

Block

2

STRUCTURE AND FUNCTION OF THE CELL

UNIT 5

Structure and Function of Subcellular Organelles	89-112
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UNIT 6

Cell Wall and Extracellular Matrix	113-138
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UNIT 7

Cytoskeleton Proteins and Cell Movement	139-160
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BLOCK 2 : STRUCTURE AND FUNCTION OF THE CELL

This block deals with the cell structure and function. It will explain you how cells perform their specific functions. As you know eukaryotic cell (animal and Plant) has membrane bound organelles with well-defined nucleus. Therefore, it is necessary to understand the structure and functions of subcellular organelles.

There are three units in this block.

Unit 5 describes the structure and function of sub-cellular organelles includes Nucleus, Endoplasmic reticulum, Lysosomes, Peroxisomes, Mitochondria and chloroplast.

Unit 6 Cell wall and Extracellular Matrix discusses the structure and functions of prokaryotic and eukaryotic cell wall and extracellular matrix along with cell-cell interactions.

Unit 7 Cytoskeleton Proteins and Cell Movement explains the structure of microtubules, actin filaments and intermediate filaments and their roles.

Expected Learning Outcomes

After reading this block, you should be able to:

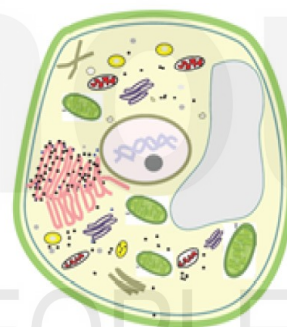
- define the nucleus;
- describe the structure and function of Nucleus;
- explain the structure, types and roles of Endoplasmic reticulum;
- explain the morphology of Golgi Body and its biological functions;
- elucidate the structure of lysosomes and its role;
- illustrate the structure and function of peroxisomes,
- describe the structure and function of mitochondria;
- explain why mitochondria is called power house of the cell;
- define the structure and function of chloroplast ;
- Identify the subcellular organelles by their schematic diagram and specific roles;
- describe the structural and biological role of cell wall and extracellular matrix;
- explain the cell-cell interactions;
- explain the structure and function of cytoskeleton proteins: Microtubules, Actin Filaments and Intermediate Filaments; and
- illustrate the structure and function of cilia and flagella.

UNIT 5

STRUCTURE AND FUNCTION OF SUBCELLULAR ORGANELLES

Structure

- | | | | |
|-----|---|------|--|
| 5.1 | Introduction | 5.6 | Peroxisomes |
| | Expected Learning Outcomes | 5.7 | Mitochondria and Chloroplast |
| 5.2 | Nucleus | | Structure and Function of Mitochondria |
| | Structure of Nuclear Envelope | | Structure and Function of Chloroplasts |
| 5.3 | Structure of Endoplasmic Reticulum (ER) | | |
| | Functions of ER | 5.8 | Summary |
| 5.4 | Structure of Golgi Apparatus | 5.9 | Terminal Questions |
| | Function of Golgi Apparatus | 5.10 | Answers |
| 5.5 | Lysosomes | 5.11 | Suggested Readings |



5.1 INTRODUCTION

In Block 1 you have learned about the organisation of prokaryotic (Archaea and Bacteria) and eukaryotic cells (Animal and plants) and role of various structures. You have also studied the principles of light microscopy, electron microscopy and centrifugation which are some of the basic tools to study cells. Both microscope and centrifuge are common laboratory equipment used to view and analyze cell structure and for isolation and purification of subcellular organelles and biomolecules, respectively.

Recall a eukaryotic cell has complex internal organisation characterised by presence of many membrane bound subcellular organelles. These organelles are structurally and functionary different and indispensable for normal cell function and survival. Therefore, in this unit you are going to learn about the structure and functions of subcellular organelles.

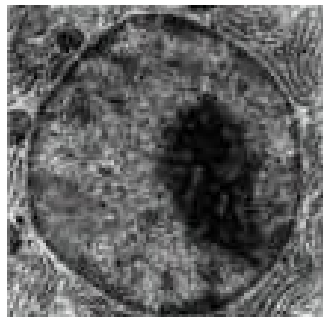
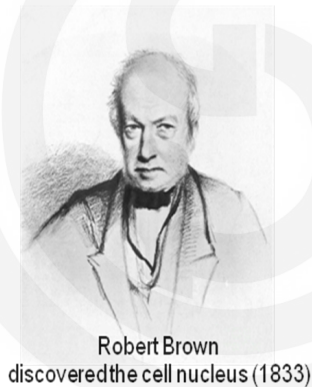
Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ define the nucleus;
- ❖ explain the structure and role of nucleus and nuclear subcompartments;
- ❖ indicate the variations in endoplasmic reticulum and role(s) of RER and SER;
- ❖ explain the structure of Golgi body and its role;
- ❖ point out the polymorphism of lysosomes and its role in intracellular digestion;
- ❖ describe the structure of peroxisomes and its role in animal and plant cells;
- ❖ describe the structure of mitochondria and chloroplast and;
- ❖ explain the role of mitochondria and chloroplast in energy metabolism.

5.2 NUCLEUS

You would have studied about nucleus in your biology course at 10+2 level. The term nucleus is derived from a Latin word which means 'kernel'. Nucleus is the most important part of a cell as it harbors the genetic material and incidentally it is the first organelle to be discovered. A well organised, membrane bound nucleus in eukaryotes is what separates them from prokaryotes (Refer to Unit 1). With few exceptions, one or more nuclei are present in all eukaryotic cells. It is mostly spherical in shape; it may become oval or elliptical in plant cells that have large vacuoles. However, a typical nucleus is usually 5-25 μm in diameter and occupies about 10% of cell volume. Generally it is present in the center of the cell. The structure of nucleus is schematically depicted in Fig. 5.1.



Micrograph of Cell **Nucleus**
Credit: Wikimedia
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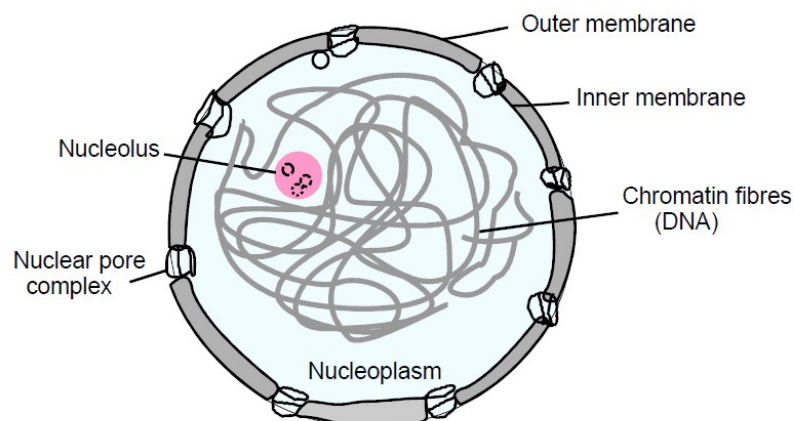


Fig. 5.1: Structure of nucleus.

The DNA in eukaryotes is complexed with histone and non-histone proteins which allow packaging of chromosomes in the limited nuclear space. You will learn details of chromatin packaging in molecular biology Course (BBCCT-117). The nucleus is no longer considered to be a diffused unorganized mass but has a well ordered internal structure. The chromatin fibers of each interphase chromosome occupy distinct territories. Genes from different chromosomes can come together in response to signals and are concentrated in regions of active transcription. The heterochromatin such as inactive X-chromosome (Barr body) in mammalian females has a peripheral localisation (nuclear lamina) while interior nuclear compartment has euchromatin. Many actively transcribed genes are also localised to the nuclear pore. A repositioning of genes away from the lamina appears to precede activation.

The nucleolus is a sub-compartment of the nucleus, not bound by lipid membrane. It is highly electron dense, irregular / circular shaped structure and is the largest nuclear body visible in an interphase nucleus. It can be visualised by light microscopy. A cell may have one or more nucleoli. The size of the nucleolus varies with functional activity. It occupies up to 25% of the total nuclear volume. During cell division, the nucleolus disappears.

A nucleolus is assembled around the **nucleolar organiser region** (NOR) in a chromosome. The NOR contains genes for pre Ribosomal RNA (rRNA). In humans, the rRNA genes are distributed in 10 clusters (five pairs of homologous chromosomes). It is the site for synthesis and processing of rRNA and ribosome biogenesis. Some non-coding RNA species (U6 snRNP, few tRNA, SRP and telomerase) are also synthesised, processed and assembled here.

In addition to nucleolus, many other **nuclear bodies** have been recognised in the nucleus. None of these nuclear bodies are membrane bound and they are highly dynamic structures. They include Cajal bodies, polycomb bodies, and nuclear speckles (inter chromatin granule cluster). We are beginning to understand their role(s) in nuclear metabolism. Nuclear speckles are storage / modification compartment for pre mRNA splicing factors; Cajal bodies (CB) contain a variety of proteins and RNA involved in the assembly and modification of small nuclear and nucleolar RNP (snRNP and snoRNP) and polycomb group of proteins are gene silencing proteins complexes that regulate the expression of a variety of genes.

The nuclear matrix or nuclear skeleton is a filamentous protein network found in nucleoplasm. The term nuclear matrix was given by Berezney and Coffey (1974) to describe a highly organised residual framework left on treatment of nuclei with salts, non ionic detergents and nucleases. It provides a structural framework for DNA metabolism and maintains shape and size of the nucleus.

The nuclear material is embedded in a viscous fluid, nucleoplasm. It is a suspension medium that supports almost all nuclear functions such as DNA replication, transcription, post transcriptional modifications and ribosome biogenesis. The nucleoplasm has nuclear proteins, enzymes, RNAs, nucleotides and dissolved ions. It also helps to maintain the shape and structure of the nucleus and plays a vital role in transportation of materials.

5.2.1 Structure of the nuclear envelope

The nuclear envelope is a double membrane enclosure of outer and inner membranes. Both membranes are parallel to one another, separated by about 10-50 nm. The outer membrane is continuous with endoplasmic reticulum (ER) while the inner membrane is bound via integral membrane proteins to nuclear lamina. The nuclear envelope is a barrier that separates the nucleus from cytoplasm, creating a distinct biochemical compartment. During cell division the nuclear envelope disappears and reappears as nuclear structures reorganise at the end of each division cycle. The major structural components of nuclear envelope are nuclear pore complex and nuclear lamina (Fig. 5.2). Let us now discuss each of these one by one.

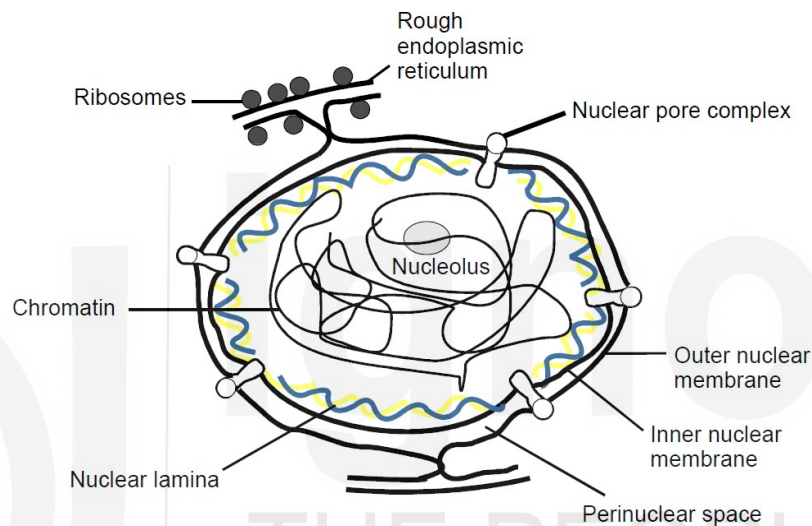


Fig. 5.2: Major structural components of nuclear envelope.

Nuclear Pore Complex (NPC)

The outer and inner nuclear membrane is fused at sites forming circular **pores** that contain complex assemblies of proteins. An average mammalian cell contains several thousand nuclear pores embedded within the nuclear envelope. The pores are enclosed by circular structures called **annuli**. The pores and annuli together constitute the pore complex (Fig. 5.3). In vertebrates, there are more than 50 different types of proteins involved in the formation of different of nuclear pores. These proteins are known as **nucleoporins**.

Electron micrographs of NPC shows that it has an eight fold symmetry of nucleoporins arranged around a central channel with cytoplasmic ring and nuclear ring. Its diameter is about 120 nm and a molecular mass of approximately 125 kilodaltons.

The nuclear pores control movement of small molecules, ions and macromolecules between nucleus and cytoplasm. There is a **bidirectional movement** of proteins and / RNA between the nucleus and cytoplasm. Mature mRNA, tRNA and ribosomal subunits are synthesized and / assembled in the nucleus and then exported to cytosol. On the other hand, small nuclear RNA

(snRNA) of spliceosome moves in both directions; they are synthesized in nucleus, assembled into RNP (ribonucleoprotein) in the cytosol and then returned back to the nucleus for RNA processing. The NPC is also involved in chromatin organization, gene expression and DNA repair.

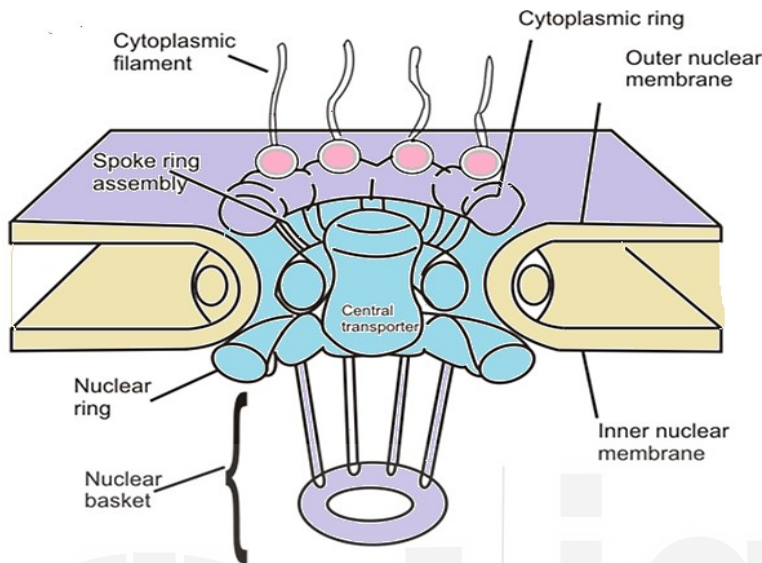


Fig. 5.3: The structure of nuclear pore complex (NPC). (Source : Cooper The Cell: A Molecular Approach)

Nuclear Lamina

Nuclear lamina (NL) is made up of a class of intermediate filament proteins called **lamins**. Lamins polymerize into 10nm fibers forming a complex protein meshwork. Nuclear lamina is on the inner side of nuclear envelope, bound to the membrane by integral membrane proteins (Fig. 5.4). The nuclear lamina disintegrates prior to cell division due to phosphorylation by specific kinases.

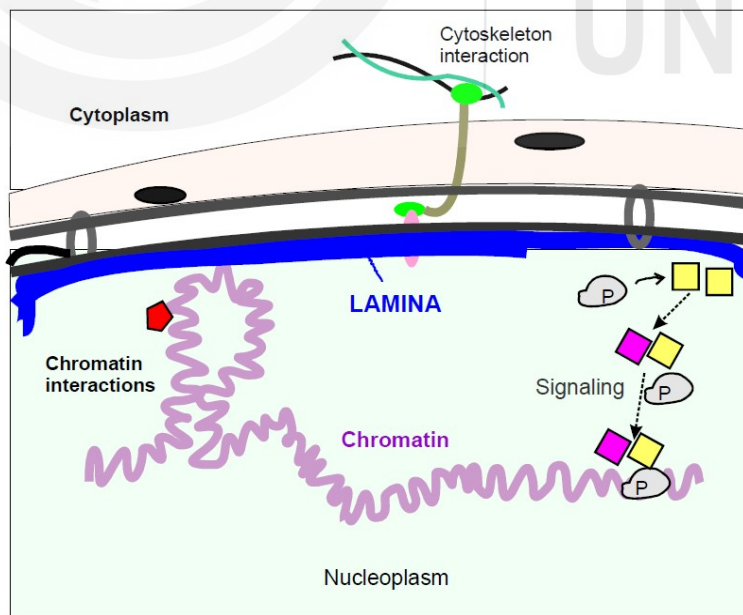


Fig. 5.4: Nuclear lamina (blue color).

The nuclear lamina provides mechanical support to nuclear envelope and serves as sites for chromatin attachment. The chromatin is bound to lamins by lamin-binding proteins and forms nuclear bodies that are involved in gene expression, DNA repair, chromatin organization, cell differentiation and signal transduction. The lamina also sequesters transcription factors.

The nucleus is a reservoir of genetic information. Table 5.1 summarizes the major structural components of nucleus and their role(s).

Table 5.1: Structure and function of nuclear sub compartments

Nuclear structures	Role(s)
DNA –protein complexes organised as multiple chromosomes	DNA is the carrier of genetic information. It can be replicated & carries in a coded language the blueprint to guide the synthesis of all cellular components.
Nucleolus	It is the site of ribosomal RNA (rRNA) synthesis and ribosome biogenesis. Few other small RNA species are also synthesized and assembled here.
Nucleoplasm	A viscous fluid that dissolves solutes and provides a medium for DNA metabolism.
Nuclear envelope / nuclear membrane	It is a double membrane enclosing the nucleus; bidirectional movement takes place via NPC. The nuclear lamina provides mechanical support to the envelope.
Nuclear matrix	A fibrillar network of proteins within nucleoplasm of that provides mechanical stability.

SAQ 1

Fill in the blanks:

- i) The sub-compartment of the nucleus that is highly electron-dense and irregularly shaped is
- ii) In chromatin, DNA is complexed with basic proteins called.....
- iii) Ribosome biogenesis and ribosomal RNA are synthesized in.....
- iv) The outer membrane of the nuclear envelope is continuous with the membrane of -----that extend up to the cell membrane.
- v) Mechanical support to the nuclear membrane is provided by
- vi) All nucleocytoplasmic movement is through the

5.3 STRUCTURE OF ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is a membrane bound organelle in all eukaryotic cells. The term 'endoplasmic' means inside the cell (cytoplasm) and 'reticulum' means a network. As the name suggests it is a network of branching tubules and flattened sacs that extend throughout the cytoplasm and is continuous with the outer membrane of nuclear envelope. It constitutes more than half of the total membrane in a cell. The membrane network forms a single continuous sheet enclosing a space or lumen within ER, allowing only selective movement of substances between the lumen and cytosol. The endoplasmic reticulum (ER) comes in two forms; **rough endoplasmic reticulum (RER)** and **smooth endoplasmic reticulum (SER)**.

The RER is characterised by the presence of ribosomes on the cytosolic side whereas they are absent in SER. The coating of ribosomes gives them a rough appearance in electron micrographs hence the name is **rough endoplasmic reticulum (RER)**. The high affinity binding of ribosomes to ER membrane is dependent on two glycoproteins, namely **ribophorins I and II**. These proteins are abundant integral membrane proteins whose cytosolic domains bind tightly to the large subunit of ribosomes. In SER, the membrane network is organized into a meshwork of branching tubules whereas RER are flattened sacs or cisternae (Fig.5.5a, b and c).

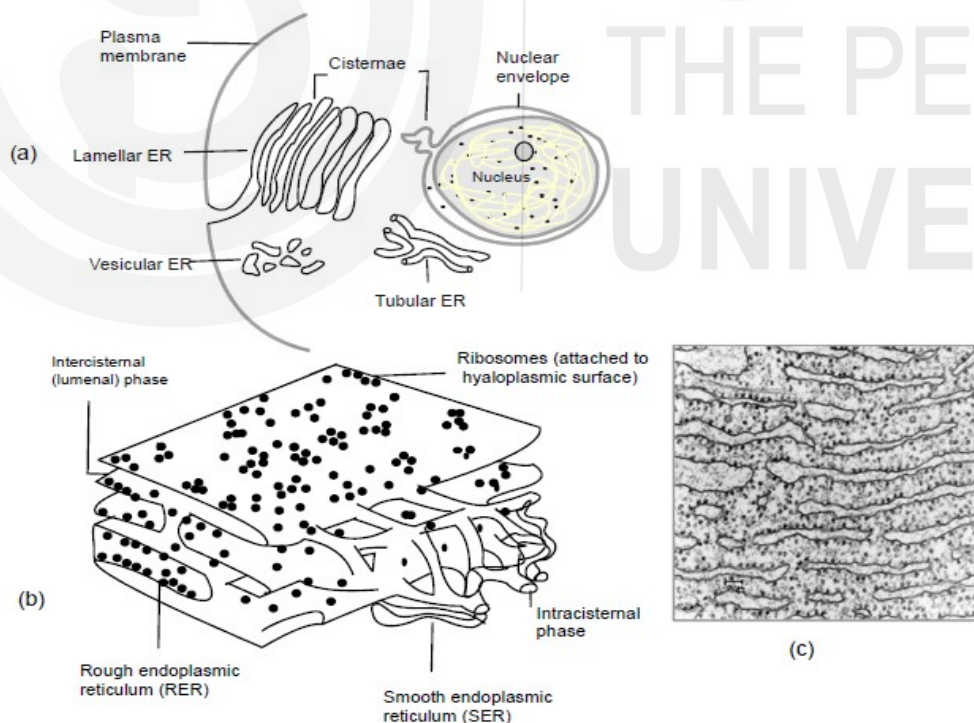


Fig. 5.5: a) Three physical forms of ER; b) The interconnected tubules and the sacs of the ER membrane form a continuous sheet that encloses a single internal space (ER lumen); c) Electron Micrograph of ER.

Most lipid bilayers are assembled in the ER. Apart from proteins, it produces nearly all lipids needed to make new membranes. The ER has enzymes for the synthesis of both phospholipids like phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol and cholesterol (**refer to Block 3 of BBCCT 101 for Phospholipids**). The phospholipids then equilibrate across the membrane and flippases help to create the asymmetric distribution of phospholipids. The ceramide backbone of sphingolipids is also made in ER and it is converted to either sphingomyelin or glycosphingolipids in the Golgi complex. The membranes of organelles like mitochondria, chloroplast and peroxisomes also acquire both proteins and lipids from ER, although they do not fall in the secretory route.

5.3.1 Function of Endoplasmic Reticulum

The two morphological variants of ER perform specialised roles (Fig. 5.6). All proteins destined to different locations along the secretory route begin their journey in RER after an initial synthesis of short signal peptide sequence by cytosolic ribosomes. The growing polypeptides are **co-translationally translocated** across the ER membrane and modified by attaching oligosaccharides to asparagine residue (**N-glycosylation**). The signal sequence is invariably removed by a peptidase on the luminal side of ER about which you will study in detail in Unit 8, Block 3 Protein Transport.

The newly synthesised proteins are **folded** to their native conformation, assisted by a combination of chaperones and protein disulphide isomerase (PDI). Those proteins that do not fold properly are destined for degradation by cytoplasmic proteasomes. The acquisition of native protein conformation is tightly coupled to its export from ER along the secretory route. This highlights an important role of RER in **quality control**.

The proteins synthesised by RER are (a) secreted proteins, (b) integral membrane proteins and (c) soluble proteins that reside within compartments of the endomembrane system that includes ER, Golgi complex and lysosomes. Some proteins such as cell adhesion molecule (N-CAM), decay acceleration factor (DAF) and human erythrocyte acetylcholine esterase are anchored to the plasma membrane via **Glycosyl phosphatidyl inositol (GPI) anchors**, attached to their C-termini. They are added post translationally in the ER.

A minor route for oxidation of fatty acids occurs in the endoplasmic reticulum. The pathway is called **ω oxidation** as oxidation of the carbon farthest (omega end) from the carboxyl end takes place. The route can serve as a way to synthesise dicarboxylic acids or for further breakdown from either end. The substrates for **ω oxidation** are generally medium chain (C10-12) fatty acids.

The endoplasmic reticulum **sequesters Ca^{+2}** from the cytosol and serves as a reservoir of calcium. The role of calcium in transducing signals and bringing about many rapid responses is well known. The release of calcium from the ER in response to signals is a common theme in mediating these cellular responses.

The SER is prominent in cells active in **lipid metabolism** such as synthesis of steroid hormones in the endocrine cells of gonads and adrenal cortex. The

liver hepatocyte cell has an abundance of SER to produce lipid components of lipoproteins and in the detoxification of lipid soluble drugs and harmful xenobiotics / products of metabolism by the **cytochromeP450** family of enzymes.

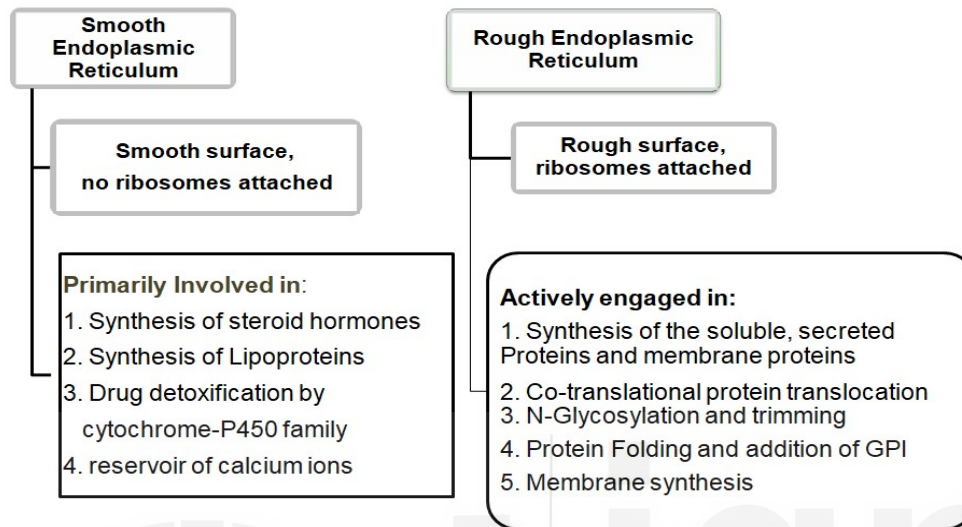


Fig. 5.6: Roles of SER and RER.

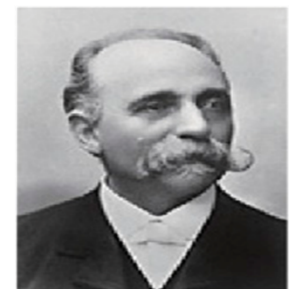
SAQ 2

- a) Which ER variant is involved in the following processes?
- i) Synthesis of membrane lipids.
 - ii) Protein folding and quality control.
- b) What are the major morphological differences between RER and SER?

5.4 GOLGI APPARATUS

Golgi apparatus is a membrane bound organelle prominent in secretory cells. The Italian scientist **Camilo Golgi** observed in 1898 a darkly reticular network located near the cell nucleus in nerve cells using silver staining. This network was later named as the Golgi complex or Golgi apparatus.

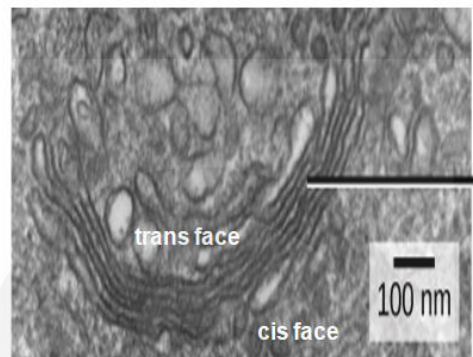
It is composed of stacks of flattened membrane enclosed cisternae. The cisternae are arranged in an orderly manner that is curved resembling a shallow bowl. The diameter of a stack is generally 0.5 to 1.0 μm . Typically a Golgi stack contains about eight cisternae in mammalian cells which are interconnected by the membranous tubules to form a single, large ribbon-like complex (Fig. 5.7). Each Golgi stack has a cis and a trans face. The convex (cis) side facing the ER is '**forming**' or '**proximal**' face, whereas the concave side oriented towards cell surface is 'releasing' or '**distal**' face and encloses a region containing large secretory vesicles.



Camillo Golgi identified the Golgi Body

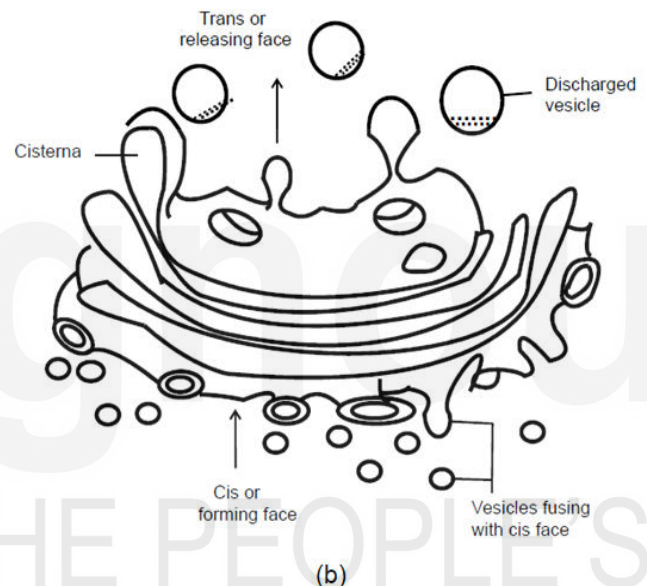
The movement of proteins from ER to Golgi and between Golgi compartments takes place through vesicles. Small vesicles are seen around the cis and the trans face of Golgi. They bud from a given location and later fuse with membranes of the other compartment. Protein trafficking from ER to Golgi and between Golgi compartments will be covered in Block 3, Unit 9 Protein Trafficking II.

The Golgi complex exhibit both structural and functional compartmentalization from cis to the trans face. Therefore, as proteins travel from one compartment to the next they become accessible to different processing enzymes. This unique feature has led biologists to appropriately label the Golgi complex as a “**processing plant.**”



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(a)



(b)

Fig.5.7: (a) Electron micrograph of Golgi apparatus (b) Schematic view of Golgi apparatus.

5.4.1 Function of Golgi Apparatus

Golgi apparatus has enzymes that step by step modify (by removal and additions) the glycosylated proteins coming from ER. Carbohydrate processing in the Golgi is termed **terminal glycosylation**. The resultant oligosaccharides may either be high mannose or have complex glycosylation with terminal sialic acid residues. It is also the site for **O-glycosylation** of glycoproteins and glycolipids. In other words, proteins acquire their final sugar decorations here and are fully equipped to perform their varied roles. Many of the plant cell wall polysaccharides (pectins and hemicelluloses) and extracellular Glycosaminoglycans in animals are also synthesised here. In plants, Golgi apparatus is also required for the formation of cell plate and cell wall.

The Golgi complex participates in **protein sorting** and packaging of cargo into separate vesicles. The proteins destined for different locations are sorted by specific signals present on them. They may be targeted to lysosomes, secretory vesicles, and plasma membrane or secreted by the cell. Some of them are also returned back to the ER.

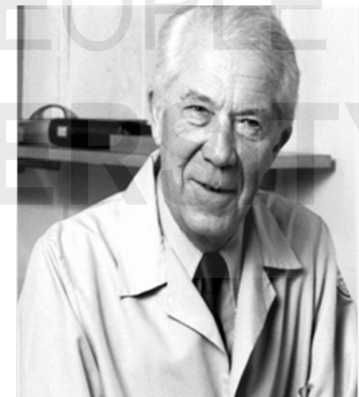
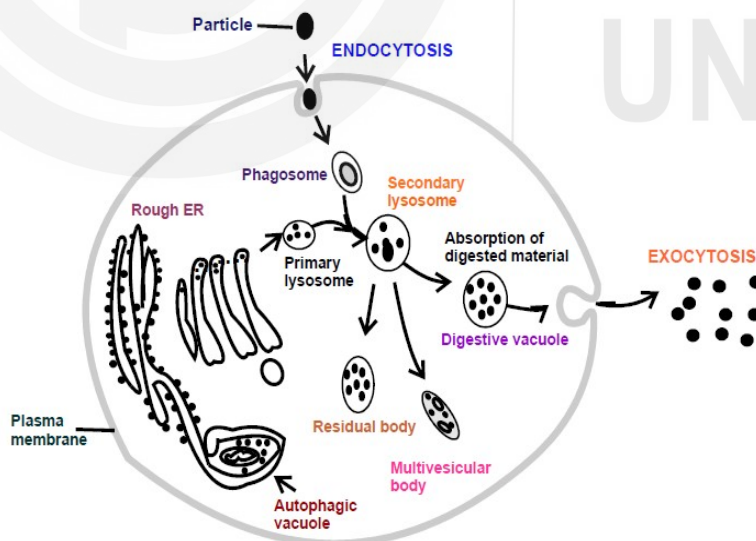
SAQ 3

Indicate whether the following statements are true (T) or false (F):

- i) Golgi apparatus is presents only in plant cells. []
- ii) The Golgi complex stores, modifies, and packages proteins for delivery to specific destination. []
- iii) The movement of cargo from ER to Golgi and between Golgi compartments is through vesicles. []
- iv) All cells have a highly developed Golgi apparatus. []
- v) The terminal glycosylation of proteins takes place in Golgi body. []
- vi) The Golgi complex has only structural differences from cis to trans face. []

5.5 LYSOSOMES

Lysosomes (Gr., lysis = dissolution + soma = body) are membrane enclosed organelles enriched in hydrolytic enzymes. Most lysosomal membrane proteins are heavily glycosylated. **Christian de Duve** discovered lysosomes as small dense spherical vacuoles within eukaryotic cells. Lysosomes are heterogeneous in their morphology, with diameter ranging from 0.05-0.5 μm (Fig.5.8). Different forms of lysosomes have been identified as **primary lysosomes**, **secondary lysosomes**, **autophagic vacuoles** and **residual bodies**.



Christian de Duve

Fig: 5.8: Lysosome Polymorphism and intracellular digestion.

The lysosomes have an assorted combination of acid hydrolases such as lipases, nucleases, proteases, glycosidases and sulphatases. These enzymes

are active around pH 5. The lysosomal membrane has a proton pump that actively pumps H^+ ions to maintain the required acidic environment. The large difference in pH between the cytosol and lysosomes will prevent auto digestion of cells in situations of leakage of lysosomal hydrolases.

Function of Lysosomes

The newly formed lysosomes are **primary lysosomes**. The **secondary lysosomes** are formed by fusion of primary lysosomes with vesicles containing material to be degraded. This form of lysosomes is involved in intracellular digestion of a variety of components such as cell debris, biomolecules and phagocytosed microorganisms. The lysosomes can receive substances for digestion via multiple routes. It can enter the cell by endocytosis or phagocytosis of microorganisms and for intra cellular digestion of cytoplasmic organelles by enclosing them in membranes enclosed autophagosomes (**autophagy**). **Autophagy** by lysosomes is required for turnover of cellular components and in the remodeling of tissues during development. It is an ongoing cellular activity. The products of hydrolysis are released by lysosomes and recycled. The indigestible substances are retained within lysosomes as **residual bodies**. Residual bodies are irregular in shape and electron dense. In some cells, they remain for a long time and play a role in the aging process.

Plants and fungi have large **vacuoles** rich acid hydrolases and they are functionally related to the lysosomes of animal cells. In addition to their role in intracellular digestion they function as storage organelles for nutrients (proteins, polyphosphates, ions, and organic acids) and secondary metabolites (rubber, pigments, etc). They also have a homeostatic role in controlling turgor pressure in plant cells.

The importance of lysosomal hydrolases is best appreciated by studying the consequences of mutation in genes encoding lysosomal enzymes. For example, Gaucher's disease is the most common lysosomal storage disorder in which β - glucosidase gene is defective. There is accumulation of glucosyl ceramide resulting in enlarged liver and spleen; erosion of long bones and mental retardation in infants. An extremely rare condition is I-cell disease in which lysosomal hydrolases are secreted from the cell. It is characterised by large inclusions of undigested proteoglycans, glycolipids and other materials.

SAQ 4

In the following statements choose the correct alternative word given in parentheses.

- i) The lysosomal hydrolases work best at (**acidic/alkaline**) pH.
- ii) The undigested material that remains within lysosomes is called (**residual bodies/ digestive bodies**).
- iii) The process for self digestion of damaged or nonfunctional cytoplasmic organelles by autophagosomes is called (**endocytosis/ autophagy**)

- iv) The vacuoles store nutrients and other secondary metabolites in (**animal / plant cells**).
- v) Most lysosomal membrane proteins are (**glycosylated/ non glycosylated**).
- vi) The large differences in pH between cytosol and lysosomes (**prevent / allow**) the autodigestion of eukaryotic cells in case of any leakage.
- vii) Gaucher's disease results from mutation in gene that encodes (**β -glucosidase/lipases**).

5.6 PEROXISOMES

Peroxisomes are single membrane bound organelle which encloses electron dense inclusions (crystalloid core) of oxidative enzymes (Fig. 5.9). Its diameter is about 0.6-0.7 μm . They were discovered in 1954 and named **microbody** but later Christian de Duve proposed the name peroxisomes due to the presence of various H_2O_2 producing oxidases and catalase that breaks down H_2O_2 . All peroxisomes are catalase positive (marker enzyme) and exhibit enormous heterogeneity both in their morphology and the overlapping set of enzymes they harbor, even in different cell types of the same organism. They can change their size and enzymes composition according to changing demands of the cell. The number of peroxisomes varies between 70 and 100 per cell.

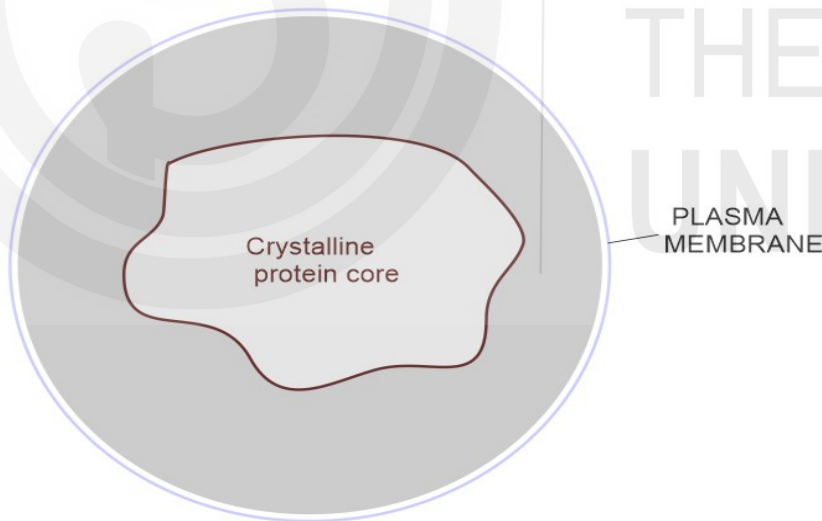


Fig. 5.9: A Peroxisome.

Peroxisomal proteins are synthesized on free ribosomes in the cytosol and then imported into preexisting peroxisomes. The import of proteins and phospholipids results in peroxisome growth, and new peroxisomes are then formed by division of old ones. They also acquire proteins and lipids from the ER. Peroxisomes are enriched in oxidases such as urate oxidase, D-amino oxidase, glycolate oxidase and fatty acyl CoA oxidase that consume oxygen and generate hydrogen peroxide. The latter is a substrate of catalase for

oxidation of compounds like phenol, alcohol and aldehydes or is broken down into water and oxygen (Fig. 5.10). In broad terms they are **respiratory organelles**. The removal of hydrogen peroxide is essential for cell survival as it is toxic and even mildly mutagenic.

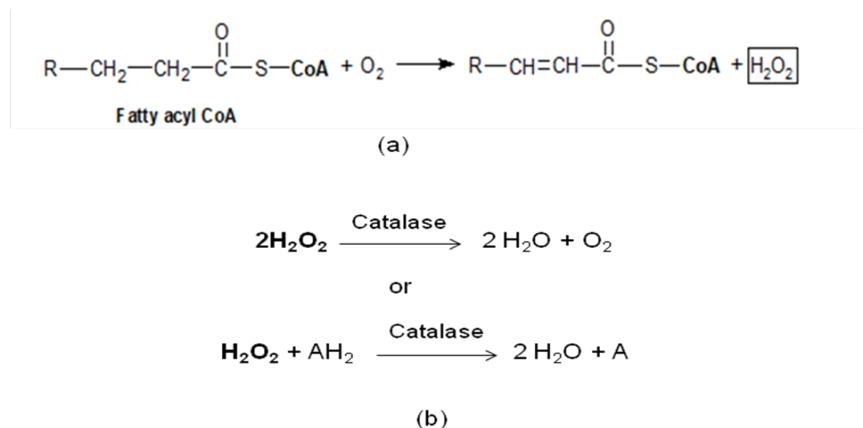


Fig. 5.10: (a) The production and (b) breakdown of hydrogen peroxide.

The catabolism of long chain fatty acids (> C-20) takes place in peroxisomes via β -oxidation spiral. In mammalian cells, the enzymes of β -oxidation are present also in the mitochondria unlike plants where they occur only in the peroxisomes / glyoxysomes. The initial breakdown of branched chain fatty acids such as phytanic acid also occurs in peroxisomes of animal cells by **α -oxidation** till a product that can be processed by β -oxidation is generated. Peroxisomes lack enzymes of both TCA cycle and electron transport chain (ETC), therefore partially processed fatty acids are either fed to anabolic routes or they enter the mitochondria for complete breakdown.

Peroxisomes also have an anabolic role as they contain enzymes responsible for the synthesis of Plasmalogens — a class of ether phospholipids in the membrane of brain and heart tissues.

In plants peroxisomes perform two particularly important roles. First, peroxisomes in germinating seeds of oil storing plants are responsible for **production of carbohydrates from the fatty acids** (gluconeogenesis), released from stored fats. This is accomplished by transient expression of enzymes of the **glyoxylate cycle** - a variant of citric acid cycle. The glyoxylate cycle essentially bypasses the two decarboxylations reactions of citric acid cycle and utilises two additional reactions catalysed by isocitrate lyase and malate synthase to have a net synthesis of a 4C gluconeogenic compound (oxaloacetate) from two acetyl CoA molecules. This cycle is critical in providing energy and raw materials for growth of germinating seedlings. These organelles are therefore called glyoxysomes.

The peroxisomes in leaves of C3 plants catalyse few steps of **photorespiration**. The role of peroxisomes is to convert glycolate to glycine in a two-step conversion and later to transform serine to glycerate that is reused by the chloroplasts. The process leads to a net loss of fixed carbon. There is consumption of oxygen and release of CO₂ as in respiration but no energy conservation. The reactions of photorespiratory cycle complete in three

organelles including peroxisome (Fig. 5.11). The details of photorespiratory pathway will be dealt later in Metabolism of Carbohydrates and Lipids (BBCCT-109).

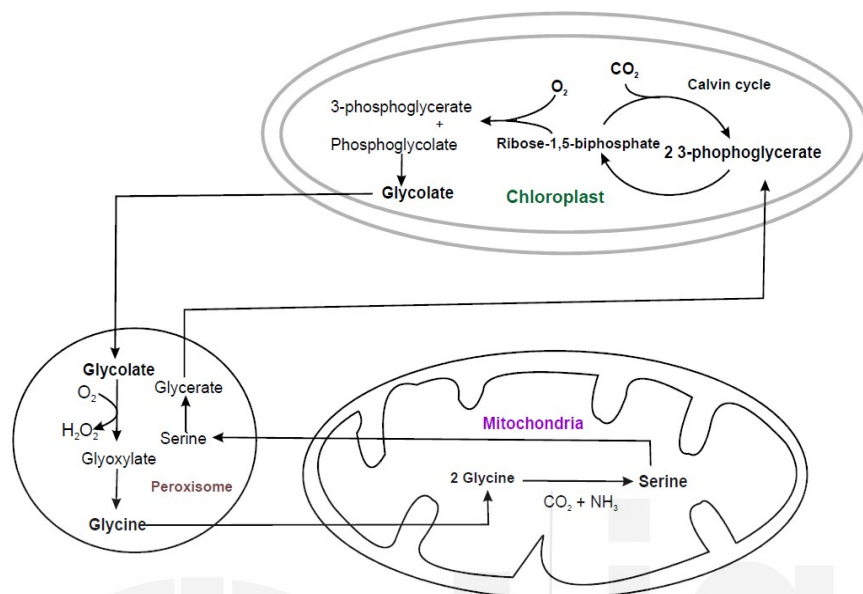


Fig. 5.11: The role of peroxisomes in photorespiration.

SAQ 5

Fill in the blanks:

- is the marker enzyme for peroxisomes.
- In mammalian cells the enzymes of β -oxidation are present in and
- The enzyme catalase breaks down toxic produced by in peroxisomes.
- The enzymes of the glyoxylate cycle are expressed transiently in
- In the leaves of C3 plants peroxisomes carry out some steps of

5.7 MITOCHONDRIA AND CHLOROPLAST

Mitochondria and chloroplasts are semiautonomous organelles. They have DNA that carries information for synthesis of some of their proteins and most of the non translatable RNA species. They have the machinery for protein synthesis and so there is no export of RNA (or even proteins) from these organelles. They are dependent on the nuclear genome for most of the proteins. Both organelles have a double membrane that defines their boundary. Mitochondria are present in all eukaryotes but only plants have chloroplast. Both are linked to energy metabolism; they generate ATP for

various cellular purposes. Both increase in size and divide by a process similar to binary fission in bacteria. Finally, both exhibit cytoplasmic (maternal) inheritance.

It is generally accepted that both organelles in eukaryotes were acquired by engulfing prokaryotic cells at different times during evolution. The **endosymbiont theory** is supported by many similarities between these organelles and present day prokaryotes such as presence of one or more circular DNA, ribosome structure and their susceptibility to prokaryotic antibiotics.

Let us learn about the structure and functions of mitochondria and chloroplast.

5.7.1 Structure and Function of Mitochondria

Mitochondria are double membrane bound organelle that plays a major role in energy generation. They are efficient in trapping energy released from the breakdown of biological fuels, primarily carbohydrates and fatty acids into ATP by a complex process of coupling electron flow through the electron transport chain (ETC) to oxidative phosphorylation. Hence, it is also known as the “**power house**” of the cell.

The term **mitochondria** was coined by a German investigator **Carl Benda** in 1898 to describe structures that sometimes looked like threads and at other times resembled granules. The word mitochondrion is derived from ‘mito’ means thread and ‘chondrion’ means granules. Now we recognise them as organelles that are constantly changing shape. Earlier they were called bioblasts by Richard Altman who incidentally was the first to consider them as separate organelles. The number of mitochondria in a cell varies widely, for example in mammalian liver cells there are between 1000 – 2000 mitochondria which accounts for almost 20% of the cytoplasmic volume. Their number and location in the cell can be correlated with energy requirements.



Carl Benda



Electron micrograph of Mitochondria

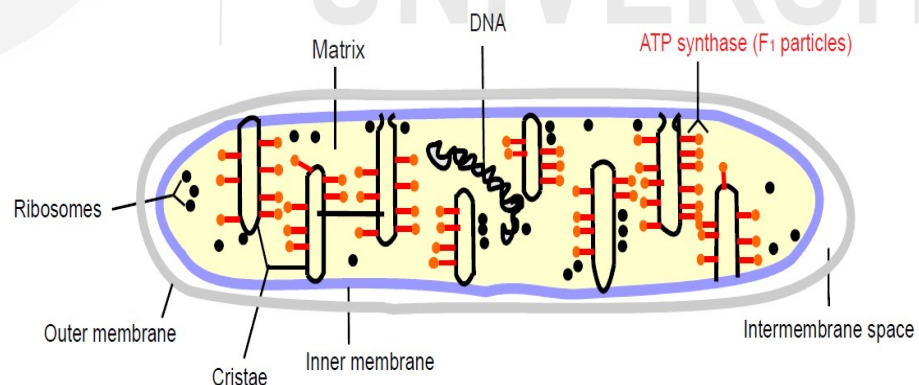


Fig.5.12: A schematic view of mitochondria.

The membranes of mitochondrion divide the organelle into two aqueous compartments; the matrix and an inter membrane space. The outer membrane has aqueous channels formed by porins that makes it permeable to molecules

up to 5000 Daltons. The inter membrane space and cytosol are therefore chemically similar for small molecules. The inner membrane has a series of infoldings called cristae that extend into the matrix and increase its surface area (Fig. 5.12). The inner membrane is selectively permeable and is the principle site of the ATP synthesis. The cristae are covered with mushroom-like **F₁ particles** (elementary particles or oxysomes) whose head contains the enzyme, ATPase. The matrix of mitochondria has enzymes of citric acid cycle; β -oxidation; few enzymes of urea cycle and ketone body synthesis (in liver); etc.

Both membranes have morphological, chemical and functional differences. The outer membrane contains about 40% lipid as compared to 20% in the inner membrane and it also has more cholesterol and phosphatidyl inositol. The inner mitochondrial membrane has **cardiolipin (or DPG)** which is a marker lipid for the membrane. This lipid is otherwise not found on eukaryotic membranes. The lipid/protein ratio is about 0.8 in the outer membrane and 0.3 in the inner membrane indicating greater degree of intercalation of different proteins (> than 100) in the inner mitochondrial membrane. The compartmentalization of representative mitochondrial enzymes is given Table 5.2.

Table 5.2: ENZYME DISTRIBUTIONS IN MITOCHONDRIA

Mitochondria	Enzymes
Outer membrane	Monoamine oxidase; Rotenone-insensitive NADH-cytochrome-c Reductase; Kynurenine hydroxylase; Fatty acid CoA ligase
Inter membrane Space	Adenylate kinase; Nucleoside-diphosphokinase;
Inner membrane	Complexes of the electron transport chain (ETC); ATP synthase; Succinate dehydrogenase (SDH; β -hydroxy butyrate dehydrogenase, Carnitine fatty acid acyl transferase
Matrix	Enzymes of the TCA cycle except SDH, β -Oxidation enzymes; some enzymes of the urea cycle; Pyruvate dehydrogenase complex (PDH)

The human mitochondrial DNA is only 16.5 kb circular DNA which encodes two rRNA and 22 tRNA and 13 proteins. The universal genetic code is not completely followed to translate mitochondrial genes. The organisation of the mammalian genome is a lesson in economy. Mitochondrial DNA sequences are used to study human migration and evolution. They are also employed to trace the ancestry of clients who are searching for their ethnic or geographic roots.

As you know, mitochondria are mainly involved in energy production (ATP) and they are responsible for fulfilling more than 95% of ATP requirements. Besides this, mitochondria also play an important role in amino acid and lipid metabolism, calcium homeostasis, regulation of programmed cell death and cell cycle regulation. Mitochondrial dysfunction has long been implicated in aging and age related diseases like atherosclerosis, arthritis, cataract, Alzheimer's disease, etc. Aging is a degenerative multi factorial process

influenced by genetic factors, nutrition and lifestyle. A defect in mitochondrial ETC leads to an increased production of reactive oxygen species (ROS); accumulation of mutations in mitochondrial DNA (mtDNA) over time; decrease in the number of mitochondria and a decline in energy production and metabolic functions.

5.7.2 Structure and Function of Chloroplast



T. Engelmann

The chloroplast is a specialised organelle found in plants and algae. It was identified as the site of photosynthesis in 1881 by the German biologist T. Engelmann. He showed a direct connection between oxygen evolution and peak of light absorption by chloroplasts in algal cultures. They are oval shaped with a diameter of 2 - 10 μm and generally there are 20-40 organelles per cell. It contains a green pigment called chlorophyll and many accessory pigments that absorb light energy to synthesize the assimilatory power (ATP and NADPH) for carbon dioxide fixation. Hence, chloroplast is also known as a light harvesting organelle.

Like the mitochondrial double membrane system, chloroplasts have outer and inner membranes. The outer membrane regulates the transport of material between the cytoplasm and the interior of the organelle while the **inner membrane** runs parallel to the outer membrane and is organized as an extensive folding into a series of internal parallel membranous sheets called lamellae. A thylakoid comprises of sac like structure of a lamellae. They are flattened vesicles arranged as a membranous network within the stroma. Some of the thylakoids are arranged in the form of discs stacked on top of each other as shown in the Fig. 5.13.

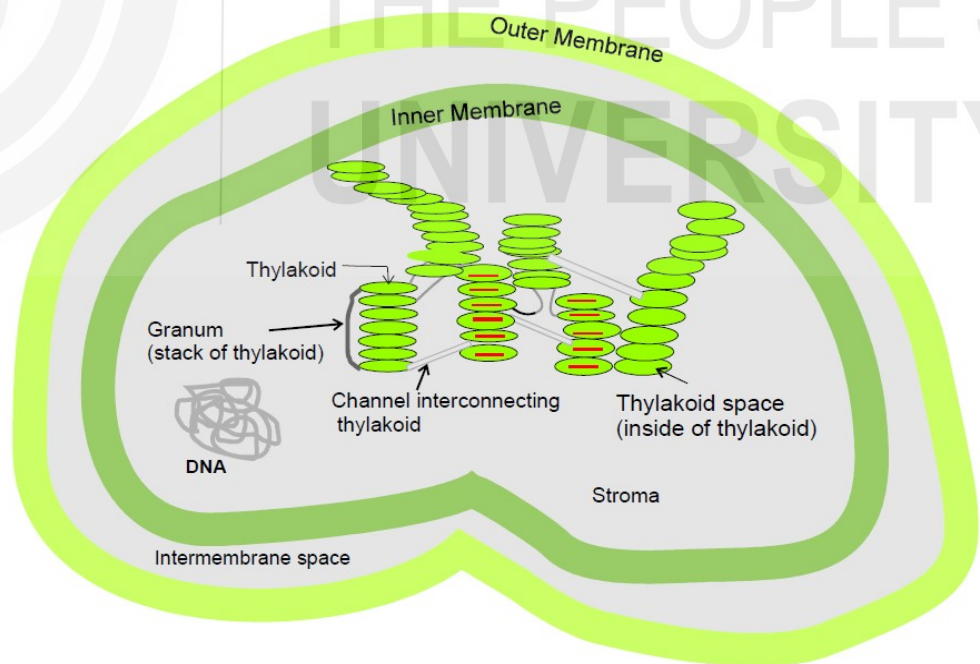


Fig. 5.13: Structure of chloroplast showing the arrangement of thylakoid lamellae and grana. The inner membrane of a chloroplast is fused to form stacks called thylakoids.

The stacked discs are called the '**grana**'. A typical chloroplast has about 40-60 grana and each granum may have 2-100 small, flattened thylakoids. Thus a chloroplast has three distinct membranes (outer, inner and thylakoid membrane) that generate three distinct compartments (intermembrane, stroma and thylakoid space).

The chloroplasts have their own DNA and machinery for proteins synthesis. In general chloroplast DNA is larger than mitochondrial DNA and their ribosomes show greater resemblance to those of bacteria. Depending on the plant species, chloroplast DNA has about 60-200 genes that include genes for tRNA, rRNA, ribosomal proteins and proteins of the thylakoid membrane complexes. The majority of proteins present in the plant chloroplast are encoded by nuclear DNA and synthesized in the cytosol.

The light absorbing chlorophyll molecules and ATP synthase are embedded in the thylakoid membranes (Fig. 5.14a). The pigment chlorophyll consists of magnesium bound (Mg^{++} ion), chlorin ring and a long phytol side chain (Fig. 5.14b). The chlorin ring resembles protoporphyrin ring of hemoglobin.

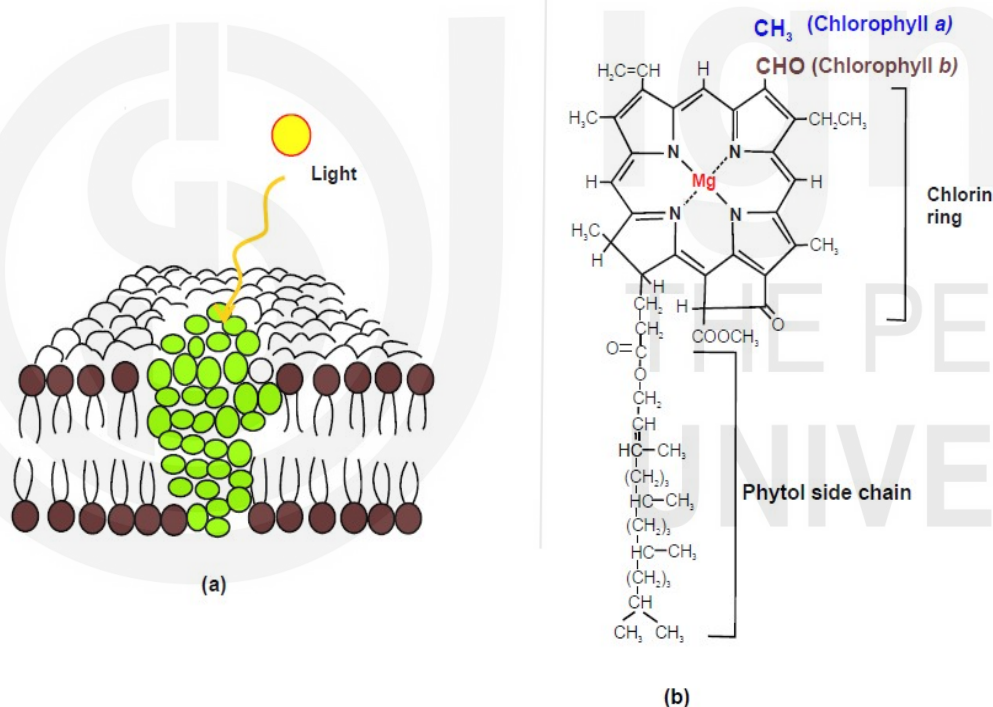


Fig. 5.14: a) Arrangement of chlorophyll in thylakoid and b) The structure of chlorophyll molecule.

It gives plants their characteristic green color and constitutes about 4% of chloroplast dry mass. There are many variants of chlorophyll known such as Chlorophyll a, Chlorophyll b, Chlorophyll c1, Chlorophyll c2, Chlorophyll d, Chlorophyll f. Among these, chlorophyll a and b are the most widely distributed form in plants. The pigment is complexed with proteins in vivo.

Plants also have **accessory pigments** called carotenoids that are present in the thylakoid membranes and as their name suggests they assist plants by absorbing light energy and transferring to chlorophyll during photosynthesis

and protect them from oxidative damage. The carotenoids include both carotenes and xanthophylls. The molecular structure of xanthophylls is similar to that of carotenes except for the presence of a hydroxyl group in xanthophylls (Fig.5.15).

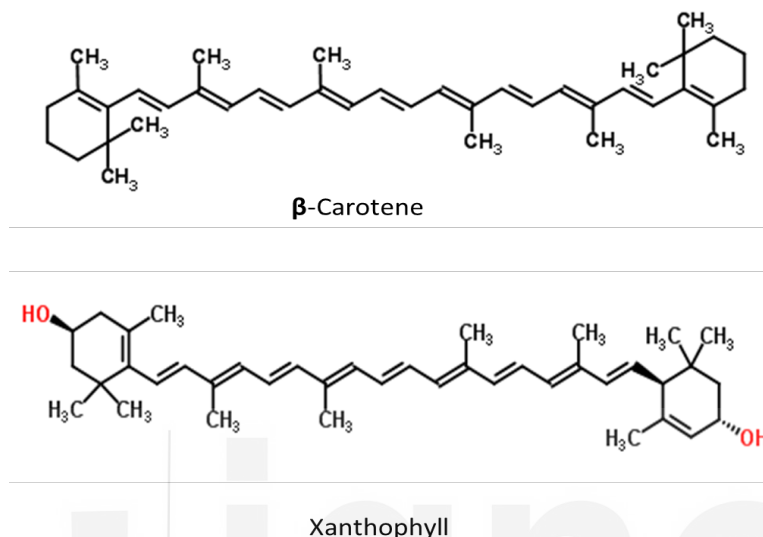


Fig. 5.15: Structure of β -carotene and Xanthophyll.

Photosynthesis is broadly subdivided into light and dark reactions. The thylakoid is the site for light reactions of photosynthesis producing ATP and NADPH by a non-cyclic flow of electrons from water to NADP^+ with the release of oxygen (a byproduct). The stroma of chloroplasts is the site for the light independent reactions or dark reactions of photosynthesis where ATP and NADPH are consumed for the reduction of CO_2 to sugar. The set of dark reactions constitute the Calvin cycle (Fig. 5.16). The chloroplasts also synthesize starch for transient storage when fixed carbon exceeds the immediate needs of the plant.

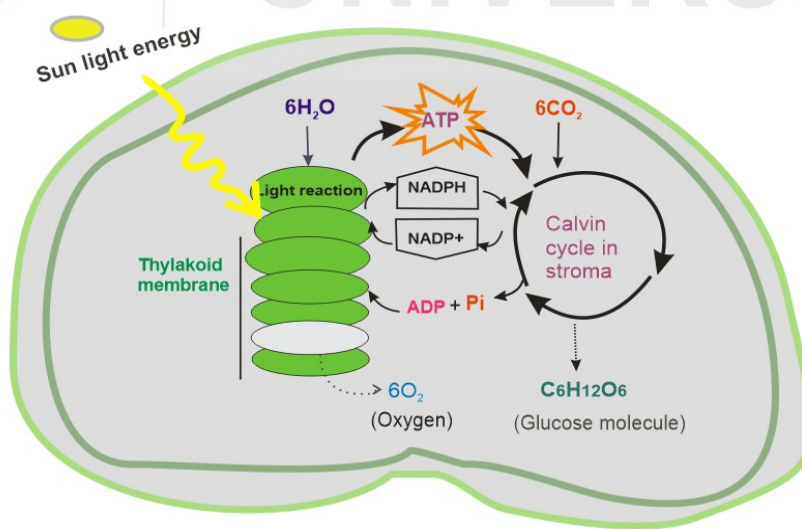


Fig.5.16: The basic photosynthetic process.

SAQ 6

Fill in the blanks:

- i) The primary function of mitochondria is
 - ii) The accessory pigments present in the chloroplast are
 - iii) Thylakoid membrane are the sites for
 - iv) Plants convert sunlight energy into energy by the process of
 - v) The marker lipid for mitochondrial inner membrane is
-

5.8 SUMMARY

In this unit you have studied that:

- Nucleus is the central organelle of eukaryotic cells, surrounded by a double membrane nuclear envelope. It has the genetic material organised into multiple chromosomes and is the site for DNA replication, transcription and processing of RNA. The bidirectional movement of proteins and / RNP between nucleus and cytoplasm is through NPC. Nucleolus is a prominent nuclear body involved in rRNA synthesis and ribosome biogenesis.
- Endoplasmic reticulum is a continuous membrane network of cisternae and tubules in eukaryotic cells that extend throughout the cytoplasm. The two morphological variants are RER and SER. The former is involved in protein synthesis, N-glycosylation and proteins folding while the latter serve as site for lipid metabolism particularly synthesis of steroid hormone and drug detoxification.
- Golgi complex is composed of stacks of flattened membrane enclosed cisternae. It plays an important role in terminal N-glycosylation of proteins and O-glycosylation of proteins and glycolipids. The major function of Golgi body is protein sorting. It receives secretory proteins from the ER and packages them into separate vesicles that carry them to their final destination.
- Lysosomes are membrane bound organelles rich in acid hydrolases that function best around pH 5. They are involved in intracellular digestion of biomolecules, autodigestion of damaged cell components and engulfed particulate matter via phagocytosis including harmful microorganisms. In Plants, the vacuoles perform a similar role but they are also sites for storage of nutrients and secondary metabolites.
- Peroxisomes are catalase positive single membrane bound organelles. They are rich in oxidases that produce H_2O_2 . They are also involved in β -oxidation of fatty acids, α -oxidation and synthesis of ether phospholipids.

In some germinating seeds they convert fat into carbohydrates. It also catalyses few steps of the photorespiratory cycle.

- Mitochondria and chloroplast are semiautonomous organelles. Their genome DNA encodes for some proteins and most of the non translatable RNA. Both are dependent on the nuclear genome for most proteins. Both are linked to energy metabolism.
- Mitochondria are also called the "Power house" of the Cell. With few exceptions, both plants and animal cells have mitochondria.
- Chloroplasts are found only in plant cells. They have pigments to trap light energy and generate assimilatory power for the reduction of CO₂ to sugars (photosynthesis).

5.9 TERMINAL QUESTIONS

1. Why is the nucleus regarded as the most important organelle?
2. State the functions of rough ER.
3. Indicate the functions of Golgi complex.
4. How do lysosomes maintain a low pH and why it is essential for their functioning?
5. Explain the term endocytosis.
6. Explain the role of peroxisomes detoxification.
7. Why mitochondria and chloroplast are considered as semiautonomous organelles?
8. Highlight important differences between mitochondria and chloroplast.

5.10 ANSWERS

Self Assessment Questions

1. i) Nucleolus ii) Histone iii) Nucleolus
iv) Endoplasmic reticulum v) Nuclear lamina vi) NPC
2. a) i) Smooth ER ii) Rough ER
b) RER consists of flattened membrane sacs. They have ribosomes on the cytosolic side and so the surface appears rough. SER has a tubular form and lacks ribosomes.
3. i) [F] ii) [T] iii) [T] iv) [F] v) [T]
vi) [F]
4. i) acidic ii) residual bodies iii) autophagy iv) plant cells
v) glycosylated vi) prevent vii) β - glucosidase
5. i) Catalase ii) Peroxisomes; mitochondria iii) H₂O₂ ; oxidases

- iv) Glyoxysomes of germinating seeds v) photorespiration
6. i) ATP synthesis ii) carotenoids iii) Light reaction of photosynthesis
iv) Chemical energy photosynthesis v) cardiolipin

Terminal Questions

1. **The nucleus** harbors genetic material (DNA) that carries information for all cellular structures in a coded language. The genetic material is replicated; transcribed and post transcriptionally modified in the nucleus. It also has many nuclear bodies including nucleolus which is the site for rRNA synthesis and ribosome biogenesis. Hence, it is regarded as the most important organelle.
2. RER is the site of protein synthesis for all proteins that fall in the secretory route. The newly synthesised proteins are N-glycosylated, trimmed and finally folded to their native forms. It is also involved in accessing the quality of folded proteins.
3. Golgi complex plays an important role in terminal N-glycosylation of proteins and O-glycosylation of proteins and glycolipids. The major function of Golgi body is in protein sorting. It receives secretory proteins from the ER and packages them into separate vesicles that carry them to their final destination. Many complex polysaccharides are also made here.
4. Lysosomes maintain low pH (around 5) by a specific H^+ ATPase that actively pumps protons across the membrane. The lysosomal enzymes work efficiently at acidic pH.
5. Endocytosis (cell eating) is a general process of uptake of either solid (biomolecules) or liquid materials (small particles). In this process, extracellular materials are internalized through invagination of membrane surface to form a large endocytic vesicle that fuse with lysosomes. The contents are degraded by acidic hydrolases. It can also be receptor mediated.
6. The peroxisomes have a number of H_2O_2 producing oxidases which is toxic and even mildly mutagenic. The removal of H_2O_2 is by catalase that breaks down H_2O_2 into H_2O and oxygen. Refer to section 5.6 and Fig. 5.11 for details.
7. Both mitochondria and chloroplasts have DNA that encodes for some of their proteins and most of the untranslatable RNA species (semiautonomous). They depend on nuclear genome for rest of the proteins needed.
8. a) Mitochondria are found in both animal and plants cells. They are the sites for oxidative metabolism and are involved in the synthesis of ATP. Chloroplasts are found only cells of plants. They trap solar energy to generate both ATP and reducing power (NADPH) for reduction of CO_2 to carbohydrates. Mitochondria have predominantly a catabolic role whereas the chloroplast has an anabolic role.

b) Chloroplasts contain pigments such as chlorophyll a, chlorophyll b and carotenoids. Mitochondria do not contain any such pigments.

c) Chloroplasts synthesise ATP only in the presence of light (photophosphorylation) whereas mitochondria generate ATP by oxidative phosphorylation // you can include more differences.

d) Mitochondrial ETC uses oxygen as the terminal electron acceptor while the light reactions of photosynthesis split water and reduce NADP^+ to NADPH.

5.11 SUGGESTED READINGS

1. The Cell: A Molecular Approach (2009) 5th ed., Cooper, G.M. and Hausman, R.E., ASM Press & Sunderland (Washington DC), Sinauer Associates, MA, ISBN: 978-0-87893-300-6.
2. Molecular Cell Biology (2012) 7th ed., Lodish, H., Berk, A., Zipursky, S.L., Matsudaira, P., Baltimore, D. and Darnell, J., W.H. Freeman & Company (New York); ISBN: 13:978-1-4641-0981-2 / ISBN: 10: 1-4641-0981-8.
3. Molecular Biology of the Cell (2008) 5th ed., Alberts, B., Johnson, A., Lewis, J., and Enlarge, M., Garland Science (Princeton), ISBN:0-8153-1619-4 / ISBN:0-8153-1620-8.
4. Cell and Molecular Biology, Concept and Experiment, (2010) 6th Edition, Gerald Karp, John Wiley & Sons, Inc.

UNIT 6

CELL WALL AND EXTRACELLULAR MATRIX

Structure

6.1	Introduction	6.4	Cell-cell interactions
	Expected Learning Outcomes		Adhesion junctions
6.2	Cell wall		Anchoring junctions
	Prokaryotic Cell Wall		Adherens junctions
	Cell Wall of Archaea		Desmosomes
	Cell Wall of Gram Positive Bacteria		Hemidesmosomes
	Cell wall of Gram Negative Bacteria		Focal adhesions
	Eukaryotic Cell Wall		Tight junctions
	Fungal Cell Wall		Communicating junctions
	Plant Cell Wall		Gap junctions
6.3	Extracellular matrix		Plasmodesmata
	Matrix Proteins	6.5	Summary
	Matrix Polysaccharides	6.6	Terminal Questions
	Cell-Matrix Interactions	6.7	Answers
		6.8	Suggested Readings

6.1 INTRODUCTION

In Unit 5, you have learned about structure and functions of subcellular organelles in eukaryotes. The boundaries of a cell are demarcated by a selectively permeable plasma membrane. However, the cells of prokaryotes (except mycoplasma), algae, plants and fungi are further covered by a tough and sometimes rigid **cell wall**. It provides structural support and protection and

acts as a filter. The animal cells on the other hand lack a cell wall and most of them are embedded in an **extracellular matrix** (ECM) consisting of secreted proteins and polysaccharides. It not only provides structural support to cells and tissues, but also plays an important role in regulating cellular interactions and cell behavior.

This unit deals with the structure and function of the cell wall in prokaryotes and eukaryotes and the ECM. It ends with a discussion on the importance of cell-cell and cell-matrix interactions in multicellular organisms.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ define the cell wall and its role;
- ❖ describe cell wall structure in all three major domains (archaea, bacteria and eukaryotes);
- ❖ compare cell wall structure of archaea and eubacteria; gram negative and gram positive bacteria, prokaryotes and eukaryotes;
- ❖ explain the structure and role of ECM and;
- ❖ appreciate the importance of cell matrix interactions and cell-cell interactions.

6.2 CELL WALL

The cells of majority of organisms are enclosed in a protective **cell wall** surrounding a fragile plasma membrane. It determines cell shape and prevents cells from shrinking and bursting due to changes in osmotic pressure. Despite a common role the cell wall of bacteria and eukaryotes are structurally divergent. This section deals with some of the variations in cell wall composition encountered in nature.

6.2.1 Prokaryotic Cell Wall

The differential staining technique developed by **Christian Gram** in 1884 broadly divided bacteria into Gram positive and Gram-negative organisms. We still do not have a complete understanding of the basis of Gram reaction although differences in cell wall structure are largely responsible for it. We begin this subsection by highlighting the major structural differences between them.

6.2.1.1 Cell Wall of Archaea

In unit 1 we have briefly discussed the cell wall structure of archaea. As you know they can be Gram positive or Gram negative like true bacteria despite lack of structural resemblance. Archaea characteristically lack muramic acid and D-amino acids. They exhibit variability in cell wall structure even within the group.

Among Gram positive archaea some have peptidoglycan like polymers (pseudomurein) and L-amino acids in cross links while others have complex polysaccharides resembling heteropolysaccharides of animal proteoglycans. The Gram negative archaea on the other hand have a layer of protein or glycoprotein.

6.2.1.2 Cell Wall of Gram Positive Bacteria

In Gram positive bacteria such as species of *Bacillus* the plasma membrane is surrounded by a thick cell wall made up of several sheets of **peptidoglycan** or murein (20-80nm) stacked one upon another (**Fig. 6.1**). Additionally, many bacteria have acidic **teichoic acid** polymers embedded in the cell wall.

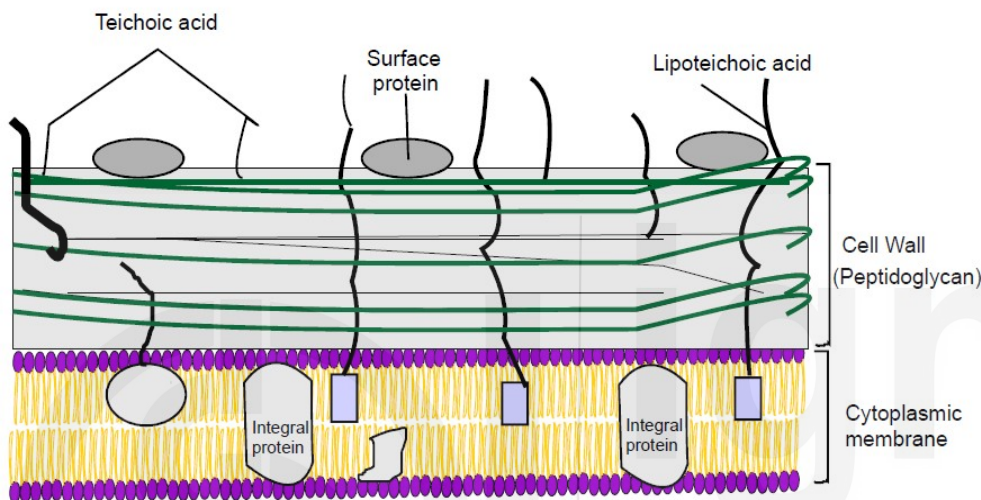


Fig. 6.1: Cell wall of Gram positive bacteria.

The peptidoglycan is a **branched polymer** of linear glycan chains made up of a repeating disaccharide, namely N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) (Fig.6.2).

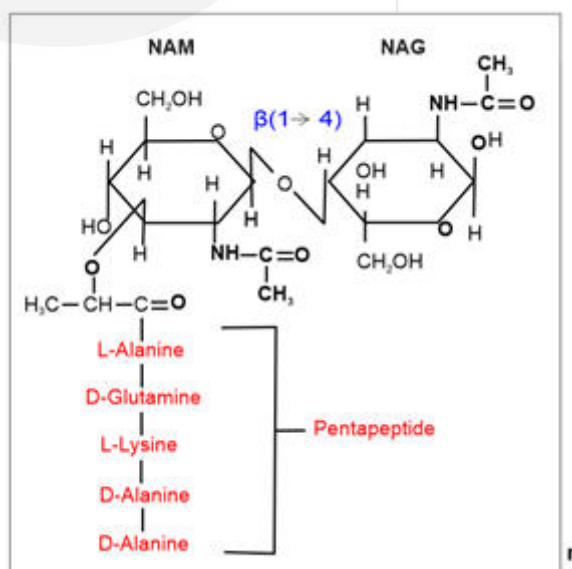


Fig. 6.2: The Repeating Unit of Peptidoglycan.

The two sugars are linked via a β (1 $\rightarrow$ 4) glycosidic linkage. Each NAM residue is linked to a tetra- or penta- peptide that generally has few D-amino acids. The peptidoglycan is cross linked either directly with the free carboxyl and amino groups of tetra peptides on different chains or via a penta glycine linker. The resultant multilayered covalently cross linked structure is much stronger than the cell wall of Gram negative bacteria. Interestingly, the unique structure of their cell walls also makes them vulnerable to some antibiotics.

The teichoic acid polymers are negatively charged phosphorylated sugar alcohols (**Fig.6.3**). The repeating units (10-50) are linked via phosphodiester bonds. Depending upon the organism the building block may vary. The 1, 3 poly (glycerol phosphate) teichoic acid are present in many species of bacteria and 1, 5 poly (ribitol phosphate) teichoic acid occur in many *Lactobacilli* and *Staphylococci*. These polymers may have D- amino acids or other sugars as substituents. Teichoic acids are attached covalently to murein through a linkage unit.

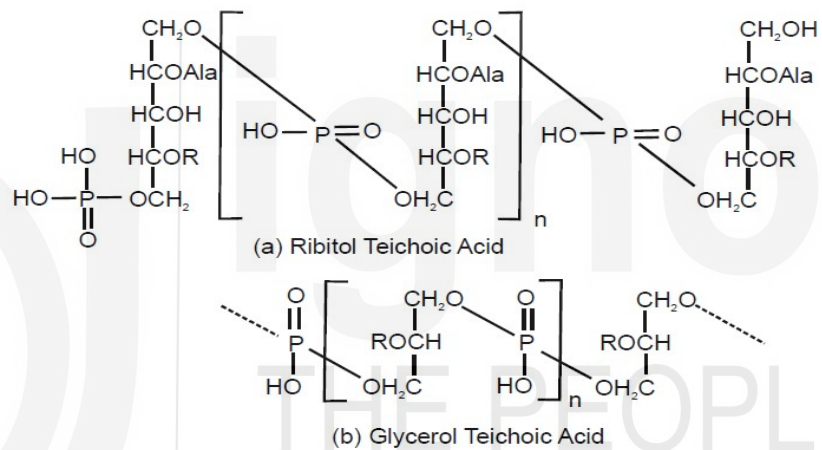


Fig. 6.3: Teichoic acid polymers.

6.2.1.3 Cell Wall of Gram Negative Bacteria

The cell wall of Gram negative bacteria is more complex than Gram positive bacteria. It has a dual membrane system consisting of an inner **peptidoglycan** and a permeable outer membrane, LPS (Fig.6.4).

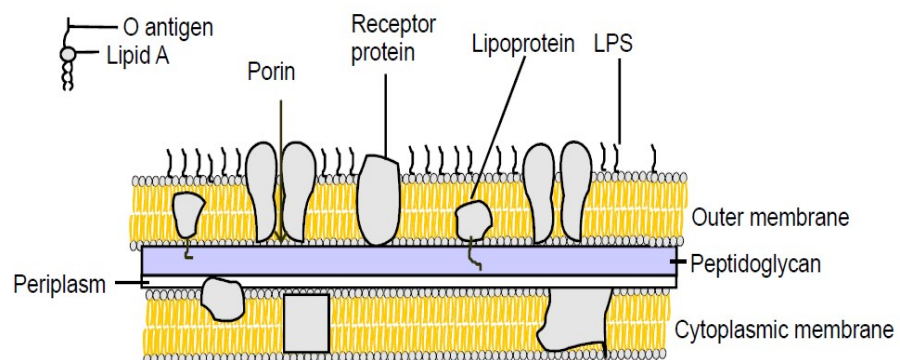


Fig. 6.4: Cell wall of Gram-negative bacterium.

The structure of peptidoglycan is similar to that of the Gram positive bacteria except that it is much thinner (2-7nm) and represents 5-10% of the wall weight. The **lipopolysaccharide (LPS)** has phospholipids, protein and lipopolysaccharides.

The structure of LPS can be subdivided into three parts – O-antigen, core polysaccharide and lipid A (Fig. 6.5). The **O-antigen** is a repeating chain of tetra or pentasaccharides and shows enormous variability. It accounts for type specific immunological reactions. The **core polysaccharide** of nearly all LPS has D-glucose, D-galactose, N-acetyl glucosamine, a heptose and an unusual octose derivative **2-keto 3-deoxyoctonate (KDO)**. Finally, **lipid A** is the least variable part of LPS and is responsible for all the ills and benefits that endotoxins (an earlier name of LPS) inflict on the host. It has two N-acetyl glucosamine (NAG) phosphates or pyrophosphate residues to which saturated fatty acids are attached. Lipid A is linked to KDO residue of the core polysaccharide. The outer membrane also has **Braun's lipoprotein** that is covalently linked to peptidoglycan. The aqueous channels (**porins**) interrupt the membrane and facilitate movement of low molecular weight hydrophilic molecules.

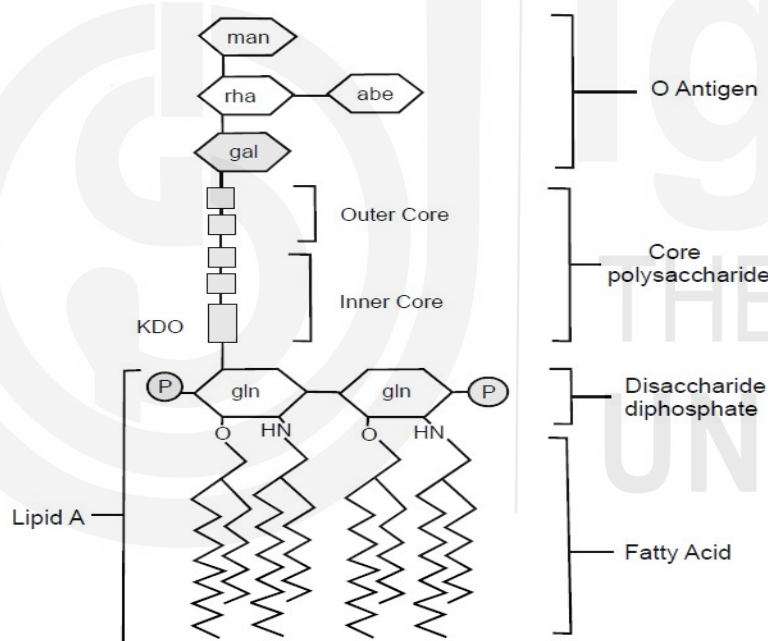


Fig.6.5: Structure of Lipopolysaccharide (LPS).

6.2.2 Eukaryotic Cell Wall

The cell wall of eukaryotes (fungi, algae, and higher plants) is composed principally of polysaccharides. In this section we will study two major structural variants seen in eukaryotes – cellulose based or largely non cellulose based.

6.2.2.1 Cell Wall of Fungi

The cell wall composition is variable among different groups of fungi and our present knowledge is incomplete. It is recognised that true fungi have chitin

and glucans in their cell wall and majority of fungi lack cellulose (Fig 6.6a). Chitin is a linear polymer of NAG residues (Fig.6.6b). In some zygomycetes fungi chitin is partially deacetylated by chitin deacetylase enzyme and so the cell wall contains **chitosan** (deacetylated chitin). Both chitin and its variant chitosan form microfibrils like cellulose and associates with non fibrillar material. The other polysaccharides include mainly **β -glucans** that are generally β (1, 3) or β (1, 6) linked. The majority of glycoproteins are mannans or **mannoproteins**. Some proteins are also anchored to the cell membrane that helps in maintaining cell wall integrity. The cell wall of yeast is composed of microfibrils of mannans and β -glucans.

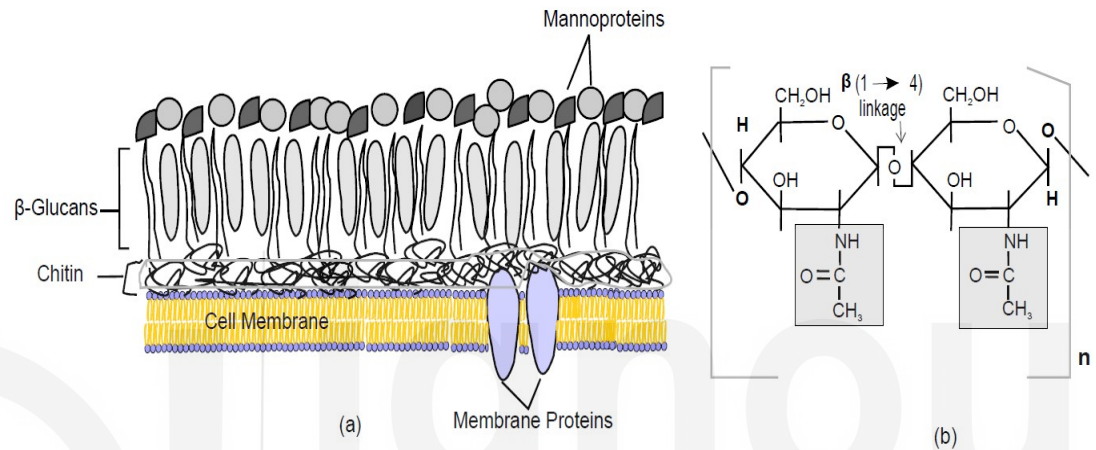


Fig. 6.6: a) The structure of fungal cell wall b) Chitin Structure

6.2.2.2 Cell Wall of Algae and Higher Plants

The cell walls of most algae and higher plants are composed of **cellulose** which is a linear homopolymer of glucose residues linked by β (1 $\rightarrow$ 4) linkage (Fig.6.7a). It is the most abundant organic substance found in nature. The most stable conformation of cellulose fibrils is an extended straight chain structure in which each monomer is turned 180° relative to the preceding one. In higher plants 30-36 chains join in the same polarity to form a single microfibril, stabilized by hydrogen bonding. The microfibrils assemble to form macrofibrils (Fig.6.7b).

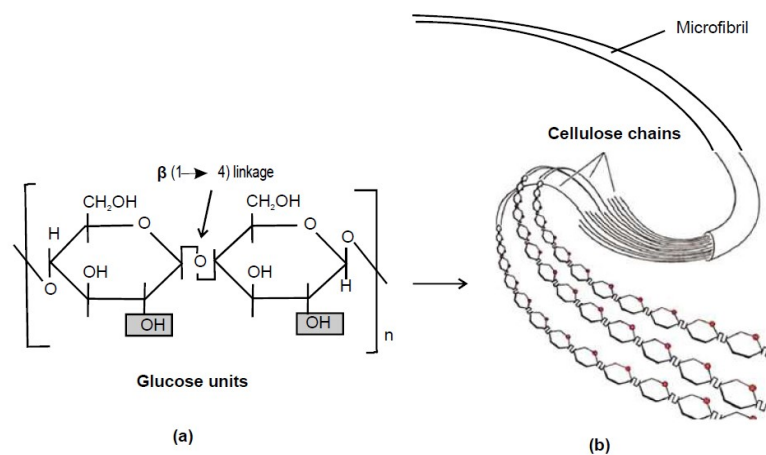


Fig.6.7: a) Building Block of Cellulose b) Cellulose Microfibrils.

(Source: M. Cooper The Cell: A Molecular Approach)

Many algae make much larger microfibrils than higher plants. The cellulose microfibrils resist stretching so their orientation governs the direction in which the growing cell enlarges.

Plant cell walls also consist of a complex mixture of polysaccharides that provide a gel like medium in which cellulose microfibrils are embedded. The matrix polysaccharides are branched heteropolysaccharides; earlier divided into **pectins** and **hemicellulose** on the basis of their solubility. Our present knowledge of the chemical complexity of these polymers has led to a nomenclature based on their structure. The pectins are acidic polysaccharides with an abundance of D-galacturonic acid residues such as homogalacturonans. The hemicelluloses are represented by xyloglucans that may be heteroxylans (arabinoxylglucans) and heteromannans (galactomannans).

Despite the apparent diversity, cell walls are classified into **primary** and **secondary walls**. Primary walls are formed by growing cells and are relatively unspecialized and similar in molecular architecture in all cell types. They are composed predominantly of polysaccharides together with lesser amount of structural glycoproteins (**extensins**), phenolic esters, minerals and enzymes. The major polysaccharides in the primary wall are **cellulose**, **hemicellulose** and **pectin** in almost equal proportions (Fig. 6.8) and cellulose fibers are randomly arranged.

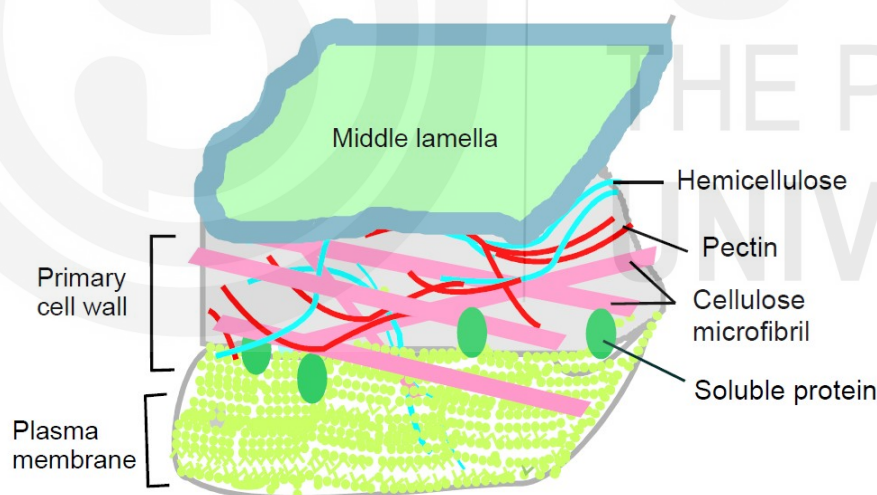


Fig.6.8: The Structure of Primary Cell Wall

The **secondary cell wall** is located between the primary cell wall and plasma membrane (Fig. 6.9). A cell starts producing secondary cell wall after primary wall is complete and cell growth (enlargement) has ceased. The secondary cell wall has a higher density of cellulose microfibrils (50-80%), along with other polysaccharides, lignin, and glycoproteins. **Lignin** largely replaces xyloglucans, glucoarabinoxylans and pectins in the matrix and it is cross linked to microfibrils that is largely responsible for much of its strength and density of wood. The secondary walls are frequently laid down in layers in which cellulose fibers differ in orientation, forming a laminated structure that greatly

increases cell wall strength. It is more rigid, and less permeable to water than the primary cell wall. It is also more resistant to degradation.

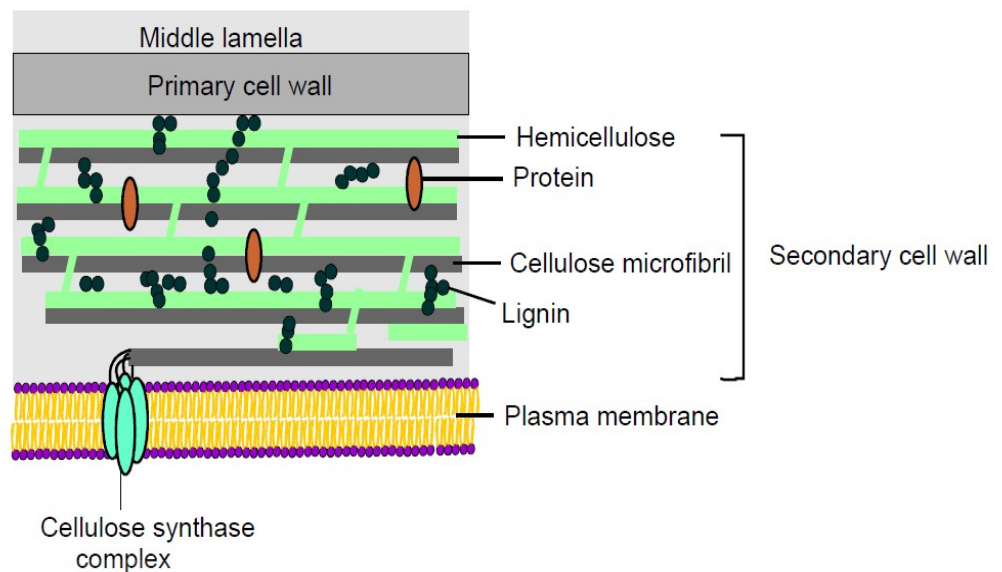


Fig. 6.9: A Model of the Secondary Cell Wall.

SAQ 1

a) Fill in the blanks:

- i) Gram positive bacterial cell wall is composed of and
- ii) The matrix polysaccharides in primary cell wall of plants are grouped into and
- iii) Cellulose is the structural constituent of
- iv) The principal polysaccharides of fungal cell walls is and
- v) The most variable part of lipopolysaccharide (LPS) is called
- vi) Chitosan is

b) Differentiate between primary and secondary plant cell walls.

6.3 EXTRACELLULAR MATRIX (ECM)

In the absence of cell wall many animal cells / tissues are surrounded by an **ECM or ground substance** — an organized network of extracellular materials present beyond the immediate vicinity of the plasma membrane. Most cells in multicellular organisms secrete proteins and heteropolysaccharides into the extracellular space where they self assemble to form an organised meshwork characteristic of the ECM. The ECM is more than inert nonspecific glue that holds cells together; it often plays a key regulatory role in determining shape and activities of the cell.

One of the best studied extracellular matrices is the **basement membrane** (or basal lamina). It is a continuous sheet that surrounds nerve fibers, muscles,

and fat cells and underlies the basal surface of epithelial tissues (epidermis of the skin), the lining of digestive and respiratory tracts and endothelial lining of blood vessels. Basement membranes provide mechanical support for attached cells, generate signals that dictate cell survival, serve as a substratum for cell migration, separate adjacent tissues within an organ, and act as a barrier to the passage of macromolecules. However, the ECM is most abundant in connective tissues. For example, the loose connective tissue beneath epithelial cell layers consists predominantly of an ECM in which fibroblasts are distributed. The ECM is mainly composed of tough fibrous proteins embedded in a gel-like polysaccharide ground substance - a design that is basically similar to that of plant cell walls. It also has adhesion proteins that link components of the matrix to one another and to attached cells (Fig. 6.10).

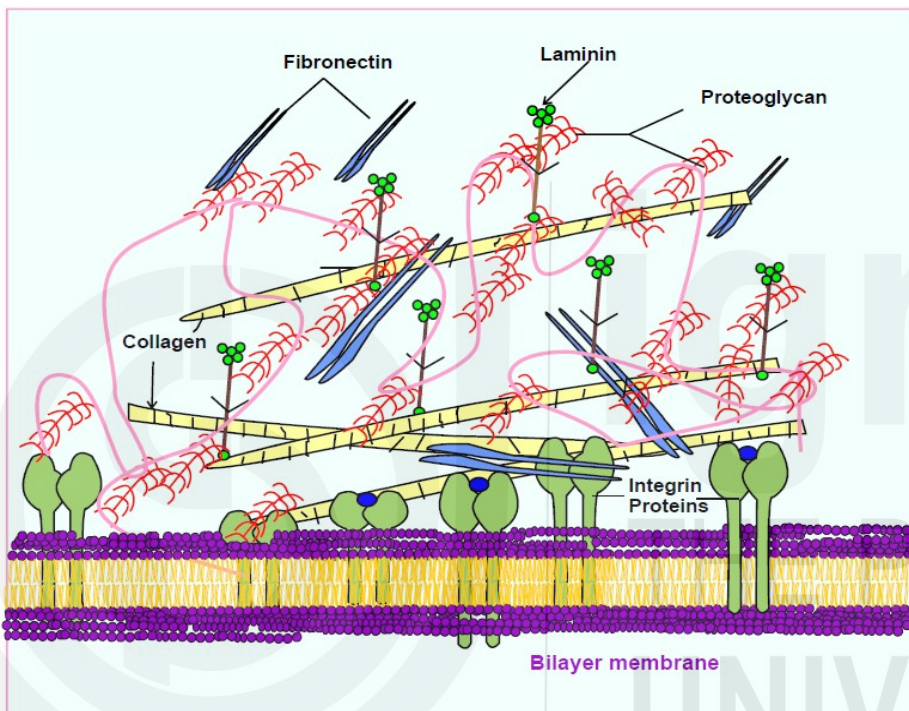


Fig. 6.10: An Overview of the Organization of the ECM.

6.3.1 Matrix Proteins

Unlike most proteins that are compact and globular in shape, those of the extracellular space are **fibrous** with extended conformation. In the reticular ECM surrounding the fibroblasts and other connective tissue cells the fibrous proteins include fibrillar collagen, elastin and fibronectin whereas basement membrane ECM underlying the epithelial cells has specialised collagens and laminin.

The major structural protein of ECM is **Collagen**. The collagens are a large family of glycoproteins containing at least 27 different members which are produced by fibroblasts, osteoblasts, chondrocytes and epithelial cells. Each collagen type is restricted to particular locations within the body, but two or more different types are often present together in the same ECM. Additional functional complexity is provided by mixing several collagen types within the same fiber.

The most abundant collagen is **type I collagen**. It is one of the fibril forming collagens. Their polypeptide chains consist of approximately 1000 amino acids or 330 **Gly-X-Pro** or **Gly-X-4-Hyp** repeats. After secretion as soluble precursors (procollagens) they are cleaved and assembled into collagen fibrils in which triple helical molecules are associated in regular staggered arrays.

All members of the collagen family share at least two important structural features. First, three separate polypeptides, called α -chains are supertwisted about each other. The superhelical twisting is right handed in collagen (Fig.6.11a and b). Second, many of the proline (and lysine) residues of each α -chain are hydroxylated. The tight wrapping of α -chains in the triple helix provides enormous tensile strength that can vary depending on the way they associate to form these supra molecular assemblies.

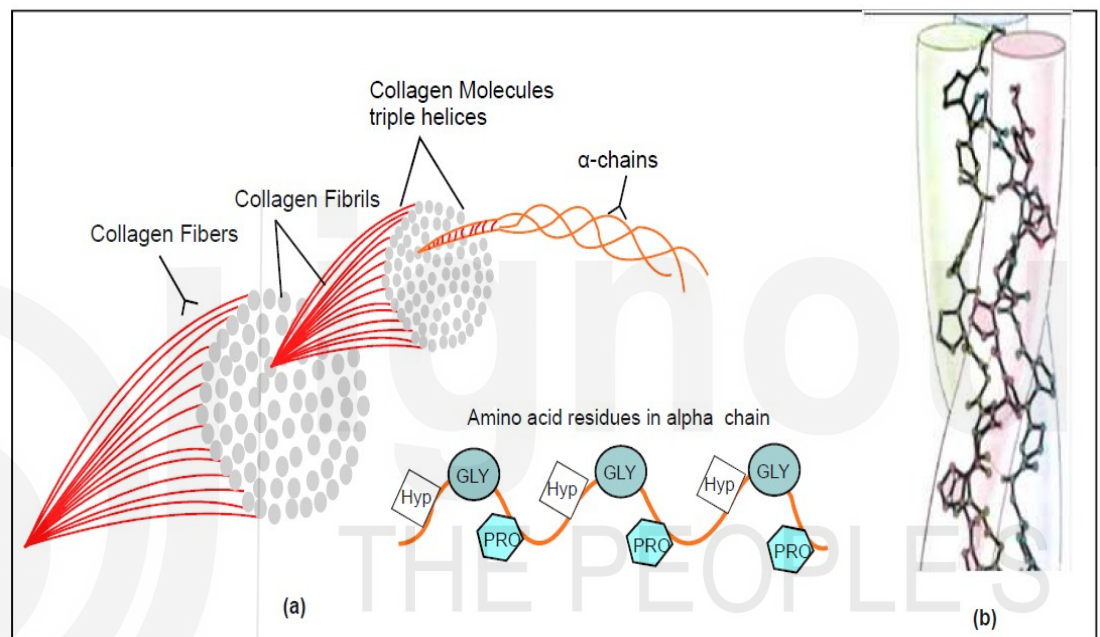


Fig. 6.11: a) The Assembly of Collagen Fibers b) Triple Helix of Collagen.
(Source : M. Cooper The Cell: A Molecular Approach)

In addition to collagen, connective tissue ECM has **elastin and fibronectin**. The former is present as bundles and provides elasticity to tissues and organs like lungs and arteries while the latter binds integrins and other ECM components such as collagen or proteoglycans and have a role in cell adhesion. The major ECM protein of the basal lamina is **laminin**. It is a heterotrimeric glycoprotein that can bind collagen, integrins and other cellular proteins. It can also bind itself to form sheets in the basal lamina and has a role in cell adhesion.

6.3.2 Matrix Polysaccharides

The most abundant heteropolysaccharides in the ECM are **Glycosaminoglycans (GAGs)**. They are a family of **unbranched polysaccharides** that are copolymers of alternating disaccharide unit. In the disaccharide unit one of the sugars is invariably an **amino sugar** that is either N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc) and the other with few exceptions is a **hexuronic acid** such as D-glucuronic acid

(GlcA) or L-idururonic acid (IdA). Some GAGs have the sugars further esterified with sulphate groups for example, Chondroitin 4-sulfate.

The carboxyl groups of uronic acids and sulphation confer a high negative charge on GAGs. They reduce the repulsive forces by assuming an extended conformation. The extended polymers are hydrated and impart high viscosity to the ECM. GAGs also have low compressibility, which makes them an ideal lubricating fluid in the joints. At the same time their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration. The major types of GAGs in animals are summarised in Table 6.1.

Table 6.1: Major Glycosaminoglycans (GAGs)

Glycosaminoglycan (GAG)	Repeating disaccharide	Distribution	Role(s)
Hyaluronic acid (hyaluronan)	GlcA and GlcNAc	Cartilage, vitreous humor of eye, cartilage and tendons	Lubricant and shock absorbers in the joints / Responsible for tensile strength & elasticity of cartilage.
Chondroitin sulfate	GlcA and GalNAc4S	Cartilage, tendons, ligaments, aorta	Contributes to tensile strength
Keratan sulfate	Gal and GlcNAc6S ; No uronic acid	Cornea, bones, hair, nails, claws	Corneal development; corneal transparency;
Dermatan sulfate	Many GlcA in Chondroitin sulfate are replaced by its epimer, L-IdoA	Skin, blood vessels, heart valves, sclera,	Pliability of the skin; structural integrity of the skin;
Heparin	IdoA2S and GlcNS3S6S	Mast cell granules	Binds to anti-thrombin & inhibit coagulation

Among the GAGs hyaluronic acid is very large with more than 50, 000 repeating units and is neither covalently attached to proteins nor sulfated. The other GAGs have much fewer number of repeating disaccharide units linked covalently to proteins. The GAG chains can bind to a variety of extracellular ligands and have the ability to modulate receptor-ligand interactions.

The majority of GAGs are linked to core proteins, forming **proteoglycans (mucopolysaccharides)**, which consists of up to 95% polysaccharide by

weight. Most proteoglycans are secreted and form the major components of the ECM while some like heparan sulphate proteoglycans are also integral membrane proteins. The linkage of GAGs to the protein core, mostly involves a specific **tetrasaccharide linker** composed of glucuronic acid (GlcA), galactose (Gal), and xylose (Xyl) residues, ($\text{GAG}_{(n)}\text{--GlcA--Gal--Gal--Xyl--Ser--protein}$). The xylose residue is linked to the protein core through an O-glycosidic bond.

The protein cores of proteoglycans are rich in serine and threonine residues which provide multiple sites of GAG attachment. Following the formation of tetra saccharide linker if the next sugar added is *N*-acetylglucosamine (GlcNAc) the GAGs attached will be either heparins or heparan sulfates but if the next sugar is *N*-acetylgalactosamine (GalNAc) instead, then the attached GAGs will be either chondroitin sulfates or dermatan sulfates.

Some proteoglycans can form proteoglycan aggregates that are very large complexes with many core proteins bound to a single molecule of hyaluronan. The core protein is called **aggrecan** and it binds covalently to several chains of GAGs to form an aggrecan monomer. It may contain as many as 30 keratan sulphate and 100 chondroitin sulfate chains.

When a large number of such core proteins bind noncovalently to hyaluronan, a hydrated aggregate of ECM is formed in cartilage type proteoglycans (Fig.6.12). On the other hand, proteoglycans found in basement membranes have only a few GAG chains attached to the core protein.

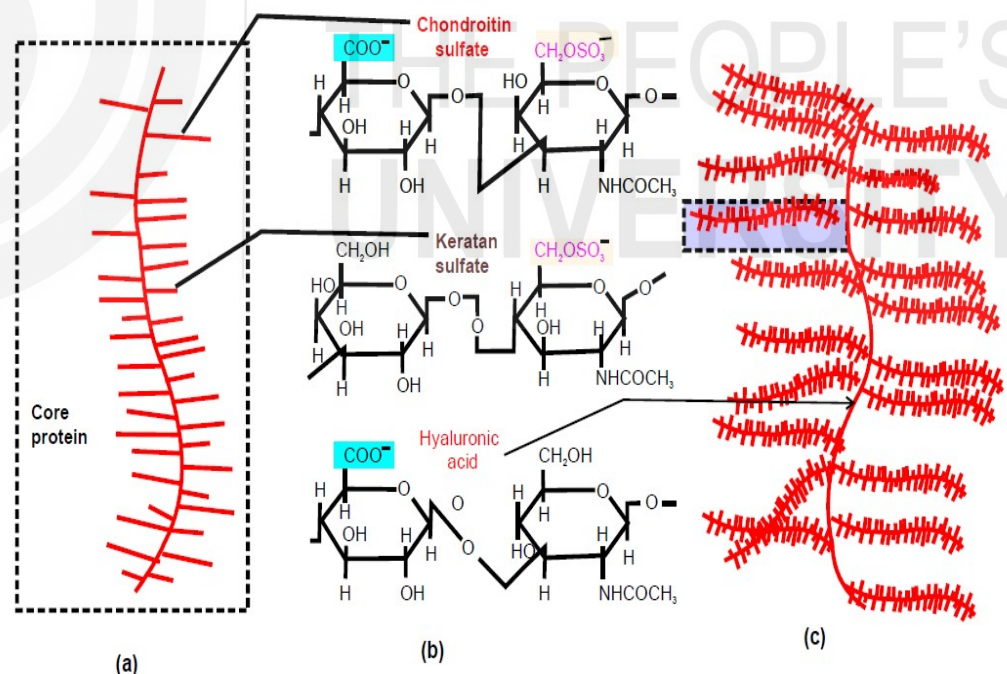


Fig. 6.12: The Structure of Cartilage type Proteoglycan Complex:

- Schematic Representation of a Single Proteoglycan with a Large Number of GAGS (shown in red) Attached to a Core Protein.
- The Repeating Disaccharides of GAGs.
- A Proteoglycan Aggregate.

6.3.3 Cell-Matrix Interactions

The structural components of the ECM such as proteoglycans bind to cell surface receptors and influence their ability to convey signals. The most important family of cell surface receptors that integrate extracellular and intracellular environments belongs to the **integrin family**. They are **heterodimers** of noncovalently linked α - and β - chains. About two dozen different integrins have been identified, each with a specific distribution. It was in recognition of their role in maintaining structural integrity of cells and tissues they were named 'integrins'. They bind to various ligands that are present in the extracellular environment and transduce signals by interacting either directly or indirectly with other proteins to influence events within the cell. Some integrins like LFA-1 (integrin) on one cell interact with LFA-3 (an ICAM) on another cell to mediate cell-cell interactions.

Integrins have been implicated in two major types of activities. First they hold cells (cellular "glue") in place by attaching at one end to the ECM components / to complementary molecules on other cells and at the other end to the cytoskeleton. Second they mediate "**outside-in**" **signaling** by relaying signals from matrix (or other cells) to the cell interior. In response to signals the cells respond in different ways such as changing shape, migration, proliferation, and differentiation.

The binding of integrin to ligands such as fibronectin or collagen induces a conformational change at the cytoplasmic end of the integrin (Fig. 6.13). This in turn alters the way the integrin interacts with cytoplasmic proteins like kinases. These kinases can then phosphorylate other proteins, initiating a chain reaction.

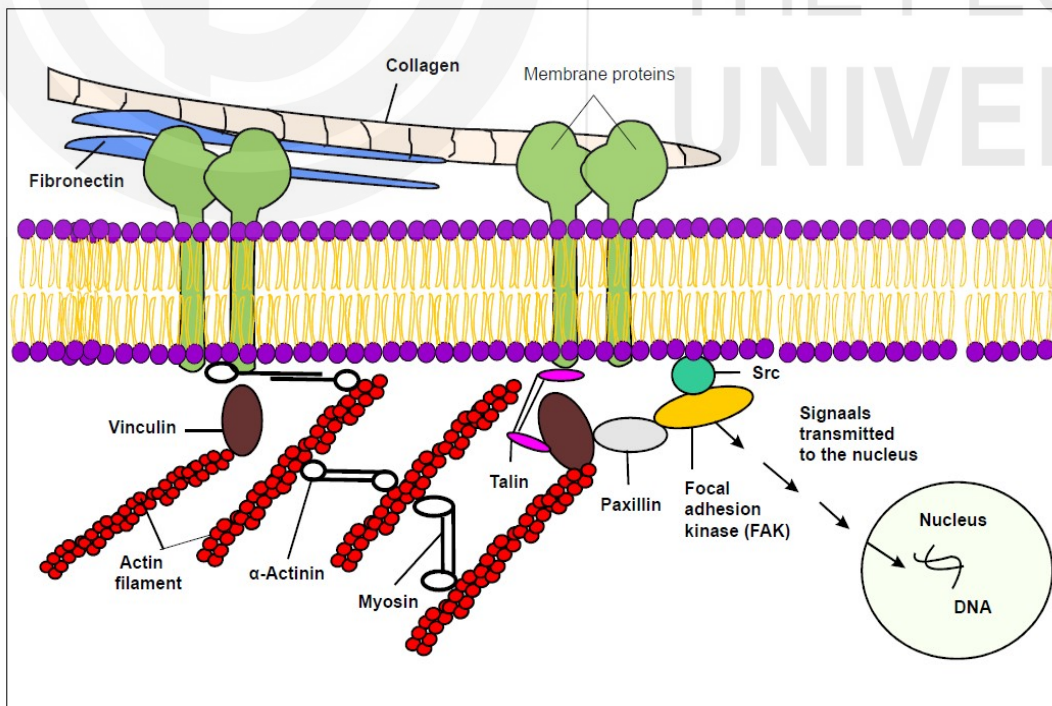


Fig. 6.13: A Focal Adhesion Showing the Concentration of Integrins.

(Source: Gerald Karp: Cell and Molecular Biology: Concepts and Experiments)

We will return to focal adhesions in the subsection on anchoring junctions. Integrins respond to messages received from inside the cell as well (**inside-out-signaling**). They can change their ligands or the strength with which they bind to them in response to signals. Inside-out-signaling is best documented in case of **blood platelets**. They circulate in blood singly and are non adherent. When the endothelial linings of blood vessels are injured, blood platelets stick to the ECM (without the help of integrins) in the exposed areas. This attachment (or the binding of thrombin) signals the cytoplasm that results in an inside-out activation of the integrins on the platelet surface. In this case integrins become more adhesive and better in picking up circulating molecules of fibrinogen that forms molecular bridges with more platelets and to the matrix. The large aggregates of platelets and proteins make a dense meshwork of cells and fibers (clots), preventing blood loss.

SAQ 2

a) Match the items in column I and column II

Column I	Column II
1. Gly-X-Pro or Gly-X-4-Hyp repeats	a. tetrasaccharide linker
2. lubricating fluid in the joints	b. Integrins
3. Links GAGs to the protein core	c. heparin
4. Cell surface receptor proteins	d. type I Collagen
5. Anticoagulant	e. hyaluronic acid

b) Fill in the blanks:

- i) The fibrous network of proteins and proteoglycans that surround animal cells/ tissues is.....
- ii) Collagen is the major component of
- iii) The repeating disaccharides units of glycosaminoglycans (GAG) are generally and
- iv) Collagen is made up of repeats.
- v) The..... family members are cell surface receptor for cell-matrix interactions.
- vi) The major extracellular protein in the basal lamina is

6.4 CELL-CELL INTERACTIONS

Cells communicate with each other also by direct contact involving intercellular junctions. There are some differences in the ways plant and animal cells do this. Direct interactions between cells are critical to the development, collaboration and coordination in multicellular organisms. Some cell-cell interactions are transient, such as the interactions between cells of the immune system while stable cell junctions play a key role in the organization of cells in tissues. Let us discuss the types of cell-cell interactions.

6.5.1 Adhesion Junctions

Cell-cell adhesion is a selective process mediated by **cell adhesion molecules (CAMs)**. They are divided into four major groups namely **selectins, integrins, immunoglobulin (Ig) super family**, and **cadherins** (**Fig. 6.14**). Cell adhesion mediated by selectins, integrins, and most cadherins requires Ca^{2+} , Mg^{2+} or Mn^{2+} .

The selectins are membrane glycoproteins with a calcium dependent lectin domain that is important for carbohydrate recognition and cell adhesion. There are three types of selectins namely, **L-selectin**, which is constitutively expressed on variety of leukocytes; **E-selectin** is expressed on activated endothelial cells; and **P-selectin**, that is expressed on platelets and activated endothelial cells. The selectin family mediates transient interactions (rolling adhesion) between leukocytes and endothelial cells for instance, during migration of leukocytes to sites of tissue inflammation. The weak adhesive interactions help to slow down circulating leukocytes allowing time to sense the signals before exiting circulation. The ligands for selectins are members of the sialomucin family.

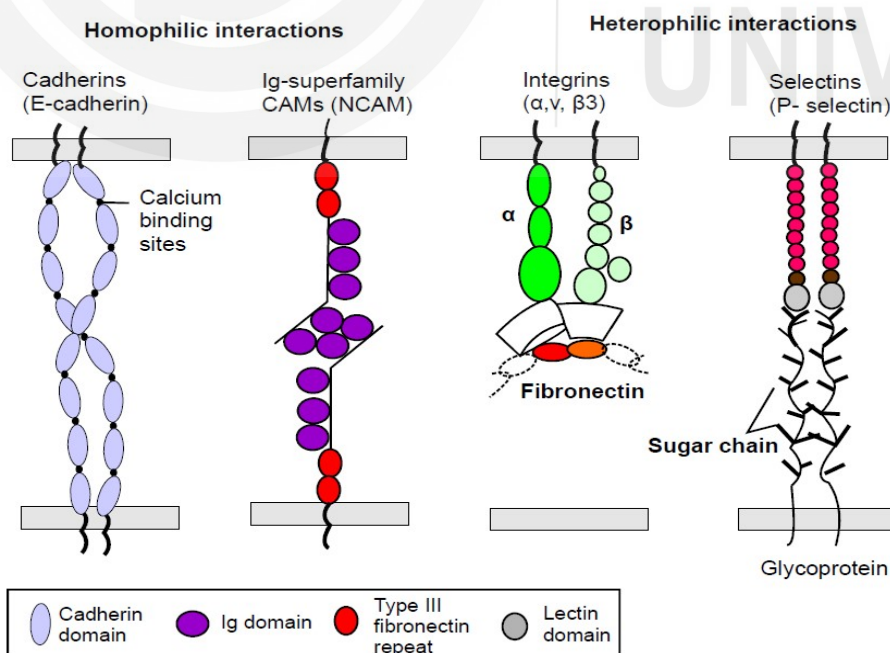


Fig. 6.14: Major Families of Cell Adhesion Molecules (CAMs) and their Receptors. (Source: Gerald Karp: Cell and Molecular Biology: Concepts and Experiments)

The weak cell-cell interactions are followed by stronger interactions involving β 2-integrins subfamily members expressed on surface of leukocytes and Ig super family CAMs such as **intercellular adhesion molecules (ICAMs)** on endothelial cells. The firm binding of leukocytes is an essential step for transendothelial migration.

Cadherins are a large family of calcium dependent transmembrane glycoproteins involved in selective adhesion between embryonic cells, formation of specific synapses in the nervous system and maintenance of stable junctions between cells in tissues. They share a highly conserved extracellular domain and mediate largely **homophilic interactions**. This means that molecules on one cell bind to molecules of the same kind on adjacent cells. For example, interactions between **E-cadherin** expressed on epithelial cells lead to the selective adhesion of epithelial cells to one another.

SAQ 3

Match the terms in column I with that in column II

Column I	Column II
1. L-selectin	a. found in both platelets and activated endothelial cells
2. P-selectin	b. non classical cadherin
3. E-selectin	c. calcium dependent cell-cell adhesion protein
4. Cadherins	d. constitutively expressed on leukocytes
5. Desmocollin	e. found in activated endothelial cells

6.5.2 Anchoring Junctions

Anchoring junctions connect the cytoskeleton of one cell to that of its neighboring cell (cell-cell junction) or to the ECM (Cell-matrix junction). They are most abundant in animal tissues that are subject to mechanical stresses. The basic components of these junctions are cell adhesion proteins and anchor proteins.

6.5.2.1 Adherens Junction

Adherens junctions are commonly found in epithelia such as lining of the intestine, where they occur as “adhesion belts” (or **zonula adherens**) to encircle cells near its apical surface. In an adherens junction, cells are held together by calcium-dependent linkages formed between extracellular domains of **cadherin molecules** (Fig. 6.15). The basic structural unit of an adherens junction includes anchor proteins **β -catenin** and **α -catenin** in addition to transmembrane cadherins. β -catenin binds directly to the cytosolic tail of the cadherins whereas, α -catenin binds to β -catenin as well as to actin filaments and actin filament-binding proteins, such as **vinculin**. Associated

with the cadherins is the β -catenin related protein p120. The cadherin clusters of an adherens junction connect the external environment to actin bundles of epithelial cells by forming a transcellular network and create a pathway for signal transduction.

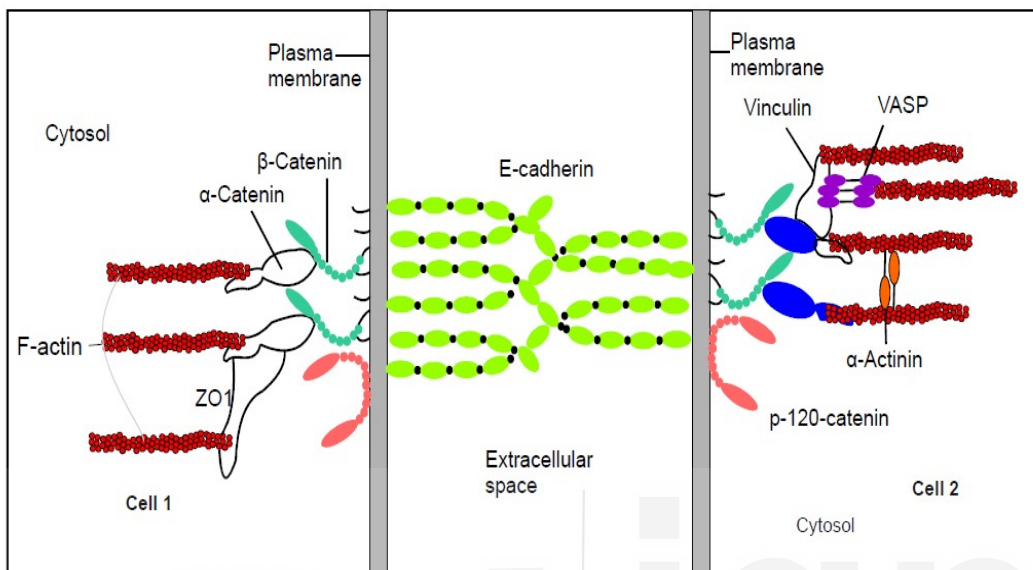


Fig. 6.15: Protein Interactions at an Adherens Junction.

6.5.2.2 Desmosomes

Desmosomes (or *maculae adherens*) are disk-shaped adhesive junctions found particularly in tissues subjected to mechanical stress such as cardiac muscle, epithelial layers of skin and uterine cervix. Desmosomes directly link the intermediate filament of adjacent cells across a narrow extracellular gap (**Fig. 6.16**) and assemble an integrated unit of great tensile strength.

The cadherins of desmosomes have a different domain structure and are called **desmogleins** and **desmocollins**. Their cytosolic tails are bound to plakoglobin and plakophilin that provide a direct link to the intermediate filament binding protein, **desmoplakin**.

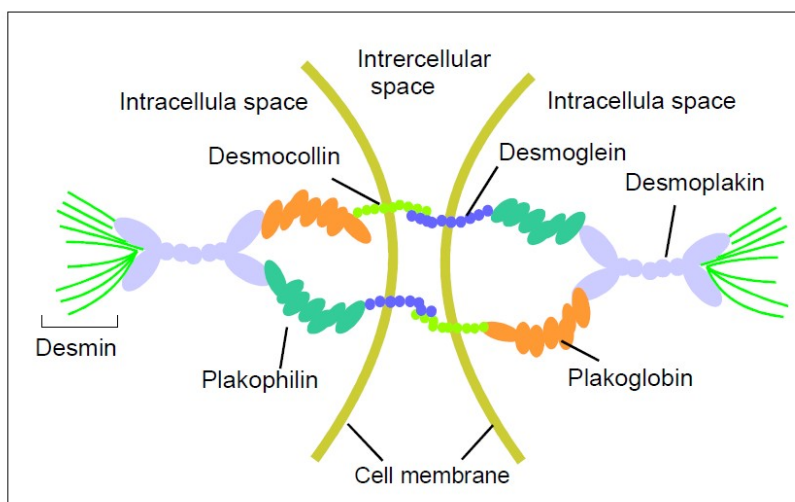


Fig. 6.16: Schematic Model of a Desmosome.

6.5.2.3 Hemidesmosomes

The basal surface of epithelial cells is anchored to the underlying basement membrane by a specialized adhesive structure called hemidesmosomes **or half desmosomes**. It resembles desmosomes and they also connect to intermediate filaments (keratin) of adjacent cells, but the transmembrane glycoproteins in hemidesmosomes are **integrins** rather than cadherins. Hemidesmosomes contain a dense plaque on the inner surface of plasma membrane with keratin filaments (intermediate filament) pointing into the cytoplasm that are linked to the ECM by integrins via an anchor protein, **plectin** (Fig. 6.17). The extracellular domains of the integrins bind to **laminin** to mediate adhesion. The hemidesmosomes facilitate association of intermediate filaments of adjacent cells into an extended network which help to distribute shearing forces across the epithelium.

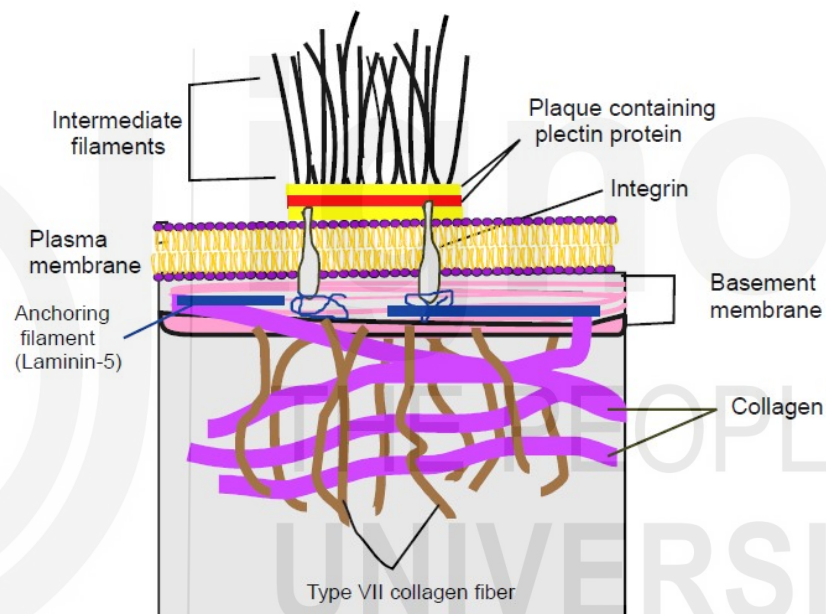


Fig. 6.17: The major Components of a Hemidesmosome.

6.5.2.4 Focal adhesions

Focal adhesions are dynamic structures that can be rapidly disassembled if the adherent cell is stimulated to move or enter mitosis. The plasma membrane in the region of a focal adhesion contains large clusters of **integrins**. The integrins can reversibly switch between active and inactive states and this dictates their ability to bind matrix components. The cytoplasmic domains of integrins are connected via adaptors to actin filaments (Fig. 6.13). Focal adhesions may act as a type of sensory structure, collecting information about extracellular environment and transmitting it to the cell interior, which may lead to changes in cell adhesion, proliferation, or survival. Focal adhesions also help in cell locomotion. They are capable of creating or responding to mechanical forces, which might be expected from a structure that contains actin and myosin.

6.5.3 Tight junctions or Zonula Occludens

Tight junctions (TJs) are the closest known contacts located at apical end of junctional complex between neighboring epithelial cells in vertebrates. They are also present between endothelial cells that line the walls of capillaries. This is particularly evident in brain where they form the *blood–brain barrier*, which prevents substances from passing from bloodstream into the brain. Essentially tight junctions perform two important functions. They form **seals** that prevent free passage of molecules (including ions) between cells of epithelial sheets. TJs also serve as “**fences**” to maintain polarized character of epithelial cells by not permitting diffusion of some membrane proteins and lipids between apical and basolateral parts of the membrane. In all cases the seal is not foolproof; they are permeable to the small molecules to varying degrees. The integral proteins of TJs form continuous fibrils that completely encircle the cell and make contacts with neighboring cells on all sides (Fig. 6.18).

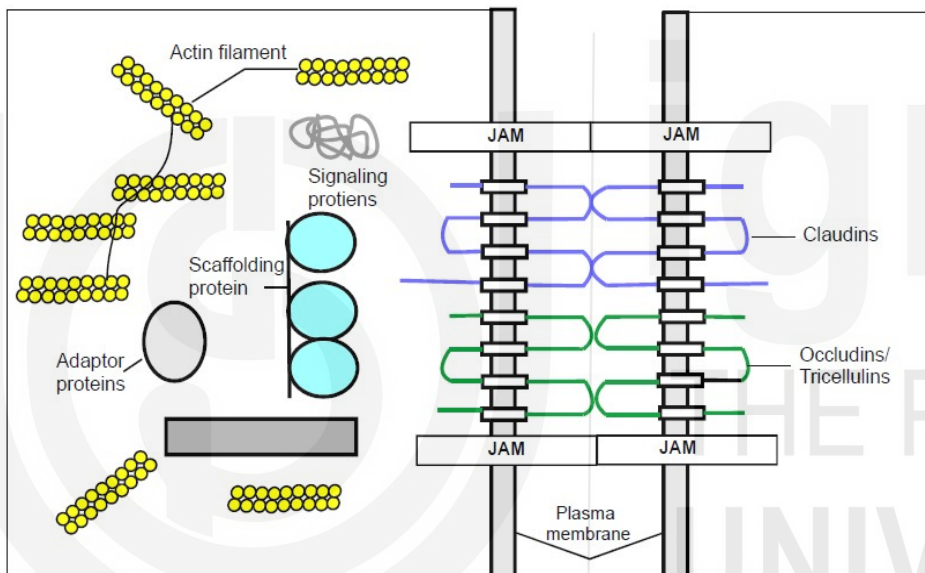


Fig. 6.18: Tight Junction Proteins Interact with Zonula Occludens (Zo) Family that Links them to Actin Cytoskeleton.

Each strand in these networks is composed of TM proteins of **claudin**, **occludin**, and **junctional adhesion molecule (JAM)** families. These proteins bind to similar proteins on adjacent cells, thereby sealing the space between their plasma membranes. The cytosolic tails of claudins, occludins, and JAMs are also associated with proteins of the zonula occludens family, which link the tight junction complex to the actin cytoskeleton and hold it in place on the plasma membrane.

6.5.4 Communicating Junctions

Communicating junctions as the name suggests will permit the movement of low molecular weight metabolites, ions, vitamins and even signaling molecules between adjacent cells. In this subsection, we will describe gap junctions and plasmodesmata.

6.5.4.1 Gap Junctions

In animal cells **gap junctions** are sites where plasma membranes of adjacent cells come very close to one another but do not make direct contact. Instead, the cleft between cells is spanned by very fine strands (molecular “pipelines”) (**Fig. 6.19**) that passes through adjoining plasma membranes and opens into the cytoplasm of neighboring cells. Consequently, gap junctions couple both metabolic activities and electric responses of the cells they connect such as epithelial cells, endothelial cells, and cells of cardiac and smooth muscles.

Gap junctions are composed of a transmembrane protein, connexins. The connexins proteins are organized into multisubunit complexes, called **connexon** that completely span the membrane. Each connexon is composed of six connexins subunits arranged in a ring around a central opening, or **annulus**. The connexon on one cell then aligns with connexon of adjacent cell, forming communicating channel between them.

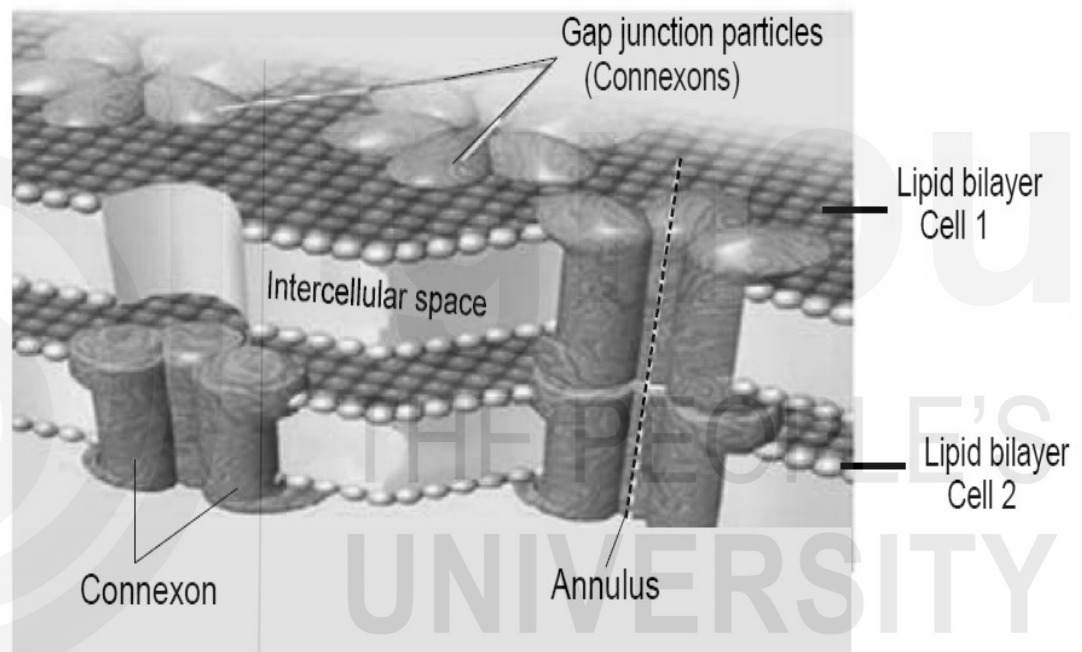


Fig. 6.19: A Gap Junction (Connexons). (Source: Gerald Karp: Cell and Molecular Biology: Concepts and Experiments)

Each cell forms a half channel that connects the cytoplasm of two adjoining cells. Most cell types express more than one type of connexins and they can assemble to form heteromeric connexons. These connexons show marked differences in conductance, permeability and regulation.

Mammalian gap junctions allow diffusion of molecules below 1000 Daltons in mass. A number of regulatory substances, such as cyclic AMP and inositol phosphates are small enough to pass through gap junction channels. As a result, gap junctions have the potential to integrate activities of individual cells of a tissue into a functional unit.

Gap junctions also allow cells to cooperate metabolically by sharing key metabolites, such as ATP, sugar phosphates, amino acids, and many coenzymes. This is particularly important in tissues such as the lens and cornea of the eye, which lack blood vessels.

6.5.4.2 Plasmodesmata

Unlike animals, plant cells are separated from one another by cell wall. Adjacent plant cells communicate through cytoplasmic connections called **plasmodesmata**. Plasmodesmata are lined by plasma membrane and usually contain a dense central structure, the **desmotubule**, derived from smooth endoplasmic reticulum of the two cells, providing continuity between endomembrane systems of adjacent cells (Fig. 6.20). Cells with thick primary and secondary walls often have pits, where the cell wall is thinner and plasmodesmata are concentrated facilitating cell-cell communication and transport. Some like stomatal guard cells and mature root hair lack plasmodesmata while some tissue boundaries (epidermis / cortex) have plasmodesmata with reduced permeability. They are dynamic structures that can open or close in response to appropriate stimuli, permitting regulated passage of macromolecules (including proteins and lipids) between adjacent cells. Thus, plasmodesmata play a key role in plant development by controlling trafficking of regulatory molecules.

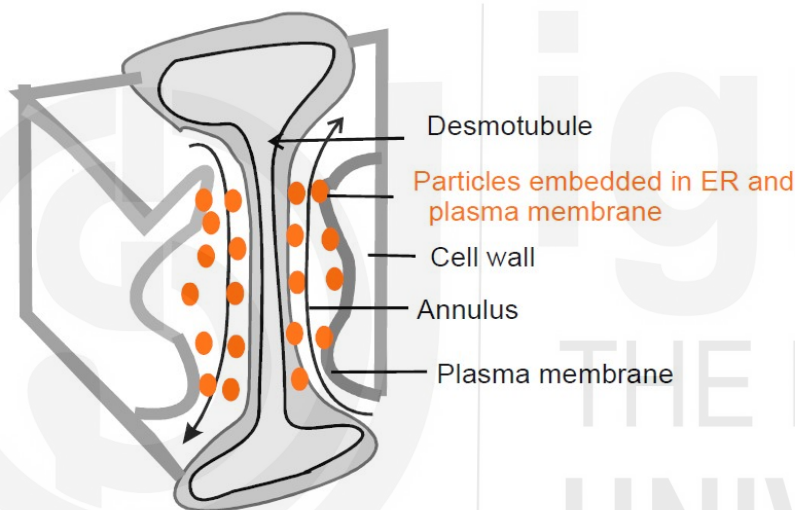


Fig. 6.20: Structure of Plasmodesmata. The Arrows Indicate the Movement through the Annulus.

SAQ 3

Indicate the role of the following:

- | | |
|----------------------|-----------------------|
| (a) Hemidesmosomes | (b) Plasmodesmata |
| (c) Zonula occludens | (d) Adherens Junction |

6.5 SUMMARY

In this unit so far, you have studied that:

- Plasma membrane of many prokaryotic and eukaryotic cells is surrounded by a cell wall that determines shape and protects the cell from bursting and shrinking due to changes in osmotic pressure. The cell wall material is chemically different among the three major domains of organisms.

- The cell wall of Gram positive and Gram negative bacteria has peptidoglycan as one of its constituents. In Gram positive bacteria, multi layered peptidoglycan is surrounded by negatively charged teichoic acid polymers whereas Gram negative bacteria have LPS covering the single layered peptidoglycan.
- The cell wall of eukaryotes (fungi, algae, and higher plants) is composed principally of polysaccharides. The composition of the cell wall is variable among different groups of fungi. It is recognised that true fungi have chitin and glucans in their cell wall and majority of fungi lack cellulose. The major component of plant cell wall is cellulose. It also consists of a complex mixture of polysaccharides that provide a gel like medium in which cellulose microfibrils are embedded.
- In the absence of cell wall many animal cell / tissues are surrounded by **ECM**. Most cells in multicellular organisms secrete fibrous proteins and heteropolysaccharides into the extracellular space where they self assemble to form an organised meshwork characteristic of the extracellular matrix.
- In mammalian cells, collagen and proteoglycans are the major structural components of ECM. They regulate a variety of cellular processes in multicellular organisms including motility, shape, survival, proliferation and differentiation. The integrin family of cell surface receptors plays a crucial role in integrating the extracellular and intracellular environment.
- Cells also communicate with each other by direct contact at intercellular junctions. There are some differences in the ways plant and animal cells do this. Plasmodesmata are junctions between plant cells, whereas animal cell contacts include gap junctions and anchoring junctions.
- Direct interactions between cells are critical to the development and functioning of multicellular organisms. Some cell-cell interactions are transient, such as the interactions that direct white blood cells to sites of tissue inflammation. In other cases, stable cell-cell junctions play a key role in the organization of cells in tissues. In addition specialized types of junctions provide mechanisms for rapid communication between cells.

6.6 TERMINAL QUESTIONS

1. Schematically compare cell wall structure of Gram positive and Gram negative bacteria.
2. Enlist the functions of extracellular matrix in animal tissues.
3. How are integrins able to link the cell surface with the ECM?
4. What is the difference between inside-out and outside-in signaling? What is the importance of each in coordinating cellular activities?

5. List two characteristics that distinguish hemidesmosomes from focal adhesions.
6. How does the structure of a tight junction contribute to its function?
7. Highlight two structural features shared by members of the collagen family.

6.7 ANSWERS

Self Assessment Questions

1. a)

- | | |
|--------------------------------------|--------------------------------------|
| i) Peptidoglycan and Teichoic Acids | ii) Hemicelluloses and pectins |
| iii) Plant and Most Algal Cell Walls | iv) β -1,3-glucans and chitins |
| v) O-antigen | vi) Deacetylated chitin |

b)

No.	Features	Primary cell wall	Secondary cell wall
1	Occurrence	In all plant cells/ relatively unspecialized	Only in mature and non-dividing cells
2	Location	Between PM and middle lamella	Between primary cell wall and PM.
3	Nature	Elastic and thinner	Inelastic, rigid and thicker
4	Major wall material	Predominantly polysaccharides: Cellulose, hemicellulose & pectin; some proteins	Cellulose; hemicellulose; Lignin, suberin or cutin and glycoproteins; lacks pectin
5	Pits	Absent	Present
6	Amount of cellulose	Low	High
7	Arrangement of cellulose fibrils	Wavy & loosely arranged	Straight parallel & closely packed.

2. a)

- 1- d; 2 - e; 3 - a; 4- b; 5- c.

b).

i) ECM

ii) Connective Tissues

iii) An Amino Sugar and Hexuronic Acid

iv) Gly-X- pro or Gly-X-4 Hyp

v) Integrin

vi) Laminin

b) 1-d; 2-a; 3-e; 4-c; 5-b

3.

- a) The hemidesmosomes facilitate the association of IF of adjacent cells into an extended network which help to distribute the shearing forces across the epithelium. They are also important in cell signaling.
- b) Plasmodesmata are communicating junctions in plant cells through which trafficking of regulatory & other molecules takes place.
- c) Zonula occludens is a tight junction that forms **seals** to prevent the free passage of molecules (including ions) between cells of epithelial sheets. TJs also serve as “fences” to maintain the **polarized character** of epithelial cells by not permitting diffusion of some membrane proteins and lipids between apical and basolateral parts of the membrane.
- d) Adherens Junction hold cells together and bridge the gap between neighboring cells especially in epithelia. The net outcome of these interactions is to link actin bundles of epithelial cells via cadherins and anchor proteins like β -catenin and α -catenin into a transcellular network.

TERMINAL QUESTIONS

1. Refer to Fig.6.1 and Fig. 6.4 for details.
2. Most animal cells secrete materials into extracellular space. They are primarily proteins and polysaccharides. Collagen is the most abundant proteins. Its fibers are interwoven with carbohydrate rich proteins molecules called proteoglycans. Collectively, these materials constitute the ECM. It performs multiple roles such as:
 - providing support,
 - segregating tissues from one another,
 - regulating intercellular communication,
 - regulating a cell's dynamic behavior,
 - sequesters a wide range of cellular growth factors and acts as a local store for them
3. Integrins are a family of cell surface receptors that function in integrating extracellular and intracellular environments. Integrins couple ECM to the cytoskeleton (in particular, the microfilaments) inside the cell. They bind to

specific ligands in the ECM depending on the type of integrin subunits. Some of the ligands include fibronectin, vitronectin, collagen, and laminin. They hold cells in place by attaching at one end to the ECM components / to complementary molecules on other cells and to the cytoskeleton in the cell interior.

4. When integrins relay signals from the matrix (or other cells) to the cell interior, it is known as “**outside-in**” **signaling**. The cells respond in different ways to these signals like changing shape, migration, proliferation, differentiation or induction of processes that prevent cells from self destructing. On the other hand, when Integrins respond to messages received from inside the cell it is referred to as **inside-out-signaling**. It is best documented in case of blood platelets that help to form blood clots (thrombus) at sites of injury.
5. **Focal adhesions** are temporal structures that can be rapidly disassembled if the adherent cell is stimulated to move or enter mitosis / provide anchorage as the cell moves in response to chemotactic signals/ the integrins are connected by adaptors to actin filaments.
Hemidesmosomes are more permanent structures that anchor epithelial cells to the underlying basal lamina / the integrins connect intermediate filaments of adjacent cells into an extended network which helps to distribute shearing forces across them.
6. The integral proteins of the tight junctions form continuous fibrils that completely encircle the entire circumference of the cell and make contact with neighboring cells on all sides (Figure 6.18). Each strand in these networks is composed of TM proteins of the **claudin**, **occludin**, and **junctional adhesion molecule (JAM)** families. These proteins bind to similar proteins on adjacent cells, thereby sealing the space between their plasma membranes. The cytosolic tails of claudins, occludins, and JAMs are also associated with proteins of the zonula occludens family, which links tight junction complex to actin cytoskeleton and hold tight junctions in place on the plasma membrane.

Such a structure prevents free passage of molecules (including ions) between cells of epithelial sheets. They also serve as “**fences**” that help maintain the polarized character of epithelial cells by not permitting diffusion of some membrane proteins and lipids between apical and basolateral parts of the membrane.

7. (a) The three separate polypeptides, called α -chains are supertwisted about each other. The superhelical twisting is right handed in collagen that is opposite to the left handed helix of the α -chains (Fig.6.11a and b).

(b) Each α -chain of collagen is enriched in proline, and many of the proline (and lysine) residues are hydroxylated. The tight wrapping of α – chains in the triple helix provides enormous tensile strength that can vary depending on the ways they associate to form these supra molecular assemblies.

6.8 SUGGESTED READINGS

1. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts and Peter Walter (2014). *Molecular Biology of the Cell*, VI Edition, Garland publishing Inc., New York and London.
2. Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell (2016). *Molecular Cell Biology*, VIII Edition, W. H. Freeman and Company; New York.
3. *Cell and Molecular Biology, Concept and Experiment*, (2010) 6th Edition, Gerald Karp, John Wiley & Sons, Inc.



UNIT 7

CYTOSKELETON PROTEINS AND CELL MOVEMENT

Structure

7.1	Introduction		Organisation and Role of Actin Filaments
	Expected Learning Outcomes		
7.2	Eukaryotic Cytoskeleton	7.5	Structure and Function of Intermediate Filaments
7.3	Structure of Microtubules	7.6	Structure and Function of Cilia and Flagella
	The Centrosome	7.7	Summary
	Assembly and Disassembly of Microtubules	7.8	Terminal Questions
	Role of Microtubules	7.9	Answers
7.4	Structure and Organisation of Actin Filaments	7.10	Suggested Readings
	Actin Filaments Polymerization and Treadmilling		

7.1 INTRODUCTION

In preceding units of Block II you have studied about structure and function of subcellular organelles; variations in cell wall structure and extracellular matrix (ECM). You would have realised that various proteins (and other biomolecules) are organised spatially at multiple levels within cells. It begins with having functional protein complexes to compartmentalisation in specific membranes or matrix and other aqueous compartments of organelles and finally a still higher level of organisation is created and maintained by the cytoskeleton. The cytoskeleton consists of three types of protein fibres and associated accessory proteins. They form a network of fibres that establish interconnected paths of communication. The cytoskeleton is a dynamic structure and reorganizes rapidly in response to changing demands. These structures help cells to

maintain or change shape; intracellular transport and positioning of subcellular organelles; cell movement; cell division and above all to withstand stress.

Therefore, in this unit you will study the structure and functions of microtubules, actin filaments and intermediate filaments. The examples cited will explain both the structural and dynamic role of cytoskeleton. At the end of the unit, structure and movement mediated by eukaryotic cilia and flagella are also discussed. In Block 3 you will study the protein transport.

Expected Learning Outcomes _____

After studying this unit, you should be able to:

- ❖ define the cytoskeleton and its classification;
- ❖ describe the structure and organisation of the three major group of cytoskeleton proteins;
- ❖ explain the assembly and disassembly of microtubules and actin filaments;
- ❖ indicate the role of GTP and ATP in polymerization of tubulin and G-actin respectively;
- ❖ explain the role of microtubules (MTs) and microfilaments in cell division;
- ❖ describe the families of MTs and Actin based motor proteins;
- ❖ describe the structure and function of cilia and flagella and;
- ❖ indicate the types of Intermediate Filaments (IF's); common structural design; assembly and role.

7.2 EUKARYOTIC CYTOSKELETON

The existence of a network of fibers or cytoskeleton within cells was postulated in 1928 by a Russian biologist, **Nikolai Koltzoff**. In eukaryotes the complex network is created from three types of protein fibers and a variety of accessory proteins. The interactions between them add to the complexity and their dynamic nature allows adaptability. A better understanding of their structure, interactions and dynamics became feasible with advances in fixation methods for EM and imaging techniques especially fluorescence microscopy. These techniques were instrumental in locating the protein at any given time and monitoring their dynamic behaviour in live cells.



Nikolai Koltzoff

It was initially assumed that the cytoskeleton was found only in eukaryotes but in 1992, a bacterial homolog of tubulin was identified. The FtsZ protein of bacteria resembles tubulin. It binds and hydrolyses GTP and has a seven amino acid tubulin signature sequence. It can also assemble into protofilaments. Later bacterial proteins like FtsA, MreB and StbA, related to actin superfamily were discovered. They can assemble into actin like filaments. The protein crescentin of *Caulobacter crescentus* bear homology to

eukaryotic proteins that assemble as intermediate filament. It is responsible for the crescent shape of *Caulobacter*.

In this unit we shall discuss the cytoskeleton of eukaryotes. The cytoskeleton is a complex network of protein filaments that is highly dynamic and reorganises continuously as the cell changes shape, divides and responds to environment. There are three major types of cytoskeleton elements namely, microtubules, microfilaments and intermediate filaments (Fig.7.1). The classification is based on their diameter and subunit structure. The diameter of microtubules, actin filaments and intermediate filaments (IF) is 25nm, 7nm and 10nm, respectively. Microtubules are polymers of tubulin heterodimer; actin filaments are assembled from G-actin and the building units of IFs varies among different cell types.

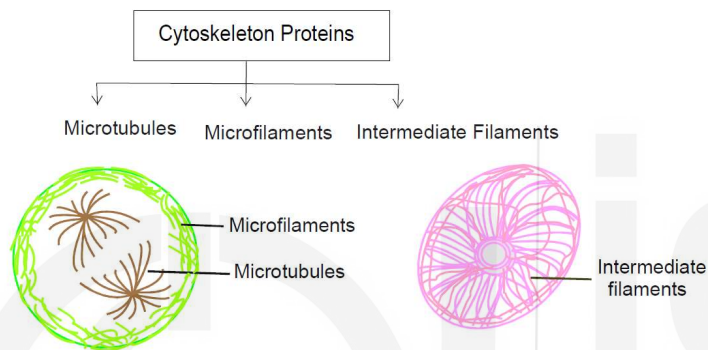


Fig. 7.1: Classification and subcellular localisation of Cytoskeleton proteins.

SAQ 1

Answer the following questions:

- Enlist three bacterial proteins that bear resemblance to cytoskeleton proteins of eukaryotes.
- What is the basis of classification of cytoskeleton proteins?

7.3 STRUCTURE OF MICROTUBULES

Microtubules (MTs) are 25nm rigid hollow cylindrical tubules of variable length. They are found in virtually all eukaryotic cells. The basic building unit of MTs is tubulin heterodimers ($\alpha\beta$) that are closely related and tightly linked globular proteins. They polymerise to form a **protofilament**. Both subunits bind GTP. In mammalian cells a cylindrical MT is formed from 13 protofilaments aligned in parallel with the same polarity (Fig.7.2). The MT is a polar structure; the two ends are plus (fast growing) and minus (slow growing). In a microtubule, α -tubulin is present at the minus (-) end of a protofilament and β -tubulin is at the plus (+) end. The minus ends are stabilised by embedding them in centrosomes. The plus ends extend throughout the cytoplasm.

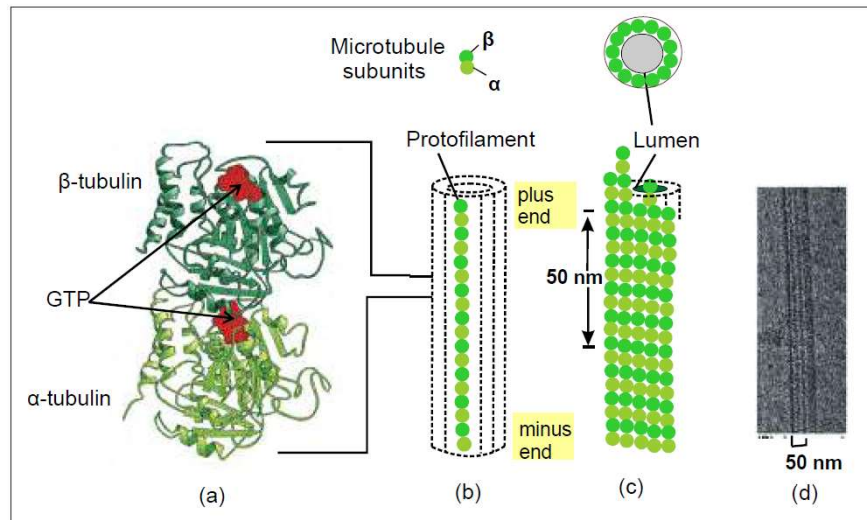


Fig. 7.2: a) GTP bound tubulin heterodimer b) structure of protofilament c) A hollow cylindrical microtubule d) Electron micrograph of microtubules

7.3.1 The Centrosome

The centrosome is somewhat shapeless body that nucleates the growth of microtubules and determines their number and distribution. The MTs in turn influence the distribution of other cytoskeletal protein fibers. Thus centrosome is considered as the master architect of cytoskeletal design. In an interphase cell, the centrosome lies close to the nucleus. It was first described independently by Theodor Boveri and Edouard van Beneden in 1897.

In animal cells each centrosome has two centrioles at right angles to each other and surrounded by pericentriolar material or centrosome matrix. Each centriole is a bundle of nine rods; each consisting of three fused MTs. They are also present in basal bodies underneath flagella and cilia. Not all microtubule organizing centres (MTOC) contain centrioles as they are not indispensable for the assembly or organisation of microtubules. The plus ends of cytoplasmic MTs emanate from the pericentriolar material. The protein that nucleates the assembly of microtubules is a highly conserved protein, γ -tubulin. Generally 10-13 γ -tubulins are complexed to form ring like structures that have diameter similar to microtubules. It is a minor species of tubulin and may remain bound to the minus end of MTs. γ -tubulin was first identified in the spindle pole body of *Aspergillus nidulans*.

7.3.2 Assembly and Disassembly of Microtubules

Now let us discuss about the assembly and disassembly of microtubules. As you know both subunits of tubulin heterodimer bind GTP but GTP bound to β -tubulin is hydrolysed during or shortly after polymerisation. This in turn weakens the affinity of tubulin for the preceding unit, resulting in depolymerisation. The dynamic behaviour of microtubules can involve treadmilling or dynamic instability. The average half life of a MT ranges from approx. 10 minutes in non dividing animal cells to as short as 20 seconds in a dividing cell.

During **treadmilling** tubulin bound to GDP are continually lost from the minus end and at the same time they are added bound to GTP from the plus end. This is more common with actin filaments. Microtubules also have cycles of growth and shrinkage. A microtubule continues to grow as long as the concentration of GTP bound tubulin is high and new ones are added more rapidly than GTP is hydrolysed. A GTP cap will be present at the growing end. The tubulin dimers are added faster at the plus end of microtubule compared to the minus end. On the other hand, a MT shrinks due to depolymerisation, if GTP is hydrolysed more rapidly than the rate at which new subunits are added (Fig 7.3). This behaviour called **dynamic instability** was described by **Mitchison and Kirschner** and it is due to delayed hydrolysis of GTP after tubulin assembly.

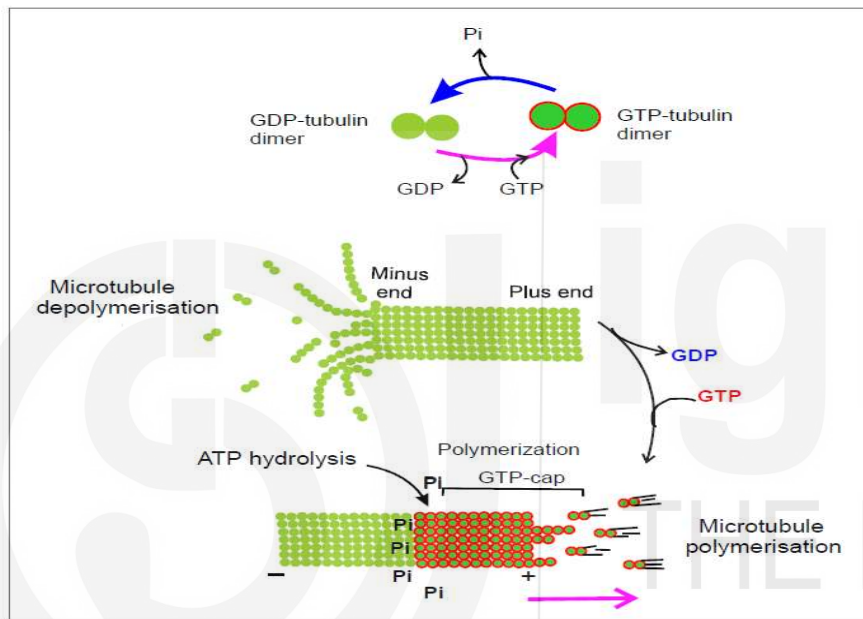


Fig. 7.3: Polymerization and depolymerisation of microtubules.

It is possible to modify dynamic instability of microtubules to suit specific needs. Microtubules can undergo post translational modifications after polymerization. Two of them are acetylation and detyrosination of α -tubulin. These modifications serve as binding sites for microtubule associated proteins (MAPs) that stabilize them. Many MAPs have been identified in different cells which perform a wide range of functions including stabilising and destabilising microtubules, guiding microtubules to specific cellular locations, and mediating interactions of microtubules with other cytoskeletal proteins in the cell.

A number of naturally occurring substances interfere with the dynamic behaviour of microtubules and are used as anti mitotic drugs. One of these is an alkaloid colchicine. It binds free tubulin and prevents its polymerization. This results in the disintegration of mitotic spindle in a dividing cell and is routinely used to induce polyploidy or to study chromosome structure. The drugs vincristine and vinblastine (similar to colchicine) are used to treat certain cancers. Another anti cancer drug, taxol binds to MTs and stabilises them. The cell division is arrested as MTs must shorten to carry chromosomes to the poles.

7.3.3 Role of Microtubules

In this subsection you will learn about some of the important roles of MTs both in a resting cell and during cell division.

❖ Intracellular transport of cell organelles and vesicles

Virtually all membrane bound organelles have a close association with microtubules. The ER extends almost to the edge of the cell whereas the Golgi apparatus is present close to the centre of the cell, near the centrosome. MTs are involved both in the intracellular transport and positioning of organelles. Similarly they transport vesicles to their destination. The motor proteins, kinesin and dynein are involved in microtubule based motility. The cytosolic face of organelles has receptors on their membranes through which they interact with specific motor proteins. These proteins require ATP to generate force and transport either along the plus (kinesin) or minus (dynein) end of MTs (Fig.7.4). The motor proteins belong to two large families of related proteins. The microtubules function as tracks for transport or positioning of cargo.

The motor protein **kinesin** consists of two heavy chains and two light chains; it has a rod-like structure composed of two globular heads, an α -helical coiled – coil stalk involved in dimer formation and a fan like tail domain while **dynein** comprises of two or three heavy chains with multiple light and intermediate chains. The heavy chains in both form globular ATP binding motor domain that facilitate movement along microtubules. They are MT-activated ATPase. The base of both proteins interacts with organelles and vesicles via a connector. The ER membrane has **kinectin** that binds kinesin. Similarly the connector complex, **dynactin** binds vesicles to dynein.

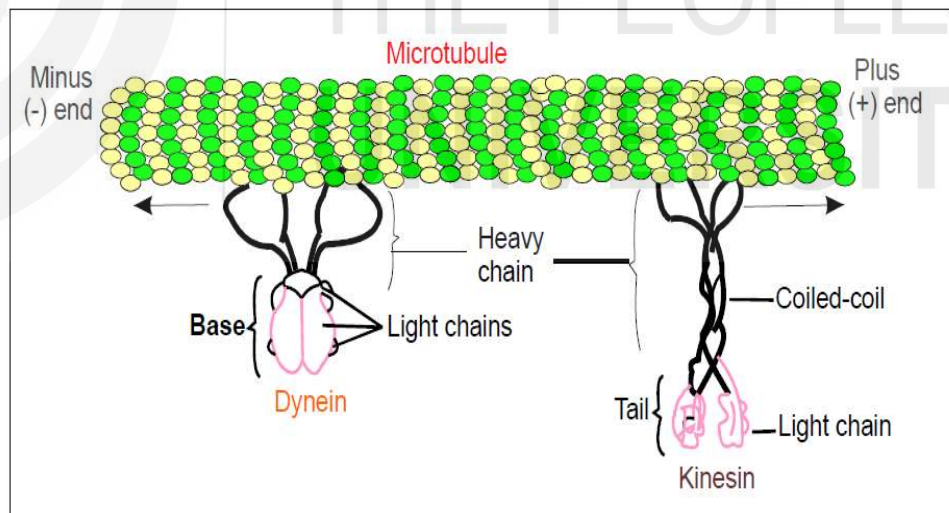


Fig.7.4: Microtubule associated motor proteins.

❖ Cell division

The dynamics of microtubule assembly and disassembly changes dramatically as the cell enters mitosis. The cytoplasmic microtubules become very unstable and the interphase array depolarises. The centrosome duplicates to form the mitotic spindle and it nucleates and

promotes the growth of many dynamic microtubules (MTs). The MTs emanating from the spindle are kinetochore MT, polar MT and astral MT (Fig 7.5).

The kinetochore MTs attach to specialised regions on condensed chromosomes called kinetochore. As MTs from both poles of mitotic spindle attach to kinetochore they will stop growing or shrinking. It is as if the centrosome sends out “feelers” in search of chromosomes. The net outcome is the alignment of all replicated chromosomes at the metaphase plate. The polar MTs are stabilized by overlapping with each other while the astral MTs extend towards the cell periphery.

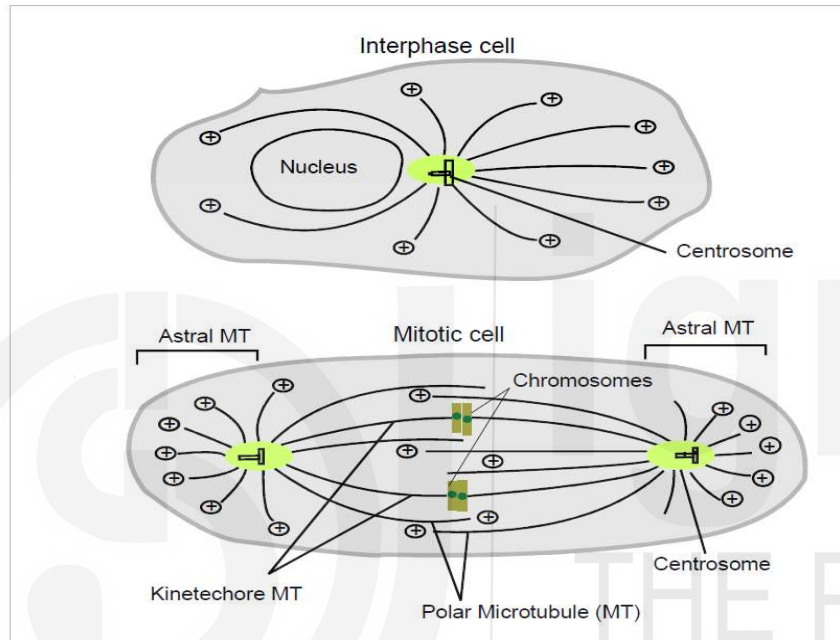


Fig. 7.5: The arrangement of MTs in a non-dividing and mitotic cell.

(Credit image: M. Cooper, *The Cell: A Molecular Approach*)

The separation of sister chromatids and movement to opposite poles is dependent both on the dynamic behaviour of MTs and kinetochore associated motor proteins. The kinetochore MTs are shortened and the polar MTs elongate. We will come back to this subject again in unit 11 Cell Cycle (Block 4).

In most cells cytokinesis follows nuclear division. In animal cells an actin and myosin based contractile ring is assembled precisely half way between the poles and perpendicular to the spindle. Even here MTs are implicated in indirectly influencing the site of cleavage furrow.

❖ Intracellular organisation

The main function of microtubules is to provide intracellular framework and maintain cell shape. In non dividing cells, network of microtubules helps to stabilize subcellular organelles in their proper position.

❖ Cell motility

The structural components of eukaryotic cilia and the flagella are bundles of microtubules. They are present on the surface of many kinds of cells and are

used for multiple purposes such as locomotion, to collect food, move mucus along with trapped particles consisting of air pollutants, dust or microorganisms up towards the mouth to be swallowed and to propel eggs. The structure and movement of cilia and flagella will be described in section 7.6.

❖ Cell polarity

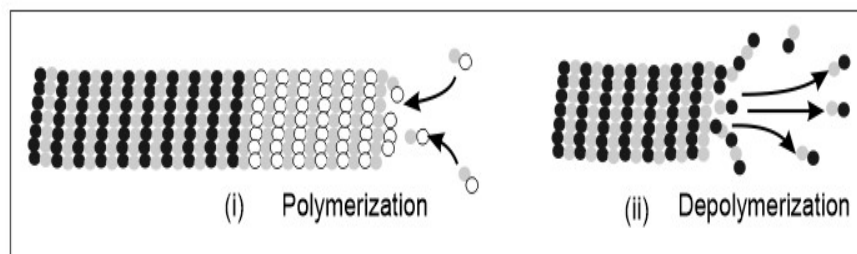
The cytoskeleton can be reorganised to polarise a cell transiently. Generally actin filaments and MTs act together. To demonstrate how these events are coordinated, we will consider the killing of a target cell by cytotoxic T lymphocyte. A cytotoxic T cell expresses antigen specific T cell receptor that looks for processed foreign antigen presented on target cells. Once antigen is recognised by a T cell it signals local reorganization of underlying actin cortex and then the centrosome reorients, moving MTs along with it. The MTs in turn position the Golgi body near site of contact allowing transport of materials needed for cytotoxicity from Golgi along MT tracks to the target cell for focused secretion.

SAQ 2

a) Fill in the blanks:

- i) The microtubules are made up of
- ii) Microtubules are polar structures because
- iii) The dynamic instability of MTs was described by and
- iv) The MTs are used as for transport and positioning of organelles and vesicle; the movement is dependent on
- v) The kinetochore microtubules are attached to during mitosis.
- vi) The plus end directed motor protein of microtubules is
- vii) The hydrolysis of GTP from β -tubulin causes of microtubules.
- viii) The cellular function of γ -tubulins is
- ix) Colchicine binds to that results in
- x) The cilia of eukaryotes are.....

b) The diagrams show growing and shrinking microtubules. Label the (+) and (–) ends of MTs in (i) and (ii).



7.4 STRUCTURE AND ORGANISATION OF ACTIN FILAMENTS

An actin filament as the name suggests is made up of actin (43Kd). It was discovered and isolated from muscle cells by Brunó Ferenc Straub in 1942. It is a major globular protein of muscle cells. An actin monomer is called G (globular) actin that binds ATP. It polymerises to form actin filaments (or F-actin). The filament has a polar structure as each subunit faces in the same direction. It has a slow growing minus or pointed end and a fast growing plus or barbed end. The polarity of actins is important in establishing the directional movement of myosin relative to actin and in their assembly.



Brunó Ferenc Straub

As compared to other cytoskeletal protein fibres they are thinner (7-9nm) and hence they are also referred to as microfilaments. Electron micrograph of actin filaments shows that they consist of two twisted chains of G-actin molecules arranged head-to-tail which makes them look like a double helix (Fig. 7.6).

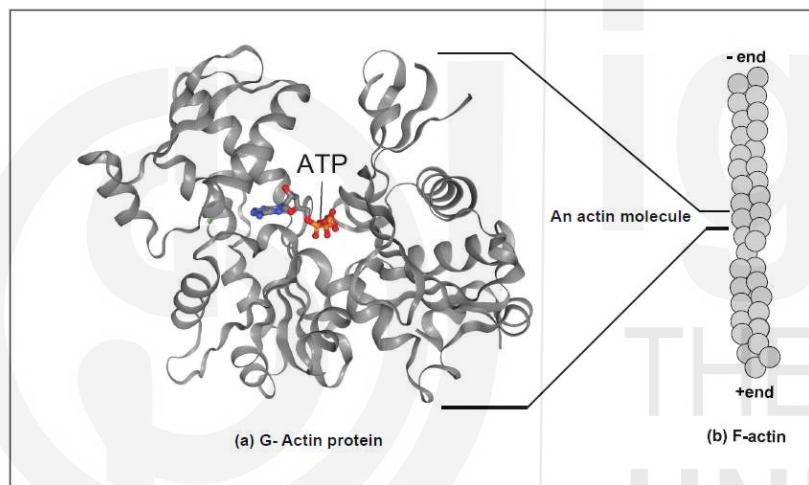


Fig.7.6: a) Molecular structure of Actin and b) Actin filament (F-actin)

Actin filament is a major cytoskeleton element of all eukaryotic cells; vertebrate skeletal muscles are a rich source (approx. 20% of total protein) and the usual source of actin for experimentation. In eukaryotes actin is encoded by a multigene family and they are divided into three classes (α - β - and γ -actins) based on their isoelectric point; α -actin is found in skeletal and cardiac muscles whereas beta (β) and gamma isoforms (γ 1-cytoplasmic) are present in non muscle cells. In spite of the occurrence of multiple forms they are highly conserved proteins. Actin filaments are generally cross linked to form actin networks and bundles which are much stronger than individual filaments.

7.4.1 Actin filament Polymerization and Treadmilling

In this subsection you will learn about reversible polymerisation of actin monomers. Look at Fig. 7.7 (a) for an overview of filament assembly and disassembly. The first step of actin polymerization is nucleation to form a small

complex consisting of three actin monomers. Then actin filament grows by addition G-actin from both ends. The two ends of the filament grow at different rates; the plus end polymerises five to ten times faster than the minus end. During the elongation phase there is a net increase in filament length. The actin subunits in a filament can hydrolyse ATP and depolymerise. The rate at which actin monomers are added into filaments is dependent on the critical concentration of free monomers.

Actin filaments engage in an ATP dependent dynamic behaviour called **treadmilling**. In this ATP-actin monomers are added from the plus end with concomitant loss of ADP-actin from the minus end (Fig. 7.7b). There is no net change in filament length but it allows a rapid exchange of subunits. This occurs at monomer concentration intermediate between the critical concentration for plus and minus ends.

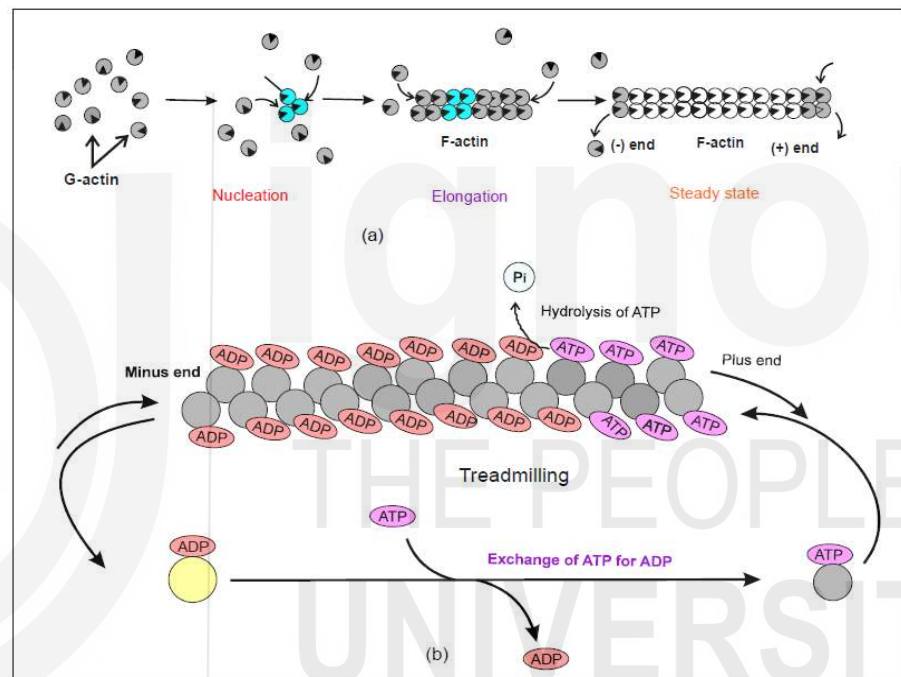


Fig 7.7: a) Assembly & disassembly of actin filaments b) Treadmilling.

The rate of polymerisation and depolymerisation is tightly regulated in the cell by actin-binding proteins. As expected these proteins can either enhance depolymerisation or stimulate the incorporation of monomers into actin filaments. In general the turnover of actin filaments is much faster in the cell and more than 40% of actin in animal cells is unpolymerised.

Look at Fig. 7.8 in which some actin binding proteins are shown to control polymerization. A G-actin sequestering protein, **profilin** stimulates exchange of ATP for ADP and its polymerisation to F-actin. A capping protein, CapZ then binds to the growing plus end to fix actin polymerization. On the other hand, **cofilin** protein binds to the minus end of actin filaments and enhances the dissociation of actin monomers. In addition cofilin can sever actin filaments and then each end can be disassembled, enhancing the overall rate. Another protein **gelsolin** fragments actin fragments in presence of Ca^{+2} . The Arp 2/3

proteins can nucleate the growth of new actin filaments. It also caps the minus end. The activities of these proteins are regulated in the cell.

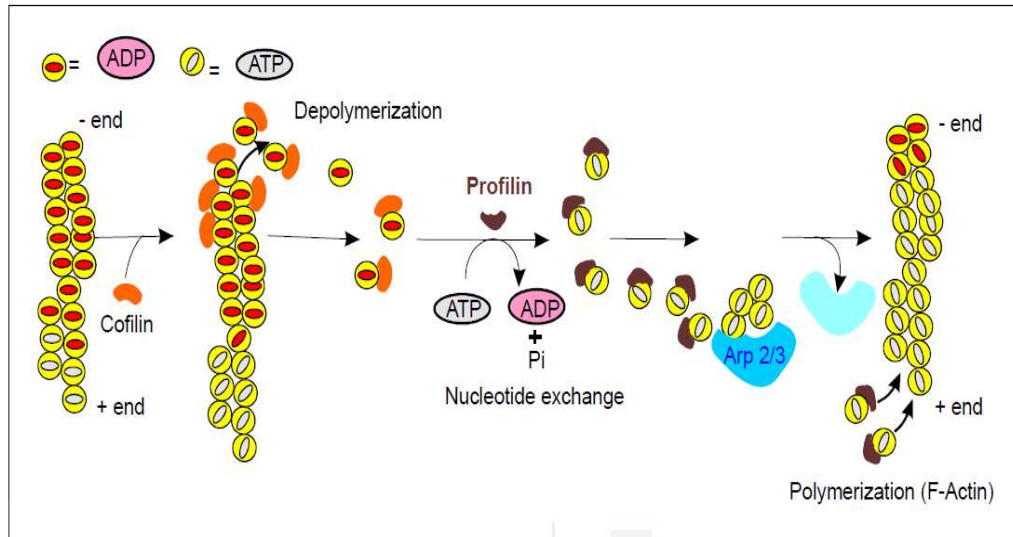


Fig. 7.8: Control of actin polymerisation by actin binding proteins
(Image source: Albert: Molecular Biology of the Cell).

Some products of biological origin are known that affect actin polymerisation. The drug **cytochalasins** are fungal products which bind to plus end of actin filaments and block further polymerisation. It is used to study cell locomotion. Similarly **phalloidin** from *Amanita* mushroom binds actin filaments tightly and stabilise them. It is generally labeled with fluorescent dyes to track actin filaments by fluorescence microscopy. These drugs result in dramatic changes in actin based dynamic structures and functions dependent on them.

7.4.2 Organisation and role of actin filaments

As mentioned earlier actin filaments are organised into bundles or networks with the help of cross linking proteins. The actin bundles may be closely packed or loosely crosslinked bundles (Fig.7.9). Some of them are also contractile (contractile ring; stress fibres).

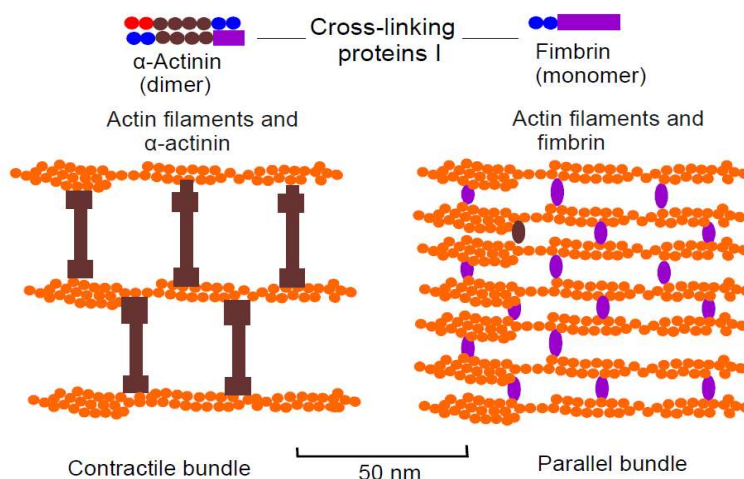


Fig. 7.9: Types of actin binding proteins.

The two cross linking proteins shown are **α -actinin** (a dimer) and **fimbrin** (a monomer). The spacing in the contractile bundle allows motor protein, myosin to interact with the filaments. An actin binding protein **filamin** has an actin binding domain at one end and a dimerisation domain at the other end of each monomer. The cross linking of actin filaments by a flexible V-shaped filamin or similar proteins results in a loose gel like 3D network of actin cortex beneath the plasma membrane (Fig. 7.10). The cortex determines cell shape and surface activities including movement as it is also linked to cell membrane that receives and signals the cell to modify the underlying cortex.

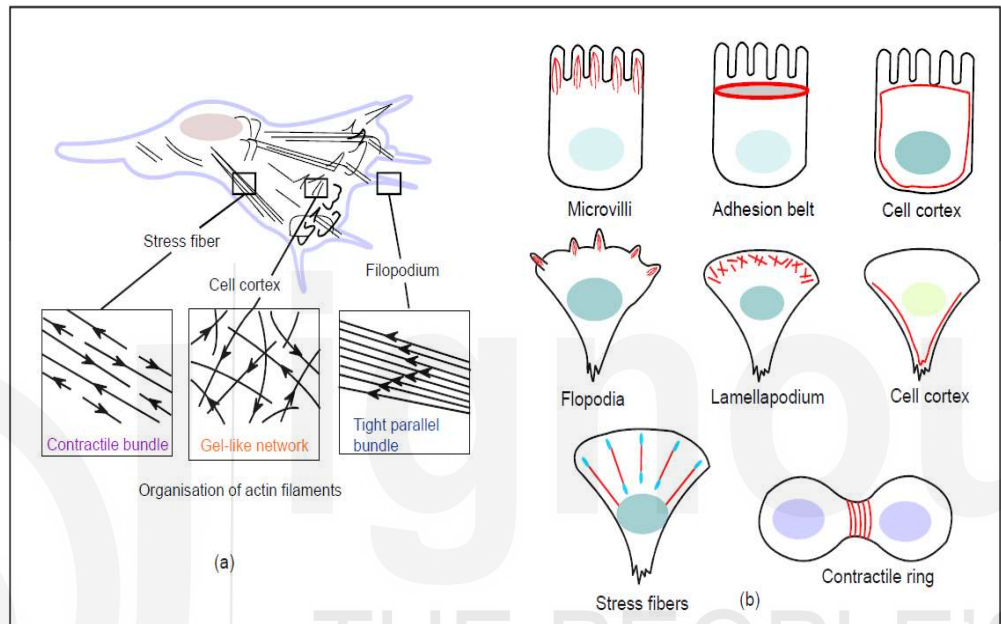


Fig. 7.10: Various actin filament based structures.

In many cells there are discrete regions of cell membrane that make contacts with adjacent cells or to the extracellular matrix. The sites of attachment include focal adhesion and adherens junctions (Refer to unit 6). Similarly microvilli are abundant on the apical surface of intestinal epithelial cells involved in absorption. It has parallel actin filaments held together by a bundling protein, **villin**.

The actin bundle is linked to the overlaying plasma membrane by lateral bridges of myosin I. In addition there are structures that are transient such as **pseudopodia** that are formed in cells during phagocytosis and movement by amoeba.

The motor protein for ATP dependent roles of actin filament belongs to myosin super family whose members are present in both muscle and non muscle actin based structures. The best studied among them is myosin I and II.

A myosin II protein is composed of two identical heavy chains and two pairs of light chains. Each heavy chain has a globular head that forms the motor domain and a long α -helical tail. The tails of both heavy chains are twisted around each other to form a coiled coil dimer. A pair of light chains is present close to the head domain of each heavy chain (Fig. 7.11).

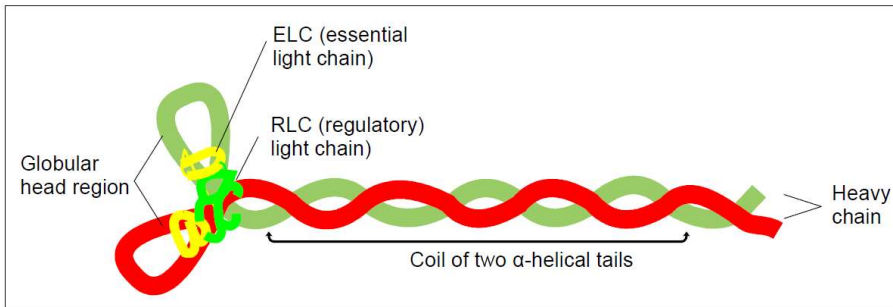


Fig. 7.11: Structure of myosin II.

Now we will learn about some examples of contractile assemblies of actin-myosin in non muscle cells. Muscle contraction will be covered in Human Physiology course of semester IV (BBCCT-115). During cytokinesis an actin –myosin II based **contractile ring** is assembled transiently in animal cells just below the plasma membrane. The contraction of the ring pulls the membrane inwards producing two daughter cells. The actin filaments disassemble as contraction progresses. The other examples include the intracellular transport of organelles and vesicles along actin filaments and **cell crawling** movements used by a variety of cells such as embryonic cells and migration of white blood cells to sites of damage.

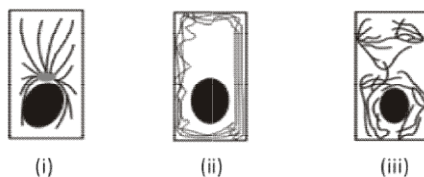
In addition to conventional bipolar myosin II, several other types of myosin such as myosin-I are found in nonmuscle cells. The myosin-I is much smaller and does not form dimers. Its tail can carry cargo along actin filaments; forms the lateral arms of microvilli and movement of plasma membrane during phagocytosis.

SAQ 3

a) Indicate whether the following statements are true and false. If false, point out the error:

- i) Actin filaments are also known as microfilaments. []
- ii) The bundling protein in microvilli is α -actinin. []
- iii) There is no net change in actin filament length during treadmilling. []
- iv) Dynein is the motor protein of actin. []
- v) Actin filaments display “dynamic instability”. []
- vi) Myosin-I forms dimers like myosin II. []

b) Which one of following figures shows the distribution of actin filaments in a cell?



7.5 STRUCTURE AND FUNCTION OF INTERMEDIATE FILAMENTS

As the name suggests intermediate filaments have a diameter (10nm) that is intermediate between actin filaments (7nm) and microtubules (25 nm). The term cytoskeleton was originally coined for the stable and insoluble fibres left behind when cells are treated with concentrated salt solutions and non ionic detergents. This fraction was actually represented by intermediate filaments (IFs). They do not bind nucleotides to regulate their assembly and disassembly. Unlike actin filaments and microtubules IFs constitute a heterogeneous group of fibrous proteins with variable expression profile. They are ubiquitous in multicellular eukaryotes. The intermediate filaments are classified based on their sequence (Table 7.1).

Table 7.1: Types of Intermediate filament (IF) proteins

Type	IF proteins	Expression profile	Remark(s)
I	Acidic keratins	Epithelial cells	Type I and II always copolymerise.
II	Basic or neutral keratins	Epithelial cells	-do-
III	Vimentin; desmin; Glial fibrillary acidic protein (GFAP)	Fibroblasts, WBC Muscle cells, Glial cells	Can polymerise either with one or two type III proteins. They do not copolymerise with keratins.
IV	Neurofilaments -NF-L; NF-M; NF-H	Neurons	The three NFs can copolymerise.
V	Nuclear lamins- A, B and C	Nuclear lamina	Assemble into 2D sheets.
VI	Nestin	Stem cells of central nervous system (CNS)	-

Although IFs differ in size, sequence and distribution yet they share a common structural design (Fig.7.12). They all possess a central α -helical rod of more than 300 amino acids flanked by non helical variable sized amino (head) - and carboxyl (tail)-terminal ends. The common central domain is largely responsible for filament assembly and the variable ends determine the specialised role of different IF proteins.

The central rod has tandem repeats of a seven amino acid sequence (heptad repeat) that promotes the formation of coiled-coil dimers between parallel α -helices. The dimer may be a homo-or hetero-dimer. Then two dimers

associate to form tetramers; the dimers have a staggered alignment and anti parallel. The resultant tetramer lacks polarity. They do not possess (+) or (-) ends. Eight of these tetramers further associate laterally to form a unit length filament. The unit length filaments are supercoiled end to end to form a mature full-length filament about 11 nm in diameter (Fig. 7.13). They form a rope like structure.

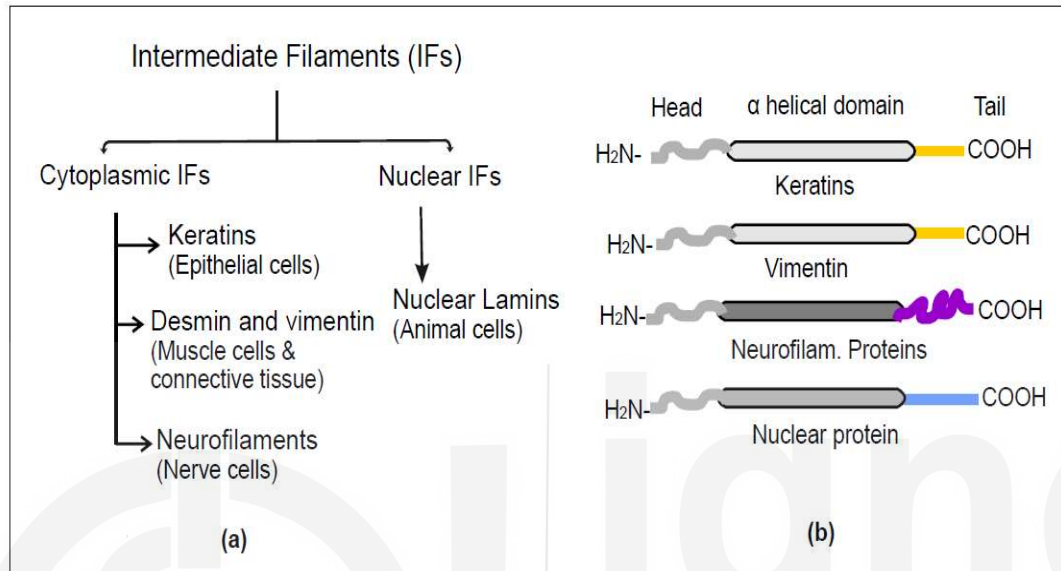


Fig. 7.12: a) Major classes of IFs b) Domain structure of IFs (helical central domain).

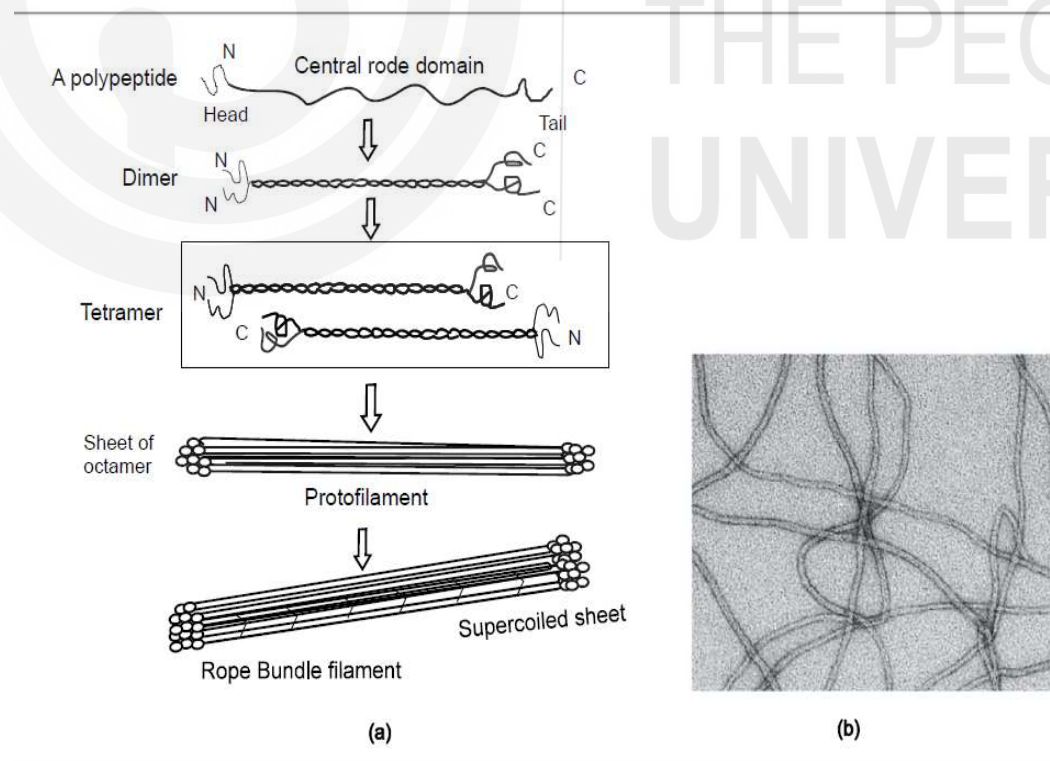


Fig. 7.13: (a) Assembly of Intermediate filaments; b) Electron micrograph of IFs.
(Source: Gerald Karp: Cell and Molecular Biology: Concepts and Experiments)

IFs in cells are dynamic and reorganise in response to cell cycle specific or differentiation specific cues. The assembly and disassembly of some intermediate filaments can be modified by phosphorylation. The nuclear lamins are disassembled by phosphorylation during mitosis that results in breakdown of the nuclear envelope. The process reverses at the end of cell division. Similarly cytoplasmic. Vimentin is also phosphorylated in a cell cycle dependent fashion. All phosphorylation do not regulate filament assembly and disassembly. The phosphorylation of NF may affect their functional interactions.

Functions of intermediate filaments

1. Intermediate filaments provide a scaffold to organize the contents of the cell. They resist mechanical and shear stresses.
2. Intermediate filaments known as lamins A, B, and C provide strength to nuclear envelope and help in distribution of chromatin. These lamins form a meshwork that reinforces the inside of the nuclear membrane.
3. Hard keratins are found in hair, scales and nails.
4. Neurofilaments along with MTs and microfilaments provide high tensile strength to compression, twisting, stretching and bending forces to stabilize the extended axons of nerve cells.
5. Desmosomes and hemidesmosomes anchor IFs to regions of cell-cell and cell-matrix contacts (Refer to unit 6).

SAQ 4

Match the cells/ sub cellular structure in column I with Ifs in Column II.

Column I	Column II
I. Nerve cells	a. Keratins
II. Glial cells	b. Neurofilaments
III. Skin cells	c. Desmins
IV. Muscle cells	d. lamins
V. Nuclear membrane	e. GFAP

7.6 STRUCTURE AND FUNCTION OF CILIA AND FLAGELLA

Recall the structure of microtubules described in section 7.2. All eukaryotic cilia and flagella are bundle of microtubules. Cilia are generally small and hair-like structures that extend from the body of a variety of cells while flagella are long hair-like structure and generally found in prokaryotic cells. They vary in

terms of length and numbers in different types of cells. The central core of doublet microtubules is called an **axoneme** in which nine pairs of outer doublet microtubules are organized in a circle around two central singlet microtubules. This arrangement of MTs is termed “9 + 2” pattern (Fig. 7.14).

At its point of attachment to the cell the minus ends of axoneme connects to basal body which like centrioles consists of nine triplets of MTs. Each triplet (A, B and C) has one complete (A tubules) and two incomplete protofilaments (B and C tubules).

The basal body initiates the growth of axonemal microtubules. The A and B tubules of each triplet extend into the axoneme and so we have a doublet of nine MTs. The outer MT doublets are connected to the central pair by **radial spokes**. The axoneme is also associated with other proteins like nexin that joins adjacent doublets and dynein to power movement. The two arms of dynein are attached to A- tubule. The central pair is surrounded by a fibrous sheath (inner sheath).

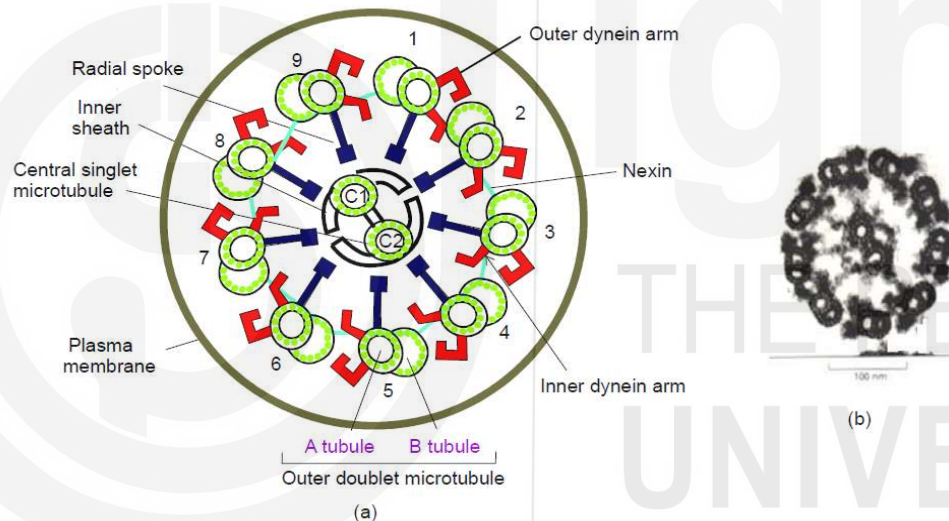


Fig. 7.14: a) Structure of cilia and flagella b) Electron micrograph of cilia.

(Image Courtesy: Gerald Karp: Cell and Molecular Biology: Concepts and Experiments)

Recall that dynein is a minus end directed motor protein. It is also responsible to drive the movement of cilia and flagella. This process requires ATP.

Look at Fig. 7.15 in which dynein arms bind to A-microtubules while dynein head interacts with B microtubules of adjacent doublets. The basis of axonemal movement is sliding of protein filaments relative to one another. As dynein heads move, it causes the A tubule of the doublet to slide towards the basal body of adjacent B tubule. Since multiple nexin links hold adjacent MT doublets together, a sliding movement is converted into a bending motion. The activity of dynein is regulated to coordinate these movements.

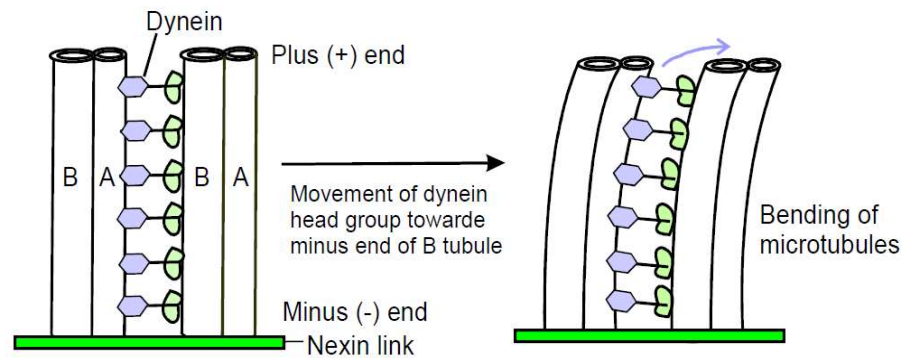


Fig. 7.15: Dynein dependent movement of cilia and flagella.

SAQ 5

Differentiate between cilia and flagella.

7.7 SUMMARY

In this unit so far, you have studied that:

- In eukaryotes the cytoskeleton consists of three types of protein fibres (MTs, microfilaments and IFs) and accessory proteins. They form a network that establishes interconnected paths of communication. The cytoskeleton is a dynamic structure and reorganises in response to changing demands.
- The cell skeleton help cells to maintain shape; in intracellular transport and positioning of subcellular organelles; cell movement; cell division and above all to withstand stress.
- Bacteria also encode proteins that bear resemblance to cytoskeletal proteins in eukaryotes. In 1992, a bacterial homolog of tubulin; FtsZ protein was shown to bind and hydrolyse GTP. It has a seven amino acid tubulin signature sequence and can also assemble into protofilaments. Later bacterial proteins (FtsA, MreB), related to actin superfamily and IFs (crescentin) were discovered. They can assemble into actin like or IFs, respectively.
- Microtubules (MTs) are 25nm rigid hollow cylindrical tubules of variable length. They are built by polymerisation of tubulin heterodimers (α and β) that are closely related and tightly linked globular proteins. In mammalian cells a cylindrical MT is formed from 13 protofilaments aligned in parallel with the same polarity. It is a polar structure with a plus (fast growing) and minus (slow growing) end. MTs exhibit dynamic instability. The centrosome nucleates the growth of microtubules and determines their number and distribution.

- MTs and associated motor proteins (dynein and kinesin) help in transport and positioning of organelles and vesicles; cell division; cell motility and cell polarity.
- Actin filaments (F actin) are thin, thread like structures, about 6-7 nm in diameter. The actin monomer (G-actin) is a globular protein (43Kd) that binds ATP and polymerises to form actin filaments. The filament has a polar structure with a slow growing minus or pointed end and a fast growing plus or barbed end. ATP hydrolysis triggers depolymerisation. Actin filaments engage in an ATP dependent dynamic behaviour called treadmilling.
- Actin filaments are cross linked with the help of actin binding proteins to form bundles and complex network. They also interact with plasma membrane and anchor the cell at regions of cell-cell contact. The actin bundles may be closely packed or loosely cross linked bundles and even contractile. The motor protein for actin filaments is a myosin family member.
- The nonmuscle myosin participates in cytokinesis, cell crawling, intracellular transport, etc.
- Intermediate filaments (IFs) are about 10nm non polar filaments. They constitute a heterogeneous group of fibrous proteins with variable expression profile. Although IFs differ in size, sequence and distribution yet they share a common structural design. These proteins do not bind nucleotides to regulate their assembly and disassembly. They provide mechanical and tensile strength to cells.
- All eukaryotic cilia and flagella are bundles of microtubules. The arrangement of microtubules is termed "9 + 2" pattern. The motor protein dynein powers the movement of cilia and flagella. They are used for multiple purposes such as locomotion, to collect food, move mucus along with trapped particles up towards the mouth to be swallowed and to propel egg.

7.8 TERMINAL QUESTIONS

1. Compare (a) intermediate filaments, (b) actin filaments and (c) microtubules in terms of size, polarity, building blocks and distribution in an animal cell.
2. What is the mode of action of the following inhibitors?
i) Colchicine ii) Cytochalasins iii) Phalloidin
3. Compare the structure and role of motor proteins associated with MTs.
4. Why MTs are polar structures? What is the difference between the plus and minus ends?
5. Indicate similarities between MTs and microfilaments.

6. Explain how ATP hydrolysis after actin addition to microfilament affects filament elongation dynamics.
7. Why are intermediate filaments more stable than other cytoskeletal proteins?
8. How do IFs differ from other cytoskeletal proteins?

7.9 ANSWERS

1. i) FtsZ protein resembles tubulin; MreB and FtsA proteins are actin like proteins; crescentin of *Caulobacter* is like IF proteins.

ii) The classification of cytoskeleton is based on their diameter and subunit structure.

2. a)

i) α β tubulin heterodimers.

ii) the two ends are asymmetric

iii) Mitchison and Kirschner

iv) tracks; motor proteins

v) kinetochore on duplicated chromosomes

vi) kinesin

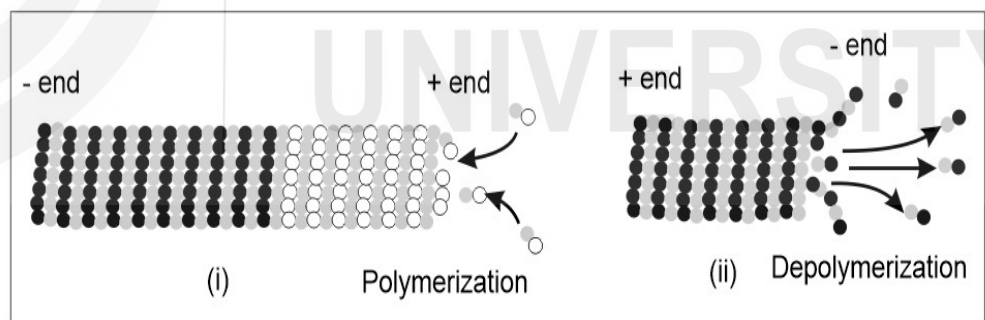
vii) depolymerisation

viii) nucleate the growth of MTs

ix) binds to free tubulin; depolymerisation of MTs

x) bundles of MTs.

b).



3. a)

i) True. ii) False iii) True iv) False v) False vi) False

b)

Fig. (ii) shows the actin filaments.

4.

I.- b; II. - e; III.- a; IV.-c; V.- d

5. Cilia are short, hair-like structures and usually more numerous that may cover the entire cell surface. In contrast, flagella are long hair-like structures, generally one or two.

TERMINAL QUESTIONS

1.

Type of cytoskeletal Proteins	Diameter	Building blocks	Filament polarity	Location in the cell
Microtubule	25 nm	Tubulin dimers	Polar with a (+) & (-) end	Present cell interior
Intermediate Filaments	7-9 nm	keratins, Vimentin; lamins; NF	Non-polar	present in the entire cell
Actin Filaments	10-12 nm	G-actin	Polar with a (+) & (-) end	Found at the inner edges of the cell

2. i) Colchicine: It binds to free tubulin and prevents polymerization. It causes the disintegration of the mitotic spindle in a dividing cell

ii) Cytochalasins: It binds to the plus end of actin filaments and block further polymerisation.

iii) Phalloidin: It binds actin filaments tightly and stabilise them.

3. i) Kinesins are relatively less complex motor proteins (MW:110,000 – 135,000) while dyneins are large and structurally complex proteins (MW: 1,000,000).

ii) They move in opposite direction along MT tracks; kinesins are plus end directed while dynein are minus end motors.

4.

The MT is a polar structure built from a related heterodimer. In a microtubule, α -tubulin is present at the minus (-) end and β -tubulin is at the plus (+) end of a protofilament. The two ends grow at different rates. The minus ends are stabilised by embedding them in centrosomes while the plus end are directed away from the MTOC.

5.

i) Both are built by polymerisation of a single globular protein encoded by multigene families; MT has tubulin dimers and actin has G-actin. (ii) Both exhibit dynamic behaviour. (iii) Both are polar structures. (iv) Both require specific motor proteins for movement related functions, for instance intracellular transport.

6.

The actin subunits in a filament can hydrolyse ATP and depolymerise by releasing ADP-actin due to a decrease in affinity with adjacent actin monomers. In the cell this process is regulated. The rate of addition and removal dictates the extent of growth or shrinkage respectively.

7.

IFs are more stable because they do not bind and hydrolyse nucleotides, responsible for the dynamic behaviour of MTs and microfilaments. IFs in cells are dynamic and reorganise in response to cell cycle specific or differentiation specific cues such as disassembly by phosphorylation of lamins.

8.

i) IFs are intermediate in diameter to MTs and actin filaments. ii) They are self assembled from a variety of fibrous proteins, either alone or in combination. iii) They do not bind NTPs. iv) They are non polar 10nm filaments. v) They are not associated with motor proteins. vi) Their dynamic behaviour is dictated by modifications or association with other proteins.

7.10 SUGGESTED READINGS

1. Gerald Karp, Janet Iwasa, Wallace Marshall (2010). *Cell and Molecular Biology: Concepts and Experiments*. VIII Edition. John Wiley and Sons. Inc. ISBN: 978-1-118-88614-4.
2. De Robertis, E.D.P. and De Robertis, E.M.F. (2006). *Cell and Molecular Biology*. VIII Edition. Lippincott Williams and Wilkins, Philadelphia.
3. Cooper, G.M. and Hausman, R.E. (2013). *The Cell: A Molecular Approach*. VI Edition. ASM Press and Sunderland, Washington, D.C.; Sinauer Associates.
4. Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore and James Darnell (2016). *Molecular Cell Biology*, VIII Edition, W. H. Freeman & Company; New York:ISBN-10:1-4641-8339-2.
5. Arshad Desai and Timothy J. Mitchison. Microtubule Polymerization Dynamics. *Annu. Rev. Cell Dev. Biol.* 1997. 13:83–117.
6. *Molecular Biology of the Cell* (© Garland Science 2008).