

Cell Biology and Genetics

Cell structure and function

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June 27, 2006, Revised Sep 12, 2006

Suggested Readings : Annexure –I

Significant Keywords :

Cell; Prokaryote; Eukaryote; Plant cell; Cell wall; Cell membrane; Nucleus; Nuclear envelope; Nucleolus; Vacuoles; Endoplasmic reticulum; Golgi apparatus; Peroxisomes.

Introduction

Cell, the smallest unit to exhibit the properties of life is too small to be directly seen or touched; yet the study of its structure as will follow in subsequent pages very beautifully reflects the evolution of science through the curiosity and creative intelligence of man.

Robert Hooke, an English microscopist, curator at Royal Society of England is credited with discovery of cells. Observing a thin slice of cork under the preliminary microscope, he saw a honeycomb. Small pores of this honeycomb were named as cells by him in 1665 as he correlated them to the cells inhabited by monks living in a monastery. As we know it today, cells of Robert Hooke are actually the empty cells of dead plant tissues.

Living cells were observed first by another microscopist-basically a cloth merchant, namely Antony Van Leeuwenhoek in 1676. He observed a drop of water under a microscope and saw small animalcules that moved here and there. He was the first to discover various types of bacteria from different sources including scraping of his teeth.

It was in 1830s that importance of cells was realized worldwide. In 1838 Mathias Schleiden, a German lawyer turned botanist concluded that despite of differences in structure of various tissues, plants were made of cells and that the plant embryo arose from single cell. In 1839, Theodor Schwann a German Zoologist and colleague of Schleiden published a comprehensive report on cellular basis of animal life. Schwann concluded that cells of plants and animals are similar structures. Both of them together proposed two tenets of the cell theory.

1. All organisms are composed of one or more cells.
2. Cell is the structural unit of life.

Schleiden & Schwann's ideas on the origin of cells however proved to be imaginative, both agreed that cells could arise from non-cellular materials.

By 1855 Rudolf Virchow, a German pathologist made a convincing case of what is now the 3rd tenet of the cell theory.

“Omnis cellula -e- cellula which means cells can arise only by division from a preexisting cell”. Using high resolution microscopes, in particular the electron microscope, internal structure of a wide variety of cells has been now studied. These studies clearly differentiate two basic classes of cells - Prokaryotes and Eukaryotes.

The structurally simple prokaryote cells are found among unicellular organisms particularly bacteria. All multicellular organisms consist of structurally complex eukaryotic cells. Eukaryotic cells are classified into plant and animal cells as these two differ slightly in their structure.

The Cell Envelopes

Each cell is separated from its neighbors and environment by a bounding membrane called as plasma membrane. This membrane is universal to all cells. Plants, bacteria and fungi have in addition a distinct envelope called cell wall.

Cell Wall

Structure:

The cells of nearly all organisms, other than animals, are enclosed in a protective outer envelope surrounding their bounding membrane. Bacteria, fungi and plants have distinctive walls termed as cell walls. Almost all plant cells have a cell wall, only sperm cells of seed plants are exception to it. It is a specialized form of extra cellular matrix that is closely applied to external surface of plant cell membrane. In evolving relatively rigid cell walls varying from 0.1 μm to many micrometers in thickness, plants were deprived of the ability to move about and therefore did not develop muscles, bones or nervous system.

The newly formed cells are small in relation to the size, they finally attain. To cope-up with this enlargement, the cell walls of young growing plant cells are thinner and semirigid and are called as **primary cell wall**. The fully grown cells may retain only primary cell wall (1-5 μ m thick) or in certain cases deposit new tough wall layers termed Secondary Cell Walls (over 10 μ m in thickness). Secondary cell wall is usually deposited between plasma membrane and the primary cell wall, sometimes in successive layers with different orientations.

Although the cell walls of higher plants vary greatly in their detailed organization, they are constructed according to a common principle; they achieve their strength from long tough fibers that are held together by a matrix of proteins and polysaccharides. Fibers are generally made up of Cellulose- a polysaccharide which forms the most abundant organic macromolecule on earth (* App. 10¹⁵ Kg of cellulose are synthesized in the biosphere every year). In matrix polysaccharides are different – these are hemicelluloses and pectins.

Chemical composition of these constituents is:

- Cellulose - 1-4 D Glucan.
- Hemicelluloses -Glucans, glucomannans, Xylans, Xyloglucans, Arabino Xylans.
- Pectins Arabinans, Galactans, Galacturonans.
- Glycoproteins Hydroxy proline rich, Proline rich, Glycine rich.
- Functional proteins Peroxidases, Pectinases, Cellulases.

The primary cell wall is composed by dry wt. of 25-40% cellulose, over 50% other polysaccharides (which include mainly hemicelluloses and pectin substances) and 5% glycoproteins also termed as structural proteins.

Secondary cell wall architecture is different. Generally the ratio of cellulose is high e.g. wood has about 60% cellulose and cotton has 98% cellulose in secondary cell walls. Also new polymers get deposited in secondary cell wall and generally replace the pectin substances. These include mainly the lignins – a complex phenolic macromolecule with molecular wt. greater than 10,000 and with monomeric units of 3 different phenyl propane alcohols i.e. coniferyl, coumaryl and linapyl alcohols. Lignification confers considerable tensile strength to secondary cell walls with a relatively low cellulose content e.g. wood.

In xylem tracheids and fibres, secondary cell wall is three layered – S₁ (thin); S₂ (wide); S₃ (thin) (Fig. 1). In Sclerenchymatous cells, secondary cell wall with many layers of nearly equal width may be deposited. An intracellular wall substance or middle lamella (ML) cements two cells together. This is mainly pectinaceous in nature. In many plant tissues the cells often round off and separate from each other along the middle lamella creating intercellular spaces. These intercellular spaces (ICS) form an interconnected system through most of the plant.

Different cells in a plant body may show some special features in their cell wall (in addition to basic architecture). The same are prominent on all plant parts exposed to the atmosphere and are meant for protection from water loss and pathogen attacks. Epidermal cells in aerial parts of plants have middle lamella and primary walls. In addition they deposit a layer of cuticle on their outer surface. Cuticle is a layer of cutin mixed with waxes and is deposited as a more or less homogenous layer. It is separated from cellulosic part of cell wall by a pectin layer. Another prominent coating is suberin which is present on underground parts, woody stems and healed wounds.

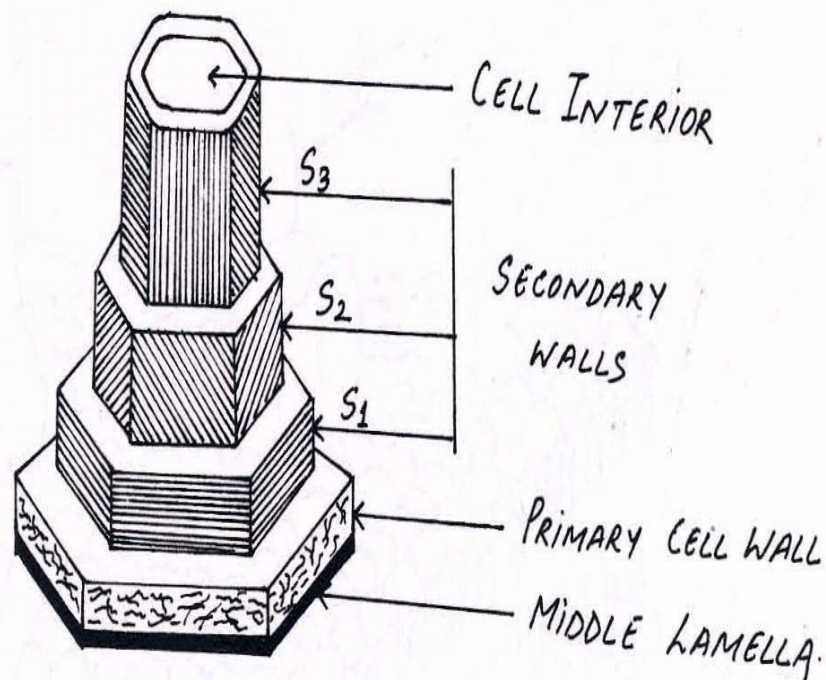


Fig: (1)

Biogenesis:

Cellulose microfibrils of the cell wall are synthesized by large protein complexes located in the plasma membrane. These complexes contain many units of enzyme **Cellulose Synthase**. The matrix components are synthesized in the golgi complex and delivered to cell wall via exocytosis. The enzymes involved in their synthesis are sugar-nucleotide polysaccharide glycosyl transferases.

Functions:

1. Cell wall is mainly responsible for giving strength and rigidity to the cell. The chemical architecture makes cell wall an envelope with great tensile strength. In water, a plant cell without a wall (protoplast) will take up water by osmosis, swell and burst. Inside the cell wall, it swells and presses against the wall creating a pressure known as turgor (like an inner tube pressing against a bicycle tyre). Turgor pressure (TP) is vital for cell expansion as well as for mechanical rigidity of young plant.
2. Intercellular adhesion provided by the middle lamella cements the plant body and at the same time allows an interconnection through most of the plant through intercellular spaces.
3. Cell wall polysaccharides may act as reserve carbohydrates. The same is seen in seeds, where cell walls of endosperm and cotyledons act as storage reservoirs. Seeds of Tamarind have cell walls rich in xyloglucan which can account for 25% of dry weight of seed. These reservoirs are used when the seed germinates. Enzymes are activated that digest these complexes into monosaccharides that can be used by embryonic plant.
4. Cell wall of the epidermal cells provides protection against dessication and pathogens.

PLASMA MEMBRANE

Structure:

An insight into the chemical nature of plasma membrane and the organization of its various components was observed in 1890s by E. Overton on plant root hair cells and R.B.Cs. He characterized this layer as solid, water insoluble – non polar barrier. Irving Langmuir, E. Gorter, F. Grendel, H. Davson, J. Danielli and J.D. Robertson contributed valuable observations.

Year	Contributor	Observation
1890:	Overton <i>et al.</i>	Plasma membrane is lipid in nature.
1900s:	Irving Langmuir	Lipids envelope the cell in form of a monolayer with polar heads projected outwards (towards water) and tails inward.
1925:	E. Gorter & F. Grendel	Lipids are in the form of a bilayer.
1935:	H. Davson & J. Danielli	Sandwich model. Plasma membrane is formed of lipids and proteins. Lipids form a bilayer while proteins (hydrophilic) line them externally.
1957	J. Robertson	Unit membrane model. Plasmamembrane is a trilamellar structure. Polar heads and proteins on either side form flanking lamella – 2-25 nm wide; tails of 2 layers form middle lamella 4nm in thickness. Proteins are different over 2 flanking layers.
1972:	S. Jonathan Singer & Garth Nicolson	Fluid mosaic model (Fig. 2)

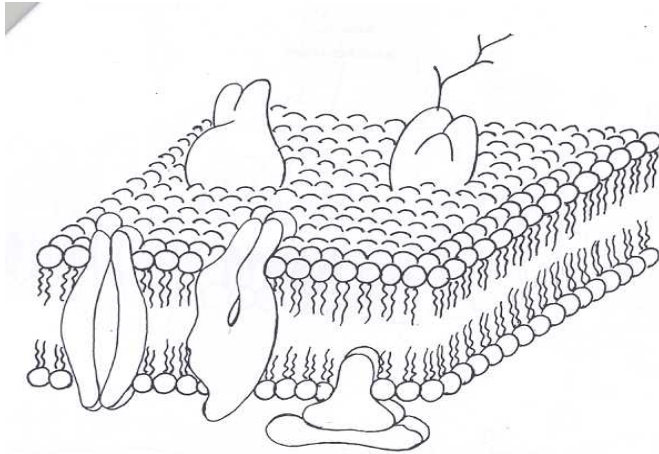


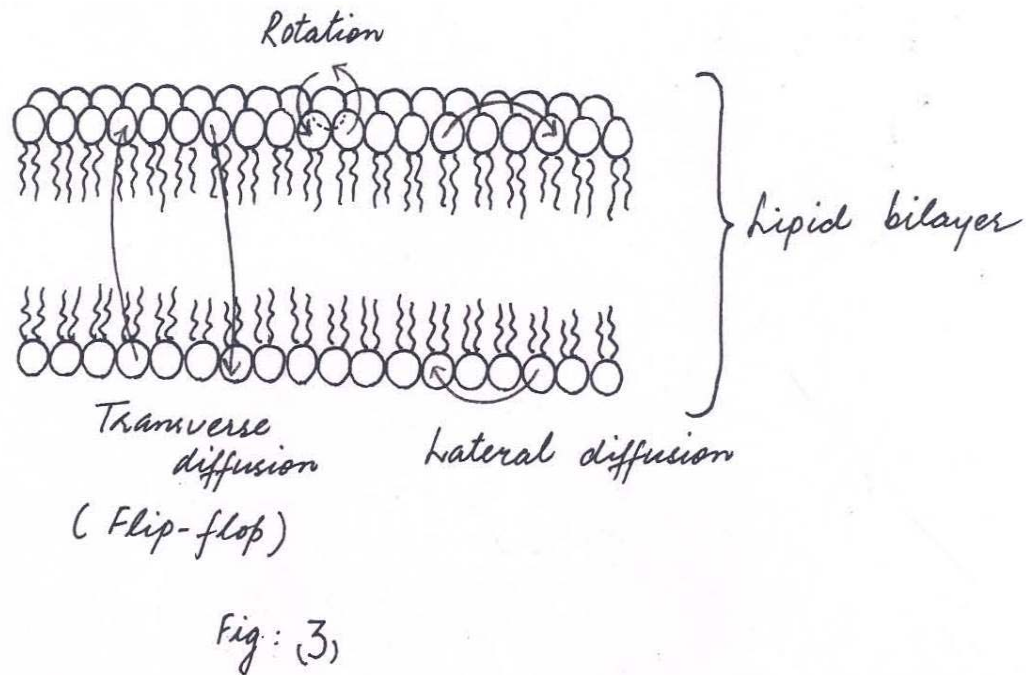
Fig. (2)

The advancements are summarized as below:

Till the advent of Electron microscope and biophysical techniques, unit membrane model was accepted widely. However, later it could not explain the distinctiveness of membranes. Isolated membranes from different sources varied greatly in protein to lipid ratio ranging from 3 in some cells to 0.23 in other. Also membrane proteins were shown to be of various shapes and sizes inconsistent with the concept of thin layers or sheets as proposed in the model. This became a basis for the formulation of a new model termed Fluid-mosaic model, which till date is a widely accepted model of cell membrane. Slight modifications made from time to time have upgraded this model a little.

Structural features of plasma membrane as are known today are summarized point wise:

1. Plasma membrane is a bilayer made up of lipids and proteins- lipids are arranged with their hydrophilic heads facing outwards and hydrophobic tails inwards. Main types of lipids are Phospholipids, followed by Glycolipids, Cholesterol and Sphingolipids. Lipid molecules are not static units but are in a fluid state i.e. each lipid molecule of the bilayer has the ability to move in the same layer or between 2 monolayers. These movements are given different names and occur with different frequencies: (Fig.3)
 - a. Lateral diffusion: when lipid molecules move or exchange places in the same monolayer. It is the most common type of movement with a diffusion coefficient of $10^{-8} \text{cm}^2 \text{sec}^{-1}$ and occurs approximately 10^7 times in a second.
 - b. Flip-flop: when movement takes place between two monolayers. It is a rare movement and occurs approximately once in two weeks.
 - c. Flexions/rotation: When a lipid molecule rotates at its place; or flexes its hydrophobic tail. These movements are too rapid to be recorded.



2. Membrane proteins are globular with both hydrophilic and hydrophobic regions. Plasma membrane is viewed as a mosaic of proteins discontinuously embedded in lipid bilayer.

Following categories of proteins are recognized:

- a. Peripheral
- b. Single pass
- c. Multipass
- d. Integral monotopic proteins
- e. Multiple subunit proteins

Last four types constitute integral proteins

Integral membrane proteins have one or more hydrophobic regions with an affinity for hydrophobic interior of lipid bilayer. They also have one or more hydrophilic regions that extend outwards from membrane on one or both sides. The three main types of integral proteins are Transport proteins, Receptor proteins and Marker proteins. The function of these is elaborated in subhead on functions of plasma membrane.

Peripheral proteins generally lack discrete hydrophobic sequences and do not penetrate into lipid bilayer. They get associated with membrane surface through weak electrostatic forces. Depending on the cell type and organelle within the cell, a membrane may contain from few (half- a dozen) to more than 50 different proteins. Starting from Nicholson's model, where membrane proteins were taken to be dispersed monomers existing in homogenous lipid bilayer, we have ended up with a more mosaic and highly dynamic picture of membrane organization where proteins form preferential associations in a crowded environment and the membrane thickness is variable (Marguet *et al* 2006).

Functions

An array of the functions have been assigned to cell membrane. Important among these include:

1. *Delineation and compartmentalization*

Most important function of membrane is to define the boundaries of cell and its organelles. It thus physically separates the cell from surrounding environment. Various intra cellular membranes serve to functionally compartmentalize the cell (in eukaryotes only) e.g. mitochondrion for respiration, chloroplast for photosynthesis. The compartmentalization thus leads to localization of functions. Also some molecules and structures responsible for a particular function are either embedded in or localized on the membranes making them active components of organelle function.

2. *Regulation of transport*

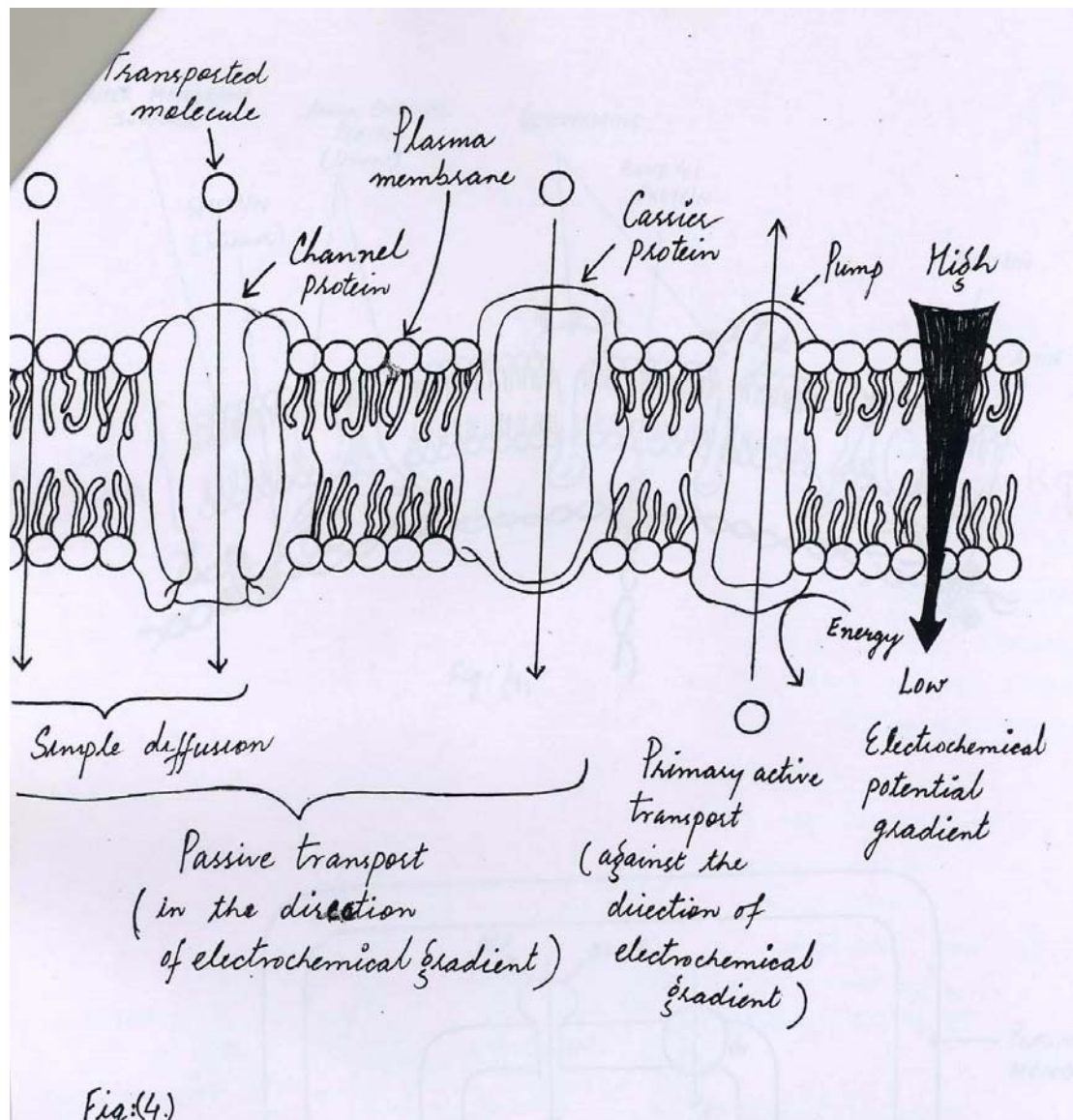
Membranes regulate the movement of substances into and out of the cells and organelles. Mode of entry for various substances may vary. These modes are categorized as:

- a. Passive transport:** Substances move in and out in a direction based on concentration/ charge gradient. Such a transport does not require energy as the movement is down the gradient. Passive transport involves either *Simple diffusion* or involvement of transport proteins. In simple diffusion, molecules e.g. H₂O, CO₂ and ethanol move across easily because of their small size and/or non polar nature. Transport proteins are specialized integral proteins (also named as permeases) located in the plasma membrane to allow the entry of either large molecules like sugars and amino acids and/or special ions. These show specificity for the solutes they transport and thus show great diversity in cells. Three types of membrane transporters enhance the movement of these substances across plasma membrane: channels, carriers and pumps (Fig.4). While the first two types are involved in passive transport, the last one allows active transport discussed in next subhead.

Channels are transmembrane proteins that function as selective pores through which molecules or ions can diffuse across the membrane. These generally have structures called gates that open and close the pore in response to external signals- which include voltage changes, hormone binding or light.

Carrier proteins do not have pores. Instead the substance being transported binds to a specific site on this protein. This binding causes a conformational change in the protein, which exposes the substance to the solution on other side of the membrane, where this substance dissociates from carrier's binding site. Passive transport on a carrier is also called facilitated diffusion.

- b. Active transport:** Substances move against a concentration and /or charge gradient. This uphill transport is generally coupled with an energy releasing event and is carried out by special type of membrane proteins called *Pumps*. Many ions like Na⁺, K⁺, Cl⁻, H⁺ and nutrients such as sugars are transported in this way. Primary active transport is generally coupled to a source of energy such as ATP hydrolysis, an oxidation - reduction reaction or the absorption of light by the carrier protein.
- c. Endocytosis:** Some large molecules also gain entry into cell by being simply engulfed and incorporated into vesicles derived from plasma membrane- a process termed as endocytosis.



3. Detection and transport of signals

Signal transduction is another important function of plasma membrane. This involves detection of specific signals on the outer surface of cells. The signal molecules (ligands) bind to specific receptors on the outer surface of the plasma membrane. Binding of such substances is followed by specific chemical events on the inner surface of the membrane. The signals are then transmitted via intracellular signaling molecules called as secondary messengers. These then generate specific responses within the cell. Signals could be hormonal, electric, chemical etc. Membrane proteins called receptors allow cells to recognize transmit and respond to a variety of these signals. These cell surface receptors are various type of integral proteins.

4. Cell to Cell Communication

It is also helped by plasma membrane e.g. Gap junctions in animals and plasmodesmata in plants.

VACUOLE

It is the largest organelle in a plant cell, occupying more than 80% of cell's inner space in some cells (range 5-95% of inner space). At least one vacuole is present per plant cell. However, number may increase also.

Size and number of this organelle depends on type of cell and its stage of development. In meristematic tissue, vacuoles are present as small structures called pro-vacuoles. As cell matures, these pro-vacuoles coalesce to form a large central vacuole and the cytoplasm gets restricted to a thin layer surrounding the vacuole.

Structure: Structurally it is the simplest organelle consisting of lumen bounded by the vacuolar membrane called *Tonoplast*. Tonoplast is typical plasma membrane with lipids and proteins as its main constituents. In sugarbeet tonoplast is composed of 40% lipids and 60% proteins. Lipids are mainly phospholipids (Phosphatidylcholine, Phosphatidyl ethanolamine) and glycolipids. There are 5-15 major polypeptides and a number of minor polypeptides. Major proteins in tonoplast work as pumps driving the inflow of ions, nutrients and other storage products into the vacuole against a concentration gradient. These also allow vacuole to act as reservoir of waste products and excess salt taken by the plant. This influx coupled with a battery of enzymes makes the lumen of vacuole more acidic (pH, 3-6) than the cytosol (pH, 7.5).

Pumps in tonoplast are categorized as H^+ pumps which include (a) V-class ATP powered pump and (b) pp_i powered pump, both of which pump H^+ ions in the vacuole. There are proteins acting as Cl^- and NO_3^- channels that transport these anions from cytosol into vacuole. This transport of anions is driven by positive potential generated by H^+ pump. Operation of proton pumps and anion channels produces an inside positive electric potential of 20mv across the vacuolar membrane and substantial pH gradient. Both are used to power the selective uptake or retention of ions and small molecules (Fig. 5).

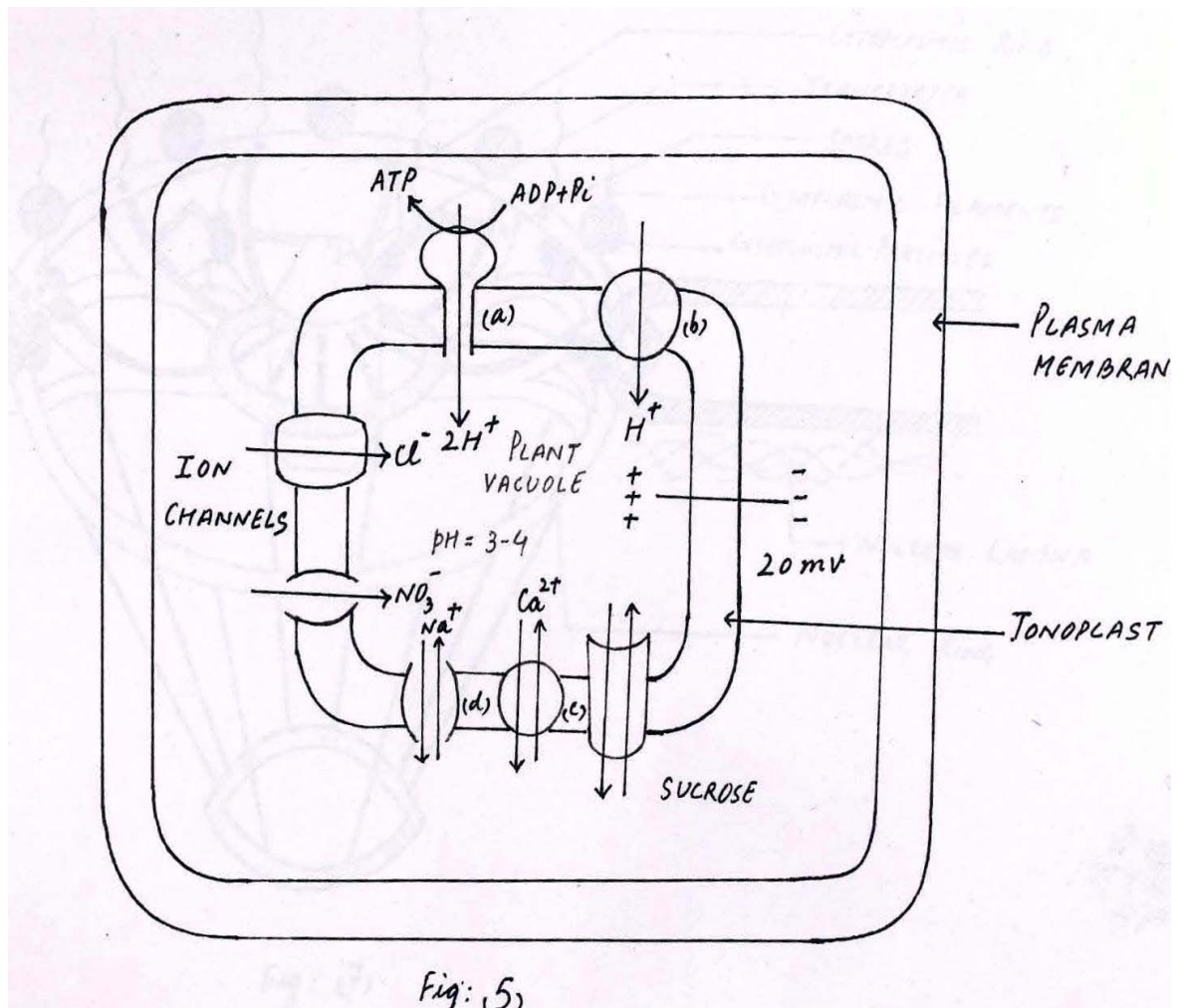
Functions

1. **Storage organelle:** vacuole is a store house for cells and stores water, ions, a number of enzymes and nutrients such as sucrose and amino acids. Substances stored in plant vacuoles include important products like rubber and opium. Anthocyanin pigments stored in vacuoles color the petals of many flowers. It also acts as a reservoir for waste products taken up by the plant. Plants like *Begonia* and *Oxalis* accumulate oxalic acid in vacuole lowering its pH to 1.2.

In leaf, excess sucrose generated during photosynthesis during day time is stored in the vacuole. During night the stored sucrose moves into cytoplasm and is metabolized into carbon dioxide and water along with the generation of ATP. A proton sucrose antiporter (a protein in tonoplast) helps in increased movement of sucrose and is powered by (a) outward movement of H^+ (b) -ve potential across the vacuolar membrane. Uptake of Ca^{2+} and Na^+ into vacuole from cytoplasm is also mediated by these types of proteins.

In seeds of many plants e.g. legumes vacuole acts as a protein storing organelle. A single vacuole in such cells becomes divided into smaller vacuoles and there is deposition of storage proteins in these small vacuoles which are then termed as *protein bodies*. In pea, in each cell of the cotyledon 175,000 protein bodies are formed per vacuole. These are 1-2 μm in diameter and bounded by a single membrane. During germination, the storage proteins are broken down into amino acids and exported into the cytoplasm for new protein synthesis. Hydrolytic enzymes required for this breakdown are stored in lytic vacuoles which fuse with the protein bodies to initiate this breakdown.

2. **Hydrostatic skeleton:** Vacuole acts as a hydrostatic skeleton for the plant. This is because it maintains turgor pressure (see cell wall) Central Vacuole filled with liquid presses the rest of cellular constituents against the cell wall and thus maintains turgor pressure.
3. **Autophagic Functions:** Vacuole helps to remove damaged / broken cell constituents. Tonoplast invaginates around the structure to be digested and it is brought inside the lumen of the vacuole, there a variety of enzymes which help in the breakdown of this broken / damaged constituent into simpler parts. The enzymes include: Acid phosphatase, Acid invertase, Acid protease, Acetyl glucosaminidase, Carboxy peptidase, Deoxyribonuclease, α -galactosidase, β -galactosidase, α -mannosidase (marker enzyme for vacuole), phosphodiesterase, phytase and ribonuclease. As such vacuoles are related to lysosomes of animal cells. However unlike lysosomes, plant vacuoles don't participate in turn over of macromolecules throughout the life of the cell. Instead, the enzyme stored leak into the cytosol as the cell under goes senescence. This helps to recycle valuable nutrients to living portion of the plant.



4. Homeostatic device: Vacuole is important as a homeostatic device enabling plant cells to withstand wide variations in their environment. It does so by helping to keep the pH in the cytosol intact e.g. when the pH in the environment of the cell drops, the flux of H^+ into the cytoplasm is balanced by increased transport of H^+ into the vacuole.

NUCLEUS

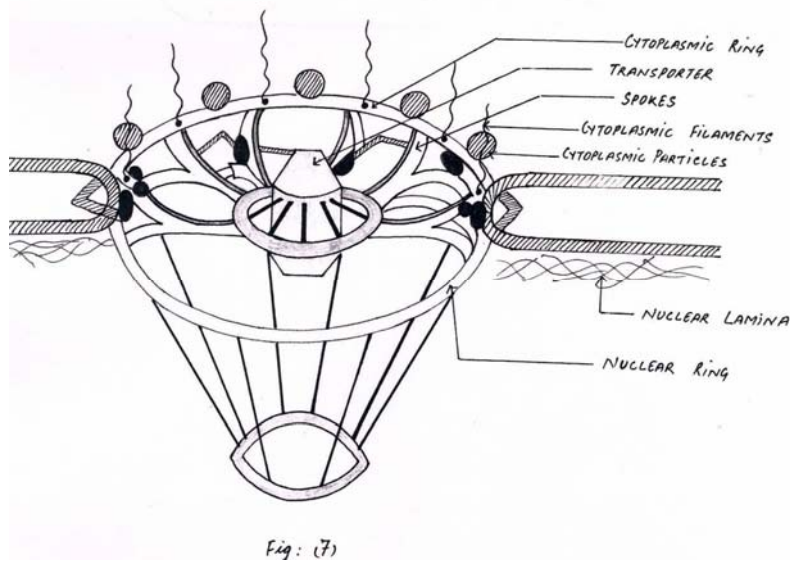
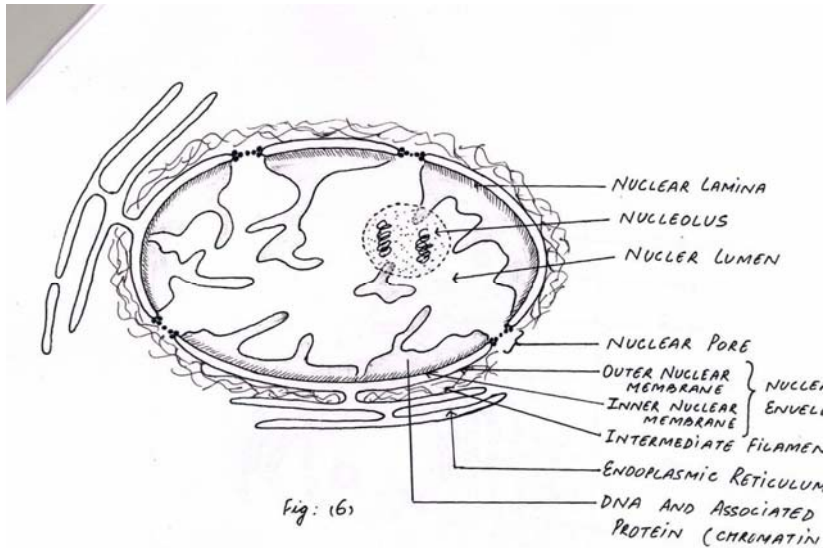
Discovered by Robert Brown in 1831, it is the most conspicuous and largest cell organelle measuring approximately $10\mu\text{m}$ in diameter. All eukaryotic cells that are metabolically active have a nucleus except for the brief period when they are dividing. Notable exceptions include mature erythrocytes in mammals and phloem cells in angiosperms. The former has a nucleus when it is formed in the bone marrow and synthesizes its haemoglobin but loses it later during the course of differentiation. Phloem cells depend on adjacent nucleated cells for their survival.

Structure: The nucleus has a rather undistinguished morphology considering its importance in the storage and utilization of genetic information. Included within the nucleus of a typical interphase (non dividing) cell are:

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

2. the **nuclear lamina** which is a protein containing fibrillar network;
3. one or more **nucleoli** which are irregular shaped electron dense structures that function in the synthesis of ribosomal RNA and the assembly of ribosomes and
4. the nucleoplasm which includes the non nucleolar regions of the nucleus and has nucleotide triphosphates, enzymes, proteins and transcription factors.

All these components are delimited from the rest of the cellular matter by a nuclear envelope or membrane (Fig.6)



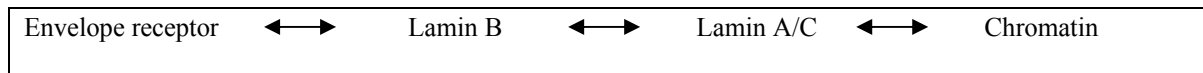
Nuclear Envelope /membrane

The nucleus is lined by a double membraned envelope. Each membrane is 6.5 nm thick and is separated by 10-50 nm intermembrane space. The two membranes are punctured at intervals by nuclear pores. In fact these membranes are fused at sites of these circular pores.

The outer membrane on its cytoplasmic site is often studded with ribosomes and is continuous with membrane of Rough Endoplasmic Reticulum (RER). The inner membrane on its nucleoplasmic surface is coated with a dense filamentous network called **nuclear lamina**. The membranes of the nuclear envelope serve as a barrier that keeps ions, solutes and macromolecules from passing freely between the nucleus and the cytoplasm.

Nuclear Lamina: It forms a layer of varying thickness typically 10-20nm thick which is absent at the site of the nuclear pore. The filaments constituting this layer are approximately 10nm in diameter and composed of polypeptides called **Lamins**. Lamins are members of the same super family of polypeptides that assembles into 10nm intermediate filaments (IF) of the cytoplasm. Three IF proteins known as lamins, A, B and C form these filaments. All these are 60,000-70,000 daltons in size. A and C have identical sequences of 566 amino acids. Lamin A has in addition an extra carboxy terminal of 98 amino acids.

Lamin B adheres to the nuclear envelope, while A and C bind both to Lamin B and chromatin material. Thus B acts as a 'nucleator' to which rest of the lamins attach. Lamins A and C bind both to Lamin B & the chromatin. The proposed relationship of lamins to the envelope and chromatin are:



Nuclear lamina provides mechanical support to the nuclear envelope and serves as a site of attachment for chromatin fibres at the nuclear periphery. It is a very dynamic structure. In a mammalian cell undergoing mitosis, phosphorylation of serine residues on the lamins causes the lamina to disassemble into lamins A & C and membrane associated lamin **B**. As the cell reenters interphase, lamins are dephosphorylated and an intact nuclear envelope reforms around separated chromosomes.

Nuclear Pores: First observed by E. Hertwig in 1876, these are membrane lined channels in which inner and outer membranes of the envelope are fused. The diameter from membrane to membrane in a pore is 60-90nm. Normally the proportion of the surface area occupied by these pores is small (less than 5%), it can be quite large, as in amphibian oocytes where it is 30% (50 million per nucleus). An average mammalian cell contains approx. 3000 nuclear pores. Looking at them one might assume that these pores are merely open channels but just the opposite is true. Nuclear pores contain a complex, basket like apparatus called the nuclear pore complex (NPC) that appears to fill the pore like a stopper, projecting into both the cytoplasm and nucleoplasm. Depending on the species, NPC's contain anywhere from 30 to 50 or more different proteins called nucleoporins (or nups), most of which are symmetrically positioned, being present on both the cytoplasmic and nuclear side of the structure. NPC itself is a huge supra molecular complex approximately 30 times the mass of a ribosome and exhibits octagonal symmetry due to 8-fold repetition of a number of structures.

The three principal components of this complex are:

1. On both the nucleoplasmic and cytoplasmic surfaces are rings of 8 (10-25nm diameter) granules that form an **annulus**.
2. Projecting from each granule of these rings is a radial spoke and these 8 spokes converge as the lining of the actual opening which is about 9nm in diameter.
3. A granular region is often seen in the center of the pore earlier thought to be the material on its way through the pore (Fig.7). Recent studies have revealed the so called materials to be actually the central plug or transporter.

Nucleolus: Nucleoli are small refractile bodies 0.5- 3.0µm in diameter forming important sub compartment/s of the interphase nuclei. These are not separated from rest of nucleus by any membrane, but appear to occupy a fixed place within nucleoplasm which is normally near the nuclear envelope. Diploid cells may have one large or a few small nucleoli.

Under electron microscope nucleolus is seen to be composed of three important components.

These include:

1. Fibrillar centers (CF) - composed of closely packed thin fibrils, 5-10nm in diameter.
2. Dense Fibrillar Centres (DFC) - which surround the fibrils.
3. Granular component (GC) - composed of particles 15-20nm in diameter, which forms the outline of the nucleolus.

Fibrils constituting fibrillar centers are made up of DNA/genes for ribosomal RNA. Hundreds of these genes are arranged in a tandem array. Dense fibrillar components contain actively transcribing rRNA genes and new rRNA transcripts and is most abundant in two proteins; nucleolin and fibrillin. Granular component (GC) forms the bulk of the nucleolus and is the site for the late processing event in biogenesis and organization of ribosomes.

Huge amounts of proteins remain associated with nucleolus, in certain cases approaching 90%. In most of the cases an approximate 3:1 mass ratio of proteins and rRNA exists. While many of these proteins are involved in the organization of ribosomes, few are nucleolar-resident proteins. Latter are non-ribosomal and include B23 which participates in the packaging of immature ribosomes and nucleolin which regulates RNA polymerase 1 transcription of **rDNA** (DNA coding for rRNA).

Functions:

Nucleus is most important cell organelle that determines cell survival as well as activities of cytoplasm that result in cell specificity. The nucleus exerts this control by virtue of its DNA organized as **chromatin material**. Two key events occur within the nucleus and both involve DNA. The first is DNA replication, which occurs during S phase. The second is transcription of DNA into RNA.

Enclosure of DNA in nuclear envelope ensures its protection and also export of its product (i.e. mRNA) becomes controllable. The surrounding cytoplasm has many ions that could alter DNA and chromosome structure e.g. the binding of proteins to DNA is largely ionic and ions in cytoplasm could change it. In fact this enclosure may be the single most important feature distinguishing eukaryotes from prokaryotes and makes the appearance of nuclear envelope a land mark in biological evolution.

Traffic into and out of the nucleus is mediated by nuclear pores. There are two main ways by which this transport occurs:

- a) Diffusion
- b) Active transport

a) Diffusion : Small molecules (5000 Daltons or less) diffuse freely in and out of the nucleus and nuclear envelope can be considered to be freely permeable to them. A protein of 17,000 Daltons equilibrates between cytoplasm and nucleus within 2 minutes, a protein of 44,000 Daltons takes 30 minutes to equilibrate, while a globular protein larger than 60,000 Daltons seems hardly able to enter the nucleus at all. Quantitative analysis of such data suggest that NPC contains a water filled cylindrical channel of 9nm diameter and 15nm long.

b) Active transport: The study of nuclear transport has helped in identifying a large number of proteins which are required for the nuclear import of important macromolecules. These include such large molecules as DNA and RNA polymerases with molecular weights of 1,00,000 and 2,00,000 daltons. Recent evidences indicate that these and many other nuclear proteins interact with receptor proteins located on the pore margins that actively transport proteins into the nucleus enlarging pore channel. A family of proteins called **karyopherins** are designated to function as transport receptors moving macro molecules across the nuclear envelope. Within this family **Importins** transport macromolecules from cytoplasm into nucleus and **Exportins** transport them in opposite direction.

ENDOPLASMIC RETICULUM

During the 19th century, it was observed that some eukaryotic cells particularly the ones involved in secretions have regions staining intensively with basic dyes. Term 'Ergastoplasm' was used for these regions. Similar network in nerve cells was named "Nissl bodies". By 1940s, J. Brachet had demonstrated that this staining was due to RNA.

K. Porter during late 1940s observed its structure under electron microscope and showed it to be an elaborated network of membrane delimited channels. He called it as 'Endoplasmic Reticulum' ('Reticulum' - network 'Endo' – within cytoplasm).

Structure: Endoplasmic Reticulum is an elaborate network of vesicles, tubules and sacs. Sacs also termed as Cisternae define a cisternal space which is continuous with the perinuclear space. ER communicates with lysosomes and Golgi via vesicles that shuttle between different structures. ER, Golgi, Lysosomes, nuclear envelope and vesicles define endomembrane system of eukaryotic cells.

Endoplasmic reticulum is designated to be of two types: Rough & Smooth (Fig. 8) Rough ER is recognized by the presence of ribosomes on its outer surface and consists of flattened sacs. Its cisternae are usually 30nm across. In smooth ER ribosomes are absent. It is more tubular component of ER with swollen cisternae. Both types seem to be continuous with each other and vary in their relative amount in different cells depending on the activity of the cell. Cells involved with synthesis of secretory proteins have more Rough ER. Smooth ER is abundant in specialized cells lacking RER e.g. endocrine glands or flower petal cells.

Many other cells contain little or no true smooth ER instead a region of ER is partly smooth and partly rough called Transitional element. Muscle cells have a specialized smooth ER like organelle called Sarcoplasmic reticulum - an internal organ that stores Ca^{2+} ions. It has a calcium pump forming more than 80% of the integral protein.

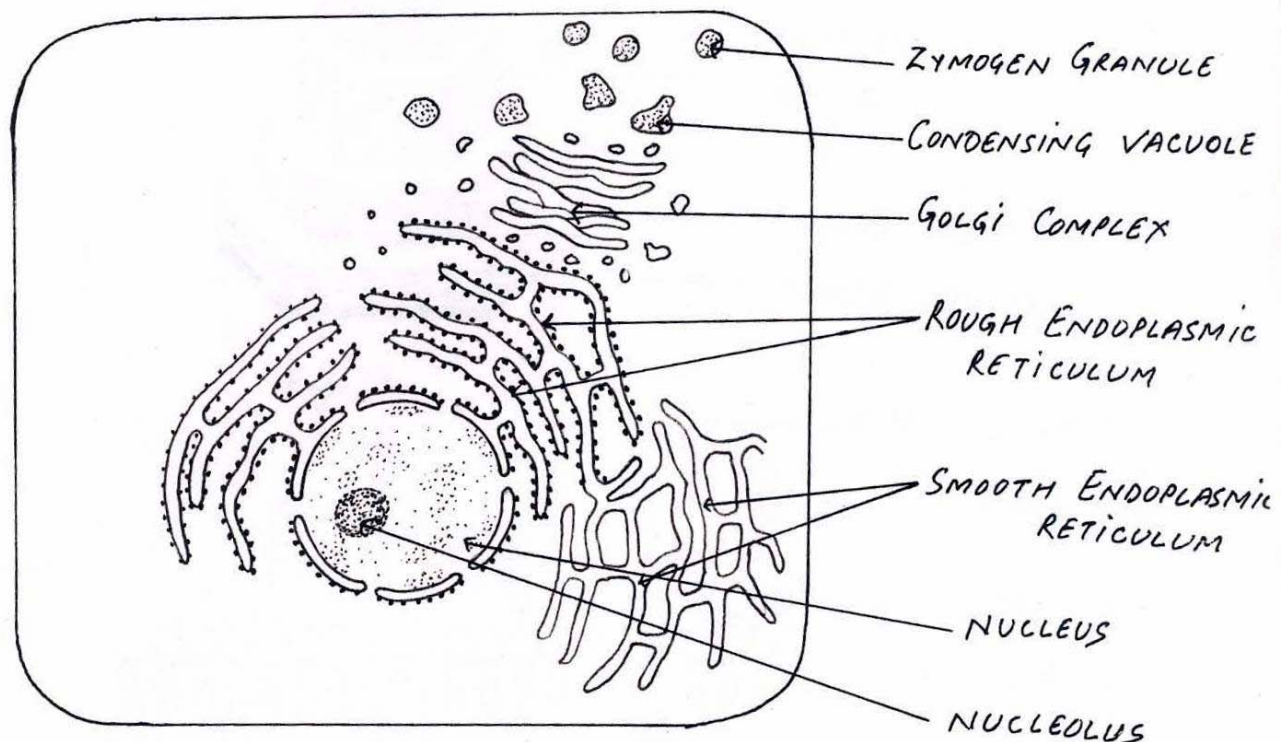


Fig: (8)

On homogenization both smooth and rough ER break down into smaller fragments which seal spontaneously into vesicles called microsomes. Most of our knowledge about the membrane and its chemical composition stems from study of these microsomes.

ER membrane is about 5-6nm thick and has a high protein to lipid ratio approximately 40% lipids and 60% protein occur by weight in this membrane. Lipid component has higher content of phosphoglycerides, lower levels of glycolipids, sphingomyelin and almost no cholesterol (smooth ER has cholesterol also). About 30 different proteins are present, many of which are enzymes and these include cytochrome B₅ reductase, Glucose – 6- phosphatase, cytochrome b₅ and cytochrome p-450.

Several proteins are permanent residents of the cisternae. These are termed “reticuloplasmins” and include PDI, Bip, reticulin, Glucuronyl transferase.

Functions

1. Protein synthesis and segregation

RER is involved in the synthesis and processing of integral membrane proteins. The proteins made on RER have a hydrophobic signal, through which they embed in the lipid bilayer. It is also a site of synthesis of a group of proteins destined for storage within other membrane bound organelles in the cell e.g. lysosomes or for export outside of the cell (e.g. digestive enzymes).

The proteins destined to be synthesized and processed in ER have the presence of signal sequence consisting of 16-30 amino acids (mostly hydrophobic) which directs the ribosome (site of synthesis of this protein) to ER membrane. Targeting involves two components (a) Signal recognition particle (SRP) and (b) SRP receptor.

SRP is a ribonucleoprotein present in the cytoplasm that transiently binds to ER signal sequence in a nascent protein growing on a ribosome. SRP receptor is a protein complex located in ER membrane that receives and is specific for complex of SRP, nascent chain and ribosome. SRP and its receptor only initiate the transfer of nascent chain across the ER membrane. Later they dissociate from the chain which is then transferred to a set of transmembrane proteins called translocon. The peptide chain continues to elongate until translation is complete. Ribosome keeps on sitting on the ER membrane during this time. Later the ribosomes are released and the protein is drawn into ER lumen, where it assumes its final configuration. Newly synthesized polypeptides in the membrane and lumen of ER undergo five principal modifications before they reach their final destination. These include:

- a) Formation and rearrangement of disulfide bonds.
- b) Proper folding.
- c) Addition and processing of carbohydrates.
- d) Specific proteolytic cleavages.
- e) Assembly into multimeric proteins.

First two and last of these modifications occur exclusively in rough endoplasmic reticulum. Addition of some carbohydrates and some proteolytic cleavages also occur in this organelle, many such modifications take place in **Golgi complex**.

2. Glycogen catabolism :

Smooth endoplasmic reticulum is involved in this. Excess sugar is stored as glycogen in liver and is released if needed by body (between meals and in response to muscular activity). This metabolism is hormone mediated and involves:

- a. stimulation of enzyme adenylate cyclase;
- b. Adenylate cyclase alleviates cAMP;
- c. Alleviated cAMP leads to activation of enzyme phosphorylase cleaving glycogen into glucose 1- phosphate;
- d. Glucose – 1- phosphate is converted to glucose – 6- P ;
- e. Glucose-6-P is converted to glucose in smooth ER involving enzyme glucose-6- phosphatase.

This last step is of significance as phosphorylated glucose cannot leave the liver (as membranes are impermeable to phosphorylated sugars).

3. *Lipid anabolism:*

It is also helped by SER as it contains a number of enzymes involved. The important and first step in the incorporation of long chain fatty acids to lipids i.e. acylation with coenzymes A occurs in SER. Fatty acyl-CoA are then used as substrates for incorporation into Phosphatides also made in ER. Glycolipids are also synthesized in SER.

Specialized lipids are also made in SER and proliferation of this organelle occurs in such cells e.g. plant cells synthesizing oils, stigma cells secreting exudates, or animal cells involved in steroid synthesis.

4. *Drug detoxification:*

Smooth ER plays an important role in a variety of hydroxylation reactions in which molecular oxygen is used to generate hydroxyl groups with electrons supplied by NADPH₂. One atom of oxygen is used to hydroxylate the substrate and other is reduced to water. Enzymes carrying out this reaction are called mono oxygenase. Transfer of electrons from NADPH₂ to oxygen involves an electron transport chain found in smooth ER with P-450 as terminal component. This hydroxylation leads to detoxification e.g. Barbiturate drugs are hydrophobic compounds. They tend to be membrane soluble and are therefore retained in body. Hydroxylation makes them water soluble and they are flushed away.

5. *Sarcoplasmic reticulum* via its Ca²⁺ pump helps in muscle contraction and relaxation. Release of Ca²⁺ from SR into cytosol causes contraction and rapid removal out of cytosol into SR causes relaxation.

GOLGI COMPLEX

Reported in 1898 by Camillo Golgi, Golgi complex is a collection of flattened membrane bound cisternae located near the cell nucleus. In animal cells these are often close to centrosome or cell center.

No. of cisternae per collection technically called *Stack* varies. On an average 4-6 cisternae also called saccules constitute one stack. Some cells however may contain only one large cisternae e.g. some fungi, other may contain 100s of very small stacks e.g. liver cells, pollen tubes. Term 'Dictyosome' (net body) is often used for individual stacks in plant cells, with Golgi apparatus reserved for the entire network in animal cells. In animal cells, many stacks remain linked by tubular connections between corresponding cisternae thus forming a single complex.

Each cisternae has a smooth membrane and is 0.5- 1µm in diameter. They are about 20nm apart in a stack. Golgi membrane is 60% lipids and 40% proteins by weight. Golgi stacks have 2 distinct faces – Cis (entry) and trans (exit) phase. 'Cis' region faces the nucleus and endoplasmic reticulum while 'Trans' region faces the plasma membrane. Between these 2 faces lie 'medial' cisternae. Proteins and lipids enter a Golgi stack on small vesicles (about 50nm in diameter) from ER on Cis side and exit for various destinations in vesicles (larger 0.5µm) from trans side. Some cisternae have 60nm diameter tubules or flat perforations (Fenestrae) extending from their periphery. These tubules are usually connected to ER or adjacent cisternae.

Golgi is prominent in cells specializing in secretions such as goblet cells of intestinal epithelium secreting large amount of mucus into the gut. In such cells usually large vesicles form from transphase which faces plasma membrane domain where the secretions occur.

Small membrane bound vesicles associated with Golgi are of three main types distinguished on the basis of a protein coat on their cytosolic surface. There are:

- a) **Clathrin coated:** Which are associated with trans phase of Golgi and are meant for transport to various destinations.
- b) **CoP II coated :** These are associated with Cis phase and involved in transport from rough ER to Golgi.

- c) **CoP I- coated** : which are involved in retrograde transport between different Golgi cisternae and form Cis Golgi back to rough ER.

Functions

Three main functions which are assigned to Golgi. These are:

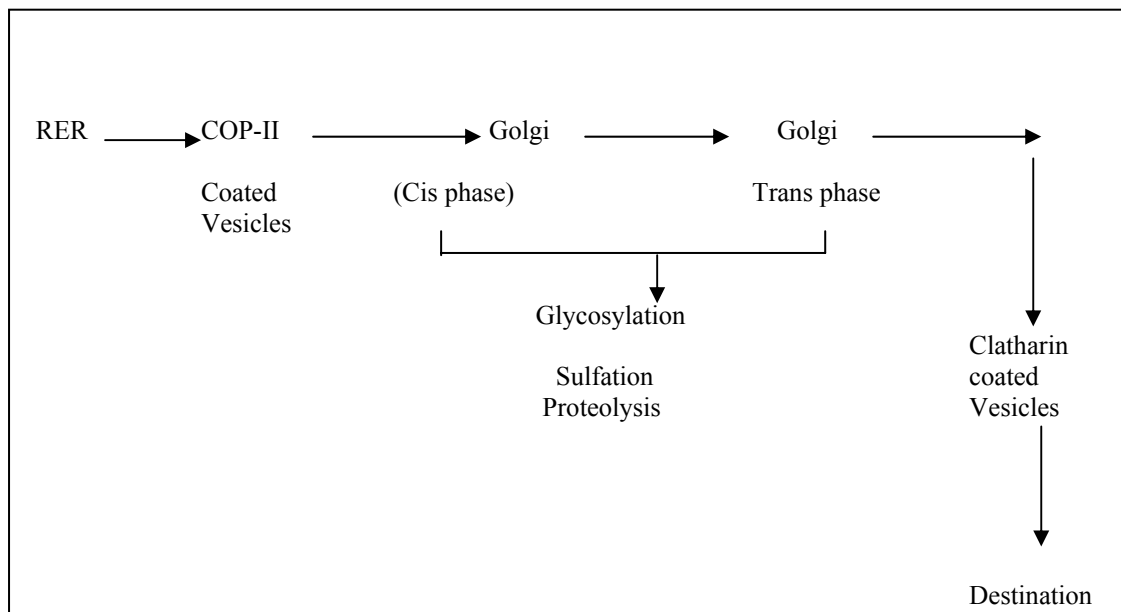
1. Protein packaging, modification and transport.
2. Polysaccharide synthesis and transport.
3. Exocytosis.

In addition, Golgi complex also participates in lipid transport and lysosome formation.

1. Protein Packaging, modification and transport:

Many proteins are synthesized on RER and are sequestered within it. They are then transferred to Cis, then to trans Golgi and finally to the vesicles for various destinations in the cell. In Golgi, a number of covalent modifications occur in these proteins, most common of these being glycosylation. The carbohydrates are added in sequence in separate compartments of the Golgi complex as protein passes through the cisternae.

Sequence of protein packaging is:



Other modifications of some secretory proteins are sulfation and proteolysis. Latter provides sulfated proteoglycans containing long carbohydrate chains with sulfated disaccharide repeating units. During proteolysis protein synthesized in RER is partly cleaved. e.g. **Insulin**: In case of insulin the initial protein formed by pancreatic cell is *Preproinsulin* which is 103 amino acid long, in RER terminal 23 residues are removed. Remaining chain called *Proinsulin* is hormonally inactive. In Golgi about 30 amino acid long internal sequence is removed leaving 21 amino acid long A-chain and 30 amino acid long B chain.

After modifications, proteins are sorted out and are either packed in separate vesicles for separate destinations or are retained as Golgi-resident proteins. Sorting is a highly specific phenomenon and is mediated by receptors – membrane proteins located in Trans Golgi elements. Membranes with their receptors attached to a specific protein are budded off from Golgi as clathrin coated vesicles.

This mechanism of sorting is very well understood for the proteins destined for lumen of **lysosomes** (membrane enclosed compartments filled with hydrolytic enzymes). Lysosome contains about 40 types of hydrolytic enzymes including proteases, nucleases, lipase, phosphatases, glycosidases and sulphatases. All these are synthesized in Rough Endoplasmic Reticulum and transported to Trans-golgi network. From Trans-golgi these enzymes are transported to late endosomes (which later form lysosomes) via transport vesicles that bud from Trans-golgi network. All these enzymes carry a unique marker in the form of Mannose-6-Phosphate. (M6P) groups which are added to these enzymes as they pass through the lumen of Cis-golgi network. These M6P groups are recognized by M6P receptor proteins in Trans-golgi network. Receptor proteins binds to lysosomes enzymes on the luminal side of the Golgi and to adaptins on outer side. Adaptins help in assembling clathrin coats. In this way M6P receptors help to package the lysosomes enzymes into clathrin coated vesicles that bud from Trans-golgi networks for destination to late endosomes.

2. Polysaccharide synthesis and transport:

Hemicelluloses and pectin substances constituting cell wall material in plants are known to be synthesized in the Golgi complex. Electron micrographs of cell active in cell wall synthesis show abundant Golgi near the plasma membrane. Large secretory vesicles budding from cisternae are often observed to contain fibrillar material similar morphologically to components of cell wall. Pulse-chase studies using radio-active glucose show that labeled polysaccharides first appear in the Golgi complex and then sequentially to secretory vesicles, plasma membrane and cell wall.

3. Exocytosis:

Secretory products of Golgi complex are released to the extracellular medium by exocytosis of secretory vesicles e.g. cell wall components. This secretion may be continuous (constitutive) or regulated by an appropriate hormonal, neural or ionic stimulus e.g. the release of vesicles of neurotransmitters from synapses.

Lipid transport

Apical domain or exposed face of almost every cell has plasma membranes rich in Glycosphingolipids protecting it from damage. Such membranes are also rich in proteins linked to lipid bilayer by Glycoside-phosphatidyl inositol (GPI) anchors.

GPI-anchored proteins are thought to be directed to the apical domains by associating with Glycosphingolipids in special structures termed as lipid rafts, formed in Trans-golgi network. Lipid rafts are microaggregates formed by self association of Glycosphingolipid and cholesterol. These rafts along with GPI-anchored proteins bud from Trans-golgi network into transport vesicles and are transported to apical plasma membrane.

PEROXISOMES

They are among the smallest cell organelles clubbed under the head **Microbodies**. Almost of universal presence, they are more abundant in kidney, liver cells, in photosynthetically active cells and in germinating seedlings. They are small round bodies 0.2 – 2µm in diameter, bounded by a single membrane and with a granular matrix. This matrix consists of enzymes urate oxidase, catalase and D-amino acid oxidase.

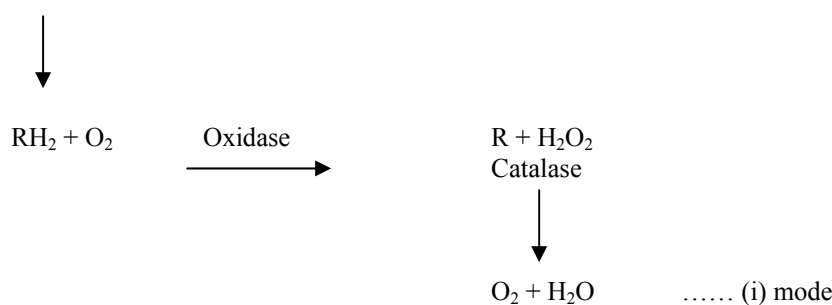
Peroxisomes are unusually diverse organelles that may contain different sets of enzymes in different cells of a single organism. Also they adapt remarkably to environment. This is exemplified by yeast cells grown on sugar or fatty acids. When grown on sugar yeast cell have small peroxisomes, but when grown on fatty acids they develop large peroxisomes. New peroxisomes are thought to arise from pre-existing peroxisomes by organelle growth and fission.

Function

1. **Detoxification** : Enzyme catalase present in the peroxisomes is involved in the breakdown of hydrogen peroxide (H_2O_2) -a potentially toxic compound formed in variety of oxidative reactions catalysed by enzyme *Oxidase* also located in the peroxisomes. Generation and degradation of hydrogen peroxide thus occurs in the same organelle protecting other parts of the cell from exposure to this toxic compound.

e.g.

RH_2 is an oxidizable substance that enters into peroxisome



Catalase can also reduce H_2O_2 to H_2O using electrons derived from an organic donor.



(where R' is an organic donor).

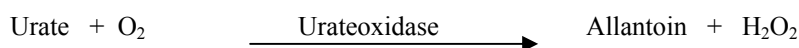
Catalase in its (ii) mode can use as its substrate a variety of substances like methanol, ethanol, formic acid, formaldehyde; phenols etc. all of which are harmful to the cell.

2. β -oxidation of fats

25-50% of oxidation of fats in animal tissues occurs in peroxisomes, remainder is localized in mitochondrion. Peroxisomal β -oxidation is important for the catabolism of fatty acids with especially long carbon chains. Fats are metabolized into sugars that can easily be consumed. It is more important in hibernating and migratory animals and in plant seedlings. In plant seedlings, fats are metabolized into sugars via *glyoxylate* cycle. All enzymes required for this cycle are located in special peroxisomes called *Glyoxysomes*. Glyoxysomes have a limited presence They are present only in endosperm / cotyledons and that too for a short period (a week or two). Glyoxysomes have been reported to appear again in senescing tissue of some plants probably to degrade lipids derived from senescent tissue.

3. Breakdown of N_2 containing substance

In peroxisomes of most animals except primates, enzyme urate oxidase is present. This is used to oxidize *Urate* a purine formed from the catabolism of nucleic acids and some proteins. Elimination of urate is must.



Allantoin is further metabolized and excreted by the organism either as allantoinic acid or as urea.

4. Breakdown of unusual substances

Some fungal peroxisomes contain enzymes which are involved in metabolism of alkanes, short chain hydrocarbon compounds found in oil and other petroleum products – such fungi are useful for cleaning oil spills.

5. Photorespiration

In plants, peroxisomes of the leaf help in photorespiration – a process that involves uptake of oxygen and release of carbon dioxide. This respiration occurs at low CO₂ concentration and high O₂ pressure and requires participation of chloroplast, mitochondrion and leaf peroxisomes.

6. Some peroxisomal enzymes called aminotransferases catalyze transfer of amino groups to α -keto acids and are important in synthesis and degradation of amino acids.
7. Another important function of animal peroxisomes is to catalyze the first reaction in the formation of plasmalogens, which are the most abundant class of phospholipids in myelin. Deficiency of plasmalogens causes abnormality in myelination of nerve cells. This leads to many neurological diseases in organisms with defunct peroxisomes. The importance of peroxisomes is demonstrated by the inherited human disease **Zellweger Syndrome**. Individuals with this syndrome contain empty peroxisomes and have severe abnormalities in their brain, liver and kidneys. They die soon after their birth. This disorder is because of disturbance in the transport of many peroxisomal proteins into peroxisomal matrix. The enzymes remain in the cytosol and are eventually degraded.
8. **Luciferase**- an enzyme present in the fire-flies and some bacteria can be localized to peroxisome in the former. In the presence of ATP and luciferin it catalyzes a light emitting reaction.

Acknowledgment:

The authors thank Miss **Renu Malpotra** for illustrations.

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