

# CELL ORGANELLES – STRUCTURE AND FUNCTIONS

## 1. PLASMA MEMBRANE

The chemical composition of membranes

Membranes are lipid-protein assemblies in which the components are held together in a thin sheet by noncovalent bonds. The core of the membrane consists of a sheet of lipids arranged in a bimolecular layer. The lipid bilayer serves primarily as a structural backbone of the membrane and provides the barrier that prevents random movements of water-soluble materials into and out of the cell. The proteins of the membrane, on the other hand, carry out most of the specific functions .

### Membrane Lipids

Membranes contain a wide diversity of lipids, all of which are amphipathic; that is, they contain both hydrophilic and hydrophobic regions. There are three main types of membrane lipids: phosphoglycerides, sphingolipids, and cholesterol.

**Phosphoglycerides:** Most membrane lipids contain a phosphate group, which makes them phospholipids. Because most membrane phospholipids are built on a glycerol backbone, they are called phosphoglycerides.

**Sphingolipids :** A less abundant class of membrane lipids, called sphingolipids, are derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain. Sphingolipids consist of sphingosine linked to a fatty acid by its amino group. This molecule is a ceramide.

**Cholesterol :** Another lipid component of certain membranes is the sterol cholesterol.

(Liposomes: An important feature of the lipid bilayer is its ability to self-assemble. If, for example, a small amount of phosphatidylcholine is dispersed in an aqueous solution, the phospholipid molecules assemble spontaneously to form the walls of fluid-filled spherical vesicles, called liposomes. The walls of these liposomes consist of a continuous lipid bilayer that is organized in the same manner as that of the lipid bilayer of a natural membrane. Liposomes have proven invaluable in membrane research.)

### Membrane Carbohydrates:

The plasma membranes of eukaryotic cells also contain carbohydrate. More than 90 percent of the membrane's carbohydrate is covalently linked to proteins to form glycoproteins, the remaining carbohydrate is covalently linked to lipids to

form glycolipids,

The addition of carbohydrate, or glycosylation, is the most complex modification of plasma membrane protein and lipid. The carbohydrate of glycoproteins is present as short, branched hydrophilic oligosaccharides, typically having fewer than about 15 sugar per chain.

Membrane Proteins:

Membrane proteins can be grouped into three distinct classes distinguished by the intimacy of their relationship to the lipid bilayer. These are;

1. Integral proteins :that penetrate the lipid bilayer. Integral proteins are transmembrane proteins; that is, they pass entirely through the lipid bilayer and thus have domains that protrude from both the extracellular and cytoplasmic sides of the membrane.
2. Peripheral proteins that are located entirely outside of the lipid bilayer, on either the cytoplasmic or extracellular side, yet are associated with the surface of the membrane by noncovalent bonds.
3. Lipid-anchored proteins that are located outside the lipid bilayer, on either the extracellular or cytoplasmic surface, but are covalently linked to a lipid molecule that is situated within the bilayer.

## Plasma Membrane – Models

Various models have been proposed to illustrate the structure of plasma membrane:

(i) Lamellar model:

(A) Lipid bilayer Hypothesis.

(B) Protein-lipid-protein hypothesis(Sandwich model)

1) Danielli and Davson model.

2) Robertson's unit membrane model.

C) Kavanau's Lipid pillar Model.

D) Models in which proteins are considered to penetrate lipid layers.

1) Benson's Model.

2) Lanard and Singer's Model.

3)Mosaic membrane concept.

(ii) Micellar (sub unit) model:

(iii) Protein crystal Model:

(iv) Modified Lamellar model:

Fluid mosaic model.

A) Lipid bilayer Hypothesis:

Overton (1895) suggested that the cell membranes contain lipids. This conclusion was based on the fact that fat solvents dissolved the membrane easily and fat soluble substances passed easily through the cell membrane. Some workers like Hober (1910) and Fricke (1925), Gorter and Grendel (1925) supported this concept.

B) Protein-lipid-protein hypothesis (Sandwich model)

1) Danielli and Davson model:

According to the bimolecular model of Danielli and Davson, the plasma membrane consists of two layers of phospholipid molecules (a bimolecular leaflet) in which phospholipid molecules are arranged in such a way that hydrophilic heads of the phospholipid molecules face outside and hydrophobic nonpolar lipid chains are associated in the inner region of leaflet.

The hypothesis also suggested that, this double layer of phospholipid molecules are sandwiched between two essentially continuous layers of globular proteins in plasma membranes.

Robertson's unit membrane model:

In 1950, David Robertson studied the cell membranes from electron micrographs of sectioned material and concluded his findings as Unit Membrane Concept.

The unit membrane model visualises the cell membrane as a trilaminar and indicates a structure consisting of two dark osmiophilic layers separated by a light osmiophilic layer. The physical appearance of this trilaminar model has led to the term unit membrane. The unit membrane concept implies a trilaminar appearance with a bimolecular lipid layer between two protein layers. Each dense osmiophilic band is made up of protein (20 Å) and the polar groups of phospholipids (5 Å) and is thus 25 Å thick. The clear osmiophilic zone 35 Å in thickness is a bimolecular layer of lipids without the polar groups. In other words, the unit membrane is 75 Å thick with a 35 Å thick phospholipid layer between two 20 Å thick protein layers.

(III) Kvanau's lipid pillar model:-

This is a modification of Danielli and Davson's trilaminar sandwich model in which the lipid layer is visualised in two forms. In one case, it is in the form of a pillar while in

others it is in the form of flattened discs. The spaces between the pillars act as pores for the passage of ions. The interior of each pillar is formed of non polar faces of phospholipids while the surface is formed of the polar heads, Protein layers are present on both sides of membrane. The main drawback of this model is that it cannot explain active transport or the differential permeability of Na<sup>+</sup> or K ions.

(IV) Models in which proteins are considered to penetrate lipid layers:-

According to some workers, proteins are considered to penetrate the lipid layer. This model can explain the low surface tension of biological membrane as properly as the trilaminar model.

1. Benson's Model:- Benson (1966) proposed a cell membrane model on the basis of a study of chloroplast membrane. According to him, the lipids and proteins form a hydrophobic association. The charged polar heads of phospholipid molecules lie on the surface of membrane and are capable of binding ions. The nonpolar hydrophobic lipid tails are linked with complementary hydrophobic regions within the interior of proteins.
2. Lanard and Singers Model:- This model was proposed in 1966. According to this model, one fourth of the proteins are in helical conformation and the rest form random coils.
3. Mosaic membrane concept:- According to Baum 1967, the backbone of membrane is possibly formed by toast shaped cuboidal units of about 80 Å diameter which are covered along the edges as well as the sides by phospholipids. The heads of phospholipids face on the outside while the tails form complexes with the hydrophobic surfaces of the proteins.
4. Fluid Mosaic Model:- S.J Singer and G.L.Nicolson, cell membranes visualised as mosaics of lipids and proteins( just as protein icebergs floating in a sea of lipids).

### Micellar Model

Hilleir and Hofman (1953) considered, membrane as a non laminar pattern and consisting of globular subunits known as micelles which have a lipid core and a hydrophobic shell of polar groups. Lipid micelles are possible building blocks of membranes. In this model protein component may form a monolayer on either side of the plane of lipid micelles.

### Protein Crystal Model

David Green and coworkers (1970) have proposed a protein crystal model for cell membrane in which proteins polymerise to form two layers of loosely packed globular protein units of 30-40 Å diameter. The proteins are visualised as having extensive nonpolar as well as polar regions on their surfaces. The nonpolar region may exhibit hydrophobic bonding with nonpolar groups of phospholipid molecules that fill the cavities between the globular protein units while the polar heads of phospholipid molecules face the membrane surface.

Functions of plasma membranes:

1. Compartmentalization:

Membranes are continuous, unbroken sheets and, as such, inevitably enclose compartments. The plasma membrane encloses the contents of the entire cell, whereas the nuclear and cytoplasmic membranes enclose diverse intracellular spaces.

2. Scaffold for biochemical activities:

Because of their construction, membranes provide the cell with an extensive framework or scaffolding within which components can be ordered for effective interaction.

3. Providing a selectively permeable barrier:

Membranes prevent the unrestricted exchange of molecules from one side to the other. At the same time, membranes provide the means of communication between the compartments they separate.

4. Transporting solutes:

The plasma membrane contains the machinery for physically transporting substances from one side of the membrane to another,

5. Responding to external signals.

The plasma membrane plays a critical role in the response of a cell to external stimuli, a process known as signal transduction. Membranes possess receptors that combine with specific molecules (ligands) having a complementary structure.

6. Intercellular interaction.

Situated at the outer edge of every living cell, the plasma membrane of multicellular organisms mediates the interactions between a cell and its neighbors.

7. Energy transduction.

Membranes are intimately involved in the processes by which one type of energy is converted to another type (energy transduction).

8. Turgor maintenance of the cells.

## 2. NUCLEUS(ROBERT BROWN):

The organelle as the control unit of an eukaryotic cell. Based on the number of nuclei cells may be uninucleate (common type), multinucleate or anucleate. The contents of the nucleus are present as a viscous, amorphous mass of material enclosed by a complex nuclear envelope that forms a boundary between the nucleus and cytoplasm.

The nucleus of a typical interphase consists of

1.the chromosomes, which are present as highly extended nucleoprotein fibres, termed chromatin .

2.one or more nucleoli which are irregularly shaped electron-dense structures that function in the synthesis of ribosomal RNA and the assembly of ribosomes .The bulk of a nucleolus is composed of nascent ribosomal subunits that give the nucleolus a granular appearance . Embedded within this granular mass are one or more rounded cores consisting primarily of fibrillar material.

3.the nucleoplasm, the fluid substance in which the solutes of the nucleus are dissolved and

4.the nuclear matrix, which is a protein containing fibrillar network.

### Nuclear Envelope:

The nucleus of a eukaryotic cell is a complex structure bounded by the nuclear envelope, which controls the exchange of materials between the nucleus and cytoplasm, maintaining the unique position of the cell's two major compartments. The nuclear envelope consists of several distinct components including an inner and outer nuclear membrane separated by a perinuclear space and a variable number of nuclear pores .Nuclear pores are sites where the inner and outer nuclear membranes are used to form circular openings that are filled with a complex structure called the nuclear pore complex (NPC) The NPC has a basket-like structure with octagonal symmetry composed of rings, spokes, and filaments. The nuclear pores are the sites through which materials pass between the nucleus and cytoplasm.

## Chromatin & Chromosomes:

German biologist Walther Flemming in the early 1880s revealed that the cytoplasmic contents were shuttled into one daughter cell or the other as a matter of chance, depending on the plane through which the furrow happened to divide the cell. In contrast, the cell appeared to go to great lengths to divide the nuclear contents equally between the daughters. During cell division, the material of the nucleus became organized into visible threads," which were named chromosomes, meaning coloured bodies. Chromosomes seem to appear out of nowhere at the beginning of mitosis and disappear once again when cell division has ended. enclosed in a nucleus. The chromosomes are capable of self-reproduction and maintaining morphological and physiological properties through successive generations and thus capable of transmitting the contained hereditary material to the next generation. Hence, these are popularly known as hereditary vehicles'.

## Different types of chromosomes:

- I. Viral chromosomes
- II. Prokaryotic genome  
Bacterial DNA, Plasmids
- III. Mitochondrial/Chloroplast chromosome
- IV. Eukaryotic chromosomes

## Different Regions Recognized in Eukaryotic chromosomes:

1. Primary Constriction or Centromere
2. Chromonemata
3. Secondary Constriction and Nuclear organizers
4. Satellite
5. Telomere
6. Chromatid

### 1) Primary Constriction or Centromere:

Each chromosome contains a site where the outer surfaces are markedly indented. The site of the constriction marks the centromere of the chromosome. In humans the centromere contains a tandemly repeated, 171-base-pair DNA sequence that extends for at least 500 kilobases. This stretch of DNA associates

with specific proteins that distinguish it from other parts of the chromosome. The centromere (C) contains highly repeated DNA sequences (satellite DNA) and a protein-containing structure called the kinetochore

It is comparatively narrower than the remaining chromosome. It is also known as primary constriction. Its position is constant for a given chromosome and forms a feature of identification. The primary constriction divides the chromosome into two arms. It shows a faintly positive Feulgen reaction, indicating presence of DNA of repetitive type. This DNA is called constitutive heterochromatin.

Normally, the chromosomes possess a single centromere i.e. they are monocentric. But, in some instances, however, the chromosome may have two centromeres i.e. dicentric chromosome or many centromeres i.e. polycentric chromosome. Sometimes the chromosome may be devoid of centromere. Such a chromosome is said to be acentric.

According to the positions of centromere the monocentric chromosomes may be designated as follows

1. Metacentric: In this the kinetochore is located at or near the mid point (median) so that the arm ratio is 1:1 or nearly so. Such chromosomes are V shaped at anaphase stage.

2. Submetacentric: When the centromere is located slightly away from the mid point so that the two arms are unequal, its position is said to be submedian. Such a chromosome appears L shaped during anaphase.

3. Acrocentric: When the centromere is located away from the mid point and is found near one end of the chromosome, its position is said to be subterminal. Acrocentric chromosomes appear J shaped during anaphase and have one long and the other very short arm.

4. Telocentric: When the centromere is located at one end of chromosome, it is said to be terminal. The chromosome with terminal centromere is called telocentric. Telocentric chromosomes appear rod-shaped during anaphase. Telocentric chromosomes are found rarely.

**Kinetochore:**

Examination of sections through a mitotic chromosome reveals the presence of a proteinaceous button-like structure called the kinetochore at the outer surface of the centromere of each chromatid. The kinetochores assemble



at the centromere during prophase.

Functions of kinetochore

- a) The site of attachment of chromosomes to the dynamic mitotic spindle microtubules
  - (b) The residence of several motor proteins involved in chromosome motility
  - c) Key component in the signaling pathway of important mitotic checkpoint
- (2) The Chromonema:

Each chromosome is formed of two identical spirally twisted delicate threads which lie so close to each other that it is difficult to see them separately by ordinary microscope. These two subunits are called chromatids. If the chromosomes are treated with trypsin to remove proteins, each chromatid is seen to consist of two or more subunits. These are termed chromonemata by Vejdovsky (1912). The number of threads may vary in different phases of cell division because at one stage the chromatid may contain one thread and at the other phase it may contain two, four or more chromonemata. The chromonemata may remain coiled with each other. The coils may be of the following types :

- (1) Paranemic coils : When the chromonemal fibrils are coiled in such a way that they are easily separable from each other, such coils are called paranemic coils.
- (2) Plectonemic coils: When the chromonemal threads are intertwined, they cannot be separated easily. This type of coils are referred to as plectonemic coils.

The chromonema of chromosome appears like a fine string or thread bearing a number of granular bodies, named as chromomeres by Balbiani. The position of chromomeres in a chromonema is found to be fixed in a given chromosome.

(3) Secondary Constriction or Nuclear Organiser:

Sometimes one or both the arms of a chromosome are marked by a constriction other than the primary constriction. During interphase, the secondary constriction is intimately associated with the nucleolus. It contains genes coding for 18S and 28S ribosomal RNA and is responsible for the formation of nucleolus, therefore, known as nucleolar organiser region (NOR). Its location in the chromosome is marked by a lightly stained constricted area and is constant for a given chromosome. In man the nucleolar organisers are located in the secondary constrictions of chromosomes 13, 14, 15, 20 or 21 and 22

#### (4)Satellite DNA:

The terminal part of a chromosome beyond secondary constriction is called satellite. It is attached to the main body of chromosome by a delicate chromatin filament. The satellite may appear as a rounded or elongated knob. It has a constant shape and size for a particular chromosome. The chromosome with satellite is known as SAT-chromosome.

#### (5)Telomeres:

Each chromosome contains a single, continuous, double-stranded DNA molecule. The tips of each DNA molecule are composed of an unusual stretch of repeated sequences that, together with a group of specialized proteins, forms a cap at each end of the chromosome called a telomere. Human telomeres contain the sequence

TTAGGG

AATCCC repeated from about 500 to 5000 times .

During each cycle of replication, due to the direction specificity of the enzyme DNA polymerase one strand overhangs the other. Thus, if cells were not able to replicate the ends of their DNA, the chromosomes would be expected to become shorter and shorter with each round of cell division . This phenomenon has been called "the end-replication problem." The primary mechanism by which organisms solve the "end replication problem," came to light in 1984 when Elizabeth Blackburn and Carl Greider of the University of California, Berkeley, discovered a novel enzyme, called telomerase, that can add new repeat units to 3' end of the overhanging strand. Telomerase is a reverse transcriptase that synthesizes DNA using an RNA template. Unlike most reverse transcriptases, the enzyme itself contains the RNA that serves as its template .

Telomeres are very important parts of a chromosome

- (1)as they are required for the complete replication of the chromosome,
- (2)they form caps that protect the chromosomes from nucleases and other destabilizing influences, and
- (3)they prevent the ends of chromosomes from fusing with one another

According to current consensus, telomere shortening plays a key role in protecting humans from cancer by limiting the number of divisions of a potential tumor cell.

Chromatid:

One of the two copies of replicated chromosome, joined by a single centromere to the other strand. Chromosomes as seen in a karyotype are only present for a brief period during cell division. Prior to replication, each chromosome has one centromere. After replication, each chromosome has two sister chromatids that appear to share a common centromere (At the molecular level, this is not true because centromeric DNA is not late replicating). (By convention, we call the same as one chromosome, because we count chromosome by counting the number of chromosomes with centromeres). Thus entering division, a human cell has 46 chromosomes composed of 92 chromatids and 46 centromeres. The chromatids from the single chromosome of a homologous pair is called sister chromatids. The chromatids from two different chromosomes of a homologous pair is known as non-sister chromatids

### Heterochromatin and Euchromatin

After mitosis has been completed, most of the chromatin in highly compacted mitotic chromosomes returns to its diffuse interphase condition. Approximately 10% of the chromatin however, generally remains in a condensed compacted form throughout the interphase. This compacted densely stained chromatin seen at the periphery of the nucleus which remains condensed during interphase is called heterochromatin to distinguish it from euchromatin which returns to a dispersed state. When a radioactively labelled RNA precursor such as [<sup>3</sup>H] Uridine is given to cells that are subsequently fixed, sectioned and autoradiographed, indicates that they have relatively little transcriptional activity. Whether euchromatic or heterochromatic, the part of the genome is stably inherited from one cell generation to the next.

Heterochromatin is divided into classes (a) Constitutive heterochromatin & (b) facultative heterochromatin

#### (a) Constitutive heterochromatin:

The part of the genome which remains in the compacted state in all cells at all times and thus represents DNA that is permanently silenced is known as constitutive heterochromatin. In mammalian cells, the bulk of the constitutive heterochromatin is found in the region that flanks the telomeres and centromere of each chromosome and in the distal arm of the Y chromosome (in males). The DNA of constitutive heterochromatin consists permanently of repeated sequences and contains relatively few genes. When genes that are normally active move into a position adjacent to heterochromatin (due to transposition or translocation) they tend to become transcriptionally silenced, a phenomenon known as position effect. [It is thought that heterochromatin

contains components whose influence can spread outward a certain distance, affecting nearby genes. Spread of heterochromatin along the chromosome is apparently blocked by specialised barrier sequences. Constitutive heterochromatin also serves to inhibit genetic recombination between homologous repetitive sequences. (This leads to DNA duplication and deletions).

(b) Facultative heterochromatin :

Chromatin that has been specifically inactivated during certain phases of an organism's life or in certain types of differentiated cells is known as facultative heterochromatin.

Eg: Barr body in mammalian females. The cells of males have a tiny Y chromosome and a much larger X chromosome. Although cells of females contain two X chromosomes, only one of them is transcriptionally active. The other X chromosome remains condensed as a heterochromatic clump called Barr body (1949).

Generally it is believed that heterochromatin has a specific role in biogenesis of ribosomes and chromosomal separation during cell division. Because of the repetitive nature of sequences within the heterochromatin, these are less susceptible to mutagens. Probably used to compartmentalize the genome into several functional parts. Also serve as the transcriptional stops and the sites of attachment of chromatin to the nuclear membrane.

The main differences between heterochromatin and listed below

- 1) Heterochromatin stains deeply while euchromatin stains lightly,
- 2) Heterochromatin is found in the condensed regions of the chromosome, whereas the euchromatin is found in the diffused or loosely coiled regions of the chromosome
- 3) Heterochromatin does not become acetylated whereas euchromatin is acetylated in its histone during interphase
- 4) Heterochromatin is inert metabolically and contains lesser number of genes than euchromatin
- 5) Heterochromatin is genetically inactive and it does not participate in transcription and protein synthesis, but euchromatin is the active part of the chromosome.
- 6) The cross over frequency is comparatively less in heterochromatin than euchromatin

## PACKAGING THE DNA

An average human cell contains about 6.4 billion basepairs of DNA divided among 46 chromosomes. Each unreplicated chromosome contains a single, continuous DNA molecule, the larger the chromosome, the longer the DNA it contains. When each base pair is about 0.34nm in length, 6 billion base pairs would constitute a DNA molecule fully 2m long. These 2 meters of DNA is well packed in a nucleus of 10  $\mu\text{m}$  diameter and is accessible to enzymes and regulatory proteins.

The lowest level of chromatin organization - Nucleosomes

The orderly packaging of eukaryotic DNA into chromosome primarily depends on the histones, a remarkable group of small proteins that possess unusually high content of basic amino acids lysine and arginine. Histones are divided into mainly 5 classes- H<sub>1</sub>, H<sub>2</sub>A, H<sub>2</sub>B, H<sub>3</sub> & H<sub>4</sub>. Histones are the highly conserved proteins in the biological world.

In 1974, using the data from nuclease digestion and other types of information, Roger Kornberg, Harvard University, proposed entirely new structure for chromatin. Kornberg proposed that DNA and histones are organized into repeating units called nucleosomes.

Each nucleosome consists of a nucleosome core particle consisting of 146 bp of supercoiled DNA wrapped almost twice around a disc-shaped complex of eight histone molecules. The histone core of each nucleosome consists of two copies, each of histones H<sub>2</sub>A, H<sub>2</sub>B, H<sub>3</sub> & H<sub>4</sub> assembled into an octamer. The remaining histone H<sub>1</sub> - reside outside the nucleosome core particle. H<sub>1</sub> is referred to as the linker histone because it binds to the linker DNA that connects one nucleosome core particle to the next. Together with the H<sub>1</sub> and histone octamer (nucleosome interacts with about 168 base pairs of DNA). When H<sub>1</sub> is depleted, chromatin is observed under electron microscope, the nucleosome core particle and linker DNA appears as beads on a string." Through X-ray crystallography it is revealed that the 8 histones in the nucleosome core particle is organized into 4 heterodimers, two H<sub>2</sub>A - H<sub>2</sub>B dimers and two H<sub>3</sub> - H<sub>4</sub> dimers.

DNA and the core histones are held together by several types of noncovalent bonds, including ionic bonds between negatively charged phosphates of DNA backbone and positively charged residues of the histones. The two molecules make contact at sites where the minor groove of the DNA faces inward toward the core, which are approximately 10 base pair intervals. Chromatin is a dynamic cellular component in which histones, regulatory proteins and a variety of enzymes move in and out of the nucleoprotein complex to facilitate the complex tasks of DNA transcription,

compaction, replication, recombination and repair.

Higher levels chromatin structure :

DNA wrapped around nucleosome 10 nm thickness is the lowest level of chromatin organisation, but in the cell chromatin does not always exist in these beads on a string state. When chromatin released from and prepared at physiologic ionic strength fiber of approximately 30 nm thickness is observed. Two models differ in the relative positioning of the nucleosome within the fiber are explained. These are

(a) Zig-Zag model:- (Recent Research favour this model).

In this model, the linker DNA is present in a straight, extended state that criss crosses back and forth between consecutive core particles, which are organised into two separate stacks of nucleosomes and are coiled into a higher-order helical structure.

(b) Solenoid model: (Conventional model)

In this model, the linker DNA gently curves and connects consecutive core particles, which are organized into a single continuous helical array containing about 6-8 nucleosomes per turn.

Maintenance of 30 nm fiber depends on the interaction between histone molecules of neighbouring nucleosomes (Both linker histones and core histones) .

The next stage in the hierarchy of DNA packaging is thought to occur as 30 nm fiber is gathered into a series of large supercoiled loops or domains, that may be compacted into even thicker (80-100nm) fiber. Presumably type II topoisomerase regulates the degree of DNA supercoiling. The topoisomerases not would also be expected to entangle the DNA molecules of different loops should they become intertwined. Normally loops of chromatin fibers are spread out within the nucleus .

The mitotic chromosome represents the ultimate in chromatin compactness. 1  $\mu\text{m}$  of mitotic chromosome length approximately contains 1cm DNA which represents the packing ratio 10,000: 1. The diameter of the chromatid of mitotic chromosome is about 700 nm.

Nucleolus:

In a nondividing (interphase) cell, the clusters of rDNA are gathered together as part of one or more irregularly shaped nuclear structures, called nucleoli (singular, nucleolus), that function in producing ribosomes. Three distinct nucleolar regions can be distinguished morphologically; Granular Component, Fibrillar Component & Dense fibrillar Component.

The bulk of the nucleolus consists of a granular component which contains

ribosomal subunits in various stages of assembly. , Embedded within the granular regions are fibrillar centers (fc) that are surrounded by a more dense fibrillar component. According to one model, the fc contains the DNA (Nuclear Organising Regions) that codes for ribosomal RNA, and the dfc contains the nascent pre-rRNA transcripts and associated proteins.

Functions of nucleolus:

1. RNA production
2. Ribosomal assembly

Nucleoplasm:

The undifferentiated protoplasm or ground substance present inside the nuclear envelope is called nuclear sap or nucleoplasm or karyolymph. The nucleoplasm seems to contain granules of various sizes and densities. During nuclear division, the nucleoplasm is of course continuous with cytoplasmic matrix. The other nuclear components such as the chromatic reticulum and nucleolus remain suspended in the nucleoplasm. Nucleoplasm contains nucleoproteins and many other inorganic and organic substances, such as, nucleic acids, proteins, enzymes, minerals and lipids.

Nuclear matrix:

When isolated nuclei are treated with nonionic detergents and high salt (e.g., 2 M NaCl), which remove lipids and nearly all of the histone and nonhistone proteins of the chromatin, the DNA is seen as a halo rounding a residual nuclear core. If the DNA fibers are subsequently digested with DNase, the structure that remains possesses the same shape as the original nucleus but is composed of a network of thin protein-containing fibrils crisscrossing through the nuclear space . This insoluble fibrillar network has been named the nuclear matrix.

Functions of nuclear matrix:

1. The nuclear matrix serves as a skeleton to maintain the shape of the nucleus
2. Act as a scaffold on which loops of chromatin are organised .
3. It also serves to anchor much of the machinery that is involved in the various activities of the nucleus, including transcription, RNA processing, and replication.

Nuclear Lamina:

The inner surface of the nuclear envelope of animal cells is bound by integral

membrane proteins to a thin filamentous meshwork, called the nuclear lamina. The nuclear lamina provides mechanical support to the nuclear envelope, serves as a site of attachment for chromatin fibers at the nuclear periphery.

### 3. The Endomembrane system:

A biosynthetic pathway can be discerned in which proteins are synthesized in the endoplasmic reticulum, modified during passage through the Golgi complex, and transported from the Golgi complex to various destinations, such as the plasma membrane, a lysosome, or the large vacuole of a plant cell. This route is also referred to as the secretory pathway.

Secretory pathway:

Secretory activities of cells can be divided into two types: constitutive and regulated. During constitutive secretion, materials are transported in secretory vesicles from their sites of synthesis and discharged into the extracellular space in a continual manner. Most cells engage in constitutive secretion, a process that contributes not only to the formation of the extracellular matrix, but to the formation of the plasma membrane itself. During regulated secretion, materials are stored as membrane-bound packages and discharged only in response to an appropriate stimulus.

Endocytic pathway:

Whereas materials move out of the cell by the secretory pathway, the endocytic pathway operates in the opposite direction. By following the endocytic pathway, materials move from the outer surface of the cell to compartments, such as endosomes and lysosomes, located within the cytoplasm.

Following internalization, vesicle-bound materials are transported to a dynamic network of tubules and vesicles known collectively as endosomes, which represent distribution centers along the endocytic pathway. The fluid in the lumen of endosomes is acidified by a H-ATPase in the boundary membrane. Endosomes are divided into two classes: early endosomes, which are typically located near the peripheral region of the cell, and late endosomes, which are typically located closer to the nucleus.

### Phagocytosis

Phagocytosis ("cell eating") is carried out extensively by a few types of cells specialized for the uptake of relatively large particles (>0.5  $\mu\text{m}$  diameter) from the environment. Many single-celled protists, such as amoebas and ciliates, make their livelihood by trapping food particles and smaller organisms and enclosing them within folds of the plasma membrane (Figure 12.45a). The folds fuse to



produce a vacuole (or phagosome) that pinches off inwardly from the plasma membrane. The phagosome fuses with a lysosome, and the material is digested within the resulting phagolysosome.

The endoplasmic reticulum, Golgi complex, endosomes, lysosomes, and vacuoles form an endomembrane system in which the individual components function as part of a coordinated unit. The organelles of the endomembrane system are part of a dynamic, integrated network in which materials are shuttled back and forth from one part of the cell to another. For the most part, materials are shuttled between organelles—from the Golgi complex to the plasma membrane

#### ENDOPLASMIC RETICULUM(PORTER)

The endoplasmic reticulum (ER) is divided into two sub compartments, the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER). Both types of ER comprise a system of membranes that enclose a space, or lumen, that is separated from the surrounding cytosol. As will be evident in the following discussion, the composition of the luminal (or cisternal) space inside the ER membranes is quite different from that of the surrounding cytosolic space.

The rough ER is defined by the presence of ribosomes bound to its cytosolic surface, whereas the smooth ER lacks associated ribosomes. The RER is typically composed of a network of flattened sacs (cisternae). The RER is continuous with the outer membrane of the nuclear envelope, which also bears ribosomes on its cytosolic surface. In contrast, the membranous elements of the SER are highly curved and tubular, forming an interconnecting system of pipelines curving through the cytoplasm.

#### Specific functions of SER

- 1) Synthesis and storage of lipids and lipoproteins.
2. Glycogenolysis. Break down of glycogen through the action of glucose 6 phosphatase occurs in SER.
3. Detoxification. Harmful materials such as drugs, pollutants as well as metabolic wastes are converted by SER into the substances suitable for excretion by the cell.
- (4) Synthetic functions. SER is involved in the synthesis of a variety of substances such as steroids, pigments and cell wall materials.
4. Sequestering  $\text{Ca}^{2+}$  ions within the cytoplasm of cells

#### Specific functions of RER

- 1) The major function of RER is the synthesis and secretion of proteins

2)Glycosylation of Secretory proteins.

3)RER is the starting point of biosynthetic pathway.

GOLGI COMPLEX(CAMILLIO GOLGI):

In 1898, Camillio Golgi applied a metallic stain to nerve cells from the cerebellum and discovered a darkly stained reticular network located near the cell nucleus. This network, which was later identified in other cell types and named the Golgi complex, helped earn its discoverer the Nobel Prize in 1906.

The Golgi complex has a characteristic morphology consisting primarily of flattened, disc-like, membranous cisternae with dilated rims and associated vesicles and tubules. The cisternae, whose diameters are typically 0.5 to 1.0  $\mu$ m, are arranged in an orderly stack, much like a stack of pancakes, and are curved so as to resemble a shallow bowl. An individual cell may contain from a few to several thousand distinct stacks, depending on the cell type. The Golgi stacks in mammalian cells are interconnected by membranous tubules to form a single, large ribbon-like complex situated adjacent to the cell's nucleus.

Functions of golgi apparatus:

1. Golgi bodies in protein targeting:

The Golgi complex is divided into several functionally distinct compartments arranged along an axis from the cis or entry face closest to the ER to the trans or exit face at the opposite end of the stack. The cis-most face of the organelle is composed of an interconnected network of tubules referred to as the cis Golgi network (CGN). The CGN is thought to function primarily as a sorting station that distinguishes between proteins to be shipped back to the ER and those that are allowed to proceed to the next Golgi station. The bulk of the Golgi complex consists of a series of large, flattened cisternae, which are divided into cis, medial, and trans cisternae.

The trans-most face of the organelle contains a distinct network of tubules and vesicles called the trans Golgi network (TGN). The TGN is a sorting station where proteins are segregated into different types of vesicles heading either to the plasma membrane or to various intracellular destinations.

2.Glycosylation:

The Golgi complex plays a key role in the assembly of the carbohydrate component of glycoproteins and glycolipids.

Vesicles:

A closer look at an individual cisterna suggests that vesicles bud from a peripheral tubular domain, many of these vesicles contain a distinct protein coat. Protein coats have at least two distinct functions: (1) they act as a mechanical device that causes the membrane to curve and form a budding vesicle, and (2) they provide a mechanism for selecting the components to be carried by the vesicle.

## LYSOSOMES (Christian de Duve)

Lysosomes are an animal cell's digestive organelles. A typical lysosome contains at least 50 different hydrolytic enzymes produced in the rough ER and targeted to different cell organelles. Taken together, lysosomal enzymes can hydrolyze virtually every type of biological macromolecule. The enzymes of a lysosome share an important property: all have their optimal activity at an acid pH and thus are acid hydrolases. The pH optimum of these enzymes is suited to the low pH of the lysosomal compartment, which is approximately 4.6. The high internal proton concentration is maintained by a proton pump (an H-ATPase) present in the organelle's boundary membrane.

Lysosomal membranes contain a variety of highly glycosylated integral proteins whose carbohydrate chains are thought to form a protective lining that shields the membrane from attack by the enclosed enzymes.

Lysosomes are of two types.

Primary lysosomes: Originate from the ER or are cut off (mostly) from the Golgi have not yet been involved in digestion process.

Secondary lysosomes: Digestive vacuoles which are or have been sites of digestive activity. Two types: Heterophagic & Autophagic.

Functions of lysosomes:

1. Extracellular digestion: The best studied role of lysosomes is the breakdown of materials brought into the cell from the extracellular environment.

2. Intracellular digestion: Autophagy and heterophagy.

Lysosomes also play a key role in organelle turnover, that is, the regulated destruction of the cell's own organelles and their replacement. During this process, which is called autophagy, an organelle, such as the mitochondrion, is surrounded by a double membrane to produce a structure called an autophagosome. The outer membrane then fuses with a lysosome to produce an autophagolysosome in which the enclosed organelle is degraded and the

breakdown products are made available to the cell.

Once the digestive process in the autophagolysosome has been completed, the organelle is termed a residual body. Depending on the type of cell, the contents of the residual body may be eliminated from the cell by exocytosis, or they may be retained within the cytoplasm indefinitely as a lipofuscin granule. Lipofuscin granules increase in number as an individual becomes older; accumulation is particularly evident in long-lived cells such as neurons, where these granules are considered a major characteristic of the aging process.

3. Lysosomal enzymes are involved in release of certain hormones from secretory cells.

4. In certain cases these enzymes also help in the penetration of sperm nucleus into the egg.

5. Lysosomal activity in relation to pathology:

6. Lysosomes of leucocytes help in defence against cell infection

#### 4. PLANT CELL VACUOLES

As much as 90 percent of the volume of many plant cells is occupied by a single membrane-bound, fluid-filled central vacuole. The membrane that bounds the vacuole, the tonoplast, contains a number of active transport systems that pump ions into the vacuolar compartment to a concentration much higher than that in the cytoplasm or the extracellular fluid. The concentrated solution inside the tonoplast is known as cell sap. Because of its high ion concentration, water enters the vacuole by osmosis. Like the proteins of the lysosome, many of the proteins of a plant vacuole are synthesized on membrane-bound ribosomes of the RER, transported through the Golgi complex, and sorted at the trans face of the Golgi before being targeted to the vacuole.

Functions of vacuoles:

1. Many of a cell's solutes and macromolecules, including ions, sugars, amino acids, proteins, and polysaccharides, are stored temporarily in the vacuole.

2. Vacuoles may also store a host of toxic compounds. Some of these compounds (such as cyanide containing glycosides and glucosinolates) are part of an arsenal of chemical weapons that are released when the cell is injured by an herbivore or fungus. Digitalis, have proven to have important clinical value.

3. Plant vacuoles are also sites of intracellular digestion, not unlike lysosomes, which are absent in plants. In fact, plant vacuoles have some of the same acid hydrolases found in lysosomes.

4. Hydrostatic (turgor) pressure exerted by the vacuole not only provides mechanical support for the soft tissues of a plant, it also stretches the cell wall during cell growth

## 5. CYTOSKELETON:

Eukaryotic cells also possess a "skeletal system"-a cytoskeleton, composed of three well-defined filamentous structures: microtubules, microfilaments, and the intermediary filaments. The three types of cytoskeletal filaments are polymers of protein subunits held together by weak, noncovalent bonds. This type of construction lends itself to rapid assembly and disassembly, which is dependent on complex cellular regulation. Each cytoskeletal element has distinct properties. Microtubules are long, hollow, unbranched tubes composed of subunits of the protein tubulin. Microfilaments are solid, thinner structures, often organized into a branching network and composed of the protein actin. Intermediate filaments are tough, ropelike fibers composed of a variety of related proteins.

### THE MAJOR FUNCTIONS OF THE CYTOSKELETON

1. **Structure and Support:** A dynamic scaffold providing structural support that can determine the shape of the cell and resist forces that tend to deform it.
2. **Spatial Organization:** An internal framework responsible for positioning the various organelles within the interior of the cell. This function is particularly evident in polarized epithelial cells, such as those depicted in Figure 12.11, in which certain organelles are arranged in a defined order from the apical to the basal end of the cell.
3. **Intracellular Transport:** A network of tracks that direct the movement of materials and organelles within cells.
4. **Contractility and Motility:** The force-generating apparatus that moves cells from one place to another.
5. **An essential component of the cell's division machinery:** Cytoskeletal elements make up the apparatus (spindle fibers) responsible for separating the chromosomes during mitosis and meiosis and for splitting the parent cell into two daughter cells during cytokinesis.

### MICROTUBULES

Microtubules are hollow, relatively rigid, tubular structures, and they occur in

nearly every eukaryotic cell. Microtubules are components of a diverse array of structures, including the mitotic spindle of dividing cells and the core of cilia and flagella. Microtubules have an outer diameter of 25 nm and a wall thickness of approximately 4 nm, and may extend across the length or breadth of a cell. The wall of a microtubule is composed of globular proteins arranged in longitudinal rows, termed protofilaments, that are aligned parallel to the long axis of the tubule.

When viewed in cross section, microtubules are seen to consist of 13 protofilaments aligned side by side in a circular pattern within the wall. . Each protofilament is assembled from dimeric building blocks consisting of one  $\alpha$ -tubulin and one  $\beta$ -tubulin subunit.

### INTERMEDIATE FILAMENTS

The second of the three major cytoskeletal elements are solid, unbranched filaments with a diameter of 10-12 nm. They were named intermediate filaments (or IFs). To date, intermediate filaments have only been identified in animal cells. Intermediate filaments are strong, flexible rope like fibers that provide mechanical strength to cells that are subjected to physical stress, including neurons, muscle cells, and the epithelial cells that line the body's cavities. Unlike microfilaments and microtubules, IFs are a chemically heterogeneous group of structures that, in humans, are encoded by approximately 70 different genes.

### MICROFILAMENTS:

Microfilaments are approximately 8 nm in diameter and composed of globular subunits of the protein actin. In the presence of ATP, actin monomers polymerize to form a flexible, helical filament. As a result of its subunit organization, an actin filament is essentially a two-stranded structure with two helical grooves running along its length. The terms actin filament, F-actin, and micro filament are basically synonyms for this type of filament.

Microfilaments are also involved in intracellular motile processes, such as the movement of vesicles, phagocytosis, and cytokinesis. In fact, plant cells rely primarily on microfilaments, rather than microtubules, for the long-distance transport of cytoplasmic vesicles and organelles.

### 6. RIBOSOMES:

A eukaryotic cell may contain millions of ribosomes, each consisting of several molecules of rRNA together with dozens of ribosomal proteins. Ribosomes are so numerous that more than 80 percent of the RNA in most cells consists of ribosomal RNA. To furnish the cell with such a large number of transcripts, the DNA sequences encoding RNA are normally repeated hundreds of times. This

DNA, called rDNA, is typically clustered in one or a few regions of the genome. The human genome has five rDNA clusters, each on a different chromosome. In a nondividing (interphase) cell, the clusters of DNA are gathered together as part of one or more irregularly shaped nuclear structures, called nucleoli (singular, nucleolus), that function in producing ribosomes,

Each ribosome consists of two subunits: a smaller subunit. 70 S ribosome particles can be split into 50 S larger subunit and 30 S smaller subunits (50S subunit consists of 23S rRNA + 5S rRNA + 34 different types of proteins and 30S subunit consists of 16S rRNA + 20 types of proteins) and 80 S ribosome particles can be split into 60 S larger subunit and 40 S smaller subunits (60S subunit consists of 28S rRNA + 5.8S rRNA + 5S rRNA + 49 different types of proteins & 40S subunit consists of 18S rRNA + 33 ribosomal proteins). The association and dissociation of these two subunits depend on  $Mg^{2+}$  ion concentration, Ribosomes are generally defined by the sedimentation coefficients or Svedberg units or 'S' values.

Function of ribosomes:

Protein Synthesis(Translation)

Polyribosomes:

When a messenger RNA in the process of being translated is examined in the electron microscope, a number of ribosomes are invariably seen to be attached along the length of the mRNA thread. This complex of ribosomes and mRNA is called a polyribosome, or polysome. Each of the ribosomes initially assembles from its subunits at the initiation codon and then moves from that point toward the 3' end of the mRNA until it reaches a termination codon. As each ribosome moves away from the initiation codon, another ribosome attaches to the mRNA and begins its translation.

## 7. THE CELL WALL:

The plant cell is always surrounded by a cell wall. The cell wall which differs from plasma membrane in being non-living is the additional layer synthesized by the living cytoplasm just on the external surface of plasma membrane. In most of the plant cells, the cell wall is made up of cellulose, hemicellulose and pectin. In many fungi the wall is formed of with fibrous polysaccharides, and chitin and in bacteria the wall contains peptidoglycans.

The cell wall is perforated at one or more places through which the cytoplasm of a cell is connected with that of adjacent cells. These cytoplasmic bridges interconnecting any two adjacent cells are termed as plasmodesmata. These are of great physiological importance because they allow flow of cytoplasmic

materials from one cell to the other. Between the walls of the any two adjacent cells, intercellular substance is deposited in the form of middle lamella . In plants the middle lamella is formed of calcium pectate. The intercellular matrix cements the cells together cell wall to cell wall or pellicle to pellicle as the case may be. The middle lamella and outer cell wall or pellicle are emphasized as non-living because of the fact that they do not play important role in the life of cell.

In plants the cell wall can be differentiated into primary, secondary and sometimes tertiary layers. The secondary layer is deposited on the inner face of the primary membrane and the tertiary layer below the secondary one.

Ultra-structure:

The primary wall and the secondary wall have the same basic structure. In both cases cellulose macro-fibrils are found embedded in the gel-like matrix. . Each cellulose chain consists of about 2000-25000 glucose units. Nearly 100 cellulose chains arranged parallel to form minute bundle called crystalline domain or micelle (1.0 nm thick). Micelle is the smallest structural unit of cell wall. About 20-40 micelles assemble in the matrix to form a micro fibril (2.6 nm thick).

Microfibrils are synthesized on the plasma membrane by protein complexes called particle rosettes. Nearly 250 micro fibrils aggregate in bigger bundles called macro fibrils (~ 0.5  $\mu\text{m}$  in diameter, may reach, 4/ $\mu\text{m}$  in length). A cotton fibre has 1500 macro fibrils. In primary wall micro fibrils are short, wavy and loosely scattered. In secondary wall micro fibrils are long, straight, close and parallel arranged.

At certain places the microfibrils of secondary wall materials are not deposited, resulting thereby depressions or perforations. Such characteristic perforations in the secondary walls are known as pits. There are microcapillary spaces between the microfibrils of cellulose in the secondary wall. In these microcapillary spaces lignin, cutin, suberin, hemicellulose, minerals and some other wall materials are deposited which make the secondary wall tensile and sometime impermeable to water and gases.

Functions of cell wall:

1. The cell wall provides rigidity and support to the cells.
2. It also protects the protoplasm from mechanical injury
3. Maintains the characteristic shape of cell.
4. Providing a porous medium for the circulation and distribution of water, minerals and small nutrient molecules.
5. Providing a storage site of regulatory molecules against pathogenic microbes.



6. In certain instances, Cutin and suberin deposits on cellwalls reduce the rate of water loss.
7. The plasmodesmata through the cell walls have cytoplasmic connections between adjacent cells

#### 8. Microbodies:

Microbodies are spherical or ovoid organelles of various sizes, 0.2 to 1.5  $\mu$ m in diameter surrounded by a single trilaminar unit membrane and containing dense or sometimes crystalline materials (matrix). Recently, they have been found to contain enzymes, particularly catalases and oxidases. They do not contain any genetic material to self replicate.

In general, two types of microbodies are distinguished; peroxisomes and glyoxysomes. The peroxisomes are so named because of their peroxidative activity. Similarly, glyoxysomes possess all or part of the enzymes involved in glyoxylate cycle in addition to some other enzymes.

Microbodies have been reported in the cells of both plants and animals. They have been isolated from protozoa, yeast, algae, mesophyll cells of plant leaves, germinating seeds and cells of other plant tissues, birds, amphibians, and a variety of mammalian tissues including liver kidney, digestive tract, brain, lung, muscles.

#### Functions of microbodies:

1. Breakdown of excess fatty acids
2. Breakdown of Hydrogen Peroxide ( $H_2O_2$ ) by catalase.
3. Participates in the synthesis of bile acids
4. Breakdown of excess purines to uric acid
5. Glyoxisomes participate in photorespiration and nitrogen metabolism in root nodules.

#### 9. Cilia and Flagella:

Cilia and flagella are hairlike, motile organelles that project from the surface of a variety of eukaryotic cells. Cilia and flagella are essentially the same structure. Most biologists use one or the other term based on the type of cell from which the organelle projects and its pattern of movement. According to this distinction, a cilium can be likened to an oar as it moves the cell in a direction perpendicular to the cilium itself. Flagella exhibit a variety of different beating patterns

(waveforms), depending on the cell type.

An electron micrograph of a cross section of a cilium or flagellum:

The entire ciliary or flagellar projection is covered by a membrane that is continuous with the plasma membrane of the cell. The core of the cilium, called the axoneme, contains an array of microtubules that runs longitudinally through the entire organelle in a 9+2 pattern (9 peripheral doublets and 2 central singlets). The peripheral doublets are seen to consist of a complete and an incomplete microtubule, whereas the two central microtubules are complete. The dynein arms are seen as "fuzzy" projections from the wall of the complete microtubule. The two types of dynein arms (three-headed outer arms and two-headed inner arms), the nexin links between the doublets, the central sheath surrounding the central microtubules, and the radial spokes projecting from the outer doublets toward the central sheath are present in the axoneme.