Course Title:	Introduction to Cytogenetics
Course Code:	Biol3121
Course Credit point:	3ECTS/ 2hrs lecture per week
Pre-requisite	Principles of Genetics (Biol3061)
Academic year	2019/2020
Target group	III year, II semester Biology major students
Course status	Elective
Instructors Name	Helen Nigussie (Helen.nigussie@aau.edu.et)
Office Number	Grad build 313
Class Schedule	Venue

1. Course Description:

Brief historical background about the development of the discipline; levels of complexity of prokaryotic and eukaryotic chromosomes in terms of genome size and organization; molecular components of the eukaryotic chromosomes; packaging of DNA into eukaryotic chromosomes; important morphological features of eukaryotic chromosomes; major chromosome bandings and their applications; chromosome numerical and structural changes, origins and consequences; main attributes of karyotypes, applications and evolution of karyotypes**.**

2. Course objectives

After completing the course, the students should be able to:

- compare the different levels of genome complexity
- enumerate that most of the eukaryotic DNA is non-coding (junk)
- describe the main morphological features of eukaryotic chromosomes
- classify chromosomes on the basis morphology and size
- discuss the major types of chromosome bandings
- explain the principles of FISH
- List the main types of numerical and structural changes in chromosomes and their consequences

3. Course outline

1. Historical development of the science of cytogenetics

2. Structure and size of chromosomes

2.1. Prokaryotic chromosomes

2.2. Eukaryotic chromosomes

3. Chromosomes in cell division

3.1 Mitosis

3.1.1. Significance of mitosis

3.1.2. Events of the cell cycle (phases in the cell cycle)

3.2 Meiosis

3.2.1. Significance of meiosis

3.2.2. Phases of meiosis

4. Special types of chromosomes

4.1. Polytene chromosomes

4.2. Lampbrush chromosomes

4.3. B chromosomes

4.4. Holokinetic chromosomes

5. Major types of chromosome banding

6. Fluorescence in situ hybridization (FISH)

6.1. Probes types and labeling of probes

6.2. Probe labels

6.3. Hybridization and methods of detection of site of hybridization

6.4. The applications/ uses of in situ hybridization

7 Chromosome structural mutations

7.1. Deficiency (deletion)

7.2. Duplication (addition)

7.3. Inversions (para- and pericentric)

7.4. Translocations

7.5. Centric fusion, fission (Robertsonian traslocation)

8. Chromosome numerical mutations

8.1. Aneuploidy (nullisomy, monosomy, polysomy)

8.2. Euploidy (monoploidy, polyploidy – autopolyploidy, allopolyploidy)

8.3. Meiotic pairing problems in numerical mutants and consequences on fertility

9. Karyotype and its evolutionary changes

9.1. Main features of a karyotype

9.2. Karyotype as species characteristics

9.3. Evolution of karyotypes through structural and numerical chromosome changes

9.4. Applications of karyotypes in systematic and phylogenetic studies

Assessment Methods:

Tests ……30 Assignements…… 20%
Final exam 50% Total ……..100%

Reference Materials:

1. Bernand, J. (1976). Population Cytogenetics. Studies in Biology No. 70. Edwa Arnold.
2. Leitch, A. R., Schwarzacher, T., Jackson, D., and Leitch, I. J. (1994). In Sit Hybridization: A Practical Guide. Bios Scientific Publishers. Oxford.
3. Sumner, A. T. (1990). Chromosome Banding. Unwin Hyman. London.
4. Sumner, A. T. (2003). Chromosomes: Organization and Function. Blackwell Publishing Company. Oxford.
5. Swanson, C. P., Merz, T. and Young, W. J. (1967). Cytogenetics. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.
6. Swanson, C. P., Merz, T. and Young, W. J. (1982). The Chromosomes in Division, Inheritance and Evolution. Prentice-Hall of India, Private Limited. New Delhi.

1. Introduction

1.1. Definition of Cytogenetics

- **Cytogenetics** is the branch of genetics that studies the structure of DNA within the cell nucleus. This DNA is condensed during cell division and form chromosomes. The cytogenetic studies the number and morphology of chromosomes. Using chromosome banding techniques (classical cytogenetics) or hybridization fluorescently labeled probes (molecular cytogenetics). The number and morphology of chromosomes in a cell of a particular species are always constant, in most cells of the body (with the exception of reproductive cells and others such as the liver). This is a characteristic of each specie, in humans such as the number of chromosomes is 46. **Cytogenetics** is the term we use to discuss looking at the genetic material through a microscope. Traditionally this was done with a light microscope and looking at chromosomes. In fact, chromosomes were visible to those using microscopes even before we knew that they were made of DNA. In modern times we can use advanced cytogenetics techniques such as fluorescence in situ hybridization (FISH) to look at the genetic material in the cell through a light microscope but at much higher resolution (Amalia S. Dutra).
- **Cytogenetics** refers to both the study of the structure of chromosome material and the study of diseases caused by structural and numerical abnormalities of chromosomes. It includes the routine analysis of G-banded chromosomes, other cytogenetic banding techniques, and molecular cytogenetics such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) (Michael, 2008).
- Cytogenetics is a gross technique for determining the presence of chromosomal loss, gain, or rearrangement (translocation) using direct inspection of chromosomes arrested in metaphase. Maxillofacial Surgery (Third Edition), 2017.
- Chromosomes are the vehicles of genes, which are the functional units of a cell's nucleus. The study of chromosome structure and function is known as cytogenetics which is historically a field of hybrid science encompassing cytology and genetics. The field of cytogenetics has undergone rapid developments over the last several decades from classical Giemsa staining of chromosomes to 3-dimensional spatial organization of chromosomes with a high resolution mapping of gene structure at the nucleotide level. Improved molecular cytogenetic techniques have opened up exciting possibilities for understanding the chromosomal/molecular basis of various human diseases including cancer and tissue degeneration (Balajee, Hande, 2018).

1.2. Historical development of the science of cytogenetics

Chromosomes are the building blocks of a cell's nucleus and they carry the hereditary genetic material in the form of genes that are comprised of deoxyribonucleic acid (DNA). The term "chromosome" originally derived from Greek language which means colour (chromo) body (some) owing to their intense staining with some dyes. In eukaryotes, the diploid number of chromosomes present in somatic

cells varies for different organisms and humans have a diploid number of 46 chromosomes with 22 pairs of autosomes and 1 pair of sex determining chromosomes (X and Y). In contrast, prokaryotes such as bacteria and archaea have a single circular chromosome organized as a nucleoid in the cell. Through a series of pioneering experiments performed since 1880s, Theodor Boveri first provided the evidence that the chromosomes are indeed the vectors of hereditary material. This fascinating finding of Boveri had inspired a number of cytologists and finally both Boveri and Sutton proposed the theory of chromosome inheritance independently in 1902. Ever since the discovery of chromosomes as hereditary vehicles of prokaryotes and eukaryotes, a plethora of techniques evolved over the last several decades to visualize and analyze the chromosome structure and function. A variety of banding techniques that evolved during 1960–1970 including Quinacrine fluorescent staining (Q-banding), centromeric banding (C-band) and iemsa banding (G-band) have been extensively used for karyotyping and for detecting constitutive chromosomal anomalies in clinical laboratories worldwide. In addition to these basic techniques, several molecular cytogenetic techniques involving non-fluorescent and fluorescent in situ hybridisation (FISH) have been developed since the early 1970s. Advancements made in the FISH technology by developing gene or chromosome locus specific DNA probes, whole chromosome specific probes, multicolor FISH for the whole genome and multi-color banding for individual chromosomes have greatly widened the use of FISH in many diverse fields of basic and applied biology: population monitoring of occupational or accidental exposure to ionizing radiation, environmental mutagens and carcinogens, prognosis and diagnosis of human cancers, detection of congenital abnormalities, prenatal diagnosis, retrospective biodosimetry and personalised medicine.

All the historical landmark developments made in the field of cytogenetics are summarised in Table 1. Among several eminent cytogeneticists who contributed to the development and application of cytogenetic techniques over the last several decades, Professor Adayapalam T. Natarajan deserves a special mention for his outstanding contributions on the mechanisms of the origin of chromosomal aberrations induced by ionizing radiation and environmental pollutants. This review summarises the past and current developments of various cytogenetic techniques and their potential prognostic and diagnostic utilities in the field of human diseases including cancer.

Ever Since the proposal of chromosome inheritance theory by Boveri and Suttonin 1902, the field of cytogenetics has undergone tremendous evolution through leaps and bounds through the tireless works of innumerable dedicated scientists over the last several decades. Currently available cytogenetic and molecular cytogenetic techniques have made possible to analyze a wide spectrum of chromosomal anomalies ranging from gene alterations, single nucleotide polymorphism, gene copy number changes, aneuploidy to structural and numerical chromosome alterations for prognosis and diagnosis of various human diseases including cancer. Future developments will certainly improve our understanding of the precise molecular mechanisms for the origin of various structural and numerical chromosomal alterations and how such alterations lead to a wide array of inherited and acquired diseases in humans.

Development of Historic Landmarks in Basic and Molecular Cytogenetics.

1842 Identification of chromosomes by Nageli in pollen mother cells of Tradescantia

1865 Mendelian laws of inheritance

1870 Behavior of meiotic chromosomes visualized by Flemming using an aniline dye

1888 Waldeyer-Hartz coined the term "Chromosomes"
1902-03 Chromosomal theory of inheritance by Boveri-Sutton
1952-59 Preparation of mammalian chromosomes by hypotonic solutions
1956 Correct identification of 46 chromosomes in humans by Tijo and Levan
1958 Detection of chromosomal abnormalities in leukaemia by Ford
1959 Identification of trisomy of one of the smallest chromosomes by Lejeune in Down's syndrome
1960 Detection of various autosomal recessive syndromes marked by numerical chromosome alterations
1960 First conference on International System for Chromosome Nomenclature (ISCN)
1961 Identification of inactive X chromosome by Lyon
1964 Use of pokeweed as a mitogen by Farnes and Barker 1968 Development of Q-banding by Casperson et al.
1969 Development of in situ hybridization using ^{3}H-labelled DNA/RNA probes by Pardue and Gall 1971 Development of centromere specific C-banding by Arrighi and Hsu
1971 Development of G-banding by Sumner et al.
1971 Development of R (Reverse) banding by Dutrillaux and Lejeune
1974 Introduction of sister chromatid exchange technique by Perry and Wolff
1975 Chromosome DNA Replication banding by Latt using Bromodeoxyuridine labelling
1975 Development of Nucleolus Organizer Region (NOR) using silver nitrate by Goodpasture and Bloom
1979 High resolution G-banding development by Yunis
1980 First use of fluorescently labelled DNA probes for in situ hybridisation (FISH) by Bauman, Wiegant, Borst and van Dujin
1981 Use of biotin-labelled probes for FISH initiated by Langer, Waldorp and Ward
1986-88 Pinkel and Gray developed interphase and metaphase FISH for clinical diagnostics
1991 Development of Comparative Genomic Hybridization by Kallioniemi and Kallioniemi
1992 Use of more than four colours in hybridisation – multicolour fluorescence in situ hybridisation (up to 24 colours) – Tanke and Raap group and Riedd and Ward group
1996 Multicolour spectral karyotyping first described by Schrock and Ried
2002 Chromosome conformation capture technique by Dekker et al.

2. Structure and size of chromosomes

2.1 Prokaryotic chromosomes

Prokaryotic cells (bacteria) contain their chromosome as circular DNA. Usually the entire genome is a single circle, but often there are extra circles called plasmids. The DNA is packaged by DNA-binding proteins. The genome of prokaryotes is usually made up of one ''chromosome'' and plasmids. Eukaryota however, contain a larger number of chromosomes - we distinguish two types of eukaryota´s chromosomes (nuclear and mitochondrial) and sometimes even plasmids.

Most of what we know about the chromosomes of prokaryotes have been obtained from studies of E.coli – it is the organism of choice for such research of prokaryotes. Chromosome consists of double–stranded circular DNA. Prokaryotes do not contain nucleus or other membrane bounded organelles. The

term "prokaryotes" actually means "before nucleus". Chromosome is stored in a special area called nucleoid.

The genome of prokaryotes is often significantly larger than the cell itself. How is it possible that the genetic information does fit into the cell? Eukaryota solves this problem by *wrapping DNA* around the histones. However, prokaryotes do not contain histones (with a few exceptions). Prokaryotes to compress their DNA using fiber rolled into small rolls – **supercoiling** (Figure.1). The fibers are twisted so tightly that the final consequences loops overlap to form one big ball. Distinguishes two types of collapse – positive (DNA turns are in the same direction as the helix) or negative (DNA is coiled in the opposite direction than the helix). Most bacteria during normal growth is **negatively coiled**.

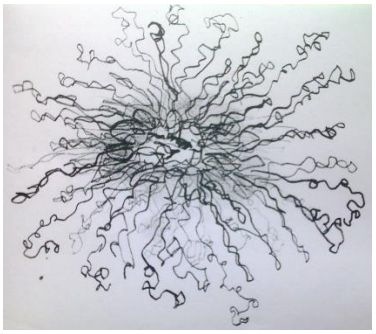


Figure 1. Supercoiling of E.Coli Chromosome

The Prokaryotic Chromosomes

- Most prokaryotes contain one circular chromosome.
- DNA communicates with the cytoplasm – so it allows direct connection to transcription and translation.
- Contain only one copy of the gene (**haploid**).
- Non-essential genes are stored outside of chromosome – in **plasmids**.
- Prokaryotic genome is very compact – contain very little non-coding DNA sequences.

2.2 Eukaryotic chromosomes

Biologists are almost certain that eukaryotes evolved from prokaryotes because:
1. Both use RNA and DNA are the genetic material
2. Both use the same 20 amino acids
3. Both have ribosomes and DNA and RNA
4. Both have a lipid bilayer cell membrane.
5. Both use L amino acids and D sugars

Biologists are also almost certain that eukaryotes evolved only once (i.e., are monophyletic-descendants of a single common ancestor) because they all share:
1. microtubules (composed of the protein tubulin) and actin molecules-cytoskeleton for support or intracellular transport.-
flagella (or cilia)

2. DNA in chromosomes (intertwined with histone protein)
3. membrane-bound organelles.
The eukaryotic chromosomes are more complicated than procaryotic. They encode more information (coding and non-coding parts) so we recognize bigger count of chromosomes in Eukaryota. Their count depends on the evolution of the species. But they structure is pretty similar.

DNA contains milions and milions of nucleotides and it is neccesary to compress them. In eukyryotic chromosomes we recognize a process similar to supercoiling in Prokaryota. This is made possible by special proteins – histones - which packed the DNA strand. Complex of DNA and these proteins is called chromatin.

DNA is turning around the histones. Sometimes it is said that it looks like the beads on a string. This is a principle, which allows to get so long DNA strand (human DNA has more than 2 metres) into the cell and chromosomes.
Compact chromosome has 3 main parts. Centromere and two arms:
Centromere: is a middle part that connects both arms together. It is very important also during cell division because it is the place where kinetochor is binding. It is a very important complex which binds a mitotic spindle. All of this serve to the separation of chromosomes.
Short and long arms

- short arm of the chromosome is also marked by letter (p), according to French "petit" (means small)
- long arm of the chromosome is marked by (q) – according to the letter which follow (p)
- the terminal parts of arms have their own name – they are called telomeres

Important morphological features of eukaryotic chromosomes

Size: The size of chromosome is normally measured at mitotic metaphase and may be as short as 0.25µm in fungi and birds to as long as 30 µm in some plants such as Trillium. However, most mitotic chromosome falls in the range of 3µm in Drosophila to 5µm in man and 8-12µm in maize. The monocots contain large sized chromosomes as compared to dicots. Organisms with less number of chromosomes contain comparatively large sized chromosomes. The chromosomes in set vary in size.
Shape: The shape of the chromosome changes from phase to phase in the continuous process of cell growth and cell division. During the resting/interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread like stainable structures, the chromatin threads. In the metaphase and the anaphase, the chromosome becomes thick and filamentous.
The outer covering or sheath of a chromosome is known as pellicle, which encloses the matrix. Within the matrix lies the chromatin. • Flemming introduced the term chromatin in 1879. The chromosome morphology changes during cell division and mitotic metaphase is the most suitable stage for studies on chromosome morphology. In mitotic metaphase chromosomes, the following structural features can be seen under the light microscope.
1. Chromatid

2. Centromere
3. Telomere
4. Secondary constriction
5. Chromomere
6. Chromonema
7. Matrix

Chromatid

• Each metaphase chromosome appears to be longitudinally divided into two identical parts each of which is called chromatid.

• Chromatids of a chromosome appear to be joined together at a point known as centromere.

• Two chromatids making up a chromosome are referred to as sister chromatids.

• The chromatids of homologous chromosomes are known as nonsister chromatids.

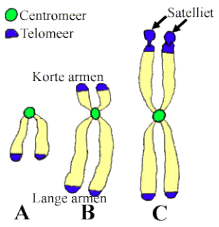


Fig. Structure of chromosomes (centromere and telomere)

Centromere:

• The region where two sister chromatids appear to be joined during mitotic metaphase is known as centromere

• It generally appears as constriction and hence called primary constriction.

• helps in the movement of the chromosomes to opposite poles during anaphase of cell division.

• The centromere consists of two disk shaped bodies called kinetochores.

• Normally chromosomes are monocentric having one centromere each.

Depending on position of the centromeres, chromosomes can be grouped as:

a) Metacentric: Centromere is located exactly at the centre of chromosome, Such chromosomes assume 'V' shape at anaphase.

b) Submetacentric: The centromere is located on one side of the centre point such that one arm is longer than the other. These chromosomes become 'J' or 'L' shaped at anaphase.

c) Acrocentric: Centromere is located close to one end of the chromosome and thus giving a very short arm and a very long arm. These chromosomes acquire ' J' shape or rod shape during anaphase.

d) Telocentric: Centromere is located at one end of the chromosome so that the chromosome has only one arm. These chromosomes are 'I" shaped or rod shaped.

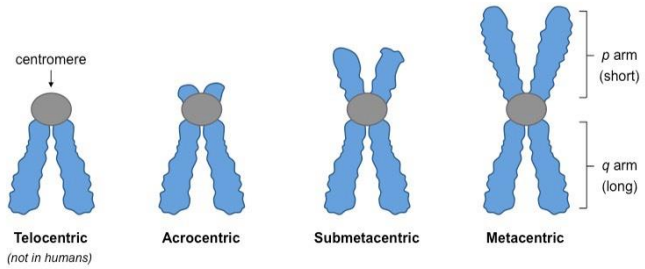


Fig. Types of chromosomes based on the position of centromere

Telomere

• The two ends of chromosomes are known as telomeres

They are highly stable and do not fuse or unite with telomeres of other chromosomes due to polarity effect.

• Any broken end of a chromosome is unstable and can join with a piece of any other chromosome.

• But the telomeres impart stability to the chromosome, which retains its identity and individuality through cell cycle and for many cell generations.

Secondary constriction

• The constricted or narrow region other than that of centromere is called secondary constriction.

• The chromosomes having secondary constriction are known as satellite chromosomes or sat chromosomes.

• Chromosome may possess secondary constriction in one or both arms of it.

• Chromosomal end distal to the secondary constriction is known as satellite.

• Production of nucleolus is associated with secondary constriction and therefore it is also called nucleolus organizer region.

• Satellite chromosomes are often referred to as nucleolus organizer chromosomes.

Chromomere

• In some species like maize, rye etc. chromosomes in pachytene stage of meiosis show small bead like structures called chromomeres.

• The distribution of chromomeres in chromosomes is highly characteristic and constant.

• The pattern of distribution being different for different chromosomes.

• They are clearly visible as dark staining bands in the giant salivary gland chromosomes.

• Chromomeres are regions of tightly folded DNA.

Chromonema

- A chromosome consists of two chromatids and each chromatid consists of thread like coiled structures called chromonema (plural chromonemata).
- The term chromonema was coined by Vejdovsky in 1912.
- The chromonemata form the gene bearing portion of chromosomes.

Matrix

• The mass of acromatic material which surrounds the chromonemata is called matrix.

• The matrix is enclosed in a sheath which is known as pellicle.

• Both matrix and pellicle are non-genetic materials and appear only at metaphase, when the nucleolus disappears.

a) Structure of Chromosome

b) Simplified structure of Chromosome

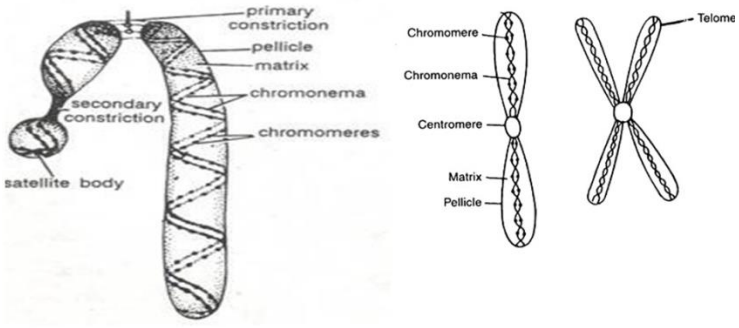


Fig. Structure of Chromosomes

Composition of chromosomes

The material of which chromosomes are composed is called chromatin.

- N.Fleming introduced the term chromatin in 1879.
- Chromatin was classified into two groups by cytologists on the basis of its affinity to basic dyes like acetocarmine or feulgen (basic fuchsin) reagent at prophase.
- The darkly stained regions were called **heterochromatin**, while lightly stained regions were called **euchromatin.**
- This differential staining capacity of different parts of chromosomes is known as 'heteropycnosis'
- Heterochromatin is further classified into two groups:
- a) Constitutive :- It is present in all cells at identical positions on both homologous chromosomes of a pair.
- b)Facultative:- It varies in state in different cell types, at different stages or sometimes, from one homologous chromosome to another.

Major molecular components of eukaryotic chromosomes

The components of eukaryotic chromosome are DNA, RNA, histone, and non-histone protein, metallic ions, etc. The DNA is the most stable molecule carrying out transfer of genetic information from one generation to the other.

RNA is transcribed over it. Most of m-RNA is transported to the cytoplasm where it is translated in terms of proteins. Some mRNA remains in the coil associated with the protein.

Proteins:

Two types of proteins, i.e., acidic and basic proteins are found associated with DNA in chromosome.

Protamines:

These are simple proteins of molecular weight less than 4000 Daltons. Rich in basic amino acid arginine, found in spermatozoa of some fish (salmon), snail, fowl, etc. They are helically rapped around DNA. Polypeptides of protamine consists of 28 amino acid residue (19 arginines, 8-9 non-basic amino acids).

Histones:

Small basic proteins associated with DNA of eukaryotic cells. They are structural proteins of chromatin and act as gene repressors also. In a wide variety of plants and animals, the ratio of DNA: Histone =1:1. There are 5 types of Histones i.e., HI (H5), H2A, H2B, H3 and H4. Histones are rich in arginine and lysine (basic amino acids). They lack tryptophan. Histones are highly modified as well as conserved proteins; with very little difference in amino acid sequences. On the basis of arginine and lysine contents, they are divided into 3 groups.

(i) High lysine rich — HI
(ii) Lysine rich — H2A, H2B
(iii) Arginine rich – H3, H4

Histones tend to depress genetic activity. They make DNA. They are structurally important in packaging DNA molecules.

Non-Histone proteins (NHP):

They have structural, enzymatic and regulatory function in chromatin. They are generally acidic proteins. The acidic to basic residues ratio in these proteins is 1.2—1.6.

The molecular weight ranges from 11000-21,500 Daltons. They may show structural variation in different species and even tissues. These proteins are synthesised throughout the cell cycle. These proteins stimulate genetic activity.

Enzymes of chromosomal metabolism like nucleic acid polymerase, nucleases, DNA pyrophosphorylases and nucleoside triphosphatases, etc., are non-histone proteins. Such proteins play important role in the interaction of steroid hormones with target cell nuclei. The chromosomes also contain metallic ions like Mg++ and Ca++, etc.

DNA Packaging in chromosome

When comparing prokaryotic cells to eukaryotic cells, prokaryotes are much simpler than eukaryotes in many of their features (Figure 1). Most prokaryotes contain a single, circular chromosome that is found in an area of the cytoplasm called the nucleoid.

The size of the genome in one of the most well-studied prokaryotes, E.coli, is 4.6 million base pairs (approximately 1.1 mm, if cut and stretched out). So how does this fit inside a small bacterial cell? The DNA is twisted by what is known as supercoiling. Supercoiling means that DNA is either under-wound (less than one turn of the helix per 10 base pairs) or over-wound (more than 1 turn per 10 base pairs) from its normal relaxed state. Some proteins are known to be involved in the supercoiling; other proteins and enzymes such as DNA gyrase help in maintaining the supercoiled structure.

Eukaryotes, whose chromosomes each consist of a linear DNA molecule, employ a different type of packing strategy to fit their DNA inside the nucleus (Figure 2). At the most basic level, DNA is wrapped

around proteins known as histones to form structures called nucleosomes. The histones are evolutionarily conserved proteins that are rich in basic amino acids and form an octamer. The DNA (which is negatively charged because of the phosphate groups) is wrapped tightly around the histone core. This nucleosome is linked to the next one with the help of a linker DNA. This is also known as the "beads on a string" structure. This is further compacted into a 30 nm fiber, which is the diameter of the structure. At the metaphase stage, the chromosomes are at their most compact, are approximately 700 nm in width, and are found in association with scaffold proteins.

In interphase, eukaryotic chromosomes have two distinct regions that can be distinguished by staining. The tightly packaged region is known as heterochromatin, and the less dense region is known as euchromatin. **Heterochromatin** usually contains genes that are not expressed, and is found in the regions of the centromere and telomeres. **The euchromatin** usually contains genes that are transcribed, with DNA packaged around nucleosomes but not further compacted

Genetic Significance of Chromosomes

The chromosomes are considered as the organs of heredity because of following reasons:

(i) They form the only link between two generations.

(ii) A diploid chromosome set consists of two morphologically similar (except the X and Y sex chromosomes) sets, one is derived from the mother and another from the father at fertilization.

(iii) The genetic material, DNA or RNA is localized in the chromosome and its contents are relatively constant from one generation to the next.

(iv) The chromosomes maintain and replicate the genetic information contained in their DNA molecule and this information is transcribed at the right time in proper sequence into the specific types of RNA molecules which directs the synthesis of different types of proteins to form a body form like the parents.

3. Chromosomes in cell division

3.1. Mitosis

3.1.1. Significance of mitosis

An important significance of mitosis is that the chromosomes of all the produced cell are identical to the mother cell. Chromosome number and their characters are just the same of the mother cell. Due to increase in mitotic cell division the organism achieves growth. Mitosis is very important phenomina for vegetative propagation.

The main purpose of mitosis in eukaryotic cells is:

- ✓ Growth of the individual,
- ✓ To repair tissue, and
- ✓ To reproduce asexually.

3.1.2. Events of the cell cycle (phases in the cell cycle)

Growth is any living organism is because of increase in number of cells without change in its characters. Such growth starts from zygote. Nucleus and cytoplasm of the zygote divide and forms two cells. In the same way each cell again divides-so the two cells forms four cells. This process goes on till the growth of the organism is not complete. This type of cell division is called mitosis. Due to mitosis in all the cells number and characters of chromosomes are identical to the mother cell. In mitosis cell division nucleus undergoes several changes. Scientists have divided the changes in to four stages such as prophase, metaphase, anaphase and telophase. Between mitotic cell division there is resting stage or also known as interphase (Fig-). All the stages are described below:

1. Interphase or Resting stage: Chromosomes appear as interwoven thin threads. Nucleolus and chromosomes are almost dark stained

2. Prophase: At prophase nucleus enlarges, chromosomes appear clearer. Chromosome becomes shorter and thicker. From beginning of prophase all chromosomes split into two. Each part is called chromatid. The chromatids are interwoven in full length. This interwovenship is known as relational coiling. Chromosomes are seen scattered in the nucleus. Nuclear membrane gradually starts disappearance.

3. Metaphase: Complete disappearance of nuclear membrane is the beginning of metaphase. Formation of spindle takes place. Cytologists differ on origin and number of spindle. The spindles arrange all chromosomes in equator.

Chromatids of each chromosome are together and through the centromere is attached to the spindle. The Centromere remains undivided. If somehow it gets split even then this acts as unit only. In mitosis cell division nucleus undergoes several changes. Scientists have divided the ,change into four stages such as prophase, metaphase, anaphase and telophase. Between mitotic cell division there is resting stage or also known as interphase . All the stages are described below: Chromatids of each chromosome are together and the centromeres are attached to the spindle. The centromere remains undivided, if somehow it gets split, even then this acts as one unit only.

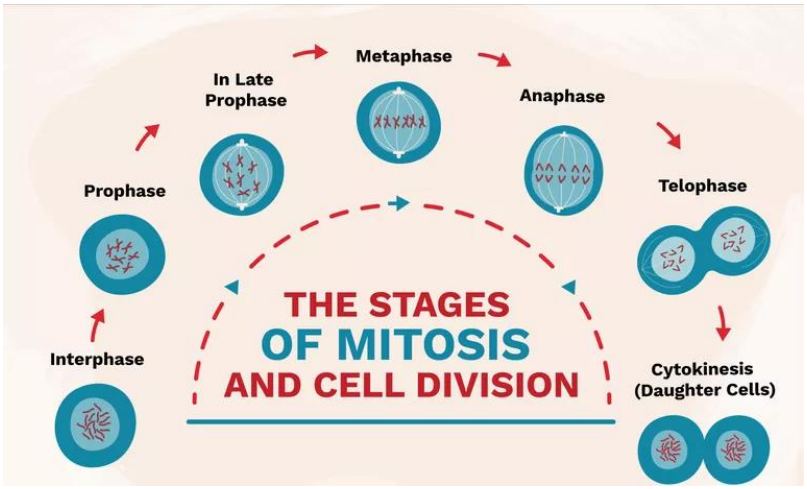


Figure. Various stages of mitosis

4. Anaphase: At this stage centromere divide into two centromeres, these have repulsive action. Due to this repulsion both the chromatids move towards poles. In absence of centromere the chromatid is unable to move and remain at the equator.

5. Telophase: At this stage all the chromatids reach to respective poles. New nucleus are formed at both the poles, in nucleus (plural nuclei) nucleolus is developed. Spindle fibres disappear. Chromosomes loose the capacity to get stained.

6. Cytokinesis (Division of cytoplasm): Cytokinesis starts forn cell plate formation. In the end of telophase in the middle of cytoplasm spindle fibers make fragmoplasts. Several fragmoplasts join and fonn a plate. Length of this plate increases and joins with cell wall. The plate is called cell plate. Over this cell plate pectin deposition starts. Afterwards this cell plate is known as middle lamella. From both side of middle lamella secretion of some compounds (ploysacharide) takes place. Afterwards this wall is called primary wall. Again deposition of cellulose, hemicellulose and polysacharide take place. Afterwards the wall is called secondary wall. This is the end of cylokinesis and the cell gets divided into two cells. In animals cytoikenesis take place. in different way. It takes place by furrowing of the cytoplasm.

3.2 Meiosis

•Meiosis is the type of cell division by which germ cells (eggs and sperm) are produced.

•Meiosis is the reduction division. It involves a replication of the genetic material followed by two successive nuclear divisions resulting in four daughter nuclei with a haploid set of genome or chromosomes.

•This process of cell division occurs in reproductive organs and is essential for the production of gametes.

- ✓ During meiosis, DNA replicates once, but the nucleus divides twice.
- ✓ Meiosis is divided into two parts: meiosis I and meiosis II**.**

3.2.1. Significance of meiosis

1. Meiosis helps in continuation of chromosome number in organismS: Due to meiosis the chromosome number gets reduced to half in gametes. when male and female gametes (with half number of chromosomes) unite and fertilize then the zygote gets the complete number of chromosomes. In this way the chromosome number remain constant
2. Helps to know breeding behaviour of organism. Investigations of meiosis helps to understand breeding behaviour of the organism.

3.2.2. Phases of meiosis

Meiosis cell division is also known as reduction division and heterotypic cell division. Gamete formations are due to meiosis only. Meiotic cell division results in reduction of chromosome number to half.

Meiosis occurs in two phases ie. two times prophase and two times metaphase, two times anaphase and two times telophase. Therefore stages of first meiosis are called prophase-1 metaphase-1, anaphase-1 and telophase-1.With telophase-1 first meiotic cell division completes. The second meiotic cell division starts. The stages are prophase-II, metaphase-II, anaphase-II and telophase -11. With telophase-II the nucleus divided two times and the meiosis (Fig-S) finishes. Meiosis is discussed in detail below.

First meiosis: 1. Prophase-1: During prophase-1 various changes in chromosomes can be described in five stages as given below.

a. Leptotene or Leptonema: Cell size increases, chromosomes are thin and long. Presence of chromomere is clear.

b. Zygotene or Zygonema: At this stage pairing or synopsis in chromosomes takes place. Every living organism has one haploid chromosome set from father (sperm or pollen) and one haploid chromosome set from mother (egg). Chromosomes obtained from father matches from chromosomes obtained from mother except to sex chromosome. Therefore in every diploid cell there are pairs of chromosomes (resembling to each other) obtained from father and mother, are called homologous chromosomes. At zygotene stage of meiosis these homologous chromosomes come together and form pairs. This process is called synopsis or pairing. In this process centromeres of homologous chromosomes come opposite to each other. Pairs of homologous chromosomes are called bivalents. Therefore if number of chromosomes is 2 at leptotene then at zygotene it appears as one bivalent.

c. Pachytene or Pachynema: At pachytene every chromosome of bivalent, splits longitudinally into two chromatids. But the chromatids are united at centromere. Chromatids do not split. Therefore each bivalent appears to have four strands at pachytene. Nucleolus is visible at'this stage. d. Diplotene or Diplonema: At diplotene stage homologous chromosome start movement from each other. But the homologous chromosomes are not completely separated i.e. those are connected at one or more places. If connected at one place then the bivalent appears to be a cross. If connected at two places then bivalent has one loop. If connected at more places then the bivalent has more loops. Each place of connection in a bivalent is called chiasma (plural chiasmata). Number of chiasma and its location

depends on length of chromosome and the type of species. Even the smallest , bivalent has one chiasma. Depending on location chiasm is of two types.

(i) Terminal chiasma: It is located at the end of chromosome.

(ii) Interstitial chiasma: Instead at end, the chiasma is located anywhere else on the chromosome. With the separation of homologous chromosomes interstitial chiasmata can get converted into terminal chiasma. The process is Simple. Due to movement in centromeres the homologous chromosomes moves apart. So interstitial chiasma moves towards end of the chromosome. Thus ultimately it becomes terminal chiasma. This movement of chiasma is known as terminalization. At diplotene stage length of chromosomes gets reduced but their thickness increases. e. Diakinesis: At diakinesis chromosome appears short and thick, Nucleolus starts disappearance and completely disappears by the and of diakinesis. Most of bivalents specialy smaller ones because of terminalization assumes nail or similar shapes,

2. Metaphase: Like metaphase stage of mitosis, in meiosis also at metaphase -1 the nuclear membrane disappears. Spindle fibres appears. Some cytologists give the name prometaphase to the phases between disappearance of nuclear membrane and formation of spindle. All bivalents come on the equator. There is a difference between metaphase of mitosis and metaphase-1 of meiosis During metaphase of mitosis every chromosome has a divided centromere and all the chromosomes lie on equator. Where as in metaphase-1 of meiosis each bivalent has two undivided centromers and bivalents do not just lie on equator but their centromeres appears to be diverted towards poles.

3. Anaphase-l: At ananphase-1 of meiosis bivalents do not have divided centromere, due to which homologous chromosomes along with two chromatids moves towards their poles in contrast to mitotic anaphase where centromeres are divided and chromatids goes towards poles The result is that at end of anaphase-1 on the poles there is only haploid number of chromosome, In other words only half the number of chromosomes at the poles.

4. Telophase-l: Telophase -1 is the last stage of first phase of meiosis. It is for a short time only. Nuclear membrane appears. Spindle fibers disappear. These nuclei are called dyad. Dyad contains only half the number of chromosome in the organism. In certain organism such as Trillium the chromosomes after reaching the poles without centering telophase1 attains second phase of meiosis.

Second Meiosis: All stage of second meiosis is similar to mitosis except the differences mentioned below. a. In comparisom to mitosis, second meiosis has only half the number of chromosomes. b. Chromatids remain at some distance from each other. Hence there is no relational coiling. c. In mitosis there is no change in the genetic sequence, whereas during first meiosis due to chiasma (plural chiasmata) there is a change in genetic sequence at second meiosis. The degree of changes depend on number of chiasma formed. Stages of second meiosis are mentioned below:

a. **Prophase-II:** It is a small stage. Nuclear membrane appears and chromosomes appear as a net work.

b. **Metaphase-II**: At this stage nuclear membrane disappears. Spindle fibres appear. Chromosomes come on the equator and their centromers get connected with the spindle fibres.
c. **Anaphase-II:** Centromers get divided. Both the chromatids of chromosomes start movement towards respective poles.
d. **Telophase-II:** At this stage chromatids reach to the respective poles and form net like structure there. Nuclear membrane gradually appears. Cell plate forms and due to this four cells are formed. Each cell has a nucleus. Number of chromosomes is only half (n) in all the four nuclei.

Cytokinesis in meiotic cells:

Cytokinesis (division of cytoplasm) mechanism varies in different organism. In some organism during first meiotic division a wall is formed in middle of cell division, dividing the cell into two cells. In second meiotic division another wall get made at right angle to the first wall. In this way the meiotic cell gives rise to four cells. In some other organism during first meiotic division cytokineSis do not take place. In the end of second meiotic division two walls get made in the cell dividing it into four cells. Each cell have only half the number of chromosomes.

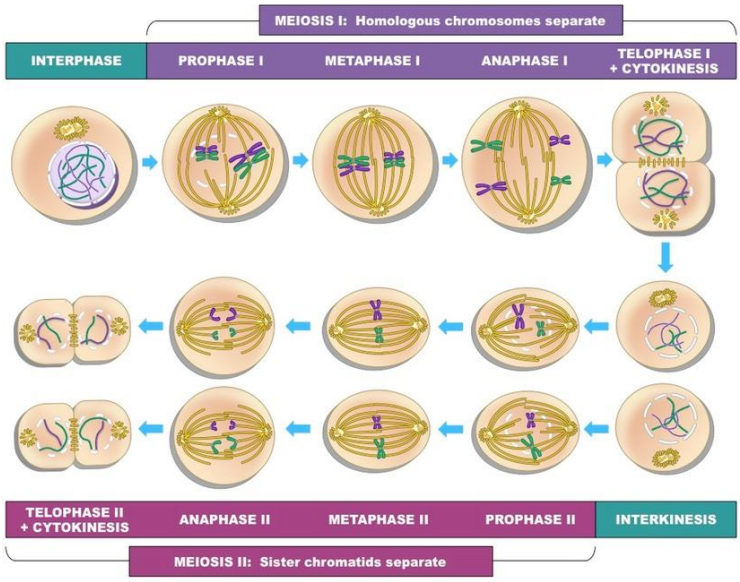


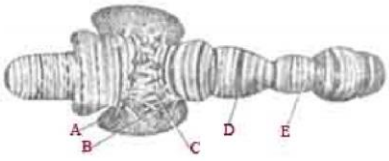
Fig. overview of stages of meiosis

4. Special Types of Chromosomes

The eukaryotes besides possessing the usual type of chromosomes in their body cells, contain some unusual and special types of chromosomes in some body cells or at some particular stage of their life cycle. The special type of eukaryotic chromosomes are following:

4.1. Polytene chromosomes

The nuclei of the salivary gland cells of the larvae of dipterans like Drosophila have unusually long and wide chromosomes, 100 or 200 times in size of the chromosomes in meiosis and mitosis of the same species. This is particularly surprising, since the salivary gland cells do not divide after the glands are formed, yet their chromosomes replicate several times (a process called endomitosis) and become exceptionally giant-sized to be called polytene or multistranded chromosomes (discovered by Balbiani (l881) and named by Koller).The endomitosis process result in the production of 2X chromosomes, where X gives the number of multiplication cycle. The polytene Chromosomes of the salivary gland cells of D. melanogaster contain 1000 to 2000 chromosomes, which are formed by nine or ten consecutive multiplication cycles and remain associated parallel to each other. Further, the polytene chromosomes have alternating dark and light bands along their length. The dark bands are comparable with the chromomeres of a simple chromosomes and are disc-shaped structures occupying the whole diameter of chromosome. They contain euchromatin. The light bands or inter bands are fibrillar and composed of heterochromatin.

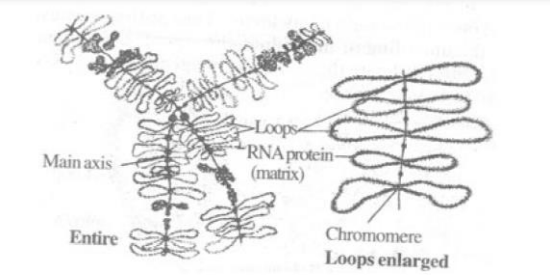


A. mRNA
B. Chromosome puff
C. Chromonemata
D. Dark band
E. Inter band

If the polytene chromosomes of dipteran larval salivary glands are examined at several stages of development; it is seen that specific areas (sets of bands) enlarge or "puff". Such puffs change location as development proceed, those at specific locations being correlated with particular developmental stages. This temporal puffing indicates changes in gene activity and involves several processes such as the accumulation of acidic proteins, despiralization of DNA, formation of chromonemal loops called Balbiani rings at the lateral sides of dark bands, synthesis of mRNA (messenger RNA) and storage (accumulation) of newly synthesized mRNA around the Balbiani rings.

4.2. Lampbrush chromosomes

In diplotene stage of meiosis, the yolk rich oocytes of vertebrates contain the nuclei with many lamp brush shaped chromosomes of exceptionally large sizes. The lampbrush chromosomes (discovered by Ruckert in 1892) are formed during the active synthesis of mRNA molecules for the future use by the egg during cleavage when no synthesis of mRNA molecules is possible due to active involvement of chromosomes in the mitotic cell division.



A lampbrush chromosome contains a main axis whose chromonemal fibres (DNA molecule) gives out lateral loops throughout its length. The loops produce the mRNA molecules of different kinds. In a mature egg, as the chromosome , contracts the lateral loops disappear.

4.3. B-chromosomes

Many plant (maize, etc.) and animal (such as insects and small mammals) species, besides having autosomes (A-chromosomes) and sex-chromosomes possess a special category of chromosomes called B-chromosomes without obvious genetic function. These Bchromosomes (also called supernumerary chromosomes, accessory chromosomes, accessory fragments, etc.) usually have a normal structure, are somewhat smaller than the autosomes and can be predominantly, heterochromatic (many insects, maize, etc.) or pro-dominantly euchromatic (rye). In maize, their number per cell can vary from 0 to 30 and they adversely affect, development and fertility only when occur, in large amount. In animals, the B-chromosomes disappear from the non-reproductive (somatic) tissue and are maintained only in the cell-lines that lead to the reproductive organs. B-chromosomes have negative consequences for the organism, as they have deleterious effect because of abnormal crossing over during the meiosis of animals and abnormal nucleus divisions of the gametoophyte plants. In animals, Bchromosomes occur more frequently in females and the basis is non-disjunction. The nondisjunction of B-chromosomes of rye plant is found to be caused due to the presence of a heterochromatic knob at the end of long arm of B-chromosome.

A. Maize **B. Rye**

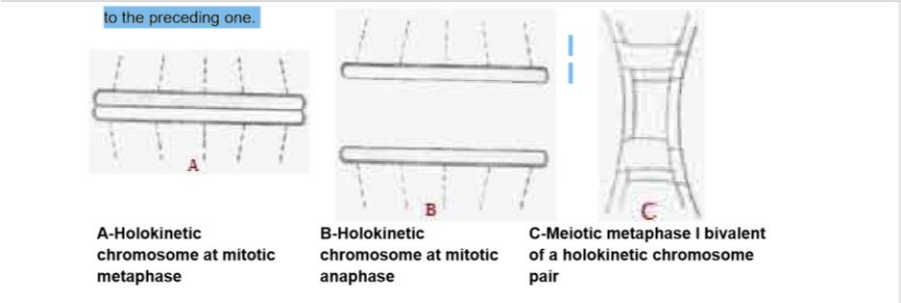
Centromere

The origin of the B-chromosomes is uncertain. In some animals they may be derivatives of sex chromosomes, but this is not the rule. They generally do not show any pairing affinity with the' A-chromosomes.

4.3. Holokinetic chromosomes

The chromosomes of most plants and animals have centromeres that are situated at one specific position in each chromosome. In a number of animals, especially in insects of the order Hemiptera and a few, mostly monocotyledonous plants (Juncales, Cyperales), the kinetic activity is distributed over the entire chromosome and such chromosomes are called Holokinetic chromosomes (Sybenga, 1972). The term -diffuse centromere bas been used as an alternative but is not quite logical. In 1966, Flach observed this type of centromere in some primitive DicotyledonS (Ranales: Myristica.Ascaris and pseudoscorpion Tityus also possess such polycentric chromosomes.

In mitotic metaphase, the chromatids of a Holokinetic chromosome orient parallel in the equator: one chromatid towards one pole the other towards the other pole. This is also the way they separate in anaphase and they maintain this orientation until they arrive at the poles. Probably kinetic activity starts at one point and proceed from there on, orienting each unit to the preceding one.



A-Holokinetic chromosome at mitotic metaphase

B-Holokinetic chromosome at mitotic anaphase

C-Meiotic metaphase I bivalent of a holokinetic chromosome pair

5. Major types of chromosome banding

Definition and History

Chromosome banding is the “lengthwise variation in staining properties along a chromosome normally independent of any immediately obvious structural variation,” and thus excludes patterns such as those seen on polytene chromosomes of Drosophila, which have a morphological component. Although the first observations of what could be called chromosome banding were made at the end of the nineteenth century, modern chromosome banding methods date from 1968 and can be applied to chromosomes of a wide variety of species with no more than slight modifications. Following the introduction of Q-banding by Caspersson and his colleagues in 1968, Pardue and Gall inadvertently produced differential staining of heterochromatin in their pioneering in situ hybridization studies, leading directly to C-banding, and in 1971 G-banding was discovered by several authors. R-banding was also introduced in 1971. Over the next few years, many other banding techniques, too numerous to mention individually, were introduced, many of them using fluorochromes. Silver staining for nucleolus organizing regions(NORs) was introduced in 1975, methods to show chromosome replication were invented, and the use of autoimmune sera to label kinetochores immunocytochemically was discovered. **Banding Patterns**

Chromosomes in metaphase can be identified using certain staining techniques, so called banding. Cells are cultured and then stopped in metaphase to maximize the number of suitable cells. They are then

spread on a slide, stained with a suitable dye and visualized in the microscope. Most conventional cytogenetic analyses depend on the karyotyping of banded metaphase chromosomes.

A band is defined as that part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or brighter with one or more banding techniques. The chromosomes are visualized as consisting of a continuous series of bright and dark bands.

The banding techniques fall into two principal groups:
1) those resulting in bands distributed along the **length of the whole chromosome**, such as G-, Q- and R-bands and
2) those that **stain a restricted number of specific bands or structures.** These latter include methods which reveal centromeric bands, C-bands, and nucleolus organizer regions, NOR's (at terminal regions of acrocentric chromosomes). C-banding methods do not permit identification of every chromosome in the somatic cell complement, but can be used to identify specific chromosomes.

G-banding, G banding, or Giemsa banding:
is a technique used in cytogenetics to produce a visible karyotype by staining condensed chromosomes. It is useful for identifying genetic diseases through the photographic representation of the entire chromosome complement. The metaphase chromosomes are treated with trypsin (to partially digest the chromosome) and stained with Giemsa stain. Heterochromatic regions, which tend to be rich with adenine and thymine (AT-rich) DNA and relatively gene-poor, stain more darkly in G-banding. In contrast, less condensed chromatin (Euchromatin)—which tends to be rich with guanine and cytosine (GC-rich) and more transcriptionally active—incorporates less Giemsa stain, and these regions appear as light bands in G-banding. The pattern of bands are numbered on each arm of the chromosome from the centromere to the telomere.This numbering system allows any band on the chromosome to be identified and described precisely. The reverse of G bands is obtained in R banding. Banding can be used to identify chromosomal abnormalities, such as translocations, because there is a unique pattern of light and dark bands for each chromosome.

It is difficult to identify and group chromosomes based on simple staining because the uniform colour of the structures makes it difficult to differentiate between the different chromosomes. Therefore, techniques like G banding were developed that made "bands" appear on the chromosomes. These bands were the same in appearance on the homologous chromosomes, thus, identification became easier and more accurate. The less condensed the chromosomes are, the more bands appear when G-banding. This means that the different chromosomes are more distinct in prophase than they are in metaphase

R-Banding (reverse Giemsa staining)
These R-bands are approximately the reverse of G-bands (the R stands for "reverse"). The dark regions are euchromatic and the bright regions are heterochromatic.

Q-bands (quinacrine) Banding :

The method of Q-banding was developed by Caspersson et al. in 1968. The chromosomes stained with Quinacrine mustard show bright and dark zones under UV light. This technique is used to identify human and mice chromosomes.

C-Bands:

The technique of C-banding originated after the work of Pardue and Gall who reported that constitutive heterochromatin can be stained specifically by Giemsa-solution. Each chromosome possesses a different degree of constitutive heterochromatin which enables the identification of individual chromosomes.

Constitutive heterochromatin is located near the centromere, at telomeres and in the nucleolar organizer regions; it is composed of highly repetitive DNA. C-banding represents the constitutive heterochromatin, and the banding is caused by differential staining reactions of the DNA of heterochromatin and euchromatin. The banding method is a complex technique that involves several treatments with acid, alkali or increased temperature. Denaturation of DNA is caused by these treatments. Subsequently, DNA renaturation occurs in treatments with sodium-citrate at 60°C.

By these treatments, the repetitive DNA (heterochromatin) re-natures but low repetitive and unique DNAs do not re-nature. This results in differential staining of the specific chromosome regions. Giemsa-C-banding technique has been used to identify chromosomes of various plant and animal species including human. The Y chromosome of mammals is mostly heterochromatic and therefore, the technique of C-banding is quite useful for its identification.

In barley chromosomes, Linde-Laursen in 1978, divided the C-bands into the following classes based on their position:

(i) Centromeric bands situated at one or both sides of the centromere,
(ii) Intercalary bands,
(iii) Telomeric bands and
(iv) Bands beside the secondary constriction in the short arm of satellited chromosomes.

He observed polymorphism in C-banding pattern in different barley lines, Giemsa-C-banding patterns may also be used to identify the extra-chromosomes of trisomies and telotrisomics.