes prior or after acetic, hydrofluoric (HF) or hydrochloric acid hydrolysis) is usually needed to initially solubilize cell wall. Further fractionation of the alkali-soluble and insoluble extracts must then be achieved with specific recombinant endo-glycosylhydrolases available to date to cleave all cell wall polysaccharides except mannan chains, which can be cleaved only internally by chemical acetolysis (Latgé et al. 1994). Carbohydrate linkages in the different fractions are then identified using specific carbohydrate chemistry methodologies (Fontaine et al. 2000).

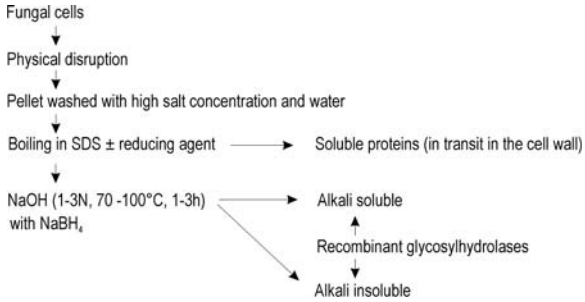

Fig. 5.2. An example of a protocol for the extraction of fungal cell wall components

B. Composition and Fungal Evolution

The major component of the cell wall is polysaccharide, which accounts for > 90% of the cell wall. Three basic components represent the major polysaccharides of the cell wall: glucans, mannans and chitin. Other carbohydrates less frequently found are galactose, galactosamine, glucosamine, xylose, fucose and hexuronic acid. A survey of the composition of the cell wall of all fungal species shows that the study of galactose or D-glucosamine

polymers (chitosan) and glucuronic acid polymers has been neglected, although they have been identified in most taxonomical groups to date. Lipids and proteins have been repeatedly reported during cell wall analysis. However, if proteins have always been considered to be a true component of the cell wall, the "unclonable" lipids have always been regarded as a contaminant. Accordingly, data on cell wall lipids are very scarce, and cell wall lipids are a research area worth revisiting.

The composition of the cell wall has been analysed precisely in very few fungal species (Table 5.1 and references therein). Less than 10 species have been analysed at the cell wall level, and in none of these species has a precise cell wall structure and organisation been given. In spite of this lack of basic knowledge, numerous attempts have been made to correlate the chemical composition of the cell wall with the major taxonomical groups. Such a cell wall taxonomy concept appeared in the 1960s. Since then, numerous reviews have reproduced and amended the table of Bartnicki-Garcia (1968), without any significant progress in the area. A comparative analysis of evolutionary trees, based on nuclear rDNA fitted to the geological time scale, indicate that the presence or absence of a cell wall component is often (but not always) associated with taxonomical clustering and fungal evolution (Fig. 5.3). The presence of an extracellular chitin matrix supports a common ancestor of fungi and animals which existed 1 billion years ago (Berbee and Taylor 2001). Associated with the segregation from the animal clade was the emergence of β1,3 glucan in the cell wall, probably as an "extracellular glycogen". The cell wall of *Blastocladiella emersonii* and of various species of *Allomyces*, belonging to the Chytridiomycetes considered distant fungal

Table. 5.1. Main discriminative carbohydrates in the cell wall composition of fungal vegetative stages (mycelium or yeast)

Main components	Chytridiomycetes		Zygomycetes		Basidiomycetes		Ascomycetes			
	Neocallimastigales + Basidiobolus[a]	Blastocladiales[b]	Mucorales[c]	Entomophthorales[d]	Mushrooms[e]	*Cryptococcus*, *Ustilago*[f]	Basal ascomycetes, *Pneumocystis*[g]	*S. pombe*[h]	*Yeasts*[i]	Filamentous ascomycetes[j]
Chitin	+	+	+	+	+	+	+	−	+	+
β1,3 glucan	+	+	−	+	+	+	+	+	+	+
α1,3 glucan	−	−	−	−	+	+	−	+	+	+
Other carbohydrates			++; chitosan, acidic polysaccharides		Xylose	Xylose		β1,6 glucan	β1,6 glucan	Galactofuranose

[a] Hoddinott and Olsen (1972), Orpin (1977)
[b] *Blastocladiella*, *Allomyces*: Skucas (1967), Kroh et al. (1977), Sikkema and Lovett (1984)
[c] Bartnicki-Garcia and Reyes (1968), Miyazaki and Irino (1970, 1971), Datema et al. (1977a,b), Tominaga and Tsujisaka (1981)
[d] Latgé et al. (1984), Latgé and Beauvais (1987)
[e] *Coprinus*, *Schizophyllum*, . . .: Michalenko et al. (1976), Schaefer (1977), Sietsma and Wessels (1977), Bottom and Siehr (1980)
[f] James et al. (1990), Fleet (1991), Cherniak et al. (1993), Ruiz-Herrera et al. (1996), Vaishnav et al. (1998)
[g] Walker et al. (1990), Nollstadt et al. (1994), M. Cushion (personal communication)
[h] Manners and Meyer (1977), Kopecka et al. (1995), Humbel et al. (2001), Perez and Ribas (2004), Sugawara et al. (2004), Grün et al. (2005)
[i] *C. albicans*: Chaffin et al. (1998), Lowman et al. (2003), Masuoka (2004); *S. cerevisiae*: Manners et al. (1973a,b), Fleet and Manners (1976, 1977), Fleet (1985, 1991), Klis (1994), Kollar et al. (1995, 1997). Other yeasts: Fleet (1985), Fukazawa et al. (1995), Nguyen et al. (1998), Bahmed et al. (2003)
[j] *Aspergillus*: Bull (1970), Zonnenveld (1971), Fontaine et al. (2000), Bernard and Latgé (2001); *Fusarium*: Bruneteau et al. (1992), Jikibara et al. (1992), Barbosa and Kemmelmeier (1993), Fukamizo et al. (1996), Schoffelmeer et al. (1999); *Neurospora*: Hiura et al. (1983), da Silva et al. (1994), Leal et al. (1996); *Penicillium*: Troy and Koffler (1969); *Trichophyton*: Reiss (1986)

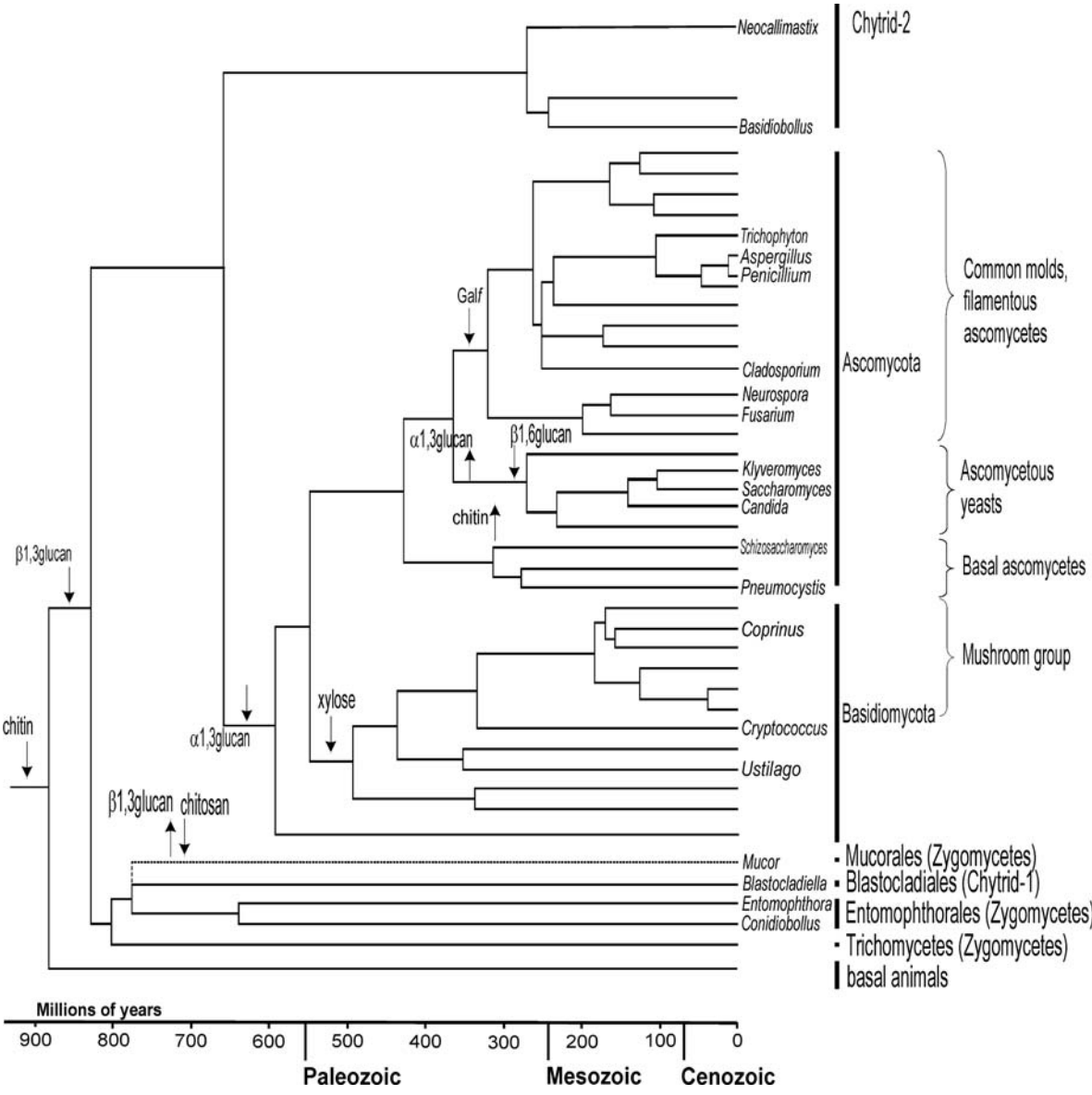

Fig. 5.3. Emergence of polysaccharides in the fungal cell wall based upon taxonomical clustering and fungal evolution fitted to the geological time scale. *Branch lengths* are proportional to the average nucleotide substitution of nuclear SSU rDNA sequences (adapted from and with J. Taylor; *arrow* ↓ polysaccharide emerging, *arrow* ↑ polysaccharide lost)

ancestors, contains both glucan and chitin, the later being present in the highest amount among all fungi. These two polysaccharides represent the first in the neosynthesis of a cell wall in the fungal kingdom, as seen especially in this class of fungi which exists mostly without a wall. Cell wall composition evolves from this central core either positively or negatively. For example, an evolutionary dead end seen in one branch of the Zygomycetes, the Mucorales, is the loss of the β1,3 glucan and the occurrence of deacetylated chitin and polymers of uronic acid. Modifications in other fungal orders consisted mainly of a more or less complex decoration of this β glucan–chitin skeleton. Some essential steps in the evolution are the appearance of α1,3 glucans both in the Ascomycetes and Basidiomycetes. Pentoses are the hallmark of Basidiomycetes. Galactofuranose seems to be present only amongst the most recently evolved fungi, the higher Ascomycetes.

Whereas the structural branched β1,3 glucan and chitin have been stable since the origin of fungi, the composition of other polysaccharides such as mannan has evolved continuously over time. For example, if short mannan chains were part of the protein *N*-glycan in ancient fungi, these mannan chains evolved over time to become a non-covalent coat on the yeast cell surface, and then a constitutive component of the cell wall in filamentous Ascomycetes (Chaffin et al. 1998; Fontaine et al. 2000; Masuoka 2004).

If qualitative differences are seen between species, quantitative differences have been also noticed. For example, both yeasts and moulds have chitin in their cell wall, but the amount of chitin in the mould mycelial cell wall is much higher than in yeast. This result is in agreement with the shape of the two fungi: more beams are necessary to support the structure of a tube such as a mycelium than that of a balloon such as yeast. Accordingly, since chitin is thought to be responsible for holding together the cell wall structure, higher amounts of chitin are expected in mycelial fungi. In dimorphic fungi, such as *Candida albicans*, hyphae contain more chitin than is the case for yeast (Chaffin et al. 1998). The number of components of yeast cell walls seems less than that in filamentous fungi: in *Saccharomyces cerevisiae* and the Hemiascomycetous yeasts, no α1,3 glucans are present whereas in *Schizosaccharomyces pombe* no chitin is found. Variations in composition are also seen in different fungal stages of one and the same species, such as spore and vegetative mycelium, suggesting a tight regulation of expression during the cell cycle. For example, in the Mucorales, glucan is present in the spore stage and absent from the mycelium (Bartnicki-Garcia 1968). Chitin is present in ascospores and absent from the yeast cell wall of *S. pombe* (Perez and Ribas 2004). Chitosan is the hallmark molecule of the ascospore cell wall of *S. cerevisiae* and is absent in yeast cells (Coluccio et al. 2004). Melanin covers the outer layer of most conidia of the Ascomycetes but hyphae are hyaline (Latgé et al. 1988, 2005).

C. Structural Organisation of the Cell Wall

Although significant variations occur in the composition of the cell wall of different species, a general scheme can be established, at least for the Ascomycetes and Basidiomycetes, which represents the vast majority of all fungi on earth. The fibrillar skeleton of the cell wall is considered to be the alkali-insoluble fraction, whereas the material in which the fibrils are embedded is alkali-soluble. It should be stressed that the linkages disturbed by the alkali treatment have not been identified yet. Figure 5.4 is an example of the polysaccharide composition of *Aspergillus* and *Saccharomyces*, which could be used to represent the putative schematic organisation of the cell wall of yeasts and moulds.

The central core of the cell wall is a branched β1,3/1,6 glucan which is linked to chitin via a β1,4 linkage; 3 and 4% β1,6 glucosidic interchain linkages have been described in *S. cerevisiae* and *A. fumigatus* respectively (Manners et al. 1973a,b; Fleet 1985; Fontaine et al. 2000). This core is present in most fungi and at least in all Ascomycetes and Basidiomycetes, but is differently decorated depending on the fungal species. In *A. fumigatus*, it is covalently bound to a linear β1,3/1,4 glucan with a [3Glcβ1-4Glcβ1] repeating unit, and a branched galactomannan composed of a linear αmannan with a repeating mannose oligosaccharide unit [6Manα1-2Manα1-2Manα1-2Manα] and short chains of β1,5 galactofuranose residues (Fontaine et al. 2000). In *S. cerevisiae*, the structure of the alkali-insoluble fraction has not been totally elucidated, but the data available suggest that in addition to chitin, β1,6 glucan is bound to the branched βglucans.

It has been known since the pioneering studies of Sietsma and Wessels (1979, 1981) that the cross-linking between β1,3 glucan and chitin is essential for the formation of a resistant fibrillar skeletal component in most Ascomycetes and Basidiomycetes. Identification of the linkage between β1,3 glucan and chitin was done later by the group of E. Cabib in *S. cerevisiae*, and confirmed in *Aspergillus fumigatus* (Kollar et al. 1995; Fontaine et al. 2000). The terminal reducing end of the chitin chain is attached to the non-reducing end of a β1,3 glucan chain by a β1,4 linkage. Older studies in *Candida albicans* have suggested that chitin and β1,3 glucan can be also linked through a glycosidic linkage at position 6 of GlcNAc (Surarit et al. 1988). The presence of chitin is, however, not always required for an organised cell wall, as seen in *S. pombe*. In the latter species, the alkali-insoluble fraction is composed of linear β1,3 glucan bound to a highly branched β1,6 glucan with β1,3 linked glucosyl branches at almost every glucose residue and α1,3 glucan chains which are too long to be solubilized by alkali (Sugawara et al. 2004).

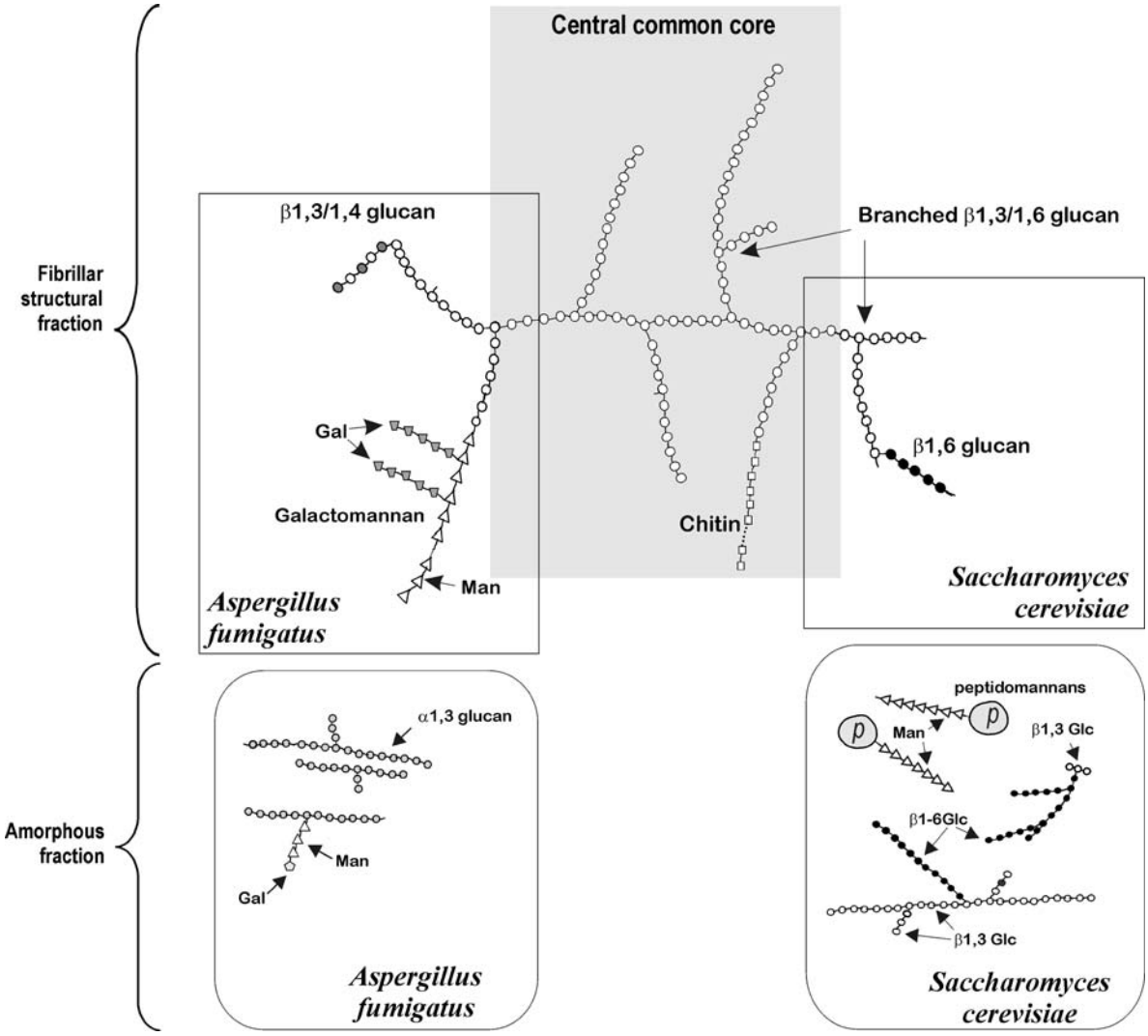

Fig. 5.4. The cell wall polysaccharides of *Saccharomyces cerevisiae* and *Aspergillus fumigatus*, showing a central core of β1,3 glucan-chitin which is common to both organisms

The fibrillar, alkali-insoluble glucan complex is embedded in an alkali-soluble amorphous cement whose composition varies with fungal species, and has been accordingly less studied. Moreover, the alkali solubility of the extracted fraction is not associated with a specific polysaccharide composition. In *Aspergillus* and *Schizosaccharomyces*, it is composed of α1,3/1,4 glucan and galactomannan with galactopyranose in *S. pombe* or galactofuranose in *A. fumigatus* (Bobbitt and Nordin 1978; Beauvais et al. 2004; Sugawara et al. 2004; Grün et al. 2005; Morelle et al., unpublished data). By contrast, in *S. cerevisiae*, it is composed of complex mannans and branched β1,3/1,6 glucan with β1,6 glucan side chains similar in organisation to that in the alkali-insoluble fraction and without cross-linking to chitin (Manners et al. 1973a,b; Hartland et al. 1994).

Proteins are also part of the alkali-soluble fraction, since they are solubilized and destroyed by alkali. In contrast to polysaccharides which are the major component of the cell wall, proteins are present in minute amounts only. Cell wall proteins are either in transit in the cell wall before being secreted or part of the cell wall structure: S-S proteins are released by reducing agents, GPI-CWPs by hydrofluorhydric acid, and PIR proteins (*p*rotein with *i*nternal *r*epeats) by 30 mM sodium hydroxide (Fleet 1991; Kapteyn et al. 1997, 2000; Moukadiri and Zueco 2001; Castillo et al. 2003). However,

their structural role in the cell wall remains controversial. In yeast, several studies have reported that numerous proteins originally anchored to the plasma membrane by a glycosylphosphatidyl inositol (GPI) anchor subsequently become covalently linked to β1,3 glucans through a β1,6 glucan linker (Kapteyn et al. 1995, 1996; Kollar et al. 1997). The putative localisation of the GPI-anchored proteins of *S. cerevisiae* in either the membrane or the cell wall has been investigated initially by Caro et al. (1997) and Hamada et al. (1998), and confirmed recently in *Candida glabrata* by Frieman and Cormack (2004). About half of the *S. cerevisiae* GPI proteins analysed were thought to be covalently bound to the cell wall. These data led the group of Klis to develop a model where the cell wall proteins would have an important role in the cross-linking of the different cell wall polysaccharides (Smits et al. 1999; Klis et al. 2002). However, the same research team showed that disruption of genes coding for the major GPI anchor proteins bound to cell wall did not affect growth, suggesting that these proteins were not essential for fungal growth (van der Vaart et al. 1995). Similarly, disruption of the PIR genes did not result in mutants with altered growth (Mrsa and Tanner 1999). Moreover, if the mannoproteins account for 40%–50% of the cell wall dry weight (Klis 1994), and if polypeptides have an average Mr of 30–60 kDa with 150 mannose unit per glycoprotein, this means that the proteins should account for 10%–20% of the cell wall dry weight. Such an amount of protein has never been found in the cell wall after SDS treatment, suggesting that the vast majority of the cell wall-associated proteins is not covalently bound. In addition, other fungi such as *A. fumigatus* do not have proteins covalently bound to the polysaccharides of the mycelial cell wall (Bernard et al. 2002). In *A. fumigatus*, some of the proteins, such as PhoAp, are tightly bound to the cell wall in the absence of covalent linkages, and can be released only as a soluble protein by a β1,3 glucanase treatment which loosens the polysaccharide net to which this protein is associated (Bernard et al. 2002; Latgé et al. 2005). Similarly, some of the PIR genes code for proteins which do not have a cell wall localisation to fulfil their biological function, although they require a mild alkali treatment to be released from the cell wall (Vongsamphanh et al. 2001). Strong non-covalent bonds could indeed occur for several putative cell wall-bound proteins in yeast, since the identification of the chemical linkages between the protein and the polysaccharide (the ultimate proof of such covalent linkages) has been assessed only for the GPI-anchored protein TIP1 and one unknown protein in *S. cerevisiae* (Kollar et al. 1997; Fuji et al. 1999). These data show also that the postulated "release by β1,3 glucanase equals covalent linkage to the cell wall polysaccharide" is evidently not always true. This chemical analysis of the cell wall proteins in *A. fumigatus* was confirmed by a recent comparative genomic analysis of the genes coding for GPI-anchored proteins. Among the GPI proteins common to *S. cerevisiae* and *A. fumigatus*, only six families of GPI proteins in *A. fumigatus* were orthologues of yeast GPI proteins: SPS2, GAS/GEL, DFG, PLB, CRH and YPS (Merkel et al. 1999; Olsen et al. 1999; Mouyna et al. 2000a; Rodriguez-Pena et al. 2000; Bernard et al. 2002; Kitagaki et al. 2002, 2004; Tougan et al. 2002; Spreghini et al. 2003; Coluccio et al. 2004; Eisenhaber et al. 2004). Five of these families were classified as membrane-bound GPI proteins in yeast (Caro et al. 1997; Hamada et al. 1998; de Groot et al. 2003), and the covalent association of the sixth one, CRH, with the cell wall could be questioned, since this family has sequence signatures suggesting a β1,3 glucanase activity (Rodriguez-Pena et al. 2000). This genomic analysis is in agreement with a proteome study of membrane GPI-anchored proteins of *A. fumigatus* (Bruneau et al. 2001). Four of the families mentioned above (SPS2, CRH, GEL/GAS and DFG) have been shown to be associated with cell wall construction, some of them having enzymatic activities such as β1,3 glucanosyltransferases (Mouyna et al. 2000a,b; Rodriguez-Pena et al. 2000; Kitagaki et al. 2002, 2004; Tougan et al. 2002; Spreghini et al. 2003) which do not require any covalent linking to the cell wall polysaccharides. By contrast, all the polysaccharide-covalently bound proteins in yeast such as Flo1p, Fig1p or Aga1p for *S. cerevisiae* or Als1p and Epa1p in *Candida albicans* and *Candida glabrata* are involved in cell–cell adhesion, mating, or adhesion to host cell surfaces (Frieman and Cormak 2004; Verstrepen et al. 2004). The covalent binding of proteins to polysaccharides is a way for the protein to remain at the surface of the cell wall where it has to bind directly to its ligand to fulfil a biological function. No genes coding for PIR proteins are found in the genome of *A. fumigatus*. The comparative chemogenomic data mentioned above suggest that proteins do not have the role of linkers between cell wall polysaccharides often proposed for the establishment of the three-dimensional polysaccharide network of the yeast cell wall (Kapteyn et al. 1999; Smits et al. 1999).

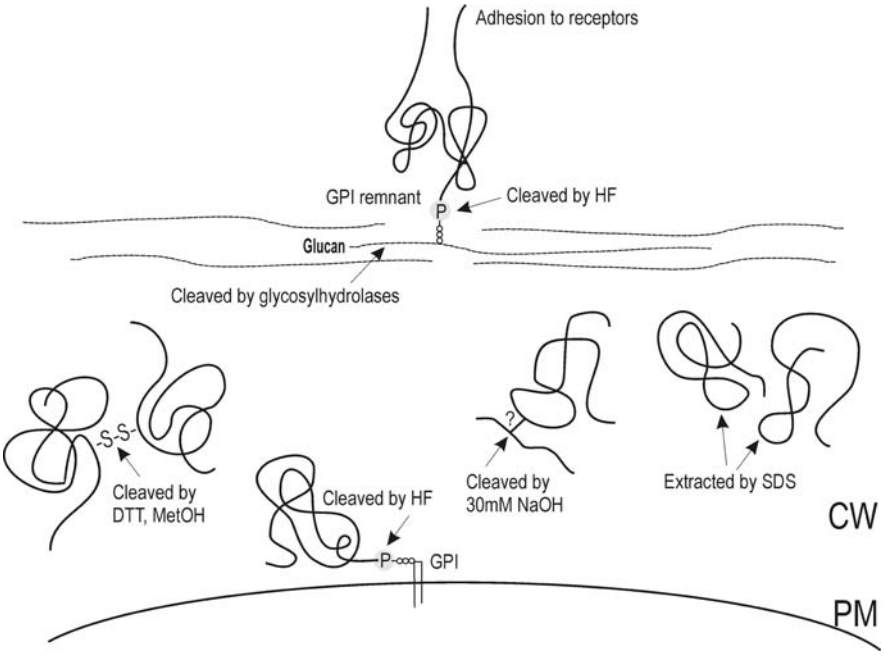

Fig. 5.5. Putative organisation of fungal cell wall proteins. GPI (glycosylphosphatidyl inositol) proteins anchored to polysaccharides or membranes are extracted by hydrofluoric acid (HF). GPI proteins on the surface are involved in cell–cell adhesion. Proteins extracted by dilute alkali are bound to the cell wall by unknown linkages. SDS-soluble proteins are secreted proteins in transit to the cell wall. Proteins extracted by reducing agents are linked by S–S bridges

A schematic view summarizing the localisation of the proteins in the yeast and mould cell wall is shown in Fig. 5.5.

III. Polysaccharide Biosynthesis

A. Chitin

1. Chitin Synthases

As mentioned above, chitin is an essential and the most ancestral structural polysaccharide in the cell wall. A family of integral membrane proteins with Mr of 100–130 kDa, called chitin synthases, are responsible for the synthesis of linear chains of β1,4 *N*-acetylglucosamine from the substrate UDP-*N*-acetylglucosamine (Valdivieso et al. 1997; Cabib et al. 2001; Munro and Gow 2001; Roncero 2002).

The unique and essential Leloir pathway leads to the synthesis of UDP-GlcNAc. All enzymes of this pathway are essential in yeast. The first enzyme in this pathway is the glucosamine-6-phosphate synthase (Gfa1p) which condenses fructose-6-phosphate and glutamine to produce glucosamine-6-phosphate and glutamate. It is considered to be a limiting factor in chitin synthesis in yeast (Lagorce et al. 2002). This enzyme has been found in all fungi sequenced to date. Alternatively, D-glucosamine-6-P and *N*-acetylglucosamine 6-P can be produced directly from D-glucosamine and *N*-acetylglucosamine. When added extracellularly, these aminosugars stimulate chitin synthesis (Bulik et al. 2003). It has never been investigated if the presence of > 15 chitinase and chitosanase genes in the genome of *A. fumigatus* and other moulds allow them to provide an intracellular source of glucosamine and *N*-acetylglucosamine for the synthesis of their own chitin (Latgé et al. 2005).

Six families of chitin synthases have been identified among fungi based on amino acid sequence analysis, among which three are specific for filamentous fungi (classes III, V and VI; Bowen et al. 1992). The significance of each of these six classes is not well understood, since mutations in members of a common family do not always result in a similar phenotype. Two groups of mutants can, however, be identified: the first has reduced chitin content but normal chitin synthase activity in vitro whereas the second group is affected in enzyme activity but has regular cell wall chitin content. The various CHS genes are usually not redundant but instead perform distinct and specific functions, even though they have very homologous sequences. A motif QRRRW present in all CHS genes from different fungi has been proposed as a catalytic domain, since mutation in this domain results in a loss of chitin synthase activity (Nagahashi et al. 1995; Cos et al. 1998). Analysis of the common domains among the

eight chitin synthases of *A. fumigatus* has shown two clusters of three and four genes and a singleton which are associated with chitin synthesis and chitin synthase activities respectively (Latgé et al. 2005). A bioinformatic analysis of these two chitin synthase families in *A. fumigatus* showed that most of the motifs are found in both families but their localisation in each family is different, suggesting that the binding domain of the UDP GlcNac will be the same and that the overall organisation of the domains inside each protein would lead to different outcomes (Latgé et al. 2005). This result fits also with the differential sensitivity of the three yeast CHS genes to nikkomycin X and Z and polyoxin D, suggesting that the active-site domains of the different CHS proteins differ appreciably (Cabib 1991).

Chitin biosynthesis is understood best in the model yeast *S. cerevisiae*. Three chitin synthases are responsible for the synthesis of *S. cerevisiae* chitin (Fig. 5.6). Chs1p acts as a repair enzyme during cell separation. It is a fairly stable protein (Choi et al. 1994), and its levels do not change significantly during the cell cycle (Ziman et al. 1996).

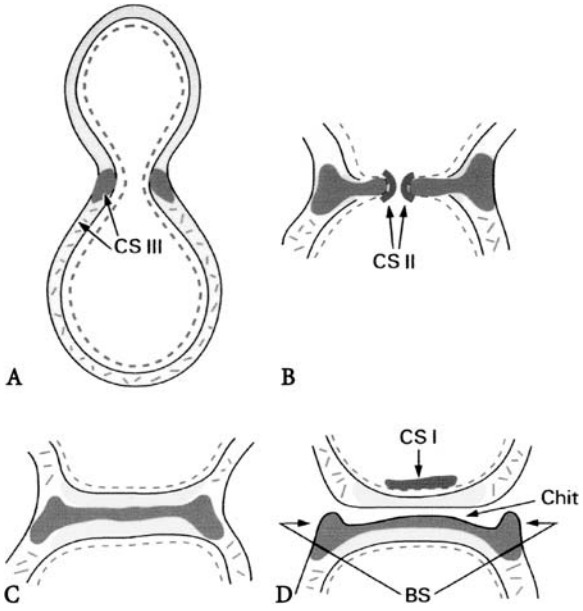

Fig. 5.6. Chitin synthesis in *S. cerevisiae* and the function of the different synthase genes (adapted from Cabib et al. 2001) Chitin is indicated by *shading*. **A** Chitin synthase 3 (*CSIII*) catalyses the synthesis of chitin in the entire cell wall and at the chitin ring. **B** Chitin synthase 2 (*CSII*) catalyses the synthesis of the primary septum chitin. **C, D** Completion of the septum and separation of the daughter cell by a chitinase (*Chit*) with extra chitin provided by chitin synthase 1 (*CSI*). *BS* Bud scar

Chs2p is responsible for septal chitin biosynthesis. Like Chs1p, Chs2p activity was originally described as zymogenic, but again there is no direct evidence for this type of regulation in vivo. Chs2p activity peaks just before cytokinesis (Choi et al. 1994). Expression of this gene is strongly reduced during mating and sporulation, two conditions in which no primary septum is formed (Choi et al. 1994). Chs2p is thought to be transported to the septum site by the general secretory pathway, where it acts in the formation of the primary septum (Shaw et al. 1991). Its function depends directly on the formation of the actomyosin ring (Schmidt et al. 2002). The non-zymogenic Chs3p is involved in the synthesis of bulk chitin of the cell wall of the mother cell and of the septum, and for the synthesis of chitin as a response to cell wall stress. Chs3p levels remain fairly constant inside the cell, mainly because of a very extended protein half-life (Ziman et al. 1996; Roncero 2002; Fig. 5.6).

In *S. cerevisiae*, none of the CHS genes are essential, although the cell wall of the triple mutant is very thick, and its survival results from the acquisition of suppressors (Schmidt 2004). This is in contrast with *C. albicans* where the CHS family comprises four genes, *CHS1* (class II), *CHS2* (class I), *CHS3* (class IV) and *CHS8* (class I), while the class II *CHS1* is essential for cell viability (Munro and Gow 2001; Munro et al. 2001).

The largest gene families of chitin synthases are found in filamentous fungi, with up to 11 genes in *Aspergillus oryzae*. The presence of the highest number of genes in filamentous Ascomycetes is correlated with a higher chitin content in the mould cell wall. In *A. fumigatus*, eight genes have been identified, among which six were inactivated. Mutants with the most altered phenotype result only from inactivation of *CHSE* and *G* genes belonging to classes III and V (Mellado et al. 1996; Aufauvre-Brown et al. 1997). The phenotypes of these mutants include a reduction in hyphal growth, periodic swellings along the length of hyphae, and a block of conidiation which is partially restored by growth in the presence of an osmotic stabilizer. A double *CHSE/CHSG* disruptant has been obtained, and the phenotype of the double mutant is only additive (Mellado et al. 2003): the cell wall still contains chitin (half of the concentration of the parental strain) and the mutant still displays zymogenic and non-zymogenic chitin synthase activities. Although the understanding of the chitin synthases in filamentous fungi remains very incomplete, cellular specialization for the different

CHSs does exist in moulds. For example, some of the CHS genes are specialized for conidiation. It has been found indeed that at least three of the five *A. nidulans* chitin synthase genes tested are regulated by *ABAA*, a transcriptional regulator of conidiation in *Aspergillus* (Park et al. 2003).

In *Wangiella dermatitidis*, mutations in chitin synthases genes led to very different phenotypes (Wang et al. 1999, 2002). Disruption of *WdCHS1* class II genes produces strains which form short chains of yeast cells. The single *WdCHS2* (class I) and *WdCHS3* (class III) mutants show no obvious phenotype. The class IV *WdChs*4 mutant is the only *chs* mutant in this organism which has a statistically significant reduction in chitin content. In addition, the *WdCHS4* mutant is hyperpigmented with melanin, and forms aggregates of yeasts. Mutants in the *WdCHS5* gene also have increased melanin, and reduced viability in late log and early stationary phases of growth.

Although no chitin has been found in *S. pombe* yeast cell walls (Perez and Ribas 2004), *CHS1* and *CHS2* and chitin synthase activity have been detected in this yeast. Chs1p is necessary for maturation of the spore wall whereas Chs2p is related to septum formation (Arellano et al. 2000). Complementing *S. cerevisiae chs* mutants with *CHS* genes from *S. pombe* has been achieved by Matsuo et al. (2004) but not by Martin-Garcia et al. (2003).

Even though CHS enzymes have been carefully analysed at the genomic and cellular levels, the biochemical activity of these enzymes remains poorly analysed. The reasons for proteolytic activation of some chitin synthases are unknown, as is the occurrence of such a phenomenon in vivo.

2. Regulation of Chitin Synthesis

The regulation of chitin synthesis in fungi is also far from being completely understood and has been studied only in yeast. No genes directly involved in the control of chitin synthase activity I and II have been described. Four yeast genes *CHS4-7* which differ at the sequence level from the catalytic chitin synthases are involved in the regulation of chitin synthase III activity (Roncero 2002; Fig. 5.7).

Chs7p acts as a specific chaperone for Chs3p, allowing its sorting from the ER. In the absence of this protein, Chs3p accumulates in the ER, producing an inactive protein both in vivo and in vitro (Trilla et al. 1999). Chs7p is unique in the *S. cerevisiae* genome, and close homologues have been described in *C. albicans*, *A. fumigatus* and *Neurospora crassa*.

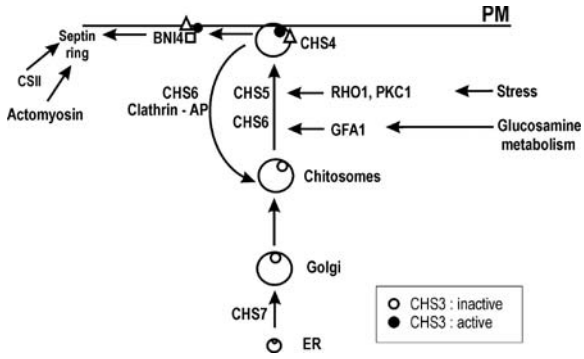

Fig. 5.7. The regulation of chitin synthesis, including both putative and experimental data. CHS3 requires CHS7 to exit from the ER, and CHS5 and CHS6 to exit from the Golgi. Inactive and phosphorylated CHS3 is transported from the endoplasmic reticulum (*ER*) to the plasma membrane (*PM*) where it is dephosphorylated and activated by CHS4. At the PM, CHS3 can be also associated with actin/myosin and septins to direct the synthesis at the chitin septum ring. More than 50% of CHS3 is stored inactive in early endosome compartments (chitosomes), and is recruited from the chitosomes during cell wall stress or glucosamine nutrition. Retrograde transport of CHS3 is regulated by CHS6 or by the clattering AP1 complex to be stored as an intracellular inactive pool of CHS3 which is immediately available to the cell when needed (protein names are in *capital letters*)

Chs5p and Chs6p are Golgi proteins required for the correct sorting of Chs3p to the membrane (Santos et al. 1997; Santos and Snyder 1997; Ziman et al. 1998; Valdivia et al. 2002). These two proteins are essential for the regulation of CSIII activity: Chs5p is responsible for the transport of Chs3p inside chitosomes (early endosomes) to the membrane where it becomes activated. Chs6p is associated with the retrograde endocytosis of Chs3p which is maintained dormant in chitosomes. This pathway regulates the activity without degradation of the enzyme. Although the function and localisation of Chs5p and Chs6p seem to be very similar, close homologues of the *ScCHS5*, but not *CHS6* genes have been found in *C. albicans*, *A. fumigatus* and *N. crassa*. These data suggest that the function of the *CHS6* gene may not be associated directly with chitin synthesis, since other homologues of Chsp such as YKR027p are not involved in chitin synthesis (Roncero 2002). In moulds, the retrograde activity could be under the control of the clathrin-associated AP-1 proteins which have been shown to have a role in yeasts and have orthologues in moulds (Valdivia and Scheckman 2003).

Characterization of the *chs4* mutant indicated that it produces functional but highly zymogenic chitin synthase III activity (Choi et al. 1994; Trilla et al. 1997). These results suggest that Chs4p could be a direct activator of chitin synthase III activity at the membrane site. *CHS4* was also identified in a synthetic lethality screening with the Cdc12p septin, and was shown to interact with Cdc10p septin through an anchor protein named Bni4p, which plays a more general role in the assembly of the septum machinery (DeMarini et al. 1997; see also Wendland and Walther, Chap. 6, this volume). The current model indicates that a complex containing Chs3p/Chs4p is positioned at the septum site through its interaction with the Bni4p/septin complex (DeMarini et al. 1997; Sanz et al. 2004). In *Aspergillus fumigatus*, where no homologues of Bni4p are found, localisation of CHS at the septum may be under a different regulation. One way for *Aspergillus* to bypass this mechanism is via a myosin–chitin linkage different to that in yeasts: at least one gene in the CHS families of moulds has a consensus domain which is homologous to kinesin or myosin motor-like domains (Horiuchi et al. 1999; Takeshita et al. 2002; Latgé et al. 2005). The product of the *CHS4* gene is required for chitin synthesis during mating but not for proper sporulation, because *chs4* mutants form completely mature ascospores (Trilla et al. 1997). *S. cerevisiae* contains a *CHS4* homologue (*SHC1*) which is required for the synthesis of the ascospore's chitosan layer, and therefore for spore maturation (Sanz et al. 2002). Both genes are functionally redundant in activating CSIII, but Shc1p lacks the localisation domain which allows Chs4p to direct Chs3p to the septum. *A. fumigatus* and *N. crassa* contain three *CHS4* homologues but their differential regulation, for example, during sporulation, has not been investigated. *C. albicans* contains only one *CHS4*-like gene, possibly related to the lack of sporulation in this organism (Roncero 2002).

3. Inhibition

The peptide nucleoside antibiotics polyoxins and nikkomycins are strong competitive inhibitors of chitin synthase and are substrate analogues of UDP-*N*-GlcNAc (Gaughran et al. 1994; Munro and Gow 2001). Polyoxin and nikkomycin are highly active in vitro but only poorly active in vivo. Reasons for this are the following: (1) as peptidyl nucleosides, these compounds are susceptible to cleavage by peptidases of microbial or host origin, and (2) nikkomycins/polyoxins must traverse the cell membrane and be transported to the active site of chitin synthase, and are poorly accumulated by fungal pathogen. Two new amphipatic compounds without peptide bonds, and with a well-defined hydrophobic domain facilitating the crossing of the hydrophobic cell membrane, have been recently described: they are a series of novel nikkomycin analogues with a hydrophobic group at the terminal amino acid (Obi et al. 2000), and a new class of inhibitors with structures differing markedly from those of the polyoxins and nikkomycins (Masubuchi et al. 2000). In spite of high activities in vitro, none of the inhibitors of chitin synthesis have been launched yet in clinical practice.

B. Glucan Synthesis

1. β1,3 Glucan Synthases

a) Synthesis

β1,3 glucans are synthesised by a plasma membrane-bound glucan synthase complex which uses UDP-glucose as a substrate, and extrudes linear β1,3 glucan chains through the membrane into the periplasmic space (Douglas 2001). The protein complex contains at least two proteins, a putative catalytic subunit encoded by the genes *FKS1* with a Mr > 200 kDa and a regulatory subunit encoded by *RH01* with an ~20 kDa Mr. The reaction adds one mole of glucose for every mole of UDP-glucose hydrolysed to produce chains of increasing length. The polysaccharide produced in the reaction was estimated to be 60–80 glucose residues long in yeasts. In *A. fumigatus*, 1500 glucose residues are produced per chain (Beauvais et al. 2001).

UDP-glucose, the substrate of β1,3 glucan synthase (GS), is synthesised from hexose phosphate precursors: glucose 6-P is utilised by the phosphoglucomutase to produce glucose 1P which is then transformed to UDP-glucose by a uridyltransferase. Genes of this pathway are present in all genomes sequenced to date and, in addition, multiple alternative pathways can be used by fungi to produce intracellularly the β-glucan synthase substrate (http://www.genome.jp/kegg/pathway/map/map00520.html). The UDP-glucose is then transported to the plasma membrane (Munoz et al. 1996; Castro et al. 1999).

In *S. cerevisiae*, three *FKS* genes exist of which only two are involved in β1,3 glucan synthesis

(Lesage et al. 2004); the third one being a putative transporter (H. Bussey, personal communication). Neither the single *FKS1* or *FKS2* disruptions was lethal. In contrast to yeasts, all filamentous Ascomycete moulds sequenced to date have one *FKS* orthologue. In *A. fumigatus*, *FKS1* is unique and essential, as shown in a diploid background after haploidization following transformation (Firon et al. 2002) or by RNAi (Mouyna et al. 2004). In *S. pombe*, four genes *BGS1-4* sharing homology with β1,3-GS catalytic subunits have been identified. Of these, Cps1p/Bgs1p is an essential gene presumably involved in the assembly of the septum β1,3 glucan (Ishiguro et al. 1997). Bgs1p is localised to the cell division site in a manner dependent on the actomyosin ring and the septation-inducing network (Liu et al. 1999). In addition, Bgs1p has been localised to the site of growth (Cortes et al. 2002; Liu et al. 2002). Bgs2p is a sporulation-specific GS required for proper ascospore wall maturation (Liu et al. 2000; Martin et al. 2000). *bgs2*Δ sporulating diploids show a defect in GS activity and fail to assemble the cell wall properly, resulting in a failure to develop viable ascospores. *bgs3* and *bgs 4* are essential for cell growth (Martin et al. 2003; Ribas, unpublished data). Bgs3p cannot be substituted by overproduction of any of the other GS homologues, suggesting a unique role for Bgs3p in the biosynthesis of β1,3 glucans where new cell wall deposition is necessary. Like Bgs3p, Bgs4p localises to the growing pole and septum. Although β1,3-GS isoforms perform a distinct function, it is also possible that all these isoforms have overlapping roles in cell wall assembly, as in *S. cerevisiae*.

Genome surveys show that the *FKS* genes are reasonably well conserved across all fungal genera. Homologues of *FKS1* have been found in all fungi. Hydropathy analysis of the large protein encoded by all FKS family members predicts a localisation within the plasma membrane, with as many as 16 transmembrane helices. A central hydrophilic domain of about 580 amino acids displays a remarkable degree of identity (> 80%) among all known FKS protein sequences (Douglas 2001). It has been proposed that this region is located on the cytoplasmic face of the plasma membrane and must have some essential, conserved function. Two aspartate residues at positions D392 and D441 in this region have been recognized as essential for function of the glucan synthase (Douglas 2001). If members of the Fks family of proteins provide the catalytic centre of β1,3 glucan synthase, then they represent a significant divergence from known glycosyltransferases which use a nucleotide-diphospho (NDP)-sugar as a substrate. Neither of two proposed UDP-glucose binding sites, (R/K)XGG implicated from glycogen synthases and D,D,D35QXXRW from hydrophobic cluster analysis, is found in Fks1p (Douglas 2001). The gaps in our knowledge of the β1,3-glucan synthase mechanism raise many questions. In the absence of purification to homogeneity of the enzyme, the first provocative question is whether Fks1p is the true catalytic subunit of the glucan synthase or only one of the members of the glucan synthase complex. Other key questions are similar to the ones which can be asked for chitin synthesis: can synthesis be initiated de novo, or does it require a glucan acceptor? Does polymerisation proceed from the non-reducing end of the growing chain? Are glucose residues added as monomers or as disaccharide units?

b) Regulation

Rho1p-GTPase, which controls β1,3 glucan synthase, is regulated by switching between a GDP-bound inactive state and a GTP-bound active state with conformational changes (Wei et al. 1997; Fig. 5.8). Only mutations in this gene lead to a growth-deficient phenotype in moulds (Guest et al. 2004). After synthesis in the ER, Rho1p is geranylgeranylated, a modification required for attachment of Rho1p to the membrane and for transport (Inoue et al. 1999). Geranylgeranylated Rho1p and Fks1p are transported to the plasma membrane as an inactive complex through the classical secretory pathways (Abe et al. 2003). Rho1p is activated on its arrival at the plasma membrane by Rom2p, the GDP/GTP exchange factor of Rho1 which is localised only at the plasma membrane. This activation, and the movement of Fks1p on the plasma membrane are required for proper cell wall β1,3 glucan localisation (Utsugi et al. 2002). Since Rho1 is a key regulator of fungal growth, it is not surprising that it is submitted to multiple regulators. Accordingly, numerous Rho1p regulators acting downstream on glucan synthesis have been identified, although their role is not fully understood. Besides the GTP-exchange factors (GEF) Rom2p and Rom1p, other GTPase activating proteins (GAPs) exist, such as Bem2p, Sac7p and Lrg1p (Heinisch et al. 1999; Watanabe et al. 2001; Schmidt and Hall 2002; Calonge et al. 2003; Fitch et al. 2004), which down-regulate β1,3 glucan synthesis. A multicopy suppressor screen

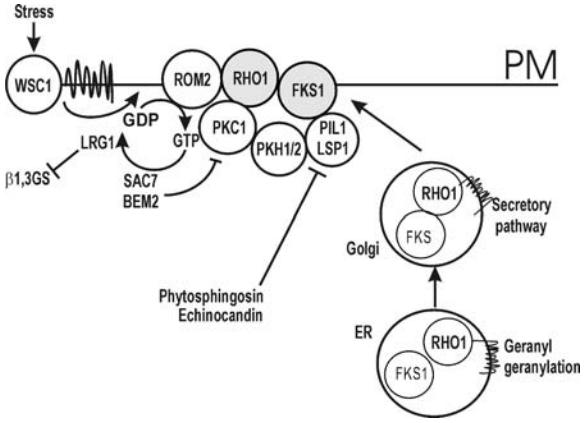

Fig. 5.8. Experimental data and hypothetical interactions in the regulation of β1,3 glucan synthesis. Fks1 and geranyl-geranylated Rho1 are transported as inactive proteins to the plasma membrane. The complex remains inactive until its contact at the plasma membrane (*PM*) with the GDP/GTP exchange factor Rom2 (activated by membrane sensors). GTPase activating proteins Lrg1, Sac7p and Bem2 negatively regulate glucan synthase activity by inhibiting either directly β1,3 glucan synthesis (Lrg1) or the PKC1 cascade (Sac7, Bem2). Besides regulating Fks1, the Rho1 GTPase also associates physically with Pkc1 to activate it and the downstream signalling cascade. A multiprotein complex Fks1-Pil1/Lsp1-Pkh1/2 could be the target for echinocandin and long chain bases such as phytosphingosine which are responsible for non-competitive inhibition of β1,3 glucan synthesis (protein names are in *capital italics*)

has also identified *LRE1*, *ZDS1* and *MSB1* to encode proteins which activate Fks1p (Sekiya-Kawasaki et al. 2002). Various cell surface sensors (Wsc1-3p, Mid2p and Mtl1p), dedicated to signalling cell wall stress during vegetative growth, interact also with Rom2p to stimulate GTP binding to Rho1p (Philip and Levin 2001; Vay et al. 2004).

In *S. pombe*, Rho1-GTP regulates β1,3 glucan synthesis and is required for maintaining polarization of the actin cytoskeleton, as in *S. cerevisiae* (Arrellano et al. 1996). As in yeast, the presence of regulatory subunits has been shown biochemically in *A. fumigatus*. However, no experiments using recombinant proteins have identified which of the four Rho proteins identified in the *A. fumigatus* genome is the regulatory subunit of the glucan synthase of *A. fumigatus*.

c) Inhibition

There are three general structural families of known natural product inhibitors of β1,3 glucan synthesis. The first family of inhibitors is the glycolipid papulacandins, which consist of a modified disaccharide linked to two fatty-acyl chains (Traxler et al. 1977). The second family includes the recently discovered acidic terpenoids (Onishi et al. 2000). The third family, the lipopeptides, comprise cyclic hexapeptides with an *N*-linked fatty-acyl side chain (Nyfeler and Keller-Schierlein 1974). Included in this group are the echinocandins currently used as anti-fungals in clinical practice. Echinocandins are non-competitive inhibitors of the β1,3 glucan synthase. Resistance to caspofungin has been associated with mutations mapping to the *FKS1* gene (Kurtz et al. 1996; Douglas 2001; Reinoso-Martin et al. 2003). An eight amino acid cluster (Phe 639-Asp646) and an amino acid in position 1357 are important for the activity, since mutations in these loci confer resistance to the echinocandins. Alterations in vacuole trafficking and function have been also associated with resistance to the echinocandin caspofungin. By contrast, fragility of the cell wall results in increased sensitivity to caspofungin (Markovitch et al. 2004). Sphingolipid metabolism has been repeatedly associated with the regulation of GS activity (El-Sherbeini and Clemas 1995; Chung et al. 2001; Feokistova et al. 2001). Recent data suggest that the association between sphingolipid-dependent regulators and *FKS1* would be the target of the echinocandin drug (Edlind and Katiyar 2004). Mutations in lipid metabolism could explain the occurrence of echinocandin-resistant mutants in *A. fumigatus* which are not associated with Fksp or efflux pumps (Gardiner et al. 2005).

2. Other Glucans

a) α1,3 Glucan Synthesis

Although the biochemical α1,3 glucan synthase activity and the substrate of this enzyme have not been described, genes encoding putative α1,3 glucan synthases have been identified (Katayama et al. 1999; Konomi et al. 2003). They are the largest genes (~8 kb) involved in cell wall polysaccharide synthesis. Each gene is characterized by two putative hydrolase and synthase domains separated by a single transmembrane domain.

In *S. pombe*, five genes encoding putative α1,3 glucan synthases have been found, but only *AGS1* (for α1,3 glucan synthase) has been well characterized in *S. pombe* (Hochstenbach et al. 1998; Katayama et al. 1999). *AGS1* is an essential gene. At restrictive temperature, the mutant *ags1* showed reduced α1,3 glucan in its cell wall,

resulting in cell lysis and the loss of cell polarity (Hochstenbach et al. 1998).

Three homologous *AGS* genes involved in α1,3 glucan synthesis have been identified in *A. fumigatus* (Beauvais et al. 2004). Their sequences are very homologous to the *S. pombe* α1,3 glucan synthase genes but, in contrast to this yeast, all genes are expressed during vegetative growth and none of the *AGS* genes is essential in *A. fumigatus*. In spite of the high homology between the three genes, a partial reduction in α1,3 glucan was seen only in the cell wall of the *AGS1* mutant. Expression study suggested that all three genes are involved in the synthesis of α1,3 glucan of *A. fumigatus*, and each is able to compensate for the lack of the other genes. *AGS1* seems to be the main responsible for synthesis at the cell wall level, as confirmed by its cell wall localisation.

Alterations in the α1,3 glucan content of other human fungal pathogens affect their pathogenicity. In *C. neoformans*, only *AGS1* was found. The *ags1* mutant grows to a very limited extent at 37 °C and does not encapsulate, because the capsule is anchored to the α1,3 glucan (Reese and Doering 2003). The lack of virulence in spontaneous α1,3 glucan mutants of *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* could be due to a higher sensitivity to phagocytes due to the total lack of α1,3 glucan (San-Blas et al. 1977; Hogan and Klein 1994; Klimpel and Goldman 1988). By contrast, in *A. fumigatus*, where the three genes are active and able to complement themselves, a limited modification of the α1,3 glucan content of the cell wall of the AGS mutants of *A. fumigatus* was not associated with a reduction in the virulence of this fungus (Beauvais et al. 2004).

α1,3 glucan synthesis is also under the regulation of the Rho2p-GTPases but it is a different Rho than that for β1,3 glucan synthesis. In *S. pombe*, Rho2-GTP acts as a positive regulator of α1,3 glucan synthase (Hirata et al. 1998; Calonge et al. 2000). The proteins activating Rho2p are not known.

b) β1,6 Glucan Synthesis

β1,6 glucan constitutes approximately 10% of the yeast cell wall. Many genes involved in β1,6 glucan synthesis have been identified based on resistance of mutants to the K1 killer toxin, which kills yeast following binding to β1,6 glucan (Shahinian and Bussey 2000). However, most of the genes identified in these screens encode proteins located along the secretory pathway from ER to plasma membrane (Shahinian et al. 1998). None of these genes have been associated with an enzymatic activity directly responsible for β1,6 glucan synthesis. It seems indeed that many mutations result in β1,6 glucan synthesis alterations (Dijkgraaf et al. 2002; Machi et al. 2004), but the role of the mutated genes in β1,6 glucan biosynthesis is only indirect (Abeijon and Chen 1998; Breinig et al. 2004). *KRE6*, one of the genes putatively involved in β1,6 glucan synthesis, has been recently found in *A. fumigatus*, indicating that its role is also indirect, since β1,6 glucan does not exist in *A. fumigatus*. Only recently, an immunoassay for β1,6 glucan synthesis in vivo has been developed. This assay shows that β1,6 glucan synthesis is produced when UDP-glucose and GTP are provided to a membrane preparation, and that β1,6 glucan synthesis is under the regulation of Rho1p, like β1,3 glucan synthesis (Vink et al. 2004).

C. Mannan Synthesis

High-MW mannan biosynthesis will be discussed since only this type is cell wall associated (Fig. 5.9). The *N*-mannan chains are usually considered as a "coating" component of the yeast cell wall. This is true in the case of the peptidomannans of yeasts, but not so in *A. fumigatus* and other moulds where mannan (as galactomannan) is an essential component of the cell wall and is bound covalently to the other polysaccharides. Although the mannan composition of the *A. fumigatus* cell wall is very different to that of yeast mannans, a comparative genomic study has indicated that orthologues of most yeast mannosyltransferase genes can be found in the genomes of moulds. *OCH1*, the gene initiating the synthesis of the long *N*-mannan chains in yeasts (Dean 1999; Stolz and Munro 2002), is present in the genome of *A. fumigatus* as a family of three proteins, whereas it is unique in *S. cerevisiae*. Other genes coding for mannosyltransferases responsible for the synthesis of linear α1,6 and α1,2 mannan in yeast have unique orthologues in *A. fumigatus*, although the end products of this mannosyltransferase complex will be structurally different in the two fungal species. This result is in agreement with the lack of specificity of the different gene products shown in vitro (Bussey, personal communication) and indicates a timely regulation, different in the two species. Regulators or specific inhibitors of high-MW mannan synthesis have not been reported yet.

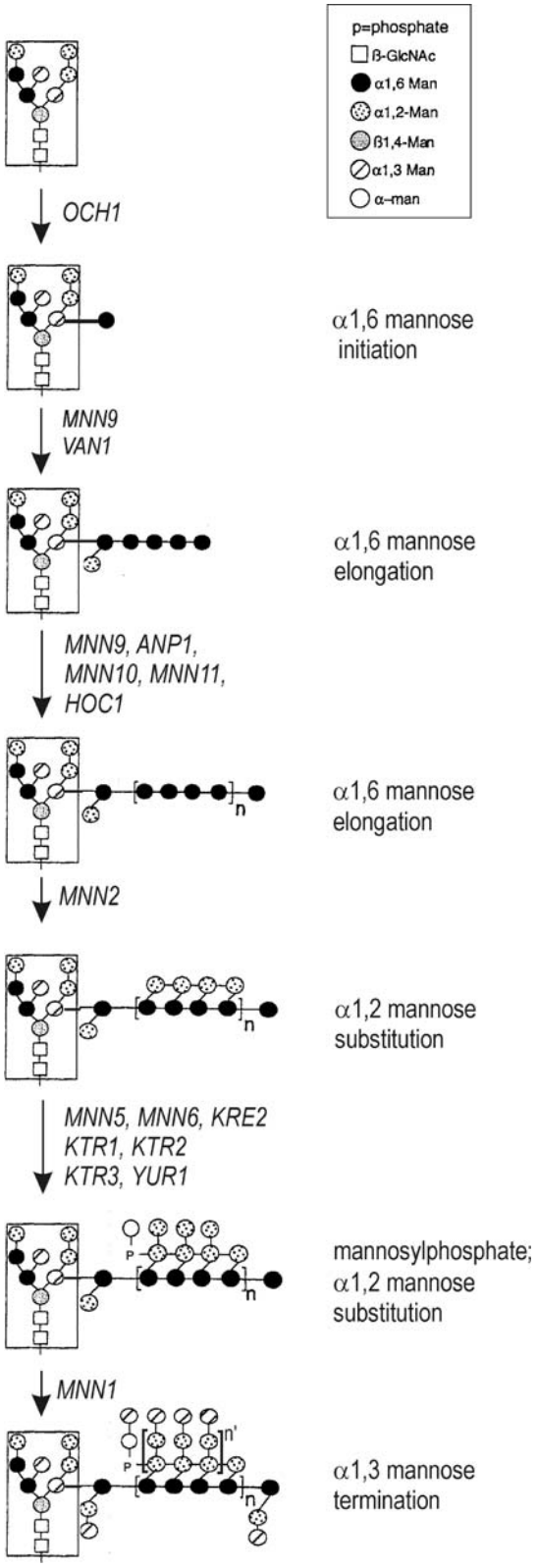

Fig. 5.9. Elongation of the mannan chain in *S. cerevisiae* (adapted from Dean 1999; Stolz and Munro 2002). *N*, *n* indicate *x* mannose (protein names are in *capital italics*)

Mannan can be linked to other polysaccharides such as side chains of galactose in *A. fumigatus* or *S. pombe* (Latgé et al. 2005). No enzymes responsible for the synthesis of these heteropolymers have been identified in fungi.

IV. Polysaccharide Remodelling

A. Polysaccharide Hydrolysis

In *S. cerevisiae*, only two chitinase genes (*CTS1* and *CTS2*) have been identified (King and Butler 1998). These hydrolases are not essential, but disruption of the encoding genes leads to defects in cell separation (Kuranda and Robbins 1991). In *C. albicans*, four chitinase genes have been identified among which only three are expressed. The role of each individual chitinase has not been totally explored, since only *cht2*, *cht3* mutants and the double *cht2/3* mutant have been obtained to date. Up-regulation of chitinase activity is seen during yeast-hypha morphogenesis, a result reinforcing the view that chitinases have an active role during the growth of this fungus (Selvaggini et al. 2004). In *A. fumigatus*, the situation is more complex, since its genome contains 18 chitinase-encoding genes. Disruption of a plant-type chitinase in *Aspergillus nidulans* resulted in reduced growth and reduced germination (Takaya et al. 1998), whereas disruption of a bacterial-type chitinase in *A. fumigatus* has no phenotype (Jacques et al. 2003). This suggests that the plant-type chitinases have a role during fungal growth and morphogenesis. An interesting feature in *A. fumigatus* is that some of the plant-type chitinases are GPI-anchored, data compatible with its putative role in cell wall morphogenesis.

Inhibition of chitin hydrolysis could represent an anti-fungal drug target, since inhibition of chitin hydrolysis by allosamidin, a specific chitinase inhibitor, blocks fungal growth (Papanikolau et al. 2003; Vaaje Kolstad et al. 2004). Cyclopeptide inhibitors of chitinases have been recently identified but their anti-fungal efficacy has not been investigated yet (Houston et al. 2002; Rao et al. 2005).

β1,3 glucan-hydrolysing enzymes have been also investigated, since β1,3 glucan is the most abundant cell wall polysaccharide and the formation of numerous free reducing and non-reducing ends is necessary for the activity of β1,3 glucanosyltransferases. In contrast to chitin-binding modules, no β1,3 glucan-binding motifs have been identified, although a high number of glucanases have been

identified in fungal genome sequences. Molecular studies have been mostly centred on endoglucanase, especially since its mode of action (endosplitting activity efficient on complex polysaccharide structure) suggested that these glycosylhydrolases could play a morphogenetic role.

Work in yeast has shown that, like chitinases, endo β1,3 glucanases are required for cell separation (Baladron et al. 2002; Martin-Cuadrado et al. 2003). In *A. fumigatus*, molecular studies have been exclusively centred on the only endo β1,3 glucanase identified to date (Eng1p). In spite of its cell wall localisation, constitutive expression occurs at all growth stages, which is independent of the presence of an insoluble and soluble β1,3 glucan substrate in the culture medium, the lack of phenotype for the null *engl1* mutant suggesting that this endo β1,3 glucanase activity does not play a morphogenetic role in *A. fumigatus* (Mouyna et al. 2002). However, although only one enzyme has been analysed, the genome of *A. fumigatus* contains > 20 putative glucanases which have to be studied before a role for endo β1,3 glucanase in fungal growth can be demonstrated.

α1,3 glucanase has been shown to have a role in cell division in *S. pombe*. Two α1,3 glucanase genes *AGS1* and *AGS2* have been identified in *S. pombe* (Dekker et al. 2004). Deletion of *AGS1* results in clumped cells which remained attached to each other. It has been proposed that the endo α1,3 glucanase Agn1p acts in concert with the endo β1,3 glucanase Eng1p to achieve efficient cell separation. *AGS2* is essential for endolysis of the ascus wall during maturation. Similarly, in *A. nidulans*, an α1,3 glucanase is expressed during sexual development. Cleistothecia were, however, formed in the mutant (Wei et al. 2001). This result is not surprising, taking into account the high number of redundant α1,3 glucanases identified in the genome of *Aspergillus* (for example, nine in *A. fumigatus*).

Expression of glucanases and chitinases is regulated by a cascade of transcription factors including Fkh1/2p and Ace2p (Dohrmann et al. 1992; Zhu et al. 2000). Moreover, recent work (Santos et al. 2003) suggests that Rho4p could be involved in the regulation of cell wall degradation in *S. pombe*. These data demonstrate that different RHO proteins regulate different glucan synthases and hydrolases.

B. β1,3 Glucan Branching and Cross-Linking Enzymes

The chronological steps in the synthesis of the core structural polysaccharides in the fungal cell wall are depicted in Fig. 5.10. β1,3 glucan chains produced by the β1,3 glucan synthase complex remain unorganised and alkali-soluble until covalent linkages occur between β1,3 glucans and other cell wall components. To date, no transglycosidases have been identified bioinformatically or biochemically which can achieve the branching of β1,3 glucans and the subsequent cross-linking of chitin and β1,3 glucan. Two β1,3 glucanosyltransferases have been identified biochemically to date but neither displays the branching activity and cross-linking activity responsible for binding chitin and β1,3 glucans.

The first β1,3 glucanosyltransferase of *A. fumigatus* (Bgl2p/Bgt1p) present in both yeasts and moulds cleaves laminaribiose from the reducing

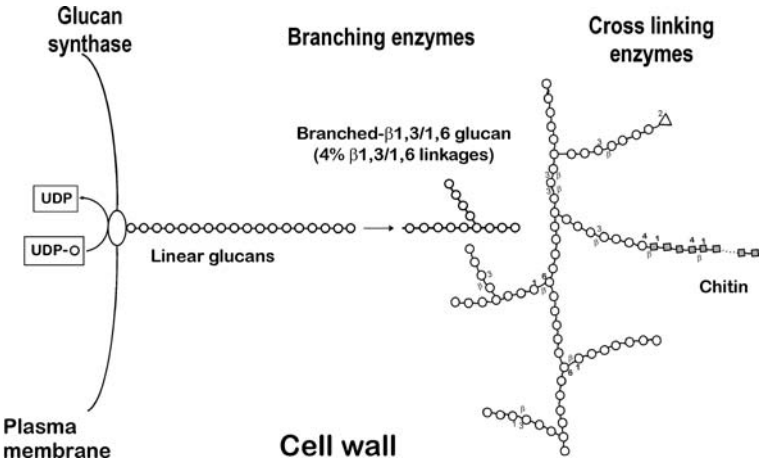

Fig. 5.10. Temporal events in the biosynthesis of the structural β1,3 glucan-chitin core. For simplification, only glucan synthase is represented but chitin synthase is active at the same time and at the same location as glucan synthase. Linear chains of glucan are modified by both branching and cross-linking enzymes

end of linear β1,3 glucans, and transfers the remaining glucan to another β1,3 glucan acceptor with a β1,6 linkage (Goldman et al. 1995; Mouyna et al. 1998). Null mutants, however, do not display a phenotype different from the wild-type parental strain. Since the gene is present as a single copy in the genome, the absence of phenotype for the null mutant suggests that this enzyme does not play a major role in cell wall morphogenesis and, in particular, in cross-linking of cell wall polysaccharides, a result expected since this enzyme requires a free reducing end to display its activity. It also indicates that not all glucanosyltransferases associated with the cell wall have an obligatory role in cell wall biosynthesis.

A second, novel β1,3 glucanosyltransferase, isolated originally from an autolysate of the cell wall of *A. fumigatus*, has been also characterized (Mouyna et al. 2000a). This enzyme splits internally a β1,3 glucan molecule and transfers the newly generated reducing end to the non-reducing end of another β1,3 glucan molecule. The generation of a new β1,3 linkage between the acceptor and donor molecules resulted in the elongation of β1,3 glucan chains. The predicted amino acid sequence of *GEL1*, encoding this enzymatic activity, is homologous to several yeast protein gene families such as *GAS1* in *Saccharomyces cerevisiae* and *PHR1* in *C. albicans*, which are required for correct morphogenesis and polar growth in these organisms (Saporito-Irvine et al. 1995; Mühlschlegel and Fonzi 1997; Popolo and Vai 1999). The biochemical assays performed on yeast recombinant proteins and complementation experiments have shown that these yeast proteins also have a β1,3 glucanosyltransferase activity similar to that of Gel1p (Mouyna et al. 2000b; Carotti et al. 2004).

V. Cell Wall Biosynthesis, Environmental Stress and Signal Transduction Pathways

A. The Cell Wall Salvage Pathway

As discussed above, genes involved in the biosynthesis of the cell wall are regulated temporally and spatially during vegetative growth. Moreover, modifications of the environment induce cell wall changes which allow the fungus to survive in its new environment. For example, in the presence of cell wall and plasma membrane perturbing agents or even in cell wall mutants, features of the cell wall compensatory response include (1) a hyperaccumulation of chitin but no significant modifications of the β1,3 glucan concentration, (2) changes in the association of the various cell wall components, i.e. less β1,6 glucan results in changes in the association of cell wall mannoproteins with glucans, (3) a redistribution of the cell wall synthesis and repair machinery throughout the cell, rather than only at active growth regions (Kapteyn et al. 2000; Lagorce et al. 2003; Fig. 5.11). These data suggest that the cell wall of fungi subjected to such stress conditions have a composition different from cells growing in the absence of a drug, for example, in vitro. Consequently, the susceptibility to drugs of fungi grown in vitro and in vivo is often different, as has been seen for cells exposed to echinocandins (Gustin et al. 1998). The re-emergence of interest in recent years in drugs which target the cell wall makes such analyses topical and important.

In *S. cerevisiae*, several genome-wide analyses were undertaken with wild-type cells in the presence of global cell wall inhibitors such as Calcofluor white or cell wall lytic enzymes such as Zymolyase, or mutants lacking biosynthetic cell wall-encoding genes or overexpressing constitutively active regulators of signalling cascades (Jung and Levin 1999; Roberts et al. 2000; de Groot et al. 2001; Lagorce et al. 2003; Boorsma et al. 2004). For example, an analysis of mutants which lacked biosynthetic cell wall-encoding genes such as *FKS1* (β1,3 glucan), *KRE6* (β1,6 glucan), *MNN9* (oligomannan synthesis), *GAS1* (β1,3 glucanosyltransferase) and *KNR4* (gene product connects with the PKC cell integrity pathway) identified a cluster of 80 genes which were up- and co-regulated, and could be considered to be associated with a transcriptional response linked with a cell wall compensatory mechanism. The 80 co-regulated genes whose transcription increased as a result of the deletions mentioned above each have pair-wise combinations of DNA-binding sites for transcriptional factors which are associated with stress and heat shock responses (Msn2/4p and Hsf1p), and two PKC cell integrity regulated transcription factors (Rlm1p and Swi4p). In addition, the sequence analysis discerning the 6-bp calcineurin-dependent response element to which the transcription factor Crz1p binds in 40% of the genes which are up-regulated in the deletion mutants recalls that Crzp1 connects with the PKC1 cell integrity pathway, most likely via calcinuerin

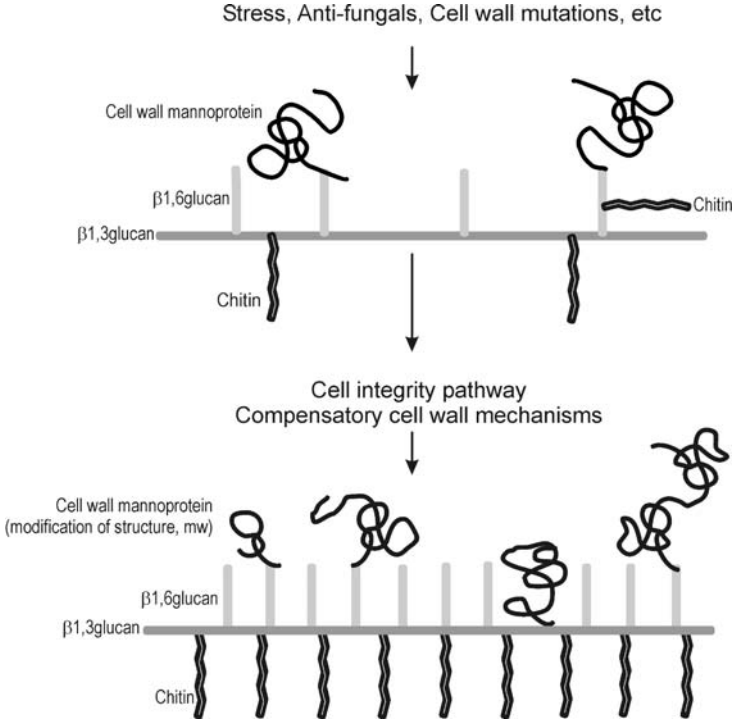

Fig. 5.11. The compensatory cell wall mechanism of fungi. A variety of stress factors cause changes in the cell wall, including an increase in chitin, changes in the ratios of β-1,3/β-1,6, shifts in the association of mannoproteins with glucans, and a redistribution of cell wall synthesis to a more global distribution, rather than an association with only the growing part of cells. The various cell wall polysaccharides coded are indicated with *shading* of different intensities

signalling (Matheos et al. 1997). In addition to the up-regulation of the Rlm1p- and STRE-controlled genes, Boorsma et al. (2004) showed an additional response with Sko1p, a downstream regulator of the HOG pathway. The cell wall compensatory mechanism to process cell wall perturbations integrates then the PKC/SLT, the global stress response pathway mediated by the Msn2p, the transcription factor downstream of the HOG pathway gene product, and the Ca^{2+}/calcineurin-dependent pathway. Direct molecular interactions between these different transduction cascades have been suggested but never demonstrated as yet.

Although the pheromone and RAS/cAMP pathways are also somehow associated with cell wall biosynthesis (Daniels et al. 2003; Calcagno et al. 2004; Fitch et al. 2004; Nelson et al. 2004), these pathways are not detailed here because they do not play a direct role in cell wall modifications (for a review of these different pathways, see Brown 2002, and Palacek et al. 2002). Only discussed below are the PKC pathway, the HOG pathway, and the Ca^{2+}/calcineurin-dependent pathway. Our understanding of the role of each pathway in modifying cell wall composition is based mainly on the analysis of the phenotype of mutants obtained by gene disruption of members of each signalling cascade.

B. Signal Transduction Cascades Responsible for Major Cell Wall Compensatory Mechanisms

The three major signal transduction cascades involved in cell wall repairs are schematised in Fig. 5.12.

1. The HOG MAP Kinase Pathway

The HOG MAPK pathway (*h*yper*o*smolarity *g*lycerol) was originally described in *S. cerevisiae* and shown to be essential for adaptation of the organism to hyperosmotic stress (O'Rourke et al. 2002). The proteins which are upstream of the MAPKKK, MAPKK and MAPK (Hog1p) signal transduction pathway proteins are two-component proteins. In *S. cerevisae*, the phosphorylation of the membrane-associated histidine kinase (HK) protein (Sln1p) results in phosphotransfer to another HK intermediate (Ypd1p), and then Ypd1p transfers its phosphate to an aspartate of the response regulator (RR) protein, Ssk1p, which acts as a transcriptional regulator of genes associated with a specific adaptive phenotype (Fig. 5.12; Tatebayashi et al. 2003).

In *C. albicans*, there is a total of five histidine kinases and response regulator proteins, includ-

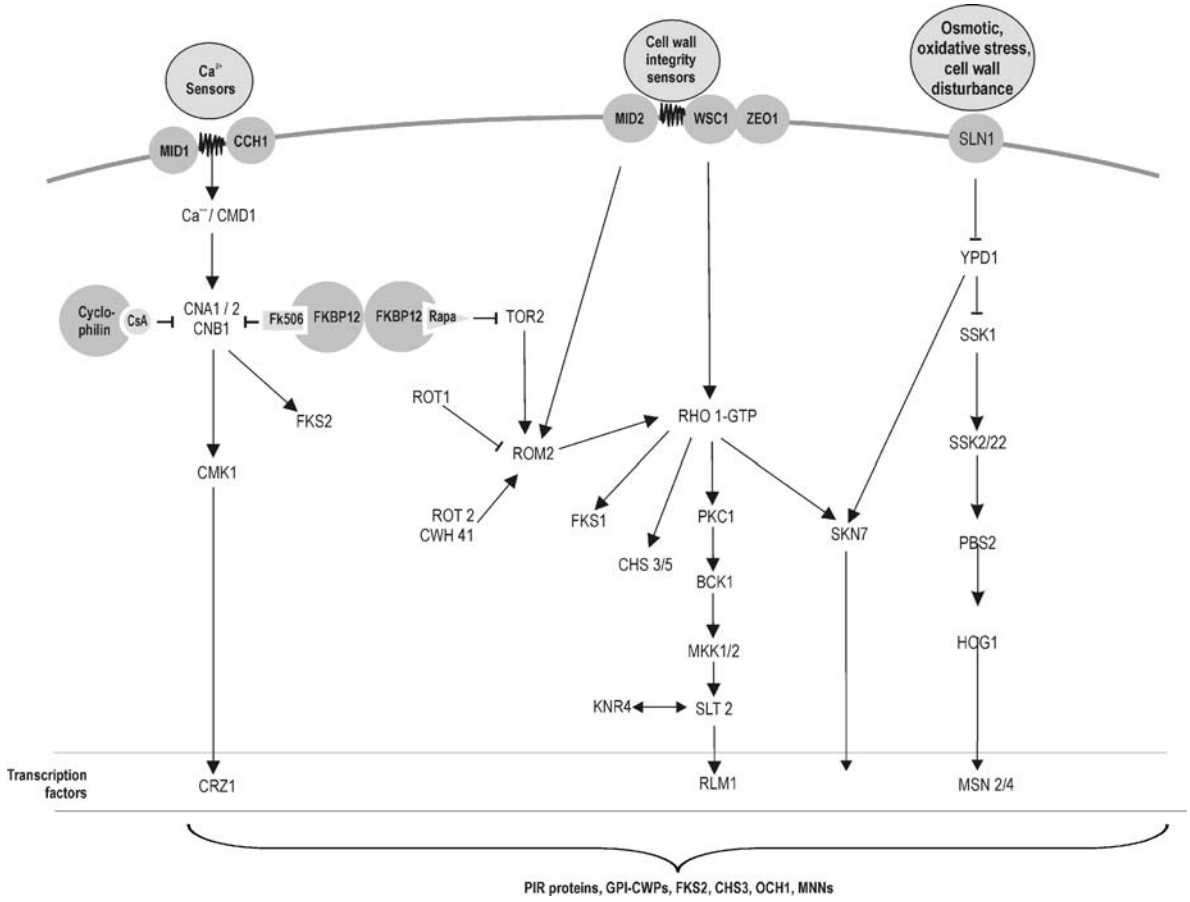

Fig. 5.12. Signal transduction in fungi. Inputs and sensors (*circles at top*) and transcription factors (*bottom*) are indicated for each of the major signal transduction pathways responsible for cell wall compensatory mechanisms. *At the bottom*, transcriptionally regulated genes encoding cell wall proteins and enzymes are shown. Only the major transcription factor is indicated by an *arrow* downstream of each signalling cascade. Interactions between the different pathways are not shown. Cross-talking is expected to occur between calcineurin (*left*) and the PKC1 cell integrity pathway (*centre*) via Rom2, Rho1, or as yet undefined interactions. Cross-talking is believed to occur with the HOG pathway (*right*) via similar proteins and Skn7, since the HOG pathway also regulates aspects of cell wall synthesis (protein names are in *capital letters*). *Rapa* Rapamycin, *FK 506* tacrolimus, *CSA* cyclosporin

ing the *S. cerevisiae* homologues Sln1p, Ypd1p and Ssk1p, as well as two proteins, Chk1p and Nik1p, which are not found in *S. cerevisiae* (Santos and Shiozaki 2001; Catlett et al. 2003). In comparison to the yeast mentioned above, the genomes of filamentous fungi encode a more extensive array of two-component proteins. For example, a survey of the recent annotation of the *A. fumigatus* genome has identified 11 HK proteins and three RR proteins (Latgé, unpublished data). The functions ascribed to the HK and RR proteins fall into four categories, i.e., oxidant and osmoadaptation, cell wall biosynthesis, morphogenesis, and virulence (Alex et al. 1996, 1998; Calera and Calderone 1999; Calera et al. 2000; Virginia et al. 2000). These functions are obviously interrelated. For example, the Chk1p HK of *C. albicans* is used for cell wall biosynthesis and, in fact, a mutant lacking the encoding gene is less adherent to human oesophageal cells in vitro, and is killed to a greater extent by human neutrophils (Li et al. 2002; Torosantucci et al. 2002). So, the interpretation is that the global function in pathogenesis relates to specific but multiple functions which have been observed with cell wall and adaptation. Of these phenotypes, the most studied is that of the change in cell wall composition. For example, in the *C. albicans chk1* histidine kinase mutant, changes in cell wall composition include a truncation in the acid-stable oligomannan, an increase in β-1,6 glucan, and a decrease in the amount of β-1,6

glucan (Kruppa et al. 2003). As with many fungal cell wall mutants, compensatory changes occur to ensure the survival of the fungus and, in the case of the *chk1* mutant, the primary phenotypic change in the cell wall which occurs as a result of the gene deletion is unknown. The response regulator Ssk1p of *C. albicans* is also linked to a cell wall function (Chauhan et al. 2003). Microarray analysis of the *ssk1* mutant has been compared to parental cells, and those genes whose expression were changed in the mutant include oxidant and other stress genes as well as genes associated with a cell wall function, including *ECP33*, *CHK1*, *MNN4* and *ALS1* (Chauhan et al. 2003). All of these genes encode a structural cell wall protein, except *CHK1* which, as mentioned above, is associated with signal transduction and cell wall biosynthesis. In *S. cerevisiae*, Skn7p regulates transcription of *OCH1*, which encodes a mannosyltransferase (Moye-Rowley 2003).

In *A. nidulans* and *A. fumigatus*, two component HK proteins have been partially characterized (Pott et al. 2000; Virginia et al. 2000; Clemons et al. 2002; Furukawa et al. 2002). The deletion of the HK *FOS1* resulted in delayed conidiophore development, and sensitivity to novozyme, the latter phenotype suggesting a role for fos1p in cell wall assembly. The *TCSB* of *A. nidulans* is the *SLN1* homologue of *S. cerevisiae*; a strain deleted of this gene was not osmosensitive and not essential for growth, unlike the corresponding *sln1* mutant of *S. cerevisiae* (Furukawa et al. 2002), while the *TSCA* of *A. nidulans* has been shown to regulate sporulation (Virginia et al. 2000). The observation on the apparent lack of a phenotype in the *tcsB* mutant may indicate reliance on the part of this fungus to use other pathways for osmoadaptation or even hyphal development. Likewise, the rather large number of HKs in filamentous fungi such as *A. fumigatus* probably indicates redundancy in gene function.

The key MAPK kinase which is downstream of many of the two-component signal relay systems is Hog1p (*S. cerevisiae*/*C. albicans*; SakAp in *A. fumigatusK*; Dixon et al. 1999; Kawasaki et al. 2002; Alonso-Monge et al. 2003). Different sensitivity of the *hog1* mutants to cell wall inhibitors suggests an effect of these genes on cell wall biogenesis. The *sakA* mutant of *A. fumigatus* was hypersensitive to β1,3 glucan inhibitors (own unpublished data) whereas the *C. albicans hog1* mutant shows an increased sensitivity to inhibitors of chitin synthesis (Alonso-Monge et al. 1999). Moreover, mutants resistant to echinocandins have an increased expression of *HOG1* (Gardiner et al. 2005). However, it remains unknown if cell wall modifications are directly regulated by the members of the HOG pathway, or if the modifications seen are part of a global metabolic response to a change in the environment.

2. The Cell Integrity Pathway

A major signal transduction pathway which is essential in the maintenance of cell wall integrity is the Pkc1p-Slt2p MAP kinase signal pathway (Popolo et al. 2001). The model organism for this pathway to which most other fungi are compared is *S. cerevisiae* (Xu 2000). *PKC1* is an essential gene, and the Pkc1p-Slt2p integrity pathway is activated in response to several conditions which cause cell wall damage, including temperature shock, hypotonic shock, perturbation by anti-fungal drugs such as papulacandin, caspofungin and nikkomycin, or other polysaccharide-interfering inhibitors such as Calcofluor white and Congo red, as well as mutations in genes implicated in cell wall biosynthesis (Xu 2000). In stressed cells of *S. cerevisae*, sensors activate Pkc1p and, in turn, a downstream MAPK pathway is activated which includes MAPKKK (Bck1p), MAPKK (Mkk1p/Mkk2p) and MAPK (Slt2p) proteins (Fig. 5.12). Pkc1p activity depends upon the binding of Rho1p to Pkc1p, which is activated by GTP (referred to as GTP loading), provided by partially redundant guanine nucleotide exchange factors, Rom1p/2p (Harrison et al. 2004).

Rho1p has a dual role: in its GTP form it activates the catalytic subunit of the glucan synthase but it also binds to *PKC1* for transcription activation and for feedback secretion. This may explain why, if Pkc1p is activated, competition for GTP will result in the lack of activation of the glucan synthase, with an associated constant level of β1,3 glucan. Another explanation comes from the study of Boorsma et al. (2004) showing a down-regulation of ribosomal and rRNA genes during cell wall stress. Accordingly, the gene products which are present in the cell, such as Chs3p, will be immediately recruited for cell wall changes. Recruitment of Chs3p to the plasma membrane is indeed induced by the activation of the PKC pathway, and consequently results in the increase in the chitin level of the cell wall (Valdivia and Scheckman 2003).

The environmental cues and regulatory events associated with the activation of this pathway have not been identified in all fungi which are reported to have homologues of the cell wall integrity pathway. In *S. cerevisiae*, nutrient levels, temperature

shock, low osmolarity as well as other factors are believed to be key environmental signals which activate this pathway. Several cell membrane proteins (Mid2p, Mtl1p, Wsc1-4p, Zeo1p) have been suggested to function as sensors for the activation of the cell integrity pathway in *S. cerevisiae* (Zu et al. 2001; Green et al. 2003). The regulation of the PKC1 signal pathway is not unexpectedly complex, and may include several other regulatory proteins. For example, in *S. cerevisiae*, the Pkh1/2p homologues of the mammalian 3-phosphoinositide protein kinase can regulate both the PKC1 and YPK1 signal pathways (Zhang et al. 2004). The regulation of these pathways requires sphingolipid long chain bases which act via two specific regulators, Pil1p and Lsp1p. There is also recent data from *S. cerevisiae* that, of these many signals activating the PKC1 pathway, not all are integrated as a "top-down" mechanism which depends upon GTP loading of Rho1p (Harrison et al. 2004). These authors show that strains lacking Pkc1p and Bck1p are still able to activate Slt2p when cells were stressed by heat shock, but activation of Slt2p occurs differently under conditions of actin depolymerisation or hyposmotic shock. The authors conclude that stress conditions provide lateral inputs into this regulatory pathway, rather than in a "top-down" linear manner. One example of this "lateral" interaction is Knr4p, whose interaction with Slt2p is essential for signalling through the cell wall integrity pathway (Martin-Yken et al. 2003).

The *MKC1* gene (*M*AP kinase from *C. albicans*) is a homologue of the *S. cerevisiae SLT2*. Although it is not essential for the growth of the organism, several phenotypes were observed which indicated a sensitivity of the deletion mutant to growth at 42 °C or heat shock at 55 °C, a sensitivity of cells to caffeine which could be reversed by the addition of 1M sorbitol and to β1,3 glucan inhibitors, and a lower susceptibility to complex lytic enzymes such as the glusulase enzyme and nikkomycin (Navarro-Garcia et al. 1995, 1998). Differences between the mutant and wild-type cells in the kinetics of cell wall precursor incorporation were not observed, and a comprehensive analysis of cell wall composition only revealed minimal changes in total chitin. Interestingly, a mannoprotein epitope (1B12) was more highly expressed in the mutant but further analysis of this phenotype was not pursued (Navarro-Garcia et al. 1995, 1998). These phenotypes indicate that the role for *MKC1* in cell wall biosynthesis is less in *C. albicans* than in *S. cerevisiae*. In *Magnaporthe grisea*, the hyphae of the *mps1/slt2* mutant are hypersensitive to cell wall-degrading enzymes, thinner and uneven, indicating that wall changes must accompany the gene deletion (Xu and Hammer 1996; Dixon et al. 1999). The deletion of *MPK1* in *C. neoformans* induced a defect in growth at 37 °C which was overcome by the addition of sorbitol. Along with this observation, the *mpk1* mutant exhibited enhanced sensitivity to nikkomycin, and partial sensitivity to caspofungin which was further enhanced in a double mutant of *mkc1/cnb1*, the latter encoding the B subunit of the calcineurin protein. In wild-type cells, Mpk1 was phosphorylated when cells were stressed with Calcofluor white. The growth phenotype, and the sensitivity of the mutants to cell wall perturbing drugs again point to the major role of the cell integrity pathway with regard to cell wall synthesis.

3. TOR and Calcineurin Signalling Pathway

The TOR (*t*arget *o*f *r*apamycin) kinases are highly conserved proteins found in organisms from yeasts to humans (Crespo and Hall 2002). They are originally characterized and named according to the observation that the immunosuppressive drugs rapamycin and tacrolimus (FK506) bind to a protein called immunophilin (Fkbp12p, *FK*506-*b*inding *p*rotein of *12* kDa), which in turn binds and inhibits the kinase TOR. In *S. cerevisiae*, there are two TOR-encoding genes, *TOR1* and *TOR2* (Crespo and Hall 2002). Both share a common function of activation of translation initiation and cell cycle progression in response to nutrients. In addition, *TOR2* mediates the cell cycle-dependent actin polarization to the bud site of dividing cells. This event is initiated through Rom2p activation of Rho1p and subsequent signalling via the PKC-MAP kinase pathway. Thus, Tor2p function cross-talks with the cell integrity pathway (Crespo and Hall 2002). The TOR kinases are members of the phosphatidylinositol 3-kinase (PI-3K) superfamily which are critical for the regulation of cell growth and differentiation.

Calcineurin is a Ca^{2+}-calmodulin-activated serine/threonine phosphatase (Lengeler et al. 2000; Rohde et al. 2001) which consists of a catalytic A subunit and a Ca^{2+}-binding regulatory subunit. This pathway connects Ca^{2+}-dependent signalling with many cellular responses, including TOR functions (Fig. 5.12; Sugiura et al. 2002). In *S. cerevisiae*, Cch1/Mid1 are putative calcium-sensitive sensors upstream of the calcineurin pathway

(Tada et al. 2003). Two redundant genes, *CNA1* and *CNA2*, encode the catalytic subunit whereas the regulatory subunit is encoded by *CNB1*. Both subunits are required for enzymatic activity.

Surprisingly, it was research efforts focused on the Ca^{2+}-calmodulin-dependent phosphoprotein phosphatase calcineurin, and the mode of action of immunosuppressive compounds such as tacrolimus (FK506) and cyclosporin A (CsA) which helped to uncover genes encoding subunits of β1,3 glucan-synthase (Douglas et al. 1994; Douglas 2001). This result was due to the presence of the proteins Fks1p and Fks2p which are alternate subunits of the β1,3 glucan-synthase enzyme complex, with an essential overlapping function (Mazur et al. 1995). When regulation of *FKS2* was studied, it was discovered that transcription is induced by Ca^{2+} in a calcineurin-dependent manner, and inhibited by tacrolimus (Zhao et al. 1998). Because calcineurin is known to be the target of tacrolimus and CsA, it follows that cells with null alleles in *FKS1* are hypersensitive to tacrolimus or CsA because they rely on Fks2p for viability.

In *C. neoformans*, the calcineurin mutants are defective in growth at 37 °C (Odom et al. 1997; Kraus and Heitman 2003), resulting in an enhanced susceptibility of a cell integrity pathway gene (*MPK1*) mutant to β1,3 glucan synthesis inhibitors (Kraus et al. 2003). Further, the same authors showed that in a *cnb1* mutant, activation of *FKS1* occurs in cells in an Mpk1-dependent manner. Thus, in *C. neoformans* which has a unique *FKS* gene (Thompson et al. 1999), both the cell integrity and calcineurin pathways interact to regulate *FKS1* transcription, and hence cell wall glucan synthesis.

Calcineurin is also critical to the morphogenesis of *C. albicans* (Bader et al. 2003; Blankenship et al. 2003; Sanglard et al. 2003). The function of the calcineurin *CNA1* in cell wall formation was suggested by the sensitivity of the mutant to cell wall-perturbing agents such as SDS, Calcofluor white and Congo red, and by evidence that the activation of *FKS2*, a glucan synthase subunit-encoding gene, is regulated by upstream events which are calcineurin-dependent (Sanglard et al. 2003).

That immunosuppressants such as rapamycin which target TOR, and FK506 (tacrolimus) and cyclosporine which target calcineurin might have anti-fungal properties has been theorized based upon the important cell functions described above. The inhibitory activity of the immunosuppressants has also been evaluated in vitro against *A. fumigatus*, *C. albicans* and *C. neoformans*. Of the three drugs, FK506 was the most inhibitory and, in agreement with its connection with glucan synthase, FK506 in combination with inhibitors of β1,3 glucan synthase resulted in the best synergy (Cruz et al. 2000; del Poeta et al. 2000; Steinbach et al. 2004).

VI. Perspectives

In spite of the essential role of the cell wall for fungi, very little is known in this field and most of our present knowledge is based on the seminal studies of Enrico Cabib for chitin and β1,3 glucan synthases. Encoding genes for these transmembrane enzymes and their enzymatic activities have been characterized but none of these enzyme activities have been demonstrated in vitro using recombinant proteins, so that there is a very limited knowledge on their mode of action. Moreover, an increasing number of studies point to the association between polysaccharide synthesis and phospholipid metabolism. Such relationships should be investigated in more detail. This could lead to specific antifungals, in a similar way to sphingolipids inhibitors. Whereas the molecular analysis of genes encoding the β1,3 glucan synthases has led to the discovery of the anti-fungal echinocandins, no compounds exist in clinical practice which target chitin synthesis in the treatment of fungal disease.

Recent comparative chemogenomic analyses of the yeast and mould cell wall described above have resulted in a major progress in delimiting the key components of the cell wall structure. More development in this area should be forthcoming following the analysis of the cell wall of ancient fungi such as the Chytridiomycetes. Data with these fungi remain very incomplete, since neither the presence of branches in the glucan nor the chitin–glucan linkages have been investigated. Genomic analysis of these fungi should give us some important clues about undiscovered cell wall genes essential for its construction.

Another area of study is the analysis of glycosyltransferases which are active in cell wall construction, since branching and cross-linking between chitin and β1,3 glucan are responsible for the formation of a resistant fibrillar skeletal component of the cell wall of all fungi. Linkages between these two polysaccharides have been shown to be essential for the construction of the fibrillar core of the fungal cell wall, providing strength and protection against adverse environmental con-

ditions. Accordingly, enzymes and regulators involved in the biosynthesis of this core structure will be key anti-fungal drug targets, in a way similar to antibiotics which target the outer bacterial wall. At this time, the enzymes responsible for the branching and cross-linking of the structural cell wall polysaccharides and the proteins controlling them are largely unknown and must be identified.

Changes in composition of the cell wall have been associated with responses to stressful conditions represented by the host environment or due to cell wall-perturbing agents. As discussed above, fungi have evolved mechanisms to sense wall damages and restore cell wall integrity but the exact modifications of the cell wall have not been described, nor is the networking of the different regulatory pathways known. Two global regulatory mechanisms are responsible for cell wall changes. A first level of cell wall compensatory mechanisms is due to the presence of many members per glycosylhydrolase or synthase family. A general trend in cell wall genes is their clustering in families of multiple members (Fig. 5.13). This is especially true in moulds where >10 homologues can be found in one and the same family. Accordingly, disruption of a gene in a family, as seen, for example, in the *AGS* genes in *A. fumigatus* (Beauvais et al. 2004), results in the overexpression of other members of the family, making the analysis of the mutant phenotype difficult to interpret. The second compensatory mechanism is the turning on of several signal transduction pathways to adjust and synthesise their cell walls. This observation reflects the dynamic nature of the biochemical and molecular events underlying this important cellular structure. The signal pathways described above also cross-talk to regulate seemingly dissimilar events. In fact, integration of signal transduction pathways is absolutely necessary for the survival of the organism. Nevertheless, it would seem equally important for an organism to conserve energy by using mechanisms which induce specificity, i.e. not all pathways are on simultaneously. How is specificity achieved? The answer to this question may involve a better understanding of scaffolding proteins, like Ste5p of *S. cerevisiae* which serves to distinguish pheromone induction of mating, rather than nitrogen sensing and pseudohyphal growth (Sprague et al. 2004). The contribution of information on the types and functions of scaffolding proteins may be paramount to understand the process of cell wall growth and survival in a stress environment.

The cell wall was considered for years to be an inert organelle. Recent data in fungi and in plants show that, on the contrary, it is a very active organelle which is able not only to protect the cell from external environment but also to sense the outside stress to modify its composition. This makes the study of the cell wall one of the most exciting challenges in mycology.

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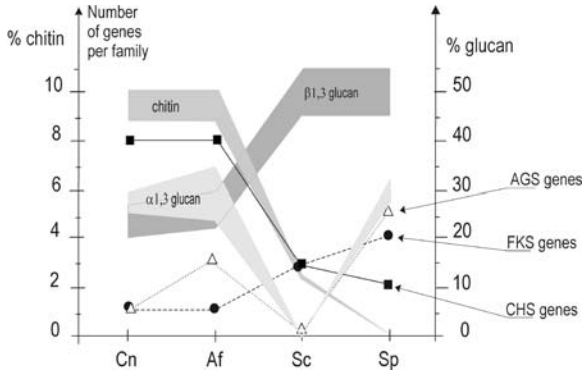

Fig. 5.13. The number of genes per AGS, FKS and CHS families responsible for α1,3, β1,3 glucan, and chitin synthesis in different fungi (*AF A. fumigatus*, *Cn C. neoformans*, *Sc S. cerevisiae*, *Sp S. pombe*) is shown. The range of cell wall chitin, α1,3 and β1,3 glucan is indicated as a % of the total cell wall weight and is roughly correlated to the importance of each polysaccharide in the different fungi

References

Abe M, Qadota H, Hirata A, Ohya Y (2003) Lack of GTP-bound Rho1p in secretory vesicles of *Saccharomyces cerevisiae*. J Cell Biol 162:85–97

Abeijon C, Chen LY (1998) The role of glucosidase I (Cwh41p) in the biosynthesis of cell wall beta-1,6-glucan is indirect. Mol Biol Cell 9:2729–2738

Adams DJ (2004) Fungal cell wall chitinases and glucanases. Microbiology 150:2029–2035

Alex LA, Borkovich KA, Simon MI (1996) Hyphal development in *Neurospora crassa*: involvement of a two-component histidine kinase. Proc Natl Acad Sci USA 93:3416–3421

Alex LA, Korch C, Selitrennikoff CP, Simon MI (1998) COS1, a two-component histidine kinase that is involved in hyphal development in the opportunistic pathogen *Candida albicans*. Proc Natl Acad Sci USA 95:7069–7073

Alonso-Monge R, Navarro-Garcia F, Molero G, Diez-Orejas R, Gustin M, Pla J, Sanchez M, Nombela C (1999) Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of *Candida albicans*. J Bacteriol 181:3058–3068

Alonso-Monge R, Navarro-Garcia F, Roman E, Negredo AI, Eisman B, Nombela C, Pla J (2003) The Hog1 mitogen-activated protein kinase is essential in the oxidative stress response and chlamydospore formation in *Candida albicans*. Eukaryot Cell 2:351–361

Arellano M, Duran A, Perez P (1996) Rho 1 GTPase activates the (1-3)β-D-glucan synthase and is involved in *Schizosaccharomyces pombe* morphogenesis. EMBO J 15:4584–4591

Arellano M, Cartagena-Lirola H, Hajibagheri MAN, Duran A, Valdivieso MH (2000) Proper ascospore maturation requires the *chs1+* chitin synthase gene in *Schizosaccharomyces pombe*. Mol Microbiol 35:79–89

Aufauvre-Brown A, Mellado E, Gow NAR, Holden DW (1997) *Aspergillus fumigatus chsE*: a gene related to *CHS3* of *Saccharomyces cerevisiae* and important for hyphal growth and conidiophore development but not pathogenicity. Fungal Genet Biol 21:141–152

Bader T, Bodendorfer B, Schroppel K, Morschhauser J (2003) Calcineurin is essential for virulence in *Candida albicans*. Infect Immun 71:5344–5354

Bahmed K, Quiles F, Bonaly R, Coulon J (2003) Fluorescence and infrared spectrometric study of cell walls from *Candida*, *Kluyveromyces*, *Rhodotorula* and *Schizosaccharomyces* yeasts in relation with their chemical composition. Biomacromolecules 4:1763–1772

Baladron V, Ufano S, Duenas E, Martin-Cuadrado AB, del Rey F, Vazquez de Aldana CR (2002) Eng1p, an endo-1,3-beta-glucanase localized at the daughter side of the septum, is involved in cell separation in *Saccharomyces cerevisiae*. Eukaryot Cell 1:774–786

Barbosa IP, Kemmelmeier C (1993) Chemical composition of the hyphal wall from *Fusarium graminearum*. Exp Mycol 17:274–283

Bartnicki-Garcia S (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu Rev Microbiol 22:87–108

Bartnicki-Garcia S, Reyes E (1968) Polyuronides in the cell walls of *Mucor rouxii*. Biochim Biophys Acta 170:54–62

Beauvais A, Bruneau JM, Mol PC, Buitrago MJ, Legrand R, Latgé JP (2001) Glucan synthase complex of *Aspergillus fumigatus*. J Bacteriol 183:2273–2279

Beauvais A, Maubon D, Park S, Morelle W, Huerre M, Perlin D, Latgé JP (2004) Two α(1-3) glucan synthases with different functions in *Aspergillus fumigatus*. Appl Environ Microbiol 71:1531–1538

Berbee ML, Taylor JW (1999) Fungal phylogeny. In: Oliver PR, Schweizer M (eds) Molecular fungal biology. Cambridge University Press, London, pp 21–77

Berbee ML, Taylor JW (2001) Fungal molecular evolutions: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota, vol VII. Springer, Berlin Heidelberg New York, pp 229–245

Bernard M, Latgé JP (2001) *Aspergillus fumigatus* cell wall: composition and biosynthesis. Med Mycol 39:9–17

Bernard M, Mouyna I, Dubreucq G, Debeaupuis JP, Fontaine T, Vorgias C, Fuglsang C, Latgé JP (2002) Characterization of a cell-wall acid phosphatase (PhoAp) in *Aspergillus fumigatus*. Microbiology 148:2819–2829

Blankenship JR, Wormley FL, Boyce MK, Schell WA, Filler SG, Perfect JR, Heitman J (2003) Calcineurin is essential for *Candida albicans* survival in serum and virulence. Eukaryot Cell 2:422–430

Bobbitt TF, Nordin JH (1978) Hyphal nigeran as a potential phylogenetic marker for *Aspergillus* and *Penicillium* species. Mycologia 70:1201–1211

Boorsma A, de Nobel H, ter Riet B, Bargmann B, Brul S, Hellingwerf KJ, Klis FM (2004) Characterization of the transcriptional response to cell wall stress in *Saccharomyces cerevisiae*. Yeast 21:413–427

Bottom CB, Siehr DJ (1980) Structure and composition of the alkali-insoluble cell wall fraction of *Coprinus macrorhizus var. microsporus*. Can J Biochem 58:147–153

Bowen AR, Chen-Wu JL, Momany M, Young R, Szaniszlo PJ, Robbins PW (1992) Classification of fungal chitin synthases. Proc Natl Acad Sci USA 89:519–523

Breinig F, Schleinkofer K, Schmitt MJ (2004) Yeast Kre1p is GPI-anchored and involved in both cell wall assembly and architecture. Microbiology 150:3209–3218

Brown AJP (2002) Morphogenetic signalling pathways in *Candida albicans* In: Calderone RA (ed) Candida and candidiasis. ASM Press, Washington, DC, pp 95–106

Bruneau JM, Magnin T, Tagat E, Legrand R, Bernard M, Diaquin M, Fudali C, Latgé JP (2001) Proteome analysis of *Aspergillus fumigatus* identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis. Electrophoresis 22:2812–2823

Bruneteau M, Perret J, Rouhier P, Michel G (1992) Structure of β-D-glucans from *Fusarium oxysporum*. Carbohydr Res 236:345–348

Bulik DA, Olczak M, Lucero HA, Osmond BC, Robbins PW, Specht CA (2003) Chitin synthesis in *Saccharomyces cerevisiae* in response to supplementation of growth medium with glucosamine and cell wall stress. Eukaryot Cell 2:886–900

Bull AT (1970) Chemical composition of wild-type and mutant *Aspergillus nidulans* cell walls. The nature of polysaccharide and melanin constituents. J Gen Microbiol 63:75–94

Cabib E (1991) Differential inhibition of chitin synthetases 1 and 2 from *Saccharomyces cerevisiae* by polyoxin D and nikkomycins. Antimicrob Agents Chemother 35:170–173

Cabib E, Roh DH, Schmidt M, Crotti LB, Varma A (2001) The yeast cell wall and septum as paradigms of cell growth and morphogenesis. J Cell Biol 276:19679–19682

Calcagno AM, Bignell E, Rogers TR, Canedo M, Muhlschlegel FA, Haynes K (2004) *Candida glabrata* Ste20 is involved in maintaining cell wall integrity and adaptation to hypertonic stress, and is required for wild-type levels of virulence. Yeast 21:557–568

Calera JA, Calderone R (1999) Flocculation of hyphae is associated with a deletion in the putative CaHK1 two-component histidine kinase gene from *Candida albicans*. Microbiology 145:1431–1442

Calera JA, Zhao XJ, Calderone R (2000) Defective hyphal development and avirulence caused by a deletion of the SSK1 response regulator gene in *Candida albicans*. Infect Immun 68:518–525

Calonge TM, Nakano K, Arellano M, Arai R, Katayama S, Toda T, Mabuchi I, Perez P (2000) *Schizosaccharomyces pombe* Rho2p GTPase regulates cell wall a-glucan biosynthesis through the protein kinase Pck2p. Mol Biol Cell 11:4393–4401

Calonge TM, Arellano M, Coll PM, Perez P (2003) Rga5p is a specific Rho1p GTPase-activating protein that regulates cell integrity in *Schizosaccharomyces pombe*. Mol Microbiol 47:507–518

Caro LH, Tettelin H, Vossen JH, Ram AF, van den Ende H, Klis FM (1997) In silicio identification of glycosylphosphatidylinositol-anchored plasma-membrane and cell wall proteins of *Saccharomyces cerevisiae*. Yeast 13:1477–1489

Carotti C, Ragni E, Palomares O, Fontaine T, Tedeschi G, Rodriguez R, Latgé JP, Vai M, Popolo L (2004) Characterization of recombinant forms of the yeast Gas1 protein and identification of residues essential for glucanosyltransferase activity and folding. Eur J Biochem 271:3635–3645

Castillo L, Martinez AI, Garcera A, Elorza MV, Valentin E, Sentandreu R (2003) Functional analysis of the cysteine residues and the repetitive sequence of *Saccharomyces cerevisiae* Pir4/Cis3: the repetitive sequence is needed for binding to the cell wall β-1,3-glucan. Yeast 20:973–983

Castro O, Chen LY, Parodi AJ, Abeijon C (1999) Uridine diphosphate-glucose transport into the endoplasmic reticulum of *Saccharomyces cerevisiae*: *in vivo* and *in vitro* evidence. Mol Biol Cell 10:1019–1030

Catlett NL, Yoder OC, Turgeon BG (2003) Whole-genome analysis of two-component signal transduction genes in fungal pathogens. Eukaryot Cell 2:1151–1161

Chaffin WL, Lopez-Ribot JL, Casanova M, Gozalbo D, Martinez JP (1998) Cell wall and secreted proteins of *Candida albicans*: identification, function, and expression. Microbiol Mol Biol Rev 62:130–180

Chauhan N, Inglis D, Roman E, Pla J, Li D, Calera JA, Calderone R (2003) *Candida albicans* response regulator gene SSK1 regulates a subset of genes whose functions are associated with cell wall biosynthesis and adaptation to oxidative stress. Eukaryot Cell 2:1018–1024

Cherniak R, Morris LC, Meyer SA, Mitchell TB (1993) Glucuronoxylomannan of *Cryptococcus neoformans* obtained from patients with AIDS. Carbohydr Res 249:405–413

Choi WJ, Santos B, Duran A, Cabib E (1994) Are yeast chitin synthases regulated at the transcriptional or the post-translational level? Mol Cell Biol 14:7685–7694

Chung N, Mao C, Heitman J, Hannun YA, Obeid LM (2001) Phytosphingosine as a specific inhibitor of growth and nutrient import in *Saccharomyces cerevisiae*. J Biol Chem 276:35614–35621

Clemons KV, Miller TK, Selitrennikoff CP, Stevens DA (2002) *fos-1*, a putative histidine kinase as a virulence factor for systemic aspergillosis. Med Mycol 40:259–262

Coluccio A, Bogengruber E, Conrad MN, Dresser ME, Briza P, Neiman AM (2004) Morphogenetic pathway of spore wall assembly in *Saccharomyces cerevisiae*. Eukaryot Cell 3:1464–1475

Cortes JC, Ishiguro J, Duran A, Ribas JC (2002) Localization of the (1,3)beta-D-glucan synthase catalytic subunit homologue Bgs1p/Cps1p from fission yeast suggests that it is involved in septation, polarized growth, mating, spore wall formation and spore germination. J Cell Sci 115:4081–4096

Cos T, Ford RA, Trilla JA, Duran A, Cabib E, Roncero C (1998) Molecular analysis of Chs3p participation in chitin synthase III activity. Eur J Biochem 256:419–426

Crespo JL, Hall MN (2002) Elucidating TOR signalling and rapamycin action: lessons from *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 66:579–591

Cruz MC, Del Poeta M, Wang P, Wenger R, Zenke G, Quesniaux VF, Movva NR, Perfect JR, Cardenas ME, Heitman J (2000) Immunosuppressive and nonimmunosuppressive cyclosporine analogs are toxic to the opportunistic fungal pathogen *Cryptococcus neoformans* via cyclophilin-dependent inhibition of calcineurin. Antimicrob Agents Chemother 44:143–149

Daniels KJ, Lockhart SR, Staab JF, Sundstrom P, Soll DR (2003) The adhesin Hwp1 and the first daughter cell localize to the a/a portion of the conjugation bridge during *Candida albicans* mating. Mol Biol Cell 14:4920–4930

Da Silva MM, Polizeli ML, Jorge JA, Terenzi HF (1994) Cell wall deficiency in "slime" strains of *Neurospora crassa*: osmotic inhibition of cell wall synthesis and β-D-glucan synthase activity. Braz J Med Biol Res 27:2843–2857

Datema R, van den Ende H, Wessels JG (1977a) The hyphal wall of *Mucor mucedo* 1. Polyanionic polymers. Eur J Biochem 80:611–619

Datema R, van den Ende H, Wessels JG (1977b) The hyphal wall of *Mucor mucedo* 2. Hexosamin containing polymers. Eur J Biochem 80:621–626

Dean N (1999) Asparagine-linked glycosylation in the yeast Golgi. Biochim Biophys Acta 1426:309–322

De Groot PWJ, Ruiz C, Vazquez de Aldana CR, Duenas E, Cid VJ, del Rey F, Rodriguez-Pena JM, Pérez P, Andel A, Caubin J et al. (2001) A genomic approach for the identification and classification of genes involved in cell wall formation and its regulation in *Saccharomyces cerevisiae*. Comp Funct Genomics 2:124–142

De Groot PW, Hellingwer KJ, Klis FM (2003) Genome-wide identification of fungal GPI proteins. Yeast 20:781–796

Dekker N, Speijer D, Grun CH, van den Berg M, de Haan A, Hochstenbach F (2004) Role of the alpha-glucanase Agn1p in fission-yeast cell separation. Mol Biol Cell 15:3903–3914

Del Poeta M, Cruz MC, Cardenas ME, Perfect JR, Heitman J (2000) Synergistic antifungal activities of bafilomycin A(1), fluconazole, and the pneumocandin MK-0991/caspofungin acetate (L-743,873) with calcineurin inhibitors FK506 and L-685,818 against *Cryptococcus neoformans*. Antimicrob Agents Chemother 44:739–746

DeMarini DJ, Adams AEM, Fares H, De Virgilio C, Valle G, Chuang JS, Pringle JR (1997) A septin-based hierarchy of proteins required for localized deposition of chitin in the *Saccharomyces cerevisiae* cell wall. J Cell Biol 139:75–93

Denning DW (2003) Echinocandin antifungal drugs. Lancet 362:1142–1151

Dijkgraaf GJ, Abe M, Ohya Y, Bussey H (2002) Mutaions in Fks1p affect the cell wall content of β-1,3- and β-1,6-glucan in *Saccharomyces cerevisiae*. Yeast 19:671–690

Dixon KP, Xu JR, Smirnoff N, Talbot NJ (1999) Independent signaling pathways regulate cellular turgor during hyperosmotic stress and appressorium-mediated plant infection by *Magnaporthe grisea*. Plant Cell 11:2045–2058

Dohrmann PR, Butler G, Tamai K, Dorland S, Greene JR, Thiele DJ, Stillman DJ (1992) Parallel pathways of gene regulation: homologous regulators SW15 and ACE2 differentially control transcription of HO and chitinase. Genes Dev 6:93–104

Douglas CM (2001) Fungal β(1,3)-D-glucan synthesis. Med Mycol 39:55–66

Douglas CM, Marrinan JA, Li W, Kurtz MB (1994) A *Saccharomyces cerevisiae* mutant with echinocandin resistant β1,3-D-glucan synthase activity. J Bacteriol 176:5686–5696

Edlind TD, Katiyar SK (2004) The echinocandin "target" identified by cross-linking is a homolog of Pil1 and Lsp1, sphingolipid-dependent regulators of cell wall integrity signaling. Antimicrob Agents Chemother 48:4491

Eisenhaber B, Schneider G, Wildpaner M, Eisenhaber F (2004) A sensitive predictor for potential GPI lipid modification sites in fungal protein sequences and its application to genome-wide studies for *Aspergillus nidulans*, *Candida albicans*, *Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. J Mol Biol 19:243–253

El-Sherbeini M, Clemas JA (1995) Cloning and characterization of GNS1: a *Saccharomyces cerevisiae* gene involved in synthesis of 1,3-beta-glucan *in vitro*. J Bacteriol 177:3227–3234

Feoktistova A, Magnelli P, Abeijon C, Perez P, Lester RL, Dickson RC, Gould KL (2001) Coordination between fission yeast glucan formation and growth requires a sphingolipase activity. Genetics 158:1397–1411

Firon A, Beauvais A, Latgé JP, Couvé E, Grosjean-Cournoyer MC, D'Enfert C (2002) Characterization of essential genes by parasexual genetics in the human fungal pathogen *Aspergillus fumigatus*: impact of genomic rearrangements associated with electroporation of DNA. Genetics 161:1077–1087

Fitch PG, Gammie AE, Lee DJ, de Candal VB, Rose MD (2004) Lrg1p Is a Rho1 GTPase-activating protein required for efficient cell fusion in yeast. Genetics 168:733–746

Fleet GH (1985) Composition and structure of yeast cell walls. Curr Topics Med Mycol 1:24–56

Fleet GH (1991) Cell walls. In: Rose AH, Harrisson JD (eds) The yeast. Academic Press, New York, pp 199–277

Fleet GH, Manners DJ (1976) Isolation and composition of an alkali-soluble glucan from the cell walls of *Saccharomyces cerevisiae*. J Gen Microbiol 94:180–192

Fleet GH, Manners DJ (1977) The enzymic degradation of an alkali-soluble glucan from the cell walls of *Saccharomyces cerevisiae*. J Gen Microbiol 98:315–327

Fontaine T, Simenel C, Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M, Latgé JP (2000) Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. J Biol Chem 275:27594–27607

Frieman MB, Cormack BP (2004) Multiple sequence signals determine the distribution of glycosylphosphatidylinositol proteins between the plasma membrane and cell wall in *Saccharomyces cerevisiae*. Microbiology 150:3105–3114

Fujii T, Shimoi H, Iimura Y (1999) Structure of the glucan-binding sugar chain of Tip1p, a cell wall protein of *Saccharomyces cerevisiae*. Biochim Biophys Acta 1427:133–144

Fukamizo T, Honda Y, Toyoda H, Ouchi S, Goto S (1996) Chitinous component of the cell wall of *Fusarium oxysporum*, its structure deduced from chitosanase digestion. Biosci Biotechnol Biochem 60:1705–1708

Fukazawa Y, Kagaya K, Shinoda T (1995) Cell wall polysaccharides of pathogenic yeasts. Curr Topics Med Mycol 6:189–219

Furukawa K, Katsuno Y, Urao T, Yabe T, Yamada-Okabe T, Yamada-Okabe H, Yamagata Y, Abe K, Nakajima T (2002) Isolation and functional analysis of a gene, *tcsB*, encoding a transmembrane hybrid-type histidine kinase from *Aspergillus nidulans*. Appl Environ Microbiol 68:5304–5310

Gardiner RE, Souteropoulos P, Park S, Perlin DS (2005) Characterization of *Aspergillus fumigatus* mutants with reduced susceptibility to caspofungin. Med Mycol 43 (in press)

Gaughran JP, Lai MH, Kirsch DR, Silverman SJ (1994) Nikkomycin Z is a specific inhibitor of *Saccharomyces cerevisiae* chitin synthase isozyme Chs3 *in vitro* and *in vivo*. J Bacteriol 176:5857–5860

Goldman RC, Sullivan PA, Zakula D, Capobianco JO (1995) Kinetics of β-1,3 glucan interaction at the donor and acceptor sites of the fungal glucosyltransferase encoded by the *BGL2* gene. Eur J Biochem 227:372–378

Green R, Lesage G, Sdicu AM, Menard P, Bussey H (2003) A synthetic analysis of the *Saccharomyces cerevisiae* stress sensor Mid2p, and identification of a Mid2p-interacting protein, Zeo1p, that modulates the PKC1-MPK1 cell integrity pathway. Microbiology 149:2487–2499

Grün CH, Hochstenbach F, Humbel BM, Verkleij AJ, Sietsma JH, Klis FM, Kamerling JP, Vliegenthart JFG (2005) The structure of cell wall a-glucan from fission yeast. Glycobiology 5:245–57

Guest GM, Lin X, Momany M (2004) *Aspergillus nidulans* RhoA is involved in polar growth, branching, and cell wall synthesis. Fungal Genet Biol 41:13–22

Gustin MC, Albertyn J, Alexander M, Davenport K (1998) MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 62:1264–1300

Hamada K, Fukuchi S, Arisawa M, Baba M, Kitada K (1998) Screening for glycosylphosphatidylinositol (GPI)-dependent cell wall proteins in *Saccharomyces cerevisiae.* Mol Gen Genet 258:53–59

Harrison JC, Zyla TR, Bardes ES, Lew DJ (2004) Stress-specific activation mechanisms for the "cell integrity" MAPK pathway. J Biol Chem 279:2616–2622

Hartland RP, Vermeulen CA, Klis FM, Sietsma JH, Wessels JG (1994) The linkage of (1-3)-β-glucan to chitin during cell wall assembly in *Saccharomyces cerevisiae*. Yeast 10:1591–1599

Heinisch JJ, Lorberg A, Schmitz HP, Jacoby JJ (1999) The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in *Saccharomyces cerevisiae*. Mol Microbiol 32:671–680

Hirata D, Nakano K, Fukui M, Takenaka H, Miyakawa T, Mabuchi I (1998) Genes that cause aberrant cell morphology by overexpression in fission yeast: a role of a small GTP-binding protein Rho2 in cell morphogenesis. J Cell Sci 111:149–159

Hiura N, Nakajima T, Matsuda K (1983) Two cell wall β-D-glucans from *Neurospora crassa*. Agric Biol Chem 47:1317–1322

Hochstenbach F, Klis FM, van den Ende H, Van Donselaar E, Peters PJ, Klausner RD (1998) Identification of a putative a-glucan synthase essential for cell wall construction and morphogenesis in fission yeast. Proc Natl Acad Sci USA 95:9161–9166

Hoddinott J, Olsen OA (1972) A study of the carbohydrates in the cell walls of some species of the Entomophthorales. Can J Bot 50:1675–1679

Hogan LH, Klein BS (1994) Altered expression of surface α-1,3-glucan in genetically related strains of *Blastomyces dermatitidis* that differ in virulence. Infect Immun 62:3543–3546

Horiuchi H, Fujiwara M, Yamashita S, Ohta A, Takagi M (1999) Proliferation of intrahyphal hyphae caused by disruption of *csm*A, which encodes a class V chitin synthase with a myosin motor-like domain in *Aspergillus nidulans*. J Bacteriol 181:3721–3729

Houston DR, Eggleston I, Synstad B, Eijsink VG, van Aalten DM (2002) The cyclic dipeptide CI-4 [cyclo-(l-Arg-d-Pro)] inhibits family 18 chitinases by structural mimicry of a reaction intermediate. Biochem J 368:23–27

Humbel BM, Konomi M, Takagi T, Kamasawa N, Ishijima SA, Osumi M (2001) *In situ* localization of beta-glucans in the cell wall of *Schizosaccharomyces pombe*. Yeast 18:433–444

Inoue SB, Qadota H, Arisawa M, Watanabe T, Ohya Y (1999) Prenylation of Rho1p is required for activation of yeast 1,3-β-glucan synthase. J Biol Chem 274:38119–38124

Ishiguro J, Saitou A, Duran A, Ribas JC (1997) *cps1*$^{+}$, a *Schizosaccharomyces pombe* gene homolog of *Saccharomyces cerevisiae* FKS genes whose mutation confers hypersensitivity to cyclosporin A and papulacandin B. J Bacteriol 179:7653–7662

Jacques AK, Fukamizo T, Hall D, Barton RC, Escott GM, Parkinson T, Hitchcock CA, Adams DJ (2003) Disruption of the gene encoding the ChiB1 chitinase of *Aspergillus fumigatus* and characterization of a recombinant gene product. Microbiology 149:2931–2939

James PJ, Cherniak R, Jones RG (1990) Cell-wall glucans of *Cryptococcus neoformans* Cap67. Carbohydr Res 198:23–38

Jikibara T, Takegawa K, Iwahara S (1992) Studies on the uronic acid-containing glycoproteins of *Fusarium* sp. M7-1. I. Isolation and some properties of the glycoproteins. J Biochem 111:225–229

Jung US, Levin DE (1999) Genome-wide analysis of gene expression regulated by the yeast cell wall integrity signalling pathway. Mol Microbiol 34:1049–1057

Kapteyn JC, Montijn RC, Dijkgraaf GJ, van den Ende H, Klis FM (1995) Covalent association of β-1,3-glucan with b-1,6-glucosylated mannoproteins in cell walls of *Candida albicans*. J Bacteriol 177:3788–3792

Kapteyn JC, Montijn RC, Vink E, de la Cruz J, Llobell A, Douwes JE, Shimoi H, Lipke PN, Klis FM (1996) Retention of *Saccharomyces cerevisiae* cell wall proteins though a phosphodiester-linked β-1,3-/β-1,6-glucan heteropolymer. Glycobiology 6:337–345

Kapteyn JC, Ram AFJ, Groos EM, Kollar R, Montijn RC, van den Ende H, Llobell A, Cabib E, Klis FM (1997) Altered extent of cross-linking of β1,6-glucosylated mannoproteins to chitin in *Saccharomyces cerevisiae* mutant with reduced cell wall β1,3-glucan content. J Bacteriol 179:6279–6284

Kapteyn JC, van den Ende H, Klis FM (1999) The contribution of cell wall proteins to the organization of the yeast cell wall. Biochim Biophys Acta 1426:373–383

Kapteyn JC, Hoyer LL, Hecht JE, Muller WH, Andel A, Verkleij AJ, Makarow M, Van Den Ende H, Klis FM (2000) The cell wall architecture of *Candida albicans* wild-type cells and cell wall-defective mutants. Mol Microbiol 35:601–611

Katayama S, Hirata D, Arellano M, Perez P, Toda T (1999) Fission yeast a-glucan synthase Mok1 requires the actin cytoskeleton to localize the sites of growth and plays an essential role in cell morphogenesis downstream of protein kinase C function. J Cell Biol 144:1173–1186

Kawasaki L, Sanchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45:1153–1163

King L, Butler G (1998) Ace2p, a regulator of CTS1 (chitinase) expression, affects pseudohyphal production in *Saccharomyces cerevisiae*. Curr Genet 34:183–191

Kitagaki H, Wu H, Shimoi H, Ito K (2002) Two homologous genes, DCW1 (YKL046c) and DFG5, are essential for cell growth and encode glycosylphosphatidylinositol (GPI)-anchored membrane proteins required for cell wall biogenesis in *Saccharomyces cerevisiae*. Mol Microbiol 46:1011–1122

Kitagaki H, Ito K, Shimoi H (2004) A temperature-sensitive *dcw1* mutant of *Saccharomyces cerevisiae* is cell cycle arrested with small buds which have aberrant cell walls. Eukaryot Cell 3:1297–1306

Klimpel KR, Goldman WE (1988) Cell walls from avirulent variants of *Histoplasma capsulatum* lack α-(1,3)-glucan. Infect Immun 56:2997–3000

Klis FM (1994) Review: cell wall assembly in yeast. Yeast 10:851–869

Klis FM, Mol P, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 26:239–256

Kollar R, Petrakova E, Ashwell G, Robbins PW, Cabib E (1995) Architecture of the yeast cell wall. The linkage between chitin and β(1-3)-glucan. J Biol Chem 270:1170–1178

Kollar R, Reinhold BB, Petrakova E, Yeh HJC, Ashwell G, Drgonova J, Kapteyn JC, Klis FM, Cabib E (1997) Architecture of the yeast cell wall: b-1-6-glucan interconnects mannoprotein, β(1-3)-glucan, and chitin. J Biol Chem 272:17762–17788

Konomi M, Fujimoto K, Toda T, Osumi M (2003) Characterization and behaviour of α-glucan synthase in *Schizosaccharomyces pombe* as revealed by electron microscopy. Yeast 20:427–438

Kopecka M, Fleet GH, Phaff HJ (1995) Ultrastructure of the cell wall of *Schizosaccharomyces pombe* following treatment with various glucanases. J Struct Biol 114:140–152

Kraus PR, Heitman J (2003) Coping with stress: calmodulin and calcineurin in model and pathogenic fungi. Biochem Biophys Res Commun 311:1151–1157

Kraus PR, Fox DS, Cox GM, Heitman J (2003) The *Cryptococcus neoformans* MAP kinase Mpk1 regulates cell integrity in response to antifungal drugs and loss of calcineurin function. Mol Microbiol 48:1377–1387

Kroh M, Knuiman B, Kirby EG, Sassen MM (1977) Cell wall formation in zoospores of *Allomyces arbuscula*. III. Carbohydrate composition of cell walls during development from meiospores to hyphae. Arch Microbiol 113:73–78

Kruppa M, Goins T, Williams D, Li D, Chauhan N, Singh P, Cutler J, Calderone R (2003) The *Chk1p* of *Candida albicans* and its role in the regulation of cell wall synthesis. FEMS Yeast Res 3:289–299

Kuranda MJ, Robbins PW (1991) Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. J Biol Chem 266:19758–19767

Kurtz MB, Abruzzo G, Flattery A, Bartizal K, Marrinan JA, Li W, Milligan J, Nollstadt K, Douglas CM (1996) Characterization of echinocandin-resistant mutants of *Candida albicans*: genetic, biochemical, and virulence studies. Infect Immun 64:3244–3251

Lagorce A, Le Berre-Anton V, Aguilar-Uscanga B, Martin-Yken H, Dagkessamanskaia A, Francois J (2002) Involvement of GFA1, which encodes glutamine-fructose-6-phosphate amidotransferase, in the activation of the chitin synthesis pathway in response to cell-wall defects in *Saccharomyces cerevisiae*. Eur J Biochem 269:1697–1707

Lagorce A, Hauser NC, Labourdette D, Rodriguez C, Martin-Yken H, Arroyo J, Hoheisel JD, Francois J (2003) Genome-wide analysis of the response to cell wall mutations in the yeast *Saccharomyces cerevisiae*. J Biol Chem 278:20345–20357

Latgé JP, Beauvais A (1987) Wall composition of the protoplastic Entomophthorales. J Invert Pathol 50:53–57

Latgé JP, Fournet B, Cole G, Dubourdiau D, Tong N (1984) Composition chimique et ultrastructurale des parois de corps hyphaux et des azygospores de *Conidiobolus obscurus*. Can J Microbiol 30:1507–1521

Latgé JP, Bouziane H, Diaquin M (1988) Ultrastructure and composition of the conidial wall of *Cladosporium cladosporioides*. Can J Microbiol 34:1325–1329

Latgé JP, Kobayashi H, Debeaupuis JP, Diaquin M, Sarfati J, Wieruszeski JM, Parra E, Bouchara JP, Fournet B (1994) Chemical and immunological characterization of the extracellular galactomannan of *Aspergillus fumigatus*. Infect Immun 62:5424–5433

Latgé JP, Mouyna I, Tekaia F, Beauvais A, Debeaupuis JP, Nierman W (2005) Specific molecular features in the organization and biosynthesis of the cell wall of *Aspergillus fumigatus*. Med Mycol 43 (in press)

Leal JA, Jimenez-Barbero J, Gomez-Miranda B, Prieto A, Domenech J, Bernabe M (1996) Structural investigation of a cell-wall galactomannan from *Neurospora crassa* and *Neurospora sitophila*. Carbohydr Res 283:215–222

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen WC, Wang P, Pan X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Lesage G, Sdicu AM, Menard P, Shapiro J, Hussein S, Bussey H (2004) Analysis of β-1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. Genetics 167:35–49

Li D, Bernhardt J, Calderone R (2002) Temporal expression of the *Candida albicans* genes esophageal epithelial candidiasis. Infect Immun 70:1558–1565

Liu J, Wang H, McCollum D, Balasubramanian MK (1999) Drc1p/Cps1p, a 1,3-β-glucan synthase subunit, is essential for division septum assembly in *Schizosaccharomyces pombe*. Genetics 153:1193–1203

Liu J, Tang X, Wang H, Balasubramanian M (2000) Bgs2p, a 1,3-beta-glucan synthase subunit, is essential for maturation of ascospore wall in *Schizosaccharomyces pombe*. FEBS Lett 478:105–108

Liu H, Wang Z, Zheng L, Hauser M, Kauffman S, Becker JM (2001) Relevance of chitin and chitin synthases to virulence in *Wangiella* (*Exophiala*) *dermatitidis* – a model melanized pathogen of humans. In: Muzzarelli RAA (ed) Chitin enzymology. Atec Edizioni, Grottammare, Italy, pp 463–472

Liu J, Tang X, Wang H, Oliferenko S, Balasubramanian MK (2002) The localization of the integral membrane protein Cps1p to the cell division site is dependent on the actomyosin ring and the septation-inducing network in *Schizosaccharomyces pombe*. Mol Biol Cell 13:989–1000

Lowman DW, Ferguson DA, Williams DL (2003) Structural characterization of (1–>3)-β-D-glucans isolated from blastospore and hyphal forms of *Candida albicans*. Carbohydr Res 338:1491–1496

Machi K, Azuma M, Igarashi K, Matsumoto T, Fukuda H, Kondo A, Ooshima H (2004) Rot1p of *Saccharomyces cerevisiae* is a putative membrane protein required for normal levels of the cell wall 1,6-β-glucan. Microbiology 150:3163–3173

Manners DJ, Meyer MT (1977) The molecular structures of some glucans from the cell walls of *Schizosaccharomyces pombe*. Carbohydr Res 57:187–203

Manners DJ, Masson AJ, Patterson JC (1973a) The structure of a β-(1-3)-D-glucan from yeast cell walls. Biochem J 135:19–30

Manners DJ, Masson AJ, Patterson JC, Bjorndal H, Lindberg B (1973b) The structure of a β-(1–6)-D-glucan from yeast cell walls. Biochem J 135:31–36

Manners DJ, Masson AJ, Patterson JC (1974) The heterogeneity of glucan preparations from the walls of various yeasts. J Gen Microbiol 80:411–417

Markovich S, Yekutiel A, Shalit I, Shadkchan Y, Osherov N (2004) Genomic approach to identification of mutations affecting caspofungin susceptibility in *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 48:3871–3876

Martin V, Ribas JC, Carnero E, Duran A, Sanchez Y (2000) *bgs2*+, a sporulation-specific glucan synthase homologue is required for proper ascospore wall maturation in fission yeast. Mol Microbiol 38:308–321

Martin V, Garcia B, Carnero E, Duran A, Sanchez Y (2003) Bgs3p, a putative 1,3-beta-glucan synthase subunit, is required for cell wall assembly in *Schizosaccharomyces pombe*. Eukaryot Cell 2:159–169

Martin-Cuadrado AB, Duenas E, Sipiczki M, Vazquez de Aldana CR, del Rey F (2003) The endo-b-1,3-glucanase eng1p is required for dissolution of the primary septum during cell separation in *Schizosaccharomyces pombe*. J Cell Sci 116:1689–1698

Martin-Garcia R, Duran A, Valdivieso MH (2003) In *Schizosaccharomyces pombe* chs2p has no chitin synthase activity but is related to septum formation. FEBS Lett 549:176–180

Martin-Yken H, Dagkessamanskaia A, Basmaji F, Lagorce A, Francois J (2003) The interaction of Slt2 MAP kinase with Knr4 is necessary for signalling through the cell wall integrity pathway in *Saccharomyces cerevisiae*. Mol Microbiol 49:23–35

Masubuchi K, Taniguchi M, Umeda I, Hattori K, Suda H, Kohchi Y, Isshiki Y, Sakai T, Kohchi M, Shirai M et al. (2000) Synthesis and structure-activity relationships of novel fungal chitin synthase inhibitors. Bioorg Med Chem Lett 10:1459–1462

Masuoka J (2004) Surface glycans of *Candida albicans* and other pathogenic fungi: physiological roles, clinical uses, and experimental challenges. Clin Microbiol Rev 17:281–310

Matheos DP, Kingsbury TJ, Ahsan US, Cunningham KW (1997) Tcn1p/Crz1p, a calcineurin-dependent transcription factor that differentially regulates gene expression in *Saccharomyces cerevisiae*. Genes Dev 11:3445–3458

Matsuo Y, Tanaka K, Nakagawa T, Matsuda H, Kawamukai M (2004) Genetic analysis of *chs1*+ and *chs2*+ encoding chitin synthases from *Schizosaccharomyces pombe*. Biosci Biotechnol Biochem 68:1489–1499

Mazur P, Morin N, Baginsky W, el-Sherbeini M, Clemas JA, Nielsen JB, Foor F (1995) Differential expression and function of two homologous subunits of yeast 1,3-β-D-glucan synthase. Mol Cell Biol 15:5671–5681

Mellado E, Aufauvre-Brown A, Gow NAR, Holden DW (1996) The *Aspergillus fumigatus chsC* and *chsG* genes encode Class III chitin synthases with different functions. Mol Microbiol 20:667–679

Mellado E, Dubreucq G, Mol P, Sarfati J, Paris S, Diaquin M, Holden DW, Rodriguez-Tudela JL, Latgé JP (2003) Cell wall biogenesis in a double chitin synthase mutant (*chsG-/chsE-*) of *Aspergillus fumigatus*. Fungal Genet Biol 38:98–109

Merkel O, Fido M, Mayr JA, Pruger H, Raab F, Zandonella G, Kohlwein SD, Paltauf F (1999) Characterization and function *in vivo* of two novel phospholipases B/lysophospholipases from *Saccharomyces cerevisiae*. J Biol Chem 274:28121–28127

Michalenko GO, Hohl H, Rast D (1976) Chemistry and architecture of the mycelial wall of *Agaricus bisporus*. J Gen Microbiol 92:251–262

Miyazaki T, Irino T (1970) Acidic polysaccharides from the cell wall of *Absidia cylindrospora*, *Mucor mucedo*, and *Rhizopus nigricans*. Chem Pharm Bull 18:1930–1931

Miyazaki T, Irino T (1971) Studies on fungal polysaccharides. IX. The acidic polysaccharide from the cell wall of *Rhizopus nigricans*. Chem Pharm Bull 19:2545–2550

Moukadiri I, Zueco J (2001) Evidence for the attachment of Hsp150/Pir2 to the cell wall of *Saccharomyces cerevisiae* through disulfide bridges. FEMS Yeast Res 1:241–245

Mouyna I, Latgé JP (2001) Cell wall of *Aspergillus fumigatus*: Structure, biosynthesis and role in host-fungus interactions. In: Cilhar R, Calderone R (eds) Fungal pathogenesis: principles and clinical applications. Dekker, New York, pp 515–537

Mouyna I, Hartland RP, Fontaine T, Diaquin M, Latgé JP (1998) A b(1-3) glucanosyltransferase isolated from the cell wall of *Aspergillus fumigatus* is an homolog of the yeast Bgl2p. Microbiology 144:3171–3180

Mouyna I, Monod M, Fontaine T, Henrissat B, Léchenne B, Latgé JP (2000a) Identification of the catalytic residues of the first family of β(1-3)glucanosyltransferases identified in fungi. Biochem J 347:741–747

Mouyna I, Fontaine T, Vai M, Monod M, Fonzi WA, Diaquin M, Popolo L, Hartland RP, Latgé JP (2000b) GPI-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. J Biol Chem 275:14882–14889

Mouyna I, Sarfati J, Recco P, Fontaine T, Henrissat B, Latgé JP (2002) Molecular characterization of a cell wall-associated β(1-3) endoglucanase of *Aspergillus fumigatus*. Med Mycol 40:455–464

Mouyna I, Henry C, Doering TL, Latgé JP (2004) Gene silencing with RNA interference in the human pathogenic fungus *Aspergillus fumigatus*. FEMS Microbiol Lett 237:317–324

Moye-Rowley WS (2003) Regulation of the transcriptional response to oxidative stress in fungi: similarities and differences. Eukaryot Cell 2:381–389

Mrsa V, Tanner W (1999) Role of NaOH-extractable cell wall proteins Ccw5p, Ccw6p, Ccw7p and Ccw8p (members of the Pir protein family) in stability of the *Saccharomyces cerevisiae* cell wall. Yeast 15:813–820

Mühlschlegel FA, Fonzi WA (1997) *PHR2* of *Candida albicans* encodes a functional homolog of the pH-regulated gene *PHR1* with an inverted pattern of pH-dependent expression. Mol Cell Biol 17:5960–5967

Munoz P, Norambuena L, Orellana A (1996) Evidence for a UDP-glucose transporter in Golgi apparatus-derived vesicles from pea and its possible role in polysaccharide biosynthesis. Plant Physiol 112:1585–1594

Munro CA, Gow NA (2001) Chitin synthesis in human pathogenic fungi. Med Mycol 39:41–53

Munro CA, Winter K, Buchan A, Henry K, Becker JM, Brown AJ, Bulawa CE, Gow NA (2001) Chs1 of *Candida albicans* is an essential chitin synthase required for synthesis of the septum and for cell integrity. Mol Microbiol 39:1414–1426

Nagahashi S, Sudoh M, Ono N, Sawada R, Yamaguchi E, Uchida Y, Mio T, Takagi M, Arisawa M, Yamada-Okabe H (1995) Characterization of chitin synthase 2 of *Saccharomyces cerevisiae*. Implication of two highly conserved domains as possible catalytic sites. J Biol Chem 270:13961–13967

Navarro-Garcia F, Sanchez M, Pla J, Nombela C (1995) Functional characterization of the MKC1 gene of *Candida albicans*, which encodes a mitogen-activated protein kinase homolog related to cell integrity. Mol Cell Biol 15:2197–2206

Navarro-Garcia F, Alonso-Monge R, Rico H, Pla J, Sentandreu R, Nombela C (1998) A role for the MAP kinase gene *MKC1* in cell wall construction and morphological transitions in *Candida albicans*. Microbiology 144:411–424

Nelson B, Parsons AB, Evangelista M, Schaefer K, Kennedy K, Ritchie S, Petryshen TL, Boone C (2004) Fus1p interacts with components of the Hog1p mitogen-activated protein kinase and Cdc42p morphogenesis signaling pathways to control cell fusion during yeast mating. Genetics 166:67–77

Nguyen TH, Fleet GH, Rogers PL (1998) Composition of the cell walls of several yeast species. Appl Microbiol Biotechnol 50:206–212

Nollstadt KH, Powles MA, Fujioka H, Aikawa M, Schmatz DM (1994) Use of β-1,3-glucan-specific antibody to study the cyst wall of *Pneumocystis carinii* and effects of pneumocandin B0 analog L-733,560. Antimicrob Agents Chemother 38:2258–2265

Nyfeler R, Keller-Schierlein W (1974) Metabolites of microorganisms. 143. Echinocandin B, a novel polypeptide-antibiotic from *Aspergillus nidulans* var. *echinulatus*: isolation and structural componenst. Helv Chim Acta 57:2459–2477

Obi K, Uda J, Iwase K, Sugimoto O, Ebisu H, Matsuda A (2000) Novel nikkomycin analogues: inhibitors of the fungal cell wall biosynthesis enzyme chitin synthase. Bioorg Med Chem Lett 10:1451–1454

Odom A, Muir S, Lim E, Toffaletti DL, Perfect J, Heitman J (1997) Calcineurin is required for virulence of *Cryptococcus neoformans*. EMBO J 16:2576–2589

Olsen V, Cawley NX, Brandt J, Egel-Mitani M, Loh YP (1999) Identification and characterization of *Saccharomyces cerevisiae* yapsin 3, a new member of the yapsin family of aspartic proteases encoded by the YPS3 gene. Biochem J 339:407–411

Onishi J, Meinz M, Thompson J, Curotto J, Dreikorn S, Rosenbach M, Douglas C, Abruzzo G, Flattery A, Kong L et al. (2000) Discovery of novel antifungal (1,3)-beta-D-glucan synthase inhibitors. Antimicrob Agents Chemother 44:368–377

O'Rourke SM, Herskowitz I, O'Shea EK (2002) Yeast go the whole HOG for the hyperosmotic response. Trends Genet 18:405–412

Orpin CG (1977) The occurrence of chitin in the cell walls of the rumen organisms *Neocallimastix frontalis*, *Piromonas communis* and *Sphaeromonas communis*. J Gen Microbiol 99:215–218

Palecek SP, Parikh AS, Kron SJ (2002) Sensing, signalling and integrating physical processes during *Saccharomyces cerevisiae* invasive and filamentous growth. Microbiology 148:893–907

Papanikolau Y, Tavlas G, Vorgias CE, Petratos K (2003) *De novo* purification scheme and crystallization conditions yield high-resolution structures of chitinase A and its complex with the inhibitor allosamidin. Acta Crystallogr D Biol Crystallogr 59:400–403

Park BC, Park YH, Park HM (2003) Activation of *chsC* transcription by AbaA during asexual development of *Aspergillus nidulans*. FEMS Microbiol Lett 220:241–246

Perez P, Ribas JC (2004) Cell wall analysis. Methods 33:245–251

Philip B, Levin DE (2001) Wsc1 and Mid2 are cell surface sensors for cell wall integrity signaling that act through Rom2, a guanine nucleotide exchange factor for Rho1. Mol Cell Biol 21:271–280

Philippe B, Ibrahim-Granet O, Prévost MC, Gougerot-Pocidalo MA, Roes J, Sanchez-Perez M, Van der Meeren A, Latgé JP (2003) Killing of *Aspergillus fumigatus* by alveolar macrophages is mediated by reactive oxidant intermediates. Infect Immun 71:3034–3042

Popolo L, Vai M (1999) The Gas1 glycoprotein, a putative wall polymer cross-linker. Biochim Biophys Acta 1426:385–400

Popolo L, Gualtieri T, Ragni E (2001) The yeast cell-wall salvage pathway. Med Mycol 39:111–121

Pott GB, Miller TK, Bartlett JA, Palas JS, Selitrennikoff CP (2000) The isolation of FOS-1, a gene encoding a putative two-component histidine kinase from *Aspergillus fumigatus*. Fungal Genet Biol 31:55–67

Rao FV, Houston DR, Boot RG, Aerts JMF, Hodkinson M, Adams DJ, Shiomi K, Omura S, van Aalten DM (2005) Specificity and affinity of natural product cyclopentapeptide inhibitors against *Aspergillus fumigatus*, human and bacterial chitinases. Chem Biol 12:65–76

Reese AJ, Doering TL (2003) Cell wall α-1,3-glucan is required to anchor the *Cryptococcus neoformans* capsule. Mol Microbiol 50:1401–1409

Reinoso-Martin C, Schyller C, Schuetzer-Muehlbauer M, Kuchler K (2003) The yeast protein kinase C cell integrity pathway mediates tolerance to the antifungal drug caspofungin through activation of Slt2p mitogen-activated protein kinase signaling. Eukaryot Cell 2:1200–1210

Reiss E (1986) Molecular immunology of mycotic and actinomycotic infections. Elsevier, Amsterdam

Roberts CJ, Nelson B, Marton MJ, Stoughton R, Meyer MR, Bennett HA, He YD, Dai H, Walker WL, Hughes TR et al. (2000) Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. Science 287:873–880

Rodriguez-Pena JM, Cid VJ, Arroyo J, Nombela C (2000) A novel family of cell wall-related proteins regulated differently during the yeast life cycle. Mol Cell Biol 20:3245–3255

Rohde J, Heitman J, Cardenas ME (2001) The TOR kinases link nutrient sensing to cell growth. J Biol Chem 276:9583–9586

Roncero C (2002) The genetic complexity of chitin synthesis in fungi. Curr Genet 41:367–378

Ruiz C, Cid VJ, Lussier M, Molina M, Nombela C (1999) A large-scale sonication assay for cell wall mutant analysis in yeast. Yeast 15:1001–1008

Ruiz-Herrera J, Leon CG, Carabez-Trejo A, Reyes-Salinas E (1996) Structure and chemical composition of the cell walls from the haploid yeast and mycelial forms of *Ustilago maydis*. Fungal Genet Biol 20:133–142

San-Blas G, San-Blas F, Serrano LE (1977) Host-parasite relationships in the yeast like form of *Paracoccidioides brasiliensis* strain IVIC Pb9. Infect Immun 15:343–346

Sanglard D, Ischer F, Marchetti O, Entenza J, Bille J (2003) Calcineurin A of *Candida albicans*: involvement in antifungal tolerance, cell morphogenesis and virulence. Mol Microbiol 48:959–976

Santos JL, Shiozaki K (2001) Fungal histidine kinases. Sci STKE 2001:RE1

Santos B, Snyder M (1997) Targeting of chitin synthase 3 to polarized growth sites in yeast requires Chs5p and Myo2p. J Cell Biol 136:95–110

Santos B, Duran A, Valdivieso H (1997) *CHS5*, a gene involved in chitin synthesis and mating in *Saccharomyces cerevisiae*. Mol Cell Biol 17:2485–2496

Santos B, Gutierrez J, Calonge TM, Perez P (2003) Novel Rho GTPase involved in cytokinesis and cell wall integrity in the fission yeast *Schizosaccharomyces pombe*. Eukaryot Cell 2:521–533

Sanz M, Trilla JA, Duran A, Roncero C (2002) Control of chitin synthesis through Shc1p, a functional homologue of Chs4p specifically induced during sporulation. Mol Microbiol 43:1183–1195

Sanz M, Castrejon F, Duran A, Roncero C (2004) *Saccharomyces cerevisiae* Bni4p directs the formation of the chitin ring and also participates in the correct assembly of the septum structure. Microbiology 150:3229–3241

Saporito-Irwin SM, Birse CE, Sypherd PS, Fonzi WA (1995) PHR1, a pH-regulated gene of *Candida albicans*, is required for morphogenesis. Mol Cell Biol 15:601–613

Schaefer HP (1977) An alkali-soluble polysaccharide from the cell walls of *Coprinus lagopus*. Arch Microbiol 113:79–82

Schmidt M (2004) Survival and cytokinesis of *Saccharomyces cerevisiae* in the absence of chitin. Microbiology 150:3253–3260

Schmidt A, Hall A (2002) Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. Genes Dev 16:1587–1609

Schmidt M, Bowers B, Varma A, Roh DH, Cabib E (2002) In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. J Cell Sci 115:293–302

Schoffelmeer EA, Klis FM, Sietsma JH, Cornelissen BJ (1999) The cell wall of *Fusarium oxysporum*. Fungal Genet Biol 27:275–282

Sekiya-Kawasaki M, Abe M, Saka A, Watanabe D, Kono K, Minemura-Asakawa M, Ishihara S, Watanabe T, Ohya Y (2002) Dissection of upstream regulatory components of the Rho1p effector, 1,3-β-glucan synthase, in *Saccharomyces cerevisiae*. Genetics 162:663–676

Selvaggini S, Munro CA, Paschoud S, Sanglard D, Gow NA (2004) Independent regulation of chitin synthase and chitinase activity in *Candida albicans* and *Saccharomyces cerevisiae*. Microbiology 150:921–928

Shahinian S, Bussey H (2000) β-1,6-Glucan synthesis in *Saccharomyces cerevisiae*. Mol Microbiol 35:477–489

Shahinian S, Dijkgraaf GJ, Sdicu AM, Thomas DY, Jakob CA, Aebi M, Bussey H (1998) Involvement of protein *N*-glycosyl chain glucosylation and processing in the biosynthesis of cell wall β-1,6-glucan of *Saccharomyces cerevisiae*. Genetics 149:843–856

Shaw JA, Mol PC, Bowers B, Silverman SJ, Valdivieso MH, Duran A, Cabib E (1991) The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J Cell Biol 114:111–123

Sietsma JH, Wessels JG (1977) Chemical analysis of the hyphal wall of *Schizophyllum commune*. Biochim Biophys Acta 496:225–239

Sietsma JH, Wessels JGH (1979) Evidence for covalent linkages between chitin and β-glucan in a fungal wall. J Gen Microbiol 1979:99–108

Sietsma JH, Wessels JG (1981) Solubility of (1,3)-β-D/(1,6)-β-D-glucan in fungal walls: importance of presumed linkage between glucan and chitin. J Gen Microbiol 125:209–212

Sikkema WD, Lovett JS (1984) Cell wall composition of the aquatic fungus *Blastocladiella emersonii*. Exp Mycol 8:225–237

Singh P, Chauhan N, Ghosh A, Dixon F, Calderone R (2004) *SKN7* of *Candida albicans*: mutant construction and phenotype analysis. Infect Immun 72:2390–2394

Skucas GP (1967) Structure and composition of the resistant sporangial wall in the fungus allomyces. Am J Bot 54:1152–1158

Smits GJ, Kapteyn JC, van den Ende H, Klis FM (1999) Cell wall dynamics in yeast. Curr Opin Microbiol 2:348–352

Sprague GF, Cullen PJ, Goehring AS (2004) Yeast signal transduction: regulation and interface with cell biology. Adv Exp Med Biol 547:91–105

Spreghini E, Davis DA, Subaran R, Kim M, Mitchell AP (2003) Roles of *Candida albicans* Dfg5p and Dcw1p cell surface proteins in growth and hypha formation. Eukaryot Cell 2:746–755

Steinbach WJ, Singh N, Miller JL, Benjamin DK Jr, Schell WA, Heitman J, Perfect JR (2004) *In vitro* interactions between antifungals and immunosuppressants against *Aspergillus fumigatus* isolates from transplant and nontransplant patients. Antimicrob Agents Chemother 48:4922–4925

Stolz J, Munro S (2002) The components of the *Saccharomyces cerevisiae* mannosyltransferase complex M-Pol I have distinct functions in mannan synthesis. J Biol Chem 277:44801–44808

Sugawara T, Takahashi S, Osumi M, Ohno N (2004) Refinement of the structures of cell-wall glucans of *Schizosaccharomyces pombe* by chemical modification and NMR spectroscopy. Carbohydr Res 339:2255–2265

Sugiura R, Sio S, Shuntoh H, Kuno T (2002) Calcineurin phosphatase in signal transduction: lessons from fission yeast. Genes Cells 7:619–627

Surarit R, Gopal PK, Shepherd MG (1988) Evidence for a glycosidic linkage between chitin and glucan in the cell wall of *Candida albicans*. J Gen Microbiol 134:1723–1730

Tada T, Ohmori M, Iida H (2003) Molecular dissection of the hydrophobic segments H3 and H4 of the yeast Ca^{2+} channel component Mid1. J Biol Chem 278:9647–9654

Takaya N, Yamazaki D, Horiuchi H, Ohta A, Takagi M (1998) Cloning and characterization of a chitinase-encoding gene (*chiA*) from *Aspergillus nidulans*, disruption of which decreases germination frequency and hyphal growth. Biosci Biotechnol Biochem 62:60–65

Takeshita N, Ohta A, Horiuchi H (2002) *csmA*, a gene encoding a class V chitin synthase with a myosin motor-like domain of *Aspergillus nidulans*, is translated as a single polypeptide and regulated in response to osmotic conditions. Biochem Biophys Res Commun 298:103–109

Tatebayashi K, Takekawa M, Saito H (2003) A docking site determining specificity of Pbs2 MAPKK for Ssk2/Ssk22 MAPKKKs in the yeast HOG pathway. EMBO J 22:3624–3634

Thompson JR, Douglas CM, Li W, Jue CK, Pramanik B, Yuan X, Rude TH, Toffaletti DL, Perfect JR, Kurtz M (1999) A glucan synthase FKS1 homolog in *Cryptococcus neoformans* is single copy and encodes an essential function. J Bacteriol 181:444–453

Tominaga Y, Tsujisaka Y (1981) Investigations of the structure of *Rhizopus* cell wall with lytic enzymes. Agric Biol Chem 45:1569–1575

Torosantucci A, Chiani P, de Bernardis F, Cassone A, Calera JA, Calderone R (2002) Deletion of the two-component histidine kinase gene (*CHK1*) of *Candida albicans* contributes to enhanced growth inhibition and killing by human neutrophils *in vitro*. Infect Immun 70:985–987

Tougan T, Chiba Y, Kakihara Y, Hirata A, Nojima H (2002) Meu10 is required for spore wall maturation in *Schizosaccharomyces pombe*. Genes Cells 7:217–231

Traxler P, Gruner J, Auden JA (1977) Papulacandins, a new family of antibiotics with antifungal activity. I. Fermentation, isolation, chemical and biological characterization of papulacandins A, B, C, D and E. J Antibiot (Tokyo) 30:289–296

Trilla JA, Cos T, Duran A, Roncero C (1997) Characterization of *CHS4* (*CAL2*), a gene of *Saccharomyces cerevisiae* involved in chitin biosynthesis and allelic to *SKT5* and *CSD4*. Yeast 13:795–807

Trilla JA, Duran A, Roncero C (1999) Chs7p, a new protein involved in the control of protein export from the endoplasmic reticulum that is specifically engaged in the regulation of chitin synthesis in *Saccharomyces cerevisiae*. J Cell Biol 145:1153–1163

Troy FA, Koffler H (1969) The chemistry and molecular architecture of the cell walls of *Penicillium chrysogenum*. J Biol Chem 244:5563–5576

Utsugi T, Minemura M, Hirata A, Abe M, Watanabe D, Ohya Y (2002) Movement of yeast 1,3-b-glucan synthase is essential for uniform cell wall synthesis. Genes Cells 7:1–9

Vaaje-Kolstad G, Houston DR, Rao FV, Peter MG, Synstad B, van Aalten DM, Eijsink VG (2004) Structure of the D142N mutant of the family 18 chitinase ChiB from *Serratia marcescens* and its complex with allosamidin. Biochim Biophys Acta 1696:103–111

Vaishnav VV, Bacon BE, O'Neill M, Cherniak R (1998) Structural characterization of the galactoxylomannan of *Cryptococcus neoformans* Cap67. Carbohydr Res 306:315–330

Valdivia RH, Schekman R (2003) The yeasts Rho1p and Pkc1p regulate the transport of chitin synthase III (Chs3p) from internal stores to the plasma membrane. Proc Natl Acad Sci USA 100:10287–10292

Valdivia RH, Baggot D, Chuang JS, Schekman R (2002) The yeast clathrin adaptor protein complex 1 is required for the efficient retention of a subset of late Golgi membrane proteins. Dev Cell 2:283–294

Valdivieso MH, Duran A, Roncero C (1997) Chitin biosynthesis and morphogenetic processes. In: Brambl R, Marzluf GA (eds) The Mycota, vol III. Biochemistry and molecular biology. Springer, Berlin Heidelberg New York, pp 275–290

Van der Vaart JM, Caro LH, Chapman JW, Klis FM, Verrips CT (1995) Identification of three mannoproteins in the cell wall of *Saccharomyces cerevisiae*. J Bacteriol 177:3104–3110

Vay HA, Philip B, Levin DE (2004) Mutational analysis of the cytoplasmic domain of the Wsc1 cell wall stress sensor. Microbiology 150:3281–3288

Verstrepen KJ, Reynolds TB, Fink GR (2004) Origins of variation in the fungal cell surface. Nat Rev Microbiol 2:533–540

Vink E, Rodriguez-Suarez RJ, Gerard-Vincent M, Ribas JC, de Nobel H, van den Ende H, Duran A, Klis FM, Bussey H (2004) An *in vitro* assay for (1,6)-β-D-glucan synthesis in *Saccharomyces cerevisiae*. Yeast 21:1121–1131

Virginia M, Appleyard CL, McPheat WL, Stark MJ (2000) A novel 'two-component' protein containing histidine kinase and response regulator domains required for sporulation in *Aspergillus nidulans*. Curr Genet 37:364–372

Vongsamphanh R, Fortier PK, Ramotar D (2001) Pir1p mediates translocation of the yeast Apn1p endonuclease into the mitochondria to maintain genomic stability. Mol Cell Biol 21:1647–55

Walker AN, Garner RE, Horst MN (1990) Immunocytochemical detection of chitin in *Pneumocystis carinii*. Infect Immun 58:412–415

Walsh TJ, Teppler H, Donowitz GR, Maertens JA, Baden LR, Dmoszynska A, Cornely OA, Bourque MR, Lupinacci RJ, Sable CA et al. (2004) Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. N Engl J Med 351:1391–1402

Wang Z, Zheng L, Hauser M, Becker JM, Szaniszlo PJ (1999) WdChs4p, a homolog of chitin synthase 3 in *Saccharomyces cerevisiae*, alone cannot support growth of *Wangiella* (*Exophiala*) *dermatitidis* at the temperature of infection. Infect Immun 67:6619–6630

Wang Q, Liu H, Szaniszlo PJ (2002) Compensatory expression of five chitin synthase genes, a response to stress stimuli, in *Wangiella* (*Exophiala*) *dermatitidis*, a melanized fungal pathogen of humans. Microbiology 148:2811–2817

Watanabe D, Abe M, Ohya Y (2001) Yeast Lrg1p acts as a specialized RhoGAP regulating 1,3-β-glucan synthesis. Yeast 18:943–951

Wei Y, Zhang Y, Derewenda U, Liu X, Minor W, Nakamoto RK, Somlyo AV, Somlyo AP, Derewenda ZS (1997) Crystal structure of RhoA-GDP and its functional implications. Nat Struct Biol 4:699–703

Wei H, Scherer M, Singh A, Liese R, Fischer R (2001) *Aspergillus nidulans* alpha-1,3 glucanase (mutanase), mutA, is expressed during sexual development and mobilizes mutan. Fungal Genet Biol 34:217–227

Xu JR (2000) Map kinases in fungal pathogens. Fungal Genet Biol 31:137–152

Xu JR, Hamer JE (1996) MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. Genes Dev 10:2696–2706

Yamaguchi I, Kubo Y (1992) Target sites of melanin biosynthesis inhibitors. In: Köller W (ed) Target sites of fungicide action. CRC Press, Boca Raton, pp 101–114

Zhang X, Lester RL, Dickson RC (2004) Pil1p and Lsp1p negatively regulate the 3-phosphoinositide-dependent protein kinase-like kinase Pkh1p and downstream signaling pathways Pkc1p and Ypk1p. J Biol Chem 279:22030–22038

Zhao C, Jung US, Garrett-Engele P, Roe T, Cyert MS, Levin DE (1998) Temperature-induced expression of yeast FKS2 is under the dual control of protein kinase C and calcineurin. Mol Cell Biol 18:1013–1022

Zhu G, Spellman PT, Volpe T, Brown PO, Botstein D, Davis TN, Futcher B (2000) Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth. Nature 406:90–94

Ziman M, Chuang JS, Schekman RW (1996) Chs1p and Chs3p, two proteins involved in chitin synthesis, populate a compartment of the *Saccharomyces cerevisiae* endocytic pathway. Mol Biol Cell 7:1909–1919

Ziman M, Chuang JS, Tsung M, Hamamoto S, Schekman R (1998) Chs6p-dependent anterograde transport of Chs3p from the chitosome to the plasma membrane in *Saccharomyces cerevisiae*. Mol Biol Cell 9:1565–1576

Zonnenveld BJM (1971) Biochemical analysis of the cell wall of *Aspergillus nidulans*. Biochem Biophys Acta 249:506–514

Zu T, Verna J, Ballester R (2001) Mutations in WSC genes for putative stress receptors result in sensitivity to multiple stress conditions and impairment of Rlm1-dependent gene expression in *Saccharomyces cerevisiae*. Mol Genet Genomics 266:142–155

6 Septation and Cytokinesis in Fungi

J. Wendland[1], A. Walther[1]

CONTENTS

I. Introduction

Fungi display either of two growth modes: yeast-like or filamentous. Yeast-like growth, as exemplified by the unicellular baker's yeast *Saccharomyces cerevisiae*, is characterized by cytokinesis, a process that results in the partial degradation of the chitin-rich septum to allow separation of daughter cells from their mother cells. Polarized growth in filamentous fungi produces hyphae that are compartmentalized by septation. Septation in filamentous fungi is carried out by proteins whose homologs act during cytokinesis in yeast cells. However, septa do not separate the cytoplasms of adjacent compartments, due to the presence of a septal pore. Chitin hydroloysis is absent at septal sites, which allows the formation of a multicellular mycelium. Cytokinesis and septation mark the ultimate step of a cell cycle and, therefore, need to be tightly controlled with other processes such as daughter cell growth/tip growth, DNA replication and mitosis. Although the concept of cell division is a fundamental problem of all cells, differing solutions have been developed in bacteria, fungi, plants, and animals. Cell division has to deal with the following tasks:

1. a site has to be chosen as division plane,
2. protein complexes assemble at this chosen site,
3. in fungal, as well as in animal cells, a contractile acto-myosin ring that has been formed earlier becomes dynamic at the end of mitosis, which
4. is accompanied by chitin deposition.

Very elaborate control mechanisms, called either mitotic exit network (MEN) or septation initiation network (SIN), have evolved to link mitosis with cytokinesis in yeast-like organisms, although in filamentous ascomycetes that generate multinucleate cellular compartments, a strict coupling of mitotic exit and septation seems questionable. Synthesis of a chitin-rich septum occurs both in yeast-like and filamentous fungi. In yeast, daughter cells actively separate from their mother cells by dissolving the chitin ring structure, using specific chitinases. In filamentous fungi, however, the septum remains intact, and the cytoplasm of apical and subapical compartments is connected via septal pores.

These differences between septation and cytokinesis, as well as similarities between fungi and animal cells have provided the basis for the use of tractable fungal model systems to elucidate the underlying mechanisms. In this chapter, we will discuss the key events leading to septation/cytokinesis. We start with the yeast model systems *S. cerevisiae* and *Schizosaccharomyces pombe*, and then compare knowledge acquired in these organisms with results from filamentous fungi, particularly *Neurospora crassa*, *Aspergillus nidulans*, and *Ashbya gossypii*. Analysis of conserved features is facilitated by the availability of genome sequences for these fungi (see Table 6.1).

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Therefore, the relevant data of fungal homologs of genes involved in the septation process will be analyzed. In the last section, open questions and new research directions will be discussed that may help to define mechanistic differences between septation and cytokinesis.

II. Selecting Sites of Septation

Fungal cells employ at least three different mechanisms for the selection of septal sites, differing in the timing and mechanism of site selection. In the budding yeasts, for example, in *S. cerevisiae*, the septal site is determined with the choice of bud site selection. In the fission yeast *S. pombe*, positioning of the division plane, and thus the septal site resembles that of animal cells. Filamentous fungi, such as *A. nidulans*, position septal sites in different ways depending on the developmental state of cells, e.g., germ cells, hyphal cells, or reproductive cells involved, for example, in conidiation.

A. Bud Site Selection in *Saccharomyces cerevisiae*

In *S. cerevisiae*, the septal site is chosen at the beginning of a new cell cycle prior to DNA replication and spindle formation. This defines the position at which a bud will be formed, and requires the correct alignment of the mitotic spindle with the mother bud axis later in the cell cycle. *S. cerevisiae* displays a cell type-specific pattern of bud site selection (Freifelder 1960). Axial budding of haploid cells (**a** or α cell types) restricts selection of bud sites to proximal cell poles with which daughter cells were connected to their mother cells (Fig. 6.1A). Bipolar budding of diploid cells (**a**/α cell type) allows buds to be formed at both cell poles (Chant and Pringle 1991, 1995). The choice of bud site is governed by landmark proteins that are localized to the cell cortex. The *BUD3*, *BUD4*, *AXL1*, and *BUD10/AXL2* genes encode specific marker proteins for the axial budding pattern. These proteins are localized in a transient manner and drive the bud site selection of the next cell cycle (Chant and Herskowitz 1991; Halme et al. 1996; Roemer et al. 1996; Sanders and Herskowitz 1996). Strikingly, upon re-feeding after a period of starvation, haploid cells can produce new buds at nonaxial/random positions, which may allow these cells to properly respond to new nutrient sources (Chant and Pringle 1995). The *AXL1* gene was identified as a key determinant for the axial budding pattern. Its expression is limited to haploid cell types, and ectopic expression of *AXL1* in diploid cells imposes the axial budding pattern on these cells (Fujita et al. 1994; Lord et al. 2002). Interestingly, single deletions of genes required for the axial budding pattern converts bud site selection to the bipolar budding pattern in haploid cell types, whereas no phenotype in diploid cells was observed. In contrast to haploid landmark proteins, diploid landmarks are positioned in a persistent manner (Madden and Snyder 1998; Ni and Snyder 2001). Relevant proteins for the bipolar budding pattern are Bud8, Bud9, and Rax1. *BUD8* encodes a protein that is localized to the distal cell pole, whereas Bud9 protein is localized to the proximal cell pole. Thus, two distinct proteins are used for marking both cell poles in diploids (Zahner et al. 1996; Taheri et al. 2000; Harkins et al. 2001). Deletion of either *BUD8* or *BUD9* leads to budding either at the proximal pole (in *bud8/bud8* mutants) or at the distal pole (in *bud9/bud9* mutants). The Bud8 and Bud9 proteins are highly similar (as a result of gene duplication and subsequent divergence), and promoter analyses indicated that the localization of the resulting proteins is dependent on the cell cycle-regulated expression of the corresponding genes (Schenkman et al. 2002). Rax1p is necessary for the establishment of the bipolar budding pattern and is required for the localization of Bud8 (Fujita et al. 2004). Rax1p and Rax2p interact with each other and with Bud8p and Bud9, suggesting a common function in the establishment of a cortical landmark used for bipolar bud site selection (Kang et al. 2004b). Signaling of the cell type-specific landmark proteins converges on a GTPase module consisting of the Bud1p/Rsr1p GTPase, its guanine-nucleotide exchange factor (GEF) Bud5p, and its GTPase-activating protein (GAP) Bud2p. Deletion of either one of these genes results in random budding in all cell types, indicating that this module is required to correctly position the cell polarity establishment machinery (Chant and Herskowitz 1991; Chant et al. 1991; Park et al. 1993; Kozminski et al. 2003). To convey positional information, the landmark proteins need to be able to specifically activate the Bud1p/Rsr1p module to implement the correct budding pattern. Since Bud5p activates Bud1p/Rsr1p by loading the protein with GTP, interactions of landmark proteins with Bud5p seem to be crucial. Such interactions have been demonstrated for Bud10p/Axl2p and Bud5p as well as for

Table. 6.1. Genomic comparison of filamentous fungal genome sequences, with *Saccharomyces cerevisiae* proteins shown to localize to the bud neck[a]

A. gossypii	*N. crassa*	*S. cerevisiae*	
Bud site selection			
AGR251C (0.0)	None	YPR122W	AXL1
AER417W (e-126)	NCU04601.1 (e-027)	YIL140W	AXL2
ABR021W (0.0)	NCU03852.1 (e-044)	YKL092C	BUD2
AAL016C (0.0)	NCU06579.1 (e-004)	YCL014W	BUD3
AGL306C (e-093)	NCU00152.1 (e-019)	YJR092W	BUD4
AFR630C (e-023)	None	YCR038C	BUD5
AFR495C (e-159)	NCU08468.1 (e-046)	YLR319C	BUD6
ACL193C (e-019)	None	YLR353W, YGR041W	BUD8, BUD9
AFR532W (e-076)	NCU08343.1 (e-021)	YOR301W	RAX1
AGR095W (0.0)	NCU03045.1 (e-045)	YLR084C	RAX2
Bud emergence/cell polarity			
AEL241W (e-146)	NCU06593.1 (e-057)	YBR200W	BEM1
AGR144C (0.0)	None	YER155C	BEM2
AGR230W (e-171)	NCU02524.1 (e-042)	YPL115C	BEM3
AGL293C (e-073)	NCU06648.1 (e-045)	YBL085W, YER114C	BOI1, BOI2
AAR145W (e-122)	NCU07481.1 (e-029)	YOR188W	MSB1
ADR232W (e-073)	NCU06668.1 (e-021)	YKR090W	PXL1
ACR205W (e-086)	NCU07688.1 (e-026)	YOR127W	RGA1
AFR616W (0.0)	NCU00553.1 (e-032)	YBR260C	RGD1
ABR241C (0.0)	NCU09537.1 (e-104)	YFL047W	RGD2
AFR585W (0.0)	NCU00668.1 (e-133)	YLR371W	ROM2
ADL022C (e-066)	NCU03115.1 (e-019)	YLL021W, YLR313C	SPA2, SPH1
Transport/secretion			
AGR027C (e-140)	None	YBR059C	AKL1
ADR342C (e-173)	NCU05232.1 (e-101)	YJR005W	APL1
ADL302W (0.0)	NCU03440.1 (e-083)	YBL037W	APL3
ADR315W (e-117)	NCU09673.1 (e-092)	YOL062C	APM4
AFL161C (e-152)	NCU06362.1 (e-040), NCU08499.1 (e-056)	YMR192W, YPL249C	APP2, GYP5
AFR370C (e-054)	NCU07989.1 (e-039)	YJR058C	APS2
AFR100W (e-175)	NCU08012.1 (e-035)	YJL085W	EXO70
ADL321W (e-155)	NCU06631.1 (e-024)	YBR102C	EXO84
AEL201W (e-173)	NCU04514.1 (e-072)	YNL293w, YOL112W	MSB3, MSB4
ABR115C (e-003), syntenic homolog	None	YNR049C	MSO1
ADR354W (0.0)	NCU01440.1 (0.0)	YOR326W, YAL029C	MYO2, MYO4
ACR191C (0.0)	NCU06544.1 (e-131)	YBL105C	PKC1
AGL162C (0.0)	NCU08312.1 (e-057)	YDR164C	SEC1
AGL130C (0.0)	NCU09313.1 (e-063)	YLR166C	SEC10
AFR251C (0.0)	NCU00117.1 (e-040)	YGL233W	SEC15
AFR670W (e-132)	NCU01911.1 (e-013)	YNL272C	SEC2
ADR012C (e-122)	NCU09869.1 (e-017)	YER008C	SEC3
AGL158C (0.0)	NCU07698.1 (e-031)	YDR166C	SEC5
ACL047W (0.0)	NCU03341.1 (e-051)	YIL068C	SEC6
ADL317C (0.0)	NCU04190.1 (e-046)	YPR055W	SEC8
Septins and their regulation			
AFR027C(e-031)	None	YNL166C	BNI5
AAR001C (e-137)	NCU03515.1 (e-109)	YCR002C	CDC10
AER445C (e-150)	NCU02464.1 (e-072)	YJR076C	CDC11
AER238C (e-169)	NCU03795.1 (e-127)	YHR107C	CDC12
AFR111C (e-149)	NCU08297.1 (e-104)	YLR314c	CDC3
ABR088C (e-051)	None	YKL048C	ELM1
AFR696C (0.0)	None	YDR507C, YCL024W	GIN4, KCC4
ABL034W (0.0)	NCU09064.1 (e-092)	YKL101W	HSL1
ABR110W (e-158)	NCU01613.1 (e-073)	YBR133C	HSL7
ABL159W (e-168)	None	YDL225W	SHS1/SEP7

Table. 6.1. (continued)

A. gossypii	*N. crassa*	*S. cerevisiae*	
Mitotic exit			
AFR035W (0.0)	NCU03242.1 (e-053), NCU07296.1 (e-146)	YNL161W	CBK1
ACL006W (0.0)	NCU09258.1 (e-063)	YMR001C	CDC5
ADR033W (0.0)	NCU09071.1 (e-135)	YGR092W, YPR111W	DBF2, DBF20
ADL149W (e-170)	NCU00622.1 (e-056)	YHR158C, YGR238C	KEL1, KEL2
ACR292W (0.0)	NCU03379.1 (e-041)	YAL024C	LTE1
ADR317C (e-175)	NCU00978.1 (e-072)	YDL028C	MPS1
AER425W (e-007), syntenic homolog	None	YNL078W	NIS1
Actin ring formation and cytokinesis			
AFR669W (0.0)	NCU01431.1 (e-085)	YNL271C	BNI1
ABL200W (e-062)	NCU00064.1 (e-014)	YNL233W	BNI4
AFR027C (e-031)	None	YNL166C	BNI5
AFR301C (e-118)	None	YIL159W	BNR1
ABL153W (0.0)	NCU04251.1 (0.0)	YNL192W	CHS1
AEL190W (0.0)	NCU05239.1 (0.0)	YBR038W	CHS2
AEL189W (0.0)	NCU09324.1 (0.0), NCU04350.1 (e-122)	YBR023C	CHS3
ACR227W (e-156)	NCU02351.1 (e-032), NCU02592.1 (e-042), NCU09322.1 (e-076)	YBL061C	SKT5/CHS4
ADL288C (0.0)	NCU04095.1 (e-045)	YDL117W	CYK3
ABR082W (e-080)	NCU04763.1 (e-017)	YMR032W	HOF1/CYK2
AFL150C (0.0)	NCU03116.1 (e-048)	YPL242C	IQG1/CYK1
AFL030C (e-051)	NCU06617.1 (e-024)	YGL106W	MLC1
AEL280W (e-022)	None	YPR188C	MLC2
ACR068W (0.0)	NCU00551.1 (0.0)	YHR023W	MYO1

[a] *Ashbya gossypii* sequences correspond to the systematic nomenclature (http://agd.unibas.ch/); *Neurospora crassa* sequences correspond to the genome annotation available at http://www.broad.mit.edu/annotation/fungi/neurospora_crassa_7/index.html. *Values in brackets* indicate the e-values generated by using BLAST-P of the fungal sequences against the *S. cerevisiae* protein. An e-value of 0.0 indicates very high sequence identity. Syntenic homology (conserved chromosomal gene order of homologs) between *Ashbya* and *S. cerevisiae* genes was used to indicate homology, despite low e-value blast scores. E-values from *Ashbya* to *S. cerevisiae* comparisons were found to be higher than those of *N. crassa* to *S. cerevisiae* comparisons, reflecting the closer relationship of the former species pair

Bud8p and Bud5p (Roemer et al. 1996; Kang et al. 2001, 2004a).

B. Placement of the Site of Cell Division in *Schizosaccharomyces pombe*

The cleavage plane, and thus the septum position in *S. pombe* is selected in a manner similar to that of animal cells, which has made *S. pombe* a model organism to study cell division. However, in animal cells, selection of the cleavage plane is dependent on spindle microtubules forming a structure termed 'midbody', whereas in *S. pombe* the mitotic spindle can be disrupted without interfering with the selection of the septal site (Chang et al. 1996). This may reflect a difference in nuclear division, which occurs as a closed mitosis in *S. pombe*, in which the nuclear envelope is not broken down (Chang 2001). In *S. pombe*, positioning of the nucleus is important for the placement of the division site, since mispositioning of the nucleus is followed by an aberrant localization of the division plane (Chang and Nurse 1996; Chang et al. 1996; Tran et al. 2001). The Mid1/Dmf1 protein was shown to play an essential role in the positioning of the cleavage plane, and may encode a landmark protein defining the septal site (Sohrmann et al. 1998). Mutants of *mid1* are still able to form actin rings, but the positioning of the ring is randomized at different angles. Mid1p is localized in the nucleus in interphase cells but exits the nucleus upon phosphorylation during mitosis, regulated via the polo-like kinase Plo1 (Ohkura et al. 1995; Bähler et al. 1998; Fig. 6.1B). Mid1p then forms a band on the cell cortex in the vicinity of the nucleus, and this localization is dependent on the position of the nucleus, since nuclear po-

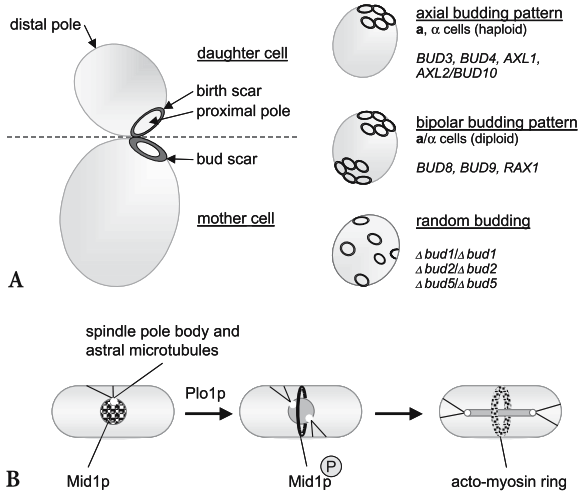

Fig. 6.1. Selection of a cell division site. **A** In *Saccharomyces cerevisiae*, the mother cell is bigger than the daughter cell. The mother cell bears a prominent bud scar at the septal site, while the daughter cell has a fainter birth scar (due to Cts1p chitinase activity – see below). Cell poles can be defined such that the proximal pole is the one with which the daughter cell was connected to the mother cell. Hence, the opposite pole is defined as the distal pole. Each budding event leads to the formation of a permanent mark – the chitin-rich bud scar. Two cell type-specific budding patterns determine bud site selection (and thus the position of the cleavage plane), and give rise to a distinctive distribution of bud scars. The axial budding pattern of haploid **a** or α cells leads to the formation of bud scar rows on the proximal cell pole, whereas diploid (a/α) cells use both poles for budding. A new bud scar in haploid cells is positioned adjacent to the bud scar of the preceding cell cycle, based on the expression of haploid-specific landmark proteins. Relevant genes for each budding pattern are listed. Lack of either *BUD1*, *BUD2*, or *BUD5* leads to loss of positional information and random budding. **B** In *Schizosaccharomyces pombe*, selection of the cleavage plane is dependent on the positioning of Mid1p. Mid1p localizes to the nucleus and, upon its phosphorylation, exits the nucleus to form a band in the vicinity of the nucleus, which serves as a landmark to assemble protein complexes for septation, e.g., the acto-myosin ring

sitioning mutants were observed to affect the localization of Mid1p (Chang 2001). The localization of Mid1p may be facilitated by the pleckstrin homology (PH)-domain of the protein that could, for example, establish interactions with lipids in the cell membrane. Based on its sequence similarity to Mid1p, another protein, Mid2p, was identified (Berlin et al. 2003). However, both proteins have divergent functions (see below; Tasto et al. 2003). Homologs of Mid1p, termed anillin, were found in *Drosophila* and human cells in which cell cycle localization patterns were similar to that in *S. pombe*, indicating that the mechanisms of cleavage plane selection in *S. pombe* and mammalian cells are based on conserved molecular machineries (Oegema et al. 2000).

C. Septation in Filamentous Fungi

In filamentous ascomycetes such as *N. crassa*, *A. nidulans* and *A. gossypii*, the search for homologs either of the *S. cerevisiae* Bud proteins or of the *S. pombe* Mid1p revealed a number of proteins with high similarity to the yeast Bud proteins but no clear homolog to Mid1p (Wendland 2003). However, selection of septation sites in filamentous fungi depends on different cell types, or the developmental stage of particular cells. In *A. nidulans* and *A. gossypii*, germination of spores produces round-shaped germ cells (Fig. 6.2A). From these germ cells, hyphae are branched off in a characteristic bipolar germination pattern similar to bipolar

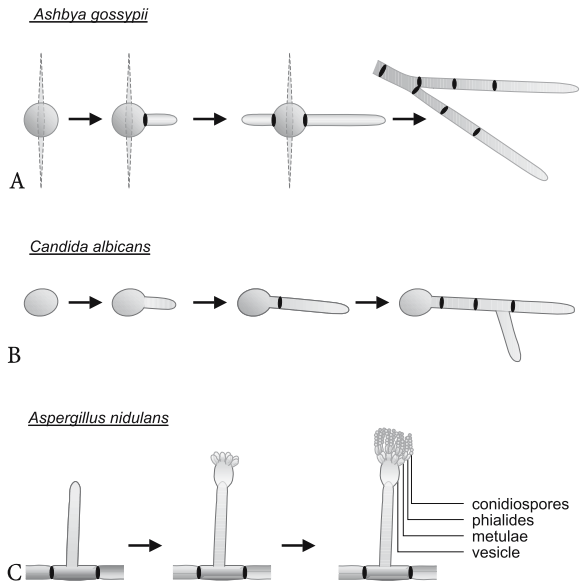

Fig. 6.2. Septation pattern in filamentous fungi. **A** Spore germination in *Ashbya gossypii* leads to the formation of a round-shaped germ cell. A bipolar germination pattern results in the successive protrusion of two hyphae. Septa are positioned at the neck between germ cell and hypha. Hyphae are compartmentalized by septation, which occurs in regular intervals. Mature mycelia of *A. gossypii* display a characteristic dichotomous tip branching pattern. This is remarkable, since both branches simultaneously form septa at their bases. **B** Hyphal induction in *Candida albicans* results in germ tube formation out of a yeast cell. The position of the first septum is peculiar, since it is not formed at the bud neck but rather 10–15 µm within the hyphal tube (reason unknown). **C** *Aspergillus nidulans* forms specialized cell types during condiogenesis. The metulae, phialides and conidiospores are uninucleate, and metulae and phialides show a unipolar budding pattern

budding in baker's yeast (Harris 1999; Wendland and Philippsen 2001). The first round of septation occurs at the neck separating the germ cell from the first hypha in both organisms (Wolkow et al. 1996; Wendland and Philippsen 2000). Mutants that interfere with the bipolar branching pattern were also obtained. *SwoA* of *A. nidulans* encodes a protein O-mannosyltransferase and is allelic to *pmtA* (Shaw and Momany 2002). This is remarkable, since O-glycosylation of the *S. cerevisiae* landmark protein Axl2p/Bud10p is required for the establishment of correct axial budding in haploid (**a**, or α) yeast cells (Sanders et al. 1999). Deletion of the *S. cerevisiae PMT4* gene resulted in unstable and mislocalized Axl2p/Bud10p, and shifted the budding pattern from axial to unipolar. A mutant branching phenotype of germ cells similar to *swoA* was observed in the *A. gossypii bem2* mutant (Wendland and Philippsen 2000). Bem2p encodes a Rho-GTPase activating protein, and in yeast is involved in bud emergence (Bender and Pringle 1991). Reminiscent of the germ cell/hypha position of the first septum, swollen *A. gossypii* hyphal tips of *rho3* mutants generate a septum between swellings and newly generated hyphae (Wendland and Philippsen 2001). Adult *A. gossypii* hyphae that underwent a process termed 'hyphal maturation' show a distinct dichotomous tip branching pattern. Characteristically, branches in *A. gossypii* become septate at their bases, so that after tip branching two septa are formed simultaneously (Ayad-Durieux et al. 2000; Wendland and Philippsen 2002).

Positioning of the first septum in the dimorphic human fungal pathogen *Candida albicans* appears to be different from that documented in *A. nidulans* and *A. gossypii* (Fig. 6.2B). *C. albicans* is a dimorphic fungus that switches between yeast and hyphal growth upon induction through environmental stimuli (Berman and Sudbery 2002). *C. albicans* yeast cells that are induced to form hyphae do not position their first septum at the bud neck, but rather move this septal site 10–15 µm into the hyphal tube (Sudbery 2001). An integrin-like protein encoded by the *C. albicans INT1* gene may play a specific role in this site selection and colocalizes to this newly formed septal site, although the biological significance of this process is unknown (Gale et al. 2001).

Similarly to the early phases of hyphal development when switching isotropic growth of germ cells to polarized hyphal growth, other developmental stages, particularly during conidiation (e.g., in *A. nidulans*), show a specific pattern of septa positioning (Fig. 6.2C). During conidiogenesis, hyphae differentiate into a stalk and a vesicle from which single cell generations called metulae, phialides, and chains of conidia emerge (Timberlake 1990). These single cells are uninucleate, and resemble elongated yeast cells with unipolar budding pattern.

Understanding the molecular mechanism of how septal sites are positioned within the growing hyphae of filamentous fungi is of central importance in understanding fungal biology. An interesting finding showed that hyphal tip regions, as well as septal sites form sterol-enriched domains, termed lipid-rafts, which may play a key upstream signaling role for the protein machinery in charge of septation (Martin and Konopka 2004). The role of the hyphal tip in positioning septal cues has been evaluated in recent reports (Kaminskyj 2000; Knechtle et al. 2003). A decrease of polarized growth rates was observed in juvenile mycelia of *A. gossypii* that at the same time formed lateral branches or septa at subapical positions. Positions at which the apical extension rate was slowed down were found to develop septa once the hyphal tip had resumed fast growth. This indicates that the hyphal tip is involved in placing cortical cues that determine the position of future septa (Knechtle et al. 2003). The molecular nature of such a mechanism is currently unknown. Pathways that may be involved are the Cdc42p Rho-GTPase module that may link a tip-generated signal via the PAK kinase Cla4p to septin family members (see below; Ayad-Durieux et al. 2000; Schmidt et al. 2003). In addition, landmark proteins such as Bud3p, for example, could play an important role in this process by linking a tip-generated signal with septum positioning, and eventually septum construction (Wendland 2003). Mining the *N. crassa* genome revealed the presence of a number of potential *BUD* gene homologs in a fungus that is more distantly related to *S. cerevisiae* than to *A. gossypii.* This suggests that the general mechanism of septum positioning is conserved in filamentous ascomycetes (Walther and Wendland 2003).

III. Protein Complexes at Septal Sites

Fungal septa are characterized by the presence of a chitin-rich septum that is absent in animal cells (see Sietsma and Wessels, Chap. 4, and Latgé and

Calderone, Chap. 5, this volume, and The Mycota, Vol. III, 2nd edn., Chap. 14). Since chitin is missing in vertebrates and is essential for fungal cell wall integrity, inhibition of the ability to synthesize chitin is a promising target for antifungal drug therapies. Polyoxins and nikkomycins are currently in use as drugs that inhibit chitin synthesis (Ruiz-Herrera and San-Blas 2003).

This section deals with the question of how an orderly assembly of protein complexes at future septal sites is accomplished, and which are the major players identified so far. During evolution, these protein complexes were apparently highly conserved between fungi and animal cells, which allows the identification of homologous proteins in various organisms, either through genomic sequencing projects or via PCR approaches using degenerate primers based on conserved protein domains.

The Rho-GTPase Cdc42p complex plays an essential role in cell polarity establishment in fungi (Johnson 1999; Wendland 2001). In *S. cerevisiae*, Cdc42p is required to establish a new site of polarized growth, resulting in bud emergence. This position also defines the new bud neck (Fig. 6.3A). Cdc42p initiates the assembly of both a septin and an acto-myosin ring, described in the following sections (Sanders and Field 1994; Gladfelter et al. 2001; Caviston et al. 2003).

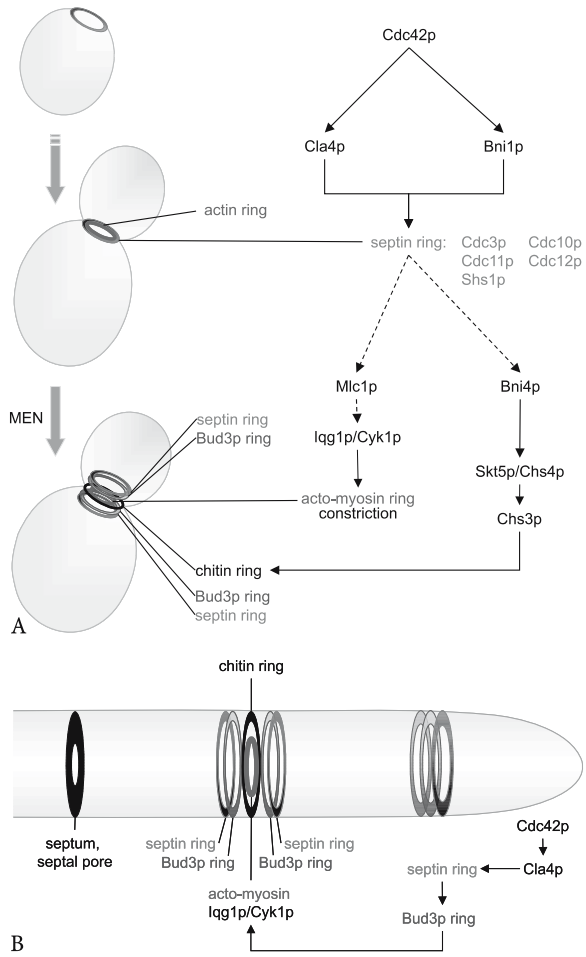

Fig. 6.3. Assembly of protein complexes during septation/cytokinesis. **A** In yeast, a signaling cascade (shown on the *right*) from Cdc42p via the effector proteins Cla4p and Bni1p leads to the formation of a septin ring (yeast cell on the *left* shows a schematic representation of the ongoing processes). Recruitment of the acto-myosin ring involves the IQGAP-related protein Cyk1p/Iqg1p. Bud3 (and other proteins) is also recruited to the bud neck to form a ring. Initially, single rings are formed. During the dynamic phase initiated by the mitotic exit network (*MEN*), ring splitting of septin and Bud3 rings occurs, while the acto-myosin ring undergoes constriction. The chitin synthase Chs3p is also localized to the septin ring via a set of protein–protein interactions, and a primary (by Chs2p) as well as secondary (by Chs3p) septum is formed. **B** Hyphae in *Ashbya gossypii* allow the view of different stages of septation, spatially separated along the hypha. Close to the hyphal tip, single rings of septins, Bud3p, and acto-myosin are formed. Dynamic events may occur at a subapical septum, showing ring splitting and acto-myosin contraction, while protein complexes at septal sites in further subapical regions have been disassembled upon septum completion. Note the different role of Bud3p in *A. gossypii* that, in contrast to *Saccharomyces cerevisiae*, is involved in recruiting Cyk1p to the septal sites

A. The Septin Ring

In *S. cerevisiae*, the septin ring is composed of a conserved family of mitotic septins encoded by *CDC3*, *CDC10*, *CDC11*, *CDC12*, and *SHS1*, and forms a cortical collar of septin filaments at the mother-bud neck (Byers and Goetsch 1976). Due to a high degree of conservation, various septins have been isolated in other organisms, too (Neufeld and Rubin 1994; Longtine et al. 1996; Field and Kellog 1999; Momany et al. 2001). Septins contain several domains including an N-terminal region with a potential phosphoinositide binding site, a core region including a P-loop guanine nucleotide binding motif, a septin-specific sequence, and a C-terminal domain that in some cases includes a predicted coiled-coil region (Casamayor and Snyder 2003). GTP/GDP binding and hydrolysis have been demonstrated for septins, although GTP turnover may be small in vivo (Field et al. 1996; Versele and Thorner 2004; Vrabioiu et al. 2004). Septins were first identified in *S. cerevisiae*

in a screen for cell division cycle (*CDC*) mutants (Hartwell 1971). Inactivation of any one of the septins, e.g., by placing temperature-sensitive alleles at the restrictive temperature, causes a failure to form a septin ring and lethality, whereas deletion of *SHS1* causes only mild defects (Carroll et al. 1998). A recent study analyzed the protein–protein interactions among septins, also with respect to the potential to form septin filaments. Based on these very elegant studies, a model has been proposed in which a heteropentameric complex of septins is polymerized into the septin ring (Versele et al. 2004).

Such a septin ring may serve multiple functions: first, as a rigid backbone that could stabilize the neck region; second, as a scaffold that could direct the assembly and/or attachment of other proteins required for septation; third, by providing a diffusion barrier that aids in localized morphogenesis of only the daughter cell, whilst the mother cell does not grow in a budding cycle; and fourth, as a positional cue for proper spindle positioning (Barral et al. 2000; Takizawa et al. 2000; Faty et al. 2002; Kusch et al. 2002; Longtine and Bi 2003). In *S. cerevisiae*, the PAK-kinase Cla4p, which is a downstream effector protein of Cdc42p, plays an essential role in septin ring assembly (Schmidt et al. 2003). This demonstrates a signaling cascade from Cdc42p via Cla4p to septins in which Cla4p directly interacts with, and also phosphorylates septins. Furthermore, besides Cla4p, another effector of Cdc42p, the formin Bni1, is involved in the assembly of the septin ring during the early phase of bud emergence. Other studies also demonstrated the involvement of GTP–GDP cycles of Cdc42p, and of the actin cytoskeleton in this process (Gladfelter et al. 2002; Kadota et al. 2004; Versele et al. 2004).

B. The Acto-Myosin Ring

The assembly and dynamic behavior (see next section) of the acto-myosin ring is a highly conserved feature in fungal and animal cytokinesis (Noguchi et al. 2001). The Cla4p kinase plays a key role in actin ring formation at septal sites, since a deletion of *CLA4* in *A. gossypii* resulted in defects in actin ring formation (Ayad-Durieux et al. 2000). The role of Cla4p may be indirect and coupled to septin function (Fig. 6.3B). Other proteins important for acto-myosin ring formation in filamentous fungi may be involved in specific transport processes to septal sites, as well as in actin filament assembly. In yeast, a cell cycle-dependent reorganization of the actin cytoskeleton directs growth either to the bud tip or to the septum. In filamentous fungi, polarization of the actin cytoskeleton can be found simultaneously at the tip and at septal sites (Wendland 2001). The *A. gossypii* Wiskott-Aldrich Syndrome protein (WASP)-homolog Wal1p is required for actin ring formation, and also for apical positioning of cortical actin patches (Walther and Wendland 2004). The requirement of Wal1p for actin ring formation may thus be due either to a failure in directed vesicle transport (as a secondary effect of correct positioning of actin patches) or to the requirement of WASP in the assembly of actin filaments needed for actin ring formation.

Two other conserved protein families are essential for actin ring formation. These are members of the formin and IQGAP protein families (Bi 2001). Formins are homologs of the limb deformity gene in the mouse, and share formin homology (FH)-domains. IQGAP-family proteins contain domains with repeated IQ-amino acid motifs – in fungi containing a consensus motif of 'QxxxRGxxxR' in which x is any amino acid (see Wendland and Philippsen 2002) – and a Ras-GTPase activating protein domain.

The *S. cerevisiae* genome contains two formins, encoded by the *BNI1* and *BNR1* genes. They interact via N-terminal G-protein binding domains with Rho-type GTPases, and are thereby activated (Dong et al. 2003; Evangelista et al. 2003). In *S. cerevisiae*, the formin-dependent pathway of actin ring formation is regulated via the Rho1-GTPase, and not via Cdc42p (Tolliday et al. 2002). In Bni1p, the central FH3- and Spa2-binding domains that may help in the localization of the protein are followed by a proline-rich FH1, a FH2-dimerization, and a Bud6-binding domain (Petersen et al. 1998; Ozaki-Kuroda et al. 2001). Spa2p, Bud6p, and Bni1p form a protein complex termed the polarisome in *S. cerevisiae*, which is required for polarized morphogenesis (Evangelista et al. 1997; Fujiwara et al. 1998; Sheu et al. 1998). Formin mutants in *S. pombe* (*cdc12*) or *Drosophila melanogaster* (*dia*) fail to form an actin ring at cleavage sites that is, however, formed in *S. cerevisiae bni1* mutants (Chang et al. 1997; Afshar et al. 2000; Vallen et al. 2000). A formin mutant in a filamentous fungus has been described so far only in *A. nidulans*. The *A. nidulans* formin SepA was shown to be required for actin ring formation (Sharpless and Harris 2002). Temperature-sensitive *sepA* mutants are aseptate at the restrictive temperature, but upon shift to the

permissive temperature, multiple septa form with a wild type-like spacing, suggesting that the correct assembly of the protein complexes at septal sites was not inhibited (Trinci and Morris 1979). The mutation in this *sepA1* mutant was found to be a single-point mutation resulting in an amino acid exchange from leucine to serine at position 1369 within the FH2-domain of the SepA protein. This, therefore, does not result in a lack of SepA protein, but may yield a protein in which essential interactions are blocked at elevated temperatures. Interestingly, SepA localizes not only to septal sites but also to hyphal tips (Sharpless and Harris 2002).

IQGAP-family members are conserved proteins that share an N-terminal calponin homology (CH)-domain required for actin bundling, central 'IQ'-repeats, and a C-terminal GTPase activating domain which is required for ring constriction (Shannon and Li 1999). The *S. cerevisiae* IQGAP, *IQG1/CYK1*, is an essential gene and Iqg1p/Cyk1p is required for actin ring assembly (Epp and Chant 1997; Lippincott and Li 1998). Iqg1p/Cyk1p does not require formins for its localization to the septal site – rather, it is localized via the interaction of its IQ-repeats with a myosin light chain (Mlc1p) that appears to be recruited by the septin ring and localizes to the bud neck prior to, and independently of Iqg1p/Cyk1p (Boyne et al. 2000; Shannon and Li 2000; Tolliday et al. 2002). These data suggest independent roles for formins and IQGAP-proteins in actin ring formation, but the mechanistic interactions are currently unclear.

In filamentous fungi, septation results in the compartmentalization of hyphae and, therefore, may not be essential. An *A. gossypii cyk1* mutant strain was found to be viable, and grew with wild-type extension rates but turned out to be aseptate. *Agcyk1* mutants failed to generate an actin ring at presumptive septal sites (Wendland and Philippsen 2002). Further analyses demonstrated that the AgBud3p homolog plays a role in the localization of AgCyk1p (see Sect. IV.).

In *S. cerevisiae*, the myosin light chain Mlc1p interacts with Iqg1p/Cyk1p, with the conventional type II myosin encoded by the *MYO1* gene, and also with the type V myosin, encoded in yeast by the *MYO2/MYO4*/genes (Stevens and Davis 1998; Boyne et al. 2000). Myo1p forms a ring at the presumptive bud site in *S. cerevisiae* prior to bud emergence. This ring formation requires septin ring assembly, and loss of *MYO1* leads to the inability to form an acto-myosin ring (Bi et al. 1998). The acto-myosin ring in *S. cerevisiae* is formed even in the absence of one of the formins (Bni1p or Bnr1p), or in strains lacking *HOF1/CYK2* (which is related to *Schizosaccharomyces pombe cdc15* – hence, its name 'homolog of fifteen'). An interesting model was postulated in which Bni1p and Myo1p form a pathway involved in acto-myosin ring constriction, and Bnr1p and Hof1p/Cyk2p are involved in linking acto-myosin ring constriction with septum formation (Kamei et al. 1998; Lippincott and Li 1998; Vallen et al. 2000). Once the assembly of protein complexes is completed, a signal needs to be generated to initiate acto-myosin ring constriction and chitin synthesis at the septum (see Sect. V.).

At this point, we can ask which set of proteins of a fungus is localized to a future site of septation. This question has been addressed in *S. cerevisiae*. In a global study including about 75% of the whole proteome, the subcellular localization of GFP-tagged proteins was analyzed (Ross-Macdonald et al. 1999; Kumar et al. 2002; Huh et al. 2003). These efforts yielded close to 100 proteins that localized either at the tip of the bud or at the septum. Different classes of proteins were distinguished, belonging to bud site-selection genes, proteins involved in bud emergence, septins, proteins required for actin ring assembly, septum formation, secretion, and exit of mitosis. Comparison of this gene set with other sequenced genomes of filamentous fungi, e.g., *A. gossypii* and *N. crassa*, revealed a large number of conserved genes, further demonstrating a generally conserved mechanism of septation/cytokinesis (Table 6.1).

IV. Dynamic Contraction of the Acto-Myosin Ring and Chitin Synthesis Lead to Septum Formation

The basic conserved feature between fungal and animal cells is the contraction of the acto-myosin ring during cytokinesis (Satterwhite and Pollard 1992; Fishkind and Wang 1995). In fungal cells, this is connected with chitin synthesis and depositioning at the septal sites (Schmidt et al. 2002). The acto-myosin ring is not essential in *S. cerevisiae* and *A. gossypii*, in contrast to animal cells and *S. pombe* (Bi et al. 1998; Lippincott and Li 1998; Wendland and Philippsen 2002). Finally, there are differences in the timing of actin ring formation in different cell systems (Vallen et al. 2000).

In filamentous ascomycetes, septation results in the generation of similarly sized hyphal compart-

ments (approx. 40 m in *A. nidulans* and *A. gossypii*; McIntyre et al. 2001; Knechtle et al. 2003). By contrast, the hyphal tip compartments, e.g., in *A. nidulans* and *A. gossypii*, can exceed this standard size several-fold. Interestingly, in the tip, compartment sites of future septation will be defined by the presence of actin rings, such that the tip compartment can harbor up to nine actin rings. In *A. gossypii*, dynamic constriction of actin rings in the tip cell is activated successively, starting with the most subapical one. Ring constriction is accompanied by the generation of the chitin-rich septum (Wendland and Philippsen 2002; see also Sietsma and Wessels, Chap. 4, and Latgé and Calderone, Chap. 5, this volume). By contrast, in *A. nidulans* septation follows the mitotic wave of nuclear division starting at the hyphal tip (Clutterbuck 1970; Westfall and Momany 2002). This allows one to use different model systems to analyze the coupling of mitotic exit with septation.

Assembly of the protein complexes at division sites is a complex and, for some proteins, dynamic process. Recently, it was shown by fluorescence recovery techniques in *S. cerevisiae* that GFP-tagged septin proteins within the septin ring form a highly stable structure with low turnover rates (Dobbelaere and Barral 2004). This is in accord with the function of the septin ring as a rigid scaffold and diffusion barrier (Schmidt and Nichols 2004). The single septin ring splits into two rings late in mitosis, producing a corral that maintains the localization of proteins (e.g., Spa2p, Sec3p, Chs2p) at the septal site by generating a specific compartment at the cell cortex (Dobbelaere and Barral 2004). Splitting of the septin ring is dependent on the activity of the mitotic exit network (MEN, see Sect. V.). Bud3p exhibits similar dynamics as that of the septin ring, as it undergoes a ring splitting as well. The role of Bud3p for septation/cytokinesis in *S. cerevisiae*, however, is unclear. Loss of *BUD3* in yeast results in a change of budding pattern in haploid cells, but does not produce a phenotype in diploid cells (Chant et al. 1995). Since *ScBUD3* is expressed in diploid cells, it may still serve a general, but redundant role. Deletion of the *BUD3* homolog in *A. gossypii* resulted in a pronounced septation defect. AgBud3p was found to be involved in localizing Cyk1p. Deletion of *AgBUD3* resulted in mislocalization of Cyk1p, which formed linear filaments attached to the cortex, rather than rings at septal sites. Subsequently, this resulted also in the formation of linear actin rings, indicating that AgBud3p plays a role in conveying positional information not directly coming from the septin filaments (Wendland 2003). Contributing additional positional information used not only for bud site selection could, therefore, be a yet uncharacterized function of Bud3p in *S. cerevisiae*. Loss of either *bud3* or *bud4* in *S. cerevisiae* has an impact on the budding pattern of haploid cells. In *S. pombe*, Mid2, a protein related to the *S. cerevisiae* Bud4p, was found to stabilize the septin ring and inhibit turnover of septin proteins (Berlin et al. 2003). The turnover of Mid2p may, therefore, be required to trigger septin ring splitting or disassembly (Tasto et al. 2003). Acto-myosin rings are rather immobile in yeast, even in the absence of septin rings, which suggests that another scaffold protein or protein complex may act upon them, and once released, the acto-myosin rings may undergo constriction. In *S. pombe* the contractile acto-myosin ring is kept in its position during cytokinesis by a postanaphase array of microtubules (Pardo and Nurse 2003). In fission yeast as in *S. cerevisiae*, it was also shown that, despite being a stable structure, the actin ring is highly dynamic. Actin and other ring components (e.g., in *S. pombe*, tropomyosin, Cdc8p, and a myosin light chain, Cdc4p) assemble into, and disassemble from the ring structure (Pelham and Chang 2002; Tolliday et al. 2002).

Two main questions are the timing of events, and the coupling of acto-myosin constriction with septum formation. The timing of ring constriction may be species specific, and either coupled or uncoupled from mitotic events. In *A. nidulans*, the *S. cerevisiae* Cdc15p homolog SepH is required early in septin and actin ring assembly, whereas in yeast Cdc15p regulates mitotic exit and is largely not involved in these early steps (Bruno et al. 2001; Westfall and Momany 2002). An intriguing example of the regulation of ring constriction is presented by the hyphal tip compartments of filamentous fungi. Multiple sites of septation within a single compartment are activated differently, which implies that diffusible factors are involved to finally trigger septum completion (Westfall and Momany 2002; Wendland and Philippsen 2002). However, such signals, and any coordination with MEN and/or the cell cycle kinase Cdc28/Cdc2 have yet to be identified.

To coordinate acto-myosin ring constriction with septum formation, there has to be a link between the generation of the forces that result in ring closure and the delivery of vesicles that transport membrane and cell wall material to the relative position of the ring once it becomes dynamic. Recent

reports suggested such a function for the myosin light chain encoded by *MLC1* in *S. cerevisiae* (Wagner et al. 2002; Luo et al. 2004). Mlc1p is a light chain for both Myo1p (in the acto-myosin ring) and Myo2p (vesicle transport), and also recruits Iqg1p/Cyk1p to septal sites (Shannon and Li 2000; Terrak et al. 2003).

Chitin at septa is synthesized by chitin synthases. In *S. cerevisiae*, a primary septum can be distinguished from secondary septa. The primary septum is laid down during acto-myosin ring constriction, forms a chitin-rich disk between mother and bud, and thus leads to the separation of the mother and daughter cytoplasms. The secondary septa are then built from both mother and daughter sides filling the space between the primary septum and cell membrane (Roncero 2002). The number of chitin synthases in fungal organisms varies. In *S. cerevisiae* three chitin synthases are present, encoded by *CHS1*, *CHS2*, and *CHS3*, but up to seven have been reported, for example, in *A. fumigatus* (Roncero 2002). There is both temporal as well as spatial regulation of chitin synthesis. Chs1p (class I) is a minor chitin synthase that is involved in repair synthesis of chitin during cytokinesis (Cabib et al. 1992). Chs2p (class II) is required for primary septum formation, whereas Chs3p (class IV) is the major chitin synthase, and contributes chitin synthesis of the chitin ring and the chitin of the cell wall during vegetative growth (for details, see Sietsma and Wessels, Chap. 4, and Latgé and Calderone, Chap. 5, this volume). Single deletions in the chitin synthase genes are viable, as are *chs1*, *chs2* and *chs1*, *chs3* double mutants, and only a *chs2*, *chs3* double deletion is lethal (Silverman et al. 1988; Shaw et al. 1991; Kollar et al. 1995; Cabib et al. 2001).

Studies with the Chs3p inhibitor nikkomycin Z in a *chs2* mutant showed that chitin synthesis for the formation of the secondary septa is important for survival to achieve septum closure between mother and daughter cells (Cabib and Schmidt 2003).

The signaling cascade leading to chitin synthesis at the septum involves the septin ring acting as a scaffold to recruit Bni4p, which interacts directly with Chs4p/Skt5p. Chs4p/Skt5p finally localizes Chs3p to the septal site. Some other proteins, namely Chs5p, Chs6p and Chs7p, are required for the transport of Chs3p from ER or chitosome to the plasma membrane (for reviews, see Valdivieso et al. 1999; Munro and Gow 2001; Roncero 2002). Septa of filamentous fungi bear one significant difference with septa of yeast-like fungi, this being the septal pore. How this pore is generated is unknown, but its presence allows the transport of material across compartments. Using time-lapse microscopy of GFP labeled nuclei, frequent movement of nuclei through septa was observed (Alberti-Segui et al. 2001). Filamentous fungi need to be able to reseal the septal pore in case of damage to the hypha, which would otherwise cause further damage along the hypha. This can be achieved by clogging the septal pore with a Woronin body (Jedd and Chua 2000; Tenney et al. 2000). The Woronin body of *N. crassa* is composed of a solid core of the Hex1 protein. Hex1 carries a peroxisomal targeting signal, suggesting that Woronin bodies are specialized peroxisomal structures (Jedd and Chua 2000; Tenney et al. 2000; Yuan et al. 2003). In *Magnaporthe grisea*, Woronin bodies were found to be essential for pathogenesis and growth under nitrogen-limiting conditions (Soundararajan et al. 2004).

Since fungal cells are surrounded by a rigid cell wall, cell growth needs to balance cell wall integrity with cell expansion (see Sietsma and Wessels, Chap. 4, this volume). Therefore, the activity of chitin synthases may be counterbalanced by chitin-degrading enzymes, the chitinases. The *S. cerevisiae* chitinase Cts1p is required for cell separation of mother and daughter cells at the end of cytokinesis. This activity is asymmetric, as the mother retains most of its chitin ring (cf. a bud scar that can be stained by calcofluor), whereas the daughter cell contains a much fainter birth scar. This asymmetry is brought about by daughter cell-specific regulatory programs in which the transcriptional activator of *CTS1*, Ace2p, is specifically localized to the daughter cell nucleus by a signaling network that has been termed RAM (cf. *r*egulation of *A*ce2p activity and cellular *m*orphogenesis; Colman-Lerner et al. 2001; Nelson et al. 2003). Filamentous fungi, on the other hand, may not require such a chitinase activity, since septation results only in the compartmentalization but not in the separation of hyphal segments. In fact, the genome of the filamentous fungus *A. gossypii* does not contain a *CTS1* homolog that we recorded during our studies on the *A. gossypii* WASP homolog (Walther and Wendland 2004).

Chitin synthase and chitinase activities may counterbalance each other to ensure polarized growth and to maintain cell wall integrity. However, specific developmental regulation of chitin synthases in *A. nidulans*, as well as the absence of chitinase activity (lack of *CTS1*) in *A. gossypii* suggest that this may not be necessary. A detailed

analysis of the regulation and activity of these enzymes was carried out recently, and in fact demonstrated the independence of both activities in *S. cerevisiae* and *C. albicans* (Selvaggini et al. 2004).

V. Coordination of the End of Mitosis with Cytokinesis

In uninucleate yeasts, cytokinesis must be strictly coordinated with mitosis to ensure that nuclear division and migration into the daughter cell occur prior to septum formation. This requires monitoring the state of mitosis, and coupling mitotic exit with control on the cell cycle to be able to delay cytokinesis until mitosis has been completed. Similar molecular mechanisms have evolved, using highly conserved protein networks that are called SIN in *S. pombe* and MEN in *S. cerevisiae*. Research in this field is vigorously pursued, and has resulted in a large advance of our knowledge that has been covered by excellent recent reviews (Balasubramanian et al. 2000; McCollum and Gould 2001; Jensen et al. 2002; Simanis 2003; Barral 2004; Stegmeier and Amon 2004). Therefore, we will present only a short overview to outline the general picture. To elucidate how mitotic exit in multinucleate compartments of filamentous fungi is coupled to septation will be a task of the future, since only one component of these networks has been analyzed in a filamentous fungus, this being the Cdc15p homolog SepH of *A. nidulans*. Since SepH functions upstream of actin ring formation in *A. nidulans*, the mitotic exit network seems to control septation at a more upstream level than in *S. cerevisiae* (Bruno et al. 2001; Harris 2001).

Generally, progression through the cell cycle is controlled by the activity of a cyclin-dependent kinase (CDKs), which in *S. cerevisiae* is encoded by *CDC28*, in *S. pombe* by *CDC2*, and in *A. nidulans* by $NIMX^{cdc2}$ (Nurse 1990; Osmani and Ye 1996). Mitotic CDK activity is eliminated by degradation via the anaphase promoting complex (APC), and CDK targets are dephosphorylated by the Cdc14 protein phosphatase (Visintin et al. 1998; Jaspersen et al. 1999; Peters 2002). Cdc14p is bound to Net1p and sequestered in the nucleolus (Visintin et al. 1999). The FEAR network (cf. Cdc fourteen early anaphase release) helps in releasing Cdc14p to the nucleus, while MEN triggers release into the cytoplasm. MEN signaling resembles a GTPase module signaling cascade. It consists of a GTPase module using the Tem1p-GTPase and its regulators (Lte1p as a putative GTP exchange factor; Bub2p-Bfa1p dimer as a GTPase activating factor; Li 2000; Lippincott et al. 2001). Nud1p acts as a scaffold protein to convey the signal from the GTPase module to the protein kinases Cdc5, Cdc15, and Dbf2p/Mob1p (Gruneberg et al. 2000). Actually, Cdc5p is also part of FEAR, since it inactivates the Bub2p-Bfa1p complex (Geymonat et al. 2002). To integrate positional information of nuclear migration into the daughter cell with mitotic exit, subcellular localization of the GTPase module components is used: Lte1p localizes to the daughter cell cortex and is kept there by the diffusion barrier set up by the septin ring, while Tem1p is bound to the spindle pole body that is directed to the daughter cell (Bardin et al. 2000; Pereira et al. 2000, 2002). This ensures that mitotic exit is activated only when the daughter nucleus has found its way into the bud. Once *S. cerevisiae* Cdc14p enters the cytoplasm, it dephosphorylates and activates Cdh1p and Sic1p. Sic1p is a CDK inhibitor while Cdh1p is an activator of the APC. This indicates that the main function of MEN in *S. cerevisiae* is to control CDK activity at the end of mitosis, whereas the *S. pombe* SIN actually initiates the contraction of the actin ring and septum formation (Simanis 2003). In *S. cerevisiae*, ring constriction depends on the degradation of Hof1/Cyk2. This is achieved upon the activation of MEN and the Skp1-Cullin-Fbox protein complex (SCF), which depends on Grr1p localization to the bud neck (Blondel et al. 2005).

Exit of mitosis was shown to be linked to the spindle pole body (SPB), since several proteins required for this process are associated with the SPB (Pereira and Schiebel 2001). Particularly, Nud1p links astral microtubule organization with the control of exit from mitosis (Gruneberg et al. 2000).

Taken together, although key components of both machineries are conserved in fungi, differences may exist to which extent they contribute to both mitotic exit and septum formation.

VI. Conclusions

Recent genome sequences have provided a wealth of information on a variety of filamentous fungi that enable comparisons with yeast-like fungi. It became evident that key elements of acto-myosin ring formation, septum formation, and the poorly

understood mechanism of mitotic exit are highly conserved. Mechanistic differences occur in the selection of a new division site, in the order of the assembly of protein complexes at the division site, and in the activation of acto-myosin ring constriction. Further research in filamentous fungi will dwell on several unique features, for example,

1. how does the hyphal tip convey the positional information to septal site placement;
2. which mechanisms ensure the timing of site selection, and thus produce regular-sized hyphal compartments that remain multinucleate or – during asexual sporulation – switch to a yeast-like program and generate uninucleate cells;
3. is one of the key mechanistic differences between septation in filamentous fungi and cytokinesis in yeasts concerned with the synthesis and degradation of chitin via chitin synthases and chitinases; and finally
4. can all this knowledge lead to the development of new drugs to combat fungal infections?

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References

Afshar K, Stuart B, Wasserman SA (2000) Functional analysis of the *Drosophila* diaphanous FH protein in early embryonic development. Development 127:1887–1897

Alberti-Segui C, Dietrich F, Altmann-Jöhl R, Hoepfner D, Philippsen P (2001) Cytoplasmic dynein is required to oppose the force that moves nuclei towards the hyphal tip in the filamentous ascomycete *Ashbya gossypii*. J Cell Sci 114:975–986

Ayad-Durieux Y, Knechtle P, Goff S, Dietrich F, Philippsen P (2000) A PAK-like protein kinase is required for maturation of young hyphae and septation in the filamentous ascomycete *Ashbya gossypii*. J Cell Sci 113:4563–4575

Bähler J, Steever AB, Wheatley S, Wang Y, Pringle JR, Gould KL, McCollum D (1998) Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. J Cell Biol 143:1603–1616

Balasubramanian MK, McCollum D, Surana U (2000) Tying the knot: linking cytokinesis to the nuclear cycle. J Cell Sci 113:1503–1513

Bardin AJ, Visintin R, Amon A (2000) A mechanism for coupling exit from mitosis to partitioning of the nucleus. Cell 102:21–31

Barral Y (2004) Mitosis: FEAR pulls them apart. Dev Cell 6:608–610

Barral Y, Mermall V, Mooseker MS, Snyder M (2000) Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. Mol Cell 5:841–851

Bender A, Pringle JR (1991) Use of a screen for synthetic lethal and multicopy suppressee mutants to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. Mol Cell Biol 11:1295–1305

Berlin A, Paoletti A, Chang F (2003) Mid2p stabilizes septin rings during cytokinesis in fission yeast. J Cell Biol 160:1083–1092

Berman J, Sudbery PE (2002) *Candida albicans*: a molecular revolution built on lessons from budding yeast. Nat Rev Genet 3:918–930

Bi E (2001) Cytokinesis in budding yeast: the relationship between actomyosin ring function and septum formation. Cell Struct Funct 26:529–537

Bi E, Maddox P, Lew DJ, Salmon ED, McMillan JN, Yeh E, Pringle JR (1998) Involvement of an actomyosin contractile ring in *Saccharomyces cerevisiae* cytokinesis. J Cell Biol 142:1301–1312

Blondel M, Bach S, Bamps S, Dobbelaere J, Wiget P, Longaretti C, Barral Y, Meijer L, Peter M (2005) Degradation of Hof1 by SCF(Grr1) is important for actomyosin contraction during cytokinesis in yeast. EMBO J 24:1440–1452

Boyne JR, Yosuf HM, Bieganowski P, Brenner C, Price C (2000) Yeast myosin light chain, Mlc1p, interacts with both IQGAP and class II myosin to effect cytokinesis. J Cell Sci 113:4533–4543

Bruno KS, Morrell JL, Hamer JE, Staiger CJ (2001) SEPH, a Cdc7p orthologue from *Aspergillus nidulans*, functions upstream of actin ring formation during cytokinesis. Mol Microbiol 42:3–12

Byers B, Goetsch L (1976) A highly ordered ring of membrane-associated filaments in budding yeast. J Cell Biol 69:717–721

Cabib E, Schmidt M (2003) Chitin synthase III activity, but not the chitin ring, is required for remedial septa formation in budding yeast. FEMS Microbiol Lett 224:299–305

Cabib E, Silverman SJ, Shaw JA (1992) Chitinase and chitin synthase 1: counterbalancing activities in cell separation of *Saccharomyces cerevisiae*. J Gen Microbiol 138:97–102

Cabib E, Roh DH, Schmidt M, Crotti LB, Varma A (2001) The yeast cell wall and septum as paradigms of cell growth and morphogenesis. J Biol Chem 276:19679–19682

Carroll CW, Altman R, Schieltz D, Yates JR, Kellogg D (1998) The septins are required for the mitosis-specific activation of the Gin4 kinase. J Cell Biol 143:709–717

Casamayor A, Snyder M (2003) Molecular dissection of a yeast septin: distinct domains are required for septin interaction, localization, and function. Mol Cell Biol 23:2762–2777

Caviston JP, Longtine M, Pringle JR, Bi E (2003) The role of Cdc42p GTPase-activating proteins in assembly of the septin ring in yeast. Mol Biol Cell 14:4051–4066

Chang F (2001) Studies in fission yeast on mechanisms of cell division site placement. Cell Struct Funct 26:539–544

Chang F, Nurse P (1996) How fission yeast fission in the middle. Cell 84:191–194

Chang F, Woollard A, Nurse P (1996) Isolation and characterization of fission yeast mutants defective in the assembly and placement of the contractile actin ring. J Cell Sci 109:131–142

Chang F, Drubin D, Nurse P (1997) cdc12p, a protein required for cytokinesis in fission yeast, is a component of the cell division ring and interacts with profilin. J Cell Biol 137:169–182

Chant J, Herskowitz I (1991) Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. Cell 65:1203–1212

Chant J, Pringle JR (1991) Budding and cell polarity in *Saccharomyces cerevisiae*. Curr Opin Genet Dev 1:342–350

Chant J, Pringle JR (1995) Patterns of bud-site selection in the yeast *Saccharomyces cerevisiae*. J Cell Biol 129:751–765

Chant J, Corrado K, Pringle JR, Herskowitz I (1991) Yeast BUD5, encoding a putative GDP-GTP exchange factor, is necessary for bud site selection and interacts with bud formation gene *BEM1*. Cell 65:1213–1224

Chant J, Mischke M, Mitchell E, Herskowitz I, Pringle JR (1995) Role of Bud3p in producing the axial budding pattern of yeast. J Cell Biol 129:767–778

Clutterbuck AJ (1970) Synchronous nuclear division and septation in *Aspergillus nidulans*. J Gen Microbiol 60:133–135

Colman-Lerner A, Chin TE, Brent R (2001) Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. Cell 107:739–750

Dobbelaere J, Barral Y (2004) Spatial coordination of cytokinetic events by compartmentalization of the cell cortex. Science 305:393–396

Dong Y, Pruyne D, Bretscher A (2003) Formin-dependent actin assembly is regulated by distinct modes of Rho signaling in yeast. J Cell Biol 161:1081–1092

Epp JA, Chant J (1997) An IQGAP-related protein controls actin-ring formation and cytokinesis in yeast. Curr Biol 7:921–929

Evangelista M, Blundell K, Longtine MS, Chow CJ, Adames N, Pringle JR, Peter M, Boone C (1997) Bni1p, a yeast formin linking Cdc42p and the actin cytoskeleton during polarized morphogenesis. Science 276:118–122

Evangelista M, Zigmond S, Boone C (2003) Formins: signaling effectors for assembly and polarization of actin filaments. J Cell Sci 116:2603–2611

Faty M, Fink M, Barral Y (2002) Septins: a ring to part mother and daughter. Curr Genet 41:123–131

Field CM, Kellogg D (1999) Septins: cytoskeletal polymers or signalling GTPases? Trends Cell Biol 9:387–394

Field CM, al-Awar O, Rosenblatt J, Wong ML, Alberts B, Mitchison TJ (1996) A purified *Drosophila* septin complex forms filaments and exhibits GTPase activity. J Cell Biol 133:605–616

Fishkind DJ, Wang YL (1995) New horizons for cytokinesis. Curr Opin Cell Biol 7:23–31

Freifelder D (1960) Bud position in *Saccharomyces cerevisiae*. J Bacteriol 80:567–568

Fujita A, Oka C, Arikawa Y, Katagai T, Tonouchi A, Kuhara S, Misumi Y (1994) A yeast gene necessary for bud-site selection encodes a protein similar to insulin-degrading enzymes. Nature 372:567–570

Fujita A, Lord M, Hiroko T, Hiroko F, Chen T, Oka C, Misumi Y, Chant J (2004) Rax1, a protein required for the establishment of the bipolar budding pattern in yeast. Gene 327:161–169

Fujiwara T, Tanaka K, Mino A, Kikyo M, Takahashi K, Shimizu K, Takai Y (1998) Rho1p-Bni1p-Spa2p interactions: implication in localization of Bni1p at the bud site and regulation of the actin cytoskeleton in *Saccharomyces cerevisiae*. Mol Biol Cell 9:1221–1233

Gale C, Gerami-Nejad M, McClellan M, Vandoninck S, Longtine MS, Berman J (2001) *Candida albicans* Int1p interacts with the septin ring in yeast and hyphal cells. Mol Biol Cell 12:3538–3549

Geymonat M, Jensen S, Johnston LH (2002) Mitotic exit: the Cdc14 double cross. Curr Biol 12:R482–R484

Gladfelter AS, Pringle JR, Lew DJ (2001) The septin cortex at the yeast mother-bud neck. Curr Opin Microbiol 4:681–689

Gladfelter AS, Bose I, Zyla TR, Bardes ES, Lew DJ (2002) Septin ring assembly involves cycles of GTP loading and hydrolysis by Cdc42p. J Cell Biol 156:315–326

Gruneberg U, Campbell K, Simpson C, Grindlay J, Schiebel E (2000) Nud1p links astral microtubule organization and the control of exit from mitosis. EMBO J 19:6475–6488

Halme A, Michelitch M, Mitchell EL, Chant J (1996) Bud10p directs axial cell polarization in budding yeast and resembles a transmembrane receptor. Curr Biol 6:570–579

Harkins HA, Page N, Schenkman LR, De Virgilio C, Shaw S, Bussey H, Pringle JR (2001) Bud8p and Bud9p, proteins that may mark the sites for bipolar budding in yeast. Mol Biol Cell 12:2497–2518

Harris SD (1999) Morphogenesis is coordinated with nuclear division in germinating *Aspergillus nidulans* conidiospores. Microbiology 145:2747–2756

Harris SD (2001) Septum formation in *Aspergillus nidulans*. Curr Opin Microbiol 4:736–739

Hartwell LH (1971) Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. Exp Cell Res 69:265–276

Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK (2003) Global analysis of protein localization in budding yeast. Nature 425:686–691

Jaspersen SL, Charles JF, Morgan DO (1999) Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. Curr Biol 9:227–236

Jedd G, Chua NH (2000) A new self-assembled peroxisomal vesicle required for efficient resealing of the plasma membrane. Nat Cell Biol 2:226–231

Jensen S, Geymonat M, Johnston LH (2002) Mitotic exit: delaying the end without FEAR. Curr Biol 12:R221–R223

Johnson DI (1999) Cdc42: an essential Rho-type GTPase controlling eukaryotic cell polarity. Microbiol Mol Biol Rev 63:54–105

Kadota J, Yamamoto T, Yoshiuchi S, Bi E, Tanaka K (2004) Septin ring assembly requires concerted action of polarisome components, a PAK Kinase Cla4p, and the actin cytoskeleton in *Saccharomyces cerevisiae*. Mol Biol Cell 15:5329–5345

Kamei T, Tanaka K, Hihara T, Umikawa M, Imamura H, Kikyo M, Ozaki K, Takai Y (1998) Interaction of Bnr1p with a novel Src homology 3 domain-containing Hof1p.

Implication in cytokinesis in *Saccharomyces cerevisiae*. J Biol Chem 273:28341–28345
Kaminskyj SG (2000) Septum position is marked at the tip of *Aspergillus nidulans* hyphae. Fungal Genet Biol 31:105–113
Kang PJ, Sanson A, Lee B, Park HO (2001) A GDP/GTP exchange factor involved in linking a spatial landmark to cell polarity. Science 292:1376–1378
Kang PJ, Angerman E, Nakashima K, Pringle JR, Park HO (2004a) Interactions among Rax1p, Rax2p, Bud8p, and Bud9p in marking cortical sites for bipolar bud-site selection in yeast. Mol Biol Cell 15:5145–5157
Kang PJ, Lee B, Park HO (2004b) Specific residues of the GDP/GTP exchange factor Bud5p are involved in establishment of the cell type-specific budding pattern in yeast. J Biol Chem 279:27980–27985
Knechtle P, Dietrich F, Philippsen P (2003) Maximal polar growth potential depends on the polarisome component AgSpa2 in the filamentous fungus *Ashbya gossypii*. Mol Biol Cell 14:4140–4154
Kollar R, Petrakova E, Ashwell G, Robbins PW, Cabib E (1995) Architecture of the yeast cell wall. The linkage between chitin and β(1→3)-glucan. J Biol Chem 270:1170–1178
Kozminski KG, Beven L, Angerman E, Tong AH, Boone C, Park HO (2003) Interaction between a Ras and a Rho GTPase couples selection of a growth site to the development of cell polarity in yeast. Mol Biol Cell 14:4958–4970
Kumar A, Cheung KH, Tosches N, Masiar P, Liu Y, Miller P, Snyder M (2002) The TRIPLES database: a community resource for yeast molecular biology. Nucleic Acids Res 30:73–75
Kusch J, Meyer A, Snyder MP, Barral Y (2002) Microtubule capture by the cleavage apparatus is required for proper spindle positioning in yeast. Genes Dev 16:1627–1639
Li R (2000) Mitosis: shutting the door behind when you leave. Curr Biol 10:R781–R784
Lippincott J, Li R (1998) Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. J Cell Biol 140:355–366
Lippincott J, Shannon KB, Shou W, Deshaies RJ, Li R (2001) The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. J Cell Sci 114:1379–1386
Longtine MS, Bi E (2003) Regulation of septin organization and function in yeast. Trends Cell Biol 13:403–409
Longtine MS, DeMarini DJ, Valencik ML, Al-Awar OS, Fares H, de Virgilio C, Pringle JR (1996) The septins: roles in cytokinesis and other processes. Curr Opin Cell Biol 8:106–119
Lord M, Inose F, Hiroko T, Hata T, Fujita A, Chant J (2002) Subcellular localization of Axl1, the cell type-specific regulator of polarity. Curr Biol 12:1347–1352
Luo J, Vallen EA, Dravis C, Tcheperegine SE, Drees B, Bi E (2004) Identification and functional analysis of the essential and regulatory light chains of the only type II myosin Myo1p in *Saccharomyces cerevisiae*. J Cell Biol 165:843–855
Madden K, Snyder M (1998) Cell polarity and morphogenesis in budding yeast. Annu Rev Microbiol 52:687–744
Martin SW, Konopka JB (2004) Lipid raft polarization contributes to hyphal growth in *Candida albicans*. Eukaryot Cell 3:675–684
McCollum D, Gould KL (2001) Timing is everything: regulation of mitotic exit and cytokinesis by the MEN and SIN. Trends Cell Biol 11:89–95
McIntyre M, Dynesen J, Nielsen J (2001) Morphological characterization of *Aspergillus nidulans*: growth, septation and fragmentation. Microbiology 147:239–246
Momany M, Zhao J, Lindsey R, Westfall PJ (2001) Characterization of the *Aspergillus nidulans* septin (asp) gene family. Genetics 157:969–977
Munro CA, Gow NA (2001) Chitin synthesis in human pathogenic fungi. Med Mycol 39 Suppl 1:41–53
Nelson B, Kurischko C, Horecka J, Mody M, Nair P, Pratt L, Zougman A, McBroom LD, Hughes TR, Boone C et al. (2003) RAM: a conserved signaling network that regulates Ace2p transcriptional activity and polarized morphogenesis. Mol Biol Cell 14:3782–3803
Neufeld TP, Rubin GM (1994) The *Drosophila* peanut gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. Cell 77:371–379
Ni L, Snyder M (2001) A genomic study of the bipolar bud site selection pattern in *Saccharomyces cerevisiae*. Mol Biol Cell 12:2147–2170
Noguchi T, Arai R, Motegi F, Nakano K, Mabuchi I (2001) Contractile ring formation in *Xenopus* egg and fission yeast. Cell Struct Funct 26:545–554
Nurse P (1990) Universal control mechanism regulating onset of M-phase. Nature 344:503–508
Oegema K, Savoian MS, Mitchison TJ, Field CM (2000) Functional analysis of a human homologue of the *Drosophila* actin binding protein anillin suggests a role in cytokinesis. J Cell Biol 150:539–552
Ohkura H, Hagan IM, Glover DM (1995) The conserved *Schizosaccharomyces pombe* kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells. Genes Dev 9:1059–1073
Osmani SA, Ye XS (1996) Cell cycle regulation in *Aspergillus* by two protein kinases. Biochem J 317:633–641
Ozaki-Kuroda K, Yamamoto Y, Nohara H, Kinoshita M, Fujiwara T, Irie K, Takai Y (2001) Dynamic localization and function of Bni1p at the sites of directed growth in *Saccharomyces cerevisiae*. Mol Cell Biol 21:827–839
Pardo M, Nurse P (2003) Equatorial retention of the contractile actin ring by microtubules during cytokinesis. Science 300:1569–1574
Park HO, Chant J, Herskowitz I (1993) BUD2 encodes a GTPase-activating protein for Bud1/Rsr1 necessary for proper bud-site selection in yeast. Nature 365:269–274
Pelham RJ, Chang F (2002) Actin dynamics in the contractile ring during cytokinesis in fission yeast. Nature 419:82–86
Pereira G, Schiebel E (2001) The role of the yeast spindle pole body and the mammalian centrosome in regulating late mitotic events. Curr Opin Cell Biol 13:762–769
Pereira G, Hofken T, Grindlay J, Manson C, Schiebel E (2000) The Bub2p spindle checkpoint links nuclear migration with mitotic exit. Mol Cell 6:1–10
Pereira G, Manson C, Grindlay J, Schiebel E (2002) Regulation of the Bfa1p-Bub2p complex at spindle pole

bodies by the cell cycle phosphatase Cdc14p. J Cell Biol 157:367–379
Peters JM (2002) The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol Cell 9:931–943
Petersen J, Nielsen O, Egel R, Hagan IM (1998) FH3, a domain found in formins, targets the fission yeast formin Fus1 to the projection tip during conjugation. J Cell Biol 141:1217–1228
Roemer T, Madden K, Chang J, Snyder M (1996) Selection of axial growth sites in yeast requires Axl2p, a novel plasma membrane glycoprotein. Genes Dev 10:777–793
Roncero C (2002) The genetic complexity of chitin synthesis in fungi. Curr Genet 41:367–378
Ross-Macdonald P, Coelho PS, Roemer T, Agarwal S, Kumar A, Jansen R, Cheung KH, Sheehan A, Symoniatis D, Umansky L et al. (1999) Large-scale analysis of the yeast genome by transposon tagging and gene disruption. Nature 402:413–418
Ruiz-Herrera J, San-Blas G (2003) Chitin synthesis as target for antifungal drugs. Curr Drug Targets Infect Disord 3:77–91
Sanders SL, Field CM (1994) Cell division. Septins in common? Curr Biol 4:907–910
Sanders SL, Herskowitz I (1996) The BUD4 protein of yeast, required for axial budding, is localized to the mother/bud neck in a cell cycle-dependent manner. J Cell Biol 134:413–427
Sanders SL, Gentzsch M, Tanner W, Herskowitz I (1999) O-glycosylation of Axl2/Bud10p by Pmt4p is required for its stability, localization, and function in daughter cells. J Cell Biol 145:1177–1188
Satterwhite LL, Pollard TD (1992) Cytokinesis. Curr Opin Cell Biol 4:43–52
Schenkman LR, Caruso C, Page N, Pringle JR (2002) The role of cell cycle-regulated expression in the localization of spatial landmark proteins in yeast. J Cell Biol 156:829–841
Schmidt K, Nichols BJ (2004) A barrier to lateral diffusion in the cleavage furrow of dividing mammalian cells. Curr Biol 14:1002–1006
Schmidt M, Bowers B, Varma A, Roh DH, Cabib E (2002) In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. J Cell Sci 115:293–302
Schmidt M, Varma A, Drgon T, Bowers B, Cabib E (2003) Septins, under Cla4p regulation, and the chitin ring are required for neck integrity in budding yeast. Mol Biol Cell 14:2128–2141
Selvaggini S, Munro CA, Paschoud S, Sanglard D, Gow NA (2004) Independent regulation of chitin synthase and chitinase activity in *Candida albicans* and *Saccharomyces cerevisiae*. Microbiology 150:921–928
Shannon KB, Li R (1999) The multiple roles of Cyk1p in the assembly and function of the actomyosin ring in budding yeast. Mol Biol Cell 10:283–296
Shannon KB, Li R (2000) A myosin light chain mediates the localization of the budding yeast IQGAP-like protein during contractile ring formation. Curr Biol 10:727–730
Sharpless KE, Harris SD (2002) Functional characterization and localization of the *Aspergillus nidulans* formin SEPA. Mol Biol Cell 13:469–479
Shaw BD, Momany M (2002) *Aspergillus nidulans* polarity mutant swoA is complemented by protein O-mannosyltransferase pmtA. Fungal Genet Biol 37:263–270
Shaw JA, Mol PC, Bowers B, Silverman SJ, Valdivieso MH, Duran A, Cabib E (1991) The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J Cell Biol 114:111–123
Sheu YJ, Santos B, Fortin N, Costigan C, Snyder M (1998) Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. Mol Cell Biol 18:4053–4069
Silverman SJ, Sburlati A, Slater ML, Cabib E (1988) Chitin synthase 2 is essential for septum formation and cell division in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 85:4735–4739
Simanis V (2003) Events at the end of mitosis in the budding and fission yeasts. J Cell Sci 116:4263–4275
Sohrmann M, Schmidt S, Hagan I, Simanis V (1998) Asymmetric segregation on spindle poles of the *Schizosaccharomyces pombe* septum-inducing protein kinase Cdc7p. Genes Dev 12:84–94
Soundararajan S, Jedd G, Li X, Ramos-Pamplona M, Chua NH, Naqvi NI (2004) Woronin body function in *Magnaporthe grisea* is essential for efficient pathogenesis and for survival during nitrogen starvation stress. Plant Cell 16:1564–1574
Stegmeier F, Amon A (2004) Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu Rev Genet 38:203–232
Stevens RC, Davis TN (1998) Mlc1p is a light chain for the unconventional myosin Myo2p in *Saccharomyces cerevisiae*. J Cell Biol 142:711–722
Sudbery PE (2001) The germ tubes of *Candida albicans* hyphae and pseudohyphae show different patterns of septin ring localization. Mol Microbiol 41:19–31
Taheri N, Kohler T, Braus GH, Mosch HU (2000) Asymmetrically localized Bud8p and Bud9p proteins control yeast cell polarity and development. EMBO J 19:6686–6696
Takizawa PA, DeRisi JL, Wilhelm JE, Vale RD (2000) Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. Science 290:341–344
Tasto JJ, Morrell JL, Gould KL (2003) An anillin homologue, Mid2p, acts during fission yeast cytokinesis to organize the septin ring and promote cell separation. J Cell Biol 160:1093–1103
Tenney K, Hunt I, Sweigard J, Pounder JI, McClain C, Bowman EJ, Bowman BJ (2000) Hex-1, a gene unique to filamentous fungi, encodes the major protein of the *Woronin* body and functions as a plug for septal pores. Fungal Genet Biol 31:205–217
Terrak M, Wu G, Stafford WF, Lu RC, Dominguez R (2003) Two distinct myosin light chain structures are induced by specific variations within the bound IQ motifs-functional implications. EMBO J 22:362–371
Timberlake WE (1990) Molecular genetics of *Aspergillus* development. Annu Rev Genet 24:5–36
Tolliday N, VerPlank L, Li R (2002) Rho1 directs formin-mediated actin ring assembly during budding yeast cytokinesis. Curr Biol 12:1864–1870
Tran PT, Marsh L, Doye V, Inoue S, Chang F (2001) A mechanism for nuclear positioning in fission yeast based on microtubule pushing. J Cell Biol 153:397–411

Trinci APJ, Morris NR (1979) Morphology and growth of a temperature sensitive mutant of *Aspergillus nidulans* which forms aseptate mycelia at non-permissive temperatures. J Gen Microbiol 114:53–59

Valdivieso MH, Duran A, Roncero C (1999) Chitin synthases in yeast and fungi. In: Jolles P, Muzarelli RAA (eds) Chitin and chitinases. Birkhäuser, Basel, pp 53–67

Vallen EA, Caviston J, Bi E (2000) Roles of Hof1p, Bni1p, Bnr1p, and myo1p in cytokinesis in *Saccharomyces cerevisiae*. Mol Biol Cell 11:593–611

Versele M, Thorner J (2004) Septin collar formation in budding yeast requires GTP binding and direct phosphorylation by the PAK, Cla4. J Cell Biol 164:701–715

Versele M, Gullbrand B, Shulewitz MJ, Cid VJ, Bahmanyar S, Chen RE, Barth P, Alber T, Thorner J (2004) Protein-protein interactions governing septin heteropentamer assembly and septin filament organization in *Saccharomyces cerevisiae*. Mol Biol Cell 15:4568–4583

Visintin R, Craig K, Hwang ES, Prinz S, Tyers M, Amon A (1998) The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol Cell 2:709–718

Visintin R, Hwang ES, Amon A (1999) Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. Nature 398:818–823

Vrabioiu AM, Gerber SA, Gygi SP, Field CM, Mitchison TJ (2004) The majority of the *Saccharomyces cerevisiae* septin complexes do not exchange guanine nucleotides. J Biol Chem 279:3111–3118

Wagner W, Bielli P, Wacha S, Ragnini-Wilson A (2002) Mlc1p promotes septum closure during cytokinesis via the IQ motifs of the vesicle motor Myo2p. EMBO J 21:6397–6408

Walther A, Wendland J (2003) Septation and cytokinesis in fungi. Fungal Genet Biol 40:187–196

Walther A, Wendland J (2004) Apical localization of actin patches and vacuolar dynamics in *Ashbya gossypii* depend on the WASP homolog Wal1p. J Cell Sci 117:4947–4958

Wendland J (2001) Comparison of morphogenetic networks of filamentous fungi and yeast. Fungal Genet Biol 34:63–82

Wendland J (2003) Analysis of the landmark protein Bud3 of *Ashbya gossypii* reveals a novel role in septum construction. EMBO Rep 4:200–204

Wendland J, Philippsen P (2000) Determination of cell polarity in germinated spores and hyphal tips of the filamentous ascomycete *Ashbya gossypii* requires a rhoGAP homolog. J Cell Sci 113:1611–1621

Wendland J, Philippsen P (2001) Cell polarity and hyphal morphogenesis are controlled by multiple rho-protein modules in the filamentous ascomycete *Ashbya gossypii*. Genetics 157:601–610

Wendland J, Philippsen P (2002) An IQGAP-related protein, encoded by *AgCYK1*, is required for septation in the filamentous fungus *Ashbya gossypii*. Fungal Genet Biol 37:81–88

Westfall PJ, Momany M (2002) *Aspergillus nidulans* septin AspB plays pre- and postmitotic roles in septum, branch, and conidiophore development. Mol Biol Cell 13:110–118

Wolkow TD, Harris SD, Hamer JE (1996) Cytokinesis in *Aspergillus nidulans* is controlled by cell size, nuclear positioning and mitosis. J Cell Sci 109:2179–2188

Yuan P, Jedd G, Kumaran D, Swaminathan S, Shio H, Hewitt D, Chua NH, Swaminathan K (2003) A HEX-1 crystal lattice required for *Woronin* body function in *Neurospora crassa*. Nat Struct Biol 10:264–270

Zahner JE, Harkins HA, Pringle JR (1996) Genetic analysis of the bipolar pattern of bud site selection in the yeast *Saccharomyces cerevisiae*. Mol Cell Biol 16:1857–1870

7 Re-Wiring the Network: Understanding the Mechanism and Function of Anastomosis in Filamentous Ascomycete Fungi

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CONTENTS

I. Introduction

A filamentous fungal colony consists of a network of interconnected multinucleate hyphae that grows by hyphal tip extension, branching and anastomosis (hyphal fusion). Vegetative hyphae within a colony, such as that of the filamentous ascomycete *Neurospora crassa*, can be separated into two morphologically distinct regions. The first is the peripheral portion of the colony, where apical hyphae grow outward and exhibit a subapical branching pattern (Buller 1933; Hickey et al. 2002). Hyphae at the periphery of the colony exhibit negative autotropism and are refractory to anastomosis. By contrast, within the interior of a colony, the hyphal morphology is distinctly different from that of the peripheral hyphae, and is highly reticulated in appearance. Branch initiation occurs at irregular intervals, and these hyphae often show positive tropic responses associated with hyphal fusion events. Both hyphal tip growth and the formation of lateral branches are associated with a membrane-dense organelle, the Spitzenkörper, found at the tips of all hyphae, branches and pegs; directionality of growth of a hypha has been associated with the position of the Spitzenkörper at the hyphal tip (Girbardt 1957; Lopez-Franco and Bracker 1996; Riquelme et al. 1998; Hickey et al. 2002; Riquelme and Bartnicki-Garcia 2004).

The ability to make an interconnected hyphal network that is characteristic of a filamentous fungal colony has certain advantages. Anastomosis between hyphae within a single colony enables fungi to establish complex functional units that show coordinated growth and exploration of their environment (Buller 1933; Rayner 1996). Cytoplasmic continuity can be restored by growth of hyphae through dead hyphal compartments, followed by hyphal fusion with living sectors (Buller 1933). Anastomosis between hyphae of genetically different, but heterokaryon-compatible genotypes can lead to genetic diversity via parasexual recombination and formation of novel genotypes (Pontecorvo 1956; Swart et al. 2001). Parasexuality is thought to contribute to the high adaptability of fungi in species that lack sexual reproduction and genetic diversity generated via meiosis.

Anastomosis between different colonies also has potential disadvantages. Hyphal fusion between different individuals increases the risk of transfer of deleterious infectious elements or resource plundering (Debets et al. 1994; Debets and Griffiths 1998; van Diepeningen et al. 1998; Chu et al. 2002; Bruggeman et al. 2003). Protection against these threats is mediated by a mechanism of nonself recognition via heterokaryon incompatibility. During heterokaryon incompatibility, hyphal fusion between individuals that have alternative specificities at nonself recognition loci,

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termed *het* loci, triggers a cell death response in the fusion cell and often in subtending hyphal compartments (Labarère and Bernet 1977; Jacobson et al. 1998; Biella et al. 2002; Sarkar et al. 2002; Marek et al. 2003).

Hyphal fusion within a fungal colony occurs in filamentous basidiomycete and ascomycete species, as well as in species in the basal lineage to the Ascomycota and the Basidomycota, the Zygomycota. Anastomosis in species of zygomycetes are apparently not common (Gregory 1984), but were observed in cultures of *Mortierella* sp. (Griffin and Perrin 1960). Recent evidence describes anastomosis in the zygomycete arbuscular mycorrhizal fungal species *Glomus mosseae*, *G. caledonium*, and *G. intraradices* (Giovannetti et al. 1999, 2001). Hyphal fusion has also been observed in species within the Oomycota, such as *Phytophthora capsici* (Stephenson et al. 1974), which grow like filamentous fungi, but are more closely related to algae.

In filamentous ascomycete species, hyphal fusion occurs during all steps of the fungal life cycle. These fusion events serve different purposes during the establishment and development of mycelial colonies. Under certain conditions and in some species, anastomosis can occur between germinating and even apparently ungerminated conidia (Köhler 1930; Mesterhazy 1973; Roca et al. 2003, 2005; Fig. 7.1A), perhaps providing cooperation during germination to establish a colony among like genotypes. Hyphal fusion within a mature colony results in the formation of a network of interlinked hyphae, which presumably facilitates communication and resource exploitation (Buller 1933; Rayner 1996; Fig. 7.1B). In the sexual phase of the life cycle, cell fusion is essential for mating in out-breeding species. Sexual reproduction can be initiated by fusion between specialized hyphae from female reproductive structures (trichogynes) and a male cell of the opposite mating type, which may be a hypha or asexual spore, such as a conidium or microconidium (Bistis 1981; Fig. 7.1C). In other species, such as *Aspergillus nidulans*, out-crossing is believed to require either transient heterokaryon formation or an undescribed fertilization event (Bruggeman et al. 2003). After initiation of the sexual cycle, cell fusion is also associated with ascogenous hyphae development and crozier formation (Fig. 7.1D), a process that occurs just prior to karyogamy and meiosis (Raju 1980; Davis 2000).

In this review, we focus on the phenomenon of germling/vegetative hyphal fusion between like genotypes in filamentous ascomycete species, particularly on *N. crassa* as a model for this process. We will describe the morphology of these events, review the known molecular factors involved in fungal anastomosis, compare them to events associated with mating cell fusion, and speculate about the possible role of anastomosis between individuals of like genotype. The genetics and molecular basis of heterokaryon incompatibility, as a consequence of hyphal fusion between individuals of unlike genotype, has recently been reviewed (Leslie 1993; Bégueret et al. 1994; Glass et al. 2000; Saupe 2000; Glass and Kaneko 2003) and thus will not be covered here.

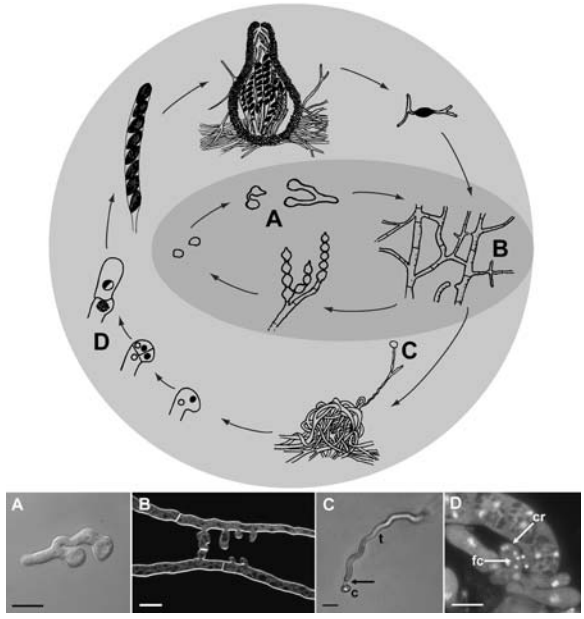

Fig. 7.1. Stages in the life cycle of *Neurospora crassa* where fusion occurs. **A** Conidia at sufficient cell density undergo fusion between germlings. *Bar* = 10 µm. **B** Hyphae within the interior of the colony show chemotropism and hyphal fusion. Adapted from (Hickey et al. 2002), reprinted with permission. Bar = 10 µm. **C** The sexual cycle is initiated by cell fusion involving a fertile receptive hypha (*t* trichogyne) emanating from a female reproductive structure, the protoperithecia (out of view). The trichogyne shows chemotropism toward a conidium of the opposite mating type (*c*). *Arrow* indicates fusion point. Bar = 10 µm. **D** Following fertilization, nuclei of opposite mating types (*mat A* and *mat a*) proliferate in the ascogenous hyphae. Opposite mating-type nuclei pair off and migrate into the crozier. In *N. crassa*, karyogamy occurs in the penultimate cell of the crozier (*cr*). Hyphal and nuclear fusion occurs between the terminal cell and the subtending cell of the crozier (*fc*). Karyogamy, meiosis and an additional mitotic division occur in the ascus, resulting in an eight-spored ascus. The ascospores are initially binucleate. Asci, ascogenous hyphae and croziers treated with DAPI, a nuclear stain. Bar = 20 µm

II. Germling Fusion

Even during the early days of mycology, microscope analysis of fusion between germinating asexual spores was described in detail. In 1930, Köhler described interactions between germinating conidia of the same or of different species. Köhler (1930) also reported that conidia of *Botrytis allii* and *Fusarium* sp. fuse by small hyphal bridges ("Fusionshyphen"), which are significantly narrower than germ tubes (Fig. 7.2A). In *Fusarium oxysporum*, Mesterhazy (1973) also observed that ungerminated macroconidia can be connected by short fusion bridges while still in asexual reproductive structures, termed sporodochia. Recent studies on *Colletotrichum* species and on *N. crassa* showed that specialized structures connect germlings as well as ungerminated conidia (Roca et al. 2003, 2005). These conidial anastomosis tubes (CATs) differ in size and growth behavior from germ tubes.

In addition to conidia, Köhler (1930) described fusion between germ tubes within single isolates of *Botrytis*, *Sclerotinia*, *Neurospora* and *Fusarium* species (Fig. 7.2B). More recent reports describe germling fusion in *Fusarium* sp., *Venturia inaequalis* and *N. crassa* (Leu 1967; Mesterhazy 1973; Pandey et al. 2004; Roca et al. 2005; Fig. 7.2C). It is currently unclear whether mechanistic or signaling differences occur between conidial fusion involving CATs versus germling fusion.

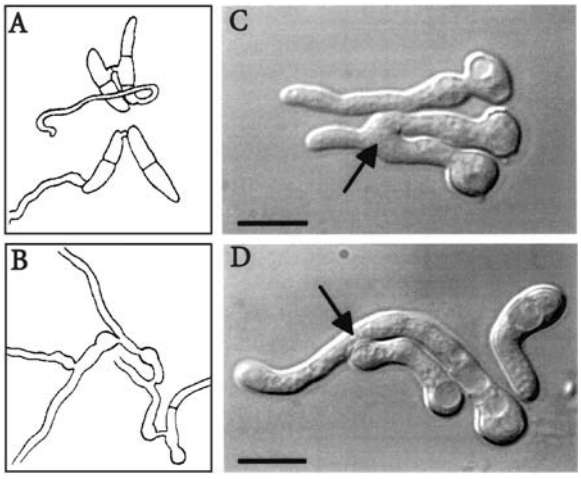

Fig. 7.2. A–D Germinating conidia in close proximity undergo germling fusion. **A** Germling fusion between conidia from *Fusarium* sp. Adapted from Köhler (1929). **B** Anastomosis between germinating conidia from *N. crassa*. Adapted from Köhler (1929). **C** Germling fusion (*arrow*) between germinating conidia of *N. crassa*. Bar = 10 µm. **D** Lack of germling fusion in the *N. crassa* anastomosis mutant *so*. Although contact between germ tubes is evident, fusion (cell wall breakdown and membrane fusion) does not occur (*arrow*)

Köhler (1930) also observed interactions between conidia of different species, although complete germling fusions were not observed. For example, conidia of *N. sitophila* and *N. tetrasperma* formed small pegs when confronted. Interactions between species that were not closely related were also observed, e.g., hyphae of *Botrytis allii* formed small pegs in response to approaching hyphal tips of *N. sitophila*. Compared to intra-species interactions, however, these interactions between different species were weak.

The frequency of germling fusion in a number of species is apparently affected by both nutritional and environmental factors. Generally speaking, the following rule applies: the fewer nutrients, the more anastomoses. In *Leptosphaeria coniothyrium*, *Sclerotinia fructigena*, *Botrytis* and *Fusarium* sp., the frequency of germling fusion was reduced on rich media (such as 1% malt or potato dextrose agar), but higher on diluted media (Laibach 1928; Köhler 1930). However, Leu (1967) reported that the number of fusions formed between germlings of *Venturia inaequalis* was significantly greater on complete medium than on basal medium or water agar. These results suggest that factors other than nutrient availability also contribute to the frequency of germling fusion between conidia.

In addition to nutritional factors that affect the frequency of germling fusion, a number of studies have suggested that the initiation of fusion events among conidia shows a conidial density-dependent function. In *V. inaequalis*, fusion was not observed between two isolated conidia mounted close to one another (Leu 1967). However, if multiple conidia were placed close to one another under the same conditions, numerous germling fusion events were observed. Fusion between an isolated conidial pair could be induced by the addition of culture filtrate from the same or different isolates. Interestingly, culture filtrates from isolates that were anastomosis deficient did not induce fusion in this assay. The observed fusion induction was apparently species-specific because culture filtrates of *V. pirina* did not induce fusions between *V. inaequalis* conidia.

III. Hyphal Fusion

Like conidial germling fusion, hyphal fusion attracted the attention of early mycologists. In

1888, Marshall Ward published his work on a *Botrytis* species found on lily (Ward 1888). He described how hyphae were attracted to each other at a distance, and showed directed growth followed by fusion. In *N. crassa* and other filamentous ascomycete species, the frequency of hyphal fusion within a vegetative colony varies from the periphery to the interior of the colony, and also within the interior of the colony itself (Hickey et al. 2002). Hyphal tips at the periphery of the colony are refractory to hyphal fusion. At the periphery, hyphae grow straight out from the colony and show subapical branching. The leading hyphae as well as the subapical branches show avoidance (negative autotropism), presumably to maximize the outward growth of the colony (Trinci 1984). Even in cases of contact between hyphae at the periphery of the colony, fusion is not observed (Hickey et al. 2002).

In the inner portion of a colony, hyphae show a different behavior. They branch and begin to fill the spaces between individual hyphae. Instead of avoidance, certain hyphae show attraction, directed growth and hyphal anastomoses (Köhler 1929; Buller 1933; Hickey et al. 2002). Within the colony, microscope observations suggest that competency plays a role in vegetative hyphal fusion, that is, not all hyphae within a colony are equally competent to respond to fusion signals. The nature of the difference in competency among hyphal types and position within a colony is unknown. It is clear that different morphologies of hyphae occur within the interior of a colony, also in *N. crassa* (for terminology, see Bistis et al. 2003). Thus, the various hyphal types within a colony may be in different physiological states with respect to hyphal fusion. The spatial frequency of anastomosis within the fusion-competent regions of a colony can also vary, although parameters that affect this process are unclear.

Similarly to germling fusion, studies on filamentous fungi have shown that the ability to undergo hyphal fusion is highly influenced by growth conditions. Investigations of nutritional conditions affecting hyphal anastomosis frequency in the basidiomycete species *Rhizoctonia solani* and *Schizophyllum commune* also showed a negative correlation between nutrient concentration and fusion frequency (Ahmad and Miles 1970; Yokoyama and Ogoshi 1988). Nitrogen levels affected hyphal fusion frequency in the basidiomycetes *R. solani* (Yokoyama and Ogoshi 1988) and *R. oryzae* (Bhuiyan and Arai 1993). The addition of nitrogen to nutrient-poor media led to the largest decrease in hyphal fusion frequency, compared to the addition of other compounds such as carbon, potassium, phosphate, magnesium or iron.

The formation of interconnected networks and the pooling of existing storage compounds may favor the survival of a fungal individual under suboptimal environmental conditions. While this theory assumes that anastomosis is beneficial, other authors speculate that fusion could be somehow connected with parasitism and pathogenicity (Laibach 1928). In this scenario, one fusion partner would benefit from the nutrient sources of the other, a type of resource plundering. A resemblance between hyphal fusion and infection of plant hosts by pathogenic fungi has also been reported (Chen and Wu 1977; Yokoyama and Ogoshi 1988). In addition, some aspects of mycoparasitism show similarity to the hyphal fusion process. Mycoparasites recognize a diffusible signal produced by the host, which results in the reorientation of a hyphal growth trajectory (Evans and Cooke 1982; Jeffries 1985). In the host–parasite system of *Absidia glauca* and *Parasitella parasitica* (both members of the Zygomycota), fusion bridges were observed between mycelia of the two species (Kellner et al. 1993). The interaction between *Absidia* and *Parasitella* was mating-type dependent; only + mating-type *A. glauca* were infected by – mating-type *P. parasitica*, and vice versa. These observations led to the hypothesis that this parasitic interaction might be an abortive attempt at sexual conjugation (Satina and Blakeslee 1926; Jeffries 1985), representing an interesting example of how basic mechanisms such as mating and cell fusion could evolve to serve new purposes. Similarly, in nematode-trapping fungi, such as *Arthrobotrys oligospora*, trap formation requires a hyphal fusion event (Nordbring-Hertz et al. 1989). Fusion occurs between a small branch growing initially perpendicular to the leading hyphae and the parental hypha; the self-communication and signaling processes that regulate the formation of the trap must occur at a very fine spatial and temporal scale.

IV. Mechanistic Aspects of Anastomosis

In 1933, Buller outlined morphological aspects of anastomosis in detail (Buller 1933). Based on

comparative observations made on different ascomycete and basidiomycete species, he concluded that vegetative fusions require two growing tips. Depending on the involvement of hyphal tips or short lateral branches, called pegs, he categorized fusion events into four types: hypha-to-hypha, hypha-to-peg, peg-to-peg and hook-to-clamp or clamp-connection fusions. Later studies proved that direct tip-to-side fusions are common in fungi; hyphal tips can fuse laterally with a hypha in the absence of a recognizable peg (Aylmore and Todd 1984; Todd and Aylmore 1985; Hickey et al. 2002). Mechanistically, the process of hyphal fusion can be divided into three steps: (1) pre-contact, (2) contact, adhesion and cell wall breakdown, and (3) pore formation and cytoplasmic flow (Glass et al. 2000, 2004; Hickey et al. 2002). In the following sections, we describe aspects of the fusion process and discuss recent literature on possible mechanisms regulating anastomosis. We also compare and contrast mating cell fusion (using *Saccharomyces cerevisiae* as a model) to germling and hyphal fusion in filamentous ascomycete species.

A. Competency

Vegetative hyphal fusion is a highly orchestrated process initiated by a physiological switch within the growing and developing mycelium that renders a portion of the colony fusion competent. What is required or involved with the developmental switch from fusion refractory hyphae (hyphae at the periphery of the colony) to fusion-competent ones (within the interior of the colony) is currently unknown. It is also not clear what controls the frequency of, or the spatial and temporal distribution of hyphal fusion events within the fusion-competent region of a fungal colony. Fusions within the interior of a colony are not uniform, indicating that microenvironmental factors within the colony may play an important role in influencing the distribution of fusion events within a colony.

B. Pre-Contact

Early observations that fusion hyphae alter their trajectory in response to proximity led to the idea that the fusion partners are capable of remote sensing. In his description of anastomosis in *Botrytis*, Ward (1888) notes: "When one sees a hypha deflected from its previous course through nearly a right angle (...) and I have seen cases in another fungus where the deflection amounts to considerably more than a right angle, it seems to me impossible to avoid the impression that some attraction is exerted" (Ward 1888). Numerous other studies have described pre-contact attraction of hyphae that eventually undergo fusion (Köhler 1930; Buller 1933; Hickey et al. 2002). Hyphae involved in fusion thus show a chemotactic response, such that reorientation of each hypha toward its neighbors results in a common growth trajectory; the presence of a fusion-competent hypha also often results in the formation of a peg in a receptive neighboring hypha.

In *N. crassa*, the reorientation of hyphae destined to fuse is associated with alterations in the position of the Spitzenkörper, or results in the formation of a new Spitzenkörper associated with peg formation in the receptive hypha (Hickey et al. 2002; Fig. 7.3A). The initiation of branching events, which is also associated with the formation of a new Spitzenkörper, has been hypothesized to be regulated by an excess in the rate of formation of vesicles associated with tip growth in relation to the rate of deposition of these vesicles at the apex (Watters and Griffiths 2001; Riquelme and Bartnicki-Garcia 2004). However, it is unlikely that such a mechanism is functioning during peg formation in a receptive fusion hypha. Presumably, a fusion hypha must be able to sense a gradient in chemotactic signals, which results in a change in Spitzenkörper localization within the hyphal apex and an alteration in growth trajectory. The localization of the Spitzenkörper in the hyphal apex has been associated with directionality of growth (Riquelme et al. 1998). Upon physical contact, the two Spitzenkörper of the fusion hyphae are juxtaposed at the point of contact (Fig. 7.3B; Hickey et al. 2002). During fusion-hypha growth and peg formation, the function of the Spitzenkörper is believed to be similar to its function at hyphal tips – secretion of cell wall and membrane material associated with growth via membrane-bound vesicles (Grove and Bracker 1970; Howard 1981; Bartnicki-Garcia et al. 1989; Gierz and Bartnicki-Garcia 2001).

1. Signaling Molecules

The nature of the substances that act at a distance and which are involved in hyphal or germling fusion is not known. Microscopically, events associated with germling and hyphal fusion are similar, although it is possible that different signaling molecules may be involved in each process. Sig-

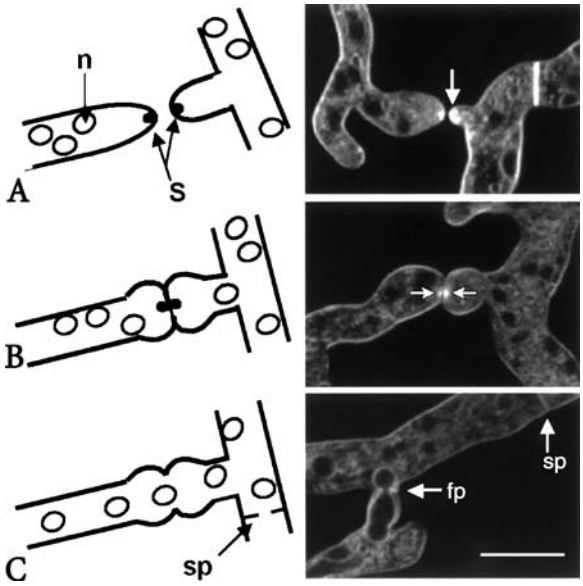

Fig. 7.3. A–C Stages of hyphal fusion. **A** The presence of a fusion-competent hypha often results in the formation of a peg in the receptive hypha. Peg formation is associated with the formation of a Spitzenkörper (*arrow*) at the tip of the new peg (*n* nuclei, *S* Spitzenkörper). **B** Contact between fusion hyphae is associated with a switch from polar to non-polar growth, resulting in a swelling of the fusion hyphae at the point of contact. The Spitzenkörper is associated with the site of the future pore in both fusion hyphae. **C** Pore formation is associated with cytoplasmic flow. A vacuole is shown traveling through the nascent pore. Nuclei and mitochondria also pass through the fusion pore. Septation is also often associated with hyphal fusion events (*fp* fusion pore, *sp* septa). Hyphae stained with FM4-64. Images adapted from Hickey et al. (2002), reprinted with permission. Bar = 10 μm

nificantly, all of the hyphal fusion mutants so far identified in *N. crassa* are also defective in germling fusion (see below).

A number of diffusible substances that mediate developmental processes occur in filamentous ascomycete fungi (see also Chap. 11, Autoregulatory signals in mycelial fungi, this volume), including peptide pheromones (Zhang et al. 1993, 1998; Pöggeler 2000; Pöggeler and Kück 2001; Kim et al. 2002; Kim and Borkovich 2004) and diffusible products required for conidiation in *A. nidulans* (Champe and el-Zayat 1989; Adams et al. 1998; Tsitsigiannis et al. 2004). In the dimorphic fungus *Candida albicans*, farnesol is a quorum-sensing molecule that prevents the switch from the yeast phase to the filamentous growth phase (Hornby et al. 2001), while tyrosol stimulates the formation of germ tubes (Chen et al. 2004). With regards to hyphal fusion, in the basidiomycete species *R. oryzae* the elimination of water-soluble substances surrounding hyphae inhibited hyphal attraction and fusion (Bhuiyan and Arai 1993), although the nature of these molecules is unknown.

The genes for peptide pheromones that are believed to play a role in mating have been identified from a number of filamentous ascomycete species (Zhang et al. 1998; Shen et al. 1999; Pöggeler 2000; Bobrowicz et al. 2002; Kim et al. 2002; Turina et al. 2003). In *N. crassa*, pheromone mutants are male-sterile, but female-fertile (Kim et al. 2002). In *N. crassa* and *Cryphonectria parasitica*, a vegetative phenotype has also been associated with the pheromone mutants, indicating a function of these substances beyond sexual development. A *C. parasitica* pheromone mutant shows reduced numbers of asexual fruiting bodies (pycnidia) and spores (Zhang et al. 1993, 1998). In *N. crassa*, a strain containing a mutation in *mfa-1* (which encodes mating factor *a-1*) shows reduced vegetative growth and aberrant sexual development (Kim et al. 2002). In *N. crassa*, the genes for the peptide pheromones are under the regulation of the mating-type (*mat*) genes (Bobrowicz et al. 2002), which encode putative transcriptional regulators (Glass et al. 1990; Staben and Yanofsky 1990). Unlike the *mfa-1* pheromone mutant, the *mat* mutants have a wild-type growth phenotype, although they are sterile as males and females. Significantly, both *mat A* and *mat a* mutants are competent to undergo hyphal fusion (Griffiths 1982).

2. Receptors

Cells recognize extracellular signaling molecules by means of different types of receptors. One class consists of 7-transmembrane G-protein coupled receptors (GCPR), such as the mating pheromone receptors in *S. cerevisiae* (Kurjan 1993; Dohlman and Thorner 2001; Fig. 7.4). In *N. crassa* and *Sordaria macrospora*, the putative pheromone receptors show amino acid identity to the **a**-pheromone GCPR of *S. cerevisiae* and to the lipopeptide pheromone receptors of basidiomycete species (Pöggeler and Kück 2001; Kim and Borkovich 2004). In *N. crassa*, mutation of the putative pheromone receptor gene, *pre-1*, results in female sterility, although the mutants are morphologically normal and form female reproductive structures; hyphal fusion in the *pre-1* mutant is normal (Kim and Borkovich 2004). Genome sequence analysis of the *N. crassa* genome has revealed a large number of additional

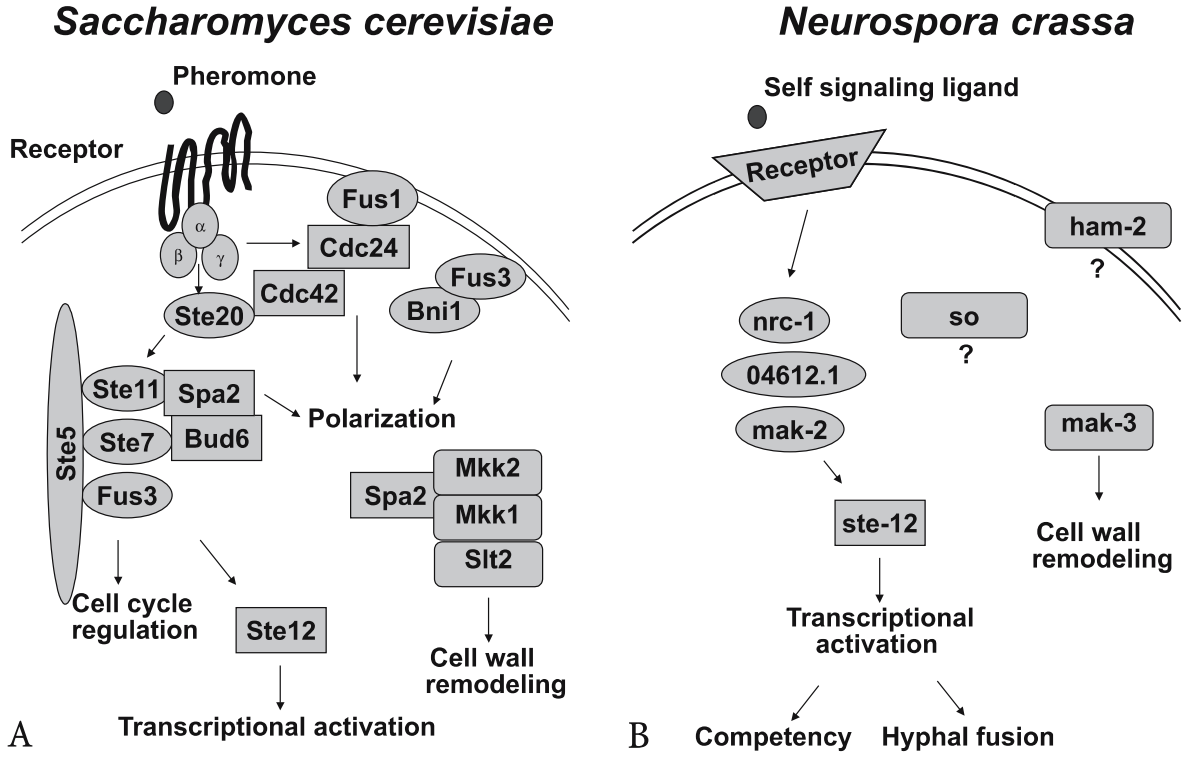

Fig. 7.4. A,**B** Comparison between mating cell fusion in *Saccharomyces cerevisiae* and anastomosis in *Neurospora crassa*. **A** In *S. cerevisiae*, reception of a mating signal via interaction of the peptide pheromones with their cognate receptors results in the dissociation of the heterotrimeric G-protein (Gα, Gβ, Gγ). Gβγ interacts with the PAK kinase Ste20, resulting in the activation of the pheromone response MAP kinase pathway (Ste11, Ste7 and Fus3, bound to the scaffold protein Ste5) and subsequent transcriptional activation of genes required for cell fusion via Ste12. Endocytosis of the receptor–ligand complex is associated with chemotropism toward an appropriate mating partner. Polarization of the cytoskeleton to the schmoo tip occurs via interactions between Gβγ and Cdc24, and subsequent recruitment and activation of Cdc42. Activated Cdc42 interacts with Fus1, which plays a role in events during cell fusion. Components of the MAP kinase pathway interact with polarization components, such as Spa2, Bud6, and the formin Bni1. Cell wall remodeling, via the Slt2 MAP kinase pathway (Mkk2, Mkk1 and Slt2), is involved in schmoo formation and mating cell fusion (for review, see Banuett 1998; Dohlman and Thorner 2001). Components not mentioned in the text are not included in the figure. **B** In *N. crassa*, mutations in orthologs of *STE11* and *FUS3* (*nrc-1* and *mak-2*, respectively) result in mutants unable to undergo germling or hyphal fusion (Pandey et al. 2004). In addition, mutations in *ham-2*, encoding a putative membrane protein, and in *so*, a putative cytoplasmic protein, result in strains unable to undergo both hyphal and germling fusion (Xiang et al. 2002; Fleissner et al. 2005). In *F. graminearum*, a strain containing a mutation in the ortholog of *SLT2* fails to form a heterokaryon (Hou et al. 2002); the *N. crassa* ortholog of *SLT2* is called *mak-3*. The nature of the receptor and ligand involved in anastomosis is unknown. NCU04612.1 is the *N. crassa* NCU number (http:/www.broad.mit.edu/annotation/fungi/neurospora/) for the predicted ortholog of *STE7*; a mutant containing a lesion in this gene is predicted to be a hyphal fusion mutant

7-transmembrane receptors (Galagan et al. 2003; Borkovich et al. 2004), including some that resemble cAMP receptors in *Dictyostelium discoideum*. *D. discoideum* shows a chemotactic phenotype in response to pulses of cAMP (Arkowitz 1999; Kay 2002).

In *S. cerevisiae*, the binding of a secreted peptide pheromone (α, or **a**-factor) to its cognate GCPR (Ste2 and Ste3, respectively) initiates mating (Cross 1988; Fig. 7.4). Oligomerization of the Ste2 pheromone receptor is required for efficient signaling (Overton and Blumer 2000), while the C-terminal portion of Ste2 is required for internalization via endocytosis. Endocytosis of the pheromone receptor–ligand complex is required for chemotropism toward an appropriate mating partner (Vallier et al. 2002). These data have led to the model that endocytosis of the receptor/ligand complex plays a role in marking the spot for polarization of the cytoskeleton, resulting in

subsequent chemotropism. It is possible that a comparable event occurs during hyphal fusion, whereby reception of a signal via a membrane-bound receptor and subsequent endocytosis act as a nucleator for Spitzenkörper formation, cytoskeletal polarization and peg formation. This is an attractive model for a multinucleate organism, where the initiation of site-specific morphogenesis via transcriptional mechanisms is difficult to envisage.

In addition to 7-transmembrane receptors, a large number of histidine kinases (HKs) are found in filamentous fungal genomes – 11 in *N. crassa* (Galagan et al. 2003) and 21 in *Cochliobolus heterostrophus* (Catlett et al. 2003). Two-component systems, such as histidine kinases, are involved in the reception of diffusible molecules and signal transduction in a number of organisms (West and Stock 2001). However, only one of the predicted histidine kinases in filamentous ascomycete genomes would have a transmembrane domain; the remaining HKs are predicted to be cytoplasmically localized proteins (Catlett et al. 2003). It is unclear whether any of these receptors or others may be involved in chemotropic interactions during anastomosis in filamentous ascomycete fungi.

3. G-Proteins

The binding of ligand to GCPRs results in the disassociation of an intracellular heterotrimeric G-protein (Gα, Gβ and Gγ), and subsequent activation of downstream processes (for review, see Dohlman and Thorner 2001). In *N. crassa*, three Gα, one Gβ and one Gγ genes are present (Galagan et al. 2003; Borkovich et al. 2004). Mutation of the gene encoding Gα (*gna-1*) results in female infertility and sensitivity to hyperosmotic media (Ivey et al. 1996). Strains containing a disruption in the gene encoding the Gβ subunit, *gnb-1*, also have a pleiotropic phenotype (Yang et al. 2002). *gna-1* and *gnb-1* mutants do not show chemotropic interactions between a trichogyne and conidium, which is required for the initiation of the sexual cycle in *N. crassa* (Fig. 7.1C; Kim and Borkovich 2004). It is currently unknown whether any of the G-protein mutants in *N. crassa*, or in other filamentous fungi such as *A. nidulans* and *Cryphonectria parasitica* (Adams et al. 1998; Segers and Nuss 2003), are required for chemotropic interactions during hyphal fusion or for other processes related to anastomosis.

4. MAP Kinase Pathways

In MAP kinase signaling pathways, mitogen-activated protein kinases are ubiquitous and evolutionary conserved enzymes that connect cell-surface receptors to regulatory targets, which results in various morphogenetic processes (Lengeler et al. 2000; Xu 2000). In *S. cerevisiae*, the binding of either **a**- or α-factor to its cognate GCPR (Ste3 and Ste2, respectively) results in the disassociation of a heterotrimeric G-protein (Gα and Gβγ). The Gβγ subunit initiates the activation of the *FUS3* MAP kinase pathway, resulting in G1 growth arrest and the transcriptional activation of genes associated with mating (Fig. 7.4; Dohlman and Thorner 2001). In addition to transcriptional activation, components of the MAP kinase pathway, such as Ste11 and Fus3, interact with proteins associated with cell polarization. For example, two components of the polarisome, Spa2 and Bud6, interact with the MAPKK kinase Ste11 (Fig. 7.4); *spa2* mutants show reduced levels of pheromone signaling (Sheu et al. 1998). The formin Bni1 is required for polarized growth during both budding and mating, by facilitating actin cable assembly (Evangelista et al. 1997). Phosphorylation of Bni1 during mating is dependent on functional Fus3 (Matheos et al. 2004). These data suggest that phosphorylated Fus3 is recruited to the cell cortex, where it activates Bni1 to promote polarization and cell fusion (Fig. 7.4).

A number of mutants in filamentous fungi that contain mutations in the putative orthologs of the *S. cerevisiae* pheromone response MAP kinase pathway are defective in anastomosis (Wei et al. 2003; Pandey et al. 2004; Fig. 7.4). In *A. nidulans*, a mutant disrupted in the *STE11* ortholog, *steC*, fails to form heterokaryons (Wei et al. 2003). The *steC* mutant also has a slower growth rate and altered conidiophore morphology. A *N. crassa STE11* mutant, *nrc-1*, is de-repressed for conidiation (*n*on-*r*epressible *c*onidiation), female-sterile and shows an ascospore-autonomous lethal phenotype (Kothe and Free 1998). The *nrc-1* mutant is also defective in germling and hyphal fusion (Pandey et al. 2004). In *N. crassa*, a strain containing a disruption of the ortholog of *FUS3* (*mak-2* in *N. crassa*) results in a mutant with a phenotype very similar to an *nrc-1* mutant, and is germling and hyphal-fusion defective (Pandey et al. 2004). Phosphorylation of MAK-2 is temporally associated with germling fusion events and is dependent on functional NRC-1, suggesting that two proteins are members of a com-

mon signal transduction pathway. In *Magnaporthe grisea*, *Colletotrichum lagenarium* and *Cochliobolus heterostrophus*, strains containing mutations in *FUS3* orthologs are defective in appressoria formation (Xu and Hamer 1996; Lev et al. 1999; Takano et al. 2000), and fail to colonize host plants when inoculated through wound sites. In addition, these MAP kinase mutants were reported to be impaired in conidiogenesis, aerial hyphae formation, and female fertility. Although unreported in the MAP kinase mutants constructed in plant pathogens, our prediction is that these mutants may also be hyphal fusion defective and fail to make an interconnected mycelial network, an aspect that may be important for pathogenesis.

One of the downstream components of the pheromone response pathway in *S. cerevisiae* is *STE12*, which encodes a transcription factor (Errede and Ammerer 1989). Activation of Ste12 by the MAP kinase pheromone response pathway culminates in the activation of a number of genes associated with mating and cell fusion (Errede and Ammerer 1989; Zeitlinger et al. 2003). In *A. nidulans*, mutations in the *ste12*-like transcription factor *steA* resulted in mutants affected in sexual development (Vallim et al. 2000). In *M. grisea*, strains containing a deletion of the *SteA* ortholog (*MST12*) formed defective appressoria, and were unable to form invasive hyphae within the plant when inoculated into wound sites (Park et al. 2002). In *N. crassa*, a strain containing a mutation in the *SteA* ortholog, *pp-1*, is very similar in phenotype to a *mak-2* mutant, and is defective in hyphal and germling fusion (Jacobson, Fleißner and Glass, unpublished data). Live cell imaging and microscope observations of the *N. crassa nrc-1/mak-2/pp-1* mutants indicate that they are blind to self (mutants neither attract nor are attracted to hyphae/conidia of identical genotype). Furthermore, the *nrc-1* and *mak-2* mutants do not form CATs (Roca et al. 2005). These data suggest that the pheromone response MAP kinase pathway is either involved early in the germling or hyphal fusion processes, or is required for rendering conidia and hyphae competent to undergo fusion. Genome comparisons show conservation of genes required for mating in *S. cerevisiae* within the genomes of filamentous fungi, such as *N. crassa* (Glass et al. 2004). However, the role of most of these genes in either mating and/or anastomosis is unknown.

In *Fusarium graminearum*, mutations in another MAP kinase, *MGV1*, the homolog of *SLT2* of *S. cerevisiae*, resulted in a mutant that was female-sterile and also failed to form heterokaryons (Hou et al. 2002). However, the step at which hyphal fusion was blocked in the *MGV1* mutant was not evaluated. In *S. cerevisiae*, the *SLT2* MAP kinase pathway is downstream of the *FUS3* MAP kinase pathway, and is required for remodeling the cell wall (Buehrer and Errede 1997; Xu 2000). The polarisome component Spa2, which interacts with Ste11 and Ste7 of the pheromone response MAP kinase pathway, also interacts with Mkk1 and Mkk2 of the Slt2 cell wall integrity MAP kinase pathway (Sheu et al. 1998; Fig. 7.4). The activation of the pheromone response MAP kinase pathway induces cells to form projections oriented toward the gradient of pheromone secreted by a mating partner, which requires new cell wall synthesis via activation of the cell wall integrity MAP kinase pathway. These data suggest that the cell wall remodeling MAP kinase pathway may be required also for anastomosis in filamentous fungi. In *A. nidulans* and *M. grisea*, mutations in the MAP kinase gene orthologous to the *slt2* gene in *S. cerevisiae* affect conidial germination, sporulation, and sensitivity to cell wall-digesting enzymes (Xu et al. 1998; Bussink and Osmani 1999). However, possible anastomosis defects in these mutants were not tested.

5. Initiation of Branch Formation/Pegs and Polarization

Initiation of branch formation is associated with the formation of an incipient Spitzenkörper at the branch point (Riquelme and Bartnicki-Garcia 2004); peg formation during hyphal fusion is also preceded by formation of a new Spitzenkörper and subsequent tip growth. In plant pathogenic rust fungi, *Uromyces* and *Puccinia*, the Spitzenkörper was associated with germ tube elongation and direction of growth, but disappeared during appressorium differentiation. However, a structure similar in appearance to the Spitzenkörper was observed during the formation of the penetration peg (Dijksterhuis 2003).

In *S. cerevisiae*, a structure termed the "polarisome" is believed to be analogous in function, and perhaps in some protein components to the Spitzenkörper described in filamentous ascomycete fungi (Harris et al. 2005). The polarisome is composed of scaffolding components (such as Spa2), signaling molecules (such as Rho proteins) and actin regulators (such as the formin Bni1; Bidlingmaier and Snyder 2004). The formation of the polarisome and subsequent polarized growth

during mating occur in a stepwise fashion and involve a series of signaling modules, the Rho- and Ras-type GTPases, which regulate the polarization of the actin cytoskeleton and the concomitant polarization of the secretory apparatus to the point of growth (Nelson 2003). In *S. cerevisiae*, a complex between Gβγ and the Cdc42 activating protein, Cdc24, localizes to the tip of the mating projection. Activation of Cdc42 is associated with alterations in cytoskeletal organization and polarization involved in chemotropic interactions (Nern and Arkowitz 1998; for review, see Etienne-Manneville 2004). In the basidiomycete *Ustilago maydis*, the yeast-like haploid sporidia respond to pheromone secreted by a compatible partner, by forming conjugation tubes; myosin-V (*myo5*) mutants were defective in this process (Weber et al. 2003). In *S. cerevisiae*, the myosin-V protein, Myo2, is involved in vesicle delivery along actin cables and is required for polarized secretion (Karpova et al. 2000).

In filamentous fungi, hyphal tip growth is also associated with highly polarized and localized exocytosis. Unlike *S. cerevisiae*, however, where secretion and polarization are primarily actin-based, a functional microtubule cytoskeleton is required for the transport of vesicles to the hyphal tip and for hyphal tip growth in filamentous ascomycete species. Disruption of microtubule function or its motors affects hyphal tip growth (Seiler et al. 1997). Within the cell, the transport and fusion of vesicles associated with secretion is accomplished by integral membrane proteins, termed SNAREs (soluble *N*-ethyl-maleimide-sensitive factor attachment protein receptor; Ungar and Hughson 2003). In *N. crassa*, transformants containing silenced copies of putative plasma membrane-localized SNARES showed reduced growth, hyphal diameter and conidiation. The defects in *nsyn1* and *nsyn2* mutants are consistent with differential impaired vesicle fusion in hyphal tips (Gupta and Heath 2002; Gupta et al. 2003).

In *N. crassa*, a number of temperature-sensitive mutants have recently been identified that show altered hyphal morphology and branching at restrictive temperatures (Seiler and Plamann 2003). The genes mutated in these morphological mutants were identified via complementation and include many that encode polarity components, such as the homolog of the Rho-type GTPase, *CDC42*, and the guanine exchange factor (GEF) for Cdc42, *CDC24*. A second class of morphological mutants has mutations in components of the secretory apparatus; the pleiotropic phenotypes may be associated with defects in intracellular vesicle trafficking, which is required for hyphal tip growth. Although the growth and branching phenotypes of these mutants have been described, a systematic microscope survey of the effect of these mutations on self-anastomosis has not been done. In addition, a number of filamentous fungal-specific genes or genes not previously suspected of having polarization defects in other organisms, when mutated, give rise to strains affected in polarization (Osherov et al. 2000; Gatherar et al. 2004; Pearson et al. 2004). These data suggest that some aspects of tip growth, polarization and, most probably, anastomosis require functions/genes that are specific to filamentous fungi.

C. Contact, Adhesion and Cell Wall Breakdown

Hyphal fusion is a temporally and spatially regulated process that involves degradation of the cell wall, followed by plasma membrane fusion. From the time of cell wall contact between fusion hyphae to membrane contact, two steps are required: adhesion of fusion hyphae, and subsequent degradation of the intervening cell wall. Via live cell imaging of the hyphal fusion process, the Spitzenkörper was observed to be associated with the site of the future pore (Hickey et al. 2002; Fig. 7.3B). These observations suggest that the Spitzenkörper plays a role in vesicle trafficking involved in the secretion of extracellular adhesives and cell wall-degrading enzymes. Upon physical contact, the hyphae (or hypha/peg) involved in fusion often switch from polar to non-polar growth, resulting in a swelling of the hyphae/hypha at the fusion point (Fig. 7.3B). The delivery of vesicles containing cell wall-degrading enzymes to the point of fusion may be analogous to the role the Spitzenkörper plays during hyphal tip growth, in which highly polarized exocytosis of vesicles at hyphal apices is an essential requirement (Grove and Bracker 1970; Howard 1981; Bartnicki-Garcia et al. 1995).

Extracellular electron-dense material associated with hyphal fusion events has been observed in *C. parasitica* (Newhouse and MacDonald 1991), which may be involved in adhesion of participating hyphae. Leakage of cytoplasm during anastomosis is not observed, suggesting that hyphae are tightly adhered to each other (Hickey et al. 2002). To complete the fusion process, the intervening cell wall between the two fusion hyphae must be dismantled. These observations indicate that a switch in

enzymes/proteins secreted to the hyphal tip must occur during hyphal fusion, from those associated with cell wall formation to others associated with cell wall degradation and adhesion. One or perhaps both participating hyphae coordinates the cellular machinery involved in hyphal adhesion and cell wall breakdown. In *S. cerevisiae*, proteins involved in adhesion, the agglutinins and enzymes involved in cell wall breakdown and re-synthesis, are associated with formation of the mating pair (Cross 1988); many of these genes are transcriptionally regulated by activation of the pheromone response MAP kinase cascade.

D. Pore Formation and Cytoplasmic Flow

The formation of a pore, resulting in cytoplasmic continuity between fusion hyphae, requires fusion of plasma membranes. Plasma membrane fusion associated with mating or with developmental processes in multicellular eukaryotes is not well understood (White and Rose 2001; Shemer and Podbilewicz 2003). In *S. cerevisiae*, a number of mutants have been identified that are capable of polarization of the cytoskeleton and schmoo formation, but fail to undergo mating cell fusion. These include strains containing lesions in *FIG1*, *FIG2*, *FUS1*, *FUS2* and *PRM1* (Elion et al. 1995; Erdman et al. 1998; Heiman and Walter 2000); *PRM1*, *FUS1* and *FIG1* encode plasma membrane proteins that are localized to the schmoo tip during mating (Trueheart and Fink 1989; Erdman et al. 1998; Heiman and Walter 2000). Fus1 also interacts with Cdc42 and the formin Bni1, and may function as a scaffold for the assembly of a complex involved in polarized secretion of septum-degrading enzymes (Nelson et al. 2004; Fig. 7.4). Fus2 is an intracellular protein that interacts with Rvs161; this complex also localizes to the schmoo tip (Elion et al. 1995; Brizzio et al. 1998). Interestingly, many of these late components, involved with septum degradation and possibly membrane fusion, such as *FUS1*, *FUS2*, *FIG1* and *FIG2*, are not conserved in the genome of filamentous ascomycete species such as *N. crassa* (Glass et al. 2004).

Following pore formation, cytoplasmic mixing occurs in the fusion region. Often, considerable cytoplasmic flow through a fusion pore is observed, possibly due to different turgor pressures between fusing hyphae (Hickey et al. 2002; http://www.icmb.ed.ac.uk/research/read/neurospora/movies.html). New septa are frequently formed close to the fusion point (Buller 1933; Hickey et al. 2002), perhaps as a response to cytoplasmic flow. Organelles, such as mitochondria, vacuoles and nuclei, are capable of being transferred between fusion hyphae as a result of hyphal fusion (Hickey et al. 2002; Glass et al. 2004; Fig. 7.3C). It is possible that hyphal compartments in the vicinity of the fusion site have a mechanism to adapt to changes in cytoplasmic flow and organelle composition. Post-contact consequences of hyphal fusion, involving physiological adaptation to cytoplasmic mixing and cytoplasmic flow, are virtually uncharacterized in filamentous fungi. The end result of hyphal fusion events is that tips that were growing along particular trajectories are changed into conduits through which the contents from different hyphal compartments mix and are subsequently shuttled in various directions. It is a process that terminates growth of specific hyphal branches at specific locations and times during the development of a mycelium.

V. Anastomosis Mutants in Filamentous Fungi of Unknown Function

A number of mutants defective in anastomosis have been identified in filamentous fungal species, although the genetic cause of the fusion defect in most cases has not been identified. These mutants have been identified primarily by heterokaryon tests. In *Gibberella fujikuroi*, a mutant (*hsi-1*) was identified that was heterokaryon self-incompatible (Correll et al. 1989). In *Verticillium albo-atrum*, four strains showed heterokaryon self-incompatibility, which was associated with the inability to undergo anastomosis (Correll et al. 1988). In *F. oxysporum* f. sp. *melonis*, self-incompatible isolates showed a highly reduced number of fusion events (Jacobson and Gordon 1988).

In *N. crassa*, a gene required for hyphal anastomosis, *ham-2* (*h*yphal *a*nasto*m*osis) encodes a putative transmembrane protein (Xiang et al. 2002). The *ham-2* mutants show a pleiotropic phenotype, including slow growth, female sterility and homozygous lethality in sexual crosses. In addition, *ham-2* mutants fail to undergo both hyphal and germling fusion and are blind to self. Recently, a function for a homolog of *ham-2* in *S. cerevisiae*, termed *FAR11*, was reported. Mutations in *FAR11* result in a mutant that prematurely recovers from

G1 growth arrest following exposure to pheromone (Kemp and Sprague 2003). Far11p was shown to interact with five other proteins (Far3, Far7, Far8, Far9 and Far10). Mutations in any of these other genes give phenotypes identical to those of the *far11* mutants (Kemp and Sprague 2003). It is possible that the Far11 complex forms part of a checkpoint that monitors mating cell fusion in coordination with G1 cell cycle arrest. Homologs of genes encoding several of the proteins in the Far11 complex are lacking in *N. crassa*, including *FAR3* and *FAR7* (Glass et al. 2004). It is unknown whether, together with *ham-2*, the other *FAR* homologs are required for anastomosis in *N. crassa*.

Another *N. crassa* mutant, *so*, shows substantial reduction in its ability to undergo both self- and non-self hyphal fusions (Fleissner et al. 2005). Compared to a wild-type strain, the *so* mutant (allelic to *ham-1*; Wilson and Dempsey 1999) shows an altered conidiation pattern, shortened aerial hyphae, and female infertility. The gene that complements the *so* mutation in *N. crassa* encodes a protein of unknown function, but that contains a WW domain. WW domains are predicted to be involved in protein–protein interactions (André and Springael 1994). Homologs of the *so* gene are present in the genomes of filamentous ascomycete fungi, but are absent in ascomycete yeast and basidiomycete species. As with *ham-2*, *mak-2* and *nrc-1* mutants, *so* mutants are deficient in both germling and hyphal fusion (Fig. 7.2D). Interestingly, fusion between the mating partners during sexual development is not impaired in the *so* mutant, indicating that *so* is specific for vegetative cell fusion events.

VI. Physiological and Morphogenetic Consequences of Anastomosis

Hyphae of ascomycete and basidiomycete fungi are divided into compartments by the formation of septa. Although septa in ascomycete species are not complete and cytoplasmic continuity remains, the presence of septa decreases the rate of cytoplasmic flow. Since a system with high internal resistance theoretically has a reduced capacity to explore, it is possible that the formation of a network can counteract the restrictive effect of septation, and thereby increase the throughput by connecting parallel hyphae (Rayner 1996). The role that anastomoses plays in the structure and functioning of a mycelium as a single, dynamic physiological entity is unclear. Conceptual paradigms that focus on hyphal tips as independent growth units become limiting when applied to understanding mycelial networks (Davidson et al. 1996; Rayner 1996; Davidson 1998).

The formation of a hyphal network may be important in influencing hyphal pattern formation and morphogenesis in filamentous fungi. Hyphal fusion may facilitate signaling within a colony (either by molecules, proteins, or perhaps electric fields; reviewed in Gow and Morris 1995), which may also affect the behavior and development of a filamentous fungal colony. Unlike the artificial situation of growth in a petri dish, in nature fungal colonies exploit diverse environments with variable distributions and types of nutrient sources. Furthermore, a single fungal individual interacts and competes for available nutrients with other microorganisms, such as fungi and bacteria, as well as insects. The ability to form a hyphal network may be needed for coordinated behavior between the different parts of a fungal colony. An example of this coordination is illustrated during growth of the basidiomycete species *Hypholoma fasciculare*. If one part of a uniformly circular colony comes into contact with a spatially discrete nutrient source, that part of the colony opposite the nutrient source ceases its extension and the colony shifts its growth toward the source. While colonizing this new resource, the portion of the colony distal to the nutrient source degenerates (Dowson et al. 1989).

In environments showing a heterogeneous distribution of resources, transfer of nutrients occurs from the place of uptake (source) to parts of the colony in need of these molecules (sinks). For example, in the basidiomycete *R. solani*, carbon from regions of the colony growing on a carbon-rich medium is translocated to regions lacking this resource (Jacobs et al. 2004). Another example for the necessity of metabolite transport across a colony is biotrophic plant pathogens, such as *Erisyphe graminis* and *Uromyces fabae*, which form haustoria within plant cells. Nutrients taken up by these specialized structures in plant cells are redistributed to the other parts of the mycelium that subsequently undergo differentiation and reproduction (Staples 2001; Voegele et al. 2001). Presumably, the formation of a hyphal network facilitates the transport of nutrients from one part of the colony to another.

In some basidiomycete species, it has been shown that the formation of a larger functional

unit (an individual colony) is advantageous in competitive environments. In a confrontation between the cord-forming wood decomposers *H. fasciculare* and *Steccherinum fimbriatum*, one species eventually replaced the other. Which species eventually dominated over the other was strongly influenced by inoculum size (Dowson et al. 1988). In confrontations between a competitor and colonies from the same isolate, but of different sizes, larger colonies had a higher success rate than smaller ones (Holmer and Stenlid 1993). These data from basidiomycete species suggest that, in ascomycete species, too, the capacity of conidial germlings or colonies of like genotype to undergo anastomosis to generate a larger colony may provide a competitive advantage.

VII. Conclusion

Anastomosis captured the imagination of early mycologists, and numerous studies describing the morphology of hyphal fusion in ascomycete and basidiomycete species have been published. However, work describing mutant phenotypes or the molecular function of genes and proteins required for anastomosis in filamentous fungi has largely been ignored. Advances in cell biology techniques and live cell imaging of the hyphal fusion process will hopefully lead to an increased awareness and interest in this important aspect of filamentous fungal biology. In addition, advances in molecular and genetic tractability of a variety of filamentous fungi, and the availability of genome sequences will lead to in-depth comparative analyses that will, hopefully, begin to reveal the rich tapestry of inter- and intrahyphal communication that accompanies both developmental and morphogenetic processes. Many questions regarding the mechanism and function of hyphal anastomosis remain unanswered. What are the diffusible chemotropic molecules responsible for causing fusion-competent hyphae to grow toward each other, and how do they regulate Spitzenkörper behavior? Is the signal transduction machinery involved in regulating hyphal homing and fusion between conidial germlings the same or different to that involved in homing and fusion between hyphae in the colony interior? How similar or different are the signaling and fusion machineries involved in vegetative stages vis-à-vis those involved in sexual stages of the life cycle? Finally, what selective advantage maintains the capacity of germlings and hyphae to undergo anastomosis, and under what conditions?

References

Adams TH, Wieser JK, Yu JH (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Ahmad SS, Miles PG (1970) Hyphal fusions in *Schizophyllum commune*. 2. Effects on environmental and chemical factors. Mycologia 62:1008–1017

André B, Springael J (1994) WWP, a new amino acid motif present in single or multiple copies in various proteins including dystrophin and the SH3-binding Yes-associated protein YAP65. Biochem Biophys Res Commun 205:1201–1205

Arkowitz RA (1999) Responding to attraction: chemotaxis and chemotropism in *Dictyostelium* and yeast. Trends Cell Biol 9:20–27

Aylmore NK, Todd RC (1984) Hyphal fusion in *Coriolus versicolor*. In: Jennings DH, Rayner ADM (eds) The ecology and functioning of the fungal mycelium. Cambridge University Press, Cambridge, UK, pp 103–125

Banuett F (1998) Signalling in the yeasts: an informational cascade with links to the filamentous fungi. Microbiol Mol Biol Rev 62:249–274

Bartnicki-Garcia S, Hergert F, Gierz G (1989) Computer simulation of morphogenesis and the mathematical basis for hyphal (tip) growth. Protoplasma 153:46–57

Bartnicki-Garcia S, Bartnicki DD, Gierz G (1995) Determinants of fungal cell wall morphology: the vesicle supply center. Can J Bot 73:S372–S378

Bégueret J, Turcq B, Clavé C (1994) Vegetative incompatibility in filamentous fungi – *het* genes begin to talk. Trends Genet 10:441–446

Bhuiyan MKA, Arai K (1993) Physiological factor affecting hyphal growth and fusion of *Rhizoctonia oryzae*. Trans Mycol Soc Jpn 32:389–397

Bidlingmaier S, Snyder M (2004) Regulation of polarized growth initiation and termination cycles by the polarisome and Cdc42 regulators. J Cell Biol 164:207–218

Biella S, Smith ML, Aist JR, Cortesi P, Milgroom MG (2002) Programmed cell death correlates with virus transmission in a filamentous fungus. Proc R Soc Lond Ser B Biol Sci 269:2269–2276

Bistis GN (1981) Chemotropic interactions between trichogynes and conidia of opposite mating-type in *Neurospora crassa*. Mycologia 73:959–975

Bistis GN, Perkins DD, Read ND (2003) Different cell types in *Neurospora crassa*. Fungal Genet Newslett 50:17–19

Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ (2002) The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Brizzio V, Gammie AE, Rose MD (1998) Rvs161p interacts with Fus2p to promote cell fusion in *Saccharomyces cerevisiae*. J Cell Biol 141:567–584

Bruggeman J, Debets AJM, Swart K, Hoekstra RF (2003) Male and female roles in crosses of *Aspergillus nidulans* as revealed by vegetatively incompatible parents. Fungal Genet Biol 39:136–141

Buehrer BM, Errede B (1997) Coordination of the mating and cell integrity mitogen-activated protein kinase pathways in *Saccharomyces cerevisiae*. Mol Cell Biol 17:6517–6525

Buller AHR (1933) Researches on Fungi, vol 5. Longman, London

Bussink H, Osmani SA (1999) A mitogen-activated protein kinase (MPKA) is involved in polarized growth in the filamentous fungus, *Aspergillus nidulans*. FEMS Microbiol Lett 173:117–125

Catlett NL, Yoder OC, Turgeon BG (2003) Whole-genome analysis of two-component signal transduction genes in fungal pathogens. Eukaryot Cell 2:1151–1161

Champe SP, el-Zayat AA (1989) Isolation of a sexual sporulation hormone from *Aspergillus nidulans*. J Bacteriol 171:3982–3988

Chen JT, Wu HK (1977) Hyphal anastomosis in *Pyricularia oryzae* Cav. Protoplasma 92:281–288

Chen H, Fujita M, Feng Q, Clardy J, Fink GR (2004) Tyrosol is a quorum-sensing molecule in *Candida albicans*. Proc Natl Acad Sci USA 101:5048–5052

Chu YM, Jeon JJ, Yea SJ, Kim YH, Yun SH, Lee YW, Kim KH (2002) Double-stranded RNA mycovirus from *Fusarium graminearum*. Appl Environ Microbiol 68:2529–2534

Correll JC, Gordon TR, McCain AH (1988) Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. Phytopathology 78:1017–1021

Correll JC, Klittich CJR, Leslie JF (1989) Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol Res 93:21–27

Cross F (1988) Conjugation in *Saccharomyces cerevisiae*. Annu Rev Cell Biol 4:429–457

Davidson FA (1998) Modelling the qualitative response of fungal mycelia to heterogeneous environments. J Theor Biol 195:281–292

Davidson FA, Sleeman BD, Rayner ADM, Crawford JW, Ritz K (1996) Context-dependent macroscopic patterns in growing and interacting mycelial networks. Proc R Soc Lond B 263:873–880

Davis RH (2000) *Neurospora*: contributions of a model organism. Oxford University Press, New York

Debets AJM, Griffiths AJF (1998) Polymorphism of *het*-genes prevents resource plundering in *Neurospora crassa*. Mycol Res 102:1343–1349

Debets F, Yang X, Griffiths AJF (1994) Vegetative incompatibility in *Neurospora* – its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. Curr Genet 26:113–119

Dijksterhuis J (2003) Confocal microscopy of Spitzenkörper dynamics during growth and differentiation of rust fungi. Protoplasma 222:53–59

Dohlman HG, Thorner J (2001) Regulation of G protein-initiated signal transduction in yeast: paradigms and principles. Annu Rev Biochem 70:703–754

Dowson CG, Rayner ADM, Boddy L (1988) The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments. New Phytol 109:423–432

Dowson CG, Springham P, Rayner ADM, Boddy L (1989) Resource relationships of foraging mycelial systems of *Phanerochaete velutina* and *Hypholoma fasciculare* in soil. New Phytol 111:501–509

Elion EA, Trueheart J, Fink GR (1995) Fus2 localizes near the site of cell fusion and is required for both cell fusion and nuclear alignment during zygote formation. J Cell Biol 130:1283–1296

Erdman S, Lin L, Malczynski M, Snyder M (1998) Pheromone-regulated genes required for yeast mating differentiation. J Cell Biol 140:461–483

Errede B, Ammerer G (1989) STE12, a protein involved in cell-type-specific transcription and signal transduction is yeast, is part of protein-DNA complexes. Genes Dev 3:1349–1361

Etienne-Manneville S (2004) Cdc42 – the centre of polarity. J Cell Sci 117:1291–1300

Evangelista M, Blundell K, Longtine MS, Chow CJ, Adames N, Pringle JR, Peter M, Boone C (1997) Bni1p, a yeast formin linking Cdc42p and the actin cytoskeleton during polarized morphogenesis. Science 276:118–122

Evans GH, Cooke RC (1982) Studies on mucoralean mycoparasites. III. Diffusable factors from *Mortierella vinacea* Dixon-Steward that direct germ tube growth in *Piptocephalis fimbriata* Richardson and Leadbeater. New Phytol 81:245–253

Fleissner A, Sarkar S, Jacobson DJ, Roca MG, Read ND, Glass NL (2005) The *so* locus is required for vegetative cell fusion and post-fertilization events in *Neurospora crassa*. Eukaryot Cell 4:920–930

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S et al. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868

Gatherar IM, Pollerman S, Dunn-Coleman N, Turner GE (2004) Identification of a novel gene *hbrB* required for polarised growth in *Aspergillus nidulans*. Fungal Genet Biol 41:463–471

Gierz G, Bartnicki-Garcia S (2001) A three-dimensional model of fungal morphogenesis based on the vesicle supply center concept. J Theor Biol 208:151–164

Giovannetti M, Azzolini D, Citernesi AS (1999) Anastomosis formation and nuclear and protoplasmic exchange in arbuscular mycorrhizal fungi. Appl Environ Microbiol 65:5571–5575

Giovannetti M, Fortuna P, Citernesia AS, Morini S, Nuti MP (2001) The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol 151:717–724

Girbardt M (1957) Der Spitzenkörper von *Polystictus versicolor* (L.). Planta 50:47–59

Glass NL, Kaneko I (2003) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 2:1–8

Glass NL, Grotelueschen J, Metzenberg RL (1990) *Neurospora crassa A* mating-type region. Proc Natl Acad Sci USA 87:4912–4916

Glass NL, Jacobson DJ, Shiu PK (2000) The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu Rev Genet 34:165–186

Glass NL, Rasmussen C, Roca MG, Read ND (2004) Hyphal homing, fusion and mycelial interconnectedness. Trends Microbiol 12:135–141

Gow NAR, Morris BM (1995) The electric fungus. Bot J Scotland 47:263–277

Gregory PH (1984) The fungal mycelium: a historical perspective. Trans Br Mycol Soc 82:1–11

Griffin DM, Perrin HN (1960) Anastomosis in the *Phycomycetes*. Nature 187:1039–1040

Griffiths AJF (1982) Null mutants of the *A* and *a* mating-type alleles of *Neurospora crassa*. Can J Genet Cytol 24:167–176

Grove SN, Bracker CE (1970) Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. J Bacteriol 104:989–1009

Gupta GD, Heath IB (2002) Predicting the distribution, conservation, and functions of SNAREs and related proteins in fungi. Fungal Genet Biol 36:1–21

Gupta GD, Free SJ, Levina NN, Keranen S, Heath IB (2003) Two divergent plasma membrane syntaxin-like SNAREs, nsyn1 and nsyn2, contribute to hyphal tip growth and other developmental processes in *Neurospora crassa*. Fungal Genet Biol 40:271–286

Harris SD, Read ND, Roberson RW, Shaw B, Seiler S, Plamann M, Momany M (2005) Polarisome meets Spitzenkörper: microscopy, genetics, and genomics converge. Eukaryot Cell 4:225–229

Heiman MG, Walter P (2000) Prm1p, a pheromone-regulated multispanning membrane protein, facilitates plasma membrane fusion during yeast mating. J Cell Biol 151:719–730

Hickey PC, Jacobson DJ, Read ND, Glass NL (2002) Live-cell imaging of vegetative hyphal fusion in *Neurospora crassa*. Fungal Genet Biol 37:109–119

Holmer L, Stenlid J (1993) The importance of inoculum size for the competitive ability of wood decomposing fungi. FEMS Microbiol Ecol 12:169–176

Hornby JM, Jacobitz-Kizzier SM, McNeel DJ, Jensen EC, Treves DS, Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl Environ Microbiol 67:2982–2992

Hou ZM, Xue CY, Peng YL, Katan T, Kistler HC, Xu JR (2002) A mitogen-activated protein kinase gene (MGV1) in *Fusarium graminearum* is required for female fertility, heterokaryon formation, and plant infection. Mol Plant Microbe Interact 15:1119–1127

Howard RJ (1981) Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. J Cell Sci 48:89–103

Ivey FD, Hodge PN, Turner GE, Borkovich KA (1996) The G alpha i homologue *gna-1* controls multiple differentiation pathways in *Neurospora crassa*. Mol Biol Cell 7:1283–1297

Jacobs H, Boswell PB, Scrimgeour CM, Davidson FA, Gadd GM, Ritz K (2004) Translocation of carbon by *Rhizoctonia solani* in nutritionally-heterogeneous microcosm. Mycol Res 108:453–462

Jacobson DJ, Gordon TR (1988) Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. Phytopathology 78:668–672

Jacobson DJ, Beurkens K, Klomparens KL (1998) Microscopic and ultrastructural examination of vegetative incompatibility in partial diploids heterozygous at *het* loci in *Neurospora crassa*. Fungal Genet Biol 23:45–56

Jeffries P (1985) Mycoparasitism within the Zygomycetes. Bot J Linnean Soc 91:135–150

Karpova TS, Reck-Peterson SL, Elkind NB, Mooseker MS, Novick PJ, Cooper JA (2000) Role of actin and Myo2p in polarized secretion and growth of *Saccharomyces cerevisiae*. Mol Biol Cell 11:1727–1737

Kay RR (2002) Chemotaxis and cell differentiation in *Dictyostelium*. Curr Opin Microbiol 5:575–579

Kellner M, Burmester A, Woestemeyer A, Woestemeyer J (1993) Transfer of genetic information from the mycoparasite *Parasitella parasitica* to its host *Absi dia glauca*. Curr Genet 23:334–337

Kemp HA, Sprague GF (2003) Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast *Saccharomyces cerevisiae*. Mol Cell Biol 23:1750–1763

Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52:1781–1798

Kim H, Metzenberg RL, Nelson MA (2002) Multiple functions of *mfa-1*, a putative pheromone precursor gene of *Neurospora crassa*. Eukaryot Cell 1:987–999

Köhler E (1929) Beiträge zur Kenntnis der vegetativen Anastomosen der Pilze I. Planta 8:140–153

Köhler E (1930) Zur Kenntnis der vegetativen Anastomosen der Pilze (II. Mitteilung). Planta 10:495–522

Kothe GO, Free SJ (1998) The isolation and characterization of *nrc-1* and *nrc-2*, two genes encoding protein kinases that control growth and development in *Neurospora crassa*. Genetics 149:117–130

Kurjan J (1993) The pheromone response pathway in *Saccharomyces cerevisiae*. Annu Rev Genet 27:147–179

Labarère J, Bernet J (1977) Protoplasmic incompatibility and cell lysis in *Podospora anserina*. I. Genetic investigations on mutations of a novel modifier gene that suppresses cell destruction. Genetics 87:249–257

Laibach F (1928) Über Zellfusionen bei Pilzen. Planta 5:340–359

Lengeler KB, Davidson RC, D'souza C, Harashima T, Shen WC, Wang P, Pan X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Leslie JF (1993) Fungal vegetative compatibility. Annu Rev Phytopathol 31:127–150

Leu LS (1967) Anastomosis in *Venturia inaequalis* (CKE) Wint. PhD Thesis, University of Wisconsin

Lev S, Sharon A, Hadar R, Ma H, Horwitz BA (1999) A mitogen-activated protein kinase of the corn leaf pathogen *Cochliobolus heterostrophus* is involved in conidiation, appressorium formation, and pathogenicity: diverse roles for mitogen-activated protein kinase homologs in foliar pathogens. Proc Natl Acad Sci USA 96:13542–13547

Lopez-Franco R, Bracker CE (1996) Diversity and dynamics of the Spitzenkörper in growing hyphal tips of higher fungi. Protoplasma 195:90–111

Marek SM, Wu J, Glass NL, Gilchrist DG, Bostock RM (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 40:126–137

Matheos D, Metodiev M, Muller E, Stone D, Rose MD (2004) Pheromone-induced polarization is dependent on the Fus3p MAPK acting through the formin Bni1p. J Cell Biol 165:99–109

Mesterhazy A (1973) The morphology of an undescribed form of anastomosis in *Fusarium*. Mycologia 65:916–919

Nelson WJ (2003) Adaptation of core mechanisms to generate cell polarity. Nature 422:766–774

Nelson B, Parsons AB, Evangelista M, Schaefer K, Kennedy K, Ritchie S, Petryshen TL, Boone C (2004) Fus1p interacts with components of the HOG1p mitogen-activated protein kinase and Cdc42p morphogenesis signaling pathways to control cell fusion during yeast mating. Genetics 166:67–77

Nern A, Arkowitz RA (1998) A GTP-exchange factor required for cell orientation. Nature 391:195–198

Newhouse JR, MacDonald WL (1991) The ultrastructure of hyphal anastomoses between vegetatively compatible and incompatible virulent and hypovirulent strains of *Cryphonectria parasitica.* Can J Bot 69:602–614

Nordbring-Hertz B, Friman E, Veenhuis M (1989) Hyphal fusion during the initial stages of trap formation in *Arthrobotrys oligospora*. Antonie van Leeuwenhoek 55:237–244

Osherov N, Mathew J, May GS (2000) Polarity-defective mutants of *Aspergillus nidulans*. Fungal Genet Biol 31:181–188

Overton MC, Blumer KJ (2000) G-protein-coupled receptors function as oligomers in vivo. Curr Biol 10:341–344

Pandey A, Roca MG, Read ND, Glass NL (2004) Role of a MAP kinase during conidial germination and hyphal fusion in *Neurospora crassa*. Eukaryot Cell 3:348–358

Park G, Xue GY, Zheng L, Lam S, Xu JR (2002) *MST12* regulates infectious growth but not appressorium formation in the rice blast fungus *Magnaporthe grisea*. Mol Plant Microbe Interact 15:183–192

Pearson CL, Xu K, Sharpless KE, Harris SD (2004) MesA, a novel fungal protein required for the stabilization of polarity axes in *Aspergillus* nidulans. Mol Biol Cell 15:3658–3672

Perkins DD, Radford A, Sachs MS (2001) The *Neurospora* compendium: chromosomal loci. Academic Press, San Diego

Pöggeler S (2000) Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. Curr Genet 37:403–411

Pöggeler S, Kück U (2001) Identification of transcriptionally expressed pheromone receptor genes in filamentous ascomycetes. Gene 280:9–17

Pontecorvo G (1956) The parasexual cycle in fungi. Annu Rev Microbiol 10:393–400

Raju NB (1980) Meiosis and ascospore genesis in *Neurospora*. Eur J Cell Biol 23:208–223

Rayner ADM (1996) Interconnectedness and individualism in fungal mycelia. In: Sutton BC (ed) A century of mycology. Cambridge University Press, Cambridge, pp 193–232

Riquelme M, Bartnicki-Garcia S (2004) Key differences between lateral and apical branching in hyphae of *Neurospora crassa.* Fungal Genet Biol 41:842–851

Riquelme M, Reynaga-Peña CG, Gierz G, Bartnicki-Garcia S (1998) What determines growth direction in fungal hyphae? Fungal Genet Biol 24:101–109

Roca MG, Davide LC, Mendes-Costa MC, Wheals A (2003) Conidial anastomosis tubes in *Colletotrichum*. Fungal Genet Biol 40:138–145

Roca MG, Arlt J, Jeffree CE, Read ND (2005) Cell biology of conidial anastomosis tubes in *Neurospora crassa*. Eukaryot Cell 4:911–919

Sarkar S, Iyer G, Wu J, Glass NL (2002) Nonself recognition is mediated by HET-C heterocomplex formation during vegetative incompatibility. EMBO J 21:4841–4850

Satina S, Blakeslee AF (1926) The *Mucor* parasite *Parasitella* in relation to sex. Proc N Y Acad Sci 12:202–207

Saupe SJ (2000) Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. Microbiol Mol Biol Rev 64:489–502

Schrick K, Garvik B, Hartwell LH (1997) Mating in *Saccharomyces cerevisiae* – the role of the pheromone signal transduction pathway in the chemotropic response to pheromone. Genetics 147:19–32

Segers GC, Nuss DL (2003) Constitutively activated Gα negatively regulates virulence, reproduction and hydrophobin gene expression in the chestnut blight fungus *Cryphonectria parasitica*. Fungal Genet Biol 38:198–208

Seiler S, Plamann M (2003) The genetic basis of cellular morphogenesis in the filamentous fungus *Neurospora crassa*. Mol Biol Cell 14:4352–4364

Seiler S, Nargang FE, Steinberg G, Schliwa M (1997) Kinesin is essential for cell morphogenesis and polarized secretion in *Neurospora crassa*. EMBO J 16:3025–3034

Shemer G, Podbilewicz B (2003) The story of cell fusion: big lessons from little worms. Bioessays 25:672–682

Shen WC, Bobrowicz P, Ebbole DJ (1999) Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet Biol 27:253–263

Sheu YJ, Santos B, Fortin N, Costigan C, Snyder M (1998) Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. Mol Cell Biol 18:4053–4069

Staben C, Yanofsky C (1990) *Neurospora crassa a* mating-type region. Proc Natl Acad Sci USA 87:4917–4921

Staples RC (2001) Nutrients for a rust fungus: the role of haustoria. Trends Plant Sci 6:496–498

Stephenson LW, Erwin DC, Leary JV (1974) Hyphal anastomosis in *Phytophthora capsici*. Phytopathology 64:149–150

Swart K, Debets AJM, Bos CJ, Slakhorst M, Holub EF, Hoekstra RF (2001) Genetic analysis in the asexual fungus *Aspergillus niger*. Acta Biol Hung 52:335–343

Takano Y, Kikuchi T, Kubo Y, Hamer JE, Mise K, Furusawa I (2000) The *Colletotrichum lagenarium* MAP kinase gene *CMK1* regulates diverse aspects of fungal pathogenesis. Mol Plant Microbe Interact 13:374–383

Todd NK, Aylmore RC (1985) Cytology of hyphal fusions in *Schizophyllum commune*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, Cambridge, UK, pp 231–248

Trinci APJ (1984) Regulation of hyphal branching and hyphal orientation. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, UK, pp 23–52

Trueheart J, Fink GR (1989) The yeast cell fusion protein Fus1 is O-glycosylated and spans the plasma membrane. Proc Natl Acad Sci USA 86:9916–9920

Tsitsigiannis DI, Zarnowski R, Keller NP (2004) The lipid body protein, PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Turina M, Prodi A, van Alfen NK (2003) Role of the *Mf1-1* pheromone precursor gene of the filamentous ascomycete *Cryphonectria parasitica*. Fungal Genet Biol 40:242–251

Ungar D, Hughson FM (2003) SNARE protein structure and function. Annu Rev Cell Dev Biol 19:493–517

Vallier LG, Segall JE, Snyder M (2002) The alpha-factor receptor C-terminus is important for mating projection formation and orientation in *Saccharomyces cerevisiae*. Cell Motil Cytoskeleton 53:251–266

Vallim MA, Miller KY, Miller BL (2000) *Aspergillus* SteA (sterile12-like) is a homeodomain-C2/H2-Zn+2 finger transcription factor required for sexual reproduction. Mol Microbiol 36:290–301

Van Diepeningen AD, Debets AJM, Hoekstra RF (1998) Intra- and interspecies virus transfer in Aspergilli via protoplast fusion. Fungal Genet Biol 25:171–180

Voegele RT, Struck C, Hahn M, Mendgen K (2001) The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. Proc Natl Acad Sci USA 98:8133–8138

Ward HM (1888) A lily-disease. Ann Bot 2:319–382

Watters MK, Griffiths AJF (2001) Tests of a cellular model for constant branch distribution in the filamentous fungus *Neurospora crassa*. Appl Environ Microbiol 67:1788–1792

Weber I, Gruber C, Steinberg G (2003) A class-V myosin required for mating, hyphal growth, and pathogenicity in the dimorphic plant pathogen *Ustilago maydis*. Plant Cell 15:2826–2842

Wei HJ, Requena N, Fischer R (2003) The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol Microbiol 47:1577–1588

West AH, Stock AM (2001) Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci 26:369–376

White JM, Rose MD (2001) Yeast mating: getting close to membrane merger. Curr Biol 11:R16–R20

Wilson JF, Dempsey JA (1999) A hyphal fusion mutant in *Neurospora crassa*. Fungal Genet Newslett 46:31

Xiang Q, Rasmussen C, Glass NL (2002) The *ham-2* locus, encoding a putative transmembrane protein, is required for hyphal fusion in *Neurospora crassa*. Genetics 160:169–180

Xu JR (2000) MAP kinases in fungal pathogens. Fungal Genet Biol 31:137–152

Xu JR, Hamer JE (1996) MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. Genes Dev 10:2696–2706

Xu JR, Staiger CJ, Hamer JE (1998) Inactivation of the mitogen-activated protein kinase *Mps1* from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA 95:12713–12718

Yang Q, Poole SI, Borkovich KA (2002) A G-protein βsubunit required for sexual and vegetative development and maintenance of normal Gα protein levels in *Neurospora crassa*. Eukaryot Cell 1:378–390

Yokoyama K, Ogoshi A (1988) Studies on hyphal anastomosis of *Rhizoctonia solani* V. Nutritional conditions for anastomosis. Trans Mycol Soc Jpn 29:125–132

Zeitlinger J, Simon I, Harbison CT, Hannett NM, Volkert TL, Fink GR, Young RA (2003) Program-specific distribution of a transcription factor dependent on partner transcription factor and MAPK signaling. Cell 113:395–404

Zhang L, Churchill AC, Kazmierczak P, Kim DH, van Alfen NK (1993) Hypovirulence-associated traits induced by a mycovirus of *Cryphonectria parasitica* are mimicked by targeted inactivation of a host gene. Mol Cell Biol 13:7782–7792

Zhang L, Baasiri RA, Van Alfen NK (1998) Viral repression of fungal pheromone precursor gene expression. Mol Cell Biol 18:953–959

8 Heterogenic Incompatibility in Fungi

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CONTENTS

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[2] This paper is dedicated to Georges Rizet (Paris) on the occasion of his 90th birthday. I am very much indebted to him for introducing me into the genetics of fungi and for his friendship lasting now more than half a century.

I. Introduction

In biology, incompatibility is usually defined as restriction of mating competence controlled by genes other than those determining sexual differentiation. It has been long recognised that incompatibility concerns not only the sexual phase but also the vegetative phase. The latter becomes apparent especially in fungi, and was first termed heterokaryon incompatibility. In both sexual and vegetative incompatibility, the action of the genetic traits involved precludes the exchange of genetic material. Thus, inhibition of recombination results by a lack of karyogamy (sexual incompatibility) as well as by an inability of the nuclei to coexist in a common cytoplasm and to undergo somatic recombination (vegetative incompatibility). Since recombination is of paramount importance in evolution, the biological significance of incompatibility as a factor controlling recombination is immediately apparent.

Nature has evolved two principal systems to control incompatibility. According to their mode of genetic determination, these have been called homogenic and heterogenic incompatibility (Esser 1962). The genetic basis of homogenic incompatibility consists in a sexual incompatibility of nuclei carrying identical incompatibility factors. **Heterogenic incompatibility consists in a genetic difference of at least one single gene which inhibits the coexistence of the nuclei concerned in a common cytoplasm**.

From these definitions, it follows that homogenic incompatibility enhances outbreeding and favours recombination and evolution of the species. Heterogenic incompatibility, however, restricts outbreeding and thereby favours the evolution of isolated groups within a single species. Both systems, despite controlling recombination in an antagonistic way, are integrated constituents of evolution.

The Mycota I
Growth, Differentation and Sexuality
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Homogenic incompatibility has been known since Darwin's time and its various mechanisms have been analysed in great detail, in both higher plants and fungi. Its actions and its distribution are the subject of many books and reviews. This type of incompatibility is described in Chaps. 15 and 17 (this volume).

The genetics of heterogenic incompatibility was first revealed 50 years ago, in studies involving the ascomycete *Podospora anserina* (Rizet and Esser 1953; Esser 1954). Meanwhile, this subject has attracted much attention, and many cases of heterogenic incompatibility, dealing with the vegetative and/or the sexual phases of fungi, have been described and analysed (see Table 8.1).

It is understandable that heterogenic incompatibility was less intensively studied than homogenic incompatibility, since the former rarely occurs within true breeding laboratory strains, but rather between geographical races differing in their genetic constituency. In addition, heterogenic incompatibility has often been overlooked and sometimes misinterpreted as "sterility", merely because of a failure to mate.

This review is a revised and updated version of the chapter on heterogenic incompatibility in the first edition of this volume (Esser and Blaich 1994).

Other pertinent reviews on heterogenic incompatibility can be found in Esser and Kuenen (1967), Esser (1971), Lemke (1973), Carlile and Gooday (1978), Lane (1981), Esser and Meinhardt (1984), Jennings and Rayner (1984), Perkins and Turner (1988), Glass and Kuldau (1992), Leslie (1993), Bégueret et al. (1994), Leslie and Zeller (1996), Worall (1997), Glass et al. (2000), Saupe (2000), and Glass and Kaneka (2003).

II. Barrage Formation

More than 100 years ago, Reinhardt (1892) first observed that, when certain fungal mycelia approached one another, sometimes an interaction phenotypically recognisable as repulsion occurred. This phenomenon was subsequently described by others (Cayley 1923, 1931; Nakata 1925), and it was probably Vandendries (1932) who introduced the term barrage to describe it. Certainly, older descriptions of barrages, based on quite different phenomena, were assigned different names. Since **barrage is a phenotypic expression of heterogenic incompatibility**, it is at first necessary to define the concept of barrage.

If fungal hyphae from different mycelia grow towards each other, in general four main types of interaction occur, and these can be easily demonstrated on agar media.

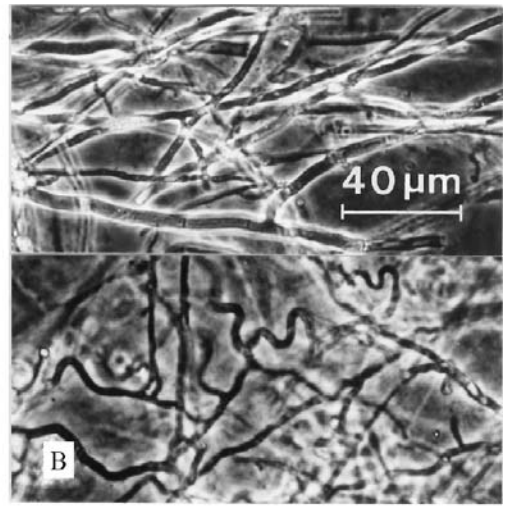
1. Mutual Intermingling = Normal Contact
   After approach, the hyphae intermingle in the zone of contact and show (with a few exceptions, as in Oomycota) numerous hyphal fusions via anastomosis. After a time, the border zone between the two mycelia becomes unrecognisable (Fig. 8.1A–D).

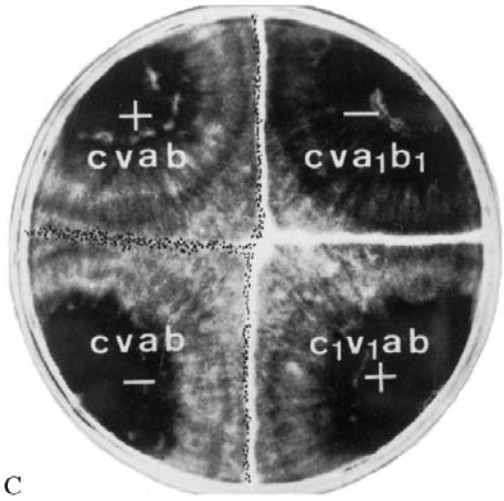
2. Inhibition
   When opposing hyphae approach each other, an inhibition zone free of hyphae is formed between the two mycelia. This phenomenon may be caused by unilateral or mutual interaction, due to the secretion and diffusion of inhibitory substances.

3. Mutual Intermingling and Inhibition = Barrage Formation
   When two mycelia grow into each other and intermingle, an antagonistic reaction ensues. In contrast to inhibition by diffusible substances, the barrage reaction requires cytoplasmic contact via hyphal fusions. The phenotype of the barrage varies depending on the species and mode of genetic control (Fig. 8.1). However, in all barrages known so far, nuclear exchange is not inhibited, but in most cases the two types of mycelia form abnormal and even lethal fusions. The hyphal tips may branch profusely. A clear line of contact appears with increasing age of the culture. Barrages are mainly found in intraspecific (inter-racial) matings. Depending on the species, the barrage may be colourless or pigmented (Fig. 8.1A,C). A recent study in *Neurospora* shows that different types of barrages may occur also within one and the same species (Micali and Smith 2003).

4. Mutual Repulsion = Border Line, Demarcation Line
   Especially noted in matings of wood-rotting basidiomycetes, a mutual repulsion and antagonistic reaction is evident which leads to the formation of a more or less strongly pigmented zone of intermingled hyphae. This demarcation line (Adams and Roth 1967) or border line (Esser and Hoffmann 1977) occurs mainly in interspecific matings. It is visible in nature on cuttings of logs (Rayner and Todd 1977; Esser and Meinhardt 1984; Fig. 8.1D) as well as in axenic cultures (Fig. 8.1C). Border lines are often used as criteria for species delineations. However, this commonly creates some problems in interpretation because, in most cases investigated to date, there are no analyses of the mi-

A





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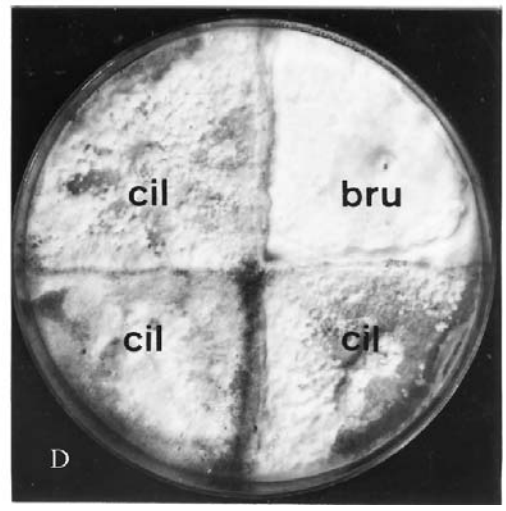

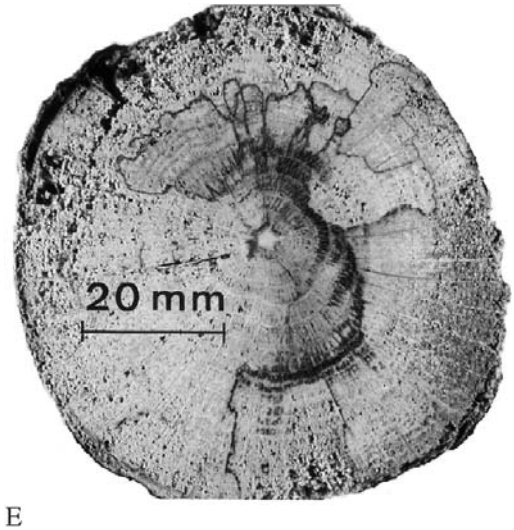

E

Fig. 8.1.A–E Compilation of mycelial interactions in fungi. **A** *Podospora anserina*. Barrage formation between different geographical races. Sexual reproduction is not affected. Perithecia are produced in any combination between different mating types. **B** *Podospora anserina*. Hyphal morphology. *Above hyphae* in the contact zone of an intra-race combination, *below hyphae* within the barrage zone. **C** *Podospora anserina*. Barrage formation linked with sexual incompatibility (for details, see Fig. 8.3 and text). **D** Intra- and interspecific interactions between monokaryons of *Polyporus ciliatus* (*cil*) and *Polyporus brumalis* (*bru*) showing normal contact (*left*), and barrage (*bottom*) and border line (*top* and *right*). All monokaryons are compatible in mating type. **E** Cross section of a log colonized by wood-destroying basidiomycetes. *Dark zones* indicate the aversion lines of mycelia. Further details are given at various sites in the text (adapted from Esser 1956c, and Esser and Meinhardt 1984a,b,d,e)

croscopic structure of these lines, nor of whether there is hyphal fusion allowing cytoplasmic contact and nuclear exchange. Therefore, it is often rather difficult to evaluate experimental reports with respect to barrage or border line formation and, consequently, to distinguish between intraspecific and interspecific matings.

Accordingly, I shall henceforth use the term barrage only if the microscopic observations show that a zone of aversion occurring between two mycelia is associated with hyphal fusions. I am well aware that this distinction is not always possible on the basis of exact data.

III. Heterogenic Incompatibility in the Ascomycete *Podospora anserina*

A. The Phenomenon

Although barrage formation is found in mycelial interactions with various higher fungi, the best analysed case concerns the ascomycete *Podospora anserina*. As in many ascomycetes, the mating competence of *P. anserina* is controlled by the bipolar mechanism of homogenic incompatibility (heterothallism) due to an interaction of the two idiomorphs + and – of the mating type locus (see Chap. 15, this volume). In analogy to the *Neurospora* terminology, these alleles were later renamed and termed *mat*+ and *mat*– respectively.

In studying various races of *P. anserina* with different geographical origins, the mycelia of which showed no recognisable macroscopic differences, Rizet (1952, 1953) found that in inter-race combinations a barrage was formed irrespective of mating type. As may be seen from Fig. 8.1A, this barrage is macroscopically characterised by a sharp white zone between two darkly pigmented (melanotic) paired mycelia. A microscopic examination revealed that in this zone the hyphae forming anastomoses become curled, swollen and degenerative (Fig. 8.1B). This barrage zone eventually consists of dead hyphae.

In some cases the barrage formation does not affect fruiting between different mating types, because perithecia are formed on both sides of the barrage (Fig. 8.1A).

This peculiar phenomenon is explained by the fact that the trichogynes of the + female sex organs (protoperithecia) fuse only with the – male gametes (spermatia), and vice versa. Spermatia, however, are never formed in the barrage zone. From this it follows that the trichogynes pass the barrage unimpaired, since no anastomoses involving trichogynes take place. Obviously, the fusion of the tip of the trichogyne with a spermatium does not necessarily bring about the incompatibility reaction occurring between two hyphae, although the reason for this remains obscure.

Nevertheless, as summarized in Fig. 8.2, a comprehensive study of the mating interactions between 19 geographical races revealed that, in addition to the barrage formation, the fruit body formation (perithecia) also was quantitatively and/or qualitatively disturbed. In the first instance, the number of perithecia was drastically reduced on one or both sides of the barrage. In the second case, one or both of the reciprocal crosses between the two mates was incompatible. In the 13 (7.6%) inter-racial combinations which showed no barrage, there was also no effect on fruiting – in other words, **sexual incompatibility in inter-race crosses is always linked with barrage formation.**

In this context, it should be noted that at least one incompatibility mechanism may also lead to distortions in meiotic segregation due to spore killer effects (van der Gaag et al. 2003; Hamann and Osiewacz 2004).

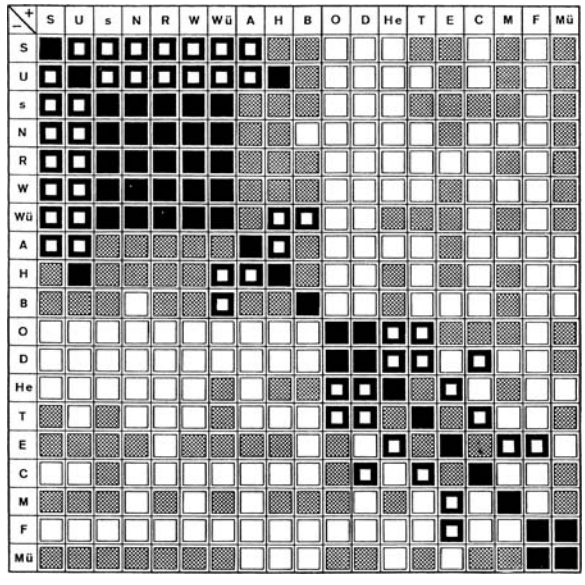

Fig. 8.2. Scheme of the mating reactions between various races of *Podospora anserina* isolated from different localities in France and Germany. +/– Mating type, *uppercase letters* designation of races, *closed squares* compatible in both vegetative and sexual phase, *closed squares with inserted open squares* sexual compatibility and vegetative incompatibility (barrage formation only), *hatched squares* vegetative incompatibility and reduced fruit body formation, *open squares* incompatibility in both vegetative and sexual phase. (Adapted from Esser 1971)

B. Genetic Control

The genetic background of heterogenic incompatibility was revealed by the analysis of the two races *s* and *M* (Rizet and Esser 1953; Esser 1954, 1956). Six loci were identified as instrumental in two different mechanisms, as summarized in Fig. 8.3 and explained below.

1. The **allelic mechanism** caused by alleles of the *t* and *u* loci does not interfere with sexual compatibility. If strains differ at either one (Fig. 8.1A) or both loci, a vegetative incompatibility is provoked, showing up as barrage formation.
2. The **non-allelic mechanism** depends on the interaction of two specific alleles at two different loci. In the inter-racial cross of *s* and *M*, four loci *a*, *b*, *c*, *v* were identified showing an incompatibility of the alleles a_1/b and c_1/v respectively, leading to barrage formation as well as to unilateral incompatibility (middle of Fig. 8.1C). If both mechanisms overlap in recombinants from the cross $s \times M$ in the combination a, b, c_1, $v_1 \times a_1$, b_1, c, v, then a complete sexual incompatibility is brought about (Fig. 8.1C, right side).

C. Physiological Expression

As mentioned above, a prerequisite for the expression of heterogenic incompatibility is that two nuclei showing a specific allelic and/or non-allelic difference are brought together in a common cytoplasm. This occurs very frequently because, in *Podospora* as in many other ascomycetes, hyphae fuse by anastomosis when they come into contact. This is followed by a mutual nuclear migration leading to heterokaryosis.

Incompatibility of heterogenic nuclei can be brought about by a unilateral or by a bilateral action. It was found that in those heterokaryons in which the allelic mechanism was effective, a destabilization took place, leading to a formation of homokaryotic sectors of either nuclear type, separated by barrage formation. In heterokaryons in which the non-allelic mechanism was instrumental, however, no sectoring occurred, because one nuclear species was eliminated. For example, in the combination $ab + a_1b_1$, only the *ab* nuclei survived. Thus, it follows that the allelic mechanism brings about a mutual interaction, whereas the non-allelic mechanism is realized through unilateral gene action (Esser 1956, 1959a,b).

This explains as well the unilateral sexual incompatibility in the non-allelic mechanism present, for instance, in the crossing of strains $ab \times a_1b_1$ (Fig. 8.3). It may be deduced from these heterokaryon experiments that *b* is the "aggressive" allele and that a_1 is its target. Since during the mating procedure in *Podospora* in the differentiated trichogynes the nuclei degenerate in the combination $♀ab \times ♂a_1b_1$, the active *b*-nuclei are no longer present and there is no inhibition for the sensitive a_1-nucleus, which may thus migrate into the ascogonial cell of the protoperithecium. In the alternate case, the active *b*-nucleus of the spermatium is able to initiate destruction when entering the trichogyne. As in the case of the allelic mechanism, there is also no explanation why the non-allelic mechanism does not become effective during the steps of sexual differentiation, and is expressed only when the ascospores germinate, as demonstrated by the assay of heterokaryons.

These findings were later confirmed by Bernet (1965), who studied the same races *s* and *M*. By analysis of other races, six more incompatibility loci were identified (Bernet 1967), one being involved in the allelic and five in the non-allelic mechanism. Unfortunately, Bernet did not use our gene designations. All these genes were later summarized under the heading *het*-genes, e.g. *het-c*. In the following, I shall give the original names of these genes in parentheses.

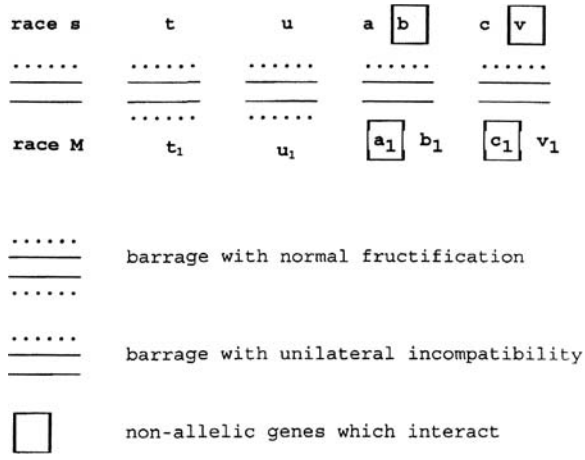

Fig. 8.3. *Podospora anserina*. Scheme of the action of the two mechanisms of heterogenic incompatibility operating in a cross of the races *s* and *M*. The different alleles are symbolized by *lowercase letters*. For further information see text. (Adapted from Blaich and Esser 1970)

D. Function of the *het*-Genes

During the last years, many efforts were undertaken to understand the function of the *het*-genes on the molecular and biochemical level.

1. The Non-Allelic Mechanism

a) Application of Biochemical Techniques

In heterokaryons, specific proteins (Esser 1959b; Blaich and Esser 1970), catabolic enzymes (Blaich and Esser 1971) and new enzyme activities, such as for several proteases, phenoloxidases, malate and NADH dehydrogenase, and amino acid oxidase (Boucherie and Bernet 1978; Boucherie et al. 1981; Paoletti et al. 1998) were found, but their involvement with the function of the *het*-genes was not evident.

b) Mutations Which Interfere with het-Genes

A series of modifier mutants (*mod*-genes) were found, most of which suppress in specific combinations the barrage formation caused by the allelic or by the non-allelic mechanism. In active combinations, these mutants also suppress the formation of female sex organs (protoperithecia). One of these *mod*-genes, *mod-E* (*a*), codes for a member of the Hsp90 family of heat-shock proteins acting in various types of stress responses (Loubradou et al. 1997). A second cloned gene, the *mod-D* gene (*v*) encodes a Gα protein. The gene displays functional interactions with *mod-E* and *Pa AC*, a gene for an adenylate cyclase. Although addition of cyclic AMP can partially suppress growth defects caused by *mod-D* mutations, a molecular connection of cAMP with the effect of *mod-D* mutations on the *het*-genes was not found (Loubradou et al. 1999).

c) Analyses of mRNA

During the incompatibility reaction, a strong decrease of mRNA synthesis and the appearance of a new set of proteins occur. Therefore, the vegetative incompatibility is regulated, at least in part, by variation of the mRNA content of specific genes. Genes induced during the incompatibility reactions have been termed *idi*.

- idi-1 is a cell wall protein and resides in the septum during normal growth (Dementhon et al. 2003).
- idi-4 is a bZIP transcripton factor regulating autophagy and cell fate, and also expression of other *idi*-genes (Dementhon et al. 2004).
- *idi-6/pspA* encodes a vacuolar protease involved in autophagy (Pinan-Lucarré et al. 2003).
- *idi-7* acts in the formation of autophagosomes, vesicles which target cytoplamsic material to the vacuole (Pinan-Lucarré et al. 2003).

Rapamycin treatment of *Podospora anserina* causes *idi*-gene expression and cellular effects typical for heterogenic incompatibility (Dementhon et al. 2003).

d) Molecular Analysis of the het-c, het-d and het-e Genes

Alleles of *het-c* and *het-e* genes correspond to the genes b/b_1 and a/a_1 in Esser's studies. Alleles of the *het-c* locus have similar ORFs but lead to protein products with some amino acid differences (Saupe et al. 1995). The *het-c* gene products are members of a family of ancestral sphingolipid transfer proteins (Mattjus et al. 2003). By inactivation of the *het-c* gene, abnormal ascospores are formed. *het-c* alleles interact in different ways with the *het-e* and *het-d* alleles (Saupe et al. 1995).

Both the *het-d* and the *het-e* genes code for proteins which display a GTP-binding site and a WD40 repeat domain, typical for a β-subunit of a G-protein. Sequence comparison of different *het-e* alleles showed that *het-e* specificity is determined by the sequence of the WD40 domain, which may confer the incompatibility interactions (for references, see Espagne et al. 2002). Physical interactions with the *het-c* proteins need to be demonstrated to clarify this.

2. The Allelic Mechanism

The first *het*-genes of all described are the alleles *het-s* and *het-S* (Rizet 1952). They cause barrage formation by the allelic mechanism but do not interfere with fruit body production. Their co-expression in a common cytoplasm causes cell death. Both alleles encode 30-kd proteins consisting of 289 amino acids. The alleles differ in 43 amino acid positions (Turcq et al. 1990, 1991).

A disruption of either gene resulted in a lack of the 30-kd protein. When mated, these strains no longer formed a barrage. Sexual compatibility was not affected. It was further shown by detailed analyses of the *het-s/S* locus of 13 wild strains that the specificity of the *s* and *S* proteins to provoke heterogenic incompatibility depends on a single amino acid difference only (Deleu et al. 1993).

Strains with the genotype *het-s* exist in two phenotypic states: the neutral phenotype *het-s** and the

active phenotype *het-s*. The neutral phenotype is characterised by the fact that it does not show the barrage reaction, when crossed with *het-S* strains. The *het-s* phenotype is infective and is able to transform, via hyphal fusions, a neutral *het-s** strain.

The *het-s* protein which provokes the transformation is a prion which adopts an amyloid structure and propagates in vivo as a self-perpetuating amyloid aggregate (Nazabal et al. 2003; Balguerie et al. 2004). Amyloid structures formed in vitro were shown to be infectious, in contrast to soluble *het-s* protein and amorphous aggregates, supporting the prion nature of the amyloid fibres (Maddelein et al. 2002).

The analysis of deletion constructs and site-directed mutants showed that a short C-terminal peptide (112 amino acids) allows the propagation of the prion-analog (Cousteau et al. 1997). This part of the protein contains the amyloid core regions of the *het-s* prion protein (Balguerie et al. 2003, 2004).

In conclusion: The many studies of heterogenic incompatibility performed with *Podospora anserina* have given deep insights into the genetic mechanism of the *het*-genes, and shown many aspects of their action. However, a complete understanding of their function in causing their mutual antagonism still needs further research.

IV. Further Examples of Heterogenic Incompatibility

As one may suppose, the discovery of heterogenic incompatibility was not through focussed research. By contrast, in the ascomycetes it was observed as a "by-product" of genetic research on breeding competence. In the basidiomycetes, as discussed below, data on heterogenic incompatibility are very often a result of studies in population genetics dealing with intergeneric and interspecific delineation and evolution. In the literature, there is a diversity of names, definitions and gene symbols for effects which can be interpreted as manifestations of heterogenic incompatibility. Thus, it is understandable that, in describing the antagonistic mycelial interactions and the various groups of natural isolates showing compatibility or incompatibility, different terms and expressions are used (as stated in the legend to Table 8.1), which I consider also as a source of information for a reader who is not familiar with this area of research and who wishes to gain more detailed information. Therefore, I shall discuss only some other cases of heterogenic incompatibility which have been studied in more detail.

Although the **myxozoa** are no longer grouped with the fungi, they have at least to be briefly mentioned, because these organisms have been the subject of intensive studies of heterogenic incompatibility. Vegetative incompatibility is widespread between geographical races of the genera and species analysed to date. In analogy with *Podospora*, plasmodial matings may lead to a visible zone of aversion, which does not allow nuclear migration, because there is either unilateral or mutual disintegration of the nuclei. Collectively from these studies, various genes acting according to the allelic mechanism have been identified. There are no data concerning the physiological actions of these genes.

For pertinent information, the reader is referred to literature on *Didymium iridis* and related species (Betterly and Collins 1984; Clark 1984, 2003), *Physarum polycephalum* (Lane and Carlile 1979; Schrauwen 1979; Lane 1981), and *Dictyostelium discoideum* (Robson and Williams 1979, 1980).

A. Oomycota

Oomycota have scarcely been used for genetic studies. This may be partially due to the fact that, in contrast to ascomycetes and basidiomycetes, they are vegetative diploids, thus complicating genetic analysis of progeny. It is not surprising that our knowledge of genetic control of their breeding systems, and especially evidence for heterogenic incompatibility, is very limited. Furthermore, anastomoses between the coenocytic hyphae having cellulose walls do not occur. The only indication for the existence of heterogenic incompatibility in Oomycota concerns the genus *Phytophthora* (for details, this is referenced in Table 8.1).

B. Glomeromycota

In the recently defined Glomeromycota, heterogenic incompatibility has been reported between isolates of the arbuscular mycorhizal fungus *Glomes mosseae* (Giovanetti et al. 2003).

C. Dikaryomycota

1. Ascomycotina

This class includes some of the most thoroughly studied saprophytic genera, for example, *Neurospora*, *Aspergillus* and *Podospora*. There are also numerous data proving the existence of heterogenic incompatibility in a great number of

Table. 8.1. Examples of occurrence of heterogenic incompatibility in fungi, as interpreted from symptoms described by the investigators
Because of the diversity involved in examples cited, there is risk of misinterpretations. I do not claim to present a complete list of all examples published. There are many papers, especially in basidiomycetes, which only vaguely indicate the existence of heterogenic incompatibility, and this needs to be further investigated. Abbreviations: v-c groups, fungal isolates which show vegetative compatibility, used mostly for ascomycetes; for the same concept in basidiomycetes, the terms intersterility (i-s) groups, biological races or biological species are used; *het*-genes, genes responsible for heterokaryon incompatibility; idiomorphs, mating type genes showing slight differences of their genetic code; bipolar and tetrapolar, homogenic incompatibility controlled by a mono- and bifactorial mechanism respectively. All these terms are also defined at appropriate places in the text. Asterisks indicate fungi treated in detail in the text

Species	Symptoms	References
Oomycota		
**Phytophthora* spp.	Restriction of mating competence	Savage et al. (1968), Boccas (1981)
Eumycota		
Glomeromycota		
Glomes mosseae	v-c groups	Giovanetti et al. (2003)
Ascomycetes, saprophytes		
**Ascobolus immersus*	Bipolar; v-c groups; barrage; sexual incompatibility	Meinhardt et al. (1984)
**Aspergillus* spp.	Self-compatible; v-c groups; up to eight *het*-genes	Grindle (1963a,b), Jinks et al. (1966), Butcher (1968, 1969), Butcher et al. (1972), Caten et al. (1971), Dales and Croft (1977), Croft and Dales (1984)
Aspergillus flavus, *A. parasiticus*, *A. tamarii*	i-c groups; morphological and physiological diversities	Horn et al. (1996)
**Aspergillus niger*	i-c groups; virus transfer inhibited	Van Diepeningen et al. (1997)
**Neurospora* spp.	Mating type idiomorphs; up to 11 *het*-genes	Garnjobst and Wilson (1956), Pittenger and Brawner (1961), Wilson (1961, 1963), Wilson et al. (1961), Pittenger (1964), Wilson and Garnjobst (1966), Newmeyer and Taylor (1967), Newmeyer (1968, 1970), Williams and Wilson (1968), Turner et al. (1969), Mylyk (1975, 1976), Leslie (1987), Perkins and Turner (1988)
**Podospora anserina*	Bipolar; v-c groups; barrage; up to nine *het*-genes; reciprocal and nonreciprocal sexual incompatibility	See Sect. II., this chapter
Ascomycetes, parasites		
**Botrytis cinerea*	v-c groups; gene *Bc-hch* homolog to *het*-genes of *N. crassa* and *P. anserina*	Fournier et al. (2003)
Cocholiobolus heterostrophus	Bipolar; v-c groups; *het*-genes	Leach and Yoder (1983)
**Cochliobolus* spp. (imperf. *Helminthosporium*)	Heterogenic incompatibility in the sexual phase	Nelson (1963, 1965a,b, 1966, 1970), Nelson and Kline (1964), Webster and Nelson (1968)
Crumenolopsis soriora	Bipolar; restriction of mating competence between subpopulations	Ennos and Swales (1987)
Diaporthe phaseolorum	Self-compatible; barrage	Ploetz and Shokes (1986)
**Endothia* (*Cryphonectria*) *parasitica*	Bipolar; barrage; v-c groups; six or seven *het*-genes identified, allelic and non-allelic mechanism	Anagnostakis (1977, 1982a,b, 1983), Nuss and Koltin (1990), Cortesi and Milgroom (1998)
Erisyphe cichoracearum	Bipolar; sexual incompatibility	Morrison (1960)
Ophiostoma (*Ceratocystis ulmi*)	Bipolar; barrage	Brasier (1984)

Table. 8.1. (continued)

Species	Symptoms	References
Gaeumannomyces graminis	v-c groups	Jamil et al. (1984)
**Gibberella fujikuori* (*Fusarium moniliforme*)	Bipolar; v-c groups; *het*-genes	Kuhlmann (1982), Puhalla and Spieth (1983, 1985), Sidhu (1986), Leslie et al. (2004)
Fusarium oxysporum	Imperfect; v-c groups	Correl et al. (1986), Jacobson and Gordon (1988), Katan and Katan (1988)
Leucocytospora kunzei	v-c groups; six *het*-loci	Proffer and Hart (1988)
Monascus purpureus	Self-fertile; six v-c groups	Chaisrisook (2002)
Nannizzia spp. (imperf. *Microsporum*)	Bipolar; heterogenic incompatibility in the sexual phase?	Padhaye and Carmichel (1971)
Sclerotinia sclerotium	v-c groups	Ford et al. (1995)
Sclerotinium rolfsii (teleomorph: *Athelia rolfsii*)	71 v-c groups	Punja and Sun (2002)
Sclerotinium delphini	Five v-c groups	
Stagonospora nodorum (teleomorph: *Leptospaeria nodorum*)	v-c groups	Newton et al. (1998)
Venturia inaequallis	Bipolar; nonreciprocal incompatibility between different mating types of geographical races	Kiebacher and Hoffmann (1981)
Verticillium dahliae	Imperfect; v-c groups	Puhalla and Hummel (1983)
Basidiomycetes (in considering the wood-destroying basidiomycetes in this group, there is no distinction between saprophytes and parasites)		
Auricularia spp.	Tetrapolar; intraspecific i-s groups; barrage formation	Wong (1993)
Agaricus spp.	Border lines between species	Anderson et al. (1984)
Armillaria mellea	Bipolar; i-s groups; partially compatible	Anderson and Ullrich (1979), Anderson et al. (1980), Anderson (1986)
Athelia (*Sclerotium*) *rolfsii*	Bipolar? Barrage; i-s groups	Punja and Grogan (1983a,b)
Bjerkandera fumosa	Bipolar; i-s groups	Lombard et al. (1992)
Ceratobasidium bicorne	Self-fertile; demarcation line; i-s groups	Hietala et al. (2003)
**Collybia dryophila*	Bipolar; i-s groups; reduced sexual compatibility	Vilgalys and Miller (1987), Vilgalys and Johnson (1987)
Collybia subnuda	Bipolar; i-s groups; barrage	Murphy and Miller (1993)
Coprinus bisporus (*Coprinellus bisporus*)	Bipolar; heterogenic incompatibility sexual phase	Kemp (1989)
**Coprinus cinereus* (*Coprinopsis cinerea*)	Tetrapolar; barrage, due to heterogenic mitochodrial DNA	May (1988)
Coriolus versicolor (*Trametes versicolor*)	Tetrapolar; i-s groups; barrage	Rayner and Todd (1977)
Cyathus spp.	Tetrapolar; unilateral dikaryotization within two species	Brodie (1970)
Fomes cajanderi	Barrage between geographical races	Adams and Roth (1967)
Ganoderma boninense	Tetrapolar incompatibility; i-s groups; border line	Pilotti et al. (2002)
Helicobasidium mompa	i-s groups; border line	Ikeda et al. (2003)
**Heterobasidium annosum*	Bipolar; i-s groups; 4–5 *het*-genes; epistatic; multiple alleles	Chase and Ullrich (1990a,b), Hansen et al. (1993a,b)
Heterobasidion insulare	Bipolar; three i-s groups	Dai et al. (2002)
Inonotus arizonicus	Self-compatible; i-s groups; barrage	Goldstein and Gilbertson (1981)
Laccaria spp.	Bipolar; i-s groups within one and between species	Fries and Müller (1984)
Lentinula edodes	Tetrapolar; i-s groups; barrage	Yindeeyoungyeon and Triratana (1992)
Marasmiellus parasiticus	Tetrapolar; i-s groups; barrage	Murphy and Miller (1993)
Marasmius spp.	Tetrapolar; i-s groups	Gordon and Peterson (1992)
Paxillus involutus	Bipolar; i-s groups	Fries (1985)

Table. 8.1. (continued)

Species	Symptoms	References
Peniophora spp.	Bipolar; host-dependent intra- and interspecific incompatibility	McKeen (1952)
Phellinus gilvus	Tetrapolar; i-s groups; one or several *het*-genes	Rizzo et al. (1996)
Phellinus pini	Bipolar; i-s groups	Fischer (1994)
Phellinus weirii	Bipolar; barrage; i-s groups	Hansen (1979)
**Phellinus torulosus*	Bipolar; barrage; i-s groups	Fischer and Bresinsky (1992)
Phlebia spp.	Bipolar; intraspecific barrage; interspecific border line; i-s groups	Boddy and Rayner (1983)
Pisolithus arhizus (syn. *P. tinctorius*)	Tetrapolar; unilateral dikaryotization	Kope (1992)
**Pleurotus ostreatus*	Tetrapolar; barrage; i-s groups	Kay and Vilgalys (1992)
**Polyporus* spp.	Interspecific crosses sterile (border line); intraspecific crosses, tetrapolar incompatibility superimposed by heterogenic incompatibility; barrage formation caused by three genes	Macrae (1967), Barrett and Uscuplic (1971), Hoffmann and Esser (1978)
**Sistrotrema brinkmanni*	Different breeding systems superimposed by heterogenic incompatibility	Lemke (1969)
**Stereum hirsutum*	Bipolar; compatibility superimposed by multiallelic *het*-genes; barrage	Coates and Rayner (1985a,b,c), Coates et al. (1985)
Stereum rugosum	Bipolar; i-s groups	Rayner and Turton (1982)
Sterum gausapatum	Bipolar; i-s groups	Boddy and Rayner (1982)
Stereum sanguinoletum	Self-compatible; i-s groups	Rayner and Turton (1982)
Stereum rameale	Self-compatible; i-s groups	Rayner and Turton (1982)
**Thanatephorus cucumeris*	Self-compatible; i-s groups; host-dependent	Stretton and Flentje (1972a,b)
Thanatephorus practicola (*Rhizoctonia solani*)	Self-compatible; i-s groups; barrage	Cubeta et al. (1993)
Typhula spp.	Tetrapolar; interspecific border line	Bruehl et al. (1975)
**Ustilago maydis*	Killer phenomenon	Puhalla (1968), Hankin and Puhalla (1971)

parasitic ascomycetes, but detailed genetic data are lacking in many cases.

a) Saprophytic Ascomycetes

Neurospora A part from *Podospora*, the genus *Neurospora* is the best analysed taxonomic entity for heterogenic incompatibility in fungi (Table 8.1).

Over 3900 isolates have been collected from nature from over 500 sampling sites. Perkins and his collaborators have classified and assessed this impressive dataset in terms of inter- and intraspecies mating relations (Perkins et al. 1976; Perkins and Turner 1988). The species most studied is *Neurospora crassa*, but the closely related species *Neurospora sitophila* is also used for investigating heterogenic incompatibility.

Heterogenic incompatibility in *Neurospora* has, according to my knowledge, been reported only as **heterokaryon incompatibility** concerning the vegetative phase. It is under polygenic control and involves **several allelic mechanisms**.

1. The Mating Type Locus

As early as 1933, Moreau and Moruzi reported "cross sterility" between opposite mating types in inter-racial crosses of *N. sitophila*. A comparable phenomenon of "cross sterility" was also described by Lindegren (1934) for *N. crassa*. In both cases, no genetic analyses were performed. A more substantial indication for the occurrence of heterogenic incompatibility originates from the classical "heterokaryon paper" of Beadle and Coonradt (1944), showing that strains of *N. crassa* must be of the same mating type in order to form a vigorous and stable heterokaryon.

Newmeyer and her collaborators (cf. Table 8.1) proved that the two mating types MAT *A* and MAT *a* are not able to coexist in a common cytoplasm. They found that the gene *tol* (linkage group IV), which is not linked with the mating type locus, suppresses this vegetative incompatibility. *tol* does not interfere with the sexual compatibility initiated in an *A/a* cross. These authors proposed that the mating type locus

is a complex genetic trait controlling both heterokaryon formation and sexual compatibility. The *tol*-gene encodes a putative reading frame for a 1011 amino acid polypeptide with a coiled-coil domain and a leucine-rich repeat. It is suggested that the TOL-locus may interact with the mating type proteins MAT *A*-1 and/or MAT *a*-1 to form a death-triggering complex (Shiu and Glass 1999).

The concept of complex mating type loci was verified by molecular analyses. The MAT *A* gene consists of 5301 bp (Glass et al. 1990) whereas the MAT *a* gene is much smaller and comprises 3225 bp only (Staben and Yanofsky 1990). However, sexual compatibility and heterokaryon incompatibility were found to be inseparable. Thus, a single gene product in both mating types MAT *A* and MAT *a* is responsible for the completion of the sexual cycle and for heterogenic incompatibility in the vegetative phase. In order to emphasize the rather strong structural dissimilarity of the mating type alleles, Metzenberg (1990) has proposed to use the term **idiomorphs**, rather than alleles.

The structure of the mating type alleles in *Podospora anserina* is very similar to those of *Neurospora crassa* (Debuchy and Coppin 1992, see also Chap. 15, this volume). However, as mentioned above, the mating idiomorphs in *P. anserina* control only homogenic incompatibility.

The involvement of mating loci in heterogenic incompatibility is rather unusual in fungi. It seems to be restricted to some of the self-incompatible *Neurospora* species. Here again, the same question as in *Podospora* is raised: why do the genes responsible for heterokaryon incompatibility not interfere with the overall sexual process? It is not possible at present to answer this question. Maybe there are additional genes which, like the abovementioned *tol*-gene, are able to act as switches to stop the interaction as soon the nuclei enter the sexual phase.

In this context, the spore killer genes of *Neurospora* should be mentioned (Raju 1979, 2002; Turner and Perkins 1979). These *sk*-genes are widely distributed in wild-type collections. They cause (albeit only in crosses with sensitive strains), after meiotic segregation, lethality of the four ascospores carrying the genes. This phenomenon does not occur in *sk/sk* matings. This observation also strengthens the idea that there are two different, genetically controlled phases in the sexual cycle of fungi: the bringing together of genetic material via cytoplasmic contact, and the true sexual cycle leading eventually to recombination.

2. The *het*-Genes

   There are some other genes, apart from the mating idiomorphs, which control heterokaryon formation in *Neurospora*. This was earlier postulated by Gross (1952) and Holloway (1955). A detailed analysis of these so-called ***het*-genes** was performed by Garnjobst, Wilson and collaborators (cf. Table 8.1). At present, 11 *het*-genes are known.

   Two unlinked allelic pairs (*C/c* and *D/d*) were identified which control heterokaryon formation according to the allelic mechanism. Heterokaryons are formed only if the two partners have identical alleles at both loci. If one or both factors is heterogenic, then there will be an incompatibility reaction, as in the barrage zone of *Podospora*, leading to a destruction of the hyphae which have anastomosed. The *C* and *D* genes are neither linked with the mating type locus nor suppressed by the *tol*-gene, nor do they interfere with sexual compatibility of different mating types. A third locus (*E/e*) was identified which resembles in its effect the *C* and *D* genes. Subsequently, these genes were given the prefix *het*.

   In analysing different geographical races, Perkins (1968) has detected multiple alleles at the *het-C* locus. The similarity of the action of the *het*-genes to that of the *Podospora* barrage genes is also supported by the observation that a clear barrage zone between the two lines of perithecia may be seen when strains of opposite mating types, but heteroallelic for the *het*-genes, meet (Griffith and Rieck 1981; Perkins 1988). Degradation of nuclear DNA indicating a form of programmed cell death has also been visualized (Marek et al. 2003).

   Another *het*-gene, but one not leading to cell death, was found by Pittenger (cf. Table 8.1). The allelic pair *I/i* controls the capacity of nuclei to divide. Allele *I* is weakly dominant over allele *i*. When the proportion of *I* nuclei in an (*I* + *i*) heterokaryon is more than 30%, the *i* nuclei are lost and the heterokaryon becomes an *I* homokaryon. By use of different marker genes, it became evident that this incompatibility is independent of the genetic background and, hence, from the action of the *C*, *D*, *E het*-genes mentioned above.

   In recent years, more detailed knowledge about the structure and function of the *het*-genes has accumulated. By deletions within *het*-genes, their action is suppressed and compatibility achieved (Smith et al. 1996). Two *het*-loci were studied in more detail.

The ***het-c* gene** encodes a 966 amino acid polypeptide with a putative signal peptide, a coiled-coil motif, and a C-terminal glycin-rich domain, found also in cell wall proteins. Deletions showed that this region is responsible for the activity of the *het-c* gene (Saupe et al. 1996). *het-c* specificities reside in a 38–48 aa domain at the N-terminal end (Saupe and Glass 1997; Wu and Glass 2001). *Het-c* alleles of *N. crassa* function also in *Podospora anserina* in cell death reaction related to heterokaryon incompatibility, whilst the *P. abserina* homolog *Pahch* causes no heterokaryon incompatibility in its host (Saupe 2000)

Three deletion mutants were identified within an open reading frame (named vib = vegetative incompatibility blocked). These mutants relieved growth inhibition and repression of conidiation caused by the *het-c* gene. Thus, it was suggested that the vib region is a regulator for conidiation (Xiang and Glass 2002). Rather often, these suppressor mutants exhibited chromosome rearrangements (Xiang and Glass 2004).

The ***het-6* gene** maps to a region of 250 kbp. Within this region, two genes were identified which show incompatibility activity. One of these shows sequence similarity to the *het-e* product of *Podospora anserina* and the *tol*-gene product. The other encodes the large subunit of the ribonucleotide reductase. Both genes are inherited as a block. Thus, it was suggested that these genes act through a non-allelic mechanism to cause heterogenic incompatibility (Smith et al. 2000; Mir-Rashed et al. 2000).

Regarding the comprehensive new data obtained for *Neurospora*, like in *Podospora*, a breakthrough in understanding the molecular mechanism of the mutual interaction of the *het*-genes requires still further research.

Aspergillus Some *Aspergillus* species were also studied for heterogenic incompatibility. The failure of heterokaryon formation between various natural isolates was already described by Gossop et al. (1940) for *Aspergillus niger* and by Raper and Fennell (1953) for *Aspergillus fonsecaeus* (both imperfect). Comprehensive studies with the perfect (telomorphic) species *Aspergillus nidulans* were performed by Jinks and his co-workers (cf. Table 8.1). A synopsis of these observations and experiments, including numerous related and unrelated isolates from all over the world, allows the following conclusions.

1. Heterokaryon incompatibility is not due to geographical isolation, since the various vegetative compatibility (v-c) groups comprise isolates from adjacent as well as from distant areas. Nor is it linked with minor morphological differences of the isolates.
2. Vegetative incompatibility does not prevent heterokaryon formation. Heterokaryons seem to have selective disadvantage and are supposed to be overgrown by the homokaryons.
3. Fruit body formation for telomorphic species is not inhibited. However, the number of fruit bodies is reduced, as observed also in *Podospora* (Fig. 8.2).
4. Eight *het*-loci were identified, two of which are multiallelic; *het-B* has four and *het-C* has three alleles. The interaction of the *het*-genes follows the allelic mechanism, as described above for *Podospora* and *Neurospora*.
5. The physiological action of these genes is not yet understood. A "killing reaction" like the one in *Podospora* and *Neurospora* seems not to take place.

Comparable results were obtained with two other telomorphic species, *A. glaucus* (Jones 1965) and *A. heterothallicus* (Kwon and Raper 1967).

Studies with some anamorphic species (*A. versicolor*, *A. terreus*, *A. amstelodami*) performed by Caten (cf. Table 8.1) in general confirmed the observations made on the telomorphic species, although without having the opportunity to identify *het*-genes.

A comprehensive study of the species *Aspergillus flavus*, *A. parasiticus* and *A. tamarii* revealed an association of morphology and mycotoxin production with the various i-c groups within each species (Horn et al. 1996).

Ascobolus In *Ascobolus immersus* (cf. Table 8.1) the mating pattern of 38 strains collected at various places in Europe and southern India has been determined. There were at least three compatibility groups: A (23 strains) and B (nine strains) comprise the European isolates, and C, the Indian isolates. Within each group sexual reproduction is, as expected, controlled by a bipolar mechanism of homogenic incompatibility. No fertile offspring are obtained in any intergroup crossing, showing that there is genetic separation by heterogenic incompatibility. However, the European group B seems to be more closely related to the Indian group (C) in that sterile fruit bodies are produced between + and – mating types. An indication for further subdivision is the occurrence of barrages between representatives of all three groups. These data thus

indicate how speciation may be initiated in *Ascobolus immersus* by means of both spatial and genetic isolation, the latter mediated by heterogenic incompatibility.

b) Parasitic Ascomycetes

For a number of plant pathogenic fungi, heterokaryon tests between different isolates led to barrage or to border line formation, and to the classification of so-called **vegetative compatibility groups (v-c)**. Partially due to the difficulty of breeding pathogens under laboratory conditions, genetic data are often not available (see Table 8.1). However, in some of the recently published papers, biochemical techniques such as RAPD analysis were used to characterise the v-c groups, e.g. Punja and Sun (2002).

More comprehensive data are available for ***Botrytis cinerea***. It was found that heterokaryon incompatibility in this fungus is caused by the gene Bc-*hch* which is homolog to Nc-*het-c* and the Pa-*hch* loci of *Neurospora crassa* and *Podospora anserina* respectively. A PCR-RFLP analysis on a 1171-bp section was used to screen for polymorphism for this locus among 117 wild isolates and revealed two allelic types, thus allowing scientists to structure the natural populations into two groups.

For some other parasites, more detailed studies are available. In the case of the chestnut blight, ***Cryphonectria*** (*Endothia*) ***parasitica***, different v-c groups characterised by barrage formation of varied intensity were detected. Weak barrages did not inhibit heterokaryon formation. Over 75 v-c groups were identified, controlled by at least seven incompatibility loci, some with multiple alleles. The heterogenic incompatibility followed mostly an allelic mechanism, but a non-allelic mechanism was also observed. Sexual compatibility was not affected by these genes.

According to Nuss and Koltin (1990), hypovirulence is related to the presence of a virus-like double-stranded RNA which can be transmitted via heterokaryosis. The efficiency of the transfer is highly reduced between incompatible strains, and leads therefore to a lack of horizontal transfer of this parasite and contributes to its biocontrol (Milgroom and Cortesi 2004).

In the ***Gibberella fujikuroi*** species complex, in addition to the mating type genes (+/−), mating groups termed A, B, C and D are recognised. They are considered varieties according to their host specificity. Within each group, heterogenic incompatibility was found. This allelic mechanism is controlled by at least 10 loci in group A, five loci in group B, and three loci each in both groups C and D.

Within parasitic ascomycetes, there are only two indications for **heterogenic incompatibility which affect the sexual phase.** Both are not linked with heterokaryon incompatibility. The genus *Cochliobolus* includes plant parasites causing leaf and inflorescence diseases in Gramineae (anamorphic: *Helminthosporium*). Nelson and collaborators have studied extensively the mating system within this genus. A detailed analysis of the mating reactions of nearly 10,000 isolates from North and South America, comprising more than 40,000 matings, has led to the following results.

1. The bipolar mechanism of homogenic incompatibility is responsible for the basic control of mating; insofar as only the combination of the alleles *A* and *a* leads to fructification.
2. Fertility in crosses between opposite mating types originating from different hosts or origin is about 29%. Most of the infertile crosses produce perithecia with immature or sterile ascospores. The others show no fruit body formation.
3. Several sterility genes blocking the normal ontogenesis at different stages were identified and were predominantly responsible for the formation of sterile perithecia.
4. The fact that some strains exhibiting incompatibility in certain combinations were compatible in all others can be explained only by the action of heterogenic incompatibility, despite the fact that the appropriate genes have not yet been identified.
5. The objection that the incompatibility might be provoked by gross genetic diversities or species differences could be excluded. Furthermore, Nelson was able to assign 92.2% of his strains to five distinct morphological types which might correspond to a single species.

2. Basidiomycotina

The first phenomena which may be attributed to heterogenic incompatibility came from this group of fungi.

Apart from the description of barrage phenomena between geographical races of *Fomes* species (Mounce 1929; Mounce and Macrae 1938), Bauch (1927) found in the smut fungus, *Microbotryum violaceum* (*Ustilago violacea*), that in inter-racial crosses additional genes interfere with the bipolar

mating system and cause unilateral or reciprocal incompatibility. Similar findings were later reported by Grasso (1955) who studied inter-racial crosses in two other species, *U. avenae* and *U. levis*, originating from Italy and the United States respectively.

Many phenomena resulting in heterokaryon incompatibility or inhibition of fruit body formation were poorly understood in older publications. They were mostly referred to as demarcation lines, barrages and/or crossing barriers. Thus, it is understandable that in a very comprehensive review (Burnett 1965), the mating restrictions in 17 species of basidiomycetes were treated only under the general heading "restrictions of outbreeding".

In this review, I distinguish between those cases in which the existence of heterogenic incompatibility is proved and supported by genetic data, and cases in which antagonistic mycelial interactions may only be interpreted as the expression of heterogenic incompatibility. Only the better-analysed cases will be presented in detail here (for others, see Table 8.1).

In this context, it needs to be stressed that, in contrast to ascomycetes where heterogenic incompatibility may concern either the vegetative and/or the sexual phase, in basidiomycetes it is instrumental only in the vegetative phase, because basidiomycetes do not form sex organs. From this it follows that, if a heterokaryon incompatibility occurs, then the sexual propagation is automatically inhibited.

An impetus to study heterogenic incompatibility stems from the interest in the population structure of saprophytic basidiomycetes, involving matings between natural isolates in order to obtain information for classification of genera and species (cf. Boidin 1986). These studies have not only revealed basic mating systems but also indicate a variety of antagonistic mycelial interactions correlated with heterokaryon and/or sexual incompatibility.

However, during the last years the interest in heterogenic incompatibility of basidiomycetes seems to have decreased, because there have been fewer publications describing this phenomenon. Instead, many comprehensive studies were published in establishing intra- or interspecific relationships by using molecular techniques. Thus, there is not much progress in understanding the genetic and physiological control of heterogenic incompatibility within this group of fungi.

In **wood-rotting fungi** the antagonistic interaction is easily recognised in cross sections from logs, as a narrow zone of interwoven hyphae in a region of relatively undecayed wood. These **border lines**, also called interaction zones or demarcation lines, are usually darkly pigmented, in contrast to the adjacent decay zones (Fig. 8.1E). They also show up on agar-grown cultures, depending on the composition of the medium. Without microscopic examination it is not possible to say whether hyphal interactions inhibiting heterokaryon formation take place, as is evident in the barrage zone of *Podospora*, or simply antagonistic repulsions occur. Thus, a distinction between delimitation of species or races is a priori not possible. In the literature, the terms **biological races** and/or **intersterility groups (i-c)** are often used to characterise the interacting mycelia.

In analogy to the evaluation of data concerning the ascomycetes, I shall start the discussion of the basidiomycetes with a subject for which information on both heterogenic incompatibility and genetic control of speciation has been obtained. This is the wood-rotting fungus *Polyporus*.

Macrae (1967) studied the mating reactions of 31 single spore isolates of the tetrapolar *Polyporus abietinus* (syn. *Hirschioporus abietinus*) collected in different places in North America and Europe. According to differences in the morphology of their hymenial surfaces, the isolates were assigned to three morphological groups. The North American strains could be subdivided into the two classes A and B which are incompatible with each other, but which are both compatible with a third class C comprising the European strains. Since geographical isolation could be excluded, Macrae concluded that genes additional to the mating type factors were involved. Similar data were also reported for *P. schweinitzii* (Barrett and Uscuplic 1971). Unfortunately, in both cases no genetic data are available.

Comparable phenomena were found and could be interpreted after comprehensive studies of other species of the genus (Hoffmann and Esser 1978). We had chosen the wood-rotting genus *Polyporus* in order to investigate, by genetic parameters, the validity of the classical species concept based on typological characters. In performing these studies, we "accidentally" detected evidence for heterogenic incompatibility.

As a result of matings of single spore-derived mycelia from 26 races of different origin, all races could unequivocally be grouped into three separate entities corresponding with the typological species *P. arcularius*, *P. brumalis* and *P. ciliatus*, on the basis of the following results (Fig. 8.4).

1. As expected, the basic breeding system in *Polyporus* is the tetrapolar mechanism of homogenic incompati-

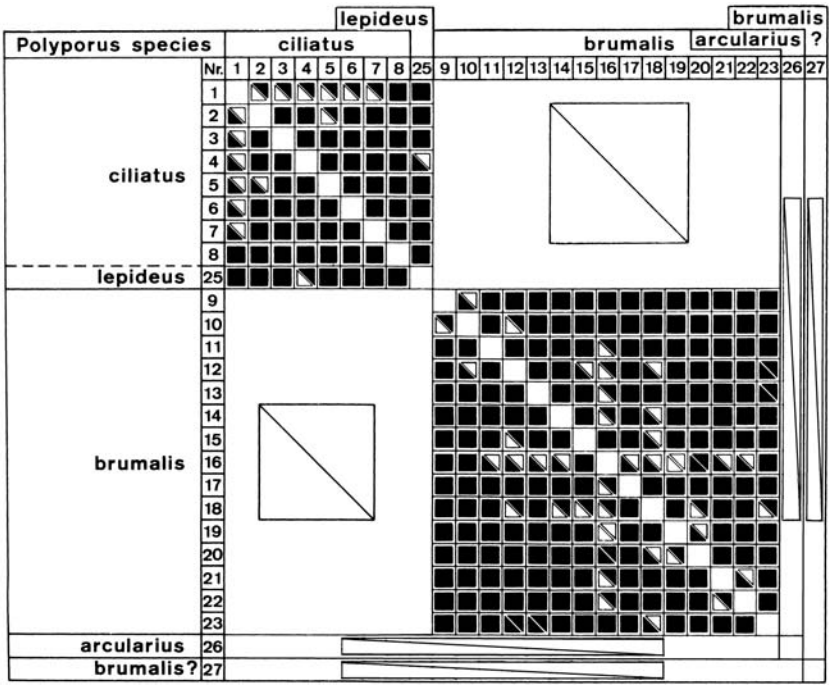

Fig. 8.4. Mating relations in intra- and interspecies combinations of monokaryons from 26 races of different species of *Polyporus*. ■ Normal contact, clamp connections and fruit bodies formed when $A \neq B \neq$ ◥; barrage formation, fruit body production delayed; ◥ barrage formation with unilateral nuclear migration (only in *dark part*); ◹ border line, neither clamp connections nor dikaryotic fruit bodies formed in any combination. (Adapted from Esser and Hoffmann 1977)

bility controlled by multiple alleles of the mating type factors *A* and *B*.

2. All intraspecific combinations were fertile. A conspicuous barrage formed in those crosses where dikaryotization and fruiting were impaired. This barrage is characterised by a clear zone, about 1–2 mm wide, free of aerial hyphae, and of reduced hyphal density in the medium (Fig. 8.1D).
3. Using two races of *P. ciliatus* as an example, it was revealed that barrage formation is induced by the specific interaction of three unlinked genes (b^+/b^- = barrage initiation, bfI_1/bfI_2 and $bfII_1/bfII_2$ = barrage formation) in a way characteristic for systems of heterogenic incompatibility. Barrage formation requires the presence of the allele bi^+ in at least one mating partner, in addition to heterogeneity of both *bf*-genes.
4. Interspecific combinations were sterile. There is no hyphal fusion between mating partners and, because of the mutual repulsion, a sharp border line is formed in the area of contact. Its formation is independent of both mating type and the nuclear status (monokaryons or dikaryons) of the confronted mycelia (see also Silveira and Wright 2002).

From the experimental data, the following conclusions may be drawn:

1. The analysis of intraspecific matings has shown that, within each species, so-called biological races (intersterility groups, i-s) exist. They are delineated by barrage formation, which, as deduced from the genetic data, is an unequivocal example of heterogenic incompatibility. The unilateral inhibition of fruiting, not caused by the mating type factors, can also be considered as an expression of heterogenic incompatibility, although genetic data for this are not yet available.
2. The analysis of interspecific matings, all characterised by a strong macroscopic border line (Fig. 8.1D), is in good agreement with the species limits derived from morphological data. This indicates the validity of both the typological and the biological species concept. The latter, however, proved superior in compensating the variability of morphological characters, at least in higher fungi.

The **biological species concept** can thus be modified as follows: **populations (races) belong to different species if the failure to interbreed and to produce viable offspring is caused by genetic mechanisms other than those operating upon completion of the sexual cycle**.

There are some more examples where genetic control of heterogenic incompatibility is available.

In *Sistotrema brinkmannii* (syn. *Corticum coronilla*), strains obtained from different geographical locations exhibit three types of basic sexual control:

1. Homokaryotic fruiting, i.e. homokaryons produce dikaryons and fruit bodies with viable spores.
2. Homogenic incompatibility determined by the bipolar mechanism, i.e. one mating type locus with multiple alleles.
3. Homogenic incompatibility determined by the tetrapolar mechanism, i.e. two incompatibility factors, each with multiple alleles (Biggs 1937).

Lemke (1969) has confirmed and extended the work of Biggs by analysing the interstrain relations of 11 isolates from different parts of the world. In using the technique of forced heterokaryons between auxotrophs, he found that there are fertility barriers within each of the three abovementioned fruiting classes as well as in interclass crosses. This phenomenon was interpreted by Lemke as heterogenic incompatibility for two reasons:

1. In incompatible inter-racial matings, the two auxotrophic partners form unbalanced mycelia with poor vegetative vigour and no clamp connections. This points to an antagonistic reaction similar to that in *Podospora* heterokaryons.
2. In one compatible inter-racial mating, recombinant types were obtained with an altered incompatibility pattern. This excluded the presence of sterility genes, which were sometimes found in other combinations.

In the oyster mushroom, *Pleurotus ostreatus*, 60 heterokaryons of a natural population were examined by pair-wise matings for mycelial antagonisms (Kay and Vilgalys 1992). Most pairings (93%–100%) between sib composed heterokaryons gave somatic incompatibility responses, showing that most isolates represent discrete individuals, with as many as 15 individuals occupying a single log. A total of 53 somatically distinct individuals were identified from the population, distributed among 21 logs. Test with homokaryons showed that genetic elements not identical with the incompatibility factors of the tetrapolar system are responsible for the formation of intersterility groups.

In *Stereum hirsutum*, mating is controlled by a bipolar mechanism of homogenic incompatibility (C-factor with multiple alleles). In analysing mating homokaryons from different geographical isolates, a mycelial aversion (bow-tie reaction) was observed. This involved the formation of a migrating or stationary band of suppressed mycelium. This was followed by partial or complete replacement of one homokaryon by another. This reaction is brought about by a heterozygosity at a single locus (B-factor), which is not linked with the mating type locus. The fact that the heterogenic nuclei reject each other, reminiscent of the barrage formation of *Podospora*, is a further example for heterogenic incompatibility as an isolation mechanism within a single species. In four other species of *Stereum*, as in *Polyporus*, all interspecific matings were sterile and delineated by strong border lines.

In the litter-decomposing bipolar *Collybia dryophila*, collected from different continents, several intersterility groups (i-s) were identified, three of which are distributed over two or more continents. In some matings within one i-c, reduced sexual compatibility was found. Genetic diversity of some strains was proved by DNA-DNA hybridisation.

In the bipolar *Heterobasidion annosum* are at least three intersterility groups, P and S (from pine and spruce) and F (from firs), the representatives of which in general show no compatibility of different mating types. However, there are exceptions, because there is a significant degree of fertility in i-s matings. Five loci were identified controlling this system, superimposed upon the mating types alleles. In contrast to the results obtained with *Podospora*, for instance, the heterogenic incompatibility between the i-s strains requires a heterogeneity of all five loci. A homogeneity at only one locus acts epistatically and suppresses the mating barrier. This does not exclude that under "fully" heterogenic conditions, inter-racial incompatibility is present. Comparable data for the *Heterobasidion insulare* complex were reported by Dai et al. (2002).

Hansen et al. (1993a,b) published data which lead to a contradictory interpretation. They suggested that mating between the incompatibility groups is controlled at 3–4 multiallelic loci. Each genotype acts independently in causing vegetative incompatibility. Thus, in accordance with the *Podospora* system, a single genetic difference would be sufficient to cause heterogenic incompatibility.

Perhaps the **control of heterogenic incompatibility is not restricted to nuclear genes**. In the tetrapolar *Coprinus cinereus* (*Copriniopsis cinerea*), barrage formation was observed in matings between heterokaryons from different geographical locations having different mitochondrial genomes but common nuclear genomes (May 1988). Unfortunately, no further details of this novel interaction were given.

Yet another example should be mentioned, which is caused by an interaction of nuclear genes and cytoplasmic genetic elements. In the tetrapolar *Ustilago maydis*, an antagonism between genetically different strains, which does not

depend on the mating type genes, was observed which is similar to the killer phenomenon of yeast (cf. Stark et al. 1990). There are three genotypes:

1. Antagonistic strains, producing a heat-labile protein which inhibits the growth of sensitive strains but does not interfere with growth of the producing strain. Genetic configurations: a nuclear gene with the alleles *s* or s^+, and cytoplasmic elements *I* and *S*. The s^+ allele confers insensitivity, the *s* allele sensitivity which is suppressed by the cytoplasmic element *S*; the element *I* is responsible for the production of the killer substance.
2. Sensitive strains, which do not produce inhibitor protein but are sensitive to it. Genetic configuration: gene *s*, but no cytoplasmic element *O*.
3. Neutral strains, which do not produce inhibitor protein and are insensitive to it. Genetic configuration: s^+ *O*, *s S* or s^+ *S*.

There are phenotypic differences to the killer system in yeasts, since only growth inhibition of the sensitive cells occurs with no cell death, and there is no interference with the fusion of different mating types; hence, sexual propagation is not prevented. Thus, this phenomenon reveals similarity to heterogenic vegetative incompatibility in *Neurospora* and *Podospora*. Moreover, in the yeast killer system the genetic determinate for killer protein is a viral-related double-stranded RNA or DNA (cf. Tipper and Bostian 1984, and Stark et al. 1990 respectively).

Conclusion: The evaluation of the experimental data obtained with fungi with respect to the occurrence, distribution and mechanisms of heterogenic incompatibility allows one to make the following statements:

1. The existence of heterogenic incompatibility is unequivocally proved among Dikaryomycota. Although *het*-genes were identified for a number of species, its genetic control certainly needs more experimental investigation.
2. This holds even more true for an understanding of the expression and functions of the *het*-genes.
3. The existence of many v-c and i-s groups in ascomycetes and basidiomycetes respectively shows the necessity to support taxonomic classification for speciation by means of comprehensive genetic data, and not just morphological criteria.

V. Correlations with Heterogenic Incompatibility in Plants and Animals, with DNA Restriction in Bacteria and with Histo-Incompatibility

As reviewed earlier (Esser and Blaich 1973), **in plants** there are also many examples for the existence of heterogenic incompatibility, manifesting as either unilateral or bilateral failures of matings between individuals of different isolates or races. Sometimes the genes responsible for homogenic incompatibility are involved, but mostly the action of other genes is superimposed. In addition, extrachromosomal genetic elements such as plastid-derived DNA have been found as determinative agents (de Nettancourt 1977; Barrett 1992). In plants, according to my knowledge, vegetative incompatibility has not been described.

In comparison to the predominantly hermaphroditic plants, sexual incompatibility of the homogenic type does not play a role in **animal breeding systems**. Increasing in outbreeding is in general achieved in animals by dioecism. There are some examples known in which karyogamy between female and male nuclei is prevented by genetic differences not identical with sex factors (cf. Esser and Blaich 1973).

The spectrum of heterogenic incompatibility comprises not only eukaryotes but also **prokaryotes**. The destruction of bacterial DNA by endonucleases, when brought into a genetically different host and as a defence mechanism to escape phage infection, is also a manifestation of this phenomenon.

Heterogenic incompatibility is not restricted to cell fusion and subsequent nuclear migration, because there is also a close correlation between heterogenic incompatibility and **histo-incompatibility**, occurring after tissue transplantation. In the latter case, however, a complicated immune-response mechanism is involved. It seems justifiable to conclude that both heterogenic incompatibility and histo-incompatibility, which seem to have convergently developed during evolution, exhibit one and the same effect, i.e. inability of genetically different material to coexist or tolerate a common physiological machinery, and simply represent different mechanisms of a fundamental biological process.

VI. Conclusions

As shown by this survey of the literature, most cases of heterogenic incompatibility can be supported by genetic data. A number of important special cases are known under different names. In other cases, however, effects indicative of heterogenic incompatibility have been attributed to other causes or relegated by investigators as inexplicable secondary effects. In any case, **heterogenic incompatibility** must be regarded as a **basic biological phenomenon** controlling the coexistence of different genetic determinants, whose impact may be summarized as follows.

1. Occurrence

Heterogenic incompatibility is widespread in both prokaryotes and eukaryotes. Special cases such as DNA restriction and histo-incompatibility may be considered different expressions of one basic biological phenomenon.

2. Nature of Genetic Determinants

The widespread occurrence is also indicative of the general importance of heterogenic incompatibility, with a varied genetic basis ranging from single to multiple nuclear genes and even to extranuclear genetic elements.

3. Biochemical Basis

There are not yet sufficient biochemical data regarding the action of the nuclear genes which bring about heterogenic incompatibility in fungi. By contrast, the molecular mechanisms of heterogenic incompatibility provoked by extranuclear genetic traits, such as bacterial DNA restriction, are well known. Evidently, many of the genetic mechanisms leading to heterogenic incompatibility have developed independently, and this may be reflected by a variety of mechanisms at the molecular level.

4. Biological Impact

The effect of heterogenic incompatibility is threefold:

1. As stated in the Introduction, heterogenic incompatibility has to be considered a **breeding system** which, in contrast to homogenic incompatibility, **favours inbreeding** by restricting the exchange of genetic material. Since there is no fundamental difference between recombinational events in the sexual and the parasexual cycle, it is not surprising that heterogenic incompatibility influences both. This also applies to the initiation of plasmogamy, which may occur either by sexual processes or simply through heterokaryosis. This mode of isolating strains or races leads to further speciation. Thus, heterogenic incompatibility must have been, and still is one of the **basic genetic events acting in evolution**.
2. Genetic isolation has a second effect which should not be overlooked. The suppression of cell fusion **stops the transfer of harmful cytoplasmic components**, such as mutated mitochondria, viruses or plasmids, between individuals and thereby inhibits the spread of cell diseases and favours the survival of uninfected cells or tissues. This is particularly important for organisms without strict cellular compartmentation, such as the majority of fungi.
3. **Consequences for taxonomy**: In many studies of natural isolates of fungi, the formation of antagonistic zones of mycelial aversion, such as barrages and/or border lines, are used by taxonomists as criteria for speciation. Although being a valuable tool, this parameter as a taxonomic criterion should be judged with great care to avoid creating taxonomic distinctions which are not valid and which could depend on only a single gene difference. This especially concerns the term "biological species", often used without any genetic basis. It is better to use the terms "race" or "geographical isolate", without a detailed evaluation of breeding patterns.

5. Practical Implications

During the last decades, concerted breeding for biotechnologically relevant fungi has gained more and more importance (Esser 1985; Esser and Mohr 1990). Breeding techniques employing new isolates from nature in order to exploit varied genetic backgrounds require a profound knowledge of the breeding systems involved. The existence of heterogenic incompatibility could be a serious handicap for any genetic exchange via either sexual or parasexual matings. Detailed experimental work would allow one in most cases to reach the desired goal, if based on alternative genetic manipulations such as DNA-mediated transformation.

6. Relation with Histo-Incompatibility and DNA Restriction

Both of these phenomena have the same effect: hostile interaction of different genetic material originating from closely related organisms. This brings up the question: will it be possible in the future, based on further experimental work, to interrelate these events and the many manifestations of heterogenic incompatibility in considering the diversity of the genetic mechanisms promulgating the **failure of coexistence of genetically different material?**

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References

Adams DH, Roth LF (1967) Demarcation lines in paired cultures of *Fomes cajanderi* as a basis for detecting genetically distinct mycelia. Can J Bot 45:1583–1589

Anagnostakis SL (1977) Vegetative incompatibility in *Endothia parasitica*. Exp Mycol 1:306–316

Anagnostakis SL (1982a) The origin of ascogenous nuclei in *Endothia parasitica*. Genetics 100:413–416

Anagnostakis SL (1982b) Genetic analyses of *Endothia parasitica*: linkage data for four single genes and three vegetative compatibility types. Genetics 102:25–28

Anagnostakis SL (1983) Conversion to curative morphology in *Endothia parasitica* and its restriction by vegetative compatibility. Mycologia 75:777–780

Anderson JB (1986) Biological species of *Armillaria* in North America: redesignation of groups IV and VIII and enumeration of voucher strains for other groups. Mycologia 78:837–839

Anderson JB, Ullrich RC (1979) Biological species of *Armillaria mellea* in North America. Mycologia 71:402–414

Anderson JB, Korhonen K, Ullrich RC (1980) Relationship between European and North American biological species of *Armillaria mellea*. Exp Mycol 4:87–95

Anderson JB, Petsche DM, Herr FB, Horgien PA (1984) Breeding relationship among several species of *Agaricus*. Can J Bot 62:1884–1889

Balguerie A, Reis SD, Ritter C, Chaignepain S, Coulary-Salin B, Forge V, Bathany K, Lascu I, Schmitter JM, Riek R et al. (2003) Domain organization and structure-function relationship of the *HET-s* prion of *Podospora anserina*. EMBO J 22:2071–2081

Balguerie A, Reis SD, Coulary-Salin B, Chaignepain S, Sabourain M, Schmitter JM, Saupe SJ (2004) The sequences appended to the amyloid core region of the *HET-s* prion protein determine higher-order aggregate organization in vivo. J Cell Sci 117:2599–2610

Barrett SCH (ed) (1992) Evolution and function of heterostyly. Monographs on Theoretical and Applied Genetics, vol 15. Springer, Berlin Heidelberg New York

Barrett DK, Uscuplic M (1971) The field distribution of interacting strains of *Polyporus schweinitzii* and their origin. New Phytol 70:581–598

Bauch R (1927) Rassenunterschiede und sekundäre Geschlechtsmerkmale beim Antherenbrand. Biol Zentralbl 47:370–383

Beadle GW, Coonradt VL (1944) Heterocaryosis in *Neurospora crassa*. Genetics 29:291–308

Bégueret J, Turc B, Clavé C (1994) Vegetative incompatibility in filamentous fungi – *het* genes begin to talk. Trends Genet 10:441–446

Bernet J (1965) Mode d'action des gènes de barrage et la relation entre l'incompatibilité cellulaire et l'incompatibilité sexuelle chez *Podospora anserina*. Ann Sci Nat Bot Biol Veg 6:611–768

Bernet J (1967) Les systèmes d'incompatibilité chez le *Podospora anserina*. C R Acad Sci Paris Ser D 256:1330–1333

Bernet J (1991) Aerial organs and cell death in *Podospora anserina* mutants: relationship with protoplasmic incompatibility. Exp Mycol 15:215–222

Bernet J (1992a) A gene suppressing the allelic protoplasmic incompatibility specified by genes at five different loci in *Podospora anserina*. J Gen Microbiol 138:2567–2574

Bernet J (1992b) In *Podospora anserina*, protoplasmic incompatibility genes are involved in cell death control via multiple gene interactions. Heredity 68:79–87

Betterley DA, Collins OR (1984) Vegetative incompatibility and myxomycete biology. Mycologia 76:785–792

Biggs R (1937) The species concept in *Corticium coronilla*. Mycologia 29:686–706

Blaich R, Esser K (1970) The incompatibility relationships between geographical races of *Podospora anserina*. IV. Biochemical aspects of the heterogenic incompatibility. Mol Gen Genet 109:186–192

Blaich R, Esser K (1971) The incompatibility relationships between geographical races of *Podospora anserina*. V. Biochemical characterization of heterogenic incompatibility on cellular level. Mol Gen Genet 111:265–272

Boccas BR (1981) Interspecific crosses between closely related heterothallic *Phytophthora* species. Phytopathology 71:60–65

Boddy L, Rayner ADM (1982) Population structure, intermycelial interactions and infection biology of *Stereum gausapatum*. Trans Br Mycol Soc 78:337–351

Boddy L, Rayner ADM (1983) Mycelial interactions, morphogenesis and ecology of *Phlebia radiata* and *P. rufa* from oak. Trans Br Mycol Soc 80:437–448

Boidin J (1986) Intercompatibility and the species concept in the saprobic basidiomycotina. Mycotaxon 76:319–336

Boucherie H, Bernet J (1978) Protoplasmic incompatibility and self-lysis in *Podospora anserina*: enzyme activities associated with cell destruction. Can J Bot 56:2171–2176

Boucherie H, Dupont CH, Bernet J (1981) Polypeptide synthesis during protoplasmic incompatibility in the fungus *Podospora anserina*. Biochim Biophys Acta 653:18–26

Bourges N, Groppi A, Barreau C, Clavé C, Bégueret J (1998) Regulation of gene the expression during the vegetative incompatibility reaction in *Podospora anserina*: characterization of three induced genes. Genetics 150:633–641

Brasier CM (1984) Inter-mycelial recognition systems in *Ceratocystis ulmi*: their physiological properties and ecological importance. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 451–498

Brodie HJ (1970) Sexuality patterns in some new and little-known species of *Cyathus*. Sver Bot Tidskr 64:44–50
Bruehl GW, Machtmes R, Kiyomoto R (1975) Taxonomic relationships among *Typhula* species as revealed by mating experiments. Phytopathology 65:1108–1114
Burnett JH (1965) The natural history of recombination systems. In: Esser K, Raper JR (eds) Incompatibility in fungi. Springer, Berlin Heidelberg New York, pp 98–113
Butcher AC (1968) The relationship between sexual outcrossing and heterokaryon incompatibility in *Aspergillus nidulans*. Heredity 23:443–452
Butcher AC (1969) Non-allelic interactions and genetic isolation in wild populations of *Aspergillus nidulans*. Heredity 24:621–631
Butcher AC, Croft J, Grindle M (1972) Use of genotype-environmental interaction analysis in the study of natural populations of *Aspergillus nidulans*. Heredity 29:263–283
Carlile MJ, Gooday GW (1978) Cell fusion in myxomycetes and fungi. In: Poste G, Nicholson GL (eds) Membrane fusion. Elsevier, Amsterdam, pp 219–265
Caten CE, Butcher AC, Croft JH (1971) Genetic control of heterokaryon formation in *Aspergillus*. Aspergillus Newslett 12:1–2
Cayley DM (1923) The phenomenon of mutual aversion between mono-spore mycelia of the same fungus (*Diaporthe perniciosa* Marchal) with a discussion of sex heterothallism in fungi. J Genet 13:353–370
Cayley DM (1931) The inheritance of the capacity for showing mutual aversion between monospore mycelia of *Diaporthe perniciosa*. J Genet 24:1–63
Chaisrisook C (2002) Mycelial reactions and mycelial compatibility groups of red rice mould (*Monascus purpureus*). Mycol Res 106:298–304
Chase TE, Ullrich RC (1990a) Genetic basis of biological species in *Heterobasidion annosum*: Mendelian determinants. Mycologia 82:67–72
Chase TE, Ullrich RC (1990b) Five genes determining intersterility in *Heterobasidion annosum*. Mycologia 82:73–81
Clark J (1984) Three-clone *Didymium iridis* crosses and plasmodial incompatibility phenotype. Mycologia 76:810–815
Clark J (2003) Plasmodial incompatibility in the myxomycete *Didymium squamulosum*. Mycologia 95:24–26
Coates D, Rayner ADM (1985a) Genetic control and variation in expression of the "bow-tie" reaction between homokaryons of *Stereum hirsutum*. Trans Br Mycol Soc 84:191–205
Coates D, Rayner ADM (1985b) Heterokaryon-homokaryon interactions in *Stereum hirsutum*. Trans Br Mycol Soc 84:637–645
Coates D, Rayner ADM (1985c) Evidence for a cytoplasmically transmissible factor affecting recognition and somato-sexual differentiation in the basidiomycete *Stereum hirsutum*. J Gen Microbiol 131:207–219
Coates D, Rayner ADM, Todd NK (1981) Mating behaviour, mycelial antagonism and the establishment of individuals in *Stereum hirsutum*. Trans Br Mycol Soc 76:41–51
Coates D, Rayner ADM, Boddy L (1985) Interactions between mating and somatic incompatibility in the basidiomycete *Stereum hirsutum*. New Phytol 99:473–483
Correl JC, Puhalla JE, Schneider RW (1986) Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396–400
Cortesi P, Milgroom MG (1998) Genetics of vegetative incompatibility in *Cryphonectria parasitica*. Appl Environ Microbiol 64:2988–2994
Cousteau V, Delau C, Saupe S, Bégueret J (1997) The protein product of the het-s heterokaryon incompatibility gene of the fungus *Podospora anserine* behaves as a prion analog. Proc Natl Acad Sci 94:9773–9778
Croft JH, Dales RBG (1984) Mycelial interactions and mitochondrial inheritance in *Aspergillus*. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 433–450
Cubeta MA, Briones-Ortega R, Vigalys R (1993) Reassessment of heterokaryon formation in *Rhizoctonia solani* anastomosis group 4. Mycologia 85:777–787
Dai YC, Vainio EJ, Hantula J, Niemelä T, Korhonen K (2002) Sexuality and intersterility within the *Heterobasidion insulare* complex. Mycol Res 106:1435–1448
Dales RBG, Croft JH (1977) Protoplast fusion and the isolation of heterokaryons and diploids from vegetatively incompatible strains of *Aspergillus nidulans*. FEMS Microbiol Lett 1:201–203
Debets F, Yang X, Griffiths AJF (1994) Vegetative incompatibility in *Neurospora*: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural population. Curr Genet 26:113–119
Debuchy R, Coppin E (1992) The mating types of *Podospora anserina*: functional analysis and sequence of the fertilization domains. Mol Gen Genet 233:113–121
Deleu C, Clavé C, Bégueret J (1993) A single amino acid difference is sufficient to elicit vegetative incompatibility in the fungus *Podospora anserina*. Genetics 135:45–52
Dementhon K, Paoletti M, Pinan-Lucarré B, Loubradou-Bourges N, Sabourin M, Saupe SJ, Clavé C (2003) Rapamycin mimics the incompatibility reaction in the fungus *Podospora anserina*. Eukaryot Cell 2:238–246
Dementhon K, Saupe SJ, Clavé C (2004) Characterization of *idi-4*, a bZIP transcription factor inducing autophagy and cell death in the fungus *Podospora anserina*. Mol Microbiol 53:1625–1640
De Nettancourt D (1977) Incompatibility in Angiosperms. Monographs on Theoretical and Applied Genetics 3. Springer, Berlin Heidelberg New York
East EM (1940) The distribution of self-sterility in flowering plants. Proc Am Philos Soc 82:449
Ennos RA, Swales KW (1987) Estimation of the mating system in a fungal pathogen *Crumenulopsis sororia* (Karst.) groves using isozyme markers. Hereditas 59:423–430
Espagne E, Balhadère P, Penin ML, Barreau C, Turcq B (2002) HET-E and HET-D belong to a new subfamily of WD40 proteins involved in vegetative incompatibility specificity in the fungus *Podospora anserina*. Genetics 161:71.81
Esser K (1954) Sur le déterminisme génétique d'un nouveau type d'incompatibilité chez *Podospora*. C R Acad Sci Paris 238:1731–1733
Esser K (1956) Die Incompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (CES) REHM I. Genetische Analyse der

Semi-Incompatibilität. Z Indukt Abstammungs Vererbungslehre 87:595–624
Esser K (1959a) Die Incompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (CES) REHM II. Die Wirkungsweise der Semi-Incompatibilitäts-Gene. Z Vererbungslehre 90:29–52
Esser K (1959b) Die Incompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (CES) REHM III. Untersuchungen zur Genphysiologie der Barragebildung und der Semi-Incompatibilität. Z Vererbungslehre 90:445–456
Esser K (1961) Incompatibilität bei Pilzen. Ber Dt Bot Ges 74:324–325
Esser K (1962) Die Genetik der sexuellen Fortpflanzung bei Pilzen. Biol Zbl 81:161–172
Esser K (1971) Breeding systems in fungi and their significance for genetic recombination. Mol Gen Genet 110:86–100
Esser K (1973) Breeding systems and recombination in fungi. In: Proc Symp Genetics of Industrial Microorganisms, Prague, 1970. Academia, Prague, pp 73–79
Esser K (1985) Potentials and prospects of genetics in biotechnology. In: Proc 3rd European Congr Biotechnology, München, 1984, vol IV. DECHEMA, Frankfurt/Main, pp 241–256
Esser K, Blaich R (1973) Heterogenic incompatibility in plants and animals. Adv Genet 17:107–152
Esser K, Hoffmann P (1977) Genetic basis for speciation in higher basidiomycetes with special reference to the genus *Polyporus*. In: Clemencon H (ed) The species concept in hymenomycetes. Cramer, Vaduz, pp 189–214
Esser K, Kuenen R (1967) Genetics of fungi. Translated by Erich Steiner. Springer, Berlin Heidelberg New York
Esser K, Meinhardt F (1984) Barrage formation in fungi. Encyclopedia of Plant Physiology, new series, vol 17. Springer, Berlin Heidelberg New York, pp 350–361
Esser K, Mohr G (1990) Stammverbesserung von Hyphenpilzen durch die Gentechnik: Fakten und Perspektiven. Bioengineering 6:44–55
Fischer M (1994) Pairing tests in the *Phellinus pini* group. Mycologia 86:524–539
Fischer M, Bresinsky A (1992) *Phellinus torulosus*: sexuality and evidence of intersterility groups. Mycologia 84:823–833
Ford EJ, Miller RV, Gray, Sherwood JE (1995) Heterokaryon formation and vegetative incompatibility in *Sclerotina sclerotium*. Mycol Res 99:241–247
Fournier E, Levis C, Fortini D, Leroux P, Giraud T, Brygoo Y (2003) Characterization of B*c-hch*, the *Botrytis cinerea* homolog of the *Neurospora crassa het-c* vegetative incompatibility locus, and its use as, a population marker. Mycologia 95:251–261
Fries N (1985) Intersterility groups in *Paxillus involutus*. Mycotaxon 14:403–409
Fries N, Mueller GM (1984) Incompatibility systems, cultural features and species circumscriptions in the ectomycorrhizal genus *Laccaria* (agaricales). Mycologia 76:633–642
Garnjobst L, Wilson JF (1956) Heterokaryosis and protoplasmic incompatibility in *Neurospora crassa*. Proc Natl Acad Sci USA 42:613–618
Giovanetti M, Sbrana C, Strani P, Agnolucci M, Rinaudo V, Avio L (2003) Genetic diversity of isolates of *Glomus mosseae* from different geographic areas detected by vegetative compatibility testing and biochemical molecular analysis. Appl Environ Microbiol 69:616–624
Glass NL, Kaneko I (2003) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 2:1–8
Glass NL, Kuldau GA (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. Annu Rev Phytopathol 30:201–224
Glass NL, Grotelueschen J, Metzenberg RL (1990) *Neurospora crassa* a mating-type region. Proc Natl Acad Sci USA 87:4912–4916
Glass NL, Jacobson DJ, Shiu PKT (2000) The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu Rev Genet 34:165–186
Goldstein D, Gilbertson RL (1981) Cultural morphology and sexuality of *Inonotus arizonicus*. Mycologia 73:167–180
Gordon SA, Petersen RH (1992) Interbreeding populations of some *Marasmius* species. Mycologia 84:204–208
Gossop GH, Yuill E, Yuill JL (1940) Heterogeneous fructifications in species of *Aspergillus*. Trans Br Mycol Soc 24:337–344
Grasso V (1955) Studio sulla genetica dei carboni dell'avena: *Ustilago avenae* e *U. levis*. Boll Staz Patol Veg Roma 12:115–126
Griffith AJF, Rieck A (1981) Perithecial distribution in standard and variant strains of *Neurospora crassa*. Can J Bot 59:2610–2617
Grindle M (1963a) Heterokaryon compatibility of unrelated strains in the *Aspergillus nidulans* group. Heredity 18:191–204
Grindle M (1963b) Heterokaryon compatibility of closely related wild isolates of *Aspergillus nidulans*. Heredity 18:397–405
Gross SR (1952) Heterokaryosis between opposite mating types in *Neurospora crassa*. Am J Bot 39:574–577
Hamann A, Osiewacz HD (2004) Genetic analysis of spore killing in the filamentous ascomycete *Podospora anserina*. Fungal Genet Biol 41:1088–1098
Hankin L, Puhalla JE (1971) Nature of a factor causing interstrain lethality in *Ustilago maydis*. Phytopathology 61:50–53
Hansen EM (1979) Sexual and vegetative incompatibility reactions in *Phellinus weirii*. Can J Bot 57:1573–1578
Hansen EM, Stenlid J, Johansson M (1993a) Somatic incompatibility and nuclear reassortment in *Heterobasidion annosum*. Mycol Res 97:1223–1228
Hansen EM, Stenlid J, Johansson M (1993b) Genetic control of somatic incompatibility in the root-rotting basidiomycete *Heterobasidion annosum*. Mycol Res 97:1229–1233
Hietala AM, Korhonen K, Sen R (2003) An unknown mechanism promotes somatic incompatibility in *Ceratobasidium bicorna*. Mycologia 95:239–250
Hoffmann P, Esser K (1978) Genetics of speciation in the basidiomycetous genus *Polyporus*. Theor Appl Genet 53:273–282
Holloway BW (1955) Genetic control of heterocaryosis in *Neurospora crassa*. Genetics 40:117–129

Horn BW, Greene RL, Sobolov VS, Dorner JW, Powell JH, Layton RC (1996) Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus* and *A. tamarii*. Mycologia 88:574–587

Ikeda K, Nakamura H, Matsumato N (2003) Mycelial incompatibility operative in pairings between single basidiospore isolates of *Helicobasodium mompa*. Mycol Res 107:847–853

Jacobson DJ, Gordon TR (1988) Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. Phytopathology 78:668–672

Jamil N, Buck KW, Carlile MJ (1984) Sequence relationships between virus double-stranded RNA from isolates of *Gaeumannomyces graminis* in different vegetative compatibility groups. J Gen Virol 65:1741–1747

Jennings DH, Rayner ADM (eds) (1984) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge

Jinks JL, Caten CE, Simchen G, Croft JH (1966) Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans*. Heredity 21:227–239

Jones DA (1965) Heterokaryon compatibility in the *Aspergillus glaucus* link group. Heredity 20:49–56

Katan T, Katan J (1988) Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78:852–855

Kay E, Vilgalys R (1992) Spatial distribution and genetic relationships among individuals in a natural population of the oyster mushroom *Pleurotus ostreatus*. Mycologia 84:173–182

Kemp RFO (1989) Incompatibility in basidiomycetes – the heterogenic pentax. FORK Publ 1:1–19

Kiebacher J, Hoffmann GM (1981) Zur Entwicklung des Perfektstadiums *Venturia inaequalis* in vitro (Development of the perfect stage of *Venturia inaequalis* in vitro). J Plant Dis Protect 88:1–8

Knowles DJC, Carlile MJ (1978a) Growth and migration of plasmodia of the myxomycete *Physarum polycephalum*: the effect of carbohydrates, including agar. J Gen Microbiol 108:9–15

Knowles DJC, Carlile MJ (1978b) The chemotactic response of plasmodia of the myxomycete *Physarum polycephalum* to sugars and related compounds. J Gen Microbiol 108:17–25

Kölreuter JG (1764) Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen, nebst Fortsetzungen 1, 2 und 3. Engelmann, Leipzig, Ostwalds Klassiker Nr 46, 1761–1766

Kope HH (1992) Interactions of heterokaryotic and homokaryotic mycelium of sibling isolates of the ectomycorrhizal fungus *Pisolithus arhizus*. Mycologia 84:659–667

Kuhlmann EG (1982) Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola*. Mycologia 74:759–768

Kwon KJ, Raper KB (1967) Heterokaryon formation and genetic analyses of color mutants in *Aspergillus heterothallicus*. Am J Bot 54:49–60

Lane EB (1981) Somatic incompatibility in fungi and myxomycetes. In: Gull K, Oliver SG (eds) The fungal nucleus. Cambridge University Press, Cambridge

Lane EB, Carlile MJ (1979) Post-fusion somatic incompatibility in plasmodia of *Physarum polycephalum*. J Cell Sci 35:339–354

Leach J, Yoder OC (1983) Heterokaryon incompatibility in the plant-pathogenic fungus, *Cochliobolus heterostrophus*. J Heredity 74:149–152

Lemke PA (1969) A reevaluation of homothallism, heterothallism and the species concept in *Sistotrema brinkmanni*. Mycologia 61:57–76

Lemke PA (1973) Isolating mechanisms in fungi – prezygotic, postzygotic, and azygotic. Persoonia 7:249–260

Leslie JF (1987) Heterokaryon incompatibility haplotypes on linkage group VII of *Neurospora crassa* (abstract). Mycol Soc Am Newslett 38:35

Leslie JF (1993) Fungal vegetative compatibility. Annu Rev Phytopathol 31:127–150

Leslie JF, Zeller KA (1996) Heterokaryon incompatibility in fungi: more than just a way to die. J Genet 75:416–424

Leslie JF, Zeller KA, Wohler M, Summerell BA (2004) Interfertility in two mating populations of *Gibberella fujikuroi*. Eur J Plant Pathol 90:1–8

Lindegren CC (1934) The genetics of *Neurospora*. VI. Bisexual and akaryotic ascospores from *N. crassa*. Genetica 16:315–320

Lombard FF, Larsen MJ, Dorworth EB (1992) Reassessment of the sexual incompatibility system and cultural characteristics of *Bjerkandera fumosa*. Mycologia 83:406–410

Loubradou G, Béguret J, Turcq B (1997) A mutation in aHSP90 gene affects the sexual cycle and suppresses vegetative incompatibility in the fungus *Podospora anseria*. Genetics 147:581–588

Loubradou G, Bégueret J, Turcq B (1999) Mod-D, a G α subunit of the fungus *Podospora anserina*, is involved in both regulation of development and vegetative incompatibility. Genetics 152:519–528

Macrae R (1967) Pairing incompatibility and other distinctions among *Hirschioporus* (*Polyporus*) *abietinus*, *H. fusco-violaceus* and *H. laricinus*. Can J Bot 45:1371–1398

Maddelein ML, Reis SD, Duvezin-Caubet S, Coulary-Salin B, Saupe SJ (2002) Amyloid aggregates of the *HET-s* prion protein are infectious. PNAS 99:7402–7407

Marek SM, Wu J, Glass NL, Gilchrist DG, Bostock RM (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 40:126–137

Mattjus P, Turcq B, Pike HM, Molotkovsky JG, Brown RE (2003) Glycolipid inermembrane transfer is accelerated by HET-C2, a filamentous fungus gene product involved in the cell-cell incompatibility response. Biochem 42:535–542

May G (1988) Somatic incompatibility and individualism in the coprophilous basidiomycete *Coprinus cinereus*. Trans Br Mycol Soc 91:443–451

McKeen CG (1952) Studies of Canadian theleophoraceae. IX. A cultural and taxonomic study of three species of *Peniophora*. Can J Bot 30:764–787

Meinhardt F, Koch H, Esser K (1984) Compatibility groups in *Ascobolus immersus*, an indication of speciation. Theor Appl Genet 68:365–367

Metzenberg RL (1990) Anecdotal, historical and critical commentaries on genetics. In: Crow JF, Dove WF (eds) Genetics 125:457–462

Micali CO, Smith ML (2003) On the independence of barrage formation and heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 38:209–219
Milgroom MG, Cortesi P (2004) The biological control of chestnut blight with hypervirolence: a critical analysis. Annu Rev Phytopathol 42:311–338
Mir-Rashed N, Jacobson DJ, Deghgany MR, Micali OC, Smith ML (2000) Molecular and functional analyses of incompatibility genes at *het-6* in a population of *Neurospora crassa*. Fungal Genet Biol 30:197–205
Moreau F, Moruzi C (1933) Sur de nouvelles irrégularités de la bipolarité sexuelle chez les Ascomycetes du genre *Neurospora*. Bull Soc Bot Fr 80:574–576
Morrison RM (1960) Compatibility of several clonal lines of *Erysiphe cichoracearum*. Mycologia 52:786–794
Mounce I (1929) Studies in forest pathology. II. The biology of *Fomes pinicola* (Sw) Cooke. Can Dept Agric Bull 111:1–77
Mounce I, Macrae R (1938) Interfertility phenomena in *Fomes pinicola*. Can J Bot 16:354–376
Murphy JF, Miller OK Jr (1993) The population biology of two litter decomposing agarics on a southern Appalachian mountain. Mycologia 85:769–776
Mylyk OM (1975) Heterokaryon incompatibility genes in *Neurospora crassa* detected using duplication-producing chromosome rearrangements. Genetics 80:107–124
Mylyk OM (1976) Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora crassa*. Genetics 83:275–284
Nakata K (1925) Studies on *Sclerotium rolfsii*. I. The phenomenon of aversion and its relation to the biologic forms of the fungus. II. The possible cause of the phenomenon of aversion in the fungus and morphological features of the phenomenon. Bull Sci Fak Terkulturen Kjusa Imp Univ 1:4
Nazabal A, Reis SD, Bonneu M, Saupe SJ, Schmitter JM (2003) Conformational transition occurring upon amyloid aggregation of the *HET-s* prion protein of *Podospora anserina* analyzed by hydrogen/deuterium exchange and mass spectrometry. Biochemistry 42:8852–8861
Nelson RR (1963) Interspecific hybridization in the fungi. Annu Rev Microbiol 17:31–48
Nelson RR (1965a) Bridging interspecific incompatibility in the ascomycetous genus *Cochliobolus*. Evolution 18:700–704
Nelson RR (1965b) Assessing biological relationship in the fungi. Phytopathology 55:823–826
Nelson RR (1966) The genetic control of conidial morphology and arrangement in *Cochliobolus carbonum*. Mycologia 58:208–214
Nelson RR (1970) Variation in mating capacities among isolates of *Cochliobolus carbonum*. Can J Bot 48:261–263
Nelson RR, Kline DM (1964) Evolution of sexuality and pathogenicity. III. Effects of geographic origin and host association on cross-fertility between isolates of *Helinthosporium* with similar conidial morphology. Phytopathology 54:963–967
Newmeyer D (1968) A suppressor of mating type incompatibility in *Neurospora*. Genetics 60:207
Newmeyer D (1970) A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. Can J Genet Cytol 12:914–926
Newmeyer D, Taylor CW (1967) A pericentric inversion in *Neurospora*, with unstable duplication progeny. Genetics 56:771–791
Newton AC, Osbourn AE, Caten CE (1998) Heterokaryosis and vegetative incompatibility in *Stagnospora nodorum*. Mycologia 90:215–225
Nuss DI, Koltin Y (1990) Significance of dsRNA genetic elements in plant pathogenic fungi. Annu Rev Phytopathol 28:37–58
Padhaye AA, Carmichel JW (1971) Incompatibility in *Microsporum cookei*. Sabouraudia 9:27–29
Paoletti M, Clave C, Begueret J (1998) Characterization of a gene from the filamentous fungus *Podospora anserina* encoding an aspartyl protease induced upon carbon starvation. Gene 210:45–52
Perkins DD (1968) Multiple alleles at a heterokaryon-compatibility locus in *Neurospora*. Genetics 61:47
Perkins DD (1988) Main features of vegetative incompatibility in *Neurospora*. Fungal Genet Newslett 35:44–46
Perkins DD, Turner BC (1988) *Neurospora* from natural populations: toward the population biology of a haploid eukaryote. Exp Mycol 12:91–131
Perkins DD, Turner BC, Barry EG (1976) Strains of *Neurospora* collected from nature. Evolution 30:281–313
Pilotti CA, Sanderson FR, Aitken AB (2002) Sexuality and interactions of monokaryotic and dikaryotic mycelia of *Ganoderma boninense*. Mycol Res 106:1315–1322
Pinan-Lucarré B, Paoletti M, Dementhon K, Coulary-Salin B, Clavé C (2003) Autophagy is induced during cell death by incompatibility and is essential for differentiation in the filamentous fungus *Podospora anserina*. Mol Microbiol 47:321–333
Pittenger TH (1964) Spontaneous alterations of heterokaryon compatibility factors in *Neurospora*. Genetics 50:471–484
Pittenger TH, Brawner TG (1961) Genetic control of nuclear selection in *Neurospora* heterokaryons. Genetics 46:1645–1663
Ploetz RC, Shokes FM (1986) Evidence for homothallism and vegetative compatibility in southern *Diaporthe phaseolorum*. Can J Bot 64:2197–2200
Proffer TJ, Hart JH (1988) Vegetative compatibility groups in *Leucocystospora kunzei*. Phytopathology 78:256–260
Puhalla EJ (1968) Compatibility reactions on solid medium and interstrain inhibition in *Ustilago maydis*. Genetics 60:461–474
Puhalla EJ, Hummel M (1983) Vegetative compatibility groups within *Verticillium dahliae*. Phytopathology 73:1305–1308
Puhalla JE, Spieth PT (1983) Heterokaryosis in *Fusarium moniliforme*. Exp Mycol 7:328–335
Puhalla JE, Spieth PT (1985) A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp Mycol 9:39–47
Punja ZK, Grogan RG (1983a) Basidiocarp induction, nuclear condition, variability, and heterokaryon incompatibility in *Athelia* (*Sclerotium*) *rolfsii*. Phytopathology 73:1273–1278
Punja ZK, Grogan RG (1983b) Hyphal interactions and antagonism among field isolates and single-basidiospore strains of *Athelia* (*Sclerotium*) *rolfsii*. Phytopathology 73:1279–1284

Punja ZK, Sun LJ (2002) Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (telemorph *Athelia rolfsii*) and *S. delphinii*. Mycol Res 105:537–546
Raju NB (1979) Cytogenetic behavior of spore killer genes in *Neurospora*. Genetics 93:607–623
Raju NB (2002) Spore killers: meiotic drive elements that distort genetic ratios. In: Osiewacz HD (ed) Molecular biology of the fungal development. Dekker, New York, pp 375–296
Raper KB, Fennell DL (1953) Heterokaryosis in *Aspergillus*. J Elisha Mitchell Sci Soc 69:1–29
Rayner ADM, Todd NK (1977) Intraspecific antagonism in natural populations of wood-decaying basidiomycetes. J Gen Microbiol 103:85–90
Rayner ADM, Turton MN (1982) Mycelial interactions and population structure in the genus *Stereum*: *S. rugosum*, *S. sanguinolentum* and *S. rameale*. Trans Br Mycol Soc 78:483–493
Reinhardt OM (1892) Das Wachstum der Pilzhyphen. Jahrb Wiss Bot 23:479
Rizet G (1952) Les phénomènes de barrage chez *Podospora anserina*. I. Analyse génétique des barrages entre souches S et s. Rev Cytol Biol Vég 13:51–92
Rizet G (1953) Sur la multiplicité des mécanismes génétiques conduisant à des barrages chez *Podospora anserina*. C R Acad Sci Paris 237:666–668
Rizet G, Esser K (1953) Sur des phénomènes d'incompatibilité entre souches d'origines différentes chez *Podospora anserina*. C R Acad Sci Paris 237:760–761
Rizzo DM, Rentmeester RM, Bursdall J (1996) Sexuality and somatic incompatibility in *Phellinus gilvus*. Mycologia 87:805–820
Robson GE, Williams KL (1979) Vegetative incompatibility and the mating-type locus in the cellular slime mold *Dictyostelium discoideum*. Genetics 93:861–875
Robson GE, Williams KL (1980) The mating system of the cellular slime mold *Dictyostelium discoideum*. Curr Genet 1:229–232
Saupe SJ (2000) Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. Microbiol Mol Biol Rev 64:489–502
Saupe S, Glass NL (1997) Allelic specificity at the *het-c* heterokaryon incompatibility locus of *Neurospora crassa* is determined by a highly variable domain. Genetics 146:1299–1309
Saupe S, Turcq B, Bégueret J (1995) Sequence diversity and unusual variability at the *het-c* locus involved in the vegetative incompatibility in the fungus *Podospora anserina*. Curr Genet 27:466–471
Saupe SJ, Kuldau GA, Smith ML, Glass NL (1996) The product of the *het-C* heterokaryon incompatibility gene of *Neurospora crassa* has characteristics of glycine cell rich wall protein. Genetics 143:1589–1600
Savage EJ, Clayton CW, Hunter JH, Brenneman JA, Laviola C, Gallegly ME (1968) Homothallism, heterothallism and interspecific hybridization in the genus *Phytophthora*. Phytopathology 58:1004–1021
Schrauwen JAM (1979) Post-fusion incompatibility in *Physarum polycephalum*. Arch Microbiol 122:1–7
Shear CL, Dodge BO (1927) Life histories and heterothallism of the red bread mould fungi of the *Monilia sitophila* group. J Agric Res 34:1019–1042
Shiu PKT, Glass NL (1999) Molecular characterization of *tol*, a mediator of mating-type-associated vegetative incompatibility in *Neurosppra crassa*. Genetics 154: 545–555
Sidhu GS (1986) Genetics of *Gibberella fujikuroi*. VIII. Vegetative compatibility groups. Can J Bot 64:117–121
Silveira RM, Borges DA, Wright JE (2002) *Polyporus* s. strictu in southern South America: mating tests. Mycol Res 106:1323–1330
Smith ML, Yang CJ, Metzenberg R, Glass NL (1996) Escape from *het*-6 incompatibility in *Neurospora crassa*. Partial diploids involves preferential deletion within ectocopic segment. Genetics 144:523–531
Smith ML, Micali OC, Hubbard SP, Mir-Rashed N, Jacobson DJ, Glass NL (2000) Vegetative incompatibility in the *het-6* region of *Neurospora crassa* is mediated by two linked genes. Genetics 155:1095–1104
Somers JM, Bevan EA (1969) The inheritance of the killer character in yeast. Genet Res 13:71–83
Staben C, Yanofsky C (1990) *Neurospora crassa* a mating-type region. Proc Natl Acad Sci USA 87:4917–4921
Stark JR, Boyd A, Mileham AJ, Romanos MA (1990) The encoded killer system of *Kluyveromyces lactis*: a review. Yeast 6:1–29
Stretton HM, Flentje NT (1972a) Inter-isolate heterokaryosis in *Thanatephorus cucumeris*. I. Between isolates of similar pathogenicity. Aust J Biol Sci 25:293–303
Stretton HM, Flentje NT (1972b) Inter-isolate heterokaryosis in *Thanatephorus cucumeris*. II. Between isolates of different pathogenicity. Aust J Biol Sci 25:305–318
Tipper DJ, Bostian KA (1984) Double stranded ribonucleic acid killer systems in yeasts. Microbiol Rev 48:125–156
Turcq B (1989) Clonage direct de gènes par complémentation chez le champignon *Podospora anserina*: application à l'étude de gènes d'incompatibilité. PhD Thesis, Université Bordeaux II
Turcq B, Denayrolles M, Bégueret J (1990) Isolation of two allelic incompatibility genes *s* and *S* of the fungus *Podospora anserina*. Curr Genet 17:297–303
Turcq B, Deleu C, Denayrolles M, Bégueret J (1991) Two allelic genes responsible for vegetative incompatibility in the fungus *Podospora anserina* are not essential for cell viability. Mol Gen Genet 228:265–269
Turner BC, Perkins DD (1979) Spore killer, a chromosomal factor in *Neurospora* that kills meiotic products not containing it. Genetics 93:587–606
Turner BL, Taylor CW, Perkins DD, Newmeyer D (1969) New duplication-generating inversions in *Neurospora*. Can J Genet Cytol 11:622–638
Vandendries R (1932) La tétrapolarité sexuelle de *Pleurotus columbinus*. Démonstrations photographiques d'un tableau de croisement. Cellule 41:267–279
Van der Gaag M, Debets AJM, Hoekstra RF (2003) Spore killing in the fungus *Podospora anserina*: a connection between meiotic drive and vegetative incompatibility? Genetics 117:59–65
Van Diepeningen AD, Debets JM, Hockstra RF (1997) Heterokaryon incompatibility blocks virus transfer among natural isolates of black *Aspergilli*. Curr Genet 32:209–217
Vilgalys RJ, Johnson JL (1987) Extensive genetic divergence associated with speciation in filamentous fungi. Proc Natl Acad Sci USA 84:2355–2358

Vilgalys RJ, Miller OK (1987) Mating relationships within the *Collybia dryophila* group in Europe. Trans Br Mycol Soc 89:295–300

Weber H (ed) (1993) Allgemeine Mykologie. Fischer, Jena

Webster RK, Nelson RR (1968) The genetics of *Cochliobolus spiciferus*. I. Genetic inhibition of perithecial and ascus formation. Can J Bot 46:197–202

Williams CA, Wilson JF (1968) Factors of cytoplasmic incompatibility. Neurospora Newslett 13:12

Wilson JF (1961) Microsurgical techniques for *Neurospora*. Am J Bot 48:46–51

Wilson JF (1963) Transplantation of nuclei in *Neurospora crassa*. Am J Bot 50:780

Wilson JF, Garnjobst L (1966) A new incompatibility locus in *Neurospora crassa*. Genetics 53:621–631

Wilson JF, Garnjobst L, Tatum EL (1961) Heterokaryon incompatibility in *Neurospora crassa* – microinjection studies. Am J Bot 48:299–305

Wong GJ (1993) Mating and fruiting studies of *Auricularia delicata* and *A. fuscosuccinea*. Mycologia 85:187–194

Worall JJ (1997) Somatic incompatibility in basidiomycetes. Mycologia 89:24–36

Wu J, Glass NL (2001) Identification of specificity determinants and generation of alleles with novel specificity at the *het-c* heterokaryon incompatibility locus of *Neurospora crassa*. Mol Cell Biol 21:1045–1057

Xiang Q, Glass NL (2002) Identification of *vib-1*, a locus involved in vegetative incompatibility mediated by *het-c* in *Neurospora crassa*. Genetics 162:89–101

Xiang Q, Glass NL (2004) Chromosome rearrangement in isolates that escape from *het-c* heterokaryon incompatibility. Curr Genet 44:329–338

Xiang Q, Rasmussen C, Glass NL (2002) The *ham* locus, encoding a putative transmission protein, is required for hyphal fusion in *Neurospora crassa*. Genetics 160:169–180

Yindeeyoungyeon W, Triratana S (1992) Hyphal interaction among dikaryotic pairings of *Lentinula edodes*. Micol Neotrop Apl 5:17–27

9 Programmed Cell Death in Fungi

B.C.K. Lu[1]

CONTENTS

I. Introduction

Cell death may be classified into three main categories: autophagy, apoptosis, and necrosis. The first two are programmed and are genetically regulated, and the third is environmentally induced by physical or chemical injuries. Programmed cell death (PCD) was first described in multicellular metazoans as a developmental strategy whereby unwanted cells are removed to make way for new cellular remodeling and differentiation. PCD is also essential for the removal of diseased or physiologically and genetically defective cells (reviewed in Ellis et al. 1991; Jacobson et al. 1997; King and Cidlowski 1998; Vaux and Korsmeyer 1999; Ranganath and Nagashree 2001).

The type I programmed cell death or apoptosis has been extensively studied initially in metazoans (reviewed in Kerr et al. 1972; Wyllie et al. 1980) and now in all organisms, such as *Caenorhabditis elegans*, *Drosophila melanogaster*, mammals, plants and even yeasts and mycelial fungi (Hengartner 1997; Metzstein et al. 1998; Matsuyama et al. 1999; Ranganath and Nagashree 2001). It may be a caspase-dependent or -independent process, and its cytological hallmarks are well defined. The type II or autophagic cell death (ACD) is not as well defined; it is a lysosome-dependent and a caspase-independent process, and it involves autophagy, vacuolization, and cell lysis (Bursch 2001).

Research on PCD in fungi is quite recent. However, cell death due to heterogenic incompatibility has been documented earlier on in *Podospora anserina* (Rizet and Esser 1953), *Neurospora crassa* (Garnjobst and Wilson 1956; Perkins 1988), and other filamentous fungi (see review in Esser and Blaich 1973; Glass et al. 2000). For further reviews, see Chap. 8 by Esser, and Chap. 7 by Glass and Fleißner (this volume). Cell aging and senescence have also been documented in *P. anserina* (Rizet 1953; Esser and Keller 1976; reviewed in Esser and Tudzynski 1980) and other fungi (reviewed in Griffiths 1992; Osiewacz 1995; see also Hamann and Osiewacz, Chap. 10, this volume). Senescence is a progressive loss of growth. Visible symptoms are hyphal tip swelling and bursting associated with dark pigmentation that is expressed only shortly before death. At the molecular level in *P. anserina*, senescence is associated with a large number of circular plDNA that are spliced off from mtDNA

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(Esser et al. 1980; Kück et al. 1981). It is a part of the intron of the cytochrome c oxidase gene that contains a 48-bp autonomous replication sequence (Osiewacz and Esser 1984). The accumulation of plDNA in mitochondria leads to mitochondrial instability and death. Interestingly, senescence can be cured by ethidium bromide that eliminates plDNA, also known as senDNA (Koll et al. 1984; see reviews in Griffiths 1992; Osiewacz 1995). In recent years, PCD is gaining recognition in fungi, which have been shown to share this vital cellular program (albeit prototypal) with all eukaryotes. In this chapter, I review some of the key findings in this field. Some important works may regrettably not have been included because of space constraints, and because they are reviewed elsewhere in this volume (Chaps. 7, 8 and 10). More details may be seen in recent reviews (Madeo et al. 2004; Ramsdale, The Mycota, Vol. XIII, Chap. 7).

II. Do Fungi Have Apoptosis, the Type I Programmed Cell Death?

From model systems such as *C. elegans* and mammalian cells, a number of proteins (and their genes) that regulate apoptosis have been identified. These are CED-3, CED-4, and CED-9 of *C. elegans*, and the *Bcl-2* (β-cell lymphocytic-leukemia protooncoprotein-2) family proteins of mammals (see reviews in Ellis et al. 1991; Green and Reed 1998; Mignotte and Vayssiere 1998; Kuwana and Newmeyer 2003). No homologs of these genes are found in the genome of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* (Koonin and Aravind 2002) and *Neurospora crassa* (Glass and Kaneko 2003). A question has been raised whether fungi have apoptosis. In recent years, evidence has been accumulating that processes of apoptosis do indeed exist in fungi.

Before I examine cases in fungi, let me take a brief look at what is known in the higher eukaryotes. Bcl-2 family proteins are key cell-death regulatory proteins in mammalian cells, and they all share one or more Bcl-2 homology (BH) domains (Zha et al. 1996; Harris and Thompson 2000). There are two opposing groups, proapoptotic and antiapoptotic. The proapoptotic group contains two subgroups: Bax and Bak contain BH1, BH2, and BH3, and Bid and Bad (also others) contain a BH3-only domain. The antiapoptotic Bcl-2 and Bcl-x_L contain all four (BH1-4) domains. All Bcl-2 family proteins can be classified as mitochondrial membrane proteins, as they are destined to mitochondria for their actions (see review in Kuwana and Newmeyer 2003). It appears that BH3 is essential for the cell-death process (Huang and Strasser 2000). When a death stimulus is received, the BH3-only Bid activates Bax/Bak while Bad inactivates Bcl-2/Bcl-x_L. Bax is then translocated to the mitochondrial outer membrane to trigger apoptosis. Bax is suggested to form membrane pores from which cytochrome c and other apoptogenic molecules can leak from mitochondrial intermembrane space into the cytosol to activate the caspases (Kuwana et al. 2002; see also references in Kuwana and Newmeyer 2003). Recently, Bax has been shown, by electron microscopy, to localize at the constricted mitochondrial fission sites to effect mitochondrial fragmentation in apoptotic Cos-7 (and HeLa) cells (Karbowski et al. 2002). In *C. elegans*, four genes have been identified, *ced9*, *ced4*, *ced3* (for cell death-defective), and *egl1* (for egg laying-defective). CED-9 is equivalent to Bcl-2, CED-4 is equivalent to mammalian Apaf1 (apoptosis protease activating factor 1), CED-3 is a caspase (a cysteine protease), and EGL-1 is a BH3-only dynamin-interacting protein that interacts with Drp-1 (dynamin-related protein 1) to mediate mitochondrial fragmentation (Jagasla et al. 2005). Other regulators of apoptosis are caspase-1/caspase-3, effectors of apoptosis, AIF (apoptosis-inducing factor), Htr-A (high-temperature resistance A), a nuclear mediator of apoptosis, BI (Bax inhibitor) and IAP (inhibitor of apoptosis protein). More details are documented below in this chapter.

A. Apoptosis Is Induced by Expression of Heterologous Proapoptotic Genes

Expression of proapoptotic genes from both mammals and *C. elegans* in the budding and fission yeasts brought about symptoms consistent with apoptotic cell death (Sato et al. 1994; Ink et al. 1997; James et al. 1997; Ligr et al. 1998; Fröhlich and Madeo 2000). For example, when Bax is expressed in the budding yeast, or when human Bak is expressed in the fission yeast, cell death is induced in these hosts. The cell killing can be prevented when the antiapoptotic gene *bcl-2*, *bcl-x_L*, or *mcl-1* is co-transformed (Sato et al. 1994; Greenhalf et al. 1996; Ink et al. 1997; Tao et al. 1997; Fröhlich and Madeo 2000). Likewise, when the mammalian

caspase-1/ICE or caspase-3/CPP32 is expressed in the fission yeast, cell death is induced and it can be rescued by co-expression of baculovirus caspase inhibitory protein p35, an *i*nhibitor of *ap*optosis (**IAP**). It is interesting to note that the Bax- or Bak-induced cell killing can be rescued by Bcl-2 but not by p35 (Jürgensmeier et al. 1997). On the other hand, the caspase-induced cell killing can be rescued by p35 but not by Bcl-2. It is possible that Bcl-2 acts upstream of caspase activation in higher eukaryotes (Ryser et al. 1999). Expression of *ced-4* from *C. elegans* in the fission yeast also results in lethal chromatin condensation, and the CED-4 protein is co-localized with the condensed chromatin, as demonstrated by immunogold labeling, suggesting that CED-4 is directly involved in chromatin condensation. Co-expression of the antiapoptotic protein CED-9 of *C. elegans* in fission yeast results in increased growth rate, complete absence of chromatin condensation and, interestingly, CED-4 protein is no longer localized to the chromatin (James et al. 1997).

B. Apoptosis Is Induced by Environmental Stimuli

Apoptotic cell death is induced in *S. cerevisiae* and in the opportunistic fungal pathogen, *Candida albicans*, by a treatment with low concentrations of acetic acid (e.g., 20–80 mM for 200 min at pH 3.0) that causes intracellular acidification (Ludovico et al. 2001; Phillips et al. 2003). A treatment with high concentrations of acetic acid (>120 mM for 200 min) causes necrosis. The apoptotic cell suicide requires active protein synthesis because the presence of cycloheximide attenuates the apoptotic effect of acetic acid and enhances the yeast survival (Ludovico et al. 2001). Acetic acid also induces apoptotic cell death in the food spoilage yeast *Zygosaccharomyces bailii*; the only difference is that this yeast can tolerate much higher concentrations (e.g., 320–800 mM for 130 min) than can baker's yeast (Ludovico et al. 2003).

Apoptotic cell death is also induced in *S. cerevisiae* as well as in *C. albicans* by oxygen stress, such as a treatment with low concentrations of hydrogen peroxide (e.g., 3–5 mM for 180–200 min). The apoptotic effect of H_2O_2 can be prevented by cycloheximide (Madeo et al. 1999; Carratore et al. 2002; Phillips et al. 2003) and by expression of antiapoptotic genes *ced-9*, *Bcl-2* and *Bcl-*x_L in yeast (Chen et al. 2003). Apoptotic cell death in yeast is also induced by low doses of viral killer toxin, such as K1, K28, and Zygocin; the cell death appears to be caspase-dependent, and the *r*eactive *o*xygen *s*pecies (**ROS**) may act as effectors (Reiter et al. 2004). Apoptotic cell death is also induced by UV irradiation (90–120 J/m^2) that causes DNA damage and cell-cycle arrest (Carratore et al. 2002). Treatments with higher concentrations of H_2O_2 (> 180 mM for 180 min), or a higher dosage of UV irradiation(> 150 J/m^2) cause severe injuries that lead to necrosis.

Apoptosis can be triggered by starvation of one or another amino acid in auxotrophic yeast mutants (Eisler et al. 2004) and by chronological aging (Herker et al. 2004). It is interesting that, in both cases, ROS is involved. There is evidence that accumulation of ROS precedes the onset of apoptosis (Eisler et al. 2004).

C. Apoptosis Is Induced by Genetic Defects

The demonstration above of apoptosis in yeast and other fungi by exogenous stimuli suggests that fungi have a molecular machinery to perform fundamental steps for type I PCD, as they do respond correctly when pro- and antiapoptotic genes from mammals and *C. elegans* are expressed in fungal cells. It is most likely that the genetic system for PCD exists and needs to be discovered. The first such genetic defect to be discovered is in *CDC48* of *S. cerevisiae*. Cdc48p is a member of the AAA family of ATPases. A specific mutant allele, *cdc*48^{S565G}, exhibits typical apoptotic phenotypes (Madeo et al. 1997). This gene has a role as a cell-cycle or an antiapoptotic regulator. It is interesting to note that an ortholog of this protein has been found in MAC-1 (member of the AAA family that binds CED-4) of *C. elegans*, *VCP* of mammals, and Smallminded protein of *Drosophila*. MAC-1 prevents *CED-4* and *CED-3* from causing apoptosis in cells that are not destined to die (Wu et al. 1999).

Cdc13p is a budding yeast telomere binding protein. It is essential for telomere replication and maintenance (Nugent et al. 1996; Qi and Zakian 2000). Inactivation of this protein, as in the ts-mutant *cdc13-1*, leads to extensive degradation of the C-strands, resulting in long single-stranded G-tails at the 3′-ends. As a consequence, the abnormal telomeres trigger the *Mec1*-dependent cell-cycle checkpoint arrest at G2/M phase (Qi et al. 2003). *Mec1* is the yeast homolog of the mammalian *ATR* and/or *ATM* checkpoint control gene (Burhans et al. 2003). When the damages are not repaired, the arrest is abrogated and the cells

enter the PCD pathway, with all the hallmarks of apoptosis. Moreover, the PCD induced in *cdc13-1* involves the mitochondrial event, because the mitochondria-deficient mutant ρ° can rescue *cdc13-1* cells and suppresses cdc13-1p-induced caspase activation (Qi et al. 2003). However, cell death of *cdc13-1* during DNA damage arrest has recently been demonstrated to be independent of caspase activation, and death by apoptosis has been called into question (Wysocki and Kron 2004). Other death pathways need to be investigated.

Cell-cycle progression is highly regulated by checkpoint controls in all organisms (see Harris, Chap. 3, this volume). For initiation of DNA replication in the budding yeast, **ORC1** and **ORC2** (*o*rigin *r*ecognition *c*omplex) are required to assemble the pre-replicative complex (preRC) on the chromatin during G1 phase (Watanabe et al. 2002; Burhans et al. 2003). Inactivation of *ORC* genes in yeast (as in *orc1-4/orc1-4* diploid cells) will trigger *RAD9*-dependent DNA-damage checkpoint control and cell-cycle arrest at G2/M boundary, and the cell-death pathway ensues when cell-cycle arrest is abrogated after the 10-h catastrophic time (Watanabe et al. 2002). Ts-mutant *orc2-1/orc2-1* diploid cells also die at non-permissive temperatures (Watanabe et al. 2002). Several checkpoint proteins (e.g., Mec1 and ScRad9 of *S. cerevisiae*, and Cdc2, Rad3 and SpRad9 of *S. pombe*) and their requirement for apoptotic pathways have been established (reviewed in Burhans et al. 2003). Interestingly, the G1/S checkpoint works by cell-cycle arrest at G1, during which pre-RC and licensing proteins are assembled on the chromatin. Defects in the checkpoint or *ORC* genes will lead to abrogation of the arrest and start of the S phase, and only then is the apoptotic pathway triggered (Burnhans et al. 2003).

Human cell-cycle gene 1 (***hCCG1***) appears to be a regulator of apoptosis and its product has been identified to be a histone acetyltransferase. The factor h**CIA1** (CCG1 interacting factor A) is a histone chaperone. The yeast homolog of h**CIA** is **ASF1** (anti-silencing factor-1). Thus, ASF1/CIA1 is a yeast histone chaperone that is essential for cell-cycle progression. The defective *asf1/cia1* mutant cells arrest and die predominantly at G2/M. The dead *asf1/cia1* cells not only exhibit characteristics of apoptosis, but also include autophagic bodies in the vacuole with some hints of necrosis, as found by Yamaki et al. (2001). These authors suggest that yeast "may have evolved a prototypal active cell death system".

D. Cytological Phenotypes of Apoptosis

The cytological phenotypes of PCD that have been found, be it induced by expression of heterologous proapoptotic genes, by external stimuli, or by genetic defects, exhibit almost all of the hallmarks of apoptosis found in metazoan organisms. These are chromatin condensation, DNA fragmentation (as demonstrated by TdT-mediated dUTP nick end labeling, or **TUNEL** assay), nuclear and cytoplasmic fragmentation, phosphatidylserine externalization and vacuolization (Sato et al. 1994; Ink et al. 1997; James et al. 1997; Madeo et al. 1997; Ligr et al. 1998; Fröhlich and Madeo 2000), activation of caspase activity (Qi et al. 2003) and increased production of ROS (Fröhlich and Madeo 2000; Gross et al. 2000; Poliaková et al. 2002; Ludovico et al. 2002; Qi et al. 2003). It should be noted that nucleosomal ladders have not been found in all cases examined, with the exception of *Mucor racemosus* (Roze and Linz 1998). It is also interesting to note that in rare cases autophagic vesicles have been associated with apoptotic cell death (Yamaki et al. 2001), suggesting a crosstalk between apoptotic and autophagic pathways (see below).

Apoptotic and necrotic cells can be distinguished by cytological criteria. Phosphatidylserine (**PS**) externalization and its localization can be detected by a fluorescent FITC-conjugate annexin V. However, annexin V can also enter the necrotic cells and bind to PS on the inner leaflet of the plasma membrane. Thus, this technique would not be discriminating. The DNA stain propidium iodite (**PI**) is not permeable to apoptotic cells but it is to necrotic cells. Thus, the apoptotic cells will be annexin V positive and PI negative, while the necrotic cells will be positive for both. The most discriminating stain for apoptotic cells will be the **TUNEL** assay, where apoptotic cells will be positive and the necrotic cells negative (Chen et al. 2003). The distinction between apoptotic and autophagic cell death is less clear cut. I attempt to define these in a following section in this chapter.

E. Caspase-Like Proteins Found

Caspases (cysteine aspartases) may have an ancient origin (Boyce et al. 2004). A caspase-like or metacaspase gene has been discovered in *S. cerevisiae*, named ***YCA1*** (for yeast caspase-1). Its protein product behaves like a bona fide caspase. Like mammalian caspases, the yeast YCA1p proenzyme (about 52 kDa) is activated by proteolysis. A 12-

kDa carboxy-subunit appears only after activation, and this activation is abrogated when the catalytic cysteine 297 of *YCA1* is mutated. As a consequence of caspase activation, apoptotic cell death is induced in yeast. Increased cell death can be prevented by the caspase-specific inhibitor zVAD-fmk (z-Val-Ala-Asp(Ome)-fluoromethyl ketone). It has been suggested that YCA1p is the executor for cell death induced by a wide range of apoptotic stimuli, as disruption of *YCA1* abrogates hydrogen peroxide-, acetic acid-, or age-induced apoptosis (Madeo et al. 2002).

Two metacaspases have also been found in *Aspergillus fumigatus*, and their activity is elevated when cultures enter the stationary phase (Amin et al. 2003). Two caspase-like (caspase 3 and caspase 8) activities have also been identified in *A. nidulans* during sporulation. These caspase-like activities are inhibited by the caspase-3-specific peptide inhibitor DEVD-fmk (aspartyl-valyl-alanyl-aspartyl-fmk) and caspase-8-specific peptide inhibitor IETD (Ile-Glu-Thr-Asp)-fmk (Thrane et al. 2004). As in animal cells, the DNA repair enzyme poly (ADP-ribose) polymerase (**PARP**) is cleaved by caspase 3 during apoptosis. From database searches in the *A. nidulans* genome, two genes for metacaspases and one gene for the PARP have been found. Here the PARP is most likely a substrate for caspase-like activity in *A. nidulans*. The same genes have also been found in the *N. crassa* genome. However, no orthologs of mammalian caspases have been found in these fungi (Thrane et al. 2004).

F. Other Regulators of Apoptosis

The Bcl-2 family proteins are regulators of apoptosis in mammalian cells. The proapoptotic Bax and Bak contain BH1, BH2 and BH3 where BH3 is critical as the death domain (Zha et al. 1996; Harris and Thompson 2000). The Rad9 protein of *S. pombe* (SpRad9) is a DNA-damage checkpoint protein, but it also contains a group of amino acids with similarity to the BH-3 death domain. It acts like Bax, it interacts with Bcl-2 and Bcl-$2x_L$, and it induces apoptosis in mammalian cells, all of which require the intact BH-3 domain, as demonstrated by Komatsu et al. (2000). These authors suggested that "SpRad9 may represent the first member of the Bcl-2 protein family in yeast".

*A*poptosis-*i*nducing *f*actor (**AIF**) is an apoptogenic mitochondrial intermembrane protein that acts beyond or independently of Bcl-2 and caspase pathways in mammalian cells (Susin et al. 1999). A yeast homolog, Aif1p, has been found showing 22% identity and 41% similarity with human AIF (Wissing et al. 2004). Like the mammalian AIF, the yeast Aif1p is localized within the mitochondrial membranes, and it is translocated to the nucleus upon induction of apoptosis. It induces apoptosis, and it has a DNase activity that requires proper cofactors, i.e., Mg^{++} and Ca^{++} (Wissing et al. 2004). Its apoptogenic function requires cyclophilin (Cyp1), just as does AIF of mammalian cells (Cande et al. 2004).

*B*ax *i*nhibitor-1 (**BI-1**) is an antiapoptotic protein; it is highly conserved in mammals, *Drosophila*, plants, and has now been found in the budding yeast. Besides being a Bax-suppressor, yBI-1 can partially rescue yeast from cell death induced by oxidative stress and heat shock, and the deletion of a C-terminal domain of yBI-1 abrogates this death protection, demonstrating a structure–function relation (Chae et al. 2003).

Inhibitor of apoptosis (**IAP**) family proteins are highly conserved suppressors of apoptosis (reviewed in Deveraux and Reed 1999). These are characterized by a ~70 amino acid Baculoviral IAP repeat (**BIR**) domain first discovered in the genome of baculoviruses (Birnbaum et al. 1994). IAP-like proteins containing BIR motifs have also been found in yeasts. The gene *BIR1* is required for efficient completion of meiosis in *S. cerevisiae*. This gene contains a putative nuclear localization sequence (KKKRKFKR 393), and BIR1-GFP is localized to the nucleus (Uren et al. 1999). The BIR1p is required for cell division, and it interacts with kinetochore proteins in controlling chromosome segregation events (Uren et al. 1999; Yoon and Carbon 1999; Li et al. 2000). It is interesting that a *ts-skp1-4* mutant has a pronounced chromosome segregation defect, and it can be rescued by over-expression of BIR1p as well as by a 3′-BIR1 fragment (C405), but not by a 5′-BIR motifs-containing fragment (Yoon and Carbon 1999). Thus, the BIR motifs play no part in chromosome segregation fidelity, and their function is still unclear. So far, BIR motif has not been linked to apoptosis as is found in higher eukaryotes, and thus remains to be discovered.

The mammalian **HtrA2** (*h*igh-*t*emperature *r*esistance *A*) protein is a mediator of apoptosis by its ability to antagonize the IAP protein, such as human XIAP. The *S. cerevisiae* HtrA-like protein has been identified recently in ORF YNL123W, which codes for a ~111 kDa protein (Fahrenkrog

et al. 2004). It is localized in the nucleus and is named **Nma111p** (for *n*uclear *m*ediator of *a*poptosis). Over-expression of Nma111 in yeast brings about cell death with all the hallmarks of apoptosis, while *nma111* and *yca1* null mutant control cells are TUNEL negative and exhibit no accumulation of ROS (Fahrenkrog et al. 2004). Nma111p belongs to the HtrA family of serine proteases, and its proapoptogenic property depends on its serine protease activity. No cell death occurs when serine 235 is mutated to cysteine by site-directed mutagenesis (Fahrenkrog et al. 2004). Since Nma111p and BIR1p are both localized in the nucleus, it remains to be discovered whether the two interact in any way.

III. Heterokaryon Incompatibility

The first case of programmed cell death in fungi extensively investigated is known as heterokaryon incompatibility, also termed vegetative incompatibility. *N. crassa* and *P. anserina* are the main model systems used (reviewed in Glass et al. 2000; Glass and Kaneko 2003). The genes that regulate this nonself recognition are named ***het*** (for heterokaryon incompatibility) and ***vic*** (for vegetative incompatibility) loci (for reviews, see Glass et al. 2000; Saupe et al. 2000; Glass and Kaneko 2003).

To understand the mechanism of incompatibility reaction and the genes involved, a *het*-R *het*-V self-incompatible strain of *P. anserina* has been used as a model system. This strain includes two incompatible genes (*het*-R and *het*-V) in the haploid nucleus, and it behaves like a temperature-sensitive mutant. At the permissive temperature (32 °C), it grows normally like the wild type whereas at the restrictive temperature (26 °C) incompatibility reaction is triggered in all cells, making it possible for unequivocal recognition (Labarère 1973). With this system, genes that are highly induced during incompatibility reaction have been identified and isolated, and these are named ***idi*** (*i*nduced *d*uring *i*ncompatibility) genes (*idi-1*, *idi-2*, *idi-3*, *idi-4*, *idi-6* and *idi-7*). These genes are also induced two- to tenfold by carbon or nitrogen starvation, indicative of their involvement in autophagy, and by rapamycin, which inhibits the **TOR** (target of rapamycin) kinase pathway (Bourges et al. 1998; Dementhon et al. 2003). The *idi-6* and *idi-7* are both directly involved in incompatibility cell death as well as in autophagy, and they may provide a link to type II programmed cell death by autophagy (Paoletti et al. 2001; Pinan-Lucarré et al. 2003). Interestingly, *idi-4* turns out to be a basic leucine zipper (bZIP) transcription factor that carries a DNA binding site (Dementhon et al. 2004; Dementhon and Saupe 2005). Its expression turns on *idi-2*, *idi-7* and *idi-4*, and causes autophagic cell death (Dementhon et al. 2004).

For *h*yphal *c*ompartmentalization and *d*eath (**HCD**), vegetative incompatibility may recruit the autophagic pathway in which the formation and accumulation of autophagic vesicles in the vacuole occur, and in which final degradation of these autophagic bodies takes place by vacuolar proteases. The strong evidence that links the *idi-6/pspA* (initially identified as *P. anserina* serine protease A) gene to autophagic cell death came from observations that inactivation of the PSPA vacuolar protease prevents degradation of autophagic bodies in the vacuole (Dementhon et al. 2003; Pinan-Lucarré et al. 2003). Also, both *pspA* and *idi-7* null mutants can survive or delay cell death (reviewed by Pinan-Lucarré et al. 2003). On the other hand, vegetative incompatibility may also recruit the apoptotic pathway to achieve cell killing, as recently demonstrated by TUNEL assay in *N. crassa* showing DNA fragmentation associated with nuclear pyknosis as well as cytoplasmic vacuolation (Marek et al. 2003).

IV. Ubiquitin-Proteasome System and Apoptosis

During cell-cycle progression, many intracellular proteins are synthesized and turned over by a cascade of events in the cytosol. This is achieved by the ubiquitin-proteasome pathway, and not by the lysosomal pathway. Proteins that are to be degraded are first tagged by an addition of multiple ubiquitin molecules, and the polyubiquitinated proteins are then transferred to the 26S proteasome for degradation (Schreader et al. 2003; Yen et al. 2003; see also Finley and Chau 1991 for review).

The 26S proteasome of *S. cerevisiae* is an ATP-dependent multicatalytic-multifunctional protease complex (Glickman et al. 1998; Naujokat and Hoffmann 2002 for review). It is made up of two subunits: a cylindrical 20S catalytic *c*ore *p*article (**CP**), and two 19S *r*egulatory *p*articles (**RP**), one on each side of the CP. Several CP proteolytic subunits (Pre1 to Pre4) have been identified (Heinemeyer et al. 1991; Gerlinger et al. 1997). Polyubiquitinated sub-

strates recognized by the proteasome are first deubiquitinated, and then unfolded before they are translocated into, and degraded by the 20S proteolytic core (Berndt et al. 2002; Naujokat and Hoffman 2002).

The ubiquitin-proteasome system may be involved in the regulation of a wide variety of cellular functions, such as DNA repairs, cell-cycle control, stress response, and apoptosis. The proteasome may have proapoptotic or antiapoptotic functions, depending on which target protein is degraded. If the target is an inhibitor of apoptosis, such as IAP or Bcl-2, it is proapoptotic, while if the target is an inducer of apoptosis, such as Bax or a caspase, it is antiapoptotic. Studies in mammalian cells have shown that inhibition of proteasome functions by a proteasome-specific inhibitor can lead to apoptosis. It can also rescue cells from apoptosis, depending on the proliferative state of the cell, suggesting that the ubiquitin-proteasome pathway may be linked to apoptosis (for reviews, see Drexler 1998; Orlowski 1999; Naujokat and Hoffmann 2002).

The discovery of apoptosis-like phenotypes induced by a gene mutation in Cdc48 of the yeast *S. cerevisiae* has provided a link between the ubiquitin-proteasome system and apoptosis. Cdc48 is a member of the family of AAA-ATPases; it is a homolog of the mammalian p97 (or VCP) and of MAC-1 of *C. elegans*. It is localized in the cytosol, the nucleus and the *e*ndoplasmic *r*eticulum (**ER**). In conjunction with different cofactors, Cdc48/p97 can perform different cellular tasks. The Cdc48-Ufd1-Npl4 complex functions to move polyubiquitinated polypeptides that are improperly assembled or misfolded from the ER into the cytosol, for their subsequent degradation by the proteasome (Ye et al. 2003). This retrotranslocation pathway is a form of quality control whereby misfolded or defective polypeptides are eliminated (Ellgaard and Helenius 2001). The failure in this process, as in the case of the yeast mutant $cdc48^{S565G}$, leads to PCD (Madeo et al. 1997).

Cell proliferation and cell death are two opposing processes that are highly regulated to maintain the integrity of the organism, and their respective effector is continuously produced and degraded depending on the status of the cell. It has been suggested that in the proliferation pathway, the activator of apoptosis being produced is identified by ubiquitination, and the polyubiquitylated proteins are rapidly degraded by the 26S proteasome. In *S. cerevisiae*, one such proteasomal substrate, **Stm1**, has been identified to participate in apoptosis-like cell death. Over-expression of *Stm1* in defective yeast *pre1-1 pre4-1* mutant background leads to cell-cycle arrest and cell death with the phenotypes of apoptosis. Stm1 is an in vivo substrate of the proteasome. This is demonstrated by the cycloheximide chase experiment where Stm1 proteins disappear in time in the wild-type cells whereas they are stable in the *pre1-1 pre4-1* mutant cells. It should be noted that cells lacking Stm1, as in *stm1-1*, survive a low-concentration H_2O_2 treatment, in contrast to the wild type. Taken all together, Stm1 is deemed to be an activator of apoptosis in yeast; it is normally degraded by the proteasome under the proliferation pathway. When the proteasome is suppressed, Stm1 is allowed to accumulate in quantities that lead to apoptosis (Ligr et al. 2001). How the proteasome is suppressed in vivo is unclear.

V. Mitochondria, Oxidative Stress, and Regulation of Apoptosis

The evolution of eukaryotic cells, by incorporating the mitochondria from an aerobic prokaryon into the power-generating system, has an enormous benefit in terms of energy production, but it comes with the cost of oxidative stress in the generation of ROS. Indeed, the mitochondrial respiratory chain is the main producer of ROS (reviewed in Green and Reed 1998; Mignotte and Vayssiere 1998). To overcome this oxidative stress, a slate of antioxidant molecules has also evolved, such as glutathione, superoxide dismutases, catalases and peroxidases. Furthermore, to maintain the homeostasis of a cell population, either in multicellular metazoans or in unicellular fungi, a system of PCD has also evolved for which modulation of ROS becomes the center for regulation of PCD.

A. Role of Mitochondria in Apoptosis

So, what is the role of mitochondria in PCD? It may be to sequester the apoptosis effector proteins, such as cytochrome c, **AIF** (apoptosis-inducing factor) or pro-caspases, etc., within the mitochondrial membrane space. In the model systems, such as *C. elegans* and mammalian cells, these proteins are normally not permanent to the outer membrane. In the process of PCD, these mediators of apoptosis are released to the cytosol either through the regulation of outer *m*itochondrial *m*embrane *p*ermeabilization

(**MMP**), through ruptures of the outer membrane, or through molecular channels. Thus, the regulation of MMP is an important control point for apoptosis, but how this regulation is achieved remains controversial (reviewed in Green and Reed 1998; Mignotte and Vayssiere 1998; Harris and Thompson 2000).

Mitochondria are the main action sites of Bcl-2 family proteins, which include proapoptotic (**Bax** and **Bak**) and antiapoptotic (**Bcl-2** and **Bcl-x_L**) proteins in mammalian systems. By using a yeast two-hybrid system to test protein–protein interactions, it has been suggested that homodimerization of Bax would lead to cell death whereas heterodimerization between Bax and Bcl-2 would lead to cell survival (Sato et al. 1994; Zha and Reed 1997). In yeast, Bax and Bcl-x_L are directly targeted to the outer mitochrondrial membrane (Gross et al. 2000; Poliaková et al. 2002; Polčic and Forte 2003). The most informative piece of evidence has been obtained by Polčic and Forte (2003) by using a system whereby Bax and Bcl-x_L in a yeast strain (CML282) can be independently and quantitatively regulated. When *BAX* is driven by *GAL1* promoter or *GAL10* promoter (*GAL-BAX*), the quantity of Bax protein produced is proportional to the amount of galactose present in the growth medium. When *BCL-XL* is driven by *tetO* promoter (*TET-BCL-XL*), in a CEN plasmid, the quantity of Bcl-x_L is proportional to the amount of doxycycline present in the growth medium. The results are most revealing – 1% of galactose gives the maximal Bax expression, but only 0.1% is sufficient to give maximum killing. Likewise, 0.5 μg/ml of doxycycline induces the maximum expression of Bcl-x_L, but only 0.1 μg/ml is needed to rescue cells from Bax-induced cell death at all concentrations of galactose used. Furthermore, the level of Bcl-x_L required for rescue does not change even if Bax expression is increased 30- to 40-fold by using multi-copy plasmids carrying *GAL-BAX* construct, making it unlikely for heterodimerization of Bcl-x_L with Bax to account for the rescue. This is consistent with the finding that a Bcl-x_L protein containing a mutation of amino acid 101 from tyrosine to lysine (Y101K), which blocks the ability of Bcl-x_L to heterodimerize with Bax, can rescue Bax-induced cell death in yeast with the same level of expression (Polčic and Forte 2003). If heterodimerization is ruled out, then what is the mechanism of cell death rescue? It would appear that Bax targeted to the mitochondrial membrane is required for killing, and indeed, when Bax alone is expressed in yeast, it is targeted to the mitochondrial membrane in a stable alkali-resistant manner. When Bax and Bcl-x_L are co-expressed in yeast, a significant amount of Bax is found in the cytosol (Polčic and Forte 2003). It is interesting to note that **VDAC** (*v*oltage-*d*ependent *a*nion *c*hannel) is not required for actions of either pro- or antiapoptotic members of the Bcl-2 family (Polčic and Forte 2003).

B. Oxidative Stress in Apoptosis

Evidence has been accumulating that ROS are regulators of apoptosis. For example, exogenous oxygen stress by application of hydrogen peroxide induces an apoptotic cell death in yeast (Madeo et al. 1999; Fröhlich and Madeo 2000; Chen et al. 2003 for review). Cell death induced by acetic acid is accompanied by a dramatic increase in production of ROS in yeast (Ludovico et al. 2002). Increased production of ROS is also found in *P. anserina* and *Coprinopsis cinerea* (*C. cinereus*) when confronted with non-self filamentous fungus (Silar 2005). The yeast ts-mutant $cdc48^{S565G}$ that exhibits the apoptotic phenotype also shows an accumulation of ROS, whereas nonapoptotic mutants of CDC48 or other cell-cycle mutants (e.g., *cdc2*, or *cdc31*) do not (Madeo et al. 1999). In yeast, oxygen stress is also induced in aging mother yeast cells (Laun et al. 2001), in chronologically aged cells (Fabrizio et al. 2004; Herker et al. 2004), and in auxotrophic mutant cells upon starvation of an essential amino acid (Eisler et al. 2004).

As discussed above, expression of the mammalian proapoptogenic Bax or Bak in yeast causes the apoptotic phenotype, and cell death is accompanied by an accumulation of ROS. This Bax-induced cell death is suppressed by co-expression of mammalian Bcl-2 or Bcl-x_L (Ligr et al. 1998; Fröhlich and Madeo 2000; Gross et al. 2000). Furthermore, expression of CED-9 of *C. elegans*, as that of Bcl-2 and Bcl-x_L of mammals, can also rescue apoptosis-like cell death induced by exogenous oxidative and heat stresses in yeast (Chen et al. 2003). There are conflicting reports with respect to the associated formation of ROS and cell death. In one case, Bcl-x_L suppresses not only Bax-induced cell death but also Bax-induced production of ROS in *S. cerevisiae* (Gross et al. 2000). In another, Bcl-x_L prevents Bax-induced cell death but not Bax-induced formation of ROS in *K. lactis* (Poliakova et al. 2002). The conflicting results suggest that there are other players in this complex problem that remain to be uncovered.

Besides ROS, mitochondrial lipid oxidation may also play a role in the Bax-induced PCD. Priault et al. (2002) have demonstrated that there is a substantial decrease in the amount of fatty acids and in the unsaturation index (unsaturated/saturated ratio) in mitochondria isolated from Bax-expressing yeast cells. This change is linked to mitochondrial respiration-induced oxidation or peroxidation of different classes of mitochondrial phospholipid. By a kinetic study, there is a strong link between Bax-induced cell death and Bax-induced mitochondrial lipid oxidation in the wild-type (**WtB1**) yeast. This link is further supported by the assay that known inhibitors of lipid oxidation (α-tocopherol and resveratrol) decrease Bax-induced cell death in yeast (Priault et al. 2002).

More recently, a direct link between ROS production and cell death has been uncovered by Pozniakovsky et al. (2005). These authors have established that elevated ROS level is essential in pheromone- and amiodarone-induced cell-death cascade in yeast, because quenching of ROS by antioxidant NAC (N-acetyl cysteine) prevented fragmentation of mitochondrial filaments and cell death. Amiodarone (2-butyl-3-benzofuranyl-4-[diethylamino]-ethoxyl-3,5-diiodophenylketone hydrochloride) is a fungicide, a Ca^{++} channel blocker, and it causes an increase of cytosolic Ca^{++} concentration that triggers mitochondrial membrane potential increase, followed by elevated ROS production (see references in Pozniakovsky et al. 2005). Moreover, glutathione is an endogenous means to overcome oxidative stress. A yeast strain (YPH98gsh1) lacking glutathione, caused by a deletion of the ***GSH1*** gene coding for G-glutamyl-cysteine synthetase, exhibits symptoms of apoptosis, and this strain is supersensitive to treatments with H_2O_2 (Madeo et al. 1999). The *gcs1/gcs1* null mutant of *C. albicans* lacking glutathione also results in increased ROS production and apoptosis (Baek et al. 2004). Besides, the apoptotic phenotype can be suppressed in yeast by oxygen radical scavengers such as free radical spin traps (e.g., 5 mM PBN or 0.5 mM TMPO), and by anaerobic growth conditions (Madeo et al. 1999).

C. Mitochondrial Respiration in Apoptosis

There is another controversy whether mitochondrial respiration is essential for PCD. Ludovico et al. (2002) have shown that PCD induced by acetic acid in *S. cerevisiae* is mitochondria-dependent: the Rho^{-}, the null *ATP10* mutant lacking mitochondrial ATPase, and the null *CYC3* mutant all fail to undergo PCD. The petite mutant of *S. cerevisiae* is also resistant to Bax-induced PCD (Greenhalf et al. 1996; Gross et al. 2000; Harris et al. 2000). Other studies have shown yeast respiration-deficient mutants to suffer a reduced and a delayed (by 12 h), but not abrogated, Bax-induced cell killing (Matsuyama et al. 1998). On the other hand, oxidative phosphorylation appears not to be a factor, because the wild-type yeast and mitochondrial mutants follow the same kinetics in Bax-induced growth arrest and cell killing (Kiššová et al. 2000). Surprisingly, in the yeast *Kluyveromyces lactis*, which is petite-negative and strictly aerobic, mitochondrial respiration is not required for Bax-induced cell killing. In fact, Rho^{-} mutant is more sensitive to Bax, with plating efficiency at 0%, compared to the wild type at 60% and the *mgi1-1* (ATPase) mutant at 20% (Poliakova et al. 2002).

Part of the controversy may rest with the way Bax is expressed in yeast. Most of the experiments reported above were done with *BAX* driven by *Gal10* promoter. This could be problematic when dealing with fermentation conditions, since *Gal10* promoter is strongly repressed by glucose, and yeast respiration-deficient mutants grow poorly in a galactose medium. A system was devised whereby the Bax expression is driven by a tetracycline-off (*tetO*) promoter that allows Bax and Bcl-x_L expression under fermentative as well as respiratory conditions (Priault et al. 1999a). There is no difference in Bax-induced growth arrest under any growth conditions using glucose or mannose as a carbon source, where glucose induces a strong catabolic repression of respiratory enzymes but mannose does not. The most revealing results are the kinetics of cell killing in different mutants under different growth conditions (Fig. 9.1). Under fermentative conditions using glucose as the carbon source, the wild-type (**WtB1**) yeast cells are resistant to Bax-induced cell killing, whereas under respiratory conditions using lactate as the carbon source, they are rapidly killed. It is interesting to note that, under respiro-fermentative conditions (cf. non-repressive sugars such as mannose), the Bax effect is intermediate. Similar result is seen in the adenine nucleotide carrier (**AtB1**) defective mutant (Priault et al. 1999a).

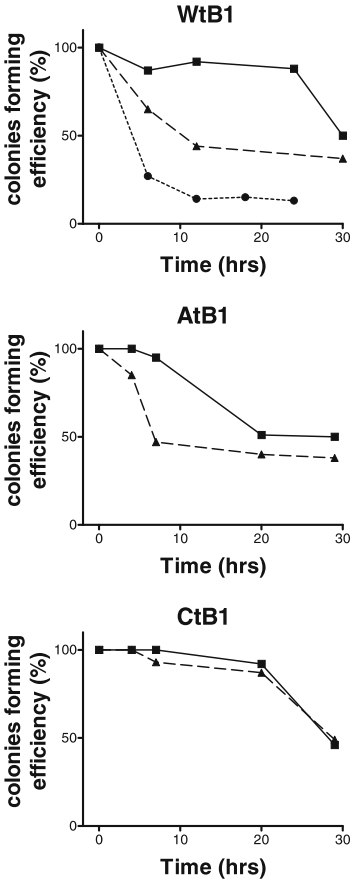

Fig. 9.1. Effects of a carbon source on Bax-induced programmed cell death in WtB1 (wild-type), AtB1 (ANC-defective), and CtB1 (cytochrome c-deficient) mutants of yeast: *squares* glucose (fermentative), *triangles* mannose (respiro-fermentative), *circles* lactate (non-fermentative). Reproduced, with permission from the authors and the publisher, from Priault et al. (1999a)

D. Cytochrome c Release and Transmembrane Potentials in Apoptosis

In multicellular organisms, an early symptom of apoptosis is the release of cytochrome c from the mitochondrial membrane space to the cytosol. This leads to the activation of the caspases, and a cascade of apoptotic events (see reviews in Mignotte and Vayssiere 1998). The release of cytochrome c from mitochondria is also found in apoptotic cell death induced by acetic acid in *S. cerevisiae* (Ludovico et al. 2002), in yeast mutant *asf1/cia1* lacking histone chaperone (Yamaki et al. 2001), and in Bax-expressing yeast cells (Manon et al. 1997). During acetic acid-induced PCD in the budding yeast, the mitochondrial transmembrane potential exhibits a transient hyperpolarization followed by depolarization (Ludovico et al. 2002). A decrease of mitochondrial transmembrane potential (by 50%) is also found in acetic acid-induced PCD in the food spoilage yeast *Z. bailii* (Ludovico et al. 2003), and in yeast lacking the histone chaperone ASF1/CIA1 (Yamaki et al. 2001). In one report, however, Bax-expressing yeast cells exhibit an increase (or hyperpolarization) in mitochondrial transmembrane potential, $\Delta\Psi m$, with no release of cytochrome c (Gross et al. 2000). The conflicting results remain unresolved. One possible suggestion could be related to the methodology of measuring transmembrane potential. The controversy has been reviewed (Ly et al. 2003).

It is clear, however, that release of cytochrome c into the cytosol is not essential for Bax-mediated cell killing in yeast (Gross et al. 2000; Roucou et al. 2000). This is demonstrated by the observation that, upon the expression of Bax in yeast cells, the cytochrome c-GFP fusion protein is not released, while the native cytochrome c is, suggesting that size is critical for its release through the selective pore. Nevertheless, cell killing occurs in both cases (Roucou et al. 2000). The question of mitochondrial *p*ermeability *t*ransition *p*ore (**PTP**) remains controversial. For Bax-mediated apoptosis in yeast, the mitochondrial F_0F_1-ATPase proton pump is required, and its inhibitor, oligomycin, partially inhibits Bax-induced cell death (Matsuyama et al. 1998). Other mitochondrial biochemistry (e.g., Δatp2, Δatp4, Δcyc1, 7; Δcyp3, Δpor1/2; ρ-) may also play a role in cell killing, albeit with variable results (evidenced in Gross et al. 2000).

As pointed out above, the release of cytochrome c from mitochondria is often associated with PCD, but massive cytochrome c relocalization appears not to be absolutely required for Bax-induced cell killing in yeast (Priault et al. 1999a). Indeed, Bax-induced cell killing is the same in the cytochrome c-less mutant as in the wild type, indicating that cytochrome c is not required for PCD. However, as demonstrated with the yeast cytochrome c-less strain, the kinetics of colony-forming efficiency is identical whether glucose or mannose is used as the carbon source (**CtB1**, Fig. 9.1). This is contrary to what is observed in the wild-type yeast (**WtB1**, Fig. 9.1); there is no rapid killing in the mutant **CtB1** when mannose is used as the carbon source. This would indicate that cytochrome c has a role in the rapid killing process in yeast, as contended by Priault et al. (1999a). After all, cytochrome c is a part of the mitochondrial respiration chain, and ATP is required for an optimal effect of Bax.

These authors concluded that a high ATP/ADP ratio of yeast is not necessary for Bax-induced growth arrest, but is required for a rapid Bax-induced cell death (Priault et al. 1999a,b).

E. Mitochondrial Fission and Apoptosis

Mitochondrial fission and fusion are normal mitochondrial function (Bossy-Wetzel et al. 2003). However, mitochondrial fission or fragmentation leading to damages to mitochondrial integrity is a key step in apoptosis in mammals and *C. elegans* (Bossy-Wetzel et al. 2003; Lee et al. 2004; Jagasla et al. 2005). Key players are dynamin-like GTPase family proteins, namely, yeast Dnm-1, its homolog Drp-1 (dynamin-related protein 1) of mammals and *C. elegans*; yeast Mgm-1 (mitochondrial genome morphology 1), its mammalian ortholog Opa-1 (optic atrophy 1) and mammalian Mfn-1 (mitofusion 1). These factors have opposing effects, Dnm-1/Drp-1 for mitochondrial fission and Mgm1/Opa-1 for fusion (Jones and Fangman 1992; Bossy-Wetzel et al. 2003; Sesaki et al. 2003; Wong et al. 2003; Sesaki and Jensen 2004; Lee et al. 2004). The function of Mgm1 in yeast requires Fzo1 (for Fuzzy onion) and Ugo1 (ugo is Japanese for fusion), a defect in any one of which leads to damages to mtDNA and cell death (Sesaki et al. 2004). Other players are Fis-1 and Mdv1/Net2 (for mitochondrial division or networks), the latter being a WD40 motif containing dynamin-interacting family protein (Cerveny et al. 2001; Cerveny and Jensen 2003; Lee et al. 2004; Fannjiang et al. 2004). Yeast yFis-1 is an 18-kDa protein that is anchored diffusely to the mitochondrial outer membrane by a C-terminal transmembrane domain characteristic of Bcl-2 (Mozdy et al. 2000). It probably mediates normal mitochondrial fission in yeast, because *Δfis-1* cells exhibit interconnected mitochondrial net-like morphology, as shown by fluorescent and electron microscopy (Fannjiang et al. 2004). Interestingly, yFis1p is antiapoptotic, as is mammalian Bcl-2, because *Δfis-1* cells are more sensitive to acetic acid-induced cell death than are *Δdnm1* cells (Fannjiang et al. 2004). It is possible that mitochondrial fission caused by yFis-1 is different from that caused by Dnm-1 in yeast, the latter leading to PCD. This above finding is in sharp contrast to that found in mammalian cells where hFis-1 is proapoptotic, and down-regulation of hFis-1 powerfully inhibits cell death (Lee et al. 2004).

In the mammalian system, hFis-1p contains signal sequence targeting it to the outer mitochondrial membrane. Bax is normally located in the cytosol, and only upon death signal is it recruited to the mitochondrial outer membrane (Lee et al. 2004). Here hFis-1p is required for Bax translocation to the outer mitochondrial membrane, and Bax for complexing with Drp-1 where Drp-1p is required for mitochondrial fragmentation and cytochrome c release (Lee et al. 2004). The expression of the dominant negative inhibitor of Drp-1, Drp-1^{K38A}, in HeLa cells prevents mitochondrial fragmentation, and inhibits apoptosis but does not prevent Bax translocation. Bax, and the fission protein complex, are co-localized to the constriction sites of mitochondrial fission in dying HeLa cells, as demonstrated by silver enhancement of immunogold labeling, and examined by electron microscopy (Karbowski et al. 2002).

Since there is no Bax or Bcl-2 in yeast, a mediator will be needed. This is found in the Dnm1-interacting factor, WD40 repeat protein Mdv1/Net2, which is required for mitochondrial fragmentation (Fannjiang et al. 2004). Interestingly, there is no mammalian homolog to this mediator. WD40 repeat protein Mdv1p/Net2p functions as a molecular adaptor by interacting with Dnm1p and yFis1p during mitochondrial fission in yeast cells (Tieu et al. 2002). Mdv1/Net2 contains a novel N-terminal extension region (NTE) that directly interacts with yFis1p, and a C-terminal region that contains seven WD repeats (WD) and directly interacts with Dnm1p. By using GFP-conjugates, GFP-Mdv1p interacts and co-localizes with Dnm1p in punctate structures associated with mitochondria in a yFis1p-independent manner, and this punctate structure is essential for mitochondrial fission function in yeast. Without Dnm1p, GFP-Mdv1p is uniformly localized to the mitochondrial outer membrane; without both Dnm1p and yFis1p, GFP-Mdv1p is diffused in the cytosol (Tieu and Nunnari 2000; Tieu et al. 2002). Yeast yFis1p has two important regulatory functions, first, to regulate assembly of Dnm1p into punctate structures and target them to mitochondria, and second, to regulate a rate-limiting, Dnm1p-dependent event during mitochondrial fission (Tieu et al. 2002). yFis1p inhibits H_2O_2-induced, and protease-dependent, cell death in yeast, much like mammalian Bcl-2 or Bcl-x_L (Fannjiang et al. 2004). Yeast yFis1p is not required for mitochon-

drial fission during cell death. Instead, yFis1p limits mitochondrial fission and death by blocking an irreversible step mediated by Dnm1p that leads to loss of function of mitochondria (Fannjiang et al. 2004). Interestingly, yFis1p has a duel function. One works for mitochondrial fission in normal growing cells, the other for regulating the cell-death pathway in much the same way as mammalian Bcl-2; over-expression of mammalian Bcl-2 or Bcl-x_L from a GAL1 expression plasmid restores viability of acetic acid-treated *Δfis1* cells as efficiently as over-expressed yeast yFis1. However, Bcl-2 or Bcl-x_L fail to replace the fission function of yFis1p (Fannjiang et al. 2004). Another gene has been identified in yeast (YHR 155W) that codes for a mitochondrial protein, **Ysp1** (*y*east *s*uicide *p*rotein 1). This gene is required for amiodarone-induced mitochondrial fragmentation (also known as the thread-grain transition), and it acts at a late stage, long after ROS levels increase (Pozniakovsky et al. 2005). Taken all together, these data led Bossy-Wetzel et al. (2003) to conclude: "mitochondrial fission per se does not result in cell death. However, cell death does not occur without mitochondrial fragmentation".

The best evidence that links mitochondrial fragmentation to cell death came from studies in *C. elegans*. Here Drop1-induced mitochondrial fission leading to PCD is *egl-1*- (egg laying-defective), *ced-4*-, and *ced-3*- (cell death-defective) dependent; the transcription activation of *egl-1* is the earliest event signaling commitment to the cell-death pathway (Jagasla et al. 2005). In *C. elegans* development, certain cells are destined to live and others are destined to die, all in one and the same animal. Jagasla et al. (2005) expressed a mitochondrial matrix-targeted green fluorescent protein (mitoGFP) under the control of the *egl-1* promoter in wild-type embryos. The appearance of GFP signal marks the onset of apoptotic process, and this only in cells destined to die. Mitrochondria are stained with the fluorescent dye rhodamine B hexyl ester and examined by confocal time-lapse microscopy. Interestingly, within 8 min 52 s of transcription activation, mitochondrial network in GFP-positive cells starts to break down, resulting in a few clusters of mitochondrial fragments located at the periphery of the cells at 16 min 8 s. In the same experiment with animals homozygous for the *egl-1* loss-of-function mutation, cells destined to die do not die, and mitochondria in GFP-positive cells retain their tubular network. Thus, mitochondrial fragmentation is an early event in the apoptotic pathway. It should be noted that EGL-1 is also a BH3-only protein, an activator of apoptosis, and its effect on mitochondrial fragmentation is specific for apoptosis (Jagasla et al. 2005).

VI. Autophagy and the Type II Programmed Cell Death

A. Autophagy Is a Cellular Recycling Program

Proteins and cell organelles like ribosomes, mitochondria and peroxisomes may be degraded for nutrient reuse. This is distinct from the ubiquitin-proteasome pathway discussed in this chapter. Autophagy is an inducible pathway; it can be triggered by a carbon or a nitrogen source starvation that inhibits **TOR** (*t*arget *o*f *r*apamycin) serine/threonine protein kinase, an upstream nutrient sensor (Raught et al. 2001). Autophagy can also be induced by a treatment with rapamycin. The terminal target site of autophagic degradation in yeasts, and probably in all fungi, is in the vacuole, which is the equivalent of lysosomes of higher eukaryotes. The loading of hydrolytic enzymes to the vacuole/lysosome is achieved by three separate pathways: (1) by *t*rans-*G*olgi *n*etwork (**TGN**), (2) by the *m*ulti-*v*esicular *b*ody (**MVB**) pathway (Khalfan and Klionsky 2002), and (3) by the *c*ytoplasm-to-*v*acuole *t*argeting (**Cvt**) pathway (Reggiori and Klionsky 2002). The Cvt pathway is a biosynthetic vacuolar trafficking pathway and it operates constitutively under nutrient-rich physiological conditions, but not induced by starvation. The formation of Cvt vesicles shares the same processes as those of autophagosomes.

There are two major catabolic multi-membrane trafficking pathways in yeast: the macroautophagy and the microautophagy pathways, and these are inducible by nutrient starvation. Macroautophagy is a generalized multi-membrane trafficking pathway for all proteins and organelles. A large number of genes involved in autophagy have been identified, and these are named ***apg***, ***aut***, and ***cvt*** genes (see the list in Klionsky and Emr 2000; Reggiori and Klionsky 2002). The last step is to deliver all cargos to the terminal acceptor compartment, the vacuole (Huang and Klionsky 2002; Meiling-Wesse et al. 2002a,b; Mizushima et al. 2002; Wang et al. 2002). The single membrane is hydrolyzed by lipases, releasing the cargos for hydrolytic degradation.

B. Autophagic Cell Death (ACD)

Autophagy is not just the machinery for intracellular recycling – it may also be recruited for PCD, in which case it is called autophagic cell death (**ACD**). The link was initially studied in dying *Drosophila* salivary gland cells (Baehrecke 2003; Lee et al. 2003) and in mammalian cell death (reviewed in Bursch 2001). The molecular evidence that provides a direct link between autophagy and ACD is found in ***idi*** genes (*i*nduced *d*uring the *i*ncompatibility reaction) of *P. anserina* (Pinan-Lucarré et al. 2003). IDI-7 is an ortholog of yeast Aut7/Apg8 that is highly conserved from yeast to mammals (Lang et al. 1998; Kim et al. 2001; Pinan-Lucarré et al. 2003). Aut7 is a key component of major pathways for autophagosome formation in yeast (Kirisako et al. 1999; Kim et al. 2001). Under nitrogen starvation, Aut7 is induced and up-regulated in yeast at the transcriptional level, and the Aut7 is relocalized from the cytoplasm to the vacuoles and to the perivacuolar structures. Likewise, GFP-IDI7 protein shows diffuse cytoplasmic distribution under a rich nutrient medium. When autophagy is induced by nitrogen starvation in *P. anserina*, GFP-IDI7 is up-regulated and relocalized from the cytoplasm to the perivacuolar structure as well as to the lumen of vacuoles. When cell death is induced in the self-incompatible *het*-R *het*-V strain of *P. anserina* under the restrictive temperature of 26 °C, the GFP-IDI7 fusion protein is also up-regulated six-fold and localized mostly in the perivacuolar structures as well as inside the vacuoles (Pinan-Lucarré et al. 2003). Here the molecular events leading to autophagy are also part of the process leading to autophagic cell death.

C. Cytological Phenotype of ACD

In higher eukaryotes, the phenotype of ACD is the presence of autophagic vesicles in the cytoplasm. ACD is caspase-independent and the cells are TUNEL negative (Bursch 2001; Cohen et al. 2002). The formation of the central vacuole system is the hallmark of autophagic cell death in yeast, and it is controlled by the ***ARL1*** (*A*DP *r*ibosylation factor-*l*ike protein-1). A deficient mutant *arl1Δ* (also known as *dlp1*) lacks central vacuoles, and instead exhibits numerous small vesicles in the cytoplasm. This mutation causes a delay in cell death in *cdc28 dlp1* mutant, and it completely abrogates Bax-induced cell death inW303 *dlp1/Bax* (Abudugupur et al. 2002). Examination by light and electron microscopy shows that the dying yeast *cdc28* cells exhibit vacuoles containing autophagic body-like particles, or a large number of vacuolar vesicles in parent cells, probably as a result of fragmentation of the central vacuoles (Fig. 9.2A–C). In addition, multiple small vesicles (or multi-vesicular bodies) are sometimes observed in the epiplasm, the space between the plasma membrane and the cell wall, in cells undergoing autophagic cell death (Abudugupur et al. 2002). The accumulation of autophagic vesicles in the central vacuole has also been demonstrated by electron microscopy in *Δidi-6/ΔpspA* mutant of *P. anserina* (Pinan-Lucarré et al. 2003).

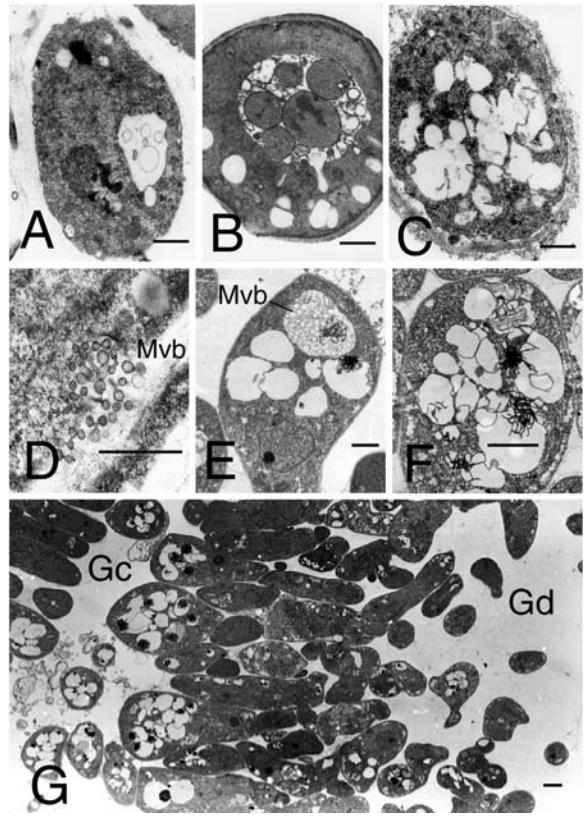

Fig. 9.2. **A–G** Electron microscopy of autophagic cell death. **A–C** Yeast showing progressive vacuolation. Reproduced, with permission of the authors and the publisher (www.nature.com/cdd), from Abudugupur et al. (2002). **D–G** Autophagic cell death in *C. cinereus*. **D** Multi-vesicular bodies (*Mvb*) in a basidium at the end of meiosis before autolysis. **E, F** Cells from the gill cavity (*Gc*) area of a fruiting primordium showing *Mvb* and progressive vacuolation. **G** Gill tissue inclusive of the gill cavity (*Gc*) area and the gill domain (*Gd*); severe vacuolation and tissue destruction are seen only in the *Gc* area. Bar = 1 μm

Although the cytological phenotype of autophagic cell death (type II PCD) is distinct from that of apoptotic cell death (type I PCD), the two pathways are not mutually exclusive. In fact, crosstalk in the same cell may occur (reviewed in Bursch 2001; Cohen et al. 2002). Examples of crosstalk is found in yeast defective of histone chaperone where dead *asf-1/cia-1* cells exhibit the phenotype of apoptosis, together with autophagic vesicles in the vacuole normally found in autophagy (Yamaki et al. 2001). Moreover, a common gene, ***Uth1***, has been found in yeast that is required for both apoptotic and autophagic pathways. ***Uth1*** is required for the late steps of Bax-induced apoptosis, namely, mitochondrial lipid oxidation, maintenance of plasma membrane integrity, and accumulation of ROS (hydrogen peroxide). It is also involved in a form of cell death that is different from apoptosis, i.e., rapamycin-induced cell death related to autophagy (Camougrand et al. 2003). For the latter, a *Δuth1* mutant is resistant to rapamycin, but only when cells are grown on a non-fermentable carbon source (lactate) under conditions in which mitochondria are fully differentiated, indicating a mitochondria connection.

Mitochondrial membrane permeabilization (**MMP**) has been suggested as a major 'checkpoint' that determines whether apoptosis occurs in higher eukaryotes. Induction of MMP at a low level, not enough to trigger apoptosis, may trigger mitochondria-specific autophagy. Here MMP may provide a link for crosstalk between apoptotic and autophagic cell-death pathways (Cohen et al. 2002). It is interesting to note that down-regulation of the antiapoptotic gene *Bcl-2*, by expression of a *Bcl-2* antisense message, causes massive autophagic cell death in human leukemic HL60 cells (Saeki et al. 2000).

In *C. cinereus* (*Coprinopsis cinerea*) and allies, mushrooms undergo deliquescence for spore dispersal (Buller 1931; Moore 2003). After completion of meiosis and spore formation, the basidia and the neighboring cells exhibit a large number of multivesicular bodies (**mvb**, Fig. 9.2D); this occurs before stipe elongation and deliquescence. When a *C. cinereus* fruiting body is pulverized in a buffer solution, the crude extract contains enzymes that can digest cell walls, proteins and all cellular components (Lu, unpublished data). Although there is no critical genetic study, it is quite possible that deliquescence is a form of autophagic cell death in which lysosomes are involved.

D. Autophagic Cell Death and Tissue Remodeling

Autophagic cell death is involved in tissue remodeling, organ morphogenesis, and cavity formation in higher eukaryotes (see review in Bursch 2001). Similar observation has been made in tissue remodeling and gill development in the mushroom of *C. cinereus* (Lu 1991). By light microscopy, at the earliest stage of gill differentiation, the hymenium appears solid, there are no gaps, and no cell disintegration. Shortly after the gills are clearly defined, the area of gill cavity appears to show gaps, suggesting cellular disintegration, as if the gills have been carved out (Lu 1991). By electron microscopy, cells in the gill cavity area exhibit prominent membrane blebbing, vacuolation, multi-vesicular systems, and residual bodies in the vacuoles, but no chromatin condensation (Fig. 9.2E–F). These observations were criticized as fixation artifacts (Moore 1996). If these were fixation artifacts, one would expect such images to be universally and randomly distributed in all tissues. However, this is not the case; they occur only in a destined area where cellular debris are found, specifically in the gill cavity, and never in the internal area of the gill domain (Fig. 9.2G). Besides, the phenotypes of vacuolation in the basidia of *C. cinereus* (Fig. 9.2F, as compared to Fig. 9.2C) provide evidence that autophagic cell death may be involved. I must admit that these are preliminary observations, and more studies are needed to establish that ACD plays a key role in gill morphogenesis in *C. cinereus*. Morphogenetic cell death has also been observed in the development of fungal fruiting bodies in *Agaricus bisporus*, *Coprinus domestica* (*Coprinellus domestica*), and other fungal species (Umar and van Griensven 1997, 1998).

VII. Meiotic Apoptosis

Meiosis is a vital link between the old and the new generation in eukaryotic organisms (see Zickler, Chap. 20, this volume). In fungi, having a haploid life cycle, it starts with the meiotic DNA replication that occurs before karyogamy (reviewed in Lu 1996), during which two compatible nuclei of a dikaryon fuse to form the only diploid nucleus in the life cycle. Karyogamy is immediately followed by meiotic division, in which events of homologous chromosome pairing and genetic recombina-

tion occur. This meiotic process will provide the genetic mechanism of random segregation and independent assortment to create genetic diversity among the progeny. The completion of meiosis will result in production of sexual spores that can be disseminated and can withstand harsh environmental conditions, an important survival and life preservation devise. In this regard, any genetic defect or physiological damage during meiosis will be detrimental to the survival of the species. Thus, to overcome such a catastrophe and to prevent defective vital genes from passing on, the evolution of meiosis-specific PCD seems plausible.

Meiotic cells are diploid, and they are not as easy to manipulate as are haploid cells when it comes to studying mutations that affect meiotic events. This complication is exacerbated by the mating-type genes in the tetrapolar sexuality of some basidiomycetes where selfing is incompatible (see Casselton and Challen, Chap. 17, this volume). To overcome this problem, a model system has been found in a homokaryon AmutBmut of *C. cinereus*, where mutation in both the *A* and the *B* mating-type loci would eliminate the need of mating to produce fruiting bodies, and to progress through meiosis and sporulation (Swamy et al. 1984). In addition, meiosis in *C. cinereus* is synchronous and its progression is regulated by light–dark cycles (Lu 1967, 2000). Thus, this homokaryon is well suited for studies in meiotic events, including meiotic apoptosis (Lu 1996; Celerin et al. 2000; Lu et al. 2003). With this strain, a large number of white-cap mutants have been created at ETH Zürich, either by restriction enzyme-mediated integration mutagenesis (called **REMI** mutants) or by **UV** irradiation (Granado et al. 1997; Kües et al., personal communication). With a simple hematoxylin staining, these white-cap mutants were discovered to exhibit meiotic PCD (Lu and Kües 1999). Further investigation, using light and electron microscopy, has revealed that these white-cap mutants can be classified into four cytologically distinguishable groups – three show defects in the meiotic prophase I, one shows defects in sporulation, and all four groups exhibit basidia-specific PCD, with the hallmarks of apoptosis (Lu et al. 2003).

Apoptosis in *C. cinereus* is basidia-specific. The phenotypes are synchronous chromatin condensation (Fig. 9.3A), DNA fragmentation as shown by TUNEL assay (Fig. 9.3B), and cytoplasmic shrinkage in basidia that are grossly deformed and DAPI negative, while the neighboring paraphyses are perfectly healthy, showing a bright nuclear stain with

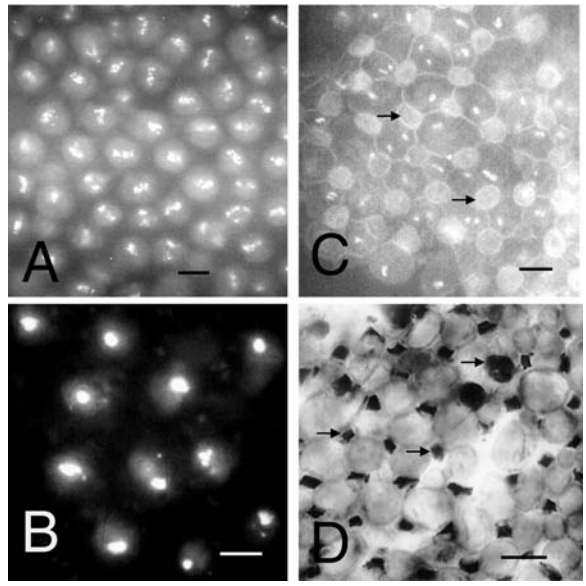

Fig. 9.3. A–D Meiotic apoptosis in *C. cinereus*. **A** Synchronous chromatin condensation (by DAPI stain) associated with meiotic arrest at meta-anaphase I. **B** TUNEL positive basidia. **C** Apoptotic (shrunken) basidia (*arrowed*) are DAPI negative whereas the neighboring paraphyses are DAPI positive. Reproduced from Lu et al. (2003). **D** The end stage of apoptosis, showing very shrunken basidia (*arrowed*) stained with propiono-iron hematoxylin. Bar = 10 µm

DAPI (Fig. 9.3C). All these are associated with specific meiotic arrest at metaphase–anaphase I. The end stage can be demonstrated with a simple hematoxylin stain (Fig. 9.3D). All meiotic mutants produce few tetrads that somehow escaped death at the end stage (see Lu et al. 2003). Some apoptotic phenotypes have also been documented in the *spo11-1* mutant, whose identity is based on DNA sequence similarity to yeast *spo11* (Celerin et al. 2000). For the sporulation mutants, apoptosis is triggered at the tetrad stage (Lu et al. 2003).

Regardless of the time of defect, all meiotic mutations trigger apoptosis in *C. cinereus* at a single entry point (Lu et al. 2003). Only when the arrest of meiosis is abrogated to enter anaphase I is apoptosis triggered. The formation of a spindle, initially well formed and then broken down, has been demonstrated in the mutant *spo11-1* by using the anti-tubuline antibody (Celerin et al. 2000). These observations strongly suggest that entry into the meiotic apoptotic pathway in *C. cinereus* is under the metaphase spindle checkpoint control. This is very different from the multiple checkpoint entries found in mice (reviewed in Lu et al. 2003). Thus, in this AmutBmut homokaryon, meiosis can

be arrested at diffused diplotene under a continuous light regime (Lu 2000), and no triggering of apoptosis will occur. When the arrest is released with a 3-h dark period, meiotic progression will resume and the timing of apoptosis can be accurately identified (Lu et al. 2003).

Why is meiosis-specific apoptosis important in some organisms, and not in others? It occurs in *C. cinereus* but not in yeasts, or *Neurospora*, where meiosis goes to completion irrespective of any genetic damages (reviewed in Lu 1996). The same dichotomy is found in higher eukaryotes. It is found in mammals (e.g., mice) but not in *Drosophila*, *C. elegans*, nor plants. Why evolution has taken a different path is not clear, and one can only speculate. As suggested by Money (2003), basidiospores of *C. cinereus* are carried to a fertile ground to compete with other microbes, to sustain growth, and then to find a mate. Chances of success are really quite remote, but particularly critical for *C. cinereus* whose sexuality is a tetrapolar mating system, whereby only one quarter of the progeny will be compatible. For altruistic reasons, evolution has selected meiotic apoptosis to ensure that all spores produced are genetically healthy for the survival of the species. Apoptosis is basidia-specific, because if the damage is induced during meiosis, apoptosis will remove the damaged cells and allow the mushroom to recover by generating new basidia, such as the case with diplotene arrest reported earlier (Lu et al. 2003). As for mammals, this is even more critical, because the litter size is small; the evolution of meiotic apoptosis is much more refined than to have a continuous molecular safety check – hence, the multiple checkpoint controls. As for yeast, *Neurospora*, *Drosophila*, *C. elegans*, and plants, they all produce a large number of progeny and their mating system is simple, but it is not at all clear why they do not have meiotic apoptosis.

VIII. Conclusion

There is no question that programmed cell death (PCD) operates in fungi, albeit at a prototypal level, and it includes apoptotic and autophagic pathways. The hallmarks of apoptosis can be induced by the expression of heterologous proapoptotic genes in yeasts. They can also be induced by exogenous stimuli, genetic defects, pheromones, oxygen stress, and even by aging, and cell death induced by all of the above can be rescued by the co-expression of antiapoptotic genes. Most importantly, the PCD is intimately connected to mitochondria and to the ubiquitin-proteasome system, in much the same way as in the metazoans. It also plays a role in tissue remodeling. The discovery of caspase-like or metacaspase genes, a possible homolog of a Bcl-2 family gene, and a homolog of AIF, BI-1, and IAP genes in yeast and other fungi makes the story compelling, and even the skeptics have begun to see the light. Meiotic apoptosis occurs in some species, and not in others. This dichotomy is found in fungi as well as in animals. This intriguing question remains to be explored.

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References

Abudugupur A, Mitsui K, Yokota S, Tsurugi K (2002) An *ARL1* mutation affected autophagic cell death in yeast, causing a defect in central vacuole formation. Cell Death Differ 9:158–168

Amin S, Mousavi A, Robson GD (2003) Entry into the stationary phase is associated with a rapid loss of viability and an apoptotic-like phenotype in the opportunistic pathogene *Aspergillus fumigatus*. Fungal Genet Biol 39:221–229

Baehrecke EH (2003) Autophagic programmed cell death in *Drosophila*. Cell Death Diff 10:940–945

Baek Y-U, KimY-R, Yim H-S, Kang S-O (2004) Disruption of G-glutamyl-cysteine synthetase results in absolute glutathione auxotrophy and apoptosis in *Candida albicans*. FEBS Lett 556:47–52

Berndt C, Bech-Otschir D, Dubiel W, Seeger M (2002) Ubiquitin system: JAMMing in the name of the lid. Curr Biol 12:R815–R817

Birnbaum MJ, Clem RJ, Miller LK (1994) An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J Virol 68:2521–2528

Bossy-Wetzel E, Barsoum MJ, Godzik A, Schwarzenbacher R, Lipton SA (2003) Mitochondrial fission in apoptosis, neurodegeneration and aging. Curr Opin Cell Biol 15:706–716

Bourges N, Groppi A, Barreau C, Clavé C, Begueret J (1998) Regulation of gene expression during the vegetative incompatibility reaction in *Podospora anserina*: characterization of three induced genes. Genetics 150:633–641

Boyce M, Degterev A, Yuan J (2004) Caspases: an ancient cellular sword of Damocles. Cell Death Diff 11:29–37

Buller AHR (1931) Researches on Fungi, vol 4. Longmans Green, London

Burhans W, Weinberger M, Marchetti MA, Ramachandran L, D'Urso GD, Huberman JA (2003) Apoptosis-like yeast cell death in response to DNA damage and replication defects. Mutat Res 532:227–243

Bursch W (2001) The autophagosomal-lysosomal compartment in programmed cell death. Cell Death Differ 8:569–581

Camougrand N, Grelaud-Coq A, Marza E, Priault M, Bessoule JJ, Manon S (2003) The product of the *UTH1* gene, required for Bax-induced cell death in yeast, is involved in the response to rapamycin. Mol Microbiol 47:495–506

Cande C, Vahsen N, Kouranti I, Schmitt E, Daugas E, Spahr C, Luban J, Kroemer RT, Giordanetto F, Garrido C et al. (2004) AIF and cyclophilin A cooperate in apoptosis-associated chromatinolysis. Oncogene 23:1514–1521

Carratore RD, Croce CD, Simili M, Taccini E, Scavuzzo M, Sbrana S (2002) Cell cycle and morphological alterations as indicative of apoptosis promoted by UV irradiation in *S. cerevisiae*. Mutat Res 513:183–191

Celerin M, Merino ST, Stone JE, Menzle AM, Zolan ME (2000) Multiple roles of *Spo11* in meiotic chromosome behavior. EMBO J 19:2739–2750

Cerveny KL, Jensen RE (2003) The WD-repeats of Net2p interact with Dnm1p and Fis1p to regulate division of mitochondria. Mol Biol Cell 14:4126–4139

Cerveny KL, McCaffery JM, Jensen RE (2001) Division of mitochondria requires a novel DNM1-interacting protein, Net2p. Mol Biol Cell 12:309–321

Chae H-J, Ke N, Kim H-R, Chen S, Godzik A, Dickman M, Reed JC (2003) Evolutionarily conserved cytoprotection provided by Bax Inhibitor-1 homologs from animals, plants, and yeast. Gene 323:101–113

Chen S-R, Dunigan DD, Dickman MB (2003) Bcl-2 family members inhibit oxidative stress-induced programmed cell death in *Saccharomyces cerevisiae*. Free Rad Biol Med 34:1315–1325

Cohen I, Castedo M, Kroemer G (2002) Tantalizing thanatos: unexpected links in death pathways. Trends Cell Biol 12:293–295

Dememthon K, Saupe SJ (2005) DNA-binding specificity of the IDI-4 basic leucine zipper factor of *Podospora anserina* defined by systematic evolution of ligands by exponential enrichment (SELEX). Eukaryot Cell 4:476–483

Dementhon K, Paoletti M, Pinan-Lucarré B, Loubradou-Bourges N, Sabourin M, Saupe SJ, Clavé C (2003) Rapamycin mimics the incompatibility reaction in the fungus *Podospora anserina*. Eukaryot Cell 2:238–246

Dementhon K, Saupe SJ, Clavé C (2004) Characterization of IDI-4, a bZIP transcription factor inducing autophagy and cell death in the fungus *Podospora anserina*. Mol Microbiol 53:1625–1640

Deveraux QL, Reed JC (1999) IAP family proteins – suppressors of apoptosis. Genes Dev 13:239–252

Drexler HCA (1998) Programmed cell death and the proteasome. Apoptosis 3:1–7

Eisler H, Fröhlich K-U, Heidenreich E (2004) Starvation for an essential amino acid induces apoptosis and oxidative stress in yeast. Exp Cell Res 300:345–353

Ellgaard L, Helenius A (2001) ER quality control: towards an understanding at the molecular level. Curr Opin Cell Biol 13:431–437

Ellis RE, Yuan J, Horvitz HR (1991) Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663–698

Esser K, Blaich R (1973) Heterogenic incompatibility in plants and animals. Adv Genet 17:107–152

Esser K, Keller W (1976) Genes inhibiting senescence in the ascomycete *Podospora anserina*. Mol Gen Genet 144:107–110

Esser K, Tudzynski P (1979) Genetic control and expression of senescence in *Podospora anserina*. In: Lemke PA (ed) Viruses and plasmids in fungi. Dekker, New York, pp 595–615

Esser K, Tudzynski P (1980) Senescence in fungi. In: Thimann KV (ed) Senescence in Plants, vol II. CRC Press, Boca Raton, pp 67–83

Esser K, Tudzynski P, Stahl U, Kück U (1980) A model to explain senescence in the filamentous fungus *Podospora anserina* by the actin of plasmid-like DNA. Mol Gen Genet 178:213–216

Fabrizio P, Battistella L, Vardavas R, Gattazzo G, Liou L-L, Diaspro A, Dossen JW, Gralla EB, Longo VD (2004) Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. J Cell Biol 166:1055–1067

Fahrenkrog B, Sauder U, Aebi U (2004) The *S. cerevisiae* HtrA-like protein Nma111p is a nuclear serine protease that mediates yeast apoptosis. J Cell Biol 117:115–126

Fannjiang Y, Cheng W-H, Lee SJ, Qi B, Pevsner J, McCaffery JM, Hill RB, Basanez G, Hardwick JM (2004) Mitochondrial fission proteins regulate programmed cell death in yeast. Genes Dev 18:2785–2797

Finley D, Chau V (1991) Ubiquitination. Annu Rev Cell Biol 7:25–69

Fröhlich K-U, Madeo F (2000) Apoptosis in yeast – a monocellular organism exhibits altruistic behaviour. FEBS Let 473:6–9

Garnjobst L, Wilson JF (1956) Heterocaryosis and protoplasmic incompatibility in *Neurospora crassa*. Proc Natl Acad Sci USA 42:613–618

Gerlinger U-M, Gückel R, Hoffmann M, Wolf DH, Hilt W (1997) Yeast cycloheximide-resistant *crl* mutants are proteasome mutants defective in protein degradation. Mol Biol Cell 8:2487–2499

Glass NL, Kaneko I (2003) Fatal attraction: nonself recognition and heterokaryon incompatibility in filamentous fungi. Eukaryot Cell 2:1–8

Glass NL, Jacobson DJ, Shiu PKT (2000) The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annu Rev Genet 34:165–186

Glickman MH, Rubin DM, Fried VA, Finley D (1998) The regulatory particle of *Saccharomyces cerevisiae* proteasome. Mol Cell Biol 18:3149–3162

Granado JD, Kertesz-Chaloupkova K, Aebi M, Kües U (1997) Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. Mol Gen Genet 256:28–36

Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309–1312

Greenhalf W, Stephan C, Chaudhuri B (1996) Role of mitochondria and C-terminal membrane anchor of Bcl-2 in Bax induced growth arrest and mortality in *Saccharomyces cerevisiae*. FEBS Lett 380:169–175

Griffiths AJF (1992) Fungal senescence. Annu Rev Genet 26:351–372

Gross A, Pilcher K, Blachly-Dyson E, Basso E, Jockel J, Bassik MC, Korsmeyer SJ, Forte M (2000) Biochemical and genetic analysis of the mitochondrial response of yeast to BAX and BCL-XL. Mol Cell Biol 20:3125–3136

Harris MH, Thompson CB (2000) The role of the Bcl-2 family in the regulation of outer mitochondrial membrane permeability. Cell Death Differ 7:1182–1191

Harris MH, van der Heiden MG, Kron SJ, Thompson CB (2000) Role of oxidative phosphorylation in Bax toxicity. Mol Cell Biol 20:3590–3596

Heinemeyer W, Kleinschmidt JA, Saidowsky J, Escher C, Wolf DH (1991) Proteinase yscE, the yeast proteasome/multicatalytic-multifunctional proteinase: mutants unravel its function in stress induced proteolysis and uncover its necessity for cell survival. EMBO J 10:555–562

Hengartner MO (1997) Apoptosis and the shape of death. Dev Genet 21:245–248

Herker E, Jungwirth H, Lehmann KA, Maldener C, Fröhlich K-U, Wissing S, Buttner A, Fehr M, Sigrist S, Madeo F (2004) Chronological aging leads to apoptosis in yeast. J Cell Biol 164:501–507

Huang W-P, Klionsky DJ (2002) Autophagy in yeast: a review of the molecular machinery. Cell Struct Funct 27:409–420

Huang DCS, Strasser A (2000) BH3-only proteins – essential initiators of apoptotic cell death. Cell 103:839–842

Ink B, Zornig M, Baum B, Hajibagheri N, James C, Chittenden T, Evan G (1997) Human Bax induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells. Mol Cell Biol 17:2468–2474

Jacobson MD, Well M, Raff MC (1997) Programmed cell death in animal development. Cell 88:347–354

Jagasla R, Grote P, Westermann B, Conradt B (2005) DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. Nature 433:754–760

James C, Gschmeissner S, Fraser A, Evan GI (1997) CED-4 induces chromatin condensation in *Schizozaccharomyces pombe* and is inhibited by direct physical association with CED-9. Curr Biol 7:246–252

Jones BA, Fangman WL (1992) Mitochondrial DNA maintenance in yeast requires a protein containing a region related to the GTP-binding domain of dynamin. Genes Dev 6:380–389

Jürgensmeier JM, Krajewski S, Armstrong RC, Wilson GM, Oltersdorf T, Fritz LC, Reed JC, Ottilie S (1997) Bax- and Bak-induced cell death in the fission yeast *Schizosaccharomyces pombe*. Mol Biol Cell 8:325–339

Karbowski M, Lee Y-J, Gaume B, Jeong S-Y, Frank S, Nechushtan A, Santel A, Fuller M, Smith CL, Youle RJ (2002) Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. J Cell Biol 159:931–938

Kerr JFR, Wyllie AM, Currie AR (1972) Apoptosis; a basic biological phenomenon with wider ranging implications in tissue kinetics. Br J Cancer 24:239–275

Khalfan WA, Klionsky DJ (2002) Molecular machinery required for autophagy and the cytoplasm to vacuole targeting (Vct) pathway in *S. cerevisiae*. Curr Opin Cell Biol 14:468–475

Kim J, Huang W-P, Klionsky DJ (2001) Membrane recruitment of Aut7p in the autophagy and cytoplasm to vacuole targeting pathways requires Aut1p, Aut2p, and the autophagy conjugation complex. J Cell Biol 152:51–64

King KL, Cidlowski JA (1998) Cell cycle regulation and apoptosis. Annu Rev Physiol 60:601–617

Kirisako T, Baba M, Ishihara N, Miyazawa K, Ohsumi M, Yoshimori T et al. (1999) Formation process of autophagosome is traced with Apg8/Aut7p in yeast. J Cell Biol 147:435–446

Kiššová I, Polčic P, Kempná P, Zeman I, Šabova L, Kolarov J (2000) The cytotoxic action of Bax on yeast cells does not require mitochondrial ADP/ATP carrier but may be related to its import to the mitochondria. FEBS Lett 471:113–118

Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. Science 290:1717–1721

Koll F, Begel O, Keller A-M, Vierny C, Belcour L (1984) Ethidium bromide rejuvenation of senescent cultures of *Podospora anserina*: loss of senescence-specific DNA and recovery of normal mitochondrial DNA. Curr Genet 8:127–134

Komatsu K, Hopkins KM, Lieberman HB, Wang H-G (2000) *Schizosaccharomyces pombe* Rad9 contains a BH3-like region and interacts with the anti-apoptotic protein Bcl-2. FEBS Lett 481:122–126

Koonin EV, Aravind L (2002) Origin and evolution of eukaryotic apoptosis: the bacterial connection. Cell Death Differ 9:394–404

Kück U, Stahl U, Esser K (1981) Plasmid-like DNA is part of mitochondrial DNA in *Podospora anserina*. Curr Genet 3:151–156

Kuwana T, Newmeyer DD (2003) Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr Opin Cell Biol 15:691–699

Kuwana T, McKey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR, Newmeyer DD (2002) Rid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 111:331–342

Labarère J (1973) Propriétés d'un système d'incompatibilité chez le champignon *Podospora anserina* et intérêt de ce système pour l'étude de l'incompatibilité. C R Acad Sci Paris 276:1301–1304

Lang E, Schaeffeler E, Bernreuther D, Bredschneider M, Wolf DH, Thumm M (1998) Aut2p and Aut7p, two novel microtubule-associated proteins are essential for delivery of autophagic vesicles to the vacuole. EMBO J 17:3597–3607

Laun P, Pichova A, Madeo F, Fuchs J, Ellinger A, Kohlwein S, Dawes I, Fröhlich K-U, Breitenbach M (2001) Aged mother cells of *Saccharomyces cerevisiae* show markers of oxidative stress and apoptosis. Mol Microbiol 39:1166–1173

Lee C-Y, Clough EA, Yellon P, Teslovich TM, Stephan DA, Baehrecke EH (2003) Genome-wide analyses of steroid-and radiation-triggered programmed cell death in *Drosophila*. Curr Biol 13:350–357

Lee Y-J, Jeong S-Y, Karbowski M, Smith CL, Youle RJ (2004) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol Biol Cell 15:5001–5011

Li F, Flanary PL, Altier DC, Dohlman HG (2000) Cell division regulation by Bir1, a member of the inhibitor of apoptosis family in yeast. J Biol Chem 275:6707–6711

Ligr M, Madeo F, Fröhlich E, Hilt W, Fröhlich K-U, Wolf DH (1998) Mammalian Bax triggers apoptotic changes in yeast. FEBS Lett 438:61–65

Ligr M, Velten I, Fröhlich E, Madeo F, Ledig M, Fröhlich K-U, Wolf D, Hilt W (2001) The proteasomal substrate

Stm1 participates in apoptosis-like cell death in yeast. Mol Biol Cell 12:2422–2432
Lu BC (1967) Meiosis in *Coprinus lagopus*: a comparative study with light and electron microscopy. J Cell Sci 2:529–536
Lu BC (1991) Cell degeneration and gill remodeling during basidiocarp development in the fungus *Coprinus cinereus*. Can J Bot 69:1161–1169
Lu BC (1996) Chromosomes, mitosis, and meiosis. In: Bos CJ (ed) Fungal genetics, principles and practice. Dekker, New York, pp 119–175
Lu BC (2000) The control of meiosis progression in the fungus *Coprinus cinereus* by light/dark cycles. Fungal Genet Biol 31:33–41
Lu BC, Kües U (1999) White-cap mutants and meiotic apoptosis in the fungus *Coprinus cinereus*. Can Genet Soc Bull (abstract) 30:47
Lu BC, Gallo N, Kües U (2003) White-cap mutants and meiotic apoptosis in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 39:82–93
Ludovico P, Sousa MJ, Silva MT, Leao C, Corte-Real M (2001) *Saccharomyces cerevisiae* commits to a programmed cell death process in response to acetic acid. Microbiology 147:2409–2415
Ludovico P, Rodrigues F, Almeida A, Silva MT, Barrientos A, Corte-Real M (2002) Cytochrome c release and mitochondria involvement in programmed cell death induced by acetic acid in *Saccharomyces cerevisiae*. Mol Biol Cell 13:2598–2606
Ludovico P, Sansonetty F, Silva MT, Corte-Real M (2003) Acetic acid induces a programmed cell death process in the food spoilage yeast *Zygosaccharomyces bailii*. Yeast Res 3:91–96
Ly JD, Grubb DR, Lawen A (2003) The mitochondrial membrane potential ($\Delta\Psi$m) in apoptosis; an update. Apoptosis 8:115–128
Madeo F, Fröhlich E, Fröhlich K-U (1997) A yeast mutant showing diagnostic markers of early and late apoptosis. J Cell Biol 139:729–734
Madeo F, Fröhlich E, Ligr M, Grey M, Sigrist SJ, Wolf DH, Fröhlich K-U (1999) Oxygen stress: a regulator of apoptosis in yeast. J Cell Biol 145:757–767
Madeo F, Herker E, Maldener C, Wissing S, Lächelt S, Herlan M, Fehr M, Lauber K, Sigrist SJ, Wesselborg S et al. (2002) A caspase-related protease regulates apoptosis in yeast. Mol Cell 9:911–917
Madeo F, Herker E, Wissing S, Jungwirth H, Eisenberg T, Fröhlich K-U (2004) Apoptosis in yeast. Curr Opin Microbiol 7:655–660
Manon S, Chaudhuri B, Guérin M (1997) Release of cytochrome c and decrease of cytochrome c oxidase in Bax-expressing yeast cells, and prevention of these effects by coexpression of Bcl-x_L. FEBS Lett 415:29–32
Marek SM, Wu J, Glass NL, Gilchrist DG, Bostock RM (2003) Nuclear DNA degradation during heterokaryon incompatibility in *Neurospora crassa*. Fungal Genet Biol 40:126–137
Matsuyama S, Xu Q, Velours J, Reed JC (1998) The mitochondrial F_0F_1-ATPase proton pump is required for function of the proapoptotic protein Bax in yeast and mammalian cells. Mol Cell 1:327–336
Matsuyama S, Nouraini S, Reed JC (1999) Yeast as a tool for apoptosis research. Curr Opin Microbiol 2:618–623
Meiling-Wesse K, Barth H, Thumm M (2002a) Ccz1p/Aut11p/Cvt16p is essential for autophagy and the cvt pathway. FEBS lett 526:71–76
Meiling-Wesse K, Barth H, Voss C, Barmark G, Muren E, Ronne H, Thumm M (2002b) Yeast Mon1p/Aut12p functions in vacuolar fusion of autophagosomes and cvt-vesicles. FEBS Lett 530:174–180
Metzstein MM, Stanfield GM, Horvitz HR (1998) Genetics of programmed cell death in *C. elegans* – past, present and future. Trends Genet 14:410–416
Mignotte B, Vayssiere JL (1998) Mitochondria and apoptosis. Eur J Biochem 252:1–15
Mizushima N, Ohsumi Y, Yoshimori T (2002) Autophagosome formation in mammalian cells. Cell Struct Funct 27:421–429
Money NP (2003) Suicidal mushroom cells. News and views. Nature 423:26–26
Moore D (1996) Inside the developing mushroom-cells tissues and tissue pattern. In: Chiu S-W, Moore D (eds) Patterns in fungal development. Cambridge University Press, Cambridge, UK, pp 1–36
Moore D (2003) Programmed cell death alive and well in fungi. Mycol Res 107:1251–1252
Mozdy AD, McCaffery JM, Shaw JM (2000) Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. J Cell Biol 151:367–379
Naujokat C, Hoffmann S (2002) Role and function of the 26S proteasome in proliferation and apoptosis. Lab Invest 82:965–980
Nugent CI, Hughes TR, Lue NF, Lundblad V (1996) Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. Science 274:249–252
Orlowski RZ (1999) The role of the ubiquitin-proteasome pathway in apoptosis. Cell Death Diff 6:303–313
Osiewacz HD (1995) Aging and genetic instabilities. In: Esser K, Martin GM (eds) Molecular aspects of aging. Wiley, New York, pp 29–44
Osiewacz HD, Esser K (1984) The mitochondrial plasmid of *Podospora anserina*: a mobile intron of a mitochondrial gene. Curr Genet 8:299–305
Paoletti M, Castroviejo M, Begueret J, Clavé C (2001) Identification and characterization of a gene encoding a subtilisin-like serine protease induced during the vegetative incompatibility reaction in *Podospora anserina*. Curr Gene 39:244–252
Perkins DD (1988) Main features of vegetative incompatibility in *Neurospora*. Fungal Genet Newslett 35:44–46
Phillips AJ, Sudbery I, Ramsdale M (2003) Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*. Proc Natl Acad Sci USA 100:14327–14332
Pinan-Lucarré B, Paoletti M, Dementhon K, Coulary-Salin B, Clavé C (2003) Autophagy is induced during cell death by incompatibility and is essential for differentiation in the filamentous fungus *Podospora anserina*. Mol Microbiol 47:321–333
Polčic P, Forte M (2003) Response of yeast to the regulated expression of proteins in the Bcl-2 family. Biochem J 374:393–402
Poliaková D, Sokolíková B, Kolarov J, Šabová L (2002) The antiapoptotic protein Bcl-x_L prevents the cytotoxic effect of Bax, but not Bax-induced formation of reactive

oxygen species, in *Kluyveromyces lactis*. Microbiology 148:2789–2795
Pozniakovsky AI, Knorre DA, Markova OV, Hyman AA, Skulachev VP, Severin FF (2005) Role of mitochondria in the pheromone- and amiodarone-induced programmed death of yeast. J Cell Biol 168:257–269
Priault M, Camougrand N, Chaudhuri B, Schaeffer J, Manon S (1999a) Comparison of the effects of bax-expression in yeast under fermentative and respiratory conditions: investigation of the role of adenine nucleotides carrier and cytochrome c. FEBS Lett 456:232–238
Priault M, Chaudhuri B, Clow A, Camougrand N, Manon S (1999b) Investigation of bax-induced release of cytochrome c from yeast mitochondria. Permeability of mitochondrial membranes, role of VDAC and ATP requirement. Eur J Biochem 260:684–691
Priault M, Bessoule JJ, Grelaud-Coq A, Camougrand N, Manon S (2002) Bax-induced cell death in yeast depends on mitochondrial lipid oxidation. Eur J Biochem 269:5440–5450
Qi H, Zakian VA (2000) The *Saccharomyces* telomere-binding protein Cdc13p interacts with both the catalytic subunit of DNA polymerase α and the telomerase-associated Est1 protein. Genes Dev 14:1777–1788
Qi H, Li T-K, Kuo D, Nur-E-Kamal A, Liu LF (2003) Inactivation of Cdc13p triggers MEC1-dependent apoptotic signals in yeast. J Biol Chem 278:15136–15141
Ranganath RM, Nagashree NR (2001) Role of programmed cell death in development. Int Rev Cytol 202:159–242
Raught B, Gingras AC, Sonenberg N (2001) The target of rapamycin (TOR) proteins. Proc Natl Acad Sci USA 98:7037–7044
Reggiori F, Klionsky DJ (2002) Autophagy in the eukaryotic cell. Eukaryot Cell 1:11–21
Reiter J, Herker E, Madeo F, Schmitt MJ (2004) Viral killer toxins induce caspase-mediated apoptosis in yeasts. J Cell Biol 168:353–358
Rizet G (1953) Sur l'impossibilité d'obtenir la multiplication végétative interrompue et illimitée de l'ascomycète *Podospora anserina*. C R Acad Sci Paris 237:838–840
Rizet G, Esser K (1953) Sur des phénomènes d'incompatibilité entre souches d'origines différentes chez *Podospora anserina*. C R Acad Sci Paris 237:760–761
Roucou X, Prescott M, Devenish RJ, Nagley P (2000) A cytochrome c-GFP fusion is not released from mitochondria into the cytoplasm upon expression of Bax in yeast cells. FEBS Lett 471:235–239
Roze LV, Linz JE (1998) Lovastatin triggers an apoptosis-like cell death process in the fungus *Mucor racemosus*. Fungal Genet Biol 25:119–133
Ryser S, Vial E, Magnenat E, Schlegel W, Maundrell K (1999) Reconstitution of caspase-mediated cell-death signalling in *Schizosaccharomyces pombe*. Curr Genet 36:21–28
Saeki K, Yuo A, Okuma E, Yazaki Y, Susin SA, Kroemer G, Takaku F (2000) Bcl-2 down-regulation causes autophagy in a caspase-independent manner in human leukemic HL60 cells. Cell Death Differ 7:1263–1269
Sato T, Hanada M, Bodrug S, Irie S, Iwama N, Boise LH, Thompson CB, Golemis E, Fong L, Wang H-G et al. (1994) Interactions among members of the Bcl-2 protein family analyzed with a yeast two-hybrid system. Proc Natl Acad Sci USA 91:9238–9242
Saupe SJ, Clavé C, Begueret J (2000) Vegetative incompatibility in filamentous fungi: *Podospora* and *Neurospora* provide some clues. Curr Opin Microbiol 3:608–612
Sesaki H, Jensen RE (2004) Ugo1p links the Fzo1p and Mgm1p GTPases for mitochondrial fusion. J Biol Chem 279:28298–28303
Sesaki H, Southard SM, Yaffe MP, Jensen RD (2003) Mgm1p, a dynamin-related GTPase, is essential for fusion of the mitochondrial outer membrane. Mol Biol Cell 14:2342–2356
Schreader BA, Wang Y, Nambu JR (2003) *Drosophila morgue* and the intersection between protein ubiquitination and programmed cell death. Apoptosis 8:129–139
Silar P (2005) Peroxide accumulation and cell death in filamentous fungi induced by contact with a contestant. Mycol Res 109:137–149
Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M et al. (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397:441–446
Swamy S, Uno I, Ishikawa T (1984) Morphogenetic effects of mutations at the A and B incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 130:3219–3224
Tao W, Kurschner C, Morgan JI (1997) Modulation of cell death in yeast by the Bcl-2 family of proteins. J Biol Chem 272:15547–15552
Thrane C, Kaufmann U, Stummann BM, Olsson S (2004) Activation of caspase-like activity and poly (ADP-ribose) polymerase degradation during sporulation in *Aspergillus nidulans*. Fungal Genet Biol 41:361–368
Tieu Q, Nunnari J (2000) Mdv1p is a WD repeat protein that interacts with the dynamin-related GTPase, Dnm1p, to trigger mitochondrial division. J Cell Biol 151:353–365
Tieu Q, Okreglak V, Naylor K, Nunnari J (2002) The WD repeat protein, Mdv1p, functions as a molecular adaptor by interacting with Dnm1p and Fis1p during mitochondrial fission. J Cell Biol 158:445–452
Umar MH, van Griensven LJLD (1997) Morphogenetic cell death in developing primordia of *Agaricus bisporus*. Mycologia 89:274–277
Umar MH, van Griensven LJLD (1998) The role of morphogenetic cell death in the histogenesis of the mycelial cord of *Agaricus bisporus* and in the development of macrofungi. Mycol Res 102:719–735
Uren AG, Beilharz T, O'Connell MJ, Bugg SJ, van Driel R, Vaux DL, Lithgow T (1999) Role for yeast inhibitor of apoptosis (IAP)-like proteins in cell division. Proc Natl Acad Sci USA 96:10170–10175
Vaux DL, Korsmeyer SJ (1999) Cell death in development. Cell 96:245–254
Wang C-W, Stromhaug PE, Shima J, Klionsky DJ (2002) The Ccz1-Mon1 protein complex is required for the late step of multiple vacuole delivery pathways. J Biol Chem 277:47917–47927
Watanabe K, Morishita J, Umezu K, Shirahige K, Maki H (2002) Involvement of RAD9-dependent damage checkpoint control in arrest of cell cycle, induction of cell death, and chromosome instability caused by defects in origin recognition complex. Eukaryot Cell 1:200–212
Wissing S, Ludovico P, Herker E, Buttner S, Engelhardt SM, Deker T, Link A, Proksch A, Rodrigues F, Corte-Real M et al. (2004) An AIF orthologue regulates apoptosis in yeast. J Cell Biol 166:969–974

Wong ED, Wagner JA, Gorsich SW, McGaffery JM, Shaw JM, Nunnari J (2000) The dynamin-related GTPase, Mgm1p, is an intermembrane space protein required for maintenance of fusion competent mitochondria. J Cell Biol 151:341–352

Wong ED, Wagner JA, Scott SV, Okreglak V, Holewinske TJ, Cassidy-Stone A, Nunnari J (2003) The intramitochondrial dynamin-related GTPase, Mgm1p, is a component of a protein complex that mediates mitochondrial fusion. J Cell Biol 160:303–311

Wu D, Chen P-J, Chen S, Hu Y, Nunez G, Ellis RE (1999) *C. elegans* MAC-1, an essential member of the AAA family of ATPases, can bind CED-4 and prevent cell death. Development 126:2021–2031

Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306

Wysocki R, Kron SJ (2004) Yeast cell death during DNA damage arrest is independent of caspase or reactive oxygen species. J Cell Biol 166:311–316

Yamaki M, Umehara T, Chimura T, Horikoshi M (2001) Cell death with predominant apoptotic features in *Saccharomyces cerevisiae* mediated by deletion of the histone chaperone ASF1/CIA1. Genes Cells 6:1043–1054

Ye Y, Meyer HH, Rapoport TA (2003) Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. J Cell Biol 162:71–84

Yen H-C, Espiritus C, Chang EC (2003) Rpn5 is a conserved proteasome subunit and required for proper proteasome localization and assembly. J Biol Chem 278:30669–30676

Yoon H-J, Carbon J (1999) Participation of Bir1p, a member of the inhibitor of apoptosis family, in yeast chromosome segregation events. Proc Natl Acad Sci USA 96:13208–13213

Zha H, Reed JC (1997) Heterodimerization-independent functions of cell death regulatory proteins Bax and Bcl-2 in yeast and mammalian cells. J Biol Chem 272:31482–31488

Zha H, Fisk HA, Yaffe MP, Mahajan N, Herman B, Reed JC (1996) Structure-function comparisons of the proapoptotic protein Bax in yeast and mammalian cells. Mol Cell Biol 16:6494–6508

10 Senescence and Longevity

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CONTENTS

I. Introduction

Most fungi, both unicellular yeasts as well as "multicellular" filamentous fungi, appear to be immortal. They propagate indefinitely either by single cell division or by hyphal tip growth. In fact, individuals of the latter group may form huge vegetation bodies with diameters of over 0.5 km (Smith et al. 1992). Remarkably, however, there are a few species which are clearly characterized by limited growth. Each individuum of these species ages and finally dies. This holds true for the yeast *Saccharomyces cerevisiae* but also for a number of filamentous fungi (Jinks 1959; Handley and Caten 1973; Caten and Handley 1978; Lazarus et al. 1980; de Vries et al. 1981, 1986; Lazarus and Küntzel 1981; Bertrand et al. 1985, 1986; Böckelmann and Esser 1986; Almasan and Mishra 1988; Court et al. 1991; Debets et al. 1995; Navaraj et al. 2000; Fox and Kennell 2001).

In budding yeast, aging is characterized by the limited ability of mother cells to divide and to produce daughter cells, a process which is characterized by the generation of bud scars on the surface of the mother cell after the newly formed daughter cell becomes separated from the mother. This allows us to microscopically distinguish between older and younger yeast cells. Today *S. cerevisiae* is one of a few models extensively studied in experimental gerontology (for review, see Guarente 1997; Guarente and Kenyon 2000; Jazwinski 2000, 2001, 2005; McMurray and Gottschling 2004).

Podospora anserina is a filamentous ascomycete in which, in contrast to yeast, senescence is visible at the macroscopic level. This and the fact that *P. anserina* is an excellent system for genetic analysis are the reasons why the aging of this species has now been investigated for more than 50 years. In this review we will focus on the mechanisms involved in senescence and life span control in *P. anserina*. Research performed on this system has stimulated investigations in other systems and clearly demonstrates that parts of the mechanisms involved in life span control have been conserved during evolution. In this chapter, we do not aim for a comprehensive description of the work on aging in *P. anserina* but draw an updated view about the current knowledge on the mechanisms of aging in this aging model. For more detailed description of earlier investigations, the reader is referred to Esser and Tudzynski (1980), Esser (1985), Griffiths (1992), Osiewacz (1995, 2002a,b), Bertrand (2000), and Silar et al. (2001).

II. Senescence Syndrome in *Podospora anserina*

A. The Senescence Phenotype

In the early 1950s, G. Rizet reported for the first time that all wild-type cultures of *P. anserina* do not grow indefinitely but senesce after a strain-specific period of time (Rizet 1953). After the germination of ascospores, the mycelial culture grows radially by hyphal tip growth. During aging, the growth rate declines and the pigmentation of the mycelium increases. In particular, the formation of

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arial hyphae, which grow into the air, is significantly reduced in aged cultures. Microscopic analyses revealed that the hyphal tips of senescent cultures show a curved and undulated phenotype and frequently burst (Delay 1963; Esser and Tudzynski 1980). In the case of the intensively studied wild-type strains, mean life span is about 25 days when grown on rich medium at 27 °C. Other wild-type strain s have other characteristic mean life spans, demonstrating that life span control is genetically determined. In addition, environmental conditions like nutrient supply or incubation temperatures affect the onset of senescence.

Aging in *P. anserina* was found to be strongly dependent on the type of carbon source supplied in the growth medium. Carbon sources like glucose have been demonstrated to shorten life span, whereas others (like acetate or glycerol) increase life span (Tudzynski and Esser 1979; Maas et al. 2004). In addition, low glucose concentration (0.05%) in the culture medium clearly increases life span. Also, metabolic inhibitors (e.g. the DNA intercalating compound ethidium bromide, and the inhibitors of mitochondrial protein synthesis chloramphenicol, kanamycin, neomycin, streptomycin, puromycin and tiamulin) which are added to the culture medium result in an increased life span (Esser and Tudzynski 1977; Tudzynski and Esser 1977; Belcour and Begel 1980; Koll et al. 1984). Antioxidants like glutathion are also capable of prolonging life span in *P. anserina* (Munkres und Rana 1978). Not only compounds added to the culture medium but also the incubation conditions have rather early been described to influence life span. Cold treatment was found to lead to the rejuvenation of cultures (Marcou 1961). A life-shortening effect of higher incubation temperature is observed not only in wild-type strains but also in mutants (Turker et al. 1987).

B. Mitochondrial DNA Instabilities

Early experimental data revealed that both nuclear as well as extrachromosomal genetic traits play a crucial role in the genetic control of senescence in *P. anserina* (Marcou 1961; Esser and Keller 1976; Tudzynski and Esser 1979). A major impact on the onset of senescence has been assigned to the occurrence of a covalently closed, circular DNA species, termed plDNA or α-senDNA (Stahl et al. 1978; Cummings et al. 1979). This element was demonstrated to accumulate in mitochondria of senescent cultures. In juvenile cultures it is an integral part of the mitochondrial DNA (mtDNA), the first intron of the gene *Cox1* coding for the cytochrome *c* oxidase (COX; Osiewacz and Esser 1984; Cummings et al. 1985). During aging of wild-type cultures, the pl-intron becomes liberated and amplified. This process is accompanied by deletions of large parts of the high-molecular weight mtDNA (Belcour et al. 1981; Kück et al. 1981). Since these reorganizations are almost quantitative, the majority of the mtDNA molecules are extensively rearranged in senescent wild-type cultures (Belcour et al. 1981; Kück et al. 1981, 1985a). In addition to these specific rearrangements which occur reproducibly during aging of wild-type strains, other types of rearrangements are observed. They occur between short dispersed repeats and are less specific and frequent, resembling recombination processes found in pathological situations and during aging in a number of different systems (Linnane et al. 1989; Osiewacz and Hermanns 1992; Wallace 1992, 2001; Cottrell et al. 2000; Samuels et al. 2004).

The term plDNA, for "plasmid-like DNA" (Stahl et al. 1978), refers to the structure of the amplified element which resembles the structure of typical circular plasmids in bacteria. senDNA refers to the stage of a culture, the senescent stage, in which this element accumulates. It should be stressed that plDNA and α-senDNA are identical elements of an oligomeric series of molecules with a size of 2539 bp of the monomer (Osiewacz and Esser 1984; Cummings et al. 1985). Other additional DNA species which are also termed senDNAs (e.g. βsenDNA, γsenDNA) are clearly different. They accumulate in senescing cultures of *P. anserina* but have a variable size and originate from a different region of the mtDNA (Jamet-Vierny et al. 1980; Belcour et al. 1981, 1986; Kück et al. 1981; Wright et al. 1982; Cummings et al. 1985, 1987; Koll et al. 1985).

On the basis of various experimental datasets, it was suggested that the age-related mtDNA rearrangements, and specifically the accumulation of the plDNA, are a prerequisite for aging of *P. anserina* cultures (Belcour et al. 1982; Stahl et al. 1982; Vierny et al. 1982; Koll et al. 1985; Schulte et al. 1988; Osiewacz et al. 1989). However, some mutant strains which, due to specific mutations, live longer than the wild-type strain were later demonstrated to senesce despite not having accumulated plDNA in the senescent stage (Borghouts et al. 1997; Silar et al. 1997). Thus, it has to be concluded that

the amplification of plDNA is not a prerequisite for senescence but rather a modulator of life span. The amplification of this element appears to speed up aging of cultures. At this point, it needs to be emphasized that long-lived strains of this plDNA-less type have not been isolated from nature but only in the laboratory. Under natural conditions, they seem not to be able to survive and, thus, the specific mtDNA rearrangements demonstrated in wild-type strains appear in fact to be the reason why the cultures age and die. These mechanisms are probably a major adaptation of *P. anserina* to its natural niche (herbivorous dung), which requires fast reproduction of cultures because the substrate dries out fast and thereafter is not suited for further growth.

The molecular mechanisms by which the amplification of plDNA leads to an acceleration of the aging process have been characterized in some detail. This is dependent on the transposition of the pl-intron. These transposition events can occur either to the position directly downstream of the first *CoxI* exon ("homing-like" transposition) or to other acceptor sites in the mtDNA molecule ("ectopic" transposition). As a result, mtDNA molecules are generated which contain two or more tandem or dispersed copies of the 2.5-kbp intron sequence (Sellem et al. 1993; Borghouts et al. 2000). Subsequent homologous recombination events between these duplicated sequences result in the formation of circular plDNA or circular DNA molecules containing other parts of the mtDNA. The intron transposition itself appears to proceed via a reverse transcriptase step and depends on the activity of a protein encoded by an open reading frame on the pl-intron (Osiewacz and Esser 1984; Kück et al. 1985b; Michel and Lang 1985; Fassbender et al. 1994).

C. Mitochondrial Functions and mtDNA Instabilities

During aging of *P. anserina* cultures, a decline of respiratory efficiency is observed (Belcour and Begel 1980; Frese and Stahl 1992). Such a decline can easily be explained by the fact that normal protein turnover, including the turnover of proteins of the respiratory chain, requires a functional set of genes encoding the corresponding proteins. Since the mtDNA encodes parts of the mitochondrial respiration chain, the age-related accumulation of deleted mtDNA molecules interferes with the turnover of respiratory chain proteins. As a consequence, the generation of ATP declines. At the same time, incomplete reduction of oxygen at impaired respiratory chains leads to an increased generation of the superoxide anion, a toxic reactive oxygen species (ROS) which is normally generated at complex I and complex III of the respiration chain (Fig. 10.1A). The superoxide anion itself is part of a reaction chain which ultimately leads to the formation of the highly toxic hydroxyl radical, capable of damaging various biomolecules in the cell (Fig. 10.1B). In principle, the level of ROS formation is rather low in undamaged juvenile mitochondria, and it is reduced by certain scavengers like the superoxide dismutases. However, during aging ROS levels increase significantly to reach thresholds which cellular scavenging systems can no longer handle. One reason is that damage of respiration chains which is not "repaired" by protein turnover leads to the generation of increasingly more ROS. A kind of "vicious circle" of reactions finally results in the death of the corresponding systems. In this scenario, there are different components involved which affect the time by which the system declines. These are (1) the level of

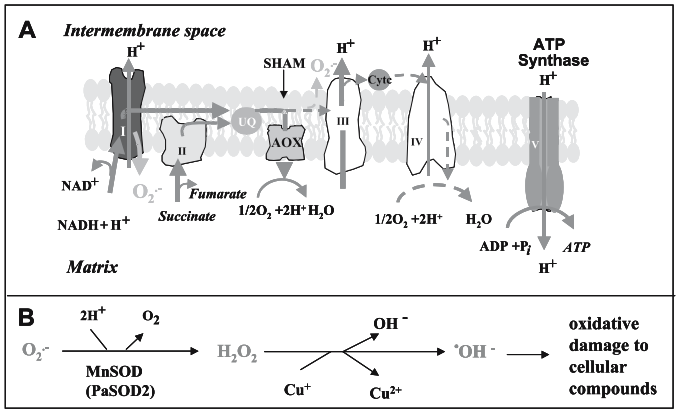

Fig. 10.1.A,B Generation of the superoxide anion in the mitochondrial respiration chain. **A** Superoxide anions are generated at complex I (NADH dehydrogenase) and at the transfer of electrons from ubiquinol (*UQ*) to complex III (cytochrome *c* reductase). Complex IV (cytochrome *c* oxidase, inhibited by cyanide) deficiency leads to the induction of an alternative oxidase (AOX, inhibited by salicylhydoxamic acid, *SHAM*) which receives the electrons directly from ubiquinol, circumventing complex III. **B** Generation of the reactive oxygen species hydroxyl anion through manganese superoxide dismutase (*MnSOD*, in *P. anserina* PaSOD2) and Fenton reaction. *Complex III* Ubiquinol–cytochrome *c* oxidoreductase, *complex V* ATP synthase, *cytc* cytochrome *c*

endogenous generation of toxic products in form of ROS during normal energy transduction at the respiratory chain, (2) scavenging systems which reduce ROS levels (e.g. superoxide dismutase), and (3) repair of damaged biomolecules (e.g. protein turnover), which depends on protein biosynthesis and a set of nuclear and mitochondrial genes. Any imbalance of these processes leads to accelerated aging. In *P. anserina* wild-type cultures, it is evident that protein turnover must be impaired in aged cultures, since the DNA rearrangements result in an almost quantitative disappearance of complete mitochondrial genomes.

In the last decades of research, a number of long-lived mutant strains of *P. anserina* have been identified and characterized which have provided important data towards the understanding of parts of the molecular network governing life span in this organism. Some of them are extrachromosomal mutants, others carry nuclear mutations.

AL2-1 is an extrachromosomal long-lived mutant in which the mtDNA rearrangements leading to the plDNA amplification are delayed. This strain contains a linear plasmid, pAL2-1, encoding an RNA and DNA polymerase, which was demonstrated to interfere with the process of mtDNA reorganization, leading ultimately to a stabilization of the mitochondrial genome which remains available for protein biosynthesis and, thus, protein turnover over a long period of growth (Osiewacz et al. 1989; Hermanns and Osiewacz 1992, 1996; Hermanns et al. 1994). Interestingly, a recent publication demonstrates an influence of this plasmid on the effect of caloric restriction, an experimental regime demonstrated to be effective in increasing life span virtually in any system analysed so far. Although the life span of wild-type strains of *P. anserina* is also increased significantly by caloric restriction (e.g. a 100-fold reduced glucose content of culture medium compared to the normal culture medium), this effect is rather low in cultures carrying the mitochondrial pAL2-1 plasmid (Maas et al. 2004). The molecular basis of this type of interference is not clear at the moment but may provide new clues into the mechanisms of aging in *P. anserina* (Maas et al. 2004).

Another way of escaping senescence is represented by extrachromosomal mutants in which parts of the mtDNA are lacking. Such mutants have been isolated as partial outgrowth of senescent cultures (Tudzynski et al. 1982; Vierny et al. 1982; Kück et al. 1985b; Belcour and Vierny 1986; Schulte et al. 1988). Deletion of the intron 1 of *CoxI* in the *mex* mutants (Vierny et al. 1982; Belcour and Vierny 1986) as well as complete deletion of *CoxI* in the *ex* mutants (Kück et al. 1985b; Schulte et al. 1988) result in longevity. This clearly stresses the importance of this part of the mtDNA which, in wild-type strains, efficiently contributes to the observed age-specific mtDNA reorganization processes. In the mutant lacking the corresponding DNA region, the mt genome is stabilized.

D. The Retrograde Response

The characterization of different long-lived *P. anserina* mutants demonstrated that severe mitochondrial dysfunction can be compensated by a "retrograde response". This pathway, leading to the induction of certain genes which under normal conditions are not or only partly expressed, has first been reported in yeast (Liao et al. 1991; Liao and Butow 1993; Sekito et al. 2000) and was found to lead to an increase in life span of yeast cells (Kirchman et al. 1999; Jazwinski 2000, 2004; Kim et al. 2004).

The retrograde response in yeast is a signalling pathway of interorganelle communication (Butow 2002), which is induced by mitochondrial dysfunction. It results in the induction of numerous nuclear genes coding for metabolic enzymes and stress proteins. The retrograde response leads to a remodelling of the cell metabolism increasing yeast life span (reviewed in Jazwinski 2004, 2005).

In *P. anserina*, it was found that different types of mutations leading to severe mitochondrial dysfunction result in the induction of the nuclear-encoded *PaAox* gene, resembling a retrograde response. One mutant of this type is the grisea mutant (Esser and Keller 1976; Prillinger and Esser 1977). In this mutant, the age-related, wild-type-specific rearrangements do not occur, due to impairments of the homologous recombination between repeated mtDNA (Borghouts et al. 2000). This type of impairment can be overcome by the supplementation of copper (Marbach et al. 1994) to the medium, indicating that the process of homologous recombination directly or indirectly depends on the availability of copper (Borghouts et al. 2000).

The relevant mutation in the grisea mutant is a loss of function of the transcription factor GRISEA. This transcription factor is involved in the tight control of cellular copper levels (Osiewacz and Nuber 1996; Borghouts et al. 1997; Borghouts and Osiewacz 1998). At high copper concentrations, it is repressed.

GRISEA is an orthologue of the yeast transcription factor MAC1 which controls cellular copper homeostasis in yeast via the control of the expression of different target genes. Among these, CTR1 encodes a yeast high-affinity copper transporter (Dancis et al. 1994). The GRISEA protein is able to rescue a *mac1*-deficiency mutant back to respiratory competence (Borghouts and Osiewacz 1998). Like MAC1, GRISEA activity is controlled by cellular copper, most likely via copper binding and conformational changes of the copper-loaded protein (Graden and Winge 1997; Zhu et al. 1998; McDaniels et al. 1999).

Upon copper depletion, GRISEA activates the transcription of different target genes including *PaGrg1*, *PaSod2* and *PaCtr3* (Borghouts and Osiewacz 1998; Kimpel and Osiewacz 1999; Borghouts et al. 2002a,b). *PaCtr3* encodes a high-affinity copper transporter which enables efficient copper transport across the plasma membrane even under low copper concentrations in the environment. In the grisea mutant, high-affinity copper uptake is impaired, leading to cellular copper deficiency which results in a pleiotropic phenotype with reduced pigmentation of mycelia and ascospores, a reduced growth rate, reduced fertility and an increased life span. The pleiotropic effect of the mutation of *Grisea* is explained by the fact that, due to cellular copper depletion, different copper-dependent cellular activities are impaired. Among others, these are the activity of tyrosinase (Fig. 10.2) which is involved in pigment synthesis. Another affected enzyme is superoxide dismutase 1 (SOD1). This enzyme is involved in ROS scavenging in both the cytoplasm and in the intermembrane space of mitochondria (Sturtz et al. 2001). In *P. anserina*, in contrast to yeast where transcription of the corresponding gene is copper dependent (Gralla et al. 1991), *Sod1* transcription is constitutive and, due to the dependency on copper as a cofactor, SOD1 activity is regulated post-transcriptionally by binding of the metal (Borghouts et al. 2002b).

In *P. anserina*, the impact of copper on life span appears to be largely the result of mitochondrial impairments. Here, a stabilization of the mtDNA in mutant grisea, which can be reversed by the addition of copper to the growth medium, is one of the factors which play a role. The reduction of ROS scavenging caused by the mitochondrial SOD2 gene not being expressed in the grisea mutant should rather have a negative effect on life span. How-

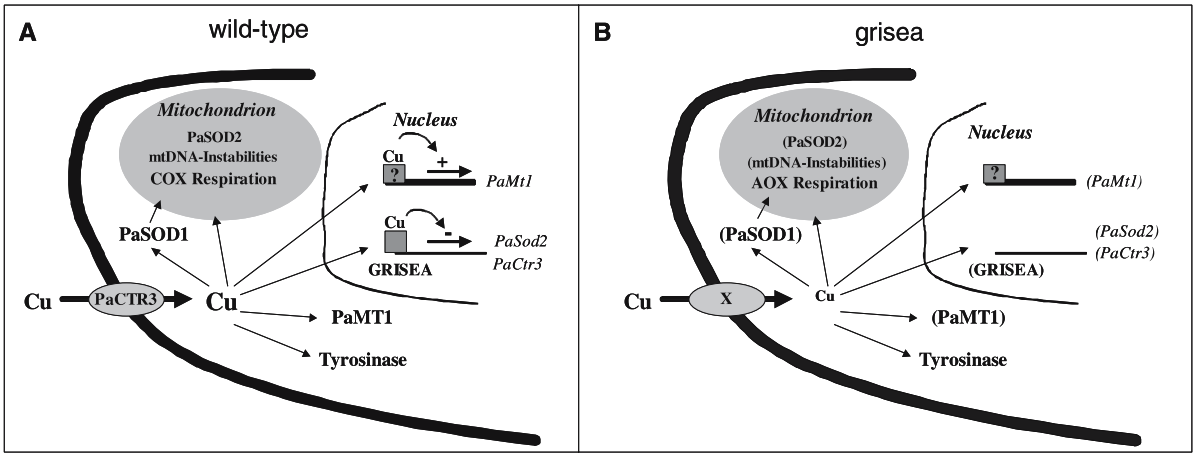

Fig. 10.2. A,B Genetic regulation of copper homeostasis in the wild type (**A**) and the long-lived grisea (**B**) mutant of *P. anserina*. Factors and processes indicated *in brackets* are missing or reduced in the mutant grisea. **A** In the wild type, copper uptake proceeds via the high-affinity transporter PaCTR3 which is transcriptionally regulated via GRISEA. The copper is bound to PaSOD1, tyrosinase and PaMt1 (metallothionein), and other proteins. In the nucleus, an unknown transcription factor induces *PaMt1* transcription under high copper concentration, whereas the expression of *PaSod2* and *PaCtr3* is repressed under these conditions. In the mitochondrion of the wild type, PaSOD2 is present (under low copper concentration), mtDNA is instable and respiration proceeds via COX. **B** In the mutant grisea, copper uptake proceeds via an unknown low-affinity copper transporter (*X*). The low copper concentration in the cell leads to PaSOD1 deficiency, and reduced PaMT1 activity in the cytoplasm. Lack of functional GRISEA inhibits *PaSod2* and *PaCtr3* expression. Consequently, PaSOD2 is missing in mitochondria of the grisea mutant, the mtDNA is stabilised and AOX is used as terminal oxidase

ever, a lowered ROS scavenging system can be tolerated in this mutant because respiration switches to being dependant on the alternative oxidase AOX, generating lower ROS than is the case for standard respiration (Borghouts et al. 2002b). This switch is a retrograde response induced by impairments of the assembly of cytochrome *c* oxidase due to copper depletion.

Cytochrome *c* oxidase binds three copper molecules per complex. One copper is bound to subunit I, and two atoms in subunit II (Capaldi 1990; Yoshikawa et al. 1998; Richter and Ludwig 2003). Depletion of copper was demonstrated to substantially impair COX assembly (Glerum et al. 1996; Nobrega et al. 2002; Barros et al. 2004).

Consequently, copper deficiency results in a failure to assemble complex IV of the respiratory chain, and therefore leads to a deficiency in standard respiration. In principle, such a situation should be lethal for a strict aerobe like *P. anserina*. However, in the grisea mutant, due to the fact that copper deficiency is not complete because low levels of copper are transported into the cell via a low-affinity copper uptake system, low standard respiration is observed. Moreover, *P. anserina* possesses a copper-independent, iron-containing alternative terminal oxidase, AOX, which can replace the COX in the respiration chain (Borghouts et al. 2001). AOX is located upstream of complex III of the respiratory chain, and therefore the formation of electron motive force is restricted to complex I. The OXPHOS complexes, organized in large supramolecular structures termed respirasomes in this mutant, clearly differ from that of the wild-type strains (Krause et al. 2004), and the generation of ATP through this kind of respiration chain is reduced (Fig. 10.1A). Most interestingly, the mutant's life span is increased about 60% compared to the wild-type strain. This life span increase can be explained by different factors: (1) a reduction of mtDNA reorganizations due to copper deficiency (see above) and (2) a reduced production of ROS. In comparison to the standard COX-dependent respiration, the alternative respiration results in the generation of a lower membrane potential (Wagner and Moore 1997). This lower membrane potential and the bypass of the superoxide anion generation at complex III result in a lower production of ROS through the alternative pathway. Which one of the two factors given above contributes most to the increase in life span remains unclear. However, it is interesting to note that, in contrast to the *ex1* mutant of *P. anserina*, the grisea mutant is not immortal, although in both mutants the alternative respiratory pathway is induced. Part of the answer appears to be that *ex1*, due to the deletion of large parts of the *CoxI* gene, respires exclusively via the alternative pathway whereas grisea uses both pathways.

In the long-lived grisea mutant, copper metabolism is affected in the whole cell. Consequently, not only mitochondrial functions are impaired. Importantly, the copper-zinc-dependent superoxide dismutase (Cu/Zn SOD, SOD1), a scavenging enzyme in the cytoplasm and the mitochondrial intermembrane space, is severely impaired, resulting in a failure to cope with accumulating oxidative stress in aging cultures (Borghouts et al. 2002b). The inability of the mutant to detoxify cytosolic ROS, and a partial respiration via COX resulting in lower ROS generation seem to be the key determinants of life span increase in this mutant.

In order to raise more specific data, transgenic strains in which more specific targets are affected have been constructed and analysed. One example is transgenic strain *Cox5*:ble in which the nuclear-encoded fifth subunit of cytochrome *c* oxidase (*Cox5*) has been replaced by a selection marker (Dufour et al. 2000). This mutant respires exclusively via AOX and has a life span which is increased at least tenfold. In another example, Stumpferl et al. (2004) replaced the endogenous *PaCox17* gene by a selection marker. The *PaCox17* gene encodes a copper transporter which is involved in the delivery of copper to complex IV of the respiratory chain (Fig. 10.3). The knockout strain respires via the alternative pathway and is characterized by a 17-fold increase in mean life span. However, apart from the switch in respiration, this type of mitochondrial copper depletion was found to lead to a stabilization of the mtDNA and to a changed profile of the two superoxide dismutases. In contrast to the wild-type strain, in which juvenile cultures express high amounts of mitochondrial superoxides dismutase 2 (SOD2) and low activity of SOD1 but senescent cultures high SOD1 and low SOD2, the transgenic strain constitutively expresses high levels of SOD1. Thus, although a specific target gene has been addressed, the outcome of modification is multiple once again (Fig. 10.4).

Induction of *PaAox* is also observed in a strain carrying a thermosensitive mutation of the gene *oxa1* (Sellem et al. 2005). The OXA1 protein is involved in the assembly and insertion of differ-

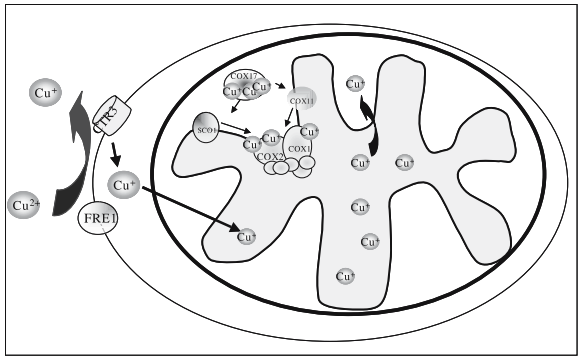

Fig. 10.3. Copper delivery to the cytochrome *c* oxidase subunits COX1 and COX2, based on data from yeast. Copper in the medium is reduced to Cu^+ by the ferric reductase FRE1. The high-affinity copper transporter CTR3 transports copper into the cell. By unknown mechanisms, copper is transported into the mitochondria, where COX17 delivers copper to the COX assembly factors SCO1 and COX11. The latter transfer the metal to the Cu_A and Cu_B site of cytochrome *c* oxidase. A recent publication (Cobine et al. 2004) demonstrates a non-proteinaceous copper pool in the mitochondrial matrix, and suggests a route of copper via the mitochondrial matrix to the respiratory chain

ent respiratory complexes (I, IV and V, compare Fig. 10.1). The mutant strain shows severe defects in complexes I and IV, consistent with an increased life span and a reduced ROS production. Interestingly, the severity of the phenotype is dependent on the allele of the nuclear *rmp1* gene, suggesting an involvement of the RMP1 protein in the OXA1-dependent protein assembly (Sellem et al. 2005). Unfortunately, the function of RMP1 is still unknown.

Overall, the investigation of different long-lived strains of *P. anserina* revealed a striking correlation of life span and respiration type. This correlation raises the question of whether it is the AOX respiration per se which is responsible for the longevity of the corresponding mutant strains. In fact, this possibility has been disproved, because the overexpression of AOX in a *Cox5*:ble background was reported to lead to an increase in ROS production, and even restores early senescence in this long-lived strain (Lorin et al. 2001). Evidently, it seems that there is a delicate tuning of different processes involved in life span control in *P. anserina*. Collectively, the data support the free radical theory of aging put forward by D. Harman almost 50 years ago, and since then modified and extended (Harman 1956, 1981, 1998). In particular, the mitochondrial free radical theory, which proposes that the generation of mitochondrial ROS is most important for aging, is supported by the available data from *P. anserina* aging. Every process leading to a decline in ROS production or availability leads to an increased life span. Processes of this kind are the lowered generation by an alternative respiration but also by the ability of remodelling damaged respiratory chains via protein turnover, a process depending on the stability of both the mitochondrial as well the nuclear genome.

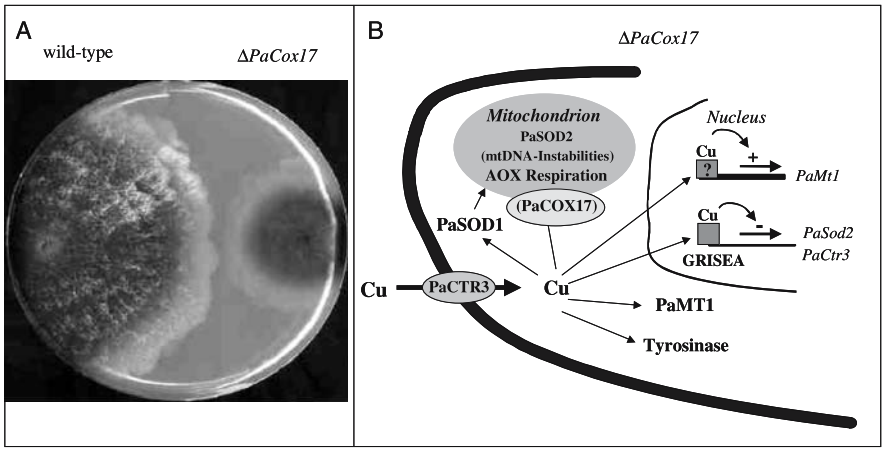

Fig. 10.4. **A,B** The *PaCox17* deletion strain *ΔPaCox17* (Stumpferl et al. 2004). **A** Phenotype of *ΔPaCox17* in comparison to wild-type strain s. **B** Copper supply within *ΔPaCox17*. Since *PaCtr3* expression is not affected in *ΔPaCox17*, the cytosolic copper concentration supports the formation of active PaSOD1. PaMT1 is induced. However, lack of PaCOX17 results in mitochondrial copper deficiency, leading to COX impairment and AOX respiration. In the mitochondrion, PaSOD2 is present and the mtDNA is stabilised

III. Concluding Remarks

As discussed above, the dynamics of "healthy" mitochondria – mitochondria which efficiently produce ATP but only low amounts of ROS – appears to be of high relevance for every organism. A shift of the population from "healthy" to damaged mitochondria leads to degeneration and senescence. Various processes, including different types of remodelling, are important pathways. The most prominent hallmark of senescence in *P. anserina* is the quantitative rearrangement of the mtDNA during aging. Assuming that the proteins of the respiration chain become damaged, mitochondrial function can be guaranteed only by successive replacement of defective molecules. Thus, remodelling of the mitochondria via the insertion of newly synthesized proteins (nuclear- and mitochondrial-encoded subunits) represents an important step in maintaining mitochondrial function.

Since mitochondria are important organelles involved in a key physiological process central in all eukaryotes – the process of energy transduction – many aspects of the biogenesis and metabolism of mitochondria are important for aging not only in *P. anserina* but also in more complex organisms, including humans. Here, also the complex molecular networks involved in cellular metal homeostasis are significant (Fig. 10.5). In *P. anserina*, various components of the network of regulatory circuits

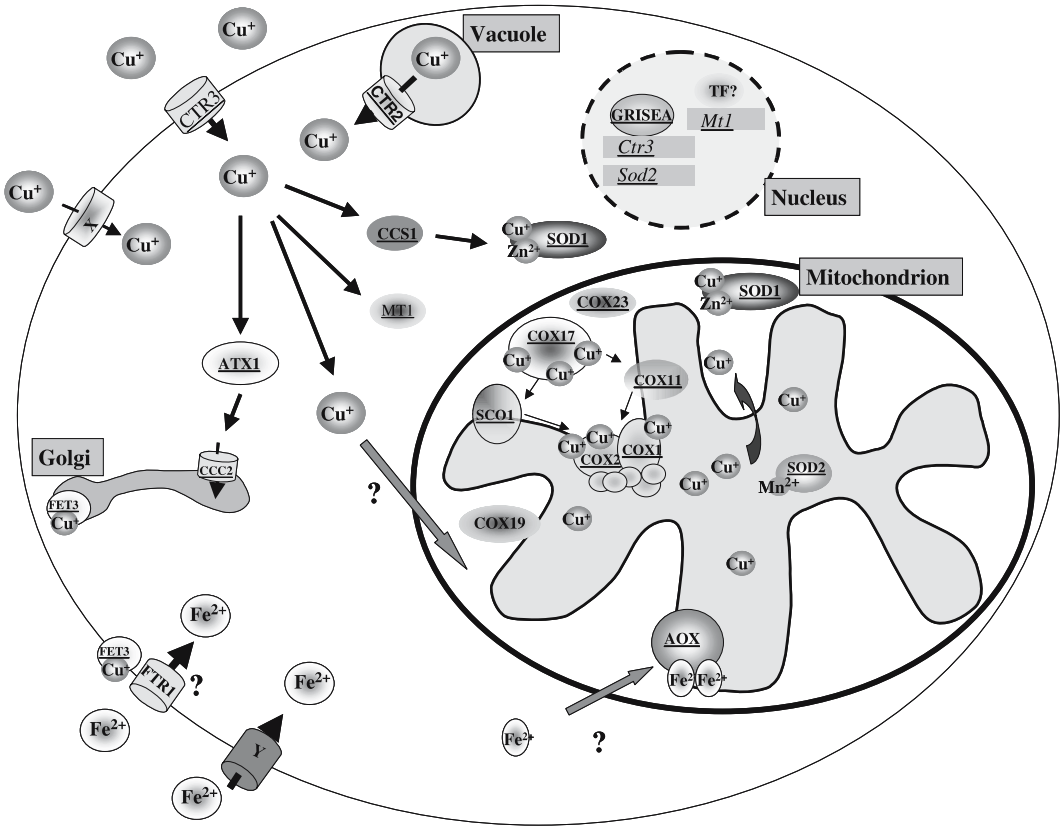

Fig. 10.5. Molecular networks controlling cellular metal homeostasis. All proteins *underlined* in the diagram have been studied in *P. anserina* in some detail (the COX subunits COX1 and COX2, the copper transporter CTR3 and CTR2, GRISEA, SOD1 and SOD2, the metallothionein MT1, COX17 and AOX) or have been deduced from the available genomic sequence with significant homology to proteins of yeast or *Arabidopsis* (the copper chaperones ATX1 and CCS1, the multi-copper oxidase FET3, the Cu(I) ATPase CCC2, and the COX assembly factors SCO1, COX11 and COX23). Others are assumed to be present but have yet not been identified in the *Podospora* sequence, presumably due to low conservation at the protein level (the iron permease FTR1, and the COX assembly factor COX19). The low-affinity copper transporter X and the iron transporter Y are speculative. In particular, the pathways delivering copper into the matrix of mitochondria and back into the intermembrane space for incorporation into COX are speculative and supported only by circumstantial evidence

have been identified, for example, AOX, CTR3, COX, SOD1 and SOD2. However, most of these have been identified only in homology searches of the *P. anserina* raw sequence available at http://podospora.igmors.u-psud.fr/index.html, and remain to be investigated in more detail. The in-depth elucidation of all pathways affecting life span is a prerequisite for the understanding of many age-related diseases, which reduce quality of life in particular in the elderly. However, today we are far from understanding these complex molecular networks in any biological system. Also in the future, research on aging models like *P. anserina* can be expected to provide important clues which are relevant for aging processes in general.

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References

Almasan A, Mishra NC (1988) Molecular characterization of the mitochondrial DNA of a stopper mutant ER-3 of *Neurospora crassa*. Genetics 120:935–945

Barros MH, Johnson A, Tzagoloff A (2004) COX23, a homologue of COX17, is required for cytochrome oxidase assembly. J Biol Chem 279:31943–31947

Belcour L, Begel O (1980) Life-span and senescence in *Podospora anserina*: effect of mitochondrial genes and function. J Gen Microbiol 119:505–515

Belcour L, Vierny C (1986) Variable DNA splicing sites of a mitochondrial intron: relationship to the senescence process in *Podospora*. EMBO J 5:609–614

Belcour L, Begel O, Mosse MO, Vierny (1981) Mitochondrial DNA amplification in senescent cultures of *Podospora anserina*: variability between the retained, amplified sequences. Curr Genet 3:13–21

Belcour L, Begel O, Keller AM, Vierny C (1982) Does senescence in *Podospora anserina* result from instability of the mitochondrial genome? In: Slominski PP, Borst P, Attardi G (eds) Mitochondrial genes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 415–422

Belcour L, Koll F, Vierny C, Sainsard-Chanet AS, Begel O (1986) Are proteins encoded in mitochondrial introns involved in the process of senescence in the fungus *Podospora anserina*? In: Courtois Y, Faucheuy B, Forette B, Knook DL, Tréton JA (eds) Modern trends in aging research. John Libbey Eurotext, London, pp 68–71

Bertrand H (2000) Role of mitochondrial DNA in the senescence and hypovirulence of fungi and potential for plant disease control. Annu Rev Phytopathol 38:397–422

Bertrand H, Collins RA, Stohl LL, Goff SC, Lambowitz AM (1985) Deletion mutants of *Neurospora crassa* mitochondrial DNA and their relationship to the "stop-start" growth phenotype. Proc Natl Acad Sci USA 77:6032–6036

Bertrand H, Griffiths AJ, Court DA, Cheng CK (1986) An extrachromosomal plasmid is the etiological precursor of kalDNA insertion sequences in the mitochondrial chromosome of senescent *Neurospora*. Cell 47:829–837

Böckelmann B, Esser K (1986) Plasmids of mitochondrial origin in senescent mycelia of *Podospora curvicolla*. Curr Genet 10:803–810

Borghouts C, Osiewacz HD (1998) GRISEA, a copper-modulated transcription factor from *Podospora anserina* involved in senescence and morphogenesis is an ortholog of MAC1 in *Saccharomyces cerevisiae*. Mol Gen Genet 260:492–502

Borghouts C, Kimpel E, Osiewacz HD (1997) Mitochondrial DNA rearrangements of *Podospora anserina* are under the control of the nuclear gene grisea. Proc Natl Acad Sci USA 94:10768–10773

Borghouts C, Kerschner S, Osiewacz HD (2000) Copper-dependence of mitochondrial DNA rearrangements in *Podospora anserina*. Curr Genet 37:268–275

Borghouts C, Werner A, Elthon T, Osiewacz HD (2001) Copper-modulated gene expression and senescence in the filamentous fungus *Podospora anserina*. Mol Cell Biol 21:390–399

Borghouts C, Scheckhuber CQ, Stephan O, Osiewacz HD (2002a) Copper homeostasis and aging in the fungal model system *Podospora anserina*: differential expression of *PaCtr3* encoding a copper transporter. Int J Biochem Cell Biol 34:1355–1371

Borghouts C, Scheckhuber CQ, Werner A, Osiewacz HD (2002b) Respiration, copper availability and SOD activity in *P. anserina* strains with different lifespan. Biogerontology 3:143–153

Butow (2002) Cellular responses to mitochondrial dysfunction: it's not always downhill. Cell Death Differ 9:1043–1045

Capaldi RA (1990) Structure and assembly of cytochrome c oxidase. Arch Biochem Biophys 280:252–262

Caten CE, Handley L (1978) Vegetative death syndrome in *Aspergillus glaucus*. Bull Br Mycol Soc 12:114

Cobine PA, Ojeda LD, Rigby KM, Winge DR (2004) Yeast contain a non-proteinaceous pool of copper in the mitochondrial matrix. J Biol Chem 279:14447–14455

Cottrell DA, Blakeley EL, Borthwick GM, Johnson MA, Taylor GA, Brierley EJ, Ince PG, Turnbull DM (2000) Role of mitochondrial DNA mutations in disease and aging. In: Toussaint O, Osiewacz HD, Lithgow G, Brack C (eds) Molecular and cellular gerontology. Ann N Y Acad Sci 908:199–207

Court DA, Griffiths AJ, Kraus SR, Russell PJ, Bertrand H (1991) A new senescence-inducing mitochondrial linear plasmid in field-isolated *Neurospora crassa* strains from India. Curr Genet 19:129–137

Cummings DJ, Belcour L, Grandchamp C (1979) Mitochondrial DNA from *Podospora anserina*. II. Properties of mutant DNA and multimeric circular DNA from senescent cultures. Mol Gen Genet 171:239–250

Cummings DJ, MacNeil IA, Domenica J, Matsuura ET (1985) Excision-amplification of mitochondrial DNA during senescence in *Podospora anserina*. DNA sequence analysis of three unique plasmids. J Mol Biol 185:659–680

Cummings DJ, Domenico JM, Turker MS (1987) Mitochondrial genomic changes during senescence in fungi. In: Thomson WW, Nothnagel EA, Huffacker RD (eds) Plant senescence: its biochemistry and physiology. American Society of Plant Physiologists, Bethesda, MD, pp 31–42

Dancis A, Haile D, Yuan DS, Klausner RD (1994) The *Saccharomyces cerevisiae* copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. J Biol Chem 269:25660–25667

Debets F, Yang X, Griffiths AJ (1995) The dynamics of mitochondrial plasmids in a Hawaiian population of *Neurospora intermedia*. Curr Genet 29:44–49

Delay C (1963) Observations inframicroscopiques sur le mycelium 'senescent' du *Podospora anserina*. C R Acad Sci Paris 256:4721–4724

De Vries H, de Jonge JC, van't Sant S, Agsteribbe E, Arnberg A (1981) A "stopper" mutant of *Neurospora crassa* containing two populations of aberrant mitochondrial DNA. Curr Genet 3:205–211

De Vries H, Alzner-DeWeerd B, Breitenberger CA, Chang DD, de Jonge JC, RajBhandary UL (1986) The E35 stopper mutant of *Neurospora crassa*: precise localization of deletion endpoints in mitochondrial DNA and evidence that the deleted DNA codes for a subunit of NADH dehydrogenase. EMBO J 5:779–785

Dufour E, Boulay J, Rincheval V, Sainsard-Chanet A (2000) A causal link between respiration and senescence in *Podospora anserina*. Proc Natl Acad Sci USA 97:4138–4143

Esser (1985) Genetic control of ageing: the mobile intron model. In: Bergener M, Emmini M, Stähelin HB (eds) The 1984 Sandoz lectures in gerontology. Academic Press, London, pp 3–20

Esser K, Keller W (1976) Genes inhibiting senescence in the ascomycete *Podospora anserina*. Mol Gen Genet 144:107–110

Esser K, Tudzynski P (1977) Prevention of senescence in the ascomycete *Podospora anserina* by the antibiotic tiamulin. Nature 265:454–456

Esser K, Tudzynksi P (1980) Senescence in fungi. In: Thiman KV (ed) Senescence in plants. CRC Press, Boca Raton, pp 67–83

Fassbender S, Bruhl KH, Ciriacy M, Kück U (1994) Reverse transcriptase activity of an intron encoded polypeptide. EMBO J 13:2075–2083

Fox AN, Kennell JC (2001) Association between variant plasmid formation and senescence in retroplasmid-containing strains of *Neurospora* spp. Curr Genet 39:92–100

Frese D, Stahl U (1992) Oxidative stress and ageing in the fungus *Podospora anserina*. Mech Ageing Dev 65:277–288

Glerum DM, Shtanko A, Tzagoloff A (1996) Characterization of COX17, a yeast gene involved in copper metabolism and assembly of cytochrome oxidase. J Biol Chem 271:14504–14509

Graden JA, Winge DR (1997) Copper-mediated repression of the activation domain in the yeast Mac1p transcription factor. Proc Natl Acad Sci USA 94:5550–5555

Gralla EB, Thiele DJ, Silar P, Valentine JS (1991) ACE1, a copper-dependent transcription factor, activates expression of the yeast copper, zinc superoxide dismutase gene. Proc Natl Acad Sci USA 88:8558–8562

Griffiths AJ (1992) Fungal senescence. Annu Rev Genet 26:351–372

Guarente L (1997) Link between aging and the nucleolus. Genes Dev 11:2449–2455

Guarente L, Kenyon C (2000) Genetic pathways that regulate ageing in model organisms. Nature 408:255–262

Handley L, Caten CE (1973) Vegetative death: a mitochondrial mutation in *Aspergillus amstelodami*. Heredity 31:136

Harman D (1956) A theory based on free radical and radiation chemistry. J Gerontol 11:298–300

Harman D (1981) The aging process. Proc Natl Acad Sci USA 78:7124–7128

Harman D (1998) Aging and oxidative stress. J Int Fed Clin Chem 10:24–27

Hermanns J, Osiewacz HD (1992) The linear mitochondrial plasmid pAL2-1 of a long-lived *Podospora anserina* mutant is an invertron encoding a DNA and RNA polymerase. Curr Genet 22:491–500

Hermanns J, Osiewacz HD (1996) Induction of longevity by cytoplasmic transfer of a linear plasmid in *Podospora anserina*. Curr Genet 29:250–256

Hermanns J, Asseburg A, Osiewacz HD (1994) Evidence for a life span-prolonging effect of a linear plasmid in a longevity mutant of *Podospora anserina*. Mol Gen Genet 243:297–307

Jamet-Vierny C, Begel O, Belcour L (1980) Senescence in *Podospora anserina*: amplification of a mitochondrial DNA sequence. Cell 21:189–194

Jazwinski SM (2000) Metabolic control and ageing. Trends Genet 16:506–511

Jazwinski SM (2001) New clues to old yeast. Mech Ageing Dev 122:865–882

Jazwinski SM (2004) Yeast replicative life span – the mitochondrial connection. FEMS Yeast Res 5:119–125

Jazwinski SM (2005) Yeast longevity and aging – the mitochondrial connection. Mech Ageing Dev 126:243–248

Jinks JL (1959) Lethal suppressive cytoplasm in aged clones of *Aspergillus glaucus*. J Gen Microbiol 21:397–409

Kim S, Ohkuni K, Couplan E, Jazwinski SM (2004) The histon acetyltransferase *GCN5* modulates the retrograde response and genome stability determining yeast longevity. Biogerontology 5:305–316

Kimpel E, Osiewacz HD (1999) *PaGrg1*, a glucose-repressible gene of *Podospora anserina* that is differentially expressed during lifespan. Curr Genet 35:557–563

Kirchman PA, Kim S, Lai CY, Jazwinski SM (1999) Interorganelle signaling is determinant of longevity in *Saccharomyces cerevisiae*. Genetics 152:179–190

Koll F, Begel O, Keller A-M, Vierny C, Belcour L (1984) Ethidium bromide rejuvenation of senescent cultures of *Podospora anserina*: loss of senescence-specific DNA and recovery of normal mitochondrial DNA. Curr Genet 8:127–134

Koll F, Belcour L, Vierny C (1985) A 1100-bp sequence of mitochondrial DNA is involved in senescence process in *Podospora*: study of senescent and mutant cultures. Plasmid 14:196–117

Krause F, Scheckhuber CQ, Werner A, Rexroth S, Reifschneider NH, Dencher NA, Osiewacz HD (2004) Supramolecular organization of cytochrome c oxidase- and alternative oxidase-dependent respiratory chains in the filamentous fungus *Podospora anserina*. J Biol Chem 279:26453–26461

Kück U, Stahl U, Esser K (1981) Plasmid-like DNA is part of mitochondrial DNA in *Podospora anserina*. Curr Genet 3:151–156

Kück U, Kappelhoff B, Esser K (1985a) Despite mtDNA polymorphism the mobile intron (plDNA) of the COI gene is present in 10 different races of *Podospora anserina*. Curr Genet 10:59–67

Kück U, Osiewacz HD, Schmidt U, Kappelhoff B, Schulte E, Stahl U, Esser K (1985b) The onset of senescence is affected by DNA rearrangements of a discontinuous mitochondrial gene in *Podospora anserina*. Curr Genet 9:373–382

Lazarus CM, Küntzel H (1981) Anatomy of amplified mitochondrial DNA in "Ragged" mutants of *Aspergillus amstelodami*: excision points within protein genes and a common 215 bp segment containing a possible origin of replication. Curr Genet 4:99–107

Lazarus CM, Earl AJ, Turner G, Künzel H (1980) Amplification of a mitochondrial DNA sequence in the cytoplasmatically inherited "Ragged" mutant of *Aspergillus amstelodami*. Eur J Biochem 106:633–641

Liao X, Butow RA (1993) RTG1 and RTG2: two yeast genes required for a novel path of communication from mitochondria to the nucleus. Cell 72:61–71

Liao XS, Small WC, Srere PA, Butow RA (1991) Intramitochondrial functions regulate nonmitochondrial citrate synthase (CIT2) expression in *Saccharomyces cerevisiae*. Mol Cell Biol 11:38–46

Linnane AW, Marzuki S, Ozawa T, Tanaka M (1989) Mitochondrial DNA mutations as an important contributor to ageing and degenerative disease. Lancet 1:642–645

Lorin S, Dufour E, Boulay J, Begel O, Marsy S, Sainsard-Chanet A (2001) Overexpression of the alternative oxidase restores senescence and fertility in a long-lived respiration-deficient mutant of *Podospora anserina*. Mol Microbiol 42:1259–1267

Maas MFPM, de Boer HJ, Debets AJM, Hoekstra RF (2004) The mitochondrial plasmid pAL2-1 reduces calorie restriction mediated life span extension in the filamentous fungus *Podospora anserina*. Fungal Genet Biol 41:865–871

Marbach K, Fernandez-Larrea J, Stahl U (1994) Reversion of a long-living, undifferentiated mutant of *Podospora anserina* by copper. Curr Genet 26:184–186

Marcou D (1961) Notion de longévité et nature cytoplasmique du déterminant de la sénescence chez quelques champignons. Ann Sci Nat Bot 12:653–764

McDaniels CPJ, Jensen LT, Srinivasan C, Winge DR, Tullius TD (1999) The yeast transcription factor Mac1 binds to DNA in a modular fashion. J Biol Chem 274:26962–26967

McMurray MA, Gottschling DE (2004) Aging and genetic instability in yeast. Curr Opin Microbiol 7:673–679

Michel F, Lang B (1985) Mitochondrial class I introns encode proteins related to the reverse transcriptases of retroviruses. Nature 316:641–643

Munkres K, Rana RS (1978) Antioxidants prolong life span and inhibit the senescence-dependent accumulation of fluorescent pigment (lipofuscin) in clones of *Podospora anserina*. Mech Ageing Dev 7:407–415

Navaraj A, Pandit A, Maheshwari R (2000) *Senescent*: a new *Neurospora crassa* nuclear gene mutant derived from nature exhibits mitochondrial abnormalities and a "death" phenotype. Fungal Genet Biol 29:165–173

Nobrega MP, Bandeira SC, Beers J, Tzagoloff A (2002) Characterization of COX19, a widely distributed gene required for expression of mitochondrial cytochrome oxidase. J Biol Chem 277:40206–40211

Osiewacz HD (1995) Aging and genetic instabilities. In: Esser K, Martin GM (eds) Molecular aspects of aging. Wiley, New York, pp 29–44

Osiewacz HD (2002a) Mitochondrial functions and aging. Gene 286:65–71

Osiewacz HD (2002b) Genes, mitochondria and aging in filamentous fungi. Ageing Res Rev 1:425–442

Osiewacz HD, Esser K (1984) The mitochondrial plasmid of *Podospora anserina*: a mobile intron of a mitochondrial gene. Curr Genet 8:299–305

Osiewacz HD, Hermanns J (1992) The role of mitochondrial DNA rearrangements in aging and human diseases. Aging (Milano) 4:273–286

Osiewacz HD, Nuber U (1996) GRISEA, a putative copper-activated transcription factor from *Podospora anserina* involved in differentiation and senescence. Mol Gen Genet 252:115–124

Osiewacz HD, Hermanns J, Marcou D, Triffi M, Esser K (1989) Mitochondrial DNA rearrangements are correlated with a delayed amplification of the mobile intron (plDNA) in a long-lived mutant of *Podospora anserina*. Mutat Res 219:9–15

Prillinger H, Esser K (1977) The phenoloxidases of the ascomycete *Podospora anserina*. XIII. Action and interaction of genes controlling the formation of laccase. Mol Gen Genet 156:333–345

Richter OM, Ludwig B (2003) Cytochrome c oxidase – structure, function, and physiology of a redox-driven molecular machine. Rev Physiol Biochem Pharmacol 147:47–74

Rizet G (1953) Sur l'impossibilité d'obtenir la multiplication végétative ininterrompue et illimitée de l'Ascomycète *Podospora anserina*. C R Acad Sci Paris 237:838–840

Samuels DC, Schon EA, Chinnery PF (2004) Two direct repeats cause most human mtDNA deletions. Trends Genet 20:393–398

Schulte E, Kück U, Esser K (1988) Extrachromosomal mutants from *Podospora anserina*: permanent vegetative growth in spite of multiple recombination events in the mitochondrial genome. Mol Gen Genet 211:342–349

Sekito T, Thornton J, Butow RA (2000) Mitochondria-to-nuclear signaling is regulated by the subcellular localization of transcription factors Rtg1p and Rtg3p. Mol Biol Cell 11:2103–2115

Sellem CH, Lecellier G, Belcour L (1993) Transposition of a group II intron. Nature 366:176–178

Sellem CH, Lemare C, Lorin S, Dujardin G, Sainsard-Chanet A (2005) Interaction between the *oxa1* and *rmp1* genes modulates respiratory complex assembly and lifespan in *Podospora anserina*. Genetics 169:1379–1389

Silar P, Koll F, Rossignol M (1997) Cytosolic ribosomal mutations that abolish accumulation of a circular intron in the mitochondria without preventing senescence of *Podospora anserina*. Genetics 145:697–705

Silar P, Lalucque H, Vierny C (2001) Cell degeneration in the model system *Podospora anserina*. Biogerontology 2:1–17

Smith ML, Bruhn JN, Anderson JB (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428–431

Stahl U, Lemke PA, Tudzynski P, Kück U, Esser K (1978) Evidence for plasmid like DNA in a filamentous fungus, the ascomycete *Podospora anserina*. Mol Gen Genet 162:341–343

Stahl U, Tudzynski P, Kück U, Esser P (1982) Replication and expression of a bacterial-mitochondrial hybrid plasmid in the fungus *Podospora anserina*. Proc Natl Acad Sci USA 79:3641–3645

Stumpferl SW, Stephan O, Osiewacz HD (2004) Impact of a disruption of a pathway delivering copper to mitochondria on *Podospora anserina* metabolism and life span. Eukaryot Cell 3:200–211

Sturtz LA, Diekert K, Jensen LT, Lill R, Culotta VC (2001) A fraction of yeast Cu/Zn superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria: a physical role for SOD1 in guarding against mitochondrial oxidative damage. J Biol Chem 276:38084–38089

Tudzynski P, Esser K (1977) Inhibitors of mitochondrial function prevent senescence in the ascomycete *Podospora anserina*. Mol Gen Genet 153:111–113

Tudzynski P, Esser K (1979) Chromosomal and extrachromosomal control of senescence in the ascomycete *Podospora anserina*. Mol Gen Genet 173:71–84

Tudzynski P, Stahl U, Esser K (1982) Development of an eukaryotic cloning system in *Podospora anserina*. I. Long-lived mutants as potential recipients. Curr Genet 6:219–222

Turker MS, Nelson JG, Cummings DJ (1987) A *Podospora anserina* longevity mutant with a temperature-sensitive phenotype for senescence. Mol Cell Biol 7:3199–3204

Vierny C, Keller AM, Begel O, Belcour L (1982) A sequence of mitochondrial DNA is associated with the onset of senescence in a fungus. Nature 297:157–159

Wagner AM, Moore AL (1997) Structure and function of the plant alternative oxidase: its putative role in the oxygen defense mechanism. Biosci Rep 17:319–333

Wallace DC (1992) Diseases of mitochondrial DNA. Annu Rev Biochem 6:1175–1212

Wallace DC (2001) Mitochondrial diseases in man and mouse. Science 283:1482–1488

Wright RM, Horrum MA, Cummings DJ (1982) Are mitochondrial structural genes selectively amplified during senescence in *Podospora anserina*? Cell 29:505–515

Yoshikawa S, Shinzawa-Itoh K, Tsukihara T (1998) Crystal structure of bovine heart cytochrome c oxidase at 2.8 A resolution. J Bioenerg Biomembr 30:7–14

Zhu Z, Labbe S, Pena MM, Thiele DJ (1998) Copper differentially regulates the activity and degradation of yeast Mac1 transcription factor. J Biol Chem 273:1277–1280

Signals in Growth and Development

11 Autoregulatory Signals in Mycelial Fungi

U. Ugalde[1]

CONTENTS

I. Introduction

The mycelium is a very successful colony form capable of orchestrating the development of different cell types in response to the microenvironments which it encounters. This attribute in particular has led to the realisation that the mycelium is governed by a sophisticated chemosensitive system.

Many of the elements of this system have probably been already inventoried after several decades of intense research, but the functional connections between them remain largely undiscovered. On the one hand, many reported colony functions have been ascribed to as yet unidentified chemical signals, whilst on the other, many thousands of molecules have been purified and characterised, of which relatively few have been credited with a biological role.

Those signals recognised so far comprise two large categories. First, those directed at relations with other organisms, such as hosts and including elicitors and inhibitors, competitors involving antibiotics, and predators where mycotoxins are implicated (for further information, see The Mycota, Vols. VA, X and XI).

The second category consists of in-house signals which formerly fell under the generic term *hormones* (Gooday 1994), and are now separately considered. They include *pheromones*, which facilitate the interaction of compatible gametes, and *developmental hormones* regulating the formation or maintenance of differentiated multicellular structures or protoorgans, such as ascomata or basidiomata. These will not be dealt with in this chapter, but individually in Chaps. 12 (Pheromone action in the fungal groups Chytridiomycota, and Zygomycota, and in the Oomycota), 17 (Mating type genes of the basidiomycetes) and 16 (Fruiting body development in ascomycetes) of this volume respectively.

In addition to these important in-house signals, there is an array of endogenous cues which convey information on environmental conditions or the status of cells within the mycelium, thus ensuring a coordinated colony function. They are transmitted by extracellular metabolites, and their signalling action results in adaptations which are advantageous to the colony as a living unit. In the past, these signals have been referred to as autoinducers, autoinhibitors, quorum sensing factors and morphogens. From a functional viewpoint, they all share the feature of regulating transitions between alternative morphogenetic and/or functional programmes, hence the proposal to term them collectively as *autoregulators*. Signalling molecules performing equivalent tasks had already been assigned this term when first discovered in filamentous bacteria (Horinouchi and Beppu 1992). However, the range of activities covered in mycelial fungi can be presumed to be wider and more complex in light of the information now available on secondary metabolites and fungal development (Calvo et al. 2002).

This review will focus on those autoregulatory signals which are involved in germination, colony morphogenesis, and asexual and sexual development in mycelial fungi. Finally, the incidence of autoregulators in dimorphism will also be covered.

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The Mycota I
Growth, Differentation and Sexuality
Kües/Fischer (Eds.)

II. Germination

The programmed outcome of every fungal spore is to land on an appropriate habitat and successfully establish a new colony. Current evidence shows that a number of chemosensory signals are involved in gauging the potential success of germination at any one circumstance. Many of these report on the nature of the substratum, be it as exudates on a host surface or a complex soil environment where, for example, phenols, carbohydrates and pH are involved. Additionally, several examples are known where autoregulatory signals are operating to prevent the spore from futile competition with members of its kin which may have followed a similar dispersion path.

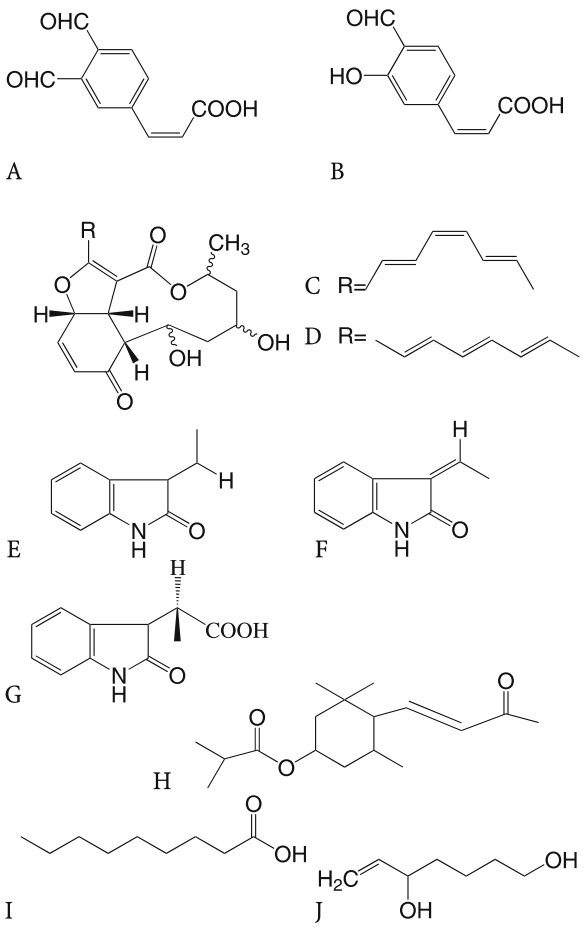


Fig. 11.1. A–J Autoregulatory signals involved in autoinhibition of germination: **A** *cis*-methyl 3,4 dimethoxycinnamate, **B** *cis*-methyl ferulate, **C** colletofragarone A, **D** colletofragarone B, **E–G** CG1, CG2 and CG3, **H** quiesone, **I** nonanoic acid, and **J** 1-octen-3-ol

The first demonstration that spores sense and respond to an overcrowded environment through self-produced chemical signals came from studies with the rust *Puccinia graminis* (Allen 1955). These autoregulatory signals have been specifically termed *autoinhibitors* or *self-inhibitors* (Macko and Staples 1973), and have been reported for more than 60 fungal species (Allen 1976). A representative collection is shown in Fig. 11.1.

Germination autoinhibitors are thought to be produced at the time of sporulation, and deposited at the outer wall layers of spores, but whether they are freely deposited or progressively pass from a bound to an unbound form is still a subject of speculation (Macko and Staples 1973). Once in contact with an aqueous medium, they diffuse through the bulk liquid surrounding the spore but also commonly partition to the gas phase, enabling the signal to spread over various diffusion barriers, to other spores in the vicinity. The signal is extremely effective, and 50% inhibition has been reported at nano molar concentrations in those cases in which it has been measured (Allen 1972).

Germination autoinhibitors of plant rusts show variability and specificity in mode of action. In the case of the bean rust *Uromyces phaseoli*, the active agent is methyl-*cis*-3,4-dimethoxycinnamate (Fig. 11.1a) whereas in the wheat stem rust counterpart *Puccinia graminis*, it is methyl-*cis*-ferulate (methyl-*cis*-4-hydroxy-3-methoxycinnamate; Fig. 11.1b). In both cases it has been shown that the *cis* isomer is the active inhibitor, although even low UV radiations result in a conversion into *trans* isomers, with both forms found at equilibrium under natural conditions (Allen 1972). Other plant pathogens, such as *Colletotrichum fragariae*, have been reported to produce colletofragarone A1 and A2 ((E)- and (Z)-(3-indoyl)propionic acid; Fig. 11.1c,d) and colletofragarone B ((2R)-(3-indoyl)propionic acid; Inoue et al. 1996). The related *C. gleosporoides* has been reported to produce CG-SI 1 and 2 ((E)- and (Z)-3-ethylidine-1,3-dihydroindol-2-one; Fig. 11.1e,f) and CG-SI 3 ((2R)-(3-indoyl)propionic acid; Fig. 11.1g; Tsurushima et al. 1995). The tobacco blight fungus *Peronospora tabacina*, in turn, uses quiesone (5-isobutyroxy-β-ionone, Fig. 11.1h) as germination autoinhibitor (Leppik et al. 1972).

In contrast to the chemical specificity displayed by plant pathogens, saprophytes appear to use a more generalised signalling cue. Nonanoic acid (Fig. 11.1i) has been shown to be produced and sensed by spores of many soil fungi (Garret

and Robinson 1969). Related compounds such as 1-octen-3-ol (Fig. 11.1j) have been recently reported to act in the cereal pathogen *Penicillium paneum* (Chitarra et al. 2004), in which the action of the autoinhibitor was species specific.

The marked differences in specificity between biotrophic pathogens and saprophytes may originate from the fact that the former occupy a specific niche in which the host may also generate molecules which play an important role in the infection process. These often comprise volatile alcohols of various chain lengths, such as nonyl alcohol (Allen 1972), *trans*-2-hexen-1-ol (Collins et al. 2001) and surface waxes (Podila et al. 1993). These stimulatory signals share molecular and physicochemical characteristics with germination autoinhibitors and must, therefore, be clearly distinguished by the spore. The general consensus is that mono-unsaturated aliphatic chain-containing self-inhibitors are *cis* isomers, whereas the stimulators are mostly in the *trans* conformation. In addition, the positioning of the unsaturated bonds is important for differentiation between stimulators and inhibitors (Wang et al. 2002).

Saprophytes need not differentiate necessarily between self and non-self, but may preferentially need to determine the extent of occupancy of the substratum in a first instance, independently of the nature of the occupants. This may be the principal reason for a less varied array of reported germination autoinhibitors in saprophytic fungi. Nevertheless, as work progresses on the chemical ecology of these signals, this current understanding may be confirmed or reformed by the weight of new evidence.

The mechanism of action of germination autoinhibitors has been examined in some detail. Nonanoic acid has been postulated to provoke alterations in membrane permeability and, consequently, in intracellular pH (Breewer et al. 1997). Other studies on the effect of germination inhibitors of mildew point to a direct involvement of K^+ channel activation and consequent loss of intracellular potassium (Wang et al. 2002). The process of germination itself appears to involve the independent participation of RAS and cAMP signalling pathways (Fillinger et al. 2002). Autoinhibitors have been shown to block calmodulin gene expression (Liu and Kalattukudy 1999) but whether this is directly or indirectly exerted by the germination effectors is not yet clear.

Although mycelial fungi are known mostly to produce germination inhibitors, the higher fungi possess self-produced germination *autoinducers*. These are also volatile substances which are produced by spores and mycelia, and which have been shown to be necessary to trigger germination. Among them, isovaleric acid is the best known autoinducer, and it is produced by spores and mycelia of *Agaricus bisporus* (Rast and Stäuble 1970).

The currently accepted interpretation of the role of germination autoinducers is that they could maximize the opportunity of compatible spores to form a dikaryon (an important feature of the life cycle of the producer). This distinguishing feature would make it advantageous for maximum spore germination in an environment crowded with compatible spores.

In all, the information available on germination autoinhibitors and autostimulators is still limited and dated. An interdisciplinary approach to the study of these molecules and their mode of action should yield important new knowledge in the future.

III. Colony Morphogenesis

Having completed the germination process, emerging hyphae extend away from the germling, eventually giving rise to a radial colony. The development of a colony from a germling to a multifunctional living body has been described as a complex process (Fig. 11.2A; Buller 1933). At the advancing edge, apical extension growth is clearly dominant, with few ramifications. Older subapical zones display lateral branches which fuse with already existing leading hyphae, or amongst themselves. This transforms the initial radial pattern into a matrix, with important functional consequences for the colony. At distal regions approaching the spatial and temporal origin of the colony, living vacuolated cells survive, support intercellular transport and the formation of long-term survival structures. There is no record of hyphal growth from the periphery into these regions. The colony hence appears to undertake a self-organising plan. The nature of the autoregulatory signals responsible for the undertaking of this plan remains largely unknown, but some information is available on the subject.

Newly formed hyphae elongate by apical extension growth, and the process by which they

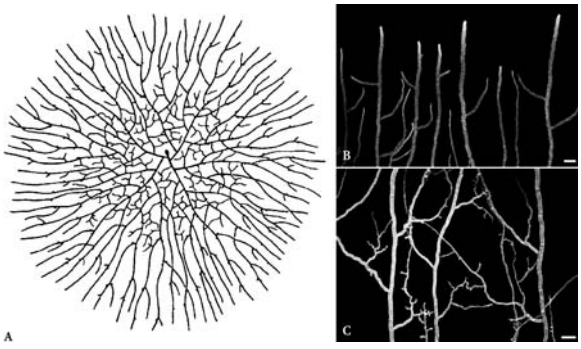


Fig. 11.2.A–C Drawing showing the typical pattern of a fungal colony resulting from a single germinated spore (**A**). The peripheral zone contains no anastomosis of hyphae, whereas the central zone shows anastomosis and the formation of a matrix. Image adapted from Buller (1933). Confocal images showing hyphal organisation at the periphery (**B**) and at distal regions (**C**) in a colony of *Neurospora crassa*, with distinctly different patterns of growth, branching and anastomosis. Images kindly provided by Hickey et al. (2002)

grow away from their origin, as well as from each other, has been the subject of much study (Glass et al. 2004). A clear depiction of this pattern is provided by Hickey et al. (2002) in *Neurospora crassa* (Fig. 11.2B) but the chemical signals driving this guiding system remain unknown. Chemotropism along increasing concentration gradients of nutrients (and therefore away from lower nutrient-containing zones) has found little supporting evidence (Gooday 1975). However, positive aerotropism (tropism towards higher oxygen concentrations) has been shown to occur in early studies (Robinson 1973a,b,c). This guiding cue would not only direct hyphae away from the centre of the colony but also away from each other. No evidence has yet emerged on the existence of autoregulatory signals guiding this growth pattern, but they cannot be ruled out at this time. Fresh biophysical, biochemical and cytochemical determinations will be required to confirm the identity of this important guiding system.

In contrast to the outward tropism shown by leading hyphae at the colony edge, subapical regions generate lateral branches which fuse with each other (Gooday 1999, and Glass and Fleißner, Chap. 7, this volume). Detailed studies with fluorophore-labelled *Neurospora crassa* hyphae (Hickey et al. 2002) show that the formation of lateral branches is induced by the remote presence of other hyphae and moreover, once formed, they attract each other (Fig. 11.2C). The chemical signals which operate in these two processes (formation of lateral branches, and their homing and fusion) could be different, and still remain unidentified. Early projections on autoregulator-mediated positive autotropism by Müller and Jaffe (1965) in *Botrytis cinerea* postulated that a diffusible growth stimulator, with a half life of 10 s and acting at a radius of 10 µm around each hypha, would fit in with their observations. This projection resembles similar ones presented later by other authors (Gooday 1975). Flow cell studies using *Aspergillus oryzae* have shown that substitution of fresh medium with medium which was re-circulated resulted in increased apical extension growth and increased branching (Spohr et al. 1998). This has supported the suggestion that a relatively stable, self-produced growth and branching inducer or inducers were present in the medium. Further studies along these lines should clarify the nature of these endogenous signals, and whether they influence colony morphogenesis.

In addition to positive autotropism (perpendicular to the growth axis) at subapical zones, a negative tropism (along the growth axis) inhibiting backgrowth towards older parts of the colony has also been reported in mycelial colonies. Studies in static agar cultures by Bottone et al. (1998) showed that cultures of *Mucor* spp. and *Aspergillus fumigatus* on membrane filters over agar plates, later removed along with the membranes, left a clear circular patch of agar, into which backgrowth was precluded. The effect of nutrient depletion on this phenomenon was ruled out by several controls, and treatment of the cleared region with chloroform removed the inhibition, leading the authors to propose that an endogenous lipidic factor was involved. In addition, backgrowth took place only when the central patch was replaced by fresh medium and separated from the surrounding colony by a "moat". This measure avoided back diffusion of the inhibitor from the surrounding biomass. In addition to this experimental evidence, theoretical models could only emulate colony morphogenesis by incorporating the action of a self-produced inhibitor of backgrowth in the kinetic algorithms (Indermitte et al. 1994). The chemical identity of this signal remains to be determined.

Previous reports by Park and Robinson (1964) had described activities of self-produced staling compounds causing vacuolation in cultures of *A. niger*. The same investigators (Robinson and Park 1966) later described that citrinin acts as a self-produced agent causing hyphal narrowing (a feature attributed to hyphae from older regions) and

senescence. However, no specific experiments on backgrowth inhibition were performed.

The combined evidence indicates that autoregulators are very probably involved at various stages of colony morphogenesis, despite our current ignorance of their chemical nature.

IV. Asexual Development

Behind the peripheral growth region, the colony not only adapts its structure for intercommunication by hyphal anastomosis but additionally, it often prepares aerial hyphae in distal regions for the production of asexual spores as dispersal propagules. The autoregulatory signals reporting on the emergence of hyphae from the substratum to the air have been assigned to secondary metabolites.

Early studies of asexual spore production point to the existence of self-produced metabolites as active agents in sporulation. Perhaps the most precise example of this comes from the work of Hadley and Harrold (1958) who described an unidentified endogenous factor believed to enhance 1–10 mM calcium-induced conidiation (asexual sporulation) in liquid cultures of *Penicillium notatum*. An equivalent factor was only recently isolated and identified in the closely related *Penicillium cyclopium*. Moreover, it was shown to be both necessary and sufficient for conidiation induction, whilst calcium was demonstrated to act as the enhancer. The factor was hence given the common name of *conidiogenone* (Roncal et al. 2002).

Conidiogenone is a diterpene (1R*,3R*,7R*,8S*, 11R*,14S*,15R*)-3,6,6,11,15 pentamethyl-tetracyclo[9.4.0.0^{1,8}.0^{3,7}]-pentadecan-12-one, Fig. 11.3a) which is produced throughout the growth phase of the mycelium under submerged conditions. On emergence to the air, the autoregulator is believed to accumulate at the surface of hyphae, thus attaining concentrations which surpass the threshold levels (350 pM). This rapid accumulation has been proposed to result in binding of conidiogenone to an as yet unidentified receptor at the cell surface, thus triggering a signal transduction pathway leading to spore production. For more details on conidiation induction, see Fischer and Kües (Asexual sporulation in mycelial fungi, Chap. 14, this volume). When the hypha is in a liquid environment, conidiogenone is diluted in the bulk medium, thereby remaining at sub-threshold levels. In the exceptional case of some *Penicillia*, the presence of calcium exerts changes at the cell surface and this reduces the threshold level fivefold, thus facilitating conidiation under submerged conditions.

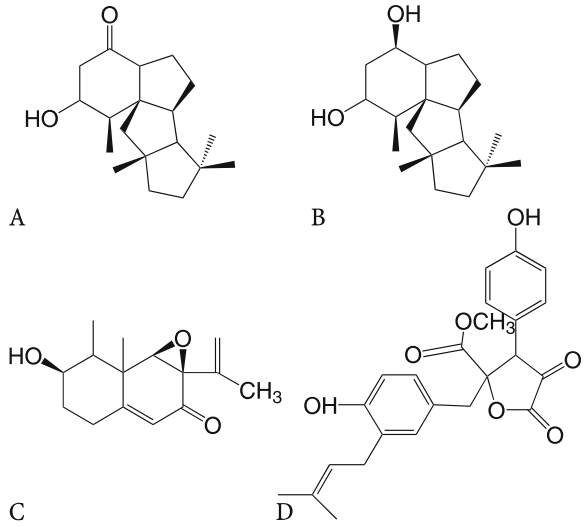


Fig. 11.3. A–D Autoregulatory signals involved in conidiation induction: **A** conidiogenone, **B** conidiogenol, **C** sporogen AO-1, and **D** butyrolactone I

In order to avoid long-term signal accumulation which would result in inappropriate conidiation induction, conidiogenone is continuously converted to an inactive derivative, conidiogenol (1R*,3R*,7R*,8S*,11R*,14S*,15R*)-14-hydroxy-3,6, 6,11,15 pentamethyl-tetracyclo[9.4.0.0^{1,8}.0^{3,7}]-pentadecan-12,14-diol, Fig. 11.3b), by the reduction of a single keto group (Roncal et al. 2002).

There are other instances in which self-produced secondary metabolites have been reported to exert a role as autoregulators in asexual spore production. The partially elucidated diterpene sporogen-PF-1 is produced by *Penicillium funiculosum* under blue light, and promotes the production of conidia (Katayama et al. 1989). The sesquiterpene sporogen-AO1 ((1aR,6R,7bR)-5,6,7,7a-tetrahydro-6-hydroxy-7,7a-dimethyl-1a-(prop-1-en-2-yl)naphtho[2,1-b]oxiren-2(1aH,4H,7bH)-one; Fig. 11.3c) has been reported to exert sporogenic effects in *Aspergillus oryzae* (Tanaka et al. 1984). Butyrolactone I (methyl 2-(4-hydroxy-3-(3-methylbut-2-enyl)benzyl)-tetrahydro-3-(4-hydroxyphenyl)-4,5-dioxofuran-2-carboxylate; Fig. 11.3d), a small γ-butyrolactone-containing metabolite, has been found in *Aspergillus terreus* cultures, with significant effects on branching and asexual spore pro-

duction (Schimmel et al. 1998). Interestingly, small γ-butyrolactone-containing molecules are known quorum-sensing factors in gram-negative bacteria, and also regulate cellular differentiation in filamentous bacteria (Horinouchi and Beppu 1992).

The autoregulatory signal responsible for the induction of conidiation in the model organism *Aspergillus nidulans* was first recognised in genetic studies which led to the isolation of a mutant defective in the *fluG* gene, which only conidiated when in contact with a wild-type colony (Lee and Adams 1994). The signalling factor which is transferred when such extracellular complementation events take place remains unidentified.

What are believed to be the principal systems which signal conidiation induction may be modulated by the action of other autoregulatory cues which exert their action under specific ecological circumstances. Recent studies by Calvo et al. (1999) have shown that linoleic acid and two oxylipin derivatives which are commonly found in seeds (9S- and 13S-hydroperoxylinoleic acid) had a sporogenic effect when administered on membrane disks over Petri dishes inoculated with 10^5 spores. The positive effects with *Aspergillus flavus*, *A. parasiticus* and *A. nidulans* were compatible with earlier reports describing similar effects on *Alternaria tomato* (Hyeon 1976), *Sclerotinia fructicola* (Katayama and Marumo 1978) and *Neurospora crassa* (Roeder et al. 1982). In the latter, fluctuations in linoleic acid levels at the hypha tips were related to oscillations in the circadian rhythm of conidiation. In *A. flavus*, linoleic acid and two of its hydroxylated derivatives produced a conidiation halo around the membrane disk, while saturated counterparts such as oleic and palmitic acid had no effect. Similar findings were recorded with the other two species but, in *A. nidulans* strains which had a mutated velvet (*veA*) gene which makes conidiation light-dependent, the effects were weak or negligible. Indeed, light was a determinant factor in the efficacy of the unsaturated fatty acid treatments in all wild-type strains (Calvo et al. 1999). The authors proposed that seed fatty acids may regulate fungal development by mimicking and/or interfering with signals which regulate fungal sporogenesis, in reference to psi factors, which are also derived from linoleic acid and are responsible for governing the balance between asexual and sexual development (see below).

The evidence accumulated in recent years supports the view that mycelial fungi use autoregulatory signals for the perception of adequate conditions to initiate asexual development. These activate signal transduction systems which also integrate exogenous cues related to stress conditions (namely, starvation and osmotic shock; Roncal and Ugalde 2003). Aside from this system, autoregulators are also involved in establishing the balance between asexual and sexual development, as discussed below.

V. Sexual Development

In addition to the formation of asexual spores, which often serve as short-term dispersal propagules, mycelial fungi also produce long-term resistance structures capable of enduring changing environmental conditions. In many cases, those structures enclose spores obtained by meiotic division, thus ensuring maximum recombination from the genetic resources available in the colony. This strategy is exemplified by the fruiting bodies of the ascomycetes, the production of which appears to be determined by a combination of environmental cues and endogenous autoregulatory signals.

Pioneering work on autoregulatory signals which determine the onset of sexual development was initiated in the early 1980s by Sewell P. Champe and collaborators, who undertook a multidisciplinary approach. For a start, a series of aconidial mutants of *Aspergillus nidulans* were isolated, which typically showed low conidiation levels, premature production of cleistothecia, and the excretion of the antibiotic diorcinol (3,3′-dihydroxy-5,5′-dimethyldiphenyl ether; Butnick et al. 1984a,b). Preliminary analysis of these mutants already led the investigators to conclude that asexual and sexual development were controlled by a common modulating pathway. It was later observed that the overproduction of a solvent-extractable chemical factor (psi factor, standing for premature sexual induction) was responsible for the phenotype. Even when administered to wild-type strains, the factor could inhibit conidiation and promote sexual development (Champe et al. 1987). Three types of hydroxylated derivatives of C18 unsaturated fatty acids were identified, based on the starting fatty acid molecule subject to hydroxylation. Those based on oleic acid (18:1) were termed psiβ, those

derived from linoleic (18:2), psiα, and those from linolenic (18:3), psiγ. The placing of the hydroxyl groups gave rise to the terms psiB (hydroxyl substitution in position 8), psiC (positions 5 and 8) and psiA (a lactoniester of psiC; Champe and El-Zayat 1989; Mazur et al. 1990, 1991).

Dosage studies with psiα oxylipins indicated that marked differences in biological activity could be obtained between applications of psiBα (8-hydroxylinoleic acid; Fig. 11.4a) and psiCα (5,8-dihydroxylinoleic acid; Fig. 11.4b), which stimulated sexual spore development to the detriment of conidiation, and psiAα (a lactonized ester of psiCα; Fig. 11.4c) which acted as an antagonist, shifting the balance towards asexual spore development.

Recent studies by Tsitsigiannis et al. (2004a,b) have further identified two *A. nidulans* dioxygenases (enzymes which catalyse the oxygenation of unsaturated fatty acids) PpoA and PpoC, which are required for the biosynthesis of psiBα and psiBβ respectively (Fig. 11.4a,d). The former oxilipin was confirmed to cause a decreased ratio of conidia to ascospores whereas the latter had a reversed effect, increasing ascospore production and decreasing conidial development. The concerted regulation of both enzymatic activities through gene expression was shown to be complex and dependent on, but also modulating the expression of *brlA* and *nsdD*, central regulator genes of conidiogenesis and ascogenesis respectively. Moreover, detailed transcriptional and biochemical analyses showed that psiBα and psiBβ possibly act as antagonist signals in regulatory feedback loops which ultimately control lipid biosynthesis.

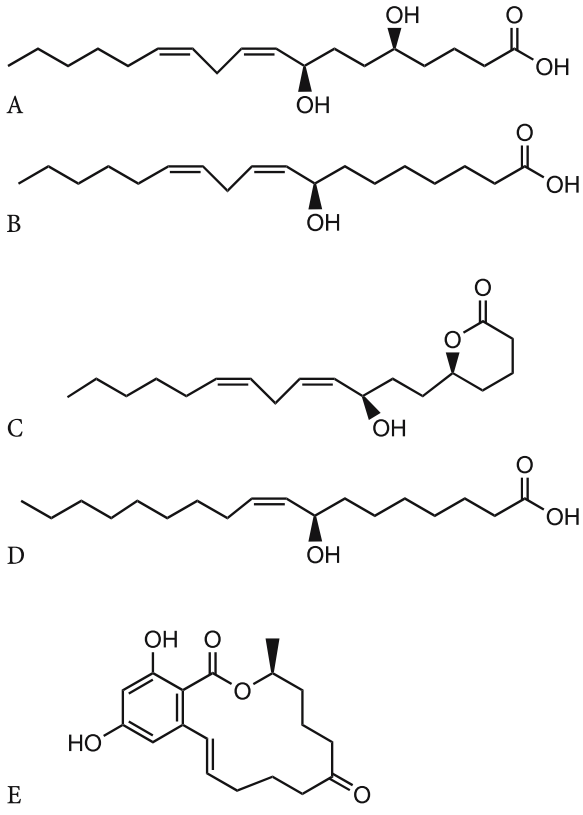


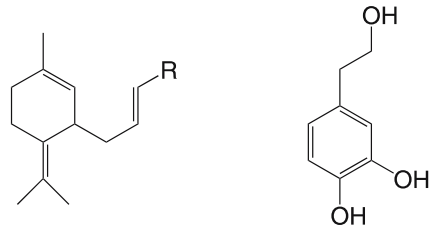
Fig. 11.4. A–E Autoregulatory signals involved in sexual development: **A** psiBα, **B** psiCα, **C** psiAα, **D** psiBβ, **E** zearalenone

The above studies appear as the initial unveiling of a sophisticated regulation system combining an array of oxylipin derivatives which may collectively modulate the balance between asexual and sexual development. The formulation of such endogenous autoregulatory signals is also known to integrate exogenous stimuli, and one environmental cue which affects the equilibrium between sexual and asexual development in wild-type cultures of *Aspergillus nidulans* is light, which stimulates conidiation and represses sexual development. An active *veA* gene, encoding for light sensitivity in this organism (Yager 1992), is essential for sensitivity to externally added linoleic acid derivatives (Champe et al. 1987), and even affects fatty acid content and composition (Calvo et al. 1999). Thus, the interconnections between the light sensory system and oxylipin signalling has been established, although the details still remain to be elucidated. The topic of sexual development and its regulation, with special reference to *Aspergillus nidulans*, is covered in the Chaps. 14 (Asexual sporulation in mycelial fungi) and 16 (Fruiting body development in ascomycetes) in this volume.

Another example of self-generated metabolites influencing sexual development is that observed in *Fusarium graminearum* by zearalenone (Wolf and Mirocha 1973). Zearalenone (3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1H-2-benzoaxacyclotetradecin-1,7(8H)-dione, Fig. 11.4e) was notorious for its oestrogenic effects on mammals which had ingested fodder contaminated with the fungus, but it was shown that its principal role was to induce perithecial formation at quantities as low as 0.1 ng per 1-cm-diameter filter disks on a Petri dish. A study of the structure–function relations of this molecule showed that the ketone in the 6′ position of the undecenil ring was necessary for activity. An inactive derivative (zearalenol) found in culture filtrates bears a hydroxyl group at the same

position, possibly indicating a time-dependent inactivation of the signal through a reduction step. Another interesting feature is that zearalenone was inhibitory at concentrations higher than 10 ng per disk, and the unsaturation at the 1′,2′ position on the undecenyl ring was associated with this feature.

Recent investigations have revealed a role of self-generated reactive oxygen species (ROS) by mycelial fungi on the onset of sexual development. Lara-Ortiz and et al. (2003) demonstrated that a NADPH oxidase (NoxA), which is a member of a family of enzymes ubiquitous to lower eukaryotes, plays a relevant role in cleistothecial development in *Aspergillus nidulans*. Expression of *NoxA* is specifically induced in Hülle cells and the outer layers of cleistothecia initials, and its deletion blocks maturation of cleistothecia at an early stage. Cleistothecia and associated Hülle cells both produce ROS (peroxide which is probably dismutated to H_2O_2) during development, and treatment with DPI (diphenyleneiodonium sulphate), a substrate inhibitor of NADPH oxidases, blocks the process. The authors therefore propose that H_2O_2 regulates the differentiation of ascogenous and peridial tissues. A homologue of *NoxA* in *Podospora anserina* (*PaNox1*) has also been shown to be involved in the differentiation of fruiting bodies (Malagnac et al. 2004), showing that ROS act as sexual development signals across a wide range of ascomycete fungi. Whether they are specific regulators of this morphogenetic process, or act as a signal within the wider context of the ageing colony (Lalucque and Silar 2003) remains to be clarified.



A R = CH_2OH
A' R = COOH B

Fig. 11.5. A,B Autoregulatory signals involved in the yeast–mycelium transition in *Candida albicans*. Farnesol (**A**) and farnesoic acid (**A**, second part) promote the yeast form and block the formation of germ tubes. Tyrosol (**B**) induces and contributes to maintain the mycelial form

VI. Dimorphism

The transition between yeast and mycelial forms is a common feature in some genera and has traditionally been associated with pathogenic species, such as *Candida albicans*, *Histoplasma capsulatum* and *Penicillium marneffei* (see The Mycota, Vol. XII), although the connection between pathogenicity and the morphogenetic transition is a controversial issue. In *C. albicans*, cellular density determines the morphological transition between yeast and mycelial growth since, at high cell densities ($>10^6$ cells ml^{-1}), the yeast form predominates whereas low cell densities favour the production of germ tubes (Hornby et al. 2001). Two molecules which were responsible for the maintenance of the yeast form at high cell densities were identified simultaneously as farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol; Fig. 11.5a; Hornby et al. 2001) and farnesoic acid (3,7,11-trimethyl-2,6,10-dodecatrienoate, Fig. 11.5a, second part; Oh et al. 2001). Both compounds are produced by yeast cells, and are effective in the micromolar concentration range.

Recent studies by Chen et al. (2004) have shown that the mycelial form, obtained at low-density cultures ($<10^6$ cells ml^{-1}), produces an autoregulatory signal which is capable of stimulating germ tube formation and extension under dilute conditions. Tyrosol (2-(4-hydroxyphenyl)ethanol, Fig. 11.5b) is continuously produced by the culture under low density, reaching concentrations around 3 µM. At this range of concentration, the signalling agent promotes mycelial growth.

The discovery of these two signalling molecules represents an innovation with respect to established concepts on yeast–mycelial transitions, since it reveals that complex autoregulatory positive and negative control systems are involved in the morphogenetic switch. More research is required in order to clarify the relationships between the two alternative signalling pathways, and their relations with other stimuli (namely, nutritional) which also influence the yeast–mycelium transition.

VII. Conclusions

The information presented in this chapter reflects on the participation of autoregulatory signals in a wide range of mycelial processes. The various

examples of this participation also indicate that the action of autoregulatory signals is combined with those of other physical and chemical signals, such as external nutrient levels, light, temperature and humidity, which have been documented in the scientific literature for many decades. The added value provided by autoregulatory signals is that of integrating responses of single cells into a higher level of organisation, representing the colony, which confers distinct selective advantage. From this standpoint, we can draw the conclusion that a considerable number of compounds produced by mycelial fungi, bearing no known functional roles to date, may eventually be found to participate in autoregulatory functions in years to come.

One evident avenue to pursue in order to assign biological roles to molecules is to define a functional assay, obtain biological extracts from cultures, and test extracts against the assay through a purification procedure. This approach has enabled the discovery of many autoregulatory signals, and should doubtless continue to prove fruitful in the future.

However, there are other approaches which can be beneficially followed and which take advantage of our current knowledge of molecular biology and natural products chemistry. For instance, autoregulators fulfilling comparable biological roles show marked similarities in their molecular structure and physicochemical properties. These properties provide some clues to the possible functions of other, similar molecules encountered within extracts of the same organism, or from different species. In this regard, new disciplines like metabolomics should contribute to the discovery of new autoregulatory signals when combined with classical approaches.

If autoregulatory signals are relevant for the understanding of functional aspects of fungal cells and colonies, they also offer a potential for the development of new biological products. One attractive aspect of autoregulatory signals arising from their fundamental role is that their long-term application cannot result in a resistance by the target organism, since it is the producer of the compound in nature.

Germination inhibitors could find a place in biological agriculture or post-harvest control of pathogens, by reducing the need for non-biological antifungal agents which often present unwanted hazards. Staling factors indicating senescent regions of colonies could potentially be used to limit the spread of certain unwanted fungal pathogens, in combination with chemical treatments or biological control agents. Conidiation autoinducers could be employed to attain high titres of conidia from large-scale fermenter cultures, where conidiation is normally weak or nonexistent. Much the same application could be found for autoregulators possibly involved in the production of microsclerotia. Both types of structures are currently in use in commercial biological control products.

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References

Adams TH, Wieser JK, Yu J-H (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Allen PJ (1955) The role of a self-inhibitor in the germination of rust uredospores. Phytopathology 45:259–266

Allen PJ (1972) Specificity of the *cis*-isomers of inhibitors of uredospore germination in the rust fungi. Proc Natl Acad Sci USA 69:3497–3500

Allen PJ (1976) Spore germination and its regulation. In: Heitefuss R, Williams PH (eds) Encyclopedia of Plant Physiology, vol 4. Springer, Berlin Heidelberg New York, pp 51–85

Bottone EJ, Nagarsheth N, Chiu K (1998) Evidence of self-inhibition by filamentous fungi accounts for unidirectional hyphal growth in colonies. Can J Microbiol 44:390–393

Breewer P, de Reu JC, Dracourt J-L, Rambouts FM, Abee T (1997) Nonanoic acid, a fungal self-inhibitor, prevents germination of *Rhizopus oligosporus* sporangiospores by dissipation of the pH gradient. Appl Environ Microbiol 63:178–185

Buller AHR (1933) Researches on Fungi, vol 5. Longman, London

Butnick NZ, Yager LN, Kurtz MB, Champe SP (1984a) Genetic analysis of mutnts of *Aspergillus nidulans* blocked at an early stage of sporulation. J Bacteriol 160:541–545

Butnick NZ, Yager LN, Hermann TE, Kurtz MB, Champe SP (1984b) Mutants of *Aspergillus nidulans* blocked at an early stage of sporulation secrete an unusual metabolite. J Bacteriol 160:533–540

Calvo AM, Hinze MII, Gardner HW, Keller NP (1999) Sporogenic effect of polyunsaturated fatty acids on development of *Aspergillus* spp. Appl Environ Microbiol 65:3668–3673

Calvo AM, Wilson RA, Bok JW, Keller NP (2002) Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev 66:447–459

Champe SP, El-Zayat AAE (1989) Isolation of a sexual sporulation hormone from *Aspergillus nidulans*. J Bacteriol 171:3982–3988

Champe SP, Rao P, Chang A (1987) An endogenous inducer of sexual development in *Aspergillus nidulans*. J Gen Microbiol 133:1383–1387

Chen H, Fujita M, Feng Q, Clardy J, Fink GR (2004) Tyrosol is a quorum-sensing molecule in *Candida albicans*. Proc Natl Acad Sci USA 101:5048–5052

Chitarra GS, Abee T, Rombouts FM, Posthumus MA, Dijksterhuis J (2004) Germination of *Penicillium paneum* conidia is regulated by octen-3-ol, a volatile self-inhibitor. Appl Environ Microbiol 70:2823–2829

Collins TJ, Moerschbacher BM, Read ND (2001) Synergistic induction of wheat stem rust appressoria by chemical and topological signals. Physiol Mol Plant Pathol 58:259–266

Fillinger S, Chaveroche M-K, Shimizu K, Keller NP, d'Enfert C (2002) cAMP and ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 63:3995–4000

Garret MK, Robinson PM (1969) A stable inhibitor of germination produced by fungi. Arch Microbiol 67:370–377

Glass NL, Rasmussen C, Roca MG, Read ND (2004) Hyphal homing, fusion and mycelial interconnectedness. Trends Microbiol 12:135–140

Gooday GW (1975) Chemotaxis and chemotropism in fungi and algae. In: Carlile MJ (ed) Primitive sensory and communication systems. Academic Press, London, pp 155–204

Gooday GW (1994) Hormones in mycelial fungi. In: Wessels JGH, Meihardt F (eds) The Mycota, vol I. Growth, differentiation and sexuality. Springer, Berlin Heidelberg New York, pp 401–411

Gooday GW (1999) Hyphal interactions. In: England R, Hobbs G, Bainton N, Roberts D (eds) Microbial signalling and communication. Symposium 57, Society for General Microbiology. Cambridge University Press, Cambridge, pp 307–334

Hadley G, Harrold CE (1958) The sporulation of *Penicillium notatum* Westling in submerged liquid culture II. J Exp Bot 9:418–425

Hickey PC, Jacobson DJ, Read ND, Glass NL (2002) Live-cell imaging of vegetative hyphal fusion in *Neurospora crassa*. Fungal Genet Biol 37:109–119

Horinouchi S, Beppu T (1992) Autoregulatory factors and communication in actinomycetes. Annu Rev Microbiol 31:377–398

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl Environ Microbiol 67:2982–2992

Hyeon B (1976) Chemical studies on the factors controlling sporulation of fungi. Chem Regul Plants 11:69–76

Indermitte C, Liebling TH, Clemencon H (1994) Culture analysis and external interaction models of mycelial growth. Bull Math Biol 56:633–644

Inoue M, Takenaka H, Tsurushima T, Miyagawa H, Ueno T (1996) Colletofragarones A1 and A2, novel germination self-inhibitors from the fungus *Colletotrichum fragariae*. Tetrahedron Lett 37:5731–5734

Katayama M, Marumo S (1978) R(-)-glycerol monolinoleate, a minor sporogenic substance of *Sclerotinia fructicola*. Agric Biol Chem 42:1431–1433

Katayama M, Yanagi M, Marumo S (1989) Isolation of sporogen-PF 1, a blue light-induced sporogenic substance from *Penicillium funiculosum*. Agric Biol Chem 53:3379–3380

Lalucque H, Silar P (2003) NADPH oxidase: an enzyme for multicellularity? Trends Microbiol 11:9–12

Lara-Ortiz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus* nidulans. Mol Microbiol 50:1241–1255

Lee BN, Adams TH (1994) The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev 8:641–651

Leppik RA, Hollomon DW, Bottomley W (1972) Quiesone: an inhibitor of the germination of *Peronospora tabacina* conidia. Phytochemistry 11:2055–2063

Lingappa BT, Lingappa Y (1969) Role of auto-inhibitors on mycelial growth and dimorphism of *Glomerella cingulata*. J Gen Microbiol 56:35–45

Liu Z-M, Kalattukudy PE (1999) Early expression of the calmodulin gene, which precedes appressorium formation in *Magnaporthe grisea*, is inhibited by self-inhibitors and requires surface attachment. J Bacteriol 181:3571–3577

Macko V, Staples RC (1973) Regulation of uredospore germination and germ tube development. Bull Torrey Bot Club 100:223–229

Malagnac P, Lalucque H, Lepere G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserine*. Fungal Genet Biol 41:982–997

Mazur P, Meyers HV, Nakanishi K, El-Kayat AAE, Champe SP (1990) Structural elucidation of sporogenic fatty acid metabolites from *Aspergillus nidulans*. Tetrahedron Lett 31:3837–3840

Mazur P, Nakanishi K, El-Zayat AEE, Champe SP (1991) Structure and synthesis of sporogenic psi factors from *Aspergillus nidulans*. J Chem Soc Chem Commun 20:1486–1487

Müller D, Jaffe L (1965) A quantitative study of cellular rheotropism. Biophys J 3:317–335

Oh KB, Miyazawa H, Naito T, Matsuoka H (2001) Purification and characterisation of an autoregulatory substance capable of regulating the morphological transition in *Candida albicans*. Proc Natl Acad Sci USA 98:4664–4668

Park D, Robinson PM (1964) Isolation and bioassay of a fungal morphogen. Nature 203:988–989

Podila GK, Rogers LM, Kolattukudy PE (1993) Chemical signals from avocado surface wax trigger germination and appresorium formation in *Colletotrichum gleosporoides*. Plant Physiol 103:267–272

Rast D, Stäuble EJ (1970) On the mode of action of isovaleric acid in stimulating the germination of *Agaricus bisporus* spores. New Phytol 69:557–566

Robinson PM (1973a) Autotropism in fungal spores and hyphae. Bot Rev 39:361–384

Robinson PM (1973b) Oxygen-positive chemotropic factor for fungi? New Phytol 72:1349–1356

Robinson PM (1973c) Chemotropism in fungi. Trans Br Mycol Soc 61:303–313

Robinson PM, Park D (1966) Citrinin-a fungistatic antibiotic and narrowing factor. Nature 211:883–884

Roeder PE, Sargent ML, Brody S (1982) Circadian rhythms in *Neurospora crassa*: oscillations in fatty acids. Biochemistry 21:4909–4916

Roncal T, Ugalde U (2003) Conidiation induction in *Penicillium*. Res Microbiol 154:539–546

Roncal T, Cordobés S, Sterner O, Ugalde U (2002) Conidiation in *Penicillium cyclopium* is induced by conidiogenone, an endogenous diterpene. Eukaryot Cell 1:823–829

Schimmel TG, Coffman AD, Parsons SJ (1998) Effect of butyrolactone I on the producing fungus, *Aspergillus terreus*. Appl Environ Microbiol 64:3707–3712

Spohr A, Dam-Mikkelsen C, Carlsen M, Nielsen J, Villadsen J (1998) On-line study of fungal morphology during submerged growth in a small flow-through cell. Biotechnol Bioeng 58:541–553

Tanaka S, Wada K, Matayama M, Marumo S (1984) Isolation of sporogen-AO1, a sporogenic substance, from *Aspergillus oryzae*. Agric Biol Chem 48:3189–3191

Tsitsigiannis DI, Zarnowski R, Keller N (2004a) The lipid body protein PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller N (2004b) Endogenous lipogenic regulators of spore balance in *Aspergillus nidulans*. Eukaryot Cell 3:1398–1411

Tsurushima T, Ueno T, Fukami H, Irie H, Inoue M (1995) Germination self-inhibitors from *Colletotrichum gleosporoides* f. sp. *jussiaea*. Mol Plant Microbe Interact 8:652–657

Wang C, Xing J, Chin C, Peters JS (2002) Fatty acids with certain structural characteristics are potent inhibitors of germination and inducers of cell death of powdery mildews. Physiol Mol Plant Pathol 61:151–161

Wolf JC, Mirocha CJ (1973) Regulation of sexual reproduction in *Gibberella zeae* (*Fusarim roseum graminearum*) by F-2 (Zearalenone)[1,2,3]. Can J Microbiol 19:725–734

Wood WF, Farquar GR, Largent DL (2000) Different volatile compounds from mycelium and sporocarp of *Pleurotus ostreatus*. Biochem Syst Ecol 28:89–90

Yager LN (1992) Early developmental events during asexual and sexual sporulation in *Aspergillus nidulans*. In: Bennet JW, Klich MA (eds) *Aspergillus*: biology and industrial applications. Butterworth-Heinemann, Boston, pp 19–41

12 Pheromone Action in the Fungal Groups Chytridiomycota, and Zygomycota, and in the Oomycota

C. Schimek[1], J. Wöstemeyer[1]

CONTENTS

I. Introduction

Fungi depend on specific intercellular communication systems for successfully managing sexual interactions. Small diffusible molecules are required for partner recognition, which comprises distinction between the same and foreign species and reliable recognition between complementary mating types. These signals need to be sent out at appropriate stages of development. They need to be sensed, differentiated from potentially interfering environmental signals, interpreted, and finally used for initiating adequate developmental programmes.

II. Recognition and Development

Looking at the chemical principles behind recognition of sexual partners reveals a surprising diversity between the major fungal groups. There is no evidence for a common communication strategy. In the phylogenetically most basal division Chytridiomycota, at least the model organism *Allomyces arbuscula* uses the sesquiterpene sirenin (Fig. 12.1) as attractant between the sexually complementary mobile gametes. All Zygomycota analysed seem to rely on trisporic acid derivates as mediators of sexual recognition (Figs. 12.2, 12.3; Schimek et al. 2003), whereas the Dikaryomycota (Ascomycotina, Basidiomycotina) rely on communication schemes based on small peptides (see Chaps. 15–17, this volume). The non-mycotan group Oomycota makes use of steroid compounds, e.g. the well-characterized compounds oogoniol and antheridiol (Fig. 12.4) in *Achlya* spp. In the plant pathogen *Phytophthora* sp., a lipid-based compatibility system provides an additional regulatory level.

The regulatory substances involved in recognition and control of sexual processes of fungi have been variously termed "hormones", indicating the activity of the substance beyond its producing cell or tissue, "pheromones", or "gamones" to describe substances involved in attraction between motile gametes. Other terms, e.g. "attractant", "morphogen", and "sex factor", are also found in the relevant literature. Throughout this text, the term "pheromone" will be used to indicate a chemical acting at a distance on members of the same, or other species. Where the importance of a substance as a mediator of specifically a sexual process is to be stressed, the term "sex pheromone" is used.

A. Chytridiomycota

1. Structures

The non-parasitic members of the Chytridiomycota occur mainly in fresh-water habitats or moist soil. Within the taxon, sexual reactions are carried out following a number of different mechanisms, all of them involving interactions between motile gametes. In the genus *Allomyces*, the two types of

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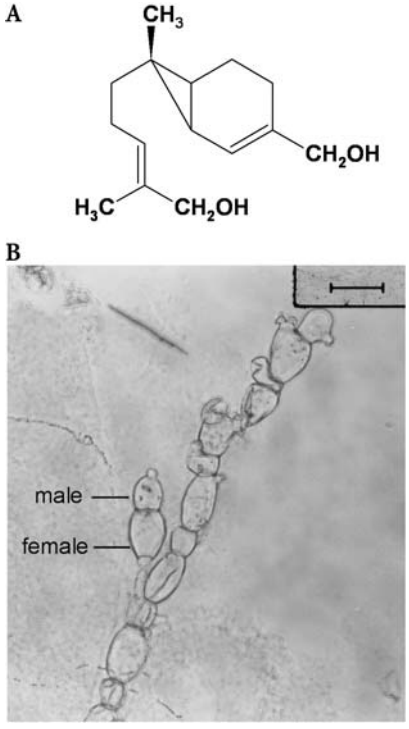

Fig. 12.1. A Structure of sirenin, the sesquiterpenoid gamete attractant produced by female gametangia of *Allomyces* sp. **B** Sexual differentiation in *A. macrogynus*: paired female and male gametagia develop on the same hypha. Bar = 50 µm (Photograph by S. Münch)

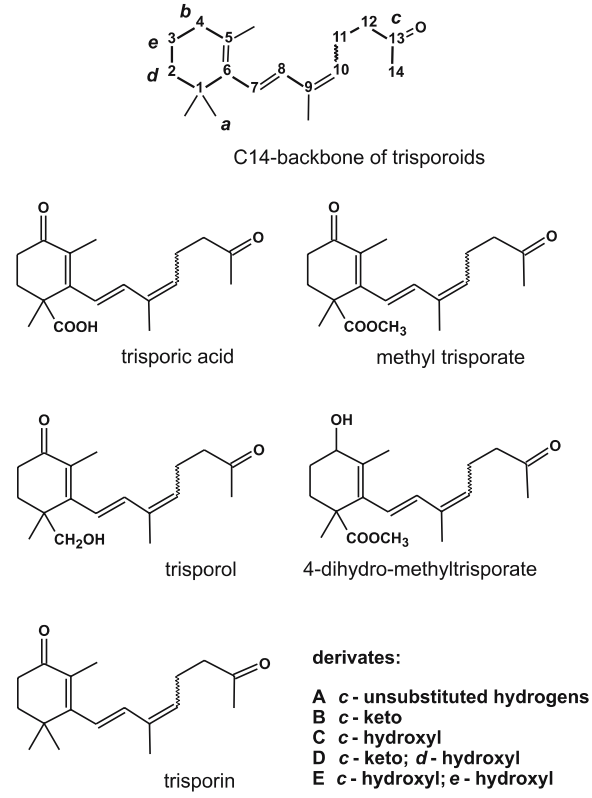

Fig. 12.2. Structures of trisporic acid and its biosynthetic precursors, sex pheromones, in Zygomycetes. Only major metabolites are shown. All structures represent the B-derivate with a keto function at C_{13}, the different substitutions and substitution sites of the other derivates being indicated in the *lower right corner*. Each species produces a differently composed mixture of these derivates

uniflagellate gametes develop in "male" and "female" gametangia, although these are usually located on the same haploid hypha. In *A. macrogynus*, the gametangia are paired, the male being terminal and the female subterminal (Fig. 12.1A). The gametes express a mutual attraction system. The larger and colourless gametes, regarded as female, attract the faster swimming, smaller male gametes (Pommerville 1978, 1981), which show an intense orange colour due to their high amount of γ-carotene. Fusion results in the formation of a biflagellate zygote which, in turn, gives rise to the diploid vegetative stage of the complex life cycle. Attraction of the gametes is mediated by a sesquiterpene, the female pheromone sirenin (Fig. 12.1; Machlis 1958). Male gametes release a different compound to the surrounding water, exerting an attractive function to female gametes. It is usually termed parisin (Pommerville and Olson 1987); its chemical structure has not been elucidated. Female gametes never react to sirenin or any of its artificial derivates (Carlile and Machlis 1965a; Pommerville and Olson 1987; Pommerville et al. 1988).

As sirenin and several derivates are available by chemical synthesis, the structural requirements for biological activity were elucidated. There are mandatory requirements for the primary hydroxyl group in the aliphatic side chain (Machlis 1973) and for the general geometry of the bicyclic ring system (Pommerville et al. 1988). The hydroxymethyl group at the bicyclic ring system can be omitted without affecting activity in the picomolar concentration range. It can not be substituted, however, by introducing large hydrophobic groups; introducing a benzyl ether ($-OCH_2-C_6H_5$) at this position drops the activity by a factor of 10^6, from 10 pM to 10 µM. A comparable drop in activity is seen when the ring system is removed (Pommerville et al. 1988).

The sirenin receptor, still not identified at the molecular level, depends on the *l*-form of sirenin (Fig. 12.1). The *d*-form is completely inactive, pos-

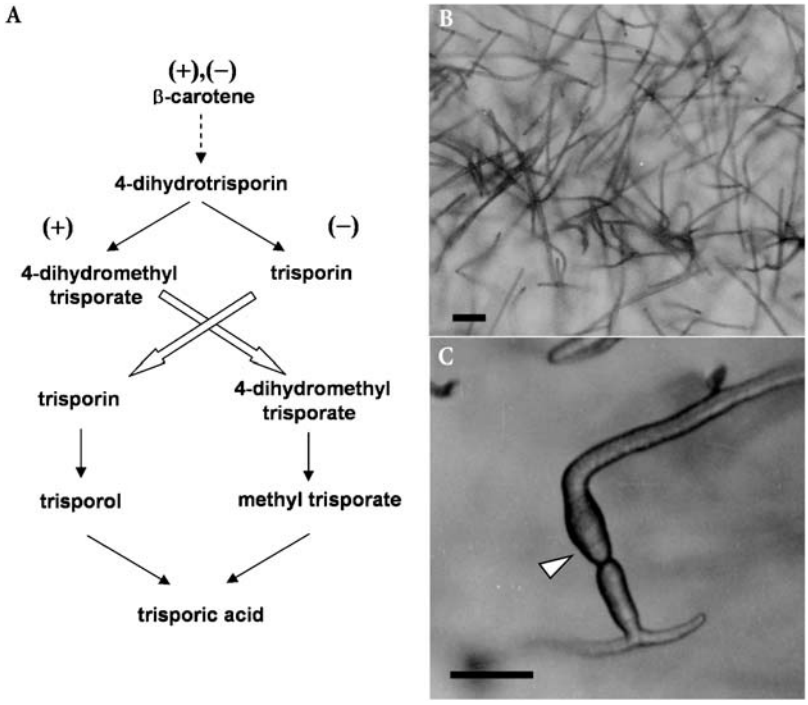

Fig. 12.3. **A** Simplified schematic representation of the cooperative biosynthesis of trisporic acid. The *open arrows* indicate exchange of metabolites between the mating partners. Only the compatible partner is able to convert the mating type-specific precursors into trisporic acid. The two mating types are indicated by (+) and (−). **B**, **C** Sexual differentiation stages in *Mucor mucedo*. **B** Zygophores produced by *M. mucedo* (−) as reaction to stimulation by either a compatible mating partner or purified trisporoids. Bar = 120 µm. **C** Upon contact between the mating types, progametangia have developed from the original zygophores. The *faint line* in the upper progametangium (*arrowhead*) indicates the formation of a septum delimiting the future gametangium. Bar = 40 µm

sibly due to displacement of the hydrophilic hydroxyl group of the ring to a region which needs to be hydrophobic in its interaction with the hypothetical receptor (Pommerville et al. 1988).

2. Mode of Action

At least under experimental conditions, the mutual and specific attraction system of the gametes

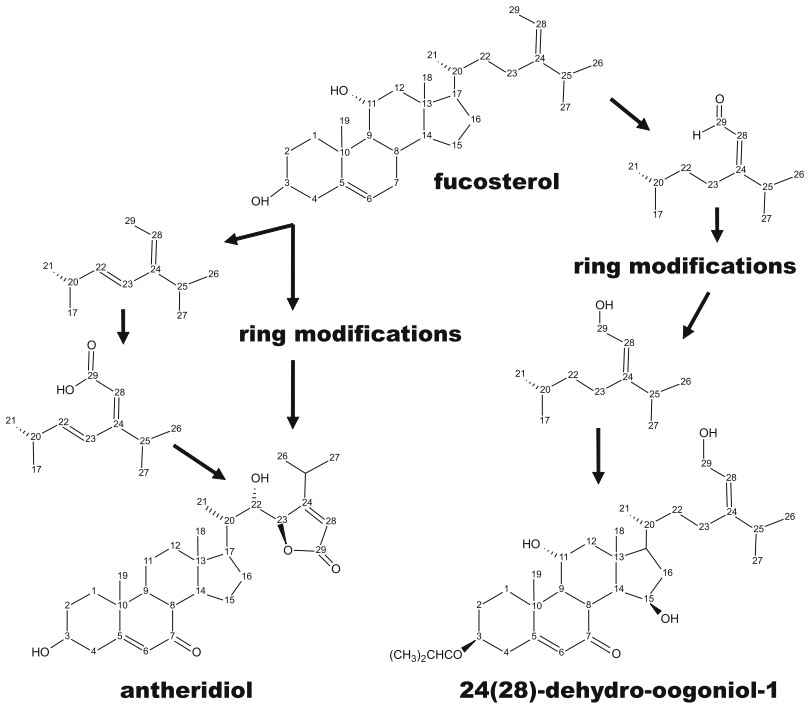

Fig. 12.4. Biosynthesis pathway of the steroid sex pheromones of *Achlya* spp., derived from the common precursor fucosterol. Antheridiol is produced by females via independently occurring modifications at the ring system and the side chain. 24(28)-dehydro-oogoniol-1 represents a number of active oogoniols produced by male strains. The unesterified oogoniol and its 24(28)-dehydro-derivate are modified by three different substituents at C_3: *oogoniol-1* $(CH_3)_2CHCO$, *oogoniol-2* $(CH_3)CH_2CO$, and *oogoniol-3* CH_3CO. In the males, biosynthesis takes place in a more strictly observed sequence of conversion steps. Ring modifications must be finished before the final modifications at the side chain may take place

ensures that essentially all female gametes are fertilised (Pommerville 1978). Sirenin is active in concentrations as low as 10 pM. However, natural concentrations seem to be considerably higher, the concentration around female gametes amounting to 1 µM, which corresponds to the peak response of male gametes (Carlile and Machlis 1965b). Sirenin modifies the chemotactic behaviour of the male gametes. Along a sirenin gradient, the duration of swimming phases increases and the frequency of directional changes is reduced, thus enhancing accuracy of motion. This is brought about by regulatory effects of sirenin on Ca^{2+} influx and intracellular concentration (Pommerville 1981). Although the system is understood at the physiological level, it has not been analysed at the level of molecular genetics.

B. Zygomycota

In Zygomycota, the two mating types cannot be discriminated based on their morphological features or behaviour. In some species, different shapes of the sexual organs occur which have been described in terms of "macrogametangia" and "microgametangia" but a certain, reliable assignment of these characteristics to one of the two mating types is not possible. Since the study of Blakeslee (1904), the mating types are classified as (+) and (−), following his arbitrary designation of a strain pair of *Rhizopus nigricans*. The mating type of newly examined strains is determined by their behaviour in mating reactions with already classified strains of the same, or different, species. Interspecific reactions occur abundantly within members of the class Zygomycetes. Recognition and regulation of the sexual process is mediated by the β-carotene derivate trisporic acid and its biosynthetic precursors. Biosynthesis and physiology of trisporic acid and its precursors have been reviewed by, amongst others, Bu'Lock et al. (1976), van den Ende (1976), Jones et al. (1981), Gooday (1983), Gooday and Adams (1993) and Sutter (1987).

1. Structures

The chemistry of trisporoids has been studied in members of three families of the Mucorales: *Blakeslea trispora* (Choanephoraceae), *Mucor mucedo* (Mucoraceae) and *Phycomyces blakesleeanus* (Phycomycetaceae). All active trisporoids are variously oxygenated C18 or C19 isoprenoid molecules and modifications of a common C14-backbone structure (Fig. 12.2). Although isomerisation is possible at the C_7 and the C_9 atom of the isoprenoid side chain, only the C_9 isomers have been found in culture extracts. Conformation at this position influences the activity of the compound, with the 9-*cis* (*9Z*) isomer being up to twice as efficient as the molecule in *trans* (*9E*) conformation (Bu'Lock et al. 1972, 1976). The influence of the stereochemistry at position C_1 has never been resolved but, in all natural isolates, the functional carboxyl, methoxy or methoxycarbonyl groups were found in *S* position. The configuration at the other chiral centre, C_4, is of critical importance: only the 4-*R*-hydroxy compounds are physiologically and metabolically active (Bu'Lock et al. 1976).

Another source of variance within the trisporoids is the occurrence of a number of derivates. These are characterized by the functional groups at C_2, C_3 and, mainly, at C_{13} (Fig. 12.2). Hydroxyl groups at C_2 and C_3 have been determined in both *R* and *S* configuration in *B. trispora* (Sutter et al. 1989). As a general rule, the substituents at C_1 and C_4 define the molecule species, those at C_2, C_3 and C_{13} define the derivate, and the configuration at the ring carbon atoms determines the biological activity as well as the metabolic specificity. Ring configuration and conformation seem therefore to affect the ability to bind to biosynthetic enzymes or to receptors, and possibly also to other binding proteins.

Trisporoids A occur only at low levels (Austin et al. 1969; van den Ende et al. 1970; Sutter et al. 1989) and, presumably for this reason, their physiological activities have never been investigated in detail. In the case of trisporic acid A, the data are ambiguous, Bu'Lock et al. (1972) indeed reporting an activity but clearly lower than that of the B and C derivates. By contrast, a fully synthetic trisporic acid A did not exhibit any biological activity (White et al. 1985). The precursors methyltrisporate A (Bu'Lock et al. 1972), trisporol A (White et al. 1985) and trisporin A (Schachtschabel et al. 2005) induce zygophore formation in *M. mucedo*. Trisporoids B and C have been isolated from all three source species, whereas the D and E forms have been established only in *P. blakesleeanus* and *B. trispora*. This might be due to the fact that, in *M. mucedo*, the production of trisporoids amounts to only a fraction of the concentrations found for the other species (Jones et al. 1981). The structural principles of the divers trisporoid forms are shown in Fig. 12.2. Of

all these compounds, the B-derivates are the most active when tested for zygophore induction in *M. mucedo* and *P. blakesleeanus*. Derivates with anhydro cyclization at the isoprenoid side chain or a shortened backbone (apotrisporoids) are inactive, indicating that the full-length backbone is one of the prerequisites for function. Based on recent analyses of various trisporoid analogues, we confirmed the importance of the overall dimensions of the trisporoid molecules for bioactivity: only compounds with side chains similar to those occurring naturally showed activity in the *Mucor* bioassay (Schachtschabel et al. 2005) which tests the induction of zygophores (Fig. 12.3B; Schimek et al. 2003). Early precursors and similar compounds such as retinyl acetate, retinol, retinal, retinoic acid, β-ionone and abscisic acid are completely inactive, too (Bu'Lock et al. 1972). A major requirement for activity is the polarity of the functional groups at the longer side chain, whereas oxygen substituents at the ring carbons are not essential. Only the early precursor 4-dihydrotrisporin, with a hydroxyl group at C_4, does not exhibit activity (Schachtschabel et al. 2005).

2. Biosynthesis

Trisporic acid, the major sex pheromone, is synthesized by a rather elaborate pathway. Neither of the mating types is able to complete the synthesis on its own – communication and exchange of metabolites between two compatible partners is indispensable. Biosynthesis starts from β-carotene, as has been ascertained by feeding experiments with radioactively labelled carotene (Austin et al. 1970). Since the very early steps of trisporoid synthesis have only recently regained the interest of researchers, reliable knowledge on this topic is scarce. Although the reaction products have never been measured directly, the consensus is that β-carotene is oxidatively cleaved by an enzyme similar to other carotene dioxygenases (monooxygenases). Whether the molecule is cleaved symmetrically or asymmetrically can currently not be answered. Retinal, the cleavage product of a symmetric 15-15′-β-carotene dioxygenase, has been spectroscopically detected in a *P. blakesleeanus* single wild-type strain culture in concentrations of 5–50 µg per gram of dry weight (Meissner and Delbrück 1968). Although the existence of retinal has never been verified by chemical analysis, its presence in Mucorales was validated by the conversion of radioactive retinyl acetate and retinol into trisporoids (Austin et al. 1970; Bu'Lock et al. 1974). It seems possible that these early conversion steps are performed by a multienzyme complex or that the substrate remains bound to protein during the conversions, so that no free metabolites can be measured. From the same studies, a probable sequence of metabolites was suggested: The next product from the C20 compound retinal or retinol would be a C18 ketone, achieved by β-oxidation.

In the only recent publication on the topic, Gessler et al. (2002), by contrast, postulated that the symmetrical, and generally enzymatic cleavage of β-carotene is but one of several possible pathways leading to trisporoids. This reaction might not be used during sexual interactions, because the concomitant increase of reactive oxygen species (ROS) and the resulting oxidative stress would damage the oxidation-sensitive carotene cleavage dioxygenase. In *B. trispora*, asymmetrical oxidative non-enzymatical cleavage of β-carotene at the C_{13-14} double bond is proposed to predominate over the formation of retinal/retinol. Oxidative cleavage would also lead to the formation of 4-hydroxy-β-carotene (isocryptoxanthine), which is also considered to be oxidatively and asymmetrically degraded. The main reaction products of the alternative cleavage pathways would be the corresponding C18 compounds β-apo-13-carotenone, the C18 ketone of Bu'Lock (Bu'Lock et al. 1974, 1976), and 4-hydroxy-β-apo-13-carotenone (4-hydroxy-β-C18-ketone). According to the scheme proposed by Bu'Lock, oxygen would subsequently be inserted at C_4 of the C18-ketone as hydroxyl, yielding 4-hydroxy-β-C18-ketone. With the reduction of the double bond between C_{11} and C_{12}, the final product of the mutual part of the synthesis pathway, 4-dihydrotrisporin, is attained.

Specific for the (−) mating type is the ability to oxidize the hydroxyl group at C_4 to form the ketone. 4-dihydrotrisporin is thus converted to trisporin, the major (−)-specific pheromone (Fig. 12.3). Depending on the species, the hydroxylation of a C_1 methyl group may occur in the (−) and in the (+) mating type, or solely in the (−) mating type. The resulting alcohols are named 4-dihydrotrisporol and trisporol respectively (Bu'Lock et al. 1973; Gooday 1983). The oxidation of the alcohol at C_1 to the carboxyl function (Fig. 12.2) is strictly specific for the (+) mating type (Fig. 12.3). Trisporin and trisporol therefore need to be transferred from the producing (−) type into the (+) mating type to complete trisporic acid synthesis.

In the (+) mating type, 4-dihydrotrisporin is converted, probably via 4-dihydrotrisporol, into 4-dihydro-methyltrisporate (Fig. 12.3). This step involves two reactions, the oxidation of the C_1 hydroxymethyl group, followed by methylation of the resulting carboxylate to methylcarboxylate. 4-dihydro-methyltrisporate and/or methyltrisporate are, in turn, converted to trisporic acid only by the mating partner, although the esterase reaction necessary for demethylation is possible in both mating types (Werkman 1976). In (+) *B. trispora*, the degradation of trisporin to apotrisporin (trisporone) was demonstrated by Sutter and Zawodny (1984). This reaction might prevent the accumulation of larger concentrations of trisporic acid by unmated (+)-type strains which would otherwise be possible (Sutter and Whitaker 1981a,b).

3. Mode of Action

Trisporoids participate in the regulation of sexual processes at different levels. In mated cultures, the amount of terpenoids is generally increased (Thomas and Goodwin 1967; Bu'Lock and Osagie 1973) but only the ergosterol content was demonstrated to be directly influenced by externally added trisporic acid (Gooday 1978; van den Ende 1978). The most conspicuous effect of trisporoids is the increase of β-carotene biosynthesis in certain species, most prominently in *B. trispora* and *P. blakesleeanus* (e.g. Caglioti et al. 1966; Thomas and Goodwin 1967; Vail et al. 1967; Govind and Cerdá-Olmedo 1986). This is generally inferred from the observation that carotene production is increased in mated cultures, compared to single mating types. The trisporoid content of these cultures was not determined. Feofila et al. (1976) described an increase of carotene production upon addition of trisporic acid in a single growing *B. trispora* (−) strain and, from the effects of cycloheximide and actinomycin D, concluded trisporic acid regulation to take place at the translation level. On the other hand, Schmidt et al. (2005) have recently shown that the transcription levels of two enzymes involved in carotene synthesis, the phytoene dehydrogenase (*carB*) and the lycopene cyclase/phytoene synthase (*carRA*) of *B. trispora*, are strongly induced in mated cultures. Neither the effects of specific trisporoids on the regulation of carotene synthesis genes, nor the influence of carotene or its precursors on trisporoid synthesis have been analysed. Purified trisporic acid B and C enhanced carotene synthesis in *B. trispora* (van den Ende 1968), as well as synthetic racemic methyltrisporate B and C, but not methyltrisporate E in *P. blakesleeanus* (Govind and Cerdá-Olmedo 1986). A series of synthetic C13 and C15 trisporoid intermediates had no effect on carotenogenesis (Yakovleva et al. 1980).

Carotene synthesis and its regulation in mucoralean fungi have been studied rather intensively in the past decades, due to the inherent biotechnological implications. Besides sexual interactions, carotene production is also regulated by light, especially blue light, and various chemicals (e.g. Cerdá-Olmedo and Hüttermann 1986; Ruiz-Hidalgo et al. 1997; Velayos et al. 2003; Quiles-Rosillo et al. 2005). Recently, its genetic regulation has been reviewed by Wöstemeyer et al. (2005).

A positive feedback regulatory cycle has been postulated, according to which trisporoids enhance their own production in mated cultures (Werkman and van den Ende 1973), probably via initially inducing carotene synthesis. This theory implies that, similarly to abscisic acid in plants (Taylor et al. 2000), the rate-limiting step of trisporoid synthesis would be the cleavage of carotene, and that only a certain amount of total carotene can be used for conversion to trisporoids. Sexual reactions and trisporoids are only two of several factors controlling carotene synthesis, whereas the presence of β-carotene is supposed to be an absolute prerequisite of trisporoid production. Mutants of *P. blakesleeanus* deficient in carotene synthesis show largely reduced sexual reactions and trisporoid production (Sutter 1975; Sutter et al. 1996). The hypothesis has, however, never been validated for species with a naturally very low carotene content. Mutants with exceptionally high carotene content are deficient in sexual morphogenesis, too (Salgado et al. 1989; Ootaki et al. 1996). However, their ability for trisporoid synthesis has never been analysed. In *P. blakesleeanus*, mutant analyses revealed that complete sexual reactions occur at β-carotene levels ranging between 5 (Salgado et al. 1991; Ootaki et al. 1996) and 3000 µg/g dry weight (Salgado and Cerdá-Olmedo 1992; Mehta et al. 1997).

The precursors trisporin, trisporol, 4-dihydro-methyltrisporate and methyltrisporate induce the development of sexually committed hyphae, the zygophores, in *M. mucedo* (Fig. 12.3B) and *P. blakesleeanus*. This reaction can be observed in actually mated cultures, and as a response to externally added trisporoids in bioassays. Trisporic acid

shows the same activity in *M. mucedo*, but not in *P. blakesleeanus* (Sutter et al. 1996). The amount of zygophores formed is usually much higher in partners of the opposite mating type. Several derivates exhibit strictly mating type-specific effects by triggering reactions only in the opposite mating type. The most active compound in bioassays using *M. mucedo* (−) is trisporic acid B, followed by 4-dihydro-methyltrisporate B, where 27 and 107 pmol (10 and 30 ng) of substance respectively are sufficient to induce a response (Sutter and Whitaker 1981b). A comprehensive model of the actual events in trisporoid action is still lacking. As zygophore induction can be observed when the mating partners are separated by a gap or barrier, volatile precursors have been postulated. 4-dihydro-methyltrisporate and trisporin are the candidates proposed to be volatile. Trisporic acid and trisporol, on the other hand, are certainly not volatile, their action depending on diffusion contact between the partners. The precursors are also said to be involved in zygotropism, i.e. the directed growth of zygophores leading to contact, adhesion and fusion of sexual structures of the two mating partners (Fig. 12.3C; Mesland et al. 1974).

As all trisporoid effects are triggered by trisporic acid as well as its precursors at more or less the same order of magnitude, the true function of trisporic acid in sexual reactions is not yet fully understood. The precursors have been described as interhyphal chemical messengers or hormones, contrasting with the trisporic acids which are intrahyphal chemical regulators (Sutter 1975). Some findings indicate that all precursors are internally converted into trisporic acid, which then would be the only regulatory molecule. Another chain of thought leads to the idea that the precursors effect their own regulatory functions, and that trisporic acid fulfils different functions or, as might also be the case, no specific function at all in the regulation of sexual events. The ability of trisporic acid to induce zygophores might be due to the inherent structural similarities with the precursor compounds. Trisporic acid and its precursors might also each cause a different set of effects. All trisporoids will certainly be involved in more general regulation networks, as is the case for the structurally similar compounds retinoic acid in animals and abscisic acid in plants, which both are involved in the regulation of gene expression on a large scale. Directly and by interaction with a number of other signal molecules and/or binding proteins, these compounds ultimately act at the transcription level (e.g. Bastien and Rochette-Egly 2004; Chung et al. 2005). A correlation to cAMP levels was observed in *M. mucedo* (Bu'Lock et al. 1976). Hitherto, no trisporoid receptor or trisporid binding protein/motif has been characterized. Retinol (vitamin A) was found to act as structural analogue to trisporoids in enhancing carotene synthesis (Eslava et al. 1974) but it is inactive in zygophore induction (Bu'Lock et al. 1976, C. Schimek, personal observation).

It should be kept in mind that trisporoid actions might be somewhat differentiated between the divers species, according to their inherent differences in structuring the mating reactions. In *Absidia glauca* and many other species, distinct zygophores are not formed and, therefore, zygotropism cannot occur. Rather, progametangia apparently develop between strong aerial hyphae of the mating partners which either get in close contact or even touch each other accidentally during vegetative growth (peg-to-peg interaction). The importance of secreted precursors, volatile or not, in these events has never been analysed at all. In other species, e.g. *P. blakesleeanus* and *B. trispora*, zygophores are formed in the upper substrate layer or at the interface between the substrate and the air. Under such circumstances, diffusing signal molecules seem much more plausible, and efficient, than volatiles.

From early studies of zygomycete sexuality, and based on numerous observations of partial interspecific reactions, it has repeatedly been argued that the same signal system is active throughout a large group of organisms. Trisporoid action was established for the sexual reactions of homothallic species (Werkman and van den Ende 1974). With the identification of trisporoids in the genera *Blakeslea*, *Mucor* and *Phycomyces*, the use of trisporic acid across family borders became a certainty. Meanwhile, trisporoid activity has also been described in a different order, the Mortierellales (Schimek et al. 2003), and preliminary data indicate the presence of these signals, although not their functions, in Chytridiomycota (S. Münch, C. Schimek, unpublished data). Moreover, trisporoids are involved in host recognition in a special type of parasitism occurring in Zygomycota. The biotrophic fusion parasite *Parasitella parasitella* (Mucorales, Mucoraceae) depends on the pheromones for identifying suitable hosts. In some cases, e.g. in crossings with *A. glauca*, this dependency is restricted to strictly mating type-specific infestations (Wöstemeyer et al. 1995).

4. Genetic Consequences

Although sexuality and the trisporoid communication system have been the focus of research on and off at least for the last five decades, only fragments are known about the genetic basis of these features. From the elucidation of the cooperative synthesis pathway, two hypotheses concerning the genetic situation have been formulated: first, a single mating type gene would exist, with the two alleles (+) and (−). The alleles would determine which of the two mating type-specific enzyme activities, i.e. the (−)-specific C_4-dehydrogenase or the (+)-specific C_1-oxygenase, would become repressed or constitutively expressed (Bu'Lock et al. 1976). The second hypothesis states that all necessary genes would exist in both mating types, but that the synthesis of one set of mating type-specific enzymes would be blocked (Nieuwenhuis and van den Ende 1975). In a side argument, the sex-specific enzymatic steps were proposed to be repressed in single growing cultures, but to become derepressed in mating situations (Bu'Lock et al. 1973, 1976). Various independent observations led to the consensus that a true mating type locus might not exist in Zygomycetes.

A genetic difference between mating types reflected at the protein level relates indeed to a plasmid encoding a non-glycosylated 15-kDa external membrane protein which was found exclusively in the (+) mating type of *Absidia glauca* (Teepe et al. 1988; Hänfler et al. 1992). Monoecious mutants, obtained by protoplast fusion of complementary mating types, do not express the protein, indicating that the protein might not be necessary for the mating process (Wöstemeyer et al. 1990).

The only gene identified from the trisporoid synthesis system in *M. mucedo* (Czempinski et al. 1996; gene referred to as *TDH*) and *P. parasitella* (Schultze et al. 2005), *TSP1*, codes for the 4-dihydro-methyltrisporate dehydrogenase (TDH), the enzyme with the strictly (−) type-specific activity (Fig. 12.3). Enzyme activity was first detected in (−) zygophores of *M. mucedo* and in the (−) equivalents of the homothallic *Zygorhynchus moelleri* (Werkman 1976). Enzyme activity was also documented in several species of *Mortierella*, belonging to the Mortierellales, thus establishing the use of trisporoids also in that order (Schimek et al. 2003). In addition, the study revealed enzyme activity throughout sexually stimulated (−)-type mycelium. Enzyme activity in mycelium beyond the sexually committed hyphae was also observed in *M. mucedo* and *A. glauca* (C. Schimek, unpublished data). As was already suggested by Mesland et al. (1974), trisporoid biosynthesis is evidently not restricted to hyphae undergoing sexual morphogenesis. Trisporoids are usually extracted from mycelia growing submerged. The biosynthesis pathway is therefore fully active under conditions where no zygophores and, in fact, no aerial structures at all are formed.

The *TSP1* gene for TDH was isolated and characterized by Czempinski et al. (1996), and was found to exist in both mating types of *M. mucedo*, *A. glauca*, *P. parasitica* and *B. trispora*. In the meantime, the gene has been identified in about 30 species, belonging to more than 10 families of three orders of the Zygomycetes, including *P. blakesleeanus* and *Thamnidium elegans* and, wherever both mating types were analysed, the gene was detected in each of these (K. Voigt, unpublished data). Another set of experiments confirms the interpretation presented by Sutter et al. (1996) concerning the existence of two independent dehydrogenase enzymes in *P. blakesleeanus*. Our data clearly show two biochemically separable enzymatic activities catalysing the conversion of 4-dihydrotrisporin and 4-dihydro-methyltrisporate respectively in *M. mucedo* (C. Walter, unpublished data). Two recent studies address the regulation of *TSP1*, encoding TDH, in *P. parasitica* (Schultze et al. 2005) and *M. mucedo* (Schimek et al. 2005). From these results, it appears that the regulation of trisporoid biosynthesis enzymes varies between the species. The *TSP1* gene is part of a complex gene cluster in both species, but the ORFs found in the *P. parasitica* gene cluster are not all present in the *M. mucedo* cluster (Schimek et al. 2005; Schultze et al. 2005). *TSP1* itself is constitutively transcribed in both species, indicating that enzyme activity is regulated post-transcriptionally. In *P. parasitica*, gene expression and regulation follow the same pattern in intraspecific sexual interactions as well as in parasitic interactions with the host *A. glauca*. With the exception of a not yet fully characterized putative *HSP* gene (K. Schultze, A. Burmester, unpublished data), the other ORFs in the gene cluster seem not to be involved directly in *TSP1* regulation. In the partially sequenced genome of *Rhizopus oryzae* (http://www.broad.mit.edu/annotation/fungi/rhizopus_oryzae/),*TSP1* is situated in a similar genetic context as that in *M. mucedo*. Whether or not the different organization of the gene cluster is related to the parasitic life style of *P. parasitica* remains to be studied in more detail in the future.

C. Oomycota

In the non-mycotan phylum Oomycota, the mating types cannot be discriminated easily because clear defining features do not exist. Moreover, the terms "sex" and "mating type", commonly understood to establish a distinct and fixed set of features and actions, do not seem to apply to all members of this group. Rather, at least within the Saprolegniales, a system of "relative sexuality" exists (besides a number of solely "male" and solely "female" strains), allowing the strain producing the highest amount of the "female" sex pheromone, antheridiol, to define the mating behaviour of the partner strain (Barksdale 1967; Barksdale and Lasure 1973). Traditionally, the mating types are termed "female" and "male". Many strains are homothallic, or self-fertile, the two types of sexual organs, oogonia and antheridia, being formed on proximal hyphae of the same thallus (Raper 1952).

Research on the sexual signal system has been performed almost exclusively on several heterothallic strains of *Achlya ambisexualis* or *A. bisexualis*, and the homothallic *A. heterosexualis* (Saprolegniales), and has been reviewed by Raper (1952), Barksdale (1969), McMorris (1978), Gooday (1983, 1994), Gooday and Adams (1993), and Mullins (1994). Since the early 1990s, little supplementary information on the biochemistry of the signal system was added. Another source of information, especially for structural considerations, addresses the sexual processes of the plant pathogens *Phytophthora* sp. (Elliott 1983) and *Pythium* sp. (Peronosporales; Knights and Elliott 1976).

1. Structures

The substance named hormone A by Raper in his detailed studies of signal exchange during sexual processes in *Achlya* in the 1930s and 1940s (reviewed by Raper 1952) was subsequently isolated from a female strain of *A. bisexualis* by McMorris and Barksdale (1967), and termed antheridiol. A molecular formula of $C_{29}H_{42}O_5$ was established, and the existence of hydroxyl and carbonyl functions as well as the presence of an α-β unsaturated γ-lactone and an α-β unsaturated ketone were deduced. A structure was proposed by Arsenault and co-workers in the following year (Arsenault et al. 1968), and these authors also first determined the steroid nature of the pheromone. This structure (Fig. 12.4) was confirmed by data derived from fully synthetic isomers (Edwards et al. 1969). Based on a standard tetracyclic steroid nucleus, with a side chain inserted at C_{17}, the major difference between antheridiol and mammalian steroid hormones lies in the length of the side chain. In antheridiol, the long (10 carbon) side chain also encompasses the lactone ring. The structure contains two C=C double bonds, one at C_{5-6} of the nucleus, the other at C_{24-25} of the side chain, as well as two carbonyl functions at C_7 and C_{26}, and two hydroxyl groups at C_3 and C_{22} respectively.

The structural feature most important for activity is the stereochemistry at C_{22} and C_{23}. From the four possible stereoisomers, only the functional antheridiol (22*S*, 23*R*) exhibits activity at 6 pg/ml, whereas the activity levels in the 22*R*, 23*S* and the 22*S*, 23*S* isomers are reduced by a factor of 1000 and activity is probably even further reduced in the 22*R*, 23*R* stereoisomer (Barksdale et al. 1974). By contrast, structural changes at the ring system seem to be of minor importance. Removal of the C_7 keto group and exchange of the free hydroxyl group at C_3 with its acetate only reduce activity by a factor of 20. By contrast, changes in the oxidation status at C_{22} or C_{23} lead to dramatic reduction of activity, whereas further structural divergence of the side chain yields completely inactive compounds. A number of other steroids, including mammalian steroid hormones, have been proved to be equally inactive (Barksdale et al. 1974).

The male pheromone, originally termed hormone B by Raper (1952), was later renamed oogoniol (McMorris et al. 1975). The oogoniols turned out to be a mixture of active steroids. A number of these have been characterized, including the unesterified oogoniol, its isobutyrate, propionate and acetate esters, oogoniol-1, -2, and -3 respectively, and their 24(28)-dehydro-analogues (McMorris et al. 1975; McMorris 1978). Similarly to antheridiol, the oogoniols are 29-carbon steroids containing a Δ^{5-7}-ketone chromophore (Fig. 12.4). They carry an ester substituent at C_3, hydroxyl functions at C_{11} and C_{15}, and a primary hydroxyl group, too, at C_{29} at the end of the side chain. Oogoniols do not contain a lactone ring (McMorris et al. 1975) but, analogously to antheridiols, the physiological activity is strongly dependent on the structure of the side chain. In fact, the minor compounds, 24(28)-dehydro-oogoniols, are about 100 times more effective than the saturated analogues and may represent the physiologically active compounds (McMorris 1978; Preus and McMorris 1979). Possibly, biosynthetic intermediates

towards dehydo-oogoniol exhibit regulatory functions; 7-deoxo-dehydro-oogoniol was described as competitive inhibitor of the pheromone (McMorris et al. 1993).

In Oomycota as well as in the Zygomcota, a common basis seems to exist for sexual signalling. All oomycete species analysed so far require sterols to perform sexual development. The signal system is, nevertheless, more restricted than in the Zygomycetes, the specific compounds being active only amongst closest neighbours, possibly within each genus. Analyses of the induction of sexual processes in other Oomycetes indicate that steroids other than the antheridiol structure are required (Knights and Elliott 1976; Musgrave et al. 1978; Kerwin and Washino 1983). The pronounced specificity of the reactions becomes apparent from the non-interchangeable character of oogoniol and antheridiol in *Achlya* species (Horgen 1977; Musgrave et al. 1978).

Self-sterile plant pathogenic species of the genera *Phytophthora* and *Pythium* are unable to synthesize sterols, and are thus sexually inactive if no external sterols are available (Kerwin and Duddles 1989). Nevertheless, these are taken up from the surroundings, presumably the host plants, and are supposedly converted internally into the necessary sexual hormones (Langcake 1974; Elliott and Knights 1981). The actual structure of these active hormones has not yet been determined. Many steroid compounds have been tested for their ability to induce sexual reactions in the genera *Phytophthora* and *Pythium*, and the results of these analyses indicate that the usefulness of external steroids is also defined largely by their configuration and stereochemistry, particularly that of the side chain (Elliott 1979).

Substances active in *Phytophthora* were found to fulfil the following criteria: they carry a hydroxyl function at C_3 of the sterol nucleus, have one C=C double bond in ring B, and contain a hydrocarbon side chain longer than five carbon atoms. Increasing length of the side chain leads to increasing activity, the exact position of C_{29} being the most important feature. All compounds with the position of C_{29} fixed by the presence of a $C_{24–28}$ double bond were of high activity (Elliott et al. 1966; Elliott 1972). Methylation at C_{24} also enhances activity; activity indeed increases concomitantly with the size of the substituent at C_{24} (Elliott 1979). Compounds lacking the double bond in the steroid nucleus and exhibiting other undesirable structural features are completely inactive (Knights and Elliott 1976; Elliott and Sansome 1977). Despite several of the structural requirements indicating a structure of the sexual pheromone similar to those of *Achlya* sp., and thus raising the possibility of a general signal mechanism, antheridiol was found to be completely inactive in *P. cactorum* (Nes et al. 1980). This possibly reflects the outcome of evolutionary adaptation to the parasitic life style. Typical plant steroids are generally far more effective than fungal or animal compounds in the plant parasitic species (Elliott et al. 1966; Nes et al. 1980).

A second signal system active in sexual reproduction of *Phytophthora* was proposed by Ko (1980, 1983, 1985, 1988) who observed intra- and interspecific sexual reactions leading to self-fertilisation in heterothallic *Phytophthora* spp. strains. The response, accordingly termed hormonal heterothallism and constituting a compatibility system, occurs only in the presence of members of both mating types, A1 and A2. It is evidently mediated by small molecules, as the resulting cross-induction of oospore formation also occurred when the two mating partners were separated by a polycarbonate membrane (Ko 1980). The putative signal compounds named hormone α1 and α2 respectively could be enriched to a limited extent, and were tentatively identified as lipid-like substances with a molecular mass between 500 and 100Dalton, α2 being more polar than α1 (Ko 1983; Chern et al. 1996). In a follow-up study, Chern et al. (1999) demonstrated that the two substances are neither phospholipids, glycolipids, glycerides nor steroids, but most probably neutral lipids with hydroxyl functional group(s).

2. Biosynthesis

Analysis and comparison of steroid content in Oomycetes, together with feeding experiments using both natural and synthetic compounds, leads to a possible sequence of events in pheromone synthesis. Common precursor for both antheridiol and the oogoniols is fucosterol, the most abundant sterol in Oomycetes (Popplestone and Unrau 1973, 1974; McMorris and White 1977). Fucosterol is also the sterol with the highest rate of uptake and the most efficient conversion in *Phytophthora* (Elliott et al. 1966; McMorris and White 1977). Saprolegniales are able to synthesize fucosterol, probably via 7-dehydrofucosterol (Lenton et al. 1971). In the following synthesis steps leading to antheridiol, modification of the steroid ring system and of the side chain occur independently. For the latter, the

following sequence of events has been established: the initial step is the dehydrogenation at C_{22}/C_{23}, followed by an oxidation series at C_{29}. Beginning with a methyl function, the final carboxyl group is synthesized via an ethyl group and the carbonyl as intermediates. After these modifications, C_{22}/C_{23} are oxidized again and, finally, cyclization occurs, yielding the unsaturated γ-lactone ring of the antheridiol side chain (Fig. 12.4; Popplestone and Unrau 1974; McMorris 1978).

The oogoniols, despite being derived from the same precursor, are synthesized by a different pathway. For a start, fucosterol is oxidized to give an aldehyde at C_{29}. In the subsequent steps, hydroxylation occurs at C_{11} and C_{15}, oxidation occurs at C_7, and the hydroxyl group at C_3 becomes esterified. Finally, after all the ring modifications have taken place, the $C_{24–28}$ double bond is reduced (McMorris and White 1977). No data whatsoever exist on the enzymatic mechanism of these conversions.

3. Mode of Action and Cellular Consequences

To fully understand the role of steroids in the regulation of sexual development in *Phytophtora* spp., it is necessary to strictly distinguish their effects from those of other steroid-mediated regulatory mechanisms. Early observations of the effects of externally added steroids promoting vegetative growth (Hendrix 1964, 1965; Elliott et al. 1966), concomitantly with these being incorporated to a large extent into the cellular membranes (Langcake 1974), emphasized the function of steroids as necessary regulatory and structural cellular components. In the case of sexual processes, studies performed on compatible male and female strains elucidated a sequence of pheromone responses. Similar reactions were observed in crossings between homothallic and one compatible heterothallic strain. For antheridiol, the same sequence was also obtained with increasing concentration of externally added pheromone. Antheridiol itself is produced constitutively at low concentrations by the female. As the first, decidedly sexual reaction, apical growth in the male stops after exposure to a female strain or within an hour after antheridiol addition (Gow and Gooday 1987). In the next step, the characteristic antheridial initials are formed in a dose-dependent manner at the proximal ends of male vegetative hyphae (Barksdale 1967). Within 30 min to exposure, certain reactions are also induced in the mating partner. So, exposure to antheridiol induces the synthesis and release of oogoniol in male-reacting strains (Barksdale and Lasure 1974; McMorris and White 1977), and the formation of oogonial initials. These release higher amounts of antheridiol and, thus, chemotrophically attract the antheridial branches (Barksdale 1963, 1967). Higher concentrations of antheridiol also effect the differentiation of the antheridia by septum formation, and are also thought to be involved in the onset of meiosis (Barksdale 1963, 1967). Continual exposure to antheridiol for at least 30 min also induces conversion of this compound into less active metabolites in the male strains of the heterothallic *A. ambisexualis* or *A. bisexualis*, and as well as in the homothallic *A. americana* and *A. conspicua* (Musgrave and Nieuwenhuis 1975). In female strains, metabolism of antheridiol does not occur, neither is any antheridiol metabolism observed in those oomycete species which are not responsive to antheridiol in their sexual reactions (Musgrave et al. 1978). The authors suggest a regulatory function of this metabolism. Inactivation of antheridiol would serve in steepening the gradient of antheridiol and, thus, facilitating directed growth and promotion of gamete formation. As the antheridia also serve to direct male gametangial nuclei through specialized tubes directly to the oospheres enclosed in the oogonium (Raper 1952), a function of antheridiol in fertilisation may also be proposed. By strongly inducing the formation of antheridial hyphae in homothallic species growing adjacent to a female *Achlya* strain, which thereby waste resources, antheridiol also acts as inhibitor to both sexual and asexual development of the homothallic strain (Barksdale 1967; Thomas and McMorris 1987).

A number of studies exist on molecular changes accompanying the onset of sexual development. Most of these observations are rather general in nature, and so the data obtained might not reflect pheromone action per se but rather general changes in cellular regulation following the switch of commitment. Thus, exposure to antheridiol was found to increase the activity and the release of cellulases in males. This increase occurs concomitantly to the formation of antheridial branches (Thomas and Mullins 1967, 1969; Mullins and Ellis 1974; Mullins 1979), implying that it is a necessary prerequisite to branching and the development of new apical growth sites. A similar increase in cellulase activity also accompanies massive

vegetative branching (Hill 1996). The same applies to all observations concerning increase in transcription rates, cellular concentrations of rRNA and mRNA, protein synthesis and histone acetylation (Horowitz and Russel 1974; Silver and Horgen 1974; Groner et al. 1976; Horgen 1977; Sutherland and Horgen 1977; Michalski 1978; Horgen et al. 1983). Most of these effects are unspecific, and reflect the expression of increasing cellular activities in the course of morphological and physiological changes during the sexual process.

The synthesis of a number of proteins is specifically and directly induced or enhanced by antheridiol (Horton and Horgen 1985), one of these possibly being cellulase (Groner et al. 1976; cf. above). This specific pheromone-mediated response has been investigated in greater detail. Over a period of almost two decades, Brunt and Silver as well as Riehl and Toft and their co-workers have elucidated the induction and participation of certain proteins in the antheridiol-mediated sexual response of *Achlya*. They found that antheridiol-regulated proteins occur at different cellular localizations, and are always to be grouped among the minor polypeptides (Brunt and Silver 1986a,b, 1987). Besides its influence on protein synthesis, treatment with antheridiol may also affect protein processing. Evidence exists that a number of glycoproteins become deglycosylated at the onset of the sexual reaction in the male *A. ambisexualis* E87 (Brunt and Silver 1986a), implicating regulation of cellular recognition events by the pheromone. A 85-kDa protein band detected both in the nuclear and the cytoplasmic fraction (Brunt and Silver 1986b) was later found to consist partially of a previously identified (Silver et al. 1983) 85-kDa heat-shock protein (Brunt et al. 1990; Brunt and Silver 1991). Based on antibody cross-reactions and sequence similarity, this protein is classified as a hsp-90 protein (Brunt et al. 1990).

A specific antheridiol receptor was identified by Riehl et al. (1984) in the cytoplasm of male cells only. As the binding activity could be recovered in fractions displaying different sedimentation coefficients, the existence of a multiprotein complex was deduced and later confirmed. At least the hsp90 protein is an integral part of that complex (Brunt et al. 1990).

Several other heat-shock proteins, e.g. three hsp-70 proteins, a 23-kDa and a 56-kDa protein (Silver et al. 1993; Brunt et al. 1998a,b) as well as other hormone-binding and non-binding polypeptides (Riehl et al. 1985; Brunt et al. 1998b) are associated with the steroid receptor-hsp complex. They constitute a multiprotein heterocomplex where some of the participating components are not necessarily present all the time or in all existing complexes.

At the transcriptional level, two similar but different transcriptional populations exist for *hsp70* and *hsp90*, and these are regulated differently (Silver et al. 1993; Brunt and Silver 2004). In each case, both transcript populations are regulated by antheridiol, but one of them also reacts to an unrelated stimulus, *hsp70* to decreased glucose concentrations (Silver et al. 1993) and *hsp90* to increased temperature (Brunt and Silver 2004). Transcript divergence from a single cDNA clone was already documented by Horton and Horgen (1989), an observation probably based on similar regulation processes.

Consistent with earlier assumptions, all authors today agree on the oomycete steroid receptor organization and its regulation strongly resembling animal steroid hormone systems (Riehl and Toft 1984; Riehl et al. 1985; Brunt et al. 1998b; Brunt and Silver 2004). This viewpoint is further supported by the identification of a diversity of putative transcription factor response elements in the 5′ region of the *hsp90* genes. Among these are motifs already known from animal steroid hormone response elements (Brunt et al. 1998a).

The *Phytophthora parasitica* and *P. infestans* mating type locus have been analysed in detail by the group around Judelson. Using RAPD markers to identify loci linked to the A1 and A2 phenotypes, genetic and physical mapping of these loci was possible. In both species, a bipolar mating system exists. Mating types are determined by heterozygosity in A1 (allele combination Aa) and by homozygosity in A2 (aa) at the single mating type locus (Judelson et al. 1995; Judelson 1996a). In *P. infestans*, an unusual segregation pattern of the mating type alleles prevails, showing a preference for two of the four possible genotypes. Despite the chromosomes bearing the A1 and A2 determinants being genetically similar, a region of structural heterozygosity, locus S1, flanks the mating type locus in A1 isolates whereas it is absent in A2. Although S1 contains no obvious open reading frames, a function of this sex chromosome-like region in the regulation of allele segregation, DNA replication or gene expression seems plausible (Judelson 1996b; Randall et al. 2003). In *Phytophthora parasitica*,

the standard Mendelian segregation pattern is realized (Fabritius and Judelson 1997). Open reading frames within the mating type locus have hitherto not been identified.

Eight genes have been characterized which are up-regulated during sexual development in *P. infestans*. As the transcription level of two of these increases during the early stages before physical contact between the partners has been established, they might be induced by the α hormones. Very low expression was also found in vegetative hyphae, which would not be uncommon in a hormone response system. Three sequences resemble that of proteins interacting with RNA: a ribonuclease activator, an RNA-binding protein of the *Drosophila melanogaster* pumilo protein family (Puf), and Rnase H, and may thus be involved in mating regulation (Fabritius et al. 2002). The Puf-like protein was later found to be transcribed not only during early sexual development but also during the early asexual development of sporangia (Cvitanich and Judelson 2003). The predicted products of two of the up-regulated genes show similarities to elicitins, and one to a glycoprotein cell surface receptor (Fabritius et al. 2002). One of the elicitin-like products belongs to a *P. infestans* multigene family of putative extracellular transglutaminases which are possibly involved in cell wall strengthening or in enhancing adhesion. The gene transcribed mating-specifically is the only one of this gene family for which an elicitin activity could not be predicted from the sequence data. The various putative enzymes are conceivably involved in cell wall-related processes within the different developmental processes leading to vegetative hyphae, zoospores, sexual organs or haustoria (Fabritius and Judelson 2003). Even with no evidence so far for a direct interaction of one of the up-regulated genes with the α hormone system, or with steroid signals, the character of all putative gene products suggests a possible involvement in mating-related events.

For the oomycete sexual pheromone systems, the complexity of the hormone response and of its regulation becomes ever more apparent. The pheromone-mediated part of the sexual reaction is strongly interwoven with other developments and influences, both intracellular and external, resulting in tightly interconnected intracellular regulatory events. Other signal compounds, the nutritional status, and the availability of phospholipids are certainly involved in the regulation of the sexual reaction, too.

III. Conclusions

In the three organismic groups here considered, one noticeable fact concerning pheromone action recurs. The sexual interactions within a larger systematic unit are mediated by the same or very similar substances, within members of a given genus in the Chytridiomycota and the Oomycota, and even within a given class in the Zygomycota. Nevertheless, different levels of specificity exist within each group, enabling recognition of a compatible mating partner of the same species. In Oomycota and Zygomycota, species-specificity is, with high probability, realized by using different derivates and isomers of a common basic compound.

Genetic analyses of the pheromone systems are largely missing, and true mating type loci are not known for nearly all of these organisms. Besides the Peronosporales, it is only in Zygomycetes that first attempts at genetic characterization have been made. In this group, however, the cooperative biosynthesis discussed above in this chapter provides an uncomplicated mating type system which essentially could do without a mating type locus governing the complete differentiation programme. Trisporic acid derivates, especially the esterised and, thus, non-charged compounds, could directly pass the cellular membrane and act immediately as ligands at the level of transcriptional regulation. Therefore, a mucoralean mating type locus could be constituted by not more than the genetic apparatus, structural and regulatory, warranting that trisporoid biosynthesis be performed obligatorily cooperatively between complementary mating types.

This idea is based on the structural similarities between trisporids and retinoids. Retinoids, which are also degradation products of β-carotene, function as intercellular communication systems in vertebrates, especially in developmental regulatory processes. Biologically active retinoids bind to nuclear retinoid receptors belonging to the steroid/thyroid hormone nuclear receptor superfamily. Ligand-bound receptor complexes function as transcription factors by binding to DNA-binding sites, the retinoic acid response elements. These are located among the transcriptional regulatory sequences of target genes (Napoli 1999). Comparable effects of intracellularly localized trisporoids, triggered by binding directly to nuclear receptor proteins and thus acting as transcription factors, are conceivable. According

to this model, membrane-associated receptors coupled to cytoplasmatic signal transduction chains would not be involved in trisporoid-mediated regulation of sexual reactions in Zygomycetes.

References

Arsenault GP, Biemann K, Barksdale AW, McMorris TC (1968) The structure of antheridiol, a sex hormone in *Achlya bisexualis*. J Am Chem Soc 90:5635–5636

Austin DJ, Bu'Lock JD, Gooday GW (1969) Trisporic acids: sexual hormones from *Mucor mucedo* and *Blakeslea trispora*. Nature 223:1178–1179

Austin DJ, Bu'Lock JD, Drake D (1970) The biosynthesis of trisporic acids from β-carotene via retinal and trisporol. Experientia 26:348–349

Barksdale AW (1963) The role of hormone A during sexual conjugation in *Achlya ambisexualis*. Mycologia 55:627–632

Barksdale AW (1967) The sexual hormones of the fungus *Achlya*. Ann N Y Acad Sci 144:313–319

Barksdale AW (1969) Sexual hormones of *Achlya* and other fungi. Science 166:831–837

Barksdale AW, Lasure LL (1973) Induction of gametangial phenotypes in *Achlya*. Bull Torrey Bot Club 100:199–202

Barksdale AW, Lasure LL (1974) Production of hormone B by *Achlya heterosexualis*. Appl Microbiol 28:544–546

Barksdale AW, McMorris TC, Seshadri R, Arunachalam T (1974) Response of *Achlya ambisexualis* E87 to the hormone antheridiol and certain other steroids. J Gen Microbiol 82:295–299

Bastien J, Rochette-Egly C (2004) Nuclear retinoid receptors and the transcription of retinoid target genes. Gene 17:1–16

Blakeslee AF (1904) Sexual reproduction in the Mucorineae. Proc Am Acad Arts Sci 40:205–319

Brunt SA, Silver JC (1986a) Steroid hormone-induced changes in secreted proteins in the filamentous fungus *Achlya*. Exp Cell Res 163:22–34

Brunt SA, Silver JC (1986b) Cellular localization of steroid hormone-regulated proteins during sexual development in *Achlya*. Exp Cell Res 165:306–319

Brunt SA, Silver JC (1987) Steroid hormone-regulated basic proteins in *Achlya ambisexualis*. Exp Mycol 11:65–69

Brunt SA, Silver JC (1991) Molecular cloning and characterization of two distinct hsp85 sequences from the steroid responsive fungus *Achlya ambisexualis*. Curr Genet 19:383–388

Brunt SA, Silver JC (2004) Molecular cloning and characterization of two different cDNAs encoding the molecular chaperone hsp90 in the oomycete *Achlya ambisexualis*. Fungal Genet Biol 41:239–252

Brunt SA, Borkar M, Silver JC (1998a) Regulation of *hsp90* and *hsp70* genes during antheridiol-induced hyphal branching in the oomycete *Achlya ambisexualis*. Fungal Genet Biol 24:310–324

Brunt SA, Perdew GH, Toft DO, Silver JC (1998b) Hsp90-containing multiprotein complexes in the eukaryotic microbe *Achlya*. Cell Stress Chaperones 3:44–56

Brunt SA, Rhiel R, Silver JC (1990) Steroid hormone regulation of the *Achlya ambisexualis* 85-kilodalton heat shock protein, a component of the *Achlya* steroid receptor complex. Mol Cell Biol 10:273–281

Bu'Lock JD, Osagie AU (1973) Prenols and ubiquinones in single-strain and mated cultures of *Blakeslea trispora*. J Gen Microbiol 76:77–83

Bu'Lock JD, Drake D, Winstanley DJ (1972) Specificity and transformations of the trisporic acid series of fungal sex hormones. Phytochemistry 11:2011–2018

Bu'Lock JD, Jones BE, Quarrie SA, Winskill N (1973) The biochemical basis of sexualitiy in Mucorales. Naturwissenschaften 60:550–551

Bu'Lock JD, Jones BE, Taylor D, Winskill N, Quarrie SA (1974) Sex hormones in Mucorales. The incorporation of C_{20} and C_{18} precursors into trisporic acids. J Gen Microbiol 80:301–306

Bu'Lock JD, Jones BE, Winskill N (1976) The apocarotenoid system of sex hormones and prohormones in Mucorales. Pure Appl Chem 47:191–202

Caglioti L, Cainelli G, Camerino B, Mondelli R, Prieto A, Quilico A, Salvatori T, Selva A (1966) The structure of trisporic-C acid. Tetrahedron Suppl 7:175–187

Carlile MJ, Machlis L (1965a) A comparative study of the chemotaxis of the motile phases of *Allomyces*. Am J Bot 52:484–486

Carlile MJ, Machlis L (1965b) The response of male gametes of *Allomyces* to the sexual hormone sirenin. Am J Bot 52:478–483

Cerdá-Olmedo E, Hüttermann A (1986) Förderung und Hemmung der Carotinsynthese bei *Phycomyces* durch Aromaten. Angew Bot 69:59–70

Chern LL, Ko WH, Tang CS (1996) Factors affecting yields of α hormones of *Phytophthora parasitica* obtained by adsorption. Can J Microbiol 42:172–176

Chern LL, Tang CS, Ko WH (1999) Chemical characterization of alpha hormones of *Phytophthora parasitica*. Bot Bull Acad Sinica 40:79–85

Chung HJ, Fu HY, Thomas TL (2005) Abscisic acid-inducible nuclear proteins bind to bipartite promoter elements required for ABA response and embryo-regulated expression of the carrot *Dc3* gene. Planta 220:424–433

Cvitanich C, Judelson HS (2003) A gene expressed during sexual and asexual sporulation in *Phytophthora infestans* is a member of the Puf family of translational regulators. Eukaryot Cell 2:465–473

Czempinski K, Kruft V, Wöstemeyer J, Burmester A (1996) 4-Dihydromethyltrisporate dehydrogenase from *Mucor mucedo*, an enzyme of the sexual hormone pathway: purification, and cloning of the corresponding gene. Microbiology 142:2647–2654

Edwards JA, Mills JS, Sundeen J, Fried JH (1969) The synthesis of the fungal sex hormone antheridiol. J Am Chem Soc 91:1248–1249

Elliott CG (1972) Sterols and the production of oospores by *Phytophthora cactorum*. J Gen Microbiol 72:321–327

Elliott CG (1979) Influence of the structure of the sterol molecule on sterol-induced reproduction in *Phytophthora infestans*. J Gen Microbiol 115:117–126

Elliott CG (1983) Physiology of sexual reproduction in *Phytophthora*. In: Erwin DC, Bartnicki-Garcia S, Tsao PH (eds) *Phytophthora*. Its biology, taxonomy, ecology and pathology. American Phytopathological Society, St Paul, pp 71–81

Elliott CG, Knights BA (1981) Uptake and interconversion of cholesterol and cholesteryl esters by *Phytophthora cactorum*. Lipids 16:1–7

Elliott CG, Sansome E (1977) The influence of sterols on meiosis in *Phytophthora cactorum*. J Gen Microbiol 98:141–145

Elliott CG, Hendrie MR, Knights BA (1966) The sterol requirement of *Phytophthora cactorum*. J Gen Microbiol 42:425–435

Eslava AP, Álvarez MI, Cerdá-Olmedo E (1974) Regulation of carotene biosynthesis in *Phycomyces* by vitamin A and β-Ionone. Eur J Biochem 48:617–623

Fabritius AL, Judelson HS (1997) Mating-type loci segregate aberrantly in *Phytophthora infestans* but normally in *Phytophthora parasitica*: implications for models of mating-type determination. Curr Genet 32:60–65

Fabritius AL, Judelson HS (2003) A mating-induced protein of *Phytophthora infestans* is a member of a family of elicitors with divergent structures and stage-specific patterns of expression. Mol Plant Microbe Interact 16:926–935

Fabritius AL, Cvitanich C, Judelson HS (2002) Stage-specific gene expression during sexual development in *Phytophthora infestans*. Mol Microbiol 45:1057–1066

Feofila EP, Fateeva TV, Arbuzov VA (1976) Mechanism of the action of trisporic acids on carotene-synthesizing enzymes of a (−) strain of *Blakeslea trispora*. Microbiologiya 45:153–155

Gessler NN, Sokolov AV, Belozerskaya TA (2002) Initial stages of trisporic acid synthesis in *Blakeslea trispora*. Appl Biochem Microbiol 38:536–543

Gooday GW (1978) Functions of trisporic acid. Philos Trans R Soc Lond B 284:509–520

Gooday GW (1983) Hormones and sexuality in fungi. In: Bennett JW, Ciegler A (eds) Secondary metabolism and differentiation in fungi. Dekker, New York, pp 239–266

Gooday GW (1994) Hormones in mycelial fungi. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol 1, 1st edn. Growth, differentiation and sexuality. Springer, Berlin Heidelberg New York, pp 401–411

Gooday GW, Adams DJ (1993) Sex hormones in fungi. Adv Microb Physiol 34:69–145

Govind NS, Cerdá-Olmedo E (1986) Sexual activation of carotenogenesis in *Phycomyces blakesleeanus*. J Gen Microbiol 132:2775–2780

Gow NAR, Gooday GW (1987) Effects of antheridiol on growth, branching and electrical currents of hyphae of *Achlya ambisexualis*. J Gen Microbiol 133:3531–3536

Groner B, Hynes N, Sippel AE, Schutz G (1976) Induction of specific proteins in hyphae of *Achlya ambisexualis* by the steroid hormone antheridiol. Nature 261:599–601

Hänfler J, Teepe H, Weigel C, Kruft V, Lurz R, Wöstemeyer J (1992) Circular extrachromosomal DNA codes for a surface protein in the (+) mating type of the zygomycete *Absidia glauca*. Curr Genet 22:319–325

Hendrix JW (1964) Sterol induction of reproduction and stimulation of growth of *Pythium* and *Phytophthora*. Science 144:1028–1029

Hendrix JW (1965) Influence of sterols on growth and reproduction of *Pythium* and *Phytophthora*. Phytopathology 55:790–797

Hill TW (1996) Electrophoretic characterization of endo-(1,4)-β-glucanases secreted during assimilative growth and antheridiol-induced branching in *Achlya ambisexualis*. Can J Microbiol 42:557–561

Horgen PA (1977) Cytosol-hormone stimulation of transcription in the aquatic fungus, *Achlya ambisexualis*. Biochem Biophys Res Commun 75:1022–1028

Horgen PA, Iwanochko M, Bettiol MF (1983) Antheridiol, RNA polymerase II and sexual development in the aquatic fungus, *Achlya*. Arch Microbiol 134:314–319

Horowitz DK, Russell PJ (1974) Hormone-induced differentiation of antheridial branches in *Achlya ambisexualis*: dependence on ribonucleic acid synthesis. Can J Microbiol 20:977–980

Horton JS, Horgen PA (1985) Synthesis of an antheridiol-inducible polypeptide during sexual morphogenesis of *Achlya ambisexualis*. Can J Biochem 63:355–366

Horton JS, Horgen PA (1989) Molecular cloning of cDNAs regulated during steroid-induced sexual differentiation in the aquatic fungus *Achlya*. Exp Mycol 13:263–273

Jones BE, Williamson IP, Gooday GW (1981) Sex pheromones in *Mucor*. In: O'Day DA, Horgen PA (eds) Sexual interactions in eukaryotic microbes. Academic Press, New York, pp 179–198

Judelson HS (1996a) Genetic and physical variability at the mating type locus of the oomycete, *Phytophthora infestans*. Genetics 144:1005–1013

Judelson HS (1996b) Chromosomal heteromorphism linked to the mating type locus of the oomycete *Phytophthora infestans*. Mol Gen Genet 252:155–161

Judelson HS, Spielman LJ, Shattock RC (1995) Genetic mapping and non-mendelian segregation of mating type loci in the oomycete, *Phytophthora infestans*. Genetics 141:503–512

Kerwin JL, Duddles ND (1989) Reassessment of the role of phospholipids in sexual reproduction by sterol-auxotrophic fungi. J Bacteriol 171:3831–3839

Kerwin JL, Washino RK (1983) Sterol induction of sexual reproduction in *Lagenidium giganteum*. Exp Mycol 7:109–115

Knights BA, Elliott CG (1976) Metabolism of Δ^5- and $\Delta^{5,7}$-sterols by *Phytophthora cactorum*. Biochim Biophys Acta 441:341–346

Ko WH (1980) Hormonal regulation of sexual reproduction in *Phytophthora*. J Gen Microbiol 116:459–463

Ko WH (1983) Isolation and partial characterization of α hormones produced by *Phytophthora parasitica*. J Gen Microbiol 129:1397–1401

Ko WH (1985) Stimulation of sexual reproduction of *Phytophthora* by phospholipids. J Gen Microbiol 131:2591–2594

Ko WH (1988) Hormonal heterothallism and homothallism in *Phytophthora*. Annu Rev Phytopathol 26:57–73

Langcake P (1974) Uptake of sterols by *Phytophthora infestans*, their intracellular distribution and metabolism. Trans Br Mycol Soc 64:55–65

Lenton JR, Hall J, Smith AR, Ghisalberti EL, Rees HH, Goad LJ, Goodwin TW (1971) The utilization of potential phytosterol precursors by *Ochromonas malhamensis*. Arch Biochem Biophys 143:664–674

Machlis L (1958) Evidence for a sexual hormone in *Allomyces*. Physiol Plant 11:181–192

Machlis L (1973) Factors affecting the stability of various sirenins and analogues and the uptake of sirenin by the sperm of *Allomyces*. Plant Physiol 52:527–530

McMorris TC (1978) Sex hormones of the aquatic fungus *Achlya*. Lipids 13:716–722
McMorris TC, Barksdale AW (1967) Isolation of a sex hormone from the water mold *Achlya ambisexualis*. Nature 215:820–821
McMorris TC, White RH (1977) Biosynthesis of the oogoniols, steroidal sex hormones of *Achlya*: the role of fucosterol. Phytochemistry 16:359–362
McMorris TC, Seshadri R, Weihe GR, Arsenault GP, Barksdale AW (1975) Structures of oogoniol-1, -2, and -3, steroidal sex hormones of the water mold, *Achlya*. J Am Chem Soc 97:2544–2545
McMorris TC, Toft DO, Moon S, Wang W (1993) Biological responses of the female strain *Achlya ambisexualis* 734 to dehydro-oogoniol and analogues. Phytochemistry 32:833–837
Mehta BJ, Salgado LM, Bejarano ER, Cerdá-Olmedo E (1997) New mutants of *Phycomyces blakesleeanus* for β-carotene production. Appl Environ Microbiol 63:3657–3661
Meissner G, Delbrück M (1968) Carotenes and retinal in *Phycomyces* mutants. Plant Physiol 43:1279–1283
Mesland DAM, Huisman JG, van den Ende H (1974) Volatile sexual hormones in *Mucor mucedo*. J Gen Microbiol 80:111–117
Michalski CJ (1978) Protein synthesis during hormone stimulation in the aquatic fungus, *Achlya ambisexualis*. Biochem Biophys Res Commun 84:417–427
Mullins JT (1979) A freeze-fracture study of hormone-induced branching in the fungus *Achlya*. Tissue Cell 11:585–595
Mullins JT (1994) Hormonal control of sexual dimorphism. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol 1, 1st edn. Growth, differentiation and sexuality. Springer, Berlin Heidelberg New York, pp 413–421
Mullins JT, Ellis EA (1974) Sexual morphogenesis in *Achlya*: ultrastructural basis for the hormonal induction of antheridial hyphae. Proc Natl Acad Sci USA 71:1347–1350
Musgrave A, Nieuwenhuis D (1975) Metabolism of radioactive antheridiol by *Achlya* species. Arch Microbiol 105:313–317
Musgrave A, Ero L, Scheffer R (1978) The self-induced metabolism of antheridiol in water moulds. Acta Bot Neerl 27:397–404
Napoli JL (1999) Interactions of retinoid binding proteins and enzymes in retinoid metabolism. Biochim Biophys Acta 1440:139–162
Nes WD, Patterson GW, Bean GA (1980) Effect of steric and nuclear changes in steroids and triterpenoids on sexual reproduction in *Phytophthora infestans*. Plant Physiol 66:1008–1011
Nieuwenhuis M, van den Ende H (1975) Sex specificity of hormones synthesis in *Mucor mucedo*. Arch Microbiol 102:167–169
Ootaki T, Yamazaki Y, Noshita T, Takahashi S (1996) Excess carotenoids disturb prospective cell-to-cell recognition system in mating responses of *Phycomyces blakesleeanus*. Mycoscience 37:427–435
Pommerville JC (1978) Analysis of gamete and zygote motility in *Allomyces*. Exp Cell Res 113:161–172
Pommerville JC (1981) The role of sex pheromones in *Allomyces*. In: O'Day DH, Horgen PA (eds) Sexual interactions in eukaryotic microbes. Academic Press, New York, pp 52–73
Pommerville J, Olson LW (1987) Evidence for a male-produced pheromone in *Allomyces macrogynus*. Exp Mycol 11:145–248
Pommerville JC, Strickland B, Romo D, Harding KE (1988) Effects of analogs of the fungal sexual pheromone sirenin on male gamete motility in *Allomyces macrogynus*. Plant Physiol 88:139–142
Popplestone CR, Unrau AM (1973) Major sterols of *Achlya bisexualis*. Phytochemistry 12:1131–1133
Popplestone CR, Unrau AM (1974) Studies on the biosynthesis of antheridiol. Can J Chem 52:462–468
Preus MW, McMorris TC (1979) The configuration at C-24 in oogoniol (24*R*-3β,11α,15β,29-tetrahydroxymast-5-en-7-one) and identification of 24 (28)-dehydrooogoniols as hormones in *Achlya*. J Am Chem Soc 101:3066–3071
Quiles-Rosillo MD, Ruiz-Vázquez RM, Torres-Martínez S, Garre V (2005) Light induction of the carotenoid biosynthesis pathway in *Blakeslea trispora*. Fungal Genet Biol 42:141–153
Randall TA, Fong AA, Judelson HS (2003) Chromosomal heteromorphism and an apparent translocation detected using a BAC contig spanning the mating type locus of *Phytophthora infestans*. Fungal Genet Biol 38:75–84
Raper JR (1952) Chemical regulation of sexual processes in the Thallophytes. Bot Rev 18:447–545
Riehl RM, Toft DO (1984) Analysis of the steroid receptor of *Achlya ambisexualis*. J Biol Chem 259:15324–15330
Riehl RM, Toft DO, Meyer MD, Carlson GL, McMorris TC (1984) Detection of a pheromone-binding protein in the aquatic fungus *Achlya ambisexualis*. Exp Cell Res 153:544–549
Riehl RM, Sullivan WP, Vroman BT, Bauer VJ, Pearson GR, Toft DO (1985) Immunological evidence that the non-hormone binding component of avian steroid receptors exists in a wide range of tissues and species. Biochemistry 24:6586–6591
Ruiz-Hidalgo MJ, Benito EP, Sandmann G, Eslava AP (1997) The phytoene dehydrogenase gene of *Phycomyces*: regulation of its expression by blue light and vitamin A. Mol Gen Genet 253:734–744
Salgado LM, Cerdá-Olmedo E (1992) Genetic interactions in the regulation of carotenogenesis in *Phycomyces*. Curr Genet 21:67–71
Salgado LK, Bejarano ER, Cerdá-Olmedo E (1989) Carotene-superproducing mutants of *Phycomyces blakesleeanus*. Exp Mycol 13:332–336
Salgado LM, Avalos J, Bejarano ER, Cerdá-Olmedo E (1991) Correlation between *in vivo* and *in vitro* carotenogenesis in *Phycomyces*. Phytochemistry 30:2587–2591
Schachtschabel D, Schimek C, Wöstemeyer J, Boland W (2005) Biological activity of trisporoids and trisporoid analogues in *Mucor mucedo* (−). Phytochemistry 66:1358–1365
Schimek C, Kleppe K, Saleem A-R, Voigt K, Burmester A, Wöstemeyer J (2003) Sexual reactions in Mortierellales are mediated by the trisporic acid system. Mycol Res 107:736–747
Schimek C, Petzold A, Schultze K, Wetzel J, Wolschendorf F, Burmester A, Wöstemeyer J (2005) 4-dihydromethyltrisporate dehydrogenase, an enzyme of the sex hormone pathway in *Mucor mucedo*, is

constitutively transcribed but its activity is differently regulated in (+) and (−) mating types. Fungal Genet Biol 42:804–812

Schmidt AD, Heinekamp T, Matuschek M, Liebmann B, Bollschweiler C, Brakhage AA (2005) Analysis of mating-dependent transcription of *Blakeslea trispora* carotenoid biosynthesis genes carB and carRA by quantitative real-time PCR. Appl Microbiol Biotechnol 67:549–555

Schultze K, Schimek C, Wöstemeyer J, Burmester A (2005) Sexuality and parasitism share common regulatory pathways in the fungus *Parasitella parasitica*. Gene 348:33–44

Silver JC, Horgen PA (1974) Hormonal regulation of presumptive mRNA in the fungus *Achlya ambisexualis*. Nature 249:252–254

Silver JC, Andrews DR, Pekkala D (1983) Effect of heat-shock on synthesis and phosphorylation of nuclear and cytoplasmic proteins in the fungus *Achlya*. Can J Biochem Cell Biol 61:447–455

Silver JC, Brunt SA, Kyriakopoulou G, Borkar M, Nazarian-Armavil V (1993) Regulation of two different *hsp70* transcript populations in steroid hormone-induced fungal development. Dev Genet 14:6–14

Sutherland RB, Horgen PA (1977) Effects of the steroid sex hormone, antheridiol, on the initiation of RNA synthesis in the simple eukaryote, *Achlya ambisexualis*. J Biol Chem 252:8812–8820

Sutter RP (1975) Mutations affecting sexual development in *Phycomyces blakesleeanus*. Proc Natl Acad Sci USA 72:127–130

Sutter RP (1977) Regulation of the first stage of sexual development in *Phycomyces blakesleeanus* and in other mucoraceous fungi. In: O'Day DH, Horgen PA (eds) Eukaryotic microbes as model development systems. Dekker, New York, pp 251–272

Sutter RP (1987) Sexual development. In: Lipson E, Cerdá-Olmedo E (eds) *Phycomyces*. Cold Spring Harbor Press, Cold Spring Harbor, pp 317–336

Sutter RP, Whitaker JP (1981a) Sex pheromone metabolism in *Blakeslea trispora*. Naturwissenschaften 68:147–148

Sutter RP, Whitaker JP (1981b) Zygophore-stimulating precursors (pheromones) of tripsoric acids active in (−)-*Phycomyces blakesleeanus*. J Biol Chem 256:2334–2341

Sutter RP, Zawodny PD (1984) Apotrisporin: a major metabolite of *Blakeslea trispora*. Exp Mycol 8:89–92

Sutter RP, Dadok J, Bothner-By AA, Smith RR, Mishra PK (1989) Cultures of separated mating types of *Blakeslea trispora* make D and E forms of trisporic acids. Biochemistry 28:4060–4066

Sutter RP, Grandin AB, Dye BD, Moore WR (1996) (−) Mating type-specific mutants of *Phycomyces* defective in sex pheromone biosynthesis. Fungal Genet Biol 20:268–279

Taylor IB, Burbidge A, Thompson AJ (2000) Control of abscisic acid synthesis. J Exp Bot 51:1563–1574

Teepe H, Böttge J-A, Wöstemeyer J (1988) Isolation and electrophoretic analysis of surface proteins of the zygomycete *Absidia glauca*. FEBS Lett 234:100–106

Thomas DM, Goodwin TW (1967) Studies on carotenogenesis in *Blakeslea trispora*. I. General observations on synthesis in mated and unmated strains. Phytochemistry 6:355–360

Thomas DS, McMorris TC (1987) Allomonal functions of steroid hormone, antheridiol, in water mold *Achlya*. J Chem Ecol 13:1131–1137

Thomas DS, Mullins JT (1967) Role of enzymatic wall-softening in plant morphogenesis: hormonal induction in *Achlya*. Science 156:84–85

Thomas DS, Mullins JT (1969) Cellulase induction and wall extension in the water mold *Achlya ambisexualis*. Physiol Plant 22:347–353

Vail WJ, Morris C, Lilly VG (1967) Hormone-like substances which increase carotenogenesis in + and − sexes of *Choanephora cucurbitarum*. Mycologia 59:1069–1074

Van den Ende H (1968) Relationship between sexuality and carotene synthesis in *Blakeslea trispora*. J Bacteriol 96:1298–1303

Van den Ende H (1976) Sexual interactions in plants. Academic Press, London

Van den Ende H (1978) Sexual morphogenesis in the Phycomycetes. In: Smith JE, Berry DR (eds) The filamentous fungi. Arnold, London, pp 257–274

Van den Ende H, Wiechmann AH, Reyngoud DJ, Hendriks T (1970) Hormonal interactions in *Mucor mucedo* and *Blakeslea trispora*. J Bacteriol 101:423–428

Velayos A, Papp T, Aguilar-Elena R, Fuentes-Vicente M, Eslava AP, Iturriaga EA, Álvarez MI (2003) Expression of the *carG* gene, encoding geranylgeranyl pyrophosphate synthase, is up-regulated by blue light in *Mucor circinelloides*. Curr Genet 43:112–120

Werkman BA (1976) Localization and partial characterization of a sex-specific enzyme in homothallic and heterothallic Mucorales. Arch Microbiol 109:209–213

Werkman TA, van den Ende H (1973) Trisporic acid synthesis in *Blakeslea trispora*. Interaction between plus and minus mating types. Arch Microbiol 90:365–374

Werkman BA, van den Ende H (1974) Trisporic acid synthesis in homothallic and heterothallic Mucorales. J Gen Microbiol 82:273–278

White JD, Takabe K, Prisbylla MP (1985) Stereoselective synthesis of trisporic acids A and B, their methyl esters, and trisporol A and B, hormones and prohormones of mucoraceous fungi. J Org Chem 50:5233–5244

Wöstemeyer A, Teepe H, Wöstemeyer J (1990) Genetic interactions in somatic inter-mating type hybrids of the zygomycete *Absidia glauca*. Curr Genet 17:163–168

Wöstemeyer J, Wöstemeyer A, Burmester A, Czempinski K (1995) Relationships between sexual processes and parasitic interactions in the host-pathogen system *Absidia glauca–Parasitella parastica*. Can J Bot 73:S243–S250

Wöstemeyer J, Grünler A, Schimek C, Voigt K (2005) Genetic regulation of carotenoid biosynthesis in fungi. In: Arora DK (ed) Applied Mycology and Biotechnology, vol 5. Genes, genomics and bioinformatics. Elsevier, Dordrecht (in press)

Yakovleva IM, Vakulova LA, Feofilova EP, Bekhtereva MN, Samokhvalov GI (1980) Influence of synthetic compounds structurally close to trisporic acids on carotenogenesis and lipogenesis of the mucor fungus *Blakeslea trispora*. Microbiologiya 49:274–278

13 Photomorphogenesis and Gravitropism in Fungi

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I. Introduction

Light and gravity are ubiquitous in nature and serve as signals to guide growth and development of many types of organisms. Fungi take advantage of the presence of ambient light, and changes of light quality and quantity to modulate several steps of their development. The induction of spore formation and the development of structures for spore dispersal are the most obvious fungal photoresponses and have attracted the attention of scientists worldwide. Many organisms sense gravity as a guiding signal for directed growth, sometimes in combination with other environmental signals such as light. Fungi use the information provided by the measurement of gravity to orient the growth of morphological structures, many of them also involved in spore development and dispersal. Light and gravity are thus environmental stimuli that are responsible independently or in coordination for the final development and appearance of many fungi.

II. Photomorphogenesis

Fungal development is often modified by the presence of light (Table 13.1). The main developmental transitions in the fungal life cycle are spore germination, hyphal growth and branching, and formation of reproductive structures for spore development and dispersal. These developmental transitions are regulated by various environmental fac-

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Table. 13.1. Effects of light on fungal development

Organism	Phenomenon	Threshold	Reference
Phycomyces	Induction of macrophores or inhibition of microphores	10^{-8} W/m^2	Corrochano and Cerdá-Olmedo (1990)
		10^{-4} J/m^2	Corrochano and Cerdá-Olmedo (1990)
		10^{-10} mol/m^2	Corrochano et al. (1988)
	Inhibition of sexual development	3×10^{-2} W/m^2	Yamazaki et al. (1996)
Neurospora	Induction of protoperithecia	4 J/m^2	Degli-Innocenti et al. (1983)
	Circadian clock resetting	10^{-5} mol/m^2	Crosthwaite et al. (1995)
Aspergillus	Induction of conidiation	135 J/m^2	Mooney and Yager (1990)
		8×10^{-4} mol/m^2	Mooney and Yager (1990)
Trichoderma	Induction of conidiation	10^{-5} mol/m^2	Horwitz et al. (1990)
Alternaria	Inhibition of photoinduced conidiation	10^{-5} mol/m^2	Kumagai (1989)
Paecilomyces	Induction of conidiation	1.8×10^{-4} mol/m^2	Sánchez-Murillo et al. (2004)

tors, including the presence or absence of light. The regulation by light of fungal development is generally referred to as fungal photomorphogenesis (Fig. 13.1), and can be measured precisely, allowing one to determine useful parameters such as thresholds (Fig. 13.2). Blue light is the type most effective in fungal photomorphogenesis but other wavelengths can also be important, as shown by the activation of conidiation by red light in *Aspergillus nidulans* (Mooney and Yager 1990), and by near-ultraviolet light in *Alternaria tomato* (Kumagai 1989). The effect of blue light is often either stimulatory or inhibitory of a developmental transition but the red-light induction of conidiation in *Aspergillus* can be inhibited by a subsequent exposure to far-red light, and the near-UV induction of conidiation in *Alternaria* can be inhibited by exposure to blue light (Kumagai 1989; Mooney and Yager 1990), suggesting the operation of complex photoreceptor systems. Fungal photomorphogenesis has been reviewed extensively (Carlile 1970; Gressel and Rau 1983; Kumagai 1988; Corrochano and Cerdá-Olmedo 1991), and the interested reader is referred to these reviews for details on a variety of experimental systems described in older literature. We have therefore decided to summarize the major discoveries regarding fungal photomorphogenesis that have been published in the last 10–15 years.

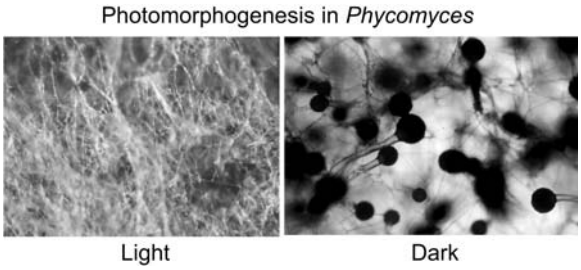

Fig. 13.1. Photomorphogenesis in *Phycomyces*. Light inhibition of microphore initiation and development in *Phycomyces*. Microphores are short sporangiophores, 1–2 mm in length, containing a *dark ball on top* (sporangia) with matured spores. The fungus was grown under continuous light (*left*) or in the dark (*right*). Microphores appeared at the mycelial surface of cultures kept in the dark only. Photographs by L.M. Corrochano

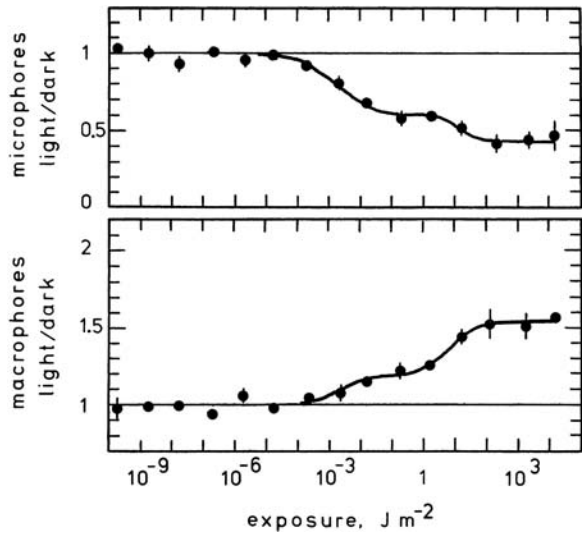

Fig. 13.2. Inhibition of microphorogenesis and stimulation of macrophorogenesis by blue-light pulses (extracted from Corrochano and Cerdá-Olmedo 1990). Standard dark cultures, 2 days old, received pulses of blue light of the fluence given in the abscissa. The number of microphores (*top*) and the dry weight of macrophores (*bottom*) were determined 2 days later, and are shown relative to the values found in dark cultures

A. Ascomycota

1. *Neurospora crassa*

The ascomycete *Neurospora crassa* has been used as an experimental organism in the laboratory for many decades, and its biology has been reviewed in detail (Davis 2000; Perkins and Davis 2000; Davis and Perkins 2002). Many *Neurospora crassa* (henceforth, *Neurospora*) mutants have been isolated and located in a detailed genetic map (Perkins et al. 2001), and the *Neurospora* genome has been recently sequenced and made available to the public (Galagan et al. 2003; Mannhaupt et al. 2003). A detailed analysis of the *Neurospora* genome has described the major gene and protein families in this model fungus (Borkovich et al. 2004). All the information available about *Neurospora*, and the possibility of detailed genetic and molecular manipulation make this organism an attractive model system for fungal photomorphogenesis.

a) The Photoreceptor System

Blue light has several effects on *Neurospora* biology, including photomorphogenesis, and all of these require the product of the *wc* genes (reviewed by Linden et al. 1997a). WC-1 is the product of the *wc-1* gene, a protein with a Zn finger, two PAS domains involved in protein–protein interactions, a putative transcriptional activation domain, a nuclear localization signal, and a chromophore-binding domain (Ballario et al. 1996). The chromophore-binding domain binds the flavin chromophore FAD, allowing WC-1 to act as a photoreceptor (Froehlich et al. 2002; He et al. 2002). WC-2 is the product of the *wc-2* gene, a protein with a Zn finger, a single PAS domain, a putative transcriptional activation domain, and a nuclear localization signal (Linden and Macino 1997). WC-1 and WC-2 interact through the PAS domains to form a white collar complex (Ballario et al. 1998; Talora et al. 1999; Cheng et al. 2001a, 2002, 2003a; Denault et al. 2001) that binds the promoter of light-inducible genes (Ballario et al. 1996; Linden and Macino 1997; Froehlich et al. 2002, 2003). Light causes a decrease in the mobility of the white collar complex bound to the promoter (Froehlich et al. 2002), suggesting a light-dependent aggregation of white collar complexes. Interestingly, mutations in the WC-1 or WC-2 Zn finger domains allows the photoactivation of several genes (Collett et al. 2002; Cheng et al. 2003a), and different *wc-1* alleles show various degrees of light sensitivity (Toyota et al. 2002; Lee et al. 2003). The structure and function of the WC proteins have been reviewed by Ballario and Macino (1997), Linden (2002), and Loros (2005).

The WC proteins are present in the dark (Talora et al. 1999; Schwerdtfeger and Linden 2000; Denault et al. 2001), and are preferentially located in the nucleus, although WC-2 is observed also in the cytoplasm and is more abundant than WC-1 (Schwerdtfeger and Linden 2000; Cheng et al. 2001a; Denault et al. 2001). Nuclear localization of either WC-1 or WC-2 is not affected by light and is not altered by mutations in *wc-2* or *wc-1*, respectively, indicating that nuclear localization does not require a complete white collar complex (Schwerdtfeger and Linden 2000). In the white collar complex, WC-1 is the limiting factor whereas WC-2 is in excess (Cheng et al. 2001b; Denault et al. 2001). However, overexpression of *wc-1* alone does not suffice to activate most light-inducible genes (Lewis et al. 2002).

WC-1 becomes phosphorylated upon light exposure, probably a primary effect of light reception (Talora et al. 1999; Schwerdtfeger and Linden 2000, 2001). The light-dependent phosphorylation of WC-1 is transient, does not require the presence of WC-2, and leads to WC-1 degradation and its replacement in the white collar complex by newly synthesized but inactive WC-1 protein. However, the amount of WC-1 and the kinetics of the light-dependent phosphorylation are altered by the presence of a mutant form of WC-2, suggesting that WC-2 is necessary to sustain the transition and magnitude of WC-1 phosphorylation (Talora et al. 1999; Schwerdtfeger and Linden 2000). Some phosphorylation of WC-1 is observed also in the dark. Five phosphorylation sites located downstream of the Zn finger domain are not responsible for the light-dependent hyperphosphorylation but rather for alterations in the circadian rhythm of conidiation (He et al. 2005). The protein kinase C, PKC, interacts in vivo with WC-1 and phosphorylates in vitro the Zn finger domain. This interaction, however, is observed only in dark mycelia or after 2 h of illumination, when gene photoactivation has ceased (Franchi et al. 2005). Regulatory mutations in PKC result in changes in the amount of WC-1, with corresponding changes in gene photoactivation, albeit without affecting the photophosphorylation pattern of WC-1, confirming that PKC is a negative regulator of WC-1 (Franchi et al. 2005). These results suggest that other kinase(s) must be responsible for the observed photophosphorylation of WC-1.

WC-2 is also phosphorylated after light exposure but the modification is more subtle, requires the presence of WC-1, does not change with longer light exposures, and is not observed in cytoplasmic WC-2 (Schwerdtfeger and Linden 2000). Light-dependent phosphorylation was not observed in WC-1 with a mutation in the chromophore-binding domain, or in WC-2 with a mutation in the Zn finger domain (Schwerdtfeger and Linden 2000).

The *wc-1* gene is induced by light but *wc-2* is expressed equally in both dark- and light-grown mycelia (Ballario et al. 1996; Linden and Macino 1997). In addition, the transcription of *wc-2* is negatively regulated by WC-1 (Cheng et al. 2003a). The photoactivation of *wc-1* requires an active white collar complex (Ballario et al. 1996), allowing the light-dependent synthesis of new WC-1 to replace the phosphorylated WC-1 when it is excluded from the white collar complex and degraded. In addition, the product of the *frequency* gene, FRQ, a master regulator of the *Neurospora* circadian clock, is required for full *wc-1* photoactivation; it activates the translation of the *wc-1* mRNA, resulting in cycles of WC-1 in dark-grown mycelia (Lee et al. 2000; Cheng et al. 2001b, 2002; Merrow et al. 2001a). WC-2 is also required for a normal steady-state amount of WC-1. The regulation of WC-2 and FRQ on WC-1 is post-transcriptional and requires the formation of a white collar complex (Cheng et al. 2002). FRQ also activates the expression of *wc-2* (Cheng et al. 2001b, 2002), and interacts with the white collar complex (Denault et al. 2001; Merrow et al. 2001a) through a coiled-coil domain (Cheng et al. 2001a) and through an interaction with WC-1 (Cheng et al. 2003a). In addition, the RNA helicase FRH is associated with the white collar complex, mediating the interaction between FRQ and the white collar complex (Cheng et al. 2005). All these protein interactions are an indication that the molecular mechanisms of light reception in *Neurospora* are more complex than originally anticipated.

VIVID is the product of the *vivid* gene, another *Neurospora* photoreceptor with a single PAS domain that binds the flavin chromophore FAD or FMN, and it has a prominent role in the transient activation of gene expression after light exposure. Mutations in *vivid* result in a sustained WC-1 photophosphorylation, a reduction of the amount of WC-1 in the cell, and a sustained gene photoactivation (Heintzen et al. 2001; Schwerdtfeger and Linden 2001, 2003; Shrode et al. 2001). Unlike WC-1 and WC-2, VIVID is not found in the *Neurospora* nuclei: VIVID is observed in the cytoplasm only after light induction (Schwerdtfeger and Linden 2003). WC-1, WC-2, and VIVID play important roles in the molecular mechanisms of the *Neurospora* circadian clock (Crosthwaite et al. 1997; Cheng et al. 2001b, 2002; Collett et al. 2001; Heintzen et al. 2001; Lee et al. 2003; He et al. 2005).

Light reception at the FAD chromophore of WC-1 should trigger the formation of a flavin-cysteinyl adduct similar to that observed in the photoreceptor VIVID (Schwerdtfeger and Linden 2003). The similarities of the flavin-binding domain of VIVID and WC-1, and the observation that these domains can be functionally interchanged (Cheng et al. 2003b) support this hypothesis. The formation of the flavin-cysteinyl adduct should result in a conformational change in the white collar complex, leading to protein phosphorylation, white collar complex aggregation, and all the subsequent events leading to gene photoactivation and the resulting photomorphogenesis.

b) Effect of Light on Hyphal Growth

Compared to cultures grown in the dark, branching in *Neurospora* hyphae increases in cultures grown in the light. As a result, colonies grown in the light are more compact than those grown in the dark (Lauter et al. 1998; Ambra et al. 2004). This effect of light on hyphal growth requires the products of the *wc* genes, and has been observed also in the related ascomycete *Tuber borchii* (Lauter et al. 1998; Ambra et al. 2004). A similar effect of light has been documented in the ascomycete *Trichoderma atroviride* but, in contrast to *Neurospora*, light inhibition of hyphal growth did not require the presence of the products of the *Trichoderma wc* genes (Casas-Flores et al. 2004). Light also promotes hyphal branching in the plant pathogen *Colletotrichum trifolii* (Chen and Dickman 2002). Mutations in the *Neurospora* gene *cot-1*, encoding a Ser/Thr protein kinase, result in high branching and colonial growth (Yarden et al. 1992). Two transcription-initiation sites in *cot-1* control the synthesis of two different transcripts. Light stimulates the transcription of the short *cot-1* transcript, and represses the synthesis of the *cot-1* long transcript. The ratio of different *cot-1* transcripts is, therefore, regulated by light. The effect of light on *cot-1* transcription depended on the carbon source employed in the culture medium. A sorbose-containing medium

stimulated *Neurospora* colonial growth and hyphal branching, and inhibited the effect of light on *cot-1* transcription (Lauter et al. 1998). However, the amount of COT-1 protein in the cell, as measured by specific antibodies, was not altered by light (Gorovits et al. 1999), a puzzling observation that suggested that the role of the short *cot-1* transcript and the effect of light on *cot-1* transcription and hyphal branching remain to be elucidated. Another, yet again puzzling, role for COT-1 has been proposed, based on the fact that a *cot-1* mutation suppressed the blind phenotype of a *wc-2* strain. Strains carrying a mutation in the gene *wc-2* and a temperature-sensitive mutation in *cot-1* showed a normal photoactivation of the clock-controlled gene *ccg-1* only when the experiments were carried out at the permissive temperature. This suppression of the *wc-2* phenotype was very specific, since the *wc-2 cot-1* strain did not show photoactivation of *al-3* or *ccg-2*, and the *cot-1* mutation did not suppress the blindness of a *wc-1* mutant (Arpaia et al. 1995). The results showed that a partially active COT-1 kinase might bypass the effect of the *wc-2* mutation for the photoactivation of some *Neurospora* genes (Arpaia et al. 1995).

c) Photoconidiation

Conidiation in *Neurospora* is a developmental process that is induced by several environmental cues such as desiccation, lack of nitrogen or carbon, and the presence of light (reviewed by Springer 1993; see Chap. 14, this volume). Light is required to obtain the maximum number of conidia, but it produces only a modest fourfold increment in the amount of conidia over that obtained in cultures kept in the dark (Lauter et al. 1997). Interestingly, the photoactivation of conidiation is 500-fold in a strain with a thermosensitive mutation in the gene *acon-2*. This strain conidiates only at the permissive temperature, 25 °C, but the mycelium remains undifferentiated when it is grown at 34 °C. At the permissive temperature, the number of conidia developed by the *acon-2* strain is very low but it reaches the amount produced by the wild type upon exposure to light (Lauter et al. 1997). It seems that a partially functional ACON2 protein represses conidiation in the dark, allowing light-dependent conidiation to be fully observed.

Other proteins and chemicals may play a role in *Neurospora* photoconidiation. A role for the flavoprotein nitrate reductase in *Neurospora* photoconidiation has been suggested, based on the lack of light-induced conidiation in mutants without detectable nitrate reductase activity (Ninnemann 1991). Additionally, a role for nitric oxide synthase and nitric oxide in *Neurospora* conidiation and its regulation by light has been proposed based on the effect of inhibitors of the enzyme and nitric oxide donors on photoconidiation (Ninnemann and Maier 1996). The photoinduction of conidiation, like all the *Neurospora* photoresponses, requires the *wc* gene products (Lauter et al. 1997), but an earlier report showed that conidiation of a *wc-1* mutant was promoted by light (Ninnemann 1991). The different nitrogen sources used in the growth medium may be responsible for the different photoconidation phenotype of the *wc-1* mutant (Lauter et al. 1997). These results suggest a complex interaction of nutritional and environmental factors in the regulation of *Neurospora* conidiation.

Conidiation in *Neurospora* is also governed by a circadian clock that can be entrained by a light exposure. The interested reader should consult the chapter on the *Neurospora* circadian clock by Dunlap et al. (2004) in this series (The Mycota, Vol. 3, 2nd edn.), and other reviews on this topic by Bell-Pedersen (2000), Merrow et al. (2001b), Liu (2003), Crosthwaite (2004), Dunlap and Loros (2004), and Lakin-Thomas and Brody (2004).

The development of conidiophores and conidia should bring about differential gene expression that could be modulated by blue light. Indeed, some genes in *Neurospora* are induced by conidiation and blue light (Lauter and Russo 1991; Lauter et al. 1992; Lauter and Yanofsky 1993; Carattoli et al. 1995), and their promoters have an array of developmental-, light-, and circadian clock-controlled DNA elements (Corrochano et al. 1995; Bell-Pedersen et al. 1996; Lee and Ebbole 1998). Mutants altered in the regulation of light-induced genes have been isolated, some having defects in the regulation of conidiation (Madi et al. 1994; Carattoli et al. 1995; Linden et al. 1997b). The genes altered in two mutants have been cloned and identified as a general repressor of gene expression (Yamashiro et al. 1996) and a glucose transporter (Madi et al. 1997), which confirmed the complex relationship between light, nutrient deprivation, and conidiation in *Neurospora*. The regulation of gene expression by light and development are likely to be primary events in the induction of conidiation by light, and are a promising avenue of research to unravel the molecular mechanism of *Neurospora* photomorphogenesis.

d) Effect of Light on Sexual Development

The female sexual structures (protoperithecia) of *Neurospora* are induced by blue-light illumination (Degli-Innocenti et al. 1983; see Chap. 16, this volume). The effect of light on protoperithecia formation is enhanced if the culture medium lacks nitrogen. Under these conditions, the formation of conidia is strongly reduced. If an inhibitor of DNA methylation (5-azacytidine) is added to the growth medium, conidiation is induced and the protoperithecia are inhibited, suggesting a role for DNA methylation of regulatory genes on the choice of developmental pathway (Kritsky et al. 2002).

The shape of fertilized perithecia culminates with a beak that is located at the top when the culture has been illuminated. However, cultures kept in the dark form beaks at a random location in each perithecium. Light-induced polarity of perithecial beaks requires the product of the *wc-1* gene (Oda and Hasunuma 1997). In addition, the perithecial beaks show a positive phototropism when illuminated with blue light (Harding and Melles 1983). Blue light induces the phosphorylation of a 15-kDa protein (Oda and Hasunuma 1994) that has been identified as a nucleoside diphosphate kinase encoded by the *ndk-1* gene (Ogura et al. 1999). A point mutation in *ndk-1* reduced the NDK-1 activity and prevented the effect of light on perithecial beak polarity, without affecting phototropism (Oda and Hasunuma 1997; Ogura et al. 2001). These results suggest that the kinase activity of NDK-1 may be an element of the signal transduction pathway for the light regulation of perithecial beak polarity (Ogura et al. 2001). Other signals may also play a role in light-induced perithecial beak polarity. A *sod-1* mutant altered in the enzyme Cu, Zn superoxide dismutase had lost the light-induced polarity of perithecia, and also showed an enhanced synthesis of carotenes and a higher expression of the *al* genes for the enzymes involved in carotene biosynthesis. These data indicate that intracellular reactive oxygen regulated by SOD-1 should have a role in the light transduction pathway (Yoshida and Hasunuma 2004). SOD reduces the level of superoxide anion. The role of oxidative stress in *Neurospora* development received support from the observation that the lack of a catalase in a *cat-3* mutant induced carotene synthesis, hyphal adhesion, and the development of more aerial hyphae and conidia than in the wild type (Michán et al. 2003). Evidently, superoxide dismutase and catalase cooperate to reduce oxidative stress. These results seem to support a proposal that microbial differentiation is a mechanism to cope with excess amounts of reactive oxygen in the cell (Aguirre et al. 2005).

e) Photoperiodism in Neurospora

Photoperiodism, the role of day or night length in biological activity, has attracted little attention in fungi (Roenneberg and Merrow 2001). In *Neurospora*, a photoperiodic response has been described for sexual and asexual reproduction, maximum protoperithecial and conidial yields having been recorded for periodic cycles of 14 and 12 h of light, respectively, followed by a dark period to complete a 24-h cycle. Light cycles with shorter or longer illuminations resulted in lower yields of reproductive structures, suggesting that the photoperiodic response did not rely on the amount of total light exposure. The involvement of the circadian clock in *Neurospora* photoperiodism was shown by the lack of a photoperiodic response in an *frq* mutant with a major defect in the circadian clock (Tan et al. 2004). Carotene biosynthesis also showed a photoperiodic maximum but, unlike photoperiodism for reproductive structures, the maximum was found with cycles of 14–20 h of light. Light/dark cycles with more hours of light yielded lower carotene accumulation (Tan et al. 2004). The photoactivation of the genes for the enzymes required for carotene biosynthesis ceases after the illumination time has been extended for a certain period of time, and further incubation in the dark is required before these genes can be photoactivated again (Baima et al. 1991; Arpaia et al. 1999; Schwerdtfeger and Linden 2001, 2003). This phenomenon, dubbed light adaptation, has been described for other light-activated genes in *Neurospora* (Arpaia et al. 1993, 1995; Lauter and Yanofsky 1993), and is modified by mutations in the gene *vivid* (Heintzen et al. 2001; Schwerdtfeger and Linden 2001, 2003; Shrode et al. 2001), by inhibitors or mutations of the protein kinase C (Arpaia et al. 1999; Franchi et al. 2005), and it requires protein synthesis (Schwerdtfeger and Linden 2001). One can speculate that light adaptation of gene expression could play a role in *Neurospora* photoperiodism. The requirement of a particular day/night duration to obtain a sustained gene photoactivation with different levels of gene expression depending on day/night duration could be one of the mechanisms used to measure day or night length for a biological response. The effect of mutations

in the gene *vivid*, the effect of inhibitors of protein kinase C, and the effect of regulatory mutations of protein kinase C on *Neurospora* photoperiodism could be interesting avenues of research to unravel the molecular mechanisms of fungal photoperiodism.

f) Photoreceptor Genes in Neurospora

Selection for mutants that have lost the *Neurospora* light responses has led to the isolation of *wc* mutants, and the cloning and characterization of the photoreceptor WC-1 (Ballario et al. 1996; Froehlich et al. 2002; He et al. 2002). A photoreceptor with a similar flavin-binding domain, VIVID, was originally identified by a mutation that promoted carotene biosynthesis but cloned only after the isolation of a cDNA segment for a protein with a PAS domain (Heintzen et al. 2001; Schwerdtfeger and Linden 2003). Genome sequencing has increased the list of *Neurospora* photoreceptors. A cDNA segment with similarity to archeal rhodopsin photoreceptors has been identified that has enabled the isolation of the full-length gene *nop-1*, but its inactivation did not result in a clear blind phenotype (Bieszke et al. 1999a), despite the characterization of the NOP-1 protein as a photoreceptor (Bieszke et al. 1999b; Brown et al. 2001; Bergo et al. 2002). In addition, the complete genome sequence included two phytochrome genes and one cryptochrome gene (Galagan et al. 2003; Borkovich et al. 2004). Cryptochromes are plant blue-light photoreceptors (Cashmore 2003; Lin and Shalitin 2003), and it is surprising that the *Neurospora* cryptochrome gene was not identified by a mutation, despite the extensive search for blind mutants (Linden et al. 1997a). It is possible that *Neurospora* rhodopsin and cryptochrome have secondary or redundant roles, and that a clear blind phenotype will be observed only with a combination of mutations in several genes. It is also possible that mutants with subtle effects in light responses (Linden et al. 1997a) are affected in these genes, or that these photoreceptors are important for the survival of *Neurospora* in nature but are redundant in the laboratory environment. The observation of light responses in *Neurospora wc-1* and *wc-2* mutants, although controversial, has suggested the presence of additional photoreceptors (Dragovic et al. 2002). More striking, however, is the identification of two genes for phytochromes that are red light-absorbing photoreceptors (Schafer and Bowle 2002), since the light responses identified and investigated in *Neurospora* are caused by blue light (Linden et al. 1997a). Interestingly, a far-red light effect on DNA stability has been reported. DNA damage and induction of mutations in conidia of *Neurospora* after X-ray treatment were increased by exposure to far-red light and were decreased by exposure to red light. The far-red light effect was reversed by a subsequent red-light exposure (Klein and Klein 1962). The possibility that *Neurospora* phytochromes are responsible for this far-red light effect deserves further investigation. Red light might also be involved in circadian clock regulation. The amount of blue and red light in nature changes throughout the day, with more red than blue light at dawn and sunset. We can speculate that red light- and blue light-absorbing photoreceptors could cooperate to measure the ratio of red and blue light received by *Neurospora*. Red light will be high and blue light low at dawn, red light will decrease throughout the day with a concomitant increase in blue light, and red light will increase again as blue light decreases at sunset. It is possible that this photoreceptor cooperation will generate two separate inputs into a molecular mechanism that will measure more precisely the time of the day for circadian clock regulation. A role for phytochromes as photoreceptors that would allow the detection of red-light variations during the day for circadian regulation has already been suggested (Hellingwerf 2002). Clearly, the relationship between red and blue light in circadian clock regulation deserves further investigation.

2. *Aspergillus nidulans*

a) Photoconidiation

Asexual development in *Aspergillus nidulans* (henceforth, *Aspergillus*) is a morphological pathway that culminates with the production of conidia, and involves a complex regulatory network of gene regulation and cell differentiation (reviewed by Adams et al. 1998; see Chap. 14, this volume). Conidiation in *Aspergillus* is induced by light, but other aspects of *Aspergillus* development are also influenced by light: *Aspergillus* produces hyphae and sexual structures in the dark, and mainly conidiophores and conidia in the light. Thus, the ratio of sexual to asexual development is changed by light.

Light is only effective if it is applied up to 6 h after conidiation has been induced. Surprisingly, only

red light is effective, and the stimulatory effect may be reversed if far-red light is applied after a red-light exposure, suggesting a phytochrome-like photoreceptor in *Aspergillus*, like those involved in plant photobiology (Mooney and Yager 1990). The similarity of the amino-end of the *Aspergillus* BRLA protein, a master regulator of conidiation, with the segment involved in light-induced conformational changes of several plant phytochromes has fuelled the idea that a phytochrome-like photoreceptor may directly regulate conidiation in *Aspergillus* (Griffith et al. 1994). The recent discovery of a phytochrome gene in the *Aspergillus nidulans* genome could give important clues to the nature of the photoreceptor involved in red-light conidiation (Blumenstein et al. 2005).

A mutation in the *velvet* (*veA*) gene allows *Aspergillus* to conidiate in the dark, suggesting a role for the *veA* gene product as a negative regulator of light-induced conidiation (Mooney and Yager 1990). The *veA1* mutant has suffered a nucleotide change in the initiation codon, resulting in a 36-amino acid shorter protein due to the presumed initiation in a secondary methionine codon (Kim et al. 2002). RNA expression and gene overexpression experiments have suggested that the *veA* gene product is also a positive activator of sexual development (Kim et al. 2002; see Chap. 16, this volume). A homolog of *velvet* has been identified in the *Neurospora* genome, suggesting that it may play a similar role for *Neurospora* photoconidiation (Galagan et al. 2003). Suppressors of the *veA1* mutations that restored a light-dependent conidiation have been isolated (Mooney et al. 1990), and three of these are alleles of the *fluG* gene (Yager et al. 1998), a gene previously identified by mutations resulting in a conidial and fluffy phenotype. Additionally, a mutant strain of *Aspergillus* that conidiates under red or blue light has been used to isolate a mutant that blocks red light-induced conidiation. Interestingly, the mutant altered in red-light conidiation is yet another allele of the *fluG* gene (Yager et al. 1998). FLUG is a protein with limited similarity with prokaryotic glutamine synthetases, and is involved in the synthesis of a diffusible, low-molecular weight factor that controls the initiation of sporulation (Lee and Adams 1994; see Chap. 11, this volume). The isolation of *fluG* alleles that modified light-dependent conidiation in *Aspergillus* suggested an important role for FLUG in the regulation of conidiation by light (Yager et al. 1998). In addition to VEA and FLUG, a role for the COP9 signalosome in light regulation of *Aspergillus* development has been suggested (Busch et al. 2003). A *csnD* deletion strain is missing one of the components of COP9 and is blind for light regulation of development, since it produced sexual development in plates grown in light or dark. The results suggest that in *Aspergillus* the COP9 signalosome, which is involved in targeting proteins for degradation, is essential for light-dependent signaling (Busch et al. 2003).

b) Effect of Light on Sexual Development and the Circadian Rhythm

Sexual development in *Aspergillus* is inhibited by red light, and is also affected by the presence of fatty acids added to the culture media or modified in the cell by mutations (Calvo et al. 1999, 2001; see Chap. 16, this volume). The *Aspergillus nidulans* genome contains a gene, *phsA*, for a protein very similar to a bacterial phytochrome. The *Aspergillus* phytochrome acts as a red-light sensor and could be a photoreceptor for the red-light inhibition of sexual development (Blumenstein et al. 2005).

Circadian rhythms have also been described in *Aspergillus*. In *Aspergillus flavus*, the development of one type of survival structures, sclerotia, is governed by a circadian clock with a period of 33 h at 30 °C (Greene et al. 2003). However, no circadian rhythm in the development of morphological structures could be observed in *Aspergillus nidulans*, but the transcription of the glyceraldehyde-3-phosphate dehydrogenase gene (*gpdA*) followed a rhythm that could be entrained by light and temperature, suggesting the presence of a circadian clock (Greene et al. 2003). The lack of an obvious circadian rhythm in *Aspergillus* development is not surprising, since the *Neurospora* circadian clock for conidia formation, now a model for circadian regulation, was fully uncovered after the isolation of a strain carrying a *band* mutation (Dunlap et al. 2004). It is thus conceivable that, similarly to *Neurospora*, a mutant strain of *Aspergillus* could be isolated showing some sort of morphological circadian clock. The *Aspergillus* genome lacks a homolog of the *Neurospora frq* gene, a key element in the *Neurospora* circadian clock, but contains homologs of the *wc* genes that are required for *Neurospora* blue-light photoresponses and that play important roles in the proper regulation of the circadian clock (Greene et al. 2003). These observations suggest that the *Aspergillus* circadian clock may be organized using some, but not all, of the elements of the *Neurospora* clock.

3. *Trichoderma*

a) Photoconidiation

Conidiation in the ascomycete *Trichoderma* is induced by a pulse of blue light (Horwitz et al. 1990). In addition, light inhibits hyphal growth, blue and red light being equally effective (Casas-Flores et al. 2004). In *Trichoderma atroviride*, the gene *phr1* for the DNA repair enzyme photolyase is transiently expressed in mycelia and conidiophores after illumination. The gene is also developmentally regulated, as the mRNA accumulates in the dark in conidiophores during conidial development. The threshold for *phr1* photoactivation is about 10 μmol/m^2, which is similar to the threshold for photoinduction of conidiation, suggesting that both photoresponses could use similar photoreceptors (Berrocal-Tito et al. 2000). However, *phr1* photoinduction is not blocked in non-conidiating mutants or in a photoreception mutant, a result that suggests that gene photoactivation does not require a fully active conidiation pathway, and that gene photoactivation and conidial photoinduction may not share all the elements of their respective phototransduction pathways (Berrocal-Tito et al. 1999). In *Trichoderma*, blue light is used as a signal to prevent the harmful effect of ultraviolet light by inducing the development of resistant conidia and the expression of the photolyase gene *phr1* (Berrocal-Tito et al. 1999, 2000). In addition, sporulation induced by light represses the gene for glyceraldehyde-3-phosphate dehydrogenase (Puyesky et al. 1997), and stimulates the expression of specific proteins (Puyesky et al. 1999). The primary effect of light in the related fungus *Trichoderma viridae* was investigated with cell-free extracts that promoted the phosphorylation of two proteins after incubation with light (Gresik et al. 1989). Light also promoted dephosphorylation of a 33-kDa protein in *Neurospora* extracts (Lauter and Russo 1990), and phosphorylation of a 15-kDa protein (Oda and Hasunuma 1994). The relationship between light and phosphorylation was further supported by the discovery of light-dependent phosphorylation and dephosphorylation of the *Neurospora* photoreceptor WC-1 and its partner WC-2 (Talora et al. 1999; Schwerdtfeger and Linden 2000, 2001). We can speculate that phosphorylation will play a similar role in *Trichoderma* photoreception.

Two genes with similarities to the *Neurospora wc* genes have been isolated in *Trichoderma atroviride*. These genes, named *blr-1* and *blr-2*, are required for the photoinduction of conidiation, and for the photoinduction of *phr1*, but not for the light-dependent inhibition of hyphal growth (Casas-Flores et al. 2004). Curiously, mutants in any of the *blr* genes have a strong light-dependent inhibition of hyphal growth. The phenotype of the *blr* mutants and the domains present in the BLR proteins indicated that they should play a major role as the photoreceptor system for the photoinduction of conidia in *Trichoderma*, as suggested for WC in *Neurospora*. The novel red-light inhibition of hyphal growth that is exacerbated in the *blr* mutants suggested the presence of additional *Trichoderma* photoreceptors showing complex interactions with the BLR photoreceptor (Casas-Flores et al. 2004).

A gene with similarities to the *Neurospora* photoreceptor gene *vivid* has been isolated in *Hypocrea jecorina* (*Trichoderma reesei*). The gene is induced by the presence of cellulose, suggesting a connection between light reception and carbon utilization in this fungus (Schmoll et al. 2004).

4. Other Ascomycetes

The ascomycete *Paecilomyces fumosoroseus* is an entomopathogenic fungus that invades insects after conidial contact and germination with the insect cuticle. Blue light has a dual role in conidial production, with a stimulatory and inhibitory effect depending on the fluence rate used. When the fungus is grown in the dark, only vegetative hyphae are present. However, 5 min of blue light is enough to induce conidial development (Sánchez-Murillo et al. 2004). A period of competence to light has been observed, since light is effective only when applied between 72 and 96 h of growth. The blue-light threshold is 180 μmol/m^2, and the maximum conidial yield was obtained with 540 μmol/m^2. Higher fluence rates decreased conidiation, suggesting the presence of a complex photosensory system (Sánchez-Murillo et al. 2004).

The ascomycete *Tuber borchii* is appreciated for the production of truffles, ascocarps developed as symbiotic mycorrhizae with host plants. Blue light inhibits colony growth in *Tuber* and in *Neurospora*. The effect of light in colony growth in *Neurospora* requires the photoreceptor WC-1 (Lauter et al. 1998; Ambra et al. 2004). The *Tuber* homolog of *wc-1* is induced by light, and its protein product may function as a photoreceptor but lacks a polyglutamine activator domain, suggesting that it may

not function as a transcriptional regulator. The inhibition of mycelial growth by light may promote the growth of *Tuber* in the soil (Ambra et al. 2004). The increase of branching by light in *Neurospora* (Lauter et al. 1998) and in *Tuber* (Ambra et al. 2004) may be responsible for the compact aspect of their colonies when they grow in the light.

Colletotrichum trifolii is a plant pathogen causing anthracnose in alfalfa. Light promotes hyphal branching in *C. trifolii* (Chen and Dickman 2002). The *C. trifolii* gene *tb-3* is the homolog of the *Neurospora cot-1* gene for a Ser/Thr protein kinase. Like *cot-1*, *tb-3* is also induced by light but TB3, unlike COT-1, may act as a transcriptional regulator for hyphal branching, as suggested by the nuclear localization of TB3 and the presence of polyglutamine repeats that serve as transcriptional activation domains in yeast (Chen and Dickman 2002).

B. Zygomycota

1. *Phycomyces blakesleeanus*

a) Photophorogenesis

The zygomycete *Phycomyces blakesleeanus* (Cerdá-Olmedo and Lipson 1987; Cerdá-Olmedo 2001) develops two types of fruiting bodies (sporangiophores) of very different size, macrophores and microphores (Thornton 1972). Blue light stimulates macrophorogenesis and inhibits microphorogenesis (photophorogenesis; Fig. 13.1; reviewed in Corrochano and Cerdá-Olmedo 1991, 1992), and has a prominent role on other aspects of *Phycomyces blakesleeanus* (henceforth, *Phycomyces*) biology, such as the phototropism of the macrophores and the stimulation of β-carotene biosynthesis in the mycelium (Galland 1990, 2001; Bejarano et al. 1991).

Sporangiophore development in *Phycomyces* is highly synchronized. Only vegetative mycelium is detected at the age of 48 h, when the mycelium is ready to develop sporangiophores and is sensitive to blue light. Sporangiophores appear soon thereafter, and can be easily collected at the age of 72 h. The maximum number of sporangiophores is obtained at the age of 96 h, and remains constant for several days. The final numbers of macrophores and microphores in the cultures depend on the blue-light fluence applied at the age of 48 h (Corrochano and Cerdá-Olmedo 1988, 1990). The effect of blue light on sporangiophore development follows a two-step stimulus-response curve with thresholds at 10^{-4} and 1 J/m^2, which suggests the presence of different photosystems optimized to operate at different light fluences (Fig. 13.2; Corrochano and Cerdá-Olmedo 1990).

b) Photophorogenesis Mutants

Mutants defective in macrophore phototropism, genotype *mad*, have been isolated and some of these, *madA* and *madB*, are also defective in photophorogenesis and other light responses, an indication that their protein products are required for all light responses in *Phycomyces* (reviewed in Cerdá-Olmedo and Corrochano 2001). A search for mutants in photomicrophorogenesis, *pim* mutants, identified three with a higher threshold for this photoresponse. The *pim* mutants had a normal phototropism and photocarotenogenesis, except for one that showed a higher threshold for photocarotenogenesis (Flores et al. 1998). A *madJ* mutant, blind for phototropism, had a higher threshold for photomicrophorogenesis whereas its photomacrophorogenesis and photocarotenogenesis remained normal (Flores et al. 1998). These results suggest the presence of a complex photosensory system with separate transduction pathways for photomicrophorogenesis and photomacrophorogenesis, as already indicated by differences in their action spectra (Corrochano et al. 1988).

c) Light Transduction Chain

The molecular basis of photophorogenesis in *Phycomyces* remains largely unknown. Experiments with chemical inhibitors have shown that heterotrimeric G proteins and protein phosphorylation may play a role in the transduction pathway for photophorogenesis (Tsolakis et al. 1999, 2004). Chemical analysis and inhibitor treatments have suggested that pteridines and NO synthase participate in blue-light signaling for photophorogenesis (Maier and Ninnemann 1995; Maier et al. 2001).

Light should activate gene transcription at the moment of sporangiophore development. The role of differential gene expression during photophorogenesis in *Phycomyces* was investigated with a method based on the polymerase chain reaction with arbitrary primers (Corrochano 2002). The method facilitated the visualization of *Phycomyces* cDNAs differentially expressed during sporangiophore development, or after light induction in 48 h-old mycelia when the fungus is sensitive to light. A segment of a cDNA from

a gene induced by blue light was isolated and sequenced. Sequence similarities identified this cDNA as a segment of the gene *hspA* encoding the heat-shock protein HSP100 (Corrochano 2002). HSP100 are ATP-binding proteins with the ability to disassemble protein complexes, and are involved in the tolerance to high temperatures, proteolysis, and the regulation of gene transcription (Maurizi and Xia 2004). The *hspA* gene is induced by light and heat shock, but about ten times more *hspA* mRNA may be observed after a heat shock than after light exposure. This observation suggests that different mechanisms may be involved in the regulation of *hspA* transcription by various environmental cues. In addition, the photoactivation of *hspA* is 10^4 times less sensitive than other mycelial light responses, which suggests differences in the photoreceptor systems involved. Transcription factors responsible for blue light-dependent and heat shock-dependent gene transcription may interact in the promoter of *hspA*, as indicated by the identification in close proximity of DNA segments that are present in other heat shock- and light-induced genes (Rodríguez-Romero and Corrochano 2004). The activation of *hspA* by light in *Phycomyces* could be required to deal with damaged proteins after an exposure to light. HSP100 could also play a role in the phototransduction pathway mediating the disaggregation of regulatory elements. The details of *hspA* photoactivation could give clues to the molecular events involved in sporangiophore development and its regulation by blue light.

The protein product of the gene *crgA* of the mucoral fungus *Mucor circinelloides* acts as a negative regulator of carotene biosynthesis, since *crgA* mutations result in increased accumulation of carotenes in mycelia grown in the dark or in the light (Navarro et al. 2000, 2001). The predicted CrgA protein contains several domains, including a RING-finger zinc-binding motif, several glutamine-rich regions, a putative nuclear localization signal, and an isoprenylation domain (Lorca-Pascual et al. 2004). A putative homolog of *crgA* has been isolated in the related zygomycete *Blakeslea trispora* (Quiles-Rosillo et al. 2005), suggesting that homologous genes could be present in *Phycomyces* and play similar roles in the regulation of photocarotenogenesis and other light-regulated phenomena, including photophorogenesis. In addition, the recent discovery of *Phycomyces* genes similar to the *Neurospora* gene *wc-1* has given support to the idea that the *Phycomyces* phototransduction pathway may be similar to that described for *Neurospora* and other fungi (Idnurm and Heitman 2005b).

d) Effect of Light on Sexual Development

The sexual development of *Phycomyces* is inhibited by light. Effective wavelengths were found to be shorter than 490 nm but the shape of the action spectra and the most effective wavelength depended on the stage of sexual development. Longer wavelengths were more effective for the inhibition of the final stages of sexual development. In addition, biphasic fluence-response curves were observed using some wavelengths, further confirming the presence of a complex photosensory system for photoinhibition of sexual development in *Phycomyces* (Yamazaki et al. 1996). The threshold for this photoresponse is unusually high for *Phycomyces* (Table 13.1). The unique shape of the action spectra with maximum efficiency at 350–410 nm suggests that the photosystems for the inhibition of sexual development would have special features not shared by other photosystems in *Phycomyces*.

C. Basidiomycota

1. *Coprinus cinereus* (*Coprinopsis cinerea*)

Basidiomycetes are characterized by the complexity of their fruiting bodies that contain and disperse the spores produced after meiosis. Perhaps as a consequence of this developmental complexity, basidiomycetes show a very intricate pattern of dark and light regulation at several steps of their developmental pathways.

The basidiomycete *Coprinus cinereus* (*Coprinopsis cinerea*) can grow as homokaryon or dikaryon mycelia. The dikaryon is formed after fusion of two homokaryon mycelia of compatible mating type, and develops a fruiting body where meiosis takes place to produce meiotic basidiospores (reviewed in Kües 2000; Kamada 2002; see Chap. 19, this volume). In addition to this sexual cycle, *C. cinereus* develops different types of reproductive and specialized cells: haploid unicellular spores (oidia) develop on oidiophores in the aerial mycelium, and large chlamydospores appear in submerged, old mycelium. Hyphal knots are areas of intense hyphal branching that can give rise to globose multicellular bodies (sclerotia) in old cultures, or serve as primordia of fruiting bodies. Different environmental conditions determine

different developmental pathways in *C. cinereus*, the presence or absence of light serving as the major signal (reviewed in Kües 2000; Fischer and Kües 2003; see Chap. 14, this volume).

a) Effect of Light on Fruiting Body Development

The presence of light determines the developmental pathway followed by hyphal knots, since sclerotia are produced in the dark, and initiation of the fruiting body occurs only in the light. Fruiting body development depends on dark/light cycles, and several steps in this development are influenced by light: (1) hyphal knot formation is inhibited by light, (2) the formation of fruiting body initials, maturation of primordia, and karyogamy are induced by light, and (3) meiosis completion is inhibited by light (reviewed in Kües 2000). Mutants altered in different steps of fruiting body development have been isolated, including blind mutants that behave under dark/light cycles as the wild type grown in the dark (Muraguchi et al. 1999). The blind mutants suffered a blockage in fruiting body development, such that only "dark stipes" were formed. This blind phenotype suggested that proteins altered in the mutants had a role in light signaling. One of the mutant genes has been cloned, and its sequence resembles that of the *Neurospora wc-1* gene for the blue-light photoreceptor, suggesting a similar role in *C. cinereus* photomorphogenesis (Terashima et al. 2003).

Of particular interest is the role of light in meiosis, since meiosis progression is controlled by light/dark cycles (Lu 2000). The timing of karyogamy and meiosis differed in different strains under the same light/dark cycle, and depended on the light intensity used. The effect of light was restricted to a period of 16 to 6 h before karyogamy but a subsequent dark period, or low-intensity exposure, was required for the completion of meiosis. The suppressing effect of light on the completion of meiosis was not observed in a specific dikaryon due to a mutation in a single gene. The effect of light/dark cycles in meiosis, and possibly of the whole fruiting body development, seems to allow the maturation of fruiting bodies for spore dispersal shortly after daybreak, regardless of night duration (Lu 2000).

b) Other Effects of Light on Development

In addition to the role of light on fruiting body development in dikaryons, it is possible to investigate several developmental pathways in *C. cinereus* monokaryons and its regulation by light after their *A* and *B* mating type pathways have been activated. Monokaryon mycelia develop oidia in high numbers after growth in the dark or in the light. However, a mutant monokaryon with both mating type pathways activated produced a small number of oidia in the dark, and a large amount of oidia when exposed to light (Polak et al. 1997; Kertesz-Chaloupková et al. 1998). Short illumination times (1–2 min) were sufficient to induce oidia above threshold levels, and the effect of light did not spread to non-illuminated mycelia. An exposure of 60 $\mu mol/m^2$ of blue light yielded a full oidia induction, with other wavelengths being less effective (Kertesz-Chaloupková et al. 1998). The role of the *A* activated pathway was further explored after transformation of monokaryons with heterologous and compatible *A* mating type genes. The resulting strains showed light induction of oidia production. In addition, light incubation repressed the formation of hyphal knots, sclerotia and chlamydospores, suggesting a major role for the *A* activated program in development and light regulation (Kües et al. 1998, 2002). This effect was partially modified by an activated *B* pathway (Kües et al. 2002).

c) Light Transduction Pathway

The primary effect of light on basidiomycetes may be transduced by G proteins. Fruiting body formation in the related basidiomycete *Coprinus congregatus* (*Coprinellus congregatus*), belonging – as does *C. cinereus* – to the Psathyrellaceae, requires light applied to dikaryon mycelia. Membrane extracts from *C. congregatus* contain G proteins showing a light-dependent nucleotide analog binding. In addition, a G-protein α subunit with similarity to transducin is expressed in light-sensitive mycelia. These results suggest a role for G proteins in light-mediated signal transduction (Kozak and Ross 1991; Kozak et al. 1995).

Several blind mutants have been isolated in *C. cinereus* (Muraguchi et al. 1999; Lu 2000) that are blocked in specific light-dependent steps of fruiting body development, without any effect on other light responses. This indicates that *C. cinereus* may use several light transduction pathways. The genome of *C. cinereus*, now available (*C. cinereus* Sequencing Project, Broad Institute of MIT and Harvard, USA, http://www.broad.mit.edu/annotation/fungi/coprinus_cinereus/), holds the possibility of identifying a whole array of photoreceptor genes that could

mediate light regulation in development. The combination of genetics and genome sequence analysis will prove invaluable for the molecular understanding of *C. cinereus* photomorphogenesis. Considering its role as model of other edible basidiomycetes, this line of research could hold an additional commercial value.

2. *Cryptococcus neoformans*

The basidiomycete *Cryptococcus neoformans* is a heterothallic yeast and a human pathogen (Casadevall and Perfect 1998; Hull and Heitman 2002). Blue light inhibits mating and haploid fruiting in *Cryptococcus neoformans* (henceforth, *Cryptococcus*). A close inspection of the *Cryptococcus* genome has allowed scientists to identify several photoreceptor genes, including one gene for an opsin, one gene for a phytochrome, and one gene for the *Neurospora* WC-1 photoreceptor. Each of these genes was disrupted but only the inactivation of the gene similar to *wc-1* showed a blind phenotype, with equal mating reactions in dark and light (Idnurm and Heitman 2005a; Lu et al. 2005). A gene with similarities to the *Neurospora wc-2* gene was identified by two independent approaches: by sequence similarities (Lu et al. 2005), and by the isolation of an insertional mutant with a blind-mating phenotype (Idnurm and Heitman 2005a). Mutations in any of the *Cryptococcus wc* genes resulted in a blind-mating phenotype (Idnurm and Heitman 2005a; Lu et al. 2005), and their overexpression resulted in a stronger light-dependent inhibition of mating (Lu et al. 2005). In addition, mutants in any of the *wc* genes were more sensitive to UV light (Idnurm and Heitman 2005a). The *Cryptococcus* WC-1 protein contains a putative chromophore-binding domain but does not contain a Zn finger domain, unlike the *Neurospora* WC-1. However, a putative Zn finger is present in the *Cryptococcus* WC-2 protein (Idnurm and Heitman 2005a; Lu et al. 2005). The *Cryptococcus wc-1* and *wc-2* genes are expressed at very low levels in the dark but the *wc-2* gene is induced by light (Idnurm and Heitman 2005a; Lu et al. 2005). In addition, the two WC proteins were shown to physically interact in a yeast two-hybrid assay (Idnurm and Heitman 2005a). These results suggest that the *Cryptococcus* WC proteins will form a complex that will bind the promoters of light-regulated genes through the WC-2 Zn finger, in a mode of action similar to the *Neurospora* white collar complex (Idnurm and Heitman 2005a; Lu et al. 2005). Surprisingly, the *Cryptococcus wc* mutants showed reduced virulence in a murine model, indicating that the WC proteins form a complex involved in the regulation of development and virulence in this pathogenic fungus (Idnurm and Heitman 2005a).

III. Gravitropism

The effect of gravity on plants and fungi is usually associated with gravitropism, i.e., the directed growth of elongating organs parallel or antiparallel to the gravity vector. While gravitropism no doubt represents the most apparent gravireaction, gravity exerts a substantial influence on morphogenesis, too – that is, on the shape and form of plants and fungi. One example is the peg formation in seedlings of several plants, including *Mimosa* and *Eucalyptus* and various Cucurbitaceae. The peg is a protuberance, a special hook-like organ, at the base of the hypocotyl that serves to remove the seed coat at the time of germination. That the morphogenesis of this organ is under control of gravity becomes apparent from the observation that clinostatted seedlings of *Cucurbita* develop two, rather than only one peg (Takahashi 1997). Prolonged clinostatting can also induce dramatic changes in flower morphology, as zygomorphic flowers can take on a radial symmetry (Rawitscher 1932). The space-filling pattern and morphology of the plant root system critically depend on the hierarchy of primary, secondary and tertiary roots, which each possess characteristic gravitropic (liminal) setpoint angles (Hart 1990). The important role of gravity for regular morphogenesis has been documented even for fungi. Under conditions of weightlessness, the agaric fungus, *Polyporus brumalis*, for example, develops flattened fruiting bodies with twisted and irregular pedicles (Zharikova et al. 1977), and the cytological fine structure of hyphae from the stem of agaric fungi changes during gravitropic bending (see below).

Graviperception is ubiquitous in the fungal kingdom, and is manifested as gravimorphogenesis and gravitropism. Fruiting bodies usually grow vertically and reorient in a few hours after displacement from the plumb line. Even though the gravitropism of different classes of fungi has been described since more than a century (Table 13.2), the cellular and molecular mechanisms underlying gravioriented growth are still poorly known, and

Table. 13.2. Examples for gravitropism in fungi

Organism	Organ	Gravitropism	Gravisusceptor	Reference
Basidiomycota				
Amanita	Stem	Negative	Unknown	Hofmeister (1863)
	Gill	Positive	Unknown	Moore (1991)
Coprinus	Stem	Negative	Unknown	Knoll (1909)
	Stem	Negative	Cytoplasm?	Gooday (1985)
	Gills	Positive	Unknown	Moore (1991)
Flammulina	Stem	Negative	Nuclei?	Monzer (1996)
	Gill	Positive	Unknown	
Fomes	Stem	Negative	Unknown	Buller (1922)
	Tube	Positive	Unknown	
Psalliota	Stem	Negative	Unknown	Buller (1909)
	Gills	Positive	Unknown	
Polyporus	Gills	Positive	Unknown	Sachs (1879)
Zygomycota				
Phycomyces	Sporangiophore	Negative	Octahedral crystals and lipid globules	Schimek et al. (1999), Grolig et al. (2004)
Pilobolus	Sporangiophore	Negative	Unknown	Horie et al. (1998)
Glomeromycota				
Gigaspora	Hyphae	Negative	Lipid globules?	Döring and Grolig (personal communication)
Glomus	Hyphae	Negative	Lipid globules?	

as a consequence our general understanding has remained largely at a phenomenological level in this field. In plants, graviperception is mediated by statoliths (usually amyloplasts), heavy cell organelles that sediment upon reorientation and that generate potential energy. The search for fungal statoliths (gravisusceptors), although a century old, has only very recently received novel input (see below). As in plant research, the hunt for the hypothetical gravireceptor has, however, remained unsuccessful and will continue.

A. Criteria for Gravisusceptors

Cell organelles or cell inclusions that function as gravisusceptors must be able to generate potential energy that exceeds the thermal noise of the cellular environment. After reorientation of the plant or fungal organ, gravisusceptors generate a gravitropic signal. To achieve this, they must have the potential either to sediment (statoliths) or to float ("buoys"), so that a particle gradient can be formed. To do this, they require a density different from that of the surrounding cytoplasm, and additionally, a critical size to overcome the effect of thermal motion, which counteracts the effect of sedimentation or buoyancy and thus the formation of a particle gradient. Whether or not a cell organelle qualifies as a gravisusceptor can be determined with a function that was originally introduced by Einstein, who modified the Boltzmann distribution by taking into account the earth's gravitational field. The function describes the ratio of sedimenting (or floating) particles separating along a distance, h:

$$N/N_0 = \exp - \left[V(\rho_c - \rho_{gs})gh/kT\right] \qquad (13.1)$$

where N_0 and N are the number of particles separated by a distance h before and after sedimentation (flotation), respectively, V is particle volume, ρ_c and ρ_{gs} are the specific densities (kg/m^3) of the cytoplasm and the gravisusceptor, respectively, g is the constant of gravitational acceleration (9.81 m/s^2), k is the Boltzmann constant (1.38×10^{-23} J/K), and T is absolute temperature (K). A plot of Eq. (13.1) is shown in Fig. 13.3 for $T = 300$ K and $\rho_{gs} = 791$ kg/m^3 (density of lipid globules of *Phycomyces*, see below). A value of $N/N_0 = 1$ indicates that the particles do not separate, so that no gradient is generated; values smaller than 1 indicate effective separation. It is apparent from such a plot that particle separation by sedimentation or by flotation over a distance h of 10 µm occurs at $1 \times g$ only for particles with diameters above 0.8 µm (Grolig et al. 2004). At $0.2 \times g$, particles of diameter >2 µm would

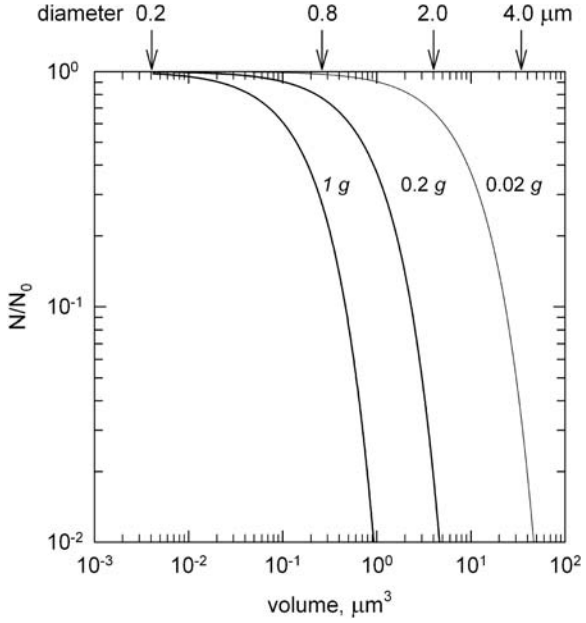

Fig. 13.3. Graphical representation of Eq. (13.1), showing the ratio N/N_0 as a function of the volume of gravisusceptors. N Number of particles per unit volume after sedimentation (flotation), N_0 number of particles per unit volume prior to sedimentation (flotation), h distance of sedimentation (flotation). Equation (13.1) was calculated for sedimenting particles of density $\rho = 1.21$ g/cm^3, or for floating particles of density $\rho = 0.79$ g/cm^3, a distance h of 10 mm, a temperature of 300 K, an assumed density of the cytoplasm of 1 g/cm^3, and for gravitational accelerations of $1 \times g$, $0.2 \times g$ and $0.02 \times g$ (gravitropic threshold of *Phycomyces*). After Grolig et al. (2004)

separate. At $0.02 \times g$, however, which represents the gravitropic threshold of *Phycomyces* (Galland et al. 2004), only particles of diameter >4 µm will form a substantial gradient.

When gravisusceptors sediment or float, they generate a force, F, which can be calculated as:

$$F = g \times V \times n \times (\rho_c - \rho_{gs}) \quad (13.2)$$

where g is the earth's gravitational acceleration (9.81 m/s^2), V the volume of a gravisusceptor, n the number of gravisusceptors, ρ_c the density of the cytoplasm, and ρ_{gs} the density of the gravisusceptor.

The potential energy, E, of a sedimenting or floating gravisusceptor is given by:

$$E = F \times d \quad (13.3)$$

where F is the static force (Newton), and d the distance (m) over which the gravisusceptors are displaced. The potential energy of a gravisusceptor needs to exceed the thermal noise ($3/2kT = 6.21 \times 10^{-21}$ J at 300 K). For example, at the gravitropic threshold of *Phycomyces*, which is near $2 \times 10^{-2} \times g$ (Galland et al. 2004), the potential energy generated by floating lipid globules would amount to 10^{-18} J, which is still 360 times above the thermal noise. The estimated potential energies are also sufficiently high to explain the adherence of *Phycomyces* to the so-called sine law of gravitropism (Galland et al. 2002). For small inclination angles of the sporangiophore of 1–2°, the gravitropic stimuli are according to the sine law 1.7 to $3.4 \times 10^{-2} \times g$, which is just above the absolute gravitropic threshold and thus above the thermal noise (see above). An energy of about 10^{-16} J could be sufficient to open 10^6 mechanosensitive Ca^{2+} channels (Howard et al. 1988). Such considerations appear relevant, in view of the reasonable possibility that the graviperception of fungi may involve ion transport and the requisite channels.

B. Basidiomycota

Gravitropism is ubiquitous among Basidiomycota, and is manifested by the lamellae as well as by the stipe. The lamellae (gills) of the pileus of agarics display positive gravitropism, i.e., they grow parallel to, and in the same direction as the vector of the earth's gravitational acceleration when they are displaced from the plumb line. A displacement of vertical lamellae by as little as 5° can reduce the spore dispersal by some 50%; an inclination angle of 30° may even completely abolish spore dispersal (Buller 1909). Stipes that are inclined bend upward, thus displaying negative gravitropism, a response that *Flammulina velutipes* completes in about 12 h (Kern and Hock 1996). Basidiomycota, such as *Polyporus brumalis* and *Flammulina*, that were cultured during microgravity in satellites or space shuttles displayed disoriented and twisted growth, and sometimes even abnormal fruiting body formation (Zharikova et al. 1977; Kern and Hock 1996).

The gravisensitive zone seems to be restricted to the apex of the stipe (Haindl and Monzer 1994), which in *Coprinus cinereus* comprises the upper 20%–30% (Greening et al. 1997). Gravitropic curvature is caused by differential elongation growth of the upper and lower flanks of the stipe. In *Flammulina*, the outer flank grows at an increased rate while the inner one shows a decreased growth rate (Monzer et al. 1994). In *Coprinus*, the outer flank shows a rapid increase in growth rate while the in-

ner flank shows a delayed increase of growth rate (Greening et al. 1997). In *Flammulina*, the gravitropic bending of horizontally placed stipes is complete in about 12 h (Kern and Hock 1996).

Inner hyphae of stipes contain large vacuoles and high turgor pressure, whereas outer hyphae contain smaller ones and moderate turgor. It is the high turgor of the inner hyphae that causes the elongation growth while the outer hyphae control the differential relaxation that causes differential growth, and thereby gravitropic curvature. Elongation growth of hyphae of the stipe differs from that of mycelial hyphae, in that cell extension occurs along the entire length of the cell; mycelial hyphae, by contrast, display apical growth (Kern and Hock 1996).

Bending stress generated by the weight of the pileus does not elicit gravitropism (Greening et al. 1993). The gravistimulus is perceived even after removal of the cap; the graviperceptive and the graviresponsive zone is thus located in the apex of the stipe (Kern and Hock 1996). However, to maintain elongation growth of the stipe for prolonged periods, the presence of the cap is necessary.

A diffusible growth promoter of unknown chemical identity plays a crucial role in the gravitropism of *Agaricus* and *Flammulina* (Hagimoto 1963; Gruen 1979, 1982). The growth promoter must be water-soluble, because stipes or segments of stipes display gravitropism in air and under silicon oil, but not, however, under water. Imbibition under water abolishes tropism, but not, however, elongation growth (Kern and Hock 1996). In order to elicit gravitropic bending, one has to postulate that the growth promoter forms a gradient in horizontal stipes that increases from the upper to the lower flank. The fact that the upper part of the stipe contains a high amount of water between the hyphae fits this hypothesis well (Kern and Hock 1996). In *Coprinus*, graviperception remains unaffected by the Ca^{2+} channel blockers verapamil or by the calmodulin inhibitor calmidazolium, although the efficiency of gravitropic bending is reduced (Novak-Frazer and Moore 1993).

1. Gravisusceptors

Sedimentation of cell organelles such as observed for plant statoliths (amyloplasts, or barium-sulfate bodies in *Chara* rhizoids) has never been observed in Basidiomycota, and their statoliths have remained elusive. Upon reorientation (inversion) of *Coprinus*, no displacement of glycogen granules could be observed, and it was thus concluded that they do not represent gravisusceptors (Borriss 1934). In horizontal stipes of *Coprinus cinereus*, the cytoplasm of hyphae was displaced to the lower, and vacuoles to the upper part. It is thus feasible that the cytoplasm functions as a gravisusceptor (Gooday 1985). A similar separation was reported for sporangiophores of *Phycomyces* (see below), but not, however, for other Basidiomycota.

In *Flammulina velutipes*, actin filaments play an important role in graviperception. Gravitropic curvature was suppressed by the actin filament-disrupting agent cytochalasin D, but not by the microtubule inhibitor oryzalin (Monzer 1995). The relatively high density of the nuclei (1.2 g/cm^3) and their close association with actin filaments suggest a role for the nuclei as gravisusceptors (Monzer 1996). The density of nuclei of *Flammulina* is lower than that of plant nuclei (1.32) but higher than that of *Neurospora* (1.08). A density of 1.2 is sufficiently high for a potential gravisusceptor Eq. (13.1), and actin-associated nuclei thus represent interesting contenders for gravisusceptors.

Reorientation of stipes of *Flammulina* causes the formation of numerous microvacuoles in the hyphae of the lower flank whereas the upper flank retains a single major vacuole. It is likely that the process of vacuolization is relevant for graviperception, as the process of vacuolization correlates with the differential growth rates of the hyphae of the upper and lower flanks (Kern and Hock 1996).

2. Kinetics, Dose Dependence and Threshold

When *Coprinus cinereus* is placed horizontally, gravitropic bending manifests itself after about 25 min (=reaction time; Kher et al. 1992). The presentation time, i.e., the minimal stimulus time for eliciting a response, for excised and clinostatted stipes of *Coprinus cinereus* is 9.6 min (Hatton and Moore 1992). In plants, the presentation time can be as short as 10–15 s or as long as 4 min, leading to gravitational threshold doses (expressed as the earth acceleration $g \times$time) ranging from 10 to 240 g s (Volkmann and Sievers 1979; Hatton and Moore 1992). The corresponding value for *Coprinus* would be 576 g s. Mechanical stress does not affect the bending reaction (Greening et al. 1993).

3. Gravimorphogenesis

Long-term space experiments have shown that the presence of a gravitational field is of paramount importance for the morphogenesis of plants and fungi. The growth pattern and the morphogenesis of Basidiomycota are severely affected under weightlessness. *Polyporus brumalis* and *Pleurotus ostreatus* raised under weightlessness showed abnormal or no formation of fruiting bodies in darkness, and irradiation-induced fruiting bodies lacked a hymenium. Stipes of *Flammulina* raised under weightlessness were flat, rather than round (Kern and Hock 1996). Similar results were also obtained in long-term space experiments with *Polyporus brumalis*. In weightlessness and darkness, albeit not in light, either the formation of fruiting bodies was arrested, or twisted stems developed that lacked a cap (Zharikova et al. 1977; Kasatkina et al. 1980).

C. Ascomycota

Even a superficial inspection of the various fructification organs of Ascomycota provides evidence for their ubiquitous capacity of graviperception. The upright-growing perithecia of *Neurospora*, the upward orientation of the apothecia of *Pezizazeae*, and the vertical fruiting bodies of *Helvellaceae*, *Helvelloidae* and *Morchellaceae* bear ample witness for gravitropism. In spite of this fact, there exists almost no literature on the graviperception of this group of organisms.

To assess whether single-celled organisms possess the potential to perceive gravity, yeasts were often employed in space experiments under microgravity conditions. Attempts to detect differences in the induction and the repair of DNA lesions under earth and microgravity conditions were without success for *Saccharomyces* (Pross et al. 2000; Takahashi et al. 2001). Cultures of *Saccharomyces* that were subjected to simulated weightlessness in a special apparatus providing low-fluid shear, the "rotating wall vessel bioreactor", displayed substantially altered expression for clusters of genes that were either up- or down-regulated. The genes contained promoter sequences with similarities to the Rap1p transcription factor binding site and the stress responsive element (STRE; Johanson et al. 2002). These experiments provide clues on how physical forces acting on the cell surface could translate into differential gene expression, and thus represent a model for gravimorphogenesis. A phenomenon of gravimorphogenesis was observed in bioreactor cultures of *Saccharomyces cerevisiae* that were maintained for 8 days under microgravity in a space laboratory. The proportion of randomly distributed bud scars was about three times higher in cells subjected to weightlessness (17%) than in those maintained on earth (5%; Walther et al. 1996).

D. Zygomycota

Sporangiophores of *Phycomyces blakesleeanus* and *Pilobolus crystallinus* display negative gravitropism (Horie et al. 1998; Schimek et al. 1999). Mycelial hyphae, zygophores and zygospores attached to suspensors are agravitropic. Sporangiophores of *Phycomyces* that grew in a satellite under weightlessness displayed completely random and disoriented growth (Parfyonov et al. 1979). The effectiveness of gravitropism depends to some extent on the developmental stage. Stage-1 sporangiophores of *Phycomyces*, which lack sporangia, bend gravitropically more slowly than do stage-4 sporangiophores, which possess sporangia (Schimek et al. 1999; Grolig et al. 2004). In *Pilobolus*, gravitropism is almost absent in stage-1, whereas it is well expressed in stage-4 sporangiophores (Horie et al. 1998). The latter observation might be explained by the fact that the growing zone in stage-1 sporangiophores of this fungus is merely 0.3 mm thick, whereas it is about 2–3 mm in *Phycomyces* (Horie et al. 1998).

1. Gravisusceptors

Flexure (bending stress) could potentially play a role in graviperception. Thus, sporangiophores respond to cell wall stress generated by flexure or compression (Dennison 1961, 1964). A unilateral force as low as 0.5 mg elicited a bending response (Dennison and Roth 1967). Stretch elicits also a transient growth response (diminution of growth rate) when sporangiophores are stretched (elongated) by a load of 5 mg; when the load is lifted, a transient increase of growth rate is observed. Both responses are adaptive, so that after a few minutes the growth rate returns to the pre-stimulus level (Dennison and Roth 1967). These data are in agreement with the assumption that stretch (flexure) occurring at the upper side of a horizontal sporangiophore influences gravitropism. The

latencies of the corresponding stretch responses are of about 1-min duration, and thus are much shorter than the gravitropic latencies obtained after reorientation of the sporangiophore.

The gravisusceptors of *Phycomyces* must be internal, because negative gravitropism persists in sporangiophores submerged in water or in fluids with a density exceeding that of the cytoplasm (Dennison 1961). Because cytoplasm sediments and the central vacuoles float slightly in horizontal sporangiophores, they might participate in gravisusception (Dennison and Shropshire 1984). The central vacuoles contain octahedral protein crystals of high density (1.27 g/cm^3) that rapidly sediment upon reorientation of the sporangiophore, and that participate in gravisusception. Mutants lacking these protein crystals are affected in gravitropism (Schimek et al. 1999; Galland et al. 2004). The crystals contain three proteins, and are associated with pterin- and flavin-like pigments (Eibel et al. 2000; Fries et al. 2002).

Beside the protein crystals, also apical lipid globules are involved in gravisusception. In stage-1 sporangiophores of *Phycomyces*, the lipid globules are clustered in a special organelle, a complex of lipid globules (CLG) that resides 110 µm below the apex. When the sporangium is subsequently formed, the lipid globules migrate into the columella. Their role in the columella is presently less clear, but it appears likely that even in this mature stage they function as gravisusceptors. Sporangiophores lacking the CLG display a greatly diminished gravitropic response (Grolig et al. 2004). The sedimentation of the octahedral crystals, and the buoyancy of the lipid globules generate a potential energy that each supersedes the thermal noise by 3–4 orders of magnitude (Schimek et al. 1999; Grolig et al. 2004). The density of the lipid globules of *Phycomyces* (0.79 g/cm^3) is much lower than that commonly found in oleosomes of plant material. The specific density of oleosomes (spherosomes) of peanuts, for example, is 0.92 g/cm^3 (Jack et al. 1967), and that of oleosomes of wheat aleuron can be as high as 1.16–1.18 g/cm^3 (Quail 1979). It is very apparent from such a comparison that the lipid globules of *Phycomyces*, which have diameters comparable to those of plant oleosomes, display traits that are indispensable for gravisusception, whereas those of plants display traits suitable only for storage.

One prominent feature of the lipid globules is the fact that they are in continual non-Brownian motion. The molecular motors are presently unknown, but the fact that they occur in a cage of dense actin mesh indicates that an acto-myosin system powers the motion. The lipid globules contain β-carotene and appear deep yellow. In addition, they carry pterin- and flavin-like pigments that emit blue and green fluorescent light upon excitation (Ogorodnikova et al. 2002; Grolig et al. 2004). Lipid globules (droplets) are ubiquitous among oleaginous fungi (e.g., *Mortierella ramanniana*; Kamisaka et al. 1999), and it appears likely that graviperception mediated by buoyancy might represent a mechanism that is rather widespread in the fungal kingdom.

2. Kinetics, Dose Dependence and Threshold

Sporangiophores of *Phycomyces* that are placed horizontally have a rather irregular gravitropic latency of about 10–30 min, or even longer when the conditions are suboptimal (Dennison 1961; Dennison and Shropshire 1984; Schimek et al. 1999). Gravitropic bending of horizontal sporangiophores is complete in 10–12 h (Schimek et al. 1999). The dose dependence was determined in long-term experiments in a clinostat centrifuge, and the threshold was found to be near $2 \times 10^{-2} \times g$ (Galland et al. 2004). A mutant that lacks the vacuolar protein crystals showed a slightly elevated threshold. Upon reorientation of the sporangiophore, the slowly ensuing bending response of *Phycomyces* is preceded by very fast molecular events that can be monitored spectroscopically. A gravitropic stimulus elicits so-called gravity-induced absorbance changes (GIACs) that occur almost instantaneously, and that are specific for early events of the transduction chain, because they are altered in a gravitropism mutant of genotype *madJ* (Schmidt and Galland 2000, 2004).

3. Cytoskeleton and Calcium

As in higher plants, the cytoskeleton appears to play a major role in the gravitropism of the *Phycomyces* sporangiophore, in which actin, myosin, spectrin and integrin have been immunodetected (Doucette et al. 1994). The apical lipid globules of stage-1 sporangiophores are encased by a dense mesh of actin filaments that fills the dome-like structure of the apex. Inhibitor studies show that it is the actin filaments that cause the non-Brownian motion of the lipid globules (Grolig et al., unpublished data). Injection of stage-4 sporangiophores

with cytochalasin D (inhibiting actin polymerization) causes a 4-h delay of the gravitropic bending, whereas rhodamin-phalloidin (inhibiting actin depolymerization) enhances the gravitropic bending rate, and sometimes also the bending angle (Edwards et al. 1997). Gadolinium chloride, an inhibitor of plant gravitropism and stretch-activated ion channels, delays the onset of gravitropism and diminishes gravitropic curvature. An asymmetric application of gadolinium, Ca^{2+}-chelators, and compound 48/80 (inhibiting calmoduline) to the growing zone of the sporangiophore elicits curvature toward the side to which the inhibitors were applied (Stecker et al. 1990; Edwards 1991). These results suggest that gravitropic curvature requires the participation of the cytoskeleton, and entails a redistribution of Ca^{2+} and calmodulin.

4. Sine Law and Exponential Law

Gravitropic bending of shoots and roots of plants obeys the so-called sine rule or sine law (Sachs 1879), which states that the gravitropic stimulus can be described by the relation:

$$S = g \times \sin \gamma \tag{13.4}$$

where S is the gravitropic stimulus, g the earth gravitational acceleration (9.81 m/s^2), and γ the inclination angle (°) of the plant organ.

Sporangiophores of *Phycomyces* obey this classical sine law (Galland et al. 2002). When sporangiophores are irradiated unilaterally, they bend toward the light source and a photogravitropic equilibrium is established. The photogravitropic bending angle is the result of two antagonistic responses, i.e., positive phototropism and negative gravitropism. The irradiance of unilateral light required to compensate the ensuing gravitropic response is well described by a novel exponential law (Grolig et al. 2000; Galland et al. 2002):

$$I = I_0 \exp -(k_\lambda \, g \sin \gamma) \tag{13.5}$$

where I is the irradiance of the unilateral light that compensates the gravitropic response elicited at an inclination angle γ, I_0 the absolute threshold irradiance (ca. 10^{-9} W/m^2, 450 nm), k_λ a wavelength-dependent constant, g the earth's gravitational acceleration, and γ the inclination angle of the sporangiophore (deviation from the vertical). The exponential law states that the light intensity that compensates a gravitropic stimulus needs to be raised exponentially when the gravitropic stimulus ($g \sin \gamma$) is raised linearly. Because this novel law is valid also for coleoptiles of *Avena*, it appears to describe a universal relationship in the interaction of gravi- and phototropic stimuli (Galland 2002).

5. Gravitropism Mutants

Mutants of *Phycomyces* with defects in the genes *madD,E,F,G,J* are gravitropically partially defective (so-called stiff mutants). They are highly pleiotropic, because they show reduced light-growth and phototropic responses, and a reduced avoidance response (Bergman et al. 1973; Campuzano et al. 1996). Mycelial responses such as photocarotenogenesis and photoinitiation of sporangiophores are, however, unaffected (see above). System analysis employing Wiener white noise or sum-of-sinusoid stimuli showed that these mutants have a lower gain than do the corresponding wild-type strains or mutants that are affected only in the phototropism genes *madA-C* (Lipson 1975; Palit et al. 1989). The threshold for photogravitropic equilibrium is raised at least 6 orders of magnitude, and the corresponding action spectra are highly abnormal, a feature indicating that these mutations directly affect the photoreceptor system (Campuzano et al. 1996). The various features indicate that (1) the photoreceptor system is affected in these gravitropism mutants, (2) photo- and graviperception interact at early steps of the transduction chain, and (3) the *madD,E,F,G* gene products interact with those of the *madA,B,C* gene products.

Another class of mutants with defects in the gene *madH* show enhanced gravitropic and phototropic bending, and also an enhanced avoidance response (Lipson et al. 1983; López-Díaz and Lipson 1983). Although the locations of the corresponding *mad* genes on the genetic map of *Phycomyces* have been determined (Alvarez et al. 1992), their molecular nature remains unknown.

E. Glomeromycota

Hyphae of the endomycorrhizal fungus *Gigaspora margarita* display either negative or positive gravitropism. The germ tubes grow upward (negative gravitropism) whereas secondary (i.e., branching) hyphae, which are the sites for the formation of enichulate vesicles, grow downward (positive gravitropism; Watrud et al. 1977; Hong et al. 2001). Calcium, an important signal element

in the graviperception of plants, plays a substantial role also in the gravitropism of *Gigaspora rosea*, because lanthanum (a Ca^{2+} blocker) and EGTA (a Ca^{2+} chelator) induce hyphal branching, and at the same time also inhibition of gravitropism (Berbara et al. 2002).

The gravisusceptor of these mycorrhizal fungi are presently unknown. In view of the fact that these oleaginous fungi contain numerous lipid droplets, it appears reasonable to assume that the buoyancy of these droplets mediates graviperception. We found that vertically growing hyphae of *Gigaspora margarita* possess an apical complex of lipid globules (CLG) that is similar to the one found in *Phycomyces* (unpublished data). Even in the CLG of *Gigaspora*, the lipid globules are in continual motion. Lateral hyphae growing horizontally do not contain apical lipid globules and are agravitropic. Thus, lipid globules occur only in segments that grow vertically upward. The close correlation between gravitropic orientation and the occurrence of lipid globules clearly indicates their role as gravisusceptors (Döring, unpublished data).

IV. Conclusions

Light and gravity are environmental stimuli responsible for the final appearance of fungi. For many decades, research on fungal photomorphogenesis and gravitropic responses has concentrated on the description and characterization of the responses to light and gravity. Genetics and molecular biology have been employed only with limited examples, but nevertheless with great success. The isolation of blind mutants has allowed the identification of relevant proteins, like the photoreceptor WC-1 from *Neurospora*, confirming the importance of a detailed genetic analysis in the dissection of a photoresponse. Additionally, the *Neurospora* genome sequence has allowed the identification of several putative photoreceptors whose functions are still unknown. The photoreceptor WC-1 binds DNA promoters of light-inducible genes, establishing a very simple transduction chain. Fungal homologs of the WC-1 photoreceptor have been identified in other ascomycetes and basidiomycetes, but their function may not be exactly the same as that of their *Neurospora* counterparts, based on the absence of some functional domains and differences in their pattern of expression. *Neurospora* photobiology is now considered a model for other fungi, but only future research will either confirm this, or will establish differences in fungal photobiology. Undoubtedly, the continuing sequencing of fungal genomes will increase the number of putative elements in light transduction pathways, and these will serve as starting points for future research in fungal photobiology. The combination of physiology with genetics and molecular biology should be employed for a fuller understanding of fungal photomorphogenesis, from the primary photoreception to the final developmental response.

The molecular basis of fungal gravitropism, however, is still rudimentary. Several examples of gravisusceptors and mechanisms for gravity sensing have been suggested and presented here. We hope that the combination of genetics and molecular biology, with detailed cytological characterization of model systems, will help to unravel the complexities of fungal gravitropism. Light and gravity interact in complex ways to direct sporangiophore growth in *Phycomyces*, possibly at the level of the photoreceptor system itself. It is thus possible that light and gravity will share some of the elements of the transduction chain in fungi. Only future research will confirm the possible relationship between light and gravity as major signals for fungal development, growth, and appearance.

References

Adams TH, Wieser JK, Yu JH (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Aguirre J, Ríos-Momberg M, Hewitt D, Hansberg W (2005) Reactive oxygen species and development in microbial eukaryotes. Trends Miocrobiol 13:111–118

Alvarez MI, Benito EP, Campuzano V, Eslava AP (1992) Genetic loci of *Phycomyces blakesleeanus*. In: O'Brien SJ (ed) Genetic maps. Locus maps of complex genomes, 6th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 3120–3126

Ambra R, Grimaldi B, Zamboni S, Filetici P, Macino G, Ballario P (2004) Photomorphogenesis in the hypogeous fungus *Tuber borchii*: isolation and characterization of *Tbwc-1*, the homologue of the blue-light photoreceptor of *Neurospora crassa*. Fungal Genet Biol 41:688–697

Arpaia G, Loros JJ, Dunlap JC, Morelli G, Macino G (1993) The interplay of light and the circadian clock. Independent dual regulation of clock-controlled gene *ccg-2*(*eas*). Plant Physiol 102:1299–1305

Arpaia G, Loros JJ, Dunlap JC, Morelli G, Macino G (1995) Light induction of the clock-controlled gene *ccg-1* is not transduced through the circadian clock in *Neurospora crassa*. Mol Gen Genet 247:157–163

Arpaia G, Cerri F, Baima S, Macino G (1999) Involvement of protein kinase C in the response of *Neurospora crassa* to blue light. Mol Gen Genet 262:314–322

Baima S, Macino G, Morelli G (1991) Photoregulation of the *albino-3* gene in *Neurospora crassa*. J Photochem Photobiol B11:107–115

Ballario P, Macino G (1997) White collar proteins: PASsing the light signal in *Neurospora crassa*. Trends Microbiol 5:458–462

Ballario P, Vittorioso P, Magrelli A, Talora C, Cabibbo A, Macino G (1996) White collar-1, a central regulator of blue light responses in *Neurospora*, is a zinc finger protein. EMBO J 15:1650–1657

Ballario P, Talora C, Galli D, Linden H, Macino G (1998) Roles in dimerization and blue light photoresponse of the PAS and LOV domains of *Neurospora crassa* white collar proteins. Mol Microbiol 29:719–729

Bejarano ER, Avalos J, Lipson ED, Cerdá-Olmedo E (1991) Photoinduced accumulation of carotene in *Phycomyces*. Planta 183:1–9

Bell-Pedersen D (2000) Understanding circadian rhythmicity in *Neurospora crassa*: from behavior to genes and back again. Fungal Genet Biol 29:1–18

Bell-Pedersen D, Dunlap JC, Loros JJ (1996) Distinct cis-acting elements mediate clock, light, and developmental regulation of the *Neurospora crassa eas* (*ccg-2*) gene. Mol Cell Biol 16:513–521

Berbara R, Fonseca H, Daft M (2002) The role of calcium in the germination and germinative hyphal growth of *Gigaspora rosea* Nicolson and Schenk. http://plantbio.berkeley.edu/~bruns/abstracts/berbara

Bergman K, Eslava AP, Cerdá-Olmedo E (1973) Mutants of *Phycomyces* with abnormal phototropism. Mol Gen Genet 123:1–16

Bergo V, Spudich EN, Spudich JL, Rothschild KJ (2002) A Fourier transform infrared study of *Neurospora* rhodopsin: similarities with archaeal rhodopsins. Photochem Photobiol 76:341–349

Berrocal-Tito G, Sametz-Baron L, Eichenberg K, Horwitz BA, Herrera-Estrella A (1999) Rapid blue light regulation of a *Trichoderma harzianum* photolyase gene. J Biol Chem 274:14288–14294

Berrocal-Tito G, Rosales-Saavedra T, Herrera-Estrella A, Horwitz BA (2000) Characterization of blue-light and developmental regulation of the photolyase gene *phr1* in *Trichoderma harzianum*. Photochem Photobiol 71:662–668

Bieszke JA, Braun EL, Bean LE, Kang S, Natvig DO, Borkovich KA (1999a) The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Proc Natl Acad Sci USA 96:8034–8039

Bieszke JA, Spudich EN, Scott KL, Borkovich KA, Spudich JL (1999b) A eukaryotic protein, NOP-1, binds retinal to form an archaeal rhodopsin-like photochemically reactive pigment. Biochemistry 38:14138–14145

Blumenstein A, Vienken K, Tasler R, Frankenberg-Dinkel N, Fischer R (2005) No sex in red light – a role for phytochromes in *Aspergillus nidulans*. Fungal Genet Newslett Suppl 52:42

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Borriss H (1934) Beiträge zur Wachstums- und Entwicklungsphysiologie der Fruchtkörper von *Coprinus lagopus*. Planta 22:28–69

Brown LS, Dioumaev AK, Lanyi JK, Spudich EN, Spudich JL (2001) Photochemical reaction cycle and proton transfers in *Neurospora* rhodopsin. J Biol Chem 276:32495–32505

Buller AHR (1909) Researches on Fungi, vol 1. Longman Green, London

Buller AHR (1922) Researches on Fungi, vol 2. Longman Green, London

Busch S, Eckert SE, Krappmann S, Braus GH (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 49:717–730

Calvo AM, Hinze LL, Gardner HW, Keller NP (1999) Sporogenic effect of polyunsaturated fatty acids on development of *Aspergillus* spp. Appl Environ Microbiol 65:3668–3673

Calvo AM, Gardner HW, Keller NP (2001) Genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans*. J Biol Chem 276:25766–25774

Campuzano V, Galland P, Alvarez MI, Eslava AP (1996) Blue-light receptor requirement for gravitropism, autochemotropism and ethylene response in *Phycomyces*. Photochem Photobiol 63:686–694

Carattoli A, Kato E, Rodriguez-Franco M, Stuart WD, Macino G (1995) A chimeric light-regulated amino acid transport system allows the isolation of blue light regulator (*blr*) mutants of *Neurospora crassa*. Proc Natl Acad Sci USA 92:6612–6616

Carlile MJ (1970) The photoresponses of fungi. In: Halldal P (ed) Photobiology of microorganisms. Wiley, London, pp 309–344

Casadevall A, Perfect JR (1998) *Cryptococcus neoformans*. ASM Press. Washington, DC

Casas-Flores S, Ríos-Momberg M, Bibbins M, Ponce-Noyola P, Herrera-Estrella A (2004) BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. Microbiology 150:3561–3569

Cashmore AR (2003) Cryptochromes: enabling plants and animals to determine circadian time. Cell 114:537–543

Cerda-Olmedo E (2001) *Phycomyces* and the biology of light and color. FEMS Microbiol Rev 25:503–512

Cerdá-Olmedo E, Corrochano LM (2001) Genetics of *Phycomyces* and its responses to light. In: Häder DP, Lebert M (eds) Photomovement. Comprehensive Series in Photosciences, vol 1. Elsevier, Amsterdam, pp 589–620

Cerdá-Olmedo E, Lipson ED (eds) (1987) *Phycomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Chen C, Dickman MB (2002) *Colletotrichum trifolii* TB3 kinase, a COT1 homolog, is light inducible and becomes localized in the nucleus during hyphal elongation. Eukaryot Cell 1:626–633

Cheng P, Yang Y, Heintzen C, Liu Y (2001a) Coiled-coil domain-mediated FRQ-FRQ interaction is essential for its circadian clock function in *Neurospora*. EMBO J 20:101–108

Cheng P, Yang Y, Liu Y (2001b) Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. Proc Natl Acad Sci USA 98:7408–7413

Cheng P, Yang Y, Gardner KH, Liu Y (2002) PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. Mol Cell Biol 22:517–524

Cheng P, Yang Y, Wang L, He Q, Liu Y (2003a) WHITE COLLAR-1, a multifunctional *Neurospora* protein involved in the circadian feedback loops, light sensing, and transcription repression of *wc-2*. J Biol Chem 278:3801–3808

Cheng P, He Q, Yang Y, Wang L, Liu Y (2003b) Functional conservation of light, oxygen, or voltage domains in light sensing. Proc Natl Acad Sci USA 100:5938–5943

Cheng P, He Q, He Q, Wang L, Liu Y (2005) Regulation of the *Neurospora* circadian clock by an RNA helicase. Genes Dev 19:234–241

Collett MA, Dunlap JC, Loros JJ (2001) Circadian clock-specific roles for the light response protein WHITE COLLAR-2. Mol Cell Biol 21:2619–2628

Collett MA, Garceau N, Dunlap JC, Loros JJ (2002) Light and clock expression of the *Neurospora* clock gene *frequency* is differentially driven by but dependent on WHITE COLLAR-2. Genetics 160:149–158

Corrochano LM (2002) Photomorphogenesis in *Phycomyces*: differential display of gene expression by PCR with arbitrary primers. Mol Genet Genomics 267:424–428

Corrochano LM, Cerdá-Olmedo E (1988) Photomorphogenesis in *Phycomyces*: dependence on environmental conditions. Planta 174:309–314

Corrochano LM, Cerdá-Olmedo E (1990) Photomorphogenesis in *Phycomyces*: competence period and stimulus-response relationships. J Photochem Photobiol B5:255–266

Corrochano LM, Cerdá-Olmedo E (1991) Photomorphogenesis in *Phycomyces* and in other fungi. Photochem Photobiol 54:319–327

Corrochano LM, Cerdá-Olmedo E (1992) Sex, light and carotenes: the development of *Phycomyces*. Trends Genet 8:268–274

Corrochano LM, Galland P, Lipson ED, Cerdá-Olmedo E (1988) Photomorphogenesis in *Phycomyces*: fluence-response curves and action spectra. Planta 174:315–320

Corrochano LM, Lauter FR, Ebbole DJ, Yanofsky C (1995) Light and developmental regulation of the gene *con-10* of *Neurospora crassa*. Dev Biol 167:190–200

Crosthwaite SK (2004) Circadian clocks and natural antisense RNA. FEBS Lett 567:49–54

Crosthwaite SK, Loros JJ, Dunlap JC (1995) Light-induced resetting of a circadian clock is mediated by a rapid increase in frequency transcript. Cell 81:1003–1012

Crosthwaite SK, Dunlap JC, Loros JJ (1997) *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origin of circadian rhythmicity. Science 276:763–769

Davis RH (2000) *Neurospora*: contributions of a model organism. Oxford University Press, New York

Davis RH, Perkins DD (2002) *Neurospora*: a model of model microbes. Nat Rev Genet 3:397–403

Degli-Innocenti F, Pohl U, Russo VEA (1983) Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem Photobiol 37:49–51

Denault DL, Loros JJ, Dunlap JC (2001) WC-2 mediates WC-1-FRQ interaction within the PAS protein-linked circadian feedback loop of *Neurospora*. EMBO J 20:109–117

Dennison D (1961) Tropic responses of *Phycomyces* sporangiophores to gravitational and centrifugal stimuli. J Gen Physiol 45:23–38

Dennison D (1964) The effect of light on the geotropic responses of *Phycomyces* sporangiophore. J Gen Physiol 47:651–665

Dennison D, Roth CC (1967) *Phycomyces* sporangiophores: fungal stretch receptors. Science 156:1386–1388

Dennison D, Shropshire W Jr (1984) The gravireceptor of *Phycomyces*: its development following gravity exposure. J Gen Physiol 84:845–859

Doucette A, Voorthuis R, Havrilla M (1994) Cytoskeletal and associated components and calcium binding proteins of *Phycomyces* gravity sensitive sporangiophores. Am Soc Gravitat Space Biol 8:82

Dragovic Z, Tan Y, Gorl M, Roenneberg T, Merrow M (2002) Light reception and circadian behavior in 'blind' and 'clock-less' mutants of *Neurospora crassa*. EMBO J 21:3643–3651

Dunlap JC, Loros JJ (2004) The *Neurospora* circadian system. J Biol Rhythms 19:414–424

Dunlap JC, Loros JJ, Denault D, Lee K, Froehlich A, Colot H, Shi M, Pregueiro A, Ruoff P (2004) Genetics and molecular biology of circadian rhythms. In: Brambl R, Marzluf GA (eds) The Mycota, vol III, 2nd edn. Biochemistry and molecular biology. Springer, Berlin Heidelberg New York, pp 209–232

Edwards KL (1991) The gravitational response of *Phycomyces* with respect to gadolinium and factors affecting calcium function. NASA Space/Gravitational Biology Accomplishments 1989–1990, NASA Tech Mem 4258, pp 32–33

Edwards KL, Chew J, Harris S, di Muzio E (1997) Effects of microinjected cytoskeletal inhibitors on the gravitropic response of the *Phycomyces* sporangiophore. Am Soc Gravitat Space Biol Bull 11:47

Eibel P, Schimek C, Fries V, Grolig F, Schapat T, Schmidt W, Schneckenburger H, Ootaki T, Galland P (2000) Statoliths in *Phycomyces*: characterization of octahedral protein crystals. Fungal Genet Biol 29:211–220

Fischer R, Kües U (2003) Developmental processes in filamentous fungi. In: Prade RA, Bohnert HJ (eds) Genomics of plants and fungi. Dekker, New York, pp 41–118

Flores R, Cerdá-Olmedo E, Corrochano LM (1998) Separate sensory pathways for photomorphogenesis in *Phycomyces*. Photochem Photobiol 67:467–472

Franchi L, Fulci V, Macino G (2005) Protein kinase C modulates light responses in *Neurospora* by regulating the blue light photoreceptor WC-1. Mol Microbiol 56:334–345

Fries V, Krockert T, Grolig F, Galland P (2002) Statoliths in *Phycomyces*: spectrofluorometric characterization of octahedral protein crystals. J Plant Physiol 159:39–47

Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. Science 297:815–819

Froehlich AC, Loros JJ, Dunlap JC (2003) Rhythmic binding of a WHITE COLLAR-containing complex to the frequency promoter is inhibited by FREQUENCY. Proc Natl Acad Sci USA 100:5914–5919

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S et al. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868

Galland P (1990) Phototropism of the *Phycomyces* sporangiophore: a comparison with higher plants. Photochem Photobiol 52:233–248

Galland P (2001) Phototropism in *Phycomyces*. In: Häder DP, Lebert M (eds) Photomovement. Comprehensive Series in Photosciences, vol 1. Elsevier, Amsterdam, pp 621–657

Galland P (2002) Tropisms of *Avena* coleoptiles: sine law for gravitropism, exponential law for photogravitropic equilibrium. Planta 215:779–784

Galland P, Wallacher Y, Finger H, Hannappel M, Tröster S, Bold E, Grolig F (2002) Tropisms in *Phycomyces*: sine law for gravitropism, exponential law for photogravitropic equilibrium. Planta 214:931–938

Galland P, Finger H, Wallacher Y (2004) Gravitropism in *Phycomyces*: threshold determination on a clinostat centrifuge. J Plant Physiol 161:733–739

Gooday GW (1985) Elongation of the stipe of *Coprinus cinereus*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, New York, pp 311–331

Gorovits R, Propheta O, Kolot M, Dombradi V, Oded Yarden O (1999) A mutation within the catalytic domain of COT1 kinase confers changes in the presence of two COT1 isoforms and in Ser/Thr protein kinase and phosphatase activities in *Neurospora crassa*. Fungal Genet Biol 27:264–274

Greene AV, Keller N, Haas H, Bell-Pedersen D (2003) A circadian oscillator in *Aspergillus* spp. regulates daily development and gene expression. Eukaryot Cell 2:231–237

Greening JP, Holden J, Moore D (1993) Distribution of mechanical stress is not involved in regulating stem gravitropism in *Coprinus cinereus*. Mycol Res 97:1001–1004

Greening JP, Sanchez C, Moore D (1997) Coordinated cell elongation alone drives tropic bending in stems of the mushroom fruit body of *Coprinus cinereus*. Can J Bot 75:1174–1181

Gresik M, Kolarova N, Farkas V (1989) Light-stimulated phosphorylation of proteins in cell-free extracts from *Trichoderma viride*. FEBS Lett 248:185–187

Gressel J, Rau W (1983) Photocontrol of fungal development. In: Shropshire W, Mohr H (eds) Encyclopedia of Plant Physiology, new series, vol 16. Springer, Berlin Heidelberg New York, pp 603–639

Griffith GW, Jenkins GI, Milner-White EJ, Clutterbuck AJ (1994) Homology at the amino acid level between plant phytochromes and a regulator of asexual sporulation in *Emericella* (=*Aspergillus*) *nidulans*. Photochem Photobiol 59:252–256

Grolig F, Eibel P, Schimek C, Schapat T, Dennison DS, Galland P (2000) Interaction between phototropism and gravitropism in the sporangiophore of *Phycomyces*. Plant Physiol 123:765–776

Grolig F, Herkenrath H, Pumm T, Gross A, Galland P (2004) Gravity susception by buoyancy: floating lipid globules in sporangiophores of *Phycomyces*. Planta 218:658–667

Gruen HE (1979) Control of rapid stipe elongation by the lamellae in fruit bodies of *Flammulina velutipes*. Can J Bot 57:1131–1135

Gruen HE (1982) Control of stipe elongation by the pileus and mycelium in fruiting bodies of *Flammulina velutipes* and other Agaricales. In: Wells K, Wells EK (eds) Basidium and basidiocarp. Springer, Berlin Heidelberg New York, pp 125–155

Hagimoto H (1963) Studies on the growth of fruit body of fungi. IV. The growth of the fruit body of *Agaricus bisporus* and the economy of the mushroom growth hormone. Bot Mag 76:256–263

Haindl E, Monzer J (1994) Elongation growth and gravitropic curvature in the *Flammulina velutipes* (Agaricales) fruiting body. Exp Mycol 18:150–158

Harding RW, Melles S (1983) Genetic analysis of phototropism of *Neurospora crassa* perithecial beaks using *white collar* and *albino* mutants. Plant Physiol 72:996–1000

Hart JW (1990) Plant tropisms and other growth movements. Unwin Hyman, London

Hatton JP, Moore D (1992) Kinetics of stem gravitropism in *Coprinus cinereus*: determination of presentation time and dosage-response relationships using clinostats. FEMS Microbiol Lett 100:81–86

He Q, Cheng P, Yang Y, Wang L, Gardner KH, Liu Y (2002) White collar-1, a DNA binding transcription factor and a light sensor. Science 297:840–843

He Q, Shu H, Cheng P, Chen S, Wang L, Liu Y (2005) Light-independent phosphorylation of WHITE COLLAR-1 regulates its function in the *Neurospora* circadian negative feedback loop. J Biol Chem 280:17526–17532

Heintzen C, Loros JJ, Dunlap JC (2001) The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. Cell 104:453–464

Hellingwerf KJ (2002) The molecular basis of sensing and responding to light in microorganisms. Antonie van Leeuwenhoek 81:51–59

Hofmeister W (1863) Über die durch die Schwerkraft bestimmten Richtungen von Pflanzentheilen. Jahrb Wissen Bot 3:77–114

Hong Y-X, Ming Z, Gao L-D, Yang XH, Zhen M, Li DG (2001) Hyphal growth of mycorrhizal fungi in time turning vertical plate culture. Mycosystema 20:358–361

Horie T, Schimek C, Fukui J, Miyazaki A, Mihara H, Tsuru T, Maki K, Ootaki T (1998) Developmental stage-dependent response of *Pilobolus crystallinus* sporangiophores to gravitative and centrifugal stimulation. Mycoscience 39:463–470

Horwitz BA, Perlman A, Gressel J (1990) Induction of *Trichoderma* sporulation by nanosecond laser pulses: evidence against cryptochrome cycling. Photochem Photobiol 51:99–104

Howard J, Roberts WM, Hudspeth AJ (1988) Mechanoelectrical transduction by hair cells. Annu Rev Biophys Chem 17:99–124

Hull CM, Heitman J (2002) Genetics of *Cryptococcus neoformans*. Annu Rev Genet 36:557–615

Idnurm A, Heitman J (2005a) Light controls growth and development via a conserved pathway in the fungal kingdom. PLoS Biol 3:e95

Idnurm A, Heitman J (2005b) White collar homologs sense blue light in basidiomycetes and zygomycetes. Fungal Genet Newslett Suppl 52:44

Jack TJ, Yatsu LY, Altschul AM (1967) Isolation and characterization of peanut sherosomes. Plant Physiol 42:585–597

Johanson K, Allen PL, Lewis F, Cubano LA, Hyman LE, Hammond TG (2002) *Saccharomyces cerevisiae* gene expression changes during rotating wall vessel suspension culture. J Appl Physiol 93:2171–2180

Kamada T (2002) Molecular genetics of sexual development in the mushroom *Coprinus cinereus*. Bioessays 24:449–459

Kamisaka Y, Noda N, Sakai T, Kawasaki K (1999) Lipid body formation in an oleaginous fungus, *Mortierella ramanniana* var. *angulispora*. Biochim Biophys Acta 1438:185–198

Kasatkina TB, Zharikova GG, Rubin AB, Palmbakh LR, Vaulina EM, Mashinsky AL (1980) Development of higher fungi under weightlessness. Life Sci Space Res 18:205–211

Kern VD, Hock B (1996) Gravitropismus bei Pilzen. Naturwissen Rdsch 49:174–180

Kertesz-Chaloupková K, Walser PJ, Granado JD, Aebi M, Kües U (1998) Blue light overrides repression of asexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 23:95–109

Kher K, Greening JP, Hatton JP, Frazer LN, Moore D (1992) Kinetics and mechanics of stem gravitropism in *Coprinus cinereus*. Mycol Res 96:817–824

Kim H, Han K, Kim K, Han D, Jahng K, Chae K (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80

Klein RM, Klein DT (1962) Interaction of ionizing and visible radiation in mutation induction in *Neurospora crassa*. Am J Bot 49:870–874

Knoll F (1909) Untersuchungen über Längenwachstum und Geotropismus der Fruchtkörperstiele von *Coprinus stiriacus*. Sitzungsber Kaiserl Akad Wissen Wien, Math-Naturwissen Klasse Abt 1 118:575–634

Kozak KR, Ross IK (1991) Signal transduction in *Coprinus congregatus*: evidence for the involvement of G proteins in blue light photomorphogenesis. Biochem Biophys Res Commun 179:1225–1231

Kozak KR, Foster LM, Ross IK (1995) Cloning and characterization of a G protein α-subunit-encoding gene from the basidiomycete, *Coprinus congregatus*. Gene 163:133–137

Kritsky MS, Russo VE, Filippovich SY, Afanasieva TP, Bachurina GP (2002) The opposed effect of 5-azacytidine and light on the development of reproductive structures in *Neurospora crassa*. Photochem Photobiol 75:79–83

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U, Granado JD, Hermann R, Boulianne RP, Kertesz-Chaloupková K, Aebi M (1998) The *A* mating type and blue light regulate all known differentiation processes in the basidiomycete *Coprinus cinereus*. Mol Gen Genet 260:81–91

Kües U, Walser PJ, Klaus MJ, Aebi M (2002) Influence of activated *A* and *B* mating-type pathways on developmental processes in the basidiomycete *Coprinus cinereus*. Mol Genet Genomics 268:262–271

Kumagai T (1988) Photocontrol of fungal development. Photochem Photobiol 47:889–896

Kumagai T (1989) Temperature and mycochrome system in near-UV light inducible and blue light reversible photoinduction of conidiation in *Alternaria tomato*. Photochem Photobiol 50:793–798

Lakin-Thomas PL, Brody S (2004) Circadian rhythms in microorganisms: new complexities. Annu Rev Microbiol 58:489–519

Lauter FR, Russo VE (1990) Light-induced dephosphorylation of a 33 kDa protein in the wild-type strain of *Neurospora crassa*: the regulatory mutants *wc-1* and *wc-2* are abnormal. J Photochem Photobiol B5:95–103

Lauter FR, Russo VE (1991) Blue light induction of conidiation-specific genes in *Neurospora crassa*. Nucleic Acids Res 19:6883–6886

Lauter FR, Yanofsky C (1993) Day/night and circadian rhythm control of *con* gene expression in *Neurospora*. Proc Natl Acad Sci USA 90:8249–8253

Lauter FR, Russo VE, Yanofsky C (1992) Developmental and light regulation of *eas*, the structural gene for the rodlet protein of *Neurospora*. Genes Dev 6:2373–2381

Lauter FR, Yamashiro CT, Yanofsky C (1997) Light stimulation of conidiation in *Neurospora crassa*: studies with the wild-type strain and mutants *wc-1*, *wc-2* and *acon-2*. J Photochem Photobiol B37:203–211

Lauter FR, Marchfelder U, Russo VEA, Yamashiro CT, Yatzkan E, Yarden O (1998) Photoregulation of *cot-1*, a kinase-encoding gene involved in hyphal growth in *Neurospora crassa*. Fungal Genet Biol 23:300–310

Lee BN, Adams TH (1994) The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev 8:641–651

Lee K, Ebbole DJ (1998) Analysis of two transcription activation elements in the promoter of the developmentally regulated *con-10* gene of *Neurospora crassa*. Fungal Genet Biol 23:259–268

Lee K, Loros JJ, Dunlap JC (2000) Interconnected feedback loops in the *Neurospora* circadian system. Science 289:107–110

Lee K, Dunlap JC, Loros JJ (2003) Roles for WHITE COLLAR-1 in circadian and general photoperception in *Neurospora crassa*. Genetics 163:103–114

Lewis ZA, Correa A, Schwerdtfeger C, Link KL, Xie X, Gomer RH, Thomas T, Ebbole DJ, Bell-Pedersen D (2002) Overexpression of White Collar-1 (WC-1) activates circadian clock-associated genes, but is not sufficient to induce most light-regulated gene expression in *Neurospora crassa*. Mol Microbiol 45:917–931

Lin C, Shalitin D (2003) Cryptochrome structure and signal transduction. Annu Rev Plant Biol 54:469–496

Linden H (2002) A white collar protein senses blue light. Science 297:777–778

Linden H, Macino G (1997) White collar 2, a partner in blue-light signal transduction, controlling expression of light-regulated genes in *Neurospora crassa*. EMBO J 16:98–109

Linden H, Ballario P, Macino G (1997a) Blue light regulation in *Neurospora crassa*. Fungal Genet Biol 22:141–150

Linden H, Rodriguez-Franco M, Macino G (1997b) Mutants of *Neurospora crassa* defective in regulation of blue light perception. Mol Gen Genet 254:111–118

Lipson ED (1975) White noise analysis of *Phycomyces* light-growth response system. III. Photomutants. Biophys J 15:1033–1045

Lipson ED, López-Díaz I, Pollock JA (1983) Mutants of *Phycomyces* with enhanced tropisms. Exp Mycol 7:241–252

Liu Y (2003) Molecular mechanisms of entrainment in the *Neurospora* circadian clock. J Biol Rhythms 18:195–205

López-Díaz I, ED Lipson (1983) Genetic analysis of hypertropic mutants of *Phycomyces*. Mol Gen Genet 190:318–325

Lorca-Pascual JM, Murcia-Flores L, Garre V, Torres-Martinez S, Ruiz-Vazquez RM (2004) The RING-finger domain of the fungal repressor *crgA* is essential for accurate light regulation of carotenogenesis. Mol Microbiol 52:1463–1474

Loros J (2005) A kinase for light and time. Mol Microbiol 56:299–302

Lu BC (2000) The control of meiosis progression in the fungus *Coprinus cinereus* by light/dark cycles. Fungal Genet Biol 31:33–41

Lu YK, Sun KH, Shen WC (2005) Blue light negatively regulates the sexual filamentation via the Cwc1 and Cwc2 proteins in *Cryptococcus neoformans*. Mol Microbiol 56:480–491

Madi L, Ebbole DJ, White BT, Yanofsky C (1994) Mutants of *Neurospora crassa* that alter gene expression and conidia development. Proc Natl Acad Sci USA 91:6226–6230

Madi L, McBride SA, Bailey LA, Ebbole DJ (1997) *rco-3*, a gene involved in glucose transport and conidiation in *Neurospora crassa*. Genetics 146:499–508

Maier J, Ninnemann H (1995) Inhibition of light-dependent photomorphogenesis of sporangiophores from *Phycomyces blakesleeanus* by application of pteridine biosynthesis inhibitors. Photochem Photobiol 61:206–209

Maier J, Hecker R, Rockel P, Ninnemann H (2001) Role of nitric oxide synthase in the light-induced development of sporangiophores in *Phycomyces blakesleeanus*. Plant Physiol 126:1323–1330

Mannhaupt G, Montrone C, Haase D, Mewes HW, Aign V, Hoheisel JD, Fartmann B, Nyakatura G, Kempken F, Maier J et al. (2003) What's in the genome of a filamentous fungus? Analysis of the *Neurospora* genome sequence. Nucleic Acids Res 31:1944–1954

Maurizi MR, Xia D (2004) Protein binding and disruption by Clp/Hsp100 chaperones. Structure 12:175–183

Merrow M, Franchi L, Dragovic Z, Gorl M, Johnson J, Brunner M, Macino G, Roenneberg T (2001a) Circadian regulation of the light input pathway in *Neurospora crassa*. EMBO J 20:307–315

Merrow M, Roenneberg T, Macino G, Franchi L (2001b) A fungus among us: the *Neurospora crassa* circadian system. Semin Cell Dev Biol 12:279–285

Michán S, Lledías F, Hansberg W (2003) Asexual development is increased in *Neurospora crassa cat-3*-null mutant strains. Eukaryot Cell 2:798–808

Monzer J (1995) Actin filaments are involved in cellular graviperception of the basidiomycete *Flammulina velutipes*. Eur J Cell Biol 66:151–156

Monzer J (1996) Cellular graviperception in the basidiomycete *Flammulina velutipes* – can the nuclei serve as fungal statolithes? Eur J Cell Biol 71:216–220

Monzer J, Haindl E, Kern V, Dressel K (1994) Gravitropism of the basidiomycete *Flammulina velutipes*: morphological and physiological aspects of the graviresponse. Exp Mycol 18:17–19

Mooney JL, Yager LN (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Mooney JL, Hassett DE, Yager LN (1990) Genetic analysis of suppressors of the *veA1* mutation in *Aspergillus nidulans*. Genetics 126:869–874

Moore D (1991) Perception and response to gravity in higher fungi – a critical appraisal. New Phytol 117:3–23

Muraguchi H, Takemaru T, Kamada T (1999) Isolation and characterization of developmental variants in fruiting using a homokaryotic fruiting strain of *Coprinus cinereus*. Mycoscience 40:227–233

Navarro E, Ruiz-Perez VL, Torres-Martinez S (2000) Overexpression of the *crgA* gene abolishes light requirement for carotenoid biosynthesis in *Mucor circinelloides*. Eur J Biochem 267:800–807

Navarro E, Lorca-Pascual JM, Quiles-Rosillo MD, Nicolas FE, Garre V, Torres-Martinez S, Ruiz-Vazquez RM (2001) A negative regulator of light-inducible carotenogenesis in *Mucor circinelloides*. Mol Genet Genomics 266:463–470

Ninnemann H (1991) Photostimulation of conidiation in mutants of *Neurospora crassa*. J Photochem Photobiol B9:189–199

Ninnemann H, Maier J (1996) Indications for the occurrence of nitric oxide synthases in fungi and plants and the involvement in photoconidiation of *Neurospora crassa*. Photochem Photobiol 64:393–398

Novak-Frazer L, Moore D (1993) Antagonists and inhibitors of calcium accumulation do not impair gravity perception though they adversely affect the gravitropic responses of *Coprinus cinereus* stipes. Mycol Res 97:11113–11118

Oda K, Hasunuma K (1994) Light signals are transduced to the phosphorylation of 15 kDa proteins in *Neurospora crassa*. FEBS Lett 345:162–166

Oda K, Hasunuma K (1997) Genetic analysis of signal transduction through light-induced protein phosphorylation in *Neurospora crassa* perithecia. Mol Gen Genet 256:593–601

Ogorodnikova U, Sineshchekov O, Galland P (2002) Blue-light perception in stage-1 sporangiophores of *Phycomyces*: a role for apical lipid globules? J Plant Physiol 159:205–209

Ogura Y, Yoshida Y, Ichimura K, Aoyagi C, Yabe N, Hasunuma K (1999) Isolation and characterization of *Neurospora crassa* nucleoside diphosphate kinase NDK-1. Eur J Biochem 266:709–714

Ogura Y, Yoshida Y, Yabe N, Hasunuma K (2001) A point mutation in nucleoside diphosphate kinase results in a deficient light response for perithecial polarity in *Neurospora crassa*. J Biol Chem 276:21228–21234

Palit A, Pratrap P, Lipson ED (1989) System analysis of *Phycomyces* light-growth response: *madC*, *madG*, and *madH* mutants. Biophys J 55:519–526

Parfyonov GP, Platanova RN, Tairbekov MG, Belenev YN, Olkhovenko VP, Rostopshina AV, Oigenblick EA (1979) Biological investigations aboard biosatellite Cosmos-782. Acta Astronaut 6:1235–1238

Perkins DD, Davis RH (2000) *Neurospora* at the millennium. Fungal Genet Biol 3:153–167

Perkins DD, Radford A, Sachs MS (2001) The *Neurospora* compendium. Chromosomal loci. Academic Press, San Diego

Polak E, Hermann R, Kües U, Aebi M (1997) Asexual sporulation in *Coprinus cinereus*: structure and development of oidiophores and oidia in an *Amut Bmut* homokaryon. Fungal Genet Biol 22:112–126

Pross HD, Casares A, Kiefer J (2000) Induction and repair of DNA double-strand breaks under irradiation and microgravity. Rad Res 153:521–525

Puyesky M, Ponce-Noyola P, Horwitz BA, Herrera-Estrella A (1997) Glyceraldehyde-3-phosphate dehydrogenase expression in *Trichoderma harzianum* is repressed during conidiation and mycoparasitism. Microbiology 143:3157–3164

Puyesky M, Benhamou N, Noyola PP, Bauw G, Ziv T, van Montagu M, Herrera-Estrella A, Horwitz BA (1999) Developmental regulation of *cmp1*, a gene encoding a multidomain conidiospore surface protein of *Trichoderma*. Fungal Genet Biol 27:88–99

Quail PH (1979) Plant cell fractionation. Annu Rev Plant Physiol 30:425–484

Quiles-Rosillo MD, Ruiz-Vazquez RM, Torres-Martinez S, Garre V (2005) Light induction of the carotenoid biosynthesis pathway in *Blakeslea trispora*. Fungal Genet Biol 42:141–153

Rawitscher F (1932) Der Geotropismus der Pflanzen. Fischer, Jena

Rodríguez-Romero J, Corrochano LM (2004) The gene for the heat-shock protein HSP100 is induced by blue light and heat shock in the fungus *Phycomyces blakesleeanus*. Curr Genet 46:295–303

Roenneberg T, Merrow M (2001) Seasonality and photoperiodism in fungi. J Biol Rhythms 16:403–414

Sachs J (1879) Über orthotrope und plagiotrope Pflanzen. Arb Bot Inst Würzburg 2:226–284

Sánchez-Murillo RI, Torre-Martínez M, Aguirre-Linares J, Herrera-Estrella A (2004) Light-regulated asexual reproduction in *Paecilomyces fumosoroseus*. Microbiology 150:311–319

Schafer E, Bowle C (2002) Phytochrome-mediated photoperception and signal transduction in higher plants. EMBO Rep 3:1042–1048

Schimek C, Eibel P, Grolig F, Horie T, Ootaki T, Galland P (1999) Gravitropism in *Phycomyces*: a role for sedimenting protein crystals and for floating lipid globules. Planta 210:132–142

Schmidt W, Galland P (2000) Gravity-induced absorbance changes in *Phycomyces*: a novel method for detecting primary reactions of gravitropism. Planta 210:848–852

Schmidt W, Galland P (2004) Optospectroscopic detection of primary reactions associated with the graviperception of *Phycomyces*: effects of micro- and hypergravity. Plant Physiol 135:183–192

Schmoll M, Zeilinger S, Mach RL, Kubicek CP (2004) Cloning of genes expressed early during cellulase induction in *Hypocrea jecorina* by a rapid subtraction hybridization approach. Fungal Genet Biol 41:877–887

Schwerdtfeger C, Linden H (2000) Localization and light-dependent phosphorylation of white collar 1 and 2, the two central components of blue light signaling in *Neurospora crassa*. Eur J Biochem 267:414–422

Schwerdtfeger C, Linden H (2001) Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. Mol Microbiol 39:1080–1087

Schwerdtfeger C, Linden H (2003) VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J 22:4846–4855

Shrode LB, Lewis ZA, White LD, Bell-Pedersen D, Ebbole DJ (2001) *vvd* is required for light adaptation of conidiation-specific genes of *Neurospora crassa*, but not circadian conidiation. Fungal Genet Biol 32:169–181

Springer ML (1993) Genetic control of fungal differentiation: the three sporulation pathways of *Neurospora crassa*. Bioessays 15:365–374

Stecker T, Joiner W, Edwards KL (1990) Inhibitors affecting gravitropism of the *Phycomyces* sporangiophore. Am Soc Gravitat Space Biol 4:98

Takahashi H (1997) Gravimorphogenesis: gravity-regulated formation of the peg in cucumber seedlings. Planta 203:164–169

Takahashi A, Ohnishi K, Takahashi S, Masukawa M, Sekikawa K, Amano T, Nakano T, Nagaoka S, Ohnishi T (2001) The effects of microgravity on induced mutation in *Escherichia coli* and *Saccharomyces cerevisiae*. Adv Space Res 28:555–561

Talora C, Franchi L, Linden H, Ballario P, Macino G (1999) Role of a white collar-1-white collar-2 complex in blue-light signal transduction. EMBO J 18:4961–4968

Tan Y, Merrow M, Roenneberg T (2004) Photoperiodism in *Neurospora crassa*. J Biol Rhythms 19:135–143

Terashima K, Yuki K, Muraguchi H, Akiyama M, Kamada T (2005) The *dst1* gene involved in mushroom photomorphogenesis of *Coprinus cinereus* encodes a putative photoreceptor for blue light. Genetics 171:101–108

Thornton RM (1972) Alternative fruiting pathways in *Phycomyces*. Plant Physiol 49:194–197

Toyota K, Onai K, Nakashima H (2002) A new *wc-1* mutant of *Neurospora crassa* shows unique light sensitivity in the circadian conidiation rhythm. Mol Genet Genomics 268:56–61

Tsolakis G, Parashi E, Galland P, Kotzabasis K (1999) Blue light signaling chains in *Phycomyces*: phototransduction of carotenogenesis and morphogenesis involves distinct protein kinase/phosphatase elements. Fungal Genet Biol 28:201–213

Tsolakis G, Moschonas NK, Galland P, Kotzabasis K (2004) Involvement of G proteins in the mycelial photoresponses of *Phycomyces*. Photochem Photobiol 79:360–370

Volkmann D, Sievers A (1979) Graviperception in multicellular organs. In: Haupt W, Feinleib ME (eds) Encyclopedia of Plant Physiology, vol 7. Physiology of movements. Springer, Berlin Heidelberg New York, pp 573–600

Walther I, Bechler B, Müller O, Hunzinger E, Cogoli A (1996) Cultivation of *Saccharomyces cerevisiae* in a bioreactor in microgravity. J Biotechnol 47:113–127

Watrud LS, Heithaus JJ III, Jaworski EG (1977) Geotropism in the endomycorrhizal fungus *Gigaspora margarita*. Mycologia 70:449–452

Yager LN, Lee HO, Nagle DL, Zimmerman JE (1998) Analysis of *fluG* mutations that affect light-dependent conidiation in *Aspergillus nidulans*. Genetics 149:1777–1786

Yamashiro CT, Ebbole DJ, Lee BU, Brown RE, Bourland C, Madi L, Yanofsky C (1996) Characterization of *rco-1* of *Neurospora crassa*, a pleiotropic gene affecting growth and development that encodes a homolog of *Tup1* of *Saccharomyces cerevisiae*. Mol Cell Biol 16:6218–6228

Yamazaki Y, Kataoka H, Miyazaki A, Watanabe M, Ootaki T (1996) Action spectra for photoinhibition of sexual development in *Phycomyces blakesleeanus*. Photochem Photobiol 64:387–392

Yarden O, Plamann M, Ebbole DJ, Yanofsky C (1992) *cot-1*, a gene required for hyphal elongation in *Neurospora crassa*, encodes a protein kinase. EMBO J 11:2159–2166

Yoshida Y, Hasunuma K (2004) Reactive oxygen species affect photomorphogenesis in *Neurospora crassa*. J Biol Chem 279:6986–6993

Zharikova GG, Rubin AB, Nemchikov AV (1977) Effects of weightlessness, space orientation and light on geotropism and the formation of fruit bodies in higher fungi. Life Sci Space Res 15:291–294

Reproductive Processes

14 Asexual Sporulation in Mycelial Fungi

R. Fischer[1], U. Kües[2]

CONTENTS

I. Introduction

The filamentous growth form is typical for many fungi. By indefinite polarized growth, fungi are able to populate enormous areas of a substrate, even though most of them are non-motile organisms. Renowned are the gigantic *Armillaria* individuals covering several hundreds of hectares of forest grounds in Northern America (Ferguson et al. 2003). However, growth of hyphae requires constant feeding with organic matter from the environment, although the mycelium can cope with short distances of nutrient-depleted areas by means of hyphal transport (Cairney 2005). One strategy with which sessile fungi adapt to conditions of limited resources at a given place is spore formation. Typically, different types of spores, sexually or asexually derived, are generated, depending on the purpose of the spores. Asexual spores are obtained from mitosis (mitospores). Usually, they are produced in high numbers as genetically identical propagules of the individual they come from. Various modes of production and different types of asexual spores have evolved in fungi, emphasizing the high significance they have within fungal life cycles, for fungal life styles and for survival in the environment. Often, asexual spores fulfil not only one distinct function but have several different roles. Asexual spores can serve as a means of reproduction and dispersal to new substratum, for the purpose of mating with other individuals and for perennation as resting bodies under unfavourable conditions (Kendrick 1979a,b; Kirk et al. 2001; see also Andrews and Harris, The Mycota, Vol. IV, Chap. 1).

II. Spore Formation

A. Variability of Spores and Spore Production

Asexual fungal spores are produced by different mechanisms (Kendrick 1979a,b; Esser 2001; Kirk et al. 2001).

1. In thallic spore formation, arthrospores or oidia are generated by fragmentation of pre-existing hyphal filaments. If production of a specific hyphal branch directly precedes fragmentation, spores are also called arthroconidia.

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2. Chlamydospores typically arise as single units endogenously within pre-existing vegetative hyphal cells, by protoplast contraction and formation of an inner, thickened secondary cell wall.
3. Sporangiospores are formed, usually in multiple numbers, by cytoplasmic cleavage within specialized spore mother cells building a sporangium.
4. Conidiospores, or conidia, are exospores which develop externally through localized bulging (budding) and subsequent constriction from a sporogenous cell.

There are many variations in conidia production – Kirk et al. (2001), for example, present schemes for 43 different conidial ontogonies. More generally, holoblastic and enteroblastic conidia are distinguished in terms of whether both cell wall layers or only the inner cell wall layer of the sporogenous cell contribute to the swelling. Enteroblastic phiallidic conidia are produced from a specialized cell called the phialide, by de novo production of a cell wall which is not linked to that of the spore-producing cell. Furthermore, spore production might occur directly in, or on cells of the vegetative mycelium or, alternatively, on sporophores produced as specialized structures on the vegetative mycelium. Based on the types of spores they produce, conidiophores and oidiophores are distinguished. Sporophores might occur individually or in compact groups such as conidiomas (Kendrick 1979a,b; Esser 2001; Kirk et al. 2001).

Fungal spores from different species, or different types of spores from one species usually vary in size and morphology, and they may differ in their nuclear load. Uninucleate spores may be haploid or diploid, or spores may carry two or more genetically identical or different nuclei. Moreover, spores may consist of only one cell or they may be bi- or multicellular (Kendrick 1979a,b; von Arx 1981; Ellis and Ellis 1998; Kirk et al. 2001). Related to their actual functions and to environmental conditions, many spores have melanin or other pigments incorporated in their thickened cell walls. Melanin incorporation gives fungal cells a better rigidity, resistance to lytic enzymes and oxidative stresses, protection against physical stresses such as cold, heat and UV, and protection against antagonistic activities from other organisms (Frederick et al. 1999; Wheeler et al. 2000). In addition, the surface of spores is often made hydrophobic by an outer rodlet layer of hydrophobins, small cysteine-rich hydrophilic secreted proteins which self-assemble into amphipathic films (Kershaw and Talbot 1998; Jeffs et al. 1999; see Chap. 19, this volume, and The Mycota, Vol. 8, Chap. 7), and may also contribute to thermotolerance (Ying and Feng 2004). Wettable spores, by contrast, lack hydrophobins (Ásgeirsdóttir et al. 1997). Spores of the Chytridiomycota are motile by presence of a flagellum (zoospores), whereas spores of all higher fungi lack flagellas and are non-motile (Esser 2001; Kirk et al. 2001).

A single fungal species may form different types of spores at the same time, or under different environmental conditions and/or at different places (von Arx 1981). For example, the cereal rust fungus *Puccinia graminis* produces four different types of asexual spores (distinctively called pycnidiospores, aecidiospores, urediospores and teliospores) which readily serve to demonstrate the wide range of spore features and functions. In the complex life cycle with host changes, the minute hyaline pycnidiospores are unicellular, uninucleate haploid conidiospores with a simple cell wall, acting as spermatia to fertilize hyphae of opposite mating type on the alternate host. From the resulting dikaryon with two haploid nuclei in the cells, aecidiospores arise as a new type of larger, thin-walled, unicellular dikaryotic exospores needed for transfer to the main host. Large, thick-walled, pigmented unicellular dikaryotic uredospores are generated as exospores on the main host for infection of other main hosts and the large two-celled, thick-walled melanized diploid teliospores for hibernation (Esser 2001, Agrios 2005).

In view of the enormous variability of modes of asexual spore production, spore morphologies and other spore features, it is not surprising that these represent commonly used criteria in fungal taxonomy, particularly for those fungi lacking a sexual state (Kendrick 1979a,b; von Arx 1981; Ellis and Ellis 1998). The variability of spores and spore formation within the fungal kingdom is far too broad to possibly be present within a single book chapter. Here, we therefore concentrate on a few model species, preferentially those for which genetic data are also available (Sect. IV.).

B. Ecological Aspects of Spore Production

Many fungal spores are airborne. It is mostly mixtures of spores from saprophytic species and phytopathogens which are detected in the air but spores

of entomopathogens and other animal and human pathogens are also present. Outdoor spore flow seems widely to correlate with indoor spore incidences but, for spores of moulds such as *Aspergillus* and *Penicillium*, numbers indoors may substantially exceed those outdoors. The relative composition of outdoor fungal aerosols is influenced by the location of a place, and also very much by the season of the year and meteorological factors such as temperature (maximum, minimum, mean), wind speed (mean, maximum), relative humidity, rainfall, snow and UV irradiation. The aerial spore contents of some species follow the same reaction pattern to changing climates, whereas others react completely differently (Li and Kendrick 1995, 1996; Angulo-Romero et al. 1999; Marchisio and Airaudi 2001; Troutt and Levetin 2001; Stennett and Beggs 2004; Ulevicius et al. 2004). Control of spore production by physical climate parameters is expedient for timely spore distribution and fungal survival in relation to life styles, availability of growth substrate, competition against other species, and actual needs of dormancy. The pea pathogen *Mycosphaerella pinodes*, for example, produces both asexual and sexual spores in the evening and night hours but specific days of production are differently determined by rainfall (Zhang et al. 2005). By contrast, conidia of the aphid pathogenic zygomycete *Erynia neoaphidis* are released after midnight up to the early morning hours, except on humid, colder days when spore release can be observed also in the late afternoons (Hemmati et al. 2001).

During the day, proportions of UV-sensitive spores commonly fluctuate. At midday and in the afternoon, amounts of UV-resistant spores are high (>50%) whereas in the evening UV-sensitive spores dominate (>90%) in the air (Ulevicius et al. 2004). These results suggest that spores are released according to temporal environmental conditions of the day, i.e. their production needs to be time-controlled. In *Neurospora crassa*, the well-understood circadian clock (see The Mycota, Vol. III, 2nd edn., Chap. 11) ensures the rhythmic appearance of spores at night (Gooch et al. 2004). Spore production in species with modes of spore distribution by animals may also correlate to specific hours of the day. For example, Brodie (1931) demonstrated that *Coprinus cinereus* (*Coprinopsis cinerea*) monokaryons produce abundantly wet, sticky oidia which are distributed by flies. This fungus lives on horse dung, a locally limited substrate, and it relies on the insects to be transferred to fresh horse dung. Since flies are active mostly in the early morning hours, completion of oidia production occurs in the early morning (Polak et al. 1997a; see Sect. IV.D), a time of day ideal also because of the morning dew which will support fast germination on new substrate. Oidia are short-lived, and germination abilities decline strongly already 2–3 days after their proliferation (Hollenstein 1997). These spores can act as spermatia to dikaryotize mycelia of opposite mating type. To this end, they stimulate hyphae to grow towards them, in a process called oidial homing (Kemp 1977). In nature, the main mycelial phase of basidiomycetes is the dikaryon with two different haploid nuclei per cell, which is formed upon fusion of two mating-compatible monokaryons (see Chap. 17, this volume). Delay of nuclear fusion upon formation of basidia within fruiting bodies (Chaps. 19 and 20, this volume) conserves the genetic status of both haploid nuclei within the vegetative mycelium. Simultaneously, the dikaryotic status enables genetic complementation, as typically shown by a diploid nucleus. By the formation of uninucleate haploid oidia, in *C. cinereus* the nuclear association of the dikaryon can break down, and the unaltered haploid nuclei can be released to find new substrate and/or new mating partners. This is advantageous for species survival under climatic conditions in which fruiting bodies cannot be formed. The balance (ratios) between the two possible oidia types formed in a dikaryon is highly in favour of a stronger nucleus. Therefore, from another viewpoint, oidia production on the dikaryon supports a selfish character of haploid nuclei which, in the dikaryon, take advantage of unrelated haploid genomes until these are abandoned in the search for new partners (Polak 1999; Kües 2002; Kües et al. 2002a). Oidia have another fascinating ecological function in the defence of resources. The spores attract not only hyphae of their own species but also hyphae from other species competing for the same limited substrate. Upon fusion, somatic incompatibility reactions are initiated, ending in the killing of the fungal rival (Kemp 1977).

Unit-restriction of individual mycelia because of limited resources, competition for these between individuals, and dynamic heterogeneous environments are clearly all integral factors controlling fungal spore production and dispersal (Gourbière et al. 1999; van Maanen and Gourbière 2000). Pine needles in coniferous litter have been chosen as a model ecosystem for spore dispersal properties from ephemeral and periodically renewed

resources. Pine needle colonisation correlates with spore dispersal. Macro- and microclimate effects, nutritional content of the substrate, and fungal successions and interactions with other organisms in suppressing or promoting spore production are seen to influence the outcome of spore dispersal (Gourbière et al. 1999, 2001; van Maanen et al. 2000; Gourbière and Debouzie 2003), demonstrating how complex even minute ecosystems are for fungal spore formation.

Spore dispersal gradients are usually steep (Gregory 1973; Horn et al. 2001), supporting the idea that the large mass of fungal spores is destined for entering new substrate and/or for long-term survival in a given biotope. Nevertheless, spores have been reported to be transferred over long distances, also across oceans, and to successfully invade new territories (Brown and Hovmøller 2002; Hovmøller et al. 2002).

C. Sexual Versus Asexual Reproduction

Verified by various mechanisms (see Sect. II.2, and The Mycota, Vol. IV, Chap. 1), asexual reproduction is very common in mycelial fungi, and asexuality even defines the class Deuteromycota. The other extreme are fungi, such as *Sordaria macrospora*, which reproduce exclusively sexually (Chap. 16, this volume). However, due to current fungal sequencing programmes, there is mounting evidence that several species previously classified as strictly asexual indeed have the genetic inventory for sexual reproduction (Tzung et al. 2001; Pöggeler 2002; Dyer et al. 2003; Johnson 2003; Wong et al. 2003; Galagan 2005). The opportunistic diploid pathogen *Candida albicans* was long believed not to have a sexual cycle. The key genes for sexual development were, however, found in the genomic sequence of this fungus, which greatly stimulated research in this field (Johnson 2003). In the laboratory, it is now well established that mating and nuclear fusion occur in this species, with formation of tetraploids (Bennett et al. 2005). Several other species considered as asexual were shown to have a mating type locus organisation with two alternate idiomorphs, as is typical for heterothallic ascomycetes (see Chap. 15, this volume). The two mating type idiomorphs of the barley pathogen *Rhynchosporium secalis*, an asexually reproducing species with no known teleomorph, have been found in equal frequencies in populations throughout the world. Such frequency-dependent selection of mating types is consistent with sexual reproduction occurring in nature, too (Linde et al. 2003). It is not clear yet how general these experimental findings are, and whether most, or even all species classified today as being asexual have the ability to reproduce sexually, or do so even in nature.

From an evolutionary point of view, it appears to be advantageous to reproduce not only asexually but also sexually, although sexual reproduction disrupts favourable gene combinations, and requires energy and time from the organism. It was proposed long ago that sexual reproduction increases genetic variation (Weismann 1904). Recently, this hypothesis was nicely tested by using strains of the yeast *Saccharomyces cerevisiae* able to reproduce sexually in competition assays with purely asexual strains in which the sexual cycle was prohibited by two mutations (Goddard et al. 2005). These experiments revealed that sexual strains adapted faster to harsh environmental conditions than asexual ones. In an earlier study with *Aspergillus nidulans*, sexual development was shown to slow down the accumulation of deleterious mutations. Thus, even for a homothallic fungus like *A. nidulans*, propagating both in a sexual and in an asexual reproduction mode, sexual development is advantageous (Bruggeman et al. 2003). Although asexual development can be regarded as very useful for the life of an organism to spread in the environment (Sect. II.B), a combination with sexual development promotes continued existence over evolutionary time.

III. Endogenous and Environmental Factors Trigger Spore Formation

Spore formation is an energy-consuming process, and requires strict regulation of the morphogenetic pathway to most efficiently use resources to reproduce and guarantee the survival of the species. Most spores are destined for distribution through the air and, in such cases, the fungus has to "ensure" that the spores are indeed delivered into the air or produced only when the sporulation structures are exposed to the air. For these reasons, spore generation is regulated by a number of endogenous and environmental factors.

A primary factor for induction of spore formation is the nutritional status of the mycelium

(Adams and Timberlake 1990). Carbon and nitrogen limitation, for example, induces spore formation in *N. crassa* and in *A. nidulans* when grown in aerated liquid culture (Guignard et al. 1984; Skromne et al. 1995; Madi et al. 1997). The phosphate status, in combination with the density of the mycelium, also influences developmental decisions in *A. nidulans.* PhoA, a kinase similar to *S. cerevisiae* Pho85, is involved in integrating these environmental signals and transducing them into morphogenetic pathways (Bussink and Osmani 1998).

A physical prerequisite for asexual sporulation in several fungi is exposure to a water–air interphase. Typically, *N. crassa* and *A. nidulans* do not form spores in submerged culture but only when they are exposed on a substrate surface (Siegel et al. 1968; Rossier et al. 1973; Adams et al. 1988; Springer and Yanofsky 1989). Nothing is known about the sensing of this factor, but it is likely that the redox status and, thus, the concentration of reactive oxygen species (ROS) of the cell changes upon air exposure. Acting as an intracellular antioxidantxs, vitamin E reduces the accumulation of ROS in *A. nidulans* and negatively affects sporulation (Emri et al. 2004). There is also good evidence in *N. crassa* that the amount of ROS plays a critical role during development, probably by affecting light signalling (Peraza and Hansberg 2002; Michan et al. 2003; Yoshida and Hasunua 2004). Starvation-induced sporulation in liquid culture by *N. crassa* is observed only under high oxygen supply through continuous agitation (Maheshwari 1991; Madi et al. 1997). CO_2, by contrast, inhibits normal conidiogenesis already at a concentration of 0.2% (Guignard et al. 1984). Fungal CO_2 sensing is considered one key factor serving to correctly place sporophores into the aerial phase (Sage 2002). Furthermore, in *N. crassa*, humidity plays a role in successful conidiation (Guignard et al. 1984). Raising CO_2 concentrations was shown to suppress conidiation in plant-pathogenic *Alternaria* species whereas an increase in humidity had no negative effect (Smart et al. 1992). In humid *C. cinereus* microslide cultures under micro-aeration (Polak et al. 1997a), oidiophore formation and oidia production arrest as soon as humidity vanishes from the cultures when lifting the cover slip (E. Polak, personal communication).

Another important physical factor which triggers several developmental decisions in fungi is light (see Chap. 13, this volume; Blumenstein et al. 2005). In *N. crassa* and many other fungi, the most effective light quality in asexual sporulation is that of blue light (Kertesz-Chaloupková et al. 1998; Liu et al. 2003). At least in *N. crassa*, blue light also regulates the biological clock which, in turn, controls conidiation (see The Mycota, Vol. III, 2nd edn., Chap. 11). On the other hand, in some ascomycetes including *A. nidulans*, red light appears to be effective (Tan 1974; Mooney and Yager 1990). Because the red-light effect is reversible by far-red light, the system is reminiscent of the plant phytochrome system (see Chap. 13, this volume; Blumenstein et al. 2005). As in *N. crassa*, also *A. nidulans* has a biological clock, but asexual sporulation appears not to be under its control (Greene et al. 2003).

Fungi often form asexual spores over a broad range of temperatures. Sometimes, but not always, there are temperature optima for spore production (Guignard et al. 1984; Kertesz-Chaloupková et al. 1998; Copes and Hendrix 2004; Parra et al. 2004). In *N. crassa*, where temperature variations in the range 4–37 °C had no effect on conidiation (Guignard et al. 1984), there is temperature compensation via the biological clock (Nouwrousian et al. 2003; Dunlap and Loros 2004). Furthermore, the biological clock of *N. crassa* has a pH compensation (Ruoff and Slewa 2002), and pH does not influence the period length of sporulation (Ruoff et al. 2000). By contrast, pH influences on sporulation have been described in some other fungi (for examples, see Murray and Walter 1991; Campbell et al. 1996; Zhang et al. 2001).

In *A. nidulans*, all external factors (depletion of nutrients, light, aeration) influencing developmental decisions are without effect if the fungus is not competent for development. This means that germinated spores need to grow vegetatively at least 18 h before they acquire this developmental competence. The fact that mutants with different competence times can be isolated demonstrates that the phenomenon is genetically controlled (Axelrod et al. 1973). Because this particular time requirement is lacking in, e.g. *N. crassa* (Siegel et al. 1968; Guignard et al. 1984), it will be interesting to see how widespread this phenomenon is.

Several low-molecular weight compounds, self-produced or from the environment, are involved in the regulation of asexual sporulation (see Chap. 11, this volume, and Roncal and Ugalde 2003), and the spectrum of pheromones ranges from terpenoid to fatty acid derivatives. In *A. nidulans*, a system of several interconvertible fatty acids, named PSI (precocious sexual induction) factors, has been described and has recently been studied on a molec-

ular level (Champe et al. 1987; Champe and Simon 1992; Tsitsigiannis et al. 2004a,b).

IV. Genetics of Asexual Spore Formation

A. *Aspergillus nidulans*

1. Cytology

More than 50 years ago, scientists chose *A. nidulans* as a model system to study the biology of eukaryotic cells (Pontecorvo et al. 1953). Asexual spore formation is a phenotype which can be easily observed with the naked eye, and therefore it represents an attractive marker to study the role and nature of developmental genes (Clutterbuck 1969; Figs. 14.1, 14.2). Asexual development starts with a non-differentiated hyphal compartment, which develops into a so-called foot. This cell produces a stalk, without any septum between the two structures. After the stalk has reached a height of about 100 µm, the tip swells into a vesicle. From this structure, up to 70 cells, named metulae, emerge in a budding-like process (Oliver 1972; Mims et al. 1988). Once a nucleus has entered the cell, a septum forms at the base of the metula. Each metula produces two to three phialides, which are also uninucleate (Fischer and Timberlake 1995). The phialides are the spore-producing cells and they generate long chains of conidia, the youngest conidium being at the bottom of the chain. In contrast to hyphal cells, nuclear division and cell division are strictly linked in metulae and phialides, and thus these cells remain uninucleate (Mims et al. 1988). In general, the arrangement of metulae and phialides largely resembles that of pseudohyphae of *S. cerevisiae*, and there is also molecular evidence that the two morphogenetic pathways, conidiophore and pseudohyphal development, share some conserved components (see below; Gimeno et al. 1992).

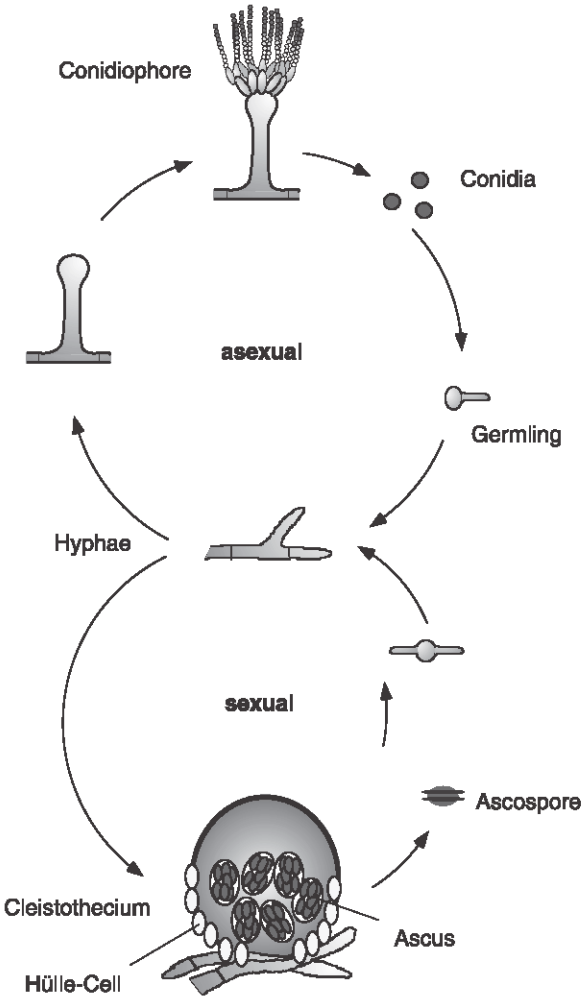

Fig. 14.1. Life cycle of *A. nidulans* (extracted from Scherer and Fischer 1998)

2. Genetics

A. nidulans has been used intensively in mutagenesis approaches, and several developmental mutants have been isolated and studied genetically (Clutterbuck 1969; Fig. 14.3). In the early 1980s, a transformation system was established, and shortly thereafter the first developmental regulator, the *brlA* gene, was cloned (Balance et al. 1983; Johnstone et al. 1985). Since then, many molecular components have been studied, and thus asexual sporulation of *A. nidulans* has become one of the best-studied morphogenetic processes in mycelial fungi (Adams et al. 1998; Fischer and Kües 2003). The isolation of regulators and developmentally regulated genes facilitated the detailed analysis of gene interactions. Known molecular components are introduced below not in chronological order but rather by functional categories. In general, genes may be sorted into three categories: genes whose deletion affects only asexual development, those whose deletion affects only sexual development, and a large group of genes whose deletion affects both asexual and sexual development. In addition, it has become clear that both spore-producing pathways are linked to vegetative hyphal growth.

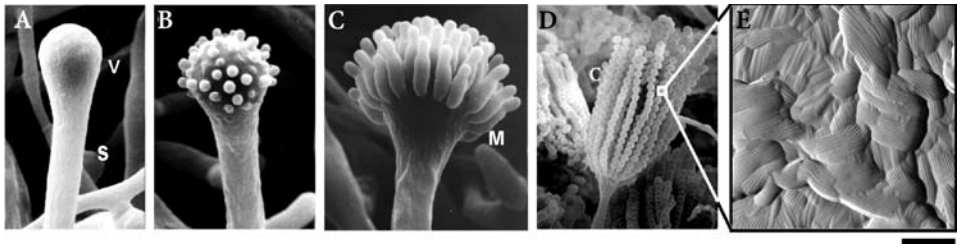

Fig. 14.2. A–E Different stages of conidiophore formation in *A. nidulans*. In **A**, a stalk (*S*) swells at the tip and forms a vesicle (*V*) from which several metulae (*M*) bud off (**B**). The small buds enlarge fairly synchronously and form the first mono-nucleate cell, the metula (**C**). In **D**, metulae produce two to three phialides, which themselves generate the long chains of conidia (*C*). Conidia are well prepared for dispersion. The spore surface is covered with a rodlet layer of hydrophobins (**E**). SEM images (**A–C**) were extracted from Fischer and Timberlake (1995). **D** was provided by R. Weber (marburg), and the rodlets (**E**) were visualized by atomic force microscopy by D. Veith (Karlsruhe). The scale bar represents 10 µm in **A–C**, 100 µm in **D**, and 0.25 µm in **E**

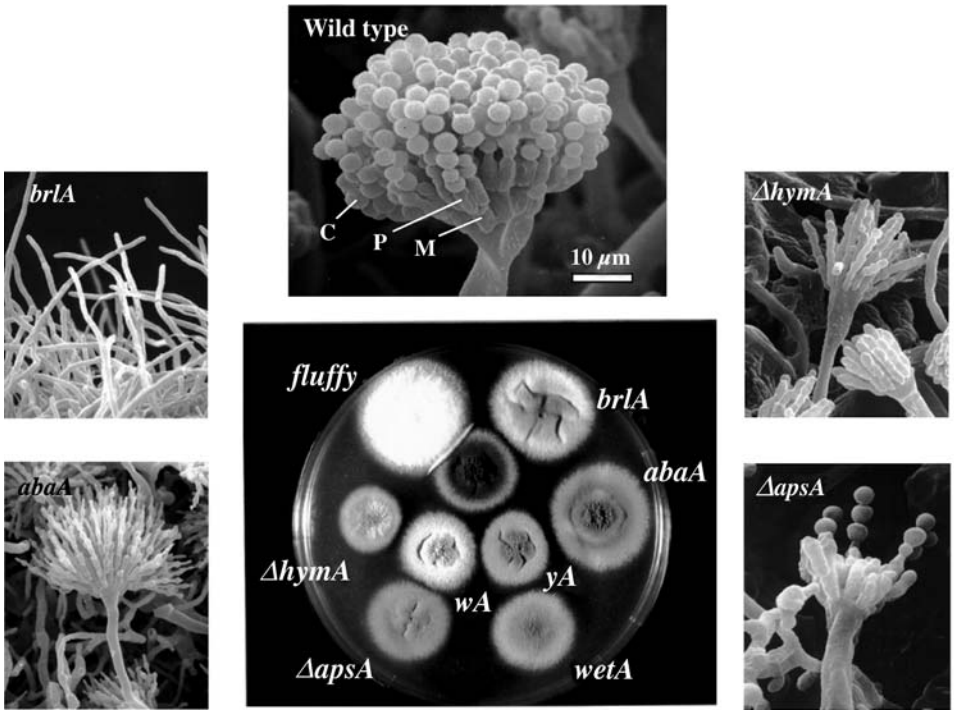

Fig. 14.3. Developmental mutant collection of *A. nidulans* (extracted from Fischer and Kües 2003)

a) Seven-Transmembrane and Other Signal Receptors

Signals, such as pheromones, are perceived at the cell surface, and thus they need to be transduced into, and through the cytoplasm. Seven-transmembrane receptors coupled to heterotrimeric G-proteins are common signalling modules for this process. In a systematic, reverse-genetic approach, the group of Yu identified nine putative seven-transmembrane *G-p*rotein *c*oupled *r*eceptors (GPCR) in the genome of *A. nidulans*, and subsequently generated loss-of-function mutations for six of these (Han et al. 2004). Deletion of *gprD* caused a severe reduction in vegetative growth and asexual development, and a massive induction of sexual development. Interestingly, environmental conditions (e.g. high salt concentrations) which repress sexual development, or mutations (e.g. Δ*nsdD*) which inhibit sexual development restored hyphal growth and asexual spore production. This is a clear example that the three morphogenetic

pathways are interconnected. By comparison, deletion of *gprA* and *gprB* affected neither asexual development nor hyphal growth, but did affect self-fertilization (Seo et al. 2004). Functions for the remaining six GPCRs have not yet been assigned.

In addition to seven-transmembrane receptors, receptors for light perception have been characterized to some extent. Two homologues of the *N. crassa* WC proteins ("white collar proteins"), acting as photoreceptors for the biological clock (Liu et al. 2003; see Chap. 13, this volume, and The Mycota, Vol. III, 2nd edn., Chap. 11), were characterized in *A. nidulans*, although deletion of the genes did not result in an obvious phenotype (H. Haas, Innsbruck, Austria, personal communication). By contrast, deletion of a phytochrome-encoding gene resulted in a slight derepression of sexual development (cf. Chap. 13, this volume; Blumenstein et al. 2005). *A. nidulans* also contains an *opsin*-related gene, whose functional analysis is under way (Schaaf and Fischer, unpublished data).

b) Heterotrimeric G-Proteins

The first indication for the involvement of G-proteins in the regulation of asexual development came from the analysis of the *fluffy* mutant *flbA* (Lee and Adams 1994a; Yu et al. 1996; Fig. 14.4). The corresponding gene encodes a *r*egulator of *G*-protein *s*ignalling (RGS protein), and negatively controls vegetative growth signalling. Mutation of the gene causes massive hyphal growth and a loss of asexual and sexual spore formation. RGS proteins contain a domain of about 130 amino acids, which interact with the switch regions of activated Gα subunits and subsequently act as GTPase-activating proteins (Chidiac and Roy 2003). Hyphal growth is so strongly stimulated in *flbA* mutants that hyphae even autolyze after prolonged incubation (Fig. 14.4). In an approach to isolate dominant mutations causing a similar autolytic phenotype, the group of Adams isolated the *fadA* (*fluffy autolytic dominant A*) mutant, which turned out to encode an α subunit of a heterotrimeric G-protein (Yu et al. 1996). The mutation was a conversion of glycine 42 to arginine, which supposedly causes a loss of GTPase activity. Deletion of *fadA* caused a reduction of vegetative growth and a stimulation of asexual sporulation. Subsequently, in a screening for extragenic suppressors of *flbA* signalling, *sfaD* was discovered, and was found to encode the β subunit (Rosén et al. 1999; Yu et al. 1999). Deletion of the gene caused hyperactive sporulation and

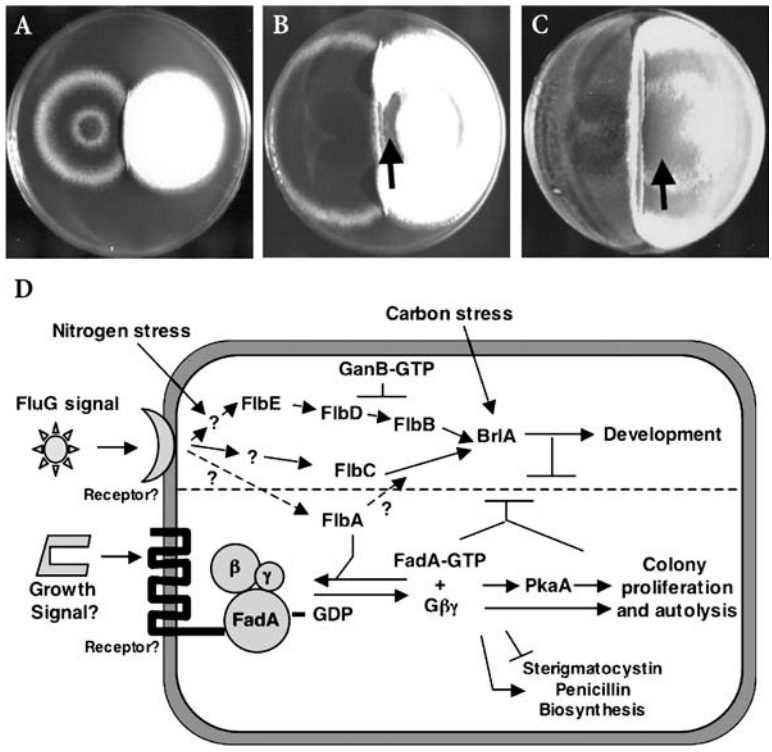

Fig. 14.4A–D *fluffy* mutants are characterized by a cotton-like appearance on agar plates. In **A**, a wild type (*left colony*) and a *fluffy* mutant (*right colony*) were inoculated on an agar plate and incubated for 3 days at 37 °C. **B** One class of *fluffy* mutants undergoes lysis of the hyphae after prolonged incubation. After 5–6 days, lysis is obvious in the middle of the colony (*arrow*), and **C** after 8 days almost all mycelium disappeared. **D** Diagram showing the regulatory interactions of some of the *fluffy* genes and the downstream components. Two antagonistic signalling pathways appear to regulate *A. nidulans* growth and development. Growth signalling is mediated by the FadA G-protein α subunit. Activation of FadA by exchange of GDP for GTP results in a proliferative phenotype and blocks sporulation. To induce conidiation, FlbA has to be activated, which in turn deactivates the growth signalling and favours the differentiation pathway. **A–C** were extracted from Fischer and Kües (2003). **D** was modified after Adams et al. (1998) and supplemented with additional data by J. Yu (Madison, USA)

a reduction of growth rate. These results show that the FadA-dependent hyphal growth signalling pathway has to be switched off, or at least the activity has to be down-regulated in order to allow asexual development to take place. In addition to FadA, two other Gα subunits were identified in the *A. nidulans* genome (Chang et al. 2004). Deletion of *ganB* caused conidiation in submerged culture, a condition under which sporulation is normally repressed. Likewise, constitutive activation of the protein caused a defect in conidiation. GanB thus appears to negatively control asexual development. It has not yet been discovered with which receptors the G-proteins may interact, and also the nature of the signals which are transduced through FadA, GanA or GanB needs to be investigated. In the case of FadA, it is possible that a low-molecular weight compound is the upstream signal, because several compounds are known to trigger developmental decisions in *A. nidulans* (see Chap. 11, this volume). In the case of GanB, Chang et al. (2004) speculate that it could be involved in carbon source sensing, because *ganB* deletion mutants germinate even in the absence of a carbon source. It will be the challenge of the near future to unravel the interconnection and interdependencies between the external signals, the GPCRs, and the G-proteins.

The analysis of *fluffy* mutants revealed another very interesting aspect, this being the regulation of development through low-molecular weight compounds. A gene was isolated, *fluG*, which encodes a protein with some similarity to prokaryotic glutamine synthetases (Lee and Adams 1994b). The *fluffy* phenotype of *fluG* mutants could be rescued by neighbour colonies, and this effect occurred even through dialysis membranes. These results suggest that a low-molecular weight molecule is absent in the mutant. The nature of the molecule has not yet been determined (see Chap. 11, this volume).

c) MAP-Kinase and Other Signalling Modules

MAP-kinase modules consist of three serine/threonine protein kinases which are sequentially activated by phosphorylation, and eventually lead to the phosphorylation of target proteins (Lengeler et al. 2000). In turn, these can regulate transcription, the cell cycle, or other cellular processes. Over the past decade, the role and functioning of MAP-kinase pathways was unravelled in a number of fungi, e.g. *S. cerevisiae* and *Ustilago maydis* (see Chap. 18, this volume). In *A. nidulans*, one MAP-kinase, SakA, was characterized which is transiently activated in early conidiogenesis, and involved in heat-shock, osmotic and oxidative stress responses (Han and Prade 2002, Kawasaki et al. 2002). Deletion of the gene resulted also in changes in cellular morphogenesis, in stress-sensitive conidia and in premature sexual development, indicating an involvement in different morphogenetic pathways. Another MAP-kinase, MpkA, appears to be involved mainly in the germination of asexual spores and in hyphal morphogenesis (Bussink and Osmani 1999). In addition to MAP-kinases, a MAPKKK, named SteC, was identified serendipitously. Deletion of the gene caused pleiotropic phenotypes, suggesting the involvement of this signalling module in several morphogenetic pathways. Δ*steC A. nidulans* strains failed to form heterokaryons and displayed a block in cleistothecium development. In addition, the gene is required for correct conidiophore and spore development (Wei et al. 2003). In about 2% of the conidiophores, secondary conidiophores developed on top of existing conidiophores, and metulae often did not mature but rather produced hyphal-like structures. Interestingly, an up-regulation of transcription was observed in metulae and phialides. This developmental stage has been compared with pseudohyphal growth of *S. cerevisiae* (see Chap. 1, this volume). The expression pattern and the mutant phenotype at the metula stage suggest a specific function during the time period that StuA and AbaA are active (see Sect. IV.A.2.f). Interestingly, *S. cerevisiae* homologues of StuA (Phd1) and of AbaA (Tec1) are required in pseudohyphal development, and are likely to be a target of cAMP-dependent protein kinase (Phd1) and a MAP-kinase cascade (Tec1; Gimeno and Fink 1994; Gavrias et al. 1996; Gancedo 2001; Chou et al. 2004). It will be interesting to see whether the activities of StuA or AbaA are fine-tuned by posttranscriptional regulation.

Another fundamental signalling module in the regulation of eukaryotic development is the COP9 signalosome. This well-conserved multiprotein complex was recently also discovered in *A. nidulans*, using an insertional mutagenesis approach which inactivated the gene for the COP9 component CsnD (Busch et al. 2003). Deletion of subunits of the COP9 multiprotein complex (CsnD, CsnE) caused a pleiotropic phenotype with defects in cell morphology, cleistothecium maturation

and conidiophore number. Similarly to the case for the MAP-kinases, it is not yet clear how the COP9 signalling module interacts with regulators of asexual development.

d) cAMP Signalling

Cyclic AMP is an important secondary messenger in pro- and eukaryotic cells, and its level is tightly controlled. Our knowledge of the role of cAMP in fungi is most advanced in *S. cerevisiae*, *Magnaporthe grisea* and *U. maydis*, in which cAMP signalling triggers development together with a MAP-kinase signalling module (see Chap. 18, this volume). The central enzyme which catalyses the formation of cAMP is adenylate cyclase, whose enzymatic activity is regulated by an upstream G-protein and which regulates, through the cAMP level, the activity of downstream protein kinase A (PkaA). cAMP binding to the regulatory subunit of PKA causes its dissociation from the catalytic subunit (PKAc). Active PKAc controls protein activities via phosphorylation of conserved Ser and Thr residues. Initial work on asexual development in *A. nidulans* already suggested that the cAMP level could play a role (Clutterbuck 1975). However, it was unclear whether the observed drop in cAMP concentration at the onset of development was the cause of conidiation, or whether this was due to an independent phenomenon linked to metabolic alterations. The adenylate cyclase gene, *cyaA*, has recently been characterized, and deletion of the gene caused severe germination phenotypes (Fillinger et al. 2002). Because of the severe impact of *cyaA* deletion on hyphal growth and colony development, a specific involvement of cAMP in the regulation of asexual development was not clearly established in the study of Fillinger et al. (2002). Better evidence for such an involvement came from the analysis of the catalytic subunit of protein kinase A, by deletion and overexpression of the gene (Shimizu and Keller 2001). Interestingly, PkaA itself appears to control the cAMP level (Fillinger et al. 2002). Whereas the lack of the PkaA catalytic subunit led to a reduced growth rate and a hypersporulation phenotype, overexpression caused an increase of aerial, *fluffy* mycelium (Shimizu and Keller 2001). These phenotypes resemble to some extent those of a *fadA* deletion or the overexpression of a dominant active allele of FadA respectively (see above). These results suggest genetic interaction between the FlbA-FadA signalling and the PkaA signalling pathways.

e) Small G-Proteins

This large superfamily of proteins has received some attention over the past years, because they are known as important regulators in higher and lower eukaryotes. The superfamily is divided into several classes, three of which are the Rho-, Ras- and Rac-type proteins. Whereas Rho and Ras proteins have been extensively studied in *S. cerevisiae* and *Schizosaccharomyces pombe*, Rac proteins are not found in these two yeasts, but rather in mycelial fungi (Boyce et al. 2003). Small G-proteins are able to bind GTP or GDP, and the GTP-bound form is the active species. The protein contains an intrinsic GTPase activity which converts triphosphate into diphosphate. The balance between the GTP and GDP form is crucial for signalling, and controlled by a number of different proteins. In *A. nidulans*, a Ras protein was identified by cross-hybridisation to the *S. cerevisiae* homologue (Som and Kolaparthi 1994). Whereas deletion of the gene was lethal, overexpression did not cause any obvious phenotype. However, the role of the protein can be studied by generating constitutively active or dominant negative alleles. The first type of mutation can be achieved by abolishing the endogenous GTPase activity, and thus the protein will be locked in the GTP-bound form. The second variety is blocked in the GDP-bound form. Overexpression of the different varieties led to the conclusion that active Ras is required at different developmental stages, and that the thresholds for active Ras are different (Som and Kolaparthi 1994; Fillinger et al. 2002). In *Aspergillus fumigatus*, the role of Ras is similar to that in *A. nidulans*, although in the pathogen two *ras* genes were identified. Dominant negative RasB caused conidiation in submersed culture, and dominant active RasA led to a reduction in conidiation (Fortwendel et al. 2004). Taken together, phenotypes of *ras* mutants are pleiotropic, and the exact interactions of Ras with the conidiation programme are not known yet. Similarly, the Rho-type small G-protein, Cdc42, appears to play several roles during the life cycle of *A. nidulans*, and is especially important for cell polarity (Boyce et al. 2003; Harris and Momany 2004; see Chap. 1, this volume).

Although not all components of the signalling cascades have yet been identified in *A. nidulans*, individual components known to date show evidence for the involvement of these pathways. The prime interest will now be the investigation of the signals feeding into the pathways, and the cellular reac-

tions at the end of the pathways. The isolation of more components of these same cascades and their detailed study will probably largely reproduce what is already known from other organisms. Therefore, it will be important to identify modifications and requirements specific for *A. nidulans* sporulation.

f) Transcription Factors and Other Regulators

Two of the most prominent transcription factors involved in asexual sporulation in *A. nidulans* are the Cys_2-His_2 Zn(II) finger transcription factor BrlA, and the ATTS/TEA DNA-binding domain transcriptional regulator AbaA. They were both identified in genetic screenings almost 40 years ago (Clutterbuck 1969; Fig. 14.3). Both corresponding mutant strains initiate the developmental programme of the wild type but fail to produce mature conidiospores. *brlA* mutant strains fail to proceed from stalk to vesicle formation, and thus the aberrant conidiophore resembles a *bristle* (Mirabito et al. 1989; Prade and Timberlake 1993). *brlA* represents one of the few genes which are necessary and sufficient for conidiation (Adams et al. 1988). Deletion of the gene prevents conidiaton from taking place, and overexpression of *brlA* in liquid culture leads to the induction of developmental genes, a block of vegetative growth, and the production of phialide-like structures and some conidia (Mirabito et al. 1989; Sewall 1994). Complex conidiophores, however, are not produced. The *brlA* gene locus is quite complex, because it encodes two overlapping transcripts and the derived proteins, BrlAα and BrlAβ, differ in 23 amino acids at the N terminus (cf. BrlAβ has the additional amino acids; Fig. 14.5). One indication for the complex transcriptional regulation may be the relatively large promoter region of about 2 kb (Han and Adams 2001). The expression of BrlAβ is not transcriptionally regulated during development, but depends on a micro-open reading frame in the upstream non-translated region (Han et al. 1993; Timberlake 1993). Factors required for the release of the translational block are not known yet. In a heterologous yeast system, Chang and Timberlake (1992) were able to define a Brl-response element (BRE) in the promoter of the *rodA* gene. It is not clear yet whether BrlAα and BrlAβ regulate different target genes.

In *abaA* mutants, development proceeds to the phialide stage (Mirabito et al. 1989; Andrianopoulos and Timberlake 1994; Fig. 14.3). These spore-producing cells fail to do so, and rather produce hypha-like structures with swellings along the hypha. These structures resemble a mechanical calculator, an *abacus*. After initial cloning and functional characterization of the *brlA* and *abaA* genes, the AbaA protein was successfully expressed in *Escherichia coli*, and promoter-binding studies were carried out (Andrianopoulos and

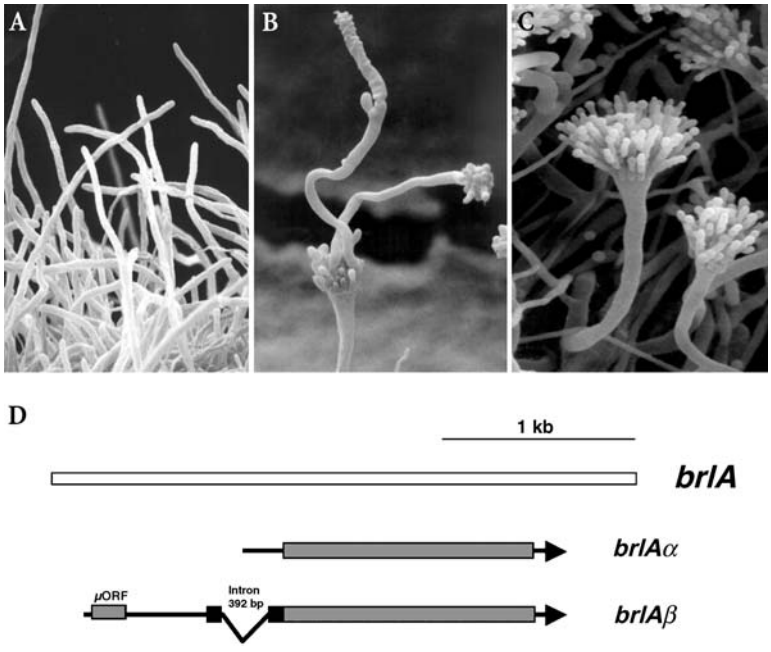

Fig. 14.5. A–D The *brlA* gene. **A** In *brlA* deletion mutants, only elongated stalks are formed. **B** In *brlA*β mutants, secondary conidiophores may arise from the vesicles of aberrant primary conidiophores. **C** In *brlA*α mutants, development proceeds further but conidia are not produced. **D** Diagram of the *brlA* locus. The transcripts are indicated with an *arrow* and the open reading frames are shown by *shaded boxes*. The N-terminal extension of the BrlAβ protein, disrupted by the intron, is indicated by a *closed box*. Modified after Prade and Timberlake (1993)

Timberlake 1994). These experiments defined the AbaA-binding sequence 5′-CATTCY-3′, where Y is a pyrimidine. They furthermore revealed direct evidence for an interaction between AbaA and several developmental genes, among which were structural genes (*rodA*, *yA*) as well as the regulators *brlA*, *wetA*, and *abaA* itself. This suggests that AbaA establishes a feedback regulatory role, which reinforces the expression of the key regulators and thus leads to fast progression of development (Fig. 14.6). It has not yet been established whether AbaA is also posttranscriptionally regulated, although there is accumulating evidence for this (see above; Schier et al. 2001). A homologue of AbaA in *S. cerevisiae*, Tec1, is required for the pseudohyphal switch (see above; Gavrias et al. 1996). Another recently identified gene with some function at the stage of phialide formation is *phiA* (Melin et al. 2003). The protein has some similarity with the *S. cerevisiae* cell wall protein Cwp1 (Melin et al. 1999), but its biochemical function has yet to be determined.

The third component of the central regulatory cascade is *wetA*, whose molecular function is less clear than that of *brlA* and *abaA*. It controls spore maturation and, in *wetA* mutant strains, spores lyze and droplets of cytoplasmic fluid are visible on top of the conidiophore heads (Sewall et al. 1990). There is some evidence that it regulates the expression of downstream genes such as *wA*, although there is no sequence motif suggesting that WetA is a DNA-binding protein or transcription factor (Marshall and Timberlake 1991).

The role and function of a basic helix-loop-helix transcriptional regulator of the APSES protein family, StuA, was studied in *A. nidulans* in similar detail as for BrlA and AbaA (Miller et al. 1992; Dutton et al. 1997). The APSES family is named after four StuA-related proteins in different fungi – *A*sm-1 (*N. crassa*), *P*hd1p (*S. cerevisiae*), *S*tuA (*A. nidulans*), *E*fg1p (*C. albicans*) and *S*ok2p (*S. cerevisiae*; Aramayo et al. 1996) – and is characterized by an APSES DNA-binding domain (Dutton et al. 1997). The gene name *stuA* reflects the short conidiophores from which some conidiospores are generated, without the employment of special cells such as metulae or phialides (Clutterbuck 1969). However, the *stuA* mutation not only affects asexual reproduction but corresponding strains are also self-sterile. *stuA* gene expression is driven from a large, 3-kb-long promoter and depends on BrlA (Wu and Miller 1997), and the large 5′ non-translated region of 1 kb suggests posttranscriptional regulation (Miller et al. 1992). Interestingly, the transcript level is not developmentally regulated, but the gene is induced during the time period in which the mycelium acquires developmental competence (Miller et al. 1991), and encodes – similarly to the *brlA* locus – two overlapping transcripts (Miller et al. 1992). The role of the two transcripts is less clear than in the case of *brlA*, because the former encode the same StuA polypeptide. The StuA protein is responsible for the correct spatial expression of the AbaA regulator. Whereas AbaA expression in *A. nidulans* wild type is restricted to metulae, phialides and immature conidia, this expression pattern changes in *stuA* null mutants, in which *abaA* expression was recorded in somatic hyphae and all cell types of the conidiophores (Miller et al. 1992). In *S. cerevisiae* and *C. albicans*, the StuA homologues are involved in pseudohyphal or hyphal growth (Gimeno and Fink 1994; Stoldt et al. 1997).

Another gene with an impact on the expression of the central transcriptional regulators BrlA and AbaA is *medA*. This gene name was given because of the morphology of the conidiophore, resembling *Medusa* in Greek mythology (Clutterbuck 1969). The conidiophores consist of branching chains of

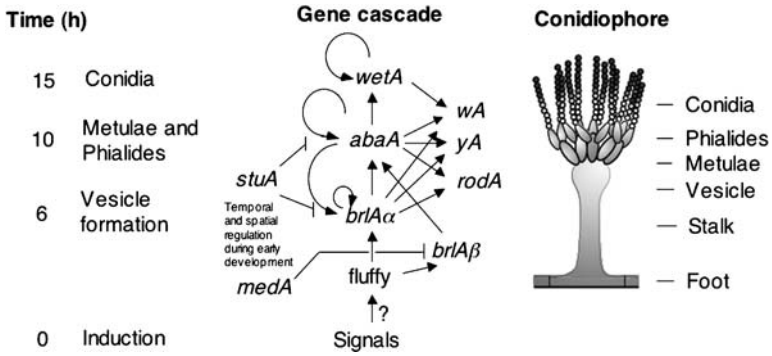

Fig. 14.6. Regulatory circuits of asexual reproduction. Modified after Andrianopoulos and Timberlake (1994), Busby et al. (1996)

replicate metulae and, frequently, secondary conidiophores on top of the primary conidiophores. Nevertheless, those aberrant conidiophores are able to produce conidia. Similarly to the *stuA* mutation, the *medA* mutation has an effect on sexual reproduction. Unlike *stuA*, however, *medA* mutants do form Hülle cells, failing to produce only cleistothecia. MedA is required for correct temporal expression of both *brlA* transcripts and it regulates, together with BrlA, the expression of *abaA*. Interestingly, the lack of functional MedA can be overcome by an additional copy of *brlA* (Busby et al. 1996). A detailed analysis of the *medA* gene locus and the interaction of MedA with putative target promoter sequences is unfortunately still pending.

The number of genes which, in one way or another, influence spore formation is continuously increasing. However, a link to the well-understood regulatory cascade and the signalling components described above is largely lacking, and mutations in these genes cause pleiotropic phenotypes. The question whether the conidiation phenotypes are primary defects of the mutations or whether the effects are secondary has to date not been fully solved. One gene which comes into consideration is *dopA*. It encodes a large protein (207 kDa) with several putative domains, including three leucine zipper-like domains (Pascon and Miller 2000). Mutant strains have abnormal vegetative hyphae, delayed and synchronous initiation of asexual development, and also a defect in the sexual cycle. Another candidate is the basic helix-loop-helix protein DevR (Tuncher et al. 2004). Conidiophores appear to have a defect at the phialide stage. There is evidence that DevR is a component of the TcsA two-component signalling pathway (Virginia et al. 2000). The last example mentioned here, is the *hymA* gene (Karos and Fischer 1996). Metulae fail to differentiate and do not produce phialides – hence, the name *hym*, *hy*pha-like *m*etulae. Expression is not developmentally regulated and the gene encodes a highly conserved protein (Karos and Fischer 1999). The corresponding protein from mouse even partially complemented the *hymA* defect in *A. nidulans*. Studies in *S. cerevisiae* showed that the yeast homologue Hym1 is involved in cell cycle regulation and polarity establishment (Nelson et al. 2003). The *hymA* mutant phenotype suggests that these functions are likely to be the primary ones in the mycelial fungus, too, because at the metula stage the cell cycle becomes strictly coordinated to cytokinesis and the reproduction mode changes to a pseudohyphal-like process (Gimeno and Fink 1994).

g) Target Genes

The presence of receptors, signalling cascades and transcription factors would not lead to any morphogenesis without the concerted actions of enzymes and structural proteins. Some of these have been isolated in the mutant screening mentioned above (Clutterbuck 1969). Two of them, *yA* and *wA*, encode enzymes for pigment biosynthesis (Aramayo and Timberlake 1990; Mayorga and Timberlake 1990, 1992). Other examples are the *rodA* and *dewA* hydrophobins, which contribute to the hydrophobic surface of the conidiospores. Whereas RodA appears to form typical rodlet structures at the spore surface, in *dewA* deletion mutants, rodlets are still visible, suggesting that the main spore-coating hydrophobin in *A. nidulans* is RodA (Stringer et al. 1991; Stringer and Timberlake 1994; Fig. 14.2). Another component of the cell wall and, thus, also of the conidiospore wall is chitin. *A. nidulans* contains several chitin synthases, which appear to be expressed to different extents in different cell types (Lee et al. 2004). Usually, deletion of a single chitin synthase gene does not cause any phenotype, because the functions are redundant. However, double deletion of, e.g. *chsA* and *chsC*, caused conidiophore morphology alterations and a reduction in spore production (Fujiwara et al. 2000). These results show that a fine regulation of the cell wall composition is essential for higher yields of spores.

Other examples for differentially regulated genes are the catalases. *A. nidulans* contains at least four of these enzymes, of which CatA is up-regulated during conidiation (Navarro and Aguirre 1998; Kawasaki and Aguirre 2001). Interestingly, up-regulation of *catA* was independent of the regulator BrlA, suggesting a regulatory cascade independent of *brlA*.

h) Interdependence of Vegetative Growth and Development

There is increasing evidence that the different developmental programmes are interlinked and, to some extent, exclude each other. As described above, the PSI factors influence the balance between asexual and sexual development (Champe et al. 1987). This phenomenon has been studied recently at the molecular level, and it was found that three fatty acid oxygenases (PpoA, PpoB and PpoC) are involved in the biosynthesis of these molecules. Deletion of *ppoC* caused a reduction of

PsiB level, and increased the ratio between asexual and sexual development (Tsitsigiannis et al. 2005).

Earlier on, it was already noticed that overexpression of the central developmental regulator *brlA* causes a block of vegetative growth, and that hyphae produce single spore-like structures at their tips (see above). By comparison, overexpression of *abaA* also causes a block in hyphal extension in liquid medium, but no differentiation of hyphae (Adams and Timberlake 1990). Vice versa, many mutants of a group named *fluffy* produce masses of vegetative hyphae and are not able to undergo asexual or sexual development (Fig. 14.4). Recent experiments suggest a similar relationship between sexual and asexual development. Wildtype *A. nidulans* (*veA*+) develops mainly sexually when grown in the dark, and asexually when grown in the light. In the absence of the *veA* gene, strains are completely asexual and develop asexually, independently of light conditions. Therefore, it has been contended that *veA* mediates the light response (Käfer 1965; Kim et al. 2002). However, another explanation could be that *veA* is required for sexual development, and that the occurrence of the sexual cycle inhibits the asexual cycle. Similarly, deletion of *nsdD* causes this shift between the two developmental cycles. Deletion mutants develop purely asexual, independently of light (Han et al. 2001; Vienken and Fischer, unpublished data).

Nice examples of how development is coupled to cellular processes are presented by a number of mutations which affect cell cycle regulation or nuclear distribution. Initial evidence for the specific requirement of genes necessary for vegetative cell functions came from the analysis of two mutants, *apsA* and *apsB*, isolated in the screen of Clutterbuck (1969). These mutants developed conidiophores until the metula stage (Fig. 14.3). However, metulae remained anucleate and, thus, development did not proceed beyond this stage. Both *aps* genes encode proteins with general functions in nuclear migration and microtubule organisation (Fischer and Timberlake 1995; Suelmann et al. 1998; Veith et al. 2005). ApsA appears to mediate microtubule cortex interactions, which are important for nuclear positioning. ApsB is a novel spindle pole-associated protein which regulates the activity of microtubule-organising centres in the cell. Mutation of either of the two genes disturbs the normal arrangement of cytoplasmic microtubules (Veith et al. 2005). Whereas in *apsA* mutants microtubules appear longer, in *apsB* mutants the number of cytoplasmic microtubules is reduced. These features appear to be crucial for nuclear migration into metulae.

Genes involved in cell cycle regulation are other examples earmarked for functional duality in basic cell biology and in development. Metulae and phialides are both uninucleate and, whereas nuclei in metuale do not divide after two or three phialides are produced, nuclei in phialides divide continuously to provide all conidia with nuclei. Nuclear division is strictly coordinated to cytokinesis. This indicates that the cell cycle of close neighbour cells may be regulated in an opposite manner, i.e. arrested in metulae and adjusted to continuous spore production in phialides. The molecular basis for this is not yet completely understood. The laboratory of Osmani found that two key regulators of the *A. nidulans* cell cycle, NimX and NimA, are adjusted in their activity during conidiophore development (Ye et al. 1999; see Chap. 3, this volume). Recently, a new cyclin, *pclA*, was identified in a developmental mutant screening (Schier et al. 2001). Deletion of this gene causes abnormal conidiophores, resembling the *abaA* mutants. Although this cyclin interacts with NimX, it is not clear yet whether the cell cylce in phialides is dependent on this interaction (Schier and Fischer 2002). In *Aspergillus oryzae*, the restriction to one nucleus per conidium does not exist, and thus conidia normally contain two nuclei, which migrate from the phialide into the conidium (Ishi et al. 2005).

B. Other Ascomycetes

Whereas *A. nidulans* asexual spore formation has been studied in detail over the past 30 years, our knowledge of the genetic regulation of sporulation in other mycelial ascomycetes is rather limited, and only some isolated components have been described. Among the better-studied species are *N. crassa* and the opportunistic human pathogenic *Penicillium marneffei*. The latter organism recently gained attention, because morphogenesis appears to be related to pathogenicity (see Chap. 1, this volume; Boyce et al. 2005). This fungus undergoes a dimorphic switch. It grows in a hyphal form at 25 °C whereas it proliferates yeast-like cells through arthroconidiation at more elevated temperatures. The hyphal form produces *Penicillium*-typical, brush-type conidiophores with four to five metulae at the tip of the stalk, each of which bears three to seven phialides producing the conidia. The yeast form, by contrast, is obtained by fission

of the double septa of hyphae (Chan and Chow 1990; Cooper and McGinnis 1997; Vossler 2001).

In a systematic approach, the group of Andrianopoulos isolated functional homologues of *A. nidulans* conidiation regulators in *P. marneffei*, and generally found conserved functions for the proteins (Borneman et al. 2000, 2002). The main regulators of conidiation, *brlA* and *abaA*, of *A. nidulans* appear to be conserved (Table 14.1; Bornemann et al. 2000; Todd et al. 2003). A *stuA* defect in *P. marneffei* shows that StuA is needed for metulae and phialide formation (Bornemann et al. 2002). Although there are conserved players, there are also variations in these processes. Contrasting with *A. nidulans rco1* mutations, a mutation in TupA (the Tup1-type transcription factor of *P. marneffei*) confers premature *brlA*-dependent asexual development (Todd et al. 2003). GasA is a close homologue to *A. nidulans* FadA and the major Gα protein blocking conidiation in *P. marneffei*. This protein seems to act through the cAMP signalling pathway in order to inhibit expression of *brlA*. The Gα protein GasC, the likely GanB homologue of *P. marneffei*, negatively affects the onset of conidiation and conidia yield but GasC is involved in sensing neither carbon nor nitrogen depletion. Moreover, GasA and GasC are not required for dimorphic switching and regulation of arthroconidiation (Zuber et al. 2002, 2003). Yeast cell production is, however, regulated by TupA. Inactivation of the *tubA* gene leads to inappropriate yeast morphogenesis at 25 °C (Todd et al. 2003). Arthrospore production in *Acremonium chrysosporium* is a process comparable to arthoconidiation in *P. marneffei*. CPCR1, a winged helix transcription factor described in *A. chrysosporium*, is the first regulator known to be required for hyphal fragmentation (Hoff et al. 2005).

In another *Penicillium* species, *Penicillium chrysogenum*, the putative regulator WetA was shown to be conserved in function in conidiogenesis (Prade and Timberlake 1994). The rice pathogen *M. grisea* has holoblastic, three-celled conidia borne sympodially on an aerial conidiophore (Shi and Leung 1995). A defect in gene *ACR1* (*acropetal*), a homologue to *A. nidulans medA*, causes indeterminate growth leading to chains of spores arranged in a head-to-tail manner (Lau and Hamer 1998; Nishimura et al. 2000). The Gα protein MAGB, homologous to *A. nidulans* GanB, is known to be a negative regulator of conidiation in this species (Fang and Dean 2000). The vascular wilt-causative *Fusarium oxysporium* produces three types of asexual spores (falcate macroconidia, ellipsoidal microconida and globose chlamydospores). Mutants of the *stuA* homologue FoSTUA lack conidiophores and produce only low amounts of macroconidia from intercalary hyphal philades whereas chlamydospore production is strongly promoted and microconidiation is not affected (Ohara and Tsuge 2004). *REN1*, the *medA* homologue in *F. oxysporium*, specifically acts in macroconidia formation. *REN1* mutants lack normal conidiophores and phialides, and form rod-shaped, conidium-like cells directly from hyphae by acropetal division (Ohara et al. 2004).

In the case of *N. crassa*, three different types of asexual spores are produced (Perkins and Barry 1977; Russo and Pandit 1992; Borkovich et al. 2004). Single-nucleate, enteroblastic ovoid microconidia emerge within vegetative hyphae (microconidiophores) as a lateral protuberance of a hyphal cell which ruptures the cell wall of the phialidic mother cell, and is released upon septum formation from the phialide, leaving behind a hole in the hyphal cell wall. Roundish, multi-nucleate holoblastic macroconidia arise in long chains through apical constriction budding at the ends of aerial hyphae. After separation of the macroconidia from the aerial hyphae, longer arthroconidia are formed by laying extra crosswalls within the aerial hyphae, and splitting the resulting hyphal sections (Springer 1993; Fig. 14.7). The formation of micro- and macroconidia depends on different environmental conditions and distinct morphogenetic pathways. Mutants in macroconidia formation may not be blocked for microconidiation (Maheshwari 1999).

A number of genes preferentially expressed during conidiation (*con* genes) have been cloned in *N. crassa* and studied in expression (*con-6*, *con-8*, *con-10*, *con-11* and *con-13*) but their exact cellular functions are not yet clear (Roberts et al. 1988; Roberts and Yanofsky 1989; Hager and Yanofsky 1990; White and Yanofsky 1993; Corrochano et al. 1995). In other cases, functions of the genes are known, some of these recurring from conidiation in *A. nidulans* whereas others are new. The *vvd* gene for the flavoprotein blue-light photoreceptor Vivid is required for light adaptation of conidiation-specific genes to daily changes in light intensity, but not for circadian conidiation (Heintzen et al. 2001; Shrode et al. 2001; Schwerdtfeger and Linden 2003). There is a structural, light- and clock-regulated gene *eas* for a spore-specific hydrophobin (Bell-Petersen et al. 1992; Lauter et al. 1992) which has now been shown to be directly

Table. 14.1. Analysis of fungal genomes for orthologues of developmental regulators known in *A. nidulans* to participate in conidiophore and conidia production (for specific regulator functions, see text)[a]

Protein/fungus	AbaA	AcoB	BrlA	FlbA	FlbC	FlbD	FluG	MedA	PhiA	StuA	WetA
Ascomycota											
Aspergillus nidulans	796 aa	327 aa	432 aa	719 aa	354 aa	314 aa	865 aa	658 aa	182 aa	622 aa	555 aa
Aspergillus fumigatus	798 aa, 2.3e-247, 460/805	322 aa, 4.2e-74, 164/332	427 aa, 8.0e-151, 281/435	719 aa, 2.2e-251, 509/730	356 aa, 3.4e-104, 211/350	315 aa, 2.9e-98, 213/330	861 aa, 0.0, 601/867	684 aa, 2.7e-214, 411/663	186 aa, 6.6e-53, 105/157	653 aa, 1.6e-90, 213/418	567 aa, 2.6e-145, 307/575
Aspergillus oryzae	833 aa, 6.0e-200, 255/450	323 aa, 6.3e-80, 166/329	422 aa, 1.3e-157, 286/433	720 aa, 2.6e-257, 507/722	353 aa, 4.7e-107, 213/347	324 aa, 1.3e-104, 224/336	864 aa, 0.0, 609/866	666 aa, 8.0e-172, 356/624	192 aa, 1.4e-41, 88/157	647 aa, 1.8e-160, 342/590	564 aa, 1.0e-143, 302/574
Penicillium marneffei	823 aa, 0.0, 407/823	?	?	421 aa, e-130, 234/421	?	?	?	?	?	?	632 aa, e-160, 334/614
Penicillium chrysogenum	?	?	?	?	?	?	?	?	?	?	499 aa, e-109, 245/522
Gibberella zeae	X	277 aa, 5e-35, 83/221	X	738 aa, e-147, 285/518	290 aa, 5e-53, 104/175	299 aa, 4e-39, 96/214	862 aa, e-171, 355/880	728 aa, 1e-83, 227/601	179 aa, 9e-29, 67/161, plus additional three	676 aa, 3e-65, 148/326	608 aa, 1e-12, 51/148
Magnaporthe grisea	X	286 aa, 4e-36, 81/205	X	X	673 aa, 3e-51, 126/343	258 aa, 4e-26, 66/148	X	862 aa, 4e-57, 151/345	189 aa, 3e-28, 72/161	612 aa, e-111, 246/563	X
Neurospora crassa	1,175 aa, 3e-04, 31/90	417 aa, 4e-26, 81/230	X	745 aa, e-137, 277/555	366 aa, 1e-46, 98/176	324 aa, 4e-30, 71/155	898 aa, e-166, 357/894	692 aa, 1e-70, 215/590	198 aa, 1e-28, 71/162	643 aa, e-114, 263/557	963 aa, 1e-11, 55/155
Candida albicans	743 aa, 9e-16, 132/542	X	X	X	X	607 aa, 3e-35, 99/302	X	1,087 aa, 3e-39, 119/349	X	549 aa, 5e-47, 139/415, plus an additional one	X
Candida glabrata	435 aa, 3e-08, 55/191	X	X	X	X	X	X	X	X	435 aa, 3e-39, 91/187	X
Debaromyces hansenii	555 aa, 1e-11, 38/86	X	X	X	X	575 aa, 8e-34, 69/109	X	652 aa, 3e-33, 89/221	X	593 aa, 5e-48, 152/422	X
Eremothecium gossypii	791 aa, 1e-06, 28/60	X	X	X	X	311 aa, 5e-33, 66/109	X	X	X	678 aa, 3e-40, 107/258	X
Kluyveromyces lactis	624 aa, 7e-07, 27/60	X	X	X	X	517 aa, 4e-27, 74/192	X	X	X	636 aa, 1e-41, 93/200	X
Saccharomyces cerevisiae	486 aa, 3e-07, 28/60	X	X	X	X	X	X	X	X	785 aa, 6e-40, 83/147	X
Yarrowia lipolytica	801 aa, 4e-19, 54/126	227 aa, 2e-07, 47/196	X	492 aa, 6e-19, 112/502	X	478 aa, 1e-36, 100/281	X	361 aa, 4e-57, 117/237	X	590 aa, 1e-59, 159/366, plus an additional one	X
Schizosaccharomyces pombe	X	X	X	481 aa, 6e-32, 117/467	X	X	X	X	X	X	X
Basidiomycota											
Cryptococcus neoformans var. *neoformans* B-3501A	X	316 aa, 6e-13, 55/205	X	624 aa, 2e-70, 184/570	X	X	X	755 aa, 1e-54, 133/318	X	X	X
Cryptococcus neoformans var. *neoformans* JEC21	X	295 aa, 3e-12, 53/205	X	624 aa, 2e-70, 184/570	X	X	X	755 aa, 6e-55, 133/318	X	X	X

Table. 14.1. (continued)

Protein/fungus	AbaA	AcoB	BrlA	FlbA	FlbC	FlbD	FluG	MedA	PhiA	StuA	WetA
Coprinus cinereus[b]	X	X	X	2e-58, 180/612	X	X	X	9e-40, 83/158	X	X	X
Ustilago maydis	1,267 aa, 4e-23, 103/383	X	X	737 aa, 3e-50, 120/348	X	X	X	716 aa, 4e-59, 125/218	X	X	X
Zygomycota											
Rhizopus oryzae[b]	X	X	X	2e-29, 78/205	X	X	X	3e-48, 105/212	X	9e-35, 72/122	X
Microsporidia Encephalitozoon cuniculi	X	X	X	X	X	208 aa, 1e-19, 44/108	X	X	X	X	X

[a] We used the *A. nidulans* proteins to search the published fungal genome sequences (http://www.broad.mit.edu/annotation/fungi/) at the Broad Institute. Because a genome sequence is not yet available for any *Penicillium* species, we searched the NCBI database for homologous proteins in *P. marneffei* and *P. chrysogenum*. The number of amino acids, the e-value and the number of overlap in relation to the entire protein are displayed. Sequences which were similar only in conserved regions, such as DNA-binding motifs, were not considered

[b] Species whose genomes are not yet annotated. Therefore, only a tblastN search was performed, without deducing the complete protein sequences. X, no hit; ?, no result, due to the lack of the genome sequence

regulated by the major regulator of sporulation, the binuclear zinc finger transcription factor Fl (Bailey-Shrode and Ebbole 2004; Rerngsamran et al. 2005). Mutations in *rco-1* coding for a Tup1 transcription factor fail to express conidiation-specific genes (Yamashiro et al. 1996; Lee and Ebbole 1998). The *ncr-1* gene encodes a MAPKK kinase and the *ncr*-2 gene a serine-threonine pro-

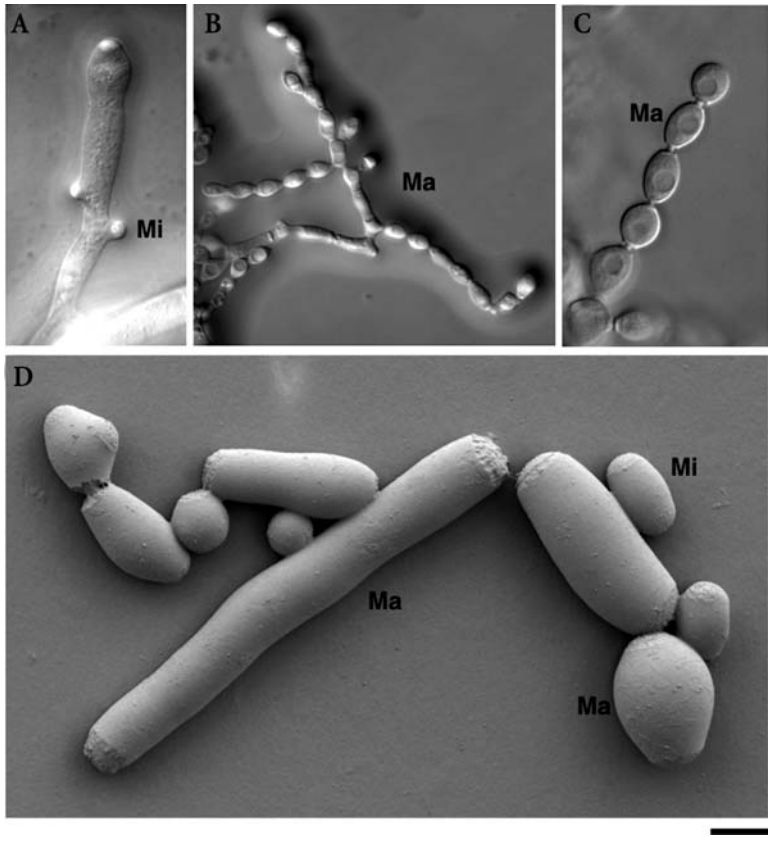

Fig. 14.7.A–D Micro- (*Mi*) and macroconidia (*Ma*) formation in *N. crassa*. **A–C** DIC (differential interference contrast) images of conidiophores, taken by D. Veith. **D** SEM image showing the three spore types in high resolution (courtesy of C.E. Jeffree, M.G. Roca and N.D. Read, unpublished data). The scale bar represents 10 µm in **A**, **C**, 20 µm in **B** and 3 µm in **D**

tein kinase, both needed for the control of entry into the conidiation programme. When defect, they cause constitutive onset of conidiation (Kothe and Free 1998). Other analysed defects leading to inappropriate sporulation in submerged culture are in genes for sugar transporters (Madi et al. 1997) and in heterotrimeric G-proteins (Yang et al. 2002; Kays and Borkovich 2004; Krystofova and Borkovich 2005). A mutant in the Gα protein GNA3 resembles in phenotype mutants in the adenylate cyclase gene *cr-1*. Phenotypic defects in both genes can be corrected by addition of cAMP, and GNA3 possibly regulates the protein levels of these enzymes. By contrast, defects by the Gα protein GNA1 are not overcome by addition of cAMP. GNA1 is necessary for response to extracellular cAMP, and the protein has been suggested to act in sensing cell densities and/or the nutritional status (Kays and Borkovich 2004). Other genes affecting conidiation have been studied, some in connection with the molecular clock. A detailed description of the role of all developmental genes characterized in *N. crassa* cannot be given in this review, and the reader is therefore referred to earlier reviews in this field (Perkins and Barry 1977; Borkovich et al. 2004; The Mycota, Vol. III, 2nd edn., Chap. 11).

C. Basidiomycetes

1. Cytology

Asexual sporulation in basidiomycetes is not uncommon (Kendrick and Watling 1979) but usually rather neglected. Only asexual sporulation of *C. cinereus* has been studied in more detail. This fungus produces two types of asexual spores, chlamydospores and oidia.

Large, thick-walled chlamydospores are produced for survival in aging cultures, preferentially in brown-coloured matting at the agar–air interface (Fig. 14.8). The spores have variable shapes, from oval to irregular inflated. There appear to be two different modes for generation – the classical endogenous chlamydospore formation within hyphal cells (see above), and an exogenous blastocyst mode where an inflated bud receives the compressed cytoplasm from a hyphal cell (in a factual sense, not chlamydospores; see definitions by Clémençon 1997). When produced on the dikaryon, chlamydospores are binucleate and germinate with either one or two germ tubes to give a new dikaryotic mycelium or two distinct monokaryons respectively (Anderson 1971; Kües et al. 1998a, 2002b).

Oidiation in *C. cinereus* in most instances starts with lateral bulging at an aerial hypha. Following nuclear division, a daughter nucleus migrates into the protrusion and a septum is laid, separating the young, elongating sporophore (oidiophore) from the hyphal foot cell. The full-grown oidiophore towers at a more or less perpendicular angle (80–85°) into the air, reaching a length of 50 µm and more (on average, about 20–30 µm). At its base, it is broader than is the case for vegetative hyphae but it tapers with length. At the tip of the matured structure, oidial hyphae bud off, one after the other. Usually, they are about 8–12 by 2 µm in size. Each oidial hypha is supplied with a nucleus coming from successive divisions of the nucleus within the oidiophore stalk. Subsequent to uptake of a nucleus, the oidial hypha separates from the stem cell

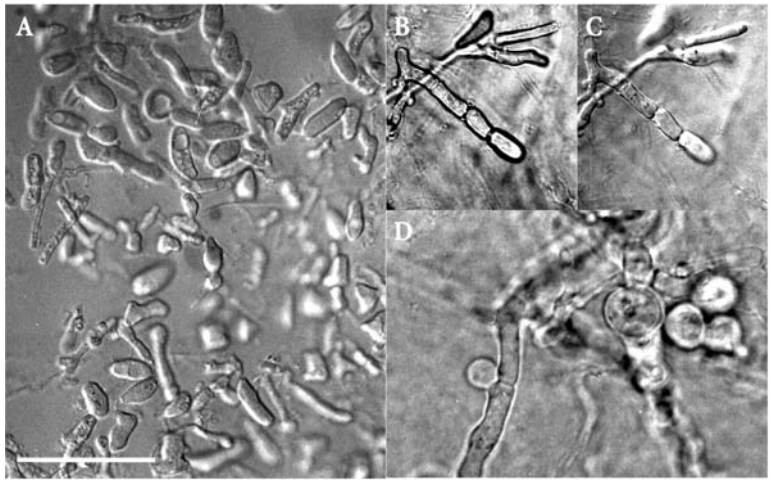

Fig. 14.8. **A–D** Chlamydospores of *Coprinus cinereus*. **A** Chlamydospores formed in the mycelial matting of a monokaryon. **B**, **C** Chlamydospores formed within hyphal cells by the classical mode of chlamodospore production (**B** bright field optic, **C** phase contrast). **D** Blastocyst mode of production. The scale bar represents 50 µm

by septation, the nucleus within the oidial hypha divides, and a septum is laid between the daughter nuclei, separating the oidial hypha into two equally sized cells. These cells split into separate uninuclear haploid arthroconidia by schizolysis at the septum within the oidial hypha, and at the septum between the oidial hypha and the oidiophore stalk. The rod-shaped, hyaline oidia (2 by 4–6 µm) are collected (up to 200) in a liquid droplet secreted from the tip of the oidiophore (Bensaude 1918; Brodie 1931; Polak et al. 1997a; Kües et al. 2002a; Fig. 14.9). Consistent with their wettable character (cf. classification "wet oidia"; Kemp 1975), oidia do not have an outer hydrophobic layer formed by hydrophobins (Ásgeirsdóttir et al. 1997). Most of the surface of the oidia is coated by double-layered primary cell walls covered with hair-like structures (Heintz and Niederpruem 1971; Polak et al. 1997a; Kües et al. 2002a) of possibly collagenous structure (Castle and Boulianne 1991; Celerin et al. 1996). This may help to keep a mucilaginous layer surrounding the oidia (Watling 1979; Polak et al. 1997a). Due to this gelatinous layer, the spores stick to surfaces (Polak et al. 1997b), including to carapaces of insects acting as vectors for their distribution (Brodie 1931).

Apart from the generalized scheme of oidiation described above (type 1 oidiophores), there is considerable morphological variation within a given *C. cinereus* strain, and also between strains. The stalk of the oidiophore may be branched (type 2A), divided into two or more cells (type 2B) or the stem cell may not elongate (type 3). In type 4, stems are not formed; rather, oidial hyphae arise directly from a cell in the vegetative mycelium, either singly (type 4A) or in bundles (type 4B). Furthermore, oidial hyphae may also branch, they may develop on one side along the length of an oidiophore or they may split into more than two spores. Usually, a single strain shows the whole spectrum of variation but, preferentially, only one or two types of oidiophores are formed (Polak et al. 2001; Kües et al. 2002a).

2. Genetics

Absolute oidia production in *C. cinereus* is influenced by the relative incidences of carbon and

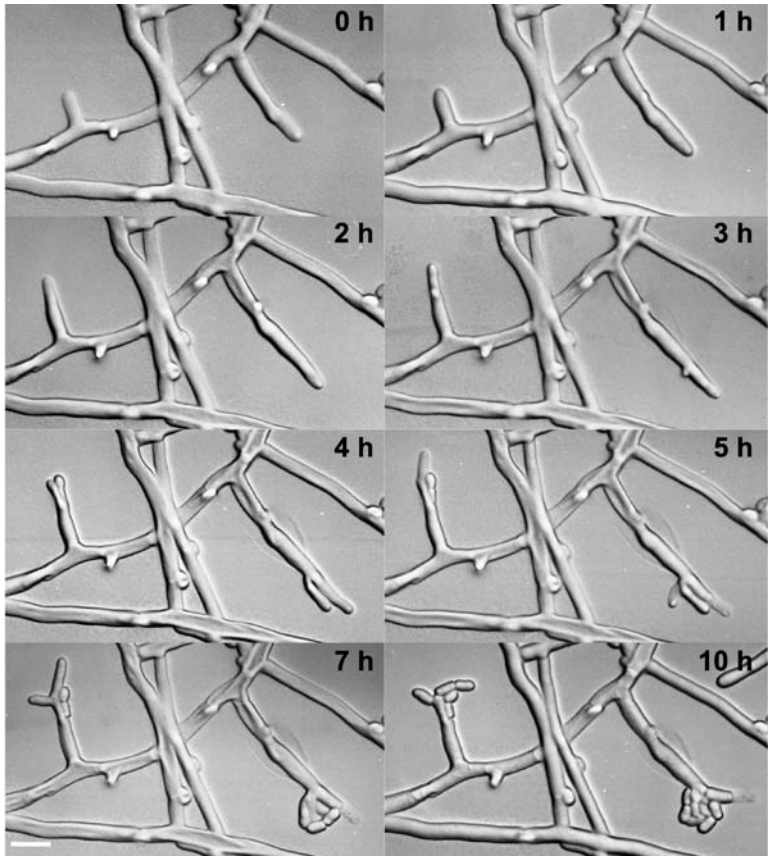

Fig. 14.9. Development of oidiophores of *Coprinus cinereus* with time. In the images to the *left*, a young bulge is seen on an aerial hypha which, during the following 3 h, elongates into a structure starting to successively produce oidial hyphae. Two hours later, the first two spores are released, followed by more within the next 5 h. In the images to the *right*, development of a larger oidiophore with more oidial hyphae and spores can be seen. The scale bar represents 10 µm. Photos taken by E. Polak

nitrogen sources (Rao and Niederpruem 1969; Walser 1997). In monokaryons, oidia production is constitutive, whilst in dikaryons oidiation is repressed in the dark. In light, repression is partially released and dikaryons indeed produce spores but in much smaller quantities (cf. factors of 100 s–1000 s) than do the parental monokaryons. Effective on the dikaryon is blue light. Elevated temperatures (37–42 °C) support spore production better than do ambient temperatures (25–30 °C; Kertesz-Chaloupková et al. 1998; Kües et al. 1998b), in an opposite manner to fruiting body development (Kües 2000; Kües et al. 2004). Negative control of oidiation on the dikaryon is exerted by the two mating type loci (Tymon et al. 1992; Kües et al. 1998a, 2002b). The genes at the *A* mating type locus encode two distinct classes of homeodomain transcription factors (HD1 and HD2), which form heterodimeric complexes functional in gene regulation when of compatible mating type specificity. The mating type genes at the *B* locus encode pheromones and pheromone receptors. Functional are pheromones in combination with pheromone receptors from different mating type specificity (for more details on mating type genes and their products, see reviews by Casselton and Olesnicky 1998; Hiscock and Kües 1999; Kües 2000; Kothe 2001; Chap. 17, this volume). In the dikaryon, *A* mating type genes have been shown to primarily be responsible for repression of oidia formation in the dark (Tymon et al. 1992). If only the *A* mating pathway is activated, then blue light is able to fully release *A*-mediated repression (Kertesz-Chaloupková et al. 1998; Kües et al. 1998a). A blue-light photoreceptor of the *N. crassa* white collar 1-type has been cloned in *C. cinereus* from a mutant defective in light regulation of fruiting body development (Terashima et al. 2005). Preliminary genetic data suggest that this receptor also takes part in overriding *A* mating type-mediated repression of oidiation (W. Chaisaena, personal communication). The *B* mating type genes counteract light-mediated release of repression, resulting in spore production in low numbers in the dikaryon. Activation of the *B* pathway on its own can cause, in some strains and on some media, reduction in general colony growth and suppression of aerial mycelium formation. However, sole activation of the *B* mating type pathway has no severe effect on oidia production (Kües et al. 2002b). There appears to be a link between the *B* mating type genes and nutritional control of growth, in particular for nitrogen (Kües et al. 2004). Constitutive activation of a Ras GTPase causes growth defects similar to those resulting from activation of the *B* mating type pathway, but oidia production is not affected by constitutive Ras activation (Bottoli 2001; S. Kilaru, personal communication).

Wessels and co-workers showed in the basidiomycete *Schizophyllum commune* that, with increasing distance of the two different haploid nuclei in aerial cells of the dikaryotic mycelium, nuclear communication eventually ceases. Consequently, repression of monokaryon-specific genes is released (Schuurs et al. 1998; see Chap. 19, this volume). In the aerial mycelium of *C. cinereus* dikaryons, there are possibly similar effects in the control of oidia formation by mating type genes. Spores produced on a wild-type dikaryon are haploid and uninucleate, as are those on monokaryons, indicating that nuclear communication is interrupted. However, from the two possible types of oidia on a dikaryon, usually one dominates. This dominance is reversed when the stronger nucleus is transformed by a compatible *A* mating type gene. The interpretation of these observations is that opposing but distinct length gradients of *A* mating type proteins exist in the dikaryotic cell, protein concentrations being highest in the nucleus which carries the genes for the corresponding proteins, and the stronger nucleus producing the longer gradient. Functional HD1-HD2 transcription factor complexes are more likely to be produced in close vicinity of the weaker nucleus which, consequently, will be repressed in oidiation (Polak 1999; Kües et al., unpublished data). The model underlines that the responsible sporulation genes are principally monokaryon-specific. It also can explain the hierarchy observed in the asexual formation of uninucleate haploid spores on dikaryons of other basidiomycetes (Hui et al. 1999; Kitamato et al. 2000). In the monokaryons of *C. cinereus*, *A* mating type genes have no effect on oidiation. Oidiophore formation is unaltered in *C. cinereus* strains lacking all *A* mating type genes, and the numbers of spores produced are similar to values recorded in wild-type monokaryons (Polak 1999).

Chlamydospores occur in aging cultures of some monokaryons of *C. cinereus* (Kües et al. 1998a) but they are much more common in dikaryons (Anderson 1971; Kües et al. 2002a). Regulation of production of chlamydospores is opposite to that for oidiation and, in some aspects, parallel to that for fruiting. Activation

of the *A* mating type pathway strongly enhances chlamydospore production when a dikaryon is kept in the dark whereas constant incubation with blue light represses chlamydospore production. Additional activation of the *B* mating type pathway promotes the process but activation on its own also has an effect (Kües et al. 1998a, 2002b). Chlamydospores are produced under conditions favouring fruiting body development, as part of the sexual mode of reproduction on the dikaryon. Fruiting body development occurs at 25–28 °C and needs repeated impulses of blue-light low energy but it is suppressed by higher light energies which are effective in oidiation (Kües 2000; Fischer and Kües 2003; Kües et al. 2004). Chlamydospores accumulate glycogen which can serve as energy source for fruiting (Madelin 1960; Moore 1998). The positive regulation in the dikaryon, and the functional linkage to fruiting corroborate that there are genes for chlamydospore production which are primarily dikaryon-specific.

Genes acting in asexual sporulation in *C. cinereus* other than the mating type genes have so far not been identified. The homokaryotic mutant strain AmutBmut has defects in both mating type loci, which makes oidiation a light-controlled process (Kertesz-Chaloupkova et al. 1998) and also leads to fruiting body production without the need to mate with another strain (Boulianne et al. 2000; Walser et al. 2003). A mutant screening approach in this strain identified many morphological changes in oidiophore and spore production and morphology. In most instances, the aerial mycelium is simultaneously affected with alterations in oidiation and, in about 85% of the cases, also fruiting body formation (Polak 1999). To separate genes acting generally in growth and development as well as nonessential or marginally important genes from functions acting specifically and/or being essential in oidiation, a detailed analysis of sexual and asexual phenotypes is required. Strain AmutBmut produces mainly the advanced type 1 and type 2A oidiophores (Polak et al. 1997a, 2001), some of the mutants rather the less-developed type 3 or type 4 structures or irregularly structured oidiophores with altered stems, branching or oidial hyphae production. In other cases, branched and elongated oidial hyphae with aberrant nuclear distributions were observed. A considerable number of mutant phenotypes, however, relate to the spores themselves. Spores occur which are much longer or shorter than normal, swollen spores, shrivelled spores and branched spores. In a flocculating strain, the outer fimbriae and the gelatinous layer were found to be absent in the spores. In one mutant, apical oidia separated not by schizolysis, the norm, but rather through ripping of the cell below (rhexolysis). In another mutant, spores are not released. There are also interesting regulatory mutants including constitutive producers and spore-negative strains unable to initiate oidiophore production (Polak 1999). The pool of available mutants holds promise for future work to identify many interesting genes.

D. Conserved Genetic Pathways

From the compilation of characterized genes from various fungi we presented in Sect. IV.B, it is clear that there are functions in asexual spore production conserved between species. Even if the mechanisms of spore formation and the environmental requirements were variable among different fungi, some genetic pathways or particular elements of these pathway may be evolutionarily conserved. How broadly will this be?

The availability of several fungal genome sequences allows us to address this question fairly easily by means of Blast searches, in contrast to less-straightforward experimental methods such as heterologous complementation or PCR-based approaches (Prade and Timberlake 1994). In such a computer-based approach, however, one should bear in mind that sequence conservation does not necessarily imply conserved cellular functions! It is obvious that all fungi contain components of the main signalling pathways, for example, various G-proteins, proteins of the MAP cascade, and certain transcription factors which can affect growth and development in more than one way (Lengeler et al. 2000), including pathways of asexual sporulation (see Sects. IV.A.2 and IV.B). Possible contributions of such regular elements to asexual spore development in a specific fungus certainly need to be addressed experimentally. However, there are other functions expected to act primarily during steps of asexual sporulation, and unlikely to play roles in other cellular pathways (see Sects. IV.A.2 and IV.B).

Here, we used genes of *A. nidulans* central to the conidiation process, and analysed the genomes of a number of different fungi. The results are summarized in Table 14.1. Probably not unexpected because of the structurally related conidiophores and the same phialidic mode of conidia produc-

tion, the *A. nidulans* key regulators BrlA, AbaA and WetA are well conserved among Aspergilli and Penicilli. Surprisingly, however, none of these regulators appear to be present in the mycelial pathogen *M. grisea*, and BrlA is not found at all in mycelial fungi and yeast outside of the Aspergilli and Penicilli. By contrast, AbaA is also found in several other ascomycetes as well as in *U. maydis*. The role of a homologue in *S. cerevisiae* has already been discussed above (see Sect. IV.A.2.f).

An example of a well-conserved protein is the transcriptional regulator MedA which is found in species of the three fungal classes ascomycetes, basidiomycetes and zygomycetes. Homologues in *M. grisea* and *F. oxysporium* were shown to function in conidiogenesis but evidently in distinct morphological patterns (Sect. IV.B). Furthermore, the molecular role of this protein is not well characterized also in *A. nidulans* (Sect. IV.A.f). Protein PhiA needed for phialide and conidia formation (Sect. IV.A.f) is detected only in mycelial ascomycetes with phialidic conidogenesis, suggesting this to be a protein with a very specific function, possibly in blastic cell wall assembly.

Many proteins of the *A. nidulans* "*fluffy*" group, FlbA, FlbC and FlbD, are also conserved among ascomycetes. However, FluG, the one protein which is likely to be involved in the generation of a signalling compound (see Sect. IV.A.2b), is found only in *Gibberellae zeae* and *N. crassa*, outside of the Aspergilli and Penicilli. Of the "fluffy" genes, only a gene for a FlbA-like RGS protein has been found in the genomes of the basidiomycetes. Interestingly, this gene was already known in *S. commune* for long years as *thn* (for "thin", due to loss of formation of aerial mycelium in the corresponding mutants), before it was cloned and shown to be a FlbA homologue (Fowler and Mitton 2000). Strikingly, in confrontations with wild-type strains, *thn* mutants can be induced by diffusible low-molecular weight molecules to produce abundant aerial mycelium (Schuren 1999). The *flbA* gene in the basidiomycete *C. cinereus* may also be needed for the production of aerial mycelium. This in turn could be a prerequisite for developing oidiophores on aerial mycelium as special aerial structures for asexual spore production. Otherwise in *C. cinereus*, from the set of tested *A. nidulans* sporulation genes, we only found a gene for a MedA transcription factor. In conclusion, in this fungus we will have to expect to find many new functions once cloned, e.g. from the available oidiation mutants, and subsequently characterized.

Similarly to the poor conservation of genes in basidiomycetes, only three of eleven tested *A. nidulans* sporulation genes were detected in *Rhizopus oryzae* (FlbA, MedA, StuA). This suggests that sporangiophore and endospore production in the zygomycetes (for description, see Esser 2001) may also follow an own, independent route.

The overall results of the rather limited gene survey presented in Table 14.1 suggest that the regulatory cascade elaborated in conidiogenesis in *A. nidulans* may work similarly in closely related species but it is not a general theme among fungi, neither in distantly related fungi from other classes, nor in all fungi belonging to the same class. The limited range of experimental data we have by now from different ascomycetes with both similar and different morphological pathways of spore production supports this interpretation. However, there is also some experimental evidence suggesting conserved gene functions which act in different morphological pathways of sporulation (Sect. IV.B).

Even if all genes were present in another fungus, and if this fungus follows a morphologically similar mechanism of spore production and if this other species were even a close relative, they may still react differently than in *A. nidulans*. A recent finding by the group of Yu (Madison, USA) suggests that assumptions and constructions of developmental pathways only in silico should be viewed with caution. The group discovered that deletion of several *A. nidulans fluffy* genes in *A. fumigatus* did not cause any severe developmental phenotype (J. Yu, personal communication). Whether this phenomenon is restricted to the *fluffy* genes, or whether it is a general complication of the understanding of the genetic regulation of sporulation in other fungi needs to be established.

V. Concluding Remarks

Our knowledge on the regulation of spore formation in mycelial fungi has expanded enormously during the past 10 years, since Navarro-Bordonaba and Adams reviewed conidia production in *A. nidulans* in the first edition of this volume (The Mycota, Vol. I, 1st edn., Chap. 20). A number of novel components have been discovered in *A. nidulans* and other fungi, and gene–function relationships have been described for many developmental genes. The involvement of the main eukaryotic signalling cascades has been demonstrated but a detailed un-

derstanding of how these perceive and transmit the signals, and how they interact is largely lacking. The availability of an increasing number of genomes, and the constant improvement of molecular methods open the possibility for reverse genetic approaches and should allow a rapid increase of our knowledge in the future.

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References

Adams TH, Timberlake WE (1990) Developmental repression of growth and gene expression in *Aspergillus*. Proc Natl Acad Sci USA 87:5405–5409

Adams TH, Boylan MT, Timberlake WE (1988) *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell 54:353–362

Adams TH, Wieser JK, Yu J-H (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Agrios GN (2005) Plant pathology. Elsevier, Amsterdam

Anderson GE (1971) The life history and genetics of *Coprinus lagopus*. Harris Biological Supplies, Weston-super-Mare, UK

Andrianopoulos A, Timberlake WE (1994) The *Aspergillus nidulans abaA* gene encodes a transcriptional activator that acts as a genetic switch to control development. Mol Cell Biol 14:2503–2515

Angulo-Romero J, Mediavilla-Molina A, Dominguez-Vilches E (1999) Conidia of *Alternaria* in the atmosphere of the city of Cordoba, Spain in relation to meteorological parameters. Int J Biometeorol 43:45–49

Aramayo R, Timberlake WE (1990) Sequence and molecular structure of the *Aspergillus nidulans yA* (laccase I) gene. Nucleic Acids Res 18:3415

Aramayo R, Peleg Y, Addison R, Metzenberg R (1996) *Asm-1*$^{+}$, a *Neurospora crassa* gene related to transcriptional regulators of fungal development. Genetics 144:991–1003

Ásgeirsdóttir SA, Halsall JR, Casselton LA (1997) Expression of two closely linked hydrophobin genes of *Coprinus cinereus* is monokaryon-specific and down-regulated by the *oid-1* mutation. Fungal Genet Biol 22:54–63

Axelrod DE, Grealt M, Pastushok M (1973) Gene control of developmental competence in *Aspergillus nidulans*. Dev Biol 34:9–15

Bailey-Shrode L, Ebbole DJ (2004) The *fluffy* gene of *Neurospora crassa* is necessary and sufficient to induce conidiophore development. Genetics 166:1741–1749

Balance DJ, Buxton FP, Turner G (1983) Transformation of *Aspergillus nidulans* by the orotidine-5′-phosphate decarboxylase gene. Biochem Biophys Res Commun 112:284–289

Bell-Pedersen D, Dunlap JC, Loros JJ (1992) The *Neurospora* circadian clock-controlled gene, *ccg-2*, is allelic to *eas* and encodes a fungal hydrophobin required for formation of the conidial rodlet layer. Genes Dev 6:2382–2394

Bennett RJ, Miller MG, Chua PR, Maxon ME, Johnson AD (2005) Nuclear fusion occurs during mating in *Candida albicans* and is dependent on the *KAR3* gene. Mol Microbiol 55:1046–1059

Bensaude M (1918) Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes. PhD Thesis, Faculté des Sciences de Paris. Imprimerie Nemourienne, Henri Bouloy, Nemours, France

Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, Frankenberg-Dinkel N, Fischer R (2005) The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr Biol 15:1833–1838

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Borneman AR, Hynes MJ, Andrianopoulos A (2000) The *abaA* homologue of *Penicillium marneffei* participates in two developmental programmes: conidiation and dimorphic growth. Mol Microbiol 38:1034–1047

Borneman AR, Hynes MJ, Andrianopoulos A (2002) A basic helix-loop-helix protein with similarity to the fungal morphological regulators, Phd1p, Efg1p and StuA, controls conidiation but not dimorphic growth in *Penicillium marneffei*. Mol Microbiol 44:621–631

Bottoli APF (2001) Metabolic and environmental control of development in *Coprinus cinereus*. PhD Thesis, ETH Zurich, Zurich, Switzerland

Boulianne RP, Liu Y, Aebi M, Lu BC, Kües U (2000) Fruiting body development in *Coprinus cinereus*: regulated expression of two galectins secreted by a non-classical pathway. Microbiology 146:1841–1853

Boyce KJ, Hynes MJ, Andrianopoulos A (2003) Control of morphogenesis and actin localization by the *Penicillium marneffei* Rac homolog. J Cell Sci 116:1249–1260

Boyce KJ, Hynes MJ, Andrianopoulos A (2005) The Ras and Rho GTPases genetically interact to co-ordinately regulate cell polarity during development in *Penicillium marneffei*. Mol Microbiol 55:1487–1501

Brodie HJ (1931) The oidia of *Coprinus lagopus* and their relation with insects. Ann Bot 45:315–344

Brown JKM, Hovmøller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537–541

Bruggeman J, Debets AJM, Wijngaarden PJ, deVisser JAGM, Hoekstra RF (2003) Sex slows down the accumulation of deleterious mutations in the homothallic fungus *Aspergillus nidulans*. Genetics 164:479–485

Busby TM, Miller KY, Miller BL (1996) Suppression and enhancement of the *Aspergillus nidulans medusa* mutation by altered dosage of the *bristle* and *stunted* genes. Genetics 143:155–163

Busch S, Eckert SE, Krappmann S, Braus GH (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 49:717–730

Bussink HJ, Osmani SA (1998) A cyclin-dependent kinase family member (PHOA) is required to link developmental fate to environmental conditions in *Aspergillus nidulans*. EMBO J 17:3990–4003

Bussink HJ, Osmani SA (1999) A mitogen-activated protein kinase (MPKA) is involved in polarized growth in the filamentous fungus *Aspergillus nidulans*. FEMS Microbiol Lett 173:117–125

Cairney JWG (2005) Basidiomycete mycelia in forest soils: dimensions, dynamics and roles in nutrient distribution. Mycol Res 109:7–20

Campbell MA, Medd RW, Brown JF (1996) Growth and sporulation of *Pyrenophora seminiperda* in vitro: effects of culture media, temperature and pH. Mycol Res 100:311–317

Casselton LA, Olesnicky NS (1998) Molecular genetics of mating recognition in basidiomycete fungi. Microbiol Mol Biol Rev 62:55–70

Castle AJ, Boulianne RB (1991) Fimbrial proteins of *Ustilago violacea*, *Coprinus cinereus*, and *Schizophyllum commune*. MSA Newslett 42:8

Celerin M, Ray JM, Schisler NJ, Day AW, Stetler-Stevenson WG, Laudenbach DE (1996) Fungal fimbriae are composed of collagen. EMBO J 15:4445–4453

Champe SP, Simon LD (1992) Cellular differentiation and tissue formation. In: Rossomando EF, Alexander S (eds) Morphogenesis: an analysis of the development of biological form. Dekker, New York, pp 63–91

Champe SP, Rao P, Chang A (1987) An endogenous inducer of sexual development in *Aspergillus nidulans*. J Gen Microbiol 133:1383–1387

Chan YF, Chow TC (1990) Ultrastructural observations on *Penicillium marneffei* natural human infection. Ultrastruct Pathol 14:439–452

Chang YC, Timberlake WE (1992) Identification of *Aspergillus brlA* response elements (BREs) by genetic selection in yeast. Genetics 133:29–38

Chang MH, Chae KS, Han DM, Jahng KY (2004) The GanB Gα-protein negatively regulates asexual sporulation and plays a positive role in conidial germination in *Aspergillus nidulans*. Genetics 167:1305–1315

Chidiac P, Roy AA (2003) Activity, regulation, and intracellular localization of RGS proteins. Receptors Channels 9:135–147

Chou S, Huang L, Liu H (2004) Fus3-regulated Tec1 degradation through SCF^{Cdc4} determines MAPK signaling specificity during mating in yeast. Cell 119:981–990

Clémençon H (1997) Anatomie der Hymenomyceten. Eine Einführung in die Cytologie und Plectologie der Krustenpilze, Porlinge, Keulenpilze, Leistlinge, Blätterpilze und Röhrlinge (Anatomy of the Hymenomycetes. An introduction to the cytology and plectology of crust fungi, bracket fungi, club fungi, Chantarelles, Agarics and Boletes). Flück-Wirth, Teufen, Switzerland

Clutterbuck AJ (1969) A mutational analysis of conidial development in *Aspergillus nidulans*. Genetics 63:317–327

Clutterbuck AJ (1975) Cyclic AMP levels during growth and conidiation. *Aspergillus* Newslett 12:13–15

Cooper CRJ, McGinnis MR (1997) Pathology of *Penicillium marneffei*: an emerging acquired immunodeficiency syndrome-related pathogen. Arch Pathol Lab Med 121:798–804

Copes WE, Hendrix FF (2004) Effect of temperature on sporulation of *Botryosphaeria dothidea*, *B. obtusa*, and *B. rhodina*. Plant Dis 88:292–296

Corrochano LM, Lauter FR, Ebbole DJ, Yanofsky C (1995) Light and developmental regulation of the gene *con-10* of *Neurospora crassa*. Dev Biol 167:190–200

Dunlap JC, Loros JJ (2004) The *Neurospora* circadian system. J Biol Rhythm 19:414–424

Dutton JR, Johns S, Miller BL (1997) StuAp is a sequence-specific transcription factor that regulates developmental complexity in *Aspergillus nidulans*. EMBO J 16:5710–5721

Dyer PS, Paoletti M, Archer DB (2003) Genomics reveals sexual secrets of *Aspergillus*. Microbiology 149:2301–2303

Ellis MB, Ellis JP (1998) Microfungi on miscellaneous substrates. An identification handbook. Richmond, Slough, England

Emri T, Molnar Z, Pusztahelyi T, Rosén S, Posci I (2004) Effect of vitamin E on autolysis and sporulation of *Aspergillus nidulans*. Appl Biochem Biotechnol 118:337–348

Esser K (2001) Kryptogamen 1. Cyanobakterien Algen Pilze Flechten, 3rd edn. Springer, Berlin Heidelberg New York

Fang EGC, Dean RA (2000) Site-directed mutagenesis of the *magB* gene affects growth and development in *Magnaporthe grisea*. Mol Plant Microb Interact 13:1214–1227

Ferguson BA, Dreisbach TA, Parks CG, Filip GM, Schmitt CL (2003) Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Orgeon. Can J For Res 33:612–623

Fillinger S, Chaveroche MK, Shimizu K, Keller N, d'Enfert C (2002) cAMP and ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 44:1001–1016

Fischer R, Kües U (2003) Developmental processes in filamentous fungi. In: Prade RA, Bohnert HJ (eds) Genomics of plants and fungi. Dekker, New York, pp 41–118

Fischer R, Timberlake WE (1995) *Aspergillus nidulans apsA* (anucleate primary sterigmata) encodes a coiled-coil protein necessary for nuclear positioning and completion of asexual development. J Cell Biol 128:485–498

Fortwendel JR, Panepinto JC, Seitz AE, Askew DS, Rhodes JC (2004) *Aspergillus fumigatus rasA* and *rasB* regulate the timing and morphology of asexual development. Fungal Genet Biol 41:129–139

Fowler TJ, Mitton MF (2000) *Scooter*, a new active transposon in *Schizophyllum commune*, has disrupted two genes regulating signal transduction. Genetics 156:1585–1594

Frederick BA, Caesar-Tonthat TC, Wheeler MH, Sheehan KB, Edens WA, Henson JM (1999) Isolation and characterisation of *Gaeumannomyces graminis* var. *graminis* melanin mutanrs. Mycol Res 103:99–1001

Fujiwara M, Ichinomiya M, Motoyama T, Horiuchi H, Ohta A, Takagi M (2000) Evidence that the *Aspergillus nidulans* class I and class II chitin synthase genes, *chsC* and *chsA*, share critical roles in hyphal wall integrity and conidiophore development. J Biochem 127:359–366

Galagan JE, Calvo SE, Cuomo C, Ma L-J, Wortman J, Batzoglou S, Lee S-I, Bastürkmen M, Spevak CC, Clutterbuck J, Kapitonov V, Jurka J, Scazzocchio C, Farman , Butler J, Purcell S, Harris SD, Braus GH, Draht O, Busch S, d'Enfert C, Bouchier C, Goldman GH, Bell-Pederson D, Griffith-Jones S, Vienken K, Pain A, Freitag M, Selker EU, Archer DB, Penalva MA, Oakley BR, Momany M, Tanaka TU, Kumagai T, Asai K, Machida M, Nierman WC, Denning DW, Caddick M, Hynes MJ, Paoletti M, Fischer R, Miller BL, Dyer PS, Sachs MS, Osmani SA, Birren B (2005) Sequencing and comparative analysis of *Aspergillus nidulans*. Nature (in press)

Gancedo JM (2001) Control of pseudohyphae formation in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 25:107–123

Gavrias V, Andrianopoulos A, Gimeno CJ, Timberlake WE (1996) *Sacchromyces cerevisiae TEC1* is required for pseudohyphal growth. Mol Microbiol 19:1255–1263

Gimeno CJ, Fink GR (1994) Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. Mol Cell Biol 14:2100–2112

Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR (1992) Unipolar cell divisions in the yeast *Saccharomyces cerevisiae* lead to filamentous growth: regulation by starvation and *RAS*. Cell 68:1077–1090

Goddard MR, Godfray CJ, Burt A (2005) Sex increases the efficacy of natural selection in experimental yeast populations. Nature 434:636–640

Gooch VD, Freeman L, Lakin-Thomas PL (2004) Time-lapse analysis of the circadian rhythms of conidiation and growth rate in *Neurospora*. J Biol Rhyth 19:493–503

Gourbière F, Debouzie D (2003) Local variations in microfungal populations on *Pinus sylvestris* needles. Mycol Res 107:1221–1230

Gourbière S, Gourbière F (2002) Competition between unit-restricted fungi: a metapopulation model. J Theor Biol 217:351–368

Gourbière F, Gourbière S, van Maanen A, Vallet G, Auger P (1999) Proportion of needles colonized by one fungal species in coniferous litter: the dispersal hypothesis. Mycol Res 103:353–359

Gourbière F, van Maanen A, Debouzie D (2001) Associations between three fungi on pine needles and their variation along a climatic gradient. Mycol Res 105:1101–1109

Greene AV, Keller N, Haas H, Bell-Perdersen D (2003) A circadian oscillator in *Aspergillus* spp. regulates daily development and gene expression. Eukaryot Cell 2:231–237

Gregory PH (1973) The microbiology of the atmosphere, 2nd edn. Leonard Hill, Aylesbury

Guignard R, Grange F, Turian G (1984) Microcycle conidiation induced by partial nitrogen deprivation in *Neurospora crassa*. Can J Microbiol 30:1210–1215

Hager KM, Yanofsky C (1990) Genes expressed during conidiation in *Neurospora crassa* – molecular characterization of *con-13*. Gene 96:153–159

Han S, Adams TH (2001) Complex control of the developmental regulatory locus *brlA* in *Aspergillus nidulans*. Mol Genet Genomics 266:260–270

Han K-H, Prade RA (2002) Osmotic stress-coupled maintenance of polar growth in *Aspergillus nidulans*. Mol Microbiol 43:1065–1078

Han S, Navarro J, Greve RA, Adams TH (1993) Translational repression of *brlA* expression prevents premature development in *Aspergillus*. EMBO J 12:2449–2457

Han K-H, Han KY, Yu J-H, Chae KS, Jahng KY, Han DM (2001) The *nsdD* gene encodes a putative GATA-type transcription factor necessary for sexual development of *Aspergillus nidulans*. Mol Microbiol 41:299–309

Han K-H, Seo J-A, Yu J-H (2004) A putative G protein-coupled receptor negatively controls sexual development in *Aspergillus nidulans*. Mol Microbiol 51:1333–1345

Harris SD, Momany C (2004) Polarity in filamentous fungi: moving beyond the yeast paradigm. Fungal Genet Biol 41:391–400

Heintz CE, Niederpruem DJ (1971) Ultrastructure of quiescent and germinated basidiospores and oidia of *Coprinus lagopus*. Mycologia 63:745–766

Heintzen C, Loros JJ, Dunlap JC (2001) The Pas protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. Cell 104:453–464

Hemmati F, Pell JK, McCartney HA, Deadman ML (2001) Airborne concentrations of conidia of *Erynia neoaphidis* above cereal fields. Mycol Res 105:485–489

Hiscock SJ, Kües U (1999) Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. Int Rev Cytol 193:165–295

Hoff B, Schmitt EK, Kück U (2005) CPCR1, but not its interacting transcription factor AcFKH1, controls fungal arthrospore formation in *Acremonium chrysosporium*. Mol Microbiol 56:1220–1233

Hollenstein M (1997) Untersuchungen zur Oidienbildung an Dikaryen von *Coprinus cinereus*. Wachstum, Oidiendifferenzierung und Lichtinduktivität der Oidienproduktion. Semesterarbeit, Institute for Microbiology, ETH Zurich, Switzerland

Horn BW, Greene RL, Sörensen RB, Blankenship PD, Dorner JW (2001) Conidial movement of nontoxigenic *Aspergillus flavus* and *A. parasiticus* in peanut fields following application to soil. Mycopathologia 151:81–92

Hovmøller MS, Justesen AF, Brown JKM (2002) Clonality and long-distance migration of *Puccinia striiformis* f.sp *tritici* in north-west Europe. Plant Pathol 51:24–32

Hui C, Tanaka Y, Takeo K, Kitamato Y (1999) Morphological and cytological aspects of oidium formation in a basidiomycete, *Pholiota nameko*. Mycoscience 40:95–102

Ishi K, Maruyama J, Juvvadi PR, Nakajima H, Kitamoto K (2005) Visualizing nuclear migration during conidiophore development in *Aspergillus nidulans* and *Aspergillus oryzae*: multinucleation of conidia occurs through direct migration of plural nuclei from phialides and confers greater viability and early germination in *Aspergillus oryzae*. Biosci Biotechnol Biochem 69:747–754

Jeffs LB, Xavier IJ, Matai RE, Khachatourians GG (1999) Relationships between fungal spore morphogenesis and surface properties for entomopathogenic members of the genera *Beauveria*, *Metarhizium*, *Paecilomyces*, *Tolypocladium*, and *Verticillium*. Can J Microbiol 45:936–948

Johnson A (2003) The biology of mating in *Candida albicans*. Nat Rev Microbiol 1:106–116

Johnstone IL, Hughes SG, Clutterbuck AJ (1985) Cloning an *Aspergillus nidulans* developmental gene by transformation. EMBO J 4:1307–1311

Käfer E (1965) The origin of translocations in *Aspergillus nidulans*. Genetics 52:217–232

Karos M, Fischer R (1996) *hymA* (hypha-like metulae), a new developmental mutant of *Aspergillus nidulans*. Microbiology 142:3211–3218

Karos M, Fischer R (1999) Molecular characterization of HymA, an evolutionarily highly conserved and highly expressed protein of *Aspergillus nidulans*. Mol Gen Genet 260:510–521

Kawasaki L, Aguirre J (2001) Multiple catalase genes are differentially regulated in *Aspergillus nidulans*. J Bacteriol 183:1434–1440

Kawasaki L, Sanchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45:1153–1163

Kays AM, Borkovich KA (2004) Severe impairment of growth and differentiation in a *Neurospora crassa* mutant lacking all heterotrimeric Gα proteins. Genetics 166:1229–1240

Kemp RFO (1975) Breeding biology of *Coprinus* species in the section *Lanatuli*. Trans Br Mycol Soc 65:375–388

Kemp RFO (1977) Oidial homing and the taxonomy and speciation of basidiomycetes with special reference to the genus *Coprinus*. In: Clémençon H (ed) The species concept in hymenomycetes. Cramer, Vaduz, Liechtenstein, pp 259–276

Kendrick B (ed) (1979a) The whole fungus, vol 1. National Museum of Natural Sciences, Ottawa, Canada

Kendrick B (ed) (1979b) The whole fungus, vol 2. National Museum of Natural Sciences, Ottawa, Canada

Kendrick B, Watling R (1979) Mitospores in basidiomycetes. In: Kendrick B (ed) The Whole Fungus, vol 2. National Museum of Natural Sciences, Ottawa, Canada, pp 473–546

Kershaw MJ, Talbot NJ (1998) Hydrophobins and repellents: proteins with fundamental roles in fungal morphogenesis. Fungal Genet Biol 23:18–33

Kertesz-Chaloupková K, Walser PJ, Granado JD, Aebi M, Kües U (1998) Blue light overrides repression of asexual sporulation by mating type genes in the basidiomcycete *Coprinus cinereus*. Fungal Genet Biol 23:95–109

Kim H-S, Han K-Y, Kim K-J, Han D-M, Jahng K-Y, Chae K-S (2002) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Dictionary of the fungi, 9th edn. CAB International, Wallingford, UK

Kitamato Y, Shishida M, Yamamoto H, Takeo K, Masuda P (2000) Nuclear selection in oidium formation from dikaryotic mycelia of *Flammulina velutipes*. Mycoscience 41:417–423

Kothe E (2001) Mating-type genes for basidiomycete strain improvement in mushroom farming. Appl Microbiol Biotechnol 56:602–612

Kothe GO, Free SJ (1998) The isolation and characterization of *nrc-1* and *ncr-2*, two genes encoding protein kinases that control growth and development in *Neurospora crassa*. Genetics 149:117–130

Krystofova S, Borkovich KA (2005) The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a Gβγ dimer required for normal female fertility, asexual development, and Gα protein levels in *Neurospora crassa*. Eukaryot Cell 4:365–378

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U (2002) Sexuelle und asexuelle Fortpflanzung bei einem Pilz mit 12000 Geschlechtern. Vierteljahrsschr Naturforsch Ges Zürich 147:23–34

Kües U, Granado JD, Hermann R, Boulianne RP, Kertesz-Chaloupková K, Aebi M (1998a) The *A* mating type and blue light regulate all known differentiation processes in the basidiomycete *Coprinus cinereus*. Mol Gen Genet 260:81–91

Kües U, Granado JD, Kertesz-Chaloupková K, Walser PJ, Hollenstein M, Polak E, Boulianne R, Bottoli APF, Aebi M (1998b) Mating type and light are major regulators of development in *Coprinus cinereus*. In: Van Griensven LJLD, Visser J (eds) Proc 4th Meet The Genetics and Cellular Biology of Basidiomycetes. Mushroom Experimental Station, Horst, The Netherlands

Kües U, Polak E, Bottoli APF, Hollenstein M, Walser PJ, Boulianne RP, Hermann R, Aebi M (2002a) Vegetative development in *Coprinus cinereus*. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 133–164

Kües U, Walser PJ, Klaus MJ, Aebi M (2002b) Influence of activated *A* and *B* mating-type pathways on developmental processes in the basidiomycete *Coprinus cinereus*. Mol Genet Genomics 268:262–271

Kües U, Künzler M, Bottoli APF, Walser PJ, Granado JD, Liu Y, Bertossa RC, Ciardo D, Clergeot P-H, Loos S et al. (2004) Mushroom development in higher basidiomycetes; implications for human and animal health. In: Kushwaha RKS (ed) Fungi in human and animal health. Scientific Publishers, Jodhpur, India, pp 431–469

Lau GW, Hamer JE (1998) *Acropetal*: a genetic locus required for conidiophore architecture and pathogenicity in the rice blast fungus. Fungal Genet Biol 24:228–239

Lauter FR, Russo VEA, Yanofsky C (1992) Developmental and light regulation of eas, the structural gene for the rodlet protein of *Neurospora*. Genes Dev 6:2373–2381

Lee BN, Adams TH (1994a) Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation, leads to activation of *brlA* and premature initiation of development. Mol Microbiol 14:323–334

Lee BN, Adams TH (1994b) The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev 8:641–651

Lee K, Ebbole DJ (1998) Tissue-specific repression of starvation and stress responses of the *Neurospora crassa con-10* gene is mediated by RCO1. Fungal Genet Biol 23:269–278

Lee JI, Choi JH, Park BC, Park YH, Lee MY, Park HM, Maeng PJ (2004) Differential expression of the chitin synthase genes of *Aspergillus nidulans*, *chsA*, *chsB*, and *chsC*, in response to developmental status and environmental factors. Fungal Genet Biol 41:635–646

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen W-C, Wang P, Pan X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Li DW, Kendrick B (1995) A year-round study on functional-relationships of airborne fungi with meteorological factors. Int J Biometeorol 39:74–80

Li DW, Kendrick B (1996) Functional and causal relationships between indoor and outdoor airborne fungi. Can J Bot 74:194–209

Linde CC, Zala M, Ceccarelli S, McDonald BA (2003) Further evidence for sexual reproduction in *Rhynchosporium secalis* based on distribution and frequency of mating-type alleles. Fungal Genet Biol 40:115–125

Liu Y, He Q, Cheng P (2003) Photoreception in *Neurospora*: a tale of two White Collar proteins. Cell Mol Life Sci 60:2131–2138

Madelin MF (1960) Visible changes in the vegetative mycelium of *Coprinus lapopus* Fr. at the time of fruiting. Trans Br Mycol Sco 43:105–110

Madi L, McBride SA, Bailey LA, Ebbole DJ (1997) *rco-3*, a gene involved in glucose transport and conidiation in *Neurospora crassa*. Genetics 146:499–508

Maheshwari R (1991) Microcycle conidiation and its genetic basis in *Neurospora crassa*. J Gen Microbiol 137:2103–2115

Maheshwari R (1999) Microconidia of *Neurospora crassa*. Fungal Genet Biol 26:1–18

Marchisio VF, Airaude D (2001) Temporal trends of the airborne fungi and their functional relations with the environment in a suburban site. Mycologia 93:831–840

Marshall MA, Timberlake WE (1991) *Aspergillus nidulans wetA* activates spore-specific gene expression. Mol Cell Biol 11:55–62

Mayorga ME, Timberlake WE (1990) Isolation and molecular characterization of the *Aspergillus nidulans wA* gene. Genetics 126:73–79

Mayorga ME, Timberlake WE (1992) The developmentally regulated *Aspergillus nidulans wA* gene encodes a polypeptide homologous to polyketide and fatty acid synthases. Mol Gen Genet 235:205–212

Melin P, Schnürer J, Wagner EGH (1999) Changes in *Aspergillus nidulans* gene expression induced by bafilomycin, a *Streptomyces*-produced antibiotic. Microbiology 145:1115–1122

Melin P, Schnurer J, Wagner EG (2003) Characterization of *phiA*, a gene essential for phialide development in *Aspergillus nidulans*. Fungal Genet Biol 40:234–241

Michan S, Lledias F, Hansberg W (2003) Asexual development is increased in *Neurospora crassa cat-3*-null mutant strains. Eukaryot Cell 2:798–808

Miller KY, Toennis TM, Adams TH, Miller BL (1991) Isolation and transcriptional characterization of a morphological modifier: the *Aspergillus nidulans* stunted (*stuA*) gene. Mol Gen Genet 227:285–292

Miller KY, Wu J, Miller BL (1992) *StuA* is required for cell pattern formation in *Aspergillus*. Genes Dev 6:1770–1782

Mims CW, Richardson EA, Timberlake WE (1988) Ultrastructural analysis of conidiophore development in the fungus *Aspergillus nidulans* using freeze-substitution. Protoplasma 44:132–141

Mirabito PM, Adams TH, Timberlake WE (1989) Interactions of three sequentially expressed genes control temporal and spatial specificity in *Aspergillus* development. Cell 57:859–868

Mooney JL, Yager LN (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge, UK

Murray TD, Walter CC (1991) Influence of pH and matric potential on sporulation of *Cephalosporium gramineum*. Phytopathology 81:79–84

Navarro RE, Aguirre J (1998) Posttranscriptional control mediates cell type-specific localization of catalase A during *Aspergillus nidulans* development. J Bacteriol 180:5733–5738

Nelson B, Kurischko C, Horecka J, Mody M, Nair P, Pratt L, Zougman A, McBroom LD, Hughes TR, Boone C et al. (2003) RAM: a conserved signaling network that regulates Ace2p transcriptional activity and polarized morphogenesis. Mol Biol Chem 14:3782–3803

Nishiura M, Hayashi N, Jwa NS, Lau GW, Hamer JE, Hasebe A (2000) Insertion of the LINE retrotransposon MGL causes a conidiophore pattern mutation in *Magnaporthe grisea*. Mol Plant Microb Interact 13:892–894

Nowrousian M, Duffield GE, Loros JJ, Dunlap JC (2003) The *frequency* gene is required for temperature-dependent regulation of many clock-controlled genes in *Neurospora crassa*. Genetics 164:923–933

Ohara T, Tsuge T (2004) *FoSTUA*, encoding a basic helix-loop-helix protein, differentially regulates development of three kinds of asexual spores, macroconidia, microconidia, and chlamydospores, in the fungal plant pathogen *Fusarium oxysporum*. Eukaryot Cell 3:1412–1422

Ohara T, Inoue I, Namiki F, Kunoh H, Tsuge T (2004) *REN1* is required for development of microconidia and macroconidia, but not of chlamydospores, in the plant pathogenic fungus *Fusarium oxysporum*. Genetics 166:113–124

Oliver PTP (1972) Conidiophore and spore development in *Aspergillus nidulans*. J Gen Microbiol 73:45–54

Parra R, Aldred D, Archer DB, Magan N (2004) Water activity, solute and temperature modify growth and spore production of wild type and genetically engineered *Aspergillus niger* strains. Enz Microb Technol 35:232–237

Pascon RC, Miller BL (2000) Morphogenesis in *Aspergillus nidulans* requires Dopey (DopA), a member of a novel family of leucine zipper-like proteins conserved from yeast to humans. Mol Microbiol 36:1250–1264

Peraza L, Hansberg W (2002) *Neurospora crassa* catalases, singlet oxygen and cell differentiation. Biol Chem 383:569–575

Perkins DD, Barry EG (1977) The cytogenetics of *Neurospora*. Adv Genet 19:133–285

Pöggeler S (2002) Genomic evidence for mating abilities in the asexual pathogen *Aspergillus fumigatus*. Curr Genet 42:153–160

Polak E (1999) Asexual sporulation in the basidiomycete *Coprinus cinereus*. PhD Thesis, ETH Zurich, Zurich, Switzerland

Polak E, Hermann R, Kües U, Aebi M (1997a) Asexual sporulation in *Coprinus cinereus*: structure and development of oidiophores and oidia in an *Amut Bmut* homokaryon. Fungal Genet Biol 22:112–126

Polak E, Kües U, Aebi M (1997b) Replica plating of *Coprinus cinereus* colonies using asexual spores. Fungal Genet Newslett 44:45–46

Polak E, Aebi M, Kües U (2001) Morphological variations in oidium formation in the basidiomycete *Coprinus cinereus*. Mycol Res 105:603–610

Pontecorvo G, Roper JA, Hemmons LM, MacDonald KD, Bufton AWJ (1953) The genetics of *Aspergillus nidulans*. Adv Genet 5:141–238

Prade R, Timberlake WE (1993) The *Aspergillus nidulans brlA* regulatory locus consists of two overlapping transcription units that are individually required for conidiophore development. EMBO J 12:2439–2447

Prade RA, Timberlake WE (1994) The *Penicillium chrysogenum* and *Aspergillus nidulans wetA* developmental regulatory genes are functionally equivalent. Mol Gen Genet 244:539–547

Rao PS, Niederpruem DJ (1969) Carbohydrate metabolism during morphogenesis of *Coprinus lagopus*. J Bacteriol 100:1222–1228

Rerngsamran P, Murphy MB, Doyle SA, Ebbole DJ (2005) Fluffy, the major regulator of conidiation in *Neurospora crassa*, directly activates a developmentally regulated hydrophobin gene. Mol Microbiol 56:282–297

Roberts AN, Yanofsky C (1989) Genes expressed during conidiation in *Neurospora crassa* – characterization of *con-8*. Nucleic Acids Res 17:197–214

Roberts AN, Berlin V, Hager KM, Yanofsky C (1988) Molecular analysis of a *Neurospora crassa* gene expressed during conidiation. Mol Cell Biol 8:2411–2418

Roncal T, Ugalde U (2003) Conidiation induction in *Penicillium*. Res Microbiol 154:539–546

Rosén S, Yu J-H, Adams TH (1999) The *Aspergillus nidulans sfaD* gene encodes a G protein β subunit that is required for normal growth and repression of sporulation. EMBO J 18:5592–5600

Rossier C, Oulevey N, Turian G (1973) Electron microscopy of selectively stimulated microconidiogenesis in wild type *Neurospora crassa*. Arch Microbiol 91:345–353

Ruoff P, Slewa I (2002) Circadian period lengths of lipid synthesis mutants (*cel*, *chol-1*) of *Neurospora* show defective temperature, but intact pH-compensation. Chronobiol Int 19:517–529

Ruoff P, Behzadi A, Hauglid M, Vinsjevik M, Havas H (2000) pH homeostasis of the circadian sporulation rhythm in clock mutants of *Neurospora crassa*. Chronobiol Int 17:733–750

Russo VEA, Pandit NN (1992) Development in *Neurospora crassa*. In: Russo VEA, Brody S, Cove D, Ottolenghi S (eds) Development. The molecular genetic approach. Springer, Berlin Heidelberg New York, pp 88–102

Sage RF (2002) How terrestrial organisms sense, signal, and respond to carbon dioxide. Int Comp Biol 42:469–480

Scherer M, Fischer R (1998) Purification and characterization of laccase II of *Aspergillus nidulans*. Arch Microbiol 170:78–84

Schier N, Fischer R (2002) The *Aspergillus nidulans* cyclin PclA accumulates in the nucleus and interacts with the central cell cycle regulator NimX(Cdc2). FEBS Lett 523:143–146

Schier N, Liese R, Fischer R (2001) A pcl-like cyclin of *Aspergillus nidulans* is transcriptionally activated by developmental regulators and is involved in sporulation. Mol Cell Biol 21:4075–4088

Schuren FHJ (1999) Atypical interactions between *thn* and wild-type mycelia of *Schizophyllum commune*. Mycol Res 103:1540–1544

Schuurs TA, Dalstra HJP, Scheer JMJ, Wessels JGH (1998) Positioning of nuclei in the secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. Fungal Genet Biol 23:150–161

Schwerdtfeger C, Linden H (2003) VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J 22:4846–4855

Seo JA, Han KH, Yu JH (2004) The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol Microbiol 53:1611–1623

Sewall TC (1994) Cellular effects of misscheduled *brlA*, *abaA*, and *wetA* expression in *Aspergillus nidulans*. Can J Microbiol 40:1035–1042

Sewall TC, Mims CW, Timberlake WE (1990) Conidium differentiation in *Aspergillus nidulans* wild-type and wet-white (*wet*) mutant strains. Dev Biol 138:499–508

Shi Z, Leung H (1995) Genetic analysis of sporulation in *Magnaporthe grisea* by chemical and insertional mutagenesis. Mol Plant Microb Interact 8:949–959

Shimizu K, Keller NP (2001) Genetic involvement of a cAMP-dependent protein kinase in a G protein signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans*. Genetics 157:591–600

Shrode LB, Lewis ZA, White LD, Bell-Pedersen D, Ebbole DJ (2001) *vvd* is required for light adaptation of conidiation-specific genes of *Neurospora crassa*, but not circadian conidiation. Fungal Genet Biol 32:169–181

Siegel RW, Matsuyama SS, Urey JC (1968) Induced macroconidia formation in *Neurospora crassa*. Experientia 24:1179–1181

Skromne I, Sánchez O, Aguirre J (1995) Starvation stress modulates the expression of the *Aspergillus nidulans brlA* regulatory gene. Microbiology 141:21–28

Smart MG, Howard KM, Bothast RJ (1992) Effect of carbon-dioxide on sporulation of *Alternaria crassa* and *Alternaria cassiae*. Mycopathologia 118:167–171

Som T, Kolaparthi VSR (1994) Developmental decisions in *Aspergillus nidulans* are modulated by ras activity. Mol Cell Biol 14:5333–5348

Springer ML (1993) Genetic control of fungal differentiation: the three sporulation pathways of *Neurospora crassa*. Bioessays 15:365–374

Springer ML, Yanofsky C (1989) A morphological and genetic analysis of conidiophore development in *Neurospora crassa*. Genes Dev 3:559–571

Stennett PJ, Begss PJ (2004) *Alternaria* spores in the atmosphere of Sydney, Australia, and relationships with meteorological factors. Int J Biometeorol 49:98–105

Stoldt VR, Sonneborn A, Leuker CE, Ernst JF (1997) Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. EMBO J 16:1982–1991

Stringer MA, Timberlake WE (1994) *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall. Mol Microbiol 16:33–44

Stringer MA, Dean RA, Sewall TC, Timberlake WE (1991) *Rodletless*, a new *Aspergillus* developmental mutant induced by directed gene inactivation. Genes Dev 5:1161–1171

Suelmann R, Sievers N, Galetzka D, Robertson L, Timberlake WE, Fischer R (1998) Increased nuclear traffic chaos in hyphae of *apsB* mutants of *Aspergillus nidulans*: molecular characterization of *apsB* and *in vivo* observation of nuclear behaviour. Mol Microbiol 30:831–842

Tan KK (1974) Red-far-red reversible photoreaction in the recovery form blue-light inhibition of sporulation in *Botrytis cinerea*. J Gen Microbiol 82:201–202

Terashima K, Yuki K, Muraguchi H, Akiyama M, Kamada T (2005) The *dst1* gene involved in mushroom photomorphogenesis of *Coprinus cinereus* encodes a putative photoreceptor for blue light. Genetics 117:101–108

Timberlake WE (1993) Translational triggering and feedback fixation in the control of fungal development. Plant Cell 5:1453–1460

Todd RB, Greenhalgh JR, Hynes MJ, Andrianopoulos A (2003) TupA, the *Penicillium marneffei* Tup1p homologue, represses both yeast and spore development. Mol Microbiol 48:85–94

Troutt C, Levetin E (2001) Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. Int J Biometeorol 45:64–74

Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller NP (2004a) Endogenous lipogenic regulators of spore balance in *Aspergillus nidulans*. Eukaryot Cell 3:1398–1411

Tsitsigiannis DI, Zarnowski R, Keller NP (2004b) The lipid body protein, PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Tsitsigiannis DI, Kowieski TM, Zarnowski R, Keller NP (2005) Three putative oxylipin biosynthetic genes integrate sexual and asexual development in *Aspergillus nidulans*. Microbiology 151:1809–1821

Tuncher A, Reinke H, Martic G, Caruso ML, Brakhage AA (2004) A basic-region helix-loop-helix protein-encoding gene (*devR*) involved in the development of *Aspergillus nidulans*. Mol Microbiol 52:364–372

Tymon AM, Kües U, Richardson WVJ, Casselton LA (1992) A fungal mating type protein that regulates sexual and asexual development contains a POU-related domain. EMBO J 11:1805–1813

Tzung KW, William RM, Scherer S, Federspiel N, Jones T, Hansen N, Bivolarevic V, Huizar L, Komp C, Surzycki R et al. (2001) Genomic evidence for a complete sexual cycle in *Candida albicans*. Proc Natl Acad Sci USA 98:3249–3253

Ulevicius V, Peciulyte D, Lugauskas A, Andriejauskiene J (2004) Field study on changes in viability of airborne fungal propagules exposed to UV radiation. Environ Toxicol 19:437–441

Van Maanen A, Gourbière F (2000) Balance between colonization and fructification in fungal dynamics control: a case study of *Lophodermium pinastri* on *Pinus sylvestris* needles. Mycol Res 104:587–594

Van Maanen A, Debouzie D, Gourbière F (2000) Distribution of three fungi colonizing fallen *Pinus sylvestris* needles along altitudinal transects. Mycol Res 104:1133–1138

Veith D, Scherr N, Efimov VP, Fischer R (2005) Role of the spindle-pole body protein ApsB and the cortex protein ApsA in microtubule organization and nuclear migration in *Aspergillus nidulans*. J Cell Sci 118:3705–3716

Virginia M, Appleyard CL, McPheat WL, Stark MJ (2000) A novel two-component protein containing histidine kinase and response regulator domains required for sporulation in *Aspergillus nidulans*. Curr Genet 37:364–372

von Arx JA (1981) The genera of fungi sporulating in pure culture. Cramer, Vaduz, Liechtenstein

Vossler J (2001) *Penicillium marneffei*: an emerging fungal pathogen. Clin Microbiol Newslett 23:25–29

Walser PJ (1997) Environmental regulation of asexual sporulation and fruit body formation in the basidiomycete *Coprinus cinereus*. Blue light induction and circadian rhythmicity of asexual sporulation. Nutritional control and carbon repression of oidiation and fruiting. MSc Thesis, ETH Zürich, Zürich, Switzerland

Walser PJ, Velagapudi R, Aebi M, Kües U (2003) Extracellular matrix proteins in mushroom development. Recent Res Dev Microbiol 7:381–415

Watling R (1979) The morphology, variation and ecological significance of anamorphs in the Agaricales. In: Kendrick B (ed) The whole fungus, vol 2. National Museum of Natural Sciences, Ottawa, Canada, pp 453–472

Wei H, Requena N, Fischer R (2003) The MAPKK-kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol Microbiol 47:1577–1589

Weismann A (1904) The evolution theory. Arnold, London, UK

Wheeler M, Guerrero-Plata A, Rico G, Torres-Guerrero H (2000) Biosynthesis and functions of melanin in *Sporothrix schenckii*. Infect Immun 68:3696–3703

White BT, Yanofsky C (1993) Structural characterization and expression analysis of the *Neurospora conidiation* gene *con-6*. Dev Biol 160:254–264

Wong S, Fares MA, Zimmermann W, Butler G, Wolfe KH (2003) Evidence from comparative genomics for a complete sexual cycle in the "asexual" pathogenic yeast *Candida glabrata*. Gen Biol 4:R10

Wu J, Miller BL (1997) *Aspergillus* asexual reproduction and sexual reproduction are differentially affected by transcriptional and translational mechanisms regulating *stunted* gene expression. Mol Cell Biol 17:6191–6201

Yamashiro CT, Ebbole DJ, Lee BU, Brown RE, Bourland C, Madi L, Yanosky C (1996) Characterization of *rco-1* of *Neurospora crassa*, a pleiotrophic gene affecting growth and development that encodes a homolog of Tup1 of *Saccharomyces cerevisiae*. Mol Cell Biol 16:6218–6228

Yang Q, Poole SI, Borkovich KA (2002) A G protein β subunit required for sexual and vegetative development and maintenance of normal Gα protein levels in *Neurospora crassa*. Eukaryot Cell 1:378–390

Ye XS, Lee S-L, Wolkow TD, McGuire S-L, Hamer JE, Wood GC, Osmani SA (1999) Interaction between developmental and cell cycle regulators is required for morphogenesis in *Aspergillus nidulans*. EMBO J 18:6994–7001

Ying SH, Feng MG (2004) Relationship between thermotolerance and hydrophobin-like proteins in aerial conidia of *Beauveria bassiana* and *Paecilomyces fumosoroseus* as fungal biocontrol agents. J Appl Microbiol 97:323–331

Yoshida Y, Hasunua K (2004) Reactive oxygen species affect photomorphogenesis in *Neurospora crassa*. J Biol Chem 279:6986–6993

Yu J-H, Wieser J, Adams TH (1996) The *Aspergillus* FlbA RGS domain protein antagonizes G-protein signaling to block proliferation and allow development. EMBO J 15:5184–5190

Yu J-H, Rosén S, Adams TH (1999) Extragenic suppressors of loss-of-function mutations in the *Aspergillus* FlbA regulator of G-protein signaling domain protein. Genetics 151:97–105

Zhang WM, Sulz M, Bailey KL (2001) Growth and spore production of *Plectosporium tabacinum*. Can J Bot 79:1297–1306

Zhang JX, Fernando WGD, Xue AG (2005) Daily and seasonal spore dispersal by *Mycosphaerella pinodes* and development of *Mycopshaerella* blight on field pea. Can J Bot 83:302–310

Zuber S, Hynes MJ, Andrianopoulos A (2002) G-protein signaling mediates development at 25 °C but has no effect on yeast-like growth at 37 °C in the dimorphic fungus *Penicillium marneffei*. Eukaryot Cell 1:440–447

Zuber S, Hynes MJ, Andrianopoulus A (2003) The G-protein α subunit GasC plays a major role in germination in the dimorphic fungus *Penicillium marneffei*. Genetics 164:487–499

15 Mating-Type Structure, Evolution, and Function in Euascomycetes

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I. Introduction

Sexual reproduction requires sensing the environment to find a mate, and mating engages a complex machinery for information exchange between individuals, cells, nuclei, chromosomes and genes. This very demanding process, called self-incompatibility or heterothallism in fungi, while conserved in general, has an intriguing history of modifications in this group of organisms, likely correlated with the wide range of natural environments. Self-incompatible individuals from the same species display different mating types. Individuals of identical mating type are defined, primarily, as individuals that cannot mate with each other, but can mate with individuals from another group that display a different mating type. This phenotypic complexity can be disentangled by genetics, which permits one to establish how many loci control mating type. To date, all self-incompatible Euascomycetes have been found to harbor only one locus (*MAT*)

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that contains one or the other of two possible forms of genetic information establishing mating type. An alternative mating strategy, called self-compatibility, is less demanding than self-incompatibility. Homokaryotic cultures of self-compatible fungi can undergo self-mating. These fungi (also known as homothallic fungi) nevertheless perform all steps of meiosis. A special case of self-compatibility (also known as pseudohomothallism or secondary homothallism) has been described for a few genera. These isolates can be resolved into homokaryotic self-incompatible cultures, indicating that isolates are heterokaryons, carrying nuclei of opposite mating type. A survey of 10,596 Ascomycetes showed that 55% may reproduce sexually, but evidence of mating is lacking for the remaining 45% of these species (Reynolds and Taylor 1993). Whether or not these apparently asexual species ever undergo sexual reproduction, or have cryptic or rare sex is often debated. A decade ago, shortly after mating-type genes were first identified in filamentous fungi, finding mating-type genes in asexual fungi was a novel discovery (first reported by Sharon et al. 1996), but is now commonplace. Whatever the reason for lack of a sexual stage (cryptic sexuality, mutations in key genes), absence of the sexual reproductive machinery is not the answer. This finding supports the notion that the asexual lifestyle arises from sexual progenitors.

This chapter emphasizes new developments in our knowledge of mating-type structure in self-compatible, self-incompatible, and asexual Euascomycetes. It presents a possible scenario for mating evolution among the three different classes of fungi, as deduced from mating-type structures, and considers functions attributed to mating-type genes. Space limitations excluded consideration of several fields that the reader can find in other reviews focusing on, for example, genetic aspects of unstable mating types (Perkins 1987), theoretical aspects of mating type (Metzenberg 1990), mating-type functions (Metzenberg and Glass 1990), vegetative incompatibility (Glass and Kuldau 1992), genetic aspects of mating systems (Coppin et al. 1997), physiology of intercellular recognition (Bistis 1998), evolution and pathology (Turgeon 1998), mating types and biotechnology (Pöggeler 2001), commonalities between Ascomycetes and Basidiomycetes (Casselton 2002), and more general aspects (Glass and Lorimer 1991; Glass and Nelson 1994; Kronstad and Staben 1997; Hiscock and Kes 1999; Shiu and Glass 2000; Souza et al. 2003).

II. Sexual Development in Mycelial Euascomycetes

The life cycle of Euascomycetes that are used as model systems, such as *Podospora anserina* and *Gibberella zeae*, is presented in Fig. 15.1. Self-incompatible homokaryotic strains of either mating type produce female reproductive structures, the ascogonia, and male cells, the spermatia. The spermatia are true differentiated sexual cells unable to germinate, in contrast to conidia. Fertilization occurs between an ascogonium of one mating type and a donor cell of opposite mating type. The donor cells may be spermatia, microconidia, macroconidia or hyphae. Ascogonia are topped by a specialized hypha, the trichogyne, which is attracted by the donor cell of opposite mating type and eventually fuses with it (Bistis 1998). Fertilization is followed by the migration of the male nucleus down the trichogyne toward the body of the female organ. During its migration, the male nucleus passes by female nuclei and when present in the body of the female organ, it undergoes a series of mitotic divisions resulting in the formation of plurinucleate cells containing nuclei of opposite mating types. Successful production of offspring depends on internuclear recognition, a process associated with cellularization whereby two nuclei, one of each mating type, migrate to form specialized dikaryotic hyphae. These hyphae, called ascogenous hyphae, maintain a strict ratio of 1:1 of each parental nucleus. Eventually, the tip of this type of hypha differentiates into a crozier, which consists of two uninucleate cells and one dikaryotic cell. Nuclei fuse and meiosis ensues immediately while the cell is elongating to form the ascus. After a post-meiotic mitosis, ascospores are delineated inside the ascus and eventually projected outside the fruiting body (see Chap. 20, this volume). In self-compatible species, the male nucleus required for fertilization is assumed to be provided to the ascogonium by its basal cell, but outcrossing indicates that external cells can occasionally be the source of a male nucleus. Variations to this description can be found in Euascomycetes, but the formation of a plurinucleate cell after fertilization and cellularization associated with internuclear recognition leading to the formation of ascogenous hyphae seems to be the common scheme.

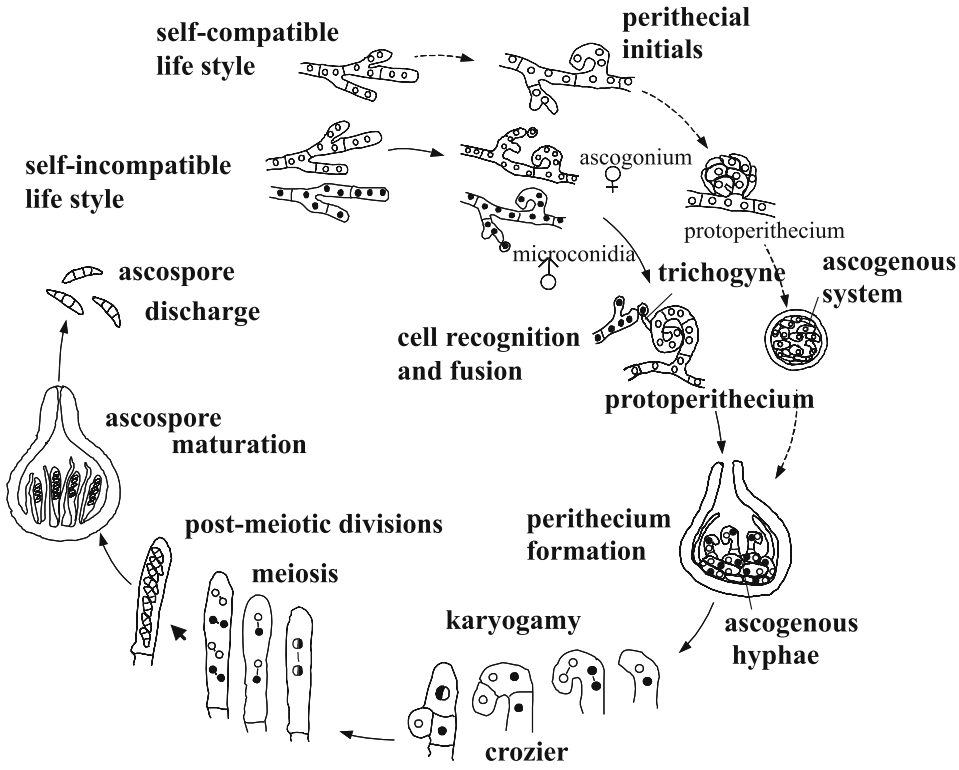

Fig. 15.1. Life cycle of Euascomycetes. *Outer circle*: self-compatible life cycle. *Inner circle*: self-incompatible life cycle (adapted from a drawing by S.-W. Lu)

III. Description of Mating-Type Structure

A. General Organization of Mating Types

In all self-incompatible filamentous Ascomycetes examined to date, the mating-type locus contains one of two different sequences occupying the same chromosomal locus in their genome. The extent of dissimilarity ranges from 1 to almost 6 kb. Metzenberg and Glass used the word "idiomorph" to denote these large sequences not obviously related by structure or common descent (Metzenberg and Glass 1990). The analysis of self-compatible species indicates that, in most cases, both mating types are contained in the haploid genome, usually tightly linked, but sometimes not, and thus these loci cannot be called idiomorphs, since there is no second strain of opposite mating type. We retain the use of the term "mating type", instead of "idiomorph", for self-compatible species. A standardized nomenclature for idiomorphs and mating-type genes has been proposed, and, in most cases adopted, to cope with the rapidly growing number of described mating-type loci from various species (Turgeon and Yoder 2000). This standardized nomenclature is based on the constant presence in one idiomorph of an ORF encoding a protein with a motif called the α1 domain, found initially in the MATα1 transcription factor of *Saccharomyces cerevisiae*. This idiomorph is called *MAT1-1*, and the corresponding gene *MAT1-1-1*. The other idiomorph is characterized by the constant presence of an ORF encoding a protein with a DNA-binding domain of the high-mobility group (HMG) type and is called *MAT1-2*. The corresponding gene is called *MAT1-2-1*. The number assigned to any particular gene within an idiomorph corresponds to that of its homolog in other fungi that have already characterized *MAT* genes. If there is no apparent homolog, the next sequential number is assigned. If a second mating-type locus is discovered in the same fungus, it would be *MAT2*. This system is adhered to by the fungal community, with the exception of members of the *Neurospora crassa* and *P. anserina* communities, since designation of genes from these genera was established before standard terminology was proposed. To date, for self-incompatible Ascomycetes, including the models *Cochliobolus heterostrophus*, *P. anserina*, and *N. crassa*, only a single

locus has been found. This locus will be described below in self-incompatible, self-compatible, and asexual species, respectively.

1. Loculoascomycetes

a) Self-Incompatible Loculoascomycetes

The simplest idiomorphs are those of self-incompatible Loculoascomycetes (Fig. 15.2). For all taxa in this group, *MAT1-1* and *MAT1-2* idiomorphs consist of one ORF corresponding to genes encoding the α1 and HMG proteins, respectively (Table 15.1). Following standard nomenclature, described above, the genes have been designated *MAT1-1-1* and *MAT1-2-1*. The size of the idiomorphs varies with species, but both encoded *MAT* genes have the same orientation with respect to their chromosome. For *C. heterostrophus*, the first described for this class, each ORF occupies almost all of the idiomorph sequence (Turgeon et al. 1993). Expression analysis revealed that both *MAT1-1-1* and *MAT1-2-1* transcripts start and stop in common sequences flanking the idiomorphs, resulting in transcripts that are almost twice the size of the corresponding ORF (Leubner-Metzger et al. 1997). Multiple transcripts, resulting from the use of at least two transcription start sites and alternative splicing of 5′ UTR introns, are produced. The 5′ UTR of the *MAT* messages contain μORFs that could control translation of the downstream coding sequence. However, the transcription initiation sites and the 5′ UTR region of the *MAT* genes have been deleted in both parents without penalty to fertility, indicating that these regions do not contain information essential for mating (Wirsel et al. 1998). By contrast, the 3′ UTR of the idiomorphic genes contains structural information that is necessary for completion of the sexual cycle. The molecular function of this sequence present in the 3′ common flanking sequence of *MAT1-1* and *MAT1-2* idiomorphs is as yet unknown. This one gene/one idiomorph structure has also been found in *C. ellisii*, *C. carbonum*, *C. victoriae*, and *C. intermedius* (Yun et al. 1999). The *MAT* idiomorphs of *Didymella rabiei* (sexual state of *Ascochyta rabiei*; Barve et al. 2003), *Mycosphaerella graminicola* (sexual form of *Septoria tritici*; Waalwijk et al. 2002) and *Phaeosphaeria nodorum* (sexual state of *Stagonospora nodorum*; Bennett et al. 2003) also contain a single ORF that starts and terminates in the idiomorph, but the sizes of the idiomorphic sequences are larger than those of *C. heterostrophus* (Table 15.1). Transcription initiation sites have not been established for these fungi. It is possible that, in contrast to *C. heterostrophus*, the promoter is localized in the *MAT*-specific sequences, raising the possibility that, unlike *C. heterostrophus*, each *MAT* gene responds to specific transcription regulatory factors.

b) Self-Compatible Loculoascomycetes

Self-compatible Loculoascomycetes display various arrangements of *MAT1-1-1* and *MAT1-2-1* genes in their haploid genome (Fig. 15.2 and Table 15.2). *C. cymbopogonis* carries complete *MAT1-1-1* and *MAT1-2-1* counterparts, in contrast to *C. luttrellii* and *C. homomorphus* that contain *MAT* gene fusions (Yun et al. 1999). These fusions consist of either *MAT1-1-1*::*MAT1-2-1* (*C. luttrellii*) or *MAT1-2-1*::*MAT1-1-1* (*C. homomorphus*) recombinant genes. Possible evolution of these self-compatible species from self-incompatible ancestors is detailed in Sect. IV. A more complex structure has been found in *C. kusanoi* (Yun et al. 1999). This fungus contains a complete *MAT1-1-1* homolog linked to a *MAT1-1-1*::*MAT1-2-1* chimeric gene that is devoid of the α1 encoding region but contains the HMG encoding sequence. The *MAT* locus from a self-compatible species outside of the genus *Cochliobolus*, *Mycosphaerella zeae-maydis* (*Didymella zeae-maydis*), has been characterized (Yun 1998). In this case, *M. zeae-maydis MAT* has complete versions of the *C. heterostrophus MAT* counterparts tandemly arranged, head to tail, separated by about one kilobase of non-coding sequence.

A total of 106 diverse isolates of *Stemphylium* have been examined to determine the structure of the mating-type loci (Inderbitzin et al. 2005). Isolates were found to contain both separate and linked *MAT* genes. Where separate, *MAT* loci contain either a *MAT1-1-1* or *MAT1-2-1* gene in the same 5′->3′ orientation as in *Cochliobolus*, between upstream *ORF1* and downstream *BGL1* genes (Fig. 15.3). Self-compatible isolates were isolated from field ascomata or conidia and demonstrated to undergo self-mating in the laboratory. These isolates had two types of *MAT* locus configuration. In the first type, the two *MAT* genes are linked. The *MAT1-1-1* gene and both flanks are inverted and connected to *MAT1-2-1* oriented in the original *MAT* gene direction (as in *Cochliobolus*), approx. 200 bp upstream of the *MAT1-2-1*

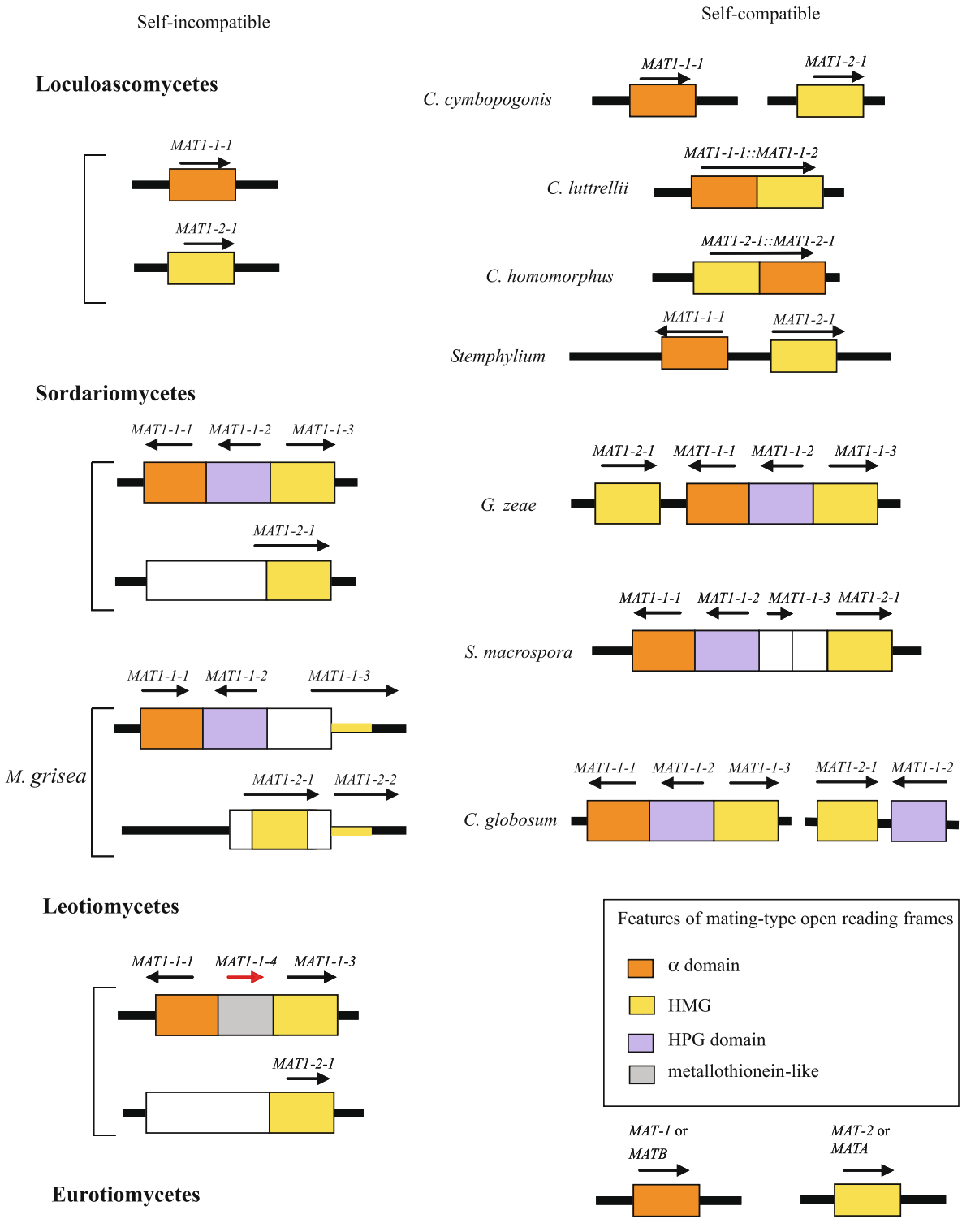

Fig. 15.2. General structure of the *MAT* locus in self-incompatible and self-compatible Euascomycetes. *Arrows* indicate position and orientation of the genes. *Black arrows* indicate genes found in all studied species in each class. *Red arrows* indicate genes that are absent in some representative in each class

Table. 15.1. Structure of the *MAT* locus in self-incompatible and related asexual Euascomycetes

	Idiomorphs	Accession number	Size of idiomorph[a]	Genes	Features
Loculoascomycetes					
Cochliobolus heterostrophus	*MAT1-1*	AF029913	1297	*MAT1-1-1*	α domain
	MAT1-2	AF027687	1171	*MAT1-2-1*	HMG
Phaeosphaeria nodorum	*MAT1-1*	AY212018	4282	*MAT1-1-1*	α domain
	MAT1-2	AY212019	4505	*MAT1-2-1*	HMG
Alternaria alternata[b]	*MAT1-1*	AB009451	1942	*MAT1-1-1*	α domain
	MAT1-2	AB009452	2256	*MAT1-2-1*	HMG
Didymella rabiei	*MAT1-1*	Barve et al. (2003)	2294	*MAT1-1-1*	α domain
	MAT1-2		2693	*MAT1-2-1*	HMG
Mycosphaerella graminicola	*MAT1-1*	AF440399	2839	*MAT1-1-1*	α domain
	MAT1-2	AF440398	2772	*MAT1-2-1*	HMG
Sordariomycetes					
Neurospora crassa	*mat A*	M33876	5736	*mat A-1*	α domain
				mat A-2	HPG domain
				mat A-3	HMG
	mat a	M54787	3235	*mat a-1*	HMG
				mat a-2	MiniORF
Podospora anserina	*mat−*	X64194	4710	*FMR1*	α domain
		X73830		*SMR1*	HPG domain
		X73830		*SMR2*	HMG
	mat+	X64195	3869	*FPR1*	HMG
Gibberella moniliformis (*Gibberella fujikuroi*)	*MAT1-1*	AF100925	4605	*MAT1-1-1*	α domain
				MAT1-1-2	HPG domain
		Kanamori and Arie, personal communication		*MAT1-1-3*	HMG
	MAT1-2	AF100926	3824	*MAT1-2-1*	HMG
Fusarium oxysporum[b]	*MAT1-1*	AB011379	4614	*MAT1-1-1*	α domain
				MAT1-1-2	HPG domain
				MAT1-1-3	HMG
	MAT1-2	AB011378	3821	*MAT1-2-1*	HMG
Cryphonectria parasitica	*MAT1-1*	AF380365	4691	*MAT1-1-1*	α domain
				MAT1-1-2	HPG domain
				MAT1-1-3	HMG
	MAT1-2	AF380364	2810	*MAT1-2-1*	HMG
Magnaporthe grisea	*MAT1-1*	AB080670	3388	*MAT1-1-1*	α domain
				MAT1-1-2	HPG domain
				MAT1-1-3	HMG
	MAT1-2	AB080671	2459	*MAT1-2-1*	HMG
				MAT1-2-2	HMG
Paecilomyces tenuipes[b]	*MAT1-1*	AB096216	3.8 kbp	*MAT1-1-1*	α domain
				MAT1-1-2	HPG domain
	MAT1-2	AB084921	4 kbp	*MAT1-2-1*	HMG
				MAT1-1-1	Pseudogene
				MAT1-2-2	None identified[c]
Leotiomycetes					
Pyrenopeziza brassicae	*MAT1-1*	AJ006073	4804	*MAT1-1-1*	α domain
				MAT1-1-4	Metallothionein
				MAT1-1-3	HMG
	MAT1-2	AJ006072	4147	*MAT1-2-1*	HMG
Rhynchosporium secalis[b]	*MAT1-1*	AJ549759	4049	*MAT1-1-1*	α domain
				MAT1-1-3	HMG
	MAT1-2	AJ537511	3153	*MAT1-2-1*	HMG

[a]In base pairs unless stated otherwise [b]Asexual species [c]This study

Table. 15.2. Structure of the *MAT* locus in self-compatible Euascomycetes

	Accession number	Genes	Features
Loculoascomycetes			
Cochliobolus homomorphus	AF129741	*MAT1-2-1*::*MAT1-1-1*	HMG+α domain
Cochliobolus luttrellii	AF129740	*MAT1-1-1*::*MAT1-2-1*	α domain+HMG
Cochliobolus kusanoi	AF129742	*MAT1-1-1*::*MAT1-2-1*	HMG
		MAT1-1-1	α domain
Cochliobolus cymbopogonis	AF129744	*MAT1-1-1*	α domain
	AF129745	*MAT1-2-1*	HMG
Sordariomycetes			
Gibberella zeae	AF318048	*MAT1-1-1*	α domain
		MAT1-1-2	HPG domain
		MAT1-1-3	HMG
		MAT1-2-1	HMG
Sordaria macrospora	Y10616	*Smt A-1*	α domain
		Smt A-2	HPG domain
		Smt A-3	None identified
		Sm a-1	HMG
Chaetomium globosum	Broad Institute	*MAT1-1-1*	α domain
		MAT1-1-2	HPG domain
		MAT1-1-3	HMG
		MAT1-2-1	HMG
		MAT1-1-2 like	HPG domain
Eurotiomycetes			
Emericella nidulans	AY339600	*MATB-1* (*MAT-1*, *MATB*)[a]	α domain
	AF508279	*MAT1-2* (*MAT-2*, *MATA*)[a]	HMG

[a] Names in parenthesis are different nomenclatures of the same gene

gene, and between it and *ORF1* (Fig. 15.2). Notably, there is also a group of self-compatible isolates carrying only *MAT1-1-1*, a situation analogous to certain *Neurospora* self-compatible isolates (e.g., *N. africana*) where only the *mat A-1* gene is detected (Glass et al. 1988, 1990). The remaining isolates of *Stemphylium* examined have undetermined sexual states and may be asexual, self-incompatible, or possibly debilitated selfers.

These analyses indicate that mating-type gene arrangements are diverse in self-compatible Loculoascomycetes. A striking observation is that *MAT1-2-1* was not detected in some *Stemphylium* isolates, which suggests that the HMG regulatory domain might be dispensable for sexual cycle control. Another possibility may be proposed, based on the recent finding that each *MAT* gene of *Cochliobolus* spp. appears to encode a protein with both HMG and α1 activities (Lu and Turgeon, unpublished data; see Sect. III.C.1); the regulatory functions of the lost *MAT1-2-1* gene may be taken on by the remaining *MAT1-1-1* gene. Evolution of the target genes of MAT1-2-1 would then be necessary to bring them under the control of the HMG domain of MAT1-1-1. A first step for the validation of this hypothesis will be to demonstrate that *Stemphylium* MAT proteins have structures similar to those of *Cochliobolus* spp.

c) Asexual Loculoascomycetes

The evolutionary conservation of *MAT* genes in sexual Loculoascomycetes prompted Turgeon and colleagues to ask if there were homologs of these genes in related species that had never been demonstrated to reproduce sexually (Sharon et al. 1996). This investigation was first conducted with *Bipolaris sacchari*, a pathogen of sugarcane. Among 21 field isolates, 19 were demonstrated to carry a *MAT1-2-1* gene and two carried a *MAT1-1-1* gene. Recently Schoch, Saenz, and Turgeon (unpublished data) have updated this study, using more isolates and several molecular characters to investigate mating-type distribution and phylogenetic relationships of asexual *B. sacchari*. Combined data from both *MAT* genes, plus the 5′ *MAT* flank, the 3′ *MAT* flank, and several additional sequences, confirm that *B. sacchari* isolates exist in the field as both mating types. All datasets indicated that the *B. sacchari* isolates fall into two well-supported groups, with representatives of both mating types in each group. The deduced MAT1-2-1 protein of

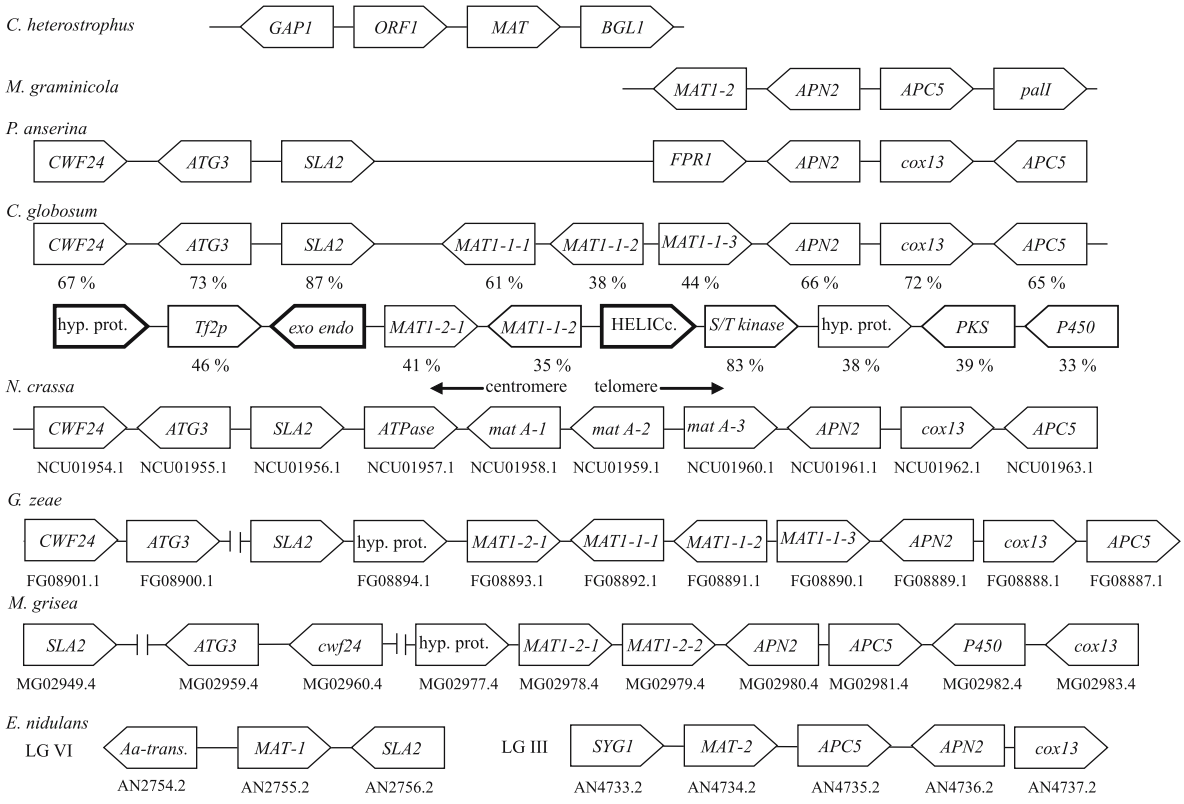

Fig. 15.3. Comparative organization of the *MAT* locus in eight Euascomycete species. Analyses are reported for *P. anserina* (LG I, supercontig G, 69,500 to 92,000), *C. globosum* (LG not determined, supercontig 1.3, bases 3,980,000 to 4,001,000 and supercontig 1.2, bases 4,630,000 to 4,665,000), *N. crassa* (LG I, contig 3.86 and 3.87), *G. zeae* (LG not determined, contig 1.359 and 1.358), *M. grisea* (LG III, contig 2.596, 2.597 and 2.600), and *E. nidulans* (LG VI, contig 1.49 and LG III, contig 1.80). Gene positions are indicated by *arrowed boxes* with gene name inside. Below each box is the *gene number*, as referred to by the sequencing project, when available. For *C. globosum*, the number under the box indicates the percentage of identity at the amino-acid level with the *P. anserina* putative homolog. *Arrowed boxes with thick lines* indicate *C. globosum* genes that have no *P. anserina* counterparts. Distances and gene sizes are not to scale; large genome sequences containing genes not relevant to the comparison have been omitted, and are symbolized by breaks in the line between genes. The number of genes present in the break can be inferred from the gene number adjacent to the break. Gene names refer to the GenBank name or to a gene encoding a protein showing best similarity to the putative gene product following Blastp analysis (Altschul et al. 1997). When no significant similarity was found, but a conserved domain was found, this domain was used for identifying the gene. Gene products with no similarity in Swissprot, nor with any identified domain, are indicated as hypothetical protein (*hyp. prot.*). *Aa_trans* Transmembrane amino-acid transporter domain, *APC5* similar to anaphase promoting factor component 5 of *S. pombe* (accession no. Q9P4W7), *ATG3* similar to autophagocytosis protein of *S. cerevisiae* (accession no. P40344), *ATPase* cation transport ATPase domain, *BGL1* beta glucosidase homolog (accession number AAB82946.1), *cox13* similar to cytochrome c oxydase polypeptide VIa, mitochondrial precursor, of *S. pombe* (accession no. O74471), *CWF24* similar to cell cycle control protein of *S. pombe* (accession number Q9P6R8), *APN2* similar to DNA (apurinic and apyrimidinic site) lyase 2 of *S. cerevisiae* (accession no. P38207), *GAP1* GTPase activating protein homolog (accession no. AAB82943.1), *HMG* high-mobility-group domain, *endo exo* endo_exo_phos domain, *ORF1* similar to *S. cerevisiae* ORF YLR456W (accession no. U22383), *palI* similar to *palI* of *E. nidulans* (accession no. CAA07588), *P450* similar to trichodiene oxygenase of *Fusarium sporotrichioides* (accession no. Q12612), *PKS* similar to conidial yellow pigment biosynthesis polyketide synthase of *E. nidulans* (accession no. Q03149), *SLA2* similar to SLA2 transmembrane protein of *S. cerevisae* (accession no. P33338), *S/T* kinase similar to serine/threonine protein kinase involved in the regulation of DNA repair in *S. pombe* (accession no. P40235), *SYG1* similar to SYG1 protein of *S. cerevisiae* (accession no. P40528), *Tf2p* similar to retrotransposable element TF2p 155-kDa protein type1 of *S. pombe* (accession no. Q05654)

B. sacchari is 97% identical to its counterpart in *C. heterostrophus* (Sharon et al. 1996). Another asexual species, *Alternaria alternata*, was found to contain *MAT1-1* or *MAT1-2* idiomorphs that are structurally similar to those of *C. heterostrophus* (Arie et al. 2000). For both asexual species, the function of the cloned asexual genes was tested in *C. heterostrophus* and shown to induce the formation of fertile pseudothecia. This test demonstrated that asexual *B. sacchari* and *A. alternata* possess functional orthologs of the mating-type genes of sexual species. The lack of sexual cycle in at least one *B. sacchari* isolate may result from a *MAT* transcriptional defect. Northern blot analyses failed to detect any *B. sacchari MAT1-2-1* transcript in isolate 764.1 of *B. sacchari*, although the heterologous *C. heterostrophus MAT1-1-1* and *MAT1-2-1* genes were expressed. A conclusive statement regarding expression of the *B. sacchari MAT* genes awaits examination of expression in additional isolates by RT-PCR, informed by the updated phylogenetic analysis described above. Expression of both *C. heterostrophus MAT* genes in *B. sacchari* does not induce the formation of fertile pseudothecia, suggesting that the sexual defect cannot be attributed only to the transcription defect of the endogenous *MAT1-2-1* gene. A transcription defect hypothesis seems to be excluded for the *A. alternata* case, since RT-PCR analyses indicated that both *MAT* genes are transcribed. These data support the argument that asexual Loculoascomycetes arise from sexual progenitors, and indicate that the cause of asexuality may be defects in any gene in the mating-type pathway, such as the target genes of the *MAT* regulatory factors.

2. Sordariomycetes

a) Self-Incompatible Sordariomycetes

Two self-incompatible Sordariomycetes, *N. crassa* and *P. anserina*, have served as model systems to investigate the structure of the idiomorphs, which were found to be similar in other self-incompatible species from this group. The *MAT1-1* idiomorph contains three genes: *MAT1-1-1*, which is the hallmark of the idiomorph, *MAT1-1-2* and *MAT1-1-3* (Fig. 15.2). These genes are designated *mat A-1*, *A-2* and *A-3* in *N. crassa*, *FMR1*, *SMR1* and *SMR2* in *P. anserina*, respectively (Table 15.1). The *MAT1-1-2* gene is located upstream of *MAT1-1-1* (Fig. 15.2). The MAT1-1-2 proteins are characterized by a HPG domain (see Sect. C) but their molecular function is not yet known. Investigation of *SMR1* indicated that this is not a bona fide mating-type gene, since mating does not display any defect whatever the location of *SMR1*, in either the one or the other parent, and even when present in both parents (Arnaise et al. 1997). *MAT1-1-2* is consistently present in all Sordariomycetes, and no homolog has been identified in species outside this taxon. These data strongly suggest that *MAT1-1-2* is a characteristic of the Sordariomycete mating-type system, and that its molecular function is linked to a specific feature of Sordariomycete mating type. A third gene, *MAT1-1-3*, is present in the *MAT1-1* idiomorph of self-incompatible Sordariomycetes. The *MAT1-1-3* gene is located at the end of the idiomorph distal to *MAT1-1-1* (Fig. 15.2). MAT1-1-3 proteins are characterized by the presence of a HMG domain.

The *MAT1-2* idiomorph contains one ORF encoding a protein with a HMG domain (Fig. 15.2), corresponding to the *MAT1-2-1* gene (*mat a-1* in *N. crassa* and *FPR1* in *P. anserina*, Table 15.1). Pöggeler and Kück performed a detailed analysis of the idiomorphic region upstream of the *mat a-1* ORF (Pöggeler and Kück 2000) in *N. crassa*, and demonstrated that the *mat a-1* transcript extended at least 1252 nucleotides upstream of the putative translational start. This transcript contained several µORFs and one miniORF of 79 residues with a 147-bp intron. The authors proposed that this miniORF corresponds to a new gene called *mat a-2*. However, *mat a-2* coding sequence does not contain any motif indicative of its molecular function, and it has no homolog in *MAT1-2* idiomorphs of other Sordariomycetes. Moreover, Chang and Staben have tested the mating behavior of *N. crassa* strains containing truncated versions of the *mat a-1* gene lacking most of the 5′ untranslated region, and notably the *mat a-2* sequence (Chang and Staben 1994). These strains mated as an *a* wild-type strain, indicating that the idiomorphic region upstream of the *mat a-1* ORF does not contain any gene or structure essential for mating in *N. crassa*.

The idiomorphs of *Magnaporthe grisea* display a structure differing from the consensus observed in other self-incompatible Sordariomycetes (Fig. 15.2 and Table 15.2; Kanamori and Arie, personal communication). The *MAT1-1-1* gene has an orientation opposite to its orientation in other Sordariomycetes. Surprisingly, the *MAT1-1-3* ORF extends from the idiomorph into the flanking sequence, and this region includes the HMG do-

main. The *MAT1-2* idiomorph contains *MAT1-2-1* and another gene, *MAT1-2-2*, which also extends into the common flanking sequence that is shared with *MAT1-1-3*, encoding the HMG domain. The *MAT1-2-2* ORF begins just at the boundary between *MAT1-2* and the flanking sequence. The function of *MAT1-2-2* is as yet unknown.

b) Self-Compatible Sordariomycetes

The structure of the mating-type genes in self-compatible Sordariomycetes has been determined for only three species: *Gibberella zeae*, *Sordaria macrospora*, and *Chaetomium globosum* (Fig. 15.2 and Table 15.2). *G. zeae* carries linked counterparts of the self-incompatible Sordiariomycete *MAT* genes, i.e., three genes structurally identical to the *MAT1-1* mating-type gene sequences in self-incompatible *G. fujikuroi* (*G. moniliformis*), separated from *MAT1-2-1* by 611 bp (Yun et al. 2000). *S. macrospora* contains a homolog of *MAT1-1-1* (called *Smt A-1*) and *MAT1-1-2* (called *Smt A-2*) whereas the counterpart of *MAT1-1-3* (called *Smt A-3*) lacks the region encoding the HMG domain specific to the *MAT1-1-3* genes found in self-incompatible Sordariomycetes (Pöggeler et al. 1997). The *Smt A-3* gene is located 813 bp upstream of the *MAT1-2-1* gene (called *Smt a-1*) and is co-transcribed with *Smt a-1* (Pöggeler and Kück 2000). This structure was proposed, by the authors, to derive from an unequal crossover between the *MAT1-1-3* gene and the *MAT1-2* idiomorph in a putative self-incompatible ancestor of *S. macrospora*. It is not known if the truncated *Smt A-3* gene still encodes a protein essential for the sexual cycle, or if it provides a promoter and a transcriptional start site for the downstream *Smt a-1* gene, or if it serves as a regulatory miniORF. The recently sequenced self-compatible *C. globosum* belongs to a group closely related to *P. anserina* (Liu and Hall 2004). It contains a *MAT1-1* mating type structurally identical to the *MAT1-1* (*mat-*) idiomorph of *P. anserina*, and a *MAT1-2-1* gene on a different supercontig. Surprisingly, *C. globosum* contains two different copies of the *MAT1-1-2* gene, one at the *MAT1-1* locus, the other 1540 bp downstream of the *MAT1-2-1* gene.

Hybridization of cloned portions of the idiomorphs of *N. crassa* to the genome of related self-compatible species has revealed three classes of hybridization pattern correlated with phylogenetic relationships (Dettman et al. 2001). The hybridization patterns of *N. terricola* provide evidence for the presence of *MAT1-1* and *MAT1-2* sequences (Glass et al. 1988), but subsequent probing with portions of the idiomorphs reveals that *MAT1-1* has lost sequences in the region corresponding to the *MAT1-1-3* gene (Beatty et al. 1994). Another distinct phylogenetic group consists of *N. africana*, *N. dodgei*, and *N. galapagosensis*, which may be individuals of the same species. *N. lineolata* represents a distinct lineage from the others, but still closely related. These four self-compatible species or isolates all contain the *MAT1-1* sequence but none of them contains a sequence that hybridizes to probes encompassing the *MAT1-2-1* (*mat a-1*) gene, suggesting that it is absent in these fungi (Glass et al. 1988, 1990). A third group corresponds to a clade containing *Gelasinospora calospora* and *N. sublineolata* (*Anixiella sublineolata*). These two self-compatible species contain both *MAT1-1* and *MAT1-2* sequences (Beatty et al. 1994). These analyses suggest that *MAT1-1-1* and *MAT1-1-2* are consistently present in all self-compatible Sordariomycetes. Some of these species have lost either *MAT1-1-3* or *MAT1-2-1*, but no species has lost both of these genes, which encode proteins with a HMG domain. We propose that the essential functions of the lost *MAT1-1-3* or *MAT1-2-1* are taken on by the remaining HMG encoding gene. The functions of the non-redundant *MAT1-1-1* and *MAT1-1-2* genes cannot be compensated for by any other gene, precluding their loss in self-compatible Sordariomycetes. Therefore, we predict that the minimal mating-type gene structure of self-compatible Sordariomycetes should be *MAT1-1-1*, *MAT1-2-1*, and at least one HMG encoding gene.

c) Asexual Sordariomycetes

The idiomorph structure of asexual Sordariomycetes has been completely established for two hypocreales: *Fusarium oxysporum* and *Paecilomyces tenuipes*. Arie et al. (2000) demonstrated that different forma specialis (f. sp.) of asexual *F. oxysporum* contain either the *MAT1-1* or the *MAT1-2* idiomorphs. Subsequent analysis of the *MAT1-1* and *MAT1-2* idiomorphs of *F. oxysporum* f. sp. *lycopersici* revealed that they contained *MAT1-1-1*, *-2*, *-3* and *MAT1-2-1* genes, respectively (Yun et al. 2000). These genes are structurally indistinguishable from the functional *MAT* genes present in sexual Sordariomycetes, notably the

close relative *G. fujikuroi* (*G. moniliformis*). Transcripts for each of the *MAT* genes of *F. oxysporum* were found by RT-PCR, suggesting that the asexual feature of *F. oxysporum* does not result from a transcriptional deficiency. A total of 22 isolates of another asexual hypocreales, *P. tenuipes* (the sexual state of *Cordyceps takaomontana*), have been tested for their mating-type structure by Yokoyama et al. (2003). Three contain the *MAT1-1* idiomorph whereas 19 have the *MAT1-2* idiomorph. The *MAT1-1* idiomorph contains *MAT1-1-1* and *MAT1-1-2* but not *MAT1-1-3*. The *MAT1-1-1* coding sequence extends beyond the idiomorph boundary into the flanking sequence, but the region encoding the α1 domain belongs to the *MAT1-1* idiomorph. Evidence for *MAT1-1-1* transcripts has been acquired by RT-PCR, but the transcriptional state of *MAT1-1-2* is unknown. The *MAT1-2* idiomorph contains the expected *MAT1-2-1* gene but Yokoyama et al. (2003) have identified an additional putative *MAT1-1-1* pseudogene in this idiomorph. This pseudogene lacks the region encoding the α1 box, and the ORF has no initiation codon and is interrupted by several termination codons. Reexamination of the sequence corresponding to the pseudogene of strain BCMUJ13 allowed us to identify a gene that includes two introns and a coding sequence of 339 residues, starting with a methionine initiation codon (*MAT1-2-2*, Table 15.1, Debuchy, unpublished data). Although Yokoyama et al. (2003) have found *MAT1-2-1* transcripts by RT-PCR, they have not searched for transcripts originating from the region corresponding to the putative *MAT1-1-1* pseudogene. Further investigations will be required to establish whether this region corresponds to a pseudogene or to a new gene.

3. Leotiomycetes

a) Self-Incompatible Leotiomycetes

The mating-type sequence has been established for one self-incompatible species of this group, *Pyrenopeziza brassicae*. A total of 30 field isolates of *P. brassicae* from different geographical locations were screened for the presence of *MAT1-1* and *MAT1-2* idiomorph sequences (Singh et al. 1999). The *MAT1-1* idiomorph was identified in 14 strains, and the *MAT1-2* idiomorph was present in 16 isolates. Although the *P. brassicae MAT* idiomorphs are structurally similar to Sordariomycete *MAT* idiomorphs, they display a distinctive feature. *MAT1-1-2* is replaced by a new gene encoding a metallothionein-like protein (Fig. 15.2 and Table 15.2). This gene is called *MAT1-1-4* but its role during the sexual cycle remains elusive because no mutant is available, nor is there any transcription analysis providing evidence that this gene is expressed during the sexual cycle. Singh and Ashby (1998) proposed that the metallothionein-like protein acts as a scavenger for metal ions that may be present at a high level during fruiting-body development on senescing plant debris. The structural organization of genes within the *MAT* idiomorphs has been investigated in another self-incompatible Leotiomycete, *Tapesia yallundae*, which is closely related to *P. brassicae* (Goodwin 2002). Genome hybridization with *P. brassicae MAT* genes as probes suggested that sequences similar to all mating-type genes were present in *T. yallundae* (Singh et al. 1999). The conservation of mating-type genes has also been tested in *Ascobolus stercorarius*, a fungus classified with *P. brassicae* in the subclass of the Discomycetes. Hybridization of *P. brassicae MAT* genes to the *Ascobolus stercorarius* genome did not reveal any mating-type-specific signal, suggesting that DNA sequences specific to mating type have diverged between different orders within the Discomycete taxon (Cisar et al. 1994; Singh et al. 1999).

b) Asexual Leotiomycetes

The structure and distribution of the mating-type genes of *Rhynchosporium secalis* have been investigated to assess if the high genetic diversity of this plant pathogenic fungus could be attributed to a cryptic sexual cycle. *R. secalis* is closely related to *T. yallundae* and *P. brassicae*, based on ITS comparisons (Goodwin 2002). Degenerate primers designed to *MAT1-1-1* and *MAT1-2-1* genes from *P. brassicae* and *T. yallundae* led to the identification of the homologous genes in *R. secalis* (Foster and Fitt 2003). Analysis of the *MAT1-2* idiomorph revealed a single ORF (*MAT1-2-1*) whereas the analysis of the *MAT1-1* sequence identified two ORFs (*MAT1-1-1* and *MAT1-1-3*). *R. secalis MAT* genes were found to be related to those of *P. brassicae*, but no *MAT1-1-4* gene could be identified between *MAT1-1-1* and *MAT1-1-3*, although the intergenic sequence has 65% identity with the corresponding region of the *P. brassicae MAT1-1* idiomorph. Translated BLAST searches revealed significant ho-

mology only with the metallothionein-like protein found within this region of *P. brassicae*, but Foster and Fitt (2003) failed to identify an ORF that would confirm the presence of this gene within *R. secalis MAT1-1* idiomorph. The mating-type frequencies in a total of 782 *R. secalis* isolates from combined geographic regions did not differ significantly, although they did deviate significantly from a 1:1 ratio in some regions (Linde et al. 2003). These overall data are in agreement with the occurrence of a sexual cycle, but the demonstration of mating awaits the identification of the sexual state of *R. secalis*, which in fact was predicted to be *Tapesia* (Goodwin 2002).

4. Eurotiomycetes

a) Self-Compatible Eurotiomycetes

Mating-type genes have been identified for only one self-compatible Eurotiomycete, *Emericella nidulans*. The name of the asexual state, *Aspergillus nidulans*, is often used instead of *E. nidulans*. The *E. nidulans* haploid genome contains two regulatory protein encoding genes homologous to *MAT1-1-1* and *MAT1-2-1* called *MAT-1* (or *MATB*) and *MAT-2* (or *MATA*), respectively. *MAT-1* and *MAT-2* map on linkage group (LG) 6 and LG3, respectively (Dyer et al. 2003). BLAST searching of the available *E. nidulans* genome sequence failed to reveal *MAT1-1-2* and *MAT1-1-3* genes.

b) Asexual Eurotiomycetes

Aspergillus fumigatus has no known sexual cycle and is closely related to the genus *Neosartorya*, which contains self-incompatible as well as self-compatible species (*N. fennelliae* and *N. fischeri*, respectively). A search of the *A. fumigatus* genome database for mating-type genes resulted in the identification of a gene encoding a protein with a HMG domain related to *MAT1-2-1* (Pöggeler 2002; Varga 2003). The α1 encoding mating-type gene was not found in the haploid genome containing *MAT1-2*, but a worldwide survey of 290 isolates allowed Paoletti et al. (2005) to discover *MAT1-1* strains, and to establish that *MAT1-1* and *MAT1-2* are present in approximately equal proportions. These authors have also found evidence, through population genetic analyses, for recombination, which suggests that this fungus with a self-incompatible structure may undergo a cryptic sexual cycle.

B. Comparative Organization of *MAT* Context in Euascomycetes

The available genomic sequences offer the possibility of investigating synteny around the mating-type loci, which furthers our understanding of mating-type evolution. Moreover, the nature of gene clusters in the *MAT* environment might reveal new pathways involved in the sexual process, since there is growing evidence that co-regulated genes may be found clustered in fungal genomes (Lee and Sonnhammer 2003).

1. *MAT* Context in Loculoascomycetes

Three genes were identified in the common DNA flanking the *MAT* locus of *C. heterostrophus*: a gene encoding a GTPase activating protein and an ORF of unknown function lie 5′, whereas a β-glucosidase encoding gene was found 3′ (Wirsel et al. 1998; Fig. 15.3). The ORF shows similarity to *S. cerevisiae* YLR456W, for which the molecular and biological function is unknown. The hypothetical corresponding gene in *C. heterostrophus*, called *ORF1*, is also present in self-incompatible *C. carbonum* and *C. ellisii* and in self-compatible *C. homomorphus* and *C. luttrellii* (Yun et al. 1999). Self-compatible *C. cymbopogonis* has two copies of *ORF1*, each linked to one *MAT* homolog. *ORF1* is also present in the common 5′ flanking sequence of asexual *A. alternata* (Bennett et al. 2003), sexual *D. rabiei* (Barve et al. 2003) and *P. nodorum* (Bennett et al. 2003), and self-compatible *M. zeae-maydis* (Yun 1998). In some cases, *ORF1* abuts or even overlaps the *MAT* idiomorphs. A search for *ORF1* and *GAP1* loci in *A. nidulans* and Sordariomycete genomes indicates that these two genes are present on the same linkage group as the *MAT* locus (Table 15.3). *BGL1*, however, is not present on the linkage groups containing the *MAT* locus, except in *C. globosum* (Table 15.3). Interestingly, *GAP1* and *ORF1* are closely linked to the *MAT-2* locus of *A. nidulans*, suggesting a common evolutionary relationship. *Leptosphaeria maculans*, *P. nodorum*, and *M. zeae-maydis* all have *ORF1* linked to *MAT*. Although the persistent linkage of *ORF1* and *MAT* might suggest that this relationship has functional meaning, Wirsel and colleagues demonstrated that *ORF1* is not essential for mating in *C. heterostrophus*. Preliminary data suggest that *GAP1* also has no detectable role in mating in this fungus (Wirsel et al. 1998).

Although *M. graminicola* is classified as a Loculoascomycete and possesses a *MAT* structure iden-

Table. 15.3. Genome position of the homologs to *C. heterostrophus* and *M. graminicola MAT* flanking genes in Sordariomycetes and Eurotiomycetes

	GAP1	*ORF1*	*MAT*	*BGL1*	*pal1*
P. anserina					
LG	I	I	I	II	I
Supercontig	E	E	G	A	F
Start	280,606	402,224	79,454	3,309,367	432,338
Stop	283,019	401,468	78,144	3,306,572	430,110
C. globosum					
Supercontig	1.3	1.3	1.3 (*MAT1-1*)		1.3
Start	2,900,141	2,736,033	3,990,896		3,892,526
Stop	2,902,311	2,736,841	3,991,867		3,894,742
Supercontig			1.2 (*MAT1-2*)	1.2	
Start			4,650,124	4,159,504	
Stop			4,651,264	4,161,935	
N. crassa					
LG	I	I	I	V	I
Supercontig	2	2	5	11	5
Contig	3.22	3.23	3.86	3.199	3.88
Gene number	NCU00553.1	NCU00590.1	NCU1960.1	NCU3641.1	NCU1996.1
G. zeae					
Supercontig	5	5	5	2	5
Contig	1.348	1.349	1.358	1.160	1.353
Gene number	FG08638.1	FG08689.1	FG08893.1	FG03570.1	FG08708.1
M. grisea					
LG	VII	VII	VII	I	I
Supercontig	4	33	4	2	2
Contig	2.611	2.1986	2.600	2.264	2.535
Gene number	MG03048.4	MG10319.4	MG02978.4	MG01441.4	MG02630.4
A. nidulans					
LG	III	III	III (*MAT-2*)	II	III
Supercontig	5	5	5	4	5
Contig	1.80	1.81	1.80	1.63	1.83
Gene number	AN4745.2	AN4780.2	AN4734.2	AN3904.2	AN4853.2

tical to the one present in the model organism *C. heterostrophus*, the genomic context of the *M. graminicola MAT* locus is different from that of *C. heterostrophus*, and appears to more closely resemble the Sordariomycete structure. Three genes were identified in the region 5′ of *MAT1-2*: *APN2*, *APC5*, and a putative homolog of the *palI* gene of *A. nidulans* (Waalwijk et al. 2002; Fig. 15.3). This difference from *C. heterostrophus* may be related to the taxonomic distance between these two species, since *M. graminicola* and *Cochliobolus* spp. are members of different, albeit sister clades, i.e., the Dothideales and Pleosporales, respectively (Liu and Hall 2004).

2. *MAT* Context in Sordariomycetes and Eurotiomycetes

The *MAT* context in Ascomycetes was investigated first by Butler et al. (2004) in different yeasts and *N. crassa*. These authors observed that the *MAT* locus of *Yarrowia lipolytica* lies between the homologs of *S. cerevisiae APN2* and *SLA2*, two genes that were identified in the left and right flanking sequences of *N. crassa*. This common structure led the authors to propose that this configuration may be the ancestral one for all Ascomycetes. We have compiled the genomic sequences available from the Broad Institute (http://www.broad.mit.edu/resources.html) and from the *Podospora* Genome Project (http://podospora.igmors.u-psud.fr/) to extend the comparison to other Euascomycetes. The results are reported in Fig. 15.3. Remarkably, homologs of *APN2* and *SLA2* were found on each side of the *MAT* locus in all Sordariomycetes. *SLA2* and *APN2* are also linked to *E. nidulans MAT-1* and *MAT-2*, respectively. Overall, these data support the hypothesis of Butler et al. (2004). Several additional

genes were found to be conserved in the *MAT* environment among Sordariomycetes, notably homologs of *ATG3*, *cox13*, and *CWF24*. Homologs of *APC5* are found close to the *MAT* locus in Sordariomycetes, *A. nidulans* and *M. graminicola* (Fig. 15.3), suggesting that *APN2*, *SLA2* and *APC5* are the ancestral companions of the *MAT* locus in Euascomycetes. Extending this analysis to other yeasts and Euascomycetes, and to a wider region around the *MAT* locus should help to specify the ancestral *MAT* locus structure.

Genome sequencing of self-incompatible Sordariomycetes reveals the structure of only one *MAT* locus, raising the question of the structure of the opposite idiomorph. The variability in the regions adjacent to each idiomorph has been investigated by Randall and Metzenberg in different self-incompatible *Neurospora* sp., by a combination of Southern and Northern blot hybridizations and sequencing (Randall and Metzenberg 1995). They observed that the region downstream of *MAT1-1-3* (*mat A-3*) has a similar hybridization pattern in both *A* and *a* strains of *N. crassa*, *N. sitophila*, *N. intermedia*, *N. discreta* and *N. tetrasperma*, suggesting that this region does not contain mating-type-specific sequences. The centromere proximal end of the *mat a* and *mat A* idiomorphs (Fig. 15.3) is followed by a 57–59 bp region that is conserved in the five above species, whatever their mating types. This short region has been called the mating-type common region. Randall and Metzenberg proposed that the striking conservation of this mating-type common region is related to a functional role, such as a pairing site for homologous chromosome during meiosis. However, this sequence appears specific to the *Neurospora* genus, as we failed to detect it in the genome of *P. anserina*, *C. globosum*, *G. zeae*, *M. grisea* and *A. nidulans*. In *a* strains, the mating-type common region is followed by an additional *a* common region of approximately 800–900 bp. This *a*-specific sequence and the mating-type common region in *A* strains are followed by a 3.5-kb region that is similar in *N. crassa* and *N. sitophila*, whatever their mating type, but extremely different in the two mating types of *N. intermedia*, *N. tetrasperma* and *N. discreta*. The 3.5-kb region in *N. intermedia*, *N. tetrasperma* and *N. discreta* has not yet been sequenced. In *N. crassa*, this 3.5-kb region is localized downstream of the ATPase domain encoding gene (Fig. 15.3). This 3.5-kb region contains a transcribed sequence corresponding to a putative pseudogene, *eat-1* (Randall and Metzenberg 1998). We failed to detect any similarity of the *eat-1* transcript in the *P. anserina*, *C. globosum*, *G. zeae*, *M. grisea* and *A. nidulans* genomes, thus indicating that *eat-1* does not encode any conserved motif. Inactivation of *eat-1* will be required to establish if it is a *N. crassa*-specific gene, or a pseudogene, as suggested by Randall and Metzenberg (Randall and Metzenberg 1998).

C. globosum is a self-compatible species that displays intriguing *MAT* structure. Unlike self-compatible *G. zeae* and *S. macrospora*, which have adjacent *MAT1-1* and *MAT1-2* mating types, *C. globosum MAT1-1* and *MAT1-2* loci are present on different supercontigs (Table 15.3). The *C. globosum MAT1-1* and *P. anserina mat+* (*MAT1-2*) locus have an identical context, in agreement with the close evolutionary relationship between these two species (Liu and Hall 2004). The *MAT1-2* environment of *C. globosum* displays several unusual features. No synteny has been detected with any *P. anserina* genome locus. Among the eight genes analyzed here in the left and right sequences flanking *MAT1-2-1* and *MAT1-1-2*, three have no homologs in *P. anserina*. Four of the five genes that have homologs in *P. anserina* display identity below 50% with their *P. anserina* counterparts. This is in contrast to the situation observed at the *MAT1-1* locus, where all genes encode proteins with more than 60% identity to their *P. anserina* counterparts (Fig. 15.3). The mating types are excluded from this comparison, as their sequences appear to be evolving at a faster rate than those of other protein-coding sequences (Pöggeler 1999). These observations suggest that the genes around the *MAT1-2* locus are not of *C. globosum* origin. Instead, the *MAT1-2* locus might result from a horizontal transfer from a phylogenetically distant fungus into a self-incompatible *MAT1-1* ancestor of *C. globosum*. Horizontal transfer of a *MAT* locus has been described in *Stemphylium* lineages, but in contrast to the large region assumed to have been transferred in *C. globosum*, the *Stemphylium MAT* locus is less than 10 kb (Inderbitzin et al. 2005). Further comparisons between the *C. globosum MAT1-2* locus and the *P. anserina* genome should establish the origin of this region.

The functions of the genes linked to the Sordariomycete *MAT* locus have not been tested, except for the ATPase encoding gene (NCU1957.1) of *N. crassa* (Randall and Metzenberg 1998). A missense mutation resulted in slow growth, mycelium colonial phenotype and no macroconidia production.

The cross of a mutant strain with an *a* mating partner containing a wild-type ATPase encoding gene gave abundant perithecia, which never developed beaks, nor produced ascospores, mature or otherwise. This ascus dominant phenotype suggests that functional genes in both sexual partners may be needed for the completion of the sexual cycle. Further analyses of this gene and of the most conserved companions of the mating-type genes should provide interesting insights in the sexual cycle.

C. Main Features of Mating-Type Genes and Proteins

1. *MAT1-1-1*

MAT1-1-1 proteins are characterized by the presence of an α1 domain showing similarity to the α1 transcription factor of *S. cerevisiae* and to the Pc polypeptide from *S. pombe* (Fig. 15.4). Although the role of the α1 protein as a DNA-binding protein has been substantiated, it has not been placed in any of the large families of sequence-specific DNA-binding proteins, such as homeodomain, zinc finger, or helix-loop-helix. Surprisingly, the Pc polypeptide contains a HMG domain that overlaps the region of similarity with the α1 domain, suggesting a close relationship between these two DNA-binding domains (Lu and Turgeon, unpublished data). Both α1 and Pc cooperate with additional regulatory proteins for the control of mating and the expression of cell type-specific genes. The α1 transcription factor forms a complex with STE12 and MCM1 to activate the α haploid-specific genes in *S. cerevisiae* (reviewed in Johnson 1995). Yuan et al. (1993) have proposed that the region, located between residues 90 and 111 of α1, interacts with MCM1, but no experimental evidence supports this interpretation. The Pc polypeptide interacts physically with Map1, a protein belonging to the MADS box family, which also includes MCM1 (Yabana and Yamamoto 1996). These data suggest that Euascomycete homologs of MCM1/Map1 may cooperate with MAT1-1-1 to control the expression of cell type-specific genes required for fertilization. This hypothesis is supported by the recent finding that the MCM1 protein is capable of

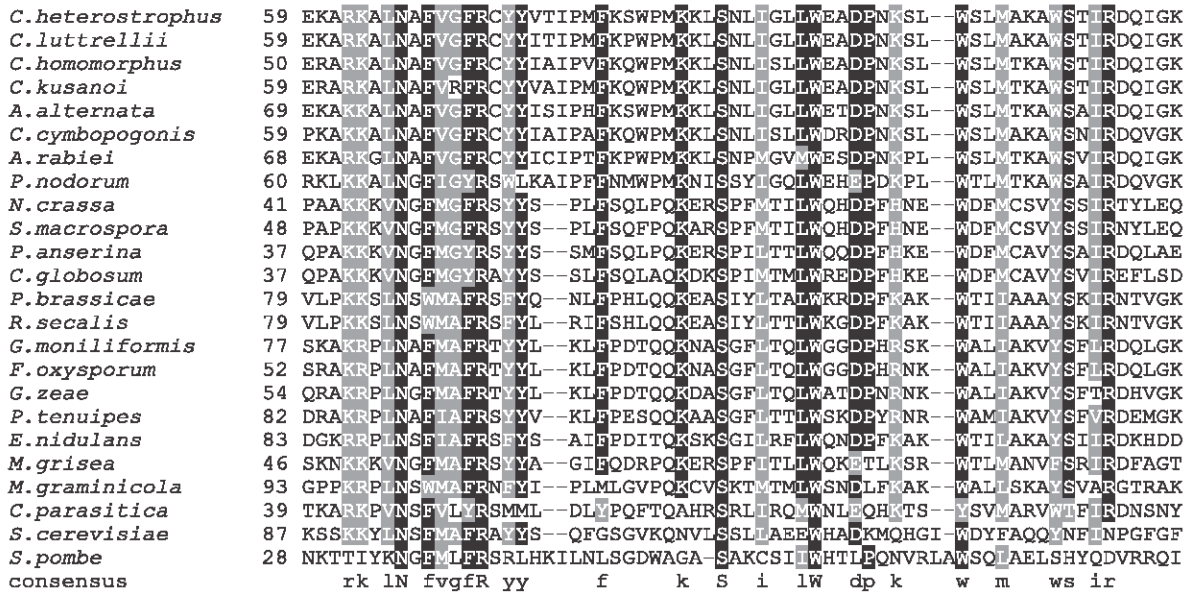

Fig. 15.4. Amino-acid alignment of the α1 box of deduced MAT1-1-1 proteins of Euascomycetes with the MATα1 protein of *S. cerevisiae*. *C. heterostrophus* (CAA48465), *C. luttrellii* (AAD33439), *C. homomorphus* (AAD33441), *C. kusanoi* (AAD33443), *A. alternata* (BAA75907), *C. cymbopogonis* (AAD33445), *D. rabiei* (Barve et al. 2003), *P. nodorum* (AAO31740), *N. crassa* (AAC37478), *S. macrospora* (CAA71623), *P. anserina* (CAA45519), *C. globosum* (Broad Institute), *P. brassicae* (CAA06844), *R. secalis* (CAD71141), *G. moniliformis/fujikuroi* /(AAC71055), *F. oxysporum* (BAA75910), *G. zeae* (AAG42809), *P. tenuipes* (BAC67541), *E. nidulans* (AAQ01665), *M. grisea* (BAC65087), *M. graminicola* (AAL30838), *C. parasitica* (AAK83346), *S. cerevisiae* α1(NP_009969), *S. pombe* Pc (P10841). Fraction of sequences that must agree for *shading*: 0.8

interacting with SMTA-1 in *S. macrospora* (Nolting and Pöggeler 2005). Interactions of MAT1-1-1 with other MAT proteins have been investigated, based on the assumption that a heterodimer involving MAT proteins from opposite idiomorphs could trigger developmental events inside the fruiting body after fertilization. A similar situation has been observed in diploid cells of *S. cerevisiae*, in which a1 and α2 encoded by the mating-type alleles form a heterodimer required for further sexual development after mating (reviewed in Souza et al. 2003). In *N. crassa*, two-hybrid assays established the ability of MAT A-1 (MAT1-1-1) to interact with MAT a-1 (MAT1-2-1; Badgett and Staben 1999). Mutations that interfere with this interaction eliminate vegetative incompatibility (reviewed in Glass and Kuldau 1992), but not mating, suggesting that this interaction is not essential for the sexual cycle. In *S. macrospora*, an interaction between homologs of the above proteins has been found, but its role during the sexual cycle has not been investigated (Jacobsen and Pöggeler 2001). No interaction between FMR1 (MAT1-1-1) and FPR1 (MAT1-2-1) has been detected in *P. anserina*. Instead, an interaction between FMR1 and SMR2 was identified (Arnaise et al. 1995). Mutations that disrupt this interaction also affect the sexual cycle (Coppin and Debuchy, unpublished data). Therefore, no conclusive data support the idea that an interaction between proteins encoded by opposite idiomorphs is essential to the sexual cycle. Nevertheless, it must be emphasized that all of these experiments rely on the yeast two-hybrid system, which failed to detect any interaction between the yeast a1 and α2, although a1/α2 heterodimer formation is well established.

Examination of all published *MAT* genes of *Cochliobolus* spp. and relatives (e.g., *Pleospora*, *Alternaria* and *Phaeosphaeria*) reveals that MAT1-1-1 and MAT1-2-1 proteins share at least two conserved motifs; Motif 1 is located in the HMG box in MAT1-2-1 and is also found 3′ of the α1 box in MAT1-1-1; motif 2 is located at the C terminus of both MAT proteins (Lu and Turgeon, unpublished data; Fig. 15.5). Motif 2 shows significant similarity to an α1 box signature motif. Amino acid alignment to known HMG box domains suggests that motif 1 is a HMG box domain. Thus, each *MAT* gene of *Cochliobolus* spp. appears to encode a protein with both HMG and α1 activities. The shorter idiomorphs and the single MAT1-1-1 proteins of the Loculoascomycetes may have evolved to include all activities provided by the three MAT1-1 proteins of the Pyrenomycetes.

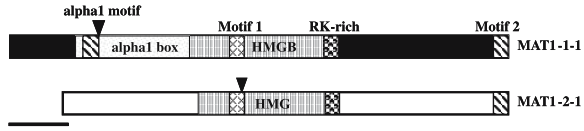

Fig. 15.5. Diagrammatic representation of the MAT1-1-1 and MAT1-2-1 proteins of taxa representing several Loculoascomycete genera including *Cochliobolus* (*Bipolaris*), *Pleospora* (*Stemphylium*), *Phaeosphaeria* (*Stagonospora*), *Leptosphaeria maculans* and asexual *Alternaria alternata*. Alignment of amino-acid sequences of the two "unlike" MAT proteins reveals shared motifs. A signature motif within the HMG box (*shaded vertical lines*) of MAT1-2-1 is also found in the MAT1-1-1 protein (motif 1, *hatched box*). A motif resembling a signature α1 box (*lightly shaded stippled*) motif is found at the C-terminal end of both MAT proteins (motif 2, *striped box*). The α1 box and the HMG box overlap slightly in MAT1-1-1. A third common stretch is RK rich (*dotted box*). Thus, although the *MAT1-1-1* and *MAT1-2-1* genes encode different transcription factors, these proteins may have dual function and may share a common evolutionary history. Introns are represented by *inverted triangles* (Lu and Turgeon, unpublished data)

Comparison of *MAT1-1-1* gene structure reveals the conserved position of an intron in all *MAT1-1-1* genes, except in *E. nidulans MATB*, which has no intron. The intron is localized after the first nucleotide of the triplet encoding the non-conserved residue in the peptide fvgfRXyy (Fig. 15.4).

2. *MAT1-1-2*

The MAT1-1-2 proteins contain a conserved region called the HPG domain, with three invariant residues, histidine, proline and glycine (Fig. 15.6). Surprisingly, mutation of these residues, in the *SMR1* gene of *P. anserina*, to alanine does not affect the sexual cycle, whereas a mutation of tryptophan 193 to alanine results in a complete arrest of the fruiting-body development at an early stage, thus confirming that this domain is essential for the sexual cycle (Coppin et al. 2005a). Based on the high isoelectric point of the conserved domain of SMR1, Debuchy et al. (1993) have proposed that it defines a new DNA-binding domain. However, subsequent investigations of SMR1 do not support this hypothesis. Subcellular localization of SMR1 by GFP tagging indicates that it has a cytosolic localization (Coppin et al. 2005a). In agreement with this observation, analysis of SMR1 with a program for identification of subcellular localization of pro-

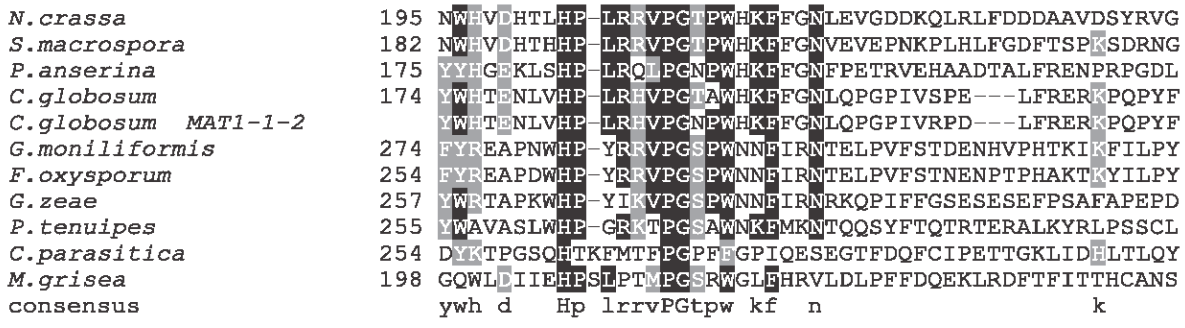

Fig. 15.6. Amino-acid alignment of the HPG domain of deduced MAT1-1-2 proteins of Sordariomycetes. *N. crassa* (AAC37477), *S. macrospora* (CAA71626), *P. anserina* (S39889), *C. globosum MAT1-1* and *MAT1-2* (Broad Institute), *G. moniliformis/fujikuroi* (AAC71054), *F. oxysporum* (Yun et al. 2000), *G. zeae* (AAG42811), *P. tenuipes* (BAC67540), *C. parasitica* (AAK83345), *M. grisea* (BAC65088). Fraction of sequences that must agree for *shading*: 0.8

teins (PSORT II, Nakai and Horton 1999) does not detect any nuclear localization signal and predicts a cytoplasmic localization. PSORT II analyses of all MAT1-1-2 proteins listed in Fig. 15.6 predict that they should have a cytoplasmic localization, except MAT1-1-2 of *M. grisea*. The latter protein was predicted to be nucleus localized, but no nuclear localization signal was detected. Taken together, these data suggest that *Podospora* SMR1 has a cytoplasmic localization, but its molecular function remains unknown.

3. *MAT1-1-3* and *MAT1-2-1*

The high-mobility-group (HMG) domain is a DNA-binding sequence found in non-histone chromosomal proteins and transcription factors. Fungal mating-type transcription factors, like MAT1-1-3 and MAT1-2-1, belong to the MATA_HMG box family, while other transcription factors, notably HMG transcription factors from animals, are classified in the SOX-TCF_HMG box family. *MAT1-2-1* and *MAT1-1-3* genes are characterized by a conserved intron inside the HMG encoding sequence. This intron is localized after the first nucleotide of the triplet encoding the invariant residue serine in the lS and neiS residues in MAT1-1-3 and MAT1-2-1, respectively (Figs. 15.7 and 15.8). Genes encoding proteins from the SOX-TCF_HMG box family do not contain an intron at this position. Although structural gene features seem in agreement with the current classification of HMG transcription factors, it may be that this classification does not correspond to their DNA-binding properties. Philley and Staben have established that the MAT a-1 HMG domain from *N. crassa* binds specific DNA sequences

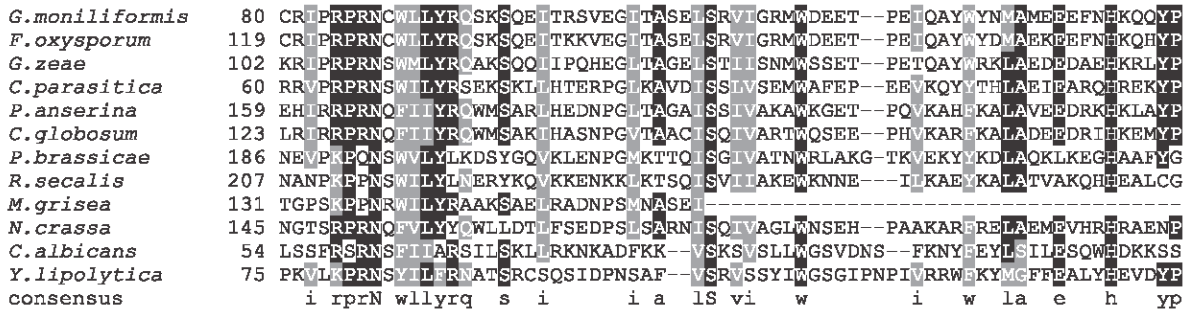

Fig. 15.7. Amino-acid alignment of the HMG domain of deduced MAT1-1-3 proteins of Euascomycetes. *G. moniliformis* (AAC71053), *F. oxysporum* (Yun et al. 2000), *G. zeae* (AAG42812), *C. parasitica* (AAK83344), *P. anserina* (CAA52051), *C. globosum* (Broad Institute), *P. brassicae* (CAA06846), *R. secalis* (CAD71142), *M. grisea*, partial sequence (Kanamori and Arie, personal communication), *N. crassa* (AAC37476), *C. albicans* (Butler et al. 2004), *Y. lipolytica* (CAA07613). Fraction of sequences that must agree for *shading*: 0.7

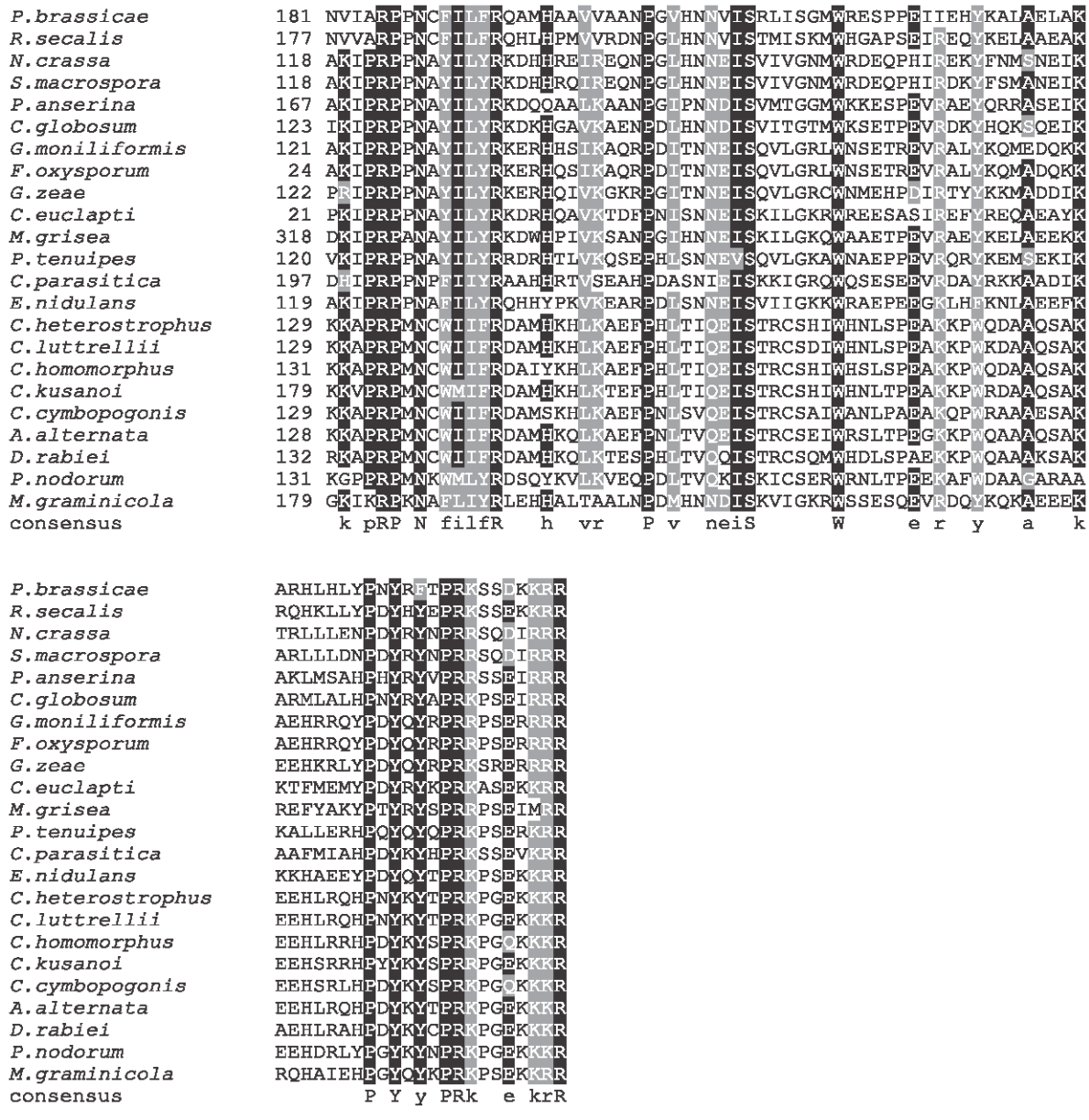

Fig. 15.8. Amino-acid alignment of the HMG domain of deduced MAT1-2-1 proteins of Euascomycetes. *P. brassicae* (CAA06843), *R. secalis* (CAD62166), *N. crassa* (AAA33598), *S. macrospora* (CAA71624), *P. anserina* (CAA45520), *C. globosum* (Broad Institute), *G. moniliformis* (AAC71056), *F. oxysporum* (BAA28611), *G. zeae* (AAO42810), *C. eucalypti* (AAF00498), *M. grisea* (BAC65090), *P. tenuipes* (BAC66503), *C. parasitica* (AAK83343), *E. nidulans* (AAQ07985), *C. heterostrophus* (CAA48464), *C. luttrellii* (AAD33439), *C. homomorphus* (AAD33441), *C. kusanoi* (AAD33442), *C. cymbopogonis* (AAD33447), *A. alternata* (BAA75908), *A. rabiei* (Barve et al. 2003), *P. nodorum* (AAO31742), *M. graminicola* (AAL30836). Fraction of sequences that must agree for *shading*: 0.8

in vitro, whose core is 5′-CAAAG-3′ (Philley and Staben 1994). This binding sequence is identical to the binding site of the SOX-TCF_HMG box, suggesting that SOX-TCF and MATA-HMG boxes may function in a very similar way, although these proteins are placed in separate families. MAT1-1-3 proteins display several features that distinguish them from MAT1-2-1, and suggest that they could have very different DNA-binding properties. The structures of the HMG boxes in MAT1-1-3 and MAT1-2-1

appear very dissimilar (see consensus in Figs. 15.7 and 15.8). Phylogenetic analysis of the HMG domains from the MATA family shows that MAT1-2-1 proteins form a distinct subfamily that does not contain any other HMG proteins, except MAT1-1-3 from *P. brassicae* and *R. secalis* (data not shown). Moreover, SMR2 from *P. anserina* was shown to interact with FMR1 (Arnaise et al. 1995), and similar phenotypes conferred by *FMR1* and *SMR2* mutations support the idea that these two proteins cooperate in the regulation of their target genes (Zickler et al. 1995; Arnaise et al. 1997, 2001). These data support the idea that MAT1-1-3 proteins bind to DNA in a very different way from that of MAT1-2-1/SOX-TCF proteins. Further investigations on DNA-binding modalities of fungal HMG transcription factors would be required to substantiate classification of these proteins, by function.

IV. Evolution of Mating Types

Two fascinating questions attend any discussion of mating-type gene evolution. The first is how dimorphic sex chromosomes or dissimilar regions (idiomorphs) of a chromosome evolved, and the second is how different reproductive lifestyles, i.e., self- or non-self-compatibility, evolve. The evolutionary origins of the dissimilar *MAT* idiomorphs of self-incompatible Ascomycetes are unknown at this point, but data are accumulating on the matter. One hypothesis is that the small pockets of identity in the otherwise unlike *MAT* idiomorphs reflect common ancestry (Coppin et al. 1997). Conceivably, these pockets are remnants of a series of mutagenic events in a single ancestral gene(s). The mutations, coupled with recombination suppression, might have led to the highly divergent extant *MAT* genes that now encode different products (Turgeon et al. 1993). A similar scenario has been proposed for the evolution of the Y chromosome. Indeed, acquisition of sex-determining genes, recombination isolation, and addition of genes not involved in mating are shared between fungal mating-type loci and mammalian X and Y chromosomes; it has been suggested that these elements may be early steps linking the evolution of sex chromosomes in diverse organisms (Lahn and Page 1999; Fraser et al. 2004). This section focuses on how Ascomycetes change from one reproductive lifestyle to another (self-incompatibility to self-compatibility, and vice versa).

A. Phylogenetic Analyses of Mating Type

1. Loculoascomycetes

a) Cochliobolus

Because *MAT* genes control the reproductive process, comparison of their sequences reflects life history and should reveal mechanisms underlying changes in reproductive mode. A combination of molecular and phylogenetic data have been used to determine the direction of evolution of reproductive lifestyle in *Cochliobolus* (Yun et al. 1999) and in *Stemphylium* (Inderbitzin et al. 2005). In both cases, examination of *MAT* structure in many species from diverse geographical locations, plus phylogenetic treatments, support the hypothesis that the direction is likely from self-incompatible to self-compatible.

To address the issue of which mode of fungal sexual reproduction is ancestral in *Cochliobolus*, Yun et al. (1999) compared extant *MAT* sequences from self-incompatible and self-compatible species. Structural organization of *MAT* loci of self-incompatible *C. heterostrophus*, *C. carbonum*, *C. victoriae*, *C. ellisii*, *C. sativus* and *C. intermedius* species is highly conserved; each strain carries a single *MAT* gene, either *MAT1-1-1* or *MAT1-2-1* (Fig. 15.2). By contrast, all self-compatible *Cochliobolus* species carry both *MAT* genes in one genome, but as described in Sect. III. and Fig. 15.2, the structural organization of each locus is unique. In two cases (*C. luttrellii* and *C. homomorphus*), the genes are fused into a single ORF; the gene order in *C. luttrellii* is reversed in *C. homomorphus*. In the remaining two cases, the genes are not fused. In *C. kusanoi*, the organization is *5′MAT1-2-13′–3′MAT1-1-15′*, and part of the sequence between the genes is similar to a portion of the β-glucosidase gene normally found 3′ of both *MAT* genes in self-incompatible *C. heterostrophus* (Fig. 15.3). To the 5′ of *MAT1-2-1* is a perfect inverted repeat of a 561-bp region containing 123 bp of the 5′ end of the *MAT1-1-1* ORF fused to the 5′ end of *MAT1-2-1*, and 145 bp of a different fragment of the β-glucosidase gene, separated from each other by 293 bp. *C. cymbopogonis* carries both homologs of the self-incompatible idiomorphs, but these are not closely linked. It is not known if they reside on the same or different chromosomes. Thus, the *MAT* genes have close physical association in three *Cochliobolus* self-compatible species, but not in the fourth. The fused *MAT* genes in self-compatible species

provide a snapshot of the genetic link between self-incompatibility and self-compatibility, and thus clues to the genetic mechanism mediating the change from one lifestyle to the other.

Inspection of the sequence at the *MAT* fusion junction in *C. luttrellii* revealed that 345 nucleotides from the 3′ end of the *MAT1-1-1* ORF and 147 nucleotides from the 5′ end of the *MAT1-2-1* ORF are missing, compared to the *C. heterostrophus* self-incompatible homologs. The deletions are consistent with the hypothesis that a crossover event occurred within the dissimilar *C. heterostrophus* genes at positions corresponding to the fusion junction. Inspection of the *C. heterostrophus* genes reveals 8 bp of sequence identity precisely at the proposed crossover site, which would explain this arrangement. A single crossover within this region would yield two chimeric products, one of which is identical to the fused *MAT* gene actually found in *C. luttrellii* (Fig. 15.2). A similar scenario can be proposed for *C. homomorphus*; in this case, the fused gene is missing 27 nucleotides from the 3′ end of *MAT1-2-1* and 21 nucleotides from the 5′ end of *MAT1-1-1*, compared with the *C. heterostrophus MAT* genes. Examination of the *C. heterostrophus MAT* sequences at positions corresponding to the *C. homomorphus* fusion junction reveals 9 bp of identity (with one mismatch), and thus a putative recombination point. The mechanism of conversion from self-incompatibility to self-compatibility is likely a recombination event between small islands of identity in the otherwise dissimilar *MAT* sequences.

To determine whether phylogenetic analyses support a convergent origin for self-compatibility, Yun et al. (1999) used maximum likelihood and parsimony trees inferred from the ribosomal gene internal transcribed spacer (ITS) and from glyceraldehyde phosphate dehydrogenase (*GPD*) datasets. All of the resulting trees show that self-compatibility is polyphyletic. None of the self-compatible species on the tree clustered together in any of the 15 most parsimonious trees or in maximum likelihood trees. Thus, phylogenetic evidence clearly supports independent evolution of self-compatibility in the four *Cochliobolus* species, and underpins the structural evidence.

b) Stemphylium

To investigate the origin of self-fertility in *Stemphylium*, Inderbitzin et al. (2005) compared the position of self-compatible species in an organismal phylogeny constructed using sequence data from four loci, unrelated to mating type, and in a phylogeny of the mating-type genes. The *MAT* datasets were analyzed using likelihood, parsimony, Bayesian and neighbor-joining methods. Several conclusions were reached:

1. Linked *MAT* regions are derived from ancestral, separate *MAT* regions, as suggested by the basal position of isolates with separate *MAT* regions in phylogenetic analyses.
2. There was 100% support from all analyses for the monophyly of *MAT1-1* from fused regions, and for the monophyly of *MAT1-2* from fused regions.
3. Organismal phylogenies support polyphyly of the isolates with fused *MAT* regions.
4. In contrast to the *Cochliobolus* history, where self-fertility appears to have originated in different species by independent mating-type gene fusions, Inderbitzin et al. (2005) found evidence for a single fusion, present in 76 self-fertile isolates, which appears to have been laterally transferred across lineages.
5. The isolates that harbor only *MAT1-1-1* may represent a third lineage that evolved selfing ability independently, with unknown genetic changes responsible for its evolution.

Evolution of fused *MAT* regions from separate self-incompatible progenitors was supported by DNA sequence comparison, showing that selfers with both *MAT* genes could have originated from a self-sterile ancestor by the inversion of an ancestral *MAT1-1-1* gene and its flanking regions, creating a crossover site and allowing the fusion of the inverted *MAT1-1-1* region to *MAT1-2-1* (Fig. 15.2; Inderbitzin et al. 2005). If the sequence of a *MAT1-1-1* locus in an incompatible strain is aligned to the same region in a self-compatible strain with both *MAT* regions, it is clear that the flanking sequence carrying *ORF1* is identical, until approx. 300 bp 3′ of *ORF1*, at which point the sequence loses identity but, in fact, is the reverse complement of the *MAT1-1-1* region for about 1740 bp. At this point, the sequence returns to identity with the 5′ flank of a *MAT1-2-1* gene, in the native orientation.

Thus, homothallism in *Stemphylium* appears to have evolved from outcrossing ancestors, by fusion of *MAT1-1* and *MAT1-2*, by lateral transfer of this fused region across lineages, and in certain cases, where *MAT1-1* is present only, by unknown means.

2. Sordariomycetes

a) Gibberella

All self-compatible, *Gibberella zeae* (nine species) isolates investigated by O'Donnell et al. (2004) carry contiguous *MAT1-1* and *MAT1-2* mating-type regions configured as initially described by Yun et al. (2000; Fig. 15.2). Five additional, related species examined by O'Donnell and coworkers, and the *G. moniliformis/fujikuroi* and *Fusarium oxysporum MAT* genes (Yun et al. 2000) carry only one of the two possible *MAT* idiomorphs. Phylogenetic analyses by O'Donnell et al. (2004) support a monophyletic origin of the linked *MAT* genes, and also suggest that the self-compatible lifestyle is derived from a self-incompatible ancestor within this group. This finding is in contrast to proposed polyphyletic evolutionary origins of self-compatibility in *Cochliobolus* (Yun et al. 1999), but in agreement with the *Stemphylium* findings (Inderbitzin et al. 2005).

There are no published data regarding an evolutionary mechanism, but the observation that the genes that flank *MAT* are faithfully conserved in *G. zeae* and most of the fungi described in Fig. 15.3 supports the notion that self-compatibility in *G. zeae* may have arisen by a recombination event that brought the *MAT1-1* and *MAT1-2* idiomorphs together in a single isolate, as proposed earlier by Yun et al. (1999). The most parsimonious event would be recombination between homologous sequences in the 3′ flanks of *MAT1-2-1* and *MAT1-1-1* of ancestral self-incompatible isolates of opposite mating type. With currently available information, it is not possible to find evidence for a candidate recombination point, since the *G. zeae MAT1-1-1* 3′ flank is unlike the *MAT1-1-1* 3′ flanks of self-incompatible isolates in the databases. The history of this stretch of sequence in *G. zeae* is unknown.

b) Neurospora and Sordaria

Pöggeler (1999) subjected *MAT* and *GPD* sequences of nine self-compatible and eight self-incompatible taxa of *Neurospora* and *Sordaria* to phylogenetic analyses, and concluded that:

1. *Neurospora* and *Sordaria* are monophyletic units, based on both *GPD* and *MAT*.
2. Self-compatible and self-incompatible species within both genera are distinct.
3. There was a single evolutionary origin of self-compatibility.
4. *Neurospora* self-compatible strains of A/a-type group separately from A-type.
5. Overall the data suggest early divergence of self-compatibility and self-incompatibility, before *Neurospora* and *Sordaria* speciated (Pöggeler 1999).

As with the Loculoascomycetes, self-compatibility in *Neurospora* and *Sordaria* appears to be derived from self-incompatible ancestors. Unlike *Cochlibolus*, but like *Stemphylium* and *G. zeae*, self-compatibility likely arose once; all extant self-compatible isolates within *Neurospora* or *Sordaria* are monophyletic. The molecular path of conversion from one lifestyle to the other is undescribed, but likely occurred by a recombination mechanism similar to that described for *Cochliobolus* (Yun et al. 1999).

3. Leotiomycetes: *Aspergillus* spp.

Geiser et al. (1996) suggested that heterothallism is the derived state in *Aspergillus* species, based on phylogenetic analyses using β-tubulin and hydrophobin sequences, and that heterothallism may have arisen once. Comparisons of three genomes, self-compatible *A. nidulans*, asexual *A. fumigatus*, and asexual *A. oryzae*, have provided insight into this question (Galagan 2005; Rydholm et al. 2005). *A. nidulans* has *MAT* genes at two unlinked loci in the genome (Dyer et al. 2003). *A. fumigatus* and *A. oryzae* each carry a single *MAT* gene – *A. fumigatus* has a *MAT1-1-1* ortholog whereas *A. oryzae* has a *MAT1-2-1* ortholog. A study of large numbers of field isolates of both indicate that both mating types are found, as with all asexual fungal populations studied to date (Sect. III.), a finding that hints, once again, to the possibility that both species may be capable of sexual reproduction.

Although the genomic data provide a glimpse of mating lifestyle evolution in *Aspergillus*, this view is based on comparison of species that are quite distantly related, unlike evaluation of closely related *Cochliobolus* taxa differing in reproductive lifestyle. Nevertheless, *MAT* structure in these three *Aspergillus* taxa supports the hypothesis of Geiser et al. (1996), and suggests that self-incompatibility is the derived state.

B. Functional Analysis of *MAT* by Heterologous Expression

1. Conversion of a Self-Incompatible to a Self-Compatible Strain

To determine whether *MAT* genes alone can control reproductive style, a sterile *C. heterostrophus MAT*-deletion strain was transformed with a construct carrying the fused *C. luttrellii MAT1-1-1*; *MAT1-2-1* gene (Yun et al. 1999). Transformants were selfed, and crossed to albino *C. heterostrophus MAT1-1* and *MAT1-2* tester strains. Abundant pseudothecia formed when the transformants were selfed or crossed, most of which were fertile (1%–10% of wild-type ascospore production). Pseudothecia and progeny from selfs were pigmented, whereas approximately half the pseudothecia and half the progeny from crosses were albino, indicating that self-incompatible *C. heterostrophus* expressing a *MAT* from a self-compatible species can both self and outcross. Thus, the *C. luttrellii MAT1-1-1*; *MAT1-2-1* gene alone conferred selfing ability to normally self-incompatible *C. heterostrophus*, without impairing its ability to cross. A similar experiment was done with the *C. homomorphus MAT1-2-1*; *MAT1-1-1* gene. In this case, transformants were able to make pseudothecia, but these were barren. Since the only difference in the *C. luttrellii* and *C. homomorphus* experiments is the *MAT* gene itself (the genetic background of *C. heterostrophus* is otherwise constant), we can conclude that differences in these *MAT* sequences determine fertility.

2. Conversion of a Self-Compatible to a Self-Incompatible Species

a) Loculoascomycete Conversion

In a reverse of the above experiment, the *C. luttrellii MAT1-1-1*; *MAT1-2-1* gene was deleted (Lu and Turgeon, unpublished data), rendering the strain completely sterile. This strain was then transformed, separately, with a *MAT1-1-1* or *MAT1-2-1* gene from self-incompatible *C. heterostrophus*, yielding a *MAT*-deleted *C. luttrellii* strain carrying *C. heterostrophus MAT1-1-1*, and a *MAT*-deleted *C. luttrellii* strain carrying *C. heterostrophus MAT1-2-1*. When these strains were crossed, it was found that:

1. A *C. luttrellii* transgenic strain carrying *ChetMAT1-1-1* and a *C. luttrellii* transgenic strain carrying *ChetMAT1-2-1* can mate in a self-incompatible manner, and the fertility of the cross is similar to that of a wild-type *C. luttrellii* self.
2. A *C. luttrellii* transgenic strain carrying *ChetMAT1-1-1* can mate with the parental wild-type *C. luttrellii MAT1-1*;*MAT1-2* strain, indicating that the latter is able to outcross, a result that was expected but has not been demonstrated previously.
3. Each transgenic *C. luttrellii* strain is also able *to self*, although all pseudothecia produced are smaller than those of wild type and fertility is low (number of asci was about 5%–10% that of the wild type, and full tetrads were found). No recombinants were found in ascospores isolated from these tiny pseudothecia, demonstrating that all sexual structures originated from a self.

These data support the argument that in *Cochliobolus* spp., and perhaps other Ascomycetes also, the primary determinant of reproductive mode is *MAT* itself, and that a self-incompatible strain can be made self-compatible, or a self-compatible strain can be made self-incompatible by exchange of *MAT* genes. This bolsters the argument that a change in reproductive lifestyle is initiated by a recombination event. The ability to self, observed in transgenic *C. luttrellii* strains generated in this study, also suggests that both MAT1-1-1 and MAT1-2-1 proteins of *Cochliobolus* spp. carry a set of equivalent transcription regulatory activities capable of promoting sexual development alone, in a suitable genetic background.

b) Sordariomycete Conversion

In a parallel study, Lee et al. (2003) demonstrated that *G. zeae* (*MAT1-1*;*MAT1-2*), a species that is naturally able to self and also outcross, can be made obligately self-incompatible by targeted deletion of either the *MAT1-1* or *MAT1-2* sequence(s). Strains (*mat1-1*;*MAT1-2* and *MAT1-1*;*mat1-2*, respectively) with these deletions are sterile – no perithecia are formed when selfed. Subsequent crossing of *MAT1-1*;*mat1-2* by *mat1-1*;*MAT1-2* strains results in fertile perithecia. This experiment should be contrasted with the *Cochliobolus* experiment, in which deletion of the entire *MAT* locus from self-compatible *C. luttrellii* results in sterility, but adding back a *single MAT* gene from self-incompatible *C. heterostrophus* can activate the full mating process. Reasons for this difference may reside in the structures of the

Loculoascomycete vs. Pyrenomycete *MAT1-1* loci, which consist of a single gene in the former, and three genes in the latter. Perhaps the finding that the Loculoascomycete proteins may be hybrid *MAT* proteins dictates these differences (see Fig 15.5).

V. Functions of Mating-Type Genes

Very little is known about the target genes to which the mating-type transcription factors bind while *MAT1-1-2* encodes proteins of unknown functions. Therefore, the developmental processes controlled by the mating-type genes cannot be directly inferred from the molecular functions of the proteins they encode. Instead, their biological role has been deduced from the effect of mating-type gene mutations on the sexual cycle. These analyses comprised qualitative and quantitative examinations of progeny, and cytological observations of the development inside the fruiting body. Deletion of the *MAT* loci from different fungi confirmed that they contain the essential regulatory genes for fertilization, but *ΔMAT* strains still produce pre-fertilization male and female reproductive structures, indicating that mating types are not involved in the developmental switch from vegetative stage to sexual reproduction (Coppin et al. 1993; Ferreira et al. 1998). In addition to the role of mating types during fertilization, genetic and cytological observations show that they are required also during the formation of the fruiting body. We will briefly summarize the main conclusions about the mating-type gene biological functions in the different model systems.

A. Functions of Mating-Type Genes During Fertilization

1. Regulatory Functions of Mating-Type Genes

Fertilization or mating requires a recognition step between sexually compatible strains, leading to the entry of a nucleus into the female organ (Fig. 15.1). In self-incompatible species, this step occurs between a female organ and a donor cell of opposite mating type. Several lines of evidence demonstrate that *MAT1-1-1* and *MAT1-2-1* genes are the master genes regulating fertilization in self-incompatible Euascomycetes. Deletion of the *MAT* locus in *N. crassa* (Ferreira et al. 1998) or *C. heterostrophus* (Wirsel et al. 1996) leads to complete absence of mating, while *P. anserina ΔMAT* strains, considered initially as completely mating deficient (Coppin et al. 1993), in fact display a very low mating ability (Arnaise et al. 2001). Transformation of the *ΔMAT* strains with wild-type *MAT1-1-1* or *MAT1-2-1* restores mating ability to a wild-type level in the three fungi (Debuchy et al. 1993; Wirsel et al. 1996, 1998; Ferreira et al. 1998). The regions required for fertilization in *mat A-1* and *mat a-1* have been determined in detail in *N. crassa*. Mutation $A^{m}99$ was localized inside the α1 encoding region of *mat A-1*, and converted the conserved tryptophan at position 86 to a stop codon (Fig. 15.4; Saupe et al. 1996). The corresponding strain is completely male sterile, but surprisingly still female fertile, albeit to a reduced level. Further analyses of a series of *mat A-1* mutations indicated that mating as male requires a complete α1 domain and residues up to position 227. These results suggest that the target genes required for female and male fertility may be regulated differently by mat A-1, for instance, by the interaction of mat A-1 with cofactors specific to female and male organs. Deletion analyses of *mat a-1* indicates that an intact HMG domain and the C-terminal tail are required for mating (Philley and Staben 1994). The sequence within the terminal 180 amino acids required for mating does not appear to be very specific; the *P. anserina FPR1* gene confers mating in *N. crassa*, although it encodes a carboxyl terminus quite different from that of *mat a-1* (Arnaise et al. 1993). Taken together, these data indicate that MAT1-1-1 and MAT1-2-1 are activators of the functions required for fertilization.

Mutations in *FMR1*, *SMR2* and *FPR1* in *P. anserina* revealed a more complex wiring (Arnaise et al. 2001). Mutations in *FPR1* that did not affect the *mat*+ mating function resulted in *mat*+ strains that were capable of selfing. Consistent with the idea that these strains became self-fertile, they produced male cells able to fertilize a *mat*+ strain. This phenotype indicates that these mutations have relieved repression of the *mat*– functions required for mating, suggesting that the wild-type *FPR1* gene represses these functions in *mat*+ sexual organs (Fig. 15.9). Similarly, mutations leading to self-fertility have been observed in *FMR1*, and more surprisingly in *SMR2*. In contrast to *FMR1*, *SMR2* is not required for the expression of *mat*– mating function, but it appears now to be necessary together with *FMR1* for the repression of *mat*+ fertilization functions in *mat*– sexual organs (Fig. 15.9).

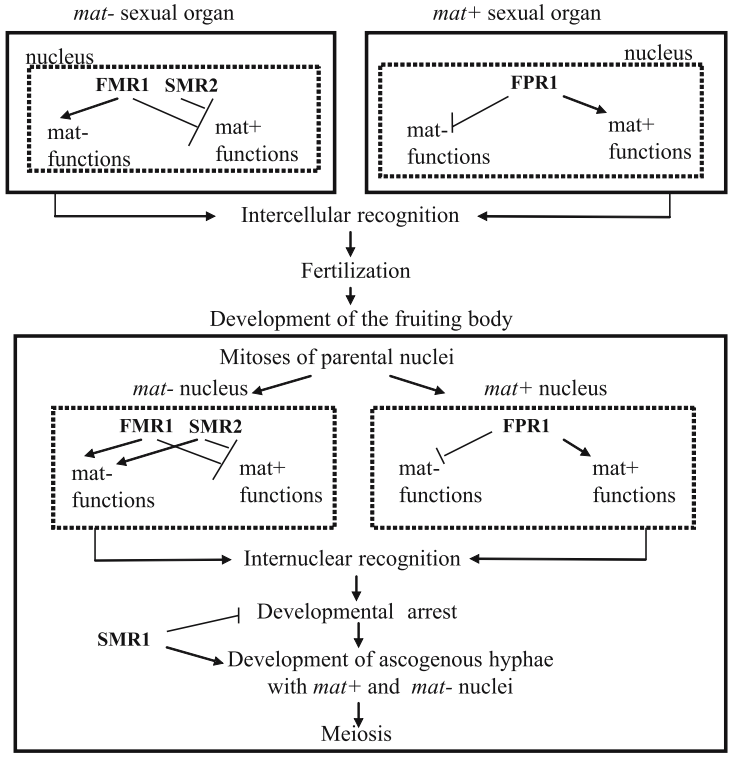

Fig. 15.9. Function of the mating-type genes of *P. anserina* during the sexual cycle. *Arrows* connote positive regulation and *lines ending in bars* connote repression. In the sexual organs, the mating-type genes control the functions required for fertilization. In the fruiting body, the mating-type genes control the functions required for internuclear recognition and the development of the ascogenous hyphae. Genetic experiments suggested that *FPR1* and *FMR1* have a nucleus-limited expression during internuclear recognition (reviewed in Coppin et al. 1997). The nucleus-limited expression of *FPR1* in the *mat+* nucleus controls the expression of a specific set of proteins that were assumed to remain in the vicinity of this nucleus and to determine a mat+ identity. A similar rationale applies to *SMR2* for the establishment of a *mat–* nuclear identity. FMR1 does not display a nucleus-limited expression in the genetic test (Arnaise et al. 1997). However, its interaction with SMR2, demonstrated in the yeast two-hybrid system, is assumed to prevent its diffusion to adjacent nuclei during internuclear recognition. Interactions between nuclei with different nuclear identity was assumed to trigger the internuclear recognition process and the developmental arrest

2. Target Genes Involved in Fertilization

The wealth of data available about mating in *S. cerevisae* (Kurjan 1993) led to the quick identification of the putative target genes required for fertilization in filamentous fungi, notably the genes encoding the pheromone precursors and the pheromone receptors. Precursor pheromone genes similar to the yeast *MFa* and *MF*α genes have been identified in *C. parasitica* (Zhang et al. 1993, 1998; Turina et al. 2003), *M. grisea* (Shen et al. 1999), *N. crassa* (Bobrowicz et al. 2002; Kim et al. 2002) and *P. anserina* (Coppin et al. 2005b; see Chap. 16, this volume). In all these self-incompatible fungi, transcripts of the prepheromone genes are present only in a mating-type-specific manner; *MF*α- and *MFa*-like genes are expressed only in *MAT1-1* or *MAT1-2* strains, respectively. Pheromone receptor genes *pre-1* and *pre-2* have been identified in *N. crassa* (Pöggeler and Kück 2001; Kim and Borkovich 2004). Kim and Borkovich (2004) observed that RNA levels of *pre-1* in *mat A* strains were more than 100-fold higher than those in *mat a* strains. Data obtained by Pöggeler and Kück (2001) suggested that transcription of the pheromone receptor genes is mating type-independent. The use of a *fluffy* mutant by this latter group, instead of a wild-type strain, may explain this discrepancy. The control of the prepheromone genes and *pre-1* by mat A-1 and mat a-1 was substantiated by the loss of transcription of these target genes in strains containing *mat A-1* and *mat a-1* mutant alleles leading to sterility (Bobrowicz et al. 2002; Kim et al. 2002; Kim and Borkovich 2004).

Genome sequences offer the possibility to search for all potential binding sites of the MAT DNA-binding proteins. This type of search has been undertaken to identify putative downstream target genes of FMR1, SMR2 and FPR1 in *P. anserina* (Hdidou, Coppin and Debuchy, unpublished data). Prepheromone genes (*mfm* and *mfp*) and pheromone receptor genes (*pre-1* and *pre-2*) of *P. anserina* were used for the identification of putative target sites of the MAT transcription factors. Comparison of the promoter sequences of the *mfm* and *pre-1* genes allows us to define two common sequences of 12 bp. Screening of the entire *P. anserina* genome with these sequences reveals only one gene, *KEX1*, unambiguously related to fertilization due to its role during α-like prepheromone processing (see Chap. 10, this volume). Screening of the *P. anserina* genome with sequences common to the promoter region of the *mfp* and *pre-2* genes of *P. anserina* does not show

any gene with an obvious relationship to fertilization. Although this study has allowed us to identify several candidate genes whose role in the sexual cycle remains to be established, it is surprising that no *STE* gene identified in yeast has been found in this search. Coppin et al. (2005b) have demonstrated that the MAT regulatory proteins control genes required for post-transcriptional modification of pheromone precursors. None of these genes, which are well defined from yeast studies, have been found, except *KEX1*. This suggests that either MAT transcription factors are not the direct activators/repressors of these genes, or that each MAT transcription factor binds different cofactors and/or has different binding sites in different target genes. This possibility would preclude the identification of common binding sites from the different target genes expressed in female organs and male cells. Biochemical evidence for the binding of MAT transcription factors to the available target genes should be the first step to resolve this question.

3. A Possible Common Scheme for Fertilization Control in Euascomycetes

The dual activator/repressor functions found for the MAT transcription factors in *P. anserina* may be extended to *N. crassa*. The prepheromone gene *ccg-4* is transcribed specifically in *mat A* strains, as demonstrated by Bobrowicz et al. (2002). This gene was also found to be transcribed in the a^{m33} mutant strain, which contains a mutant *mat a-1* allele but has wild-type mating behavior. Bobrowicz and coworkers proposed that the *mat a-1*^{m33} allele retains its ability to activate the a-like pheromone gene, but has lost a repressing function for *ccg-4*. This situation is reminiscent of the fertilization control evidenced in *P. anserina*, although the a^{m33} strain does not display the self-fertile phenotype observed in *P. anserina mat* mutants. It must be noted that the self-fertile phenotype is hardly detectable in the absence of mutations that increase the production of male cells, and had escaped our initial examination of *mat* mutants.

The deletion of the mating-type locus of the self-compatible *G. zeae* (Fig. 15.2) resulted in one half of the strains being completely sterile but the other half produced perithecium-like structures (Desjardins et al. 2004). These structures were smaller in size and more variable in shape than perithecia produced by wild-type strains, and did not contain any ascospores. We would suggest that these perithecium-like structures result from the basal expression of the mating-type target genes in a context where they are neither activated nor repressed. This basal expression of the target genes may be sufficient for fertilization, but appropriate regulatory proteins would be required for further development such as internuclear recognition. The perithecia-like structures were not produced in strains carrying a deletion of either of the *MAT1-1* or *MAT1-2* mating-type sequences. This observation suggests that MAT1-2-1 represses the *MAT1-1* target genes required for fertilization while the MAT1-1 products have a similar effect on *MAT1-2* target genes, according to the regulatory pathway evidenced in *P. anserina*. Therefore, even in self-compatible species, the mating-type transcription factors may have a repressor activity on the target genes required for fertilization. The compatibility between the MAT1-1-1 and MAT1-2-1 activator and repressor functions in the same genome could be obtained by an epigenetic event, silencing either the one or the other mating-type information, thus resulting in a functionally self-incompatible mechanism. A similar hypothesis was initially proposed by Metzenberg and Glass (1990).

It appears that in self-incompatible *Cochliobolus* species, *MAT1-1-1* encodes a protein that contains an alpha box domain and a partial HMG box domain, and *MAT1-2-1* encodes protein containing a HMG box domain plus a partial alpha box domain (Lu and Turgeon, unpublished data; see Sect. III.C.1 and Fig. 15.5). Both MAT1-1-1 and MAT1-2-1 proteins alone are capable of promoting sexual development in a heterologous genetic background. However, both proteins may be inactive in their respective self-incompatible genetic background due to the presence of a common repressor. A mutation that blocks the repressor activity would allow *MAT* to initiate the early stages of sexual development (e.g., fruiting-body formation) in the absence of the opposite mating type. A *C. heterostrophus* REMI mutant, generated in a *MAT1-2* strain, is available that produces abundant, but barren pseudothecia when selfed. This type of mutant, although self-compatible, is able to form fertile pseudothecia when mated to a *MAT1-1* but not a *MAT1-2* albino tester, indicating the self-incompatible mating specificity is maintained. The native *MAT1-2-1* gene is intact in the REMI mutant, and unlinked to the mutation site (Turgeon and Lu, unpublished data). These data suggest that both *MAT1-1* and *MAT1-2* haploid strains could initiate sexual development if a protein is blocked

in the self-incompatible genetic background. The REMI mutated gene may correspond to the putative repressor hypothesized above.

B. Functions of Mating-Type Genes During Fruiting-Body Development

Mating-type genes are required for the post-fertilization development of fruiting bodies, but the specific stages controlled by these genes remain elusive, although in most cases a common feature seems to be the control of internuclear recognition.

1. *C. heterostrophus*

Complementation of *ΔMAT* strains with various fragments of each idiomorph leads to the identification of a sequence that is not necessary for pseudothecia formation, but is required for the completion of the sexual cycle after mating (Wirsel et al. 1998). This 160-bp sequence is localized in the 3′ UTR of *MAT1-1-1* and *MAT1-2-1* transcripts, outside the idiomorph sequence. This sequence is therefore identical in *MAT1-1-1* and *MAT1-2-1* transcripts, but must be present in each transcript for ascospore production. The authors have proposed that this sequence is required for proper localization of *MAT* mRNAs, leading to recognition between nuclei of opposite mating type and to normal meiosis. No cytological observations are available to allow specification of the defect resulting from the absence of the 160-bp region in the *MAT* loci.

2. *G. zeae*

Deletion of either *MAT1-1* or *MAT1-2* mating-type sequences from self-compatible *G. zeae* (Fig. 15.2) resulted in two strains that have a self-incompatible mating-type structure and behavior (Lee et al. 2003). In crosses of the *mat1-1*; *MAT1-2* strain with a wild-type strain, the perithecia contain *mat1-1*; *MAT1-2* nuclei and nuclei of the self-compatible parent. Lee et al. (2003) analyzed the progeny to establish what the proportion is of the two possible nuclear recognition events: self-recognition of wild-type nuclei, and internuclear recognition between a wild-type nucleus and a *mat1-1*; *MAT1-2* nucleus. Surprisingly, they found only asci contributed by both parents, suggesting that wild-type and *mat1-1*; *MAT1-2* nuclei recognize each other preferentially. This preferential recognition might be attributed to an undefined mechanism that favors recruitment of nuclei with different genetic backgrounds, a phenomenon known as "relative heterothallism" in *E. nidulans* (Hoffmann et al. 2001). This explanation is excluded here, however, as the two strains used for crossing differ only by the deletion of the *MAT1-1* mating-type genes. Similar results were obtained in crosses of *MAT1-1*; *mat1-2* strains with wild-type strains. The finding of Lee et al. (2003) clearly demonstrates that the partnering of nuclei is not random during perithecium development, but it remains to be established why wild-type nuclei do not contribute to the progeny while they do so in a wild-type cross. A possible explanation is that their crossing partner induces the wild-type nuclei to switch to the opposite mating type by an epigenetic control of mating-type gene expression (Metzenberg and Glass 1990). It would be interesting to test if wild-type progeny issued from a first cross with *MAT1-1*; *mat1-2* or *mat1-1*; *MAT1-2* strains of *G. zeae* are still able to cross with both *MAT1-1*; *mat1-2* and *mat1-1*; *MAT1-2* partners, or if they have conserved the putative epigenetic switching of their first cross, as proposed by Metzenberg and Glass (1990).

3. *N. crassa*

In *N. crassa*, several mutations in *mat A-1* or *mat a-1* prevent mating but none was found to impede fruiting-body development after mating, precluding the investigation of the role of these genes after fertilization. Surprisingly, mutations in either *mat A-2* or *mat A-3* do not affect fruiting-body development, although these mutations result in stop codons before the HPG and HMG domains of mat A-2 or mat A-3, respectively (Ferreira et al. 1998). However, Glass and Lee (1992) have isolated a strain called A^{IIRIP} that displayed very low fertility. Subsequent RT-PCR experiments show that in A^{IIRIP}, *mat A-2* and *mat A-3* are not transcribed, probably as a result of RIP mutations and methylation (Ferreira et al. 1998). This leads to the intriguing conclusion that *mat A-2* and *mat A-3* are redundant genes, since inactivation of both is required to observe a defect during the sexual cycle. Examination of developing $A^{IIRIP} \times a$ perithecia showed that formation of ascogenous hyphae is infrequent, although occasional croziers can be observed (Glass and Lee 1992). Glass' group proposed that these genes are involved in orchestrating the multiple mitotic divisions of opposite mating-type nuclei before cellularization, or in the control of the internuclear

recognition during cellularization (Glass and Lee 1992; Ferreira et al. 1998). Progress on mating-type function in *N. crassa* has been impeded by meiotic silencing by unpaired DNA (MSUD), a process whereby an unpaired gene and all homologous copies are silenced during meiosis (Shiu et al. 2001). Idiomorphs at the *mat* locus are immune to MSUD, although they are unpaired during meiosis. By contrast, ectopic copies of *mat a* or *mat A* idiomorphs trigger MSUD and result in barren perithecia (Glass et al. 1988; Shiu and Metzenberg 2002). The action of MSUD on ectopic copies of the idiomorphs reveals that their information is required during meiosis, or shortly after this step. Therefore, we propose that *mat a-1* has an essential function during meiosis. A similar conclusion could be proposed for the *mat A* idiomorph, but the specific requirement for each *mat A* gene during meiosis still remains to be established.

4. *P. anserina*

The interpretation of the data obtained on the mating-type function in *P. anserina* suggests that mating-type proteins control at least three steps during fruiting-body development: internuclear recognition, a developmental arrest, and a recovery from developmental arrest. This model is presented in Fig. 15.9, and recent data supporting this model are presented below.

Several lines of evidence support the idea that the mating-type genes encode the master regulators of internuclear recognition. Crosses of *FPR1* mutant strains with a *mat–* tester strain gave the expected biparental asci, but surprisingly some of the asci contain ascospore progeny carrying genetic markers from the *mat+* mutant strain only (Zickler et al. 1995; Arnaise et al. 2001). The proportion of these latter asci and their ascospores, called uniparental progeny, is constant for a given mutant allele, but varies depending on the allele. Moreover, high proportions of uniparental progeny correlate always with greatly reduced numbers of progeny relative to a wild-type cross. Interpretation of the uniparental progeny is based on the model proposed for the self-fertility of these mutants. The *FPR1* mutant allele has retained the ability to activate the *mat+* functions required for internuclear recognition, but has lost a repressing ability for the *mat–* functions required for internuclear recognition (Fig. 15.9). Therefore, both *mat+* and *mat–* functions required for internuclear recognition are expressed in the same nucleus, and trigger self-recognition of this nucleus. This selfish nucleus can engage itself in the developmental events followed by wild-type pairs of nuclei. It eventually yields a uniparental progeny, although with a much lower efficiency. Microscope observations of *FPR1*$^-$ × *mat–* perithecia showed uninucleate ascogenous hyphae and haploid meioses, which are consistent with the proposed model (Zickler et al. 1995; Arnaise et al. 2001). A similar rationale applies to *FMR1* and *SMR2* mutant strains, which contribute to the formation of a uniparental progeny when crossed to *mat+* tester strains (Fig. 15.9). Genetic data indicate that *FMR1* and *SMR2* are both necessary for the control of internuclear recognition, in agreement with the FMR1/SMR2 interaction found in the yeast two-hybrid system. The similarity of the regulation of fertilization and internuclear recognition prompted Coppin et al. (2005b) to test if the pheromone/receptor systems may be involved in nuclear partnering. These authors found that crosses between strains devoid of pheromone genes were fertile, indicating that these genes are not involved in the fruiting-body development after fertilization.

Further investigation on the expression of mating-type genes suggested that internuclear recognition is associated with a developmental arrest (Fig. 15.9). While trying to construct a strain expressing, vegetatively, the three genes involved in internuclear recognition, Coppin and Debuchy (2000) observed that the expression of *FMR1*, *SMR2* and *FPR1* in the same nucleus inside ascospores resulted in a lethal phenotype. This lethal phenotype can be suppressed by the expression of *SMR1*. These effects suggest that *FMR1*, *SMR2* and *FPR1* expression during internuclear recognition triggers a developmental arrest that is overcome by the action of *SMR1*. According to this hypothesis, a cross involving a *mat–* strain with an inactive *SMR1* gene should reveal the putative developmental arrest. In agreement with this prediction, crosses involving different *SMR1*$^-$ mutant strains and a *mat+* strain result in complete arrest of the development of the fruiting body at a stage preceding the formation of the ascogenous hyphae (Coppin, Robelet, Arnaise, Bouhouche, Zickler and Debuchy, unpublished data; Arnaise et al. 1997). This phenotype is reminiscent of the $A^{IIRIP} \times a$ phenotype in *N. crassa* (Glass and Lee 1992), except that in the latter case, the perithecia contained some croziers and yielded a reduced number of progeny, whereas in *P. anserina* the *SMR1*$^-$ × *mat+* cross was completely sterile. Nevertheless, it must

be noted that the A^{IIRIP} phenotype was attributed to a transcription extinction of *mat A-2* and *mat A-3*. A very low level of transcription of one of these two genes cannot be ruled out and may contribute to the formation of a few asci. For these reasons, we would suggest that the A^{IIRIP} and $SMR1^{-}$ mutant alleles are in one and the same pathway. *SMR1* is required each time a biparental progeny is formed, namely, each time two different nuclei of opposite mating type recognize each other. It is supposed that SMR1 biological function is to overcome the developmental arrest resulting from internuclear recognition, and to regulate the differentiation of the biparental ascogenous hyphae (Fig. 15.9).

VI. Conclusion

The past several years have seen a rapid rise in the number of cloned and characterized mating-type loci from an ever-expanding group of filamentous Ascomycetes. However, the available mating-type gene database still lacks some representatives of functionally or taxonomically important groups. No complete mating-type sequences are available for the Euascomycetes that undergo mating-type switching, or for lichen-forming fungi. The analysis of the evolution of mating types is at the beginning, focusing first on the evolutionary relationship between self-compatible and self-incompatible species from the same genus. A comparative evolutionary history of the mating-type loci of organisms with more distant connections, such as those of *Candida albicans*, *N. crassa*, and *Cryptococcus neoformans*, will require much effort to be understood. Finally, investigations into the role of mating-type proteins during development of the fruiting body must be scaled up. The major challenge in the mating-type field is to identify the target genes of the mating-type transcription factors, and to determine the function of these target genes, as well as the function of the MAT1-1-2 and MAT1-1-4 proteins themselves. Until recently, this topic was limited due to intractable difficulties with genetical approaches, or tedious and uncertain molecular methods for finding target genes. Now, entire genomes are available for all model systems, and microarrays have been made or are under construction for most of them. Whole-genome methods will accelerate the discovery of target genes, provided that microarray strategies include careful selection of the mutants to be profiled, to avoid including candidate genes not related to mating-type function.

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References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Arie T, Kaneko I, Yoshida T, Noguchi M, Nomura Y, Yamaguchi I (2000) Mating-type genes from asexual phytopathogenic ascomycetes *Fusarium oxysporum* and *Alternaria alternata*. Mol Plant Microbe Interact 13:1330–1339

Arnaise S, Zickler D, Glass NL (1993) Heterologous expression of mating-type genes in filamentous fungi. Proc Natl Acad Sci USA 90:6616–6620

Arnaise S, Coppin E, Debuchy R, Zickler D, Picard M (1995) Models for mating type gene functions in *Podospora anserina*. Fungal Genet Newslett Suppl 42:79

Arnaise S, Debuchy R, Picard M (1997) What is a bona fide mating-type gene? Internuclear complementation of *mat* mutants in *Podospora anserina*. Mol Gen Genet 256:169–178

Arnaise S, Zickler D, Le Bilcot S, Poisier C, Debuchy R (2001) Mutations in mating-type genes of the heterothallic fungus *Podospora anserina* lead to self-fertility. Genetics 159:545–556

Badgett TC, Staben C (1999) Interaction between and transactivation by mating type polypeptides of *Neurospora crassa*. Fungal Genet Newslett Suppl 46:73

Barve MP, Arie T, Salimath SS, Muehlbauer FJ, Peever TL (2003) Cloning and characterization of the mating type (*MAT*) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a *MAT* phylogeny of legume-associated *Ascochyta spp*. Fungal Genet Biol 39:151–167

Beatty NP, Smith ML, Glass NL (1994) Molecular characterization of mating-type loci in selected homothallic species of *Neurospora*, *Gelasinospora* and *Anixiella*. Mycol Res 98:1309–1316

Bennett RS, Yun SH, Lee TY, Turgeon BG, Arseniuk E, Cunfer BM, Bergstrom GC (2003) Identity and conservation of mating type genes in geographically diverse

isolates of *Phaeosphaeria nodorum*. Fungal Genet Biol 40:25–37
Bistis GN (1998) Physiological heterothallism and sexuality in Euascomycetes: a partial history. Fungal Genet Biol 23:213–222
Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ (2002) The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804
Butler G, Kenny C, Fagan A, Kurischko C, Gaillardin C, Wolfe KH (2004) Evolution of the *MAT* locus and its Ho endonuclease in yeast species. Proc Natl Acad Sci USA 101:1632–1637
Casselton LA (2002) Mate recognition in fungi. Heredity 88:142–147
Chang S, Staben C (1994) Directed replacement of *mt A* by *mt a-1* effects a mating type switch in *Neurospora crassa*. Genetics 138:75–81
Cisar CR, TeBeest DO, Spiegel FW (1994) Sequence similarity of mating type idiomorphs: a method which detects similarity among the Sordariaceae fails to detect similar sequences in other filamentous ascomycetes. Mycologia 86:540–546
Coppin E, Debuchy R (2000) Co-expression of the mating-type genes involved in internuclear recognition is lethal in *Podospora anserina*. Genetics 155:657–669
Coppin E, Arnaise S, Contamine V, Picard M (1993) Deletion of the mating-type sequences in *Podospora anserina* abolishes mating without affecting vegetative functions and sexual differentiation. Mol Gen Genet 241:409–414
Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61:411–428
Coppin E, Arnaise S, Bouhouch K, Robellet X, Zickler D, Debuchy R (2005a) Functional study of *SMR1*, a mating type gene which does not control self/non-self recognition in *Podospora anserina*. Fungal Genet Newslett Suppl 52:176
Coppin E, de Renty C, Debuchy R (2005b) The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Eukaryot Cell 4:407–420
Debuchy R, Arnaise S, Lecellier G (1993) The *mat–* allele of *Podospora anserina* contains three regulatory genes required for the development of fertilized female organs. Mol Gen Genet 241:667–673
Desjardins AE, Brown DW, Yun SH, Proctor RH, Lee T, Plattner RD, Lu SW, Turgeon BG (2004) Deletion and complementation of the mating type (*MAT*) locus of the wheat head blight pathogen *Gibberella zeae*. Appl Environ Microbiol 70:2437–2444
Dettman JR, Harbinski FM, Taylor JW (2001) Ascospore morphology is a poor predictor of the phylogenetic relationships of *Neurospora* and *Gelasinospora*. Fungal Genet Biol 34:49–61
Dyer PS, Paoletti M, Archer DB (2003) Genomics reveals sexual secrets of *Aspergillus*. Microbiology 149:2301–2303
Ferreira AV, An Z, Metzenberg RL, Glass NL (1998) Characterization of *mat A-2*, *mat A-3* and *ΔmatA* mating-type mutants of *Neurospora crassa*. Genetics 148:1069–1079
Foster SJ, Fitt BD (2003) Isolation and characterisation of the mating-type (*MAT*) locus from *Rhynchosporium secalis*. Curr Genet 44:277–286
Fraser JA, Diezmann S, Subaran RL, Allen A, Lengeler KB, Dietrich FS, Heitman J (2004) Convergent evolution of chromosomal sex-determining regions in the animal and fungal kingdoms. PLoS Biol 2:e384
Galagan J (2005) Comparative analysis of filamentous fungi. Fungal Genet Newslett Suppl 52:29
Geiser DM, Timberlake WE, Arnold ML (1996) Loss of meiosis in *Aspergillus*. Mol Biol Evol 13:809–817
Glass NL, Kuldau GA (1992) Mating type and vegetative incompatibility in filamentous ascomycetes. Annu Rev Phytopathol 30:201–224
Glass NL, Lee L (1992) Isolation of *Neurospora crassa A* mating type mutants by repeat induced point (RIP) mutation. Genetics 132:125–133
Glass NL, Lorimer IAJ (1991) Ascomycete mating types. In: Bennett JW, Lasure LS (eds) More gene manipulations in fungi. Academic Press, New York, pp 193–216
Glass NL, Nelson MA (1994) Mating type genes in Ascomycetes. In: Wessels JGH, Meinhardt F (eds) Growth, differentiation and sexuality. Springer, Berlin Heidelberg New York, pp 295–306
Glass NL, Vollmer SJ, Staben C, Grotelueschen J, Metzenberg RL, Yanofsky C (1988) DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. Science 241:570–573
Glass NL, Metzenberg RL, Raju NB (1990) Homothallic Sordariaceae from nature: the absence of strains containing only the *a* mating type sequence. Exp Mycol 14:274–289
Goodwin SB (2002) The barley scald pathogen *Rhynchosporium secalis* is closely related to the discomycetes *Tapesia* and *Pyrenopeziza*. Mycol Res 106:645–654
Hiscock SJ, Kües U (1999) Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. Int Rev Cytol 193:165–295
Hoffmann B, Eckert SE, Krappmann S, Braus GH (2001) Sexual diploids of *Aspergillus nidulans* do not form by random fusion of nuclei in the heterokaryon. Genetics 157:141–147
Inderbitzin P, Harkness J, Turgeon BG, Berbee ML (2005) Lateral transfer of life history strategy in *Stemphylium*. Proc Natl Acad Sci USA 102:11390–11395
Jacobsen S, Pöggeler S (2001) Interaction between mating-type proteins from the homothallic ascomycete *Sordaria macrospora*. Fungal Genet Newslett Suppl 48:140
Johnson AD (1995) Molecular mechanisms of cell-type determination in budding yeast. Curr Opin Genet Dev 5:552–558
Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52:1781–1798
Kim H, Metzenberg RL, Nelson MA (2002) Multiple functions of *mfa-1*, a putative pheromone precursor gene of *Neurospora crassa*. Eukaryot Cell 1:987–999
Kronstad JW, Staben C (1997) Mating type in filamentous fungi. Annu Rev Genet 31:245–276
Kurjan J (1993) The pheromone response pathway in *Saccharomyces cerevisiae*. Annu Rev Genet 27:147–179
Lahn BT, Page DC (1999) Four evolutionary strata on the human X chromosome. Science 286:964–967

Lee J, Sonnhammer EL (2003) Genomic gene clustering analysis of pathways in eukaryotes. Genome Res 13:875–882

Lee J, Lee T, Lee YW, Yun SH, Turgeon BG (2003) Shifting fungal reproductive mode by manipulation of mating type genes: obligatory heterothallism of *Gibberella zeae*. Mol Microbiol 50:145–152

Leubner-Metzger G, Horwitz BA, Yoder OC, Turgeon BG (1997) Transcripts at the mating type locus of *Cochliobolus heterostrophus*. Mol Gen Genet 256:661–673

Linde CC, Zala M, Ceccarelli S, McDonald BA (2003) Further evidence for sexual reproduction in *Rhynchosporium secalis* based on distribution and frequency of mating-type alleles. Fungal Genet Biol 40:115–125

Liu YJ, Hall BD (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proc Natl Acad Sci USA 101:4507–4512

Metzenberg RL (1990) The role of similarity and difference in fungal mating. Genetics 125:457–462

Metzenberg RL, Glass NL (1990) Mating type and mating strategies in *Neurospora*. Bioessays 12:53–59

Nakai K, Horton P (1999) PSORT: a program for detecting the sorting signals of proteins and predicting their subcellular localization. Trends Biochem Sci 24:34–35

Nolting N, Pöggeler S (2005) Characterization of transcription factors from the filamentous ascomycete *Sordaria macrospora* and their implications on fruiting-body development. Fungal Genet Newsletter Suppl 52:184

O'Donnell K, Ward TJ, Geiser DM, Corby Kistler H, Aoki T (2004) Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet Biol 41:600–623

Paoletti M, Rydholm C, Schwier EU, Anderson MJ, Szakacs G, Lutzoni F, Debeaupuis JP, Latg JP, Denning DW, Dyer PS (2005) Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr Biol (in press)

Perkins DD (1987) Mating-type switching in filamentous ascomycetes. Genetics 115:215–216

Philley ML, Staben C (1994) Functional analyses of the *Neurospora crassa* MT a-1 mating type polypeptide. Genetics 137:715–722

Pöggeler S (1999) Phylogenetic relationships between mating-type sequences from homothallic and heterothallic ascomycetes. Curr Genet 36:222–231

Pöggeler S (2001) Mating-type genes for classical strain improvements of ascomycetes. Appl Microbiol Biotechnol 56:589–601

Pöggeler S (2002) Genomic evidence for mating abilities in the asexual pathogen *Aspergillus fumigatus*. Curr Genet 42:153–160

Pöggeler S, Kück U (2000) Comparative analysis of the mating-type loci from *Neurospora crassa* and *Sordaria macrospora*: identification of novel transcribed ORFs. Mol Gen Genet 263:292–301

Pöggeler S, Kück U (2001) Identification of transcriptionally expressed pheromone receptor genes in filamentous ascomycetes. Gene 280:9–17

Pöggeler S, Risch S, Kück U, Osiewacz HD (1997) Mating-type genes from the homothallic fungus *Sordaria macrospora* are functionally expressed in a heterothallic ascomycete. Genetics 147:567–580

Randall TA, Metzenberg RL (1995) Species-specific and mating type-specific DNA regions adjacent to mating type idiomorphs in the genus *Neurospora*. Genetics 141:119–136

Randall TA, Metzenberg RL (1998) The mating type locus of *Neurospora crassa*: identification of an adjacent gene and characterization of transcripts surrounding the idiomorphs. Mol Gen Genet 259:615–621

Reynolds DR, Taylor J (1993) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. Oxford University Press, Wallingford, UK

Rydholm C, Paoletti M, Dyer PS, Lutzoni F (2005) Mating system transition in the euascomycete genus *Aspergillus* subgenus *Fumigati* section *Fumigati*. Fungal Genet Newslett Suppl 52

Saupe S, Stenberg L, Shiu KT, Griffiths AJ, Glass NL (1996) The molecular nature of mutations in the *mt A-1* gene of the *Neurospora crassa A* idiomorph and their relation to mating-type function. Mol Gen Genet 250:115–122

Sharon A, Yamaguchi K, Christiansen S, Horwitz BA, Yoder OC, Turgeon BG (1996) An asexual fungus has the potential for sexual development. Mol Gen Genet 251:60–68

Shen WC, Bobrowicz P, Ebbole DJ (1999) Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet Biol 27:253–263

Shiu PK, Glass NL (2000) Cell and nuclear recognition mechanisms mediated by mating type in filamentous ascomycetes. Curr Opin Microbiol 3:183–188

Shiu PK, Metzenberg RL (2002) Meiotic silencing by unpaired DNA: properties, regulation and suppression. Genetics 161:1483–1495

Shiu PK, Raju NB, Zickler D, Metzenberg RL (2001) Meiotic silencing by unpaired DNA. Cell 107:905–916

Singh G, Ashby AM (1998) Cloning of the mating type loci from *Pyrenopeziza brassicae* reveals the presence of a novel mating type gene within a discomycete *MAT 1-2* locus encoding a putative metallothionein-like protein. Mol Microbiol 30:799–806

Singh G, Dyer PS, Ashby AM (1999) Intra-specific and inter-specific conservation of mating-type genes from the discomycete plant-pathogenic fungi *Pyrenopeziza brassicae* and *Tapesia yallundae*. Curr Genet 36:290–300

Souza CA, Silva CC, Ferreira AV (2003) Sex in fungi: lessons of gene regulation. Genet Mol Res 2:136–147

Turgeon BG (1998) Application of mating type gene technology to problems in fungal biology. Ann Rev Phytopathol 36:115–137

Turgeon BG, Yoder OC (2000) Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet Biol 31:1–5

Turgeon BG, Bohlmann H, Ciuffetti LM, Christiansen SK, Yang G, Schafer W, Yoder OC (1993) Cloning and analysis of the mating type genes from *Cochliobolus heterostrophus*. Mol Gen Genet 238:270–284

Turina M, Prodi A, Alfen NK (2003) Role of the *Mf1-1* pheromone precursor gene of the filamentous ascomycete *Cryphonectria parasitica*. Fungal Genet Biol 40:242–251

Varga J (2003) Mating type gene homologues in *Aspergillus fumigatus*. Microbiology 149:816–819

Waalwijk C, Mendes O, Verstappen EC, de Waard MA, Kema GH (2002) Isolation and characterization of the mating-type idiomorphs from the wheat septoria leaf blotch fungus *Mycosphaerella graminicola*. Fungal Genet Biol 35:277–286

Wirsel S, Turgeon BG, Yoder OC (1996) Deletion of the *Cochliobolus heterostrophus* mating-type (*MAT*) locus promotes the function of *MAT* transgenes. Curr Genet 29:241–249

Wirsel S, Horwitz B, Yamaguchi K, Yoder OC, Turgeon BG (1998) Single mating type-specific genes and their 3′ UTRs control mating and fertility in *Cochliobolus heterostrophus*. Mol Gen Genet 259:272–281

Yabana N, Yamamoto M (1996) *Schizosaccharomyces pombe map1+* encodes a MADS-box-family protein required for cell-type-specific gene expression. Mol Cell Biol 16:3420–3428

Yokoyama E, Yamagishi K, Hara A (2003) Structures of the mating-type loci of *Cordyceps takaomontana*. Appl Environ Microbiol 69:5019–5022

Yuan YO, Stroke IL, Fields S (1993) Coupling of cell identity to signal response in yeast: interaction between the α1 and STE12 proteins. Genes Dev 7:1584–1597

Yun SH (1998) Molecular genetics and manipulation of pathogenicity and mating determinants on *Mycosphaerella zeae-maydis* and *Cochliobolus heterostrophus*. PhD Thesis, Cornell University, Ithaca, NY

Yun SH, Berbee ML, Yoder OC, Turgeon BG (1999) Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proc Natl Acad Sci USA 96:5592–5597

Yun SH, Arie T, Kaneko I, Yoder OC, Turgeon BG (2000) Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. Fungal Genet Biol 31:7–20

Zhang L, Churchill AC, Kazmierczak P, Kim DH, van Alfen NK (1993) Hypovirulence-associated traits induced by a mycovirus of *Cryphonectria parasitica* are mimicked by targeted inactivation of a host gene. Mol Cell Biol 13:7782–7792

Zhang L, Baasiri RA, van Alfen NK (1998) Viral repression of fungal pheromone precursor gene expression. Mol Cell Biol 18:953–959

Zickler D, Arnaise S, Coppin E, Debuchy R, Picard M (1995) Altered mating-type identity in the fungus *Podospora anserina* leads to selfish nuclei, uniparental progeny, and haploid meiosis. Genetics 140:493–503

16 Fruiting-Body Development in Ascomycetes

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I. Introduction

The primary morphological character that distinguishes members of the phylum Ascomycota from all other fungi is the ascus. This sac-like meiosporangium contains the meiospores (ascospores) and is produced only during the sexual life cycle. The taxonomic classification of members of the phylum Ascomycota is complex, because these comprise a multitude of species showing high diversity in morphology, habitat and life history. However, the ability to produce a dikaryon separates members of the Ascomycota into two distinct groups: (1) the saccharomycetes, which are mostly unicellular, and (2) mycelial ascomycetes, the majority of which share several characteristics including certain cell wall constituents, septal pores with Woronin bodies, and a dikaryotic phase as part of their life cycle. Mycelial ascomycetes typically form fruiting bodies called ascomata or ascocarps (Alexopoulos et al. 1996; Barr 2001). These are highly complex, multicellular structures composed of many different cell types that surround the asci in a characteristic manner (Bistis et al. 2003). Whereas ascospores arise from dikaryotic hyphae after karyogamy and meiosis, all fruiting body-forming tissues rise from haploid, non-dikaryotic hyphae. This feature clearly distinguishes fruiting-body formation in ascomycetes from that in basidiomycetous fungi, in which dikaryotic hyphae are involved in both the meiotic cycle and fruiting-body formation (for review, see Moore 1998). Fruiting-body development in filamentous ascomycetes is a complex cellular differentiation process that requires special environmental conditions and is controlled by many developmentally regulated genes. This chapter gives a concise overview on the basics of fruiting-body development in hyphal ascomycetes, focusing on aspects relevant for taxonomy and morphology, and it summarizes environmental

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as well as intrinsic signals influencing ascocarp formation. Finally, we highlight recent molecular genetic datasets from forward and reverse genetic approaches, which give further insight into presumptive signal transduction pathways determining this multicellular differentiation process.

A. Induction of Sexual Development and Fruiting-Body Morphology

The sexual life cycle of ascomycetes can be either heterothallic (self-incompatible) or homothallic (self-compatible). Heterothallic fungi exist in two mating types, designated, for example, *A* and *a* (or + and −), and mating occurs only between sexual structures of opposite mating type. In a homothallic fungus, every strain is able to complete the sexual cycle without a mating partner.

Sexual reproduction is typically controlled by genes that reside in the mating-type locus. Mating type-encoded proteins are putative transcription factors that are thought to act as transcriptional regulators on pheromone and pheromone receptor genes (see Sect. III.C, and Debuchy and Turgeon, Chap. 15, this volume). In most cases, the single mating-type locus conferring mating behavior consists of dissimilar DNA sequences (idiomorphs) in the mating partners (Coppin et al. 1997; Kronstad and Staben 1997; Pöggeler 2001).

The first step in the sexual reproduction of mycelial fungi is to combine two compatible nuclei in the same cell. This occurs by fusion of morphologically similar or morphologically differentiated gametangia, the male antheridia and female ascogonia. In some species, the ascogonium is surrounded by sterile hyphae to form a pre-fruiting body. It then bears a specialized hypha, the trichogyne, which receives the male nucleus. A functional male gamete may be a uninucleate spermatium or microconidium or a multinucleate macroconidium, which can be formed on the mycelium or in specialized structures called spermogonia. Subsequent to contact with a male gamete, the trichogyne recruits a fertilizing nucleus from the male gamete, which migrates to the ascogonium (Bistis 1981). An alternative method of fertilization, referred to as somatogamy, involves the fusion of unspecialized somatic hyphae of two compatible mycelia. Fertilization is not immediately followed by karyogamy. The nuclei migrate in pairs to the developing, hook-shaped ascogenous hyphae (croizers), and divide mitotically in synchrony. The two nuclei remain in close association and undergo successive divisions that result in dikaryotic cells (ascogenous hyphae), in which each cell has two sexually compatible haploid nuclei. During the dikaryotic phase, a dikaryotic mycelium grows within, and draws nourishment from, the haploid ascoma tissue. Inside the ultimate branches of the dikaryotic hyphae (the young asci), of which there may be millions in larger ascomata, fusion of the male and female nucleus takes place. Immediately after karyogamy, the diploid nucleus of the zygote undergoes meiosis, which is in many species followed by a post-meiotic mitosis, resulting in the formation of eight nuclei that will become incorporated into eight ascospores (see Zickler, Chap. 20, this volume).

Depending on the number of post-meiotic mitoses and the degeneration of nuclei after meiosis, the number of ascospores within an ascus varies between one and many thousands. The sizes and shapes of ascospores and asci vary in a species-specific manner (Esser 1982; Alexopoulos et al. 1996). Based on light microscope studies, and irrespective of size and shape, different types of asci can be defined (Kirk et al. 2001). In a large number of hyphal ascomycetes, the spores are discharged from the ascus. Inside the ascoma (fruiting body), the asci may be arranged in a scattered fashion or within a definite layer, which is called the hymenium.

Four major types of the multicellular ascomata (fruiting bodies) can be distinguished: cleistothecia, perithecia, apothecia and pseudothecia (Fig. 16.1). These produce the asci, and act as the platforms from which the meiospores are launched. The cleistothecium is a completely closed fruiting body, whereas the perithecium is a more or less closed, globose or flask-like fruiting body with a pore (ostiole) through which the ascospores escape. The apothecium is a cup- or saucer-like ascoma in which the hymenium is exposed at maturity. In ascomycetes with a pseudothecium (or ascostroma), the fruiting body is initiated by the formation of a locule within a stroma, which is a matrix of vegetative hyphae, with or without tissue of a host. In contrast to cleistothecia, perithecia and apothecia, the sexual organs of fungi producing pseudothecia are formed from hyphae already within the developing ascocarp. In addition to asci, many fruiting bodies contain sterile hyphae of various types that are important taxonomic characters. Amongst these are paraphyses, elongated hy-

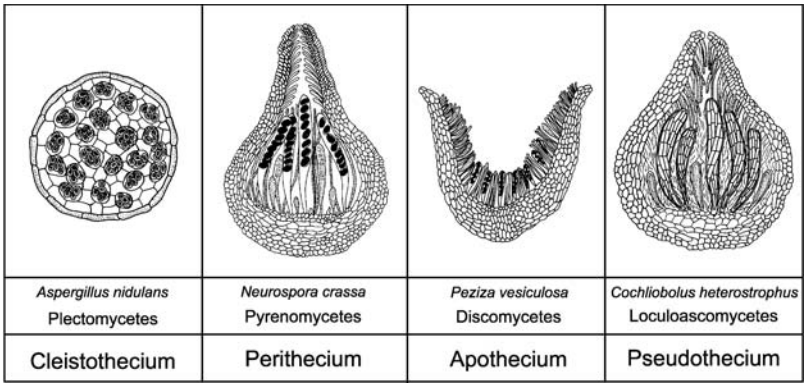

Fig. 16.1. Fruiting bodies of filamentous ascomycetes

phae originating from the base of an ascocarp, periphyses, short unbranched hyphae in the ostiolar canal of perithecia, and pseudoparaphyses, sterile hyphae originating above the level of asci in a pseudothecium (Alexopoulos et al. 1996).

Traditionally, fruiting-body morphology and the ascus structure have been used for a general taxonomic classification of the hyphal ascomycetes, which have thereby been grouped into four classes. The typical ascocarps of plectomycetes, pyrenomycetes, discomycetes and loculoascomycetes are cleistothecia, perithecia, apothecia and pseudothecia, respectively. However, this traditional classification has been questioned in recent years, due to the availability of molecular markers based on DNA sequencing data (Berbee et al. 2000). From this follows that some groups, such as the pyrenomycetes and plectomycetes, can indeed be defined by their ascocarp and ascus structure. However, others like the discomycetes and loculoascomycetes appear not to be monophyletic (Liu et al. 1999; Lumbsch 2000; Lumbsch et al. 2000; Lindenmuth et al. 2001).

B. Model Organisms to Study Fruiting-Body Development

In recent years, classical and molecular genetic approaches have been used to gain a detailed insight into the mechanism directing the cellular processes of fruiting-body development.

Classical genetic studies have demonstrated that fruiting-body development is under polygenic control. Much information has come from mutagenesis assessments in which mutants have been obtained that are blocked at one specific stage of development. Fungal model organisms used to study fruiting-body development are mainly the pyrenomycetes *Neurospora crassa* and *Sordaria macrospora*, and the plectomycete *Aspergillus nidulans*, which will be described in detail in the following sections. However, molecular genetic analyses of many other hyphal ascomycetes, including the pyrenomycetes *Podospora anserina*, *Magnaporthe grisea* and *Cryphonectria parasitica* as well as the loculoascomycete *Cochliobolus heterostrophus*, have also led to the isolation of fruiting-body developmental genes (Table 16.1).

1. *Neurospora crassa*

Since the 1940s, the pyrenomycete *N. crassa* has been used as model organism for genetic and biochemical analyses (Perkins and Davis 2000; Davis and Perkins 2002). *N. crassa* is heterothallic with two mating types, designated *mat a* and *mat A*, and is able to propagate by both sexual and asexual spores. Haploid strains are hermaphroditic but self-sterile. The formation of protoperithecia is induced by nitrogen limitation. However, the sexual cycle is initiated only when a protoperithecium of one mating type is fertilized via a trichogyne by a male cell of another mating type. In *N. crassa*, there are no specialized male cells that function exclusively as spermatia; rather, macroconidia, microconidia or hyphal fragments can fertilize the protoperithecia (see Glass and Fleißner, Chap. 7, this volume). Fertilized protoperithecia develop into perithecia within which asci with eight linearly ordered, homokaryotic ascospores are formed. Over 200 mutants that affect sexual development have been isolated in *N. crassa*. These include male-sterile mutants that are fertile as female parents, female-sterile mutants that are fertile as male parents, mutants that are both female- and male-sterile, and mutants that affect ascus development in various ways

Table. 16.1. Genes involved in fruiting-body development

Organisms	Gene	Gene product	Defect of mutants	Reference
Neurospora crassa	*asd-1*	Rhamnogalacturonase	No ascospores	Nelson et al. (1997b)
	ndk-1	Nucleoside diphosphate kinase	Light-dependent positioning of perithecia	Ogura et al. (2001)
	sod-1	Superoxid dismutase	Light-dependent positioning of perithecia	Yoshida and Hasunuma (2004)
	nuo12.3, *nuo20.8*, *nuo30.4*, *nuo78*, *nuo51*, *nuo24*, *nuo2*	Subunit of respiratory chain NADH dehydrogenase	No ascospores	Fecke et al. (1994), Duarte et al. (1998), Almeida et al. (1999), Duarte and Videira (2000)
	mfa-1	Hydrophobic peptide pheromone	Male sterility, aberrant female sexual development and ascospore production	Kim et al. (2002a)
	pre-1	GPRC, pheromone receptor	Female sterility	Kim and Borkovich (2004)
	gna-1	G protein α subunit	Female-sterile, no perithecia	Ivey et al. (1996), Yang and Borkovich (1999)
	gna-3	G protein α subunit	Smaller perithecia with no beaks, reduced number of ascospores	Kays et al. (2000)
	gnb-1	G protein β subunit	Female-sterile, no perithecia	Yang et al. (2002)
	gng-1	G protein ɣ subunit	Female-sterile, no perithecia	Krystofova and Borkovich (2005)
	krev-1	GTP/GDP-binding protein; member of the ras superfamily	No perithecia	Ito et al. (1997)
	cr-1	Adenylyl cyclase	Delayed perithecia and ascospore formation	Perkins et al. (1982), Ivey et al. (2002)
	mak-2	MAPK, related to *S. cerevisiae* Fus3p and Kss1p	Female-sterile, no protoperithecia	Pandey et al. (2004), Li et al. (2005)
	nrc-1	MAPKKK, similar to *S. cerevisiae* Ste11p	Female-sterile, no protoperithecia	Kothe and Free (1998)
	nrc-2	Serine-threonine protein kinase	Female-sterile, no protoperithecia	Kothe and Free (1998)
	rgb-1	B regulatory subunit of the type 2A Ser/Thr phosphatase	Female-sterile, no prothoperithecia	Yatzkan and Yarden (1999)
	ham-2	Putative transmembrane protein	Female-sterile, no protoperithecia	Xiang et al. (2002)
	eat-2	Highly similar to a domain present in the plasma membrane ATPase	Female-sterile, no prothoperithecia	Randall and Metzenberg (1998)
	cel-2	β subunit of fatty acid synthase	Fewer peritecia, rare ascospores	Goodrich-Tanrikulu et al. (1999)
	asm-1	Transcription factor with APSES domain	Female-sterile, no protoperithecia	Aramayo et al. (1996)
	vma-1	Subunit A of the V-ATPase	Female-sterile, no protoperithecia, can not donate nuclei in a cross	Bowman et al. (2000)
	cpc-2	WD-repeat protein required to repress general amino acid control, scaffold protein	Female-sterile, no protoperithecia	Müller et al. (1995)
	rco-1	Multidomain protein that mediates transcriptional repression	Female-sterile, no protoperithecia	Yamashiro et al. (1996)
	asd-4	GATA-type zinc finger transcription factor	No asci, no ascospores	Feng et al. (2000)
	wc-1	GATA-like zinc finger transcription factor	No phototropism of perithecial beaks	Ballario et al. (1996), Oda and Hasunuma (1997)

Table. 16.1. (continued)

Organisms	Gene	Gene product	Defect of mutants	Reference
	wc-2	GATA-like zinc finger transcription factor	No phototropism of perithecial beaks	Linden and Macino (1997), Oda and Hasunuma (1997)
	pp-1	Transcription factor homeodomain, C_2H_2 zinc finger, Ste12p homolog	No protoperithecia	Li et al. (2005)
Podospora anserina	*car1*	Peroxisomal membrane protein	Impaired karyogamy, no ascospores	Berteaux-Lecellier et al. (1995)
	cro1	Cytosolic protein with C_2H_2 zinc finger motif	Impaired meiosis, no ascospores	Berteaux-Lecellier et al. (1998)
	AS4	Translation-elongation factor EF-1α, eF1A	No ascospores, no perithecia	Silar et al. (2001)
	su1	Translation-termination factor eRF3	Female-sterile, no protoperithecia	Gagny and Silar (1998)
	su2	Translation-termination factor eRF1	Female-sterile, no protoperithecia	Gagny and Silar (1998)
	PaCox17	Chaperone targeting copper to cytochrome c	Delayed perithecial formation and reduced ascus formation	Stumpferl et al. (2004)
	rmp1	No homology, functions in nucleus–mitochondria cross-talk	Lethal or without mitochondrial targeting sequence, no ascospores	Contamine et al. (2004)
	PaNox1	NADPH oxidase	No perithecia	Malagnac et al. (2004)
	PaNox2	NADPH oxidase	No ascospore germination	Malagnac et al. (2004)
	mod-A	Proline-rich protein with SH3-binding motif	Female-sterile, no protoperithecia	Barreau et al. (1998)
	mod-E	Heat-shock protein HSP90	Sterile perithecia, no ascospores	Loubradou et al. (1997)
	mfp	Hydrophobic peptide pheromone	Male-sterile	Coppin et al. (2005)
	mfm	Peptide pheromone	Male-sterile	Coppin et al. (2005)
	mod-D	G protein α subunit	Female-sterile, no protoperithecia	Loubradou et al. (1999)
	PaAsk1	MAPKKK, similar to *S. cerevisiae* Bck1p	No perithecia	Kicka and Silar (2004)
	grisea	Copper-activated transcription factor, ortholog of the yeast transcription factor MAC1	Female-sterile, no protoperithecia	Osiewacz and Nuber (1996), Borghouts and Osiewacz (1998)
	fle1	Transcription factor, C_2H_2 zinc finger	Fewer microconidia, more abundant protoperithecia	Coppin (2002)
	pah1	Transcription factor, homeobox protein	Enhanced number of microconidia, delayed protoperithecia development	Arnaise et al. (2001)
	ami1	Homolog of *A. nidulans* APS-A	Male-sterile, delayed fruiting-body formation	Graia et al. (2000)
Sordaria macrospora	*pro11*	WD-repeat protein	No perithecia	Pöggeler and Kück (2004)
	pro4	Leucine biosynthesis	No perithecia	Kück (2005)
	acl1	Subunit of ATP-citrate-lyase	No ascospores	Nowrousian et al. (1999)
	spo76	Chromosomal protein	Impaired meiosis, few ascospores	van Heemst et al. (1999)
	pro1	C_6 zinc finger transcription factor	No perithecia	Masloff et al. (1999)
Aspergillus nidulans	*trpB*	Tryptophane biosynthesis	No cleistothecia	Eckert et al. (1999, 2000)
	hisB	Histidine biosynthesis	No cleistothecia	Busch et al. (2001)
	tubB	α-tubulin	No ascospores	Kirk and Morris (1991)
	uvsC	DNA-repair enzyme homolog of *S. cerevisiae* RAD51	No karyogamy, no ascospores	van Heemst et al. (1997)
	lsdA	No sequence similarity, unknown function	No inhibition of cleistothecia development under high salt conditions	Lee et al. (2001)

Table. 16.1. (continued)

Organisms	Gene	Gene product	Defect of mutants	Reference
	phoA	Cyclin-dependent kinase	Enhanced number of cleistothecia under phosphorus-limited conditions	Bussink and Osmani (1998)
	pho80	Putative cyclin-dependent kinase inhibitor	Promotes sexual development	Wu et al. (2004)
	csnD	Subunit of COP9 signalosome	No cleistothecia	Busch et al. (2003)
	veA	No homology, unknown	No cleistothecia	Kim et al. (2002b)
	noxA	NADPH oxidase	No cleistothecia	Lara-Ortíz et al. (2003)
	ppoA	Putative fatty acid dioxygenase	Increased ratio of asexual to sexual development	Tsitsigiannis et al. (2004)
	odeA	Δ-12 desaturase	Delayed ascosporogenesis	Calvo et al. (2001)
	gprA	GPRC, pheromone receptor	Fewer cleistothecia and ascospores	Seo et al. (2004)
	gprB	GPRC, pheromone receptor	Fewer cleistothecia and ascospores	Seo et al. (2004)
	gprD	GPRC	No cleistothecia	Han et al. (2004)
	fadA	G protein α subunit	No cleistothecia	Rosèn et al. (1999)
	sfdA	G protein β subunit	No cleistothecia, more Hülle cells	Rosèn et al. (1999)
	flbA	RGS protein	No cleistothecia	Han et al. (2001)
	sakA	MAPK, similar to *S. cerevisiae* Hog1p	Premature sexual development, more cleistothecia	Kawasaki et al. (2002)
	steC	MAPKKK, similar to *S. cerevisiae* Ste11p	No cleistothecia	Wei et al. (2003)
	steA	Transcription factor homeodomain, C_2H_2 zinc finger, Ste12p homolog	No cleistothecia, only Hülle cells	Vallim et al. (2000)
	nsdD	Transcription factor GATA-type	No cleistothecia, no Hülle cells	Han et al. (2001)
	stuA	Transcription factor with APSES domain	No cleistothecia, no Hülle cells	Wu and Miller (1997)
	dopA	Putative transcription factor, leucine zipper-like domains and similarity to C/EBP transcription factor	No cleistothecia, no Hülle cells	Pascon Castiglioni and Miller (2000)
	medA	Transcriptional regulator	No cleistothecia, only Hülle cells	Busby et al. (1996)
	cpcA	Transcription factor, c-Jun-like leucine zipper, required to activate general amino acid control	When overexpressed, no cleistothecia	Hoffmann et al. (2001b)
	cpcB	WD-repeat protein, required to repress general amino acid control, scaffold protein	No cleistothecia	Hoffmann et al. (2000)
Magnaporthe grisea	*magA*	G protein α subunit	No ascospores	Liu and Dean (1997)
	magC	G protein α subunit	No ascospores	Liu and Dean (1997)
	magB	G protein α subunit	Female-sterile, no perithecia	Liu and Dean (1997)
	mac-1	Adenylyl cyclase	Female-sterile, no perithecia	Choi and Dean (1997)
	mps-1	MAPK, similar to *S. cerevisiae* Sltp	Female-sterile, no perithecia	Xu et al. (1998)
Cryphonectria parasitica	*Mf2-2*	Hydrophobic peptide pheromone	Female sterility	Zhang et al. (1993)
	Mf1-1	Peptide pheromone	Male-sterile	Turina et al. (2003)
	cpg-1	G protein α subunit	Female-sterile	Gao and Nuss (1996)
	cpg-2	G protein α subunit	Enhanced perithecial development	Gao and Nuss (1996)
Fusarium graminearum	*mgv-1*	MAPK, similar to *S. cerevisiae* Sltp	Female-sterile, no perithecia	Hou et al. (2002)
Cochliobolus heterostrophus	*cga-1*	G protein α subunit	Female sterility	Horwitz et al. (1999)
	chk-1	MAPK, related to *S. cerevisiae* Fus3p and Kss1p	Female sterility	Lev et al. (1998)

(Raju 1992). When male-sterile mutants are used as fertilizing parent, perithecial development is initiated, but then arrested at an early stage. In *N. crassa*, numerous female-sterile mutants that do not form functional protoperithecia or display a reduced fertility have been described. Because of this high frequency of sterility in female strains, it was suggested that at least 400 genes are required for perithecium and ascospore development in *N. crassa* (Johnson 1978; Leslie and Raju 1985). However, many of these female-sterile mutants show abnormal vegetative growth, and thus this female sterility may be a consequence of a morphological defect, rather than a mutation in a gene specifically needed for fruiting-body differentiation (Raju 1992).

Effective molecular techniques developed for *N. crassa*, such as the transformation and creation of cosmid genomic libraries involving, for example, phenotypic complementation, have led to the cloning and functional characterization of regulatory genes that affect morphology (Bailey and Ebbole 1998). Other molecular genetic approaches used subtractive hybridization to isolate *N. crassa* sexual developmental genes.

With this attempt, Nelson and Metzenberg (1992) succeeded in identifying 14 genes transcribed only under nitrogen-depleting growth conditions. One of these, *asd-1*, has been shown to encode a putative rhamnogalacturonase necessary for ascus development (Nelson et al. 1997b). In large-scale analyses, fruiting body-specific expressed sequence tags (ESTs) from *N. crassa* were sequenced for further molecular characterization (Nelson et al. 1997a). In such experimental approaches, isolated candidate genes have to be inactivated to obtain detailed information about mutant phenotypes and gene function.

Inactivation of developmental genes in *N. crassa* was achieved either by homologous recombination or by gene silencing via repeat-induced point mutation (RIP). The *N. crassa* RIP process efficiently detects and mutates both copies of a sequence duplication. RIP acts during the dikaryotic stage of the sexual cycle, causing numerous C:G-to-T:A transitions within duplicated sequences, and is frequently used to inactivate genes in *N. crassa* (Galagan and Selker 2004). Finally, the whole genome sequence of *N. crassa* has become available, opening the opportunity for analyzing genes involved in fruiting-body development by means of reverse genetic approaches (Galagan et al. 2003; Borkovich et al. 2004).

2. *Sordaria macrospora*

In the heterothallic species *N. crassa*, mutations conferring male and/or female sterility can be detected directly because of their sterility effects in heterozygous crosses. However, recessive mutations that affect post-fertilization perithecial development will remain undetected in heterothallic species until the mutant allele is available in both mating types, thus allowing homozygous crosses. In contrast to *N. crassa*, the homothallic pyrenomycete *S. macrospora* is self-fertile, which means that recessive mutations can directly be tested for defects in fruiting-body development. Moreover, *S. macrospora* produces only meiotically derived ascospores, whereas asexual spores, such as conidia, are absent. Thus, there is no interference between two different developmental programs, which makes it easier, for example, to analyze differentially expressed genes involved in ascocarp development. Under laboratory conditions, perithecia and ascospores reach maturity within 7 days after ascospore germination. During this development, distinct reproductive structures, such as ascogonia, protoperithecia (young fruiting bodies), and perithecia, can be distinguished (Fig. 16.2). Because *S. macrospora* represents such a favorable genetic system for scientists, this homothallic pyrenomycete was used to generate numerous mutants that are blocked at various stages of perithecial development (Esser and Straub 1958; Masloff et al. 1999). Wild-type strains of *S. macrospora* are self-fertile and produce perithecia. However, fertile perithecia are also formed in crosses between sterile strains, when nuclei are interchanged by hyphal anastomoses at the contact zones of two sterile mycelia. These crosses facilitate the analysis of epistatic relationships among developmental mutants. The establishment of molecular tools provides the basis for studying fruiting-body development in *S. macrospora* (Walz and Kück 1995; Pöggeler et al. 1997).

3. *Aspergillus nidulans*

The plectomycete *Aspergillus nidulans* (teleomorph: *Emericella nidulans*) propagates by the formation of spores that can be either asexual or sexual, and has long served as a model system for understanding the genetic regulation of asexual development in ascomycetes (Adams et al. 1998). The asexual cycle is characterized by the produc-

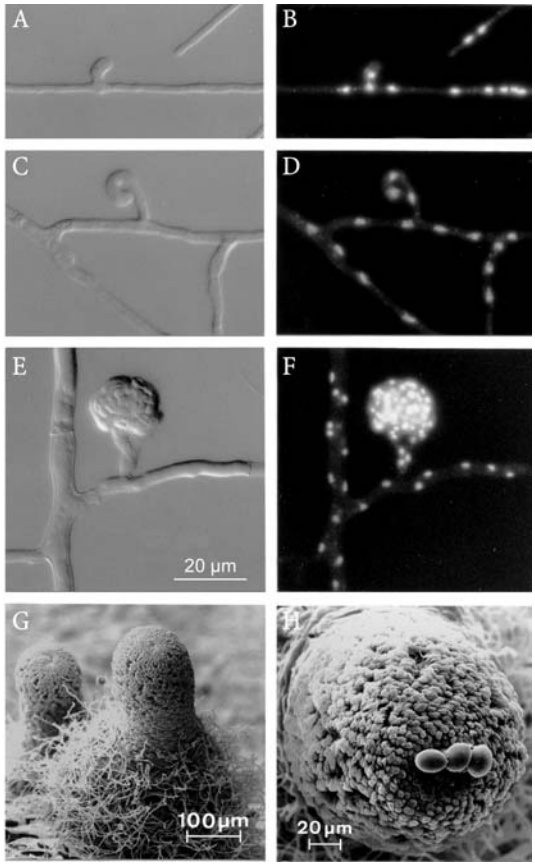

Fig. 16.2. Sexual development of *Sordaria macrospora*. **A**,**B** Young ascogonium; **C**,**D** ascogonium; **E**,**F** protoperithecium; **G** perithecium; **H** top view of perithecium, with ascospores. **A**,**C**,**E** Differential interference contrast light micrographs DIC. **B**,**D**,**F** Fluorescence micrographs of **A**,**C**, and **E** with DAPI staining of nuclei. **G**,**H** Scanning electron micrographs

tion of haploid conidiophores that bear asexual, single-celled spores called conidia (Fischer 2002, see Fischer and Kües, Chap. 14, this volume). Sexual development of *A. nidulans* starts after conidiophore differentiation. The conidiophores are produced by mitotic division in 3 days after germination, whereas the sexual ascospores are formed after at least 7 days (Pontecorvo 1953). *A. nidulans* is homothallic, and a single colony can produce cleistothecia filled with up to 1000 ascospores by self-fertilization.

In *A. nidulans*, no obvious antheridium or ascogonium structures can be observed (Benjamin 1955). It was, however, assumed by Kwon and Raper (1967) that in *Aspergillus heterothallicus*, a heterothallic relative of *A. nidulans*, a coiled structure equivalent to an ascogonium fuses to a second cell equivalent to an antheridium.

In contrast to pyrenomycetes, the presumptive ascogonium of *A. nidulans* is not surrounded by sterile hyphae, and pre-fruiting bodies are not formed. Only after fertilization is the ascogonium surrounded by growing, unordered hyphae, which form an increasingly packed "nest" and then differentiate globose, multinucleate Hülle cells, which support the development of cleistothecia (Ellis et al. 1973). The surrounding hyphae that form the "nest" later differentiate into the cleistothecial envelope. The developing spherical cleistothecia are filled with ascogenous hyphae that differentiate into asci. After karyogamy, the zygote stage is immediately followed by meiosis. Directly after meiosis, the four resulting nuclei pass through a first post-meiotic mitosis. The eight nuclei are then separated by membranes, and give rise to eight red-pigmented ascospores within each ascus. As second post-meiotic mitosis results in eight binucleate, mature ascospores (Braus et al. 2002). Similarly to the homothallic pyrenomycete *S. macrospora*, self-fertile *A. nidulans* strains can be used in crossing experiments. In this case, hyphae from two different strains can form anastomoses and exchange nuclei when growing sufficiently close to each other (Hoffmann et al. 2001a).

The coexistence of sexual and asexual reproduction within one and the same individual has made *A. nidulans* a popular genetic model organism to compare fitness effects of sexual and asexual reproduction. Because of its homothallism, the sexual and asexual offspring of *A. nidulans* have largely identical genotypes. As was shown by Bruggeman et al. (2003, 2004), slightly deleterious mutations accumulate at a lower rate in the sexual than in the asexual pathway.

In addition to classical genetics, various tools required for molecular biology have been developed, and recently the entire genome of *A. nidulans* has been sequenced (http://www.broad.mit.edu/annotation/fungi/aspergillus), thus making *A. nidulans* an excellent model for studying various biological questions, including the multicellular cleistothecium development.

For the genetic dissection of sexual sporulation in *A. nidulans*, a collection of ascospore-less mutants was isolated by Swart et al. (2001). To understand the sexual reproduction of *A. nidulans* in more detail, Han et al. (1990) identified numerous mutants that were defective in sexual development in a forward genetic screen. These were classified into two groups:

1. *nsd* (*n*ever in *s*exual *d*evelopment) mutants that are unable to form any sexual structures; and
2. *bsd* (*b*lock in *s*exual *d*evelopment) mutants that show differences in the amount or timing of sexual organ production, compared to the wild type.

Several *nsd* mutants were analyzed for their genetic and morphological characteristics (Han et al. 1994, 1998). Finally, the *nsdD* gene has been cloned and was shown to encode a GATA-type transcription factor (Han et al. 2001). Beside the *nsdD* gene, several other *A. nidulans* genes that affect sexual development have been cloned, not only by complementation of developmental mutants but also by reverse genetic approaches (Table 16.1).

II. Physiological Factors Influencing Fruiting-Body Development

Most fungi do not form fruiting bodies continuously, but require special environmental conditions. Additionally, the vegetative mycelium has to acquire a certain stage of "competence" before differentiating fruiting bodies. The external factors needed to promote fruiting-body formation are mostly organism-specific, and depend on the ecological niche the fungus occupies. Most prominent among these are nutrients, light, temperature, aeration, pH, and the presence of a partner or host for symbiotic or pathogenic fungi, respectively. Endogenous factors that have been identified as relevant for fruiting-body formation often involve components of primary metabolism as well as pheromones or hormone-like substances (Dyer et al. 1992; Moore-Landecker 1992). Perception of these factors and initiation of an appropriate reaction require complex regulatory networks; in recent years, genetic and molecular biology methods have helped to start unraveling the molecular basis of fruiting-body development.

A. Environmental Factors

The influence of environmental factors on fruiting-body development is well-studied on the physiological level in economically important fungi, most of which are basidiomycetes (Kües and Liu 2000), and to some degree in several ascomycetes, which have been used to investigate the basic principles of fruiting-body development (Moore-Landecker 1992). In this section, a brief overview of some of these factors is given, focusing on cases where at least some genes involved in the perception of these factors have been identified.

1. Nutrients and Related Factors

The requirements for certain nutrients and other chemical substances for fruiting-body formation vary in different ascomycetes. In many species, fruiting bodies are formed preferentially at much lower nutrient concentrations than those promoting vegetative growth (Moore-Landecker 1992). One reason might be that the vegetative mycelium accumulates nutrients that can be used for the formation of fruiting bodies once a critical amount is reached. Thus, the mycelium would, in effect, nurture the developing fruiting bodies. Another reason could be that sexual development is initiated when the growth substrate is depleted of nutrients, and durable ascospores are produced that can lie dormant until more suitable conditions occur.

Some ascomycetes require specific factors, such as vitamins, for sexual development, but not for vegetative growth. *S. macrospora*, for example, needs biotin to complete the sexual cycle (Molowitz et al. 1976). Furthermore, it has been shown in *S. macrospora* that addition of arginine to the growth medium enhances fruiting-body formation (Molowitz et al. 1976). This finding, together with the observation that amino acid synthesis mutants of both *A. nidulans* and *S. macrospora* show defects in fruiting-body development, indicates an influence of amino acid metabolism in sexual development, an aspect discussed in more detail in Sect. II.B.1. In some ascomycetes, fruiting-body formation can be increased by addition of fatty acids to the growth medium. In *Ceratocystis ulmi* and *Nectria haematococca*, production of perithecia can be increased by exogenous linoleic acid, whereas in *N. crassa*, both oleate and linoleate enhance fruiting-body formation (Nukina et al. 1981; Marshall et al. 1982; Dyer et al. 1993; Goodrich-Tanrikulu et al. 1998). Mutants with defects in lipid metabolism often have developmental defects, too (see Sect. II.B.1).

Environmental factors that influence sexual development (and each other) are the pH of the growth medium, aeration (CO_2 pressure), osmotic pressure, and the presence and availability of mineral salts. In *N. crassa*, mutants in a gene encoding a subunit of the vacuolar H(+)-ATPase do not grow

in medium with a pH of 7.0 and above, and they are female-sterile, most likely due to insufficient cellular homeostasis (Bowman et al. 2000). It has long been known that in *A. nidulans*, increased partial pressure of carbon dioxide favors fruiting-body formation, whereas aeration, and thereby the removal of carbon dioxide, shifts the balance toward asexual sporulation (Champe et al. 1994).

In *A. nidulans*, high concentrations of potassium, sodium or magnesium ions inhibit sexual development and promote asexual sporulation. Regulation of this salt-dependent developmental balance requires the *lsdA* gene, which does not have any homology to previously characterized genes (Lee et al. 2001). Also involved in this regulation process is *veA*, a gene that integrates a number of signals and that is described more fully in Sect. II.A.2.

Another gene involved in salt-dependent developmental decisions between asexual and sexual development in *A. nidulans* is *phoA*, a gene for a cyclin-dependent kinase. Depending on both pH and the initial phosphorus concentration, *phoA* mutants can switch from asexual to sexual development, or do not show any form of spore differentiation (Bussink and Osmani 1998). Interacting with PHOA is the cyclin An-PHO80, which also is involved in regulating the balance between asexual and sexual differentiation. Effects of the *An-pho80* deletion also depend on phosphate concentration, but in contrast to *phoA* mutants, *An-pho80* mutants increase the production of conidia and do not form mature cleistothecia (Wu et al. 2004).

Many pathogenic or mycorrhizal fungi form fruiting bodies only in contact with their hosts or symbiont partners, which makes analysis of these differentiation processes difficult. For a taxol-producing *Pestalotiopsis microspora* isolate that lives as an endophyte of yew, it was found that a methylene chloride extract of yew needles induces the formation of perithecia (Metz et al. 2000). Most likely, one or more lipid-like compounds present in yew needles stimulate sexual differentiation in the fungus.

Lipid-derived factors produced by fungi themselves, as well as lipid metabolism in general have also been found to be involved in sexual development (see Sect. II.B.1).

For organisms which cannot easily be cultivated under laboratory conditions, but where RNA extraction is possible from field isolates, large-scale expression analysis techniques such as microarrays have great potential to help unraveling molecular mechanisms of development (Nowrousian et al. 2004). Lacourt and coworkers compared the expression of 171 genes in vegetative tissue and different stages of developing fruiting bodies of the mycorrhizal ascomycete *Tuber borchii*, using cDNA macroarrays. These investigations revealed that metabolism and cell wall synthesis are substantially altered during development (Lacourt et al. 2002), but how the plant partner influences fungal development at the molecular level remains to be elucidated.

2. Physical Factors

Most ascomycetes form fruiting bodies on the surface of their growth substrate to facilitate ascospore dispersal, the most notable exception being truffles and related fungi. This means that the fungus somehow has to organize the correct place and orientation of the fruiting bodies. Possible mechanisms for this process include the perception of gravity or of air/substrate interfaces. There has been some research into the effects of gravity on fungal sexual development that has established that at least some fungal fruiting bodies, or parts of fruiting bodies exhibit gravitropism, but the genetic and biochemical processes needed to perceive and respond to gravity remain enigmatic (see Corrochano and Galland, Chap. 13, this volume). Little more is known about the recognition of air/surface interfaces, or other means of spatial control of fruiting-body formation in ascomycetes. In basidiomycetes, a class of small secreted proteins called hydrophobins has been shown to be essential for breaching an air/water interface, and hydrophobins coat many fruiting-body surfaces (see Chap. 19, this volume). Hydrophobins have been identified in several ascomycetes, too, but whether they play a role in fruiting-body formation is not yet clear (see Sect. IV.B). Another effect that has been observed in several ascomycetes is the so-called edge effect – fruiting bodies are formed preferentially at the edges of a petri dish or other culture vessels. Investigation of this effect in *S. macrospora* has revealed that the determining parameter is not a recognition of edges or other surface structures, but rather an increased hyphal density, which can be due to mechanical obstacles and also to nutrient availability (Molowitz et al. 1976; Hock et al. 1978); similar results have been reached with other ascomycetes (Moore 1998).

Related to the "edge effect" might be the observation that fruiting bodies are often formed at

regular intervals; both effects require the fungus to sense hyphal or fruiting-body spacing, but the genetic basis behind this regulation remains to be elucidated.

Another possible signal for the production and orientation of fruiting bodies is light (see also Chap. 13, this volume). Light has been shown to influence fruiting-body formation in many ascomycetes from different phylogenetic groups. Influences of light range from complete light-dependence of fruiting-body formation to orientation of fruiting bodies or, in reverse, a preferential formation of fruiting bodies in the dark. Several genes involved in light perception have been identified in various fungi.

Early reports of light effects on fruiting-body formation have been for *Pyronema confluens* and *Pyronema domesticum*, where apothecium formation is completely light-dependent, and dark-grown mycelia are sterile (Claussen 1912; Moore-Landecker 1979). In other fungi, like *N. crassa*, fruiting bodies are formed in the dark and are placed on the surface of the growth substrate even without illumination, but perithecial necks are normally oriented toward the light, and in darkness point to various directions (Harding and Melles 1983). Furthermore, not only the orientation but also the position of the neck on the perithecium is light-dependent (Oda and Hasunuma 1997). Additionally, the number of protoperithecia that are formed is greatly increased upon blue-light illumination (Degli Innocenti and Russo 1983). All three effects were shown to depend on the *white collar* genes *wc-1* and *wc-2* (Harding and Melles 1983; Degli Innocenti and Russo 1984; Oda and Hasunuma 1997). WC-1 has been identified as a blue-light photoreceptor as well as a transcription factor, and it interacts with WC-2 to regulate the transcription of target genes in a light-dependent manner (Ballario et al. 1996; Linden and Macino 1997; Froehlich et al. 2002; He et al. 2002).

Another gene that plays a role in certain aspects of light-dependent morphogenesis is *ndk-1* (Ogura et al. 2001; Yoshida and Hasunuma 2004). *ndk-1* encodes a nucleoside diphosphate kinase, and is required for light-dependent neck positioning on the perithecia but not for orientation of the neck itself. Nucleoside diphosphate kinase is a conserved enzyme that is involved in signal transduction cascades in various organisms.

In *N. crassa*, NDK-1 is autophosphorylated after blue-light illumination, which makes it a likely candidate for a light signal transduction pathway component (Ogura et al. 2001).

Studies with double mutants have shown that *ndk-1* depends on the presence of functional *wc* genes (Yoshida and Hasunuma 2004). Both WC proteins are also part of the circadian clock of *N. crassa* (Loros and Dunlap 2001), and transcription of the pheromone precursor genes that are involved in sexual development of *N. crassa* (see Sect. B.2) is regulated by the endogenous clock (Loros et al. 1989; Bobrowicz et al. 2002). These findings indicate that in *N. crassa*, sexual development is not only light-regulated but the circadian clock may also contribute to the control of fruiting-body formation (see also The Mycota, Vol. III, Chap. 11).

The influence of light on developmental processes was also investigated extensively in *A. nidulans*. In this ascomycete, red light shifts the ratio of asexual to sexual reproduction structures (conidiophores vs. cleistothecia) toward asexual reproduction, whereas fruiting-body formation is favored in the dark. One gene that is necessary for maintaining this light-dependent balance is *veA* (Mooney and Yager 1990; Kim et al. 2002b). *veA*, which does not have homology to any genes with known function, is essential for fruiting-body formation; *veA* deletion mutants do not form any cleistothecia (Kim et al. 2002b). The *veA1* allele, a partially deleted form of *veA* that is present in many laboratory strains of *A. nidulans*, causes a less severe phenotype with a preference for conidiation, even without illumination (Kim et al. 2002b).

Another gene involved in controlling the light-dependent balance of asexual versus sexual reproduction of *Aspergillus* is *csnD*. This gene encodes a subunit of the COP9 signalosome, a conserved eukaryotic protein complex that regulates developmental processes by targeting proteins for ubiquitinylation and subsequent degradation by the 26S proteasome (Busch et al. 2003). In contrast to *veA* mutants, a *csnD* deletion mutant predominantly induces (but does not complete) the sexual cycle, irrespective of the light signal (Busch et al. 2003). However, both *veA* and *csnD* mutants have other, light-independent phenotypes, and may be part of signal transduction networks that integrate many signals controlling fruiting-body development (Kim et al. 2002b; Busch et al. 2003).

Another physical factor influencing fruiting-body development is temperature. For those ascomycetes in which this aspect has been investigated, it was found that the temperature range that controls fruiting-body formation is usually more

restricted than that controlling vegetative growth (Moore-Landecker 1992), but the genetic basis for this is not yet clear. *mod-E*, a heat-shock protein HSP90 homolog, was found to be involved in both sexual development and vegetative incompatibility in *Podospora anserina* (Loubradou et al. 1997). *mod-E* transcripts are accumulated after a shift from 26 to 37 °C, but effects of different temperatures on fruiting-body formation in the wild type versus *mod-E* mutants were not reported. Therefore, it remains to be determined whether *mod-E* or other (heat-shock) proteins are involved in temperature-dependence of fruiting-body development.

B. Endogenous Factors

The transition from vegetative growth to sexual development requires a physiologically "competent" mycelium. This competence often depends on nutrient availability, but the nutrients also have to be processed by the fungal metabolism; and genetic analyses have shown that fruiting-body formation requires metabolic reactions different from those of vegetative growth (see Sect. II.B.1). In several fungal species, pheromones or hormone-like substances are necessary for completion of the sexual cycle, as described in Sect. II.B.2 and in Chap. 11 (this volume).

1. Metabolic Processes

In several ascomycetes, it was found that mutations in genes for primary metabolism often interfere with sexual development under conditions where vegetative growth remains more or less normal. Examples for this are mutants blocked in amino acid biosynthesis pathways, and fatty acid biosynthesis mutants. Many effects of mutations leading to amino acid auxotrophy on fruiting-body morphogenesis have been investigated in *A. nidulans*. Deletion of the tryptophan synthase-encoding gene *trpB*, or the histidine biosynthesis gene *hisB* leads to loss of cleistothecia production on medium with low levels of tryptophan or histidine, respectively (Eckert et al. 1999, 2000; Busch et al. 2001). Both genes are regulated by the cross-pathway control system, a regulatory network that activates a variety of amino acid biosynthesis genes when the amounts of a single amino acid are low. Besides regulating amino acid biosynthesis, this cross-pathway network also comprises a control point for progression of sexual development (Hoffmann et al. 2000). This was demonstrated by investigating the functions in fruiting-body formation of two members of the cross-pathway network, *cpcA* and *cpcB*. *cpcA* encodes a transcriptional activator homologous to the yeast Gcn4p protein, which is the activating transcription factor for cross-pathway control (termed general control of amino acid biosynthesis) in *Saccharomyces cerevisiae* (Hoffmann et al. 2001b). CpcB is homologous to mammalian RACK1 (receptor for activated C-kinase 1), a scaffold protein involved in many cellular signaling processes (McCahill et al. 2002). *cpcA* and *cpcB* play antagonistic roles in cross-pathway control as well as in sexual development: *cpcA* activates amino acid biosynthesis gene transcription under conditions of amino acid deprivation, whereas *cpcB* represses the cross-pathway control network when amino acids are present. Overexpression of *cpcA* in the presence of amino acids leads to a block in sexual development, thereby mimicking a lack of amino acids, and the same effect can be reached by deletion of *cpcB* (Hoffmann et al. 2000). The connection between cross-pathway control and sexual development seems to be widespread in filamentous ascomycetes, as a mutant in the *N. crassa cpcB* homolog, *cpc-2*, is female-sterile (Müller et al. 1995). Also, a sterile mutant of *S. macrospora* was shown to have a defect in a gene for leucine biosynthesis (Kück 2005). This mutant, as well as the *A. nidulans* amino acid biosynthesis mutants mentioned above, grow normally on media with moderate amounts of the amino acid they are auxotrophic for, but if at all, fertility can be restored only by much higher amounts. These findings indicate that fungi are able to integrate nutrient availability and cellular metabolism, and react properly with respect to the initiation of energy-demanding processes such as fruiting-body formation.

Similar regulatory events can be proposed for fatty acid metabolism and fruiting-body development, although the evidence here is more spurious and signal transduction pathways have yet to be identified. Nevertheless, data from mutants in diverse genes involved in different aspects of fatty acid metabolism indicate that appropriate amounts and composition of fatty acids and their derivatives are essential for sexual development. *N. crassa* mutants of a fatty acid synthase subunit are sterile in homozygous crosses, and *A. nidulans* mutants of several desaturase genes show changes in

the balance between sexual and asexual development (Goodrich-Tanrikulu et al. 1999; Calvo et al. 2001; Wilson et al. 2004). In *A. nidulans*, several fatty acid-derived factors, so-called psi factors, are necessary for correct developmental decisions, and changes in fatty acid composition also influence psi factor composition, which could explain morphological defects in the mutants (see Chap. 11, this volume). It remains to be determined if similar fatty acid-derived specific developmental factors are present in other ascomycetes. Analyses of other mutants point to a more general requirement for fatty acids, probably as energy source for fruiting-body formation. In *S. macrospora*, the sterile mutant per5 was found to harbor a defect in the *acl1* gene that encodes a subunit of ATP citrate lyase (Nowrousian et al. 1999). This enzyme is involved in the production of cytosolic acetyl-CoA, which is used mainly for the synthesis of fatty acids and sterols, and the mutant can be partially rescued by addition of exogenous oleate, thereby indicating that it suffers from a lack of lipid biosynthesis products. The *acl1* gene is expressed mainly during vegetative growth prior to sexual development, which fits a model of the vegetative mycelium acquiring nutrients that are mobilized later during fruiting-body formation (Nowrousian et al. 1999, 2000). Another mutation with implications for fatty acid metabolism in fruiting-body development is *car1* of *Podospora anserina*. CAR1 is a peroxisomal protein necessary for peroxisome biogenesis, and interestingly, the *car1* mutant has defects in karyogamy, and therefore is sterile (Berteaux-Lecellier et al. 1995). In peroxisomes, mobilization of fatty acids by β-oxidation takes place, and the phenotype of the *car1* mutant might be due to a disturbed fatty acid metabolism.

Higher metabolic demands of fruiting-body formation versus vegetative growth or asexual sporulation can also be inferred by analyses of various complex I mutants of *N. crassa* (Videira and Duarte 2001). Complex I of the respiratory chain is a multi-subunit, proton-pumping NADH ubiquinone oxidoreductase that is found in most eukaryotic mitochondria. Mutants in several complex I subunits are sterile in homozygous crosses. This might be due to the subunits having other, non-respiratory functions, but the fact that mutants in subunits with different functions, such as complex assembly or reductase activity, display similar phenotypes with respect to sexual differentiation indicates that probably a lack of energy due to complex I malfunction is responsible for the developmental phenotype (Duarte and Videira 2000). This is consistent with an increased demand for energy and metabolites during fruiting-body formation.

Another aspect of increased respiration is the generation of reactive oxygen species (ROS), and thereby oxidative stress. It has long been known that ROS can have a deleterious effect on many cellular compounds, e.g., DNA and proteins, but only lately has it become acknowledged that the generation of ROS can be an actively regulated process, and that ROS can have diverse roles in cell physiology and signaling.

In *N. crassa*, it was shown recently that *sod-1*, which encodes a superoxide dismutase, is necessary for light-dependent positioning of perithecial necks (Yoshida and Hasunuma 2004). Superoxide dismutases catalyze the conversion of oxygen radicals that are generated during aerobic metabolism to hydrogen peroxide, thereby protecting the organism from damage by ROS. A possible explanation for the fact that *sod-1* is necessary for correct fruiting-body morphology is that SOD-1 is involved in generating a light-dependent ROS gradient that controls neck positioning.

In *Podospora anserina*, a mutant of the transcription factor GRISEA that is involved in cellular copper homeostasis is female-sterile (Osiewacz and Nuber 1996). Copper is an essential cofactor for several enzymes, among these some superoxide dismutases as well as cytochrome oxidase (COX), which is involved in the generation of ROS. Another mutant, in which the gene encoding a mitochondrial chaperone targeting copper to COX was deleted, showed delayed perithecial formation and reduced ascus production (Stumpferl et al. 2004). In contrast to the *grisea* mutant, this *PaCox17* mutant still had superoxide dismutase activity but respired via an alternative oxidase pathway, which might indicate that the balance between the generation and degradation of ROS is disturbed in both mutants, albeit not in the same way (Stumpferl et al. 2004). Further evidence for a participation of ROS in fruiting-body formation comes from investigation of *A. nidulans*. In this fungus, mutants of the NADPH oxidase gene, *noxA*, are sterile. NoxA generates superoxide, and is induced during sexual development of *A. nidulans*. At this time, superoxide can be detected in Hülle cells and cleistothecia (Lara-Ortíz et al. 2003). A similar expression pattern can be observed for the catalase-peroxidase gene *cpeA* (Scherer et al. 2002). Catalase-peroxidases are enzymes that convert ROS to harmless

compounds, thereby protecting the cell from oxidative damage. It is conceivable that *noxA* and *cpeA* act in concert to generate the correct amount of self-induced oxidative stress during fruiting-body formation. *noxA* expression is dependent on the MAP kinase SakA. *sakA* mutants show premature sexual development, and *noxA* is expressed in *sakA* mutants much earlier than in the wild type (Kawasaki et al. 2002; Lara-Ortíz et al. 2003).

Additional information on the involvement of *nox* genes in fruiting-body formation was recently gained from an investigation of *P. anserina*, where a mutant in the *noxA* ortholog *PaNox1* no longer differentiates mature fruiting bodies. Additionally, a mutant in a second member of the NADPH oxidase family, *PaNox2*, is blocked in ascospore germination, and both *PaNox1* and *PaNox2* are required for the controlled production of superoxide as well as peroxide during sexual development (Malagnac et al. 2004). These findings indicate that the generation of ROS is tightly regulated by a signal transduction network, and is an integral part of fruiting-body formation (see also Chap. 10, this volume).

2. Pheromones

In heterothallic ascomycetes, mating and subsequent fruiting-body development occur only after fusion of mycelial structures of opposite mating type. In *N. crassa*, diffusible pheromones have been suggested to be involved in the mating process, and to be the cause for the directional growth of trichogynes toward the male fertilizing cells of the opposite mating type (Bistis 1981, 1983). In *N. crassa*, this directional growth of the trichogynes did not occur when the recipient male cells harbored mutations at the mating-type locus, thus suggesting that the mating-type locus regulates the pheromone production (Bistis 1981). Pheromone precursor genes encoding two different types of pheromones have been isolated from the heterothallic filamentous ascomycetes *Cryphonectria parasitica*, *Magnaporthe grisea*, *N. crassa* and *P. anserina*, as well as from the homothallic ascomycete *S. macrospora* (Zhang et al. 1998; Shen et al. 1999; Pöggeler 2000; Bobrowicz et al. 2002; Coppin et al. 2005). One of the precursor genes encodes a polypeptide containing multiple repeats of a putative pheromone sequence bordered by protease processing sites, and resembles the α-factor precursor gene of *S. cerevisiae* (Fig. 16.3). The other gene encodes a short polypeptide similar to the *S. cerevisiae* **a**-factor precursor. The short precursor has a C-terminal CaaX (C = cysteine, a = aliphatic, and X = any amino acid residue) motif, expected to produce a mature pheromone with a C-terminal carboxy methyl isoprenylated cysteine (Fig. 16.3). The two types of pheromone precursor genes are present in the same nucleus. In heterothallic ascomycetes, pro-

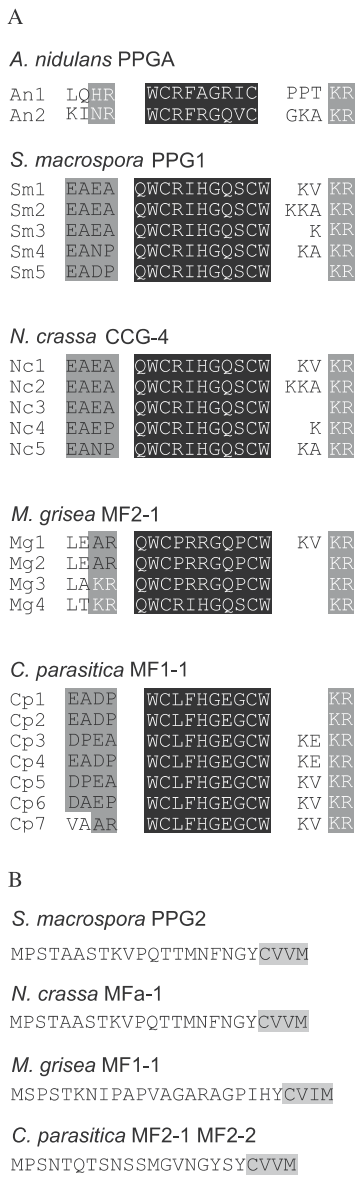

Fig. 16.3. **A**,**B** Pheromones of filamentous ascomycetes. **A** Sequences of predicted α-factor-like pheromones from filamentous ascomycetes. Repeats are shown in *white* and *boxed in black*, Kex2 processing sites (KR) in *white* and *boxed in gray*, STE13 processing sites in *black and boxed in gray*. **B** Sequences of predicted **a**-factor-like pheromones from filamentous ascomycetes. The prenylation signal motifs (CaaX) are *boxed in grey*

duction of either pheromone is directly controlled by transcription factors encoded by mating-type genes, and expression of pheromone genes seems to occur in a mating type-specific manner (see Debuchy and Turgeon, Chap. 15, this volume; Herskowitz 1989; Zhang et al. 1998; Shen et al. 1999; Bobrowicz et al. 2002; Coppin et al. 2005).

In contrast to all other hyphal ascomycetes, only one pheromone precursor gene, encoding a hydrophilic pheromone similar to the *S. cerevisiae* α-factor, was identified in the homothallic *Aspergillus nidulans* (Dyer et al. 2003).

In the heterothallic *N. crassa*, it was shown that pheromone precursor genes are highly expressed under conditions that favor sexual development (Bobrowicz et al. 2002). Interestingly, an elevated transcript level of the *N. crassa mfa-1* gene encoding the hydrophobic lipopeptide pheromone was observed in 7–9 day old perithecia (Kim et al. 2002a).

Furthermore, the expression of the *N. crassa* pheromone genes is regulated by the endogenous circadian clock in a time-of-day specific fashion. Both genes are repressed by RCO-1, a homolog of the *S. cerevisiae* transcriptional co-repressor Tup1p (Bobrowicz et al. 2002).

Recently, it was demonstrated that male and female fertility of heterothallic mycelial ascomycetes depends on interactions of pheromones with their specific receptors. When pheromone genes were deleted, spermatia were no longer able to fertilize the female partner, proving that pheromones are crucial for the fertility of male spermatia (Kim et al. 2002a; Turina et al. 2003; Coppin et al. 2005). In *P. anserina*, the function of pheromones is restricted to fertilization, while the *N. crassa* lipopeptide-pheromone gene *mfa-1* has also been shown to be involved in female sexual development, ascospore production, and vegetative growth of both mating types (Kim et al. 2002a; Coppin et al. 2005).

Similarly, in *C. parasitica*, deletion of only one of the two copies of CaaX-type pheromone genes (*Mf2-2*) was sufficient to prevent female fertility (Zhang et al. 1993). It was therefore speculated that *Mf2-2* of *C. parasitica* is required for a developmental phase after fertilization, and that the CaaX-type pheromone acts in a dosage-specific manner in post-fertilization events (Turina et al. 2003). Kim et al. (2002a) postulated an additional role for CaaX-type pheromones, in "conglutination" in perithecial development, or in the cementation of hyphae to stabilize the sclerotial structure of the maturing perithecium.

In contrast to the heterothallic mycelial ascomycetes *N. crassa*, *M. grisea* and *C. parasitica*, in the homothallic *S. macrospora* both pheromone precursor genes encoding structurally different pheromones are expressed in the same mycelium during the entire life cycle (Pöggeler 2000). It has recently been demonstrated that the disruption of the *S. macrospora ppg1* gene, encoding the α-factor-like peptide pheromone, prevents production of the peptide pheromone. However, this affects neither vegetative growth nor fruiting-body and ascospore development (Mayrhofer and Pöggeler 2005).

III. Signal Transduction Cascades

The molecular mechanisms underlying the development of fruiting bodies in ascomycetes are only poorly understood. However, there is increasing evidence that the external and internal stimuli are linked to genetically programmed cellular events. Usually, protein-mediated transduction of signals from the cell membrane to the nucleus is responsible for changes in gene expression. Components supposedly involved in signal transduction pathways can be subdivided into (1) receptors that percept the signal, (2) components that transmit the signal into the cell, and (3) nuclear transcription factors regulating gene expression. In the ascomycetous yeast *S. cerevisiae*, key components of the signaling cascade from the cell surface to the nucleus have been genetically characterized. In recent years, it has became evident that principles elucidated in yeast are applicable also to the more complex developmental programs of mycelial ascomycetes (Lengeler et al. 2000). However, analysis of the *N. crassa* genome demonstrated that filamentous ascomycetes encode classes of sensing molecules not found in *S. cerevisiae*, suggesting that alternative signaling cascades are involved in development processes of mycelial ascomycetes (Borkovich et al. 2004).

A. Perception of Environmental and Endogenous Signals

Central to many signaling pathways in eukaryotes is a cell surface receptor, which percepts an external chemical stimulus. Among the best characterized signaling systems are those mediated by guanine nucleotide binding (G)-protein-coupled seven-transmembrane-spanning receptors (GPCRs;

Neves et al. 2002). Typically, a ligand-bound GPCR activates or inhibits heterotrimeric G proteins, and transmits the signal to downstream effectors including adenylyl cyclases and protein kinases. Genome sequence analysis has shown that *N. crassa* possesses at least 10 predicted seven-transmembrane helix proteins that are potential G-PRCs (Galagan et al. 2003; Borkovich et al. 2004). Three of these were characterized at the molecular level. The *nop-1* gene encodes a seven-transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Analysis of *nop-1* deletion strains did not reveal obvious defects in light-regulated processes or fruiting-body development under normal laboratory conditions (Bieszke et al. 1999).

Two genes, designated *pre-1* and *pre-2*, encoding putative pheromone receptor genes similar to *S. cerevisiae* **a**-factor receptor (Ste3p) and α-factor receptor (Ste2p), respectively, have been identified in *N. crassa* and *S. macrospora* (Pöggeler and Kück 2001). Using a heterologous yeast system, it has been shown that the *S. macrospora* receptor PRE2 facilitates all aspects of the yeast pheromone response in *S. cerevisiae MAT***a** cells lacking the Ste2p receptor, when activated by the *S. macrospora* peptide pheromone. Therefore, one may conclude that the receptor encoded by the *pre2* gene functions as a GPCR in *S. macrospora*, too (Mayrhofer and Pöggeler 2005).

Northern and reverse transcription-polymerase chain reaction analyses indicate that in the heterothallic *N. crassa*, in contrast to pheromone precursor genes, expression of the receptor genes does not occur in a mating type-specific manner (Pöggeler and Kück 2001; Kim and Borkovich 2004). Recently, Kim and Borkovich (2004) demonstrated that deletion of the *N. crassa pre-1* gene does not affect vegetative growth or male fertility. However, protoperithecia from *Δpre1 mat A* mutants were shown to be female-sterile, because their trichogynes are unable to recognize and fuse with *mat a* cells. In the genome of the homothallic *A. nidulans*, nine genes (*gprA-gprI*) for potential seven-transmembrane spanning G-PRCs have been identified. Six of nine putative GPCRs have been disrupted and three genes, *gprA*, *gprB* and *gprD*, were found to play a central role in coordinating hyphal growth and sexual development (Han et al. 2004; Seo et al. 2004). Putative G-PRCs similar to the *S. cerevisiae* pheromone receptors Ste2p and Ste3p were shown to be encoded by *gprA* and *gprB*, respectively. Deletion of *gprA* or *gprB* resulted in the production of a few small cleistothecia carrying a reduced number of ascospores. Under homothallic conditions, an *A. nidulans* double-receptor-knockout strain *ΔgprA/ΔgprB* was completely abolished in fruiting-body and ascospore formation. Interestingly, outcrossing of *A. nidulans* receptor-mutant strains (*ΔgprA/ΔgprB*×*ΔgprA/ΔgprB*) resulted in fruiting-body and ascospore formation at wild-type level, suggesting that in *A. nidulans* the pheromone receptors GprA/B are specifically required for self-fertilization, and not for sexual development per se (Seo et al. 2004). By contrast, deletion of the GPRD-encoding *gprD* gene causes extremely restricted hyphal growth, delayed conidial germination, and uncontrolled activation of sexual development. Since elimination of sexual development rescues both growth and developmental abnormalities in *ΔgprD* strains, it was suggested that the primary role of GprD is to negatively regulate sexual development (Han et al. 2004). Moreover, it was demonstrated that deletion of pheromone receptor genes *gprA* and or *gprB* suppressed growth defects caused by the deletion of the *gprD* gene. This result implies that pheromone receptors GprA and GprB function downstream of GprD-mediated negative control of sexual development (Seo et al. 2004).

As has been described in the section above, fruiting-body development in filamentous ascomycetes is influenced by a variety of environmental stimuli and endogenous factors. In *N. crassa*, known elements in light sensing involved in fruiting-body development include the white collar complex, which acts as a blue-light photoreceptor as well as a transcription factor to regulate transcription of target genes in a light-dependent manner (see Sect. II.A.2, and Corrochano and Galland, Chap. 13, this volume). In addition to these two proteins, numerous other putative light-sensing genes have been identified in the genomic sequence of *N. crassa*, but their role in fruiting-body development has still to be elucidated (Borkovich et al. 2004).

Other environmental signals influencing fruiting-body development, such as osmolarity, nutrient levels, oxygen levels, and cellular redox status, might be sensed by two-component signal transduction pathways.

Two-component regulatory systems are composed of an autophosphorylating sensor histidine kinase and a response regulator. In contrast to yeasts, filamentous ascomycetes encode an extensive family of two-component signaling proteins

(Catlett et al. 2003). Eleven genes encoding putative histidine kinases have been identified in the *N. crassa* genome. So far, only two of them, *nik-1*/*os-1* and *nik-2*, have been characterized, but seem not to be involved in fruiting-body development. Whereas NIK-1 is an osmosensing histidine kinase that plays an important role in the regulation of cell wall assembly and cell responses to changes in external osmolarity, *nik-2* deletion mutants exhibit no obvious phenotypes (Alex et al. 1996; Schumacher et al. 1997; Borkovich et al. 2004). Similarly, two histidine kinases analyzed in *A. nidulans* were either shown to be involved in the formation of asexual spores, or disruption yielded no obvious clues to their function (Virginia et al. 2000; Furukawa et al. 2002). The involvement of the other two-component systems in ascocarp development awaits testing.

B. Signal Transduction Pathways

In response to extracellular stimuli, two cytoplasmic signaling branches defined by cAMP-dependent protein kinases (PKA) and mitogen-activated protein kinases regulate gene expression that finally leads to ascocarp formation (Lengeler et al. 2000). Upstream of these two signaling cascades, either heterotrimeric G proteins or ras and ras-like proteins act to relay extracellular ligand-stimulated signals to the cytoplasm. In addition to these four classes of proteins, several highly conserved proteins of unknown function have been identified by complementation of fungal developmental mutants (Fig. 16.4). These proteins are supposedly part of signal transduction pathways that lead to ascocarp formation (Table 16.1).

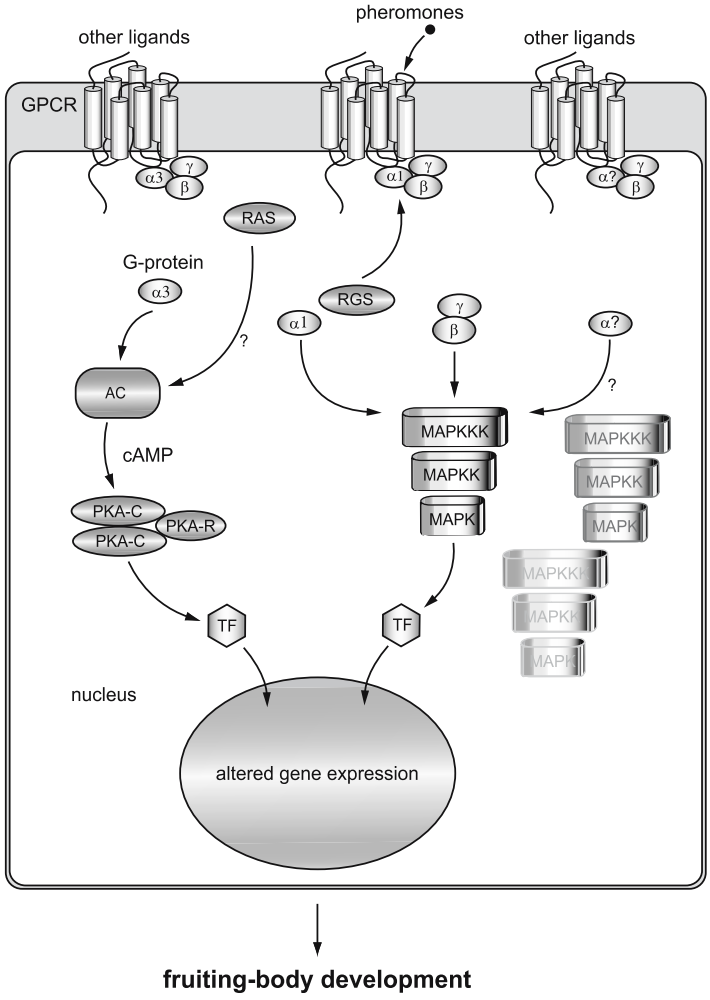

Fig. 16.4. Signal transduction pathways leading to fruiting-body development in filamentous ascomycetes

1. Heterotrimeric G Proteins

Upon activation of G-PRC receptors, heterotrimeric G proteins catalyze the exchange of GDP for GTP on the G protein α subunit, which leads to its dissociation from the βγ subunits. Either Gα, or Gβγ, or both are then free to activate downstream effectors (Dohlman 2002). In *N. crassa* and *A. nidulans* as well as other filamentous ascomycetes, it was demonstrated that subunits of G proteins are important for hyphal growth, conidiation, and fruiting-body development (Table 16.1). The genome of *N. crassa* contains three genes (*gna-1*, *gna-2* and *gna-3*) encoding Gα subunits, a single gene encoding the Gβ (*gnb-1*) subunit, and a single gene encoding the Gγ (*gng-1*) subunit (Borkovich et al. 2004). During the sexual cycle, GNA-1 is necessary for female fertility, whereas GNA-3, which is also important for asexual sporulation, mediates ascospore maturation (Ivey et al. 1996; Yang and Borkovich 1999; Kays et al. 2000). Loss of *gna-2* has no obvious effect on *N. crassa* growth and development; however, the double mutant *Δgna-1/Δgna-2* was shown to have more pronounced defects in female fertility than was the case for *Δgna-1* strains. This suggests that *gna-1* and *gna-2* have overlapping functions in sexual development (Baasiri et al. 1997; Kays and Borkovich 2004). *Δgnb-1* and *Δgng-1* strains of *N. crassa* are able to function as males during crosses with wild-type strains. When used as female parents, however, protoperithecia do not develop normally after fertilization, and they produce only small perithecia with aberrant fertilized reproductive structures (Yang et al. 2002; Krystofova and Borkovich 2005).

In the closely related pyrenomycete *Podospora anserina*, the *mod-D* gene encoding a Gα subunit and cloned as a suppressor of nonallelic heterokaryon incompatibility was shown to be involved in protoperithecial development (Loubradou et al. 1999).

Similar to *N. crassa*, the rice pathogen *Magnaporthe grisea* encodes three Gα subunits. Female fertility is mediated by MAG-B, whereas the other two subunits MAG-A and MAG-C are required for the production of mature asci (Liu and Dean 1997; Fang and Dean 2000). Targeted disruption of two Gα subunit genes in the chestnut blight fungus *Cryphonectria parasitica* revealed roles for the subunit CPG-1 in fungal reproduction, virulence, and vegetative growth. Disruption of the second Gα subunit gene, *cpg-2*, was found to enhance perithecial development (Gao and Nuss 1996). Mutation of the Gα subunit gene *cga-1* of the maize pathogen *Cochliobolus heterostrophus* leads to defects in several developmental pathways. Conidia from *cga-1* mutants germinate as abnormal, straight-growing germ tubes that form few appressoria, and the mutants are female-sterile (Horwitz et al. 1999; Degani et al. 2004). In *A. nidulans*, the Gα subunit FadA, along with the Gβ subunit SfdA, was shown to be an active participant in signaling pathways that govern critical decisions in the ascocarp development. *A. nidulans* strains carrying a deletion of the *fadA* gene fail to form cleistothecia. By contrast, strains carrying a dominant-negative mutation in the *fadA* ($fadA^{G203R}$) gene display an increase in Hülle cell formation but no cleistothecia formation. A similar phenotype was observed when the *sfaD* gene was deleted (Rosén et al. 1999). In addition to FadA, FlbA, a member of the regulator of G protein signalling (RGS) proteins that function as GTPase activating proteins, was shown to be involved in cleistothecia formation in *A. nidulans* (Han et al. 2001).

2. RAS and RAS-Like Proteins

RAS and RAS-like proteins are small GTP-binding proteins, which reside on the inner surface of the plasma membrane. They are able to transduce signals to the cytoplasmic signaling cascades, and control a variety of essential cellular processes (Vojtek and Der 1998).

Small GTP-binding proteins are generally grouped into five subfamilies named after their prototypical member: RAS, RHO, RAN, RAB, and ARF. The RAS superfamily of GTP-binding proteins comprises over 100 members, which act as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state (Takai et al. 2001).

The pyrenomycete *N. crassa* encodes two RAS proteins and three RAS-like proteins (Borkovich et al. 2004). To date, an involvement in ascocarp development has been demonstrated only for the RAS-like *N. crassa* protein KREV-1, a homolog of the mammalian RAP protein, and the *S. cerevisiae* Rsp1p protein. Disruption of the *N. crassa krev-1* gene by RIP was not correlated with growth abnormalities, but mutants overexpressing a constitutively active KREV-1 were shown to have defects in the sexual cycle. Mutations that are thought to lock the KREV-1 protein into the active GTP-bound form completely inhibit the development of protoperithecia into perithecia, whereas a dominant

negative mutation of KREV-1 resulted in some enlargement of protoperithecia (Ito et al. 1997).

In *A. nidulans*, mutations in the *rasA* gene cause aberrations in conidial germination and asexual development, but the role of RAS- and RAS-like proteins in cleistothecium formation has not yet been determined (Som and Kolaparthi 1994; Osherov and May 2000; Fillinger et al. 2002).

3. cAMP Activated Protein Kinases PKA

Activated RAS, RAS-like proteins, Gα or Gβγ subunits can regulate downstream effectors such as adenylyl cyclase and mitogen-activated protein kinase (MAPK) cascades (see Sect. III.B.4). Adenylyl cyclase is a membrane-bound enzyme that produces cyclic AMP (cAMP) from ATP. cAMP is a ubiquitous secondary messenger in prokaryotic and eukaryotic cells. In filamentous ascomycetes, cAMP signaling is involved in such diverse cellular processes as stress response, metabolism, pathogenicity, and sexual development (Kronstad et al. 1998; Lengeler et al. 2000). A well-characterized intracellular target of cAMP is the regulatory subunit of protein kinase A (PKA). PKA is a tetrameric enzyme that is composed of two regulatory subunits and two catalytic subunits. Binding of cAMP to the regulatory subunits releases the catalytic subunits. The latter phosporylate target proteins involved in cAMP-regulated processes (Dickman and Yarden 1999; Taylor et al. 2004).

In the plant pathogen *M. grisea*, it was shown that the sexual cycle is dependent on cAMP. *M. grisea* mutants lacking the adenylyl cyclase gene *mac-1* are female-sterile, and mate only when exogenous cAMP is supplied (Choi and Dean 1997; Adachi and Hamer 1998). By contrast, the *N. crassa cr-1* mutant, lacking adenylyl cyclase activity and cAMP, is able to function as male and female partner in sexual crosses, but exhibits delayed perithecial and ascospore production, compared to the wild type (Perkins et al. 1982; Ivey et al. 2002).

Female sterility of the *cr-1* mutant was observed only in a *Δgna-1* background. Unlike the *Δgna-1* mutant, the *Δgna-1*/*Δcr-1* double mutant does not form protoperithecia, suggesting that cAMP may be required for protoperithecial development. A recent study explored the contribution of all three Gα subunits on the cAMP level. The effects of mutating *gna-1* and *gna-3* were shown to be additive with respect to the adenylyl cyclase activity, whereas loss of *gna-2* did not appreciably affect adenylyl cyclase activity (Kays and Borkovich 2004).

The involvement of catalytic and regulatory subunits of PKA has so far been demonstrated only with respect to the asexual development, hyphal growth, and pathogenicity of *N. crassa*, *A. nidulans* and *M. grisea*, respectively (Mitchell and Dean 1995; Bruno et al. 1996; Fillinger et al. 2002). However, effects of PKA on fruiting-body formation have not yet been analyzed. Due to the presence of genes in the *N. crassa* genome encoding G-PRCs similar to slime mold cAMP receptors, it was recently suggested that cAMP may also serve as an environmental signal and G-PRC ligand in *N. crassa* (Borkovich et al. 2004).

4. Mitogen-Activated Protein Kinase (MAPK) Pathways

Mitogen-activated protein kinase (MAPK) pathways regulate eukaryotic gene expression in response to extracellular stimuli. The basic assembly of MAPK pathways is a three-component module conserved from yeast to humans.

The MAPK module includes three kinases that establish a sequential activation pathway comprising a MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK (Widmann et al. 1999). MAPK is activated by phosphorylation on conserved tyrosine and threonine residues by MAKK. In turn, MAPKK is activated by phosphorylation on conserved serine and threonine residues by MAPKKK. MAPKKK become activated by phosphorylation in response to various extracellular stimuli. The activated MAPK can translocate to the nucleus where it phosphorylates, and thus activates transcription factors (Dickman and Yarden 1999).

In the filamentous ascomycete *N. crassa*, three MAPK modules have been identified by genome sequence analysis (Borkovich et al. 2004). These correspond to those for pheromone response/filamentation, osmosensing/stress and cell integrity pathways in the yeasts *S. pombe* and *S. cerevisiae*. Three different MAPKs and two different MAPKKKs have been shown to be involved in fruiting-body development in different mycelial ascomycetes (Table 16.1). Mutation of the corresponding genes always leads to multiple phenotypic defects, including defects in ascocarp formation. Deletion of the *N. crassa mak-2* gene encoding a Fus3p MAPK was shown to result in loss of hyphal fusion and female sterility (Pandey et al. 2004; Li et al. 2005).

The MAPK Hog1p plays an essential role in osmotic stress signaling and osmoadaptation in the

yeast *S. cerevisiae* (Hohmann 2002). In *A. nidulans*, the Hog1p homolog SakA/HogA was demonstrated to be involved not only in stress signal transduction but also in sexual development. *A. nidulans ΔsakA* mutants display premature sexual development, and produce two times more cleistothecia at least 24 h earlier than wild-type strains (Kawasaki et al. 2002). In the plant pathogens *M. grisea* and *Fusarium graminearum*, it was demonstrated that homologs of the *S. cerevisiae* Slt2p MAPK are required for plant infection, and also for female fertility (Xu et al. 1998; Hou et al. 2002).

The MAPKKK NRC-1 of *N. crassa* functions to repress the onset of conidiation and is required for female fertility, because a *N. crassa nrc-1* mutant is unable to make protoperithecia (Kothe and Free 1998). It was recently demonstrated by Pandey et al. (2004) that an *nrc-1* mutant shares many of the same phenotypic traits as the *mak-2* mutant, and is also a hyphal fusion mutant, implying that NRC-1 acts upstream of MAK-2 during fruiting-body development. Similarly to the *N. crassa* NRC-1, the *A. nidulans* SteC regulates conidiophore development and is essential for cleistothecial development (Wei et al. 2003).

5. Other Putative Signaling Proteins

In addition to the components of conventional signal transduction pathways, forward genetic screens in filamentous ascomycetes identified several conserved proteins that may play key roles in fruiting-body development, but have so far not been shown to be involved in signal transduction processes in other eukaryotes. During a screen for suppressors of vegetative incompatibility in *N. crassa*, Xiang et al. (2002) isolated a deletion mutant that displayed pleiotropic defects including suppression of vegetative incompatibility, altered conidiation pattern, lack of hyphal fusion, and female sterility. A single gene termed *ham-2* was shown to complement hyphal fusion and female fertility, but not vegetative incompatibility and condiation pattern. The *ham-2* gene encodes a putative transmembrane protein that is highly conserved, but of unknown function among eukaryotes. Since the *ham-2* mutant forms only few protoperithecial-like structures, it was speculated that a HAM-2-dependent hyphal fusion process may be required for the formation of female reproductive structures (Xiang et al. 2002). In addition, the *S. cerevisiae* homolog of HAM-2, Far11p, was shown to be transcriptionally induced by exposure to pheromone, and to participate in the signaling pathway leading to pheromone-mediated G1 arrest (Kemp and Sprague 2003).

Similarly, in the related pyrenomycete *Podospora anserina*, there is evidence for a link between genes involved in incompatibility and perithecial development. A screen for suppressors of vegetative incompatibility led to the isolation of the *P. anserina mod-A* gene, which is not only responsible for growth arrest in the self-incompatible strains, but also involved in the control of the development of female organs. The MODA-encoded polypeptide is rich in proline residues, which are clustered in a domain containing a motif that displays similarity to SH3-binding motifs (Barreau et al. 1998).

In the homothallic pyrenomycete *Sordaria macrospora*, the sterile mutant pro11 carries a defect in the *pro11* gene encoding a multimodular WD40-repeat protein. PRO11 shows significant homology to several vertebrate WD40 proteins, such as striatin or zinedin, which seem to be involved in Ca^{2+}-dependent signaling in cells of the central nervous system, and supposedly function as scaffolding proteins linking signaling and eukaryotic endocytosis. Most importantly, a cDNA encoding the mouse striatin caused a functional substitution of the *S. macrospora* wild-type gene with a restoration of fertility in the *pro11* mutant, suggesting that an evolutionary conserved cellular process in eukaryotic cell differentiation may regulate fruiting-body formation (Pöggeler and Kück 2004).

C. Transcription Factors

From the preceding section, it is evident that diverse signal transduction pathways interpret environmental or intrinsic signals. Eventually, signal transduction initiates morphogenesis by activating transcription factors that in turn activate or repress cell- or tissue-specific expression of morphogenetic genes. Most transcription factors involved in ascocarp formation have been identified in *A. nidulans* (Table 16.1). Some of these are thought to be direct targets of signaling pathways.

In *A. nidulans*, SteA, a homolog of the *S. cerevisiae* homeodomain transcription factor Ste12p, was isolated and shown to be required for cleistothecia formation. In the budding yeast, Ste12p plays a key role in coupling signal transduction

through MAP kinase modules to cell-specific or morphogenesis-specific gene expression required for mating and pseudohyphal filamentous growth. An *A. nidulans ΔsteA* strain is sterile and differentiates neither ascogenous hyphae nor cleistothecia. However, the development of sexual cycle-specific Hülle cells and asexual conidiation is unaffected (Vallim et al. 2000). A similar phenotype has been observed in *N. crassa*. *pp-1* mutants of *N. crassa* fail to develop protoperithecia. In addition, ascospores carrying null mutations of the *pp-1* gene are nonviable (Li et al. 2005).

In a forward genetic screen of *A. nidulans*, Han et al. (2001) isolated the *nsdD* (*n*ever in *s*exual *d*evelopment) gene encoding a GATA-type transcription factor. Deletion of *nsdD* resulted in loss of cleistothecia or Hülle cell formation, even under conditions that preferentially promote sexual development, indicating that NsdD is necessary for ascocarp formation. Transcription factors StuA, MedA, and DopA are also required during sexual reproduction in *A. nidulans*. Whereas *ΔstuA* and *ΔdopA* strains fail to differentiate Hülle cells and cleistothecia, *ΔmedA* strains form extensive masses of unorganized Hülle cells, but fail to differentiate cleistothecia.

In contrast to *steA* and *nsdD*, *stuA*, *medA* as well as *dopA* are developmental modifier genes that are also involved in asexual development of *A. nidulans* (Busby et al. 1996; Dutton et al. 1997; Wu and Miller 1997; Pascon Castiglioni and Miller 2000). The *N. crassa* homolog of the *A. nidulans stuA* gene, *asm-1*, has also been shown to be involved in sexual development, since deletion of *asm-1* destroys the ability to make protoperithecia, but does not affect male-specific functions (Aramayo et al. 1996).

So far, information about the sexual regulatory network in *A. nidulans* is limited. However, Vallim et al. (2000) reported that *steA* lies upstream of *medA* in the same regulatory pathway, and that SteA directly or indirectly represses *medA* transcription (see Fischer and Kües, Chap. 14, this volume). Upregulation of *nsdD* resulted in the production of barren cleistothecia in the *ΔgprA*/*ΔgprB* double mutant. This result suggests that NsdD can partially rescue the developmental defects caused by the deletion of GPCRs, and that GprA/B-mediated signaling may activate other genes necessary for the maturation of cleistothecia (Seo et al. 2004). In turn, *nsdD* expression was shown to be under control of FadA-mediated signaling (Han et al. 2001). As summarized in Table 16.1, other transcription factors of different classes from hyphal ascomycetes have been identified that regulate fruiting-body formation. Amongst these is the C_6 zinc finger transcription factor PRO1, isolated in a forward genetic screen from the homothallic pyrenomycete *Sordaria macrospora*. The developmental mutant pro1 forms only protoperithecia, and is unable to perform the transition into mature perithecia (Masloff et al. 1999). Functional analysis of the PRO1 transcription factor revealed that the $Zn(II)_2Cys_6$ binuclear cluster is a prerequisite for the developmental function of the protein. Fertility of the *S. macrospora pro1* mutant can be restored by the *pro1* homolog from *N. crassa* (Masloff et al. 2002). Recently, it was shown that the putative $Zn(II)_2Cys_6$ transcription factor RosA from *A. nidulans*, sharing 38% sequence similarity to the *S. macrospora* PRO1, is a negative regulator of sexual development in *A. nidulans* (Vienken et al. 2004).

Besides the transcription factors described above, mating type-encoded transcription factors are also involved in fruiting-body development of filamentous ascomycetes (reviewed by Debuchy and Turgeon, Chap. 15, this volume). Mating proteins, as master regulatory transcription factors, control pathways of cell speciation as well as sexual morphogenesis in heterothallic and homothallic ascomycetes. They are not only necessary for fertilization, but are also required for subsequent development of the fertilized pre-fruiting bodies (Coppin et al. 1997; Pöggeler 2001).

IV. Structural Components Involved in Fruiting-Body Development

Fruiting-body formation involves the differentiation of many morphologically distinct cell types. Besides the cells that participate directly in karyogamy and meiosis, many more specialized cell types are formed that comprise the mature fruiting body. Of the 28 recognized cell types of *N. crassa*, 15 occur only during fruiting-body formation (Bistis et al. 2003). As the cell wall is the main form-giving structure of the fungal cell, it can be expected that enzymes involved in cell wall biogenesis and metabolism are required and coordinately regulated during sexual development. In addition, genes for cytoskeleton structure and organization have in some cases been shown to be specifically involved in sexual development, and these and the cell wall-related genes will be discussed in this section.

A. The Cytoskeleton in Fruiting-Body Development

Genes encoding components or regulators of the cytoskeleton have been shown to contribute to sexual development in *A. nidulans* and *P. anserina*. *A. nidulans* has two genes encoding α-tubulin, *tubA* and *tubB*. Whereas *tubA* is necessary for mitosis and nuclear migration, *tubB* is essential for ascospore formation (Kirk and Morris 1991). In *P. anserina*, it was shown that an ami1 mutant is male-sterile and displays delayed fruiting-body formation (Bouhouche et al. 2004). *ami1* encodes a homolog of the *A. nidulans apsA* gene, which is a protein necessary for nuclear positioning, most likely by regulating components of the dynein pathway. Thus, the cytoskeleton-related genes involved in sexual development have so far turned out to be important for nuclear migration and cell cycle events.

B. The Cell Wall in Fruiting-Body Development

The function of fruiting bodies is the protection and discharge of the ascospores, and this is achieved by developing morphologically distinct structures that are often characterized by cells with rigid and heavily pigmented cell walls. Thus, it has long been proposed that enzymes involved in cell wall biogenesis and metabolism are essential for sexual development in ascomycetes. Analyses have concentrated on several classes of genes considered to have a role in development, namely, genes involved in pigmentation, chitin synthases, lectins, hydrophobins, and genes involved in carbohydrate metabolism of the cell wall. Some of the results are described below (see also Chaps. 4 and 5, this volume).

1. Chitin

One of the characteristic components of the fungal cell wall is chitin, and central enzymes in chitin biosynthesis are chitin synthases. Fungi usually possess several chitin synthase genes, e.g., the genome of *N. crassa* contains seven chitin synthase genes (Borkovich et al. 2004). Chitin synthases that might be specifically required for fruiting-body formation have not been identified with certainty. However, chitin synthase genes that are expressed preferentially in fruiting-body tissue have been found in *A. nidulans* and *T. borchii*. In *A. nidulans*, the three chitin synthase genes *chsA*, *chsB* and *chsC* have been investigated. *chsA* was expressed solely during asexual sporulation, and *chsB* during all stages of growth and development, whereas *chsC* was expressed during early vegetative growth as well as sexual development. In the latter case, *chsC* expression was restricted to cleistothecia and ascospores (Lee et al. 2004). In *T. borchii*, expression of three genes for chitin synthases was analyzed in vegetative hyphae and fruiting bodies. All three were constitutively expressed in vegetative mycelium, but two of them were additionally expressed in fruiting bodies. One of these was found in sporogenic tissue, and the other in the vegetative tissue of the fruiting body (Balestrini et al. 2000). These examples indicate that, most likely, chitin synthases have specific but overlapping functions during different stages of fungal development.

2. Carbohydrates

After chitin, carbohydrates are another important constituent of the fungal cell wall, one main component being β-1,3-glucan (Walser et al. 2003). Apart from being structural elements, it has been proposed that carbohydrates are stored during vegetative growth to be mobilized as a carbon source for sexual development. One of these potential storage carbohydrates is α-1,3-glucan. It was shown to accumulate during vegetative growth of *A. nidulans*, and is degraded at the onset of sexual development (Zonneveld 1972). This finding correlates well with the fact that the gene for α-1,3-glucanase, *mutA*, is expressed during sexual development and degrades α-1,3-glucan to yield glucose.

Interestingly, MUTA is mainly found in Hülle cells whereas α-1,3-glucan is located mostly in the walls of cells other than Hülle cells. This might indicate that Hülle cells produce glucanase, which then degrades glucan in the cell walls of storage hyphae to yield carbohydrates that can be absorbed by the still intact Hülle cells (Zonneveld 1972; Wei et al. 2001).

However, *mutA* mutants are still able to form fruiting bodies, which indicates that other forms of carbohydrates can be used during fruiting-body development (Wei et al. 2001). One of these might be rhamnogalacturonan, as it has been shown that a putative rhamnogalacturonase encoded by the *asd-1* gene is essential for ascospore formation in *N. crassa* (Nelson and Metzenberg 1992; Nelson et al. 1997b). It is not yet clear whether rhamnogalacturonan inhibits sexual development and is degraded by ASD-1, or if the rhamnogalacturonan degradation products are needed for development.

The fact that fruiting-body formation requires high amounts of energy in the form of carbohydrates initially stored in vegetative hyphae, and subsequently released mostly in the extracellular space, would imply the existence of effective transport systems for nutrient uptake into the cells of the developing fruiting body. One such transporter for hexoses that is localized in ascogenous hyphae was described in *A. nidulans* (Wei et al. 2004). The gene for this transporter, *hxtA*, is not essential for sexual development, but as there are at least 17 putative hexose transporter genes in the *A. nidulans* genome, this might be due to the existence of functionally redundant proteins (Wei et al. 2004).

3. Pigments

Other common components of fungal cell walls are pigments, e.g., brown or black melanins. Melanins stabilize the cell wall and offer protection against UV light-induced DNA damage, but also have additional functions. For example, they are necessary for appressorial penetration of plant tissue in several fungal plant pathogens, and for pathogenicity of several human pathogens (Howard and Valent 1996; Perpetua et al. 1996; Jacobson 2000). Two biochemical pathways have been recognized for fungal melanin production, the DHN (dihydroxynaphtalene)- and the DOPA (dihydroxyphenylalanine)-melanin biosynthesis pathways (Langfelder et al. 2003). Both pathways contain laccases for the first (DOPA) or last (DHN) biosynthetic step, respectively. The DHN pathway involves a polyketide synthase in its initial step, but laccases and polyketide synthases also participate in a variety of biosynthetic pathways not necessarily involved in pigment formation. Laccase and polyketide synthase genes have been identified in several fungi, and have been implied in fruiting-body pigmentation of ascomycetes, but so far evidence for a genetic control of melanin biosynthesis in black or darkly pigmented fruiting bodies is missing.

It was shown for *A. nidulans* that laccase activity is located in young cleistothecia and Hülle cells, and that a sterile mutant had reduced laccase activity, but the corresponding laccase gene(s) have not yet been identified (Kurtz and Champe 1981; Hermann et al. 1983). In the plant pathogen *Nectria haematococca*, a polyketide synthase, PKSN, was shown to be essential for the synthesis of the red perithecial pigment by complementation of a mutant with white perithecia (Graziani et al. 2004). The mutant, however, is fertile. Therefore, the lack of perithecial pigmentation does not preclude ascospore maturation (Babai-Ahary et al. 1982), although it might be interesting to determine performance of the mutant strain under natural growth conditions.

4. Cell Wall Proteins

In addition to carbohydrates and pigment molecules, proteins comprise a sizeable part of the fungal cell wall. Two classes of proteins, hydrophobins and lectins, have been characterized extensively in higher basidiomycetes where they are implied in mushroom formation (see also Chap. 19, this volume). Hydrophobins can self-assemble at water/air interfaces, and form highly insoluble amphipathic films. These films can attach to the hydrophilic cellular surfaces, thereby orienting the hydrophobic side to the outside, which allows the fungus to break a water/air interface and grow into the air (Wösten et al. 1999). Lectins are carbohydrate-binding, mostly extracellular proteins that occur in virtually all classes of organisms. They have been implicated in the interaction of fungi with other organisms as well as fruiting-body formation in basidiomycetes (Walser et al. 2003). Genes encoding hydrophobins and lectins have been found in ascomycetes, too, but whether any of these play a role in sexual development is not yet clear.

Hydrophobins from *N. crassa*, *A. nidulans* and *M. grisea* have been shown to be involved in the formation of the water-repellent coat of conidiospores, but not in fruiting-body development (Stringer et al. 1991; Bell-Pedersen et al. 1992; Stringer and Timberlake 1995; Talbot et al. 1996).

As fungi can contain several members of the hydrophobin family in their genomes (Segers et al. 1999; Fuchs et al. 2004), addressing the question whether any of these are involved in fruiting-body morphogenesis might be a complex task involving the generation of mutants in more than one hydrophobin gene. Similarly to hydrophobins, lectins are thought to be involved in fruiting-body formation, but a requirement for lectins has yet to be shown in any fungus. The only described mutant in a lectin-encoding gene of an ascomycete is the aol mutant of *Arthrobotrys oligospora* (Balogh et al. 2003), but as no perfect stage of *A. oligospora* is known, the question of an involvement of AOL in sexual development cannot be addressed.

5. Are Multiple Genes with Overlapping Functions Involved in Cell Wall Metabolism?

The information about the molecular and genetic basis of cell wall metabolism during fruiting-body formation in ascomycetes is rather limited. A recurring theme are mutants in "candidate genes" that surprisingly do not have any discernible phenotype (see Sect. IV.B.2–4). Especially with the availability of whole genome sequences, it has become increasingly clear that one of the reasons for these findings might be the existence of large gene families most likely with partly overlapping functions. This does seem to be the case for most of the genes involved in the biosynthesis of cell wall components. A possible explanation for this might be the fact that the cell wall is a highly important structure for fungi – it is form-giving as well as protecting – but makes assessments of single components somewhat tedious. However, analysis of whole genome data, especially cross-species comparisons as well as large-scale expression studies, could help to identify candidates for future gene inactivation projects.

V. Conclusions

Fruiting-body formation in filamentous ascomycetes is a highly complex differentiation process that in some cases produces up to 15 different cell types. This complexity is further enhanced by morphogenic signals, such as light, temperature, and nutrients as well as species-specific cell communication factors such as pheromones and other signaling molecules. The many parameters determining this process may explain why several genes encoding G proteins, receptors, pheromones, and transcription factors have been identified as being involved in this developmental process. Mainly two different signal transduction pathways, MAPK cascades, and a cAMP-PKA cascade, function coordinately to regulate sexual cell differentiation processes by activating or repressing numerous transcription factors of different classes. Most probably, these regulate the transcription of genes encoding enzymes involved in cell wall biogenesis and metabolism, and genes for cytoskeleton structure and organization. Classical genetic studies have shown that ascocarp development involves a series of developmentally regulated genes. Therefore, it seems not surprising that currently no "main stream" signal transduction pathway is obvious that predominantly directs fruiting-body development in ascomycetes.

In summary, it has become evident that multicellular ascocarp development requires precise integration of a number of fundamental biological processes. The sexual pathway in ascomycetes provides a valuable experimental system for studying the mechanism and regulation of developmental processes that are usually more complex in animal and plant development. The analysis of fungal genomes is a starting point for further understanding developmental processes in mycelial fungi and other multicellular eukaryotes. Post-genomic analysis, including transcriptome and proteome assessments, will hopefully decipher components directing multicellular differentiation processes in ascomycetes, and finally as a long-term objective will help to understand eukaryotic differentiation processes at the molecular level.

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References

Adachi K, Hamer JE (1998) Divergent cAMP signaling pathways regulate growth and pathogenesis in the rice blast fungus *Magnaporthe grisea*. Plant Cell 10:1361–1374

Adams TH, Wieser JK, Yu J-H (1998) Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev 62:35–54

Alex LA, Borkovich KA, Simon MI (1996) Hyphal development in *Neurospora crassa*: involvement of a two-component histidine kinase. Proc Natl Acad Sci USA 93:3416–3421

Alexopoulos CJ, Mims CW, Blackwell M (1996) Introductory mycology. Wiley, New York

Almeida T, Duarte M, Melo AM, Videira A (1999) The 24-kDa iron-sulphur subunit of complex I is required for enzyme activity. Eur J Biochem 265:86–93

Aramayo R, Peleg Y, Addison R, Metzenberg R (1996) *Asm-1*$^{+}$, a *Neurospora crassa* gene related to transcriptional regulators of fungal development. Genetics 144:991–1003

Arnaise S, Zickler D, Poisier C, Debuchy R (2001) *pah1*: a homeobox gene involved in hyphal morphology and microconidiogenesis in the filamentous ascomycete *Podospora anserina*. Mol Microbiol 39:54–64

Baasiri RA, Lu X, Rowley PS, Turner GE, Borkovich KA (1997) Overlapping functions for two G protein α subunits in *Neurospora crassa*. Genetics 147:137–145

Babai-Ahary A, Daboussi-Bareyre MJ, Parisot D (1982) Isolation and genetic analysis of self-sterility and perithecial pigmentation mutants in a homothallic isolate of *Nectria haematococca*. Can J Bot 60:79–84

Bailey LA, Ebbole DJ (1998) The *fluffy* gene of *Neurospora crassa* encodes a Gal4p-type C6 zinc cluster protein required for conidial development. Genetics 148:1813–1820

Balestrini R, Mainieri D, Soragni E, Garnero L, Rollino S, Viotti A, Ottonello S, Bonfante P (2000) Differential expression of chitin synthase III and IV mRNAs in ascomata of *Tuber borchii* Vittad. Fungal Genet Biol 31:291–232

Ballario P, Vittorioso P, Magrelli A, Talora C, Cabibbo A, Macino G (1996) White collar-1, a central regulator of blue-light responses in *Neurospora*, is a zinc finger protein. EMBO J 15:1650–1657

Balogh J, Tunlid A, Rosen S (2003) Deletion of a lectin gene does not affect the phenotype of the nematode-trapping fungus *Arthrobotrys oligospora*. Fungal Genet Biol 39:128–135

Barr ME (2001) Ascomycota. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota, vol 7A. Systematics and evolution, part A. Springer, Berlin Heidelberg New York, pp 161–177

Barreau C, Iskandar M, Loubradou G, Levallois V, Begueret J (1998) The mod-A suppressor of nonallelic heterokaryon incompatibility in *Podospora anserina* encodes a proline-rich polypeptide involved in female organ formation. Genetics 149:915–926

Bell-Pedersen D, Dunlap JC, Loros JJ (1992) The *Neurospora* circadian clock-controlled gene, *ccg-2*, is allelic to *eas* and encodes a fungal hydrophobin required for formation of the conidial rodlet layer. Genes Dev 6:2382–2394

Benjamin CR (1955) Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47:669–687

Berbee ML, Carmean DA, Winka K (2000) Ribosomal DNA and resolution of branching order among the Ascomycota: how many nucleotides are enough? Mol Phylogenet Evol 17:337–344

Berteaux-Lecellier V, Picard M, Thompson-Coffe C, Zickler D, Panvier-Adoutte A, Simonet JM (1995) A non-mammalian homolog of the PAF1 gene (Zellweger syndrome) discovered as a gene involved in caryogamy in the fungus *Podospora anserina*. Cell 81:1043–1051

Berteaux-Lecellier V, Zickler D, Debuchy R, Panvier-Adoutte A, Thompson-Coffe C, Picard M (1998) A homologue of the yeast SHE4 gene is essential for the transition between the syncytial and cellular stages during sexual reproduction of the fungus *Podospora anserina*. EMBO J 17:1248–1258

Bieszke JA, Braun EL, Bean LE, Kang S, Natvig DO, Borkovich KA (1999) The *nop-1* gene of *Neurospora crassa* encodes a seven transmembrane helix retinal-binding protein homologous to archaeal rhodopsins. Proc Natl Acad Sci USA 96:8034–8039

Bistis GN (1981) Chemotrophic interactions between trichogynes and conidia of the opposite mating type in *Neurospora crassa*. Mycologia 73:959–975

Bistis GN (1983) Evidence for diffusible mating-type specific trichogyne attractants in *Neurospora crassa*. Exp Mycol 7:292–295

Bistis GN, Perkins DD, Read ND (2003) Different cell types in *Neurospora crassa*. Fungal Genet Biol 50:17–19

Bobrowicz P, Pawlak R, Correa A, Bell-Pedersen D, Ebbole DJ (2002) The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. Mol Microbiol 45:795–804

Borghouts C, Osiewacz HD (1998) GRISEA, a copper-modulated transcription factor from *Podospora anserina* involved in senescence and morphogenesis, is an ortholog of MAC1 in *Saccharomyces cerevisiae*. Mol Gen Genet 260:492–502

Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108

Bouhouche K, Zickler D, Debuchy R, Arnaise S (2004) Altering a gene involved in nuclear distribution increases the repeat-induced point mutation process in the fungus *Podospora anserina*. Genetics 167:151–159

Bowman EJ, Kendle R, Bowman BJ (2000) Disruption of *vma-1*, the gene encoding the catalytic subunit of the vacuolar H(+)-ATPase, causes severe morphological changes in *Neurospora crassa*. J Biol Chem 275:167–176

Braus GH, Krappmann S, Eckert SE (2002) Sexual development in ascomycetes – fruit body formation of *Aspergillus nidulans*. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 215–244

Bruggeman J, Debets AJ, Wijngaarden PJ, deVisser JA, Hoekstra RF (2003) Sex slows down the accumulation of deleterious mutations in the homothallic fungus *Aspergillus nidulans*. Genetics 164:479–485

Bruggeman J, Debets AJ, Hoekstra RF (2004) Selection arena in *Aspergillus nidulans*. Fungal Genet Biol 41:181–188

Bruno KS, Aramayo R, Minke PF, Metzenberg RL, Plamann M (1996) Loss of growth polarity and mislocalization of septa in a *Neurospora* mutant altered in the regulatory subunit of cAMP-dependent protein kinase. EMBO J 15:5572–5582

Busby TM, Miller KY, Miller BL (1996) Suppression and enhancement of the *Aspergillus nidulans medusa* mutation by altered dosage of the *bristle* and *stunted* genes. Genetics 143:155–163

Busch S, Hoffmann B, Valerius O, Starke K, Düvel K, Braus GH (2001) Regulation of the *Aspergillus nidulans hisB* gene by histidine starvation. Curr Genet 38:314–322

Busch S, Eckert SE, Krappmann S, Braus GH (2003) The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 49:717–730

Bussink HJ, Osmani SA (1998) A cyclin-dependent kinase family member (PHOA) is required to link developmental fate to environmental conditions in *Aspergillus nidulans*. EMBO J 17:3990–4003

Calvo AM, Gardner HW, Keller NP (2001) Genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans*. J Biol Chem 276:25766–25774

Catlett NL, Yoder OC, Turgeon BG (2003) Whole-genome analysis of two-component signal transduction genes in fungal pathogens. Eukaryot Cell 2:1151–1161

Champe SP, Nagle DL, Yager LN (1994) Sexual sporulation. In: Martinelli SD, Kinghorn JR (eds) *Aspergillus*: 50 years on. Elsevier, Amsterdam, pp 429–454

Choi W, Dean RA (1997) The adenylate cyclase gene MAC1 of *Magnaporthe grisea* controls appressorium formation and other aspects of growth and development. Plant Cell 9:1973–1983

Claussen P (1912) Zur Entwicklungsgeschichte der Ascomyceten. *Pyronema confluens*. Zeitschr Bot 4:1–63

Contamine V, Zickler D, Picard M (2004) The *Podospora rmp1* gene implicated in nucleus-mitochondria cross-talk encodes an essential protein whose subcellular location is developmentally regulated. Genetics 166:135–150

Coppin E (2002) The *fle1* gene encoding a C_2H_2 zinc finger protein co-ordinates male and female sexual differentiation in *Podospora anserina*. Mol Microbiol 43:1255–1268

Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61:411–428

Coppin E, de Renty C, Debuchy R (2005) The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Eukaryot Cell 4:407–420

Davis R, Perkins DD (2002) *Neurospora*: a model of model microbes. Nat Rev Genet 3:7–13

Degani O, Maor R, Hadar R, Sharon A, Horwitz BA (2004) Host physiology and pathogenic variation of *Cochliobolus heterostrophus* strains with mutations in the G protein alpha subunit, CGA1. Appl Environ Microbiol 70:5005–5009

Degli Innocenti F, Russo VEA (1983) Photoinduction of protoperithecia in *Neurospora crassa* by blue light. Photochem Photobiol 37:49–51

Degli Innocenti F, Russo VEA (1984) Isolation of new white collar mutants of *Neurospora crassa* and studies on their behavior in the blue light-induced formation of protoperithecia. J Bacteriol 159:757–761

Dickman MB, Yarden O (1999) Serine/threonine protein kinases and phosphatases in filamentous fungi. Fungal Genet Biol 26:99–117

Dohlman HG (2002) G proteins and pheromone signaling. Annu Rev Physiol 64:129–152

Duarte M, Videira A (2000) Respiratory chain complex I is essential for sexual development in *Neurospora* and binding of iron sulfur clusters are required for enzyme assembly. Genetics 156:607–615

Duarte M, Mota N, Pinto L, Videira A (1998) Inactivation of the gene coding for the 30.4-kDa subunit of respiratory chain NADH dehydrogenase: is the enzyme essential for *Neurospora*? Mol Gen Genet 257:368–375

Dutton JR, Johns S, Miller BL (1997) StuAp is a sequence-specific transcription factor that regulates developmental complexity in *Aspergillus nidulans*. EMBO J 16:5710–5721

Dyer PS, Ingram DS, Johnstone K (1992) The control of sexual morphogenesis in the Ascomycotina. Biol Rev 67:421–458

Dyer PS, Ingram DS, Johnstone K (1993) Evidence for the involvement of linoleic acid and other endogenous lipid factors in perithecial development of *Nectria haematococca* mating population VI. Mycol Res 97:485–496

Dyer PS, Paoletti M, Archer DB (2003) Genomics reveals sexual secrets of *Aspergillus*. Microbiology 149:2301–2303

Eckert SE, Hoffmann B, Wanke C, Braus GH (1999) Sexual development of *Aspergillus nidulans* in tryptophan auxotrophic strains. Arch Microbiol 172:157–166

Eckert SE, Kübler E, Hoffmann B, Braus GH (2000) The tryptophan synthase-encoding *trpB* gene of *Aspergillus nidulans* is regulated by the cross-pathway control system. Mol Gen Genet 263:867–876

Ellis TT, Reynolds DR, Alexopoulus CJ (1973) Hülle cell development in *Emericella nidulans*. Mycologia 65:1028–1035

Esser K (1982) Cryptogams. Cambridge University Press, Cambridge

Esser K, Straub J (1958) Genetische Untersuchungen an *Sordaria macrospora* Auersw.: Kompensation und Induktion bei genbedingten Entwicklungsdefekten. Z Vererbungslehre 89:729–746

Fang EG, Dean RA (2000) Site-directed mutagenesis of the *magB* gene affects growth and development in *Magnaporthe grisea*. Mol Plant Microbe Interact 13:1214–1227

Fecke W, Sled VD, Ohnishi T, Weiss H (1994) Disruption of the gene encoding the NADH-binding subunit of NADH: ubiquinone oxidoreductase in *Neurospora crassa*. Formation of a partially assembled enzyme without FMN and the iron-sulphur cluster N-3. Eur J Biochem 220:551–558

Feng B, Haas H, Marzluf GA (2000) ASD4, a new GATA factor of *Neurospora crassa*, displays sequence-specific DNA binding and functions in ascus and ascospore development. Biochemistry 39:11065–11073

Fillinger S, Chaveroche MK, Shimizu K, Keller N, d'Enfert C (2002) cAMP and ras signalling independently control spore germination in the filamentous fungus *Aspergillus nidulans*. Mol Microbiol 44:1001–1016

Fischer R (2002) Conidiation in *Aspergillus nidulans*. In: Osiewacz HD (ed) Molecular biology of fungal development. Dekker, New York, pp 59–86

Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. Science 297:815–819

Fuchs U, Czymmek KJ, Sweigard JA (2004) Five hydrophobin genes in *Fusarium verticillioides* include two required for microconidial chain formation. Fungal Genet Biol 41:852–864

Furukawa K, Katsuno Y, Urao T, Yabe T, Yamada-Okabe T, Yamada-Okabe H, Yamagata Y, Abe K, Nakajima T (2002) Isolation and functional analysis of a gene, *tcsB*, encoding a transmembrane hybrid-type histidine kinase from *Aspergillus nidulans*. Appl Environ Microbiol 68:5304–5310

Gagny B, Silar P (1998) Identification of the genes encoding the cytosolic translation release factors from *Podospora anserina* and analysis of their role during the life cycle. Genetics 149:1763–1775

Galagan JE, Selker EU (2004) RIP: the evolutionary cost of genome defense. Trends Genet 20:417–423

Galagan JE, Calvo SE, Borkovich KA, Selker EU, Read ND, Jaffe D, FitzHugh W, Ma LJ, Smirnov S, Purcell S et al. (2003) The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859–868

Gao S, Nuss DL (1996) Distinct roles for two G protein α subunits in fungal virulence, morphology, and reproduction revealed by targeted gene disruption. Proc Natl Acad Sci USA 93:14122–14127

Goodrich-Tanrikulu M, Howe K, Stafford A, Nelson MA (1998) Changes in fatty acid composition of *Neurospora crassa* accompany sexual development and ascospore germination. Microbiology 144:1713–1720

Goodrich-Tanrikulu M, Jacobson DJ, Stafford AE, Lin JT, McKeon TA (1999) Characterization of *Neurospora crassa* mutants isolated following repeat-induced

point mutation of the beta subunit of fatty acid synthase. Curr Genet 36:147–152
Graia F, Berteaux-Lecellier V, Zickler D, Picard M (2000) *ami1*, an orthologue of the *Aspergillus nidulans apsA* gene, is involved in nuclear migration events throughout the life cycle of *Podospora anserina*. Genetics 155:633–646
Graziani S, Vasnier C, Daboussi MJ (2004) Novel polyketide synthase from *Nectria haematococca*. Appl Environ Microbiol 70:2984–2988
Han DM, Han YK, Lee YH, Jahng KY, Jahng SH, Chae KS (1990) Inhibitory conditions of asexual development and their application for the screening of mutants defective in sexual development. Korean J Mycol 18:225–232
Han DM, Han YK, Kim JH, Jahng KY, Chung YS, Chae KS (1994) Isolation and characterization of NSD mutants in *Aspergillus nidulans*. Korean J Mycol 22:1–7
Han KY, Cheong SS, Hoe HS, Han DM (1998) Characterization of several NSD mutants of *Aspergillus nidulans* that never undergo sexual development. Korea J Genet 20:257–264
Han KH, Han KY, Yu JH, Chae KS, Jahng KY, Han DM (2001) The *nsdD* gene encodes a putative GATA-type transcription factor necessary for sexual development of *Aspergillus nidulans*. Mol Microbiol 41:299–309
Han KH, Seo JA, Yu JH (2004) A putative G protein-coupled receptor negatively controls sexual development in *Aspergillus nidulans*. Mol Microbiol 51:1333–1345
Harding RW, Melles S (1983) Genetic analysis of phototropism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. Plant Physiol 72:996–1000
He Q, Cheng P, Yang Y, Wang L, Gardner K, Liu Y (2002) White collar-1, a DNA binding transcription factor and a light sensor. Science 297:840–843
Hermann TE, Kurtz MB, Champe SP (1983) Laccase localized in Hülle cells and cleistothecial primordia of *Aspergillus nidulans*. J Bacteriol 154:955–964
Herskowitz I (1989) A regulatory hierarchy for cell specialization in yeast. Nature 342:749–757
Hock B, Bahn M, Walk RA, Nitschke U (1978) The control of fruiting body formation in the ascomycete *Sordaria macrospora* Auersw. by regulation of hyphal development. Planta 141:93–103
Hoffmann B, Wanke C, Lapaglia SK, Braus GH (2000) c-Jun and RACK1 homologues regulate a control point for sexual development in *Aspergillus nidulans*. Mol Microbiol 37:28–41
Hoffmann B, Eckert SE, Krappmann S, Braus GH (2001a) Sexual diploids of *Aspergillus nidulans* do not form by random fusion of nuclei in the heterokaryon. Genetics 157:141–147
Hoffmann B, Valerius O, Anderman M, Braus GH (2001b) Transcriptional autoregulation and inhibition of mRNA translation of amino acid regulator gene *cpcA* of filamentous fungus *Aspergillus nidulans*. Mol Biol Cell 12:2846–2857
Hohmann S (2002) Osmotic stress signaling and osmoadaptation in yeasts. Microbiol Mol Biol Rev 66:300–372
Horwitz BA, Sharon A, Lu SW, Ritter V, Sandrock TM, Yoder OC, Turgeon BG (1999) A G protein alpha subunit from *Cochliobolus heterostrophus* involved in mating and appressorium formation. Fungal Genet Biol 26:19–32
Hou Z, Xue C, Peng Y, Katan T, Kistler HC, Xu JR (2002) A mitogen-activated protein kinase gene (MGV1) in *Fusarium graminearum* is required for female fertility, heterokaryon formation, and plant infection. Mol Plant Microbe Interact 15:1119–1127
Howard RJ, Valent B (1996) Breaking and entering: host penetration by the fungal rice blast pathogen *Magnaporthe grisea*. Annu Rev Microbiol 50:491–512
Ito S, Matsui Y, Toh-e A, Harashima T, Inoue H (1997) Isolation and characterization of the *krev-1* gene, a novel member of *ras* superfamily in *Neurospora crassa*: involvement in sexual cycle progression. Mol Gen Genet 255:429–437
Ivey FD, Hodge PN, Turner GE, Borkovich KA (1996) The $G\alpha_i$ homologue *gna-1* controls multiple differentiation pathways in *Neurospora crassa*. Mol Biol Cell 7:1283–1297
Ivey FD, Kays AM, Borkovich KA (2002) Shared and independent roles for a $G\alpha_i$ protein and adenylyl cyclase in regulating development and stress responses in *Neurospora crassa*. Eukaryot Cell 1:634–642
Jacobson ES (2000) Pathogenic roles for fungal melanins. Clin Microbiol Rev 13:708–717
Johnson TE (1978) Isolation and characterization of perithecial developmental mutants in *Neurospora*. Genetics 88:27–47
Kawasaki L, Sanchez O, Shiozaki K, Aguirre J (2002) SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. Mol Microbiol 45:1153–1163
Kays AM, Borkovich KA (2004) Severe impairment of growth and differentiation in a *Neurospora crassa* mutant lacking all heterotrimeric Gα proteins. Genetics 166:1229–1240
Kays AM, Rowley PS, Baasiri RA, Borkovich KA (2000) Regulation of conidiation and adenylyl cyclase levels by the Gα protein GNA-3 in *Neurospora crassa*. Mol Cell Biol 20:7693–7705
Kemp HA, Sprague GFJ (2003) Far3 and five interacting proteins prevent premature recovery from pheromone arrest in the budding yeast *Saccharomyces cerevisiae*. Mol Cell Biol 23:1750–1763
Kicka S, Silar P (2004) PaASK1, a mitogen-activated protein kinase kinase kinase that controls cell degeneration and cell differentiation in *Podospora anserina*. Genetics 166:1241–1252
Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type-specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52:1781–1798
Kim H, Metzenberg RL, Nelson MA (2002a) Multiple functions of *mfa-1*, a putative pheromone precursor gene of *Neurospora crassa*. Eukaryot Cell 1:987–999
Kim H, Kyu-Yong H, Kim KJ, Han DM, Jahng KY, Chae KS (2002b) The *veA* gene activates sexual development in *Aspergillus nidulans*. Fungal Genet Biol 37:72–80
Kirk KE, Morris NR (1991) The *tubB* alpha-tubulin gene is essential for sexual development in *Aspergillus nidulans*. Genes Dev 5:2014–2023
Kirk PM, Cannon PF, David JC, Staplers JA (2001) Ainsworth and Bisby's dictionary of the fungi. CAB International, Wallingford, Oxon

Kothe GO, Free SJ (1998) The isolation and characterization of *nrc-1* and *nrc-2*, two genes encoding protein kinases that control growth and development in *Neurospora crassa*. Genetics 149:117–130

Kronstad J, Staben C (1997) Mating type in filamentous fungi. Annu Rev Genet 31:245–276

Kronstad J, de Maria AD, Funnell D, Laidlaw RD, Lee N, de Sa MM, Ramesh M (1998) Signaling via cAMP in fungi: interconnections with mitogen-activated protein kinase pathways. Arch Microbiol 170:395–404

Krystofova S, Borkovich KA (2005) The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a Gβγ dimer required for normal female fertility, asexual development, and Gα protein levels in *Neurospora crassa*. Eukaryot Cell 4:365–378

Kück U (2005) A *Sordaria macrospora* mutant, lacking the *leu1* gene, shows a developmental arrest during fruiting body formation. Mol Gen Genomics DOI:10.1007/s00438-005-0021-8

Kües U, Liu Y (2000) Fruiting body production in basidiomycetes. Appl Microbiol Biotechnol 54:141–152

Kurtz MB, Champe SP (1981) Dominant spore color mutants of *Aspergillus nidulans* defective in germination and sexual development. J Bacteriol 148:629–638

Kwon KJ, Raper KB (1967) Sexuality and cultural characteristics of *Aspergillus heterothallis*. Am J Bot 54:36–48

Lacourt I, Duplessis S, Abbà S, Bonfante P, Martin F (2002) Isolation and characterization of differentially expressed genes in the mycelium and fruit body of *Tuber borchii*. Appl Env Microbiol 68:4574–4582

Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38:143–158

Lara-Ortíz T, Riveros-Rosas H, Aguirre J (2003) Reactive oxygen species generated by microbial NADPH oxidase NoxA regulate sexual development in *Aspergillus nidulans*. Mol Microbiol 50:1241–1255

Lee DW, Kim S, Kim SJ, Han DM, Jahng KY, Chae KS (2001) The *lsdA* gene is necessary for sexual development inhibition by a salt in *Aspergillus nidulans*. Curr Genet 39:237–243

Lee JI, Choi JH, Park BC, Park YH, Lee MY, Park HM, Maeng PJ (2004) Differential expression of the chitin synthase genes of *Aspergillus nidulans*, *chsA*, *chsB*, and *chsC*, in response to developmental status and environmental factors. Fungal Genet Biol 41:635–646

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen WC, Wang P, Pan X, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Leslie JF, Raju NB (1985) Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. Genetics 111:759–777

Lev S, Sharon A, Hadar R, Ma H, Horwitz BA (1998) A mitogen-activated protein kinase of the corn leaf pathogen *Cochliobolus heterostrophus* is involved in conidiation, appressorium formation, and pathogenicity: diverse roles for mitogen-activated protein kinase homologs in foliar pathogens. Proc Natl Acad Sci USA 96:13542–13547

Li D, Bobrowicz P, Wilkinson HH, Ebbole DJ (2005) A MAP kinase pathway essential for mating and contributing to vegetative growth in *Neurospora crassa*. Genetics (in press)

Linden H, Macino G (1997) White collar 2, a partner in blue-light signal transduction, controlling expression of light-regulated genes in *Neurospora crassa*. EMBO J 16:98–109

Lindenmuth R, Wirtz N, Lumbsch HT (2001) Phylogeneic analysis of nuclear and mitochondrial rDNA sequences supports the view that loculoascomycetes (Ascomycota) are not monophyletic. Mycol Res 105:1176–1181

Liu S, Dean RA (1997) G protein α-subunit genes control growth, development, and pathogenicity of *Magnaporthe grisea*. Mol Plant Microbe Interact 10:1075–1086

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 16:1799–1808

Loros JJ, Dunlap JC (2001) Genetic and molecular analysis of circadian rhythms in *Neurospora*. Annu Rev Physiol 63:757–794

Loros JJ, Denome SA, Dunlap JC (1989) Molecular cloning of genes under the control of the circadian clock in *Neurospora*. Science 243:385–388

Loubradou G, Bégueret J, Turcq B (1997) A mutation in an *HSP90* gene affects the sexual cycle and suppresses vegetative incompatibility in the fungus *Podospora anserina*. Genetics 147:581–588

Loubradou G, Bégueret J, Turcq B (1999) MOD-D, a Gα subunit of the fungus *Podospora anserina*, is involved in both regulation of development and vegetative incompatibility. Genetics 152:519–528

Lumbsch HT (2000) Phylogeny of filamentous ascomycetes. Naturwissenschaften 87:335–342

Lumbsch HT, Lindemuth R, Schmitt I (2000) Evolution of filamentous ascomycetes interferred from LSU rRNA sequence data. Plant Biol 2:525–529

Malagnac F, Lalucque H, Lepere G, Silar P (2004) Two NADPH oxidase isoforms are required for sexual reproduction and ascospore germination in the filamentous fungus *Podospora anserina*. Fungal Genet Biol 41:982–997

Marshall MR, Hindal DF, MacDonald WL (1982) Production of perithecia in culture by *Ceratocystis ulmi*. Mycologia 74:376–381

Masloff S, Pöggeler S, Kück U (1999) The *pro1*$^+$ gene from *Sordaria macrospora* encodes a C_6 zinc finger transcription factor required for fruiting body development. Genetics 152:191–199

Masloff S, Jacobsen S, Pöggeler S, Kück U (2002) Functional analysis of the C_6 zinc finger gene *pro1* involved in fungal sexual development. Fungal Genet Biol 36:107–116

Mayrhofer S, Pöggeler S (2005) Functional characterization of an α-factor-like *Sordaria macrospora* peptide pheromone and analysis of its interaction with its cognate receptor in *Saccharomyces cerevisiae*. Eukaryot Cell 4:661–672

McCahill A, Warwicker J, Bolger GB, Houslay MD, Yarwood SJ (2002) The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. Mol Pharmacol 62:1261–1273

Metz AM, Haddad A, Worapong J, Long DM, Ford EJ, Hess WM, Strobel GA (2000) Induction of the sexual stage of *Pestalotiopsis microspora*, a taxol-producing fungus. Microbiology 146:2079–2089

Mitchell TK, Dean RA (1995) The cAMP-dependent protein kinase catalytic subunit is required for appressorium formation and pathogenesis by the rice blast pathogen *Magnaporthe grisea*. Plant Cell 7:1869–1878

Molowitz R, Bahn M, Hock B (1976) The control of fruiting body formation in the ascomycete *Sordaria macrospora* Auersw. by arginine and biotin: a two-factor analysis. Planta 128:143–148

Mooney JL, Yager LN (1990) Light is required for conidiation in *Aspergillus nidulans*. Genes Dev 4:1473–1482

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge

Moore-Landecker E (1979) Effect of light regimens and intensities on morphogenesis of the discomycete *Pyronema domesticum*. Mycologia 71:699–712

Moore-Landecker E (1992) Physiology and biochemistry of ascocarp induction and development. Mycol Res 96:705–716

Müller F, Krüger D, Sattlegger E, Hoffmann B, Ballario P, Kanaan M, Barthelmeß IB (1995) The *cpc-2* gene of *Neurospora crassa* encodes a protein entirely composed of WD-repeat segments that is involved in general amino acid control and female fertility. Mol Gen Genet 248:162–173

Nelson MA, Metzenberg RL (1992) Sexual development genes of *Neurospora crassa*. Genetics 132:149–162

Nelson MA, Kang S, Braun EL, Crawford ME, Dolan PL, Leonard PM, Mitchell J, Armijo AM, Bean L, Blueyes E et al. (1997a) Expressed sequences from conidial, mycelial, and sexual stages of *Neurospora crassa*. Fungal Genet Biol 21:348–363

Nelson MA, Merino ST, Metzenberg RL (1997b) A putative rhamnogalacturonase required for sexual development of *Neurospora crassa*. Genetics 146:531–540

Neves SR, Ram PT, Iyengar R (2002) G protein pathways. Science 296:1636–1639

Nowrousian M, Masloff S, Pöggeler S, Kück U (1999) Cell differentiation during sexual development of the fungus *Sordaria macrospora* requires ATP citrate lyase activity. Mol Cell Biol 19:450–460

Nowrousian M, Kück U, Loser K, Weltring KM (2000) The fungal *acl1* and *acl2* genes encode two polypeptides with homology to the N- and C-terminal parts of the animal ATP citrate lyase polypeptide. Curr Genet 37:189–193

Nowrousian M, Dunlap JC, Nelson MA (2004) Functional genomics in fungi. In: Kück U (ed) The Mycota, vol II. Springer, Berlin Heidelberg New York, pp 115–128

Nukina M, Sassa T, Ikeda M, Takahashi K, Toyota S (1981) Linoleic acid enhances perithecial production in *Neurospora crassa*. Agric Biol Chem 45:2371–2373

Oda K, Hasunuma K (1997) Genetic analysis of signal transduction through light-induced protein phosphorylation in *Neurospora crassa* perithecia. Mol Gen Genet 256:593–601

Ogura Y, Yoshida Y, Yabe N, Hasunuma K (2001) A point mutation in nucleoside diphosphate kinase results in a deficient light response for perithecial polarity in *Neurospora crassa*. J Biol Chem 276:21228–21234

Osherov N, May G (2000) Conidial germination in *Aspergillus nidulans* requires RAS signaling and protein synthesis. Genetics 155:647–656

Osiewacz HD, Nuber U (1996) GRISEA, a putative copper-activated transcription factor from *Podospora anserina* involved in differentiation and senescence. Mol Gen Genet 252:115–124

Pandey A, Roca MG, Read ND, Glass NL (2004) Role of a mitogen-activated protein kinase pathway during conidial germination and hyphal fusion in *Neurospora crassa*. Eukaryot Cell 3:348–358

Pascon Castiglioni R, Miller BL (2000) Morphogenesis in *Aspergillus nidulans* requires Dopey (DopA), a member of a novel family of leucine zipper-like proteins conserved from yeast to humans. Mol Microbiol 36:1250–1264

Perkins DD, Davis R (2000) *Neursopora* at the millenium. Fungal Genet Biol 31:153–167

Perkins DD, Radford A, Newmeyer D, Bjorkman M (1982) Chromosomal loci of *Neurospora crassa*. Microbiol Rev 46:426–570

Perpetua NS, Kubo Y, Yasuda N, Takano Y, Furusawa I (1996) Cloning and characterization of a melanin biosynthetic THR1 reductase gene essential for appressorial penetration of *Colletotrichum lagenarium*. Mol Plant Microbe Interact 9:323–329

Pöggeler S (2000) Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. Curr Genet 37:403–411

Pöggeler S (2001) Mating-type genes for classical strain improvements of ascomycetes. Appl Microbiol Biotechnol 56:589–601

Pöggeler S, Kück U (2001) Identification of transcriptionally expressed pheromone receptor genes in filamentous ascomycetes. Gene 280:9–17

Pöggeler S, Kück U (2004) A WD40 repeat protein regulates fungal cell differentiation and can functionally be replaced by the mammalian homologue striatin. Eukaryot Cell 3:232–240

Pöggeler S, Nowrousian M, Jacobsen S, Kück U (1997) An efficient procedure to isolate fungal genes from an indexed cosmid library. J Microbiol Methods 29:49–61

Pontecorvo G (1953) The genetics of *Aspergillus nidulans*. In: Demerec M (ed) Advances in genetics. Academic Press, New York, pp 141–238

Raju BB (1992) Genetic control of the sexual cycle in *Neurospora*. Mycol Res 96:241–262

Randall TA, Metzenberg RL (1998) The mating type locus of *Neurospora crassa*: identification of an adjacent gene and characterization of transcripts surrounding the idiomorphs. Mol Gen Genet 259:615–621

Rosén S, Yu JH, Adams TH (1999) The *Aspergillus nidulans sfaD* gene encodes a G protein β-subunit that is required for normal growth and repression of sporulation. EMBO J 18:5592–5600

Scherer M, Wei H, Liese R, Fischer R (2002) *Aspergillus nidulans* catalase-peroxidase gene (*cpeA*) is transcriptionally induced during sexual development through the transcription factor StuA. Eukaryot Cell 1:725–735

Schumacher MM, Enderlin CS, Selitrennikoff CP (1997) The *osmotic-1* locus of *Neurospora crassa* encodes a putative histidine kinase similar to osmosensors of bacteria and yeast. Curr Microbiol 34:340–347

Segers GC, Hamada W, Oliver RP, Spanu PD (1999) Isolation and characterisation of five different hydrophobin-encoding cDNAs from the fungal tomato pathogen *Cladosporium fulvum*. Mol Gen Genet 261:644–652

Seo JA, Han KH, Yu JH (2004) The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol Microbiol 53:1611–1623

Shen WC, Bobrowicz P, Ebbole DJ (1999) Isolation of pheromone precursor genes of *Magnaporthe grisea*. Fungal Genet Biol 27:253–263

Silar P, Lalucque H, Haedens V, Zickler D, Picard M (2001) eEF1A controls ascospore differentiation through elevated accuracy, but controls longevity and fruiting body formation through another mechanism in *Podospora anserina*. Genetics 158:1477–1489

Som T, Kolaparthi VS (1994) Developmental decisions in *Aspergillus nidulans* are modulated by Ras activity. Mol Cell Biol 14:5333–5348

Stringer MA, Timberlake WE (1995) *dewA* encodes a fungal hydrophobin component of the *Aspergillus* spore wall. Mol Microbiol 16:33–44

Stringer MA, Dean RA, Sewall TC, Timberlake WE (1991) Rodletless, a new *Aspergillus* developmental mutant induced by direct gene inactivation. Genes Dev 5:1161–1171

Stumpferl SW, Stephan O, Osiewacz HD (2004) Impact of a disruption of a pathway delivering copper to mitochondria on *Podospora anserina* metabolism and life span. Eukaryot Cell 3:200–211

Swart K, van Heemst D, Slakhorst M, Debets F, Heyting C (2001) Isolation and characterization of sexual sporulation mutants of *Aspergillus nidulans*. Fungal Genet Biol 33:25–35

Takai Y, Sasaki T, Matozaki T (2001) Small GTP-binding proteins. Physiol Rev 81:153–208

Talbot NJ, Kershaw MJ, Wakley G, de Vries O, Wessels JG, Hamer JE (1996) *MPG1* encodes a fungal hydrophobin involved in surface interactions during infection-related development of *Magnaporthe grisea*. Plant Cell 8:985–999

Taylor SS, Yang J, Wu J, Haste NM, Radzio-Andzelm E, Anand G (2004) PKA: a portrait of protein kinase dynamics. Biochim Biophys Acta 11:259–269

Tsitsigiannis DI, Zarnowski R, Keller NP (2004) The lipid body protein, PpoA, coordinates sexual and asexual sporulation in *Aspergillus nidulans*. J Biol Chem 279:11344–11353

Turina M, Prodi A, Alfen NK (2003) Role of the *Mf1-1* pheromone precursor gene of the filamentous ascomycete *Cryphonectria parasitica*. Fungal Genet Biol 40:242–251

Vallim MA, Miller KA, Miller BL (2000) *Aspergillus* SteA (Sterile12-like) is a homeodomain-C_2/H_2-Zn^{2+} finger transcription factor required for sexual reproduction. Mol Microbiol 36:290–301

Van Heemst D, Swart K, Holub EF, van Dijk R, Offenberg HH, Goosen T, van den Broek HW, Heyting C (1997) Cloning, sequencing, disruption and phenotypic analysis of uvsC, an *Aspergillus nidulans* homologue of yeast RAD51. Mol Gen Genet 254:654–664

Van Heemst D, James F, Pöggeler S, Berteaux-Lecellier V, Zickler D (1999) Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programs. Cell 98:261–271

Videira A, Duarte M (2001) On complex I and other NADH:ubiquinone reductases of *Neurospora crassa* mitochondria. J Bioenerg Biomembr 33:197–203

Vienken K, Scherer M, Fischer R (2004) The $Zn(II)_2Cys_6$ putative *Aspergillus nidulans* transcription factor repressor of sexual development inhibits sexual development under low-carbon conditions and in submersed culture. Genetics 169:619–630

Virginia M, Appleyard CL, McPheat WL, Stark MJ (2000) A novel 'two-component' protein containing histidine kinase and response regulator domains required for sporulation in *Aspergillus nidulans*. Curr Genet 37:364–372

Vojtek AB, Der CJ (1998) Increasing complexity of the Ras signaling pathway. J Biol Chem 273:19925–19928

Walser PJ, Velagapudi R, Aebi M, Kües U (2003) Extracellular matrix proteins in mushroom development. Recent Res Dev Microbiol 7:381–415

Walz M, Kück U (1995) Transformation of *Sordaria macrospora* to hygromycin B resistance: characterization of transformants by electrophoretic karyotyping and tetrad analysis. Curr Genet 29:88–95

Wei H, Scherer M, Singh A, Liese R, Fischer R (2001) *Aspergillus nidulans* α-1,3 glucanase (mutanase), *mutA*, is expressed during sexual development and mobilizes mutan. Fungal Genet Biol 34:217–227

Wei H, Requena N, Fischer R (2003) The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol Microbiol 47:1577–1588

Wei H, Vienken K, Weber R, Bunting S, Requena N, Fischer R (2004) A putative high affinity hexose transporter, *hxtA*, of *Aspergillus nidulans* is induced in vegetative hyphae upon starvation and in ascogenous hyphae during cleistothecium formation. Fungal Genet Biol 41:148–156

Widmann C, Gibson S, Jarpe MB, Johnson GL (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 79:143–180

Wilson RA, Chang PK, Dobrzyn A, Ntambi JM, Zarnowski R, Keller NP (2004) Two *Δ*9-stearic acid desaturases are required for *Aspergillus nidulans* growth and development. Fungal Genet Biol 41:501–509

Wösten HA, van Wetter MA, Lugones LG, van der Mei HC, Busscher HJ, Wessels JG (1999) How a fungus escapes the water to grow into the air. Curr Biol 9:85–88

Wu J, Miller BL (1997) *Aspergillus* asexual reproduction and sexual reproduction are differentially affected by transcriptional and translational mechanisms regulating *stunted* gene expression. Mol Cell Biol 17:6191–6120

Wu D, Dou X, Hashmi SB, Osmani SA (2004) The Pho80-like cyclin of *Aspergillus nidulans* regulates development independently of its role in phosphate acquisition. J Biol Chem 279:37693–37703

Xiang Q, Rasmussen C, Glass NL (2002) The *ham-2* locus, encoding a putative transmembrane protein, is required for hyphal fusion in *Neurospora crassa*. Genetics 160:169–180

Xu JR, Staiger CJ, Hamer JE (1998) Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA 95:12713–12718

Yamashiro CT, Ebbole DJ, Lee BU, Brown RE, Bourland C, Madi L, Yanofsky C (1996) Characterization of *rco-1* of *Neurospora crassa*, a pleiotropic gene affecting growth and development that encodes a homolog of Tup1 of *Saccharomyces cerevisiae*. Mol Cell Biol 16:6218–6228

Yang Q, Borkovich KA (1999) Mutational activation of a $G\alpha_i$ causes uncontrolled proliferation of aerial hyphae and increased sensitivity to heat and oxidative stress in *Neurospora crassa*. Genetics 151:107–117

Yang Q, Poole SI, Borkovich KA (2002) A G-protein β-subunit required for sexual and vegetative development and maintenance of normal Gα protein levels in *Neurospora crassa*. Eukaryot Cell 1:378–390

Yatzkan E, Yarden O (1999) The B regulatory subunit of protein phosphatase 2A is required for completion of macroconidiation and other developmental processes in *Neurospora crassa*. Mol Microbiol 31:197–209

Yoshida Y, Hasunuma K (2004) Reactive oxygen species affect photomorphogenesis in *Neurospora crassa*. J Biol Chem 279:6986–6993

Zhang L, Churchill ACL, Kazmierczak P, Kim DH, van Alfen NK (1993) Hypovirulence-associated traits induced by a mycovirus of *Cryphonectria parasitica* are mimicked by targeted inactivation of a host gene. Mol Cell Biol 13:7782–7792

Zhang L, Baasiri RA, van Alfen NK (1998) Viral repression of the fungal pheromone precursor gene expression. Mol Cell Biol 18:953–959

Zonneveld BJM (1972) Morphogenesis in *Aspergillus nidulans*. The significance of α-1,3-glucan of the cell wall and α-1,3-glucanase for cleistothecium development. Biochim Biophys Acta 273:174–187

17 The Mating Type Genes of the Basidiomycetes

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CONTENTS

I. Introduction

The basidiomycete fungi are a diverse group that includes the mushrooms, many of which are valuable crop species, the plant pathogenic smuts and rusts, and yeast-like species such as the saprophytic *Rhodosporidium toruloides* and *Cryptococcus neoformans*, an opportunist pathogen in humans. Understanding the mating systems of these fungi has applications in the design of breeding programmes for crop improvement and in the elucidation of pathogenicity determinants. Mating generally leads to a dramatic change in cellular morphology, and the mating type genes themselves are directly involved in bringing about these changes. Significantly, the genes encode members of protein families and signalling molecules that are ubiquitous in eukaryotic cells. Mating pathways thus present unique opportunities to study the functions of these proteins and to gain a general insight into how they regulate eukaryotic cellular development.

The function of the mating type genes is to impose barriers on self-mating and thereby promote outbreeding, which maintains variability within the population. In ascomycete fungi this is achieved by having two mating types determined by alternative forms of a mating type locus that have no DNA homology and contain genes encoding functionally different proteins (see Chap. 15, this volume). In the basidiomycetes, typically each mating type locus contains similar genes, and compatible mates are those that have different alleles of the same set of genes (reviewed by Casselton and Olesnicky 1998). The genes may be multiallelic, so that there can be many more than two versions of the mating type locus. There may be two mating type loci, each containing a different set of multiallelic genes. This enormous diversity, found particularly in mushroom species, can generate several thousands of different mating types. Although superficially complex, the underlying mechanisms that permit the recognition of compatible mating partners are highly conserved between ascomycetes and basidiomycetes, as we attempt to show here. The best described basidiomycete models are the homobasidiomycete mushrooms *Coprinus cinereus* (*Coprinopsis cinerea*, Redhead et al. 2001) and *Schizophyllum commune*, the hemibasidiomycete corn smut *Ustilago maydis* and *C. neoformans*. Conservation in genome organisation in homobasidiomycetes (Kües et al. 2001) and degenerate PCR cloning strategies now make it possible to isolate the genes from other species, and it is of considerable interest to evolutionary biologists to compare mating type gene sequences because they are considered to have evolved more rapidly than genes encoding conserved metabolic functions (May et al. 1999; James et al. 2004a).

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The Mycota I
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A. Breeding Systems

Historically, heterothallic basidiomycete fungi were classified as bipolar or tetrapolar, depending on whether mating type was determined by one or two loci. Where there is just a single locus in bipolar mushroom species, this was designated *A* and in tetrapolar species with two loci, these were designated *A* and *B* (see Raper 1966 for a historical account of the discovery of mating type systems, in particular the pioneering studies of Kniep and Bensaude). In most species, the two loci are unlinked. For compatibility, mates must have different alleles of all genes, and the names bipolar and tetrapolar derive from the fact that two mating types segregate at meiosis in the former, and four in the latter. The mating type loci were recognised as being complex long before this was confirmed by molecular analyses, and the terms *A* factor and *B* factor will be found in older literature. Hence, these breeding systems are also called unifactorial and bifactorial. Estimates of the frequencies of the two types of breeding systems in homobasidiomycetes are that 49%–65% are tetrapolar, 25% are bipolar and 10%–15% are homothallic (Whitehouse 1949; Quintanilha and Pinto-Lopes 1950). Because of the complex structure and high levels of sequence dissimilarity at the mating type loci, we no longer equate different versions of *A* and *B* with alleles, as assumed in the classical literature (Raper 1966), and the term specificity is now more generally used to describe their different versions. The more mating types there are, the greater the chance of finding a compatible partner – outbreeding efficiency is high; ten *A* specificities in bipolar species are sufficient to generate 90% outbreeding efficiency whereas it requires 20 specificities of both *A* and *B* to give the same potential in tetrapolar species (based on the calculation one gene $(nA-1)/nA$; two genes $(nAnB-nA-nB+1)/nAnB$, Koltin et al. 1972). The numbers of different specificities are surprisingly high in the species studied to date; for example, *S. commune* is estimated to have 288 *A* and 81 *B* specificities (generating more than 20,000 mating types; Raper 1966), *C. cinereus* 164 *A* and 79 *B* specificities (generating more than 12,000 mating types; Raper 1966), and *Pleurotus populinus* 126 *A* and 354 *B* specificities (generating nearly 45,000 mating types; James et al. 2004b). *U. maydis* is also a tetrapolar species, the mating type loci in this fungus are designated *a* and *b*, and there are just two alleles of the *a* locus and some 25 of the *b* locus (Christensen 1931; Rowell and de Vay 1954).

The genes found at the mating type loci of *U. maydis* and the tetrapolar homobasidiomycetes are highly conserved in function. At one locus, there are genes that encode transcription factors belonging to the homeodomain family, and at the other, genes encoding pheromones and receptors (see Sect. II.A). Locus designations predate a knowledge of the functions of the genes, and it is now rather unfortunate to discover that the *A* locus of homobasidiomycetes is equivalent to the *b* locus of *U. maydis*, and the *B* locus of homobasidiomycetes is equivalent to the *a* locus of *U. maydis*! In *C. neoformans*, the morphological changes that occur during mating are very similar to those occurring in homobasidiomycetes (Kwon Chung 1975) but remarkably, the organisation at the mating type locus is totally different (Lengeler et al. 2002; Hull et al. 2005). There are two versions of the locus, *MATa* and *MAT*α; both extend over more than 100 kb and contain some 20 genes, some are relevant to mating, others are not, and some encode enzymes normally associated with downstream mating events (see Sect. II.).

B. Developmental Pathways

To understand the role of the mating type genes, we need to look at the developmental pathways they regulate. Relevant stages in the life cycles of *U. maydis*, *C. cinereus* and *C. neoformans* are illustrated in Figs. 17.1, 17.2 and 17.3. Life cycles of these fungi have been reviewed by Banuett (1995), Kües (2000), and Hull and Heitman (2002) respectively. One feature of the basidiomycete life cycle that is crucial to understanding mating type gene function is the extended period between mating cell fusion and nuclear fusion. In the model species illustrated, mating cell fusion generates a specialised mycelium known as a dikaryon, in which the nuclei of both mates remain paired in each cell and divide in synchrony. In many basidiomycetes, the dikaryon has structures known as clamp connections, which we can see in both *C. cinereus* and *C. neoformans*, and these are formed every time the tip cell divides. The paired nuclei in each cell fuse only in cells that are specialised for meiosis.

U. maydis is a dimorphic fungus that is pathogenic on *Zea mays*. The asexual stage is saprophytic and composed of unicellular, yeast-like cells that divide by budding. When cells are mating, they secrete small pheromones that bind receptors on the surface of compatible mating partners. The

Ustilago maydis

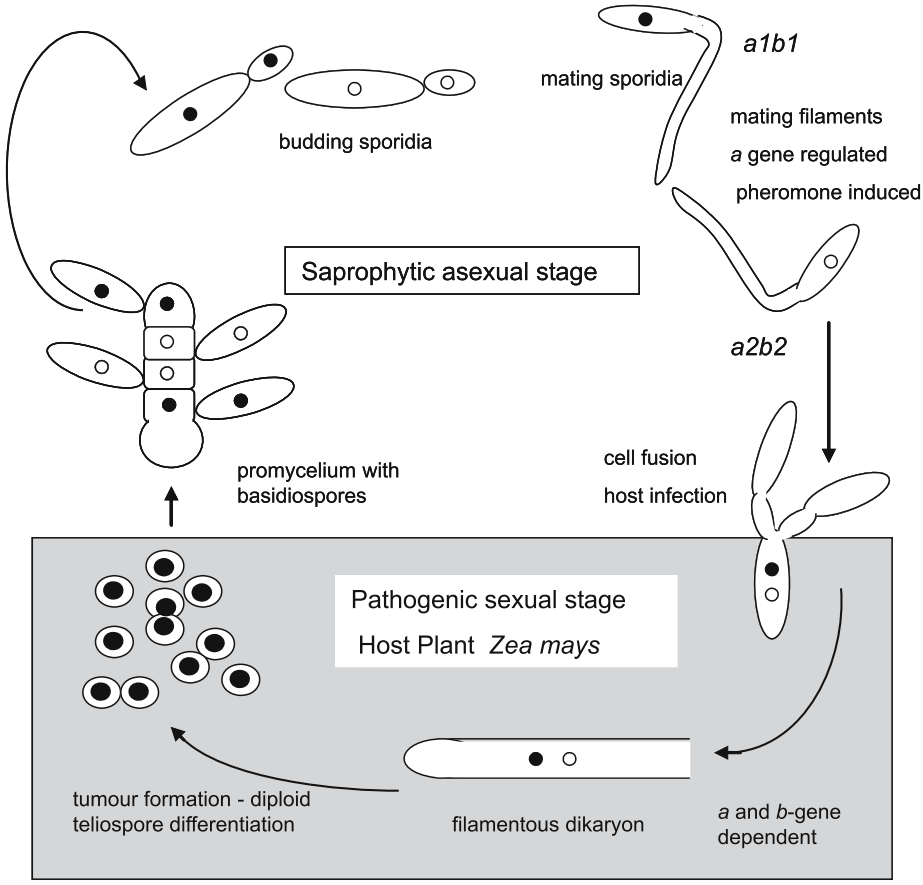

Fig. 17.1. Life cycle of the dimorphic plant pathogen *Ustilago maydis*

pheromone signal induces the formation of long, thin mating filaments that fuse at their tips. Once the cells have fused, there is a switch to filamentous mycelial growth and obligate pathogenicity. The filamentous dikaryotic mycelium invades the tissues of the host plant where it induces tumours, which are filled with black diploid teliospores, the smut from which the fungus takes its name. These germinate to give a promycelium that buds off basidiospores. Classical studies established that mating filament formation was dependent on compatible *a* genes whereas filamentous dikaryotic growth required compatible *b* genes (see Banuett 1995; Kahmann et al. 2000).

C. cinereus is a saprophytic fungus that has a typical mushroom life cycle. The roles of the mating type genes in the mating pathway were elucidated by Swiezynski and Day (1960). In all homobasidiomycetes, hyphal fusion is sufficient to initiate mating. There is no evidence that pheromones are secreted into the environment to attract mates; unlike *U. maydis* and *C. neoformans*, cell fusion is mating type-independent in *C. cinereus*, and pheromone signalling is activated only after cells have fused (Olesnicky et al. 1999). The asexual stage in *C. cinereus* is known as the monokaryon (homokaryon) because the cells are uninucleate. Provided mates have different alleles of the *B* genes, following cell fusion there is an exchange of nuclei and extensive migration of the donor nucleus through the established cells of each recipient monokaryon. Nuclear migration involves major restructuring within the cell; the complex dolipore septa separating the cells in the hyphae normally preclude movement of organelles but these are dissolved away, so that nuclear movement is facilitated (Giesy and Day 1965). Once the tip cells have both nuclei, this triggers a complex division leading to the formation of the clamp connection. The clamp cell forms on the side of the

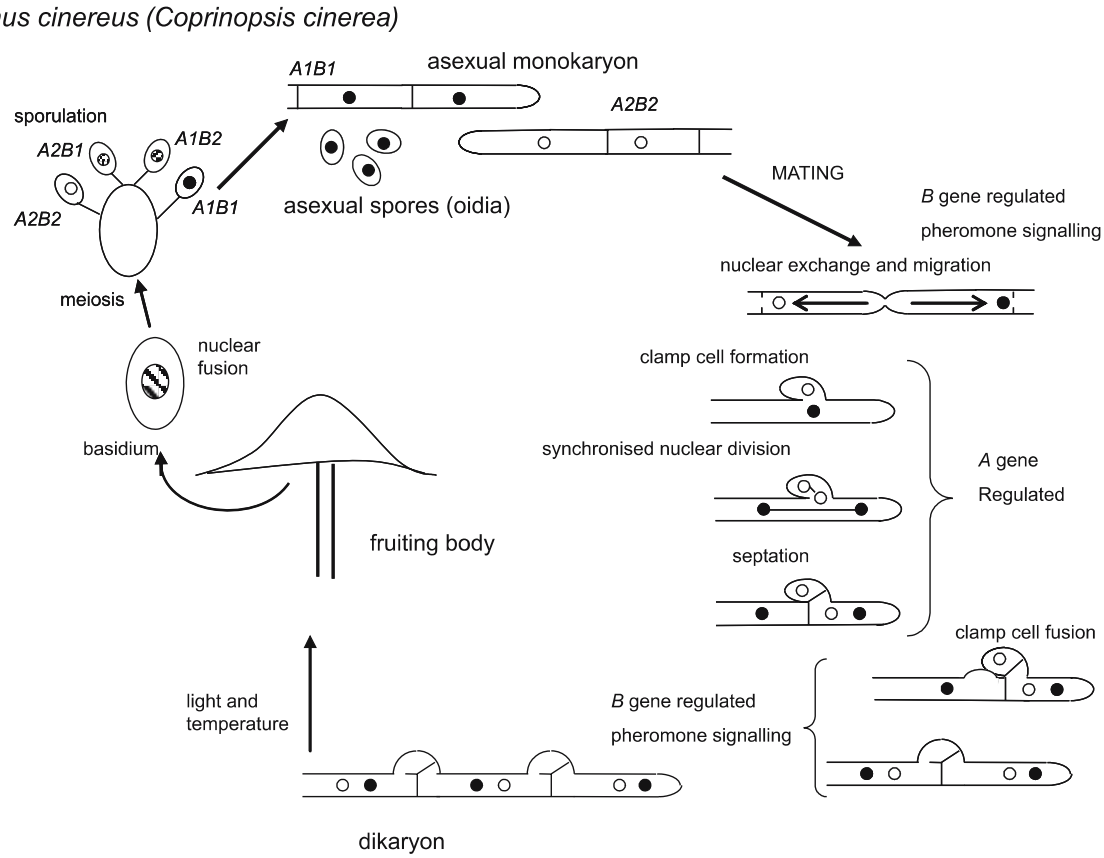

Fig. 17.2. Life cycle of the saprophytic mushroom *Coprinus cinereus* (*Coprinopsis cinerea*)

tip cell, and both nuclei take up positions adjacent to the entrance to this cell, one inside and one in the main cell (Iwasa et al. 1998). Both nuclei divide in synchrony; new cell walls are laid down, creating three cells, a dikaryotic tip cell, and uninucleate clamp and subterminal cells. The clamp cell grows backwards to fuse with the subterminal cell, and the clamp cell nucleus is released to join its partner. An observation of Buller (1931), of which we are reminded by Badalyan et al. (2004), is that the subterminal cell produces a projection that grows towards the clamp cell tip and fuses with it. This is reminiscent of the mating projections produced by mating *Saccharomyces cerevisiae* cells in response to pheromone stimulation and, since clamp cell fusion is a *B*-dependent step, it is assumed to require pheromone signalling (Brown and Casselton 2001). As well as being necessary for clamp cell fusion, nuclear migration following the initial mating cell fusion is also *B* gene- and, thus, pheromone-dependent. Formation of the clamp cell and synchronised nuclear division are *A* gene-dependent (Sweizynski and Day 1960).

C. neoformans has a more complex life cycle than do *U. maydis* and *C. cinereus*, in that there are haploid, diploid and dikaryotic developmental pathways (see Hull and Heitman 2002). Like *U. maydis*, it is a dimorphic fungus and can exist as yeast-like cells that divide by budding, or as filaments. The dikaryon of this fungus is not the pathogenic stage – it is the yeast form that infects humans and causes meningoencephalitis and, interestingly, it is the α mating type cells that are the most virulent and occur more frequently in the population (Kwon-Chung and Bennett 1978; McClelland et al. 2004). The yeast-like *MATa* and *MAT*α cells can mate; pheromone signalling between mates induces *MAT*α cells to form conjugation tubes. *MATa* cells do not form conjugation tubes but may become enlarged in some strains (Moore and Edman 1993; Davidson et al. 2000; McClelland et al. 2004; Heitman, personal communication). Cell fusion gives rise to a dikaryon with binucleate cells and fused clamp connections, similar to those we see in *C. cinereus*.

Cryptococcus neoformans

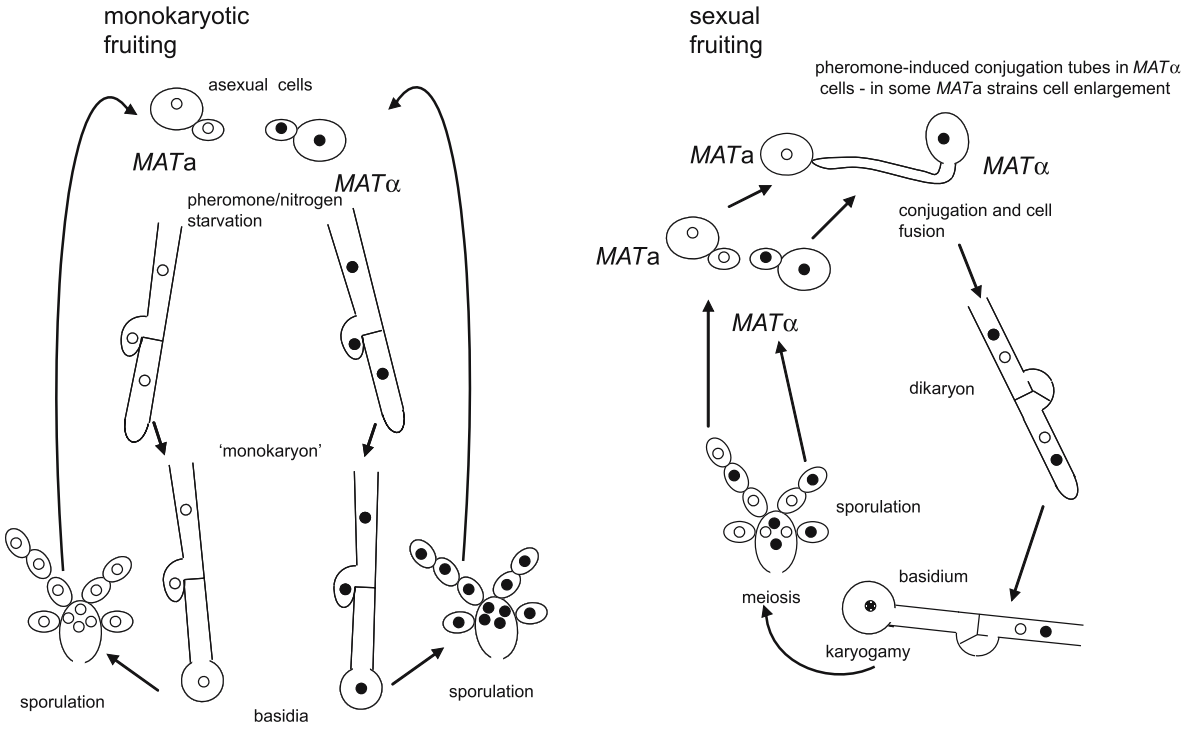

Fig. 17.3. Life cycle of the dimorphic human pathogen *Cryptococcus neoformans*, based on Kwon-Chung (personal communication). The filamentous phase is also known as *Filobasidiella neoformans* (Kwon-Chung 1975)

The tip cells of these filaments swell to form the basidia in which meiosis occurs (Kwon-Chung 1975).

A second developmental pathway occurs in both *MAT*α and *MATa* cells under conditions of desiccation and nitrogen limitation (Wickes et al. 1966; Tscharke et al. 2003), which is asexual and known as monokaryotic or haploid fruiting (Wickes et al. 1966). Cells of either mating type produce filaments, which typically produce branch-like projections at each septum. According to Kwon-Chung (personal communication), these projections are nucleate and appear to be potential branches, as shown in Fig. 17.3, but other authors maintain they are anucleate, and refer to them as unfused clamp cells (see Hull and Heitman 2002). Haploid fruiting in both mating types is stimulated by pheromone signalling, suggesting that it may have a role in promoting mate fusion (Wang et al. 2000). Occasionally, mating gives rise to diploid cells that are thermally dimorphic – at 37 °C the cells are yeast-like but at 25 °C they are filamentous and resemble the haploid filaments produced by *MAT*α and *MATa* cells (Sia et al. 2000). All mycelial filaments differentiate basidia as swellings of the hyphal tip cells. In matings, the basidium is the cell in which meiosis occurs; the four nuclei produced by meiosis remain in the basidium, and nuclei from subsequent mitotic divisions are incorporated into chains of up to 40 basidiospores that are a mixture of *MAT*α and *MATa* mating types (Kwon-Chung 1980). In haploid fruiting, the spores produced are all of one mating type and, until recently, it was assumed that these were derived from entirely mitotic events. However, Lin et al. (2005) have now shown that both diploidisation and meiosis may occur during monokaryotic fruiting in *MAT*α strains. Significantly, different *MAT*α cells can fuse, so that this so-called monokaryotic pathway can lead to genetic recombination between individuals of the same mating type, something that the authors emphasise as being particularly important, in view of the greater virulence of

*MAT*α cells and their near-clonal distribution in the wild.

II. Molecular Analysis of Mating Type Genes

The organisation of the mating type loci of *U. maydis*, *C. cinereus*, *S. commune* and *C. neoformans* are illustrated in Figs. 17.4, 17.5 and 17.6.

A. Tetrapolar Species

1. The *b* and *A* Genes Encode a Transcription Factor

The genes at the *b* and *A* loci of *U. maydis* and *C. cinereus* encode two dissimilar proteins, each characterised by a homeodomain DNA-binding motif (Fig. 17.4). The genes and proteins belong to two subfamilies that have been termed HD1 and HD2 on the basis of conserved but distinctly different homeodomain sequences. These genes/proteins are, in fact, the homologues of the **a**1 and α2 mating type genes/proteins of *S. cerevisiae* (Kües and Casselton 1992). In *S. cerevisiae*, the two classes of proteins are encoded by genes in different haploid cells, and it is only after cell fusion that they are present together in the resulting diploid cell, and they then heterodimerise to form a diploid cell-specific transcription factor (Herskowitz 1988). In the basidiomycetes, by contrast, every cell produces an HD1 and an HD2 protein. The *b* locus of *U. maydis* spans some 4 kb and contains a single pair of genes, *bW* and *bE*, that are divergently transcribed from a common promoter sequence (Gillissen et al. 1992). The *HD1* gene (*bE*) corresponds to α2 and the *HD2* gene (*bW*) corresponds to **a**1 (Gillissen et al. 1992). Like **a**1 and α2, a compatible cell fusion leads to heterodimerisation between an HD1 and an HD2 protein, to generate a transcription factor complex unique to mated cells – in this case, the dikaryon. Self-mating is prevented because the genes found together at the *b* locus (e.g. *bW1-bE1*) encode proteins that are unable to heterodimerise. Heterodimerisation is possible

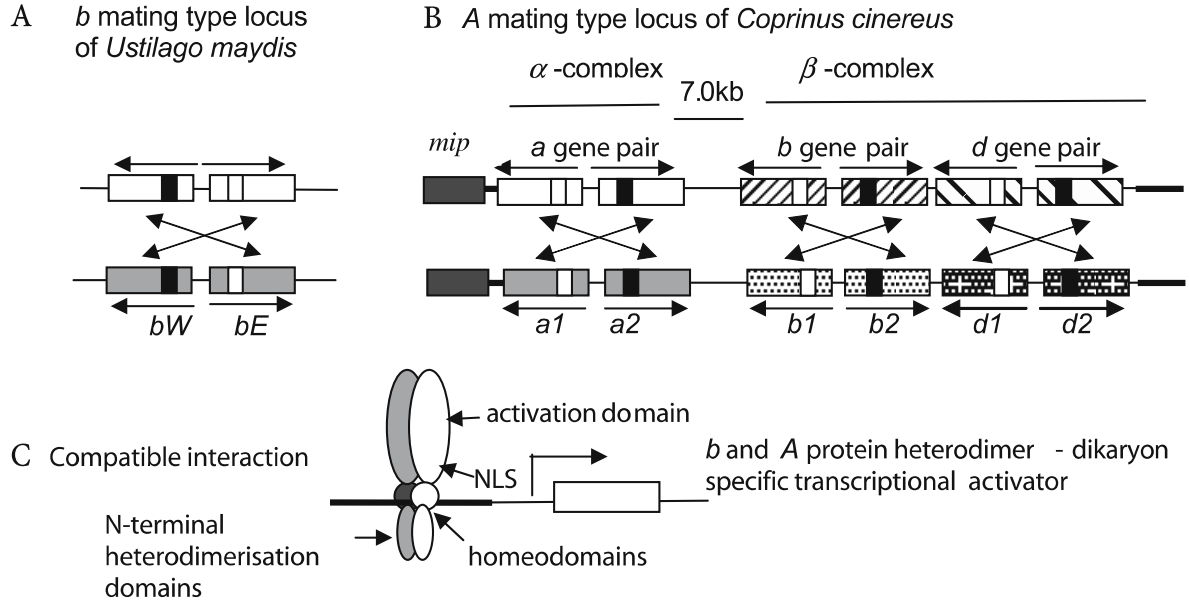

Fig. 17.4.A–C Organisation of the homeodomain transcription factor genes at the **A** *b* and **B** *A* mating type loci of *Ustilago maydis* and *Coprinus cinereus*. **C** Hypothetical heterodimer that results from a compatible protein interaction on mating. The HD1 and HD2 homeodomain-encoding sequences within the genes are represented by *open* and *closed boxes* respectively. Different alleles of the paired genes are represented by different *fill motifs*. *Horizontal arrows* indicate direction of transcription whereas *diagonal arrows* indicate compatible gene combinations that lead to heterodimerisation of the corresponding proteins. The *A* locus is flanked by the *mip* gene encoding an intermediate mitochondrial endopeptidase that is highly conserved in position in other homobasidiomycete species (Kües et al. 2001; James et al. 2004a)

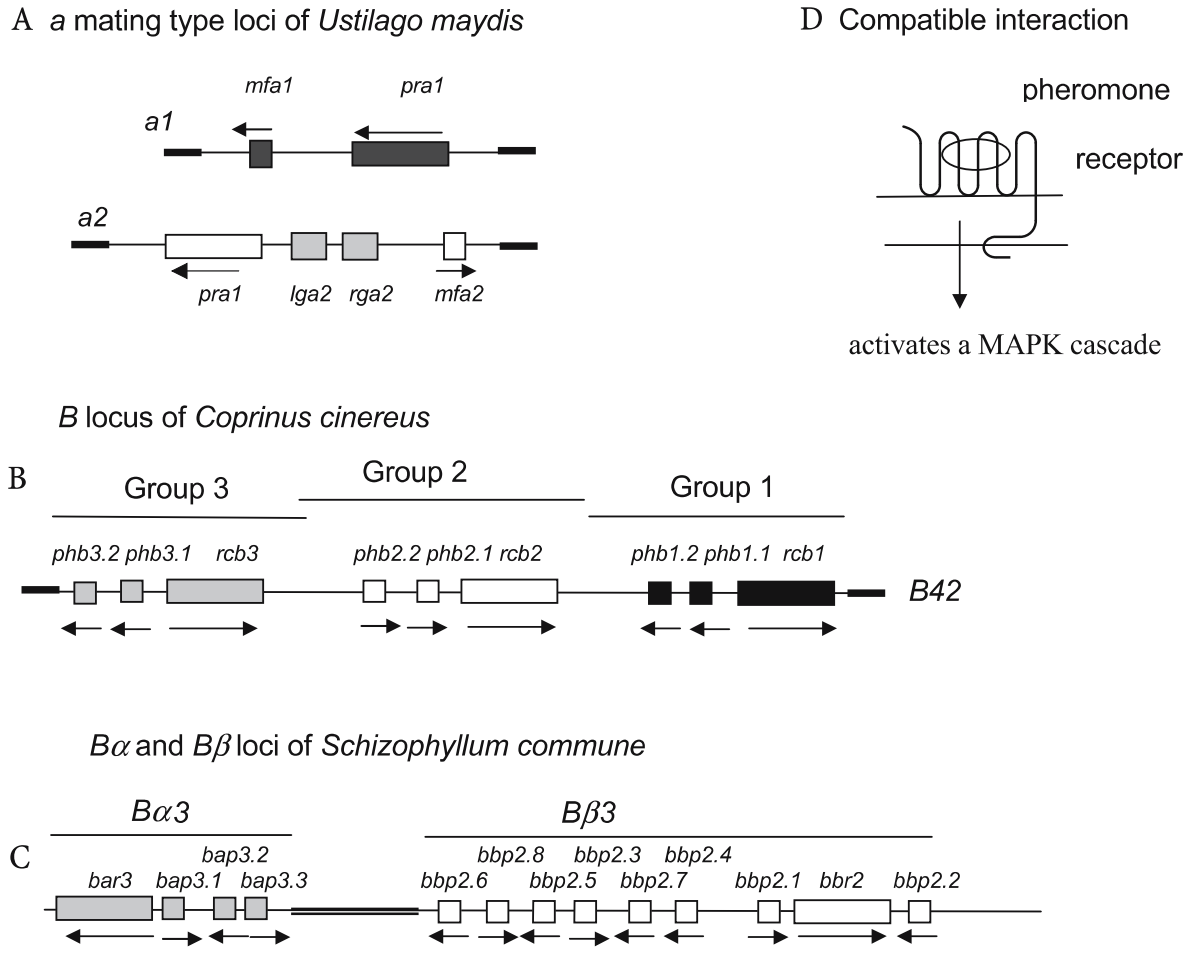

Fig. 17.5.A–D Organisation of the pheromone and receptor genes at the *a* and *B* mating type loci of **A** *Ustilago maydis*, **B** *Coprinus cinereus* and **C** *Schizophyllum commune*. The receptor genes are designated *pra1* and *pra 2* in *U. maydis*, *rcb1*, *rcb2* and *rcb3* in *C. cinereus*, and *bar* and *bbr* in *S. commune*. The prefix *mfa* is used to denote the pheromone genes of *U. maydis*, *phb* in *C. cinereus*, and either *bap* or *bbp* in *S. commune*. Different *fill motifs* indicate different alleles of the genes in *U. maydis* or paralogous sets of genes in *C. cinereus* and *S. commune*. A *double line* separating the *B*α and *B*β loci of *S. commune* indicates a region of unusual DNA sequence that suppresses recombination. **D** Consequence of a compatible combination of pheromones and receptors during mating

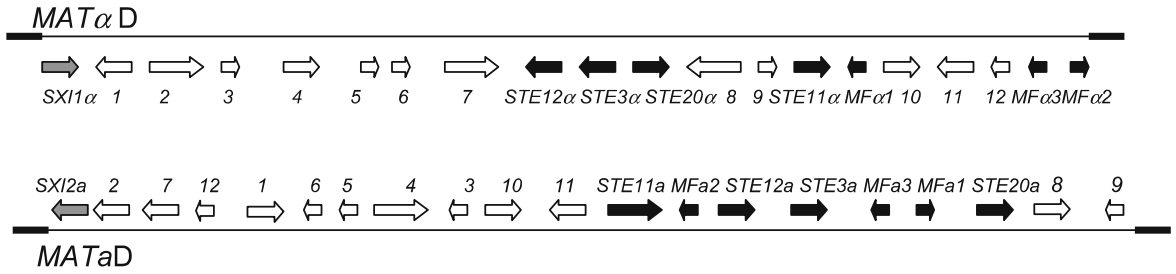

Fig. 17.6. Alternative forms of the mating type locus of *Cryptococcus neoformans* serotype D. Genes and their orientation are indicated by *arrows*. Genes encoding mating pheromones and receptors and elements of the MAPK pathway are shown in *bold* and homeodomain protein-encoding genes as *shaded*. *Numbers* are used to indicate other genes present at *MAT*; all genes are present in both loci but the alleles vary in sequence, order and orientation (based on Hull et al. 2005)

only between proteins encoded by different alleles of the genes. In any one mating in *U. maydis* (e.g. *bW1-bE1*×*bW2-bE2*), two functionally equivalent heterodimers form (bW1/bE2 and bW2/bE1; Kämper et al. 1995). The 5′ regions of the genes are highly variable, as is the promoter region, so a compatible gene combination cannot be generated by recombination. Such combinations can be made experimentally, and are sufficient without further mating to activate *b*-regulated development (Gillissen et al. 1992).

In *C. cinereus*, we see the same pairs of *HD1/HD2* genes, but the locus now extends over some 25 kb and contains representative members of three different pairs of genes (designated *a*, *b* and *d*), all of which are multiallelic (Kües et al. 1992; Pardo et al. 1996). These three groups of genes have clearly arisen by duplication but are now functionally independent (i.e. they are paralogous). Mating partners are compatible provided they have different alleles of just one pair of genes. Because of the redundancy in function, it is common to find that few *A* loci contain all six genes – only one such locus was identified amongst nine investigated (Pardo et al. 1996). The *A5* and *A3* loci, for example, have only one gene of the *d* pair, *A3* has the *HD1* gene, and *A5* has the *HD2* gene; these encode compatible proteins that can heterodimerise in *A*3 × *A*5 matings. There are inactive pseudogenes in some loci; the first locus sequenced, *A42*, contained an additional *HD1* gene which was thought to represent a *c* gene pair (Kües et al. 1992) – hence, the designation *d* for the third gene pair! If mating partners contained different alleles of all three gene pairs, six different but functionally equivalent heterodimers would be formed. Locus organisation is maintained because the DNA sequences that comprise allelic versions of the genes (both coding and flanking sequence) are sufficiently different to prevent homologous recombination between alleles and, thus, bring compatible combinations of genes together. During evolution of this complex, there has been random mixing of the three groups of genes to generate many different allele combinations (May and Matzke 1995; Pardo et al. 1996). Large numbers of *A* mating specificities can be generated by having relatively few alleles of the three groups of genes. A population analysis of the *C. cinereus A* locus identified four alleles of the *a* genes, ten of the *b* genes and three of the *d* genes, sufficient to generate 120 genetically different *A* loci (May and Matzke 1995). Recombination between the different groups of genes at the *C. cinereus A* locus can still occur with a frequency of 0.07% (Day 1960, 1963b), which is due to a short 7.0-kb sequence of homology that exists between the *a* and the *b* gene pairs in all versions of the *A* locus (Kües et al. 1992; May and Matzke 1995). Recombination has the beneficial effect of generating non-parental *A* mating specificities but the disadvantage that these are fully compatible with all other sibs from the mating. Increasing sib-compatibility reduces the efficiency of an outbreeding system, so evolutionary pressure will have acted to maintain close linkage of the gene pairs. The *a* gene pair in the *C. cinereus* locus corresponds to the *A*α locus (hence, α-complex in Fig. 17.4), and the *b* and *d* genes to the *A*β locus (hence, β-complex in Fig. 17.4) described by Day (1960, 1963b). In *S. commune*, where recombination between *A* genes was first described (see Raper 1966), the two groups of genes are separated at much greater distance (1-17cM), depending on the strains (Raper et al. 1960), and the *A*α and *A*β genes clearly reside at two distinct loci. The *A*α locus contains a single pair of *HD1/HD2* genes for which there are nine alleles (Stankis et al. 1992). As yet, only a single gene of the *A*β complex has been identified (Shen et al. 1996) but there are a predicted 32 *A*β specificities (Raper 1966), and it is likely that at least two pairs of genes would be needed to generate this number.

2. Homeodomain Protein Interactions

An important property of the homeodomain proteins encoded by the *HD1* and *HD2* genes is their ability to discriminate between large numbers of potential dimerisation partners. With 25 alleles of the *b* genes of *U. maydis*, we can calculate that there are potentially 625 heterodimer interactions; 25 are self-interactions that are incompatible whereas the remaining 600 are predicted to be possible and all equally capable of activating *b*-regulated development. In *C. cinereus*, there are many more incompatible interactions because proteins encoded by paralogous genes are also unable to heterodimerise (Pardo et al. 1996). Studies with *U. maydis* b proteins and *C. cinereus* and *S. commune* A proteins have shown that specificity resides in the N-terminal domains; exchanges between these domains generated altered specificities, and these domains are sufficient to mediate protein–protein dimerisation in vitro (Banham et al. 1995; Kämper et al. 1995; Magae et al. 1995). The N-terminal

domains of the HD1 proteins are predicted to contain coiled-coil α-helices that mediate protein dimerisation in other transcription factors. Two such domains were predicted in the *C. cinereus* proteins and, significantly, the relative positions of these were different in proteins coded by paralogous *a*, *b* and *d* genes (Banham et al. 1995). For the *U. maydis* proteins, it was shown that single amino acid substitutions were sufficient to convert a normally incompatible protein pair into a pair that could dimerise and, significantly, these substitutions caused either an increase in hydrophobicity or a change in charge, both consistent with changes affecting coiled-coil interactions (Kämper et al. 1995).

Heterodimerisation plays an important role in regulating transcription factor function. Studies on DNA binding by the *S. cerevisiae* a1/α2 heterodimer illustrated the importance of dimerisation in determining DNA-binding specificity (Johnson 1995). In *C. cinereus*, it has been shown that the HD1 protein provides the likely activation domain and the nuclear targeting sequences, but has a dispensable DNA-binding domain whereas the HD2 protein provides the essential DNA-binding domain (Asante-Owusu et al. 1996; Spit et al. 1998). Here, separation of functional domains into two proteins represents an elegant strategy to ensure that mating-dependent developmental pathways are activated only after fusion between compatible mates.

3. The *a* and *B* Genes Encode Pheromones and Receptors

Pheromone signalling plays an essential role in mating in both ascomycete and basidiomycete fungi (see also Chap. 16, this volume) but it is only in the latter that the genes have become mating type determinants, and only in homobasdiomycetes are these genes multiallelic. The two alleles of the *a* locus of *U. maydis*, first described by Bölker et al. (1992), consist of very dissimilar DNA sequences bordered by regions of homology, and each contains two genes that encode a mating type-specific pheromone and the corresponding receptor. The *a1* locus spans 4.5 kb whereas the *a2* locus spans more than 8.0 kb. The pheromones are unable to activate the receptors with which they are found, and the dissimilarity in sequence throughout the two different versions of this locus ensures that recombination cannot generate compatible receptor–pheromone combinations. (There are two additional genes, *lga1* and *rga1*, in the *a2* locus that encode mitochondrial functions activated by mating but not relevant to it; Bölker et al. 1992; Bortfeld et al. 2004.)

In *C. cinereus*, the corresponding *B* locus spans some 17 kb and is far more complex than the *U. maydis a* locus (O'Shea et al. 1998; Halsall et al. 2000). As at the *A* locus, we find three tandemly arranged groups of genes that are functionally redundant. These have been designated groups 1, 2 and 3. In the *B42* locus illustrated in Fig. 17.5, each group comprises a receptor gene and two pheromone genes but, in other loci, pheromone gene numbers range from 1 to 3 and the orders of the genes and their orientation are variable (Riquelme et al. 2005). As with the *A* genes, locus integrity is maintained by the dissimilarity in sequence between allelic versions of the genes and the flanking sequence in which they are embedded, so that recombination cannot bring together compatible gene combinations. Mating partners are compatible if they bring together different alleles of just one group of genes, these genes encoding a compatible complement of pheromones and receptors (O'Shea et al. 1998). As with the *A* locus, evidence points to large numbers of *B* specificities being generated by the different allele combinations of these three sets of genes. Molecular characterisation of 13 *B* specificities in *C. cinereus* has identified sufficient alleles to generate 70 unique combinations and, hence, different *B* specificities, close to the 79 predicted by population studies (Riquelme et al. 2005). It is not clear why there are so many pheromone genes in *C. cinereus*; few show any difference in specificity, and though they cannot activate a self-receptor, most appear to activate all other receptors within the same group, i.e. group 1 pheromones activate group 1 receptors, group 2 pheromones activate group 2 receptors and group 3 pheromones activate group 3 receptors (Riquelme et al. 2005).

In *S. commune*, the pheromone and receptor genes are separated into two groups that correspond to the *B*α and *B*β loci identified in classical recombination analyses, when it was established that there are nine alleles of each locus (Raper et al. 1958). *B*α and *B*β have each been shown to contain a receptor gene (*bar* and *bbr*) and a variable number of pheromone genes (*bap* and *bbp*; Wendland et al. 1995; Vaillancourt et al. 1997; Fowler et al. 2004). Not all crosses between *B*α and *B*β specificities, however, yielded recombinants (Stamberg and Koltin 1971). Molecular analysis of the *B*α3–*B*β2 complex illustrated in Fig. 17.5 provided the answer to this puzzle and a surprising twist to our

understanding of the ways in which multiple *B* mating specificities have evolved (Fowler et al. 2004). In this fungus, the two groups of genes at the *B*α and *B*β loci are not entirely functionally independent. *B*α3 and *B*β2 are closely linked but separated by an unusual DNA sequence that suppresses recombination. There are 11 pheromone genes in this complex, three in the *B*α complex and eight in the *B*β complex, and together these can activate all the receptors in the eight other variants of *B*α and *B*β. Each pheromone has a distinct specificity and can activate only some of the 18 receptors but, remarkably, five of these pheromonesxs were found to activate *B*α and *B*β receptors (Fowler et al. 2004). *S. commune* and *C. cinereus* appear to have evolved different strategies for creating large numbers of *B* mating specificities, both equally efficient.

4. The Pheromones and Receptors

In ascomycetes, the mating type-specific pheromones belong to two distinct subfamilies, typified by *S. cerevisiae* α-factor and **a**-factor (see Chap. 16, this volume). These pheromones have different precursors and are secreted by different pathways. All basidiomycete pheromone genes identified to date are predicted to encode lipopeptides belonging to the **a**-factor family (Vaillancourt and Raper 1996). The active peptides are some 9–15 amino acids long and are derived from longer precursor molecules that have a C-terminal CaaX motif, a signal for C-terminal truncation, and modification of the terminal cysteine residue by carboxymethylation and farnesylation. The sequences of the mature pheromones of *U. maydis* were obtained by sequencing purified peptides (Spellig et al. 1994). As far as is known, homobasidiomycete pheromones are not secreted into the surrounding environment. Thus, there is no way at present to purify them. The mature peptide sequence has, however, been predicted by comparing the sequences of several different precursors (Casselton and Olesnicky 1998). If one aligns the precursor sequences of the *C. cinereus* and *S. commune* pheromones, they are remarkably variable but there is a conserved glutamic/arginine (ER) or aspartic/arginine (DR) motif 12–15 amino acids upstream of the CaaX motif in most sequences, which suggests a likely N-terminal processing site. The pheromone receptors of basidiomycetes belong, as do all pheromone receptors of ascomycete and basidiomycete fungi, to the G-protein-coupled (GPCR) family with seven transmembrane-spanning domains. The basidiomycete receptors are activated by **a**-factor-like pheromones and, not surprisingly, all are closely related to the *S. cerevisiae* **a**-factor receptor Ste3p (http://www.gpcr.org/7tm/).

The *C. cinereus* and *S. commune* pheromones and receptors have been expressed heterologously in *S. cerevisiae* (Fowler et al. 1999; Olesnicky et al. 1999, 2000). Yeast strains have been developed in which the intracellular pheromone signalling pathway is engineered to link receptor activation to reporter gene expression. The reporter gene may be *HIS3* which, when expressed in response to pheromone stimulation, permits *his3* mutants to grow without histidine. For quantitative assays, the reporter gene is the bacterial gene encoding β-galactosidase. When *C. cinereus* or *S. commune* receptor and pheromone genes are expressed in such cells, a pheromone species is secreted that can activate the compatible mushroom receptor expressed on the surface of the yeast cell. Significantly in experiments with *C. cinereus* genes, pheromone secretion occurred only in *MAT***a** cells that express **a**-factor processing enzymes and the **a**-factor secretion pathway (Olesnicky et al. 1999). It is remarkable that *S. cerevisae* can process the mushroom pheromone precursors, because they show no sequence similarity to the **a**-factor precursor. It suggests, however, that the processing and secretion pathways are highly conserved in ascomycetes and basidiomycetes, and that the enzyme machinery recognises secondary structural features of the precursor molecules. Using the yeast assay, synthetic peptides have been tested for their ability to activate a receptor, thus making it possible to test predictions as to the likely native structures of the *C. cinereus* pheromones. These are farnesylated peptides with the conserved ER motif at the N terminus (Olesnicky et al. 1999).

The pheromones and receptors of the homobasidiomycetes are truly remarkable in the specificity they display. A single receptor may be activated by more than one pheromone, and a single pheromone can activate several different receptors. Current interest is in where the specificity determinants lie within these molecules, and how these large families of proteins and peptides evolved.

B. Bipolar Species

Bipolar species have just a single mating type locus. Few species have been investigated at the molecu-

lar level but two patterns emerge, one exemplified by *Ustilago hordei* and the other by *Coprinus* (*Coprinellus*) *disseminatus*. In *U. hordei*, a close relative of *U. maydis*, there are only two mating types (*MAT1* and *MAT2*). The alternative loci each contain genes found at the *a* and *b* loci of *U. maydis*, a pair of *bW/bE* genes, and a pheromone and receptor gene. There are only two alleles of the *a* and *b* genes but different allelic versions of these are necessary for a compatible mating, in the same way as they are in *U. maydis* (Bakkeren and Kronstad 1993, 1994; Anderson et al. 1999). A bipolar mating behaviour arises because the *a* and *b* genes of *U. hordei* are linked on the same chromosome. Remarkably, the genes are separated by 430–500 kb of non-mating type-specific sequence but recombination suppression means that only two allele combinations exist in nature (Lee et al. 1999).

In the homobasidiomycetes, bipolar species are found in all phylogenies, indicating that these have arisen independently many times (James et al. 2003). Unlike smuts, however, there is no evidence that the two classes of mating type genes have become linked. It appears that the *A* locus is sufficient to confer mating type identity, and the *B* genes have lost their role as mating type determinants. Two pairs of closely linked *HD1/HD2* genes have been found in *C. disseminatus* (James, personal communication) and a single pair in the commercial mushroom *Agaricus bisporus* (Li et al. 2004). Receptor and pheromone genes have been found in *C. disseminatus* but these are not linked to the *A* locus, and they are not polymorphic (James et al. 2003). It is unlikely that pheromone signalling is dispensable in bipolar mushrooms, because the MAPK pathway that it activates plays such an essential role in dikaryosis. Indeed, nuclear migration occurs during mating in *C. disseminatus*, and the dikaryon has fused clamp connections (James, personal communication), developmental steps that are pheromone-dependent in tetrapolar species. A pheromone or receptor gene mutation or a recombination event could bring a compatible receptor/pheromone gene combination together, and would replace the need for mating to do this. A compatible combination of genes might constitutively activate the MAPK pathway; alternatively, the receptor and pheromone genes may be induced only once a compatible *A* gene interaction is established. The latter is more likely, since mutation studies (described below) show that a constitutive pheromone response can severely disadvantage growth of the monokaryon.

C. Homothallic Species

In ascomycete fungi, several homothallic species have been looked at and found to contain functional mating type genes. In some species, the genes that are normally found in the alternative forms of the *MAT* locus are combined at a single, complex mating type locus, as in *Sordaria macrospora* (Jacobsen et al. 2002; see Chap. 15, this volume). In some cases, compatible genes have become fused and encode chimeric proteins that are effective in promoting sexual development (reviewed by Turgeon 1998). It is not surprising that mating type genes are essential for sexual development in homothallic species, since we know that they are required to activate the genes that bring about the same developmental programme in heterothallic species. At present, we know little about homothallic basidiomycetes but preliminary studies with *Agaricus* species indicate that the *A* mating type genes at least are present (Burrow, Casselton and Challen, unpublished data). A scan of the recently published *Phanerochaete chrysosporium* genome sequence identified five potential pheromone receptor genes and an unlinked pair of *HD1-HD2* genes (Martinez et al. 2004). Assuming this species is homothallic, as originally reported (Alic et al. 1987), the finding of all the mating type genes would confirm our predictions that they are still essential for sexual development. However, other authors suggest that this species is heterothallic and has a bipolar mating system (James et al. 2003).

Mutations that lead to self-compatibility have long been known in *S. commune* and *C. cinereus*, and one could ask whether these might give clues to the origin of homothallism. Strong selection was needed to recover the mutants; incompatible matings between monokaryons with the same *A* specificities or the same *B* specificities were used to select for rare mutations that would convert these mycelia into fertile dikaryons (Parag 1962; Raper et al. 1965; Day 1963a; Haylock et al. 1980). The mutations obtained were predominantly dominant and mapped within the *A* or the *B* loci. Mutants characteristically showed constitutive expression of the pathway normally induced by a compatible combination of the corresponding non-mutant genes. *A* mutants produce mycelia with unfused clamp connections and *B* mutants, at least in *S. commune*, produce mycelia in which nuclear migration is actively occurring (see Raper 1966). Mutant *B* strains in *S. commune* are slow growing and lack aerial mycelium, a phenotype known as 'flat'

(see Raper 1966). In *C. cinereus*, in some but not all backgrounds, such phenotype is induced in transformants carrying extra *B* genes (Kües et al. 2002). Strains with mutations at both the *A* and *B* loci are constitutive for the entire mating pathway; they resemble in most respects true dikaryons, and produce fertile fruiting bodies in which meiosis is normal (Swamy et al. 1984). These strains have proved invaluable in many genetic selection programmes because they permit the selection of recessive mutations that affect all stages of development, from dikaryosis to sporulation, and which would not be detectable in normal matings with two genetically different nuclei (Granado et al. 1997; Cummings et al. 1999; Inada et al. 2001; Kamada 2002).

Molecular analysis of two *A* mutants of *C. cinereus* revealed that there had been a major deletion within the *A* locus, such that only a single gene remained, and this was a chimeric gene consisting of the major part of an *HD2* gene fused to the 3′ end of an *HD1* gene (Kües et al. 1994; Pardo et al. 1996). Such fusion genes are sufficient to constitutively activate the clamp cell pathway in the mutant and in any other background into which the gene is introduced (Kües et al. 1994). As mentioned above, heterodimerisation brings together different functional domains of an active transcription factor, the chimeric *A* gene is predicted to encode a minimal heterodimer that has the essential HD2 homeodomain for DNA binding and sufficient of the HD1 protein to provide the nuclear targeting sequences and the activation domain (Asante-Owusu et al. 1996). Self-compatible mutations in the *B* genes may occur in either the receptor genes or the pheromone genes and, in either case, lead to constitutive activation of the pheromone-dependent pathway, nuclear migration and clamp cell fusion. Receptor gene mutations may result in constitutive activation of the receptor or altered specificity towards a normally incompatible pheromone whereas pheromone mutations alter receptor specificity (Olesnicky et al. 1999, 2000; Fowler et al. 2001).

D. *Cryptococcus neoformans* Mating Type Locus

The two versions of the *C. neoformans* mating type locus are totally dissimilar to the basidiomycete loci described so far. Each locus extends over some 100 kb (Lengeler et al. 2002; Hull et al. 2005). The alternative loci of serotype D strains are illustrated diagrammatically in Fig. 17.6. As can be seen, within these sequences there are mating type-specific genes encoding the pheromone receptors (*STE3a* and *STE3*α) and the corresponding pheromones (*MF*α*1*, *MF*α*2* and *MF*α*3* in the *MAT*α locus and *MFa1*, *MFa2* and *MFa3* in the *MATa* locus). Pheromone signalling is important in mating and, as shown in Fig. 17.3, it induces mating structures in mating cells in the same way as it does in *U. maydis*. Once the mating cells have fused, and by analogy with homobasidiomycetes, pheromone signalling is expected to be important in maintaining the dikaryophase, particularly clamp cell fusion. Based on our understanding of mating in homobasidiomycetes, one would expect that heterodimerisation between an HD1 and an HD2 protein would be required for mate recognition and sexual development in *C. neoformans*. This is indeed so. A gene encoding an HD1 protein (SXI1α) resides at the border of the *MAT*α locus (Hull et al. 2002), and a gene encoding an HD2 protein (SXI2a) resides at the corresponding position of the *MATa* locus (Hull et al. 2005). As in *S. cerevisiae*, mating is necessary to bring this compatible pair of proteins together. Sxi1α has been implicated in a/α-cell identity (Hull et al. 2002), and a direct interaction between the proteins (as determined by a two-hybrid analysis) is required for sexual development (Hull et al. 2005).

In addition to the pheromone and receptor genes and the *HD1* and *HD2* genes, the *MAT* loci contain mating type-specific genes encoding elements of the MAPK phosphorylation pathway, Ste20p, the first kinase in the pathway, Ste11p, the first kinase in the MAPK module (MAPKKK) and the homologue of the *S. cerevisiae* pheromone response pathway target transcription factor, Ste12p. There are many other genes within these extended loci that have no obvious role in mating; the different alleles of these genes are variable in position, sequence and orientation, variability which, together with transposons and remnants of transposons, acts to generate extended regions of homologous chromosomes where recombination is suppressed. Fraser and Heitman (2004) have pointed out that the unusual organisation of genes around the extended *MAT* loci in both *C. neoformans* and *U. hordei*, and the fact that there are just two mating types, rather than the many seen in other basidiomycetes, have remarkable parallel with sex chromosomes in other organisms, and they present models to explain how these complex loci may have evolved.

III. Downstream Regulation of Development

A. Pheromone Signalling

The yeast *S. cerevisiae* provides us with the most completely understood model of how pheromone binding activates an intracellular phosphorylation cascade and leads to induction of genes required for mating (see recent review by Lengeler et al. 2000). The corresponding signalling pathway in basidiomycetes is dealt with in Chap. 19 of this volume, and only a brief mention is made here with the object of seeing, with examples from *U. maydis* and *C. cinereus*, how the transcription factors targeted by pheromone signalling can regulate different subsets of genes to bring about the different developmental responses that we see during sexual development.

In *S. cerevisiae*, receptor binding by a pheromone activates a heterotrimeric G protein coupled to its internal surface and initiates a phosphorelay through a MAPK cascade to activate the target transcription factor Ste12p, a member of the homeodomain family of transcription factors. Ste12p binds pheromone response elements (PREs) in the promoters of genes required for mating. The first kinase in the pathway is Ste20p, and the MAPK module is composed of the three kinases Ste11p (MAPKKK), Ste7p (MAPKK) and Fus3p (MAPK). Several components of a similar *U. maydis* MAPK pathway have been identified (see Lengeler et al. 2000), in particular homologues of the MAPK module and a target transcription factor Prf1 (pheromone response factor, Hartmann et al. 1996). Pheromone stimulation leads to induction of both the pheromone and receptor genes, and within the promoters of these genes are elements (PREs) that have been shown to bind Prf1. Prf1 is also essential for induction of the *b* genes, and PREs can be found in the DNA sequences that contain these genes (Urban et al. 1996). Inactivation of components of the MAPK module leads to inability to make conjugation tubes; cell fusion can still occur but cells cannot induce the *b* genes necessary for filamentous growth and, even if these are driven from a constitutive promoter, the hyphae are non-pathogenic (Kaffarnik et al. 2003; Müller et al. 2003).

Another highly conserved signalling pathway implicated in mating in *U. maydis* is the cAMP pathway, with key elements a Gα protein that initiates downstream activation of adenylate cyclase which, together with cAMP, phosphorylates cAMP-dependent protein kinase A (PKA; Gold et al. 1994, 1997; Regenfelder et al. 1997; Dürrenberger et al. 1998; see Chap. 15, this volume). Prf1 integrates signals from these two pathways to coordinate the activities of the *a* and *b* genes (Hartmann et al. 1996, 1999). It has been shown recently that both PKA and the MAPK phosphorylate Prf1, and that this permits promoter discrimination whilst still using the same PRE DNA-binding sites. Depending on its phosphorylation status, Prf1 is able to activate either *a* or *b* genes; induction of *a* genes requires PKA sites to be phosphorylated but not the MAPK sites, whereas induction of *b* genes requires the integrity of both PKA and MAPK sites (Kaffarnik et al. 2003). Evidence points to pheromone signal activating both pathways.

Prf1, unlike *S. cerevisiae* Ste12p, is a member of a transcription factor family that has an HMG DNA-binding domain (Hartmann et al. 1996). Two transcription factors have been identified in *C. cinereus* that like Prf1 of *U. maydis*, have an HMG DNA-binding domain, Hmg1 (Milner, Aime and Casselton, unpublished data) and Pcc1 (Murata et al. 1998), and are both required for pheromone-induced mating functions. Hmg1 is necessary for nuclear migration in mating but not for clamp cell fusion. Deletion of the *hmg1* gene in one mate means that it can no longer receive nuclei but it can still donate nuclei into its non-mutant partner that has a functional transcription factor. When both mates lack a functional copy of *hmg1*, nuclear migration is totally blocked, but hyphal fusion leads to formation of a dikaryon with perfectly fused clamp cells (Milner, Aime and Casselton, unpublished data). Pcc1 is required for clamp cell fusion but not nuclear migration (own unpublished data). There are thus two different transcription factors for different outputs of the pheromone signal – Hmg1 for nuclear migration and Pcc1 for clamp cell fusion; discrimination between them depends on whether or not the *A* (clamp cell) pathway has been activated.

B. Heterodimer Targets

Many different morphological and physiological changes may accompany the switch from an asexual to a dikaryotic phase of the life cycle, other than fruiting, and overall regulation of some of these can be attributed to one or other set of mating type genes (Brachmann et al. 2001;

Kües et al. 2002). Several interesting genes have been characterised (see Chaps. 15 and 19, this volume) but we are a long way from understanding the downstream pathways in any of the fungi that we have described above. Progress is likely to change dramatically now that the genome sequences of these fungi are becoming available (http://www.broad.mit.edu/annotation/fungi/fgi/), and microarray technology will aid identification of major subsets of genes whose transcription alters in response to mating.

As yet, few genes have been identified that are direct targets of the pheromone response factor (other than the mating type genes themselves) or the homeodomain protein heterodimer. Fortuitously, a direct target gene of the b heterodimer of *U. maydis* resides in the *a2* mating type locus, *lga2* (Romeis et al. 1997) but its function appears to be irrelevant to mating (Bortfeld et al. 2004). The *lga2* promoter has a sequence motif that binds the b proteins, and this is remarkably similar to the binding site of the corresponding a1/a2 heterodimer of *S. cerevisiae*. The b proteins of *U. maydis* are much longer than the yeast proteins and, unlike a1/α2, the b proteins are transcriptional activators. Another *b*-target gene (*frb52*), encoding a putative DNA polymerase, was identified in a major screen to detect genes activated or repressed by the b heterodimer (Brachmann et al. 2001). Several interesting gene functions were identified that act downstream of *b*, one being a MAPK (Kpp6) required for fungal penetration of the host tissues (Brachmann et al. 2003). In *C. cinereus*, a potential target of the A protein heterodimer was identified by the classical genetic approach of looking for mutants blocked in clamp cell formation. The *clp1* gene is induced by activating the *A* pathway, and has an essential sequence motif in its promoter that resembles the b protein binding sites in *lga2* and *frb52* (Inada et al. 2001).

Expressed off a constitutive promoter, *clp1* is sufficient to activate the entire clamp cell programme, showing that although there may be many promoters that bind the HD1/HD2 heterodimer, *clp1* may be the only critical target required to activate the pathway in *C. cinereus*. Makino and Kamada (2004) have identified several mutants that are defective in nuclear migration; these could be potential targets of the predicted pheromone response factor Hmg1. The Pcc1 protein has been implicated in regulating clamp cell fusion (see above) but the *pcc1* mutant has an unusual phenotype, which is why it was identified; it constitutively produces unfused clamp cells, as demonstrated by Murata et al. (1998). These authors suggested that Pcc1 might act as a repressor of the clamp cell pathway. This may well be an essential, if indirect function of an activated pheromone response. The *A* and *B* regulated pathways alternate during dikaryotic cell growth and are mutually exclusive. The *A* pathway leads to tip cell extension and nuclear division, whereas the *B* pathway requires cell–cell recognition which, as in mating *S. cerevisiae* cells, is likely to require cell cycle arrest before cell fusion.

IV. Concluding Remarks

The mating type genes are not conserved between basidiomycetes and ascomycetes (see Chap. 15, this volume, and Hiscock and Kües 1999 for a detailed account of both ascomycete and basidiomycete mating types) but this does not mean that the pathways they regulate are different. The mating type locus is simply a strategy for preventing constitutive sexual development by requiring mates to bring together the full complement of necessary genes (see Casselton 2002); the genes that are sequestered there are the result of evolutionary accident. Pheromone signalling is an essential part of mating in both groups of fungi, and we see a strong conservation in the response induced by pheromone signalling, once we look beyond the apparent differences in life style. There are only two mating types in ascomycetes, and the pheromones and receptors are not mating type genes – they are regulated by the mating type genes (see Coppin et al. 1997, and Chap. 16, this volume). As in *U. maydis* and *C. neoformans*, pheromones can act as chemoattractants, and are secreted into the surrounding environment where binding to a compatible receptor may induce the formation of mating structures that aid cell fusion. *S. cerevisiae*, *U. maydis* and *C. neoformans* all change shape in response to pheromone stimulation, increasing in size or forming pegs or filaments that orient towards the source of pheromone and promote cell fusion. In the filamentous ascomycetes such as *Neurospora crassa*, both mating types may differentiate male and female cells, the female cells being the protoperithecia which, when fertilised, develop into the fruiting bodies in which the sexual cycle is completed. Emerging from the protoperithecia is a filament known as the trichogyne

that is attracted by pheromone stimulation to fuse with the conidia that act as male gametes (Chap. 16, this volume). The conidial nucleus must migrate through the trichogyne cells (Chap. 20, this volume), in much the same way that the donor nucleus migrates through recipient monokaryotic cells in *C. cinereus* mating (Fig. 17.2). At the base of the trichogyne in *N. crassa*, nuclear sorting occurs to establish binucleate cells (ascogenous hyphae) that divide in exactly the same way as do dikaryotic cells of homobasidiomycetes and *C. neoformans*. These cells contain one nucleus from each mate, and tip cell division involves a structure analogous to the clamp connection (crozier; Chap. 20, this volume). It is not surprising that, in *N. crassa*, pheromone signalling has been shown to be essential after trichogyne fusion (Kim and Borkovich 2004) – it is required to maintain the dikaryophase.

Why do so many of these fungi have a long dikaryophase? Diploidy is not a normal feature of the filamentous fungal life cycle, and nuclear fusion generally occurs only in cells specialised for meiosis. The dikaryophase provides a way of amplifying a compatible pair of nuclei so that large numbers of meiotic products can finally be derived from them. For example, a single fruiting body of *C. cinereus* may produce 10^7 meiocytes (Pukkila and Casselton 1991), all having a diploid nucleus derived from the same dikaryotic nuclear pair. In filamentous ascomycetes, the dikaryophase is of limited duration but in mushrooms it probably represents the predominant mycelial state in nature, and can exist for hundreds of years, colonising entire woodlands (Smith et al. 1992).

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References

Alic M, Letzring C, Gold MH (1987) Mating system and basidiospore formation in the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 53:1464–1469

Anderson CM, Willits DA, Kosted PJ, Ford EJ, Martinez-Espinoza AD, Sherwood JE (1999) Molecular analysis of the pheromone and pheromone receptor genes of *Ustilago hordei*. Gene 240:89–97

Asante-Owusu RN, Banham AH, Böhnert HU, Mellor EJC, Casselton LA (1996) Heterodimerization between two classes of homeodomain proteins in the mushroom *Coprinus cinereus* brings together potential DNA-binding and activation domains. Gene 172:25–31

Badalyan SM, Polak E, Hermann R, Aebi M, Kües U (2004) Role of peg formation in clamp cell fusion of homobasidiomycete fungi. J Basic Microbiol 44:167–177

Bakkeren G, Kronstad JW (1993) Conservation of the *b*-mating-type gene-complex among bipolar and tetrapolar smut fungi. Plant Cell 5:123–136

Bakkeren G, Kronstad JW (1994) Linkage of mating-type loci distinguishes bipolar from tetrapolar mating in basidiomycetous smut fungi. Proc Natl Acad Sci USA 91:7085–7089

Banham AH, Asante Owusu RN, Göttgens B, Thompson SAJ, Kingsnorth CS, Mellor EJC, Casselton LA (1995) An N-terminal dimerization domain permits homeodomain proteins to choose compatible partners and initiate sexual development in the mushroom *Coprinus cinereus*. Plant Cell 7:773–783

Banuett F (1995) Genetics of *Ustilago maydis*, a fungal pathogen that induces tumors in maize. Annu Rev Genet 29:179–208

Bölker M, Urban M, Kahmann R (1992) The *a* mating type locus of *U. maydis* specifies cell signaling components. Cell 68:441–450

Bortfeld M, Auffarth K, Kahmann R, Basse CW (2004) The *Ustilago maydis a2* mating-type locus genes *Iga2* and *rga2* compromise pathogenicity in the absence of the mitochondrial p32 family protein Mrb1. Plant Cell 16:2233–2248

Brachmann A, Weinzierl G, Kämper J, Kahmann R (2001) Identification of genes in the *bW/bE* regulatory cascade in *Ustilago maydis*. Mol Microbiol 42:1047–1063

Brachmann A, Schirawski J, Müller P, Kahmann R (2003) An unusual MAP kinase is required for efficient penetration of the plant surface by *Ustilago maydis*. EMBO J 22:2199–2210

Brown AJ, Casselton LA (2001) Mating in mushrooms: increasing the chances but prolonging the affair. Trends Genet 17:393–400

Buller AHR (1931) Researches on Fungi, vol 4. Longmans, Green, London

Casselton LA (2002) Mate recognition in fungi. Heredity 88:142–147

Casselton LA, Olesnicky NS (1998) Molecular genetics of mating recognition in basidiomycete fungi. Microbiol Mol Biol Rev 62:55–70

Christensen JJ (1931) Studies on the genetics of *Ustilago zeae*. Phytopathol Z 4:129–188

Coppin E, Debuchy R, Arnaise S, Picard M (1997) Mating types and sexual development in filamentous ascomycetes. Microbiol Mol Biol Rev 61:411–428

Cummings WJ, Celerin M, Crodian J, Brunick LK, Zolan ME (1999) Insertional mutagenesis in *Coprinus cinereus*: use of a dominant selectable marker to generate tagged, sporulation-defective mutants. Curr Genet 36:371–382

Davidson RC, Moore TDE, Odom AR, Heitman J (2000) Characterization of the MF α pheromone of the human fungal pathogen *Cryptococcus neoformans*. Mol Microbiol 38:1017–1026

Day PR (1960) The structure of the *A* mating-type locus in *Coprinus lagopus*. Genetics 45:641–650

Day PR (1963a) Mutations affecting the *A* mating-type factor in *Coprinus lagopus*. Genet Res Camb 4:55–64

Day PR (1963b) The structure of the *A* mating type factor in *Coprinus lagopus*. Wild alleles. Genet Res Camb 4:323–325

Dürrenberger F, Wong K, Kronstad JW (1998) Identification of a cAMP-dependent protein kinase catalytic subunit required for virulence and morphogenesis in *Ustilago maydis*. Proc Natl Acad Sci USA 95:5684–5689

Fowler TJ, DeSimone SM, Mitton MF, Kurjan J, Raper CA (1999) Multiple sex pheromones and receptors of a mushroom-producing fungus elicit mating in yeast. Mol Biol Cell 10:2559–2572

Fowler TJ, Mitton MF, Vaillancourt LJ, Raper CA (2001) Changes in mate recognition through alterations of pheromones and receptors in the multisexual mushroom fungus *Schizophyllum commune*. Genetics 158:1491–1503

Fowler TJ, Mitton MF, Rees EI, Raper CA (2004) Crossing the boundary between the *B*α and *B*β mating-type loci in *Schizophyllum commune*. Fungal Genet Biol 41:89–101

Fraser JA, Heitman J (2004) Evolution of fungal sex chromosomes. Mol Microbiol 51:299–306

Giesy RM, Day PR (1965) The septal pores of *Coprinus lagopus* (Fr.) sensu Buller in relation to nuclear migration. Am J Bot 52:287–293

Gillissen B, Bergemann J, Sandmann C, Schroeer B, Bolker M, Kahmann R (1992) A 2-component regulatory system for self non-self recognition in *Ustilago maydis*. Cell 68:647–657

Gold S, Duncan G, Barrett K, Kronstad J (1994) cAMP regulates morphogenesis in the fungal pathogen *Ustilago maydis*. Genes Dev 8:2805–2816

Gold SE, Brogdon SM, Mayorga ME, Kronstad JW (1997) The *Ustilago maydis* regulatory subunit of a cAMP-dependent protein kinase is required for gall formation in maize. Plant Cell 9:1585–1594

Granado JD, Kertesz-Chaloupková K, Aebi M, Kües U (1997) Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. Mol Gen Genet 256:28–36

Halsall JR, Milner MJ, Casselton LA (2000) Three subfamilies of pheromone and receptor genes generate multiple *B* mating specificities in the mushroom *Coprinus cinereus*. Genetics 154:1115–1123

Hartmann HA, Kahmann R, Bölker M (1996) The pheromone response factor coordinates filamentous growth and pathogenicity in *Ustilago maydis*. EMBO J 15:1632–1641

Hartmann HA, Krüger J, Lottspeich F, Kahmann R (1999) Environmental signals controlling sexual development of the corn smut fungus *Ustilago maydis* through the transcriptional regulator *prf1*. Plant Cell 11:1293–1305

Haylock RW, Economou A, Casselton LA (1980) Dikaryon formation in *Coprinus cinereus*: selection and identification of *B* factor mutants. J Gen Microbiol 121:17–26

Herskowitz I (1988) The life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol Rev 52:536–553

Hiscock SJ, Kües U (1999) Cellular and molecular mechanisms of sexual incompatibility in plants and fungi. Int Rev Cytol Surv Cell Biol 193:165–295

Hull CM, Heitman J (2002) Fungal mating: *Candida albicans* flips a switch to get in the mood. Curr Biol 12:R782–R784

Hull CM, Davidson RC, Heitman J (2002) Cell identity and sexual development in *Cryptococcus neoformans* are controlled by the mating-type-specific homeodomain protein Sxi1α. Genes Dev 16:3046–3060

Hull CM, Boily MJ, Heitman J (2005) Sex-specific homeodomain proteins Sxi1α and Sxi2a coordinately regulate sexual development in *Cryptococcus neoformans*. Eukaryot Cell 4:526–535

Inada K, Morimoto Y, Arima T, Murata Y, Kamada T (2001) The *clp1* gene of the mushroom *Coprinus cinereus* is essential for *A*-regulated sexual development. Genetics 157:133–140

Iwasa M, Tanabe S, Kamada T (1998) The two nuclei in the dikaryon of the homobasidiomycete *Coprinus cinereus* change position after each conjugate division. Fungal Genet Biol 23:110–116

Jacobsen S, Wittig M, Pöggeler S (2002) Interaction between mating-type proteins from the homothallic fungus *Sordaria macrospora*. Curr Genet 41:150–158

James T, Kües U, Cullen D, Vilgalys R (2003) The origins of unifactorial mating systems in *Coprinellus disseminatus* and *Phanerochaete chrysosporium* from bifactorial ancestors. Inoculum (MSA abstr) 54:28

James TY, Kües U, Rehner SA, Vilgalys R (2004a) Evolution of the gene encoding mitochondrial intermediate peptidase and its cosegregation with the *A* mating-type locus of mushroom fungi. Fungal Genet Biol 41:381–390

James TY, Liou SR, Vilgalys R (2004b) The genetic structure and diversity of the *A* and *B* mating-type genes from the tropical oyster mushroom, *Pleurotus djamor*. Fungal Genet Biol 41:813–825

Johnson AD (1995) Molecular mechanisms of cell-type determination in budding yeast. Curr Opin Genet Dev 5:552–558

Kaffarnik F, Müller P, Leibundgut M, Kahmann R, Feldbrügge M (2003) PKA and MAPK phosphorylation of *Prf1* allows promoter discrimination in *Ustilago maydis*. EMBO J 22:5817–5826

Kahmann R, Steinberg G, Basse C, Feldbrügge M, Kämper J (2000) *Ustilago maydis*, the causative agent of corn smut disease. In: Kronstad JW (ed) Fungal pathology. Kluwer, Dordrecht, pp 347–371

Kamada T (2002) Molecular genetics of sexual development in the mushroom *Coprinus cinereus*. BioEssays 24:449–459

Kämper J, Reichmann M, Romeis T, Bölker M, Kahmann R (1995) Multiallelic recognition nonself-dependent dimerization of the *bE* and *bW* homeodomain proteins in *Ustilago maydis*. Cell 81:73–83

Kim H, Borkovich KA (2004) A pheromone receptor gene, *pre-1*, is essential for mating type specific directional growth and fusion of trichogynes and female fertility in *Neurospora crassa*. Mol Microbiol 52:1781–1798

Koltin Y, Stamberg J, Lemke P (1972) Genetic structure and evolution of the incompatibility factors in higher fungi. Bacteriol Rev 36:156–171

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U, Casselton LA (1992) Homeodomains and regulation of sexual development in basidiomycetes. Trends Genet 8:154–155

Kües U, Richardson WV, Tymon AM, Mutasa ES, Göttgens B, Gaubatz S, Gregoriades A, Casselton LA (1992) The combination of dissimilar alleles of the *A*α and *A*β gene complexes, whose proteins contain homeo domain motifs, determines sexual development in the mushroom *Coprinus cinereus*. Genes Dev 6:568–577

Kües U, Göttgens B, Stratmann R, Richardson WVJ, Oshea SF, Casselton LA (1994) A chimeric homeodomain protein causes self compatibility and constitutive sexual development in the mushroom *Coprinus cinereus*. EMBO J 13:4054–4059

Kües U, James TY, Vilgalys R, Challen MP (2001) The chromosomal region containing *pab-1*, *mip*, and the *A* mating type locus of the secondarily homothallic homobasidiomycete *Coprinus bilanatus*. Curr Genet 39:16–24

Kües U, Walser PJ, Klaus MJ, Aebi M (2002) Influence of activated *A* and *B* mating-type pathways on developmental processes in the basidiomycete *Coprinus cinereus*. Mol Genet Genomics 268:262–271

Kwon-Chung KJ (1975) A new genus *Filobasidiella*, the perfect state of *Cryptococcus neoformans*. Mycologia 67:1197–1200

Kwon-Chung KJ (1980) Nuclear genotypes of spore chains in *Filobasidiella neoformans* (*Cryptococcus neoformans*). Mycologia 72:418–422

Kwon-Chung KJ, Bennett JE (1978) Distribution of α and *a* mating types of *Cryptococcus neoformans* among natural and clinical isolates. Am J Epidemiol 108:337–340

Lee N, Bakkeren G, Wong K, Sherwood JE, Kronstad JW (1999) The mating-type and pathogenicity locus of the fungus *Ustilago hordei* spans a 500-kb region. Proc Natl Acad Sci USA 96:15026–15031

Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen WC, Wang P, Pan XW, Waugh M, Heitman J (2000) Signal transduction cascades regulating fungal development and virulence. Microbiol Mol Biol Rev 64:746–785

Lengeler KB, Fox DS, Fraser JA, Allen A, Forrester K, Dietrick FS, Heitman J (2002) Mating-type locus of *Cryptococcus neoformans*: a step in the evolution of sex chromosomes. Eukaryot Cell 1:704–718

Li Y, Challen MP, Elliott TJ, Casselton LA (2004) Molecular analysis of breeding behaviour in *Agaricus* species. Mushroom Sci 16:103–109

Lin X, Hull CM, Heitman J (2005) Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. Nature 434:1017–1021

Magae Y, Novotny C, Ullrich R (1995) Interaction of the Aα Y and Z mating-type homeodomain proteins of *Schizophyllum commune* detected by the two-hybrid system. Biochem Biophys Res Commun 211:1071–1076

Makino R, Kamada T (2004) Isolation and characterization of mutations that affect nuclear migration for dikaryosis in *Coprinus cinereus*. Curr Genet 45:149–156

Martinez D, Larrondo LF, Putnam N, Gelpke MDS, Huang K, Chapman J, Helfenbein KG, Ramaiya P, Detter JC, Larimer F et al. (2004) Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. Nat Biotechnol 22:695–700

May G, Matzke E (1995) Recombination and variation at the *A* mating-type of *Coprinus cinereus*. Mol Biol Evol 12:794–802

May G, Shaw F, Badrane H, Vekemans X (1999) The signature of balancing selection: fungal mating compatibility gene evolution. Proc Natl Acad Sci USA 96:9172–9177

McClelland CM, Chang YC, Varma A, Kwon-Chung KJ (2004) Uniqueness of the mating system in *Cryptococcus neoformans*. Trends Microbiol 12:208–212

Moore TDE, Edman JC (1993) The α-mating type locus of *Cryptococcus neoformans* contains a peptide pheromone gene. Mol Cell Biol 13:1962–1970

Müller P, Weinzierl G, Brachmann A, Feldbrügge M, Kahmann R (2003) Mating and pathogenic development of the smut fungus *Ustilago maydis* are regulated by one mitogen-activated protein kinase cascade. Eukaryot Cell 2:1187–1199

Murata Y, Fujii M, Zolan ME, Kamada T (1998) Molecular analysis of *pcc1*, a gene that leads to *A*-regulated sexual morphogenesis in *Coprinus cinereus*. Genetics 149:1753–1761

Olesnicky NS, Brown AJ, Dowell SJ, Casselton LA (1999) A constitutively active G-protein-coupled receptor causes mating self-compatibility in the mushroom *Coprinus*. EMBO J 18:2756–2763

Olesnicky NS, Brown AJ, Honda Y, Dyos SL, Dowell SJ, Casselton LA (2000) Self-compatible *B* mutants in *Coprinus* with altered pheromone-receptor specificities. Genetics 156:1025–1033

O'Shea SF, Chaure PT, Halsall JR, Olesnicky NS, Leibbrandt A, Connerton IF, Casselton LA (1998) A large pheromone and receptor gene complex determines multiple *B* mating type specificities in *Coprinus cinereus*. Genetics 148:1081–1090

Parag Y (1962) Mutations in the *B* incompatibility factor of *Schizophyllum commune*. Proc Natl Acad Sci USA 48:743–750

Pardo EH, Oshea SF, Casselton LA (1996) Multiple versions of the *A* mating type locus of *Coprinus cinereus* are generated by three paralogous pairs of multiallelic homeobox genes. Genetics 144:87–94

Pukkila PJ, Casselton LA (1991) Molecular genetics of the agaric *Coprinus cinereus*. In: Bennett JW, Lasure LL (eds) More gene manipulations in fungi. Academic Press, London, pp 126–150

Quintanilha A, Pinto Lopes J (1950) Aperçu sur l'état actuel de nos connaissances concernant la 'conduite sexuelle' des espèces d'Hymenomycetes. Bol Soc Broteriana 24:115–290

Raper JR (1966) Genetics of sexuality in higher fungi. Ronald, New York

Raper JR, Krongelb GS, Baxter MG (1958) The number and distribution of incompatibility factors in *Schizophyllum commune*. Am Nat 92:221–232

Raper JR, Baxter MG, Ellingboe AH (1960) The genetic structure of the incompatibility factors of *Schizophyllum commune*: the *A* factor. Proc Natl Acad Sci USA 46:833–842

Raper JR, Boyd DH, Raper CA (1965) Primary and secondary mutations at the incompatibility loci in *Schizophyllum*. Proc Natl Acad Sci USA 53:1324–1332

Redhead SA, Vilgalys R, Moncalvo JM, Johnson J, Hopple JS (2001) *Coprinus* Pers. and the disposition of *Coprinus* species *sensu lato*. Taxon 50:203–241

Regenfelder E, Spellig T, Hartmann A, Lauenstein S, Bölker M, Kahmann R (1997) G proteins in *Ustilago maydis*: Transmission of multiple signals? EMBO J 16:1934–1942

Riquelme M, Challen MP, Casselton LA, Brown AJ (2005) The origin of multiple *B* mating specificities in *Coprinus cinereus*. Genetics 170:1105–1119

Romeis T, Kämper J, Kahmann R (1997) Single-chain fusions of two unrelated homeodomain proteins trigger pathogenicity in *Ustilago maydis*. Proc Natl Acad Sci USA 94:1230–1234

Rowell JB, DeVay JE (1954) Genetics of *Ustilago zeae* in relation to basic problems of its pathogenicity. Phytopathology 44:356–362

Shen GP, Park DC, Ullrich RC, Novotny CP (1996) Cloning and characterization of a *Schizophyllum* gene with *Aβ6* mating-type activity. Curr Genet 29:136–142

Sia RA, Lengeler KB, Heitman J (2000) Diploid strains of the pathogenic basidiomycete *Cryptococcus neoformans* are thermally dimorphic. Fungal Genet Biol 29:153–163

Smith ML, Bruhn JN, Anderson JB (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428–431

Spellig T, Bölker M, Lottspeich F, Frank RW, Kahmann R (1994) Pheromones trigger filamentous growth in *Ustilago maydis*. EMBO J 13:1620–1627

Spit A, Hyland RH, Mellor EJC, Casselton LA (1998) A role for heterodimerization in nuclear localization of a homeodomain protein. Proc Natl Acad Sci USA 95:6228–6233

Stamberg J, Koltin Y (1971) Selectively recombining *B* incompatibility factors of *Schizophyllum commune*. Mol Gen Genet 113:157–165

Stankis MM, Specht CA, Yang HL, Giasson L, Ullrich RC, Novotny CP (1992) The *Aα* mating locus of *Schizophyllum commune* encodes two dissimilar multiallelic homeodomain proteins. Proc Natl Acad Sci USA 89:7169–7173

Swamy S, Uno I, Ishikawa T (1984) Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 130:3219–3224

Swiezynski KM, Day PR (1960) Heterokaryon formation in *Coprinus lagopus*. Genet Res (Camb) 1:114–128

Tscharke RL, Lazera M, Chang YC, Wickes BL, Kwon-Chung KJ (2003) Haploid fruiting in *Cryptococcus neoformans* is not mating type alpha-specific. Fungal Genet Biol 39:230–237

Turgeon BG (1998) Application of mating type gene technology to problems in fungal biology. Annu Rev Phytopathol 36:115–137

Urban M, Kahmann R, Bölker M (1996) Identification of the pheromone response element in *Ustilago maydis*. Mol Gen Genet 251:31–37

Vaillancourt LJ, Raper CA (1996) Pheromones and pheromone receptors as mating-type determinants in basidiomycetes. Genetic Engineering, vol 18. Plenum Press, New York, pp 219–247

Vaillancourt LJ, Raudaskoski M, Specht CA, Raper CA (1997) Multiple genes encoding pheromones and a pheromone receptor define the *Bβ1* mating-type specificity in *Schizophyllum commune*. Genetics 146:541–551

Wang P, Perfect JR, Heitman J (2000) The G-protein β-subunit GPB1 is required for mating and haploid fruiting in *Cryptococcus neoformans*. Mol Cell Biol 20:352–362

Wendland J, Vaillancourt LJ, Hegner J, Lengeler KB, Laddison KJ, Specht CA, Raper CA, Kothe E (1995) The mating-type locus *Bα1* of *Schizophyllum commune* contains a pheromone receptor gene and putative pheromone genes. EMBO J 14:5271–5278

Whitehouse HLK (1949) Multiple allelomorph heterothallism in the fungi. New Phytol 48:212–244

Wickes BL, Mayorga ME, Edman U, Edman JC (1996) Dimorphism and haploid fruiting in *Cryptococcus neoformans*: Association with the α-mating type. Proc Natl Acad Sci USA 93:7327–7331

18 Regulatory and Structural Networks Orchestrating Mating, Dimorphism, Cell Shape, and Pathogenesis in *Ustilago maydis*

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CONTENTS

I. Introduction

The heterobasidiomycete smut fungi are characterized by their narrow host range on defined groups of grasses including valuable crop species such as maize, sugar cane, barley and wheat. Most smuts enter a long systemic phase before symptoms develop in the inflorescences of their respective hosts. Disease symptoms are sori in which the flower tissue is replaced by black teliospores. It is in these organs that massive proliferation of the fungus takes place and that the diploid teliospores are eventually produced. Teliospores represent a resting stage which can survive harsh environments. Under appropriate conditions teliospores germinate, undergo meiosis and produce haploid progeny. *Ustilago maydis*, the focus of this article, is a member of the smut fungi and infects maize plants. In contrast to the other smuts, prominent tumors are induced on all parts of its host, except the roots. Disease symptoms develop early and independently of flowering, which is one of the reasons why *U. maydis* has become an important model organism for the study of fungal pathogenicity (Banuett 1995; Bölker 2001; Kahmann and Kämper 2004).

In its haploid form, *U. maydis* is yeast-like and proliferates by budding (Fig. 18.1, step 1). It can be propagated in the laboratory but is unable to cause disease when injected into the host as pure culture. The infectious form is generated when compatible haploid strains fuse and generate a dikaryotic filament (Fig. 18.1, steps 2–4). In *U. maydis*, this process is controlled by a tetrapolar mating system consisting of the biallelic *a* locus and the multiallelic *b* locus (Feldbrügge et al. 2004; see Chap. 17, this volume). The *a* locus encodes a pheromone/receptor system which allows haploid cells to sense each other's presence and to fuse (Bölker et al. 1992). The *b* locus codes for a pair of homeodomain transcription factors, bE and bW, which dimerize when derived from

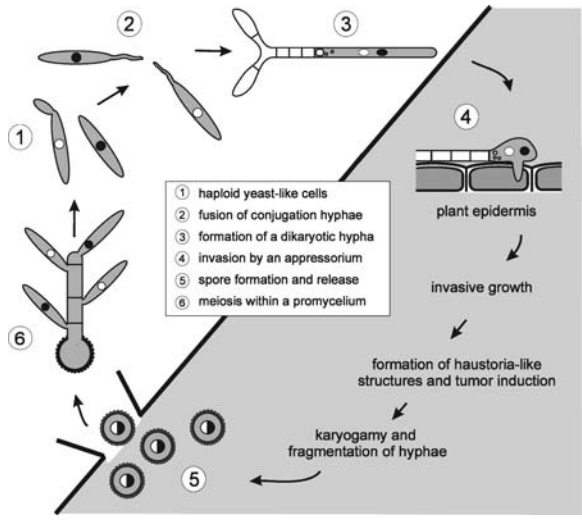

Fig. 18.1. Life cycle of *U. maydis*. For details, see Introduction

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different alleles (Kämper et al. 1995). Under natural conditions, the heterodimeric bE/bW complex is formed after fusion of compatible cells, and it is this complex which triggers all subsequent steps of pathogenic as well as sexual development. *U. maydis* was the first basidiomycete in which the mating type loci were cloned and their mode of function elucidated. Subsequently, it was shown that the mating type loci of all basidiomycete fungi consist of variations of this general scheme: pheromone and pheromone receptors on the one hand, and homeodomain gene pairs on the other. However, the developmental processes controlled by these loci are quite distinct, and the structure of these loci in the homobasidiomycete fungi is much more complex than in *U. maydis*. This topic is reviewed elsewhere in this volume (see Chap. 17, this volume). The filamentous dikaryon of *U. maydis* requires the plant for sustained growth, and this phase is therefore termed biotrophic. On the plant surface, prior to penetration, the hyphae have a peculiar growth modus: while the filament expands at the tip, the cytoplasm from the rear moves forwards. The distal region of the tip cell becomes vacuolated and eventually collapses to leave empty sections behind, which are sealed off by regularly spaced septa (Fig. 18.1, steps 3 and 4). Stimulated by an unknown signal on the plant surface, hyphae stop polar growth. Their tips swell and non-melanized appressoria are formed. Subsequently, infection hyphae are formed which penetrate the plant tissue (Fig. 18.1, step 4). Plant penetration is presumably aided by lytic enzymes. The penetrating hyphae are never in direct contact with the host cell cytoplasm but appear surrounded by invaginated plasma membrane of the plant. Within the plant, dikaryotic hyphae undergo a number of developmentally regulated morphological transitions which have been described in detail by earlier workers (Snetselaar 1993; Snetselaar and Mims 1994; Banuett and Herskowitz 1996). Massive fungal proliferation is a late event occurring only 5–6 days after infection within the tumour tissue, and is followed by sporogenesis (Fig. 18.1, step 5). Except for the induction of anthocyanin pigmentation, there are no apparent host responses, suggesting that *U. maydis* either shields itself from being recognized or is actively suppressing plant defence pathways. When the heavily melanized diploid spores germinate, they produce a promycelium in which meiosis takes place and from which haploid sporidia are released by budding (Fig. 18.1, step 6).

U. maydis is not only a fully developed genetic system but is also amenable to efficient reverse genetics. The emphasis in the beginning of the molecular era has been to unravel the events triggered by the mating type loci. More recently, these studies have been extended to the cell biological level, as it became increasingly apparent that morphological transitions are a prerequisite for disease. Publication of the 20.5-Mb genome sequence of *U. maydis* in 2003 through the Broad Institute and Bayer CropScience (http://www.broad.mit.edu/annotation/fungi/ustilago_maydis/) has created a wonderful resource for comparative genetics and has opened up new avenues for research. In this review, we will emphasize the current status of signalling networks underlying mating and pathogenic development, summarize our current understanding of the complex morphological transitions which govern disease, and indicate which challenges lie ahead.

II. Signalling Networks

Distinct stages of the *U. maydis* life cycle are regulated by intricate signalling networks. These not only trigger fusion of haploid cells during mating but coordinate all morphological transitions necessary for pathogenic development.

A. Signalling Network During Mating

The biallelic *a* locus encodes components of an intercellular recognition system consisting of lipopeptide pheromones (mating factor *a1* and *a2*; Mfa1 and Mfa2) and cognate seven-transmembrane pheromone receptors (pheromone receptor *a1* and *a2*; Pra1 and Pra2; Froeliger and Leong 1991; Bölker et al. 1992). The ability of cells to fuse is dependent solely on the *a* locus. The expression of all mating type genes is pheromone-inducible, and this is conferred by pheromone response elements in the regulatory regions of these genes (Urban et al. 1996b). The key transcription factor determining basal as well as pheromone-responsive expression of mating type genes is the pheromone response factor (Prf1), a transcription factor which recognizes pheromone response elements via its sequence-specific HMG (high mobility group) box DNA-binding domain (Hartmann et al. 1996). Comparable to pheromone signalling in other

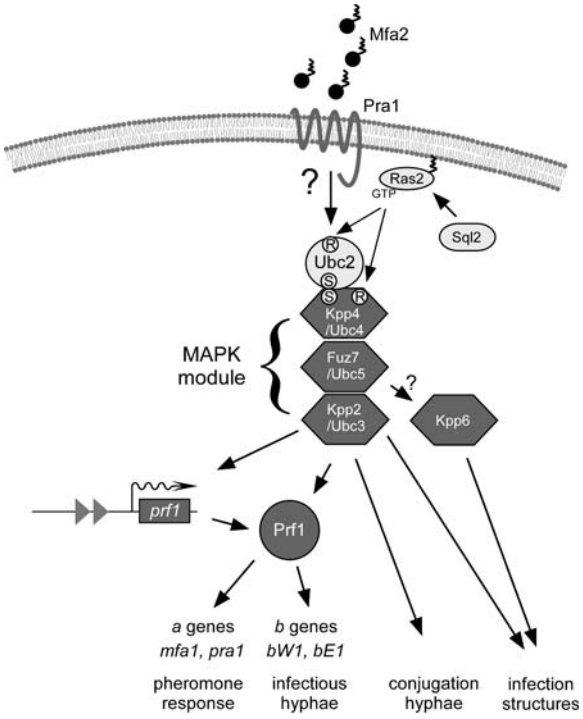

Fig. 18.2. Basic outline of the MAP kinase module transmitting the pheromone signal. Components of the MAP kinase module (*hexagons*)-regulate pheromone response, functional infectious hyphae, conjugation hyphae as well as infection structures. Upstream components are shown as *ovals* (Ras association domain and sterile alpha motive are symbolized by *encircled R* and *S* respectively). The pheromone-responsive MAPK module regulates Prf1 (*circle*) which, in turn, induces *a* and *b* gene expression, as well as expression of the *prf1* gene (*rectangle*). Possible autoregulation of *prf1* is indicated by the presence of pheromone response elements (*triangles*) upstream of the transcriptional start site (*wavy line*)

model fungi such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, an evolutionarily conserved pheromone-responsive mitogen-activated protein kinase module (MAPK) has been identified which transduces the pheromone signal towards Prf1 (Kaffarnik et al. 2003; Müller et al. 2003b; Fig. 18.2). This module consists of the MAP kinase Kpp2/Ubc3, the MAPK kinase Fuz7/Ubc5, and the MAPKK kinase Kpp4/Ubc4 (Banuett and Herskowitz 1994; Mayorga and Gold 1999; Müller et al. 1999, 2003b; Andrews et al. 2000; Fig. 18.2). In addition to direct activation of Prf1 by phosphorylation via Kpp2, the pheromone-activated MAPK module triggers transcriptional activation of the *prf1* promoter as well as formation of conjugation hyphae (Kaffarnik et al. 2003; Müller et al. 2003b). Conjugation hyphae are the first morphological response to be seen after pheromone stimulation. They usually develop at one pole of the cell, grow towards the pheromone source, and fuse at their tips (Snetselaar et al. 1996). The formation of conjugation hyphae is independent of Prf1 function, indicating that this morphological program is controlled by a different transcription factor or may be triggered through direct modification of components of the cytoskeleton (Müller et al. 2003b; Fig. 18.2).

At present it is not known how the signal from the pheromone-bound activated receptor is transduced to the MAPKKK Kpp4/Ubc4. A factor likely to be involved in this signalling step is the putative adapter protein Ubc2 (Fig. 18.2), which shares similarity with the pheromone signalling component Ste50p from *S. cerevisiae*. Ubc2 contains several protein interaction domains such as a sterile alpha motif (SAM), a Ras association domain (RA), and a Src homology domain (SH3). Since *ubc2Δ* strains are impaired in pheromone response and virulence, it has been suggested that Ubc2 funnels signalling information to the MAPK module (Mayorga and Gold 2001; Fig. 18.2). The interaction between Ubc2 and MAPKKK Kpp4/Ubc4 which could be mediated by the SAM domain of Kpp4/Ubc4 has been described for the Ste50p/Ste11p interaction in *S. cerevisiae* (Müller et al. 2003b; Grimshaw et al. 2004; Fig. 18.2). Further components potentially involved in pheromone signalling are the small G protein Ras2 and the guanine nucleotide exchange factor (GEF) Sql2. Ras2 is epistatic to components of the pheromone-responsive MAPK module, and expression of a constitutively active version $Ras2^{G16V}$ elicits increased *mfa1* expression. Thus, Ras2 could feed into MAPK signalling via the Ras association (RA) domains of Ubc2 or Kpp4/Ubc4 (Lee and Kronstad 2002; Müller et al. 2003a; Fig. 18.2). Genetic evidence indicates that the GEF Sql2 might function as cognate activator of Ras2 (Müller et al. 2003a; Fig. 18.2).

Research during the last decade has revealed that the pheromone signalling pathway in *U. maydis* is not a simple linear pathway consisting of insulated signalling components which function only during pheromone response. On the contrary, intensive crosstalk with other signalling pathways exists and is essential to orchestrate mating and subsequent pathogenic development (Feldbrügge et al. 2004; see below). A connection between pheromone signalling and nutritional and cell cycle signalling is well established in *S. pombe* and *S. cerevisiae* (Davey 1998; Elion 2000). For *U.*

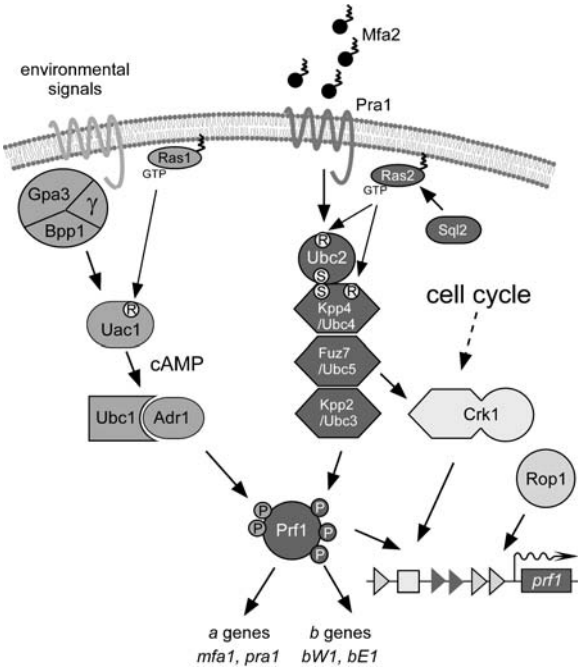

Fig. 18.3. Signalling network during mating. Components of the MAP kinase module (*hexagons*) communicate with a conserved cAMP signalling pathway (indicated on the *left*) as well as with putative cell cycle components (indicated on the *right*). The same symbols are used as those described in the caption of Fig. 18.2. The cAMP signalling pathway consists of a heterotrimeric G protein (Gpa3, Bpp1, and a γ subunit), adenylate cylase (Uac1) and protein kinase A (Ubc1 and Adr1). Phosphorylation of Prf1 through PKA and MAPK signalling (*encircled P*) is used to differentiate between *a* and *b* gene expression (see text for details). The novel MAPK Crk1 as well as the HMG box transcription factors Rop1 and Prf1 regulate *prf1* expression transcriptionally

maydis, the input of environmental signals might be particularly important to achieve temporal and local control of pheromone signalling to allow mating on the plant surface. One of these communicating signalling pathways required for pheromone response and further pathogenic development is an evolutionarily conserved cAMP signalling pathway which consists of a heterotrimeric G protein, containing Gpa3 and Bpp1 as α and β subunit respectively (Regenfelder et al. 1997; Müller et al. 2004). G protein-mediated stimulation of adenylate cyclase Uac1 (Gold et al. 1994) leads to the production of cAMP. This secondary messenger regulates the cAMP-dependent protein kinase A (PKA), consisting of the regulatory subunit Ubc1 and the catalytic subunit Adr1 (Gold et al. 1997; Dürrenberger et al. 1998; Lee et al. 2003; Fig. 18.3). The connection to pheromone signalling has been revealed by finding that the mating defect of compatible *gpa3Δ* strains can be rescued by addition of external cAMP (Krüger et al. 1998). How this crosstalk operates was revealed by investigating *mfa1* gene expression. Initially, it was observed that strains carrying deletions in genes encoding components of the cAMP pathway, such as *gpa3Δ* or *uac1Δ*, exhibit strongly reduced *mfa1* expression. Conversely, strains expressing constitutively active versions of Gpa3 or protein kinase A ($gpa3^{Q206L}$ or *ubc1Δ*, respectively) showed elevated *mfa1* expression. This indicated that high internal cAMP levels induce *mfa1* gene expression (Krüger et al. 1998). In *U. maydis*, a second Ras protein, Ras1, has been described whose deletion has no significant effect on cellular morphology. A constitutive active version of Ras1 is likely to activate *mfa1* expression via the cAMP cascade, and it has been hypothesized that this might occur via an RA domain in Uac1 (Müller et al. 2003a; Fig. 18.3).

The observations that Prf1 binding sites are necessary and sufficient for cAMP-mediated induction of *mfa1*, and the finding that PKA phosphorylation sites in Prf1 are essential for *mfa1* expression provided compelling evidence that PKA phosphorylation of Prf1 is the key signalling node mediating crosstalk between cAMP and MAP kinase pathways (Kaffarnik et al. 2003; Fig. 18.3). Although phosphorylation of Prf1 through Adr1 appears sufficient for the induction of the genes in the *a* locus, transcriptional activation of the *b* genes requires Prf1 to be phosphorylated by Adr1 as well as Kpp2 (Kaffarnik et al. 2003; Fig. 18.3).

In addition to its regulation by phosphorylation, Prf1 activity is also intensively regulated on the transcriptional level (Fig. 18.3). As mentioned above, the pheromone-responsive MAPK module mediates pheromone-induced *prf1* expression. This is likely to operate by positive autoregulation via two PREs which are present in the *prf1* promoter and are recognized by Prf1 *in vitro* (Hartmann et al. 1999; Brefort et al. 2005). However, recent results indicate a more complex transcriptional regulation of *prf1*, involving at least two additional proteins. One of these is the sequence-specific HMG box protein Rop1 (regulator of *prf1*) which is essential for *prf1* expression during fungal growth in axenic culture. This regulation is mediated by direct binding of Rop1 to three distinct response elements present in the *prf1* promoter (Fig. 18.3). In the absence of *rop1*, *prf1* transcrip-

tion is barely detectable and results in a mating defect (Brefort et al. 2005; Fig. 18.3). Surprisingly, on the plant surface *rop1Δ* strains express sufficient amounts of *prf1* to fuse and cause pathogenic development (Brefort et al. 2005). This indicates the presence of an independent signalling program which is able to activate *prf1* transcription on the plant surface (see below). A potential signalling component for this alternative branch is the recently identified MAP kinase Crk1 (Garrido et al. 2004). The N-terminal domain of this protein functions as MAPK, and the C-terminal part has activities so far unknown. Crk1 constitutes the founding member of a novel class of MAPKs and is also involved in crosstalk during morphogenesis (see below; Garrido and Pérez-Martín 2003; Garrido et al. 2004). *crk1Δ* strains are impaired in mating because they are severely attenuated in basal and pheromone-induced *prf1* expression. Crk1 requires activation by the pheromone-responsive MAPKK Fuz7 as well as the presence of its C-terminal domain for *in vivo* activity (Garrido et al. 2004, Fig. 18.3). Crk1 mediates *prf1* transcription via an unknown transcription factor acting on the upstream activating sequence (UAS), a distal promoter element located approximately 1600 bp upstream of the transcriptional start site (Fig. 18.3). The UAS was shown previously to be involved in carbon source sensing (Hartmann et al. 1999). At present, it is not clear which signals determine Rop1- or Crk1-dependent *prf1* expression. One attractive possibility would be that nutritional or cell cycle regulation feeds into the pheromone signalling network. The latter assumption rests on the findings that (1) Crk1-related proteins from other organisms are involved in cell cycle regulation (Garrido and Pérez-Martín 2003; Garrido et al. 2004); (2) pheromone stimulation induces a cell cycle arrest in G2 (Garcia-Muse et al. 2003); and (3) strains deleted in the cell cycle regulator Cru1 are impaired in pheromone-induced *mfa1* expression (Castillo-Lluva et al. 2004). Possibly, *U. maydis* needs to sense the appropriate environment as well as the correct phase of its cell cycle to mate successfully on the plant surface.

B. Signalling Network During Morphogenesis

An additional level of complexity in the PKA/MAPK signalling network has been uncovered by the observation that most of the components involved in pheromone signalling are also of functional importance during regulation of filamentous growth and pathogenicity (Feldbrügge et al. 2004). Initially, it was found that haploid *uac1Δ* strains grow filamentously, indicating that cAMP signalling suppresses filamentous growth (Gold et al. 1994). Crosstalk between cAMP signalling and the pheromone-responsive MAPK module became apparent when suppressors of filamentous growth of *uac1Δ* strains (*ubc1*, *ubc2*, *ubc3*, *ubc4* and *ubc5*: *U. maydis* bypass of cyclase mutants; Barrett et al. 1993) were characterized. Ubc2 turned out to be the adapter protein for the MAPK module (see above), and Ubc4, Ubc5 and Ubc3 are identical to components of the pheromone-responsive MAPK module Kpp4, Fuz7 and Kpp2 respectively (Mayorga and Gold 1999; Andrews et al. 2000; Figs. 18.2 and 18.3). Thus, the MAPK module appears to antagonize cAMP signalling. A potential candidate for mediating PKA and MAPK signalling crosstalk is the MAPK Crk1 (see above), whose activity is also regulated on the transcriptional and posttranscriptional level (Garrido and Pérez-Martín 2003; Garrido

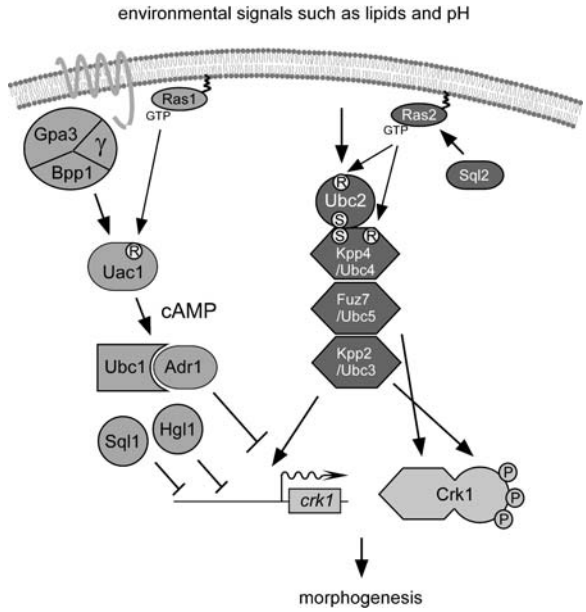

Fig. 18.4. Signalling network during dimorphic transition and pathogenesis. MAP kinase signalling antagonizes cAMP signalling in order to regulate *crk1* expression during morphogenesis. The same symbols are used as those described in the captions of Figs. 18.2 and 18.3. Potential transcriptional regulators (Sql1 and Hql1) which could mediate inhibitory functions of cAMP signalling are indicated as *lightly shaded circles*. *Arrows* indicate regulation of the Crk1 N and C terminus by MAPKK Fuz7 and MAPK Kpp2 (*encircled Ps*) respectively

et al. 2004; Fig. 18.4). Transcription of *crk1* is regulated antagonistically by PKA and MAPK signalling, and overexpression of *crk1* is sufficient to trigger filament formation (Garrido and Pérez-Martín 2003; Fig. 18.4). Potential transcriptional regulators for *crk1* could be Hgl1 and Sql1. Hgl1, a PKA target *in vitro*, was identified as suppressor for the filamentous phenotype of *adr1Δ* mutants (Dürrenberger et al. 2001). Sql1, a functional homologue of the transcriptional repressor Ssn6p from *S. cerevisiae*, has been shown to suppress the phenotype of an activated cAMP pathway (Loubradou et al. 2001; Fig. 18.4).

The induction of filaments by Crk1 is also regulated posttranscriptionally by Fuz7/Ubc5- and Kpp2/Ubc3-dependent phosphorylation of the N- and C-terminal domains of Crk1 respectively. Interestingly, the MAPK sites in the C terminus are dispensable for Crk1 function during mating (Garrido et al. 2004; Figs. 18.3 and 18.4). This results in a complex mode of regulation in which the N-terminal part of Crk1, which has MAPK activity, is a MAPKK target whereas the C terminus is a MAPK target (Fig. 18.4).

Although extensive progress has been made in identifying components of the PKA/MAPK signalling network, the signals perceived via these pathways, besides pheromones, are waiting to be uncovered. Promising candidates for such signals are specific lipids and the pH of the environment, which were shown to stimulate this signalling network during filament formation (Klose et al. 2004; Martinez-Espinoza et al. 2004; see below).

C. Signalling Network During Pathogenic Development

In addition to their role in regulating mating and morphogenesis, PKA and MAPK signalling are important determinants of pathogenic development. With the exception of the G protein β subunit Bpp1, all components of cAMP signalling identified to date are essential for pathogenicity (Garcia-Pedrajas et al. 2004; Müller et al. 2004). Mutants with different levels of perturbation of cAMP signalling arrest at specific points of the pathogenicity program. Filamentously growing mutant strains affected in the cAMP pathway, such as *gpa3Δ*, *uac1Δ* and *adr1Δ*, do not cause any disease symptoms (Gold et al. 1994; Regenfelder et al. 1997; Dürrenberger et al. 1998). Thus, the induction of filamentous growth through low PKA activity is not sufficient to trigger pathogenic development, stressing the need for an active bE/bW heterodimer during this process (see below).

Mutants such as $ubc1^{R312Q}$, $gpa3^{Q206L}$ or *hgl1Δ* which reflect increased PKA activity elicit the formation of tumours devoid of black teliospores (Krüger et al. 2000; Dürrenberger et al. 2001). Interestingly, $gpa3^{Q206L}$-expressing strains form abnormal tumours with shoot-like structures (Krüger et al. 2000). These results indicate that fungal development during growth *in planta* requires tightly regulated levels of PKA activity, and inadequate levels of PKA activity affect communication with the host during tumour differentiation.

Given the fact that MAPK signalling is a central regulator for mating and morphogenesis, it was no surprise that mutants affected in MAPK signalling are affected also in pathogenicity. *kpp4Δ* and *fuz7Δ* strains are non-pathogenic (Banuett and Herskowitz 1994; Müller et al. 2003b), whereas *kpp2Δ* mutants are reduced in virulence (Müller et al. 1999). This apparent discrepancy was resolved when a third MAP kinase, *kpp6*, was identified as bE/bW-regulated MAPK with partially overlapping functions to *kpp2* (Fig. 18.2). *kpp2Δ*/*kpp6Δ* double mutants are non-pathogenic, supporting the notion that these two MAPKs exert important but redundant functions during pathogenic development (Brachmann et al. 2003). By expressing mutant versions of *kpp2* and *kpp6* which can no longer be activated by their corresponding MAPKK, it was furthermore demonstrated that Kpp2 is required for appressorium formation whereas Kpp6 functions at a later stage during plant penetration (Brachmann et al. 2003). Given the finding that pheromones and receptors are dispensable for pathogenic development after cell fusion, the MAP kinase module, which transmits the pheromone signal, must be activated by different input signals during pathogenesis. It was recently shown that *U. maydis* cells respond to the presence of fatty acids or triglycerides by switching to filamentous growth. Since mutations in *ras2*, *fuz7* and *ubc3* block this reaction (Klose et al. 2004), the MAP kinase module transmitting the pheromone signal must be required for this response. In addition, low pH signalling was also demonstrated to involve this module (Martinez-Espinoza et al. 2004). It will be a future challenge to determine whether these signals are indeed used in communication with the plant.

III. Transcriptional Cascades for Pathogenic Development

The multiallelic *b* locus encodes a pair of homeodomain transcription factors (bE and bW) which are active only as heterodimers with monomers encoded by different alleles (e.g. bE1/bW2; Kronstad and Leong 1990; Schulz et al. 1990; Gillissen et al. 1992; Kämper et al. 1995). The active bE/bW heterodimer is formed after two haploid cells have fused, and triggers a transcriptional cascade resulting in the formation of infectious dikaryotic hyphae. Since structural aspects of the *b* locus and the encoded proteins are covered in the article by Casselton and Challen (Chap. 17, this volume), we will restrict ourselves to the regulatory cascade triggered by the bE/bW complex (Fig. 18.5).

The central role of the bE/bW heterodimer for pathogenic development was demonstrated by the construction of haploid strains with a hybrid *b* locus expressing different alleles of *bE* and *bW* (Bölker et al. 1995). Such strains are solopathogenic, i.e. they are able to infect the host plant without a mating partner. Solopathogenic haploid strains have become an invaluable resource for studying gene functions independently of fusion events. At the same time, such strains serve

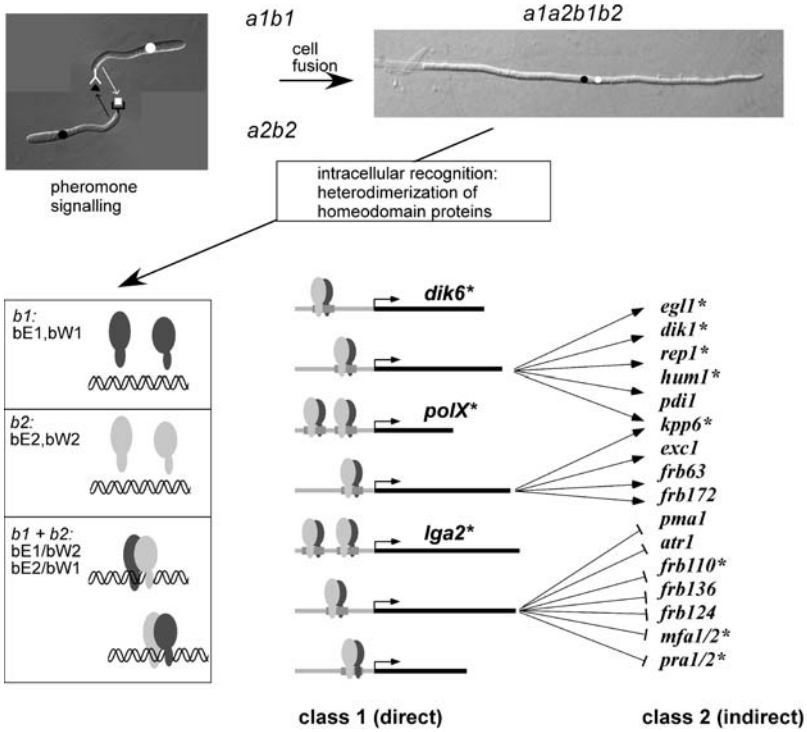

Fig. 18.5. Function of the *a* and *b* mating type loci during mating. The *a1* and *a2* alleles encode pheromones and receptor molecules which regulate directed growth and fusion of sporidia. After cell fusion, the bE and bW homeodomain proteins encoded by the multiallelic *b* locus form a heterodimeric complex, but only when derived from different alleles. The bE/bW heterodimer binds to a conserved sequence motif (*bbs*) in the promoter regions of class 1 genes. Class 2 genes are indirectly regulated by *b* via regulatory proteins which are encoded by class 1 genes. *b*-induced and *b*-repressed genes are indicated by *arrowheads* and *bars* respectively. An *asterisks* indicates that deletion mutants have been generated. With the exception of *kpp6*, none of these genes are required for pathogenicity. The following functions can be assigned to proteins encoded by *b*-dependent genes: *polX* (*frb 52*), hypothetical DNA polymerase X; *dik6*, no similarities; *lga2*, interferes with mitochondrial fusion; *egl1*, endoglucanase; *dik1*, no similarities; *rep1*, repellent; *hum1*, hydrophobin; *pdi1* (*frb23*), hypothetical protein disulfide isomerase; *kpp6*, MAPK; *exc1* (*frb133*), hypothetical exochitinase; *frb63*, unknown; *ant1* (*frb172*), hypothetical K^+/H^+ antiporter; *pma1* (*frb323*), hypothetical plasma membrane ATPase; *atr1* (*frb34*), hypothetical acyl transferase; *frb110*, unknown (homology to potential polypeptide from *Neurospora crassa*); *cap1* (*frb136*), unknown homology to capsule-associated protein from *Cryptococcus neoformans*, *frb124*, unknown; *mfa1/2*, pheromone precursor; *pra1/2*, pheromone receptor (Bohlmann et al. 1994; Schauwecker et al. 1995; Urban et al. 1996a,b; Wösten et al. 1996; Brachmann et al. 2001; Bortfeld et al. 2004)

for unbiased genetic approaches to pathogenesis (Bölker et al. 1995).

Initial approaches to isolate genes regulated through the bE/bW heterodimer relied on differential methods in which the *b*-induced filamentous form was compared with the yeast-like form. With the construction of *U. maydis* strains which harbour an inducible combination of *bE1/bW2* genes, it became possible to time-resolve the *b*-dependent regulatory cascade (Brachmann et al. 2001). Using such *b*-inducible strains, a large number of *b*-regulated genes have been identified by RNA fingerprinting. So far, only three of the *b*-dependent genes have been shown to possess binding sites for the bE/bW heterodimer in their respective promoter regions, implicating a direct regulation by the bE/bW heterodimer: *lga2*, a gene located in the *a2* mating type locus which appears to be involved in mitochondrial fusion, *polX*, encoding a protein with weak similarities to DNA polymerase X, and *dik6*, a putative membrane protein without similarity to known proteins (Bohlmann et al. 1994; Romeis et al. 2000; Brachmann et al. 2003; Bortfeld et al. 2004). The finding that the majority of *b*-controlled genes are indirectly regulated suggests that the bE/bW heterodimer triggers a regulatory cascade with only a limited number of genes being direct targets (termed class 1 genes, Fig. 18.5). It follows that within this class there must be genes with regulatory function which are in turn required for the induction or repression of the indirect bE/bW targets (termed class 2 genes, Fig. 18.5). Deletion analysis revealed that neither the three directly regulated genes, nor any of the other, indirectly regulated genes affected pathogenicity, with the exception of the MAP kinase *kpp6* (see above). One explanation is that *b*-regulated genes required for cell wall structure, like the repellent Rep1 (Wösten et al. 1996), the hydrophobin Hum2 (Bohlmann 1996), cell wall-modifying enzymes like the potential exochitinase Exc1 (Brachmann et al. 2001) and the endoglucanase Egl1 (Schauwecker et al. 1995), are members of gene families with redundant functions. In this scenario, single gene knockouts will be without effect. However, class 1 genes with a regulatory function are expected to play crucial roles for pathogenic development, as they would presumably co-regulate larger subsets of b-regulated genes.

With the availability of the genomic sequence of *U. maydis* (http://www.broad.mit.edu/annotation/fungi/ustilago_maydis/), it became possible to design genome-wide DNA arrays (J. Kämper and R. Kahmann, unpublished data). The currently available Affymetrix high-density arrays represent approximately 93% of all *U. maydis* genes. These arrays were used to monitor changes in gene expression after *b* induction over a 12-h time course leading to the identification of about 250 *b*-responsive genes, of which about 50% are either up-regulated or down-regulated (M. Scherer and J. Kämper, unpublished data). The functional classification of these genes allowed to visualize that cellular processes like the restructuring of the cell wall as well as lipid metabolism are controlled by the *b* mating type locus. Another large number of *b*-regulated genes are involved in the cell cycle, mitosis and DNA replication, which is consistent with the observation that *b* induction leads to cell cycle arrest until the fungus has infected the plant. Systematic analysis of *b*-regulated genes with potential regulatory function has already allowed identification of new pathogenicity factors. Of particular interest is a *b* dependently expressed transcription factor required for both filamentous growth as well as pathogenic development. Microarray experiments revealed that this transcription factor indeed controls expression of a subset of class 2 genes (M. Scherer and J. Kämper, unpublished data).

Insight into *b*-dependent processes was also possible by comparing sequences from cDNA clones obtained from two different developmental stages, namely, germinating teliospores and a filamentous diploid strain heterozygous for both mating type loci. From differences in expression in these stages, it can be inferred, for instance, that the filament is involved in nutrient acquisition, and that germinating teliospores may be utilizing stored metabolites. Similarly, more ESTs corresponding to ABC transporters were found in the filament-specific library, which could indicate that some of these proteins may be required to eliminate toxic compounds produced by the host (Sacadura and Saville 2003; Nugent et al. 2004).

Independent approaches to identify pathogenicity factors have relied on the identification of fungal genes differentially regulated during fungal growth in planta. This has led to the discovery of chromosomal loci containing co-expressed genes. The so-called *pig* (plant-induced gene) locus, identified by an enhancer-trapping mutagenesis approach, consists of 11 genes of which five are differentially regulated in planta (Aichinger et al. 2003). *pig4* and *pig6* encode proteins with similarities to a membrane transporter

and a multi-drug resistance protein respectively (Aichinger et al. 2003). The *mig1* and *mig2* (maize-induced) gene clusters comprise two and five highly homologous co-regulated genes respectively, which are highly expressed during the biotrophic stage and encode small, cysteine-rich secreted proteins sharing no homologies to known proteins (Basse et al. 2002b). *udet1* was also identified as being up-regulated during fungal growth in planta, and was subsequently shown to encode a steroid 5α reductase by complementing a *det2* mutant of *Arabidopsis thaliana*. In plants, these enzymes are needed for the synthesis of brassinosteroids, hormones which control plant development. However, *udet1* mutants were unaffected in pathogenic development (Basse et al. 2002a), which rules out that Udet1 is involved in synthesis of the trigger for tumour induction. Deletion of either the entire *pig* or the entire *mig2* cluster was also without effect on pathogenicity (Basse et al. 2002b; Aichinger et al. 2003).

The *mig* genes as well as *ssp1*, a gene encoding a potential dioxygenase predominantly expressed in teliospores (Huber et al. 2002), are negatively regulated by the histone deacetylase Hda1 (Huber et al. 2002; Torreblanca et al. 2003). Hda1 is thought to form a complex with a previously identified regulatory protein, Rum1 (Quadbeck-Seeger et al. 2000), and was shown to regulate a distinct set of genes presumably by modulation of their chromatin structure (Reichmann et al. 2002). The deletion of either *rum1* or *hda1* leads to a defined developmental block prior to the formation of teliospores (Quadbeck-Seeger et al. 2000; Reichmann et al. 2002). It is likely that modulation of chromatin structure represents an additional level of global gene regulation which is used for co-regulating specific sets of genes.

Preliminary data from array analyses indicate that a set of more than 500 genes is plant-regulated. This set includes various genes which encode transporters for carbon compounds and amino acids, possibly reflecting adaptation of *U. maydis* to conditions encountered in the plant environment (M. Vranes and J. Kämper, unpublished data). Research on those genes whose expression is confined to the biotrophic phase is currently focused on their regulators (Farfsing et al. 2005), as it is anticipated that deletions in the corresponding regulatory genes will affect pathogenic development. We expect that the systematic analysis of these genes will provide important insights into the process of fungal adaptation during biotrophic growth.

IV. Small GTPase Networks for Cytokinesis and Dimorphism

GTP-binding proteins of the Ras superfamily act as molecular switches and are involved in diverse biological processes (Bourne et al. 1990). They exist in two different states, the active GTP-bound conformation and the inactive GDP-bound form. Switching between active and inactive forms is regulated by interaction with specific accessory proteins. Guanine nucleotide exchange factors (GEFs) activate Ras-like GTPases by catalysing the release of GDP, which is then immediately replaced with GTP (Cherfils and Chardin 1999). Inactivation of these molecular switches requires interaction with GTPase activating proteins (GAPs) which stimulate the low intrinsic GTPase activity resulting in the hydrolysis of GTP to GDP (Bernards and Settleman 2004). Most members of the family of Ras-like proteins are associated with cellular membranes (Fig. 18.6). Membrane binding is mediated by hydrophobic membrane anchors consisting of a C-terminal farnesylation, as in the case of Ras, or geranylgeranylation, as in the case of Rho/Rac proteins (Zhang and Casey 1996). An additional level of regulation occurs by Rho/Rac-specific guanine nucleotide dissociation inhibitors (Rho-GDIs) which can bind to the prenylated C terminus. Binding of Rho-GDI results in the relocalization of GTPases from the membrane to the cytoplasm, by sequestering the hydrophobic membrane anchor (Olofsson 1999).

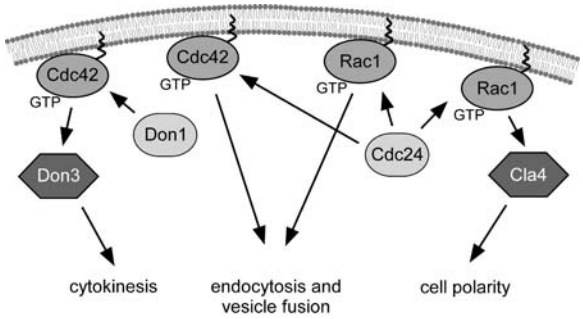

Fig. 18.6. Signalling network involving small GTPases of the Rho/Rac family in *U. maydis*. The highly related GTPases Rac1 and Cdc42 trigger both specific and common pathways. Potential GEFs (Don1 and Cdc24) and target kinases (Don3 and Cla4) are indicated by *ovals* and *hexagons* respectively. The distinct phenotypes of Cdc42 and Rac1 deletion mutants stress their involvement in specific as well as overlapping signalling events

In *U. maydis*, two members of the Ras family, Ras1 and Ras2, and four Rho/Rac proteins, Rho1, Rho3, Rac1 and Cdc42, have been described to date (Lee and Kronstad 2002; Weinzierl et al. 2002; Müller et al. 2003a). Inspection of the genome sequence revealed the presence of a third Rho protein, Rho2. Whereas Ras proteins in *U. maydis* appear to be mainly involved in MAP kinase- and cAMP-dependent signalling (see above), small GTPases of the Rho/Rac family have been implicated in signalling modules regulating cytokinesis, cell polarity, and vesicle trafficking (Fig. 18.6).

A specific function for members of the Rho family of small GTPases became apparent when mutants were identified which displayed a dramatic cytokinesis defect. *don1* and *don3* mutants have a normal cell shape and are not affected in nuclear division. However, the last step of cytokinesis, the physical separation of mother and daughter cells, is completely blocked. Mutant cells form tree-like clusters in liquid medium and donut-shaped colonies on agar plates (Weinzierl et al. 2002). Closer inspection of this peculiar phenotype revealed that cell separation in *U. maydis* requires the consecutive formation of two septa. Between these two septa, normally a vacuolar fragmentation zone is delimited in which the breakdown of cell wall occurs (O'Donnell and McLaughlin 1984; Weinzierl et al. 2002). Complementation of the *don1* and *don3* mutants allowed the identification of the Rho/Rac-specific GEF Don1 and the Ste20-like protein kinase Don3 (Weinzierl et al. 2002). In yeast two-hybrid assays, Cdc42 showed a weak but significant interaction with both Don1 and Don3, suggesting that Cdc42 acts as a central regulator in this signalling module (Weinzierl et al. 2002; Fig. 18.6). This interpretation was substantiated by the observation that expression of dominant active Cdc42 was able to suppress the phenotype of *don1* but not that of *don3* mutant cells (Mahlert et al., 2005). Surprisingly, deletion of *cdc42* did not affect viability of *U. maydis* cells, although *cdc42* was essential in most organisms tested so far. *cdc42Δ* mutants displayed a cell separation defect identical to that of the previously described *don* mutants, supporting the view that a Cdc42-containing signalling module plays a pivotal role in the regulation of cytokinesis. *rac1* mutants are viable but are affected in cell morphology as well as cell polarity and display a phenotype similar to that of mutants deleted for the Ste20-like kinase Cla4 (Leveleki et al. 2004). However, the *cdc42*/*rac1* double mutant was synthetically lethal, indicating that these proteins share at least one essential function (Mahlert et al., 2005). Interestingly, expression of constitutive active Rac1 also proved to be lethal. Upon induction, cells stop budding, become highly vacuolated, and eventually burst. These events are accompanied by massive isotropic deposition of cell wall material, indicating that active Rac1 most probably directly stimulates cell wall growth. Surprisingly, overexpression of wild-type Rac1 in haploid cells induced a switch to filamentous growth, similar to that seen after *b* induction (Mahlert et al., 2005). Moreover, it was shown that Rac1 is required for filamentous growth and pathogenicity induced by the active bW/bE heterodimer (Mahlert et al., 2005). Thus, Rac1 is both necessary and sufficient for filament formation in *U. maydis*. These results can be explained by assuming that a Rac1-specific GEF is located at the very tip of cells. The basal activity of this GEF could be sufficient to activate Rac1 if this protein is present at sufficiently high concentrations. Rac1 would stimulate deposition of cell wall material, and this would elicit hyphal tip growth. For the wild-type situation, this would imply that accumulation of activated Rac1 at the tip is directly or indirectly regulated through the bE/bW heterodimer.

The existence of both a Cdc42 and a Rac1 homolog in *U. maydis* allows one to study their distinct contributions to cellular regulatory networks (Fig. 18.6). The biological function of small GTPases can be determined either by specific activation through upstream regulators or at the level of downstream effectors. With the exception of Don1, only little is known about Rho/Rac-specific GEFs. The *U. maydis* genome contains a homolog of the yeast Rho/Rac-specific guanine nucleotide exchange factor (GEF) *cdc24* gene, which appears to be essential (M. Bölker, unpublished data). Since single deletions of either *cdc42* or *rac1* are viable, Cdc24 is likely to be responsible for the activation of both GTPases (Fig. 18.6). Several candidates for downstream targets of Rho/Rac GTPases have been identified in the *U. maydis* genome by the presence of the characteristic CRIB domain. This conserved protein motif has been first identified as a minimum Cdc42/Rac interactive binding (CRIB) region in the mammalian effector protein p65PAK, and was found subsequently in many other potential effectors of Cdc42 or Rac1 (Burbelo et al. 1995). Among these are the previously described Cla4 kinase, which has been isolated in a screen for morphological mutants (Leveleki et al. 2004), and the

Ste20 homolog Smu1 (Smith et al. 2004). *cla4* mutants display a phenotype similar to that of *rac1* mutants, implying that Cla4 might act as effector for Rac1 (Leveleki et al. 2004). Deletion of *smu1* resulted in a delayed mating response in a mating type-specific manner, and also in a substantial reduction of disease (Smith et al. 2004). The genome of *U. maydis* contains two additional genes coding for proteins with a CRIB domain. These are homologues of the Skm1 protein kinase from yeast, and a protein related to the human N-WASP protein which has been identified in mammalian systems as Wilskott-Aldrich syndrome protein involved in organizing the actin cytoskeleton (Stradal et al. 2004). Testing isolated CRIB domains of all these proteins in the yeast two-hybrid system revealed that the CRIB domain of Cla4, Smu1 and Skm1 are recognized by both Cdc42 and Rac1, whereas the CRIB domain of the *U. maydis* WASP protein is specific for Cdc42 (Leveleki et al. 2004). Since *cdc42* and *rac1* deletion mutants are viable in *U. maydis*, this organism provides a unique opportunity to study biological functions of individual players in this important regulatory network.

V. Cytoskeletal Networks for Morphology

In *U. maydis*, the transition from a budding yeast form to the filamentous conjugation hyphae marks the beginning of the pathogenic cycle (Banuett 1995; Kahmann et al. 1999). Conjugation hyphae grow in a directed and polarized fashion towards each other, fuse their cytoplasm and form the dikaryotic hypha, which consists of a single tip-growing cell which leaves empty cell sections behind while scanning the plant surface for a site of invasion (see above). Polarized growth is the key factor in these morphogenetic transitions, and is therefore essential for successful infection of the host plant.

Tip growth of polarized fungal hyphae requires anterograde membrane traffic to the apical cell pole (Gow 1995; Geitmann and Emons 2000). It is thought that intracellular vesicle and organelle transport is mediated by filaments of the cytoskeleton which provide the tracks along which mechanoenzymes, the so-called molecular motors, transport their cargo for delivery to the hyphal tip (Steinberg 2000; Xiang and Plamann 2003). In fungi, indications exist that F-actin as well as microtubules participate in membrane transport (Heath 1995), though the importance for each system is weighed differently in each fungus. Numerous lines of evidence indicate that F-actin has fundamental roles in polarized growth of fungi (Harold 1990; Heath 1995). Transport along the actin cytoskeleton allows the delivery of wall vesicles to the growing apex where exocytosis takes place (Gow 1995). F-actin is also involved in fungal endocytosis (Kübler and Riezman 1993; Kaksonen et al. 2003; Huckaba et al. 2004).

U. maydis cells contain long actin cables (Banuett and Herskowitz 2002; Weber et al. 2003) which are generally thought to support the tipward traffic of wall compounds needed for proper hyphal growth (Harold 1990; Heath 1995; Ayscough et al. 1997). Disruption of F-actin by latrunculin A led to severe defects in morphogenesis in yeast-like sporidia, and affected the formation and directed growth of conjugation hyphae as well as the development of dikaryotic hyphae. Moreover, it was found that F-actin is essential for cell fusion during mating of compatible cells (Fuchs et al. 2005). In addition, class V myosin Myo5, which most likely transports membranous vesicles along F-actin filaments, was found to be required for hyphal growth as well as pathogenic development (Weber et al. 2003). In higher eukaryotes as well as in the yeasts *S. cerevisiae* and *S. pombe*, myosin V is thought to participate in vesicle transport (Johnston et al. 1991; Win et al. 2001), suggesting that Myo5 might deliver vesicles containing cell wall components to the growing tip. In *S. cerevisiae*, myosin V is responsible for transport of a chitin synthase (Santos and Snyder 1997), and enzymes of this class might also be required for tip growth of *U. maydis* hyphae. Indeed, it was recently demonstrated that five of the eight chitin synthases present in *U. maydis* localize to the growth region of yeast-like and hyphal cells, and their localization depends on an intact F-actin cytoskeleton (Weber et al. 2005). These data reinforce the essential role for F-actin-based transport in polarized growth of *U. maydis*.

In contrast to the well-established role of F-actin in fungal morphogenesis, the role of microtubules in fungi is less clear (Heath 1995). Microtubules have no obvious function in *Candida albicans* (Yokoyama et al. 1990) and *S. cerevisiae* (Huffaker et al. 1988), whereas microtubule integrity is necessary for fast tip growth in *Aspergillus nidulans* (Horio and Oakley 2005). Moreover, disrupting microtubules induces multiple growth sites in

Neurospora crassa germlings (That et al. 1988), and leads to branching in *S. pombe* (Sawin and Nurse 1998).

In *U. maydis*, yeast-like interphase cells contain long microtubules which reach from the mother cell into the growing bud (Steinberg et al. 2001; Banuett and Herskowitz 2002; Fig. 18.7). Live cell imaging of GFP-α-tubulin revealed that microtubules are surprisingly dynamic, and suggested that the fast-growing plus-ends extend to the cell poles whereas the minus-ends are focused at the bud neck (Steinberg et al. 2001). Further studies demonstrated that gamma-tubulin, a tubulin-isoform which participates in the formation of microtubules (Oakley and Akkari 1999), localizes at two spherical structures which are often in contact with microtubules (Steinberg et al. 2001; Straube et al. 2003). Observation of a YFP-fusion to Peb1, an EB1-like protein which labels growing plus-ends of microtubules, indicated that microtubules are nucleated at the bud neck and elongate towards the cell poles (Straube et al. 2003). These data suggest that the neck region contains cytoplasmic microtubule-organizing centres.

However, the situation is more complicated than this, as molecular motors participate in microtubule organization. In a screen for polarity mutants, an ER-resident SERCA (sarcoplasmic-endoplasmic calcium ATPase) was identified which is thought to shuffle cytoplasmic calcium into the ER stores (Adamikova et al. 2004). In *eca1* null mutants, this calcium transport into the ER is impaired, resulting in increased cytoplasmic calcium concentration, which most likely affects dynein activity. Dynein is localized at the plus-ends of microtubules (I. Manns and G. Steinberg, unpublished data), where it is thought to regulate the dynamics of tubulin assembly and disassembly. It was found that *eca1Δ* mutants contain longer and unordered microtubules, which is the likely cause for disturbed secretion leading, in turn, to the observed defects in cellular morphogenesis (Adamikova et al. 2004).

In *U. maydis*, microtubules were found to affect ER motility (Wedlich-Söldner et al. 2002a) and participate in the opening of the nuclear envelope during "open" mitosis (Straube et al. 2005). In addition, it was shown that early endosomes move along microtubules, and that proper function of these endosomes is required for cellular morphogenesis in yeast-like cells and hyphae (Wedlich-Söldner et al. 2000). The motility of endosomes depends on Kin3, which is a fungal member of the kinesin-3 family (Wedlich-Söldner et al. 2002b), and cytoplasmic dynein. Both motors utilize the polarity of microtubules to transport endosomes in opposite directions, and it was demonstrated that Kin3-dependent transport to microtubule plus-ends is required for cell separation and bud site selection (Wedlich-Söldner et al. 2002b). However, although *kin3* null mutants are heavily impaired in motility of early endosomes, they maintain their mor-

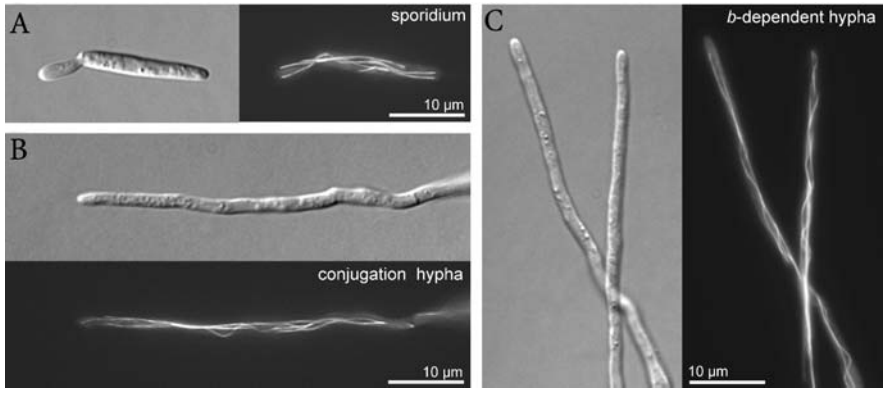

Fig. 18.7. Microtubules in sporidia and hyphae of *U. maydis*. **A** Yeast-like cells grow by polar budding and microtubules, stained with GFP-α-tubulin, reach from the mother cell into the growing daughter cell. **B** Upon pheromone treatment, yeast-like sporidia switch from budding to filamentous growth. This switch depends on the *a* locus (*a*-dependent hypha = conjugation hyphae or mating tube). Again, long microtubules reach into the growing apex, suggesting that they participate in tip growth. **C** Under natural conditions, compatible conjugation hyphae fuse to form a bE1/bW2 transcription factor-dependent dikaryotic hypha. This growth mode can be induced by experimental induction of *bE1/bW2* expression (Brachmann et al. 2001), which turns yeast-like cells directly into *b*-dependent hyphae which also contain long microtubule tracks. *Bars*: 10 µm

phology and are affected only in cell separation (Wedlich-Söldner et al. 2002b). This indicates that functional endosomes are required for morphogenesis whereas their motility is not. Indeed, recent studies clearly demonstrate that microtubules have only minor roles in determining shape and bud formation in yeast-like cells (Fuchs et al. 2005), whereas they are essential in nuclear migration and mitosis (Steinberg et al. 2001; Straube et al. 2001). With respect to the filamentous growth forms, however, the situation is different. The overall distribution of microtubules in yeast-like cells, conjugation hyphae and bE/bW-induced filaments appears very similar (Fig. 18.7). However, in the absence of microtubules, growth of conjugation hyphae stops at a length of about 50–60 µm, indicating that microtubules are specifically required for long-distance transport. Kin2, a kinesin-1 member in *U. maydis* (Lehmler et al. 1997), cooperates with Myo5 in polar growth, and evidence for a role of both motors in polarized secretion exists (Schuchardt et al. 2005). These data suggest that microtubules and F-actin, in combination with associated components such as molecular motors, need to cooperate in a functional network to promote filamentous growth.

VI. Concluding Remarks

The elucidation of signalling networks which operate during mating and morphogenesis in *U. maydis* has solved a number of old mysteries but has raised even more new questions. The most prominent ones concern the biotrophic phase: what are the signalling inputs provided by the plant, and how is the fungus able to juggle with three different types of MAP kinases likely to be connected to the same upstream components in determining temporal and spatial control of signalling? How are the signalling pathways interconnected during pathogenic development? How is the plethora of different shapes adopted by the fungus during the biotrophic phase determined and controlled by cytoskeletal elements? What is the trigger for the change in growth direction during the initial phase of infection? Which are the genes allowing *U. maydis* to adapt to its new environment in the host, and why is it that *U. maydis* needs the host plant at all to complete its sexual cycle? Although these questions all focus on the fungal partner, it is quite clear that the plant host is still a black box and needs to be included much more extensively in future investigations. How is host specificity determined? Which compounds are provided by the plant for the proliferation of dikaryotic hyphae? What are the molecular events underlying tumour development? It is expected that the available genome sequence of both partners will greatly speed up the discovery of genes involved in these processes. Since mutants can be efficiently generated in *U. maydis* through reverse genetics (Brachmann et al. 2004; Kämper 2004), and since large collections of transposon-tagged maize lines exist, the chances are high for new insights into this fascinating interaction of a fungal pathogen with its host.

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References

Adamikova L, Straube A, Schulz I, Steinberg G (2004) Calcium signaling is involved in dynein-dependent microtubule organization. Mol Biol Cell 15:1969–1980

Aichinger C, Hansson K, Eichhorn H, Lessing F, Mannhaupt G, Mewes W, Kahmann R (2003) Identification of plant-regulated genes in *Ustilago maydis* by enhancer-trapping mutagenesis. Mol Gen Genomics 270:303–314

Andrews DL, Egan JD, Mayorga ME, Gold SE (2000) The *Ustilago maydis ubc4* and *ubc5* genes encode members of a MAP kinase cascade required for filamentous growth. Mol Plant Microbe Interact 13:781–786

Ayscough KR, Stryker J, Pokala N, Sanders M, Crews P, Drubin DG (1997) High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. J Cell Biol 137:399–416

Banuett F (1995) Genetics of *Ustilago maydis*, a fungal pathogen that induces tumors in maize. Annu Rev Genet 29:179–208

Banuett F, Herskowitz I (1994) Identification of *fuz7*, a *Ustilago maydis* MEK/MAPKK homolog required for *a*-locus-dependent and -independent steps in the fungal life cycle. Genes Dev 8:1367–1378

Banuett F, Herskowitz I (1996) Discrete developmental stages during teliospore formation in the corn smut fungus, *Ustilago maydis*. Development 122:2965–2976

Banuett F, Herskowitz I (2002) Bud morphogenesis and the actin and microtubule cytoskeletons during budding in the corn smut fungus, *Ustilago maydis*. Fungal Genet Biol 37:149–170

Barrett KJ, Gold SE, Kronstad JW (1993) Identification and complementation of a mutation to constitutive filamentous growth in *Ustilago maydis*. Mol Plant Microbe Interact 6:274–283

Basse CW, Kerschbamer C, Brustmann M, Altmann T, Kahmann R (2002a) Evidence for a *Ustilago maydis* steroid 5α-reductase by functional expression in *Arabidopsis det2-1* mutants. Plant Physiol 129:717–732

Basse CW, Kolb S, Kahmann R (2002b) A maize-specifically expressed gene cluster in *Ustilago maydis*. Mol Microbiol 43:75–93

Bernards A, Settleman J (2004) GAP control: regulating the regulators of small GTPases. Trends Cell Biol 14:377–385

Bohlmann R (1996) Isolierung und Charakterisierung von filamentspezifisch exprimierten Genen aus *Ustilago maydis*. PhD Thesis, Fakultät für Biologie, Ludwig-Maximilians-Universität München, Germany

Bohlmann R, Schauwecker F, Basse C, Kahmann R (1994) Genetic regulation of mating and dimorphism in *Ustilago maydis*. In: Daniels MJ (ed) Advances in Molecular Genetics of Plant-Microbe Interactions, vol 3. Kluwer, Dordrecht, pp 239–245

Bölker M (2001) *Ustilago maydis* – a valuable model system for the study of fungal dimorphism and virulence. Microbiology 147:1395–1401

Bölker M, Urban M, Kahmann R (1992) The *a* mating type locus of *U. maydis* specifies cell signaling components. Cell 68:441–450

Bölker M, Genin S, Lehmler C, Kahmann R (1995) Genetic regulation of mating, and dimorphism in *Ustilago maydis*. Can J Bot 73:320–325

Bortfeld M, Auffarth K, Kahmann R, Basse CW (2004) The *Ustilago maydis a2* mating-type locus genes *lga2* and *rga2* compromise pathogenicity in the absence of the mitochondrial p32 family protein Mrb1. Plant Cell 16:2233–2248

Bourne HR, Sanders DA, McCormick F (1990) The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348:125–132

Brachmann A, Weinzierl G, Kämper J, Kahmann R (2001) Identification of genes in the bW/bE regulatory cascade in *Ustilago maydis*. Mol Microbiol 42:1047–1063

Brachmann A, Schirawski J, Müller P, Kahmann R (2003) An unusual MAP kinase is required for efficient penetration of the plant surface by *Ustilago maydis*. EMBO J 22:2199–2210

Brachmann A, König J, Julius C, Feldbrügge M (2004) A reverse genetic approach for generating gene replacement mutants in *Ustilago maydis*. Mol Gen Genomics 272:216–226

Brefort T, Müller P, Kahmann R (2005) The high-mobility-group domain transcription factor Rop1 is a direct regulator of *prf1* in *Ustilago maydis*. Eukaryot Cell 4:379–391

Burbelo PD, Drechsel D, Hall A (1995) A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases. J Biol Chem 270:29071–29074

Castillo-Lluva S, Garcia-Muse T, Pérez-Martín J (2004) A member of the Fizzy-related family of APC activators is regulated by cAMP and is required at different stages of plant infection by *Ustilago maydis*. J Cell Sci 117:4143–4156

Cherfils J, Chardin P (1999) GEFs: structural basis for their activation of small GTP-binding proteins. Trends Biochem Sci 24:306–311

Davey J (1998) Fusion of a fission yeast. Yeast 14:1529–1566

Dürrenberger F, Wong K, Kronstad JW (1998) Identification of a cAMP-dependent protein kinase catalytic subunit required for virulence and morphogenesis in *Ustilago maydis*. Proc Natl Acad Sci USA 95:5684–5689

Dürrenberger F, Laidlaw RD, Kronstad JW (2001) The *hgl1* gene is required for dimorphism and teliospore formation in the fungal pathogen *Ustilago maydis*. Mol Microbiol 41:337–348

Elion EA (2000) Pheromone response, mating and cell biology. Curr Opin Microbiol 3:573–581

Farfsing JW, Auffarth K, Basse CW (2005) Identification of *cis*-active elements in *Ustilago maydis mig2* promoters conferring high-level activity during pathogenic growth in maize. Mol Plant Microbe Interact 18:75–87

Feldbrügge M, Kämper J, Steinberg G, Kahmann R (2004) Regulation of mating and pathogenic development in *Ustilago maydis*. Curr Opin Microbiol 7:666–672

Froeliger EH, Leong SA (1991) The *a* mating-type alleles of *Ustilago maydis* are idiomorphs. Gene 100:113–122

Fuchs U, Manns I, Steinberg G (2005) The cytoskeleton has essential roles in dimorphic transition in the plant pathogen *Ustilago maydis*. Mol Biol Cell 16:2746–2758

Garcerá-Teruel A, Xoconostle-Cázares B, Rosas-Quijano R, Ortiz L, León-Ramírez C, Specht CA, Sentandreu R, Ruiz-Herrera J (2004) Loss of virulence in *Ustilago maydis* by *Umchs6* gene disruption. Res Microbiol 155:87–97

Garcia-Muse T, Steinberg G, Pérez-Martín J (2003) Pheromone-induced G2 arrest in the phytopathogenic fungus *Ustilago maydis*. Eukaryot Cell 2:494–500

Garcia-Pedrajas MD, Klostermann SJ, Andrews DL, Gold SE (2004) The *Ustilago maydis*–maize interaction. In: Talbot NJ (ed) Plant-Pathogen Interactions, vol 11. CRC Press, London, pp 166–201

Garrido E, Pérez-Martín J (2003) The *crk1* gene encodes an Ime2-related protein that is required for morphogenesis in the plant pathogen *Ustilago maydis*. Mol Microbiol 47:729–743

Garrido E, Voß U, Müller P, Kahmann R, Pérez-Martín J (2004) The induction of sexual development and virulence in the smut fungus *Ustilago maydis* on Crk1, a novel MAPK protein. Genes Dev 18:3117–3130

Geitmann A, Emons AM (2000) The cytoskeleton in plant and fungal cell tip growth. J Microsc 198:218–245

Gillissen B, Bergemann J, Sandmann C, Schroeer B, Bölker M, Kahmann R (1992) A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. Cell 68:647–657

Gold SE, Duncan G, Barrett K, Kronstad J (1994) cAMP regulates morphogenesis in the fungal pathogen *Ustilago maydis*. Genes Dev 8:2805–2816

Gold SE, Brogdon SM, Mayorga ME, Kronstad JW (1997) The *Ustilago maydis* regulatory subunit of a cAMP-dependent protein kinase is required for gall formation in maize. Plant Cell 9:1585–1594

Gow NAR (1995) Tip growth and polarity. In: Gow NAR, Gadd GM (eds) The growing fungus. Chapman and Hall, London, pp 99–134

Grimshaw SJ, Mott HR, Stott KM, Nielsen PR, Evetts KA, Hopkins LJ, Nietlispach D, Owen D (2004) Structure of the sterile alpha motif (SAM) domain of the *Saccharomyces cerevisiae* mitogen-activated protein kinase pathway-modulating protein STE50 and analysis of its interaction with the STE11 SAM. J Biol Chem 279:2192–2201

Harold FM (1990) To shape a cell: an inquiry into the causes of morphogenesis of microorganisms. Microbiol Rev 54:381–431

Hartmann HA, Kahmann R, Bölker M (1996) The pheromone response factor coordinates filamentous

growth and pathogenicity in *Ustilago maydis*. EMBO J 15:1632–1641

Hartmann HA, Krüger J, Lottspeich F, Kahmann R (1999) Environmental signals controlling sexual development of the corn smut fungus *Ustilago maydis* through the transcriptional regulator Prf1. Plant Cell 11:1293–1306

Heath IB (1995) The cytoskeleton. In: Gow NAR, Gadd GM (eds) The growing fungus. Chapman and Hall, London, pp 99–134

Horio T, Oakley BR (2005) The role of microtubules in rapid hyphal tip growth of *Aspergillus nidulans*. Mol Biol Cell 16:918–926

Huber SM, Lottspeich F, Kämper J (2002) A gene that encodes a product with similarity to dioxygenases is highly expressed in teliospores of *Ustilago maydis*. Mol Gen Genomics 267:757–771

Huckaba TM, Gay AC, Pantalena LF, Yang HC, Pon LA (2004) Live cell imaging of the assembly, disassembly, and actin cable-dependent movement of endosomes and actin patches in the budding yeast, *Saccharomyces cerevisiae*. J Cell Biol 167:519–530

Huffaker TC, Thomas JH, Botstein D (1988) Diverse effects of beta-tubulin mutations on microtubule formation and function. J Cell Biol 106:1997–2010

Johnston GC, Prendergast JA, Singer RA (1991) The *Saccharomyces cerevisiae* MYO2 gene encodes an essential myosin for vectorial transport of vesicles. J Cell Biol 113:539–551

Kaffarnik F, Müller P, Leibundgut M, Kahmann R, Feldbrügge M (2003) PKA and MAPK phosphorylation of Prf1 allows promoter discrimination in *Ustilago maydis*. EMBO J 22:5817–5826

Kahmann R, Kämper J (2004) *Ustilago maydis*: how its biology relates to pathogenic development. New Phytol 164:31–42

Kahmann R, Basse C, Feldbrügge M (1999) Fungal-plant signalling in the *Ustilago maydis*-maize pathosystem. Curr Opin Microbiol 2:647–650

Kaksonen M, Sun Y, Drubin DG (2003) A pathway for association of receptors, adaptors, and actin during endocytic internalization. Cell 115:475–487

Kämper J (2004) A PCR-based system for highly efficient generation of gene replacement mutants in *Ustilago maydis*. Mol Gen Genomics 271:103–110

Kämper J, Reichmann M, Romeis T, Bölker M, Kahmann R (1995) Multiallelic recognition: nonself-dependent dimerization of the bE and bW homeodomain proteins in *Ustilago maydis*. Cell 81:73–83

Klose J, de Sa MM, Kronstad JW (2004) Lipid-induced filamentous growth in *Ustilago maydis*. Mol Microbiol 52:823–835

Kronstad JW, Leong SA (1990) The *b* mating-type locus of *Ustilago maydis* contains variable and constant regions. Genes Dev 4:1384–1395

Krüger J, Loubradou G, Regenfelder E, Hartmann A, Kahmann R (1998) Crosstalk between cAMP and pheromone signalling pathways in *Ustilago maydis*. Mol Gen Genet 260:193–198

Krüger J, Loubradou G, Wanner G, Regenfelder E, Feldbrügge M, Kahmann R (2000) Activation of the cAMP pathway in *Ustilago maydis* reduces fungal proliferation and teliospore formation in plant tumors. Mol Plant Microbe Interact 13:1034–1040

Kübler E, Riezman H (1993) Actin and fimbrin are required for the internalization step of endocytosis in yeast. EMBO J 12:2855–2862

Lee N, Kronstad JW (2002) *ras2* controls morphogenesis, pheromone response, and pathogenicity in the fungal pathogen *Ustilago maydis*. Eukaryot Cell 1:954–966

Lee N, D'Souza C, Kronstad JW (2003) Of smuts, blasts, mildews, and blights: cAMP signalling in phytophatogenic fungi. Annu Rev Phytopathol 41:399–427

Lehmler C, Steinberg G, Snetselaar KM, Schliwa M, Kahmann R, Bölker M (1997) Identification of a motor protein required for filamentous growth in *Ustilago maydis*. EMBO J 16:3464–3473

Leveleki L, Mahlert M, Sandrock B, Bölker M (2004) The PAK family kinase Cla4 is required for budding and morphogenesis in *Ustilago maydis*. Mol Microbiol 54:396–406

Loubradou G, Brachmann A, Feldbrügge M, Kahmann R (2001) A homolog of the transcriptional repressor Ssn6p antagonizes cAMP signalling in *Ustilago maydis*. Mol Microbiol 40:719–730

Mahlert M, Leveleki L, Hlubek A, Sandrock B, Bölker M (2005) Rac1 and Cdc42 regulate hyphal growth and cytokinesis in the dimorphic fungus *Ustilago maydis*. Mol Microbiol (in press)

Martinez-Espinoza AD, Ruiz-Herrera J, Leon-Ramirez CG, Gold SE (2004) MAP kinase and cAMP signaling pathways modulate the pH-induced yeast-to-mycelium dimorphic transition in the corn smut fungus *Ustilago maydis*. Curr Microbiol 49:274–281

Mayorga ME, Gold SE (1999) A MAP kinase encoded by the *ubc3* gene of *Ustilago maydis* is required for filamentous growth and full virulence. Mol Microbiol 34:485–497

Mayorga ME, Gold SE (2001) The *ubc2* gene of *Ustilago maydis* encodes a putative novel adaptor protein required for filamentous growth, pheromone response and virulence. Mol Microbiol 41:1365–1379

Müller P, Aichinger C, Feldbrügge M, Kahmann R (1999) The MAP kinase Kpp2 regulates mating and pathogenic development in *Ustilago maydis*. Mol Microbiol 34:1007–1017

Müller P, Katzenberger JD, Loubradou G, Kahmann R (2003a) Guanyl nucleotide exchange factor Sql2 and Ras2 regulate filamentous growth in *Ustilago maydis*. Eukaryot Cell 2:609–617

Müller P, Weinzierl G, Brachmann A, Feldbrügge M, Kahmann R (2003b) Mating and pathogenic development of the smut fungus *Ustilago maydis* are regulated by one mitogen-activated protein kinase cascade. Eukaryot Cell 2:1187–1199

Müller P, Leibbrandt A, Teunissen H, Cubasch S, Aichinger C, Kahmann R (2004) The Gß-subunit-encoding gene *bpp1* controls cyclic-AMP signaling in *Ustilago maydis*. Eukaryot Cell 3:806–814

Nugent KG, Choffe K, Saville BJ (2004) Gene expression during *Ustilago maydis* diploid filamentous growth: EST library creation and analyses. Fungal Genet Biol 41:349–360

Oakley BR, Akkari YN (1999) Gamma-tubulin at ten: progress and prospects. Cell Struct Funct 24:365–372

O'Donnell KL, McLaughlin DJ (1984) Postmeiotic mitosis, basidiospore development, and septation in *Ustilago maydis*. Mycologia 76:486–502

Olofsson B (1999) Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. Cell Signal 11:545–554

Quadbeck-Seeger C, Wanner G, Huber S, Kahmann R, Kämper J (2000) A protein with similarity to the human retinoblastoma binding protein 2 acts specifically as a repressor for genes regulated by the *b* mating type locus in *Ustilago maydis*. Mol Microbiol 38:154–166

Regenfelder E, Spellig T, Hartmann A, Lauenstein S, Bölker M, Kahmann R (1997) G proteins in *Ustilago maydis*: transmission of multiple signals? EMBO J 16:1934–1942

Reichmann M, Jamnischek A, Weinzierl G, Ladendorf O, Huber S, Kahmann R, Kämper J (2002) The histone deacetylase Hda1 from *Ustilago maydis* is essential for teliospore development. Mol Microbiol 46:1169–1182

Romeis T, Brachmann A, Kahmann R, Kämper J (2000) Identification of a target gene for the bE-bW homeodomain protein complex in *Ustilago maydis*. Mol Microbiol 37:54–66

Sacadura NT, Saville BJ (2003) Gene expression and EST analyses of *Ustilago maydis* germinating teliospores. Fungal Genet Biol 40:47–64

Santos B, Snyder M (1997) Targeting of chitin synthase 3 to polarized growth sites in yeast requires Chs5p and Myo2p. J Cell Biol 136:95–110

Sawin KE, Nurse P (1998) Regulation of cell polarity by microtubules in fission yeast. J Cell Biol 142:457–471

Schauwecker F, Wanner G, Kahmann R (1995) Filament-specific expression of a cellulase gene in the dimorphic fungus *Ustilago maydis*. Biol Chem Hoppe Seyler 376:617–625

Schuchardt I, Aßmann D, Thines E, Schuberth C, Steinberg G (2005) Myosin-V, Kinesin-1, and Kinesin-3 cooperate in long-distance transport in hyphal growth of the fungus *Ustilago maydis*, Mol Biol Cell (in press)

Schulz B, Banuett F, Dahl M, Schlesinger R, Schäfer W, Martin T, Herskowitz I, Kahmann R (1990) The *b* alleles of *U. maydis*, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell 60:295–306

Smith DG, Garcia-Pedrajas MD, Hong W, Yu Z, Gold SE, Perlin MH (2004) A *ste20* homologue in *Ustilago maydis* plays a role in mating and pathogenicity. Eukaryot Cell 3:180–189

Snetselaar KM (1993) Microscopic observation of *Ustilago maydis* mating interactions. Exp Mycol 17:345–355

Snetselaar KM, Mims CW (1994) Light and electron microscopy of *Ustilago maydis* hyphae in maize. Mycol Res 98:347–355

Snetselaar KM, Bölker M, Kahmann R (1996) *Ustilago maydis* mating hyphae orient their growth toward pheromone sources. Fungal Genet Biol 20:299–312

Steinberg G (2000) The cellular roles of molecular motors in fungi. Trends Microbiol 8:162–168

Steinberg G, Wedlich-Söldner R, Brill M, Schulz I (2001) Microtubules in the fungal pathogen *Ustilago maydis* are highly dynamic and determine cell polarity. J Cell Sci 114:609–622

Stradal TE, Rottner K, Disanza A, Confalonieri S, Innocenti M, Scita G (2004) Regulation of actin dynamics by WASP and WAVE family proteins. Trends Cell Biol 14:303–311

Straube A, Enard W, Berner A, Wedlich-Söldner R, Kahmann R, Steinberg G (2001) A split motor domain in a cytoplasmic dynein. EMBO J 20:5091–5100

Straube A, Brill M, Oakley BR, Horio T, Steinberg G (2003) Microtubule organization requires cell cycle-dependent nucleation at dispersed cytoplasmic sites: polar and perinuclear microtubule organizing centers in the plant pathogen *Ustilago maydis*. Mol Biol Cell 14:642–657

Straube A, Weber I, Steinberg G (2005) A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. EMBO J 24:1674–1685

That TC, Rossier C, Barja F, Turian G, Roos UP (1988) Induction of multiple germ tubes in *Neurospora crassa* by antitubulin agents. Eur J Cell Biol 46:68–79

Torreblanca J, Stumpferl S, Basse CW (2003) Histone deacetylase Hda1 acts as repressor of the *Ustilago maydis* biotrophic marker gene *mig1*. Fungal Genet Biol 38:22–32

Urban M, Kahmann R, Bölker M (1996a) The biallelic *a* mating type locus of *Ustilago maydis*: remnants of an additional pheromone gene indicate evolution from a multiallelic ancestor. Mol Gen Genet 250:414–420

Urban M, Kahmann R, Bölker M (1996b) Identification of the pheromone response element in *Ustilago maydis*. Mol Gen Genet 251:31–37

Weber I, Aßmann D, Thines E, Steinberg G (2005) Development-specific roles of polar localizing chitin syntheses in the plant pathogen *Ustilago maydis*, Plant Cell (in press)

Weber I, Gruber C, Steinberg G (2003) A class-V myosin required for mating, hyphal growth, and pathogenicity in the dimorphic plant pathogen *Ustilago maydis*. Plant Cell 15:2826–2842

Wedlich-Söldner R, Bölker M, Kahmann R, Steinberg G (2000) A putative endosomal t-SNARE links exo- and endocytosis in the phytopathogenic fungus *Ustilago maydis*. EMBO J 19:1974–1986

Wedlich-Söldner R, Schulz I, Straube A, Steinberg G (2002a) Dynein supports motility of endoplasmic reticulum in the fungus *Ustilago maydis*. Mol Biol Cell 13:965–977

Wedlich-Söldner R, Straube A, Friedrich MW, Steinberg G (2002b) A balance of KIF1A-like kinesin and dynein organizes early endosomes in the fungus *Ustilago maydis*. EMBO J 21:2946–2957

Weinzierl G, Leveleki L, Hassel A, Kost G, Wanner G, Bölker M (2002) Regulation of cell separation in the dimorphic fungus *Ustilago maydis*. Mol Microbiol 45:219–231

Win TZ, Gachet Y, Mulvihill DP, May KM, Hyams JS (2001) Two type V myosins with non-overlapping functions in the fission yeast *Schizosaccharomyces pombe*: Myo52 is concerned with growth polarity and cytokinesis, Myo51 is a component of the cytokinetic actin ring. J Cell Sci 114:69–79

Wösten HA, Bohlmann R, Eckerskorn C, Lottspeich F, Bölker M, Kahmann R (1996) A novel class of small amphipathic peptides affect aerial hyphal growth and surface hydrophobicity in *Ustilago maydis*. EMBO J 15:4274–4281

Xiang X, Plamann M (2003) Cytoskeleton and motor proteins in filamentous fungi. Curr Opin Microbiol 6:628–633

Yokoyama K, Kaji H, Nishimura K, Miyaji M (1990) The role of microfilaments and microtubules in apical growth and dimorphism of *Candida albicans*. J Gen Microbiol 136:1067–1075

Zhang FL, Casey PJ (1996) Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 65:241–269

19 The Emergence of Fruiting Bodies in Basidiomycetes

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CONTENTS

I. Introduction

Fruiting bodies are adaptations for aerial dissemination of sexual spores by assimilative mycelia, which grow within moist substrata. Most species of the homobasidiomycetes produce large fruiting bodies, also called basidiocarps, carpophores or basidiomes (Moore 1998). It is generally the case that, in these fruiting bodies, specialised cells, basidia, are generated in which genetically different haploid nuclei, derived from a mating event and coexisting in a common cytoplasm (a heterokaryon), fuse to form diploid nuclei. These diploid cells immediately undergo meiosis to form haploid basidiospores. In some cases, diploid nuclei are already formed in the vegetative mycelium, as in *Armillaria* species (Ullrich and Anderson 1978; Grillo et al. 2000).

After discharge, the haploid basidiospores can germinate and generate recombinant homokaryotic mycelia which, depending on an often complex system of mating-type genes, can fuse to produce a new, fertile heterokaryotic mycelium (see Chap. 17, this volume). Basidiomycete species behaving according to this scheme are in the majority and are called heterothallic (i.e. self-incompatible). From a teleological point of view, this makes sense because it ensures that the diploid basidia produce recombinant meiotic progeny. It is less clear why a minority of basidiomycetes (about 10%; Whitehouse 1949) is homothallic (i.e. self-compatible). Mycelia resulting from basidiospores of such species are capable of directly forming fruiting bodies. Homothallic forms can arise from heterothallic forms by various mechanisms (see Sect. III.B).

This review discusses the regulation of fruiting-body formation in basidiomycetes and the role structural proteins and enzymes play in this process. Related topics, including morphogenesis, cytology and mathematical modelling, are well treated in Wells and Wells (1982), Moore et al. (1985), Wessels (1993a), Chiu and Moore (1996), Moore (1998), Kües (2000), and Meskauskas et al. (2004). Fruiting of commercially important species for mushroom cultivation is treated in Chang and Hays (1978), Flegg et al. (1985), Wuest et al. (1987), van Griensven (1988), Kües and Liu (2000) and Kothe (2001).

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The Mycota I
Growth, Differentation and Sexuality
Kües/Fischer (Eds.)

II. Development of Emergent Structures

A. Formation of a Feeding Submerged Mycelium

Fruiting bodies develop from a vegetative submerged mycelium. Formation of this submerged mycelium starts with the germination of an asexual or sexual spore. Hyphae growing out of these spores grow at their tips, while branching subapically (Wessels 1986, 1990). Since hyphae can also fuse by a process called anastomosis, a mycelium is formed which represents a network of hyphae, allowing for translocation of molecules in different directions (see The Mycota, Vol. VIII, Chap. 6). The vegetative mycelium colonizes and assimilates the substrate, often by degrading polymeric components by extracellular enzymes which are secreted at tips of growing hyphae (Wösten et al. 1991; Moukha et al. 1993; Wessels 1993b). The degradation products can serve as nutrients not only for those hyphae which have taken up these molecules but also for the mycelium as a whole. This explains why some fungi can grow for considerable distances over non-nutritive surfaces, their growth being supported by transport of water and nutrients from a food base (Jennings 1984; see The Mycota, Vol. I, 1st edn., Chap. 9). Translocation of water and nutrients, together with tip growth, are also instrumental for development of aerial structures, the most conspicuous being the fruiting bodies. It is clear that growth of such emergent structures requires massive transport of materials from the substrate mycelium. Mathematical models have been constructed which take most of these activities of the whole mycelium into account (Edelstein and Segel 1983). In such models, initiation of fruiting bodies is viewed as the development of focal points of high-density growth of branching hyphae with their growth axis away from the substrate (Edelstein 1982). Recently, a mathematical model was described which visualises the formation of the fruiting body. Remarkably, formation of this complex fungal structure can be simulated by successive waves of hyphal attraction and repulsion. As long as all filaments behave in the same way at the same time, there is no need for a global or organ level control in formation of the mushroom. The shape of the mushroom would be determined by genes activating the repulsion and attraction, and which would operate in a clockwork fashion (Meskauskas et al. 2004; Money 2004).

Redistribution of active cytoplasm has been considered as most characteristic for fungal mycelia (Gregory 1984). In *Schizophyllum commune* (Wessels 1965) and *Coprinus cinereus* (*Coprinopsis cinerea*) (Moore 1998), fruiting-body primordia can arise at the expense of polymeric constituents of the supporting mycelium, whereas expanding fruiting bodies grow at the expense of polymeric constituents of both supporting mycelium and abortive fruiting-body primordia. This is accompanied by redistribution of active cytoplasm (Ruiters and Wessels 1989a). At the moment, it is not clear how much of this apparent translocation of cytoplasm is due to movement of cytoplasm as such or to degradation and resynthesis of cellular components. Cell turnover, involving translocation of breakdown products, becomes very evident in the later stages of fruiting-body development, which can occur in the absence of external nutrients (see Sect. IV.F).

Jennings (1984) has suggested that mass flow occurs through hyphae because of the existence of a gradient in hydrostatic potential created by sources and sinks of assimilates. It is clear that rapidly growing structures emerging into the air represent powerful sinks for assimilates and water. In fact, such structures are generally quite isolated from the environment by the presence of hydrophobic coatings, a point emphasized by Rayner (1991). Hydrophobic coatings are the result of self-assembly of hydrophobins (see Sect. IV.A) but also may be due to other proteins or phenolic substances polymerised by the action of phenoloxidases or peroxidases (see Sect. IV.E).

B. Formation of Fruiting Bodies from the Submerged Mycelium

Formation of fruiting bodies is a highly complex developmental process. A generalized scheme for formation of agaric fruiting bodies such as those of *C. cinereus* (Moore 1998; Kües 2000) is as follows: after a "critical mass" of submerged mycelium has been formed, hyphae escape the substrate to grow into the air. These hyphae form aggregates, which are called hyphal knots or nodules. These knots may result from a single hypha which branches intensely or they arise from branches of neighbouring aerial hyphae which grow towards and alongside each other. Within the knots hyphae aggregate, forming a fruiting-body initial. These initials (also referred to as secondary hyphal knots; Walser et al.

2003; Kües et al. 2004) are the first fruiting body-specific structures, and this stage can be seen by the naked eye using the vital stain Janus green (Sánchez and Moore 1999; Sánchez et al. 2004). Within the core of the initial, differentiation of cells occurs (Moore 1998; Kües 2000; Kües et al. 2004). The lower part will develop into the stipe, while the cap will be formed from the upper part. Within the cap, different tissues develop, which are not formed from meristems, as in plants, but result from the interaction of individual hyphae. By growing at their apices, they form an interwoven structure called a plectenchyma. The outer part of the cap is called the veil. In the inner part, the pileus trama and gills (or pores) with a hymenium can be distinguished (Fig. 19.1). In the hymenium, different cell types are formed, among which the basidia. In the basidia, karyogamy and meioses take place, ultimately resulting in basidiospores.

That development of fruiting bodies is complex is also exemplified by the fact that formation of the different tissues overlaps in time. Moreover, cells in the developing mushroom differ in diameter, length, the number of septa, nuclei and vacuoles as well as the molecular composition (e.g. the content of reserve carbohydrate; Moore 1998). The different cell types are not only the result of localized growth but also apoptosis is involved (Umar and van Griensven 1997; Chap. 9, this volume). Fruiting bodies of other basidiomycetes, such as those of *S. commune*, follow a completely different morphogenetic pathway (Fig. 19.2). These fruiting bodies result from indeterminate growth of fruiting-body primorida. Expansion of the cup-shaped primordia is not the result of intercalary growth but is due to continued apical growth and differentiation of hyphae in the primordium (Wessels 1993a). The variety in developmental programmes makes it even more difficult to understand processes involved in fruiting-body formation in the homoba-

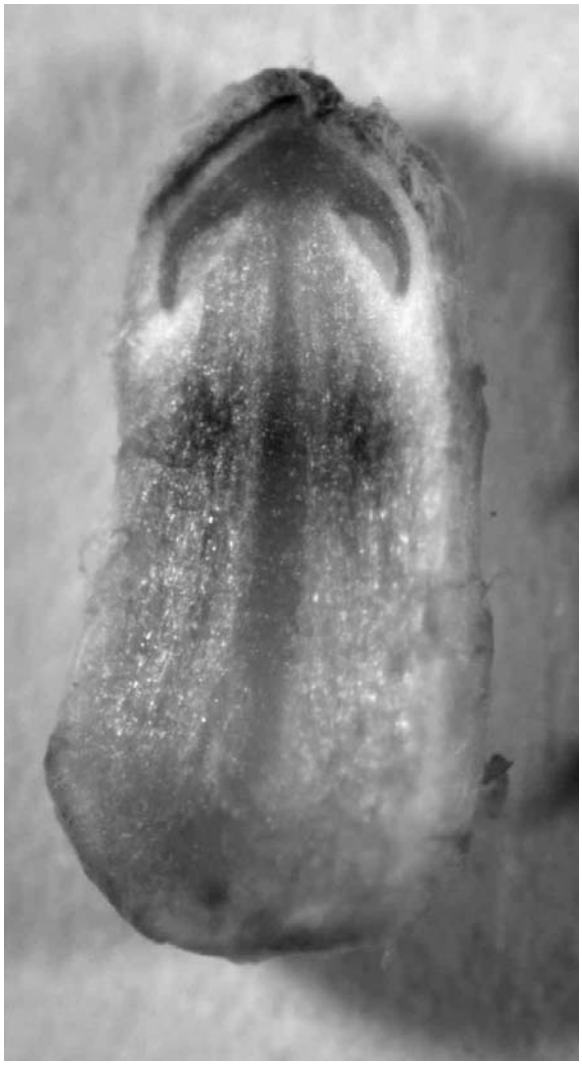

Fig. 19.1. Within the primordium of *C. cinera*, all parts and tissues of the fruiting body can already be distinguished. The mature fruiting body results from intercalary cell expansion (courtesy of M. Navarro-González)

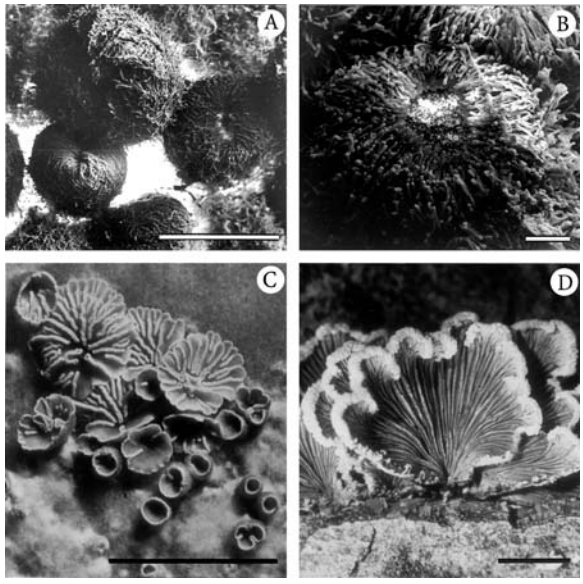

Fig. 19.2. A–D Fruit-body development of *S. commune* (extracted from Wessels 1996). **A** Cryo-scanning electron micrograph of an early stage of fruiting-body formation. Hyphae which extend at their tips aggregate and grow upwards. **B** As in **A** but at a later stage of development. **C** Macrograph of fruiting bodies at the cup stage. Note the formation of the split gills. **D** Full-grown fruiting bodies. *Bars* represent 1 mm (**A**), 0.1 mm (**B**) and 10 mm (**C**, **D**)

sidiomycetes. For reviews in developmental patterns, see Wessels (1965), Reijnders and Stafleu (1992), Watling (1996) and Clémonçon (1997).

In colonies of *S. commune* (Perkins 1969; Raudaskoski and Vauras 1982) and *Coprinus* (*Coprinellus*) *congregatus* (Ross 1982; Durand 1983), it was shown that light induction of primordia occurs in the youngest growth zone, immediately behind the advancing front of the colony. In *S. commune*, light induction alone leads to the immediate appearance of primordia (Perkins 1969; Yli-Mattila et al. 1989b). In *C. congregatus*, some additional stimulus emanating from the whole mycelium is required to realize formation of primordia. Ross (1982) noted that primordia formed only in the growth zone after the colony front had reached the edge of the Petri dish. Durand (1983) saw immediate formation of primordia after light induction in the growth zone of a half-colony growing on non-nutritive medium, while the other part had already fully colonized a nutrient medium. Thus, in some cases, as in *S. commune*, induced initials may immediately act as a sink for translocation of materials from the vegetative mycelium whereas in other cases, as in *C. congregatus*, vegetative mycelium has to be checked in its growth before such a translocation system becomes operative. Only part of the hyphal aggregates eventually form mature fruiting bodies. Possibly, stochastic processes and competition for translocated materials determine which initials will grow into primordia and, subsequently, into mature fruiting bodies.

III. Regulation of Fruiting-Body Formation

A. Environmental Signals

Much effort has gone into identifying environmental factors conducive to fruiting in basidiomycetes. On the one hand, such studies were done to provide inroads to establish causative mechanisms of fruiting. On the other hand, they have been very important in establishing optimal conditions for commercial mushroom growing. Apart from studies on normal environmental conditions, a few studies have been concerned with compounds in fungal extracts which enhance fruiting. Sphingolipids and cerebrosides appear to be effective inducers of fruiting in *S. commune* and *C. cinereus* (Kawai and Ikeda 1982; Kawai et al. 1986; Mizushina et al. 1998), while cAMP was shown to stimulate fruiting in *C. cinereus* (Uno and Ishikawa 1971, 1973, 1982). Also in *S. commune* (Schwalb 1978; Yli-Mattila 1987; Kinoshita et al. 2002), *Phanerochaete chrysosporium* (Gold and Cheng 1979) and *Lentinula edodes* (Takagi et al. 1988), a relation was found between high levels of endogenous cAMP and fruiting. High levels of intracellular cAMP could be obtained by expressing dominant active heterotrimeric G protein alpha subunits (SCGP-A and SCGP-C) in *S. commune* (Yamagishi et al. 2002, 2004). However, this resulted in reduced, rather than increased fruiting in the dikaryon. Future research should elucidate the exact role of cAMP and the heterotrimeric G proteins.

It is self-evident that the emergence of fruiting bodies is accompanied by a drastic change in exposure to oxygen (limited availability in the substrate, high in the air), carbon dioxide (high in the substrate, low in the air) and light. It is therefore not surprising that these environmental factors can exert a profound influence on fruiting-body development (Manachère 1980). Moreover, environmental conditions like temperature, humidity and availability of nutrients may play a decisive role (Madelin 1956; Kües and Liu 2000). Of the environmental factors least studied is the availability of oxygen. In some basidiomycetes, higher mycelial biomass production has been observed at 5% than at 20% O_2 (White and Boddy 1992). The high oxidative activity of fruiting bodies of *S. commune* (Wessels 1965) suggests that a high concentration of O_2 may be necessary for their development. Indeed, at 2% O_2, half of the maximum amount of mycelium was formed but neither fruiting bodies nor aerial hyphae appeared (J.G.H. Wessels, unpublished data). A possible regulatory effect of oxygen on emergent growth is the more interesting, in view of the hypothesis that oxygen may induce a hyperoxidant state involving oxidized proteins which may operate a switch to aerial differentiation (Hansberg and Aguirre 1990; Toledo and Hansberg 1990).

Light has been most intensively studied as a modulating factor in fruiting-body development (Lu 1974, 2000; Eger-Hummel 1980; Manachère 1980, 1988; Durand 1985). The effect of light on *S. commune* is limited to induction of primordia (Perkins 1969; Raudaskoski and Yli-Mattila 1985); often, illumination for a few minutes suffices. This inducing effect of light can sometimes be bypassed, such as in *C. cinereus*, by low-temperature treatment (Tsusué 1969). In, for example, *C. congregatus*

(Manachère 1988), *C. cinereus* (Tsusué 1969; Lu 1974; Kamada et al. 1978) and *Flavolus arcularius* (Kitamoto et al. 1974), light is also required for normal stipe and pileus (cap) development. In fact, at least five light-sensitive phases can be distinguished in fruiting-body formation in *C. cinereus* (Kües 2000; Lu 2000). Light is needed for the formation of initials, for maturation of primordia and for karyogamy but it has a negative effect on hyphal knot formation and completion of meioses. Thus, for fruiting bodies to develop, cycles of light and darkness are required. Light effects are local and are not spread systemically (Madelin 1956; Kertesz-Chaloupková et al. 1998).

Action spectra of light have been established for several of these systems. The spectra show differences, but all exhibit peaks in the UV-A (320–400 nm) and blue (400–520 nm) regions, suggesting that a flavin is the photoreceptor (Lu 1974; Elliott 1994). Recently, progress has been made in *C. cinereus* in isolating a gene encoding a putative blue-light receptor. The gene *dst1* has high similarity to the candidate photoreceptor WC-1 of *Neurospora crassa* (Yuki et al. 2003).

Light caused formation of short, heavily branched hyphal compartments in dikaryotic strains of *S. commune*, an effect completely absent from sealed cultures which do not develop primordia, possibly due to accumulation of carbon dioxide (Raudaskoski and Viitanen 1982; Raudaskoski and Salonen 1983). The effect of sealing cultures on fruiting-body formation in *S. commune*, attributed to accumulation of carbon dioxide, was originally detected by Niederpruem (1963). However, some caution is necessary because this fungus also releases large amounts of methylmercaptan and dimethylsulfide (Birkinshaw et al. 1942), to the extent that nearly all of the sulphate in the medium which is not assimilated is converted into these volatile compounds (O.M.H. de Vries and J.G.H. Wessels, unpublished data).

In basidiomycete fruiting-body initiation, the most rapid effects of blue light detected were increases in contents of cAMP in *C. cinereus* (Uno et al. 1974) and *S. commune* (Yli-Mattila 1987). The light stimulus, combined with sufficient aeration, also leads to activation of specific genes. Between 6 and 24 h after illumination of dark-grown colonies of *S. commune*, levels of fruiting-associated mRNAs rise (Yli-Mattila et al. 1989a). However, these increases may have been a consequence, rather than a cause of formation of fruiting-body primordia (Wessels 1992). This is suggested from the fact that these genes are also activated in the dikaryon when fruiting is suppressed by darkness or by a high concentration of carbon dioxide (Wessels et al. 1987). However, fruiting-body primordia maintain high concentrations of these mRNAs, in contrast to the vegetative mycelium (Mulder and Wessels 1986; Ruiters and Wessels 1989b).

B. Mating-Type Genes as Master Regulators

In the heterothallic basidiomycetes, fruiting is most regularly observed in the heterokaryon, which is also called the secondary mycelium. The heterokaryon arises from a mating between two compatible homokaryons, that is, between homokaryons carrying different mating-type genes (in older literature referred to as incompatibility factors; for further details, see Chap. 17, this volume). With respect to morphological differences between homokaryons and the derived heterokaryon, there is a great deal of variation. The most regular pattern is that exemplified by the two most intensively studied species, namely, *S. commune* and *C. cinereus*. In these species the homokaryon contains one nucleus in each hyphal compartment, and is therefore called a monokaryon. The established heterokaryon contains two (genetically different) nuclei in each hyphal compartment, and is therefore called a dikaryon. These dikaryons are typified by the presence of a clamp connection at each septum, which is formed during synchronous mitotic division of the two nuclei (Chap. 17, this volume). To cite a few deviating examples: in the occasionally cultivated *Agaricus bitorquis*, the homokaryon is multikaryotic, whereas the fertile heterokaryon is dikaryotic but without clamp connections (Raper 1976). In the commonly cultivated *Agaricus bisporus*, the fertile heterokaryon grows directly from a basidiospore which contains two nuclei of different mating types. The heterokaryon is multikaryotic and has no clamp connections (Raper et al. 1972).

The mating-type genes are the master regulators of sexual development (see also Chap. 10, this volume). When two homokaryons with different *A* and *B* mating-type genes (in *S. commune*, here called *MATA* and *MATB*) fuse, a heterokaryon is formed with the propensity to develop fruiting bodies (see Fig. 19.3A,C). Following hyphal fusion, nuclei are exchanged. These nuclei migrate to the apical compartment of the recipient hypha, which

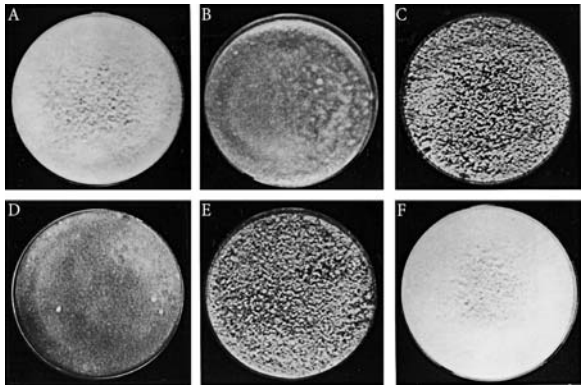

Fig. 19.3. **A–F** Morphological appearances of 4-day-old surface cultures of co-isogenic *Schizophyllum commune* strains, inoculated as a lawn from a mycelial homogenate, variously exhibiting the ability to produce aerial hyphae and fruit bodies. **A** *MATA41MATB41*, **B** *MATA41MATB41 thn*, **C** *MATA41/A43 MATB41/B43*, **D** *MATA41/A43 MATB41/B43 thn/thn*, **E** $MATA^{con}$ $MATB^{con}$, **F** $MATA^{con}$ $MATB^{con}$ *fbf*

is accompanied by septal dissolution. In the apical compartment, the donated and recipient nuclei pair and hyphal dissolution is switched off. In fact, new septa are formed which are more resistant to dissolution and which physically prevent nuclear migration (Wessels and Marchant 1974). The nuclei in the dikaryotic hyphae divide synchronously. Nuclear division is accompanied by the formation of clamps. As a result, the apical and the subapical compartments contain nuclei of both mating type. The heterokaryotic dikaryon is stable and, unlike the monokaryon, forms fruiting bodies under appropriate environmental conditions. Coordination of the mating-type regulation also manifests itself in control of cAMP production (Swamy et al. 1985a), in cAMP-dependent protein kinase activity (Swamy et al. 1985b) and in fruiting-body initiation (Swamy et al. 1984).

The molecular structure of the mating-type loci has been identified in, for instance, *S. commune* and *C. cinereus* (for a detailed overview and references, see Chap. 17, this volume). The *A* genes of these fungi encode homeodomain proteins of the HD1 and HD2 type. In heterokaryons with nuclei containing different *A* genes, these proteins form heterodimers which are active in development. On the other hand, the *B* genes of *C. cinereus* and *S. commune* encode pheromones and G-coupled receptors for pheromones. In heterokaryons with nuclei containing different *B* genes, pheromones from one nucleus can interact with receptors encoded by the other nucleus, and vice versa. Homokaryotic mutant strains have been isolated with a constitutive active *A* mating-type gene (referred to as $MATA^{con}$ in *S. commune*, *Amut* in *C. cinereus*) and/or *B* mating-type gene (referred to as $MATB^{con}$ in *S. commune*, *Bmut* in *C. cinereus*; Raper et al. 1965; Koltin 1970; Swamy et al. 1984). Activity of these loci is thus independent of a compatible locus donated by a sexual partner. The *S. commune* $MATA^{con}MATB^{con}$ homokaryon (Fig. 19.3E) is a dikaryon which shares with the heterokaryotic dikaryon (Fig. 19.3C) the propensity to form fruiting bodies. Likewise, *AmutBmut* homokaryons of *C. cinereus* do form fruiting bodies as dikaryons (Swamy et al. 1984; Boulianne et al. 2000).

C. Other Regulatory Genes

Relatively little is known about genes involved in establishment of the dikaryotic mycelium and emergence of fruiting bodies, other than the mating-type genes. Yet, many genes are expected to play a role. Both establishment of the dikaryon and emergence of fruiting bodies encompass a number of complex processes which have to be coordinated (see above). Before 1990, only mutations were known and most of them have hardly been studied. For instance, Raper and Krongelb (1958) detected dominant alleles in a natural population of *S. commune* affecting the morphology of fruiting bodies, described as corraloid (highly involuted hymenium), medusoid (long stipes) and bug's ear (numerous small fruiting bodies without gills). By chemical and UV mutagenesis of a normal dikaryon (Takemaru and Kamada 1972) or a *AmutBmut* homokaryon (Kanda and Ishikawa 1986), a remarkably high number of variants of *C. cinereus* were isolated. Many of the mutants had developmental defects in initiation of fruiting-body development, in primordia and fruiting-body maturation and/or were defective in sporulation. More recently, REMI (restriction enzyme mediated integration) mutagenesis in homokaryotic fruiting strains gave rise to similarly large collections of mutations with defects in fruiting-body development and sporulation (Granado et al. 1997; Cummings et al. 1999; Muraguchi et al. 1999). Temperature-sensitive sporulation mutants of *S. commune* have also been isolated (Bromberg and Schwalb 1977). In the last decade, several genes other than the mating-type genes have been isolated which are involved in establishment of the dikaryotic mycelium and

emergence of fruiting bodies. In the following sections, these genes are described. Moreover, a few fruiting mutants are discussed which were studied in some detail.

1. Haploid Fruiting

In haploid fruiting, the control by the mating-type genes is bypassed. It must be distinguished from homokaryotic fruiting, as it occurs in mating type-activated homokaryons (Raper et al. 1965; Koltin 1970; Swamy et al. 1984), diploid monokaryons (Koltin and Raper 1968), and in homokaryons after a mating-type switch (Labarère and Noël 1992). In these cases, the mating-type genes do control fruiting. By studying segregation of naturally occurring alleles for haploid fruiting in *Polyporus ciliatus* (Stahl and Esser 1976) and *Agrocybe aegerita* (Esser and Meinhardt 1977), Esser and his co-workers implicated the alleles fi^+ and fb^+, operating in sequence. In the presence of fi^+, only stipes were formed; the additional presence of fb^+ led to the formation of normal but small fruiting bodies. In *S. commune* (Esser et al. 1979), the alleles fi_1^+ and fi_2^+ were each found to lead to formation of fruiting-body initials, whereas the presence of both alleles led to formation of stipes without spores. The additional presence of a third allele, fb^+, resulted in abnormally shaped but gilled fruiting bodies with two-spored basidia. The presence of exclusively fi_1, fi_2 and *fb* alleles permitted fruiting in the dikaryon but the presence of the fruiting alleles tended to shorten the time required for fruiting. Fruiting alleles in *S. commune*, bypassing the control of the mating-type genes, have also been studied by Leslie and Leonard (1979a,b). These authors implicated two alleles, *hap-5* and *hap-6*, working in sequence, in spontaneous haploid fruiting. In the absence of these alleles, they implicated four other *hap* alleles in haploid fruiting, as this occurred after mechanical injury, and again two other *hap* alleles, in the absence of any of the others, in the occurrence of haploid fruiting after adding (unidentified) fruiting substances. Yli-Mattila et al. (1989b) found that inbreeding of haploid fruiters led to enhancement of haploid fruiting, indicating the polygenic character of this trait. A cross between inbred monokaryotic fruiters resulted in a dikaryon which fruited profusely even in the dark. They also found that mRNAs characteristic for dikaryotic fruiting accumulated during haploid fruiting.

It has been suggested that the haploid fruiting pathway operates independently from that operating during dikaryotic fruiting (Raper and Krongelb 1958), but the aforementioned, clear effects of the presence of haploid fruiting alleles on dikaryotic fruiting argues against this. One possibility is that these naturally occurring alleles represent relaxed versions of regulatory DNA sequences within regulatory circuits by which mating-type genes control normal dikaryotic fruiting. This can be likened to mating-type control of sporulation in *Saccharomyces cerevisiae*. In this yeast, mutations in secondary regulatory genes resulted in sporulation in the absence of mating-type gene control (Kassir et al. 1988; see The Mycota, Vol. I, 1st edn., Chap. 13). In *C. cinereus*, mutations in the *pcc1* gene can lead to fruiting in monokaryons, abolishing the need for mating-type gene actions (Uno and Ishikawa 1971; Muraguchi et al. 1999). *pcc1* is thought to act as a negative regulator gene in the *A* mating-type pathway (Kamada 2002; see below).

Except for *pcc1* in *C. cinereus*, none of the haploid fruiting genes have been cloned. However, the *FRT1* gene of *S. commune* which has been isolated may be considered a haploid fruiter gene. Certain homokaryons with introduced copies of this gene started to fruit independently of the mating-type loci (see below).

2. Regulatory Genes in Establishment of the Dikaryotic Mycelium and in Fruiting-Body Formation

The assumption that the heterodimers formed by the homeodomain proteins encoded by the *A* genes are transcription factors implies that there are target genes for this protein complex. This hypothesis was strengthened by the identification of target genes for the orthologous protein complexes in *Ustilago maydis* (Romeis et al. 2000; Brachmann et al. 2001). Recently, a putative target gene of *C. cinereus* has been identified (Inada et al. 2001). This gene, *clp1*, was isolated by complementation of a mutant of an *AmutBmut* homokaryon which did not form clamps. The gene encodes a novel protein of 365 amino acids without known structural motifs. Several pieces of evidence support the hypothesis that *clp1* is a target for the homeodomain proteins encoded in the *A* genes. First, expression of *clp1* depends on the A protein heterodimer. Second, when *clp1* was expressed from the constitutive promoter of the β1 tubulin gene, clamps formed independently of an active A complex. Moreover, the promoter of *clp1* contains a conserved *hsg* motif. This motif has been shown to bind heterodimeric

homeodomain complexes of MATa1 and MATα2 of *S. cerevisae* (Goutte and Johnson 1988) and the bE and bW heterodimers of *U. maydis* (Romeis et al. 2000).

Another gene which seems to be part of the *A*-regulated pathway is *pcc1* (Murata et al. 1998). A homokaryotic strain with a mutated copy of this gene formed pseudoclamps. Moreover, after prolonged time, it formed fully differentiated fruiting bodies. From this and the fact that *pcc1* is expressed in a wild-type homokaryon, it was concluded that this gene may be a repressor of the fruiting pathway in the absence of a functional *A* complex. The *pcc1* gene encodes a protein with a HMG box motif and a nuclear localization signal, indicating that it is a transcription factor. The HMG box is also present in *ste11* of *Schizosaccharomyces pombe* (Sugimoto et al. 1991) and *prf1* of *U. maydis* (Hartmann et al. 1996). These genes encode pheromone response factors which bind to a pheromone-responsive element in the promoter of target genes. Among these genes are *prf1* and *ste11* themselves (Sugimoto et al. 1991; Aono et al. 1994; Hartmann et al. 1996; Urban et al. 1996). A pheromone-responsive element similar to those of *U. maydis* and *S. pombe* was found in the promoter of the *pcc1* gene. This, and the fact that *pcc1* is up-regulated by a compatible *B* mating interaction suggest that *pcc1* is a possible pheromone response factor of *C. cinereus* (Murata et al. 1998; see Chap. 17, this volume). Since the gene is also up-regulated by an activated *A* gene, Murata et al. (1998) suggested that *pcc1* plays an important role in coordinating the activities of the *A* and *B* genes. This interpretation is strengthened by the fact that the *pcc1* mutant homokaryon formed fully differentiated fruiting bodies, albeit after a prolonged time. It was suggested that the repressor activity of *pcc1* is released by a compatible *A* gene interaction via *clp1* (Kamada 2002).

Like *pcc-1* and possibly *clp1*, the *FBF* gene of *S. commune* is involved in clamp formation and in fruiting (Springer and Wessels 1989). A recessive mutation of *FBF* (*fbf*) completely blocks dikaryotic fruiting. The mutation occurs spontaneously at high frequency, causing sterility in about 10% of mycelia regenerated from protoplasts of a $MATA^{con}MATB^{con}$ homokaryon. The *fbf* mutation is also responsible for the frequent occurrence of sterile sectors in fruiting colonies of this homokaryon. The phenotype of the mutation is particularly clear when mycelia are grown as a lawn from mycelial fragments. Under these conditions, the homokaryotic $MATA^{con}MATB^{con}$ forms numerous fruiting bodies and few aerial hyphae (Fig. 19.3E), its phenotype being similar to that of the normal heterokaryotic dikaryon (Fig. 19.3C). The $MATA^{con}MATB^{con}$ *fbf* mycelium forms no fruiting bodies but instead produces copious aerial mycelium (Fig. 19.3F). The *fbf* mutation, which has no phenotype in monokaryons, completely suppresses fruiting when homozygous in a *MATA*-on *MATB*-on heterokaryon. Notably, it also affects the formation of clamp connections; the clamps do not fuse. *fbf* may be related to a spontaneously occurring recessive mutation, called *coh1* (Perkins and Raper 1970), because no complementation occurred in an *fbf* × *coh1* cross (Springer and Wessels 1989). Wessels (in The Mycota, Vol. I, 1st edn., Chap. 21) suggested that *fbf* could also be related to *FRT1* of *S. commune* (Horton and Raper 1991). However, this seems not to be the case (Horton et al. 1999).

FRT1 was initially identified as a gene which induced fruiting when transformed into certain homokaryons of *S. commune* (Horton and Raper 1991). Experimental evidence indicated that the strains which fruited upon introduction of *FRT1* contained an endogenous *FRT1* allele of a different kind (designated *FRT1-2* as opposed to *FRT1-1*; Horton et al. 1999). By contrast, strains possessing a similar allele did not fruit when transformed with *FRT1-1* (Horton and Raper 1991). Fruiting in heterokaryotic dikaryons derived from these fruiting homokaryons was also accelerated. *FRT1-1* encodes a putative nucleotide-binding protein of 192 amino acids with a P-loop motif (Horton and Raper 1995). This P-loop motif was demonstrated to be essential for the fruiting-inducing activity of the protein. Surprisingly, homokaryotic strains in which the *FRT1* gene was disrupted were more fluffy, compared to wild-type strains. The aerial hyphae of the disruptant strains were aggregated (Horton et al. 1999), resembling the first stages of fruiting-body development (van der Valk and Marchant 1978; Raudaskoski and Vauras 1982). Indeed, not only were levels of the monokaryon-specific *SC3* mRNA increased in the haploid fruiter but also those of the dikaryon-specific mRNAs of *SC1*, *SC4* and *SC7* (see below). From these results, it was hypothesized that *FRT1* is part of a signal transduction pathway which represses expression of dikaryon-specific genes in the monokaryon (Horton and Raper 1995). The expression of the dikaryon-specific genes in a homokaryon in which the *FRT1* gene was deleted is apparently not sufficient to initiate fruiting-body formation.

Rather, development is arrested in the initial stages of hyphal aggregation. Still, how can the haploid fruiting be explained when *FRT1-1* is transformed into a *FRT1-2* strain? Horton et al. (1999) proposed that in this case these proteins dimerise, relieving the repression of the dikaryon-specific genes. Moreover, the heterodimer would activate some other genes, resulting in the formation of fruiting bodies. However, fruiting in the dikaryon was not affected when the *FRT1* gene was deleted (Horton et al. 1999). This suggests that *FRT1* is not a crucial component, if a component at all, of the pathways encompassing the mating-type genes which lead to formation of fruiting bodies in the dikaryon.

3. Regulatory Genes in Fruiting-Body Formation but not in Establishment of the Dikaryotic Mycelium

A mutation in the *THN* (*THIN*) gene of *S. commune* occurs spontaneously and has pleiotropic effects (Raper and Miles 1958; Schwalb and Miles 1967; Wessels et al. 1991b). The scanty presence of aerial hyphae, the perfectly round edge of colonies, the wavy or corkscrew-like appearance of submerged hyphae, and a pungent smell easily score the mutation. The suppression of formation of aerial hyphae is best seen in cultures grown as a lawn from mycelial fragments (Fig. 19.3B); in a dikaryon homozygous for *thn*, formation of both aerial hyphae and fruiting bodies is suppressed (Fig. 19.3D).

The *THN1* gene was cloned (Fowler and Mitton 2000). It encodes a putative RGS protein (regulator of G protein signalling) which is homologous to Crg1 of *Cryptococcus neoformans* (Fraser et al. 2003; Wang et al. 2004), Sstp2p of *S. cerevisae* (Dietzel and Kurjan 1987) and FlbA of *Aspergillus nidulans* (Lee and Adams 1994). These proteins share a domain of about 120 amino acids (de Vries et al. 1995) assumed to interact with the Gα subunit of heterotrimeric G proteins. The interaction modulates the conversion of GTP bound to the Gα subunit to GDP. In this way, it regulates signals from an activated receptor protein which are transferred via a heterotrimeric G protein to downstream effector molecules. It was hypothesized (Fowler and Mitton 2000) that *THN1* regulates a heterotrimeric G protein signalling pathway which, in turn, regulates hydrophobin expression (see below). Schuren (1999) reported that most of the pleiotropic effects of the *thn* mutation were overcome by growing the mutant near wild-type hyphae. A diffusible molecule smaller than 8 kDa would be responsible for this effect, and may be part of the signalling cascade. It is not yet clear whether this signalling pathway is directly linked to the pheromone receptor encoded by the *MATB* genes or whether it activates, for example, cAMP production.

In contrast to *thn* of *S. commune*, aerial growth is not affected in the *ich1-1* mutant of *C. cinereus*. Rather, cap differentiation is blocked at an early stage of fruiting-body differentiation (Muraguchi and Kamada 1998). In contrast to wild-type primorida, no rudimentary pileus could be observed in the primordial shaft of the *ich1-1* mutant. *ich1* mRNA accumulates in the cap of the wild-type fruiting body. The precise role of the gene, however, is not yet known. The *ich1-1* gene encodes a novel protein of 1353 amino acids containing nuclear targeting signals. Also notable, the protein contains a S-adenosyl-L-methionine (SAM) binding motif (Kües 2000), being characteristic for the enzyme family of methyltransferases (Faumann et al. 1999).

D. Nuclear Positioning

The *SC1*, *SC4* and *SC6* hydrophobin genes as well as *SC7* and *SC14* of *S. commune* (for their function, see below) are expressed in dikaryons (*MATA*-on *MATB*-on) but not in monokaryons (*MATA*-off *MATB*-off) and *MATA*-on *MATB*-off or *MATA*-off *MATB*-on mycelia (Mulder and Wessels 1986; Wessels et al. 1995). By contrast, the *SC3* hydrophobin gene is active in the monokaryon but it is downregulated in a *MATA*-off *MATB*-on mycelium (Ásgeirsdóttir et al. 1995). From this, it is expected that *SC3* would also be inactive in dikaryons (i.e. *MATA*-on *MATB*-on). Indeed, *SC3* mRNA was lowered in a fruiting dikaryon. However, under non-fruiting conditions (e.g. high CO_2 and darkness), high *SC3* expression did occur in the dikaryon, whereas expression of the dikaryon-specific hydrophobin genes and the *SC7* and *SC14* genes was relatively low (Wessels et al. 1987). Apparently, the *MATB* pathway and, possibly, also the *MATA* pathway are not active in at least part of the dikaryotic mycelium. How can this be explained? It was shown that the distance between nuclei in the dikaryotic hyphae varies. Aerial hyphae have a large nuclear distance (>8 µm), correlating with high *SC3* expression. By contrast, the nuclear distance in hyphae within the fruiting body is small (<2 µm), correlating with high *SC4* and low *SC3* expression. This led to the hypothesis that disruption of the binucleate state (i.e. an increased distance between the

nuclei) inactivates the *MATB*-on pathway (Ásgeirsdóttir et al. 1995), and possibly also the *MATA*-on pathway. This would thus result in a monokaryon-like gene expression. The binucleate state can be experimentally disrupted by growing the dikaryon in liquid shaken cultures at high rpm (round per minute) or by growing the hyphae on a hydrophobic solid. This is accompanied by cessation of SC4 and SC7 secretion. Instead, SC3 is now produced (Schuurs et al. 1998). It appears that when the nuclear distance exceeds 3–4 µm, gene expression in the dikaryon shifts to the monokaryotic type (Wessels et al. 1998).

Although these findings are relevant for the interaction of *MATB* genes (Schuurs et al. 1998; Wessels et al. 1998), they also bear on the role of the mating-type genes in regulating fruiting. A dikaryotic type of gene expression (e.g. of *SC4* and *SC7* but not *SC3*) conducive to fruiting-body formation is possible only in the binucleate state. Aerial hyphae formed by the dikaryon are typically non-clamped, have widely separated nuclei and produce SC3. Non-clamped hyphae also form the outer layer of the fruiting bodies (Fig. 19.2), and these produce SC3 (Ásgeirsdóttir et al. 1995). By contrast, the central plectenchyma of the fruiting bodies produces SC4 and SC7 but not SC3.

IV. Proteins Involved in Fruiting

When 4-day-old surface cultures of co-isogenic *S. commune* monokaryons (only aerial hyphae, Fig. 19.3A) and dikaryons (mainly fruiting bodies, Fig. 19.3C) were compared, clear differences were seen in the proteins synthesised at this stage (de Vries and Wessels 1984). Among 400 proteins, pulse-labelled with [^{35}S] sulphate and analysed on two-dimensional gels, only eight proteins appeared to be synthesised exclusively in the monokaryon whereas the fruiting dikaryon synthesised 37 abundant proteins not detected in the monokaryon. Total RNA::cDNA hybridisations and in vitro translations of total RNA showed the presence of about 30 unique, abundant mRNAs in the dikaryon, accounting for about 5% of the mRNA mass (Hoge et al. 1982). No mRNAs unique to the monokaryon were detected; apparently, an additional set of genes is activated in the dikaryon during fruiting.

Complementary DNA (cDNA) synthesised on poly(A)RNA of the fruiting dikaryon of *S. commune* was cloned, and clones containing sequences expressed in this dikaryon, and not in the co-isogenic monokaryons, were selected (Dons et al. 1984; Mulder and Wessels 1986). Among the mRNAs detected with these clones, those for hydrophobins SC1, SC4 and SC6 (see below) were most abundant, as was the mRNA for the SC3 hydrophobin, which was expressed in both monokaryon and dikaryon (Mulder and Wessels 1986). It was found that formation of these mRNAs is controlled at the transcriptional level (Schuren et al. 1993a,b).

In other basidiomycetes, a number of other genes have been cloned which are specifically expressed during fruiting-body development. However, only few of these genes have been studied in detail, and a function has been assigned to even fewer of them. For instance, future research should establish the role of PRI3 and PRI4 in fruiting-body development of *A. aegeritae* (Sirand-Pugnet and Labarère 2002; Sirand-Pugnet et al. 2003) and of the cysteine-rich PRIA of *L. edodes* (Kajiwara et al. 1992). These genes encode proteins without homologues in the databases. Recently, it was established that over-expression of the *priA* gene in *L. edodes* led to decreased intracellular zinc ion accumulation (Ishizaki and Shishido 2000). It is not clear why a role in regulation of intracellular zinc concentration is important in the early stages of fruiting-body development.

A. Hydrophobins

Hydrophobins are secreted proteins which fulfil a wide spectrum of functions in fungal growth and development in general, and in fruiting-body formation in particular (Wessels 1997; Wösten and Wessels 1997; Wösten 2001; see The Mycota, Vol. VIII, Chap. 7). Based on their hydropathy patterns and their solubility characteristics, class I and class II hydrophobins were distinguished (Wessels 1994). In the basidiomycetes, only class I hydrophobins have been identified and, thus, class II hydrophobins seem not to be involved in fruiting-body formation in this phylum of the fungal kingdom.

Hydrophobins can function in a soluble state by affecting hyphal wall composition (van Wetter et al. 2000b). However, the mechanism underlying most functions is based on the property of hydrophobins to self-assemble at a hydrophilic/hydrophobic interface in an amphipathic membrane (Wösten et al. 1993, 1994a,b, 1995, 1999). This membrane of about

10 nm thick is highly insoluble and characterized by an ultrastructure of a mosaic of parallel rods, called the rodlet layer (Wösten et al. 1993). The rodlets, which have an amyloid-like nature (for structural aspects of hydrophobins, see Wösten and de Vocht 2000), are generally observed at surfaces of aerial structures (see Wösten and Wessels 1997; Wösten 2001). Upon self-assembly at the interface between the hydrophilic cell wall and a hydrophobic environment (the air or the hydrophobic surface of a host), the hydrophilic side of the amphipathic membrane will orient and attach itself to the cell wall, whereas the hydrophobic side becomes exposed to the hydrophobic environment. Aerial hyphae and spores thus become hydrophobic, whereas hyphae which grow over a hydrophobic substrate become attached.

The role of hydrophobins in fungal growth and development has been best studied in *S. commune*. This fungus has at least four hydrophobin genes; i.e. *SC1*, *SC3*, *SC4* and *SC6* (Mulder and Wessels 1986; Schuren and Wessels 1990; Wessels et al. 1995; de Vocht et al. 1998). The *SC1*, *SC4* and *SC6* hydrophobin genes are expressed in the dikaryon and regulated by the mating-type genes (Ruiters et al. 1988), the *FBF* gene (Springer and Wessels 1989) and the *THN* gene (Wessels et al. 1991b). The *SC3* gene is expressed both in the monokaryon and the dikaryon (see above). It is regulated by *THN* but not by *FBF*. Hydrophobins constitute some 6%–8% of all proteins synthesised in *S. commune* at the time of emergent growth (Wessels et al. 1991a,b). Despite several attempts, we were unable to isolate SC1 and SC6. By contrast, SC3 was isolated in quantity from the medium, and was present in walls of aerial hyphae (Wessels et al. 1991a,b) and hyphae which coat fruiting bodies (Ásgeirsdóttir et al. 1995). On the other hand, SC4 was found in the medium and in walls of hyphae forming the context of fruiting bodies (Wessels et al. 1991a,b).

Monokaryons of *S. commune* are sterile. Yet, formation of aerial hyphae by monokaryons can serve as a model system to study the first stages of fruiting-body formation. Monokaryotic strains express only *SC3* of the hydrophobin genes. Its expression is induced after a feeding submerged mycelium has been established (Mulder and Wessels 1986). SC3 secreted by submerged hyphae self-assembles at the medium–air interface. Self-assembly is accompanied by a strong drop in water surface tension, enabling hyphae to breach the interface of the aqueous environment and the air to form aerial hyphae (Wösten et al. 1999; Fig. 19.4). In the absence of *SC3* expression, due to disruption of the gene (van Wetter et al. 1996), the water surface tension remains high and only few hyphae can escape the aqueous environment – in other words, most hyphae are forced to remain growing in the aqueous substrate. The onset of *SC3* gene expression is thus a regulatory switch for aerial growth. How the mycelium senses that the feeding mycelium is large enough to be able to support aerial growth by switching on SC3 production remains to be solved (Wösten and Willey 2000). SC3 secreted by aerial hyphae cannot diffuse into the medium but is confronted with the cell wall–air interface. As a result, SC3 assembles on these hyphae. The hydrophilic side of the SC3 film orients itself to the cell wall, whereas its hydrophobic side is exposed (Wösten et al. 1994a; Fig. 19.4). Aerial hyphae thus become hydrophobic.

In the dikaryon, SC3 also lowers the water surface tension, allowing aerial hyphae to grow into the air (van Wetter et al. 2000a). The amount of SC4 secreted into the medium is too low to compensate for the absence of SC3 in a *ΔSC3* dikaryon. SC3 also coats aerial hyphae of the dikaryon and hyphae at the outer surface of fruiting bodies (Ásgeirsdóttir et al. 1995). SC4, but not SC3, is located in the fruiting-body context, in which it lines air channels which traverse the plectenchyma (Lugones et al. 1999). A dikaryon in which the *SC4* genes were inactivated did form normal sporulating fruiting bodies. However, the air channels in the fruiting bodies readily filled with water in the absence of a hydrophobic coating (van Wetter et al. 2000a). From this, it was concluded that the SC4 coating serves to ensure gas exchange in the fruiting-body tissue under moist conditions.

The ABH1/HypA hydrophobin of *A. bisporus* (de Groot et al. 1996; Lugones et al. 1996) can be considered to be the orthologue of SC4 of *S. commune*. It not only coats the outer surface of the fruiting body (Lugones et al. 1996) but also lines air channels in the fruiting-body tissue (Lugones et al. 1999). Another hydrophobin, HYPB, seems to be located at the border of the cap and the stipe tissue, and was proposed to protect the mushroom against bacterial infection (de Groot et al. 1999).

Why should *S. commune*, *A. bisporus* and other fungi have more than one hydrophobin gene? It was suggested that this enables the fungus to express hydrophobins at different stages of development (Kershaw et al. 1998) but also that hydrophobins are tailored to fulfil specific functions (van Wet-

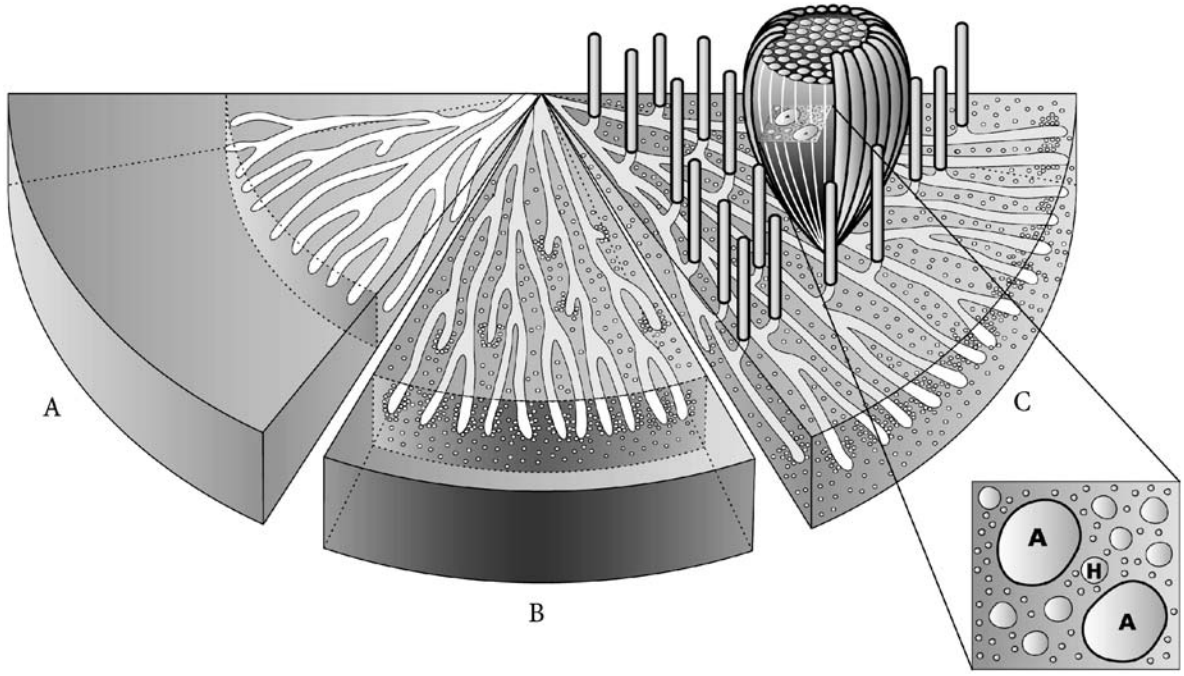

Fig. 19.4. A–C Schematic presentation of the role of hydrophobins in development of emergent structures of a *S. commune* dikaryon (a similar scheme can be drawn for the monokaryon which only produces the SC3 hydrophobin and aerial hyphae). **A** In juvenile cultures, the hydrophobin genes are silent and a substantial amount of submerged mycelium can be produced. **B** After a feeding submerged mycelium is formed, the hydrophobin genes are switched on. The SC3 hydrophobin (and, to a lesser extent, the SC4 hydrophobin) is secreted into the medium in a water-soluble form (*stipples*) at tips of growing hyphae, whereas the walls of these hyphae are virtually devoid of hydrophobins (walls of substrate hyphae drawn as *thin lines*). SC3 self-assembles at the water–air interface, thus reducing the surface tension. This allows hyphae to breach the interface to grow into the air (**C**). While continuing apical secretion, surfaces of aerial structures in contact with air are coated with an insoluble hydrophobin membrane due to self-assembly at the cell wall–air interface (hyphal walls drawn as *thick lines*). SC3 coats aerial hyphae, the hyphae surrounding the fruit bodies (here represented by a fruit body in which the pileus has not yet expanded) and the hymenium. SC4 coats air channels within the fruiting body, thus ensuring gas exchange (*inset*)

ter et al. 2000a). By swapping promoters, it was shown that SC4 can substitute for SC3 in formation of hydrophobic aerial hyphae. However, hyphal attachment to hydrophobic surfaces is only partially restored because the hydrophilic side of the SC4 membrane has a lower affinity for the cell wall of emergent hyphae than does SC3. Possibly, this is related to the different lectin specificities of these hydrophobins (van Wetter et al. 2000a). The exposed carbohydrates of cell walls of aerial hyphae and hyphae in fruiting-body tissue may be different, requiring different lectin specificities to ensure strong binding to the cell wall.

That hydrophobins are tailored to fulfil specific functions is also indicated by sequence analysis. SC3 of *S. commune* (de Vocht et al. 1998), ABH3 of *A. bisporus* (Lugones et al. 1998), COH1 of *C. cinereus* (Ásgeirsdóttir et al. 1997) and POH1 of *Pleurotus ostreatus* (Ásgeirsdóttir et al. 1998) have all been implicated to be involved in formation of aerial hyphae. These hydrophobins are more related to each other than are SC3 and ABH3 to the other hydrophobins of *S. commune* and *A. bisporus* respectively. Similarly, the fruiting body-specific hydrophobins of *S. commune* cluster with *HypB* (de Groot et al. 1999), *ABH1/HypA* (de Groot et al. 1996; Lugones et al. 1996) and *ABH2/HypC* (de Groot et al. 1996; Lugones et al. 1996) of *A. bisporus*. This suggests that functional similarity is reflected in the primary sequence of hydrophobins.

With the established roles of SC3, SC4 and ABH1, we are only at the beginning of our understanding of the functions of these proteins in fruiting. Dikaryons seem to express several hydrophobins which may have specific properties or are expressed at a particular place. For instance,

it has been suggested that hydrophobins could be involved in aggregating aerial hyphae during fruiting-body morphogenesis (see The Mycota, Vol. I, 1st edn., Chap. 21).

B. SC7 and SC14

SC7 and *SC14* were isolated as genes highly expressed during fruiting of *S. commune* (Mulder and Wessels 1986). Like the *SC1*, *SC4* and *SC6* hydrophobin genes, these genes are regulated by the mating-type genes (Ruiters et al. 1988), the *FBF* gene (Springer and Wessels 1989) and the *THN* gene (Wessels et al. 1991b). The coding sequences of *SC7* and *SC14* are 70% identical at the nucleotide level, while the encoded proteins have 87% similarity in amino acids (Schuren et al. 1993c). SC7 and SC14 are rather hydrophilic proteins with homology to pathogenesis-related proteins of plants, testis-specific proteins from mammals, and venom allergen proteins from insects. By using an antibody against a bacterial fusion protein, it was shown that SC7 is secreted in the medium, and is loosely bound to the extracellular matrix which binds hyphae in the fruiting body together (Schuren et al. 1993c). The precise role of SC7 and SC14 remains to be established.

C. Lectins

Lectins have been implicated in fruiting-body development of various basidiomycetes (Wang et al. 1998). Although a large number of these carbohydrate-binding proteins have been isolated, their role in fruiting is still unclear. The best-studied lectins with respect to fruiting-body development are CGL1 and CGL2 of *C. cinereus*. These β-galactoside binding lectins, called galectins, show 87% sequence identity (Charlton et al. 1992; Cooper et al. 1997) and have been shown to be specifically produced during fruiting-body formation (Boulianne et al. 2000). CGL2 is produced in the dark in aerial mycelium which forms the primary hyphal knots. Production of the lectin proceeds until completion of tissue differentiation in the primordia. Expression of *cgl2* is regulated by the *A* mating-type genes, is inhibited by constant light and is subject to carbon and/or nitrogen repression. The promoter of *cgl2* contains a cAMP response element with the consensus sequence TGCGTC (Bertossa et al. 2004), which is in agreement with a role of this second messenger in fruiting-body development (see above). Within the promoter sequence of *cgl2*, no motif could be defined acting in light or *A* mating-type regulation (Bertossa et al. 2004). Possibly, regulation is not direct but acts via transcription factors which have not yet been identified.

CGL1 production starts later than that of CGL2. It is produced at the moment the light-induced compact secondary hyphal knots are produced. Expression of *cgl1* continues throughout primordial development, reaching its maximum just prior to meiosis (Charlton et al. 1992; Boulianne et al. 2000). CGL1 and CGL2 are located in cell walls and in the extracellular matrix in the outer layers of the cap and stipe of primordia (Boulianne et al. 2000). By incubating fluorescently labelled galectins on sections of mushroom, it was shown that ligands of the galectins are located mainly in the gill tissue (Walser et al. 2005). This was rather surprising, although ligands were also localized at the outer surface layers. These outer layers are subjected to strong forces during stipe elongation and cap expansion (see The Mycota, Vol. I, 1st edn., Chap. 22). It was proposed that the galectins and their receptors are involved in resisting these stretching forces by attaching hyphal cells to each other (Boulianne et al. 2000). Recently, the structure of CGL2 was determined (Walser et al. 2004). The protein forms a clover leaf-shaped tetramer. Each monomer has a galectin fold composed of two antiparallel, six-stranded β-sheets forming a β-sandwich. The orientation of the binding sites in the tetramer makes CGL2 an excellent candidate to function as a cross-linker. Experimental evidence showed that β-galactosides derivatised at the 2′ and 3′ positions most strongly interact with CGL1 and CGL2 (Walser et al. 2004, 2005). Such derivatised galactosides are found in lipids of basidiomycetes (Jennemann et al. 1999, 2001).

Galectins have also been isolated from other basidiomycetes. A galectin called ACG, with 37% identity to CGL1, was isolated from *Agrocybe cylindracea* (synonym *A. aegerita*) (Yagi et al. 2001). Walser et al. (2003) suggested that this lectin is the same as the lectin AAL from *A. aegerita* (Sun et al. 2003). AAL has the property to promote differentiation of fruiting-body primordia of *A aegerita* as well as *Auricularia polytricha*. This indicates that galectins could also function as a signal for fruiting-body initiation.

D. Haemolysins

Aegerolysin of *A. aegerita*, which is probably encoded by the *Aa-pri1* gene of *A. aegerita* (Fernandez Espinar and Labarère 1997; Berne et al. 2002), and ostreolysin of *P. ostreatus* are acidic proteins of about 16 kDa which are specifically expressed in primordia and immature fruiting bodies (Berne et al. 2002). Both proteins have haemolytic activity at nanomolar concentrations. By aggregating in the plasma membrane, ostreolysin can permeabilize cell membranes of erythrocytes and artificial lipid bilayers by forming pores of about 4 nm (Sepčić et al. 2003, 2004). To form a transmembrane core complex for cytolysis, the closely related, 17-kDa pleurotolysin A assembles with the 59-kDa protein pleurotolysin B of *P. ostreatus* (Sakurai et al. 2004; Tomita et al. 2004). Evidence indicates that haemolysis works according to the colloid osmotic mechanism, i.e. it can be prevented by the presence of osmotically active solutes with a diameter exceeding that of the pore. Permeabilization of membranes by ostreolysin is inhibited by lysophospholipids (Sepčić et al. 2003). These lipids are well-known signalling molecules involved in a variety of processes (Corda et al. 2002), including differentiation (Spiegel et al. 2002). Therefore, it was suggested that ostreolysin has a role in cell signalling. Sepčić et al. (2003) suggested that the level of lysophopholipids in the cell could regulate membrane binding and pore-forming ability of ostreolysin. By this action, it may be involved in apoptosis (Walser et al. 2003), now known to be involved in development of the basidiomycete fruiting body (see above, and Chap. 9, this volume). Notably, no permeabilization was observed of membranes made of total lipid extract from fruiting bodies of *P. ostreatus*. These membranes were strong inhibitors of haemolysis of erythrocytes. This suggests that membranes of fruiting-body hyphae of *P. ostreatus* contain lysophospholipids (Sepčić et al. 2003). It remains to be established whether there are differences in the amounts of these lipids in hyphae making up the fruiting body, which is to be expected if ostreolysin fulfils a role in apoptosis of specific cells.

E. Oxidative Enzymes

A role for laccases in oxidative cross-linking was first proposed for polypores (Bu'Lock 1967; Bu'Lock and Walker 1967), which become pigmented and woody by oxidation of phenolic compounds. Since then, intracellular and extracellular laccase activity has been reported in primorida and fruiting bodies of a number of basidiomycetes such as *S. commune* (Leonard and Phillips 1973; Phillips and Leonard 1976; de Vries et al. 1986), *L. edodes* (Leatham and Stahmann 1981; Leatham 1985; Zhao and Kwan 1999; Nagai et al. 2003) and *Volvariella volvacea* (Chen et al. 2003, 2004a,b). This supports a role for these enzymes in fruiting-body development, for instance, by oxidative cross-linking of hyphae in fruiting bodies (Leatham and Stahmann 1981; Wessels et al. 1985). Alternatively, the deposition of oxidized phenolics resulting from laccase activity in the walls may increase surface hydrophobicity and thus aid in the occurrence of emergent growth. Unfortunately, these roles have still not been verified, for instance, by gene inactivation.

The *eln2-1* mutant of *C. cinereus* was isolated in a screen for developmental mutants (Muraguchi et al. 1999). This mutant is characterized by dumpy fruiting-body primordia. Cell morphogenesis and tissue organization in the primordial shaft are affected. As a result, the mature fruiting bodies of this strain have short stipes (Muraguchi and Kamada 2000). The *eln2* gene is constitutively expressed and encodes a novel type of cytochrome P450 enzyme. These enzymes are involved in the oxidative, peroxidative and reductive metabolism of numerous compounds. A deletion of 18 amino acids at the C terminus is the cause of the mutant phenotype. Possibly, this deletion results in a modified mode of catalytic activity. How can this explain the mutant phenotype? Muraguchi and Kamada (2000) gave three explanations. First, a changed catalytic activity may produce a toxic compound which affects development in the primordial shaft. Second, activity of the truncated enzyme may not result in a product which is normally instrumental in development. Finally, the mutant enzyme may overproduce a normal metabolite or produce superoxide radicals. A disruption of the *eln2* gene should establish if this gene is indeed involved in fruiting-body development.

Expression of cytochrome P450 genes has also been shown in mushrooms of *A. bisporus* and *L. edodes* (de Groot et al. 1997; Akiyama et al. 2002; Hirano et al. 2004) Interestingly, the cytochrome P450 genes of *L. edodes*, *Le.cyp1* and *Le.cyp2*, were shown to be differentially expressed. MRNA levels were higher in primorida and in the stipe of the premature fruiting body (Akiyama et al. 2002). The

expression in the stipe suggests that it could have a function similar to that of the *eln2* gene of *C. cinereus*.

F. Enzymes Involved in Carbohydrate Metabolism

Due to substrate limitation in most laboratory cultures of *S. commune*, only a few primordia can grow into typical, fan-shaped fruiting bodies (Fig. 19.2), which can measure a few centimetres across. During enlargement, here based on proliferation of hyphae (Wessels 1993a) and not on hyphal inflation, as in agarics (Hammad et al. 1993, see The Mycota, Vol. I, 1st edn., Chap. 22), nitrogenous compounds and carbohydrates are retrieved from preformed substrate mycelium and from abortive primordia (Wessels 1965; Niederpruem and Wessels 1969; Wessels and Sietsma 1979). Glycogen degradation occurs mainly by a glucoamylase (Yli-Mattila and Raudaskoski 1992) while degradation of water-soluble β-(1,3)/β(1,6)-glucan occurs by a β-(1,3)-glucan glucohydrolase (Wessels 1969). The latter glucan occurs as a jelly material around hyphae and in the medium (Wessels et al. 1972). In addition, a glucan of similar structure, but occurring as a major alkali-insoluble component of hyphal walls (R-glucan) due to linkage to chitin (Sietsma and Wessels 1977, 1979), is degraded in substrate hyphae and in abortive primordia to provide for the needs of growing fruiting bodies. Breakdown of this glucan is initiated by a catabolite-repressed β-(1,6)-glucan glucanohydrolase (Wessels 1966, 1969). Because other cell components are also degraded and reused for growth of the fruiting bodies, most of what remains of the supporting structures are empty hyphae with walls containing mainly (1,3)-α-glucan, notwithstanding the apparent possibility of this fungus to make 1,3-α-glucanase under other circumstances (Reyes et al. 1980). A mutant in which R-glucan degradation is blocked is deficient in outgrowth of primordia (Wessels 1965, 1966). In the wild-type strain, high concentrations of carbon dioxide lead to synthesis of an altered R-glucan which is less susceptible to enzymatic degradation and, thus, cannot sustain outgrowth of primordia (Sietsma et al. 1977). Also spore production in *S. commune* depends on degradation of previously synthesised polymers (Bromberg and Schwalb 1976). Even in the presence of a carbon source in the medium, 30% of the total material in spores derives from previously synthesised material. In agarics, such as *C. cinereus* (Madelin 1960; Ji and Moore 1993), *Flammulina velutipes* (Kitamoto and Gruen 1976; Gruen and Wong 1982) and *C. congregatus* (Robert 1977), fruiting-body formation has also been shown to be associated with breakdown of polysaccharides in the substrate mycelium.

During transition from vegetative growth to fruiting-body development in *S. commune*, the respiratory quotient of cultures changes from above 2 to around unity, indicating the operation of purely oxidative metabolism in the fruiting bodies but some fermentative activity in the substrate mycelium (Wessels 1965). This may be a consequence of the greater oxygen availability to hyphae in the emerging fruiting bodies. Of enzymes involved in respiratory activity, Schwalb (1974) noted a marked decrease in the activity of phosphoglucomutase in the fruiting bodies. This was linked to the appearance of specific proteases in fruiting bodies which inactivated the enzyme (Schwalb 1977).

V. Conclusions

Establishment of the dikaryotic mycelium and formation of fruiting bodies are highly complex developmental programmes. A wide variety of proteins are expected to regulate and coordinate these programmes or to fulfil enzymatic conversions or structural roles. With the identification of the first genes during the last two decades, we are only at the beginning of understanding fruiting-body formation. A number of genes have been isolated which have no homologues in the protein databases, and for which functions are unknown. Other genes have been studied in more detail and, based on these studies, functions have been proposed or assigned.

Gene expression during establishment of the dikaryon and emergence of fruiting bodies in basidiomycetes has been linked to the combinatorial activities of the mating-type genes. These genes encode DNA-binding proteins and pheromones and their receptors. The regulation of fruiting genes by the mating-type genes seems to be quite indirect, and other regulatory genes such as *FRT1* and *THN* of *S. commune* and *pcc1* and *clp1* of *C. cinereus* have been shown to influence emergent growth, and fruiting-body formation in particular. The latter gene appears to be a direct target of the homeodomain protein encoded in the *A* mating-type

locus. Interestingly, basidiomycetes have a way of regulating gene expression by varying the nuclear distance in the vegetative mycelium of the dikaryon. There are indications that this phenomenon also takes place in the fruiting bodies.

Among the fruiting genes are members of the hydrophobin gene family which enable fungi to escape the aqueous environment to allow fruiting-body development. In fact, their formation may potentiate the mycelium to form such emergent structures. The signals leading to hydrophobin expression have to be determined. Hydrophobins also coat surfaces of fruiting bodies and spores. The hydrophobic coating irreversibly directs growth of hyphae into the air, allows aerial dispersal of spores, and ensures gas exchange in fruiting bodies under humid conditions. Apart from hydrophobins, phenolics polymerised by the action of laccases may contribute to surface hydrophobicity of fruiting bodies. These enzymes have also been proposed to cross-link cell walls of hyphae in the fruiting bodies.

The role of a variety of other proteins specifically expressed during fruiting-body development is not yet clear. Among these proteins are the lectins, cytochrome P450 and the haemolysins. Lectins may be involved in aggregation of hyphae, whereas haemolysins may be involved in signalling, particularly to induce apoptosis of selected hyphae in the fruiting body.

Although environmental factors profoundly influence fruiting, in general little is known about mechanisms by which this is achieved. Some studies indicate transduction of the light signal through elevation of cyclic AMP. This may be related to initiation of the extensive translocation processes, accompanied by degradation of previously formed polymers, during the emergence of fruiting bodies.

References

Akiyama R, Sato Y, Kajiwara S, Shishido K (2002) Cloning and expression of cytochrome P450 genes, belonging to a new P450 family, of the basidiomycete *Lentinula edodes*. Biosci Biotechnol Biochem 66:2183–2188

Aono T, Yanai H, Miki F, Davey J, Shimoda C (1994) Mating pheromone-induced expression of the *mat1-Pm* gene of *Schizosaccaromyces pombe*: identification of signalling components and characterization of upstream controlling elements. Yeast 10:757–770

Ásgeirsdóttir SA, van Wetter MA, Wessels JGH (1995) Differential expression of genes under control of the mating-type genes in the secondary mycelium of *Schizophyllum commune*. Microbiology 141:1281–1288

Ásgeirsdóttir SA, Halsall JR, Casselton LA (1997) Expression of two closely linked hydrophobin genes of *Coprinus cinereus* is monokaryon-specific and down-regulated by the *oid-1* mutation. Fungal Genet Biol 22:54–63

Ásgeirsdóttir SA, de Vries OMH, Wessels JGH (1998) Identification of three differentially expressed hydrophobins in *Pleurotus ostreatus* (oyster mushroom). Microbiology 144:2961–2969

Berne S, Križaj I, Pohleven F, Turk T, Maček P, Sepčić K (2002) *Pleurotus* and *Agrocybe* hemolysins, new proteins hypothetically involved in fungal fruiting. Biochem Biophys Acta 1570:153–159

Bertossa RC, Kues U, Aebi M, Kunzler M (2004) Promoter analysis of *cgl2*, a galectin encoding gene transcribed during fruiting body formation in *Coprinopsis cinerea* (*Coprinus cinereus*). Fungal Genet Biol 41:1120–1131

Birkinshaw JH, Findlay, WPK, Webb RA (1942) Biochemistry of the wood-rotting fungi. The production of methyl mercaptan by *Schizophyllum commune*. Biochem J 36:526–529

Boulianne RP, Liu Y, Aebi, M, Lu BC, Kües U (2000) Fruiting body development in *Coprinus cinereus*: regulated expression of two galectins secreted by a non classical pathway. Microbiology 146:1841–1853

Brachmann A, Weinzierl G, Kämper J, Kahmann R (2001) Identification of genes in the bW/bE regulatory cascade in *Ustilago maydis*. Mol Microbiol 42:1047–1063

Bromberg SK, Schwalb MN (1976) Studies in basidiospore development in *Schizophyllum commune*. J Gen Microbiol 96:409–413

Bromberg SK, Schwalb MN (1977) Isolation and characterization of temperature sensitive sporulationless mutants of the basidiomycete *Schizophyllum commune*. Can J Genet Cytol 19:477–481

Bu'Lock JD (1967) Essays in biosynthesis and microbial development. In: Squibb ER (ed) Essays in biosynthesis and microbial development, lectures on chemistry and microbial products. Wiley, New York, pp 1–18

Bu'Lock JD, Walker DC (1967) On chagi. J Chem Soc Sect C, pp 336–338

Chang ST, Hayes WA (eds) (1978) The biology and cultivation of edible mushrooms. Academic Press, New York

Charlton S, Boulianne R, Chow YC, Lu BC (1992) Cloning and differential expression during the sexual cycle of a meiotic endonuclease-encoding gene from the basidiomycete *Coprinus cinereus*. Gene 122:163–169

Chen SC, Ma DB, Ge W, Buswell JA (2003) Induction of laccase activity in the edible straw mushroom, *Volvariella volvacea*. FEMS Microbiol Lett 218:143–148

Chen SC, Ge W, Buswell JA (2004a) Molecular cloning of a new laccase from the edible straw mushroom *Volvariella volvacea*: possible involvement in fruit body development. FEMS Microbiol Lett 230:171–176

Chen SC, Ge W, Buswell JA (2004b) Biochemical and molecular characterization of a laccase from the edible straw mushroom, *Volvariella volvacea*. Eur J Biochem 271:318–328

Chiu S-W, Moore D (eds) (1996) Patterns in fungal development. Cambridge University Press, Cambridge, UK

Clémonçon H (1997) Anatomy of the hymenomycetes. An introduction to the cytology and plectology of crust fungi, bracket fungi, club fungi, Chantarelles, Agarics and Boletes. Flück-Wirth, Teufen, Switzerland

Cooper DNW, Boulianne RP, Charlton S, Farell EM, Sucher A, Lu BC (1997) Fungal galectins, sequence and specificity of two isolectins from *Coprinus cinereus*. J Biol Chem 272:1514–1521

Corda D, Iurisci C, Berrie CP (2002) Biological activities and metabolism of lysophophatidylinositols and glycerophosphoinositols. Biochim Biophys Acta 1582:52–69

Cummings WJ, Celerin M, Crodian J, Brunick LK, Zolan ME (1999) Insertional mutagenesis in *Coprinus cinereus*: use of a dominant selectable marker to generate tagged, sporulation-defective mutants. Curr Genet 36:371–382

De Groot PWJ, Schaap PJ, Sonnenberg ASM, Visser J, van Griensven LJLD (1996) The *Agaricus bisporus hypA* gene encodes a hydrophobin and specifically accumulates in peel tissue of mushroom caps during fruit body development. J Mol Biol 257:1008–1018

De Groot PWJ, Schaap PJ, van Griensven LJLD (1997) Isolation of developmentally regulated genes from the edible mushroom *Agaricus bisporus*. Microbiology 143:1993–2001

De Groot PWJ, Roeven RT, van Griensven LJ, Visser J, Schaap PJ (1999) Different temporal and spatial expression of two hydrophobin-encoding genes of the edible mushroom *Agaricus bisporus*. Microbiology 145:1105–1113

De Vocht ML, Scholtmeijer K, van der Vegte EW, de Vries OMH, Sonveaux N, Wösten HAB, Ruysschaert J-M, Hadziioannou G, Wessels JGH, Robillard GT (1998) Structural characterization of the hydrophobin SC3, as a monomer and after self-assembly at hydrophobic/hydrophilic interfaces. Biophys J 74:2059–2068

De Vries OMH, Wessels JGH (1984) Patterns of protein synthesis in non-fruiting monokaryons and a fruiting dikaryon of *Schizophyllum commune*. J Gen Microbiol 130:145–154

De Vries OMH, Kooistra WHCF, Wessels JGH (1986) Formation of an extracellular laccase by a *Schizophyllum commune* dikaryon. J Gen Microbiol 132:2817–2826

De Vries L, Mousli M, Wurmser A, Farquhar MG (1995) GAIP, a protein that specifically interacts with the trimeric G protein Giα3, is a member of a protein family with a highly conserved core. Proc Natl Acad Sci USA 92:11916–11920

Dietzel C, Kurjan J (1987) Pheromonal regulation and sequence of the *Saccharomyces cerevisae SST2* gene: a model for desensitisation to pheromone. Mol Cell Biol 7:4169–4177

Dons JJM, Springer J, de Vries SC, Wessels JGH (1984) Molecular cloning of a gene abundantly expressed during fruiting body initiation in *Schizophyllum commune*. J Bacteriol 157:802–808

Durand R (1983) Effects of inhibitors of nucleic acid and protein synthesis on light-induced primordial initiation in *Coprinus congrgatus*. Trans Br Mycol Soc 81:553–558

Durand R (1985) Blue U.V.-light photoreception in fungi. Review. Physiol Végét 23:935–943

Edelstein L (1982) The propagation of fungal colonies: a model for tissue growth. J Theor Biol 98:679–701

Edelstein L, Segel LA (1983) Growth and metabolism in mycelial fungi. J Theor Biol 104:187–210

Eger-Hummel G (1980) Blue-light photomorphogenesis in mushrooms (basidiomycetes). In: Senger H (ed) The blue light syndrome. Springer, Berlin Heidelberg New York, pp 555–562

Elliot CG (1994) Reproduction in fungi. Chapman and Hall, London

Esser K, Meinhardt F (1977) A common genetic control of dikaryotic and monokaryotic fruiting in the basidiomycete *Agrocybe aegerita*. Mol Gen Genet 155:113–115

Esser K, Saleh F, Meinhardt F (1979) Genetics of fruit-body production in higher basidiomycetes. II. Monokaryotic and dikaryotic fruiting in *Schizophyllum commune*. Curr Genet 1:85–88

Faumann EB, Blumenthal RM, Cheng XD (1999) Structure and evolution of AdoMet-dependent methyltransferases. In: Cheng XD, Blumenthal RM (eds) S-adenosylmethionine-dependent methytransferases: structures and functions. World Scientific, Singapore, pp 1–38

Fernandez Espinar MT, Labarère J (1997) Cloning and sequencing of the *Aa-pri1* gene specifically expressed during fruiting initiation in the edible mushroom *Agrocybe aegeritae*. Curr Genet 32:420–424

Flegg PB, Spencer DM, Wood D (1985) The biology and technology of the cultivated mushroom. Wiley, Chichester

Fowler TJ, Mitton MF (2000) *Scooter*, a new active transposon in *Schizophyllum commune*, has disrupted two genes regulating signal transduction. Genetics 156:1585–1594

Fraser JA, Subaran RL, Nichols CB, Heitman J (2003) Recapitulation of the sexual cycle of the primary fungal pathogen *Cryptococcus neoformans* var. *gattii*: implications for an outbreak on Vancouver Island, Canada. Eukaryot Cell 2:1036–1045

Gold MH, Cheng TM (1979) Conditions for fruit body formation in the white rot basidiomycete *Phanerochaete chrysosporium*. Arch Microbiol 121:37–41

Goutte C, Johnson AD (1988) **a1** protein alters the DNA binding specificity of α2 repressor. Cell 52:875–882

Granado JD, Kertesz Chaloupková K, Aebi M, Kues U (1997) Restriction enzyme-mediated DNA integration in *Coprinus cinereus*. Mol Gen Genet 256:28–36

Gregory PH (1984) The fungal mycelium: an historical perspective. Trans Br Mycol Soc 82:1–11

Grillo R, Korhonen K, Hantula J, Hietala AM (2000) Genetic evidence of somatic haploidization in developing fruit bodies of *Armillaria tabscens*. Fungal Genet Biol 30:135–145

Gruen HE, Wong WM (1982) Distribution of cellular amino acids, protein, and total organic nitrogen during fruitbody development in *Flammulina velutipes*. II. Growth on potato-glucose solution. Can J Bot 60:1342–1351

Hammad F, Ji J, Watling R, Moore D (1993) Cell population dynamics in *Coprinus cinereus*: co-ordination of cell inflation throughout the maturing basidiome. Mycol Res 97:269–274

Hansberg W, Aguirre J (1990) Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. J Theor Biol 142:201–221

Hartmann HA, Kahmann R, Bölker M (1996) The pheromone response factor coordinates filamentous growth and pathogenicity in *Ustilago maydis*. EMBO J 15:1632–1641

Hirano T, Sato T, Enei H (2004) Isolation of genes specifically expressed in the fruit body of the edible basidiomycete *Lentinula edodes*. Biosci Biotechnol Biochem 68:468–472

Hoge JHC, Springer J, Wessels JGH (1982) Changes in complex RNA during fruit-body initiation in the fungus *Schizophyllum commune*. Exp Mycol 6:233–243

Horton JS, Raper CA (1991) A mushroom-inducing DNA sequence isolated from the basidiomycete *Schizophyllum commune*. Genetics 129:707–716

Horton JS, Raper CA (1995) The mushroom-inducing gene *FRT1* of *Schizophyllum commune* encodes a putative nucleotide binding protein. Mol Gen Genet 247:358–366

Horton JS, Palmer GE, Smith WJ (1999) Regulation of dikaryon-expressed genes by *FRT1* in the basidiomycete *Schizophyllum commune*. Fungal Genet Biol 26:33–47

Inada K, Morimoto Y, Arima T, Murata Y, Kamada T (2001) The *clp1* gene of the mushroom *Coprinus cinereus* is essential for *A*-regulated sexual development. Genetics 157:133–140

Ishizaki T, Shishido K (2000) Decreased zinc ion accumulation by the basidiomycete *Lentinus edodes* overexpressing *L. edodes priA* gene. FEMS Microbiol Lett 193:111–115

Jennemann R, Bauer BL, Bertalanffy H, Geyer R, Gschwind RM, Selmer T, Wiegandt H (1999) Novel glycoinositolphosphosphingolipids, basidiolipids, from *Agaricus*. Eur J Biochem 259:331–338

Jennemann R, Geyer R, Sandhoff R, Gschwind RM, Levery SB, Grone HJ, Wiegandt H (2001) Glycoinositolphosphosphingolipids (basidiolipids) of higher mushrooms. Eur J Biochem 268:1190–1205

Jennings DH (1984) Water flow through mycelia. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of fungal mycelia. Cambridge University Press, Cambridge, pp 143–164

Ji J, Moore D (1993) Glycogen metabolism in relation to fruit body maturation in *Coprinus cinereus*. Mycol Res 97:283–289

Kajiwara S, Yamaoka K, Hori K, Miyazawa H, Saito T, Kanno T, Shishido K (1992) Isolation and sequence of a developmentally regulated putative novel gene, *priA*, from the basidiomycete *Lentinus edodes*. Gene 114:173–178

Kamada T (2002) Molecular genetics of sexual development in the mushroom *Coprinus cinereus*. BioEssays 24:449–459

Kamada T, Kurita R, Takemaru T (1978) Effects of light on basidiocarp maturation in *Coprinus macrorhizus*. Plant Cell Physiol 19:263–275

Kanda T, Ishikawa T (1986) Isolation of recessive developmental mutants in *Coprinus cinereus*. J Gen Appl Microbiol 32:541–543

Kassir Y, Granot D, Simchen G (1988) IME1, a positive regulator gene of meiosis in *Saccharomyces cerevisiae*. Cell 52:853–862

Kawai G, Ikeda Y (1982) Fruiting-inducing activity of cerebrosides observed with *Schizophyllum commune*. Biochim Biophys Acta 719:612–618

Kawai G, Onishi M, Fujino Y, Ikeda Y (1986) Stimulatory effect of certain plant sphingolipids on fruiting of *Schizophyllum commune*. J Biol Chem 261:779–784

Kershaw MJ, Wakley G, Talbot NJ (1998) Complementation of the *mpg1* mutant phenotype in *Magnaporthe grisea* reveals functional relationships between fungal hydrophobins. EMBO J 17:3838–3849

Kertesz-Chaloupková K, Walser PJ, Granado JD, Aebi M, Kües U (1998) Blue light overrides repression of asexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 23:95–109

Kinoshita H, Sen K, Iwama H, Samadder PP, Kurosawa S, Shibai H (2002) Effects of indole and caffeine on CAMP in the ind1 and cfn1 mutant strains of *Schizophyllum commune* during sexual development. FEMS Microbiol Lett 206:247–251

Kitamoto Y, Gruen HE (1976) Distribution of cellular carbohydrates during development of the mycelium and fruitbodies of *Flammulina velutipes*. Plant Physiol 58:485–491

Kitamoto Y, Horikoshi T, Suzuki A (1974) An action spectrum for photoinduction of pileus formation in a Basidiomycete *Flavolus arcularius*. Planta 119:81–84

Koltin Y (1970) Development of the *AmutBmut* strain of *Schizophyllum commune*. Arch Microbiol 74:123–128

Koltin Y, Raper JR (1968) Dikaryosis genetic determination in *Schizophyllum commune*. Science 60:85–86

Kothe E (2001) Mating-type genes for basidiomycete strain improvement in mushroom farming. Appl Microbiol Biotechnol 56:602–612

Kües U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Kües U, Liu Y (2000) Fruiting body production in basidiomycetes. Appl Microbiol Biotechnol 54:141–152

Kües U, Künzler M, Bottoli APF, Walser PJ, Granado JD, Liu Yi, Bertossa RC, Ciardo D, Clergeot P-H, Loos S et al. (2004) Mushroom development in higher basidiomycetes; implications for human and animal health. In: Kushwaha RKS (ed) Fungi in human and animal health. Scientific Publishers, Jodhpur, India, pp 431–469

Labarère J, Noel T (1992) Mating type switching in the tetrapolar basidiomycete *Agrocybe aegerita*. Genetics 131:307–319

Leatham GF (1985) Growth and development of *Lentinus edodes* on chemically defined medium. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, Cambridge, pp 403–427

Leatham GF, Stahmann MA (1981) Studies on the laccase of *Lentinus edodus*: specificity, localization and association with the development of fruiting bodies. J Gen Microbiol 125:147–157

Lee BN, Adams TH (1994) Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation, leads to activation of *brlA* and premature initiation of development. Mol Microbiol 14:323–334

Leonard TJ, Phillips LE (1973) Studies of phenoloxidase activity during the reproductive cycle in *Schizophyllum commune*. J Bacteriol 114:7–10

Leslie JF, Leonard TJ (1979a) Three independent genetic systems that control initiation of a fungal fruit body. Mol Gen Genet 171:257–260

Leslie JF, Leonard TJ (1979b) Monokaryotic fruiting in *Schizophyllum commune*: genetic control of the response to mechanical injury. Mol Gen Genet 175:5–12

Lu BC (1974) Meiosis in *Coprinus*. Role of light on basidiocarp initiation, mitosis, and hymenium differentiation in *Coprinus lagopodus*. Can J Bot 52:299–308

Lu BC (2000) The control of meiosis progression in the fungus *Coprinus cinereus* by light/dark cycles. Fungal Genet Biol 31:33–41

Lugones LG, Bosscher JS, Scholtmeijer K, de Vries OMH, Wessels JGH (1996) An abundant hydrophobin (ABH1) forms hydrophobic rodlet layers in *Agaricus bisporus* fruiting bodies. Microbiology 142:1321–1329

Lugones LG, Wösten HAB, Wessels JGH (1998) A hydrophobin (ABH3) secreted by the substrate mycelium of *Agaricus bisporus* (common white button mushroom). Microbiology 144:2345–2353

Lugones LG, Wösten HAB, Birkenkamp KU, Sjollema KA, Zagers J, Wessels JGH (1999) Hydrophobins line air channels in fruiting bodies of *Schizophyllum commune* and *Agaricus bisporus*. Mycol Res 103:635–640

Madelin MF (1956) The influence of light and temperature on fruiting of *Coprinus lagopus* Fr. in pure culture. Ann Bot 20:467–480

Madelin MF (1960) Visible changes in the vegetative mycelium of *Coprinus lagopus* Fr. at the time of fruiting. Trans Br Mycol Soc 43:105–110

Manachère G (1980) Conditions essential for controlled fruiting of macromycetes – a review. Trans Br Mycol Soc 75:255–270

Manachère G (1988) Regulation of sporophore differentiation in some macromycetes, particularly in Coprini: an overview of some experimental studies from fruiting initiation to sporogenesis. Cryptogamie Mycol 9:291–323

Meskauskas A, McNulty LJ, Moore D (2004) Concerted regulation of all hyphal tips generates fungal fruit body structures: experiments with computer visualizations produced by a new mathematical model of hyphal growth. Mycol Res 108:341–353

Mizushina Y, Hanashima L, Yamaguchi T, Takemura M, Sugawara F, Saneyoshi M, Matsukage A, Yoshida S, Sakaguchi K (1998) A mushroom fruiting body-inducing substance inhibits activities of replicative DNA polymerases. Biochem Biophys Res Commun 249:17–22

Money N (2004) Theoretical biology – mushrooms in cyberspace. Nature 431:32

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge, UK

Moore D, Casselton LA, Wood DA, Frankland JC (eds) (1985) Developmental biology of higher fungi. Cambridge University Press, Cambridge UK

Moukha SM, Wösten HAB, Asther M, Wessels JGH (1993) In situ localization of lignin peroxidase excretion in colonies of *Phanerochaete chrysosporium* using sandwiched mode of culture. J Gen Microbiol 139:969–978

Mulder GH, Wessels JGH (1986) Molecular cloning of RNAs differentially expressed in monokaryons and dikaryons of *Schizophyllum commune*. Exp Mycol 10:214–227

Muraguchi H, Kamada T (1998) The *ich1* gene of the mushroom *Coprinus cinereus* is essential for pileus formation in fruiting. Development 125:3133–3141

Muraguchi H, Kamada T (2000) A mutation in the *eln2* gene encoding a cytochrome P450 of *Coprinus cinereus* affects mushroom morphogenesis. Fungal Genet Biol 29:49–59

Muraguchi H, Takemaru T, Kamada K (1999) Isolation and characterization of developmental variants in fruiting using a homokaryotic fruiting strain of *Coprinus cinereus*. Mycoscience 40:227–235

Murata Y, Fujii M, Zolan ME, Kamada T (1998) Molecular analysis of *pcc1*, a gene that leads to *A*-regulated sexual morphogenesis in *Coprinus cinereus*. Genetics 149:1753–1761

Nagai M, Kawata M, Watanabe H, Ogawa M, Saito K, Takesawa T, Kanda K, Sato T (2003) Important role of fungal intracellular laccase for melanin synthesis: purification and characterization of an intracellular laccase from *Lentinula edodes* fruit bodies. Microbiology 149:2455–2462

Niederpruem DJ (1963) Role of carbon dioxide in the control of fruiting of *Schizophyllum commune*. J Bacteriol 85:1300–1308

Niederpruem DJ, Wessels JGH (1969) Cytodifferentiation and morphogenesis in *Schizophyllum commune*. Bacteriol Rev 33:505–535

Perkins JH (1969) Morphogenesis in *Schizophyllum commune*. I. Effects of white light. Plant Physiol 44:1706–1711

Perkins JH, Raper JR (1970) Morphogenesis in *Schizophyllum commune*. III. Mutation that blocks initiation of fruiting. Mol Gen Genet 106:151–154

Phillips LE, Leonard TJ (1976) Extracellular and intracellular phenoloxidase activity during growth and development in *Schizophyllum commune*. Mycologia 68:268–276

Raper CA (1976) Sexuality and life cycle of the edible, wild *Agaricus bitorquis*. J Gen Microbiol 95:54–66

Raper JR, Krongelb GS (1958) Genetic and environmental aspects of fruiting in *Schizophyllum commune*. Mycologia 59:707–740

Raper JR, Miles PG (1958) The genetics of *Schizophyllum commune*. Genetics 43:530–546

Raper JR, Boyd DH, Raper CA (1965) Primary and secondary mutations at the incompatibility loci in *Schizophyllum*. Proc Natl Acad Sci USA 53:1324–1332

Raper CA, Raper JR, Miller RE (1972) Genetic analysis of the life cycle of *Agaricus bisporus*. Mycologia 64:1088–1117

Raudaskoski M, Salonen M (1983) Interrelationships between vegetative development and basidiocarp initiation. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 291–322

Raudaskoski M, Vauras R (1982) Scanning electron microscope study of fruit body differentiation in *Schizophyllum commune*. Trans Br Mycol Soc 78:89–96

Raudaskoski M, Viitanen H (1982) Effects of aeration and light on fruit-body induction in *Schizophyllum commune*. Trans Br Mycol Soc 78:89–96

Raudaskoski M, Yli-Mattila T (1985) Capacity for photoinduced fruiting in the dikaryon of *Schizophyllum commune*. Trans Br Mycol Soc 85:145–151

Rayner AD (1991) The challenge of the individualistic mycelium. Mycologia 83:48–71

Reijnders AFM, Stafleu JA (1992) The development of the hymenophoral trama in the Aphylophorales and Agaricales. Stud Mycol 34:1–109

Reyes F, Lahoz R, Vasques C (1980) Lytic enzymes in the autolysis of *Schizophyllum commune* with special reference to 1,3-α-glucanase. Can J Microbiol 26:1120–1125

Robert JC (1977) Fruiting of *Coprinus congregatus*: relationship to biochemical changes in the whole culture. Trans Br Mycol Soc 68:389–395

Romeis T, Brachmann A, Kahmann R, Kämper J (2000) Identification of a target gene for the bE-bW homeodomain protein complex in *Ustilago maydis*. Mol Microbiol 37:54–66

Ross IK (1982) Location of carpophore initiation in *Coprinus congregatus*. J Gen Microbiol 128:2755–2762

Ruiters MHJ, Wessels JGH (1989a) *In situ* localization of specific RNAs in whole fruiting colonies of *Schizophyllum commune*. J Gen Microbiol 135:1747–1754

Ruiters MHJ, Wessels JGH (1989b) *In situ* localization of specific RNAs in developing fruit bodies of the basidiomycete *Schizophyllum commune*. Exp Mycol 13:212–222

Ruiters MHJ, Sietsma JH, Wessels JGH (1988) Expression of dikaryon-specific mRNAs of *Schizophyllum commune* in relation to incompatibility genes, light, and fruiting. Exp Mycol 12:60–69

Sakurai N, Kaneko J, Kamio Y, Tomita T (2004) Cloning, expression, and pore-forming properties of mature and precursor forms of pleurotolysin, a sphingomyelin-specific two-component cytolysin from the edible mushroom *Pleurotus ostreatus*. Biochim Biophys Acta Gene Struct Expression 1679:65–73

Sánchez C, Moore D (1999) Conventional histological stains selectively stain fruit body initials of basidiomycetes. Mycol Res 103:315–318

Sánchez C, Tellez-Tellez M, Diaz-Godinez G, Moore D (2004) Simple staining detects ultrastructural and biochemical differentiation of vegetative hyphae and fruit body initials in colonies of *Pleurotus pulmonarius*. Lett Appl Microbiol 38:483–487

Schuren FHJ (1999) Atypical interactions between the *thn* and wild-type mycelia of *Schizophyllum commune*. Mycol Res 103:1540–1544

Schuren FHJ, Wessels JGH (1990) Two genes specifically expressed in fruiting dikaryons of *Schizophyllum commune*: homologies with a gene not regulated by mating-type genes. Gene 90:199–205

Schuren FHJ, van der Lende T, Wessels JGH (1993a) Fruiting genes of *Schizophyllum commune* are transcriptionally regulated. Mycol Res 97:538–542

Schuren FHJ, Harmsen MC, Wessels JGH (1993b) A homologous gene-reporter system for the basidiomycete *Schizophyllum commune* based on internally deleted genes. Mol Gen Genet 238:91–96

Schuren FHJ, Ásgeirsdóttir SA, Kothe EM, Scheer JMJ, Wessels JGH (1993c) The *Sc7/Sc14* gene family of *Schizophyllum commune* codes for extracellular proteins specifically expressed during fruit-body formation. J Gen Microbiol 139:2083–2090

Schuurs TA, Dalstra HJP, Scheer JMJ, Wessels JGH (1998) Positioning of nuclei in secondary mycelium of *Schizophyllum commune* in relation to differential gene expression. Fungal Genet Biol 23:150–161

Schwalb MN (1974) Changes in activity of enzymes metabolizing glucose-6-phosphate during development of the basidiomycete *Schizophyllum commune*. Dev Biol 40:84–89

Schwalb MN (1977) Developmentally regulated proteases from the basidiomycete *Schizophyllum commune*. J Biol Chem 225:8435–8439

Schwalb MN (1978) Regulation of fruiting. In Schwalb MN, Miles PG (eds) Genetics and morphogenesis in the basidiomycetes. Academic Press, New York, pp 135–165

Schwalb MN, Miles PG (1967) Morphogenesis of *Schizophyllum commune*. I. Morphological variation and mating behaviour of the *thin* mutation. Am J Bot 54:440–446

Sepčić K, Berne S, Potrich C, Turk T, Maček P, Menestrina G (2003) Interaction of ostreolysin, a cytolytic protein from the edible mushroom *Pleurotus ostreatus*, with lipid membranes and modulation by lysophospholipids. Eur J Biochem 270:1199–1210

Sepčić K, Berne S, Rebolj K, Batista UK, Plemenitas A, Sentjurc M, Maček P (2004) Ostreolysin, a pore-forming protein from the oyster mushroom, interacts specifically with membrane cholesterol-rich lipid domains. FEBS Lett 575:81–85

Sietsma JH, Wessels JGH (1977) Chemical analysis of the hyphal wall of *Schizophyllum commune*. Biochim Biophys Acta 496:225–239

Sietsma JH, Wessels JGH (1979) Evidence for covalent linkages between chitin and β-glucan in a fungal wall. J Gen Microbiol 114:99–108

Sietsma JH, Rast D, Wessels JGH (1977) The effect of carbon dioxide on fruiting and on the degradation of a cell-wall glucan in *Schizophyllum commune*. J Gen Microbiol 102:385–389

Sirand-Pugnet P, Labarère J (2002) Molecular characterization of the PRI3 gene encoding a cysteine-rich protein, specifically expressed during fruiting initiation within the *Agrocybe aegerita* complex. Curr Genet 41:31–42

Sirand-Pugnet P, Santos C, Labarère J (2003) The *Aa-Pri4* gene, specifically expressed during fruiting initiation in the *Agrocybe aegerita* complex, contains an unusual CT-rich leader intron within the 5′ uncoding region. Curr Genet 44:124–131

Spiegel S, English D, Milstien S (2002) Spingosine-1-phosphate signalling: providing cells with sense of direction. Trends Cell Biol 12:236–242

Springer J, Wessels JGH (1989) A frequently occurring mutation that blocks the expression of fruiting genes in *Schizophyllum commune*. Mol Gen Genet 219:486–488

Stahl U, Esser K (1976) Genetics of fruit-body production in higher basidiomycetes I. Monokaryotic fruiting and its correlation with dikaryotic fruiting in *Polyporus ciliatus*. Mol Gen Genet 148:183–197

Sugimoto A, Iino Y, Maeda T, Watanabe Y, Yamamoto M (1991) *Schizosaccharomyces pombe ste11*$^{+}$ encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. Genes Dev 5:1990–1999

Sun H, Zhao CG, Tong X, Qi YP (2003) A lectin with mycelia differentiation and antiphytovirus activities from the edible mushroom *Agrocybe aegerita*. J Biochem Mol Biol 36:214–222

Swamy S, Uno I, Ishikawa T (1984) Morphogenetic effects of mutations at the *A* and *B* incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 130:3219–3224

Swamy S, Uno I, Ishikawa T (1985a) Regulation of cyclic AMP metabolism by the incompatibility factors in *Coprinus cinereus*. J Gen Microbiol 131:3211–3217

Swamy S, Uno I, Ishikawa T (1985b) Regulation of cyclic AMP-dependent phosphorylation of cellular proteins by the incompatibility factors in *Coprinus cinereus*. J Gen Appl Microbiol 31:339–346

Takagi Y, Katayose Y, Shishido K (1988) Intracellular levels of cyclic AMP and adenylate cyclase activity during mycelial development in fruiting body formation in *L. edodes*. FEMS Microbiol Lett 55:275–278

Takemaru T, Kamada T (1972) Basidiocarp development in *Coprinus macrorhizus*. I. Induction of developmental variations. Bot Mag (Tokyo) 85:51–57

Toledo I, Hansberg W (1990) Protein oxidation related to morphogenesis in *Neurospora crassa*. Exp Mycol 14:184–189

Tomita T, Noguchi K, Mimuro H, Ukaji F, Ito K, Sugawara-Tomita N, Hashimoto Y (2004) Pleurotolysin, a novel sphingomyelin-specific two-component cytolysin from the edible mushroom *Pleurotus ostreatus*, assembles into a transmembrane pore complex. J Biol Chem 279:26975–26982

Tsusué YM (1969) Experimental control of fruit-body formation in *Coprinus macrorhizus*. Dev Growth Diff 11:164–178

Ullrich RC, Anderson JB (1978) Sex and diploidy in *Arnillaria mellea*. Exp Mycol 2:119–129

Umar MH, van Griensven LJLD (1997) Morphogenetic cell death in developing primordia of *Agaricus bisporus*. Mycologia 89:274–277

Uno I, Ishikawa T (1971) Chemical and genetical control of induction of monokaryotic fruiting bodies in *Coprinus macrorhizus*. Mol Gen Genet 113:228–239

Uno I, Ishikawa T (1973) Purification and identification of the fruiting inducing substances in *Coprinus macrorhizus*. J Bacteriol 113:1240–1248

Uno I, Ishikawa T (1982) Biochemical and genetic studies on the initial events of fruitbody formation. In: Wells K, Wells EK (eds) Basidium and basidiocarp. Springer, Berlin Heidelberg New York, pp 113–123

Uno I, Yamaguchi M, Ishikawa T (1974) The effect of light on fruiting body formation and adenosine 3′,5′-cyclic monophosphate metabolism in *Coprinus macrorhizus*. Proc Natl Acad Sci USA 71:479–483

Urban M, Kahmann R, Bölker M (1996) Identification of the pheromone response element in *Ustilago maydis*. Mol Gen Genet 251:31–37

Van der Valk P, Marchant R (1978) Hyphal ultrastructure in fruit body primordial of the basidiomycetes *Schizophyllum commune* and *Coprinus cinereus*. Protoplasma 95:57–72

Van Griensven LJLD (ed) (1988) The cultivation of mushrooms. Darlington Mushroom Laboratories, Rustington, Sussex, UK

Van Wetter MA, Schuren FHJ, Wessels JGH (1996) Targeted mutation of the *Sc3* hydrophobin gene of *Schizophyllum commune* affects formation of aerial hyphae. FEMS Microbiol Lett 140:265–270

Van Wetter MA, Wösten HAB, Wessels JGH (2000a) SC3 and SC4 hydrophobins have distinct functions in formation of aerial structures in dikaryons of *Schizophyllum commune*. Mol Microbiol 36:201–210

Van Wetter MA, Wösten HAB, Sietsma JH, Wessels JGH (2000b) Hydrophobin gene expression affects hyphal wall composition in *Schizophyllum commune*. Fungal Genet Biol 31:99–104

Walser PJ, Velagapudi R, Aebi M, Kües U (2003) Extracellular matrix proteins in mushroom development. Rec Res Dev Microbiol 7:381–415

Walser PJ, Haebel PW, Kunzler M, Sargent D, Kues U, Aebi M, Ban N (2004) Structure and functional analysis of the fungal galectin CGL2. Structure 12:689–702

Walser PJ, Kues U, Aebi M, Kunzler M (2005) Ligand interactions of the *Coprinopsis cinerea* galectins. Fungal Genet Biol 42:293–305

Wang H, Ng TB, Ooi VEC (1998) Lectins from mushrooms. Mycol Res 102:897–906

Wang P, Cutler J, King J, Palmer D (2004) Mutation of the regulator of G protein signaling Crg1 increases virulence in *Cryptococcus neoformans*. Eukaryot Cell 3:1028–1035

Watling R (1996) Patterns in fungal development – fruiting patterns in nature. In: Chiu S-W, Moore D (eds) Patterns in fungal development. Cambridge University Press, Cambridge, UK

Wells K, Wells EK (eds) (1982) Basidium and basidiocarp. Springer, Berlin Heidelberg New York

Wessels JGH (1965) Morphological and biochemical processes in *Schizophyllum commune*. Wentia 13:1–113

Wessels JGH (1966) Control of cell-wall glucan degradation during development in *Schizophyllum commune*. Antonie van Leeuwenhoek J Microbiol Serol 32:341–355

Wessels JGH (1969) A β-1,6-glucan glucanohydrolase involved in hydrolysis of cell wall glucan in *Schizophyllum commune*. Biochim Biophys Acta 178:191–193

Wessels JGH (1986) Cell wall synthesis in apical hyphal growth. Int Rev Cytol 104:37–79

Wessels JGH (1990) Role of cell wall architecture in fungal tip growth generation. In: Heath IB (ed) Tip growth of plant and fungal cells. Academic Press, San Diego, pp 1–29

Wessels JGH (1992) Gene expression during fruiting in *Schizophyllum commune*. Mycol Res 96:609–620

Wessels JGH (1993a) Fruiting in the higher fungi. Adv Microb Physiol 34:147–202

Wessels JGH (1993b) Wall growth, protein excretion and morphogenesis in fungi. New Phytol 123:397–413

Wessels JGH (1994) Developmental regulation of fungal cell wall formation. Annu Rev Phytopathol 32:413–437

Wessels JGH (1996) Fungal hydrophobins: proteins that function at an interface. Trends Plant Sci 1:9–15

Wessels JGH (1997) Hydrophobins: proteins that change the nature of the fungal surface. Adv Microb Physiol 38:1–45

Wessels JGH, Marchant JR (1974) Enzymatic degradation in hyphal wall preparations from a monokaryon and a dikaryon of *Schizophyllum commune*. J Gen Microbiol 83:359–368

Wessels JGH, Sietsma JH (1979) Wall structure and growth in *Schizophyllum commune*. In: Burnett JH, Trinci APJ (eds) Fungal walls and hyphal growth. Cambridge University Press, Cambridge, pp 27–48

Wessels JGH, Kreger DR, Marchant R, Regenburg BA, de Vries OMH (1972) Chemical and morphological characterization of the hyphal wall surface of the basidiomycete *Schizophyllum commune*. Biochim Biophys Acta 273:346–358

Wessels JGH, Dons JJM, de Vries OMH (1985) Molecular biology of fruit body formation in *Schizophyllum commune*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology of higher fungi. Cambridge University Press, Cambridge, pp 485–497

Wessels JGH, Mulder GH, Springer J (1987) Expression of dikaryon-specific and non-specific mRNAs of *Schizophyllum commune* in relation to environmental conditions and fruiting. J Gen Microbiol 133:2557–2561

Wessels JGH, de Vries OMH, Ásgeirsdóttir SA, Schuren FHJ (1991a) Hydrophobin genes involved in formation of aerial hyphae and fruit bodies in *Schizophyllum*. Plant Cell 3:793–799

Wessels JGH, de Vries OMH, Ásgeirsdóttir SA, Springer J (1991b) The *thn* mutation of *Schizophyllum commune*, which suppresses formation of aerial hyphae, affects expression of the *Sc3* hydrophobin gene. J Gen Microbiol 137:2439–2445

Wessels JGH, Ásgeirsdóttir SA, Birkenkamp KU, de Vries OMH, Lugones LG, Scheer JMJ, Schuren FHJ, Schuurs TA, van Wetter M-A, Wösten HAB (1995) Genetic regulation of emergent growth in *Schizophyllum commune*. Can J Bot 73:S273–S281

Wessels JGH, Schuurs TA, Dalstra HJP, Scheer JMJ (1998) Nuclear distribution and gene expression in the secondary mycelium of *Schizophyllum commune*. In: Gow N, Robson G, Gadd G (eds) The fungal colony. Cambridge University Press, Cambridge, pp 302–325

White NA, Boddy L (1992) Differential extracellular enzyme production in colonies of *Coriolus versicolor*, *Phlebia radiata* and *Phlebia rufa*. J Gen Microbiol 138:2589–2598

Whitehouse HLK (1949) Multiple allelomorph heterothallism in the fungi. New Phytol 48:212–244

Wösten HAB (2001) Hydrophobins: multipurpose proteins. Annu Rev Microbiol 55: 625–646

Wösten HAB, de Vocht ML (2000) Hydrophobins, the fungal coating unravelled. Biochim Biophys Acta Rev Biomembranes 1469:79–86

Wösten HAB, Wessels JGH (1997) Hydrophobins, from molecular structure to multiple functions in fungal development. Mycoscience 38:363–374

Wösten HAB, Willey JM (2000) Surface active proteins enable microbial aerial hyphae to grow into the air. Microbiology 146:767–773

Wösten HAB, Moukha SM, Sietsma JH, Wessels JGH (1991) Localization of growth and secretion of proteins in *Aspergillus niger*. J Gen Microbiol 137:2017–2024

Wösten HAB, de Vries OMH, Wessels JGH (1993) Interfacial self-assembly of a fungal hydrophobin into a hydrophobic rodlet layer. Plant Cell 5:1567–1574

Wösten HAB, Ásgeirsdóttir SA, Krook JH, Drenth JHH, Wessels JGH (1994a) The Sc3p hydrophobin self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. Eur J Cell Biol 63:122–129

Wösten HAB, Schuren FHJ, Wessels JGH (1994b) Interfacial self-assembly of a hydrophobin into an amphipathic membrane mediates fungal attachment to hydrophobic surfaces. EMBO J 13:5848–54

Wösten HAB, Ruardy TG, van der Mei HC, Busscher HJ, Wessels JGH (1995) Interfacial self-assembly of a *Schizophyllum commune* hydrophobin into an insoluble amphipathic membrane depends on surface hydrophobicity. Colloids Surfaces B Biointerfaces 5:189–195

Wösten HAB, van Wetter MA, Lugones LG, van der Mei HC, Busscher HJ, Wessels JGH (1999) How a fungus escapes the water to grow into the air. Curr Biol 9:85–88

Wuest PJ, Royce DJ, Beelman RB (eds) (1987) Cultivating edible fungi. Elsevier, Amsterdam

Yagi F, Hiroyama H, Kodama S (2001) *Agrocybe cylindracea* lectin is a member of the galectin family. Glycoconjugate J 18:745–749

Yamagishi K, Kimura T, Suzuki M, Shinmoto H (2002) Suppression of fruit-body formation by constitutively active G-protein alpha-subunits ScGP-A and ScGP-C in the homobasidiomycete *Schizophyllum commune*. Microbiology 148:2797–2809

Yamagishi K, Kimura T, Suzuki M, Shinmoto H, Yamaki KJ (2004) Elevation of intracellular cAMP levels by dominant active heterotrimeric G protein alpha Subunits ScGP-A and ScGP-C in homobasidiomycete, *Schizophyllum commune*. Biosci Biotechnol Biochem 68:1017–1026

Yli-Mattila T (1987) The effect of UV-A light on cAMP in the basidiomycete *Schizophyllum commune*. Physiol Plant 69:451–455

Yli-Mattila T, Raudaskoski M (1992) Glucoamylase activity and water-soluble polysaccharides during monokaryotic and dikaryotic fruiting in *Schizophyllum commune*. Mycol Res 96:597–604

Yli-Mattila T, Ruiters MHJ, Wessels JGH (1989a) Photoregulation of dikaryon-specific mRNAs and proteins by UV-A light in *Schizophyllum commune*. Curr Microbiol 18:289–295

Yli-Mattila T, Ruiters MHJ, Wessels JGH, Raudaskoski M (1989b) Effect of inbreeding and light on monokaryotic and dikaryotic fruiting in the basidiomycete *Schizophyllum commune*. Mycol Res 93:535–542

Yuki K, Akiyama M, Muraguchi H, Kamada T (2003) The *dst1* gene responsible for a photomorphogenetic mutation in *Coprinus cinereus* encodes a protein with high similarity to WC-1. Fungal Genet Newslett Suppl 50 abstr 147

Zhao J, Kwan HS (1999) Characterization, molecular cloning, and differential expression analysis of laccase genes from the edible mushroom *Lentinula edodes*. Appl Environ Microbiol 65:4908–4913

20 Meiosis in Mycelial Fungi

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CONTENTS

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I. Introduction

Meiosis is a highly conserved process, occupying a central role in most eukaryote life cycles. The meiotic process, in contrast to the mitotic process, reduces the diploid cellular genome complement by half, as required for sexual reproduction and fecundation. The reduction in chromosome number is achieved by one round of DNA replication, followed by two rounds of divisions with no intervening replication. The absolute need for each gamete to inherit a copy of the genome is achieved by the fact that paternal and maternal homologous chromosomes segregate to opposite poles during the first meiotic division, whereas their sister chromatids segregate at the second division.

Mycelial fungi provide several positive attributes for studying meiosis. First, they have a brief life cycle during which several hundred meiocytes and the resulting gametes (asco- and basidiospores) can be analyzed.

Second, the four products of a single meiosis are held together in a single cell (ascus or basidium). This allows the determination of the genetic constitution of each of the DNA strands involved in meiosis. Moreover, in species with linearly arranged ascospores, the position of each ascospore reflects the preceding nuclear divisions. When in some of these species a mitotic division occurs after meiosis, each of the resulting eight haploid nuclei represents the genetic character of one of the eight DNA strands of the chromosomes produced by meiosis (e.g., Perkins 1974, 1997). The popularity of *Neurospora crassa*, *Sordaria fimicola*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus nidulans*, *Ascobolus immersus*, *Podospora anserina*, *Sordaria macrospora*, *Schizophyllum commune* and *Coprinus cinereus* (now called *Coprinopsis cinerea*) for a wide variety of genetic studies on meiotic recombination reflects these exceptional advantages (review in Esser and Kuenen 1967; Whitehouse 1982; Lamb 1996).

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Third, a large pool of meiotic mutants have been isolated in *A. nidulans*, *N. crassa*, *P. anserina*, *S. macrospora* and *C. cinereus*, and molecular tools are now available to characterize them (see below).

Fourth, publication of the genome sequences of five ascomycetes (*A. nidulans*, *Fusarium graminearum*, *N. crassa*, *Magnaporthe grisea*, *P. anserina*) and four basiomycetes (*C. cinerea*, *Cryptococcus neoformans*, *Phanerochaete chrysosporium*, *Ustilago maydis*) provides a powerful basis for genome comparison with other organisms, and especially with the budding and fission yeasts, in which a large spectrum of meiotic genes are already characterized (e.g., Borkovich et al. 2004). Homologies among genes involved in the meiotic and sporulation processes establish rapid ways to identify genes in the fungus of interest (even when the sexual cycle is unknown, e.g., Pöggeler 2002). They offer also a starting point for the identification of fungal-specific genes and/or evolutionary gene requirements.

Fifth, the small chromosomal size of most fungi was long considered a handicap for the general requirement for good chromosome morphology to study meiosis. However, since chromosome organization can be investigated using tools such as three-dimensional reconstruction from serial sections, fluorescence in situ hybridization (FISH) and green fluorescent protein (GFP) tagging, fungi became model systems for investigating both meiotic pairing and segregation (cf. references below, in corresponding paragraphs).

Finally, everyone who has observed fungal chromosomes knows that Barbara McClintock was right when she said in her 1961 conference exposé "You may think they (chromosomes of *N. crassa*) are small when I show you pictures of them. But when you look at them, they get bigger and bigger and bigger" (cited in Perkins 1992).

The major purpose of the present review is to explore similarities and differences in the better known organisms, in order to identify key conserved features of the meiotic process. Recent studies based on genetic, molecular and cytological approaches, combined with a rapidly growing arsenal of mutations, have yielded a great deal of new information about how pairing and synapsis relate to other processes such as recombination and segregation in budding and fission yeasts as well as in *C. cinereus* and *S. macrospora*. In fact, the major task of the prolonged meiotic prophase is orderly recognition plus juxtaposition of the homologous chromosomes and establishment of stable connections via crossing-over. Throughout this period, events at the DNA level and events at the chromosome axis level are spatially and temporally coordinated. Most of the review will therefore be dedicated to these critical steps. Differences found when mutants of different organisms are analyzed shed light on our understanding of meiosis, and although only few mycelial fungi are used as model organisms, it is clear that they underline already both the diversity and the potentiality of these organisms. A clear example is given by the variety of gene silencing mechanisms developed by some fungi before and during meiosis. Differences underline also the fact that in this vast group of organisms there may be interesting "obscure" species awaiting the opportunity to give important clues. Interestingly also, mycelial fungi have developed a great variety of methods for separating or bringing together the pairs of nuclei that are issued from both meiotic divisions and, when present, the postmeiotic mitosis. Such movements imply a strict regulation of how spindles are located, which is also discussed here. Finally, an understanding of the meiotic process is not only pivotal to furthering research on fertility but also has important implications for fungal and human disease curing.

II. Entering Meiosis: Mycelial Fungi Devote Significant Resources to Make Sure that the Two Nuclei that Will Fuse Before Entering Meiosis Have Identical Genomes

Mycelial fungi are self-fertile (also called self-compatible or homothallic) or self-sterile, cross-fertile (self-incompatible or heterothallic). In primary homothallic species, a homokaryotic mycelium established from a single haploid nucleus has the potentiality to progress through both the dikaryotic stage and meiosis to complete the sexual cycle. In secondary homothallic species (also called "pseudoheterothallic"), a fertile dikaryotic mycelium is established from a single spore carrying two haploid nuclei of different mating types. In heterothallic species, mating between homokaryotic mycelia of different mating types is required to complete the sexual cycle (Fig. 20.1A; see also Debuchy and Turgeon, and Casselton and Challen, Chaps. 15 and 17, respectively, this volume). In all cases, there is a more (cf. mycelial basidiomycetes) or less (cf. mycelial

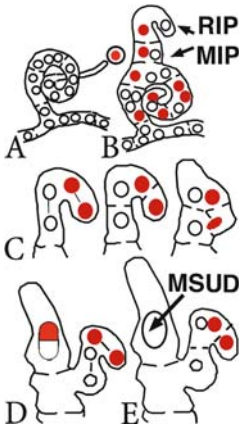

Fig. 20.1. A–E Diagrammatic representation of ascus formation, with indication of the sites for RIP/MIP and MSUD. The stages cartooned have all been demonstrated cytologically (e.g., Raju 1980; Zickler et al. 1995). The two nuclei of opposite mating types are shown as *white* and *red* nuclei. **A** Mating between polynucleated ascogonium (*left*) and uninucleated conidium through the trichogyne. **B** After fertilization, the haploid nuclei proliferate in the ascogonium. This heterokaryotic ascogonim then forms binucleated dikaryotic cells containing nuclei of opposite mating type, in which will occur RIP or MIP. In homothallic species, this results in genetically identical binucleate daughter cells. The tip cell bends to form a hook-shaped cell called a crozier. **C** Different steps of the crozier development, from one-celled to a three-celled structure (see text). **D** The two upper nuclei of the three-celled crozier fuse, giving rise to the only diploid nucleus of the fungal life cycle. The two lower nuclei divide again in the basal cell, and give rise to a second crozier. **E** As the upper crozier cell elongates into an ascus, karyogamy is immediately followed by meiosis. MSUD occurs during the early steps of meiotic prophase

ascomycetes) prolonged dikaryotic phase between fertilization and karyogamy. The mechanism by which formation of ascomycetal dikaryotic cells is regulated remains unknown (e.g., Hoffmann et al. 2001).

A. Karyogamy and Premeiotic Replication

The two nuclei issued from a unique nucleus (homothallic species) or the two nuclei of opposite mating type (heterothallic species) divide synchronously several times before fusing (karyogamy) and entering meiosis (Fig. 20.1B–E). Karyogamy of most ascomycetes is preceded by the formation of a hook-shaped crozier cell containing two haploid nuclei (Fig. 20.1B). These undergo a simultaneous mitosis, with spindles positioned such that one daughter nucleus from each parent is present in the crook portion of the cell (Fig. 20.1C). Septa form on each side of the crook, resulting in a basal and a lateral cell flanking the binucleate ascus-mother cell (Fig. 20.1C). Karyogamy takes place as the ascus-mother cell begins to elongate (Fig. 20.1D), followed immediately by the long prophase of the first meiotic division (Fig. 20.1E; review in Read and Beckett 1996). In *P. anserina*, elongation of this upper cell, and therefore karyogamy, requires wild-type levels of peroxisomes (Berteaux-Lecellier et al. 1995). Meiosis can be induced in the absence of karyogamy: haploid meiosis proceeds up to ascospore formation in monokaryotic asci of *P. anserina* (Zickler et al. 1995). Diploidy per se is also not required: tetraploid nuclei issued from diploid crosses of *A. nidulans* as well as the highly polyploid (over 8n) nuclei formed after karyogamy in the *cro1-1/she4* mutants of *P. anserina* go through both meiotic divisions (Elliot 1960; Berteaux-Lecellier et al. 1995). The rosette of over 100 asci formed in a wild-type fruiting body results usually from the establishment of one dikaryon made by a single "male" and a single "female" nucleus (e.g., Johnson 1976), but exceptions are also observed (e.g., Hoffmann et al. 2001).

Premeiotic replication is closely analogous to its mitotic counterpart. In budding yeast, replication utilizes the same specific origins that fire, with the same relative frequencies and the same general order, in both meiosis and mitosis (Collins and Newlon 1994). However, a common feature of meiotic S-phase is its extended duration, compared to mitotic S-phases in the same organism (e.g., Cha et al. 2000). The mechanism of this prolongation remains unknown. Premeiotic replication is also a critical step for the meiotic recombination process. DNA double-strand breaks (DSBs), which initiate meiotic recombination and premeiotic replication, are tightly coupled, at least in budding yeast: DSBs do not form when replication is blocked, and delaying replication in a region causes a corresponding delay in DSB formation in this region (Borde et al. 2000). Despite the general realization of the importance of premeiotic S-phase, timing of S-phase remains questionable in mycelial ascomycetes (e.g., Farman 2002). Based on microspectrophotometric quantitation of DNA (a technique potentially subject to artifacts), replication was found to precede karyogamy in *Neottiella rutilans*, *N. crassa* and *S. fimicola* (Rossen and Westergaard 1966; Iyengar et al. 1976; Bell and Therrien 1977). By contrast, timing of S-phase is

clearly defined in *C. cinereus*, in which replication also occurs before karyogamy (Kanda et al. 1990; Pukkila 1994). Strikingly, *C. cinereus* goes through meiosis even if replication is defective (Kanda et al. 1990; Pukkila et al. 1995; Merino et al. 2000).

B. Premeiotic "Checking and Cleaning" Mechanisms

The period between fertilization and meiosis is also one during which several mycelial fungi "check and clean their genomes". To do so, they have developed different premeiotic mechanisms that scan the genome for DNA sequences present in more than a single copy and larger than 450 bp. These mechanisms, which occur before premeiotic S-phase (likely during or just before the dikaryotic stage; Fig. 20.1B), are triggered by the duplication itself and mutate or silence extra DNA sequences. They include:

1. The irreversible repeat-induced-point mutation (RIP) mechanism, which is associated with de novo methylation of cytosine residues and converts C/G base pairs to A/T pairs in the duplicated sequences of *N. crassa*, *P. anserina*, *M. grisea* and *Leptosphaeria maculans* (Cambareri et al. 1989; Hamann et al. 2000; Graia et al. 2001; Ikeda et al. 2002; Selker 2002; Idnurm and Howlett 2003; Bouhouche et al. 2004). RIP operates on linked and unlinked DNA sequences, and is more or less frequent in the different organisms studied to date. A cytosine methyltransferase-homologue gene (*rid*) is essential for RIP in *N. crassa* (Freitag et al. 2002). In *N. crassa* and *P. anserina*, the frequency of progeny affected by RIP is highly increased in late-expelled ascospores vs. early-expelled ascospores (Singer et al. 1995; Bouhouche et al. 2004). Similar increases are also observed in *P. anserina* when the steps between fertilization and karyogamy are delayed in the absence of *ami1/apsA*, a gene required for correct nuclear movements and positioning (Bouhouche et al. 2004).
2. The methylation induced premeiotically mechanism (MIP), found in *A. immersus* (Rhounim et al. 1992) and *C. cinereus* (Freedman and Pukkila 1993). MIP methylates de novo all gene-sized duplications at their cytosine residues, and maintains this methylation without further requirement for the methylated sequence to remain duplicated. The inactive state segregates in a Mendelian way (Rhounim et al. 1992). As no mutation is associated with MIP, the gene inactivation process is reversible, and inhibitors of DNA methylation accelerate the gene reactivation (reviewed in Colot and Rossignol 1999; Faugeron 2000). This de novo methylation is triggered by the putative C5-DNA-methyltransferase *masc1* (Malagnac et al. 1997).
3. Mechanisms that lead to gene/sequence losses, rather than silencing. Premeiotic recombination between cis-duplicated sequences leads to deletion of the interstitial sequence in *N. crassa* and *P. anserina* (e.g., Selker et al. 1987; Coppin-Raynal et al. 1989). Chromosome de novo deletions were found in *Nectria haematococca* (Miao et al. 1991), *Cochliobolus heterostrophus* (Tzeng et al. 1992) and *C. carbonum* (Pitkin et al. 2000). Changes in the size of the *N. crassa* nucleolar organizer region (Butler and Metzenberg 1989) as well as *m*eiosis-associated *d*eletion in *h*eteroallelic *r*epeats (MDHR) of *M. grisea* are likely due to intrachromosomal recombination events (Farman 2002).

In order to trigger RIP and MIP, the two elements of the duplicated sequence must be in the same haploid nucleus, and tetrad analyses show that the "checking for duplications" mechanism occurs before karyogamy and prior to the premeiotic S-phase (Fig. 20.1B; review in Rossignol and Faugeron 1995; Selker 2002). However, the precise step at which inactivation occurs remains unknown. Interestingly, MIP strongly reduces the frequency of allelic crossing-over in the hotspot *b2* gene of *A. immersus* when *b2* is methylated in one or both parents (Maloisel and Rossignol 1998). In these crosses, methylation transfer between homologous chromosomes occurs during meiotic prophase, and the methylated derivative of a gene can be transferred to the unmethylated active *b2* parental allele at frequencies comparable to those of the gene conversion frequencies seen within *b2*. Interestingly also, methylation transfer and gene conversion are mechanistically related: (1) they share the same 5′ to 3′ polarity along the *b2* gene (decrease of frequency from one end of the gene to the other), and (2) they are similarly reduced by ectopic positioning and nucleotidic divergence (Colot et al. 1996). This suggests that, aside from inactivating possible transposons, methylation and MIP likely contribute to the stabilization of the genome by preventing homologous recombination

between dispersed DNA repeats. In *N. crassa*, duplications of chromosome segments of the order of megabases are subject to RIP, implying that RIP may also play an evolutionary role in "weeding out" spontaneous chromosome rearrangements (Perkins et al. 1997).

C. A "Checking" Mechanism that Operates After Karyogamy

In addition to prekaryogamy mechanisms, *N. crassa* has developed ways of controlling the integrity of its genome during meiotic prophase (Fig. 20.1E). When during pairing an asymmetrical situation is detected, such as a deletion or an insertion in one of the homologous chromosomes, the presence of this unpaired sequence of DNA activates a process called *m*eiotic *s*ilencing by *u*npaired *D*NA (MSUD). This, in turn, silences all paired and unpaired copies of the sequence/gene in the genome; silencing holds also for even numbers of copies, but inserted at different positions along the chromosomes (Shiu et al. 2001; Shiu and Metzenberg 2002). The mechanism appears to be post-transcriptional, based on the observation that mutations in the suppressor of ascus dominance-1 (*sad-1*), coding for an RNA-dependent RNA polymerase (RdRP), suppress the ascus dominance exerted by unpaired copies of reporter genes (Shiu et al. 2001; Shiu and Metzenberg 2002). The post-transcriptional gene-silencing nature of meiotic silencing may involve at least two more genes in the pathway: the suppressor of meiotic silencing *sms-2* coding for an Argonaute-like protein, and the suppressor of meiotic silencing *sms-3* coding for a Dicer-like protein (Kutil et al. 2003; Lee et al. 2003). Production of the silencing signal (e.g., small interfering RNAs) does not affect the expression of adjacent genes, as the aberrant transcript seems restricted to the unpaired sequence (Kutil et al. 2003). Also, both size and degree of homology directly correlate with the silencing efficiency but the presence of promoter elements in the unpaired DNA is not required to induce silencing (Lee et al. 2004).

MSUD potentially plays a role in development: if the unpaired gene encodes a protein required for the completion of meiosis and/or sporulation, MSUD will arrest development at that particular stage when the product of the gene is required (Shiu et al. 2001). Interestingly, meiotic silencing is also important from an evolutionary point of view. Shiu et al. (2001) showed that *sad-1* mutants could suppress the interbreeding inability between *N. crassa* and three related species: *N. sitophila*, *N. tetrasperma*, and *N. intermedia*. A significant increase in fertility was observed in crosses between the tested species, suggesting that an important barrier between two closely related species is, in fact, the existence of numerous small rearrangements in the genome.

III. Meiotic Recombination

The unique feature of meiosis to generate offspring that are genetically different from their parents is important to maintain genetic diversity, but is actually an indirect consequence of the fact that homologous recombination is mechanically indispensable for meiosis. During mitosis, the two sister chromatids separate from one another by releasing cohesion along their entire length. During meiosis, by contrast, homologous chromosomes separate at division I: they must, therefore, be connected to one another to avoid that the pulling force of the spindle will segregate them randomly. This is achieved by programmed high levels of DNA double-strand breaks (DSBs), which give rise to recombinant non-sister chromatids that ultimately will join homologues together via chiasmata. The need for inter-homologue links is illustrated by phenotypes of recombination-deficient mutants: non-recombinant chromosomes are single when they are captured by microtubules, and thus undergo missegregation leading to aneuploid, sterile gametes (e.g., review in Lichten 2001; Bishop and Zickler 2004).

A. Initiation

Recombination occurs during the long prophase of the first meiotic division, at a much higher frequency than during vegetative/somatic growth (review in Pâques and Haber 1999). It is initiated by the programmed formation of a large number of DSBs – thus, in a risky way. Repair of these DSBs differs from that of DSBs occurring in mitotic cells because meiotic recombination uses a homologue non-sister chromatid for repair, whereas mitotic recombinational repair uses the sister chromatid or repeated sequences on the same chromosome (e.g., review in Käfer 1977; Pâques and Haber 1999; van Heemst and Heyting 2000).

In a wide range of organisms including the fungi budding and fission yeasts*C. cinereus* and *S. macrospora*, DSBs are induced by the evolutionarily conserved and meiosis-specific topoisomerase II-related Spo11 protein (Celerin et al. 2000; Keeney 2001; Sharif et al. 2002; Storlazzi et al. 2003). Spo11p is transiently covalently attached to the 5′ ends of the DNA fragments and after removal, DSBs are rapidly resected on their 5′-strand termini to produce 3′-single-stranded tails, which bind to a protein complex that includes the RecA analogs Rad51 and Dmc1 (Keeney 2001). This strand transfer complex promotes later recombination steps that occur during zygotene and early pachytene (see below). DSBs are formed in haploid meiosis of budding yeast, and thus independently of homologous interactions (de Massy et al. 1994).

All known *spo11* mutants strongly reduce CO and chiasma formation, but exhibit also interesting differences in their pairing and progression phenotypes. In the fungal, plant and mammal species studied so far, *spo11* mutants display severe pairing defects (Celerin et al. 2000; Keeney 2001; Storlazzi et al. 2003 and references therein). Irradiation of budding yeast, *C. cinereus* and *S. macrospora*, mutant meiocytes partially corrects these defects, consistent with a requirement for break-induced events to ensure both recombination and pairing (Celerin et al. 2000; Storlazzi et al. 2003; Tesse et al. 2003). By contrast, pairing is normal in *spo11* mutants of *Drosophila melanogaster* and *Caenorhabditis elegans* (review in McKee 2004). The most striking result to emerge from these studies concerns the role of Spo11p in meiotic progression. Chromosomes segregate randomly at both divisions in *spo11* mutants of plants, yeasts and *S. macrospora*, leading to reduced fertility (Sharif et al. 2002; Storlazzi et al. 2003). By contrast, in *C. cinereus* and mice, *spo11* mutants undergo programmed cell death after prophase (Celerin et al. 2000; Romanienko and Camerini-Otero 2000).

Spo11p does not act alone: in budding yeast, there are at least nine other proteins required for DSBs (Arora et al. 2004 and references therein) and one of these, called Rec103/Ski8, is also found to be a direct partner of Spo11p in *S. macrospora* (Tesse et al. 2003). *SKI8* was originally identified in budding yeast on the basis of its *s*uper*ki*ller (of RNA viruses) mutant phenotype, and has likely roles in RNA metabolism during the vegetative cycle in yeasts and *S. macrospora* (Tesse et al. 2003; Arora et al. 2004 and references therein). Interestingly, Ski8p redistributes from cytoplasm to nuclei only at meiosis, and localizes to chromosomes specifically at early meiotic prophase. The localizations of Ski8p and Spo11p are mutually interdependent (Tesse et al. 2003; Arora et al. 2004). Four of the other proteins (Rec102, Rec104, Mer2 and Mei4) have so far no obvious homologues in other organisms, including fission yeast and *N. crassa* (Borkovich et al. 2004). This may reflect a species difference in some aspects of recombination initiation or a lack of sequence conservation for proteins with conserved functions. The same is true for the meiosis-specific RecA homologue Dmc1, absent in *N. crassa*, but present in *C. cinereus* and *Pleurotus ostreatus* (Nara et al. 2000; Mikosch et al. 2001; Borkovich et al. 2004). Also, in contrast to yeasts and *N. crassa*, *Ustilago maydis* has an orthologue (Brh2) of BRCA2, the tumor suppressor essential for the error-free repair of DSBs. In plants and *Ustilago*, BRCA2 is required for meiotic DSB repair (Kojic et al. 2002).

B. From Initiation to Recombination Products

Some DSBs mature into inter-homologue crossingovers (COs), also called reciprocal exchanges (CRs), in which the arrangement of flanking regions is modified because the recombination intermediates resolve in such a way that each of the two interacting chromatids is cleaved and religated to its homologue (Fig. 20.2, right). The remaining DSBs do also interact with their homologous partner but the two molecules will be resolved back without accompanying exchange of their flanking regions (Fig. 20.2, left). This type of recombination is called noncrossovers (NCOs) or non-reciprocal recombinations (NCRs), and will result in non-Mendelian segregation of the studied marker, also defined as "gene conversion". In heterozygous crosses of eight-spored ascomycetes, gene conversions are seen as two wild-type and six mutant ascospores or six wild-type and two mutant ascospores (Fig. 20.2, left), in contrast to the 4:4 parental marker segregation issued from crossovers (Fig. 20.2, right). Gene conversion was first described in 1934 by H. Zickler in the ascomycete *Bombardia lunata*, "rediscovered" in budding yeast by Lindegren in 1953 and definitively demonstrated by Mitchell in 1955 with the use of linked markers on either side of the studied locus. When 6:2 segregation was observed at the *pyridoxine* locus of *N. crassa*, the linked markers segregated with normal 4:4 ratios, indicating that

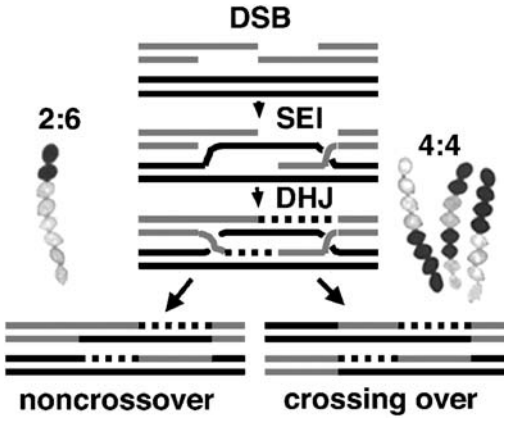

Fig. 20.2. Meiotic recombination. Shown are two homologous DNA duplexes (of the four involved in the process), one in *gray* and one in *black*. Meiotic recombination initiates with a double-strand break (*DSB*) in one duplex. Processing of the ends results in single-stranded tails (*SEI*). A single-stranded tail then invades the homologous duplex. Repair synthesis (*dotted lines*) results in a double Holliday junction (*DHJ*) that can be resolved to generate either crossing-over (*right*) or noncrossover (*left*) products. Crossovers will result as 4 : 4 asci (four wild-type spores, *black*, and four mutant ascospores, *white*) whereas noncrossovers will result in 2 : 6 (or 6 : 2) asci in *S. macrospora* heterozygous crosses (shown above the corresponding drawings)

the aberrant segregation occurred at the *pyridoxin* mutant site, and not as a consequence of aberrant chromosome segregation.

However, most progress in our understanding of the conversion of DSBs into CO and NCO has been associated with the development of physical assays of recombination in completely synchronous budding yeast meiocytes (e.g., Padmore et al. 1991; Schwacha and Kleckner 1995; Hunter and Kleckner 2001). These approaches have defined several key events occurring from leptotene to diplotene. DSB formation is followed by the sequential appearance of two stable strand exchange intermediates (Fig. 20.2). One strand at one end of the DSB undergoes stable exchange with one strand of the homologous DNA duplex to give a single-end invasion intermediate (SEI), which will be converted to double Holliday junction (DHJ; Fig. 20.2). DHJs will in turn be converted into CO products at late pachytene (Schwacha and Kleckner 1995; Allers and Lichten 2001; Hunter and Kleckner 2001; Börner et al. 2004).

In budding yeast, DHJs were shown to be precursors specifically of COs, eliminating the hypothesis that the CO/NCO differentiation is made by alternate resolution of Holliday junctions (Allers and Lichten 2001). Also, the CO versus NCO decision is made prior to, or concomitant with formation of SEIs (Börner et al. 2004). Allers and Lichten (2001) and Börner et al. (2004) proposed therefore that CO/NCO control might be imposed as soon as DSBs are engaged in nascent interactions with the homologous DNA duplex region of their homologue partner (Fig. 20.2, and detailed drawings in Bishop and Zickler 2004). Moreover, recent studies of the endonuclease Mus81 indicate that, in addition to DHJ resolution, some COs may be formed by the processing of non-DHJ intermediates. Interestingly, these latter COs do not show interference (review in Hollingsworth and Brill 2004). The *MUS81* pathway accounts for a relatively minor fraction of COs in budding yeast, and likely in both *S. macrospora* and *N. crassa*, according to the presence of *MSH4* and *MSH5*, encoding meiosis-specific MutS homologues required to promote COs. By contrast, the *MUS81* pathway is responsible for most, if not all, of the COs in fission yeast and *C. elegans* (review in Hollingsworth and Brill 2004). Thus, the decision of which CO pathway to use appears to vary between organisms.

The considerable recent progress made in elucidating the meiotic recombination process is the extension of an almost 80-year-long development of studies on recombination, in which mycelial fungi played a central and often pioneering role. Detailed analyses of CO and NCO events, together with stimulating models, allowed the discovery of key steps of the process (for excellent reviews, see Esser and Kuenen 1967; Catcheside 1977; Whitehouse 1982; Rossignol et al. 1988; Nicolas and Petes 1994).

C. Meiotic Exchanges Are Highly Regulated

An additional, prominent feature of meiotic recombination is the tight regulation of both the number and distribution of exchanges along and among chromosomes. Although a large number of recombinational interactions are initiated, only a selected subset is designated for maturation into COs, the remaining interactions maturing primarily or exclusively into inter-homologue NCOs. In the absence of any regulation, COs and their cytological counterparts, chiasmata and recombination nodules (RNs; see Sect. V.), should be randomly distributed among and along chromosomes. Analysis of their distribution in several organisms shows clearly that their distribution is non-random, as reflected by three main features.

- First, chiasma and RNs never occur uniformly along chromosome arms, with a lesser tendency to occur around centromeres and a greater tendency to occur in the middle of arms and/or in sub-telomeric regions (for mycelial fungi, see Zickler 1977; Holm et al. 1981; Bojko 1989; Zickler et al. 1992; reviewed in von Wettstein et al. 1984; Zickler and Kleckner 1999). Also, short chromosomes have higher number of COs per physical length than do longer chromosomes (Mautino et al. 1993; Kaback et al. 1999). This non-random distribution along chromosomes is already reflected by the distribution of DSBs. They occur at higher frequencies in some genomic regions called hotspots, and at lower frequencies in other regions called coldspots (review in Petes 2001). Position of hotspots/DSBs may be regulated by chromatin structure. They are located in accessible (DNaseI-sensitive) regions of the chromatin and in chromosome domains that are GC-rich, whereas coldspots are often associated with centromere, telomere and AT-rich regions (Baudat and Nicolas 1997; Gerton et al. 2000). This is confirmed by moving hotspot domains at various places along a chromosome; formation of DSBs is also controlled by distant cis and trans interactions (e.g., Wu and Lichten 1995). Most hotspots are intergenic, rather than intragenic. Meiotic recombination can also be regionally regulated, as in *N. crassa* and *S. pombe* (e.g., de Veaux and Smith 1994; Yeadon et al. 2004).
- Second, each pair of homologous chromosomes will have at least one CO/chiasma. This "obligatory chiasma" occurs irrespective of chromosome length, and is likely related to the fact that at least one chiasma is necessary to ensure regular segregation of the maternal and paternal homologous chromosomes at division I. The average number of chiasmata per bivalent being rather small in most organisms (see King and Mortimer 1990 for examples), this obligatory event is achieved by tight regulation. There are, however, two known exceptions: *S. pombe* and *A. nidulans*. In both organisms, the number of COs per meiosis and per chromosome is high enough, so that the probability that the smallest chromosome gets no CO is extremely low (2×10^{-5} for *S. pombe*; review in Kohli and Bähler 1994).
- Third, when two genetic intervals are considered, the presence of a CO at one position is accompanied by a decreased probability that another will occur nearby (for fungi, see Strickland 1958; Perkins 1962; Perkins et al. 1993; Munz 1994). Also, if two or more chiasmata are present along a bivalent, they exhibit interference and, therefore, a tendency for even spacing. The strength of this interference is inversely correlated with distance (review in Jones 1984; Foss et al. 1993). By contrast, NCOs do not show interference, regardless of whether they are associated with COs (Mortimer and Fogel 1974). Also, chromatid interference (the fact that the choice of a non-sister chromatid involved in an exchange might influence the choice to be involved in another close exchange) was found to be absent or very rare (Perkins 1962; Esser and Kuenen 1967; Mortimer and Fogel 1974).

The mechanism that regulates interference is not understood, although several models have been proposed. The counting model proposes that a fixed number of NCOs separate COs (Foss et al. 1993). Another model suggests that interference signals initiate at sites of COs and spread outward along the synaptonemal complex (SC; see Sect. V.), repressing additional COs in the neighborhood, in agreement with the fact that interference is not observed between intervals that are far apart (King and Mortimer 1990). The SC model has received support from two findings: (1) the parallel absence of CO interference and SC in *A. nidulans* and *S. pombe* (Strickland 1958; Egel-Mitani et al. 1982; Bähler et al. 1993; Munz 1994), and (2) the parallel absence of SC and CO interference in the *zip1* mutant of budding yeast, Zip1p being an essential structural component of the SC central region (Sym and Roeder 1994; Fung et al. 2004). However, recent studies indicate that the SC is not required for interference in budding yeast and *Drosophila*, leaving the subject open for further explanations (see Bishop and Zickler 2004).

IV. Homologue Recognition and Pairing

Juxtaposition of homologous chromosomes is a prominent universal feature of meiosis. It involves a long-distance recognition of homology, which occurs no later than leptotene, in advance of SC formation. Pairing, namely, the distinction between "self" and "non-self", is therefore a fundamental biological problem. However, the

mechanism whereby homologues locate each other in the nucleus is not yet understood. The term juxtaposition or pairing is used to refer to the coming together of the homologous chromosomes, whereas the term synapsis is used to mean the intimate juxtaposition that culminates with the formation of the SC.

A. Mycelial Fungi Offer Unique Opportunities for the Analysis of Homologous Pairing

First, contrary to most eukaryotes, the two sets of homologous chromosomes are in separated nuclei before meiosis starts (Fig. 20.1C). The first contacts occur after karyogamy, thus after or during S-phase (e.g., Lu and Raju 1970; Zickler 1977; Raju 1980; Li et al. 1999). The progression of homologue juxtaposition can therefore be conveniently described with respect to two major landmarks: karyogamy and SC formation.

Second, although premeiotic pairing is often presumed to simplify the process of meiotic pairing, in mycelial fungi, homologue juxtaposition does not involve simple retention of premeiotic pairing plus reinforcement by meiosis-specific processes (review in Zickler and Kleckner 1999).

Third, the SC axial elements are formed just after karyogamy, which allows an accurate analysis of chromosome disposition and movements during the recognition and juxtaposition processes (see Holm et al. 1981; Rasmussen et al. 1981; Pukkila and Lu 1985; Pukkila 1994; Pukkila et al. 1995; Celerin et al. 2000; Gerecke and Zolan 2000; Merino et al. 2000 for *C. cinereus*; Zickler 1977; Zickler et al. 1992; Storlazzi et al. 2003; Tesse et al. 2003 and Fig. 20.3 for *S. macrospora*; Gillies 1979; Bojko 1988, 1989, 1990; Lu 1993 for *N. crassa*). Moreover, ascus growth and nuclear volume increase give easy landmarks for staging.

Fourth, the development of molecular assays based on FISH and GFP tagging of single loci, combined with mutant analyses of *C. cinereus* and *S. macrospora*, have yielded a great deal of new information about meiotic pairing (e.g., Li et al. 1999; van Heemst et al. 1999; Cummings et al. 2002; Storlazzi et al. 2003; Tesse et al. 2003).

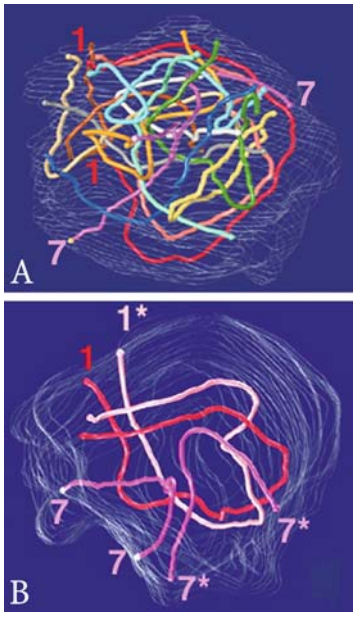

Fig. 20.3. A,B Computer EM reconstruction from a serially sectionedleptotene nucleus of *S. macrospora*. **A** The 14 chromosomes can be distinguished by their size and centromere location. No obvious alignment can be seen. **B** Rotation of the nucleus allows us to see that homologue pair 1-1* (*red* and *pink*) is, in fact, completely aligned whereas pair 7-7* (*dark* and *light violet*) is only partially aligned.

B. How Does Pairing Occur?

Three-dimensional EM reconstructions of post-karyogamy and leptotene nuclei of *S. macrospora* reveal that homologous chromosomes come together in three distinct steps (Figs. 20.3 and 20.4). In very early leptotene, they are far apart and show no evidence of any specific relationship (Fig. 20.4A). By mid-leptotene, each homologous pair has moved from its previous configuration into a spatial domain of the nucleus (Figs. 20.3B and 20.4B). Then, all homologous pairs progressively co-align along their entire length at a distance of ~400 nm (Figs. 20.3B and 20.4D). Upon completion of SC formation, homologues are uniformly 100 nm apart (see Sect. V.). In budding yeast, homologue recognition and alignment occurs in the absence of Zip1p, the SC central component (Fung et al. 2004). Also, in polyploids the unsynapsed homologues remain mostly in parallel association with their synapsed partners (e.g., triploid *C. cinereus*; Rasmussen et al. 1981).

Recombination is an important determinant of stable homologue juxtaposition. Absence of DSBs results in a dramatic reduction in homologous alignment in budding and fission yeasts, *S. macrospora* and *C. cinereus* (Celerin et al. 2000; Keeney 2001; Storlazzi et al. 2003). Interestingly, there is a quantitative correlation between DSB

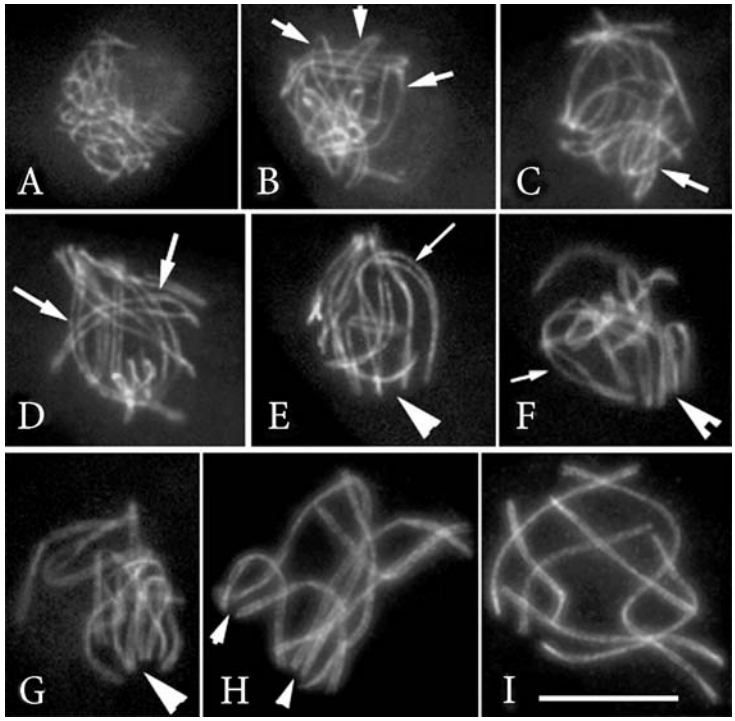

Fig. 20.4. A–I Pairing, bouquet formation and synapsis in wild-type *S. macrospora*. Chromosome axes are visualized by Spo76-GFP (see Sect. VI.), and nuclei are staged by progression of ascus growth. **A** Early leptotene. **B,C** Mid-leptotene nuclei showing progressive recognition of homologues, initially at telomere regions in **B** (*arrows*) and then along most chromosomes in **C** (*arrow*). **D** Late leptotene: all homologues are aligned (*arrows*). **E** Early zygotene, with partially unsynapsed regions (*arrow*) and synapsed telomere regions that are grouped into a loose bouquet formation (*arrowhead*). **F,G** Zygotene nuclei (*arrow* points to a non-synapsed region) with a loose (**F**) and a tight (**G**) bouquet conformation (*arrowheads*). **H** Early pachytene, with seven synapsed bivalents and releasing bouquet (*arrowheads* point to the bouquet area). **I** Telomere clustering is completely released at mid-pachytene. Bar = 5 µm

defects and pairing defects: *S. macrospora* and budding yeast null and partial loss of function alleles of *spo11* and *ski8* mutants show different levels of pairing and synapsis (Tesse et al. 2003; Henderson and Keeney 2004). Also, modulated doses of irradiation of *spo11* and *ski8* mutants of *S. macrospora* showed that the three steps of pairing (described above) are distinguishable by their differential dependence on DSBs (Tesse et al. 2003). Therefore, recombination need not be initiated by Spo11p to promote pairing: DSBs induced by ionizing radiation can rescue both recombination and pairing defects in *spo11* mutants (Celerin et al. 2000; Storlazzi et al. 2003). However, this coupling is not universal. It is required in fungi, plants and mammals, but DSB formation is not required for efficient pairing in *C. elegans* and in *D. melanogaster* female. Rather, pairing and synapsis are likely established through chromosomal pairing centers, even in mutant situations where *SPO11*/DSBs are absent (review in McKee 2004).

C. The Bouquet Stage: a Specific Configuration of Meiosis

From end of leptotene through early pachytene, chromosome ends are attached to the inner surface of the nuclear envelope and are grouped together within a limited area, generally facing the microtubule-organizing center (MTOC) in organisms with a clearly defined MTOC. This telomere polarization takes the form of a "bouquet", so named for its resemblance to the stems from a bouquet of cut flowers (Fig. 20.4E–G). In most species, the bouquet formation is rapid and transient (e.g., nuclei with a bouquet never exceed 5%–10% of prophase nuclei in *S. macrospora*; Storlazzi et al. 2003). The bouquet formation is unique to meiosis and highly conserved (review in Bass et al. 1997; Zickler and Kleckner 1998; Scherthan 2001). Fission yeast provides the most extreme example of the bouquet arrangement: during telomere clustering, the nucleus moves back and forth between the cell poles for the entirety of meiotic prophase, with the spindle pole body (SPB) located at the leading edge of the moving nucleus (called "the horsetail stage"). Telomere clustering and the subsequent nuclear oscillation likely facilitate alignment and pairing of homologues (Chikashige et al. 1994; Kohli and Bähler 1994). When telomere clustering is impaired by depleting the dynein heavy chain Dhc1 or in the absence of protein components of either the telomere (Taz1 or Rap1) or the SPB (Kms1), both homologous pairing and recombination are reduced (review in Ding et al. 2004).

An important component of the bouquet configuration is the existence of a robust physical association between chromosome ends and the inner surface of the nuclear envelope (NE). At early leptotene, chromosome ends attach to the NE but are still distributed throughout the nuclear volume (Fig. 20.4B). At late leptotene, ends start to cluster into the bouquet, either after chromosome alignment, like in *S. macrospora* (Fig. 20.4C), or coincidently with pairing, like in maize (Bass et al. 1997; Storlazzi et al. 2003). At the leptotene–zygotene transition stage, the telomeres form a relatively tight cluster (Fig. 20.4E) that persists throughout all of zygotene (Fig. 20.4F,G), and during early pachytene, after synapsis is completed (Fig. 20.4H). In middle pachytene, the telomere cluster disperses (Fig. 20.4I), but chromosome ends remain attached at the NE until the end of pachytene (review in Zickler and Kleckner 1998). This sequence of events implies that NE association and bouquet formation are two functionally distinct aspects of telomere behavior.

Both the mechanism of bouquet formation and its possible function remain unknown. A common suggestion is that the bouquet might promote telomere-initiated inter-homologue pairing by restricting the freedom of telomere movement (review in Scherthan 2001). However, in many organisms including *S. macrospora*, *N. crassa* and higher plants, homologues are substantially aligned before their telomeres become organized into the bouquet (see Zickler 1977; Loidl 1990; Lu 1993). Also, the bouquet forms independently of DSBs, and independently of Spo11p, Rad50p and Ski8p in *S. macrospora* and budding yeast (Trelles-Sticken et al. 1999; Storlazzi et al. 2003; Tesse et al. 2003). Thus, the bouquet formation requires neither DSBs nor the downstream processes of recombination and SC formation. Conversely, the failure of homologue pairing in *spo11* and *ski8* mutants cannot be attributed to a failure to make a bouquet. Additionally, the bouquet forms in haploid meiosis, and thus in the absence of a homologue (review in Zickler and Kleckner 1998). Interestingly, timely exit from the bouquet stage is delayed in the absence of DSBs, and this delay is abrogated by exogenous DSBs (Trelles-Sticken et al. 1999; Storlazzi et al. 2003). Therefore, whereas it is often considered that the functionally critical feature of the bouquet stage is its formation, the yeast and *S. macrospora* results show that it is the bouquet exit that is important for recombination/chiasma formation, and not the bouquet entry. For example, the accompanying chromosome dispersal might help to relocate unsynapsed or entangled homologues and/or abrogate inappropriate nascent recombinational interactions (e.g., Niwa et al. 2000).

D. Chromosome Interlocking: a Universal Complication of Pairing

EM reconstructions that trace the three-dimensional paths of chromosomes via their SC axial elements reveal that unrelated chromosomes can be entangled with one another at zygotene. When synapsis of two homologous chromosomes is initiated at two different sites, other chromosomes located between their unpaired regions will be trapped, and an interlocking is formed. Such entanglement concerns either one unsynapsed chromosome (or one homologue of a partially synapsed bivalent) or a partially or fully synapsed bivalent that has become trapped in a loop between two synapsed stretches of another bivalent. Interlockings are routinely seen in zygotene nuclei (i.e., before SC is completed) but they are almost never detected at pachytene or later stages (review in von Wettstein et al. 1984; Zickler and Kleckner 1999). Zygotene interlockings are rare in species with small chromosomes (e.g., *C. cinereus*, Holm et al. 1981; *Sordaria humana*, Zickler and Sage 1981; *N. crassa*, Bojko 1988), and rather numerous per nucleus in species with long chromosomes (von Wettstein et al. 1984). Also, in species where interlocks are rare, higher frequencies are found in translocation heterozygotes, with almost all irregularities involving the translocation quadrivalents (e.g., Holm et al. 1981). Because frequent axis or SC interruptions are seen in association with interlocks, it was suggested that interlocks are resolved by breakage and rejoining of chromosomes, with type II topoisomerase being a possible mediator (von Wettstein et al. 1984).

V. The Synaptonemal Complex and Synapsis

Pairing of homologous chromosomes culminates in the formation of a proteinaceous structure called the synaptonemal complexes (SCs), which is as highly evolutionarily conserved as meiosis itself (Fig. 20.5A). The SC consists of two dense, parallel structures called lateral elements (LEs), separated by a less dense central region (Fig. 20.5A).

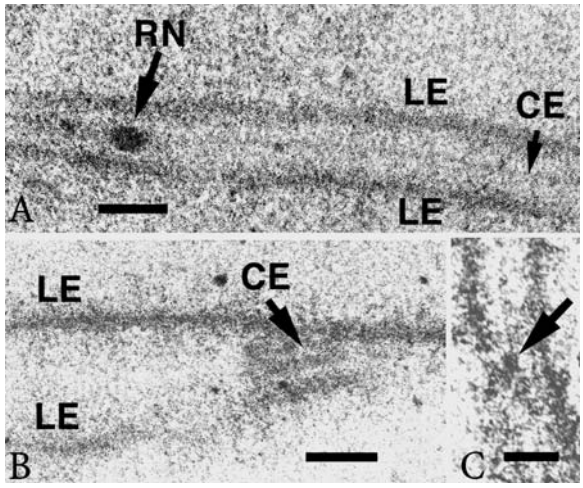

Fig. 20.5. A–C Synaptonemal complex and recombination nodules of *S. macrospora*. **A** Pachytene synaptonemal complex. *LE* indicates maternal and paternal lateral elements, and *CE* the central element of the complex. The *arrow* points to a late recombination nodule (*RN*) located on the CE. **B** At zygotene, the central element (*CE*) initiates between converging lateral elements (*LE*). **C** The *arrow* points to an early recombination nodule. Note the difference in size and density, compared to the late nodule shown in **A**. Bar = 100 nm

Transverse filaments span the width of the central region, and the central element (CE) runs longitudinally along the SC, equidistant between the LEs (Fig. 20.5A). This morphology and the distance between the LEs are remarkably conserved: the two LEs are held in register about 100 nm apart in all studied organisms (review in Zickler and Kleckner 1999). Only one LE is formed along the two sister chromatids, and corresponds to a rod-like structure along which sister-chromatid loops are organized and co-oriented (e.g., illustrations in Pukkila and Lu 1985; Moens and Pearlman 1988; Lu 1993). LE and CE proteins have been identified in rat, mouse, *Drosophila*, *C. elegans* and budding yeast. Intriguingly, despite sharing broad secondary structure, they lack sequence conservation (review in Page and Hawley 2004). The condensin complex, required for metaphase axial compaction, is also required for efficient assembly of the SC (Yu and Koshland 2003). SC is shed progressively from the homologues in the transition from pachytene to diplotene when homologues start to separate.

Because of the universal conservation of SC along each pair of homologues, reconstruction of SC complements is a powerful tool to estimate the chromosome number and to detect chromosome aberrations in organisms, such as fungi, with small chromosomes (e.g., Carmi et al. 1978; Zickler et al. 1984; Leblon et al. 1986; review in Pukkila 1994).

A. Synaptonemal Complex Formation

SCs form progressively between homologues during zygotene (Fig. 20.5B) and are seen along their entire lengths during pachytene (Fig. 20.5A). Despite a general tendency for SC to initiate near chromosome ends, SC can also initiate interstitially, excluding a zipper-like mechanism from the telomeres (examples in Zickler et al. 1992). SCs form preferentially between homologous chromosomes but they can also form between axial elements (AEs) of non-homologous regions or chromosomes. In heterozygotes for chromosome inversions, duplications and reciprocal translocations, SC formation is initially strictly homologous. At mid-pachytene, homologous SCs formed in inversion or duplication loops as well as in the typical translocation crosses can be gradually eliminated to form straight SCs with regions of non-homologous synapsis (examples in Bojko 1990 for inversion loops of *N. crassa*, and in Rasmussen et al. 1981 for reciprocal translocations of *C. cinereus*). Extensive non-homologous SC formation is also observed in haploid meioses and in plant hybrids with chromosomes of different lengths (review in Zickler and Kleckner 1999). At least two proteins, Hop2p and Mnd1p, are required for preventing synapsis between non-homologous chromosomes (Leu et al. 1998; Chen et al. 2004 and references therein). DNA replication is not required for SC formation in *C. cinereus*: SCs form between unreplicated homologues in the *spo22/msh5* mutant (Pukkila et al. 1995).

B. Ascomycetes Exhibit Several Peculiarities in the Synaptonemal Complex Formation and Morphology

Although evolutionarily conserved with respect to basic structure, some organisms show clear substructures in either the CEs or the LEs. LE substructures are particularly prominent in discomycetes, which exhibit a characteristic, banded pattern with species-specific replicate units of alternating thick and thin bands (von Wettstein 1971; Gillies 1972; Zickler 1973). Interestingly, the spacing of the bands is constant at about 25 per micron of LE, and the same periodicity is also

observed when the mammal LE component SCP3 is expressed in somatic cells (Yuan et al. 1998; Zickler and Kleckner 1999). In *Sordaria humana*, LEs are tubular and form numerous bulges that are variable in size and location along chromosomes. Bulges are more frequent at the junctions between synapsed and unsynapsed regions and are gone at pachytene, but their role, if any, remains unknown (Zickler and Sage 1981).

Five fungi (*S. pombe*, *A. nidulans*, *Schizosaccharomyces octosporus*, *Schizosaccharomyces japanicus* and *Ustilago maydis*) are among the very few exceptions (with *Drosophila* male) that do not form SCs (Olson et al. 1978; Fletcher 1981; Egel-Mitani et al. 1982; Bähler et al. 1993; Kohli and Bähler 1994). Detailed analyzes by EM, combined with FISH in time-course experiments of synchronized cells, showed clearly that no classical SC is formed in *S. pombe* (Bähler et al. 1993; Scherthan et al. 1994; Molnar et al. 2003). Fission yeast, however, forms linear structures that are likely functionally analogous to AEs (review in Molnar et al. 2003). Differences with standard continuous AEs/LEs are nevertheless observed: although their total length per nucleus varies with ploidy, both length and number are highly variable from one nucleus to another. FISH with telomeric, centromeric and interstitial region probes reveal that homologues occupy distinct territories, with maximum pairing of probes during the stage when the linear elements are longitudinally parallel in the horsetail elongated nucleus (Scherthan et al. 1994). Those examples clearly illustrate the importance of studying meiosis in a variety of different organisms.

C. Synaptonemal Complex and the Recombination Process

Studies of synchronous budding yeast meiocytes have allowed to draw a parallel between SC formation and the recombination steps (Padmore et al. 1991; Hunter and Kleckner 2001). DSBs occur at early leptotene – thus, before pairing and SC. The next step, namely, the appearance of single-end invasion intermediates (see Sect. III.), is concomitant with the initiation of the central element and is completed by the end of SC formation. Double Holliday junctions formation occurs during pachytene, and the resolution of DHJs to mature crossovers occurs at the end of pachytene (Börner et al. 2004 and references therein). Complete SC along each pair of homologues likely both promotes the maturation of recombination intermediates and stabilizes homologous associations throughout the period when crossovers are being formed.

Accordingly, budding yeast *C. cinereus* and *S. macrospora* mutants that are impaired in recombination steps show various SC formation defects. For example, neither AEs nor SCs are complete in a *C. cinereus* mutant defective for the nuclease Mre11p involved in several steps of DNA repair and homologous recombination (Gerecke and Zolan 2000). No SCs are formed in the absence of DSBs (Celerin et al. 2000; Storlazzi et al. 2003). Also, SC appears to be nucleated at sites of recombination interactions that eventually mature into crossovers. In wild-type *S. macrospora*, numbers of interstitial SC initiation sites correspond well to the number of COs, chiasmata and recombination nodules, and are decreased in two mutants with decreased COs (Zickler et al. 1992). As CO interference precedes initiation of SC, it is possible that the spreading of interference may license SC polymerization.

D. Recombination Nodules, the Substructures of the Synaptonemal Complex that Correlate with Crossover and Noncrossover Exchanges

Recombination nodules are electron-dense structures associated with forming or completed SC in all investigated organisms (Fig. 20.5A). They were termed recombination nodules (RNs) by Carpenter (1975), on the basis of their correlation with COs in *Drosophila* oocytes. Further investigations identified two types of RNs, early nodules and late nodules, which can be distinguished from one another on the basis of stage of appearance, frequency, shape, size, and staining properties. Early nodules (ENs) are spherical or ellipsoidal structures associated with axial elements and the forming SCs from late leptotene until early pachytene (Fig. 20.5C). Late nodules (LNs) are denser, less variable in shape, and appear during pachytene (Fig. 20.5A) and in lower numbers; they sometimes persist through diplotene (review in von Wettstein et al. 1984; Carpenter 1987; Zickler and Kleckner 1999). Distributions of both types of nodules were extensively investigated in four mycelial fungi: *N. crassa* (Gillies 1972, 1979; Bojko 1988, 1989), *S. macrospora* (Zickler 1977; Zickler et al. 1992), *S. commune* (Carmi et al. 1978) and *C. cinereus* (Holm et al. 1981).

The mean number of LNs per mid-pachytene nucleus matches the species number of COs deduced from genetic maps and/or chiasmata (review in von Wettstein et al. 1984; Zickler and Kleckner 1999). LNs match also the COs and chiasmata location along bivalent arms (e.g., Bojko 1989; Zickler et al. 1992, for fungi). Like COs, LNs show both intra-arm as well as inter-arm interference, as indicated by their non-Poisson distribution (review in Carpenter 1987). Also, the frequency of LNs per micron length of SC is higher in small than in larger chromosomes, as are the number of chiasmata (review in Jones 1984; Zickler and Kleckner 1999). Mutants known to reduce the frequency of meiotic exchanges and/or alter their distribution alter also the number and location of the late RNs in *Drosophila* and *S. macrospora* (Carpenter 1979; Zickler et al. 1992). The mismatch repair Mlh1, Mlh3, Msh4 and Msh5p proteins are components of LNs (Moens et al. 2002). A group of proteins (Zip1p, Zip2p, Zip3p) specifically involved in crossover maturation (but not in gene conversion) are likely components of LNs in budding yeast (Fung et al. 2004).

In contrast to LNs, ENs are distributed uniformly and do not exhibit interference (e.g., Holm et al. 1981 for *C. cinereus*). Their number per nucleus is two to over 100 times the number of LNs. The available evidence strongly suggests that ENs represent recombinational interactions that are at the DSBs stage or just later. First, their time of appearance, along unsynapsed AEs or at the sites of convergence between synapsing homologues, corresponds to the transition of DSB into single-end invasion intermediates formation (see Sect. III.). By contrast, LNs appear later and are exclusively associated with the SC central region.

Second, Rad51p assembles in large amounts into ENs but not into LNs. Also, ENs are known to occur in two stability classes, implying the occurrence of a functionally transition that precedes any obvious transition reflected in EM morphology (review in Anderson et al. 2001).

Third, whereas LNs are of uniform appearance within a given organism, ENs are notable for their diversity of form, even in a given nucleus (review in Zickler and Kleckner 1999). This diversity is fully consistent with developmental progression of a complex protein/DNA assembly through a series of biochemical steps that are accompanied by changes in both DNA and protein components. However, the exact relationship between ENs and LNs remains unknown.

VI. Meiotic Chromosome Segregation or how to Resolve Sister-Chromatid Cohesion in Two Steps

Successful execution of the two meiotic divisions has three prerequisites. First, each pair of homologous chromosomes must be connected by at least one physical link achieved by crossover between one sister chromatid of each homologue. Chiasmata thus establish an inherent polarity that will direct chromosome segregation: by staying together, homologues are constrained such that their kinetochores capture microtubules from opposite poles. In addition, the link established by chiasmata plus sister cohesion at the centromeric region counteracts the pulling forces of the spindle, thereby generating tension across the kinetochores and signaling of a stable bipolar attachment. In the absence of chiasmata, chromosomes are free to attach and travel to either pole randomly (e.g., Moreau et al. 1985; Storlazzi et al. 2003).

Second, sister kinetochores must orient to the same spindle pole at division I, and to opposite poles at division II. In budding yeast, the meiosis-specific protein Mam1 collaborates with the nucleolar proteins Csm1 and Lrs4 to ensure co-orientation of sister kinetochores during meiosis I. Their dissociation from kinetochores during early anaphase I is likely to be part of the events that allow sister kinetochores to bi-orient during meiosis II (Toth et al. 2000; Rabitsch et al. 2003).

Third, cohesion must be released in two steps: along arms, in order to permit the release of chiasmata at metaphase I, and at centromeres at metaphase II. Retention of centromeric cohesion between sister chromatids beyond anaphase I is critical for proper segregation of sister chromatids during division II (review in Lee and Orr-Weaver 2001; Nasmyth 2001). Premature sister-chromatid separation (e.g., in *rec8* or *spo76* mutants, see Klein et al. 1999; van Heemst et al. 1999) or the inability to separate sister chromatids in a timely fashion (e.g., in *spo12* and *spo13* mutants, Katis et al. 2004) lead to unequal chromosome segregation, and thus chromosome imbalance in gametes. Chromosome segregation is a central aspect of meiosis. Errors in the transmission of chromosomes produce aneuploid gametes/spores bearing too many or too few chromosomes, the major cause of infertility in all organisms, including fungi (review in Lee and Orr-Weaver 2001).

A. Chromosome and Sister-Chromatid Segregation Are Mediated by the Cohesin Complex

Proper sister-chromatid cohesion is a fundamental aspect of chromosome segregation. Both in mitosis and meiosis, cohesion is mediated by evolutionarily conserved chromosomal proteins, termed "cohesins". The cohesin complex consists of two long, coiled-coil proteins, Smc1 and Smc3, and two regulatory subunits called Scc1/Mcd1/Rad21 and Scc3. All members of the complex are essential for cohesion, since mutations result in precocious separation of sister chromatids (review in Nasmyth 2001). Several lines of evidence suggest that cohesins are loaded onto chromosomes during or immediately following S-phase in meiosis as in mitosis. They are removed by proteolysis of the Scc1/Rad21 subunit at anaphase onset, which in turn is thought to trigger chromatid separation (review in Nasmyth 2001). Chromatin immunoprecipitation analyses along budding yeast chromosomes show that cohesins tend to associate with AT-rich regions, and an even higher enrichment is seen in the pericentric region. This pattern of sites is very similar in mitotic and meiotic cells (review in Glynn et al. 2004). A negative correlation is found between the location of meiotic cohesins and that of the DSBs that initiate meiotic recombination: DSBs tend to occur in GC-rich regions where cohesins are absent, and cohesins tend to be located in regions that contain low levels of DSBs (Glynn et al. 2004). Cohesins function not only in chromosome segregation but also in DNA repair by homologous recombination (review in Jessberger 2002).

At least three cohesin subunits have meiosis-specific variants. Rec8 replaces largely Scc1/Rad21 in all species analyzed so far (e.g., Klein et al. 1999; Watanabe and Nurse 1999). The ability of meiotic centromeric cohesion to resist the endopeptidase Esp1 (called separase) requires Rec8: when Scc1 is expressed instead of Rec8 during meiosis, cohesion is destroyed in both arm and centromere at metaphase I (Buonomo et al. 2000). In fission yeast, both Rec8 and Rad21 are present, but show distinct localization patterns in the centromeric region, and determine the manner of kinetochore attachment to microtubules (Yokobayashi et al. 2003). Scc3 is partially replaced by Rec11/STAG3, along arms but not at centromeres (Prieto et al. 2001). Mammalian spermatocytes also express a meiosis-specific version of SMC1, called SMC1β (Revenkova et al. 2004). Requirement for meiosis-specific cohesins is a clear indication of a reinforcement of their functions during meiosis (review in Nasmyth 2001).

Cohesins have at least four meiotic functions, as shown by the fact that cohesin mutants have multiple phenotypes.

1. They are required for the regulation of DSB repair. In the absence of *REC8*, *SMC3* or *SCC3*, DSBs are normally formed but not properly repaired, as seen by the accumulation of Rad51 foci, severely reduced recombination, and from the presence of broken chromosomes at diplotene (Klein et al. 1999; Pasierbek et al. 2001, 2003).
2. The cohesin complex stabilizes chiasmata along chromosome arms: cleavage of Rec8 is needed for homologue segregation only if they are joined by chiasmata (Buonomo et al. 2000).
3. Cohesins co-localize with SC axial elements (AEs) and are implied in SC assembly (Klein et al. 1999; Pelttari et al. 2001; Eijpe et al. 2003; Revenkova et al. 2004 and references therein). First, SCs are not formed in the absence of either Rec8 or Scc3 (Klein et al. 1999; Pasierbek et al. 2003). Second, the cohesin core provides a scaffold for binding both AE and recombination proteins. The central region protein SCP1 forms SCs in the absence of the AE components SCP3 and SCP2, showing that the organization of the cohesin complex is sufficient for SCP1 fixation to the chromosome axes (Pelttari et al. 2001). Third, co-immunoprecipitation experiments suggest a physical association between cohesins and both SCP2 and SCP3 (Eijpe et al. 2000). Fourth, reciprocally, AE components appear to be required to reinforce sister-chromatid cohesion: mutations in genes encoding AE components cause premature separation of the sister chromatids during meiosis I in budding yeast and *C. elegans* (review in Lee and Orr-Weaver 2001; Eijpe et al. 2003). Also, the persistent localization of AE proteins along arms up to the anaphase I onset strongly suggests a role in sister cohesion (Eijpe et al. 2003).
4. The meiosis-specific cohesin SMC1β is also required for orderly chromosome processing: AEs and SCs are shorter, and chromosome loops are enlarged in the absence of SMC1β (Revenkova et al. 2004).

B. Other Proteins Important for Sister-Chromatid Cohesion and Segregation

Besides cohesins, several proteins have essential roles in generating or maintaining sister cohesion during meiosis. Five proteins have been implicated in protecting centromeric cohesion during division I.

1. The MEI-S332 and ORD proteins of *D. melanogaster* are required to maintain sister cohesion until anaphase II (Lee and Orr-Weather 2001; Balicky et al. 2002). A protein related to MEI-S332, called Sgo1 (for "*shugo*shin", which means "protector" in Japanese), binds to centromeric DNA during both meiotic divisions in fission and budding yeasts. Sgo1 is necessary both for protecting centromeric Rec8p from separase, and for proper sister centromere disjunction at division II (Kitajima et al. 2004). Through its regulation of microtubules, Sgo1 may also influence the spindle checkpoint, and is thus a crucial link between centromere cohesion and kinetochore/microtubule interaction (Salic et al. 2004). Sgo1 function is likely conserved, as orthologues are found in *N. crassa* and *M. grisea* (Rabitsch et al. 2004).
2. The conserved Bub1 spindle-checkpoint kinase is also required for both the retention of Rec8 at centromeres and their correct disjunction at division II (Bernard et al. 2001).
3. The budding yeast meiosis-specific protein Spo13 (which so far has no known orthologue) is necessary to prevent sister kinetochore bi-orientation during metaphase I, by facilitating the recruitment of Mam1 to kinetochores (review in Katis et al. 2004).

The complex composed of Scc2/Mis4/Rad9 and Scc4, required for the loading of the cohesin complex onto chromosomes, is also implied in sister cohesion during meiosis. *C. cinereus rad9* mutants are impaired in cohesion, homologous pairing, and in chromosome condensation (Seitz et al. 1996; Cummings et al. 2002). However, as the *rad9* mutant still shows a partial defect in homologous pairing in a *spo22/msh5* background (thus, in the absence of a sister chromatid), the role of Rad9p in homologous pairing may not entirely derive from its role in sister cohesion (Cummings et al. 2002).

The Spo76/BIMD/Pds5 protein family members are conserved components of the basic chromosome structure that are recruited from the mitotic cycle and functionally adapted for use in the meiotic program (van Heemst et al. 1999, 2001). They are likely needed for the morphological transformations in chromosome structure that lead to condensed metaphase chromosomes, as shown by the fact that *S. macrospora* and budding yeast *spo76/pds5* mutants show defects in both chromosome cohesion and condensation (van Heemst et al. 1999; Hartman et al. 2000; Panizza et al. 2000). Although bound to the same chromosome sites as the cohesin complex, Spo76/Pds5 is not part of the cohesin complex, and Scc1/Mcd1 is needed for chromosomal localization of Pds5 (Hartman et al. 2000, Panizza et al. 2000). Aside from their role in chromosome morphogenesis, they are also involved in cell cycle progression: human orthologue AS3 is a possible tumor suppressor (Geck et al. 2000), and *A. nidulans* BIMD is a negative regulator of normal mitotic cell cycle progression, with a G1 arrest when over-expressed (Denison et al. 1993; van Heemst et al. 2001). All mutants are hypersensitive to DNA damage, and Spo76p is also required for meiotic inter-homologue recombination, likely at post-initiation stages (van Heemst et al. 1999). The *bimD6* mutant shows reduced homologous recombination but normal intra-chromosomal recombination, suggesting that BIMD/Spo76 is not involved in the enzymology of recombinational repair per se (van Heemst et al. 2001). The specific defects of the *S. macrospora spo76-1* mutant at both mitotic prometaphase and meiotic zygotene, with cohesion and condensation coordinately affected on a regional basis, suggest that Spo76p plays a crucial role at this critical chromosome transition point for both divisions. Also, Spo76-GFP makes stronger lines during meiotic prophase (Fig. 20.4) than during mitotic prophase, showing that Spo76p is used to reinforce the sister cohesion along meiotic axes, which fits with the mutant phenotypes. Maintenance of meiotic chromosome axes integrity and formation of the SC are also dependent on Spo76p (van Heemst et al. 1999). During meiosis, Spo76/Pds5p likely plays the role of a spring-clip, allowing local destabilization at sites of recombination and chiasma formation, while maintaining chromosome axis integrity elsewhere (Storlazzi et al. 2003).

VII. From Meiosis to Sporulation

Genetically defined mating types impose developmental constraints in most mycelial fungi, and

ascus plus ascospore morphogenesis involves features of cell differentiation that are unique among fungi (review in Raju and Perkins 1994; Read and Beckett 1996). For example, within the single cell of the ascus, meiotic divisions and *post**m**e*iotic *m*itosis (PMM) lead to the formation of four or eight new cells. Therefore, spindles must be regularly spaced – initially parallel to the long axis and widely separated for the two meiotic spindles, and then regularly spaced across the long axis of the cell for the PMM spindles. Absence or deficiency of actin and microtubule polymerization leads to abnormally shaped asci with irregular spindle orientation (Thompson-Coffe and Zickler 1992, 1993; Shiu et al. 2001). In asci of secondary homothallic species where two nuclei of opposite mating type must be included together in each ascospore, cross-talk between the two nuclei is critical. This requires a strict regulation of how spindles are located during both meiosis and PMM. Moreover, even among these species, different mechanisms are used to package nuclei of opposite mating type into ascospores. *N. tetrasperma*-type species, in which mating type segregates at meiosis I, align meiosis II spindles so that positional overlap will result in pairs of nuclei of opposite mating type in close proximity for PMM. In other secondary homothallic species such as *P. anserina*, mating type is segregated at the second meiotic division; each nucleus must then associate with another of opposite mating type to produce two widely separated pairs of PMM spindles (Thompson-Coffe and Zickler 1993; review in Raju and Perkins 1994).

Immunofluorescence and drug disruption studies indicate that formation of asci, and proper migration of single nuclei or nuclear pairs are less dependent on intact microtubules than on actin microfilaments and actin–myosin interactions (Thompson-Coffe and Zickler 1993, 1994). From PMM to spore enclosure, the two nuclei of opposite mating type, which will form the binucleate ascospore, remain in very close proximity. Moreover, SPBs migrate and orient with their asters connected, and this connection is released only after spore closure (Thompson-Coffe and Zickler 1994). The critical orientation of spindles and nuclei during and after meiosis is supported by the extreme phenotypes of sporulation-deficient mutants (e.g., Srb et al. 1973; Zickler and Simonet 1980; Raju 1992). Genetic and cytological analyses of *P. anserina* strains carrying mating-type mutations showed that mating-type genes are required to ensure biparental dikaryotic ascospores. Altered mating type leads to the formation of either monokaryotic asci resulting in haploid meiosis, or dikaryotic uniparental ascospores when wild-type strains give only biparental dikaryotic ascospores (Zickler et al. 1995). Ultrastructural analyses of basidiomycetes do also underline the obvious relationship between the spatial arrangement of both SPB and spindles, and the correct spore formation (e.g., O'Donnell and McLaughin 1984; O'Donnel 1992). However, questions such as how do nuclei distinguish self from non-self and maintain their own identities when located in a common cytoplasm, as well as how are the precise nuclear movements controlled, remain unanswered.

VIII. Concluding Remarks

For several years, analysis of meiosis was confined to cytological descriptions, which clearly delineated the various phases of chromosome morphogenesis as well as the crucial role of the spindle apparatus. In recent years, significant progress has been made in understanding meiotic pairing and recombination, and the field is progressing at a rapid speed (review in Dernburg 2003; Bishop and Zickler 2004; Hollingsworth and Brill 2004). It is, however, humbling to realize that, despite major advances in our understanding of pairing and synapsis during the past 40 years (cf. the SC was discovered by both Moses and Fawcett in 1956), we have remained rather ignorant of the mechanisms that regulate both homologous chromosome recognition and SC function. Though many of the proteins mediating SC formation and sister-chromatid cohesion have been identified, we still have a poor understanding as to the mechanism by which they function. Also, though we have a rather clear picture of some of the recombination steps, much remains to be discovered. For instance, it is now clear that the outcome of recombination events is determined very early during the recombination process (Börner et al. 2004; Fung et al. 2004), but the signal that triggers a DSB to be a crossover or a non-crossover remains unknown. Two central questions remaining to be answered are how do crossover interference and the obligate chiasma occur? Recent studies using chromatin immunoprecipitation experiments revealed complex organizational and functional interplay among changes at DNA and the axis levels (Blat et al. 2002). However, a key unanswered question concerns the

mechanism by which chiasmata are formed and maintained through chromosome condensation. Another important aspect of meiosis to be solved is cell progression. Several studies indicate that the cell cycle machinery that controls progression through mitosis is also used to regulate progression through meiosis (review in Lee and Amon 2001). However, most meiosis-specific modifications of the mitotic cycle remain largely uncharacterized. Also, very little is known concerning the metabolic requirements ensuring correct meiotic and/or sporulation progression. For example, why do *P. anserina* mutants defective for the mitochondrial citrate synthase arrest at the diffuse stage, a major landmark of oogenesis (Ruprich-Robert et al. 2002)? In short, the discovery of new proteins involved in the meiotic process often simultaneously provides satisfying answers to long-standing questions in chromosome biology, while revealing a new set of puzzles to solve.

The most important research progress has been made in budding yeast because genetic, physiology, cytology and biochemistry were used in combination. Although meiosis is a highly conserved process, recent comparative studies (e.g., with *S. pombe*, *C. cinereus*, *S. macrospora*, among the fungi) have provided informative variations in the mechanisms used by different organisms. Also, recent findings in *N. crassa* support the notion that an important barrier between two closely related species is, in fact, the existence of numerous small rearrangements in the genome (Shiu et al. 2001). Future studies will likely make use of genome-sequencing projects to identify homologues to important proteins from other species (e.g., Borkovich et al. 2004). With the availability of yeast homologues to fungal, plant and animal genes, it will be possible to use immunolocalization and newly established reverse genetic strategies to determine whether there are functional similarities across organisms. Although promising, it is also clear that several structural proteins with analogous functions do not share apparent sequence similarities (e.g., SC components and proteins that initiate recombination or protect centromeric cohesins, see above). A complete understanding of the mechanisms of chromosome recognition and segregation will continue to require synergistic approaches, integrating structural information with biochemical experimentation. In addition, it will be important to pursue forward genetic approaches in combination with high-resolution cytological analyses to identify genes that have conserved roles but also genes that have roles unique to fungal meiosis. After we have defined more precisely the function of several genes, we will have to solve the mystery of how the meiotic process evolved. Whether we will ever be able to answer this question may depend on the finding of missing links that may exist in the vast fungal kingdom.

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References

Allers T, Lichten M (2001) Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106:47–57

Anderson LK, Hooker KD, Stack SM (2001) Distribution of early recombination nodules on zygotene bivalents from plants. Genetics 159:1259–1269

Arora C, Kee K, Maleki S, Keeney S (2004) Antiviral protein Ski8 is a direct partner of Spo11 in meiotic DNA break formation, independent of its cytoplasmic role in RNA metabolism. Mol Cell 13:549–559

Bähler J, Wyler T, Loidl J, Kohli J (1993) Unusual nuclear structures in meiotic prophase of fission yeast: a cytological analysis. J Cell Biol 121:241–256

Balicky EM, Endres MW, Lai C, Bickel SE (2002) Meiotic cohesion requires accumulation of ORD on chromosomes before condensation. Mol Biol Cell 13:3890–3900

Bass HW, Marshall WF, Sedat JW, Agard DA, Cande WZ (1997) Telomeres cluster de novo before the initiation of synapsis: a three-dimensional spatial analysis of telomere positions before and during meiotic prophase. J Cell Biol 137:5–18

Baudat F, Nicolas A (1997) Clustering of meiotic double-strand breaks on yeast chromosome III. Proc Natl Acad Sci USA 94:5213–5218

Bell WR, Therrien CD (1977) A cytophotometric investigation of the relationship of DNA and RNA synthesis to ascus development in *Sordaria fimicola*. Can J Genet Cytol 19:359–370

Bernard P, Maure JF, Javerzat JP (2001) Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation. Nat Cell Biol 3:522–526

Berteaux-Lecellier V, Picard M, Thompson-Coffe C, Zickler D, Panvier-Adoutte A, Simonet J-M (1995) A non-mammalian homologue of the *PAF1* gene (Zellweger

syndrome) discovered as a gene involved in caryogamy in the fungus *Podospora anserina*. Cell 81:1043–1051
Berteaux-Lecellier V, Zickler D, Debuchy R, Panvier-Adoutte A, Thompson-Coffe C, Picard M (1998) A homologue of the yeast *SHE4* gene is essential for the transition between the syncytial and cellular stages during sexual reproduction of the fungus *Podospora anserina*. EMBO J 17:1248–1258
Bishop DK, Zickler D (2004) Early decision; meiotic crossover interference prior to stable strand exchange and synapsis. Cell 117:9–15
Blat Y, Protacio RU, Hunter N, Kleckner N (2002) Physical and functional interactions among basic chromosome organizational features govern early steps of meiotic chiasma formation. Cell 111:791–802
Bojko M (1988) Presence of abnormal synaptonemal complexes in heterothallic species of *Neurospora*. Genome 30:697–709
Bojko M (1989) Two kinds of "recombination nodules" in *Neurospora crassa*. Genome 32:309–317
Bojko M (1990) Synaptic adjustment of inversion loops in *Neurospora crassa*. Genetics 124:593–598
Borde V, Goldman AS, Lichten M (2000) Direct coupling between meiotic DNA replication and recombination initiation. Science 290:806–809
Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N et al. (2004) Lessons from the genome sequence of *Neurospora crassa*: tracing the path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev 68:1–108
Börner GV, Kleckner N, Hunter N (2004) Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117:29–45
Bouhouche K, Zickler D, Debuchy R, Arnaise S (2004) Altering a gene involved in nuclear distribution increases the repeat-induced point mutation process in the fungus *Podospora anserina*. Genetics 167:151–159
Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K (2000) Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell 103:387–398
Butler DK, Metzenberg RL (1989) Pre-meiotic change of nucleolus organizer size in *Neurospora*. Genetics 122:783–791
Cambareri EB, Jensen BC, Schabtach E, Selker EU (1989) Repeat-induced G-C to A-T mutations in *Neurospora*. Science 244:1571–1575
Carmi P, Holm PB, Koltin Y, Rasmussen SW, Sage J, Zickler D (1978) The pachytene karyotype of *Schizophyllum commune* analyzed by three dimensional reconstruction of synaptonemal complexes. Carlsberg Res Commun 43:117–132
Carpenter ATC (1975) Electron microscopy of meiosis in *Drosophila melanogaster* females. II. The recombination nodule a recombination-associated structure at pachytene? Proc Natl Acad Sci USA 72:3186–3189
Carpenter ATC (1979) Recombination nodules and synaptonemal complex in recombination-defective females of *Drosophila melanogaster*. Chromosoma 75:259–292
Carpenter ATC (1987) Gene conversion, recombination nodules, and the initiation of meiotic synapsis. Bioessays 6:232–236
Catcheside DG (1977) The genetics of recombination. University Park Press, Baltimore, MD
Celerin M, Merino ST, Stone JE, Menzie AM, Zolan ME (2000) Multiple roles of Spo11 in meiotic chromosome behavior. EMBO J 19:2739–2750
Cha RS, Weiner BM, Keeney S, Dekker J, Kleckner N (2000) Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev 14:493–503
Chen YK, Leng CH, Olivares H, Lee MH, Chang YC, Kung WM, Ti SC, Lo YH, Wang AH, Chang CS et al. (2004) Heterodimeric complexes of Hop2 and Mnd1 function with Dmc1 to promote meiotic homolog juxtaposition and strand assimilation. Proc Natl Acad Sci USA 101:10572–10577
Chikashige Y, Ding DQ, Funabiki H, Haraguchi T, Mashiko S, Yanagida M, Hiraoka Y (1994) Telomere-led premeiotic chromosome movement in fission yeast. Science 264:270–273
Collins I, Newlon CS (1994) Chromosomal DNA replication initiates at the same origins in meiosis and mitosis. Mol Cell Biol 14:3524–3534
Colot V, Rossignol J-L (1999) Eukaryotic DNA methylation as an evolutionary device. Bioessays 21:402–411
Colot V, Maloisel L, Rossignol J-L (1996) Interchromosomal transfer of epigenetic states in *Ascobolus*: transfer of DNA methylation is mechanistically related to homologous recombination. Cell 86:855–864
Coppin-Raynal E, Picard M, Arnaise S (1989) Transformation by integration in *Podospora anserina*. III. Replacement of a chromosome segment by a two-step process. Mol Gen Genet 219:270–276
Cummings WJ, Merino ST, Young KG, Li L, Johnson CW, Sierra EA, Zolan ME (2002) The *Coprinus cinereus* adherin Rad9 functions in Mre11-dependent DNA repair, meiotic sister-chromatid cohesion, and meiotic homolog pairing. Proc Natl Acad Sci USA 99:14958–14963
De Massy B, Baudat F, Nicolas A (1994) Initiation of recombination in *Saccharomyces cerevisiae* haploid meiosis. Proc Natl Acad Sci USA 91:11929–11933
Denison SH, Kafer E, May GS (1993) Mutation in the *bimD* gene of *Aspergillus nidulans* confers a conditional mitotic block and sensitivity to DNA damaging agents. Genetics 134:1085–1096
Dernburg AF (2003) Meiosis researchers exchange information in the Alps. Dev Cell 5:691–693
De Veaux LC, Smith GR (1994) Region-specific activators of meiotic recombination in *Schizosaccharomyces pombe*. Genes Dev 8:203–210
Ding DQ, Yamamoto A, Haraguchi T, Hiraoka Y (2004) Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. Dev Cell 6:329–341
Egel R (1978) Synaptonemal complex and crossing-over: structural support or interference? Heredity 41:233–237
Egel-Mitani M, Olson LW, Egel R (1982) Meiosis in *Aspergillus nidulans*: another example for lacking synaptonemal complexes in the absence of crossover interference. Hereditas 97:179–187
Eijpe M, Heyting C, Gross B, Jessberger R (2000) Association of mammalian SMC1 and SMC3 proteins with meiotic chromosomes and synaptonemal complexes. J Cell Sci 113:673–682

Eijpe M, Offenberg H, Jessberger R, Revenkova E, Heyting C (2003) Meiotic cohesin REC8 marks the axial elements of rat synaptonemal complexes before cohesins SMC1β and SMC3. J Cell Biol 160:657–670

Elliot CG (1960) The cytology of *Aspergillus nidulans*. Genet Res 1:462–476

Esser K, Kuenen R (1967) Genetics of fungi. Springer, Berlin Heidelberg New York

Farman ML (2002) Meiotic deletion at the *BUF1* locus of the fungus *Magnaporthe grisea* is controlled by interaction with the homologous chromosome. Genetics 160:137–148

Faugeron G (2000) Diversity of homology-dependent gene silencing strategies in fungi. Curr Opin Microbiol 3:144–148

Fawcett DW (1956) The fine structure of chromosomes in the meiotic prophase of vertebrate spermatocytes. J Biophys Biochem Cytol 2:403–406

Fletcher HL (1981) A search for synaptonemal complexes in *Ustilago maydis*. J Cell Sci 50:171–180

Foss E, Lande R, Stahl FW, Steinberg CM (1993) Chiasma interference as a function of genetic distance. Genetics 133:681–691

Freedman T, Pukkila PJ (1993) *De novo* methylation of repeated sequences in *Coprinus cinereus*. Genetics 135:357–366

Freitag M, Williams RL, Kothe GO, Selker EU (2002) A cytosine methyltransferase homologue is essential for repeat-induced point mutation in *Neurospora crassa*. Proc Natl Acad Sci USA 99:8802–8807

Fung JC, Rockmill B, Odell M, Roeder GS (2004) Imposition of crossover interference through the nonrandom distribution of synapsis initiation complexes. Cell 116:795–802

Geck P, Maffini MV, Szelei J, Sonnenschein C, Soto AM (2000) Androgen-induced proliferative quiescence in prostate cancer cells: the role of AS3 as its mediator. Proc Natl Acad Sci USA 97:10185–10190

Gerecke EE, Zolan ME (2000) An *mre11* mutant of *Coprinus cinereus* has defects in meiotic chromosome pairing, condensation and synapsis. Genetics 154:1125–1139

Gerton JL, DeRisi J, Shroff R, Lichten M, Brown PO, Petes TD (2000) Global mapping of meiotic recombination hotspots and coldspots in the yeast *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 97:11383–11390

Gillies CB (1972) Reconstruction of the *Neurospora crassa* pachytene karyotype from serial sections of synaptonemal complexes. Chromosoma 36:119–130

Gillies CB (1979) The relationship between synaptonemal complexes, recombination nodules and crossing over in *Neurospora crassa* bivalents and translocation quadrivalents. Genetics 91:1–17

Glynn EF, Megee PC, Yu HG, Mistrot C, Unal E, Koshland DE, DeRisi JL, Gerton JL (2004) Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*. PLoS Biol 2:E259

Graia F, Lespinet O, Rimbault M, Dequart-Chablat M, Coppin E, Picard M (2001) Genome quality control: RIP (repeat-induced point mutation) comes to *Podospora*. Mol Microbiol 40:586–595

Hamann A, Feller F, Osiewacz HD (2000) The degenerate DNA transposon Pat and repeat-induced point mutation (RIP) in *Podospora anserina*. Mol Gen Genet 263:1061–1069

Hartman T, Stead K, Koshland D, Guacci V (2000) Pds5p is an essential chromosomal protein required for both sister chromatid cohesion and condensation in *Saccharomyces cerevisiae*. J Cell Biol 151:613–626

Henderson KA, Keeney S (2004) Tying synaptonemal complex initiation to the formation and programmed repair of DNA double-strand breaks. Proc Natl Acad Sci USA 101:4519–4524

Hoffmann B, Eckert SE, Krappmann S, Braus GH (2001) Sexual diploids of *Aspergillus nidulans* do not form by random fusion of nuclei in the heterokaryon. Genetics 157:141–147

Hollingsworth NM, Brill SJ (2004) The Mus81 solution to resolution: generating meiotic crossovers without Holliday junctions. Genes Dev 18:117–125

Holm PB, Rasmussen SW, Zickler D, Lu BC, Sage J (1981) Chromosome pairing, recombination nodules and chiasma formation in the basidiomycete *Coprinus cinereus*. Carlsberg Res Commun 46:305–346

Hunter N, Kleckner N (2001) The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination. Cell 106:59–70

Idnurm A, Howlett BJ (2003) Analysis of loss of pathogenicity mutants reveals that repeat-induced point mutations can occur in the Dothideomycete *Leptosphaeria maculans*. Fungal Genet Biol 39:31–37

Ikeda K, Nakayashiki H, Kataoka T, Tamba H, Hashimoto Y, Tosa Y, Mayama S (2002) Repeat-induced point mutation (RIP) in *Magnaporthe grisea*: implications for its sexual cycle in the natural field context. Mol Microbiol 45:1355–1364

Iyengar GA, Deka PC, Kundu SC, Sen SK (1976) DNA synthesis in course of meiotic development in *Neurospora crassa*. Genet Res 29:1–8

Jessberger R (2002) The many functions of SMC proteins in chromosome dynamics. Nat Rev Mol Cell Biol 3:767–778

Johnson TE (1976) Analysis of pattern formation in *Neurospora* perithecial development using genetic mosaics. Dev Biol 54:23–36

Jones GH (1984) The control of chiasma distribution. Symp Soc Exp Biol 38:293–320

Kaback DB, Barber D, Mahon J, Lamb J, You J (1999) Chromosome size-dependent control of meiotic reciprocal recombination in *Saccharomyces cerevisiae*: the role of crossover interference. Genetics 152:1475–1486

Käfer E (1977) Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv Genet 19:33–131

Kanda T, Arakawa H, Yasuda Y, Takemaru T (1990) Basidiospore formation in a mutant of incompatibility factors and in mutants that arrest at meta-anaphase I in *Coprinus cinereus*. Exp Mycol 14:218–226

Katis VL, Matos J, Mori S, Shirahige K, Zachariae W, Nasmyth K (2004) Spo13 facilitates monopolin recruitment to kinetochores and regulates maintenance of centromeric cohesion during yeast meiosis. Curr Biol 14:2183–2196

Keeney S (2001) Mechanism and control of meiotic recombination initiation. Curr Topics Dev Biol 52:1–53

King JS, Mortimer RK (1990) A polymerization model of chiasma interference and corresponding computer simulation. Genetics 126:1127–1138

Kitajima TS, Kawashima SA, Watanabe Y (2004) The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. Nature 427:510–517

Klein F, Mahr P, Galova M, Buonomo SB, Michaelis C, Nairz K, Nasmyth K (1999) A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 98:91–103

Kohli J, Bähler J (1994) Homologous recombination in fission yeast: absence of crossover interference and synaptonemal complex. Experientia 50:295–306

Kojic M, Kostrub CF, Buchman AR, Holloman WK (2002) BRCA2 homolog required for proficiency in DNA repair, recombination, and genome stability in *Ustilago maydis*. Mol Cell 10:683–691

Kutil BL, Seong KY, Aramayo R (2003) Unpaired genes do not silence their paired neighbors. Curr Genet 43:425–432

Lamb BC (1996) Ascomycete genetics: the part played by ascus segregation phenomena in our understanding of the mechanisms of recombination. Mycol Res 100:1025–1059

Leblon G, Zickler D, Le Bilcot S (1986) Most UV induced reciprocal translocations in *Sordaria macrospora* occur in or near the centromere regions. Genetics 112:183–204

Lee B, Amon A (2001) Meiosis: how to create a specialized cell cycle. Curr Opin Cell Biol 13:770–777

Lee JY, Orr-Weaver TL (2001) The molecular basis of sister-chromatid cohesion. Annu Rev Cell Dev Biol 17:753–777

Lee DW, Pratt RJ, McLaughlin M, Aramayo R (2003) An argonaute-like protein is required for meiotic silencing. Genetics 164:821–828

Lee DW, Seong KY, Pratt RJ, Baker K, Aramayo R (2004) Properties of unpaired DNA required for efficient silencing in *Neurospora crassa*. Genetics 167:131–150

Leu JY, Chua PR, Roeder GS (1998) The meiosis-specific Hop2 protein of *S. cerevisiae* ensures synapsis between homologous chromosomes. Cell 94:375–386

Li L, Gerecke EE, Zolan ME (1999) Homolog pairing and meiotic progression in *Coprinus cinereus*. Chromosoma 108:384–392

Lichten M (2001) Meiotic recombination: breaking the genome to save it. Curr Biol 11:253–256

Lindegren CC (1953) Gene conversion in *Saccharomyces*. J Genet 51:625–637

Loidl J (1990) The initiation of meiotic chromosome pairing: the cytological view. Genome 33:759–778

Lu BC (1993) Spreading the synaptonemal complex of *Neurospora crassa*. Chromosoma 102:464–472

Lu BC, Raju NB (1970) Meiosis in *Coprinus*. II. Chromosome pairing and the lampbrush diplotene stage of meiotic prophase. Chromosoma 29:305–316

Malagnac F, Wendel B, Goyon C, Faugeron G, Zickler D, Rossignol J-L, Noyer-Weidner M, Vollmayr P, Trautner TA, Walter J (1997) A gene essential for de nove methylation and development in *Ascobolus* reveals a novel type of eukaryotic DNA methyltransferase structure. Cell 91:281–290

Maloisel L, Rossignol J-L (1998) Suppression of crossing-over by DNA methylation in *Ascobolus*. Genes Dev 12:1381–1389

Mautino MR, Haedo SD, Rosa AL (1993) Physical mapping of meiotic crossover events in a 200-kb region of *Neurospora crassa* linkage group I. Genetics 134:1077–1083

McKee BD (2004) Homologous pairing and chromosome dynamics in meiosis and mitosis. Biochim Biophys Acta 1677:165–180

Merino ST, Cummings WJ, Acharya SN, Zolan ME (2000) Replication-dependent early meiotic requirement for Spo11 and Rad50. Proc Natl Acad Sci USA 97:10477–10482

Miao VP, Covert SF, VanEtten HD (1991) A fungal gene for antibiotic resistance on a dispensable ("B") chromosome. Science 254:1773–1776

Mikosch TS, Sonnenberg AS, Van Griensven LJ (2001) Isolation, characterization, and expression patterns of a DMC1 homolog from the basidiomycete *Pleurotus ostreatus*. Fungal Genet Biol 33:59–66

Mitchell MB (1955) Aberrant recombination of pyridoxine mutants of *Neurospora*. Proc Natl Acad Sci USA 41:216–220

Moens PB, Pearlman RE (1988) Chromatin organization at meiosis. Bioessays 9:151–153

Moens PB, Kolas NK, Tarsounas M, Marcon E, Cohen PE, Spyropoulos B (2002) The time course and chromosomal localization of recombination-related proteins at meiosis in the mouse are compatible with models that can resolve the early DNA-DNA interactions without reciprocal recombination. J Cell Sci 115:1611–1622

Molnar M, Bahler J, Sipiczki M, Kohli J (1995) The *rec8* gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister chromatid cohesion during meiosis. Genetics 141:61–73

Molnar M, Doll E, Yamamoto A, Hiraoka Y, Kohli J (2003) Linear element formation and their role in meiotic sister chromatid cohesion and chromosome pairing. J Cell Sci 116:1719–1731

Moreau PJF, Zickler D, Leblon G (1985) One class of mutants with disturbed centromere cleavage and chromosome pairing in *Sordaria macrospora*. Mol Gen Genet 198:189–197

Mortimer RK, Fogel S (1974) Genetical interference and gene conversion. In: Grell R (ed) Mechanisms in recombination. Plenum Press, New York, pp 263–275

Moses MJ (1956) Chromosome structures in crayfish spermatocytes. J Biophys Biochem Cytol 2:215–218

Munz P (1994) An analysis of interference in the fission yeast *Schizosaccharomyces pombe*. Genetics 137:701–707

Nara T, Yamamoto T, Sakaguchi K (2000) Characterization of interaction of C- and N-terminal domains in LIM15/DMC1 and RAD51 from a basidiomycete, *Coprinus cinereus*. Biochem Biophys Res Commun 275:97–102

Nasmyth K (2001) Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. Annu Rev Genet 35:673–745

Nicolas A, Petes TD (1994) Polarity of meiotic recombination in fungi: contrasting views. Experientia 50:242–252

Niwa O, Shimanuki M, Miki F (2000) Telomere-led bouquet formation facilitates homologous chromosome pairing and restricts ectopic interaction in fission yeast meiosis. EMBO J 19:3831–3840

O'Donnel K (1992) Ultrastructure of meiosis and the spindle pole body cycle in freeze-substituted basidia of the smut fungi *Ustilago maydis* and *Ustilago avenae*. Can J Bot 70:629–638

O'Donnel KL, McLaughlin DJ (1984) Ultrastructure of meiosis in *Ustilago maydis*. Mycologia 76:468–485

Olson LW, Eden U, Egel-Mitani M, Egel R (1978) Asynaptic meiosis in fission yeast? Hereditas 89:189–199

Padmore R, Cao L, Kleckner N (1991) Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell 66:1239–1256

Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Cell Dev Biol 20:525–558

Panizza S, Tanaka T, Hochwagen A, Eisenhaber F, Nasmyth K (2000) Pds5 cooperates with cohesin in maintaining sister chromatid cohesion. Curr Biol 10:1557–1564

Pâques F, Haber JE (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 63:349–404

Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J (2001) A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev 15:1349–1360

Pasierbek P, Fodermayr M, Jantsch V, Jantsch M, Schweizer D, Loidl J (2003) The *Caenorhabditis elegans* SCC-3 homologue is required for meiotic synapsis and for proper chromosome disjunction in mitosis and meiosis. Exp Cell Res 289:245–255

Pelttari J, Hoja MR, Yuan L, Liu JG, Brundell E, Moens P, Santucci-Darmanin S, Jessberger R, Barbero JL, Heyting C et al. (2001) A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. Mol Cell Biol 21:5667–5677

Perkins DD (1962) Crossing-over and interference in a multiply marked chromosome arm of *Neurospora*. Genetics 47:1253–1274

Perkins DD (1974) The manifestation of chromosome rearrangements in unordered asci of *Neurospora*. Genetics 77:459–489

Perkins DD (1992) *Neurospora* chromosomes. In: Federoff N, Botstein D (eds) The dynamic genome. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp 33–44

Perkins DD (1997) Chromosome rearrangements in *Neurospora* and other filamentous fungi. Adv Genet 36:239–398

Perkins DD, Lande R, Stahl FW (1993) Estimates of the proportion of intermediates that are resolved with crossing over in *Neurospora crassa*. Genetics 133:690–691

Perkins DD, Margolin BS, Selker EU, Haedo SD (1997) Occurrence of repeat induced point mutation in long segmental duplications of *Neurospora*. Genetics 147:125–136

Petes TD (2001) Meiotic recombination hot spots and cold spots. Nat Rev Genet 2:360–369

Pitkin JW, Nikolskaya A, Ahn J-H, Walton JD (2000) Reduced virulence caused by meiotic instability of the *TOX2* chromosome of the maize pathogen *Cochliobolus carbonum*. Mol Plant Microbe Interact 13:80–87

Pöggeler S (2002) Genomic evidence for mating abilities in the asexual pathogen *Aspergillus fumigatus*. Curr Genet 42:153–160

Prieto I, Suja JA, Pezzi N, Kremer L, Martinez AC, Rufas JS, Barbero JL (2001) Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis I. Nat Cell Biol 3:761–766

Pukkila PJ (1994) Meiosis in mycelial fungi. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol I, 1st edn. Growth, differentiation and sexuality. Springer, Berlin Heidelberg New York, pp 267–281

Pukkila PJ, Lu BC (1985) Silver staining of meiotic chromosomes in the fungus, *Coprinus cinereus*. Chromosoma 91:108–112

Pukkila PJ, Yashar BM, Binninger DM (1984) Analysis of meiotic development in *Coprinus cinereus*. Symp Soc Exp Biol 38:177–194

Pukkila PJ, Shannon KB, Skrzynia C (1995) Independent synaptic behavior of sister chromatids in *Coprinus cinereus*. Can J Bot 73 Suppl 1:S215–S220

Rabitsch KP, Petronczki M, Javerzat JP, Genier S, Chwalla B, Schleiffer A, Tanaka TU, Nasmyth K (2003) Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I. Dev Cell 4:535–548

Rabitsch KP, Gregan J, Schleiffer A, Javerzat JP, Eisenhaber F, Nasmyth K (2004) Two fission yeast homologs of *Drosophila* MEI-S332 are required for chromosome segregation during meiosis I and II. Curr Biol 14:287–301

Raju NB (1980) Meiosis and ascospore genesis in *Neurospora*. Eur J Cell Biol 23:208–223

Raju NB (1992) Genetic control of the sexual cycle in *Neurospora*. Mycol Res 96:241–262

Raju NB, Lu BC (1970) Meiosis in *Coprinus*. III. Timing of meiotic events in *C. lagopus*. Can J Bot 48:2183–2186

Raju NB, Perkins DD (1994) Diverse programs of ascus development in pseudohomothallic species of *Neurospora*, *Gelasinospora*, and *Podospora*. Dev Genet 15:104–118

Rasmussen SW, Holm PB, Lu BC, Zickler D, Sage J (1981) Synaptonemal complex formation and distribution of recombination nodules in pachytene trivalents of triploid *Coprinus cinereus*. Carlsberg Res Commun 46:347–360

Read ND, Beckett A (1996) Ascus and ascospore morphogenesis. Mycol Res 100:1281–1314

Revenkova E, Eijpe M, Heyting C, Hodges CA, Hunt PA, Liebe B, Scherthan H, Jessberger R (2004) Cohesin SMC1β is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. Nat Cell Biol 6:555–562

Rhounim L, Rossignol JL, Faugeron G (1992) Epimutation of repeated genes in *Ascobolus immersus*. EMBO J 11:4451–4457

Romanienko PJ, Camerini-Otero RD (2000) The mouse *Spo11* gene is required for meiotic chromosome synapsis. Mol Cell 6:975–987

Rossen JM, Westergaard M (1966) Studies on the mechanism of crossing over. II. Meiosis and the time of meiotic chromosome replication in the ascomycete *Neottiella rutilans*. Carlsberg Res Commun 35:233–260

Rossignol J-L, Faugeron G (1995) MIP: an epigenetic gene silencing process in *Ascobolus immersus*. Curr Top Microbiol Immunol 197:179–191

Rossignol J-L, Nicolas A, Hamza H, Kalogeropoulos A (1988) Recombination and gene conversion in *Ascobolus*. In: Low B (ed) The recombination of genetic material. Academic Press, New York, pp 23–72

Ruprich-Robert G, Zickler D, Berteaux-Lecellier V, Velot C, Picard M (2002) Lack of mitochondrial citrate synthase discloses a new meiotic checkpoint in a strict aerobe. EMBO J 21:6440–6451

Salic A, Waters JC, Mitchison TJ (2004) Vertebrate Shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis. Cell 118:567–578

Scherthan H (2001) A bouquet makes ends meet. Nat Rev Mol Cell Biol 2:621–627

Scherthan H, Bähler J, Kohli J (1994) Dynamics of chromosome organization and pairing during meiotic prophase in fission yeast. J. Cell Biol 127:273–285

Schwacha A, Kleckner N (1995) Identification of double Holliday junctions as intermediates in meiotic recombination. Cell 83:783–791

Seitz LC, Tang K, Cummings WJ, Zolan ME (1996) The *rad9* gene of *Coprinus cinereus* encodes a proline-rich protein required for meiotic chromosome condensation and synapsis. Genetics 142:1105–1117

Selker EU (2002) Repeat-induced gene silencing in fungi. Adv Genet 46:439–450

Selker EU, Cambareri EB, Jensen BC, Haack KR (1987) Rearrangement of duplicated DNA in specialized cells of *Neurospora crassa*. Cell 51:741–752

Sharif WD, Glick GG, Davidson MK, Wahls WP (2002) Distinct functions of *S. pombe* Rec12 (Spo11) protein and Rec12-dependent crossover recombination (chiasmata) in meiosis I; and a requirement for Rec12 in meiosis II. Cell Chromosome 1:1–14

Shiu PK, Metzenberg RL (2002) Meiotic silencing by unpaired DNA: properties, regulation and suppression. Genetics 161:1483–1495

Shiu PK, Raju NB, Zickler D, Metzenberg RL (2001) Silencing of unpaired DNA during meiosis. Biological consequences and identification of a suppressor that encodes an RNA-dependent RNA polymerase. Cell 107:905–916

Singer MJ, Kuzminova EA, Tharp A, Margolin BS, Selker EU (1995) Different frequencies of RIP among early vs. late ascospores of *Neurospora crassa*. Fungal Genet Newslett 42:74–75

Srb AM, Basl M, Bobst M, Leary J (1973) Mutations in *Neurospora crassa* ascus and ascospore development. J Heredity 64:242–246

Storlazzi A, Tesse S, Gargano S, James F, Kleckner N, Zickler D (2003) Meiotic double-strand breaks at the interface of chromosome movement, chromosome remodeling, and reductional division. Genes Dev 17:2675–2687

Strickland WN (1958) An analysis of interference in *Aspergillus nidulans*. Proc R Soc Ser B 149:82–101

Sym M, Roeder GS (1994) Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell 79:283–292

Tesse S, Storlazzi A, Kleckner N, Gargano S, Zickler D (2003) Localization and roles of Ski8p protein in *Sordaria* meiosis and delineation of three mechanistically distinct steps of meiotic homolog juxtaposition. Proc Natl Acad Sci USA 100:12865–12870

Thompson-Coffe C, Zickler D (1992) Three microtubule-organizing centers are required for ascus growth and sporulation in the fungus *Sordaria macrospora*. Cell Motil Cytoskeleton 22:257–273

Thompson-Coffe C, Zickler D (1993) Cytoskeletal interactions in the ascus development and sporulation of *Sordaria macrospora*. J Cell Sci 104:883–898

Thompson-Coffe C, Zickler D (1994) How the cytoskeleton recognizes and sorts nuclei of opposite mating type during the sexual cycle in filamentous ascomycetes. Dev Biol 165:257–271

Toth A, Rabitsch KP, Galova M, Schleiffer A, Buonomo SB, Nasmyth K (2000) Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I. Cell 103:1155–1168

Trelles-Sticken E, Loidl J, Scherthan H (1999). Bouquet formation in budding yeast: initiation of recombination is not required for meiotic telomere clustering. J Cell Sci 112:651–658

Tzeng TH, Lyngholm LK, Ford CF, Bronson CR (1992) A restriction fragment length polymorphism map and electrophoretic karotype of the fungal maize pathogen *Cochliobolus heterostrophus*. Genetics 130:81–96

Van Heemst D, Heyting C (2000) Sister chromatid cohesion and recombination in meiosis. Chromosoma 109:10–26

Van Heemst D, James F, Pöggeler S, Berteaux-Lecellier V, Zickler D (1999) Spo76p is a conserved chromosome morphogenesis protein that links the mitotic and meiotic programs. Cell 98:261–271

Van Heemst D, Kafer E, John T, Heyting C, van Aalderen M, Zickler D (2001) *BimD/Spo76* is at the interface between cell cycle progression, chromosome morphogenesis and recombination. Proc Natl Acad Sci USA 98:6267–6272

Von Wettstein D (1971) The synaptinemal complex and four-strand crossing over. Proc Natl Acad Sci USA 68:851–855

Von Wettstein D, Rasmussen SW, Holm PB (1984) The synaptonemal complex in genetic segregation. Annu Rev Genet 18:331–413

Watanabe Y, Nurse P (1999) Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature 400:461–464

Whitehouse HLK (1982) Genetic recombination: understanding the mechanisms. Wiley, Chichester

Wu TC, Lichten M (1995) Factors that affect the location and frequency of meiosis-induced double-strand breaks in *Saccharomyces cerevisiae*. Genetics 140:55–66

Yeadon PJ, Bowring FJ, Catcheside DE (2004) Alleles of the hotspot *cog* are codominant in effect on recombination in the *his-3* region of *Neurospora*. Genetics 167:1143–1153

Yokobayashi S, Yamamoto M, Watanabe Y (2003) Cohesins determine the attachment manner of kinetochores to spindle microtubules at meiosis I in fission yeast. Mol Cell Biol 23:3965–3973

Yu HG, Koshland DE (2003) Meiotic condensin is required for proper chromosome compaction, SC assembly, and resolution of recombination-dependent chromosome linkages. J Cell Biol 163:937–947

Yuan L, Pelttari J, Brundell E, Bjorkroth B, Zhao J, Liu JG, Brismar H, Daneholt B, Hoog C (1998) The synaptonemal complex protein SCP3 can form multistranded, cross-striated fibers in vivo. J Cell Biol 142:331–339

Zickler H (1934) Genetische Untersuchungen an einer heterothallischen Askomyzeten (*Bombardia lunata* nov. spec.). Planta 22:573–613

Zickler D (1973) Fine structure of chromosome pairing in ten Ascomycetes: meiotic and premeiotic (mitotic) synaptonemal complexes. Chromosoma 40:401–416

Zickler D (1977) Development of the synaptonemal complex and the "recombination nodules" during meiotic prophase in the seven bivalents of the fungus *Sordaria macrospora* Auersw. Chromosoma 61:289–316

Zickler D, Kleckner N (1998) The leptotene-zygotene transition of meiosis. Annu Rev Genet 32:619–697

Zickler D, Kleckner N (1999) Meiotic chromosomes: integrating structure and function. Annu Rev Genet 33:603–754

Zickler D, Sage J (1981) Synaptonemal complexes with modified lateral elements in *Sordaria humana*: development of and relationship to the "recombination nodules". Chromosoma 84:305–318

Zickler D, Simonet J-M (1980) Identification of gene-controlled steps of ascospore development in *Podospora anserina*. Exp Mycol 4:191–206

Zickler D; Leblon G, Haedens V, Collard A, Thuriaux P (1984) Linkage group-chromosome correlations in *Sordaria macrospora*: chromosome identification by three dimensional reconstruction of their synaptonemal complex. Curr Genet 8:57–67

Zickler D, Moreau PJ, Huynh AD, Slezec AM (1992) Correlation between pairing initiation sites, recombination nodules and meiotic recombination in *Sordaria macrospora*. Genetics 132:135–148

Zickler D, Arnaise S, Coppin E, Debuchy R, Picard M (1995) Altered mating-type identity in the fungus *Podospora anserina* leads to selfish nuclei, uniparental progeny and haploid meiosis. Genetics 140:493–503

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