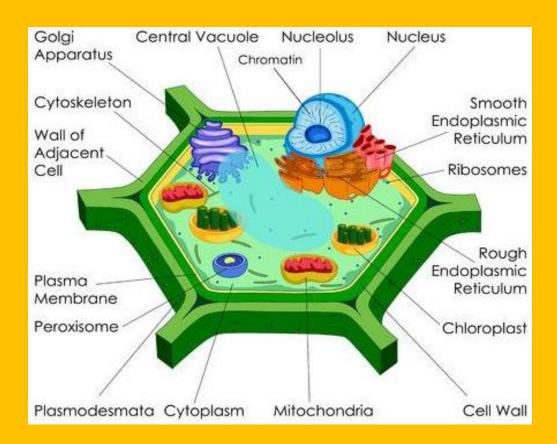


MSCBOT-506

M.Sc. II Semester CELL BIOLOGY OF PLANTS



DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

CELL BIOLOGY OF PLANTS



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BLOCK-1 PLANT CELL

UNIT-1 PROKARYOTIC AND EUKARYOTIC

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1.1 OBJECTIVES

The present topic provides an overview of structure, organization and chemical composition of the prokaryotic as well as eukaryotic organisms. After reading this topic you will learn about the following:

- Fundamental characteristics of prokaryotic and eukaryotic organisms.
- Able to differentiate between prokaryotic and eukaryotic organisms
- Cell structure and organization of organisms
- Know about cellular structure of plant and animal cell
- Specialized plant cell types
- Chemical composition of cell

1.2 INTRODUCTION

Cell "building blocks of life" is the smallest unit of all organisms. The cell was first discovered by Robert Hooke in 1665 after building of the microscope. The word cell is derived from a Latin word "cella" which means small room. Cell is considered as basic biological, structural and functional unit of all living organisms. Cells consist of cytoplasm enclosed within a membrane, which contain many vital macromolecules such as proteins and nucleic acids. The cell is a unit of biological activity delimited by a semipermeable membrane and capable of self-reproduction in a medium free of other living systems. However, this definition is not applicable for the viruses because they are capable to self-multiply only using the cellular machinery of other organisms.

All the living organisms, except virus and certain group of plants (*Rhizopus*, *Vaucheria* etc.) possess the well-organized cellular body which may contain one or more cells. According to the number of cells, the organisms can be divided into two categories. The living organisms which are made up of a single cell are known as unicellular organisms. All blue green algae, some higher algae (diatoms, *Cosmarium*, *Chlorella*, *Microcystis*, *Pinnularia*, *Haematococcus* etc.) and group of protozoa are the good examples of unicellular organisms. On the other hand, the organisms made up of more than one cell is called multicellular (other group of plants and animals).

The cells are found in different varieties of shapes, sizes and numbers which indicate their evolutionary adaptation at different environmental conditions. Cells range in size from the smallest bacteria, only a few tenths of µm in diameter to certain large marine algae and to various bird eggs with dimension of centimeters. Besides all its apparent diversity, however, cells have many common characteristics, for example, potential for an independent existence. Thus cells have the ability to continue living in the absence of any other cell. In the same reference, the cell theory explains that all living organisms are constructed by one or more cells,

and the functions of a multicellular organism are a consequence of the types of cells it has. According to this theory, each living beings on this earth begin its life as a single cell. Some living being, including bacteria, remain single-celled in the process of evaluation, while the other living things (including plants and animals) grow and develop into many cells. Collectively, all the living cells are broadly classified into two category i.e., prokaryotic cells and eukaryotic cells, according to whether their genetic materials are enclosed by a nuclear envelope or not. Eukaryotic cells possess this envelope while a prokaryotic cell does not. Eukaryotic cells may have evolved from prokaryotic cells but contain different types of organelles like well-organized nucleus, endoplasmic reticulum, golgi body, mitochondria etc, which are specific in their functions. But features like growth, response, and most importantly giving birth to the young ones are the commonly shared by all living organisms.

1.3 STRUCTURE AND ORGANIZATION OF CELLS

Life exhibits varying degrees of organization. Based upon the origin and development, cells fall into two major groups: prokaryotes and eukaryotes. The term prokaryotic and eukaryotic were coined by Hans Ris in the early 1960's. Any cellular organism may have only one kind of cell either prokaryotic or eukaryotic. Prokaryotic cells are usually more primitive, smaller, simple, and lack the internal compartmentalization while eukaryotic cells have a great variety of organelles and have internal organization. No matter, which type of cell we are considering; all cells have certain features in common, such as a cell membrane, genetic material, cytoplasm, and ribosomes.

1.3.1 Prokaryotic cells

Prokaryotic cells may be defined as the cells which do not have a true nucleus and the membrane-bound organelles. Organisms that possess such kind of cells are known as prokaryotes and they are typically unicellular in organization (Nelson and Cox 2005). For example: Archaea, bacteria, pleuropneumonia like organisms (PPLO), blue green algae, protozoa etc.

The prokaryotic (Gr., *pro*- primitive, *karyon*- nucleus) cells are the most primitive type of cells that exist on this earth from the morphological point of view. These are the simplest and mostly small sized (1-10 µm) cells. It is essentially one envelope system. As its name indicates, the nucleus are primitive type but true nucleus is absent. The cells comprise of a central nuclear components, sometime called nucleoid (resemble like nucleus, but not true nucleus) which contain their vital biomolecules (nuclear protein and DNA). Neither the nuclear apparatus nor the respiratory enzyme systems are separately enclosed by membrane. However, the inner surface of the plasma membrane itself may serve for enzyme attachment. The cytoplasm of such kind of cells lack in well-defined cytoplasmic organelles (endoplasmic reticulum, mitochondria, centrioles, golgi bodies etc).

Reproduction takes place by the vegetative means. Binary fission and budding are the common methods of reproduction in prokaryotes. Sexual reproduction is usually absent; however, if present it is unidirectional transfer of genes from donor to recipient. The mode of nutrition is principally absorption type. However, some blue green algae synthesize their own food due to the presence of photosynthetic pigments. Some examples of the typical prokaryotic cells and cell organization are given below.

(i) Pleuropneumonia like organisms (PPLO)

The Pleuropneumonia like organisms (PPLOs) are the simplest known cells devoid of cell wall. They are small in size and having deformable triple layer plasma membrane around the cell. PPLO resemble with the bacteria cell which differ with only in the absence of cell wall and mesosomes. Therefore, they are excluded from the group of bacteria. The diameters of the smallest PPLO are 0.1-0.3 µm, and of the largest on the order of a micron. Thus, in size, the PPLOs overleaped with the largest viruses and with the smallest bacteria (Verma and Agarwal, 2008). *Mycoplasma* is the widely studies genus of the PPLO.

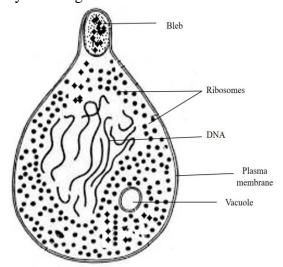


Fig. 1.1: Diagram of a typical prokaryotic cell (PPLO)

The cell of PPLO (**Fig. 1.1**) is restricted by a thick (75 A°) plasma membrane. The granulated ground material called cytoplasm contains vacuoles of undefined significance. At the end of the cell, a bud like structure called bleb is present with less known function. The genetic material (DNA=fibrils form or a single circular double helix) is limited in nuclear region and not enclosed by nuclear membrane. The nuclear region is encircled by many ribosomes and other components involved in protein synthesis.

PPLOs are free living and do not required host cell for its multiplication. In PPLO the reproduction takes place by the method of budding, binary fission, formation of tiny spore like bodies and growth of large branched filament that fragmented ultimately and produced as new organisms.

(ii) Bacteria cell

Cell organization of a typical bacterial cell (Fig.1.2) is given as below:

1. Outer covering: The outer covering of the bacterial cell possess three layers i.e.:

Layer 1: Capsule: It is the outermost, additional protective layer of the bacterial cell which is slimy in nature and made up of polysaccharides. It may or may not be present around the cell wall.

Layer 2: Cell wall: After the capsule, a rigid and strong layer is present which is known as cell wall. The cell wall besides containing protein, lipids, carbohydrates, phosphorus and certain inorganic salts, also contains an amino acid diaminopimelic acid (only in bacteria and blue green algae) and a derivative of glucose known as muramic acid. The bacterial cell walls are made of peptidoglycan which is made up of polysaccharide chains cross-linked by unusual peptides containing D-amino acids (van Heijenoort 2001; Koch 2003). Therefore, the cell wall of bacteria is different from the plant and fungi. In plant and fungi, cell walls are made up of cellulose and chitin, respectively.

The bacterial cell wall can be divided into two categories i.e., Gram positive and Gram negative according to differences in their cell wall. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan, teichoic acids and a traces of RNA. In contrast, Gramnegative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. They do not contain teichoic acids and RNA.

Layer 3: Plasma membrane: After cell wall, a thin bilayer surrounds the whole cytoplasm. This thin layer membrane is called plasma membrane or cytoplasmic membrane. This layer functioned as a selective permeability barrier that regulates the passage of substances into and out of the cell. The bacterial membrane allows passage of water and uncharged molecules up to molecular weight of about 100 Daltons, but does not allow passage of larger molecules. This membrane also contains the oxidative or respiratory enzymes which have the similar functions as mitochondria in eukaryotic cells. The plasma membrane is composed of a phospholipid bilayer. Bacterial membranes are composed of 40% phospholipid and 60% protein. The phospholipids are amphiphilic molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environment.

2. Cytoplasm: The bacterial cytoplasm is a viscous, dense, colloidal and granulated material. All the cell organelles, which are usually present in the eukaryotic organisms, are absent in it. However, no membrane bound, organelles occur in cytoplasm. The ribosomes function in the protein synthesis.

DNA molecule or bacterial chromosome is considered as the genetic material which is confined in a particular region called nucleoid. Certain bacteria are able of photosynthesis with the help of a pigment called bacteriochlorophyll. This photosynthesis pigment and some other enzymes are associated with the internal membranes that are arranged as lamellae, tubules or vesicles in different species.

Many bacteria are able to swim freely with the help of thread like structure called flagella, which usually help the bacteria in locomotion. Generally, rod and spiral shaped bacteria regularly contain flagella while it is entirely absent in spherical bacteria.

Some bacteria also possess a hair like out growth called pili or fimbriae. Pili somewhat resemble with the flagella including a basal body anchor within the cytoplasm. In most bacteria pili are responsible for recognizing and attaching to the host. In some bacteria, sex pili in male strain play a role to make a bridge with the female cell during mating.

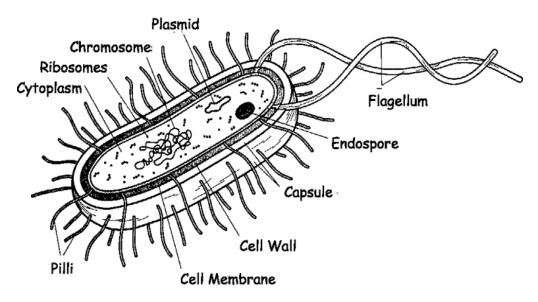


Fig. 1.2: A typical bacterial cell

1.3.2 Eukaryotic cells

Eukaryotes represent a tiny minority of all living things (Whitman et al. 1998). However, due to their much larger size, their collective worldwide biomass is estimated to be about equal to that of prokaryotes (Whitman et al. 1998). Eukaryotes evolved approximately 1.6–2.1 billion years ago, during the Proterozoic eon. These are the advanced type of cells which may be derived or evaluated from the prokaryotic cells. There is a hudge differences between pro and eukaryotic cells as given below:

	Properties	Prokaryote	Eukaryote
Basics	Size	Generally small $< 2 \mu m$ in diameter	Usually larger. 2 to 100µm in diameter
	Origin	Most primitive	Relative new, or evolved from the prokaryote
	Phylogenetic group	Bacteria, Archaea	Algae, fungi, protozoa, plnt and animal cell
motility	Flagellar movement	Flagella composed of single type of protein arrange in a fiber; flagella rotate	Flagella or cillia; composed of microtubues; do not rotate
Forms of motility	Nonflagellar movement	Gliding motility; gas vesicle mediated	Cytoplasmic streamlining and ameboid movement; gliding motility
	Nuclear membrane	Absent	Present
ction	Nucleolus	Absent	Present
and func	DNA	Single molecule generally covalently closed and circular, not complexed with histones	Linear, present in several chromosomes, usually complexed with histones.
ructur	Division	No mitosis	Mitosis, mitotic apparatus with microtubular spindle
Nuclear structure and function	Sexual reproduction	Fragmentary process, unidirectional, no meiosis, usually only portions of genetic complement reassorted	Regular process, meiosis reassortment whole chromosome complement
	Introns in genes	Rare	Common
Cytoplasmic structure and organization	Cytoplasmic membrane	Usually lacks sterols: hopanoids may be present	Sterols usually present; hopanoids absent
	Internal membrane	Relative simple, limited to specific group	Complex, endoplasmic reticulum and Golgi complex
	Ribosomes	70S in size	80S, excepts for ribosome of mitochondria and chloroplasts, which are 70S
Cyt	Membranous organelles	Absent	Several present

Resp Cell	Photosynthetic pigments	In internal membranes of chromosomes, chloroplast are absent	In chloroplasts
	Respiratory system	Part of cytoplasmic membrane	In mitochondria
	Cell wall	Present (in most), composed f peptidoglycan (bacteria), other polysaccharides, protein, glycoprotein	Present in plant, algae, fungi, usually polysaccharid, absent in animals and most protozoa
	Endospores	Present (in some), very heat resistant	Absent
	Gas vesicles	Present (in some)	Absent

The eukaryotic (Gr., *eu*- well or true, *Karyotic*-nucleus) cells are those cells which contain a well-constructed nucleus (nucleus enclosed within the membrane) and membrane bounded cell organelles (**Fig. 1.3**). Organisms that possess such kind of cells are known as eukaryotes. They are mostly multicellular in organization (Nelson and Cox 2005) which include organisms consisting of many cell types forming different kinds of tissue. For example, animal and plant cells.

Difference between animal and plant cells:

- 1. **Shape and Size:** Plant cells are 10-100 micrometers in length, typically rectangular or cubic in shape, however animal cells are 10-30 micrometers in length and irregular in shape.
- 2. **Energy store:** Animals cells store energy in the form of the complex carbohydrate glycogen. Plant cells store energy as starch.
- 3. **Differentiation:** In animal cells, only stem cells are capable of converting to other cell types. Most plant cell types are capable of differentiation.
- 4. **Growth:** Animal cells increase in size by increasing in cell numbers. Plant cells mainly increase cell size by becoming larger by absorbing more water into the central vacuole.
- 5. **Cell Wall:** Animal cells do not have a cell wall but have a cell membrane. Plant cells have a cell wall composed of cellulose as well as a cell membrane.
- 6. **Centrioles**: Animal cells contain these cylindrical structures that organize the assembly of microtubules during cell division. Plant cells do not typically contain centrioles.
- 7. **Lysosomes:** Animal cells possess lysosomes which contain enzymes that digest cellular macromolecules. In plant cells vacuole handles molecule degradation.

8. **Plastids:** Animal cells do not have plastids. Plant cells contain plastids such as chloroplasts, which are needed for photosynthesis.

- 9. Plasmodesmata: Animal cells do not have plasmodesmata. Plant cells have plasmodesmata, which are pores between two plant cell walls that allow molecules and communication signals to pass between individual plant cells.
- 10. **Vacuole:** Animal cells may have many small vacuoles. Plant cells have a large central vacuole that can occupy up to 90% of the cell's volume.

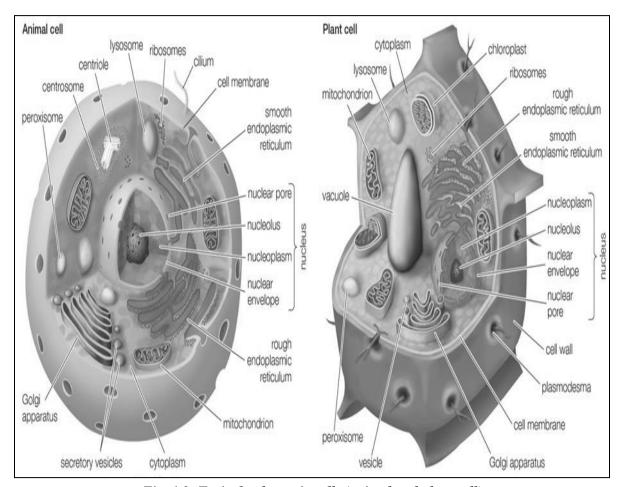


Fig. 1.3: Typical eukaryotic cells (animal and plant cell)

Eukaryotes possess the true nuclear, which contains chromatin fibres, nucleoplasm, nucleolus etc. remain separated from the cytoplasm by the thin perforated nuclear membranes. Apart from the true nucleus, eukaryotic cells also possess some other membrane-bound organelles such as endoplasmic reticulum, golgi apparatus, mitochondria etc. In addition, group of photosynthesis plants also contain chloroplasts.

The process of reproduction takes place by both asexual and sexual methods in eukaryotes. They can reproduce asexually through mitosis and sexually through meiosis and gamete fusion. Before

going to the detail of the eukaryotic cells, it is advisable to known the general characteristics of different types of eukaryotic cells.

1.3.2.1 Shape

The eukaryotic cells exhibited various forms and shapes (**Fig. 1.4**). The shape may vary with animal to animal or organ to organ. Even the cells of the same organ may display variations in the shapes. The shape of the cells may depend upon the following;

- (i) Functional adaptations of the cell.
- (ii) Internal or external environment of the cell.
- (iii) Mechanical stress or pressure and surface tension of the cell.
- (iv) Viscosity of the protoplasm.
- (v) Mechanical action exerted by the adjoining cell.
- (vi) Rigidity of the cell membrane.

Typically the animal cell is spherical in shape but irregular, cylindrical, cuboidal, triangular, tubular, polygonal, oval, round elongated etc have also been seen in different organisms. Some cells (*Amaeba* and leucocytes) able to change their body shape very frequently.

1.3.2.2 Size

Generally, the eukaryotic cells are microscopic in nature but some animals and plant cells are easily visible by the necked eyes. For example: the egg of ostrich is the largest cell in the diameter, some nerve cells are considered more than 3-5 feet in length (Verma and Agrawal 2008). Usually, the size of cells may vary between 1 µm and 175000 µm (175 mm).

1.3.2.3 Number

The body of the unicellular and acellular organisms (protozoa and protophyta) is composed by a single cell. Most of the eukaryotic cells have many cells in the body therefore known as multicellular organisms. The numbers of the cells in particular organisms depend upon the size of the organisms. Usually, larger in size have the great number of the cells in their body. The number of cells in eukaryotic organisms varies from single cell to trillions. For example in the unicellular organisms a single cell is present however, the human body contain approximately 26 trillions cells, 5 million erythrocytes cells per cubic ml in blood and 10 billion neurons in nervous system.

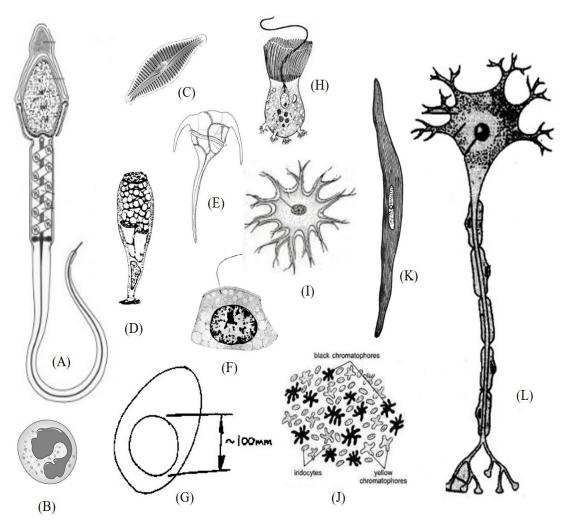


Fig. 1.4: Various types of eukaryotic cells exhibiting diverse shapes; (A). human sperm, (B). leucocyte, (C). diatom, (D). goblet cell, (E). ceratium, (F). epithelial cell, (G). Ostrich egg, (H). choanocyte, (I). osteocyte, (J). chromatophore, (K). muscle cell, (L). nerve cell

1.3.2.4 Structure

The eukaryotic cells are well defined cell. It can be categorized in the following components: (A) Cell wall and plasma membrane, (B) Cytoplasm and (C) Nucleus

(A) Cell wall and plasma membrane: Cell wall is the characteristic feature of the plant cells which is completely absent in animal cells. The protoplasm of the plant cells become separated from the external environment by a laminated, semi rigid and the layer of non-living cells known as cell wall. The composition of the cell wall depends upon the species, type of cell, function and developmental process. Usually, the plant cell wall is composed of the polysaccharide cellulose, hemicellulose and pectin. Some polymers (lignin, suberin and cutin) are also embedded in plant cell wall. Glycoproteins and polysaccharides such as carrageenan and agar exclusively occur in the cell wall of few algae which are completely lacking in land plants. The

cell wall of diatoms is composed of biogenic silica. The composition of fungal cell wall is quite different from the plant which is made up of N-acetylglucosamine polymer chitin.

The main functions of cell wall are to provide the shape, support, strength, rigidity, and protection against mechanical/ biological stress. The cell wall of certain plants possesses the pit like small apparatus known as plasmodesmata which help to connect the cell to the adjacent cell.

Most plant and animal cells have an external covering of living, thin, porous and semipermeable cells known as plasma membrane or cell membrane. This membrane is composed of three layers in which the inner and outer layers are constituted by protein and middle layer by lipid. Technically, plasma membrane gives the mechanical strength to the cell. It is selectively permeable to ions and organic molecules thus regulate the movement of substances in the cells.

- **(B) Cytoplasm:** The plasma membrane is followed by a cytoplasm which can be divided into two parts i.e., (1) cytoplasmic matrix and (2) cytoplasmic structures.
- (1) Cytoplasmic matrix: The space between the plasma membrane and the nucleus is occupied by the gel like, translucent and homogenous colloidal liquid known as cytoplasmic matrix or hyaloplasm. The 90 % of the cytoplasm is constituted by water. Remaining 10% is constituted by various inorganic compounds including salts of Na, K and minerals, organic compounds such as carbohydrates, lipids, fats, proteins, vitamins, nucleoproteins, nucleic acids (RNA and DNA) and enzymes. The peripheral region of cytoplasmic matrix is comparatively non granular, viscous, clear and rigid as known as the plasmagel, cortex or cortical layer and ectoplasm. On the other hand the inner region is granular, less viscous and known as endoplasm.

The main function of the cytoplasmic matrix is to conduct the various vital activities of the cells. Some important functions are listed as:

- Biosynthesis of biochemical substances (proteins, carbohydrates, proteins, nucleic acid etc).
- The process of glycolysis, anaerobic respiration and pentose pathway type of respiration occur in the matrix part of cytoplasm.
- The cell organelles are usually unconnected. They exchange materials through the cytoplasmic matrix.
- The products of cell organelles are passed out into the matrix.
- The cytoplasmic matrix is always in motion. It is autonomic and is called cytoplasmic or protoplasmic streaming. This helps in distribution of various materials inside the cell.
- (2) Cytoplasmic structures: In the cytoplasmic matrix, some non-living and living substances are suspended. The non-living substance grouped under paraplasm, deutoplasm or cytoplasmic inclusions while the living, membrane bounded structure are called organoids or organelles (**Fig. 1.5**).
- (i) Cytoplasmic inclusions: The stored food and secretory substances of the cell remain suspended in the cytoplasmic matrix in the form of granules which form the cytoplasmic

inclusions. It includes oil drops, yolk granules, pigments, secretory granules, starch granules in plant whereas glycogen granules in animal cells.

- (ii) Cytoplasmic organelles: The organelles are the living structure of the cytoplasm having double membrane. They perform various important biosynthesis and metabolic activities such as respiration, transportation, support, storage and reproduction. Some most important cell organelles are discussed below:
- **a. Microtubules:** The cytoplasm of eukaryotic cells is traversed by many ultrafine tubes of tubulin protein called microtubules (**Fig. 1.5A**). It is complex structure generally comprised of thirteen individual protofilaments arranged to form a hollow cylinder. Microtubules are filamentous intracellular structures that are responsible for various kinds of movements including transportation of water, ions or small molecules, cytoplasmic streaming in all eukaryotic cells. Microtubules are also involved in asters formation in the mitotic and meiotic spindle during cell division. Moreover, they form the structural units of the centrioles, basal granules, cilia and flagella.
- b. Cytoplasmic filaments: The cytoplasm is fastened with ultrafine, tube like, proteinous and soil filaments of various sizes. There are three types of cytoplasm filaments. The smallest is microfilaments around 40 to 60 A° in diameter that occur next to plasma membrane where they form the web in ectoplasm. The second class is myosin filament. It is also called actin filaments. Microfilaments are usually about 7 nm in diameter and made up of two strands of actin. This filament is involved in many kinds of cell movements including division, various extension of the cell surface, such as microvilli. In addition of both the above mentioned filaments, a third class of filament simply called 100 A° filaments. These filaments are involved in movement in both the cell itself and of material within the cell.
- Centrosome: The centrosome possesses thick cytoplasm and located adjacent to nucleus. This cell organelle is found only in the animal cells, not in the plant and fungal cells (Fig. 1.5 B). The centrosome is composed of two centrioles at right angles to each another. They are surrounded by a shapeless mass of protein. A centrosome is the site where microtubules are organized. Other than this, it plays an important role in cell division in animal cell especially where one cell divining into two daughter cells.

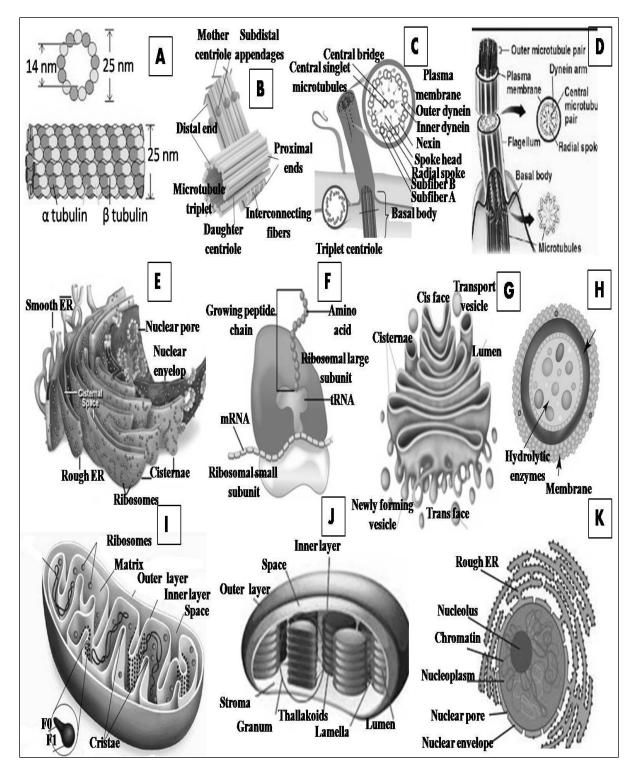


Fig. 1.5: Various types of eukaryotic cell organelles; (A). Microtubues, (B). Centrosome, (C). Cilia, (D). Flagella, (E). Endoplamic reticulum, (F). Ribosome, (G). Golgi bodies, (H). Lysosomes, (I). Mitochondria, (J). Chloroplast, (K). Nucleus

d. Cilia and flagella: The cells of the many unicellular organisms and ciliated epithelium of multicellular organisms consist of some hair like cytoplasmic outgrowth on the surface of the cell. These are known as cilia or flagella. There is some difference between cilia and flagella. Cilia are typically 2-10 μm long and 0.5 μm in diameter, however, flagella are longer (100-200 μm) in size (Fig. 1.5 C & D). Usually there are no more than one or two flagella in a single cell. Both cilia and flagella help in the locomotion.

- Endoplasmic reticulum: In a eukaryotic cell, endoplasmic reticulum (ER) is a reticulated organelle of the cytoplasm. They form an interconnected network of flattened, tubular structures known as cisternae (Fig. 1.5 E). Usually, endoplasmic reticulum present in all the eukaryotic cells, however, some cells (spermatozoa and red blood cells) do not contain it. ER continues to the outer membrane of the nucleus. Endoplasmic reticulum can categorize into two types according to variation in its structure; (i) Rough Endoplasmic Reticulum (RER) (ii) Smooth Endoplasmic Reticulum (SER). The ribosomes remain attached to the outer surface of RER which gives a rough appearance; therefore, it is called rough endoplasmic reticulum. It frequently occurs in cells such as hepatocytes and site of protein synthesis. On the other hand, the smooth endoplasmic reticulum lacks ribosomes and functions in lipid synthesis but not metabolism, the production of steroid hormones and detoxification. The smooth ER is especially abundant in mammalian liver and gonad cells. Other than the above mentioned functions, endoplasmic reticulum forms the ultrastructure skeleton framework of the cytoplasmic network and provides mechanical support.
- f. Golgi complex: The Golgi complex or Golgi body, or Golgi apparatus, or Golgi is a cytoplasmic organelle found in most eukaryotes (Fig. 1.5 G). It was discovered by Camillo Golgi in 1897. Golgi complex is made up of a series of compartments and is a collection of fused, flattened membrane-enclosed disks known as cisternae, originating from vesicular clusters that bud off the endoplasmic reticulum. Each Golgi is composed of many lamellae, tubules, vesicles and vacuoles. The functions of Golgi complex are storage of proteins and enzymes secreted by ribosomes and transported by endoplasmic reticulum. In plants cells the Golgi complex is known as dictyosome that secretes necessary materials for cell wall formation during cell division. It is of particular function of storage of proteins and enzymes which are secreted by ribosomes and transported by endoplasmic reticulum. Further, it has most important secretory function is secreting many secretory granules and lysosomes. 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.
- **g. Lysosomes:** The cytoplasm of animal cell possessing numerous membrane-bound organelles originated from the Golgi complex is called lysosomes (**Fig. 1.5 H**). They are spherical or irregular vesicles that contain hydrolytic enzymes which help to break

down the biomolecules (carbohydrates, lipids, proteins). They are not only accountable for breaking down of biomolecules but also help to get rid of waste products of the cell. A lysosome has a specific composition, of both its membrane proteins, and its lumenal proteins. Besides degradation of biomolecules, they are involved in secretion, plasma membrane repair, cell signaling, and energy metabolism. It is commonly called "suicide bags of cell". Lysosomes are known to contain more than 60 different enzymes, and have more than 50 membrane proteins. Enzymes of the lysosomes are synthesised in the rough endoplasmic reticulum. The lysosomes of plant cells are storage granules containing hydrolytic enzymes and are comprised of spherosomes, aleurone grain and vacuoles.

- h. Cytoplasmic vacuoles: The vacuole is a membrane-bound, closed sacs of membranes filled with organic or inorganic molecules. They do not have a certain size and shape, wither cell can change them. The entire vacuole contains a watery substance called cell sap. They are much more important in plant and fungus cells than in animal cells. Some common functions of a vacuole are to grasp waste products, maintain amount of water in plant cells, balance internal hydrostatic pressure or turgor steady in a cell, maintain pH inside of the cell and hold small molecules. Vacuoles are also important in autophagy. In protists, vacuoles also store and help digested food. The vacuoles of the plant cells are bounded by a single, semipermeable membrane known as tonoplast. These vacuoles contain water, phenol, flavonols, anthocyanins, alkaloids, and stored product such as sugar and proteins.
- i. Microbodies: The cytoplasmic matrix of many kind of cells (yeast, protozoa, cells of higher plants etc) contains certain roughly spherical, membrane bounded particles (0.3-1.5 µm diameter). These particles have a central granular or crystalloid core containing some enzymes; occur in intimate relation with endoplasmic reticulum, mitochondria and chloroplast are called microbodies. It contains enzymes that participate in the preparatory or intermediate stages of biochemical reactions within the cell. This facilitates the breakdown of fats, alcohols and amino acids. Generally, microbodies are involved in detoxification of peroxides and in photorespiration in plants.
- **j. Ribosomes:** The ribosomes are the small, spherical structured, minute organelle of cytoplasm (**Fig. 1.5 F**). It is found in all the living organisms (including prokaryotes and eukaryotes). The ribosomes are originated in the nucleolus and consist of mainly the ribonucleic acids (RNA) and proteins. In eukaryotic cells they are attached with the membrane of endoplasmic reticulum, or occur freely in the cytoplasm. The eukaryotic ribosomes are differ structurally from the prokaryotic ribosomes, however, in both kind of cells, it is the site of protein synthesis. In prokaryotic cell, the ribosomes (70S type) consist of with two ribosomal subunits: the small subunits (30S) and large subunits (50S). On the other hand, the eukaryotic ribosomal subunits (80S type) are composed of 40S as small

subunit and 60S as large subunit. In ribosomes, the small ribosomal subunits read the mRNA, and the large subunits join amino acids to form a polypeptide chain. Each subunit consists of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins (Weiler and Nover 2008).

- k. Mitochondria: The shape, size and number of mitochondria varied from cell to cell. Generally, they are rod and round shaped having double membrane bound structure, occurring in most eukaryotic cells, however, some cells lack of them (for example, mature mammalian red blood cells). Both the inner and outer layer along with the central region are filled with a viscos fluid known as mitochondrial matrix. The outer membrane forms a bag like structure around the inner membrane which gives out many fingers like folds in the lumen of the mitochondria. The folds of the inner membrane are known as cristae (Fig. 1.5 I). The matrix, outer and inner membranes possess many oxidative enzymes and coenzymes. The main functions of mitochondria are respiration, oxidation of food and metabolized the energy. They store the energy and release when needed in the various vital activities of life. Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy (Campbell et al. 2006); therefore, named the "power house of the cell".
- **Plastids:** Plastids are known as the "kitchen of the cell". The plastids exhibit only in plant cells and completely absent in animal cells. They give the particular colour to the plants. The size of plastids range from 4 to 6 μm and colour from colorless to many colours. The colourless plastids are known as leucoplast. They functions specially for the storage of starch, lipids and proteins, therefore, they are called amyloplasts, lipoplasts and proteinoplasts, respectively. The coloured or pigmented plastids commonly called as chromoplast occur in many colours, in which the green is the most common. The green coloured plastid is known as chloroplast (Fig. 1.5 J) which helps in the biosynthesis of food by the process of photosynthesis. The chloroplasts have complicated organization and contain DNA, ribosomes and complete protein synthesis machinery.
- (C) Nucleus: The nucleus is well defined, centrally located and spherical cellular component which regulate all the vigorous process of the cell. Nucleus is constituted by the three structures (Fig. 1.5 K).
- a. Nuclear membrane: Nuclear membrane is the outer most layer of the nucleus and occurs in both plant and animal cells. The nuclear membrane is made of two layer of lipoprotein. It forms an envelope like structure around the nucleus known as nuclear envelop. The nuclear envelop contain many tiny pore which regulate the movement of the chemical substance. The outer nuclear membrane of nuclear remains continues with the membrane of endoplasmic reticulum and plasma membrane. The main function of the nuclear membrane is to create a barrier that physically protects the genetical material of the cell from the chemical reactions that are occurring elsewhere in the cell.

b. Nucleoplasm and chromosomes: Like the cytoplasm, the nucleus contains a watery substance called nucleoplasm or karyoplasm. The space between the nuclear membrane and nucleolus is filled by nucleoplasm. It contains dissolved phosphorus, ribos sugar, proteins, nucleotides and nucleic acids. The nucleoplasm contains some threads like structure called the chromosomes. The chromosomes appear only during the cell division otherwise they occur in the form of chromatin granules. The chromosomes and chromatin granules contain the genetic materials along with the many nucleoproteins.

c. Nucleolus: The nucleoplasm contains a conspicuous darkly stained spherical body known as the nucleolus. Chemically, nucleolus is composed of large amount of ribosomal protein and ribosomal RNA. The nucleolus stores the rRNA molecules synthesized by nucleolar organizer region of DNA and provides the raw material such as different kind of rRNA's and ribosomal proteins for the biogenesis of ribosomes.

1.4 SPECIALIZED PLANT CELL TYPES

The multicellular organism are composed of different type of cells which are designed to perform the specific activates. The cells are distinguished from each other by their shape, size, properties of the cell wall and protoplast. The similar type of cells performed the similar type of work and organized into tissues, which ultimately forms the organs. A tissue system may be constitute by many specialized cells to perform a particular function or may carries out different functions or few cells are found in more than one tissue systems.

Many specialized cells are present in plants (**Fig.1.6**) which makes them unique from others. For example, in plants, cell wall is the prominent distinguishing features of the different kinds of specialized cells. The primary cell wall of plants is made up of cellulose and carbohydrates. Furthermore, a thick, rigid secondary cell wall is also present which is made up of cellulose impregnated with lignin. Other than the cell wall many other specialized cells are also present in the plants. Therefore, for the suitability, the specialized cell types in plant (roots stems, leaves, stem appendages and fruits) can be categorized into three different tissue systems;

1. Ground tissue system: Provides support, photosynthesis and storage.

2. Dermal tissue system: Provides protection and gaseous exchange.

3. Vascular tissue system: Transports water and solutes over long distances within the

plant.

1.4.1 Cells of the ground tissue system

The ground tissue system is originated by the ground meristems and function to synthesize the organic compounds that support, and protect the plants. In some cases, the ground tissue also stores

food in the form of starch. Basically, the ground tissue system is made up of three types of cells; parenchyma, collenchyma and sclerenchyma.

- **a. Parenchyma cells:** These are the living, generalized, multipurpose, thin walled cells and range from spherical to barrel-like in shape. The parenchymatic cells of leaves are adapted for photosynthesis due to the presence of many chloroplasts, therefore, they are called chlorenchyma cells. In some species, parenchyma cells often store food reserves. For example, in potato and apple the cells store starch and sugar, respectively. Sometime, these cells are specialized for transportation of solute across the membrane and called as the transfer cells. Transfer cells are common in nectaries.
- b. Collenchyma cells: These are the living, elongated and irregularly thickened primary cell walls composed of cellulose. The secretory apparatus (ER and Golgi) proliferates to secrete additional primary wall. Collenchyma cells develope from meristem cells that initially resemble parenchyma, but differentiate quickly becoming apparent. Plastids do not develop in it, therefore, these cell do not help in the photosynthesis. The main functions of these cells are in the support of growing tissues especially the stem and leaves. Collenchyma cells form long cables of thousands of cells that together can provide mechanical support during stem and leaves elongation. Collenchyma is common in the veins of leaves and forms the strings of celery stalks.
- c. Sclerenchyma cells: The sclerenchyma cells are dead, elongated, rigid, heavily thickened secondary walls containing lignified cells. These cells frequently occur in those meristematically inactive regions (bark or old trunk) of the plant. Unlike both parenchyma and collenchyma cells, sclerenchyma cells become dead at maturity. These cells provide mechanical support to tissues that are no longer expanding. Sclerenchyma fibers make up the bulk of woody tissues and also form long strands in the leaves and stems of many plants. Natural fiber ropes (hemp or sisal plants) are made up of sclerenchyma fibers. Some sclerenchyma cells called sclereids are much shorter than the fibers; these form the hard layers of walnut shells and peach pits, and small clusters of sclereids form the grit in pear fruits.

1.4.2 Cells of the dermal system

The dermal tissue system protects the soft tissues of plants and controls interactions with the plants to the surroundings. The examples of specialized plant cell of dermal system are given below;

a. Epidermal cells: These cells comprise of numerous types of cells which constitute the outer covering of plants called epidermis layer. Usually the cells of epidermis are flat and form a continuous sheet with no spaces between the cells. Most epidermal cells also secrete waxes on the surface of the cutin, which further reduces transpiration, as well as wettability of the leaf surface. The cells of epidermis vary in shape and size. They may be irregular

wavy shape (in *Arabidopsis thaliana*), interlocking jigsaw puzzle pieces like (in the leaves of many dicots), and rectangular (in stem and elongated plant organs). Therefore, the shape of the epidermis depends upon the functions and organs where they are located. Epidermis layer serves numerous important functions in plant. The main function is to provide the protection to the plant cell from a variety of external harmful factors (environmental stressors) including microbes, chemical compounds as well as physical influences. Apart from the protection, these layers prevent the loss of water from the exposed area of plants.

- b. Guard cells: These are the specialized epidermal cells that function to open small pores in the plant surface, allowing the CO₂ needed for photosynthesis to diffuse from the external atmosphere into the chlorenchyma tissue. Guard cells are usually crescent-shaped, contain green chloroplasts, and are able to rapidly change their shape in response to changes in water status. As guard cells take up water, the pore opens as they lose water the pore closes. The two guard cells and pore are termed a stomate. They surround each stoma and help to regulate the rate of transpiration by opening and closing the stomata.
- **c. Trichomes:** The word trichome is derived from a Greek word meaning hair. These are like a fine outgrowths or appendages on plants, algae, lichens, and certain protists which project from the surface of the body of the organisms. They may vary in shape and size according to the species. They are reported as simple, straight, spiral, hooked, glandular, peltate and etellate in various organisms. In plants, they function to reduce the water loss through the trapping of water vapor near the plant surface. In some plants trichomes serve protection by secreting sticky or toxic substances that repel insect herbivores.

1.4.3 Cells of the vascular system

The vascular system of the plant is composed of xylem and phloem cells. Both the components regulate the supply of food and water in the plant.

- **a. Xylem cell:** Xylem is made up of several types of cells i.e., xylem tracheids, xylem vessel and xylem parenchyma. Xylem tracheids are the long elongated cells that help transport xylem sap and also provide structural support. Vessels are shorter than tracheids, but also help to conduct water. They are generally found in angiosperm plants (flowering plants) but not in gymnosperms. Vessel elements have perforation plates that connect each vessel element to form one continuous vessel. Xylem also contains parenchyma, a tissue that makes up most of the soft parts of plants, and long fibers that help support the plant.
- **b. Phloem:** Phloem tissue functions to transport the prepared food after process of photosynthesis to the various part of the plant where energy-rich carbohydrates are required for storage or growth. Sieve elements are the conducting cells of the phloem which are elongated and thick primary wall. Sieve elements have large, conspicuous pores on the end

walls, forming a sieve plate. The sieve plate pores allow the phloem sap to travel from cell to cell along the file of cells called a sieve tube. Each sieve element is living with an intact plasma membrane; the differential permeability of the membrane prevents solutes from leaking out of the sieve tube. Sieve-tube elements lack a nucleus and some other components of the cytoplasm; this feature functions to keep the pores unplugged. Companion cells are small parenchyma cells associated with each sieve element. The nucleus of the companion cell must direct the metabolism of both the companion cell itself and of its sister sieve element.

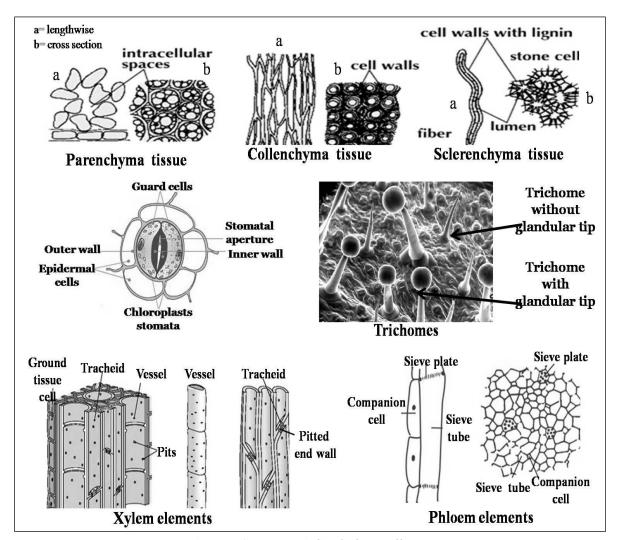


Fig. 1.6: Some specialized plant cell types

1.5 CHEMICAL COMPOSITION

The chemical composition of a cell can be categorized into two parts: inorganic compounds and organic compounds.

1.5.1 Inorganic compounds

The inorganic compounds normally occur in physical, non living universe such as elements, metals, non metals and their compounds like water, salts and the verities of the electrolytes and non electrolytes. From the 104 elements of the universe, the cytoplasmic matrix contains only 36 elements (Verma and Agrawal, 2008). Generally they are grouped into three categories:

Major constituentOccur predominantly in matrix, e.g., oxygen, carbon, hydrogen and nitrogen.

Occur in low percentage in matrix, e.g., calcium, phosphorus,

chlorine, sulphur, potassium, sodium, magnesium, iodine and iron.

Ultrastructure elementOccur in the matrix in 0.756%. The remaining 23 elements are copper, cobalt, manganese, zinc, molybdenum, boron, silicon etc.

These inorganic compounds play the vital role in the various biological and chemical activities in the cell. Various physiological activities such as osmosis, diffusion, impulse conduction etc are influenced by the trace inorganic compounds. Ultrastructure elements play an important role as cofactors in various chemical reactions.

Water: It is the most abundant inorganic compound of the cell and constitute about 70-80% of the matrix. They occur in the two forms viz., free water and bound water. About 95% water of the matrix is free water and functions as the solvent for many substances. The remaining 5% of the cellular water remains loosely linked with protein molecules by hydrogen bonds and known as bound water.

Functions of water

- 1. Water is the best solvent for the various cellular substances.
- 2. It is the main component of the process of photosynthesis in the plant. Without it, plant cannot synthesize their food.
- 3. Water forms the good dispersion medium for the colloidal systems of the matrix.
- 4. Water serves as a natural stabilizer for the atmospheric temperature.
- 5. It has greater importance for the various metabolic functions because most of them require the exclusively aqueous medium.
- 6. Water is used by the cell as a transporting media for the food, nitrogen waste and other necessary substances.

1.5.2 Organic compounds

The chemical substances which contain the carbon in combination with one or more other elements as hydrogen, nitrogen, sulphur etc are called organic compound. Usually they are the large molecules formed by the similar or dissimilar unit structure known as the monomers.

The four main classes of molecules (viz., carbohydrates, lipids, proteins and nucleic acids) occurring in the cells are called biomolecules (Slabaugh and Seager, 2013) in a typical cell. Many small micromolecules get linked together to prepare a large macromolecule known as polymers and this process is known as dehydration synthesis. The detail description of chemical composition (organic) of the cell is given below:

1.5.2.1 Carbohydrates

Carbohydrates are the main source of the energy in all living organisms on the Earth. Only green plants and few groups of microbes are able to synthesize the carbohydrate from the water and carbon dioxide in the presence of the Sun light and chlorophyll pigment. Therefore, all the animals, non-green plants (fungi) and bacteria depend on green plants for the supply of the carbohydrates. Carbohydrates can be represented by the stoichiometric formula $(CH_2O)n$, where n is the number (at least 3) or $C_nH_{2n}O_n$. The ratio of carbon to hydrogen to oxygen represents as 1:2:1 in carbohydrate molecules. They are called carbohydrate because as the formula explains itself that it is buildup of the components of carbon (carbo) and water (hydrate). Carbohydrates are classified into three subtypes: monosaccharaides, disaccharides, and polysaccharides.

(a) Monosaccharides: Monosaccharides (*mono*= one, *sacchar*= sweet) are the simplest type of the carbohydrate. Here the number of carbon is restricted three to seven in number and their names end with the suffix "-*ose*". Depending upon the numbers of carbon molecules they may be known as **trioses** (C=3, e.g., glyceraldehydea and dihydroxy acetone), **tetrose** (C=4 e.g., erythrose), **pentoses** (C=5, e.g., ribose, deoxyribose, arabinose and xylulose), **hexoses** (C=6, e.g., glucose, fructose and galactose) and **heptose** (C=7, e.g., sedoheptulose). If the sugar has an aldehyde group (the functional group with the structure R-CHO), it is known as an aldose, and if it has a ketone group (the functional group with the structure RC(=O)R'), it is known as a ketose (**Fig. 1.7**).

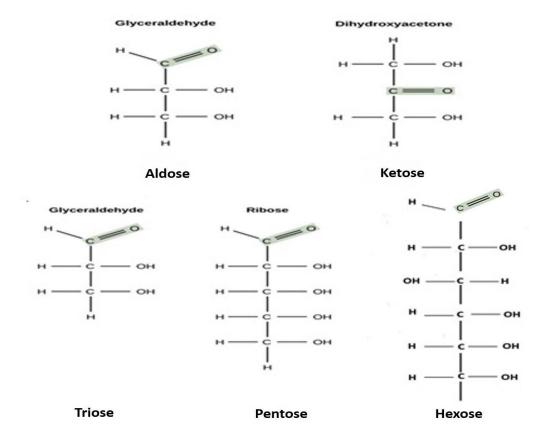


Fig. 1.7: Showing the Aldoses (R-CHO at the end of the carbon chain) and keto (R-CO at the middle of the carbon chain) group in the monosaccharide. Triose, Pentose and Hexose have three, five and six carbon, respectively

Example: Glucose, galactose and fructose are the example of monosaccharide. They all are hexose sugars having similar chemical formula $(C_6H_{12}O_6)$, therefore, known as isomers. However, chemically and structurally, they are differ from each other because of arrangement of functional groups around the asymmetric carbon (Fig. 1.8). Glucose is one of the most important monosaccharides, fructose, the sugar commonly associated with the sweet taste of fruits, and galactose occurs in the milk.

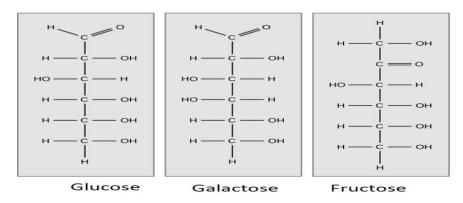


Fig.1.8: Isomers of hexose sugar (Glucose, Galactose and fructose)

(b) Oligosaccharides: The oligosaccharides consist of 2 to 10 monosaccharides in a single molecule of oligosaccharide. The monosaccharides are pooled together by the specific covalent bond called glycosidic bond and a molecule of water is released. In this process, OH group of one monosaccharide joins with the hydrogen group of another molecule of monosaccharide and form a water molecule. Therefore, one molecules of disaccharide is made up of two molecules of monosaccharides. Therefore, when reverse reaction takes place the glycosidic bond of a disaccharide broken into two monosaccharides is termed hydrolysis, for example, the molecules of disaccharides (contain two monomers). The most abundant oligosaccharides of the animal and the plant cells are disaccharides such as sucrose, maltose and lactose. The sucrose (Fig.1.9) and maltose occur mainly in the plant cells, whereas the lactose occurs exclusively in the animal cells.

Fig. 1.9: Formation a molecule of sucrose (oligosaccharides) by joining one molecules of glucose and one molecules of fructose

Certain important oligosaccharides are: **disaccharides** (two monomers, *e.g.*, sucrose, maltose, lactose etc), **trisaccharides** (tree monomers *e.g.*, *raffinose*, *mannotriose*, *rabinose*, *rhaminose*, *gentianose* and *melezitose*), **tetrasaccharides** (four monomers *e.g.*, *stachyose*, *scordose*), **pentasaccharides** (five monomers *e.g.*, verbascose).

(c) Polysaccharide: The polysaccharides are the group of carbohydrate composed of ten to many thousands monomers of monosaccharides in their macromolecules. The macromolecules of this group contain colloidal size with high molecular weights. *Polysaccharide* can be subdivided into two categories. i.e., **homo-polysaccharide** (contain similar type of monosccharides in their molecules, e.g., starch, cellulose, glycogen etc), **hetro-polysaccharide** (contain different type of monosccharides and amino-nitrogen or sulphuric or phosphorus acids in their molecules, *e.g.*, chitin, heparin etc).

Functions of Carbohydrate

- 1. The glucose, a hexose sugar is the main source of energy in all living organisms. Fructose is also an important source of energy.
- 2. Carbohydrate functions as the main structural elements in plants in two forms i.e., cellulose and hemicellulose. Cellulose, a polysaccharide, is used to build the cell wall.

- 3. Carbohydrates give the mechanical support to the plant body.
- 4. The pentose sugar, ribose is the important constitution of the ribonucleic acid (RNA) and certain co enzymes as Nicotinamide Adenine Dinucleotide (NAD), Nicotinamide Adenine Dinucleotide Phosphate (NADP), Adinosine triphosphate (ATP) and coenzyme A (CoA). Another pentose sugar dioxyribose is an important constitute of genetic material, dioxyribonucleic acid (DNA).
- 5. Polysaccharides such as starch serve as storage molecules. Plants store starch in root, tuber and leafy parts mainly during photosynthesis activity.

1.5.2.2 Lipids (Fats)

The lipids (Gr., *lipos*=fats) are the organic compound which are insoluble in water whereas soluble in organic compound (chloroform, ether, alcohol, benzene and other organic compound) (**Fig. 1.10**). Lipids are non polar and hydrophobic in nature. The lipids are classified into three classes.

- (a) **Simple lipids:** These are the esters of the alcohols or the triglycerides containing fatty acids and alcohols. The composition of this type of class may be **saturated**, *e.g.*, palmatic acid, stearic acid etc. or **unsaturated**, *e.g.*, oleic acid, linolenic acid, arachedonic and clupanadonic acid. The simple lipids of the matrix are given:
- **i. Natural fats:** These are the naturally occurring fats in animal and plant cells in the form of stored food substances.
- **ii.** Wax: These are the esters of fatty acids of high molecular weight with the alcohol except the glycerol. The most important constituent alcohol of the molecules of wax is the cholesterol, bee wax.
- **(b) Compound lipids:** The compound lipids contain fatty acids, alcohols and other compounds such as phosphorus, amino-nitrogen carbohydrates etc in its molecules. Steroids, phospholipids, glycolipids, lipoproteins and carotenoids are some of the examples of the compound lipids.
- **(c) Derived lipids:** These are the group of lipids which are derived from the simple or compound lipids by hydrolysis. These include fatty acids, alcohols, monoglycerides and diglycerides, steroids, terpenes, and carotenoids. The most common derived lipids are steroids, terpenes and carotenoids.

Fig. 1.10: Structure of some common lipids. At the top are cholesterol and oleic acid. The middlestructure is triglyceride composed of oleoyl, stearoyl and palmitoyl chains attached to a glycerolbackbone. At the bottom is the common phospholipid phasphatidylcholine (Maitland, 1998)

Functions of lipids

- 1. The lipids are important constituents of the cellular membrane, hormones and vitamins of the cells.
- 2. They serve as the source of energy in the cells.
- 3. Lipids, especially phospholipids, are also used in various pharmaceutical products either as co-solubilisers (e.g., in parenteral infusions) or else as drug carrier components (e.g., in a liposome or transfersome).
- 4. Protective wax coating found in certain group of plants.
- 5. In plants, seed oils such as triacylglycerols (TAGs) provide food storage for seed germination and growth in both angiosperms and gymnosperms.
- 6. Lipids provide to the plants the necessary energy for their metabolic processes and signals between cells.

1.5.2.3. Proteins

Proteins are very large molecules (macro-biopolymers) made up of monomers called amino acids. An amino acid consists of an alpha carbon atom attached to an amino group, -NH₂, a carboxylic acid group, -COOH (although these exist as -NH₃⁺ and -COO⁻ under physiologic conditions), a simple hydrogen atom, and a side chain commonly denoted as "-R". The side chain "R" is different for each amino acid of which there are 20 standard ones. It is this "R" group that makes each amino acid different, and the properties of the side-chains greatly influence the overall three-dimensional conformation of a protein. Amino acids can be joined via a peptide bond. In this dehydration synthesis, a water molecule is removed and the peptide bond connects the nitrogen of one amino acid's amino group to the carbon of the other's carboxylic acid group. The resulting molecule is called as *dipeptide*, and short stretches of amino acids (usually, fewer than thirty) are called *peptides* or polypeptides. Longer stretches merit the title *proteins*. The structure of proteins is traditionally described in a hierarchy of four levels.

- **a. Primary structure:** Primary structure of a protein consists of its linear sequence of amino acids found in the protein. It is determined by the covalent peptide bonding between amino acids. Primary structure also includes the other covalent bonds in proteins.
- **b. Secondary structure:** Secondary structure is concerned with local morphology. It is any regular repeating organization of the polypeptide chain.
- **c. Tertiary structure**: Tertiary structure is the entire three-dimensional shape of the protein. This shape is determined by the sequence of amino acids. In fact, a single change can change the entire structure. Tertiary structure is defined as any irregular loops or bends in the polypeptide chain and is typical of globular protein structure.
- **d. Quaternary structure:** The highest type of the protein structure called quaternary refers to the subunit structure of proteins. Proteins that have more than one polypeptide chain may also have more than one independently folded unit, each of at least on chain. The independently folded units are called subunits. The manner in which subunits are arranged to form the intact functional protein is called the quaternary structure.

Functions of protein

- 1. Enzymes are proteins that aid the thousands of biochemical reactions taking place within and outside of the cells.
- 2. Some proteins are hormones, which are chemical messengers that aid communication between your cells, tissues and organs.
- 3. Some proteins are enzymes, which catalyse the chemical reactions.
- 4. Protein contains four calories per gram, the same amount of energy that carbohydrates provide. Fats supply energy as nine calories per gram.
- 5. Growth, maintenance and repairing of the cells.
- 6. The structural proteins which forms various structures of the cell.

1.5.2.4. Nucleic acids

The nucleic acids are the complex macromolecular organic compounds of immense biological importance. They control the important biosynthetic activities of the cells and carry hereditary information from generations to generations. There are two type of nucleic acids in animal as well as in the plant cells viz., deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Both are the polymers of nucleotides. A nucleotide is composed of nucleoside and phosphoric acid. Even the nucleoside is composed of the pentose sugar and nitrogen based (purines or pyrimidines). The purines are adenine and guanine and the pyrimidines are the cytosine, thymine and uracil. The cytoplasmic matrix contains only RNA, while DNA exclusively remains concentrated in the nucleus. The DNA and RNA almost have the similar chemical compositions except few differences (**Fig. 1.11**).

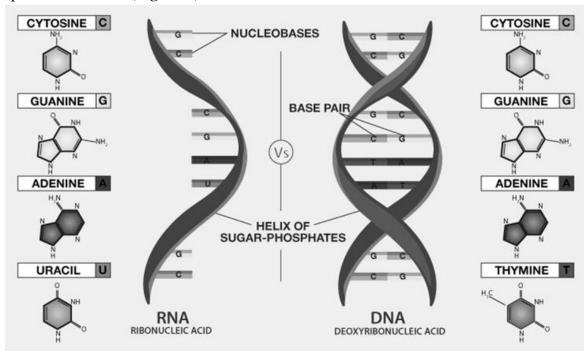


Fig. 1.11: Structure of DNA and RNA (Nucleic acids)

Functions of nucleic acids

- 1. Nucleic acids play an essential role in mitosis and meiosis.
- 2. Certain sections of nitrogenous bases along the strand of DNA form a *gene* which contain genetic information or codes for a particular product and transmits hereditary information to the next generation. In simple words, nucleic acids are reserve bank of genetic information.
- 3. Nucleic acids are the basic information pathway and control the cellular function of the organisms.
- 4. Responsible for the maintaining the identity of different species of organisms over millions of years.

1.6 SUMMARY

• The cell is a unit of biological activity delimited by a semipermeable membrane and capable of self-reproduction in a medium free of other living systems.

- Cells emerged on the Earth at least 3.5 billion years ago.
- Collectively, all the living cells are broadly classified into two category i.e., prokaryotic cells and eukaryotic cells, according to whether their genetic materials are enclosed by a nuclear envelope or not.
- The shape may vary with animal to animal or organ to organ. Even the cells of the same organ may display variations in the shapes.
- Typically the animal cell is spherical in shape but irregular, cylindrical, cuboidal, triangular, tubular, polygonal, oval, round elongated etc have also been seen in different organisms. Some cells (*Amaeba* and leucocytes) are able to change their body shape very frequently.
- Generally, the eukaryotic cells are microscopic in nature but some animals and plant cells are easily visible by the necked eyes. For example, the egg of ostrich is the largest cell in the diameter and some nerve cells are considered more than 3-5 feet in length. Usually, the size of cells may vary between 1µm and 175000 µm (175 mm).
- The numbers of the cells in particular organisms depend upon the size of the organisms. Larger size has the great number of the cells in its body.
- The animal and plant cells are different from each other. The plant cells possess cell wall containing cellulose, hemicelluloses and pectin and large vacuole that regulates turgor pressure and allows plants to grow tall, while animal cell do not.
- The cell provides the structure and protection to the cell.
- The plasma it is selectively permeable to ions and organic molecules and thus regulates the movement of substances in the cells.
- The cytoplasm conducts various vital activities of life.
- The microtubules help and support in transportation to the cell.
- Plastids are the characteristic features of the plant cell. They functions specially for the storage of starch, lipids and proteins, therefore, they are called amyloplasts, lipoplasts and proteinoplasts, respectively.
- The green coloured plastid is known as chloroplast which helps in the biosynthesis of food by the process of photosynthesis.
- Mitochondria are called the power house of the cell which provides the energy to conduct the various vital activities of the cell.
- Endoplasmic reticulum provides the mechanical support to the cell and sites for a number of enzymes and cytochromes to carry out specific reactions.
- Golgi complex carries out the processing of proteins generated in endoplasmic reticulum and also transports protein to the different parts of cell.

• Cytoplasmic vacuole is also the characteristic feature of the plant cell. Vacuoles are important in autophagy, store and help the digested food, contain water, phenol, flavonols, anthocyanins, alkaloids, and stored product such as sugar and proteins.

- Ribosomes are the site of protein synthesis.
- Nucleus controls all the functions of the cell. It regulates the heredity characters, synthesis of
 particular enzymes, responsible for protein synthesis, cell division, growth and
 differentiation.
- Many specialized cells are present in plants which makes them unique from others. For example, in plants, cell wall is the prominent distinguishing features of the different kinds of specialized cells.
- For the suitability, the specialized cell types in plant may be divided into three; Ground tissue system, dermal tissue system and vascular tissue system.
- The chemical composition of the cell can be categorized into two parts viz., inorganic compounds and organic compounds.
- The inorganic compounds normally occur in physical, non living universe such as elements, metals, non metals and its compounds like water, salts and the verities of the electrolytes and non electrolytes.
- Water is the best solvent and plays a major role in the biochemical rections.
- The chemical substance which contain the carbon in combination with one or more other elements as hydrogen, nitrogen, sulphur etc are called organic compound.
- Carbohydrates can be represented by the stoichiometric formula $(CH_2O)n$, where n is the number (at least 3) or $C_nH_{2n}O_n$.
- Carbohydrate functions as the main structural elements in plants in two forms i.e., cellulose and hemicellulose. Cellulose, a polysaccharide, is used to build the cell wall.
- The lipids are the organic compound which are insoluble in water, whereas, soluable in organic compound (chloroform, ether, alcohol, benzene and other organic compound).
- The lipids are important constituents of the cellular membrane, hormones and vitamins of the cells.
- Proteins are very large molecules (macro-biopolymers) made from monomers called amino acids.
- Proteins play a crucial role in the various biochemical activities of the cell.
- The nucleic acids are the complex macromolecular organic compounds of immense biological importance. They control the important biosynthetic activities of the cells and carry hereditary information from generation to generations.
- Nucleic acids play an essential role in mitosis and meiosis.
- Nucleic acids are the basic information pathway and control the cellular function of the organisms.
- Nucleic acid carried the genetic information to generation to generation.

1.7 GLOSSARY

Amyloplast: Organelle found in some plant cells that helps store and synthesize starch. When a plant is in need of energy, amyloplasts can also convert its starch back into sugar for food. Many amyloplasts can be found in starchy plants like potato tubers.

ATP: Adenosine triphosphate. An adenine molecule, or a nucleotide, attached to three linearly connected phosphate groups ($-H_2PO_4R$, where R is a functional group). ATP basically shuffles energy around to support metabolism and a bunch of super important cellular processes, like photosynthesis.

Cell Membrane: A thin semi-permeable membrane that surrounds the cytoplasm of a cell.

Cell theory: The scientifically supported idea that the basic structural unit of life is the cell and that all cells arise from other cells.

Cell wall: A rigid, but often flexible, layer containing cellulose or chitin, pectin, and other polymers. The cell wall is the outermost structure of plant, algal, fungal, and some prokaryotic cells.

Centriole: A tubular structure that is made of protein and found only in animal cells. It is involved in cell division and the formation of flagella and cilia.

Chloroplast: The organelle (see definition; think "mini organ") in plant cells, and a few other eukaryotic cells, which contains chlorophyll, the magical green pigment, and carries out the process of photosynthesis.

Chromatin: The mass of genetic material composed of DNA and proteins that condense to form chromosomes during eukaryotic cell division.

Chromosome: A long, stringy aggregate of genes that carries heredity information (DNA) and is formed from condensed chromatin.

Cilia and Flagella: A rotrusions from some cells that aid in cellular locomotion.

Collenchyma: Tissue composed of cells with unevenly thickened walls.

Cytoplasm: All of the contents outside of the nucleus and enclosed within the cell membrane of a cell.

Cytoplasm: The cytosol (fluid inside cells), organelles (except the nucleus), and other particles enclosed within the cell membrane. The cytoplasm is the site of most cellular activities, including glycolysis, production of energy from carbohydrates, and cell division, or the way cells reproduce.

Cytoskeleton: A network of fibers throughout the cell's cytoplasm that helps the cell maintain its shape and gives support to the cell.

Cytosol: The fluid component of the cytoplasm (collective name for the stuff within the boundaries of the cell membrane) composed of cytoskeleton filaments, dissolved molecules, and water. The cytosol is the part of the cytoplasm between the cell membrane and organelle membranes.

DNA: Deoxyribonucleic acid. DNA is a macromolecule ("macro" = big) also known as a nucleic acid that is composed of phosphate groups, deoxyribose sugar groups, and the nucleotides adenine, guanine, cytosine, and thymine. DNA contains the genetic code needed by all cells to produce proteins and other molecules necessary to sustain life. He seems to make into every one of Shmoop's Biology glossaries.

Endoplasmic Reticulum: A network of tubules and flattened sacs that serve a variety of functions in the cell.

Enzymes: A protein that catalyzes, or increases the rate of, a chemical reaction in a cell. Eukaryote: An organism whose cells contain a membrane-bound nucleus.

Flagellum: A protrusion of the cell membrane in some eukaryotic and prokaryotic cells that spins or lashes back and forth to aid in cellular locomotion.

Genes: Segments of DNA located on chromosomes that exist in alternative forms called alleles.

Genome: All of an organism's heredity information encoded in either DNA or RNA.

Golgi body: An organelle in eukaryotic cells containing between three and seven flattened membrane disks called cisternae. The Golgi body packages and processes proteins and lipids, and is also called the "Golgi apparatus."

Histone: Large protein complexes that control the messages sent from the DNA to the rest of the cell.

Lysosome: A spherical, membrane-bound organelle in eukaryotic cells that contains enzymes (catalysts) and other proteins that digests, or break down, substances that have been taken into a cell by phagocytosis.

Meiosis: A two-part cell division process in organisms that sexually reproduces, resulting in gametes with one-half the number of chromosomes of the parent cell.

Microtubules: Fibrous, hollow rods that function primarily to help support and shape the cell.

Mitochondrion (plural *mitochondria*): A membrane-bound organelle that provide the usable energy (in the form of ATP) from lipids, sugars, and proteins in a process known as cellular respiration. The mitochondrion is the cell's powerhouse.

Mitosis: A phase of the cell cycle that involves the separation of nuclear chromosomes followed by cytokinesis.

Nucleus: A membrane-bound structure that contains the cell's hereditary information and controls the cell's growth and reproduction.

Organelles: Tiny cellular structures, that carries out specific functions necessary for normal cellular operation. E.g., Golgi bodies, lysosomes, mitochondria, chloroplasts, endoplasmic reticulum, vacuoles, and vesicles.

Plant Cells: Eukaryotic cells that contain various membrane-bound organelles. They are distinct from animal cells, containing various structures not found in animal cells.

Plasma membrane: A phospholipid (see previous definition) bilayer separating the interior of a cell from the surrounding environment. The membrane does lots of stuff, including protecting the cell, transporting materials into and out of the cell, and helping the cell communicate to other cells.

Polar Fibers: Spindle fibers that extend from the two poles of a dividing cell.

Prokaryote: An organism whose cells lack nuclei and membrane-bound organelles. Prokaryotes are generally single-celled.

Prokaryotes: Single-celled organisms that are the earliest and most primitive forms of life on earth.

Protein: A chain, or chains, of amino acids specifically folded to take on a certain shape, one that determines the protein's function.

Ribosome: A complex structure made of proteins and ribosomal RNA, or rRNA. Ribosomes are found in all cells, both prokaryotic and eukaryotic. Together with messenger RNA (mRNA) and transfer RNA (tRNA), ribosomes synthesize proteins from amino acids (the building blocks of proteins). Ribosomes are not generally considered organelles because they are not membrane-bound.

RNA: Ribonucleic acid. RNA is a macromolecule composed of phosphate groups (aka – H₂PO₄R, where R is a functional group), ribose sugars, and the nucleotides adenine, guanine, cytosine, and uracil.

Rough endoplasmic reticulum (RER): A highly membranous organelle in eukaryotic cells, with a membrane-bound nucleus, dotted with ribosomes. The dots inspired the "rough" part of the name. The RER is the major site of protein synthesis.

Schlerenchyma: Tissue composed of thick-walled cells containing lignin for strength and support.

Sclereid: A type of schlerenchyma, made up of gritty cells, often called "stone cells."

Sieve element: Cell in the phloem tissue concerned with longitudinal conduction of food materials. In flowering plants, it is called a sieve-tube element.

Sieve tube: A series of sieve-tube elements arranged end to end and interconnected through sieve plates.

Smooth endoplasmic reticulum (SER): A highly membranous organelle in eukaryotic cells that is not associated with ribosomes. The SER is the major site of lipid and steroid synthesis, and is continuous with the nuclear membrane and rough endoplasmic reticulum, or RER.

Stroma: Dense fluid found between grana (stacks of thylakoid disks) of a plant cell's chloroplast. Stroma is where carbohydrate forming reactions occur during photosynthesis.

Vacuole: A large, membrane-bound organelle found in most plant and fungal cells, as well as some animal and bacterial cells. The vacuole's job varies with cell type, but in many cases, the vacuole is involved in water regulation, waste removal, and pH balance within the cell.

Vesicle: A small, sac-like organelle involved in the transport and storage of cellular substances, especially proteins marked for secretion from the cell.

Vessel element: Individual cells that make up vessels.

Vessel: A tube-like series of vessel elements with open ends. The walls that join the members have perforations or holes in them to allow water to pass through freely.

Xylem: The water-conducting tissue of a vascular plant. Minerals are also transported through the xylem.

1.8 SELF ASSESSMENT QUESTIONS

1.8.1 Multiple Choice Questions:

- 1. Name an organelle which serves as a primary packaging area for molecules that will be distributed throughout the cell?
- (a) Mitochondria

(b) Golgi apparatus

(c) Vacuole

- (d) None
- 2. Which among the following sentences is not correct about the organelles?
- (a) They are found in multicellular organisms.
- (b) They are found in all Eukaryotic cells.
- (c) They are small sized and mostly internal.
- (d) They coordinate to produce the cell.
- 3. Blue green Algae are:
- (a) Eukaryotic

(b) Prokaryotic

(c) Neither a nor b

- (d) Both a or b
- 4. Which of the followings has a perforated cell wall?
- (a) Vessel

(b) Fibre

(c) Tracheid

(d) Sclereid

5 Fibras associated with phloam	
5. Fibres associated with phloem	(h) Doct fibros
(a) Wood fibres	(b) Bast fibres
(c) Hard fibres	(d) Surface fibres
6. Collenchyma occurs in	
(a) Herbaceous climbers	(b) Woody climbers
(c) Climbing stems	(d) Water plants
7. Which biomolecule is distributed	more widely in a cell?
(a) Chloroplast	(b) RNA
(c) DNA	(d) Spaherosomes
8. Which is a reducing sugar?	
(a) Galactose	(b) Gluconic acid
(c) Sucrose	(d) None of the above
9. Which of the following is a phosp	holipid?
(a) Sterol	(b) Cholesterol
(c) Lecithin	(d) Steroid
10. RNA does not possess	
(a) Uracil	(b) Thymine
(c) Adenine	(d) Cytosine
1.8.2 Fill in the blanks:	
1. Cell "building blocks of life" is th	e unit of all organisms.
2. The cell was first discovered by	_
	as the cells which do not have a and organelles.
	and do not required host cell for
5. The bacterial cytoplasm is a	
	otosynthesis with the help of a pigment called
-	reticulated organelle of the cytoplasm.
-	zed, multipurpose, thin walled cells and range from spherical
to barrel-like in shape.	zea, manipurpose, unii wanca cens ana range ironi sphericar
<u>*</u>	norgania compound of the call and constitute about 70, 900/
7 is the most abundant 1	norganic compound of the cell and constitute about 70-80%

1.8.3 True and False:

of the matrix.

1. Many bacteria are able to swim freely with the help of thread like structure called flagella.

10. are very large molecules (macro-biopolymers) made from monomers called amino acids.

- 2. Some bacteria also possess a hair like out growth called pili or fimbriae.
- 3. The word trichomes is derived from a Greek word meaning hair.

4. The eukaryotic cells do not contain a well-constructed nucleus (nucleus enclosed within the membrane) and membrane bounded cell organelles.

- 5. Animal cells true have a cell wall.
- 6. Vessel elements have perforation plates that connect each vessel element to form one continuous vessel.
- 7. The cytoplasm of animal cell possesses numerous membrane-bound organelle originated from the Golgi complex is called lysosomes.
- 8. The sclerenchyma cells are dead, elongated, rigid, heavily thickened secondary walls containing lignified cells.
- 9. Carbohydrates can be represented by the stoichiometric formula $(C_2H_4O)n$.
- 10. The lipids are the organic compound which are soluble in water whereas insoluble in organic compound.
- **1.8.1 Answers Key:** 1(b); 2(a); 3(b); 4(a); 5(b); 6(c); 7(b); 8(a); 9(c); 10(b)
- **1.8.2** Answers Key: 1. Smallest; 2. Robert Hooke 1665; 3. True nucleus, membrane-bound;
- 4. Duplication, 5. Viscous, dense, colloidal and granulated; 6. Bacteriochlorophyll; 7. Endoplasmic reticulum (ER); 8.Parenchyma; 9.Water; 10. Proteins
- **1.8.3 Answers Key:** 1. True; 2. True; 3. True; 4. False; 5. False; 6. True; 7. True; 8. True; 9. False; 10. False

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1.10 SUGGESTED READINGS

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1.11 TERMINAL QUESTIONS

1.11.1 Short answer type questions:

- 1. What are difference between prokaryotic cells and eukaryotic cells?
- 2. Write the differences between animal and plant cells
- 3. Discuss the structure of PPLO.
- 4. Explain the functions of special tissues in plants?
- 5. Write the difference between xylem and phloem
- 6. Discuss the functions of water in cell.
- 7. What are the difference between DNA and RNA?
- 8. Describe the functions of carbohydrate in cell
- 9. Write the functions of protein in cell?
- 10. Discuss the types of lipids in brief.

1.11.2 Long answer type questions:

- 1. Write in detail about structure of prokaryotic cell.
- 2. What do you understand by eukaryotic cell?
- 3. Write a detailed note on specialized plant cell types.
- 4. Discuss the chemical composition of the cell.
- 5. Write a detailed note on carbohydrates, types and functions in cell.

UNIT-2 CELL DIVISION

Contents

- 2.1- Objectives
- 2.2- Introduction
- 2.3- Mitosis
- 2.4- Meiosis
- 2.5- Summary
- 2.6- Glossary
- 2.7- Self Assessment Questions
- 2.8- References
- 2.9- Suggested Readings
- 2.10-Terminal Questions

2.1 OBJECTIVES

After reading this unit you will be able to:

- Understand the basic concept of cell cycle
- Mitosis, its different phases and significance
- Meiosis, its different phases and significance and cytokinesis.

2.2 INTRODUCTION

Cell cycle or cell division is a series of changes which occur in the dividing cells. It includes growth, development and division. Its detail was given by **Howard and Pelc** in 1953.

It consists of interphase (I phase) and dividing M phase.

(A) Interphase

It is non-dividing resting phase of the cell but the nucleus is metabolically most active. It is the most important stage of the cell, without completing I-phase a cell cannot divide. During this period the cell prepares itself for next division. The dividing cell synthesizes and stores all those substances which are essential for cell division. This period is also known as **preparatory phase**. It is a growth phase in which biosynthetic activities of the cell are at their maximum level or point, that's why it is also called **metabolic** or **biosynthetic phase**.

The duration of this phase varies in different tissues. Normally it is the longest phase that persists about 20 to 22 hours of duration. Cell cycle consists of three phases (Fig, 2.1):

- (i) **G**₁ **Phase** (post mitotic phase)
- (ii) **S Phase** (synthetic phase)
- (iii) **G₂ Phase** (pre mitotic phase)
- (i) G₁ phase (I growth phase): It is the longest phase which takes 30-50% of total time and is most variable phase in which maximum growth occurs. It is also called I-growth period. During this phase, cell grows in size and active synthesis of RNA and protein take place. All the enzymes and amino acids are accumulated without any change in DNA content. In human cell cycle, its duration is about six hours. The non-dividing cells such as nerve cells remain permanent in this phase. Circumstances which induce a cell to divide arise in G₁ under the influence of some cytological clock. Decision for cell division is taken in G₁ phase. The point of no return (restriction point or R point) occurs in late G₁. Once the cells have passed this point, they will complete the rest of the cycles at their normal rate. Cells need a certain level of trigger I protein called u-Protein in order to pass through R point. Overcrowding, presence of inhibitors, short supply of nutrients and growth factors results in slowing down or stopping of cell division in G₁ phase or cell cycle.

G1 phase involves transcription of three types of RNAs (rRNA, mRNA and tRNA), synthesis of proteins like regulatory proteins, enzymes (DNA polymerase) nonhistone and some histone proteins. G_1 is absent in Amoeba, slime molds and fission yeasts. The non-dividing cells remain in G_1 stage.

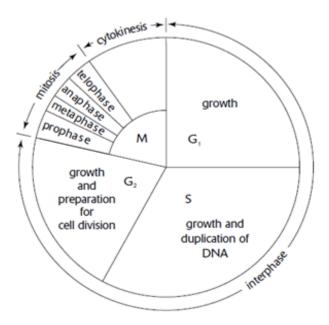


Fig. 2.1: The cell cycle

- (ii) S phase (synthesis phase): DNA replication takes places in this phase. Each chromosome is formed of two sister chromatids and holds together by centromere so that the amount of DNA may double. It initiates the mechanism of cell division. Here the synthesis of basic histones and formation of new nucleosome occur. S phase takes 30-40% of total time (approx. 7 hours in human cell cycle).
- (iii) G_2 phase (II growth phase): Damaged DNA is repaired in G_2 phase. All the organelles including centrioles and ribosomes are doubled in G_2 phase. Synthesis of RNA and proteins continues which is required for cell growth. It is signified by synthesis of some non-histone and tubulin protein required for spindle formation. It takes 10-20% of total time (approximately 3 hours in human cell cycle). The cells which have been arrested in the stable state and may undergo differentiation are said to have entered **Go phase**.

(B) M phase (dividing phase)

It is the last phase of cell cycle. It is of short duration and takes 5-10% of total time. It involves division of nucleus (**karyokinesis**) and cytoplasm (**cytokinesis**). Karyokinesis consists of prophase, metaphase, anaphase and telophase. M-phase is absent is larval cells of diptera insects. Hence, there is permanent interphase system e.g., salivary gland chromosomes. Strassburger has described cell division in plant cells.

2.3 MITOSIS OR MITOTIC DIVISION

The mitosis was first observed in plants by Strasburger. The term mitosis was coined by **W. Flemming** in 1879. Mitotic division occurs in somatic or vegetative cells (haploid or diploid). The two daughter cells, which are formed, are identical to their parents. Chromosomes number remains same in the daughter cells. The chromosomes are distributed equally both quantitatively and qualitatively. The best material is onion root tips to study mitosis in lab orating. Cells of bone marrow, base of nails and skin are also used for this study. In mitosis two divisions take place:

- (A) Karyokinesis: In this process, one nucleus divides into two daughter nuclei. It takes about 90 minutes in one onion root cells and is completed in the following stages:
- **1. Prophase**: It is the first and longest phase of cell division and takes 2-3% of total time of cell division. Condensation of the chromosomes takes place and each chromosome, which has become shorter and thicker, appears a double structure consisting of two identical threads called **chromatids**. The two chromatids are joined together at small clear area which does not stain dark with basic dyes, it is called **centromere**. Nucleolus and nuclear membrane disappear at late prophase. In animal cells, centrosome divides into two centrioles which move to the opposite poles and initiate spindle formation.
- **2. Metaphase:** The chromosomes are shortest in length. The structure, size and number of chromosomes in a cell (karyotype) are best studied at metaphase stage. Spindle is astral (amphiastral) and arises from centriole in animal cell but in plant cell it is **anastral** and arises from cytoplasmic proteins. Spindle consists of microtubules made up of sulphur-rich tubulin protein. Spindle becomes visible with polarising microscope only. Each spindle fibre is made up of 4- 20 microtubules. Mitotic spindle contains following three main types of fibres (Fig. 2.2a)
- (a) Continuous fibres: It appears linked from pole to pole.
- **(b) Discontinuous fibres**: Arise from pole but do not reach to other pole.
- (c) Chromosomal fibres: Arise from pole and attached to kinetochore of centromere of chromosome.

In higher plants spindle forms without the aid of centrioles and lacks asters. The fast growing polymerising plus end (+ end) of microtubules of spindle faces towards the center, while slow growing non-polymerising minus end (-end) is oriented towards the poles.

The chromosomes scattered in the cytoplasm begin to move, get arranged on equator of cell and form an equatorial plate. Therefore, one chromatid of each chromosome faces one pole and other faces the opposite pole. The two spindle fibers are attached to the centromere of each

chromosome one on either side of it. The orientation of chromosomes on equator at metaphase by tightening of chromosomes fibres is called congression (Fig. 2.2 a, b).

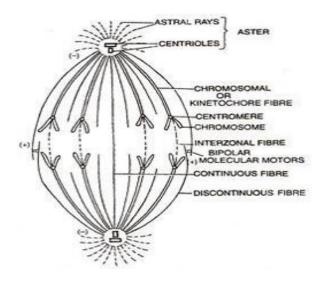


Fig. 2.2(a): Types of nuclear fibres

- **3. Anaphase:** It is of shortest duration and takes less than 0.5% of total time. At this phase centromere divides and disjunction occurs. The spindle fibers contract and the two chromatids of chromosomes are pulled apart and move towards the opposite poles. Each chromatid is known as daughter chromosome. About 30 molecules of ATP are needed to move one chromosome from equator to pole. Acentric chromosomes (without centromere) do not move as they do not have chromosomal fibres. The entire nuclear matter has been divided into two equal and similar halves. Therefore, mitosis is known as equational division. The separating chromosomes or chromatids can be seen under the phase contrast microscope. Shape of chromosome is best studied at anaphase. Mitotic anaphase differs from metaphase in possessing same number of chromosomes and half number of chromatids.
- **4. Telophase:** Nuclear membrane reappears in late telophase from ER and nucleolus reappear from NOR. Chromosomes become less compact and the chromonemata coils unwind and finally converted into chromatin network. Thus, it is just reverse of prophase ad takes more than 1 % of total time of cell division. It results in formation of two daughter nuclei which are identical in number of chromosomes and amount of DNA.

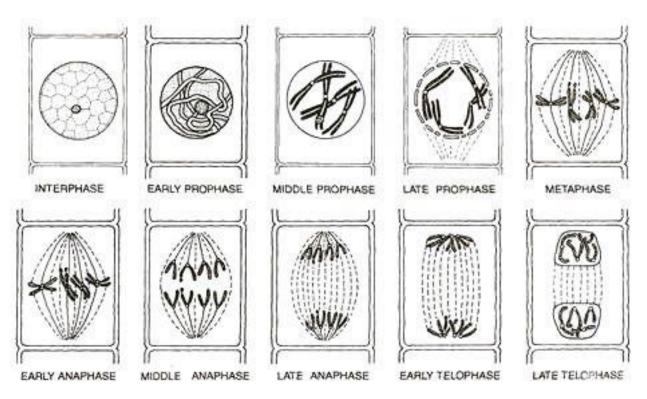


Fig. 2.2(b): Different stages of mitosis in plant cell

- **(B) Cytokinesis:** It is the division of cytoplasm which occurs during early telophase in animal cells and late anaphase in plant cells (Fig. 2.2c). It is accomplished in the two methods:
- (i) Cleavage (furrow) method: It can be seen in animal cells, lower plants and bacteria cells. The cell membrane invaginates (constricts) from two sides near equator, grows centripetally (from periphery to centre) to form cleavage and finally meets in the centre to form two daughter cells.

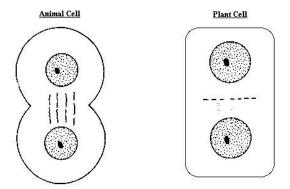


Fig. 2.2(c): Cytokinesis in animal and plant cells

(ii) Cell plate method: It occurs in plant cells. The spindle fibres do not degenerate but persist and are called phragmoplasts (precursor of cell plate). The secretary vesicle, derived mainly from

Golgi apparatus fuse with persisting spindle fibers to form a cell plate. The cell plate grows centrifugally (from centre to periphery), divides the cell into two and forms middle lamella. On both the sides of middle lamella, cell wall materials get deposited and form primary cell wall.

Significance of mitosis:

- (i) Mitotic division helps in the production of genetically identical cells which have same number and types of chromosomes.
- (ii) Mitotic division is method of asexual reproduction in unicellular organisms.
- (iii) It helps in growth and development in multicellular organisms by forming new somatic cells.
- (iv) It keeps chromosome number constant so that heredity character of an organism or cell is maintained.
- (v) Due to mitosis, old, decaying and dead cells are replaced by new cells.

Useful Information

- Mitotic division involves one chromosomal and one nuclear division.
- In prokaryotes, cell division (mitosis and meiosis) is absent due to lack of nucleus.
- Cell membrane helps in separating the replicated DNA as spindle is absent in prokaryotes.
- Any agent that stimulates cell division is called mitogen e.g. temperature, cytokinin, auxin, gibberellin, insulin and steroids.
- Inhibitors of cell divisions or mitotic poisons are **Azides** and **cyanides** that inhibit prophase, **Colchicine** checks spindle formation at metaphase, ribonuclease blocks prophase.

2.4 MEIOTIC OR MEIOSIS DIVISION

The term meiosis was used by **Farmer and Moore** in 1905. It occurs in mature diploid reproductive or germ cells in which nucleus divides twice but chromosome replicates only once to form four haploid cells. Four haploid cells are produced after this division, not identical to their parent cell. The chromosome number gets reduced to half; hence, it is called as 'reduction division'. The meiosis includes two nuclear and one chromosome division. It occurs only once in a cell. Best material for study of meiosis in laboratory is anthers of a flowering plant, e.g., *Tradescantia* (*Rheo discolor*). Meiosis is completed in the following two successive divisions:

(A) **Meiosis I:** It is the reductional division or heterotypic division (two chromatids of a chromosome became genetically different due to crossing over). It consists of I-phase and M-phase. M phase involves karyokinesis and cytokinesis.

Karyokinesis: It consists of following stages:

(1) **Prophase I:** It is the longest phase. It can be further divided into the following five substages (Fig.2.3a):

- (a) **Leptotene:** At this phase, chromosomes become visible as the long filamentous structures and bear chromomeres, the beaded structures on chromosomes. The chromosomes have a definite polarized orientation with all their ends directed towards one small area, on one side of nucleus. This peculiar arrangement is known as **bouquet stage**.
- **(b) Zygotene:** Pair of chromosomes having similar genes which control the same characters are called as homologous chromosomes. One member of a pair of homologous chromosomes comes from male parent and the other from female parent. The homologous chromosomes begin to pair length wise due to force of attraction. The pairing proceeds from one end to another. The pairing of homologous chromosomes is known as **synapsis** or **zipping**. The pair of homologous chromosome is known as bivalent. The two chromosomes in bivalents are surrounded by ribonucleoproteins. Synapsis provides mechanical basis of heredity and variations. It produces a complex called as **synaptinemal complex**. The number of bivalents is half the number of total chromosome in a diploid cell. e.g., If 2n = 50, the number of bivalents is 25.
- (c) **Pachytene:** At this phase, the longitudinal splitting occurs in the cell; therefore, chromosomes consist of two chromatids. Now each bivalent consists of 4 chromatids and 2 centromere and they are called **tetrad**. At this, 4 strand stage crossing over occurs (exchange of segments of chromatids). Chromatids belonging to the two different chromosomes of homologous pair are termed as **non-sister chromatids**.

The breakage of chromatids is brought by endonuclease enzymes and broken chromatids unite with non-sister chromatids with the help of an enzyme called as **R proteins** (exchange of non-sister chromatids). This exchange of genetic material between nonsister chromatids at four strand stage in a bivallent at pachytene stage is called **crossing over** which is the major source of continuous type of genetic variations in sexually reproducing organisms. Pachytene is the longest stage of prophase I. The synaptinemal complex stabilizes the pairing and helps in smooth crossing over process.

- (d) **Diplotene**: Due to dual nature of bivalent it is called diplotene. The ribonucleoprotein of synaptinemal complex dissolves and the homologous chromosomes start separating. During the separation or **poleward movement of chromosome** (PMC) the homologous chromosomes are held together at certain point that is known as **chiasmata** (look like cross). Chiasmata can be observed at diplotene. Chiasmata are regarded as expressions of crossing over. Chiasmata were first observed by **Janssen** (1909).
- **(e) Diakinesis:** At this stage, nucleolus and nuclear membrane disappear. Chiasmata move from centromere towards the ends of chromosomes. It is called as **terminalization.** Chromosomes in bivalent separate completely. Spindle fibers start to develop with or without centriole.

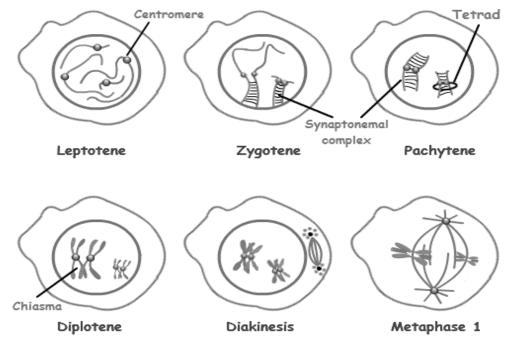


Fig. 2.3(a): Different stages of prophase I of meiosis

(2) **Metaphase I**: Centrioles reach to their opposite poles in animal cells in pair. A bipolar spindle apparatus is formed. The bivalent chromosomes get oriented themselves at equator of spindle forming two equatorial or metaphasic plates, one by the centromeres of maternal and the second by centromeres of paternal chromosomes.

Each chromosome of bivalent is connected to spindle pole of its side by single chromosomal fibre. The distribution of bivalents on metaphasic plate is random (independent assortment can align the chromosome in different ways). This independent assortment is the second source of genetic variations which is necessary for evolution.

- (3) Anaphase I: One chromosome from each bivalent goes to opposite pole or segregation of homologous chromosomes occurs, hence, it is called as reductional or disjunctional division. At the end of anaphase I, two groups of chromosomes (one at each pole) are produced. Each such group possesses half the original number of chromosomes in the parent nucleus. Anaphase I results in the reduction of chromosome number to half and segregation of Mendelian factors (Aa) which are essential for sexual reproduction. Due to independent assortment, it induces genetic variability.
- (4) **Telophase I:** Nuclear membrane and nucleolus reappear and karyokinesis occurs which is followed by cytokinesis.

(B) Meiosis II: It is the equational division or homotypic division. It is similar to as mitotic cell division. Meiosis II is essential to separate out the chromatids of dyad chromosomes which are different from each other due to crossing over and to bring real haploidy in the amount of DNA.

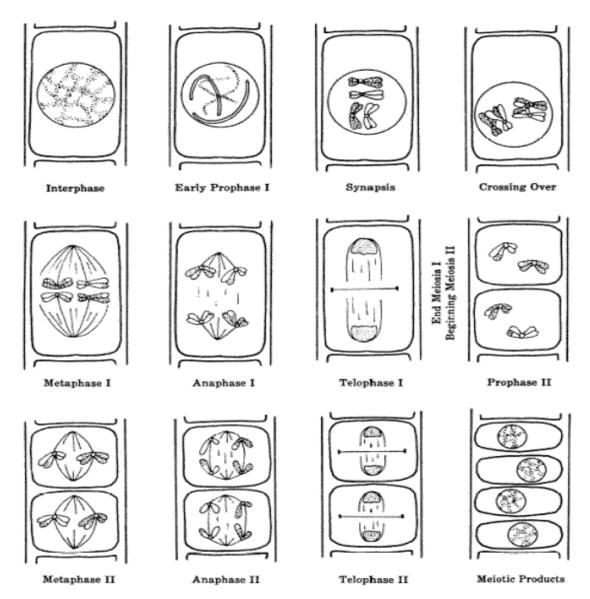


Fig. 2.3 (b): Meiosis in plant cell

Significance of meiosis

- 1. It is responsible for recombination of characters in the progeny due to crossing over (major source of continuous type of genetic variations).
- 2. It maintains the original number of chromsomes or constant number of chromosomes.
- 3. The distribution of bivalents is at random in metaphase I which provides the second source of genetic variations essential for speciation and evolution in the organisms.

	Mitosis	Meiosis
(i)	It occurs in all somatic cells and may continue throughout life.	It occurs in reproductive cells and at specific times.
(ii)	It involves a single division, resulting in two daughter cells only.	It involves two successive divisions, resulting in four daughter cells.
(iii)	Subsequent mitotic divisions are similar to the earlier ones.	Two meiotic divisions are dissimilar, first is reductional while the second is equational.
(iv)	Prophase is relatively short and simple.	Prophase I is very long and elaborate, comprising 5 subphases.
(v)	There is no pairing of homologous chromosomes.	Homologous chromosomes pair and often undergo crossing over in prophase I.
(vi)	Chromatids are genetically similar to chromosomes they arise from.	Chromatids may differ genetically from the chromosomes they arise from due to crossing over.
(vii)	No synaptonemal complex forms.	Synaptonemal complex forms between synapsed homologous chromosomes.
(viii)	Chromosomes do not unfold, and no transcription and protein synthesis occur in prophase.	Chromosomes unfold, and transcription and protein synthesis may occur in diplotene of prophase I (oocytes of certain animals).
(ix)	Daughter cells have diploid number (2N) of chromosomes like the parent cell.	Daughter cells have haploid number (N) of chromosomes unlike the parent cell.

Table 1: Differentiate between mitosis and meiosis

Useful Information

- Karyokinesis without cytokinesis results in a multinucleate **coenocytic** cell.
- Colchicine, an alkaloid isolated from *Colchicum autumnale* (liliaceae), is used in plant
 breeding for inducing polyploidy. It inhibits or interferes the formation of spindle at
 metaphase. Therefore, chromosomes fail to separate in anaphase. Thus, the number of
 chromosomes gets doubled. This nucleus with double sets of chromosomes is called
 restitution nucleus.
- Sometimes, there is replication of DNA as well as the chromosome but without nuclear division. So the somatic number of chromosomes in nucleus gets increased. This process is called as **endopolyploidy** or **endomitosis** or **e-mitosis**. Endopolyploidy can be induced by colchicines.
- X-rays and U.V. rays inhibit or retard cell division.
- Folic acid is metaphasic inhibitor.
- Mitosis without asteral rays (due to absence of centromere) is called **anastral** mitosis e.g., angiospermic plants (higher plants).

2.5 SUMMARY

When **cell division** is observed under a microscope, a series of changes, ranging from **chromosome condensation** to chromosome alignment and segregation, take place within just 1

hour in human cells such dramatic changes. However, are not frequently repeated. More than 20 hours are necessary for the next dynamic change to occur.

When cells multiply, the process in which the structural components of the cell such as chromosomes, are doubled and segregated into two cells is repeated. This process is called **cell cycle**. During the cell cycle, the phase in which cells divide, is called the mitosis phase (**M phase**), whereas the phase in which DNA is replicated is called the synthesis phase (**S phase**). Between M and S phases, there is the gap 1 phase (**G₁ phase**), and between the S and M phases is the gap 2 phase (**G₂ phase**). Assuming the G₁ and G₂ phases are the preparative phases for DNA synthesis and cell division, respectively, the cell cycle can be described as a repeated series of events in which cell division and replication alternate in the following order: M phase, G₁ phase, S phase, and G₂ phase (and back to the next M phase). Multicellular organisms including human beings have countless cells in the gap 0 phase (**G₀ phase**), which are cells in their quiescent state (a state in which cells stop multiplying even though they have proliferating potency). The cell cycle, for human cells to multiply takes about 1 day, during which the S phase lasts for 6–8 hours and the M phase, for about 1 hour.

2.6 GLOSSARY

Amitosis: Nuclear division occurs by simple constriction into two halves. No formation of chromosomes and spindle.

Chromatid: one of two threads like structures are formed in the duplication of a chromosome to form daughter chromosomes.

Chromosome: A structural unit in the nucleus, which carries the genes in the linear constant order; it preserves its individuality from one cell generation to the next and it's typically consistant in number in any species.

Cytokinesis: The process of division of cytoplasm.

Cytoplasm: The protoplasm of a cell excluding the nucleus.

Dicentric: Chromosome having two centromeres.

Disjunction: Refers to the separation of homologous chromosomes.

Euchromatin: Light stained portion of chromatin.

Filiform: Like a filament or thread.

Fission: The splitting of a cell into two, without any spindle formation.

Heterochromatin: Dark stained portion of chromatin.

Homologous chromosomes: Chromosomes that synapse or pair at the first division in meiosis. Each member of a pair has a corresponding sequence of gene loci and is derived from different parent.

Kariyokinesis: Refers to division of nucleus.

Meiosis: Reduction division of the diploid nucleus resulting in haploid nuclei.

Mitosis: A process of nuclear division in which the chromosomes are duplicated longitudinally forming two daughter nuclei each having a chromosome complement equal to that of the original nucleus.

Nucleus: Complex spheroidal mass essential to life of most cells.

Zygote: Diploid cell forms after the fusion of male and female gamete cells.

2.7 SELF ASSESSMENT QUESTIONS

2.7.1 Multiple Choice Questions	:
1- The meiotic division takes place in-	

(a) Meristematic cells

b) Conductive cells (c) Reproductive cells (d) Vegetative cells

2. Synapsis takes place between-

(a) Spindle fibre and centromere (b) mRNA and ribosomes

(c) a female and a male gamete (d) Two homologous chromosomes

3. The stage of prophase I wherein crossing over occurs is-(a) Zygotene (b) Diplotene

(c) Leptotene (d) Pachytene

4- The RNA and protein synthesis occurs in-

(a) M phase (b) S phase (c) G1 phase (d) G2 phase

5- Chromosomes break during?

(a) Prophase (b) Anaphase (c) Metaphase (d) Telophase

6-Number of chromatids at metaphase is:

(a) two each in mitosis and meiosis (b) two in mitosis and one in meiosis

(d) one in mitosis and two in meiosis (c) two in mitosis and four in meiosis

7-The sequence of cell cycle is-

(a) S, M, G1 and G2 (b) M, G1, G2 and S (c) G1, G2, M and S (d) G1, S, G2 and M

8-In mitosis, chromosomes duplication occurs during-

(a) Interphase (b) Prophase

(c) Late prophase (d) Late telophase

9-Cytokinesis is:

(a) division of nucleus (b) division of cytoplasm

(c) division of chromosomes (d) none of these

10- In which of the following stage, the chromosome is thin and like long thread-

(a) Leptotene(b) Zygotene(c) Pachytene(d) Diakinesis

2.7.1 Answer Key: 1-(c), 2-(d), 3-(d), 4-(c), 5-(b), 6-(a), 7-(d), 8-(a), 9-(b), 10-(a)

2.8 REFERENCES

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- Verma, P.S. (2001). Cell Biology, Genetics, Molecular Biology, Evolution and Ecology. pp. 1-1291. ISBN: 97-88121-94429.
- Verma, P.S and Agarwal, V.K. (2016). Cell Biology (Cytology, Biomolecules and Molecular Biology). pp. 1-1191. ISBN: 978-93-856-7614-7.

2.9 SUGGESTED READINGS

- Rastogi, S.C. (2008). Cell Biology. pp. 1-548, ISBN: 13-978-8122416886.
- Verma, P.S and Agarwal, V.K. (2016). Cell Biology (Cytology, Biomolecules and Molecular Biology). pp. 1-1191. ISBN: 978-93-856-7614-7.

2.10 TERMINAL QUESTIONS

2.10.1. Short answer type questions

- Q1. Define cell cycle?
- Q2. Name the phases of cell cycle?
- Q3. What is the G_1 phase of the interphase?
- Q4. Which phase follows the S phase in the cell cycle?
- O5. What is mitosis?
- Q6. What is meiosis?
- Q7. In which phase of cell division crossing over takes place?
- Q8. What term is used for a full set of DNA instructions in a cell?
- Q9. In which phase of the cell division the chromosomes are set free in the cytoplasm?
- Q10. By which method cytokinesis occurs in plant cells?
- Q11. What is the significance of pachytene?
- Q12. At what stage of mitosis, chromosomes arrange themselves around the equator?

- Q13. What is karyokinesis?
- Q14. What is cytokinesis?
- Q15. What is quiescent phase (G_0) ?
- Q16. Why mitosis is called equational division?
- Q17. What are bivalents?
- Q18. What is the synapse?
- Q19. What is chaismata?
- Q20. Why meiosis is called reductional division?
- Q21. What is termilization?
- Q22. What is cell plate?
- Q23. Mention the significance of chaismata.
- Q24. What is mean by recombination?
- Q25. What is a metaphasic plate?

2.10.2 Long answer type questions

- 1- Comment on the statement telophase is reverse of prophase.
- 2- What are the various stages of meiotic prophase-I? Enumerate the chromosomal events during each stage?
- 3- Differentiate between the events of mitosis and meiosis.
- 4- Write brief notes on the following:
 - a) Synaptonemal complex
 - b) Metaphase
 - c) Spindle fibres.
- 5- Write briefly the significance of mitosis and meiosis in multicellular organism.
- 6- An organism has two pair of chromosomes (i.e., chromosome number = 4). Diagrammatically represent the chromosomal arrangement during different phases of meiosis-II.

UNIT-3 CELL SENESCENCE

Contents

- 3.1-Objectives
- 3.2-Introduction
- 3.3- Cell Senescence
 - 3.3.1. What is Cell Senescence?
 - 3.3.2 Why does senescence occur?
 - 3.3.3 Characteristics of senescent cells
 - 3.3.4 Biomarkers of senescent cells
 - 3.3.5 How to combat senescence?
 - 3.3.6 Is senescence beneficial or harmful activity in cell?
 - 3.3.7 Physiological changes during senescence in plants
- 3.4- Summary
- 3.5- Glossary
- 3.6- Self Assessment Question
- 3.7- References
- 3.8- Suggested Readings
- 3.9- Terminal Questions

3.1 OBJECTIVES

As you all know that no life is immortal may it be any microbial form, animal or plant. After reading this unit students will be able:

- to know about the cellular senescence, what it is and why it happens?
- to understand Various theories proposed by scientists, why does it occur.
- to understand about the mechanism of cell senescence in simple language.

3.2 INTRODUCTION

Any living organism has three types of cells, mitotic, non-mitotic or post mitotic, and germ cells. The mitotic cells are actively dividing cells; non-mitotic cells do not undergo mitosis where as germ cells play role in reproduction. The mitotic cells divide into two daughter cells by cell cycle which has G1, S, G2 and M phase. Any cell can have many fates as apoptosis, necrosis and autophagy. Apoptosis is also known as programmed cell death and it helps in removal of unwanted cells and death signal may come from outside or inside the cell. The cell DNA breaks, cell shrinks, blebs are formed, nucleus also breaks and these blebs are then engulfed by macrophages. In necrosis, the cells are chemically or physically damaged (trauma). It is a non-programmed death in which cell swells, organelles swells, water is absorbed and finally cells rupture. While in third type of fate, damage takes place within the cell, starvation or infection may be the reason, cell organelles lysosomes or suicidal bags play the main role.

Despite of above routes, cells undergo another state known as senescence. As there is increase in cell's age, they do not divide and enter into senescence state. They also secrete certain chemicals which encourages nearby cells to enter into senescence. They can cause cancer, damages tissue, effects tissue function, increases inflammation, etc. Senescence can beat cellular, tissue, organ and organism levels. In the present chapter, you will study about cell senescence in detail.

3.3 CELL SENESCENCE

For the sake of easiness and complexity of the topic, students will study cell senescence under the following sub headings:

3.3.1. What is cell senescence?

Cellular senescence is a phenomenon characterized by the cessation of cell division. It refers to a state of stable cell cycle arrest in which the proliferating cells become resistant to growth-promoting stimuli, in response to DNA damage. This phenomenon was first described by Hayflick and Moorhead (1960) who found that normal human fetal fibroblasts stopped dividing, but remained viable and metabolically active after prolonged time in culture. They reached a

maximum of approximately 50 cell population doublings before becoming senescent in culture. This process is known as "replicative senescence" or the Hayflick limit. It was observed that *in vitro*, cells stops dividing, after approximately fifty divisions and it was termed as the Hayflick limit. Senescent cells are distinct from both quiescent cells which can reenter the cell cycle and from terminally differentiated cells.

Senescence causes an abrupt termination of life at a given stage in life cycle. These deteriorative processes may terminate in death either gradually or abruptly. Senescence is a normal energy dependent developmental process which is controlled by cell's own genetic programme and the death of the cell is called as programmed cell death (PCD). Senescence is closely associated with the phenomenon of aging and both are sometimes considered as the same by many workers. According to Medawar (1957) the term 'senescence' should be used to refer to natural changes towards termination of life, while aging is a process in which maturity is attained with the passage of time. The terms aging and cellular senescence cannot be used interchangeably. Aging is a progressive decline with time, whereas senescence occurs throughout the lifespan, including embryogenesis. The number of senescent cells increases with age, but senescence also plays an important role during development and wound healing.

Senescent cells destroy themselves through a process called apoptosis by which they are removed by the immune system. But as the age increases the immune system gets weak and the senescent cells gets accumulated and results in progression of aging.

3.3.2Why does senescence occurs?

Cellular senescence can be initiated by a wide variety of factors as described below:

3.3.2.1 Stress response and DNA damage

Cells can also be induced to senescence by DNA damage. The DNA damage can be caused by stress which can be due to internal or external environment. The senescent cells are characterized by DNA damage response (DDR) including ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related) kinase signaling which ultimately arrests cell cycle progression through activation of p53/21 and p16/pRb pathways. The prolonged DDR activates DNA damage kinases resulting in cell cycle arrest.

Senescent cells display persistent DDR that appears to be resistant to endogenous DNA repair activities. Depending on the severity of the DNA damage, the cells may no longer be able to undergo repair and either go through apoptosis or cell senescence. It has been proposed that retained DSBs are major drivers of the aging process.

Although senescent cells can no longer replicate; they remain metabolically active and commonly adopt an immunogenic phenotype. Senescent cells can undergo conversion to an immunogenic phenotype that enables them to be eliminated by the immune system. The nucleus of senescent cells is characterized by senescence associated heterochromatin foci (SAHF) and

DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS). Senescent cells affect tumour suppression, wound healing and possibly embryonic/placental development and a pathological role in age related diseases.

3.3.2.2 Role of telomeres

Telomeres are DNA tandem repeats at the end of chromosomes that shorten during each cycle of cell division. The successive shortening of the chromosomal telomeres with each cell cycle is also believed to limit the number of divisions of the cell, contributing to aging. Some cells do not age and are described as being "biologically immortal". The length of the telomere strand has senescent effects; telomere shortening activates extensive alterations in alternative RNA splicing that produce senescent toxins such as progerin, which degrades tissue and makes it more prone to failure. Critically short, uncapped telomeres initiate a DNA damage response which triggers sensescence.

3.3.2.3 Role of oncogenes

The transitions to a state of senescence, due to oncogene mutations, are irreversible and have been termed oncogene induced senescence.

There are several reports on signaling pathways that lead to cellular senescence including the p53 and p16 pathways. Both of these pathways are activated in response to cellular stressors and lead to cell cycle inhibition. The p53 activates p21, which deactivates Cdk2 (cyclin dependent kinase). Due to the deactivation of Cdk2 the pRB (retinoblastoma protein) remains in active state, and binds to E2F1 (transcription factor), thus arresting the cell cycle after G1 phase.

3.3.3 Characteristics of senescent cells

The secretome of senescent cells is very complex. The products are mainly associated with inflammation, proliferation and changes in the extracellular matrix. A senescence associated secretory phenotype (SASP) consisting of inflammatory cytokines, growth factors, and proteasesis another characteristic feature of senescent cells. SASP molecules, IL-6 and IL-8, are likely to cause senescence without affecting the healthy neighbor cells. Growth factors, GM-CSF and VEGF also serve as SASP molecules.

Proteins, p53, p21, p16ink4a, and Bmi-1, have been termed as major senescence signaling factors, allowing them to serve as markers. Other markers includes: morphology changes as cells becomes flat, enlarged, vacuolized sometimes with multiple or enlarged nuclei, cytoplasmic bridges, reorganization of chromatin, apoptosis resistance, altered metabolism, enlarged cytoplasm and abnormal shape of the nucleus.

SASP is associated with many age related diseases, including type 2 diabetes and atherosclerosis. The nucleus of senescent cells is characterized by senescence associated heterochromatin foci (SAHF) and DNA segments with chromatin alterations causing senescence (DNA-SCARS).

Senescent cells affect tumor suppression, wound healing and possibly embryonic/placental development. The removal of aggregated p16 INK 4A positive senescent cells can delay tissue dysfunction and ultimately extend life.

3.3.4 Biomarkers of senescent cells

Senescent cells can be identified by stable cell cycle arrest, morphological and metabolic changes, chromatin reorganization, altered gene expression and SASP (senescence associated secretory phenotype). Though some of these markers are observed in other cells also as in quiescent cells. These biomarkers are discussed here in brief to make you understand well.

Phospho-histone H2A.X (Ser139), also known as γ -H2A.X, is a commonly used as the marker of cell senescence. Two proteins, senescence associated beta-galactosidase and p16, are regarded as biomarkers of cellular senescence.

(a) Stable cell cycle arrest

Senescent cells are characterized by stable cell cycle arrest. They do not reenter cell cycle. Cell cycle arrest is mediated by the $p53/p21^{CIP1}$ and $p16^{INK4A/pRb}$ tumor suppressor pathways. $p16^{INK4A}$ is a very useful biomarker and is frequently observed in senescent cells, though it is expressed in other cells also.

(b) Morphological and metabolic changes

Senescent cells are generally largein size and flattened in shape as compared to its dividing cell counterparts. Senescent cells display extensive vacuolization and are, sometimes, multinucleated. In addition, disrupted nuclear envelope integrity is observed due to a loss oflamin B1 expression. Mitochondria become dysfunctional and also show increased levels of reactive oxygen species (ROS). The lysosomes become more active due to which β -galactosidaseactivitygets increased at pH 6.0. This is another widely adopted biomarker of cellular senescence.

(c) Chromatin reorganization and altered gene expression

Extensive chromatin reorganization is the another characteristic of cell senescence. Senescence-associated heterochromatin foci (SAHF) are formed. These sites of facultative heterochromatin play a role in silencing genes that promote proliferation including E2F target genes like cyclin A. Senescent cells typically contain 30-50 SAHF which are characterized by bright DAPI(4',6-diamidino-2-phenylindole)staining and macroH2A, heterochromatin protein 1 (HP1), and lysine 9 di-or-tri-methylated histone H3 (H3K9Me2/3) immunoreactivity. Although SAHF is frequently observed during senescence, some cells undergo senescence without forming SAHF.

(d) DNA damage and persistent DNA damage response (DDR)

As already discussed, DNA damage, such as DNA double strand breaks, is a prominent feature of senescence. These cells show a persistent DDR which ultimately arrests cell cycle. Senescent cells contain nuclear foci called DNA segments with chromatin alterations reinforcing

senescence (DNA-SCARS), which associate with PML nuclear bodies and accumulate DDR proteins such as activated p53, ATR, and ATM. DNA-SCARS that occur at uncapped telomeres are called telomere dysfunction-induced foci (TIF). Another indicator of DNA damage isγ-H2A.X, which is the phosphorylated form of H2A.X, a variant histone required for checkpoint-mediated cell cycle arrest and DNA repair following double-stranded DNA breaks. DNA damage, caused by ionizing radiation, UV-light, or radiomimetic agents, results in rapid phosphorylation of H2A.X at Ser139.

(e) Senescence-associated secretory phenotype (SASP)

The SASP is comprised of a highly complex mixture of secreted cytokines, chemokines, growth factors, and proteases, with the precise composition varying markedly by cell and tissue context and the senescence-inducing stimulus. These secreted factors facilitate communication with neighbouring cells and the immune system, which ultimately influences the fate of the senescent cell.

3.3.5 How to Combat senescence?

Senolytics is the proposed method to combat senescence. It is a drug that leads to destruction of senescent cells. It has been found that reducing senescent cells by even 30% slows down the ageing process.

3.3.6 Is senescence beneficial or harmful activity in cell?

The senescence shows both protective as well as deleterious effects. In simple language it can be understood as a mechanism which avoids malignant transformation of damaged cells and on the other hand it may lead to many age related diseases. Many senescent cells acquire a proinflammatory senescence-associated secretory phenotype (SASP) that mediates non-cell autonomous effects of senescence, both beneficial and deleterious. For example, the SASP recruits immune cells to senescent cells, thereby facilitating their elimination, which serves a tumor suppressor function. Paradoxically, however, the SASP has been shown to promote tumor cell progression through secretion of factors that promote angiogenesis (development of new blood cells), extracellular matrix remodeling, or epithelial-mesenchymal transition (EMT). Additionally, chronic senescence-induced inflammation induce systemic can immunosuppression, potentially leading to the onset of diseases including cancer. This chronic inflammation may also drive tissue damage and degeneration associated with aging.

3.3.7 Physiological changes during senescence in plants:

Since we are dealing with plants we should also know the effect of senescence on the physiology of plants. Generally all the physiological processes decline during senescence and many changes lead to death. Some of these processes are discussed below:

a) Photosynthesis: The net photosynthetic rate increases as leaves grow and then declines gradually at the time of maximum leaf expansion. During the rapid phase of whole plant

senescence young and old leaves degenerate almost together (Fig. 3.3.7.). Simultaneously senescence of leaves occur not only in monocarpic senescence but also in autumnal senescence of polycarpic plants. Chlorophyll loss during senescence offers a visual method for estimating the degree of senescence as with chlorophyll content the synthesis and activities of chloroplast enzymes (e.g. RuBPcase) decline after the cessation of leaf growth which is parallel to the loss of photosynthetic activity. Thus yellowing of leaves is the most obvious change during the senescence.

In Calvin cycle, the enzymes synthesized on chloroplast ribosomes decline earlier during senescence than those synthesized primarily on cytoplasmic ribosomes. It has been shown that conformational changes and loss of active site lead to the loss of RuBPcase. Thus, it appears that the overall decrease in photosynthetic rate in senescing leaves results from a decline of CO₂ reduction reactions. The stomatal conductance also decreases during senescence. The stomata of senescing leaves open less in response to light than those of young leaves.

- **b) Respiration**: The respiratory apparatus of senescing tissue remains active until late in senescence and then declines rapidly. Loss of respiratory capacity can be a factor in the initial stages. In the later stages mitochondria swells and decreases in number. It appears that cellular energy is required during senescence possibly for the synthesis of the degradative enzymes.
- **c) Nitrogen fixation and mineral uptake**: Depodding increases the availability of photosynthate which can cause appreciable increase in nitrogen fixation and delay nodule senescence. Symbiotic nitrogen fixation decreases with the ageing and senescence of leguminous plants.
- **d) Protein and nucleic acids:** The decline in proteins and nucleic acid levels is the most basic step during senescence. Protein, DNA and RNA do not decline at the same rate. Protein and RNA declines gradually early but DNA declines in the last.
- e) Membranes and organelles: The chloroplast shows the earliest symptoms of physiological decline and in later stages of senescence there are ultrastructural changes in thylakoids. Some other changes also occur including swelling, vesiculation and disappearance of endoplasmic reticulum, ER related ribosomes and Golgi bodies. The phospholipids, galactolipids and sulpholipids decreases with onset of senescence. In the final stage of cellular degradation the plasmalemma disintegrates and the nucleus undergoes massive alteration, whereas the mitochondria may persist with intact shape. Membrane plays an important role in plant senescence.

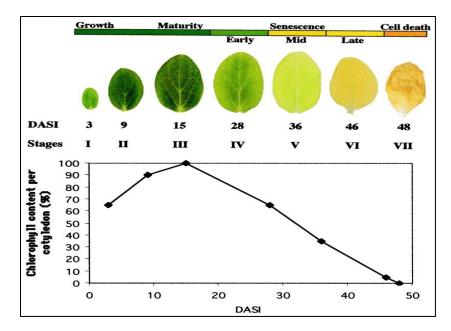


Fig. 3.3.7 Different stages of leaf senescence

There are many theories about plant cell senescence some of them have been discussed below:

1. Nutritional or Plant self pruning theory

It was proposed by H. Molisch (1920) that senescence was caused by nutritional deficiency. There is a speculative hypothesis on how and why a plant induces part of itself to die off. The theory holds that leaves and roots are routinely pruned off during the growing season whether they are annual or perennial. This is done mainly to mature leaves and roots and is for one or two reasons; either both the leaves and roots that are pruned are no longer efficient enough nutrient acquisition wise or that energy and resources are needed in another part of the plant.

- Poor productivity reasons for plant self pruning
- Shortage/need-based reason for plant self pruning

It has also been found that application of fertilizers to mature plants also delays senescence. But this does not apply to annual plants that have flowered or fruited even if heavy doses of fertilizers were applied.

2. Theory of hormonal induction of senescence

There is little theory on how plants induce themselves to senescence, although it is reasonably widely accepted that some of it is done hormonally. Plant scientists generally concentrate on ethylene and abscisic acid (ABA) as culprits in senescence, but neglect gibberellin and brassinosteroid which inhibits root growth if not causing actual root pruning. Parallels to cell division - the theory, asserts that just as both auxin and cytokinin seem to be needed before a plant cell divides, in the same way perhaps ethylene, gibberellic acid, ABA and strigolactones are needed before a cell senesce.

L.D. Nooden and A. C. Leopold (1978) proposed a term "death hormone" for the causative agent of senescence in monocarpic species. The death hormone is a chemical which is supposed to be produced in developing seeds, from where it is translocated through the xylem to the vegetative parts. Aging and senescence are two processes that are associated with hormonal interactions. The external applications of plant hormones have been found either to promote or to retard senescence. The well known senescence promoting hormones are ethylene and abscisic acid, where as senescence retarding hormones include auxin, cytokinins, gibberellins etc.

It was found by Richmond and Lang (1957) that cytokinins inhibit senescence through the maintenance of protein and nucleic acid levels, delaying chlorophyll breakdown, increases chloroplast DNA, promotes chloroplast replication and grana formation and maintain pigment levels. It has been found that both natural and synthetic auxins delays senescence by altering processes such as chlorophyll loss, RNA degradation, RNA synthesis, protein degradation, protein synthesis, wilting and membrane breakdown. Gibberellins when applied exogenously inhibit senescence by inhibiting processes like RNA and protein breakdown. Endogenous gibberellin activity has been found to decline during senescence in a wide variety of tissues.

After senescence signaling there is massive degradation of membrane in structure as well as function. The loss in membrane is followed by leakage of solutes. This may be due to peroxidation of lipid components of the membrane by free radicals. The vacuolar membrane, called as tonoplast, breaks and functions as lysosomes. The hydrolytic enzymes located in them are released and digests the cellular materials.

3.4 SUMMARY

Cellular senescence is a phenomenon characterized by the cessation of cell division after a limited number of divisions known as "replicative senescence" or the Hayflick limit. This limit was found to be about 50-60 cell divisions. These cells are different from mitotic, post mitotic and germ cells. The process of senescence should not be confused with ageing.

Senescence causes an abrupt termination of life at a given stage in life cycle. The number of senescent cells increases with age of organism. Cellular senescence can be initiated by a wide variety of factors as DNA damage, telomeres, oncogenes, etc. Telomeres are DNA tandem repeats at the end of chromosomes that shorten during each cycle of cell division. The successive shortening of the chromosomal telomeres with each cell cycle is also believed to limit the number of divisions of the cell, contributing to aging.

The senescent cells can be identified by various biomarkers. One of them is secretomewhich is associated mainly with inflammation, proliferation and changes in the extracellular matrix. A Senescence Associated Secretory Phenotype (SASP), consisting of inflammatory cytokines, growth factors and proteases, is another characteristic feature of senescent cells. Proteins p53,

p21, p16ink4a, and Bmi-1have been termed as major senescence signalling factors, allowing them to serve as markers. The senescent cells also show changes in cell morphology.

The biomarkers which can be used in identification of senescent cells stable cell cycle arrest, morphological and metabolic changes, chromatin reorganization, altered gene expression and SASP (senescence associated secretory phenotype).

Senolytics is one of the methods to combat senescence. It is a drug that leads to destruction of senescent cells. The senescence shows both protective as well as deleterious effects.

Senescence affects physiological processes like photosynthesis, respiration, nitrogen fixation and mineral uptake, protein and nucleic acids synthesis. Nutrient deficiency syndrome has been found in monocarpic plants in which the flowers and fruit development imposes a great demand on the vegetative body, thus causing nutrient deficiency.

There are various theories about plant cell senescence as:

- 1. Nutritional or plant self-pruning theory proposed by H. Molisch suggests that senescence is caused by nutritional deficiency.
- 2. Theory of hormonal induction of senescence according to which hormones are responsible for it, as ethylene and abscisic acid and in some cases gibberellin and brassinosteroid also.

Nooden and Leopold (1978) proposed a term "death hormone" for the causative agent of senescence in monocarpic species. Factors that influence senescence are growth hormones, light/dark, water stress, temperature and nutrients like nitrogen, calcium etc.

3. The vacuoles and membranes also disintegrate and digest the cellular materials.

3.5 GLOSSARY

Auxin: it is a class of plant growth hormones with some morphogen like characteristics. They play major role in growth and behavioural processes in plant life cycles.

Cytokinins: It is a class of plant growth substances that promotes cell division, in roots and shoots. They affect cell growth, differentiation, apical dominance, axillary bud growth, leaf senescence, etc.

Death hormone: The 'death hormones' or monocarpic senescence factors are hypothetical substances transported from the fruits to the vegetative parts of the mother plant, where they may stop growth, activate senescence, remobilize nutrients and finally lead to the deathof the plant.

Deciduous: tree or shrub shedding its leaves annually.

Death hormone: it is the causative agent of senescence in monocarpic species.

Ethylene: it is a plant growth retardant present in trace amounts and regulates ripening of fruits, opening of flowers and abscission of leaves.

Gibberellin: these are plant hormones that regulate various developmental processes including stem elongation, germination, dormancy, flowering, flower development, leaf and fruit senescence. These are one of the largest known classes of plant hormone.

Senescence: It is also called as biological ageing. It is the gradual deterioration of functional characteristics. The word senescencecan refer either to cellular senescence or to senescence of the whole organism. Organismal senescence involves an increase in death rates and/or a decrease in fecundity with increasing age, at least in the later part of an organism's life cycle.

Tonoplast: the membrane surrounding the vacuole is called as tonoplast.

3.6 SELF ASSESSMENT QUESTIONS

3.6.1 Answer the following true/false

- 1) Senescence results in ageing.
- 2) Ethylene triggers senescence
- 3) Cytokinins delays senescence
- 4) Abscission is not the final phase of senescence

3.6.2 Multiple choice questions

1- Yellowing and shedding of leaves during autumn is an example of	эf
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(a) Overall (b) Top

(c) Deciduous (d) Progressive

2- Which of the following increases during senescence?

(a) Chlorophyll(b) Protein(c) Photosynthesis(d) Respiration

3- The factors which influences senescence most are

(a) Hormones (b) Light/dark

(c) Water (d) Temperature

4- Which of the following hormone is growth retardant?

(a) Auxin (b) Gibberellins

(c) Cytokinins (d) Ethylene

- 5- When does a cell become senescent?
- (a) After three years
- (b) Always after DNA damage
- (c) When the telomeres have become too short
- (d) When the telomeres have become too long

- 6- What does cellular senescence cause?
- (a) Tumor suppression
- (b) Tumor growth

(c) Tissue repair

(d) All of the above

7- Why does cellular senescence prevent tumor growth in young organisms?

- (a) Because it kills cancer cells.
- (b) Because it prevents cancer cells from dividing and spreading.
- (c) Because it promotes cell division.
- (d) Because DNA is damaged.

Answer Key: 3.6.1. 1- True, 2- True, 3- True, 4- False

Answer Key: 3.6.2. 1-(c), 2-(d), 3-(a), 4-(d), 5-(c), 6-(d), 7-(b)

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3.9 TERMINAL QUESTIONS

- 1-Define senescence. Describe various types of senescence. What is the significance of senescence in plant life?
- 2- Give a brief account of physiological and biochemical changes during leaf senescence. Describe the effect of cytokinins on these changes.
- 3-Describe various factors effecting senescence.
- 4- Write short notes on the following:
 - (a) Nutritional theory of senescence
 - (b) Death hormone of senescence
 - (c) Factors retarding senescence
 - (d) Role of light in senescence

CELL BIOLOGY OF PLANTS	MSCBOT-506

BLOCK-2 EXTRANUCLEAR ORGANELLES

UNIT-4 CELL WALL

Contents

- 4.1-Objectives
- 4.2-Introduction
- 4.3-Structure and function of Cell wall
- 4.4-Structure and function of Cell membranes
- 4.5-Summary
- 4.6-Glossary
- 4.7-Self Assessment Question
- 4.8-References
- 4.9-Suggested Readings
- 4.10-Terminal Questions

4.1 OBJECTIVES

After reading this unit students will be able-

- To study about structure of Cell wall.
- To have preliminary idea about the composition of Cell wall.
- Role and major functions of Cell wall.
- To understand about the function and components of the cell membrane.
- Differences between Cell membrane & Cell wall.

4.2 INTRODUCTION

Cell is described as fundamental unit and "building blocks" of an organism. It is functionally and structurally working unit of life. An English natural philosopher 'Robert Hooke' in the year 1665 identified and coined the term *Cell*. It was first seen in cork cells and named (simply as a "wall") by Hooke. According to its functions and compositions, the cells vary in its shape and size. Its thickness varies in different types of cells from 0.1 µm to 10 µm. There are various types of cells which can be differentiated on the basis of presence or absence of few cell organelles.

Cells of plant, fungi, bacteria and many protists are surrounded by rigid structure called cell walls, an integral part of the cell. The eukaryotic cell wall (including fungi and plants) are primarily composed of polysaccharides. *Chitin* is the basic structural polysaccharide of fungal cell walls. The plant cell wall is mainly composed of *cellulose*, a single most abundant polymer on earth. Along with cellulose (long fibers of carbohydrates), plant cell wall also contains hemicelluloses, pectin, suberin or chitin and lignin.

The cell wall is a structural layer which surrounds various types of cell and present outside the cell membrane. Cell wall can be flexible or tough, and sometimes rigid in its texture. It is the outermost covering of plant, fungal and bacteria cells. It provides strength, protection and structural support to the cell, and can also control the movement of molecules entering or leaving the cell to some extent, acts as a filtering mechanism. All cells have an outer plasma membrane (or cell membranes) which are selectively permeable, but usually only fungi, algae, plants and some archaea and bacteria have cell walls. Depending on the type of organism, the materials that make up the cell wall varies. During the course of evolution, cell wall has evolved many times and develops into various types. Structural variation can be seen among different groups of organisms.

Cell wall is a non-living, extracellular secretion or matrix of the cell which is closely apprised to it. Cell wall act as pressure vessels, preventing the cell membrane from bursting in a hypotonic medium (i.e., resists water pressure) or we can say it prevents over-expansion of the cell when water enters. Cell membranes or cytoplasmic membrane on the other hand organize cells and gave them protection as well. All cells have an outer plasma membrane (Cell membrane) which regulates the movement of any given substance in and out of the cell.

Eukaryotic cells also have internal membranes which enclose their organelles and control the exchange of vital cell components. Both types of membranes have a specialized structure that facilitates their protector function.

4.3 STRUCTURE AND FUNCTION OF CELL WALL

Cell walls evolved independently in many groups, including within the photosynthetic eukaryotes. In these lineages, the cell wall is closely related to the evolution of multicellularity, terrestrialization and vascularization. Cells of many organisms like fungi, bacteria, plants and many protists are enclosed by the inflexible cell wall, which gives protection and strength to the cell and being an integral part of it. The cell wall has mechanical strength, and defines the shape and size of the cell. The cell walls of eukaryotic organisms like fungi and plants are principally composed of polysaccharides. The basic structural polysaccharide in fungal cell wall is *chitin*, which is a long-chain polymer of *N*-acetylglucosamine (derivative of glucose). The cell wall of plant cell is principally composed of *Cellulose*, which is a polysaccharide consisting of a linear chain of β linked D-glucose units.

CELL WALL OF PLANT

As cellulose being the major component of the plant cell wall, forms the long fibers and help plants to remain stiff and upright and gives rigidity to the cell. Plant cell expansion is limited by the rigidity of the cell wall, and auxin brings about cell wall loosening. Cellulose is a linear

polymer of glucose residue, which is joined by β (1-4) linkages and parallel association of several chains with one another to form bundles of cellulose microfibrils, which having a diameter of ~ 30 nm. Other essential carbohydrates include pectin and hemicellulose, with a chief non-carbohydrate constituent of lignin (complex wood, organic polymers), consisting various of aromatic alcohols and it gives structural support to the tissues of plant and few algal cell. Cell wall is formed by a complex network of carbohydrates and lignin along with structural proteins.

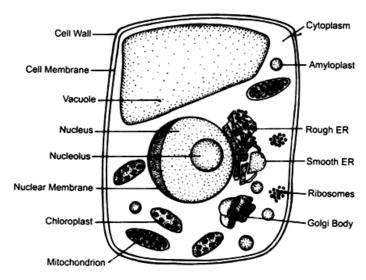


Fig.4.3.a - A plant cell showing different organelles

Growing and dividing plant cells

have primary cell walls, and once the cells get fully grown, they develop into secondary cell walls. Primary walls get less rigid and thinner, to allow cell wall development during growth. A secondary cell wall is formed on the inner side of primary cell wall which is a thick layer and

this layer is generally referred to a plant cell wall. Middle lamella is as well another layer in between plant cells which is pectin rich and helps the plant cells stick together. The cell wall of plant cells help them to maintain the turgor pressure (which give plant cells a typical square shape), and cell wall holds water in efficiently so that the cell does not rupture. Preferably, plants cells must have a lot of water within them (a condition of high turgidity). But a plant cell without a cell wall cause bulging and get rupture, since much water diffuses into it. Plants need to be in hypotonic solution to maintain the turgor pressure and their structural shape, but when turgor pressure is diminished the plant gets start to wilt.

CELL WALL OF ALGAE

Algae are one of the diverse groups in eukaryotes, and having a vast diversity in their cell walls as well. This diverse range of cell walls exhibited in a variety of algal group which is a sign of ecological pressures of present earth habitats and primitive evolutionary origins. Some algae like green algae have cell walls that are alike in structure to those of plants. The algae, such as red algae and brown algae, have cellulose along with other fibrils or polysaccharide.

Diatoms, a foremost group of algae (distinctively microalgae) have cell wall made up of *silica* (hydrated silicon dioxide), called a frustules (or valves), which require less energy to synthesize the organic cell walls. The biogenic silica composing the cell wall is synthesized intracellularly by the polymerization of silicic acid monomers. Potentially, silica frustules are most important saving on the overall cell energy account and probably an explanation for higher growth rates in diatoms. Cell walls of algae have polysaccharides like cellulose (Glucan) or a range of glycoproteins or sometimes both. The important molecules in algal cell walls include mannan, xylan, and alginic acid or alginate.

CELL WALL OF FUNGI

The fungal cell wall is a multi-polymeric structure that balances rigidity and strength to contest internal turgor pressure with enough flexibility for the deposition of new material at the active zones of growth. The fungal cell wall is composed of glycoproteins like mannoproteins, chitins (polysaccharide), and alpha $(\alpha$ -) and beta $(\beta$ -) linked glucans and perform many functions, such as providing cell shape and rigidity, ion exchange, metabolism, and interactions with host defense mechanisms.

Chitin is a glucose derivative which is alike in structure to cellulose and has very tough layers. It is similar to the molecule present in the rigid exoskeletons of organisms like crustaceans, arthropods and insects. The other glucose polymers i.e. **Glucans**, are also found in the fungal cell wall along with lipids and proteins. Hydrophobins (cysteine-rich proteins) are found only in the cell walls of filamentous fungi, which help them adhere to surfaces, give strength to the cells, and help in controlling the movement of water into the cells.

CELL WALL OF BACTERIA AND ARCHAEA

The bacterial cell wall generally contains the polysaccharide peptidoglycan (also known as *murein*), a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the

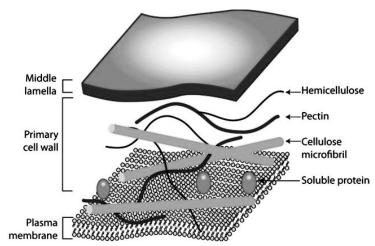
plasma membrane of most bacteria, forming the cell wall. Cell walls provide mechanical strength to bacteria and guard them from interior turgor pressure. Bacterial cells have high concentration of molecules (like proteins) inside themselves as compared to their environment; hence water stops rushing into the cell. It is possible to make Gram-staining due to the differences in the thickness of cell wall. Gram staining is used for the general identification of bacteria. There is a difference between thicknesses of cell wall; bacteria with thin cell walls are gram-negative, while those with thick cell walls are Gram-positive.

Archaea are very much alike to bacteria in many ways. They were initially classified as bacteria, receiving the name Archaebacteria, but this classification is outdated, and barely any archaeal walls have peptidoglycan. There are several different types of cell walls in archaea. Some are composed of pseudopeptidoglycan (also known as *pseudomurein*) which is a major cell wall component of some Archaea that differs from bacterial peptidoglycan in chemical structure. But it resembles the bacterial peptidoglycan in function and physical structure. Some bacteria have polysaccharides, while the others have glycoproteins (proteins which contain oligosaccharide chains, glycans), and some others have surface-layer proteins (known as Slayer), which can also be found in bacteria.

COMPOSITION

Depending on developmental stage and cell type, the compositions of cell walls differ from species to species. The growing (primary) cell wall consists of the polysaccharides such ashemicelluloses, pectin, and cellulose along with the other polymers like cutin or suberin and

lignins, are embedded in or anchored to plant cell walls. Algae acquire cell wall which is made up of polysaccharides such as *carrageenan* (a substance extracted from red and purple seaweeds, consisting of a mixture of polysaccharides), glycoproteins, and *agar*, which are not present in land plants. The cell wall of bacteria is consists of peptidoglycan, whereas, cell walls of



archaea have different compositions. Archaea may have made up of

Fig.4.3.b Structural Composition of cell wall

Pseudopeptidoglycan or Polysaccharides and Glycoprotein S-layers. The cell walls of fungi are composed of chitin, a polymer of N-acetylglucosamine, while diatoms have a cell wall made up of biogenic silica.

In plants primary cell walls typically grow or extended by a mechanism known as **acid-growth** (ability of cell to expand rapidly at low pH), mediated by non-enzymatic proteins called *expansins*. An acidic condition brings about the activation of extracellular proteins which

modify the hydrogen bonds between cellulose and pectin. This process functions to increase cell wall extensibility. Secondary cell walls have a broad range of supplementary compounds which gives permeability to cell wall and modify their mechanical properties. There are many polymers which contribute to the formation plant secondary cell wall and provide rigidity and give strength to the plant. The most important polymers which compose wood (mainly secondary cell walls) and structural components of plant cell wall comprise of cellulose, hemicelluloses, lignin, proteins etc.

1. Cellulose microfibrils (35-50%)

Cellulose is a linear polymer of glucose residue which is insoluble, chemically stable and relatively resistant to chemical and enzymatic attack. The cellulose microfibrils are associated through hemicellulose tied up to form the cellulose-hemicellulose network, which is implanted in the pectin matrix. Cellulose is synthesized by a cell membrane enzyme complex called cellulose synthase. In developing and expanding cells, the newly synthesized cellulose microfibrils are deposited parallel to cortical microtubules underlying the plasma membrane.

2. *Matrix polysaccharides* (20-35%)

Cellulose microfibrils are embedded in a matrix having polysaccharides and proteins. The major polysaccharides of the matrix are synthesized by membrane bound enzymes in the Golgi apparatus and are delivered to the cell wall through exocytosis of tiny vesicles. Matrix materials are hetero-polymers that are conventionally grouped into two families of polysaccharides i.e., hemicelluloses (cross-linking glycans) and pectins (galacturonic acid). Hemicellulose (polyose) is a heterogeneous group of highly branched polysaccharide (xyloglucan, xylan and glucomannan) that are hydrogen-bonded to the surface of cellulose microfibrils. Pectins are gel forming components of the matrix which contain acidic sugar such as galacturonic acid and play an important role in forming connections between plant cells, ion balancing, adjusting pH, establishing cell wall porosity and recognizing foreign molecules to alert the cell to the presence of microbes. Xyloglucan and pectin in grass cell walls are reduced in great quantity and somewhat replaced by another type of hemicellulose known as glucuronarabinoxylan.

3. *Lignin* (10-25%)

Lignin is a highly branched complex phenolic polymer having three phenyl propylene lignin monomers- *coumaryl alcohol*, *coniferyl alcohol*, and *sinapyl alcohol*, also known as *monolignols*. Precursors of lignin are synthesized from phenylalanine and are secreted to the wall. Lignin, which fills the spaces between the components like hemicellulose, cellulose and pectin in the cell wall, especially in vascular and support tissues: xylem tracheids, vessel elements and sclereid cells. It is covalently linked to hemicellulose and therefore, cross-links different plant polysaccharides, conferring mechanical strength to the cell wall and by extension the plant as a whole. It is particularly abundant in compression wood but scarce in tension woods, which are types of reaction wood.

Lignin plays a vital part in conducting water in plant stems, driving out water and strengthening the wall. The polysaccharide components of plant cell walls are highly hydrophilic and thus permeable to water, whereas lignin is more hydrophobic. The cross-linking of polysaccharides by lignin is an obstacle for water absorption to the cell wall. Thus, lignin makes it possible for the plant's vascular tissue to conduct water efficiently.

4. Structural proteins

The other structural components of plant cell wall consist of several classes of structural proteins which constitute about 1-5% of cell wall and are found in most plants cell wall. These proteins are generally classified according to their predominant amino acid composition, such as HRGP (hydroxyproline-rich glycoproteins), GRPs (glycine-rich proteins), PRPs (proline-rich proteins), and AGP (arabinogalactan proteins). Most of these proteins are glycosylated and have hydroxyproline (Hyp) with an exception of glycine-rich protein. Each class of glycoprotein becomes cross-linked in the cell wall, distinct by a feature, well repetitive protein sequence.

Extensin (family of HRGPs), is a key structural protein in the cell walls of higher plant. Cell walls also contain some functional proteins such as expansin, which are responsible for long term acid induced cell wall expansion and can acts by disrupting non-covalent interactions and operates by disrupting H-bonding between cellulose microfibrils and hemicellulose. These proteins are frequently concentrated in dedicated cells and in cell corners. Cell walls of epidermis and periderm cell of the higher plants may have cutin, while casparian strip in the endodermis roots and cork cells of plant bark have suberin. Both are cell wall-associated cutin and suberin is glycerolipid polyesters with a functional role as permeability barriers to the movement of water. The composition of carbohydrate, proteins and other compounds vary from one type of cell to another.

Plant cell walls also include many enzymes- esterases (splits esters into alcohol and acid), peroxidase (catalyzes the oxidation), hydrolases (catalyze the cleavage of a covalent bond using water), and transglycosylases (catalyze the transformation of one glycoside to another), which functions as to cut, trim and cross-link wall polymers. In some plant tissues cell wall also act as storage units for carbohydrates which later on is broken down and used in metabolic and growth of the plant, e.g. endosperm cell walls in seeds of some plant species like cereal grasses, nasturtium, that are rich in glucans and other polysaccharides. It is easily digested by enzymes (like amylase) during seed germination to form simple sugars that promote the growing embryo.

CELL WALL FORMATION

During cytokinesis, middle lamella is constituted first formed by the cell plate, and later the primary cell wall is deposited within the middle lamella. The cell wall is made during cell division when the cell plate is formed between daughter cell nuclei. This plate undergoes chemical and physical changes and then finally converted into the intercellular substance, the middle lamella. A series of vesicles formed by the Golgi apparatus makes cell plate. The vesicles travel along the cytoskeleton and move to the cell equator, where it mobilizes and deposits their contents. These vesicle membranes now turn into the new cell membrane. The Golgi apparatus synthesizes the non-cellulosic polysaccharides, which is composed of about 25–35% of plant cell walls. Golgi vesicles typically have pectic polysaccharides that are used to make the middle lamella.

The protoplast goes on secreting cell wall materials on the middle lamella, and ultimately a soft, delicate and plastic wall is formed, called primary cell wall. This is really the first formed cell wall, which may persist in many cells as the only wall. It consists mainly of cellulose and pectic compounds and may also contain other polysaccharides. In plants, cellulose is synthesized at the plasma membrane by rosette terminal complexes (RTCs) that contain the cellulose synthase enzymes which synthesize the individual cellulose chains. Each RTC floats in the cell's plasma membrane and "spins" a microfibril into the cell wall. Initially, cellulose synthase prepared by the ribosomes (of rough ER) is formed and travel through vesicles to Golgi and finally to cell

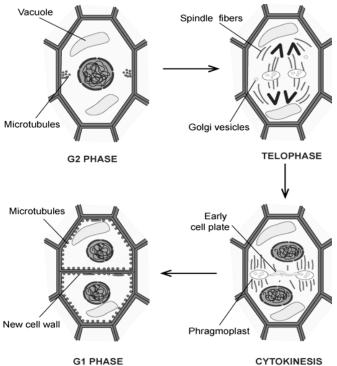


Fig.4.3.c Development of new wall

membrane. Notably, the wall is made from the outside in and when the wall gets thicker, the lumen gets smaller. The actual structure of the cell wall is not clearly defined, and several models exist such as the covalently linked cross model, the tether model, the diffuse layer model and the stratified layer model. However, the primary cell wall can be defined as composed of cellulose microfibrils aligned at all angles and are joined together by H-bonds to give high flexible strength. The cells are retained and share a gelatinous membrane called the middle lamella, which contains salts of pectic acid, calcium and magnesium pectates. Cell to cell interaction are made via plasmodesmata, the inter-connecting channels of cytoplasm which connect to the protoplasts of neighboring cells across the cell wall.

In some plants and cell types, after attaining full size or position in development, a secondary wall is formed between the primary wall and the plasma membrane. The microfibrils are associated generally in the same direction dissimilar to the primary wall, and with each added layer, there is slight change in the direction. Cell interactions are made via pits in the secondary cell wall (a rigid structure) which allow plasmodesmata to connect with adjacent cells.

STRUCTURE OF CELL WALL

A multilayer plant cell wall is primarily made up of three parts i.e., middle lamella (outer layer of cell wall), primary cell wall and secondary cell wall (inner layers of cell wall). Primary cell wall and middle lamella are found in all plant cells but secondary cell wall is not found in all. In some tissues a tertiary cell wall is formed on the inner surface of the secondary cell wall. This layer is very thin and is found in the xylem tracheids of gymnosperms, it is not found in all the cells. It is mainly composed of chemical substances xylan instead of cellulose. Typically, it does not contain any cellulose microfibrils.

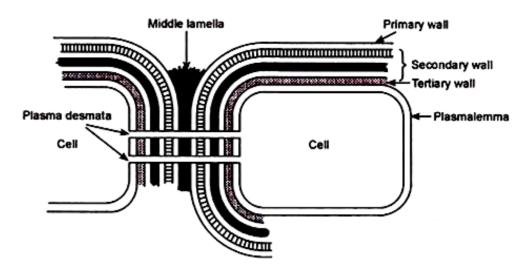


Fig.4.3.d Organization of plant cell wall

1. Middle Lamella

Middle lamella is the first layer which is deposited at the time of cytokinesis. It is the outermost layer of the cell, which forms the boundary between the neighboring cells of the plant and holds them together. It is a thin, amorphous and cementing layer between two adjacent cells, just like brick work of the common wall between two adjacent rooms. Middle lamella contains polysaccharides called **pectins** (also known as pectic polysaccharides), is rich in galacturonic acid and facilitate in cell adhesion by serving the cell walls of neighboring cells to join to one another. It is made up of calcium and magnesium pectates. The softening of ripe fruits is caused by partial solubilization of pectic compounds to produce jelly-like consistency.

In plant biology, pectin consists of a complex set of polysaccharides that are present in most primary cell walls and are particularly abundant in the non-woody parts of terrestrial plants. Pectin is a major component of the middle lamella, where it helps to bind cells together, but is

also found in primary cell walls. Pectin is deposited by exocytosis into the cell wall via vesicles produced in the Golgi. It is an important cell wall polysaccharide that allows primary cell wall extension and plant growth.

2. Primary Cell Wall

Primary cell wall layer is built up between plasma membrane and the middle lamella in a growing plant cells. It is typically a flexible, thin (\sim 0.1 to 3.0 μ m), and extensible layer formed while the cell is rising. The primary cell wall provides the strength and flexibility needed to allow for cell growth. It is found in all plant cells and is formed during the early stage of growth and development. It is principally composed of **cellulose microfibrils** enclosed inside a gel-like matrix of pectin polysaccharides and hemicellulose fibers. The chief components are hemicellulose (53%), cellulose (30%), pectin (5%), and lipid (7%).

The primary cell wall is elastic and undergoes extension with the growth of the cell. In many roots, fleshy stems, fruits and leaves the cells contain only the primary cell wall and middle lamella. Cellulose molecules are nothing but long chains of about 3000 glucose units. In the cell wall, these molecules are arranged parallel to one another to form microfibrils. Each microfibril contains about 2000 cellulose molecules and has a diameter of 100-250 angstrom (Å) and a length of several microns. Cellulose microfibrils are the units of cell wall structural organization. A layer of pectin is also seen in between two layers of microfibrils.

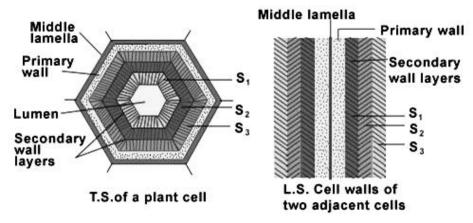


Fig.4.3.e Arrangement of different cell walls in plant cell and microfibrils in the common wall between two adjacent cells

3. Secondary Cell Wall

The secondary cell wall is a thick layer that forms between the plasma membrane and the primary cell wall after the cell membrane is fully developed in some cells. Secondary wall may be absent it is not found in all cell types, irregularly deposited or formed uniformly in the cells, which results in differentiation of cells: parenchyma, collenchyma, sclerenchyma, tracheids and vessels. It consists of mainly cellulose with glycoprotein, lignin and other polysaccharides and provides additional protection to cells and rigidity and strength to the larger plant. At one stage

when the primary cell walls stop growing and dividing, they become thick to form a secondary cell wall, which is an inflexible layer that strengthens and supports the cell.

These walls are constructed of layered sheaths of cellulose microfibrils, wherein the fibers lie close, parallel within each layer and at an angle to the longitudinal axis of the cell. It sometimes consists of three distinct layers - S1, S2 and S3 - where the direction of the cellulose microfibrils differs between the layers. A number of different materials may be deposited in the wall. Along with hemicellulose and cellulose, several secondary cell walls also contain lignin. The incorporation of lignin reduces the flexibility of secondary cell walls, and makes it less permeable to water than the primary cell wall. Lignin provides strength to the cell wall and helps them to conduct water to the vascular tissue cells of the plant. In addition to giving more resistant against cell degradation, the hydrophobic nature of lignin is essential for keeping water within the vascular tissues transported throughout the plant.

The innermost layer of secondary wall is sometimes distinct both chemically as well as in staining properties due to the presence of xylans (a group of hemicelluloses). It is then called **tertiary wall**, e.g., tension wood in gymnosperms. The secondary cell wall has different ratios of constituents compared to the primary wall. For example, secondary wall in wood contains polysaccharides called *xylan*, whereas the primary wall contains the polysaccharide xyloglucan. The cellulose fraction in secondary walls is also higher. Pectins may also be absent from the secondary wall, and unlike primary walls, no structural proteins or enzymes have been identified. Because of the low permeability through the secondary cell wall, cellular transport is carried out through openings in the wall called **pits**.

Primary Wall Secondary Wall Extensible layer Non-extensible layer The dispersed texture of microfibril Parallel texture of microfibrils (to the long axis) Smallest cellulosic unit micelle or microfibril Smallest cellulose unit microfibril or fibril Cellulose (5.2%) Cellulose (50 to 94%) Lipid content (5 to 10%) Normally no lipid Proteins (5%) Proteins deficient Hemicellulose (50%) Hemicellulose (6-25%)

Table- 4.3.A Difference between primary and secondary walls

PLASMODESMATA

Plasmodesmata (singular: plasmodesma) are intercellular organelles found only in plant and algal cells. The animal cell "equivalent" is called the gap junction. Plasmodesmata are narrow channels that act as intercellular cytoplasmic bridges to facilitate communication and transport of materials between adjacent plant cells. The plasmodesmata consist of minute pores or channels lying between individual plant cells, and connect the symplastic space in the plant.

They form a protoplasmic continuum called **symplast**. Cell wall and intercellular spaces of cells form a non-living component of the plant body called **apoplasm**. The actual air space

separating the cells is called the **desmotubule**. The desmotubule possesses a rigid membrane that runs the length of the plasmodesma. Cytoplasm lies between the cell membrane and the desmotubule. Plasmodesmata are extremely specialized channels that allow for intercellular movement of water, various nutrients, and other molecules (including signalling molecules). They are located in narrow areas of cell walls called **primary pit** fields, and they are so dense in these areas that they make up one percent of the entire area of the cell wall. The plasmodesmata separate the outer cell membranes of the plant cells. The entire plasmodesma is covered with the smooth endoplasmic reticulum of the connected cells.

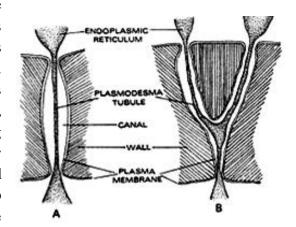


Fig.4.3.f - Structure of plasmodesmata; A- Simple, B- Branched (as between sieve tube cells and companion cells)

Plasmodesmata are formed during the periods of cell division and during plant development. They form when parts of the smooth endoplasmic reticulum from the parent cells become trapped in the newly formed plant cell wall. Primary plasmodesmata are formed while the cell wall and endoplasmic reticulum are formed; secondary plasmodesmata are formed afterward. Secondary plasmodesmata are more complex and may have different functional properties in terms of the size and nature of the molecules able to pass through.

PLANT CELL WALL PITS

They are un-thickened areas in the secondary walls of plant cells. Therefore, they appear as depressions. Generally pits occur in pairs on the wall of two adjacent cells. A pit has a cavity or and a pit membrane which consists of middle lamella and primary wall. There are two types of pits, simple and bordered (Fig. 4.3.g). Simple pit has uniform width of the pit chamber. In bordered pit, the pit chamber is flask-shaped because the secondary wall overarches its mouth. Pit membrane is permeable. It may have minute sub-zmicroscopic pores. Therefore, pits help in rapid translocation between two adjacent cells.

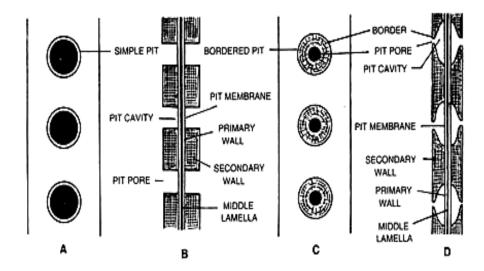


Fig.4.3.g Pits (A) Surface view of simple pits, (B) Simple pits pairs in section, (C) Surface view of bordered pits, and (D) Bordered pits pair in section.

CELL WALL FUNCTIONS

The cell wall gives rigidity and strength to the cells, providing protection from mechanical stress. The important function of a cell wall is to build a framework, which is used to avoid cell expansion. The cell wall helps in forming and holding a fixed shape in multicellular organisms. Cell wall structure allows numerous minute molecules to pass through it, but limits the entry of large molecules into the cell which can be harmful for it. The cell wall can also provide protection from pathogens such as bacteria that are trying to attack the cell. In addition, they allow the formation of a stable osmotic environment to avoid cell bursting (osmotic lysis) and to hold water. Their properties, form and composition change during the cell cycle, and depend on the state of development. The cell wall serves a variety of functions which includes the following-

- **Protection:** It protects the cell against physical damage, the protoplasm against mechanical injury and protects the cell from attack of invading pathogens. Cell wall also helps to stop water loss and acts as a physical obstacle to guard against viruses and other plant pathogens.
- **Support:** It provides structural support and maintains the shape of the cell. The cell wall gives mechanical strength and rigidity, as well as controls the orientation of cell growth. It gives strength to the land plants to withstand gravitational forces.
- Withstand turgor pressure: Turgor pressure is the force exerted on the cell wall, and has a major role in plant growth by promoting cell volume expansion. Its magnitude is determined by osmotic potential and water potential, as the turgor pressure increases, the cytoplasm pushes the cell membrane against the cell wall. It helps a plant to remain rigid and straight,

but it can also cause a cell to burst. The cell wall prevents rupture of the cell membrane by preventing excessive endosmosis in a hypotonic medium.

- **Regulate growth:** It controls the direction of cell growth and regulate cell volume. The cell wall has some enzymatic activity along with metabolism. It also sends a signal to the cell, which enters the cell cycle to divide and grow.
- **Regulate diffusion:** The porous cell wall allows certain substances, including proteins, to pass into the cell, keeping the other substances out. It, therefore, acts as a selective permeable membrane that allows the small molecules to enter and also counteracts the osmotic pressure. Cutin and suberin of the cell wall reduce the loss of water through transpiration.
- **Communication:** Cells communicate via plasmodesmata; these are pathways or pores found in plant cell walls, which allow communication signals and molecules to pass from one cell to another.
- **Storage:** It also functions as a storage component as it stores carbohydrates like starch and glycogen, which are mainly used at growing period of a plant, especially in germination of seeds.

4.4 STRUCTURE AND FUNCTION OF CELL MEMBRANES

The cell membrane (plasma membrane), which defines the boundaries of the cytoplasm or cell organ, also known as the cytoplasmic membrane, it is a membrane found in all cells, which divides the cell's interior from the external environment. It surrounds a cell and consists of a lipid bilayer with embedded proteins, and is sometimes referred to as the *plasmalemma* (given by Mast, 1924) to the outer region of the cell. It is selectively permeable to molecules and ions, meaning that it allows only a few molecules to pass through it, and controls the motion of substances. It performs various cellular processes like cell signalling where it transmit signals to other cells via receptors, ion conductivity which controls the exchange of electrically charged ions across the cell membrane, and cell adhesion in that cells which interact and attach to the neighbouring cells. It also provides an add-on surface for various extracellular structures, such as cell wall, including glycocalyx (carbohydrate coating of glycoprotein and glycolipid of oligosaccharide side-chains), and the cytoskeleton (a complex, dynamic intracellular network of protein filaments).

Structure of the Cell Membrane

The cell membrane having structures that assist uptake of required solutes into the cell. It is mainly made of a combination of proteins and lipids. Typically at the plasma membrane, lipids form a barrier and avoid mixing, while proteins facilitate polar substances and charges across the membrane. Depending on the different functions and locations of cell membranes in the body,

lipids can constitute about 20 to 80% of the membrane, and the rest are proteins. Proteins observe and maintain the chemical environment of cells and also help in the transport of membrane molecules, while lipids provide flexibility to the cell membrane. A carbohydrate is also present in plasma membranes and comprises of only 5 to 10% of membrane mass. Carbohydrates bound either to proteins as constituents of glycoproteins or to lipids as constituents of glycolipids. Carbohydrates are especially abundant in the plasma membranes of eukaryotic cells.

Fluid Mosaic Model

The structure of cell membranes has been explained by scientists using the fluid mosaic model. Different models were proposed to explain the structure and composition of plasma membrane. In 1972, *Jonathan Singer* and *Garth Nicolson* proposed fluid-mosaic model, which is now the most accepted model. In this model, membranes are viewed as *quasi-fluid* structures in which proteins are inserted into lipid bilayer. It describes both the *mosaic* arrangement of proteins embedded throughout the lipid bilayer as well as the fluid movement of lipids and proteins equally.

This fluid model reveals the basic structure of a cell membrane as a mosaic of constituents like cholesterol, phospholipids, proteins, and carbohydrates- that give a fluidity to the membrane and specifies that the cell membrane is more gel-like, not a solid. Being made up of a large fraction of phospholipids, cell membrane showing flexibility and they have a liquid character similar to vegetable oil, and because of this, all individual molecules (such as proteins) could pass freely across the its surface. The massive amount of diverse lipids and proteins in the cell membrane, gives it the appearance of a mosaic. Mosaic refers to something that contains many different parts. The plasma membrane is a mosaic of phospholipids, cholesterol molecules, proteins and carbohydrates.

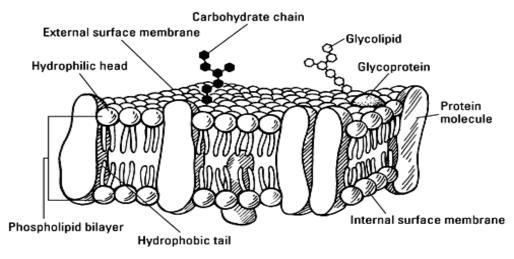


Fig.4.4.a Structure of fluid mosaic model showing lipid bilayer of cell membrane

Compositions of Cell Membrane

(A) Cell Membrane Lipids

1. Phospholipids

Phospholipids are the major components which make up the basic structure of the cell membrane. A single phospholipid molecule has two different ends, i.e. a head and a tail. The head end contains a phosphate group which is **hydrophilic** (likes or attracted to water molecules) and instinctively position to face the aqueous extracellular fluid and the cytosol. The tail end is made up of two strings of hydrogen and carbon atoms called fatty acid chains. These chains are **hydrophobic** (repelled by water), or do not like to mingle with water molecules and faces away from the extracellular fluid and cytosol. This is just like what happens when you pour vegetable

oil in water. This arrangement of phospholipid molecules, let them to instinctively form a double-layered membrane, termed as phospholipids bilayer. This phospholipid bilayer is a semi-permeable which allows only specific molecules to diffuse across the membrane. The cell membrane of nearly all organisms is composed of a lipid bilayer, except for several viruses, archaea, and bacteria, but they have a cell nucleus that is surrounded by a nuclear membrane (composed of phospholipid bilayer). Many subcellular structures also have other membranes around them.

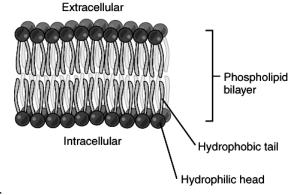


Fig.4.4.b Structure of lipid-bilayer showing the orientation of phospholipid head and tail

2. Sterols and Glycolipids

In addition, the cell membrane contains glycolipids and sterols. The basic structure of sterol is a steroid nucleus, consisting of four fused rings, three with six carbons and one with five. It is planar, and relatively a rigid structure. Cholesterol is one of the essential sterols present in the cell membrane of an animal cell, which controls the fluidity of the cell membrane.

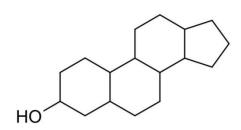


Fig.4.4.c- Sterol chemical structure

Cholesterol molecules are specifically scattered in between phospholipid membrane, and plays a distinctive role in membrane structure by providing flexibility to the cell membrane. It does not allow the close packing of phospholipids and preventing stiffness of the structure. The amount of cholesterol controls the fluidity of the membrane, and helps to maintain permeability allowing the appropriate amount of molecules to enter the cell. Plasma membrane of plant cells lacks cholesterol, but they contain other sterols like stigmasterol, sitosterol. With rare exceptions like mycoplasma, bacterial plasma membrane also lacks cholesterol.

Glycolipids are lipids randomly embedded on the surfaces of cell membrane and have a chain of carbohydrate sugar attached to it by a glycosidic bond. They played an important role in maintaining the stability of cell membranes, and this ensures cellular identification, i. e., it helps the cell recognize other cells. This is essential for establishing connections between cells to connect with each other to form tissues and it is very crucial for the immune response as well as for signal transduction.

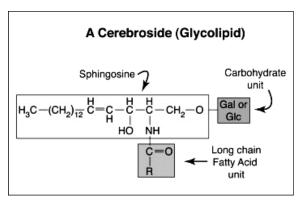


Fig.4.4.d- A cerebroside (Glycolipid)

Glycolipids contain carbohydrates that are covalently attached to the lipids. It covers a variety of compounds, such as glycoglycerolipids, glycophosphospholipids, glycosphingolipids (e.g. Cerebroside, Gangliosides), glycosylated sterols, etc. These can be derived from glycerol or sphingosine. The simplest glycolipid called cerebroside, contains a single sugar residue, either glucose or galactose. Gangliosides are more complex glycolipids, containing a branched chain of as many as seven sugar residues. The glycolipids are found exclusively in the outer leaflet of the plasma membrane, with its carbohydrate portions exposed on the cell surface.

(B) Membrane Proteins

The cell membranes also have a variety of proteins which make up around half of its content. Many of the proteins are transmembrane that surrounds the membrane and attach on both sides. Some proteins act as receptors, which bind to signal molecules, while other proteins function as ion channels allowing the specific ions to enter or exit the cell.

Cell membranes are divided into two main types of associated proteins, namely, peripheral and integral membrane proteins, which are based on the interactions between membrane and protein. **Peripheral** membrane proteins (also called *extrinsic* proteins) are externally attached to the membrane as they are not inserted into the bilayer, but are attached to the membrane with the help of interactions with other proteins. It is secreted by the cell to form a extracellular matrix, which plays a role in cell identification, and it also provides a connection site for transmembrane proteins by forming a filamentous network under the membrane.

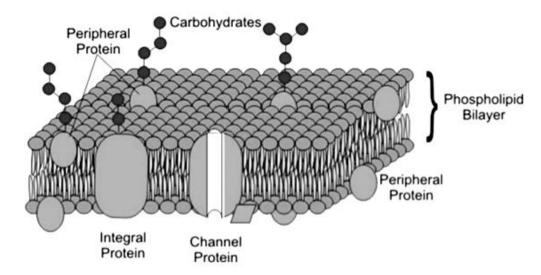


Fig.4.4.e- Cell membrane proteins (peripheral and integral)

The **integral** membrane protein (*intrinsic* protein) is embedded in the lipid bilayer membrane and can be further subdivided into type-I and type-II membrane proteins. Integral proteins expresses carbohydrate residue on outer side of the membrane. Some of them pass through the membrane, may exist as transmembrane proteins, interfacing with both external and internal sides of membrane. They generally make up of several α -helices with hydrophobic side chains of amino acids and interact with the membrane lipids through these chains. They function as transport or receptors proteins and add negative (-) charge to the cell surface. There are several functions performed by cell membrane proteins which are given below:

- 1. The structural protein helps to give shape and support the cell.
- 2. The receptor proteins of the cell membrane help in communication with surrounding cells using signaling molecules such as neurotransmitters and hormones.
- 3. Transport molecules across cell membranes facilitate diffusion such as transportation of globular proteins.
- 4. Glycoproteins have a carbohydrate chain attached to it. They are embedded in the cell membrane and help in cell to cell communications and molecule transport across the membrane.

Functions of Plasma Membrane

The cell membrane covers the cytoplasm of living cells, and physically separates the intracellular components from the external environment, as it acts as inhibitors (or barrier) and as a gatekeeper. It plays an important role in anchoring the cytoskeleton that shapes the cell and also controls the motion of materials entering and exiting the cell. The cell membrane controls the exchange of vital gases between the surrounding and cytoplasm, the oxygen (required for respiration), and carbon dioxide, can easily enter and exit through the cell membrane. It controls the rate of diffusion as some molecules and ions can easily pass through the membrane but some

of them enter the cell via transmembrane proteins that are embedded in the lipid bilayer membrane.

By the process of endocytosis (phagocytosis and pinocytosis), cell membrane brings molecules inside the cell membrane and removes them in the vesicle form by the **exocytosis** process. The cell membrane participates in cell signaling and communication. The cell membrane serves as both an insulator and a diffusion barrier to the movement of ions. Transmembrane proteins, also known as ion transporter or **ion pump** proteins actively push ions across the membrane and establish concentration gradients across the membrane, and ion channels allow ions to move across the membrane down those concentration gradients. Membrane potential (also transmembrane potential or membrane voltage) is the difference in electric potential between the interior and the exterior of a biological cell. Some important functions of the cell membrane are summarized below:

- 1. It maintains the integrity of cells interior by guarding the cell from external environment.
- 2. It acts as selective permeable membrane that allows only specific molecules to diffuse across the membrane and enter the cell, while keeping other substances out.
- 3. It maintains the cell shape and support by anchoring the cytoskeleton. It also plays a role in forming a tissue by connecting with the extracellular matrix and with other cells to group together.
- 4. It regulates cell growth by balancing exocytosis and endocytosis. In endocytosis, lipids and proteins are eliminated, as the material is internalized, whereas in exocytosis vesicles contain proteins and lipids, which fuse along the cell membrane increasing the cell size.
- 5. It maintains the cell potential as it has a small voltage or "potential" across the membrane.
- 6. It serves a vital role in cell signaling and communication, by interacting with the cell membrane of adjacent cells e.g. to form plant and animal tissues.
- 7. Cell membrane lipids provide flexibility to the membrane structure, while membrane proteins probe the chemical environment of the cell and also help to move molecules across the membrane.

Table-4.4.A Difference between cell wall and cell membrane

S.No.	CELL WALL	CELL MEMBRANE
1-	The cell wall is present mainly in plant cell along with bacteria, fungi, and algae. It is absent in an animal cell and protozoa.	The cell membrane is present in almost all types of cells, in plant, animal, bacterial, etc.
2-	The cell wall is the non-living outer most covering of the cell. The cell wall covers the cell membrane and provides a fixed shape.	The cell membrane is a semi-permeable, flexible biological membrane which allows the passage of certain substances through them. It covers the cytoplasm and provides shape to the cell.

3-	The composition of the cell wall varies in prokaryotic and eukaryotic cell. In prokaryotes; peptidoglycans, lipoproteins, and lipopolysaccharides. In eukaryotes, cellulose, pectins (polysaccharides) and lignin. Fungi contain a polysaccharide called chitin.	The cell membrane is a lipid bilayer made up of proteins, carbohydrates (glycoprotein) and lipids (glycolipids, phospholipids and steroids). Three types of proteins are found integral proteins (trans-membrane protein), lipid anchored proteins, peripheral proteins. Other sugars like galactose and sialic acid also found.
4-	It is slightly elastic, controls turgidity, and metabolically inactive.	It is an elastic, thin and delicate structure and metabolically active.
5-	The function of the cell wall is to provide strength and rigidity to the cell. It protects the cell against mechanical forces and external environment. It also helps in retaining the water of the cell, thus a stable osmotic environment is created in the cell	The function of the cell is to separates the components inside the cell from the outside, provides support to the cytoskeleton of the cell, allows the passage of a certain substance, maintains cell potential, helps in communication with other cells, and act as molecular signals.
6-	Cell wall lack receptors.	The cell membrane has receptors to receive signals from external chemicals.

4.5 SUMMARY

Cell wall is a rigid, outer covering and protective layer in plants, fungi, algae and some other organism's cells but are absent in animal cells. The cell membrane is a semipermeable, which allows specific molecule to pass through the membrane and perform the major functions in cells, such as in maintaining shape and structural, support internal structures, and provides protection against external environment. Cell wall composition differs from one species to other depending on their development and type of cell, such as in plants; it is primarily composed of stronger fibers of *cellulose* (polysaccharide polymer).

Cell wall of plants are multilayered having three segments starting from the outermost these layers are recognized as- *middle lamella* (the first formed layer), *primary cell wall* (the cellulose-containing layer), and *secondary cell wall* (having cellulose, lignin, and glycoprotein). Most of the plants cell consists of both middle lamella and primary cell wall, while secondary cell wall occurs in certain cell types (like sclerenchyma and tracheids), and not found in all plant. The middle lamella contains *pectin* (a polysaccharide), which assists neighboring cells to attach to cell walls.

Primary cell wall is composed of mainly cellulose microfibrils (gel matrix of hemicellulose fibers and pectin), which are formed between the plasma membrane and middle

lamella in developing plant cells. It provides the flexibility and strength to the cell, which is required for cell growth. Secondary cell wall, formed between the plasma membrane and primary cell wall in some plant cells. Once the primary cell wall stops developing and dividing or expanding, the cell becomes thicker, and begins to produce a rigid secondary cell wall layer, which provides strength and support to the cell. Secondary cell walls include lignin as well as hemicellulose and cellulose, which help plants to conduct water in vascular tissue cells. Cell walls are important character for plant cells that serve a number of vital functions. It provides structural framework or shape to various cell types, required for tissue formation and plant growth. The cell wall plays an important role in intercellular communication by building connection between neighboring cells also. It acts as the first line of defense responses against potential pathogens.

Cell membranes are very important structures to cells because they function as an obstacle between interior of the cell and its external surrounding. The cell membrane is not only responsible for creating a wall between inside and outside the cell, it also act as a threshold through which select molecules can enter and exit the cell when necessary. The cell membrane is what defines the cell and keeps its components separate from outside cells or organisms. The modern understanding of the plasma membrane is referred to as the fluid mosaic model. The plasma membrane is composed of a bilayer of phospholipids, with its hydrophobic, fatty acid tails in contact with each other. The landscape of the membrane is studded with proteins, some of which span the membrane. Some of these proteins serve to transport materials into or out of the cell. Carbohydrates are attached to some of the proteins and lipids on the outward-facing surface of the membrane. The fluid nature of the membrane owes itself to the configuration of the fatty acid tails, the presence of sterol embedded in the membrane, and the mosaic nature of the proteins and protein-carbohydrate complexes, which are not firmly fixed in place. Plasma membranes enclose the borders of cells, but rather than being a static bag, they are dynamic and constantly in flux.

4.6 GLOSSARY

Apoplasm: Non-protoplasmic component of a plant, including the cell walls and intercellular material

Carrageenan: A substance extracted from red and purple seaweeds, consisting of a mixture of polysaccharides, which is used as a thickening or emulsifying agent in food products.

Cell Membrane: The semipermeable membrane enclosing the cytoplasm of a cell, enclosing its contents.

Cell Wall: An outer covering of a rigid structure external to the plasma membrane which protects the plant cell and gives it a shape. In plants it contains cellulose and lignin; in fungi it contains chitin; and in bacteria it contains peptidoglycans.

Cellulose: A polysaccharide, which is composed of glucose monomers and is the main constituent of the cell walls of plants. It is used in the manufacture of numerous products, including paper, textiles, pharmaceuticals, and insulation.

- **Centrioles:** A small set of microtubules arranged in a specific way. It is a cylindrical organelle occurring in flagellated or ciliated cells, where it acts as a precursor to the basal body of each flagellum or cilium.
- **Chitin:** A nitrogen-containing polysaccharide that is a tough, protective, semitransparent substance and is the principal component of arthropod exoskeletons and the cell walls of certain fungi.
- **Chloroplasts:** A plastid containing chlorophyll and other pigments, occurring in plants and algae that carry out photosynthesis.
- Cytokinesis: The division of the cytoplasm of a cell following the division of the nucleus.
- **Cytoplasm:** Cell substance (gel-like) between the cell membrane and the nucleus, containing the cytosol, organelles, cytoskeleton, and various particles.
- Cytoskeleton: The cytoskeleton is a network of fibers throughout the cytoplasm.
- **Desmotubule:** A tube of appressed (flattened) endoplasmic reticulum that runs between two adjacent cells.
- **Endocytosis:** A process of cellular ingestion by which the plasma membrane folds inward to bring substances into the cell.
- **Endosmosis:** The inward flow of a fluid through a permeable membrane toward a fluid of greater concentration.
- **Exocytosis:** A process of cellular secretion or excretion in which substances contained in vesicles are discharged from the cell by fusion of the vesicular membrane with the outer cell membrane.
- **Glycans:** Any of a group of sugar molecules that are freestanding as oligosaccharides or polysaccharides or are combined in conjugates, as in glycoproteins.
- **Glycocalyx:** The outer layer usually made up of bound polysaccharides on the cell surface and superficial layer of unbound proteoglycans and glycoproteins.
- **Glycoprotein:** Any of a group of conjugated proteins having a carbohydrate as the non-protein component.
- **Hemicellulose:** Any of several branched polysaccharides that are composed of a variety of different monosaccharides and form a matrix with cellulose and lignin or pectin in plant cell walls.
- **Hydrophobins:** A group of small cysteine-rich proteins that are expressed only by filamentous fungi. They are known for their ability to form a hydrophobic (water-repellent) coating on the surface of an object.
- **Lignin:** A complex polymer, the chief non-carbohydrate constituent of wood, which binds to cellulose fibers and hardens and strengthens the cell walls of plants.
- **Membrane potential:** The difference in electric potential between the interior and the exterior of a biological cell.

Microfibrils: A microtubule or microfilament within the cell; an extremely small, submicroscopic cellular fiber.

- **Middle lamella:** A pectin layer which cements the cell walls of two adjoining plant cells together. It is the first formed layer which is deposited at the time of cytokinesis.
- **Morphogenesis:** Formation of the structure of an organism or part; differentiation and growth of tissues and organs during development.
- **Murein:** Any of several polymers containing sugars and amino acids which help to make up the cell walls of certain bacteria.
- **Mycoplasma:** Any of various extremely small bacteria of the genus Mycoplasma that lack cell walls, are usually non-motile, and are often pathogenic or parasitic in mammals.
- **Pectin:** Any of a group of water-soluble colloidal carbohydrates of high molecular weight found in ripe fruits, such as apples, plums, and grapefruit, and used to jell various foods, drugs, and cosmetics.
- **Peptidoglycan:** Also known as murein is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of most bacteria, forming the cell wall.
- **Pinocytosis:** Introduction of fluids into a cell by invagination of the cell membrane, followed by formation of vesicles within the cell.
- **Plasmalemma:** A plasma membrane which bounds a cell, especially one immediately within the wall of a plant cell.
- **Plasmodesmata:** Any minute strands, pores, or channels of cytoplasm that extend through plant cell walls and connect adjoining cells. It allows communication signals and molecules to pass between individual plant cells.
- **Polysaccharide:** A carbohydrate (e.g. starch, cellulose, or glycogen) whose molecules consist of a number of sugar molecules bonded together.
- **Protoplasts:** A cell of a plant, fungus, bacterium, or archaeon from which the cell wall has been removed, leaving the protoplasm and plasma membrane.
- **Symplast:** The continuous system of protoplasts linked by plasmodesmata and bounded by the cell wall.
- **Xylan:** A group of hemicelluloses that represents third most abundant biopolymer on Earth. It is found in plants, in the secondary cell walls of dicots and all cell walls of grasses.
- **Xyloglucan:** A hemicellulose that occurs in the primary cell wall of all vascular plants; however, all enzymes responsible for xyloglucan metabolism are found in *Charophyceae* algae. In many dicotyledonous plants, it is the most abundant hemicellulose in the primary cell wall.

4.7 SELF ASSESSMENT QUESTIONS

4.7.1 Multiple Choice Questions

- 1. What is the function of the cell wall?
 - (a) Provide support to the cell

	(b) Control what molecules enter and exit the cell		
	(c) Maintain turgor pressure		
	(d) All of the above		
2.	Which of the following organism lacks a cell wall?		
	(a) Fungi	(b) Archaea	
	(c) Bacteria	(d) Animal	
3.	Which of the following is not a component of the cell membrane?		
	(a) Nucleic Acids	(b) Proteins	
	(c) Phospholipids	(d) Sterols	
4.	Hydrophilic part of a phospholipid molecule is?		
	(a) Fatty acid tails	(b) Phosphate group head	
	(c) Neither 'A' nor 'B'	(d) Both 'A' and 'B'	
5.	Which plasma membrane component can be either found on its surface or embedded in the membrane structure?		
	(a) Protein	(b) Cholesterol	
	(c) Carbohydrate	(d) Phospholipid	
6.	The tails of the phospholipids of the plasma membrane are composed ofand are?		
	(a) Phosphate groups; hydrophobic	(b) Fatty acid groups; hydrophilic	
	(c) Phosphate groups; hydrophilic	(d) Fatty acid groups; hydrophobic	
7.	Which of the following groups has cell wall?		
	(a) Bacteria, plant and animals	(b) Bacteria, fungi and plants	
	(c) Bacteria, fungi, plants and animals	(d) Bacteria and plants only	
8.	Plant cell wall is made up of?		
	(a) Cellulose, hemicelluloses and pectin	(b) Cellulose and chitin	
	(c) Cellulose, hemicelluloses and chitin	(d) Cellulose only	
9.	Chitinous cell wall is present in?		
	(a) Plants	(b) Bacteria	
	(c) Protists	(d) Fungi	
10.	Which of the following organelle is involved in cell wall synthesis?		
	(a) Mitochondria	(b) Chloroplast	
	(c) Golgi apparatus	(d) Lysosome	

CELL BIOLOGI OF FLANTS	IVISCEO I -SU
11. Secondary cell wall of plants is?(a) Located outside the primary wall(b) Located inside the plasma membrane(c) Located inside the primary wall(d) Located just beneath middle lamellae	
12. Which of these statements is incorrect regard(a) Primary and secondary walls are present(b) Secondary cell wall consists of 3 concent(c) In certain plants, tertiary cell wall is also(d) Middle lamella is made up of pectin and lamella	in meristematic cells ric layers present which has xylan beside cellulose
13. Which of these is a function of the cell members.(a) Receive signal molecules.(b) Control the amount of certain molecules of the cell members.(c) Control what types of molecules enter and (d) All of the above.	that enters and exits the cell
14. Clathrin coated pits are associated with?(a) Phagocytosis(c) Receptor mediated endocytosis	(b) Pinocytosis(d) Exocytosis
15. The role of carbohydrates in cell membrane?(a) Cell adhesion(b) Cell-cell recognition(c) Assisting transport across cell membrane(d) Cell storage reserve	
6. Fine cytoplasmic connections between neighbouring cells through the cell wall for cell to communication is called?	
(a) Plasmosome(c) Mesosome	(b) Plasmodesmata(d) All of these
17. Lipid bilayer is(a) Hydrophilic(c) Hydrophilic and hydrophobic	(b) Hydrophobic(d) Depends on the surrounding medium
18. Which of the following is the most abundant(a) Sterols(c) Phospholipids	component of the cell membrane? (b) Proteins (d) Carbohydrates

- 19. Who proposed the fluid mosaic model of plasma membrane?
 - (a) Camillo Golgi

(b) Schleiden and Schwann

(c) Singer and Nicolson

(d) Robert Brown

- 20. What is a function of proteins in cell membranes?
 - (a) Cellular transport

(b) Photosynthesis

(c) Cellular respiration

(d) None of the above

4.7.2 Short Answer Type Questions

- 1. What is middle lamella? Give its role in cell wall.
- 2. What is primary wall? Give its composition.
- 3. What is secondary wall? Give its composition.
- 4. What are plasmodesmata? Give their function.
- 5. What are pits? Where are they present?
- 6. Define cell membrane. What is the major component of it?
- 7. What is the role of carbohydrate in cell membrane?
- 8. What is pinocytosis?
- 9. Define phagocytosis
- 10. What is exocytosis?

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4.10 TERMINAL QUESTIONS

- 1. Describe plant cell wall and how it is different from fungi cell wall. Also write about the formation of cell wall.
- 2. Give an account on the structure of cell wall. What is the role of lignin in cell wall?
- 3. Give detail account on structural composition of cell wall. What are the major polymers that make up wood?
- 4. Write short notes on:
 - (a) Plasmodesmata
 - (b) Functions of cell wall
 - (c) Primary and secondary cell wall
 - (d) Cell membrane lipids.
- 5. Describe cell membrane? Where did the fluid mosaic name come from in cell membranes? Why is it advantageous for the cell membrane to be fluid in nature?
- 6. Write in detail about the composition of cell membrane. Also give the various function of cell membrane protein.
- 7. Explain how the structure and properties of phospholipids which help to maintain the structure of cell membranes.
- 8. Give a detail account on functions of cell membrane. How it helps in maintaining the cell potential?

9. Describe proteins are arranged in a plasma membrane and the part they play in transporting substances into and out of cells.

- 10. Differentiate between:
 - (a) Primary cell wall and secondary cell wall
 - (b) Cell wall and cell membrane

UNIT-5 MITOCHONDRIA

Contents

- 5.1 Objectives
- 5.2 Introduction
- 5.3 Mitochondria
 - 5.3.1 Morphology
 - 5.3.2 Structure of Mitochondria
 - 5.3.3 Autonomy of Mitochondria
 - 5.3.4 Evolution of Mitochondria
 - 5.3.5 Chemical Composition
 - 5.3.6 Functions of Mitochondria
- 5.4 Summary
- 5.5 Glossary
- 5.6 Self Assessment Questions
- 5.7 References
- 5.8 Suggested Readings
- 5.9 Terminal Questions

5.1 OBJECTIVES

After reading this unit students will be able to-

- Have brief idea about the meaning and history of mitochondria
- Understand the morphology and chemical composition
- Study the detailed structure of mitochondria
- Know various functions performed by mitochondria in a cell

5.2 INTRODUCTION

The mitochondrion (plural mitochondria) derived from Greek words; *mitos*, thread, and *chondrion*, "granule" or "grain-like", they are the granular or filamentous cell organelles that is present in the cytoplasm of aerobic cells of higher animals, plants and some microorganisms including protozoa, algae and fungi. Mitochondria are found in nearly all eukaryotic cells and occupy a substantial portion of the cytoplasm. They are absent in prokaryotic cells and anaerobic eukaryotes.

Almost all the eukaryotic cell has mitochondria, though they are lost in the later stages of development of cell like in the red blood cells or in elements of phloem sieve tube. Like in other eukaryotic cells, the mitochondria in plants play an important role in the production of ATP via the process of **oxidative phosphorylation**. Mitochondria also play essential roles in other aspects of plant development and performance. It also has various properties which allow the mitochondria to interact with special features of metabolism in plant cell.

Mitochondria are well-defined cytoplasmic organelles of the cell which take part in a variety of cellular metabolic functions. Survival of the cells requires energy to perform different functions. The mitochondria are important as the fact that these organelles supply all the necessary biological energy of the cell, and they obtain this energy by oxidizing the substrates of the Krebs cycle. Energy of the cell is got from the enzymatic oxidation of chemical compounds in the mitochondria. Hence, the mitochondria are referred to as the 'power house' of the cell. Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy.

They contain a 'battery' of enzymes and coenzymes that interact to catalyze cellular energy transformation. The enzymes also produce specific DNA for the cytoplasmic inheritance and ribosomes for protein synthesis. The energy is derived by the breakdown of carbohydrates, amino acids and fatty acids and is used in the formation of energy rich molecules the ATP (often referred to as the energy currency of the cell) by the process of oxidative phosphorylation. 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. Although most of a cell DNA is contained in the cell nucleus, the mitochondrion has its own independent genome that shows substantial similarity to bacterial genomes.

Mitochondrial proteins (proteins transcribed from mitochondrial DNA) vary depending on the tissue and the species. Mitochondria have their own circular DNA and synthesize some of their proteins. Thus, they are said to be "semi-autonomous" organelles. Most of the proteins required by the mitochondria, however, are encoded by the nuclear genes and are imported from the cytosol. The organelle is believed to have originated by the process of endosymbiosis.

The first observations of intracellular structures that probably represented mitochondria were published in the 1840s. **Rudolf Kolliker** (1880) was the first who observed the granules (mitochondria) in striated muscle cell of insects. **Richard Altmann**, (1890) established them as cell organelles and called them "bioblasts". The term "mitochondria" was coined by **Carl Benda** (1898). In the 1920s, a biochemist Warburg found that oxidative reactions takes place in most tissues in small parts of the cell. 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 water lily, *Nymphaea alba* and in 1908, along with Claudius Regaud, suggested that they contain proteins and lipids.

5.3 MITOCHONDRIA

Mitochondria are cell organelles of aerobic eukaryotes which take part in oxidative phosphorylation and Krebs cycle of aerobic respiration. They are called power houses of a cell because they are the major centers of release of energy in the aerobic respiration. Cells of dormant seeds have very few mitochondria. But that of germinating seeds have several mitochondria. In general, green plant cells contain less number of mitochondria as compared to non-green plant and animal cells.

The position of mitochondria in a cell depends upon the requirement of energy and amino acids. unspecialized cells they are randomly distributed throughout the cytoplasm. In absorptive and secretory cells, they lie in peripheral cytoplasm. During nuclear division, more of mitochondria come to lie around the spindle. Mitochondria are more abundant at the bases of cilia or flagella to provide them energy for movements. In muscle fibres they occur in rows in the regions of light contractile bands in between the elements.

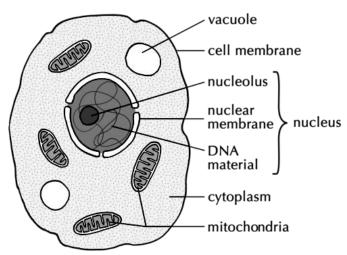


Fig.5.1 Structure of plant cell showing mitochondria

5.3.1 Morphology

Mitochondria are very dynamic organelle and may interact extensively with the other cellular structures. These are bean-shaped organelle that occurs free in the cytoplasm. The shape of mitochondria varies according to the functional stages of the cell. In general, these organelles are filamentous or granular in shape. A long mitochondrion may swell at one end to assume the form of a club or be hollowed out to take the form of a tennis racket. At other times, mitochondria may become vesicular by the appearance of a central clear zone.

The size of mitochondria is also variable; however, in most cells the width is relatively constant (0.5 to 3 μ m), but vary considerably in length (reaching a maximum of 10 μ m) and structure. They are large enough to be resolved in the light microscope (though ultra structure can be studied only under electron microscope) but unless specifically stained, they are generally not visible due to lack of contrast structure. Special stains are used to make them visible, for example Janus Green B. The mitochondria are highly plastic and constantly change their shape and position. In some cells, however, they remain in a fixed position and provide ATP, e.g., the muscle cells and around the flagellum of a sperm.

They are uniformly distributed throughout the cytoplasm, but there may be exceptions. For example, in certain muscle cells, mitochondria are grouped like rings or braces around the I-band of the myofibril or in sperm they are wrapped tightly around the flagellum. The number of mitochondria in a cell varies from one to several and depends upon the type and functional state of the cell (cellular activities). A normal liver cell contains between 1,000 and 1,600 mitochondria but this number diminishes during regeneration and also in cancerous state. The number may be as high as 3, 00,000 in some oocytes, but some algal cells may contain only one mitochondrion.

5.3.2 Structure of Mitochondria

Mitochondria are double membranous organelle; each mitochondrion is bounded by two highly specialized membranes. An outer limiting membrane of about 6 nm thick is surrounding the organelle. Within this membrane, separated by a space of about 6 to 8 nm, is an inner membrane. The outer membrane is smooth and continuous but the inner membrane projects into the mitochondrial cavity and forms complex infoldings called mitochondrial crests. It folds into finger-like projections called **cristae** that project into the matrix. The two chemically and physiologically different membranes in mitochondria create two separate mitochondrial compartments; the **matrix** and the **intermembrane space**. The two membranes have different properties. Because of this double-membrane organization, there are five distinct parts of a mitochondrion.

- i Outer mitochondrial membrane,
- ii Intermembrane space (the space between the outer and inner membranes),
- iii Inner mitochondrial membrane,
- iv Cristae space (formed by infoldings of the inner membrane), and
- v Matrix (space within the inner membrane).

1. The 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 cell membrane (about 1:1 by weight). The outer membrane is a simple phospholipid bilayer, containing 50% lipid and 50% proteins by weight. A mixture of enzymes is involved in degradation of tryptophan and the elongation of fatty acids. The outer mitochondrial membrane is homologous to an outer membrane present in as part of the cell wall of certain bacterial cells.

In contrast to the inner membrane, outer mitochondrial membrane is highly permeable as it contains large numbers of integral membrane proteins or special protein called **porins**. It forms an aqueous channel which allows free diffusion of molecules of 5000 Daltons or less. A major trafficking protein is the pore-forming voltage-dependent anion channel (**VDAC**). The VDAC is the primary transporter of nucleotides, ions and metabolites between the cytosol and the intermembrane

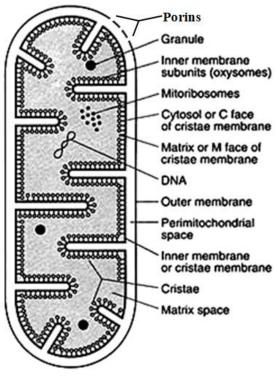


Fig.5.2 A detailed structure of Mitochondria (section view)

The outer membrane also contains enzymes involved in such diverse activities as the elongation of fatty acids, mitochondrial lipid synthesis, oxidation of epinephrine, and the degradation of tryptophan. Outer membrane contains many other proteins such as the receptor proteins that recognize the import signal and also the enzymes that are involved in the division and fusion of the mitochondria. Mitochondria stripped of their outer membrane are called **mitoplasts**.

2. Intermembrane space

space.

The outer chamber or intermembrane is the space between the outer membrane and the inner membrane, usually 60–75 Å wide and filled with watery fluid having a few enzymes. It is also known as **peri-mitochondrial space** and can be increased by placing the isolated mitochondria in a sucrose solution. 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; therefore 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 way

is **cytochrome c**. It has an important role in the primary function of mitochondria, which is oxidative phosphorylation. The intermembrane space contains the enzymes that use the ATP to phosphorylate other nucleotides.

3. Inner Membrane

As mentioned earlier the inner membrane is impermeable to most ions and small charged molecules and thus forms a functional barrier to the free passage of molecules between the cytosol and the matrix. The inner membrane contains more than 100 different polypeptides; it has high protein-to-lipid ratio (80:20). It is rich in unusual phospholipids named **cardiolipins** (which have four fatty acids rather than two), which is characteristic of bacterial membrane.

Presence of phospholipid cardiolipin makes the inner membrane impermeable to ions though oxygen, carbon dioxide and water can move freely through this layer. This property enables the membrane to maintain the proton gradient that drives the oxidative phosphorylation. The inner membrane is thus the principal site of ATP synthesis. Another structural feature that enables ATP synthesis is that the inner membrane is folded into finger-like projections called cristae (singular-crista). The folding of the inner membrane increases the total surface area. The number and shape of the cristae is highly variable. Greater the demand of ATP will leads to more the number of cristae.

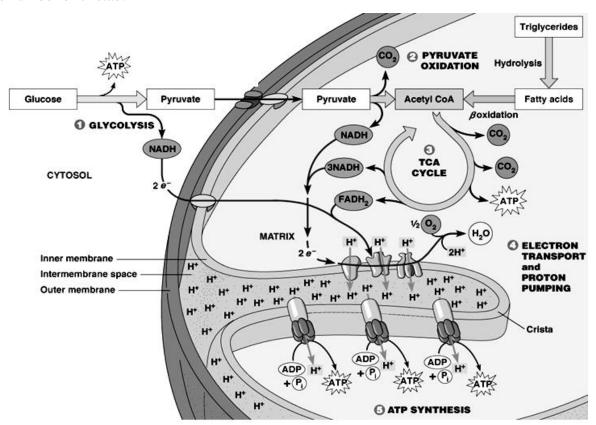


Fig. 5.3. Production of ATP in the inner membrane of Mitochondria

The inner membrane is highly complex, containing complexes of the electron transport chain, the ATP synthase and transport proteins (**Fig. 5.3**). The proteins present in the inner membrane can be divided into three principal types, those that are part of the **electron transport chain** and carry out oxidation reaction, **ATP synthase** (the enzyme that is involved in synthesis of ATP) and **transport proteins** involved in the transport of molecules like fatty acids and pyruvate between the cytosol and mitochondria). The main functions of the inner mitochondrial membrane proteins are:

- Oxidative phosphorylation
- ATP synthesis
- Regulation of protein transport
- Protein import
- Mitochondria fusion and fission

Inner membrane divides the mitochondrion into two chambers; the **outer chamber** contained between the two membranes and in the core of the crests and the **inner chamber** filled with a relatively dense proteinaceous material usually called the mitochondrial matrix.

4. Cristae

In general, the mitochondrial crests are incomplete septa or ridges that do not interrupt the continuity of the inner chamber; thus the matrix is continuous within the mitochondrion. The cristae of animal cells are usually lamellar or plate-like, but in many protozoa and in steroid synthesizing cells including the adrenal cortex and corpus luteum, they occur as regularly packed tubules.

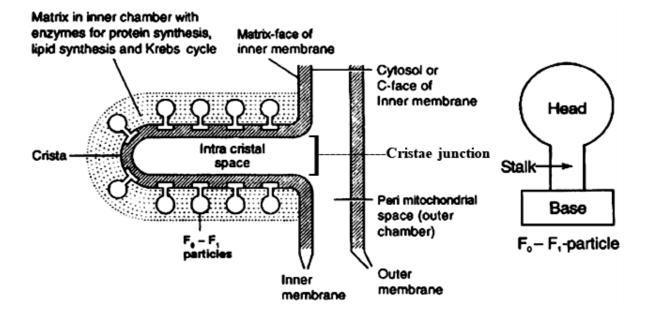


Fig. 5.4 Detailed structure of crista showing elementary particles (F0-F1) and matrix

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 the outer membrane. This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. Cristae and the inner boundary membranes are separated by junctions called **cristae junctions**. The ends of cristae are partially closed by transmembrane protein complexes that bind head to head and link opposing crista membranes in a bottleneck-like fashion.

Cristae membranes folds are studded with small round protein complexes bodies known as **F1 particles** or **oxysomes**, the site of proton-gradient driven ATP synthesis (**Fig.5.3.**). These are not simple random folds but rather invaginations of the inner membrane, which can affect overall chemiosmotic function of mitochondria. The presence of F1 particles on the matrix side (M-face) confers to the inner mitochondrial membrane, a characteristic asymmetry that is of fundamental importance to its function i.e. formation of ATP.

F1 particles: If a mitochondrion is allowed to swell and break in a hypotonic solution and is then immersed in phosphotungstate, the inner membrane in the crest appears covered by particles of 8.5 nm. These particles have a stem linking each with the membrane (Fig.5.3.). These particles are called **elementary particles** (F1 or F0-F1 particles) and are regularly spaced at intervals of 10 nm on the inner surface of these membranes. There may be 104-105 elementary particles per mitochondrion. Actually these particles correspond to a special ATP synthase involved in the coupling of oxidation and phosphorylation.

Electron micrographs revealed that the inner membrane bound F1 particle or the enzyme ATP synthase consists of two major portions; the **F1 head** piece, which consists of five different subunits, alpha (α), beta (β), gamma (γ), delta (δ) and epsilon (ϵ), with the probable ratio of $3\alpha : 3\beta : 1\delta : 1\gamma : 1\epsilon$; and

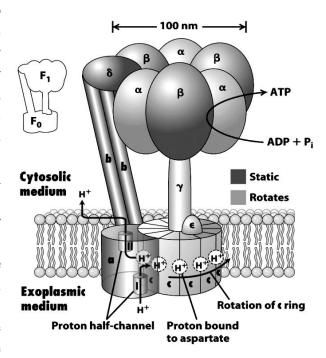


Fig.5.5 Schematic diagram of ATP synthase occurring on the inner mitochondrial membrane

the **F0 base** piece. The F0 base piece remains embedded in the membrane and consists of three different subunits in the ratio of 1a:2b:12c. The b subunit of F0 extends into the head piece and forms the stalk (stem).

5. Matrix

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion. It is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane. The matrix components can easily diffuse to inner membrane or inner chamber because of the folds of the cristae. Matrix forms the core of the mitochondrion which has a pH of about 7.8. The matrix contains a highly concentrated mixture of hundreds of enzymes required for the expression of mitochondrial genes, insoluble inorganic salts, special mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome. In addition, the matrix also contains the enzymes required for the oxidation of pyruvate and fatty acids and for the citric acid cycle.

Cytoplasmic matrix of mitochondria contains the DNA molecules responsible for cellular respiration. Mitochondrial DNA was discovered by **Margit** and **Sylvan Nass** in 1963. One to several double stranded, mainly circular DNA is present in mitochondrial matrix. Mitochondrial DNA is 1% of total DNA of a cell, rich in guanine and cytosine content. The DNA molecules are packaged into nucleoids by proteins. Mitochondrial ribosomes ranging from 55 S to 70 S in nature, For example mammals have 55 S ribosomes. They thus resemble the ribosomes of prokaryotes. Mitochondrial double standard DNA is commonly circular but can be linear. The size of mitochondrial DNA also varies greatly among different species. Mitochondria have their own genetic material, and the machinery to manufacture their own RNAs and proteins. DNA makes the mitochondrion semi-autonomous.

5.3.3 Autonomy of Mitochondria

Mitochondria show a large degree of autonomy or independence in their functioning. Mitochondria are considered as autonomous cell organelle due to the following counts:

- Mitochondria have their own DNA which can replicate independently.
- The mitochondrial DNA produces its own mRNA, tRNA and rRNA.
- The organelle posses their own ribosomes called mitoribosomes.
- Mitochondria synthesize some of their own structural proteins. However, most of the mitochondrial proteins are synthesized under instructions from cell nucleus.
- The organelles synthesize some of the enzymes required for their functioning, e.g. succinate dehydrogenase.
- They show hypertrophy, i.e. internal growth.
- New mitochondria develop by division or binary fission of pre-existing mitochondria.

However, mitochondria are not fully autonomous, both their structure and functioning are partially controlled by nucleus of the cell and availability of materials from cytoplasm. Hence, they are termed as the 'semi-autonomous' cell organelles. Mitochondria are believed to be symbionts in the eukaryotic cells which became associated with them quite early in the evolution.

5.3.4 Evolution of Mitochondria

Mitochondria are thought to have evolved from free-living bacteria that developed into a symbiotic relationship with a prokaryotic cell, providing it energy in return for a safe place to live. It eventually became an organelle, a specialized structure within the cell, the presence of which is used to distinguish eukaryotic cells from prokaryotic cells. This occurred over a long process of millions of years, and now the mitochondria inside the cell cannot live separately from it. The idea that mitochondria evolved this way is called **endosymbiotic theory**.

5.3.5 Chemical Composition

Composition of mitochondrial membrane is just like the plasma membrane, i.e. phospholipids and proteins. Protein is present on two surfaces and a bimolecular layer of lipid in between the two. Outer membrane contains more cholesterol and is higher in phosphatidyl-inositol. The gross chemical composition of the mitochondria varies in different animal and plant cells. In general, mitochondria are found to contain 65 to 70% proteins, 25 to 30% lipids, 0.5% RNA and small amount of DNA.

The lipid content of mitochondria is composed of 90% phospholipids, 5% or less cholesterol and 5% free fatty acids and triglycerides. The inner membrane is rich in the phospholipid, called cardiolipin that makes the membrane impermeable to various ions and small molecules. **Cardiolipin** (tetra-acyl-diphophatidyl-glycerol) is a unique phosphoglyceride, which is important in all systems involving electron transport and essential for membranes involved in coupled (oxidative) phosphorylation.

Mitochondria contain sulphur, iron, copper and some vitamins in traces which are mostly related to enzyme activities. When mitochondria are separated from a cellular environment and ruptured, some of the enzymes associated with matrix are released as soluble proteins while the other enzymes remain firmly bound to the membranes. Mitochondria contain about 70 enzymes and about a dozen coenzymes and numerous cofactors. Soluble enzymes from mitochondria include all enzymes of the tricarboxylic acid cycle (TCA) except some dehydrogenases, those that catalyze β -oxidation of fatty acids and others that catalyze transamination of amino-acids and synthesis of protein. Membrane bound enzymes of mitochondria are the essential for electron transport chain and oxidative phosphorylation. Mitochondria do not contain the enzymes for anaerobic glycolysis which occurs in the groundplasm.

Mitochondrial enzymes

All the three major components of food (carbohydrates, proteins and lipids) degraded in cytoplasm, enter mitochondrial Krebs cycle and undergo oxidation. The electrons emitted during Krebs cycle are transported to **electron transport system** (ETS). Several enzymes and coenzymes are involved in the oxidative phosphorylation and electron transfer mechanism. **Lehninger** (1969) classifies them as follows:

Table-1: Enzyme composition of mitochondrial compartments and membranes are-

S.No.	Part of	Enzymes distribution in mitochondria	
	Mitochondria		
1-	Outer membrane	Monoamine oxidase, fatty acid CoA ligase, NADH-	
		cytochromes-C reductase, kynurenine hydroxylase.	
2-	Outer compartment	Adenylate kinase and nucleoside diphosphokinase	
3-	Inner membrane	Respiratory chain enzymes, ATP synthetase oxidase,	
		succinate dehydrogenase, β-Hydroxybutyrate dehydrogenase,	
		carnitine fatty acid acyl transferase, etc.	
4-	Matrix	Malate and Isocitrate dehydrogenase, fumarase and aconitase	
		Citrate synthetase, α-keto acid dehydrogenases, β-oxidation	
		enzymes, enzymes required for expression of mitochondrial	
		genes.	

Electron Transfers in Oxidative Phosphorylation

- 1- An electron from NADH is first accepted by the protein complex **NADH-Q reductase**, also known as the NADH dehydrogenase complex. The NADH-Q reductase complex accepts an electron from NADH and passes the electron to the next electron carrier, Ubiquinone, which has a higher reduction potential.
- 2- Ubiquinone, abbreviated as Q, is an organic molecule (not a protein) dissolved in the hydrophobic region of the inner membrane of the mitochondrion. It can move freely within the hydrophobic region of the membrane, by diffusion. It has a higher reduction potential than the NADH-Q reductase. Hence, when ubiquinone in the oxidized form comes in contact with the NADH-Q reductase complex (by a random collision), this mobile electron carrier accepts an electron from NADH-Q reductase.
- 3- The reduced form of ubiquinone then continues to move through the hydrophobic region of the membrane by diffusion. When the ubiquinone comes in contact with the next carrier in the electron-transport chain, the electron is transferred to this protein complex, known as **cytochrome reductase**.
- 4-From cytochrome reductase, the electron is picked up by another mobile electron carrier, cytochrome C (not to be confused with the cytochrome c1 subunit of cytochrome reductase). Cytochrome C is a small protein containing one heme group. When the oxidized form of cytochrome C contacts the cytochrome reductase complex by a random collision, its heme group can accept an electron from the heme group of the cytochrome c1 subunit (in cytochrome reductase).

Cytochrome C then carries this electron until the carrier collides with the final protein carrier in the electron-transport chain, cytochrome oxidase. Like NADH-Q reductase, cytochrome reductase acts as both an electron carrier and a proton pump. As the electron is spontaneously transferred from one group to another in the protein complex, free energy is released. This free energy is used to pump protons from the matrix, across the inner mitochondrial membrane (through cytochrome reductase), and into the intermembrane space. Hence, the proton gradient is increased further.

5-Cytochrome oxidase is the best understood of all the electron-carrier proteins involved in oxidative phosphorylation. Cytochrome oxidase accepts an electron from cytochrome C, and passes it to O_2 , the final electron acceptor in this chain. As with the other proteins, the free energy from the spontaneous oxidation-reduction reaction is used to pump more protons into the intermembrane space, increasing the proton gradient even further.

5.3.6 Functions of Mitochondria

Mitochondria supply nearly all the required biological energy. Only mitochondria are fully capable of converting pyruvic acid to carbon dioxide and water. They are the respiratory centers of the cell. The ATP molecules produced as a result of cellular respiration accumulate in the mitochondria. The enzymes for Krebs cycle are found in the matrix of the mitochondrion. The enzymes for electron transport are located in the inner membrane of mitochondrion. A set of enzymes that control synthesis of lecithin and phosphatidyl ethanolamine from fatty acids, glycerol and nitrogenous bases is present in most mitochondria. Mitochondrial genes control some hereditary characters, for example male sterility in maize. An organism generally receives mitochondria from its mother (maternal inheritance).

S.No.	Mitochondrial Part	Functions in Cellular Respiration
1-	Outer Mitochondrial Membrane	Separates the contents of the mitochondrion from the rest of the cell.
2-	Matrix	Internal cytosol-like area that contains the enzymes for the link reaction & Krebs Cycle.
3-	Cristae	Tubular regions surrounded by membranes increasing surface area for oxidative phosphorylation.
4-	Inner mitochondrial membrane	Contains the carriers for the ETC & ATP.
5-	Space between Inner and Outer membrane	Reservoir for hydrogen ions (protons), the high concentration of hydrogen ions is necessary for chemiosmosis.

Table-2: Functions of mitochondrial parts in cellular respiration

Mitochondria are miniature biochemical factories where food stuffs or respiratory substrates are completely oxidized to carbon dioxide and water. The energy liberated in the process is initially

stored in the form of reduced coenzymes and reduced prosthetic groups. The latter soon undergo oxidation and form energy rich ATP molecule, comes out of mitochondria and helps perform various energy requiring processes of the cell like muscle contraction, nerve impulse conduction, biosynthesis, membrane transport, cell division, movement, etc. Because of the formation of ATP, the mitochondria are called power houses of the cell. Some of the major functions of mitochondria are-

1-Production of ATP: The most important function of the mitochondria is to produce energy. These charged molecules combine with oxygen and produce ATP molecules. This process is known as oxidative phosphorylation. Mitochondria function as energy-transducing organelle into which the major degradation products of cell metabolism penetrate and are converted into chemical energy (ATP) that is used in various activities of the cell. The processes of energy transformation that occur in mitochondria are based on three coordinated steps:

- **Krebs cycle**, carried out by a series of soluble enzymes present in the matrix, which produce CO₂ by decarboxylation and removes electrons from the metabolites.
- The **respiratory chain** or electron transport system, which captures the pairs of electrons and transfers them through a series of electron carriers, which finally leads by combination with activated oxygen to the formation of H₂O.
- A **phosphorylation system**, tightly coupled with the respiratory chain, which at three points gives rise to ATP molecules.
- **2-Synthesis of fatty acid:** The matrix or inner chamber of the mitochondria has enzymes for the synthesis of fatty acids. Enzymes required for elongation of fatty acids have been reported in the outer mitochondrial chamber. In the mitochondria of all cells, the outer membrane enzymes mediate the movement of free fatty acids into the mitochondrial matrix. In the matrix, each fatty acid molecule is broken down completely by a cycle of reactions, called β -oxidation that trims two carbons from its carboxyl end, generating one molecule of acetyl-CoA in each turn of cycle. This acetyl-CoA is fed into Krebs cycle for further oxidation.
- **3-Synthesis of amino acid:** Synthesis of many amino acids occurs in the mitochondria. The first formed amino acids are glutamic acid and aspartic acid. They are synthesized from α -ketoglutaric acid and oxaloacetic acid respectively. Other amino acids are produced by transformation and transamination or transfer of amino group (-NH₂) from glutamic acid and aspartic acid.
- **4-Synthesis of biochemicals:** Mitochondria provide important intermediates for the synthesis of several biochemicals like chlorophyll, cytochromes, pyrimidines, steroids, alkaloids, etc. Besides the ATP production, mitochondria perform certain biosynthetic or anabolic functions also.
 - It contains DNA and the machinery needed for protein synthesis. Therefore, it can make different proteins of its own. These proteins include subunits of ATP synthase, portions of the reductase and three of the seven subunits in cytochrome oxidase.

• The synthesis of **haeme** (needed for myoglobin, haemoglobin) begins with a mitochondrial reaction catalyzed by the enzyme delta or d-aminolevulinic acid synthetase.

- Likewise, some early steps in the conversion of cholesterol to steroid hormones in the adrenal cortex are also catalyzed by mitochondrial enzymes.
- **5**-Sometimes, the mitochondria assume storage functions, for example the mitochondria of ovum store large amounts of yolk proteins and transform them into yolk platelets. Mitochondria help the cells to maintain proper concentration of calcium ions within the compartments of the cell. Mitochondria may store and release calcium when required.
- **6-**The mitochondria also help in building certain parts of blood and hormones like testosterone and estrogen.
- **7-**The liver cells mitochondria have enzymes that detoxify ammonia.
- **8-**The mitochondria also play important role in the process of apoptosis or programmed cell death. Abnormal death of cells due to the dysfunction of mitochondria can affect the function of organ.

S.No. **Membrane or Compartment Metabolic Functions** Outer membrane Phospholipid synthesis 1-Fatty acid desaturation Fatty acid elongation Electron transport 2-Inner membrane Oxidative phosphorylation Pyruvate import Fatty acyl CoA import Metabolite transport 3-Pyruvate oxidation Matrix TCA cycle β-oxidation of fats DNA replication RNA synthesis (transcription) Protein synthesis (translation)

Table-3: Localization of Metabolic Functions within the Mitochondrion

5.4 SUMMARY

Mitochondria, the granular or filamentous cell organelles present in the cytoplasm of aerobic cells of higher animals, plants and some microorganisms including protozoa, algae and fungi. These are bean-shaped organelles, occur free in the cytoplasm, and can vary greatly in both size and number per cell. However, regardless of their size, number per cell, plant or animal origin,

they have very similar structures. Mitochondria are found in the cytoplasm of nearly all eukaryotic cells and occupy a substantial portion of the cytoplasm. They are generally not visible as they lack contrast.

Mitochondria are membrane bound cell organelles, associated with cellular respiration, the source of energy, being termed as power houses of cell. Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP) used as a source of chemical energy. Energy is derived by the breakdown of carbohydrates, amino acids and fatty acids and is used in the formation of energy rich molecules the ATP (often referred to as the energy currency of the cell) by the process of oxidative phosphorylation. The central set of reactions involved in ATP production is collectively known as citric acid cycle or the Krebs cycle. However, the mitochondrion has many other functions in addition to the production of ATP. They are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining the control of cell cycle and cell growth.

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. Mitochondria have their own circular DNA and synthesize some of their proteins. Mitochondrial proteins (proteins transcribed from mitochondrial DNA) vary depending on the tissue and the species. Most of the proteins required by the mitochondria, however, are encoded by the nuclear genes and are imported from the cytosol. Thus, they are said to be "semi-autonomous" organelles. The organelle is believed to have originated by the process of endosymbiosis.

Unlike other organelles (miniature organs within the cell), they have two membranes, an outer and an inner one. Each membrane has different functions. Mitochondria split into different compartments or regions, each of which carries out distinct roles. Some of the major regions include outer membrane, through which small molecules can pass freely. This outer portion includes proteins called porins which form channels that allow proteins to cross. The outer membrane also hosts a number of enzymes with a wide variety of functions.

Intermembrane space is the area between the inner and outer membranes. Inner membrane holds proteins that have several roles because there are no porins in the inner membrane. It is impermeable to most molecules. Molecules can only cross the inner membrane in special membrane transporters. The inner membrane is where most ATP is created. Cristae, the folds of the inner membrane increase the surface area of the membrane; therefore increase the space available for chemical reactions. Matrix, the space within the inner membrane containing hundreds of enzymes. It is important in the production of ATP. Mitochondrial DNA is housed here.

5.5 GLOSSARY

Amino acid: An organic molecule that is made up of a basic amino group (-NH2), an acidic carboxyl group (-COOH), and an organic R group (or side chain) that is unique to each amino acid.

Anabolic: The phase of metabolism in which simple substances are synthesized into the complex materials of living tissue.

ATP synthase: An enzyme that catalyzes the formation of ATP from the phosphorylation of ADP with inorganic phosphate, using a form of energy, such as the energy from a proton gradient.

ATP: Adenosine triphosphate, coenzyme used as an energy carrier in the cells of all known organisms; the process in which energy is moved throughout the cell.

Biosynthesis: The production of a complex chemical compound from simpler precursors in a living organism, usually involving enzymes to catalyze the reaction and energy source (ATP)

Cardiolipin: An important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. A phospholipid used in combination with phosphatidylcholine and cholesterol as an antigen to diagnose syphilis.

Cristae junction: A tubular structure of relatively uniform size that connects a mitochondrial crista to the mitochondrial inner boundary membrane.

Cristae: One of the inward projections or folds of the inner membrane of a mitochondrion.

Cytochrome c: A type of cytochrome, a protein which carries electrons, that is central to the process of respiration in mitochondria

Cytosol: The liquid component of the cytoplasm surrounding the organelles and other insoluble cytoplasmic structures in an intact cell where a wide variety of cell processes take place.

Electron transport chain: A group of compounds that pass electron from one to another via redox reactions coupled with the transfer of proton across a membrane to create a proton gradient that drives ATP synthesis

Endosymbiosis: A symbiotic association in which one or more organisms live inside another, such as bacteria normally present in human intestines.

Enzyme: Any of numerous compounds that are produced by living organisms and function as biochemical catalysts.

Fatty acid: Any of the group of a long chain of hydrocarbon with a carboxylic acid at the beginning and a methyl end, and derived from the breakdown of fats.

Krebs cycle: Also known as citric acid cycle or TCA(tricarboxylic acid) cycle is a series of enzymatic reactions in aerobic organisms involving oxidative metabolism of acetyl units and producing high-energy phosphate compounds such as ATP, which serve as the main source of cellular energy.

Matrix: The substance occupying the space enclosed by the inner membrane of a mitochondrion. It contains enzymes, filaments of DNA, granules, and inclusions of protein crystals, glycogen and lipid.

Metabolite: A substance that is a product of metabolic action or involved in a metabolic process **Mitochondrion:** A spherical or elongated organelle in the cytoplasm of nearly all eukaryotic cells, containing genetic material and many enzymes important for cell metabolism, including those responsible for the conversion of food to usable energy.

Mitoplasts: A mitochondrion that has been stripped of its outer membrane leaving the inner membrane intact.

Oxidative phosphorylation: The process in cell metabolism by which respiratory enzymes in the mitochondria synthesize ATP from ADP and inorganic phosphate during the oxidation of NADH by molecular oxygen.

Oxysomes: Oxysomes are the structures which are present on the surface of the folded Inner membrane of the mitochondria. They are also called F0-F1 particles or ATP synthase.

Porins: Proteins found in the outer membrane of a double membrane that allow permeability in most small molecules.

Pyruvate: A salt, ester or anion of pyruvic acid. Pyruvate is the end product of glycolysis and may be metabolized to lactate or to acetyl CoA.

Respiration: A process in living organisms involving the production of energy, typically with the intake of oxygen and the release of carbon dioxide from the oxidation of complex organic substances.

Tryptophan: A naturally occurring, one of the essential amino acids; it is a precursor of serotonin. Adequate levels in the diet may mitigate pellagra by compensating for deficiencies of niacin.

VDAC: Voltage-dependent anion channel is a pore located at the outer membrane of the mitochondrion. It allows the entry and exit of numerous ions and metabolites between the cytosol and the mitochondrion.

5.6 SELF ASSESSMENT QUESTIONS

5.6.1 Multiple Choice Questions:

- 1. Where is the mitochondrial matrix located?
- (a) Within the inner membrane
- (b) Between the inner and outer membrane

(c) Inside the mtDNA

- (d) In the intermembrane space
- 2. The term "mitochondria" was coined by?
- (a) Altmann

(b) Kolliker

(c) Robert Hooke

- (d) Benda
- 3. Which is NOT a reason why mitochondria are thought to have evolved from free-living bacteria?
- (a) Mitochondria have their own DNA.
- (b) Mitochondria reproduce through binary fission.
- (c) Mitochondrial DNA is inherited matrilineally.
- (d) The genome is similar to that of bacterial DNA.
- 4. Mitochondria are the store houses or power house of?

(a) Fats	(b) ATP
(c) Glucose	(d) Glycogen
5. The number of mitochondria in a cell depo	ends on?
(a) pH of the cell	(b) Shape of the cell
(c) Both a and b	(d) Functional state of the cell
6. The inner membrane of the mitochondria infolding known as?	a is usually, highly convoluted forming a series of
(a) Thylakoids	(b) Lamellae
(c) Cristae	(d) Grana
7. F 1 particles are also called?	
(a) Electron transport particles	(b) Elementary particles
(c) Cytochromes	(d) Cristae
8. Mitochondria supply most of the necessar	y biological energy by?
(a) Breaking down of sugar	(b) Breaking down of protein
(c) Reducing NADP	(d) Oxidizing substrates of TCA cycle
9. First plant cell in which mitochondria wer	re observed?
(a) Nymphea	(b) Lily
(c) Nelumbium	(d) Nerium
10. Mitochondria are usually found in?	
(a) Reproductive cells	(b) Vegetative cells
(c) Both reproductive and vegetative cells	(d) None of these
11. F0-F1 particles meant for ATP synthesis a	are present on?
(a) Outer mitochondrial membrane attached to	o the cytosolic or C face
(b) Outer mitochondrial membrane attached to	o the Matrix or M face
(c) Inner mitochondrial membrane attached to	the Matrix or M face
(d) Inner mitochondrial membrane attached to	the cytosolic or C face
12. Which is a function of mitochondria?	
(a) Regulating metabolism	(b) Producing ATP
(c) Storing calcium	(d) All of the above
13. In which part of mitochondria, ATP is ger	nerated
(a) Oxysomes	(b) Matrix

- (c) Cristae
- (d) Outer membrane
- 14. In which of the following parts of mitochondria, succinate dehydrogenase enzyme is located
- (a) Outer membrane

(b) Inner membrane

(c) Peri mitochondrial space

- (d) Matrix
- 15. The site of aerobic respiration in eukaryotic cells is______
- (a) Peroxisome

(b) Plastid

(c) Mitochondria

- (d) Cilia
- 16. How do the small molecules pass through the outer membrane of mitochondria?
- (a) ATP pump

(b) Carrier protein

(c) Channels

- (d) Porins
- 17. In mitochondria, cristae act as sites for?
- (a) Protein synthesis

- (b) Phosphorylation of flavoproteins
- (c) Breakdown of macromolecules
- (d) Oxidation reduction reaction
- 18. Mitochondrial inner membrane is rich in phospholipid
- (a) Cardiolipin

(b) Phosphatidylinositol

(c) Phosphatidylserine

- (d) Phosphatidylcholine
- 19. Which of the following is a mobile electron carrier in the mitochondrial electron transport system?
- (a) NADH dehydrogenase

(b) FADH Dehydrogenase

(c) Ubiquinone

- (d) Succinate dehydrogenase
- 20. Which of the following statement regarding the distribution of cholesterol is true in the membrane system of mitochondria?
- (a) Cholesterol is completely absent in mitochondrial membrane
- (b) Cholesterol is absent in the inner membrane
- (c) Cholesterol is present only in the inner membrane
- (d) Cholesterol is present in both inner and outer membrane

5.6.1 Answers key: 1-(a), 2-(d), 3-(c), 4-(b), 5-(d), 6-(c), 7-(b), 8-(d), 9-(a), 10-(c), 11-(c), 12-(d), 13-(a), 14-(b), 15-(c), 16-(d), 17-(d), 18-(a), 19-(c), 20-(b)

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5.9 TERMINAL QUESTIONS

5.9.1 Short Answer Type Questions:

- 1. Why mitochondria called as powerhouse of the cell?
- 2. What is endosymbiosis?
- 3. What is aerobic respiration?
- 4. Define mitoplasts?
- 5. Why mitochondria considered as semiautonomous in nature?
- 6. Define matrix of mitochondria?

- 7. What is porins?
- 8. Define Peri-mitochondrial space?
- 9. What is cardiolipin?
- 10. Write about the evolution of mitochondria.
- 11. What is the cytochrome c?
- 12. Define cristae junction?
- 13. What are oxysomes?
- 14. What is the role of Ubiquinone in electron transfer?
- 15. Define citric acid cycle?

5.9.2 Short Answer Type Questions:

- 1. Write an essay note on mitochondria? Discuss the historical events about the discovery of mitochondria.
- 2. Explain the morphology of mitochondria. Write about the stain used to make them visible.
- 3. Discuss the structure of mitochondrion. Draw a detail diagram of mitochondria showing various parts.
- 4. Give a detailed account on inner membrane and outer membrane of mitochondria.
- 5. How the Krebs cycle and electron transport chain works in mitochondria? What is the role of inner mitochondrial membrane in the production of ATP?
- 6. Write a short note on the followings:
 - (a) Mitochondrial matrix
 - (b) Autonomy of mitochondria
 - (c) Mitochondrial enzymes
 - (d) Cardiolipin and Ubiquinone
- 7. Write an essay note on the chemical composition of mitochondria.
- 8. What are the functions of mitochondrial parts in cellular respiration? Write in detail about the various functions performed by mitochondria.
- 9. What are F1 particles or elementary particle? With the help of diagram explain the role of F1 particles in ATP synthase.
- 10. Discuss the role of electron transport transfer in oxidative phosphorylation.

UNIT-6 CHLOROPLAST AND ENDOPLASMIC RETICULUM

Contents

- 6.1 Objectives
- 6.2 Introduction
- 6.3 Chloroplast
- 6.4 Endoplasmic reticulum
- 6.5 Summary
- 6.6 Glossary
- 6.7 Self assessment questions
- 6.8 References
- 6.7 Suggested readings
- 6.9 Terminal questions

6.1 OBJECTIVES

After reading this unit students will be able-

- to know the Brief idea of chloroplast and endoplasmic reticulum
- To understand structures and evolution of chloroplasts
- to understand the Role of chloroplasts in conversion of light energy
- to know about the Description of endoplasmic reticulum and its structure
- to understand types and various functions of endoplasmic reticulum

6.2 INTRODUCTION

When learning about the living cells, one should have a basic understanding that all eukaryotic cells contain numerous organelles, which are subunits within cells with different and specific function. Both types of cells i.e., plant and animal, have very similar organelles, but there are distinct differences between animal and plant cells. Firstly, the animals lack a cell wall and chloroplast but the plants contain both. Plants form the basis of all life on earth and are known as producers. Plants, especially the green ones prepare their own food by a process called photosynthesis and hence, they are known as autotrophs. A specialized structure known as chloroplast that acts as the site of photosynthesis. It consists of photosynthetic pigments called chlorophyll that are responsible for the food preparation.

Plant cells contain structures known as plastids which are absent in animal and bacterial cells. **Plastids** are double-membrane organelles which are found in the cells of plants and algae. They are responsible for manufacturing and storing of food. These often contain pigments used in photosynthesis and can change the colour of the cell. There are three types of plastids-chromoplast, chloroplast, and leucoplast. Chromoplasts are the colored plastids other than the green ones i.e. chloroplasts which are the green colored plastids. The colorless plastids are known as leucoplasts.

Chloroplasts are the organelles present in plant cells. They are the most important plastids found in plant cells where the photosynthesis occurs. A chloroplast is an organelle unique to plant cells that contains chlorophyll (which is what makes plants green) and is responsible for enabling photosynthesis to occur so that plants can convert sunlight into chemical energy. They absorb sunlight and convert into sugar molecules and also produce free energy like ATP through photosynthesis. Therefore, without chloroplasts, plants could not create energy.

Another organelle in the cell is the **endoplasmic reticulum** (ER). While the function of the nucleus is to act as the cell brain, the **endoplasmic reticulum** functions as a packaging and manufacturing system. It also works closely with the ribosomes, mRNA, tRNA and Golgi apparatus. Structurally, the endoplasmic reticulum is a network of membranes found throughout the cell and connected to the nucleus. The membranes are slightly different from cell to cell and function of cell determines the structure and size of the ER, e.g., some cells, like RBCs (red

blood cells) and prokaryotes, do not have **endoplasmic reticulum**. A large amount of ER is needed for the cells that synthesize and release the proteins. You might look at a cell from the pancreas or liver for good examples of cells with large ER structures.

Several functions are performed by the cells, which include the transport and folding of various proteins, specifically carrying them to the Golgi apparatus. Mostly, the glycoproteins and some other proteins, moves across the ER's membrane. Endoplasmic reticulum is also responsible for marking these proteins which they transport, with a signal sequence. Proteins are headed outside the endoplasmic reticulum, so they are packed into transport vesicles and moved out from the cell via the cytoskeleton. Hence, ER acts as a transportation system of the eukaryotic cell, which contained proteins within it until they are needed to move outside.

6.3 CHLOROPLAST

The plastid (Greek-*plastos* meaning formed, molded – plural plastids) is a membrane-bound organelle found in the cells of plants, algae, and some other eukaryotic organisms. Important chemical compounds used by the cells of autotrophic eukaryotes are manufactured and stored by plastids. They often contain pigments used in photosynthesis, and the types of pigments in a plastid determine the cell colour. Plastids that contain chlorophyll carry out photosynthesis and are called **chloroplasts**.

All plastids are derived from proplastids, which are present in the meristematic regions of the plant. In plants, plastids may differentiate into several forms, depending upon which function they play in the cell. Undifferentiated plastids (proplastids) may develop into any of the following variants:

- 1. **Chloroplasts:** green plastids for photosynthesis; etioplasts are the predecessors of chloroplasts.
- 2. **Chromoplasts:** coloured plastids for pigment synthesis and storage e.g., rhodoplast, a chromoplast containing a red pigment, phycoerythrin, found in red algae.
- 3. **Gerontoplasts:** control the dismantling of the photosynthetic apparatus during plant senescence.
- 4. **Leucoplasts:** colourless plastids for monoterpene synthesis; leucoplasts sometimes differentiate into more specialized plastids:
 - **Amyloplasts** for starch storage and detecting gravity (for geotropism).
 - Elaioplasts- for storing fat.
 - **Proteinoplasts** for storing and modifying protein.
 - **Tannosomes** for synthesizing and producing tannins and polyphenols.

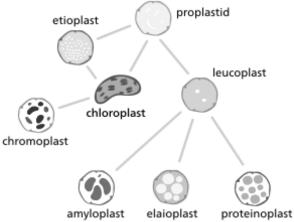


Fig. 6.3.a – Types of plastids

The Chloroplasts

The chloroplast derived from the Greek word 'chloros' meaning *green* and 'plastes' mean *the one who forms*. The chloroplasts are cellular organelles of green plants. There are no chloroplasts in animal or bacterial cells, as it is only present in photosynthetic cells like plant cells and algae. Chloroplasts have a high concentration of chlorophyll, the molecule that captures light energy, and gives many plants and algae a green color. All green plant take part in the process of photosynthesis which converts solar energy into sugars and the byproduct of the process is oxygen, which is consumed by all animals for breathe. Chloroplast number per cell varies from 1 to 100 in Algae (unicellular) as well as plants (like *Arabidopsis* and wheat), respectively. Typically a plant cell might contain about 50 chloroplasts per cell.

Chloroplast is a type of plastid which is characterized by its membranes and great concentration of chlorophyll. The leucoplast and the chromoplast are the other plastid types which contains small amount of chlorophyll and do not carry out photosynthesis. Chloroplast behavior is highly influenced by environmental factors such as light intensity and color. The distribution of chloroplasts is homogeneous in the cytoplasm of the cells and in certain cells chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. They are highly dynamic, circulate and moved around within the plant cells, and occasionally pinch in two to reproduce.

Chloroplasts conduct photosynthesis, where the photosynthetic pigment chlorophyll receives the sunlight. These pigments capture and convert the sunlight energy (photons) and store it in the form of ATP and NADPH (energy-storage molecules) while releasing oxygen from water in autotrophic plant and algal cells. Further, they use the ATP and NADPH to make organic molecules from CO₂ in a process known as Calvin cycle. Chloroplast performs many other functions like amino acid synthesis, fatty acid synthesis, and the immune response in plants.

Chloroplasts are unique organelles and are said to have originated as endosymbiotic bacteria. Like mitochondrion, the chloroplast is thought to have evolved from free-living bacteria. Chloroplasts have its own DNA just like the mitochondria, which are thought to be inherited from its ancestor, a photosynthetic cyanobacteria engulfed by an early eukaryotic cell. Chloroplasts are not made by the plant cell that's why it must be inherited by each daughter cell during cell division. The circular DNA of chloroplast is referred to as **cpDNA** and helps to regulate how the organelle functions.

Chloroplasts Evolution

Chloroplasts were thought to be a part of certain eukaryotic cells in a similar way as mitochondria was incorporated into all eukaryotic cells. They existed as free-living cyanobacteria which had the symbiotic relationship with a cell, where they make energy for the cell in return of a safe place to live, and eventually evolving into a form that could no longer exist separately from the cell. This process of evolution of mitochondria is called the **endosymbiotic theory**.

Evidence which support chloroplasts were evolved from the bacteria is very similar to the evidence that mitochondria evolved from bacteria. Chloroplasts have its own, separate circular DNA, similar to that of a bacterial cell, and inherited maternally. New chloroplasts are formed through binary fission or splitting, which is how bacteria reproduce. These forms of evidence are also found in mitochondria. Chloroplasts are believed to have evolved from cyanobacteria, while mitochondria evolved from aerobic bacteria. Mitochondria cannot photosynthesize but the process of cellular respiration occurs instead. The structure of chloroplasts is similar to that of cyanobacteria; both have double membranes, circular DNA, ribosomes, and thylakoids.

Morphology

Chloroplasts found in higher plants are generally biconvex or planoconvex shaped. In land plants, chloroplasts are generally lens-shaped, and have an average sizes, 3–10 µm in diameter and 1–3 µm thick. The size of the chloroplast varies from species to species and it is constant for a given cell type. They are vesicular and have a colorless center. The chloroplasts, where photosynthesis occurs, are in the mesophyll cells. There are two kinds of mesophyll cells in a typical leaf; the first type is in the palisade parenchyma region (where most photosynthesis is done) and the other region is called the spongy parenchyma region. Chloroplasts can be found in the mesophyll cells and bundle of sheath cells of plant leaves. In different plants, chloroplasts have different shapes, they vary from spheroid, filamentous saucer-shaped, discoid or ovoid shaped e.g., they may appear spherical or ovoid in maize plant. Some chloroplasts are in shape of club, they have a thin middle zone and the ends are filled with chlorophyll. In algae a single huge chloroplast is seen that appears as a network, a spiral band or a stellate plate e.g., they appear as spiral coils in *Spirogyra* (water silk).

Structure of Chloroplasts

The chloroplast is bounded by a double membrane layer and having at least three membrane systems i.e., the outer membrane of chloroplast, inner membrane of chloroplast, and the thylakoid system. Chloroplasts are the product of secondary endosymbiosis and may have additional membranes surrounding these three membrane system. Inside of the chloroplast membranes there is a presence of stroma (semi gel-like fluid) that makes up most volume of chloroplast. The thylakoid system (stacked grana and stroma thylakoids) floats in stroma.

There are two distinct regions present inside the chloroplasts. One is grana while the other is stroma. **Grana** are made up of stacks of disc-shaped structures known as thylakoids. These contain the molecule chlorophyll and are the functional units of chloroplasts. **Stroma** is the matrix which contains grana and is similar to the cytoplasm of cells in which all the organelles are embedded. Stroma also contains various enzymes, DNA, ribosomes, and other substances. Stroma lamellae connect the stacks of thylakoid sacs. There are two types of reactions by which photosynthesis occur, light reaction and dark reaction. Light reaction occurs in grana while dark reaction takes place in the stroma of chloroplasts.

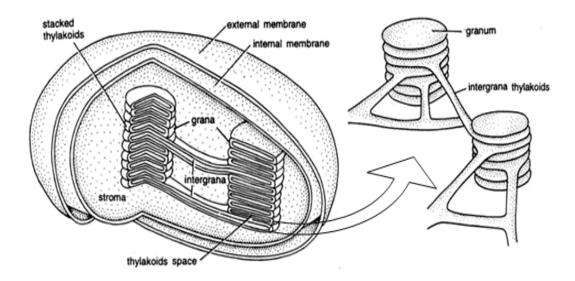


Fig. 6.3.b - A typical structure of a chloroplast

Sometimes the double membrane of chloroplast is often compared to the double membrane of mitochondria. Since the inner membrane of mitochondria is used to run proton pumps and carry out oxidative phosphorylation to generate ATP; therefore, it is not valid to compare these two. Internal thylakoid system is the only chloroplast structure which consider analogous to mitochondria. But even so, in terms of 'in-out', the direction of H⁺ flow is in the opposite direction compared to oxidative phosphorylation in mitochondria. In terms of function, the inner membrane of chloroplast, which regulates metabolite passage and synthesizes materials, has no counterpart in the mitochondria.

Parts of Chloroplasts

- **1. Outer membrane** which forms the external surface of the chloroplast, is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.
- **2. Intermembrane Space** It is usually a thin intermembrane space which is about 10-20 nanometers wide and it is present between the outer membrane and the inner of chloroplast.
- **3. Inner membrane** It forms a border to the stroma and regulates in-out passage of materials of the chloroplast. In addition to regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.
- **4. Stroma -** The space within the inner membrane and outside the thylakoid space is called the **stroma.** It is an alkaline, aqueous fluid which is protein rich and present within the inner membrane of the chloroplast. The thylakoid system, ribosomes, chloroplast DNA, starch granules and many proteins are found be floating around in the stroma.

5. Thylakoid System – It is suspended within the chloroplast stroma. Thylakoid system, a highly dynamic collection of membranous sacks called thylakoid, where chlorophyll is present and light reactions of photosynthesis takes place. In most vascular plants and green algae, the thylakoids are stacked on top of one another, and a stack of thylakoids is called a **Granum** (plural-Grana), but in the chloroplasts of some C4 plant and algae, they are free floating. Thylakoids contain pigments like chlorophylls and carotenoids, which absorb light during the process of photosynthesis.

Light-absorbing pigments are associated with various proteins to form complexes known as **photosystems**. There are two different types of photosystems present in a thylakoid membrane i.e., photosystems I (PSI) and photosystem II (PSII), which have roles in light-dependent reactions. Inside the chloroplast i.e. in stroma, enzymes make the complex organic molecules such as carbohydrates which are used to store energy.

Thylakoids

The word thylakoid is coined from the Greek word *thylakos* meaning "sac" or "pouch". Thus, thylakoid means "sac-like" or "pouch-like" structures. The thylakoid system is suspended in the stroma. Thylakoids are interconnected small sacks, the membranes of which are the site for the light reactions of the photosynthesis. Thus a collection of membranous sacks is called thylakoids. They are arranged in a stacks known as granum and each of it contains about 10-20 thylakoids.

Important protein complexes which carry out light reaction of photosynthesis are embedded in the membranes of the thylakoids. The PSI and the PSII are complexes that harvest light in the present of chlorophyll and carotenoids. They absorb the light energy and use to energize the electrons. Complex molecules present in the membrane of thylakoid energize the electrons to pump hydrogen ions (H⁺) into the lumen of thylakoid which decrease causing acidic pH. **ATP synthase** is a large protein complex that uses the H⁺ concentration gradient in the lumen to generate ATP as energy resulting hydrogen ions flow back to the stroma.

There are two types of thylakoids, the granal and stromal thylakoids. Granal thylakoids are circular discs, which are about 300-600 nm in diameter and are arranged in the grana, whereas stromal thylakoids are present in the form of helicoids sheets spiral around grana and are in contact with the stroma. The inward flattened membranes of the grana thylakoids contains only relatively flat PSII protein complex, which allows them to stack tightly and ultimately forming grana with multiple layers of tightly appressed membrane (granal membrane). This structure increases the stability and surface area for the capturing light. The Photosystem I and ATP synthase protein complexes are present in the stroma side of thylakoids and act as spacers between the sheets of stromal thylakoids.

Light has a significance impact on thylakoid number and the total area covered by the thylakoids. Both are influenced by light exposure in such a way that shaded chloroplasts contain larger and more grana, hence more thylakoid membrane area than chloroplasts exposed to bright light, which have smaller and fewer grana and less thylakoid area. Within a minute exposure or removal of light thylakoid extent can be changed.

Specialized chloroplasts in C₄ plants

The process of photosynthesis where CO₂ is fixed into sugar molecules, chloroplasts use an enzyme called RuBisCO (Ribulose-1, 5-bisphosphate carboxylase/oxygenase) which gets trouble distinguishing between CO₂ and O₂. Therefore at high concentration of oxygen, RuBisCO starts adding oxygen to sugar precursors accidentally which has an end result where CO₂ is released and ATP energy is being wasted, all with no sugar produced. This will creates a problem, since O₂ is produced by the initial light reactions of photosynthesis, causing issues down the line in the Calvin cycle which uses RuBisCO.

A C₄ plant has evolved a way to solve this problem by spatially separating the light reactions and the Calvin cycle. The light reactions which form ATP and NADPH occur in the mesophyll cells, whereas the Calvin cycle, which uses stored energy to make sugar with the help of RuBisCO takes place in the bundle sheath cells of a C₄ plant leaf, respectively. As a result, chloroplasts in C₄ mesophyll cells and bundle sheath cells are specialized for each stage of photosynthesis.

Chloroplasts of mesophyll cells lack the enzyme RuBisCO, and have normal grana and thylakoids, which they use to make energy, as well as oxygen. They store CO₂ in the form of 4-carbon compound, that's why the process is called **C**₄ **photosynthesis**. The 4-carbon compound is then transported to the bundle sheath chloroplasts, where it drops off CO₂ and returns to the mesophyll. Bundle sheath chloroplasts do not carry out the light reactions, preventing oxygen from building up in them and disrupting RuBisCO activity. Therefore, they lack thylakoids organized into grana stacks though bundle they still have free-floating thylakoids in the stroma where they still carry out cyclic electron flow, a light-driven method of synthesizing ATP to power the Calvin cycle without generating oxygen. They have only PSI, the only protein complex needed for cyclic electron flow.

Photosynthesis: Mechanism involved in Thylakoid System

Basically, photosynthesis is the process through which plants (and other primary producers) convert energy from sunlight to chemical energy that is in turn is used to convert water, carbon-dioxide and minerals into organic compounds (glucose). This takes place on the thylakoid membrane and stroma. Here, the photosynthetic pigments are embedded in the thylakoid membrane.

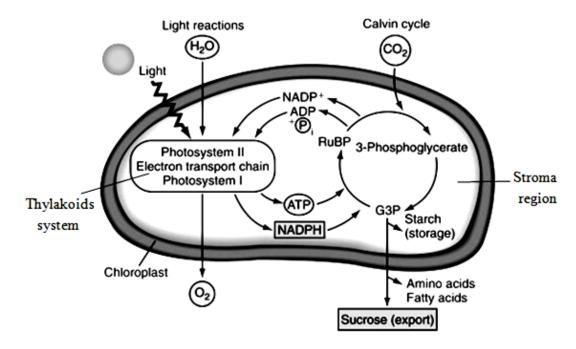


Fig. 6.3.c Process of light and dark reaction (Calvin cycle) during photosynthesis

The process of photosynthesis involves two major stages including the light phase (light reactions) and the dark phase (dark reactions). The light reactions are involved in the production or synthesis of energy generating molecules ATP and NADPH, whereas dark reaction is involved in the production of the organic compound by using these energy molecules. Light reactions occur in the thylakoid membrane while dark reactions take place in the stroma.

Light Reactions

Photosystems I and II (PSII and PSI) are the most important transmembrane protein complexes involved in electron transfer in light reactions. These photosystems contain chlorophyll pigments that absorb light energy. When sunlight is absorbed by the peripheral chlorophyll molecules (in photosystem II), it is transported (through Resonance Energy Transfer) to the reaction center, which is the central pair of chlorophyll molecules.

In the process, the energy causes the electrons to be exited at a higher state and the subsequent loss of electrons from the photosystem II. These electrons then enter into the electron transfer chain where they are required for the synthesis of ATP and NADPH. Every electron lost from photosystem II is replaced by electrons obtained from split water molecules. Every time this photosystem absorbs light photons, it is able to split water molecules to replace lost electrons (of both PSII and PSI).

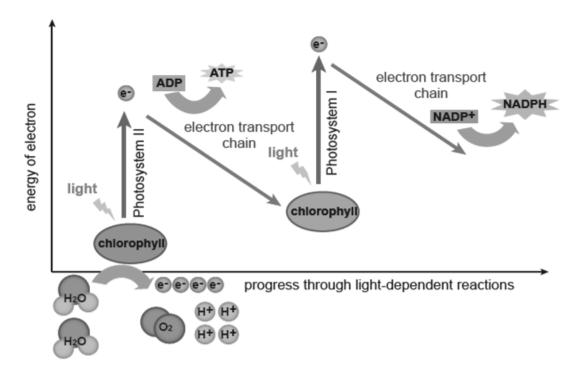


Fig.6.3.d Z-scheme electron transport of the light reactions

Five Protein Complexes involved in Electron Transfer

- (a) **PSII and PSI** These two photosystems are the five protein complexes. Since they contain chlorophyll (pigment that absorbs sunlight energy) they release electrons that are then transported through the electron transfer chain.
- (b) Plastoquinone (PQ) Before the electrons arrive at the cytochrome b6f complex, they are to be carried by carriers to this destination. This role is carried out by plastoquinone. When the electrons are released from the photosystems (PSII), they are accepted by plastoquinone (it also accepts hydrogen ions from the stroma). Electrons from the photosystems are then transported by plastoquinone to the cytochrome b6f complex while the hydrogen ions (protons) are transported to the lumen (thylakoid lumen) which is also important or synthesis and production of ATP.
- (c) Cytochrome b6f complex Electrons carried by plastoquinone are transported to the cytochrome bf complex, which in turn transfers these electrons (as well as protons from stroma) to the plastocyanin. During photosynthesis, this complex enzyme and contributes in the transfer of electrons to PSI, while mediating in the pumping of protons (into thylakoid lumen space) to contribute in the synthesis of ATP.
- (d) Plastocyanin (PC) From the cytochrome b6f complex, electrons are transferred to the plastocyanin, which acts as a carrier that in turn transports these electrons to PSI. As with PSII, photons cause the electrons to become excited and act at higher energy level. Here, the reaction center belonging to PSI moves these electrons to a small protein known as ferredoxin located in the thylakoid membrane (stromal side) where NADP reductase (an

enzyme) helps to synthesize NADPH by moving the electrons in this protein (ferredoxin) to NADP ion.

Ferredoxin acts as a carrier that accepts the electrons and consequently reduced to give up the electrons for synthesis of NADPH. This transport process is also involved in the production of ATP. Here, the protons (hydrogen ion), transported in the electron transfer chain, provides the energy required to produce ATP from the phospholylation of ADP (adenosine diphosphate). ATP synthase enzyme uses this energy to catalyze the ATP from ADP.

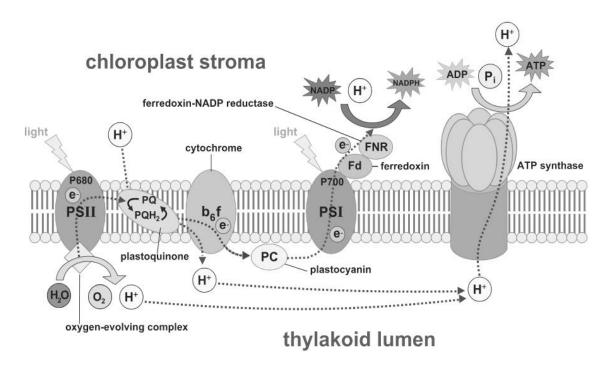


Fig.6.3.e-Electron transport chain and Photophosphorylation during light reaction

The light reaction involves the two important steps which include photolysis and photophosphorylation. Photolysis is the process involved in water splitting (releasing oxygen, hydrogen and electrons), whereas photophosphorylation uses these components to produce ATP energy, which is a chemical energy. Photophosphorylation may occur through non-cyclic as well as cyclic process. Non-cyclic photophosphorylation involves the production of ATP and NADPH, whereas cyclic-photophosphorylation (cyclic electron flow) only gives ATP as the end product.

Dark Reactions

Unlike light dependent reactions, light-independent reactions take place in the stroma of the chloroplast which is filled with fluids. As the name suggests, dark reactions do not require light energy and thus take place in the absence of light as such, they are also referred to as light

independent reactions. By using the Calvin cycle, it becomes easier to understand the light independent reaction.

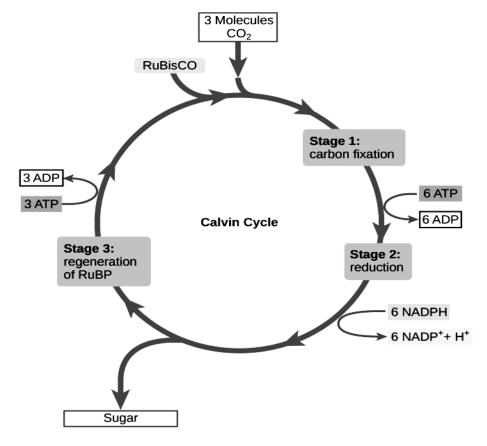


Fig. 6.3.f – The stages involved in Calvin cycle (Dark reaction)

Calvin cycle is named after **Calvin and Benson**, who discovered it and explains the reactions that produce carbohydrate molecules. This process takes place in the absence of light (in the dark) it starts with the plant taking in carbon-dioxide through the stomata (pores on the surface of leaves) which moves to the stroma. The processes that follow are divided into three main phases which includes: fixation, reduction and regeneration.

- (a) **Fixation** During fixation, an enzyme known as RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) in the stroma acts as the catalysts in the reaction between the carbondioxide and a molecule known as ribulose-1,5-bisphosphate (RuBP) which is also present in the stroma of the chloroplast. This reaction results in the production of a six carbons compound that is then converted into two 3-Phosphoglyceric acid, a compound with three carbons.
- **(b) Reduction -** In this phase, energy from the light dependent phase (in form of ATP and NADPH) is used to convert the 3-Phosphoglyceric acid molecule into glyceraldehyde 3-phosphate (G3P) which also contains three carbons. In this phase, reduction occurs with

NADPH donating electrons to produce the G3P, a three-carbon sugar. Here, ATP is also reduced to ADP.

(c) Regeneration - While some of the G3P molecules form such carbohydrates as glucose, the remains of which are recycled to regenerate RuBP to start fixation again.

Function of Chloroplasts

Chloroplasts are essential for the growth and survival of plants and photosynthetic algae. Like solar panels, chloroplasts take light energy and convert it into a usable form that powers activities. The role of chloroplasts in photosynthesis has been illustrated below followed by the roles of different components of chloroplasts:

- 1. The envelope of the chloroplasts is semi-permeable, and it regulates the entry and exit of molecules from the chloroplast absorb. The outer and inner membranes have specialized intermembrane proteins for the transport of large molecules in and out of the chloroplasts. In addition, they are the site for synthesis of certain lipid molecules as well as pigments like carotenoids that are required for light harvesting.
- 2. Chloroplasts are the sites for photosynthesis, which comprises of a set of light-dependent and light-independent reactions to harness the solar energy and convert it into chemical energy (stored in the form of sugar and other organic molecules) that the plant or alga uses as food.
- 3. The light-dependent reactions of photosynthesis occur in the grana and the associated photosystems. This is where photosynthetic pigments like chlorophyll a, chlorophyll b, carotenoids, etc. absorb light energy, which is then used to break down the water molecules, and ultimately give rise to ATP, NADPH and oxygen.
- 4. The stroma of chloroplasts is the site for the dark or light-independent reactions of photosynthesis. The enzymes in the stroma utilize carbon dioxide from the atmosphere, as well as the ATP and NADPH molecules released from grana, to synthesize sugar molecules and starch. This process is also known as carbon dioxide fixation, and occurs through a series of reactions collectively called Calvin cycle.
- 5. Chloroplasts participate in several regulatory functions of the cell as well as in photorespiration (light-dependent oxygen fixation). A part of the reactions which take place in the process of photorespiration, also occur in the stroma of the chloroplasts. Other reactions of photorespiration take place in the mitochondria and peroxisomes. Photorespiration has been speculated to play a protective role during drought stress and exposure to high amounts of radiation.

Some of the important functions of chloroplasts are summaries below:

1- Absorption of light energy and conversion of it into biological energy through the process of photosynthesis which take place in grana of chloroplast.

2- NADPH and ATP are the assimilatory powers of photosynthesis. Evolution of oxygen through the process of photolysis of water, and the production of ATP by photophosphorylation occur in chloroplasts. NADPH is produced by ferredoxin-NADP+ reductase in the last step of the electron chain of the light reactions of photosynthesis. It is used as reducing power for the biosynthetic reactions in the Calvin cycle to assimilate carbon dioxide and help turn the carbon dioxide into glucose.

- 3- Carbon fixation occurs in the stroma of chloroplasts. The CO₂ obtained from the atmosphere combines with the five-carbon compound ribulose 1,5-bisphosphate (RuBP) and water to yield two molecules of the three-carbon compound 3-phosphoglycerate (3-PGA). The breaking of six-carbon atom compound into two molecules of phosphoglyceric acid (PGA) is done by the utilization of assimilatory powers (ATP and NADPH). Finally, the PGA is converted into different sugars and store as starch.
- 4- The most important function of chloroplast is to syntesize food in the form of sugars through the process of photosynthesis, during the process of photosynthesis, sugar and oxygen are made using light energy, water, and carbon dioxide. The chloroplast acts as a cooking place for all the green plants. The heterotrophs, which are unable to prepare their own food, also depend on chloroplasts for food.
- 5- In plants all the cells participate immune response as they lack specialized immune cells. The chloroplast along with the nucleus, cell membrane and ER are the key organelles of pathogen defense.
- 6- Light reaction takes place on the membranes of the thylakoids. Like mitochondria, chloroplast uses the potential energy of the H^+ ions to generate energy in the form of ATP.

6.4 ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is the largest single membrane bound intracellular organelle found in eukaryotic cells that forms an extensive interconnected network of close and flattened membrane sacs or tube-like structures known as cisternae. The enclosed compartment is called the lumen of ER. The membranes of the ER are contiguous with the outer nuclear membrane, even though their compositions can be different. The ER occurs in most of the eukaryotic cells, but is lack in red blood cells and spermatozoa. The ER was observed with light microscope by **Garnier** in 1897, who coined the term "ergastoplasm". With electron microscopy, the lacy membranes of the endoplasmic reticulum were first seen in 1945 by **Keith R. Porter**, Albert Claude, and Ernest F. Fullam. Later, the word "reticulum", which means "network", was applied by Porter in 1953 to describe this fabric of membranes.

The ER performs many essential cellular functions protein transport is one of them. Most of the cellular proteins are imported or integrated into the lumen of the ER. It retains some of these imported proteins for their own functions, whereas some are degraded and others are exported into the secretory pathway for targeting to other compartments within the cell. There are special membrane-embedded proteins present in ER that stabilize its structure and

curvature. This organelle acts as an important regulator of various cell functions because it interacts closely with a number of other organelles, from the plasma membrane and Golgi network, to mitochondria and peroxisomes. It can even influence mitochondrial fission, and change the aerobic status of the cell.

The ER is a dynamic structure that serves many roles in the cell including protein synthesis and transport, protein folding, lipid and steroid synthesis, lipid transfer and signaling to other organelles, compartmentalization of the nucleus, carbohydrate metabolism, detoxification of compounds, and calcium storage. It

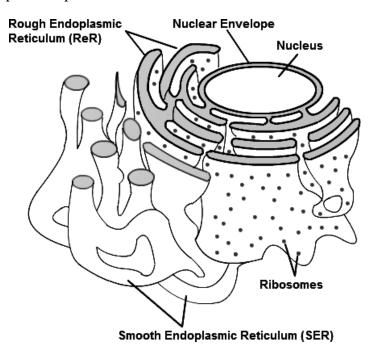


Fig. 6.4.a - Diagram of Endoplasmic reticulum

also has roles in the biogenesis of the Golgi apparatus and helps mitochondria to divide. The multi-functional nature of this organelle requires a myriad of proteins, unique physical structures and coordination with and response to changes in the intracellular environment. The diverse functions of the ER are performed by distinct domains; consisting of tubules, sheets and the nuclear envelope.

Structure of the Endoplasmic Reticulum

Endoplasmic reticulum is a network of tubules, vesicles and cisternae within the cells and they are differentiated into rough and smooth regions which differ in both structure and function. Rough Endoplasmic Reticulum (RER) region contains ribosome attached to the cytoplasmic side of the membrane and are the series of flattened sacs. Smooth Endoplasmic Reticulum (SER) region lacks the attached ribosome and they have tubular network. The double membranes of smooth and rough ER form sacs called **cisternae**. Protein molecules are synthesized and collected in the cisternal space or lumen. When enough proteins have been synthesized, they collect and are pinched off in vesicles. The vesicles often move to the Golgi apparatus for additional protein packaging and distribution.

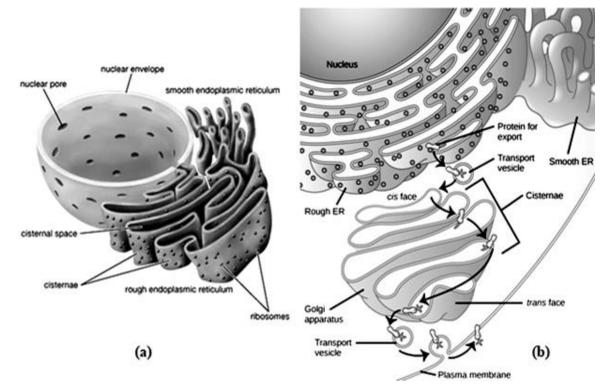


Fig. 6.4.b – (a) Structure of ER and (b) Transport of protein from ER to Golgi

Proteins produced by the ER, flow in transport vesicles to the Golgi. Transport vesicle carries proteins to plasma membrane for secretion. During their subsequent transport, from the ER to the Golgi apparatus and from the Golgi to the cell surface and elsewhere, these proteins pass through a series of compartments, where they are successively modified. Some transport vesicles select cargo molecules and move them to the next compartment in the pathway, while others retrieve escaped proteins and return them to a previous compartment where they normally function. Thus, the pathway from the ER to the cell surface involves many sorting steps, which continually select membrane and soluble luminal proteins for packaging and transport-in vesicles or organelle fragments that bud from the ER and Golgi apparatus.

The membrane of ER are morphologically divided into two structures—cisternae and sheets. Cisternae are tubular structure, and form a 3D polygonal network, whereas ER sheets are membrane-enclosed, 2D flattened sacs structure that extends across the cytoplasm. They are frequently associated with ribosomes and **translocons** (a translocator or translocation channel), necessary for protein translation within RER.

The extensive membrane networks of cisternae are held together by the cytoskeleton. The surface of the RER is studded with the protein manufacturing ribosome, which gives it a rough appearance whereas SER consists of tubules, which are located near the cell periphery. These networks increase the surface area for storing key enzymes and the products of these enzymes. The ER varies, extending from the cell membrane through cytoplasm and forming a continuous connection with nuclear envelope.

Types of Endoplasmic Reticulum

There are two types of ER within each cell, smooth endoplasmic reticulum and rough endoplasmic reticulum. Both rough ER and smooth ER have the same types of membranes but they have different shapes and functions. RER synthesizes proteins, while SER synthesizes lipids and steroids, metabolizes carbohydrates and regulates calcium concentration, drug detoxification, and attachment of receptors on cell membrane proteins.

(a) Smooth Endoplasmic Reticulum (SER)

It is continuous disk-shaped tubular membrane vesicles within the cytoplasm. SER is involved in the synthesis and storage of lipids like phospholipids and cholesterol, which are used in making of new cellular membrane. The smooth endoplasmic reticulum is distinguished from the rough endoplasmic reticulum as its lack ribosomes, which synthesis protein located to the outer surface of the RER. Smooth endoplasmic reticulum is often located near the periphery of the cell and occurs both in animal and in plant cells, especially abundant in mammalian liver and gonad cells.

Functions of SER

Depending on cell type, smooth endoplasmic reticulum performs various functions. Smooth ER is abundant in the liver cell, where it contains enzymes that detoxify various lipid-soluble compounds. It renders drugs, metabolic wastes, and harmful chemicals thereby contributing to detoxification or removal from the body. Drug detoxification often involves enzyme-catalyzed hydroxylation because addition of hydroxyl groups to hydrophobic drugs making them more soluble and easier to excrete from the body.

Another important function performed by SER, through the action of an enzyme present, is the conversion of glycogen to glucose along with glucose-6-phosphatase, catalyzing the final step in glucose production in the liver. Synthesis of steroid hormones from cholesterol in adrenal gland and some other endocrine glands is a key role performed by SER. Smooth ER is important in the creation and storage of lipids and steroids and thus acts as a storage organelle.

It is also involved in storing calcium for the cells, which is essentially needed for contraction of muscle cells, where calcium ions are used for contraction. Sarcoplasmic reticulum (SR), a specialized membrane structure found in skeletal muscle cells, is a variation of the Smooth ER. The sarcoplasmic reticulum helps to regulate calcium ion concentrations, since it acts as storage site for calcium ions by taking up the ions from the cytoplasm. When the muscle cell is triggered by the action of nerve impulse occurred by some kind of stimuli, it releases calcium ions resulting in muscle contraction. When a cell needs to do something immediately, it doesn't make sense to search the environment for extra ions that may or may not be floating around, so it is necessary to have them stored as a pack for easy use. For example, when you are running around and your muscle cells are active, it needs calcium (Ca) ions. The SR can release those ions immediately. When you are resting, they are able to store them for later use. Summary of the functions are:

- Lipid synthesis and Glycogen synthesis.
- Steroid synthesis like cholesterol, progesterone, testosterone, etc.

- Detoxification function.
- Metabolism of carbohydrates.
- Major storage and released calcium ions.

(b) Rough Endoplasmic Reticulum (RER)

The RER is an extensive organelle composed of highly convoluted but flattish sealed sacs, attached to the nuclear envelope that surrounds the nucleus. This direct connection between the perinuclear space and the lumen of the ER allows for the movement of molecules through both membranes. The outer (cytosolic) face of it is studded with ribosomes which gives it a characteristic 'rough' appearance. These ribosomes synthesize proteins that are destined for the lumen of the ER and further moved into the organelle as they are translated. The synthesis of protein starts when mRNA starts moving from the nucleus towards a ribosome embedded on the surface of the Rough ER. These proteins are pinches off as vesicle after being collected in RER. The vesicle is a small membrane bubble, which can move to the cell membrane or the Golgi apparatus. Some of the proteins will be used in the cell and some will be sent out into intercellular space. Ribophorins are transmembrane glycoproteins which are located in the membrane of the rough endoplasmic reticulum, but are absent in the membrane of the smooth endoplasmic reticulum. There are two types of ribophorins, ribophorin I and II, which are present only in eukaryote cells. Both types of ribophorins develop a key role in the binding of ribosomes to the rough endoplasmic reticulum as well as in the co-translational processes that depend on this interaction. Important functions of RER are:

- Providing site for protein synthesis.
- Protein translocation, folding and transport of protein.
- Involvement in membrane synthesis and glycosylation and disulfide bond formation
- Providing precursors of enzymes for the formation of lysosomes in Golgi complex.
- Synthesis of Zymogens of lysosome enzymes by RER.

Functions of Endoplasmic Reticulum

- 1. ER plays an important role in the formation of the skeletal framework. The membranous network of endoplasmic reticulum provides mechanical support. It holds various cell organelles in its position.
- 2. ER membranes possess sites for a number of enzymes and cytochromes to carry out specific reactions.
- 3. It provides the increased surface area for cellular reactions. The larger surface area is useful for rapid synthesis of biochemicals.
- 4. With the help of desmotubules, ER of one cell communicates with ER of adjacent cells.
- 5. It conducts information from outside to inside of cell and between different organelles of the same cell.

6. ER functions as circulating system of the cell for quick transport of materials, such as transportation of proteins and carbohydrates to other organelle including lysosomes, Golgi apparatus, plasma membrane, etc.

- 7. During telophase, part of the nuclear envelope is formed by endoplasmic reticulum, thus helping in the formation of nuclear membrane during cell division.
- 8. It forms vacuoles. ER provides membranes to Golgi bodies for production of vesicles and Golgian vacuoles.
- 9. It provides precursors to Golgi bodies for complexing and elaboration of biochemicals for internal use as well as secretion
- 10. Synthesis of proteins, glycogen, lipids and other steroids such as cholesterol, testosterone progesterone.

6.5 SUMMARY

Chloroplasts are the energy-converting structures found in the cells of plants and eukaryotic algae. Chloroplasts are not found in animal cells and are the most distinguishing feature of a plant cell. Chloroplasts found in higher plants are generally biconvex or planoconvex shaped. The size of the chloroplast varies from species to species and it is constant for a given cell type. Chloroplasts can be found in the mesophyll cells and bundle of sheath cells of plant leaves. Chloroplasts are unique organelles and are said to have originated as endosymbiotic bacteria. Like the mitochondrion, the chloroplast is thought to have evolved from once free-living bacteria. Chloroplast organelles are responsible for photosynthesis, the process by which sunlight is absorbed and converted into fixed chemical energy in the form of simple sugars synthesized from carbon dioxide and water.

There are four layers or zones that define the structure of a chloroplast. The chloroplast is enclosed by two membranes with a narrow intermembrane space, known as the chloroplast envelope. Raw material and products for photosynthesis enter in and pass out through this double membrane, the first layer of the structure. Inside the chloroplast envelope is the second layer, with in which an area is filled with a fluid is called stroma. A series of chemical reactions involving enzymes and the incorporation of carbon dioxide into organic compounds occur in this region. The third layer is a membrane-like structure of thylakoid sacs. Stacked like poker chips, the thylakoid sacs form grana. These grana stacks are connected by membranous structures. Thylakoid sacs contain a green pigment called chlorophyll. In this region the grana, absorb light energy using this pigment. Chlorophyll absorbs light between the red and blue spectrums and reflects green light, making leaves appear green. Once the light energy is absorbed into the final layer, the intra-thylakoid sac, the important process of photosynthesis can begin.

Photosynthesis occurs in two stages i.e., light dependent and light independent reactions. Light reaction occurs in grana while dark reaction takes place in the stroma of chloroplasts. In the first stage, the reactions capture sunlight to form ATP, the energy currency of the cell and NADPH, which carries electrons. The Photosystem I and the Photosystem II are complexes that

harvest light with chlorophyll and carotenoids; it absorbs the light energy and uses it to energize the electrons. The second stage which is also known as the Calvin cycle, where the electrons carried by NADPH convert inorganic carbon dioxide to an organic molecule in the form of a carbohydrate. This process is known as CO₂ fixation. The production of NADPH molecules and oxygen are done as a result of photolysis of water. Carbohydrates and other organic molecules can be stored and used at a later time for energy.

The chloroplast is very important as it is the cooking place for all the green plants and performs various functions. In plants, all the cells participate in plant immune response as they lack specialized immune cells. The chloroplasts with the nucleus, cell membrane and ER are the key organelles of pathogen defense. The most important function of chloroplast is to make food by the process of photosynthesis. During the process of photosynthesis, sugar and oxygen are made using light energy, water, and carbon dioxide.

Endoplasmic reticulum is the interconnected network of flattened sacs or tubes enclosed within the membranes. The membrane is contiguous, joining with the outer membrane of the nuclear membrane. It is present within the cytoplasm, transpiring either with a smooth surface which is called smooth endoplasmic reticulum (SER) or studded with ribosomes called the rough endoplasmic reticulum (RER), which involves the transportation of materials. They occur in almost every type of eukaryotic cell except red blood cells and sperm cells.

Endoplasmic reticulum is morphologically divided into cisternae and sheets, where cisternae are tubular in structure whose membrane networks are held together by the cytoskeleton, on the other hand ER sheets are membrane-enclosed flattened sacs that extend across the cytoplasm. The ER sheets are associated with ribosomes and **translocons** (special protein), that are necessary for protein translation within the RER. The double membranes of smooth and rough ER form sacs called cisternae. Protein molecules are synthesized and collected in the cisternal space or lumen. When enough proteins have been synthesized, they collect and are pinched off in vesicles. The vesicles often move to the Golgi apparatus for additional protein packaging and distribution. Common functions of both Smooth ER and that of the Rough endoplasmic reticulum are as below:

- Formation of the skeletal framework.
- Formation of nuclear membrane during cell division.
- Active transport of cellular materials.
- Metabolic activities due to presence of different enzymes.
- Providing increased surface area for cellular reactions.

6.6 GLOSSARY

ATP synthase: An enzyme that catalyzes the conversion of phosphate and adenosine diphosphate into adenosine triphosphate during oxidative phosphorylation in mitochondria and bacteria or phosphorylation in chloroplasts.

ATP: Adenosine triphosphate molecule is the nucleotide known as the "molecular currency" of intracellular energy transfer. It is present in all cells, where it is used to store and transport needed for biochemical reactions.

- **C4 plants:** A plant that cycles carbon dioxide into four-carbon C₄ sugar compounds to enter into the Calvin cycle. These plants are very efficient in hot and dry climates and make a lot of energy.
- **Calvin cycle:** A set of chemical reactions that take place in chloroplasts during photosynthesis. The cycle is light-independent because it takes place after the energy has been captured from sunlight.
- **Chlorophyll:** Green pigment that gives most plants their color and enables them to carry on the process of photosynthesis.
- **Chloroplasts:** A plastid that contains chlorophyll and is found in the cells of green plants and algae. They absorb light to make sugar in a process called photosynthesis.
- **Chromoplasts:** A coloured plastid other than a chloroplast, typically containing a yellow or orange pigment.
- Cisternae: A flattened membrane disk of the endoplasmic reticulum and Golgi apparatus.
- **Cytochrome:** Any of a class of usually colored proteins that contain a heme group, is electron carriers, and catalyzes oxidation-reduction reactions during cellular respiration.
- **Cytoskeletal:** The internal framework of a eukaryotic cell composed of protein filaments that provide structural support and drive the movement of the cell and its internal components. It is typically divided into three categories (microfilaments, intermediate filaments, and microtubules) based on the diameter and composition of the filaments.
- **Desmotubule:** A desmotubule is an endomembrane derived structure of the plasmodesmata that connects the endoplasmic reticulum of two adjacent plant cells. The desmotubule is not a tubule, but a compact, cylindrical segment of ER that is found within the larger tubule structure of the plasmodesmata pore.
- **ER:** A network of membranous tubules within the cytoplasm, continuous with the nuclear membrane of a eukaryotic cell.
- **Ferredoxin:** An iron-containing protein present in green plants and certain anaerobic bacteria that functions in electron transport reactions in biochemical processes, such as photosynthesis.
- **Glycosylation:** A highly regulated mechanism of secondary protein processing within cells. It plays a critical role in determining protein structure, function and stability.
- **Leucoplasts:** The colorless plastids mostly found in the cells of higher plants and in a number of lower plants.
- **Lumen:** The interior space within a tubular structure, such as within a blood vessel, a duct, or the intestine.
- Mesophyll: The photosynthetic tissue of a leaf, located between the upper and lower epidermis.
- **NADPH:** Nicotinamide adenine dinucleotide phosphate hydrogen, a molecule which plays a crucial role in some of the chemical reactions that makes up the process of photosynthesis.

Parenchyma: A simple plant tissue, composed of thin-walled cells and forming the greater part of leaves, roots, the pulp of fruit, and the pith of stems.

Perinuclear space: The luminal space between the inner and outer nuclear envelope bilayer that separate the nucleus from the cytoplasm.

Phosphorylation: A chemical process in which a phosphate group is added to an organic molecule. In living cells phosphorylation is associated with respiration and extremely important in most cellular processes such as protein synthesis

Photolysis: The decomposition or separation of molecules by the action of light.

Photophosphorylation: The addition of a phosphoryl group (PO₃) to a molecule and is induced by radiant energy in photosynthesis.

Photorespiration: Oxidation of carbohydrates in plants with the release of carbon dioxide during photosynthesis.

Photosynthesis: The process in green plants and certain other organisms by which carbohydrates are synthesized from carbon dioxide and a source of hydrogen (usually water), using light as an energy source. Most forms of photosynthesis release oxygen as a byproduct.

Photosystem: Either of two pigment-containing systems, photosystem I or II, in which the light-dependent chemical reactions of photosynthesis occur in the chloroplasts of plants.

Plastids: Site of manufacturing and storage of important chemical compounds used by the cells of autotrophic eukaryotes. They often contain pigments used in photosynthesis, and the types of pigments in a plastid determine the cell's color.

Plastocyanin: A copper-containing protein that plays a role in the electron transport process associated with photosynthesis. It serves as an electron transfer agent between the cytochrome complex which follows Photosystem II and the entry point to Photosystem I of the non-cyclic electron transfer process.

Plastoquinone: One of the electron acceptors associated with Photosystem II in photosynthesis. It accepts two electrons and is reduced to plastoquinol and as such acts as an electron and energy carrier in the electron transport process.

Proplastids: A cytoplasmic organelle from which a plastid develops.

RER: An organelle found in eukaryotes and a parts of ER to which the ribosomes are attached on the cytoplasmic side, involves biosynthesis of proteins and enzymes.

Ribophorins: Ribosome receptor proteins that interact specifically with the large ribosomal subunit and aid in translocation of newly synthesized proteins across the endoplasmic reticulum.

RuBisCO: Ribulose-1, 5-bisphosphate carboxylase/oxygenase, is an enzyme involved in the first major step of carbon fixation, a process by which atmospheric carbon dioxide is converted by plants and other photosynthetic organisms to energy-rich molecules such as glucose.

Sarcoplasmic reticulum: A membrane-bound specialized type of smooth ER which has a main role in regulating the calcium ion concentration in the cytoplasm of striated muscle cells.

SER: Smooth endoplasmic reticulum, is an organelle found in eukaryotes like plants and animals, which has main functions in the synthesis of lipids and steroid hormones as well as

the detoxification of harmful metabolic byproducts and the storage and metabolism of ca²⁺ within the cell.

Translocons: A complex of proteins associated with the translocation of polypeptides across membranes and commonly known as a translocator or translocation channel.

Vesicles: A membrane-bound structure within a cell in which materials such as enzymes are transported or stored.

6.7 SELF ASSESSMENT QUESTIONS

	6.7.1	Multiple	Choice	Questions:
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1.	In which part of the chloroplast of	does photosynthesis occur?
(a)	Thylakoid	(b) Outer membrane
(c)	Stroma	(d) Intermembrane space

- 2. What are chloroplasts thought to have evolved from?
 (a) Algae (b) Cyanobacteria
 (c) Aerobic bacteria (d) *Rafflesia* plant
- 3. Plastids are absent in?
- (a) Animals and plants(b) Fungi and animals(c) Animals, bacterium and fungi(d) None of these
- 4. All are colorless plastids (leucoplasts) except?
 (a) Rhodoplasts
 (b) Amyloplasts
 (c) Proteinoplasts
 (d) Elaioplasts
- 5. Which of the statements are true regarding chloroplast?
- (a) A double membrane bound organelle
- (b) Chloroplasts are site of photosynthesis
- (c) Chloroplasts are responsible for the synthesis of carbohydrates
- (d) All of these
- 6. The site of light reaction is?
- (a) Stroma (b) Grana
- (c) Thylakoid lumen (d) Outer membrane
- 7. Which of the following pigment is most abundant in green plants?
- (a) Chlorophyll a(b) Chlorophyll b(c) Carotene(d) Xanthophylls

8. Which of the following statements are incorrect regarding light reaction?

- (a) Light reaction is called as Hill reaction
- (b) Light reaction takes place in the grana of the chloroplast
- (c) CO₂ fixation to carbohydrate is the major event in light reaction
- (d) ATP and NADPH are produced in light reaction
- 9. Chloroplast is similar to mitochondria in having a?
- (a) Double layered membrane
- (b) Circular DNA

(c) 70S ribosomes

- (d) All of these
- 10. Photosynthetic Pigments are located on the?
- (a) Inner membrane

(b) Thylakoid membrane

(c) Thylakoid lumen

- (d) Outer membrane
- 11. Which of the following is an accessory pigment?
- (a) Carotenoids

(b) Chlorophyll a

(c) Chlorophyll b

- (d) None of these
- 12. Photolysis (water splitting complex) is present in
- (a) Grana

(b) Stroma

(c) Thylakoid lumen

- (d) Outer membrane
- 13. Which of the following statements are incorrect regarding ER?
- (a) RBC lacks both RER and SER
- (b) Plasma cells has RER only
- (c) The adipose tissue has both SER and RER
- (d) Hepatocytes has both RER and SER
- 14. Which of the statement is correct regarding endoplasmic reticulum?
- (a) Contiguous with the plasma membrane and ideal for protein secretion
- (b) Made of tubular cisternae
- (c) Cytoskeleton dependent
- (d) All of the above
- 15. Which of the following is the function of SER?
- (a) Synthesis and secretion of steroid hormones
- (b) Maintenance and regeneration of the plasma membrane
- (c) Storage and release of Ca2⁺ ions
- (d) All of the above

16. Which of the following organelles has a continuous connection with nuclear membrane?

(a) Golgi apparatus

(b) Lysosome

(c) RER

(d) SER

17. In RER, ribosomes are located on?

(a) Luminal side

(b) Cytoplasmic side

(c) Both a and b

- (d) All through out
- 18. Smooth endoplasmic reticulum (SER) is involved in?
- (a) Protein synthesis
- (b) Phospholipid biosynthesis
- (c) Phospholipid biosynthesis and protein synthesis
- (d) Phospholipid biosynthesis and detoxification reaction
- 19. The functions of RER include?
- (a) Protein synthesis only
- (b) Protein synthesis and phospholipid biosynthesis
- (c) Protein synthesis and post translational modification
- (d) Protein synthesis and detoxification
- 20. Which of the following statements are true regarding endoplasmic reticulum?
- (a) ER provides structural framework to the cell
- (b) ER acts as an intracellular transporting system
- (c) SER is involved in synthesis of lipids
- (d) All of these
- 21. The transport of secretory proteins takes place trough organelles in the order-
- (a) RER > Cis-Golgi > Trans-Golgi > secretory vesicles
- (b) SER > Cis-Golgi > Trans-Golgi > secretory vesicles
- (c) RER > Trans-Golgi > Cis-Golgi > secretory vesicles
- (d) RER > Trans-Golgi > Cis-Golgi > secretory vesicles
- 22. Rough Endoplasmic Reticulum is called 'rough' because?
- (a) Rough texture of the surface
- (b) surface is studded with membrane proteins
- (c) surface is studded with ribosomes
- (d) All of these
- 23. RER is involved in the synthesis of
- (a) Membrane proteins and secretory proteins
- (b) Different proteins of the cell
- (c) Membrane proteins, secretory proteins and lysosomal proteins
- (d) Membrane proteins and secretory proteins and nuclear proteins

- 24. Protein glycosylation occurs in the lumen of?
- (a) Mitochondria (b) Rough endoplasmic reticulum
- (c) Smooth endoplasmic reticulum (d) Lysosomes
- 25. Ribophorins are?
- (a) Luminal proteins on SER (b) Transmembrane glycoprotein on SER
- (c) Luminal proteins on RER (d) Transmembrane glycoprotein on RER

6.7.1 Answers key: 1-(a), 2-(b), 3-(c), 4-(a), 5-(d), 6-(b), 7-(a), 8-(c), 9-(d), 10-(b), 11-(a), 12-(b), 13-(c), 14-(a), 15-(d), 16-(c), 17-(b), 18-(d), 19-(c), 20-(d), 21-(a), 22-(c), 23-(a), 24-(b), 25-(d)

6.7.2 Short Answer Type Questions:

- 1. Calvin cycle or dark reaction occurs in which part of chloroplast?
- 2. What is ribophorins?
- 3. What are Chromoplasts?
- 4. What are the various types of leucoplasts?
- 5. What are thylakoids?
- 6. What is photosystem?
- 7. What is cisternae and cisternal space?
- 8. What are translocons?
- 9. How plastoquinone help in electron transport?
- 10. What are stroma and granum?
- 11. How photolysis takes place?
- 12. What is Rough Endoplasmic Reticulum?
- 13. What is plastocyanin?
- 14. What is the role of cytochrome B6f complex?
- 15. What is Smooth Endoplasmic Reticulum?

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6.10 TERMINAL QUESTIONS

- 1. What are plastids? Write a descriptive essay on chloroplast and its morphology.
- 2. Explain the endosymbiotic theory? How chloroplast evolved from bacteria?
- 3. Write in detailed about the structure of chloroplast with the help on diagram.
- 4. Explain the role of RuBisCO in photosynthesis? Also write about the specialized chloroplasts in C4 plants.
- 5. Differentiate between light and dark reaction. What are the five protein complexes involved in electron transfer?
- 6. Write short notes on the following.
 - (a) Thylakoid
 - (b) Mechanism in the thylakoid system
 - (c) Protein synthesis and folding
 - (d) Sarcoplasmic reticulum
- 7. Describe endoplasmic reticulum with the help of a well labeled diagram.

8. Write about the structure of endoplasmic reticulum. How the protein is transported through ER to Golgi?

- 9. What are the difference between SER and RER? Also write their major functions.
- 10. Give an account on the various functions performed by endoplasmic reticulum.

UNIT-7 RIBOSOME AND GOLGI APPARATUS

Contents

- 7.1 Objective
- 7.2 Introduction
- 7.3 Ribosome
 - 7.3.1 Endosomes
 - 7.3.2 Lysosomes
 - 7.3.3 Peroxisomes
 - 7.3.4 Hydrogenosome
- 7.4 Golgi apparatus
 - 7.4.1 Structure
 - 7.4.2 Genome organization
- 7.5 Summary
- 7.6 Glossary
- 7.7 Self assessment questions
- 7.8 References
- 7.9 Suggested readings
- 7.10 Terminal question

7.1 OBJECTIVES

After reading this unit students will be able:

- To understand the ribosomes, its types and function
- To give structural description and functions of endosome, lysosomes, peroxisomes and hydrogenosomes
- To understand general description of Golgi apparatus and its role in protein transport
- To develop understanding of genome organization and its importance

7.2 INTRODUCTION

Protein is needed for many cell functions such as repairing damage or directing chemical processes. **Ribosomes** are the cell structure that makes protein. Ribosome is a ribonucleoprotein complex found in all living cells that is responsible for the translation of the mRNA into protein. It can be found floating within the cytoplasm or attached to the endoplasmic reticulum. Ribosomes were first observed in the mid-1950s by cell biologist **George Emil Palade**, using an electron microscope, as dense particles or granules. The term "*ribosome*" was proposed by **Richard B. Roberts** during the end of 1950s. The location of the ribosomes in a cell determines what kind of protein it makes. If the ribosomes are floating freely throughout the cell, it will make proteins that will be utilized within the cell itself. When ribosomes are attached to endoplasmic reticulum, it is referred to as rough endoplasmic reticulum (ER) and proteins made on the rough ER are used for usage inside the cell or outside the cell.

The ability of cells to interact with one another and with their environment relies on a large number of proteins that need to be in the right proportion and in the right location. For this reason, eukaryotic cells have evolved complex sorting machineries to ensure the dynamic and strictly controlled flow of proteins between cellular compartments. This machinery exists in both plants and animals, and it involves vesicular structures called endosomes. **Endosomes** are a heterogeneous collection of organelles that function in the sorting and delivery of internalized material from the cell surface and the transport of materials from the Golgi to the lysosome or vacuole. **Lysosomes** are an important cellular organelle that receive and degrade macromolecules from the secretory, endocytic, autophagic and phagocytic membrane-trafficking pathways. The main function of lysosomes is the digestion of internal (non-functional cell organelles) and external (food, bacteria, leukocytes, debris) material. The processed material is either released to the cytoplasm, secreted or stored in lysosomes.

The **peroxisome** is another membrane-bounded vesicle. It contains oxidative enzymes such as catalase, D-amino acid oxidase, and urate oxidase. Like the mitochondria, the peroxisomes are a major site of O_2 utilization. The peroxisome detoxifies foreign chemicals and metabolizes fatty acids. Beta-oxidation, a process in which fatty acids are shortened by two carbons to form acetyl-coenzyme A, occurs in both mitochondria and peroxisomes.

Hydrogenosomes are very interesting organelles found in non-mitochondrial organisms. They display similarities and differences with mitochondria. Some single-celled eukaryotes live in places where oxygen is scarce or non-existent and use hydrogenosomes instead of mitochondria to produce ATP. Mitochondria do not function with little or no oxygen, but hydrogenosomes are able to produce ATP from most of the same fuels without using oxygen. Hydrogenosomes produce ATP by fermentation of acetate and do not usually contain a genome. Dysfunctional hydrogenosomes are removed by an autophagic process and further digested in the lysosomes, which is produced by Golgi.

The Golgi apparatus plays essential roles in intracellular trafficking, protein and lipid modification, and polysaccharide synthesis in eukaryotic cells. It is well known for its unique stacked structure, which is conserved among most eukaryotes. The Golgi apparatus gathers simple molecules and combines them to make molecules that are more complex. It then takes those big molecules, packages them in vesicles and either stores them for later use or sends out of the cell. 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.

In humans, nearly two meters of genomic material must be folded to fit inside each micrometer-scale cell nucleus while remaining accessible for gene transcription, DNA replication and DNA repair. This fact highlights the need for mechanisms governing **genome organization** during any activity and to maintain the physical organization of chromosomes at all times. Application of visual techniques such as 'fluorescence in situ hybridization' (**FISH**) and molecular approaches including chromosome conformation capture (**3C**) technologies helps to visualize in detail. Recent developments in both types of approaches now offer the possibility of exploring the folded state of an entire genome and maybe even the identification of how complex molecular machines govern its shape.

7.3 RIBOSOME

Ribosomes are small, rounded and dense particles of ribonucleoprotein. They occur either freely in the cytoplasm and the matrix of mitochondria and chloroplast or remain attached with the membranes of the endoplasmic reticulum and nucleus. They are the sites of protein synthesis in both prokaryotic and eukaryotic cells. The cells in which active protein synthesis takes place (for example, pancreatic cells, hepatic parenchymal cells, plasma cells and thyroid cells), ribosomes remain attached with the membranes of RER. Cells which synthesize specific proteins for the intracellular utilization and storage like erythroblasts, skin, etc. contain large number of free ribosomes in the cytoplasm. Ribosomes are usually designated according to their rate of sedimentation. The sedimentation coefficient is expressed in the **Svedberg unit** or S unit (S is related with the size and molecular weight of the ribosomal particles).

Ribosomes are important cell organelle having a large complex of RNA and protein. It does RNA translation, building proteins from amino acids using messenger RNA (mRNA) as a template. Ribosomes are found in all living cells, prokaryotes as well as eukaryotes. A ribosome is a mixture of protein and RNA that starts its formation in the nucleolus of a cell. The nucleolus is found in the center of the nucleus. The job of the ribosome is to make new proteins; it does this by moving along a strand of mRNA and building a protein based on the code it reads. Making a protein is called **translation**.

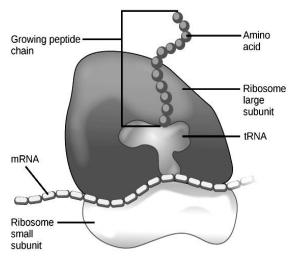


Fig.7.1- A ribosome translating a protein

Ribosome is an important part in the course of decoding the genetic message reserved in the genome into protein. The essential chemical step of protein synthesis is peptidyl transfer, where the developing or nascent peptide is moved from one tRNA molecule to the amino acid together with another tRNA. Amino acids are included in the developing polypeptide in line with the arrangement of codons of an mRNA. The ribosome, therefore, has necessary sites for one mRNA and no less than two tRNAs, performing protein synthesis. Ribosomes are made up of two subunits i.e., the big and the small subunit, which comprises a couple of rRNA molecules and an irregular number of ribosomal proteins. Numerous protein factors catalyze distinct impression of protein synthesis. The translation of the genetic code is of essential significance for the manufacturing of useful proteins and for the growth of the cell.

Biogenesis of Ribosomes

Ribosomes are not self-replicating particles. The synthesis of various components of ribosomes such as rRNAs and proteins are under genetic control. In bacterial cells, ribosomes are synthesized in the cytoplasm through the transcription of multiple ribosome gene operons. In *E.coli* the synthesis of ribosomal proteins is controlled at the translational level. Some of the ribosomal proteins that bind directly to rRNA can also bind to similar structure in their own mRNA. In eukaryotes, the process takes place both in the cell cytoplasm and in the nucleolus. In eukaryotes, the biogenesis of ribosomes is the result of the coordinated assembly of several molecular products that converge upon the nucleolus.

The assembly process involves the coordinated function of over 200 proteins in the synthesis and processing of the four rRNAs, as well as assembly of those rRNAs with the ribosomal proteins. The 18S, 5.8S and 28S RNAs are synthesized as part of a much longer precursor molecule in the nucleolus, 5S RNA is synthesized on the chromosomes outside the nucleolus, and ribosomal proteins are synthesized in the cytoplasm. All these components

migrate to the nucleolus, where they are assembled into ribosomal subunits and transported to the cytoplasm.

Locations of Ribosomes

Ribosomes are classified as being either "free" or "membrane-bound". Free and membrane-bound ribosomes differ only in their spatial distribution; they are identical in structure. Whether the ribosome exists in a free or membrane-bound state depends on the presence of an ER-targeting signal sequence on the protein being synthesized, so an individual ribosome might be membrane-bound when it makes one protein, but free in the cytosol when it makes another protein. Ribosomes are sometimes referred to as organelles, but the use of the term organelle is often restricted to describing sub-cellular components that include a phospholipid membrane, which ribosomes, being entirely particulate, do not. For this reason, ribosomes may sometimes be described as "non-membranous organelles".

Free ribosomes can move anywhere in the cytosol, but are excluded from the cell nucleus and other organelles. Proteins formed from free ribosomes are released into the cytosol and used within the cell. Since the cytosol contains high concentrations of glutathione (antioxidants) and is, therefore, a reducing environment, proteins containing disulfide bonds cannot be produced within it. When a ribosome begins to synthesize proteins that are needed in some organelles, the ribosome making this protein can become "membrane-bound". In eukaryotic cells this happens in a region of the ER called the "rough ER". The newly produced polypeptide chains are inserted directly into the ER by the ribosome and are then transported to their destinations, through the secretory pathway. Bound ribosomes usually produce proteins that are used within the plasma membrane or are expelled from the cell via exocytosis.

Ribosomes Size and Types:

cytoplasmic Ribosomes are non-membranous ribonucleoprotein granules of 150 -200 Å diameters. They have a typical binary and constricted structure with the two units being unequal in size. The prokaryotic eukaryotic and ribosomes differentiated on the basis of the sedimentation coefficient. Ribosomes are measured in Svedberg units (S), refers to sedimentation coefficient which shows how fast cell organelle sediments in an ultracentrifuge. Heavier particles sediment faster and have higher S values. These are of two basic types, 70S and 80S ribosomes found in prokaryotes and eukaryotes, respectively. The 70S ribosome of prokaryotes is relatively smaller and consists of a large 50S subunit and a small 30S subunit. The 80S

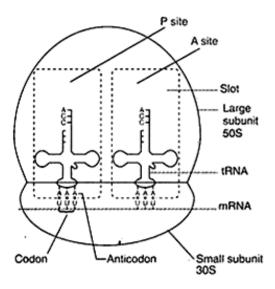


Fig.7.2- Ribosome showing A and P sites and the location of mRNA and tRNA in two subunits, 30S and 50S

ribosomes of eukaryotes are heavier and made up of large 60S subunit and small 40S subunit.

Ribosomes comprise of two subunits that are suitably composed and function as one to translate the mRNA into a polypeptide chain during protein synthesis. Due to the fact that they are made from two subunits of differing size, they are a little longer in the hinge than in diameter. They vary in size between prokaryotic cells and eukaryotic cells. The prokaryotic is comprised of a 30S and a 50S subunit (Fig.7.3.b), meaning 70S for the entire organelle equal to the molecular weight of 2.7×10^6 Daltons. The 70S ribosomes are found in prokaryotic cells of blue green algae and bacteria. On the other hand, eukaryotes comprise of a 40S and a 60S subunit which means 80S for the entire organelle which is equal to the molecular weight of 4×10^6 Daltons. The 80S ribosomes occur in eukaryotic cells of plants and animals.

Prokaryotic ribosomes are about 20 nm (200 Å) in diameter and are made of 35% ribosomal proteins and 65% rRNA, whereas, the eukaryotic are 25 and 30 nm (250–300 Å) in diameter. Small subunit of eukaryotes has a 16S RNA sub-unit (consisting of 1540 nucleotides) bound to 21 proteins. The large subunit has a 5S RNA (120 nucleotides), a 28S RNA (4700 nucleotides), a 5.8S RNA (160 nucleotides) subunits and 46 proteins. The small ribosomal subunit reads the mRNA, while the large subunit joins amino acids to form a polypeptide chain.

	70S ribosome	80S ribosome
Occurrence	Prokaryotes, Plastids, mitochondria	Eukaryotes
Sedimentation coefficient	70S	80S
Size	Smaller	Larger
Molecular weight	3 million	4-5 million
Subunits	Small 30S and large 50S	Small 40S and large 60S
RNA	3 molecules	4 molecules
	30S subunit : 16S	40S subunit : 18S
	50S subunit : 23S, 5S	60S subunit : 28S, 5.8S and 5S
Number of proteins	30S subunit : 21	40S subunit : 33
	50S subunit : 31	60S subunit : 49
	Total: 50-60	Total: 70-80

Table-1: Properties of 70S ribosome and 80S ribosomes

One notable difference between prokaryotic and eukaryotic ribosomes is their size. According to the Svedberg unit, the larger the number, the larger the molecule. Eukaryotic ribosome are larger size and usually having 80S, whereas, prokaryotes are smaller size contain only one kind of ribosomes i.e. 70 type. The 80S and 70S ribosomes can be further distinguished by their sensitivity to Chloramphenicol (CAP) and Cycloheximide (CHI), respectively. The 70S ribosomal mediated protein synthesis is inhibited by chloramphenicol, while 80S ribosomal

protein synthesis is inhibited by cycloheximide. The differences are subtle as the ribosomes of each operate in much the same way.

S.No. Eukaryotic 80S ribosome Prokaryotic 70S ribosome These are larger than prokaryotic ribosomes Comparatively smaller size 1-They contain 70-80 types of proteins Contains 53 types of proteins 2-They have four types of RNA molecules They have three types of RNA 3-(28S rRNA, 18S rRNA, 5.8S rRNA and 5S molecules (23S rRNA, 16S rRNA rRNA) and 5S rRNA) The RNA protein ratio is nearly 1:1 The RNA protein ratio is 2:1 4-Protein synthesis by these ribosome is Protein synthesis by these ribosome 5is inhibited by Chloramphenicol inhibited by Cycloheximide

Table-2: Comparison of 70S and 80S ribosomes

Structure of Ribosomes

Ribosomes have an incredibly similar structure throughout all forms of life. Scientists attribute this to the ribosome being a very effective and efficient way of synthesizing proteins. Thus, early in the evolution of the various forms of life, the ribosome was universally adopted as the method for translating RNA into proteins. Ribosomes, therefore, change very little between different organisms. Ribosomes consist of a large and small subunit, which come together around an mRNA molecule when translation takes place. Each subunit is a combination of proteins and RNA, called ribosomal RNA (rRNA). This rRNA is exists in various strands of different length, and is surrounded by the many proteins that create a ribosome. The rRNA acts both to secure the mRNA and tRNA in the ribosome, and as a catalyst to speed the formation of peptide bonds between amino acids.

The small subunits help to hold the mRNA in place as the ribosome translates it into protein. The larger subunit has various sites involved with different parts of the protein synthesis process. It has two functional sites amino acyl or **acceptor** (**A**) **site** and the peptidyl or **donor** (**P**) **site** (Fig.7.3). The acceptor sites receive the tRNA amino acid complex, and the donor site binds the growing polypeptide tRNA. As such, they perform most important function in protein synthesis. When the tRNA first binds to the mRNA, the P site can bind to these molecules. The P site is named after the **polymerization** or construction of polymers that occurs in ribosome. Conformational changes occur in the proteins of the ribosome which causes it to change shapes during the various steps of protein synthesis. As amino acids are added to the chain, tRNAs move from the A site to the P site, and eventually they exit the ribosome without their amino acid. The rRNA that is associated with the ribosome helps attach to the tRNAs as they move

through the ribosome, and has been found to help catalyze the formation of peptide bonds. This RNA is known as a **ribozyme**, or **RNA catalyst**.

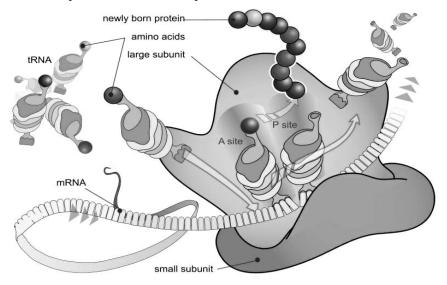


Fig.7.3- Structure of Ribosome involved in the synthesis of protein

Structurally, the large subunit, named 50S in prokaryotes, is spherical with a prominent "stalk" and a "central protuberance". It contains the peptidyl transferase centre that catalyses the peptide bond formation between the incoming amino acid and the growing peptide chain. The 50S particle is thick and monolithic, whereas, the small subunit, named 30S in prokaryotes, is thin and flexible. The 30S accommodates the mRNA decoding centre and is divided into three domains (head, body and platform). Each of these domains contains one of the principal secondary structure domains of 16S rRNA, namely the 3' minor, 3' major, 5' and central domains. The 3' minor domain of the 16S rRNA forms an extended helix that runs down the long axis of the 30S subunit surface that interacts with the 50S subunit. All four domain of the 30S particle join at a narrow neck region. The two "active sites" (the decoding centre and the peptidyl transferase centre) face each other across the subunit interface and are functionally linked by the two ends of the tRNA molecule.

The **Shine-Dalgarno** (**SD**) **Sequence** is a ribosomal binding site in bacterial and archaeal messenger RNA, generally located around 8 bases upstream of the start codon AUG. The RNA sequence helps recruit the ribosome to the messenger RNA (mRNA) to initiate protein synthesis by aligning the ribosome with the start codon. The SD sequence exists both in bacteria and archaea. It is also present in some chloroplast and mitochondrial transcripts. 16S ribosomal RNA (or 16S rRNA) is the component of the 30S small subunit of a prokaryotic ribosome that binds

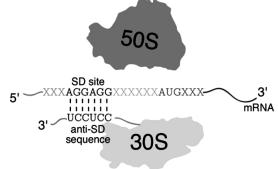


Fig.7.4- Shine-Dalgarno Sequence (SD site) involved in initiation of protein synthesis by ribosome.

to the SD sequence.

The genes coding for it are referred to as **16S rRNA gene** and are used in reconstructing phylogenies, due to the slow rates of evolution of this region of the gene. Multiple sequences of the 16S rRNA gene can exist within a single bacterium. Several functions are performed by 16S ribosomal RNA:

- (i) Like the large (23S) ribosomal RNA, it has a structural role, acting as a scaffold defining the positions of the ribosomal proteins.
- (ii) The 3' end contains the *anti-Shine-Dalgarno sequence*, which binds upstream to the AUG start codon on the mRNA. The 3'-end of 16S RNA binds to the proteins **S1** and **S21** known to be involved in initiation of protein synthesis.
- (iii) Interacts with 23S, aiding in the binding of the two ribosomal subunits (50S+30S)
- (iv) Stabilizes correct codon-anticodon pairing in the A site, via a hydrogen bond formation between the **N1** atom of adenine residues and the 2'OH group of the mRNA backbone

The general structures of prokaryotic and eukaryotic ribosomes are similar. Each ribosome is porous, hydrated and composed of two subunits. One subunit is large in size and has a dome-like shape, while the other subunit is smaller in size and occurring above the larger subunit and forming a cap-like structure. Both subunits remain separated by a narrow cleft. The two subunits are united with each other due to high concentration of the Mg⁺⁺. When the concentration of Mg⁺⁺ reduces in the matrix, both ribosomal subunits get separated. In bacterial cells, the two subunits are found to occur freely in the cytoplasm and they unite only during protein synthesis. During protein synthesis in both prokaryotes and eukaryotes, many ribosomes bind to an individual mRNA molecule. The ribosomes are spaced as close as 80 nucleotides apart along a single messenger RNA molecule. The structures are called polyribosomes or **polysomes**, a number of ribosomes translating the same transcript (Fig.7.5).

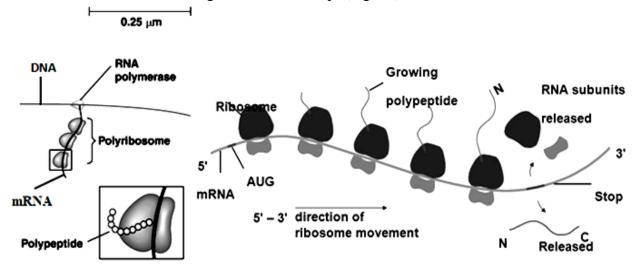


Fig. 7.5- Formation of Polyribosome during the protein translation

Functions of Ribosome

Ribosomes are the site of protein synthesis and are string together by mRNA. Two or more ribosomes simultaneously engaged in protein synthesis on the same mRNA-strand forming polysomes or polyribosomes. Interaction of the tRNA-amino acid complex with mRNA, which brings about translation of the genetic code, is coordinated by the ribosomes. During protein

synthesis. ribosomes play protective function. The mRNA strand which passes or moves through a channel between two subunits of the ribosome protected from the action nucleases. Similarly the nascent chains polypeptide passing through the tunnel or channel of the larger subunit of ribosome are protected against the action of protein digesting enzymes.

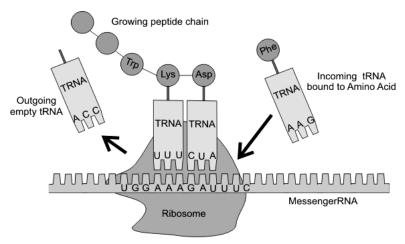


Fig. 7.6 – Ribosome involved in the peptide synthesis

The main function of ribosomes is to translate the genetic information encoded in the mRNA into proteins. During protein synthesis, ribosomes move along an mRNA molecule, reading one codon at a time. Reading of each codon by the ribosome results in the incorporation of one amino acid into a gradually lengthening protein chain. The amino acids are brought to the ribosome by the tRNA molecules, i.e. the adapter molecules in the translation mechanism. Each of the 20 amino acids used in protein synthesis is linked to a specific kind of tRNA. Ribosomes are thus capable of recognizing and binding the right nucleic acid code word specified by the anticodon of the tRNA with its attached amino acid. Once the ribosome reaches a stop codon on the mRNA, translation stops, the ribosomal subunits separate and detach from the mRNA and the completed protein is released (Fig.7.6). Role of ribosomes are summarized below:

- (i) They assemble amino acids to form specific proteins, which are essential to carry out cellular activities. The process of production of proteins, the DNA produces mRNA by the process of **transcription**.
- (ii) The genetic message from the mRNA is translated into proteins during translation. The sequences of protein assembly during protein synthesis are specified in the mRNA.
- (iii) The mRNA is synthesized in the nucleus and is transported to the cytoplasm for further process of protein synthesis.
- (iv) In the cytoplasm, the two subunits of ribosomes are bound around the polymers of mRNA; proteins are then synthesized with the help of transfer RNA.
- (v) The proteins that are synthesized by the ribosomes present in the cytoplasm are used in the cytoplasm itself. The proteins produced by the membrane bound ribosomes are transported outside the cell.

7.3.1-Endosomes

The ability of cells to interact with one another and with their environment relies on a large number of proteins that need to be in the right proportion and in the right location. For this reason, eukaryotic cells have evolved complex sorting machineries to ensure the dynamic and strictly controlled flow of proteins between cellular compartments. This machinery exists in both plants and animals, and it involves vesicular structures called **endosomes**. They are heterogeneous collection of organelles that function in the sorting and delivery of internalized material from the cell surface and the transport of materials from the Golgi bodies to the lysosome or vacuole. Plant endosomes have some unique features, with an organization distinct from that of yeast or animal cells.

Endosomes are primarily intracellular sorting organelles. They regulate trafficking of proteins and lipids among other subcellular compartments of the secretory and endocytic pathway, specifically the plasma membrane Golgi bodies, trans-Golgi network (TGN), and vacuoles/lysosomes. Endosomes receive cargo (proteins and lipids) from both the biosynthetic and the endocytic pathways. For instance, proteins that are destined for the vacuoles/lysosomes are synthesized at the endoplasmic reticulum, trafficked through the Golgi bodies, sorted at the TGN, and then sent to endosomes for their final vacuolar/lysosomal delivery (Fig.7.7).

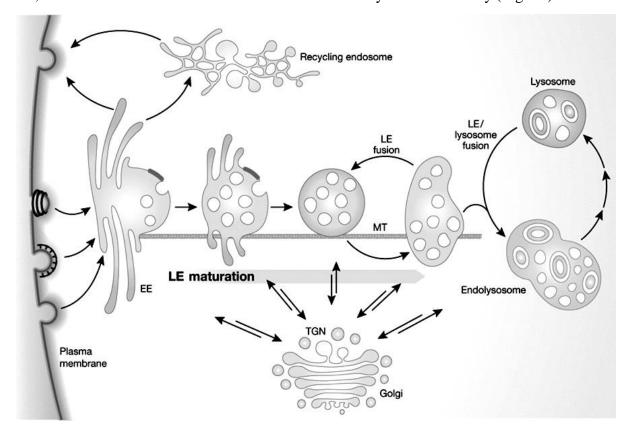


Fig.7.7 – Endosome system showing formation of various types; early endosome (EE), late endosome (LE) along with microtubules (MT), recycling endosome, and endolysosome

Types of Endosomes

There are three different types of endosomes, early endosomes, late endosomes, and recycling endosomes. They are distinguished by the time it takes for endocytosed material to reach them, and by markers such as **Rabs** (membrane-bound proteins involved in vesicular fusion and trafficking, e.g. Rab2A). They also have different morphology. Once endocytic vesicles have uncoated, they fuse with early endosomes and then mature into late endosomes before fusing with lysosomes. Endosomes also receive plasma membrane proteins that are internalized by **endocytosis**. At endosomes, these proteins are either recycled back to the plasma membrane or are sorted for degradation. The recycling of plasma membrane proteins mostly occurs at early and recycling endosomes. Their degradative sorting is achieved in intermediate/late endosomes, which are also called multivesicular bodies or multivesicular endosomes.

Endomembrane system

The eukaryotic endomembrane system functions in the synthesis, sorting, delivery and degradation of macromolecules within the cell. The system is composed of a variety of membrane-bound organelles that are connected either directly or through a series of transport vesicles. The main organelles of the endomembrane system are the endoplasmic reticulum, the Golgi complex and trans-Golgi network, endosomes, and lysosomes or vacuoles. Endosomes have a core sorting function within the endomembrane system. These organelles are the first point of fusion for endocytic vesicles, which are used to internalize the extracellular materials. They are also involved in the transport of materials either from the Golgi to the lysosome or vacuole, or their return from lysosomes to the Golgi. Endosomes provide intermediate compartments where materials can be stored, sorted and then sent to a designated target organelle within the cell. Two clearly defined endosomal compartments have been studied in plant cells, the trans-Golgi network (equivalent to the early endosome) and the multivesicular body (equivalent to the late endosome).

In plants, lysosomes are generally absent in favor of a large central vacuole, although there is evidence that lysosome-like organelles can co-exist with the vacuole in some cells. The vacuole is responsible for degradative functions, in common with lysosomes, and also acts as a large storage compartment for proteins, water, ions and some defense compounds. Appropriate sorting of proteins at the **Trans-Golgi network** (TGN) is essential for proper maintenance of the central vacuole and cell wall. In plants, as in other organisms, endosomes serve as an entry point for the endocytosis of external materials and an intermediate compartment in the transport of macromolecules to the vacuole. However, the organization of the endosomal system in plants has some unique features when compared with that in animal cells.

In animal and yeast cells, an array of different types of endosomes have been identified that can be classified according to their function during endocytosis. **Early endosomes** (EEs) are the first site of deposition of internalized components upon endocytosis. The internalized components might then be transported to a recycling endosome for subsequent recycling to the cell surface, or might enter a **multivesicular body** (MVB), designated as an intermediate or **late**

endosome (LE), for transport to a lysosome or vacuole, often for degradation. The MVB is generally considered to be the point at which endocytic and biosynthetic vacuolar trafficking meet, and is therefore often also termed the **prevacuolar compartment** (PVC) in both yeast and plants. A third major endosome type, the **recycling endosome** (RE), receives internalized material from the EE and directs it back to the cell surface or by a retrograde pathway to the TGN. Although endosomes perform similar functions of cargo sorting and transport in plants, yet they cannot be classified using the same criteria.

Function of Endosomes

The endosomes perform the following functions:

- (i) Endosomes provide an environment for material to be sorted before it reaches the degradative lysosome.
- (ii) The composition and structure of the plasma membrane and cell wall require proper endosomal sorting function.
- (iii) Endosomes have also been linked to activation of several signaling pathways.
- (iv) Endosomes serve functions beyond the sorting of cargo from endocytosis and biosynthesis. Plant endosomes are important in the maintenance of the vacuole and for cell growth, including the growth of specialized cells such as pollen tubes.
- (v) Despite being small and structurally simple organelles, endosomes perform an amazing range of controlled events that affect a multitude of signaling processes in cells.
- (vi) In fact, endocytosis and endosomal trafficking are of paramount importance in key plant processes such as embryo differentiation, gravitropism (responses of roots and shoots to gravity), guard cell movement during stomata opening, cell wall remodeling, the regulation of hormone auxin and ion transport, self-incompatibility responses during pollen tube growth, and defense responses against pathogens.

7.3.2-Lysosomes

In the cytoplasmic matrix of the cells, there occur variously shaped bodies usually bounded by a single surface membrane and containing hydrolytic enzymes. These are called lysosomes. These enzyme containing bodies play important role in the digestion or lysis of intracellular substances, so they are called **lysosomes**. Lysosomes are specialized vesicles within cells that digest large molecules through the use of hydrolytic enzymes. Vesicles are small spheres of fluid surrounded by a lipid bilayer membrane, and they play roles in transporting molecules within the cell. Lysosomes are only found in animal cells; a human cell contains around 300 lysosomes. Not only do they digest large molecules but also they are responsible for breaking down and getting rid of waste products of the cell. Lysosomes contain over 60 different enzymes that allow them to carry out these processes.

Lysosomes were first reported by **Christian de Duve** and co-workers in Belgium in 1955 following their extensive work on the biochemical identification of certain hydrolytic enzymes in the liver cells of rats. Lysosomes are ultra structural particles of the cell containing hydrolytic

enzymes responsible for digestion. Though these are common in animal cell, but in plants these are found in the lower groups, such as euglenoids, and some saprophytic fungi. In plants and fungi, lysosomes are called acidic vacuoles. Particles isolated from tobacco and maize seedlings contain several types of hydrolases found in animal lysosomes. They are called so because they contain lytic or destructive enzymes. A damaged or infected cell fails to do its functions due to metabolic irregularities or other reasons. In such situations, the lysosomes inside a cell burst out and engulf their own cell. Thus, it protects the other cells of the

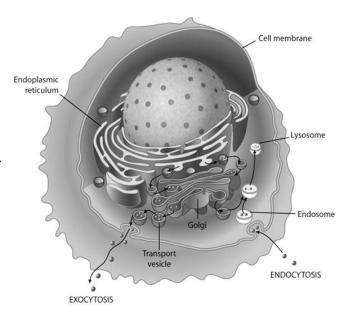


Fig.7.8-Synthesis of lysosomes by RER

body from further infections and damages. Since lysosomes engulf their own cell, they are known as "suicide bags" of a cell. Hence, it has a great role in immunity of the body. Rough endoplasmic reticulum (RER) in the cell is responsible for the synthesis of these enzymes.

Lysosomes digest many complex molecules such as carbohydrates, lipids, proteins, and nucleic acids, which the cell then recycles for other uses. The pH of lysosomes is acidic (around pH 5) because their hydrolytic enzymes function best at this pH instead of at the neutral pH of the rest of the cell. Hydrolytic enzymes specifically break down large molecules through **hydrolysis**. During the process of hydrolysis, a water molecule is added to a substance, causing its cleavage. Like the digestive system of the human body, which breaks down food using enzymes, the lysosome can be thought as the "**digestive system**" of the cell because it breaks down molecules using enzymes.

Lysosomes digest several different kinds of molecules. They can digest food molecules that enter the cell into smaller pieces if an endocytic vesicle (a vesicle that brings particles into the cell) fuses with them. They can also perform **autophagy**, which is the destruction of improperly functioning organelles. In addition, lysosomes have a role in **phagocytosis**, when a cell engulfs a molecule in order to break it down; it is also known as "cell eating". For example, the white blood cells called phagocytes ingest the invading bacteria in order to break and destroy it, and the bacteria are enclosed by a vesicle that lysosomes fuse with it. These lysosomes then break down the bacterial cells.

Structure of Lysosome

Lysosomes are generally very small, ranging in size from 0.1 to 0.5 μm , though they can reach upto 1.2 μm . Lysosomes are spherical bodies or vacuoles that are enclosed by a single membrane

consisting of a phospholipid bilayer that can fuse with some other membrane-bound organelles. The membrane serves as a protectorate to the cell, since lysosomes contain harsh digestive enzymes, which would cause significant damage if exposed to cell content. Lysosomes are formed by the fusion of vesicles that have budded off from the trans-Golgi apparatus, and the hydrolytic enzymes within them are formed in the endoplasmic reticulum.

The sorting system recognizes address sequences in the hydrolytic enzymes and directs them to the growing lysosomes. Vesicles formed in this way that contains enzymes such as proteases and lipases are the primary lysosomes. Secondary lysosomes are formed when primary lysosomes fuse with the other membrane-bound vesicles.

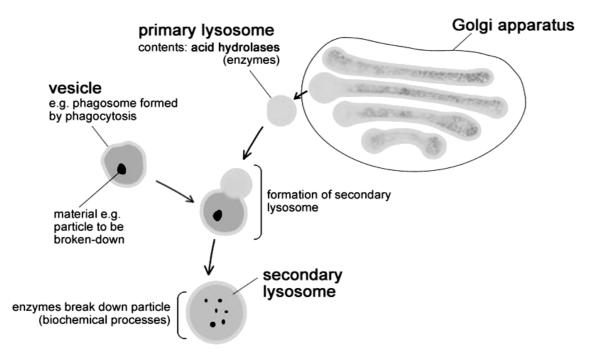


Fig.7.9 – Formation of primary and secondary lysosomes

A simple description of lysosomes is that they are tiny sacs filled with fluid containing enzymes which enable the cell to process its nutrients and are also responsible for destroying the cell after its death. The enzymes are tagged with the molecule mannose-6-phosphate, transported to the Golgi apparatus in vesicles, and then packaged into the lysosomes. There are many different types of enzymes in lysosomes including proteases, amylases, nucleases, lipases, and acid phosphatases. Enzymes are usually named for the molecules that they break down; for example, proteases break down proteins and nucleases break down nucleic acids. Amylases break down starches into sugars.

Function of Lysosomes

The functions of lysosomes concern the different ways in which the enzymes contained within the membrane affect other materials, which can originate from either outside or inside the cell.

1. Extracellular digestion: Lysosomes are small bags containing digestive enzymes. They behave like tiny time bombs waiting for their explosion in the cytoplasm. Whenever the limiting membrane ruptures, the digestive enzymes is released and taking part in the digestion. Exocytosis: Release of enzymes outside the cell which may serve the purpose of destroying materials around the cell. Sometimes lysosomal enzymes may be released outside the cell where they digest extracellular substances. Saprophytic fungi and other micro-organisms utilize extracellular digestion of complex substrates in the habitat and degrade them into simpler soluble forms which are then absorbed.

- **2. Intracellular digestion:** The digestive enzymes released in the cytoplasm may be involved in autophagy or heterophagy.
- I. **Autophagy:** It refers to digestion of endogenous materials or breakdown of molecules and pieces of cytoplasmic materials within the cell. The simpler substances formed after the digestions are then utilized in the synthesis of some other substances. This recycling of cell component is called turnover. Actually the digestive materials are non-functional part of other organelles like mitochondria, endoplasmic tubules, enclosed in a vesicle or digestive vacuole called autophagic vesicle or **autophagosome** (Fig.7.10). Autophagy may bring about cellular digestion after the death of a cell and so it brings about the self-clearance of dead cells.

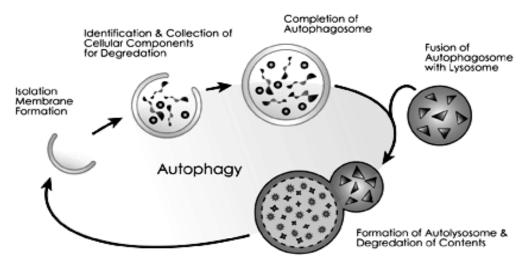


Fig.7.10 – Autophagy perform by lysosome

- II. **Heterophagy:** It refers to intake of extraneous matter into the cell and subsequent breakdown of that material by acid hydrolases. Specific mechanisms of heterophagy can be:
 - (a) Endocytic: The bulk intake of exogenous material is called **endocytosis**, in which cells take-up particles such as molecules that have become attached to the outer-surface of the cell membrane. The material to be internalized is surrounded by an area of plasma membrane, which then buds off inside the cell to form a vesicle containing the ingested material. Endocytosis includes pinocytosis and phagocytosis.

(b) Pinocytosis: The intake of extracellular liquid material is called pinocytosis, by which cells engulf extracellular fluid.

(c) Phagocytosis: The intake of extracellular solid matter is referred to as phagocytosis, by which cells engulf extracellular debris, bacteria or other particles. It only occurs in certain specialized cells.

In general, the functions of lysosomes involve breakingdown of useless and potentially harmful materials such as old worn-out parts of the cell or potential threats such as harmful bacteria. Lysosomes can, therefore, be thought

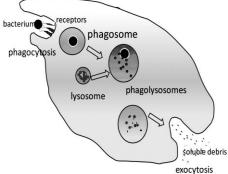


Fig.7.11 – Phagocytosis by lysosome

of as the rubbish disposal units within cells. Some of the important roles of lysosomes are listed below.

- (i) Hormone release: Lysosomal acid hydrolases are involved in release of certain hormones from secretory cells of certain glands, for example, thyroid hormones are released by hydrolysis of thyroglobulin.
- (ii) Fertilization: The enzymes released from acrosome vesicle, the giant lysosomes of sperms, dissolve the cortical granules, the structure surrounding the egg nucleus and help in the penetration of sperm nucleus into the egg.
- (iii) Metamorphosis: During the development of embryo, several tissues become functionless which are digested by the enzymes released from lysosomes and the digested materials are then absorbed by the surrounding cells.
- (iv) Protection: Lysosomes of leucocytes help in defense against infection by bacteria and other microbes and guard against toxic molecules by digesting them. A mature leucocyte or white blood cell entering the circulation contains many lysosomes.
- (v) Lysosomal Storage Diseases: Lysosomal storage diseases are caused by the accumulation of macromolecules (proteins, polysaccharides, lipids) because of a genetic failure to manufacture an enzyme needed for their breakdown. Neurons of the central nervous system are particularly susceptible to damage. Most of these diseases are caused by the inheritance of two defective alleles of the gene encoding one of the hydrolytic enzymes, For examples, Tay-Sachs disease and Gaucher's disease. Both of diseases are caused by a failure to produce an enzyme needed to break down sphingolipids (fatty acid derivatives found in all cell membranes).
- (vi) Malfunctioning: Several recent pathological studies have indicated that irregularities in lysosomal activity may cause fever, congestive heart failure, hepatitis, pyelonephritis, hypertension, joint injuries, leucocyte granules and tissue injuries.
- (vii) Inflammation: Accumulation of certain indigestible materials such as silica, asbestos particles, and crystals of sodium urate in the cells under certain conditions may result in cell inflammation. The inflammations of cells in such cases results in due to the release of enzymes after lysosomal break down in the cells containing ingested particles.

7.3.3 - Peroxisomes

A peroxisome is a type of organelle known as a microbody, found in virtually all eukaryotic cells. They are small, membrane-enclosed organelles which contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism. Although peroxisomes are morphologically similar to lysosomes, they are assembled like mitochondria and chloroplasts, from proteins synthesized on free ribosomes and then imported into peroxisomes as completed polypeptide chains. Although peroxisomes do not contain their own genomes yet they are similar to mitochondria and chloroplasts in that they replicate by division.

The liver is responsible for many vital functions in the normal anatomical functioning of the human body including blood detoxification, protein synthesis and producing chemicals necessary for digestion. Peroxisomes are vital to the healthy function of the liver. These tiny vesicles are found surrounding the liver cells and contain enzymes responsible for many metabolic reactions including energy metabolization and holding the digestive enzymes necessary for breaking down of the toxic matter in the cell.

Structure of Peroxisomes

Peroxisomes are enclosed in a single membrane and have a size of about 0.5-1.5 μ m in diameter. In some mammalian tissues, peroxisomes form an extensive network. Often they are compared to lysosomes, but peroxisomes differ in that they hold antioxidative enzymes. Peroxisomes contain at least 50 enzymes and self-replicate by enlarging and dividing. They contain H_2O_2 producing enzymes like oxidases and catalases as well as oxidative enzymes like peroxidase, catalase, glycolic acid oxidase and some other enzymes. Proteins are selectively imported into peroxidases. Peroxisomes contain no DNA or ribosomes and have no means of producing proteins. Instead, all of these proteins are imported across the membranes.

Function of Peroxisomes

Peroxisomes are involved in a variety of biochemical pathways in different types of cells. Peroxisomes originally were defined as organelles that carry out oxidation reactions leading to the production of hydrogen peroxide. Because hydrogen peroxide is harmful to the cell, peroxisomes also contain the enzyme catalase, which decompose hydrogen peroxide either by converting it to water or by using it to oxidize another organic compound. A variety of substrates are broken down by such oxidative reactions in peroxisomes, including uric acid, amino acids, and fatty acids.

The oxidation of fatty acids is a particularly important example, since it provides a major source of metabolic energy. In animal cells, fatty acids are oxidized in both peroxisomes and mitochondria, but in yeasts and plants fatty acid oxidation is restricted to peroxisomes. In addition to providing a compartment for oxidation reactions, peroxisomes are involved in lipid biosynthesis. In animal cells, cholesterol and dolichol are synthesized in peroxisomes as well as in the ER. In the liver, peroxisomes are also involved in the synthesis of bile acids, which are derived from cholesterol.

Peroxisomes are unusually diverse organelles, and even in the various cell types of a single organism they may contain different sets of enzymes. They can also adapt remarkably to changing conditions. Yeast cells grown on sugar, for example, have small peroxisomes. But when some yeast is grown on methanol, they develop large peroxisomes that oxidize methanol. But when grown on fatty acids, they develop large peroxisomes that break down fatty acids to **acetyl-CoA** by β -oxidation. Some of the major functions performed by peroxisomes are as followed:

- (i) A major function of the oxidative reactions performed in peroxisomes is the breakdown of fatty acid molecules in a process called β -oxidation. In this process, fatty acids are converted into acetyl-CoA, which is then exported from the peroxisomes to the cytosol for reuse in biosynthetic reactions. In mammalian cells, β -oxidation occurs in both mitochondria and peroxisomes; in yeast and plant cells, however, this essential reaction occurs exclusively in peroxisomes.
- (ii) An essential biosynthetic function of animal peroxisomes is to catalyze the first reactions in the formation of **Plasmalogens** (class of phospholipids), mainly found in nervous tissue (i.e. in myelin sheath). **Myelin** is an insulating layer or sheath that forms around nerves, including those in the brain and spinal cord, and is made up of protein and fatty substances. Plasmalogens protect internodal myelin from oxidative damage and deficiency of which causes profound abnormalities in the myelination of nerve cells, which is one reason why many peroxisomal disorders lead to neurological disease.
- (iii) Peroxisomes are involved in the formation and decomposition of hydrogen peroxide and the word peroxisome is actually derived from hydrogen peroxide. They contain oxidative enzymes, such as catalase, D-amino acid oxidase and uric acid oxidase, which use molecular oxygen to remove hydrogen atoms from a specific organic substrate (R) in an oxidative reaction that produces hydrogen peroxide (H_2O_2) , a toxic byproduct of cellular metabolism.

$$RH_2 + O_2 ==>> R + H_2O_2$$

Catalases use H_2O_2 generated by other enzymes in the organelle to oxidize the other substrates like phenols, formic acid, formaldehyde, and alcohol by the peroxidative reaction:

$$H_2O_2 + R'H_2 ==>> R' + 2H_2O$$

This type of oxidative reaction is important in liver and kidney cells where the peroxisomes detoxify various toxic molecules that enter the bloodstream. In addition, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O through this reaction:

$$2 H_2O_2 ==>> 2H_2O + O_2$$

(iv) Peroxisomes play particularly two important roles in plants. First, peroxisomes in germinating seeds are responsible for the conversion of stored fatty acids to carbohydrates, which is critical to providing energy and raw materials for growth of the young plant. This occurs via a series of reactions termed the **glyoxylate cycle**, which is a variant of the citric acid cycle. The glyoxylate cycle does not occur in animal cells, and animals are therefore unable to convert the fatty acids in fats into carbohydrates. The peroxisomes in which this reaction takes

place are sometimes called **Glyoxysomes**. Second, peroxisomes in leaves are involved in photorespiration, which serves to metabolize a side product formed during photosynthesis.

7.3.4-Hydrogenosome

Hydrogenosome are membrane-bound organelle found in the cytoplasm of eukaryotic cells of some anaerobic ciliates, trichomonads, fungi, and animals. Hydrogenosomes are so named because it releases molecular hydrogen (H₂) as a by-product of energy generation under anaerobic conditions. In hydrogenosome-containing organisms, the hydrogenosomes takes the place of the energy-producing mitochondria, and, similar to organisms with mitochondria, organisms with hydrogenosomes make use of the by-products of metabolic reactions that occur in the cell cytoplasm.

Hydrogenosomes are approximately 1 micrometer in diameter but under stress conditions can reach up to 2 micrometers. Like mitochondria, they are bound by distinct double membranes and one has an inner membrane with some cristae-like projections. In most cases, hydrogenosomes are genomeless, though genomes have persisted in some lineages such as *Neocallimastix*, *Trichomonas vaginalis* or *Tritrichomonas foetus*. However, a hydrogenosomal genome has been detected in the cockroach- ciliate *Nyctotherus ovalis*, and the heterokont or stramenopile *Blastocystis*.

The term hydrogenosome was introduced to describe a unique structure found in *Tritrichomonas foetus*, a parasite that lives in the gastrointestinal tracts of cats and in the reproductive tracts of cattle in 1973 by **Lindmark** and **Muller** as subcellular compartments that produce hydrogen and ATP. Since then, these organelles (or variations of them) have been described in a number of different unicellular eukaryotes adapted to microaerobic or anoxic environments. It includes multiple species of flagellated trichomonads (the most studied of the hydrogenosome-containing microorganisms), many of which are parasitic in animals; several free-living anaerobic ciliates, such as *Trimyema*, *Plagiopyla*, and *Metopus*; and some anaerobic chytridiomycete fungi, including *Neocallimastix*, which lives in the rumen of herbivores.

Hydrogenosome-like organelles have been found in several small multicellular marine organisms known as loriciferans, mainly members of the genera *Pliciloricus*, *Spinoloricus*, and *Rugiloricus*.

The hydrogenosomes of **trichomonads** produce molecular hydrogen, acetate, dioxide and ATP by the combined actions of pyruvate: ferredoxin oxidoreductase, hydrogenase, acetate: succinate CoA transferase and succinate thiokinase. Superoxide dismutase, malate dehydrogenase (decarboxylating), ferredoxin. adenylate kinase and NADH: ferredoxin

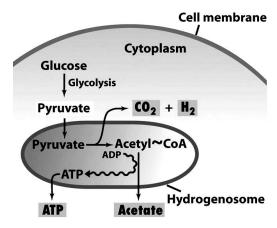


Fig.7.12-Functioning of hydrogenosome

oxidoreductase is also localized in the hydrogenosome. It is nearly universally accepted that hydrogenosomes evolved from mitochondria by loss of aerobiosis-related features in several

lineages (not all hydrogenosomes are directly related). The hydrogenosome appears to have a common ancestry with mitochondria based on similarities in protein import. However, major differences exist between hydrogenosomes and mitochondria in that hydrogenosomes lack cytochromes, mitochondrial respiratory chain enzymes and DNA.

Hydrogenosomes are very interesting organelles found in non-mitochondrial organisms. They display similarities and differences with mitochondria. Hydrogenosomes are spherical or slightly elongated organelles, although very elongated hydrogenosomes are also found. Hydrogenosomes divide in three different ways like mitochondria, segmentation, partition, and the heart form. They may divide at any phase of the cell cycle. Nucleoid or electron-dense deposits are not considered part of the normal structure of the hydrogenosome. They are surrounded by two closely apposed membranes and present a granular matrix. Hydrogenosomes have one or multiple peripheral vesicles, which incorporate calcium. The peripheral vesicle can be isolated from the hydrogenosomal matrix and is considered as a distinct hydrogenosomal compartment. Dysfunctional hydrogenosomes are removed by an autophagic process and further digested in lysosomes.

7.4 GOLGI 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. The Golgi apparatus was observed in 1897 by Italian cytologist **Camillo Golgi**. In Golgi's early studies of nervous tissue, he had established a staining technique that he referred to as *reazione nera*, meaning "black reaction"; today it is known as the **Golgi stain**. In this technique, nervous tissue



is fixed with potassium dichromate and then suffused with silver nitrate. While examining neurons that Golgi stained

Fig.7.13 Camillo Golgi (1843-1926)

using his black reaction, he identified an "internal reticular apparatus". This structure became known as the Golgi apparatus, though some scientists questioned whether the structure was real and attributed the find to free-floating particles of Golgi's metal stain. In the 1950s, however, when the electron microscope came into use, the existence of the Golgi apparatus was confirmed.

Golgi apparatus, also called **Golgi complex** or **Golgi body**, membrane-bound organelle of eukaryotic cells that is made up of a series of flattened, stacked pouches called **cisternae**. It is located in the cytoplasm next to the endoplasmic reticulum and near the cell nucleus. The Golgi apparatus is responsible for transporting, modifying, and packaging proteins and lipids into vesicles for delivery to targeted destinations. 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. While many types of cells contain only one or several Golgi apparatus, plant cells can contain hundreds.

Plant cells usually contain smaller arrays of Golgi-type vesicles, called dictyosomes. They are stacks of flat, membrane-bound cavities (cisternae) that together comprise of the Golgi apparatus. Within the dictyosomes, proteins are stored, modified, sorted, and packed into vesicles (which are then closed off as Golgi vesicles) for further transport. Dictyosomes in animal cells are stacked tightly together, whereas, the dictyosomes in plant cells are dispersed in the cytoplasm, making them difficult to identify as the Golgi apparatus.

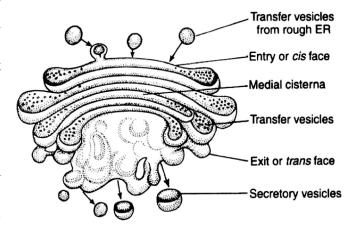


Fig.7.14 - Diagrammatic structure of Golgi apparatus

The Golgi apparatus functions as a factory in which proteins received from the endoplasmic reticulum (ER) are further processed and sorted for transport to their eventual destinations- lysosomes, the plasma membrane or secretion. In addition, glycolipids and sphingomyelin are synthesized within the Golgi. In plant cells, the Golgi apparatus further serves as the site at which the complex polysaccharides of the cell wall are synthesized. The Golgi apparatus is thus involved in processing the broad range of cellular constituents that travel along the secretory pathway. The Golgi complex is capable of both disassembly and reassembly during mitosis. In the early stages of mitosis, it disassembles while it reassembles in the telophase stage.

Subcellular localization

The subcellular localization of the Golgi apparatus varies among eukaryotes. In mammals, a single Golgi apparatus 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. In yeast, multiple Golgi apparatuses are scattered throughout the cytoplasm (e.g. *Saccharomyces cerevisiae*). 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.

In animal cells Golgi complex or apparatus is either single or consists of a single connected complex. The two conditions are respectively called localized (most vertebrate cells) and diffused (most invertebrate cells, liver and nerve cells of vertebrates). The localized organelle is compact. It generally occurs at one end between the nucleus and the periphery. The diffused organelle is found to form a network, e.g., around the nucleus in nerve cells. In plant cells, Golgi apparatus is formed of a number of unconnected units i.e., dictyosomes. Their

number is highly variable, from 1 in certain simple algae to 25000 in rhizoidal cell of *Chara*. Commonly there are 10-20 dictyosomes per plant cell. A liver cell may possess up to 50 units of Golgi apparatus called **Golgisomes**.

7.4.1-Structure of Golgi apparatus

In most eukaryotes, the Golgi apparatus is made up of a series of compartments and is a collection of fused, flattened membrane-enclosed disks known as cisternae (singular: cisterna, also called "dictyosomes"), originating from vesicular clusters that bud off the endoplasmic reticulum. In general, the Golgi apparatus is made up of approximately four to eight cisternae, although in some single-celled organisms (like in protists) it may consist of as many as 60 cisternae. The cisternae are held together by matrix proteins, and the whole of the Golgi apparatus is supported by cytoplasmic microtubules.

The apparatus has three primary compartments, known generally as "cis" (cisternae nearest the endoplasmic reticulum), "medial" (central layers of cisternae), and "trans" (cisternae farthest from the endoplasmic reticulum). Two networks, the cis Golgi network (CGN) and the trans Golgi network (TGN), which are made up of the outermost cisternae at the cis and trans faces, are responsible for the essential task of sorting proteins and lipids received (at the cis face) or released (at the trans face) by the organelle.

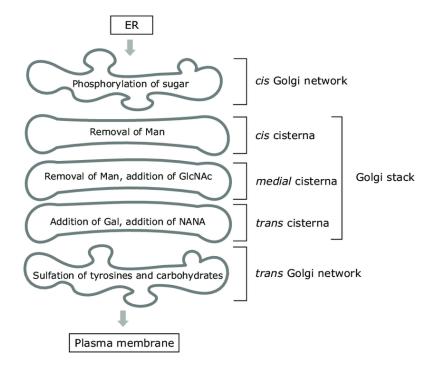


Fig. 7.15 – Different regions of Golgi performing essential biochemical modifications

The CGN is the first cisternal structure, and the TGN is the final, from which proteins are packaged into vesicles destined to lysosomes, secretory vesicles, or the cell surface. The TGN is usually positioned adjacent to the stack, but can also be separated from it. 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 yeast, Golgi stacking is not observed like *Saccharomyces cerevisiae*, while in *Pichia pastoris* (methylotrophic yeast) does have stacked Golgi. 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.

Morphology: The plant animal cells have Golgi complexes of similar morphology. The shape and size of Golgi complex are not fixed. The size and morphology of individual plant Golgi stacks vary tremendously between different cell types and species. The cisternal membranes and associated vesicles can be from 0.5 to 2.0 μm in diameter. They depend upon the physiological state of the cells. Usually Golgi complex is made up of four parts- cisternae, tubules, vesicles and vacuoles.

(a) Cisternae: A cisterna is a membrane bound sac filled with the fluid matrix. The Golgi complex consists of about 3 to 7 such membrane bound saccules or cisternae, which may be flat, tubular or filamentous, held together in parallel bundles or in a stack, one above the other. In insects, the number of cisternae may go up to 30 or more. Unicistemal dictyosomes are found in fungi. The cisternae are differentiated into three types based on the location as *cis* (near ER), medial (in middle) and *trans* (near membrane). In a stack of cisternae, there are 100-150 Å intercisternal spaces separating the two cisternae. In certain cells, this space is maintained and strengthened by a layer of parallel fibre celled intercisternal element. Each cisterna further consists of 0.5-1 nm diameter central region fenestrated at the peripheral margins.

The cisternae are frequently curved to give a definite polarity to the Golgi apparatus. One face of the apparatus is convex while the other is concave. The convex side is called **forming face** (cisface), while the concave side of the apparatus is known as **maturing face** (trans-face). The membranes of the maturing face are 7-8 nm in thickness while those of the forming face are about 4 nm in thickness. The forming face receives (transitional) vesicles from endoplasmic reticulum. Their contents pass through various cisternae with the help of coated vesicles and intercisternal connectives. They ultimately reach the maturing face where they are budded off as secretion, coated or Golgian vesicles or vacuoles. While passing through the apparatus, biochemicals are variously transformed.

(b) Tubules: They form a complicated network towards the periphery and maturing face of the apparatus. Actually tubules arise due to fenestrations of the cisternae. They have a diameter of 30-50 nm (or 300-500Å). The tubules interconnect the different cisternae.

- (c) Vesicles: The vesicles are small droplet-like sacs which remain attached to tubules at the periphery of the cisternae. They are of two types of vesicles, smooth and coated.
 - (i) **Smooth vesicles:** They are small sacs of 20-80 nm diameters and have a smooth surface. They contain secretory material (hence known as secretory vesicles), and are budded off from the ends of cisternal tubules within the net.
 - (ii) Coated vesicles: They are spherical protuberances, about 50 nm in diameter and have a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules and are morphologically quite distinct from the secretory vesicles. They elaborate membrane proteins.
- (d) Golgian Vacuoles: These are large rounded sacs present on the maturing face of Golgi. These are formed either by the expanded cisternae or by the fusion of secretory vesicles and have become modified to form vacuoles. The vacuoles develop from the concave or maturing face. Golgian vacuoles contain amorphous or granular substance. Some of the Golgian vacuoles function as lysosomes.

Organization of the Golgi

Morphologically the Golgi is composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles. A striking feature of the Golgi apparatus is its distinct polarity in both structure and function. Proteins from the ER enter at its *cis* face (entry face), which is convex and usually oriented toward the nucleus. They are then transported through the Golgi and exit from its concave *trans* face (exit face). As they pass through the Golgi, proteins are modified and sorted for transport to their eventual destinations within the cell. The distinct processing and sorting events appear to take place in an ordered sequence within different regions of the Golgi complex. The Golgi is most commonly viewed as consisting of four functionally distinct regions; the *cis* Golgi network, the Golgi stack (which is divided into the medial and *trans* subcompartments), and the *trans* Golgi network.

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, depending upon the signal sequence they carry.

- **1. Exocytosis vesicles (constitutive):** Vesicle contains proteins destined for extracellular release. After packaging, the vesicles bud off and immediately move towards the plasma membrane, where they fuse and release the contents into the extracellular space in a process known as constitutive secretion e.g., antibody release by activated plasma B cells.
- **2. Secretory vesicles (regulated)**: Vesicles contain proteins destined for extracellular release. After packaging, the vesicles bud off and are stored in the cell until a signal is given for their release. When the appropriate signal is received, they move toward the membrane and fuse to release their contents. This process is known as regulated secretion e.g., neurotransmitter release from neurons.
- **3. Lysosomal vesicles:** Vesicles contain proteins and ribosomes destined for the lysosome, a degradative organelle containing many acid hydrolases or to lysosome-like storage organelles. These proteins include both digestive enzymes and membrane proteins. The vesicle first fuses with the late endosome, and the contents are then transferred to the lysosome via unknown mechanisms e.g., digestive proteases destined for the lysosome.

Proteins, as well as lipids and polysaccharides, are transported from the Golgi apparatus to their final destinations through the secretory pathway. This involves the sorting of proteins into different kinds of transport vesicles, which bud from the trans Golgi network and deliver their contents to the appropriate cellular locations. Some proteins are carried from the Golgi to the plasma membrane by a **constitutive secretory pathway**. It accounts for the incorporation of new proteins and lipids into the plasma membrane, as well as for the continuous secretion of proteins from the cell. Other proteins are transported to the cell surface by a distinct pathway of **regulated secretion** or are specifically targeted to other intracellular destinations, such as lysosomes in animal cells or vacuoles in yeast.

Proteins that function within the Golgi apparatus must be retained within that organelle, rather than being transported along the secretory pathway. In contrast to the ER, all of the proteins retained within the Golgi complex are associated with the Golgi membrane rather than being soluble proteins within the lumen. The signals responsible for retention of some proteins within the Golgi have been localized to their transmembrane domains, which retain proteins within the Golgi apparatus by preventing them from being packaged in the transport vesicles that leave the trans Golgi network.

In yeasts and plant cells which lack lysosomes, proteins are transported from the Golgi apparatus to an additional destination, the vacuole. **Vacuoles** assume the functions of lysosomes in these cells as well as performing a variety of other tasks, such as the storage of nutrients and the maintenance of turgor pressure and osmotic balance. In contrast to lysosomal targeting, proteins are directed to vacuoles by short peptide sequences instead of carbohydrate markers.

Functions of Golgi apparatus

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, and 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 together. Individual stacks have different assortments of enzymes, allowing for progressive processing of cargo proteins as they travel from the cisternae 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.

Cis cisternae are associated with the removal of mannose residues, whereas, addition of N-acetylglucosamine occur in medial cisternae and 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 (GAGs), thus create proteoglycans.

Protein Sorting and Export from the Golgi apparatus

The proteins and lipids received at the *cis* face arrive in clusters of fused vesicles. These fused vesicles migrate along microtubules through a special trafficking compartment, called the **vesicular-tubular cluster**, which lies between the endoplasmic reticulum and the Golgi apparatus. When a vesicle cluster fuses with the *cis* membrane, the contents are delivered into the lumen of the *cis* face cisternae. As proteins and lipids progress from the *cis* face to the *trans* face, they are modified into functional molecules and are marked for delivery to specific intracellular or extracellular locations.

Some modifications involve cleavage of oligosaccharide side chains followed by attachment of different sugar moieties in place of the side chain. Other modifications may involve the addition of fatty acids or phosphate groups (phosphorylation) or the removal of monosaccharides. The different enzyme-driven modification reactions are specific to the compartments of the Golgi apparatus. For example, the removal of mannose moieties occurs primarily in the *cis* and medial cisternae, whereas the addition of galactose or sulfate occurs primarily in the *trans* cisternae.

In the final stage of transport through the Golgi apparatus, modified proteins and lipids are sorted in the Trans Golgi network and are packaged into vesicles at the *trans* face. These vesicles then deliver the molecules to their target destinations, such as lysosomes or the cell membrane. Some molecules, including certain soluble proteins and secretory proteins, are carried in vesicles to the cell membrane for exocytosis (release into the extracellular environment). The exocytosis of secretory proteins may be regulated, whereby a ligand must bind to a receptor to trigger vesicle fusion and protein secretion.

7.4.2-Genome organization

Human genomic DNA was identified in 1869 by **Friedrich Miescher** while searching for new proteins in the pus of wounded soldiers. Thereafter, it was recognized that DNA is the genetic material containing all the information essential for life and the basis for heredity. The human genome is divided into 46 chromosomes, consisting of pairs of chromosomes 1 to 22 (autosomes), numbered sequentially according to their size, and two sex chromosomes that determine whether an individual is male or female. Together, these chromosomes contain over 6 billion letters that when joined would measure ~2 m in length. It stands to reason that the human genome must be extensively packaged in order to fit inside the nucleus, the size of which is in the micrometer range.

Genome is the sum total of all genetic material (haploid chromosomes) of an organism which store biological information. The nature of the genome may be either DNA or RNA. All eukaryotes and prokaryotes always have a DNA genome, but viruses may either have DNA or RNA genome. The eukaryotic genome consists of two distinct parts: nuclear genome and organellar (mitochondria and chloroplast) genome, and consists mostly of linear dsDNA. The amount of DNA present in the genome of a species is called **C-value**, which is characteristic of each species. The value ranges from $<10^6$ bps as in smallest prokaryote, *Mycoplasma* to more than 10^{11} bps for eukaryotes such as amphibians. The genome of higher eukaryotes contains a large amount of DNA.

Genomic organization

Genomic organization refers to the linear order of DNA elements and their division into chromosomes. It can also refer to the 3D structure of chromosomes and the positioning of DNA sequences within the nucleus. Thus, genome organization is the sequential, not the structural organization of the genome. The hereditary material i.e. DNA of an organism is composed of a sequence of four nucleotides in a specific pattern, which encode information as a function of their order. Infact, in humans only 1.5% of the entire genome length corresponds to coding DNA. This 1.5% codes for about 27,000 genes which in turn code for proteins that are responsible for all the cellular processes. Besides the **coding exons**, the non-coding DNA in eukaryotes may fall in the following classes:

1. **Introns**: They are DNA sequences inserted between the exons and found in the open reading frame (ORF). They are enzymatically spliced after the first level of transcription. Most of introns are junk inserted within genes.

- 2. **Pseudogenes**: They are 'dead' or non-functional copies of genes present elsewhere in the genome, but no longer of any use.
- 3. **Retropseudogenes**: They are like pseudogenes, but have been processed, i.e. lack introns, produced by the action of reverse transcriptase (RT) on mRNA, and subsequent incorporation of the cDNA into the genome.
- 4. **Transposons:** They are jumping genes, which splice themselves in and out of the genome (in DNA form) randomly by the action of transposase.
- 5. **Retrotransposons**. They are transcribed into an mRNA, which encodes an reverse transcriptase (RT) enzyme, which then copies the mRNA back to DNA and incorporates it into the genome.

Description

Organisms have a vast array of ways in which their respective genomes are organized. A comparison of the genomic organization of various model organisms like *S. cerevisiae* with size 2kb gene (14mb) & -6,000 genes, (16chr.), *Arabidopsis* with size 2kb gene (125mb) and >25,000 genes, (5chr.), and *H. sapiens* with size 50kb genes (2.4gb) and 6,000 genes, (23chr.), shows size expansion with the increase of complexity of the organism. There is a more than 300-fold difference between the genome sizes of yeast and mammals, but only a modest 4- to 5-fold increase in overall gene number. However, the ratio of coding to non-coding and repetitive sequences is indicative of the complexity of the genome. The largely "open" genomes of unicellular fungi have relatively little non-coding DNA as compared with the highly heterochromatic genomes of multicellular organisms.

In particular, mammals have accumulated considerable repetitive elements and non-coding regions, which account for the majority of their DNA sequences (52% non-coding and 44% repetitive DNA). Only 1.2% of the mammalian genome thus encodes for protein function. This massive expansion of repetitive and non-coding sequences in multicellular organisms is most likely due to the incorporation of invasive elements, such as DNA transposons, retrotransposons, and other repetitive elements. The expansion of repetitive elements has even infiltrated the transcriptional units of the mammalian genome. This results in transcription units that are frequently much larger (30-200 kb), commonly containing multiple promoters and DNA repeats within untranslated introns. The vast expansion of the genome with non-coding and repetitive DNA in higher eukaryotes implies more extensive epigenetic silencing mechanisms. Studies of the genomic organization are thought to be the future of genomic medicine, which will provide the opportunity for personalized prognoses in clinics.

Repetitive Sequences

There are multiple classes of repetitive DNA, two of these classes include: highly repetitive and moderately repetitive DNA. The function of repetitive DNA is not really known but approximately 30% of the human genome consists of repetitive DNA.

- (a) **Highly Repetitive DNA:** It consists of several different sets of short repeated polynucleotides; generally the repeats range from 5 to 500 base pairs in length and exist in tandem arrays. Highly repetitive DNA comprises of about 10-15% of the total genomic DNA, is present in over a million copies and is transcriptionally inactive. Some of the highly repetitive DNA is clustered in structural regions of chromosomes particularly in the centromeric and telomeric regions.
- (b) Moderately Repetitive DNA: It contains a large variety of repeated sequences ranging from a few hundred to tens of thousands of base pairs with different characteristics. Moderately repetitive DNA can be clustered at specific chromosomal locations or distributed throughout the genome. One type of moderately repetitive human DNA sequence is the rRNA precursor gene. Each rRNA precursor gene is contained in a DNA segment of about 43,000 base pairs.

Chromatin organization (Chromosomal packaging): The human haploid genome consists of about $3x10^9$ base pairs of DNA. Genomic DNA exists as single linear pieces of DNA that are associated with a protein called a **nucleoprotein complex**. The DNA-protein complex is the basis of the formation of chromosomes. Virtually the entire genomic DNA is distributed among the 23 chromosomes that reside in the cellular nucleus. A very small fraction of the genome is also found in a 16,000 base pair circular piece of DNA found in the mitochondria.

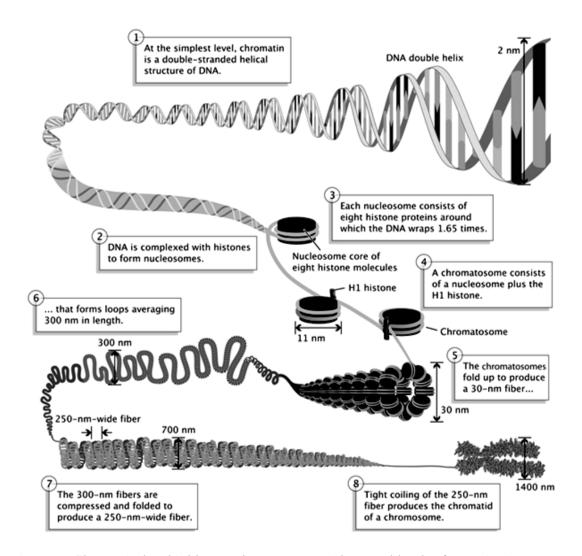


Fig. 7.16 - Chromatin has highly complex structure with several levels of organization

The double helical DNA of the chromatin replicates with the chromatin fiber which further condenses into discrete bodies, the chromosomes, each consisting of two identical chromatids. The two sister chromatids separate, one moving to each pole of the cell, where they become part of the newly formed nucleus of each daughter cell. The cells that make up most of the body of a multicellular organism, the somatic cells, have two copies of each chromosome and are said to be diploid (2n). Egg and sperm, produced by meiosis and having only one copy of each

chromosome are haploid (n). The DNA of chromatin and chromosomes is bound tightly to a family of positively charged proteins, the **histones**, which associate strongly with the many negatively charged phosphate groups in DNA. The histones and DNA associated in complexes are called nucleosomes in which the DNA strand winds around core of histone molecules.

The physiological state of genomic DNA is in the form of **chromatin**, where it is bound to histone and non-histone proteins. Histones are the most abundant proteins in chromatin and bind DNA mainly as **nucleosomes** composed of two copies each of H2A, H2B, H3, and H4. Wrapping of DNA around nucleosomes represents the first level in packaging, which effectively shortens the length of chromosomes by 7-fold. The positive charge (basicity) of the histones allows the negatively charged DNA to "wrap" around it forming a nucleosome. DNA wraps around the octamer in a left-handed supercoils in about 1.75 turns which encloses about 150 bp. Histone **H1** is a linker histone that, along with linker DNA (the DNA in between two nucleosome core particles), physically connects the adjacent nucleosome core particles. The length of linker DNA varies with species and cell types.

Usually, nucleosome core particle and linker DNA on both sides of the core encompasses between 180- and 200-bp DNA. Between the nucleosome unit structure and the metaphase chromosome structure contain two chromatids, there are several levels of organization and compaction of the chromatin. The nucleosomes are compacted into a **solenoid fiber** structure of 30 nm called as 30 nm fiber. The 30-nm solenoid fibers are compacted into a 300-nm **filament** and finally, the 300-nm filaments are further compacted into a 700-nm **chromosome**. During cell division, when the chromosomes duplicate, a 1,400-nm metaphase chromosome is produced containing two chromatids, each chromatid being 700 nm.

7.5 SUMMARY

The **ribosome** is a complex molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation). A ribosome is made from complexes of RNAs and proteins and is therefore a ribonucleoprotein. Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules. Ribosomes consist of two major components: the small ribosomal subunits, which read the RNA, and the large subunits, which join amino acids to form a polypeptide chain. Each subunit consists of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins (r-protein). The ribosomes and associated molecules are also known as the **translational apparatus**. When a ribosome finishes reading an mRNA molecule, these two subunits split apart. Ribosomes are ribozymes, because the catalytic peptidyl transferase activity that links amino acids together is performed by the ribosomal RNA. Ribosomes are often associated with the intracellular membranes that make up the rough endoplasmic reticulum.

The sequence of DNA, which encodes the sequence of the amino acids in a protein, is copied into a messenger RNA chain. Ribosomes can bind to a messenger RNA chain and use its sequence for determining the correct sequence of amino acids for generating a given protein.

Amino acids are selected, collected and carried to the ribosome by transfer RNA (tRNA) molecules, which enter one part of the ribosome and bind to the messenger RNA chain. It is during this binding that the correct translation of nucleic acid sequence to amino acid sequence occurs. For each coding triplet in the messenger RNA there is a distinct transfer RNA that matches and which carries the correct amino acid for that coding triplet. The attached amino acids are then linked together by another part of the ribosome. Once the protein is produced, it can then fold to produce a specific functional three-dimensional structure although during synthesis some proteins start folding into their correct form.

Endosomes are primarily intracellular sorting organelles. They regulate trafficking of proteins and lipids among other subcellular compartments of the secretory and endocytic pathway, specifically the plasma membrane Golgi, trans-Golgi network (TGN), and vacuoles/lysosomes. Endosomes receive cargo (proteins and lipids) from both the biosynthetic and the endocytic pathways. Endosomes provide an environment for material to be sorted before it reaches the degradative lysosome. Lysosomes are small membrane-bound sac-like structures which release digestive enzymes that break down food. They act as a waste bin of the cell and keep the cell clean.

Lysosomes are present in eukaryotic cells but a prokaryotic cell lacks them. When a foreign matter enters the cell, they release their enzymes which break the foreign substance into tiny pieces and kill them. They also remove the old and damaged or dead organelles from the cell and thus, protect the cell from further damages and consequences. Lysosomes are also responsible for the digestion of food which we eat. They release an enzyme which is powerful enough to break any organic matter.

A **peroxisome** is a type of organelle known as a microbody, found in virtually all eukaryotic cells. They are small, membrane-enclosed organelles which contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism. Peroxisomes are involved in a variety of biochemical pathways in different types of cells. Peroxisomes originally were defined as organelles that carry out oxidation reactions leading to the production of hydrogen peroxide. **Hydrogenosome** are membrane-bound organelle found in the cytoplasm of eukaryotic cells of some anaerobic ciliates, trichomonads, fungi, and animals. They are very interesting organelles found in non-mitochondrial organisms. They display similarities and differences with mitochondria. The hydrogenosomes takes the place of the energy-producing mitochondria.

The Golgi apparatus is part of the membrane system that also contains the ER. It consists of stacked membrane-coated cavities, called dictyosomes. The Golgi apparatus is located close to the nucleus and can be very large in secretory cells, where it fills almost the complete cytoplasm. The convex side facing the ER or nucleus is called cis-Golgi. The concave side facing the cytoplasm is called trans-Golgi. From the Golgi apparatus, small vesicles transport products to other cellular sites or the exterior. Inside the structure, complex biochemical operations are performed most of them resulting in post-translational modifications of synthesized proteins.

The Golgi apparatus plays essential roles in intracellular trafficking, protein and lipid modification, and polysaccharide synthesis in eukaryotic cells. It is well known for its unique stacked structure, which is conserved among most eukaryotes. Proteins from the ER are transported to the ER-Golgi intermediate compartment and then enter the Golgi apparatus at the CGN, then progress to the medial and trans compartments of the Golgi stack, within which most metabolic activities of the Golgi apparatus take place. The modified proteins, lipids, and polysaccharides then move to the TGN, which acts as a sorting and distribution center, directing molecular traffic to lysosomes, the plasma membrane, or the cell exterior.

Genomic organization refers to the linear order of DNA elements and their division into chromosomes. In humans, nearly two meters of genomic material must be folded to fit inside each micrometer-scale cell nucleus while remaining accessible for gene transcription, DNA replication, and DNA repair. This fact highlights the need for mechanisms governing genome organization during any activity and to maintain the physical organization of chromosomes at all times.

Chromosomes are much shorter than the DNA molecules that they contain. A highly organized packaging system is therefore needed to fit a DNA molecule into its chromosome. DNA in the nucleus exists mainly in combination with histone proteins; the DNA-histone complex is called "chromatin". Chromatin can undergo changes in its structure in response to various cellular metabolic demands. Chromatin can be envisioned as a repeat of structural units called "nucleosomes". The nucleosome core particle is composed of histone octamer plus the DNA that wraps around it. Between the nucleosome unit structure and the metaphase chromosome structure containing two chromatids, there are several levels of organization and compaction of the chromatin.

7.6 GLOSSARY

Amino acid: An organic molecule that is made up of a basic amino group (-NH2), an acidic carboxyl group (-COOH), and an organic R group (or side chain) that is unique to each amino acid.

Autophagosome: An intra-cytoplasmic vacuole containing elements of a cell's own cytoplasm; it fuses with a lysosome and the contents are subjected to enzymatic digestion.

Autophagy: The degradation of unnecessary or improperly functioning components within a cell.

CGN: Cis-Golgi network is an extensive tubule-vesicular network bound to the cis face of the Golgi stack and which function is to receive process the biosynthetic output from the ER.

Chloramphenicol: A broad-spectrum antibiotic with specific therapeutic activity against rickettsiae and many different bacteria.

Chloroplast: The photosynthetic unit of a plant cell, containing all the chlorophyll.

Chromosome: A thread-like structure of nucleic acids and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.

Cis face: The side of the Golgi apparatus sacs closest to the endoplasmic reticulum.

Cisternae: A flattened membrane disk of the endoplasmic reticulum and Golgi apparatus.

Codon: A series of three adjacent bases in one polynucleotide chain of a DNA or RNA molecule, which codes for a specific amino acid.

Dictyosome: A stack of flat, membranous cisternae which, with the vesicles, make up the Golgi apparatus. Plant cells usually contain smaller arrays of Golgi-type vesicles, called dictyosomes.

DNA: (Deoxyribonucleic acid), the chemical molecule that is the basic genetic material found in all cells.

Endocytosis: The uptake by a cell of material from the environment by invagination of the plasma membrane; it includes both phagocytosis and pinocytosis.

Endoplasmic reticulum: A network of membranous tubules within the cytoplasm of a eukaryotic cell, continuous with the nuclear membrane.

Endosome: A membrane-bound compartment inside eukaryotic cells. It is a compartment of the endocytic membrane transport pathway originating from the trans Golgi membrane.

Enzyme: Any of numerous compounds that are produced by living organisms and function as biochemical catalysts.

Eukaryotes: A eukaryote is any organism whose cells contain a nucleus and other organelles enclosed within membranes.

Exon: A nucleotide sequence that is found in a gene, codes information for protein synthesis, and is transcribed to messenger RNA.

Ferredoxin: An iron-containing protein present in green plants and certain anaerobic bacteria that functions in electron transport reactions in biochemical processes, such as photosynthesis.

Genome: A full haploid set of chromosomes with all its genes; the total genetic constitution of a cell or organism.

Glyoxysomes: A specialized type of peroxisome found in plant tissues that is bounded by a single membrane and contains a broad spectrum of enzymes, including those of the glyoxylate cycle and the β -oxidation cycle in addition to catalase and oxidase.

Golgi apparatus: A complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport.

Heterophagy: The taking into a cell of exogenous material by phagocytosis or pinocytosis and the digestion of the ingested material after fusion of the newly formed vacuole with a lysosome.

Histone: Any of several basic proteins found in association with the DNA in the chromatin of eukaryotes.

Hydrogenosome: An ATP and hydrogen producing organelle in anaerobic eukaryocytes, including *Trichomonas*, having a double membrane and thought by some to be a mitochondrial homologue.

Hydrolysis: The cleavage of a compound by the addition of water, the hydroxyl group being incorporated in one fragment and the hydrogen atom in the other.

Hydrolytic enzyme: A molecule that speeds up a chemical reaction involving hydrolysis.

Intron: A portion of DNA that lies between two exons, is transcribed into RNA, but does not appear in that mRNA after maturation because the intron is removed and the exons spliced together, and so is not expressed (as protein) in protein synthesis.

Lysosomes: A membrane-bound organelle in the cytoplasm of most cells containing various hydrolytic enzymes that function in intracellular digestion.

Macromolecule: A large molecule formed by the combination of smaller molecules.

Metamorphosis: Change in the form and often habits of an animal during normal development after the embryonic stage.

Microtubule: An ultrafine cylindrical structure in the cell cytoplasm, involved in shape and transport.

M-RNA: A single-stranded molecule of RNA that is synthesized in the nucleus from a DNA template and then enters the cytoplasm, where its genetic code specifies the amino acid sequence for protein synthesis.

Nucleic acid: a complex organic substance present in living cells, especially DNA or RNA, whose molecules consist of many nucleotides linked in a long chain.

Nucleotides: Nucleotides are the building blocks of nucleic acids; they are composed of three subunit molecules: a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group.

Peroxisome: A cell organelle containing a large number of enzymes, including catalase and oxidase that break down long-chain fatty acids and other organic molecules. The hydrogen peroxide produced by these reactions is also broken down within the peroxisome.

Phagocytosis: The engulfing of microorganisms and destruction of particulate matter, such as a bacterium or other cells and foreign particles by phagocytes.

Pinocytosis: Introduction of fluids into a cell by invagination of the cell membrane, followed by formation of vesicles within the cell.

Polymerization: The combining of several simpler molecules (monomers) to form a polymer.

Polysome: (Also called polyribosome), a group of ribosomes joined by a molecule of messenger RNA containing a portion of the genetic code that is to be translated. Polysomes are found in the cytoplasm during protein synthesis.

Prokaryotes: A prokaryote is a unicellular organism that lacks a membrane-bound nucleus, mitochondria, or any other membrane-bound organelle.

Proteases: (Also called peptidase), any of various enzymes, including the endopeptidases and exopeptidases, that catalyze the hydrolytic breakdown of proteins.

Proteoglycans: Any of various glycoproteins that have glycosaminoglycan chains attached by covalent bonds to the protein, usually found in the extracellular matrix of connective tissue.

Ribonuclease: Any of the class of enzymes that catalyze the hydrolysis of RNA.

Ribosomal RNA: RNA molecules associated with ribosomes, some of which are ribozymes and catalyze reactions.

Ribozyme: RNA that acts as a biological catalyst, which in a ribosome helps form peptide bonds.

RNA: Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation, and expression of genes.

TGN: The trans-Golgi network is a major secretory pathway sorting station that directs newly synthesized proteins to different subcellular destinations. The TGN also receives extracellular materials and recycled molecules from endocytic compartments.

Trans face: The side of the Golgi apparatus sacs furthest from the endoplasmic reticulum.

Transcription: The synthesis of RNA using a DNA template catalyzed by an RNA polymerase; the base sequences of the RNA and the DNA template are complementary.

Translation: Translation is the process of translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis.

Transport Vesicle: A small sac made of cell membrane, used to transport various cellular products.

T-RNA: A small RNA molecule, consisting of a strand of nucleotides folded into a clover-leaf shape that picks up an unattached amino acid within the cell cytoplasm and conveys it to the ribosome for protein synthesis.

Vacuole: A membrane-bound organelle in the cytoplasm of most cells, especially plant cells, containing water and dissolved substances such as salts, sugars, enzymes, and amino acids.

Vesicles: A membrane-bound structure within a cell in which materials such as enzymes are transported or stored.

7.7 SELF ASSESSMENT QUESTIONS

7.7.1 Multiple Choice Questions:

- 1. Ribosomes are present in?
- (a) Eukaryotes only
- (c) Prokaryotes only
- 2. In 70S ribosomes, 'S' stands for?
- (a) SI unit

(b) Solubility factor

(c) Svedberg unit

(d) All of these

- 3. 80 S ribosomes occur in?
- (a) Eukaryotic cells of animals

(b) Eukaryotic cells of animals and plants

(b) Eukaryotes and prokaryotes

(d) Eukaryotes, prokaryotes and viruses

- (c) Both Eukaryotic cells and prokaryotic cells
- (d) All of these
- 4. The subunits of 80S ribosome include-
- (a) 40S and 50S

(b) 30S and 50S

(c) 40S and 60S

- (d) 20S and 60S
- 5. Which of the following ions are required for binding of ribosomal subunits?

(a) Na ⁺	(b) Fe ⁺⁺			
(c) Mn ⁺⁺	(d) Mg ⁺⁺			
6. 70S ribosome occur in				
(a) Viruses	(b) Prokaryotes			
(c) Prokaryotes and eukaryotic plant cells	(d) All of these			
7. The larger and smaller subunit of 70S ri	ibosome is?			
(a) 50S and 30S	(b) 30S and 40S			
(c) 40S and 20S	(d) 20S and 50S			
8. Polysomes are?				
(a) Multiple units of ribosomes				
(b) Attachment of many mRNA to commor	ribosomes			
c) Attachment of many ribosomes to a common mRNA				
(d) Lysosomal aggregations				
9. Chloramphenicol inhibits?				
(a) Bacterial ribosomes	(b) Plant ribosomes			
(c) Both a and b	(d) Animal ribosomes			
10. Ribosomes are made up of?				
(a) RNA only	(b) RNA and proteins			
(c) RNA, DNA and proteins	(d) Nucleic acids, proteins and lipids			
11. Which of the following organelle lack r	ibosomes?			
(a) Mitochondrion	(b) RER			
(c) Chloroplast	(d) Nucleus			
12. Protein synthesis by eukaryotic ribosom	nes is inhibited by?			
(a) Chloramphenicol	(b) Penicillin			
(c) Cycloheximide	(d) Cinchonine			
13. Which of the following statements are t	rue-			
(a) Ribosomes are self-replicating organelle	es			

(b) Ribosomal components are coded by DNA

(d) Ribosomes are deoxy ribonucleoproteins

14. The mRNA links up with ribosomes to start?

(c) Ribosomes are double membrane bound organelles

(a) Translation	(b) Transcription
(c) Replication	(d) Splicing
15. Which of the following cell organelle lacks Γ	ONA and bounding membrane?
(a) Plasmid	(b) Ribosome
(c) Nucleolus	(d) Plastid
16. Which of the following is the function of lyso	osomes?
(a) Autophagy	(b) Autolysis
(c) Digestion	(d) All of these
17. Lysosomes are involved in?	
(a) Extracellular digestion	(b) Intracellular digestion
(c) Both a and b	(d) None of these
18. The cell organelle showing extensive polymo	orphism is?
(a) Dictyosomes	(b) Chloroplasts
(c) Lysosomes	(d) Ribosomes
19. Organelles that absorb and neutralize drugs a	and toxins are?
(a) Peroxisomes	(b) Lysosomes
(c) Smooth Endoplasmic reticulum	(d) Endocytic vesicles
20. The H ₂ O ₂ clearance inside the cell is carried	out by?
(a) Glyoxysome with enzyme Isocitrate lyase	(b) Peroxisome with enzyme amino oxidase
(c) Glyoxysome with enzyme catalase	(d) Peroxisome with enzyme catalase
21. Which of the following organelle is called as	the 'traffic police' of the cell?
(a) Lysosome	(b) SER
(c) Golgi apparatus	(d) RER
22. Dictyosomes are?	
(a) Golgi apparatus of plant cells	
(b) Golgi apparatus of plant cells and lower inver-	rtebrates
(c) Golgi apparatus of plant cells, lower inverteb	rates and animal cells
(d) Golgi apparatus like structure in prokaryotes	
23. The functions of Golgi apparatus include all,	except?
(a) GA is the sorting centre of the cell	
(b) GA is involved in post translational modifica	tion

- (c) GA is involved in cell plate formation
- (d) GA is involved in secretory protein synthesis

24. The region around Golgi apparatus where other organelles are absent is called as the?

(a) Zone of inhibition

(b) Zone of exclusion

(c) Both a and b

(d) Organelle inhibition zone

25. Golgi complex was first recognized in?

(a) Nerve cell

(b) Root cell

(c) Blood cell

(d) None of the above

26. Besides giving out secretary vesicles, the Golgi apparatus is also concerned with the formation of?

(a) Ribosomes

(b) Plastids

(c) Nucleus

(d) Lysosomes

27. Golgi apparatus is absent in?

(a) Higher plants

(b) Yeast

(c) Bacteria and blue green algae

(d) None

- 28. After synthesis of secretory protein in RER, it moves through-
- (a) Cis Golgi > median Golgi > trans Golgi > secretory vesicle
- (b) Trans Golgi > median Golgi > cis Golgi > secretory vesicle
- (c) Secretory vesicles > cis Golgi > median Golgi > trans Golgi
- (d) All of these
- 29. Which of the following statements are true regarding Golgi apparatus?
- (a) GA has polarity
- (b) Cis-face is located close to either nucleus or transitional ER
- (c) Trans-face is located near plasma membrane
- (d) All of these
- 30. Which of the following is not the function of the Golgi apparatus?
- (a) Amino acid metabolism

(b) Lipid metabolism

(c) Carbohydrate metabolism

(d) Processing and shorting of glycoprotein

7.7.1 Answers key: 1-(b), 2-(c), 3-(b), 4-(c), 5-(d), 6-(b), 7-(a), 8-(c), 9-(a), 10-(b), 11-(d), 12-(c), 13-(b), 14-(a), 15-(b), 16-(d), 17-(c), 18-(c), 19-(a), 20-(d), 21-(c), 22-(b), 23-(d), 24-(b), 25-(a), 26-(d), 27-(c), 28-(a), 29-(d), 30-(a)

7.7.2 Short Answer Type Questions:

- 1. Define ribosome?
- 2. What are P-site and A-site of ribosome?
- 3. What are polyribosomes?
- 4. What is the role of peroxisomes?
- 5. What is autophagy and phagocytosis?
- 6. What are lysosomes?
- 7. What is the main function of endosomes?
- 8. What are hydrogenosomes?
- 9. What is Golgi apparatus?
- 10. What are Pseudogenes and Retropseudogenes?
- 11. What are transposons and retrotransposons?
- 12. What are codon and anticodon?
- 13. What is endocytosis and exocytosis?
- 14. What is Shine-Dalgarno sequence?
- 15. What is genome and C-value?

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7.10 TERMINAL QUESTIONS

- 1. Describe ribosomes and its biogenesis. What are the two subunits of ribosome and what are their roles?
- 2. How ribosomal structure help in the synthesis of protein? With the help of a well labelled diagram describe the process of protein synthesis.
- 3. How prokaryotic ribosomes different from eukaryotic ribosomes? Differentiate between 70S and 80S ribosome.
- 4. How endosome controls the trafficking of proteins and lipids? What are the types of endosome and how they work in endomembrane system?
- 5. Write about the autophagy and phagocytosis process performed by lysosomes. Describe the structure and functions of lysosomes.
- 6. Discuss the Golgi apparatus and its discovery. Also write about the subcellular localization varies among eukaryotes.
- 7. Give detailed account on morphology and structure of Golgi apparatus.
- 8. Write about the vesicular transport performed by Golgi. Also discuss the difference between constitutive and regulated secretory pathways.
- 9. What is genome and how the size of an organism related to its complexity? What are the various levels of chromatin organization? Also write about the role of histone in DNA wrapping.
- 10. Write a short notes on the following:
- (a) Functions of ribosomes
- (b) Peroxisomes
- (c) Hydrogenosomes
- (d) Genome organization
- (e) Repetitive sequences

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BLOCK-3: INTRANUCLEAR ORGANEI	LLES

UNIT-8 NUCLEUS

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8.1 OBJECTIVES

After reading this unit students will be able-

- To understand the overview of the plant cell nucleus
- To know about the structure and function of plant cell nucleus
- Explaining the importance of nucleus

8.2 INTRODUCTION

Nucleus is present in all eukaryotic cells except mature sieve tube cells of plants and red blood cells (RBC) of mammals. It contains genetic material, which is tightly and neatly packaged into an area of about one-tenth size of the cell and able to be accessed for replication, transcription and repair of DNA. The observation of this central structure common to most cell types was made in the early 1830's, by Robert Brown. Nucleus is a microscopic spherical or oval shaped body embedded in the cytoplasm of the cell. Diameter of nucleus ranges from 5 to 10 µm. Generally, cells are uninucleate but binucleate cells are also found in some protistans (e.g. *Paramecium*) while in multinucleate cells it is found in latex vessels, some member of algae (*Vaucheria*), fungi and animals. However, the multinucleate condition in plants is called coenocytic while in animals it is called syncytia (e.g. malarial parasite). Microscopic details of cellular and nuclear morphology quickly became an interest to many biologists, and concepts of our modern day cell theory were formally established.

8.3 NUCLEUS

The nucleus is the membrane bound cell organelle occuring in eukaryotic cells. It contains all of the cell genome, excluding small parts of the mitochondrial DNA, organized as a linear DNA molecule with complex of large protein such as a histone protein and forming the chromosomes. The gene in the chromosomes are arranged and structured properly to promote the cell function. Nucleoplasm and nuclear envelope are the two main structural components that form nucleus of cell (Taddei *et al.*, 2004).

The nucleoplasm (karyoplasm) comprises of chromatin decondensed into chromosomes, which occupy the chromosomal domains, interchromosomal domains, the nucleolus, and the other nuclear bodies such as Cajal bodies and nuclear speckles. The nucleoplasm having a variety of different enzymes, molecules and structures participates in packing of DNA (Lanctot et al., 2007).

The Interphase Nucleus

Normally the nucleus remains in resting, metabolic, or in interphase. It is the phase of cell cycle in which a typical cell spends most of its life. The chromosomes of a cell are contained within its

nucleus during interphase. However, interphase does not describe a cell that is merely resting; rather, the cell is living and preparing for later cell division; therefore the name is changed. A common misconception is that interphase is the first stage of mitosis. Since mitosis is the division of the nucleus, prophase is actually the first stage. During this phase, the cell copies its DNA for preparation of mitosis. Interphase is considered as the metabolic stage of the cell in which the cell possesses nutrients and metabolizes it, grows, reads its DNA, and conducts other normal functions of cell.

Chemical Composition

The nucleus of the cell contains about 9-12% DNA, 5% RNA, 3% lipids, 15% simple basic proteins like histone, about 65% complex protein, including enzymes like polymerases used in the synthesis of DNA and RNA, organic phosphates and inorganic salts like Mg++, Ca++ and Fe++.

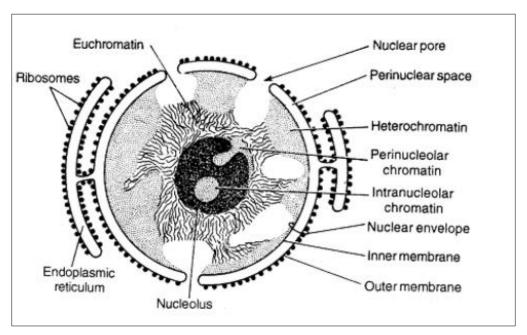


Fig 8.1: Structure of Nucleus

8.3.1 The Structure and function

8.3.1.1 Nuclear Envelope

The nuclear envelopes are also known as nuclear membrane; it is a double membrane layer which separates the nucleus from the rest of the cell. It is occurs in both plant as well as animal cells. Cell has many different jobs, such as synthesis of proteins, converting molecules into energy, and removing waste products of the cells. It has many different proteins which are used in organizing DNA and regulating the genes.

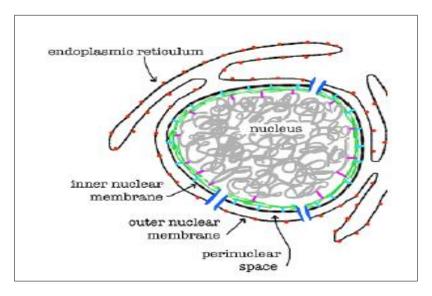


Fig.8.2. Structure of Nuclear Envelope

Nucleus of the plant cells contain majority of the hereditary material (Fig.8.2), a multifunctional organelle which are surrounded by the nuclear envelope and have several subcompartments. Nuclear envelope is formed by the inner and outer nuclear membranes (INM and ONM, respectively), nuclear pore complexes (NPCs), and in metazoa, the nuclear lamina (Hetzer et al., 2005). Nuclear envelope controls trafficking of macromolecules between the cytosol and nucleoplasm, and anchors the chromatin and cytoskeleton. Those chromatin which are associated with the nuclear envelope has been characterized as silent chromatin (Akhtar and Gasser, 2007; Kalverda et al., 2008) and interacts with the nuclear lamina. The active chromatin interacts with nuclear pore proteins at inner side of the nucleus; proteins of the inner nuclear membranes (INM) interact with lamina and/ or with chromatin. The inner and outer nuclear membranes are a dynamic structure regularly rebuilt during the cell division, and its components participate in mitosis (Kutay and Hetzer, 2008).

Nuclear membrane is a barrier that protects cell's DNA from the chemical reactions found elsewhere in the cell. If any molecules that stay in the cytoplasm are to move toward the nucleus, they could destroy part of the cell's DNA, which would stop it from functioning appropriately and could even lead to cell death. This envelope having a network of proteins that keep the genetic material in place inside of the nucleus. It manages what kind of materials can enter and exit from the nucleus. It does so by being selectively permeable. Certain proteins can physically transfer through the double layer membrane. This protects genetic information from mixing with other parts of the cell, and allows different cellular activities to occur inside the nucleus and outside the nucleus in the cytoplasm, where all other cellular structures are present.

Outer Membrane

The nuclear membrane contains lipid bilayer, two layers of lipid molecules, similar to the cell membrane. Outer layer of lipids has ribosomes, which is involved in synthesis of proteins, on its surface and it remains connected with the endoplasmic reticulum (ER), a cell structure at packages and transports proteins.

Inner Membrane

It contains those proteins which help in the organization of genetic material in nucleus. Network of fibers and proteins attach to the inner membrane known as nuclear lamina. Structurally it supports the nucleus and plays an important role in repairing DNA, and regulates the cell cycle events such as cell division and DNA replication. The nuclear lamina is found only in animal cells, although plant cells may have some similar proteins on the inner membrane.

8.3.1.2 Nuclear Pores

The nuclear pore passes through both the outer and inner membranes of the nuclear membrane. It is made up of large complexes of proteins and allows certain molecules to pass through the nuclear membrane.

8.3.1.3 Chromatin/Chromosomes

Chromosomes are formed by DNA tightly-bound around histones. Chromosomal DNA is packaged inside microscopic nuclei with the help of histones. These are positively-charged proteins that strongly adhere to negatively-charged DNA and form a series of bead-like structures, known as nucleosomes. Each nuclesome is made up of DNA and bound 1.65 times around eight histone proteins. The nucleosomes fold up and formed a chromatin fiber about 30-nanometer which forms loops averaging 300 nanometers in length.

When the cell enters metaphase and prepares to divide, the chromatin changes dramatically. First, all the chromatin strands make copies of themselves through the process of DNA replication. Then they are compressed to an even greater degree than at interphase, a 10,000-fold compaction into specialized structures for reproduction, termed as chromosomes. As the cell divides to become two cells, the chromosomes separate, giving each cell a complete copy of the genetic information contained in the chromatin.

Euchromatin and heterochromatin are two types of chromatin present in the nucleus. However, the genetically active portion of euchromatin are involved in transcribing RNA to produce proteins which are used in cell functioning, while heterochromatin possesses inactive DNA and the portion of that is most condensed chromatin since it not being used. Throughout the life of a cell, chromatin fibers take on different forms inside the nucleus.

8.3.1.4 Nuclear matrix

Nuclear matrix is the network of fibres found throughout inside of a cell nucleus and is somewhat analogous to the cell cytoskeleton. However, in contrast to the cytoskeleton, the

nuclear matrix has been proposed to be a highly dynamic structure, perhaps more like a dynamic sponge with open compartments for free diffusion of molecules in the nucleus. The nuclear matrix, along with the nuclear lamina aid in organizing the genetic information within the cell.

The main functions of the nuclear matrix are to maintain the shape of nucleus. Chromatin fibers are anchored to nuclear matrix. However, the machinery is involved in the various nuclear activities, such as replication and transcription which is associated with the matrix. It has also been implicated in the processing of newly formed RNA molecules and its transport through the nucleus.

8.3.2 Nucleolus

Nucleolus is a membrane-less cell organelle located within the nucleus that manufactures the ribosomes, and is known as the protein-producing structures of the cell (Fig.8.3). Under the microscope, the nucleolus looks like a large dark spot within the nucleus. A nucleus may possess up to four nucleoli, but within each species the number of nucleoli is fixed. After a cell divides, a nucleolus is produced when chromosomes are brought together into nucleolar organizing regions. During the time of cell division, the nucleolus disappears. Some studies suggest that the nucleolus may be involved with cellular aging and, therefore, may affect and involved in the aging of an organism.

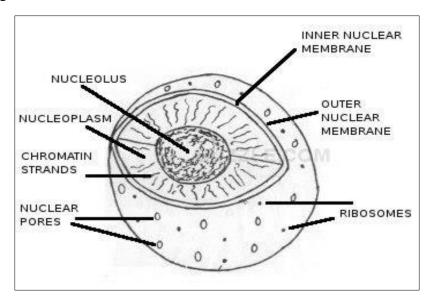


Fig.8.3: Structure of nucleolus

The nucleolus is comprised of granular and fibrillar components. Granular material consists of ribosomal subunits and is waiting to be exported to the cytoplasm. Thread like fibrillar part of a nucleolus is predominantly composed of rRNA molecules and associated proteins that have joined together to form fibrils.

8.3.3 Importance of Nucleus

Nucleus is the brain of cell which is characterized by the presence of hereditary material, i.e. DNA. In addition, nucleus is the site where DNA gets duplicated/replication before it gets divided equally into daughter cells. The genetic information stored in DNA has to reach the cytoplasm, where this information gets translated to proteins. The molecules, which carry this information from nucleus to cytoplasm, are messenger RNA (mRNA). Nucleus is the place in the cell, where mRNAs are synthesized (transcription), before they carry the genetically encoded information of DNA to cytoplasm.

8.4 SUMMARY

- 1. Nucleus is present in the eukaryote cell, where DNA is replicated and genetic information for protein synthesis is transcribed in the form of mRNA. This mRNA is translated for protein synthesis and carries the information from nucleus to cytoplasm.
- 2. In eukaryotes, these activities are separated in the cell by presence of nuclear envelope. These are well-organized structure in which nuclear pores are present.
- 3. Through the nuclear pores, exchange of molecules occurs in regulated way in between nucleoplasm and cytoplasm.
- 4. Nucleolus is present within nucleus and is not surrounded by any membrane. These are responsible for the organization of ribosomes. Ribosomal RNAs are synthesized within nucleolus while 5S rRNA is synthesized outside nucleolus. Ribosome subunits are organized in the nucleolus.
- 5. Nuclear lamina is a proteinaceous structure found just below the nuclear envelop. Having a protein known as lamins it provides skeletal support to nuclear envelope.
- 6. There are specific biochemical areas known as chromosome neighborhood areas, which help in switching on/off the genes.
- 7. The chromosomes are not present randomly only in the nucleus. It occupies specific territories.
- 8. In eukaryotic cell, DNA is complexed with histones. It is known as chromatin. Some of the chromatin remains condensed even during interphase and is known as heterochromatin, while the other type of chromatin gets decondensed. This latter type of chromatin is known as euchromatin.
- 9. Histones help in the packaging of DNA in an organized way; therefore a very large amount of DNA gets fitted in the nucleus of eukaryotic cell.
- 10. Base sequences present in the region of centromere and telomeres of chromosomes consist of repeat sequences. Centromere sequences encode for the proteins CENP, which is necessary for the attachment of chromosomes to spindle fibers during mitosis, but the base sequence is present at telomere in a specific way to protect the chromosome ends.

8.5 GLOSSARY

Chromatids: Copies of chromosomes produced in cell division.

Chromatin: The material of chromosomes composed of DNA and chromosomal proteins.

Codon A 3-base sequence in mRNA that causes the insertion of a specific amino acid into protein or causes termination of translation.

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

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

Genomics: The study of the structure and function of whole genomes.

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

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

Mitosis: Cell division that produces two daughter cells having nuclei identical to the parental cell.

Nucleolus: A cell organelle found in the nucleus that disappears during part of cell division.

Nucleosome: A repeating structural element in eukaryotic chromosomes, composed of a core of eight histone molecules with about 200 bp of DNA wrapped around the outside and one molecule of histone H1, also bound outside the core histone octamer.

8.6 SELF ASSESSMENT QUESTIONS

8.6.1 Mutiple Choice Questions:

- 1. Name the control center of the eukaryotic cell?
- (a) Nucleus (b) Ribosome
- (c) Cytoplasm (d) Golgi complex
- 2. Which of the following microorganism have two nuclei?
- (a) Slime molds (b) Cyanobacteria
- (c) Amoeba (d) Paramecium
- 3. Which of the following is not a component of the nucleus?
- (a) Chromosome (b) Nucleolus
- (c) Cytoplasm (d) Nuclear envelope
- 4. Mark the incorrect statement about nuclear lamina.
- (a) Filaments present in the inner membrane of the nucleus

- (b) Made up of lamin proteins
- (c) Provide mechanical support to the nucleus
- (d) It has bounded with the ribosomes

5. Non-membrane bound body of the nucleus which disappears in the late prophase and reappears in telophase

(a) Nucleolus

(b) Chromosome

(c) Nucleoplasm

(d) Nuclear pore

- 6. Which region of chromatin is transcriptionally silent?
- (a) Nucleoid

(b) Centromere

(c) Euchromatin

(d) Heterochromatin

- 7. Which of the following is not true for chromatin?
- (a) Organized structure of DNA and protein

(b) These are highly condensed DNA

(c) It is found in the nucleus

(d) It contains a single dsDNA

8. Name the structure which is used to transfer macromolecules between the cytoplasm and nucleus.

(a) Microtubules

(b) Nuclear pores

(c) Cilia

(d) Centrioles

8.6.2 Very Short answer type Questions

- 1. What is an interphase nucleus?
- 2. Who discovered the nucleus?
- 3. Name two types of chromatin.
- 4. Give the role of DNA present in nucleolus?
- 5. Which region of chromatin is not transcribed?
- 6. Nucleolus disappears during cell division and reappears again during which stage of cell division?
- 7. Who was described nucleolus?
- 8. Give three essential characteristics of cell?
- 9. The prokaryotic cells are characterized by?
- 10. What is Nucleoid?

8.6.1 Answer Key: 1-(a), 2-(d), 3-(c), 4-(d), 5-(a), 6-(d), 7-(a), 8-(b)

8.6.2 Answer Key

- 1. Nucleus of non-dividing cell.
- 2. Robert Brown in 1831.
- 3. Heterochromatin and Euchromatin.

- 4. Transcription of r RNA.
- 5. Heterochromatin is darkly stained and highly condensed region of chromatin which is generally believed to be transcriptionally silent.
- 6. Metaphase
- 7. Fontana
- 8. Cell membrane, cytoplasm, nuclear material.
- 9. Absence of nuclear membrane.
- 10. Nuclear envelope is absent in prokaryotic cell and the genetic material lies directly into the cytoplasm. Such nuclear material is known as nucleoid.

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8.10 TERMINAL AND MODEL QUESTIONS

- 1. Discuss the morphology, chemical organization and functions of the nucleus.
- 2. Give detailed account of nuclear envelope.
- 3. What is nucleosome? Write down about the structure of nucleosome.
- 4. Describe the nuclear pore.
- 5. What is chromatin? Name the types of chromatin present in the nucleus of eukaryotes cell. Discuss their role in the cell.

UNIT-9 CHROMOSOMES

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- 9.2. Introduction
- 9.3. Chromosomes
 - 9.3.1. Structure
 - 9.3.2. Genes
 - 9.3.3. Nucleic Acid
- 9.4. Lampbrush Chromosomes
 - 9.4.1. Structure
 - 9.4.2. Behavior
 - 9.4.3. Significance
- 9.5. B-Chromosomes
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9.1 OBJECTIVES

After reading this unit students will be able:

- To define chromosomes
- To study about the structure, behavior and significance of chromosomes
- To study details of nucleic acid
- To study Describe various types of chromosomes
- To Describe with structure of lampbrush and B-chromosomes

9.2 INTRODUCTION

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

9.3 CHROMOSOMES

In 1875, E. Strasburger discovered a thread like structure which appeared during the process of cell division. Such kind of thread like structure is called Chromosomes (chroma = color) due to its great affinity with basic dyes. W.S. Sutton and T. Boveri suggested the role of chromosomes in heredity in 1902, which was confirmed by Morgan in 1933. In all type of higher eukaryota, nucleus bears a number of chromosomes of definite size and shape. Normally, chromosomes are invisible in the nucleus, but can be seen easily during the process of cell division, whether meiosis or mitosis. In the Leptotene stage of meiotic prophase, the structure of chromosomes appears as bead like structure called chromosomes.

9.3.1. Structure

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

1. Viral Genome: 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. Viral genomes exhibit significant diversity in structure. Some viruses have genomes that consist of DNA as their genetic material. This DNA may be single stranded, as exemplified by M13 human parvoviruses, or double stranded, as seen in the herpesviruses, adenoviruses and poxviruses. Additionally, although all

cellular life uses DNA as its genetic material. Some viral genomes are made of either single-stranded or double-stranded RNA molecules, for example SARS (corona virus), and paramyxoviruses. Viral genomes are typically smaller than most bacterial genomes, encoding only a few genes, because they rely on their hosts to carry out many of the functions required for their replication.

- **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 cells 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.

9.3.1.1. Shape, Size and Number of Chromosomes

In eukaryotes, during interphase stage the chromosomes are extended into long and thin chromatin fibers, where is called chromatin reticulum. During the S phase of cell cycle it replicates and become double. In cell division, chromosomes condense and tightly coil up and become distinct at metaphase stage. Chromosomes condense and tightly coil up and become distinct at metaphase stage. Eukaryotic chromosomes vary in number, size, shape and position but they have remarkably uniform structure:

- a) Chromosome number- Each species contains a fixed number of chromosomes, however change in chromosome number can be seen in a species and is called as polyploidy (euploidy and aneuploidy). Normally gamete or gametophyte cells contain one set of chromosomes called genome and the cells are called haploid. The somatic cells of animals and sporophytes have two haploid sets or genomes and are called diploid cells.
- b) Chromosome size- The size of chromosome is normally measured at mitotic metaphase. The length may vary from $0.1-50.0/\mu m$, while the diameter may vary from 0.2- $3/\mu m$. In general monocots among plants have large chromosomes, while Orthoptera (Grasshopper) and Amphibia among animals have larger chromosomes.
- **c) 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 shapes, depending upon the position of the centromere.

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

9.3.1.2. Morphology of Chromosomes

During the cell cycle, size and shape of chromosomes are occurred changed and chromosomes remaining, chromatin reticulum forms during the interphase. In cell division, the chromatin reticulum condenses, so that by the end of prophase different thread like structures appear called chromonemata. In metaphase and anaphase the chromonemeta become fully condensed and converts in the shapes of chromatids in eukaryotic nuclear chromosomes. Such kind of changes in the chromosomes during the cell cycle is called chromosomal cycle.

Morphology of chromosome can be understood better during at metaphase and anaphase because of their high degree of condensation. For this purpose, one can use chiefly shoot or root apex having meristematic tissue or pollen mother cells (PMC) of plants, and tissue from sex organs. A typical chromosome has following parts:

(a) Centromere (Primary constriction): In primary constriction, the metaphase chromosomes have two identical sister chromatids, are attached to each other at a point called centromere/primary constriction. At anaphase stage the centromere splits the sister chromatids separate to become two anaphasic chromosomes. Thus, anaphase chromosome is a half metaphase chromosome. The parts of chromosome on either side of centromere are called arms (Fig.9.1).

Based on the position of centromere, chromosomes are called:

- (i) Telocentric (centromere terminal),
- (ii) Acrocentric (centromere subterminal and capped by telomere),
- (iii) Sub-metacentric (centromere is submedian),
- (iv) Metacentric (centromere median).

So metaphase chromosome has 4 arms while anaphase chromosome has 2 arms, which are equal to isobrachial chromosomes and unequal to heterobrachial chromosomes. When the arms are unequal, the short arm is nominated as 'p' and the long arm is nominated as 'q'.

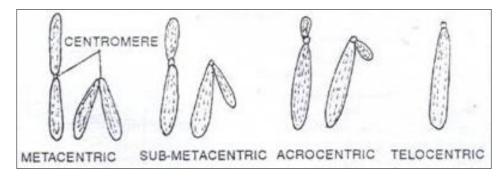


Fig.9.1. Types of chromosomes based on position of centromere

(b) Secondary Constrictions: Besides centromere, achromosome may be containing more than one secondary constriction. The part of chromosome beyond secondary constriction is called 'satellite' of which remaining is attached to the main portion of chromosomes with a thread of chromatin. The chromosome with satellite is known as sat chromosome. The secondary constriction mainly are of two types NOR and joint which are always constant in their positions and often can used as markers.

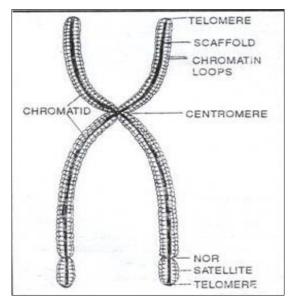


Fig.9.2. Structure of metaphase chromosomes

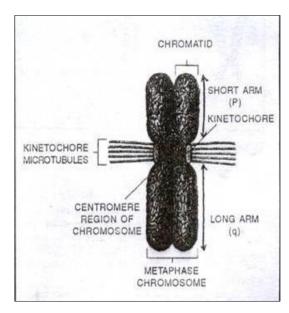


Fig.9.3. Structure of a mitotic chromosomes. Kinetochore microtubules are anchored to proteins at the centromere

Nucleolar organizer region (NOR) are very specialized to produce nucleolus and rRNA. While the joints sometimes develop due to the breaking and fusion of chromosome segments (Fig.9.2).

- **(c) Kinetochore:** Surface of centromere, possess a specialized multi-protein complex known as kinetochore and spindle fibers (microtubules) attached to it. Centromere of a metaphase chromosome possessing two kinetochores which faces in opposite direction. In lower plants kinetochore is trilamilar type while ball and cup type found in higher plants (Fig. 9.3).
- (d) **Telomeres:** The terminal ends of chromosomes are known as telomeres. Telomere is a short repeated GC rich DNA sequence complex with proteins. They are synthesized separately and later add to the chromosomal tips.

The telomeres help is various ways:

- (i) It provides stability by preventing end fusions of chromosomes.
- (ii) It acts as initiators of synapsis.
- (iii) Shortening of telomeres causes senescence and aging.

9.3.1.3. Functions of Chromosomes:

- 1. It contains genetic information in the form of genes and act like as hereditary vehicle.
- 2. It controls cellular differentiation, metabolism, cell division and growth.
- 3. Ploidy in chromosomes determines the expression of gametophyte or sporophyte generation.
- 4. Sex chromosomes determine sex of the individuals.
- 5. Crossing over and aberrations of chromosomes introduce variations in population.
- 6. They transmit hereditary information from generation to generation.
- 7. They maintain the continuity of life by replication.
- 8. They produce variations through changes in their genes and contribute to the evolution of the organisms.

9.3.1.4. Packaging of DNA in a metaphase chromosome: The nucleus is the site in which storage and replication of the chromosomes occur, where DNA is associated with proteins.

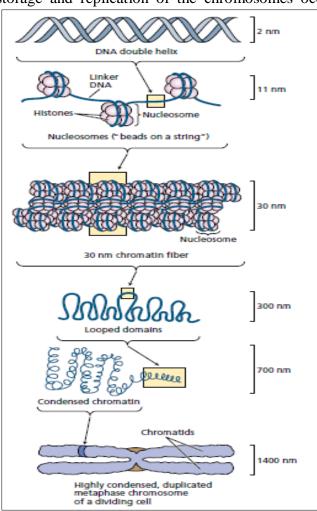


Fig.9.4. Packaging of DNA in a metaphase chromosome

Collectively, this DNA-protein complex is called chromatin. The linear length of the entire DNA within any plant genome is usually millions of times greater than the diameter of the nucleus in which it is found. To solve the problem of packaging this chromosomal DNA within the nucleus, segments of the linear double helix of DNA are coiled twice around a solid cylinder of eight histone protein molecules, forming a nucleosome. Nucleosomes are arranged like beads on a string along the length of each chromosome. During mitosis, the chromatin condenses, first by coiling tightly into a 30 nm chromatin fiber, with six nucleosomes per turn, followed by further folding and packing processes depend that interactions between proteins and nucleic acids. At interphase, two types of chromatin visible: heterochromatin euchromatin. About 10% of the DNA consists of heterochromatin, highly compact and transcriptionally inactive form of chromatin. The rest of the DNA consists of euchromatin, the dispersed, transcriptionally active form. Only about 10% of the

euchromatin is transcriptionally active at any given time.

The DNA is first aggregated into nucleosomes and then wound to form the 30 nm chromatin fibers. The remainder exists in an intermediate state of condensation, between heterochromatin and transcriptionally active euchromatin (Fig. 9.4).

9.3.2. Genes

The term "gene" was defined as a segment of a chromosome that determines a single character or phenotype (visible property), such as eye color. Beadle and Tatum proposed a molecular definition of a gene in 1940. After exposing, the spores of the fungus *Neurospora crassa* to x-rays and other agents known to damage DNA and cause alterations in DNA sequence (mutations), they detected mutant fungal strains that lacked one or another specific enzyme, sometimes resulting in the failure of an entire metabolic pathway.

Beadle and Tatum concluded and defined that a gene is a segment of genetic material that determines or codes for one enzyme: 'the one gene—one enzyme hypothesis'. Later this concept was broadened to one gene—one polypeptide, because many genes code for proteins that are not enzymes or for one polypeptide of a multisubunit protein.

The modern biochemical definition of a gene is even more precise. A gene is the 'entire DNA that encodes the primary sequence of some final gene product', which can be either a polypeptide or RNA with a structural or catalytic function. DNA also contains other segments or sequences that have a purely regulatory function. Regulatory sequences provide signals that may denote the beginning or the end of genes, or influence the transcription of genes, or function as initiation points for replication or recombination. Some genes can be expressed in different ways to generate multiple gene products from one segment of DNA.

How many genes are found in a single chromosome? The *E.coli* chromosome, one of the prokaryotic genomes that have been completely sequenced, is a circular DNA molecule with 4,639,221 bp. These base pairs encode about 4,300 genes for proteins and another 115 genes for stable RNA molecules. While in eukaryotes, the approximately 3.2 billion bp of the human genome include 30,000 genes on 24 distinct chromosomes (Table.1).

Organisms	Total DNA (bp)	Number of chromosomes	Approximate number of genes
Bacterium (Escherichia coli)	4,639,221	1	4,405
Yeast (Saccharomyces cerevisiae)	12,068,000	16	6,200
Nematode (Caenorhabditis elegans)	97,000,000	12	19,000
Plant (Arabidopsis thaliana)	125,000,000	10	25,500
Fruit fly (<i>Drosophila</i> melanogaster)	80,000,000	18	13,600

Table-1: DNA, gene, and chromosome content in some genomes

Plant (Oryza sativa; rice)	480,000,000	24	57,000
Mouse (Mus musculus)	2,500,000,000	40	30,000–35,000
Human (Homo sapiens)	3,200,000,000	46	30,000–35,000

9.3.2.1. Eukaryotic genes and chromosomes are very complex

In many bacterial species have only one chromosome per cell and each chromosome having only one copy of each gene. The organization of genes in eukaryotic DNA is structurally and functionally more complex. In eukaryotic genomes, many, if not most, eukaryotic genes have a distinct and complex structural feature occurred. Their nucleotide sequences possess more than one intervening segments of DNA that do not code for the amino acid sequence of polypeptide product.

Such kind of non-translated inserts interrupt the otherwise collinear relationship between the nucleotide sequence of the gene and the amino acid sequence of the polypeptide it encodes. Such non-translated DNA segments in genes are known as intervening sequences or 'introns' and the coding segments are known 'exons'.

9.3.3. Nucleic acid

Nucleotides have a variety of roles in cellular metabolism. They are the energy currency in metabolic transactions, the essential chemical links in the response of cells to hormones and other extracellular stimuli, and the structural components of an array of enzyme cofactors and metabolic intermediates. And they are the constituents of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the molecular repositories of genetic information. The structure of every protein, and ultimately of every biomolecule and cellular component, is a product of information programmed into the nucleotide sequence of a cell's nucleic acids. The ability to store and transmit genetic information from one generation to the next is a fundamental condition for life.

9.3.3.1. Nucleotides and nucleic acids have characteristic bases and pentoses

Nucleotides have three characteristic components: (i) a nitrogenous (nitrogen-containing) base, (ii) a pentose, and (iii) a phosphate. The molecule without the phosphate group is called a nucleoside. The nitrogenous bases are derivatives of two parent compounds, pyrimidine and purine. The bases and pentoses of the common nucleotides are heterocyclic compounds. The carbon and nitrogen atoms in the parent structures are conventionally numbered to facilitate the naming and identification of the many derivative compounds. The base of a nucleotide is joined by covalently (at N-1 of pyrimidines and N-9 of purines) in an *N*-glycosyl bond to the 1 carbon of the pentose, and the phosphate is esterified to the 5 carbon.

The *N*-glycosyl bond is formed by elimination of the water (a hydroxyl group from the pentose and hydrogen from the base), as in *O*-glycosidic bond formation. Both DNA and RNA contain two major purine bases, adenine (A) and guanine (G), and two major pyrimidines. In

both DNA and RNA one of the pyrimidines is cytosine (C), but the second major pyrimidine is not the same in both: it is thymine (T) in DNA and uracil (U) in RNA (Fig. 9.5).

Fig. 9.5. General Structure showing the pyrimidine and purine bases of nucleotides and nucleic acids

9.3.3.2. Phosphodiester Bonds Link Successive Nucleotides in Nucleic Acids

The successive nucleotides of both DNA and RNA are covalently linked through phosphate-group "bridges," in which the 5'phosphate group of one nucleotide unit is joined to the 3' droxyl group of the next nucleotide, creating a **phosphodiester linkage**. Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals. The backbones of both DNA and RNA are hydrophilic. The hydroxyl groups of the sugar residues form hydrogen bonds with water. The phosphate groups, with a pKa near 0, are completely ionized and negatively charged at pH 7, and the negative charges are generally neutralized by ionic interactions with positive charges on proteins, metal ions, and polyamines (Fig.9.6).

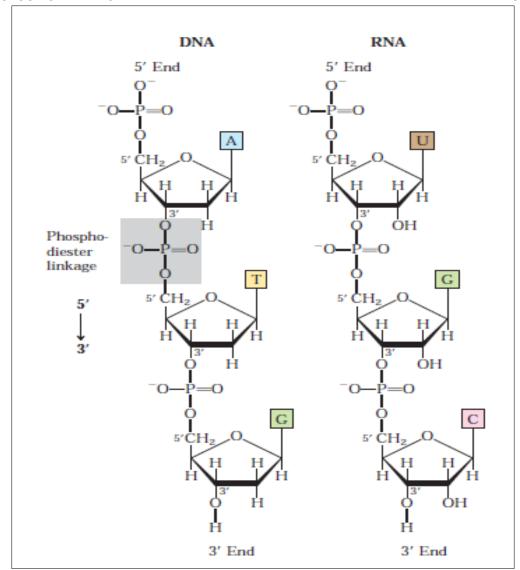


Fig.9.6. Phosphodiester linkages in the covalent backbone of DNA and RNA

The phosphodiester bonds (one of which is shaded in the DNA) link successive nucleotide units. The backbone of alternating pentose and phosphate groups in both types of nucleic acid is highly polar. The 5' end of the macromolecule lacks a nucleotide at the 5' position, and the 3' end lacks a nucleotide at the 3' position.

All the phosphodiester linkages have the same orientation along the chain, giving each linear nucleic acid strand a specific polarity and distinct 5' and 3'ends. By definition, the **5'end** lacks a nucleotide at the 5'position and the **3'end** lacks a nucleotide at the 3'position. Other groups (most often one or more phosphates) may be present on one or both ends. The covalent backbone of DNA and RNA is subject to slow, non enzymatic hydrolysis of the phosphodiester bonds. In the test tube, RNA is hydrolyzed rapidly under alkaline conditions, but DNA is not; the 2'-hydroxyl groups in RNA (absent in DNA) are directly involved in the process. Cyclic 2', 3'

monophosphate nucleotides are the first products of the action of alkali on RNA and are rapidly hydrolyzed further to yield a mixture of 2' and 3'nucleoside monophosphates.

9.4 LAMPBRUSH 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.

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** which were first discovered by **Flemming** in 1882 and were described in shark oocytes by **Ruckert** (1892).

9.4.1. Lampbrush chromosome structure

Lampbrush chromosome structure has been studied mainly in urodele amphibia (Box 14.1), from which the following description is largely derived, although the essential features are the same in all organisms. The subject has been reviewed by Macgregor (1980, 1993) and Callan (1982, 1986) and most recently by Morgan (2002). Lampbrush chromosomes are diplotene bivalents; therefore, they consist of two axis connected at the chiasmata, and the loops extend on both sides of the axis.

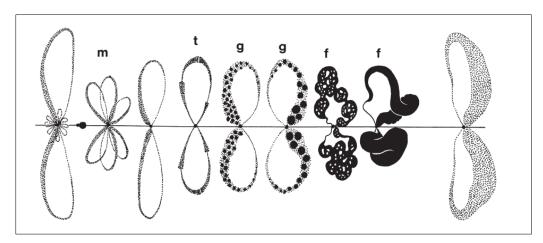


Fig. 9.7. Different types of loops on lampbrush chromosomes

The axis of a lampbrush chromosome consist of a series of dense granules, 0.25–2.0 mm in diameter and 1–2 mm apart; there may be in the region of 5000 of these granules in the haploid genome. These granules consist of deoxyribonucleoprotein and are often, confusingly, referred to as chromomeres. These granules are clearly not the same as the chromomeres of

pachytene chromosomes, which are much larger and many fewer in number. On the other hand, the number of these granules is similar to that of the 'chromomeres' of polytene chromosomes (Fig. 9.7).

9.4.2. Behavior

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. The 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.9.8).

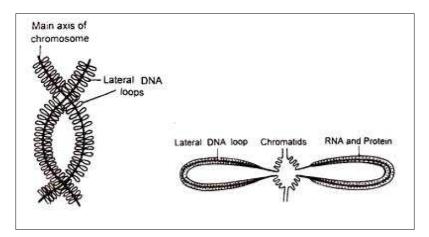


Fig. 9.8. Detailed structure of lampbrush chromosome

9.4.3. Significance

Significance of lampbrush chromosomes is as below:

- (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.

9.5. B-CHROMOSOMES

The B-Chromosomes, also referred as to as supernumerary or accessory chromosomes, are the additional (extra) chromosomes which are present in some individuals in certain species. In eukaryotic cells, normal chromosomes are termed as A-chromosomes. Most of the B-Chromosomes are chiefly or entirely heterochromatic and genetically inert. They are thought to be selfish genetic material with no defined function. The evolutionary origin of B chromosomes is not clear, but presumably they must have been derived from heterochromatic segment of normal A-chromosoems (Fig.9.9).

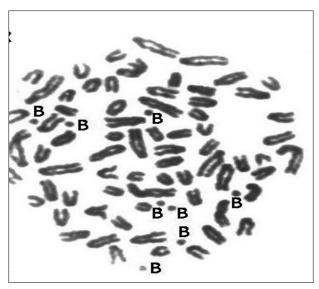


Fig. 9.9. A mitotic metaphase plate showing the presence of B Chromosomes

9.5.1. Structure

B chromosomes are smaller than A chromosomes except in a few cases, in which they are of equal size. They often have distinct centromere positions and can be readily identified at mitosis. Variants include B chromosomes as isochromosomes or telocentrics, and in a few species, they appear as microchromosomes.

9.5.2. Behavior

In addition to the normal karyotype wild populations of many animal, plant and fungi species contain B chromosomes. These chromosomes are not essential for the life of a species, hence they are lacking in some of the individuals.

9.5.2.1. Gene content and sequence organization

No B-chromosome-localized gene has been isolated in plants yet but they might have ribosomal DNA, some organize nucleoli and some have genetic information controlling their own transmission (e.g. in rye and maize). The strongest evidence to date for genes on B chromosomes

comes from the fungal plant pathogen *Nectria haematococca*, which infects peas. Several genes determining pathogenicity have been identified and located on the 1.6 Mb supernumerary B chromosomes in this fungus, and electrophoretic karyotypes indicate that several fungal species carry dispensable supernumerary B chromosomes. The chromosomes of these fungi are too small to be resolved by light microscopy and so we have no information about how they behave at meiosis.

9.5.2.2 Inheritance

The inheritance of B chromosomes is non-mendelian and irregular owing to vagaries in the levels of paring, to degrees of meiotic elimination and to various drive processes. Drive is mostly caused by directed non disjunction of sister chromatids at the first pollen mitosis, such that the generative nucleus carries the unreduced number, which then forms the sperm (e.g. many species of Gramineae). In rye, unusually, this drive happens on both the male (Fig. 2d) and the female side, and in maize, the no disjunction happens at the second pollen mitosis, followed by preferential fertilization by the B-chromosome containing sperm. Meiotic drive and accumulation at earlier developmental stages in the germ line operate in a few cases. These irregularities in transmission generate a numerical polymorphism in populations, with a spectrum of B chromosome numbers including individuals with none. There is usually a modal number and an equilibrium frequency based on a balance between drive and the harmful effects caused by high numbers. Drive is by no means a universal process: it is known in 60% of species for which transmission data are available. In the others, there is no known drive and no real understanding of how the population equilibrium frequencies are maintained.

9.5.3. Significance of B-chromosomes

The significance of B chromosomes is to be found in their widespread occurrence in hundreds of flowering plants, and also in gymnosperms and in some lower forms such as ferns, bryophytes and fungi (they are also common in animals, including mammals). Owing to their particular properties, B chromosomes have been used to elucidate the function of post-translational histone modifications, such as histone H3 phosphorylation and methylation. They are of particular interest in maize (*Zea mays*), in which they have been extensively used in genetic analysis involving A–B translocations for mapping for the identification of centromere structure and size. In other species, there is interest in their capacity to behave as diploidizing agents for chromosome pairing in certain allopolyploid hybrids and their influence on recombination through the modulation of chiasma frequency and distribution in the A chromosomes (e.g. in rye).

There is evidence of deleterious effects of supernumeraries on pollen fertility and favourable effects or associations with particular habitats are also known in a number of species. B chromosomes may play a positive role on normal A chromosomes in some circumstances. In wheat, an allopolyploid, the B chromosomes suppress homologous pairing which reduces multiple pairing between homologous chromosomes. Bivalent pairing is ensured by a gene on

chromosome 5 of the B genome Ph locus (Jones and Houben, 2003). The B chromosomes also have the following effects on A chromosomes:

- increases asymmetry chiasma distribution
- increases crossing over and recombination frequencies: increases variation
- cause increased unpaired chromosomes: infertility

B chromosomes have tendency to accumulate in meiotic cell products resulting in an increase of B number over generations, thereby acting as selfish genetic elements. However this effect is counterbalanced for selection against infertility.

9.6 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, 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, aelocentric 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) and lampbrush chromosomes (Fleming 1882). The former occurs 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. The Lampbrush chromosomes are found in yolk rich oocytic nuclei of certain vertebrates. They bear fine lateral loops arising from the chromosomes during the first prophase of meiosis.

9.7 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.

9.8 SELF ASSESSMENT QUESTIONS

9.8.1. Multiple Choice Questions

1	The	DNA	threads	which	annear	inside	the	nucleus	at the	time	of cel	1 division
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(a) Spindle fibers

(b) Centrioles

(c) Asters

(d) Chromosomes

2. Which of the following is not a major class of chromatin proteins?

(a) Histones

(b) Topoisomerases

(c) SMC proteins

(d) Cohesins

3. Which of the following plays a substantial role in linking together sister chromatids immediately after replication?

(a) Cohesins

(b) Condensins

(c) Histones	(d) Topoisomerases			
4. Chromatin is composed of				
(a) DNA	(b) DNA and proteins			
(c) DNA, RNA and proteins	(d) None			
5. Which of the following statements is true?				
(a) The template strand matches the sequence of	the RNA transcript			
(b) The two strands of DNA run parallel to each				
(c) G-C bonds are much more resistant to denatu	ration than A-T rich regions			
(d) The common form of DNA is left handed				
6. Number of hydrogen bonds between adenine a	and thymine?			
(a) 1	(b) 2			
(c) 3	(d) 4			
7. Which of the following is the correct order of smallest?	organization of genetic material from largest to			
(a) Genome, chromosome, gene, nucleotide				
(b) Nucleotide, gene, chromosome, genome				
(c) Gene, nucleotide, chromosome, genome				
(d) Chromosome, genome, nucleotide, gene				
8. Which of the following are not the component	s of RNA?			
(a) Thymine	(b) Adenine			
(c) Guanine	(d) Cytosine			
9. DNA replication occurs in which stage of cell	division?			
(a) G1 phase	(b) G2 phase			
(c) S phase	(d) M phase			
10. Bacterial genes lack				
(a) Exons	(b) Introns			
(c) Promoters	(d) Operators			
9.8.2. Fill in the blank:				
1. The composition of nucleotide is a base + a su	gar +			
2. In DNA, group of adjacent nucleotides are join				
3. The sister chromatids separate at				
4. The sugar in RNA is, the sugar	-			

5. Which pyrimidine base contains an amino group at carbon 4 is.....

9.8.3. Very Short Questions:

- 1. How are alleles of particular gene different? Explain its significance.
- 2. Which component of the chromosomes is responsible for heredity?
- 3. Explain heterochromatin.
- 4. Name the part of a chromosome separated by a secondary constriction?
- 5. How does DNA replication differ between eukaryotes and prokaryotes?
- 6. How do RNA molecules structurally differ from DNA molecules?
- 7. Distinguish between introns and spacer DNA.
- 8. What is metacentric and telocentric region in chromosomes?
- 9. What is the role of kinetochores?
- 10. Explain B-chromosomes?

Answer keys:

9.8.1. Multiple choice questions

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

9.8.2. Fill in the blanks:

1. phosphate, 2. Phosphodiester bond, 3. Anaphase, 4. Ribose, deoxyribose, 5. Cytosine

9.8.3. Very Short Questions:

- 1. Alleles of a particular gene differ from each other on the basis of certain changes (i.e., mutations) in the genetic material (segment of DNA or RNA). Different allele of a gene increases the variability or variation among the organisms.
- 2. DNA
- 3. Darkly stained regions of chromosomes are called heterochromosome
- 4. Satellite
- 5. The basic mechanisms of prokaryotic and eukaryotic DNA replication are similar. However, eukaryotes have numerous linear chromosomes, each with many oriR sites whereas prokaryotes have only one replication origin on a single circular chromosome. Eukaryotes have more DNA polymerases than prokaryotes. Different proteins bind to the origins to initiate unwinding of the DNA prior to replication.
- 6. RNA contains uracil rather than thymine, has ribose rather than deoxyribose as the pentose sugar, and is usually single-stranded, whereas DNA is usually double stranded.
- 7. In more complex eukaryotes such as humans, it is estimated that over 95% of the genome consists of noncoding DNA. Much of the noncoding DNA is found between genes. This is referred to as spacer DNA. Introns are located within genes and are transcribed by RNA

polymerase. They are later removed and the exons (coding regions) are spliced together to produce the final mRNA.

- 8. A centromere is a highly constricted region of a mitotic or meiotic chromosome where the spindle fibers attach. Complex sequences of DNA constitute centromeres. If the centromere is in the middle of the chromosome, the chromosome is said to be metacentric. If the centromere is near the tip, it is called telocentric.
- 9. Protein complexes associated with the centromeric regions are called kinetochores. Kinetochores bind microtubules of the spindle bundle and function to distribute chromosomes as cells proliferate.
- 10. B-chromosomes (Bs) are dispensable components of the genome exhibiting non-Mendelian inheritance and have been widely reported on over several thousand eukaryotes.

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9.11 TERMINAL QUESTIONS

1. Describe lampbrush chromosomes present in spermatocyte nucleus of *Drosophila* with diagram.

- **2.** Classify the eukaryotic chromosomes on the basis of the position of centromere. How is it related with karyotype asymmetry?
- **3.** Describe the structure of eukaryotic chromosomes, and discuss the compositions and organization of DNA and histone proteins within chromosomes.
- **4.** Discuss about the structure of B-chomrosomes and also describe the significance of B-chromosomes?
- **5.** What is nucleic acid? Describe their structure and function.

UNIT-10 CELL SIGNALING AND CELL RECEPTORS

Contents

- 10.1 Objectives
- 10.2 Introduction
- 10.3 Cell Signaling
- 10.4 Cell Receptors
- 10.5 Summary
- 10.6 Glossary
- 10.7 Self Assessment Question
- 10.8 References
- 10.9 Suggested Readings
- 10.10 Terminal Questions

10.1 OBJECTIVES

After reading this unit students will be able-

- To understand the overviews of the cell signaling and cell receptors
- To know about the characteristics of signal perception, transduction and integration in plants

10.2 INTRODUCTION

Plant cells detect a wide range of signals arising within the plant or the surrounding environment, and they use this information to regulate their behavior. Cell signaling is one of the important processes required for the normal growth and development of cell. It is the basic process that helps the cells to sustain through various environmental cues and develop tolerance against stress conditions. The basic cell signaling machinery involves a receptor molecule that perceives the signal: (i) The signal or primary stimulus could be light, hormone, antigen, neurotransmitter or the surface molecules of another cell, which are transported into the cell via membrane receptor, through signal transduction triad (receptor/transducer/effector).

- (ii) The second messenger could be Ca²⁺ (for ion channels), cAMP and cGMP (for adenylyl and guanlyl cyclases), inositol-1, 4, 5-triphosphate (IP3), diacyl glycerol (DAG) and arachidonic acid (for phospholipases).
- (iii) The triad is responsible for converting the signal from first to second messenger, which could be further regulated by protein kinases or phosphatases in the cytoplasm. The target of the signal may be enzymes, intracellular receptors, special transport vehicles and finally transcription factors, which ultimately controls the gene expression.

10.3 CELL SIGNALING

Plants perceive the environmental signals such as light and chemicals and respond by changing their morphologies. Signaling pathways utilize a complex network of biochemical and physiological responses such as flowering, fruit ripening, germination, photosynthetic regulation, and shoot or root development. Plants regulate their biology in accordance with diverse environmental and internal cues.

In under physical environment, plant cells also detect gravity, temperature, local distributions of soil nutrients and water and mechanical forces such as wind, pressure between the root and soil, and internal tension or compression. They also sense the concentrations of gases such as CO₂, O₂, and ozone as well as the intensity, direction, and spectral quality of light. In biotic environment, cells also detect the presence of pathogens, herbivores, neighboring plants, and symbiotic bacteria and fungi. Internally, plants monitor their developmental stage, health, and water, nutrient, and photosynthate status. In addition, signaling interactions between cells in developing regions of the plant that determine patterns of cells, tissues, and organs, while long-range signals also control larger patterns of development (Fig.10.1).

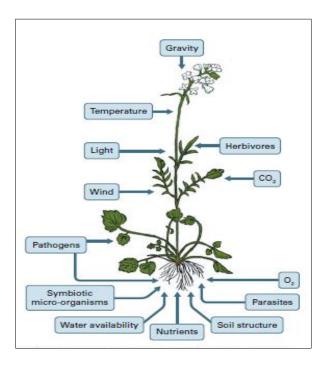


Fig.10.1. External factors that affects plant physiology, defense, and development comes from many aspects of the plant's physical and biological environments

Cell signaling can be understood by following stages/model:

- **1. Receptors:** A cell detects a signaling molecule from the outside of the cell. A signal is detected when a ligand binds to a receptor protein on the surface of the cell or inside the cell.
- **2. Transduction:** When the signaling molecule binds to the receptor, it changes the receptor protein. This change initiates the process of transduction. Each relay molecule in the signal transduction pathway changes the next molecule in the pathway.
- **3. Response:** Finally, the signal triggers a specific cellular response (Fig. 10.2).

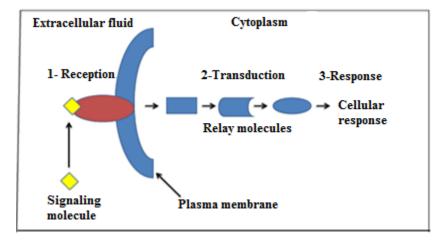


Fig.10.2.Cell signalling stages

10.3.1. Chains of signal-transducing molecules connect perception to response

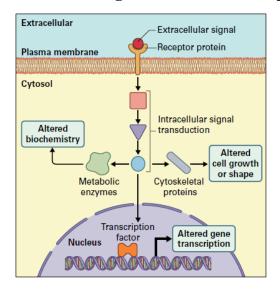


Fig. 10.3. Plant signal perception, transduction and response

An extracellular signal is shown to bind a plasma membrane receptor protein. The receptor controls the activity of an intracellular transduction pathway that regulates cell responses to the signal. Overall, information inherent to the signal is transferred from the site of perception to sites of response within the cell.

Plants perceive environmental information that is may be physical (e.g. light intensity and temperature) and chemical (e.g. water availability and nutrient concentration). Signals operating between the plant cells are mostly chemical in nature although exceptions can be made for electrical events at membranes and physical forces generated by tissue growth. Regardless of signal type, plant cells perceive most environmental information and intercellular signals at dedicated receptor proteins. Signal perception results in a change in the activity of receptor protein to regulate an intracellular signal transduction pathway.

Operation of the transduction pathway then depends on interactions between its component signal-transducing molecules, which often result in covalent modifications and/or allosteric changes. For example, some receptor proteins respond to signal perception by activating intracellular protein kinases, enzymes that phosphorylate proteins. Phosphorylation typically causes an allosteric change in the target protein that can change its activity to induce either a change in cell behavior or downstream signaling. Interactions within a transduction pathway often also lead to movement of signaling molecules within the cell, for example through the release of a signaling protein from an anchoring complex or opening of ion channels. In such ways, transduction pathways transfer information inherent to the perceived signal through the cell to the sites of response (Fig.10.3).

Many pathways also eventually regulate the synthesis, activity, or stability of transcription factors, proteins that regulate gene transcription. This may reflect a central role for transcriptional control in establishing overall patterns of cellular behavior.

10.3.2. The plasma membrane is a major site of signal perception

Plant cells have mainly two routes through which they communicate to each other: (i) the apoplast pathway, formed by the interconnected cell walls of the plant, while the symplast, (ii) the cytoplasmic continuum created by strands of cytoplasm connecting neighboring cells via plasmodesmata. With respect to intercellular signaling, the apoplast is involved with the transmission of signals such as the plant hormones and signal peptides, which require conventional receptor proteins. Signaling via the symplast allows regulated movement of RNAs and transcription factor (TFs) proteins, which can induce responses in the receiving cell more directly.

10.4 CELL RECEPTORS

The plasma membrane is a major site of perception for signals arriving at the cell through the apoplast; indeed, large and hydrophilic molecules that lack a membrane import channel can only be perceived by plasma membrane receptors. Receptors at the membrane also perceive some physical signals, for example mechanical force and blue light.

Receptors are divided into three major groups distinguished by how they interact with intracellular signaling components:

- (i) Receptor kinases
- (ii) G protein-coupled receptors
- (iii) Ion channel receptors

10.4.1. Receptor kinases

The receptor kinases are the largest groups of membrane receptors in plants, and they transduce extracellular signals through phosphorylation of intracellular targets. Phosphorylation is the most common posttranslational modification used by cells to regulate protein activity. Catalyzed by kinases, phosphorylation can alter protein stability, subcellular location, binding properties, enzyme activity, and susceptibility to subsequent modifications. Furthermore, single proteins may be phosphorylated at multiple amino acid residues, sometimes by different kinases and with different effects, enabling integration of separate signal transduction pathways. Consequently, it is not surprising that kinases and phosphatases (which dephosphorylate proteins) play the vital roles in signal transduction in all organisms.

10.4.1.1. Receptor-like Kinases (RLKs) in Plants

In plants, signal perception appears to rely heavily on plasma membrane via receptor like kinases (RLKs), a family of over 600 proteins reported in *Arabidopsis* and over 1,100 in rice (*Oryza sativa*) that typically phosphorylate to target proteins on serine and threonine residues of amino acid. RLKs possess an extracellular domain that provides potential ligand-binding sites, a transmembrane and intracellular kinase domain.

Based on the predicted structure of their extracellular domains, this super family at kinases is divided into 20 families and many more subfamilies also. Out of this two common motifs are: (i) the S domain and, (ii) the leucine- rich repeat (LRR). They act in a wide range of processes, including hormone perception, development, defense, symbiosis, and pollen tube germination. The best understood of the signal transduction mechanisms mediated by RLKs is the perception of brassinosteroids (BRs) which is a plant hormone. RLKs also function in the perception of CLE peptides (CLAVATA3/ENDOSPERM SURROUNDING REGION).

The LRR motif is characterized by several tandem repeats of amino acid sequences rich in leucine with a conserved core sequence Leu-x-x-Leu-x-Leu-x-Asnx- Leu. This repeat may be interrupted by gaps or insertions within or between repeats. The LRR motifs occur in many other proteins besides transmembrane RLKs and are thought to mediate protein-protein interactions. Extracellular domains of RLKs with an LRR motif show considerable variations outside of the LRR region (Fig.10.4).

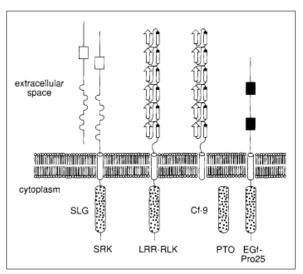


Fig.10. 4. Receptor-like kinases (RLKs) and RLK-related proteins; a S-locus glycoprotein (SLG) with an S domain; a S-locus RLK (SRKl); an RLK with a leucine-rich repeat (LRR) motif; Cf-9, a protein conferring disease resistance to a leaf mold of tomato; PTO, a Ser/Thr kinase that confers resistance in tomato to a bacterial pathogen; and Pro25 with an epidermal growth factor-like domain (based on Braun and Walker, 1996).

10.4.2. G protein-coupled receptors (GPCR)

There are several signaling mechanisms present in the cell to carry out normal functions. One of an important signaling cascade is formed by GTP binding proteins, simply known as G proteins, because of their ability to bind to guanine nucleotide. G protein-coupled receptors regulate trimeric GTP-binding proteins (G proteins) located on the inner surface of the membrane. One more molecule that is involved in this signaling cascade and forms an important part of the cascade is G Protein Coupled Receptor (GPCR). It is known that the signals are mostly perceived at the level of membrane and, therefore, transmembrane (TM) events are the likely routes for signal generation and transduction.

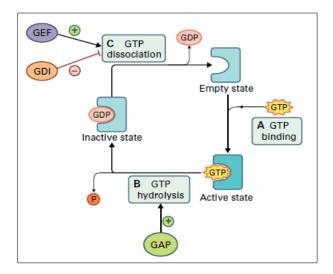


Fig. 10.5. Hydrolysis of GTP

The monomeric G proteins are converted from an empty state to an active state by GTP binding. B shows that they are inactivated by GTP hydrolysis, which is stimulated by a GTPase activating protein (GAP). C shows GDP release, which is inhibited by GDP dissociation inhibitors (GDIs) and stimulated by guanine nucleotide exchange-factors (GEFs), returns the G protein to its empty state and allows reactivation (Fig. 10.5).

10.4.2.1. Heterotrimeric G-proteins

Heterotrimeric G-proteins are so called because they are a complex of three separate proteins, $G\alpha$ (Mw - 35-45 kDa), $G\beta$ (Mw- 35-36 kDa), and $G\gamma$ (Mw- 8- lOkDa). The $G\alpha$ subunit acts as a GTPase that binds and hydrolyzes the GTP; the $G\beta$ and $G\gamma$ subunits are members of a family of dimeric proteins that have been recruited for functioning of the $G\alpha$ subunit.

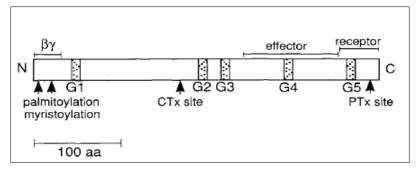


Fig. 10.6. Schematic structure of a Ga protein

The N and C termini of the polypeptide are indicated along with an approximate scale in amino acids (Fig.10.6). The five conserved regions (Gl through G5) loop together form the guanine nucleotide-binding domain. G α subunits are lipid modified at their N termini through the addition of a myristoyl and a palmitoyl moiety, which tethers them to the plasma membrane. Regions believed to bind to the G γ dimer, downstream effectors, and C terminus of the cognate

7TM receptor, are indicated. Sites that are targets for ADP-ribosylation by cholera (CTx) and pertussis (PTx) toxins are also indicated. They are present in some, but not all, Ga subunits.

 $G\alpha$ proteins, like other GTPases, are structurally highly diverse, except for five conserved domains, Gl through G5, which are involved in GTP binding and hydrolysis. The variable parts include the C terminus, which interacts with the 7TM receptor and with the downstream effectors, and the N terminus, which interacts with the GP7 dimer.

 $G\beta$ subunits belong to the WD40 family of proteins. Members of this family have a variable N-terminal region followed by multiple copies of a repeat motif, 4 to 16 copies of which can be present in a single protein. The motif consists of amino acid sequences of more or less constant length (Mw~ 44 to 60 residues) which typically end with a tyrosine-aspartic acid (WD) pair at the C terminus of the sequence; hence, the "WD" repeat (Fig. 10. 7).

 $G\gamma$ subunits are more diverse in structure. Their internal sequences determine their association with different $G\beta$ units. Each $G\gamma$ subunit is prenylated and carries a lipid chain at the C terminus, which anchors the $G\gamma$ dimer and the inactive trimer to the plasma membrane.



Fig. 10.7. Schematic illustration of a protein with WD repeats. Only three repeats are shown

G-Protein coupled receptors (GPCRs) regulate the activity of trimeric GTP-binding proteins (G proteins. These consist of $G\alpha$, $G\beta$, and $G\gamma$ subunits, and as trimers they are inactive and bound at the $G\alpha$ subunit by a molecule of GDP. GPCRs possess an extracellular ligand-binding domain, a transmembrane domain composed of seven hydrophobic helices, and an intracellular domain that interacts with inactive G protein trimers. Ligand binding by the receptor induces the $G\alpha$ subunit of the trimer to release its bound GDP molecule and bind a molecule of GTP instead. This activates the G protein, which uncouples from the receptor and dissociates into the $G\alpha$ subunit and a $G\beta/G\gamma$ dimer.

The $G\alpha$ and $G\gamma$ both have short, covalently attached lipid tails that anchor them to the inside of the plasma membrane so that $G\alpha$ and the $G\beta/G\gamma$ dimer diffuse on the membrane to activate downstream signaling proteins. A single ligand bound GPCR can amplify a signal by activating multiple G proteins. Signaling is terminated by the hydrolysis of bound GTP to GDP by $G\alpha$ (which has GTPase activity), and this process is stimulated by GTPase activating proteins (GAPs). GDP-bound $G\alpha$ reassociates with the $G\beta/G\gamma$ dimer to form an inactive trimer, which then binds a GPCR to complete the cycle. The alternation of $G\alpha$ between an active GTP-bound and an inactive GDP-bound form is a general feature of the GTP-binding protein super family. The superfamily includes GTP-binding initiation and elongation factors that act in protein

synthesis, and also the small or monomeric G proteins, whose functions include signal transduction, organelle trafficking, and cytoskeleton assembly.

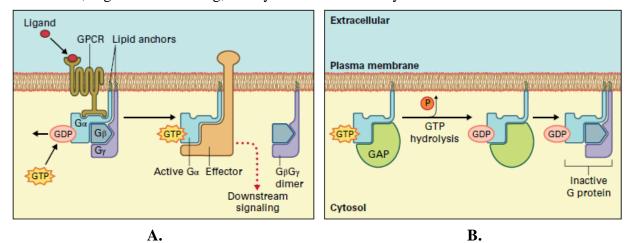


Fig.10.8. Regulation of heterotrimeric G proteins. (A) Ligand binding by a G protein coupled receptor (GPCR) induces the Ga subunit of the heterotrimeric G protein to exchange GDP for GTP. This leads to the dissociation of the G protein into active Ga and G β /G γ subunits that regulate the activity of downstream effector proteins (G β /G γ activity not shown). (B) Ga is inactivated by the hydrolysis of bound GTP to GDP, which is stimulated by a GTPase activating protein (GAP). Inactive Ga binds to the G β /G γ dimer, reconstituting the inactive heterotrimeric G protein

The regulation of monomeric G proteins has parallels with the regulation of G α activity. Monomeric G proteins are activated by guanine nucleotide exchange factors (GEFs), which induce the exchange of GDP for GTP, a function equivalent to that of GPCRs. As is the case for regulation of G α activity, the hydrolysis of GTP to GDP by monomeric G proteins is stimulated by GAPs. Lastly, GDP dissociation inhibitors (GDIs) prevent the spontaneous release of GDP by monomeric G proteins, a role played by the G β /G γ dimer in trimeric G proteins (Fig.10.8).

10.4.2.2. Effector Enzymes and Secondary Messengers

Effector enzymes are responsible for regulating the intracellular concentrations of secondary messengers, such as cyclic AMP (cAMP), cyclic GMP (cGMP), diacylglycerol (DAG), inositol-l,4,5-triphosphate (IP3), and calcium (Ca⁺), which in turn regulate the activities of many secondary messenger-dependent protein kinases or phosphatases. Nucleotide cyclases and phosphodiesterases regulate the intracellular levels of cAMP and cGMP. Adenylate cyclase produces 3'5'-cyclic AMP from ATP, whereas adenylate phosphodiesterase degrades it to 5' AMP (Fig10.9).

The intracellular levels of DAG and calcium are regulated by the coupling of G-protein signaling and the phosphoinositide pathway. Phosphatidylinositol (PI) is an abundant metabolite in most plant cells. It is phosphorylated in succession to give phosphatidylinositol phosphate (PIP) and phosphatidylinositiol-4, 5-biphosphate (PIP2). Phospholipase C (PLC) is an effector enzyme, which occurs in multiple isoforms, both membrane bound and soluble. The P form, here

called PLC, is the isoform activated by Ga signaling. It occurs bound to the inner leaflet of the plasma membrane and, on activation, cleaves the phosphodiester bond between PIP2 and DAG to give inositol-l, 4, 5-triphosphate. DAG is hydrophobic and stays bound to the inner leaflet of the plasma membrane, but IP3 is water soluble and freely mobile. On release from PIP2, it diffuses to its receptor sites on vacuolar and ER membranes. The IP3 receptor is a tetrameric transmembrane protein, which forms a Ca⁺ specific channel; the channel opens on binding of the ligand IP3. The NH₂-terminal in the cytoplasm carries the IPs-binding site, while the transmembrane carboxy terminus forms the channel through which stored Ca⁺ moves out into the cytoplasm. PLC thus regulates the intracellular levels of free Ca⁺ and DAG (Fig.10.10).

10.4.3. Ion channel receptors

Ion channels are proteinaceous pores in the plasma membrane or vacuolar membrane, or membrane of an organelle (e.g., chloroplast, mitochondria or endoplasmic reticulum). They span the membrane and occur in "open" or "close" conformational states. They may be specific to an ion, or nonspecific, and may be stretch, voltage, or receptor activated. As mentioned earlier, pharmacological studies using bacterial toxins and inactive GTP/GDP homologues have provided evidence that heterotrimeric G-proteins are involved in regulation of the inward-directed K⁺ channel in guard cells. Similar data are beginning to accumulate with regard to Ca⁺ channel activity in plants.

Fig 10.9. Synthesis and degradation of 3'5'-cyclic AMP

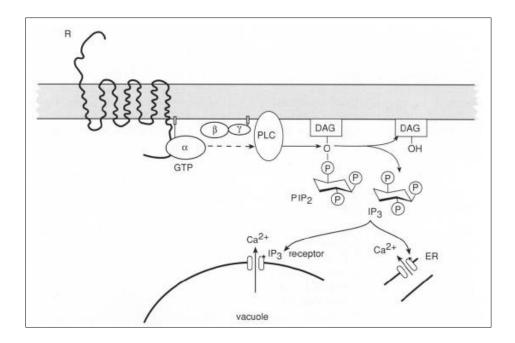


Fig. 10.10. Phosphoinositide pathway and its coupling to heterotrimeric G-protein. The GTP-Ga complex activates phospholipase C, which in turn breaks the phosphodiester bond between phosphatidylinositol- 4,5-biphosphate and diacylglycerol to give rise to inositol-1,4,5-triphosphate (IP3). IP3 is freely mobile and migrates to its receptors, on ER and vacuolar membranes, which act as ligand-gated Ca⁺channels, leading to an increase in the level of free Ca⁺.

10.4.4. The gibberellin signal transduction pathway is similar for stem growth and α -amylase production

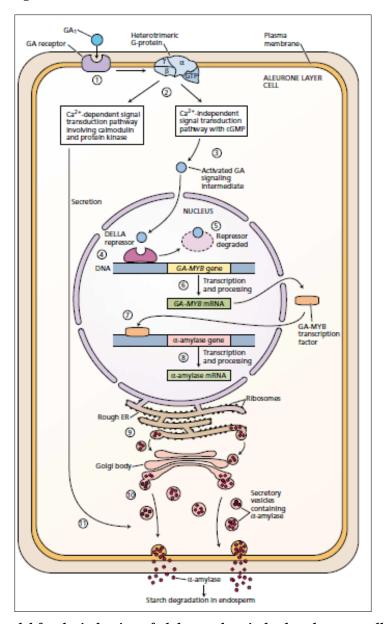


Fig.10.11. A model for the induction of alpha amylase in barley aleurone cells by gibberellin

It is widely believed that gibberellin initially acts through a common pathway or pathways in all of its effects on development. As we have seen, the genetic approaches applied to the study of gibberellin-stimulated growth led to the identification of the *SPY/GAI/RGA* negative regulatory pathway.

The proteins SPY, GAI and RGA act as repressors of gibberellins responses. Gibberellin deactivates these repressors. Because the aleurone layers of gibberellin-insensitive dwarf wheat are also insensitive to GA, the same signal transduction pathways that regulate growth appear to

regulate gibberellin-induced alpha amylase production. Indeed a *SPY*-type gene associated with alpha -amylase production has been isolated from barley (*HvSPY*), and its expression is able to inhibit gibberellin-induced alpha-amylase synthesis, while GA-MYB-type factors are also implicated in the gibberellins transduction chain regulating stem growth.

- (i) GA1 from the embryo first binds to a cell surface receptor.
- (ii) The cell surface GA receptor complex interacts with a heterotrimeric G protein, initiating two separate signal transduction chains.
- (iii) A calcium independent pathway, involving cGMP, results in the activation of a signaling intermediate.
- (iv) The activated signaling intermediate binds to DELLA repressor proteins in the nucleus.
- (v) The DELLA repressors are degraded when bound to the GA signal.
- (vi) The inactivation of the DELLA repressors allows the expression of the *MYB* gene, as well as the other genes, to proceed through transcription, processing, and translation.
- (vii) The newly synthesized MYB protein then enters the nucleus and binds to the promoter genes for a-amylase and other hydrolytic enzymes.
- (viii) Transcription of a-amylase and other hydrolytic genes is activated.
- (ix) a-Amylase and other hydrolases are synthesized on the rough ER.
- (x) Proteins are secreted via the Golgi.
- (xi) The secretory pathway requires GA stimulation via a calcium-calmodulin dependent signal transduction pathway.

10.5 SUMMARY

Cell signaling and transduction is the fundamental process by which specific information is transferred from cell surface to cytosol and ultimately the nucleus, leading to change in gene expression. Plant development, physiology, and defense are flexible processes regulated by numerous internal and external cues. At the cellular level, detection of physical and chemical signals by receptor proteins initiates intracellular transduction pathways that control cell behavior. In addition, transduction pathways initiated by separate signals and perceived by distinct receptors interact to form a signaling network that integrates the acquired information. Above the cellular level, short and long-range intercellular signaling coordinates the activity of tissues, organs, and the plant as a whole.

Investigations into the roles of GTPases in plant signal transduction has been progressed considerably and several small GTP binding proteins have been implicated in these processes. Cyclic nucleotides also appear to act as second messengers in plant cells and most likely interact with another second messenger, cytosolic calcium. Calcium channels and other calcium transporters form the basis of a complex Ca²⁺ signaling network in plants. Protein kinases are the most common transduction components interpreting signal in plant cells. Various classes of protein kinase act in concert with protein phosphatases to mediate plant cell signaling and control

metabolism. So conclusively, advances in signal transduction research are rapidly expanding our understanding of how plant cells communicate and cooperate.

10.6 GLOSSARY

Acetylcholine (**ACh**): Neurotransmitter that functions at vertebrate neuromuscular junctions and at various neuron-neuron synapses in the brain and peripheral nervous system.

Adenylyl cyclase: Membrane-bound enzyme that catalyzes formation of cyclic AMP (cAMP) from ATP; also called *adenylate cyclase*. Binding of certain ligands to their cell-surface receptors leads to activation of adenylyl cyclase and a rise in intracellular cAMP.

Cadherin: Protein belonging to a family of Ca²⁺-dependent cell-adhesion molecules that play roles in tissue differentiation and structure.

Calmodulin: A small cytosolic protein that binds four Ca²⁺ ions; the Ca²⁺⁻calmodulin complex binds to and activates many enzymes.

AMP-dependent protein kinase (cAPK): Type of cytosolic enzyme that is activated by cAMP and functions to regulate the activity of numerous cellular proteins, also called *protein kinase A*. Generally is activated in response to a rise in cAMP level resulting from stimulation of G protein—coupled receptors

Cyclic AMP (cAMP): A second messenger, produced in response to hormonal stimulation of certain G protein – coupled receptors, that activates cAMP-dependent protein kinases.

Cyclin: Any of several related proteins whose concentrations rise and fall during the course of the eukaryotic cell cycle. Cyclins form complexes with cyclin-dependent kinases, thereby activating and determining the substrate specificity of these enzymes.

Diacylglycerol (**DAG**): Intracellular signaling molecule produced by cleavage of phosphoinositides in response to stimulation of certain cell-surface receptors; functions as a membrane-bound second messenger in inositol-lipid signaling pathways.

Domain: Region of a protein with a distinct tertiary structure (e.g., globular or rodlike) and characteristic activity; homologous domains may occur in different proteins.

Enhancer: A regulatory sequence in eukaryotic DNA (rarely in prokaryotic DNA) that may be located at a great distance from the gene it controls. Binding of specific proteins to an enhancer modulates the rate of transcription of the associated gene.

G protein: Any of numerous heterotrimeric GTP-binding proteins that function in intracellular signaling pathways; usually activated by ligand binding to a coupled seven-spanning receptor on the cell surface.

G protein-coupled receptor (GPCR): Member of an important class of cell-surface receptors that have seven transmembrane α helices and are directly coupled to a trimeric G protein.

Gene: Physical and functional unit of heredity, which carries information from one generation to the next, is called gene. In molecular terms, it is the entire DNA sequence — including exons, introns, and noncoding transcription-control regions — necessary for production of a functional protein or RNA.

GTP (guanosine 5-triphosphate): A nucleotide is a precursor in RNA synthesis and also plays a special role in protein synthesis, signal-transduction pathways, and microtubule assembly.

GTPase superfamily: It is a group of GTP-binding proteins cycle between an inactive state with bound GDP and an active state with bound GTP. These proteins — including G proteins, Ras proteins, and certain polypeptide elongation factors — function as intracellular switch proteins.

Kinase: An enzyme that transfers the terminal (γ) phosphate group from ATP to a substrate. Protein kinases, which phosphorylate specific serine, threonine, or tyrosine residues in target proteins, play a critical role in regulating the activity of many cellular proteins.

NADP+ (**nicotinic adenine dinucleotide phosphate**): NAD⁺ is phosphorylated form of NAD+, which is used extensively as an electron carrier in biosynthetic pathways and during photosynthesis.

Nuclear receptor: General term for intracellular receptors that bind lipid-soluble hormones (e.g., steroid hormones) is also called *steroid receptor superfamily*. Following ligand binding, the hormonereceptor complex translocates to the nucleus and functions as a transcription factor.

Operator: It is short DNA sequence in a bacterial or viral genome that binds a repressor protein and controls transcription of an adjacent gene.

pH: A measure of the acidity or alkalinity of a solution defined as the negative logarithm of the hydrogen ion concentration in moles per liter: pH=-log [H⁺]. Neutrality is equivalent to a pH of 7; values below this are acidic and those above are alkaline.

Phosphatase: It is an enzyme that removes a phosphate group from a substrate by hydrolysis. Phosphoprotein phosphatases act with protein kinases to control the activity of many cellular proteins.

Phosphodiester bond: A covalent bond in which two hydroxyl groups form ester linkages to the same phosphate group; joins adjacent nucleotides in DNA and RNA.

Phosphoinositides: A family of membrane-bound lipids containing phosphorylated inositol derivatives that are important in signal-transduction pathways in eukaryotic cells.

Plasmodesmata (singular plasmodesma): It is a tube like cell junctions that interconnect the cytoplasm of adjacent plant cells and are functionally analogous to gap junctions in animal cells.

Ras protein: It is a monomeric GTP-binding protein that functions in intracellular signaling pathways and is activated by ligand binding to receptor tyrosine kinasesand other cell-surface receptors.

Receptor: Any protein that binds a specific extracellular signaling molecule (ligand) and then initiates a cellular response is called receptor. Receptors for steroid hormones, which diffuse across the plasma membrane, are located within the cell; receptors for water-soluble hormones, peptide growth factors, and neurotransmitters are located in the plasma membrane with their ligand-binding domain exposed to the external medium.

Second messenger: It is an intracellular signaling molecule whose concentration increases (or decreases) in response to binding of an extracellular ligand to a cell-surface receptor. Examples include cAMP, Ca², diacylglycerol (DAG), and inositol 1, 4, 5-trisphosphate (IP₃).

Signal transduction: Conversion of a signal from one physical or chemical form into another is signal transduction. In cell biology commonly refers to the sequential process initiated by binding of an extracellular signal to a receptor and culminating in one or more specific cellular responses.

Signaling molecule: General term for any extracellular or intracellular molecule involved in mediating the response of a cell to its external environment or other cells.

Transcription factor (TF): General term for any protein, other than RNA polymerase, is required to initiate or regulate transcription in eukaryotic cells. General factors, required for transcription of all genes, participate in formation of the transcription-initiation complex near the start site. Specific factors stimulate (or repress) transcription of particular genes by binding to their regulatory sequences.

10.7 SELF ASSESSMENT QUESTION

10.7.1. Multiple Choice Questions:

1.	. Which of th	e following sig	nal molecule is	NOT used for	extracellular	signaling	<u>, ?</u>
							•

(a) Autocrine (b) Endocrine

(c) Paracrine (d) Cyclic AMP

2. Arrange the following sequence of extracellular signaling in the correct order?

- 1) Transport of signal to a target
- 2) Start of signal transduction pathways
- 3) Signaling cell synthesize and release signaling molecules
- 4) Binding of the signal to the specific receptor

(a) 2, 3, 4, 1 (b) 3, 1, 4, 2

(c) 1, 2, 3, 4 (d) 1, 3, 4, 2

3. Name the signaling which requires physical contacts between the cells involved.

(a) Paracrine signaling (b) Intracellular signaling

(c) Autocrine signaling (d) Juxtacrine signaling

4. Name the largest family of cell surface receptor?

(a) GPCR (b) Ion-channel receptor

(c) Enzyme-linked receptor (d) Nuclear receptor

5. Small charged molecules, often biogenic amines function as-

(a) Hormones (b) Neurotransmitters

(c) Both (a) and (b) (d) None of these

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6. Which of the following is not a type of signa	ling molecule?				
(a) Testosterone	(b) Insulin				
(c) Thyroxin	(d) Adenylate cyclase				
7. Self-phosphorylation is an excellent mechan	nism for triggering specific catalytic function				
of the proteins involved in signal cascades beca	ause it-				
(a) Changes the shape and thus the enzymatic act	• •				
b) Makes the receptor more likely to capture the signaling, molecule					
(c) Allows hydrophilic signaling molecules to cro	oss the plasma membrane				
(d) None of the above					
8. Which of the following statements about G _j	proteins is false?				
(a) They are involved in signal cascades					
(b) They bind to and are regulated by guanine nucleons.	cleotides				
c) They become activated when bound to GDP					
(d) They must be active before the cell can make	needed cAMP				
9. cAMP and cGMP are derived from					
(a) ATP and GTP by the actions of adenylate cyc	lase and guanylate cyclas respectively				
(b) GTP and ATP by the actions of adenylate cyc	clase and guanylate cyclase respectively				
(c) ATP and GTP by the actions of guanylate cyc	lase and adenylate cyclase respectively				
(d) None of the above					
10. In the signal transduction mechanism kno	wn as protein phosphorylation				
(a) The signaling molecule binds to a surface rece	eptor				
(b) Receptor kinases play a key role in triggering	the signal cascade				
(c) Phosphorylated proteins act with enzymes to	trigger the signal cascade				
(d) All of the above					
10.7.2. Fill in the blank					
1. When a, there	is a specific means of receiving it and acting on				
the message. This task is the responsibility of spe	cialized proteins called				
2. The enzyme that catalyzes the splitting of Pl	IP2 into two molecules of inositol triphosphate				

10.7.3. Very Short Questions:

(IP3) and diacylglycerol in cell signaling, is____.

3. lnositol 1,4,5-triphosphate is_____Messenger.

4. G β subunits belong to the _____ family of proteins.

5. The hormone or ligand can be considered as _____ messenger.

- **1.** Describe the function of Ca²⁺ as a second messenger.
- **2.** Explain the enzyme which is dependent on G-protein activation?
- 3. How cAMP involve in signal transduction pathway?
- **4.** What are receptors?
- **5.** How many types of receptors are present on the cell?
- **6.** What are cytosolic receptors?
- **7.** What are nuclear receptors?
- **8.** What is membrane bound receptors?
- **9.** What is signal transduction?
- **10.** What is calmodulin?

Answer Key:

10.7.1. Multiple Choice Questions:

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

10.7.2. Fill in the blank

1. Signaling molecule; target cell; receptors, 2. Phospholipase C, 3. Second, 4. WD40, 5. First messenger

10.7.3. Very Short Questions:

- 1. The roles of calcium include regulating enzyme activity, permeability of ion channels, activity of ion pumps, and components of the cytoskeleton. Many of Ca^{2+} -mediated events occurs when the released Ca^{2+} binds to and activates the regulatory protein calmodulin.
- 2. GTPases.
- 3. Adenosine 3',5'-cyclic monophosphate (cAMP) is a nucleotide that acts as a key second messenger in numerous signal transduction pathways. cAMP regulates various cellular functions, including cell growth and differentiation, gene transcription and protein expression.
- 4. Any protein that binds a specific extracellular signaling molecule (ligand) and then initiates a cellular response.
- 5. Receptors are protein molecules in the target cell or on its surface that bind ligands. There are two types of receptors: internal receptors and cell-surface receptors.
- 6. Cytosolic receptors are receptors located inside the cell rather than on its cell membrane. Examples are the class of nuclear receptors located in the cell nucleus and cytoplasm and the IP3 receptor located on the endoplasmic reticulum.
- 7. In the field of molecular biology, nuclear receptors are a class of proteins found within cells that are responsible for sensing steroid and thyroid hormones and certain other molecules. In response, these receptors work with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism.

8. Membrane bound receptors are usually transmembrane proteins. Transmembrane proteins with part of their mass on both sides of the membrane are poised structurally to transmit information from one side of the membrane to the other.

- 9. Conversion of a signal from one physical or chemical form into another. In cell biology commonly refers to the sequential process initiated by binding of an extracellular signal to a receptor and culminating in one or more specific cellular responses.
- 10. Calmodulin is a small cytosolic protein that binds four Ca²⁺ ions; the Ca²⁺-calmodulin complex binds to and activates many enzymes.

10.8 REFERENCES

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10.10 TERMINAL QUESTIONS

1. What is meant by the term signal transduction? What are some of the steps by which signal transduction can occur?

2. How is the [Ca⁺²] of the cytosol maintained at such a low level? How does the concentration change in response to stimuli?

- **3.** What is the mechanism of formation of the second messenger IP3? What is the relationship between the formation of IP3 and an elevation of intracellular $[Ca^{2+}]$?
- **4.** What is the role of G proteins in a signaling pathway?
- 5. Describe the model of signal transduction for induction of α -amylase during the seed germination is caused by gibberellins?





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