BASIC GENETICS

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INTRODUCTION

Dear Student,

In the last few years remarkable achievements in the field of human genome project and gene therapy have made the basic subjects of genetics an important part in the advanced medical curriculum of many medical schools. The genetic information is important *not only* in understanding and analyzing the molecular basis of genetic diseases, *but also* for selective breeding of plants and animals.

This work is intended to explain the essential information about the different subjects of basic genetics and gene expression, such as cell division, principles of genetics, structure of hereditary material, gene expression and control, genetic engineering, and human genetics. Also, this book presents the required information in field of genetics for building the basic knowledge of students in the preparatory year of medical sciences and medicine.

This book includes a lot of simple illustrations which will help you to understand the text. In addition, a lot of multiple choice questions have been added at the end of every chapter to enable you to test yourself.

Generally, this book is prepared not only to students who will study the basic medical genetics, but also to students of different branches of medical sciences. We hope this book will be useful and interesting for you while studying the course of basic genetics.

With best wishes,

The Authors

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CELL DIVISION

Cell Cycle, Mitosis and Meiosis

Chapter 1:

CELL DIVISION

Cell Cycle, Mitosis and Meiosis

Objectives

After studying a cell cycle, mitosis and meiosis you should be able to:

- *Differentiate between the genetic material, chromatin, chromosome and gene.*
- *Explain the stages of eukaryotic cell cycle and how the cell cycle is regulated.*
- *Define homologous chromosomes and distinguish between haploid and diploid cells.*
- *Describe the phases of cell divisions and explain the significance of meiosis.*
- *Explain how synapses and crossing over cause genetic variations.*
- *Explain the differences between the phases of mitosis and meiosis*

Introduction

On the basis of presence or absence of nuclei, living organisms are classified into: **Prokaryotes** and **eukaryotes**. Prokaryotes are characterized by absence of membrane – bound nuclei, while eukaryotes contain membrane bound nuclei. The cells are formed of two separate cytoplasmic and nuclear compartments. Genetic material or *chromatin* within the nucleus is separated from cytoplasmic components by nuclear envelope.

The chromatin is a darkly stained material that is formed of highly folded chromatin threads. It is a complex structure formed of about 60% protein (*histones*), 35% deoxyribonucleic acid (*DNA*) and 5% ribonucleic acid (*RNA*).

When a cell begins division, the chromatin fibers, which are highly folded, condense to form *chromosomes which* are different in number and their informational content from one species to another.

Chromatin threads are formed of complexes in the form of bead–like structures which are called *nucleosomes. Each nucleosome is* composed of a core of globular basic proteins of histone type and surrounded by DNA strand *(see chapter 3)*. DNA carries genetic information which is organized in the form of units called *genes.* Each gene is an informational unit that affects some characteristics of an organism such as color of eyes and hair.

The genetic material is composed of pairs of chromosomes of the same length which are called **homologous chromosomes**. One of each pair is inherited from the mother, and the other from the father.

The inherited chromosomes from mother (maternal) form set (*1n*) and that inherited from father (paternal) form homologous set (*1n*). Therefore each cell contains two sets (*2n*) and called *diploid cell*; one is maternal and the other is paternal set.

Each pair of homologous chromosomes carries genes for the same biological features. They carry similar but not identical genetic information. For example, in a carrier person for sickle cell anemia, if a gene of abnormal RBCs is inherited from a diseased mother and located on the maternal chromosome, a gene of normal RBCs is inherited from a normal father and

located on paternal chromosome. So, maternal and paternal chromosomes carry genes controlling the RBCs formation but the genetic information are different; one is responsible for normal RBCs and the other codes for RBCs of sickle cell anemia.

The chromosomes are classified into: Sex chromosomes; X and Y (XY in male and XX in female) and autosomes which are 22 pairs of chromosomes representing all other chromosomes in human being.

Cell Cycle

The cell cycle is defined as the period from the beginning of one cell division to the beginning of the next cell division. There are many factors that affect the cell cycle such as type of tissue, the condition of environment, the cell function and degree of specialization. So, the cell cycle is variable from one cell type to another (fig. 1-1).

The cell cycle process consists of *mitotic division and interphase.* Mitotic division occurs in all multicellular organisms and most unicellular ones. For most organisms, mitotic cell division is required for growth and repair of body tissues. This process ensures that exact copies of DNA in chromosomes are transferred to the daughter cells.

Phases of the cell cycle

During the cell cycle, the cells pass through two different phases:

1. Cell division (M-phase)
2. Interphase

Cell division

It is called M-phase and involves both the nuclear and cytoplasmic divisions. There are two types of cell division; *mitosis* and *meiosis*.

Meiotic division is different form mitotic division, while mitotic division occurs in somatic cells and produces two identical cells that may start a new cell cycle, meiotic cell division occurs only in reproductive organs and results in formation of gametes with half number of chromosomes which are required for sexual reproduction.

Karyokinesis is the process of *nuclear division.* In mitosis, nuclear division produces two nuclei, each new nucleus receives the same number and type of chromosomes – the same genetic information as in the original cell (fig. 1-2).

Cytokinesis is the process of cytoplasmic division. It follows the nuclear division and results in formation of two daughter cells each of which contains one nucleus. If karyokinesis is not followed by cytokinesis, the mitotic division results in formation of binucleated or multinucleated cells.

Interphase

It is the stage between two successive cellular mitotic divisions (fig. 1-2 in animal cell and fig. 1-3 in plant cell). Interphase is formed of three phases:

1. G_1 -phase (1^{st} gap phase).
2. S -phase (synthesis phase).
3. G_2 –phase (2^{nd} gap phase).

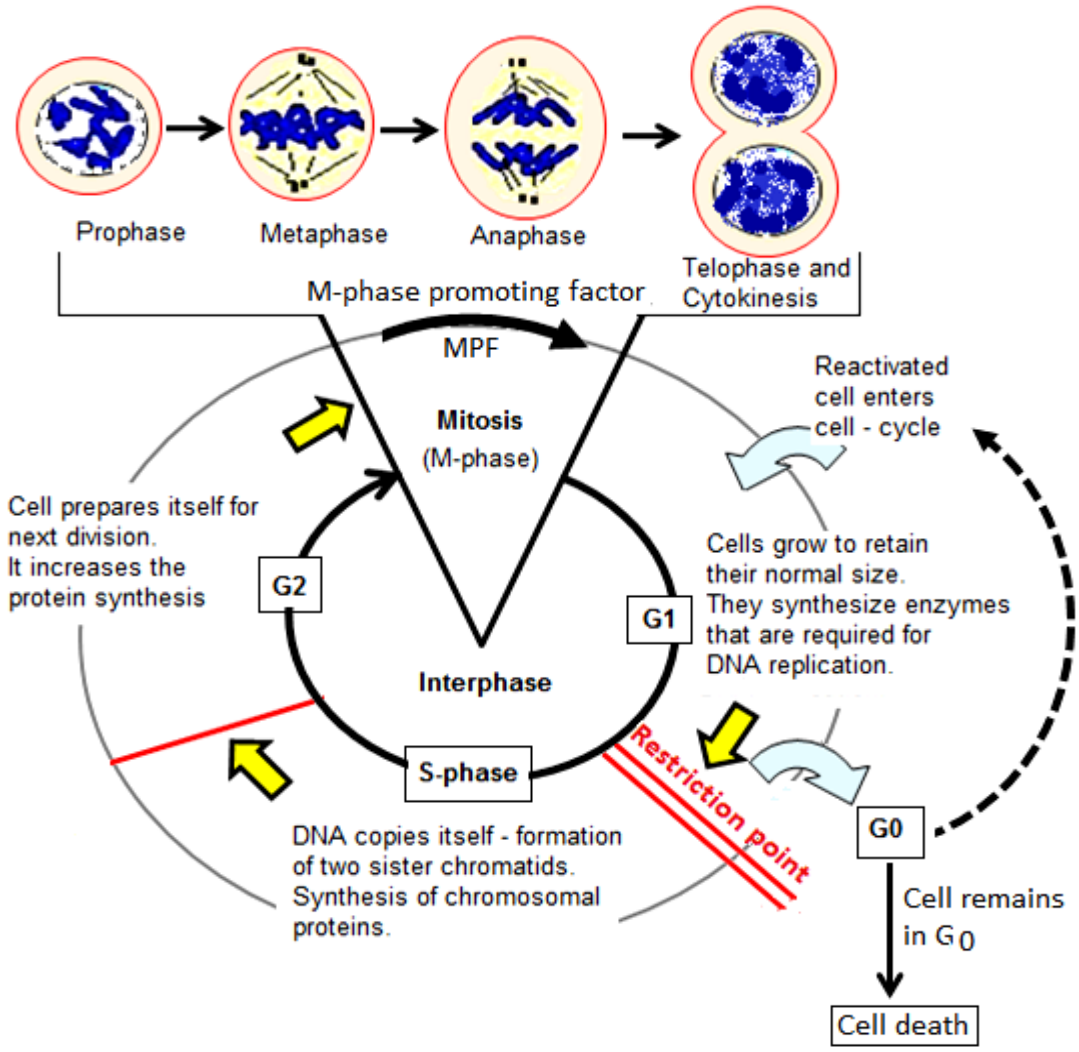


Fig.1-1: Diagram showing life cycle of the cell. It includes: Interphase in which the cell prepares itself for division and is formed of G_1 (1^{st} gap phase), S-phase (synthesis phase) and G_2 (2^{nd} gap phase). Mitosis is divided into four successive phases; prophase, metaphase, anaphase and telophase. It is accompanied by cytokinesis and results in the formation of 2 new daughter cells. Under certain conditions the cells may exit from the cell cycle and enter G_0–phase to become non-cycling cells and other cells may be reactivated to enter the cycle.

G_1–phase

After mitosis, cells enter G_1 –phase; first gap phase of interphase. In this phase, cells begin to grow and retain their normal size. Each cell, in order to prepare itself for the next phase (synthesis phase), increases its activity and synthesizes the enzymes that are required for DNA replication. G_1-phase is usually the longest period of the cell cycle. Its duration ranges from 6 to 12 hours in cycling animal cell.

The cells which perform the ordinary metabolic activities and do not prepare themselves for mitotic division are non-cycling cells and stay in **G_0 –phase**, such as neurons in brain, cardiac muscle fibers in heart and red blood cells.
Under certain conditions or stress; such as any physical damage or injury, reactivation of cellular mitotic activity occurs and results in some non-cyclic cells in a quiescent stage, such as liver cell and Schwann's cell, become active and begin new cell cycle to repair the damaged tissue. *Some other cells lose their ability to proliferate to repair the damaged tissue such as the oligodendroglia of central nervous system.*

S -phase

2nd phase of interphase is called S –phase that means *synthesis phase*. In this phase, DNA copies itself; duplicates and each chromosome becomes formed of two sister chromatids connected together at *centromeres* (fig. 1-4). In addition, cells synthesize actively chromosomal proteins that are required mainly for DNA replication. Duration of S-phase extends from 6 to 8 hours in cycling animal cell.

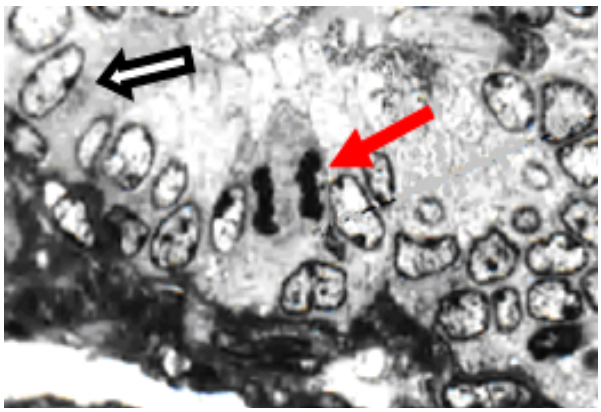


Fig. 1-2: Semithin section of animal cells of intestine shows telophase of mitosis (red arrow) and non-dividing cells contain nucleus with intact nuclear envelope (white arrow). Light microscopic picture.

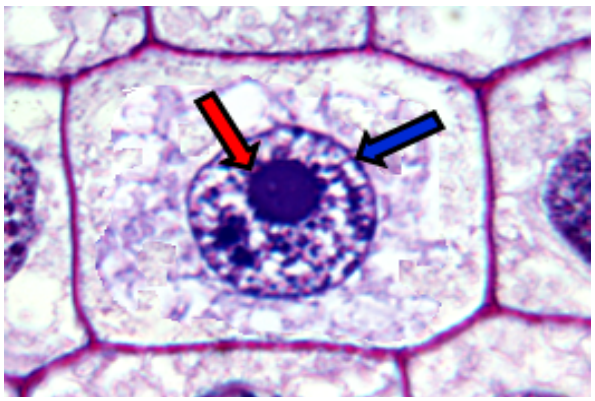


Fig. 1-3: Plant cell in interphase period. The centrally located nucleus shows chromatin substance and prominent nucleolus (red arrow), and is surrounded by intact nuclear envelope (blue arrow). ©Carolina Biological Supply Company, Burlington, N.C., Used with permission.

G_2 –phase

In*G_2 –phase*, *second gap phase,* the cell prepares itself for the next cell division, M-phase. The cell increases its protein synthesis that is required during cell division. It is the shortest period of interphase.

The time that is required for the cell to complete one cycle is called **generation time.** It ranges from 18 to 24 hours for the actively cycling somatic animals cells.

All the cells that share in building the structure of plants or animals, except the reproductive cells are called somatic cells. These somatic cells do not participate in the production of gametes. Somatic cells are produced from other preexisting cells.

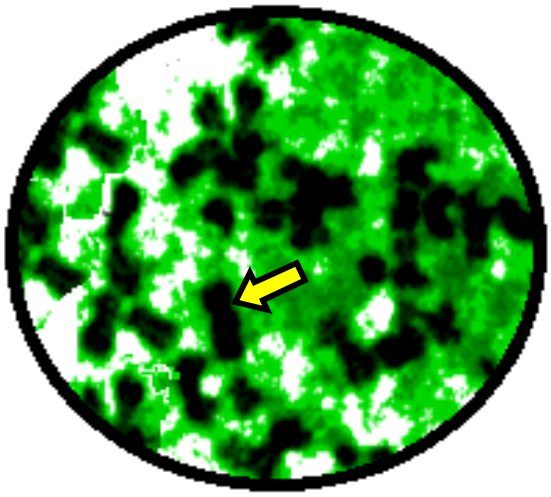


Fig. 1-4: Chromosomes of metaphase of mitosis are prepared by karyotyping technique. Each one is formed of two sister chromatids attached at centromeres, constricted area (arrow).

Mitosis

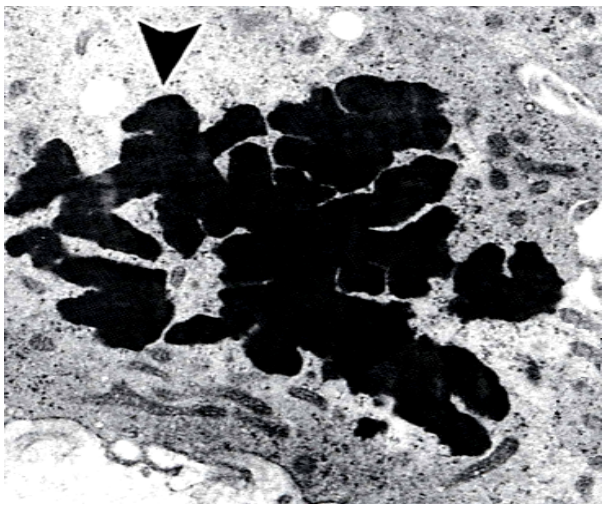
Mitotic division is a *nuclear division* by which a eukaryotic cell separates the chromosomes in its nucleus into two identical sets in two nuclei. For forming two separate new daughter cells the nuclear division is followed immediately by *cytokinesis*, which divides the cytoplasm, organelles and cell membrane into two cells containing roughly equal cellular components. **Mitosis** and **cytokinesis** form together the **mitotic (M) phase** of the cell cycle.

Mitosis in the animal somatic cells is a brief period that is usually less than 1 hour in duration.

Mitosis occurs exclusively in eukaryotic cells, but occurs in different ways in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

Mitotic division is formed of the following four successive phases:

1. Prophase
2. Metaphase
3. Anaphase
4. Telophase (telophase is followed by cytokinesis)



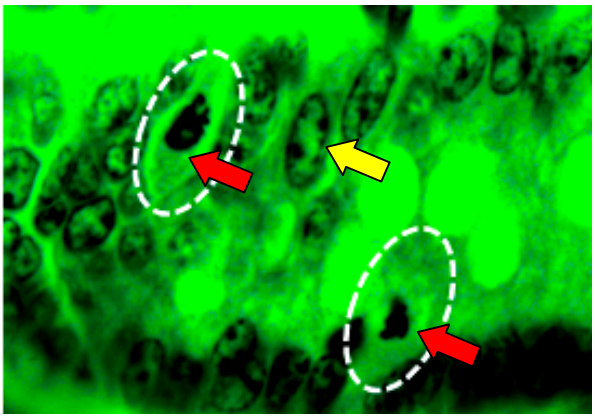


Fig. 1-5: *Left*: Condensed chromatin fibers (arrow head) in prophase as appear under EM. *Right*: Yellow arrow indicates non-dividing cell in the intestinal gland showing a nucleus with intact envelope, while red arrows indicate dividing cells in prophase of mitosis. In prophase nuclear envelope disappears and chromatin threads condense to form short and thick chromosomes.

Prophase

Cellular mitotic division begins when cell initiates prophase; the first stage of mitosis (figs.1-5 and 1-6).

In early prophase:

The *chromatin threads* of genetic substance gradually shorten and thicken to form clearly defined bodies called chromosomes.

In middle prophase:

Centrioles, the organelles of mitotic spindle formation are duplicated during the interphase forming a pair of centrioles. They move apart and begin to synthesize the mitotic spindle. Each centriole migrates to a pole of the cell in late prophase. During mitotic spindle synthesis, each centromere of sister chromatids attaches itself to the microtubules of the developing mitotic spindle.

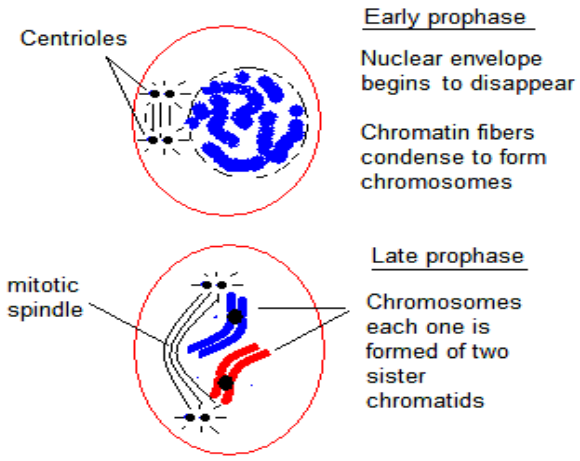


Fig. 1-6: Diagrams illustrate the early and late prophases in mitosis.

In late prophase:
Centrioles become nearly at opposite poles of the cell. Nuclear envelope and nucleolus disappear. Each chromosome is formed of two sister chromatids attached together at centromeres.

Metaphase
In metaphase (figs.1-7 and 1-8), the chromosomes appear attaching the mitotic spindle and line up along the equatorial plane of the cell. Each chromatid is completely condensed. It is the shortest phase of mitosis and the best stage for studying chromosomes. In metaphase, synthesis of the *mitotic spindle* is completed.

Mitotic spindle is responsible for separation of sister chromatids or chromosomes in anaphase of cell division (fig. 1-9). It is composed of assembled microtubules by polymerization of tubulin protein subunits. Assembling of the microtubules takes place by microtubule organizing centers (MTOC) containing a pair of small bodies, centrioles.
Microtubules of the mitotic spindle grow out from the pericentriolar material and differentiate into three types (fig. 1-9):

a. Polar microtubules
b. Kinetochore microtubules
c. Astral microtubules

- Polar microtubules:
  They overlap at equatorial plane, the positive ends, over the polar ends of chromosomes. Kinesins operating between overlapping polar fiber are believed to drive the centrosome to opposite poles of the cell for building mitotic spindle.

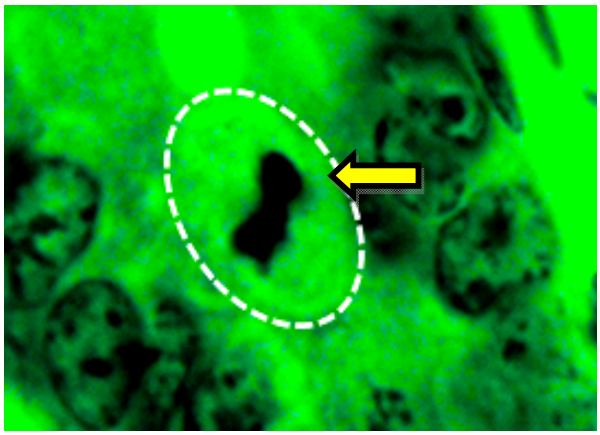


Fig. 1-7: In cells lining the basal portion of intestinal gland, the chromosomes are lined up along the equatorial plane in metaphase of mitosis (arrow). In this phase they attach to the mitotic spindle.

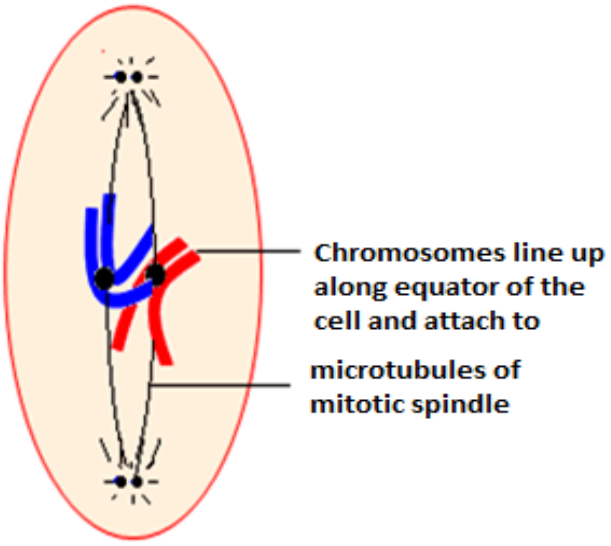


Fig. 1-8: Diagram shows metaphase of mitosis. Chromosomes line up along the cell equator and attach to the mitotic spindle.

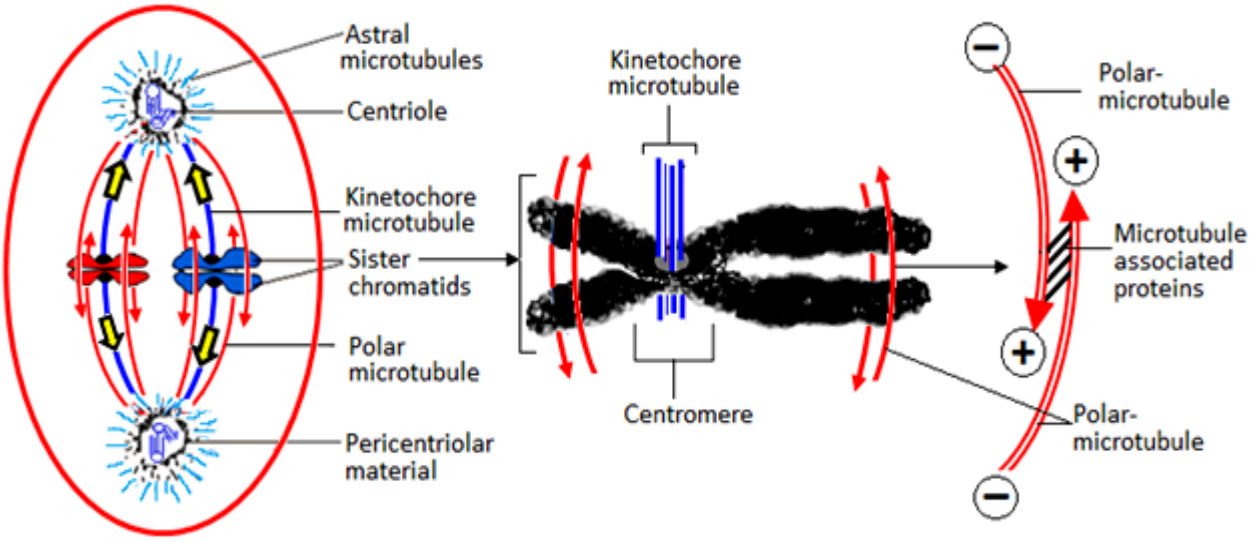


Fig. 1-9: Mitotic spindle is formed of kinetochore microtubules attaching the centromeres of each chromatid and polar microtubules which overlap at their positive ends. They are originated from microtubule organizing center (MTOC; a pair of polar centrioles). Disassembling of kinetochore microtubules (yellow arrows) and assembling of polar microtubules (red arrowheads) move the separated chromatids toward the respective poles. Polar microtubules are held together by microtubule associated proteins.

- Kinetochore microtubules:
  They attach to the kinetochore region in centromeres of each chromatid.

- Astral microtubules:
  They radiate out from pericentriolar material in many directions. Molecular motors associated with the astral fibers pull the centrosome apart during the spindle formation. Their role in cell division is unknown but it is postulated that they may play a role in cytokinesis.

Kinetochore is formed of a special type of proteins which is associated with the centromere – a constricted region in each chromosome (fig.1-9).

It is the site of attachment of kinetochore microtubules of mitotic spindle. It may be also the site for disassembling of kinetochore microtubules which is responsible for migration of chromosomes toward cell poles.

Anaphase

In early anaphase two sister chromatids are separated from each other. Each chromatid moves away from equatorial plane toward the opposite cell poles (fig. 1-10 and 1-11), and each chromatid is called now chromosome.

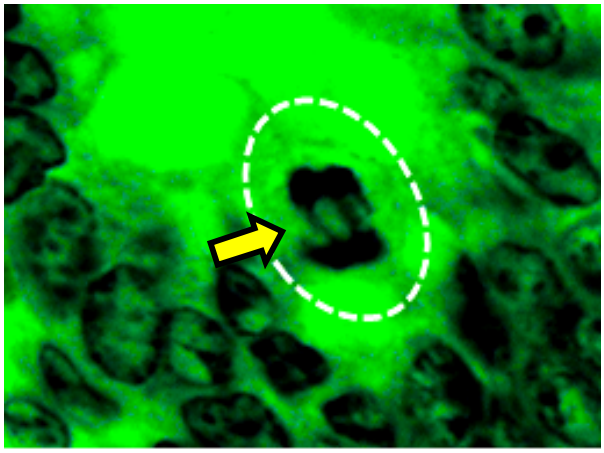


Fig. 1-10: Early anaphase in dividing Cell of the basal portion of intestinal gland. The sister chromatids separate and start to move away from the equatorial plane toward opposite poles (arrow)

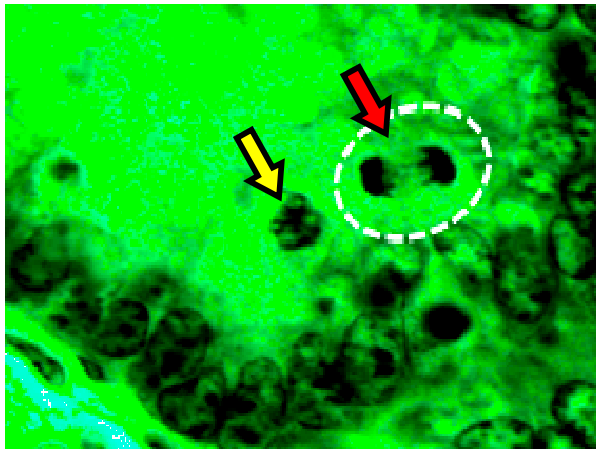


Fig. 1-11: Late anaphase in a cell of epithelium lining the basal portion of intestinal gland. Yellow arrow indicates that two groups of chromosomes are located at their respective poles. Red arrow indicates another cell in the prophase of mitosis.

In late anaphase each cell pole receives a newly formed set of chromosomes (fig. 1-12). Each set is formed of the same original number of chromosomes which carry the same genetic material of parent cell.

The cells possess mechanisms to correct the errors that happen during anaphase. Presence of free kinetochore as a result of disrupting attached spindle fibers causes an *immediate block* to the process during anaphase.

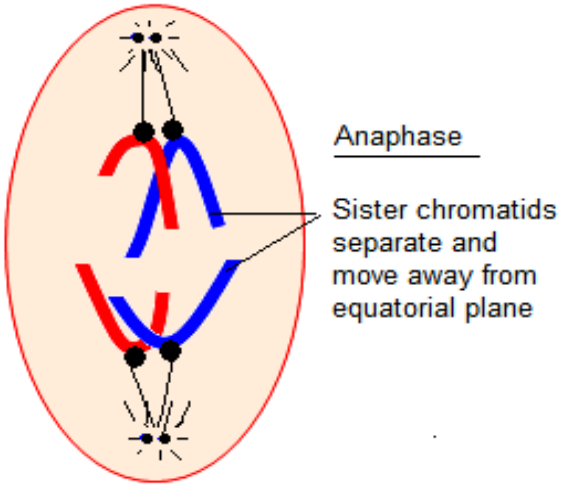


Fig. 1-12: Diagram illustrates anaphase of mitosis. Two sister chromatids separate and move away from equator toward the opposite poles of the cell.

Mechanism of sister chromatids separation in anaphase:

a) They are separated by decomposition of protein that holds the centromeres together.

b) The separated chromatids move to opposite poles by:

1. Shortening of the kinetochore microtubules (MTs) takes place by disassembling of tubulin subunits at point of attachment to chromosomes.

2. Elongation of the polar MTs occurs by addition new tubulin subunits at plus end. They slide past one another at the equator to maintain the correct orientation of chromosomes during the movement toward the cell poles.

Telophase

Telophase starts when each group of chromosomes reaches its respective cell pole and begins to reform nucleus. Around each group reformation of the nuclear envelope is assembled, and nucleolus within each nucleus is gradually developed.

Also, the mitotic spindle disappears and the chromosomes become gradually longer and thinner, and transform to chromatin threads (fig. 1-13).

Cytokinesis

It is a cytoplasmic division which begins during telophase and results in the formation of two separate daughter cells (figs. 1-13 and 1-14). Both daughter cells, which appear smaller in size compared with the parent cells, are genetically identical and contain the equal number of chromosomes as that of parent cells.

Steps of cytoplasmic division in animal cells are different when they are compared with steps of cytoplasmic division in plant cells, because the plant cells are supported by cell wall.

Steps of cytokinesis:

In animal cells (fig. 1-15), cytokinesis begins by formation of a contractile ring of actin and myosin microfilaments around the equatorial region. This ring narrows gradually to form a cleavage furrow. The furrow increases until it divides the cytoplasm into two daughter cells. Each one contains a nucleus. Organelles are distributed randomly to the daughter cells.

- Mitochondria have their own DNA and divide during interphase.
- In plant cells (fig. 1-16), cytokinesis starts by formation of small membrane-bound vesicles along the cell equator. Gradually, they fuse together. Their fusion begins from inside and continue outward, forming double membrane across the cell.

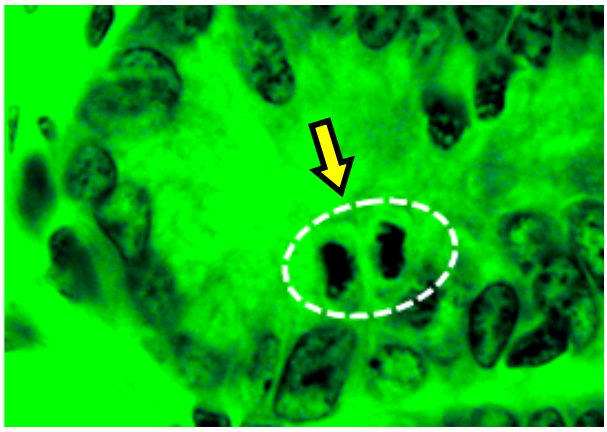
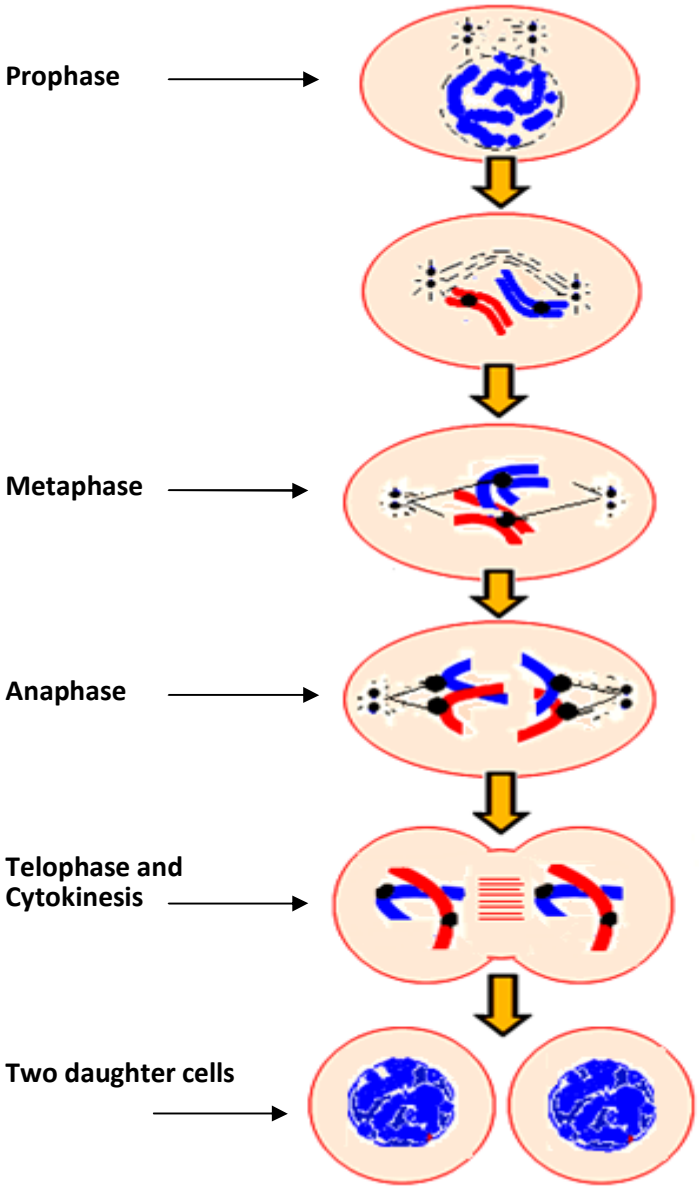


Fig. 1-13: Cell division shows Telophase of karyokinesis and cytokinesis in epithelial cell of the intestinal gland (arrow). Each new cell has received a collection of daughter chromosomes. The chromosomes begin to return to their interphase form. A nuclear envelope starts to reform around each set of chromosomes.



In early prophase:
Nuclear envelope begins to Disappear. Chromatin fibers condense to form chromosomes.
Each chromosome is formed of 2 sister chromatids.

In late prophase:
Centrioles reach cell poles. Nuclear envelope and nucleolus disappear.

Chromosomes line up along the equator of the cell. They attach the microtubules of mitotic spindle.

Sister chromatids separate and become independent chromosomes that move away from equatorial plane toward the cell poles.

Each group of chromosomes reaches its respective pole and begins to reform nucleus.

Cytokinesis results in formation of two genetically identical daughter cells containing equal number of chromosomes as that in parent cell.

Fig. 1-14: Diagram illustrates successive phases of mitotic division in anima cell. Parent cell produces 2 genetically identical daughter cells.

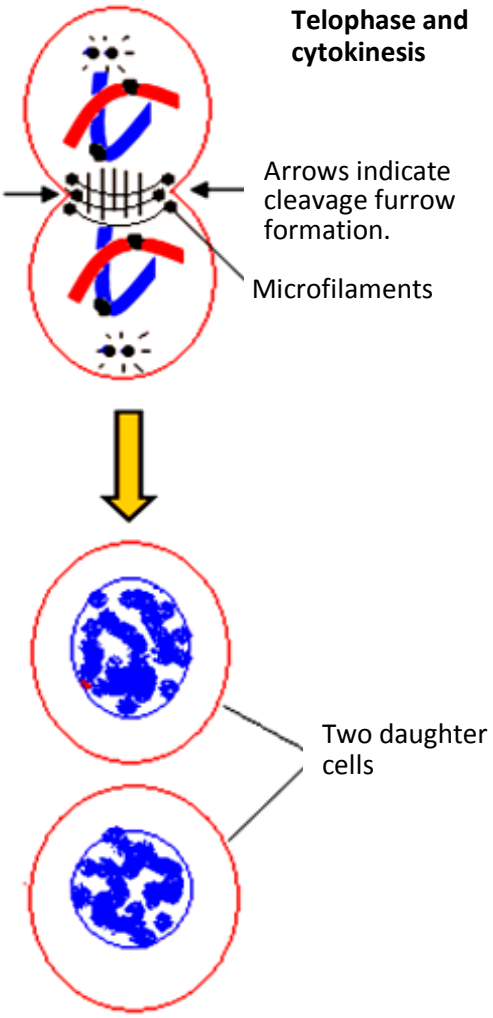


Fig. 1-15: Diagram demonstrates mechanism of cytokinesis in animal cells. Cytokinesis begins by formation of a ring of microfilaments which contracts gradually from outside toward inside. Thus results in cleavage furrow formation (arrows). Cleavage furrow increases until it divides the cytoplasm; cytokinesis.

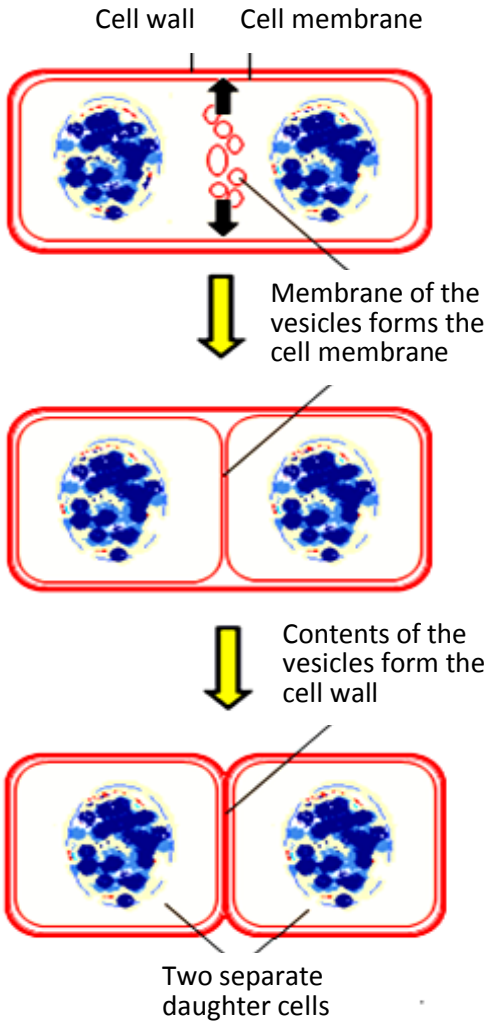


Fig. 1-16: Diagram illustrates the mechanism of cytokinesis in plant cell. It begins from inside by formation of membrane –bound vesicles (arrows) along the equator of the cell. The vesicles fuse gradually together forming double membrane across the cell which forms the cell plate.

Endomitosis

It is called ***inner indirect mitosis,*** a type of mitosis that occurs in certain plant and animal cells in which the chromosomes within intact nucleus surrounded by nuclear envelope multiply but not followed by nuclear division and cytokinesis. Endomitotic division produces cells containing polyploidic nuclei (containing more than two sets of chromosomes).

Endomitosis may indicate high cellular differentiation. It may occur under the effect of a specific gene expression. The polyploidic nuclei have the capability to go in mitosis.

Endomitosis occurs during development of polyploid megakaryocytes in bone marrow.

Amitosis

It is the simple direct method of nuclear and cytoplasmic division, without formation of chromosomes, in which the nuclear division takes place without mitotic spindle formation (fig. 1-17). It is classified into:

1) Amitotic nuclear division: It is responsible for formation of binucleated or multinucleated cells in order to achieve an increase in their metabolic activities, such as binucleated liver cell and multinucleated osteoclast and skeletal muscle fibers.
2) Amitotic cell division: Division includes not only nucleus but also the cytoplasm. This amitotic division is rare, and takes place in tissue regeneration after physical damage to the body caused by violence, accident or fracture.

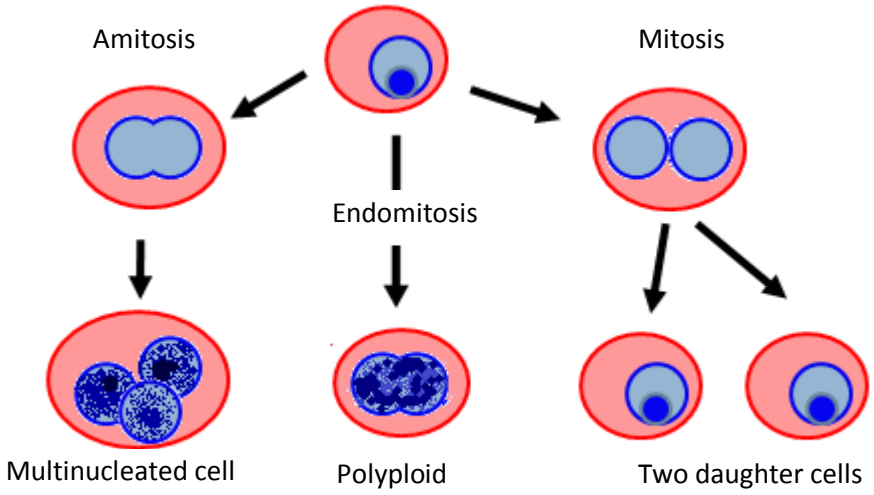


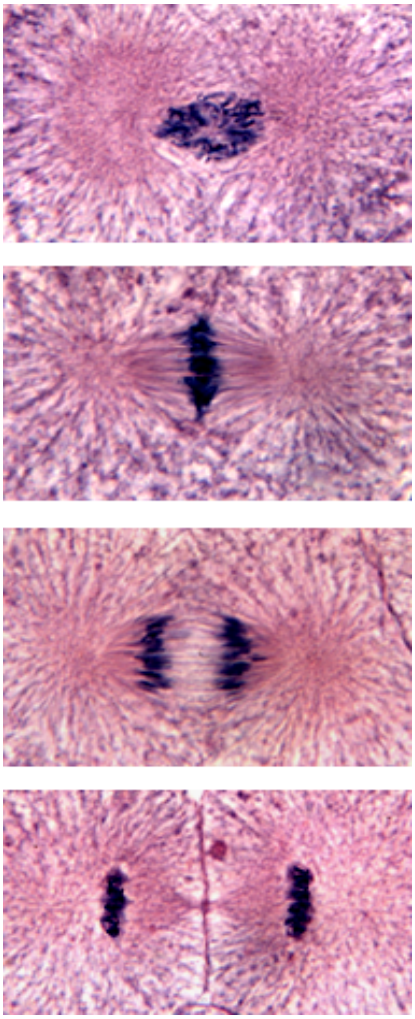
Fig. 1-17: Diagram showing the differences between amitosis, endomitosis and mitosis. In amitosis nuclear division nuclear division takes place from outside by contractile actin filaments without mitotic spindle formation. In endomitosis; multiplication of chromosomal material occur, but not followed by nuclear division and cytokinesis, and in mitosis nuclear division is followed by cytokinesis.

Prophase
Nuclear envelope begins to disappear. Nucleolus disappears, and the chromatin threads begin to condense forming short and thick chromosomes. Centrioles move to the opposite poles and form the spindle.

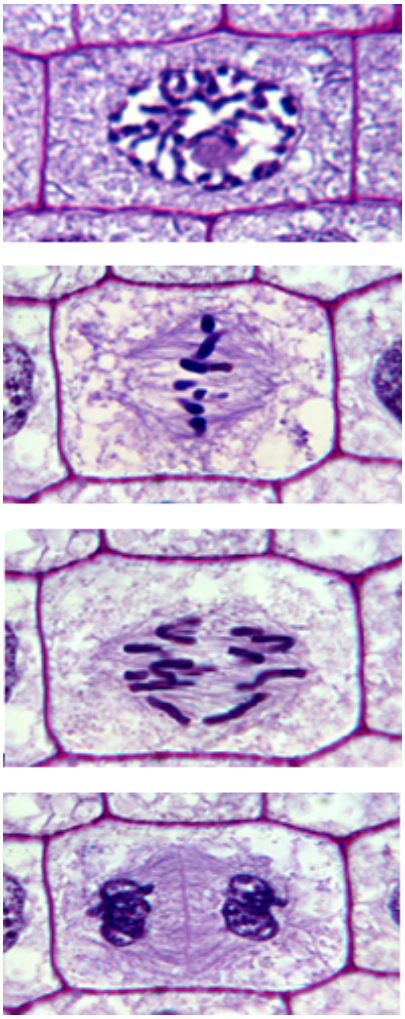
Metaphase
The chromosomes are lined up along the equatorial plane and attached to the mitotic spindle.

Anaphase
The sister chromatids of each chromosome separate and start to move away from the equatorial plane toward the opposite poles.

Telophase and cytokinesis
The chromosomes return to their interphase form. A nuclear envelope begins to reconstitute and encircle each set of chromosomes.



Phases of mitosis as appear in cells of fish embryo.



Phases of mitosis as appear in cells of onion root tip.

Fig. 1-18: Light microscopic photomicrographs of the different phases of mitosis in plant cells of onion root tips (left column) and animal cells of fish embryo (right column). ©Carolina Biological Supply Company, Burlington, N.C., Used by permission.

Cell Cycle Regulation

The cell cycle is regulated by different factors which control the rate of cell division. Cell cycle regulation is necessary in order to avoid occurrence of an error that may result in development of cancerous cells. Cancer is a disease where regulation of cell cycle and cell growth is lost.

There are two main ***check-points*** in cell cycle control:
a. The point at the end of G1
b. The point at end of S-phase

- At the end of G_1 is the most important restriction point, at which the cells stop until they receive signals to continue and enter the S-phase where DNA will duplicate. If the cell condition is not ready, or external environment is not appropriate, the cell may enter G_0 -phase, a *quiescent stage*. For example lack of growth factors causes arrest of some cells at the restriction point.

- The check-point at end of S-phase; DNA duplication, is important to detect any alterations in DNA composition during replication. It may stop the continuation of cell cycle until the repair processes for any DNA-alteration are completed.

Cell division varies from one cell to another. Stem cells in bone marrow are always dividing to produce RBCs. Neuron in brain and muscle cells in heart are arrested in G_0. Liver cells are arrested in G_0, but can be reactivated to re-enter the cell cycle and divide when liver cells become damaged.

Extracellular factors
They are environmental promoting or inhibiting factors for the cell division such as nutrition, temperature, pH, cell-cell interaction, hormones and growth factors.

Intracellular factors

- **Cyclins**

They are regulatory proteins that have a fluctuated level during the different phases of cell cycle.

- **Protein kinases**

The kinases are enzymes that activate or inactivate other protein by addition of phosphate to certain locations.
Phosphorylation of histone H1 could be concerned with condensation of chromatin at mitosis.
Cyclins and Cdk, Cyclin–dependent protein kinases, are the major intracellular factors which are working as switchers for cell cycle. They are responsible for moving the cell from G_1 to S or G_2 to M.
When Cdk forms with a specific cyclin an active complex, it becomes able to phosphorylate some proteins. The phosphorylation activates some enzymes which are required for mitosis. Mitosis promoting factor, MPF is an example of active Cyclin-Cdk complex that stimulates the continuation of the cell cycle (fig. 1-19).

- **Protein 53 (p53)**

Active protein p53 is function to block the cell cycle when DNA damages due to, for example, chemotherapy and radiotherapy.

If the damage is severe, the **p53**; it is a transcription factor tetramer, destroys the damaged cell by activation of a cell death program or apoptosis.
The action of **p53** is mediated by **p21,** an inhibitor of **Cdk4**. Activation of p53 causes stop of the cell cycle at G1 to enable cells to correct DNA damage before they enter to the S-phase.

Ap53 is a product of expression of a tumor suppressor gene. A **p53 mutation** is the most frequent mutation that results in occurrence of cancer. Mutations of the p53 gene are observed in 50% of human cancers. The loss of p53 gene expression is responsible for a multicancer disease known as **Li Fraumeni syndrome**. Many clinical studies correlate also the inactivation of p53 gene with resistance of cancer cell to chemotherapeutic agents.

- **Protein 27 (p27)**

Binding **p27** to cyclin and Cdk causes blocking entry of the cell into S -phase. Recent research suggests that breast cancer prognosis is determined by **p27** levels. Reduced levels of p27 predict a poor outcome for breast cancer patients.

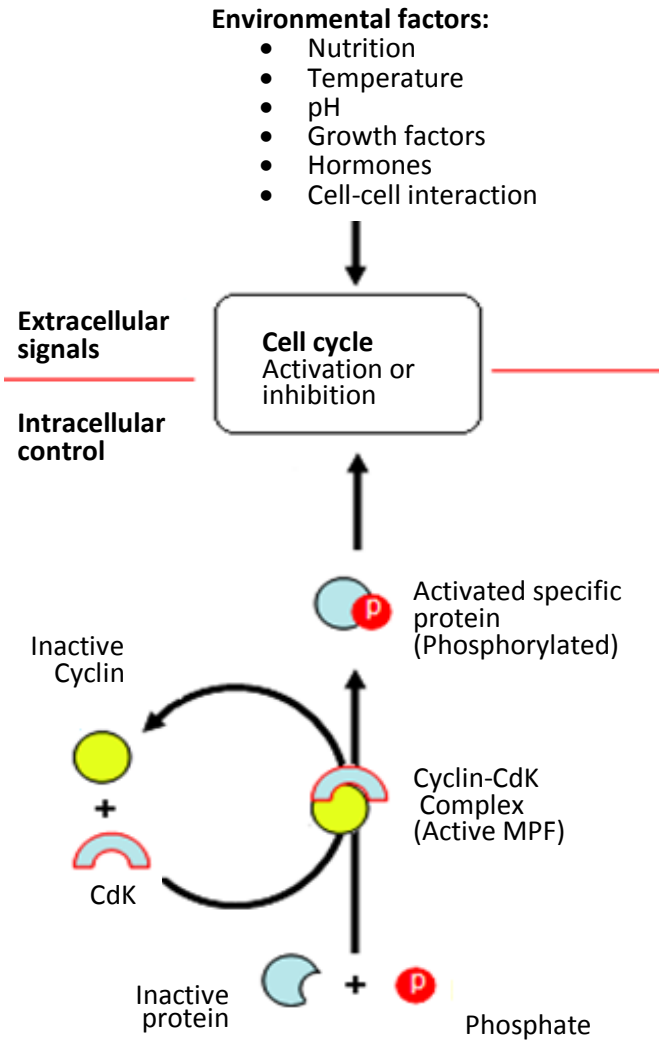


Fig. 1-19: Cell cy[illegible]n is regulated by extracellular signals such as nutrition, and temperature and intracellular factors; Cyclin-CdK complex (mitosis promoting factor, MPF). It phosphorylates specific proteins which activate or inhibit the cell cycle.

Applications on Cell Cycle Control

- Many drugs have been used for treatment of cancer; uncontrolled cell division. For example, anticancer drugs for treatment of diseased patient. Colchicine that binds unpolymerized tubulin is used to prevent the mitotic spindle formation and to stop the cell division. **Colchicine** is used widely in laboratory examination of genetic composition. It arrests cell division at metaphase as the chromosomes are highly condensed to study the chromosomal abnormalities in karyotyping technique.

- Drugs stimulate mitosis: There are many growth promoting factors which can be used to stimulate cell division such as erythropoietin for RBCs, epidermal growth factor (EGF) and some steroid hormones.

According to the number of chromosomes carrying the genetic information, the cells are classified into:

a. Haploid cells:
They contain a single set of chromosomes; such as male and female human gametes - each one contains 23 chromosomes (1n).

b. Diploid cells:
They contain two sets of chromosomes (2n) for example all somatic cells human cells contain 46 chromosomes (two set of chromosomes).

c. Polyploid cells:
They contain three sets (3n) or more set of chromosomes. They are common in plants and rare in animal cells.

Meiosis

Meiotic division is a complex process of conversion a diploid cell to haploid gametes containing the half number of chromosomes; chromosomal number reduction.

The word "meiosis" comes from the Greek **meioun**, meaning "to make smaller," since it results in a reduction of chromosome number in the gamete cell. Meiosis takes place only in the gametes producing organ, ovary in female and testis in male. Meiosis is formed of two successive divisions; **meiosis-I** and **meiosis-II** which are separated by a short interphase period called **interkinesis.**

Goals of meiosis are production of haploid gametes which are required to achieve the sexual reproduction.

Reproduction is a process of generating offspring. There are two types of reproduction:

i) Asexual reproduction

It is a form of duplication using only mitosis as in figure 1-20.

Asexual reproduction is characterized by:

- A single parent divides by mitosis into two cells or more forming a clone of genetically identical organisms.
- In the clone each one is an exact copy of the original organism.
- This type of reproduction is rapid and effective.
- This type of reproduction doesn't cause genetic diversity within the population.

ii) Sexual reproduction

- In sexual reproduction, a new individual is formed by a combination of two haploid sex cells, two gametes, as in figure 1-21.

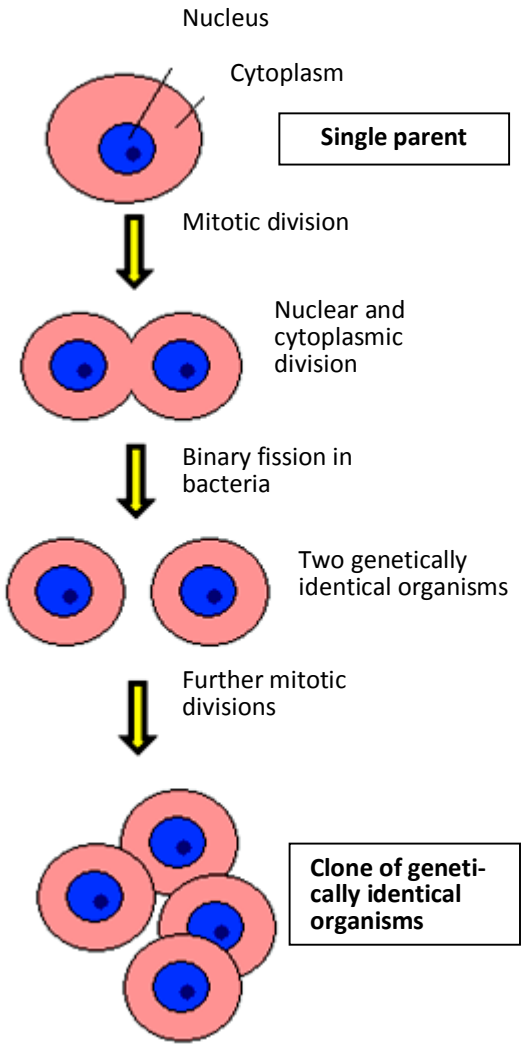


Fig. 1-20: In asexual reproduction, a single parent reproduces to form clone of genetically identical organisms.

- When two gametes or sex cells, one from male, sperm, and the other from female organism, ovum, fuse together to form zygote.
- The offspring are not genetically identical organisms.

Meiosis-I

It begins with a cell that has a diploid number of chromosomes; 23 pairs of homologous chromosomes in somatic cells, and ends with the formation of two haploid cells each one contains 23 chromosomes; gametes.
It causes the random segregation of chromosomes in a diploid cell and results in production of two haploid daughter cells. This step of meiosis is responsible for genetic diversity in the offspring.

In meiosis–I, dividing cell passes through the following four successive phases:

1. Prophase-I
2. Metaphase-I
3. Anaphase-I
4. Telophase-I

Telophase-I is followed by **cytokinesis** and a short period called **interkinesis** separating between meiosis-I and meiosis-II.

Prophase– I

The events of prophase–I (fig. 1-22) are similar to that of mitosis. The nuclear envelope and nucleolus disappear, except in prophase-I, homologous chromosomes **synapse** (fig.1-23) forming **tetrad** which allows for crossing over - exchange of genetic material to occur (fig. 1-23). This is the remarkable evidence which differentiates meiosis from mitosis.

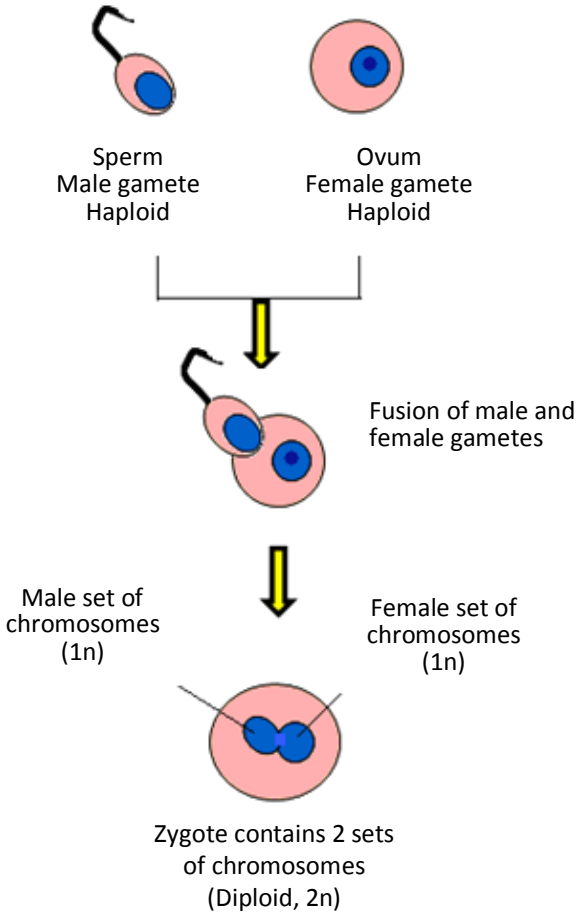


Fig. 1-21: Sexual reproduction. Male and female gametes (haploid cells) fuse together to form zygote (diploid cell). Sexual reproduction doesn't produce identical offspring.

Each pair of homologous chromosomes contains genes for the same biological features, such as eye color, at the same locations on the chromosome. Chromosomes of homologous pair are similar in length, except for sex chromosomes, where the X -chromosome is larger than the Y -chromosome. These chromosomes share only small regions of homology.

Synapsis and Crossing over

Synapsis is a process of the side by side pairing of homologous maternal and paternal chromosomes in prophase-I.

Every two homologous chromosomes that are formed of four chromatids lie side by side to form a complex structure called **tetrad**. Also the associated pairs of homologous chromosomes in synapsis called **bivalent**. They are held together by synaptonemal complex that is formed of specific protein. It is important for crossing over and exchange of genetic material (fig.1-23).

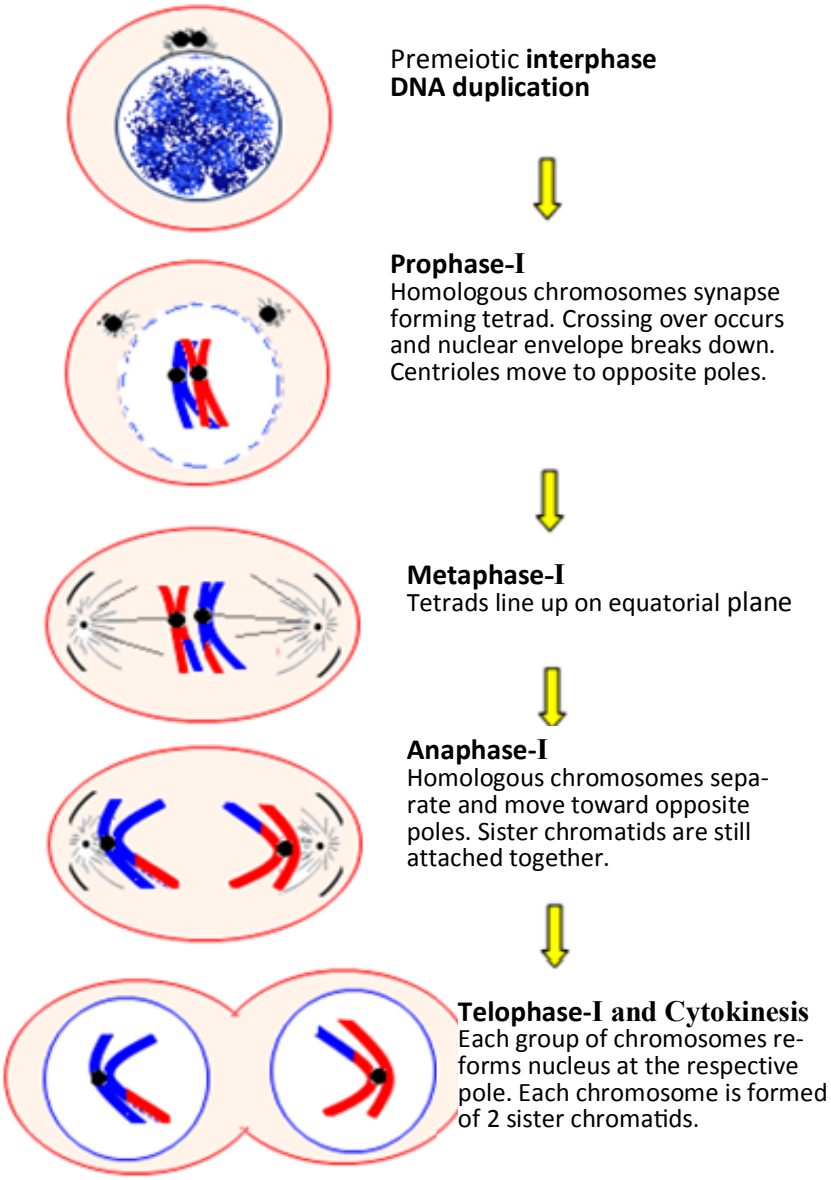


Fig. 1-22: Phases of meiosis-I. It produces two haploid cells each one contains a half number of chromosomes, each chromosome is formed of two sister chromatids.

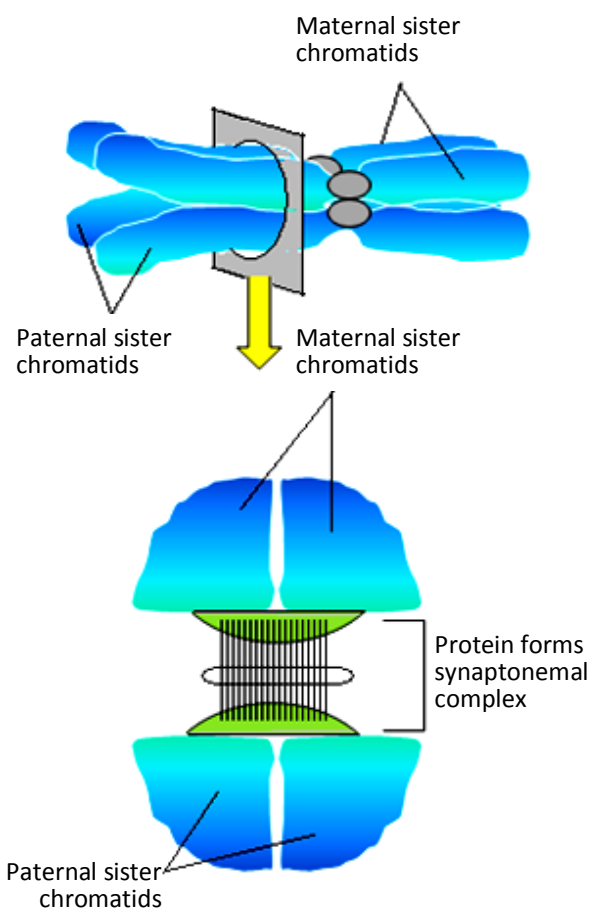


Fig. 1-23: In prophase-I: Each one of the homologous chromosomes; maternal and paternal, is formed of two sister chromatids. They are placed side by side forming complex called tetrad. They are attached together by synaptonemal complex

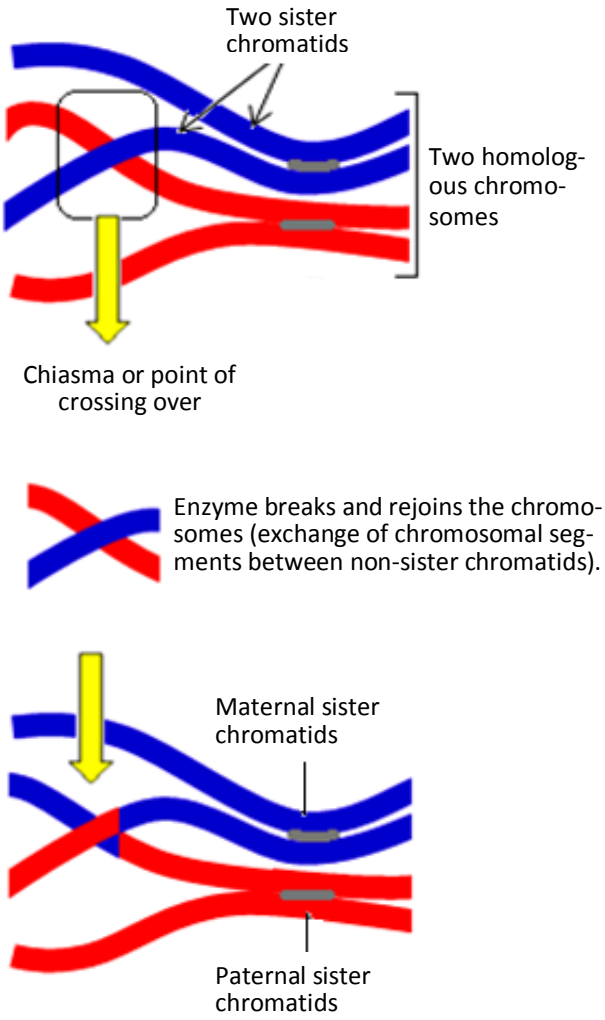


Fig. 1-24: Diagram illustrates the process of crossing over in prophase-I. Exchange of the genetic material takes place between non-sister chromatids of homologous chromosomes at region which appears as X-shape called chiasma under light microscope

Crossing over is a complex process which involves an exchange of the genetic material between the non-sister chromatids of the bivalent homologous chromosomes.
As illustrated in figure 1-24, the exchange of genetic material between non-sister chromatids takes place by enzymes that cut the chromosomes and rejoin them at specific regions called chiasmata. This process results in formation of a new genetic recombination. Each region of crossing over– **chiasma** (pl. chiasmata) between homologous chromosomes appears as X –shape under light microscope.

Steps of synapsis and crossing over in prophase have been classified into the following five stages:

i) **Leptotene stage:** It is the first stage of the prophase-I of meiosis, during which each chromosome becomes visible as two fine threads (chromatids). The long chromatin filaments condense and attach the nuclear envelope.
ii) **Zygotene stage**: It is the second stage of the prophase-I of meiosis, during which the homologous chromosomes begin to pair, so it is called stage of pairing of homologous chromosomes, and formation of bivalent.
iii) **Pachytene stage:** It is the third stage of the prophase-I of meiosis, during which the paired chromosomes shorten and thicken. The two sister chromatids separate, and exchange of chromosomal segments between non-sister chromatids may occur, so it is a stage of points of crossing over formation (points of chiasmata).
iv) **Diplotene stage:** It is the fourth stage of the prophase-I of meiosis, during which the paired chromosomes of each bivalent begin to separate. The chiasmata decrease in number and disappear gradually.
v) **Diakinesis stage:** It is the last stage of prophase-I. In which chromosomes of each bivalent separate from each other. Chiasmata disappear and centrioles also separate and move apart toward cell poles. The mitotic spindle is formed. In which the nuclear envelope disappears.

Metaphase– I
The tetrads line up along the equatorial plane. The sister kinetochores are attached to the kinetochore microtubules of the spindle of one pole. The random orientation allows an equal chance for the daughter cells to get either the maternal or paternal chromosome of each pair of homologous chromosome.

Anaphase– I
Each one of homologous chromosomes is formed of two sister chromatids. The homologous chromosomes separate and move away from the equator toward the opposite poles. In human being each cell pole receives 23 chromosomes, but each chromosome is formed of two sister chromatids.

Telophase– I
The two sister chromatids of each chromosome decondense; they extend to form chromatin threads. The nuclear envelope reorganizes. Each

of the daughter cells is now haploid containing half number of chromosomes, but each one is still formed of two sister chromatids. Telophase-I and **cytokinesis** are followed by **interkinesis**; a very short period separates between meiosis-I and meiosis-II, but there is **no S –phase.**

Meiosis–II

Meiosis-II is the second maturation division of meiosis, in which each of the two haploid resulting cells of meiosis–I produces **two** haploid cells**.** Meiosis-II is similar to mitosis (fig.1-25), and formed of four successive phases:

1. Prophase -II
2. Metaphase-II
3. Anaphase -II
4. Telophase-II

Telophase-II is followed by division of cytoplasmic; **cytokinesis** to produce 4 haploid gametes.

Prophase– II

In this phase, each centriole divides into two which move to the opposite poles. Nuclear envelope begins to disappear. Each chromosome is still formed of two attached sister chromatids and becomes associated with the formed spindle microtubules.

Metaphase– II

In metaphase II, condensed chromo-somes line up along the equatorial plane of the cell and attach the microtubules of spindle apparatus.

Anaphase– II

In anaphase II, two sister chromatids separate from each other and move toward the corresponding poles of the cell.

Each separated chromatid becomes an independent chromosome.

Telophase– II

In telophase II, each set of chromosomes are grouped together near the corresponding pole. Chromosomes transform into thin uncoiled chromatin fiber.

- Nuclear envelope is reformed around the chromatin threads forming nucleus containing half the number of chromosomes.

- The chromatids of each chromosome are no longer identical because of the formation of new genetic recombination and exchange of the chromosomal segments between non-sister chromatids in meiosis-I.

- Meiosis -II separates the two sister chromatids and produces two new daughter cells, gametes. Each gamete contains 23 chromosomes in human, haploid, where each chromatid is named chromosome. So, meiosis –II results in 4 haploid cells, each contains one of each kind of chromosomes. Each resulting cell has different combinations of genes.

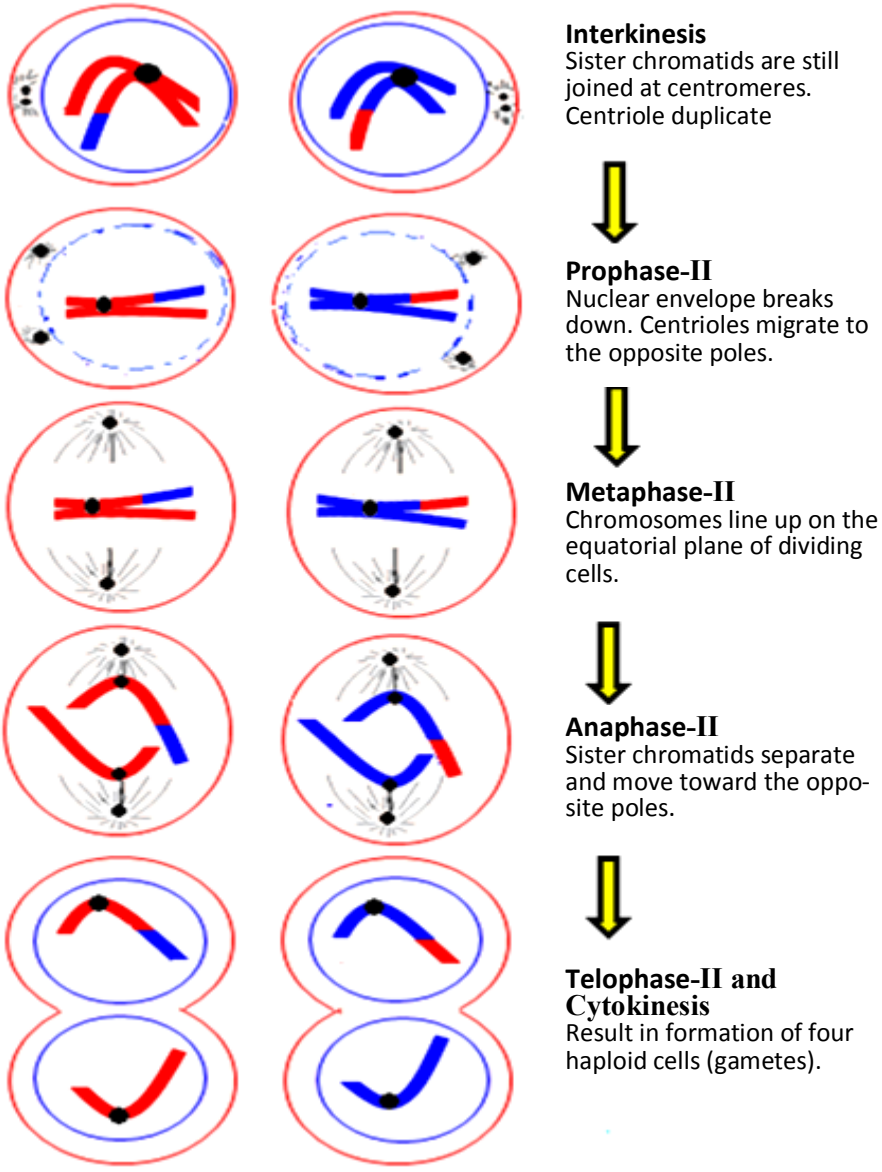


Fig. 1-25: Diagrammatic illustration of the phases of meiosis -II. Separation of sister chromatids results in formation of 4 haploid cells. Each cell contains half number of chromosomes.

Gametogenesis in Human

Oogenesis

- It occurs in female's ovaries.
- Oogenesis starts in germinal epithelium by creation of oogonia. Oogonial transformation into primary oocytes is completed either before or shortly after birth (fig.1-26).
- So, there are **no** additional primary oocytes created in oogenesis, in contrast to male spermatogenesis where gametocytes are continuously created.

- Meiosis-I begins during the embryonic life. It divides primary oocyte containing 2 sets (2N= 46 chromosomes, each one 2 sister chromatids) into secondary oocyte containing one set (1N= 23 chromosomes, each is formed of 2 sister chromatids) and first polar body.

- Secondary oocyte initiates meiosis – II that produces ovum containing one set (1N= 23 chromosomes) and second polar body. Therefore, ovaries contain a fixed number of oocytes (fig. 1-26).

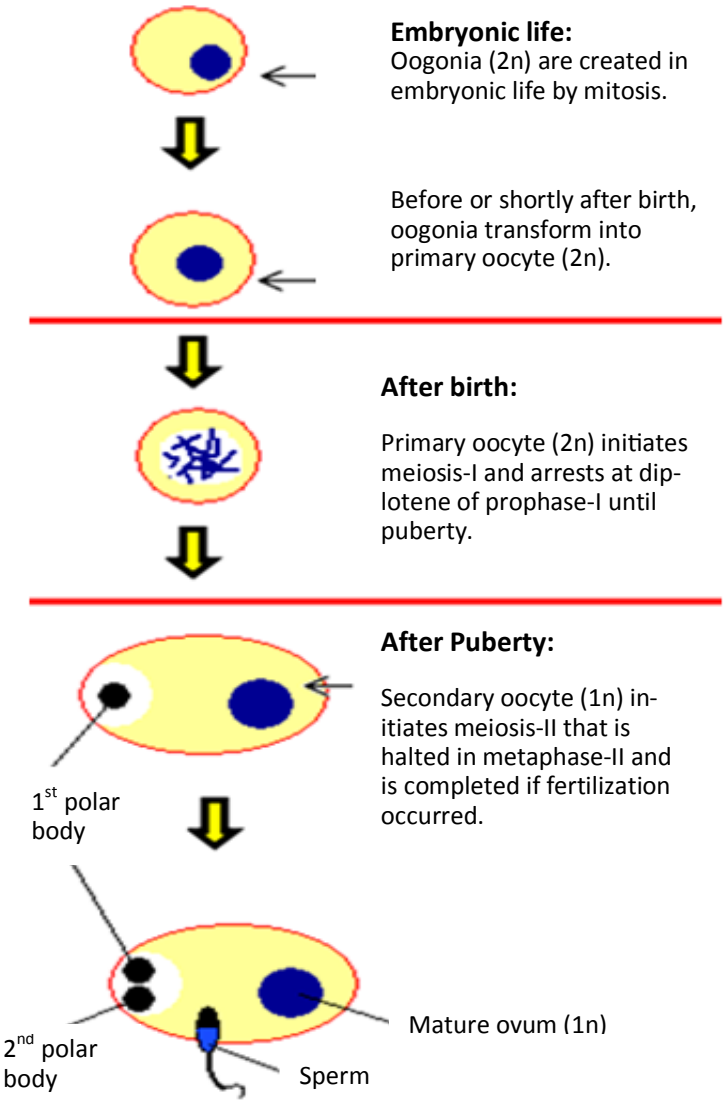


Fig. 1-26: Diagrammatic illustration of oogenesis. It occurs in female's ovaries. It includes meiosis-I and meiosis-II. It results in the formation of single mature oocyte and two polar bodies.

Spermatogenesis

- It takes place in the seminiferous tubules of testes (fig.1-27 & fig.1-28) where spermatogonia are created.
- After sexual maturation spermatogonium continues to multiply by mitosis then followed by meiosis to produce an unlimited number of spermatozoa.
- Diploid spermatogonium (2n = 46 chromosomes) which occupies the basal compartment of seminiferous tubules, divides mitotically to produce two diploid intermediate cell called a primary spermatocyte. Then each primary spermatocyte moves into the adluminal compartment of the seminiferous tubules and duplicates its DNA (2n = 46 chromo-somes, each one is formed of 2 chromatids).
- Primary spermatocyte passes through meiosis-I to produce two haploid secondary spermatocytes (1n = 23 chromosomes, each one is formed of 2 sister chromatids).
- The secondary spermatocytes enter rapidly into meiosis-II and divide to produce two haploid spermatids (1n), which grow and transform into spermatozoa.

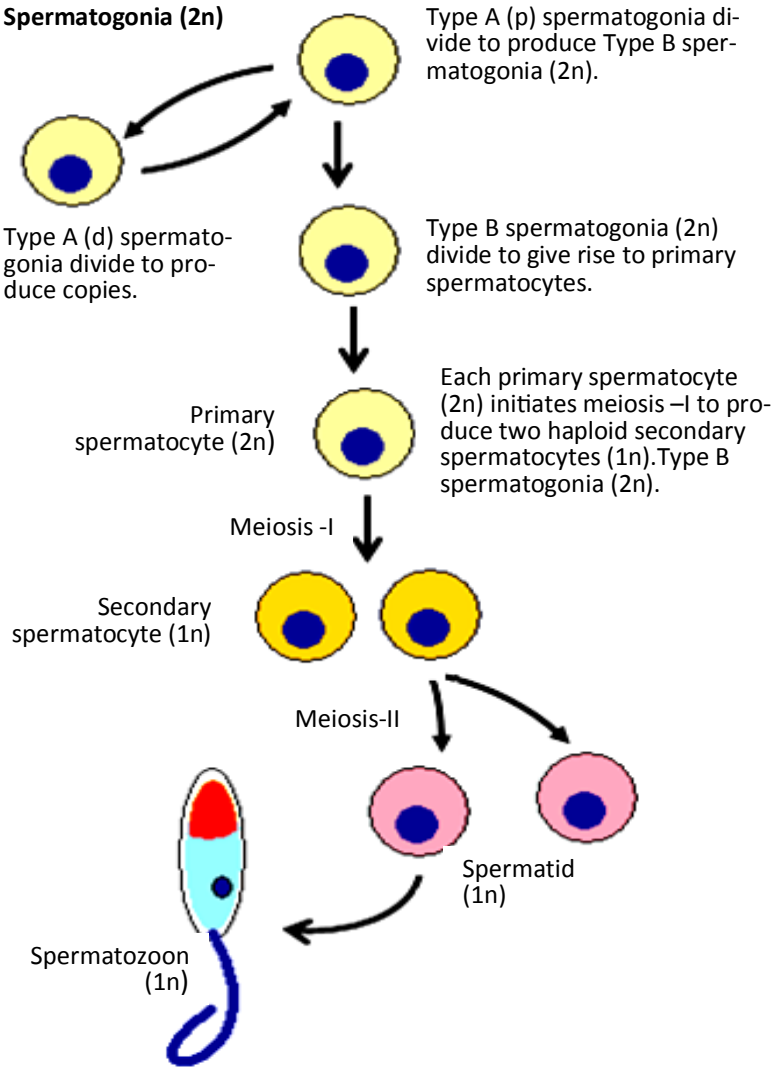


Fig. 1-27: Diagrammatic illustration of spermatogenesis. In spermatogenesis, type B spermatogonia divide mitotically to produce primary spermatocytes, which duplicate their DNA. Meiosis-I divides each primary spermatocyte into 2 haploid secondary spermatocytes, while meiosis II divides the secondary spermatocyte into 2 haploid spermatids, which develop into spermatozoa.

Genetic variations

Individuals of many offspring that result from sexual reproduction exhibit genetic variations because:

- One member of each pair of homologous chromosomes is randomly distributed to the poles in anaphase-I due to independent segregation of the maternal and paternal homologous chromosomes.

- Exchange of chromosomal segments during crossing-over produces new genetic recombinations of linked genes.

The general features of meiosis:

- It is formed of two successive divisions, meiosis-I and meiosis-II.
- It produces four cells each one contains haploid number of chromo-somes.
- DNA is duplicated only once during interphase preceding meiosis-I.
- The genetic information is mixed up.
- Synapsis and crossing over occur in prophase-I.

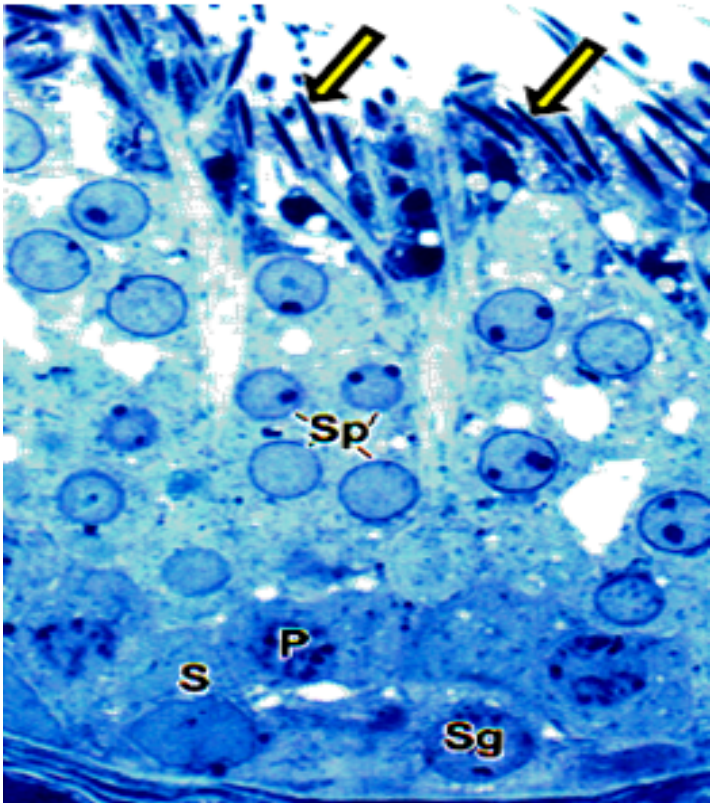


Fig. 1-28: Semithin section of seminiferous epithelium in tests shows different stages of spermatogenesis. Spermatogonia (Sg) are located at the basal portion of seminiferous epithelium, primary spermatocyte (P) and spermatids (Sp) are found in the middle part, while spermatozoa (arrows) are attached to the luminal surface of Sertoli cell (S).

Additional reading

Albert B et al. (2002): Molecular biology of the cell; 4th Ed. New York and London: Garland Science.

Austin C and Short R (1992): Reproduction in mammals: 1. Germ cells and fertilization, 2nd ed. Cambridge: Cambridge University Press.

Beach D, Basil co C and Newport J (1988): Cell cycle control in eukaryotes. Cold Spring Harbor, NY: Spring Harbor Press.

Glover DM, Gonzalez C, and Raff JW (1993): The centrosome. Sci. Amer. 268 (6): 32-9.

Goodman SR (1998): Medical cell biology. 2nd ed. Lippincott Williams & Wilkins: A Walters Kluwer Company. Philadelphia.

Lewin B (2002): Cell cycle and growth regulation. Genes VII. Oxford University Press Inc., New York, 835-874.

Lodish H et al. (2000): Molecular cell biology 4th ed. W.H. Freeman and Co., New York.

Manson AL, Jones E and Morris A (2002): Cell biology and genetics, 2nd ed. Mosby, New York, 71-75.

Michaelis C, Ciosk R, and Nasmyth K (1997): Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. Cell 91, 35 – 45.

Murray AW, Solomon MJ and Kirschner MW (1989): The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. Nature 339, 280-286.

Nasmyth K (1996): Viewpoint: Putting the Cell Cycle in Order. Science, Vol. 274. Dec 6; 274 (5293): 1643-5.

Nicklas RB (1997): How cells get the right chromosomes. Science, Vol. 275. Jan 31; 275 (5300): 632-7.

Solomon EP, Berg LR and Martin DW (2002): Chromosomes, mitosis and meiosis in Biology 6th ed. Thomson Learning Academic Resources Center, United States, 189-215.

Rubenstein, Irwin, and Susan M. Wick: "Cell." World Book Online Reference Center. 2008. 12 January 2008.

http://www.worldbookonline.com/wb/Article.
De Souza CP, Osmani SA: "Mitosis, not just open or closed". Eukaryotic Cell, **6**: 9 (2007)1521–7

http://www.wikipedia.com (A web based encyclopedia)

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer:

1) In which phase of the following does eukaryotic cell withdraw usually from cell cycle?
   a. G1-phase
   b. S-phase
   c. M-phase
   d. G2-phase

2) Which of the following enzymes are involved in the cell cycle regulation?
   a. Peptidases
   b. Transferases
   c. Kinases
   d. Nucleases

3) In which phase of the following does DNA replicate?
   a. G1-phase
   b. S-phase
   c. M-phase
   d. G2-phase

4) By which process of the following does the growth of human offspring take place?
   a. Amitosis
   b. Mitosis
   c. Meiosis
   d. Endomitosis

5) At which phase of mitosis in plant cell, does formation of cell plate begin?
   a. Prophase
   b. Metaphase
   c. Anaphase
   d. Telophase

6) How many chromatids are found in prophase of a cell containing 22 pairs of homologous chromosomes?
   a. 22
   b. 44
   c. 66
   d. 88

7) In which phase of the following does the disjunction of sister chromatids take place?
   a. Prophase- I
   b. Metaphase- I
   c. Anaphase- I
   d. Telophase- I

8) Absence of which protein of the following may be expected in Li Fraumeni syndrome?
   a. P53
   b. P27
   c. Cdk
   d. MPF

9) In breast cancer outcome a high level of which protein of the following may be expected?
   a. Cyclin
   b. P27
   c. P53
   d. CdK

10) In which process of the following may the mitosis **not** be expected?
   a. Tissue repair
   b. Growth
   c. Gamete formation
   d. Clone formation

11) During which stage of mitosis may cytokinesis start?
   a. Prophase
   b. Metaphase
   c. Anaphase
   d. Telophase

12) How many chromosomes after mitosis does each new daughter cell contain, if the parent cell has 12 pairs of homologous chromosomes?
   a. 24 chromosomes
   b. 36 chromosomes
   c. 48 chromosomes
   d. 60 chromosomes

13) In which phase of meiosis do homologous chromosomes separate from each other?
   a. Prophase- I
   b. Prophase- II
   c. Anaphase- I
   d. Anaphase- II

14) Which event of the following occurs in mitotic division?
   a. Bivalent formation
   b. Synapse of homologous chromosomes
   c. Formation of genetically identical cells
   d. Formation of chiasmata

15) In which phase of the following may chromosomal non-disjunction be expected?
   a. Prophase-I
   b. Metaphase- I
   c. Anaphase- I
   d. Telophase- I

16) Which process of the following results in formation of zygote??
   a. Binary fission
   b. Development
   c. Cellular Growth
   d. Fusion of gametes

17) In which phase of meiosis may exchange of genetic material occur?
   a. Prophase- I
   b. Prophase- II
   c. Anaphase-I
   d. Anaphase-II

18) How many chromosomes are found in each gamete after meiosis, if the parent cell contains 24 pairs of homologous chromosomes?
   a. 12 chromosomes
   b. 24 chromosomes
   c. 36 chromosomes
   d. 48 chromosomes

19) Which of the following is a characteristic event of prophase- I?
   a. Separation of sister chromatids
   b. Chromosomes are located in equator
   c. Chromosomes move toward cell poles
   d. Pairing of homologous chromosomes

20) Which process of the following results in formation of haploid gametes?
   a. Endomitosis
   b. Asexual reproduction
   c. Sexual reproduction
   d. Mitotic division

21) Which phase of the following separates meiosis–I from meiosis–II?
   a. G1-phase
   b. G2-phase
   c. M-phase
   d. Interkinesis

22) Which event of the following results from damage of mitotic spindle microtubules?
   a. Chromosomes move quickly to the poles.
   b. Separation of sister chromatids.
   c. Exchange of genetic material.
   d. An immediate block to the process.

23) In which phase of the following does cycling cell enter when the cycling cell arrests mitosis?
   a. G_0 - phase
   b. G_1-phase
   c. G_2-phase
   d. S –phase

24) In which phase of the following does genetic material appear in form of the chromatin fibers?
   a. Prophase
   b. Anaphase
   c. Telophase
   d. Interphase

25) Which condition of the following **doesn't** occur in mitosis of plant cells?
   a. Condensation of chromosomes.
   b. Disappearance of nucleolus.
   c. Formation of mitotic spindle
   d. Cleavage furrow formation

26) Which event of the following does shortening of kinetochore microtubules in mitosis cause?
   a. Formation of chiasmata
   b. Separation of sister chromatids
   c. Formation of tetrad
   d. Exchange of genetic material

27) In which phase of the following does formation of the synaptonemal complex occur?
   a. Prophase
   b. Prophase- I
   c. Prophase- II
   d. Telophase

28) In which phase of meiosis do homologous chromosomes form bivalent?
   a. Prophase- I
   b. Prophase- II
   c. Anaphase- I
   d. Anaphase- II

29) In which process of the following can the chiasmata be seen by microscope?
   a. Endomitosis
   b. Amitosis
   c. Meiotic division
   d. Mitotic division

30) Which process of the following results in the genetic variations between individuals?
   a. Cytokinesis during telophase.
   b. Replication in interphase.
   c. Chromosomal disjunction in mitosis.
   d. Crossing over in meiosis.

31) Which of the following cells show uncontrolled mitotic division?
   a. Stem cell
   b. Cancerous cells
   c. Nerve cells
   d. Embryonic cells

32) Which of the following can be used to stop the cell division in labs?
   a. Growth factor
   b. Colchicines
   c. Epidermal growth factor
   d. Erythropoietin

33) If a cell passes the restriction point at end of G1- phase, which of the following will occur?
   a. Stop cell division.
   b. Enter in S-phase.
   c. Become inactive.
   d. Enter in the G_0 phase

34) Which structure of the following forms the mitotic spindle?
   a. Microfilaments
   b. Myosin filaments
   c. Microtubules
   d. Intermediate filaments

35) At the end of which phase of mitosis is the main ***check-point*** located in the cell cycle?
   a. M -phase
   b. G2-phase
   c. G1-phase
   d. Cytokinesis

36) Which structures of the following separate in anaphase -II of meiosis?
   a. Homologous chromosomes.
   b. Sister chromatids.
   c. Sex chromosomes.
   d. Non-sister chromatids.

37) Which phase of mitosis is the best phase for studying karyotype of human chromosomes?
   a. Prophase
   b. Metaphase
   c. Anaphase
   d. Telophase

38) Which process of the following moves the chromosomes in anaphase toward the cell poles?
   a. Shortening of kinetochore MT
   b. Assembling of astral microtubules
   c. Disassembling of polar microtubules.
   d. Gravity of cell poles.

39) Which of the following cells are arrested in the G_0 phase?
   a. Embryonic cells
   b. Stem cells
   c. Cancerous cells
   d. Nerve cell

40) In which phase of cell cycle does chromatin fiber condense to form visible chromosomes?
   a. G_1 –phase
   b. S- phase
   c. G_2 –phase
   d. M-phase

41) In which phase of the following the chromosomal non-disjunction may occur and result in abnormalities in chromosomal number?
   a. Prophase
   b. Metaphase
   c. Anaphase
   d. Telophase

42) In which phase of the following does exchange of genetic material in spermatogenesis take place?
   a. Leptotene
   b. Zygotene
   c. Pachytene
   d. Diplotene

43) Which cell of the following results from fusion of normal male and female gametes?
   a. Haploid cell
   b. Diploid cell
   c. Triploid cell
   d. Polyploid cell

44) By which product of the following spermatogenesis is characterized?
   a. Limited number of spermatozoa.
   b. Genetically identical spermatozoa.
   c. Unlimited number of spermatozoa.
   d. Production of Diploid cells

45) Which factor of the following may have influence on cell cycle?
   a. Nutrition.
   b. Temperature.
   c. Kinases
   d. All of above

46) In which situation of the following does formation of mature ovum occur?
   a. During embryonic life
   b. During ovulation
   c. Before ovulation
   d. After fertilization

47) Why do women have a fixed number of oocytes? Because primary oocytes:
   a. Multiply after birth.
   b. Develop after birth.
   c. Create during prenatal period.
   d. Develop after ovulation.

Answer of MCQs:

1) A	7) C	13) C	19) D	25) D	31) B	37) B	43) B
2) C	8) A	14) C	20) C	26) B	32) B	38) A	44) D
3) B	9) B	15) C	21) D	27) B	33) D	39) D	45) D
4) B	10) C	16) D	22) D	28) A	34) C	40) D	46) B
5) D	11) D	17) A	23) A	29) C	35) C	41) C	47) C
6) B	12) A	18) B	24) D	30) D	36) B	42) C	

Part II: Short answer questions.

1) What is the relationship between chromatin, chromosomes and genes?

_____
_____
_____

2) What is the importance of RNA for gene - expression?

_____
_____
_____

3) The chromosomes begin to move away from equator of the cell in phase _____

4) The chromosomes move toward the cell poles by: _____and_____

5) What are the types of microtubules that form the mitotic spindle?
a._____
b._____
c. _____

6) What is the function of mitotic spindle?
_____.

7) Metaphasic chromosome is composed of two _____chromatids.

8) In which stage of mitosis can you study the chromosomes and why? In_____ phase, because:
_____.

10) When are the sister chromatids exactly called chromosomes? _____

11) Chromatin fibers are located within_____ of eukaryotic cell and are chemically composed of _____and _____

12) The gene is a part from a DNA molecule which carries and controls _____of an organism.

13) The life cycle of a cell means:
_____

14) The cell cycle is formed of:
_____ and _____

15) When the nuclear division is not followed by cytokinesis, it results in formation of:
_____

16) Cell cycle includes: _____ and _____

17) Cytokinesis starts in animal cell by formation of _____ around the cell equator as a result of contraction of a ring of microfilaments.

18) Cytokinesis is a process of _____
_____

19) After cell division, the non - cycling cells enter _____phase.

20) DNA replicates (duplicates) in _____ phase of the cell cycle.

21) Time that is required to complete one cell cycle is called_____

22) Interphase is the stage between two successive _____, while interkinesis is the stage between _____and _____

23) _____is an example of non-dividing cell.

24) Microtubules of mitotic spindle are developed from _____

25) Two sister chromatids of metaphasic chromosome are tightly attached at_____

26) There are external signaling molecules or factors which regulate the cell cycle such as: _____ and _____.

27) The intracellular factors that regulate the cell-cycle are _____ and _____

28) CdK becomes active when it conjugates with _____

29) Dividing cells pass across the restriction points of cell cycle by a help of an active complex formed of _____and _____

30) Why does colchicine prevent the mitotic spindle formation and stop the cell cycle?
Because it _____
_____

PRINCIPLES OF HEREDITY

Chapter 2:
PRINCIPLES OF HEREDITY

Objectives

After you have studied this chapter you should be able to:

- *Define the terms: Gene, allele, locus, genotype, phenotype, dominant, recessive, homozygous, heterozygous, and test cross.*
- *Explain Mendel's principles.*
- *Explain the differences between codominance and incomplete dominance.*
- *Discuss the differences between mono- and dihybrid crosses and test cross, and relate them to Mendel's laws.*
- *Explain gene mapping and dosage compensation.*
- *Define the meaning of the following terms: Sex- influenced genes, X-linked genes, sex-linked traits and sex–limited traits.*
- *Explain the types of gene interactions.*

Mendelian Inheritance

The term heredity refers to a process of genetic transmission of a physical or mental characteristic that is carried by the genes, from the parents to the offspring. For example the color of the eye transfers from the parents to their children by the genetic inheritance of a particular gene that is responsible for the color of the eye.

The first scientist who had applied the basic rules of experimental science in genetics was ***Gregor Mendel.*** He is called *Father of Genetics*, began his experiments in 1856 to reveal how traits are inherited from parents to offspring in pea plants. He worked to count and categorize the data from a large number of samples and analyzed his results to recognize the pattern of appearance of traits in offspring. His experimental studies lead to discover and explain the following basic principles of heredity: *Principle of dominance, principle of segregation and principle of independent assortment.* These principles represent foundation of the science of basic genetics. Genetically, animals (including humans) and plants have two copies of gene, known as alleles, in their genome, one inherited from each parent.

- **True breeding line** is formed of genetically pure individuals. For example animal's color is carried by two alleles. If **B** is the allele of black and **b** is the allele of brown color, the individuals are genetically pure for black or brown, as they have **BB** for black and **bb** for brown, and produce one type of gametes (**B**) or (**b**).
- **The pure parents** produce only offspring expressing the same phenotype generation after generation. Black parent produces black offspring and a brown parent produces brown offspring.

- **Parental generation** (**P**) is formed of the parents of sons and daughters of F1; the first filial generation.
- **Filial generations** are the sons and daughters. *First filial generation* (**F1**) is formed of offspring that resulted from mating individuals of parental generation (fig. 2-1).
  Offspring of F1 result from mating of two genetically dissimilar pure parents; one pure black **BB** (genetically called **homozygous dominant**) and the other is pure brown **bb** (genetically called **homozygous recessive**), they will be genetically **Bb** and called **heterozygous or hybrid**.

  Second filial generation (**F2**) is formed of the offspring that result from mating two individuals from F1 generation.

- **Phenotype** is the physical appearance of an organism. Black and white colors in figure 2-1 are the phenotypes of mated individuals.

- **Genotype is** genetic constitution of individuals. For example: **BB (homozygous dominant)** is the genotype of black individual (black is the dominant color) and **bb (homozygous recessive)** is the genotype of brown individual (brown is the recessive color) in figure 2-1. While **Bb (heterozygous)** indicates the genetic composition of non-pure individual or **hybrid** organism.

Principle of Dominance

Dominance refers to the relationship between the effects of two genes controlling the same trait. The ***dominant*** gene from one parent masks the expression of the ***recessive*** gene of the other in a heterozygous individual, **hybrid**.

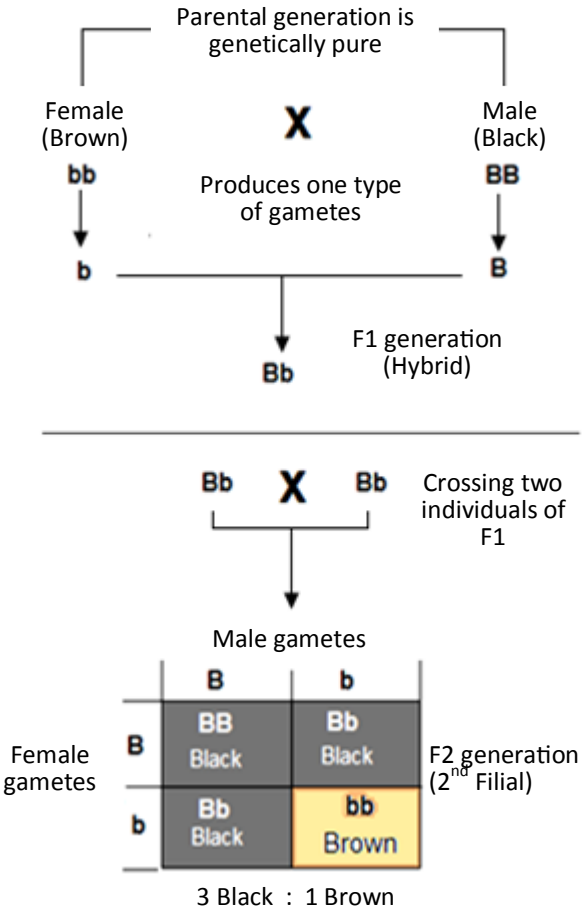


Fig. 2-1: Diagram demonstrates mating of brown female (bb) and black male (BB) individuals. If black color is dominant and brown color is recessive, all F1 generation will be black (Bb). F2 generation is formed of a mixture of black and brown individuals. The phenotypic ratio is 3 black (BB, Bb, Bb) : 1 brown (bb).

As demonstrated in fig. 2-1, if two dissimilar genetically pure parents; a black colored male (***homozygous***, BB) and a brown colored female (***homozygous***, bb) from true breeding lines are mated, results of this mating can be summarized in:

- All of progeny of F1 generation, ***hybrid*** will be genetically ***heterozygous*** black colored offspring *(**Bb**) because the gene of black color masks the expression of the gene of brown color in heterozygous (Bb) offspring.*
- Black color is the expressed trait; observed trait, so it is called *a **dominant trait***.
- Brown color is the hidden trait, so it is called a ***recessive trait***.
- The recessive trait, ***brown*** reappears in about 25% of individuals of F2 generation (fig. 2-1).

Generally, the ***dominant trait*** or observed trait in hybrid is expressed in capital letters (***B*** for black color), while the ***recessive trait*** or hidden trait is expressed in small letters (***b*** for brown).

Loci and Alleles

Gene is a segment of the DNA molecule. It carries genetic information required to control some specific characteristics of organism.

Locus (*pl., Loci*),
It refers to the location of a particular gene on a chromosome. Genes that occupy corresponding loci on a pair of homologous chromosomes are called *allelic genes* (fig. 2-2).

Alleles
There are alternative forms of genes called alleles. They represent the genes that control the traits, and are used to study and explain the genotype of individuals.

Notes:

- Each allele is represented by a ***capital letter*** with the dominant trait and ***small letter*** with the recessive trait.
- **Allelic *genes*** *do not necessarily carry the same information. For example;* gene of black and gene of brown color are allelic genes, because they control one trait (animal's color).
- *Genes which control different traits such as genes that cause hemophilia and color blindness are X-chromosome linked genes, they are **non –allelic genes**.*
- If alleles on homologous chromosomes are similar for a single locus (***BB*** *or* ***bb***), the individual genotype is called ***homozygous*** (fig. 2-3).
- If the alleles are different for a locus (***Bb****; one is dominant & the other is recessive*), the individual genotype is called ***heterozygous*** (fig. 2-3).
- According to the principle of dominance, the dominant allele (***B***) masks the expression of the recessive allele *(**b***) in heterozygous individual, hybrid (***Bb***).

H: Allele of normal blood.
h: allele of hemophilia.
B: Allele of black color.
b: Allele of brown color.
C: Allele of normal vision.
c: Allele of color blindness.

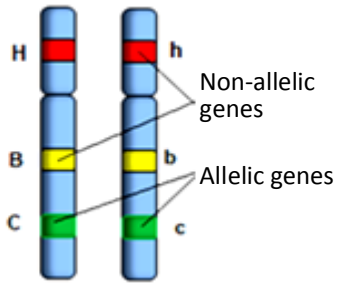


*Fig. 2-2: A pair of homologous chromosomes, one is paternal (from father) and the other is maternal (from mother), shows the allelic genes **C** & **c**, **B** & **b** and **H** & **h** that control one trait, and non-allelic genes B & A that control different traits.*

H: Allele of normal blood.
h: Allele of hemophilia.
B: Allele of black color.
b: Allele of brown color.
C: Allele of normal vision.
c: Allele of color blindness.

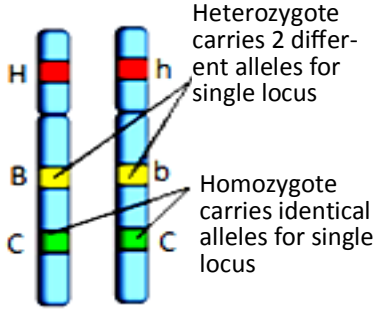


*Fig. 2-3: A pair of homologous chromosomes illustrates the difference between homozygous and heterozygous genotypes. **Homozygous** is formed of similar alleles **C** and **C** at corresponding loci, and **heterozygous** genotype is formed of two dissimilar alleles **B** & **b** for the corresponding loci.*

Incomplete dominance

If the offspring *express a mixture* of the traits of parents, the genes that control these traits are incomplete dominant alleles.

For example: When a homozygous black animal (*BB*) is mated with a homozygous white animal (*WW*), and all their offspring are hybrid gray color (*BW*) - displaying a mixture of both traits (fig. 2-4), The alleles for white and black colors are incomplete dominant genes for each other.

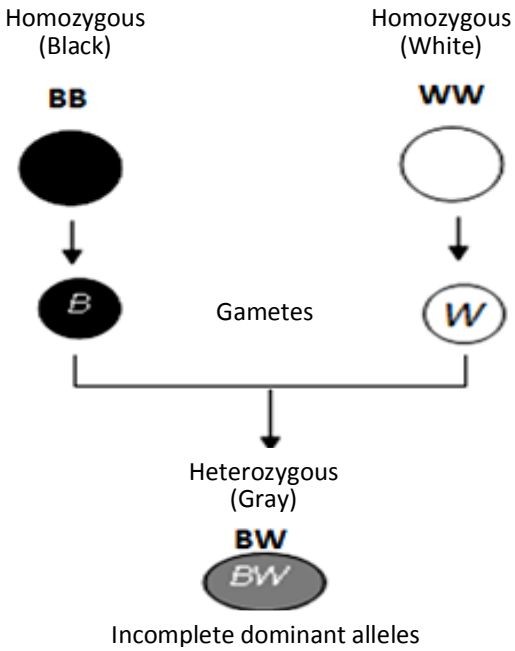


Fig.2-4: Diagram illustrates incomplete dominance. If mating of black and white individuals produces gray offspring that express a mixture of both traits. These traits are carried by incomplete dominant alleles. In this case neither white nor black color is a dominant trait.

Codominance

When two alleles for single locus are expressed in hybrid; heterozygous individuals, they are called codominant alleles.

For example: Alleles of AB blood group are co-dominant. In blood groups of human, there are four different blood types: A, B, AB, O determined by the presence of specific antigens (A-antigen & B- antigen) on the surface of RBCs.
These antigens are coded by two allelic genes: I^A gene for blood group A and I^B gene for blood group B (fig. 2-5).

- When both alleles are present in a person, they express themselves and the RBCs of this person express both antigen –*A* and antigen –*B* on their *surfaces* (fig. 2-5).
- Therefore, I^A and I^B are known as codominant alleles in AB -blood group.

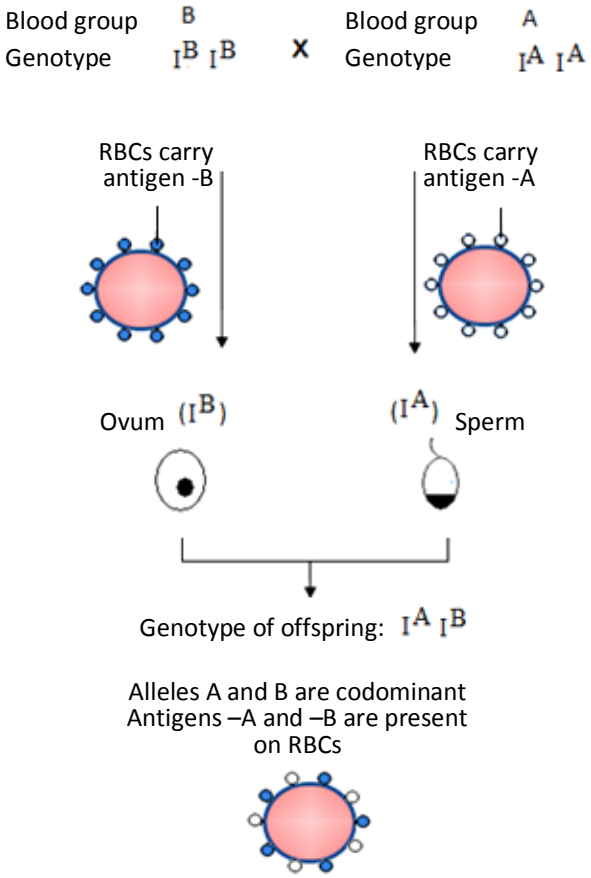


*Fig. 2-5: Diagrammatic illustration of codominant alleles. **I^A** and **I^B** are codominant alleles. They express their antigens on the surface of RBCs of **AB** blood group of heterozygous individuals.*

Principle of Segregation

(Segregation means separation)

Each trait is controlled by two alleles (for example **Bb** control color of eye). According to principle of segregation, these alleles segregate (separate) during meiosis, as a result of separation of the homologous chromosomes. This separation produces haploid gametes, each one carries only one allele, (allele **B** or allele **b**).

Principle of segregation has been demonstrated in figure 2-6. The basis of segregation of alleles during meiosis-I and II can be explained in the following steps:

- Each one of a pair of homologous chromosomes is formed of 2 sister chromatids.
- Meiosis-1 separates ***the homologous chromosomes*** from each other to produce two **haploid** cells.
- Meiosis-II separates ***the sister chromatids*** from each other and results in formation of **haploid** gametes:
  1. Each gamete carries one chromosome of each homologous pair.
  2. Each gamete carries only one allele, either (**B**) or (**b**).
  3. Each gamete has **one set** of chromosomes (n, **haploid**).

If maternal and paternal gametes are fused together, they form zygote that has 2 sets of homologous pairs of chromosomes.

What are the genetic characters of zygote?

Answer:
Zygote is a diploid cell which contains two sets of chromosomes (*2n*); one set (n) from mother (maternal) and the other set (n) from father (paternal).
A member of each set can be paired with a member of the other (fig. 2-7).

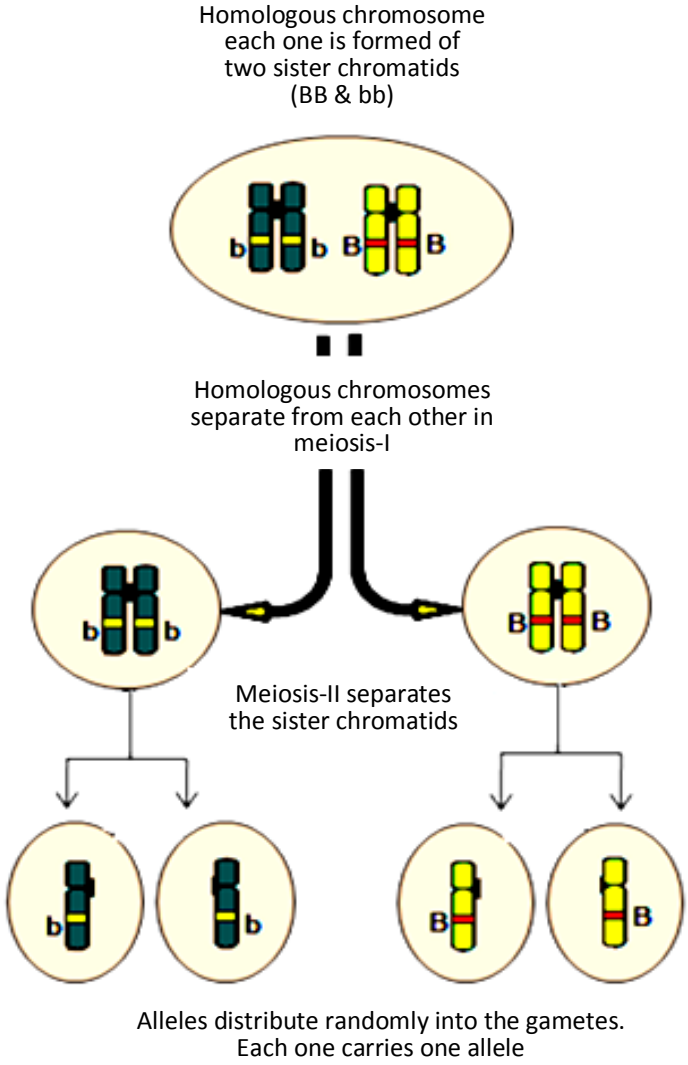


Fig. 2-6: Diagram demonstrates principle of segregation. Meiosis-I separates the homologous chromosomes, and in meiosis-II separates the sister chromatids. This separation produces haploid gametes. Each one carries only one allele. Two alleles for each trait segregate during meiosis.

What are the characters of homologous chromosomes?

Answer:

- Members of each pair of homologous chromosomes are similar in shape, pattern of bands, position of centromere and type of genetic information, and equal in size.
- Genes that are located at corresponding loci on a pair of homologous chromosomes are called allelic genes.
- Genes that are not located at corresponding loci on a pair of homologous chromosomes are called non-allelic genes (fig. 2-2).

What are the characters of allelic genes?

Answer:

- They occupy the same locus on each pair of homologous chromosomes.
- They control the same kind of trait.
- In heterozygous (Bb), they contain non-identical information. For example, two allelic genes, one controls black color and the other for brown.
- In homozygous (bb or BB) they contain similar genetic information (figure 2-3).

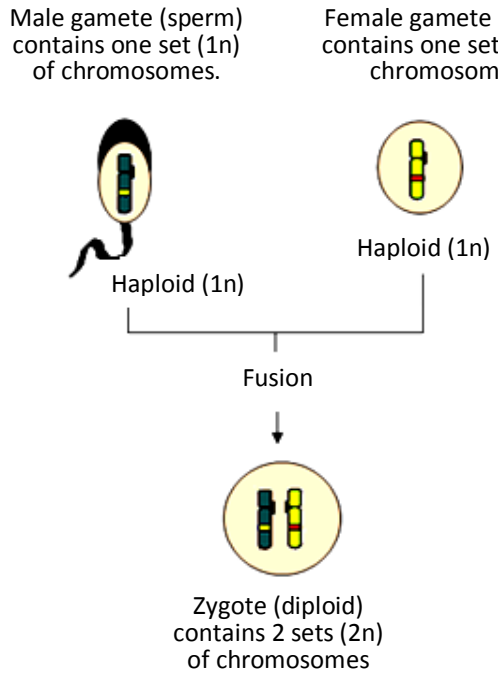


Fig. 2-7 (right): Diagram shows the genetic characters of gametes and zygote. Zygote is a diploid cell, receives one set of chromosomes from male (sperm) and other set from female (ovum). A member of each set can pair with a member of the other set.

Monohybrid Cross

It occurs between two organisms carrying different alleles for single locus. This cross is used to study the inheritance pattern of the different alleles that control a single trait.

For example to study the relationship between the alleles that control the color (black and brown) of hair, pure parental generation is required. The *steps* of monohybrid cross have been illustrated in figure 2-8.

- It begins by crossing two individuals of genetically pure parental generation. One is homozygous **black BB** (black is **dominant** trait) and the other is homozygous **brown bb** (brown is a **recessive** trait).

- This crossing produces offspring of F1 generation which are **hybrid**, genetically **heterozygous** (***Bb***). They express the dominant **black phenotype**.

- When two black heterozygous hybrid individuals (Bb) of F1 generation are crossed, this cross yields offspring of F2 generation. Genetically gametes of male or female individuals from F1 generation divide into 50% carrying allele (*B*) and 50% carrying allele (*b*). This means that: ½ of male gametes carries allele ***B*** and the other half carries allele ***b***. Also ½ of female gametes carries allele ***B*** and the other half carries ***b***.

After mating, phenotypically 75% of offspring of F2 express the ***dominant trait*** (black) while the rest (25%) express the ***recessive trait*** (brown). So, the phenotypic ratio of F2 offspring is 3 black to 1 brown.

Offspring of F2 generation in this cross are genetically different, and they also can be classified genetically into: 25% ***BB*** *(black), 50%* ***Bb*** *(black) and 25%* ***bb*** *(brown).*

Examples of monohybrid cross:

1) If two tall individuals were mated and their offspring is formed of 30 tall and 10 short individuals, this means that:

- *The short character is recessive*
- *The tall character is dominant*
- *The genotype of both parents is Tt (T= tall, t= short) heterozygous individuals.*

2) If two heterozygous individuals for single locus are mated and produced 400 offspring, we expect that:

- *The phenotypic ratio of offspring can be classified into:*
  - *The individuals that have the phenotype of dominant allele are 300.*
  - *The individuals that have the phenotype of recessive allele are 100.*
- *The genotypic ratio of offspring can be classified into:*
  - *The individuals that have the genotype of the homozygous recessive (tt) are 100.*
  - *The individuals that have the genotype of the homozygous dominant (TT) are 100.*
  - *The individuals which have the genotype of the heterozygous (Tt) are 200.*

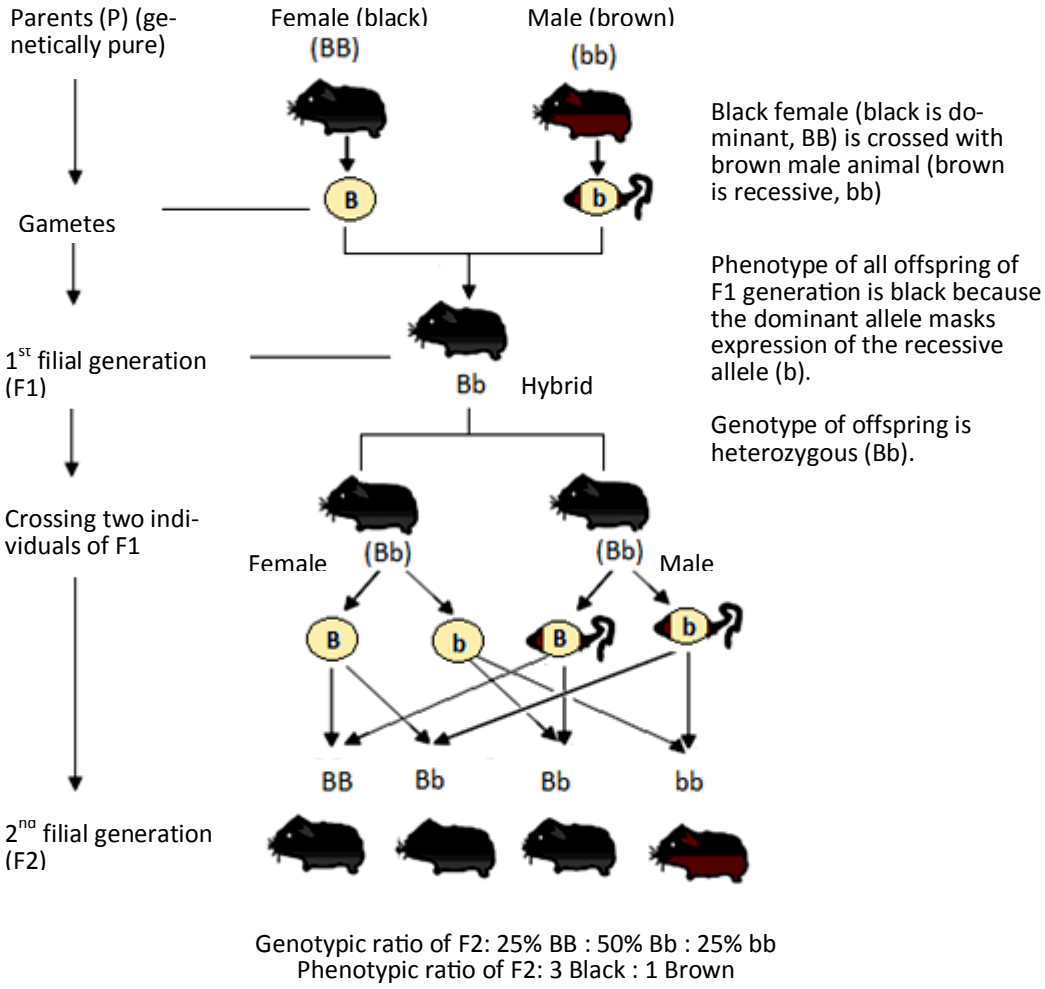


*Fig. 2-8: A monohybrid cross shows the inheritance pattern of alleles for single locus. A homozygous black guinea pig (black color is dominant, **BB**) and homozygous brown guinea pig (brown color is recessive, **bb**) represent pure parental generation. Offspring of F1 are black heterozygoushhybrid. Offspring of F2 generation iare formed genetically of **1 BB : 2 Bb : 1 bb**, and their phenotypic ratio is 3 black : 1 brown.*
***Note that:** Black phenotype could be either BB or Bb genetically, so the phenotype doesn't always reveal genotype.*

Monohybrid Test Cross

The phenotype of heterozygous individuals doesn't always reveal the genotype, for example the genotype of a black animal may be **homozygous dominant *BB* or heterozygous *Bb*.** For detection of the genotype of an unknown individual *a monohybrid test cross* can be carried out between ***homozygous recessive*** individual and the individual of unknown genotype.

Monohybrid test cross is important during the breeding processes. To improve productivity, test cross is used to detect and exclude the recessive alleles that result in a decrease in animal or plant production.

- To identify the unknown genotype of a black animal that could have the genotype *BB or Bb*, the animal with unknown genotype is mated with a homozygous recessive brown animal (*bb*).
  - If all offspring are black as in figure 2-9, the genotype of unknown is homozygous dominant (BB).
  - If offspring are divided into about 50% black and 50% brown, as in figure 2-10, the unknown is heterozygous (Bb).

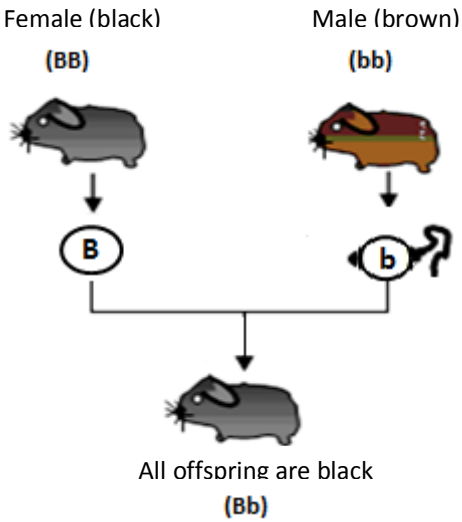


Fig. 2-9: Monohybrid test cross (crossing of two individuals (one with unknown genotype and the other is homozygous recessive). If all offspring express the same phenotype of unknown, the genotype of the unknown is homozygous dominant.

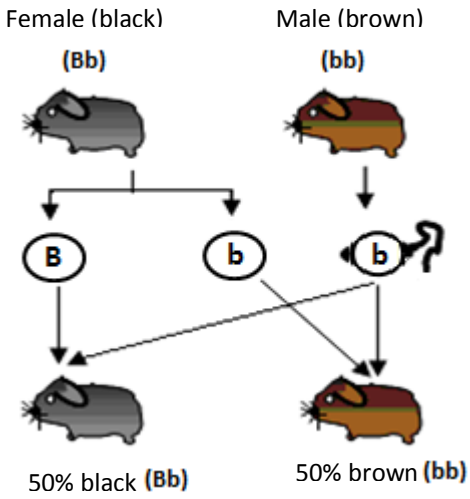


Fig. 2-10: Monohybrid test cross. If about 50% of offspring express the phenotype of the unknown individual and 50% of offspring express the other phenotype, the genotype of unknown is heterozygous.

Law of Independent Assortment

According to law of independent assortment, the alleles of different loci on non-homologous chromosomes segregate (separate) during meiosis independently of each other.

As in figure 2-11, there are two loci for heterozygous animal; one controls hair length (**Ss**) and the other controls hair color (**Bb**) are found on two different pairs of homologous chromosomes of germinal diploid cells that divide by meiosis to produce haploid gametes.
Chromosomes carrying alleles of these loci duplicate during interphase of cell cycle. In meiosis-I (fig. 2-11) the random distribution of these chromosomes may result in formation of chromosomal composition as in (1) or as that chromosomal composition in (2). Because there are equal chances for occurrence of chromosomal composition in (1) or that in (2), the probability for occurrence of the composition in (1) is 50% and 50% for that in (2).

Anaphase-I separates the homologous chromosomes from each other, while anaphase-II separates the sister chromatids of each chromosome.

This division produces equal ratios of four types of gametes: **BS, Bs, bS and bs**, because the chromosomes distribute randomly into gametes and the allelic genes are inherited independently of each other.

Independent assortment of alleles results in formation of parental gametes which are identical to that of parents (**BS** and **bs**) and gametes which contain new chromosomal combinations, new mixture of genetic information (**bS** and **Bs**).

Therefore, in addition to exchange of genetic material at crossing over, the random distribution of chromosomes in meiosis-I plays an important role in occurrence of variations in genetic constitution between individuals.

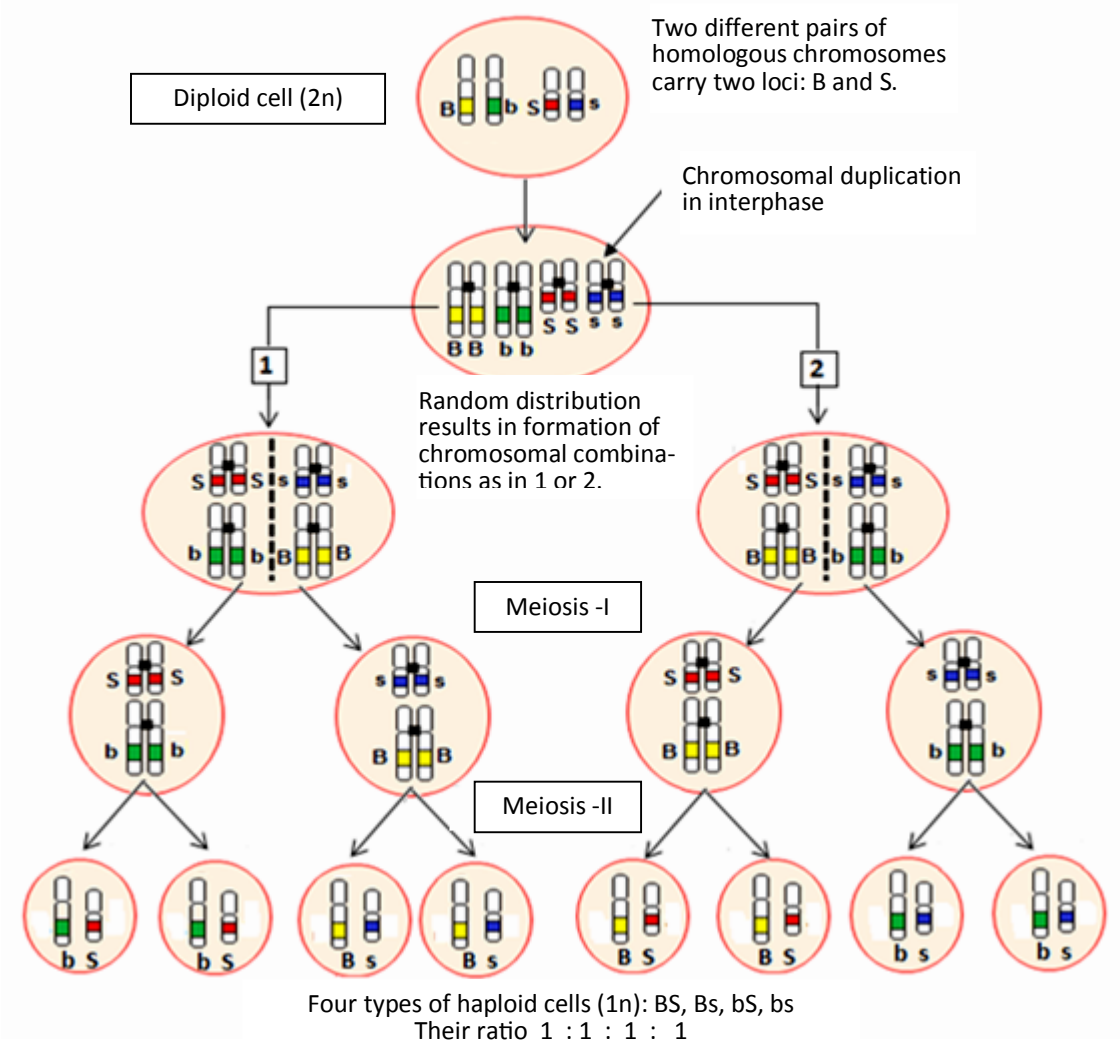


*Fig. 2-11: Alleles located on different pairs of homologous chromosomes assort independently in meiosis. They distribute randomly to form different chromosomal combinations as in (1) or (2). In meiosis-I they separate independently from each other. The ratio of the four resulted gametes **BS, Bs, bS** and **bs**, is 1:1:1:1. The ratio of parental gametes **BS** & **bs** to gametes with new mixture of chromosomal combination **Bs** & **bS** is 1:1.*

Dihybrid Cross

When male and female individuals are crossed to study the hereditary pattern of different alleles for two or more loci controlling different traits, this type of crossing is called dihybrid cross.

- The dihybrid cross can be considered as two simultaneous and independent monohybrid crosses.
- Figure 2-12 illustrates the inheritance pattern of different alleles in dihybrid cross.
- One pair of alleles (**BB**) is located on one pair of homologous chromosomes and the other pair (**SS**) of alleles is located on a different pair of homologous chromosomes.

- At crossing for example (fig. 2-12) a homozygous black short-haired male rabbit (**BBSS**) and a homozygous brown long-haired female rabbit (**ssbb**), if black color is dominant over brown, and short hair is dominant over long hair. Because **BBSS** male rabbit produces one type of sperms (**BS**), and **bbss** female rabbit produces one type of ova (**bs**) this crossing will produce offspring of F1 generation which express only the phenotypes of dominant traits (black short-hair), but they are genetically heterozygous; hybrid (**BbSs**).

Punnett square of Sir Reginald; grid structure in figure 2-12, is used to illustrate all possible combination of sperms and ova, when individuals (Bb Ss) of F1 generation are crossed. It facilitates counting the individuals and studying the inheritance pattern of the alleles in F2 generation in dihybrid cross.

The phenotypic ratio of F2 offspring is:
9 : 3 : 3 : 1.
- 9 short-haired black
- 3 long-haired black
- 3 short-haired brown
- 1 long-haired brown

This phenotypic ratio is only true for **unlinked genes.** They are located on different pairs of homologous chromosomes and their inheritance pattern follows the rules of principle of independent assortment of Mendel.

Write the differences between linked and unlinked genes?

……
……
……
……

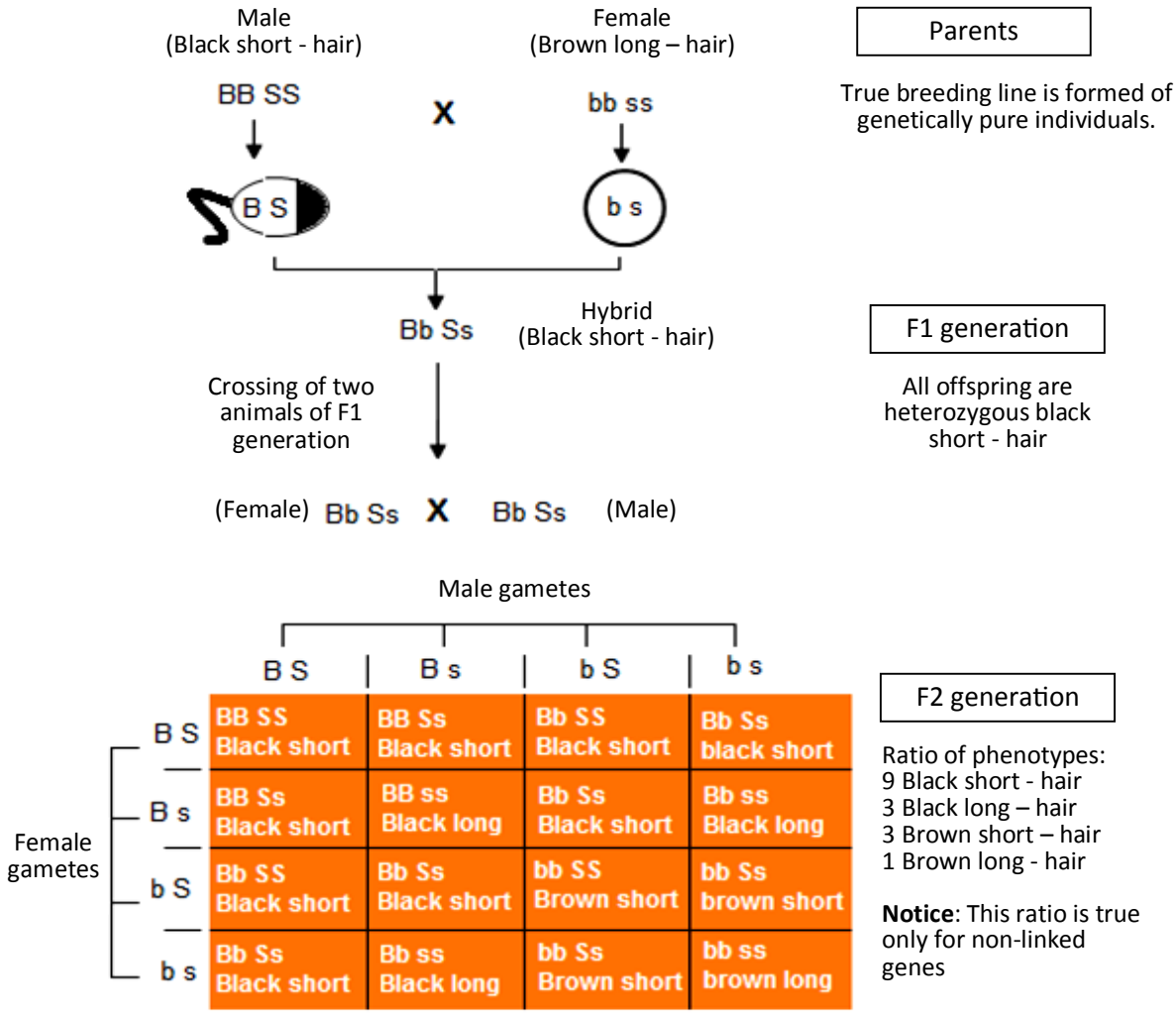


Fig. 2-12: Punnett square illustrates F2 offspring of a dihybrid cross. The alleles of two different loci on non-homologous chromosomes are inherited independently from each other. Phenotypic ratio of F2 generation is: ***9 black short-hair : 3 black long-hair : 3 brown short- hair : 1 brown long-hair****. This 9:3:3:1 ratio is known as a* ***di-hybrid ratio*** *of independent assortment and random fertilization.*

Linked Genes

Genes that occupy loci distributing on the same chromosome are called linked genes. For example, humans have 46 groups of linked genes because human somatic cell contains 46 chromosomes, and bacteria have single group of linked genes, because the bacterial cell contains single folded circular genophore if the small bacterial plasmids have been excluded.

The inheritance pattern of linked genes

Linked genes don't follow the inheritance pattern of Mendel's law of independent assortment because they are located on the same pair of homologous chromo-somes and tend to be inherited together, unless they are separated by crossing over (fig. 2-13).

- Alleles of two linked loci (**H** & **B**) in figure 2-13 are located on a pair of homologous chromosomes.
- If one chromosome carries the alleles **B** & **h**, and **b** & **H** are located on the other, the produced gametes will only be parental gametes (**bH**) and (**Bh**).
- If the crossing over takes place between non-sister chromatids, it results in formation of new recombinant gametes (**BH**) and (**bh**).

- Therefore results of mating heterozygous (**Bb Hh**) and homozygous recessive (**bb hh**) for two linked loci will deviate from those expected for two non-linked loci because the frequency of recombinant gametes formation depends on the distance between the linked genes.

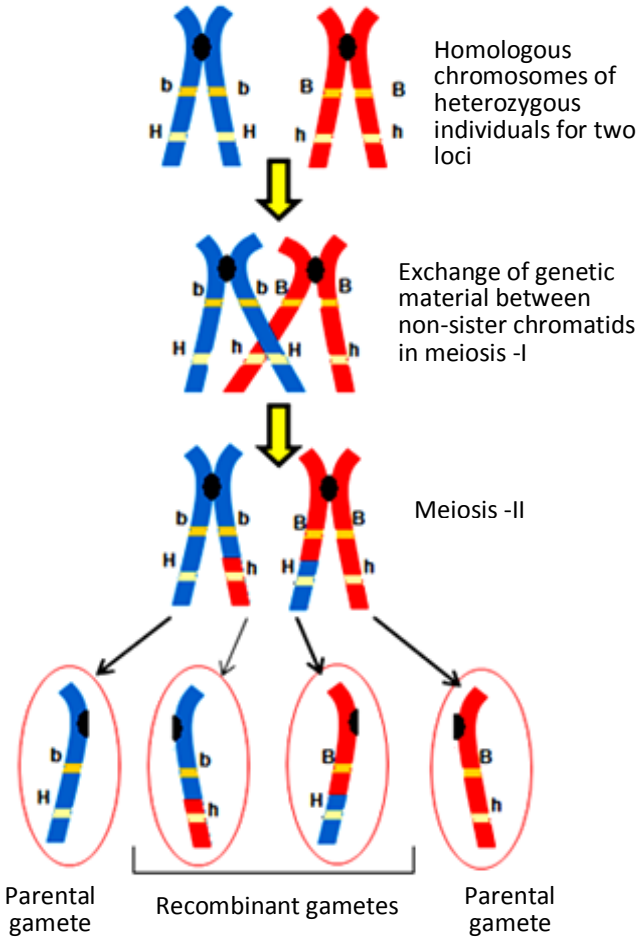


Fig. 2-13: Exchange of segments between non-sister chromatids of a pair of homologous chromosomes in crossing over may cause separation of linked genes and formation of the new recombination. Genes located away from each other have larger probability to separate than genes that are closer to each other.

- If the distance between linked genes allows occurrence of crossing over (fig.2-15a & 2-15b), the appearance of recombinant individuals in offspring occurs but their ratio will be varied, depending on the frequency of recombination. The genes are linked with recombination.
- As the distance between two genes increases, the probability of occurrence of crossing over will be high (fig. 2-13). Crossing over results in formation of a new recombination (new group of genes on chromosome) - mixing of genetic material.
- Both crossing over between linked genes and random distribution of non-linked genes into the gametes are considered the major sources of variation in the sexual reproduction.

Two-Point Test Cross

To differentiate linked from unlinked genes a *two-point test cross* is used. It is called **dihybrid test cross** - it involves two different loci (fig. 2-14 and 2-15).

After crossing between homozygous recessive individual and individual of unknown genetic constitution for two different loci; **B and H**, the following results will be obtained:

- If the genes are unlinked, they follow the inheritance pattern of principle of independent assortment (fig. 2-14). In this case the offspring is formed of recombinant and parental individuals and their ratio will be 1 : 1 : 1 : 1 (50% parental and 50% recombinant).
- If the genes are completely linked and inherited together as a unit, in this case the offspring will be free of the recombinant individuals due to production of recombinant gametes will not occur.
- When the linear distance between two linked loci increases and becomes enough to separate them by crossing over, recombinant individuals will appear in offspring. Their percentage depends on the frequency of recombination; it increases with increase in the distance between the linked loci.

DNA-marker:

It is an example of linked genetic DNA- sequence that is closely linked to mutant gene and inherited along with it. For many genetic diseases such as Huntington's disease, detection of the linked DNA–marker is used for diagnosis.

What kinds of crosses result in the following phenotypic ratios?

- **Ratio 3:1**?

Answer: Both parents are heterozygous for single locus (monohybrid cross).

- **Ratio 1:1**?

Answer: One parent is heterozygous and the other is homozygous recessive for single locus (monohybrid test cross).

- **Ratio 9:3:3:1**?

Answer: Both parents are heterozygous for two unlinked loci (dihybrid cross).

- **Ratio 1 : 1 :1 :1**

Answer: One parent is heterozygous and the other is homozygous recessive for two unlinked loci (dihybrid test cross).

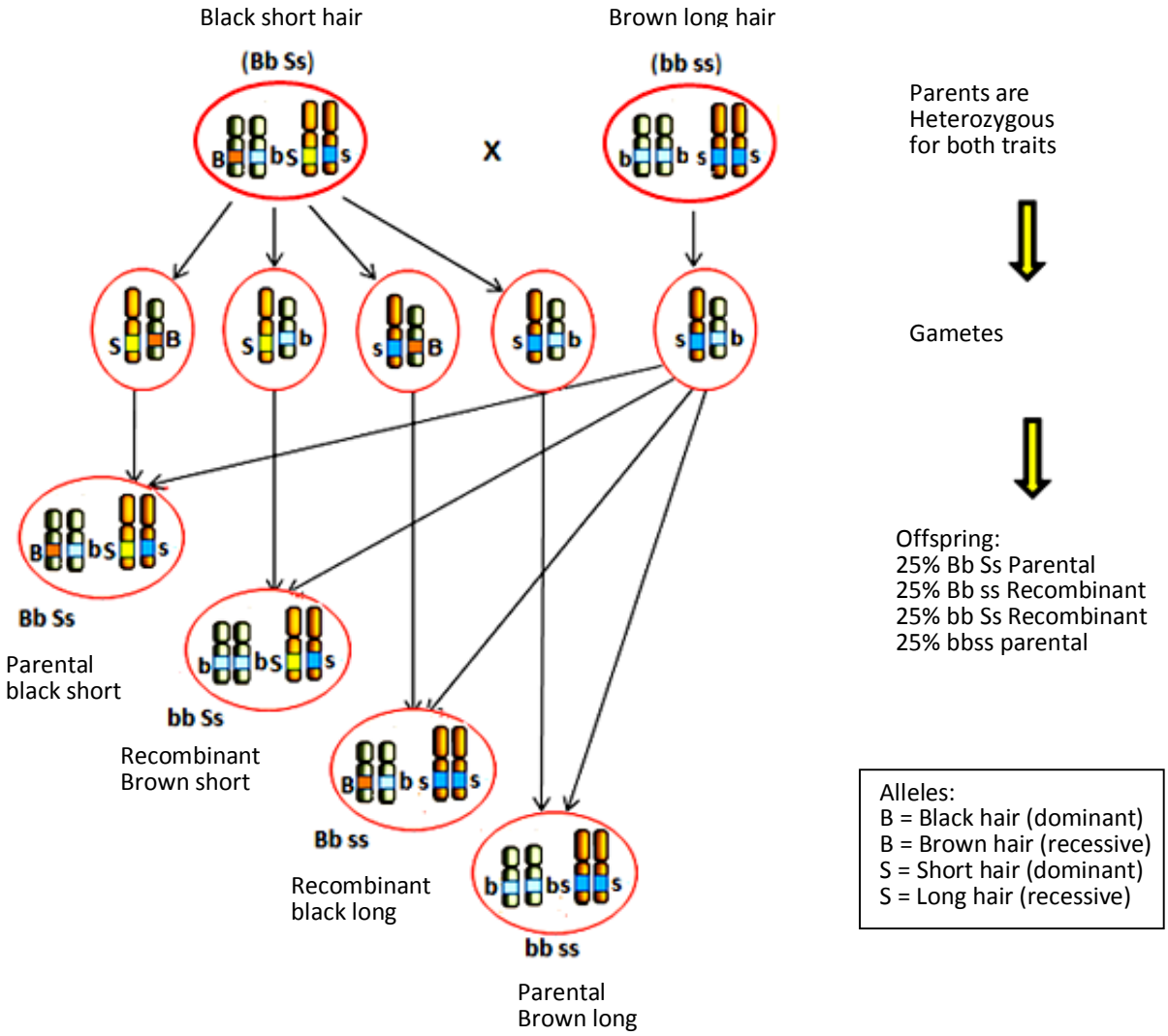


Fig. 2-14: Detection of linked genes by the two point test crosses. When the genes are unlinked, they are located on two different pairs of homologous chromosomes and follow the law of independent assortment. At crossing two individuals one is heterozygous and the other is homozygous recessive for both traits, it produces parental and recombinant individuals with the following ratio 1 : 1 : 1 : 1.

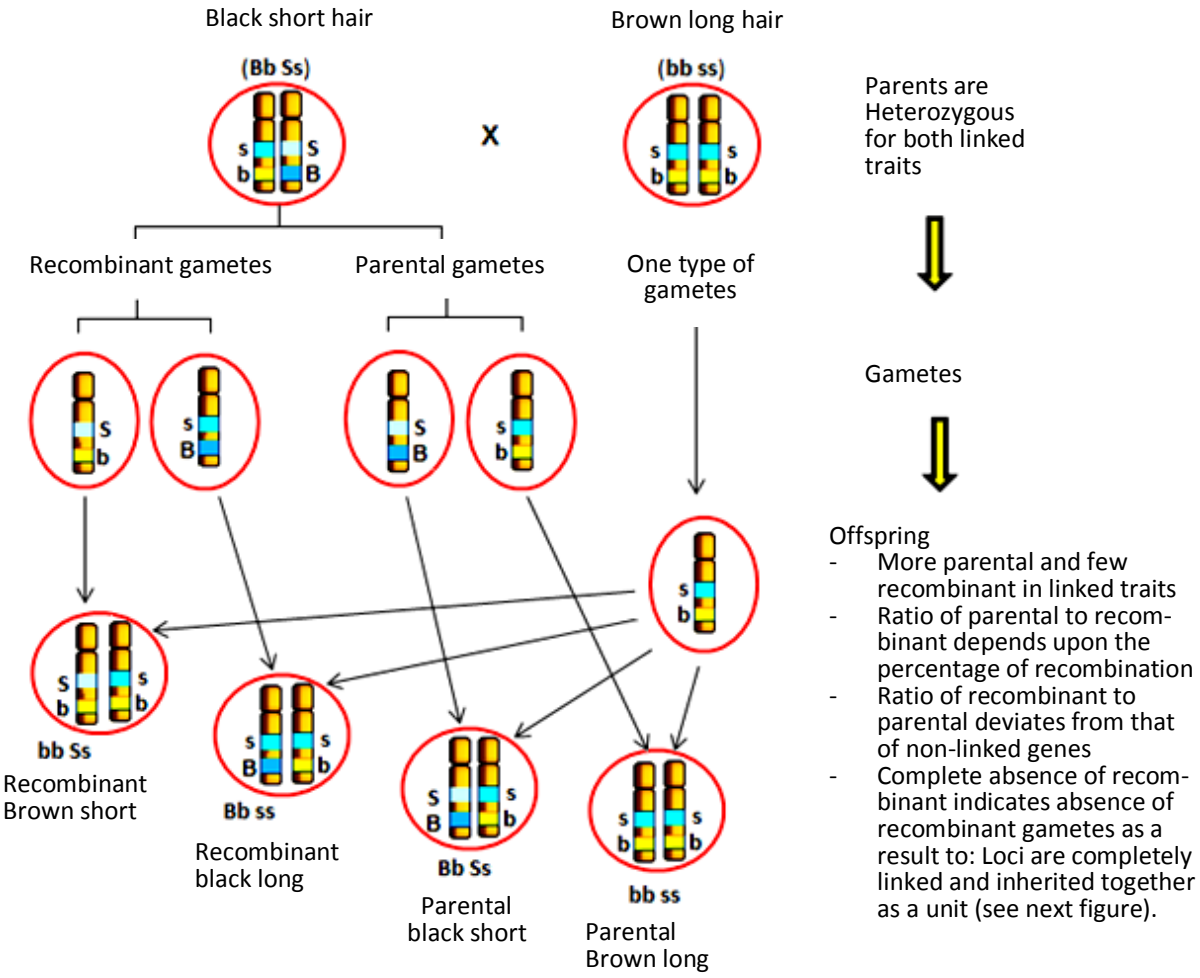


Fig. 2-15a: Detection of linked genes by a two-point test crosses. If the genes are linked, they are located on the same pair of homologous chromosomes and affected by crossing over. Mating of homozygous recessive and heterozygous for both traits produces more parental individuals and few recombinant individuals and their ratio depends upon the percentage of recombination.

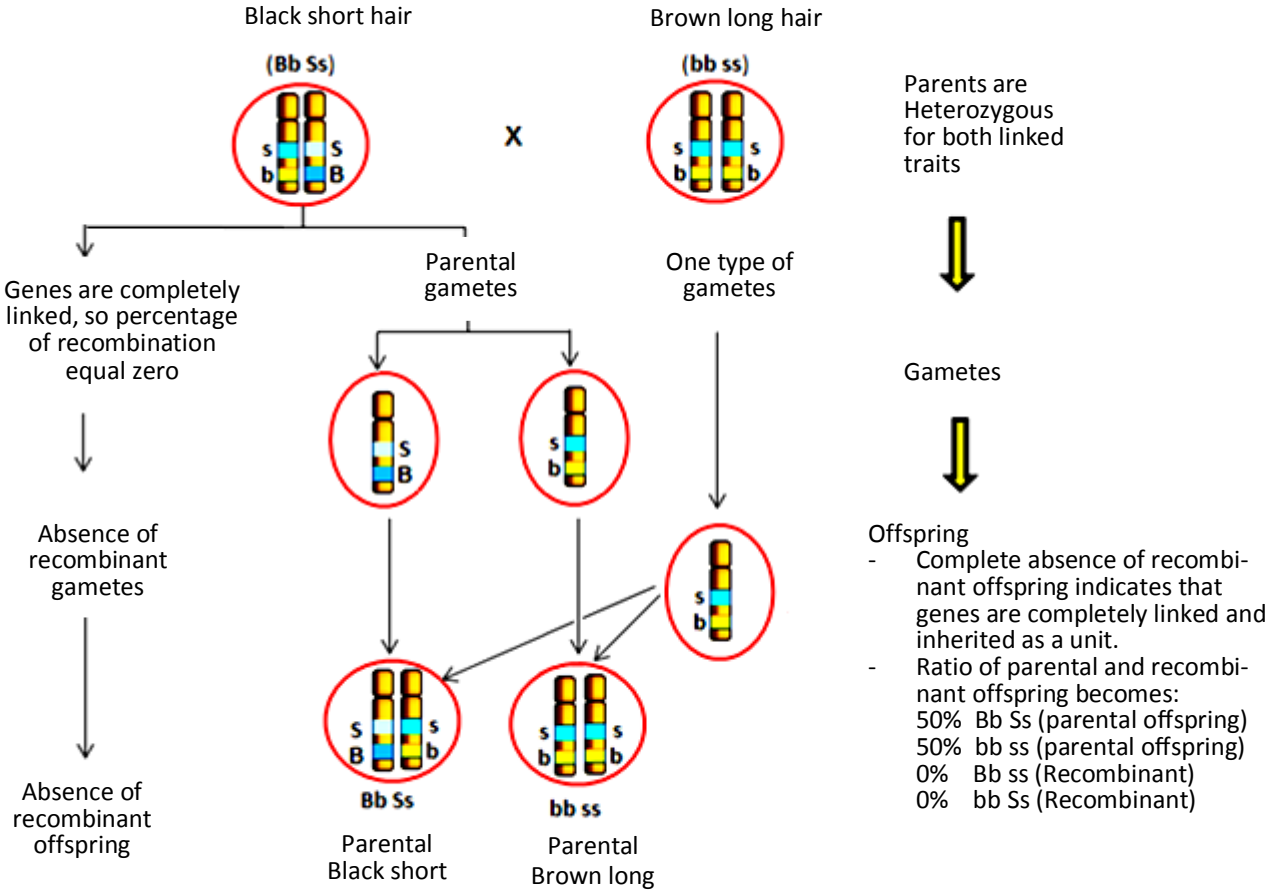


Fig. 2-15b: Detection of linked genes by a two-point test -cross. If genes of two loci are located on the same pair of homologous chromosomes and completely linked, the percentage of recombination equals zero as a result to absence of the recombinant gametes. So recombinant offspring are not produced and the offspring is only formed of parental individuals.

Gene Mapping

Gene mapping is a process of determination the linear distance between linked genes which occupy neighboring loci and distribute along the chromosome.

- The rate of recombination is determined by the rate of occurrence of crossing over between genes on homologous chromosomes. It has been used to determine the linear order of linked genes.
- So, calculating the frequency of crossing-over or the percentage of recombination for two loci determines the linear order of linked genes.
- If one map unit distance on a chromosome is the distance which allows for recombination to occur, it is called the rate of recombination is 1% of the time.

- If the genes A, B and C are linked and the frequency of crossing over for A and B is 40%, and for A and C is 15%, and for C and B is 25%, the gene mapping from this data is **A C B; gene C is located in the middle between the genes A** and **B.**

How can you calculate the frequency of recombination?

To calculate of the frequency of recombination between two loci (for example: locus **A** and locus **B**) is used the following equation.

Percentage of recombination= $\frac{\text{Recobinant individuals (A + B) x 100}}{\text{Total number of offspring}}$ = map units

.Example for calculation of linear distance between two loci:

- If an ***AaBb*** individual is mated with an ***aabb*** individual, and their offspring are classified into ***Aabb***= 10, ***aaBb***= 5, ***AaBb***= 30 and ***aabb***= 40, calculate the percentage of recombination.

Answer:

- Two parental classes= **AaBb** and **aabb**
- Two recombinant classes= **Aabb** and **aaBb**
- The recombination% = (10 + 5) x 100 /85 = 17.6%
- The linear distance between the two loci = 17.6 map units.

- Recombination is high between loci that are located far apart and low between loci that are found close to each other.

- Calculate the linear distance between locus A and locus B, if the percentage of recombination between the loci A and B equals 10%.

Answer:

1% of recombination between 2 loci = 1 map unit. Therefore, the linear distance between loci A and B = 10 map units apart.

Sex–Chromosomes

The somatic cells of human have 2 sex chromosomes (**X** and **Y**) and 44 (22 pairs) autosomal chromosomes. The sex (male or female) of the organism is determined by sex chromosomes (X and Y).

- The composition of **X** and **Y** chromosomes is morphologically and genetically different.
- Human males have 22 pairs of autosomes plus one **X**-chromosome and one **Y**-chromosome.
- Human females have 22 pairs of autosomes plus 2 **X**-chromosomes.
- **X**-chromosome contains many loci that are required in both sexes.
- **Y**-chromosome contains only few genes, including one or more genes for maleness.

In mammals females are ***homogametic*.** They contain identical sex chromosomes, **2 X**-chromosomes and produce one type of gametes; **X**-gametes (ova), while males are ***heterogametic*** their cells contain two different sex chromosomes (a single **X**-chromosome and a smaller **Y**-chromosome) and produce two different types of sperms; **X**- and **Y**-sperms.

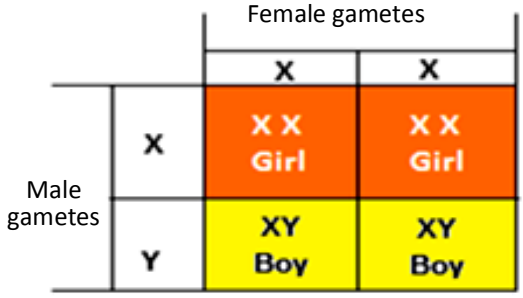
Sex determination

The sex of the embryo is determined by which sperm the ovum is fertilized (**X-** or **Y**- sperm) as in the next figure 2-16.

X- Linked traits

They are controlled by alleles for loci carried by X-chromosomes, such as *alleles of* ***color blindness*** and ***hemophilia***. They are genetic disorders that follow the transmission pattern of X-chromosome.

- The most common abnormal X-linked traits; hemophilia and color blindness, are recessive.
- Females become only diseased if they are homozygous recessive (allele of the disease is carried by both X-chromosomes.
- Males become diseased if they have the recessive allele on X-chromosome.
- They are more common in males than females.



Ratio of male to female gametes in every pregnancy is 1 : 1 (primary sex ratio).

Fig. 2-16: Sex of the embryo is determined by which sperm the ovum is fertilized (X or Y-sperm).

Color blindness

Alleles of color vision are carried by X- chromosome, X-linked alleles. Allele of normal color vision is dominant and allele for color blindness is recessive.

To be color blind (fig. 2-17), a female must inherit alleles of color blindness from both parents. A male inherits an allele of color blindness from a carrier mother or a color blind mother.

Inactivation of X-chromosome

Inactivation of X-chromosome takes place in a process known as **dosage compensation** which involves inactivation of one of the 2 X-chromosomes in female in order to equalize the gene - products of X-chromosomes in female and male.

- The inactive X- chromosome forms dark spot of chromatin at the edge of the nucleus in cells of female called *Barr body*. *Canadian scientist Murray Barr (1908-1995) was the first who saw Barr body in the cells.*
- Number of Barr bodies equal number of the inactive X-chromosomes.
- Inactivation of X-chromosome occurs randomly in female; in one type of embryonic cells one X is inactivated while the other X chromosome is inactivated in different cells of the same type.

Variegation is an example of X -chromosome deactivation in female cat when the coat expresses different colors. The alleles for black and yellow coat (X^B and X^Y) are carried on X chromosome. Randomly deactivation results in development of **patches of black and yellow color** from embryonic cell. Black coat is expressed in embryonic cells with deactivated X^Y and the yellow color is expressed in other embryonic cells with deactivated X^B.

Random deactivation of X –chromosome in embryonic cells causes development of **patches of skin lacking sweat glands** within the normal skin in human female carrying X-linked mutant recessive gene that causes absence of sweat glands.

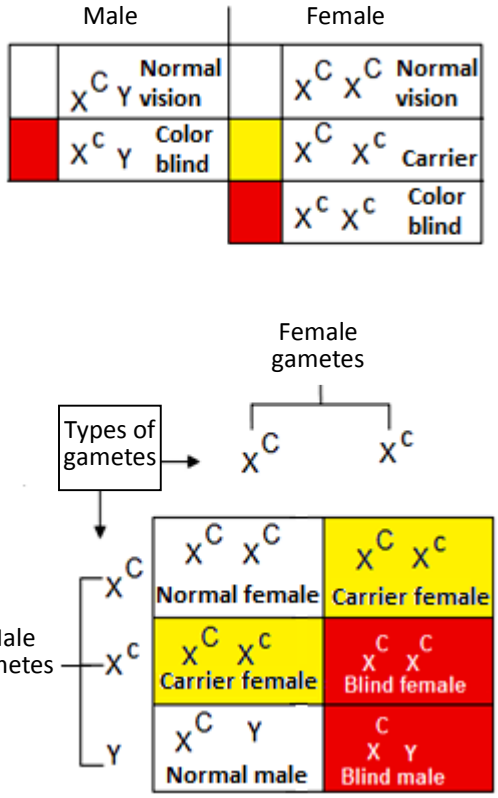


Fig. 2-17: Color blindness inheritance.
-Above: The genotype of both diseased and normal male and female.
-Below: Table shows the probabilities of color blindness transmission form parent into offspring.

What are the differences between Y and X chromosomes?

……

……

Sex –Influenced Genes

They are responsible for appearance of specific characters in either males or females.

- They are autosomal genes. Their trait-expression is affected by the individual's sex.
- The gene expression in female is different from that in male, although both sexes have the same genotype.
- The sex-influenced trait is more effective in males than females because the male sex hormone, testosterone, is involved strongly in the trait -expression.

Baldness which is the premature loss of hair on front and top of the head is an example of sex-influenced trait. It is more common among males than females, see table 2-1.

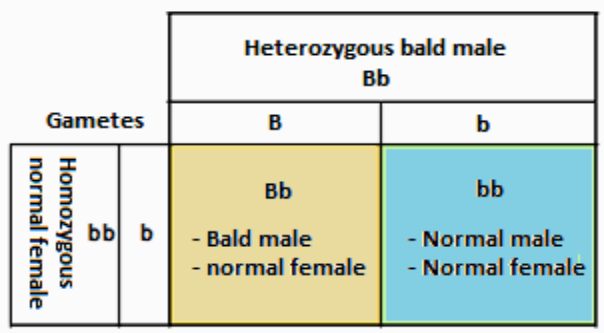
Table 2-1: It shows the influence of sex on inheritance pattern of baldness.

	Genotype	Phenotype
Female	**BB** **Bb (recessive)*** **bb**	**Bald** **normal** **normal**
Male	**BB** **Bb (dominant) *** **bb**	**Bald** **Bald** **normal**

* In heterozygous, the allele is recessive in female and dominant in male

What is the result of mating a heterozygous bald man and a homozygous normal woman?

Answer:
Gene of baldness is dominant (B) in male and recessive (b) in female.
½ of the sons will be bald and the other ½ will be normal, while all females will be normal



Sex –Limited Genes

They are responsible for the secondary sexual characters in either male or female. They are not necessarily located on the sex chromosomes, but may be found on autosomal chromosomes.

- The **beard** producing genes are found not only in man but also in women, but women don't usually have a beard, unless some abnormalities occur in hormone secretion.
- Also, the **breast** is characteristic of women, but if a man has hormonal secretion disturbance, he will have a feminine breast.

Gene Interaction

Pleiotropy

It occurs when a single gene influences multiple phenotypic traits. A new mutation in the gene may have an effect on some or all traits simultaneously. *Ability* of one single gene to affect many characteristics of an organism is called pleiotropy. (***Pleio*n** means "**more**" and ***tropy*** means "to **turn**, to **convert**").

Some examples of pleiotropy:

- ***Marfan syndrome:*** In *Marfan syndrome a single defective gene results in abnormality of the eyes, skeleton, and the large blood vessels.*
- ***Cystic fibrosis****: In cystic fibrosis genetic mutation codes for protein that is responsible for production of thick mucus substance. Accumulation of the thick mucus secretion causes tissue damage in gastrointestinal tract and respiratory system.*

Epistasis

It refers to a condition in which the effect of one gene is modified by one or several other genes, which are sometimes called **modifier genes**. Genes occupy different loci and one of them masks or changes the expression of the other gene (**quantitative loci**).

Epistasis can be contrasted with dominance, which is an interaction between alleles at the same gene locus. The gene that masks the expression of the other is considered *epistatic gene* to the masked gene.

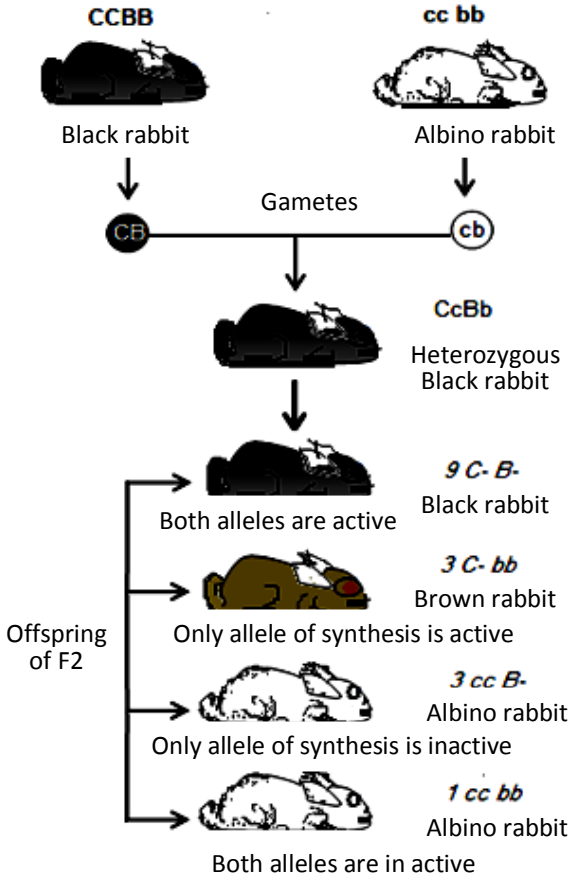


*Fig. 2-18: Epistasis. Two alleles control synthesis (**CC**) and deposition (**BB**) of melanin pigments. If the gene of production is absent, the gene of deposition cannot deposit the melanin, and the animal becomes albino.*

For example, a gene for melanin production is epistatic to other for the melanin deposition (fig. 2-18). How does the epistatic gene; gene of synthesis, change the expression of another gene; gene of melanin deposition?

- Alleles of melanin deposition (B and b):
  If the tyrosinase enzyme is synthesized, alleles of melanin deposition produce a black coat in both homozygous dominant (**BB**) and heterozygous (**Bb**) individuals, while in homozygous recessive (**bb**) individuals they produce a brown coat.
- Alleles of tyrosinase synthesis (C and c)**:**
  A large amount of tyrosinase is synthesized in homozygous dominant (CC) individuals and a low amount of tyrosinase is synthesized in heterozygous individuals (Cc), but tyrosinase is absent in homozygous recessive (cc) individuals.
- Tyrosinase is required for synthesis of melanin; it converts a colorless precursor into melanin.
- Individuals have BBcc, Bbcc, bbcc are albino (colorless) because the second gene is homozygous recessive, so no tyrosinase synthesis, and so the first gene can only synthesize and cannot deposit the melanin.

Polygenic inheritance

Two or more independent pairs of alleles at different loci have similar and additive effects on the same trait (fig. 2-19). It means the inheritance of a phenotypic characteristic that varies in degree and can be attributed to the interactions between two or more genes and their environment. It is determined by a group of genes which act together to produce observable variation.

When many characteristics such as the skin color, hair color and height result from mixing several separate traits, they are polygenic inherited characteristics.

For example: Two parents who have black skin are carrying more genes for black color than white skin. If they have a child who inherits more genes for white skin than for black skin from both parents, he will be a white child of black parents.

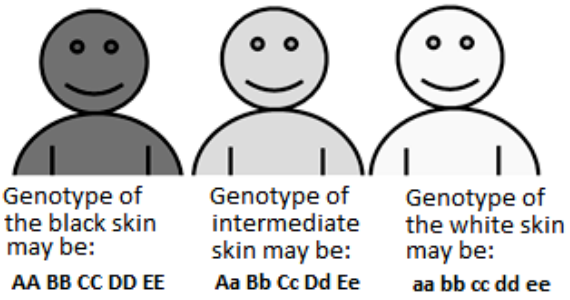


*Fig. 2-19: In polygenic inheritance, hypothetically if the skin color is controlled by several alleles **(AA BB CC DD EE)** which have additive effect, the skin color will be varied according to the ratio of participation of inherited expressed genes.*

Outbreeding

When the individuals of totally unrelated strains are mated, the mating is called *outbreeding*. It increases the proportion of heterozygotes and leads to offspring much better adapted for sur-

vival than both parents, the offspring is called *hybrid vigor*.

Inbreeding

When two closely related individuals are mated, this mating is called inbreeding. It increases not only the proportion of dominant and recessive homozygous, but also it decreases the proportions of heterozygous, so it leads to increase in the proportions of genetic undesirable traits or disorders within offspring.

Overdominance

It is a condition where the phenotype of the heterozygote is found outside of the phenotypical range of both homozygote parents. *A* heterozygous individual for a particular locus expresses a more pronounced phenotype than both of parental homozygotes. If the heterozygote has a phenotype that is more desirable, this is called ***heterozygote advantage*** *(fig. 2-20)*.

For example the **mutant gene of sickle cell anemia** is incompletely recessive, so carriers can produce a few sickled red blood cells, not enough to cause symptoms, but enough to give **resistance to malaria** and may confer a degree of protection against malaria. Thus conferring is a selective survival advantage for carriers (known as heterozygous advantage).

Multiple alleles

An allele of a genetic locus has more than two allelic forms within a population. Three or more allelic forms of a single gene can potentially occupy a particular locus and control one trait. The phenotypes (polymorphism) are controlled by multiple alleles at one locus. In human there are four different blood types: A, B, AB and O. Therefore, there are three alleles for a single locus, A, B, and O – multiple alleles determine the four different blood groups (fig. 2-21).

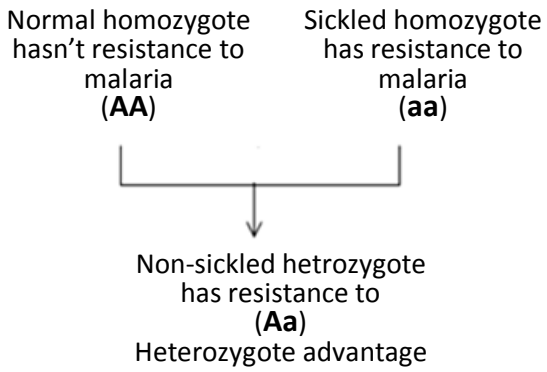


Fig. 2-20: Heterozygote advantage can produce a few sickled red blood cells, not enough to cause symptoms, but enough to give resistance to malaria.

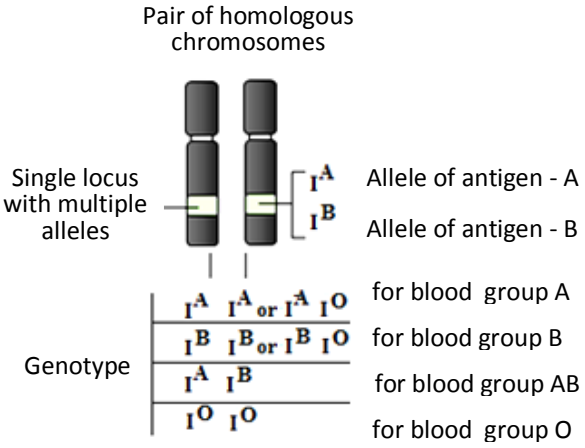


Fig. 2-20: Multiple alleles. Three allelic forms of a single gene of human blood groups can potentially occupy a locus that determines the blood group type.

Additional readings

Albert B et al. (2002): Molecular biology of the cell; 4th Ed. New York and London: Garland Science. Part II: Basic genetic mechanisms

Atherly AG, Girton JR and McDonald JF (1999): The science of genetics, 1st ed. Philadelphia: Saunders College Publishing.

Bowler PJ (1989): The Mendelian revolution: The emergence of hereditarian concepts in modern science and society. Baltimore, Md.: Johns Hopkins University.

Crocus A and Monaghan F (1993): Mendel's experiments on plant hybrids: A guided study. New Brunswick: Rutgers University Press.

Heim, W. G. 1991. What is a recessive allele? The American Biology Teacher 53:94–97.

Edelson E (1999): Gregor Mendel and the roots of genetics. New York, NY: Oxford University Press.

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, and Gelbart WM (2000): An introduction to genetic analysis. New York: W.H. Freeman and Company.

Manson AL, Jones E and Morris A (2002): Cell biology and genetics, 2nd ed. London, New York: Elsevier Science Limited.

Reece J, Campbell N (2002): Biology. San Francisco: Benjamin Cummings (ISBN 0 – 8053 – 6624 - 5).

Russell P J (1998): Genetics, 5th ed. Menlo Park, CA. Benjamin/Cummings Publishing Company, Inc.

Solomon EP, Berg LR and Martin DW (2002): DNA: The carrier of genetic information in Biology, 6th ed. United States: Thomson Learning Academic Resources Center, 243-260.

Thomson Learning Academic Resources Center, United States, 216-242.

Watson J, Hopkins N, Roberts J, Seitz J and Weiner A. (1987): Molecular biology of the gene. Calif, USA: Benjamin/Cummings, Menlo Park.

Activity:

Part I: Multiple Choice Questions

Choose single correct answer:

1. Which cross of the following is a monohybrid test cross?
   a. 2 homozygous recessive individuals.
   b. 2 homozygous dominant individuals.
   c. 2 dissimilar pure parents
   d. Unknown genotype and homozygous recessive individuals.

2. Which offspring of the following are produced by cross of two genetically dissimilar pure parents?
   a. Homozygotes.
   b. Heterozygotes.
   c. Homo- and heterozygotes.
   d. Unknown genotype.

3. If offspring express the same phenotype of parent generation after generation, which genotype has the parent?
   a. Both are hetrozygous
   b. Homozygous and heterozygous
   c. Both are homozygous dominant
   d. Homozygous recessive and homozygous dominant.

4. Which of the following genes are expressed in all offspring of F1 generation?
   a. Dominant genes
   b. Recessive genes
   c. Linked genes
   d. Allelic genes

5. Which of the following genes are hidden in F1 generation and reappears in F2 generation of monohybrid cross?
   a. Dominant genes
   b. Recessive genes
   c. Allelic genes
   d. Codominant genes

6. Which genes of the following occupy corresponding loci on homologous chromosomes?
   a. Linked genes
   b. Recessive genes
   c. Dominant genes
   d. Allelic genes

7. In which type of cross, the ratio of the phenotype of F2 -generation is 3:1?
   a. Monohybrid cross
   b. Dihybrid cross
   c. Monohybrid test cross
   d. Dihybrid test cross

8. Which of the following genes assort independently in meiosis?
   a. Linked genes
   b. Dominant genes
   c. Recessive genes
   d. Non-linked genes

9. Which of the following genes are inherited together as a unit?
   a. Dominant genes
   b. Complete linked genes
   c. Codominant genes
   d. Incomplete dominant genes

10. By which cross of the following can linkage between two loci be recognized?
    a. Two homozygous individuals.
    b. Heterozygous and homozygous dominant.
    c. Two heterozygous individuals
    d. Heterozygous and homozygous recessive.

11. Which term of the following refers to the site of a gene on chromosome?
    a. Allele
    b. Locus
    c. Chiasma
    d. Centromere

12. Which ratio of the following is the phenotypic ratio of F2 generation in dihybrid cross?
    a. 3 : 3 : 3 : 1
    b. 5 : 3 : 3 : 1
    c. 7 : 3 : 3 : 1
    d. 9 : 3 : 3 : 1

13. Which cross of the following is used to study the inheritance pattern of alleles for single locus?
    a. Monohybrid cross
    b. Dihybrid cross
    c. Monohybrid test cross
    d. Dihybrid test cross

14. Which term of the following refers to the genetic constitution of an individual?
    a. Phenotype
    b. Locus
    c. Alleles
    d. Genotype

15. Which cross of the following is used to study different alleles representing two or more loci?
    a. Monohybrid cross
    b. Dihybrid cross
    c. Monohybrid test cross
    d. Dihybrid test cross

16. Which genes of the following do not follow the law of independent assortment?
    a. Linked genes
    b. Non-linked genes
    c. Allelic genes
    d. Non-allelic genes

17. Which term of the following refers to cross of two totally unrelated individuals?
    a. Inbreeding
    b. Outbreeding
    c. Overdominance
    d. Codominance

18. If two hybrid individuals for single locus are crossed, what is the percentage of hybrid individuals in offspring?
    a. 10%
    b. 25%
    c. 50%
    d. 75%

19. If a cell has 3 loci: ***Aa Bb Cc*** on 3 different pairs of homologous chromosomes, how many different haploid gametes does this cell produce?
    a. 4
    b. 6
    c. 8
    d. 10

20. If ***A*** and ***B*** are codominant, and ***O*** is the recessive allele for a single locus, which of the following **could not** be the parents of a recessive individual ***OO***?
    a. A and B
    b. A and A
    c. O and O
    d. AB and O

21. How many linkage groups have diploid cells containing 36 chromosomes?
    a. 9
    b. 18
    c. 27
    d. 36

22. If a man has X-linked allele, he will pass it on to whom of the following?
    a. All his daughters
    b. All his sons
    c. ½ of his daughters
    d. ½ of his sons

23. Which process of the following includes Barr - body formation in female?
    a. Gene amplification
    b. Gene activation
    c. Gene mutation
    d. Dosage compensation

24. Which term of the following means the ability of one single gene to affect many characteristics?
    a. Pleiotropy
    b. Gene interaction
    c. Epistasis
    d. Polygenic inheritance

25. Which gene of the following can mask or change the expression of other unrelated gene?
    a. Allelic gene.
    b. Non-allelic gene.
    c. Epistatic gene.
    d. Autosomal gene.

26. Which of the following refers to three or more alleles that can potentially occupy a particular locus, and control one trait?
    a. Linked genes
    b. Non-linked genes
    c. Multiple alleles
    d. Allelic genes

27. Which condition of the following refers to a character resulting from blending several traits?
    a. Incomplete dominant
    b. Codominant trait
    c. Over dominant trait
    d. Dominant trait

28. Baldness is one of which traits of the following?
    a. X- linked traits
    b. Sex limited traits
    c. Sex influenced traits.
    d. Y-linked traits

29. Which trait of the following follows the transmission pattern of X-chromosome?
    a. Klinefelter syndrome
    b. Baldness
    c. Turner syndrome
    d. Hemophilia

30. In which process of the following does mating two closely related individuals occur?
    a. Pleiotropy
    b. Inbreeding
    c. Outbreeding
    d. Overdominance

31. Which of the followings is an X-linked trait?
    a. Color blindness
    b. Turner syndrome
    c. Baldness
    d. Epistasis

32. X-chromosome carries many loci. For which of the following they are required?
    a. For both sexes.
    b. Only for females.
    c. Only for males.
    d. Not required for both sexes.

33. Which chromosomal composition of the following is a karyotype of Klinefelter's syndromes?
    a. 47, XXY
    b. *47, XY, + 17*
    c. *47, XX,+21*
    d. 45, XO

34. Which chromosome of the following is responsible for male phenotype?
    a. X-chromosome.
    b. Y-chromosome.
    c. Autosomes.

35. Which of the following disorders is a sex-limited trait?
    a. Color blindness
    b. Hemophilia
    c. Baldness
    d. Breast

36. Which of the following traits include all secondary sex characters in both sexes?
    a. Sex-influenced traits
    b. Sex-limited traits
    c. Sex-linked traits

37. If a woman is a carrier of X-linked recessive allele, she will pass it on to whom of the following?
    a. All her daughters
    b. All her sons
    c. all her children
    d. ½ of her children

38. Which term of the following refers to *a* heterozygous individual who expresses a more desirable phenotype than that of parental homozygotes?
    a. Dominance
    b. Codominance
    c. Overdominance
    d. Heterozygote advantage

39. How many paternal chromosomal set does a human somatic cell contain?
    a. Single set
    b. 2 sets
    c. 3 sets
    d. Variable number

40. If two hybrid individuals for single locus are crossed, what is the percentage of recessive individuals in the resulting offspring?
    a. 10%
    b. 25%
    c. 50%
    d. 75%

41. Which ratio of the following is a result of cross of Bb and Bb individuals?
    a. 1 BB : 1 Bb : 2 Bb
    b. 1 BB : 2 Bb : 1 Bb
    c. 2 BB : 1 Bb : 1 Bb
    d. 1 BB : 1 Bb : 1 Bb

Answer of MCQs:

1)	D	11)	D	21)	D	31)	A
2)	B	12)	B	22)	A	32)	A
3)	C	13)	C	23)	D	33)	A
4)	A	14)	A	24)	A	34)	A
5)	B	15)	B	25)	C	35)	D
6)	D	16)	D	26)	C	36)	B
7)	A	17)	A	27)	A	37)	D
8)	D	18)	D	28)	C	38)	D
9)	B	19)	C	29)	D	39)	B
10)	D	20)	D	30)	B	40)	B
						41)	B

Part II: Short Answer Questions.

1. When black color is dominant over brown, what are the expected genotype and phenotype of offspring, if homozygous black coat and homozygous brown coat individuals are mated?

   _____
   _____

2. When black color is dominant over brown, what is the expected ratio of offspring expressing phenotype of the dominant allele, if hybrid black, heterozygous individuals are mated?

   _____
   _____

3. After crossing a male and a female, if offspring are divided into 75% who express long hair and 25% who expresss short hair, answer the following questions:
   a. The genotype of parent is:_____
   b. The genotype of offspring is:_____
   c. The dominant trait is:_____
   d. The recessive trait is:_____

4. A and B are two linked loci. If homozygous recessive (aa bb) and heterozygous (Aa Bb) individuals are mated, write the expected ratio of parental and recombinant individual, if these two genes are completely linked.

   _____
   _____

5. If long (T) is dominant over short (t), and black color (B) is dominant over blond (b) for mice hair, complete the following table when these two loci follow the law of independent assortment:

Parents	Female with long black hair **X** Male with short blond hair
Genotype of parents	.....
Genotype of F1	.....
Genotype of F2	....
Phenotype of F1	.....
Phenotype of F2	....

6. After crossing individuals of genotype *Dd Cc and genotype dd cc,* they produced 1000 offspring. If their offspring are classified into:150 *Dd cc;* 200 *dd Cc;* 320 *Dd Cc; 330 dd cc*, answer the following questions:

   a. What are the parental classes and the recombinant classes of offspring?

   _____

   _____

   b. Calculate the percentage of recombination between these two loci.

   _____

   _____

7. The X-linked allele (b) is responsible for a certain disorder. If a normal male is mated to a diseased female, what will be the ratio of both diseased and normal of male and female progeny?

   _____

   _____

8. Genes A and B are 10 map units apart, and A and C are 8 map units apart. Which gene is in the middle, if B and C are 18 map units apart?

   _____

   _____

MOLECULAR BASIS OF GENETIC MATERIAL (Part -I)

Chapter 3:

MOLECULAR BASIS OF GENETIC MATERIAL (Part -I)

Discovery of Genetic Material

Objectives

After you have studied this chapter (part I), you should be able to:

- *Outline the history of the search for the heritable material.*
- *Explain: DNA is responsible for heredity and carries the genetic information.*
- *Explain how the genetic diseases have been discovered.*
- *Discuss the bacterial transformation, semi-conservative model of DNA replication, and one gene-one enzyme concept.*

History of Genetic Materials

Chromosomes are Chromatin Threads

German chemist *Walter Flemming in 1879* discovered tiny *thread-like structures* within the cellular nuclei. He coined the term *chromatin*, "coloured structure", as indication to the coloured components of cellular nuclei observed after treatment with various chemical stains. *Wilhelm Roux in 1883* proposed that these colored structures carry the genetic information. In 1888, *Wilhelm Waldeyer* used the term *chromosome*, "colour body", to describe the threads of stainable material found within the nucleus**.**

Chromosomes Carry Genes.

American scientist *Walter Sutton and German* scientist *Theodor Boveri* both *in* the late 1880 and early 1890described the process of meiosis and the connection between chromosomes and heredity theoretical units called *now genes*:

- Both chromosomes and these units, genes, are present as pairs in cells.
- They separate in the same way during meiosis or gametes formation; sperm or ovum.
- They reappear as pairs in fertilized egg.

Their theory is known as the ***Boveri–Sutton chromosome theory***. It is a fundamental unifying theory of genetics which refers to chromosome as the carrier of genetic information. It also states that chromosomes are linear structures with genes located at specific sites along them.

Gene is the Unit of Heredity

Danish botanist *Wilhelm Ludwig Johansson (1857-1927) in 1911* coined the term *gene* to describe the unit of heredity. He used the term

genotype to define the gene which determines a particular trait, and *phenotype* to define the physical form of the trait.

DNA is the Genetic Material

Frederick Griffith *(1879-1941),* a British medical officer, discovered in 1928 accidentally a **bacterial phenomenon** that is called ***bacterial transformation.*** The experiment is known as the *transformation experiment*, and the factor of transformation was called the ***transforming principle***.

During his experiment to develop a vaccine for pneumonia by using several strains of bacteria streptococcus *pneumonia*, he discovered that some harmless bacteria are transformed into lethal bacteria. He observed (fig. 3-1) that when virulent bacteria (S-strain) are killed by heat treatment, they lose their ability to harm animals. But when they are killed by heat and then are mixed with R-strain and injected together, they have a quite different effect. They caused pneumonia that resulted in death of experimental animal.

In this experiment, some components of the S-strain can transform the harmless R-strain into virulent strain called *transforming principle*. The smooth (S -strain) of *pneumococcal bacteria* is virulent; lethal. It has protective polysaccharide capsule which is absent in the harmless, non-lethal, rough strain (R -strain). Absence of polysaccharide capsule allows the animal to destroy the R -strain. Therefore, the R -strain of *pneumococcus* does not cause pneumonia.

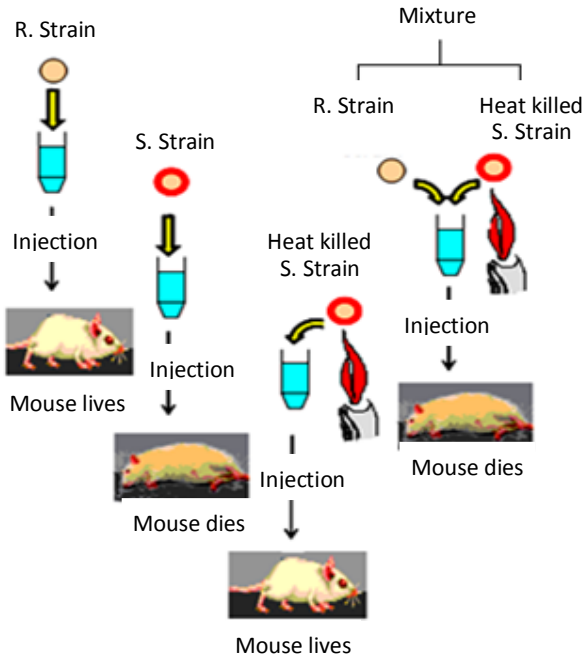


Fig. 3-1: Bacterial transformation experiments of Griffith. Mouse dies after injection of smooth strain (S. strain) and mixture of DNA of smooth strain and rough strain (R. Strain), while rough stain alone cannot kill the mouse.

Avery T, C.M. Macleod, and M. McCarty *in 1944* repeated the transformation experiment of *Frederick Griffith* (fig. 3-2), to clarify the **principles of bacterial transformation**.

They isolated the different structural components such as carbohydrate, lipid, protein, DNA and RNA from the virulent strain. Then they added each of these fractions to a separate sus-

pension of harmless non-virulent pneumococcal bacteria.
They discovered that:

- Bacteria which have been exposed to DNA became virulent.
- Bacteria which have been treated with the other fractions are not affected as demonstrated in figure 3-3.

They concluded that:

- Griffith transforming principle that is responsible for the phenomenon of bacterial transformation is DNA.
- DNA can transform the phenotype of bacteria.

It was the first experimental evidence which indicates that ***DNA is the carrier of the genetic material.***

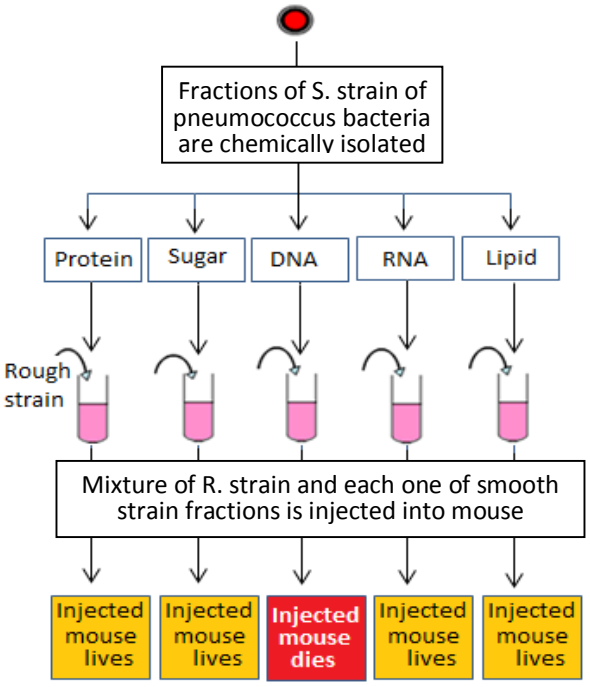


Fig. 3-2: Avery, MacLeod, and McCarty repeated the bacterial transformation experiments of Frederick Griffith and concluded that DNA can change the phenotype of bacteria.

One Gene – One Enzyme Concept

The Nobel-prize winner ***George Beadle*** *(1903-1989) and* ***Edward Tatum*** *(1909-1975)* showed that each individual step in metabolism is controlled by a single gene.

In 1945 they studied different mutant strains of fungus *Neurospora* and presented additional experimental evidence that **DNA is the carrier of genetic information.**
They have used special genetic crossing experiments to identify the existing relations between functioning genes and their corresponding proteins (fig. 3-3).

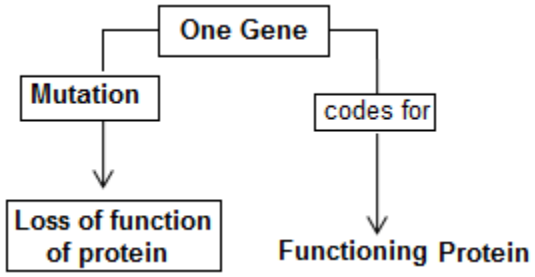


Fig. 3-3: One gene –one enzyme concept of Beadle and Tatum (1945) is a conclusion of the fact that each gene codes for functioning protein and gene mutation may cause loss of its function.

They came to the conclusion that for each mutation in only one gene locus; that affected on the gene function, only one enzyme was affected. This one to one correspondence between gene and enzyme was known as **One Gene – One Enzyme (One Protein) Concept**. This work of Beadle and Tatum proved that the genes code for proteins.

DNA Carries Genetic Information of Protein and DNA Synthesis

Hershey A.D. and H. Chase in 1952 found that DNA is responsible for both protein- and DNA-synthesis in viral multiplication.
They performed experimental infection of the bacteria by bacteriophage in order to answer which is responsible for viral reproduction, viral DNA or viral protein?

They labeled the viral protein coat of bacteriophage with radioactive ^{35}S and the viral DNA with radioactive isotope ^{32}P. Then they infected E.coli by the virus and let the virus multiply within the bacteria. They found that:

- The protein coat could be separated from the infected bacteria without interfering with the viral reproduction.
- The viral DNA could not be separated from infected bacteria.
- They concluded that viral DNA is required for viral reproduction and synthesis of both viral protein coat and viral DNA (fig. 3-4).

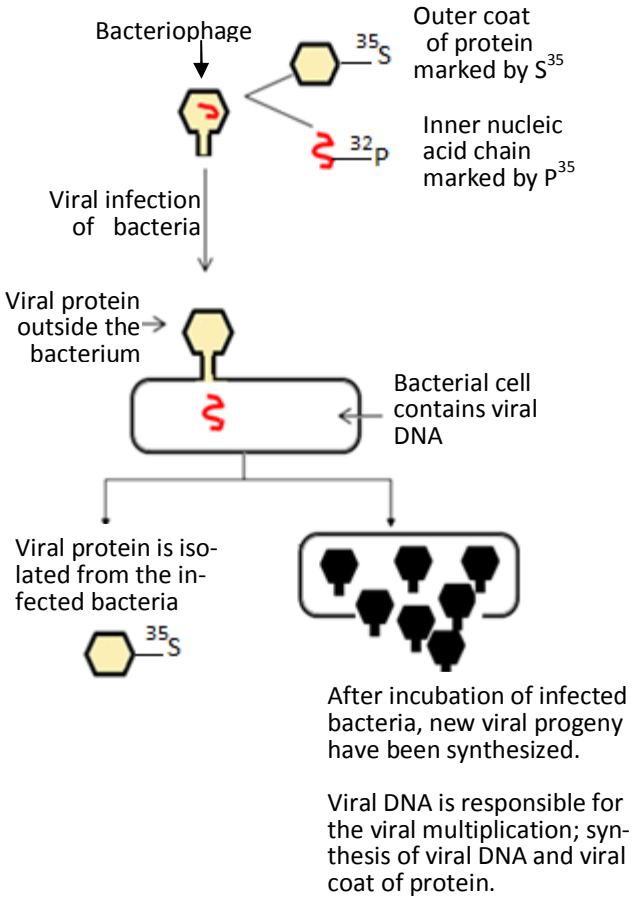


Fig. 3-4: Hershey and Chase experiment proved that DNA of virus is responsible for viral multiplication within the bacteria and synthesis of new viral progeny.

DNA is a Double Helix Molecule

- ***Rosalind Franklin*** *(1920-1958) in 1950,* while she was working in *Maurice Wilkins* lab, carried out an X-ray crystallography analysis of DNA. Her analysis showed that DNA molecule is a double helix structure.

- ***Watson and Crick*** *in 1953,* while they were working at Cambridge University, they published in *Nature* the double helix structure of DNA in a one page paper.

Watson and Crick used the biochemical information about DNA of ***Erwin Chargaff*** *(1905-2002)* and the results of X-ray diffraction of *Rosalind Franklin* to build their model of DNA structure. Today, this model of DNA structure has been accepted and became the foundation to understand how DNA could replicate itself and preserve the genetic information generation after generation.

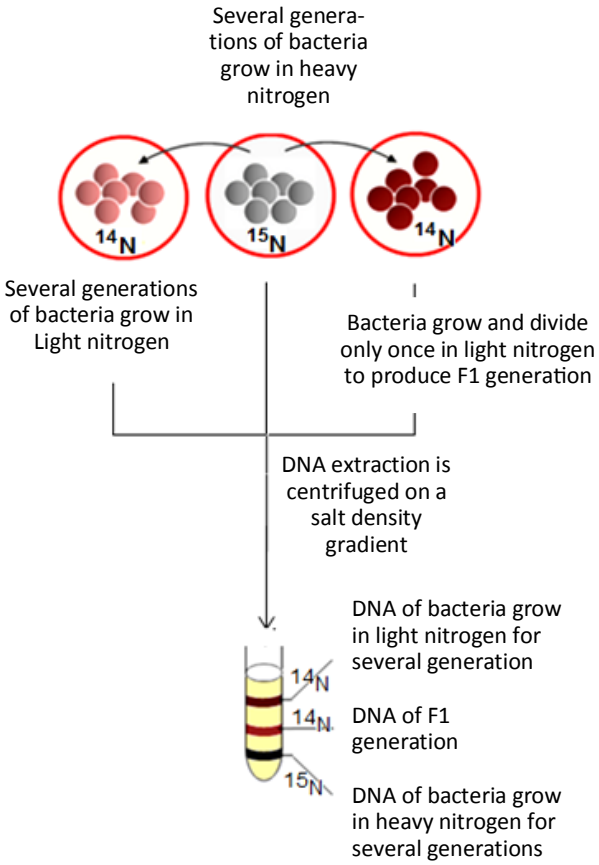


Fig. 3-5: F1 generation of E.col is in midway between the bacteria in heavy nitrogen and those in light nitrogen, because they contain DNA formed of one heavy strand and one light strand.

Semiconservative Replication of DNA

Mathew Meselson *and* ***Franklin Stahl*** *in 1958*proved that DNA replicates itself in s*emiconservative* manner (fig. 3-5 and fig. 3-6).Semiconservative replication means that when the double stranded DNA helix replicates, it produces two strands of DNA double helix, each of the two double stranded DNA helices consisted of ***one strand coming from the original helix and one newly synthesized.***

Meselson - Stahl experiment

- *E. coli* are grown in a medium containing heavy nitrogen (^{15}N, heavier than the common nitrogen) for several generations. The heavy nitrogen incorporates into the bacterial DNA. Then these bacteria in heavy nitrogen were transferred to medium containing light nitrogen (^{14}N) and let to divide only once producing F1 generation.
  Other *E. coli* are grown in light nitrogen (^{14}N) for several generations.

- DNA is extracted from *E.coli* grow in light nitrogen, heavy nitrogen, as well as from those divided only once in light nitrogen.

- DNA extract is centrifuged on salt density gradient. After centrifugation the following results and conclusion have been obtained(fig.3-5):

1. DNA of *E. coli* that multiplies in heavy nitrogen is formed only of heavy strands, and present in the base of test tube.
2. DNA of *E. coli* that multiplies in light nitrogen is formed only of light strands and present in the top of test tube.
3. DNA of *E.coli* F1 generation that contains 50% light strands and 50% heavy strands, present in the middle of test tube. It is found in midway between DNA -molecules containing heavy nitrogen located near the base and DNA –molecules containing light nitrogen located near the top.

Replication of bacterial DNA strands follows the semiconservative model in figure 3-6. According to the semiconservative model DNA strands of F1 generation are formed of one heavy strand and one light strand.

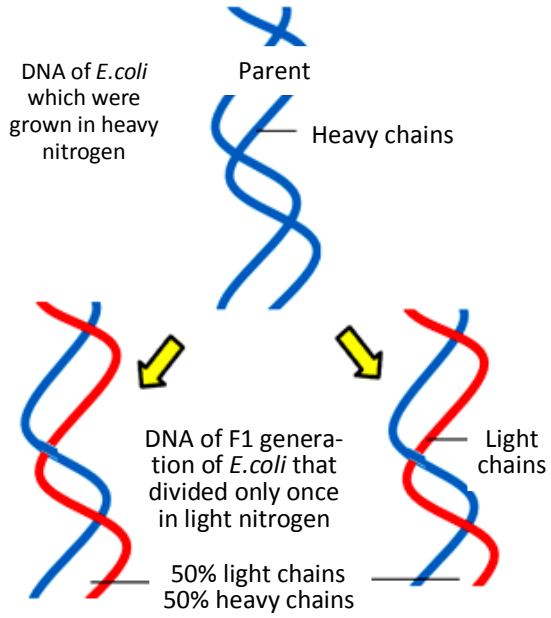


Fig. 3-6: Semi-conservative model of DNA replication. After one generation, the double stranded DNA is intermediate density, one strand heavy (from the parent) and one strand light (newly synthesized). This result is predicted by semi-conservative replication.

Discovery of Genetic Diseases

English physician ***Archibold Garrod*** (1857-1936) who found the biochemical genetics, in 1909 suggested that a*lkaptonuria* is a genetic disease, which is a result of a defective enzyme.

He observed that the excreted urine of patients with a disorder called *alkaptonuria* is darkened on standing (fig. 3-7), because their urine contains homogentisic acid that is the common name for 2, 5 –dihydroxyphenyl acetic acid. It is formed as an intermediate product of metabolism of tyrosine and phenylalanine.

This genetic disorder has a family distribution. The incidence of this disease is high between individuals resulting from marriages of close related parents**.**

Garrod postulated that alkaptonuria or black urine disease is an inherited genetic disorder. He published his findings in 1909 under the title **"**Inborn errors of metabolism".

The **"one gene, one enzyme"** hypothesis is based on studies of Archibald Garrod on the nature and inheritance of alkaptonuria.

Inborn errors of metabolism comprise a large class of genetic diseases involving disorders of metabolism. The majority are due to defects of individual genes coding for enzymes that facilitate conversion of various substrates into products.

Alkaptonuria is a rare inherited genetic disorder of phenylalanine and tyrosine metabolism. This is an autosomal recessive condition that is due to a defect in the enzyme which participates in the degradation of tyrosine. Absence of the enzyme activity results in a toxic tyrosine byproduct called homogentisic acid (or *alkapton*) accumulates in the blood and is excreted in urine in large amounts. Excessive homogentisic acid causes damage to cartilage (leading to osteoarthritis) and heart valves as well as precipitating as kidney stones.

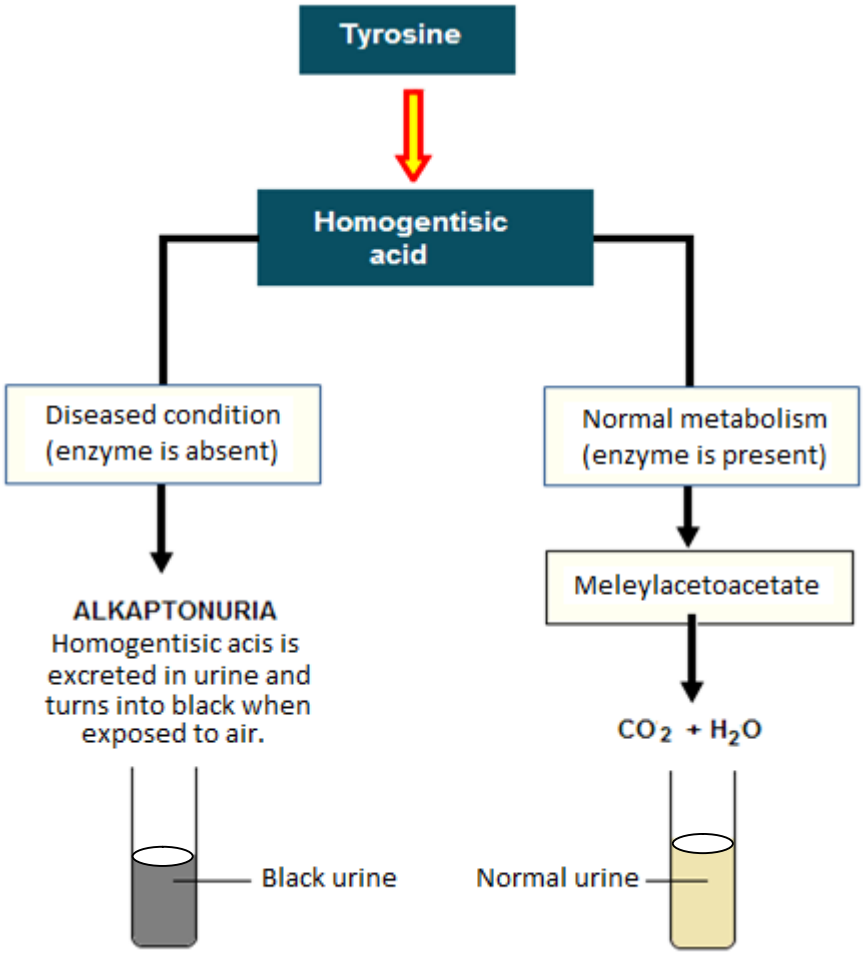


Fig. 3-7: Alkaptonuria allele causes the absence of enzyme in pathway of tyrosine catabolism that result in accumulation of homogentisic acid in blood. It is excreted in urine and turns black when exposed to air.

Additional reading

Avery OT, MacLeod CM and McCarty M (1944): Studies on the chemical nature of the substance inducing transformation of *pneumococcal* types. J. Exp. Med.98, 451-460.

Crick RHC et al. (1961): General nature of the genetic code for proteins. *Nature* 192, 1227-1232.

Griffith F (1928): The significance of pneumococcal types. J. Hyg. 27, 113-159.

Judson HF (1996): The eighth day of creation. Plainview, NY; CSHL Press

Hershey AD and Chase M (1952): Independent functions of viral protein and nucleic acid in growth of bacteriophage. I. Gen. Physiol.36, 39-56.

Lagerkvist U (1998): DNA pioneers and their legacy. New Haven CT: Yale University Press.

Meselson M and Stahl RW (1958): The replication of DNA in *E. coli.* Proc. Nat. Acad. Sri. USA 44,671-682.

Mirsky AE (1968): The discovery of DNA. Scientific American 218 (June): 78-88.

Portugal FH and Cohen JS (1977): A century of DNA. MIT Press Cambridge MA

Tracy K (2005): Friedrich Mescher and the story of nucleic Acid. Mitchell Lane Publishers

Solomon EP, Berg LR and Martin DW (2002): DNA: The carrier of genetic information in Biology, 6th ed. United States: Thomson Learning Academic Resources Center, 243-260.

Wilkins MRH, Stokes AR and Wilson HR (1953): Molecular structure of DNA. Nature 171, 738-740.

Watson JD and Crick FHC (1953): A structure for DNA. Nature 171*, 737-738*.

Watson JD and Crick FHC (1953): Genetic implications of the structure of DNA. Nature 171, 964-967.

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer:

1) The threads of chromatin in the nucleus have been firstly discovered by:
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Avery, MacLeod, and McCarty
   d. Walter Fleming

2) By whom was the term "chromosome" firstly used to describe the chromatin threads?
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Walter Sutton and Theodor Boveri
   d. Waldeyer

3) Who used the term "gene" for the first time to describe the unit of heredity?
   a. Hershey and Chase
   b. Wilhelm Ludwig Johansson
   c. Walter Sutton and Theodor Boveri
   d. Walter Flemming

4) Which scientist of the following discovered accidentally principle of bacterial transformation?
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Avery, MacLeod, and McCarty
   d. Frederick Griffith

5) Which of the following scientists have experimentally proven that bacterial transformation may occur?
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Avery, MacLeod, and McCarty
   d. Walter Sutton and Theodor Boveri

6) Who stated that one gene – one enzyme concept?
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Avery, MacLeod, and McCarty
   d. Walter Sutton and Theodor Boveri

7) Which scientists of the following have published a model of DNA double helix?
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Avery, MacLeod, and McCarty
   d. Watson and Crick

8) Which scientists of the following have experimentally tested semi-conservative model of DNA –replication?
   a. Hershey and Chase
   b. Beadle & Tatum
   c. Meselson and Stahl
   d. Watson and Crick.

9) Which process of the following is **not** consistent with results of Griffith's experiments?
   a. Mixture of S-strain& R-strain kills the mouse.
   b. Mixture of heat killed S-strain &R-strain doesn't kill the mouse.
   c. Heat-killed S-strain **doesn't** kill the mouse.
   d. Mixture of heat-killed S-strain and R-strain kills the mouse.

10) When and which scientist firstly used the term chromosomes to describe the chromatin fibers?
   a. 1879 by Walter Flemming
   b. 1883 by Wilhelm
   c. 1888 by Waldeyer
   d. 1911 by Johansson

11) Which scientist of the following published firstly data about alkaptonuria as a genetic disease?
   a. Garrod
   b. Flemming
   c. Waldeyer
   d. Johansson

12) Which of the following is **not** correct about alkaptonuria or black urine disorder?
   a. A family distribution disorder.
   b. Inherited as a dominant condition.
   c. An inborn error of metabolism.
   d. Detected by Archibold Garrod.

13) Which of the following is responsible for synthesis of viral DNA and coat?
   a. Viral DNA only
   b. Viral coat only
   c. Both Viral DNA and coat
   d. Non of above

14) Which of the following **is not** consistent with the fact that chromosomes carry genes?
   a. Occurrence of both as pairs.
   b. They separate in a similar fashion during gametes formation
   c. They repair during fertilization.
   d. Chromosomes synapse in meiosis.

15) Which of the following is the principle of bacterial transformation?
   a. DNA
   b. RNA
   c. Protein
   d. Carbohydrate

16) Which of the following is responsible for black urine disorder?
   a. Mutant recessive gene
   b. High activity of specific enzyme.
   c. Accumulation of tyrosine in blood.
   d. Low level of homogentisic acid in urine.

17) When a culture of *E.coli* contains radioactive phosphate, which of the following may occur for E.coli?
   a. They lose their pathogenic character.
   b. They die after few minutes.
   c. Their DNA becomes radioactive.
   d. Their protein coat becomes radioactive.

Part II: Fill in the spaces:

1. W. Flemming discovered _____ In 1879
2. W. Roux proposed the chromatin carrying _____ in 1883.
3. W. Waldeyer used the name _____ to describe the chromatin fibers in 1888.
4. The parallels between chromosomes and genes have been observed in 1890 in meiosis by _____.
5. W.L. Johansson used the term _____ to describe the unit of heredity in the year _____.
6. The terms genotype & phenotype have been used in 1911 by _____.

Answer of MCQs:
1) D 2) D 3) B 4) D 5) C 6) B 7) D 8) C 9) B 10) C
11) A 12) B 13) A 14) D 15) A 16) A 17) C

MOLECULAR BASIS OF GENETIC MATERIAL (Part -II)

Chapter 3:

MOLECULAR BASIS OF GENETIC MATERIAL (Part -II)

Structure of Hereditary Material

Objectives

After you have studied this chapter (part II), you should be able to:

- *Explain the structure of DNA molecule.*
- *Describe the linkages within DNA double helix.*
- *Explain the base – pairing rule.*
- *Describe how complementary bases link to each other.*
- *Discuss the general features of DNA double helix.*
- *Discuss the relationship between purine and pyrimidine within DNA molecules.*

Structure and Organization of Hereditary Material

The hereditary material appears as a scattered fine granular substance and aggregation of dark-stained material in nuclei of eukaryotes. This material forms the chromatin fibers which carry the genetic information (fig.3-8). Chromatin has a compact organized structure in which most DNA elements form heterochromatin and are functionally inactive.

Within this mass of compact chromatin, the minority of active sequences is found. Chromatin fibers condense to form chromosome during cell division. The chromatin fiber is a complex structure formed of DNA and proteins.

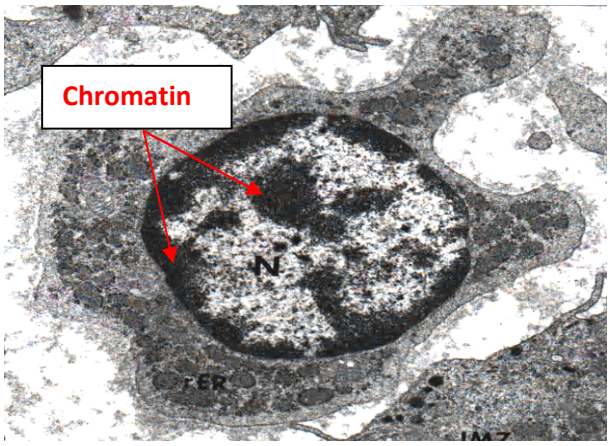


Fig. 3-8: An electron micrograph of nucleus of a mononuclear cell demonstrates the structure of chromatin substance. It appears as fine granules, or dark chromatin substance in the form of islands or nuclear membrane attaching chromatin.

DNA associated proteins

They are classified into: *Histones and Non-histones*

A) Histones:

They are formed of relatively small protein molecules containing a large amount of lysine and arginine. They are *positively* charged and bound strongly to DNA, which is *negatively* charged. They can be classified into:

a) *Histones of nucleosomes* (102-135 amino acids) H2A, H2B, H3 and H4.
b) *H1–histone or peripheral histone* (220 amino acids). It may be responsible for the aggregation of nucleosomes in the form of chromatin fibers of about 30 nm thick.

Binding of histones to DNA molecule protects it against degradation with endonucleases. *The regulatory regions of genes are highly sensitive to restriction endonuclease, because the regulatory proteins that attach the regulatory regions of genes prevent binding histone molecules to the regulatory regions of genes.*

B) *Non-histones:*

The non-histone protein molecules of chromosomes are necessary for controlling the processes of DNA replication. They function also in the regulation of DNA transcription.

DNA organization:

The hereditary material is organized in the form of

a. Nucleosomes
b. Linker DNA (internucleosomal DNA)
c. Scaffolding protein

Nucleosomes:

DNA is formed of two polymer chains of nucleotides in the form of a double helix. DNA organization begins by formation of *nucleosomes* that are *the 1st level of DNA organization*. They are the basic structural units of chromatin fibers (fig.3-9, 3-10).

Each nucleosome consists of a strand of *DNA double helix and a core of globular basic histone protein.* DNA strand which is formed of about 146 base pairs is wrapped around the core of globular basic protein.

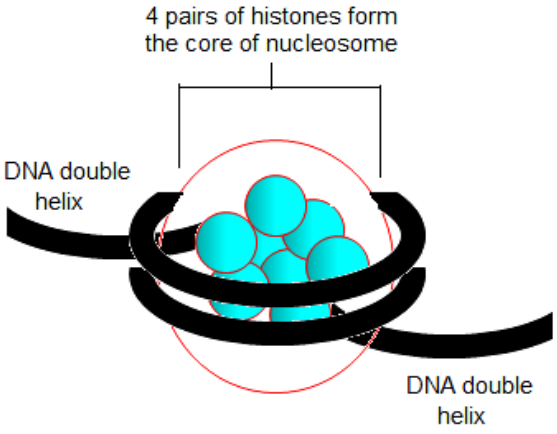


Fig. 3-9: Structure of nucleosomes. It consists of core of 4 pairs of histones, which is surrounded by a strand of DNA double helix of about 146 base pairs.

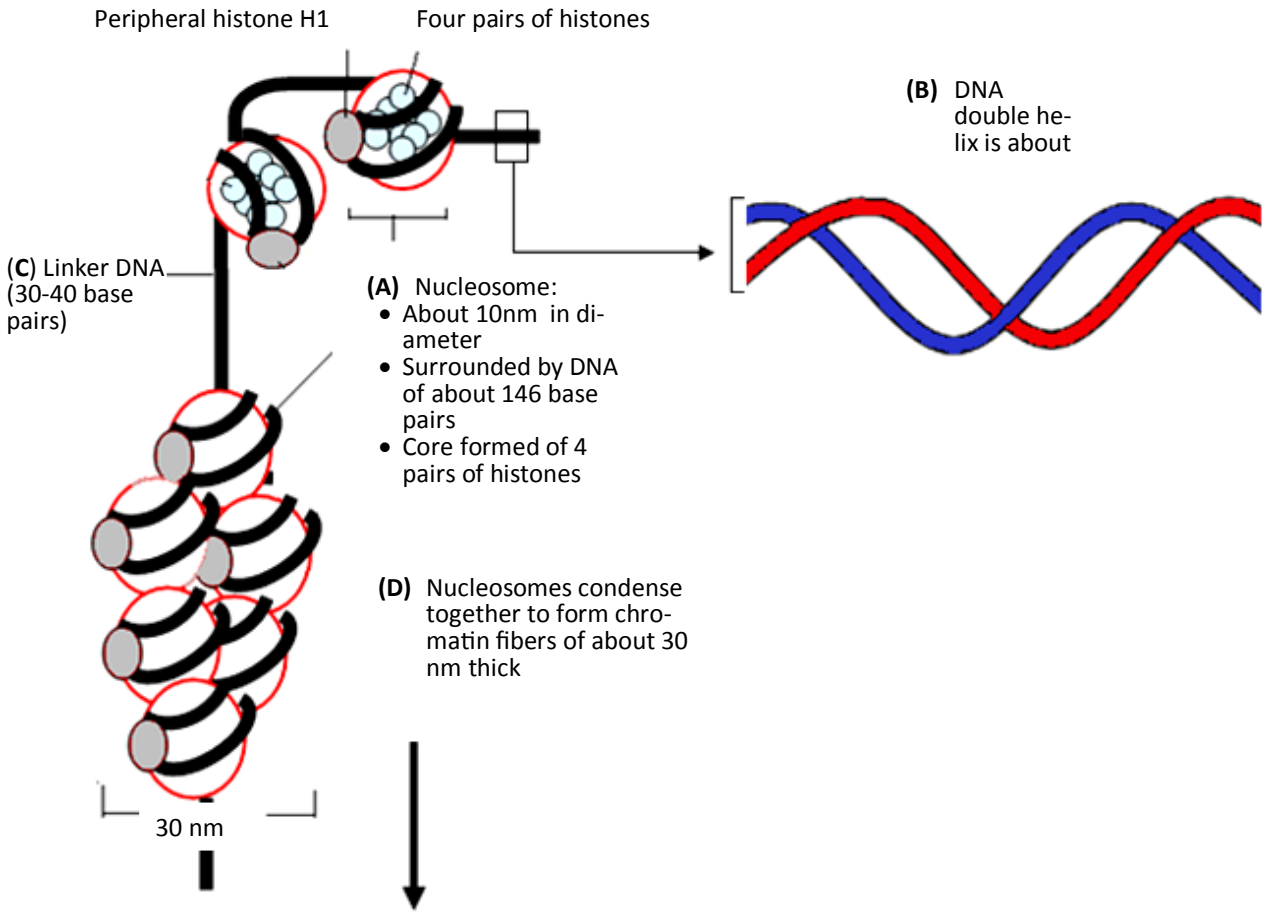


*Fig. 3-10 A: Diagram illustrates the organization of hereditary material. DNA double helix (**A**) of about 2.0 nm thick is wrapped around a core of 4 pairs of histone molecules to form nucleosomes (**B**) with diameter of about 10 nm thick. Linker DNA (C) connects between nucleosomes. Nucleosomes attach together by peripheral histone (H1) and condense forming a fiber of about 30 nm thick (D).*

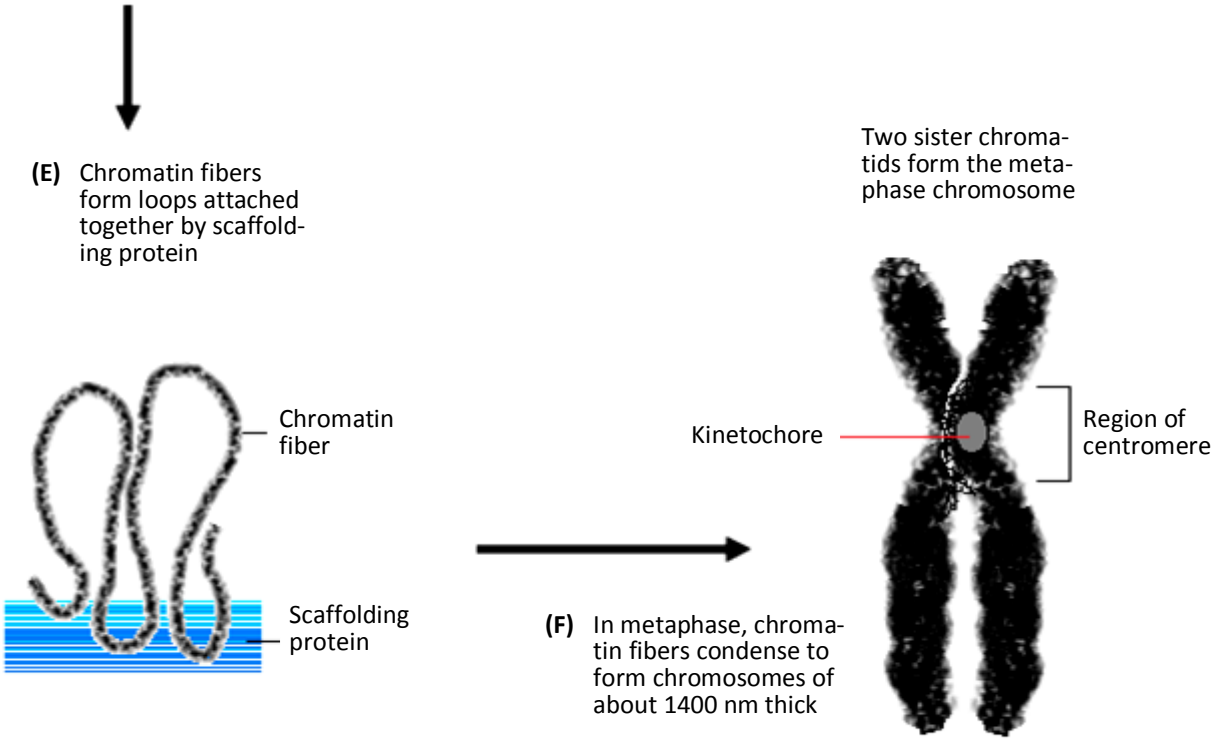


Fig. 3-10B: The nucleosomal fibers form loops (E) radiating from scaffolding non-histone protein to form the DNA protein complex of chromatin fibers. The chromatin fibers condense to form chromosomes (F) during cell division. As a result of DNA replication, each chromosome becomes two sister chromatids attaching at centromere and of about 1400 nm in thickness in metaphase.

The core of globular basic protein consists of four pairs of histones. Each pair is formed of two molecules of nucleosomal histones; H2A, H2B, H3 and H4.

Nucleosomes are connected together by *internucleosomal* strand of *DNA double helix*. By peripheral histone (H1) the adjacent nucleosomes attach together and condense forming fibers of about 30 nm thick which is *the 2nd level of organization.* This level of 30 nm thick chromatin

structure is thought to be the form of euchromatin, which contains actively transcribed genes.

In *the 3rd level of DNA organization,* nucleosomes are packed together forming large *coiled loops of DNA by scaffolding protein (fig 3-10B)*. The loops of DNA is radiating from central scaffolding non-histone protein. These loops may form the transcriptional units as mentioned by some scientists.

Deoxyribonucleic Acid (DNA)

The structure of DNA is especially important to understand:

- How the genetic information is preserved!
- How it can be duplicated and passed on during the cell division!
- How it can be transcribed during gene expression!

DNA molecule has indefinite length. Analysis of purified DNA molecule using *X-ray diffraction* has demonstrated that:

- DNA molecule is a **twisted double helix** (fig. 3-10) of two strands moving in a circular direction around an axis and running in opposite directions, ***antiparallel***.
- DNA is composed of a polymer of repeating units of nucleotides, *deoxyribose polynucleotides* in the form of chain.

Nucleotide Molecule:

Hydrolysis of DNA nucleotide molecule (fig. 3-11) produces:

- A heterocyclic base (purine or pyrimidine).
- A sugar of 5- carbon monosaccharide (D-ribose or 2'-deoxy-D-ribose).
- A phosphate group.

There are four types of nucleotides (fig. 3-12) forming DNA molecule according to type of nitrogenous bases:

- Adenine nucleotide (A)= Deoxyadenylic acid
- Thymine nucleotide (T)= Deoxythymidylic acid
- Guanine nucleotide (G) = Deoxyguanylic acid
- Cytosine nucleotide (C)= Deoxycytidylic acid

Of the four bases adenine and guanine are purines, while thymine and cytosine are pyrimidines (fig. 3-12).

Medical applications for purine derivatives and related compounds, including nucleoside, have been published. They are used in combination with other chemotherapeutic agent to treat acute leukemia in children, and reported that almost 80% of the treated children are now cured. Also allopurinol, purine derivative, is a standard therapy for treatment of gout.

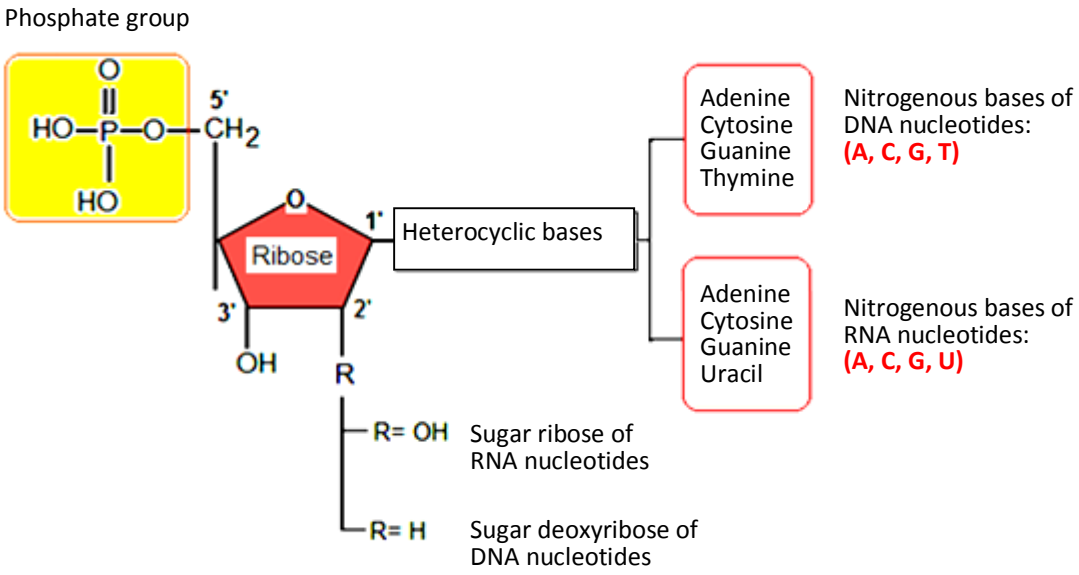


Fig. 3-11: General structure of nucleotide. It is formed of pentose sugar, phosphate group and one nitrogenous base. Nucleotides obtained from RNA are formed of sugar ribose, phosphate group and nitrogenous base (A, U, G, and C). Nucleotides obtained from DNA are formed of sugar 2-deoxyribose, phosphate group and nitrogenous base (A, T, G, and C); –OH at position 2 is replaced by -H.

Nucleotides and Nucleosides

Nucleotides consist of a nucleoside and one or more phosphate groups, and named in several ways: Adenylic acid is for example called:

- 5' –adenylic acid or
- Adenosine 5'-phosphate or
- Adenosine monophosphate (AMP).

Nucleoside is a nucleotide after removal of phosphate group (fig. 3-13). They, nucleotides, are present in other structures than nucleic acids RNA and DNA.

For example adenosine triphosphate (ATP) is the important source of energy.

Phosphate group links to # 5' -carbon of pentose sugar. The nitrogenous base links to # 1' -carbon of pentose sugar.

The bases of two polynucleotide chains pair together. They attach together by two hydrogen bonds between A=T and three hydrogen bonds between C ≡ G to form DNA double helix.

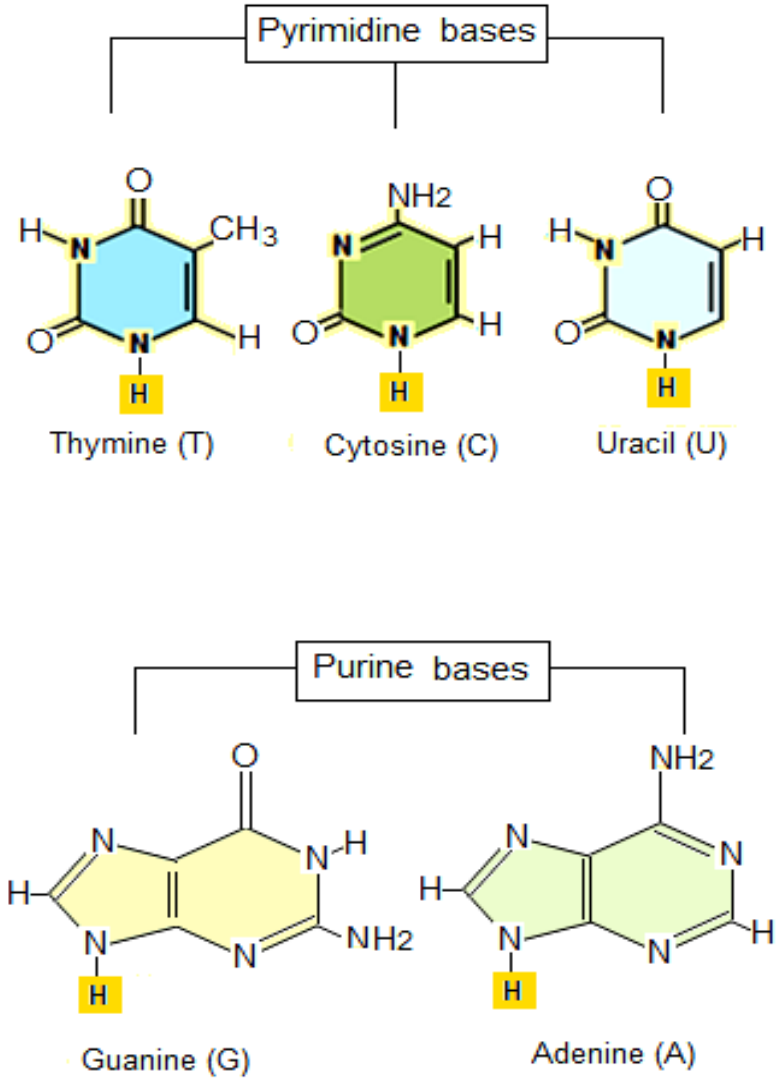


Fig. 3-12: Three major pyrimidine and two purine bases are found in nucleotides of DNA and RNA. Adenine, thymine, guanine and cytosine are found in DNA. Uracil is a pyrimidine nitrogenous base. It replaces the thymine in RNA molecules.

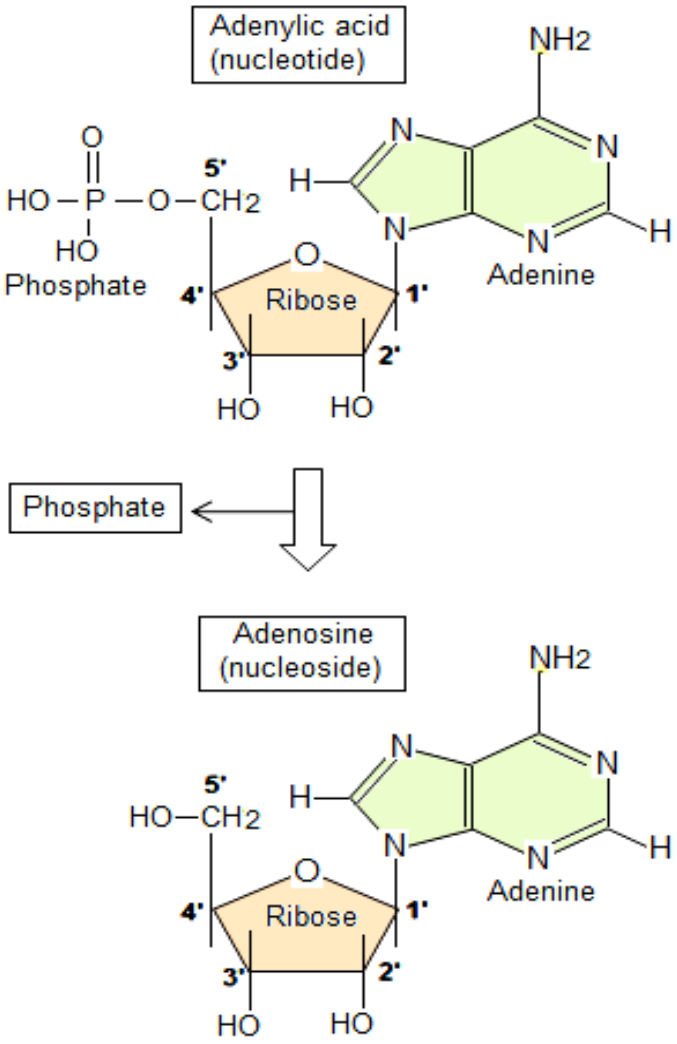


Fig. 3-13: Removal of a phosphate molecule of nucleotide monophosphate results in conversion of nucleotide into nucleoside.

Linkages within DNA Double Helix

A) 3, 5 –phosphodiester linkage. Each nucleotide attaches to the next by 3', 5' phosphodiester linkage; *covalent-bond*. Phosphate esters link the 3'-OH of deoxyribose with the 5'-OH of another (fig. 3-14).

This linkage makes the polynucleotide chain a long stable unbranched chain and supported by backbone of sugar - phosphate units with heterocyclic bases protruding from the chain at regular intervals (figs. 3-15 and 3-16).

Characters of polynucleotide chain:

- Long stable unbranched chain
- Supported by backbone of sugar - phosphate units
- Heterocyclic bases protruding from the chain at regular intervals (0.34 nm)

B) Hydrogen –bonds. According to the model of DNA double helix of Watson and Crick, two polymer nucleotide chains of DNA (fig. 3-16) are held together by hydrogen -bonds between the nitrogenous bases on opposite strands. These nitrogenous bases form ***base-pairs***.

The two polynucleotide chains move in a circular direction around an axis forming a double helix (both chains sharing the same axis), and they run in opposite directions, ***antiparallel,*** and in 5' to 3' direction. The base pairs are on the inside of the helix, and the sugar-phosphate backbone is on the outside.

The length of every complete turn is 3.4 nm and includes 10 successive base pairs. The exterior width of the spiral is about 2 nm.

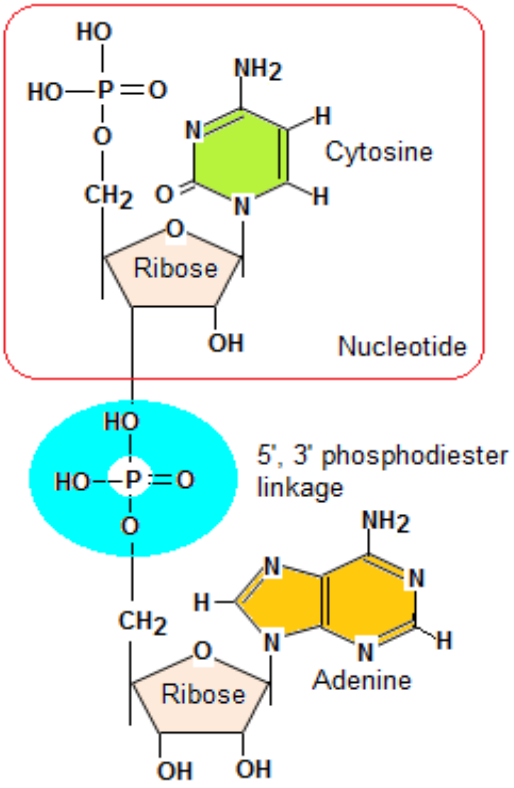


Fig. 3-14: Nucleotides are joined together by 3`, 5` phosphodiester linkage that makes nucleotides form a linear chain. Each linkage consists of a phosphate group and covalent bonds that attach it to the sugar of adjacent nucleotide. This linkage is important for formation of a stable polynucleotide chain as a result of formation of sugar phosphate backbone.

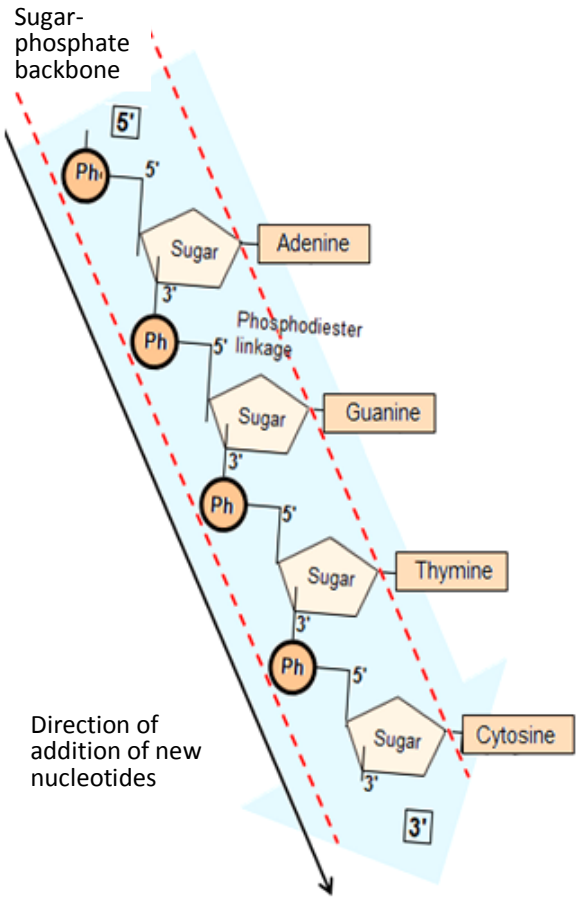


Fig. 3-15: Polynucleotide chain shows phosphate ester groups link the 3'-OH and 5'-OH groups of deoxyribose units. Sugar-phosphate backbone is formed by attaching each nucleotide and the next by 3', 5' phosphate linkage. (Ph = phosphate group).

Base Pairing Rules

Base pairs mean that two nitrogenous bases are linked together by hydrogen bonds within DNA double helix. It is a completely automatic process requiring no catalysis. Because the hydrogen bonds are weak, they are easily broken.

For example, DNA double helix is denatured by thermal energy. Mild heat can cause un-pairing and re-association occurs by cooling.
In base pairing rules (fig. 3-16):

- Adenine (A) pairs only with thymine (T) by two hydrogen bonds (2H).
- Cytosine (C) pairs only with guanine (G) by three hydrogen bonds (3H).
- The two chains are complementary.
- Wherever adenine appears in one chain, thymine must appear opposite to it in the other; wherever guanine appears in one chain, cytosine must appear opposite to it in the other.
- Distance between two successive base pairs in DNA double helix equals 0.34nm. In twisted DNA double helix (fig. 3-17) each turn repeats every 10 base pairs. This means that the distance between two successive turns (Ten / Turn) equals = 10 x 0.34 = 3.4 nm.
- The internal distance between deoxyribose units on opposite chains is about 1.1nm, such internal distance allows only a purine – pyrimidine type of hydrogen bonding between base pairs.

- Purine –purine base pairs do not occur because they would be too large to fit, and pyrimidine – pyrimidine base pairs do not occur because they would be too far apart to form effective hydrogen bonds. Therefore, Hydrogen bonds can occur in only a specific way: adenine (A) with thymine (T) and cytosine (C) with guanine (G).

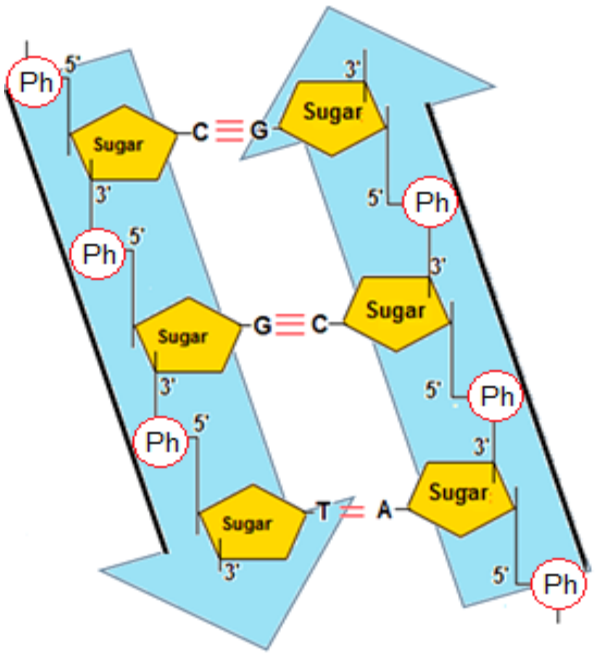


Fig. 3-16: Two polynucleotide chains form DNA double helix. Their nitrogenous bases attach together by hydrogen bonds to form base -pairs inside DNA double helix. Ph (phosphate), T (thymine), C (cytosine), A (adenine) and G (guanine).

Chargaff's rule

Erwin Chargaff (1905-2005) and his coworkers have studied the base composition of DNA from a number of organisms.

Chargaff pointed out that:

1) Total mole percentage of purines is approximately equal to that of the pyrimidines that is (%G + %A) = (%C +%T).

2) The mole percentage of thymine is nearly equal to that of adenine (%T / %A=1).

1) The mole percentage of cytosine is nearly equal to that of guanine (%G / %C=1).

This ratio is the same for DNA of different types of tissues of the same animal and does not differ with the age within the same species.

***General characters** of DNA double helix (fig.3-17) can be summarized in the following points:*

- *Antiparallel*: The two polynucleotide chains run in opposite.
- *Complementary*:
  The sequence of nitrogenous bases (A, T, C, and G) in one chain determines (dictates) the complementary sequence of nitrogenous bases in the other chain.
- The two polynucleotide chains of DNA are not identical.
- The nitrogenous bases form complementary *base pairs*.

- The outer wall of DNA double helix is formed of *sugar-phosphate back-bone.* Phosphate groups form 3', 5' phosphodiester linkage between each nucleotide and the next.
- A turn of DNA double helix repeats every 10 base pairs (every 3.4 nm).
- The width of DNA double helix equals 2.0

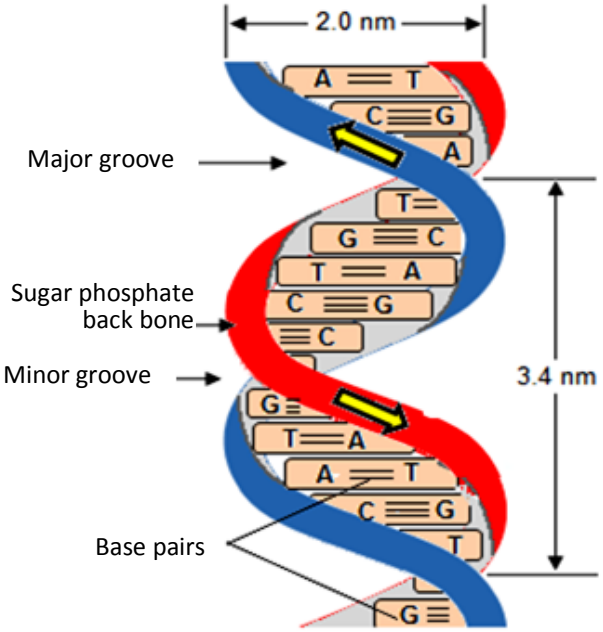


Fig. 3-17: DNA double helix showing the minor and major grooves. Each turn is 3.4 nm in length. Arrows indicate the antiparallel character of DNA double helix.

Additional reading

Arents G et al. (1991): The nucleosomal core histone octamer at 3.1 A resolution: A tripartite protein assembly and a left-handed super helix. Proc Natl. Acad Sci USA 88:10148-10152

Felsenfeld G and McGhee J D (1986): Structure of the 30 nm chromatin fiber. Cell, 44: 375-377.

Kornberg RD (1974): Chromatin structure: A repeating unit of histones and DNA, Science, 184, 868-871.

Kornberg RD (1977): Structure of chromatin, Ann. Rev. Biochem, 46, 931-954.

Kornberg RD and Lorch Y (1992): Chromatin structure and transcription, Ann. Rev. Cell Biol. 8, 563-587.

Krick FHC (1954): "The Structure of the hereditary material". Sci. Am., 91 (10), 20, 54-61.

Lewin B (2002): DNA in Genes VII. Oxford University Press, 347-378.

McGhee JD and Felsenfeld G (1980): nucleosome structure. Ann. Rev. Biochem. 49, 1115-1156.

Richmond T J. et al. (1984): Structure of the nucleosome core particle at 7 A resolutions. Nature, 311, 532-537.

Solomon EP, Berg LR and Martin DW (2002): DNA: The carrier of genetic information in Biology, 6th ed. United States: Thomson Learning Academic Resources Center, 243-260.

Wang J (1982): The path of DNA in the nucleosome. Cell 29, 724-726.

Workman L and Kingston RE (1998): Alteration of nucleosome structure as a mechanism of transcriptional regulation. Ann. Rev. Biochem. 67, 545-580.

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer:

1) What is the expected percentage of RNA in chromatin substance of eukaryotic cell?
   a. 5%
   b. 10%
   c. 20%
   d. 40%

2) By which molecule of the following is carbon #1 of sugar ribose connected in the nucleotide molecule?
   a. phosphate molecules
   b. Nitrogenous base
   c. Histones
   d. Non-histones

3) To which of the following does the antiparallel character of DNA strands refer?
   a. DNA strands run parallel to each other.
   b. DNA is a double helix structure.
   c. Bases of DNA are complementary.
   d. Parallel DNA strands run in opposite directions.

4) To which of the following does term of **base-pairing** refer?
   a. DNA contains 4 types of bases.
   b. DNA contains 2 types of bases.
   c. DNA is formed of 2 polynucleotide chain.
   d. Nitrogenous bases form pairs within DNA.

5) How many nanometers is the distance between two successive base– pairs in DNA double helix?
   a. 10 nm
   b. 0.34 nm
   c. 3.4 nm
   d. 34 nm

6) Which of the following is the basic structural unit of chromatin fibers?
   a. Peripheral histone
   b. Globular basic histone
   c. Nucleosome
   d. Nucleotide

7) How many base pairs in 2 DNA successive turns?
   a. 5 base pairs
   b. 10 base pairs
   c. 20 base pairs
   d. 30 base pairs

8) Which nitrogenous base of the following is **not** found in DNA?
   a. Adenine
   b. Guanine
   c. Thymine
   d. Uracil

9) Of which molecules of the following is DNA backbone formed?
   a. Phosphate and nitrogenous bases
   b. Nitrogenous bases and sugar ribose
   c. Sugar deoxyribose and nitrogenous bases
   d. Phosphate and sugar deoxyribose

10) DNA molecule contains equal amounts of adenine and which base of the following?
   a. Adenine
   b. Guanine
   c. Thymine
   d. Cytosine

11) Which structures of the following are complementary to each other within DNA molecule?
   a. Nitrogenous bases
   b. Phosphate molecules
   c. Sugar ribose molecules
   d. Globule of Histones

12) Which part of the gene is particularly sensitive to endonuclease?
   a. Non-coding region
   b. Protein coding region
   c. Regulatory region
   d. Termination region

13) Which of the following is free of histone particles?
   a. Protein coding region of gene
   b. Regulatory region of gene
   c. Upstream leader region
   e. Termination region of gene

14) How many histone particles form the core of nucleosome?
   a. One pair
   b. 2 pairs
   c. 4 pairs
   d. 8 pairs

15) Nucleosomes are connected together by:
   a. Cytosine molecules
   b. Phosphate groups
   c. Scaffolding proteins
   d. Strand of DNA

16) Ratio of cytosine in DNA equals to ratio of which nitrogenous base of the following?
   a. Adenine
   b. Guanine
   c. Thymine
   d. Ribose

17) In DNA double helix, which of the following is attached by 3 hydrogen bonds with cytosine?
   a. Adenine
   b. Thymine
   c. Uracil
   d. Guanine

18) Nucleoside is a nucleotide after removal of:
   a. Sugar pentose
   b. Nitrogenous base
   c. Phosphate groups
   d. Sugar ribose

19) Between which molecules of the following does phosphodiester linkage connecting DNA molecule?
   a. Nitrogenous bases
   b. Phosphate molecules
   c. Nucleotide molecules
   d. Deoxyribose molecules

20) How many base pairs does nucleosomal DNA molecule contain?
   a. 100 base pairs
   b. 120 base pairs
   c. 146 base pairs
   d. 170 base pairs

21) Which structure of the following functions in regulation of DNA transcription and replication?
   a. Nitrogenous bases of DNA
   b. Non –histone proteins
   c. Peripheral histone
   d. Globular histone protein

22) Why **doesn't** DNA double helix allow formation of base pairs from adenine and guanine?
   a. Adenine -guanine is too small to fit
   b. Adenine attaches only with cytosine
   c. Guanine attaches only with thymine
   d. Internal distance is so small.

23) Loops of chromatin fiber are held together by:
   a. Histone protein
   b. Non-histones protein
   c. Nitrogenous bases
   d. Scaffolding protein

24) Which protein of the following works to held nucleosomes of chromatin fibers together?
   a. Globular Histone
   b. Non-histone protein
   c. Peripheral histone
   d. Scaffolding protein

25) If a sample of DNA contains 20 nitrogenous bases of thymine, what is the percentage of nitrogenous bases connected by 3 hydrogen bonds in this sample?
   a. 10%
   b. 20%
   c. 40%
   d. 60%

26) Which nitrogenous base of the following is purine?
   a. Uracil
   b. Cytosine
   c. Adenine
   d. Thymine

Answer of MCQs:

1) A	7) C	13) B	19) D	25) D
2) B	8) D	14) C	20) C	26) C
3) D	9) D	15) D	21) B	
4) D	10) C	16) B	22) D	
5) B	11) A	17) D	23) D	
6) C	12) C	18) C	24) C	

Part II: Short Answer Questions.

1. Define :

a) Nucleosome:

_____

b) Nucleotide:

_____

2. Fill in the spaces:

a) Scaffolding protein of genetic material hold _____together.

b) Condensation of chromatin fibers in cell division results in formation of _____.

c) DNA associated proteins are classified into: _____and_____

d) Histones protect DNA against _____.

e) Non-histones are necessary for_____ and _____ of DNA.

f) How many bases in a piece of 340 nm length of DNA double helix?

_____

g) If Adenine forms 10%, calculate the percentage of other bases?
   1. Thymine:_____
   2. Cytosine:_____
   3. Guanine :_____

h) If the percentage of bases that are bound by 2 hydrogen bonds is 40% in a sample of DNA, what is the percentage of the other base pairs of 3 hydrogen bonds?

_____

MOLECULAR BASIS OF GENETIC MATERIAL (Part -III)

Chapter 3:

MOLECULAR BASIS OF GENETIC MATERIAL (Part -III)

DNA Replication

Objectives

After you have studied this chapter (part III) you should be able to:

- *Describe the process of semi- conservative replication of DNA.*
- *State the features and significance of DNA–replication.*
- *Explain how DNA replication preserves genetic information generation after generation.*
- *Contrast the chromosomal organization and DNA replication in both prokaryotic and eukaryotic cells.*
- *Explain the relation between DNA replication and cell aging.*

***DNA** replication* is a complex process, in which DNA molecule replicates itself (copies itself) into two molecules of double stranded DNA. Each one is formed of *one new* and *one old* strand. It occurs in *S-phase* of the cell cycle. DNA replication is a process of semidiscontinuous synthesis of two complementary chains *(fig. 3-18).*

Requirements of DNA –replication:

- ***Nucleoside triphosphates.***
  They are the substrate of DNA-replication, so they are the sources of nucleosides mono-phosphate that build the new complementary nucleotide chains.
- ***DNA-helicase.***
  Enzyme which unwinds and separates the two strands of DNA double helix at origin of replication in order to form the replication forks.
- ***SSB; single strand binding protein, DNA destabilizing proteins.***
  They prevent the reformation of DNA double helix by attaching the separated DNA strands at replication forks.
- ***DNA polymerases***
  They are enzymes of replication, which are responsible for repairing DNA errors in replication, synthesis and elongation of new DNA strands in replication by addition of nucleotides to 3'-OH end of RNA-primer.
- ***Topoisomerase.***
  It is an ATP –dependent enzyme. It works to prevent knotting and tangling during DNA replication by cutting and rejoining DNA strands. It relaxes super-coils and separates interlinked circles of DNA molecules.
- ***DNA ligase.***
  Enzyme which is responsible for sealing the nicks between adjacent nucleotides after replacing RNA-primers with DNA fragments.

- ***RNA –primer.***

It is a sequence of RNA, ~10 bases long, that provides the 3'-OH end for extension of the new DNA strand by DNA polymerase. It is synthesized by primase, a special RNA polymerase

- ***Telomerase.***

It is an enzyme that is responsible for extending the chromosomal end to its original length after each cell cycle in order to delay cell aging

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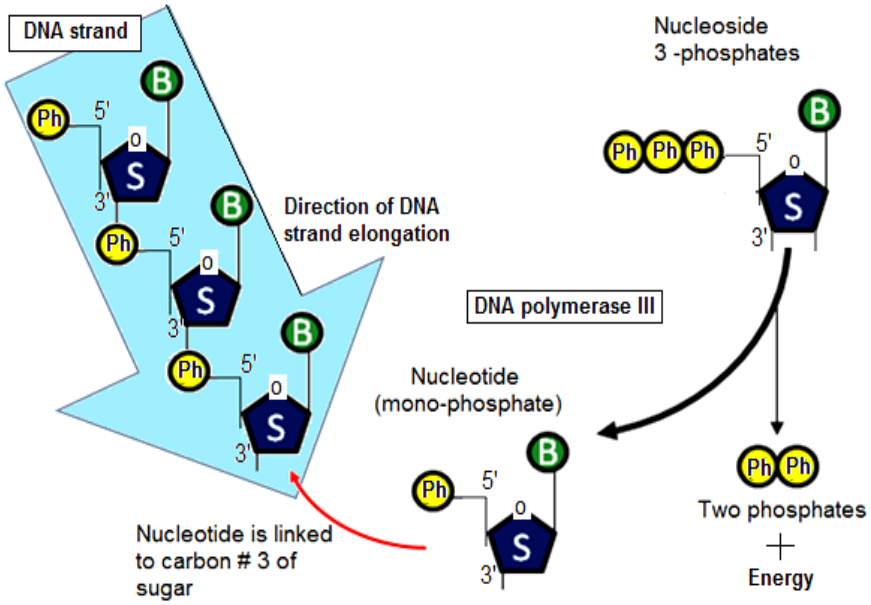


Fig. 3-18: In DNA replication the elongation process of DNA proceeds by addition of nucleoside triphosphate, where two phosphate groups are released when the nucleotide monophosphate are linked to carbon # 3 of sugar molecule in the last nucleotide at end-3'of DNA strand. This process is performed by enzyme of replication DNA polymerase III.

DNA polymerase III of replication in prokaryotes is a complex formed of several components: A catalytic core, β -subunits (sliding clamp) that keep the polymerase on DNA, and clamp loader that places β -subunits on DNA (fig. 3-19).It is responsible for DNA elongation in bacteria. The catalytic core enzyme contains:

1. α-subunit has ability to synthesize DNA.
2. ε -subunit has 3'-5' proofreading exonuclease.
3. Θ -subunits may be required for assembly.

Steps of DNA –Replication

In semi-conservative DNA replication, each strand of DNA duplex is used as a template for the formation of a new one using complementary nucleoside monophosphate.

- Replication begins by binding components of the ***replication complex***; DNA -helicase, DNA –polymerase and DNA –destabilizing protein, to a specific sequence on DNA duplex called *origin of replication.*
- *DNA –helicases* start the replication *by* unwinding and separation of DNA –strands that

result in formation of *two replication forks*, *Y-shaped regions*.

- Binding of *destabilizing proteins (SSB; single -strand binding proteins)* to single strands of DNA at the replication forks prevents rewinding the separated DNA -strands.
- At binding of *DNA –polymerases* to the two replication forks, two *replication complexes are formed at each origin of replication. Each one contains two polymerases.*
- DNA -polymerase starts the synthesis of complementary strands by linking nucleoside mono-phosphate to the free 3'-OH end of **pre-existing complementary *RNA –primer***. DNA polymerases use also a magnesium ion for catalytic activity.

Primase is required for the replication process because it is responsible for formation of the complementary RNA –primer (10-20 nucleotides).

- DNA -polymerases continue in their work to elongate the new DNA strands by addition of nucleosides mono-phosphate to free 3'–OH end of the last nucleotide.
- As the new strands grow in one replication fork, another replication begins in opposite direction at opposite replication fork; *replication is bidirectional at origin of replication*.
- At the end of replication, two new daughter strands are formed. These two new strands are complementary attaching to their templates; *old DNA strand.*

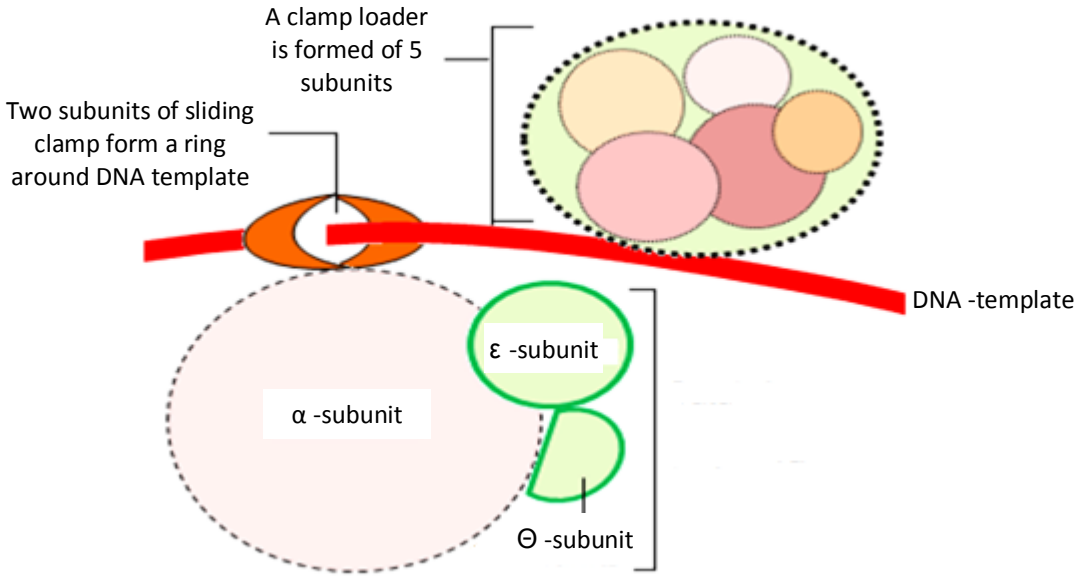


Fig. 3-19: Diagram showing the different component of DNA polymerase III and clamp loader.

- The following diagrams (figs. 3-20, 3-21, 3-22, and 3-23) illustrate a summary of the steps of DNA replication.

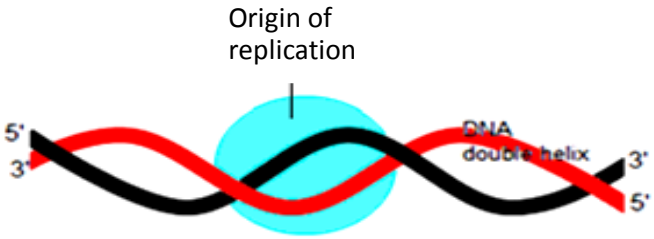


Fig. 3-20: DNA replication begins by binding of replication complex formed of DNA helicase, SSB and DNA polymerase to origin of replication

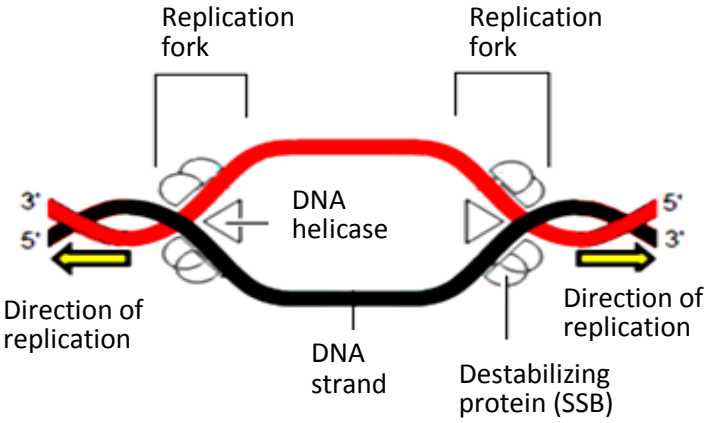


Fig. 3-21: Replication forks formation: DNA helicase unwinds and separates DNA strands at origin of replication. Attaching of SSB prevents their rewinds.

Fig. 3-22: Replication complexes formation and synthesis of new complementary strands: The replication process begins when formation of replication complexes is accomplished by binding of DNA polymerases to replication forks. DNA polymerases add nucleotides to preexisting RNA primers

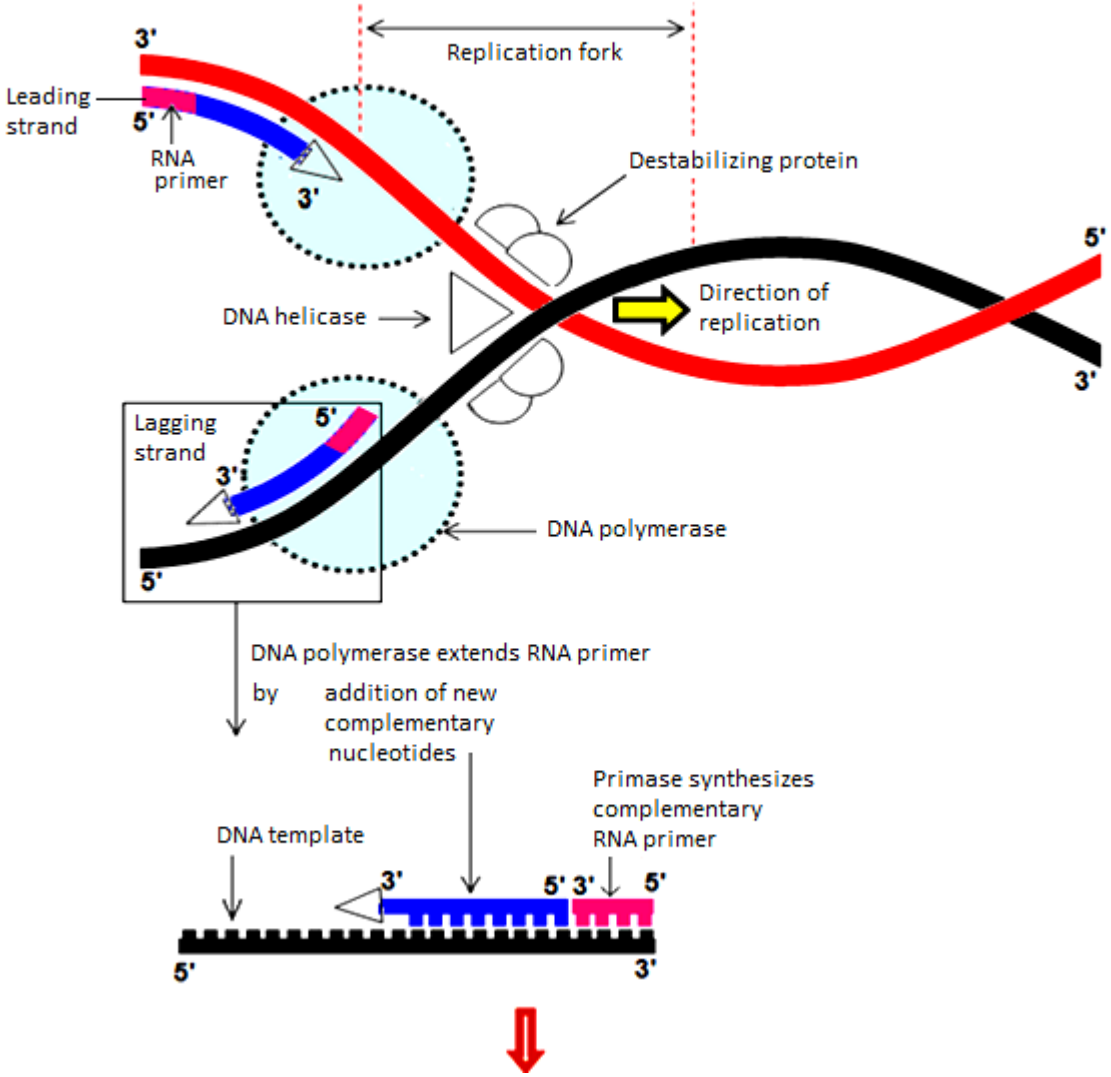


Fig. 3-23: Formation of leading and lagging strands.

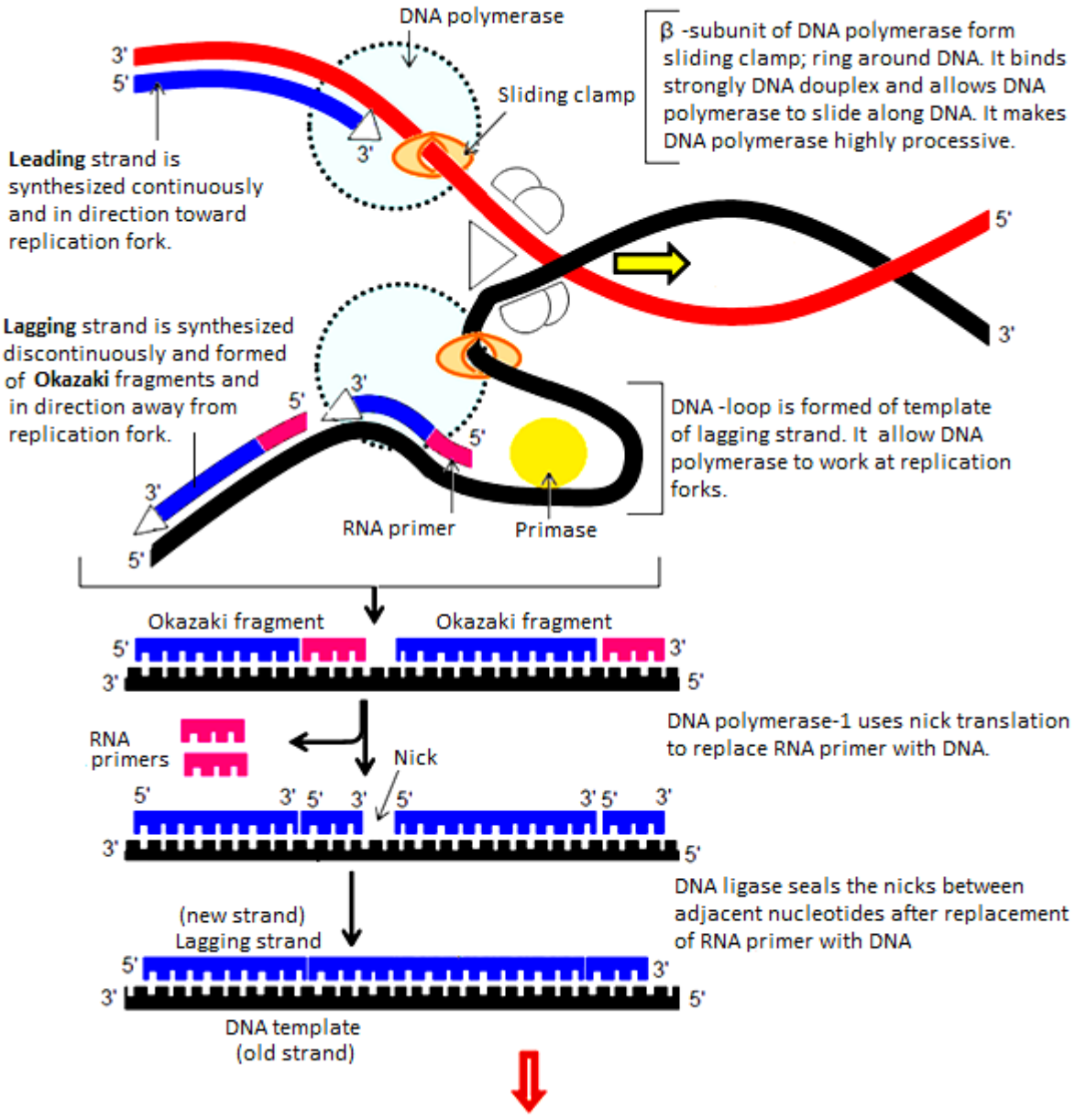
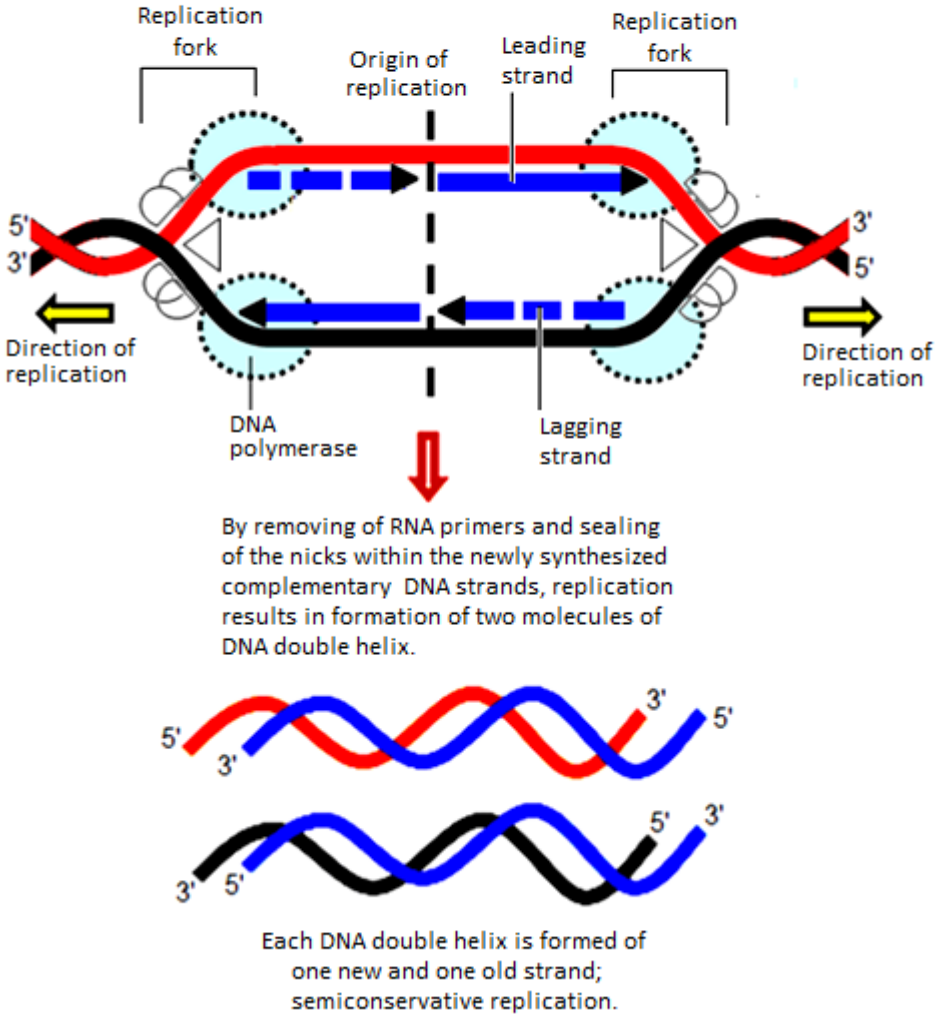


Fig. 3-24: Formation of two DNA double helices is the end of replication. DNA polymerases continue to complete the processes of synthesis of leading and lagging strands.



The **semidiscontinuous DNA replication** results in formation of two newly synthesized strands:

1) **Leading strand:**
   It is formed as a continuous strand in direction toward replication fork.
2) **Lagging strand:**
   It is formed discontinuously in direction away from replication fork as short fragments called **Okazaki fragments**. Each one fragment ranges from 100 to 200 nucleotides in length.

Why Lagging strand is synthesized as short separate fragments/

DNA polymerase works at replication fork– site of synthesis and lagging strand grows in direction away from replication fork. So, DNA polymerase becomes unable to continue in its function as the fragment of lagging strand moves away from the site of synthesis, so it repetitively dissociates itself from the synthesized fragment and reassociates with the template of lagging strand to start its synthesis of another fragment at replication fork. These dissociation and reassociation result in synthesis of short separate fragments of discontinuous lagging strand.

DNA polymerase-I replaces RNA-primer of Okazaki –fragments with DNA-fragment and ***DNA-ligase*** seals the nicks between the adjacent fragments.

For coordinating synthesis of leading and lagging strands, fragments of lagging strand and its template form a loop close to the replication forks. This **loop formation** results in:

i) DNA polymerase of works at the replication fork.
ii) Synthesis of leading and lagging strands occurs at the same time.
iii) It may accelerate the replication process.

DNA-polymerases:

- They are enzymes of replication - responsible for synthesizing of DNA- strands.
- They use nucleoside triphosphate in building the growing new complementary DNA -chains.
- Their function is break down of nucleoside triphosphates into nucleoside monophosphate, energy and two Phosphate molecules.
- They add up the nucleotide (monophosphate) to the free 3'-OH end of the last nucleotide in the new polynucleotide chain.
- They carry out proofreading function to correct any error during replication.
- They move in 5' to 3' direction by using the energy resulting from break down of nucleosides triphosphates.
- Polymerase requires primers to start its function.

- In **prokaryotes** five types of **DNA polymerases** have been identified, from which:
  Pol I: It is implicated in proofreading DNA -repair and RNA primer removal. It replaces RNA-primer of Okazaki–fragments with DNA-fragment.
  Pol II: It is involved in repairing damaged DNA.
  Pol III: It is responsible for DNA elongation in bacteria.

- At least 15 **Eukaryotic DNA polymerases** have been recognized, from which:
  Pol α: Acting as a primase (synthesizing an RNA primer) and involved in synthesizes the lagging strand.
  Pol δ: It is involved in synthesis of the leading strand.
  Pol β: it is implicated in repairing DNA, in base excision repair and gap-filling synthesis.
  Pol γ: It replicates and repairs mitochondrial DNA and has proofreading activity.

Features of DNA -Replication in Eukaryotic Cell:

- Replication initiates at multiple origins of replication at the same time.

- Heterochromatin replicates later in S-phase than euchromatin.
- After replication, chromatin structure is re-formed by the addition of new histones.
- Chromosomal histones reduce the rate of replication and make the Okazaki fragments shorter in comparison with prokaryotes.
- The eukaryote chromosomes have a linear shape and vary in length.
- *Telomerase* is required to add *protective telomeres* because the linear eukaryotic chromosome could be shorter after successive rounds of replication.
- The haploid human cell contains 23 chromosomes which form large coiled loops and carry about 20,000 - 22,000 determined genes and are estimated to be about *three billion base pair long*.

Features of DNA-Replication in Prokaryotic Cell:

Genome of prokaryotic organisms is localized in the *nucleus-like* region; **nucleoid** which is an irregularly-shaped region without a nuclear membrane. Generally it is a circular, double-stranded piece of DNA, of which multiple copies may exist at any time. The length of a genome widely varies, but generally is at least a few million base pairs. Prokaryotic genome is stored within a nucleoid.

The DNA of a prokaryote; genophore is compacted through a mechanism known as supercoiling. It is commonly referred to as a prokaryotic chromosome which lacks chromatin of Eukaryote. The genophore is generally of a much smaller size than Eukaryotic chromosomes. The genophore is circular in most prokaryotes, and linear in very few. The circular nature of the genophore allows replication to occur without telomeres.

- There is one origin of replication which is a DNA sequence formed of 245 base pairs.
- Bacteria can copy all DNA in few minutes. Under optimal conditions, bacteria can grow and divide extremely rapidly, and bacterial populations can double as quickly as every 9.8 minutes. In cell division, two identical clone daughter cells are produced.
- Bacterial cell contains extra chromosomal elements called **plasmid.** It is a small circular DNA double helix molecule.

Plasmids

They are capable of autonomous replication. Plasmid is typically a circular and double-stranded DNA molecule. It usually occurs in bacteria, sometimes in eukaryotic organisms. Size of

plasmids varies from 1 to over 400 kilobase pairs (Kbp).
A single bacterial cell may contain one copy for large - sized plasmids, or many copies of the small -sized plasmids. *It* carries 5 to 100 genes that are responsible for:

1. Synthesis of toxins.
2. Synthesis of proteins required for transfer of plasmid to other bacterial cell.
3. Formation of transposons.

Plasmids can be a part of the mobilome; the total of all mobile genetic elements in a genome (transposons), since they are often associated with bacterial conjugation; a mechanism of horizontal gene transfer.

Transposon

It is a mobile DNA segment that can move between plasmids and bacterial DNA molecule. Transposons are also called "jumping genes", and are examples of mobile genetic elements.
If it carries antibiotic resistance genes, it becomes responsible for distribution of the antibiotic resistance character between bacterial populations.
It is responsible for the ability of plasmid to be integrated into the bacterial chromosome.

Notes:

- To finish the replication of all DNA, eukaryotic cell requires a few hours.
- All cellular organisms, from bacteria to human, contain a single type of genetic material, DNA molecule.
- Gene mutation, gene replication and gene recombination are the same for all life forms.
- The prototypic organism which has been used in microbial genetic studies for the past 50 years is *E.coli*, the enteric gram negative *Escherichia coli.*

Ways of Transfer of exogenous Genetic Material into Bacteria Cell

There are three processes by which exogenous genetic material may be introduced into bacterial cell (fig. 3-20):

1) Transformation
2) Transduction
3) Conjugation

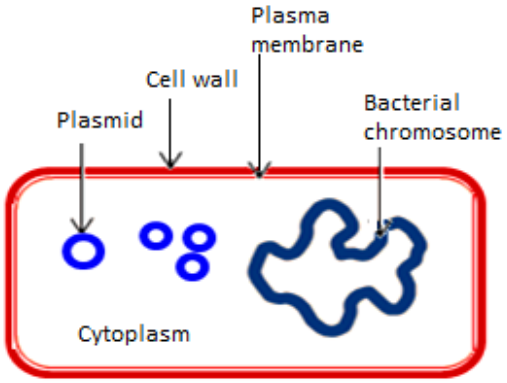


Fig. 3-19: The hereditary material of a bacterial cell is formed of single bacterial chromosome; folded strand of DNA double helix and circular bacterial plasmids.

1) **Transformation** is the genetic alteration of a cell which results from the direct uptake and expression of exogenous genetic material, exogenous DNA, from its surrounding. Exogenous DND is taken up through the cell membrane receptors. Transformation can also be effected by artificial means. Bacteria that are capable of being transformed, whether naturally or artificially, are called competent.

About 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; many more are able to take it up in their natural environments. Such bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane.

Introduction of DNA into animal cells is usually called **transfection**.

2) **Conjugation.** It is a process of transfer of genetic material between two bacterial cells in direct contact.

- *E. coli* bacteria can sexually conjugate and exchange genetic information.
- The genes that are required for *conjugation* are carried on the *F –factor DNA (plasmid) of the male bacterium.*
- *These genes code for the pili* on the surface of the male bacterium, and the *conjugation tube* that is used for one-directional transfer of a copy of male DNA to the female cell.
- During conjugation, a copy of the *F -factor DNA is transferred from the male to the female.* The female becomes male following conjugation. An *F* -factor, is also called an ***F -episome***. Transfer of an *F* -episome is a special -type of conjugation known as ***sexduction***. Conjugation and mixing of DNAs of two living bacterial cells has been considered as a special sexual process.

3) Transduction. It means injection of foreign DNA by a bacteriophage into the host.

Telomerase and Cellular Aging

- A **telomere** is a region of repetitive DNA at the end of eukaryotic chromosomes, which protects the end of the chromosome from destruction. In eukaryotic cell replication, the linear form of chromosomes makes that DNA -polymerase leaves a small portion at the end of DNA unreplicated, so it causes a small single stranded DNA to be lost and the chromosomal ends shorten slightly with each cell cycle.
- Telomeres are extended after each cycle by special enzymes called ***telomerases***, part of a protein subgroup of specialized reverse transcriptase enzymes known as TERT (*telomerase reverse transcriptases*). They use RNA template to maintain the length of telomeres. It is involved in synthesis of telomeres in humans and many other, but not all, organisms.
- Function of telomerase is maintaining and protecting the length of the telomeres in actively proliferating cells after each cycle such as germ cells, hemopoietic stem cells in bone marrow and cancerous cell.

Active telomerase has been observed in animal and human germ cell line but not in somatic cells which show evidence of cellular aging in tissue culture.

If the number of cell divisions is determined by the age of an individual, scientists reported that *cells from a 70 year old-individual can divide only 20 to 30 times as compared with those from an infant which can divide 80 to 90 times*

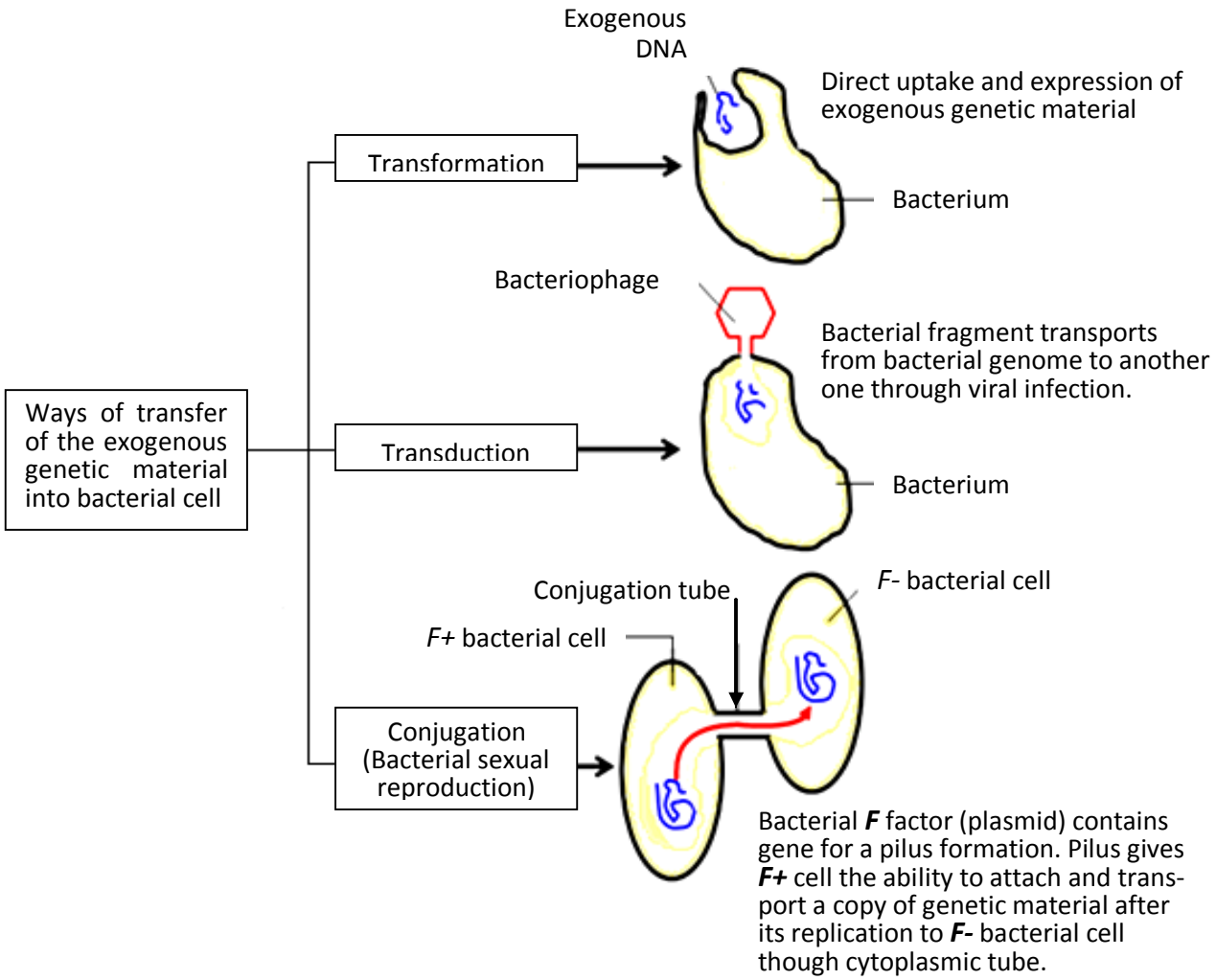


Fig. 3-19: Diagram illustrates the mechanisms of transfer of exogenous genetic material into bacterial cells: Transformation, transduction and conjugation.

Additional reading

Eiu B, Wong M E and Alberts B (1994): A transcribing RNA polymerase molecule survives DNA replication without aborting its growing RNA chain. Proc. Natl. Acad. Sci. USA 91, 10660-10664.

Elliott WH and Elliott DC (2002): Biochemistry and molecular biology, 2nd ed. UK: Oxford University Press.

Featherstone C and Jackson SP (1999): Double strand break repair. Current Biology9: R759-761.

Judson HF (1979): The eight day of creation: Makers of the revolution in biology. New York: Simon & Schuster.

Johnson FB, Marcniak RA and Guarrente L (1998): Telomeres, the nucleolus and ageing. Current Opinion in Cell Biology: 10: 332 – 328.

Hayes B (1998): The invention of the genetic code, American Scientist, Vol. 86, No.1.

Hewish DR and Burgoyne LA (1973): Chromatin structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. Biochem Biophys. Res. Comms., 52, 504-510.

Kierszenbaum AL (2002): Histology and cell biology: An introduction to pathology. St. Louis, Missouri: Mosby Inc., Elsevier Science.

Lewin B (2002): Genes VII. Chapter 13: DNA replication. UK: Oxford University Press, 385-408.

Li JJ (1995): Once and once only. Current Biology, Vol. 5, 472-475.

Lodish H. et al. (2000): Molecular cell biology, 4th ed. New York: WH Freeman and Co.

Marahrens Y and Stillman B (1992): A yeast chromosomal origin of DNA replication defined by multiple functional elements. *Science 255,* 817-823.

Naktinis V, Turner J and O'Donnell M (1996): A molecular switch in a replication machine defined by an internal competition for protein rings. Cell 84, 137-145.

Solomons G and Fryhle C (2000): Organic chemistry, 2nd ed. New York: John Willy & Sons, Inc.

Solomon EP, Berg LR and Martin DW (2002): Biology, 6th ed. DNA: The carrier of genetic information. United States: Thomson Learning Academic Resources Center, 243-260

Waga S and Botcham M (1999): The DNA replication fork in eukaryotic cells. Ann. Rev. Biochem. 67: 721

Willetts N and Skurray R (1987): Structure and function of the F factor and mechanism of conjugation, In *E. coli* and *S. typhimurium*, (Ed. F. C. Neidhart, American Society for Microbiology, Washington DC, 1110-1131).

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer:

1. According to which model of the following does DNA molecule replicate?
   a. Conservative.
   b. Overconservative.
   c. Semiconservative.
   d. Random replication.

2. Which of the following molecules are used to synthesize the new strands in DNA replication?
   a. Nucleotides
   b. Nitrogenous bases
   c. Nucleosomes
   d. Polypeptide molecule

3. Which molecule of the following is the source of energy that is required for linking nucleotides of new strands together in DNA replication?
   a. Nucleotide
   b. Sugar ribose
   c. Nucleoside
   d. Nucleosomes

4. Which enzyme of the following is responsible for linking nucleotides together in DNA replication?
   a. Primase
   b. DNA -polymerase
   c. Topoisomerase
   d. DNA ligase

5. DNA replication proceeds by addition of new nucleotides to which carbon atom of sugar deoxyribose?
   a. 1'
   b. 2'
   c. 3'
   d. 4'

6. Which enzyme of the following unwinds DNA double helix at the beginning of replication?
   a. DNA polymerase
   b. Primase
   c. DNA ligase
   d. DNA helicase

7. Which of the following works to prevent re-winding of DNA strands during the replication process?
   a. DNA polymerase
   b. Topoisomerase
   c. Destabilizing proteins
   d. DNA helicase

8. How many replication forks are formed at two origins of replication?
   a. 2
   b. 4
   c. 6
   d. 8

9. Which enzyme of the following requires primers to work during replication?
   a. DNA polymerase
   b. RNA polymerase
   c. Topoisomerase
   d. DNA helicase

10. To correct any error in DNA replication a proofreading function is performed by which of the following?
    a. RNA primase
    b. Topoisomerase
    c. Telomerase
    d. DNA polymerase

11. How many leading and lagging strands are synthesized at 2 origin of replication?
    a. 2
    b. 4
    c. 6
    d. 8

12. In which form of the following lagging strand is synthesized during replication?
    a. Continuous strand.
    b. Discontinuous strand.
    c. Coiled strand.
    d. Irregular strand.

13. Why does DNA polymerase synthesize lagging strand as separate fragments? Because:
    a. Synthesis occurs toward replication fork.
    b. Its template forms DNA -loop.
    c. Synthesis requires RNA primers.
    d. Dissociation & reassociation of DNA polymerase.

14. Which enzymeof the following seals the nicks between Okazaki fragments?
    a. Telomerase
    b. DNA ligase
    c. DNA Polymerase
    d. Topoisomerase

15. The loop at replication fork is formed of:
    a. Leading strand and its template
    b. Lagging strand only
    c. Lagging strand and Its template
    d. Lagging and leading strands

16. Which of the following enzymes replaces the RNA primer with DNA?
    a. RNA primase
    b. RNA polymerase
    c. DNA polymerase
    d. DNA ligase

17. Which enzyme of the following is required for extending the ends of chromosomes after each replication?
    a. Telomerase
    b. DNA ligase
    c. DNA Polymerase
    d. RNA primase

18. Why do lagging strand and its template form loop at the replication fork?
    a. For working of DNA polymerase at replication fork.
    b. To avoid tangling of DNA strand.
    c. To synthesize lagging strand before leading strand.
    d. For formation of discontinuous strand.

19. Which enzyme of the following prevents tangling and knotting of DNA during replication?
    a. DNA ligase
    b. DNA Polymerase
    c. Topoisomerase
    d. RNA polymerase

20. How many plasmids are present in a bacterial cell?
    a. 2
    b. 4
    c. 5
    d. variable

21. All the following are consistent with the characters of plasmid ***except***:
    a. It is a circular DNA molecule.
    b. It carries genes for synthesis of toxins
    c. It carries genes for bacterial conjugation
    d. It is present only in eukaryotic cell

22. In which process of the following does DNA duplicate?
    a. Translation
    b. Replication
    c. Transcription
    d. Transformation

23. In which process of the following may DNA be taken up by a bacterial cell from environment?
    a. Transformation
    b. Transcription
    c. Conjugation
    d. Transduction

24. In replication, DNA polymerase works to do which process of the following?
    a. Separate strands of DNA double helix
    b. Synthesize the RNA- primer
    c. Build a new complementary strand
    d. Twist of DNA double helix

25. Which of the following is not used in replication of DNA?
    a. RNA primer
    b. Nucleoside
    c. DNA ligase
    d. RNA polymerase

26. In 2ndgeneration of mitotic division, what is the percentage of the newly synthesized DNA strands in the total DNA molecules of the new daughter cells?
    a. 0%
    b. 25%
    c. 50%
    d. 75%

27. For building of which structure of the following does F –factor in bacteria carry genetic information?
    a. Synaptonemal complex
    b. Mitotic spindle
    c. Conjugation tube
    d. RNA -primers

28. Formation of cytoplasmic –tube between male and female bacterial cells takes place in which process of the following?
    a. Transformation
    b. Transcription
    c. Conjugation
    d. Transduction

29. All the followings are ways of exchange of genetic material between bacteria ***except*:**
    a. Transformation
    b. Transduction
    c. Conjugation
    d. Binary fission

30. Transfer of an F' -factor episome from one bacterial cell to another is known as:
    a. Transformation
    b. Transduction
    c. Sexduction
    d. Conjugation

31. Which of the following molecules form a sliding clamp that maintains binding and movement of DNA polymerase on DNA strand during replication?
    a. α –subunits of DNA polymerase
    b. β- subunits of DNA polymerase
    c. δ- subunits of DNA polymerase
    d. π- subunits of DNA polymerase

32. In DNA molecule, phosphate group links between which of the following carbon atoms of deoxyribose molecules?
    a. Carbon #1& 2’
    b. Carbon #3 & 4’
    c. Carbon #5 & 3’
    d. Carbon #5 & 1’

33. Which of the following enzymes move in opposite direction during the replication process?
    a. DNA ligases
    b. DNA helicases
    c. Topoisomerases
    d. Telomerases

34. How many DNA polymerase work at 2 replication forks?
    a. 2
    b. 3
    c. 4
    d. 5

Answer of MCQs:

1)C	9) A	17)A	25)D	32)C
2)A	10)D	18)A	26)B	33)B
3)C	11)B	19)C	27)C	34)C
4)D	12)B	20)D	28)C	
5)C	13)D	21)D	29)D	
6)D	14)B	22)B	30)C	
7) C	15)C	23)A	31)B	
8) D	16) C	24)C		

Part II: Short Answer Questions.

1. **Define** each term of the following:
    a. Origin of replication:
    _____
    _____

    b. Replication complex:
    _____
    _____

    c. Bacterial sexduction:
    _____
    _____

    d. Antiparallel:
    _____
    _____

    e. Semiconservative replication:
    _____
    _____

    f. Bacterial conjugation:
    _____
    _____

2. What are the differences between the following structures:
    a. Leading & Lagging strands.
    _____
    _____

    b. Transformation & transduction
    _____
    _____

c. Primase & ligase
_____
_____

Fill in the spaces:

1. Ways of transfer of exogenous genetic material into bacterial cell are:
   a. _____
   b. _____
   c. _____

2. Requirements for DNA replication are :
   _____
   _____
   _____
   _____

3. DNA polymerase III is a complex composed of the following 3 components:
   a) _____
   b) _____
   c) _____

4. The catalytic core of DNA polymerase III is formed of:
   a) _____
   b) _____
   c) _____

5. Plasmid carries genes that are responsible for:
   a) _____
   b) _____
   c) _____

Explain each statement of the following:

a. Lagging strand is synthesized as discontinuous strand while leading strand is synthesized as continuous DNA strand.
   _____
   _____
   _____

b. Sliding clamp makes DNA polymerase highly processive.
   _____
   _____
   _____

c. Role of F- factor in sexduction in bacteria.
   _____
   _____
   _____

EXPRESSION OF GENETIC INFORMATION

Chapter 4:
EXPRESSION OF GENETIC INFORMATION

Objectives

After you have studied this chapter you should be able to:

- *Outline the flow of genetic information from DNA up to protein synthesis.*
- *Differentiate the types of RNA.*
- *Describe the structure of DNA and RNA.*
- *State the general characteristics of genetic code and describe the transcription process.*
- *Explain the importance of tRNA, mRNA and rRNA for translation process.*
- *Diagram the steps of protein synthesis.*
- *Outline the process of eukaryotic mRNA modification and processing.*
- *Discuss eukaryotic and prokaryotic mRNA.*
- *Describe the types of post-translation modifications of synthesized protein.*

Flow of Genetic Information

The genetic information that is carried by nuclear genetic material in the form of genes is required to flow from nucleus into the cytoplasm to be expressed.

The genetic informational units, genes, are generally defined as informational units that are required to carry out one or more cellular functions.

- Human chromosomes carry about 22000 recognized genes and contain about 3 billion base pairs. They are formed of a specific sequence of bases; adenine (A) – thymine (T) – cytosine (C) – guanine (G). Each gene has a specific location on DNA double helix.
- The 4 bases A, T, C and G form the 4 letters of alphabet of the genetic language.
- The genetic word which specifies any amino acid is called the genetic code.
- The genetic code (fig. 4-1) is formed of 3 letters from the alphabet of gene language representing 3 bases of 3 successive nucleotides. So the genetic code is called the ***triplet code***. It *is a sequence of three successive nucleotides on a DNA or RNA molecule codes for a specific amino acid in protein synthesis.*
- Genes have a different number of triple codes that determine the complementary codons of messenger RNA (mRNA) - during *transcription (fig. 4-1).*
- Each *codon* of mRNA - 3 successive bases, determines the complementary *anti-codon* of transfer RNA (tRNA) during *translation.*
- Each tRNA carries specific *amino acid*, so the codons of mRNA determine the amino acids that share in synthesis of the polypeptide chain of protein.

The General Features of Genetic Codes:

- It is a triplet code formed of three bases and is nearly universal for all organisms - UUU is coding for phenylalanine in all organisms.
- It is redundant. The **redundancy** of genetic code is due to more than one code which specifies one amino acid; there are 64 possible codons, 61 of which specify the 20 amino acids that make up proteins and are carried by 40 tRNA.
- It is read as a series of nucleotide.
  The start codon of mRNA determines the starting point of reading frame, while the stop codon of mRNA is the ending point of reading frame - frame of translation of genetic message.
- The genetic codes could undergo mutation.

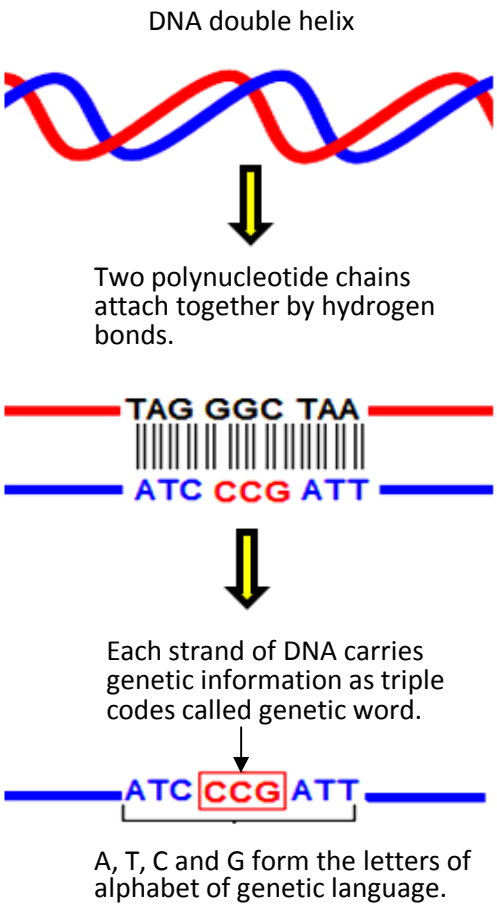


Fig. 4-1: Diagram illustrates a single strand of DNA. The genetic word (triple code) of a gene is formed of three letters from the four letters; C, G, T, and A of alphabet of the genetic language.

Expression of Genetic Information

It is a complex process which includes decoding the genetic information in DNA to be used for synthesizing of a specific protein in the cell cytoplasm (fig. 4-2).Expression of genetic information includes two steps:

1. *Transcription*
2. *Translation*

Transcription

It is a process of synthesis of RNA molecules; mRNA, tRNA, and rRNA, as a single complementary strand on DNA template, gene. The process is catalyzed by DNA dependent RNA polymerase.

Translation or Protein Synthesis

Translation is a process of conversion of the genetic information on DNA molecule which is copied and carried by mRNA molecule, in form of *codons*, into amino acid chain in the cytoplasm. The process of translation in cytoplasm requires: mRNA, ribosome, GTP; guanosine triphosphate as a source of energy, initiation factors and elongation factors.

RNA -Molecules

There are three types of RNA molecules:

a) Messenger RNA(**mRNA**)
b) *Transfer RNA*(**tRNA**)
c) *Ribosomal RNA*(**rRNA**)

Structure of RNA molecules:

- It is a folded single strand.
- They are formed as a single strand of polynucleotides like DNA, but it has some differences:

– It forms a short double stranded segment.
– Deoxyribose sugar of DNA becomes ribose in RNA molecules.
– The base thymine in DNA is replaced by uracil in RNA.

Ribosomes

They are the machine of protein synthesis. **Composition**: It is formed of two subunits; large and small subunits, which are made up of proteins and rRNAs. Large subunit contains *two binding* sites: ***P****-site* for binding **p**eptidyl-tRNA that holds the polypeptide chain, while ***A*** *–site* for binding of **a**minoacyl-tRNA that delivers the amino acid.

In *E. coli, large ribosomal subunits* appear as solid particles, each one is composed of3 rRNA - molecules and 34 proteins*. Small ribosomal subunit* is formed of one rRNA molecule and 21 proteins. Small subunit carries the mRNA binding site (fig. 4-3).

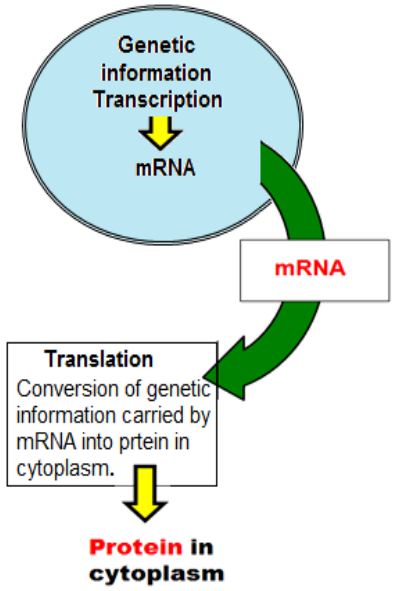


Fig. 4-2: Process of expression of genetic information includes two processes.

1. *Transcription: Synthesis of mRNA.*
2. *Translation: Conversion of genetic information into proteins of specific functions.*

Ribosomes are present in an *inactive* state when the two subunits are found as separate entities scattering in cytoplasm.
Ribosomes become *active* when their subunits are linked to mRNA during translation of genetic message.
Polyribosomes (polysomes) are composed of mRNA -linked clusters of ribosomes during protein synthesis.

Ribosomal RNA is synthesized on DNA as a complementary strand in nucleolar organizing region (NOR) of eukaryotic cell, while the **ribosomal proteins** are synthesized in cytoplasm and then migrate to the site of ribosomal synthesis inside the nucleus.

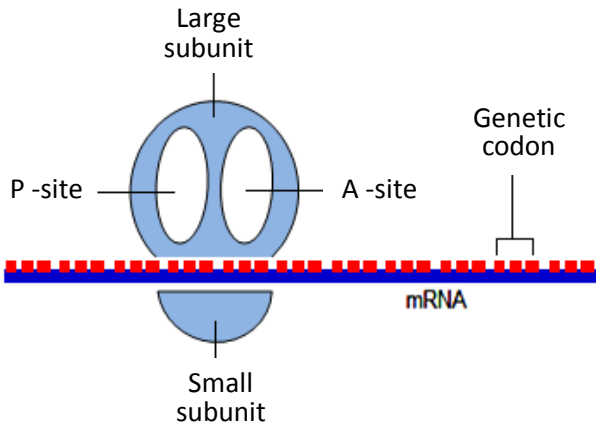


Fig. 4-3: Ribosome is formed of two subunits; large and small subunits. They attach to mRNA during protein synthesis.

Functions of ribosomes:

- They translate the genetic information which is carried by mature mRNA into protein.
- They are responsible for holding mRNA, amino-acyl tRNA and polypeptide chain in a correct orientation during translation.
- Active ribosomes form the peptide bonds between amino acids of polypeptide chains.

Transfer RNA *(tRNA)*
They are small molecules, less than 100 nucleotides in length, which are synthesized by transcription of **tRNA genes**.

All *transfer RNAs* have **common features**. Their secondary structure can be diagramed in the form of *clover-leaf* (fig. 4-4), in which the complementary bases pair forming stems for single stranded loops which form the 4 arms of tRNA.

- These tRNA -molecules are recognized by both *amino acyl- tRNA synthetase* and *ribosomes*.
- They have an amino acid acceptor part which has CCA base sequence with 3'-OH end of the terminal adenine nucleotide for binding *a specific amino acid*.
- They have specific *anti-codons* that recognize and link the complementary codons of mRNA.
- There are **40** different tRNAs carrying **20** amino acids that are specified by **61** different codons could be on mRNA.
- The active tRNA molecule functions to transfer its amino acid into the site of protein synthesis.

Transfer RNAs are classified into:

1. ***Initiator tRNA***
2. ***Regular tRNA***

Initiator tRNA carries amino acid called *methionine*. It recognizes and links the start codon (***AUG***) of mRNA.
Regular-tRNAs are specific for other amino acids.

At gene expression the following processes occur in nucleus and cytoplasm of eukaryotic cell. These processes are associated with the gene expression and necessary for complete gene expression (fig. 4-5).

In nucleus

- Transcription of ribosomal RNA takes place by *RNA -polymerase-I* in nucleolar organizing region (NOR).
- Synthesis of mRNA takes place by *RNA -polymerase -II*. It transports the genetic information into the cytoplasm in the form of a specific sequence of triplet codons.
- Transfer RNA (tRNA) is transcribed by *RNA -polymerase –III.*
- *In prokaryotes, they are transcribed by a single type of RNA – polymerase*

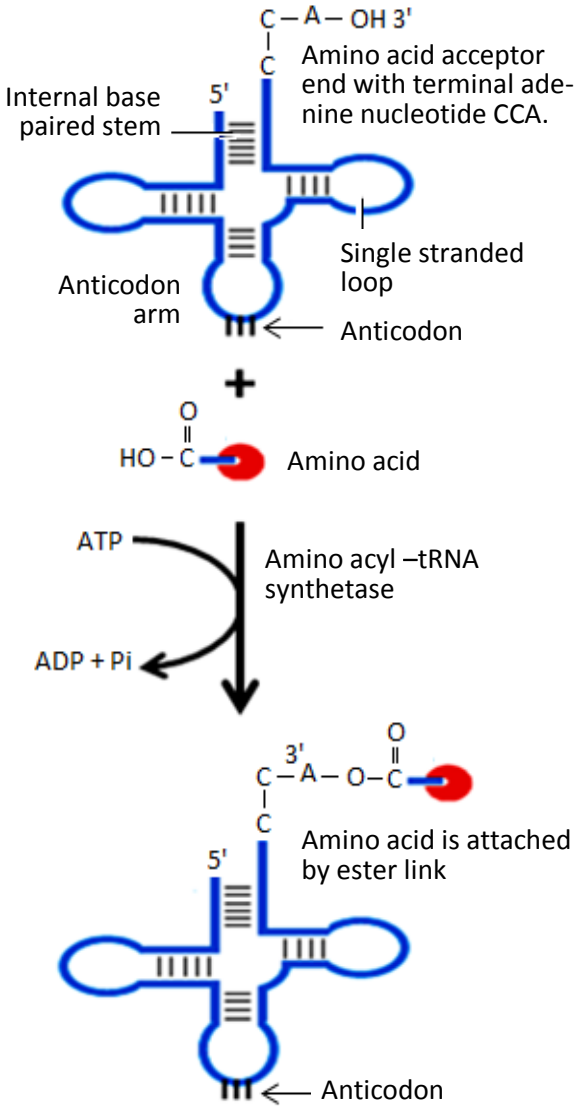


Fig. 4-4: The cloverleaf structure of tRNA. Formation of active tRNA is catalyzed by aminoacyl –tRNA synthetase that adds aminoacyl; a specific amino acid to the 3' end of tRNA.

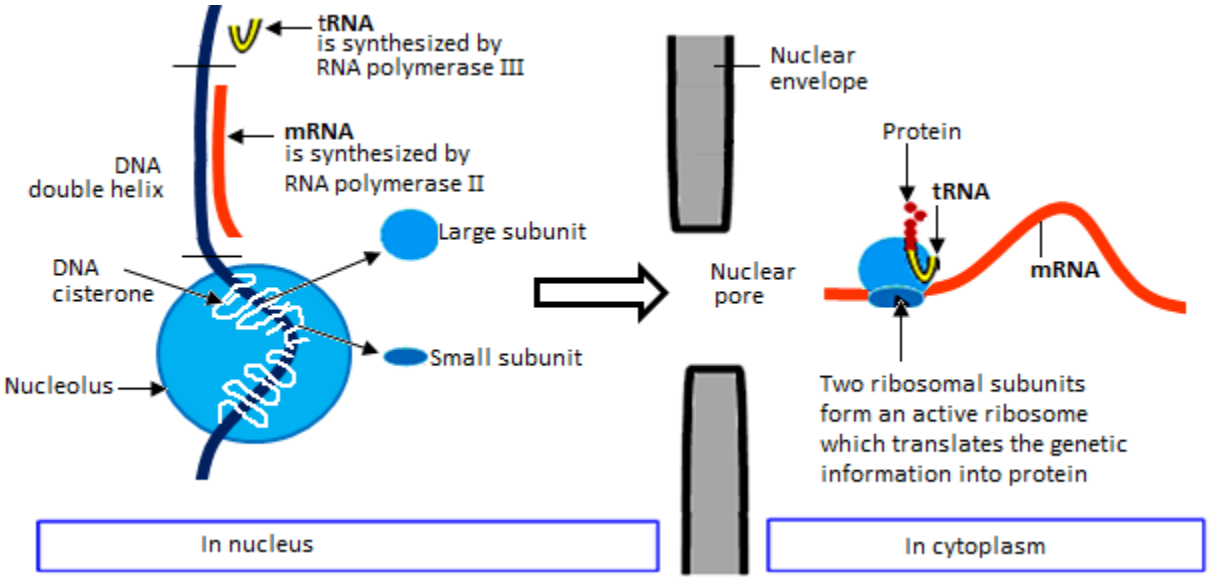


Fig 4-5: The processes that occur in nucleus and cytoplasm of eukaryotic cell during gene expression. Transcription of mRNA, rRNA and tRNA, and formation of large and small ribosomal subunits occurs in nucleus, while protein synthesis takes place in cytoplasm by cooperation of mRNA, ribosome and tRNA.

In cytoplasm

Ribosomal subunits and tRNA form a complex; *active ribosome* which reads and translates the genetic message on mRNA into polypeptide chain.

Transcription of Genetic Information

It is a process of synthesis of a single complementary RNA -strand (mRNA, tRNA and rRNA) on DNA –template; *gene transcription*. Understanding of the transcription process begins by explanation of gene composition (figs. 4-6& 4-7).

Gene Composition

The gene is composed of 3 regions (fig. 4-6):

i) Promoter
ii) Transcribed region
iii) mRNA termination region

Promoter

A specific sequence is located at the beginning of the gene. It is the binding site of RNA polymerase. It is found upstream of a base–pair sequence where gene transcription begins, known as **the transcription initiation site**. It is formed of 2 regions:

1. **TATA –box**

It is the binding site of RNA –polymerase. Many eukaryotic promoters, between 10 and 20% of all genes, contain a TATA box (sequence TA-TAAA).

It is required for attaching a TATA binding protein which assists in the formation of *RNA polymerase transcriptional complex*.

2. **UPEs** (upstream promoter elements).

They are components of eukaryotic promoter that are found upstream of RNA polymerase–binding site. Each one is formed of a sequence of 8 to 12 bases. They tend to contain primary regulatory elements approximately 250 specific transcription factor binding sites.

The **strength** of gene promoter is affected by the number and type of UPEs. A weakly expressed constitutive gene contains only one UPE, while the much more actively transcribed gene contains usually five or six UPEs.

In *prokaryotes*, the **promoter** consists of two short sequences at -10 and -35 positions *upstream* from the transcription start site. *Sigma factors* not only help in enhancing RNA polymerase binding to the promoter but also they help RNA polymerase target which genes to be transcribed.

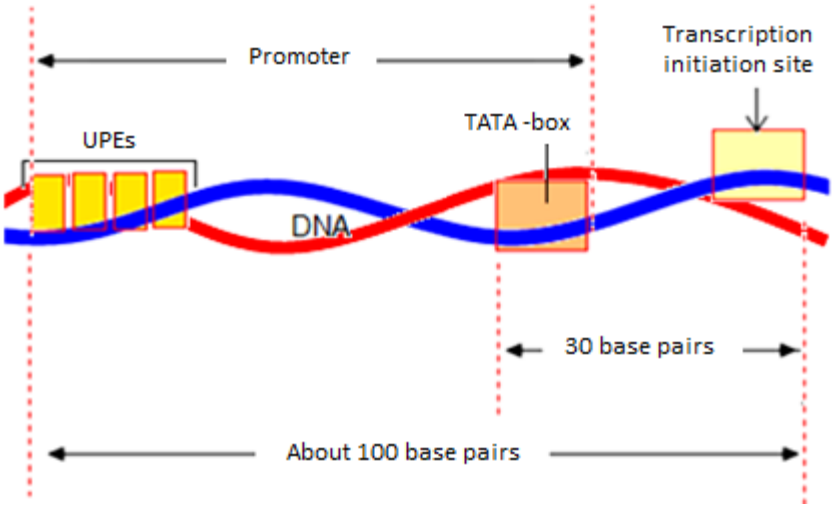


Fig. 4-6: Structure of promoter of gene in eukaryotic cell. It is formed of TATA –box and UPEs (upstream promoter elements). Efficiency of promoter depends upon the number and type of UPEs.

A **sigma factor** (**δ factor**) is a prokaryotic transcription initiation factor that enables specific binding of RNA polymerase to gene promoters. Different sigma factors are activated in response to different environmental conditions. Every molecule of RNA polymerase contains exactly one sigma factor subunit.

The number of sigma factors varies between bacterial species; *E. coli* has at least eight sigma factors.

Transcribed region

It is divided into 3 sequences:

1. Upstream leader sequence.
2. Protein –coding sequence (**exons**) and non-coding sequence (**introns**).
3. Downstream trailing sequence.

mRNA termination region

A specific sequence that is located at the end of the gene. It signals the RNA-polymerase to stop process of transcription and release the mRNA.

Steps of Gene Transcription

The process can be summarized in the following points (fig. 4-8):

1. Transcription begins by binding of RNA polymerase to promoter of the gene.

   In ***bacteria*,** the promoter is recognized by RNA polymerase and an associated sigma factor. They are often brought to the promoter DNA by binding an activator protein.

   In ***eukaryotes***, the process is more complicated, and at least seven different factors are necessary for the binding of an RNA polymerase -II to the promoter.

2. DNA double helix is gradually unwound by RNA -polymerase resulting in formation of a region for transcription which is formed of two separate strands:
   a. *Sense or active strand* of DNA which is transcribed by RNA –polymerase is the template strand.
   b. Non-sense strand of DNA is the inactive strand of DNA.

3. *RNA-polymerase* uses the complementary nucleotides in the transcription process (fig. 4-8), after converting nucleoside triphosphate to nucleotide monophosphate by removing two phosphate molecules.
4. RNA-polymerase links the remaining phosphate of nucleotide monophosphate to carbon #3of the1st nucleoside triphosphates at the beginning of RNA molecule synthesis.
5. Addition of the complementary nucleotides continues in 5’ - 3’ direction.
6. When RNA-polymerase reaches RNA- termination region of DNA sense strand, RNA-polymerase –enzyme of transcription receives signal to stop transcription and release the transcribed RNA molecule (tRNA, mRNA and rRNA).
7. RNA molecule leaves the DNA template and DNA reforms its double helix.

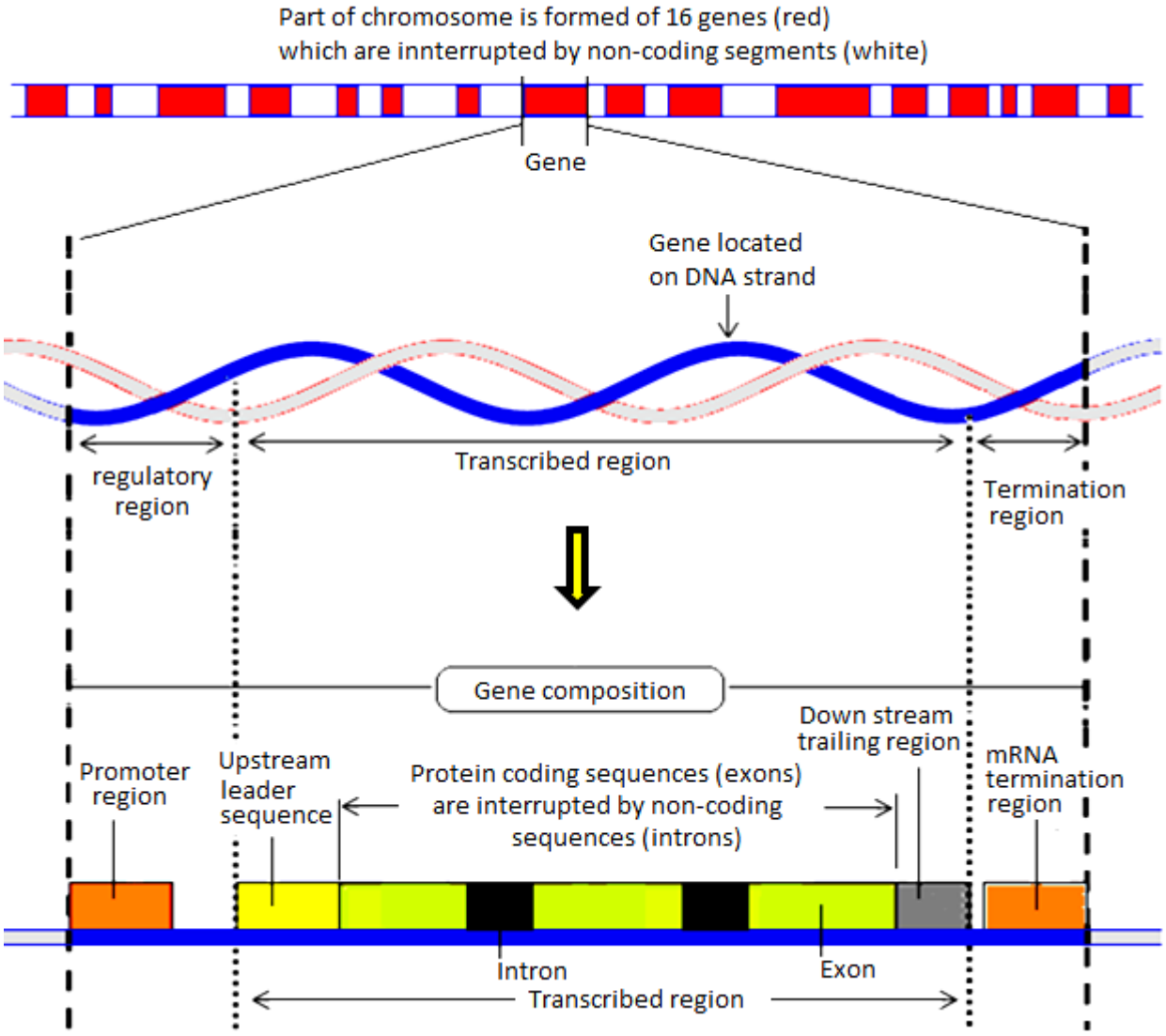


Fig. 4-7: Arrangement and structure of eukaryotic genes on chromosome. It is formed of a regulatory region (promoter), transcribed region (upstream leader sequence, introns, exons and downstream trailing region) and mRNA - termination signal. Blue strand of DNA double helix is the active strand and shows the different components of the gene.

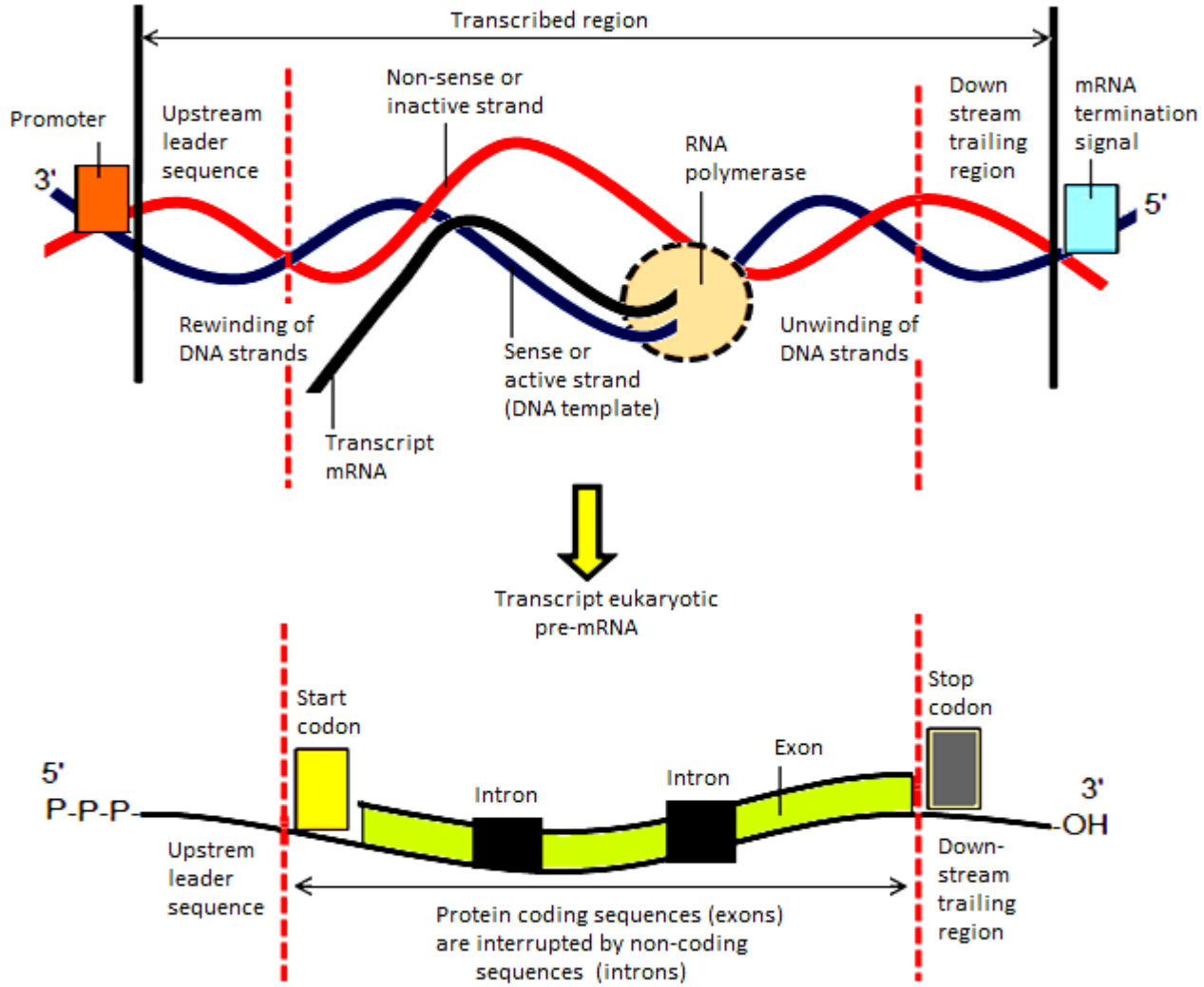


Fig. 4-8: Diagram showing the transcription process of mRNA. It begins by binding of RNA –polymerase to promoter of gene. Transcription starts at upstream leader sequence by unwinding of DNA double helix. RNA-polymerase moves along the sense strand (gene) from upstream leader sequence toward downstream trailing region, and links the complementary nucleotides to construct the mRNA. At mRNA –termination region RNA –polymerase stops transcription and releases mRNA, which is formed of upstream leader sequence, protein-coding and non- coding sequences and downstream trailing region.

Transcription in Prokaryotic Cell

- Transcription in *E. coli* is carried out by one type of RNA polymerase that is formed of α, β and δ -subunits.
- δ -subunit recognizes and binds to the promoter TATA -box.
- β –subunit forms catalytic centre.
- α -subunit is important in the assembly of the complex.
- Binding of δ-subunit to promoter causes association of nucleosides triphosphate with β–subunit and initiation of the synthesis of polynucleotides chain in 5'-3' direction.
- Elongation of the RNA molecule continues by dissociation of α-factor and association of elongation -factors.

Transcription in Eukaryotic Cell

- There are 3 types of RNA polymerases I, II and III which recognize different promoters and transcribe different types of RNA molecules.
- Transcription requires several transcription factors. They are required to initiate and regulate the transcription process.
- Physiological rate of transcription requires the presence of enhancer, activator proteins and transcription factors.

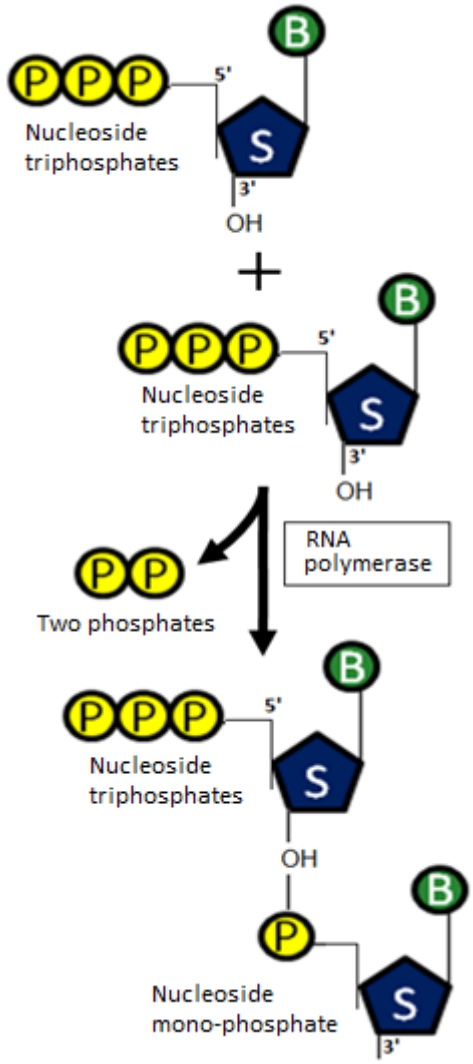


Fig. 4-8: RNA polymerase removes 2 phosphate molecules (p) from nucleoside triphosphates to use the released energy in building the polynucleotides chain of RNA molecules from the nucleoside mono-phosphate. Sugar (S) and nitrogenous base (B).

General Features of DNA Transcription

DNA transcription processes have the following characteristics:

- It proceeds in 5' to 3' direction.
- The active template DNA strand is called sense strand, while the inactive DNA strand is called non-sense strand.
- RNA polymerase uses nucleoside triphosphates as a source of energy, in building mRNA.
- The 1st nucleotide of mRNA retains its 3 phosphate molecules at 5' –end.
- The transcription process doesn't require a RNA-primer
- Transcribed region is formed of:

1. Upstream leader region.
2. Protein coding sequence which extends from start codon (AUG) to stop codon at the beginning of downstream trailing region.
3. Downstream trailing region which starts by stop codon (UAA, UAG, and UGA).

- Non-transcribed regions are:

1. Promoter.
2. Termination sequence.

- Kinds of transcript RNA are mRNA, tRNA and rRNA.

Post-transcriptional Modification

In eukaryotic cells, the transcribed -mRNA is called pre-mRNA or immature mRNA (hnRNA; heterogenous nuclear ribonucleic acid). It requires processing and modifications for the following reasons:

a) It contains non-coding regions called *introns.*

Introns: They are polymorphic regions scattering between protein coding regions called **exons**. Introns are highly variable from one individual to the other. Analysis of these regions produces **DNA-finger prints** that look like a bar code. The DNA-finger print is unique for every individual, although all humans are genetically almost identical.

b) It needs modifications to become *competent for transport and translation in cytoplasm.* Figure 4-10 explains the processing and modifications of pre-mRNA in eukaryotic cells.

Fig. 4-9: Diagram illustrates a composition of an example for a bar code.

Types of Processing and Modifications

1) Capping:

It begins when mRNA is about 20-30 nucleotides long. Capping of mRNA takes place by addition of a 7-methyl guanosine to the 5' end of mRNA.

Importance of capping:

- Non-capped pre-mRNA cannot be recognized by ribosome; the cap of 7-methyl guanosine is required for recognition process.
- It protects mature mRNA against degradation.
- It increases the stability or life span of mature mRNA. Due to absence of modification in bacteria, half the life is about two minutes in prokaryotic cell while it is about 10 hours in eukaryotic cells.

2) Polyadenylated tail:

Polyadenylated tail or *poly-A tail* is added to the 3`-OH end of completed mRNA. Poly-adenylated tail is formed of *100 – 250 adenine nucleotides.*

Importance of the addition of Poly-adenylated tail (poly-A tail):

- It helps in the passage of mRNA from nucleus into the cytoplasm.
- It protects the mRNA against degradation.
- It increases the stability or life span of mRNA.

3) Splicing:

It is a complex process which includes the removing of introns (*non-coding regions)* and joining of the exons together forming a continuous protein-*coding message* (figs. 4-10 & 4-11).

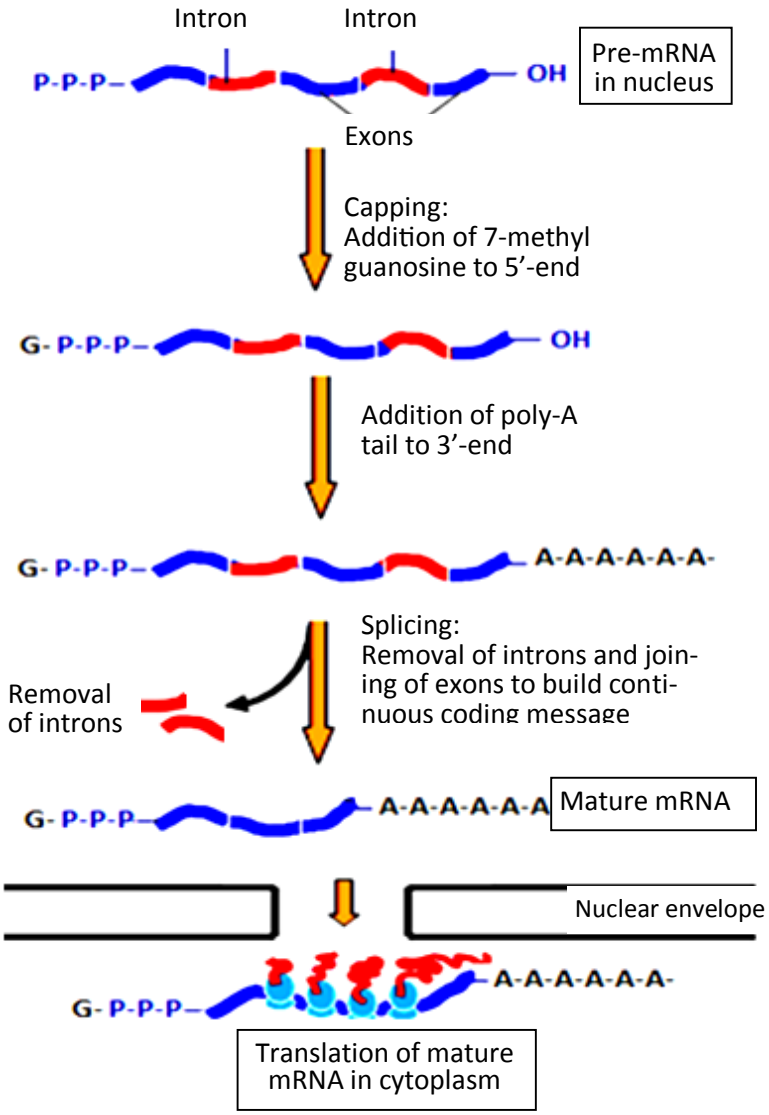


Fig. 4-10: Modification and processing of mRNA in eukaryotic cell; capping by addition of 7 –methyl guanosine cap to 5' end, addition of poly -A tail to 3' –OH end of mRNA and removal of introns and splicing of exons.

Steps of splicing as performed by spliceosome in figure 4-11:

- Assembling of spliceosomes on the splicing sites.
- A cut takes place at the 5'-splice site for separating the left exon.
- Cutting occurs at the 3'-splice site to release the free intron in lariat form.
- Right exon is ligated (spliced) to left exon.

Splicing is important for formation of a continuous protein coding message, mature mRNA.

Introns can be divided into 3 general classes:

1. Introns of nuclear pre-mRNA are found in structural genes of eukaryotic cells. *They are removed by spliceosome.*

2. Introns of group-I class. They are found in rRNA genes in lower eukaryotes and in structural, tRNA and rRNA genes of mitochondrial DNA in fungi and plants as well as in chloroplast DNA. *They are removed by self-splicing*.

3. Introns of group-II class. They are found in a few structural, tRNA, rRNA genes of mitochondrial DNA and chloroplast DNA. *They are removed by self-splicing*.

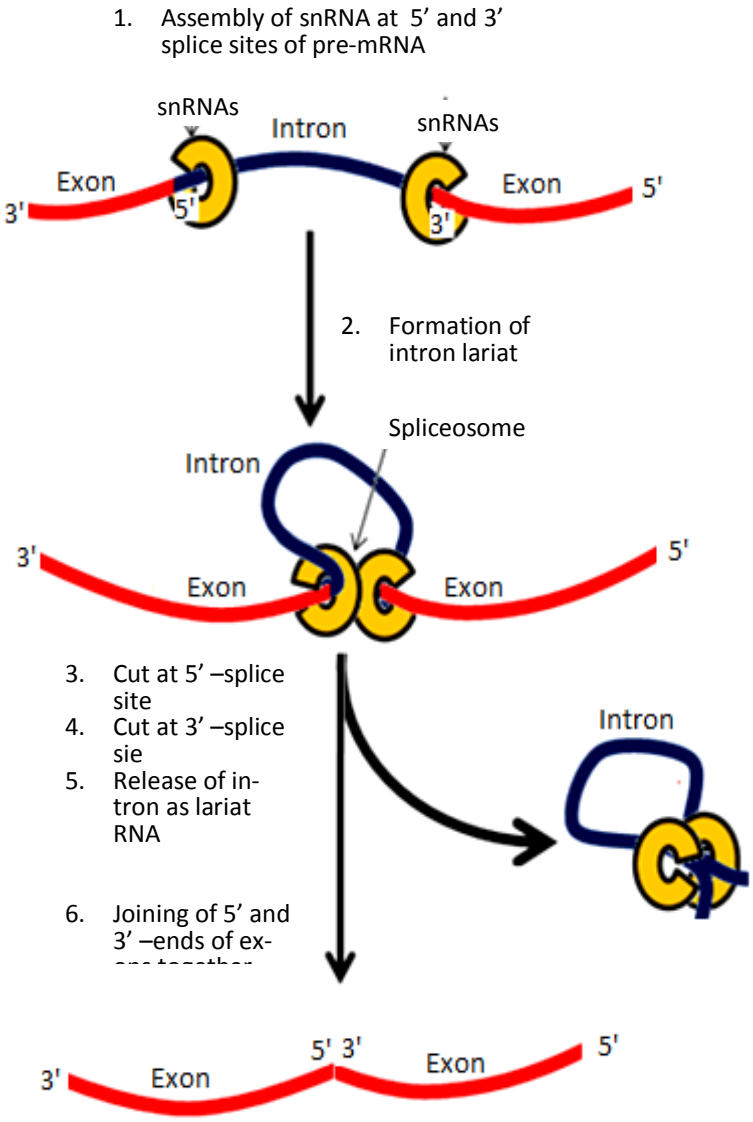


Fig. 4-11: Steps of removing introns and joining of exons together by spliceosome (snRNPs). They include cleavage and ligation.

Introns of the group I and II possess the ability to splice themselves out of the pre-mRNA that contains them. They function as ***ribozymes***. Their *catalytic activity* is capable of catalyzing all steps of introns removal and joining of exons together. Removal of nuclear pre-mRNA introns is performed by a small nuclear ribonucleoprotein complex ***(snRNPs)*** which possess enzymatic activities.

The snRNPs assemble on the pre-mRNA to form the ***spliceosome*** (fig. 4-11).

After modifications and processing, mature mRNA becomes able to carry and transport the genetic information, and is competent for the process of translation in cytoplasm.

In prokaryotic cell, expression of genetic information differs from that in eukaryotic cells. Firstly there is no post-transcriptional modification of mRNA and secondly translation of genetic information is coupled with transcription, because the nuclear envelope that separates the genetic material from cytoplasm is absent in prokaryotic cells.

Translation in prokaryotes begins while the transcription of mRNA occurs when multiple ribosomes (polyribosomes) attach to the synthesized mRNA to manufacture of the polypeptide chain (fig. 4-12).

Both Ribosomal–RNA and transfer–RNA show a degree of post-transcriptional modification. Their continuous strands undergo post-transcriptional cleavage with a nuclease which may followed by base modification.

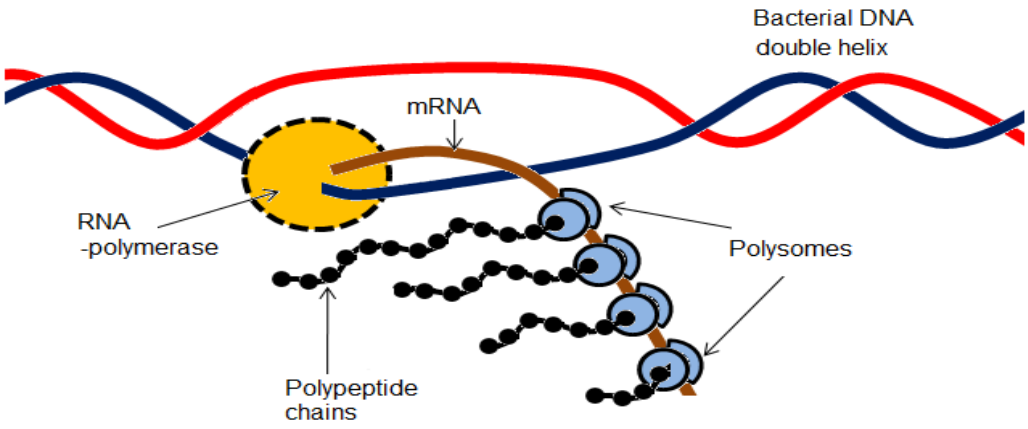


Fig. 4-12: Gene expression in bacteria. Translation is coupled with transcription in bacteria as the genetic materials are not separated from cytoplasm. There are no modification and processing for mRNA.

Translation of Genetic Information

Protein synthesis means translations; transformation of genetic information into polypeptides chains.

It is the second step of the gene expression process which begins with transcription of genetic information within the nucleus.

Protein synthesis includes **three steps:**

1. **Initiation.**
2. **Elongation.**
3. **Termination.**

1. Initiation

Translation of genetic information begins by formation of the ***initiator complex*** (Fig. 4-13), when *initiator tRNA carrying methionine and small ribosomal subunit*, binds the *start codon* ***AUG*** of mRNA.

Then *functioning ribosome* is formed by binding of the large ribosomal subunit to the initiator complex.

On formation of active ribosome, the ***P-site*** *is occupied by initiator tRNA* and ***A-site*** *is free* and ready to receive active tRNA carrying complementary anticodon to the next codon of mRNA.

2. Elongation

The elongation cycle (fig.4-14) *takes place in 3 successive steps:*

a) Occupying A- site.
b) Peptide bond formation.
c) Translocation.

Occupying A- site:

If the next triplet codon of mRNA that follows directly the start codon is **CCG** that codes for amino acid proline, elongation cycle begins by occupying **A-site** of a large ribosomal subunit (at ***CCG*** *codon* of mRNA) by active tRNA carrying *amino acid* proline and ***GGC*** *–anticodon.* This step occurs as follows:

- *Complex is formed of aminoacyl- tRNA, GTP and elongation factor*
- *Codon CCG -anticodon GGC bond formation by hydrolysis of GTP.*

Peptide bond formation:

Amino acid methionine is detached from tRNA in P-site and joined by peptide bond to amino acid proline that is specified by CCG codon and linked to aminoacyl- tRNA in A-site. Peptide bond formation is catalyzed by a peptidyl transferase of large ribosomal subunit.

Translocation:

Ribosome moves one codon in direction 5’ to 3’ by the help of *elongation factor and GTP* that leads to:

- Release tRNA from the P -site into cytoplasm. Transfer RNA (tRNA) and the peptide chain move from A -site to P -site.
- A -site becomes open to receive another suitable tRNA.
- Then release of GDP, elongation factor and phosphate ion (Pi).

By repeating steps of the *elongation cycle*; occupying A –site by suitable active tRNA, peptide bond formation and translocation, the peptide chain elongates till the ribosome reaches the stop codon.

3. Termination

When ribosome reaches stop codon (the termination codons are ***UAA, UAG, and UGA***), a release factor recognizes and binds the mRNA - stop codon. It terminates the protein synthesis and causes (fig. 4-15):

- A free polypeptide chain.
- Dissociation of ribosomal subunits; Large and small ribosomal subunits from mRNA.
- Separation of tRNA molecule from polypeptide chain.
- Separation of releasing factor from mRNA.

Types of releasing factors (RF):

- RF-I recognizes UAA and UAG.
- RF-II recognizes UAA and UGA.
- RF-III recognizes and binds GTP and stimulates ribosomal binding of RF-I and RF-II.

In both prokaryotic and eukaryotic mRNA carries the coding sequence which contains the actual messages for protein synthesis besides the regulatory sequences. Amino acids that are required for protein synthesis are demonstrated in table 4-1.

- The table 4-1 also shows which codons of mRNA or genetic codes specify each amino acid of the required polypeptide chain of protein molecule.

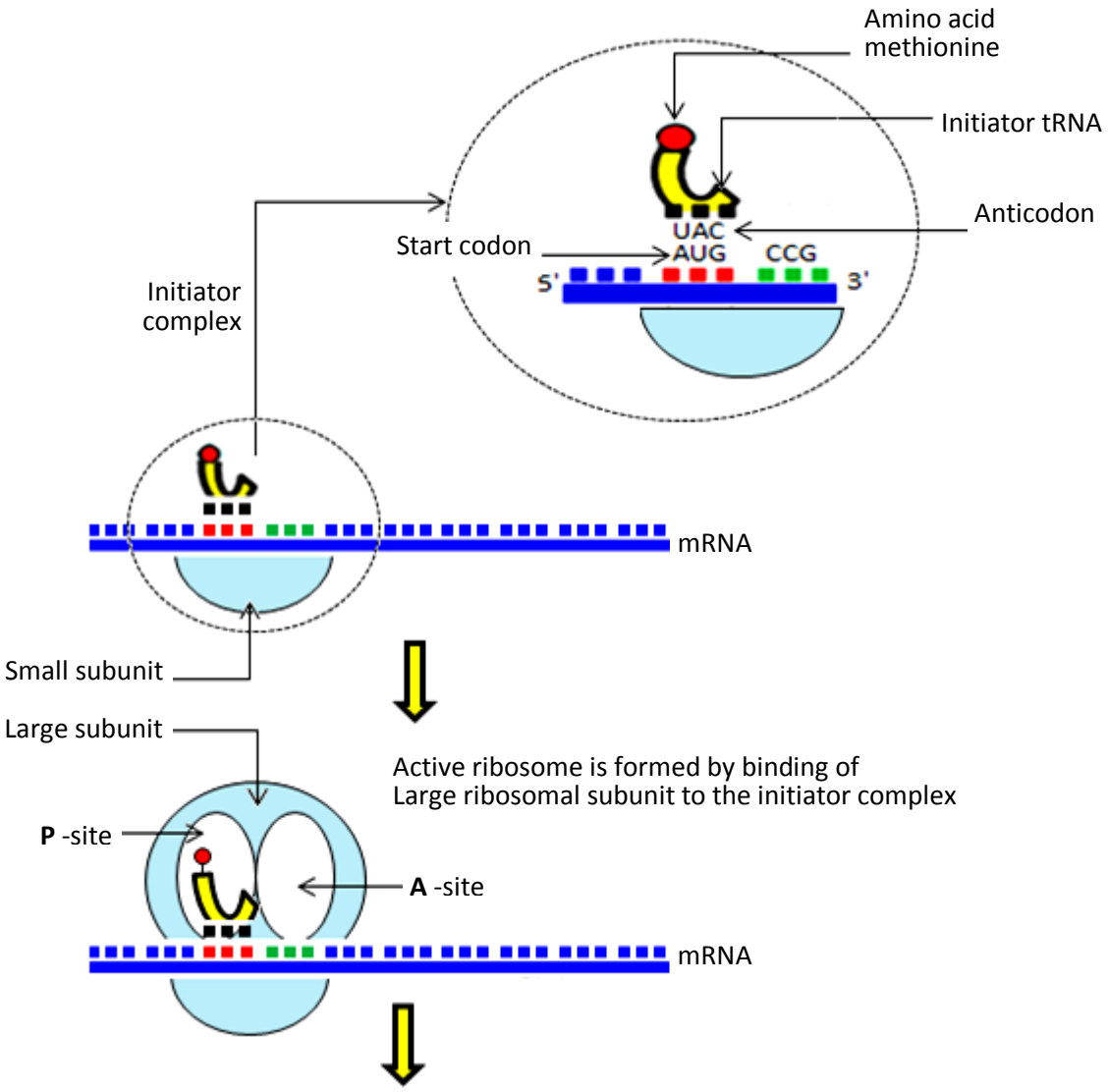


Fig. 4.13: Initiation of protein synthesis. Translation process begins by formation of initiator complex when initiator -tRNA and small ribosomal subunit bind the start codon (AUG) of mRNA. Active ribosome or functioning ribosome is formed by binding of large ribosomal subunit to initiator complex.

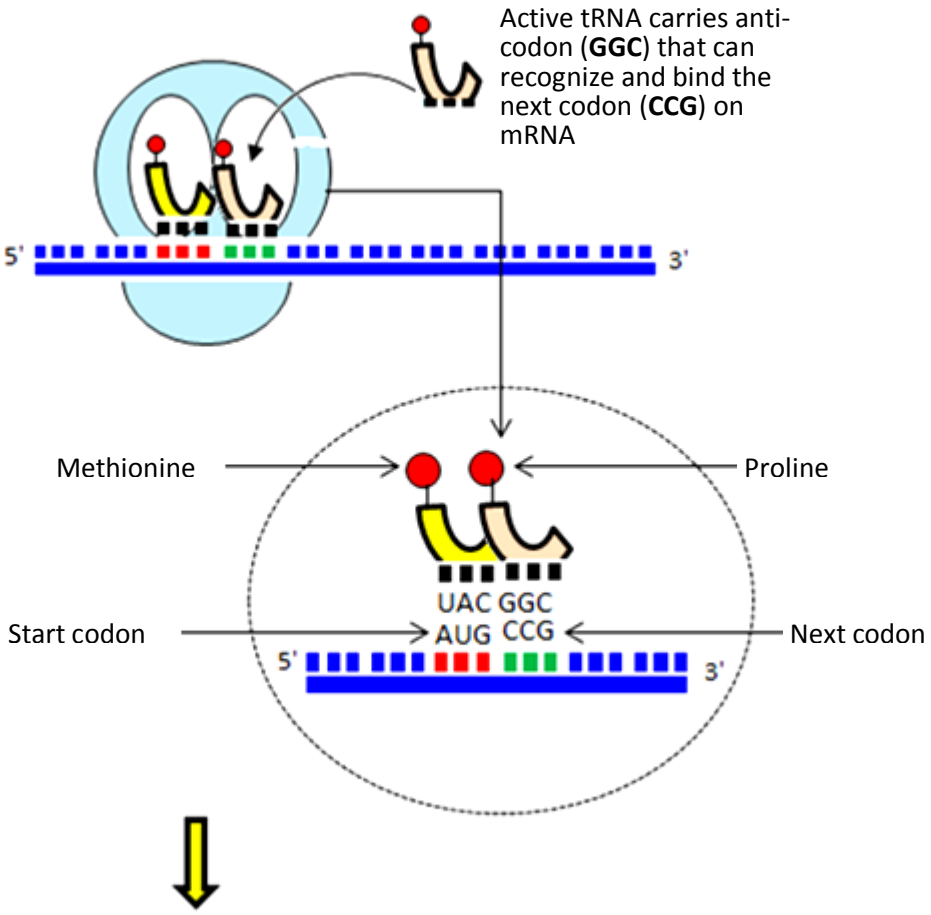


Fig. 4.14 A: Elongation cycle of protein synthesis. 1st Step: Occupying A -site by suitable active tRNA that carries anticodon (GGC) and amino acid proline. It can recognize and bind the next codon (CCG) on mRNA.

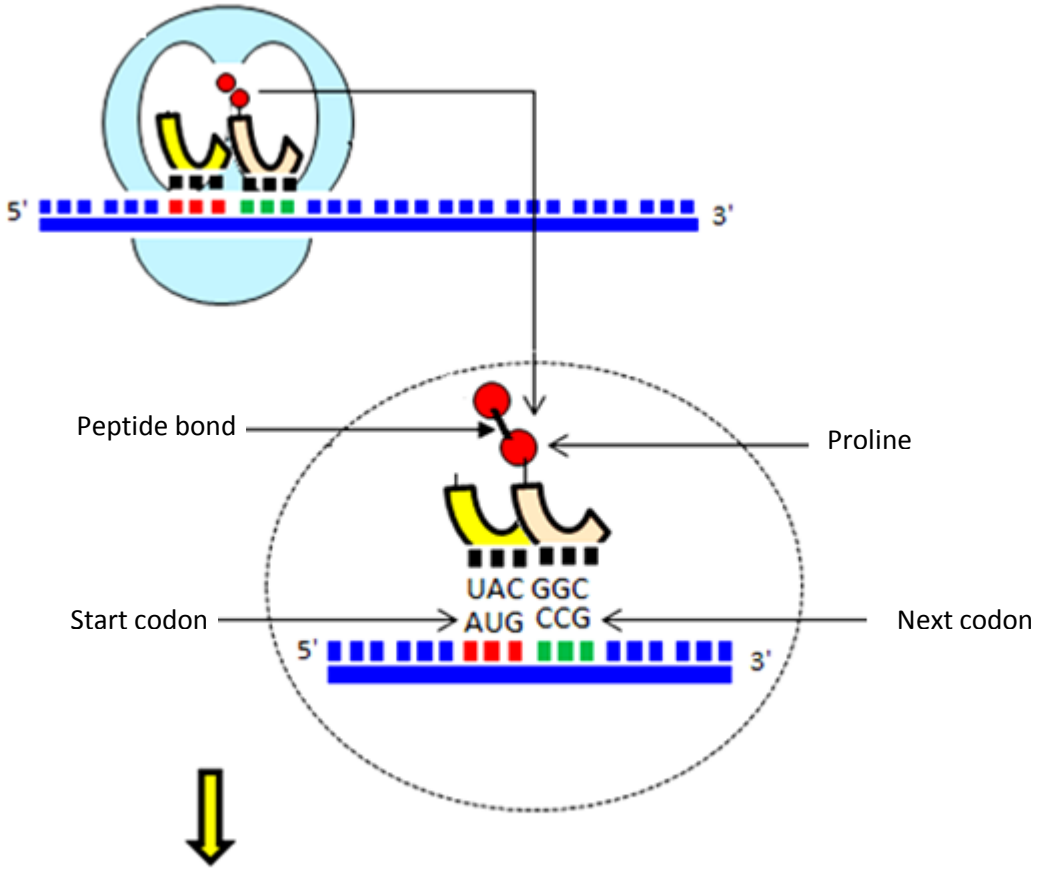


Fig. 4.14 B: Elongation cycle of protein synthesis. 2nd Step: Peptide bond formation by ribosome between amino acid methionine and proline (the next amino acid).

Ribosome moves for one codon on mRNA in direction from 5' to 3' end. Translocation of ribosome requires energy by hydrolysis of GTP.
This translocation of ribosome results in:

- ***P*** –site becomes occupied by tRNA carrying the synthesized polypeptide chain
- ***A*** –site becomes open and ready to receive suitable active tRNA
- Inactive tRNA leaves the ribosome into the cytoplasm

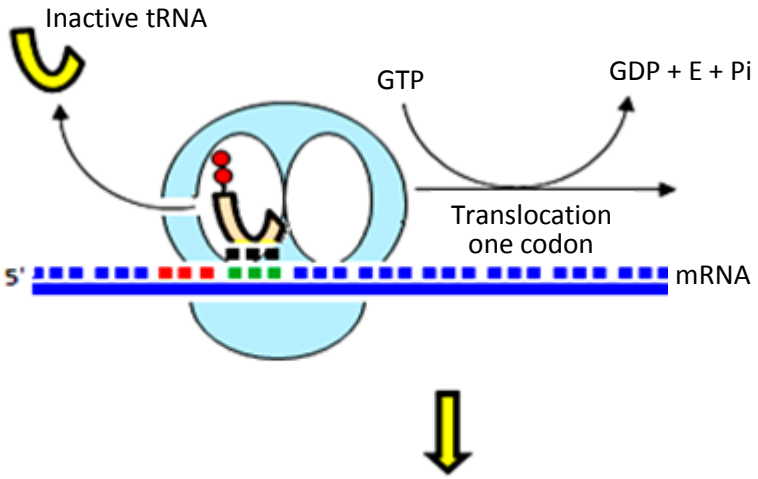


Fig. 4.14 C: Elongation cycle of protein synthesis. 3rd Step: Translocation of ribosome one mRNA codon in direction toward 3'-end.

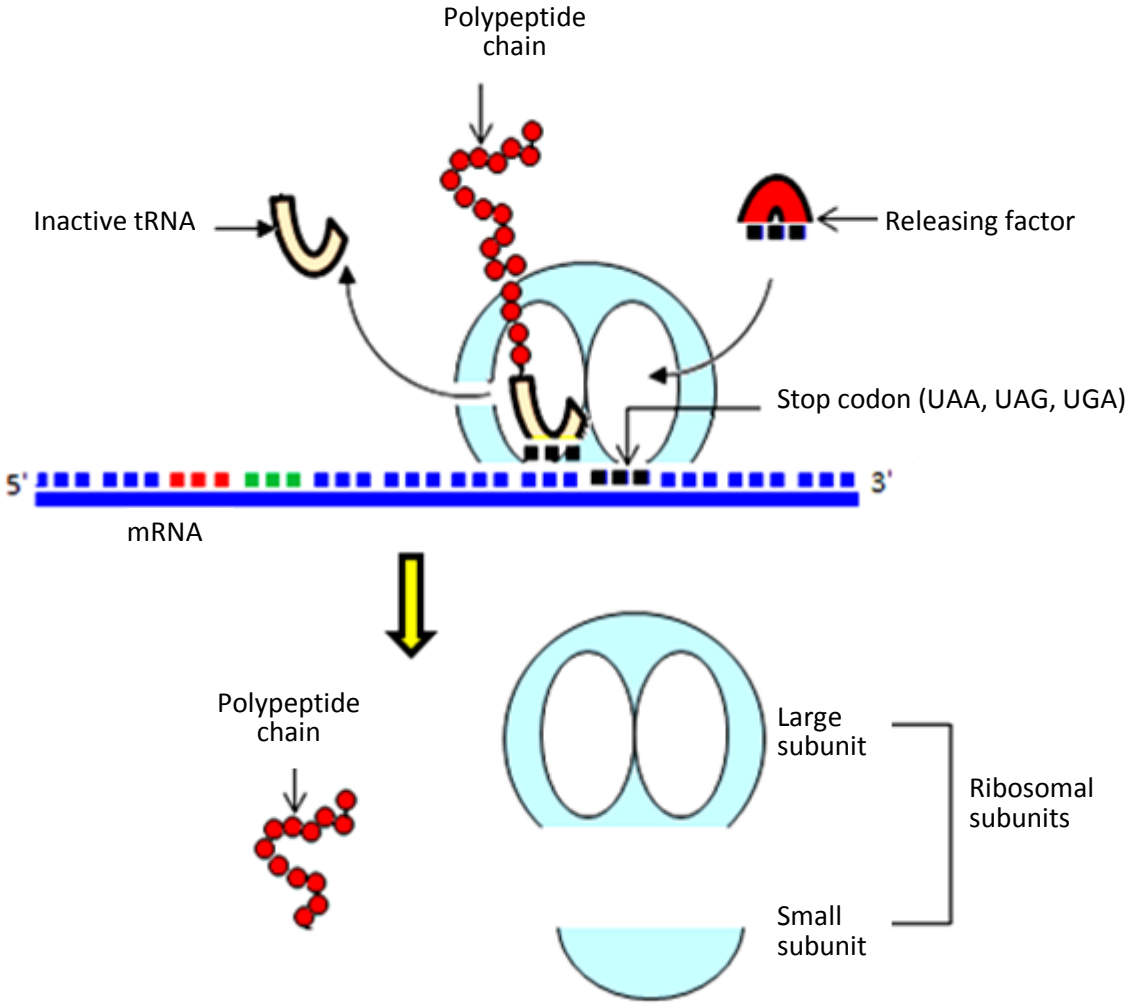


Fig. 4.15: termination of protein synthesis. Ribosome continues in synthesis of polypeptide chain until reaches the stop codon (one of the codons: UAA, UAG, UGA). Stop codon can be recognized by a release factor that binds it and terminates the protein synthesis by releasing the ribosomal subunits and the polypeptide chain.

Proteins

There are 20 different amino acids which form the protein molecules in all life forms. The types of proteins are different because they are formed not only of a different number of amino acids but also in the way of assembling or ordering the amino acids.

Types of proteins

- **Simple proteins:** They are formed of only amino acids.

- **Compound proteins**: They contain non-protein components. For example: lipo-, glyco-, nucleo-, chromo- and metallo-proteins).

1st base	2nd base	3rd base U	3rd base C	3rd base A	3rd base G
U	U	UUU **Phenylalanine** (Phy)	UUC **Phenylalanine** (Phy)	UUA **Leucine** (Leu)	UUG **Leucine** (Leu)
	C	UCU **Serine (Ser)**	UCC **Serine (Ser)**	UCA **Serine (Ser)**	UCG **Serine (Ser)**
	A	UAU **Tyrosine (Tyr)**	UAC **Tyrosine (Tyr)**	UAA **Stop**	UAG **Stop**
	G	UGU **Cysteine (Cys)**	UGC **Cysteine (Cys)**	UGA **Stop**	UGG **Tryptophan**
C	U	CUU **Leucine (Leu)**	CUC **Leucine (Leu)**	CUA **Leucine (Leu)**	CUG **Leucine (Leu)**
	C	CCU **Proline (Pro)**	CCC **Proline (Pro)**	CCA **Proline (Pro)**	CCG **Proline (Pro)**
	A	CAU **Histidine (His)**	CAC **Histidine (His)**	CAA **Glutamine (Gln)**	CAG **Glutamine (Gln)**
	G	CGU **Arginine (Arg)**	CGC **Arginine (Arg)**	CGA **Arginine (Arg)**	CGG **Arginine (Arg)**
A	U	AUU **Isoleucine (Ile)**	AUC **Isoleucine (Ile)**	AUA **Isoleucine (Ile)**	AUG **(start) Methionine**
	C	ACU **Threonine (Thr)**	ACC **Threonine (Thr)**	ACA **Threonine (Thr)**	ACG **Threonine (Thr)**
	A	AAU **Asparagine (Asn)**	AAC **Asparagine (Asn)**	AAA **Lysine (Lys)**	AAU **Lysine (Lys)**
	G	AGU **Serine (Ser)**	AGC **Serine (Ser)**	AGA **Arginine (Arg)**	AGG **Arginine (Arg)**
G	U	GUU **Valine (Val)**	GUC **Valine (Val)**	GUA **Valine (Val)**	GUG **Valine (Val)**
	C	GCU **Alanine (Ala)**	GCC **Alanine (Ala)**	GCA **Alanine (Ala)**	GCG **Alanine (Ala)**
	A	GAU **Aspartic acid (Asp)**	GAC **Aspartic acid (Asp)**	GAA **Glutamic acid (Glu)**	GAG **Glutamic acid (Glu)**
	G	GGU **Glycine (Gly)**	GGC **Glycine (Gly)**	GGA **Glycine (Gly)**	GGG **Glycine (Gly)**

Table 4-1: The codons of mRNA are formed of three bases that specify amino acids of polypeptide chain. Amino acids are classified into: Hydrophobic amino acids (black), hydrophilic amino acids (blue) and ambiphilic amino acids (red).

***Proteins form different structures**:*

- **Fibrillar proteins:** They dissolve in water. They form structural parts of organisms.

- **Globular proteins:** They are water soluble (for example: Enzymes, plasma protein, hemo- and myoglobin).

- **Globulo-fibrillar proteins**: They are composed of globular and fibrillar parts, for example: Receptors of membrane proteins.

Proteins represent an important way to study the genes. HOW?

- An important way to discover the genes is studying the proteins for which the genes code.

- DNA directs RNA to synthesize protein that is rapidly changing to form sequence which implies the structure and the structure implies the function.

Computer programs can now analyze the structure of proteins, determine how they fold and their functions. This data is used to identify the genes in chromosome which are responsible for production of these proteins.

Example for Gene Expression:
If DNA strand has the following base sequence:

3'-TACGCCTAACGCGATAATACT-5'

Give the mRNA base sequence and the polypeptide chain of the synthesized protein, when this mRNA doesn't require modification or processing.
Answer:

- *DNA base sequence:*

  *3'-**TAC**GCC**TAA**CGC**GAT**AAT**ACT**-5'*

- *mRNA base sequence:*
  *5'-**AUG**CGG**AUU**GCG**CUA**UUA**UGA**-3'*
  - AUG start codon
  - UGA stop codon

- *Codons of mRNA and the amino acids which are specified by them:*
  1. ***AUG** – Methionine*
  2. *CGG – Argenine*
  3. ***AUU** – Isoleucine*
  4. *GCG – Alanine*
  5. ***CUA** – Leucine*
  6. *UUA – Leucine*

  ***UGA** of mRNA – Stop codon*

- *Polypeptide chain:*

*- **Meth** – Arg – **Isoleu** – Ala – **Leu** – Leu –*

Types of Post-translation Modification of Proteins:
The post-translation modifications of proteins are necessary for maturation of synthesized proteins. They give the mature protein its functional activity.

1) Glycosylation:
Oligosaccharides are added to specific amino acid residues in rough ER and Golgi –complex.

2) Phosphorylation:
It targets serine and tyrosine. It regulates enzyme activity, e.g. Kinases phosphorylate serine and tyrosine

3) **Sulphation**:
It targets tyrosine. It is used for export and biological activity.

4) Hydroxylation:
It targets lysine and proline. It is essential for collagen formation.

5) Lipidation:
It targets cysteine and glycine. It is important for anchoring of protein to cell membrane.

6) Acetylation:
It targets lysine. It changes the charge that alters the binding character of protein.

7) Cleavage:
It activates some enzymes and hormones.

Additional readings

Brennan RG and Matthews BM (1989): Structural basis of DNA-protein recognition. Trends in: Biochem. Sci., Vol. 14, 287-290.

Clayton D A (1984): Transcription of the mammalian mitochondrial genome. *Ann. Rev. Biochem.* 53, 573-594.

Davidson E H and Britten R J (1973): Organization, transcription, and regulation in the animal genome. *Quart Rev. Biol.* 48, 565-613.

Elliott WH and Elliott DC (2002): Biochemistry and molecular biology. 2nd ed., New York: Oxford University Press, 343-399.

Hastie NB and Bishop J O (1976): The expression of three abundance classes of mRNA in mouse tissues. Cell, 9, 761-774.

Holstege FCP et al. (1998): Dissecting the regulatory circuitry of a eukaryotic genome. Cell, 95, 717-728.

Johnson FB, Marcniak RA and Guarrente L (1998): Telomeres, the nucleolus and ageing. Current Opinion in: Cell Biology, 10: 332 – 328.

Featherstone C and Jackson SP (1999): Double strand break repair. Current Biology, 9, R759-761

Lewin B (1975): Units of transcription and translation: Sequence components of hnRNA and mRNA. *Cell,* 4, 77-93.

Lewin B (2002): Genes VII. Part 2 and 3. New York: Oxford University Press Inc.

Lodish H. et al. (2000): Molecular cell biology. 4th ed. New York: W. H. Freeman and Co.

Mikos GLG and Rubin GM (1996): The role of the genome project in determining gene function: Insights from model organisms. Cell, 86, 521-529.

Osawa S et al. (1992): Recent evidence for evolution of the genetic code. Microbiol Rev. 56, 229-264.

Ruff M et al. (1991): Class II aminoacyl tRNA synthetases: Crystal structure of yeast as partyl-tRNA synthetase complexes with tRNA. Science 252, 1682-1689.

Solomon EP, Berg LR and Martin DW (2002): Biology, 6th ed. Chapter 12: the expression of genetic information. United States: Thomson Learning Academic Resources Center, 261-283.

Velculescu VE et al. (1997): Characterization of the yeast transcriptosome. Cell, 88, 243-251.

Waga S and Botcham M (1998): The DNA replication Fork in eukaryotic cells. Ann. Rev. Biochem. 67, 721

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer.

1. Which structure of the following is required to carry out one or more cellular functions?
   a. DNA strand
   b. DNA fragment
   c. DNA sequence
   d. Gene

2. Which of the following array of letters forms the alphabet of gene language?
   a. A, B , C, D
   b. A, T, C, M
   c. A, T, C, G
   d. A, T, G, D

3. How many successive nucleotides form the genetic code?
   a. 2
   b. 3
   c. 4
   d. 5

4. How many amino acid form the polypeptide chain if a GENE is formed of this base-sequence *3'*-TACGCCTAACGCGATAATACT-5'?
   a. 4
   b. 5
   c. 6
   d. 7

5. Which process of the following is the 1st step of gene expression?
   a. Transcription
   b. Translation
   c. Replication
   d. Transformation

6. In which process of the following does RNA – polymerase make messenger RNA?
   a. Translation
   b. Replication
   c. Transcription
   d. Sexduction

7. In which process of the following does genetic language convert into polypeptide chain or amino acid language?
   a. Translation
   b. Replication
   c. Transcription
   d. Termination

8. Which of the following is not consistent with character of RNA molecules?
   a. Folded strand
   b. Single strand
   c. Contains uracil
   d. Contains deoxyribose

9. Which of the following is not consistent with character of genetic code?
   a. A triple code
   b. Nearly universal
   c. Specifies one amino acid
   d. Complementary for anticodon of tRNA

10. In which part of functioning ribosome is the peptidyl- tRNA located?
    a. Small subunit of ribosome
    b. A-site of large ribosomal subunit
    c. P- site of large ribosomal subunit
    d. Messenger RNA

11. Which process of the following modifies mRNA to carry continuous protein coding message?
    a. Glycosylation
    b. Splicing
    c. Phosphorylation
    d. Cleavage

12. Within which place of the following is ribosomal RNA synthesized?
    a. Cytoplasm
    b. RER
    c. Nucleolus
    d. Golgi cisternae

13. In which compartment of the following is ribosomal protein synthesized?
    a. Cytoplasm
    b. Chromatin
    c. Nucleolus (NOR)
    d. Golgi cisternae

14. Which sequence of the following is the start codon of mRNA?
    a. AUG
    b. TAA
    c. UUA
    d. AAU

15. Which tRNA of the following can recognize and link the start codon?
    a. Inactive tRNA
    b. Regular tRNA
    c. Initiator tRNA
    d. Active tRNA

16. Which process of the following occurs in cytoplasm during gene expression in eukaryotic cell?
    a. Replication
    b. Transduction
    c. Translation
    d. Transcription

17. Which region of the following forms the regulatory region of eukaryotic genes?
    a. Promoter
    b. Protein coding region
    c. Downstream trailing regions
    d. Upstream leader sequences

18. Which amino acid of the following is carried by initiator tRNA?
    a. Methionine
    b. Argenine
    c. Lucine
    d. Alanine

19. Which anticodon on tRNA can recognize and link the codon UUA on mRNA?
    a. AUU
    b. TAA
    c. AAT
    d. AAU

20. Which mRNA base sequence of the following can be recognized and linked by releasing factor?
    a. AGA
    b. UTU
    c. UAU
    d. UAA

21. Which gene sequence of the following codes for proteins?
    a. Intron
    b. Promoter
    c. Termination region
    d. Exons

22. Which fragment of the followings is the correct complementary DNA strand for the DNA strand 5'- GTAATTACGAT -3'?

    a. 3'- TACGATCATAT -5'
    b. 3'- CATTAATGCTA -5'
    c. 3'- AUGCUAGUAUA -5'
    d. 3'- GCATATACGCG -5'

23. Which structure of the following is the machine of translation of genetic information into protein?
    a. mRNA
    b. Ribosome
    c. tRNA
    d. RNA–polymerase

24. Which process of the following is the 1st step in modification of pre-mRNA?
    a. Capping
    b. Translation
    c. Poly-A tail addition
    d. Splicing

25. By which enzyme of the following is tRNA synthesized?
    a. RNA polymerase I
    b. RNA polymerase II
    c. RNA polymerase III
    d. DNA polymerase

26. A messenger RNA is formed of 333 nucleotides long, including the initiator and termination codons. The number of amino acids in the protein translated from this mRNA is
    a. 990
    b. 660
    c. 330
    d. 110

27. The following sequence is a protein coding message carried by a mRNA:
    5' – ACGCGAACGCGAACGCGA … ,

    If protein synthesis can begin without the need for a start codon, this synthesized protein is formed of:
    a. A single type of amino acid
    b. Two different types of amino acids
    c. Three different types of amino acids
    d. Four different types of amino acids

28. How many letter forms the genetic code?
    a. 2 letters
    b. 3 letters
    c. 4 letters
    d. 5 letters

29. If the polypeptide chain begins by methionine amino acid, which protein would be coded by the following mRNA: *5'CCU CAU AUG CGC CAU UAU AAG UGA CAC ACA-3'?*
    To answer this question use table 4-1:
    a. Pro his met arg his tyr lys cys his thr
    b. Met arg his tyr lys cys his thr
    c. Met arg his tyr lys
    d. Met pro his met arg his tyr lys cys his thr

29. Which mRNA codes for the following polypeptide *chain: met arg ser leu glu try*? *To answer use table 4-1.*

    a. 3'- AUGCGUAGCUUGGAGUGA -5'
    b. 3'- AGUGAGGUUCGAUGCGUA -5'
    c. 5'- AUGCGUAGCUUGGAGUGG -3'
    d. 5'- AUCGGUAGCUUGGAGUGA -3'

31. Which structure of the following is added to the 3'-end of pre-mRNA to be able to pass from nucleus into cytoplasm?
    a. Introns
    b. A poly A tail
    c. Cap of 7- guanosine
    d. Exons

32. Which of the following mRNA can be recognized by ribosome?
    a. mRNA provided with a guanosine cap
    b. Pre –mRNA.
    c. mRNA provided with poly-A tail
    d. mRNA free of introns

33. Of which structure of the following does mature mRNA become free after processing & modifications of pre-mRNA?
    a. Poly-A tail
    b. Exons
    c. Guanosine cap
    d. Introns

34. Which two successive processes of the following does eukaryotic gene expression include?
    a. Transcription and modification
    b. Transcription & translation
    c. Replication& modification
    d. Modification & translation

35. Posttranslation modification of proteins **doesn't** include which process of the following?
    a. Glycosylation
    b. Phosphorylation
    c. Degradation
    d. Cleavage

Part II: Short Answer Questions.

1. What are the types of post-translation modifications of the newly synthesized proteins?
   _____
   _____

2. What are the reasons that make proteins an important way to study genes?
   _____
   _____

3. What is the importance of editing ploy-A tail to pre-mRNA?
   _____
   _____
   _____

4. What is the importance of addition of a cap of 7-methyl guanosine to pre-mRNA?
   _____
   _____
   _____

5. What are the types of base sequence that can stop the mRNA translation?
   a) _____
   b) _____
   c) _____

6. What are the components of initiator complex of mRNA translation?
   a) _____
   b) _____

7. What are the types of active transfer RNA?
   a) _____
   b) _____

8. What are the processes that occur in the nucleus during gene expression?
   a) _____
   b) _____
   c) _____

9. What are the steps of mRNA translation?
   a) _____
   b) _____
   c) _____

10. **Define each term of the following**:

a. Gene expression:
_____
_____

b. Gene transcription:
_____
_____

c. Splicing of mRNA exons:
_____
_____

11. **Fill in the spaces**:

a) Gene promoter in eukaryotic cell is formed of:
_____ and _____.

b) The transcribed regions of gene are:
_____
_____ and
_____

c) Non-transcribed region of the gene are:
_____ and
_____

12. **Explain the following statement**:
Transcription and translation in prokaryotes are coupled.
_____
_____

Answer of MCQs:

1) D	8) D	15) C	22) B	29) ...
2) C	9) D	16) C	23) B	30) …
3) B	10) C	17) A	24) A	31) B
4) C	11) B	18) A	25) C	32) A
5) A	12) C	19) D	26) D	33) D
6) C	13) A	20) D	27) B	34) B
7) A	14) A	21) D	28) B	35) C

CONTROL OF
GENE EXPRESSION (Part -I)

Chapter 5:

CONTROL OF GENE EXPRESSION (Part -I)

Control of Gene Expression in Prokaryotes

Objectives

After you have studied this chapter you should be able to:

- *Describe the concept of the control of gene expression.*
- *Explain the operon model of prokaryotic gene regulation.*
- *Explain genetic sequences which are involved in the gene regulation.*
- *Explain the role of genetic inducible and repressible factors in gene regulation.*

General Considerations

Gene regulation in prokaryotic cells is an ***economical*** *process* associated with the bacterial requirement. Prokaryotes synthesize only the gene products which are required for maintaining the cellular structure at any time. *For example, for energy production, E. coli* contains active constitutive genes, usually switched on, which encode for enzymes that are needed for glycolysis.

Activation of other genes occurs only under special conditions such as absence of glucose and presence of lactose in the media. This situation results in activation of lactose operon – a system of structural gene complex, which is required to catabolize the lactose into glucose for production of energy.

General Levels of Gene Expression Control

Transcriptional level

There are different regulatory factors (*proteins*). Their binding to RNA -polymerases makes the RNA -polymerases able to initiate or activate transcription of a different set of genes.

Translation level

Gene expression is affected by increases or decreases in the rate of translation, rate of ribosomal function.

Post-translation level

Activation or inhibition of the function of the synthesized enzymes takes place by the feedback inhibition mechanism.

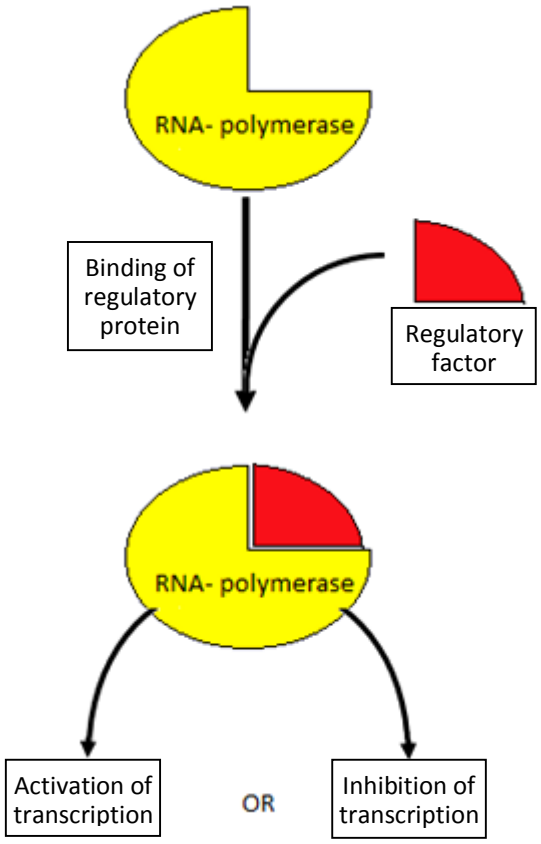


Fig. 5-1: Binding of the regulatory proteins to RNA polymerases may results in activation or inhibition of gene transcription.

Lactose Operon (*Lac* Operon)

Lac operon is an inducible system of gene complex (fig. 5-1) that is composed of:

a) Structural genes
b) Operator
c) Promoter

Structural genes

They have the following general characters:

- They are a group of linked genes with related functions.
  In lactose operon there are three linked genes; Z -gene, Y-gene, and α-gene that code for three enzymes: ß-galactosidase, permease and transacetylase respectively.
- These genes form a unit on bacterial DNA and code for a group of enzymes with related functions.
- Their transcription produces a single mRNA. Because each enzyme is marked by an initiation codon (start codon) and a termination codon (stop codon) on mRNA, translation of mRNA results in production of three separate enzymes:
  - Lactose permease,
  - ß- galactosidase,
  - Galactoside transacetylase.

These enzymes (fig.5-2) are necessary for the catabolism of lactose:

- Lactose permease is responsible for transporting of lactose across the plasma membrane of *E.coli*.

- ß-galactosidase breaks down lactose inside the cell into glucose and galactose to be used as a source of energy in absence of glucose in the media.

- The physiological role of galactoside trans-acetylase is still not completely clear. It may be involved in removal of toxic by-products of lactose digestion from the bacterial cell.

- **Promoter:**

It is a specific nucleotide sequence on DNA strand. It is recognized by RNA -polymerase and is the binding site of RNA-polymerase.

- **Operator:**

It is formed of a specific DNA–sequence which switches the transcription on or off. It overlaps the promoter.

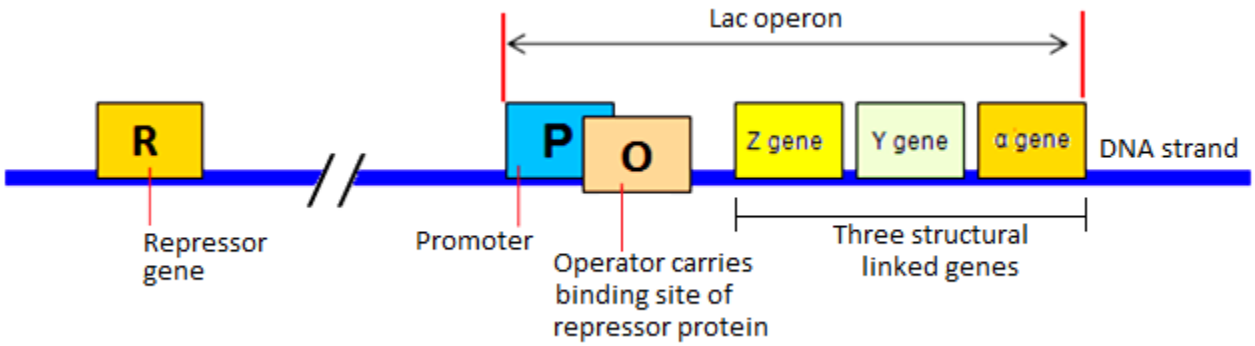


Fig. 5-2: Diagram illustrates the composition of Lac operon. It is formed of promoter, operator and 3 structural linked genes. Repressor gene is responsible for synthesis of a repressor protein which controls Lac operon transcription.

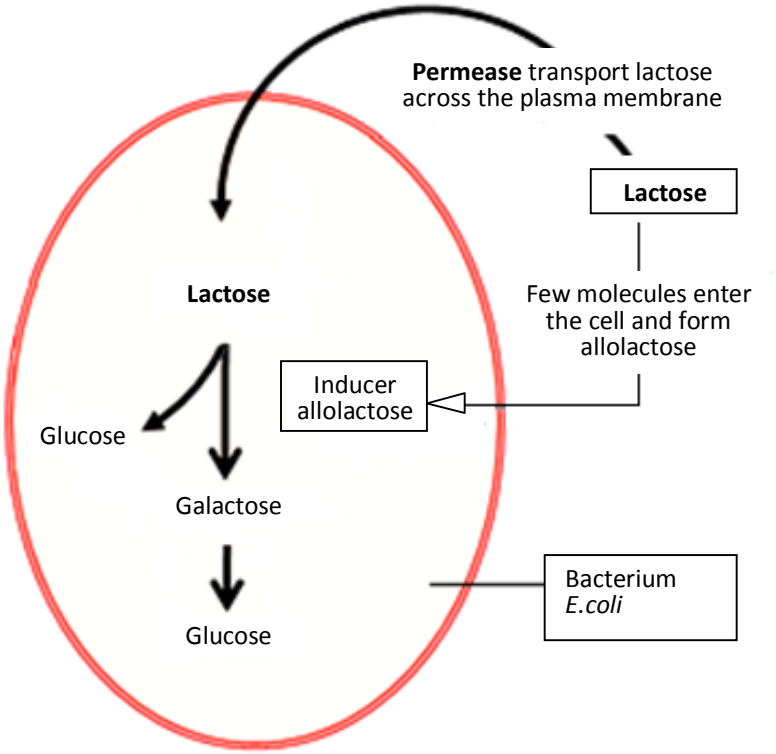


Fig. 5-3: E. coli grow in a culture containing only lactose. They work to activate Lac operon which breaks down the disaccharide lactose into galactose and glucose as a source of energy.

How does Lac operon work?

Lac operon is an inducible system of genes complex (fig. 5-3).

- The inducible genes are usually not transcribed, inactivated by repressor protein, unless a specific inducer inactivates their repressor protein.
- This system of inducible genes becomes active under certain conditions such as absence of glucose and presence of lactose in case of Lac operon.
- Lac operon works to transform lactose into glucose to be used as a source of energy at absence of glucose.
- The metabolic pathway of Lac operon is catabolism of lactose.
- The repressor protein recognizes and binds to operator, so it switches the transcription off.
- The repressor protein is encoded by a gene called repressor gene that is a constitutive gene. It is always on, so it produces continuously a small amount of repressor protein. It is located upstream from the promoter site.
- Inducer is required for inactivation of repressor protein. Inactive repressor cannot link operator, therefore inducer works to switch the transcription on. Inducer is a lactose isomer called allolactose. It recognizes and binds to repressor protein. It's binding to repressor results in inactivation of repressor protein.

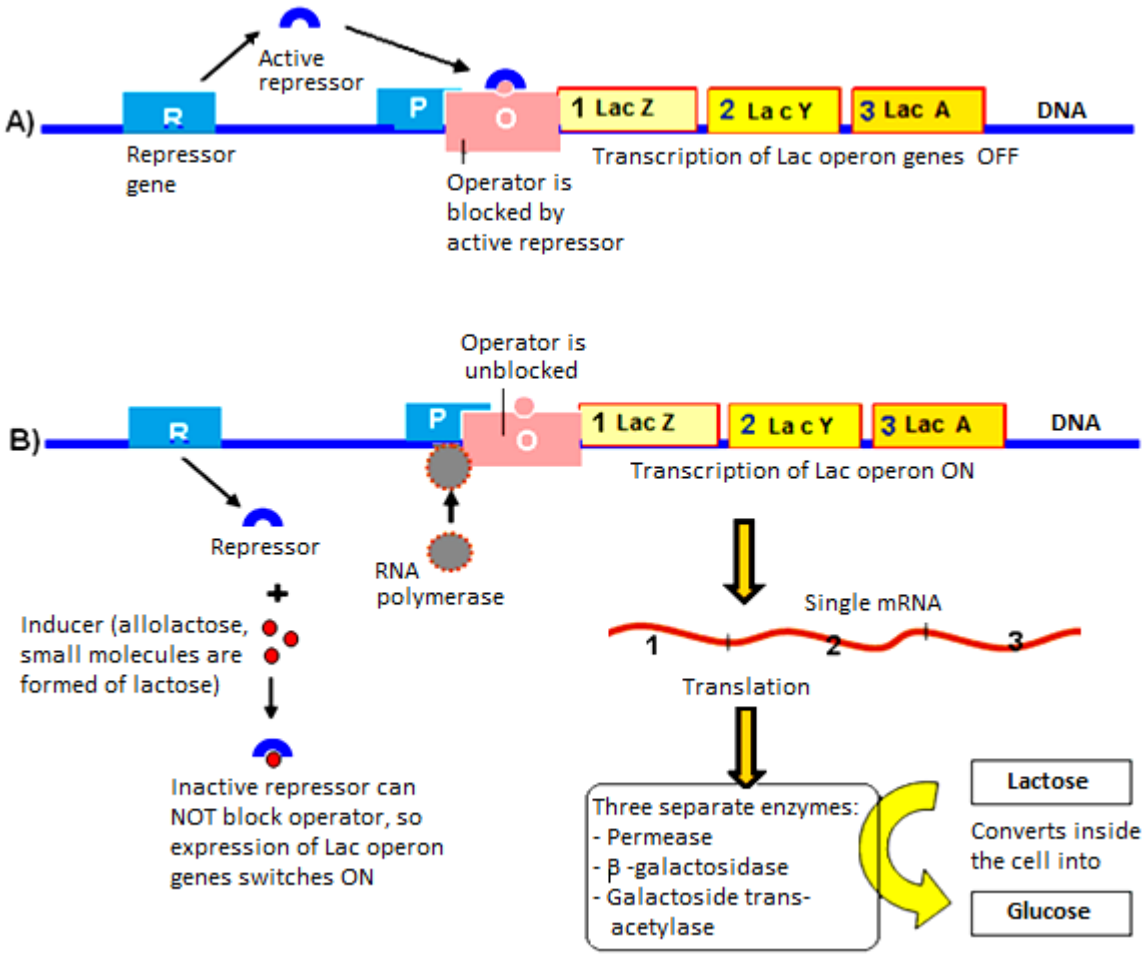


Fig. 5-4: Diagram illustrates Lac operon control. A) Negative control: Inhibition of Lac operon begins by binding of an active repressor to the operator that switches transcription OFF. B) Positive control: Activation of Lac operon begins at binding of the inducer to repressor that inactivates the repressor, so it becomes unable to recognize and bind the operator. RNA polymerase binds to the unblocked promoter and starts the genes transcription. The synthesized enzymes are responsible for conversion of lactose into glucose.

Under which conditions does Lac operon become active? Is the Lac operon always transcribed when glucose is scarce in the media?

- Inhibition of *Lac* operon transcription is an economical process as a result of the cellular refusal to consume their energy in catabolism of lactose to produce a source of energy, glucose, when they have already glucose in the media (fig. 5-3 and 5-4).
- This inactivation of the transcription process by blocking the operator is called *negative control*.
- The controlling element of Lac operon is the repressor protein that switches mRNA transcription off when it binds the operator and blocks the transcription process.
- Activation of lactose catabolism is called *positive control*. It requires presence of lactose as a secondary source of energy and absence of glucose, primary source of energy in the media.

What is happening in the absence of glucose and presence of lactose in the media?

When lactose is present and glucose is absent in the media what is the mechanism of switching Lac operon from inactive into the active state?

Steps of activation:

- Few molecules of lactose enter the cell and form allolactose, an inducer.
- Inducer inactivates the repressor protein (fig. 5-3 and 5-5).

Allolactose is a minor side product of the ß-galactosidase reaction and is formed in sufficient amount within the cell only when a significant quantity of lactose is present in media.

- Because the promoter of Lac operon has low affinity for RNA-polymerase, although the repressor protein is inactivated by allolactose, Lac operon remains inactive. Activation of Lac operon requires addititional factors more than just presence of allolactose.

- Activation of Lac operon requires binding of catabolic activator protein, **CAP**.
  CAP is always inactive, but it becomes active as it combines with cyclic adenosine monophosphate (**cAMP)** as a co-activator, forming **active CAP- cAMP- complex.**
  Cyclic AMP, cAMP, is regulated by glucose level in the medium. *It is inversely proportional to glucose concentration***.**

- Presence of lactose and insufficient glucose in the media causes **increase** of cAMP level and formation of active CAP-cAMP complex that increases the affinity of promoter for RNA-polymerase. When that occurs, activation of Lac operon is achieved by the inducer that inactivates the repressor and turns the process of transcription on (fig. 5-5).

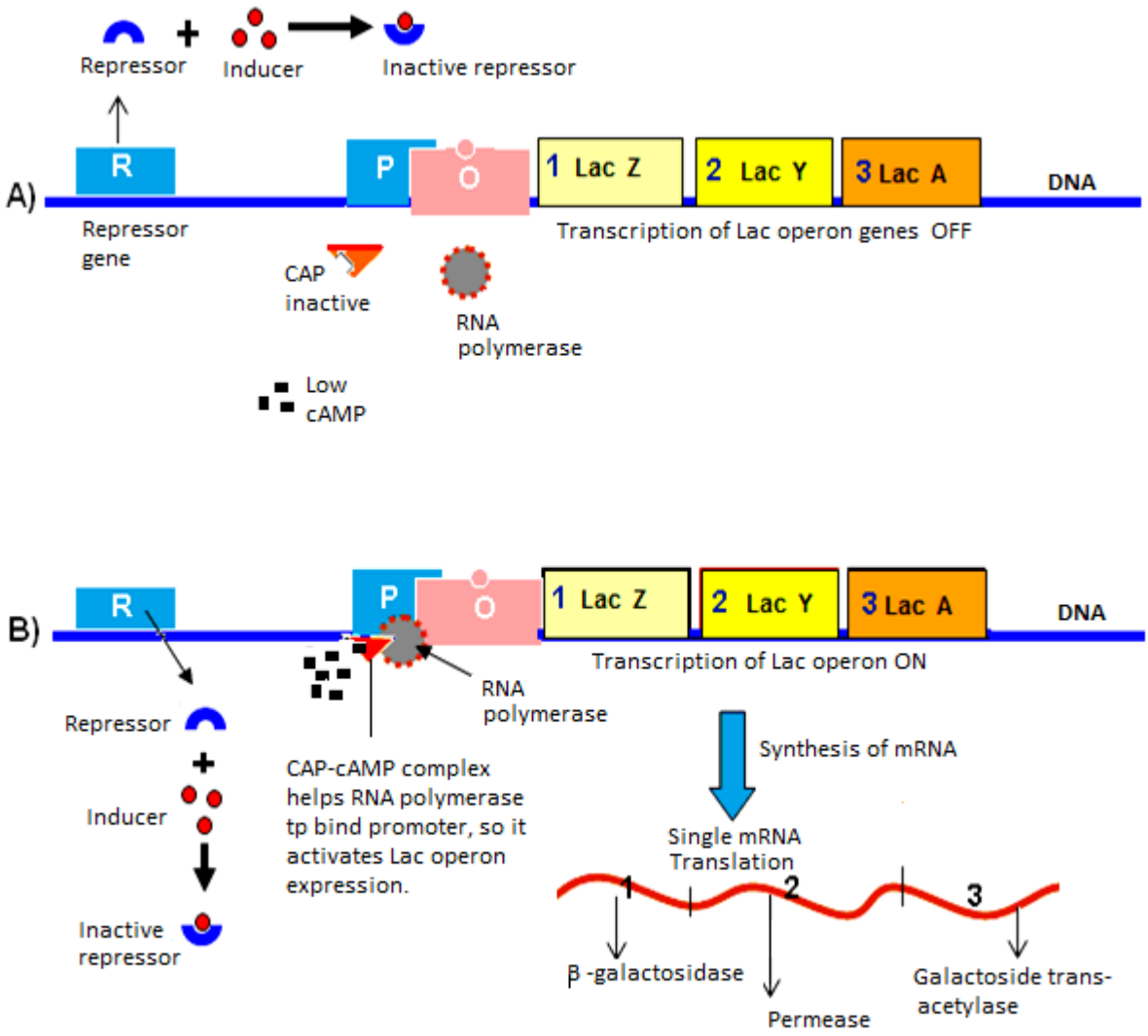


Fig. 5-5: Mechanism of Lac operon activation. A) In presence of high lactose and sufficient glucose in the media the operator becomes unblocked, but Lac operon is inactive because low affinity of RNA-polymerase for promoter, where binding of RNA -polymerase to the promoter requires active CAP. B) In presence of high lactose and insufficient glucose in the media, the level of co-activator cAMP becomes high and binds the activator to form CAP-cAMP complex. CAP-cAMP helps RNA polymerase to bind promoter and turns Lac operon ON.

Mutations of Lac Operon Genes

- Lac Z gene encodes ß-galactosidase.
- Lac Y gene encodes galactose permease and Lac A gene encodes galactoside transacetylase.
- When Lac Z - gene is defective or absent due to mutation, the *E. coli* cell becomes unable to synthesize the Lac Z - gene product, ß-galactosidase. Without this enzyme, cells cannot metabolize lactose, and fail to grow if lactose is the only source of energy in the media.

There are several types of mutation that can cause a *Lac* Z⁻ (Z minus) phenotype – defective or absent *Lac Z* gene.

a) ***Non-sense mutation***
A codon for an amino acid is mutated to a termination codon (*UAA, UAG, UGA*), causing premature termination of the protein synthesis process. This mutation produces non-functioning protein.

b) ***Missense mutation***
A codon for an amino acid is mutated to a codon for a different amino acid. The new amino acid substitution renders the enzyme inactive.

c) ***Deletion mutation***
A portion of DNA of the *Lac* Z gene is absent. With absence of enzyme coding information, a functional enzyme cannot be synthesized.

General characters of Lac operon can be summarized in the following:

- It is an inducible gene complex.
- Its metabolic pathway is a catabolism.
- It converts lactose into glucose and galactose.
- It contains 3 structural linked genes.
- It produces 3 inducible separate enzymes.
- It is usually OFF, repressor protein is active.
- High lactose turns transcription ON, when glucose level in media is low;
- Lactose acts as an inducer allolactose. It inactivates repressor protein.
- It is active under certain conditions; economic regulation.
- Cyclic adenosine monophosphate (cAMP) is necessary for CAP to switch transcription ON.

Tryptophan Operon
(*Trp* Operon)

General features:

- Tryptophan operon (*Trp operon*) is another type of bacterial operon.
- The mechanism of regulation of *Trp operon* depends on the level of intracellular amino acid tryptophan that is required for cell growth.
- Its repressor protein is inactive - *unable to bind and turn operator off without help of co-repressor*. So, it is usually on – it synthesizes the enzymes required for anabolism of amino acid tryptophan.
- It is a repressible system of gene complex.
- Similar to *Lac* operon, it is formed of 5 repressible linked structural genes. These genes form together a transcriptional unit with a single promoter for binding of RNA – polymerase and a single operator for switching transcription ON or OFF.
- The genes transcription produces a single mRNA, and translation of mRNA produces 5 separate enzymes. Three of them are required for synthesis of amino tryptophan.
- In comparison with Lac –operon, the metabolic pathway of *Trp* -operon is anabolism – it synthesizes the tryptophan amino acid.

Trp operon composition and mechanism of regulation:

- Intracellular tryptophan level regulates the transcription of *Trp* operon.

- Tryptophan operon is usually ON because repressor protein is inactive.

- When the end-product of anabolic path-way, tryptophan, reaches a high-level, tryptophan acts as co-repressor to activate the repressor protein which binds to DNA-operator and turns operator OFF, represses the genes; inactivates transcription.

- When tryptophan level is decreased under the level of required cellular amount inside the cell, tryptophan becomes insufficient to activate the repressor protein that becomes inactive and unable to bind and turn the operator OFF. So, *Trp operon switches* ON to synthesize the required enzymes for manufacturing of amino acid tryptophan (fig. 5-6).

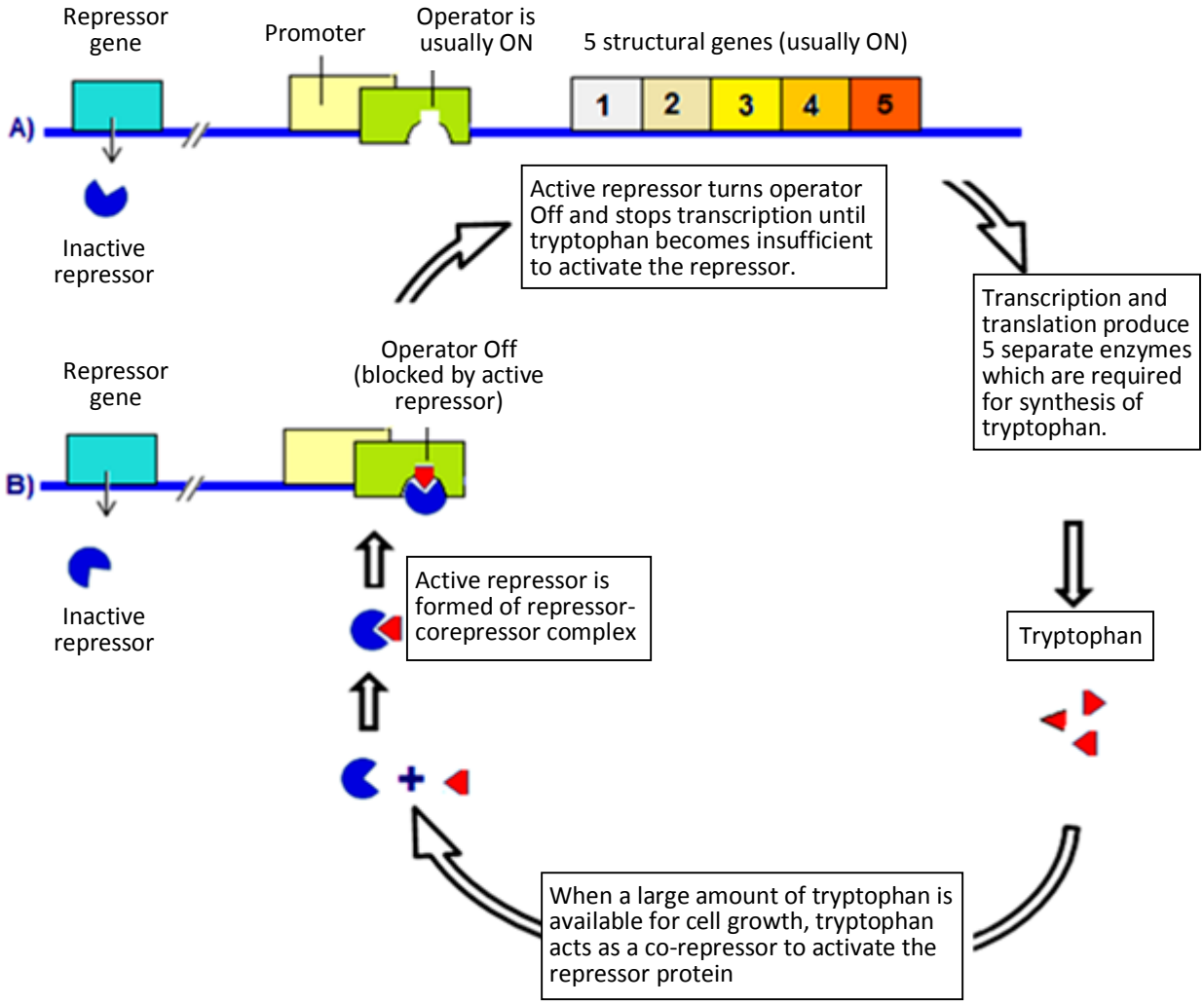


Fig. 5-6: Composition of tryptophan operon and the mechanism of regulation. Trp operon is regulated by the level of amino acid tryptophan within the cell. A) At low level of intracellular tryptophan, usually all enzymes required for synthesis of tryptophan are actively produced, and the repressor protein is inactive. B) Increase of intracellular tryptophan level results in formation of active repressor-corepressor complex that turns operator Off.

In comparison with Lac operon Trp operon is different in the following:

- It is a repressible gene complex.
- An anabolic system; works to synthesize amino acid tryptophan.
- Contains 5 structural genes.
- The product of the repressible genes of Trp operon is formed of five enzymes. Three of them are required for synthesis of amino acid tryptophan.
- The repressor protein is inactive and encoded by a regulatory constitutive gene.
- It has one type of gene regulation, negative control.
- Usually ON, and becomes inactive at high level of tryptophan.
- The cAMP, cyclic adenosine monophosphate, is not necessary for activation of tryptophan operon.
- High level of cytoplasmic tryptophan turns transcription OFF.
- Tryptophan acts as co-repressor. It combines with repressor to form an active repressor-corepressor complex which changes its conformation to be able to bind the operator and turns transcription OFF, when the cell has a sufficient amount of intracellular tryptophan.

CONTROL OF
GENE EXPRESSION (Part -II)

Chapter 5:
CONTROL OF GENE EXPRESSION (Part -II)

Control of Gene Expression in Eukaryotic Cells

Objectives

After you have studied this chapter you should be able to:

- *Describe the levels of gene regulation control in eukaryotic cells.*
- *Contrast the gene expression control in prokaryotic and eukaryotic cells.*

General features: In eukaryotic cells, gene expression control is more complicated in comparison with prokaryotic cells.

- *Environmental regulation:* The changes in cellular environment turn a set of genes ON or OFF such as infection by bacterial or viral disease.
- *Tissue regulation:* Specialization and organization of the cells in organs cause activation of some genes and inactivation of others.
- *Temporal regulation:* Some genes are active only during a certain period of life, inducible genes.
- In eukaryotic cells genes are not organized in form of operons as in prokaryotic cell.
- *House-keeping enzymes* are usually encoded by constitutive genes.

Levels of Control of Gene Expression:

1. Level of DNA Organization

i) Multiple copies of genes:

The cell may contain a variable number of transcription units according to the cellular activity and the required gene product. For example the cell may contain 150 to 450 transcriptional units of genes.

i) Gene amplification.

Some genes amplify themselves. They do self – replication in order to increase their product under certain circumstances. This amplification of genes depends upon the amount of gene products which are required by the cells.

iii) Chromatin structure.

Genes become inactive if they are transformed into a compact mass of inactive heterochromatin, such as transformation of X-chromosome into inactive ***Barr-body***, in order to compensate the products of the X-linked gene in both female and male individuals.

iv) DNA -methylation.

Gene is inactivated by addition of methyl group to DNA-cytosine forming methyl cytosine. Methyl cytosine combines with specific protein to form methyl cytosine protein complex which blocks transcription (fig. 5-7).

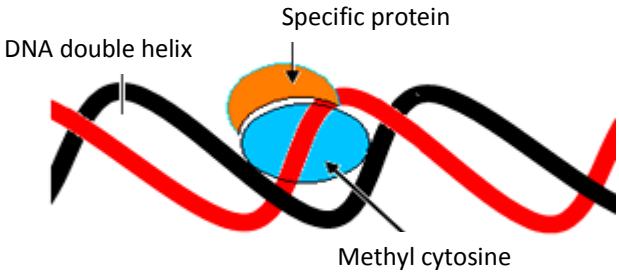


Fig. 5-7: Formation of methyl-cytosine–protein complex which blocks transcription of DNA.

2. Level of Gene Transcription

It takes place by acceleration of the rate of transcription or blocking gene transcription. Generally, gene transcription in eukaryotic cells requires multiple regulatory proteins which are called *general transcription machinery or regulatory protein complex.*

The rate of gene transcription depends on the following:

a) Efficiency of promoter
b) Regulatory protein
c) Enhancer sequence
d) Activator protein

Efficiency of Promoter

Promoter is a regulatory region of DNA located upstream of a gene, providing a control point for regulated gene transcription. In *eukaryotic gene* (fig.5-8), the promoter is *composed of:*

i) TATA –box
ii) UPEs

TATA –box:

It has the core DNA sequence 5'-TATAAA-3'.The adenine and thymine -rich sequence facilitates easy unwinding (due to 2 hydrogen bonds between bases).

The TATA box usually forms the binding site of RNA polymerase-II. After binding several transcription factors to the TATA box, and its upstream and downstream parts, the RNA polymerase can then recognize this multi-protein complex and bind to it.

Transcription process is then initiated, and the polymerase moves along the DNA strand, leaving transcription factors bound to the TATA box. These can then facilitate the binding of additional RNA polymerase -II molecules.

This cluster of RNA polymerase -II and various transcriptional factors is known as the *transcriptional machinery complex* (TMC). In this case, it gives only a low level of transcription. Other factors must stimulate the TMC to increase transcription rate.

- TATA -box is located at about 30 base pairs upstream from transcription initiation site.
- It is formed of thymine and adenine nucleotides.

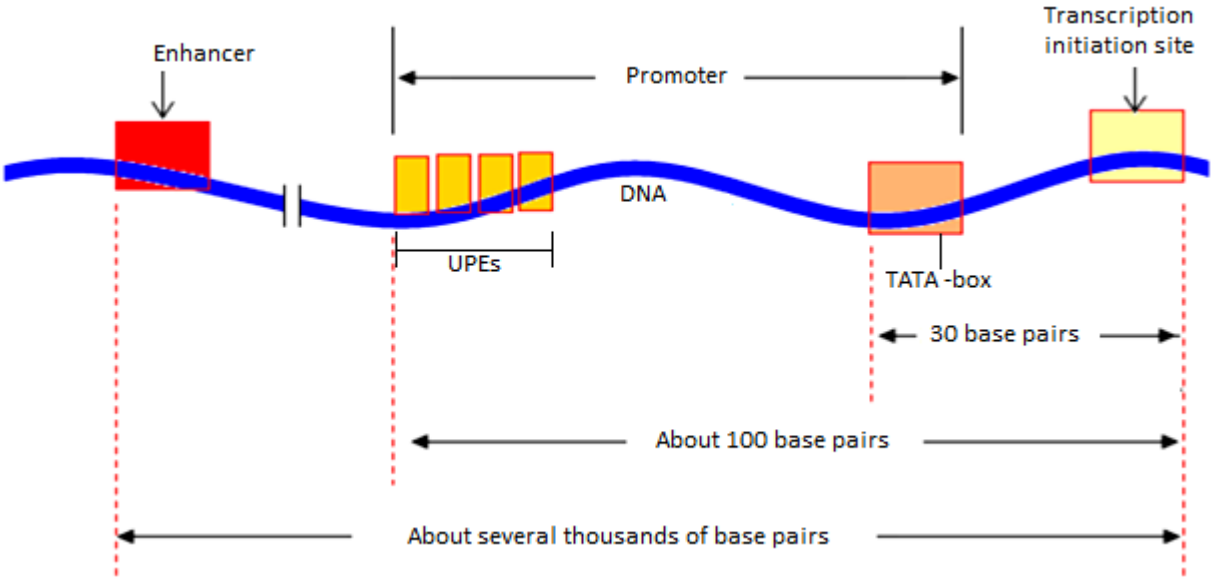


Fig. 5-8: Structure of promoter of gene in eukaryotic cell. It is formed of TATA –box and UPEs (upstream promoter elements). Efficiency of promoter depends upon the number of UPEs.

UPEs (upstream promoter elements):

- Each one is formed of about 8 to 12 base pairs.
- They are found at about 100 base pairs upstream from transcription initiation site.
- They influence the process of gene transcription because their number and type affect the efficiency of promoter.

Efficiency of promoter depends on the *number* and *type* of UPEs. While *weak promoter* contains few UPEs, a *strong promoter* contains several UPEs.

- UPEs are required for accurate and efficient initiation of mRNA synthesis.

Regulatory proteins

It may be an *activator or repressor* protein. The transcription process requires many regulatory proteins which form a *regulatory protein complex* (fig. 5-9).

Binding of a regulatory protein complex to TATA –box of promoter is necessary for formation of the *transcription machinery complex*.

Formation of transcription machinery complex is required for *binding of RNA-polymerase, enzyme of transcription.*

Enhancer sequence

An enhancer is a short segment of specific DNA base sequence that can bind proteins called activators.

Binding of activators to this enhancer base sequence region can initiate the transcription process of a gene that may be located some distance away from the enhancer sequence, or can even be on *a different chromosome*.

- Using transcription factors by the activators, which enhances the binding of RNA polymerase, increases the gene transcription.
- Enhancer base sequence works to increase the rate of gene transcription, when it interacts with *transcription machinery complex* in presence of the *activator protein.*
- It is found at several thousands of base pairs upstream from transcription initiation site.

Activator protein

An activator protein is a DNA-binding protein that regulates one or more genes by increasing the rate of transcription.

The activator may increase transcription by a connected domain which assists in the formation of the RNA polymerase holo-enzyme (the active form); some enzymes require binding of *cofactors* to show full activity. Holo-enzyme is a complete complex containing all the subunits needed for full activity.

- A particular activator may bind one or more specific co-activators.
- It has 2 functional domains, one binds to enhancer and the other connects the transcription machinery complex.

It increases the rate of gene transcription and mRNA synthesis

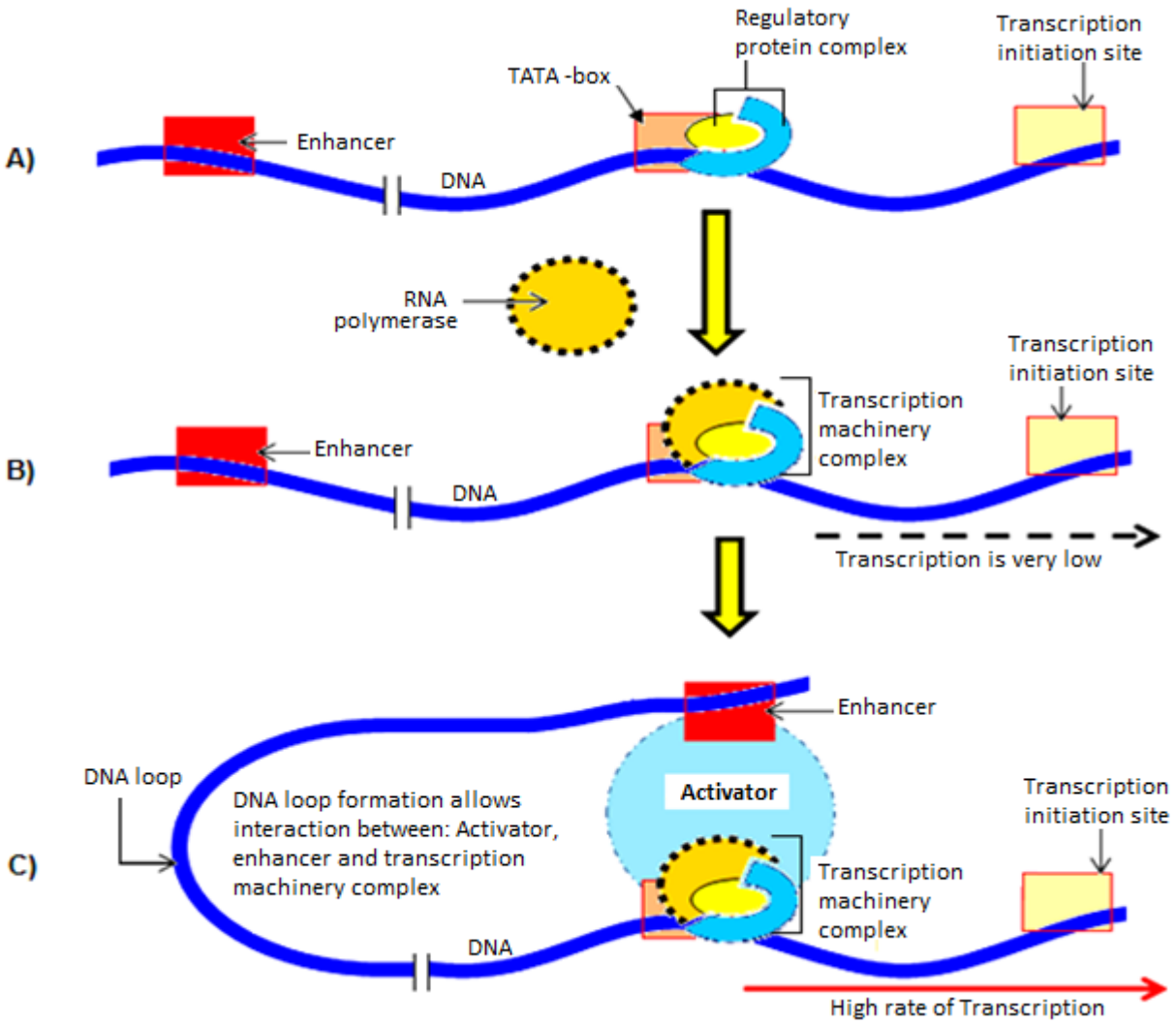


Fig. 5-9: Diagram illustrates steps of gene transcription activation in eukaryotic cells. A) Binding of regulatory protein complex to TATA -box is required for binding of RNA polymerase. B) Formation of transcription machinery complex by binding of RNA polymerase to the regulatory protein complex at TATA-box is not at all enough to activate gene transcription. C) Increasing the rate of gene transcription requires interaction between activator protein, enhancer and transcription machinery complex.

3. Level of Post-transcription

The post-transcription control of gene can be achieved within the tissue. Some genes produce pre-mRNA which has ***multiple splicing patterns***.

- *Pre-mRNA* can be spliced by more than one way depending on the type of tissue and the required gene products.
- So according to the way of splicing, the same gene may produce one protein in one type of tissue and another type of protein in a different type of tissue.

4. Level of mRNA Translation

Gene control at level of mRNA translation is obtained by regulation of mRNA modification, processing, and translation.

The rate and efficiency of mRNA translation are usually regulated by several factors such as:

- The efficiency of the mRNA leader sequence.
- The number of polyribosomes in the translation process.
- The rate of translation besides the number of ribosomes depends upon the number of tRNA and other elongation factors.

5. Level of Post-translation

Gene control at level of post-translation may be accomplished by modifications of the synthesized protein.

- Many eukaryotic proteins are modified after they are synthesized within rough endoplasmic reticulum and Golgi-complex
- Chemical modifications of proteins by addition or removal of functional groups; such as phosphate group, can alter the activity of an enzyme.

  Phosphorylation - addition of phosphate group to the adenosine diphosphates (ADP) results in formation of active adenosine triphosphates (ATP) that is the source of energy within the cell.

  Examples of protein modifications that may regulate the gene function at level of post-translation include:

- Conversion of the protein which is synthesized as inactive form into active protein by removal of a portion from the polypeptide chain. This process is called the proteolytic process. For example inactive pepsinogen is converted into active pepsin.

- A selective degradation of cellular proteins by proteasomes is an important process to regulate the amount of intracellular content of protein and remove the unrequired non-functioning or malformed proteins.

Additional readings

Brennan RG and Matthew BM (1989): Structural basis of DNA-protein recognition. Trends in Biochem Sci., Vol.14, 287-290.

Botsford J L and Harman J G (1992): Cyclic AMP in prokaryotes. Microbiol. Rev. 56, 100-122.

Brown CE et al. (2000): The many HATs of transcription co-activators. Trends Biochem Sci., 25, 15-28

Condon C, Squires C, and Squires C L (1995): Control of rRNA transcription in E. coli. Microbiol. Rev. 59, 623-645.

Elliott WH and Elliott DC (2002): Biochemistry and molecular biology. 2nd ed. New York: Oxford University Press

Fedor M J (1998): Ribozyme. Current Biology, Vo. 8, R441-443

Fiering S, Whitelaw E, and Martin DIK (2000): To be or not to be active: The stochastic nature of enhancer action. Bio - Essays, Vol. 22, 381-387.

Friedman A M, Fischmann TO and Steitz TA (1995): Crystal structure of lac repressor core tetramer and its implications for DNA looping. Science, 268, 1721-1727.

Gold L (1988): Posttranscriptional regulatory mechanisms in E. coli. Ann. Rev. Biochem. 57, 199-223.

Greenblatt J, Nodwell J R and Mason S W (1993): Transcriptional anti-termination. Nature, 364, 401-406.

Kornberg, R.D. (1999): Eukaryotic transcriptional control. Trends Gen., 15 (12), 46- 49.

Kolb A et al. (1993): Transcriptional regulation by cAMP and its receptor protein. Ann. Rev. Biochem. 62, 749-795.

Lewin B (2002): Genes VII: Part 5. New York: Oxford University Press Inc.

Li JJ (1995): Once and once only. Current Biology, 5, 472-475.

Lodish, H., et al. (2007): Molecular Cell Biology, 6th ed. Oxford: W.H. Freeman,

Oehler S (1990): The three operators of the lac operon cooperate in repression. EMBO, 9, 973-979.

Orgel LE (1994): The origin of life on the earth. Sci. Amer., 271 (4), 52-61.

Ptashne, M. (1992): A Genetic Switch: Phage Lambda and Higher Organisms, 2nd ed. Cambridge: Cell Press and Blackwell Scientific, 192pp

Solomon EP, Berg LR and Martin DW (2002): Biology, 6th ed. Gene regulation. United States: Thomson Learning Academic Resources Center, 284-300.

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer.

1. Which statement of the following is **not** consistent with gene expression control in eukaryotic cell?
   a. Environmental changes turn a set of genes ON or OFF
   b. Organization of the cells in organs activates or inhibits of some genes.
   c. Constitutive genes code for house-keeping enzymes.
   d. Genes are organized in operon

2. Which structure of the following is absent in Lac operon?
   a. Structural genes
   b. Operator
   c. Promoter
   d. Enhancer

3. Which structure of the following is inactivated by inducer protein of Lac operon?
   a. Repressor protein
   b. Activator protein
   c. Cyclic AMP
   d. Regulatory protein

4. In which process of the following bacteria utilize glucose first, even if other sugars are present?
   a. Operon repression
   b. Catabolic repression
   c. Gene regulation
   d. Glucose utilization

5. Which condition of the following **doesn't** occur in presence of high amounts of glucose?
   a. Lac operon will be inactive.
   b. Cyclic-AMP level will be low.
   c. Catabolism of lactose will be high.
   d. Repressor protein will be active

6. Which of the following growth media activates transcription of Lac operon?
   a. High glucose, high lactose
   b. High glucose, no lactose.
   c. High glucose, low lactose.
   d. No glucose, high lactose.

7. Which of the following is **not *true*** about tryptophan operon?
   a. Repressible gene complex.
   b. Catabolic system
   c. Contains 5 structural genes.
   d. Produces 5 separate enzymes.

8. Binding which of the following to operator switches tryptophan operon OFF?
   a. Repressor only
   b. Repressor-corepressor
   c. Corepressor only
   d. Cyclic AMP

9. If the media is free of glucose and contains lactose, it causes which process of the following?
   a. Decrease level of cyclic-AMP.
   b. Inactivation of CAP.
   c. Activation of Lac operon.
   d. Increase cell division

10. Methyl cytosine protein complex formation is a type of gene control at the level of:
    a. DNA organization
    b. DNA transcription
    c. DNA replication
    d. mRNA translation

11. Upon which structure of the following does efficiency of promoter in eukaryotic cell depend?
    a. TATA boxes
    b. Number of UPEs
    c. Activator proteins
    d. Enhancers

12. Which of the following accelerate (s) the rate of gene transcription?
    a. TATA boxes
    b. UPEs
    c. Regulatory protein
    d. Enhancer

13. Which region of the following is the binding site of RNA-polymerase in eukaryotic gene?
    a. TATA box
    b. Operator
    c. UPE s
    d. Transcription initiation site

14. Barr body formation is a type of gene regulation at which level of the following?
    a. DNA replication
    b. DNA transcription
    c. DNA organization
    d. Post-transcription

15. Enhancer increases the rate of gene transcription when it interacts with which of the following?
    a. Activator protein only
    b. Activator protein and TATA box
    c. Activator protein and transcription machinery complex
    d. Activator protein and promoter

16. Which of the following codes for housekeeping enzymes in eukaryotic cell?
    a. Structural genes
    b. Constitutive gene
    c. Temporal genes
    d. Catabolic genes

17. To which system of the following does bacterial operon refer?
    a. A respiratory system
    b. A system of transport protein
    c. A system of gene complex
    d. A system for nutrition

18. Which of the following acts as an inducer in the *Lac* operon?
    a. Lactose
    b. Repressor
    c. Operator
    d. Promoter

19. Which site of the following is the binding site for RNA polymerase in prokaryotes?
    a. Structural gene
    b. Repressor gene
    c. Operator
    d. Promoter

20. How many enzymes are produced by tryptophan operon?
    a. 2
    b. 3
    c. 4
    d. 5

21. How many genes form Lac operon?
    a. 2
    b. 3
    c. 4
    d. 5

22. Which process of the following is **not** happened by regulatory proteins?
    a. Activation of gene
    b. Inhibition of gene.
    c. Formation of transcription machinery.
    d. Translation of gene.

23. In gene expression, transcription machinery complex is formed when RNA polymerase binds to which structure of the following?
    a. Enhancer.
    b. Cyclic AMP.
    c. Regulatory protein complex
    d. Activator protein.

24. Which of the following statements about promoters of eukaryotic genes is **not** correct?
    a. Complicated than prokaryotic promoters
    b. Formed of TATA box and UPES.
    c. Activated by multiple transcription factors.
    d. The binding site of DNA polymerase.

25. Which function of the following does DNA methylation perform?
    a. Enhances gene transcription.
    b. Blocks gene transcription.
    c. Inhibits mRNA modification.
    d. Accelerates gene expression.

26. Which type of the following genes become active only during a certain period of life?
    a. Repressible genes
    b. Inducible genes
    c. Recessive genes
    d. Dominant genes

27. Modification of mRNA is a type of gene regulation at which level of the following?
    a. DNA organization.
    b. Gene transcription.
    c. Post- transcription.
    d. Post-translation.

28. What is the type of gene expression regulation at the organization of cells in organs?
    a. Temporal regulation
    b. Environmental regulation.
    c. Tissue regulation.
    d. DNA organization regulation.

29. Gene amplification (self-replication) is a type of gene regulation at which level of the following?
    a. Gene transcription.
    b. DNA organization
    c. Posttranslation.
    d. Gene replication.

30. Modification of protein is a type of gene control at which level of the following?
    a. DNA organization
    b. RNA translation
    c. DNA transcription
    d. Post-translation

31. Which factor of the following regulates the rate and efficiency of mRNA translation?
    a. Number of polysomes
    b. Number of DNA polymerase
    c. Number of RNA polymerase
    d. Rate of transcription

32. Z-gene of Lac operon encodes for which enzyme of the following?
    a. ß-galactosidase.
    b. Adenosine triphosphatase.
    c. Galactoside transacetylase.
    d. Galactose permease.

Part II: Short Answer Questions.

Fill in the spaces:

1. Lac operon is formed of _____, _____ and _____
2. Repressor gene is responsible for synthesis of _____ which control Lac operon transcription.
3. Gene regulation in bacteria is a (an) _____ associated with the bacterial requirements.
4. Lac operon is a (an) _____ system of gene complex.
5. DNA –sequence which switches transcription of Lac operon ON or Off is called _____.
6. In Lac operon the binding site of RNA polymerase is _____.
7. Some genes are active only during certain period of life, these genes are called _____. This type of gene regulation in eukaryotic cells is called _____.
8. Specialization and organization of the cells in organs cause activation of some genes and inactivation of the others. This type of gene regulation is called _____.
9. Change of chromatin structure by formation of _____ is a type of gene regulation in eukaryotic cells because it causes inactivation of genes carried by X-chromosome _____ in to compensate the gene product in both male and female.
10. DNA –loop formation allows interaction between _____, _____ and _____ to increase the rate of _____.

Explain:

a) Lac operon is inactive in presence of sufficient amount of glucose in medi-
di-
um._____
_____

b) Gene regulation in eukaryotes is more complicated than prokaryotes.
_____
_____

c) Environmental regulation of gene expression in eukaryotic cells.
_____
_____

Answer of MCQs:

1) D	8) B	15) C	22) D	29) B
2) D	9) C	16) B	23) C	30) D
3) A	10) A	17) C	24) D	31) A
4) C	11) B	18) A	25) B	32) A
5) C	12) D	19) D	26) B	
6) D	13) A	20) D	27) C	
7) B	14) C	21) B	28) C	

GENETIC VARIATIONS

Chapter 6:
GENETIC VARIATIONS

Objectives

After you have studied this chapter you should be able to

- *Enumerate different kinds of changes in the base sequences of DNA.*
- *Explain different types of mutation and how the mutation changes the phenotype.*
- *Enumerate the mutagenic agents*
- *Explain the structure of transposon and its role in spreading the antibiotic resistance gene in bacterial populations.*

Mutation

There are about 4000 hereditary diseases that may be caused by a defect in a single gene somewhere in the human genome. The defect could be an addition, a deletion or a repeat of one or more base pair.

There are two classes of mutations: Spontaneous mutations (**molecular decay**) and **induced mutations** caused by mutagens.

For example, ***gene of cystic fibrosis*** contains 250,000 base pairs which form base sequence similar to that of the normal gene, except that one base pair is missing out in the disease–causing gene.

Definition:

Mutations are any changes in the nucleotide sequence; base pair sequence of DNA molecules of an organism. Mutations can be caused by copying errors during DNA replication, as a result of exposing to radiation, chemical mutagens or viruses, or can be produced by organism itself in *hypermutation*. **Hypermutation** is a mechanism inside the cells which allows immune cells to adapt its response to new foreign antigens during the life time of an organism.

Classifications of Mutations

a. According to the **stability of mutations**, they can be classified into:
   1. **Unstable mutations,** which revert -back to its original sequence.
   2. **Stable mutations,** which result in changes of the phenotype of organism.

b. According to **situation of Mutations** in multicellular organism, they can be subdivided into:
   1. **Germ line mutations**: They can be passed on to the offspring through the reproductive cells.
   2. **Somatic mutations**, which involve the cells outside the reproductive organs. This type of mutations is not usually transmitted to the offspring.

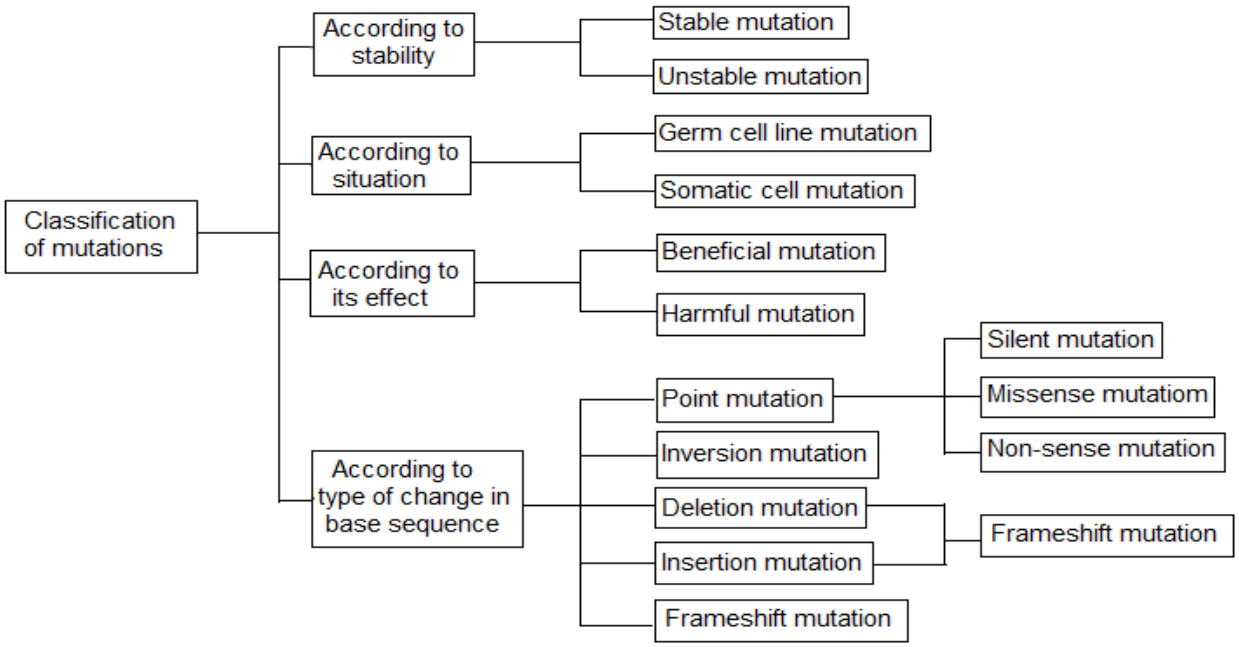


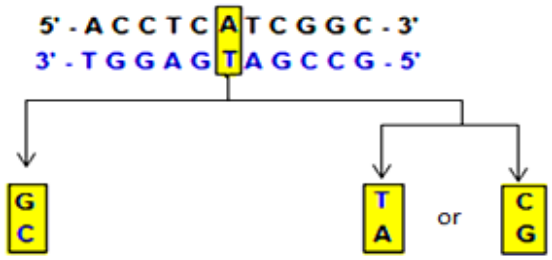
Fig. 6-1: Classification and types of mutations.

c. *According to the **effect of mutations**, they can be classified into:*

1. **Harmful mutations** which can cause errors in protein sequence, creating partially or completely non-functional proteins. When a mutation alters a protein that plays a critical role in the body, it may cause a medical condition called a genetic disorder.
2. **Beneficial mutations** which may have a positive effect. For example, when a base deletion occurs in human gene that codes for CCR5, a receptor on T –cells which is used by HIV to enter the cell, this deletion results in delay of the onset of AIDS manifestations in heterozygotes and confers HIV resistance to homozygotes.

d. *According to **type of change in the structure of base sequence**, mutations are classified into:*

1. Point mutations. There are 3 types:
   a. Silent mutations
   b. Missense mutations
   c. Nonsense mutations
1. Insertion
2. Deletion
3. Inversion
4. Frameshift mutations



Transition substitution:
Purine is replaced by purine
Pyrimidine is replaced by pyrimidine

Transverse substitution:
Purine is replaced by pyrimidine
Pyrimidine is replaced by purine

Fig. 6-1: In point mutations transition base substitution is the most common while transverse base substitution is a less common mutation.

1. Point Mutations

Point mutations result when a single base pair in DNA is replaced of by another in base substitution mutations (fig.6-1).

Point mutations that may occur within the protein coding region can be classified into:

a) Missense mutations, which cause a re-placement of a single nucleotide by another, that codes for another amino acid in the synthesized protein (fig. 6-2).

Their effect depends upon the position of the replaced amino acid.

- If the amino acid is not a part of the active site of the enzyme, the mutation does not cause any change in the enzyme activity.
- If the amino acid is located at or near the active site, the mutation causes reduction or absence of the enzyme activity. Such mutations result in diseases such as ***epidermolysis bullosa and sickle-cell disease.***
- In ***sickle cell disease***, a base pair in the gene that is found on chromosome 11 and codes for *the beta chain of hemoglobin* is replaced by another base pair. Because of this replacement, the codon G**A**G (containing nitrogenous base adenine and coding for **glutamic acid**) is changed to G**U**G (containing nitrogenous base uracil and coding for **valine**).

b) Silent mutations, which don't change the amino acid sequence in the active area, thereby do not alter protein function. They occur in a non- coding region (outside of a gene or within an intron), or they may occur within an exons in non-effective protein coding sequence.

c) Nonsense mutations, which create an *internal stop codon in mRNA. This mutation* will terminate prematurely the encoded protein that is usually non-functional protein product (fig. 6-3). Some genetic disorders, such as ***thalassemia,*** result from non-sense mutations.

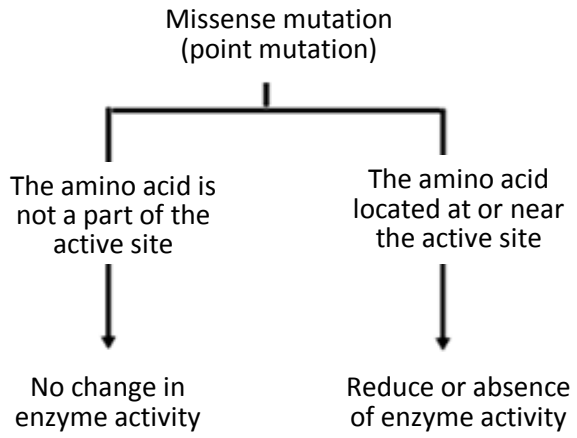


Fig. 6-2: Diagram illustrates the missense mutation. The effect of mutation depends upon the position of the replaced amino acid.

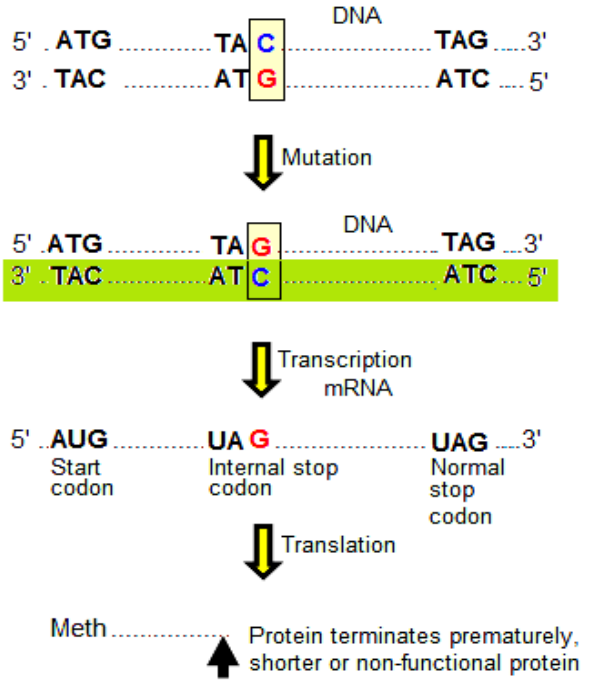


Fig. 6-3: Non-sense mutation. Point mutation results in formation of an internal stop codon which terminates the protein synthesis prematurely.

2. Insertion and deletion mutations

Insertion mutation results from addition of one or more nucleotide base pairs into a DNA sequence (figs. 6-4 & 6-5).

This can happen due to unequal crossing over during meiosis. It may involve addition of non-coded nucleotides during recombination, or insertion of palindromic sequences.

They are usually caused by transposable elements or errors occurring during replication of repeating elements. *Insertions of transposable elements may be harmful because* when they are inserted in an exon, the amino acid coding region of gene. Their insertion changes the base sequence and causes an alteration in the normal reading frame of a gene (*frame shift mutation, fig 6-6*).

Deletion mutations remove one or more nucleotides from the DNA (figs. 6-3 and 6-4). Like insertions these mutations can alter the reading frame (*frame shift mutation, fig 6-6*) of the gene. They are generally irreversible.

• *Cri-du-chat syndrome:* Small chromosomal deletions may cause genetic disorders such as *Cri-du-chat syndrome* (cry of cat). It results from the loss of a short arm of chromosome # 5.

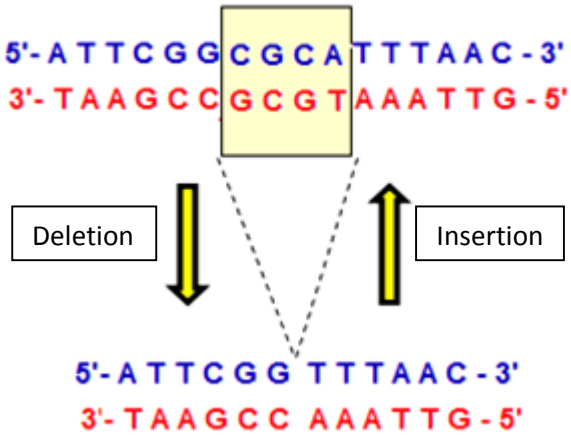


Fig. 6-4: Diagram illustrates an insertion or deletion of a group of base pairs.

3. Frame shift mutations

They are a result of insertion or deletion mutations that cause insertion or deletion of one or more base pairs. This mutation results in misreading of all codons from the point of deletion or insertion to the end of the message and changes the amino acid sequence of encoded polypeptide chain (fig. 6-6).

4. Inversion mutations

Inversion mutations cause a string of bases is deleted and followed by reinsertion of the same base sequence in opposite direction (fig. 6-7).

An *inversion mutation* is a chromosome rearrangement in which a segment of a chromosome is reversed end to end.

An i*nversion* occurs when a single chromosome undergoes breakage and rearrangement within itself.

Inversions usually do not cause any abnormalities in carriers as long as the rearrangement doesn't cause extra or missing genetic information.

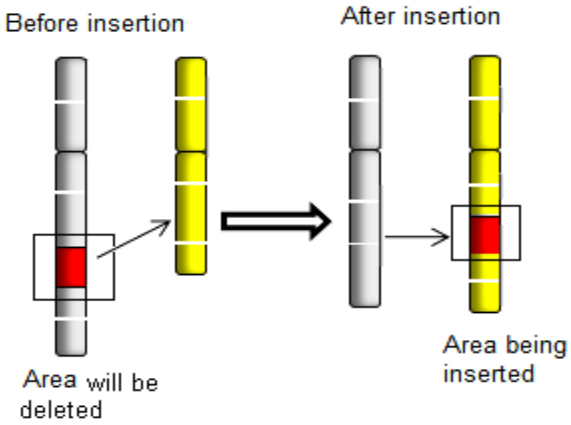


Fig. 6-5: Diagram illustrates area deletion from one chromosome and its insertion into another.

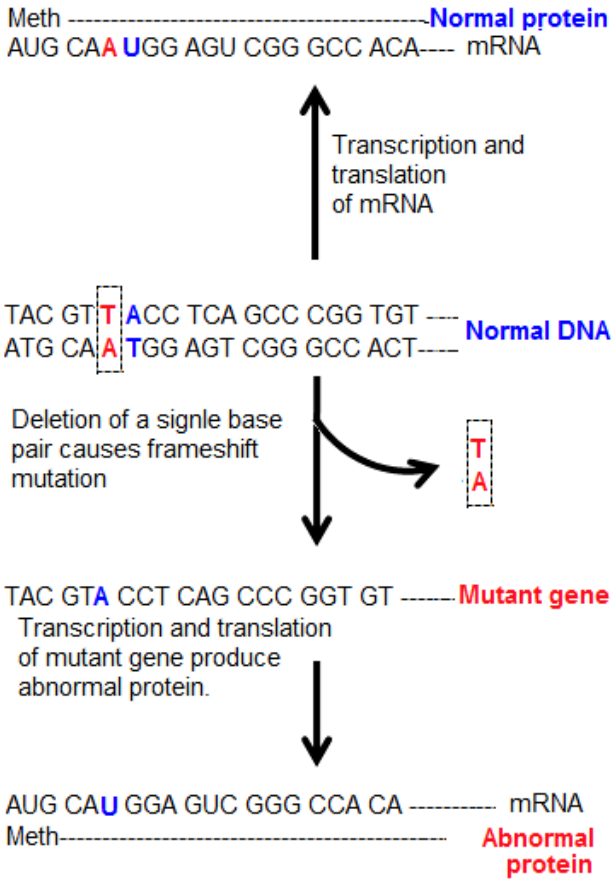


Fig. 6-6: Diagram showing frame shift mutation which results in formation of abnormal new polypeptide chain; non-functional protein. 1. Normal synthesized protein. 2. Abnormal synthesized protein.

Mutant

An individual or organism that shows deviation in some characters or phenotypes, whose progeny maintains this variation, is called a mutant.

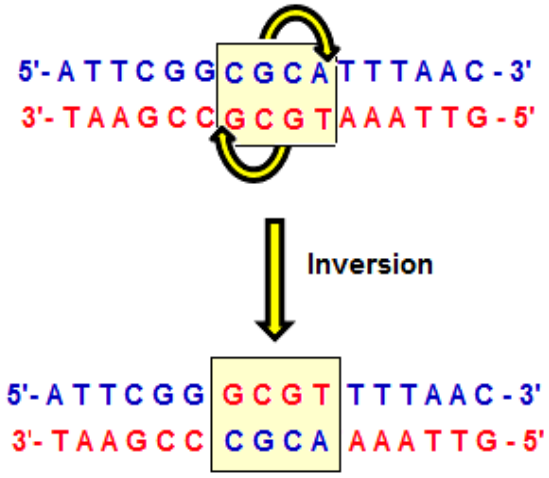


Fig. 6-7: Inversion mutation causes a string of bases which is deleted and followed by reinsertion of the same base sequence in opposite direction.

This new genetic character arises or results from an occurrence of mutation which causes change of base-pair sequence within the DNA of an organism and results in the appearance of a new character or trait.

Temperature Sensitive Mutation

It destroys the function of DNA polymerase at high temperature over 42°C. It causes misfolding of the protein product of DNA polymerase gene, when the organism is exposed to high temperature over 42°C. Therefore this is a lethal ***conditional mutation***.

For example: In a high temperature environment, where molecules are moving more quickly and hitting each other, this results in the protein

losing its structure and failing to function, but in a low temperature environment, the protein's structure is stable and functions normally. On this basic information, ***thermotherapy*** has been used by scientists to destroy the cancerous cells. Thermotherapy may render the diseased cell to undergo apoptosis in direct response to local applied heat. The local applied temperatures should be under 44 °C to avoid damage to surrounding tissues.

Mutagenic Agents

In nature, mutations occur spontaneously at low frequency. This rate can be increased by many mutagens, mutagenic agents (fig 6-8).

Mutagenic agents which induce mutations on the molecular level can be classified into:

1. Chemical modifiers
2. Base analogs
3. Radiation
4. Frame shift mutagens
5. Mobile genetic elements
6. Viral infection

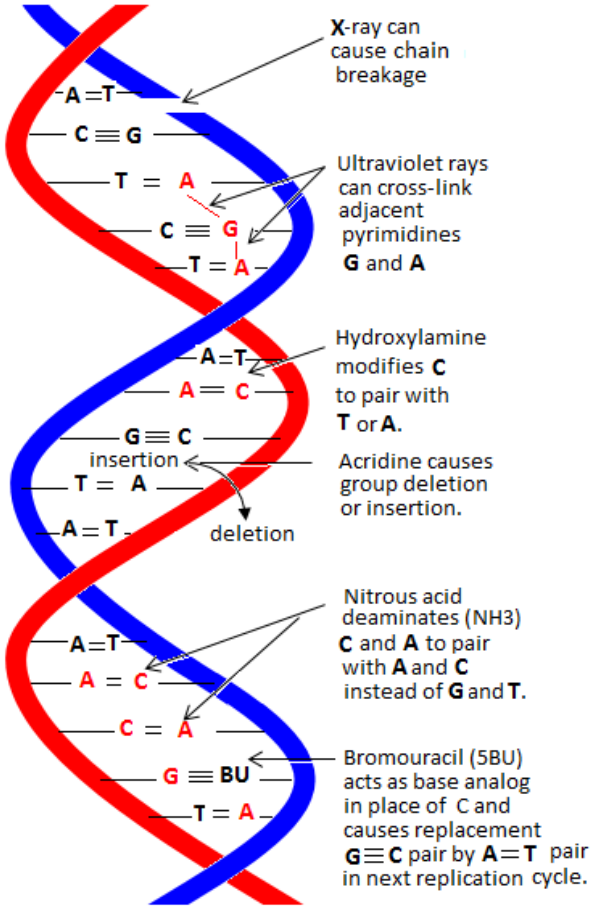


Fig. 6-8: Diagram illustrates some mutagenic agents that affect the base sequence of DNA molecule and cause mutations.

Chemical Modifiers

They are chemical reagents that can modify the normal bases. The modifications result in their mis-pair that increase the frequency of mutations. From the chemical modifiers, for example:

- ***Nitrous acid*** modifies amine groups of cytosine and adenine; altering their hydrogen bonding patterns which leads to incorrect base pairing during replication.
- ***Hydroxylamine*** has been used by biologists to introduce random mutations by switching base pairs from A to G, or from C to T. This is to study functional areas of genes, and to illustrate what happens if their functions are stopped. It can modify cytosine to pair with adenine.

Base Analogs

Natural or synthetic purines or pyrimidines can substitute for the normal 4 bases of DNA. For example, when a nucleotide containing ***5-bromouracil (5-BU)*** *molecule* is incorporated into the DNA, it is most likely to pair with adenine. If this happens during DNA replication, a guanine will be inserted opposite the base analog, and allows for guanine to pair with a cytosine in the next DNA replication. This results in a transition mutation (figs. 6-8 and 6-9).

Radiation

- ***Ultraviolet rays*** are non-ionizing radiation. However, UV light can induce mutation by creating bonds between adjacent thymine bases in a DNA strand.
- ***X -rays*** which consist of subatomic particles and electromagnetic waves, are ionizing radiations. So, they are able to ionize atoms or molecules when they are energetic enough to detach electrons from them. Exposure to ***X–rays or radiation*** causes damage to living tissue, resulting in skin burns and radiation sickness at high doses, and causes cancer, tumors and genetic damage at low doses.

Frame Shift Mutagens

Molecules about the size of nucleotides are able to slip in between the bases of DNA double helix during replication causing insertion or deletion of a group of bases. For example ***Ethidium bromide and acridine*** belong to DNA intercalators; a type of molecule that binds to DNA and inserts itself into the DNA structure.

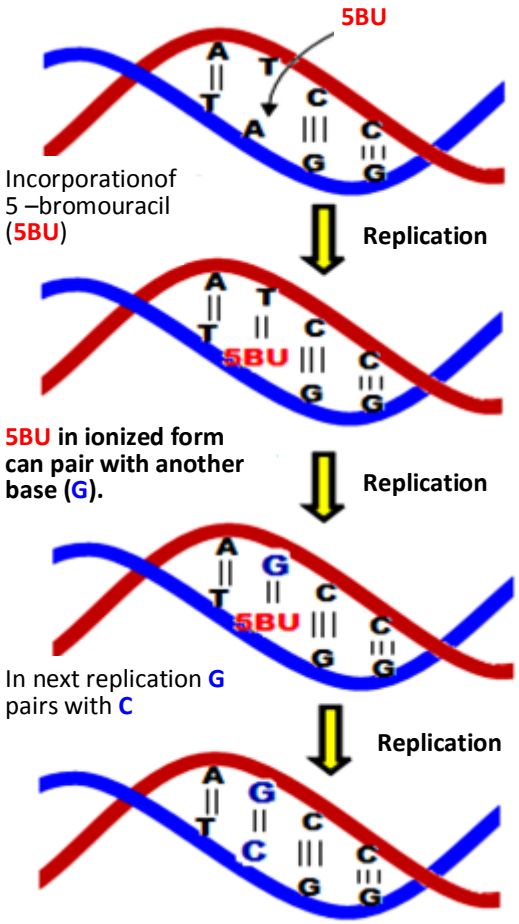


Fig. 6-9: Base analog 5-bromouracil is incorporated into DNA double helix at replication and results in a transition mutation.

Some intercalators are used as treatments for cancer. These ligands induce structural DNA distortions when they fit themselves in between the base pairs of DNA. These structural modifications can lead to functional changes, often to the inhibition of transcription, replication and repair processes of DNA double helix, which makes intercalators potent mutagens and carcinogenic agents.

Mobile Genetic Elements

DNA segments called transposons (fig. 6-10) are able to move from place to place on the chromosome (genome).

There are 2 types:

i) ***Replicative transposon****, which* leaves a copy of itself at the original location by being transcribed to RNA and then back to DNA by reverse transcriptase.

ii) ***Non-replicative transposon*** *which* does not leave a copy. It moves directly from one position to another within the genome using a transposonase. *Insertion of a transposon into protein coding regions, exons, of a functional gene leads to destruction of the gene function.*

Transposons are generally responsible for the genetic variation in bacterial populations and spreading of antibiotic resistance genes.

Viral Infection

Viruses are (*virus* meaning *toxin* or *poison*) infectious agents which are unable to grow or reproduce outside a host cell. Viruses infect all forms of cellular life.

Viruses have genetic material formed of either DNA or RNA, long molecules that carry genes. These genes are protected by protein coat. Some have an envelope of fat that surrounds them when they are outside a cell. They are about 100 times smaller than bacteria.

Viral infection results in changing the genetic constitution of the infected cells.

Viral infection increases the tendency of transformation of the infected cells into cancerous cells. Viruses are an established cause of cancer in humans and other species. The main viruses associated with human cancers are ***human papilloma virus, hepatitis B virus, Epstein-Barr virus, and human T-lymphotropic virus.***

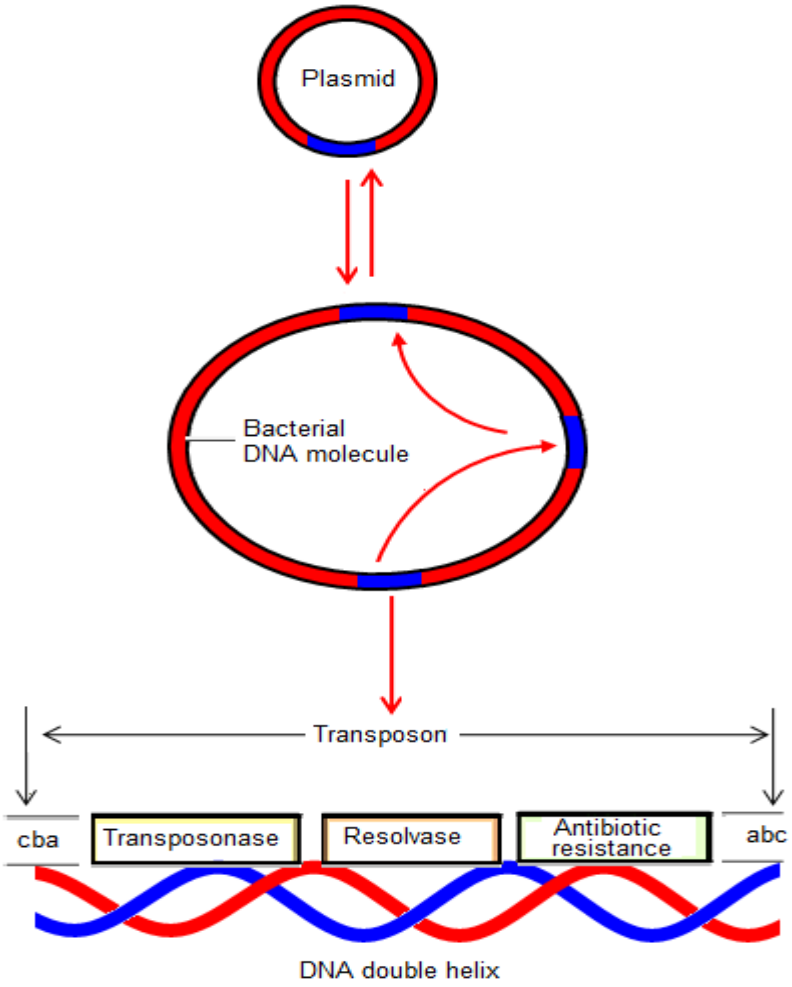


Fig. 6- 10: Diagram shows structure of transposon molecule.
Abc: *Left and right inverted repeat. It is formed of about 50 bases. It is recognized by transposonase as it initiates transposition.* ***Transposonase*** *and* ***Resolvase*** *code for enzymes involved in transposition process.*

Additional reading

Aldaz H, Schuster E, and Baker TA (1996): The interwoven architecture of the Mu transposase couples DNA synthesis to catalysis. Cell, 85, 257-269.

Craig NL (1997): Target site selection in transposition. Ann. Rev. Biochem. 66, 437-474.

Friedberg E C, Walker GC, and Siede W (1995): DNA repair and mutagenesis, American Society for Microbiology, Washington DC.

Grindley ND et al. (1982): Transposon-mediated site-specific recombination: Identification of three binding sites for resolvase at the res sites of 78 and Tn3. Cell, 30, 19-27.

Kleckner N (1990): Regulation of trans-position in bacteria. Ann. Rev. Cell Biol, 6,297-327.

Lewin B (2002): Genes VII. Part: 4. Transposon. Oxford University Press Inc. 457-479

Mizuuchi K (1992): Transpositional recombination: mechanistic insights from studies of Mu and other elements. Ann. Rev. Biochem. 61, 1011-1051.

Reznikoff WS (1993): The Tn5 transposon. Ann. Rev. Microbiol, 47, 945-963.

Pilon L, Langelier Y and Royal A (1986): "Herpes simplex virus type 2 mutagenesis: Characterization of mutants induced at the hprt locus of non-permissive XC cells". Mol. Cell. Biol. 6 (8): 2977–83.

Scott JR and Churchward GG (1995): Conjugative transposition. Ann. Rev. Microbiol. 49, 367-397.

Savilahti H and Mizuuchi K (1996): Mu transpositional recombination: Donor DNA cleavage and strand transfer by the Mu transpose. Cell 85, 271-280.

Moffat AS (2000): Transposons help sculpt a dynamic genome. Science, 289: 1455-1457.

Nachman M W and Crowell S L (2000) Estimate of the mutation rate per nucleotide in humans. Genetics, 156(1): 297-304.

Newcomb R D et al. (1997) A single amino acid substitution converts a carboxylesterase to an organo-phosphorus hydrolase and confers insecticide resistance on a blowfly. Proceedings of the National Academy of Science USA 94: 7464-7468.

Oliver A et al. (2000): High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science, 288: 1251-1253.

Schiffman M et al. (2007): "Human papillomavirus and cervical cancer". Lancet 370 (9590): 890–907.

Walker GC (1984): Mutagenesis and inducible responses to DNA damage in E. coli. Microbiol Rev. 48, 60 - 93.

Activity:

Part I: Multiple Choice Questions.

Choose a single correct answer.

1. Mutation that reverts back to its original sequence is known as:
   a. Stable
   b. Unstable
   c. Inversion
   d. substitution

2. Mutation which results from replacement of a single base-pair by another is called:
   a. Stable mutation
   b. Point mutation
   c. Inversion mutation
   d. Insertion mutation

3. Mutation that involves replacement of single base-pair of purine by another of pyrimidine is called:
   a. Transition substitution
   b. Transverse substitution
   c. Inversion mutation
   d. Deletion mutation

4. What type of mutagenic agent can act as base analog instead of cytosine?
   a. 5-Bromouracil
   b. Nitrous acid
   c. X -rays
   d. Hydroxylamine

5. What type of mutation occurs if a string of bases is removed and replaced by the same sequence of bases but in the opposite direction?
   a. Deletion
   b. Substitution
   c. Inversion
   d. Deletion

6. What is the effect of temperature sensitive mutation on mutant bacteria? It causes:
   a. Replication of DNA
   b. Inactivation of DNA
   c. Improvement of DNA transcription
   d. Mis-folding of DNA polymerase

7. Mutation that results in reduction or absence of enzyme activity due to one amino acid is replaced by another one is known as:
   a. Non-sense mutation
   b. Mis-sense mutation
   c. Frame shift mutation
   d. Unstable mutation

8. What type of mutation that may result in sickle cell anemia?
   a. Non-sense
   b. Missense
   c. Frame shift
   d. Deletion

9. Which mutation of the following **doesn't** alter the protein function?
   a. Non-sense
   b. Missense
   c. Silent
   d. Frame shift

10. Which type of the following mutation results in misreading of all codons from the point of deletion or insertion to the end of the message?
    a. Non-sense
    b. Missense
    c. Frame shift
    d. Inversion

11. Which mutagenic agent of the following can change the reading frame of mRNA?
    a. 5-Bromouracil
    b. Nitrous acid
    c. Acridine
    d. Hydroxylamine

12. Which of the following is responsible for spreading of antibiotic resistance gene in bacterial populations?
    a. Plasmid only
    b. Virus only
    c. Transposon only
    d. Plasmid & transposon

13. What type of amino acid that replaces glutamic acid of β – chain Hemoglobin in sickle cell anemia?
    a. Leucine
    b. Methionine
    c. Valine
    d. Serine

14. If this base -sequence **CGAACGGT** has been rearranged in the linear sequence **CGATGGCA,** this change in base sequence is known as which type of the following mutations?
    a. Translocation
    b. Deletion
    c. Insertion
    d. Inversion

15. Which of the following causes chain breakage of DNA double helix?
    a. Bromouracil
    b. Nitrous acid
    c. Acridine
    d. X- rays

16. Bromouracil is one of which type of the following mutagens?
    a. Base analog
    b. Frame shift mutagenic agent
    c. Mutagenic mobile element
    d. Agent of radiation

17. Which process of the following results from insertion of transposon into protein coding region of a functional gene?
    a. Gene transcription
    b. Gene activation
    c. Gene amplification
    d. Gene destruction

Part II: Short Answer Questions.

Define each term of the following:

1. Mutation.

_____
_____

3. Point mutation.

_____
_____

4. Hypermutation.

_____
_____

5. Mutagenic agents.

_____
_____

Answer of MCQs:

1) B	6) D	11) C	16) A
2) B	7) B	12) D	17) D
3) B	8) B	13) C	
4) A	9) C	14) D	
5) C	10) C	15) D	

GENETIC ENGINEERING

Chapter 7:
GENETIC ENGINEERING

Objectives

After you have studied this chapter, you should be able to:

- *Define genetic engineering, recombinant DNA, restriction enzyme and palindromic sequence.*
- *Explain the properties and types of biological vectors.*
- *Explain:*
  How a specific gene is isolated?
  How a specific gene is cloned?
- *Outline the steps of construction of a genomic library.*
- *Discuss the applications of recombinant DNA technology.*
- *Explain the gel electrophoresis, blot hybridization and PCR applications.*
- *Describe the requirements for gene expression in bacteria.*
- *Outline the importance of DNA sequencing.*

Genetic engineering refers to the processes that are used to change or modify the genetic composition (the nucleotide sequences of DNA double helix) of an organism, for producing new genes with new characteristics or genetically modified organisms (GMO).

It is one of the practical applications of the recombinant DNA -technology.

Importance of Genetic Engineering

In biology:

Genetic engineering is used to study the ways of transport of information within cells and between generations, as well as to identify the mechanism of working of the living cells.

In agriculture and animal production:

Genetic engineering is used to improve the genetic characteristics of animals and plants in order to increase the production of milk, meat, eggs and grains.

In medicine:

Genetic engineering is used for producing new strains of bacteria that can be used for synthesizing and producing useful substances, such as: Insulin, growth hormone and vaccines.

Genetic engineering is also used in diagnosis of genetic diseases and gene therapy.

Recombinant DNA technology

Recombinant refers to a cell or organism in which genetic recombination has occurred. Recombinant DNA is combined DNA molecule containing fragments of different organisms such as human and bacteria.

Building of this recombinant DNA –molecule takes place by the addition of *relevant DNA fragment* of human or other organisms into an

existing bacterial DNA, such as *plasmid*, to code for a synthesis of specific required useful protein. Genetic engineering technology has started by developing the recombinant DNA – technology especially by discovery of the restriction endonucleases, which are used to construct a recombinant DNA.

The following components are the **requirements** for constructing and carrying a recombinant DNA molecule:

1. **DNA molecules**
2. **Restriction enzyme**
3. **Vector**
4. **DNA ligase**

DNA Molecule Extraction

DNA can be extracted from any living organism. In human sufficient DNA can be extracted from cells of *buccal mucosa, peripheral leucocytes or from liver cells.* In eukaryotic cells,
DNA is located within an envelope that surrounds the nucleus. Each cell is formed of the cytoplasm and the nucleus. To obtain the DNA, breaking down cell membrane and nuclear envelope, is required**.**

Steps of DNA extraction:

For extraction of DNA for example from onion or liver cells, the following steps should be used (fig. 7-1):

1st step: A cell mixture preparation:

- A cell-mixture can be prepared from small amount of the cell source (such as onion cells). Put in blender:
  - Coarsely chopped onion (source of DNA).
  - 0.9% Na Cl (less than 1ml).
  - 1 cup cold water.
- After blending for 5-10 seconds you have a mixture of cells. Pour the mixture through strainer into another clear suitable container or test tube.
- Blender will break and open the tough plant cell wall.

2nd step: Cell lysis:

- To dissolve the cell and nuclear membranes, add to the cell mixture an equal amount of detergent –based cell-lysis solution containing enzyme and gently mix.
- The enzyme will cut the proteins away from the DNA double helix.
- After 5 -10 minutes in water bath at 60 °C, cell contents flow out forming *cell -liquid.*
- By filter receive the clear cell-liquid in a test tube.

3rd Step: DNA separation:

- DNA can be separated from lysed cell-mixture by addition of an equal amount of ice-cold 70-95% isopropyl or ethyl alcohol.
- After 30-60 minutes DNA will rise into the alcohol layer from the cell liquid layer forming a white cloud of fine stringy fibers (DNA is insoluble in alcohol). By using a wooden stick or other hook DNA fibers can be separated.

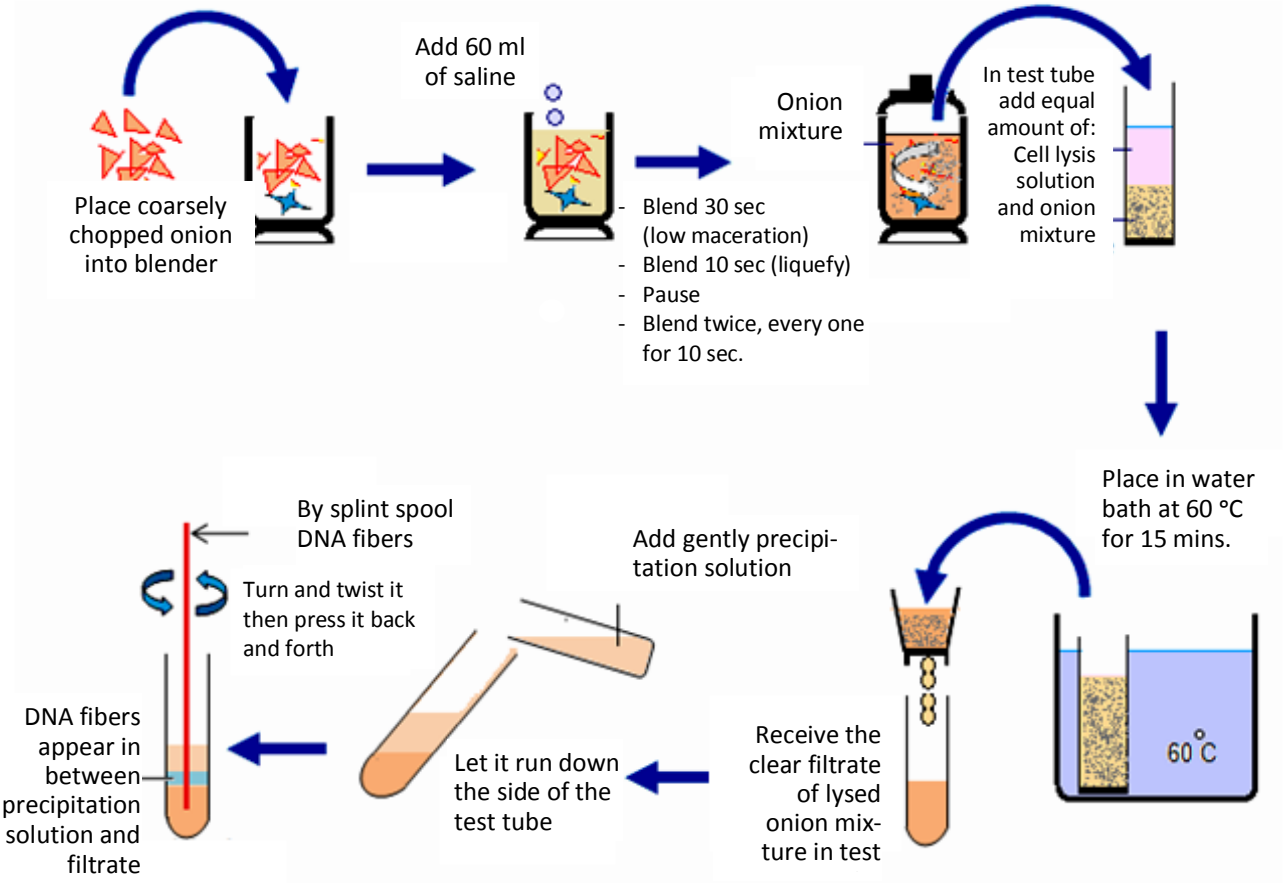


Figure 7-1: A diagram illustrates how DNA molecule is extracted from onion cells by using saline, blender, detergent – based cell lysis solution, hot water bath, and alcohol-based DNA precipitation solution. Spooling the DNA (separation of DNA fibers) occurs by winding of DNA fibers onto a splint.

Notes:

- *Using the ice-cold water protects the DNA by slowing down enzymes that can break it apart. Normally these enzymes destroy the DNA of viruses that may invade our bodies and make us sick.*
- *The ice-cold alcohol helps the DNA precipitation and separation, more quickly.*
- *The cell liquid should be opaque, meaning that you can't see through it.*
- *Salty water helps the DNA precipitation and separation, when alcohol is added.*
- *The DNAase enzymes are denatured at a temperature of 60 °C, while DNA is denatured at a temperature from 60 to 80 °C.*
- *NaCl is also used to neutralize the normal negative charge of DNA.*

Restriction Enzymes

- They are called molecular scissors or nuclear endonucleases. They are bacterial enzymes such as **Hind III** which is isolated from Hemophilus influenzae, and **Eco RI** and **Eco RII** which are isolated from *Escherichia coli*, E. coli.
- They are able to cut DNA strands by catalyzing the hydrolysis of the phosphodiester bonds.
- They cut DNA molecule only at a specific base sequence that is called ***palindromic sequence*** (figs. 7-2 and 7-3).
- Bacteria use the restriction endonucleases to protect themselves against viruses. By these enzymes they digest the viral DNA.

Palindromic Sequence

It is a base sequence that is read the same as its complementary strand but in the opposite direction. When DNA molecule is cut by restriction enzyme at palindromic sequence (figs. 7-2 & 7-3), two **sticky ends** are formed**.**

- **Sticky end** is an end of DNA in which one strand of the double helix extends a few units beyond the other. (Sticky means it have the properties of an adhesive.)
- Each one is complementary to the other and to any sticky ends of DNA -fragments that have been cut by the same restriction enzyme.

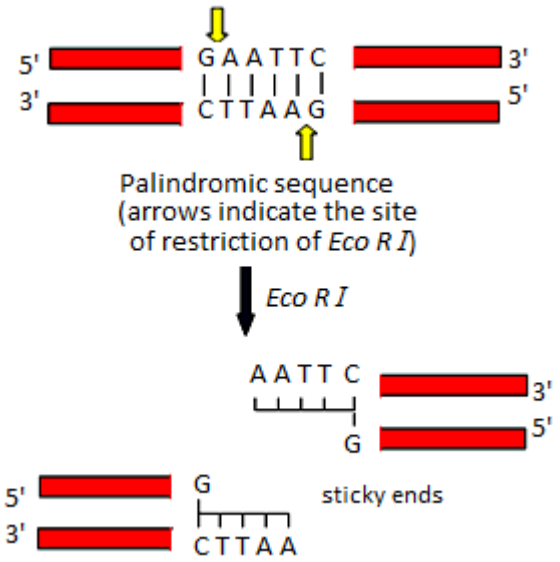


*Fig. 7-2: A diagram illustrates the palindromic sequence that is cut by restriction enzyme, **Eco RI**, producing two sticky ends.*

For example, **Eco RI** can recognize and cut the DNA –palindromic sequence **5'– GAATTC –3'** (fig. 7-2), while **Hind-III** restriction enzyme recognizes and cuts the palindromic sequence in figure 7-3.

Characters of the sticky ends:

- *They are single stranded unpaired ends of DNA fragments.*
- *They result from palindromic sequences that are cut by restriction enzymes.*
- *They are able to pair with other sticky ends of DNA -fragments that have been cut by the same restriction enzyme; complementary sticky ends.*

DNA Ligase

It is an enzyme that is necessary for building *stable* recombinant DNA –molecule. It can repair the phosphodiester linkages between the nucleotides of cut or broken DNA fragments. These DNA–fragments are products of DNA digestion by the same restriction enzyme and have complementary sticky ends (fig. 7-4).

- Base-pairing between nitrogenous bases of sticky ends of the complementary sequences enables two fragments to be joined or "spliced" by a DNA ligase.
- Sticky-ends of fragments can be ligated not only to the fragment from which it was originally cleaved, but also to any other complementary fragments.

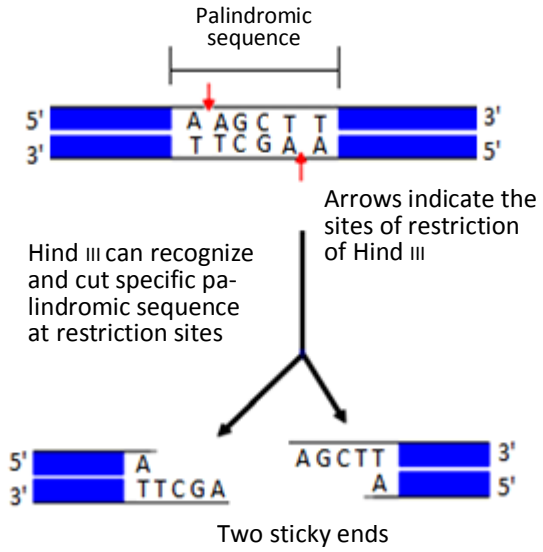


Fig. 7-3: Palindromic sequence is recognized and cut by restriction enzyme Hind III forming two single stranded sticky ends at the restriction site.

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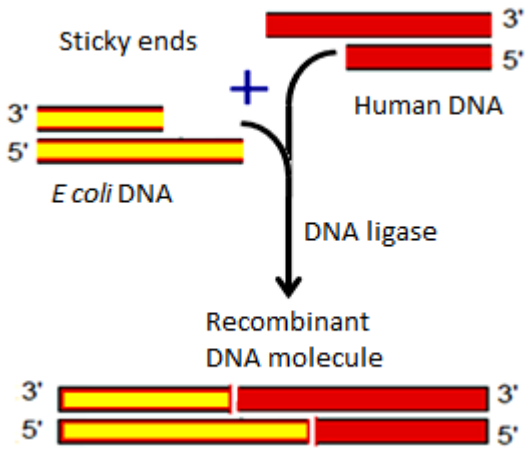


Fig. 7-4: DNA ligase links DNA fragments that have been cut by the same restriction enzyme – with complementary sticky ends.

Vectors

A biological vector is defined as a DNA molecule of plasmid or bacteriophage that acts as a *carrier and transmitter* for restricted DNA fragments. It is used to deliver DNA fragment to a cell. Types of vectors:

1. Biological vectors: **Plasmid** of bacteria and bacteriophage (**virus**).
2. Manufactured vector (**liposome**).

Plasmid:

- It is a small circular DNA molecule and capable of autonomous replication. It carries at least one origin of replication (fig. 7-5).
- It can be inserted into a bacterial cell by ***transformation***; the uptake of foreign DNA by bacterial cell.
- It is able to replicate inside bacterial cell and distribute to the daughter cells during the bacterial cell multiplication.
- By cloning of bacteria containing recombinant plasmid, the DNA -fragment that is carried by plasmid can be amplified.
- Bacterial plasmid carries a fragment smaller than 10 kb (1kb = 1000 bases).

Bacteriophage:

- *Virus* has also been used as a biological vector.
- It carries recombinant DNA into the mammalian cell where it incorporates into the cell genome, but it must be *unable to kill* the mammalian cell.
- It carries large DNA fragment about 15 Kb (kilo bases).

Liposomes

They are used as a manufactured carrier for DNA fragment.

In order to avoid using biological vectors recently synthesized liposomes have been developed as carriers for DNA fragment to introduce recombinant DNA into mammalian cells.

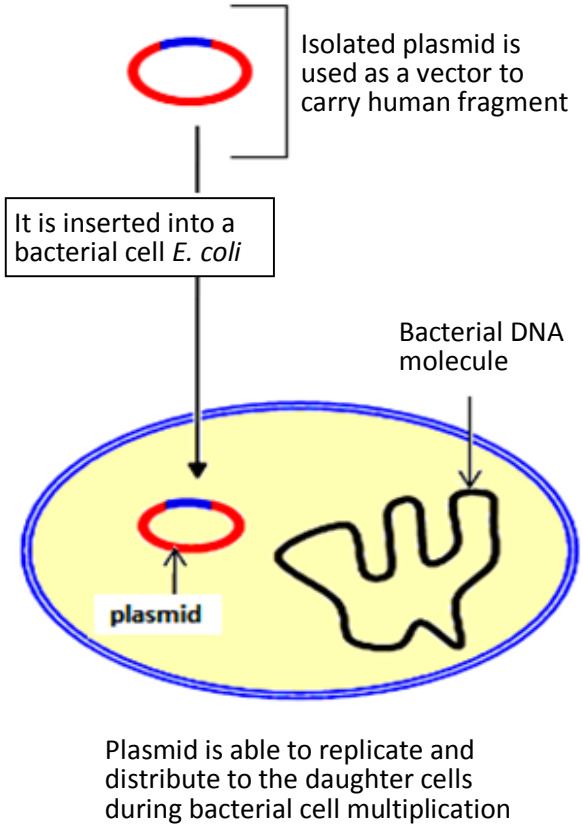


Fig. 7-5: Plasmid can be used as a biological vector. It can be inserted into a bacterial cell as recombinant DNA carrying a human DNA fragment.

Formation of Recombinant DNA

Splicing DNA-fragment into plasmid:

Steps *of the splicing process have been demonstrated in figure* 7-6*:*

- Bacterial plasmid and DNA from another organism, such as human, are cut by the same restriction enzyme.
- The products of restriction are linear molecules with complementary single stranded sticky ends.
- Addition of DNA -ligase to a mixture of DNA fragments of both plasmid and the other organism.
- DNA ligase links together the complementary sticky ends of DNA fragments to build a stable recombinant DNA molecule called recombinant plasmid.
- If DNA -fragment that is carried by plasmid represents a particular gene of interest, it *can be amplified* when bacterial cells multiply *forming colony* (fig. 7-7).

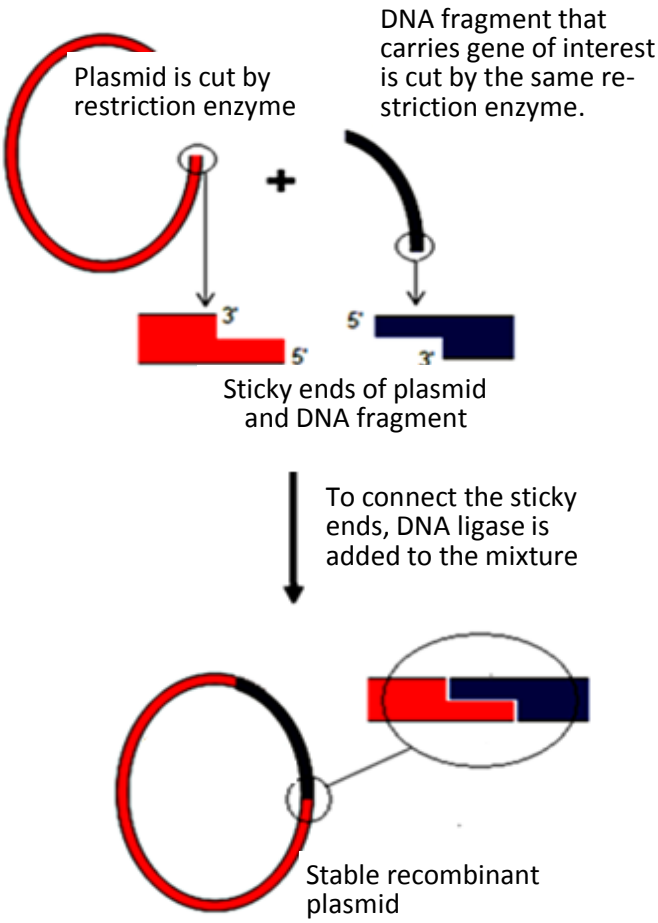


Fig. 7-6: Diagrammatic demonstration of how recombinant DNA plasmid is made.

Recombinant DNA technologies have been used to develop a genetically engineered recombinant plasmid from several DNA fragments. This genetically recombinant plasmid can be used in studying and analysis of cloned DNA in laboratories, such as the gene regulation sequence, gene transcription and translation.

Genomic Library and Gene Bank

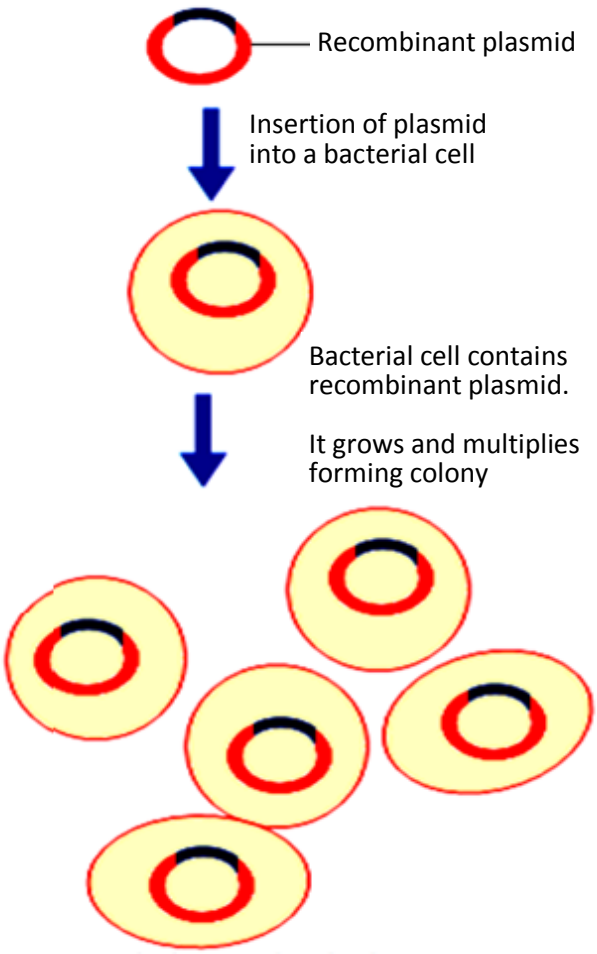
Genomics is the science of studying the DNA sequences and properties of entire genomes.

Genomic library is defined as a collection of DNA -fragments that represent more or less all the DNA in human genome.

Genetic materials of different plant and animal species are collected and preserved in what is called **the gene bank.** In plants, this could be by freezing cuts from the plant, or stocking the seeds. In animals, this is the freezing of sperm and eggs until further need.

Genome: The word *genome* dates to 1930. It was cobbled from the German *Gen*, gene and *–om* from the Greek *soma*, body. Genome refers to the total DNA per cell.

For example human genome is formed of all genetic information in the DNA of our chromosomes as well as that in our mitochondrial DNA. Therefore Human genome represents all of genes within our cell plus all of junk DNA; non-coding sequences of the DNA.



During bacterial multiplication, recombinant plasmid replicates and distributes into the daughter bacterial cells

Fig. 7-7: Steps of making multiple copies of recombinant DNA molecules; recombinant plasmids carrying human DNA fragments are inserted into bacteria E.coli that have been let to grow and multiply.

Construction of Human Genomic Library

Steps of construction of a human genomic library (fig.7-8):

E. coli is the bacterial type that can be used as a host organism for making multiple copies of DNA fragments of the human genome.

- Human DNA is cut by a restriction enzyme into fragments.
- The fragments are spliced into plasmids that are cut by the same restriction enzyme and carry antibiotic resistance gene.
- Recombinant plasmids are inserted into the antibiotic sensitive *E. coli.*
- Bacterial cells are incubated on antibiotic-containing medium.
  Only the bacterial cells which have received the plasmids survive, grow and multiply because the antibiotic resistance genes code for enzymes that destroy the antibiotics.
- Bacterial cells with recombinant plasmids multiply forming many colonies.
- These bacterial colonies are used to construct the *genomic library* of DNA -fragments.

How can you identify the gene of interest in a genomic library?

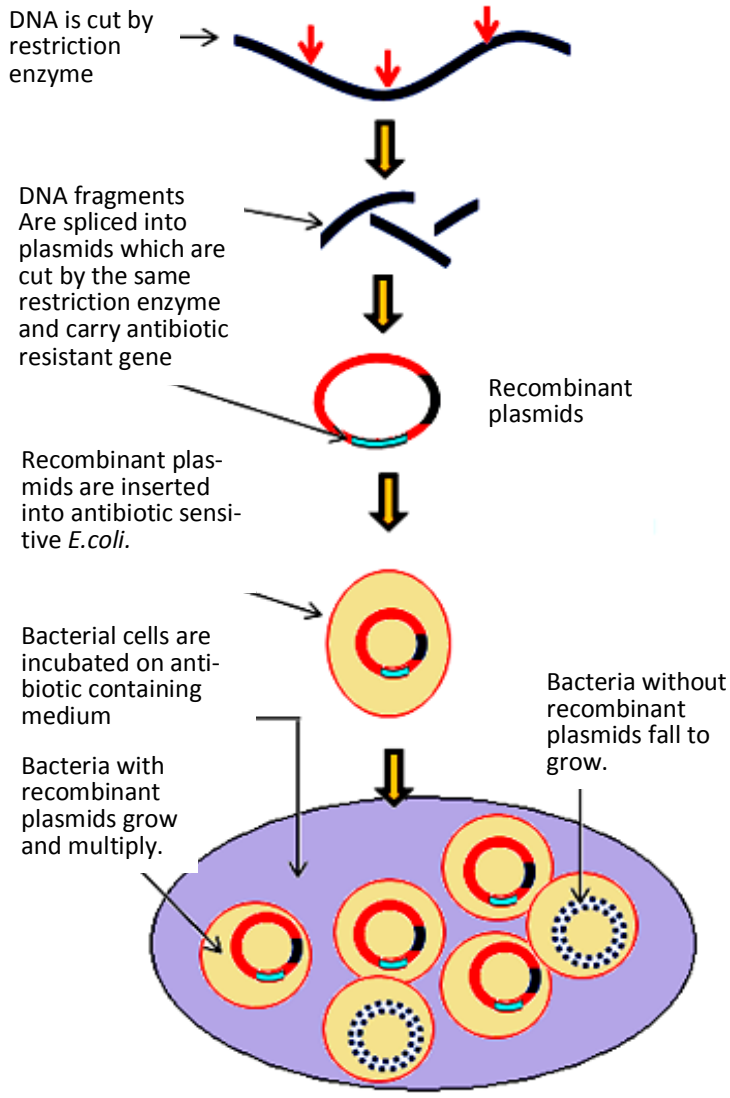


Fig.7-8: Steps of construction of a genomic library from multiple copies of recombinant DNA inserted into bacterial cells; E.coli.

Gene Isolation from Genomic Library

To identify a specific gene and isolate it from the human genomic library, hybridization with a radioactive genetic probe is used.

Genetic probe:
It is a radioactive labeled or marked **RNA** strand or single stranded **DNA** that can be used in lab to identify a certain gene by complementary pairing with it.

Hybridization:
In molecular biology, nucleic acid hybridization is the process of joining two complementary strands of DNA.
Both DNA and RNA are able to pair in solution with other DNA or RNA molecules that have complementary base-pairing.

- When a DNA–strand hybridizes with another DNA-strand, adenine pairs with thymine and guanine pairs with cytosine.

- The most common source of DNA complementary to an mRNA is the DNA coding strand that was the template for synthesis of the mRNA.

In process of hybridization; DNA-RNA hybrid formation, *guanine* of DNA pairs with *cytosine* of RNA, *adenine* of DNA pairs with *uracil* of the RNA, *cytosine* of DNA pairs with *guanine* of RNA, and *thymine* of DNA pairs with *adenine* of RNA molecule.

- *Double stranded DNA can be "**denatured**" by heating to a high temperature. If the resulting single stranded DNA is slowly cooled, the separated DNA strands can reanneal to reform the DNA double helix.*

Steps of identification of a certain gene are illustrated in figure 7-9.

- Cellular copies from *E.coli* colonies containing recombinant plasmids are transferred to nitrocellulose filter.
- Chemically, cellular samples on the filter are lysed and DNA denatured.
- Filter carrying denatured DNA is incubated with radioactive genetic probe.
- Genetic probe hybridizes with the gene of interest and the gene becomes radioactive.
- By using a special X- ray film, the target gene can be identified by auto-radiography.

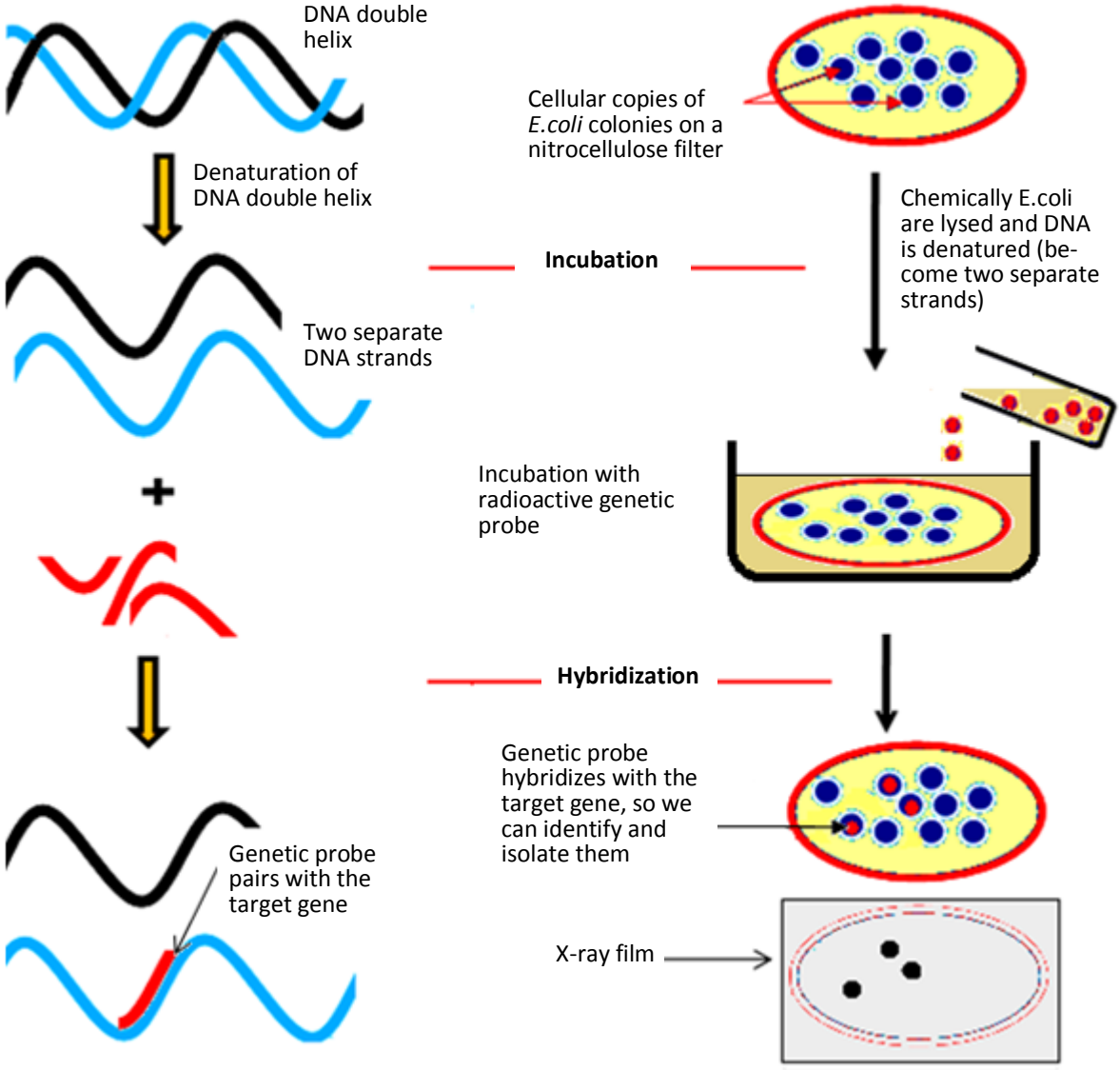


Fig. 7-9: Steps of identification of a specific gene in genomic library by radioactive genetic probe. The process begins by denaturation of DNA double helix and incubation of denatured DNA with a radioactive genetic probe which will pair with the target gene. By using autoradiography the hybridized target gene can be identified.

Complementary DNA Library (cDNA Library)

Complementary DNA (*cDNA*) is a DNA fragment that has been synthesized by performing reverse transcription of mature mRNA.

Reverse Transcription

It is a process of making a double stranded cDNA molecule from a single stranded RNA template.

Why cDNA-library is required? Because:

- Construction of cDNA-library is required to avoid the non-coding sequences (**introns**) on DNA.
- *If a gene as cDNA – free of introns is inserted downstream of an appropriate bacterial promoter, it can be transcribed and translated in bacterial cell.*

How can you make a cDNA?

To construct a library of cDNA copies of eukaryotic mature mRNA represent all cellular mRNAs. It is required to know firstly how a cDNA is synthesized.

It is called reverse transcription because it acts in the opposite or reverse direction to transcription. Figure 7-10 illustrates the process of making cDNA from mature mRNA by using *reverse transcriptase enzyme.*

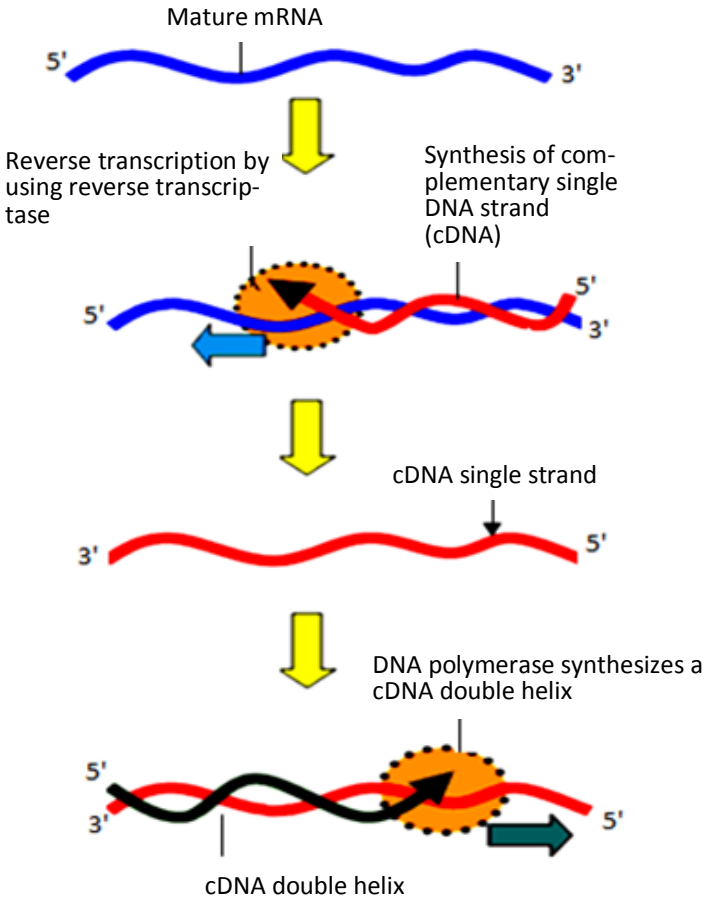


Fig. 7-10: Steps of formation of a double stranded cDNA from a mature mRNA by using reverse transcription technique. It starts by synthesis of a single stranded cDNA from a mature mRNA by reverse transcriptase. Next, single stranded cDNA copies itself by DNA polymerase forming double stranded cDNA.

Steps of cDNA –Library Construction

Construction of a cDNA -library begins by the synthesis of cDNA (fig. 7-10). After synthesis of cDNA, the following steps are used to construct cDNA library:

- Cutting of both cDNA and plasmid by the same restriction enzyme.
- Adding DNA-ligase to a mixture of cDNA–fragments and plasmids which carry antibiotic resistance gene. DNA-ligase form stable recombinant DNA by pairing the sticky ends.
- Recombinant DNA is inserted into *antibiotic sensitive E.coli*.
- *E. coli* cells are incubated on antibiotic -containing medium.
- Only the cells which have received the plasmid survive, grow and multiply. Multiplication of bacterial cell results in amplification of recombinant DNA.
- Amplification of cDNA fragments by bacterial multiplication is required for construction of cDNA –library.

What are the requirements for recombinant eukaryotic gene to be expressed in bacteria?

Expression of Recombinant Eukaryotic Gene in a Bacterial Cell

If a gene has been introduced into *E. coli*, *what are the requirements which are necessary for human gene expression in bacterial cell?*

Eukaryotic gene can't be expressed in a bacterial cell due to:

1. The regulatory regions cannot be recognized by bacterial RNA polymerase.
2. It contains introns.
3. The ways of gene activation are different in prokaryotic and eukaryotic cells.
4. Posttranslation modification of the synthesized Proteins is different in pro-karyotic and eukaryotic organisms (For example: Insulin needs posttranslation modification by proteolytic enzymes in eukaryotes).

Therefore, eukaryotic gene needs the following additional genetic engineering modifications to be expressed in prokaryotes:

1. Using regulatory regions that can be recognized by bacterial RNA polymerase.
2. Using cDNA copy of gene to avoid non-coding region and regulatory regions of eukaryotic gene.
3. Activation of gene transcription by using suitable environmental economical conditions.
4. For production of active posttranslation enzymes, if bacteria *lack* the specific enzymes which are required for post-translation modification of proteins, additional genetic engineering becomes required for synthesis of enzymes that perform posttranslation modification of protein within the bacterial cell.

Techniques Essential for Developing of Genetic Engineering

There are two very important laboratory techniques; **gel electrophoresis** and **polymerase chain reaction (PCR).** These laboratory techniques have been used for developing the science of genetic engineering.

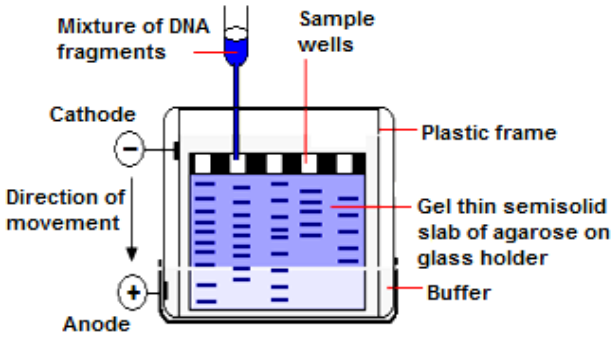


Fig. 7-11b: Diagram illustrates the gel electrophoresis apparatus. Samples are placed in wells at the top of the gel. Application of electric current results in migration of the samples through the gel from the negative toward the positive pole of the electric field. The samples move in parallel lanes in rates depending on the molecular weight.

Gel Electrophoresis

Gel electrophoresis is the most widely used technique to separate macro-molecules such as polypeptides, DNA and RNA fragments. It is used to separate DNA fragments, after digestion by restriction enzymes.

This technique is based on:

- The nucleic acids and polypeptides are negatively charged. In electric field, these molecules migrate through the gel from negative toward the positive pole of the electric field.
- The **gel retards** the movement of large molecules more than small molecules.
- So, the rate of movement through the gel is **inversely proportional** to the length of molecules (molecular weight)**.**
- The rate of migration of charged molecules through the gel in an electrical field depends upon their **molecular weights** (fig. 7-11).

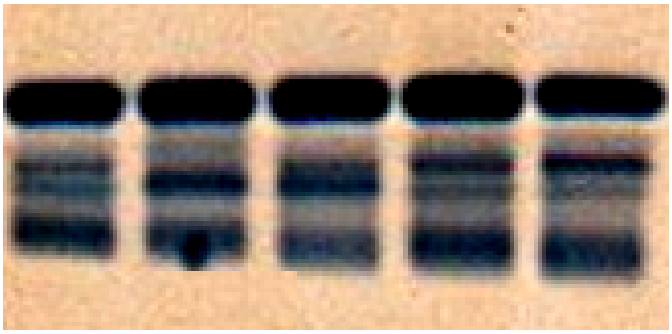


Fig. 7-11a: Separation of proteins by using gel electrophoresis technique. Proteins migrate toward the positive pole in electric field through gel. The samples move in parallel lanes.

Steps of DNA –Fragments Separation:

Figure 7-12 illustrates steps of DNA-fragments separation *by using gel electrophoresis.*

1. A mixture of DNA fragments of different sizes is loaded in wells at the negative pole of the gel (gel is a thin, semisolid slab of agarose on a glass holder).
2. The electric current is applied to the buffer in the glass holder.
3. The DNA–fragments separate from each other and move toward the positive pole.
4. The small DNA fragments move faster (move for a longer distance) than the large ones.
5. The DNA-fragments arrange in rows, according to their molecular weights.
6. DNA–fragments can be differentiated from other macromolecules by staining the gel with *ethidium bromide* (a specific dye that binds to DNA and is fluorescent under UV light).

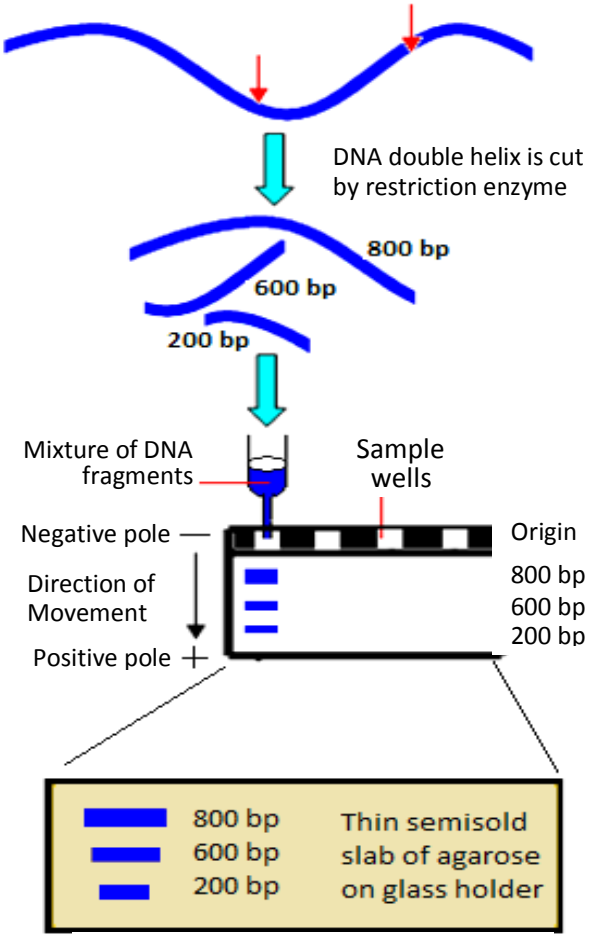


Fig. 7-12: Steps of separation of DNA fragments by using gel electrophoresis. The DNA-fragments separate from each other, move and arrange in rows according to their molecular weights.

The Polymerase Chain Reaction (PCR)

Importance: PCR technique is used for amplifying tiny sample of DNA template *in vitro.*

What are the reasons that make PCR is necessary for amplifying DNA fragment *in vitro* instead of amplifying DNA fragments by bacterial cloning*?*

Reasons of amplification of DNA fragment by using PCR:

1. Amplifying of DNA fragments by bacterial cloning consumes time and requires an adequate DNA sample.
2. PCR technique amplifies a tiny sample of DNA million times in a few hours.

For purposes of genetic testing or DNA finger-printing, it is necessary to examine only a small region of the genomic DNA.

The requirements are:

1) Heat-resistant polymerase (Taq-polymerase). Special DNA polymerase will not denature at high temperature required for PCR.

2) DNA primers-1 and-2synthesized single stranded DNA that are complementary to both ends of the DNA template or target gene to be copied. When a primer binds to DNA it attracts the DNA polymerase to begin copying the DNA.

3) Deoxynucleotide (Equal amounts of nucleotides A, T, C, G). These are used as building blocks for the new DNA copies.

4) Extracted genome DNA as a DNA –template.

Figure 7-10 illustrates ***steps of*** amplification of a tiny DNA fragment by using the PCR technique.

- In PCR, a number of double-stranded DNA molecules are duplicated each time. If the cycle of heating and cooling is repeated 100 times, the process yields 2^{100} =…… copies of the target sequences.

- By PCR -technique millions of DNA copies can be produced to be used for different purposes such as diagnosis of genetic diseases and DNA finger-prints.

- PCR technique is almost too sensitive, so a tiny amount of contaminated DNA in a sample will become amplified and lead to an incorrect conclusion.

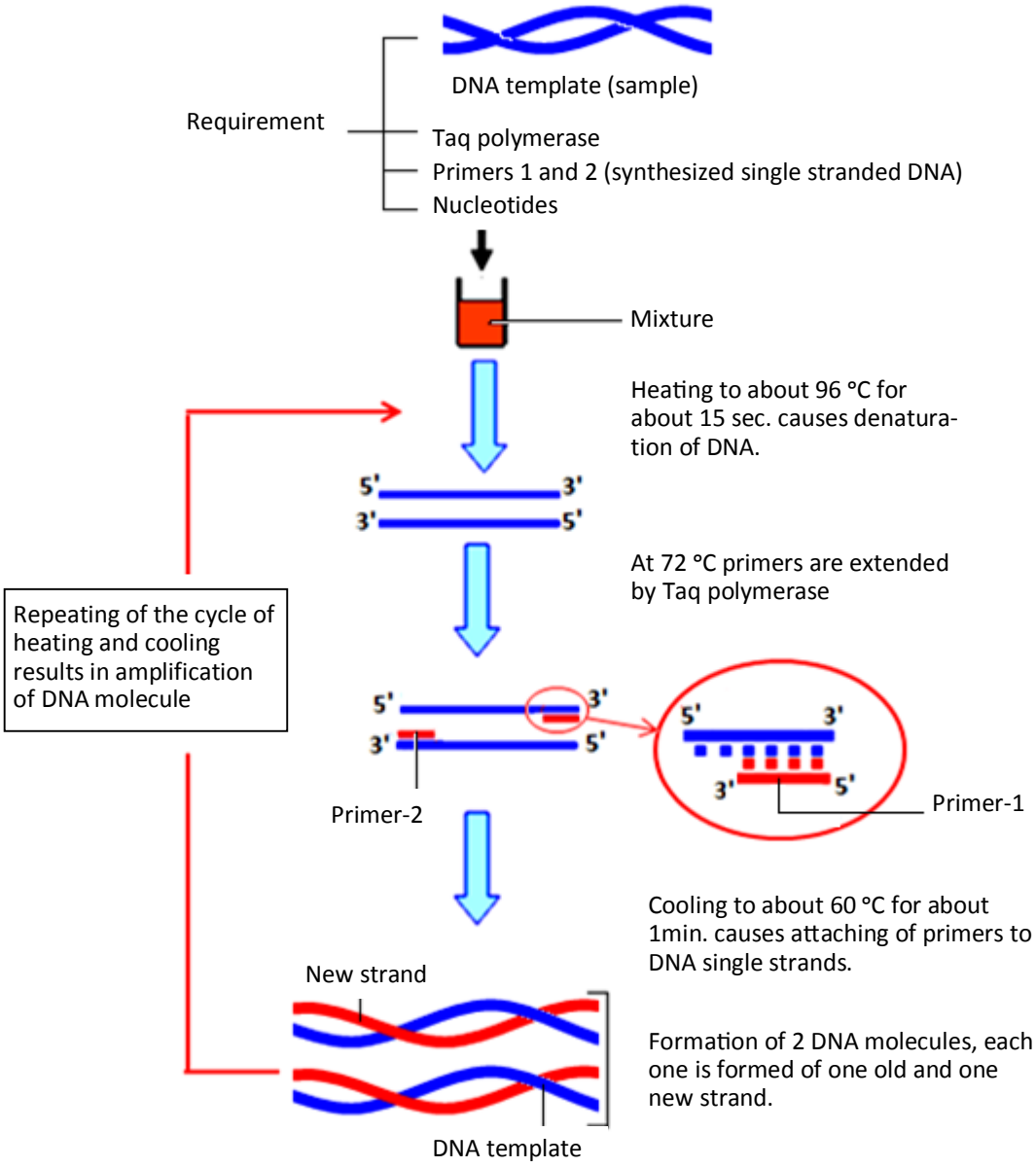


Fig. 7-10: Diagram illustrates the requirements and process of amplification of a DNA fragment in PCR. Every cycle that is formed of successive heating and cooling, causes duplication of DNA.

Medical Applications of Genetic Engineering Techniques

Genetic engineering is used in the following diagnostic techniques:

1) **Blot hybridization**
2) **DNA sequencing**
3) **DNA Fingerprints, RFLPs -Technique**
4) **DNA microarray**

Blot Hybridization

There are two types:
a) Southern blot technique
b) Northern and western blot techniques

Southern Blot Technique:
It is used to diagnose certain types of genetic disorders. In blot hybridization, the DNA fragments separated by gel electrophoresis are transferred to a nitrocellulose filter, then denatured and incubated with specific radioactive genetic probe to identify the gene of interest.

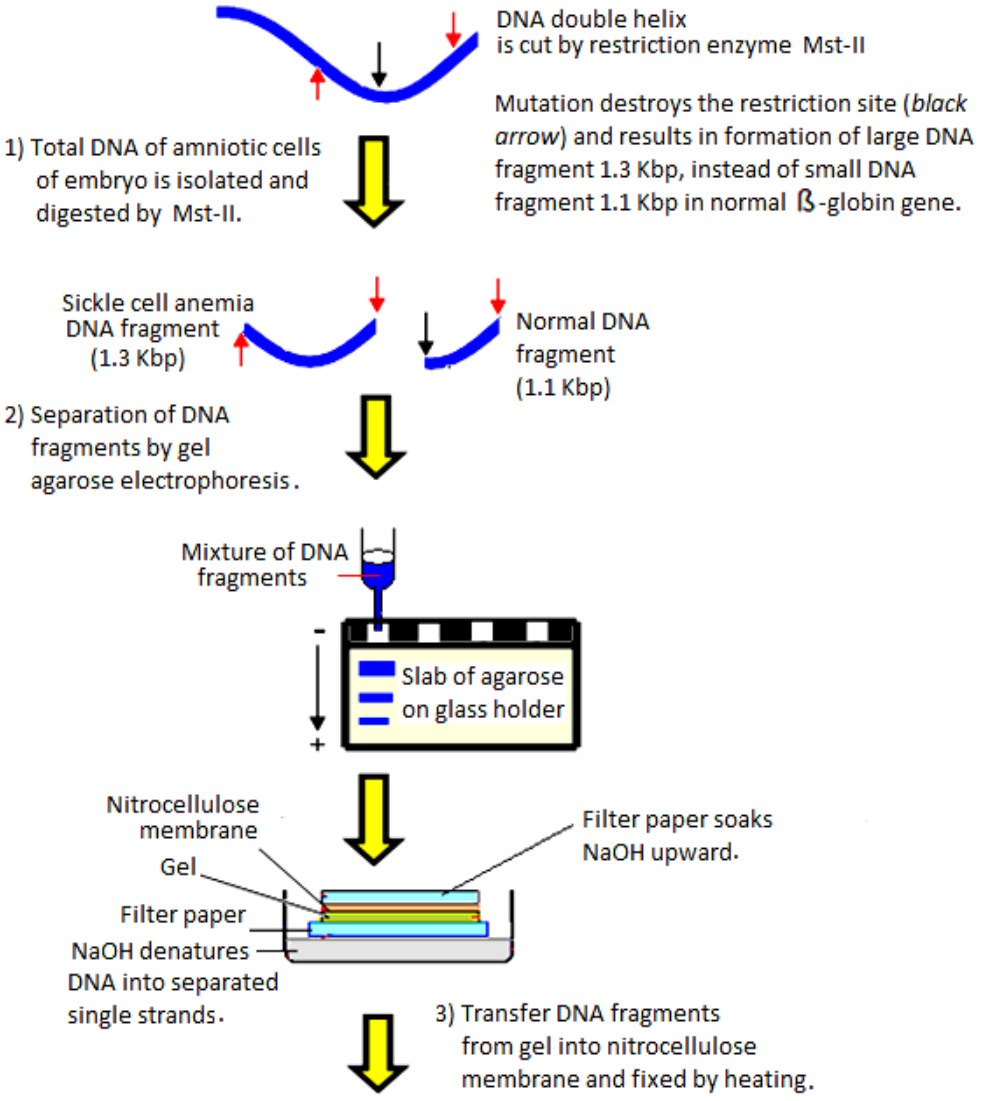
Figure 7-13 illustrates detection of gene of ***sickle cell anemia.*** It is about 1.15 Kbp (kilo base pair). Cutting the normal gene by *Mst-II* restriction enzyme produces small DNA –fragments, while cutting the mutant gene by *Mst-II* restriction enzyme produces large DNA segment of about 1.3 Kbp, because mutation destroys the restriction site of *Mst-II*.

How can you differentiate between a normal gene and that of sickle cell anemia?

- The fragments of a normal gene can be identified by using a radioactive genetic probe.
- The mutant gene of sickle cell anemia can be identified by the following:
  a) Failure of hybridization
  b) Its digestion by *Mst-II* produces a large fragment of β- globin.

DNA- Marker
It is a DNA sequence that is closely linked to a mutant gene and inherited along with it. For many genetic diseases such as Huntington's disease, this DNA sequence, DNA-marker, can be identified by blot hybridization methods. So, DNA marker is used for diagnosis and detection of these genetic diseases.

Northern and Western Blot Techniques:
These techniques are used to study *RNA and proteins* respectively which are separated by gel electrophoresis. In Western blot technique, the polypeptides chains of proteins can be recognized by *radioactive antibodies.*


DNA double helix
is cut by restriction enzyme Mst-II
Mutation destroys the restriction site (*black arrow*) and results in formation of large DNA fragment 1.3 Kbp, instead of small DNA fragment 1.1 Kbp in normal ß-globin gene.
1) Total DNA of amniotic cells of embryo is isolated and digested by Mst-II.
Sickle cell anemia DNA fragment (1.3 Kbp)
Normal DNA fragment (1.1 Kbp)
2) Separation of DNA fragments by gel agarose electrophoresis.
Mixture of DNA fragments
Slab of agarose on glass holder
Nitrocellulose membrane
Gel
Filter paper
NaOH denatures DNA into separated single strands.
Filter paper soaks NaOH upward.
3) Transfer DNA fragments from gel into nitrocellulose membrane and fixed by heating.

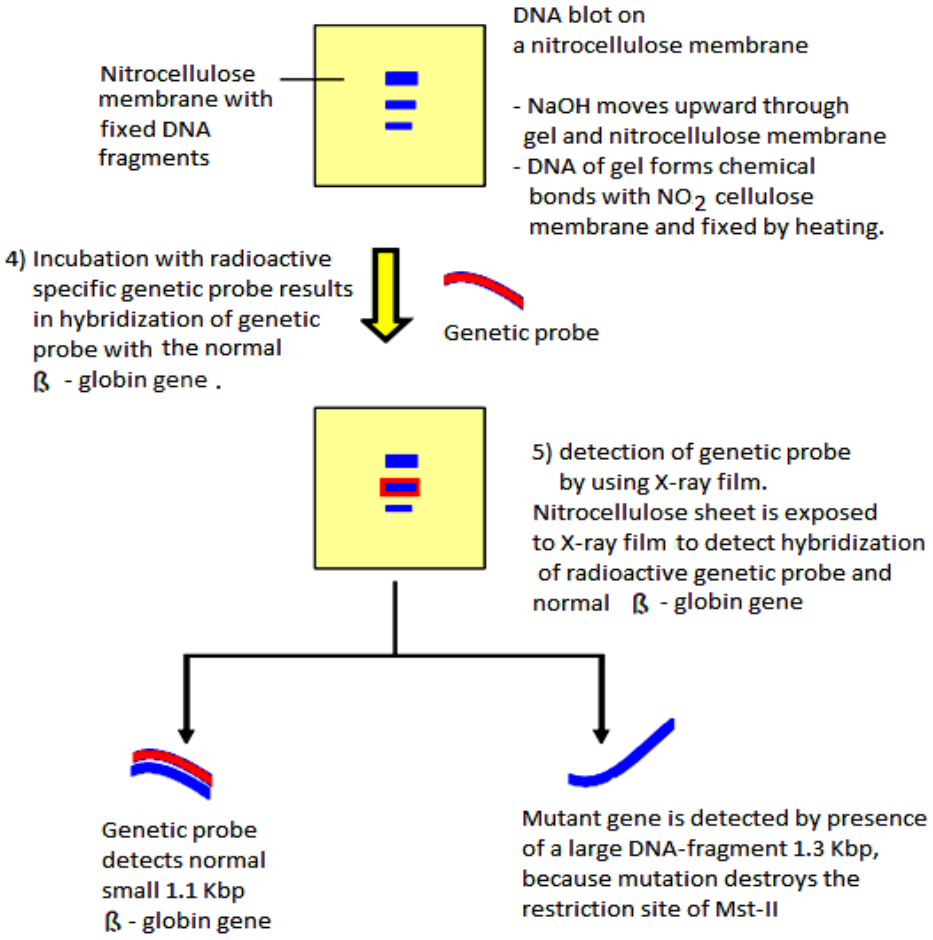


Fig. 7-13: Diagram illustrates the steps of differentiation between gene of the normal β-globin and mutant gene of the sickle cell anemia by using of restriction enzyme Mst-II and radioactive genetic probe: 1) DNA digestion. 2) DNA fragments separation. 3) Transfer of DNA –fragments onto a membrane. 4) Incubation with a specific DNA genetic probe. 5) Detection of genetic probe on X-ray film.

DNA–Sequencing

This technique is used to determine the base sequence of nucleotides forming DNA of a gene of interest.

The widely used DNA sequencing technique is *the chain termination technique* (*Sanger or di-deoxy sequencing*). It is illustrated in figure 7-14.

This DNA-sequencing technique is based on:

- Dideoxynucleotides; ddATP, ddCTP, ddGTP and ddTTP, block the elongation of the new DNA chain due to absence of a hydroxyl group at the 3' end.
- The hydroxyl group at the 3' end is necessary for attaching the next nucleotide during amplifying the DNA fragment in PCR.
- Gel electrophoresis and autoradiography make it possible to distinguish between fragments that differ in length by only one single nucleotide.
- DNA sequencing is read directly from an X-ray film that demonstrates the separated fragments by using gel electrophoresis for the products of each radioactive reaction.
- The base sequencing of DNA fragment in (fig. 7-14) begins with the shorter fragment that is formed in this case of one nucleotide (one base):
  a. The shortest fragment is G lane. So, the first base is G toward the 5' end.
  b. The next shortest fragment is also G lane. Then the next base is G.
  c. The third shortest fragment is in the A lane. Then the next base is A. and so on, until the last base at the 3' end of the DNA–fragment.

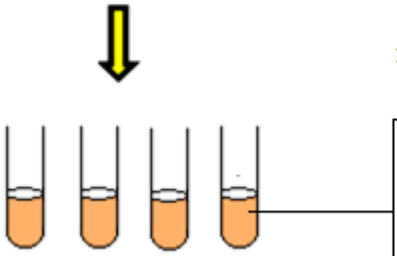
Importance of DNA –Sequencing

Sequencing of human chromosome is a part of human genome project (HGP), which is an international project for mapping and base sequencing of DNA in human genome that contains about 3 billion base-pairs. So, DNA sequencing is used to identify*:*

- The protein coding sequence on DNA
- The regulatory regions involved in gene expression.
- Signals involved in mRNA processing and modifications.
- Amino acid sequence of encoded protein.

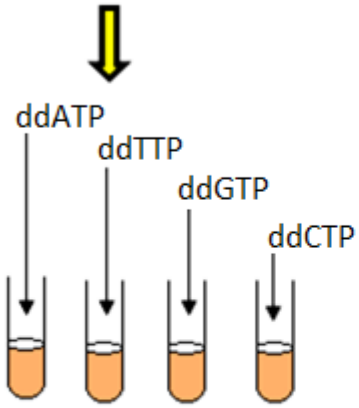
For example, if the base sequence of this single stranded DNA fragment is unknown, the following steps illustrate the process of sequencing:

3' A C G T A C T G A G T C 5'



1. DNA fragments are added into four test tubes containing a mixture formed of:

- Radioactive primers
- DNA polymerase
- Large amount of deoxynucleotides triphosphates (dATP, dTTP, dCTP, dGTP)



2. Add a small amount of dideoxynucleoside triphosphates into each test tube:

(ddNTP lacks 3' hydroxyl group, so it blocks the elongation of DNA strand)

3. Amplification of DNA fragment in PCR produces fragments of variable lengths . the shortest one is formed of one nucleotide.

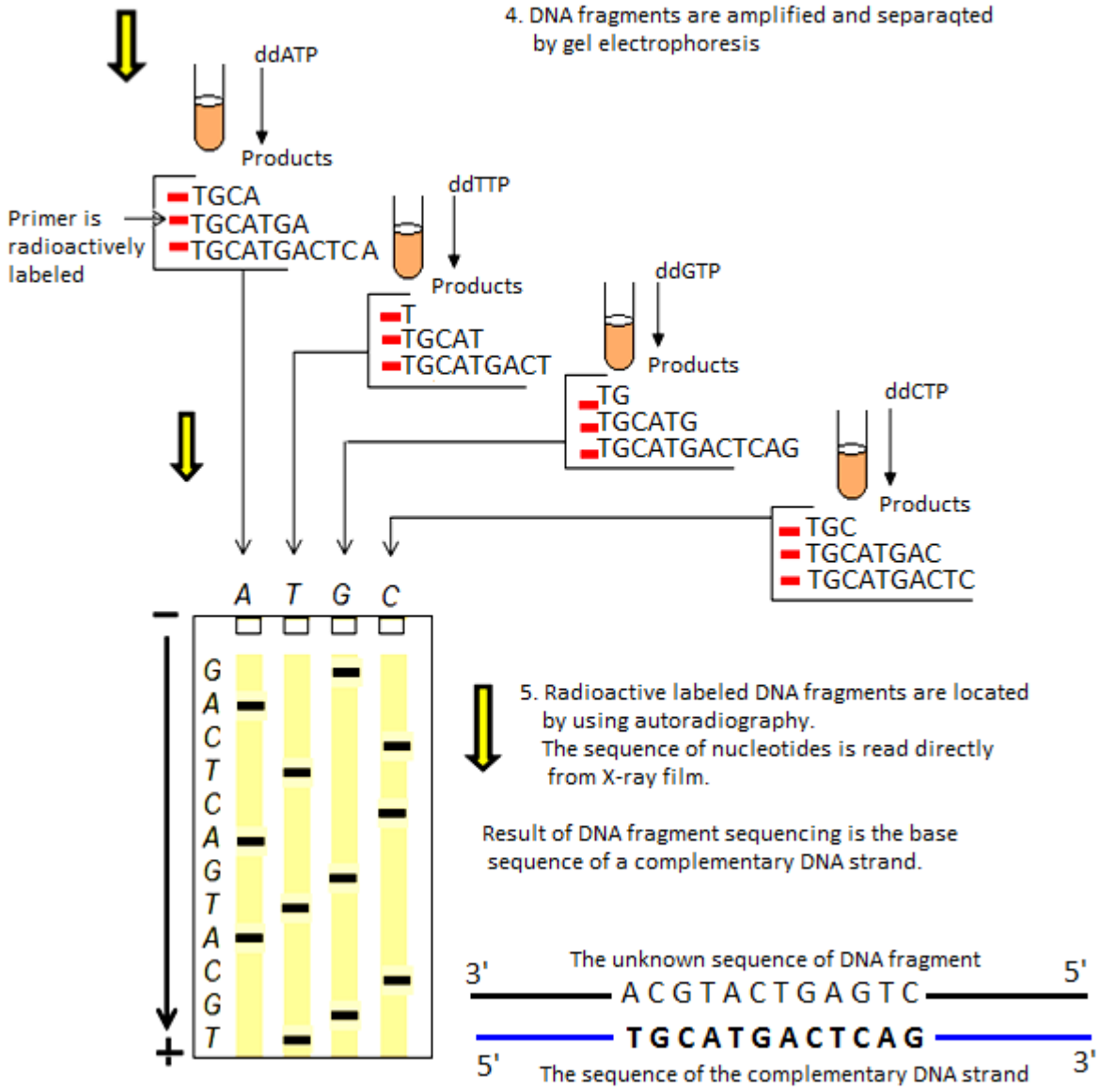


Fig.7-14: Steps of DNA sequencing: It is based on that dideoxynucleosides triphosphates (ddATP, ddCTP, ddGTP and ddTTP) block the elongation of the new DNA chain in PCR.

Automated DNA Sequencing

DNA sequencing using *Sanger chain termination technique* takes time, particularly when a DNA sequencing for a lot of samples are required such as human genome project and in diagnosis. Now, automated DNA sequencing is used, in which a laser beam is used to detect both florescent primers and the four different fluorochromes that are used for each ddNTP.

Human Genome

A working draft of the human genome sequence was completed in 2000. Over the next three years this draft sequence was converted into a "finished sequence." The finished sequence covered about 99% of the human genome's active gene-containing regions, and it had been sequenced to an accuracy of 99.99% by April 2003. It consists of about 3 billion base-pairs of DNA and about 22000 genes which have been identified until now.

- Only 3% of human DNA codes for proteins and the rest which is about 97% called **junk or non-coding DNA.**
- Some non-coding DNA form **regulatory sequences** that regulate gene expression and some others are **introns** that interrupt genes.
- Most of non-coding DNAs are repetitive sequences.
- Some of genetic diseases such as Huntington's disease are caused by abnormal stretches of **tandem repeats** (back-to-back repeats of variable lengths) within affected genes.
- Many of these tandem repeats are formed of satellite DNA that is located at telomeres (fig. 7-15).

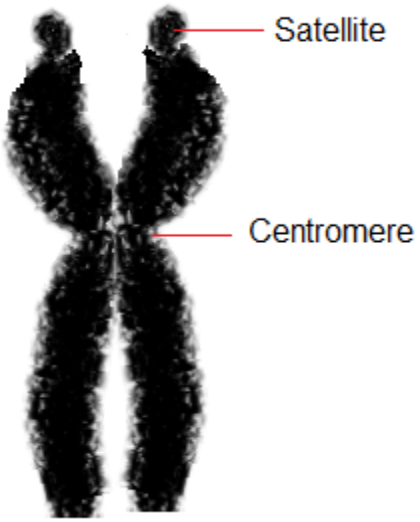


Fig. 7-15: A chromosome showing constricted area of centromere and satellite attaching the telomere.

DNA Fingerprint and RFLPs – Technique

It is a technique used by forensic scientists to assist in the identification of individuals on the basis of their respective DNA profiles. It involves restriction enzyme digestion, followed by southern blot analysis and can also be used as the person's identifier. This technique is called *restriction fragment length polymorphism* (***RFLPs or Riflips***); polymorphisms - the genetic variation within a population can exist in the restriction enzyme cleavage sites.

Medical applications of RFLPs technique:

- This technique can also be used as the person's identifier.
- It is used to measure the degree of close relation between individuals, parental testing.
- It is used as evidence in criminal law cases.

DNA family relationship analysis

Each person's DNA contains two copies of chromosomes—one copy inherited from the father and one from the mother. While a lot of DNA contains information for a certain function, there is some called junk DNA, which is currently used for human identification. At some special locations in the junk DNA, predictable inheritance patterns were found to be useful in determining biological relationships. These locations contain specific DNA markers that can be used to identify individuals.

The combination of inherited marker sizes found in each person makes up his/her unique genetic profile. When determining the relationship between two individuals, their genetic profiles are compared to see if they share the same inheritance patterns at a statistically conclusive rate.

Procedure:

- DNA from both the children and their parents are cut by the same restriction enzyme (fig. 7-16).
- The DNA fragment is separated by gel electrophoresis for each individual in a separate lane. The patterns of bands are referred to as *DNA fingerprints*.
- Then lanes of the children and their parents can be compared.

Results:

*Every band present in one of the children is also found in at least one of the parents***.**

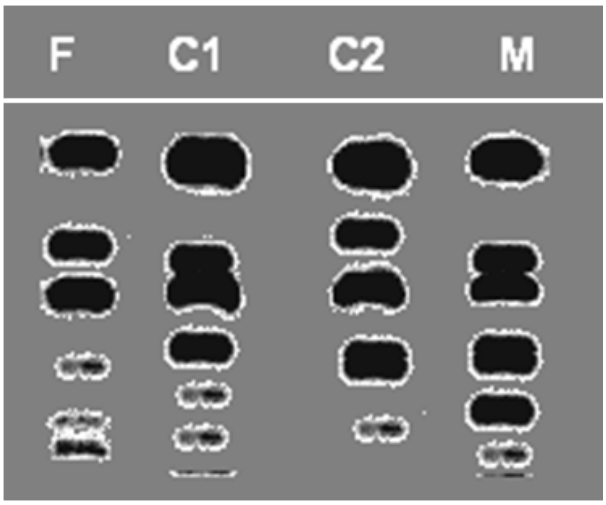


Fig. 7-16: DNA finger-prints for two children (C1 and C2) and their parents; father (F) and mother (M). Each band present in one of the children is also found in at least one of the parents.

DNA–Microarray

- DNA microarray is a multiplex technology used in molecular biology and in medicine.
- It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing picomoles of a specific DNA sequence.
- These thousands of spots are arrayed in ordered rows and columns on a solid surface which is usually glass (fig. 7-17).
- This piece of glass can be the size of microscopic slide. Each spot represents one gene; single strands of DNA fragments. The precise location and sequence of each spot is recorded in a computer database.
- In the traditional solid-phase array, a collection of orderly microscopic "spots", called **features**, each with a specific genetic probe attached to a solid surface, such as glass, plastic or silicon biochip (commonly known ***gene chip***, ***genome chip***, ***DNA chip*** **or** ***gene array***). Thousands of them can be placed in known locations on a single DNA microarray.

The human genome contains approximately 22,000 genes. When a DNA microarray contains all genes of human genome, it allows us to perform an experiment on thousands of genes in the same time.

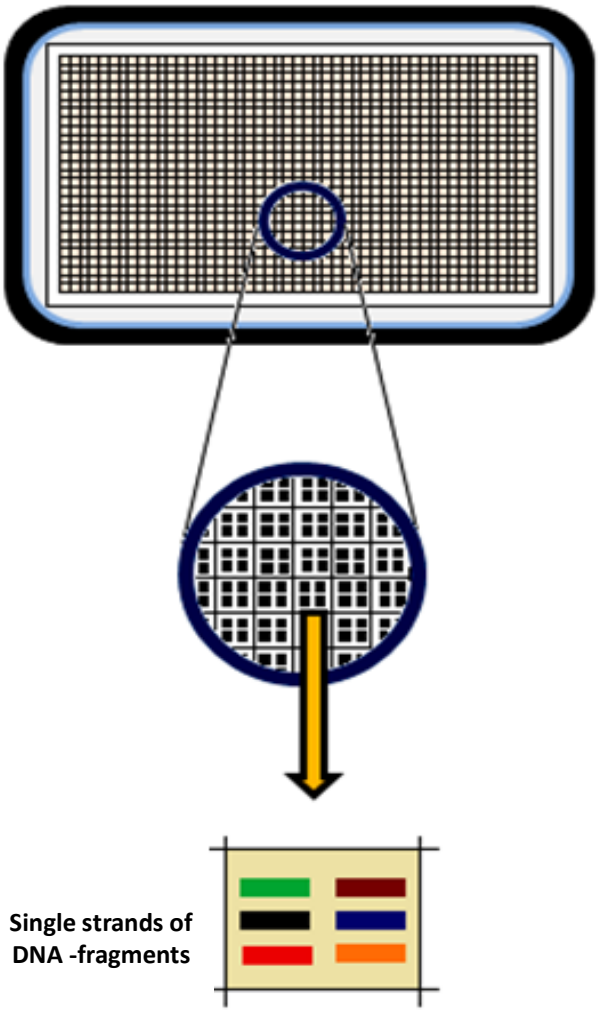


Fig. 7-17: Example of a spotted oligo microarray. The source of this picture is Wikimedia Commons.

Applications of DNA-Microarray Analysis in Medicine:

- It is used to determine whether specific genes which are responsible for certain disorder are ON or OFF.
- It is used to **diagnose** certain genetic diseases.
- It is used to detect the **susceptibility of a person**, who has the microarray, if he will suffer from a genetic disease in future.
- It allows us to perform an experiment on **thousands of genes at the same time**.

DNA Microarray is used to identify the active genes in the cell. How?

Only genes that are active produce messenger RNA (mRNA).

- *The first step is isolation of messenger RNA of active genes from the cell sample.*
- *Make cDNA copies of mRNA and label them to be identified by computerized scanner.*
- *Apply cDNA samples to microarray.*
- *cDNA will bind the spot that contains the specific active gene.*
- *The data of scanning the microarray is analyzed by computer to identify the active gene.*

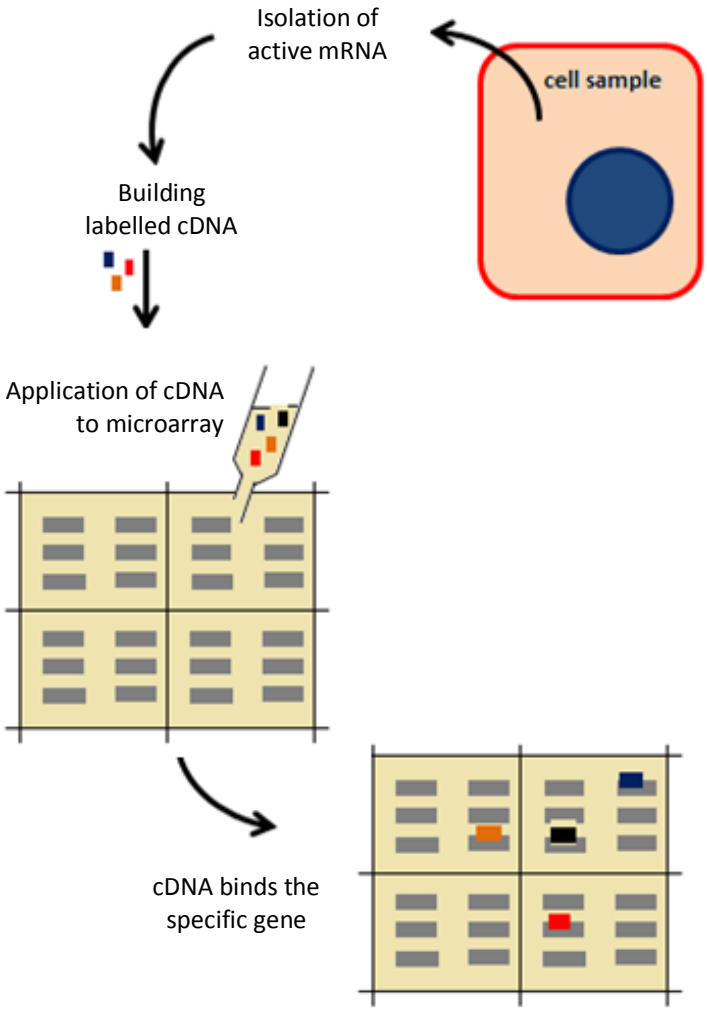


Fig.7-18: Steps of identification of the many active genes by using the microarray.

1. *Isolation of active mRNA from tissue sample.*
2. *Synthesis of labeled cDNA for the mRNA.*
3. *Application of cDNA to the microarray*
4. *cDNA will bind and recognize the specific gene.*

Additional reading

Aberger F et al. (2001): Analysis of gene expression using high-density and ifn-γ-specific low-density cDNA arrays.Genomics, 77(1-2):50-57.

Ahn J-I (2004): Comprehensive transcriptome analysis of differentiation of embryonic stem cells into midbrain and hindbrain neurons. Developmental Biology, 265:491-501.

Barrett J C and Kawasaki E S (2003): Microarrays: The use of oligonucleotides and cDNA for the analysis of gene expression. Drug Discovery Today, 8 (3):134-141.

Brown K (2001): Seeds of Concern. Scientific American, 284, (4), 52-57

Blattner F et al. (1997): The complete genome sequence of *E. coli* K12. Science, 277, 1453-1462.

Boyer HW (1971): DNA restriction and modification mechanisms in bacteria. Annu. Rev. Microbiol., 25, 153–176.

Dujon B et al. (1994): Complete DNA sequence of yeast chromosome XL Nature, 369, 371-378.

Eichner ER (2007): "Sickle cell trait". J Sport Rehabil 16 (3): 197–203.

Holstege FCP et al. (1999): Dissecting the regulatory circuitry of a eukaryotic genome. Cell, 95, 717-728.

Johnston M et al. (1994): Complete nucleotide sequence of Saccharomyces cerevisiae chromosome VIII. Science, 265, 2077-2082.

Langridge, W.H. (2000): Edible Vaccines. Scientific American, 283: 66-71.

Lewin B (1999): Genes VII. Part: 1 and Part: 5. Oxford University Press.

Marvie M (2001): Ecology of Transgenic Crops. Scientific American, 89: 160-167.

Miller, R.V. (1998): Bacterial gene swapping in nature, Scientific American, 278(1):46–51.

Mitchell BL (2007). "Sickle cell trait and sudden death--bringing it home". J Natl Med Assoc,99 (3): 300–5

Oliver SG et al. (1992): The complete DNA sequence of yeast chromosome III. Nature, 357, 38-46.

Solomon EP, Berg LR and Martin DW (2002): Biology. 6th ed. genetic engineering, United States: Thomson Learning Academic Resources Center, (301-339).

Somerville C and Dangel J (2000): Plant Biology in 2010. Science,. 290: 809-812

Strasser BJ (1999): "Sickle Cell Anemia, a Molecular Disease", Science, 286, 1488-1490.

Sturn A, Quackenbush J, and Trajanoski Z (2002): Genesis: Cluster analysis of microarray data. Bioinformatics, 18(1):207-208.

Weiner DB and RC Kennedy (1999): Genetic Vaccines. Scientific American, 281(1): 50-57.

Activity:

Part I: Multiple Choice Questions

Choose one best correct answer.

1. To which statement of the following does human genome refer?
   a. Human sex chromosomes
   b. Human homologous chromosomes
   c. Human autosomal chromosomes
   d. Total DNA per human cell

2. How many genes have been recognized in chromosome of *E. coli*?
   a. 1000
   b. 2000
   c. 3000
   d. 4000

3. How many genes have been identified in a human genome until now?
   a. 22.000 genes
   b. 30.000 genes
   c. 50,000 genes
   d. 100,000 genes

4. Which percentage of the following is the amount of non-coding DNA in a human genome?
   a. 25%
   b. 50%
   c. 75%
   d. 97%

5. Which character of the following is incorrect about tandem repeats?
   a. Back to back repeats
   b. Form satellite structure
   c. Located at telomeres
   d. Form active genes

6. Which term of the following means a collection of DNA fragments representing more or less the total DNA per human cell?
   a. Coding DNA
   b. Non-coding DNA
   c. Genomic library
   d. Recombinant DNA

7. Of which fragments of the following is the recombinant DNA formed?
   a. DNA and RNA fragments
   b. DNA-fragment of different organisms
   c. DNA fragment and genetic probe.
   d. Two Complementary DNA strands.

8. Which of the following is ***not*** one of the steps of construction of a human genomic library?
   a. Splicing of DNA fragments into plasmid
   b. Analysis of human karyotype
   c. Plasmid is inserted into bacteria
   d. Bacteria multiply in a media.

9. Which technique of the following is used for modifying of DNA base sequence?
   a. DNA Microarray
   b. Pedigree analysis
   c. Genetic engineering
   d. Gene therapy

10. Which of the following is ***not*** used in recombinant DNA–technology?
   a. Restriction enzyme
   b. DNA –ligase
   c. Biological vector
   d. Telomerase

11. Which enzyme of the following is used to cut Palindromic DNA–sequence?
    a. Endonuclease
    b. RNA polymerase
    c. DNA –ligase
    d. DNA -polymerase

12. What are the characters of ends of DNA fragments that have been cut by a restriction enzyme?
    a. Double stranded ends.
    b. Non-complementary ends.
    c. Unequal single stranded ends.
    d. Complementary sticky ends

13. Which technique of the following is used for studying of base pairs in DNA fragments?
    a. Sequencing
    b. PCR
    c. Gel electrophoresis.
    d. Microarray technique

14. With which molecule of the following can protein coding sequence of eukaryotic gene hybridize?
    a. Intron sequence
    b. Complementary mRNA
    c. Promoter
    d. Radioactive antibody

15. With which structure of the following is RNA able to hybridize in solution?
    a. Recombinant plasmid
    b. DNA double helix
    c. Complementary gene
    d. Genetic probe

16. Which molecule of the following is used as template for synthesizing complementary DNA?
    a. Pre-mRNA
    b. Regulatory DNA sequence
    c. DNA double helix
    d. Mature mRNA

17. Which enzyme of the following is used for synthesis of cDNA?
    a. Topoisomerase
    b. RNA polymerase
    c. Reverse transcriptase
    d. Endonuclease

18. Which of the following is ***not*** required for amplifying DNA fragment in PCR?
    a. RNA-polymerase
    b. Nucleotides
    c. RNA primers
    d. Taq polymerase

19. After repeating heating and cooling one DNA fragment for 20 times, the expected number of copies of DNA fragments in PCR will be:
    a. >Million
    b. <Million
    c. 900,000
    d. 700,000

20. In which of the following is gel electrophoresis ***not*** used?
    a. DNA sequencing
    b. DNA fingerprints
    c. Separation of DNA fragments
    d. Karyotyping technique

21. To which bacterial component of the following *does Eco –R1* refer?
    a. Nucleoid
    b. Protoplasm
    c. Bacterial DNA
    d. Endonuclease

22. Which process of the following is ***not*** required for eukaryotic gene to be expressed in *E.coli*?
    a. Removal of introns.
    b. Using regulatory regions can be recognized by bacterial RNA polymerase.
    c. Cut of DNA by restriction enzymes.
    d. Activation of bacterial operon.

23. All of the following are true about gel electrophoresis ***except*:**
    a. Used to separate DNA fragments
    b. The gel retards the migration of large fragment more than smaller one
    c. DNA fragments migrate to -v pole
    d. The gel is covered by buffer

24. Which of the following is (are) used as a vector in recombinant DNA technology?
    a. Plasmids
    b. mRNA
    c. rRNA
    d. cDNA

25. Exons are DNA sequences that are:
    a. Regions of translation
    b. Regions of termination
    c. Regulatory regions
    d. Origin of replication

26. Which of the following is the process of synthesis of RNA?
    a. Translation
    b. Modification
    c. Replication
    d. Transcription

27. Of which sequence of the following is complementary DNA formed?
    a. Introns
    b. Exons
    c. Anticodons
    d. Promoter

28. Which primer of the following is complementary to this DNA sequence:
    5' - ATCCACCGGTT -3' ?
    a. 5' TCGUA
    b. 3' UAGGU
    c. 5' TGGUC
    d. 5' UACGG

29. Which tools of the following is ***incorrect*** to be used for the corresponding function?
    a. Endonuclease - restriction of DNA
    b. DNA ligase-creating sticky ends.
    c. DNA polymerase - copying DNA
    d. Reverse transcriptase - production of cDNA

30. cDNA-library contains genes free of which sequence of the following?
    a. Exons
    b. Introns
    c. Both exons & introns
    d. Termination region

32. Which technique is used for isolation of DNA fragment in Southern blot technique?
    a. Hybridization with radioactive antibodies.
    b. Incubation with radioactive genetic probe.
    c. Application of histological stain.
    d. Using differential centrifugation.

32. In which place of the following are the genetic materials of different species collected and preserved?
    a. Bacterial colonies.
    b. Biological centers.
    c. Genomic library.
    d. Gene bank.

33. Which organisms of the following have been used for amplification of recombinant DNA?
    a. Parasites
    b. Viruses
    c. Bacteria
    d. Fungi

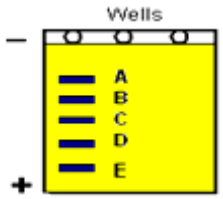
34. In which technique of the following are polypeptide chains hybridized with radioactive antibodies?
    a. Gene cloning
    b. Southern blot technique
    c. Western blot technique
    d. Recombinant DNA

35. Which of the following will be the palindromic sequence of 5'- CCGGCCGG - 3'?
    a. 3' GGCCGG AA 5'
    b. 3' GGCCGGCC 5'
    c. 3' GGCCGGGC 5'
    d. 3' GG AA GGAA 5'

36. In which of the following can gel electrophoresis be used?
    a. Amplify small pieces of DNA
    b. Cut DNA into small pieces
    c. Synthesis of recombinant DNA
    d. Separation of DNA fragments.

37. Which molecule of the following ***doesn't*** hybridize with complementary nucleic acids?
    a. DNA single strand
    b. Mature mRNA
    c. Genetic probe
    d. Antibodies

38. In the following diagram which DNA fragment is the largest in gel electrophoresis?
    a.
    b.
    c.
    d.
    e.



39. All the following molecules are used in DNA sequencing technique **except**:
    a. ddTDP
    b. ddATP
    c. ddCTP
    d. ddGTP

40. Which of the following is used during DNA extraction to dissolve the cell membranes and to separate the DNA from DNA conjugated protein?
    a. Liquid detergent.
    b. Enzyme Papain.
    c. Hot water bath.
    d. A, B and C together

41. Which technique of the following is used to measure the degree of close relation between individuals?
    a. Karyotyping technique
    b. Gene mapping
    c. Pedigree analysis
    d. RFLPs - technique

42. DNA-fragments on gel can be differentiated from other components of proteins by using:
    a. 5-Bromouracil.
    b. Acridine.
    c. Nitrous acid.
    d. Ethidium bromide.

43. Which of the following is used to recognize polypeptide chain in bolt hybridization?
    a. 5-bromouracile.
    b. Nitrous acid
    c. Ethidium bromide.
    d. Radioactive antibodies

44. Which technique can be used to determine the nucleotides forming DNA molecule?
    a. Southern blot hybridization
    b. DNA sequencing
    c. DNA fingerprints
    d. DNA microarray

45. Which technique of the followings can be used to separate RNA and proteins by hybridization?
    a. DNA sequencing
    b. DNA fingerprints
    c. DNA microarray
    d. Northern and Western blot

46. DNA sequencing is used to study all the following, ***except:***
    a. The protein coding sequence on DNA
    b. The regulatory regions involved in gene expression.
    c. Amino acid sequence of encoded protein.
    d. DNA conjugated proteins.

47. All the following are the characters of DNA-microarray of an individual ***except:***
    a. Possess a size of a microscopic slide.
    b. Formed of ordered array of spots containing multiple identical strands of DNA.
    c. Each spot represents one gene.
    d. Each spot contains multiple DNA fragments.

48. Which technique of the following is used for amplifying a tiny sample of DNA in lab?
    a. Hybridization
    b. PCR
    c. DNA sequencing
    d. DNA fingerprints

49. Which step of the followings causes denaturation of DNA strands in PCR technique?
    a. Addition of Taq polymerase.
    b. Addition of primers
    c. Cooling toabout60°C for 1 min.
    d. Heating to about 96°C for 15 sec.

50. Which of the following is the enzyme of DNA replication in PCR –technique?
    a. Taq DNA-polymerase.
    b. Primase
    c. RNA polymerase
    d. DNA –ligase

51. Which molecule of the following can be used as a genetic probe for gene identification?
    a. Long fatty acid chains
    b. Single DNA strand.
    c. Glycolipid molecules
    d. Polysaccharide chain.

52. Hybridization means a process of joining which two molecules of the following?
    a. Ribosome and mRNA
    b. Two sticky ends of DNA fragments
    c. Genetic probe and single strand of DNA
    d. Two Okazaki fragments

53. All the followings are required for making cDNA molecule, ***except:***
    a. Reverse transcriptase
    b. Mature mRNA
    c. RNA polymerase
    d. DNA polymerase

54. Which technique of the following is used to analyze the expression of large numbers of genes at the same time?
    a. DNA sequencing
    b. DNA fingerprints
    c. DNA microarray
    d. Hybridization

55. Which of the following is unique – distinctive for each individual?
    a. DNA sequencing
    b. DNA fingerprints
    c. DNA microarray
    d. Karyotype

Answer of MCQs:

1) D	10)D	19)B	28)B	37)D
2) C	11)A	20)D	29)B	38)A
3) A	12)D	21)D	30)B	39)A
4) D	13)A	22)A	31)D	40)D
5) D	14)B	23)C	32)D	41)D
6) C	15)C	24)A	33)C	42)D
7) B	16)D	25)A	34)B	
8) B	17)C	26)D	35)B	
9) C	18)D	27)B	36)D	

Part II: Short Answer Questions.

Define each of the following:

1. Genetic probe
   _____
   _____
2. Blot hybridization
   _____
   _____
3. Human genome
   _____
   _____
4. Gene bank
   _____
   _____
5. Genomic library
   _____
   _____
6. DNA –marker
   _____
   _____
7. Recombinant DNA
   _____
   _____
8. DNA microarray
   _____
   _____
9. Riflips technique
   _____
   _____

10. Palindromic sequence

_____
_____

What are the requirements for recombinant eukaryotic gene to be expressed in bacteria?

_____
_____
_____
_____
_____
_____
_____
_____
_____

HUMAN GENETICS

Chapter 8:
HUMAN GENETICS

Objectives:
After you have studied this chapter you should be able to:

- *Explain the methods of studying the human genetics.*
- *Discuss the goals of human genome project.*
- *Enumerate the causes of human genetic diseases, and discuss the role of chromosomal non-disjunction in genetic disorders.*
- *Explain the chromosomal abnormalities in number and structure.*
- *Identify the genetic causes of birth defects.*
- Describe the methods which are used in prenatal diagnosis of genetic disorders.
- *Explain* the genetic basis of *ABO blood groups, blood transfusion and Rh – factor.*
- *Illustrate the genetic basis of cancer.*

The main attention for human genetics is directed toward studying the inherited variations and disorders in human. So, the human genetics has been in the last few years greatly promoted by medical attention which has been given to diagnosis and treatment, gene therapy of genetic diseases in human.
The new information in this field has developed several scientific branches.

For example: *bioinformatics, pharmaco-genetics* and *DNA microarray.*
In DNA microarray, genetic information about human genome is placed on a chip. This genetic information allows comparing the activities of thousands of genes in normal and diseased cells. Diseased cells exhibit an abnormal pattern of gene expression.

Methods of Studying Genetic Disorders

To identify and study the mode of inheritance of any human trait, there are 3 main methods:

1. Pedigree analysis of human traits.
2. Studying of human chromosomes by karyotyping technique.
3. DNA sequencing and mapping of genes

Pedigree Analysis of Human Traits:
It is a process of genetic analysis of the history of families to detect the inheritance pattern of a certain trait. Figures 8-1A and 8-1B illustrate two cases of studying the pedigree analysis of human genetic diseases recorded in the children of 3rd generation to detect their inheritance pattern in two families, as *sickle cell anemia* in figure 8-1A, and *color blindness* in figure 8-1B.

A girl # 2 in figure 8-1A is diseased by sickle cell anemia, while her parents are phenotypically normal. Genetic analysis of the history of this family indicates that cause of this disease cannot be autosomal dominant allele or X-linked recessive allele because:

- None of her parents is sickled , but
- Both her father and her mother are carriers.

Therefore, the cause of this disease is defective autosomal recessive allele. So, two phenotypically normal parents could have diseased offspring when they are carriers of recessive defective allele on autosomal chromosomes.

Genetic analysis of the history of the phenotypically normal family which has a color blind boy # 1 (fig. 8-1B) indicates that the cause of this disease cannot be autosomal dominant or autosomal recessive alleles because:

- His parents are phenotypically normal, and
- Only his mother is a carrier, and his father is normal.

Therefore, the cause of this disease is a defective recessive allele carried by X –chromosome. So, this color blind boy inherited the recessive allele from his carrier mother.

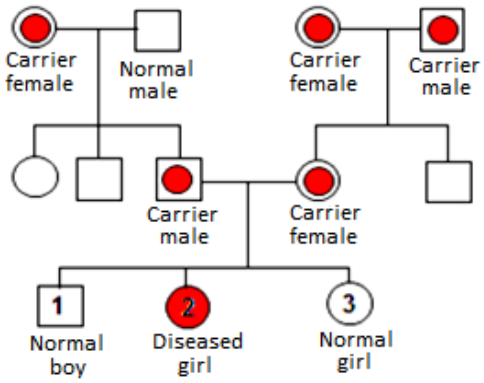


Fig. 8-1A: Pedigree of an autosomal recessive disorder. Two phenotypically normal parents could produce a diseased offspring, when they are carriers of autosomal recessive defective allele, such as sickle cell anemia.

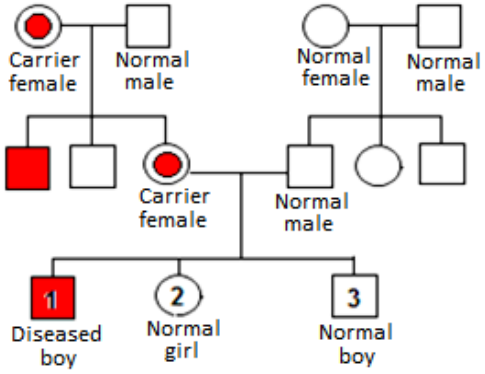


Fig. 8-1B: Pedigree of X-linked recessive disorder. A boy is diseased because he inherited the recessive alleles from his diseased or carrier mother, such as color blindness.

Studying of Human Chromosomes

Studying of chromosomes and their role in inheritance in order to do correlation between specific types of genetic disorders and certain corresponding alterations in human chromosomal structure is a very important step for diagnosis of some genetic diseases. These techniques are called *cytogenetic techniques.*

According to position of centromere the metaphase chromosomes can be anatomically classified into: Metacentric, submetacentric and acrocentric (fig. 8-2).

Methods of studying human chromosomes are:

a. Karyotyping
b. G-banding
c. Fluorescence in situ hybridization (FISH).
d. Gene mapping

Karyotyping

Karyotype refers to both the chromosomal composition (total number of chromosomes per cell) of an individual, and the photomicrograph that shows this composition.

In human **cytogenetics**, the normal human karyotype is 46 chromosomes - total number of chromosomes - formed of 44 autosomes (22 pairs) and 2 sex chromosomes (1 pair).

For example, a normal male is identified as 46, XY (46 chromosomes including the XY - chromosomal pair), and the female as 46, XX (46 chromosomes including the XX chromosomal pair).

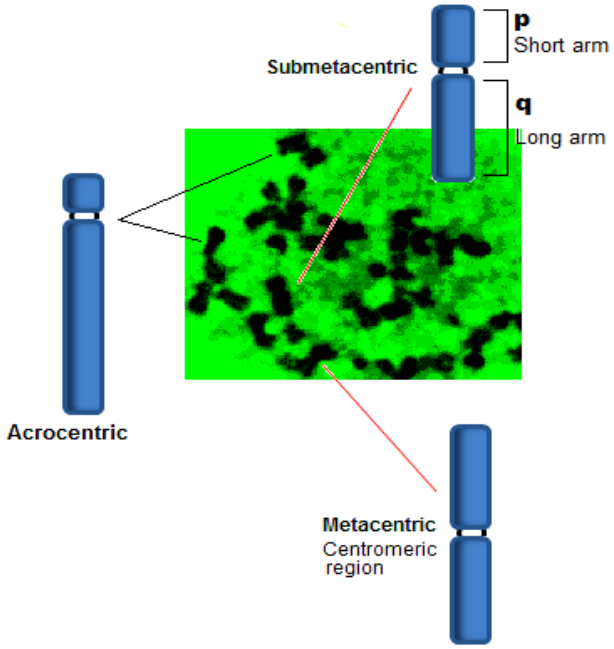


Fig. 8-2: Human Metaphase chromosomes. According to position of centromere, chromosomes can be classified into metacentric, submetacentric and acrocentric. (p= short arm, q= long arm in sub-metacentric chromosome).

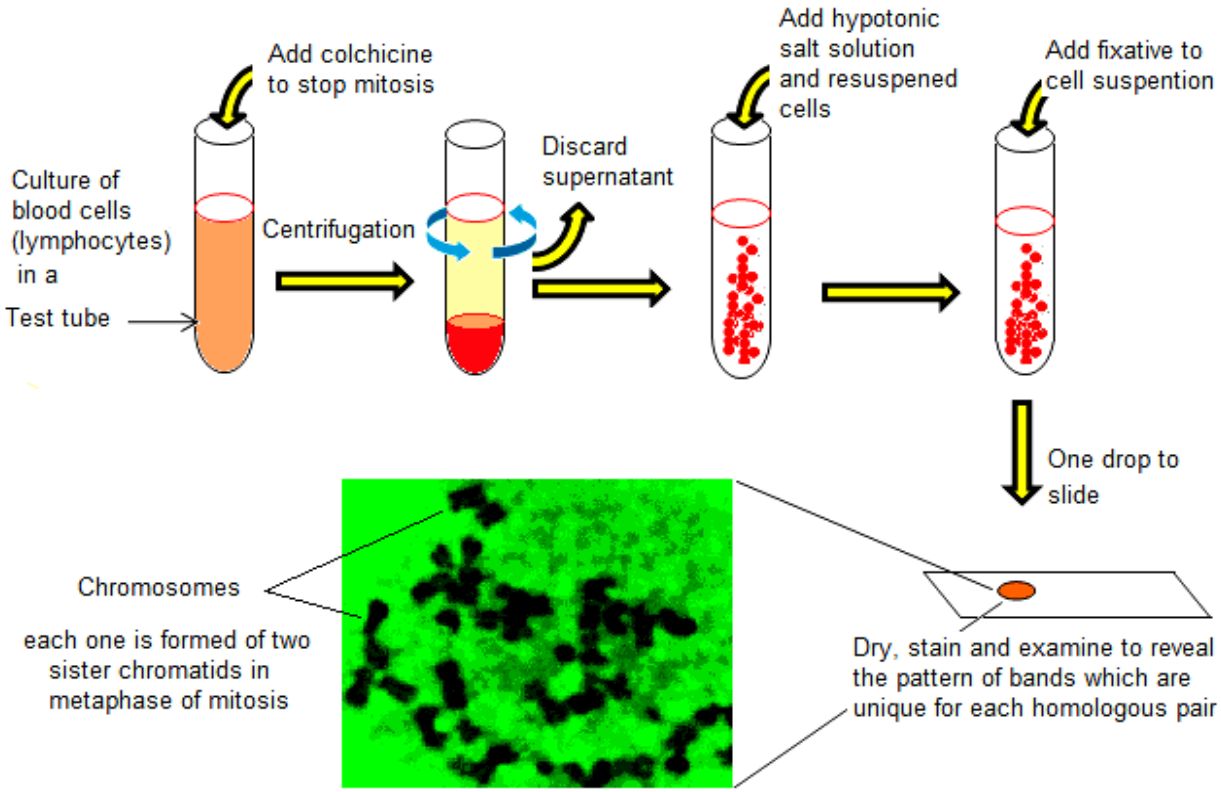


Fig. 8-3: Karyotyping technique illustrates how a sample of blood cell lymphocytes is prepared for chromosomal examination. Bottom picture shows the metaphase chromosomes, each is formed of two sister chromatids attached at centromere, constricted region.

Extra autosomal chromosome is indicated by placing the number of extra chromosomes after the sex chromosomes with a plus (+) sign. For example: 47, XX, +21 is the karyotype of a female with trisomy 21 (Down syndrome), 47, XXY is the karyotype of a male with an extra X-chromo-some (Klinefelter syndrome). A plus or minus sign is placed following a chromosomal symbol to indicate the increase or decrease in arm length. The letter p indicates the short arm and q indicates the long arm. For example, 47, XY, + 17 p+ is the karyotype of a male with 47 chromosomes including extra chromosome 17 with an increase in length of its short arm.

Karyotyping Technique

Steps of karyotyping are demonstrated in figure 8-3. The microscopic picture of metaphase chromosomes is scanned into a computer and the homologous pairs are matched and placed together.

Homologous pairs of chromosomes can be differentiated from the others by their length, position of centromeres, pattern of banding and satellite (refer to tiny knobs of chromosomal material at the tip of certain chromosomes).

- The homologous chromosomes are arranged according to their length into (fig. 8-4):
  - The largest chromosome (No.1).
  - The intermediate chromosomes.
  - The smallest chromosome (No.22).
  - The sex chromosomes (XY)

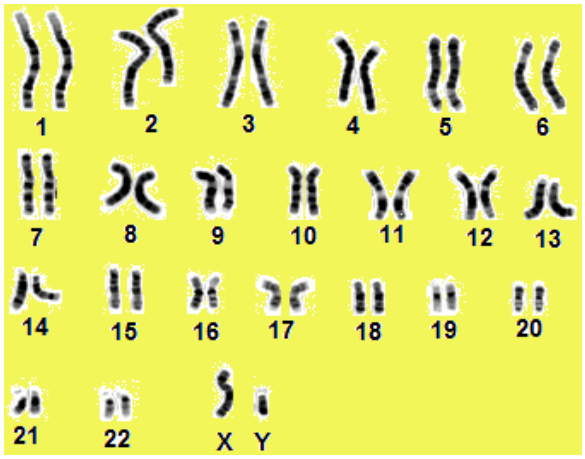


Fig. 8-4: Arrangement of metaphase homologous chromosomes of human genome in karyotype. (The photo is adopted from illustrations of National Human Genome Research Institute, USA. With the mention that: all of the illustrations in the talking glossary of genetics are freely available and may be used without special permission*).*

G - Banding

G-banding is obtained by ***Giemsa stain*** that is the most commonly used staining technique for metaphase chromosomes after chromosomal digestion with trypsin (fig. 8-4). It demonstrates a pattern of dark and light bands that is specific for each chromosome. The dark bands tend to be heterochromatic and rich in both adenine and thymine bases. The light regions tend to be formed of euchromatin and rich in guanine and cytosine bases. This method produces 300 - 400 bands in normal human genome.

Recently, a new method for karyotyping, called spectral karyotype (fig. 8-5A), has been developed. It increases the ability to detect altered chromosomes in pre- and postnatal diagnostics, in cancer and in other diseases. The new karyotyping method uses fluorescent dyes that bind to specific regions in chromosomes, instead of the traditional method of karyotyping; *G- banding*, by adding a dye, *Giemsa*, to metaphase chromosomes (fig. 8-5B).

Fluorescence in *Situ* Hybridization (FISH)

FISH is a specific technique that has been used to identify specific regions of metaphase chromo-somes.

DNA is fixed on microscopic slide and denatured, and then is hybridized with fluorescently labeled DNA-probe. The region of chromosome that hybridizes with the genetic probe can be seen under a fluorescent microscope (for hybridization technique see chapter 7).

FISH and G-banding techniques are used diagnostically to detect a variety of chromosomal abnormalities such as:

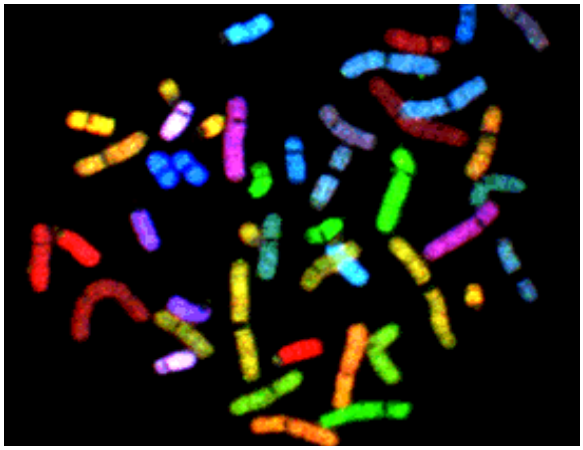
- Monosomies and trisomies
- Translocation
- Macro- and micro-deletions or insertions

DNA -Sequencing

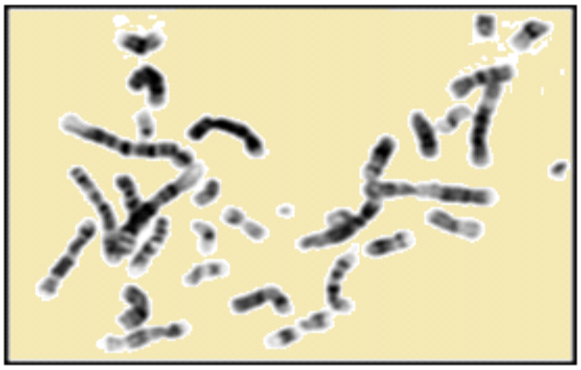
It is the determination of order of bases in a piece of DNA in order to detect the abnormalities of base sequence or mutant genes.

The human genome sequencing provides valuable information about the genes and chromosomal location of genetic disorders in human genome.

DNA sequencing technique has been discussed in chapter 7.



A



B

Fig. 8-5: A) Spectral karyotype. It is a new method ***for karyotyping by using fluorescent dyes that bind to s****pecific chromosomal regions. B) Traditional method for karyotyping by using Giemsa stain. (The source of photos is Schröck E et al., National Human Genome Research Institute, USA, with the mention:* All of the illustrations in the Talking Glossary of Genetics are freely available and may be used without special permission.*).*

Gene Mapping

It is a process of determining the location of certain genes on the chromosome.

Mapping includes:

- Dividing the chromosomes into smaller fragments that can be propagated and characterized.
- Ordering the fragments to be matched with their respective locations on chromosomes.

Importance:

It will help us to understand the physical and functional relationships among genes and groups of genes on chromosomes. It will help us to study genes interaction with each other, what each gene does, and how gene expression is regulated in different tissues

There are two types of maps:

1) Genetic maps.
2) Physical maps.

Genetic Maps:

They are based on the frequency of crossing over (percentage of recombination) between paternal and maternal derived chromosomes at meiosis. Distances of linear order are calculated in recombination units (*centi-Morgans*) or map unit. One centi-Morgan (1cM) is equivalent to a 1% chance of recombination (*see chapter 2*).

Physical Maps:

There are different types of physical maps.

- The *cytogenetic map* which is based on the banding pattern of stained chromosomes observed through light microscope.
- A *cDNA map* shows the locations of expressed DNA regions (exons) on chromosomal map.
- A *cosmid map* describes the order of overlapping DNA fragments spanning the genome.
- A *macrorestriction map* illustrates the order and distance between enzyme cleavage sites.
- A *base-pair sequence map* shows the highest-resolution of physical map. It demonstrates the complete DNA base-pair sequence of each chromosome.

Human Genome Project (HGP):

The HGP is an international project to understand the hereditary instructions that make each individual of us unique.

Gene mapping of human chromosome is one of the goals of Human Genome Project. The HGP had identified about 22.000 genes are located in DNA.

The specific goals of HGP include:

- *Mapping of human genes.*
- *Sequencing of human genome.*
- *Comparing human genome with that of other organisms such as E. coli.*
- *Developing of new studying technologies such as automated sequencing and micro-array.*
- *Developing bioinformatics which is a system for collecting, storing and analyzing the information about human genome.*

Human Genetic Disorders

Human genetic disorders are classified according to the factors that are responsible for causing these genetic disorders into 3 types. They are summarized in figure 8-6.

A) Inheritance of Single Gene Disorders:

Human genetic diseases according to the hereditary pattern of single gene disorders can be classified into:

1. Classical inherited disorders.
   They are disorders that follow the principles of hereditary pattern of Mendel. They are called *Mendelian inherited disorders*.

2. Non-classical inherited disorders.
   They are the genetic disorders that don't follow the principles of hereditary pattern of Mendel and called *Non-Mendelian inherited* disorders.

B) Chromosomal Abnormalities:

They are classified into:

1. Chromosomal abnormalities in number.
2. Chromosomal abnormalities in structure

C) Multi-Factorial Disorders:

They are human genetic diseases resulting from a combination of environmental factors and genetic variations.

Single Gene Disorders (SGD)

1) Classical Inherited Single Gene Disorders:

These inherited genetic disorders are caused by a single defective gene; individual mutant gene and frequently follow the characteristic patterns of inheritance of Mendel's laws.

There are approximately 6000 single gene disorders. Single gene disorders are usually affecting about 1% of the population.
The phenotype of certain inherited genetic disorders requires an environmental signal to be expressed.

According to patterns of inheritance of the single gene that causes the disease, and by which chromosome it is carried, the classical single gene disorders are classified into 4 types:

i) Autosomal recessive (AR).
ii) Autosomal dominant (AD).
iii) X-linked recessive (XLR)
iv) X-linked dominant (XLD)

Autosomal Recessive Disorders (AR):

A recessive trait or gene is expressed only in homozygotes. In figure 8-7, the inheritance pattern of autosomal recessive disorders is explained.

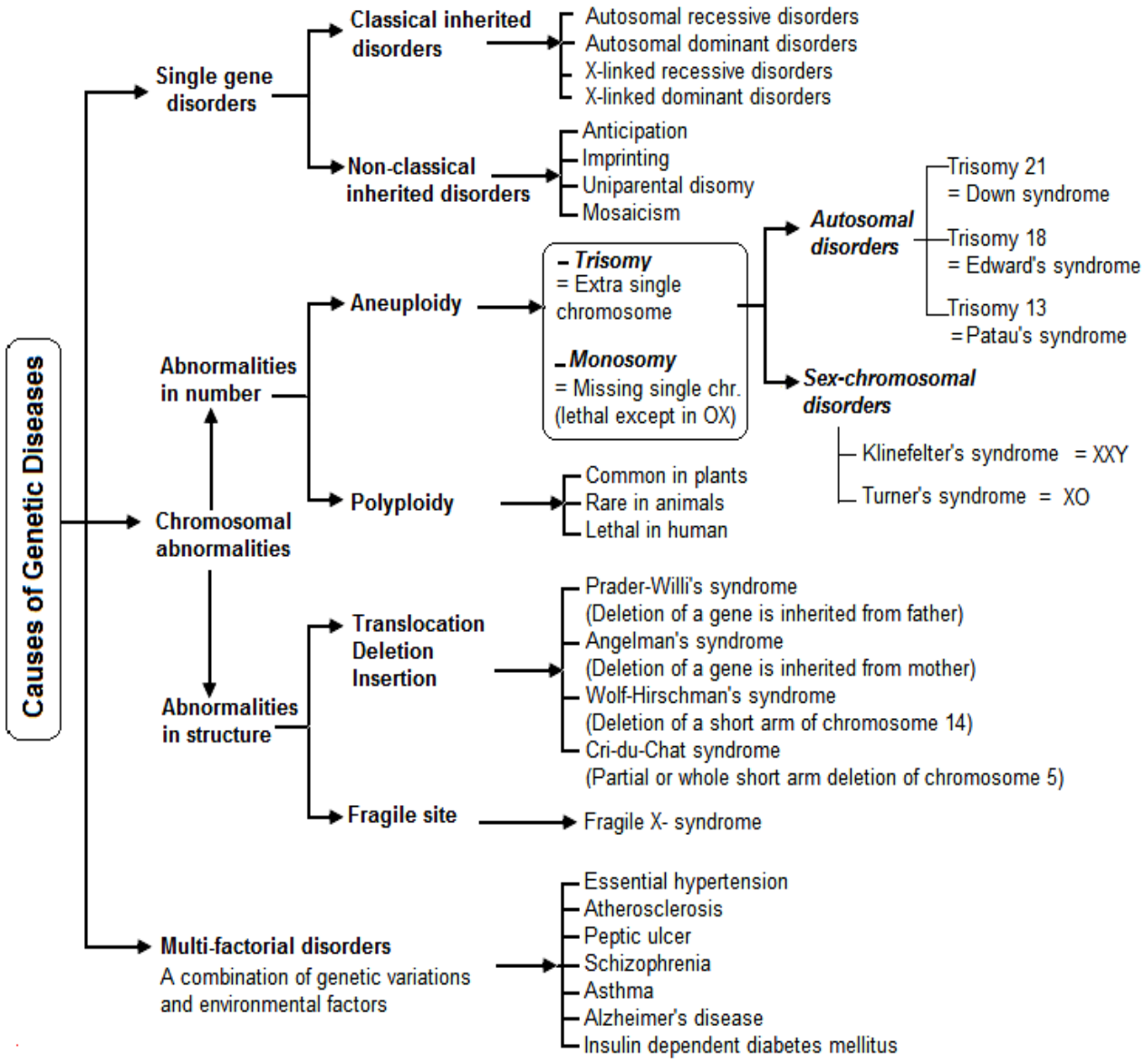


Fig. 8- 6: Classification of human genetic disorders according to their causes.

- Phenotypically normal parents may have diseased offspring if they are genetically carriers.
- If a carrier has a normal partner, a chance of children to be carriers is 50%.
- Mating two heterozygotes will produce diseased individuals, with a probability of ratio of 25% (1/4).
- Both male and female are equally affected.
- Autosomal recessive genes may be sex influenced genes.

Examples of autosomal recessive disorders:

- Phenylketonuria
- Sickle cell anemia
- Tay – Sachs disease
- Cystic fibrosis
- β- thalassemia
- Congenital adrenal hyperplasia
- Friedreich's ataxia
- Gaucher's disease
- Hemochromatosis

Phenylketonuria (PKU)

- *The defective allele* is located on chromosome # **12**.
- It encodes for phenylalanine hydroxylase.
- Enzyme phenylalanine hydroxylase is responsible for converting phenylalanine into tyrosine. Its deficiency causes accumulation of ***toxic phenylketones*** *in blood* and results in *severe mental retardation*.
- Detection of PKU is done by analysis of a blood sample in childhood.
- A diseased person is treated by a low phenylalanine diet.

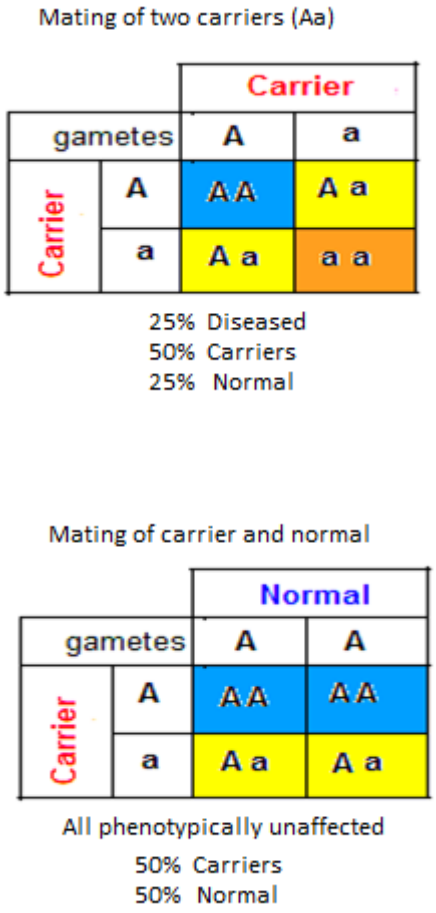


Fig. 8-7: The expected phenotype of offspring after mating of two carriers or carrier and normal, in autosomal recessive inheritance of single gene disorders, AR, (a, disease allele).

Sickle cell anemia

- Its cause is a defective autosomal recessive gene located on chromosome #**11**.
- It causes replacement of glutamic acid by valine in hemoglobin β-chain which results in formation of abnormal hemoglobin molecules. It is less soluble and forms crystals that causes:
  - Formation of distorted sickle shaped *half-moon* RBCs due to abnormal polymerization of *hemoglobin ß- chain*.
  - Obstruction of small blood vessels and capillaries by abnormal RBCs that prevent oxygen from being delivered to tissues.
  - *It may cause* hemolytic anemia; anemia results from destruction of erythrocytes.
  - Infarction and localized necrosis result from obstruction of the blood supply (e.g. hand and foot syndrome) and splenomegaly.

Thalassemia

Both α and β thalassemia are inherited autosomal recessive blood disorders that originated in the Mediterranean region. The genetic defect, which could be either mutation or deletion, results in reduced rate of synthesis or no synthesis of one of the globin chains that make up hemoglobin. This can cause the formation of abnormal hemoglobin molecules, thus causing anemia.

The genetic defect in thalassemia causes a quantitative problem of too few globins synthesized, whereas it causes in sickle-cell disease a qualitative problem of synthesis of an incorrectly functioning globin (abnormal polymerization of *hemoglobin ß- chain)*.

- Slowed growth rates: anemia can cause a child's growth to slow. Puberty also may be delayed in children with thalassemia.
- Heart problems: such as congestive heart failure and abnormal heart rhythms (arrhythmias), may be associated with severe thalassemia.
- The thalassemia trait may confer a degree of protection against malaria, which is or was prevalent in the regions where the trait is common, thus conferring a selective survival advantage on carriers (known as heterozygous advantage), and perpetuating the mutation. In that respect, the various thalassemias resemble another genetic disorder affecting hemoglobin, sickle-cell disease.

Cystic fibrosis

- *The defective gene* is located on chromosome # **7**.
- It causes defect in *chloride ion transport* across the cell membrane that results in:
  - Secretion of *abnormal thick viscous mucous* in *respiratory, digestive, urinary and genital tracts*.
  - Chronic obstructive respiratory airways.
  - Difficulty of breath.
  - Pancreatic insufficiency.
  - Malabsorption in gastro-intestinal tract (GIT).
  - May cause infertility.

Tay-Sachs disease

- *The defective allele* is an *autosomal recessive mutant* gene *located* on chromosome #**15**.
- It is a deadly disease of the nervous system passed down through families.
- It causes abnormal accumulation of **gangliosides;** gangliosides are more complex glycosphingolipids in which oligosaccharide chains containing N-acetylneuraminic acid (NeuNAc) are attached to lipid molecules forming lipid bilayer of cell membrane.
- Synthesis of gangliosides occurs in cells, especially nerve cells.
- In brain cells absence of lysosomal enzyme hexosaminidase that digests the gangliosides results in:
  - *Blindness,*
  - *Deafness and*
  - *Severe mental retardation*.

- Symptoms can be observed within the 1st year of life and result in death before the age of five years.
- The disease is most common among the Ashkenazi Jewish population. About 1 in every 27 members of the Ashkenazi Jewish population carries the Tay-Sachs gene.

- *Treatment: no effective treatment is available until now.*

Autosomal Dominant Disorders (AD)

Autosomal dominant traits are expressed in heterozygotes and homozygotes.

The hereditary pattern of autosomal dominant disorders:

- Diseased parents have diseased children.
  -Heterozygotes diseased parent produce 75% diseased children and 25% normal children.
  - Homozygous diseased parents produce 100% diseased children.
- Normal families (homozygous recessive) have usually normal children.
- Mating of heterozygous diseased and normal individuals produces a 1 : 1 ratio of normal and diseased children.
- Mating of homozygous diseased and normal individuals produces diseased offspring.
- Both sexes are usually equally affected by autosomal dominant disorders.
  Examples of autosomal dominant disorders:
  - Huntington's disease
  - Marfan syndrome
  - Von Willebrand's disease

Huntington's disease (HD)

- The cause is an abnormal autosomal dominant allele that affects nervous system. An unusual form of mutation known as trinucleotides repeat expansion (TNRE) repeats CAG from 40 to more than 150 times, while the normal allele repeats CAG to 35 times.

- In some references, the increase in the number of repeats is associated with greater severity.
- It causes:
  - Severe mental retardation,
  - Physical deterioration,
  - Uncontrolled muscular spasm and
  - Ultimately death.
- Symptoms begin relatively late in life in persons over 30 or 40 years of age.
- No treatment is available.

Marfan syndrome

It results from mutant gene coding for connective tissue factors.

A Marfan syndrome diseased person suffers from long spidery limbs and fingers, high arched palate, lens dislocation and aortic incompetence.

X–linked Recessive Disorders (XLR)

The X-linked recessive disorders follow the inheritance pattern of X-chromosome (fig. 8-8). The inheritance patterns of XLR disorders are:

- Males are hemizygous (XY) and females are homozygous (XX).
- The disease is transmitted by a female carrier.
- 50% of male offspring of a female carrier will be affected, while her daughters have a 50% chance of being carriers.
- There is no male to male transmission.

Color blindness

Color blindness is caused by a recessive allele on the X-chromosome. Color-blindness is expressed in a *homozygous* recessive female or a *hemizygous* male, who received the allele of color blindness from a carrier mother. Color blindness has been discussed in chapter 2.

Hemophilia

Hemophilia results from mutation of a recessive allele on X- chromosome.

- It causes lack of blood clotting factor VIII – a gene product is involved in intrinsic blood coagulation pathway, and results in recurrent bleeding into soft tissue and joints.
- A male inherits hemophilia from a carrier mother.

Treatment of Hemophilia:

- Blood transfusion.
- Administration of blood clotting factor produced by recombinant DNA -technology.

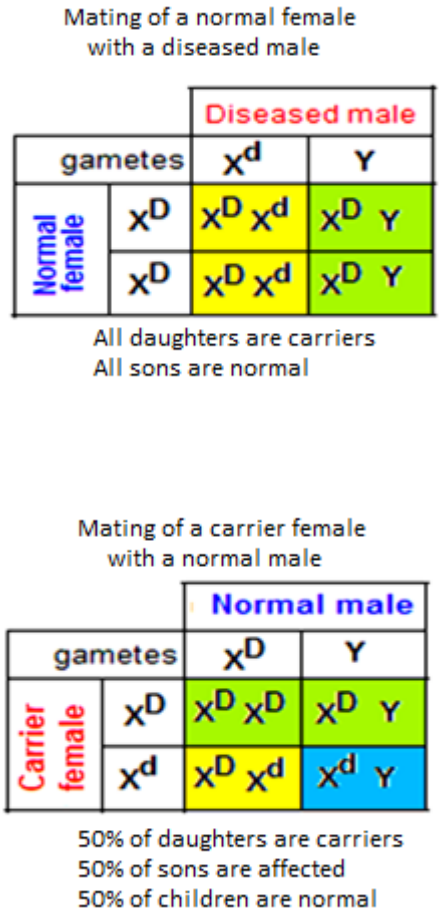


Fig. 8-8: The expected phenotypes of offspring after mating of two individuals for inheritance of X-linked recessive single gene disorders, XLR, allele X^d (allele causes disease).

X-linked Dominant Disorders (XLD):
They are rare and difficult to differentiate from autosomal dominant inherited disorders, except that: In X-linked disorders the affected males have normal sons, but all daughters are diseased.

Example: ***Rett syndrome***
It is a neurodevelopmental disorder that is classified as a spreading developmental disorder. It is characterized by delays in the development of multiple basic functions including socialization and communication.

The clinical features include a decrease in the rate of head growth that causes microcephaly in some, and small hands and feet in others. Repetitive hand movements such as mouthing or twisting can also be noted. Socialization typically improves by the time they enter school.

Girls with Rett syndrome have a tendency to gastrointestinal disorders, and up to 80% have seizures. They typically have few or no verbal skills, and about 50% of females are not ambulatory.

2. Non-classical Inherited Single Gene Disorders

Some genetic diseases don't follow the classic patterns of the principles of inheritance. Rather, they follow other ways of inheritance:

Anticipation

- It is a genetic state in which the severity of an inherited disease tends to be more defective in the successive generations. This phenomenon occurs due to a dynamic genetic mutation.
- This mutation results from trinucleotides repeat expansions. The expansions have a tendency to increase gradually in successive generations such as *Huntington's chorea, several degenerative nervous disorders, and myotonic dystrophy*.

Genomic imprinting

- It is a pattern of inheritance that involves a change in a single gene or chromosomal structure during gamete formation.
- These chromosomal modifications occur during spermatogenesis in male or oogenesis in female. For example: If a fetus (46, XX) has an extra maternal set and lacks the paternal set. This results in either an undeveloped fetus or an abnormal placenta. An extra paternal set or extra maternal set causes poor embryonic development.
- It may be a cause of *Prader-Willi and Angelman syndromes.*

Uniparental disomy

- It means duplication of a chromosome from one parent that is accompanied with loss of the corresponding homologous chromosome from the other parent. Uniparental of chromosome # 7 of maternal origin may result in few cases of cystic fibrosis with severe growth retardation.

Mosaicism

- Mosaicism results from post-zygotic mutation which occurs during mitotic division of the cells of embryo.
- A diseased person shows different phenotypes because he has different types of growing cells which have been developed during the fetal life.
- Mosaicism may cause mild cases of Down syndrome and a tendency to have cancerous cells.
- Mosaicism of germ cell line in gonads may results in that the normal parents produce a child with genetic disorders.

Chromosomal Abnormalities

At least 17% to 20% of pregnancies end in spontaneous abortion, half of these aborted embryos have major chromosome abnormalities.
Chromosomal abnormalities are classified into:

1. **Abnormalities in number of chromosomes.**
2. **Abnormalities in structure of chromosomes.**

Abnormalities in Number of Chromosomes

The main cause of chromosomal abnormalities in number is the chromosomal non-disjunction (fig. 8-9).
Chromosomal non-disjunction means that chromosomes fail to separate at anaphase in meiosis or mitosis which causes abnormalities in chromosomal number.

In Meiosis:
Chromosomal non-disjunction may occur during the 1st or 2nd meiotic division and results in:

- Formation of gametes which contain an extra chromosome or extra set of chromosomes.
- Formation of gametes lacking a single chromo-some or lacking a set of chromosomes.

In Mitosis:
Chromosomal non-disjunction may occur in anaphase of mitosis and results in establishment of a clone of abnormal cells in a normal organism.

Tyes of chromosomal abnormalities in number:

a. Polyploidy
b. Aneuploidy

Polyploidy
It is the condition of being polyploid, in which an organism or cell having more than double the haploid number of chromosomes - multiple chromosomal sets form the genetic material within the cells (normal diploid organisms have 2 sets of chromosomes).

- Polyploidy is common in plants and rare in animals and lethal in humans.
- Presence of one extra set is called *triploidy* (3n) and presence of 2 extra sets is called *tetra-ploidy* (4n).
- Triploidy (3n) causes early abortion of pregnancy and tetraploidy (4n) causes death in few days after birth.
- *Usually, a polyploid plant is much bigger, more robust and healthy than its parental diploid stock!*
- *Causes of ploypoidy*:

- Occurrence of chromosomal non-disjunction in anaphase- I and II of meiosis.
- In rare cases *complete mitotic non-disjunction may occur*; all the chromosomes can undergo mitotic non-disjunction and migrate to one of the daughter cells.
- Fertilization of an egg by more than one sperm is one of the causes of polyploidy.

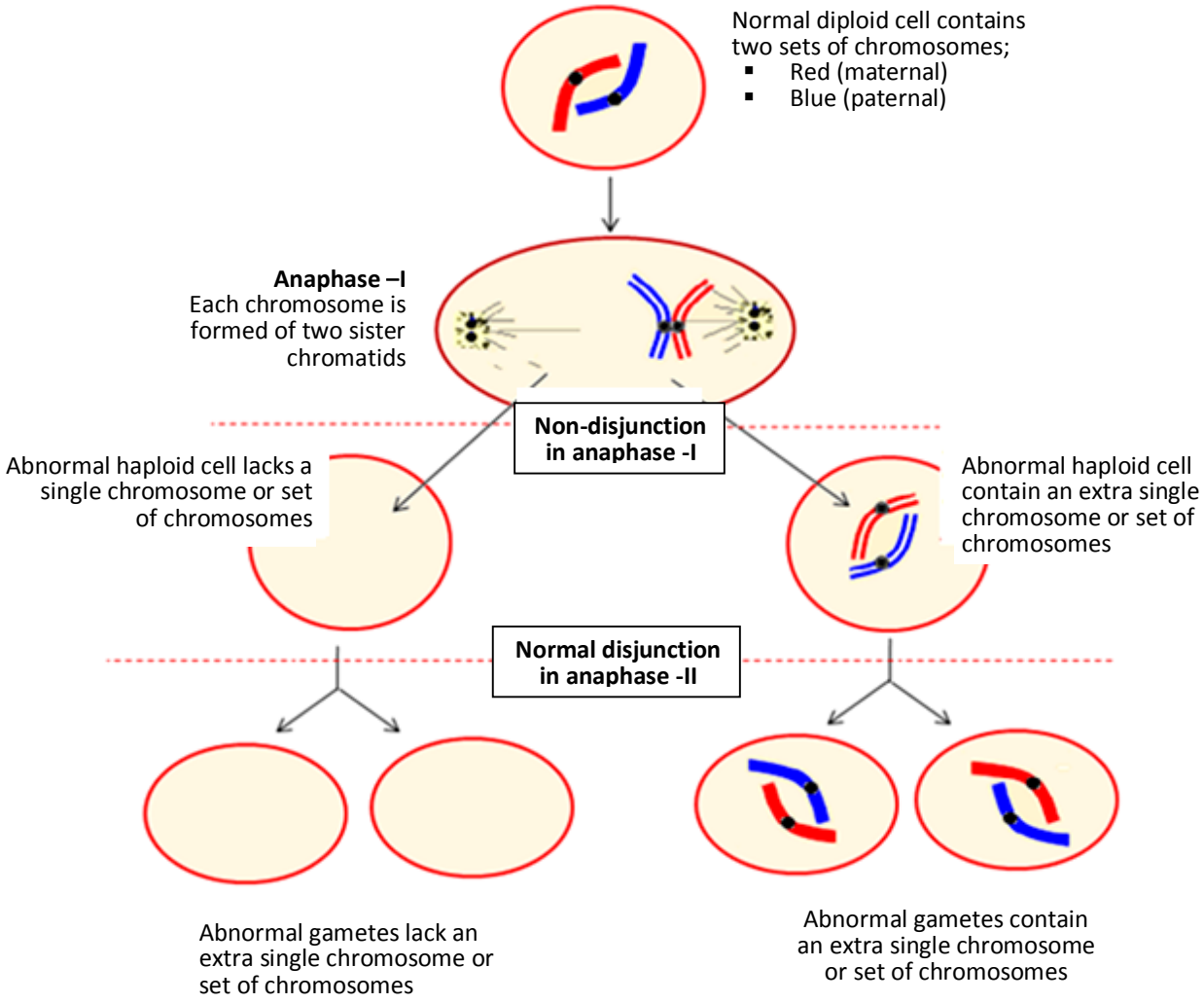


Fig. 8-9: Diagram illustrates how non-disjunction of homologous chromosomes in meiosis–I divisions may result in formation of gametes containing an extra single chromosome or an extra set of chromosomes.

Aneuploidy

It is a condition of existing abnormal chromosomal number within the cell or organism that results from presence or absence of one or more extra chromosome in the cells of plants or animals.

Also, it refers to an abnormality in a chromosome number, in which one chromosomal set is incomplete.

- Presence of a single extra chromosome within cells of an organism is called *trisomic*.
- Absence of one chromosome (*one member of a pair is absent*) is called *Monosomic.* It is usually the cause of a prenatal death
- Presence of two copies of a chromosome within cells of an organism is called *Disomic*, while presence of four copies of chromosome is called *tetrasomic*.
- Almost, for an organism, it is more harmful to have aneuploidy than to have polyploidy.
- Also, monosomies are much worse than trisomies.

The harmful effect of aneuploidy is probably due to that it changes the gene balance and gene interaction required for proper development of an organism. So, the normal development of an organism depends not only on the absolute quantity of transcriptions of a particular gene, but also on the balance between the amount of gene product and the products of other genes in a normal cell.

X-chromosome inactivation in case of dosage compensation in female is an example of this phenomenon.

Aneuploidies are classified into:

1. Autosomal aneuploidies
2. Sex- chromosome aneuploidies

Autosomal Aneuploidies

There are three autosomal genes aneuploidies that are able to survive after birth. These aneuploidies are the following syndromes:

a. Trisomy **21** – Down syndrome
b. Trisomy **18** – Edward's syndrome
c. Trisomy **13** – Patau syndrome

The published data related to these three aneuploidies has been summarized in the following short sentences:

Down syndrome

Cause: Trisomy 21

The diseased person has an extra chromosome 21, and its rate is about 0.15% of live-borns. *It is the most common chromosomal abnormality among children of older mothers (over 40 year of age).*

Symptoms:

- Patient has slanted eyes (mongoloid).
- Flat round face, flat occipital region.
- Low-set ears and a protruding tongue.
- May suffer from congenital heart disease and mental retardation (but highly variable).
- Later, he may have lymphoblastic leukemia, Alzheimer's disease by 30-40 years of age.
- **Relevant literature** indicates that he may have an increasing risk for respiratory infections, secretory otitis media, cataracts and squints, hypothyroid, epilepsy and diabetes.

Edward's syndrome

Cause: Trisomy 18

The patient has an extra chromosome 18, and its rate of prevalence is about 0.01% of live births.

Symptoms:

- Elongated skull, a small jaw and malformed ears with large lobes, congenital heart disease, malformed kidneys and developmental delay.
- A *clinically* suspected person is confirmed by chromosomal analysis. 90% of patients **die within the first years**, most of them in first few weeks.

Patau syndrome

Cause: Trisomy 13

The patient has an extra chromosome # 13. The rate of prevalence is about 0.007% of live births.

Symptoms:

- Severe bilateral cleft lip, narrow temples, deformed ears, and deafness, structural brain defect, congenital heart disease.
- Other common symptoms are malformed kidneys, and malformed small widely set eyes. Death mostly occurs by the age of 1 to 3 months.

Sex- Chromosomes Aneuploidies

Sex chromosome aneuploidies are mostly less severe than autosomal aneuploidies. From genetic disorders of sex chromosomal aneuploidies, there are 3 distinct syndromes:

1. Klinefelter's syndrome
2. Turner syndrome
3. Jacob's syndrome

Klinefelter's syndrome

Karyotype: 47, XXY

Cause:

The patient has an extra X-chromosome. Prevalence rate is about 0.2% of live born males.

Symptoms:

- Clinically, Male with a female body shape and female distribution of body hair.
- When he reaches puberty, he is sterile (adequate quantities of sperm are not produced). He has hypogonadism.

Treatment:

Using long-term testosterone implants to improve the sperm production and body hair distribution.

Turner syndrome

Karyotype: 45, XO

Cause:

A normal female has two X-chromosomes while an affected female has only one X-chromosome.

Symptoms:

- Clinically, she has swollen extremities, short

fingers and toes (especially fourth metatarsal), frail nails, gastro-intestinal bleeding, and widely spaced nipples.
- *No secondary sexual characteristics*, *sterile* (gonads degenerate to connective tissue).

Jacob's syndrome

Karyotype: 47, XYY

Cause:
- Male with an extra Y -chromosome. Often, the extra Y -chromosome causes no unusual physical features or medical problems. The rate of distribution is about 1 per 1000 live-born males.

Symptoms:
- Show difficulties in learning and speech skills. The probability of they having a risk of learning difficulties is high in up to 50%. They also show behaviour problems in childhood**.**

Abnormalities in Structure of Chromosomes

Chromosomal disorders result from chromosomal damage that may be caused by exposure to some mutagenic agents such as:
- Environmental factors,
- Chemicals modifiers,
- Base analogs and
- Radiation.

Also chromosomal abnormalities in structure may result from genetic chromosomal instability due to exposure to mobile genetic elements.

Mechanisms that lead to abnormalities in chromosomal structure are classified into:
1) Fragile site
2) Translocation
3) Deletion and insertion

Fragile site

A weak point is found near the tip of X-chromosome (fig.8-10) due to presence of a defective allele which causes *fragile X- syndrome.*

Symptoms:

It causes mild-learning and attention disability or severe mental retardation and hyperactivity. It is more remarkable in males than females.

Translocation

Exchange of chromosomal segments between non-homologous chromosomes may occur if they are attached in meiosis (fig. 8-11).

This translocation between two non-homologous chromosomes is called *reciprocal translocation*.

Translocation may lead to:

a. Exchange of chromosomal parts.
b. Missing of some genes (deletion).
c. Formation of extra copies of genes (duplication).

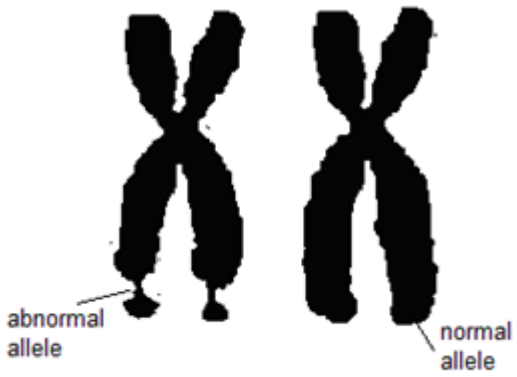


Fig. 8-10: Fragile X- syndrome. The normal allele at the tip of X -chromosome repeats the nucleotide triplet CCG up to 50 times. The defective allele repeats CCG from 200 to more than 1000 times.

Deletion

In case of deletion, a segment of DNA or chromosome is missing. The chromosome loses a part because the chromosome breaks but fails to rejoin (fig.8-11).

- Large chromosomal deletions may be lethal and result in death.

- Small chromosomal deletions may cause genetic disorders such as *Cri-du-chat syndrome* (cry of cat) that result from the loss of a short arm of chromosome # 5.

Symptoms foci-du-chat Syndrome:

Infants have a small head (moon face), severe mental retardation, and die in infancy or childhood.

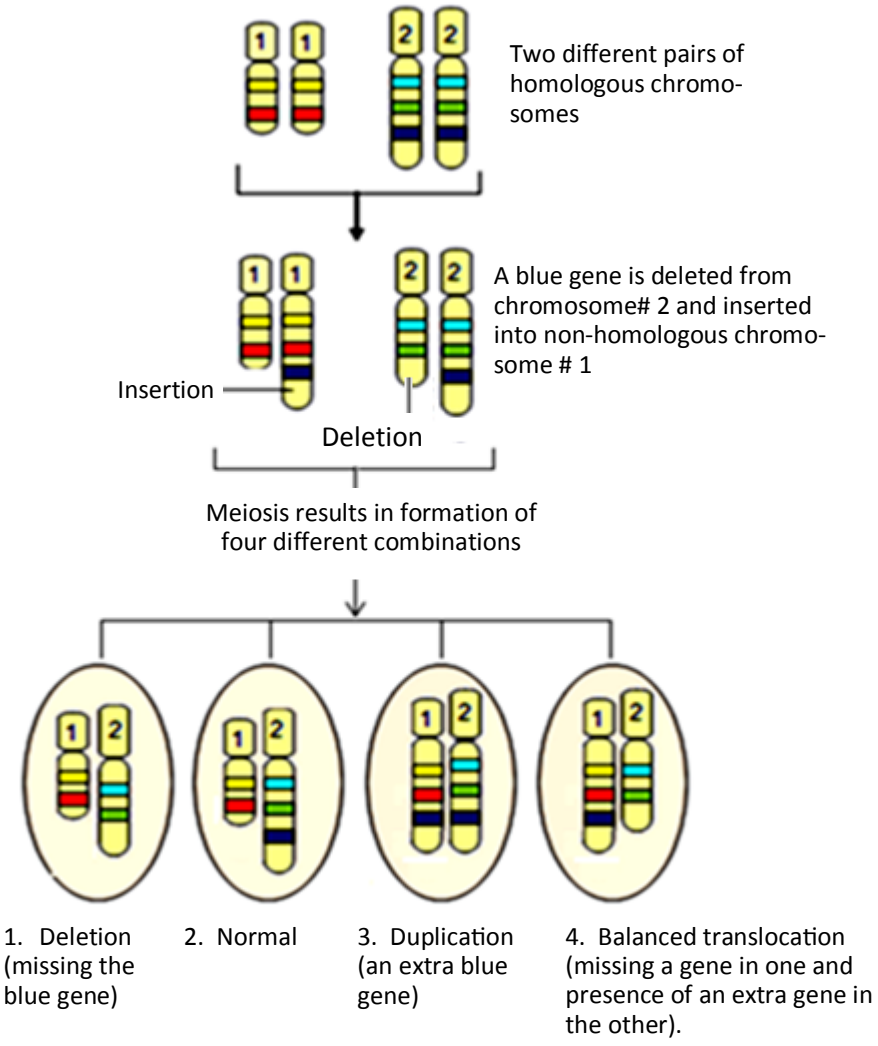


Fig. 8-11: Deletion and insertion (translocation) between non-homologous chromosomes may result in formation of 4 different types of gametes containing different combinations of genes.

Multi-Factorial Disorders

The *phenotypic variations* among individuals, that make an individual susceptible to be diseased by a certain disorder, may be due to genetic, **heritability**, and/or environmental factors.

Heritability is the proportion of phenotypic variations within a population that is attribute-able to genetic variations among individuals.
Heritability analysis estimates the relative contribution of differences in genetic and non-genetic factors to the total phenotypic variance in a population.

Examples of multi-factorial disorders:

Diabetes mellitus (Insulin dependent)
Genetic factors are important in susceptibility of an individual to *insulin dependent diabetes mellitus*. Only 50% of non-diabetics have the major histocompatibility complex (MHC) alleles; a family of fifty or more genes on the sixth human chromosome that code for proteins on the surfaces of cells and that play a role in the immune response, while about 98% of insulin-dependent diabetes mellitus have these alleles of MHC.

Essential hypertension
The heritability of essential hypertension is 62%. Genes are important in the incidence of hypertension and the response to treatment.

Atherosclerosis
The heritability of atherosclerosis is about 65%. Genetic factors are important causes of hypertension, diabetes mellitus and premature ischemic heart disease.

Peptic ulcer
Peptic ulcer is inherited as an autosomal recessive disease. The heritability of peptic ulcers is 37%. 50% of affected families have genetically a high level of increased pepsinogen-I.

Schizophrenia
The heritability of schizophrenia is 85%. It may be associated with a locus on chromosome # 5, but some references suggest alternative loci.

Asthma
The heritability of asthma is 80%, and it is associated with human leukocyte antigen (HLA) A23.

Alzheimer's disease
Alzheimer's disease is inherited as a monogenic autosomal disorder in about 10% of patients. Mutations in the amyloid precursor protein (APP) gene on chromosome # 21 and mutations in other genes on chromosomes # 14 and 1 are also involved in ascending the susceptibility to Alzheimer.

Genetics of Cancer

Cancer is characterized by abnormal cellular growth and proliferation. It develops in the majority of cases as a result of multi-factorial disorder. *Both genetic and environmental factors interact to initiate carcinogenesis.*

- The normal cell division and proliferation are controlled by growth promoting *proto-oncogenes* and *growth inhibiting tumour suppressor genes.* Defects or mutations in these genes may cause cancer.

- The process of carcinogenesis initiates when mutations accumulate in both oncogenes and tumour suppressor genes (fig. 8-12).

Oncogenes

Mutations activate *oncogenes* which cause abnormal cell proliferation. They develop from proto-oncogenes. Proto-oncogenes are normal genes which promote the normal cell growth.

Causes of oncogenes activation:

- Translocation
- Amplification
- Point mutation

Tumor Suppressor Genes

They inhibit the carcinogenesis or tumor development. They are known as *anti- oncogenes*.

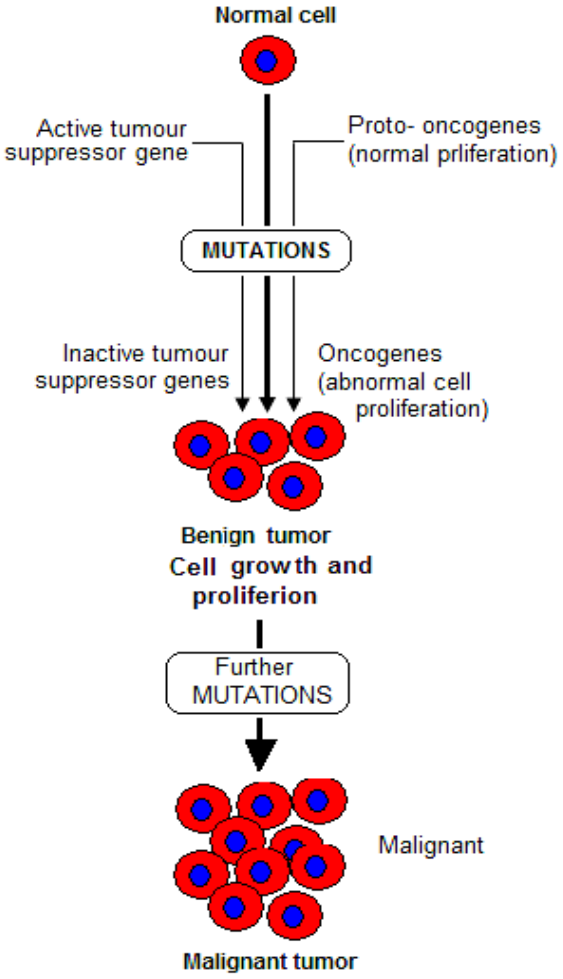


Fig. 8-12: Genetic basis of cancer. This diagram illustrates how mutations may result in developing of a tumor under effect of oncogenes that code for abnormal cell proliferation, and inactivation of tumor suppressor genes.

Tumour suppressor genes are recessive at the cellular level. They could lose their activity by:

- *Mutation,*
- *Gene conversion,*
- *Mitotic recombination or*
- *Interaction with cellular or viral proteins which cause damage to the genes.*

Tumor Chemotherapy

Treatment of carcinogenic tissue (tumor) by using chemotherapeutic agents as well as by application of radiotherapy is effective in control of tumor growth.

***Mode of action** of the chemotherapeutic drugs:*

- They can chemically cross-link DNA.
- They also inhibit enzymes that are required for DNA synthesis.
- They unfortunately, have a toxic effect on other sensitive tissues and mitotically active organs such as bone marrow, the intestinal epithelium, the kidney and the nervous tissue.
- Chemotherapeutic drugs as *colchicine, colcemid, vincristine and vinblastine* block cell division by inhibiting the process of assembling of microtubules of mitotic spindle.
- *Taxol* is a chemotherapeutic drug that also blocks cell mitosis by stabilizing the microtubules of mitotic spindle.

Genes of Chemotherapy Resistance:

- Mutation in the *p53 gene.*
  Inactivation of p53stopscell apoptosis in response to drug that causes DNA damage.
- Multidrug-resistance (mdr) gene.
  It prevents intracellular action of drug. It increases pumping of drug outside the cancer cell.

  The *multi-drug-resistance (mdr) gene family* encodes ATP-dependent carrier protein that is involved in pumping therapeutic drug outside the cell.

Birth Defects

Birth defects or congenital defects are abnormalities in physical or mental structure present at birth. It may or may not be an inherited disorder (fig. 8-13). The major birth defects occur in approximately 3% of all births.

Defects may be genetic in origin, as in Down syndrome, Tay-Sachs disease, sickle cell anemia and hemophilia or may be the result of infections, such as rubella, German measles and sexually transmitted diseases. Incidence of some disorders is elevated when the mother or father is older, which increases the age-related gene mutations or gene abnormalities.

- Defects may be environmental in origin, as exposure to teratogenic, malformation-causing agents that include drugs or hormones taken by the mother, and maternal illnesses (e.g., diabetes).
- The mother's nutrition, drinking (fetal alcohol syndrome), smoking and drug abuse, as well as exposure to toxic chemicals and radiation, can also affect the developing fetus. Smoking, drugs, and toxic chemicals can also damage the father's sperm, which may pass on the defect to the embryo in fertilization.

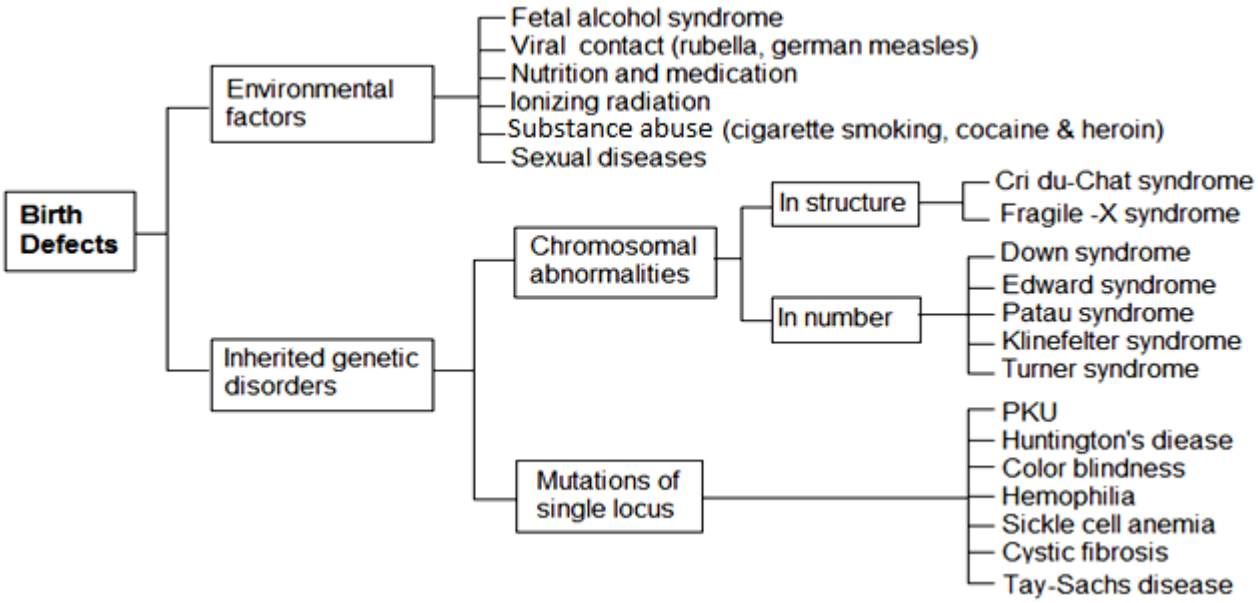


Fig. 8-13: Factors that may cause birth defects are classified into environmental factors and inherited genetic disorders.

Prenatal Diagnosis of Genetic Diseases

- Early diagnosis of chromosomal abnormalities particularly in prenatal period increases the possibilities for prevention of the effect of genetic disorders.
- Techniques which are used in diagnosis of prenatal genetic diseases:
  1. Amniocentesis
  2. Chorionic villus sampling

Amniocentesis

- In amniocentesis the amniotic fluid that contains cells of the fetus is obtained by a needle through the abdominal wall of pregnant women.
- The collected fetal cells (Fig. 8-14) can be cultured and the structure of chromosomes is analyzed to detect the chromosomal abnormalities.

○ ***Importance of Amniocentesis***

- Detection of Down syndrome in pregnant women over 35 years of age, because their fetuses have high risk of Down syndrome.
- Detection of genetic disorders which cause enzyme deficiencies by incubation of cells in media containing suitable substrate and measurement of the product. This test is useful in prenatal diagnosis of Tay-Sachs disease.
- Analysis of DNA is used for prenatal diagnosis of defective genes in order to detect several genetic diseases including sickle cell anemia and cystic fibrosis.
- It is useful in detection of spina bifida (congenital defect in which a vertebra is malformed and spinal cord does not close properly). This birth defect is associated by a high level of α-fetoprotein in amniotic fluid.

Chorionic Villus Sampling (CVS)

- Placenta is formed of maternal and fetal parts. Chorionic villus sampling involves obtaining and studying fetal cells from the fetal part of placenta.
- The advantage of CVS is that it can be performed in the first trimester; the first three months of pregnancy.

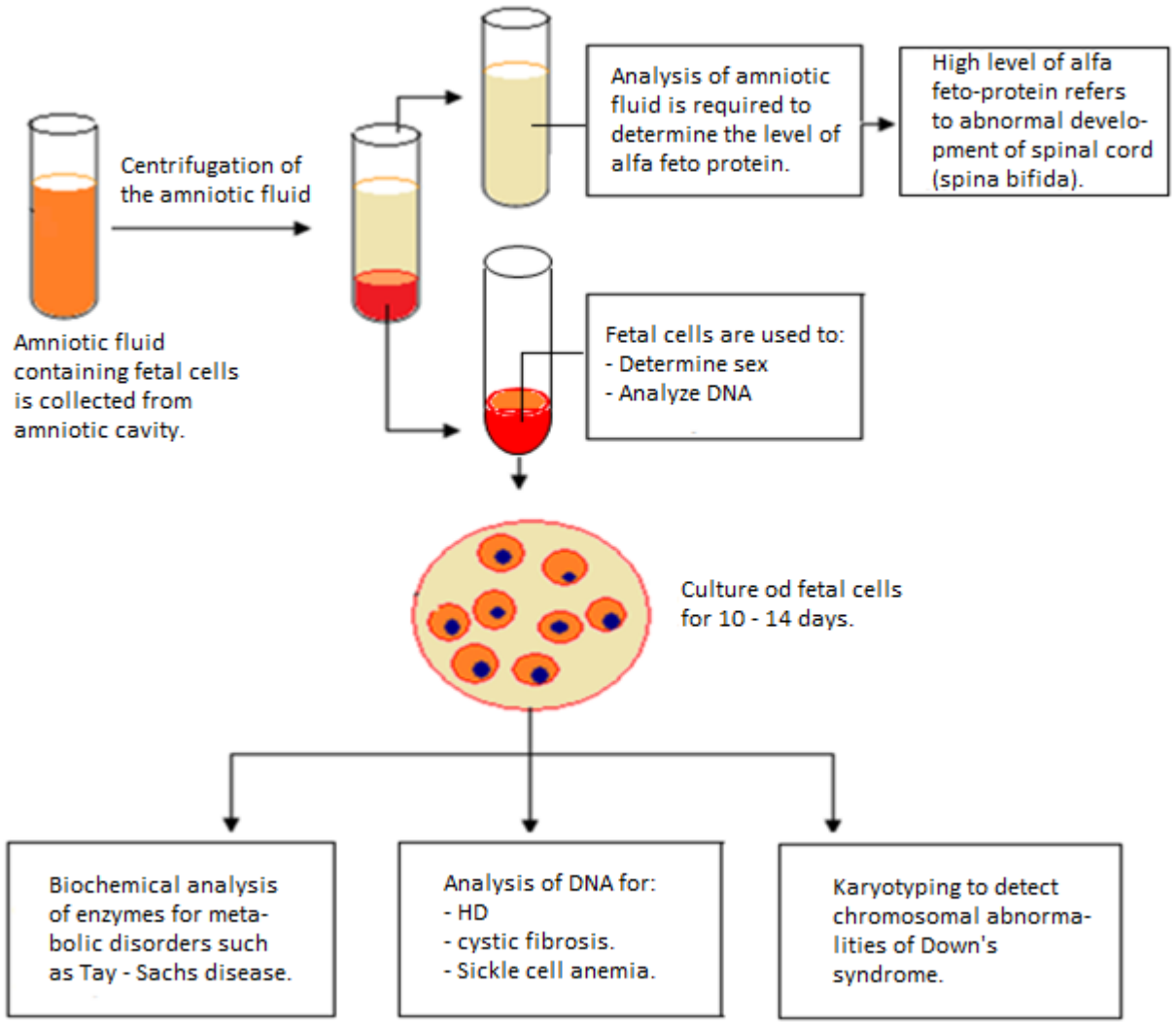


Fig. 8-14: Amniocentesis. Analysis of both amniotic fluid and fetal amniotic cells is used for prenatal detection of chromosomal abnormalities and diagnosis of many metabolic disorders and genetic diseases such as cystic fibrosis, sickle cell anemia and Huntington's disease (HD).

Gene Therapy

Gene therapy is an experimental treatment used for a variety of medical conditions.
This treatment is based on using viruses and other carriers, *vectors*, to transport healthy genes into human cells that contain a defective or missing gene, in order to restore cell function, or give the cell a new function.
The possible goals for gene therapy in the treatment of medical conditions are that the gene therapy may be used to:

- Change or fix an abnormal gene so that it functions normally.
- Insert healthy genes into cells to replace an absent gene or to compensate for one gene that is poorly functioning.
- Replace an abnormal gene with a normal one.

Many diseases could be treated in this way such as:

- Immune deficiency,
- Hemophilia,
- Cystic fibrosis,
- Phenylketonuria,
- Muscular dystrophy,
- Thalassemia and sickle cell anemia.

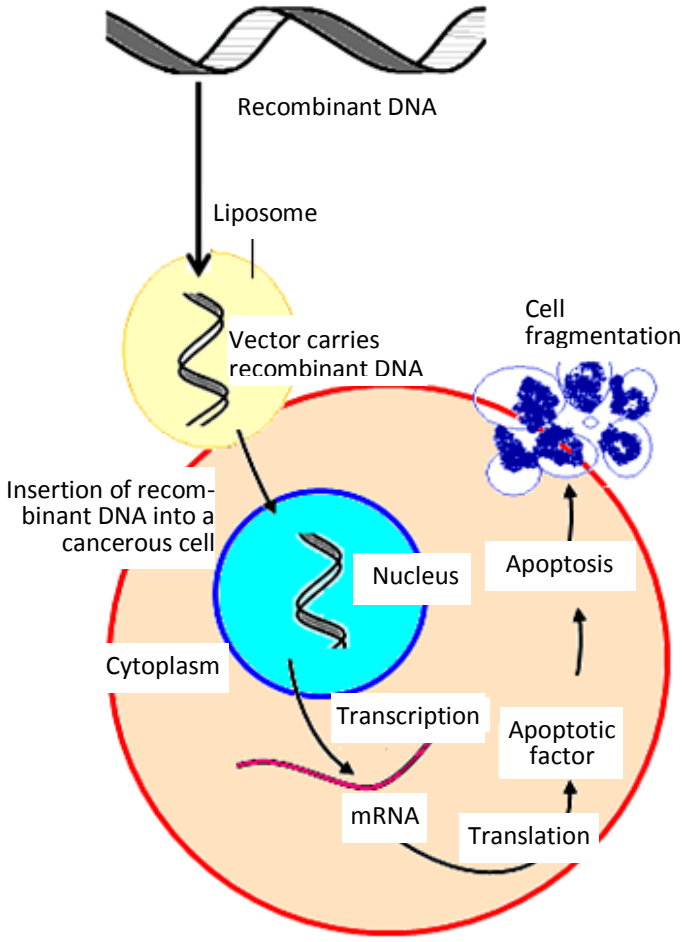


Fig. 8-15: Diagram illustrates gene therapy technique. It uses viruses or other non-biological vectors to transport DNA fragment into abnormal human cell, for example to initiate apoptosis or programmed cell death of cancer cell.

Although gene therapy has shown great promise, it is important to remember that much more research is needed to produce safe, reliable genetic treatment and determine the proper therapeutic effective dosage of gene for treatments of genetic diseases. *For example in cancer:*

- Gene therapy must be a tumor suppressor gene.
- Gene therapy causes what is known as "apoptosis" or programmed cell death of cancer cells.
- Gene therapy activates the immune system to fight cancer cells.

Gene therapy requires a system of vectors by which DNA is delivered to the target cells. The delivered DNA can override and change the defective genes of the target cells.

There are two main types of vector system:

i. Non-viral vector (*liposome*).
ii. Viral vector *(adenovirus, retrovirus, lentivirus),* by replacing the genetic components by therapeutic gene.

What are the characters of the vector that can be used in gene therapy?

The general characters of the vector that can be used in gene therapy are:

- It can be easily produced and immunologically inactive.
- It is able to deliver the gene to the required tissue only.
- It is able to multiply and transport to progeny.

Genetics of Human Blood

Discovery of Blood Groups

The human blood groups have been discovered by the Austrian *Karl Landsteiner in 1901*. He also explained why mixing blood from two individuals or the transfer of blood or blood components into a person's blood stream can lead to blood clumping or agglutination.

Blood clumping is an immunological reaction which occurs when the receiver of a blood transfusion has antibodies against the donor's blood cells.

Composition of Blood

- A human adult has about 4–6 liters of blood circulating in the body. Among other things, blood transports oxygen to various parts of the body.
- Blood consists of fluid called plasma in which several types of cells are floating:

- ***The red blood cells*** contain hemoglobin, a protein that binds oxygen. Red blood cells transport oxygen from the lung to body tissues, and carbon dioxide from the body tissue to the lung.
- ***The white blood cells*** fight infection.
- ***The platelets*** help blood clot formation to reduce blood loss at an accidental injury.
- ***The plasma*** contains salts and various kinds of proteins.

Because of the presence or absence of certain protein molecules in blood which are called *antigen* and *antibodies*, the human blood is different from one person to another.

- The antigens are located on the surface of the red blood cells. *Antigen is a substance capable of stimulating an immune system.*

The antibodies are found in the blood plasma. *Antibodies (anti-A and anti-B) are proteins that appear in plasma of persons lacking corresponding antigens on their RBCs. They are produced by the immune system and combine with the specific antigen (agglutination) for example Anti-A combines with antigen A.*

- Individuals have different types and combinations of antigens and antibodies. The blood group you have depends on what you have inherited from your parents.
- There are more than *20 genetically determined blood group systems* known today, but the *AB0 and Rh systems* are the most important ones used for blood transfusions.

ABO Blood Grouping System

There are four different kinds of human blood types: A, B, AB or 0 (null).

- The human *O, A, B, AB* blood types are inherited through *multiple alleles representing single locus.*
- *Allele* I^A codes for synthesis of glycoprotein called *antigen A* which is expressed on the cell surface**.**
- *Allele* I^B is responsible for production of a glycoprotein called *Antigen B*.
- *Allele* i^O does not code for an antigen and is *recessive* to other alleles.
- An individual with genotype I^AI^A *or* I^Ai^O has *blood type A phenotype***,** his RBCs carry A-antigens and his plasma contains B-antibodies.
- An individual with genotype I^BI^B *or* I^Bi^O has *blood type B,* his RBCs carry B-antigens and his plasma contains A-antibodies.
- An individual with genotype *iOiO* has*blood type O,* his RBCs don't carry A or B-antigens and his plasma contains A and B -antibodies.
- An individual with genotype I^AI^B has *blood group type AB***,** his RBCs carry both A and B -antigens and his plasma contains neither A nor B -antibodies.

Mixing incompatible blood groups leads to blood clumping or agglutination, which is dangerous for blood-receivers.

Determination of Parentage

The methods, which are used to determine the parent of a particular child, are:

1. Blood type test
2. DNA fingerprinting
3. Tissue typing

Blood Type Test

Blood type tests can never prove that a certain person *is* the parent of a particular child. They can determine whether he or she *could be*.

Please answer the following questions:

- Could a man with blood type- AB be the father of a child with blood type- O?
- Could a woman with blood type- O be the mother of a child with blood type- AB?
- Could a type-B child with a type-A mother have a type-A father or type- O father?

DNA -fingerprinting

DNA fingerprinting has been discussed in chapter – 7 (restriction fragment length polymorphisms, RFLPs).

Tissue Typing

- Tissue typing means examination of inherited antigens which are found on the surfaces of the body's cells.
- Tissue typing tests are used now in organs and tissue transplantation to determine whether the tissue is suitable for recipient.
- Only identical twins have the same DNA fingerprint and the same tissue type. These tests have greater than 99% certainty (sureness) and can come close to proving parentage.

Transfusion of ABO blood types

Blood transfusion will work if a person who is going to receive blood has a blood that doesn't have any *antibodies against the donor blood's antigens.* But if a person who is going to receive blood has antibodies matching the donor blood's antigens, *the red blood cells in the donated blood will clump*.

- People with blood group O are called "universal donors" and people with blood group AB are called "universal receivers" (table 8- 1 and fig. 8-16). But it is recommended that it will be safe if a person receives blood only from other having the same blood type.

- The agglutinated red blood cells can close blood vessels and stop the circulation of the blood to various parts of the body. Agglutination breaks down the red blood cells. The red blood cells contain hemoglobin which becomes toxic when it is present outside the cell. This can have fatal consequences for the recipient.

Rh -system

- There are more than 40 types of Rh blood group antigens.
- The most important *Rh allele* is that responsible for formation of D -antigen on the surface of red blood cell. People who have it are called Rh^+ and those who don't have it are called Rh^-.
- A person with Rh^- blood does not have Rh antibodies in the blood plasma. But a person with Rh^- blood can *develop* Rh antibodies in the blood plasma if he or she receives blood from a person with Rh+ blood, whose Rh antigens can stimulate the production of Rh antibodies.
- A person with Rh^+ blood can safely receive blood from a person with Rh^- -blood.

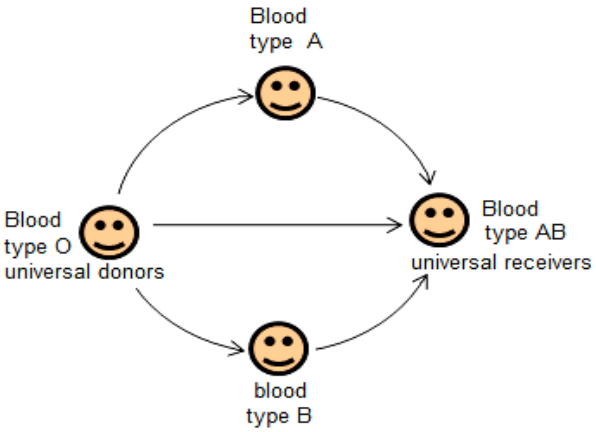


Fig. 8-16: Blood transfusion. An individual of blood type O is a universal donor and an individual of blood type AB is a universal receiver

Phenotype (blood type)	Genotype	Antigens on RBCs	Antibodies in plasma	Can donate blood to	Can receive blood from
A	I^AI^A, I^AiO	A	Anti- B	A, AB	A, O
B	I^BI^B, I^BiO	B	Anti- A	B, AB	B, O
AB	I^AI^B	AB	None	AB	A, B, AB, O
O	ii	None	Anti- A, Anti- B	A, B, AB, O	O

Table 8-1: Blood transfusion: People with blood type O can donate their blood to people carrying the other types of blood. People with blood group AB can receive blood from all other types of blood.

Summary of the role of Rh allele in Rh incompatibility or hypersensitivity

- Rh allele controls the expression of RBCs-antigens. The most important of these is that responsible for antigen –D.
- Rh system has at least 8 different kinds.
- Rh allele is responsible for formation of antigen –D on surface of RBCs.
- Rh^+ person carries antigen - D.
- Rh^- person does not contain antibodies against antigen - D, but can produce antibodies if he or she is exposed to Rh^+ blood.
- The Rh^+ allele is dominant over allele of Rh^- allele.
- Rh^- persons are homozygous recessive (Rh^- Rh^-).
- Rh^+ persons are homozygous dominant or heterozygous (Rh^+ Rh^+ or Rh^+ Rh^-).

Rh - incompatibility

When an Rh^- -woman is married to an Rh^+-man and becomes pregnant in an Rh^+ - fetus, she will have a disease called Rh incompatibility disease or *erythroblastosis fetalis***.**

In *erythroblastosis fetalis* (fig. 8-17), some fetal blood reaches the mother blood circulation in late pregnancy or during birth. The immune system of an Rh^- mother synthesizes anti-D against the fetal RBCs. Anti-D passes on into fetal circulation in next pregnancy and causes hemolysis of fetal RBCs. It may cause abortion.

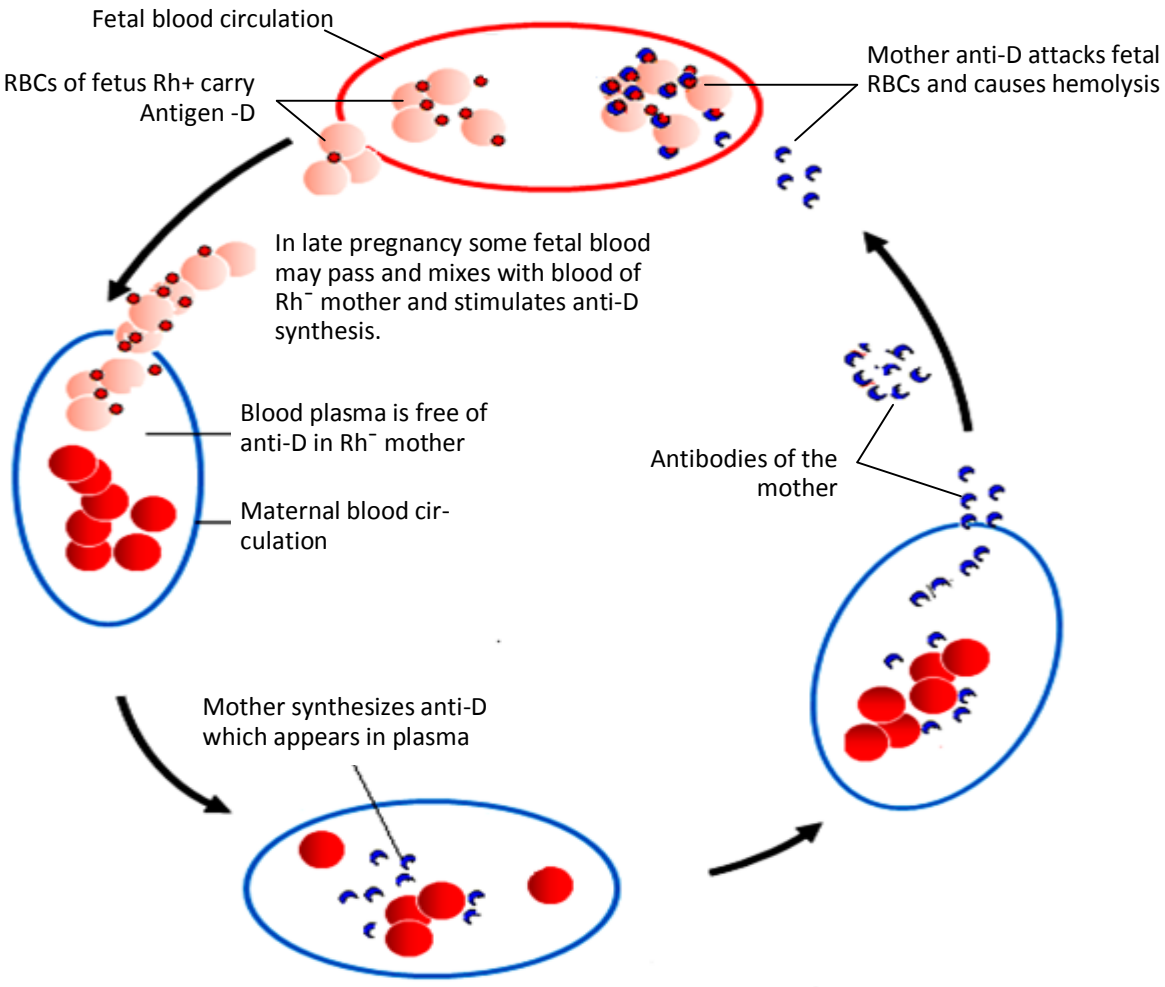


Fig. 8-17: Diagram illustrates how the immune system of an Rh⁻ mother synthesizes anti-D against the fetal RBCs. Anti-D passes on into fetal circulation in next pregnancy and causes hemolysis of fetal RBCs. Rh incompatibility is the reason for hemolysis of Rh+ RBCs of fetus. It causes a disease in extreme cases known as Erythroblastosis Fetalis. Hemolysis leads to release of toxic hemoglobin which damages organs including brain and may kill the fetus.

Additional reading

Brown K (2000): The human genome business today. Scientific American, 283, (1), pp 50-58

Emanuel BS and Saitta SC (2007): From microscopes to microarrays: Dissecting recurrent chromosomal rearrangements. Nat. Rev. Genet. Nov; 8 (11): 869-83

Epple JM et al. (2007): Strainer: Software for analysis of population variation in community genomic datasets. BMC Bioinformatics, 8(1): 398, 17.

Ezzell C (2000): Beyond the human genome. Scientific American, 283, (1) 64-69.

Friedmann T (2000): Principles for human gene therapy. Science, 287: 2163-4.

Howard K (2000): The Bioinformatics Gold. Scientific American, 283, (1), 58-63

Lee YS, Poh LK, Kek BL, Loke KY (2008): Novel melanocortin 4 receptor gene mutations in severely obese children. Clin Endocrinol. (Oxf) 68: 529-35.

Lewin B (2002): Genes VII: The definitive textbook of molecular genetics. New York: Oxford University Press Inc.

Mange EJ, Mange AP (1999): Human pedigrees. In: Basic Human Genetics, 2nd edition. Sunderland, MA: Sinauer Associates Inc., 67-94.

Marshall CJ (1999): Tumor suppressor genes. Cell, 64, 313-326.

McKusick, VA (1998). Mendelian Inheritance in Man; A Catalog of Human Genes and Genetic Disorders.

Baltimore, Maryland: The Johns Hopkins University Press.

Pedraza-Farina LG (2006): Mechanisms of oncogenic cooperation in cancer initiation and metastasis. Yale J Biol Med. 79(3-4): 95-103.

Repsilber D et al. (2005): Tutorial on microarray gene expression experiments. An introduction, Methods Inf. Med. 44(3): 392-9.

Schröck E et al. (1996): Multicolor spectral karyotyping of human chromosomes. Science, 273 (5274): 494.

Solomon EP, Berg LR and Martin DW (2002): Biology. 6th ed. Human genome, United States: Thomson Learning Academic Resources Center.

Sorensen KD et al. (2007): Chromosomal deletion, promoter hypermethylation and down regulation of FYN in prostate cancer. Int. J. Cancer, 17. 71 - 76.

Strasser BJ (1999): Sickle Cell Anemia, a Molecular Disease. Science, 286 (5444): 1488-1490.

Zoller H et al. (2007): CFTR gene mutations in pancreatitis: Frequency and clinical manifestations in an Austrian patient cohort. Wien Klin Wochen-schr. 119: 527-533

Activity:

Part I: Multiple Choice Questions

Choose one best correct answer.

1. Which of the following is used to study thousands of genes in normal and diseased cells?
   a. Bioinformatics
   b. Pharmacogenetics
   c. Pedigree analysis
   d. DNA microarray

2. For studying which of the following is pedigree analysis used?
   a. Genotype of an organism
   b. History of a particular trait in the families
   c. Heterozygosity of unknown genotype
   d. Chromosomal abnormalities

3. Karyotyping technique is used for studying which structure of the following?
   a. Chromosomes
   b. DNA molecules
   c. RNA molecules
   d. Cell cytoplasm

4. Which of the following is the normal female human karyotype?
   a. 45, XO
   b. 46, XY
   c. 46,XX
   d. 47, XXY

5. Which of the following **isn't/aren't** used in matching homologous chromosomes?
   a. Length of chromosomes
   b. Position of centromeres
   c. Pattern of banding
   d. Kinetochores

6. Which chromosomes of the following occupy the 1st position in the picture of human karyotype?
   a. The largest homologous pair
   b. The intermediate pair
   c. The smallest homologous pair
   d. The sex chromosomes

7. Which of the following is the goal of human genome project?
   a. Detection of sex chromosomes.
   b. Sequencing of all DNA in human genome.
   c. Studying of karyotype of human.
   d. Detection of heterozygosity.

8. Which situation of the following **doesn't** result from chromosomal non-disjunction?
   a. Translocation
   b. Tetraploidy
   c. Trisomy
   d. Triploidy

9. In which phase of the following may formation of a clone of abnormal cells in a normal organism appear as a result to non-disjunction?
   a. Anaphase- I
   b. Anaphase- II
   c. Prophase- I
   d. Anaphase

10. Which case of the following refers to presence of a single extra chromosome in cell or organism?
    a. Trisomy
    b. Monosomy
    c. Polyploidy
    d. Triploidy

11. Which case of the following refers to presence of an extra set of chromosomes?
    a. Trisomic
    b. Monosomic
    c. Triploidy
    d. Tetraploidy

12. Which disease of the following results from loss of a part of chromosome #5?
    a. Fragile x –syndrome
    b. Huntington disease
    c. Color blindness
    d. Cri-du-chat syndrome

13. Which of the following is ***not*** a type of abnormalities in chromosomal structure?
    a. Translocation
    b. Deletion
    c. Fragile x syndrome
    d. Triploidy

14. Which disease of the following is ***not*** an autosomal aneuploidy?
    a. Patau syndrome
    b. Edward syndrome
    c. Down syndrome
    d. Cri-du-chat syndrome

15. Sex chromosomal aneuploidy may be the cause of which syndrome of the following?
    a. Patau syndrome
    b. Down syndrome
    c. Edward syndrome
    d. Klinefelter's syndrome

16. If a woman has the karyotype 45, XO, you will expect she has which disorder of the following?
    a. Turner syndrome
    b. Klinefelter's syndrome
    c. Hemophilia
    d. Cystic fibrosis

17. Which genetic disorder of the following results from defective recessive autosomal allele?
    a. PKU
    b. Huntington disease
    c. Hemophilia
    d. Color blindness

18. Which genetic disorder of the following results from defective dominant autosomal allele?
    a. Cystic fibrosis
    b. Tay-Sachs disease
    c. Sickle cell anemia
    d. Huntington's disease

19. Which genetic disease of the following results from accumulation of phospholipid in neurons due to absence of specific lysosomal enzymes?
    a. Cystic fibrosis
    b. Tay-Sachs disease
    c. Sickle cell anemia
    d. Huntington disease

20. Which disease of the following causes abnormal formation of hemoglobin?
    a. Tay-Sachs disease
    b. Cystic fibrosis
    c. Sickle cell anemia
    d. Cri-du-Chat

21. Which technique of the following is used in prenatal diagnosis of genetic diseases?
    a. Amniocentesis
    b. Cell fractionation
    c. Reverse transcription
    d. DNA hybridization

22. Blood groups have been discovered by:
    a. Gregor Mendel
    b. Watson and Crick
    c. Karl Landsteiner
    d. Archibold Garrod

23. Which blood type person of the followings is a universal receiver?
    a. A
    b. B
    c. O
    d. AB

24. Which blood type person of the followings is a universal donor?
    a. A
    b. B
    c. O
    d. AB

25. Which of the following is the genotype of a blood type -B person?
    a. AA, AO
    b. BB, BO
    c. AB
    d. AO

26. Which type of antibodies in blood plasma is carried by a blood type AB person?
    a. Anti –A only
    b. Anti –B only
    c. Anti–A and anti-B
    d. None of the above

27. Which defective allele of the following causes Huntington's disease?
    a. A sex-linked recessive allele
    b. An autosomal recessive allele
    c. A sex-linked dominant allele
    d. An autosomal dominant allele

28. Rh -incompatibility may cause a serious problem for pregnant Rh^- woman, if she has which pregnancy of the following?
    a. Rh^- -offspring in 1^{st} pregnancy.
    b. Rh+ -offspring in 1^{st} pregnancy.
    c. Rh^- -offspring in 2^{nd} pregnancy.
    d. Rh+ -offspring in 2^{nd} pregnancy.

29. Erythroblastosis fetalis is a disease resulting from which condition of the following?
    a. Blood transfusion
    b. Hypersensitivity of fetal blood
    c. Anemia of vitamin B12 deficiency
    d. Rh -incompatibility

30. All the following genetic diseases are caused by mutant autosomal recessive allele, ***except*:**
    a. PKU
    b. Klinefelter's syndrome
    c. Cystic fibrosis
    d. Tay-Sachs disease

31. Which mutation of the following results in replacement of glutamic acid by valine in sickle cell anemia results from?
    a. Frame shift mutation
    b. Non-sense mutation
    c. Silent mutation
    d. Missense mutation

32. For a man to be color blind, he must have which allele of the following?
    a. X-linked dominant allele
    b. X-linked recessive allele
    c. Y-linked dominant allele
    d. Y-linked recessive allele

33. If the parents have AO and BO blood types, which blood type of the following may their offspring have?
    a. AO and BO
    b. AA, BB and OO
    c. AO and BB
    d. AO, BO, AB and OO

34. Which condition of the following is an X-linked disorder?
    a. Sickle cell anemia
    b. Huntington disease
    c. Albinism
    d. Hemophilia

35. Under which condition could a woman have a color blind daughter?
    a. If her husband is normal.
    b. If her husband is color blind.
    c. If she and her husband are color blind.
    d. If she is a carrier and her husband is normal.

36. Which karyotype of the following refers to Turner syndrome?
    a. 47, XXY
    b. 46, XY
    c. 45, XO
    d. 45, OY

37. A person with Down syndrome has three copies of which chromosome of the following?
    a. 2
    b. 13
    c. 18
    d. 21

38. For safe gene therapy, the process should cause which of the following?
    a. Cellular hypotrophy
    b. Cellular hypertrophy
    c. Delivering of functioning target gene
    d. Increase of cell growth

39. Which of the following can be safely used in gene therapy as a vector?
    a. liposome
    b. Plasmid
    c. E.coli
    d. Bacteriophage

40. All the following are multi-factorial disorders ***except*:**
    a. Insulin dependent diabetes mellitus
    b. Schizophrenia
    c. Essential hypertension
    d. Sickle cell anemia

41. Which genes of the following may cause abnormal cell proliferation?
    a. Proto-oncogenes
    b. Oncogenes
    c. Active suppressor genes
    d. Inactive mutant genes

42. Which karyotype of the following refers to Klinefelter's syndrome?
    a. 47, XXY
    b. *47, XY, + 17 p+*
    c. *47, XX,+21*
    d. 45, XO

43- All the followings are environmental causes of birth defects ***except***:
    a. Viral contact
    b. Medication
    c. Radiation
    d. Chromosomal abnormalities

Part II: Short Answer Questions.

C) Fill in the spaces:

1. In human karyotype, the chromosomal abnormalities can be classified into:
   a. _____
   b. _____

2. The causes of chromosomal abnormalities in number are:
   a. _____
   b. _____

3. Chromosomal non-disjunction means :
   _____
   _____

4. Chromosomal non-disjunction in meiosis results in production of gametes containing _____ _____or lacking_____

5. Non disjunction in mitosis results in formation of _____ in a normal organism.

6. What are the types of chromosomal abnormalities in number?
   a. _____
   b. _____

7. Polyploid means:_____
   _____

8. Types of polyploidy are:
   a. _____
   b. _____

9. Polyploid is common in _____and rare in _____and lethal in_____.

10. Triploidy (3n) causes_____, while tetraploidy (4n) causes_____ in human.

11. Polyploidy is healthy in _____ and harmful in_____.

12. Causes of polyploid are :
    a. _____
    b. _____

13. Aneuploidy is much harmful than _____, while monosomy is much worse than _____.

14. Fill in the spaces:
    a. Trisomy 21 causes _____disease.
    b. Trisomy18 causes _____ disease.
    c. Trisomy 13 causes _____disease.

15. The most common autosomal aneuploidy among children of mothers over 40 years of age is _____that causes _____ _____.

16. An example for disorders due to sex chromosomal aneuploidies are:
    b. Karyotype _____causes _____syndrome.
    c. Karyotype _____causes _____syndrome.

17. Exchange of chromosomal segments between non homologous chromosome (translocation) leads to:
    a. _____
    b. _____
    c. _____

18. Balanced translocation means:
    _____
    _____

19. Large chromosomal deletions may be lethal and result in_____.

20. Small deletions may cause genetic disorders such as _____that result from the loss of a short arm of chromosome # 5.

21. Fragile site is _____
    _____. It causes _____syndrome.

22. What are the types of Aneuploidy?
    a. _____
    b. _____
    c. _____
    d. _____

23. What are the methods of detecting the genetic disorders?
    a) _____
    b) _____
    c) _____

24. When could 2 phenotypically normal parents produce a diseased offspring?
    _____

25. A boy becomes sick with x - linked recessive disorder if his mother is _____ and his father is_____.

26. The normal female human karyotype is
    _____

27. - Chromosomes are classified anatomically into:
    _____.
    _____.
    _____.

28. Which technique is used to study the metaphase chromosomes in blood cell lymphocyte?
    _____.

29. Which phase of mitosis is used to study human chromosomes? _____.

30. - Metaphase chromosome is formed of _____attached at_____.

31. In karyotyping, chromosomes arrange according to_____, while the largest chromosome is _____, the smallest chromosome is_____.

32. Why does aneuploidy have a harmful effect!
    _____
    _____
    _____

D) Define each term of the following:

a. Polyploidy:
   _____
   _____

b. Aneuploidy:
   _____
   _____

c. Trisomy:
   _____
   _____

d. Monosomy:

_____

_____

e. Tetrasomy:

_____

_____

g. Pedigree analysis

_____

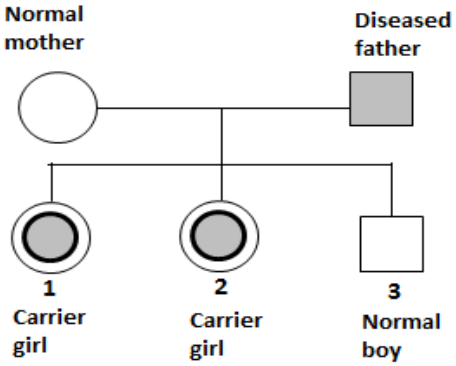
_____

h. Karyotype

_____

_____

E) The following diagram shows a family which is formed of a normal mother, a diseased father, two carrier girls and a normal boy.



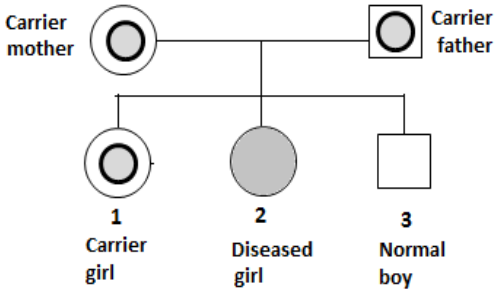
- By which allele of the following this genetic disease is carried?_____

  a. Autosomal dominant allele
  b. Autosomal recessive allele
  c. X-linked recessive allele

- Determine the genotype for each member of the family (homozygous or heterozygous)

  - Genotype of mother is:_____
  - Genotype of father is:_____
  - Genotype of girl # 1 is: _____
  - Genotype of girl # 2 is: _____
  - Genotype of boy # 3 is: _____

F) The following diagram shows a family which is formed of carrier parents, a diseased girl, a carrier girl and a normal boy.



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  - Genotype of father is:_____
  - Genotype of girl # 1 is: _____
  - Genotype of girl # 2 is: _____
  - Genotype of boy # 3 is: _____

Answer of MCQs:

1)D	10) A	19) B	28) D	36) C
2) B	11) C	20) C	29) D	37) D
3) A	12) A	21) A	30) B	38) C
4) C	13) D	22) C	31) D	39) A
5) D	14) D	23) D	32) B	40) D
6) A	15) D	24) C	33) D	41) B
7) B	16) A	25) B	34) D	42) A
8) A	17) A	26) D	35) C	43) D
9) D	18) D	27) D		

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