ZO(N)-102 & ZO(N)-102L

B. Sc. Semester- 2
Cell and Molecular Biology and Lab Work

DEPARTMENT OF ZOOLOGY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

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Course 1: Cell and Molecular Biology and Lab Work

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UNIT 1 CELL TYPE

Contents

1.1 Objectives

Study of this unit will let the students to:

- Define Prokaryotic cell;
- Explain the structure of prokaryotic cell;
- Write about Eukaryotic cell;
- Elucidate the structure of Eukaryotic cell;
- Differentiate between prokaryotic and eukaryotic cell.

1.2 Introduction

A structure containing a mass of cytoplasm surrounded by semi-permeable membrane called plasma membrane is called a cell. It encloses cytoplasm, many cell organelles along with nucleus or nuclear material. On the basis of organization of membranes, variety and structure of cytoplasmic organelles and complexity of nuclear region, the cells are classified into two types: Prokaryotic cell and Eukaryotic cell. These terms were suggested by **Hans Ris** in **1960s**.

1.3 History and Origin

A cell was defined as **"**unit of biological activity delimited by a semi permeable membrane and capable of self-reproduction in a medium free of other living systems" by **Loewy and Siekevitz (1963)**.

The study of cell has been made possible with the help of light microscope. **Robert Hooke (1665)** with the help of light microscope discovered that a section of cork is made up of small cavities surrounded by firm walls. He used the term **"cell"** for the first time to describe his investigations on the "texture of a piece of cork". Later on **A. Van Leeuwenhoek (1632-1723)** observed various unicellular organisms and cells like bacteria, protozoan's, red blood cells and sperm etc. He observed nucleus in some erythrocytes and all this was made possible with the improved microscopes. In **1809, Mirble M.** stated that all plant tissues are composed of cells. In the same year, importance of cells in living organisms was described by **J.B. Lamarck**. **Robert Brown** in **1831** observed nucleus in certain plant cells. *Mimosa* cells were boiled in nitric acid by **Dutrochet (1837)** to separate the cells to conclude that all organic tissues are composed of globular cells, united by simple adhesive forces. "All living organism are composed of cells" was stated by **Schwann, T. (1839)** after examining a variety of animals and plant tissues.

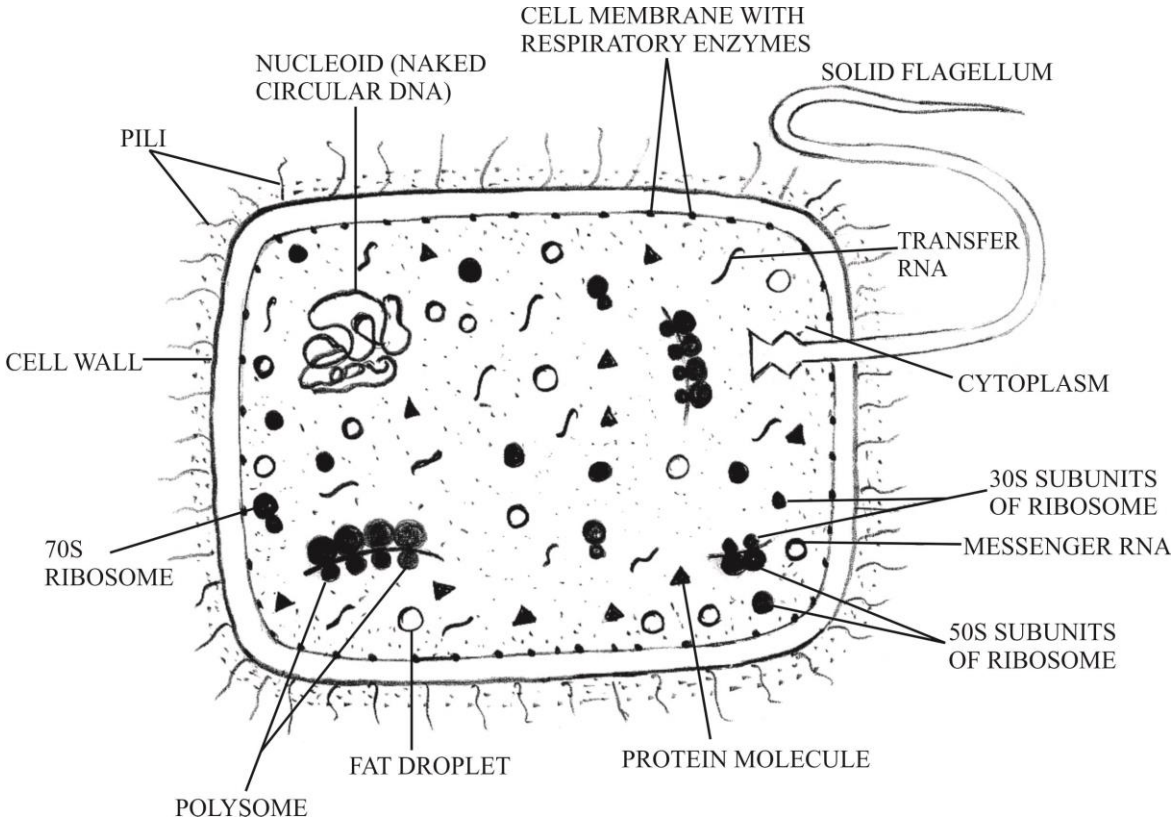

Fig. 1.1: A Bacterial Cell

1.4 BASIC COMPONENTS OF PROKARYOTIC AND EUKARYOTIC CELL

1.4.1 Prokaryotic Cells

Prokaryotic cells are the most primitive cells and have simple structural organization. It has a single membrane system. They include bacteria, viruses, blue-green algae, mycoplasmas, rickettsias, spirochetes etc. Cyanobacteria or blue green algae are the largest and most complex prokaryote, in which photosynthesis of higher plants type have evolved. **Prokaryotes** are included in the kingdom **Monera** and the super kingdom **Prokaryota**. The Prokaryotes have the following characters:

1. The size of prokaryotic cells ranges between 1 to 10 µm. They occur in a variety of forms.

2. Prokaryotic cell consists of three main components:

(I) **Outer covering:** It is composed of inner cell or plasma membrane, middle cell wall and outer slimy capsule.

a. **Cell membrane:** Cell membrane made up of lipids and proteins, is thin and flexible and controls the movement of molecules across the cell. Respiratory enzymes are carried by it for energy releasing reactions. **Mesosomes**, the in-folds of plasma

membrane bears respiratory enzymes and these are considered analogous to mitochondria of eukaryotic cells. Similarly, the pigments and enzymes molecules that absorb and convert the light into chemical energy in photosynthetic cells are also associated with the plasma membrane's in-folds called **photosynthetic lamella**. These lamellae are analogous to the chloroplast of eukaryotic cells. Plasma membrane plays role in replication and division of nuclear material. Since the in-folds remain continuous with the cell membrane, they are not considered as separate compartments. Thus, prokaryotic cell is non-compartmentalized.

b. **Cell wall :** It is a rigid or semi-rigid non-living structure that surrounds the cell membrane and its thickness ranges between 1.5 to 100 µm. Chemically it is composed of **peptidoglycans**. . Some bacteria such as mycoplasmas lack cell wall.

c. **Slimy capsule:** A gelatinous coat outside the cell wall is the slimy capsule. It is composed of largely of polysaccharides and sometimes it may have polypeptides and other compounds also. It protects the cell against desiccation, virus attacks, phagocytosis and antibiotics

(II) **Cytoplasm:** Prokaryotic cytoplasm contains proteins, lipids, glycogen and inorganic ions along with enzymes for biosynthetic reactions and ribosomes, tRNA and mRNA for protein synthesis. Prokaryotic cytoplasm has some special features as follows:

a. It lacks cell organelles like endoplasmic reticulum, mitochondria, Golgi apparatus, Centrosomes, vacuoles, Lysosomes, microfilaments, intermediate filaments and microtubules.

b. The only cytoplasmic organelle found in prokaryotic cells is the **ribosomes**. They are smaller than eukaryotic ribosomes i.e., 70S and lie free in the cytoplasm. They form poly-ribosomes at the time of protein synthesis. They are the sites of protein synthesis.

c. Like eukaryotic cells, the cytoplasm of prokaryotic cell does not show streaming movement or cyclosis.

d. Gas vacuoles are also formed in some prokaryotic cells.

e. The cell does not show phagocytosis, pinocytosis and exocytose, substances enter and leave the cell through the cell membrane.

f. They may contain deposits of polysaccharides or inorganic phosphates.

(III) **Nucleoid:** Nuclear envelope is absent in prokaryotic cell and the genetic material lies directly into the cytoplasm. Such nuclear material is known as **nucleoid**. **Nucleoid** consists of greatly coiled single pro-chromosome. It shows the following special features:

a. A short and simple pro-chromosome is present which is attached at least at one point on cell membrane.

b. Mostly there is single copy of chromosome, the prokaryotic cell is haploid.

c. **The DNA is naked** as it is not associated with basic histone proteins. It is double stranded, helical and circular.

d. The amount of DNA is lesser than eukaryotic cell and it codes fewer proteins. Replication of DNA is continuous throughout the cell cycle. Transcription and translation occurs in cytoplasm and processing of mRNA is not required.

e. The processes like meiosis, gamete formation or fertilization are absent. Conjugation is seen in some bacteria.

f. Mitotic apparatus absent.

g. There is no nucleolus.

h. Cell membrane folds or mesosomes help to segregate the replicated products of chromosomes into daughter cells.

3. **Plasmids:** In some prokaryotic cells, in addition to nucleoid, a small circular double stranded DNA molecule is present. It is called **plasmid**. Plasmids have 1000 to 30,000 base pairs and they generally encode proteins required by the organism to resist antibiotic and other toxic material.

4. **Flagellum:** It is a whip like locomotory structure found in many bacteria. It is 150Å thick and 10 to 15µm long. As the flagellum does not have any surrounding membrane, it grows at the tip.

It has two main parts: Filament and basal body.

(i) **Filament-** Filament extends out of cell into the medium and it is composed of many intertwined spiral chains of the subunits of a protein called **flagellin**. Flagellin differs from actins or tubulin.

(ii) **Basal Body-** The basal body attaches the flagellum to the cell and generates the force to rotate it. It is composed of many components and numerous proteins. It has two parts: shaft and hook.

5. **Pili:** These are short, rod like non-motile processes or fimbriae present on many bacteria. These are formed of pilin protein. They are usually less than 10 nm thick. They help in attachment of bacteria to surfaces or food or to one another. Tubular sex Pili are present in some bacteria.

Prokaryotic cells have all the biochemical mechanisms required to synthesize complex organic materials from simple organic precursors necessary for life. Thus,

inspite of being simple in structure prokaryotes are more versatile in their synthetic activities than eukaryotes.

1.4.2 Eukaryotic Cells

The internal organization of eukaryotic cell is more developed than prokaryotic cells from which they are believed to have been evolved. They are evolved to have double membrane system. Primary membranes are the one that surrounds the cell, celled cell or plasma membrane and the secondary membrane surround the nucleus and other cellular organelles. Eukaryotic cells occur in protists, fungi, plants and animals. Eukaryotic cells have the following characteristics:

1. **Number-** In multicellular organisms the numbers of cells are correlated with the body size. The human blood contains about 30 quadrillion (3×10^{15}) corpuscles and a 60 kg human being has about 60×10^{15} cells. All multicellular organisms begin their life with a single cell "Zygote" and then become multicellular by its mitotic division during development.

2. **Shape-** A cell may be spherical, cuboidal, oval, disc-like, polygonal, columnar, spindle like or irregular. Thus, cells acquire a variety of shapes not only in various organisms but also in different tissues of the same organism. The shape of cell is correlated with its functions like the shape of muscles and nerve cells are well adapted to their functions. Many factors such as cell functions, age of cell, presence or absence of cell wall, viscosity of cytoplasm etc. are responsible for various shapes of cells.

3. **Size-** Most of the eukaryotic cells is microscopic and their size ranges between 10 to 100µm. Sporozoits of malaria parasite (*Plasmodium vivax)* is among the smallest cells having the size equal to 2µm long. While the Ostrich egg measures 175 × 120mm. Nerve cells are the longest having the size of its fiber to be of few meters long. Human cells generally range from 20 to 30µm.

4. **Components of a cell-** Three main components of the eukaryotic cells are cell membrane, cytoplasm and nucleus. The cytoplasm and the nucleus further have several components. Various cell components are discussed below:

(i) **Cell membrane-** Cell membrane, plasma membrane or plasmalemma is a thin elastic living covering that surrounds the cell keeping the cell contents in place, provides shape to the cell and controls the transfer of materials across it. It is composed of lipid-protein complex. It lacks respiratory enzymes. In many protists and animal cells it allows endocytosis and exocytosis.

In certain protists, many fungi and all plant cells, the cell membrane is covered by a thick, rigid non-living cell wall that protects and supports the cell. In prokaryotes the cell wall surrounding the plasma membrane has a different structure in comparison to eukaryotes.

(ii) **Cytoplasm-** The cytoplasm or the cytosome is a semi-fluid, homogeneous, translucent ground substance known as cytoplasmic matrix or cytosol which is present between the cell membrane and the nucleus. In the protozoan cell the outer firm layer of cytoplasm is called ectoplasm and the inner layer around the central fluid mass is called the endoplasm. The cytosol shows "cyclosis" or the streaming movement. The eukaryotic cytoplasm has the following features:-

a. Organelles*:* The organized structures having the specific functions and capacity of growth and multiplication in some cases are known as organelles. Mitochondria, centrosomes, Golgi bodies, plastids and vacuoles are the organelles that can be observed under light microscope, while endoplasmic reticulum, ribosome, microfilaments, microtubules, intermediate filaments and micro bodies can only be seen under electron microscope. These organelles are often described as protoplasmic structures. The cells having cilia or flagella have their basal bodies at the bases are in the cytoplasm while rest of its part extends out of cytoplasm. These organelles are described as follows:

I. Mitochondria: The rod like or globule shaped structures scattered in the cytoplasm are found singly or in groups. They are bounded by **double membrane** of lipoproteins. The inner membrane gives out finger like structure known as **cristae** which partially subdivide the inner chamber of mitochondrion. On the inner surface of cristae are present mushroom like structures, **oxysomes that** are related to phosphorylation. The space between the membranes and its lumen is filled with mitochondrial **matrix**. Both the membranes and the matrix contain many oxidative enzymes and coenzymes. Since mitochondria contain DNA molecules and ribosomes, they synthesize certain proteins. They produce the energy and reserve it in the form of **adenosine triphosphate (ATP).** Due to the presence of its own DNA and ability of protein synthesis along with its duplication, the mitochondria are called **semi autonomous organelle**. The DNA of mitochondria resembles that of bacterial cell; hence it is also called as **endo-symbiotic organelle**.

II. Centrosomes: (9+0) there is a clear zone around centrioles, near the nucleus, that includes a specialized portion of cytoplasm, called **centrospheres**. Its matrix is called kinoplasm that bears two rounded bodies the "centrioles". Each centriole consists of **nine fibrillar** units and each of them is found to contain **three microtubules** arranged in a circle. Both the centrioles are arranged at right angle to each other. Centrioles form the spindles of microtubules at the time of cell division. Centrioles are absent in plant cell and the spindle is formed without their help.

III. Golgi bodies: These are the stack of flattened parallel-arranged **sacs** and **vesicles** found in association of endoplasmic reticulum. They are composed of many **lamellae, tubules, vesicles and vacuoles**. Their membranes are supposed to be originated from ER and are composed of lipoproteins. In plant cells the Golgi complex is called **dictyosome** that secretes required materials for the formation of cell

wall at the time of cell division. It helps in the formation of acrosome of sperms, release of hormones, enzymes and other synthetic materials.

IV. Plastids: These organelles are found in plant cells and are absent in animal cells. They may be colored like chloroplast or chromoplasts or colorless like leucoplast. Since the leucoplast store and metabolise the starch and lipids, they are called amyloplast and lipoplast respectively. Chloroplast contains the green pigment the chlorophyll that helps in photosynthesis and protein storage. Chloroplast has a **double outer membrane**, the **stroma**, that bears many soluble enzymes, and a complex system of membrane bound compartments called **thalakoids** constituting **granna**. Like mitochondria, chloroplast also has their own DNA, ribosomes and complete protein synthetic machinery. Hence these are also called endo-symbiotic and semi-autonomous organelle.

V. Metaplasm: The particles like vacuoles, granules and other cytoplasmic bodies such as ribonucleoprotein molecules are represented by it.

VI. Cilia, basal bodies and flagella: Cilia are the minute structures covering the surface in some cells. Both cilia and flagella originate from the **basal bodies or blepharoplast** lying in cytoplasm. They consist of nine outer fibrils with the two larger fibrils in the centre. Each fibril consists of two microtubules, or has **9+2** arrangement. Cilia and Flagella are the structure born by certain cells. They are composed of microtubules made of the protein **tubulin**. They have 9 + 2 plan of microtubule. Both grow at the base. They act as locomotory organelles, moves by their beats or undulations for they get the energy by breakdown of ATP molecule.

VII. Microtubules: The ultra fine tubules of protein (**tubulin**) traversing the cytoplasm of plant and animal cells providing the structural framework to the cell, determine the cell shape and general organization of the cytoplasm are known as microtubules. Tubules are made up of **13 individual filaments**. Microtubules help in transport of water and ions, cytoplasmic streaming (cyclosis) and the formation of spindles during cell division.

VIII. Basal granules: The spherical bodies found at the base of cilia and flagella are called the basal bodies. Each of them is composed of **nine fibrils** and each fibril consists of the three microtubules, out of which two enter the cilia or flagella.

IX. Ribosome's: Ribosome is the minute spherical structures that originate in nucleolus and are found attached with the membrane of endoplasmic reticulum and in the cytoplasm. They are mainly composed of **ribonucleic acids (RNA) and protein**. They are mainly responsible for **protein synthesis**.

b. Inclusions: These are the **non-living or deutoplasmic structures** which are incapable of growth and multiplication. Common cell inclusions are stored organic materials such as starch grains, glycogen granules, aleuron grains, fat droplets, pigment granules and inorganic crystals.Cytoplasm is stores raw materials needed for

the metabolism in both the cytoplasm and the nucleus. Many metabolic processes like biosynthesis of fatty acids, nucleotides, proteins and oxidation take place in cytoplasm. It distributes the nutrients, metabolites and enzymes in a cell and brings about exchange of materials between the organelles as well as with the environment or extracellular fluid also.

c. Nucleus: In a eukaryotic cell the genetic material is enclosed by a distinct **nuclear envelope** that forms a prominent spherical organelle the "Nucleus". The nuclear envelope bears **pores** for the exchange of materials between the cytoplasm and the nucleoplasm.

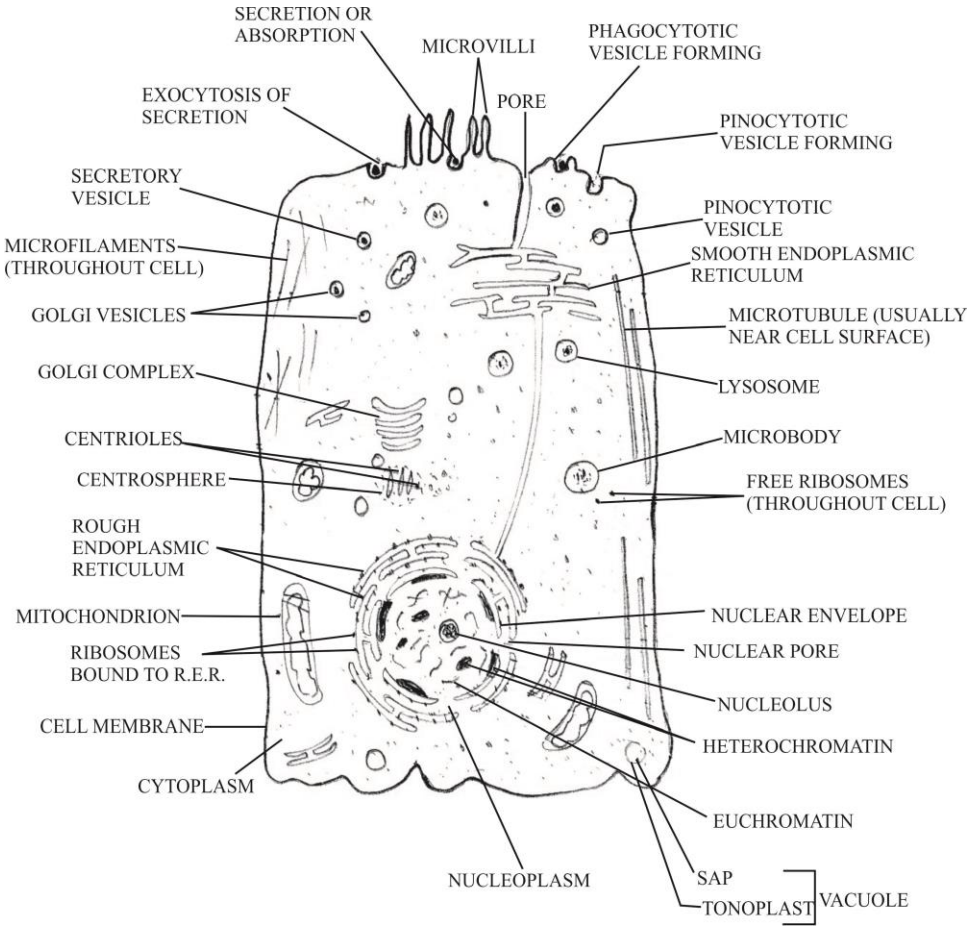

Fig. 1.2: An animal cell as shown by electron microscope

1.4.3 Differences between Prokaryotic Cells and Eukaryotic Cells

The internal organization of eukaryotic cell is more developed than prokaryotic cells from which they are believed to have been evolved.

S. No.	Prokaryotic Cells	S. No.	Eukaryotic Cells
1.	A prokaryotic cell is surrounded by a single membrane layer.	1.	A eukaryotic cell is surrounded by a double membrane layer.
2.	In most cases the cell wall surrounds the plasma membrane and it is composed of carbohydrates, lipids proteins and certain amino acids.	2.	Cell wall is present in protists, most fungi and plants and is composed of chitin in most fungi and or cellulose in others.
3.	Respiratory enzymes are present on cell membranes.	3.	Absent on the cell membrane
4.	Thalakoids occurs free in cytoplasm.	4.	They occur within the chloroplast.
5.	Cytoplasm lacks organelles like centrosomes, endoplasmic reticulum, mitochondria, Golgi apparatus, microfilaments, intermediate filaments, microtubules and micro bodies. While ribosomes are present	5.	All the cell organelles are present in the cell along with ribosomes.
6.	Gas vacuoles may occur while sap vacuoles are absent.	6.	Sap vacuoles are commonly present.
7.	70S ribosomes are present that lie free in cytoplasm or attached to mRNA.	7.	80S ribosome's are present, either free or bound to ER and nuclear envelope or mRNA.

S. No.	Prokaryotic Cells	S. No.	Eukaryotic Cells
8.	Endocytosis and exocytose do not occur.	8.	These processes take place in many protists and in animals.
9.	Process of meiosis or gamete formation or true fertilization does not occur.	9.	In these cells the process of meiosis, gamete formation and true fertilization occur in most cases of sexual reproduction.
10.	Cells are haploid.	10.	Cells are diploid, while haploid cells also occur.
11.	Nuclear envelope is absent and nuclear material lie in cytoplasm and is called nucleoid. Nucleoid contains a single chromosome.	11.	Nuclear envelope surrounds the nuclear material. The structure is called nucleus. It contains two to many chromosomes.
12.	Nucleolus absent.	12.	One or more nucleoli are present within the nucleus.
13.	Circular DNA is present without associated proteins.	13.	Nuclear DNA is linear and is associated with proteins, while extra nuclear DNA is present without proteins.
14.	Flagella if present are simple, consist of a single fibril and are formed of a protein flagellin.	14.	Flagella, if present are complex, have 9+2 pattern of microtubules formed of a protein tubulin.
15.	Plasmids and pili occur in many prokaryotic cells.	15.	These structures are absent.
16.	Most prokaryotes are	16.	Most of them are

S. No.	Prokaryotic Cells	S. No.	Eukaryotic Cells
	asexual organisms.		sexual organisms.

1.5 Summary

Robert Hook (1665) for the first time described the texture of a piece of cork as "cell". Similar structures were observed by many scientists while studying many living organisms. It was Schwann T. (1839) who stated that all living organisms are composed of cells after examining a variety of plant and animal tissues. Basically two types of cells are there, "Prokaryotic" and "Eukaryotic". Prokaryotic cells are the primitive cells that include bacteria, blue-green algae, viruses and photosynthetic cells cyanobacteria etc. Their size varies from 1 to 10 um and they consist of mainly three components: the outer covering that includes all cell membrane, cell wall and a slimy capsule. Another component is cytoplasm which lacks cell organelles except ribosomes. The processes like phagocytosis and endocytosis are absent. The third component is nucleoid that lacks nuclear membrane. Additional small circular DNA the plasmid may also be present. Flagella and pili like structure are also seen in some prokaryotic cells. Eukaryotic cells are more developed and are surrounded by double membranes. Shape and size of these cells and their number in multicellular organisms varies. It is also composed of three main components. Cell membrane or plasma membrane is a thin elastic living covering. The cytoplasm is a semi fluid, homogenous, translucent consisting of many cell organelles, inclusions, cilia, flagella, basal bodies and microtubules.

1.6 Glossary

Cytoplasm: Gel like substance enclosed within the cell membrane excluding nucleus.

Plasma membrane: It is the biological membrane that separates the interior of the cell from the outside environment.

Prokaryote: The cell that lacks a distinct nucleus and other specialized membrane bound organelles.

Eukaryote: an organism whose cell contains a membrane bound distinct nucleus along with other specialized organelles enclosed in membranes.

Mesosome: The in-folding of plasma membrane in some bacterial cells that carry respiratory enzymes.

Poly-ribosome: It is a group of ribosomes associated with a single messenger RNA during the translation process.

Phagocytosis: The process by which a cell engulfs a solid particle to form an internal vesicle known as phagosome is called phagocytosis, also called eating of cell.

Pinocytosis: The process of intake of liquid into a cell by the budding of small vesicles from the cell membrane is called pinocytosis, also called drinking of cell.

Exocytosis: In the process of exocytosis materials are exported outside the cell by using energy from ATP molecules.

Conjugation: When the genetic material is transferred from one bacterial cell to other either by direct contact or by a bridge like connection between two cells is called conjugation.

1.7 Self Assessment Questions and Possible Answers

1.7.1 Multiple Choice Questions:

1. There is no organized nucleus in:

   (a) Bacterial cell (b) Green algae cell

   (c) Animal cell (d) Plant cell

2. The prokaryotic cells are characterized by:

   (a) A distinct nuclear membrane (b) Absence of chromatin material

   (c) Distinct chromosome (d) Absence of nuclear membrane

3. In a prokaryotic cell, DNA is:

   (a) Enclosed by nuclear envelop (b) Lacking

   (c) Not a genetic material (d) without a membrane

4. Cell wall is found around the:

   (a) Prokaryotic cells (b) Algal cells

   (c) Plant cells (d) All the above

5. Chemical energy of food stuffs is converted into biologically useful forms by:

   (a) Ribosomes (b) Golgi complex

   (c) Mitochondria (d) Plastids

6. Sun radiant energy is converted into chemical energy of organic compound by:

(a) Mitochondria (b) Chloroplast

(c) Ribosomes (d) Centrosomes

7. Which structure is present only in animal cell?

(a) Cell membrane (b) Lysosomes

(c) Centrioles (d) Ribosomes

8. Single envelope system is characteristic of:

(a) Prokaryotic cell (b) Eukaryotic cell

(c) None (d) Both

9. Prokaryote and eukaryotes have the common:

(a) Mitotic apparatus (b) Histone

(c) Genetic code (d) Mitochondria

10. Unicellular microscopic organisms were first studied by:

(a) Robert Hooke (b) Priestley

(c) Pasteur (d) Leeuwenhoek

ANSWERS:-

1. (a) 5.(c) 9. (c)
2. (d) 6.(b) 10.(d)
3. (d) 7.(c)
4. (d) 8.(a)

1.7.2 Very Short Questions:

1. What are prokaryotes? Give an example.
2. What are eukaryotes? Give few examples.
3. Cell is an open dynamic system. Is it correct?
4. Prokaryotic cells are haploid. Is it so?

5. What are cyanobacteria?
6. Give three essential characteristics of cell?
7. Where is nucleolus found?
8. What are the power houses of the cell?
9. Name the protein factories of prokaryotic and eukaryotic cells?
10. What is the control centre of a cell?

Answer:-

1. Organisms without an organized nucleus e.g., Bacteria
2. Organisms with an organized nucleus. Plants, yeast;
3. Yes
4. Yes
5. Blue green algae
6. Cell membrane, cytoplasm, nuclear material
7. Nucleus
8. Mitochondria
9. Ribosome
10. Nucleus

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1.9 Terminal and Model Questions

1. What is a cell? Draw a neat and labeled diagram of prokaryotic and eukaryotic cells.
2. Describe the structure of prokaryotic cells.
3. Give the salient features of eukaryotic cell.
4. Tabulate the differences between prokaryotic and eukaryotic cells.
5. What are cytoplasmic inclusions? Describe them in brief.

UNIT 2 PLASMA MEMBRANE

Contents

2.1 Objectives

After reading this unit the readers should be able to:

- Define plasma membrane
- Describe the ultra structure of plasma membrane
- Explain the chemical composition of plasma membrane
- Outline the various theories of plasma membrane
- Discuss the functions of plasma membrane

2.2 Introduction

Every cell, prokaryotic or eukaryotic, is surrounded by a thin layer of outermost boundary called the **plasma membrane or cell membrane or plasma - lemma**. The plasma membrane is a discrete structure and is remarkably complex in its molecular organization. It maintains the difference of the internal environment of the cell from its external environment by controlling the entrance and exit of the molecules and ions. It checks the loss of metabolically useful substances and encourages the release of toxic metabolic byproducts of the cell. Thus, it functions as **semi-permeable or selectively permeable membrane**. It is about 70-100Å in thickness. In plant cells plasma lemma is further covered by cellulosic cell wall. It is an important cell organelle composed of lipids and proteins. It possesses devices for attachment to other cells for cell-to-cell communications, ion pumps for controlling internal milieu of the cell, receptors for hormones and mechanisms for the production of secondary messengers that activates the cell's physiological response.

2.3 History

It had been shown by **Karl W. Nageli** (1817-1891) that the cell membrane is semi-permeable and is responsible for the osmotic and other related phenomena exhibited by living cells. Before 1855, he used the term zellen membrane in his early papers. The term plasma membrane was used in 1855 by him to describe the membrane as a firm protective film that is formed by out flowing cytoplasm of an injured cell when protein rich cell sap came in contact with water.

2.4 Plasma Membrane

2.4.1 Ultra Structure of Plasma Membrane

2.4.1.1 Symmetrical Molecular Structure of Plasma Membrane:-

Plasma membrane is a tripartite structure and is made up of three layers, having total thickness of 75Å. Two di-electronic layers are there, each of 25Å thickness, enclosing a middle dielectronic layer which is also 25Å thick. The middle layer is a tri-molecular layer of lipids having its non-polar hydrophobic groups facing inwards, whereas polar hydrophilic groups facing outwards. The hydrophilic polar groups are covered by a protein layer which is 20 to 25Å thick. The protein chains lie at right angles to the lipids.

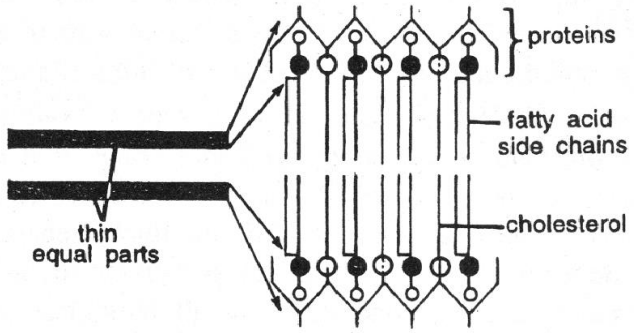

Fig. 2.1: Symmetrical pattern of molecules in plasma membrane (Source: Singh and Tomar, 2008)

2.4.1.2 Asymmetrical Molecular Structure of Plasma Membrane

It is also a tripartite structure having a thick inner dielectronic component of 35-40 Å, a narrow outer dielectronic component of 25Å thickness, and a central dielectronic layer (bimolecular layer of lipids) which is 30Å wide; thus total thickness comes to 90-95Å.

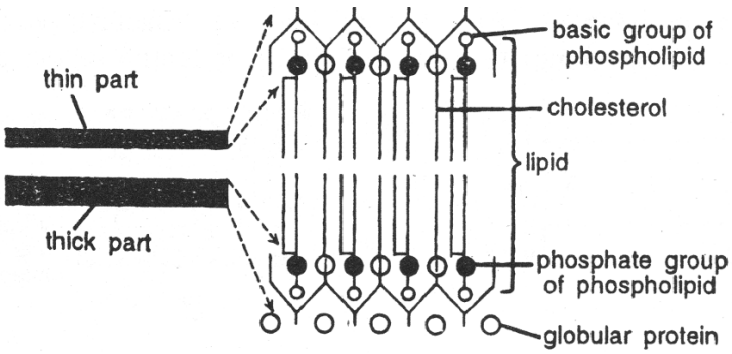

Fig. 2.2: Asymmetrical pattern of plasma membrane

In different types of cells the thickness of plasma membrane varies. For example, in red blood corpuscles of rabbit, the plasma membrane is about 215 Å thick whereas, in intestinal epithelial cells it is 105 Å in thickness. Very small pores measuring about 10Å in diameter (smaller than pores of nuclear membrane) have been discovered in the membranes.

2.4.2 Chemical Composition of the Plasma Membrane

Plasma membrane is primarily composed of protein and lipid, although carbohydrate is often present in association with protein (as glycoprotein) or lipid (as glycolipid). However, the relative proportions of protein and lipid vary considerably in membranes from different sources.

2.4.2.1 Lipids

The plasma membrane contains about 20 to 79% lipids mainly of three types like phospholipids, cholesterol and glycolipids. The phospholipids which make up between 55% and 75% of the total lipid content, consists chiefly of lecithin and cephalin. The remainder consists of sphingolipids (with an amino group) and glycolipid conjugates with carbohydrates. Phospholipids derived from glycerol are called phosphoglycerides.

A phosphoglycerides is made up of two fatty acid chains, a glycerol backbone and a phosphorylated alcohol. The outer layer of phospholipids consists mainly of lecithin and sphingomyeline, while the inner layer is composed mainly of phosphatidyl ethanolamine and phosphatidyl serine (both are phosphoglycerides). The glycolipids (sugar containing lipids) are mainly in the outer half of the bilayer.

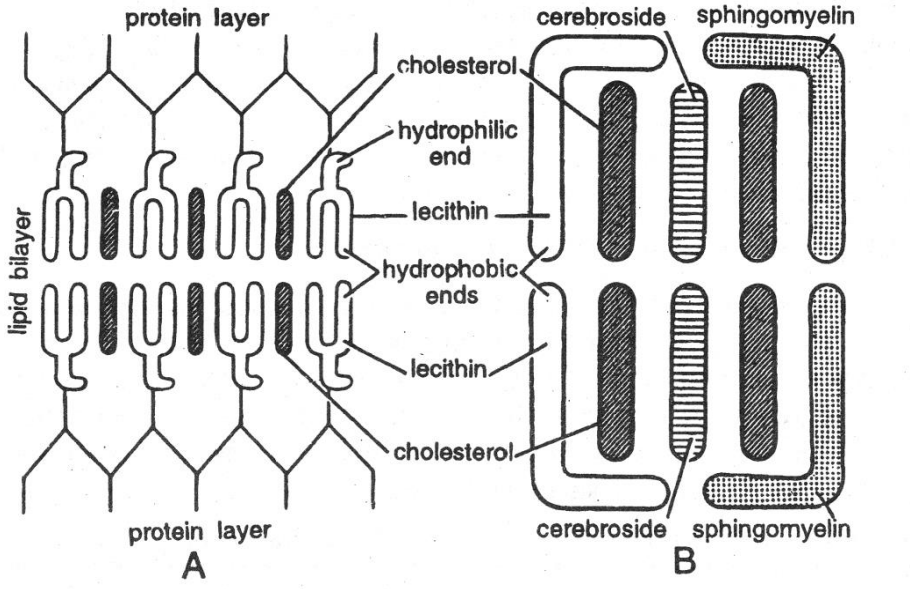

Fig. 2.3: A phospholipids cholesterol complex of cell membrane

Cholesterol is present in eukaryotes but not in prokaryotes. Plasma membrane of cells such as erythrocyte, liver cells and myelinated nerve cells are rich in cholesterol.

Membrane lipids are amphipathic molecules. They contain both a hydrophobic and hydrophilic moiety. Hydrophilic unit is also called the polar head groups, is represented by a circle and their hydrocarbon tails are depicted by straight or wavy lines. Polar head groups have affinity for water, whereas their hydrocarbons tails avoid water. This can be accomplished by forming a micelle, in which polar head groups are on the surface and hydrocarbon tails are directed inside.

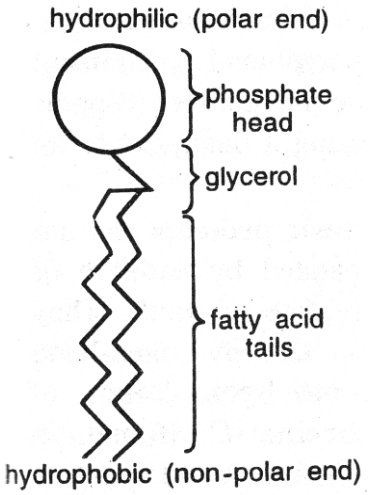

Fig.2. 4: A phospholipids molecule

Another arrangement of lipid molecule in a membrane is a bimolecular sheet, which is also called a lipid bilayer. Phospholipids and glycolipids are key membrane constituents of bimolecular sheets. Hydrophobic interactions are the major driving force for the formation of lipid bilayer. The lipid bilayer of the membrane is interrupted only by the proteins that traverse it. This bilayer consists primarily of:

(a) *Neutral Phospholipids and Cholesterol*: These include phosphatidylenoline, lecithin cerebroside, and sphingomyeline and phosphatidyl ethanolamine. They are without any electric charge at neutral pH and are closely packed in the bilayer along with cholesterol.

(b) *Acidic Phospholipids*: These constitute about 5% to 20% fractions of the total phospholipids of plasma membrane. They are **negatively charged** and are associated with proteins by way of lipid-protein interactions. Common examples are phosphatidyl inositol, phosphatidylserine, sulpholipids, phosphatidyl glycerol and Cardiolipin.

In plasma membrane, lipid fractions form permeability barrier and structural framework.

2.4.2.2 Proteins

Proteins are the main component of plasma membrane. Myelin sheath (membrane surrounding some nerve axons) is composed of about 80% lipids and 20% protein and

presence of lipid makes myelin an excellent insulator. Eukaryotes membrane which serves primarily as permeability barriers possesses about 50% proteins and 50% lipid. Plasma membrane that are actively involved in energy transfer, such as inner membrane of mitochondria, chloroplasts and membranes of aerobic prokaryotes have large amounts of proteins i.e. about 75%. They not only provide mechanical support but also act as carriers or channels, serving for transport. In addition numerous enzymes, antigens and various kinds of receptor molecules are present in plasma membranes. Membrane proteins are classified as **integral (intrinsic) or peripheral (extrinsic**) according to the degree of their association with the membrane (Singer, 1971).

(a) *Peripheral Proteins*: They are also called extrinsic proteins associated with membrane surface. These can be separated by addition of salts, soluble in aqueous solutions and usually free of lipids. They are bound to the surface by electrostatic and hydrogen bond interactions. They form outer and inner layers of the lipid bilayer of plasma membrane. Common examples are cytochrome-C found in mitochondria, acetyl cholinesterase in electroplax membrane and spectrin found in erythrocytes.

(b) *Integral or Intrinsic Proteins*: These proteins penetrate the lipid layer wholly or partially and represent more than 70% of the two protein types. Their polar ends protrude from the membrane surface while non-polar regions are embedded in the interior of the membrane. Usually they are insoluble in water solutions and can be separate them from the membrane by detergents or organic solvents. The major integral proteins span the thickness of the membrane and have a small amount of carbohydrates on the pole at the outer surface. This protein appears to be involved in the diffusion of anions across the membrane. Integral proteins may be attached to the oligosaccharides to form glycoprotein or to phospholipid to form lipoproteins or proteolipids. Common intrinsic proteins are rhodopsin found in retinal rod cells and cytochrome oxidase found in mitochondrial membranes.

Every protein in the cell membrane is distributed asymmetrically with respect to the lipid bilayer.

2.4.2.3 Enzymes

About 30 enzymes have been found in various membranes. Those most constantly found are 5'-nucleotidase, Na^+-K^+ activated ATPase, alkaline phosphatase, adenylcyclase, RNAse and acid phosphomonoestrase. Na^+-K^+ activated Mg^+ ATPase plays an important role in the ionic exchange and may also act as carrier protein or permease across the plasma membrane. Some enzymes have a preferential localization. For example, alkaline phosphatase and ATPase are more abundant in bile capillaries, while disaccharides are present in microvilli of the intestine. Enzymes are asymmetrically distributed, for example in the outer surface of erythrocytes there are acetylcholinestrase, nicotinamide adenine dinucleotidase and Na^+-K^+ ATPase. In the inner surface there is NADH-diaphorase, G3PD, adenylate cyclase, protein kinase and ATPase.

2.4.2.4 Carbohydrates

The membranes of eukaryotic cells usually contain 2% to 10% carbohydrates in the form of glycolipids and glycoproteins. Hexose, hexosamine, fucose and sialic acid are the commonest carbohydrates found in the membrane. Plasma membranes of neuronal surface contain gangliosides (Lapertina, 1967) and are probably involved in the ion transfers. The distribution of oligosaccharides is also highly asymmetrical.

2.4.2.5 Salts and water

They are also present in cell membranes. Water in cell membranes forms parts of membrane structure as it does in all cell constituents.

2.4.3 Lamella-model of plasma membrane (Danielli-Davson model)

Danielli-Davson model (1934) suggested that the plasma membrane consists of two layers of lipid molecules arranged radially with their hydrophobic hydrocarbon chains toward each other and with their respective polar groups arranged outwardly and inwardly throughout the entire double layer of lipid molecules. The polar ends of the lipid molecules are associated with a monomolecular layer of polar globular protein molecule. The entire structure thus consisted of double layer of lipid molecule sandwiched between two continuous layers of protein. The lipid molecules are set at right angles to the surface and are so arranged in two layers that their non-polar hydrophobic fatty acid tails face each other and their polar hydrophilic phosphate heads face the protein layer. The proteins involved were thought to be globular. Moreover, lamellar theory assumed the cell membrane to be a stable structure with little functional specificity and variability.

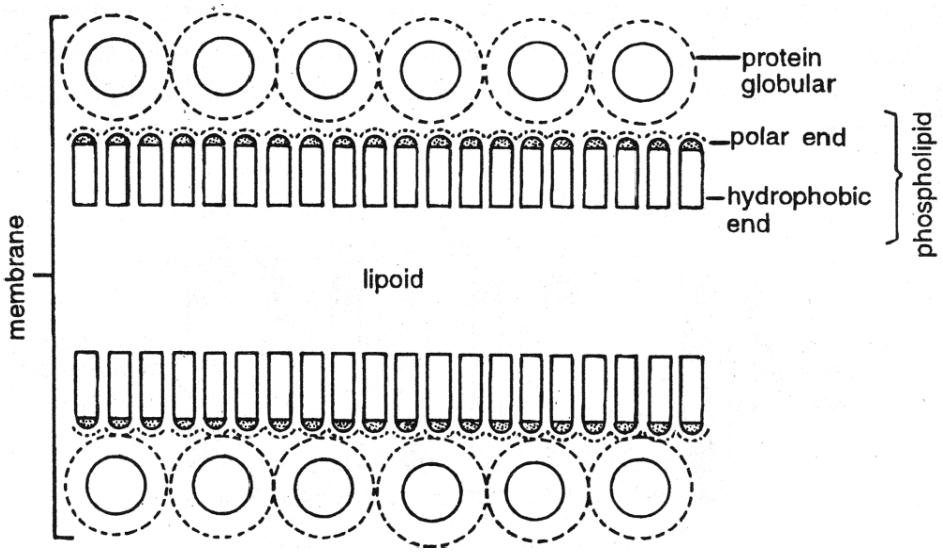

Fig. 2.5: A schematic diagram of Davson-Danielli model of membrane structure

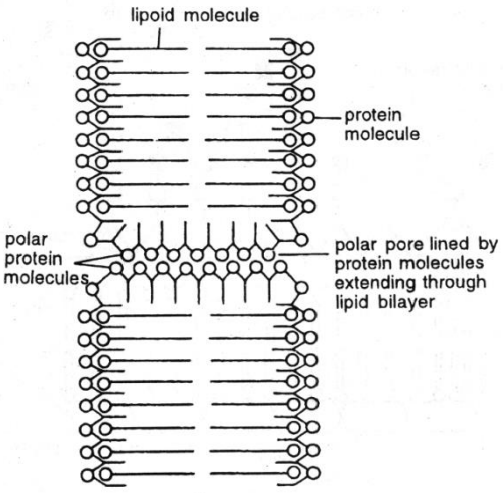

Fig. 2.6: A modification of original Danielli-Davson model, showing pores lined by polar protein molecules extending through the lipid bilayer

2.4.4 Miceller model of plasma Membrane

According to the view **of Hiller and Hoffman (1953)**, plasma membrane consists of a mosaic of globular subunits or micelles. If fatty acid molecules are completely surrounded by water, they may form aggregate called micelles in which the hydrophobic regions of fatty acid molecules are oriented toward the interior of the micelle away from the aqueous phase and their hydrophilic groups are at the surface in contact with the surrounding water. Micelles may be in the form of small spheres of bimolecular layers. These micelles are closely packed together having a central core of lipid molecules and hydrophilic shell of polar groups. Each lipid micelle measures 40Å to 70Å in diameter. Protein component of the plasma membrane forms a monolayer on either side of the lipid micelles and is represented by globular type. The spaces between the globular micelles are thought to represent water filled pores which measures about 4Å in diameter. These pores are bounded partly by the polar groups of micelles and partly by the polar groups of associated protein molecules.

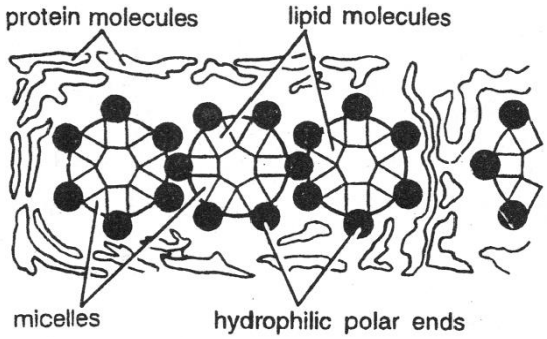

Fig.2.7: Plasma membrane based on Miceller theory (diagrammatic)

2.4.5 Fluid Mosaic Model of plasma membrane

It was proposed by **Singer and Nicholson (1972**). The lipids are thought to be arranged primarily in a bilayer in which proteins are embedded to varying degrees. Singer classifies membrane proteins as peripheral or integral. The proteins varied in size and dissolved to varying degrees in the lipid matrix are able to diffuse laterally in the plane of membrane, and the entire structure is hence dynamic. In this model, lipid molecules may exhibit intra molecular movement or may rotate about their axis or may display flip-flop movement including transfer from one side of bilayer to the other.

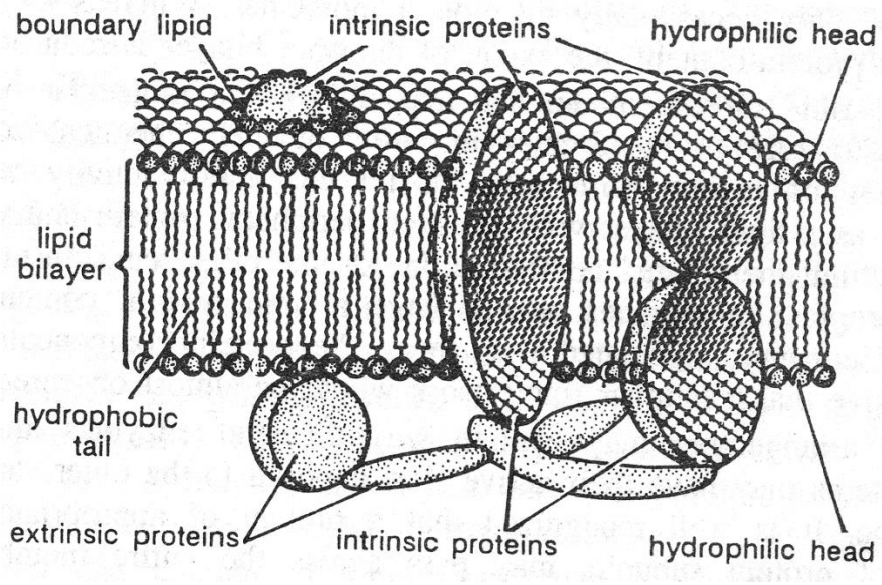

Fig. 2.8: Plasma membrane based upon Fluid-mosaic model

The lipids, glycoprotein and many of the intrinsic proteins of the membranes are amphipathic molecules. These amphipathic molecules constitute liquid crystalline aggregates in which the polar groups are directed toward the water phase and the non-polar groups are situated inside the bilayer. The lipid bilayer forms the structural matrix which serves as the permeability barrier of the membrane. In membranes with high lipid content, lipid bilayer is extensive and interrupted only occasionally by protein molecules, whereas in membranes with high protein content, the extent of lipid bilayer is reduced. Thus, fluid mosaic model may describe the chemical composition of the molecular organization and ultra structure of plasma membranes. This arrangement allows various enzymes and antigenic glycoprotein to have their active sites exposed to the outer surface of the membrane. The fluidity of membrane also implies that both the lipid and the protein have considerable freedom of movement within the bilayer. The fluidity of the lipid depends on the degree of saturation of the hydrocarbon chains and on the ambient temperature. A considerable proportion of the lipids in the membrane are unsaturated, so that melting point of the bilayer is below body temperature.

2.5 Functions of Plasma Membrane

The plasma membrane serves many functions such as:

- It maintains the individuality and form of the cell.
- It keeps the cell contents in place and distinct from the environmental materials.
- It protects the cell from injury.
- It regulates the flow of materials into and out of the cell to maintain the concentration and kinds of molecules and ions in the cell. A cell remains alive as long as the cell membrane is able to determine which materials should enter or leave the cell.
- It forms organelles within the cytoplasm.
- Its junctions keep the cells together.
- It's infolds help in the intake of materials by endocytosis (pinocytosis and phagocytosis).
- It's out folds (microvilli) increase the surface area for absorption of nutrients. The out folds also form protective sheaths around cilia and flagella.
- Its receptor molecules permit flow of information into the cell.
- Its oligosaccharide molecule helps in recognizing self from non-self.
- By controlling flow of material and information into the cell, the plasma membrane makes metabolism possible.
- It permits exit of secretions and wastes by exocytosis.
- It controls cellular interactions necessary for tissue formation and defense against microbes.
- It helps certain cells in movement by forming pseudopodia as in Amoeba and leucocytes.

The bio-membranes around the organelles help the latter to:

(1) Maintain their identity and functional individuality.

(2) Receive and turn out required material.

2.6 Summary

The plasma membrane constitutes the outermost boundary of the cell and it is remarkably complex in its molecular organization. It is composed of almost equal parts of proteins and lipids. It allows only selected ions and macromolecules to enter or leave the cell, thus it functions as a semi permeable membrane.

Ultra structure of plasma membrane may be of symmetrical or asymmetrical molecular structure in nature. Plasma membrane is a tripartite structure in both of the above types, the difference lies in the thickness of the three layers. In symmetrical molecular structure all the

three layers, the outer and inner adielectronic along with the middle di-electronic layer are of 25Å thickness each having total thickness of 75Å. While in asymmetrical structure the inner adielectronic component is of 35Å to 40Å thickness, the outer dielectronic component is of 25Å thick and the central dielectronic layer is 30Å wide, thus total thickness becomes 90-95Å.

Plasma membrane is primarily composed of proteins and lipids, although carbohydrate is often present in association with proteins (as glycoproteins) or lipids (as glycolipids). However, the relative proportions of proteins and lipids vary considerably in membranes. Enzymes are also found in plasma membranes which play an important role in ionic exchange. Besides, salt and water are also present. The arrangement of lipids and proteins molecules is explained through various theories.

Lamella model of plasma membrane is consisted of a double layer of lipid molecules arranged radially with their hydrophobic hydrocarbon chains towards each other and with their respective polar groups arranged outwardly and inwardly. The double layer of lipids is sandwiched between two continuous layers of proteins. According to miceller theory, plasma membrane consists of a mosaic of globular subunits or micelles. These micelles are closely packed together having the lipid molecules in the central core. Protein components form a monolayer on the entire surface of the lipid micelles forming a globule. The widely accepted theory is fluid mosaic models of membrane as it can be used to describe the structure of different membranes. In this model the lipids are arranged in a bilayer in which proteins are embedded as peripheral or integral. The proteins varied in size and dissolved to varying degrees in the lipid matrix, diffuse laterally in the plane of membranes and the entire structure is hence dynamic.

Plasma membrane performs variety of functions as they impart shape to the cell and protects the cell contents. It regulates the cellular semi permeability, resorption, excretion and secretion. It contributes to the formation of various cell organelles within the cell. Its junctions keep the cells together.

2.7 Glossary

Plasma membrane - A microscopic membrane made up of lipids and proteins which forms the external boundary of the cytoplasm of a cell or encloses a vacuole, and regulates the passage of molecules in and out of the cytoplasm.

Permeability- The ability of a barrier to let any substance pass through it.

Ions- An atom or molecule with a net electric charge due to the loss or gain of one or more electrons.

Semi permeable- Allowing certain substances especially small molecules or ions to pass through it but not others, especially allowing the passage of a solvent but not of certain solutes.

Receptor- A receptor is a protein molecule in a cell or on the surface of a cell to which a substance such as a hormone, a drug, or an antigen can bind, causing a change in the activity of the cell.

Dielectric- Having the property of transmitting electric force without conduction.

Hydrophobic- The substances that have an affinity for water due to the formation of hydrogen bonds.

Hydrophilic- Hydrophilic molecules typically have polar groups enabling them to readily absorb or dissolve in water as well as in other polar solvents.

Micelles- It is an aggregate of molecules in a colloidal solution, such as those formed by detergents.

Peripheral proteins/extrinsic proteins- Peripheral membrane proteins are proteins that adhere only temporarily to the biological membrane with which they are associated. These molecules attach to integral membrane proteins, or penetrate the peripheral regions of the lipid bilayer.

Integral proteins/intrinsic proteins- An integral membrane protein (IMP) is a type of membrane protein that is permanently attached to the biological membrane. All trans membrane proteins are IMPs, but not all IMPs are trans membrane proteins.

Amphipathy- It is the property of a molecule having both polar (water-soluble) and non polar (not water-soluble) affinities in its structure.

Enzyme- The proteins which acts as catalysts within living cells and increases the rate of biochemical reactions.

2.8 Self Assessment Questions and Possible Answers

2.8.1 Multiple Choice Questions:

1. According to Fluid mosaic model, the correct sequences of substances in plasmalemma is:

   (a) L-P-P-L (b) P-L-L-P

   (c) P-P-L-L (d) L-P-L-P

2. Membrane occurs in:

   (a) Chromosomes, nuclei and mitochondria

   (b) Cytoplasm, chloroplasts and mitochondria

   (c) Cytoplasm, nuclei and starch grains

   (d) Chromosomes, chloroplasts and starch grains

3. Plasma membrane is:

   (a) Non-selective barrier (b) Selective barrier

   (c) Impermeable (d) made of cellulose

4. What limits Animal cells from outside?

(a) Cell wall (b) Basement membrane

(c) Shell membrane (d) Plasma membrane

5. Cell membrane consists of:

(a) Protein double layer (b) Phospholipid proteins

(c) Phosphoproteins (d) Glycoproteins

6. Non-membranous cell organelles are:

(a) Ribosomes (b) centrioles and ribosomes

(c) E.R. (d) Mitochondria

7. Which of the following theories explain that plasma membrane is selectively permeable:

(a) Unit membrane theory (b) Cascade theory

(c) Sandwich theory (d) Fluid Mosaic theory

8. The hydrophobic ends of phospholipid molecules are:

(a) Polar (b) Non-polar

(c) Neutral (d) Bipolar

9. The membrane protein that extend through both sides of lipid bilayer.

(a) Acidic protein (b) Glycoprotein

(c) Intrinsic protein (d) Glycolic acid

10. Two plant cells are connected with the help of:

(a) Cell wall (b) Plasma membrane

(c) Plasmodesmata (d) None of these

M.C.Q:- ANSWERS

1. (b) 2. (b) 3. (b) 4. (d) 5. (b) 6. (b) 7. (d) 8. (b) 9. (c) 10.(c)

2.8.2 Very short questions

1. What is the thickness of plasma membrane?
2. Who proposed the fluid mosaic hypothesis for the molecular structure of cell membrane?
3. What is the structure of plasma membrane?

4. What are the main lipid components of the plasma membrane?
5. What are the two types of proteins of the plasma membrane on the basis of their association with the membrane and their solubility?
6. What are tunnel proteins?
7. Why Na^+-K^+ ATPase enzyme is most important?
8. Who proposed that plasma membrane contained a lipid bilayer and protein adhering to both lipid aqueous interfaces?
9. Who gave the unit membrane model of plasma membrane?
10. Give the two alternative name of cell membrane.

Answers:-

1. 70 - 100Å.
2. Singer and Nicolson.
3. It is formed of bilayer of lipids into which protein complexes are embedded in a kind of mosaic arrangement.
4. Phospholipids, cholesterol and galactolipids.
5. Integral or intrinsic proteins and peripheral or extrinsic proteins.
6. Large integral protein molecules that lie throughout the phospholipid matrix and projects on both the surfaces.
7. It helps in ion transfer across the plasma membrane. This enzyme is dependent on the presence of lipids and is inactivated when all lipids are extracted.
8. Danielli and Davson in 1935.
9. Robertson, 1959.
10. Plasma membrane and plasmelemma.

2.9 References

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2.10 Suggested Readings

Kimball's Biology pages, Cell Membranes.

1. *Alberts B, Johnson A, Lewis J, et al. (2002). Molecular Biology of the Cell (4th ed.). New York: Garland Science. ISBN 0-8153-3218-1.*
2. Kleinzeller, A. 1999. Charles Ernest Overton's concept of a cell membrane. In: *Membrane permeability: 100 years since Ernest Overton* (ed. Deamer D.W., Kleinzeller A., Fambrough D.M.), pp. 1–18, Academic Press, San Diego.
3. Sharp, L. W. (1921). *Introduction to Cytology*. New York: McGraw Hill, p. 42.
5. *Singer SJ, Nicolson GL (Feb 1972). "The fluid mosaic model of the structure of cell membranes". Science.* ***175*** *(4023): 720–31.*

2.10 Terminal and Model Questions

1. Define plasma membrane.
2. Describe the structure and functions of plasma membrane.
3. Write notes on:
   a. Fluid Mosaic Theory
   b. Miceller Model of Plasma Membrane
   c. Lamella model of Plasma membrane
   d. Define phagocytosis and pinocytosis.
4. Explain in detail the ultra structure of plasma membrane.
5. Differentiate between integral and peripheral proteins.

UNIT 3 MITOCHONDRIA

Contents

3.1 Objectives

After reading this unit the readers will be able to:

- Define mitochondria
- Illustrate the morphology and ultra structure of mitochondria
- Describe the biogenesis of mitochondria
- Explain the functions of mitochondria
- Elucidate the respiratory chain complex and electron transport mechanism.

3.2 Introduction

Mitochondria (Gr., *mito*, thread; *chondrion*, granule) are thread like or granular structures of eukaryotic cells. These may assume rod-like shape called chondriosomes which may enlarge or aggregate to form massive spheroidal bodies called chondriospheres. These are not present in bacterial cells. Mitochondria are the **'power plants'** which by oxidation release the energy contained in the fuel molecules or nutrients and make other forms of chemical energy. The main function of mitochondria is oxidative phosphorylation, which is an exergonic reaction, meaning that it releases energy. In prokaryotes, oxidation of organic material is carried out by plasma membrane enzymes.

3.3 History

Kölliker (1880) was the first who observed the mitochondria in insects muscle cells. He called them as 'sarcosomes'. **Flemming** (1882) named the mitochondria as 'fila'. **Altmann** in 1894 observed them and named them Altmann's granules or bioblasts. The term '**mitochondria**' was applied by **Benda** (1897-98). They were recognized as the sites of respiration by Hogeboom and his coworkers in 1948. **Lehninger and Kennedy** (1948) reported that the mitochondria catalyze all the reactions of the citric acid cycle, fatty acid oxidation and coupled phosphorylation.

3.4 Structure of Mitochondria

3.4.1 Morphology of Mitochondria

Morphologically mitochondria may be in the form of filaments or small granules. These may assume rod-like shape called **chondriosomes** which may enlarge or aggregate to form massive spheroid bodies called **chondriospheres**.

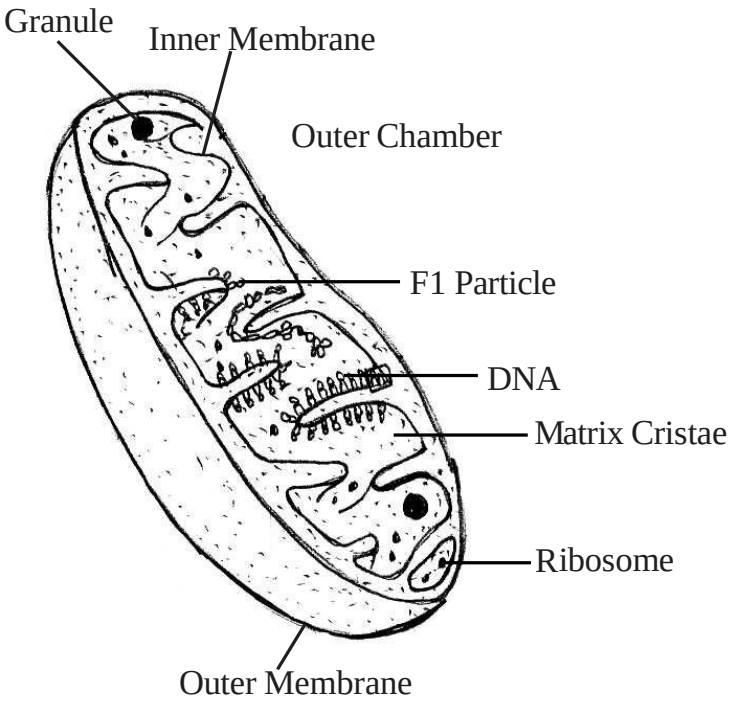


Fig. 3.1: Structure of a Mitochondrion

1. **Position-** Mitochondria lie freely in cytoplasm, possessing power of independent movement and may take the form of filaments. In some cells they can move freely, carrying ATP where needed, but in others they are located permanently near the region of the cell where more energy is needed. E.g., in the rod and cone cells of retina mitochondria are located in the inner segment, in cells of kidney tubules they occur in the folds of basal regions near plasma membrane, in neurons they are located in the transmitting region of impulse, in certain muscle cells (e.g. diaphragm), mitochondria are grouped like rings or bracers around the I-band of myofibril. During cell division they get concentrated around the spindle.
2. **Number-** The number of mitochondria varies a good deal from cell to cell and from species to species. A few algae and some protozoan have only single mitochondria. Their number is related to the activity, age and type of the cell. Growing, dividing and actively synthesizing cells contain more mitochondria than the other cells. In Amoeba (***Chaos chaos),*** there may be as many as 50,000 mitochondria. In rat liver cells, these are few in number, about 1000 to 1600. Some Oocytes contain as many as 3, 00,000 mitochondria.
3. **Size-** The average size of mitochondria is 0.5-1.0μ in diameter and about 2-8 μ in length. In exocrine cells of mammalian pancreas they are about 10 μ long and in oocytes of amphibian ***Rana pipiens*** are 20-40μ long. Yeast cells have the smallest mitochondria.

3.4.2 Ultra structure of Mitochondria

The electron microscope shows the mitochondrion as the vesicles bounded by an envelope of two unit membranes and filled with a fluid matrix.

1. **Membranes-** Both the inner and the outer mitochondrial membranes resemble the plasma membrane in molecular structure. Each of them is 60-70Å, trilamellar and composed of two layers of phospholipid molecules sandwiched between two layers of protein molecules. However, the two membranes differ in the kinds of protein and lipids they have and also in their properties. Both the outer and the inner membranes contain specific pumps or channels, for the transport of molecules through them. The membranes may be connected at adhesion sites through which proteins are transferred from the outer to the inner membrane. The outer and the inner membrane are separated from each other by a narrow space called the inter-membrane space or outer chamber or peri-mitochondrial space. It is about 80Å wide. It contains a clear homogeneous fluid.

   (i) ***Outer Membrane-*** The outer membrane is smooth permeable to most small molecules, having trans-membrane channels formed by the protein '**porin**'. It consists of about 50% lipid, including a large amount of cholesterol. It contains some enzymes but is poor in protein.

   (ii) ***Inner Membrane-*** The inner membrane is selectively permeable and regulates the movement of materials into and out of the mitochondrion. It is rich in enzymes and carrier proteins **permease**. It has a very high protein/lipid ratio (about 4:1 by weight). It lacks cholesterol. Cardiolipin is closely associated with certain integral proteins and is apparently required for their activity.

2. **Matrix-** The space between the cristae called the inner chamber is filled with a gel like material termed the mitochondrial matrix. It contains proteins, lipids, some ribosomes, RNA, one or two DNA molecules and certain fibrils, crystals and dense granules.

3. **Cristae-** The inner mitochondrial membrane bears plate like infoldings called the cristae. They extend inwards to varying degrees, and may fuse with those from the opposite side, dividing the mitochondrion into compartments. They are arranged in a characteristic manner in different cells. Normally they run at right angles to the long axis of the rod shaped mitochondria. In cells of the proximal parts of the kidney tubules, the cristae are longitudinal folds parallel to the long axis of mitochondrion. In many protozoans, in insect flight muscles cells and in adrenal endocrine cells the cristae are tubular. Cristae are lamellar in hepatocytes. In heart muscle cells cristae are zig-zag.

   They also vary in number. The active cells may have numerous cristae whereas the inactive cells may have only a few. The cristae have in them a narrow intra-crista space. It is continuous with the inter-membrane space. The cristae greatly increase the inner surface of the mitochondrion to provide enough space for housing enzyme assemblies. The cristae also allow for expansion or swelling of mitochondria under different metabolic and environmental conditions.

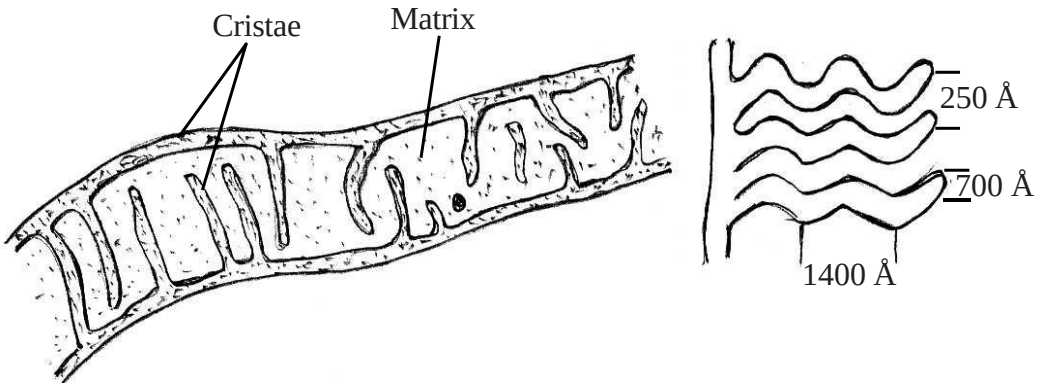


Fig. 3.2: Cristae in a mitochondrion of an endothelial cell of human being

4. **Oxysomes-** The inner mitochondrial membrane bears minute regularly spaced particles known as the inner membrane subunits or **elementary particles (EP) or oxysomes**. An oxysome consists of three parts- a rounded **head piece or F_1 subunit** joined by a short stalk to a **base piece or F_0 subunit** located in the inner membrane. There may be 100,000 to 1000,000 oxysomes in a single mitochondrion.

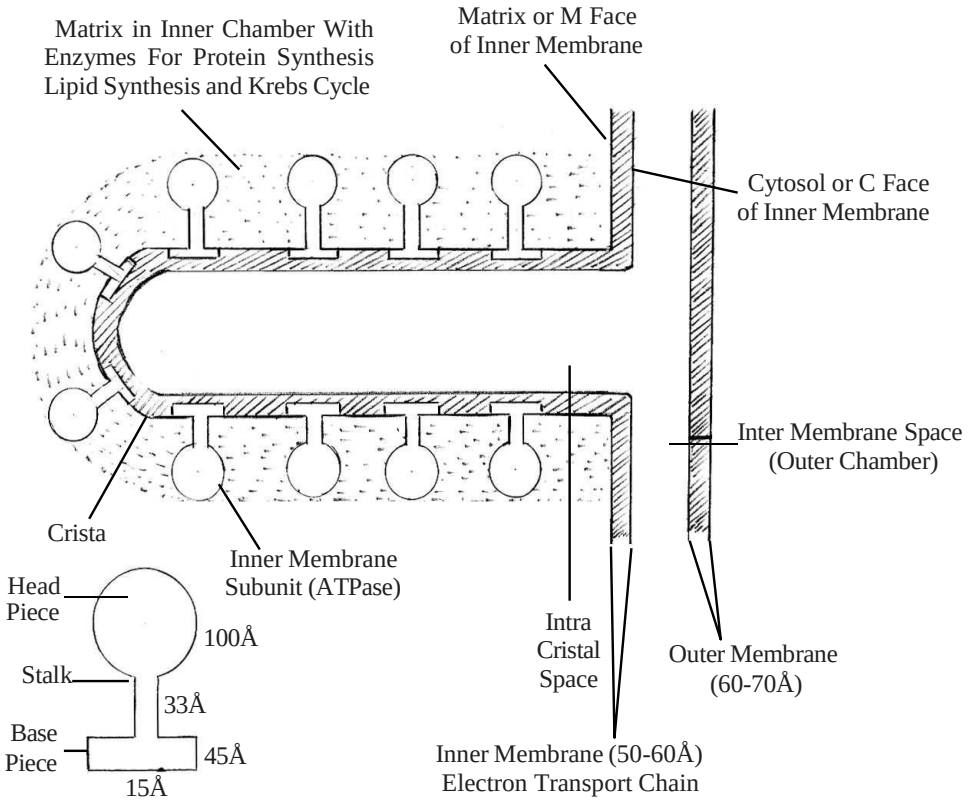


Fig. 3.3: Detailed structure of a crista and an oxysome

3.5 Biogenesis of Mitochondria

The formation of new mitochondria has been explained with the following hypothesis.

1. **De Novo Synthesis-** According to this hypothesis mitochondria arises de novo from precursors in the cytoplasm.
2. **Origin from membrane-** This hypothesis proposes that the mitochondria arises from the invaginations of plasma membrane, endoplasmic reticulum, Golgi apparatus or nuclear envelop. The membrane invaginates and extends into the cytoplasm as a tubular structure. It gradually becomes curved and folded and forms a double walled structure, the mitochondrion.
3. **Develop from Micro bodies-** It is held that they mitochondria are developed by the accumulation of micro bodies in the cytoplasm. A micro body consists of a single outer membrane and a dense matrix with a few cristae which eventually develops into fully formed mitochondria.
4. **Prokaryotic Origin-** It is believed that mitochondria are originated from bacteria. It is supported by many evidences.
    (i) First is the localization of enzymes of respiratory chain, which in case of bacteria, are localized in plasma membrane which can be compared with the inner membrane of the mitochondrion.
    (ii) In some bacteria, plasma membrane forms membranous projections (called mesosomes) like cristae of mitochondria. These mesosomes possess respiratory chain enzymes.
    (iii) The mitochondrial DNA is circular as it is in bacteria. Replication process of mitochondria is similar to bacteria.
    (iv) Ribosomes in mitochondria are smaller and similar in size to that of bacterial ribosomes.
    (v) Chloramphenicol inhibits the synthesis of protein in mitochondria as well as in bacteria. Furthermore, in the process of protein synthesis, mitochondria depend partially on mitochondrial matrix and DNA and partially on nucleus and cytoplasm of the eukaryotic cells. It exhibits the symbiotic nature of mitochondria. These evidences support the prokaryotic origin of mitochondria.
5. **Replication-** It is held that mitochondria are self-replicating organelles. New mitochondria arise by some type of splitting process from pre-existing mitochondria.

The last hypothesis seems probable. Since the mitochondria have their own DNA and ribosomes, they can replicate new mitochondria. However, there is a nuclear control over the process as the mitochondria synthesize some of their proteins themselves and get others from the cytoplasm of the cell formed under the direction of the nuclear DNA.

3.6 Functions of Mitochondria

Mitochondria perform the following functions:-

1. **Cell respiration** takes place in mitochondria and so they are known as the **'power house' of the cell.** They bring about stepwise oxidation of food stuffs or "low-grade" fuel of the cell and transfer the energy so released to the energy carrier ATP, the "high-grade" fuel of the cell. ATP is used to bring about the energy-requiring activities in the cells, namely, biosynthesis, active transport, transmission of nerve impulse, muscle contraction, cell growth and division and bioluminescence.
2. Mitochondria provide **intermediates** for the **synthesis of important biomolecules** such as chlorophyll, cytochromes, steroids etc.
3. Some **amino acids** are also formed in the mitochondria.
4. Mitochondria actively **accumulate calcium ions** as calcium phosphate precipitate. They regulate the calcium ions concentration in the cytoplasm by storing and releasing Ca^{+}. The calcium ions regulate numerous biochemical activities in the cell.

3.7 Respiratory Chain Complex or Electron Transport System

Respiratory chain complex or electron transport system consists of a series of complex proteins, which take part in the respiratory chain. There are **five complexes formed of lipoproteins and two mobile electron carriers** — coenzyme Q (CoQ) or ubiquinone (UQ) and cytochrome C.

3.7.1 Complexes

Complexes are the sites where hydrogen ions released during Krebs's cycle are oxidized and their energy is trapped in ATP.

1. **Complex I (NADH-CoQ reductase).** It consists of the following components.
   (a) **NADH dehydrogenase**- It consists of flavoprotein with FMN as prosthetic group. The protein is a single polypeptide chain with molecular weight 70,000.
   (b) **Non-heme iron (NH_I)-** Protein with iron-sulphur centers (Fe-S).There are six Fe-S centers, i.e., Fe-SN1a, Fe-SN1b, Fe-SN2, Fe-SN3, Fe-SN4 and Fe-SN5. It is the largest complex with molecular weight 8, 50,000 and includes a flavoprotein containing FMN. This is the first step in the electron transport chain. Electrons are taken into this complex by NAD+ which is located at the matrix side of the membrane.
2. **Complex II (Succinate-CoQ reductase).** It has the following components.

(a) Succinic dehydrogenase with the molecular weight 70,000 it has covalently bound FAD as prosthetic group and two Fe-S centers, i.e., Fe-SS1 and Fe-SS2.

(b) Fe-SS 3 protein of molecular weight 27, 000 and

(c) Cytochrome b with absorbance 557.5 nm

Coenzyme Q (CoQ) or Ubiquinone (UQ) - It is mobile carrier between complex I and III, and II and III. Complex II precedes the electron transport chain and is coupled to succinate by way of FAD (flavinadenine dinucleotide).

3. **Complex III (CoQH2-Cyt.C-reductase).** This complex contains:

(a) **Cytochrome b** of molecular weight 30,000

(b) **Cytochrome e** of molecular weight 50,000

(c) **Cytochrome c_1** having two polypeptides of molecular weight 29,000 and 15,000.

(d) **NH_1** protein with Fe-S centre and molecular weight 26,000

(e) Core proteins

(f) **Antimycin-binding protein.**

Cytochrome c- It is mobile carrier between complexes III and IV with molecular weight 13,000.

4. **Complex IV (Cytochrome C-Oxidase).** It contain cytochrome a (Cyt. a) not inhibited by CO, cytochrome a3 (Cyt. a3) inhibited by CO and two atoms of copper (Cu and Cu). The final oxidation of hydrogen occurs in it, resulting in water (H_2O) formation.

5. **Complex V (ATPase complex).** It contains head piece, stalk and base piece. Head piece (F1) consists **of 5 subunits** and inhibitor of molecular weight 3, 60,000.

α — Subunits 2 or 3 with molecular weight 53,000

β — Subunits 2 or 3 with molecular weight 50,000

γ — Subunits 1 or 2 with molecular weight 33,000

δ — Subunits 1 or 2 with molecular weight 7,500

E — Subunits 1 or 2 with molecular weight 7,000

F1 inhibitor protein I with molecular weight 10,000

Stalk has F_5 or oligomycin sensitivity conferring protein (OSCP) of molecular weight 18,000 and F_6 (Fe_2) of molecular weight 8,000.

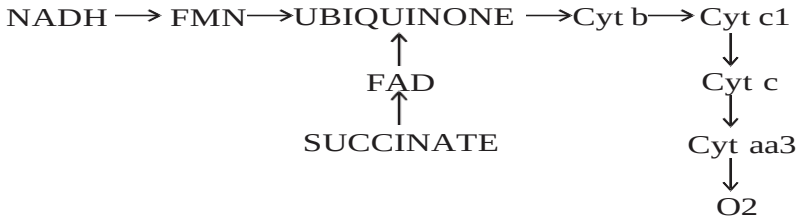
Base piece (F_0) is made of proteolipids — a hydrophobic protein complex forming proton channel. There are four proteins of molecular weight 29,000, 22,000, 12,000 and 7,800.

All these complexes and the phosphorylation system are organized within the inner mitochondrial membrane in an asymmetrical manner. The electron transport system is only accessible to NADH and succinate from matrix side of the membrane, while cytochrome c is reached from cytoplasm side of the membrane. This molecular organization is consistent with the transfer of proton (H+) across the membrane from matrix side to cytoplasm side of the membrane.

The respiratory chain is coupled at three points with the system in which phosphorylation of ADP to ATP takes place. The six protons that originated in the respiratory chain are translocated across the inner mitochondrial membrane from matrix side to cytoplasm side, and these six protons will give rise to three molecules of ATP through the use of mitochondrial ATPase.

3.8 Electron Transport Mechanism

In the electron transport chain electrons are transferred from a donor molecule to an acceptor molecule, thus, it consists of a several electron receptors. Molecular oxygen is the final hydrogen acceptor. The respiratory chain is located in the inner mitochondrial membrane. In the respiratory chain, the electron transfer is done in stepwise fashion in which the electron pairs are passed from one acceptor to another, thus, delivering energy more gradually. Flow of electrons in mitochondria occurs as follows:



3.9 Summary

The term Mitochondria was coined by Benda (1897-98). Mitochondria are the 'power house' which by oxidation, release the energy contained in the fuel molecules or nutrients and make other forms of chemical energy. Mitochondria are lacking in bacterial cells, where oxidation of organic material is carried out in plasma membrane. They may move freely in the cytoplasm in some cells or they are fixed permanently in others depending upon the requirement of ATP energy in that particular part of the organ.

Ultra structure of mitochondria reveals that it is a double membrane bounded organelle. The outer membrane is smooth contoured and is freely permeable while, the inner membrane is selectively permeable. It regulates the movement of materials into or out of the mitochondria. The salient feature of the inner membrane is that it is thrown into a series of infoldings in the cavity of mitochondrion. These infoldings are known as cristae. Between the outer and the inner membrane is a space called peri-mitochondrial space or intracristal space. It contains a homogeneous fluid of low density. The cavity of mitochondria is filled with dense fluid known as mitochondrial matrix. In the matrix are present proteins, lipids few

ribosomes, one or two DNA molecules, RNA and certain other granules. A larger chunk of the mitochondrial proteins represent enzymes.

A number of functions are performed by mitochondria; these include oxidation, dehydrogenation, oxidative phosphorylation and respiratory activity. A large number of enzymes and numerous cofactors and metals essential to mitochondrial functions, work together in an orderly fashion. Besides, oxygen the only fuel that a mitochondrial needs is phosphate and adenosine diphosphate (ADP). The principal final products are ATP plus CO_2 and H_2O.

The respiratory chain takes in succinic acid (succinate) and NADH from Krebs's cycle enzymes. These together with oxygen, respiratory chain produce many molecules of ATP and finally CO_2 and water. As the electrons carried by NADH and succinic acid travel down the chain they give up their energy, which is used up for the conversion of ADP to ATP. During respiratory chain a series of pigments, chemicals and enzymes are involved. In the major pathway chief line of oxidation-reduction reactions of the cell is the removal of hydrogen from substrate by dehydrogenases. Hydrogen is usually picked up by the coenzyme part of dehydrogenase from substrate and carried to flavoprotein, which act as a hydrogen carrier (i.e. FAD-flavin adenine dinucleotide). From FAD, each hydrogen is discharged as ion in the cell fluid and electrons are passed on to the pigments — cytochromes which are a, b, c, c_1 and c_3 types mainly. From cytochromes, electrons are given to the enzymes cytochrome oxidase, which finally discharges electrons to oxygen. This oxygen unites with hydrogen ions to form water.

3.10 Glossary

Plasma membrane: The membrane forming the surface of cytoplasm and consisting of a bimolecular phospholipids layer between an inner and outer layer of protein molecules.

Neuron: The nerve cell with its outgrowths, structural unit of nervous system.

Adenosine Triphosphate (ATP): a molecule containing high energy bonds that provides energy for many biochemical cellular processes by undergoing enzymatic hydrolysis.

Diaphragm: a muscular or ligamentous partition that separates the thorax from the abdomen in mammals.

I-band: In a sarcomere, I-band is the zone of thin filaments that is not superimposed by thick filaments.

Myofibril: Myofibrils are the rod like units of muscle cells. They are composed of repeating sections of sarcomere, which appear under the as dark and light bands.

Oocytes: Oocyte is a female gametocyte or an immature ovum involved in reproduction. It is produced in the ovary during female gametogenesis and it undergoes meiotic division to form an ovum.

Vesicles: A vesicle is a small structure within a cell, consisting of fluid enclosed by a lipid bilayer membrane.

Porin: The beta barrel proteins that acts as transport protein which cross a cellular membrane and act as a pore through which molecules can diffuse. Porins are large enough to act as channels that are specific to different types of molecules.

Permease: The permease is membrane transport proteins that facilitate the diffusion of a specific molecule in or out of the cell by passive transport.

Cardiolipin: Cardiolipin is an important component of the inner mitochondrial membrane where it constitutes about 20% of the total lipid composition. It is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism.

Cristae: A cristae is a fold in the inner membrane of the mitochondrion. It provides a large amount of surface area for the chemical reactions to occur on.

Oxysomes: It is a structural unit of cellular cristae.

***De novo*:** *De novo* is a Latin expression meaning "from the beginning," "afresh," "anew," "beginning again."

Microbody: A microbody is a type of organelle that is found in the cells of plants, protozoa and animals and microbody include peroxisome, glyoxysome and glycosome.

3.11 Self Assessment Questions and Possible Answers

3.11.1 Multiple Choice Questions

1. Cell's power houses are its:
   (a) Lysosomes (b) Mitochondria
   (c) Ribosomes (d) Golgi apparatus

2. Mitochondrion is bounded by:
   (a) A single unit membrane (b) Two unit membranes
   (c) No membranes (d) Plasma membranes

3. New mitochondria arise:
   (a) De novo (b) By replication
   (c) From plasma membrane (d) from nuclear envelop

4. The ATPase enzyme is located in the mitochondria in:
   (a) Oxysomes (b) Outer membrane
   (c) Inner membranes (d) Matrix

5. The name mitochondria were given by:
   (a) Altman (b) Flemming
   (c) Benda (d) Kollikar

6. ETS is located in:
   (a) Outer mitochondrial membrane (b) Inter membrane space
   (c) Inner mitochondrial membrane (d) mitochondrial matrix

3.11.2 Very short questions

1. Where are ETS enzymes located in mitochondria?
2. Give the function of mitochondria.
3. What are cristae?
4. What type of DNA do mitochondria have?
5. Mention three parts of oxysome.
6. Who named mitochondria?
7. What kind of enzymes is present in the mitochondria?
8. Name the enzymes oxysomes represent.
9. Which is the most common energy carrier in cells?
10. Give alternative names of oxysomes.

ANSWERS

3.11.1:-

1. (b)	3. (b)	5. (c)
2. (b)	4. (a	6. (c)

3.11.2:- ANSWERS

1. Inner membrane
2. ATP formation
3. Infolds of inner mitochondrial membrane
4. Circular, single molecule and double stranded
5. Head piece, stalk and base piece (FO & F_1)
6. Benda
7. Respiratory enzymes
8. ATPase (ATP Synthetase)
9. ATP
10. Elementary particles, inner membrane subunits, F0-F1 Complex.

3.12 References and Suggested Readings

1. Flemming, W. (1882). Zellsubstanz, Kern and Zellteilung, Leipzig (quoted from Cowdry).

2. Hogeboom, G.H., Schneider, W.C. and Pallade, G.E. (1948). Cytochemical studies on mammalian tissues. I. Isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. *J. Biol. Chem.,* **172**: 619-636.

3. Kölliker, R.A. (1880). Report on the Pennatulida dredged by H.M.S. Challenger during the years 1873-1876. *Challenger Reports Zool.*, **1**(2): 1-41.

4. Lehninger, A.L. and Kennedy, E.P. (1948). The requirements of the fatty acid oxidase complex of the rat liver. *J. biol. Chem.*, **173(2)**: 753-771.

3.13 Terminal and Model Questions

1. Give an account of history and structure of endoplasmic reticulum.
2. Show protein trafficking in a cell with the help of a labeled diagram.
3. Describe types and functions of ER.
4. Describe structure and functions of ribosomes.
5. Show the structure of 80S ribosome with the help of labeled diagram.
6. Give an account of history, structure and functions of Golgi bodies.
7. Describe the morphology of Golgi bodies.

UNIT 4 ENDOPLASMIC RETICULUM, RIBOSOME, GOLGI BODIES

Contents

4.9 References and suggested readings.

4.10 Terminal and Model Questions.

4.1 Objectives

After reading this unit the readers will be able to:

- Define endoplasmic reticulum (ER)
- Discuss the structure and functions of endoplasmic reticulum
- Explain the importance of ER
- Discuss the structure and functions of ribosome
- Write the importance of ribosome
- Explain the structure and functions of Golgi bodies
- Tell the importance of Golgi bodies.

4.2 Introduction

The matrix of cell contains various particles of different sizes called cytoplasmic constituents or organelles. They include rounded, globular, filamentous or granular mitochondria, network of endoplasmic reticulum, elongated secretary particles of Golgi apparatus, ribosomes, plastids, centrosomes and lysosomes. Endoplasmic reticulum is a complex, finely divided vacuolar or tubular system, extending from nucleus through cytoplasm to the margins of the cells. This system is enclosed by double membrane. Ribosomes are small dense and granular ribonucleoprotein (i.e. RNA and proteins) particles found attached to outer surface of endoplasmic reticulum and nucleus as well as freely scattered in cytoplasm, mitochondrial matrix and chloroplast. Golgi bodies may consist of many flattened sacs. In plant cells they are collectively called as **'dictyosome'**. They are found scattered throughout the cytoplasm. Golgi complex occupies different positions in different kinds of cells. In secretary and absorptive cells, it usually lies between the nucleus and the cell surface where secretion and absorption occurs. In nerve cells it surrounds the nucleus, and lies elsewhere in other cells.

4.3 Endoplasmic Reticulum

4.3.1 General History of Endoplasmic Reticulum

Early cytologists held that some sort of supporting network or cytoskeleton was present in the cells. It was given various names — **Nissil substance**, **ergastoplasm**, **basophilic bodies**, etc. In 1945, **Porter, Claude and Fullman** with the help of electron microscope noted a delicate membranous network in the cytoplasm. It was later called **endoplasmic reticulum** (ER) by **Keith Porter** in 1953. The ER originally seemed to be confined to the endoplasm of the cell, hence its name.

4.3.2 Structure of Endoplasmic Reticulum

In eukaryotic cells endoplasmic reticulum is generally the largest membrane which forms extensive system of intercommunicating membranous sacs or channels. It represents 30 to 60% of total membrane in a cell. The membrane of endoplasmic reticulum may or may not have ribosomes attached to their outer membrane. Accordingly these are classified as rough (RER) or smooth endoplasmic reticulum (SER). Rough endoplasmic reticulum is characterized by the presence of ribosomes of about 150Å in diameter and rich in protein and RNA. Smooth endoplasmic reticulum lacks ribosomes. It comprises three types of elements: cisternae, tubules and vesicles (Fig. 4.1).

Cisternae- These are flattened, unbranched, sac like elements with about 40-50μm in diameter. They lie in stacks (piles) parallel to but interconnected with one another. They are separated from one another by cytosolic spaces. The small granular structures called the ribosomes may or may not be present on the surface of cisternae.

Tubules- These are irregular, branching elements, which form a network along with other elements. They are about 50-100μm in diameter, and are often free of ribosomes.

Vesicles- These are oval, vacuole like elements, about 25-500μm in diameter. They often occur isolated in the cytoplasmic matrix. They are also free of ribosomes. A fluid called the endoplasmic matrix is present in the lumen of ER. All the elements of ER freely communicate with one another

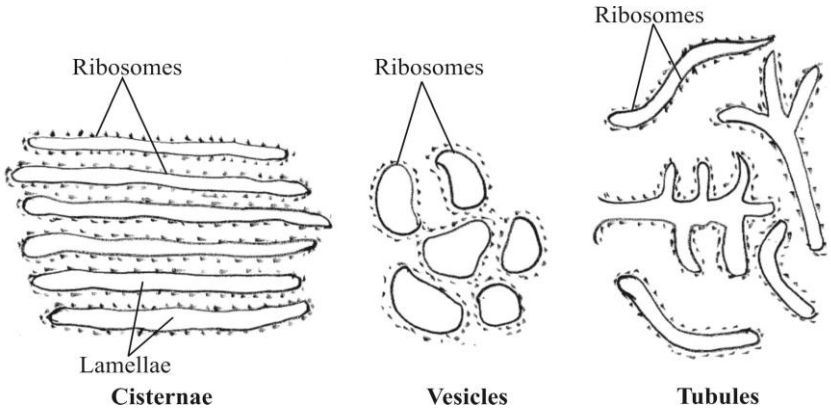


Fig. 4.1: Various forms of ER.

4.3.2.1 Ultra structure of Endoplasmic Reticulum

The membrane bounding the cisternae, tubules and vacuoles of the ER is similar to the cell membrane. It is 50-60Å thick. The membranes of endoplasmic reticulum are composed of two layers of phospholipids molecules sandwiched by two layers of protein molecules like other membranes in the cell (Robertson, 1959). The ER membrane has a relatively high protein/lipid ratio. It is continuous with the cell membrane, Golgi membranes and outer membrane of the nuclear envelope. Certain cisternae open out by pores in the cell membrane. In the lumen of endoplasmic reticulum, secretary granules were observed by Palade (1956). The lumen acts as a passage for the secretary products. About 30-40 different enzymes are associated with the ER for the various synthetic activities. These may be located on the cytoplasmic surface or luminal surface or both. Membrane bound endoplasmic reticulum spaces varies in shape and sizes in different cell types (Fig. 4.2).

On the basis of absence or presence of ribosomes, two kinds of ER are found in cells.

1. **Smooth Endoplasmic Reticulum:** Ribosomes are absent on the walls of ER and so it appears smooth and hence called **smooth or agranular ER**. It mainly occurs as tubular forms. The tubules forms irregular lattices and measures about 500-1000Å in diameter. Smooth ER is commonly found in the cells involved in the synthesis of **steroids or lipids i.e. non protein type of synthesis** (Christensen and Fawcett, 1961) such as adrenal or sebaceous glands, gonadial interstitial cells. Certain cells with carbohydrate metabolism (e.g. liver cells), impulse conduction (e.g. muscle cells), with pigment production (e.g., retinal pigment cell) and electrolyte excretion (e.g., chloride cells of fish gills) are also have more of SER in them.

2. **Rough Endoplasmic Reticulum (RER):** It is characterized by the presence of ribosomes on the surface of reticulum and so it is also known as **granular ER**. It is in the form of flattened cisternae with the width of 400-500Å. RER occurs largely in the cells that are actively involved in **the synthesis of proteins such as enzymes** (e.g. pancreatic cells, plasma cells and liver cells) or mucus (goblet cells). In exocrine cells of pancreas, RER consists of reticular sheets and fenestrated cisternae in the basal region of the cell. These cisternae measures about 5-10 micron in length and their

groups are 400-1000Å in diameter. In apical region of the cells, granular reticulum occurs in the form of vesicles. Granular and agranular ER are in continuity of their membranes in the regions of contact.

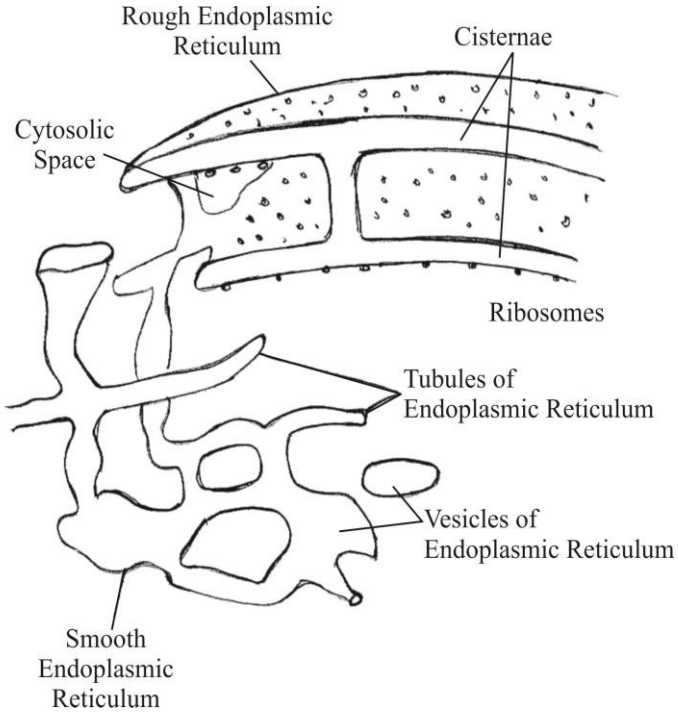


Fig. 4.2: Various types of elements of endoplasmic reticulum.

4.3.3 Functions of Endoplasmic Reticulum

ER serves many functions. These may be listed as follows.

4.3.3.1 Functions of smooth endoplasmic reticulum

1. **Surface for Synthesis-** The SER provides surface for the synthesis of fatty acids, phospholipids, glycolipids, steroids and visual pigments.
2. **Glycogen Metabolism-** The SER carries enzymes for glycogen metabolism in liver cells. Glycogen granules are attached in larger numbers to the outside of the SER's membranes in liver cells.
3. **Detoxification-** The SER has enzymes that are involved in the detoxification in the liver, i.e., converts harmful materials such as carcinogens and pesticides, into harmless ones for excretion by the cell.
4. **Formation of organelles-** The SER produces Golgi apparatus, lysosomes, micro bodies and vacuoles.
5. **Transport route-** The proteins shift from RER through SER to Golgi apparatus for further processing.

6. **Skeletal Muscle Contraction-** The sarcoplasmic reticulum in skeletal muscle cells release Ca^{2+} ions to cause contraction and absorbs Ca^{2+} ions to bring about relaxation.

7. **Fat Oxidation-** The SER membranes carry out the initial reactions in the oxidation of fats.

4.3.3.2 Functions of rough endoplasmic reticulum

1. **Surface for Ribosomes-** The RER provides a large surface for the attachment of ribosomes.

2. **Surface for synthesis-** The RER offers extensive surface on which protein synthesis can be conveniently carried on by ribosomes. The newly formed proteins may enter the ER membranes, becoming a part of the membrane structure or pass into the ER lumen. The proteins becoming a part of ER membrane eventually move from the ER via membranes of other cell organelles, namely Golgi apparatus, secretary vesicles to become permanent plasma membrane proteins. The proteins entering ER lumen are packed for export.

3. **Packaging-** The proteins in ER lumen are processed and get enclosed in spherical membrane bound vesicles which get pinch off from the ER. These vesicles have various fates. Some remain in the cytoplasm as storage vesicles while others migrate to the plasma membrane and expel their contents by exocytosis. Some fuse with Golgi apparatus for further processing of their proteins for storage or release from the cell.

4. **Smooth ER Formation-** The RER gives rise to the smooth ER by loss of ribosomes.

5. **Formation of Nuclear Envelope-** The RER forms nuclear envelope around daughter cells in cell division.

6. **Formation of Glycoproteins-** The process of linking sugars to proteins to form glycoproteins starts in the RER and is completed in Golgi apparatus.

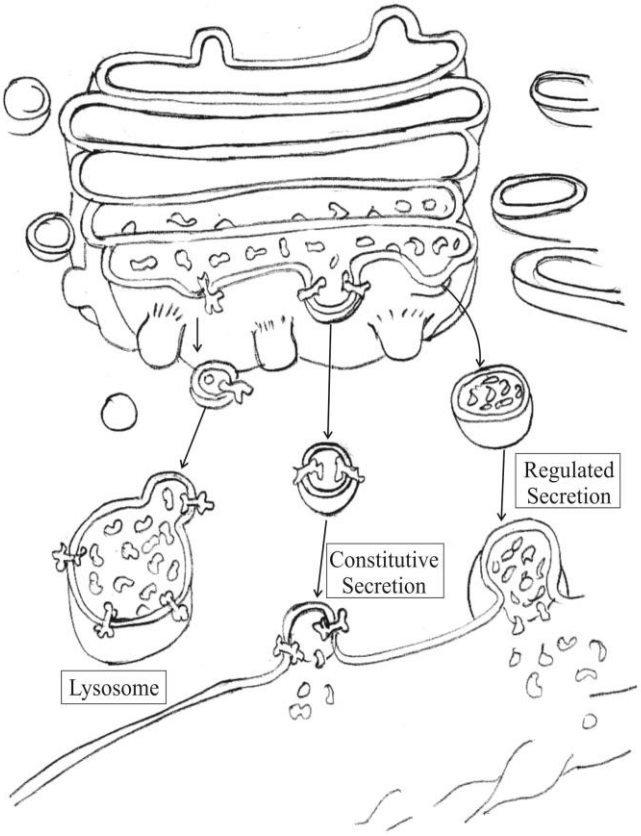


Fig. 4.3: Transport of proteins from Golgi apparatus. Proteins are sorted and transformed in Golgi network and transported in vesicles to their final destination.

4.3.4 Importance of Endoplasmic Reticulum

1. **Transport of Materials-** The ER facilitates transport of materials from one part of the cell to another thus forming the cell's circulatory system.
2. **Formation of Desmotubule-** Tubular extension, called desmotubule, extends through plasmodesmata to make ER continuous in the two adjacent plant cells.
3. **Support-** The ER acts as an intracellular supporting framework, the cytoskeleton that also maintains the form of the cell.
4. **Localization of Organelles-** It keeps the cell organelles properly stationed and distributed in relation to one another.
5. **Surface for Synthesis-** The ER offers extensive surface for the synthesis of a variety of materials.
6. **Storage of Materials-** The ER provides space for temporary storage of synthetic products such as proteins and glycogen.
7. **Exchange of materials-** The ER helps in the exchange of materials between the cytoplasm and the nucleus.
8. **Location of Enzymes-** A variety of enzymes is located in the ER membranes to catalyze the biochemical reactions.

4.4 Ribosome's

4.4.1 General History of Ribosome

George E. Palade (1953) was the first to observe dense particles or granules in animal cells under electron microscope. These were thus called as Palade's Particles. Later **Richard B. Roberts** named them "**ribosomes"** in 1958. **Tissieres and J.D. Watson** (1958) isolated ribosomes from E. coli for the first time. It was shown that ribosomes contain approximately equal amount of RNA and proteins.

4.4.2 Structure of Ribosome

Ribosomes are of two types **70S and 80S. 'S'** is **Svedberg unit**, a measure of particle size dependent on the speed with which the particles sediment in the ultracentrifuge. The **70S** ribosomes are found in the **prokaryotic cells** and in the **mitochondria and plastids** of eukaryotic cells. The **80S** ribosomes occur in the cytoplasm of the eukaryotic cells. Both the 70S and 80S ribosomes are similar in structure. They are small, spherical structures of which 70S ribosomes are around 200Å in diameter, while 80S are 250 to 300Å in diameter. They are porous and hydrated having two subunits, one is larger (140-160Å in diameter) having dome shaped structure and the other is smaller in size, found over the larger subunit, forming a cap like structure. The two subunits are separated by clefts (Palade and Kuff, 1966). **Membrane is absent around them**. The subunits occur separately in the cytoplasm, and join to form ribosomes only at the time of protein synthesis. Many ribosomes line up and join the mRNA chain. After the synthesis of protein, the ribosomes leave the mRNA chain and dissociate into subunits.

1. **70S Ribosome:** These are found in bacterial cells and have the molecular wt. 2.7×10^{-6} daltons and sedimentation coefficient 70S. 70S ribosome consists of a large 50S subunit and a small 30S subunit. Each subunit is composed of rRNA and several basic proteins. The 50S subunit has two species of RNA: 23S and 5S and about 34 different ribosomal proteins. The 30S subunit has only one species of rRNA, i.e., 16S and about 21 different ribosomal proteins. They also occur in mitochondria and chloroplasts of eukaryotic cells (Fig. 4.4a).

2. **80S Ribosome:** Having the sedimentation coefficient 80S, these are somewhat larger and contain more RNA and proteins than 70S ribosomes. An 80S ribosome is over 250 to 300Å in diameter. Their mol. wt. is 4×10^{-6} daltons. It consists of a large 60S subunit and a small 40S subunit. Each subunit is composed of rRNA and several specific basic proteins. The 60S subunit has three species of rRNA: 28S, 5.8S and 5S and over 45 different ribosomal proteins. The 40S subunit has only one species of rRNA, i.e., 18S and over 33 different ribosomal proteins. They are found in eukaryotic cells (Fig. 4.4b).

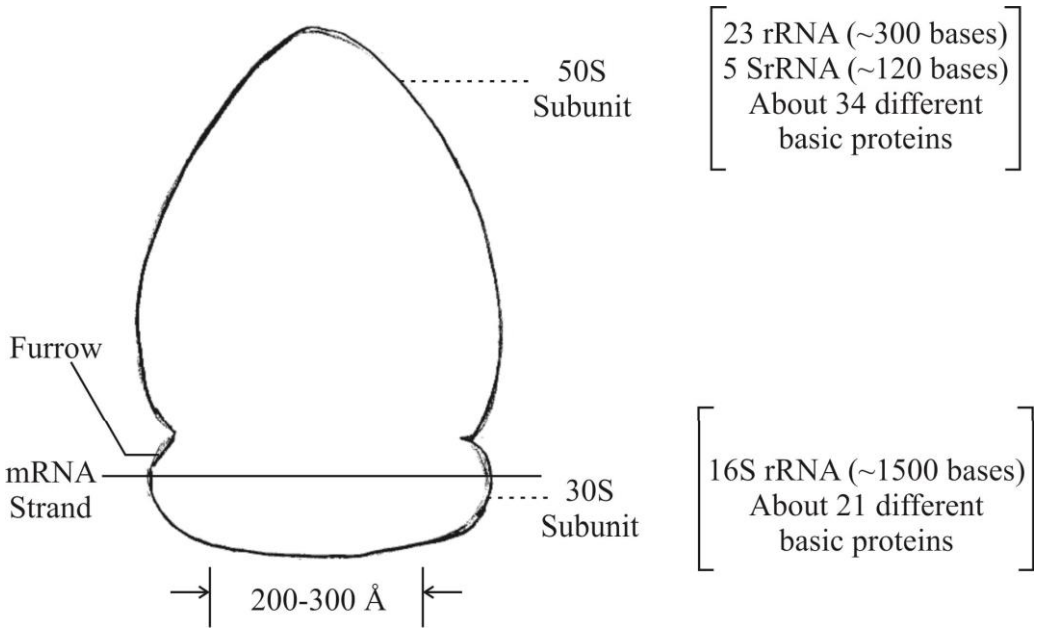


Fig. 4.4a: Structure of 70S ribosome of Escherichia coli, a colon bacilia

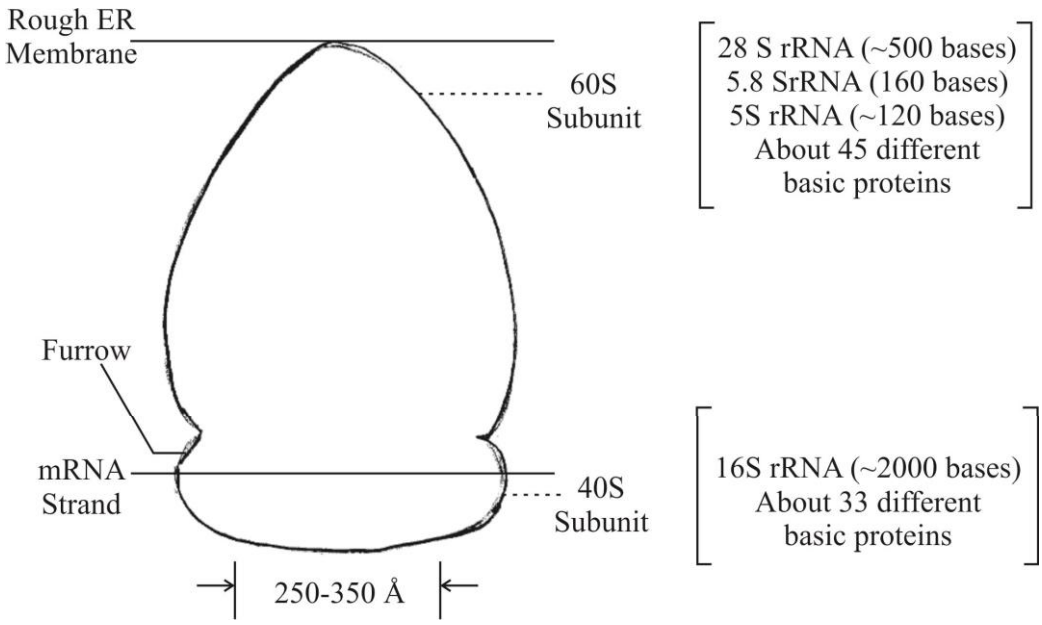


Fig. 4.4b: Structure of 80S ribosome of eukaryotic cell

4.4.2.1 Ultra structure of ribosome

The ribosomes are composed of two subunits (one subunit is almost twice in size than the other) fitted together to form a complete unit of about 300Å in diameter. In 70S ribosome the 50S subunit is pentagonal compact particle of 160 to 180Å bearing a round concave area in its center of about 40 to 60Å that accommodates the small subunit. A small pore like transparent area is also present that inhibits the entrance of enzyme ribonuclease. Similar pores are present in 60S subunit of 80S ribosomes. The smaller subunits 30S of 70S and 40S of 80S ribosomes have irregular forms and are often divided into two portions which are

interconnected by a strand of 30 to 60 Å thicknesses. Ribosomes have a groove at the junction of large and small subunits. The mRNA is seated in the gap between both ribosomal subunits, where the ribosome protects a stretch of some 25 nucleotides of mRNA from degradation by ribonuclease. From this groove, a canal or tunnel extends through the large subunit and opens into the lumen of the endoplasmic reticulum. Polypeptides are synthesized in the groove between the two ribosomal subunits and pass through the tunnel of the large subunit into the endoplasmic reticulum (Fig. 4.5).

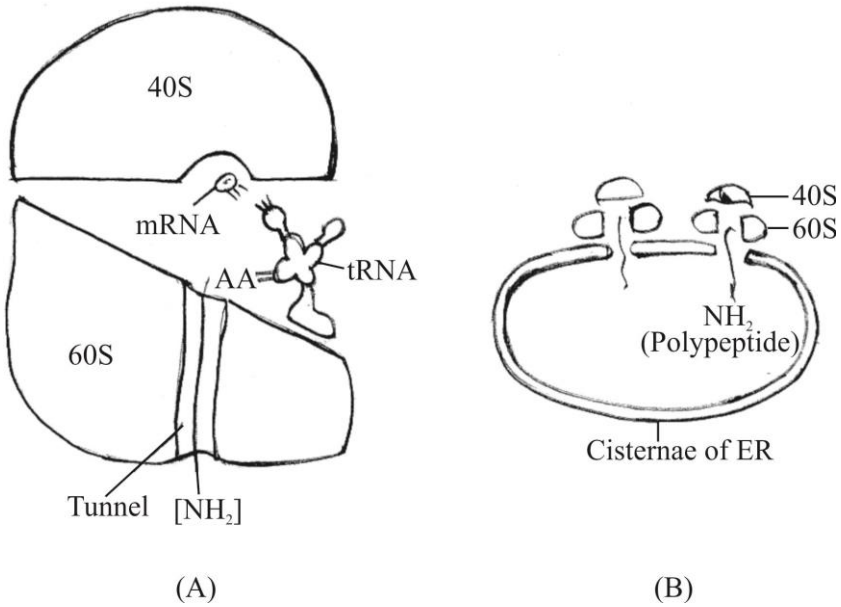


Fig. 4.5: Ultra structure of ribosomes showing two subunits

4.4.3 Functions of Ribosome

1. **Attached Ribosomes-** The ribosomes provide space and enzymes for the synthesis of proteins in the cell. The ribosomes bound to the ER membranes synthesize: (i) integral proteins for cellular membranes, (ii) lysosomal proteins and (iii) secretary proteins for export as secretions.
2. **Free Ribosomes-** The free ribosomes produce structural and enzymatic proteins for use in the cell itself. These proteins include glycolytic enzymes and most extrinsic membrane proteins, such as spectrin.

4.4.4 Importance of Ribosome

- Ribosomes are known as protein factories. Ribosomal RNA molecules possibly serve as a skeletal framework in the ribosomes.
- Smaller ribosomal subunit is required for the formation of initiation complex at the start of the protein synthesis. Whereas larger ribosomal subunit is necessary for peptide bond formation and the elongation for the polypeptide.
- The ribosome function as a template in order to bring together various components involved in the synthesis of proteins. Ribosomes co-ordinate the interaction of t-RNA-

amino acid complex with m-RNA. This co-ordination results in the translation of genetic code forming specific proteins.

- Since free ribosomes are not involved in protein synthesis, they are transported through endoplasmic reticulum membranes and assembled into globules within the cisternae and canals in the cells that produce 'proteins for transport'. Proteins later appear in the form of granules outside the Golgi complex.

4.5 Golgi Complex

4.5.1 General History of Golgi Bodies

Camillo Golgi in 1898 discovered the Golgi apparatus in the nerve cells of barn owl and cat by metallic impregnation method. After it's discoverer's name, the Golgi apparatus has been variously named as **Golgisome**, **Golgi material**, **Golgi membranes**, **Golgi body**, etc.

4.5.2 Structure of Golgi Bodies

Golgi bodies varies in size and form in different types of cells, but they have similar organization in all kinds of cells. For example, it is well developed in secretory and nerve cells, but is rather small in muscle cells. Golgi bodies are compiled as a central stack (pile) of flattened sacs or cisternae and many peripheral tubules and vesicles.

1. **Cisternae-** The cisternae vary in number from 3 to 7 in most animal cells and from 10 to 24 in plant cells. They are usually equally spaced in pile so that they are nearly parallel to one another, having 200-300Å wide inter-cisternal spaces containing a layer of parallel fibers called inter-cisternal elements. These support the cisternae and maintain regular spacing between them. The cisternae may be flat, but are often curved, having a distinct polarity with a convex face towards the cell membrane and concave face towards the nucleus. They are free of ribosomes and have swollen ends. They look like the smooth endoplasmic reticulum and are continuous with it at certain places. This suggests that the Golgi apparatus is derived from the smooth endoplasmic reticulum. A cisterna is about 0.5-1 μm in diameter and its cavity is about 100Å wide. It is fenestrated at the margin as here it passes into tubules. All the cisternae have a continuous lumen filled with a fluid.

2. **Tubules-** Short tubules arise from the periphery of the cisternae. Some of these enlarge at their ends to form vesicles.

3. **Vesicles-** The vesicles lie near the ends and concave surface of the Golgi complex. They are pinched off from the tubules of the cisternae. They are of three types: transitional, smooth or secretary and coated vesicles.

a. **Transitional Vesicles:** These are the small outgrowths formed from the transitional ER. They migrate to, converge and coalesce to cis face of Golgi, where they form new cisternae.

b. **Smooth Vesicles:** These have smooth surface and contain secretions of the cell and so they are also called secretary vesicles. They arise from the ends of the cisternae tubules.

c. **Coated vesicles:** These have rough surface and they also arise from the cisternae tubules. They play a role in intracellular traffic of secretary protein molecules.

The Golgi complex has 3 functional regions: **cis region** that lies nearest the ER, **medial region** in the middle, and **Tran's region** with trans Golgi reticulum nearest to the plasma membrane. These regions have different enzymes which introduce different modifications to secretary and membrane proteins passing through them. The principal modification is glycosylation, i.e., addition of sugars to proteins, forming glycoproteins. Glycosylation starts in the ER and is completed in the Golgi complex. Modification of proteins in the Golgi apparatus also involves addition of lipids, forming lipoproteins (liposylation), and even the addition of other groups (Fig. 4.6).

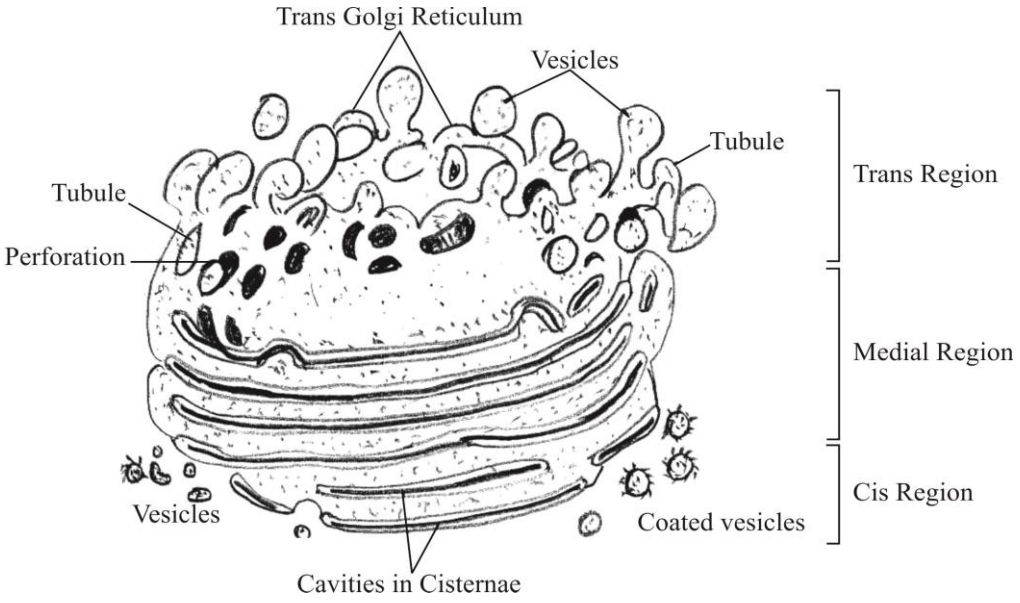


Fig. 4.6: Thee-dimensional view of Golgi apparatus

4.5.3 Functions of Golgi Bodies

Golgi apparatus is metabolically very active. Many functions have been assigned to it:

1. **Formation of secretary vesicles-** The Golgi complex processes and packages proteins and lipids coming from the ER for transport to other parts of the cell or out of

the cell. Packaging involves wrapping the materials by a membrane, forming secretary vesicles. The materials so packed includes zymogen in pancreatic cells, mucus in goblet cells, lactoprotein in mammary gland cells, pigment granules in pigment cells, collagen in connective tissue cells, hormones in endocrine cells, etc.

2. **Synthesis of carbohydrates-** The Golgi apparatus synthesizes certain mucopolysaccharides from simple sugars.

3. **Formation of Glycoproteins-** The Golgi apparatus links the sugars with proteins coming from rough ER to form glycoproteins.

4. **Formation of Lipoproteins-** Lipids and proteins coming from the ER are complexed into lipoproteins in the Golgi apparatus.

5. **Addition to Cell Membrane-** The Golgi apparatus provides membrane material for the plasma membrane when the later must enlarge for the formation of pinocytotic and phagocytotic vesicles and for the formation of cleavage furrow during the division of animal cells. As the secretary vesicles discharge their contents by exocytosis, their membranes are incorporated into the cell membrane. This enlarges the cell membrane. Since, endocytosis removes segments of the cell membrane, the latter's enlargement by exocytosis is temporary, rather compensatory. The transfer of membrane from the ER via transition vesicles, Golgi complex and secretary vesicles to the plasma membrane is called **membrane flow**.

6. **Membrane Transformation-** The Golgi apparatus changes one type of membrane into another type. Membranes are gradually modified from the ER type to one with characteristics of the plasma membrane as they shift through the Golgi complex.

7. **Formation of cell wall-** In some algae, cellulose plates for cell wall is synthesized in Golgi complex. In higher plants the Golgi complex (a) synthesizes pectin and some carbohydrates necessary for the formation of cell wall and (b) produces some secretions such as mucilage, gums, etc.

8. **Formation of lysosomes-** The Golgi complex gives rise to primary lysosomes by budding. The lysosomes may also arise from ER.

9. **Acrosome Formation-** The Golgi complex gives rise to the acrosome in a sperm.

10. **Formation of Yolk and Cortical Granules-** The Golgi complex produces yolk and cortical granules in the eggs. Formation of yolk is called vitellogenesis.

11. **Formation of Nematocysts and Trichocysts-** The Golgi apparatus gives rise to the nematocysts in Hydra and perhaps also in other coelenterates, and trichocysts in ciliates such as Paramecium.

12. **Storage of Secretions-** The Golgi complex stores cell secretions such as proteins and lipids.

13. **Absorption of Materials-** Golgi apparatus absorbs materials from the environment. For example, cells of the intestinal lining use Golgi apparatus to absorb lipids from the intestine.

14. **Location of Enzymes-** A variety of enzymes is localized in the Golgi complex to help in the cell's biochemical reactions.

4.5.4 Importance of Golgi Bodies

The Golgi apparatus is often referred to as the "traffic police" of the cell because its enzymes sort out and modify cell's secretary proteins passing through its lumen and membrane proteins in its membranes and directs them to their proper destination.

4.6 Summary

The ER is present in almost all eukaryotic cells except ova, embryonic cells and mature RBC. The prokaryotic cells lack ER. It comprises three types of elements: cisternae, tubules and vesicles. Various functions are performed by them, such as transport of materials, formation of desmotubule. They form supporting framework; provide surfaces for synthesis, storage and exchange of various materials. Similarly ribosomes are present in all types of cells prokaryotic as well as eukaryotic. Although they occur freely floating in the cytoplasm of prokaryotic cells, they are present free in cytoplasmic matrix and also attached to the outer surface of RER and nuclear envelop of eukaryotic cells. Each ribosome consists of two structurally and functionally distinct subunits: one large, dome shaped and the other smaller and ovoid. The subunits occur separately in the cytoplasm, and join to form ribosome only at the time of protein synthesis. The 70S ribosome is found in prokaryotic cells and in the mitochondria and plastids of the eukaryotic cells. The 80S ribosome occurs in the cytoplasm of eukaryotic cells. The ribosomes provide space and enzymes for the synthesis of proteins in the cells.

The Golgi apparatus is a system of membranes like ER. It is present in all eukaryotic cells, except a few cell types such as the mammalian RBC, sperm cells of bryophytes and pteridophytes and sieve tubes of plants. It is absent in prokaryotic cells. It is composed of cisternae, tubules and vesicles and they perform various functions like formation of secretary vesicles, carbohydrates, glycoproteins, lipoproteins etc, cell walls in plant cells, lysosomes, acrosomes in sperms, yolk and cortical granules in eggs etc. They also store secretions and absorb various materials and many enzymes are located in them.

4.7 Glossary

Endoplasmic reticulum: It is a network of membranous tubules within the cytoplasm of a eukaryotic cell, continuous with the nuclear membrane. It usually has ribosomes attached and is involved in protein and lipid synthesis.

Ribosome: A ribosome is a protein synthesizing machine found within all living cells that serves as the site of biological protein synthesis.

Golgi Bodies: The Golgi bodies also called Golgi complex or Golgi apparatus is a system of membranes like ER. It receives proteins and lipids from rough endoplasmic reticulum, modifies some of them and sorts, concentrates and packs them into vesicles.

Mitochondria: It is an organelle bounded by double membrane in which the biochemical processes of respiration and energy production occur.

Plastids: Plastids are double membrane organelle found in the cells of plants and algae. They are the site of manufacture and storage of important chemical compounds like pigments used in photosynthesis.

Centrosomes: It is an organelle where cell microtubules get organized. It regulates the cell division cycle, the stage which lead up to cell division. They occur only in animal cells.

Lysosomes: A lysosome is a membrane bound cell organelle found in most animal cells. They are spherical vesicles containing hydrolytic enzymes capable of breaking down all kinds of biomolecules, including proteins, nucleic acids, carbohydrates, lipids and cellular debris.

Dictyosome: In invertebrates and plant cells, Golgi complex usually consists of many isolated units called dictyosome. They are scattered throughout the cytoplasm.

Cisternae: Cisternae refer to a flattened membrane discs lying stacked upon each other like pancakes.

Tubules: These are the short structures arising from the periphery of the cisternae. Some of these enlarge at their ends to form vesicles.

Vesicles: The vesicles are the spherical structures that lie near the ends and concave surface of the Golgi complex. They are pinched off from the tubules of the cisternae.

Interstitial cells: Any cells that lie between other cells are called interstitial cells. For e.g. Leydig cells that produce testosterone are found adjacent to the seminiferous tubules of testicle.

Electrolyte: An electrolyte is a substance that produces an electrically conducting solution when dissolved in a polar solvent, such as water. The dissolved electrolyte separate into cations and anions and are dispersed uniformly through the solute.

Goblet cells: A goblet cell is a glandular, columnar epithelial cell whose function is to secrete gel-forming mucins, the major components of mucus.

Metabolism: It is the process by which body converts the food into energy. During this complex biochemical process, calories in food are combined with oxygen to release the energy for the body to function.

Oxidation: Oxidation is the loss of electrons by a molecule, atom, or ion.

Exocytosis: Exocytosis is a process by which a cell directs the contents of secretary vesicles

out of the cell membrane and into the extracellular space.

Desmotubules: The desmotubule is a tube of appressed endoplasmic reticulum that runs between two adjacent cells. Some molecules are known to be transported through this channel, but it is not thought to be the main route for plasmodesmatal transport.

Dalton: Dalton is the standard unit that is used for indicating mass on an atomic or molecular scale (atomic mass). One unified atomic mass unit is approximately the mass of one nucleon (either a single proton or neutron) and is equivalent to 1 g/mol.

Sedimentation Coefficient: The sedimentation coefficient of a particle is used to characterize its behavior in sedimentation processes, notably centrifugation. It is defined as the ratio of a particle's sedimentation velocity to the acceleration that is applied to it.

Ribonuclease: Ribonuclease is a type of nuclease that catalyzes the degradation of RNA into smaller components.

4.8 Self Assessment Questions and Possible Answers

4.8.1 Multiple Choice Questions

1. Endoskeleton of the cell is made of:

   (a) Endoplasmic reticulum (b) Mitochondria

   (c) Cell Wall (d) Cytoplasm

2. Metabolic enzymes bringing about synthesis of chemical components of unit membrane in cell occur in:

   (a) Rough Endoplasmic reticulum (b) Smooth Endoplasmic reticulum

   (c) Lysosomes (d) Mitochondria

3. What part of the cell forms the nuclear envelope during telophase?

   (a) Cytoskeleton (b) Centriole

   (c) Golgi complex (d) Endoplasmic reticulum

4. Pores in the cell membrane and outer membrane of nuclear envelope open into:

   (a) Golgi apparatus (b) Mitochondria

   (c) ER (d) Lysosome

5. A ribosome consists of:

   (a) Four subunits (b) Six subunits

   (c) Two subunits (d) three subunits

6. 70S ribosomes are found in:

   (a) Prokaryotic cells (b) Eukaryotic cells

   (c) Both of these (d) None of these

7. Ribosomes are composed of:

(a) rRNA and proteins (b) rRNA and lipids

(c) rRNA and carbohydrates (d) Proteins and lipids

8. The 80S ribosomes of eukaryotes break into:

(a) 50S and 30S (b) 40S and 40S

(c) 60S and 40S (d) 60S and 50S

9. Ribosome was discovered by:

(a) Kollicker (b) Palade

(c) de Duve (d) Porter

10. Ribosome helps in:

(a) Lipogenesis (b) Cellular digestion

(c) Protein synthesis (d) Photosynthesis

11. Golgi apparatus occurs in:

(a) Bacteria

(b) Human RBC

(c) All the cells

(d) All the cells except bacteria and RBC

12. Dictyosome is called:

(a) Lysosome (b) Mitochondria

(c) Golgi body (d) Ribosome

13. Cell secretion is carried out by:

(a) Nucleolus (b) Plastids

(c) E.R. (d) Golgi complex

14. Materials enter Golgi complex at:

(a) Cis region (b) Medial region

(c) Trans region (d) Trans Golgi reticulum

15. Proteins are modified in:

(a) ER (b) Golgi complex

(c) Both a and b (d) Neither in a nor in b

4.8.2 Very Short Questions:

1. Who introduce the term Endoplasmic Reticulum?

2. Name two types of ER.
3. How does ER arise?
4. Which type of cells possesses smooth ER?
5. In which cells rough ER is well developed?
6. What are ribonucleoprotein particles?
7. Name two types of ribosomes.
8. Where are 70S ribosome found in eukaryotic cells?
9. Name the protein factories of the cell.
10. Which ribosomes produce proteins for export from the cell?
11. Name the chemical components of a ribosome.
12. Who discovered Golgi bodies?
13. Golgi apparatus in plants and invertebrate cells consists of several separate units. What are these called?
14. Name three types of elements that form the Golgi apparatus.
15. From where do the vesicles of Golgi apparatus arise?
16. What is the origin of Golgi bodies?
17. Name the organelle commonly referred to as the "traffic police" of the cell.
18. What is glycosylation?

ANSWERS

4.8.1 :-

1. (a)	6. (c)	11.(d)
2. (b)	7. (a)	12.(c)
3. (d)	8. (c)	13.(d)
4. (c)	9. (b)	14.(a)
5. (c)	10.(c)	15.(c)

4.8.2 :-

1. Porter
2. Rough or granular ER and Smooth or agranular ER
3. By out folding of nuclear envelope
4. Those cells which are engaged in lipid metabolism, such as adipose, brown fat and adrenocortical cells
5. Those cells which are engaged in protein synthesis (enzymes) such as

pancreatic cells

6. Ribosomes
7. 70S and 80S
8. In mitochondria and plastids
9. Ribosomes
10. that is attached to ER
11. rRNA and proteins
12. Camillo Golgi
13. Dictyosomes
14. Cisternae, tubules and vesicles
15. from Golgi tubules
16. Smooth ER
17. Golgi apparatus
18. Linking sugars with proteins

4.9 References and Suggested Readings

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4.10 Terminal and Model Questions

1. Give an account of history and structure of endoplasmic reticulum.
2. Show protein trafficking in a cell with the help of a labeled diagram.
3. Describe types and functions of ER.
4. Describe structure and functions of ribosomes.
5. Show the structure of 80S ribosome with the help of labeled diagram.
6. Give an account of history, structure and functions of Golgi bodies.
7. Describe the morphology of Golgi bodies.

UNIT 5 LYSOSOME, CENTRIOLE, MICROTUBULE

Contents

5.1 Objectives

After reading this unit the readers will be able to:

- Explain the structure of lysosome
- Tell the functions and importance of lysosome
- Describe the structure and functions of centriole
- Discuss the importance of centriole
- Define microtubules
- Explain the structure and functions of microtubules

5.2 Introduction

The **lysosomes** are important products of the secretary pathway in cells. Lysosomes are also known as **"suicidal bags"**. They are rounded, elliptical or highly irregular in shape. They are single membrane bounded bodies having a multiple hydrolytic enzymes capable of digesting all kinds of materials inside or outside the cell. **Centrioles** are also another cytoplasmic bodies found in most animal cells. These are located at one pole of the cell just outside the nuclear envelop. Higher plant cells lack centrioles, and the spindle is formed without their aid though lower plants do have centriole. They are usually **hollow cylinders** 3000 to 5000Å long and 1200 to 1500Å in diameter composed of nine sets of hollow triple microtubules arranged in a circle and embedded in a dense granule or amorphous, electron dense matrix. These may appear to be a granular disc, called satellites, around the centriole. Each triplet formed of three microtubules run oblique towards the centre. These nine triplets are considered to form the wall of the cylinder since centriole has no outer membrane.

5.3 Lysosomes

5.3.1 General history of Lysosomes

Lysosome is an organelle which unlike other organelles, first became known through the biochemical studies and thereafter their morphological identifications were made. **Christian de Duve,** a Belgian cytologist and biochemist, in 1955 reported the presence of lysosomes in the cells by biochemical studies. Later on, **Novikoff** in 1956 observed these lysosomes as distinct cell organelles with the help of electron microscope.

5.3.2 Structure of Lysosomes

Lysosomes are round tiny bags filled with dense material rich in acid phosphatase (tissue dissolving enzymes) and other hydrolytic enzymes. They consist of two parts: (i) limiting membrane and (ii) inner dense mass.

1. **Limiting membrane:** This membrane is single and is composed of lipoprotein.

Chemical structure is homologous with unit membrane of plasmalemma, consisting of bimolecular layer.

2. **Inner dense mass:** This enclosed mass may be solid or of very dense contents. Some lysosomes have a very dense outer zone and a less dense inner zone. Some others have cavities or vacuoles within the inner granular material. Lysosomes are of various types and they help in intracellular digestion. Their contents vary with the stage of digestion.

5.3.2.1 Kinds of Lysosomes

There are four types of lysosomes: primary, secondary, residual bodies and cytolysosome or autophagosome.

1. **Primary Lysosome (storage granules):** It is a small sac like body. Its enzymatic contents are synthesized by ribosomes and accumulated in ER. From there, they enter the Golgi region, where acid phosphatase reaction takes place. The GERL region, i.e., acid phosphatase rich region of Golgi maturing face is thought to be involved in the production of lysosomes. The primary lysosome comprises only one type of enzyme or another.

2. **Secondary Lysosome (digestive vacuole or heterophagosome)**: These are produced either from phagocytosis or pinocytosis of foreign material by the cell. Actually within the cell, after phagocytosis or pinocytosis, the foreign bodies or extra-cellular substances are enclosed within the membrane and these membranes bound structures are known as **phagosome or pinosomes.** These ultimately fuse with primary lysosomes, thus forming secondary lysosome. This body having engulfed material within membrane has also full complements of acid hydrolases (hydrolytic enzymes). The digested material of these lysosomes passes through the lysosomal membrane and is incorporated into the cell so that they may be reused in metabolic pathways.

3. **Residual bodies*:*** These are formed in case the digestion is incomplete. In some cells, such as Amoeba and other protozoa, these residual bodies are eliminated by defecation. Hence, lysosomes **having undigested material or debris** are called residual bodies. These bodies are formed due to lack of certain enzymes in lysosomes. These are rejected from the cell by exocytosis and some time in certain cells these bodies remain in cells for long time causing ageing. These residual bodies also cause diseases in man such as **fever, hepatitis, polynephritis, hypertension, congested heart failure** etc. If the debris which is mostly lipid in nature may accumulate and condense into concentric lamella, it forms myelin figure.

4. **Autophagic vacuole (cytolysosome or autophagosome):** In this case, the lysosome **digests a part of cell** (e.g., mitochondria or portion of ER) by the process of autophagy. For example, liver cell shows numerous autophagosome during starvation among which remnants of mitochondria occur. This is a mechanism by which the cell can achieve degradation of its own constituents without irreparable damage (Fig. 1).

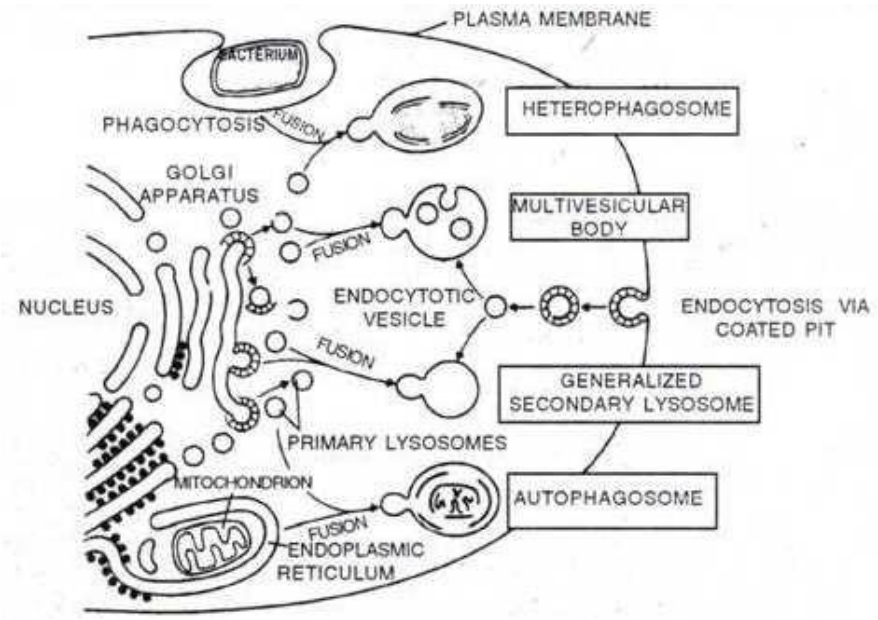

Fig. 5.1: Formation of lysosomes and intracellular digestion in them

5.3.2.2 Chemical Nature of Lysosomes

Chemically lysosomes are defined as a body rich in **acid hydrolases**. Acid phosphatase has been found in many cells of plant roots, fungi, liver, kidney and endocrine glands. The lysosomal enzymes can break down all major biological macromolecules present in the cells or entering the cells from outside into their building block subunits by adding water. The common enzymes in the lysosomes are proteases, nucleases (deoxyribonuclease and ribonuclease), glycosidase, lipases, sulphatases and phosphatase, which hydrolyses proteins, nucleic acids, polysaccharides, lipids, organic sulphatases and organic phosphates respectively.

5.3.3 Functions of Lysosomes

1. **Digestion of useful materials:** Intracellular digestion is a regular feature in protozoans and in lower invertebrates such as sponges and coelenterates. In this process the organic substances (food particles) taken up by the cells in vacuoles (pinosomes or phagosome) from the environment are digested.
2. **Digestion of harmful materials*:*** The foreign particles, such as viruses, bacteria and toxic molecules, are disposed of by hydrolyzing them in certain leucocytes and macrophages. This is called natural defense of the body. This activity of lysosomes is characteristic of higher animals.
3. **Digestion of unwanted materials:** The dead cells and debris that accumulate at the sites of injury are destroyed in some white blood cells. This is called natural scavenging of the body.
4. **Renewal of cells and organelles:** The old worn out cells and cell organelles are broken down to make the component molecules available for formation of new cells

and cell organelles. Thus, the lysosomes facilitate the turn-over of cells in normal tissues and of organelles in normal cells.

5. **Feeding of starving animals*:*** Food to a starving animal is provided by digesting the stored food materials (proteins, lipids and glycogen) and even the cells. This is called autophagy.

6. **Autolysis*:*** Autolysis caused by the lysosomal enzymes plays a role in normal developmental changes in both animals and plants. E.g., in the breakdown and absorption of tail during the metamorphosis of frog's tadpole. In autolysis, lysosome membrane ruptures and releases the enzymes into the surrounding cytoplasm. This kills and lyses the cell.

7. **Aid in fertilization*:*** The lysosome of sperms releases their enzymes to dissolve the egg membranes for the entry of the sperm into the ovum in fertilization. This is called extracellular digestion.

5.3.4 Importance of Lysosomes

As lysosomes store the hydrolyzing enzymes of the cell, they digest the incoming food materials and remove the foreign bodies and their organelles no longer required. Their membrane prevents the enzymes from escaping into the cytoplasm and destroying it.

Malfunctioning of lysosomes may lead to diseases. Abnormal rupturing of lysosomal membrane and release of enzymes may cause blood cancer, sunburn and genetic disorders. The degenerative changes in bones and joints associated with arthritis are suspected to be the result of abnormal release of enzymes from the lysosomes of the bone cells or lymph cells into the extracellular fluid.

5.4 Centriole

General History of Centriole

Van Benden in 1880 discovered centrosome in cells of certain parasites of cephalopods.

Centrosome is the area of cytoplasm, often a clear zone, around the centriole. It is found lying in the center of the cell, near the nucleus, in the cytoplasm. In Metazoa, centrosome lies outside the nucleus, but in Protozoa it lies within the nucleus.

It is lacking in some plant cells. **T. Boveri** in 1888 described centrosome in detail. The substance of centrosome also called kinoplasm consists of two parts:

- Smaller bodies or centrioles
- Surrounding mass or centrosphere

5.4.1 Structure of Centriole

The centrioles usually occur as paired hollow cylinders which are about 0.2μm in diameter and 0.3 to 0.5 μm in length. The two centrioles usually lie at right angles to each other.

The centriole is composed of nine sets of microtubules triplets arranged in a ring and embedded in a dense granular or amorphous, electron dense matrix (Fig. 2). There are no microtubules at the center of the ring giving the **"9+0"** pattern for the centriole. Each microtubule in a triplet is about 250Å wide. The triplets are tilted in such a way that each forms an angle of about 30 to 40° to the circumference of the cylinder, with the A sub tubule of each set nearest the center of the ring. Membrane around the centrioles is absent. Sometimes a granular disc, called satellites, appears around the centriole.

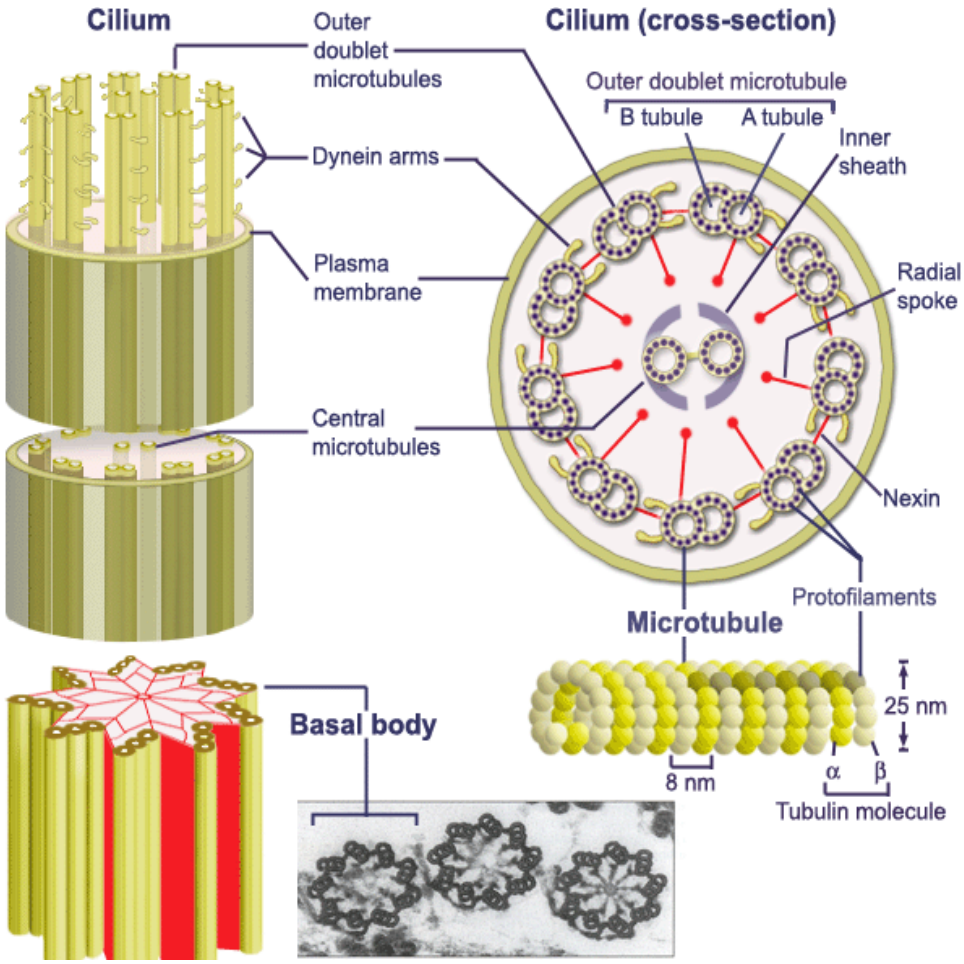

Fig. 5.2: T.S. Centriole, cilium and microtubule (showing faint 'cartwheel' pattern of fibrils)

All the triplets of centriole are similar and indistinguishable from one another. The three microtubules often called sub-tubules, of a triplet are named A, B and C, beginning from the inside of the cylinder. A dense strand called A-C linker, connects the A sub-tubule

of each triplet to the C sub-tubule of the adjacent triplet. These A-C linkers cause the tilt of the triplets from the radii of the cylinder. A fine radial fiber or spoke joins each A sub-tubule to the central hub of the cylinder. Each radial fiber has a dense thickening, the foot, near the A sub-tubule. This "cart-wheel" configuration though not always presents and when present it is often confined to the denser proximal end of the centriole. The C sub-tubules stop short of near upper ends and the peripheral tubules become doublet. B and C sub-tubules are C-shaped and their wall is completed by adjacent sub-tubules. Only 'A' sub-tubules are complete. The wall of 'A' sub-tubule is composed of 13 parallel proto-filaments which are made up of a row of α-β tubulin dimers (Fig. 3). A few proto-filaments are shared with the B-sub-tubule, which, in turn, shares a few of its proto-filaments with the C sub-tubule (Fig. 4).

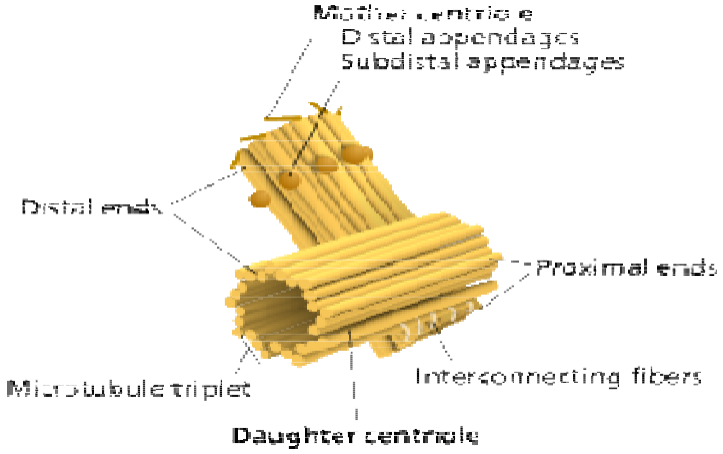

Fig. 5.3: A schematic view of centriole or basal body

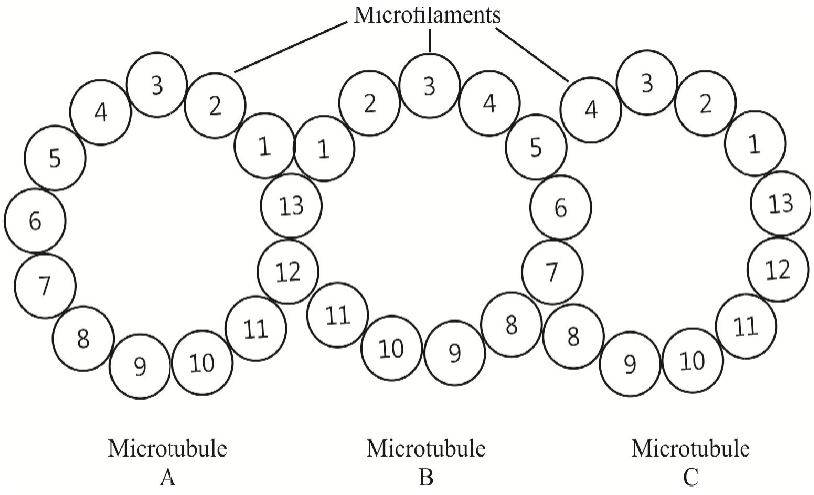

Fig.5.4: Subunits of A, B and C microtubules in T.S.

Nine amorphous shapes of electron dense material with poorly defined outer limits are present around the centriole. These are called **pericentriolar satellites**.

5.4.1.1 Chemical Composition

The microtubule of the centriole is composed of a protein tubulin and some lipids having a high concentration of ATPase enzymes. They seem to contain RNA and a small DNA molecule. Proteins encoded by this DNA are presumably translated on cytosolic ribosomes and then incorporated into the centriole.

5.4.2 Functions of Centriole

The centriole serves the following functions:

(i) They help in organizing spindle fibers and astral rays during mitosis and meiosis.

(ii) They provide basal bodies giving rise to cilia and flagella.

(iii) Pericentriolar material acts at the MTOC (microtubule-organizing centre) for the cytoplasmic microtubules.

5.4.3 Importance of Centriole

Centriole is involved in the **formation of spindle and astral rays** which are responsible for the chromosomal movements during cell division. Also, centrioles give rise to basal bodies (kinetosome) or cilia or flagella.

5.5 Microtubules

5.5.1 General History of Microtubule

The cytologists like **Freud** (1882), **Ballowitz** (1890) and **Meves** (1910) observed filamentous components of the cytoplasm and referred these as fibrils. Later, with the improved microscopic techniques along with advancement made in the field of sectioning and staining, the ultra structure of these components was revealed. These were found to be tubular in nature (Burgos and Fawcett, 1955; Palay, 1960; Harris, 1962). **De Robertis** and **Franchi** (1953) reported the presence of microtubules in the axons of medullated nerve fibers and called them neurotubules. Slautterback in 1963 describes them to be associated with the developing nematocysts of Hydra and he proposed the name microtubules to these components.

5.5.2 Structure of Microtubule

The microtubules are hollow, unbranched cylinders, generally about 200 to 270 Å thick and several micrometers long. They may occur singly or in bundles, and radiate from the centriole to the periphery of the cell. The microtubule is composed of 13 parallel proto-filaments that run its entire length and enclose a central lumen about 150 Å wide (Fig. 5). Each proto-filament is made up of a row of globular subunits that have a diameter of about 40 to 50 Å. There may be cross bridges between adjacent microtubules.

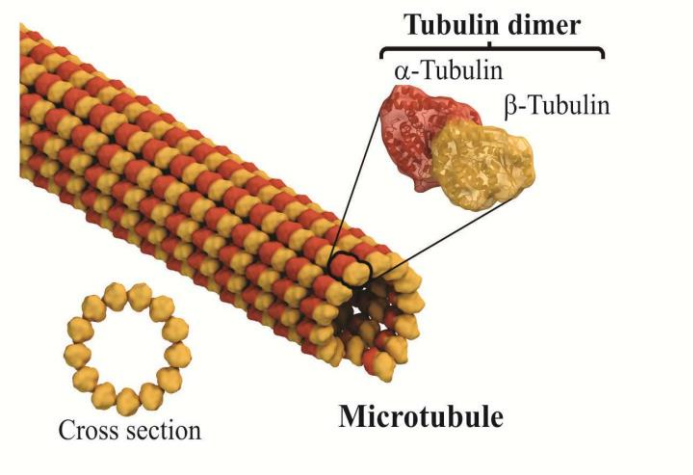

Fig. 5.5: A microtubule in surface view and in cross section

5.5.2.1 Chemical Composition

The microtubules are formed of a protein called **tubulin**. A tubulin subunit contains one α-tubulin molecule and one β-tubulin molecule. This αβ dimer is 80-100 Å long. The α- and β-tubulin molecules are arranged alternately in a helical manner. Many other proteins, called MAPs (microtubule associated proteins), form some 5 to 10 percent of the proteins of microtubules. These proteins promote tubulin polymerization. A tubulin dimer has two GTP molecules bounded to it. One GTP is hydrolyzed to GDP when a tubulin dimer is incorporated into a microtubule. The α-β-α-β arrangement of the tubulin subunits gives polarity to the microtubule.

5.5.3 Functions of Microtubules

1. **Form and support-** The microtubules form a part of cytoskeleton which (a) maintains the shape of the cell and (b) provides mechanical support to the cell. This role of microtubules is especially evident in cells having long processes such as the axopodia of certain protozoans and axons of nerve cells. Red blood corpuscles of non-mammalian vertebrates are kept flat by peripheral band microtubules.
2. **Movement-** The microtubules form the motile elements of cilia and flagella. These bring about locomotion in protists and cause currents in the environment of animals.
3. **Components of centriole and basal bodies-** The microtubules are components of centriole and basal bodies. The centriole give rise to the mitotic spindle and the basal bodies produce cilia and flagella.
4. **Formation of mitotic spindle-** The microtubules forms the spindle and astral rays in cell division.
5. **Chromosome movement-** The chromosome fibers of spindle bring about movement of the chromosomes to the opposite poles of the cell in the anaphase.

6. **Cell differentiation-** The microtubules play a role in cell differentiation and determination of polarity.

7. **Intracellular transport-** Vesicles and protein molecules in the cell move along the "tracks" of microtubules. The movement is brought about by motor proteins kinesin and MAPIC (cytoplasmic dynin) powered by ATP.

5.5.4 Importance of Microtubules

Microtubules are very important for the cells as they provide internal framework serving as cytoskeleton to determine and maintain the cell form. They also define pathway along which the particles move in cell. The mitotic apparatus consisting of spindle fibers and astral rays is in fact bundles of microtubules. The generation of bending movements in cilia and flagella is attributed to a sliding microtubule mechanism.

5.6 Summary

Lysosomes which are also known as suicidal bags are the secretary pathway in the cells. They are rounded tiny bags consisting of two parts, one part made of single limiting membrane composed of lipoproteins and the other part is inner dense mass. Various types of lysosomes are present in a cell which is characterized according to their functions. Primary lysosomes act as storage granules, secondary lysosomes functions as digestive vacuoles or heterophagosome. Third type of vacuole is residual bodies which is formed in case of digestion is incomplete and the fourth type is autophagic vacuole which digest a part of cell itself like a portion of ER or mitochondria. Thus lysosomes store the hydrolysisng enzymes of the cell and they digest the incoming food materials and remove the foreign bodies and the cell organelles which are no longer required by the cell. Malfunctioning of lysosomes may lead to certain diseases. The centrioles occur in pairs as hollow cylinder and lie at right angles to each other. It is composed of nine sets of microtubule triplets and in the centre the microtubule is absent giving rise to the pattern as “9+0”. Microtubules are hollow unbranched cylinders having length of several micrometers which may occur single or in bundles. These are composed of a protein tubulin and some lipids having high concentration of ATPase enzymes. They perform various functions like they help in organizing spindle fibers and astral rays during mitosis and meiosis. They also provide basal bodies for the emergence of cilia and flagella. The wall of microtubule is composed of 13 parallel protofilaments which is made up of a row of globular subunits. Microtubules form a part of cytoskeleton which maintains the shape of the cell. They define pathway along which particles move. They play vital role in cell differentiation and determination of polarity.

5.7 Glossary

Lysosome: an organelle in the cytoplasm of eukaryotic cells containing degrading or hydrolysing enzymes enclosed in a membrane.

Matrix: matrix is the material in animal or plant cells, in which more specialized structures

are embedded. A specific part of the mitochondrion that is the site of oxidation of organic molecules is also called matrix.

Plasmalemma: Plasmalemma is the cell membrane that surrounds the cytoplasm of living cells, physically separating the intracellular components from the extracellular environment.

Centriole: It is a cylindrical cell structure composed mainly of a protein called tubulin.

Phagocytosis: Phagocytosis is the process by which a cell engulfs a solid particle to form an internal vesicle known as a phagosome.

Pinocytosis: The ingestion of liquid into a cell by the budding of small vesicles from the cell membrane.

Autophagosome: Autophagy allows the orderly degradation and recycling of cellular components. During this process, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membrane vesicle known as an autophagosome.

Leucocytes: White blood cells are also called leucocytes. These are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders.

Macrophages: Macrophages are the type of white blood cells that engulf and digest cellular debris, foreign substances, microbes, cancer cells, and anything else that does not have the types of proteins specific of healthy body cells on its surface.

Metamorphosis: Metamorphosis is a biological process by which an animal physically develops after birth or hatching, involving a conspicuous and relatively abrupt change in the animal's body structure through cell growth and differentiation.

Kinetosome: Kinetosome forms the base of the flagellum, consisting of a circular arrangement of microtubules.

Dimer: A dimer is a chemical structure formed from two similar subunits.

Metabolic pathway: A sequence of chemical reactions undergone by a compound or class of compounds in a living organism.

Electron microscope: A microscope that uses a beam of accelerated electrons as a source of illumination is known as electron microscope. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, the electron microscope has a higher resolving power than a light microscope and can reveal the structure of smaller objects.

Pericentriolar satellite: Pericentriolar satellites are electron-dense granules that are concentrated around the centrosome. They are involved in the recruitment of centrosomal proteins and microtubule organization the interphase stage of the cells.

5.8 Self Assessment Questions and Possible Answers

5.8.1 Multiple Choice Questions

1. Lysosomes arise from:

   (a) Smooth ER (b) Golgi complex

   (c) Both of these (d) None of these

2. Autophagic vesicles digest:

   (a) Pinosome contents (b) Cell organelles

   (c) Phagosome contents (d) Micro-organisms

3. Lysosome was discovered by:

   (a) de Duve (b) Robert Brown

   (c) Hooke (d) Robinson

4. Lysosomes are considered suicide bags because they contain:

   (a) Parasitic activity (b) Food vacuole

   (c) Catabolic enzymes (d) Hydrolytic enzymes

5. The pattern of organization in centriole is:

   (a) 9 + 0 (b) 9 + 1

   (c) 9 + 2 (d) 9 + 3

6. Centriole occurs:

   (a) Singly (b) In pairs

   (c) In threes (d) In fours

7. Function of centriole is related with:

   (a) Initiation of cell division (b) Formation of cell plate

   (c) Formation of spindle fibers (d) Formation of nucleolus

8. Microtubules in cilia and flagella are formed of:

   (a) Actin (b) Myosin

   (c) Elastin (d) Tubulin

9. Arms of A sub-units are composed of:

   (a) Tubulin (b) Actin

   (c) Myosin (d) Dynein

10. The supporting framework of a cell consists of:

(a) Microtubules (b) Intermediate filament

(c) Microfilaments (d) All the above

5.8.2 Very Short Questions

1. Who gave the name lysosome?
2. How the cell is protected from the destructive effect of lysosomal enzymes?
3. Name different types of lysosomes.
4. Give the popular name for the lysosomes.
5. Name the protein of which microtubules in centriole, basal bodies, cilia and flagella are formed.
6. Who discovered the microtubules?
7. What are Kinetosomes?
8. Give the main function of centrioles.
9. Microtubules are hollow. Is it true?
10. What MTOC stands for?

ANSWERS

5.8.1

1. (c)	5.(a)	9.(d)
2. (b)	6.(b)	10.(d)
3.(a)	7.(c)	
4.(d)	8.(d)	

5.8.2

1. De Duve in 1955 because they contain hydrolytic enzymes
2. Lysosomal enzymes does not allow the enzymes to go out of the lysosome
3. Primary lysosomes, secondary lysosomes, residual bodies and autophagic
4. Suicide bags or disposal units
5. Tubulin
6. Robertis and Franchi
7. Basal bodies
8. Help organize mitotic apparatus during cell division
9. Yes
10. Microtubule organizing centre

5.9 References and Suggested readings

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5.10 Terminal and Model Questions

1. Define Lysosomes along with their detailed chemical structure.
2. Describe various types of lysosomes.
3. Write notes on:
    a. Functions of lysosomes
    b. chemical structure of centriole
    c. functions of centriole
    d. structure and function of microtubules
    e. types of lysosomes
4. Explain in detail the ultra structure centriole.
5. Explain in detail the ultra structure microtubules.

UNIT 6 NUCLEUS

Contents

6.1 Objectives

After reading this unit the readers will be able to:

- Define nucleus
- Explain the structure of nucleus
- Mention the functions of nucleus
- Describe the importance of nucleus

6.2 Introduction

Nucleus is usually the most conspicuous organelle of eukaryotic cell. However, well defined nucleus is absent in prokaryotic cells. Nucleus is the repository of genome and the source of informational macromolecules that govern the synthetic activities of the cytoplasm. It is surrounded by a bilaminary nuclear envelop having pore complexes that permit the nuclear-cytoplasm transport of materials. In the animal cells, it generally lies in the centre, surrounded on all sides by the cytoplasm. However, in plant cells it is often pushed to one side of the cell due to the presence of large central sap vacuole.

The shape of nucleus is variable according to cell type. It is generally spheroid but ellipsoid or flattened nuclei may also occur in certain cells. In certain WBC (white blood cells) the nucleus is dumbbell shaped. In human neutrophil it is trilobed.

Most cells contain a single nucleus, known as **mono or uninucleate** cells. Cells with two nuclei are known as **binucleate cells e.g. Paramecium**. Sometimes more than two nuclei are present in a single cell. Such cells are called **polynucleate or multinucleated cells**. Such cells in animals are called **syncytial cells (e.g. osteoblast**) and such plants are termed **coenocytes (e.g. siphonal algae).** Cells having distinct nucleus are called eukaryotic cells, whereas cells without definite nucleus are called prokaryotic cells (e.g. bacteria). The latter possess scattered chromatin material (DNA) in the cytoplasm called nucleoid. The mature mammalian erythrocytes also do not possess any nucleus.

Size of nucleus is not constant and is generally correlated with DNA content. The nuclear size is variable depending upon the number of chromosomes (DNA content).

6.3 Nucleus

6.3.1 General History of Nucleus

Nucleus was observed by a Dutch Microscopist, **Antonie van Leeuwenhoek in 1710**, as a centrally placed clear area in the blood cells of amphibians and birds. **Fontana** (1781) recorded an ovoid structure in each of the isolated epidermal cells of eel's skin. However, **Robert Brown (1831)** was the first to use the term nucleus for a prominent body present in the orchid cell. He stated that nucleus was the regular feature of the cells and initiated the concept of nucleated cells.

6.3.2 Structure of Nucleus

The nucleus consists of various parts. It is bounded by a thin but clearly defined covering, the nuclear envelop or karyotheca. Within the envelope is a clear fluid substance called nucleoplasm or nuclear sap or karyolymph is present in which the solutes of the nucleus are dissolved. Suspended in the nucleoplasm are network of protein-containing fibrils called nuclear matrix; fine intermingled nucleoprotein filaments collectively referred to as the chromatin; and one or more spherical bodies known as nucleoli (singular, nucleolus). There are no membranes or microtubules inside the nucleus. Protozoans that form a mitotic spindle within the nuclear envelop, however, have microtubules in their nuclei (Fig. 1).

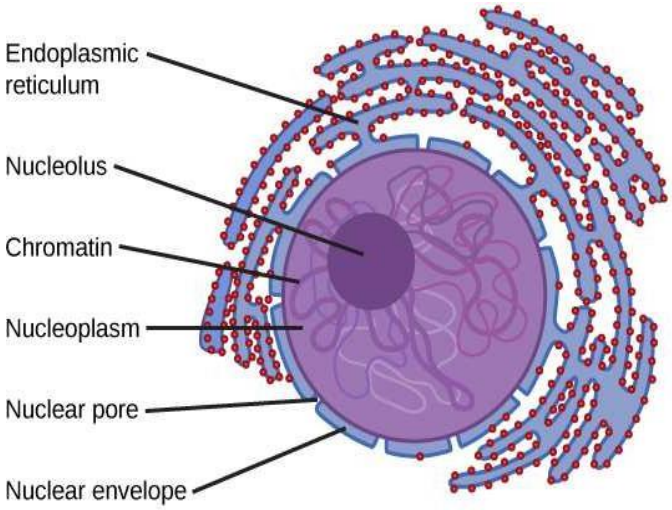

Fig. 6.1: Structure of nucleus

- **Chemical Composition:** The nucleus is composed of about 9-12% DNA, 5% RNA, 3% lipids, 15% simple basic proteins such as histone or protamines, about 65% complex acid or neutral proteins, including enzymes such as polymerases for the synthesis of DNA and RNA, organic phosphates and inorganic salts or ions such as Mg^{++}, Ca^{++} and Fe^{++}.
- **Functions:** The nucleus acts as a control center of the cell. It serves the following main functions:
  - It maintains the cell by directing the synthesis of structural proteins.
  - It regulates cell metabolism by directing the synthesis of enzymatic proteins.
  - It contains genetic information for reproduction, development and behavior of the organism besides for structure and metabolism.
  - It brings about cell replication when needed.
  - It is the site for the formation of ribosome subunits.
  - It brings about cell differentiation by keeping only certain genes operational.
  - It develops genetic variations that result in evolution.

6.3.2.1 Nuclear Envelope

The nuclear envelop separates the nucleoplasm from the cytoplasm. It consists of two unit membranes: outer and inner. Each unit membrane is about 75Å thick, and is a trilaminar lipoprotein like the plasma membrane. The two unit membranes are separated by a space called the inter membrane or perinuclear space. It is about 250Å wide. The outer or cytoplasmic surface of the outer membrane is studded with ribosomes and polysomes and is rough. These ribosomes carry on protein synthesis. The outer membrane is continuous with RER at certain places. Thus, the perinuclear space is continuous with the channels of the RER. The inner membrane of the nuclear envelope is free of ribosomes, but has a dense layer, the nuclear lamina, closely associated with its inner or nucleoplasmic surface. The nuclear lamina is a 30 to 100 nm thick network of filaments composed of proteins named lamina A, B and C. The nuclear lamina supports the inner membrane and gives shape to it. It connects chromatin to the inner membrane, keeping most of the chromosomes in the periphery of the nucleus. It also plays a role in the breakdown and reformation of nuclear envelope during mitosis (Fig. 2).

Nuclear Pores: The nuclear envelope is generally perforated by minute apertures, the nuclear pores that control the passage of some molecules and particles. The pores are formed by fusion of the inner and outer membranes of the nuclear envelope. There may be 1000 to 10,000 pores per nucleus.

Each nuclear pore is fitted with an apparatus called the **pore complex** which fills considerable part of the pore. The pore complex is nearly cylindrical, projects into both cytoplasm and nucleoplasm, and projects beyond the rim of the pore over the nuclear envelope. The pore complex consists of two rings, the annuli, one located at the cytoplasmic rim of the pore and the other at the nucleoplasmic rim. Each annulus comprises eight symmetrically arranged subunits, and sends a spoke into the pore. The spoke encloses a channel about 100 to 200 Å wide. Ions and small molecules of the size of monosaccharide, disaccharides or amino acids pass freely between the nucleus and cytoplasm. The pore complexes do control the passage of larger molecules, such as RNA and proteins, and of ribosomal subunits. The pore complexes also act as a barrier to some molecules such as DNA of chromosomes.

Functions:

- It maintains the shape of the nucleus.
- It keeps the nuclear contents in place and distinct from cytoplasm.
- It regulates the flow of materials into and out of the nucleus by active transport and out pocketing.
- Its pores allow the exit of ribosomal subunits formed in the nucleolus and tRNA and mRNA synthesized on the chromosomes.

6.3.2.2 Nucleoplasm

Nucleoplasm is a transparent fluid material in the nucleus. The chromatin fibers and nucleoli are suspended in it. It contains raw materials (nucleotides), enzymes (polymerases) and metal ions (Mn^{++}, Mg^{++}) for the synthesis of DNA and RNA. It also contains proteins and lipids. The proteins include basic histones and acidic or neutral non-histones that associate with the DNA molecules. There are proteins for the formation of ribosomal subunits also. The RNAs (rRNAs, tRNAs, mRNAs) and ribosomal subunits synthesized in the nucleoplasm pass into the cytoplasm via nuclear pores (Fig. 2).

Functions:

- It is the seat for the synthesis of DNA, RNAs, ribosomal subunits, ATP and NAD.
- It supports the nuclear matrix, chromatin material and nucleoli.
- It provides turgidity to the nucleus.

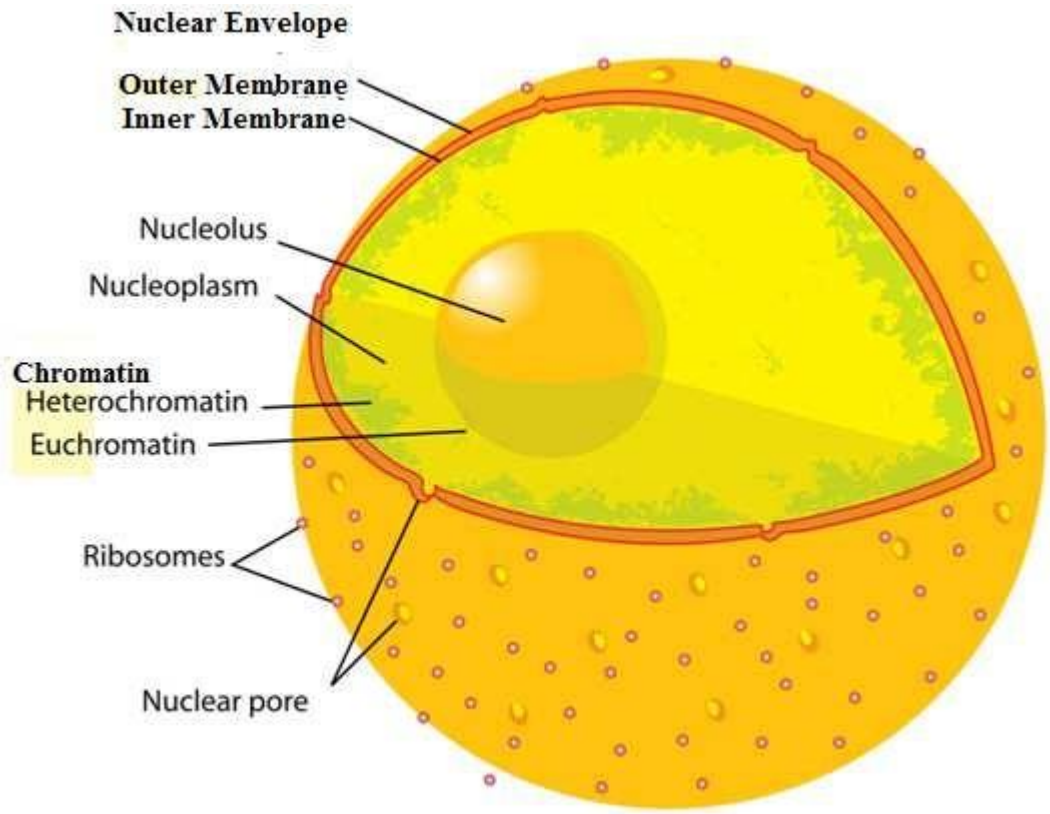

Fig. 6.2: Ultra structure of nucleus

6.3.2.3 Nuclear Matrix

The nuclear matrix is a network of thin, criss-crossing, protein- containing fibrils that are connected at their ends to the nuclear envelope. It forms a sort of nuclear skeleton. It remains intact after the chromatin and DNA have been removed.

Functions:

- It maintains the shape of the nucleus.
- Chromatin fibers are anchored to nuclear matrix.
- The machinery for various nuclear activities, such as transcription and replication, is associated with the matrix.
- It has also been implicated in the processing of newly formed RNA molecules and their transport through the nucleus.

6.3.2.4 Chromatin

The term chromatin was first coined **by Flemming in 1879.** The chromatin occurs in an interphase (non-dividing) nucleus as fine filaments, the **chromatin fibers**. The fibers lie criss-cross so as to give the appearance of a diffuse network often referred to as the nuclear or chromatin reticulum. The chromatin occupies most of the nucleus. The chromatin fibers are simply extremely extended chromosomes. A chromatin fiber is normally about 100Å in diameter. A fiber thicker than 100Å appears to be coiled or folded, a fiber thinner than 100Å seems to have less protein content associated with it. Chromatin fibers typically appear approximately 250Å in diameter. During cell division, the chromatin fibers, by condensing and tight coiling, form short, thick, rod like bodies known as **chromosomes.**

Upon staining, this diffuse network of chromatin material shows light stained and dark stained areas. After cell division, the chromosomes change back into chromatin fibers. Most of the chromatin fibers become uncoiled, extended and scattered in the nucleoplasm. These represent the **euchromatin** (true chromatin) of the interphase nucleus. They are stained lightly.

The term **heterochromatin** is applied to those chromosomal regions that stain darker than others. They remain c**o**iled and compacted in the interphase too. Heterochromatin represents relatively inactive parts of the chromosomes. It contains less DNA and more RNA than the euchromatin. Few mutations occur in this region. Little or no mRNA is synthesized here. Most of the DNA in heterochromatin is highly repeated DNA which is never, or very seldom, transcribed. Heterochromatin is of two types: **constitutive and facultative**. The DNA of constitutive heterochromatin is permanently inactivated and remains in the condensed state at all times. It occurs at several places: adjacent to the centromere of the chromosome, at the ends (telomeres) of the chromosomes, at certain portions within the euchromatin, and adjacent to the nuclear envelope. Facultative heterochromatin is partly

condensed and inactivated. **One X-chromosome in female mammals is condensed to form the heterochromatic Barr body.**

Nucleosomes: In 1974, Kornberg and Thomas proposed that a chromatin fiber is a chain of similar subunits called nucleosomes (Fig. 3). The nucleosome consists of a core particle wrapped by DNA strand. The core particle is an octamer of **8 histone molecules**, two each of the histones H2A, H2B, H3 and H4. The DNA strand forms 1½ or 1¾ turns around the core and consists of 140 nucleotides. Each nucleosome is connected to the next by a short DNA linker of 60 nucleotides. A nucleosome and a linker together have a total average length of 200 nucleotides and are together referred to as a chromatosome. A molecule of histone H1 is associated with each DNA linker and it serves to pack nucleosomes together. Thus, a chromatin fiber is a chain of beads, a bead (nucleosome) is about 100Å wide and DNA linker is about 140Å long. Nucleosomes represent the lowest level of chromatin organization. Chromatin fiber appears about 250Å thick in electron micrographs. which suggests that the 100Å thick chromatin fiber is either packed into a spiral or solenoids, containing 6 nucleosomes per turn or 6 nucleosomes are organized into a cluster, or super bead, thereby increasing the DNA packing by 5 folds. The thicker filament is maintained by H1 histone protein. The non-histone proteins do not occur in the nucleosome structure of chromatin. Nucleosomes are not formed in prokaryotes.

Functions:

- The chromatin fibers form chromosomes during cell division.

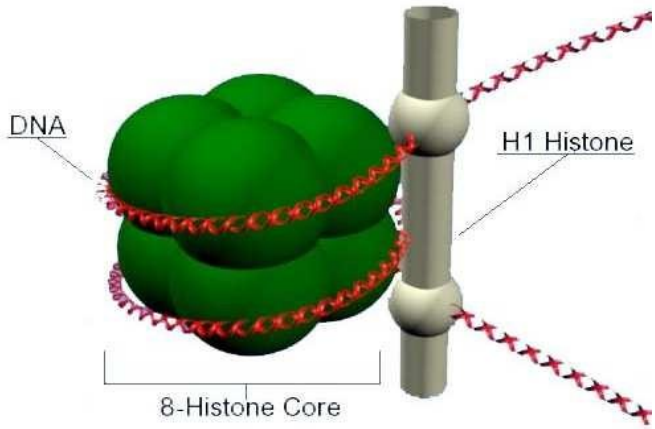

Fig. 6.3: Nucleosome

6.3.2.5 Nucleolus (Little Nucleus)

The nucleolus was discovered in 1781 by **F. Fontana** in the slime from the eel skin. It is present in the nucleus of most cells, but is inconspicuous or absent in sperm cells and in muscle cells. It is usually spherical, but may have other forms. The number of nucleoli in a nucleus varies in different species. The nucleoli disappear during cell division, and are

reformed at specific sites, the nucleolar organizers or nucleolar organizer regions (NORs), of certain chromosomes, the nucleolar chromosomes, at the end of cell division before the chromosomes become diffuse. Position of the nucleolus in the nucleus is often eccentric. However, it occupies a specific position on its chromosome.

The nucleolus is a dense, somewhat rounded, dark staining organelle. It is without a limiting membrane. Calcium ions keep it intact. It consists of four regions.

1. **Fibrillar Region or Nucleolonema-** It contains indistinct fibrils about 50-100Å in diameter. The fibrils represent the long rRNA precursor molecules in early stages of processing before the processing enzymes have cut off segments from them.
2. **Granular Region-** It contains spherical, electron dense particles, about 150-200 Å in diameter and with fizzy outline. The granules are ribosomal subunits (rRNA + ribosomal proteins) that are nearly ready for transport to the cytoplasm.
3. **Amorphous Region or Pars Amorpha-** It is a structure-less proteinaceous matrix in which the granular and fibrillar regions are suspended.
4. **Nucleolar Chromatin-** It consists of 100 Å thick chromatin fibers. The latter are a part of the nucleolar chromosome which follows a tortuous path through the granular and fibrillar components of the nucleolus. This part contains many copies of DNA that directs the synthesis of ribosomal RNA. The rest of the nucleolar chromosome lies in the nucleoplasm.

Functions-

- The nucleolus synthesizes and stores rRNA.
- It also stores ribosomal proteins received from the cytoplasm.
- It forms ribosomal subunits by wrapping the rRNA by ribosomal proteins. The ribosomal subunits pass out through the nuclear pores into the cytoplasm. Here the subunits join to form ribosomes when needed. Thus, it is the nucleolus which provides machinery (ribosomes) for protein synthesis.
- The nucleolus also plays a role in cell division.

6.4 Importance of Nucleus

The nucleus is the control center of a cell. It regulates all metabolic activities of the cell and stores entire hereditary information. A cell without nucleus cannot survive.

6.5 Summary

Nucleus is absent in prokaryotic cells but it is the most conspicuous organelle of eukaryotic cell. Whole of the genome is present in the nucleus thus; it is the source of informational macromolecules. It is surrounded by bilaminary nuclear envelop having pore complexes that permit the nuclear cytoplasmic transport of materials. The size of nucleus is not constant and it is correlated with the DNA content. Nucleus consists of nuclear envelope that separates nucleoplasm from the cytoplasm and it consists of two unit membranes, the

outer and the inner and each unit membrane is a trilaminar lipoprotein sandwich like plasma membrane and the two unit membranes are separated by perinuclear space. Nuclear pores present in the nuclear envelope are loaded with an apparatus called the pore complex, which act as a barrier to some molecules such as a DNA of chromosome. A transparent fluid the "nucleoplasm" is present inside the nucleus that contains raw materials, enzymes and metal ions. It provides turgidity to the nucleus and supports the matrix, chromatin material and nucleoli. The nuclear matrix is a network of thin criss-crossing protein containing fibrils that forms a sort of nuclear skeleton. The fine filaments present in the non-dividing nucleus are the chromatin fiber that occupies most of the nucleus and the nucleosome consists of a core particle wrapped by DNA strand. Nucleolus is present in various forms. It disappears during cell division and is reformed at specific sites known as nucleolar organizer regions of certain chromosomes at the end of the cell division. The nucleolus synthesizes and stores RNA and ribosomal proteins received from the cytoplasm. It plays important role in cell division.

6.6 Glossary

Neutrophil: Neutrophil are the most abundant type of granulocyte and the most abundant type of white blood cell in most mammals. They form an essential part of the innate immune system.

Karyotheca: A double membrane at the boundary of the nucleoplasm is called karyolymph. It has regularly spaced pores covered by a disc-like nuclear pore complex and a space between the two membranes; the outer membrane is continuous at intervals with the rough endoplasmic reticulum.

Karyolymph: It is the fluid or gel-like substance of the nucleus in which the chromatin material, nucleolus, and other particulate elements of the nucleus are suspended.

Annuli: It is a ring-shaped object, structure, or region.

Heterochromatin: Heterochromatin represents relatively inactive parts of the chromosomes. They stain darker than others and remain coiled and compacted in the interphase.

Euchromatin: The uncoiled chromatin fibers, extended and scattered in the nucleoplasm represent the euchromatin (true chromatin) of the interphase nucleus. They are stained lightly.

Constitutive heterochromatin: The DNA of constitutive heterochromatin is permanently inactivated and remains in the condensed state at all times.

Facultative heterochromatin: Heterochromatin that is partly condensed and inactivated is called facultative heterochromatin.

DNA linker: Linker DNA is double-stranded DNA in between two nucleosome cores that, in association with histone H1, holds the cores together.

Histone proteins: Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes.

6.7 Self Assessment Questions and Possible Answers

6.7.1 Multiple Choice Questions

1. Nucleus is separated from cytoplasm by nuclear membrane which is:
   (a) Double, non-porous (b) Single, non-porous
   (c) Single, porous (d) Double, porous
2. Nucleolus is especially rich in:
   (a) DNA and proteins (b) DNA and lipids
   (c) RNA and proteins (d) RNA and lipids
3. Nuclear membrane facilitates:
   (a) Synapses of homologous chromosomes
   (b) Nucleocytoplasmic exchange of materials
   (c) Anaphasic separation of daughter chromosomes
   (d) Organization of spindles
4. Nucleoplasm is continuous with cytoplasm through:
   (a) Centriole (b) Nucleopores
   (c) E.R. (d) Golgi Body
5. The major component of the nucleus is:
   (a) DNA (b) RNA
   (c) Lipids (d) Proteins
6. Chief role of nucleolus in a nucleus concerns:
   (a) Organization of chromosomes (b) DNA replication
   (c) Ribosomal synthesis (d) Chromatid separation
7. Nucleus was discovered by:
   (a) Robert Brown (b) Robert Hook
   (c) Virchow (d) De Duve
8. Nucleolar organizer is associated with:
   (a) Synthesis of plasma membrane (b) Ribosome formation
   (c) G6PD (d) Disappearance of nuclear membrane

6.7.2 Very Short Questions

1. What the study of nucleus is called?
2. Who discovered the nucleus?
3. How many types of histones are found associated with DNA?

4. What is the composition of chromatin?
5. What are nucleosomes?
6. What is an interphase nucleus?
7. Give the role of DNA present in nucleolus?
8. Which has more DNA and less RNA, euchromatin or heterochromatin?
9. Where are nucleoli formed at the end of cell division?
10. Name two types of chromatin.

ANSWERS

6.7.1

1. (d)
2. (c)
3. (b)
4. (b)
5. (d)
6. (c)
7. (a)
8. (b)

6.7.2

1. Karyolgy
2. Robert Brown in 1831
3. 5 types: H1, H2A, H2B, H3, H4
4. Chromatin is viscous, gelatinous substance and contains DNA, RNA, histones (basic proteins) and non-histone proteins (acidic proteins)
5. Bead like enlargements of interphase chromatin fibers
6. Nucleus of non-dividing cell
7. Transcription of r RNA
8. Euchromatin
9. At nucleolar organizing regions of nucleolar chromosomes
10. Heterochromatin and Euchromatin

6.8 References and Suggested Readings

1. Fontana, F. (1781). "Traite sur le Venin de la Viper, sur les Poisons Americains, sur le Laurier Cerise et sur quelques autres Poisons Vegetaux". Gibelin, Florence.

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3. Kornberg, R.D. and Thomas, J.O. (1974). Chromatin structure: oligomers of the histones. *Science*, 184: 865-868.

6.9 Terminal and Model Questions

1. Discuss the morphology, chemical organization and functions of the nucleus.
2. Give detailed account of nuclear envelope.
3. Give an account of nuclear matrix.
4. Describe the nuclear pore.
5. Write a short account of the ultra-structure of the nucleus. Mention its chemical composition.

UNIT 7 CHROMOSOMES

Contents

7.1 Objectives

Reading of the unit will let the readers to:

- Define chromosomes
- Describe various types of chromosomes
- Mention the functions of chromosomes
- Explain Giant chromosomes
- Describe with structure polytene and lampbrush chromosomes

7.2 Introduction

The word chromosome has been derived from two Greek words **"Chroma"** meaning colour and **"Soma"** meaning body. They are the unique cell organelles made up of chromatin material which is the most important and permanent constituent of the cell nucleus. They are capable of self-reproduction. They control cell's structure and metabolism, and play an important role in the differentiation, heredity, mutation and evolution.

7.3 Chromosomes

7.3.1 General History of Chromosomes

W. Hofmeister in 1848, discovered nuclear filaments in the nuclei of pollen mother cells of ***Tradescantia***. First accurate count of chromosomes was made by W. Flemming in 1882, in the nucleus of a cell. In 1884**, W. Flemming, Evan Beneden and E. Strasburger** demonstrated that the chromosomes double in number by longitudinal division during mitosis. Beneden in 1887 found that the number of chromosomes for each species was constant. The term **"Chromosomes"** was coined in 1888 by **W. Waldeyer** for the nuclear filaments. **W.S. Sutton and T. Boveri** suggested the role of chromosomes in heredity in 1902, which was confirmed by **Morgan** in 1933.

The structure of chromosomes varies in viruses, prokaryotes and eukaryotes.

1. **Viral chromosome-** In viruses there is a single chromosome bearing a single nucleic acid molecule **(DNA or RNA)** surrounded by a protein coat called **Capsid**. It may be linear or circular. The viruses having DNA as genetic material are called **DNA viruses** and those having RNA as genetic material are known as **RNA viruses**. A limited amount of genetic information is present in the viral chromosome which codes for little more than the production of more virus particles of the same kind in the host cell. In RNA viruses, often the RNA directs the synthesis of DNA complementary to itself by reverse transcription in the host. The RNA is then transcribed by the DNA for the formation of new virus particles. Such ribovirus is called **retrovirus**. The AIDS causing virus is a retrovirus.
2. **Prokaryotic chromosomes-** Prokaryotic chromosome (e.g., bacteria) has a **single**

and circular two-stranded DNA molecule which is not enveloped by any membrane. It lacks proteins and is in direct contact with the cytoplasm. The bacterial chromosome is packed into the nucleoid by some RNA that appears to form a core. It is attached to plasma membrane permanently at least at one point. In addition to the main chromosome some **extra-chromosomal DNA** molecules may also be present in most of the bacterial cells they are also double stranded and circular, but are much smaller in size. They are known as **plasmids**. The plasmid may occur independently in the cytoplasm of calls or may also be found in association of main chromosomal DNA and called as **episome.**

3. **Eukaryotic chromosomes-** The eukaryotic chromosomes are present in **nucleus** and in certain other organelles, like **mitochondria and plastids**. These chromosomes are called nuclear and extra nuclear chromosomes respectively.

Nuclear chromosomes are **double stranded long DNA** molecules of linear form. Proteins are associated with them. They are surrounded by nuclear envelope. More DNA is involved in coding far more proteins than the prokaryotic chromosomes.

Extra nuclear chromosomes are present in mitochondria and plastids. They are double stranded short DNA molecules of circular form. They lack protein association. Less genetic information is available for the synthesis of only some particles of proteins for the organelles containing them. Other proteins are received from the cytoplasm where they are synthesized under the direction of nuclear chromosomes.

7.3.2 Morphology of Chromosomes

During the interphase stage, the eukaryotic chromosomes are extended into long and thin chromatin fibers where they lie criss-cross to form the **chromatin reticulum**. They replicate in the S-phase and become double. At this stage they consist of two chromatids that are held together at one point called **centromere**. At the time of cell division, the chromosomes condense and tightly coil up and become distinct at metaphase stage. The eukaryotic chromosomes vary in number, size, shape and position but they have remarkably uniform structure.

1. **Number-** Eukaryotic chromosomes vary in number from two to a few hundred in different species. In a species all the individuals have same number of chromosomes in all of their cells, except the gametes. Since **the chromosome number is constant for a species,** it is helpful in determining the phylogeny and taxonomic position of the species.

2. **Size-** In a **species all the chromosomes are not of the same size.** Their size also varies from species to species. The particular chromosome of a species however has more or less a constant size. The organisms having fewer chromosomes have large sized chromosomes than those having many. Generally, **plant chromosomes are larger than animal chromosomes** and among plants the **monocots have larger chromosomes than the dicots.**

3. **Shape-** The chromosomes at metaphase stage look like slender rods that may be

straight or curved to form an arc or a letter S. In anaphase stage they may assume J or V shapes, depending upon the position of the centromere.

4. **Position-** In a nucleus each chromosome is independent of all the other chromosomes in its location. Thus, they may occupy any region of the nucleus.

5. **Structure-** At **metaphase stage**, since the chromosome is **a highly condensed nucleoprotein filament, it contains two greatly coiled sister chromatids.** These chromatids that lie side by side along their length, are held together at a point called centromere, an area of the narrow region also called **primary constriction** of the metaphase chromosome. At the centromere each chromatid has a darkly staining, disc like, fibrous structure, called **kinetochore**, to which spindle microtubules attach during cell division. Kinetochores are the sites where force is exerted to pull the chromatids towards the poles. One or more chromosomes may have additional narrow regions called the **secondary constrictions**. The part of the chromosome separated by secondary constrictions is termed as **satellite**. A chromosome with a satellite is called **sat chromosome**. The size and the shape of the satellite remain constant for a species. Secondary constrictions are associated with the nucleoli, and are known as the **nucleolar organizers**. The chromosomes which have nucleolar organizing regions are known as the **nucleolar chromosomes** (Fig. 7.1).

   Ends- The ends of chromosomes are called **telomeres**. The function of telomere varies from the rest of the chromosome. On exposure to X-rays a chromosome may break and its pieces may rejoin, but no segment connects to the telomere, showing that the telomere has a polarity, and it, somehow "seals" the end (Fig. 7.1).

6. **Ultra structure-** A chromatid contains a very fine filament called chromonema which is a single, long, double stranded DNA molecule. It is wrapped around histones to form **nucleosomes**. The nucleosome and non-histone proteins together form the chromatin fiber. The chromatin fiber has reactive groups, probably H1 histone molecules, which act as "folders" and crosslink the chromatin fiber changing it into a great coiled, compact metaphase chromatid.

7. **Chemical composition-** The chromatin in the eukaryotic chromosome consists chemically of about 35% DNA, about 60% proteins, about 5% RNA, some metal ions and certain enzymes.

8. **Types of chromosomes-** On the basis of the position and number of centromeres, the chromosomes are classified as below (Fig. 7.2):

   (i) **Metacentric-** In metacentric chromosomes the centromere is at the middle of the chromosome, and the arms are equal. In anaphase the chromosome appears V-shaped. For example: human chromosome no. 3.

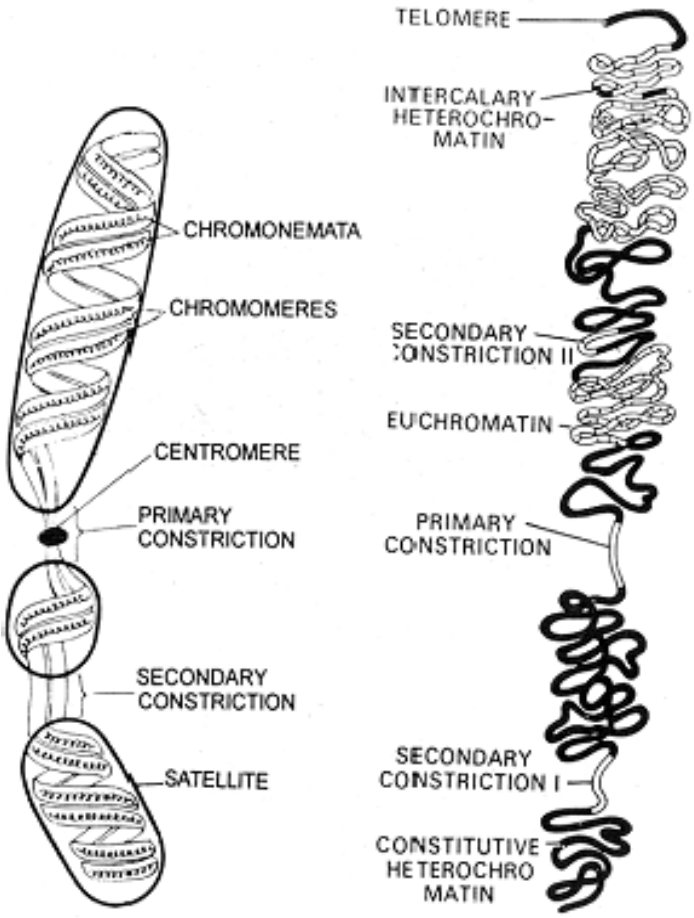

Fig. 7.1: Detailed schematic structure of chromosomes

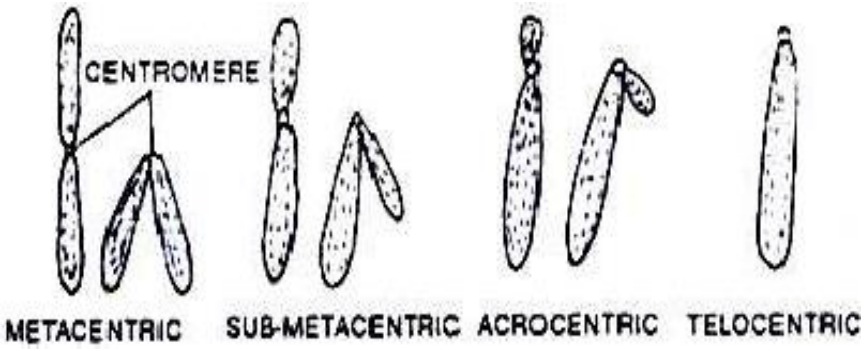

Fig. 7.2: Types of chromosomes based on centromere position

(ii) **Submetacentric-.** In such chromosome, the centromere is near the centre of the chromosome, and the arms are slightly unequal and in anaphase the chromosome appears J or L shaped. For example: Human chromosome No. 1.

(iii) **Acrocentric-** In this type the centromere is near one end of the chromosome, and the arms are very unequal. For example: Human chromosome No. 4 & 5.

(iv) **Telocentric-** The centromere is at one end in such chromosomes, and the arms are on one side only. The chromosome remains rod shaped in anaphase also.

Depending upon the number of centromeres there are three types of chromosomes:

(i) **Acentric-** The chromosome is without a centromere, which is formed by breakage of the chromosome. It does not attach to spindle microtubules so it is lost in the cell division.

(ii) **Monocentric-** It is the chromosome with a single centromere and it is the most common type.

(iii) **Dicentric-** It is the chromosome with two centromeres and is formed by the fusion of two chromosome segments each having a centromere. It is unstable and may break when the two centromeres are pulled to opposite poles in mitosis.

7.3.3 Functions of Chromosomes

(i) Chromosomes carry hereditary characters from parents to offspring.

(ii) They direct the synthesis of structural proteins and thus, help the cell grow, divide and maintain itself.

(iii) By directing the formation of necessary enzymes, they control metabolism.

(iv) They guide cell differentiation during development.

(v) They form nucleoli at nucleolar organizer sites in daughter cells.

(vi) They produce variations through changes in their genes and contribute to the evolution of the organisms.

(vii) They play role in sex determination.

(viii) They maintain the continuity of life by replication.

7.4 Giant Chromosomes

Giant chromosomes are special, enormously enlarged chromosomes about 100 times thicker than the ordinary mitotic chromosomes. These are seen in certain tissues of varied groups of animals and plants. They are easily visible under light microscope. The giant chromosomes are of two types: polytene and lampbrush.

7.5 Polytene Chromosomes

Polytene chromosomes were first observed by **Balbiani (**1881) in **Chironomus** (a dipteran larva). Because of their large size showing numerous strands these are named as polytene chromosomes by **Kollar**. These banded chromosomes occur in the larval salivary glands, midgut epithelium, and rectum and Malpighian tubules of various genera of dipterans. These are also known as **salivary gland chromosomes** because they have been best studied in the salivary gland cells of fly larvae (Fig. 7.3).

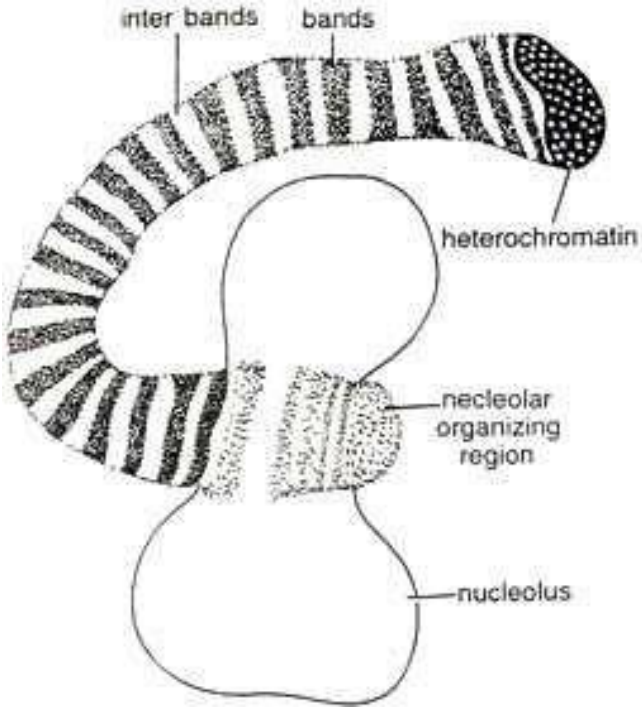

Fig. 7.3: Structure of polytene chromosome showing nucleolar part

These chromosomes are about 100-200 times larger than those of somatic chromosomes. They are roughly cylindrical and exhibit a distinct pattern of transverse striated structures consisting of alternate **darkly staining band** and **light staining interbands**. Dark bands are rich in DNA along with a small amount of RNA and basic proteins. They are genetically active. The inter-bands contain less of DNA but more acidic proteins and hence they are less active. The polytene chromosomes are formed by repeated replication of DNA without division of chromosome into daughter chromosomes. This amplification without separation is called **polytenization**. As a result, a thick bundle of parallel DNA molecules all having the same banding pattern across them is produced. Thus, there can be as many as several thousands of chromonemata in a giant chromosome.

During the initial stages of development the bands or inter-bands of chromosomes exhibit swellings or puffs. During development the **puffs** appear and disappear in definite patterns in response to the needs of developing larvae for the RNAs. The puffs are genetic sites active in RNA synthesis. In some regions of polytene chromosomes the chromonemata may give out a number of loops at certain places. Such loops are known as the **Balbiani rings**. These rings are formed by the lateral stretching of loops. They are rich in mRNA like the chromosomal puffs (Fig. 7.4).

7.5.1 Functions of the Giant Polytene Chromosomes

(i) Polytene chromosomes carry genes which ultimately control physiology of an organism. These genes are formed of DNA molecules.

(ii) These chromosomes also help in protein synthesis indirectly. The RNA present in the nucleolus serves as a means of transmission of genetic information to the cytoplasm, leading to the formation of specific protein.

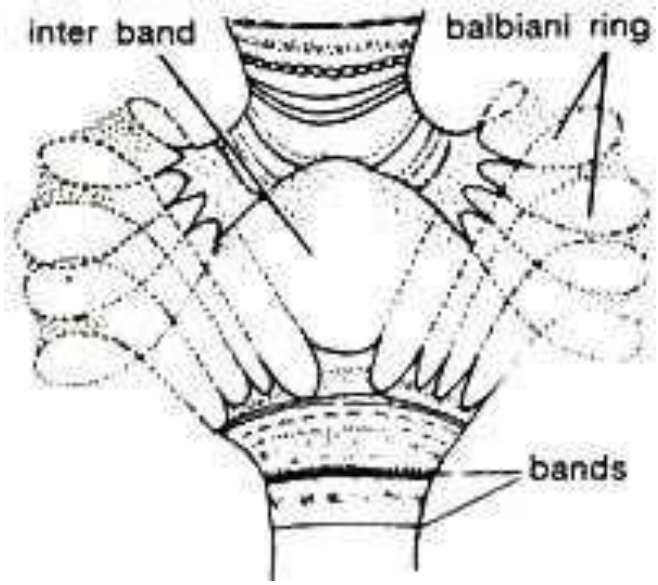

Fig. 7.4: Structure of Balbiani ring of polytene chromosome

7.6 Lampbrush Chromosomes

These are the largest chromosomes which can be seen with naked eyes and are found in yolk rich **oocytic nuclei** of certain vertebrates such as fishes, amphibians, reptiles and birds. They are characterized by the fine lateral loops, arising from the chromomeres, during first prophase of meiosis. Because of these loops they appear like brush; that is why they are called **lampbrush chromosomes** first discovered by **Flemming** in 1882 and were described in shark oocytes by **Ruckert** (1892).

Lampbrush chromosome consists of longitudinal axis formed by a single DNA molecule along which hundreds of bead like chromomeres are distributed. Two symmetrical lateral loops (one for each chromatid) emerge from each chromomere, which are able to expand or contract in response to various environmental conditions. About 5 to 10% of the DNA is in the lateral loops. The axis having compacted DNA and tightly associated proteins is transcriptionally inactive. The loops consist of uncompacted DNA and proteins but have a good amount of RNA and they are transcriptionally active. A chromomere and its associated loop correspond with one gene.

In lampbrush chromosomes the DNA loops are the sites of intensive RNA synthesis. rRNA and mRNA are synthesized in large amount and the transcription of rRNA causes the enlargement of nucleolus, or formation of numerous additional nucleoli. Due to the synthesis of large amounts of proteins, fats, carbohydrates and other molecules in the cytoplasm needed for further development of the embryo, the oocyte grows in size. Synthesis of proteins occurs near the loops (Fig. 7.5).

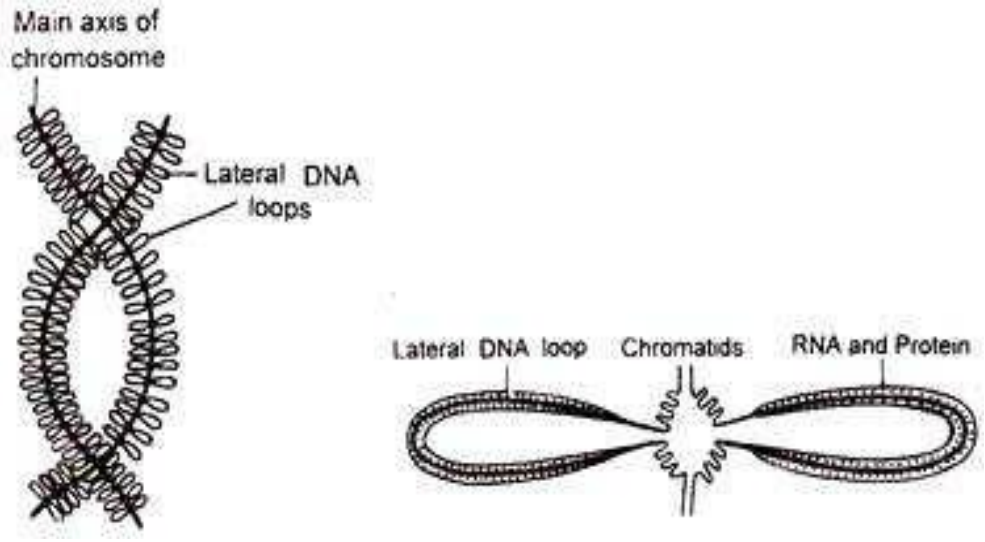

Fig. 7.5: Detailed structure of lampbrush chromosome

7.6.1 Functions of Lampbrush Chromosome

(i) These chromosomes are involved in the synthesis of RNA and proteins by their loops.

(ii) Lampbrush chromosomes probably help in the formation of certain amount of yolk material for the egg.

7.7 Summary

Chromosomes are made up of chromatin material and are capable of self-reproduction. They control cell's structure and metabolism and play an important role in the differentiation, heredity, mutation and evolution. Their structure varies in viruses, prokaryotes and eukaryotes. In viruses there is a single chromosome bearing a single nucleic acid molecule i.e. DNA or RNA, surrounded by a protein coat, which may be linear or circular, while prokaryotic chromosomes have a single and circular two stranded DNA molecule which is not enveloped by any membrane. The eukaryotic chromosomes are present in nucleus and are called nuclear chromosomes which are double stranded long DNA molecules of linear forms. When they are present in certain other organelles like mitochondria and plastids, then they are called extra nuclear chromosomes, which are double stranded short DNA molecules of circular forms. The eukaryotic chromosomes vary in number, size, shape and position, but they have remarkably uniform structures. The ends of chromosomes are known as "telomeres". A chromatin contain very fine chromonema which is single, long, double stranded DNA molecule wrapped around histones to form nucleosomes. Chemically a chromosome consists of DNA, proteins, RNA, some metal ions and some enzymes. Chromosomes on the basis of position and number of centromeres can be classified as metacentric, Submetacentric, Acrocentric, Telocentric and Acentric, Monocentric and Dicentric respectively. Giant chromosomes are special enormously enlarged chromosomes about 100 times thicker than the ordinary mitotic chromosomes. They are of two types- Polytene chromosomes (Balbiani, 1881). These occur in the larval salivary glands,

midgut, epithelium, rectum and malphigian tubules of various genera of dipterans. They carry genes which control physiology of an organism and they also help in protein synthesis indirectly. Second type is Lampbrush chromosomes (Flemming, 1882) found in yolk rich Oocytic nuclei of certain vertebrates. They bear fine lateral loops arising from the chromosomes during first prophase of meiosis.

7.8 Glossary

Chromatin fiber: A complex of macromolecules found in cells consisting of DNA, RNA and proteins.

Nucleic acid: The biopolymers, which include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), made from nucleotides are known as nucleic acids.

Ribovirus: Any of a group of viruses whose nucleic acid core is composed of RNA, including the retroviruses and picornaviruses is known as ribovirus.

Nucleoid: The nucleoid is an irregularly shaped region within the cell of a prokaryote that contains all or most of the genetic material and it is not surrounded by a nuclear membrane.

Extra nuclear Chromosomes: Extra chromosomal DNA is any DNA that is found outside of the nucleus of a cell like in mitochondria and plastids.

Centrosome: The centrosome is an organelle that is the main place where cell microtubules get organized.

Kinetochore: A kinetochore is a protein structure that forms on a chromatid during cell division and allows it to attach to a spindle fiber on a chromosome.

Sat-chromosome: A satellite chromosome or SAT chromosome has a chromosome segment that is separated from the main body of the chromosome by a secondary constriction.

Nuclear organizer: A nucleolar organizer is a chromosomal region around which the nucleolus forms.

Telomere: At each end of a chromosome there is a region of repetitive nucleotide sequences which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes. This region is known as telomere.

Malpighian tubule: The Malpighian tubule system is a type of excretory and osmoregulatory system found in some insects, myriapods, arachnids, and tardigrades. It consists of branching tubules extending from the alimentary canal that absorbs solutes, water, and wastes from the surrounding haemolymph.

Chromomere: A chromomere is one of the serially aligned beads or granules of a eukaryotic chromosome, resulting from local coiling of a continuous DNA thread.

7.9 Self Assessment Questions and Possible Answers

7.9.1 Multiple Choice Questions

1. Chromosomes are best seen in:

   (a) Interphase (b) Metaphase

   (c) Prophase (d) Telophase

2. A chromosome with terminal centromere is called:

   (a) Metacentric (b) Telocentric

   (c) Submetacentric (d) Acrocentric

3. A chromatid has:

   (a) One chromonema (b) Four chromonemata

   (c) Two chromonemata (d) Numerous chromonemata

4. In bacterial chromosomes, nucleic acid polymers are:

   (a) Linear RNA molecule (b) Linear DNA molecule

   (c) Two types of DNA and RNA (d) Circular DNA molecule

5. The component of chromosomes that controls heredity is:

   (a) Proteins (b) RNA

   (c) DNA (d) Metal ions

6. In which of the following organisms were discovered polytene chromosomes?

   (a) *Musca* *(b)* *Cimex*

   (c) *Drosophila* *(d)* *Chironomus*

7. Lampbrush chromosomes are found during:

   (a) Interphase (b) Metaphase of meiosis

   (c) Prophase of mitosis (d) First prophase of meiosis

8. Balbiani rings occur in:

   (a) Polytene chromosomes (b) Lampbrush chromosomes

   (c) Polysomes (d) Heterosomes

9. Chromosomes with equal arms are called:

(a) Submetacentric (b) Metacentric

(c) Telocentric (d) Acrocentric

10. An octamer of four histones complexed with DNA is called:

(a) Nucleosome (b) Centrosome

(c) Chromosome (d) Endosome

7.9.2 Very Short Questions

1. Who discovered the chromosomes?
2. Name the part of a chromosome separated by a secondary constriction?
3. What is a SAT-Chromosome?
4. What are nucleolar chromosomes?
5. Name the four types of chromosomes with regard to the position of a centromere.
6. Give the terms used for a chromosome with numerous chromonemata.
7. Which component of the chromosomes is responsible for heredity?
8. Explain heterochromatin.
9. Define nucleolar organizing region?
10. Who discovered salivary gland chromosomes?

ANSWERS

7.9.1

1.(b)	5.(c)	9.(b)
2.(b)	6.(d)	10.(a)
3.(a)	7.(d)	
4.(d)	8.(a)	

7.9.2

1. W. Hofsmeister
2. Satellite
3. Chromosome with a satellite
4. Which form nucleoli on them?
5. Metacentric, Submetacentric, Acrocentric and Telocentric
6. Polytene chromosome
7. DNA

8. Darkly stained regions of chromosomes are called heterochromosome

9. Chromosomal regions that contains the genes for ribosomal RNAse and induces formation of nucleolus

10. E. G. Balbiani in Chironomus.

7.10 References and Suggested Readings

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7.11 Terminal and Model Questions

1. Describe the structure and functions of chromosomes.
2. Write an account of special type of chromosomes.
3. Give an account of Giant chromosomes.
4. Write down the properties and functions of chromosomes.
5. Describe the morphology and chemical composition of chromosomes.

UNIT 8 CELL DIVISION

Contents

8.1 Objectives

After reading this unit the readers will be able to:

- Define mitosis and meiosis.
- Elucidate stages of cell cycle.
- Explain cytokinesis.
- Describe reproductive cycle stages and synaptonemal complex.
- Discuss recombination nodules.
- Compare between mitosis and meiosis.

8.2 Introduction

A multicellular organism starts its life as a single cell and it undergoes repeated division, thus, the growth and development of every living organism depends on the growth and multiplication of its cells. The cell increase in size due to growth and it is the characteristic feature of all the living organisms. After the cell attains maximum growth, it begins to divide. The vegetative growth of an organism takes place by an increase in the number of cells through cell divisions which follows the geometrical progression. The cell division is a continuous and dynamic process and it involves the following three stages:

1. DNA or genome replication
2. Nuclear division or karyokinesis
3. Cytoplasmic division or cytokinesis

The cell division is of two types on the basis of number of genomes present in the daughter cells in comparison to the dividing parent cell — **mitosis** and **meiosis**.

1. Mitosis- The term mitosis was coined by **W. Flemming** in 1882. The multiplication of a body cell into two daughter cells of equal size and containing the same number of chromosomes as in the parent cell is called mitosis or **somatic division**.

2. Meiosis- The term meiosis was first coined by **J. B. Farmer (1905) with J. E. Moore.** Meiosis occurs only in gonads (in germ mother cells) during the formation of gametes like sperm and ovum. Meiosis is a process by means of which double number or 2N or diploid chromosomes is reduced to its half number or N or haploid. It is also called **reduction process.**

8.3 Cell Cycle Stages, Mitosis & Cytokinesis

8.3.1 Cell Cycle:-

Every cell having the capacity to divide passes through a regular cycle of changes

known as cell cycle. A cell starts its cycle in diploid condition.

8.3.1.1 Phases of cell cycle:-

Cell cycle consists of two stages: A long un-dividing stage called **interphase or I-phase** and a short dividing stage called **mitotic or M-phase** (Fig. 8.1).

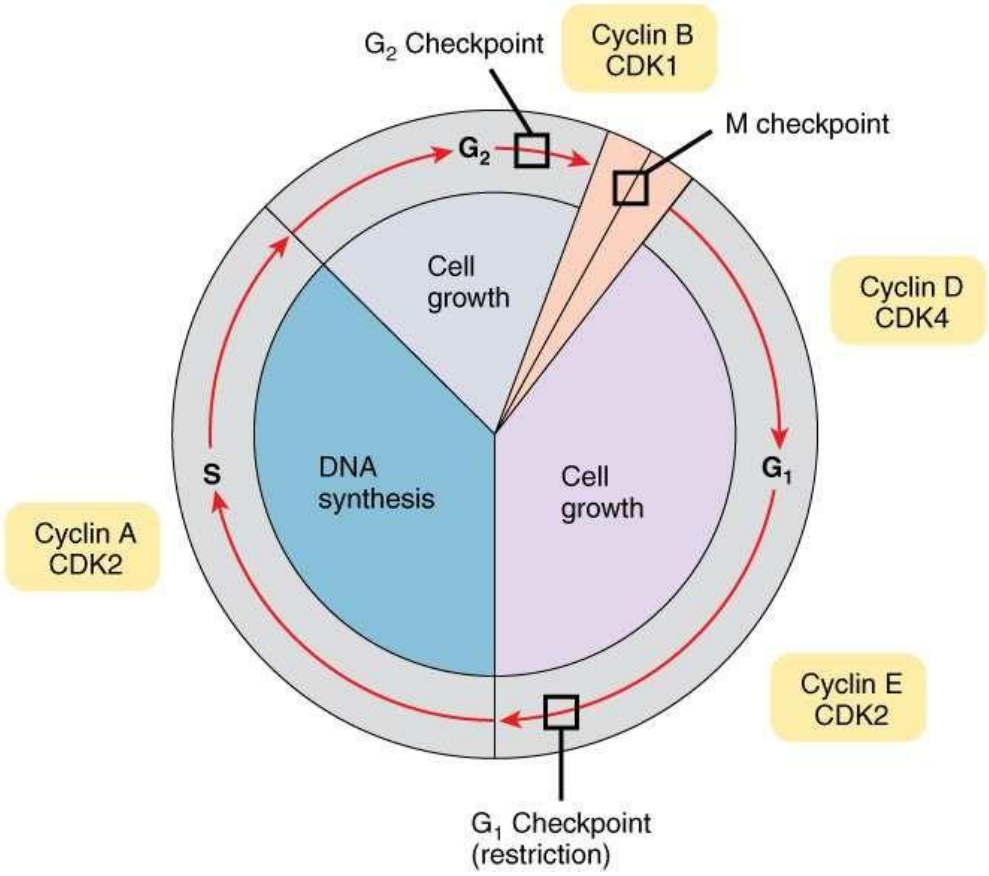


Fig. 8.1: Cell Cycle checkpoints

1. ***Interphase-*** The time between the end of telophase and the beginning of the next M-phase is called the interphase. It is a long stage that lasts for 10 to 30 hours. During this phase the cell grows by synthesizing biological molecules such as lipids, proteins, carbohydrates, nucleic acids.

   Interphase is further divided into three sub phases or periods: first gap or G1 phase, synthetic or S phase and second gap or G2 phase.

   (i) **G_1 phase-** The **gap between previous mitosis and beginning of DNA synthesis** is represented by G_1 phase. In this stage initial growth of a newly formed cell takes place. Various biological molecules (carbohydrates, proteins, lipids, including some non-histones, RNAs) are synthesized in this phase. Normal metabolism is carried out for the preparation for DNA

replication that is to take place next to it. DNA synthesis does not occur in this phase.

(ii) **S Phase-** During this **phase duplication of each chromosome** take place by replication of new DNA molecule on the template of the existing DNA. Synthesis of histone proteins and their mRNA, some non-histone proteins and formation of new nucleosome also occur in S-phase only. In most of the eukaryotes the S-phase lasts for 6 to 8 hours.

(iii) **G_2 Phase-** G_2 phase is the gap between DNA synthesis and nuclear division. RNA transcription and protein synthesis continues during this phase. Further growth of the cell and preparation for its division also takes place in this stage. During this stage the cytoplasmic organelles such as centrioles, mitochondria and Golgi apparatus are doubled, proteins for spindle and asters are synthesized and active metabolism stores energy for the next mitosis. The G_2 phase in most cells lasts for 2 to 5 hours.

2. ***Mitotic Phase-*** Interphase is followed by mitotic phase. During mitotic phase the already **duplicated chromosomes are equally distributed to the daughter cells** which contain exactly the same hereditary information as the parent cell. Though, the other cell components (organelles and molecules) are also divided approximately equally between the daughter cells, but not as precisely as the DNA. After the mitosis is over, the daughter cells enter the G_1 phase of the next cell cycle.

During mitosis many structural and physiological changes take place in the cell, as the chromatin of the nucleus is packed into visible chromosomes, which are set free by breakdown of nuclear envelope. An extensive reorganization of the membranous components and cytoskeletal elements takes place. Endoplasmic reticulum and Golgi apparatus break down into small vesicles and stops the protein movement. Microtubules dissociate into tubulin dimers and are assembled into the spindle which occupies most of the cell and helps in the distribution of chromosomes into the daughter cells. Actin filaments get reorganized and form a contractile ring for the cytoplasmic division.

8.3.1.2 Control of Cell Cycle:-

1. ***Nucleo-cytoplasmic Ratio-*** In 1910**, Hertwig** proposed that the **cell division starts when the ratio between the volume of the nucleus and the volume of the cytoplasm is upset.** As the cell grows, the synthesis of proteins, nucleic acids, lipids and other cellular components takes place. During synthesis of these molecules, the back and forth movements of materials through the nuclear and the cell membranes occurs. With the growth of the cell, its volume increases more than the surface of the nucleus and the cell, and at a critical point, the surface of the nucleus become inadequate for the exchange of materials between the nucleus and the cytoplasm required for further growth. The cell divides at this stage and regains the optimum and

efficient nucleo-cytoplasmic ratio that allows the growth. Although the cell division usually occurs after a cell has grown to a certain size, there are important exceptions to this pattern.

2. ***Surface-Volume Ratio-*** With the growth of the cell size, its volume increases more than its surface area. All the materials of the cell required for its maintenance and growth are drawn through its surface. A stage will reach when the surface area is insufficient to supply the large volume of the cell. It is thought that there is a critical point at which the cell division starts and the division of the cell greatly increases the surface without increasing the volume. This theory fails in case of starved cells, which may divide without doubling their size and form smaller daughter cells.

3. ***Nucleolus-*** Damage to nucleolus at a certain critical time (telophase or mid prophase) stops cell division.

4. ***Cyclic Nucleotides-*** Concentration of cAMP and cGMP vary regularly during the cell division. Concentration of cAMP is high during G_1 phase, but it falls as the cell enters the S phase and mitosis. However the concentration of cGMP often varies in the reverse pattern. Thus, addition or removal of any of these nucleotides can start or stop entry of many cells into S phase and the subsequent M phase. The concentration of these cyclic nucleotides remains constant throughout the cell cycle in many cells.

   Also, plant cells do not have cyclic nucleotides. On the basis of these facts, cyclic AMP and GMP are no longer thought to regulate the cell cycle.

5. ***Phosphorylation-*** During cell cycle the phosphate groups are added to the histone groups particularly to H_1 as the cell enters S phase, increases during M phase, and are removed on the completion of mitosis before G_1 starts. Phosphate groups are also added and removed to non-histone proteins during cell cycle. Thus, it is believed that the changes in the histones and non-histones may have a role in the control of cell cycle because these proteins have been found to regulate the activity of genes in RNA transcription during interphase.

6. ***Cyclin****:* The concentration of the protein called cyclin appears to control mitosis as it builds up during interphase and is degraded during mitosis.

8.3.2 Mitosis

A German biologist **Eduard Strasburger** described mitosis for the first time in 1875. Same was described later in 1879 by **Walther Flemming** who also termed it "mitosis" in 1882.

It is the most common method of cell division in eukaryotes that takes place in somatic cells of the body and hence it is also known as somatic division. However in gonads it occurs in undifferentiated germ cells. In plants it takes place in the cells of meristematic tissues. The duration of mitosis on an average is from 30 minutes to 3 hours.

Mitosis is defined as the division of a parent cell into two identical daughter cells each with a nucleus having the same amount of DNA, the same number and kind of chromosomes and the same hereditary instructions as the parent cell. Therefore, it is also known as the equational division. There are two main events involved in mitosis: **Karyokinesis or division of the nucleus and cytokinesis or division of cytoplasm.**

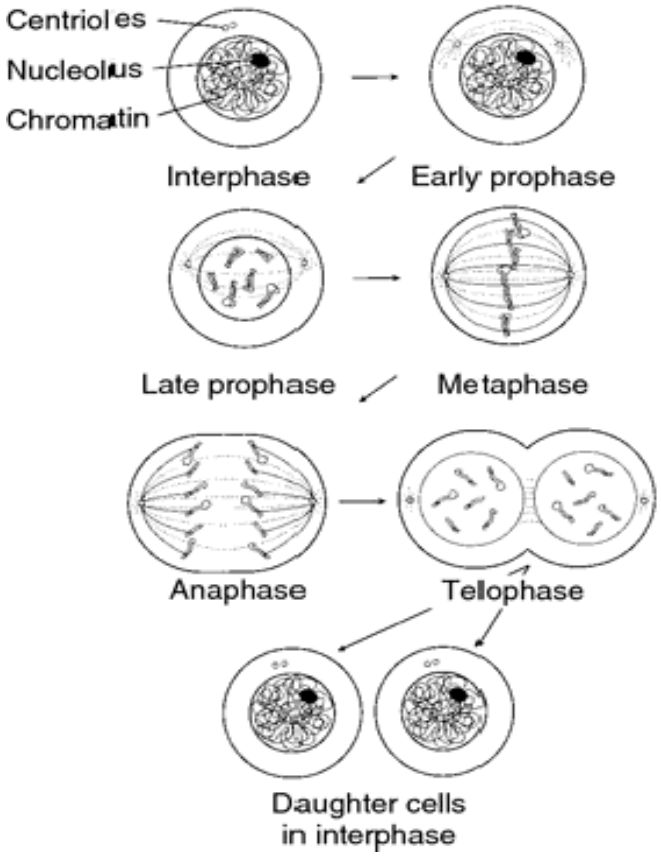


Fig. 8.2: Stages of mitosis in animal cells

8.3.2.1 Karyokinesis

In eukaryotes, karyokinesis is a complex process due to the presence of many chromosomes. It is a continuous process which may be divided into four stages: prophase, metaphase, anaphase and telophase.

1. ***Prophase-*** In an interphase cell the chromosomes are greatly extended and spread throughout the space in the nuclear compartment. Approximately 4 meters of DNA is organized into 46 duplicated chromosomes is present in the nucleus of a human G_2 cell. The prophase is long and complex that lasts for about 50 minutes. It may be divided into 3 sub stages: early prophase, middle prophase and late prophase.

   A) Early prophase- During the early prophase of mitosis the following events take place:

   (i) The shape of cell becomes almost rounded and the cytoplasm becomes viscous.

(ii) The centrioles lie close to the nucleus and around them assembles the short radiating microtubules by polymerization of the tubulin dimers. Both pairs of centrioles also called **diplosomes**, start moving to the opposite ends of the cell. The microtubules surrounding each pair of centrioles appear like a star body, and are called the **aster**. The microtubules which are also termed as **astral rays**, are not in contact with the centrioles, but are separated from them by an amorphous zone of cytoplasm known as **pericentriolar cloud**. The microtubules stretching between the diplosomes moving apart increase in number and length by incorporating more tubulin dimers. Thus, asters shift the duplicated centrioles to the opposite ends of the cell from where the centriole pair will pass into separate daughter cells when cytokinesis occurs. Though the centrioles have no role in the formation of the spindle but they may be concerned with orienting the spindle.

(iii) Long microtubules assemble on one side of the nucleus to form mitotic spindle. **Microtubules are arranged in bundles called spindle fibers** and at each pole of the spindle lies the mother-daughter centriole pair.

(iv) The chromosomes that appear like threads in the nucleus gradually change into short, thick rods by loss of water and progressive coiling and become visible. Due to the duplication of DNA and chromosomal proteins during the interphase, each chromosome appears longitudinally double, consisting of two identical sister chromatids which are held together at the narrow region called **primary constriction or centromere**. Each chromatid has a disc like structure at centromere, where the spindle microtubules join it. This disc is called as **kinetochore**.

B) Middle prophase- It includes the following events:

(i) The chromosomes further get shorter, thicker and their chromatids become uncoiled and finally they assume their characteristics sizes and become distinguishable individually.

(ii) **Nucleoli** progressively become smaller and **finally disappear.** Nuclear envelope begins to breakdown into small vesicles which disperse into the cytoplasm. The lamina dissociates into its protein subunits.

C) Late Prophase- This phase involves the following events:

(i) The nuclear envelope breaks completely thus, releasing the chromosomes and other nuclear contents into the cytoplasm.

(ii) The spindle gains their proper shape and size.

(iii) The growing spindles push the centriole pairs to the opposite ends of the cell.

2. ***Metaphase-*** The metaphase being short and simple lasts for 2 to 10 minutes and it involves the following events:

A. The spindle occupies the region of the nucleus.

B. The chromosomes move to the **equatorial plane** of the spindle.

C. Some spindle microtubules extend to and join the chromosomes. These are called chromosomal or kinetochore microtubules.

D. The chromosomes get aligned at the middle of the spindle in the form of a plate called **equatorial or metaphase plate**. This plate is formed by the kinetochores, the arms of the chromatids trailing away on the sides. It is at the right angles of the long axis of the spindle. During metaphase the chromosomes have fully aligned into a plate and await the separation of their chromatids.

3. ***Anaphase-*** Anaphase lasts only 2 to 3 minutes and it comprises the following events:

A. The **sister chromatids of each chromosome slightly separate** at the primary constriction so that their kinetochores stretch towards the opposite poles of the spindle. In all the chromosomes separation of chromatids occurs almost simultaneously. The **chromatids are now referred to as chromosomes** because they are no longer held to their duplicates.

B. After a short time, the chromatids separate completely from their former mates, and start moving to opposite poles of the spindle. As each chromosome is being pulled by its attached microtubules, its kinetochore leads and arms trail behind. As a result the chromosomes are pulled into V, J and I shapes, depending upon the position of the kinetochore. (Metacentric, sub metacentric or telocentric respectively)

C. As the chromosomes move toward their respective poles, the two poles move farther apart by elongation of spindle.

The anaphase ends when all the chromatids reach the opposite poles. Each pole of the spindle receive one chromatid from every metaphase chromosome, the two groups of chromatids have exactly the same hereditary information.

4. ***Telophase-*** The telophase is long and complex and lasts for an hour or so. In this phase nucleus is reconstructed from each group of chromosomes. It involves the following events:

A. The **chromosomes** at each pole **unfold, and become long and slender**. Finally, they become indistinguishable as were in an interphase cell.

B. **Nuclear envelope** is **reconstructed** around each group of chromosomes gradually. First, the membrane vesicles associate with the individual unfolding chromosomes, partially enclosing each chromosome. Then they fuse to form an envelope surrounding the entire set of chromosomes at each pole. The lamina proteins re-associate simultaneously with the reconstruction of nuclear envelope and form a complete lamina within the nuclear envelope

C. Nucleolar material, composed of partially processed ribosomal subunits and processing enzymes, dispersed into the cytoplasm in the prophase return to the

nucleolar organizer site and forms a small nucleolus. Processing of this preexisting material then continues. Transcription of new rRNA also begins at this time; it gradually speeds up until it attains the high level of characteristic of interphase cell. Along with this, the nucleolus grows and attains its normal size. The nucleolus reformed at telophase, thus contains both old and new rRNA and ribosomal proteins.

With the transformation of chromosomes into chromatin and reconstruction of nucleoli, transcription of all the three RNA types gradually becomes normal.

The spindle begins to disappear and the asters become small by depolymerization of microtubules and the centrioles take up their characteristic interphase position close to the one side of the nucleus. Short spindle microtubules persist for sometime at the spindle equator to mark the region where the cytoplasm will later divide.

8.3.2.2 Cytokinesis

Cytokinesis is the division of cytoplasm. It encloses the daughter nuclei formed by the karyokinesis in separate cells, thus completing the process of cell division. Cytokinesis is signaled at the metaphase by cytoplasmic movements that bring about equal distribution of mitochondria and other cell organelles in the two halves of the cell. Division occurs differently in animal cells and the plant cells.

8.3.2.3 Significance of Mitosis

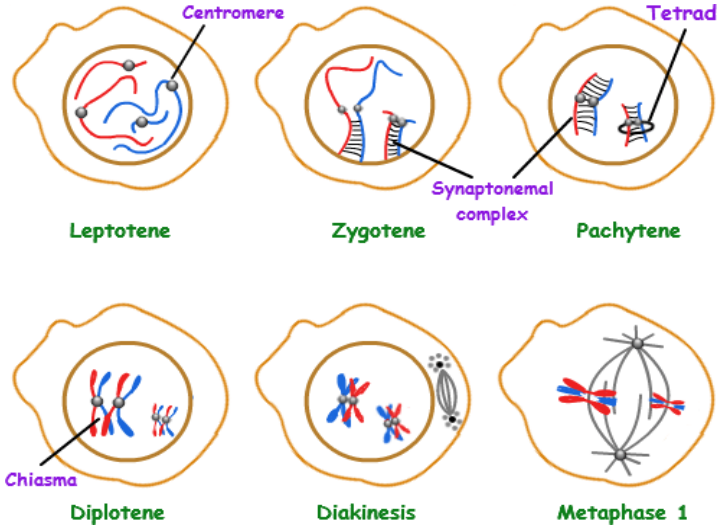
Mitosis has manifold significance-

- **Maintenance of Size**- Mitosis helps maintaining the size of the cell. A cell, when full grown, divides by mitosis instead of growing further.
- **Growth**- A fertilized egg develops into an embryo and finally into an adult by repeated mitotic cell division.
- **Maintenance of Chromosome Number**- Mitosis keeps the number of chromosomes equal in all the cells of an individual. Thus mitosis provides a complete set of genetic information to each cell, since DNA is duplicated in S phase prior to mitosis.
- **Repair**- Mitosis provides new cells to replace the old worn out and dying cells.
- **Healing and Regeneration**- Mitosis produces new cells for the healing of wounds and regeneration.
- **Reproduction-** Mitosis brings about multiplication in the acellular organisms. In multicellular organisms also, it plays an important role in reproduction, asexual as well as sexual.
- **Evidence of Basic Relationship of Organisms-** Mitosis, being essentially similar in many kinds of organisms, supports the basic relationship of all living things.

8.4 Meiosis

In 1887, August Weismann predicted on theoretical grounds that the number of chromosomes must be reduced by one-half during gamete formation. **Edouard Van Beneden** demonstrated reduction division in1887. **J.B. Farmer and Moore** introduced the term "meiosis" in 1905.

Mitosis occurs in all kinds of eukaryotic cells, while meiosis is confined to certain cells and takes place at a particular time. Only the cells of sexually reproducing organisms undergo meiosis, and only special cells in the multicellular organisms switch over from mitosis to meiosis at the specific time in the life cycle. Meiosis produces gametes or gametic nuclei in animals, some lower plants, and various protists and fungus groups. Meiosis forms spore in higher plants. The spores give rise to gamete producing structure called gametophytes, which produces gametes by mitosis.



Meiosis consists of two divisions that take place in rapid succession, with the chromosomes replicating only once. Thus, a parent cell produces four daughter cells, each having half the number of chromosomes and half of the nuclear DNA amount present in the parent cell. Meiosis is therefore also known as **reduction division**. The two divisions of meiosis are known as the first and the second meiotic divisions or **meiosis-I and meiosis-II.**

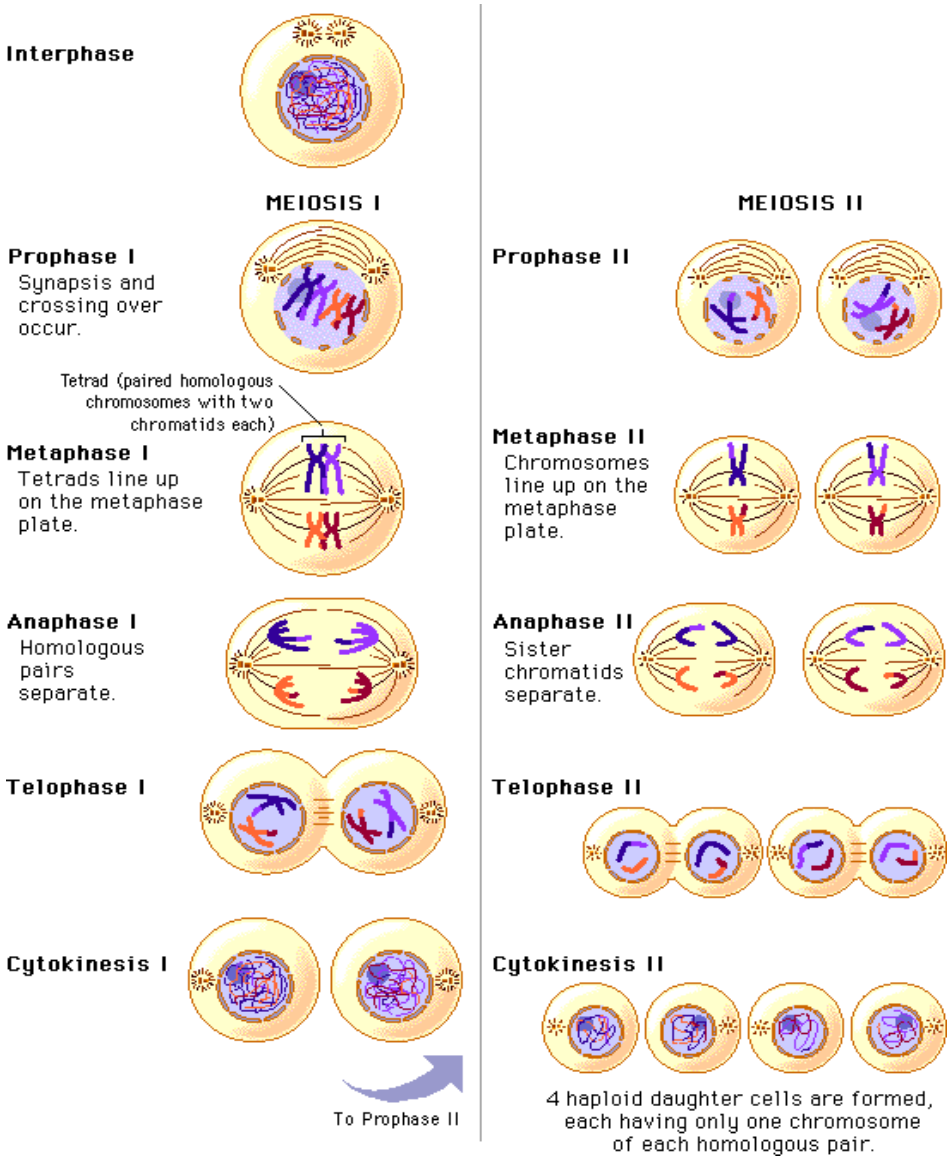


Fig.8.3: Stages of meiosis in animal cells

8.4.1 Divisions of Meiosis

8.4.1.1 First meiotic division or Meiosis-I :-

During the first meiotic division, the two homologous chromosomes of each pair separate from each other and go to separate daughter cells. This reduces the number of chromosomes from diploid to haploid condition**. Meiosis-I** is therefore known as **heterotypic division**. The four phase of this division are called Prophase-1, metaphase-1, anaphase-1 and telophase-1.

1. ***Prophase--***. The meiotic prophase-1 is **more complex** than the mitotic prophase because of the process of recombination that occurs in it. It also lasts **much longer** than the mitotic prophase in the same organism. It may extend over weeks, months or

even years. Although it is more or less a continuous process, it is divided into 5 sub-stages: leptotene, zygotene, pachytene, diplotene and diakinesis.

(a) ***Leptotene-*** Leptotene begins when chromosomes appear as thin threads by condensation. The chromosomes become thicker as condensation proceeds. They lie jumbled up so that it is not possible to trace individual chromosomes. Each chromosome is double, consisting of two chromatids due to DNA replication during premeiotic interphase. However, the chromatids are closely adhered together and are not distinguishable.

(b) ***Zygotene-*** The homologous chromosomes come to lie side by side in pairs. The pairing of homologous chromosomes is called **Synapsis or conjugation**. A pair of homologous chromosome lying together is termed as a **bivalent**. Pairing is so through that the corresponding ends and all the corresponding genes of the two homologous chromosomes lie exactly opposite to each other. The centrosome of the chromosomes also lies adjacent to one another. The chromatids are still not visible. A regular space of about 0.15 to 0.2 µm wide exists between the synapsed homologous chromosomes, bearing a highly specialized fibrillar organelle, **the synaptonemal complex**. The synaptonemal complex consists of three parallel and equally spaced longitudinal filaments flanked by chromatin and interconnected by short transverse filaments. The complex contains DNA and some specific proteinaceous material. It was discovered by Montrose J. Moses in 1955 in crayfish.

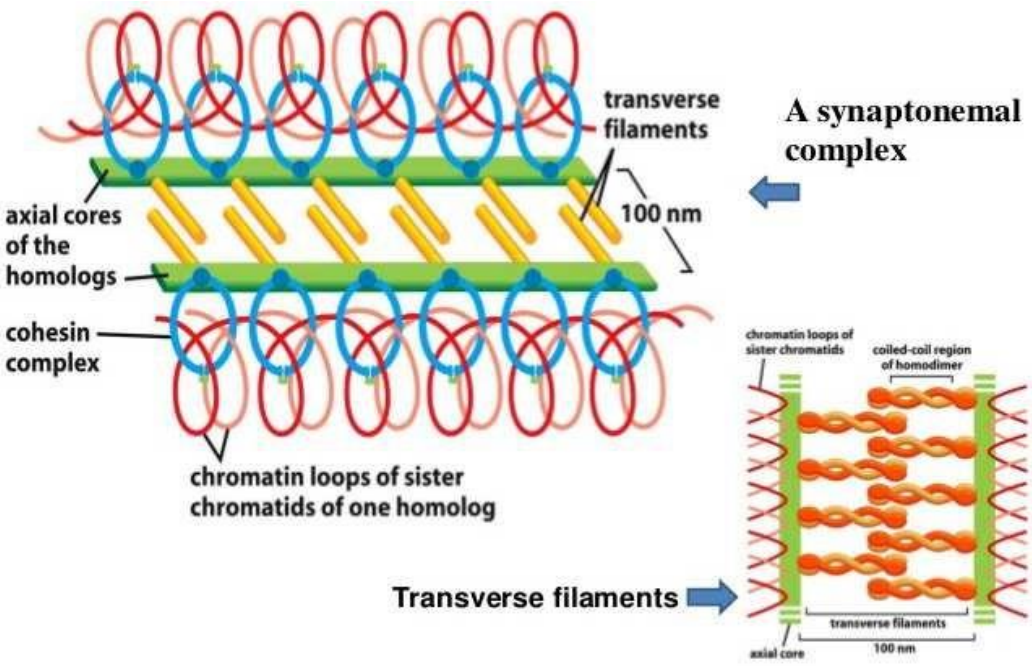


Fig. 8.4: Synaptonemal complex

(c) ***Pachytene-*** The synapsed chromosomes continue to become short and thick. The chromatids of each synapsed chromosome slightly separate and become

visible. A chromosome with two visible chromatids is known as **dyad**. A group of four homologous chromatids (two dyads) is called a **tetrad**. The number of tetrads equals the haploid number of chromosomes. The two chromatids of the same chromosomes are called are called sister chromatids and those of the two homologous chromosomes are called non-sister chromatids. The leptotene and the zygotene stages last for a few hours, the pachytene may take weeks, months or even years. It is prolonged because recombination or crossing over occurs in it.

Recombination involves mutual exchange of the corresponding segments of non-sister chromatids of homologous chromosomes. It occurs by breakage and reunion of non sister chromatid segments. Certain structures mediate the meiotic recombination by marking the sites of crossing over. These are known as **recombination nodules** (RNs). They are multicomponent proteinaceous ellipsoids found in association with the synaptonemal complex during prophase-I of meiosis (Carpenter, 1975b). The **synaptonemal complex**, a protein structure, helps in recombination by keeping the homologous chromosomes in paired state for the required period and also by containing and aligning the enzymes needed for breakage and union.

(d) ***Diplotene***- At this stage the **homologous chromosomes separate** at many places. This is called **disjunction**. It occurs because the synaptic forces and the synaptonemal complex disappear. The chromatids become more distinct and tetrads seem very clear. The homologous chromosomes do not separate at certain points. These points are called **chiasmata**. The chiasmata mark the sites where the exchange of chromatids occurred during pachytene. The number of chiasmata is related to the length of the chromosomes. Longer chromosomes have more chiasmata than the shorter ones. In case of single chiasmata, the bivalent looks like a cross; in case of two chiasmata, it looks like a ring; and in case of many it shows series of loops.

(e) ***Diakinesis***- In this stage the chromosomes condense again into short, thick rods. The chiasmata disappear by sliding towards the tips of chromosomes due to tight condensation. This process is called **terminalization**. The centrioles already duplicated in premeiotic interphase, move apart in pairs to the opposite ends of the cell. Asters form around each centriole pair. Spindle develops between the centriole pairs. The nucleolus disintegrates. The nuclear envelope breaks down into vesicles. The tetrads are released into the cytoplasm.

2. ***Metaphase-*** The spindle shifts to the position that is earlier occupied by the nucleus. The tetrads scattered in the cytoplasm move to the equator of the spindle. Here, they **align in two parallel metaphase plates**, one formed by chromosomes and other by their homologous. The attachment of the tetrads to the spindle microtubules in metaphase-I is different from that of mitotic metaphase chromosomes. Each homologous chromosome has two kinetochores, one for each of its two chromatids.

Both the kinetochores of a homologous chromosome connect to the same spindle pole. The two kinetochores of its homologue join the opposite spindle pole.

3. ***Anaphase-I-*** From each tetrad, two chromatids of a chromosome move as a unit (dyad) to one pole of the spindle, and the other two chromatids of its homologue migrate to the opposite pole. Thus, the two homologous chromosomes of each pair are separated in the anaphase-I of meiosis. The process is also called as **disjunction.** As a result half of the chromosomes, which appear in early prophase, go to each pole. Thus, it is during anaphase-I that the real reduction in the chromosome number occurs. Each chromosome at the pole is still double and consists of two chromatids. Thus, the group of chromosomes at each pole though has only one member of each homologous pair still contains twice the haploid amount of DNA.

4. ***Telophase-***During telophase-I, the chromosome at each pole of the spindle partly unfold and elongate, and form a nucleus with nucleolus and nuclear envelope. The spindle and asters disappear.

   The cytoplasm divides at its middle by constriction in an animal cell and by cell plate formation in a plant cell. This produces, two daughter cells, each with one nucleus. The nucleus of each daughter cell has received only one chromosome from each homologous pair. Thus, it has **half the number of chromosome, but double the amount of nuclear DNA as each chromosome is double.**

8.4.1.2 First meiotic division or Meiosis-II

The meiosis-II is similar to mitosis as in this division, the two chromatids of each chromosome separate from each other and go to separate daughter cells. With the result, the number of chromosomes remains the same as produced by meiosis-I. Meiosis-II is, therefore, known as **homotypic division.** The four stages of this division are called prophase-II, metaphase-II, anaphase-II and telophase-II.

1. ***Prophase-I-*** When there is no interkinesis, the telophase-I spindle is replaced by two new spindles; and the centrioles and asters, if present, duplicate and one copy of each comes to lie at each pole of the new spindles. The telophase-I chromosomes move from the poles of the old spindle to the equators of the new spindles. If decondensation has occurred during telophase-I, the chromosome recondense to short rod lets as they migrate to the metaphase-II spindles.

   If interkinesis is present, centrioles move apart and asters are formed around them. A spindle is formed between the centrioles. Chromosomes each consisting of two chromatids, appear in the nucleus. They are set free in the cytoplasm by breakdown of the nuclear envelope. Nucleus disappears.

2. ***Metaphase-II***- The chromosomes get arranged at the equator of the spindle as a metaphase plate. The chromatids of each chromosome are joined at their kinetochores by chromosomal microtubules extending from the opposite poles of the spindle as in mitosis.

3. ***Anaphase-II***- The two chromatids of each chromosome separate and move to the opposite poles of the spindles. Here they are called chromosomes. Each pole has **haploid number of chromosomes and haploid amount of DNA**. This amount is one-fourth of the DNA present in the original cell which entered meiosis.

4. ***Telophase-I-:*** The chromosome at each pole decondenses, and nuclear envelope develops around them. This produces two nuclei. Nucleolus is formed in each nucleus. Spindle and asters disappear. In cases that lack interkinesis, four nuclei are formed in telophase-II.

8.4.2 Cytokinesis

Cytoplasm divides at its middle by constriction in an animal cell and by cell plate formation in a plant cell. This produces two daughter cells. The later have half the number of chromosomes, and half the amount of nuclear DNA, i.e., in Reduction division is complete when this point is reached. The cells formed by meiosis-II in animals are mature gametes. They do not divide further. A gamete must fuse with another suitable gamete before a new individual can develop. The cells formed by meiosis-II in plants are the spores. The spores can develop into new individuals without fusing in pairs. In fact the main difference between a spore and a gamete is the ability of the spore to develop directly into a new individual.

8.5 Comparison between Mitosis and Meiosis

Mitosis and meiosis can be differentiated through following points:-

S. No.	Mitosis	S. No.	Meiosis
1.	It occurs in all kinds of cells and may continue throughout life.	1.	It occurs only in special cells(gamete mother cells or spore mother cells) and at specific times
2.	It involves a single division, resulting in two daughter cells only.	2.	It involves two successive divisions, resulting in four daughter cells.
3.	A cell can repeat mitosis almost indefinitely.	3.	Meiosis takes place only once in a cell.
4.	All mitotic divisions are alike.	4.	Two meiotic divisions are dissimilar, first is reductional and second equational.
5.	Each mitotic division is preceded by an interphase	5.	The second meiotic division is generally not preceded by an interphase.
6.	Chromosomes replicate before	6.	Chromosome do not replicate before

	each mitotic division.		second meiotic division.
7.	Prophase is relatively short and simple.	7.	Prophase-1 is very long and elaborate, comprising 5 sub phases.
8.	Prophase chromosomes appear double from the very start.	8.	Prophase-1 chromosomes do not look double in the beginning.
9.	There is no pairing of homologous chromosomes, hence no chance of crossing over.	9.	Homologous chromosomes pair and often undergo crossing over in prophase-1.
10.	No chiasmata are formed.	10.	Chiasmata form temporarily where crossing over occurs.
11.	Chromatids are genetically similar to chromosomes they arise from	11.	Chromatids may differ genetically from the chromosomes they arise from due to crossing over.
12.	No synaptonemal complex forms between chromosomes.	12.	Synaptonemal complex forms between synapsed homologous chromosomes
13.	Chromosomes do not unfold, and no transcription and protein synthesis occur in prophase.	13.	Chromosomes unfold and, transcription and protein synthesis may occur in diplotene of prophase-I.
14.	All chromosomes form a single plate in metaphase.	14.	Chromosomes form two parallel plates in metaphase-I and one plate in metaphase-II.
15.	The two kinetochores of a chromosome connect to both the poles of the spindle.	15.	The kinetochores of a chromosome connect to the same spindle pole in metaphase-I and to both the poles in metaphase-II.
16.	Anaphase involves separation of chromatids of each chromosome.	16.	Anaphase-I involves separation of homologous chromosomes. The chromatids move apart in anaphase-II.
17.	Telophase occurs in all cases.	17.	Telophase-I is eliminated in some cases.
18.	Daughter cells have diploid number of chromosomes like the parent cell.	18.	Daughter cells have haploid number of chromosomes unlike the parent cell.
19.	Daughter cells have 2n amount of DNA unlike 4n amount in	19.	Daughter cells have 1n amount of DNA unlike the 4n amount in the parent cell.

the parent cell.

20.	Daughter cells divide again after interphase.	20.	Daughter cells, if gametes, do not divide further.
21.	Mitosis brings about growth, repair and healing.	21.	Meiosis forms gametes or spores, helps maintain the number of chromosomes constant from generation to generation, and introduces variation.
22.	Mitosis is much shorter than meiosis in the same animal.	22.	Meiosis is much longer than mitosis in the same animal.
23.	Cytokinesis usually follows karyokinesis.	23.	Cytokinesis often doesn't occur after meiosis-I, but always occur after meiosis-II, forming four cells simultaneously.
24.	Mitosis may occur in haploid or diploid cells	24.	Meiosis always occurs in diploid cells.
25.	Chromosomes do not show chromomeres.	25.	Chromosomes may show chromomeres.

8.6 Summary

Cell division is a continuous and dynamic process that involves replication of DNA, karyokinesis and cytokinesis. Mitosis and meiosis are the two types of cell division. In mitosis somatic cells are divided in two daughter cells of equal size and containing equal number of chromosomes, while meiosis is a reductional cell division that takes place in germ cells. Cell cycle undergoes various phases like long interphase (time between the end of telophase and beginning of next phase); G_1-Phase (time between previous mitosis and beginning of DNA synthesis; S-Phase during which duplication of each chromosomes takes place; G_2-Phase, the gap between DNA synthesis and nuclear division and a short mitotic phase during which the already duplicated chromosomes are equally distributed to the diploid daughter cells. The cell cycle is controlled by various parameters like nucleo-cytoplasmic ratio; cyclic nucleotides; phosphorylation and the protein cyclin. Mitosis or the equational cell division involves various stages like prophase, metaphase, anaphase and telophase followed by cytokinesis. It is a vital process as it maintains the size, growth, chromosome number of the cell along with carrying out repairs, healing and regeneration and reproduction of cell. Meiosis involves two stages, meiosis-I and meiosis-II that takes place in rapid succession, with the chromosomes replicating only once. During meiosis-I two homologous chromosomes of each pair separate from each other and go to separate daughter cells, reducing the number of chromosomes from diploid to haploid condition. Its first stage is prophase-I that is further divided into 5 sub stages: i) Leptotene (during leptotene condensation of chromosomes takes place), ii) zygotene (during zygotene homologous

chromosomes pair and synaptonemal complex is formed. iii) pachytene is the third sub-stage in which two chromatids of synapsed chromosomes becomes visible and is known as dyad. Recombination also takes place during this stage. iv) At the stage of diplotene disjunction at many points takes place on homologous chromosomes. v) Diakinesis: During diakinesis terminalization takes place. Meiosis-II is similar to mitotic division in which two chromatids of each chromosome separate from each other and go to separate daughter cells. Various stages involved are prophase-II, metaphase-II, anaphase-II and telophase-II. At the end of the cell division cytoplasm divides at its middle by constriction in an animal cell and by cell plate formation in a plant cell. This process is called cytokinesis.

8.7 Glossary

Karyokinesis: It is the division of nucleus during cell cycle.

Cytokinesis: Division of cytoplasm that separates the daughter cells following division of parent cells.

Genome: Genome is defined as complete set of gene or genetic material present in a cell.

Spindles: A protein structure that divides the genetic material in a cell. The spindle is necessary to equally divide the chromosomes in a parental cell into two daughter cells during both types of nuclear division: mitosis and meiosis.

Asters: Asters are star like cellular structures, formed around each centrosome during mitosis in an animal cell. Astral rays, composed of microtubules, radiate from the centrospheres.

Meristematic tissues: A meristematic tissue in most plants contains undifferentiated cells and is found in zones of the plant where growth can take place.

Gonads: The organs that produces gametes (sperms and ovum); i.e. testis or ovary.

Nucleosomes: In eukaryotic cells the chromosomes consisting of a length of DNA are coiled around a core of histones. This structural unit is called nucleosome.

Cytoskeleton: It is a microscopic network of protein filaments and tubules in the cytoplasm of many living cells.

Somatic cell: The cells of a living organism other than the reproductive cells are known as somatic cells.

Germ cell: These are haploid cells that have the capacity to unite with the germ cell of the opposite sex and reproduce new individual. These are also called gametes.

Kinetochore: It is a protein structure present on chromosomes and is a by which they are attached to spindle fibers.

Spores: It is a minute, one-celled, reproductive unit that is capable of giving rise to a new individual without sexual fusion and is the characteristic of protozoans, fungi and lower plants.

Bivalent: A pair of homologous chromosomes.

Chiasmata: During the first metaphase of meiosis, chromosomes remain in contact at certain points at which crossing over and exchange of genetic material occur between the strands. These points are called chiasmata.

8.8 Self Assessment Questions and Possible Answers

8.8.1 Multiple Choice Questions

1. The proper sequence of cell cycle is:

   (a) S, M, G1, G2 (b) M, G1, G2, S

   (c) S, G1, G2, M (d) G1, S, G2, M

2. Karyokinesis refers to the division of:

   (a) Cytoplasm into two (b) Nucleus into two

   (c) Protoplasm into two (d) None of them

3. The spindle fibers attach chromosomes with:

   (a) Chromo center (b) Centriole

   (c) Kinetochore (d) Telocentric

4. Who proposed the term mitosis?

   (a) Farmer and Moore (b) Flemming

   (c) Nigeli (d) Brown

5. Chromosomes reach equator during cell division at:

   (a) Prophase (b) Metaphase

   (c) Anaphase (d) Telophase

6. Mitosis occurs in:

   (a) Roots (b) Shoots

   (c) Germ cells (d) Somatic cells

7. Nuclear membrane disappears at which stage:

   (a) Metaphase (b) Anaphase

   (c) Early prophase (d) Late prophase

8. Chromosomes move towards different poles, during cell division, due to:

   (a) Centrioles (b) Vacuoles

   (c) Cytokinesis (d) Microtubules

9. In cell cycle DNA replication takes place in:

(a) M-phase (b) S-phase

(c) G1 -phase (d) G2-phase

10. Anaphase of mitosis differs from metaphase in:

(a) Half the number of chromosomes

(b) Half the number of chromatids in each chromosome

(c) Half the number of chromosomes but doubles the number of chromatids in each chromosome

(d) Half the number of chromosomes and half the number of chromatids in each chromosome.

11. Synaptonemal complex is associated with:

(a) Mitotic chromosomes (b) Paired meiotic chromosomes

(c) Lampbrush chromosomes (d) polytene chromosomes

12. The term meiosis was coined by:

(a) Leeuwenhoek (b) Beadle and Tatum

(c) Hooke and Brown (d) Farmer and Moore

13. During meiosis exchange of paternal and maternal chromosomes is called:

(a) Recombination (b) Linkage

(c) Segregation (d) Crossing over

14. Crossing over and unzipping of homologous chromosomes in meiosis occurs at:

(a) Diplotene (b) Pachytene

(c) Zygotene (d) Leptotene

15. Synapsis occurs during:

(a) Leptotene (b) Zygotene

(c) Pachytene (d) Diplotene

16. Crossing over occurs at:

(a) One stranded stage (b) Two stranded stage

(c) Three stranded stage (d) four stranded stage

17. Advantage of crossing over is that it causes:

(a) Linkage (b) Stability

(c) Inbreeding (d) Variation

18. At the end of first meiotic division, number of chromosomes is:

(a) Halved (b) Doubled

(c) Remains same (d) tripled

19. Second meiotic division results:

(a) Separation of homologous chromosomes

(b) Separation of chromatids and centromeres

(c) Synthesis of fresh DNA

(d) Separation of sex chromosomes

20. Anaphase in second meiotic division is characterized by:

(a) Separation of non-homologous chromosomes

(b) Separation of homologous chromosomes

(c) Separation of chromatids

(d) All of them

8.8.2 Very short questions

1. In which period of interphase DNA duplicates?
2. What is G1 period?
3. In which cell mitosis occurs?
4. Who proposed the term mitosis?
5. What are different stages of mitosis?
6. Which stage of mitosis is of longest duration?
7. What is cytokinesis?
8. At which stage centrioles replicate?
9. In which cell meiotic divisions occur?
10. What are the various sub stages of meiotic prophase?
11. Who gave the term meiosis?
12. In which stage of meiosis, homologous chromosomes form pair?

ANSWERS

8.8.1

1.(d)

2.(b)

3.(b)

4.(b)

5.(b)

6.(d)

7.(c)

8.(d)

9.(b)

10.(b)

11.(b)

12.(d)

13.(d)

14.(a)

15.(b)

16.(d)

17.(d)

18.(a)

19.(b)

20.(c)

8.8.2 Answers

1. S-phase (synthetic phase).

2. Period between end of mitosis and start of DNA synthesis.

3. Somatic cells.

4. W. Flemming in 1882.

5. Prophase, metaphase, anaphase, telophase.

6. Prophase.

7. Division of cytoplasm.

8. Interphase.

9. Gonadian cells (spermatozoa and ovum).

10. Leptonema, Zygonema, Pachynema, Diploma and diakinesis.

11. J. B. Farmer and J. E. Moore in 1905.

12. Zygotene or zygonema.

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8.10 Terminal and model questions

1. Explain in details cell cycle.
2. Describe the various phases involved in the mitotic division of an animal cell.
3. Elucidate the process of mitosis with neat and labeled diagram.
4. What is the significance of mitosis?
5. Give an account of meiotic type of cell division.
6. Describe the changes that occur in nucleus during meiosis.
7. Write about synaptonemal complex and chiasma formation.
8. Differentiate between the mitotic and meiotic division.

UNIT 9 STRUCTURE AND TYPES OF DNA

Contents

9.1 Objective

Study of this unit will let the students to:

- Structure, functions and type of DNA
- Watson and Crick's structural model of DNA
- Chemical composition of DNA
- Replication of DNA
- Recombinant DNA.

9.2 Introduction

Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) the principal **genetic materials** of living organisms are chemically called **nucleic acids**. Nucleic acid especially the DNA, a universal genetic material of most of the organisms, is having all the features required to be a good genetic materials. DNA is a macromolecule and is a helically twisted double chain of poly deoxyribonucleotides.

In **prokaryotes** it occurs in **nucleoid** and also as **plasmids**, both are **double stranded circular DNA**. In **Eukaryotes** most of the DNA is found in **chromatin of nucleus**. It is **linear**. Some small quantitative of DNA are found in **mitochondria and plastids** which is generally double stranded and circular RNA also acts as genetic material in majority of plant viruses.

Features of DNA to act as genetic material:

- Genetic material is able to **store information** used to control both the development and metabolic activities of cell
- It should be **chemically stable** so that it can be replicated accurately during cell division
- It should be **transmitted for generations**
- It should be able to undergo **mutations providing genetic variability** required for the evolution.

9.3 Structure of DNA

Nucleic acid (DNA or RNA) first called **nuclein** by a Swiss chemist **Friedreich Miescher** (1869) as he removed nuclei from pus cells and isolated DNA i.e., "nuclein" from it. Nucleic acid (DNA or RNA) are macromolecules composed of repeating sub unit called **nucleotides.**

Constitution of a nucleotide:

- A phosphate group
- A five carbon sugar (ribose in RNA and deoxyribose in DNA)
- A cyclic nitrogen containing compound called a base (purines and pyrimidines)

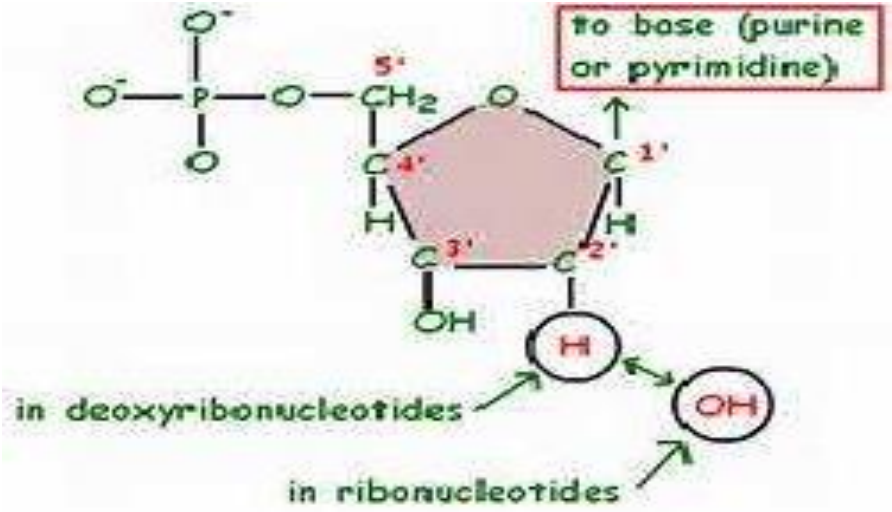


Fig. 9.1 Structure of a nucleotide (in general)

.Most commonly DNA occurs as a **double helix.** The two spiral strands of DNA are collectively called DNA duplex. Two separate and anti parallel chains of DNA are wound around each other in a **right handed helical manner**. The DNA double helix comes to have two types of alternate **grooves major** and **minor** with the sugar phosphate backbone on the outer sides. The bases paired by hydrogen bonding are stocked on each other.

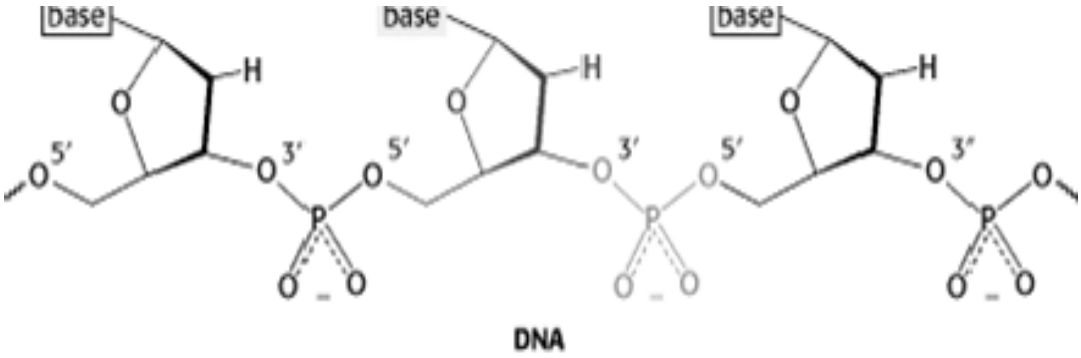


Fig. 9.2 Backbone of DNA. [The backbones are formed by 3 -to-5 phosphodiester linkages

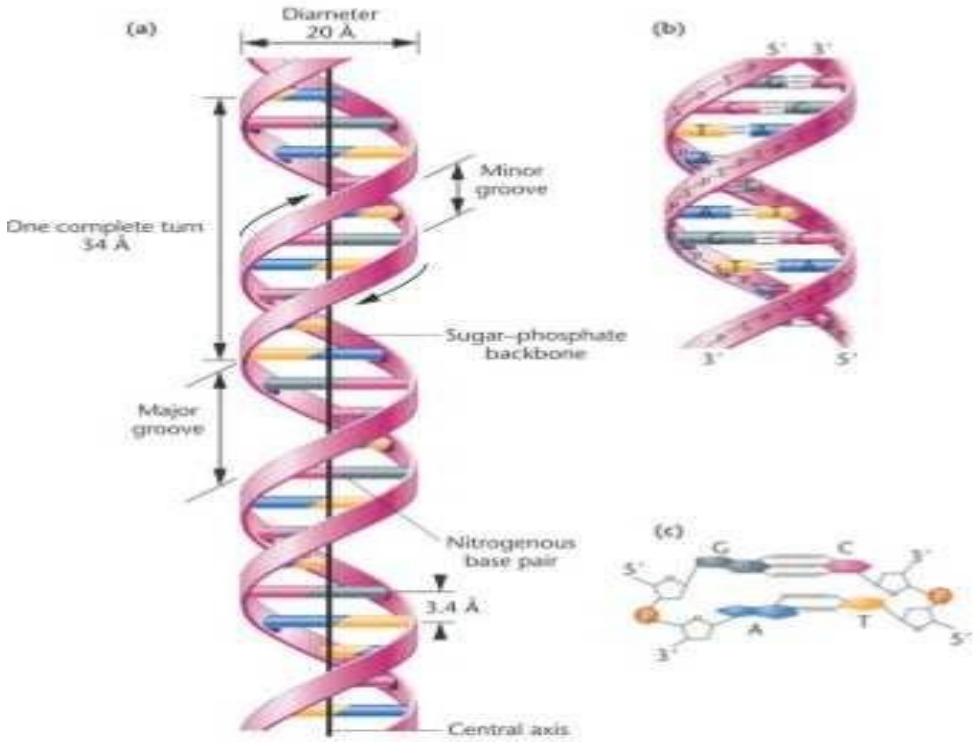


Fig. 9.3 DNA Double Helix Right Handed Helix Model

9.4 Chemical Composition of DNA

Deoxyribonucleotides (monomer) of DNA are composed by three different types of chemicals.

(1) **Phosphoric acid (H_3PO_4)** has three reactive (-OH) groups of which two are involved in forming sugar phosphate back bone of DNA.

(2) **Pentose sugar ($C_5H_{10}O_4$)** - DNA contains 2'-deoxy-D-ribose, hence the name deoxyribose.

(3) **Nitrogen bases**- DNA contained four different nitrogen bases (**A, G, C & T).** These four bases are grouped in to two classes on the basis their chemical structure.

(a) Purine base – Adenine and Guanine

(b) Pyrimidine bases- Cytocine and uracil

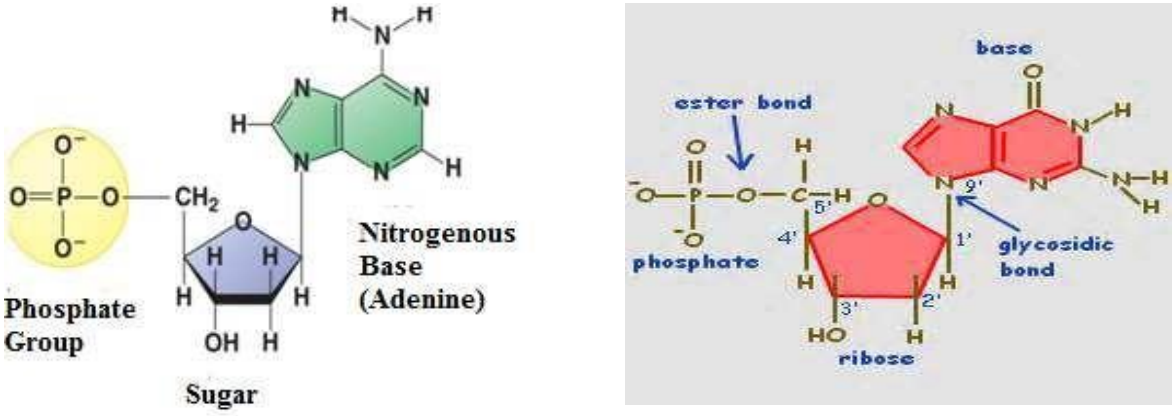


Fig. 9.4 Chemical constituents of a nucleotide

(a) Purine bases - DNA has two types of purines (**adenine and guanine**). Each purine is a type of nitrogen base having a **double ring structure** (i.e. 9 member double rings with nitrogen at 1, 3, 7 and 9 positions).
Some of the common names of these bases reflect the circumstances of their discovery. Guanine, for example, was first isolated from guano (bird manure), and thymine was first isolated from thymus tissue.

(b) Pyrimidine bases- DNA has two types of pyrimidine bases (**cytosine and thymine**). Each pyrimidine is a type of nitrogen containing base having a **single ring structure** (i.e. 6 member rings with nitrogen at 1 and 3 positions).

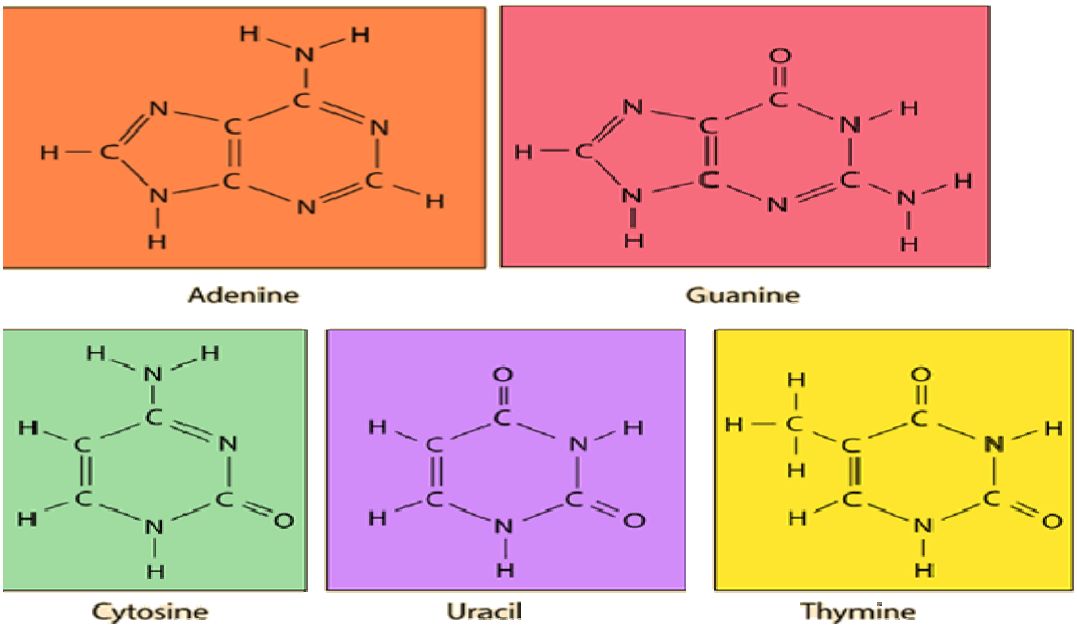


Fig. 9.5 Nitrogen bases of nucleic acids

(**A, G and C** is common to DNA and RNA, **U** is present in RNA and **T** in DNA)

Nucleosides- A nitrogenous base with a molecule of deoxyribose sugar (without phosphate group) is known as nucleosides. In nucleic acids, the nitrogen bases are covalently attached to the 1'-position of a pentose sugar ring with the help of glycosidic bond.

Nitrogen base + sugar = nucleoside.

- Adenine + deoxyribose = deoxyadenosine
- Guanine + deoxyribose = deoxyguanosine
- Cytosine + deoxyribose = deoxycytidine
- Thymine + deoxyribose = deoxythymidine

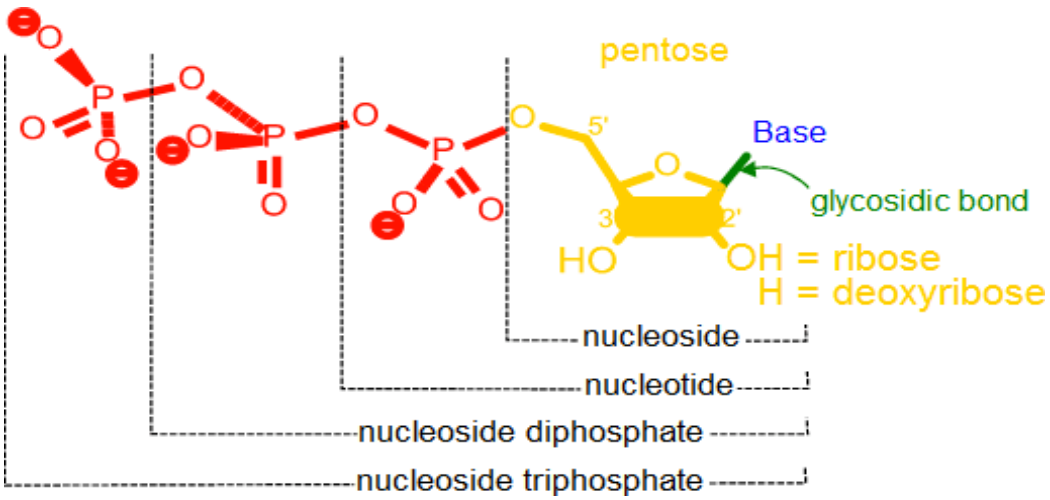


Fig. 9.6 Progressive formation of nucleoside to nucleotide (from lower to higher energy compounds)

Nucleotides- A nucleotide is formed of one molecule of deoxyribose sugar, one molecule of phosphoric acid and anyone of the nitrogen base. Phosphoric molecule is attached to the 5^{th} – carbon atom of deoxyribose ring with the help of phosphoesterbond.

Nucleosides + phosphoric acid = nucleotides

Different nucleotides of DNA are as follows:

(1)Adenine + deoxyribose + phosphoric acid = deoxyadenylic acid or deoxyadenylate / dAMP

(2) Guanine + deoxyribose + phosphoric acid = deoxyguanylic acid or deoxyguanylate / dGMP

(3)Cytosine + deoxyribose + phosphoric acid = deoxycytidylic acid or deoxycytidylate / dCMP

(4)Thymine+deoxyribose+phosphoric acid = deoxythymidylic acid or deoxythymidylate / dTMP

Nitrogen base	**Nucleoside (nitrogen base + sugar)**	**Nucleotide (nucleocide +phosphate gp.)**
Adenine (A)	A+S= Adenosine	Adenylic acid adenosine monophosphate (AMP)
Guanine (G)	G+S= Guanosine	Guanylic acid Guanosine monophosphate (GMP)
Thyamine (T)	T+S = Thyamidine	Thyamidylic acid Thyadine monophosphate (TMP)
Cytosine (C)	C+S = Cytidine	Cytidylic acid Cytidine monophosphate (CMP)

Table- 1 Nitrogen bases, their respective nucleosides and nucleotides of DNA

9.5 Watson and Crick Double Helix Model of DNA

The structure of DNA was deduced by American **J. D. Watson and F.H.C. Crick in** 1953 for which they received the Nobel Prize in 1962. Their double- helix model of DNA structure model is widely accepted. Their double helix model of DNA was based on the data and information given by so many workers like **E. Chargaff, M.H.F. Wilkins, R. Franklin and their coworkers.** Main contributions in deducing this model was of:

Chargaff's rule, Franklin's X-ray diffraction patterns and Kornberg's results

James Watson

Francis Crick

Rosalind Franklin

Maurice Wilkins.

Chargaff's rule- In 1940's **Erwin Chargaff** analyzed base content of DNA using new chemical techniques and their observations and generalizations were called as Chargaff's rule. Chargaff's rule strongly suggested that thymine and adenine as well as cytosine and guanine were present in DNA, always bonded to each other by H-bonds and shows some fixed inter relationship

- The proportion of A always equals that of T, and the proportion of G always equals that of C or **A = T and G = C.**
- The amount of A, T, G, and C in DNA vary from species to species but **A+T/G+C = constant for a particular species.**

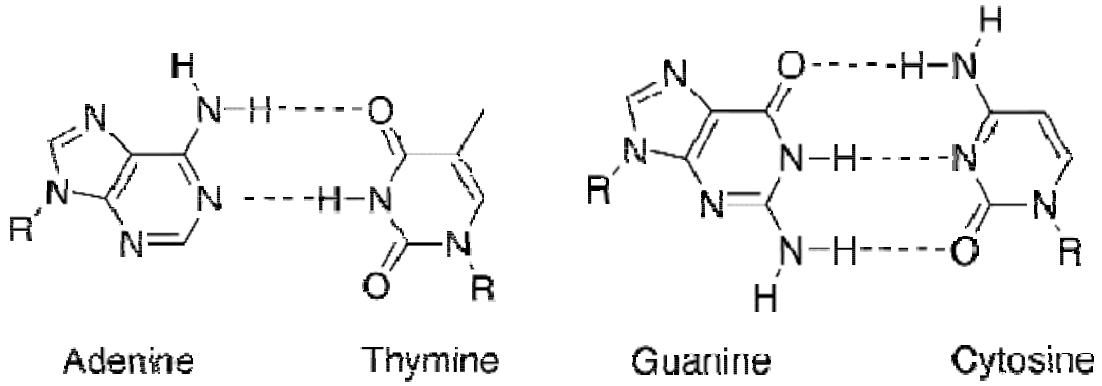


Fig. 9.7 Structures of the Base Pairs (Proposed by Watson and Crick)

Franklin's X-ray diffraction patterns- Watson and Crick made use of the data of x-ray crystallographic of DNA structure from the studies of **M.H.F. Wilkins, R. Franklin** and their coworkers. According to their data, DNA was a highly ordered, multiple stranded structure with repeating sub structure spaced every 3.4A^{o} along the axis of the molecule.

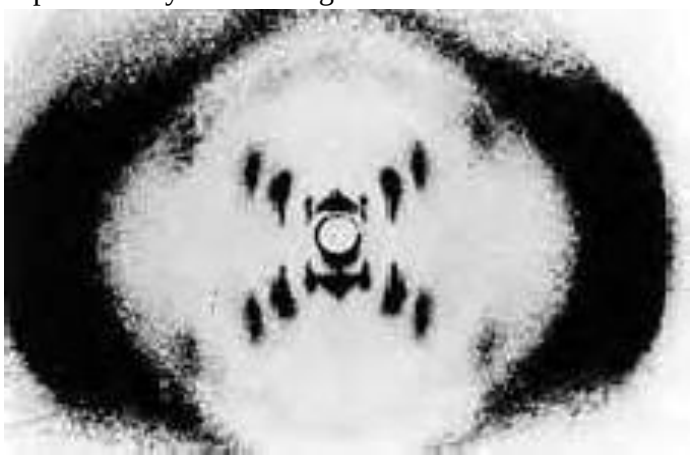


Fig. 9.8 X-Ray Diffraction Photograph of a Hydrated DNA Fiber
The central cross is diagnostic of a helical structure

Korenberg's results- Korenberg and his associates tried to synthesize DNA in a medium free of DNA but in the presence of enzyme **DNA polymerase** and nucleotides-the building blocks of DNA. They found that in a DNA free medium with all necessary compounds DNA synthesis does not occur but the same happens i.e., DNA synthesis starts only when some DNA was added as a primer to the same medium.

The important features of their model of DNA are-

(a) Two helical polynucleotide chains are coiled around common axis, where the backbone is constituted by sugar phosphate and the bases project inside.

(b) The polynucleotide chains run in opposite directions. It means, if one chain has the polarity 5'P → 3'OH, the other has 3'OH→5'P.

(c) The two chains are he d together by hydrogen bonds between their bases. Three hydrogen bonds occur between cytosine and guanine (C≡G) and two hydrogen bonds between adenine and thymine (A=T).

(d) The diameter of the helix is $20A^0$ and bases are separated by 3.4 A^0 along the helix axis and related by a rotation of 36^0.

(e) The helical structure repeated after 10 residues on each chain, and intervals of 34 A^0 .

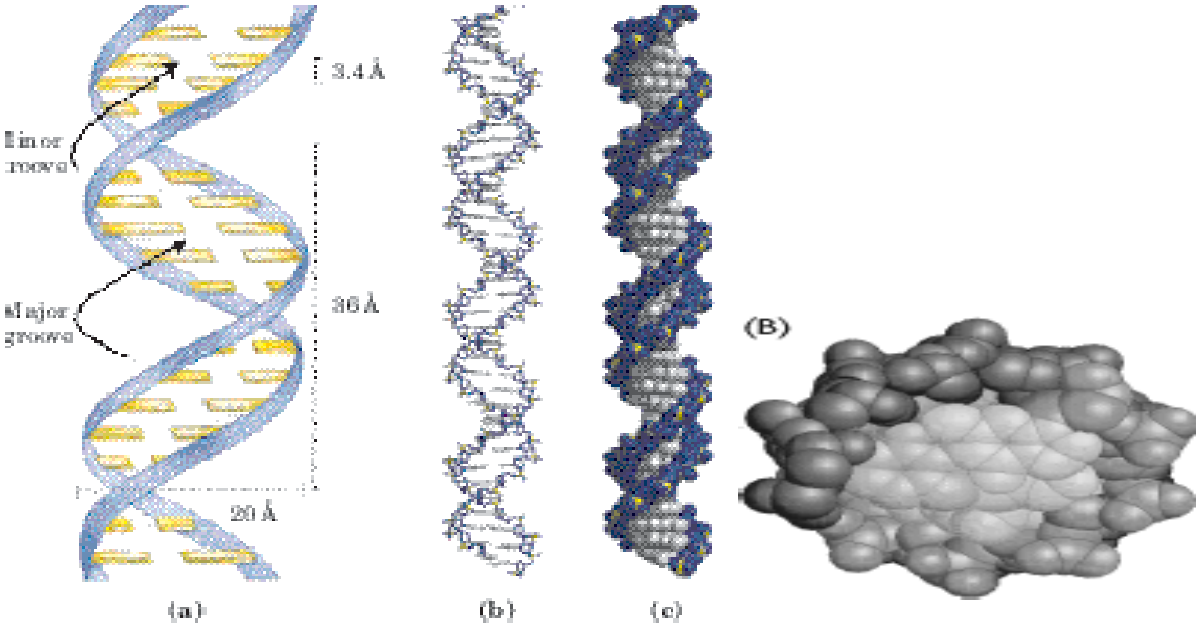


Fig. 9.9.Watson-Crick model for the structure of DNA. The original model proposed by Watson and Crick had 10 base pairs, or 34 Å (3.4 nm), per turn of the helix; subsequent measurements revealed 10.5 base pairs, or 36 Å (3.6 nm), per turn. ***(a)*** *Schematic representation, showing dimensions of the helix.* ***(b)*** *Stick representation showing the backbone and stacking of the bases.* ***(c)*** *Space-filling model* ***(B)*** *Radial view, looking down the helix axis*

9.6 Types of DNA

The vast majority of the DNA molecules present in the aqueous protoplasm of living cells almost certainly exist in the Watson – Crick double helix form is the B-form of DNA. B-DNA shows right handed coiling. Intracellular B-DNA appears to have an average of 10.4 nucleotide pairs per turn.In high concentration of salt or in a dehydrated state, DNA exits in the A-form. A- DNA is also a right handed helix and contains 11 base pairs per turn. Recently DNA sequences have been shown to exist in a unique left handed structure also called double helical Z-DNA. It contains 12 base pairs per turn. In Z-DNA, the sugar-phosphate backbone follows a zigzagged path giving it the name Z-DNA or Z-form. The helices of A and B form DNA are wound in a right handed manner. Specific segments of DNA molecules can undergo conformational shift from the B-form to the Z-form and vice-versa. These changes may be brought about by some specific regulatory proteins. The Z-form DNA is postulated to play a role in gene regulation.

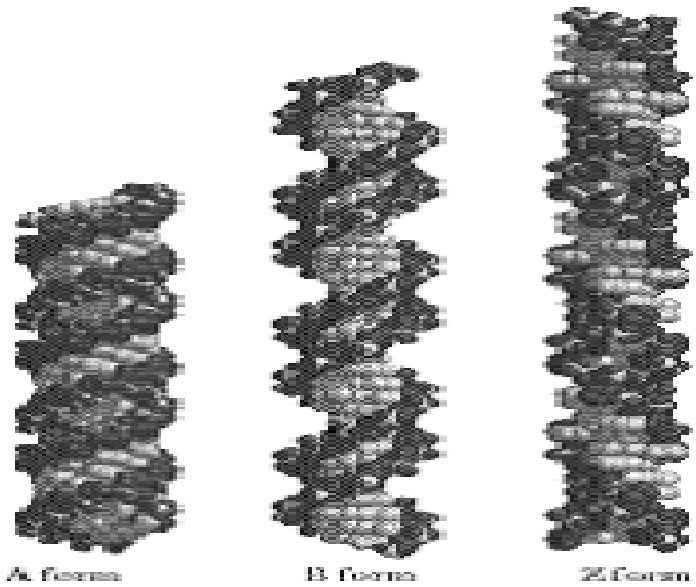


Figure 9.10 Comparison of A, B, and Z forms of DNA, each structure shown here has 36 base pairs.

Features	A-DNA	B-DNA	C-DNA	Z-DNA
Helical sense	Right handed	Right handed	Right handed	Left handed
Diameter (nm)	-2.6nm	-2.0nm	-	-1.8nm
Base-pairs per helical turn (n)	11	10	10	12 (6 dimers)
Helical twist per bp (360/n)	33^0	36^0	39^0	60^0 (per dimer)
Helix rise per bp (nm)	0.26nm	0.34nm	-	0.37nm
Base tilt to helix axis	20^0	6^0	-	7^0
Major groove	Narrow/deep	Wide/deep	-	Flat
Minor groove	Wide/shallow	Narrow/deep	-	Narrow/deep
Helix pitch (nm)	2.8nm	3.4nm	-	4.5nm
Condition	75% relative humidity, Na^+ K^+, Cs^+ ions.	92% relative humidity, low ionic strength	66% relative humidity, Li^+ ions	Very light salt concentrations

Table- 2 Comparison of different type of DNA

There are certain other forms of DNA such as D-form and E-form, both of which are found as rare extreme variants and contain only 8 and 7.5 base pairs per turn respectively.

9.7 Function of DNA

1. DNA is genetic material which able to store information used to control both the development and metabolic activities of cells.
2. DNA can be replicated accurately during cell division and transmitted for generations.
3. Crossing over during meiosis produces natural recombination of DNA which is passed on to next generation to produce variants in all sexually reproducing organisms.
4. DNA able to undergo mutations providing genetic variability required for evolution.

5. Differentiation of various body parts is due to differential functioning of specific parts of DNA.
6. Developmental stages occur in the life cycle of an organism by an internal clock of DNA functioning.

9.7.1 Evidence for DNA is Genetic Material

9.7.1 (a) Griffith's experiment on bacteria

The **transformation** was first studied by a British doctor S. F. Griffith (1928). Griffith observed that ***Diplococcus pneumonia*** known as ***Pneumococcus*** has two strains

(a) **Virulent or S-III- or smooth or capsulated type**-in which mucous coat produce shiny colonies and cause pneumonia

(b) **Non Virulent or R-II- or rough or non-capsulated** – in which mucous coat is absent and do not cause pneumonia.

Summary of Griffith's experiments on transformation

a) Smooth type bacteria were injected into mice. The mice died as a result of pneumonia caused by virulent.
b) Rough type bacteria were injected in to mice. The mice lived and pneumonia was not produced.
c) Smooth type bacteria which cause disease were heat killed and then injected in to the mice. The mice lived and pneumonia was not caused.
d) Rough type bacteria and smooth type heat killed bacteria were injected together in to mice. The mice died due to pneumonia and virulent smooth type living bacteria could also be recovered from their bodies.

The occurrence of living S-type virulent bacteria is possible only by their transformation from R-type or non virulent bacteria which pick up the trait of virulent from dead or heat killed S-type bacteria. The phenomenon is called **Griffith effect or transformation**.

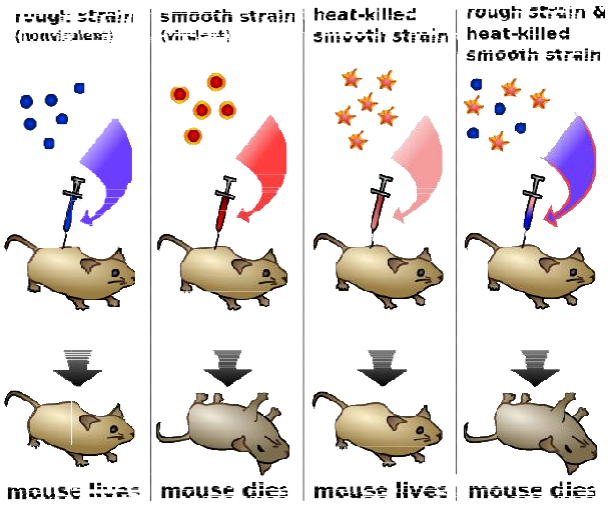


Fig. 9.11 Diagrammatic representation of Griffith's effect of transformation

But Griffith effect or experiment can't prove the following points:

- ✓ Whether or not mice were essential for transformation of R-type into S-type
- ✓ Whether the character of virulence belong to polysaccharide of mucilage, protein or DNA of S-type bacteria that resulted in the transformation

9.7.1 (b) Avery, Macleod and Mc Carty Experiment

In 1940, **Avery, Macleod and Mc Carty** did various experiments to show and prove DNA to be transforming agents in Griffith's observations. They showed that if highly purified DNA from type III S Pneumococci was present with type II R Pneumococci, some of the types 11 R Pneumococci were transferred to type III S. This is known **as transforming principle**. Finally the results obtained by Avery and coworkers clearly established that the genetic information in pneumococcus was present in DNA.

Summary of Avery, Macleod, and Mc Carty's experiment-

1. Type II R → II R colonies.
   &
   DNA extract type III S heat killed →no colonies.
2. Type II + DNA extract type III S heat killed + serum that precipitates II R cells from mixture → III S colonies.
3. Type II + DNA extract type III S heat killed + serum that precipitate II R cells from mixture +RNase→ III S colonies.
4. Type II + DNA extract type III S heat killed + serum that precipitates II R cells from mixture + protease→ III S colonies.

5. Type II + DNA extract type III S heat killed + serum that precipitates II R cells from mixture + DNAase → no colonies

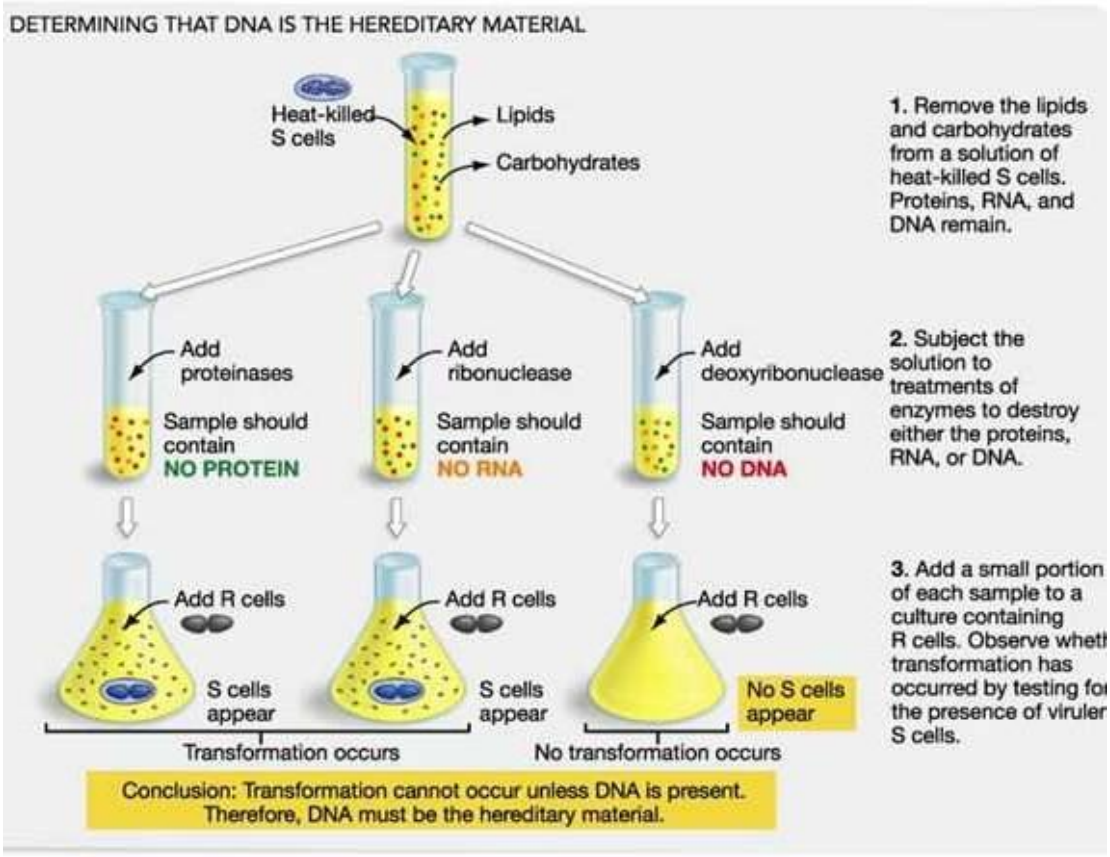


Figure 9.12 The Avery-MacLeod-McCarty experiments

9.7.1 (c) Hershey and Chase Experiment

Hershey and Chase in 1950 conducted an experiment with phage T_2 inside the common bacterium ***Escherichia coli*** and proved that the DNA is the genetic material in bacteriophage T_2. His experiment goes as follows:

- Escherichia coli cells were infected with P^{32} labeled phage (DNA labeled) and after being allowed time for infection, they were agitated in a blender which sheared off the phage coats.
- The phage coats and the infected cells were then separated by centrifugation. Radioactivity was measured in the cell pellet and in the phage coat suspension.
- Most of the radioactivity was found in the cells.
- The same experiment was repeated using phage with S^{35} (labeled proteins) and found that the results were very different.

- The bacterial cells showed the presence of radioactive DNA labeled with P^{32} while radioactive protein labeled with S^{35} appeared on the outside of bacteria cells.
- Labeled DNA was also found in the next generation of phage. This experiment showed that only DNA enters the bacterial host and not the protein which helps in phage multiplication.
- This provided the unequivocal proof that DNA is the genetic material.

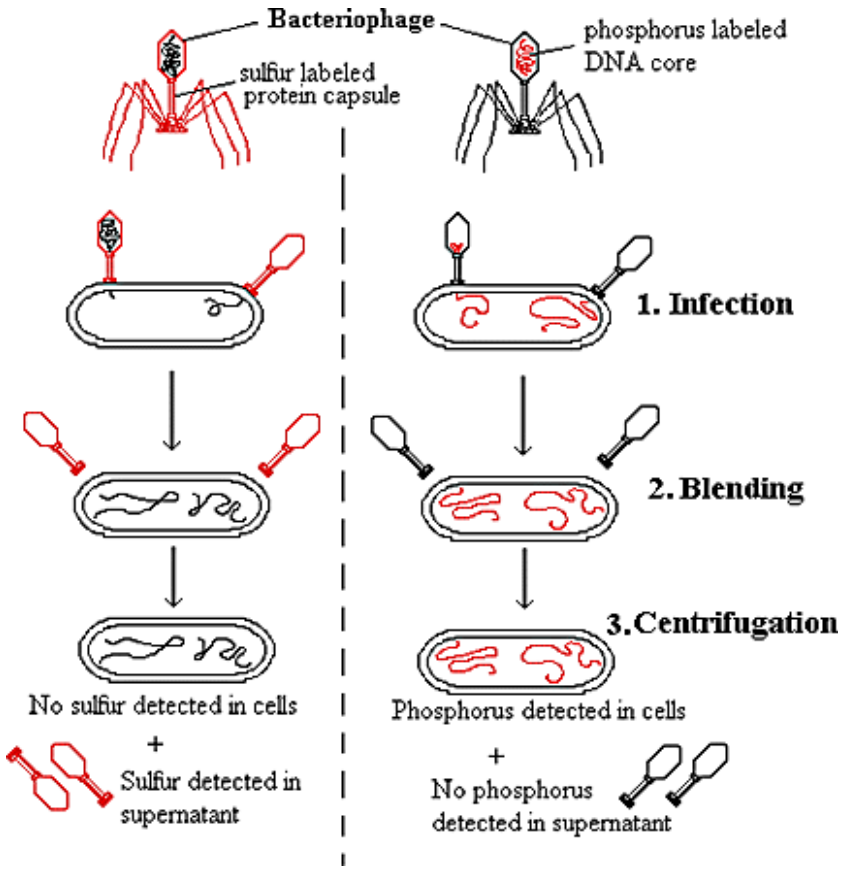


Fig. 9.13 Hershey and Chase Experiment

9.8 Replication of DNA

Replication is the process of formation of carbon copies on DNA. DNA functions as its own template. DNA replication is an autocatalytic function of DNA. During DNA replication the weak hydrogen bonds between nitrogen bases of the nucleotides separate so that the two polynucleotide chains of DNA separate and uncoil. The chains thus separated are complementary to one another. Each stand acts as a **template** and makes its own complimentary copy over it so that the new formed DNA duplex has **one parental stand and one newly formed strand.** This method of formation of new daughter DNA molecules is called **semi-conservative method of replication.**

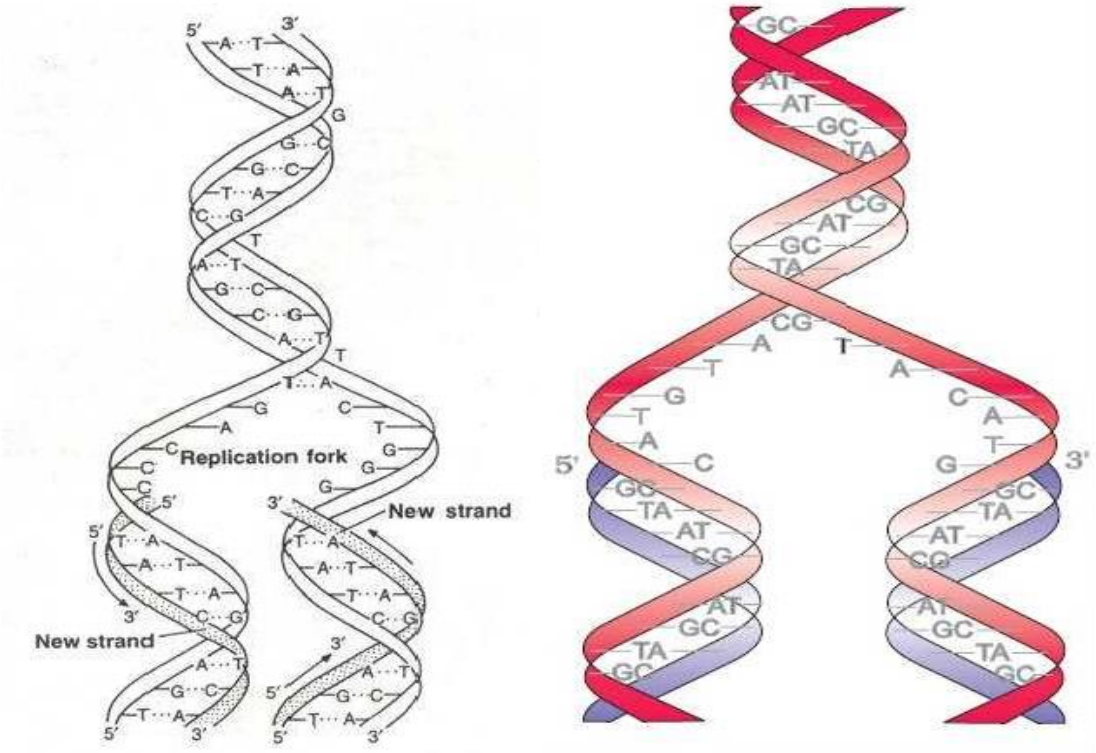


*Fig. 9.14 Replication of DNA as suggested by **Watson and Crick**.*
[The parent strands become separated; each is the template for biosynthesis of a complementary "daughter" strand]

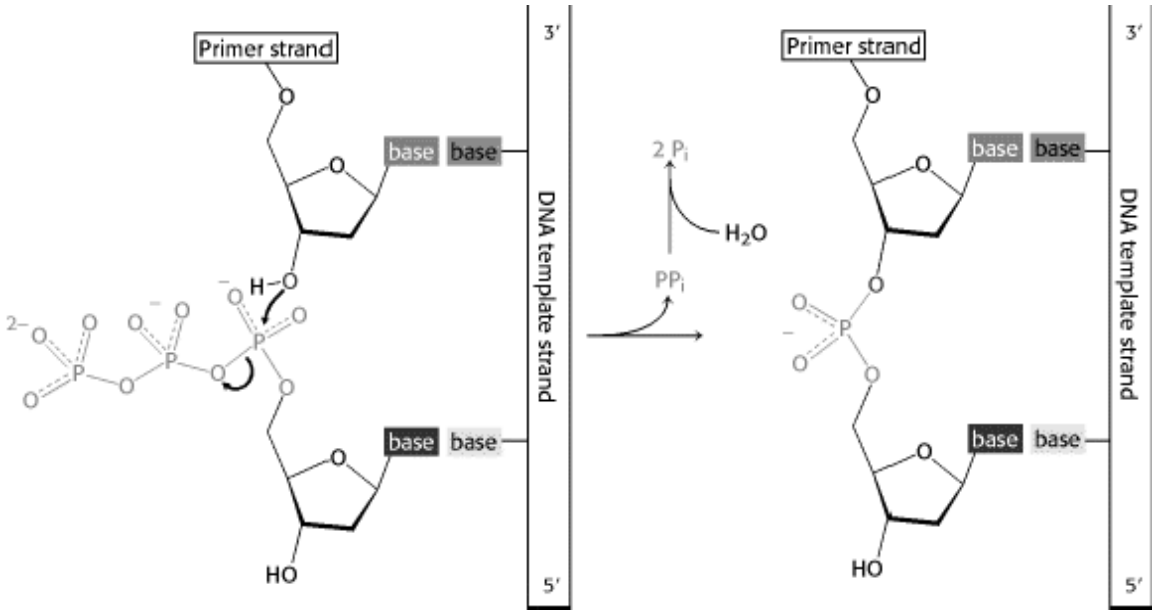
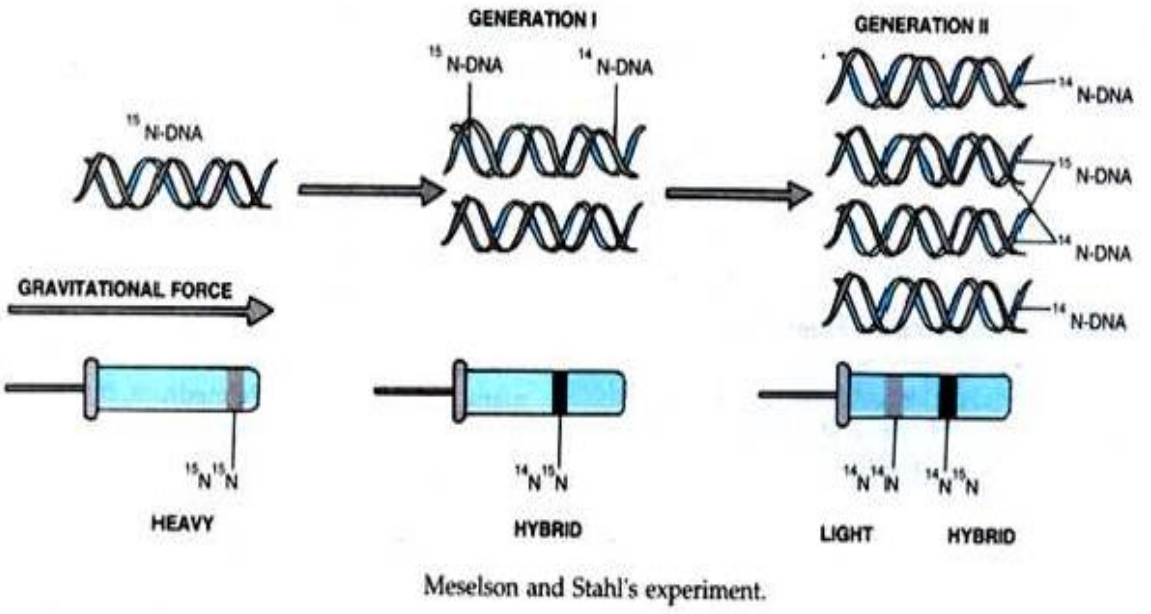


Fig. 9.15 DNA Replication (Phosphodiester Bridge is catalyzed by DNA polymerases]

9.8.1 Experiment to prove semi-conservative mode of DNA duplication

The Meselson- Stahl Experiment- The result of the first critical test of Watson and Crick's proposal that DNA replicates semi conservatively were published in 1958 by M.S. Meselson and F.W. Stahl. Their experiment was as follows:

- They grew Escherichia coli for many generations in a medium having heavy isotopes of nitrogen, N^{15} till the bacterial DNA becomes completely labeled with heavy isotope.
- The labeled bacteria were then shifted to fresh medium having normal or N^{14}.
- After each cell division DNA was separated from a sample of the cells and analyzed on a CsCl (cesium chloride) gradient using the technique of equilibrium density gradient centrifugation, which separates molecules according to differences in buoyant density.
- Meselson and Stahl found that DNA of the first generation was hybrid or intermediate between N^{15} and N^{14}.
- The second generation of bacteria contained two types of DNA, 50% light and 50% hybrid.
- There were exactly the results to be expected if DNA replication is semi conservative



Meselson and Stahl's experiment.

Fig. 9.16 Diagram of Semi-conservative Replication.
[After M. Meselson and F. W. Stahl. Proc. Natl. Acad. Sci. U.S.A. 44(1958):671.]

9.8.2 Mechanism of DNA Replication

DNA replication is the process of copying a DNA molecule and involves following four major steps-

1. Initiation of DNA replication
2. Unwinding of helix
3. Formation of primer strand
4. Elongation of new strand.

1. Initiation of DNA replication- Replication is regulated by the rate of initiation. Replication of DNA in E. coli always begins at a definite site called **origin of replication**. The ***E. coli,*** origin of replication lies within the genetic locus **'ori'** and is bond to the cell membrane. 'Ori' contains four 9bp binding sites for the initiator protein (DnaA-ATP). The helicase DnaB (or mobile promoter) binds and extends the single-stranded region for copying.

2. Unwinding of helix- Unwinding of DNA molecule into two strands results in the formation of Y shaped structure called **replication fork.** Due to unwinding positive super coiling has to be relieved by the **enzyme topoisomerase or DNA Gyrase**.

3. Formation of Primer strand- As the newly formed replication fork displaces the parental lagging strand, a mobile complex called a **primosome**, which includes the DnaB, Helicase and DNA primase help in the synthesizes **of RNA primers**. Both leading and lagging strand primers are elongated by **DNA polymerase III.** Need of primer is there to facilitate the action of DNA polymerase III as this enzyme cannot initiate the process but can add activated deoxyribonucleotides to the 3' OH end of primer.

4. Elongation of new strand – after the formation of primer strand, DNA replication occurs in 5'→3'direction and complementary deoxyribonucleotides are added only to the free 3'OH end of the primer. A dimer of DNA polymerase III elongates both leading (3'→5') and lagging strands. The leading strand shows continuous replication while the lagging strand shows discontinuous replication. These short pieces of DNA replicated against lagging strand are known as **Okazaki fragments.** Okazaki fragments are 1000-2000 nucleotides long in prokaryotes. A separate RNA primer is used for the synthesis of each Okazaki fragments which, after replacing the RNA primers from deoxyribonucleotides, are later joined together with the help of **DNA ligase** or **DNA synthetase** forming a continuous lagging strand. Hence DNA replication is semi-discontinuous as the leading strand is synthesized continuously and the lagging strand is formed discontinuously in short pieces that join later.

Important features of Prokaryotic replication→

i. Bacteria have a single loop of DNA that must replicate before the cell divides.
ii. Replication proceeds in one direction from 5'→3'.

iii. Replication may be bidirectional or directional.
iv. One cycle of DNA replication gets completed in 40 minutes.
v. Prokaryotes are able to replicate their DNA at a rate of about 106 base pairs/min

Important features of Eukaryotic replication→

i. Replication starts at many points of origin and spreads with many replication bubbles. These bubbles are the places where the DNA strands are separating and replication is occurring.
ii. Replication forks are the V shaped ends of the replication bubbles.
iii. Eukaryotes replicate their DNA at slower 500-5000 base pairs per minutes.
iv. These cells can complete **DNA replication in one hour.**

9.9 Recombinant DNA

The tools and technologies of molecular biology **for breaking and rejoining DNA sequences** from two or more different organisms are known as DNA recombinant technologies. These modified DNA fragments are called recombinant DNA. A recombinant DNA molecule is a vector in which the desired DNA fragment has been inserted to enable its cloning in an appropriate host. This is achieved by using specific enzymes (**restriction enzymes**) for cutting the DNA into suitable fragments and then for joining together the appropriate fragments by ligation.

9.9.1 Steps of recombinant DNA technology

i. **Identification and isolation of the desired gene** or DNA fragment to be recombined with other DNA or cloned.
ii. **Insertion of the isolated gene in a suitable vector** (a vector is a plasmid- a small accessory ring of DNA in the cytoplasm of bacteria or virus which is used to transfer foreign genetic material in to a cell)
iii. **Introduction of recombinant DNA in to host**- ***E. Coli, Bacillus subtitles*** and **yeast** are used as hosts for the recombinant DNA. Three methods are used for introduction or recombinant DNA into the host.
    a. **Transformation**- it is the process by which a cell takes up naked DNA segment from the environment and incorporate it into its own chromosomal DNA.
    b. **Transduction**- it is the transfer of DNA from one organism to another through a bacteriophage.
    c. **Vector less gene transfer-** gene transfer can be affected by certain means that do not use vectors. It may be done by microinjection needles or gene gun or biolistic.
iv. **Multiplication /expression/integration** followed by expression of the introduced gene in the host.

9.9.2 Biological tools for RDT (Recombinant DNA technology)

Three biological tools are used for RDT-

A. Enzymes-

i. Lysing enzymes- lysozyme.

ii. Cleaving enzymes-
   a. exonucleases - ƛ exonucleases, exonuclease III
   b. Endonucleases
   c. Restriction endonucleases- EcoB, EcoK, EcoRI

iii. Synthesizing enzymes- reverse transcriptase

iv. Joining enzymes- ligases

v. Alkaline phosphateses

B. Vehicle DNA –
   a. plasmids – pBR322, pBR324
   b. Bacteriophage DNA- SV40, phase ƛ

C. Passenger DNA-
   a. complementary DNA
   b. synthetic DNA
   c. Random DNA.

9.10 Summary

Many lines of evidence show that DNA bears genetic information. In particular, the Avery-MacLeod-McCarty experiment showed that DNA isolated from one bacterial strain can enter and transforms the cells of another strain, endowing it with some of the inheritable characteristic of the donor. The Hershey-Chase experiment showed that the DNA and not its protein coat of a bacterial virus carries the genetic message for replication of the virus in a host cell.

Putting together much published data, Watson and Crick postulated that native DNA consists of two antiparallel chains in a right-handed double-helical arrangement. Complementary base pairs, A-T or A-U and G-C are formed by hydrogen bonding within the helix. Pairs are stacked perpendicular to the long axis of the double helix, 3.4 Å apart, with 10.5 base pairs per turn. DNA can exist in several structural forms. Two variations of the Watson-Crick form or B-DNA are A and Z-DNA. Some sequence dependent structural variations cause bends in the DNA molecule. DNA strands with appropriate sequences can form hairpin/cruciform structures or triplex or tetraplex DNA.

Replication is the process of formation of carbon copies. DNA functions as its own template. DNA replication is an autocatalytic function of DNA. This method of formation of new daughter DNA molecules is called semi-conservative method of replication.

The tools and technologies of molecular biology for breaking and rejoining DNA sequences from two or more different organisms are known as recombinant DNA technology. These modified DNA fragments are called recombinant DNA. This is achieved by using specific enzymes (restriction enzymes) for cutting the DNA into suitable fragments and then for joining together the appropriate fragments by ligation.

9.11 Glossary

DNA- Deoxyribonucleic acid, the information carrying genetic material that comprises the genes

Genetics- the science of heredity and variation

Gene- a hereditary determinant of a specific biological function, a unit of inheritance located in a fixed place on the chromosome.

Nucleic acid- a macromolecule composed of phosphoric acid, pentose sugar, and organic bases, DNA and RNA.

Nucleotide- a unit of DNA and RNA molecules containing a phosphate, a sugar, and an organic base

Replication- a duplication process that is accomplished by copying from a template

9.12 Self Assessment Question

1. DNA is acidic due to the presence of:

(a) Nitrogen bases (b) Sugar

(c) Phosphate group (d) double helix structure

2. DNA double helix model was proposed by

(a) Watson (b) Watson and Franklin

(c) Franklin and Crick (d) Watson and crick

3. Double Helix model of DNA was based on the observations of:

(a) Watson (b) Wilkins and Franklin

(c) Franklin and Crick (d) Watson and crick

4. DNA replication is:

(a) Dispersive (b) Conservative

(c) Non conservative (d) Semi conservative

5. DNA replication enzyme is:

(a) DNA Gyrase (b) DNA polymerase

(c) Restriction Endonuclease (d) all of these

6. Who proposed the concept of transformation?

(a) Hershey and Chase (b) Griffith

(c) Avery, Macleod, and Mc Carty's (d) none of these

7. Who proved chemical basis of transformation?

(a) Harshey and Chase (b) Griffith

(c) Avery, Macleod and Mc Carty's (d) Watson and Crick

8. Recombinant DNA technology is primarily based of the discovery of which enzyme?

(a) DNA Polymerase (b) DNA Ligase

(c) DNA Endonuclease (d)) DNA Restriction Endonuclease

9. Vector in RNA recombinant Technology helps in:

(a) Infecting host cell with bacteria (b) Transferring target DNA in host cell

(c) Transferring desired gene for recombination (d) transferring any type of DNA in host cell

10. When E.coli is cultured in N^{15}, for two cell cycles, how many DNA molecules of DNA after two cycles will have heavy N:

(a) All but 2 molecules will be pure heavy (b) All but no molecules will be pure heavy

(c)All DNA molecules will have N^{15} (d) 50% heavy and 50% light

ANSWER

9.12:-

1-c	5- b	9-b
2- d	6- b	10-a
3- d	7- c	
4- d	8-d	

9.12.1 Fill in the Blanks

1. Two types of nucleic acids differ from each other in ----- as well as ---- --.
2. DNA and RNA has similar --- but different ----.
3. Any types of DNA molecules will always follow -----rule, which states that total amount of --- are always equal to the total amount of ---- .
4. While proving the chemical responsible for transformation of bacteria, ---- enzyme was used to prove it as it could digest --- which was found responsible for causing transformation while other enzymes like ---, ---- and were found ineffective.
5. Endonuclease can cut DNA from ----- site but restriction Endonuclease at some sites also called as sequences.

9.12.1 Answer:

1. Sugar, nitrogen base
2. Purines, pyrimidines
3. Chargaff's , purines, pyrimidines
4. DNase, DNA, RNase, Lipase, protease
5. Any non specific, specific, Palindromic

9.14 Terminal Questions

A- Long answerer questions-

i) What is DNA? Explain their types and function.
ii) Write an essay on Watson and Crick structural model of DNA.
iii) Discuss the chemical composition of DNA.

B- Short answerer questions-

i) Differentiate between B-DNA & Z-DNA.
ii) What is recombinant DNA?
iii) What do you mean by replication of DNA?

C- Fill in the blanks-

i) Prokaryotic replication proceeds in…..direction from…….
ii) Most commonly DNA occurs as a …….helix.
iii) Replication is the process of formation of ……

ANSWER

9.14(C)

(i) One, 5'→3'

(ii) Double

(iii) Carbon copies.

9.13 References and Suggested Readings

i) Molecular biology-P.C. Turner, A.G. McLennen, A.D. Bates & M.R.H. white.

ii) Principles of Genetics- D.Peter Snustad, Michael J. Simmons.

iii) Hand Book of Life Science- Sunil Patel, Rukum. S. Tomer, Harsukin Gazera, B.A. Golakiya & Manoj Parakhia.

iv) Cell Biology, Genetics, Molecular Biology, Evolution & Ecology- P.S.Verma, V.K. Agarwal.

v) Lehninger- Principles of Biochemistry. 4th edition- David L. Nelson, Michael M. Cox.

vi) Color Atlas of Biochemistry-2nd edition – J. Koolman, K. H. Roehm

vii) Genetics- Benjamin A. Pierce.

viii) Genetics & Molecular Biology- 2nd edition.-Robert Schleif.

UNIT 10 STRUCTURE OF RNA

Contents

10.1 Objective

Study of this unit will let the students to:

- Structure of RNA
- Types of RNA: Transfer RNA, Messenger RNA, Ribosomal RNA
- Structure of : Transfer RNA, Messenger RNA, Ribosomal RNA
- Biosynthesis of RNA: Transfer RNA, Messenger RNA, Ribosomal RNA
- Function and importance of RNA: Transfer RNA, Messenger RNA, Ribosomal RNA

10.2 Introduction

RNA is the genetic material of some plants, animal and bacterial viruses. Except some viruses (e.g. reoviruses), most cellular RNA is single stranded called as a single chain poly – ribonucleotide. A variety of RNA molecules performing varied functions are found in the cell. rRNA constitute the ribosomes, tRNA helps in aligning amino acids against the mRNA, thus helps in decoding the genetic message of polypeptide formation while mRNA (messenger RNA) functions as carrier of coded genetic or hereditary information from DNA to cytoplasm for taking part in structural protein and functional proteins like enzyme. All types of RNA are transcribed from nuclear DNA except rRNA which is transcribed from nucleolus DNA. Inside the cytoplasm RNA molecules may occur freely as well as in association with the ribosomes. These are also found in mitochondria, chloroplasts and eukaryotic chromosomes. These are key intermediary molecule between DNA and polypeptide.

Chemically RNA differs from DNA in three ways-

- The sugar molecule found in RNA is ribose, rather than the deoxyribose of DNA.
- It is generally consists of only one polynucleotide strand or single stranded.
- Three nitrogen bases (A, G, C) in RNA are identical to those in DNA, the fourth base in RNA is Uracil (U), which is similar to thymine but lacks the methyl ($-CH_3$) group.

RNA is generally involved in protein synthesis but in majority of plant and some animal viruses it also acts as genetic material. There are two major types of RNA:

1. **Genetic RNA-** H. Fraenkel-Conrat showed that RNA present in **Tobacco Mosaic Virus** is its genetic material and this RNA is responsible for the infection in tobacco plant.

2. Non- genetic RNA- Prokaryotes and Eukaryotes where genetic information is contained in the DNA molecule, functions of such cells are performed by a different kind of nucleic acids called non- genetic ribonucleic acid. Non-genetic RNA is synthesized on DNA template. Such non genetic RNAs can be of many types like mRNA, r RNA, & t RNA.

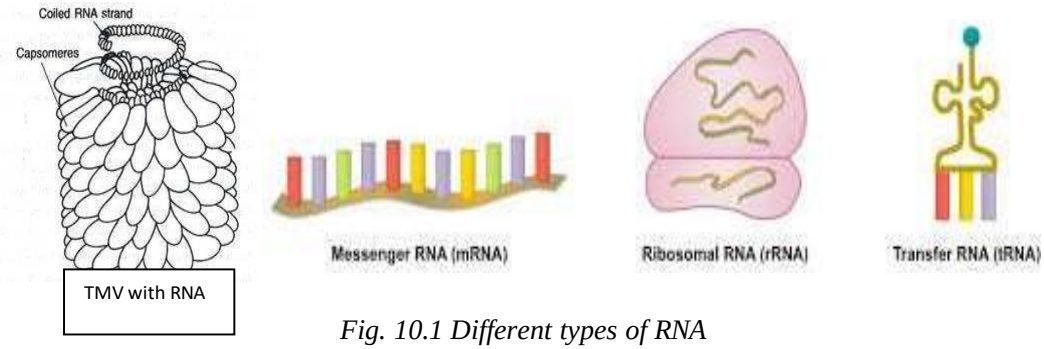


Fig. 10.1 Different types of RNA

10.3 Structure of RNA

RNA is single stranded polyribonucleotide. Each ribonucleotide is made of:

- Phosphoric acid- H_3PO_4
- Ribose sugar- $C_5H_{10}O_5$
- Nitrogen base- Adenine (A), Guanine (G), Cytocine (C) and Uracil (U)

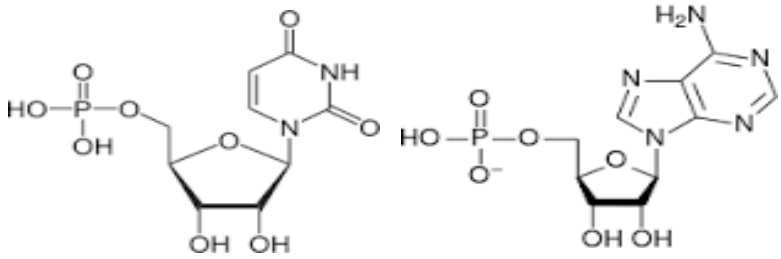


Fig. 10.2 Components of a ribonucleotide

Many ribonucleotides join with each other by phosphor-ester bonds to make a linear chain of polyribonucleotides. The chain will remain straight under all conditions in mRNA, may fold randomly in r-RNA or specifically to form t-RNA.

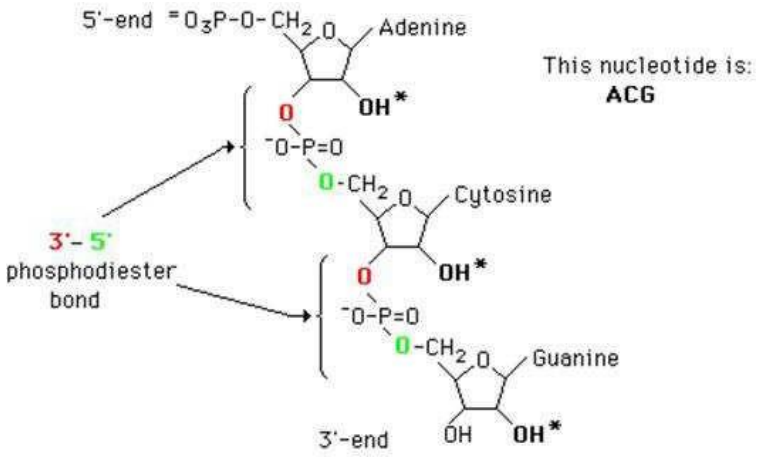


Fig. 10.3 A chain of ribonucleotides to form polyribonucleotides

10.4 Types of RNA

The RNA is of following three major types: t RNA, mRNA and r RNA

10.4.1 t-RNA or Transfer RNA

It is also called **soluble or s-RNA**. There are over 100 types of t-RNA. t-RNA is the smallest RNA with 70-85 nucleotides and sedimentation co-efficient of 4S. It is about 10-15% of the total weight of tRNA of the cell. Each tRNA has a corresponding **anticodon** that can recognize the codon on mRNA and exhibit high affinity for specific activated amino acids combine with them and carry them to the site of protein synthesis .

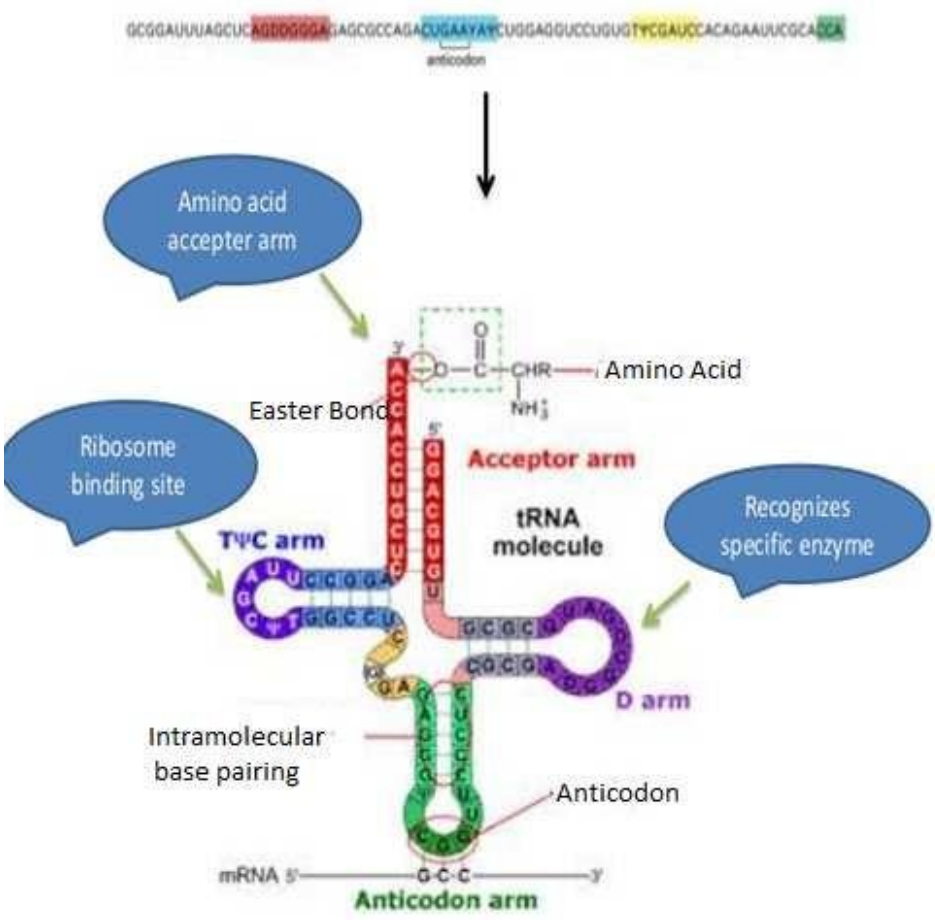


Fig. 10.4. t-RNA structure

STRUCTURE OF tRNA

Robert Holley (1965) and his colleagues reported the complete nucleotide sequence of alanine tRNA of yeast. R. Holley (1965) first of all proposed a **clover leaf model for yeast tRNA^{ala}**. The clover leaf model of tRNA was widely accepted because it explains several of the known functions of tRNA. Nucleotide sequences are now known for more than 100 different "species" of tRNA.. The number of nucleotide varies from 77 (tRNA alanine) to 207 (tRNA tyrosine). A tRNA molecule commonly has a guanine residue at its 5' terminal end. At its 3' end, unpaired-CCA sequence is present and this end acts as amino acid carrier end.

3-dimensional structure of tRNA- The three dimensional structure of this tRNA was proposed to be **L-shaped by Kim and Klug.** A. Klug, the noble laureate of 1982 has contributed much to the three dimensional structure of tRNA. He proposed L-shape model of tRNA molecule with thickness of 20 A^{o}. Each arm of the L doubled over by bonds holding complementary base together. L-shaped easily derived from 2D clover leaf model. S.H. Kim (1973) proposed a most

acceptable 3-D structure model of tRNA. Three dimensional structures were worked out by the help of X-ray crystallography study.

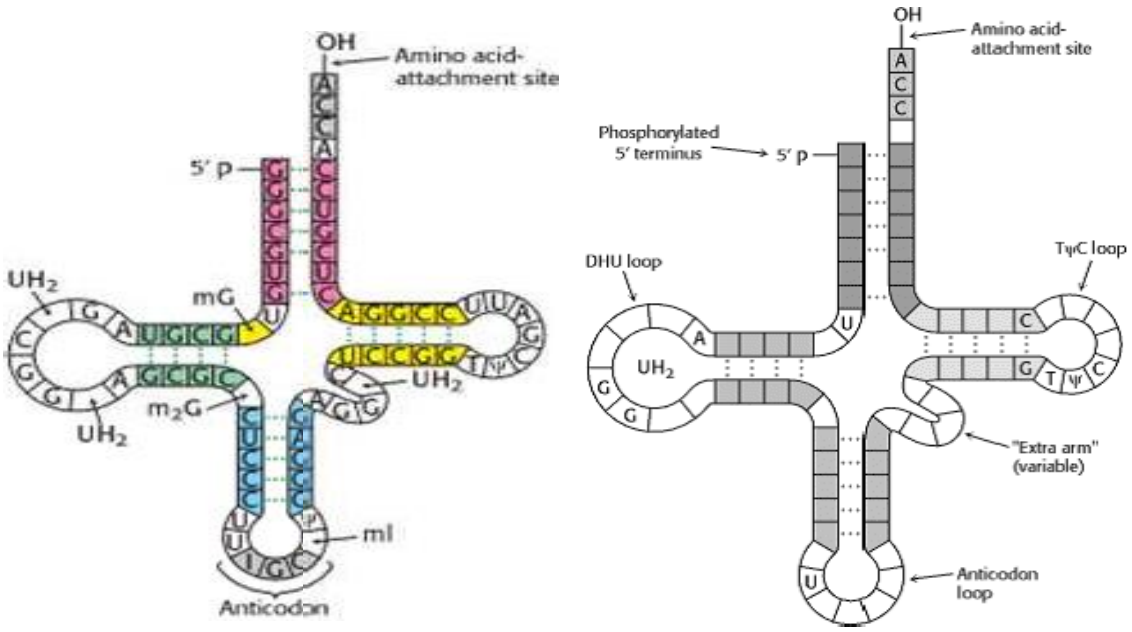


Fig. 10.5 -1 Alanine-tRNA Sequence- The base sequence of yeast alanyl-tRNA and the deduced cloverleaf secondary structure are shown.

[Modified nucleosides are abbreviated as follows: methylinosine (mI), dihydrouridine (UH2),ribothymidine (T), pseudouridine (□), methylguanosine (mG), and dimethylguanosine (m2G). Inosine (I), another modified nucleoside, is part of the anticodon.]

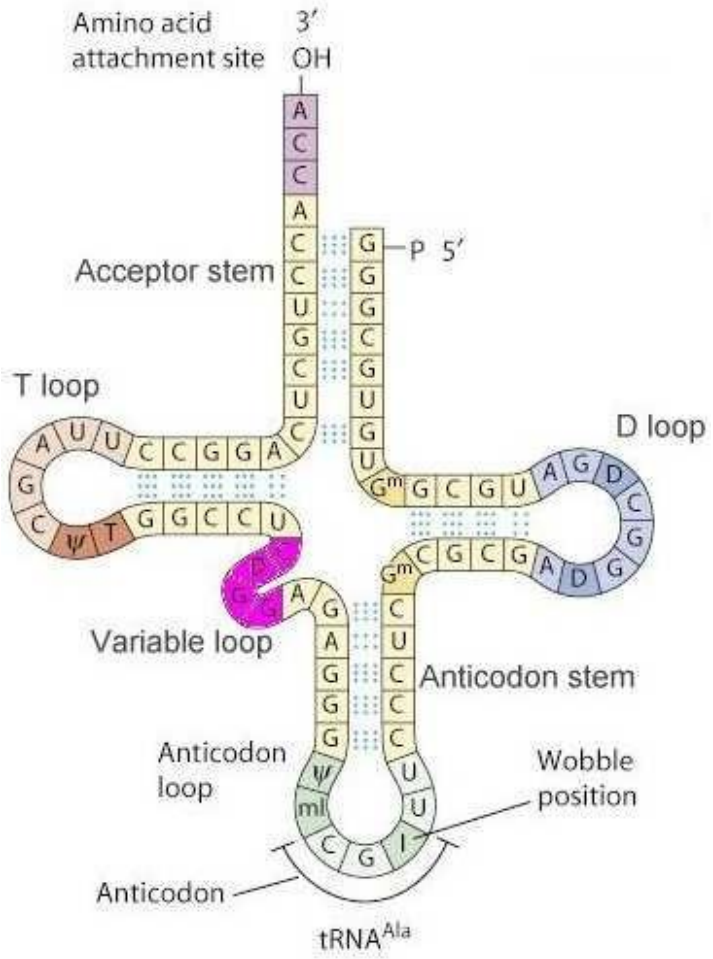


Fig. 10.5 -2 Schematic of t-RNA (t-RNA^{Alanine}) secondary structure.

Cloverleaf structure-. Five parts or arms of cloverleaf structure are:

a) Acceptor stem or arm - this is a region of the tRNA which acts as a site of attachment for the appropriate amino acid. It is also called **amino acid carrier arm**. It is formed by seven regular Watson & Crick base pairs between the 5' and 3' end of the tRNA. The **3' terminal end** of all tRNA is **always CCA-OH**. It is not base- paired and is the site of attachment of the amino acid. The amino acid is covalently bound through an ester linkage between the carboxyl group of the amino acid and the 3' hydroxyl group of the ribose of the tRNA.

b) Anti-codon loop or arm - The anti-codon loop contains **the three nucleotide sequence** that is complementary to the codon of mRNA to which it corresponds. It consists of a total of 7 unpaired bases, three of which constitute the anti codon. With this site **tRNA attaches to mRNA** and helps in the transport of amino acids to the site of protein synthesis

c) DHU loop or D loop or arm - The DHU loop is composed of three or four base pairs. It is depending on the species of tRNA. It is also variable in size containing 8 to 12 unpaired bases. The D-loop helps in binding of **amino-acyl synthetase**. It has modified bases called **dihydrouridine** hence named so.

d) T φC loop or arm- is named so because of the presence of triplet sequence **of pseudouridine (φ).** It acts as **ribosome recognize arm**, help in determining the site of ribosome (A, P or E site) where the tRNA has to come and attach during translation.

d) The extra arm- is variable in nucleotides composition and is lacking entirely in some tRNA.

10.4.2 m-RNA or Messenger RNA

Messenger RNA is a long unfolded RNA which constitutes 3-**5% of the total RNA content**. It brings instruction from the DNA for the information of particular type of polypeptide to be synthesized, having base sequence complementary to DNA at the sites of protein synthesis-the ribosomes, to which they become associated to participate in codon-anticodon interaction with tRNA. These are also called **informational or messenger or template RNAs** (mRNA). RNA is synthesized inside the nucleus as a complementary strand to DNA and serves to carry genetic information from chromosomal DNA to the cytoplasm for the synthesis of proteins. Out of the two strands of DNA only **template or noncoding or antisense strand transcribes mRNA**. The name, messenger RNA, has been proposed by Jacob and Monod (1961). It may constitute up to 10% of the total RNA present in the cell, when the cell is actively engaged in protein synthesis.

THE STRUCTURE OF mRNA

m-RNA is **always single stranded** having normal bases like A, G, U and C along with only a few unusual substituted bases. There is never base pairing in mRNA. It functions as a template for protein synthesis it carries genetic information from DNA to a ribosome and helps to assemble amino acids in their correct order. Each amino acid in a protein is specified by a set of three nucleotides in the mRNA called **codons.** Both prokaryotic and eukaryotic mRNA contains three primary regions:

a) 5' untranslated region (5'UTR) - the 5' untranslated region is a sequence of nucleotides at the 5' end of the mRNA that does not code for the amino acid sequence of a protein. In **prokaryotic** (bacterial cell) mRNA contains a consensus sequence called the **Shine-Dalgarno sequence** (5'AGGAGGU3'), which serves as **the ribosome binding site during translation**, it is formed of approximately 7 nucleotides upstream of the first or start codon. Eukaryotic mRNA has no such equivalent sequences in its 5' untranslated region. This is the sequence of the mRNA extending from the 5' end of the mRNA to the initiation codon. It is not translated into

polypeptide sequence. It has a **function analogous to the function of a promoter on a gene**. It will direct the binding of the ribosome to the initiation codon.

b) Protein coding region- this region comprises the codon that specify the amino acid sequence of the protein. This region **begins with a start codon and ends with a stop codon.** This region has 3 regions namely initiation codon, coding region, stop codon.

- **Initiation codon-** it is always **AUG** and codes for a **methionine.** This is the triplet codon at which polypeptide synthesis begins. All polypeptides are synthesized with an amino terminal methionine.
- **Coding region-**this is the sequence of mRNA that contains **the consecutive triplet codons** that direct polypeptide synthesis. This region starts from the start codon and continue up to the stop codon. The coding region is often referred to as the open reading frame or ORF.
- **Stop codon-**this is the triplet codon that signals the **termination of translation**. There are three possible stop codon sequences **UAA, UAG, UGA**. Stop codons have no corresponding tRNA or amino acid.

c) 3' Untranslated region (3'UTR)-This region of mRNA is the 3' un-translated region, a sequence of nucleotides at the 3'end of mRNA that is not translated into protein. This is the nucleotide sequence downstream from the stop codon. It extends from the stop codon to the 3' end of the mRNA. It does not code for amino acid sequence. It may function in stabilizing the mRNA. In eukaryotes it is transcribes as hnRNA which is converted into functional mRNA in the cytoplasm by removing introns (intervening sequences) and joining together exons (expressible sequences)

For the convenience the mRNA structure can be summarized as:

1. **Cap**- at 5' end, has methylated structure, does not translate
2. **Noncoding region-1**- has 10-100 nucleotides, rich in U and A bases, does not translate
3. **The inititation codon**- AUG, codes for methionine amino acid
4. **The coding region**-about 1500 nucleotides on an average, translate protiens
5. **Termination codon**- either of UAA, UAG or UGA ie present, helps in termination of translation
6. **Noncoding region-2**- made of 50-150 nucleotides, does not translate, has sequence like AAUAAA
7. **Poly(A) sequence**- 200-250 A nucleotides, does not translate, makes tail of mRNA

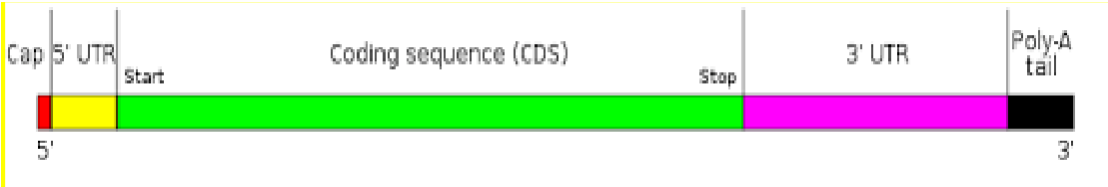


Fig. 10.6 mRNA showing different regions

10.4.3 r-RNA or RIBOSOMES RNA

Ribosomal, stable or insoluble RNA constitutes the largest part (up to 80%) of the total cellular RNA. It was reported by **Kuntz.** It is found primarily in the cytoplasm as well as organelle. In prokaryotes it is transcribed from ribosomal DNA which is a part of nuclear DNA but in eukaryotes ribosome is formed on nucleolar DNA. The genetic instruction contained in mRNA is translated into the amino acid sequences of polypeptides only with the help of ribosomes. Thus ribosomes play an integral part in the transfer of genetic information from genotype to phenotype. R-RNA is most stable type of RNA.

Structure and processing of ribosome RNA- it forms about 80% of the total cellular RNA. r-RNA consists of a single stranded RNA which gets twisted over itself in certain regions due to complementary base pairing. R-RNA strand unfold on heating and refold on coiling. It is one the most stable RNA among all types of RNAs. R-RNA and ribo-proteins constitute ribosomes.

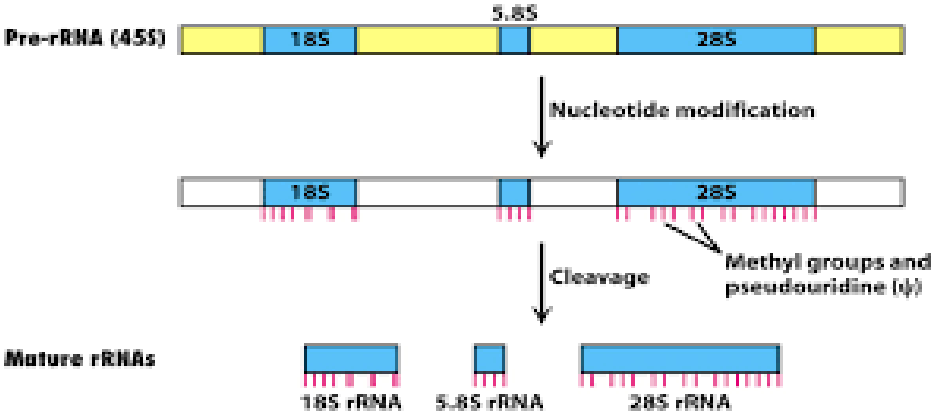


Fig. 10.7 processing of rRNA in a eukaryotic cell

In prokaryotes, 70S ribosome is made of two sub units- 30S and 50S. p30S subunit has 16SrRNA while 50S has 23S and 5S rRNA. An initial 30s transcript is made in ***E. coli*** by RNA polymerase. During processing p30S transcriptional unit is cleaved by RNase 111 into 25S

and18S segments. Which are further reduced to p23S and p16S, further trimming results in functional 23Sa and 16S. Some modification of bases like methylation also occurs during processing of rRNA.

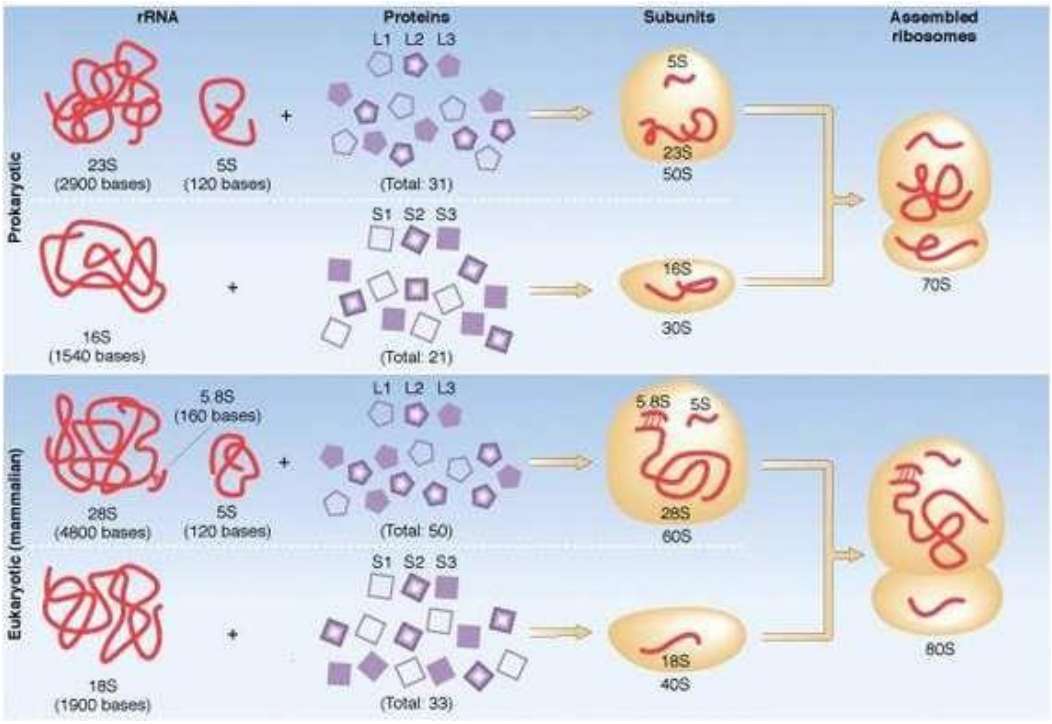


Fig. 10.8 rRNA and riboprotien association

In **eukaryotes** 4 types of rRNAs found are **28s, 18s, 5.85s, and 5s.** In the nucleolus of eukaryotes, RNA polymerase-I transcribes the rRNA genes, which usually exit in tandem repeats to yield a long, single pre-rRNA which contains one copy each of the 18s, 5.8s and 28s sequences. Various spacer sequences are removed from the long pre-rRNA molecule by a series of specific cleavages. Many specific ribose methylations take place directed by small ribonucleoprotein particles (snRNPs) and the mature rRNA molecule fold and complex with ribosomal proteins. RNA pol. III synthesizes the 5srRNA from unlinked genes.

Base composition of rRNA- rRNA differs in base constant from tRNA and mRNA. It is relatively **rich in guanine and cytosine**. The base components in rRNA of E.coli have a molar ratio of adenine 21: uracil 17: guanine 36: cytosine 23.

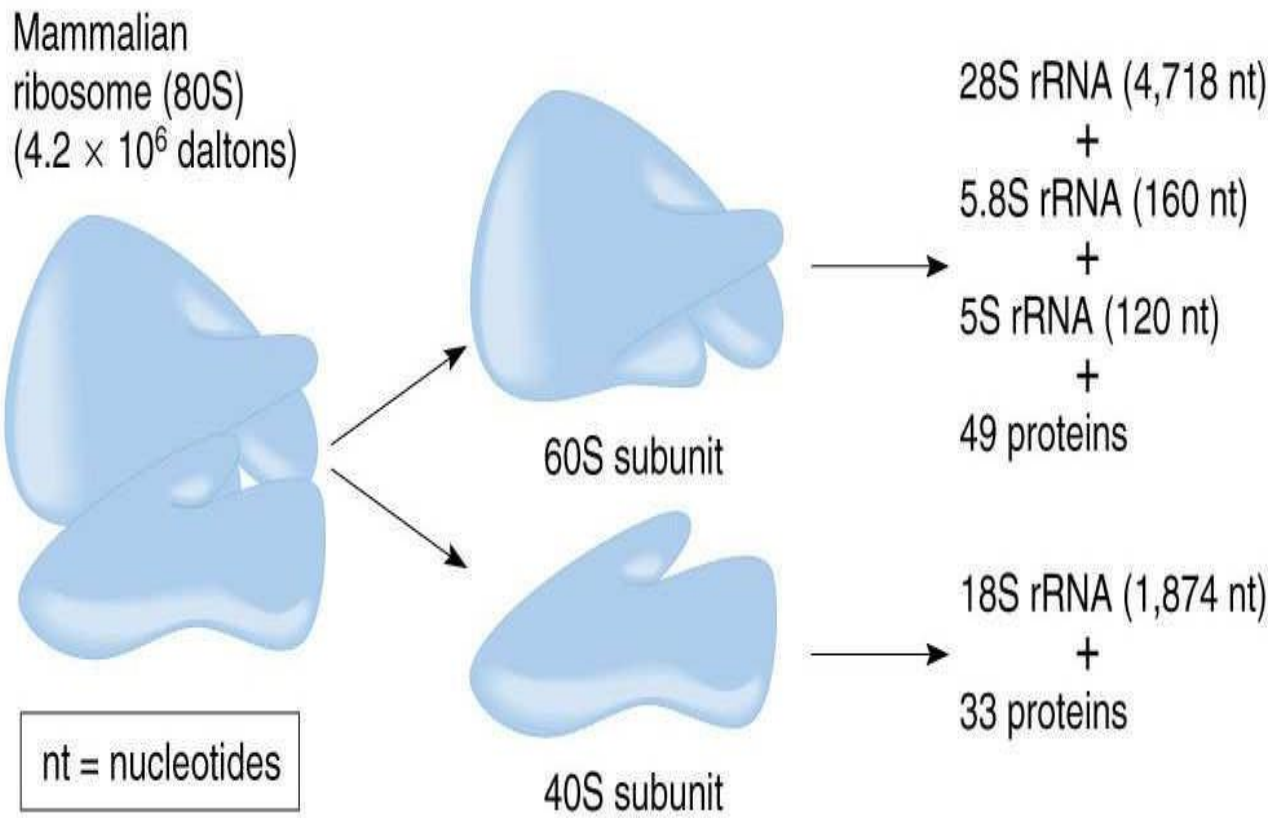


Fig. 10.9 Different types of rRNA in a eukaryotic cell

Composition of ribosomes in bacterial and eukaryotic cells-

Cell type	Ribosome size	Subunit	rRNA component	Proteins
Bacterial	70S (Svedberg unit)	Large (50S)	23S (2900 nucleotides)	31
			5S (120 nucleotides)	
		Small (30S)	16S (1500 nucleotides)	21
Eukaryotic	80S	Large (60S)	28S (4700 nucleotides)	49
			5.8S (160 nucleotides)	
			5S (120 nucleotides)	
		Small (40S)	18S (1900 nucleotides)	33

10.5 Biosynthesis of RNA

10.5.1 Biosynthesis of tRNA

t-RNA is synthesized by **RNA polymerase III in eukaryotes**. Primary transcript is a precursor that generally has extra nucleotide on both the 5' and 3' ends. The general precursor of tRNA molecules is like:

5'P-----leader--- (tRNA-----spacer) n tRNA-----trailer-------- OH3'

Some tRNA genes also have introns but its splicing is done differently than mRNA. The extra nucleotides are removed from the ends and then 3 nucleotides (-CCA, there are not encoded by the gene) are added to the 3' end in a post-transcriptional method. The bases of tRNA undergo extensive **post-transcriptional modification**; up to 10% of the nucleotide can be modified. Mature tRNA has extensive secondary and tertiary structure that is important for their function.

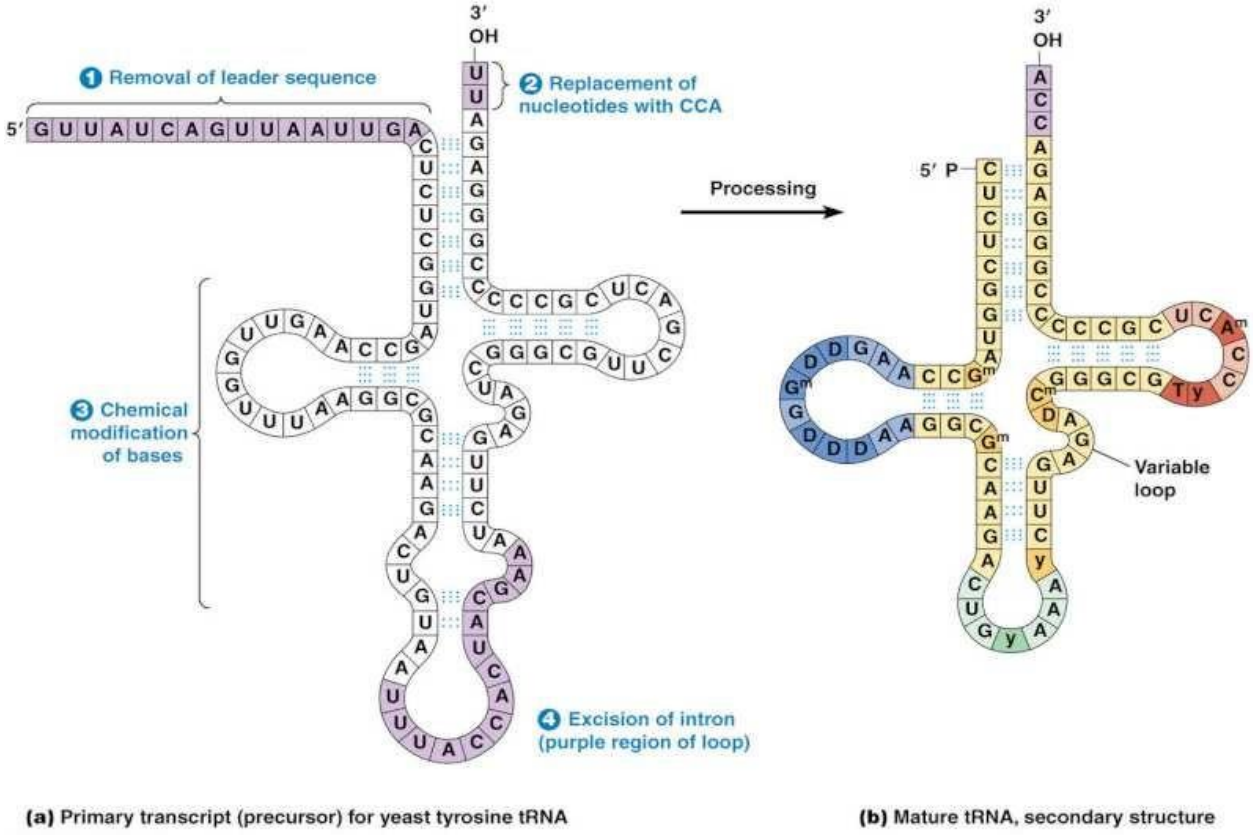


Fig. 10.10 Post transcriptional changes in tRNA

10.5.2 Biosynthesis of m-RNA

Synthesis of messenger RNA is accomplished by using one of the two DNA strands called template or non coding strand. It is carried out from the 5' end towards 3' end. RNA polymerase attaches to the initiator or promoter end of the structural gene and catalyze RNA synthesis. This phenomenon of synthesis of mRNA from DNA template is called **transcription**. In prokaryotes mRNA undergoes very little post transcriptional processing. There is hardly some time gap between transcription and translation, most of the times two processes occur simultaneously. While in eukaryotes handsome processing mechanism results in the formation of functional mRNA. Removal of introns and rejoining of exons is an important step in the transformation of mRNA into functional mRNA. Processes like polyadenylation of bases at 3' end, capping and methylation of some bases are the most important ones.

10.5.3 Biosynthesis of r-RNA

It is synthesized by genes present on DNA of several chromosomes found within a region known as **nucleolar organizer**. R-DNA associated with the nucleolus is responsible for coding rRNA. This part of DNA is known as nucleolar recognizer. RNA although present in ribosomes but is formed inside the nucleus. In bacteria (prokaryotic) about 10-200 cistrons are concerned with the rRNA synthesis whereas in higher organisms 200-2000 tightly clustered cistrons are involved in rRNA synthesis. Fragmentation is the most common and important step in rRNA synthesis.

10.6 Functions of RNA

10.6.1 Functions of t-RNA

The tRNA plays important role in protein synthesis. T-RNA picks up a specific amino acid from the cytoplasm carries it to the site of protein synthesis and attaches itself to ribosome in accord with the sequence specified by mRNA. It transmits its amino acid to the polypeptide chain. In protein synthesis tRNA acts an adaptor molecule which is meant for transferring amino acids to ribosomes for synthesis of polypeptides. There are different tRNAs for different amino acids. Codons are recognized by anticodons of tRNA. They hold peptidyl chains over the mRNAs.

10.6.2 Functions of m-RNA

m-RNA carries coded information to be translation into polypeptide. It directly takes part in protein synthesis in a cell. In some viruses having RNA as genetic material, it may undergo reverse transcription to from compact genes which are used in genetic engineering. The phenomenon also occurs in nature and has added certain genes in the genomes.

10.6.3 Functions of r-RNA

r-RNA binds to protein molecules and give rise to ribosomes. 3'end of 18s rRNA (16s in prokaryotes) has unpaired nucleotides complementary to those of region or m-RNA, it is the site where ribosomes bind to mRNA during translation. 5s rRNA and surrounding protein complex provide binding site for tRNA.

10.7 Important features of RNA

- RNA is copied from one strand of the double helix called the template strand.
- RNA differs from DNA in that it is single stranded, has uracil inserted of thymine and has ribose sugar instead of deoxyribose ribose.
- Messenger RNA (mRNA) carries the genetic information that specifies a particular amino acid sequence of protein synthesized.
- mRNA bases constitute codons, each codon is made of three consecutive bases in a row.
- rRNA joins certain proteins to form ribosomes. Ribosomes physically support the other structures involved in protein synthesis, and some rRNA catalyses formation of peptide bonds.
- tRNA is clover leaf-shaped and connects mRNA codon to an amino.
- In prokaryotes, RNA is translated as soon as it is transcribed while in eukaryotes, RNA is often altered (or modified) before it is actively translated.
- mRNA gains a modified nucleotide cap and a poly A tail.
- Many genes have intervening sequences called introns, which are not transcribed and cutout from the mRNA. The protein encoding sequences in mRNA, exons, are then reattached. Ribozymes are small RNAs with catalytic activity that can splice introns. They join proteins to form snurps, which associate to form spliceosomes.
- After being processed the RNA must be exported from the nucleus before it is translated.

10.8 Summary

RNA is the genetic material of some plants, animal and bacterial viruses. Except some viruses (e.g. reoviruses) most cellular RNA is single stranded. RNA is generally involved in protein synthesis but in majority of plant viruses it acts as genetic material too. There are two major types of RNA- Genetic RNA and Non-genetic RNA. Non genetic RNA is of three major types- mRNA, rRNA, & tRNA. T-RNA is also called soluble or sRNA. In prokaryote all types of RNAs are synthesized by single RNA polymerase but in eukaryotes there are three different RNA polymerases for three types of RNA polymerase.

All types of non genetic RNAs participate in protein synthesis. M-RNA carrier genetic information from DNA to cytoplasm to determine the specific sequence of amino acids in a

protein. Messenger RNA is a long RNA which constitutes 2-5% of the total RNA content tRNA picks up a specific amino acid from the cytoplasm carries it to the site of protein synthesis and attaches itself to ribosome in accord with the sequence specified by mRNA.

r-RNA, stable or insoluble RNA constitutes the largest part (up to 80%) of the total cellular RNA. It was reported by Kuntz. It is found primarily in the ribosome. Along with cytoplasm ribosomes are also found in some organelle like mitochondria and plastids. It is synthesized by genes present on DNA of several chromosomes found within a region known as nucleolus organizer.

Transfer RNA is clover leaf-shaped and connects and mRNA codon to an amino. In prokaryotes, RNA is translated as soon as it is transcribed. In eukaryotes all types RNAs is often altered (or modified) before it is active.

10.9 Glossary

RNA- (Ribonucleic acid) the genetic information carrying material in some viruses, non genetic RNA is generally derived from DNA by transcription that may carry information (mRNA), provide sub-cellular structure (rRNA), transport amino acids (tRNA), or facilitate the biochemical modification of itself or other RNA molecules (enzymes).

Ribosome- cytoplasmic organelle on which proteins are synthesized

Intron- non translatable part of mRNA in eukaryotic cells

Exons- translatable part of mRNA in eukaryotic cell

Transcription- formation of RNA from one strand of DNA

Splicing- removal of introns to join together all exons to form functional mRNA in eukaryotes

SnRNPs- are small nuclear ribonucleo proteins that combine with unmodified PmRNA and other proteins to form spliceosomes

Spliceosome- is a complex of snRNAs and proteins, found in eukaryotic cells. It helps in removing introns from PmRNA

10.10 Self Assessment Question

1. On the basis of functions RNA is of ------ types:

(a) 2 (b) 3

(c) 1 (d) 4

2. Formation of RNA from DNA is called as:

(a) Replication (b) Duplication

(c) Transcription (d) Translation

3. The genetic material of some of the viruses is constituted of:

(a) Protiens (b) Ribonucleic acid

(c) Deoxyribonucleic acid (d) Any of these

4. t-RNA acts as an:

(a) Adaptor molecule (b) molecule to transfer amino acids to the site of protein synthesis

(c) Soluble RNA (d) all of these

5. The genetic information of protein synthesis is carried by:

(a) RNA (b) r-RNA

(c) m-RNA (d) t-RNA

6. Amino acid is attached to tRNA by its ----- arm:

(a) Anticodon arm (b) 3'end of arm opposite to anticodon arm

(c) Any arm (d) DHU arm

7. r-RNA formation takes place in:

(a) Cytoplasm (b) Nucleus

(c) Nucleolus (d) Golgi body

8. Eukaryotic ribosomes are of ------S, having smaller and bigger unit made of ---- and ----- S.

(a) 70S, 30S & 40S (b) 70S, 30S & 50S

(c) 80S, 50S &30S (d) 80S, 60S & 40S

9. Codons are present on:

(a) DNA (b) RNA

(c) t-RNA (d) mRNA

10 Clover leaf shape is attained by------molecule after maturation:

(a) t-RNA (b) m-RNA

(c) r-RNA (d) DsDNA

10.10 ANSWER

1. (a) 2. (c) 3. (b) 4. (d)

5. (c) 6. (b) 7. (c) 8.(d)

9.(d) 10.(a)

10.10.1 Fill up the following blanks

1. t- RNA has a special shape, called as--------- and helps in carrying amino acids from ---- to the----- of protein synthesis.
2. RNA plays the role of both ---- and----- genetic molecule.
3. RNA may also act as a functional molecule by acting as----- --.
4. m-RNA carries ------- information from nucleus to ----- for -------synthesis.
5. m-RNA of eukaryotic cell undergoes processing by ------ and -------.

10.10.1 Answer

1. clover leaf shape, cytoplasmic pool, site
2. genetic, non genetic
3. enzyme
4. genetic, cytoplasm, protein
5. capping, tailing

10.12 Terminal Questions

A- Long answerer questions-

i) Write an essay on types of RNA.
ii) Describe the biosynthesis of mRNA.
iii) Discuss the function of RNA.

B- Short answerer questions-

i) Differentiate between tRNA and mRNA.
ii) Explain the functions of rRNA.
iii) Draw the structure of tRNA.

C- Fill in the blanks-

i) RNA is the genetic material of……...
ii) The three dimensional structure of tRNA was proposed to L-shaped by …….
iii) t-RNA synthesized by ……in eukaryotes.

Ans- (i) some plants, animal and bacterial viruses, (ii) Kim and Klug.
(iii) RNA polymerase III.

6.

10.12 References and Suggested Readings

ix) Molecular biology-P.C. Turner, A.G. McLennen, A.D. Bates & M.R.H. white.
x) Principles of Genetics- D.Peter Snustad, Michael J. Simmons.
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xiii) Lehninger- Principles of Biochemistry. 4th edition- David L. Nelson, Michael M. Cox.
xiv) Color Atlas of Biochemistry-2nd edition – J. Koolman, K. H. Roehm
xv) Genetics- Benjamin A. Pierce.
xvi) Genetics & Molecular Biology- 2nd edition.-Robert Schleif.

UNIT 11 PROTEIN SYNTHESIS AND REGULATION

Contents

11.1 Objectives

Study of this unit will let the students to:

- Protein Synthesis and mechanism (initiation, elongation and termination
- Gene regulation
- Operon hypothesis: regulator gene, promoter gene, operator gene, structural gene, repressor gene, co-repressor gene and inducer gene
- Regulation at transcription
- Regulation by gene arrangement and reversible phosphorylation
- Types of control mechanisms
- Regulation of gene activity in eukaryotes.

11.2 Introduction

The replication of DNA serves to carry genetic information from cell to cell and from generation to generation. This information is translated in to protein that determines the phenotype of cell by controlling its biochemical reactions. Protein synthesis is the vital function of the cell where in the genetic information stored in DNA is passed on to RNA, especially mRNA by the process of transcription. All the three types of RNA i.e., mRNA, tRNA and rRNA together help in translating the coded information in the form of a polypeptide. The linear chain of amino acids translated is the primary protein which undergoes configurationally changes to form secondary, tertiary or quaternary proteins.

11.3 Protein Syhthesis and its Mechanism

A gene expresses itself by protein synthesis. Protein synthesis is under direct control of DNA in most cases or else under the control of genetic RNA where DNA is absent. Information for structure of a polypeptide is stored in a polynucleotide chain of DNA or RNA.

In 1958 **F.Crick** proposed that the concept of **central dogma**, which states that when a particular gene is expressed (control a function or a reactions) its information is copied into another nucleic acid (mRNA) which in turn directs the synthesis of specific proteins. So the central dogma was proposed as unidirectional flow of molecular information from DNA to mRNA and finally to polypeptide. Later a reverse of central dogma was also found in retroviruses. **H. Temin and D. Baltimore** (1970) reported that retro viruses operate a central dogma in reverse manner (inverse flow of information) or **teminism** inside host cells. This

discovery was important in understanding cancer and hence, these two scientists were awarded Nobel Prize.

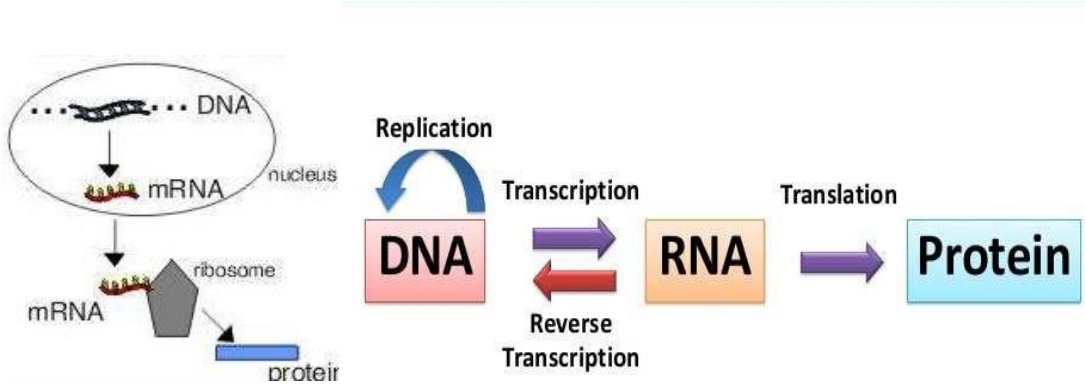

Fig. 11.1 Linear flow of Central Dogma and Reverse of Central Dogma

Genetic RNA of these viruses first synthesizes DNA through reverse transcription. This process is catalyzed by the enzyme **reverse transcriptase**. DNA then transfers information to messenger RNA which takes part in translation of the coded information to from polypeptide.

11.3.1 Minimum necessary Materials

i. **Amino acids**- there are some 20 amino acids and amides which constitute building blocks or monomers of proteins. They are found in the cellular pool or cytoplasm.

ii. **Ribosome**- ribosome comprises two sub units which exists as separate subunits prior to the translation of mRNA and contain following sites:

- **P site (peptidyl site or D site- donor site) -** P site is jointly contributed by the two ribosomal subunits, most frequently occupied by peptidyl-tRNA or the tRNA carrying growing peptide chain. . The P-site is also referred to as the puromycin sensitive site. Puromycin is an antibiotic which shows similarities with a part of amino acyl-tRNA
- **A site (amino acyl site) -** A site is situated on the larger subunit of ribosome. It faces the tunnel between the two subunits, frequently occupied by amino acyl-tRNA, functions as acceptor for growing protein during peptide bond formation.
- **E-site** – the exit site, the ribosomal site harboring decylated tRNA on transit out from the ribosome.

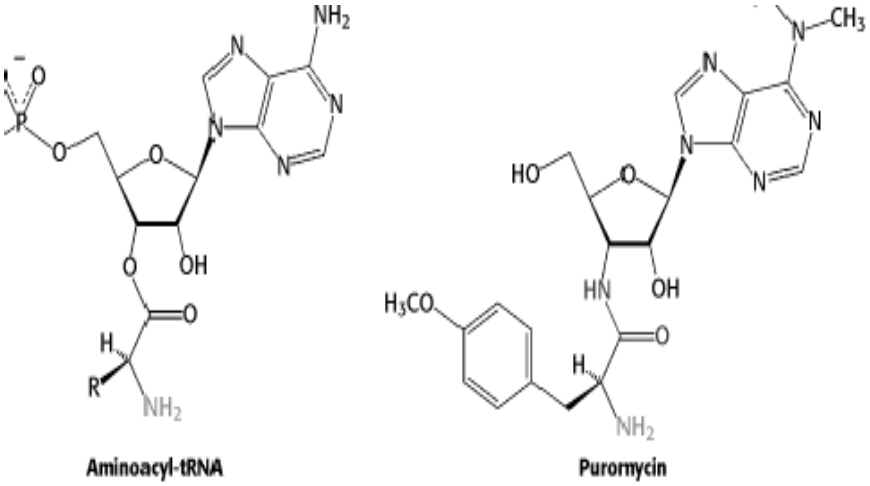

Fig.11.2 Antibiotic Action of Puromycin resembles the aminoacyl terminus of an aminoacyl-t-RNA.

The different parts of ribosomes, connected with protein synthesis are-

a- **A tunnel**- It lies between the two subunits, acts as a place for mRNA

b- **The longitudinal groove**- is part of the longer subunits which acts as a passage of newly synthesized polypeptide

c- **Reactive sites**- P, A and E-site

d- **P-site**- acts as a donor of peptide chain to the newly coming tRNA

e- **A-site-** acts as a binding site for new tRNA with its amino acid for the elongation of polypeptide chain

f-

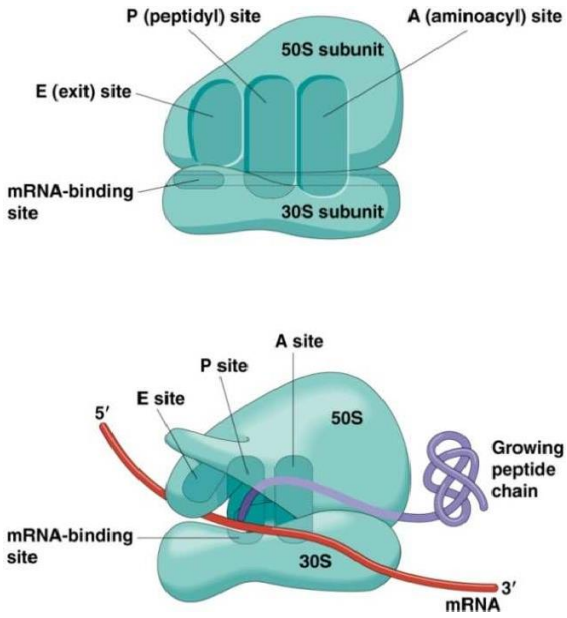

Fig. 11.3 Different sites of ribosome [each with specified function]

iii. **mRNA-** carrying genetic information of DNA into cytoplasm for its translation

iv. **tRNA-** to transport the respective amino acids as per their anticodons against the codons of mRNA

v. **Enzymes**- amino acid activating system **(aminoacyl- tRNA synthetase),** Peptide polymerase system
vi. **ATP-** as energy source
vii. **GTP**- for synthesis of peptide bonds
viii. **Soluble protein initiation and transfer factors**
ix. **Variousinorganic cations** (K^+, NH_4^+, Mg^{++} or Mn^{++})

11.3.2 Mechanism of protein Synthesis

Two major steps are involved in protein synthesis are:-

- **Transcription**- involving transfer of genetic information from DNA to mRNA
- **Translation**- involving translation of the language of nucleic acid into that of a polypeptide

11.4 Transcription

The transfer of genetic information from DNA to mRNA in general is known as transcription. The segment of DNA that takes part in transcription is called transcription unit. It has three components-

a) A promoter
b) The structural gene
c) A terminator

a) A promoter- promoter sequences are present upstream (5'end) of the structural genes of a transcription unit. The binding sites for RNA polymerase lies within the promoter sequence. In prokaryotes 10bp upstream from the start point lies a conserved sequence described as 10 nucleotide sequences**TATAAT or "pribnow box**" and 35 nucleotide sequences**TTGACA as "recognition sequence".**

b) **The structural gene**- structure gene is part of that DNA strand which has 3'→5' polarity as transcription occur in 5'→3' direction. The strand of DNA that directs the synthesis of mRNA is called **template or non-coding strand**. The complementary strand is called **non-template or coding strand,** it is identical in base sequence to RNA transcribed from the gene, only with U in place of T.

c) **A terminator**- terminator is present at 3' end of coding strand and defines the end of the process of transcription.

- The base sequence of the mRNA molecule is complementary to that of the antisense strand which served as it template.
- Like DNA synthesis RNA synthesis also proceeds from 5' to 3' direction (5'→3').

11.4.1 Transcription in Prokaryotes

In bacteria there is **single RNA polymerase** which catalyses synthesis of different types of RNAs i.e., mRNA, tRNA and rRNA. RNA polymerase is a **holoenzyme** that is represented as **(α β β^1 α_2)** which constitutes core enzyme and a sigma factor (σ). The core enzyme is capable of transcribing DNA into RNA but cannot specify the starting point of transcription. It is **σ subunit which confers specificity**. Rho factor (ρ) is required for the termination of transcription.

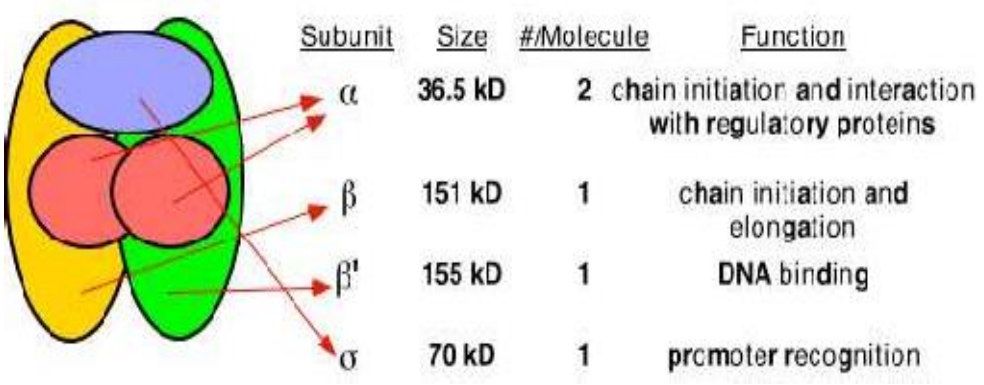

Fig. 11.4 Component of RNA polymerase enzyme (holoenzyme with sigma factor)

The mechanism of transcription in prokaryotes thus involves the following steps-

1. Binding of RNA polymerase, a holoenzyme, to a promoter site. The promoter sites are generally present before the start point of transcription.
2. The specificity of the binding of enzyme with a specific promoter is helped by sigma factor.

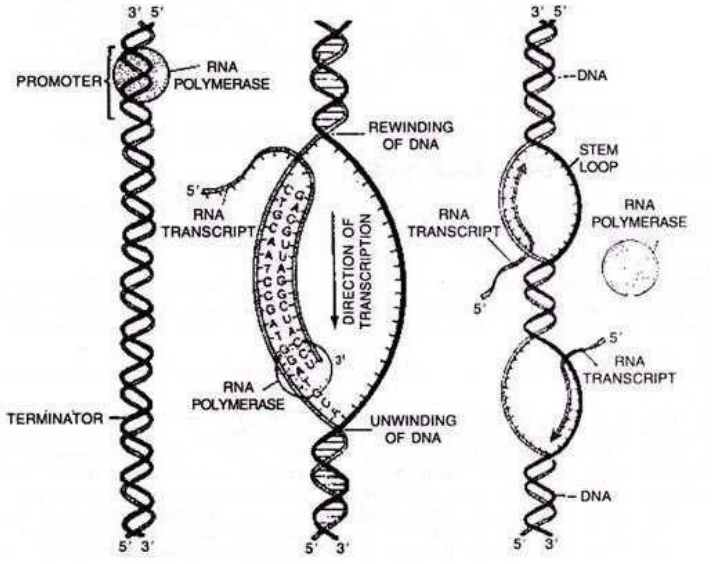

Fig. 11.5 Overall process of transcription

3. Unwinding of DNA, leading to separation of two strands of which only one is transcribed.
4. Dissociation sigma factor (σ).
5. Elongation of mRNA transcript with the help of core enzyme i.e., RNA polymerase
6. Termination of mRNA synthesis is brought about by termination gene on DNA. In bacteria this termination signal is recognized by the factor rho (ρ).
7. Its amino group joins the carbonyl group of the growing polypeptide chain to form an adduct that dissociates from the ribosome. This adduct is stable because puromycin has an amide rather than an ester linkage.

11.4.2. Transcription of mRNA in Eukaryotes

Eukaryotes-total 4 types of RNA polymerase, 3 types of RNA polymerase in nucleus, one in organelles,

- **RNA-Polymerase-1**= transcribes**rRNA (28S, 18S & 5.8S)**
- **RNA polymerase-11=** transcribes **precursor of mRNA** (**hnRNA**-heterogeneous nuclear RNA)
- **RNA polymerase-111**= transcribes **tRNA, 5SrRNA &snRNAs** (small nuclear RNAs)

1. **Initiation**- binding of **RNA polymerase** to the **promoter region** with the help of an **Initiation Factor- Sigma factor** (binding of σ-factor alter the property of enzyme; make to function as an initiation enzyme).
2. **Elongation**- RNA polymerase will keep on making a complementary strand against template strand with the help of ribonucleotides. The newly transcribed strand keeps separating and the DNA duplex keep on folding back instantaneously. During elongation, same RNA polymerase acts as elongation enzyme due to separation of σ-factor from it. **The direction of transcription is also from 5' 3'like replication**. So the template against which it is transcribed has polarity of 3'—5'.
3. **Termination**- after reaching the terminator region newly formed or nascent RNA falls off along with RNA polymerase. Termination is assisted by Rho-factor(ρ-factor)

In eukaryotes the promoter site is recognized by presence of specific nucleotide sequence called **TATA box or Hogness box or Pribnow Box** (7 base pair long- TATAAA or TATAATs) located 19-27bp upstream to the start point. Another sequence is CAAT box present between -70 and -80bp. The nucleotide sequence at the two ends of all mRNA molecules is the same. Normally mRNA carries the codons of signal complete protein molecule (monocistronic mRNA) in eukaryotes, but in prokaryotes, it carries codons from several adjacent DNA cistron and becomes much longer in size (polycistronic mRNA).

11.4.3 Processing of Eukaryotic Transcript

- **Splicing-** removal of **non-functional introns** and joining of all **functional exons** to make it a functional transcript. Splicing is important to remove the non-functional part of genetic information the DNA has kept but RNA does not need it. During copying from DNA, RNA does receive this non informative part in the form of introns, but remove it with the help of some enzymes to make it functional.

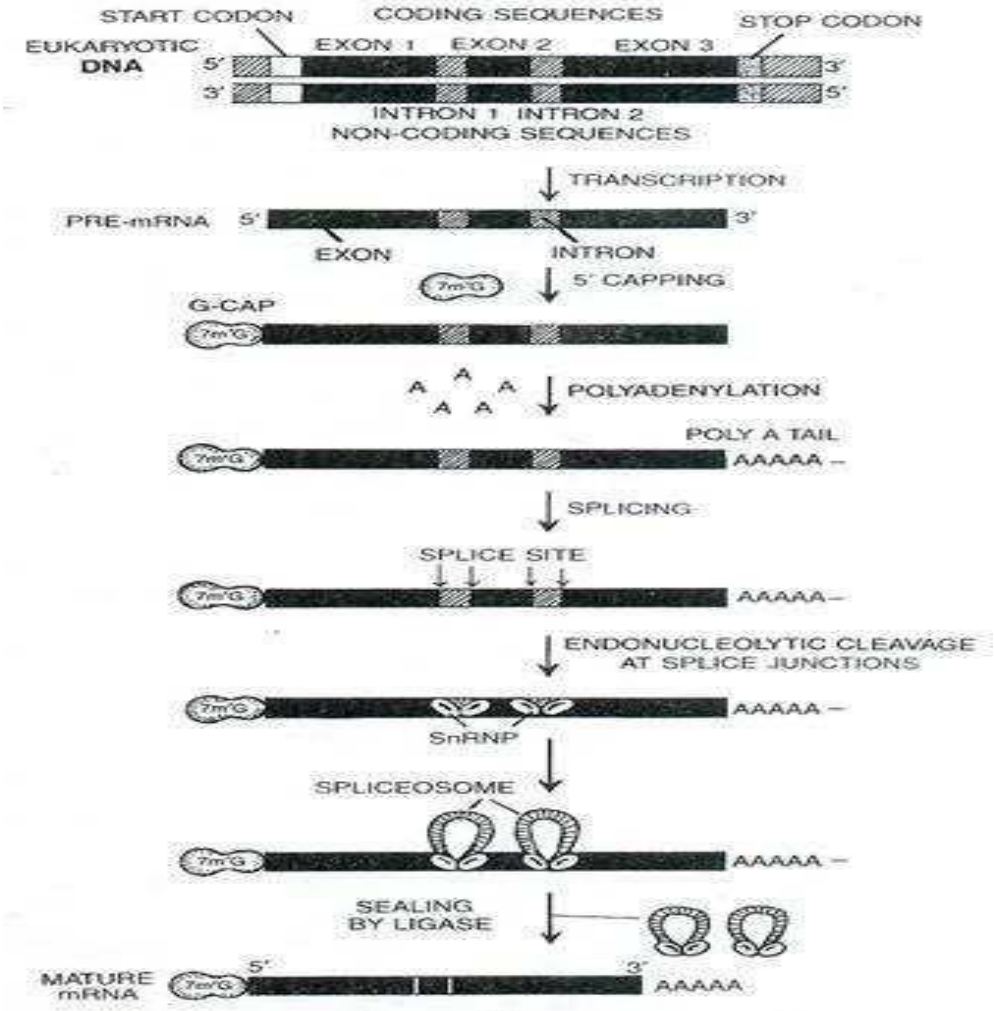

Fig. 11.6 Process of maturation of transcript [form hnRNA to functional mRNA]

- **Capping-** addition of methyl-guanosine triphosphate at 5' end of hnRNA
- **Tailing-** addition of 200-300 adenylated nucleotides at 3'end of hnRNA, addition of these nucleotides has no relation with the template
- The fully processed hnRNA is called mRNA, transported to the cytoplasm for translation.

11.5 Translation

11.5.1 Components of Translation:-

- **mRNA**– the mRNA serves at the template that will determine the sequence of amino acids in the new polypeptide.It has following components:
    - 5’ un translated region or 5’UTR
    - Initiation codon
    - Coding region
    - Stop codon
    - 3’ un translated region or 3’UTR
- **t-RNA-** tRNA, a clover leaf shaped molecule, delivers the correct amino acid to the ribosome as directed by the codon on the mRNA for incorporation into the polypeptide. It has following arms, each with specified function:
    - 3’amino acid carrier arm or acceptor arm with –CCA sequence
    - Ribosome recognizing arm-to recognize A or P or E-site
    - Anticodon arm- with 3 nucleotides to bind to complementary codon
    - Enzyme recognizing arm- to recognize specific animoacyl synthetase
    - 5énd with G
- **Ribosome-** protein synthesizing machinery, help in holding mRNA and tRNA for specific codon translation, has following components:
    - Smaller subunit (30S or 40S)
    - Larger subunit (50S or 60S)
    - Groove or tunnel between two subunits to hold mRNA
    - Three sites- P, A and E-site
    - Enzyme, peptidyl transferase , helps in peptide bond formation

11.5.2 General steps of Translation

The translation step involves the translation of the language of nucleic acids into the language of protein. Translation is the process by which a polypeptide chain is synthesized by ribosomes using the sequence of codons in an mRNA to direct the sequence of amino acids.

a) **Activation of amino acids or Charging of amino acids**-Lipmann and co-working showed during 1950s that amino acids attachment to the tRNA moleculesis an active process and requires a lot of energy. In the presence of ATP, an amino acid combines with its specific amino acyl-tRNA synthetase; Mg2+ is also required in this reaction. It produces amino acyl-adenylate enzyme complex.

AA* + ATP + E** → AA-AMP**-E +PPi***

[*AA-amino acid, **E- aminoacyl tRNA synthetase, ***PPi- pyrophosphate]

b) **Aminoacylation of tRNA or Charging of tRNA-**It is the loading of tRNA with the activated amino acid. Amino acid molecule is transferred to a specific tRNA molecule and the AMP (adenosine monophosphate) molecule is released.

AA-AMP-E* + tRNA → AA-tRNA + AMP + E

[*AA-AMP-E – aminoacyladenylate enzyme]

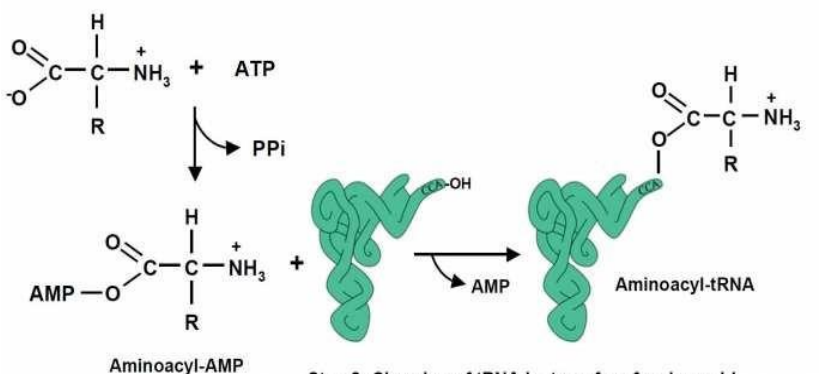

Fig. 11.7 Charging or animoacylation of tRNA

c) **Initiation of translation**- In the first step there is binding of mRNA with smaller sub unit of ribosome. Translation of Initiation codon (AUG) by a charged tRNA with Methionine (n-formyl methionine, f-Met, in prokaryote) amino acids takes place. It is followed by the translation of second codon by 2nd charged tRNA. After the translation of first two codons, the association of bigger subunit of ribosome takes place to form a complete translational complex. When two such charged tRNA comes close, the peptide bond between two amino acids, they carry, will take place with the help of a ribozyme called- Peptidyltransferase (23SrRNA molecule) enzyme. Formation of peptide bond between 1st& 2nd amino acid takes place. UTR- (Un-Translated-Regions) is the flanks of mRNA before Initiation and after the stop codon, which are not to be translated, but they play role in efficient translation .There are three initiation factors in prokaryotes- IF3, IF2, IF1. Eukaryotes have 9 initiation factors – eIF2, eIF3, eIF1, eIF4A, eIF4B, eIF4C, eIF4D, eIF5, eIF6.

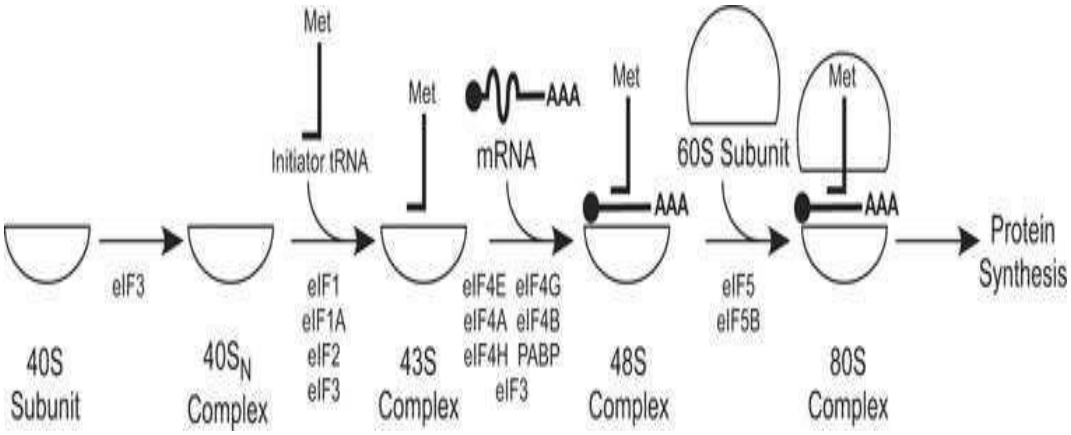

Fig. 11.8 Steps in translation in eukaryotic cell

d) **Elongation-** The translated part of mRNA translocates out and ribosome moves fromone to next codon. Regular addition of new amino acids takes place at A-site. Polypeptide chain (PPC) keeps elongating at the expense of energy provided by GTP. PPC hangs in the groove of bigger sub unit of ribosome on the P-site.

e)

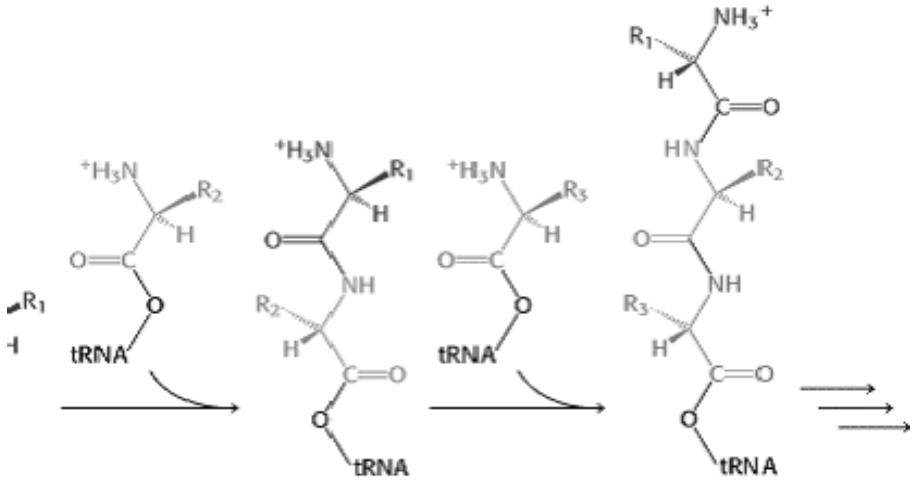

Fig. 11.9 Polypeptide-Chain Growth [Proteins are synthesized by the successive addition of amino acids to the carboxyl terminus]

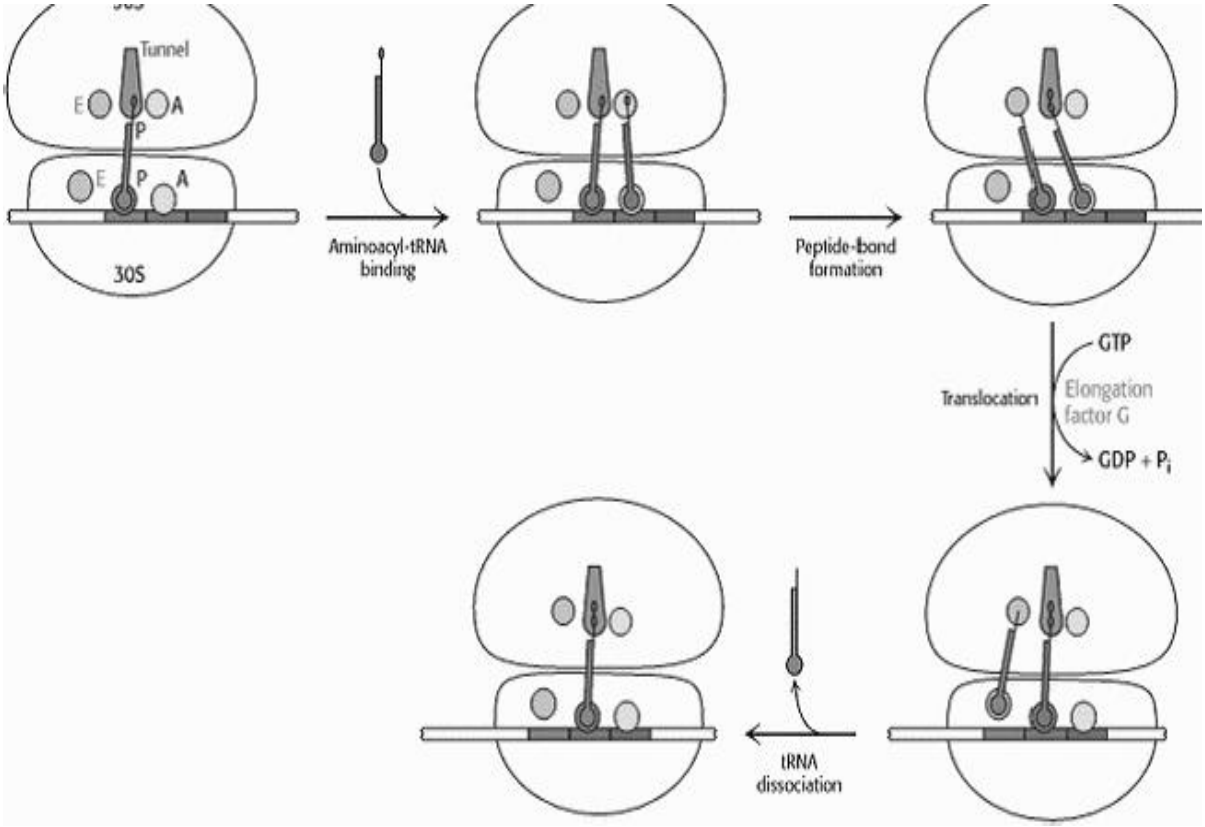

Fig. 11.10 Mechanism of Protein Synthesis

[The cycle begins with peptidyl-tRNA in the P site. An aminoacyltRNA binds in the A site. With both sites occupied, a new peptide bond is formed. The t-RNAs and the mRNA are translocated through the action of elongation factor G, which moves the deacylated tRNA to the E site. Once there, it is free to dissociate to complete the cycle.]

e) Termination- Binding of releasing factors to the stop codon helps in the release of polypeptide and terminates translation. Synthesis of polypeptide terminates when a nonsense codon of mRNA reaches the A-site. There are three nonsense codons- UAA, UAG & UGA. These codons are not recognized by any of the tRNAs. There is no tRNA having anticodon complementary to stop codon i.e., none of the tRNA has AUU, AUC or ACU anticodon. Finally the ribosome encounters a stop codon. The polypeptide, tRNA and mRNA are released. The small and large subunits dissociate from one another.

Some special features-

1. Translation is the ultimate step in gene expression.
2. The energy cost for protein synthesis is very high-
   a. Only a small fraction of the energy input of translation is needed to form the peptide bond.
   b. The majority of energy is invested to assure that the sequence of the polypeptide is correct.
   c. If incorrect polypeptides (e.g. enzymes) are made by the cell it could have divesting effect affects cell fraction.
3. The mRNA is always read from 5' to 3'. The polypeptide is always synthesized in the direction of amino terminus to carboxyl terminus.

Prokaryote cell	Eukaryote cell	Function
Initiation factors		
IF1, IF3	eIF3, eIF4c, eIF6 eIF4b eIF4f	Bind to ribosome subunits Bind to mRNA
	eIF2b, eIF2	Initiate tRNA delivery
IF2	eIF5	Displacement of other factors
Elongation factors		
EF-Tu	eEF1α	Aminoacyl tRNA delivery
EF-Ts	eEF1βy	Recycling of EF-Tu/eEF1α
EF-g	eEFF2	Translocation
Termination factors RF, RF2, RF3	eRF	Polypeptide chain release

Table.1Comparison of factors controlling translation in Prokaryote and Eukaryote cell

11.6 Prokaryotic Translation

a) **Initiation –** the purpose of the initiation step is to assemble a complete ribosome on to an mRNA molecule at the correct start points. The components involved are the large and small ribosome subunit the mRNA, the initiator tRNA in its charged form three initiation factors and GTP. The initiation factors IF1, IF2, and IF3 are all just over 1/10 as abundant as ribosome. The overall sequence of events is as follows three main steps to initiation-

- **m-RNA binds to 30S-** ribosome aligned by base pairing of a region of 16s rRNA of the 30S ribosomal subunit to a region on the mRNA 6-

10bases upstream of the initiation codon. The region is called the shine/dalganno sequence.

- **Methionyl-tRNA binds to 30SmRNA complex**- in prokaryotes the first amino acyl-tRNA is always formylmethionyl tRNA (f-met-tRNAfMet). All proteins in prokaryotes are synthesized with formyl methionine as their first amino acid. This complex is called the 30s pre-initiation complex.
- **50S subunit binds to 30S-tRNA-mRNA complex (initiation complex)**- two sites for amino acyl-tRNA binding on 50s subunit, there are called A-site (amino acyl site) and P-site (peptidyl site). The A-site is where incoming amino acyl-tRNA molecules bind and the P-site is where the growing poly peptide chain is usually found. At initiation f met-tRNA is in the P-site and the 2^{nd} codon is positioned at the A-site.

b) **Elongation**- after the formation of 70s-mRNA f-met-tRNA f-met complex, the elongation of polypeptide chain occurs by the regular addition of amino acids. Elongation involves the three factorsEf-Tu, Ef-Ts and Ef-G, GTP, charged t-RNA and the 70s initiation complex. It takes places in three steps-

- A charged t-RNA is delivered as a complex with Ef-Tu and GTP. The GTP is hydrolyzed. GDP is released which can be re-used with the help of EF-Ts and GTP (via the Ef-Tu-Ef-TS exchange cycle).
- Peptidyl transferase makes a peptide bond by joining the two adjacent amino acids without the input of more energy.
- Translocase (EF-G), with energy from GTP, moves the ribosome one codon along the mRNA, ejecting the uncharged tRNA and transferring the growing peptide chain to the P-site

c) **Termination**- it is brought about by the presence of any of the three termination codons, **UAA, UAG and UGA**. When the ribosome encounters a UGA, UAG or UAA codon, no amino acid is added to polypeptide. There codons are called termination codons. These termination codons are recognized by are of the two release factors RF1 and RF2 in E. coli. Function of release factors catalyze hydrolysis of peptidyl-tRNA and promote dissociation of 50s subunit. RF1 recognize UAA and UAG, while RF2 recognizes UGA and UAA. They help the ribosome to recognize there triplets. 30s dissociates or moves to the next start codon on the poly cistronic mRNA.

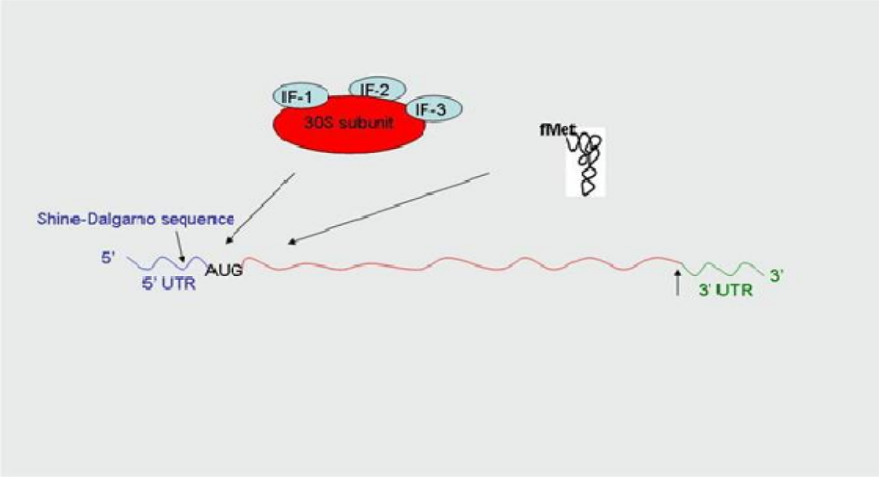

Fig. 11.11 Formation of initiation complex in prokaryotes

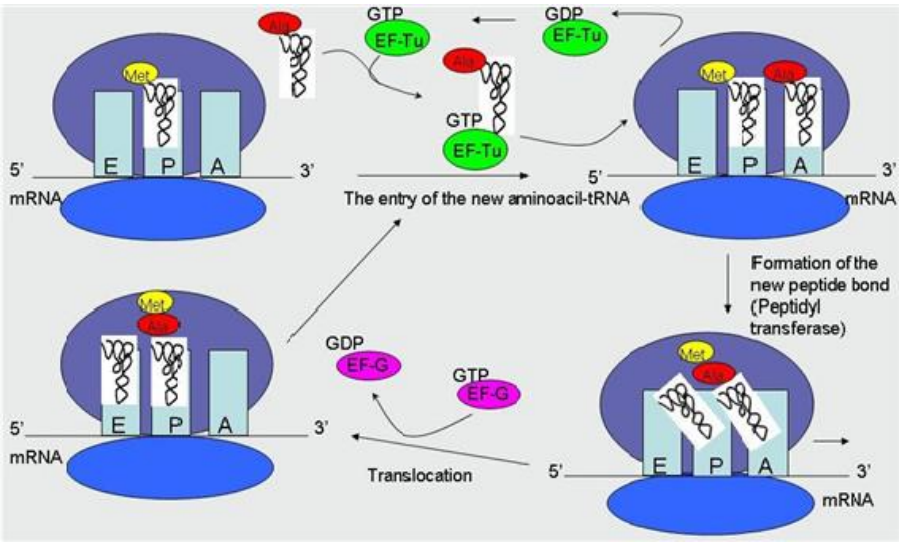

Fig. 11.12 Role of Various Factors during Translocation in Translation

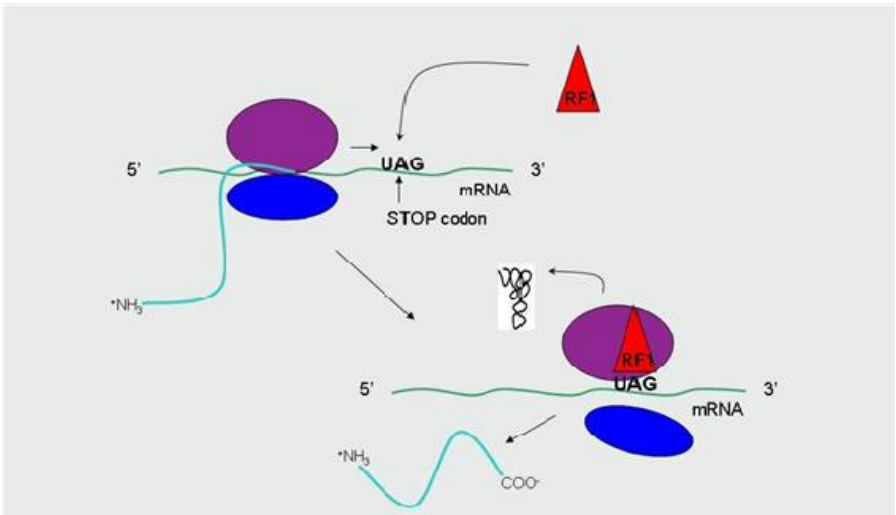

Fig. 11.13 Role of Various Factors during Termination in Translation

11.7 Eukaryotic Translation

Initiation- this is the major point of difference between prokaryotic and eukaryotic protein synthesis, there being at least **nine eIFs** involved. Functionally there factors can be grouped-

a) Binding to ribosomal subunits- eIF6, eIF3, eIF4c.
b) Binding to the mRNA- eIF4B, eIF4F, eIF4A, eIF4E.
c) Involved in initiator tRNA delivery-eIF2, eIF2B.
d) Displace other factors –eIF5.

In contrast to the events in prokaryotes initiation involves the initiator tRNA binding to the 40s subunit before it can bind to the mRNA.

Some differences from prokaryotes in the initiation stage-

1. Initiation takes place at 1st AUG on the mRNA within Kozak sequence (gccRccAUGG).
2. Methonyl-tRNA met is used to initiate translation.
3. There is no shine/dalganno sequence in eukaryotes. The 40S ribosome subunit binds to the 5' cap structure of the mRNA and scans to Kozak sequence.
4. Initiation complete with association of 60S subunit

Elongation and termination is very similar in prokaryotes and eukaryotes. Eukaryotes use only are release factor (eRF), which requires GTP for termination of protein synthesis. It can recognize all three stop codon.

11.8 Gene Regulation and Operon Hypothesis

The synthesis of particular gene products is controlled by mechanism which is collectively called gene regulation. Genes whose products are required at all times or remain switch on all the times, such as those for the enzyme of glycolysis metabolism, are expressed at a more or less constant level in virtually every cell of a species or organism and such genes are called **constitutive genes or housekeeping genes**. Non constitutive genes are not always expressing themselves in a cell. These are called **luxury or non-constitutive genes**. They are switched on or off according to the requirement of cellular activities. E.g., Genes for nitrate reeducates is plants and lactose digestion in E.coli.

Two French scientists (microbiologists) **Jacob and Monad** (1961) found that the genetic material possesses group of regulatory gene units called **operons in prokaryotes for which they received Nobel Prize too**. Jacob and Monad proposed that the transcription of a set of contiguous structural genes is regulated by two controlling elements.

(a) Inducible- this is a process of gene regulation where addition of a substrate or inducer switch on the synthesis of enzymes needed for the breakdown of inducer.

(b) Repressible- in this process of gene regulation addition or accumulation of end product stops the synthesis of enzymes needed for its formation. This phenomenon is also known as feedback repression.

11.9 Operon Model

According to the operon model, several gene codes for an enzyme in some metabolic pathway are located in sequence on chromosome. The expressions of structural genes are controlled by some regulatory genes. The **Operon means a unit of gene expression** and regulation which typically includes-

(i) The structural genes- also called cistron are any gene/s other than the regulatory genes, whose products or enzymes are involved in a specific biosynthetic pathway and whose expression is coordinately controlled.

(ii) Operator sequence- control elements such as an operator sequence, which is a DNA sequence that regulates transcription of the structural genes.

(iii) Regulator gene (s) –the genes, whose products recognize the control elements e.g., a repressor which binds told regulates the operator sequence of the same operon.

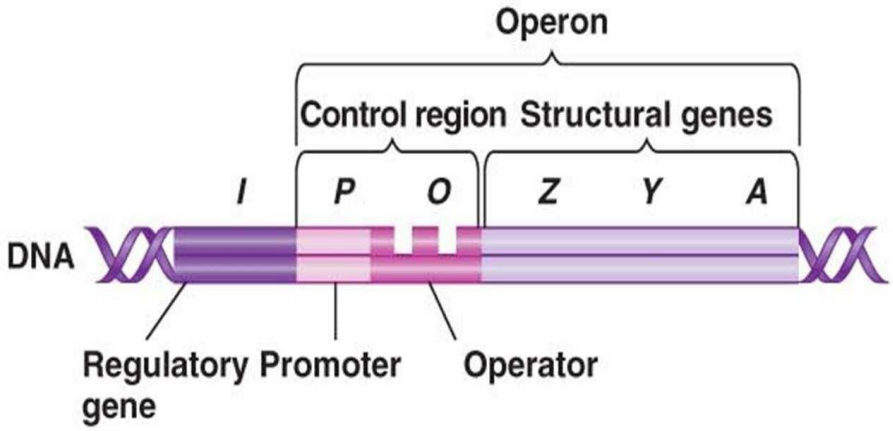

Fig. 11.14 Components of Operon

Operon has structural and regulatory genes that function as a single unit, it includes the following-

- A regulator gene is located outside the operon codes for a repressor or Apo-repressor protein molecule.
- A promoter is a sequence of DNA where RNA polymerase attaches when a gene is to be transcribed.

- An operator is a short sequence of DNA where repressor binds, preventing RNA polymerase from attaching to the promoter.
- Structural genes code for enzymes of a metabolic pathway and are transcribed as a unit.

11.9.1 Inducible Operon

In ***E.coli*** break down of lactose requires three enzymes. These enzymes are synthesized together in a coordinated manner via a regulatory system known as **lac operon**. The addition of lactose itself stimulates the production of required enzymes hence the gene regulation system is also known as **inducible system**. Lac operon regulatory machine includes:

I. **Structural genes**- are those genes which actually synthesize mRNAs. mRNA controls metabolic activity of cytoplasm through the formation of protein or enzyme. The lactose or lac-operon of E. coli contains three structural genes.-
   - Lac a- gene coding for enzyme transacetylase
   - Lac y- gene coding for enzyme permease
   - Lac z- gene coding for enzyme β–galactosidase

II. **Operator gene-** it interacts with a protein molecule (the regulator molecule), which promotes (induce) or prevents (repress) the transcription of structural genes. The gene then directs the structural genes to transcribe. Operator gene of lac operon is made of only 27 base pairs.

III. **Promoter gene-** this gene is the **recognition point** where RNA polymerase remains associated. When the operator gene is functional, the polymerase moves over it and reaches the structural genes to perform transcription.

IV. **Regulator gene**- this is generally known as **inhibitory gene**. The regulator gene codes for a lac operon repressor protein that binds to the operator and prevents transcription of the three genes.

V. **Repressor-** the lac repressor, an **inhibitory protein**, is made up of four identical protein subunits. It therefore has a symmetrical structure and binds to a palindromic 28bp operator DNA sequence. Bound repressor blocks transcription of lac Z,Y,A transcription.

VI. **Inducer** – an inducer is any substance, like lactose in the case of the lac operon that can bind to a particular repressor protein, **preventing the repressor** from binding to a particular operator, consequently permitting RNA polymerase to bind to the promoter, causing transcription of structural genes.

VII. **cAMP-** it exerts a **positive control** in lac-operon because in its absence RNA polymerase is unable to recognize promoter gene. cAMP itself requires catabolic activator protein or CAP for its functioning.

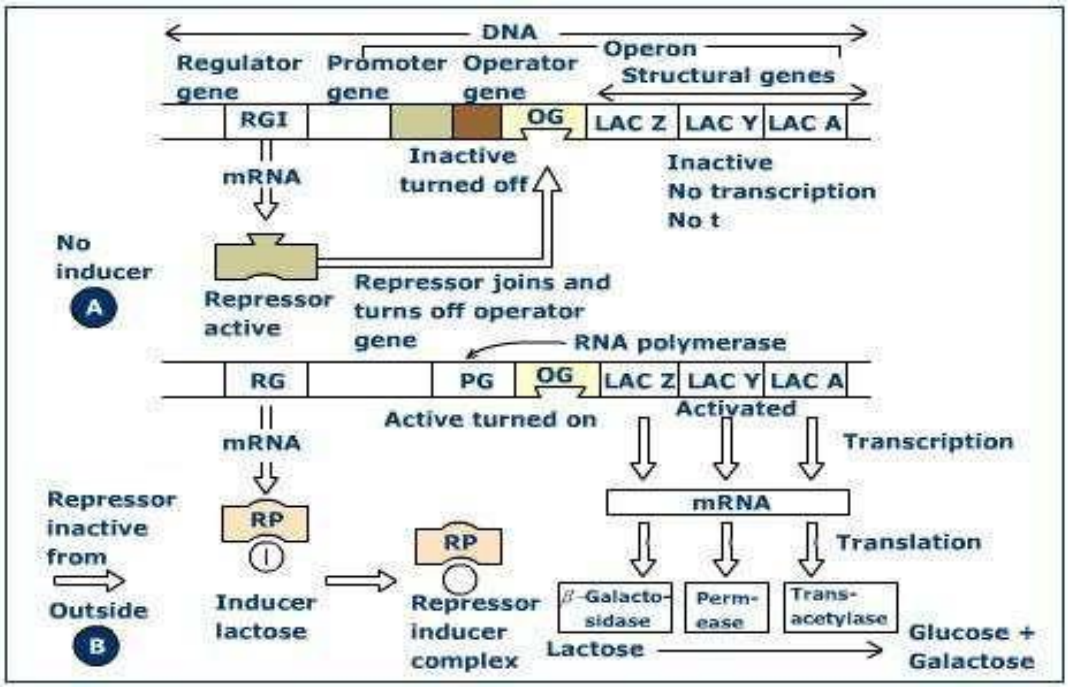

Fig. 11.15 Lac-Operon (in the absence and presence of inducer)

11.9.2 Further control of the lac operon

i) When **glucose is absent**, cyclic AMP (cAMP) accumulates. Cytosol contains catabolism activator protein (CAP). When cAMP binds to CAP, the complex attaches to the lac promoter that makes RNA polymerase bind to the promoter.

ii) When **glucose is present**, there is little cAMP in the cell. CAP is inactive and the lactose operon does not function maximally. CAP affects other operons when glucose is absent. This encourages metabolism of lactose and provides backup system for when for when glucose is absent.

11.9.3 Negative control vs. positive control

Use of both positive and negative controls allows cells to fine tune its control of metabolism. Active repressors shut down the activity of an operon, they are negative control. CAP is example of positive control when molecule is active, it promotes activity of operon.

11.10 Regulation of Gene Activity in Eukaryotes

In Eukaryotic cells gene expression can be regulated primarily at four distinct levels-

11.10.1 Regulation at Transcriptions Level

Regulation of gene expression at transcription level determines which gene will be transcribed following type-

- Selective gene amplification
- Rearrangement of DNA sequence
- Decondensation of chromatin
- Methylation of DNA

11.10.2 Regulation at Processing of mRNA Level

Gene expression is also regulated at the level of processing of primary RNA transcription to functional mRNA. This may involve differential processing of preliminary mRNA before it leaves the nucleus.

11.10.3 Post transcriptional Control

Post transcriptional control involves differential processing of preliminary mRNA before it leaves the nucleus and regulation of transport of mature mRNA. Differential excision of introns and splicing of mRNA can change the type of mRNA that leaves nucleus. Speed of transport of mRNA from nucleus into cytoplasm affects amount of gene product realized. There is difference in length of time it takes various mRNA molecules to pass through nuclear pores.

11.11 Summary

The replication of DNA serves to carry genetic information from cell to cell and from generation to generation. This information is translated in to protein that determines the phenotype and the results of biochemical reactions that occur in the cell. In 1958 F.Crick proposed that when a particular gene is expressed its information is copied into another nucleic acid (mRNA) which in turn directs the synthesis of specific proteins. This concept forms the central dogma of molecular biology.

Two major steps are involved in protein synthesis, transcription (involving transfer of genetic information from DNA to mRNA) and translation (involving translation of the language of nucleic acid into that of proteins).Mechanism of translation- the translation process may be divided in to the following distinct steps-Initiation, Elongation and Termination.Initiation is the assembly of a ribosome on an mRNA molecule. The small and large subunits of the ribosome bind at the initiation codon and the methionine tRNA anticodon pairs with the start codon.Elongation repeated cycles of amino acid addition.

The ribosome proceeds down the mRNA one triplet codon at a time, positioning the correct amino-acyl-tRNA with the codon and catalyzing polypeptide synthesis. Termination results in the release of the new protein chain. Synthesis of polypeptide terminates when a nonsense codon of mRNA reaches the A-site. There are three nonsense codons - UAA, UAG & UGA. Genes for products that are required at all times, such as those for the enzyme of control metabolic pathways are expressed at a more or less constant level in virtually every cell of a species or organism.

The synthesis of particular gene products is controlled by mechanism collectively called gene regulation. In 1961, French microbiologist Francs Jacob and Jacques Monod proposed operon model to explain regulation of gene expression in prokaryotes. According to Operon model, several gene codes for an enzyme in same metabolic pathway and are located in sequence on chromosome, expression of structural genes controlled by same regulatory genes. The Operon means a unit of gene expression and regulation which typically includes- Structural genes, Operator sequence, and Regulator gene.

11.12 Glossary

Inducer- a substance of low molecular weight that inactivates a repressor by combining with it, thereby stimulating gene expression

Inducible enzyme- an enzyme that is synthesized only in the presence of the substrate that acts as an inducer.

Inhibitor- any substance or product that retards a chemical reaction or modifier gene that interferes with a reaction

Promoter- a nucleotide sequence to which RNA polymerase binds and initiates transcription, Also, a chemical substance that enhances the transformation of benign cells into cancerous cells

Operator- a part of an operon that controls the activity of one or more structural genes by binding a regulatory protein

Operon- a group of genes making up a regulatory or control unit, includes an operator, a promoter, and structural genes.

Repressible enzyme- an enzyme whose synthesis is diminished by a regulatory molecule

Regulator gene- a gene that controls the rate of expression of another gene or genes, Example- the lac I gene produces a protein that controls the expression of the structural genes of the lac operon in ***Escherichia coli.***

11.13 Self Assessment Questions & Possible Answers

D- Long answer questions-

iv) Discuss the protein synthesis and its mechanism.
v) What is operon hypothesis? Explain it.
vi) Write an essay on regulation of gene activity in eukaryotes.

E- Short answer questions-

iv) What is protein synthesis?
v) Differentiate between repressor and co- repressor gene.
vi) What do you mean by gene regulation?

F- Fill in the blanks-

iv) P site is jointly contributed by the two.....subunits.

v) The transfer of genetic information from DNA to mRNA is known as. …….

vi) The Operon means a unit of……. and…….

Ans- (i) ribosome (ii) transcription (iii) gene expression, regulation

11.14 Reference and Suggested Readings

i) Molecular biology-P.C. Turner, A.G. McLennen, A.D. Bates & M.R.H. white.

ii) Principles of Genetics- D.PeterSnustad, Michael J. Simmons.

iii) Hand Book of Life Science- Sunil Patel, Rukum. S. Tomer, HarsukinGazera, B.A. Golakiya&ManojParakhia.

iv) Cell Biology, Genetics, Molecular Biology, Evolution & Ecology- P.S.Verma, V.K. Agarwal.

v) Lehninger- Principles of Biochemistry. 4th edition- David L. Nelson, Michael M. Cox.

vi) Color Atlas of Biochemistry-2nd edition – J. Koolman, K. H. Roehm

vii) Genetics- Benjamin A. Pierce.

viii) Genetics & Molecular Biology- 2nd edition.-Robert Schleif.

11.15 Terminal Questions

1. Transcript is:

a) A chain of ribonucleotides
b) Any type of RNA
c) The copy of DNA template
d) All the above

2. RNA formation takes place in:

a) Cytoplasm
b) Nucleus
c) Golgi complex
d) Endoplasmic reticulum

3. In eukaryotic cell, transcription occurs in:

a) Nucleus
b) Mitochondria
c) Plastids
d) All the above

4. In prokaryotes, transcription of all three types of RNAis controlled by:

a) RNA Polymerase
b) RNA Polymerase-1
c) RNA Polymerase-11
d) RNA Polymerase-111

5. mRNA in eukaryotic cell is transcribed by:

a) RNA Polymerase
b) RNA Polymerase-1

c) RNA Polymerase-11
d) RNA Polymerase-111

6. Bigger subunit of ribosomes has ----sites:
   a) 2
   b) 3
   c) 1
   d) 4
7. Operon model of regulation of translation was proposed by:
   a) Watson
   b) Crick
   c) Jacob
   d) Morgan
8. In Lac-operon model, -------acts as inducer to switch on operon:
   a) Galactose
   b) Permease
   c) Lactose
   d) None of the above
9. The expressible part of hnRNA are:
   a) mRNA
   b) Exons
   c) Introns
   d) Cistrons
10. Which enzyme helps in the loading and activation of tRNA?
   a) Ribozyme
   b) Peptidyl transferase
   c) Aminoacyl synthetase
   d) RNA polymerase

Answer:-

1. D 3.-D
2. B 4.-A
5. B 6.-B
7. C 8.-C
9. C 10.C

Unit 12 GENETIC CODE

Contents

12.1 Objective

Study of this unit will let the students to:

- Properties of genetic code
- Codons and anti codon
- "The Wobble Hypothesis"
- Mutation and the triplet code

12.2 Introduction

The genetic code is the way in which the nucleotide sequence in nucleic acids specifies the amino acid sequence in proteins. It is a triplet in nature, where the codons or the groups of three nucleotides lying in adjacent codes for the respective sequence of amino acid in a polypeptide. The number of amino acids are very limited i.e., only 20 but the number of codons are 64.Most of the codons are meant for a specific amino acid but some of the amino acids are coded by more than one code, so genetic code do show degenerate nature.

The relationship between the sequence of amino acids in a polypeptide and nucleotide sequence of amino acids in a polypeptide and nucleotide sequence of DNA or mRNA is called **genetic code**. The theory which is widely accepted now days were proposed by F.H.C. Crick is the theory of triplet nature of code. First two nucleotides of a code determine the specificity of code; change in third position of nucleotide is sometimes ignored, which is supported by Wobble Hypothesis and degenerate nature of code.

12.3 Properties of Genetic Code

The genetic code has the following general properties-

i) The code is a **triplet codon**. The nucleotides of mRNA are arranged as a linear sequence of codons and each codon consists of three successive nitrogenous bases.

ii) The code is **universal**. The genetic code is applicable universally i.e. a codon specifies the same amino acid from a virus to a tree or human being. Thus mRNA from chick oviduct introduced in E. coli produces ovalbumen in the bacterium exactly similar to one formed in chick.

iii) The code is **non-overlapping** but is read sequentially.

iv) The code is **comma less** which means that no codon is reserved for punctuations.

v) The code is **non-ambiguous** means that a particular codon will always code for the same amino acid.

vi) The code has **polarity**. The code is always read in a fixed direction (5'→3'direction).

vii) The code is **degenerate**. In degenerate codons the first two nitrogen bases are similar while the third one is different. As the third nitrogen base has no effect on coding. The same is called **wobble position**.

viii) Some codes act **as start codons**. Most of the times **AUG** codon is the start or initiation codon but sometimes even **GUG a**lso acts the same.

ix) Some codes act **as stop codons**. Three codons **UAG, UAA and UGA** are the chain stop or termination codons.

x) **Cistron-polypeptide parity**- the genetic system should have as many cistrons as the types of polypeptides found in the organism.

		Second or middle base of codon					
		U	**C**	**A**	**G**		
First base of codon (5'end)	**U**	UUU *(phe)* UUC *(phe)* UUA *(Leu)* UUG *(Leu)*	UCU *(Ser)* UCC *(Ser)* UCA *(Ser)* UCG *(Ser)*	UAU *(Tyr)* UAC *(Tyr)* UAA " UAG "	UGU *(Cys)* UGC *(Cys)* UGA" UGG *(Trp)*	**U** **C** **A** **G**	**Third base of codon (3'end)**
	C	CUU *(Leu)* CUC *(Leu)* CUA *(Leu)* CUG *(Leu)*	CCU *(Pro)* CCC *(Pro)* CCA *(Pro)* CCG *(Pro)*	CAU *(His)* CAC *(His)* CAA *(Gln)* CAG *(Gln)*	CGU *(Arg)* CGC *(Arg)* CGA *(Arg)* CGG *(Arg)*	**U** **C** **A** **G**	
	A	AUU *(Ile)* AUC *(Ile)* AUA *(Ile)* AGU**(Met)*	ACU *(Thr)* ACC *(Thr)* ACA *(Thr)* ACG *(Thr)*	AAU *(Asn)* AAC *(Asn)* AAA *(Lys)* AAG *(Lys)*	AGU *(Ser)* AGC *(Ser)* AGA *(Arg)* AGG *(Arg)*	**U** **C** **A** **G**	
	G	GUU *(Val)* GUC *(Val)* GUA *(Val)* GUG* *(Val)*	GCU *(Ala)* GCC *(Ala)* GCA *(Ala)* GCG *(Ala)*	GAU *(Asp)* GAC *(Asp)* GAA *(Glu)* GAG *(Glu)*	GGU *(Gly)* GGC *(Gly)* GGA *(Gly)* GGG *(Gly)*	**U** **C** **A** **G**	

Fig.12.1 The genetic code Dictionary (Chain initiation codons," Chain termination codons)*

12.4 Codon and Anticodon

The codon words of DNA would be complementary to the mRNA code words (i.e. DNA codes run in 3'→5' direction and mRNA code words run in 5'→3' direction) and so thereby the three bases forming the anticodon of tRNA (bases of anticodons run in 3'→5' direction). Three bases of anticodon pair with the mRNA attached to the ribosomes at the time of aligning the amino acids during protein synthesis (translation of mRNA into protein which proceeds in N_2→COOH direction).

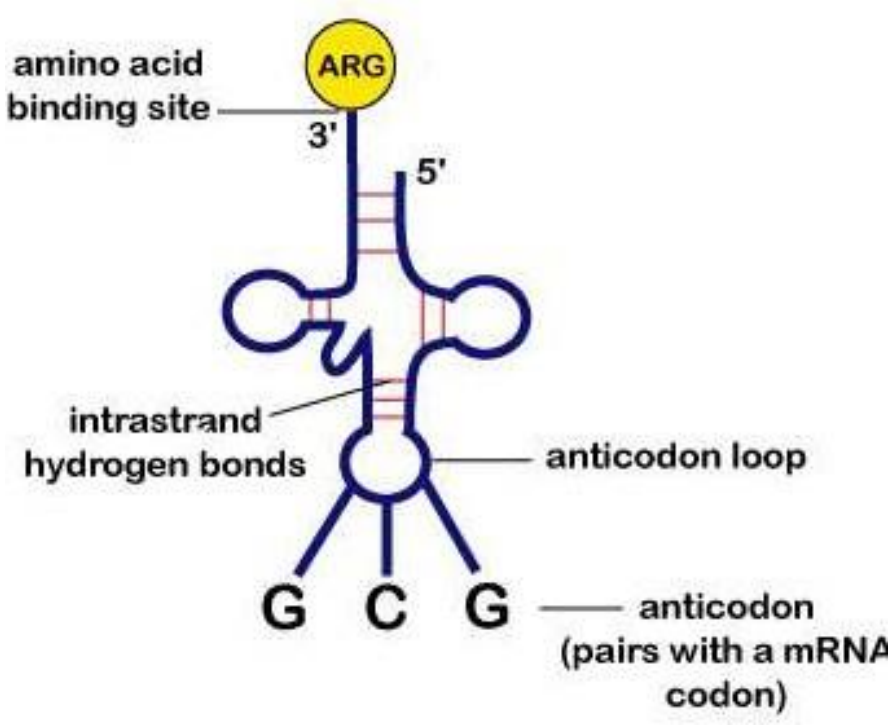

Fig. 12.2 t-RNA molecule with anticodon GCG for specific amino acid Arginine

12.5 Wobble Hypothesis

Wobble means to sway or move unsteadily. To explain the possible cause of degeneracy of codons, Crick (1966) proposed the Wobble hypothesis. A change in nitrogen base at the third position of a codon does not normally cause any change in the expression of the codon because the codon is mostly read by the first two nitrogen bases.

Thus Cricks Wobble hypothesis states that the base at 5' end of the anticodon is not spatially confined as the other two bases allowing it to form hydrogen bonds with any of several bases located at the 3' end of a codon.

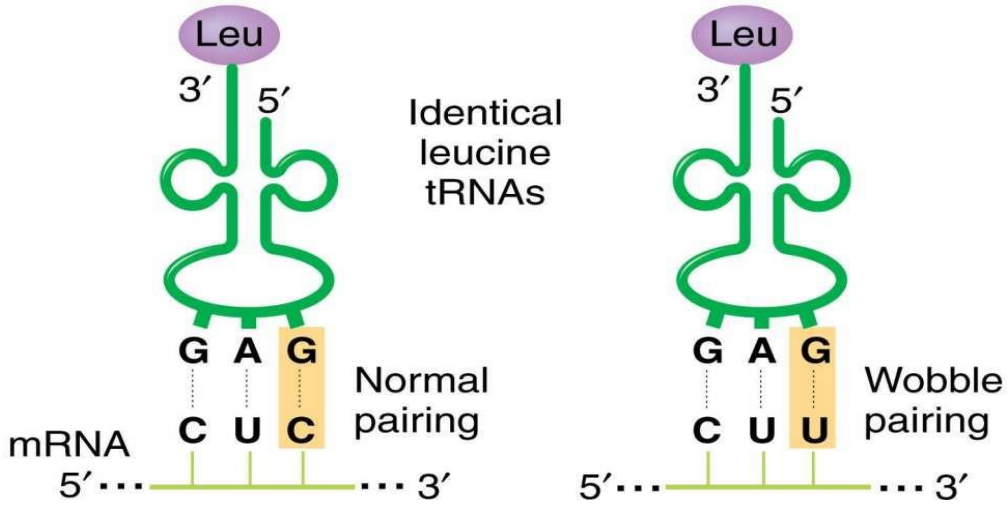

Fig. 12.3 tRNA molecules with same anticodon for decode different code, showing 3rd Wobble position

Allowed base pairing combinations according to the Wobble hypothesis (source King 1986)-

5’ bases of codon	3’ base of anticodon
C	G
A	U
U	A orG
G	U,C or A
T	U,C or A

Mutation and the Triplet code-

It is generally considered that the genetic code evolved in such a way as to minimize the effect of mutations. The most common type of mutation is a transition where either a purine is mutated to the other purine or a pyrimidine is changed to the other pyrimidine.

- Transversion are where a pyrimidine changes to a purine or vice versa.
- In the third position, transitions usually have no effect, but can cause change between Met and Ile, or Trp and stop.
- Just over half of transversion in the third position have no effect and the remainder usually results in a similar type of amino acid being specified
- In the second position transitions will usually result in a similar chemical type of amino acid being used but transversion will change the type of amino acid.
- In the first position mutations (both transition and transversion) usually specify a similar type of amino acid and in a few cases it is the same amino acid.

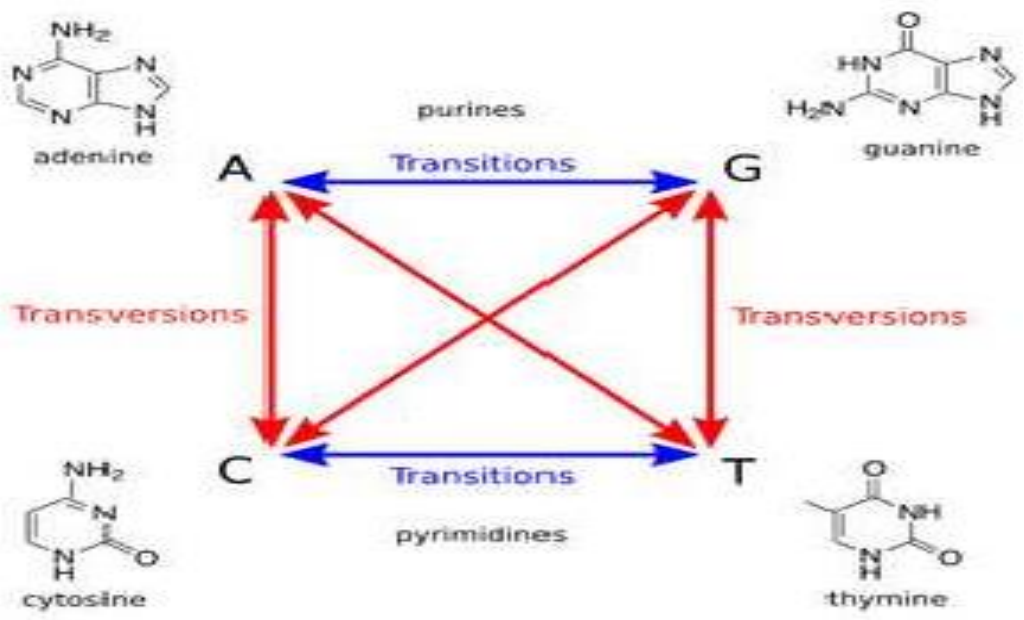

Fig. 12.4 Common mutation due change in bases

The genetic code is redundant but not ambiguous

- After subtracting start (codes for methionine) and stop codons, the remaining 60
- codons code for 19 different amino acids
- This means that many amino acids have more than one codon. Thus the code is redundant. But the code is *not* ambiguous.
- Each codon is assigned only one amino acid, not two or three possible amino acids.
- The genetic code is nearly universal, applying to all species on our planet.
- This common genetic code has great implications in genetic engineering. In mitochondria of Drosophila, yeast, higher plants UGA is codon for tryptophan rather than stops
- In mammalian mitochondria including human, AGG and AGA, they are stop codon instead of arginine.
- Minor variations are found within mitochondria and chloroplasts; other exceptions are few and slight.

12.6 Summary

The relationship between the sequence of amino acids in a polypeptide and nucleotide sequence of amino acids in a polypeptide and nucleotide sequence of DNA or mRNA is called genetic code. The codon words of DNA would be complementary to the mRNA code words (i.e.

DNA codes run in 3'→5' direction and mRNA code words run in 5'→3' direction) and so thereby the three bases forming the anticodon of tRNA (bases of anticodons run in 3'→5' direction). Crick (1966) proposed the Wobble hypothesis. A change in nitrogen base at the third position of a codon does not normally cause any change in the expression of the codon because the codon is mostly read by the first two nitrogen bases.

12.7 Glossary

Anti codons- three bases in a transfer RNA molecule that are complementary to the three bases of a specific codon in messenger RNA.

Codons- are set of three adjacent nucleotides in an mRNA molecule that specifies the incorporation of an amino acid in to a polypeptide chain or that signals the end of polypeptide synthesis. Codons with the latter function are called termination codons.

Mutation-is a change in the DNA at a particular locus in an organism. The term is used loosely to include point mutations in a population.

Wobble hypothesis- hypothesis to explain how one tRNA may recognize two codons. The first two bases of the mRNA codon and anti-codon pair properly, but the third base in the anticodon has some play (or wobble) that permits it to pair with more than one base.

12.8 Self Assessment Questions and Possible Answers

12.8.1 Multiple Choice Questions

1. Mutation means:
   a) Any change in organism
   b) Any non inheritable genetic change
   c) Any environmental induced change in genes
   d) A genetic change, natural of induce but inheritable
2. Wobble Hypothesis is applicable to :
   a) Entire codon of mRNA
   b) Second nucleotide of a codon on mRNA
   c) First nucleotide of a codon
   d) Third nucleotide of a codon
3. If mutation cause a change in nucleotide of a codon at 3rd position, it results into:
   a) Entire defective protein chain translation
   b) No translation of mRNA at all

c) No change in translational product
d) Except for that amino acid, rest of the polypeptide chain will be normal

4. Codons are made of 3 nucleotides, so the codon is called as:
a) Triplet
b) Singlet
c) Uniform
d) Triple

5. The main feature of codons are :
a) These are triplet and continuous
b) These are universal and made of deoxyribonucleotides
c) Present on DNA are translate into polypeptide
d) Present on RNA

6. The initiation codon is :
a) AUG
b) AUG or GUG
c) UUA
d) UUA, UAG or UGA

7. Some of the codons are degenerate in nature, it means:
a) At the time of translation they disintegrate into nucleotides
b) More than one codon can code for same amino acid
c) Same codon can code for more than one amino acid
d) None of the above

8. The termination of translation occur, when the codon ready for translation is:
a) AUG
b) AUG or GUG
c) UUA
d) UUA, UAG or UGA

9. For translating a codon, its corresponding anticodon is present on:
a) mRNA
b) tRNA
c) rRNA
d) All of them

10. The reading of codon starts from:
a) 5’ end
b) 3’ end
c) Any end
d) Anywhere in between

12.8.2 Very Short Questions

1. Define genetic code.
2. Write important properties of genetic code
3. How was it deduced that the code is triplet in nature?
4. What is meant by Wobble Hypothesis?
5. How Wobble Hypothesis in silencing the incidences of mutations?
6. How inversion or transversion influence the result of mutations during translation?
7. Justify the statement "codons and anticodons are complementary to each other**.**

12.8.1 ANSWERS:-

1. D
2. D
3. C
4. A
5. A
6. B
7. B
8. D
9. B
10. A

12.9 References and Suggested Readings

i) Molecular biology-P.C. Turner, A.G. McLennan, A.D. Bates & M.R.H. white.

ii) Principles of Genetics- D.Peter Snustad, Michael J. Simmons.

iii) Hand Book of Life Science- Sunil Patel, Rukum. S. Tomar, Harsukin Gazera, B.A. Golakiya & Manoj Parakhia.

iv) Cell Biology, Genetics, Molecular Biology, Evolution & Ecology- P.S.Verma, V.K. Agarwal.

v) Lehninger- Principles of Biochemistry. 4th edition- David L. Nelson, Michael M. Cox.

vi) Color Atlas of Biochemistry-2nd edition – J. Koolman, K. H. Roehm

vii) Genetics- Benjamin A. Pierce.

viii) Genetics & Molecular Biology- 2nd edition.-Robert Schleif.

12.10 Terminal Questions

G- Long answer questions-

vii) What is genetic code? What are the essential qualities for a universal genetic code?

viii) Discuss the properties of genetic code.

ix) Write an essay on genetic code.

H- Short answer questions-

vii) What is wobble hypothesis?

viii) Differentiate between codon and anticodon.

ix) What do you mean by a nonsense codon?

I- True and False-

vii) Genetic code was given by Watson & Crick. ( )

viii) Those codons which code for amino acids are called sense codons. ( )

Answer

(i) False, (ii) True

LAB WORK ZO(N)-102L

UNIT 1 PERMANENT SLIDE PREPARATIONS

Contents

1.1 Objectives

The study of Permanent slide preparation of Obelia colony: Pharyngeal and septal nephridium of earthworm,parapodia of Nereis and Heteronereis,gill,radula and osphradium of Pila,salivary gland, mouth parts and trachea of cockroach, gills lamina of Uniostaocyst and hastate plate of prawn.

1.2 Introduction

A microscope slide is a thin flat piece of glass, typically 75 by 26 mm (3 by 1 inch) and about 1 mm thick, used to hold objects for examination under a microscope. Typically the object is placed or secured ("mounted") on the slide, and then both are inserted together in the microscope for viewing. This arrangement allows several slide-mounted objects to be quickly inserted and removed from the microscope, labeled, transported, and stored in appropriate slide cases or folders. Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips, slide clamps or a cross-table which is used to achieve precise, remote movement of the slide upon the microscope's stage.

The following points highlight the seven main processes involved in preparation of permanent slides. The processes are: 1. Killing 2. Fixing and Hardening 3. Staining 4. Dehydration 5. Clearing 6. Mounting 7. Labelling.

1.3 Method of Microscopic Preparations

For microscopic studies the specific material tissue organs or small organism) is mounted on a glass slide. There are two methods of mounting the material on slide.

I. Temporary
II. Permanent

I. Temporary mounting:-

The temporary mount is prepared either in glycerin, water or normal saline. The material is first washed in tap, then stained and differentiated. Drop of mounting medium (glycerine and water)is placed on center of the slide. The material is then transferred into that drop. It is then covered neatly with a cover slip. The excess of glycerine or water is absorbed by piece of blotting paper. Mount prepared by this method can be used for study only for few hours, after which material loses its original form due to diffusion and other post mortem changes.

II. Permanent mounting:-

But for the study of microorganisms, smaller animals and histological studies of tissues, an elaborate technique is employed for making their permanent preparations. These smaller objects are mounted in balsam on a slide. There is a series of processes by which a living organism or its tissue is made fit for microscopic examination in a permanent state. The utility of permanent preparation is that the animal cell or tissue remains as such without undergoing major changes. The permanent preparation includes:

(1) Killing and narcotization
(2) Fixing
(3) Washing
(4) Staining
(5) De-staining or removal of excess of stain.
(6) Clearing or de-alcoholization.
(7) Mounting on slide

1. Killing and Narcotization :-

The first step in permanent preparation is killing instantaneously in order to prevent the change in form of the object as it has in living condition and immediately fixing the objet. Sometimes killing is preceded by narcotization. The narcotics used are chloroform, menthol, ether, alcohol, acetone, etc. the purpose of narcotization and killing in important as to have the same form and chemically constructed tissue or organisms as it had during its lifetime. In certain cases, for smaller animals killing is heating done by the slide.

2. Fixing :-

Fixing is done with various fixative agents for histological elements. Fixative is essential in every type of microscopic preparations, either for sections or for whole mounts and also in larger specimens. The function of fixation is manifold:

1) The tissues become hard and hardening resists further post-mortem changes.
2) Fixative agent coagulates and renders insoluble elements of tissues which are dissolved in further processing.
3) The fixative agent renders insoluble the various constituent elements of cells, alters their refractive indices and thus makes them optically differentiated under the microscope. Because of Brownian motion there is no possibility of material but we must bear in mind that fixed details are the coagulation artifact of the living structures.

   Various fixative agents generally used are absolute alcohol, 90% alcohol plus

glycerine, picric acid, corrosive sublimate, formal, osmium tetra oxide and nitric acid with or without water. Depending upon the material, corrosive sublimate or alcohol Carnoy's fluid for cytological studies and other fixative for histochemical studies.

3. Washing :-

Washing is essential as by this process the uncombined and excess of fixative agent is removed. The presence of fixative agent in tissues or cells will inhibit good staining. The washing agent depends upon the type of fixative agent used. As alcoholic picric acid in waterіs removed by 70% alcohol. Formal and corrosive sublimate are washed with water distillate. Sublimate is washed in alcohol.

4. Staining:-

The tissue or cell components are stained in various dyes. The dye makes the tissues distinctin its histological sphere. The various dyes are Orange G. Bordeaux red, Sudan's Congo red, Alizarins oxyquinoine, methylene blue, neutral red, borax carmine, heamatoxylin,e picro- indigo carmine, eosin and Gower's carmine. Mainly two kinds of stains are used.

1. Nuclear stains. Stains the nuclear parts of the cells, such as Delafield's or Erhlich's haematoxylin.
2. Cytoplasmic stains such as borax carmine, picro-indigo carmine, Gower's carmine and eosin, etc., which stain cytoplasm.

For general staining borax carmine is used aqueous stains are prepared in water whereas alcoholic stains are prepared in alcohol. When a single stain is used the process is called assimple or single staining. In some cases two stains, i.e., nuclear and cytoplasmic are used mand this is called as double staining. Generally single stain is used for whole mounts but for protozoan's etc., both cytoplasmic and nuclear stains are used.

5. Destaining:-

The removal of excess of stain is called as destaining or differentiation. De-staining agents are acid alcohol or acid water. The acid alcohol is used with alcoholic stains while acid water is used with aqueous stains.

6. Dehydration :-

This process is meant for removal of water from the tissues. The dehydration prevents putrefaction or decaying and maintains the same shape and size of tissues or cells. The moisture or water in tissues absorbs various germs of destructive nature so that the tissuemay be destroyed, hence the passing the mounting material through various grades of alcohol, such as 30, 50, 70, 90 and 100% alcohols. The tissue is soaked in gradually increasing strengths of alcohol. The lower grads prepared either from 90% or absolute alcohol. The dehydration is carried out in corked or glass-stopper tubes.

7. De-alcoholization or clearing :-

After dehydration, transparency in tissues is obtained by treating with a clearing agent, which removes alcohol and makes the tissue clear and transparent. The clearing agents are wood oil, clove oil, xylol and benzol, etc. Xylol is most commonly employed and it makesthe tissues hard and brittle. Clove oil is a superior clearing agent especially in the whole mounts. It also possesses higher index of refraction than balsam mounting media.

8. Mounting:-

Mounting forms the end of permanent preparation the choice of mounting media is not much but they should have the same refractive index as that of the cleared tissue. The refractive index of such a stained, dehydrated and cleared cells is 1.54. Canada balsam or D.P.X has almost the same refractive index. Mounting is an easy process. The tissue is kept over glass slide in a drop of balsam and cover-slip is lowered slightly. After mounting, the excess of balsam on the slide, as generally happens with beginners, should be removed with cotton soaked with the balsam has dried. For much better finishing the edge of the cover-glass may be ringed with cement such as gold seal or a varnish. The air bubbles present in balsam under cover-glass should be removed by gentle heating.

During all the chemical bathing of tissues, two changes of each reagent are necessary. The time of keeping tissue in various reagents may vary from 5 to 15 minute.

9. Precautions and Instructions:-

1. The articles, such as slide, cover slips and instruments should be perfectly cleaned.
2. The working place should be kept in order.
3. During dehydration, the tissues should be kept in tightly closed cork or glass stopper tubes. The opened tube will spoil material by absorbing moisture from atmosphere. Even breathing closely with dehydrating tube is undesirable.
4. The change of solution is done very quickly, reducing time of exposure to atmosphere to minimum.
5. The chemicals used once should not be reutilized.

6. The Canada balsam used should be clean, dust-free and not viscous.

1.4 Methods for slide preparation

11.4.1 Protozoa (Paramecium)

Classification:-

Phylum …… Protozoa → Unicellular

Sub-Phylum …... Ciliophora → Ciliary movement in all stages.

Class ……Ciliata → Cilia present throughout life.

Sub Class ……. Euciliata → Cytopharynx, contractile vacuole, mega and micronucleus present

Order.....Holotricha → Equal cilia.

Sub-order ……. Trichostomata → Mouth leads in cytopharynx.

Family.....Paramecidae → Oral groove present

Genus.....Paramecium

Culture preparation of Paramecium:-

It is found abundantly in the ponds and ditches in decaying vegetation. For culturing paramecia boil 20 grains of wheat plus 20-25 hay steams in 500 cc of distilled water for about 10 minutes. Keep it in dark and cool place for about four days and inoculate it with few paramecia by a micropipette, within little days. The culture will found to contain numerous paramecia.

Examination in living condition:-

Take a clean slide .Through the micropipette put a drop of water from the culture medium of Paramecium Examine the slide under low magnification of compound microscope .Observe the fast moving Paramecia and their cytopharynx.

Many protozoan's' move very fast. So, they must be slowed down for proper examination. This is done in three ways:

1. Protozoan's are slowed in 10per methyl cellulose solution. Dissolve 10 gm of methyl cellulose solution50cc of water. Boil, cool and make up to 100 cc .The solution slows down the movement.

2. 2. % sodium carboxymethyl cellulose solution is also good for slowing down protozoan movement. Boil 2gm of sodium methyl cellulose. Cool.

3 .Nickel sulphate acts as anaesthetic .By keeping the animal for 15 min can restrict their movement.

Permanent preparation:-

For the free living and fast moving protozoans, they are first made non motile on a glass slide coated with albumin. Then the small drop of culture containing Paramecium is fixed with an equal drop of 1% of Agar solution melted (1gm of Agar in 100 cc of water distillate) at 45^0 C
.The solution become jelly like. The animal may survive for 30 min. They are fixed with 90% alcohol or by a drop of Schaudinn's fixative.

Pass the slide through descending grade of alcohol 90%, 70%, 50% and 30% and distilled water. Stain both nuclei and cytoplasm by double staining .Stain first with Ehrlich's haemotoxylin .Destain in acid water and wash in tap water. Again dehydrate in ascending gradeof alcohol. After 90% alcohol stain in cytoplasm Eosin .Keep in 100% alcohol, Clear in xyloland mount on D.P.X.

1) **Feeding experiment**: As *Paramecium* is a ciliary and selective feeder. The cilia direct the food particles into the cytopharynx or gullet. Its food particles consist of bacterial etc. The food is collected into membranous vesicle which is formed just below the gullet. When the vesicle is filled with food it is detached and is called food vacuole.In paramecium food particle is circulated in the body by more or less definite path by slow streaming movement of endoplasm called cyclosis. Digestion and assimilation take place during the journey of food vesicle, First it is alkaline and then acidic and again alkaline.

2) **For observing cyclosis: Take** a drop of culture medium of Paramecia over a slide. Add a little yeast Congo red in a drop of water. The Congo red is taken into the food vacuole .Observe under low magnification along with the movement of Congo red in Foodvacuole.

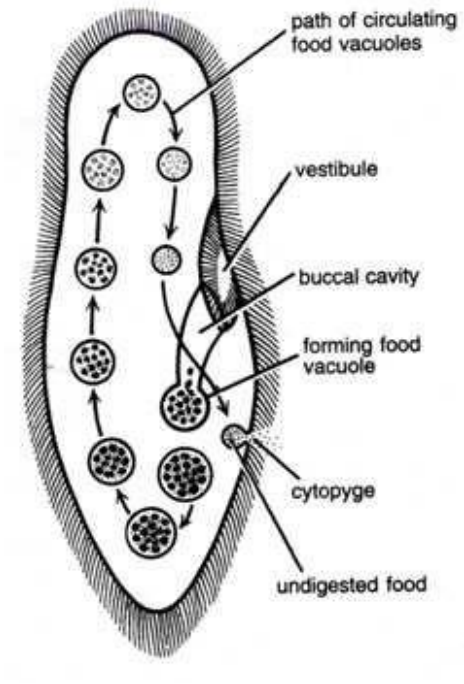

Fig.1.1 Paramecium showing Cyclosis

Distribution: It has cosmopolitan distribution.

Habit and Habitat:

Paramecium caudatum is commonly found in freshwater ponds, pools, ditches, streams, lakes, reservoirs and rivers. It is specially found in abundance in stagnant ponds rich in decaying matter, in organic infusions, and in the sewage water. *Paramecium caudatum* is a free-living organism and this species is worldwide in distribution.

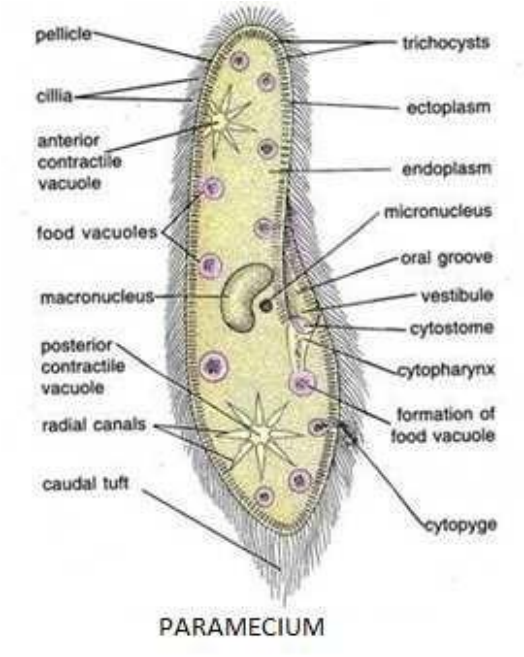

Fig.1.2

Comments:-

1. Commonly called as slipper animalcule, being microscopic, elongated slipper-shaped, cigar-shaped or spindle shaped.
2. Most familiar and extensively studied protozoans.
3. Anterior end is bluntly rounded, while posterior end is pointed.
4. *P. caudatum* measures 80 to 350 microns, while *P. aurelia* 170 to 290 microns.
5. Pellicle covers the body. It is clear, firm and elastic cuticular membrane. Pellicle has series of polygonal or hexagonal depressions for trichocysts.
6. Cilia cover the entire animal. They are hair-like projections of uniform length, except at posterior end where they are longer and at cytopharynx where they form undulating membrane.
7. Infraciliary system consists of basal bodies and kinetodesmata.
8. Cytoplasm contains ecto- and endoplasm. Ectoplasm has myonemes and rod-shaped trichocysts. Endoplasm contains food vacuoles, granules, meganucleus, micronucleus, anterior contractile, posterior contractile vacuole, fat and glycogen.
9. Trichocysts are rod-shaped bodies consisting of lower trichocyst shaft, basal body and projecting cilium. Cilium project through the hexagonal areas. Trichocysts are discharged to anchor with substratum.
10. Reproduction is by binary fission, conjugation, endomixis, hemixis and automixis.
11. Locomotion is ciliary. Nutrition is holozoic and it shows response to light and temperature, etc.

Identification: Since the animal contains slipper-shaped body and 2 contractile vacuoles whichare star-shaped and has all above features, hence it is Paramecium.

1.4.2 Porifera (Sponge and Gemmules)

Spicules of Sponges

Introduction

The body wall of sponges is supported by various minute crystalline and calcareous bodies calledas spicules. These are secreted by special mesenchymal cells called scleroblasts.

Spicules provide taxonomic characters and are classified according to the axas and rayscalled as axon, actine and actinal respectively. These are of two types:

1) Megascleres-Support skelton
2) Microscleres-Smaller and none supporting.
3) These are of following types

a) Monaxon-consist of single axix,straight or curved
b) Tetraxon-Consist of four rays
c) Triaxon-consists of three axes

d) Polyaxon-Having several equal rays

Spicules generally support and protect the body and helps in identification classification and metabolism.

Method for Slide preparation:-

For extraction of spicules, boil a small portion of sponge in 15-20%potassium hydroxide solution in a test tube till cells are dissolved. The spicules settle in the bottom. Decant the KOH solutionand wash the spicules several times in tap water. Pass the spicules in ascending series of alcohol, 30%, 50%, 70%, 90% and 100% alcohol. Dealcoholize or clear with xylol and mount on a slide after pipetting the spicules. There is no need of staining.Study under the microscope and note different type of spicules as monaxon, triaxon, tetraxon etc.

Comments:

1. Sponge body wall is supported by various minute, crystalline and calcareous bodies called as spicules, which are secreted by special mesenchymal cells called as scleroblasts.
2. Spicules provide taxonomic character and are classified according to the axes and rays, spoken of as axon, actine or actinal respectively.
3. Spicules are of two types: (i) Megascleres or supporting skeleton, (ii) Microscleres small and non supporting. Kinds of Megascleres are as follows;

   (i) **Manaxon** consists of a single axis, straight or curved. They may be styles, rhabds and tylots.
   (ii) **Tetraxon** consists of four rays. It also includes triradiate or triactinal spicules.
   (iii) **Polyaxon** having several equal rays. Amphidisk spicules are found in fresh water sponges. In this type, the rhabdom contains disks at both ends. The arrangement of different types of spicules could be seen in Sycon.
4. M**icroscleres** are found throughout the mesenchyme and include spires and asters.
5. Spicules support and protect the body. They are helpful in identification, classification and metabolism.

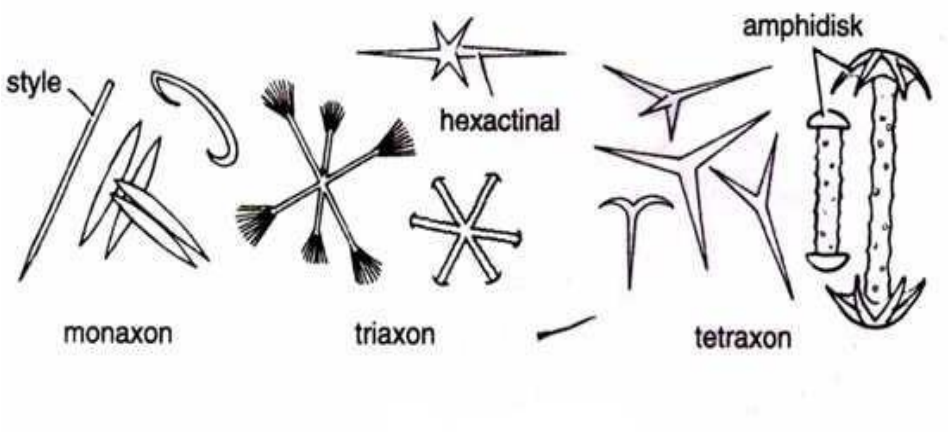

Fig.1.3 Spicules

Identification:-

The clear transparent monaxon or triaxon spicules indicate **Spicule** of sponges.

Gemmules:-

Gemmules are asexual reproductive bodies forming a part of regular life cycle.These are endogenous buds which are diagnostics of Porifers,especially of freshwater and a few marine sponges.Gemmulation or endogenous budding is a peculiar mode of reproduction under unfavourable condition such as excessive cold or drought.

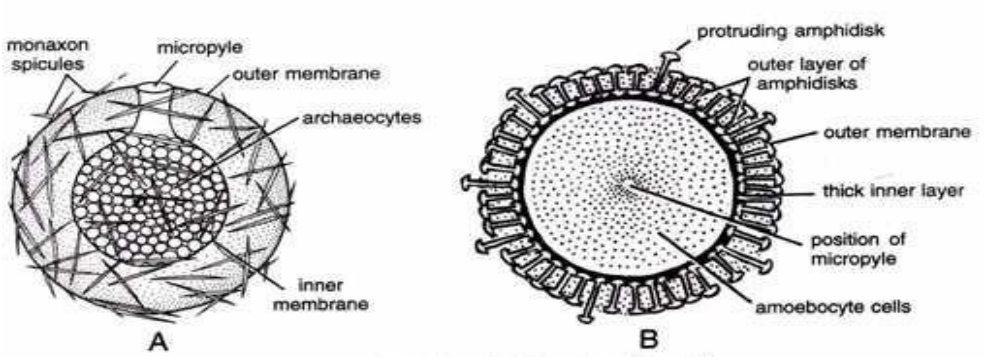

Fig 1.4 A. Gemmule of Ephydatia B. Gemmule of Spongilla

Comments:

1. Gemmules are asexual reproductive bodies forming a part of regular life-cycle .
2. Gemmules or endogenous buds are diagnostic of Porifera and especially of fresh-water and a few marine sponges.
3. Gemmulation or endogenous budding is a peculiar mode of reproduction under unfavourable conditions such as excessive cold or draught.
4. Gemmules contain outer and inner membrane.
5. Gemmule is rounded structure formed by the aggregation of archaeocytes into groups accompanied by trophocytes which are impregnated with food particles of glycoproteins or lipoproteins.
6. **Scleroblasts** secretes the **amphidisk spicules**, which forms a row in columnar layer between outer and inner membrane
7. Gemmules are resistant to external factors such as freezing and drying. Gemmules of fresh water sponge can be kept for 2 years.
8. They hatch at a temperature of 13-21◦C in about 3 days. After hatching, a gemmule gives rise to a young sponge.
9. A full grown gemmule is usually pierced by opening on one side, called a **micropyle**.

Identification: Since the material has micropyle in mature and amphidisk spicules in immature gemmules and has above all features,hence it is Gemmule whole mount.

1.5 Glossary

Aboral	Opposite the mouth.
Amoeboid	Cell movements resembling those of the amoeba.
Angstrom	One thousand of a micron.
Archenteron	Primitive digestive tract of a metazoan embryo, formed during gastrulation.
Autotrophic	Nutrition. Process of nutrition in which an organism manufactures its own food.
Basal disc	Foot of some Cnidaria which is flattened and attaches to a substratum by secretion of a sticky substance.
Binary fission	the type of asexual reproduction by means of which the organism divides into two approximately equal halves.
Buccal	Pertaining to the mouth or oral cavity.
Cnidaria or Coelenterata.	Phylum of animals all possessing cnidoblast structures.
Cnidoblast	Type of cell in which nematocyst is found.
Coelom	The body cavity lined with tissue of mesodermal origin in whichthe digestive and other organs lie.
Conjugation	A method of sexual reproduction in which two unicellular animals untie, exchange nuclear material and then divide as in the Paramecium.
Contractile vacuole	A space in the cytoplasm of certain species of protozoa where fluids collect before being periodically discharged to the outside.
Ctenophora	Radiate phylum of animals possessing comb- such as comb-jellies.
Cuticle	Thin non-cellular outermost secreted by the underlying epidermis.
Cyst	The stage of an organism where it is enclosed in a resistant wall.
Cytopharynx	Pharynx or gullet of a protozoan such as Paramecium.
Cytostome	Cell mouth, for example in Paramecium.
Diplobastic	Derived from two embryonic germ layers, ectoderm and endoderm.
Enteron	Digestive tract, especially in Cnidaria.

Entoprocta	Pseudocoelomate, sessile phylum with U-shaped intestine, mouth surrounded by circle of ciliated tentacles, and opening within cirle.
Extracellular	Outside of the cell or cells.
Exumbrella	Convex, aboral surface of the medusa.
Fission	Asexual method of reproduction by division into two or more approximately equal in size.
Food vacuole	Intra-cellular digestive organelle.
Free-living	Capable of independent existence.
Gastrodermis	Lining of coelenterate digestive cavity.
Gastrulation	Process by which two germ layers, ectoderm and endoderm.
Holophytic	Type of nutrition, found in green plants and in some mastigophores, which involves photosynthesis.
Holozoic	Type of nutrition found in most animals, that involves ingestionand digestion of organic material.
Hydranth	Expand end of a branch of a hydroid colony specialized for vegetative function.
Hydrocaulus	Basal portion of a hydroid colony often branched and root-like used for attachment to substratum.
Hydrotheca	Transparent membrane that extends from the perisarc and surrounds the main part of the hydranth.
Hypostome	Region surrounding the mouth in coelenterates.
Inter-cellular	Between cells.
Intra-cellular	Within cells.
Isogamy	Sexual reproduction involving fusion of two similar gametes but from opposite sexes.
Kinetosome	The basal body of a flagellum or cilium.
Lophophore	Anterior tentacle- bearing area of certain coelomates; serves in food capture.
Mesogloea	Non-cellular jelly-like substance lying between the ectoderm and endoderm in coelenterates.
Metagenesis	Alternation of sexual with an asexual generation in reproduction in the life cycle of a coelenterate such as Obelia.

Myoneme	Type of contractile fibril in certain Protozoa.
Nephridiopore	External opening of an excretory tubule or
nephridium.**Pedal**	Pertaining to father.
Pellicle	The protective layer on the surface of some protozoans, for example, Paramecium.
Penetrant	Largest type of cnidarians nematocyst, containing a coiled tube and spines, used in prey capture.
Peristome	Region around the mouth of a radially symmetrical animal such ashydra.
Phagocyte	Type of white blood cell that engulfs and digests bacteria and other foreign materials.
Pinocytosis	Cellular drinking or intake of fluid.
Plankton	Floating or drifting aquatic organisms, mostly microscopic.
Plasmasol	Relatively liquid cytoplasm.
Pneumoatophore	Air-filled float of siphonophoran hydroids.
Polyp	A tubular coelenterate form.
Prosopyle	One of the surfaces pores opening into a sponge chamber.
Protozoa	A phylum of acellular animals.
Proximal	Near the point of attachment of an organ.
Pseudocoel	A body cavity not completely lined with mesoderm as found in round worms.
Pseudopodia	Blunt temporary protoplasmic projections found in amoeba or in some ameba like cells.
Schizocoel	The coelom formed by the splitting of embryonic mesoderm.
Sedentary	Staying in one place.
Siliceous	Containing silicon dioxide or silica.
Spicule	One of many solid structures that composed the structural framework of a sponge.
Spongocoel	Paragastric or central cavity of a sponge.
Syngamy	Union of gametes in sexual reproduction forming a zygote.

Taxis	A movement response
Tentacle	A flexible arm likes extension from the body of many invertebratessuch as hydra. Used in grasping and movement.
Tentaculocyst	Sense organs of some cnidarians.
Triploblastic	Derived from three primary germ layers-ectoderm, mesoderm, and endoderm.
Vestibule	An outer cavity with an entrance to a (usually) larger, deeper cavity.
Zooid	One of the members of a hydroid or siphonophore colony.

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UNIT 2 PERMANENT SLIDE PREPARATIONS

Contents

2.1 Objectives

The study of Permanent slide preparation of Obelia colony: Pharyngeal and septal nephridium of earthworm,parapodia of Nereis and Heteronereis,gill,radula and osphradium of Pila,salivary gland, mouth parts and trachea of cockroach, gills lamina of Uniostaocyst and hastate plate of prawn.

2.2 Introduction

A microscope slide is a thin flat piece of glass, typically 75 by 26 mm (3 by 1 inch) and about 1 mm thick, used to hold objects for examination under a microscope. Typically the object is placed or secured ("mounted") on the slide, and then both are inserted together in the microscope for viewing. This arrangement allows several slide-mounted objects to be quickly inserted and removed from the microscope, labeled, transported, and stored in appropriate slide cases or folders. Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips, slide clamps or a cross-table which is used to achieve precise, remote movement of the slide upon the microscope's stage.

The following points highlight the seven main processes involved in preparation of permanent slides. The processes are: 1. Killing 2. Fixing and Hardening 3. Staining 4. Dehydration 5.
Clearing 6. Mounting 7. Labelling.

2.3- Method of Microscopic Preparations

For microscopic studies the specific material tissue organs or small organism) is mounted on a glass slide. There are two methods of mounting the material on slide.

III. Temporary
IV. Permanent

III. Temporary mounting:-

The temporary mount is prepared either in glycerin, water or normal saline. The material is first washed in tap, then stained and differentiated. Drop of mounting medium (glycerine and water)is placed on center of the slide. The material is then transferred into that drop. It is then covered neatly with a cover slip. The excess of glycerine or water is absorbed by piece of blotting paper. Mount prepared by this method can be used for study only for few hours, after which material loses its original form due to diffusion and other post mortem changes.

IV. Permanent mounting:-

But for the study of microorganisms, smaller animals and histological studies of tissues, an elaborate technique is employed for making their permanent preparations. These smaller objects are mounted in balsam on a slide. There is a series of processes by which a living organism or its tissue is made fit for microscopic examination in a permanent state. The utility of permanent preparation is that the animal cell or tissue remains as such without undergoing major changes. The permanent preparation includes:

(1) Killing and narcotization
(2) Fixing
(3) Washing
(4) Staining
(5) De-staining or removal of excess of stain.
(6) Clearing or de-alcoholization.
(7) Mounting on slide

10. Killing and Narcotization :-

The first step in permanent preparation is killing instantaneously in order to prevent the change in form of the object as it has in living condition and immediately fixing the objet. Sometimes killing is preceded by narcotization. The narcotics used are chloroform, menthol, ether, alcohol, acetone, etc. the purpose of narcotization and killing in important as to have the same form and chemically constructed tissue or organisms as it had during its lifetime. In certain cases, for smaller animals killing is heating done by the slide.

11. Fixing :-

Fixing is done with various fixative agents for histological elements. Fixative is essential in every type of microscopic preparations, either for sections or for whole mounts and also in larger specimens. The function of fixation is manifold:

1) The tissues become hard and hardening resists further post-mortem changes.
2) Fixative agent coagulates and renders insoluble elements of tissues which are dissolved in further processing.
3) The fixative agent renders insoluble the various constituent elements of cells, alters their refractive indices and thus makes them optically differentiated under the microscope. Because of Brownian motion there is no possibility of material but we must bear in mind that fixed details are the coagulation artifact of the living structures.

Various fixative agents generally used are absolute alcohol, 90% alcohol plus glycerine, picric acid, corrosive sublimate, formal, osmium tetra oxide and nitric acid with or without water. Depending upon the material, corrosive sublimate or alcohol

Carnoy's fluid for cytological studies and other fixative for histochemical studies.

12. Washing :-

Washing is essential as by this process the uncombined and excess of fixative agent is removed. The presence of fixative agent in tissues or cells will inhibit good staining. The washing agent depends upon the type of fixative agent used. As alcoholic picric acid in water is removed by 70% alcohol. Formal and corrosive sublimate are washed with water distillate. Sublimate is washed in alcohol.

13. Staining:-

The tissue or cell components are stained in various dyes. The dye makes the tissues distinct in its histological sphere. The various dyes are Orange G. Bordeaux red, Sudan's Congo red, Alizarins oxyquinoine, methylene blue, neutral red, borax carmine, heamatoxylin,e picro- indigo carmine, eosin and Gower's carmine. Mainly two kinds of stains are used.

3. Nuclear stains. Stains the nuclear parts of the cells, such as Delafield's or Erhlich's haematoxylin.
4. Cytoplasmic stains such as borax carmine, picro-indigo carmine, Gower's carmine and eosin, etc., which stain cytoplasm.

For general staining borax carmine is used aqueous stains are prepared in water whereas alcoholic stains are prepared in alcohol. When a single stain is used the process is called as simple or single staining. In some cases two stains, i.e., nuclear and cytoplasmic are used mand this is called as double staining. Generally single stain is used for whole mounts but for protozoan's etc., both cytoplasmic and nuclear stains are used.

Destaining:-

The removal of excess of stain is called as destaining or differentiation. De-staining agents are acid alcohol or acid water. The acid alcohol is used with alcoholic stains while acid water is used with aqueous stains.

14. Dehydration :-

This process is meant for removal of water from the tissues. The dehydration prevents putrefaction or decaying and maintains the same shape and size of tissues or cells. The moisture or water in tissues absorbs various germs of destructive nature so that the tissue may be destroyed, hence the passing the mounting material through various grades of alcohol, such as 30, 50, 70, 90 and 100% alcohols. The tissue is soaked in gradually increasing strengths of alcohol. The lower grads prepared either from 90% or absolute alcohol. The dehydration is carried out in corked or glass-stopper tubes.

15. De-alcoholization or clearing :-

After dehydration, transparency in tissues is obtained by treating with a clearing agent, which removes alcohol and makes the tissue clear and transparent. The clearing agents are wood oil, clove oil, xylol and benzol, etc. Xylol is most commonly employed and it makes the tissues hard and brittle. Clove oil is a superior clearing agent especially in the whole mounts. It also possesses higher index of refraction than balsam mounting media.

16. Mounting:-

Mounting forms the end of permanent preparation the choice of mounting media is not much but they should have the same refractive index as that of the cleared tissue. The refractive index of such a stained, dehydrated and cleared cells is 1.54. Canada balsam or D.P.X has almost the same refractive index. Mounting is an easy process. The tissue is kept over glass slide in a drop of balsam and cover-slip is lowered slightly. After mounting, the excess of balsam on the slide, as generally happens with beginners, should be removed with cotton soaked with the balsam has dried. For much better finishing the edge of the cover-glass may be ringed with cement such as gold seal or a varnish. The air bubbles present in balsam under cover-glass should be removed by gentle heating.

During all the chemical bathing of tissues, two changes of each reagent are necessary. The time of keeping tissue in various reagents may vary from 5 to 15 minute.

17. Precautions and Instructions:-

7. The articles, such as slide, cover slips and instruments should be perfectly cleaned.
8. The working place should be kept in order.
9. During dehydration, the tissues should be kept in tightly closed cork or glass stopper tubes. The opened tube will spoil material by absorbing moisture from atmosphere. Even breathing closely with dehydrating tube is undesirable.
10. The change of solution is done very quickly, reducing time of exposure to atmosphere to minimum.
11. The chemicals used once should not be reutilized.
12. The Canada balsam used should be clean, dust-free and not viscous.

2.4 Methods for slide preparation

2.4. 1- Coelenterate (Obelia Colony & Obelia Medusa)

OBELIA:-

Obelia is colonial, mainly sedentary hydrozoan zoophyte attached to the seaweed, hills and rocks.It is mostly found in shallow water and also up to approximately 250 ft.deep.

Method for slide preparation:-

Coelenterates are first narcotized in water mixed with menthol crystal or Magnesium sulphate. After decanting the narcotizing liquid,fix the animal by adding drop by drop formol.(commercial preparation).These are then preserved in 70 %alcohol or 5%formalin solution.

For making permanent mount ,keep the material in 70%alcohol,then stain in borax carmine,if overstain ,destain with acid alcohol. Dehydrate in 70%, 90% and 100% alcohol. Clear in xylol or benzene and finally mount on Canada balsam. Then study under the microscope, draw the diagram, label them and note down the characteristic features.

OBELIA COLONY:-

Classification:

Phylum ……. Coelenterata - Tissue grade, diploblastic and acoelomate.Class ……. Hydrozoa - Hydroids: medusa with velum.

Order …… Hydroidea - Polypoid generation well developed

Sub order …….. Calyptoblastea - Hydranths have hydrotheca and gonophores with gonotheca.

Genus..... Obelia

Habit and habitat:

Obelia is colonial, marine, sedentary hydrozoan zoophyte, attached to seaweeds, shells and rocks.

Distribution:-

Its range is from the Arctic region to the Gulf of Mexico and the Pacific coast, and from

Southern California to Oregon. it is found in shallow watter and also upto approximately 250 feet deep.

Comments:-

1) It is a dimorphic colony in the form of small seaweed filaments, measuring several cm in height. The filaments may be horizontal and vertical. The colony consists of several parts.
2) **Hydrorhiza**: It is basal or horizontal portion called as stolon or rhizostome, which is meant for attachment to substratum. Hydrorhiza gives vertical branches called hydrocaulus.
3) **Hydrocaulus** gives alternate branches that terminate into individual zooids called as polyps and medusa.
4) **Coenosarc:** Stems and zooids are made of a living hollow, cellular tube called as coenosarcs. It is made up or ectoderm, endoderm and mesogloea.
5) Stems and zooids are made up of two components : (i) Outer protective tough, transparent non-cellular covering called as **perisarc** (ii) **mesogloea** (iii) inner living hollow cellular tube called **coenosarcs**.
6) Zooids consist of polyp and medusa.
7) Medusa grows at the base of polyp-bearing branches and is enclosed in blastostyles. Medusa is composed of upper exumbrellar and lowr sub-umbrellar surfaces, manubrium and gonads. Free medusa occurs in the life cycle. It is a reproductive zooid.
8) Polyp is a bell-shaped cup made up of lower cub-shaped hydrotheca and upper hypostome. Hypostome is a feeding zooid having circlet of 24 nematocyst bearing tentacles.
9) Growth of the colony is sympodial, i.e., each new hydranth arises as bud from the stem, just proximal to the next youngest polyp.
10) It reproduces asexually and sexually.

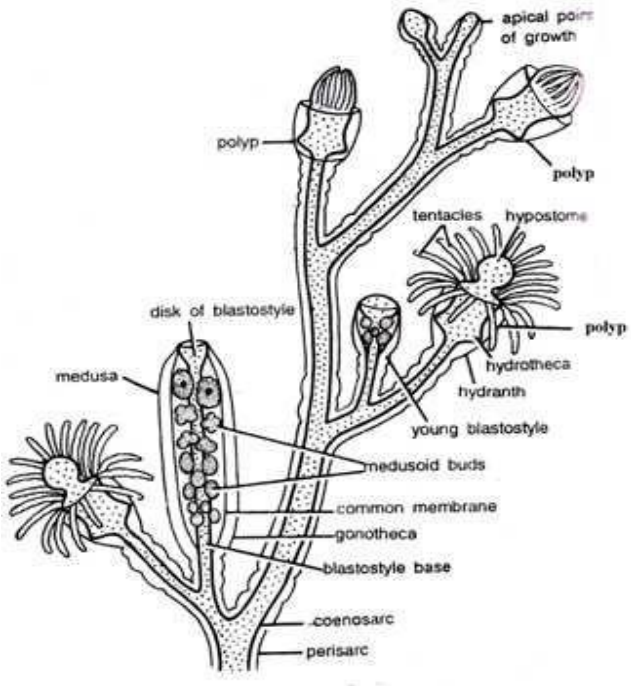

Fig. 2.1 Obelia colony

Identification:

The colony has alternate branches of polyps, blastostyles and all above features, hence it is **Obelia**.

Obelia: Medusa:-

Comments:

1) Medusa is a modified zooid for sexual reproduction.
2) It is a solitary free-swimming zooid, originating from blastostyles.
3) Medusa is umbrella-like and has convex exumbrellar and concave sub-umbrellar surfaces with well defined radial symmetry.
4) Umbrellar edge contains radially symmetrical tentacles.
5) Base of fully grown tentacle is thickened to tentacular bulb which contains a number of stinging cells.
6) In the four radial positions each tentacular bulb contains two otocysts, which are hollow and balancing organs containing calcareous otoliths.
7) Manubrium hangs from the centre of sub-umbrella, having mouth.
8) Mouth communicates with 4 radial canals which join with circular canal lining umbrellar margin which all around contains velum.
9) Beneath the radial canals are gonads lying in Sub-umbrellar ectoderm.

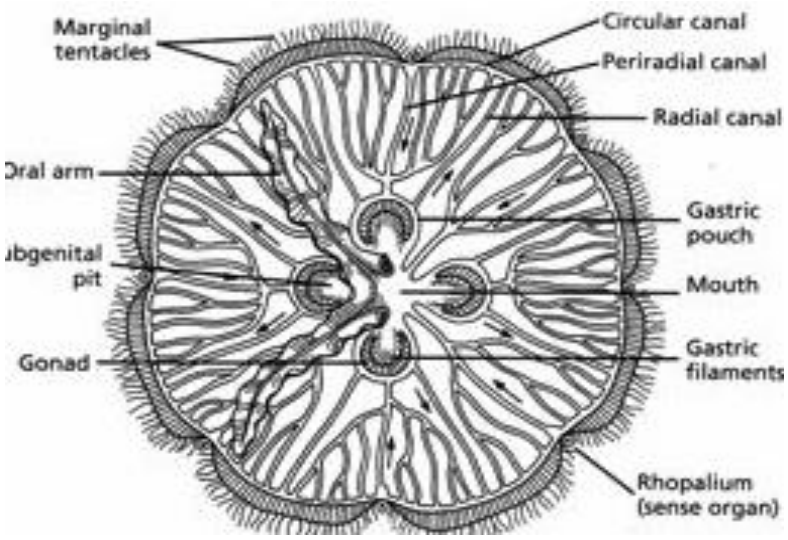

Fig.2.2 Medusa: Obelia

Identification:

Since the mount has circular tentaculated body, 4-radial rounded gonads and all above features,hence it is obelia medusa a very favourite slide-spot.

2.4.2- Arthropoda (Mouth parts of Honey Bee, Butterfly, Cockroach and Grasshopper)

MOUTH PARTS OF INSECTS:-

Insects constitute the largest group of animals in the Animal Kingdom. They have developed different feeding habits as their food differs variously. So for this purpose, they have got certain appendages in their head around the mouth; these appendages together constitute the mouth parts. The mouth parts of insects, therefore, grouped into two main categories; chewing or mandibulate type and sucking or suctorial type

Basically, the mouth parts of insects include a pair of mandibles, a pair of labium or first maxillae and the lower lip represented by and the maxillae and the lower lip represented by fused second pair of maxillae. In chewing type of mouth parts, the mandibles are well developed and the maxillae are simple as found in Orthopterans like cockroaches and grasshopper. These mouth parts are adapted for cutting or biting and chewing or crushing the food. In suctorial type of mouth parts, the mandibles are vestigial, e.g., lepidopteron or absent, e.g., housefly or blade-like, e.g., honeybee or in the form of piercing needles or stylets, e.g., mosquito. The maxillae, however, exhibit modifications in various ways for piercing and sucking the food.

The mouth parts of insects are, however, classified into following five types:

Chewing type:-

These consist of the labrum forming upper lip, mandibles, first maxillae, second maxillae forming lower lip, hypopharynx and the epipharynx.The labrum is median, somewhat rectangular flap-like. The mandibles are paired and bear toothed edges at their inner surfaces; they work transversely by two sets the first maxillae are paired and lie one on either side of the head capsule behind the mandibles. Each possesses a five-jointed maxillary palp which is atactile organ. The first maxillae help in holding the food. The second maxillae are paled but fused to from the lower lip. Its function is to push the masticated food into the mouth. The hypopharynx is dingle median tongue-like process at whose base the common salivary duct opens. The epipharynx is a single small membranous piece lying under the labrum and bears taste buds. This type of mouth parts are found in Orthopteran insects like cockroaches, grasshoppers, crickets, etc. These are also found in silver fish, termites, earwigs, beetles, some hymenopterans and in caterpillars of Lepidoptera.

2. Chewing and lapping type:-

This type of mouth parts are modified for collecting the nectar and pollen from flowers and also for moulding the wax, as is found in honeybees. They consist of the labrum, epipharynx,

mandibles, first pair of maxillae and second pair of maxillae. The labrum lies below the clypeus,

below the labrum is a fleshy epipharynx which is an organ of taste. Mandibles are short, smooth and spatulated, situated one, either side of the labrum; used in moulding wax and making the honeycomb. The labium (second pair of maxillae) has reduced paraglossae, the glossae are united and elongated to from the so called retractile tongue, at its tip is a small labellum or honey spoon. The labial palps are elongated. The glossa is used for gathering honey spoon. The labial palps are elongated. The glossa is used for gathering honey and it is an organ of touch and taste. The first pair of maxillae are placed at the sides of labium, they bear small maxillary palps,lacinia is very much reduced but galea are placed at the sides of labium, they bear small maxillary palps, lacinia is very much reduced but galea are elongated and blade-like; The galea and labial palps form a tube enclosing the glossae which moves up and down to collect nectar from flower nectarines. The nectar is sucked up the through the tube, so formed, by the pumping action of the pharynx. The labrum and mandibles help in chewing the food

3. Piercing and sucking type:-

This type of mouth parts are adapted for piercing the tissues of animals and plants to suck blood and plant juice, and found in dipteran insects like mosquitoes and hemipteran insects like bugs, aphids, etc. They usually consist of labium, labrum and epipharynx, mandibles, maxillae (1^{st}pair) and hypopharynx.

4. Sponging type:-

This type of mouth parts are adapted for sucking up liquid or semiliquid food and found in houseflies and some other flies. They consist of labrum-epipharynx, maxillae, labium and hypopharynx; mandibles are entirely absent.In fact, in this type of mouth parts, the labium, i.e., lower lip is well developed and modified to form a long, fleshy and retractile proboscis. The proboscis is divisible into three distinct parts: (i) rostrum or basiproboscis: it is broad, elongated and cone-shaped basal part of proboscis articulated proximally with the head and bears a pair of elongated and cone-shaped basal part of proboscis articulated proximally with the head and bears a pair of unjointed maxillary palps representing the maxillae, (ii) haustellum or mediproboscis; it is the middle part of proboscis bearing a mid-dorsal oral groove and a ventral weakly chitinized plate-like theca or mentum. A duct and closes the grooved of labrum epipharynx form below. The labrum-epipharynx is a long, somewhat firmed and grooved structure covering the oral groove. The food canal or channel is, thus, formed by labium – epipharynx and the hypopharynx and (iii) labella or distiproboscis; it is the distal part of proboscis and consists of two broad, flattened and oval spongy pads having a series of channels pseudotracheae. These open externally by a double row of tiny

holes through which liquid food is taken in.

5. Siphoning type:-

This type of mouth parts are adapted wonderfully for sucking flower nectar and fruit juice, found in butterflies and moths belonging to the order Lepidoptera of class- Insecta. They consist of small labrum, coiled proboscis, reduced mandibles and labium. The hypopharynx and epipharynx are not found.

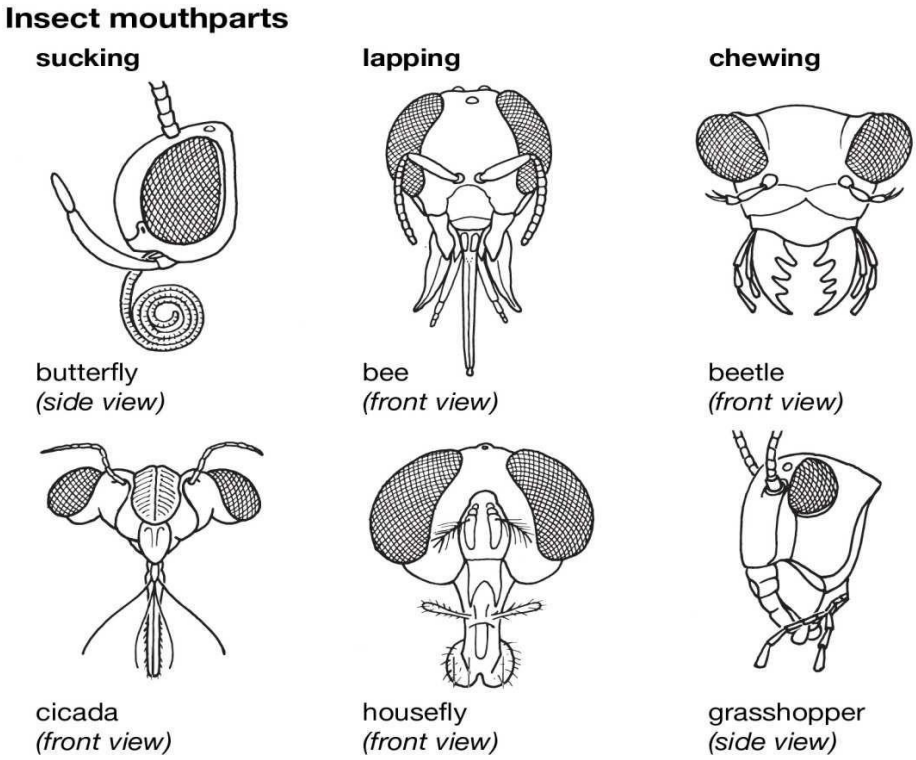

Fig.2.3 Mouth parts of Insects

Slide preparation method:-

For making permanent mount of mouth parts of honey bee, butterfly and cockroach, first cut the head of the above insects. Boil the head in 5% KOH for some time, till the chitin is dissolved.

Then wash in water, dehydrate in 30%,50%,70% alcohol. Stain in picro-indigo carmine or acid fuchsin, dehydrate in 90% and absolute alcohol. Clear in xylol or benzene and finally mount in Canada balsam. Study under the microscope draw the diagram and note down the characteristic features,

Butterfly: - Head and Mouth Parts

Comments:-

1) Butterfly, belonging to order Lepidoptera, contains siphoning or sucking mouth parts. Head may be examined under binocular microscope for mouth parts.

2) Head of butterfly is composed of large compound eyes and antennae. It is broad and contains siphoning type of mouth parts.

3) Mouth parts are composed of small labrum in front of clypeus, triangular labium and coiled proboscis.

4) Mandibles are absent

5) Proboscis is composed of elastic cuticle and greatly elongated galeae of maxillae, grooved internally forming food canal for nectar.

6) Proboscis lies in coiled stage, but it immediately uncoils and protrudes in response to a food stimulus, due to rise in blood pressure.

7) Labium is triangular and plate-like containing labial palps.

8) Other joints of maxillae and maxillary palps are reduced or vestigial.

9) Head contains ventral groove for proboscis.

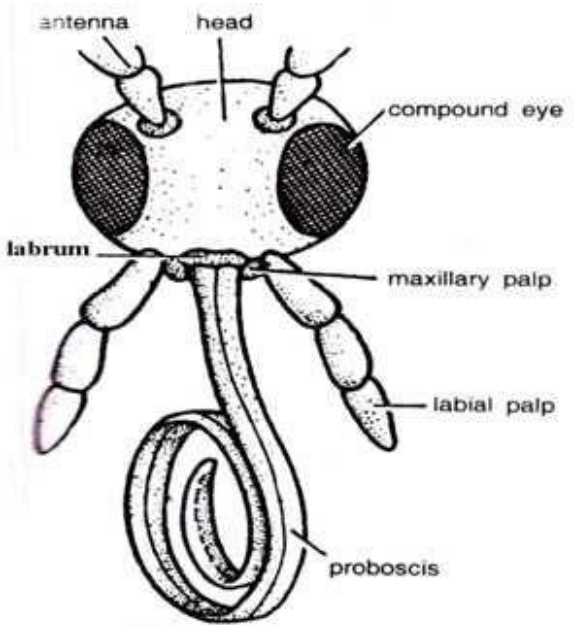

Fig 2.4 Head and mouth parts of butterfly

Identification:-

Since the mouth contains coiled proboscis, hence it is the mouth parts of butterfly.

APIS:-

Honey – Bee: Mouth Parts of

Worker:-Comments:

1) Honey-bee belonging to the order Hymenoptera contains rasping and lapping mouth parts, adapted for collection of nectar and pollen.
2) Head is triangular, containing large compound eyes, 3 ocelli antennae and mouth parts.
3) Mouth parts are composed of spoon shaped mandibles, labrum and maxillae devoid of lacinia.
4) Mandibles are smooth and spatulate type, food on either side of the labrum.
5) It contains vestigial maxillary palps and blade-like galea.
6) Labellum is spoon shaped, grooved internally forming a tube and is called as tongue.
7) Epipharynx is soft and triangular lying below the labrum. Cardo and stipes are well developed.
8) Liquid food taken along tongue is converted into honey in honey-sac by enzymes from salivary glands.
9) Prementum contains segmented labial palps, Paraglossae and glossae.
10) Honey-bee also moulds waxes in its hive.

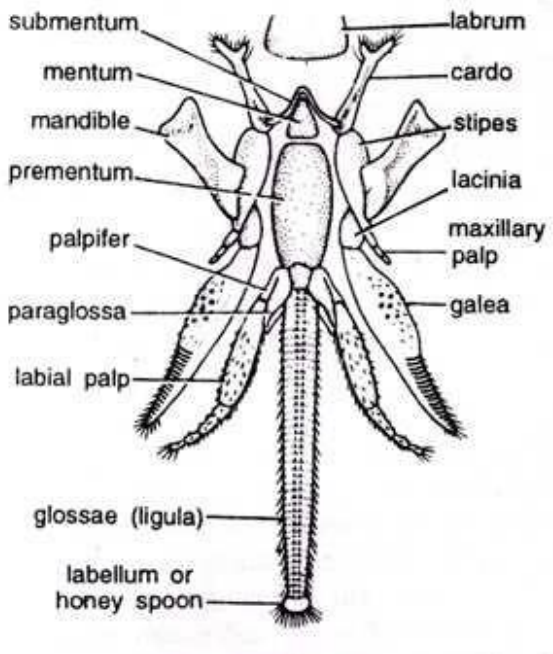

Fig 2.5 Apis Honey –bee Mouth parts of worker

Identification: -

Since the mount contains spoon-shaped labellum, hence these are mouth parts of works, honey-bee.

COCKROACH: HEAD AND MOUTH PARTS

Comments:-

1) Cockroach, belonging to order- Orthoptera, contains chewing mouth parts.
2) Head is dorso-ventrally elongated and is composed of antennae, large compound eyes and mouth parts.
3) Mouth parts consist of (i) labrum, (ii) mandibles and (iii) maxillae.
4) Labrum protects the mouth. Mandibles are simple and toothed.
5) Maxilla has two part-cardo and stipes. Stipes contains internally lacinia, medially galea and extemally maxillary palp.
6) Labium is composed of submentum, postmentum and prementum.
7) Prementum carries glossa internally, Paraglossa medially and palpiger externally.
8) Maxillary and labial palps are tasting organs.

Identification:-

Since the mount shows definitely arranged various parts especially labium maxilla and all abovefeatures, hence it is mouth parts of cockroach.

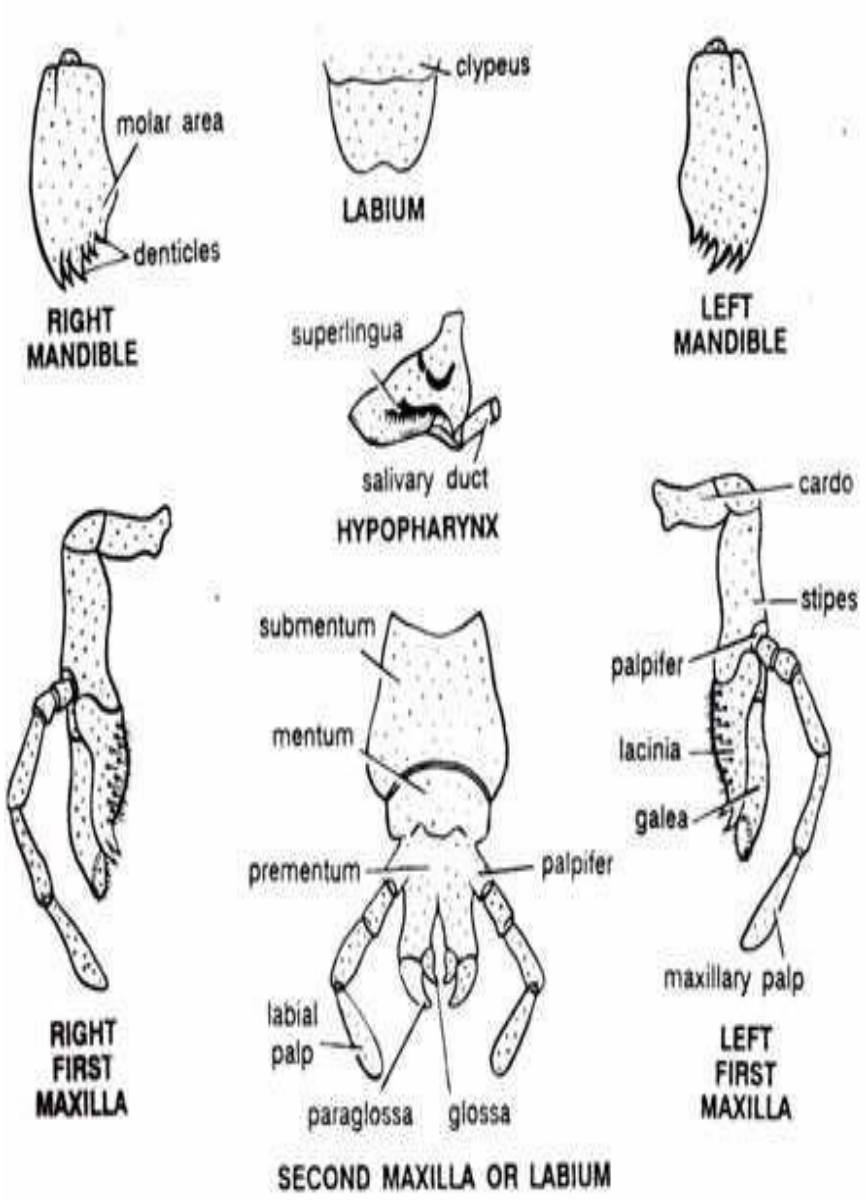

Fig.2.6 Mouth parts of Cockroach

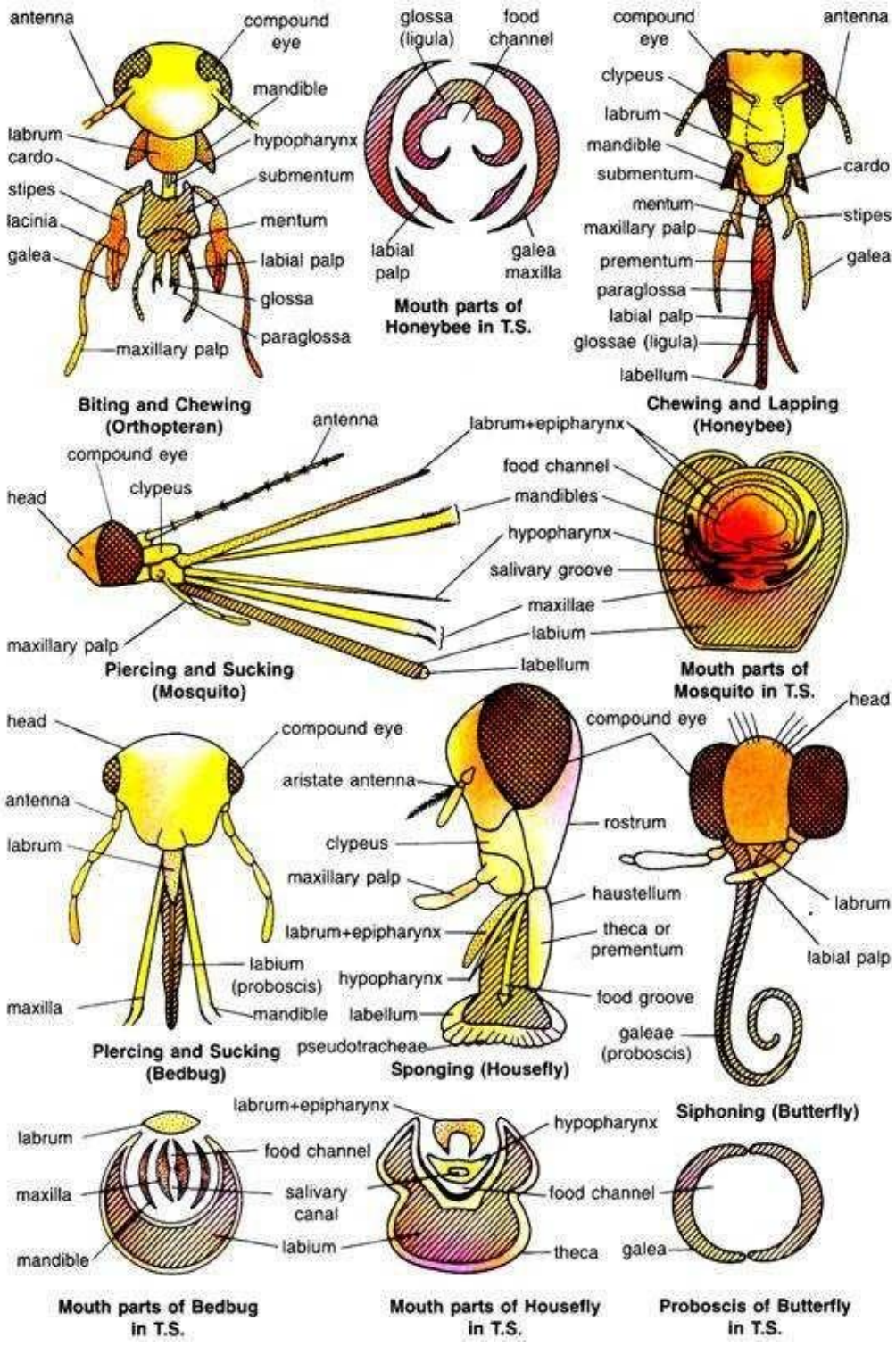

Fig 2.7.Mouth parts of insects

APPENDAGES IN COCKROACH:-

(1) Thorax contains 3 pairs of waling legs, which bear dense hairs. Each leg is composed of coxa, **trochanter, femur, tibia** and **5-jointed tarsus,** which ends in a **pulvillus** (Fig. 147

***A. first pairs of legs*:**

(2) It is found in the prothoracic region; inner surface of **tibia** bears **pollen brush; posterior surface of tibia** contains a velar process, which fits into the tarsal notch; and the bristles of the tarsal notch form **antenna comb.**

(3) Anterior edge of the first tarsal segment contains **eye brush** to remove particles from the eyes.

B Second pair of legs:

(4) It originates from **mesothorax** containing 5 **podomers.**

(5) Inner end of Tibia bears spine-like **pollen spur** for removing pollen from the pollen basket. The outer surface bears **pollen brush**. Terminal part of tarsus contains **pulvilus** and **claw**

C. Third pair of leg

(6) It originates from the **metathorax.** The proximal tarsus contains stiff hair, which help in removing the pollen from the body. The tibial podomere is slightly concave and is fringed with long hairs to forming **pollen basket** or **corbicula.**\

(7) Distal end of tibia has stiff bristles called as **pecten.** Just below pecten is a plate like structure called **auricle.** Pecten and auricle form **wax pincher** for removing wax from **abdomen.**

(8) Outer surface of tarsus has **pollen brush** while inner surface has **pollen comb** or **scopa**. Terminal segment of tarsus contain **claw** and **pulvillus.**

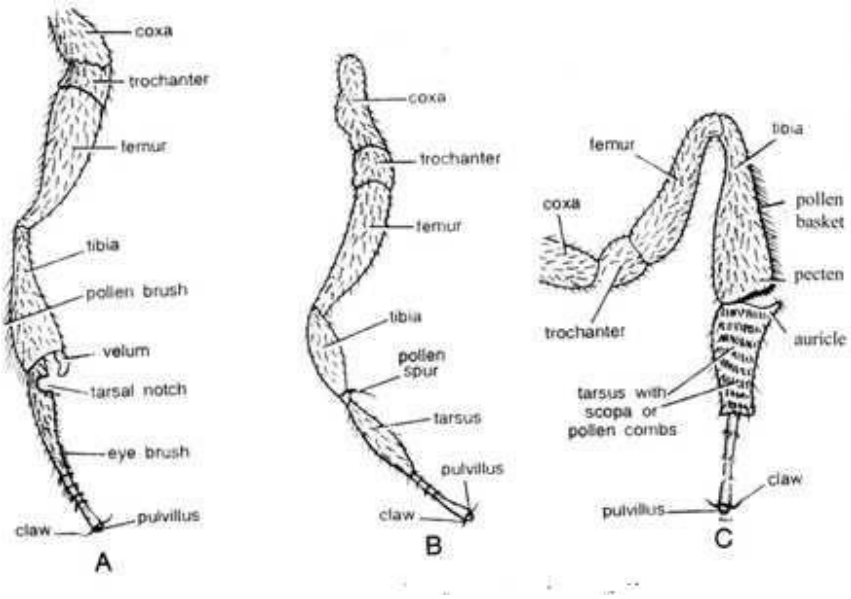

Fig.2.8 Leg of Honey Bee A.First Leg B.Second Leg C.Third Leg

HONEY BEE-STING APPARATUS:-

Comment

(1) **Sting apparatus** of honey-bee is a modified **ovipositor**, found at the posterior extremity of abdomen in queens and workers)
(2) It is composed of **sting** or **terebra, bulb, levering plates** and **glans.**

(3) Sting is made up of 2 pairs of **gonapophyses :** those of the 8th segment forming **stylets** an of the 9th segment **stylet sheath,** which enclose **poison canal.**

(4) Distally the stylet sheath and **stylet contain pointed spines** or **barbs.**

(5) Stylet sheath is expanded into the bulb at the base of the sting.

(6) There are 3 pairs of plates. The anterior one is triangular **fulcral** plate, the **postero-dorsal** is **quadrate plate** and the innermost is **oblong** plate bearing sting palp.

(7) There are two glands namely **poison gland,** opening into the poison-sac and a small **alkaline gland,** opening into sting bulb. The bite of the **sting** causes burning sensation, pain and swelling of the part concerned.

Identification: Since the mount contains sting and poison gland hence it is sting apparatus of ***Apis.***

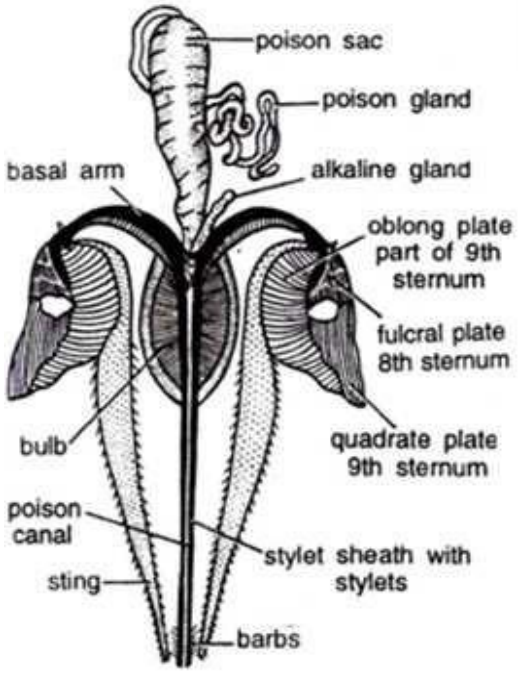

Fig 2.9 Sting apparatus of Honey Bee

Minor Dissections:-

Nervous system of cockroach:-

Procedure: (i) Take a freshly-killed cockroach for dissection; remove wings, cut off antennaeand legs close to their bases

(ii) Hold cockroach in left hand and cut the lateral membranes between terga and sterna up to the anterior edge of pronotum.

(iii) Lay the insect in the dissecting dish with dorssl side uppermost and pin it in abdominal stena and coxae of legs. (Another better procedure of fixing cockroach is to float it in petridish containing had melted wax. Allow it to cool and in due course the animal will be embedded and dissection can be done.) Fix the head by pinning between mandibles by means of fine scissors make a rectangular cut in the head around clypeus and anterior epicranium to expose two cerebral ganglia.

(iv)Make a transverse cut along the posterior edge of the ninth segment (tergum) and gently remove other segment very carefully, so that the underlying organs and tissues are not disturbed.

(v) Uncoil intestine and stretch alimentary canal on one side. Remove fat bodies, tracheae and other muscles to expose internal organs. Study and draw the following parts:

(1) Heart : -

13 **chambers** in number (3 thoracic and 10 abdominal narrow chambers). Note inter-segmental alary muscles.

(2) **Alimentary canal : -**

(3) Is divided into three parts:

(a) Foregut: It comprises of **mouth, buccal cavity, oesophagus, crop and gizzard.** The buccal cavity, receives the common salivary duct. Crop is meant for storing food. The gizzard has chitinous lining, which is internally produced into six teeth for masticating the food and setae for straining the food.

(b) Mesenteron *or* ***midgut:*** It is a narrow duct originating from gizzard and midgut there are 7 to 8 **hepatic** or **mesenteric caeca**. (Their function is to increase the absorptive area).

(c) Hindgut or ***proctodaeum:*** It includes ileum, colon and rectum. The beginning of ileum is marked by 60-70 fine and long **greenish yellow Malpighian tubules** (excretory in function).

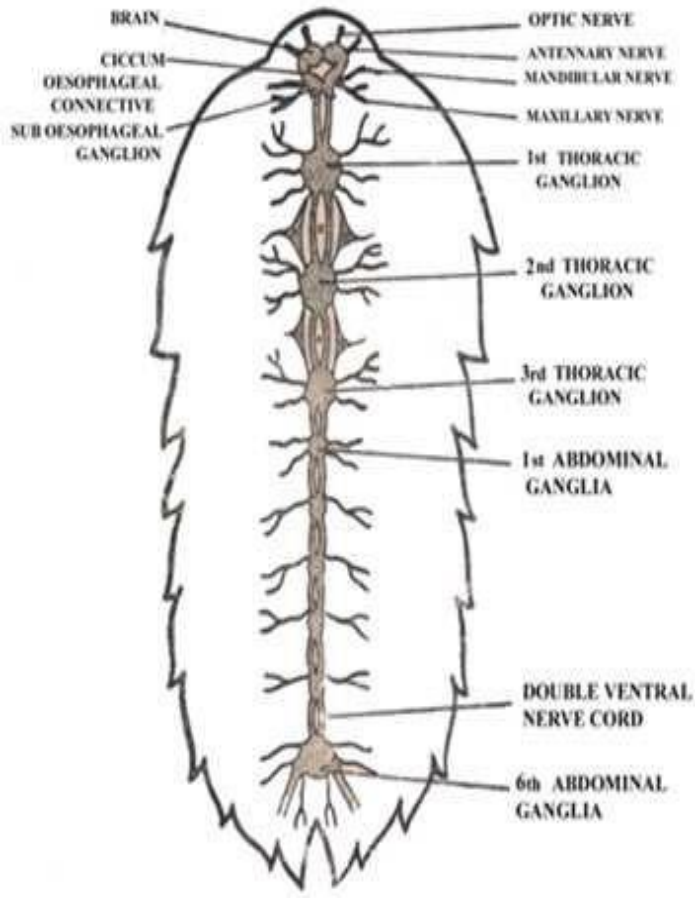

Fig. 2.10 Nervous System of Cockroach

Nervous system of Grasshopper:-

Procedure:

Take a freshly chloroformed or preserved grasshopper, cut wings and fix the animal with dorsal side upwards. Make incision in pleura and remove the targal sclerites. Remove terga in head region. Now carefully remove the viscera and expose as clearly as possible the entire nervous system. Start from posterior side and gradually trace the nerve cord up to brain. Observe the following parts:

Entire nervous system is divided into 3 years:-

1. The central nervous system:

It consists of a dorsal brain or supra-oesophageal ganglia situated above oesophagus between eyes and connected to ventral sub-oesophageal ganglion by circum-oesophageal connectives (Fig. 32).

Sub-oesophageal ganglion is formed by the fusion of mandiblur, maxillary and labial ganglia. It gives rise to double ventral nerve cord which extends upto posterior region and shows the following thickenings or ganglia:

(1) First thoracic ganglion.

(2)Second thoracic ganglion.

(3) **Third thoracic ganglion,** and

(4) Five pairs of abdominal ganglia.

2. Peripheral nervous system: The following nerves arise from central nervous system:

(1) A pair of **optic nerves** originates from optic lobes and supplies to **antennules.**

(2) **Ocellary nerves:** They innervate ocelli.

(3) A pair of **antennary nerves** originates from thoracic ganglia.

(4) **Walking leg nerves.** They originate from thoracic ganglia.

(5) **Abdominal nerves** arise from abdominal ganglia and supply to various organs.

3. Sympathetic nervous system: it includes occipital ganglion, frontal ganglion and ingluvial ganglion, which are associated with brain and control involuntary actions of alimentation, heart ganglion, frontal aorta and genital organs.

Instructions: Draw the diagram of your dissection with the help of the practical book.

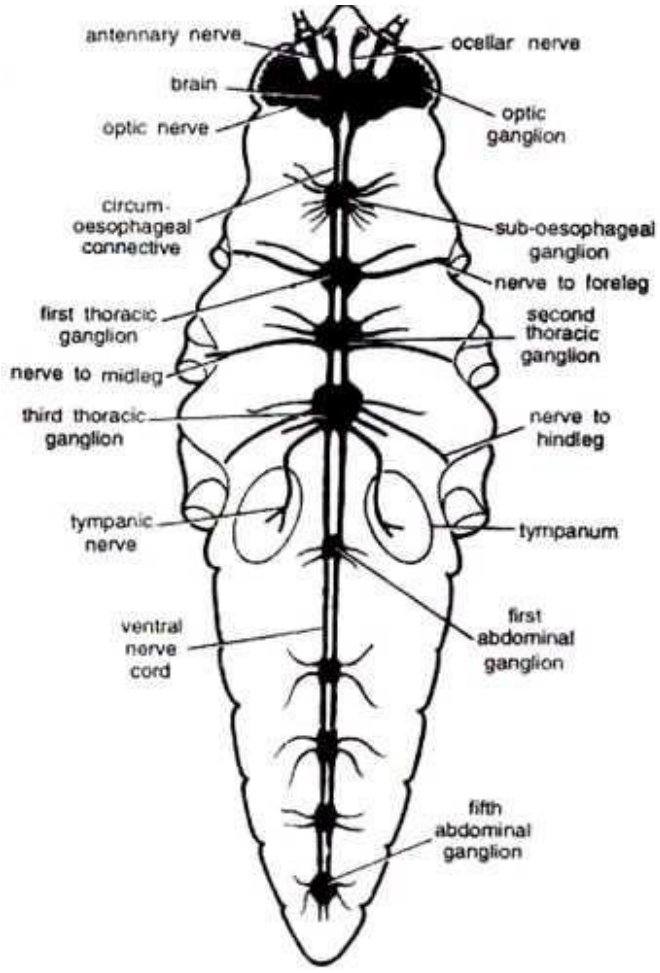

Fig 2.11 Nervous System of Grasshopper

2.5 Glossary

Aboral	Opposite the mouth.
Amoeboid	Cell movements resembling those of the amoeba.
Angstrom	One thousand of a micron.
Archenteron	Primitive digestive tract of a metazoan embryo, formed during gastrulation.
Autotrophic	Nutrition. Process of nutrition in which an organism manufactures its own food.
Basal disc	Foot of some Cnidaria which is flattened and attaches to a substratum by secretion of a sticky substance.
Binary fission	the type of asexual reproduction by means of which the organism divides into two approximately equal halves.
Buccal	Pertaining to the mouth or oral cavity.
Cnidaria or Coelenterata.	Phylum of animals all possessing cnidoblast structures.
Cnidoblast	Type of cell in which nematocyst is found.
Coelom	The body cavity lined with tissue of mesodermal origin in whichthe digestive and other organs lie.
Conjugation	A method of sexual reproduction in which two unicellular animals untie, exchange nuclear material and then divide as in the Paramecium.
Contractile vacuole	A space in the cytoplasm of certain species of protozoa where fluids collect before being periodically discharged to the outside.
Ctenophora	Radiate phylum of animals possessing comb- such as comb-jellies.
Cuticle	Thin non-cellular outermost secreted by the underlying epidermis.
Cyst	The stage of an organism where it is enclosed in a resistant wall.
Cytopharynx	Pharynx or gullet of a protozoan such as Paramecium.
Cytostome	Cell mouth, for example in Paramecium.
Diplobastic	Derived from two embryonic germ layers, ectoderm and endoderm.
Enteron	Digestive tract, especially in Cnidaria.

Entoprocta	Pseudocoelomate, sessile phylum with U-shaped intestine, mouth surrounded by circle of ciliated tentacles, and opening within cirle.
Extracellular	Outside of the cell or cells.
Exumbrella	Convex, aboral surface of the medusa.
Fission	Asexual method of reproduction by division into two or more approximately equal in size.
Food vacuole	Intra-cellular digestive organelle.
Free-living	Capable of independent existence.
Gastrodermis	Lining of coelenterate digestive cavity.
Gastrulation	Process by which two germ layers, ectoderm and endoderm.
Holophytic	Type of nutrition, found in green plants and in some mastigophores, which involves photosynthesis.
Holozoic	Type of nutrition found in most animals, that involves ingestionand digestion of organic material.
Hydranth	Expand end of a branch of a hydroid colony specialized for vegetative function.
Hydrocaulus	Basal portion of a hydroid colony often branched and root-like used for attachment to substratum.
Hydrotheca	Transparent membrane that extends from the perisarc and surrounds the main part of the hydranth.
Hypostome	Region surrounding the mouth in coelenterates.
Inter-cellular	Between cells.
Intra-cellular	Within cells.
Isogamy	Sexual reproduction involving fusion of two similar gametes but from opposite sexes.
Kinetosome	The basal body of a flagellum or cilium.
Lophophore	Anterior tentacle- bearing area of certain coelomates; serves in food capture.
Mesogloea	Non-cellular jelly-like substance lying between the ectoderm and endoderm in coelenterates.
Metagenesis	Alternation of sexual with an asexual generation in reproduction in the life cycle of a coelenterate such as Obelia.

Myoneme	Type of contractile fibril in certain Protozoa.
Nephridiopore	External opening of an excretory tubule or
nephridium.**Pedal**	Pertaining to father.
Pellicle	The protective layer on the surface of some protozoans, for example, Paramecium.
Penetrant	Largest type of cnidarians nematocyst, containing a coiled tube and spines, used in prey capture.
Peristome	Region around the mouth of a radially symmetrical animal such ashydra.
Phagocyte	Type of white blood cell that engulfs and digests bacteria and other foreign materials.
Pinocytosis	Cellular drinking or intake of fluid.
Plankton	Floating or drifting aquatic organisms, mostly microscopic.
Plasmasol	Relatively liquid cytoplasm.
Pneumoatophore	Air-filled float of siphonophoran hydroids.
Polyp	A tubular coelenterate form.
Prosopyle	One of the surfaces pores opening into a sponge chamber.
Protozoa	A phylum of acellular animals.
Proximal	Near the point of attachment of an organ.
Pseudocoel	A body cavity not completely lined with mesoderm as found in round worms.
Pseudopodia	Blunt temporary protoplasmic projections found in amoeba or in some ameba like cells.
Schizocoel	The coelom formed by the splitting of embryonic mesoderm.
Sedentary	Staying in one place.
Siliceous	Containing silicon dioxide or silica.
Spicule	One of many solid structures that composed the structural framework of a sponge.
Spongocoel	Paragastric or central cavity of a sponge.
Syngamy	Union of gametes in sexual reproduction forming a zygote.

Taxis	A movement response
Tentacle	A flexible arm likes extension from the body of many invertebratessuch as hydra. Used in grasping and movement.
Tentaculocyst	Sense organs of some cnidarians.
Triploblastic	Derived from three primary germ layers-ectoderm, mesoderm, and endoderm.
Vestibule	An outer cavity with an entrance to a (usually) larger, deeper cavity.
Zooid	One of the members of a hydroid or siphonophore colony.

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Unit 3 CYTOLOGICAL STUDY

Contents

3.1 Objectives

To study the Meiosis and describe the chromosomal makeup of a cell using the terms chromosome, sister chromatid, homologous chromosome, diploid, haploid, and tetrad and also recognize the function and products of mitosis and meiosis. Compare and contrast the behaviors of chromosomes in mitosis and meiosis. Recognize when cells are diploid vs. haploid and Predict the DNA content of cells in different phases of mitosis and meiosis stage because meiosis is a specialized and rather complicated type of cell division and we have to recall and describe the phases of the cell cycle co-relate the cell cycle stages to changes in DNA content.

3.2 Introduction

Meiosis is a specialized and rather complicated type of cell division, occurring only in the diploid reproductive cells and results in the formation of haploid sex-cells of gametes. The gametes, formed as a result of meiosis, possess half the number of chromosomes as found in the parent cells and their chromosome number is represented by **n,** whereas the zygote formed by the fusion (fertilization) of male and female gametes and the cells derived from it are known as **diploid** and their chromosome number is symbolized **by 2n.** The two similar chromosomes of diploid cells are known homologous chromosomes or homologous pair."The chromosomes of a homologous pair are brought together in the zygote by the union of male and female gametesfrom the parents.

3.3 Study of Mitosis And Meiosis

Meiosis occurs in the life cycle of each and every living being whether a plant or an animal, butits period of occurrence varies in different groups. In majority of cases it occurs prior to gamete formation. The cells undergoing meiosis are known as **meiocytes.** In animals, the **meiocytes** are the **primary spermatocytes** and **primary oocytes** while in plants these are represented by **sporocytes.** The relative amounts of RNA and DNA are supposed to initiate meiosis in some way. If the ratio of RNA to DNA is high, the cell will undergo meiosis but if reverse is the caseit will lead to mitosis.

3.4 Study of Permanent Slide

Process of Meiosis:-

The process of meiosis is separated into a sequence of events similar to those of mitosis but these events or stages are repeated twice, i.e., in meiosis, two complete cell divisions follow in close sequence, with or without a short interphase between them. The first meiotic division is known as **reduction division or heterotypic division.** In it the diploid parent cell divides into two daughter cells having haploid chromosome number. The second division is known as

homoeotypic division and it is a simple mitotic division in which the two haploid cells formedas result of h eterotypic division divide forming four haploid cells. The two meiotic cell divisionis further distinguished into phases. These are:-

A. Heterotypic Division or First Meiotic Division or Reduction Division

I FIRST PROPHASE:-

The prophase of first meiotic division is of longer duration and profoundly modified. It is distinguished into following **five** phases or sub stages –

(a) Leptotene
(b) Zygotene
(c) Pachytene
(d) Diplotene and
(e) Diakinesis

1. Proleptotene :

The meiocytes or the meiotic cell is comparatively larger in size and possesses a large nucleus. It contains diploid number of chromosomes which form a network. In the beginning, the movement of centrioles, the formation of astral rays and the gradual condensation of the chromatin material proceed in a similar fashion as in the prophase of mitosis. These preliminary steps constitute proleptotene.

2. Leptotene or Leptonema :

The leptotene stage initiater meiosis. Due to the condensation of chromatin matter the chromosomes appear in diploid number as long, thin and uncoiled threads or slender filaments longitudinally single rather than double as in mitosis. These threads correspondto the chromonema of the anaphase of mitotic division. Their arrangement is often irregular but they might exhibit some definite orientation. Each chromosome parents a beaded appearance due to the presence of a longitudinal series of dense, bead- like swelling called **chromomeres.** The chromomeres **are** of different sizes and occur in definite sequence on each chromosome. The homologous chromosomes display the same sequence of chromosomes. The DNA and histone synthesis and the chromosomes duplication either starts in this substage or occurs in the later substage but in most cells the duplication is completed by the end of next substage, i.e., **zygotene.** The nucleolus is well marked and increases in size in leptotene and zygotene.

3. Zygotene or Zygonema

The zygotene commences with the movement of chromosomes. It is affected by the

forces of attraction between the two homologous of a chromosome pair. Thus, the chromosomes of a pair approach each other and each chromosome shortly takes a

position along the side of its partner to form a bivalent. The pairing of homologous is known as **synapsis** and is very intimate and precise, the chromomere to chromomere.

Once the pairing has started at some point along the homologues it proceeds from there in zipper-like fashion. This indicates that the homologous chromosomes are not only similar in appearance, but they also carry the same genes in the same sequence.

The pairing may be completed in any of the following methods:

- The two homologues start pairing progresses towards centromere region - **proterminal apis.**
- The pairing may start near the centromere and then progresses towards the ends -**procentric synapis**
- The pairing starts at random either at one point or at many points simultaneously- **random synapsis**.

In organisms with definitely oriented or polarized chromosomes, pairing usually commences at the ends nearest the nuclear membrane and progresses onwards till completion. This peculiar state of orientation, polarization and association is known as bouquet **stage**.

As the pairing proceeds, the chromosomes continue to condense and become shorter and thicker. Two views have come forward to explain the possible initiation of synapsis.

According to precocity theory put forward by **Darlington,** the chromosomes pair due to their singleness. But this theory does not explain the extra synthesis of DNA and chromosomes duplication at leptotene stage.

The **retardation theory** by Sax and others explains that the pairing of homologous isdue to the retardation of cessation of metabolic activities of the cell.

At zygotene the nucleolus increases in size and the centrioles move apart initiating the spindle formation.

4. Pachytene Stage or pachynema

With the pairing or synapsis of homologues the nucleus enters the pachytene stage. It represents the stable period in cell division. During this stage the paired chromosomes of bivalent get shortened and thickened due to gradual condensation of chromatin and appear as thick rods of different shapes and sizes, so that the chromosomes are more readily distinguished.

The homologous chromosomes now twist or twin around each other forming relational

coils. Each chromosomes starts splitting into two sister chromatids by a vertical or longitudinal furrow. As a result the bivalent is now converted into tetrad.

The time of duplication varies in different types of cells. In some it is said to occure in leptotene, while in others in pachytene.

Their relational coiling gets further complicated due to the coiling of two chromatids of each chromosome. This vigorous coiling exerts considerable starin upon the chromosomes. As a result the weaker chromatids break down at points.

These transverse breaks occur in the non-sister chromatids of a pair at corresponding points. The broken ends are then interchanged between the matching chromatids and are attached to their respective remaining portions.

This exchange and recombination of chromosomal parts is known as **crossing over**. Its completion marks the end of pachytene.

5. Diplotene or Diplonema

The separation of homologous chromosomes initiates diplotene. The synaptic forces of attention between them lapse due to breakage at one or more points so that the homologous chromosome uncoil and starts separating.

But the separation is none the less incomplete since the homologous are in contact are known as **chiasmata** (sing. **Chiasma**, meaning, **cross**) which present cross-shaped appearance.

The chiasma is the bivalent varies in the same pair of chromosomes and in different cellsof the same individual. By the end of diplotene the chiasmata begin to move along the length of chromosomes from the centromere towards the end.
This displacement of chiasmata is termed as *terminalization.* When the terminalization of chiasmata. The degree of terminalization is generally expressed as coefficient of termination *(T).*

$$T = \frac{\text{Number of terminal chiasmata}}{\text{Total number of chiasmata}}$$

The average number of chiasmata in bivalent is known as frequency of chiasmata

9Fq)Frequency (Fq) = $\frac{\text{Total number of chiasmata}}{\text{Total number of bivalents}}$

According to Darlington, two types of repelling forces operate on the chromosome at

diplotene. One of the forces is electro negatively charged and operates on the surface of the chromosome throughout its length and the other with electropositive charge is localized on the centromere. The former controls the repulsion of the chromosomes andthe latter cause's distal movement of the chiasmata.

6. Diakinesis:

The bivalents still contract and get thickened into deeply stained bodies. These migrate to the periphery of the nucleus. The two chromatids of each chromosome become closely oppressed together losing their individual identity.

At the same time the homologues move still apart due to the force of repulsion developed between their centromeres. In doing so the chiasmata move towards the ends.

At this stage the nucleolus and nuclear memebrane disappear and the formation of nuclear spindle starts.

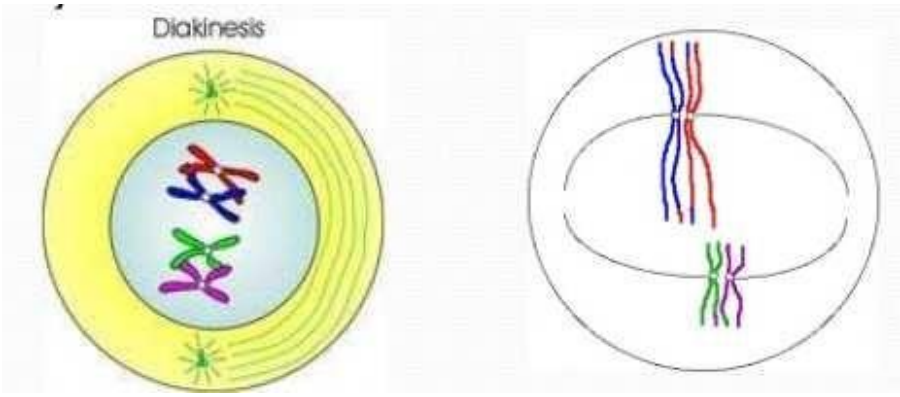

Fig.3.1 Diakinesis

II FIRST METAPHASE (METAPHASE I):-

The metaphase of meiosis is very similar to that of meiosis. At the close of diakiness the nuclear membrane disappears and the formation of amphiaster or achromatic figure or the spindle is completed. In metaphase the bivalents move the equator.

Later on, they orient themselves on the equator in such a way that their centromeres lieone on either side or equidistant from the equatorial plate.

Their centromeres face the pole of the spindle and the arms are directed towards the equator and rest on the equator.

III FIRST ANAPHASE (ANAPHASE I):-

During this stage the bivalents move apart towards the opposite poles of the spindle.

The tetrad which was having four chromatids now separates into two dyads due to the complete separation of maternal and paternal chromosomes of the bivalent.

Therefore, each separated half consists of two sister chromatids attached together by a common centromere. This process of separation is known as **disjunction** and this involves the separation of those homologous chromosomes which were brought togetherin the zygote stage.

By this time the two chromatids of a dyad also separate except at the points of centromere, so that they present V-shaped appearance.

IV FISRT TELOPHASE (TELOPHASE I)

The first telophase commences with the formation of nuclear wall around the haploid group of chromosomal dyads which have already reached the poles of the spindle.

The chromosomes elongate the uncoil. Nucleolus is also formed.

The cell cytoplasm also segments into two. Thus two daughter cells are formed, each of which contains haploid number of chromosomes.

V INTERPHASE:-

It is the resting stage of dividing meiocytes and its duration depends upon the species involved. It may be totally absent and the chromosomes of first anaphase directly passinto second prophase omitting the telophase.

In this condition the nuclear material remains unchanged and the nuclear membrane isnot formed. If the interphase is present the nucleus assumes its original form by the development of nuclear net and nuclear membrane. But if at all interphase is present it isof a very short duration.

B. Homeotypic Division:-

The second meiotic division is essentially mitosis, occurring independently in both the haploid sister cells. It may follow immediately after first meiotic division or may not occur until much later.

1. Second Prophase or Prophase II

During second prophase the nucleus and nuclear membrane disappear in both the daughter haploid cells and the formation of spindle starts.

The chromatids are coiled and the dyad has X-shaped appearance having chromatids joined by centromere and arms radiating.

2. Second Metaphase or Metaphase II

The second metaphase is of short duration. The chromatids move towards the centre ofthe spindle and orient on the equator.

Their centromeres touch the equator but the arms radiate out toward poles. Later on, thecentromere in each dyad divides into two.

3. Second Anaphase or Anaphase II

The chromatids with their independent centromeres from sister chromosomes and moveapart towards the opposite poles of the spindle.

The chromatids of second anaphase are not short and compact bodies like those of firstanaphase but are very similar to the chromosomes of anaphase in mitotic division.

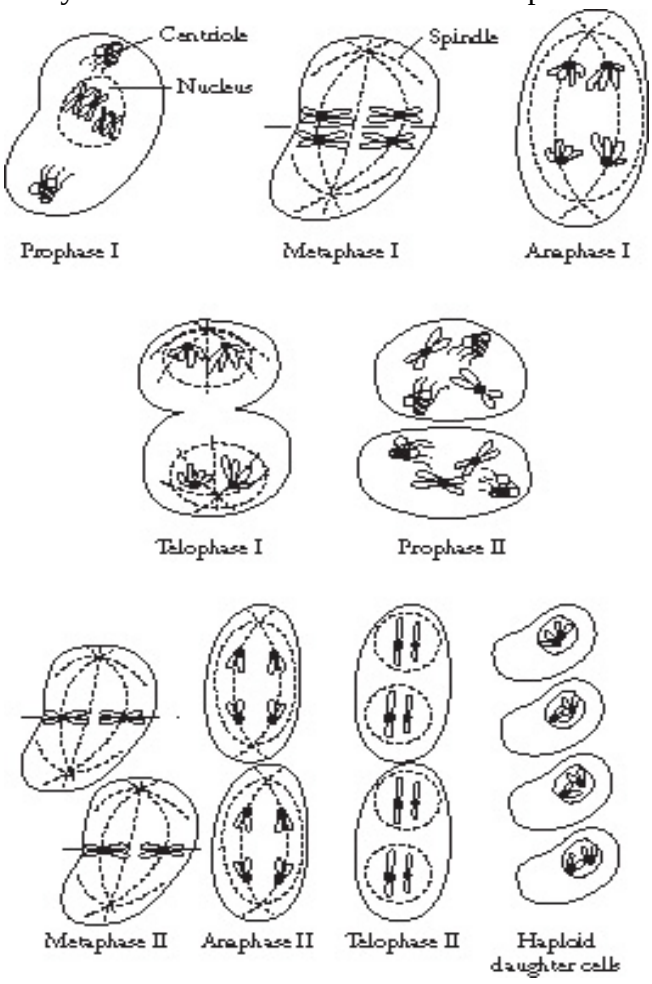

Fig.3.2 Homeotypic Division

4. Second Telophase or Telophase II

The chromosomes at each pole uncoil and thin out to form the nuclear net. Each group gets surrounded by a nuclear membrane. Nucleolus reappears. Thus two nuclei are recognized in each cell.

Significance of Meiosis

The significance of meiosis is threefold:-

1. The meiosis is a logical and necessary part in the life cycle of sexually reproducing animals since it leads to the formation of gametes or sex cells that participates in fertilization. These are haploid cells having only one member of each homologous pair.

2. The meiosis is concomitant of doubling chromosome number due to gametic fusion. The gametes formed as a result of meiosis are haploid and the zygote formed by their fusion is diploid. Thus, it is only means for restoring the chromosome number characteristics of the species.

3. Meiosis results new combination of genetic material. During crossing over, the hereditary factors from male and female parents get mixed due to breakage and exchange of chromatids in pachytene. Thus, the gametes produced are not all alike but with variable combination of genes. The random segregation of chromosomes and the new alignments of genes in them resulting from crossing over ensure genetic variations in the population. The inherited variability leads to the evolution of organisms.

Cytological Study Exercise:-

(1) - Object: To study the meiosis by using available material**.**

Requirement: - Living grasshoppers, Chloroform, normal saline, Carnoy's fluid, acetocarmine, slides, cover-slip, blotting paper and microscope.

Procedure: - Take a chloroformed grasshopper and dissect it in normal saline. Take out its testis and fix them in Carnoy's fluid for 2-12 hours. Take a small lobe of testis and stain it in acetocarmine. Put the stained lobe on a clean slide and cover it with a cover slip. Warm the slide over the flame of a sprit lamp and then put a blotting paper over it, press it smoothly by your thumb. Examine the slide under microscope.

Result: The cells of testis lobes are spread out and became distinct. Carefully observe differentstages of meiosis under microscope and draw them in practical notebook.

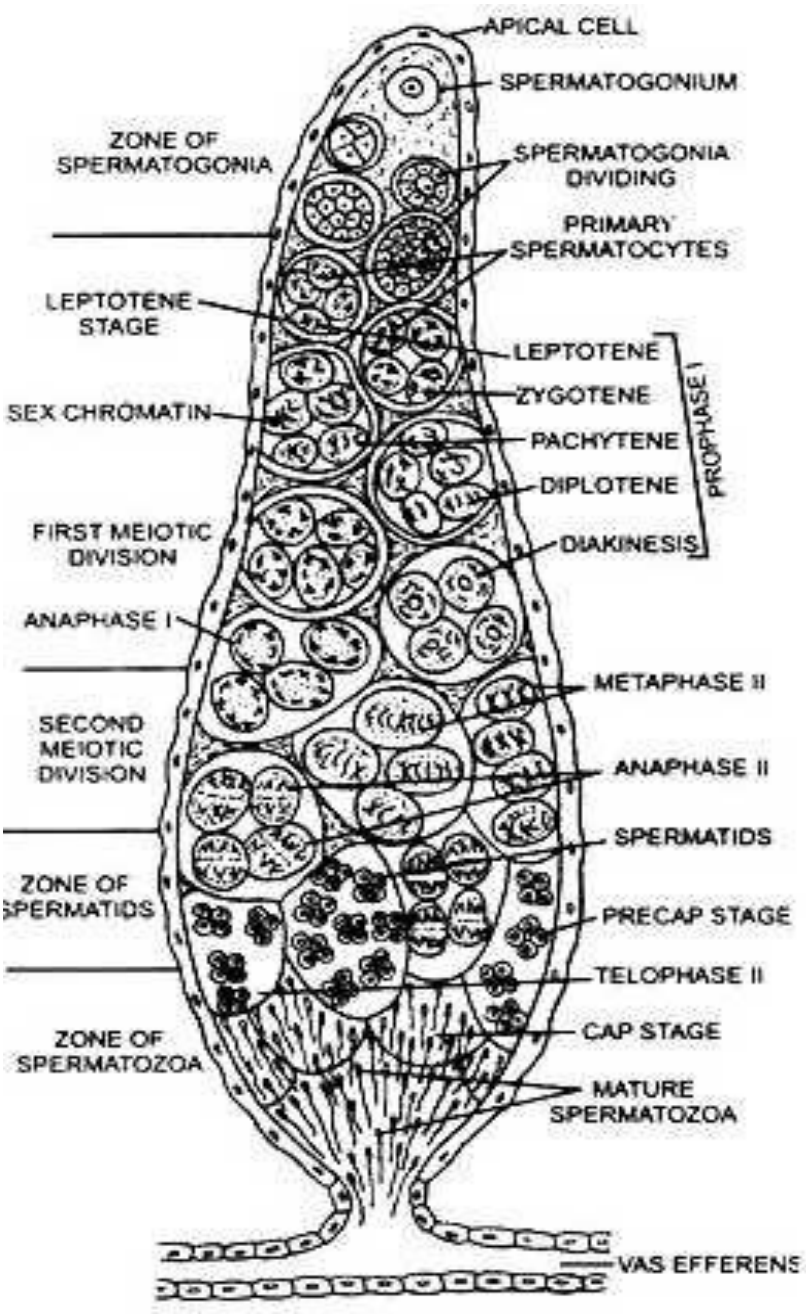

Fig. 3.3 T.S of one follicle of testis of Grasshopper to show the stage of meiosis

Cell Division :-

The process of cell division is found to be essentially the same in all living organisms and theevents are chiefly centered in the nucleus. Three types of cell divisions have been distinguished.

(i) Amitosis or direct cell division.
(ii) Mitosis or indirect cell division.
(iii) Meiosis or reduction division.

Amitosis:-

Amitosis or direct type of cell division is characterized by the splitting of the nucleus followed by that of cytoplasm. It is seen in unicellular organism like protozoan's and the cells of foetal membranes.

The beginning is marked by the elongation of the nucleus. Due to the appearance of depression or constriction in the middle line, the nucleus assumes dumb- bell –shaped appearance. The depression increases in size and splits the nucleus into two.

Simultaneously, the cell body or the cytoplasm is also constricted into two equal or approximately similar halves. During the process of amitotic cell division there is complete absence of nuclear events and the mechanism is very simple.

Mitosis:-

Objective: (B) To study the Mitosis

Definition:-

Mitosis involves the exact replication of parent cell followed by its division into two daughter cells which are identical and contain the same number of chromosomes as found in the parent cell.

Introduction:-

This nuclear division was first observed by **Straburger (1870)** in plant cell and **Flemming (1882)** in animal cells. Flemming used the **term mitosis** (**Gr. Mitos, thread**) for this process with reference to the thread-like appearance of chromosomes early in the cell division. An illustrated account of behavior of chromosomes during the period of cell division has been given by **Darlington**. The cell division where chromosomal duplication (i.e. longitudinal splitting of chromosomes) is followed by the nuclear division so that each daughter cell possesses the same number of chromosomes as present in the parent cell.

Mitosis, division of a living cell nucleus (control centre), leading to the production of two offspring or daughter cells, normally with the same genetic information. Mitosis is the standard way that cells multiply. It occurs all the time in the human body and other multi-cellular living things, especially during growth to make more cells, and during maintenance to replace damaged and worn-out cells. In single-celled organisms, it represents asexual reproduction. In plants, it isthe basis of asexual or vegetative reproduction (making cells for sexual reproduction involves another type of cell division). Genes exist as chemical codes on lengths of the chemical deoxyribonucleic acid (DNA) inside the nucleus. During a cell's "resting" period, or interphase, the DNA copies or replicates itself to form two complete sets. Mitosis then occurs in four main stages.

Process of Mitosis

The process of mitosis is characterized by the duplication of chromosomes, their separation intotwo and then their movement to opposite poles so as to construct two daughter nuclei.

It is followed by the constriction of cytoplasm to form two daughter cells.

The replication and distribution of chromosomes is known as **karyokinesis** while the division of cell cytoplasm and separation into two daughter cells is called **cytokinesis.**

It means cell division can be separated into two categories:

- The nuclear division or **karyokinesis,** and
- The division of the cytoplasm or **cytokinesis.**

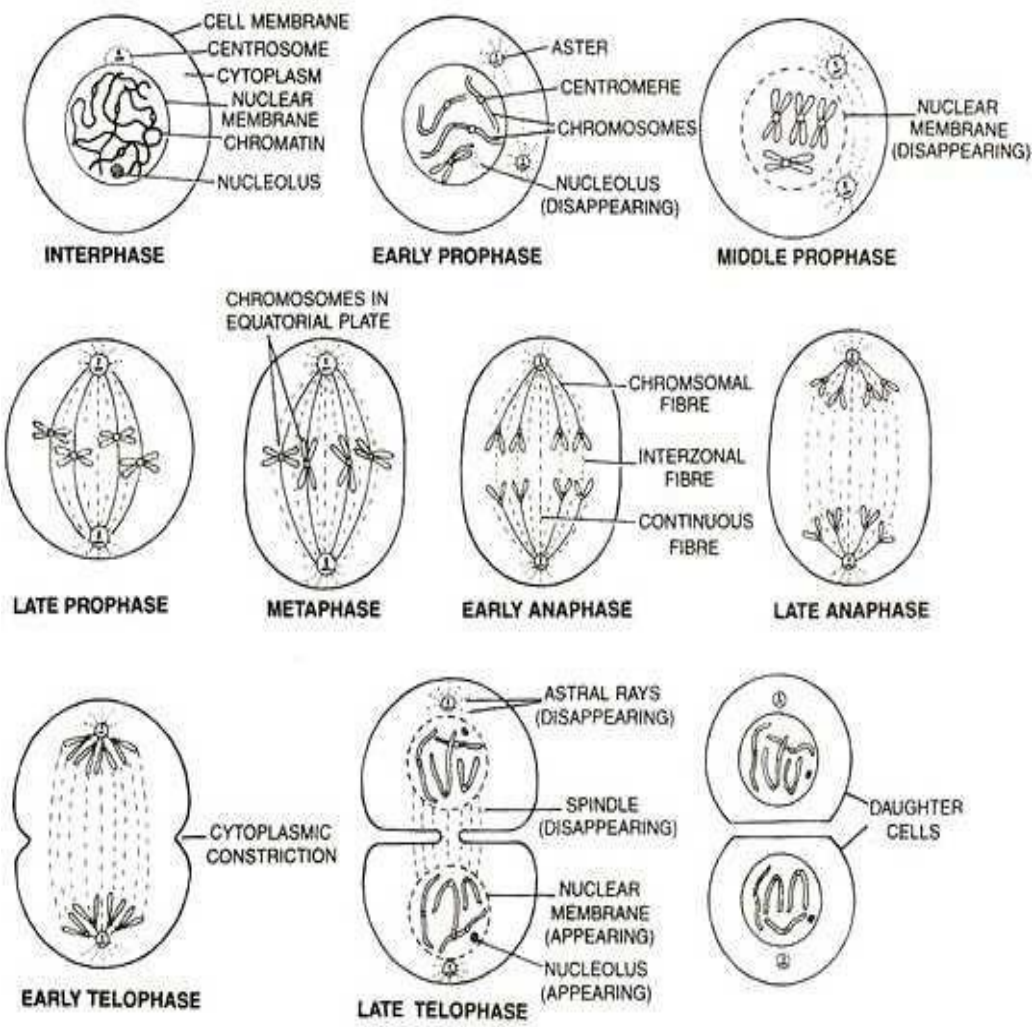

Fig. 3.4 cell division in Animal Cell

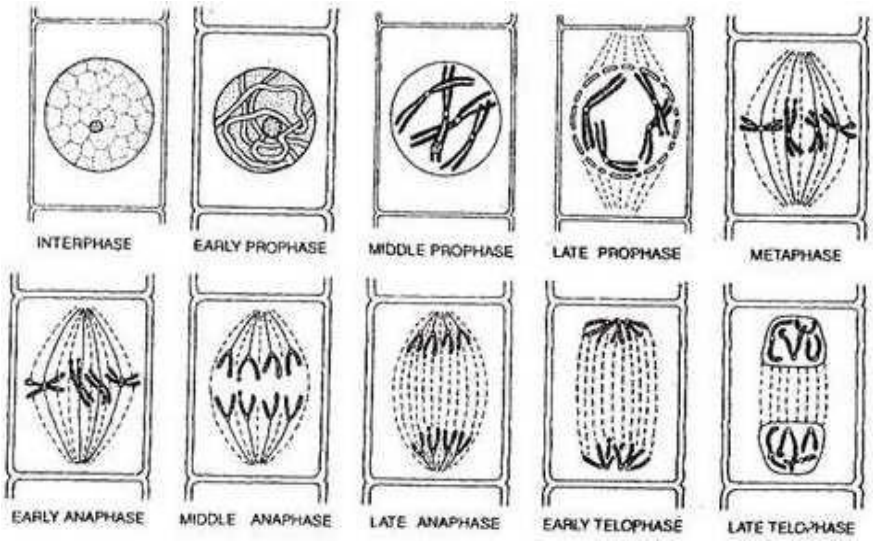

Fig. 3.4 cell division in plant Cell

Karyokinesis:-

The process of karyokinesis includes the division of cell nucleus into two daughter nuclei, It isdivided into prophase, metaphase, anaphase and telophase.

1. **Prophase**

The nuclear division (mitosis) begins with prophase. The important events during thisphase are given below-

(A) Nuclear Changes:

(a) The chromatin materials of nucleus gradually condense into distinct chromatin thread by losing water.

(b) The chromatin threads coil like cylindrical spring and in so doing they gradually become shorter and thicker and form the chromosomes.

(c) The proteinous matrix gets deposited around the chromosomes, so that these gradually become shorter and thicker and form chromosomes.

(d) Each chromosome is already doubled due to the doubling of DNA contents in interphase.

(e) By the end of prophase the two chromatids of each chromosome become more distinct and each chromosome appears to be splitted up lengthwise.

(B) Cytoplasmic Events:

(a) The centriole divides into two and then one of the daughter centrioles moves towards the opposite pole.

(b) Astral rays radiate out from each daughter centriole.

(2) Metaphase

The metaphase is marked by the appearance of spindle and arrangement of chromosomeson the equator of spindle.

a) The microtubules in the cytoplasm of the cell orient in between the centrioles of the opposite poles and form the spindle. Such a spindle is known as **amphiaster.**

b) The chromosomes from periphery of the nucleus migrate towards equator of the spindle, lie on the equator and are attached to the chromosomal fibres of the spindle, whereas the arms are orient towards the poles.

c) Each chromosome becomes more compact and short and its two chromatids separate except at the centromere which has not divided so far.

(3) Anaphase

(a) The centromere of each chromosome divides and allow the separation of two sister chromatids into two daughter chromosomes.
(b) The daughter chromosomes move apart and migrate towards opposite poles.
(c) The movement of chromosomes is governed by the contraction of spindle fibres, the centromere is pulled first towards the pole of the spindle and the arms of chromosomes are dragged behind.
(d) In anaphase, the arms of daughter chromosomes are directed towards the equator and centromeres towards the poles of the equator.

(4) Telophase

(a) Chromosomes reach poles of the spindle and form two groups.
(b) Chromosomes begin to uncoil and form chromatin net.
(c) The nuclear wall and nucleolus reappear.

Mitotic apparatus or mitotic spindle:-

The mitotic spindle is formed of spindle fibres extending between the two centrioles and the astral rays radiating out from each centriole.

Structure of Spindle Fibres:-

Spindle fibres are formed of microtubules, arranged in parallel bundles. These are about 250- 270Å in diameter and with a 50-70 Å thick wall. The number of microtubules composing the spindle of yeast cells (Moor, 1967).

Chemical Composition of Spindle Fibres:-

The spindle fibres represent long chain protein molecules oriented in longitudinal direction between the two poles. The protein chain are linked by bonding of protein monomers by –SH and-S-S bonds. These contain 90% proteins and 5% RNA.

Formation of Spindle Fibres:-

- Spindle fibres are cytoplasmic in origin and about 15% of the cytoplasmic proteins form the spindle.
- The formation of spindle starts in the late prophase and is completed in metaphase.
- Commonly it begins outside the nuclear membrane more or less simultaneously with the disappearance of nuclear membrane.
- During the formation of microtubules of the spindle, the polymerization of protein monomers to from amorphous gel and the formation of secondary bonds through-SH- and-S-S-groups takes places.
- The process is initiated by the release of RNA from the nucleus.

Types of Spindle Fibres: -

The spindle fibres of the three types-

- **Continuous Fibres:** These extend from one pole of the spindle to the other poles.
- **Chromosomal Fibres:** these fibres extend from pole of the spindle to the centromereof chromosomes. These are also called **kinetochore microtubules.**
- **Interzonal Fibre :** These appear in anaphase and telephase and extend between the centromeres of separating chromatids (daughter chromosomes)

Role of Spindle Fibres: -

Spindle fibres help in the movement of chromosomes from equator to the pole of spindle.

Chromosomal Movement during Cell Division:-

Cell division is characterized by the movement of chromosomes and of a number of other cellular structures. These movements are:

(1) Movement of spindle poles or centrioles to the opposite sides of the cell during prophase.
(2) Oscillatory movement of chromosomes to the equator of spindle during prometaphase.
(3) Movement of chromosomes from the equator of spindle towards poles during anaphase A.
(4) Elongation of spindle during anaphase B.

Duration of Mitosis:-

- The time required for mitosis differs with species and environment.
- Temperature and nutrition, in particular, are important factors.
- The entire sequence of phases may be completed in 6 minutes to many hours.
- Normally the entire cycle of cell division takes approximately 18 hours, about 45 minutes from prophase to the end of telophase and about 17 hours for the interphase.
- Different phases of mitosis are of different duration.
- Anaphase is the shortest, the prophase and telophase the most prolonged, and the metaphase of intermediate duration.

Mitotic Poisons: -

- There are certain substances that affect the cells in mitosis or prevent them from entering it.
- These are commonly known as mitotic poisons.
- The **colchicine** inhibits spindle formation and holds the cells in metaphase**.**
- The enzymes ribonuclease is prophase poison.
- Mustard gas fragments and agglutinates the chromosomes.
- Higher concentration of some of these poisons may lead to the immediate death of the cells.

Significance of Mitosis:-

- Mitosis is a significant aspect in the growth of living matter.

- It ensures that the new cytoplasm is accompanied by an appropriate amount of governing nuclear material.
- Individual cells cannot grow indefinitely and their size remains within economical limits with respect to the intake of foodstuffs and their transformation into energy and new cytoplasm.
- As a result of mitosis each new cell receives a set of chromosomes to regulate the activities of the cytoplasm.
- Mitosis ensures a continuous succession of similarity endowed cells, because from one dividing cell two daughter cells with exactly the same number and the same type of chromosomes are formed.
- Thus, no matter how many consecutives cell divisions have taken place, all the cells have an array of chromosomes identical to the parent cell from which they have descended by division.
- Mitotic divisions help not only in the increase of size by cell accumulation but also in replacing the old and damaged tissue by the new cells.
- In plants these do not cease to divide even when the plant is mature but continuously go on cutting new cells from the cambium.

Cytological Exercise:-

The study of cells necessarily involves sophisticated equipments and techniques.

Following is very simple and elementary methods are being described here to study cell divisionand making preparations of certain cell components.

(1) Objective: To observe the stages of mitosis using onion root tips.

Requirements:

- Onion root tips fixed in Carnoy's fluid.
- Microscope glass slide.
- Cover-slip.
- Acetocarmine.
- Sprit lamp.
- Blotting paper and
- Microscope.

Procedure:

- Take a drop of acetocarmine on a clean microscopic slide and put on it one or two tips.
- Place a cover slip over it and tap it gently by a needle.
- Warm the slide over the flame of a sprit lamp and then put a blotting paper over it, pressit smoothly by your thumb.

- Examine the slide under microscope.

Result:-

The cells and their chromosomes are spread out and become distinct. Observe carefully the different stages of mitosis.

Cytological Exercise

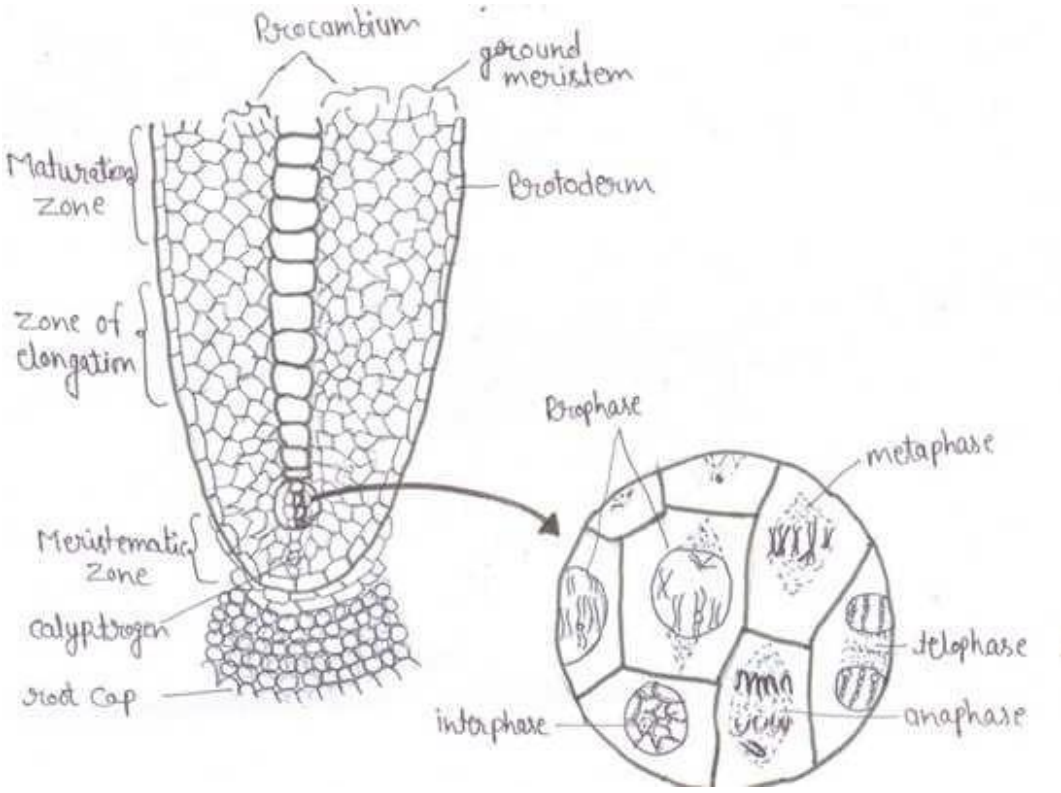

Fig.3.5 onion root tip showing different stage of mitosis

3.4 Study of permanent Slide

What is a cell?

Unicellular organisms are capable of:-

(i) Independent existence and
(ii) Performing the essential functions of life.

Anything less than a complete structure of a cell does not ensure independent living. Hence, cellis the fundamental structure and functional unit of all living organisms.

Anton Von Leeuwenhoek first saw and describe a live cell. Robert Brown later discovered the nucleus.

The invention of the microscope and its important leading to the electron microscope revealed all the structural details of the cell.

Introduction:-

During cell division oxidative process are minimum and the deficiency of oxygen has no visible effect on the process and speed of mitosis. It is, therefore, presumed that the dividing cell obtains energy by **glycolysis**.

No doubt prior to division, when DNA synthesis takes place in the nucleus, the oxygen consumption is normal. Swan (1957) has suggested that an energy reservoir is created inside the cell before it enters cell division. Recently, **Allfrey and** coworkers (1975) and later have suggested that nucleus synthesis ATP through oxidative phosphorylation mechanism.

There are several evidences in support of the view that the oxidative processes are maximum in premitotic period and minimum during active mitosis:

(a) Oxygen consumption is minimum during cell division and deficiency of O2 or concentration of carbon monoxide has no effect on the process of mitosis.

(b) Certain glycolytic enzymes (lactic acid dehydrogenase, triphosphate dehydrogenase, aldolase) are present in high concentration in the nucleus, whereas enzymes associated with respiration and oxidative phosphorylation in cytoplasm are absent from the nucleus.

(c) Mitochondria fragment into granules which completely disappear during mitosis (Agrell, 1955; Chevermont and Fredric, 1952). This reconstitution starts during terminal stages of mitosis.

The body of multicelluar organism is formed of different types of cells and tissues. These cannot be studied directly by the microscope. The required tissue is separated from the body and is prepared in a wway that it becomes suitable for fixing them properly. The following techniquesare often employed for the study of tissues or cells:

1. Teasing or Dissociation

The tissue to be studied is teased either directly in the stain or saline solution with the help of needles on a microscopic slide. It is covered with a cover slip and studied under microscope. The muscular tissue is studied by this method.

2. Smear Technique

Fluid tissue containing cells (blood) or small fragments of tissue such as aspirated bone marrow are smeared on the microscopic slide so that a thin film is formed on the slide. It is then fixed immediately, stained and maintained. The smear technique is very popular in **exfoliate cytology** (study of superficial cells shed from mucous membrane) or in the study of chromosomes and cell division.

3. Sectional Method

In this method the specimen is cut into very thin sections. For this purpose tissue is first fixed by immersion in some fixative and is embedded in paraffin wax or colloidin. Thereafter, is cut into thin sections which are stained and then studied under the microscope.

Cytological Study of Preserved Cells: -

Fixation:

As soon as the living processes of a tissue are grossly disturbed either by death or by the removal of tissue from animal body, some changes begin.

These changes are introduced by the onset of **autolysis (auto digestion), by** the attack of bacteria and moulds and by drying or due to osmotic effect. Some of these changes can be minimized by fixation.

The fixation is the process that brings about sudden death of the cells or tissues in such a manner that their morphological and chemical composition is retained either by the use of chemicals or by freezing.

Aims and Effects of Fixation:

(a) Fixation hardens the tissues and gives them a consistent from.
(b) It prevents autolysis and bacterial decomposition.
(c) It coagulates the tissue, renders the contents insoluble and prevents loss of easily diffusiable substances.
(d) It avoids cell shrinkage and distortion in form d ue to postmortem changes.
(e) Improves the optical differentiation of cell components by changing refractive indicesand thus increases their visibility.

(f) Prepares the tissue for staining.

(g) It fortifies tissue against the harmful effects of various stages in the preparation of sections.

A. Chemical Fixation: The tissue is fixed by some chemical compounds such as formaldehyde, mercuric chloride, picric acid, chromic acid, osmium tetraoxid, acetic acid and ethyl alcohol. These are called fixative.

1. Simple Fixatives

(a) Formalin: 4-10% formalin solution is used for fixing golgi apparatus, mitochondria and enzymes. It fixes and hardens the tissue but causes little or no shrinkage.

(b) Mercuric Chloride: It is an intolerable fixative and is used only in combination with some other fixatives. It hardens and causes shrinkage in the tissue but does not distort it. It precipitates the proteins and fixes lipids**.**

(c) Picric Acid: it precipitates proteins and nucleoproteins. It produces shrinkage. Commonly it is not used for cytological studies.

(d) Chromic Acid: 0.5-1% chromic acid is used to fix those tissues which are studied for Golgi complex and mitochondria. It precipitates all proteins and fixes carbohydrates.

(e) Osmium Tetraoxide: 0.5-2% solution of osmium tetraoxide is used for fixing cytoplasm, golgi complex, mitochondria and fat. It fixes lipids and causes their blackening. It forms additive compounds with proteins, its penetration is poor but fixation is very nice. It is extensively used for electron microscopy.

(f) Potassium dichromate: 2.5-5% potassium dichromate solution is used in conjunction with some other chemical substance. It renders protein insoluble in water and fixes lipids. It is used for the fixation of chromosomes.

(g) Acetic Acid: Glacial acetic acid is never used alone because of its swelling effect. It is used along with other fixative to counteract their shrinkage effect. It precipitates nucleoproteins but not the cytoplasmic proteins. It destroys golgi complex and mitochondria. It is, therefore, used for the fixation of nucleus and chromosomes.

(h) Ethanol: 70% to absolute alcohol is used as fixative.

2. Compound Fixatives

Since each of the primary fixative listed above has its virtues and defects, none ofthem is ideal to preserve and allows the observation of every component of the tissues and cells.

As a practice a mixture of two or more reagent is used as a fixative to make use of the special properties of each.

The most essential feature of a fixative should be its quick penetration power. Someof them are mentioned below:

(a) 19% formal saline: It is mixture of formaline and normal saline solution.

(b) Formal alcohol (FAA): A mixture of 10ml formaline, 90 ml of 90% alcohol and 5ml glacial acetic acid is called formal alcohol. It is used as a fixative for polysaccharides and nucleoproteins.

(c) Carnoy's solution: It is mixture of ethanol (absolute alcohol) 60ml and glacial acetic acid 10 ml and 30ml chloroform. It fixes nucleoprotein and chromosomes. It combines the properties of ethanol and acetic acid.

(d) Bouin's fluid: It is mixture of 75 parts picric acid, 25parts formalin and 5 parts glacial acid. It precipitates all proteins, penetrates rapidly and produces little shrinkage. It is used for the histological studies. It fixes chromosomes.

Procedure of Fixation:-

When a piece of tissue is immersed in the fixative, cellular death does not occur instantaneously and "post-mortem" changes due to anoxia, changes in the concentration of hydrogen ions and enzymatic action (autolysis) may occur.

The fixative penetrates the tissue by diffusion in such a way that the most external cells are fixed more rapidly and better than the central ones. Thus, every fixed tissue has a gradientof fixatation, progressive dilution with the liquid of the cells.

The rate of penetration of the fixative depends upon the type of protein barrier of precipitation produced at the periphery of the tissue. If the precipitate is very fine as in the case of osmium tetraoxide, it forms a barrier preventing further passage of the fixative.

Mechanism of staining:-

It is a well known fact that proteins, certain polysaccharides and nucleic acids have the property of ionization. But the ionization of proteins depends upon pH of the medium.

At pH values above isoelectric point, acid groups become ionized and below isoelectric point, all the basic groups dissociate. Thus, at a pH above isoelectric point, the proteins react with basic dyes and exhibit basophilic property.

The intensity of staining depends upon the degree of acidity or alkalinity of the medium. The basophilic or acidophilic property of cell components also depends on the fixative used.

3.4.1 Study of permanent Slide showing stage of cell division

The onion cell which is a typical plant cell has a distinct **cell wall** as its outer boundary and just within it is the **cell membrane**.

The cells of human cheek have an **outer membrane** as the delimiting structure of the cell. Inside each cell is a dense membrane bound structure **called nucleus**. The nucleus contains the **chromosomes** which in turn contain the gentle material, **DNA**. Cells that have membrane bound nuclei are called **eukaryotic** whereas cells that lack of a membrane bound nucleus are **prokaryotic**.

In both prokaryotic and eukaryotic cells, a semi-fluid matrix called cytoplasm occupies the volume of the cell. The cytoplasm is the main area of cellular activities in both the plant andanimal cells. Various chemical reactions occur in it to keep the cell in the "living state".

Besides the nucleus, the eukaryotic cells have other memebrane bound distinct structure called **organelles** like the endoplasmic reticulam (ER), the **golgi complex, lysosomes, mitochondria, micro bodies and vacuoles.** The prokaryotic cells lack such membrane bound **organelles.**

Ribosomes are non-membrane bound organelles found in all cells both eukaryotic as well as prokaryotic. Within the cell, ribosomes are found not only in the cytoplasm but also within the two organelles **chloroplasts (in plants)** and **mitochondria** and on **rough ER.**

Animal cells contain another non-membrane bound organelle called **centrioles which helps in cell division.**

Cells differ greatly in size, shape and activities. For example, **Mycoplasmas, the** the smallest cells, are only 0.3µm in length while bacteria could be 3 to 5 µm. The largest isolated single cellis the egg of an ostrich. Among multicellular organism, human red blood cells are about 7.0 µmin diameter.

Nerve cells are some of the longest cells. Cells also vary greatly in their shape. They

may be disc-like, polygonal, columnar, cuboid, thread like, or even irregular. The shape of the cell may vary with the function they perform.

The ability to grow and reproduce is a fundamental property of living organisms. However, growth of single cells is fundamentally limited. As new proteins, nucleic acids, carbohydrates, and lipids are synthesized, their accumulation causes the volume of a cell to increase, forcing the plasma membrane to expand to prevent the cell from bursting.

But cells cannot continue to enlarge indefinitely; as a cell grows larger, there is an accompanying decrease in its surface area/volume ratio and hence in its capacity for effective exchange with the environment.

Therefore, cell growth is generally accompanied by cell division, whereby one cell gives rise to two new daughter cells. (The term daughter is used by convention and does not indicate that cells have gender.)

For single-celled organisms, cell division increases the total number of individuals in a population. In multicellular organism, cell division either increases the number of cells, leading to growth of the organism, or replace cells that have died.

In an adult human, for example about 2 million stem cells in bone marrow divide every second to maintain a constant number of red blood cells in the body.

Although often cell growth and cell division are coupled, there is a notable exception. A fertilized animal egg typically undergoes many divisions without the growth of its cells, dividing the volume of the egg into smaller and smaller parcels. Here as well, however, tight regulation of where and when cells divide is crucial.

When cells grow and divide, the newly formed daughter cells are usually genetic duplicates ofthe parent cells, containing the same (or virtually the same) DNA sequences.

Therefore, all the genetic information in the nucleus of the parent cell must be duplicated and carefully distributed to the daughter cells during the division process. In accomplishing this task,a cell passes through a series of discrete stages, collectively known as the **cell cycle**.

3.4.2- Study of permanent Slide showing stage of giant chromosomes

Chromosome is single large DNA molecules and its associated proteins, containing many genes, stores and transmits genetic information. These are popularly known as hereditary vehicle.

Lamp brush Chromosomes

In the oocytic nuclei of those animals which have large yolky eggs, the prophase of first meiotic division is extremely extended.

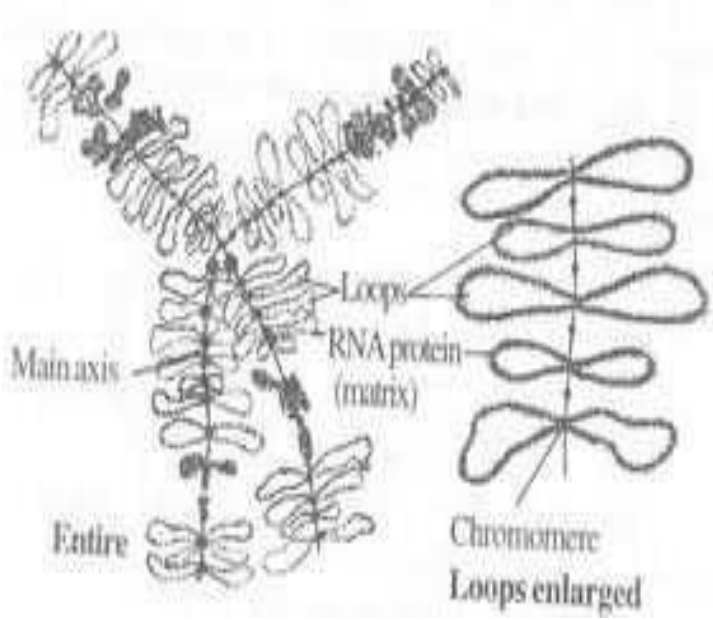

Fig.3.6 Lamp brush *Chromosomes*

During this phase the oocyte grows and synthesizes nutrients for the future embryo. In them, the chromosomes become greatly enlarged and assume unusual configuration. A large number of loops project out from the chromatid axis, giving a lampbrush appearance. Hence, these chromosomes are called **lampbrush chromosomes.**

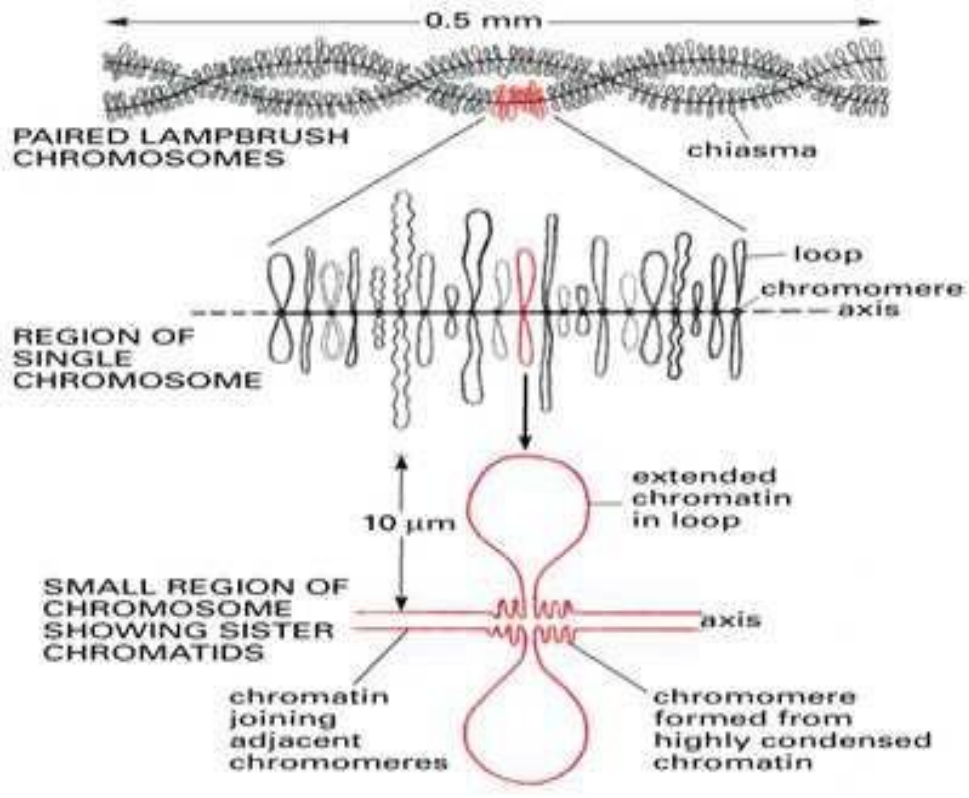

Fig.3.7 Lampbrush *chromosome*

The lampbrush chromosomes are bivalent each consisting of two chromatids. This persists during the prolonged diplotene phase of first meiotic prophase.

History: -

Lampbrush chromosomes were first observed by **Flemming (1882)** in amphibian occyte.A detailed study was made by **J. Rucert** (1892) in the oocytes of sharks.

Occurrence:-

Lampbrush chromosomes are found in the oocytes of insects, sharks, ambhibians, reptiles and birds which produce large and yolky eggs. These have also been found in plants and invertibrates like Sagitta, Sepia and Echinaster.

Size:- Lampbrush chromosomes are enough to be seen under light microscope. These may be as long as 1,000 µm or more and about 20 µ in width. In salamander oocyte these may attain a length of about 5,900 µ.

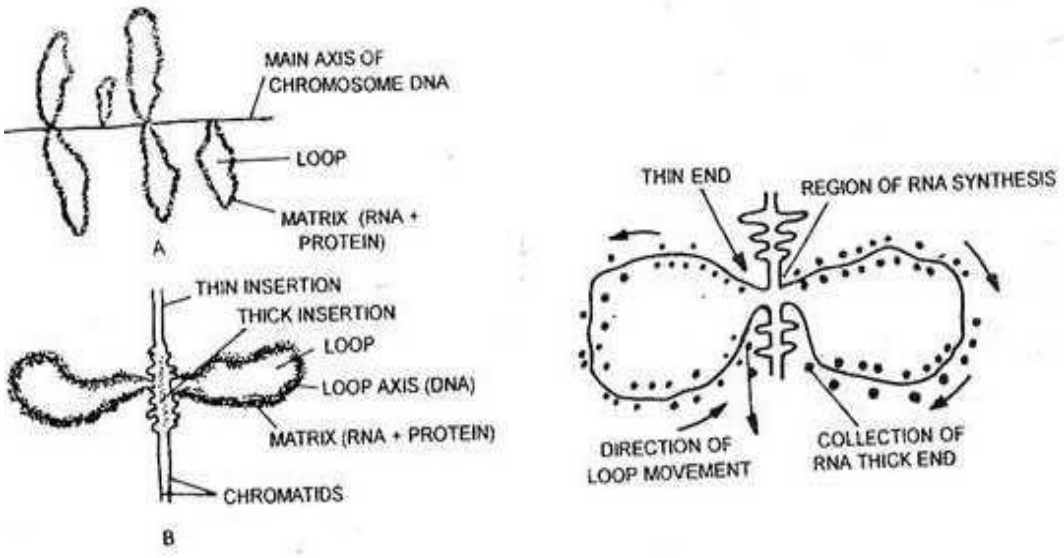

Fig 3.8 Lampbrush chromosome (A) Gross structure (B) Enlarged view (C) Synthesis of RNA in a loop of lampbrush chromosome

Structure:- A lampbrush chromosome (in diplotene stage) consist of two homologous chromosome of the pair is formed of two chromatids which lie parallel and form of high density loops which are lightly coloured, and arise on both sides of the chromosomal axis.

The chromosomal axis, the chromomeres and the loop axis all are formed of DNA. The chromomeres are found in pairs, one chromosome on each chromatid.

These are about 0.25 to 2.0 µm in diameter and are spaced about 2 µm from entire to centre along chromatid axis. These probably represent heterochromatic regions where axial filament remains tightly coiled.

The lateral loops arise from the chromomeres either 2 or in multiple of two. These extended on either side of the chromosomal axis about 550 µm and are about 30-50Å (3-5nm) in diameter. Each loop consists of an axial fibre formed of DNA.

It is surrounded with the matrix composed of RNA and proteins. This gives fuzzy appearance to lateral loops.

Electron Microscopic Structure:-

Electron microscope studies by Miller and Beaty (1969) on Lampbrush chromosomes of salamander oocyte have shown the presence of dense granules on the loop axis of DNA.

These dense granules represent large molecules of enzyme RNA polymerase. On getting attached to DNA, these initiate RNA synthesis. Arising from these RNA polymerase molecules are seen fine fibrils of RNA.

Each loop is considered to be long operon consisting of a series of identical copies of the same structural genes (cistrone) rseparated by spacer DNA. Each gene locus probably produces a very long RNA molecule. This interacts with protein to form ribonucleoprotein.

- According to Callan and Liyod (1960) a chromosome is the master gene with solenoid which produces several identical copies of its own. These extended out as a lateral loop formed of linear strand of nucleosomes, representing the transcriptionally active stage. These are called Salve gene copies.

- According to spinning out and retraction hypothesis, a chromomere is fully transcribed from end to end by spinning out a transient loop. The new loop material spins out on one side of a chromomere at the end of loop and returns to a condenced stage on the other side after completing the synthesis of RNA.

- These are associated with the rapid synthesis of yolk and protein in the maturing ovum. These disappear by the end of first prophase when chromosomes become thick and more condensed.

Polytene Chromosome:-

Polytene chromosomes are one of the giant chromosomes found in animals and plants. In animals it is found in the salivary glands, Malpighian tubules, the epithelium cell lining of thegut and in the fatty cells of the larvae of certain Diptera.

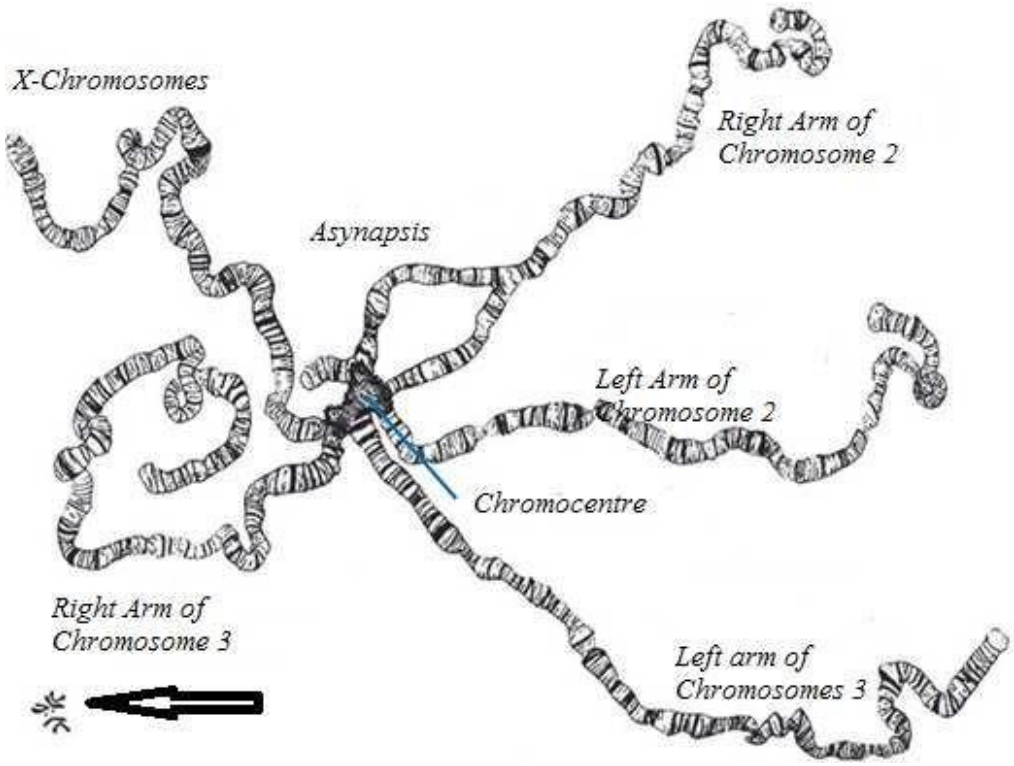

Fig 3.9 Polytene chromosome

The polytene chromosomes of salivary glands in Drosophila larvae of certain Diptera

The polytene chromosomes of salivary glands in Drosophila larvae can be demonstratedeasily in laboratory. It can also be demonstrated in the larvae of Chironomous fly.

Preparation of Polytene Chromosomes from Drosophila

Larvae:-Preparation of Culture: -

- Take a small specimen jar. Make a mixture of the pulp of apple, banana and lemon.
- Take 2 gm of moldex add it in 40 ml boiling water.
- Cool it and add in pulp of fruits prepared.
- Place the jar in the culture of Drosophila flies, keep it open for 2-3 days during which some of the flies will visit the jar for feeding and lay eggs.
- Now cover the jar with fine muslin cloth. Within a week larvae will appear.
- Observe carefully for 3rd instar larvae which will be white coloured.

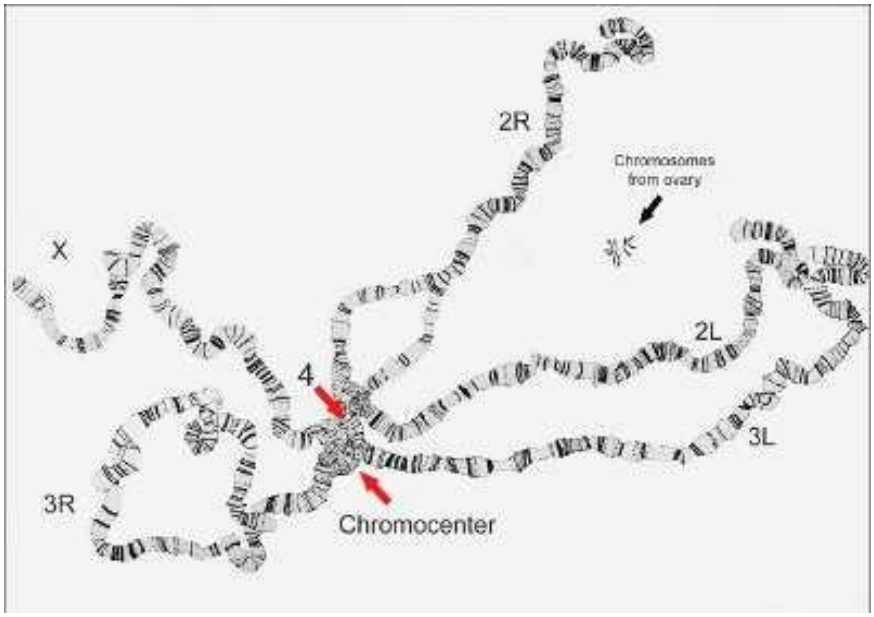

Fig.3.10 Polytene chromosome in the salivary gland of Drosophila

Dissection of 3rd instar larvae for salivary gland: -

- Take a few drop of saline on a clean slide and put the 3rd instlar larvae in it.
- Locate the junction of thorax and abdomen.
- Take two needles, one in each hand. Press the first needle firmly on the posterior end of thorax and other needle at the junction of thorax and abdomen.
- Pull the second needle so that abdomen is separated from head and thorax.
- Then press the thorax with a needle and observe that the salivary glands are seen floating in the saline water on the slide.

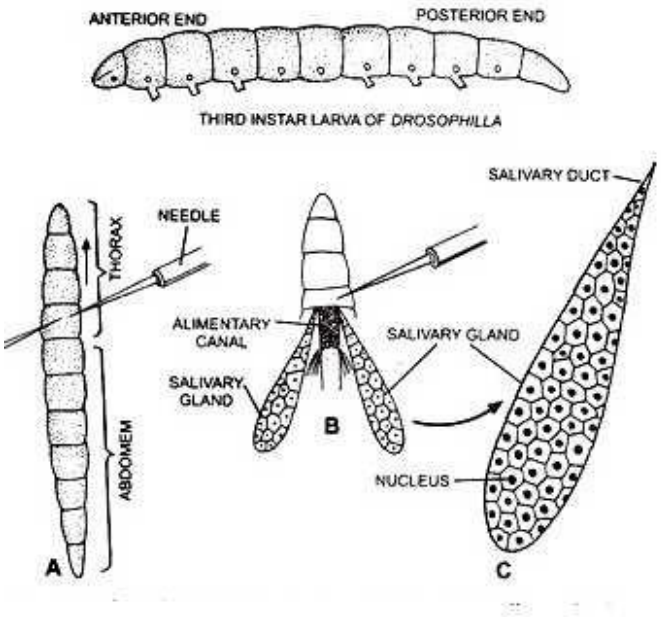

Fig 3.11 Dissection of 3 rd instar Drosophila larva for salivary gland

Preparation of Slide: -

- Take a clean slide; put a drop of acetocarmine on it.
- Transfer the salivary glands in acetocarmine on slide and cover it with a cover slip.
- Leave it for 10 minutes and then warm it gently and then put a blotting paper over it press it smoothly by your thumb.
- Observe the slide under microscope and for details observe under high power of microscope.

Comments:-

1. These are large-sized, hence, called giant chromosomes.

2. These chromosomes present alternate pattern of dark bands and light inter-bands.

3. The dark bands contain rich amount of DNA and RNA, and composed of much coiled chromonemal thread.

4. The light bands contain rich amount of proteins and little amount of DNA and RNA.

5. A polytene chromosomal is multistranded; it is formed of large number of chromosomal thread or strands.

6. A polytene chromosomes exhibits puffs and Balbiani rings at certain points.

7. The puffs are made of lateral extension of bands of chromosomal starands into side loops.

8. The puffs and Balbiani rings are related with the metabolic activities of the chromosomes.

9. These Chromosomes help in the synthesis of proteins, nucleic acids and formation of nuclear material.

10. These were discovered by Balbiani in 1881.

3.4.3 Study of permanent Slide showing stage of Mitochondria

The main components of a typical animal cell are as follows:-

1. Nucleolus
2. Nucleus
3. Ribosome
4. Vesicle
5. Rough endoplasmic reticulum
6. Golgi apparatus (or "Golgi body")
7. Cytoskeleton
8. Smooth endoplasmic reticulum
9. **Mitochondrion**
10. Vacuole
11. Cytosol (fluid that contains organelles, comprising the cytoplasm)
12. Lysosome
13. Centrosome.
14. Cell membrane

The **mitochondrion** (plural **mitochondria**) is a double membrane-bound organelle found in all eukaryotic organisms, although some cells in some organisms may lack them (e.g. Red blood cells). A number of organisms have reduced or transformed their mitochondria into other structures. To date, only one eukaryote is known to have completely lost its **mitochondria**.

The word mitochondrion comes from the Greek, *mitos,* i.e. "thread", and, *chondrion,* i.e. "granule" or "grain-like". Mitochondria have been described as "the powerhouse of the cell" because they generate most of the cell's supply of adenosine tri-phosphate (ATP), used as a source of chemical energy.

Mitochondria are commonly between 0.75 and 3μm in diameter but vary considerably in size and structure. Unless specifically stained, they are not visible.

In addition to supplying cellular energy, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth. Mitochondrial biogenesis is in turn temporally coordinated with these cellular processes. Mitochondria have been implicated in several human diseases, including mitochondrial disorders, cardiac dysfunction, heart failure and autism.

The number of mitochondria in a cell can vary widely by organism, tissue, and cell type. For instance, red blood cells have no mitochondria, whereas liver cells can have more than 2000. The organelle is composed of compartments that carry out specialized functions.

These compartments or regions include the outer membrane, the inter membrane space, the inner membrane, and the cristae and matrix. Mitochondrial proteins vary depending on the tissue and the species. In humans, 615 distinct types of protein have been identified from cardiac mitochondria, whereas in rats, 940 proteins have been reported. The mitochondrial proteome is thought to be dynamically regulated. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome that shows substantial similarity to bacterial genomes.

History:-

The first observations of intracellular structures that probably represented mitochondria were published in the 1840s. Richard Altman, in 1894, established them as cell organelles and called them "bioblasts". The term **"mitochondria"** was **coined by Carl Benda in 1898.**

Leonor Michaelis discovered that Janus green can be used as a supravital stain for mitochondria in 1900. In 1904, Friedrich Meves, made the first recorded observation of mitochondria in plants in cells of the white waterlily, *Nymphaea Alba* and in 1908, along with Claudius Regaud, suggested that they contain proteins and lipids.

Benjamin F. Kingsbury, in 1912, first related them with cell respiration, but almost exclusively based on morphological observations. In 1913, particles from extracts of guinea-pig liver were linked to respiration by Otto Heinrich Warburg, which he called "grana".

Warburg and Heinrich Otto Wieland, who had also postulated a similar particle mechanism, disagreed on the chemical nature of the respiration. It was not until 1925, when David Keilin discovered cytochromes, that the respiratory chain was described.

In 1939, experiments using minced muscle cells demonstrated that cellular respiration using one oxygen atom can form two adenosine triphosphate (ATP) molecules, and, in 1941, the concept of the phosphate bonds of ATP being a form of energy in cellular metabolism was developed by Fritz Albert Lipmann. In the following years, the mechanism behind cellular

respiration was further elaborated, although its link to the mitochondria was not known.

The introduction of tissue fractionation by Albert Claude allowed mitochondria to be isolated from other cell fractions and biochemical analysis to be conducted on them alone. In 1946, he concluded that cytochrome oxidase and other enzymes responsible for the respiratory chain were isolated to the mitchondria.

Eugene Kennedy and Albert Lehninger discovered in 1948 that mitochondria are the site of oxidative phosphorylation in eukaryotes. Over time, the fractionation method was further developed, improving the quality of the mitochondria isolated and other elements of cell respiration were determined to occur in the mitochondria.

The first high-resolution electron micrographs appeared in 1952, replacing the Janus Green stains as the preferred way of visualizing the mitochondria. This led to a more detailed analysis of the structure of the mitochondria, including confirmation that they were surrounded by a membrane.

It also showed a second membrane inside the mitochondria that folded up in ridges dividing upthe inner chamber and that the size and shape of the mitochondria varied from cell to cell. The popular term **"powerhouse of the cell"** was coined by **Philip Siekevitz in 1957**.

In 1967, it was discovered that mitochondria contained ribosomes. In 1968, methods were developed for mapping the mitochondrial genes, with the genetic and physical map of yeast mitochondrial DNA being completed in 1976.

Origin and evolution:-

There are two hypotheses about the origin of mitochondria, **endosymbiotic and autogenous**. The endosymbiotic hypothesis suggests that mitochondria were **originally prokaryotic** cells, capable of implementing oxidative mechanisms that were not possible for eukaryotic cells; they became endosymbionts living inside the eukaryote.

In the autogenous hypothesis, mitochondria were born by splitting off a portion of DNA from the nucleus of the eukaryotic cell at the time of divergence with the prokaryotes; this DNA portion would have been enclosed by membranes, which could not be crossed by proteins. Since mitochondria have many features in common with bacteria, the most accredited theory at presentis endosymbiosis.

A mitochondrion contains DNA, which is organized as several copies of a single, circular chromosome. This mitochondrial chromosome contains genes for redox proteins, such as those of the respiratory chain.

The CoRR hypothesis:

CoRR is short form of co-location for redox regulation. CoRR hypothesis proposes that this co-

location is required for redox regulation. The mitochondrial genome codes for some RNAs of ribosomes, and the 22 t-RNAs necessary for the translation of messenger RNAs into protein. The circular structure is also found in prokaryotes. The proto-mitochondrion was probably closely related to the Rickettsia.

However, the exact relationship of the ancestor of mitochondria to the alphaproteobacteria and whether the mitochondrion was formed at the same time or after the nucleus remain controversial.

The ribosome's coded for by the mitochondrial DNA are similar to those from bacteria in size and structure. They closely resemble the bacterial 70S ribosome and not the 80S cytoplasmic ribosomes, which are coded for by nuclear DNA.

The endosymbiotic relationship of mitochondria with their host cells was popularized by Lynn Margulis. The endosymbiotic hypothesis suggests that mitochondria descended from bacteria that somehow survived endocytosis by another cell, and became incorporated into the cytoplasm.

The ability of these bacteria to conduct respiration in host cells that had relied on glycolysis and fermentation would have provided a considerable evolutionary advantage. This symbiotic relationship probably developed 1.7 to 2 billion years ago.

A few groups of unicellular eukaryotes have only vestigial mitochondria or derived structures: the microsporidians, metamonads, and archamoebae. These groups appear as the most primitive eukaryotes on phylogenetic trees constructed using r-RNA information, which once suggested that they appeared before the origin of mitochondria.

However, this is now known to be an artifact of long-branch attraction—they are derived groups and retain genes or organelles derived from mitochondria (e.g., mitosomes and hydrogenosomes).

Structure:-

A mitochondrion has a double membrane; the inner one contains its chemiosmotic apparatus and has deep grooves which increase its surface area. While commonly depicted as an "orange sausage with a blob inside of it" (like it is here), mitochondria can take many shapes and their inter-membrane space is quite thin.

A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins. The two membranes have different properties. Because of this double-membraned organization, there are five distinct parts to a mitochondrion. They are:

1. The outer mitochondrial membrane.
2. The intermembrane space (the space between the outer and inner membranes),
3. The inner mitochondrial membrane,
4. The cristae space (formed by in-folding of the inner membrane), and

5. The matrix (space within the inner membrane).

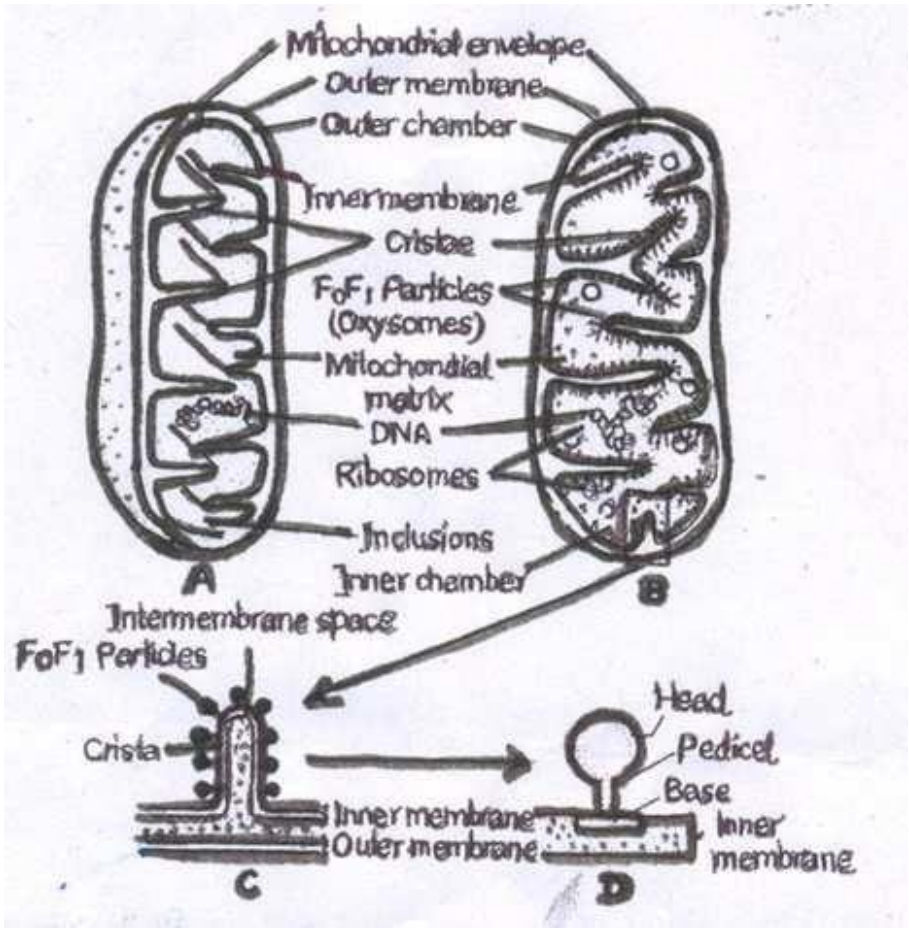

Fig.3.12 Section of Mitochondria showing membrane Chamber and cristae

Mitochondria stripped of their outer membrane are called mitoplasts.

Outer membrane:-

The **outer mitochondrial membrane**, which encloses the entire organelle, is 60 to 75 angstroms (Å) thick.

It has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of integral membrane proteins called porins. Theseporins form channels that allow molecules of 5000 daltons or less in molecular weight to freely diffuse from one side of the membrane to the other.

Larger proteins can enter the mitochondrion if a signaling sequence at their N-terminus binds

to a large multisubunit protein called translocase of the outer membrane, which then actively moves them across the membrane.

Mitochondrial pro-proteins are imported through specialised translocation complexes. The outer membrane also contains enzymes involved in such diverse activities as the elongation of fatty acids, oxidation of epinephrine, and the degradation of tryptophan.

These enzymes include monoamine oxidase, rotenone-insensitive NADH-cytochrome c-reductase, kynurenine hydroxylase and fatty acid Co-A ligase. Disruption of the outer membrane permits proteins in the intermembrane space to leak into the cytosol, leading to certain cell death. The mitochondrial outer membrane can associate with the endoplasmic reticulum (ER) membrane, in a structure called MAM (mitochondria-associated ER-membrane).

This is important in the ER-mitochondria calcium signaling and is involved in the transfer of lipids between the ER and mitochondria. Outside the outer membrane there are small (diameter: 60Å) particles named sub-units of Parson.

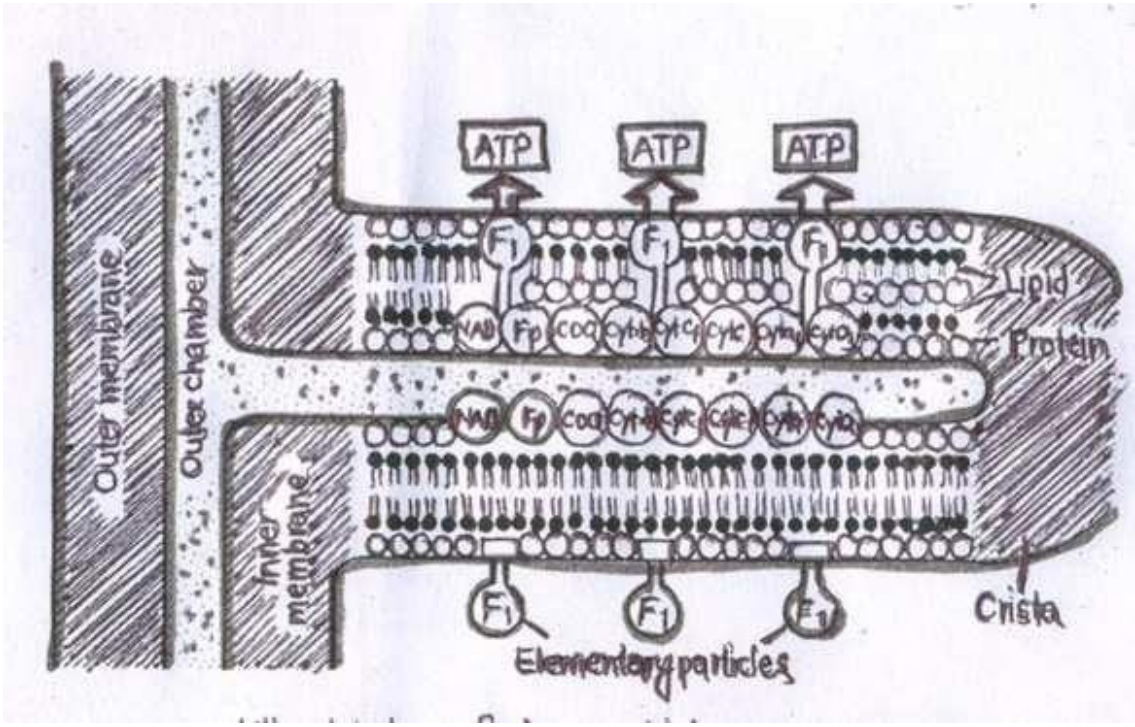

Fig.3.13 Ultra structure of mitochondrial crest showing F1 particles

Intermembrane space:-

The intermembrane space is the space between the outer membrane and the inner membrane. It is also known as perimitochondrial space. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules, such as ions and sugars, in the intermembrane space is the same as in the cytosol.

However, large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the cytosol. One protein that is localized to the intermembrane space in this wayis cytochrome c.

Inner membrane:-

The inner mitochondrial membrane contains proteins with five types of functions:

1. Those that perform the redox reactions of oxidative phosphorylation
2. ATP synthase, which generates ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery
5. Mitochondrial fusion and fission protein

It contains more than 151 different polypeptides, and has a very high protein-to-phospholipid ratio (more than 3:1 by weight, which is about 1 protein for 15 phospholipids). The inner membrane is home to around 1/5 of the total protein in a mitochondrion. In addition, the inner membrane is rich in an unusual phospholipid, cardiolipin.

This phospholipid was originally discovered in cow hearts in 1942, and is usually characteristic of mitochondrial and bacterial plasma membranes. Cardiolipin contains four fatty acids rather than two, and may help to make the inner membrane impermeable. Unlike the outer membrane, the inner membrane doesn't contain porins, and is highly impermeable to all molecules. Almost all ions and molecules require special membrane transporters to enter or exit the matrix.

Proteins are ferried into the matrix via the translocase of the inner membrane (TIM) complex orvia Oxa1. In addition, there is a membrane potential across the inner membrane, formed by the action of the enzymes of the electron transport chain.

Cristae:-

The inner mitochondrial membrane is compartmentalized into numerous cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. For typical liver mitochondria, the area of the inner membrane is about five times as large as theouter membrane.

This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. These folds are studded with small round bodies knownas F_1 particles or oxysomes. These are not simple random folds but rather invaginations of the inner membrane, which can affect overall chemiosmotic function.

One recent mathematical modeling study has suggested that the optical properties of the

cristae in filamentous mitochondria may affect the generation and propagation of light within the tissue.

Matrix:-

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total proteinin a mitochondrion. The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane.

The matrix contains a highly concentrated mixture of hundreds of enzymes, special mitochondrial ribosome's, t-RNA, and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.

Mitochondria have their own genetic material, and the machinery to manufacture their own RNAs and proteins. A published human mitochondrial DNA sequence revealed 16,569 base pairs encoding 37 genes: 22 t-RNA, 2 r-RNA, and 13 peptide genes.

The 13 mitochondrial peptides in humans are integrated into the inner mitochondrial membrane, along with proteins encoded by genes that reside in the host cell's nucleus.

Mitochondria-associated ER membrane:-

The mitochondria-associated ER membrane (MAM) is another structural element that is increasingly recognized for its critical role in cellular physiology and homeostasis. Once considered a technical snag in cell fractionation techniques, the alleged ER vesicle contaminants that invariably appeared in the mitochondrial fraction have been re-identified as membranous structures derived from the MAM—the interface between mitochondria and the ER. Physical coupling between these two organelles had previously been observed in electron micrographsand has more recently been probed with fluorescence microscopy.

Such studies estimate that at the MAM, which may comprise up to 20% of the mitochondrialouter membrane, the ER and mitochondria are separated by a mere 10–25 nm and held togetherby protein tethering complexes.

Purified MAM from sub cellular fractionation has been shown to be enriched in enzymes involved in phospholipids exchange, in addition to channels associated with Ca^{2+} signaling. These hints of a prominent role for the MAM in the regulation of cellular lipid stores and signal transduction have been borne out, with significant implications for mitochondrial-associated cellular phenomena, as discussed below.

Not only has the MAM provided insight into the mechanistic basis underlying such physiological processes as intrinsic apoptosis and the propagation of calcium signaling, but it also favors a more refined view of the mitochondria.

Though often seen as static, isolated 'powerhouses' hijacked for cellular metabolism through an ancient endosymbiotic event, the evolution of the MAM underscores the extent to which

mitochondria have been integrated into overall cellular physiology, with intimate physical and functional coupling to the endomembrane system.

Phospholipids transfer:-

The MAM is enriched in enzymes involved in lipid biosynthesis, such as phosphatidylserine synthase on the ER face and phosphatidylserine decarboxylase on the mitochondrial face. Because mitochondria are dynamic organelles constantly undergoing fission and fusion events, they require a constant and well-regulated supply of phospholipids for membrane integrity.

But mitochondria are not only a destination for the phospholipids they finish synthesis of; rather, this organelle also plays a role in inter-organelle trafficking of the intermediates and products of phospholipids biosynthetic pathways, ceramide and cholesterol metabolism, and glycosphingolipid anabolism.

Such trafficking capacity depends on the MAM, which has been shown to facilitate transfer of lipid intermediates between organelles. In contrast to the standard vesicular mechanism of lipid transfer, evidence indicates that the physical proximity of the ER and mitochondrial membranes at the MAM allows for lipid flipping between opposed bilayers.

Despite this unusual and seemingly energetically unfavorable mechanism, such transport does not require ATP. Instead, in yeast, it has been shown to be dependent on a multiprotein tethering structure termed the ER-mitochondria encounter structure, or ERMES, although it remains unclear whether this structure directly mediates lipid transfer or is required to keep the membranes in sufficiently close proximity to lower the energy barrier for lipid flipping.

The MAM may also be part of the secretory pathway, in addition to its role in intracellular lipid trafficking. In particular, the MAM appears to be an intermediate destination between the rough ER and the Golgi in the pathway that leads to very-low-density lipoprotein, or VLDL, assembly and secretion. The MAM thus serves as a critical metabolic and trafficking hub in lipid metabolism.

Calcium signaling:-

A critical role for the ER in calcium signaling was acknowledged before such a role for the mitochondria was widely accepted, in part because the low affinity of Ca^{2+} channels localized to the outer mitochondrial membrane seemed to fly in the face of this organelle's purported responsiveness to changes in intracellular Ca^{2+} flux.

But the presence of the MAM resolves this apparent contradiction: the close physical association between the two organelles results in Ca^{2+} microdomains at contact points that

facilitate efficient Ca^{2+} transmission from the ER to the mitochondria. Transmission occurs in response to so-called "Ca^{2+} puffs" generated by spontaneous clustering and activation of IP3R, a canonical ER membrane Ca^{2+} channel.

The fate of these puffs—in particular, whether they remain restricted to isolated locales or integrated into Ca^{2+} waves for propagation throughout the cell—is determined in large part by MAM dynamics. Although reuptake of Ca^{2+} by the ER (concomitant with its release) modulates the intensity of the puffs, thus insulating mitochondria to a certain degree from high Ca^{2+} exposure, the MAM often serves as a firewall that essentially buffers Ca^{2+} puffs by acting as a sink into which free ions released into the cytosol can be funneled. This Ca^{2+} tunneling occurs through the low-affinity Ca^{2+} receptor VDAC1, which recently has been shown to be physically tethered to the IP3R clusters on the ER membrane and enriched at the MAM. The ability of mitochondria to serve as a Ca^{2+} sink is a result of the electrochemical gradient generated during oxidative phosphorylation, which makes tunneling of the cation an exergonic process.

Normally, mild calcium influx from cytosol into the mitochondrial matrix causes transient depolarization that is corrected by pumping out protons.

But transmission of Ca^{2+} is not unidirectional; rather, it is a two-way street. The properties of the Ca^{2+} pump SERCA and the channel IP3R present on the ER membrane facilitate feedback regulation coordinated by MAM function. In particular, the clearance of Ca^{2+} by the MAM allows for spatio-temporal patterning of Ca^{2+} signaling because Ca^{2+} alters IP3R activity in a biphasic manner.

SERCA is likewise affected by mitochondrial feedback: uptake of Ca^{2+} by the MAM stimulates ATP production, thus providing energy that enables SERCA to reload the ER with Ca^{2+} for continued Ca^{2+} efflux at the MAM. Thus, the MAM is not a passive buffer for Ca^{2+} puffs; rather it helps modulate further Ca^{2+} signaling through feedback loops that affect ER dynamics.

Regulating ER release of Ca^{2+} at the MAM is especially critical because only a certain window of Ca^{2+} uptake sustains the mitochondria, and consequently the cell, at homeostasis. Sufficient intraorganelle

Ca^{2+} signaling is required to stimulate metabolism by activating dehydrogenase enzymes critical to flux through the citric acid cycle. However, once Ca^{2+} signaling in the mitochondria passes a certain threshold, it stimulates the intrinsic pathway of apoptosis in part by collapsing the mitochondrial membrane potential required for metabolism.

Studies examining the role of pro- and anti-apoptotic factors support this model; for example, the anti-apoptotic factor Bcl-2 has been shown to interact with IP3Rs to reduce Ca^{2+} filling of the ER, leading to reduced efflux at the MAM and preventing collapse of the mitochondrial membrane potential post-apoptotic stimuli. Given the need for such fine regulation of Ca^{2+} signaling, it is perhaps unsurprising that disregulated mitochondrial Ca^{2+} has been implicated in several neurodegenerative diseases, while the catalogue of tumor

suppressors includes a few that are enriched at the MAM.

Molecular basis for tethering:-

Recent advances in the identification of the tethers between the mitochondrial and ER membranes suggest that the scaffolding function of the molecular elements involved is secondary to other, non-structural functions. In yeast, ERMES, a multiprotein complex of interacting ER- and mitochondrial-resident membrane proteins, is required for lipid transfer at the MAM and exemplifies this principle.

One of its components, for example, is also a constituent of the protein complex required for insertion of transmembrane beta-barrel proteins into the lipid bilayer. However, a homologue ofthe ERMES complex has not yet been identified in mammalian cells.

Other proteins implicated in scaffolding likewise have functions independent of structural tethering at the MAM; for example, ER-resident and mitochondrial-resident mitofusins form heterocomplexes that regulate the number of inter-organelle contact sites, although mitofusins were first identified for their role in fission and fusion events between individual mitochondria.

Glucose-related protein 75 (grp75) is another dual-function protein. In addition to the matrix pool of grp75, a portion serves as a chaperone that physically links the mitochondrial and ER Ca^{2+} channels VDAC and IP3R for efficient Ca^{2+} transmission at the MAM. Another potential tether is Sigma-1R, a non-opioid receptor whose stabilization of ER-resident IP3R may preserve communication at the MAM during the metabolic stress response.

Function:-

The most prominent roles of mitochondria are to produce the energy currency of the cell, ATP(i.e., phosphorylation of ADP), through respiration, and to regulate cellular metabolism.

The central sets of reactions involved in ATP production are collectively known as the citric acid cycle, or the Krebs cycle. However, the mitochondrion has many other functions in addition tothe production of ATP.

Energy conversion:-

A dominant role for the mitochondria is the production of ATP, as reflected by the large numberof proteins in the inner membrane for this task. This is done by oxidizing the major products of glucose: pyruvate, and NADH, which are produced in the cytosol.

This type of cellular respiration known as aerobic respiration, is dependent on the presence of oxygen. When oxygen is limited, the glycolytic products will be metabolized by anaerobic fermentation, a process that is independent of the mitochondria.

The production of ATP from glucose has an approximately 13-times higher yield during aerobic respiration compared to fermentation. Recently it has been shown that plant mitochondria can produce a limited amount of ATP without oxygen by using the alternate substrate nitrite.

ATP crosses out through the inner membrane with the help of a specific protein, and across the outer membrane via porins. ADP returns via the same route.

Additional functions:-

Mitochondria play a central role in many other metabolic tasks, such as:

- Signaling through mitochondrial reactive oxygen species
- Regulation of the membrane potential
- Apoptosis-programmed cell death
- Calcium signaling (including calcium-evoked apoptosis)
- Regulation of cellular metabolism
- Certain heme synthesis reactions Steroid synthesis.
- Hormonal signaling

Mitochondria are sensitive and responsive to hormones, in part by the action of mitochondrial estrogen receptors (mtERs). These receptors have been found in various tissues and cell types, including brain and heart

Some mitochondrial functions are performed only in specific types of cells. For example, mitochondria in liver cells contain enzymes that allow them to detoxify ammonia, a waste product of protein metabolism. A mutation in the genes regulating any of these functions canresult in mitochondrial diseases.

Mitochondrial diseases:-

Damage and subsequent dysfunction in mitochondria is an important factor in a range of human diseases due to their influence in cell metabolism. Mitochondrial disorders often present themselves as neurological disorders, including autism.

They can also manifest as myopathy, diabetes, multiple endocrinopathy, and a variety of other systemic disorders. Diseases caused by mutation in the mtDNA include Kearns-Sayre syndrome, MELAS syndrome and Leber's hereditary optic neuropathy.

In the vast majority of cases, these diseases are transmitted by a female to her children, as the zygote derives its mitochondria and hence its mtDNA from the ovum. Diseases such as Kearns- Sayre syndrome, Pearson syndrome, and progressive external ophthalmoplegia are thought to be due to large-scale mtDNA rearrangements, whereas other diseases such as MELAS syndrome, Leber's hereditary optic neuropathy, myoclonic epilepsy with ragged red fibers (MERRF), and others are due to point mutations in mtDNA.

In other diseases, defects in nuclear genes lead to dysfunction of mitochondrial proteins. This is the case in Friedreich's ataxia, hereditary spastic paraplegia, and Wilson's disease.

These diseases are inherited in a dominance relationship, as applies to most other genetic diseases. A variety of disorders can be caused by nuclear mutations of oxidative phosphorylation enzymes, such as coenzyme Q10 deficiency and Barth syndrome. Environmental influences may interact with hereditary predispositions and cause mitochondrial disease.

For example, there may be a link between pesticide exposure and the later onset of Parkinson's disease. Other pathologies with etiology involving mitochondrial dysfunction include schizophrenia, bipolar disorder, dementia, Alzheimer's disease, Parkinson's disease, epilepsy, stroke, cardiovascular disease, chronic fatigue syndrome, retinitis pigmentosa, and diabetes mellitus.

Mitochondria-mediated oxidative stress plays a role in cardiomyopathy in Type 2 diabetics. Increased fatty acid delivery to the heart increases fatty acid uptake by cardiomyocytes, resulting in increased fatty acid oxidation in these cells. This process increases the reducing equivalents available to the electron transport chain of the mitochondria, ultimately increasing reactive oxygen species (ROS) production. ROS increases uncoupling proteins (UCPs) and potentiate proton leakage through the adenine nucleotide translocator (ANT), the combination of which uncouples the mitochondria.

Uncoupling then increases oxygen consumption by the mitochondria, compounding the increase in fatty acid oxidation. This creates a vicious cycle of uncoupling; furthermore, even though oxygen consumption increases, ATP synthesis does not increase proportionally because the mitochondrion is uncoupled.

Less ATP availability ultimately results in an energy deficit presenting as reduced cardiac efficiency and contractile dysfunction. To compound the problem, impaired sarcoplasmic reticulum calcium release and reduced mitochondrial reuptake limits peak cytosolic levels of the important signaling ion during muscle contraction.

The decreased intra-mitochondrial calcium concentration increases dehydrogenase activation and ATP synthesis. So in addition to lower ATP synthesis due to fatty acid oxidation, ATP synthesis is impaired by poor calcium signaling as well, causing cardiac problems for diabetics.

3.4.4- Study of permanent Slide showing stage of Golgi body

The **Golgi body** also known as the **Golgi complex**, **Golgi apparatus**, or simply the **Golgi**, is an organelle found in most eukaryotic cells. It was identified in 1897 by the Italian scientist Camillo Golgi and named after him in 1898.

Part of the cellular endomembrane system, the Golgi apparatus packages proteins into

membrane-bound vesicles inside the cell before the vesicles are sent to their destination. The Golgi apparatus resides at the intersection of the secretory, lysosomal, and endocytic pathways.

It is of particular importance in processing proteins for secretion, containing a set of glycosylation enzymes that attach various sugar monomers to proteins as the proteins move through the apparatus.

Owing to its large size and distinctive structure, the Golgi apparatus was one of the first organelles to be discovered and observed in detail. It was discovered in 1898 by Italian physician Camillo Golgi during an investigation of the nervous system. After first observing it under his microscope, he termed the structure the internal reticular apparatus. Some doubted the discovery at first, arguing that the appearance of the structure was merely an optical illusion created by the observation technique used by Golgi.

With the development of modern microscopes in the 20th century, the discovery was confirmed. Early references to the Golgi referred to it by various names including the "Golgi–Holmgren apparatus", "Golgi–Holmgren ducts", and "Golgi–Kopsch apparatus". The term "Golgi apparatus" was used in 1910 and first appeared in the scientific literature in 1913.

Among eukaryotes, the sub cellular localization of the Golgi apparatus differs. In mammals, a single Golgi apparatus complex is usually located near the cell nucleus, close to the centrosome. Tubular connections are responsible for linking the stacks together. Localization and tubular connections of the Golgi apparatus are dependent on microtubules. If microtubules are experimentally depolymerized, then the Golgi apparatus loses connections and becomes individual stacks throughout the cytoplasm. In yeast, multiple Golgi apparatuses are scattered throughout the cytoplasm.

In plants, Golgi stacks are not concentrated at the centrosomal region and do not form Golgi ribbons. Organization of the plant Golgi depends on actin cables and not microtubules. The common feature among Golgi is that they are adjacent to endoplasmic reticulum (ER) exit sites.

Structure:-

In most eukaryotes, the Golgi apparatus is made up of a series of compartments consisting of two main networks: the cis Golgi network (CGN) and the Trans Golgi network (TGN). The CGN is a collection of fused, flattened membrane-enclosed disks known as cisternae, originating from vesicular clusters that bud off the endoplasmic reticulum.

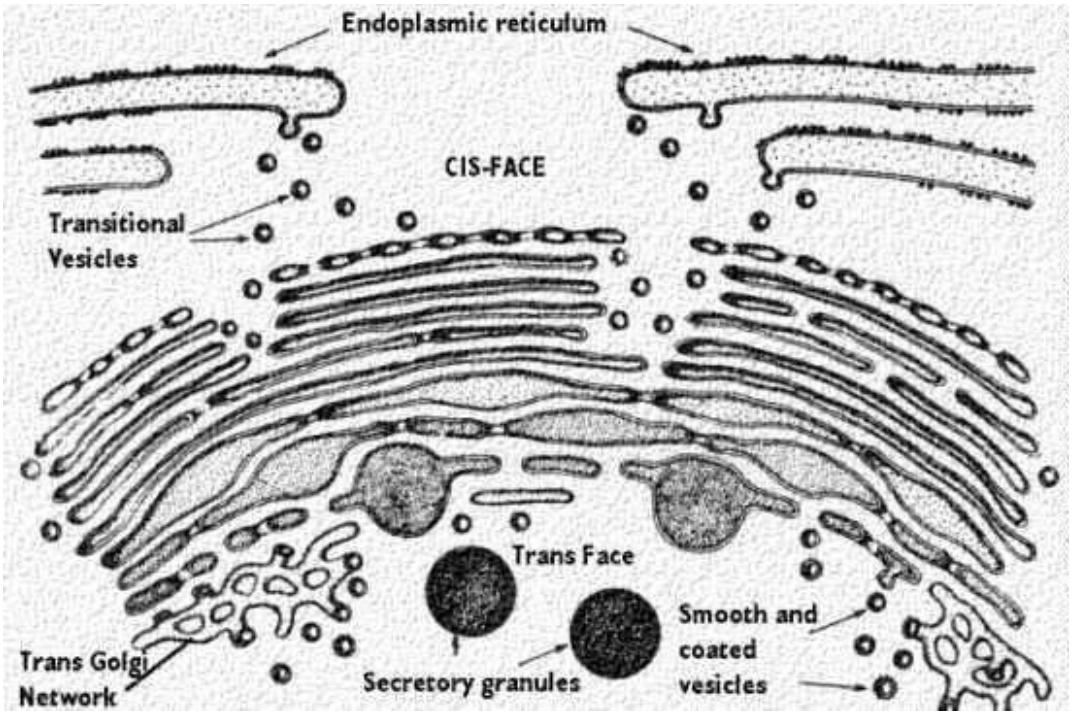

Fig.3.14 Relation between different component of Golgi body & their relation with secretion

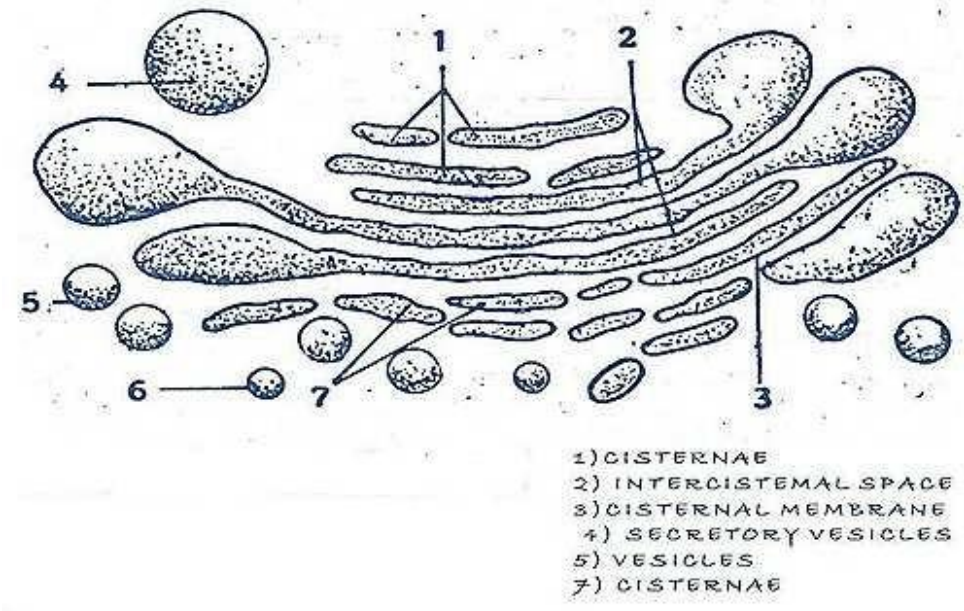

Fig.3.15 Ultrastructure of Golgi complex

A mammalian cell typically contains 40 to 100 stacks. Between four and eight cisternae are usually present in a stack; however, in some protists as many as sixty cisternae have been observed. This collection of cisternae is broken down into cis, medial, and Trans compartments.

The TGN is the final cisternal structure, from which proteins are packaged into vesicles destined to lysosomes, secretory vesicles, or the cell surface. The TGN is usually positioned adjacent to the stacks of the Golgi apparatus, but can also be separate from the stacks. The TGN may act as an early endosome in yeast and plants.

There are structural and organizational differences in the Golgi apparatus among eukaryotes. In some yeasts, Golgi stacking is not observed. Pichia pastoris does have stacked Golgi, while Saccharomyces cerevisiae does not. In plants, the individual stacks of the Golgi apparatus seem to operate independently.

The Golgi apparatus tends to be larger and more numerous in cells that synthesize and secrete large amounts of substances; for example, the antibody-secreting plasma B cells of the immune system have prominent Golgi complexes.

In all eukaryotes, each cisternal stack has a cis entry face and a trans exit face. These faces are characterized by unique morphology and biochemistry. Within individual stacks are assortments of enzymes responsible for selectively modifying protein cargo.

These modifications influence the fate of the protein. The compartmentalization of the Golgi apparatus is advantageous for separating enzymes, thereby maintaining consecutive and selective processing steps: enzymes catalyzing early modifications are gathered in the cis face cisternae,and enzymes catalyzing later modifications are found in Trans face cisternae of the Golgi stacks.

Function:-

The Golgi apparatus is a major collection and dispatch station of protein products received from the endoplasmic reticulum (ER). Proteins synthesized in the ER are packaged into vesicles, which then fuse with the Golgi apparatus.

These cargo proteins are modified and destined for secretion via exocytosis or for use in the cell. In this respect, the Golgi can be thought of as similar to a post office: it packages and labels items which it then sends to different parts of the cell or to the extracellular space. The Golgi apparatus is also involved in lipid transport and lysosome formation.

The structure and function of the Golgi apparatus are intimately linked. Individual stacks have different assortments of enzymes, allowing for progressive processing of cargo proteins as they travel from the cis to the trans Golgi face. Enzymatic reactions within the Golgi stacks occur exclusively near its membrane surfaces, where enzymes are anchored.

This feature is in contrast to the ER, which has soluble proteins and enzymes in its lumen. Much of the enzymatic processing is post-translational modification of proteins. For example,

phosphorylation of oligosaccharides on lysosomal proteins occurs in the early CGN. Cis cisternais associated with the removal of mannose residues. Removal of mannose residues and additionof N-acetylglucosamine occur in medial cisternae.

Addition of galactose and sialic acid occurs in the trans cisternae. Sulfation of tyrosines and carbohydrates occurs within the TGN. Other general post-translational modifications of proteins include the addition of carbohydrates (glycosylation) and phosphates (phosphorylation). Protein modifications may form a signal sequence that determines the final destination of the protein. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes.

Another important function of the Golgi apparatus is in the formation of proteoglycans. Enzymes in the Golgi append proteins to glycosaminoglycans, thus creating proteoglycans. Glycosaminoglycans are long unbranched polysaccharide molecules present in the extracellular matrix of animals.

Vesicular transport:-

The vesicles that leave the rough endoplasmic reticulum are transported to the cis face of the Golgi apparatus, where they fuse with the Golgi membrane and empty their contents into the lumen. Once inside the lumen, the molecules are modified, and then sorted for transport to their next destinations.

Those proteins destined for areas of the cell other than either the endoplasmic reticulum or the Golgi apparatus are moved through the Golgi cisternae towards the trans face, to a complex network of membranes and associated vesicles known as the trans-Golgi network (TGN). This area of the Golgi is the point at which proteins are sorted and shipped to their intended destinations by their placement into one of at least three different types of vesicles, dependingupon the signal sequence they carry.

Current models of vesicular transport and trafficking:-

Model 1: Anterograde vesicular transport between stable compartments:-

- In this model, the Golgi is viewed as a set of stable compartments that work together. Each compartment has a unique collection of enzymes that work to modify protein cargo. Proteins are delivered from the ER to the cis face using COPII-coated vesicles. Cargo then progress toward the trans face in COPI-coated vesicles. This model proposes that COPI vesicles move in two directions: anterograde vesicles carry secretory proteins, while retrograde vesicles recycle Golgi-specific trafficking proteins.

  Strengths: The model explains observations of compartments, polarized distributionof enzymes, and waves of moving vesicles. It also attempts to explain how Golgi-specific enzymes are recycled.

Weaknesses: Since the amount of COPI vesicles varies drastically among types of cells, this model cannot easily explain high trafficking activity within the Golgi for both small and large cargoes. Additionally, there is no convincing evidence that COPI vesicles move in both the anterograde and retrograde directions.

- This model was widely accepted from the early 1980s until the late 1990s.

Model 2: Cisternal progression/maturation:-

In this model, the fusion of COPII vesicles from the ER begins the formation of the first cis-cisterna of the Golgi stack, which progresses later to become mature TGN cisternae. Once matured, the TGN cisternae dissolve to become secretory vesicles. While this progression occurs, COPI vesicles continually recycle Golgi-specific proteins by delivery from older to younger cisternae. Different recycling patterns may account for the differing biochemistry throughout the Golgi stack. Thus, the compartments within the Golgi are seen as discrete kinetic stages of the maturing Golgi apparatus.

Strengths:

The model addresses the existence of Golgi compartments, as well as differing biochemistry within the cisternae, transport of large proteins, transient formation and disintegration of the cisternae, and retrograde mobility of native Golgi proteins, and it can account for the variability seen in the structures of the Golgi.

Weaknesses:

This model cannot easily explain the observation of fused Golgi networks, tubular connections among cisternae, and differing kinetics of secretory cargo exit.

Model 3: Cisternal progression/maturation with heterotypic tubulartransport:-

This model is an extension of the cisternal progression/maturation model. It incorporates the existence of tubular connections among the cisternae that form the Golgi ribbon, in which cisternae within a stack are linked. This model posits that the tubules are important for bidirectional traffic in the ER-Golgi system: they allow for fast anterograde traffic of small cargo and/or the retrograde traffic of native Golgi proteins.

Strengths: This model encompasses the strengths of the cisternal progression/maturation model that also explains rapid trafficking of cargo, and how native Golgi proteins can recycle independently of COPI vesicles

Weaknesses: This model cannot explain the transport kinetics of large protein cargo, such

as collagen. Additionally, tubular connections are not prevalent in plant cells. The roles that these connections have can be attributed to a cell-specific specialization rather than a universal trait. If the membranes are continuous, that suggests the existence of mechanisms that preserve the unique biochemical gradients observed throughout the Golgi apparatus.

Model 4: Rapid partitioning in a mixed Golgi:-

This rapid partitioning model is the most drastic alteration of the traditional vesicular trafficking point of view. Proponents of this model hypothesize that the Golgi works as a single unit, containing domains that function separately in the processing and export of protein cargo. Cargo from the ER moves between these two domains, and randomly exits from any level of the Golgi to their final location. This model is supported by the observation that cargo exits the Golgi in a pattern best described by exponential kinetics. The existence of domains is supported by fluorescence microscopy data.

Strengths: Notably, this model explains the exponential kinetics of cargo exit of both large and small proteins whereas other models cannot.

Weaknesses: This model cannot explain the transport kinetics of large protein cargo, such as collagen. This model falls short on explaining the observation of discrete compartments and polarized biochemistry of the Golgi cisternae. It also does not explain formation and disintegration of the Golgi network, nor the role of COPI vesicles.

Model 5: Stable compartments as cisternal model progenitors:-

This is the most recent model. In this model, the Golgi is seen as a collection of stable compartments defined by Rab (G-protein) GTPases.

Strengths: This model is consistent with numerous observations and encompasses some of the strengths of the cisternal progression/maturation model. Additionally, what is known of the Rab GTPase roles in mammalian endosomes can help predict putative roles within the Golgi. This model is unique in that it can explain the observation of "megavesicle" transport intermediates.

Weaknesses: This model does not explain morphological variations in the Golgi apparatus, nor define a role for COPI vesicles. This model does not apply well for plants, algae, and fungi in which individual Golgi stacks are observed (transfer of domainsbetween stacks is not likely). Additionally, megavesicles are not established to be intra- Golgi transporters.

Though there are multiple models that attempt to explain vesicular traffic throughout the Golgi, no individual model can independently explain all observations of the Golgi apparatus. Currently, the cisternal progression/maturation model is the most acceptedamong

scientists, accommodating many observations across eukaryotes. The other models are still important in framing questions and guiding future experimentation. Among the fundamental unanswered questions are the directionality of COPI vesicles and role of Rab GTPases in modulating protein cargo traffic.

3.6 Summary

Cytology, branch of biology concerned with the study of the structure and function of cells as individual units, supplementing histology, which deals with cells as components of tissues. Cytology is concerned with the structure and activities of the various parts of the cell and cell membrane; the mechanism of cell division; the development of sex cells, fertilization, and the formation of the embryo; cell derangements, such as those occurring in cancer; cellular immunity; and the problems of heredity.

Until modern times, cytology was concerned primarily with the microscopic observation of stained dead cells and the correlation of such observations with known physiological phenomena. Recently, new procedures have been introduced by which the living cell can be observed and studied. The phase-contrast microscope provides a means of studying the living cell in action without the use of dyes. Micro dissection, microinjection, and microchemistry furnish methods for drawing off minute amounts of living protoplasm through tubes a half micron in diameter, and subjecting them to analysis.

Cytology is important in modern medicine, especially in the diagnosis of diseases by examination of the cells occurring in the various body fluids. The determination of the number and proportion of the different types of cells in the blood, by a blood count, is important in diagnosing acute infections and other diseases. Variations in the size and shape of the red blood cell indicate the presence of: sickle-cell anemia if the cell is half-moon shaped; pernicious anemia if it is very large; or iron-deficiency anemia if it is very small. The type of disease may also be determined through cytology, as, for example, in distinguishing the various types of meningitis by examination of the cells present in the cerebrospinal fluid.

3.7 Glossary

Active Transport: The movements or ions or molecules of a substance through the plasma membrane from a solution of low concentration to a solution of high concentration i.e. against electro-chemical gradient. The process needs energy.

Amino Acid Organic compounds with acidic (-COOH) and amino (-NH2), groups; 20of which, different in organic chain attached to carbon atom, are the structural units of protein macromolecules.

Amitosis Director division of nucleus into two, without differentiation of chromosomes and formation of spindle etc.

Anaphase A stage in nuclear division immediately after metaphase and is followed by telophase. It is characterized by the movement of sets of daughter

	chromosomes from the equatorial plate towards the opposite poles of the spindle.
Aneuploids	The organism having chromosomes of a set parent in different numbers
Autolysis	Disintegration of cells by the action of their own enzymes
Budding	A mode of asexual reproduction in which new organism develops from the parent body in the form of an outgrowth or projection.
Catalase	An enzyme which facilitates conversion of hydrogen peroxide to water and oxygen
Cell division	The process of division of pre-existing (parental) cell into two newdaughter cells.
Chromatin	Deeply stained part of of the nuclear reticulum mostly of DNA, which condenses into chromosomes during cell division.
Chromomere	Irregular masses of heterochromatin
Coenzyme	Organic compound which activates the enzyme.
Colloid	Substances having particles which range from 1mμ to 100 mμ in size
Conjugation	Temporary association between the organisms of two different strains so as to facilitate nuclear exchange
Cytolysis	Dissolution or disintegration of a cell
Deletion	Loss of segment from a chromosome
Diplotene	A stage in the first prophase of meiosis, in which each of the synaptic chromosomes get doubled by splitting. It comes after pachytene and is followed by diakinesis
Germ cell	As gamete
Gonad	Gamete producing organ
Haploid	Having half the number of chromosomes that are present in the diploid organism. Usually the gametes..
Heterogamy	Darkely stained part of the chromatin in the interphase nucleus which represents the condenced chromatin and results due to failure of its conversion into a nuclear reticulum.
Matrix	Intercellular substances in which animal cells are embedded
Micron	A unit of measurement: 1/1000 mm usually designated by the Greek letterμ.
Operon	A group of genes that are transcribed into a single length messenger RNA for a single character

Osmosis	The passage of a fluid through a semi-permiable membrane due to osmotic pressure
Pachytene	Midprophase stage of first reduction division (or meiosis) in which the chromosomes are visible as long paired threads
Polar body	Bodies extruded out during oogenesis one after each maturation division
Promotor	A site on a chromosome where RNA-polymerase binds and initiates RNA synthesis
Recombination	The appearance in an individual of alleles for different characters thatwere not present together in either parent
Spindle	The chromatic figure formed during cell division by the differentiation of cytoplasm into radiating fibres which are diposed in such a manner that these form a spindle figure. The equator of spindle provides surface for the orientation of chromosomes
Tetrad	The group of four chromatids resulting from the pairing of homologous chromosomes and their splitting during 1st prophase of meiosis
Triploids	Organism having three haploid sets of chromosomes i.e.3n
Unit membrane	The membrane formed of two layers of lipid molecules sandwiched between the two layers of protein molecules. It forms the outer boundaryof almost all the cell organelles.

3.7 Self assessment Questions

1. Who proposed the cell theory?
2. Mitochondria are generally called the power houses of cell. Why?
3. What are the special types of chromosomes?
4. What are the difference between mitosis and meiosis?
5. What do you understand by meiotic division?

3.8 References

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3.9 Suggested Readings

Cell Biology-Harvey Lodish,

Dr. S.P. Singh, Cytology – Prof. P.K.Gupta

3.10 Terminal Questions

1. Describe the structure and function of Mitochondria.
2. What are Lampbrush Chromosomes? Mention its structure and significance.
3. What is cell theory? Give an illustrated account of the structure and function of golgi body.
4. Discuss the methods of preparation and dissection of 3rd instar larvae for salivary gland.
5. Write short notes any two of the following.

(a) Polytene Chromosome (b) Cristae © Karyokinesis

Unit 4 Cell Biology

Learners will get familiar with different structure of biomolecules, types of cells and various biological processes with the help of models and charts present in the laboratory.