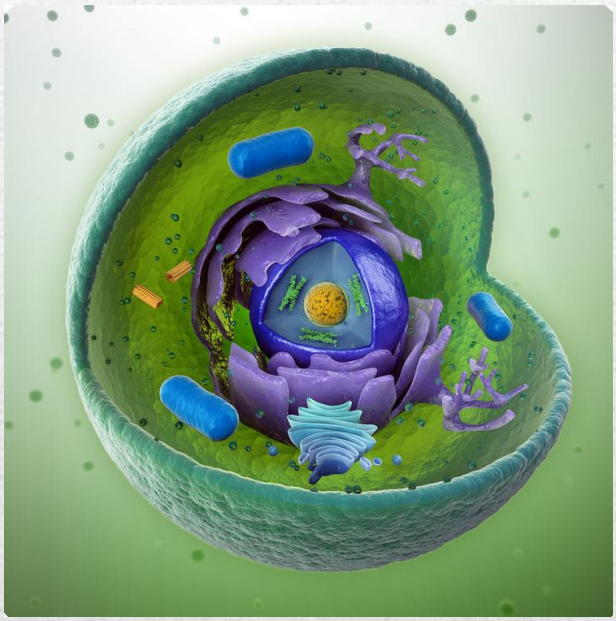
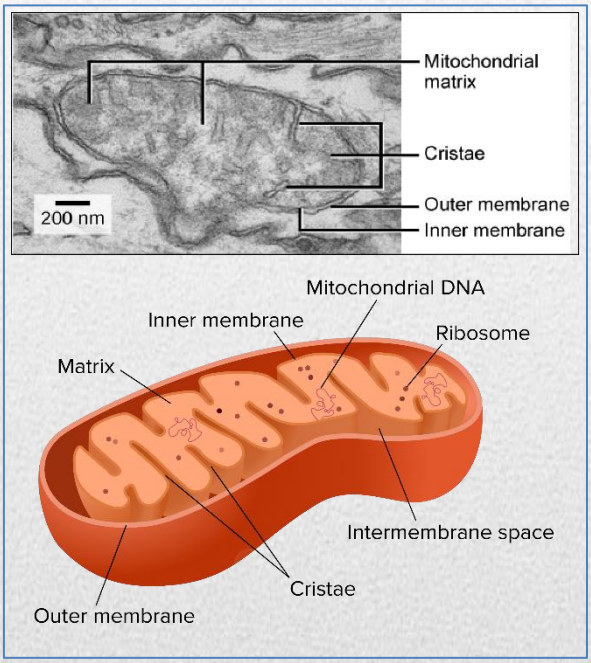
Cell Biology





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2023-2024

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An introduction to cells and their organelles

❖ What is the mean of Cell biology?

Cell biology is a branch of biology that studies the structure, function, and behavior of cells.

The term cell, as first used by **Robert Hooke** in **1665** (Fig.1), meant a hollow chamber surrounded by a definite wall. The nucleus was discovered by **Robert Brown** in **1831**.

One of the most important concepts in biology is that a cell is a basic structural and functional unit of living organism. This is known as **a cell theory** and was proposed jointly by two scientists in 1833. A Belgian Botanist called **Schleiden** and the German zoologist called **Schwan**. They studied the plant cell and animal cell respectively and come up with the idea that plants and animals are made up by small individuals which perform different functions of the whole organism. They finally come up with what they say cell theory.

The **cell theory** embraces four ideas, these are:

1. Living organisms are made up of smallest sufficient unit of living matter called cell.
2. The new cell is derived from pre-existing ones by cell division.
3. Each cell is independent with others but function as integral part of the whole organism.
4. The cell contains the hereditary material which is passed from generation to generation.

Von Mohl and **Nagelli**, working independently distinguished the two main parts of cell: the cell wall and the cell content. **De bary** and **Max Schultz** around 1861 established that cells consist of tiny masses of

protoplasm each containing a nucleus, thus founding what is known as the protoplasm theory.

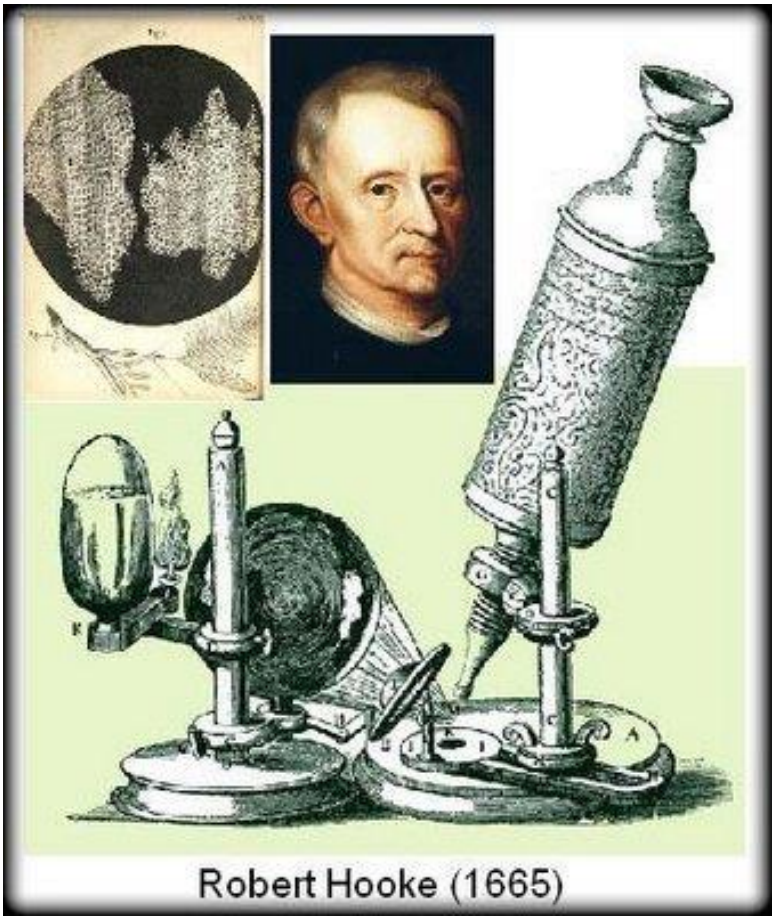


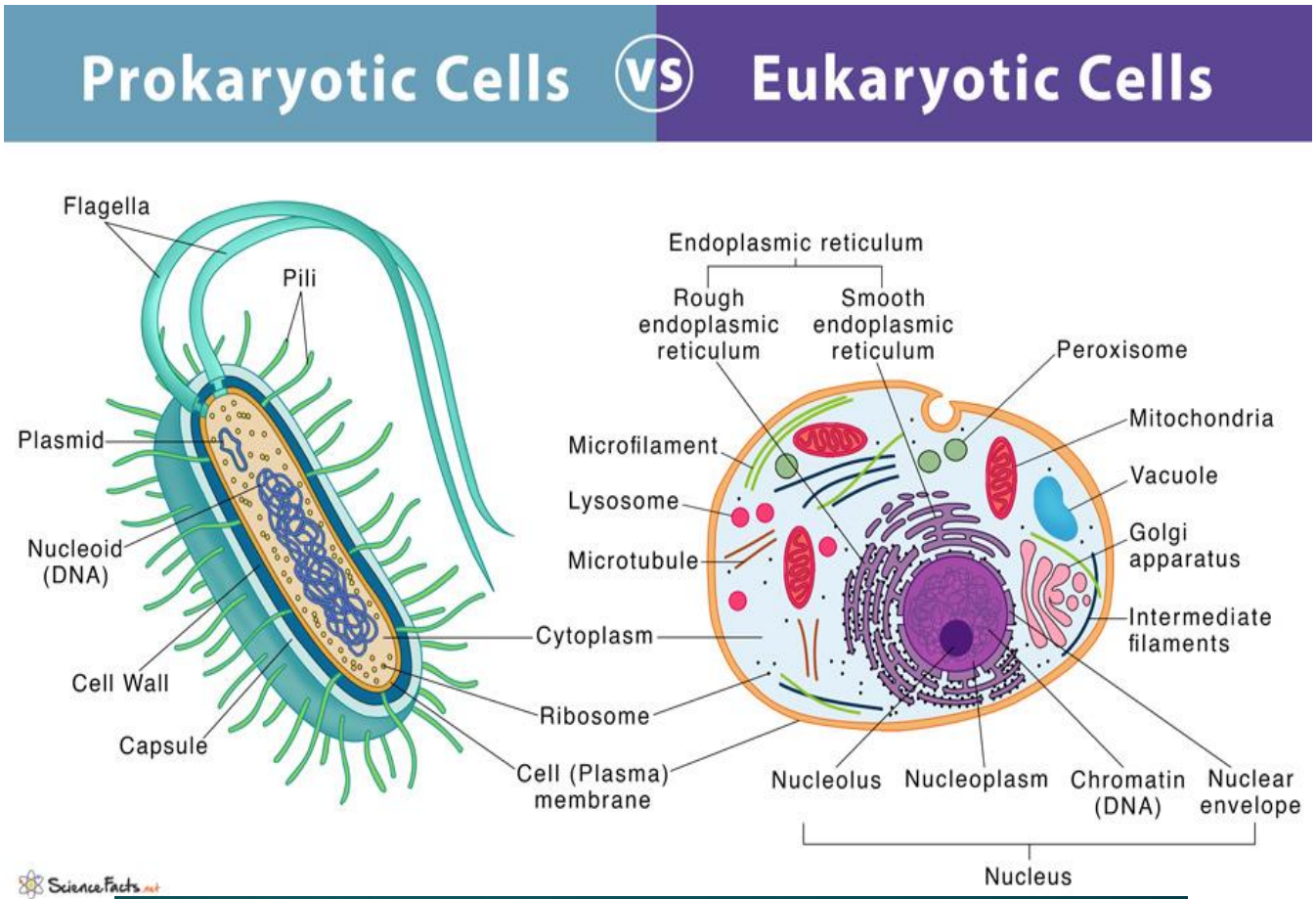
Fig.1: Robert Hook and his microscope (1665).

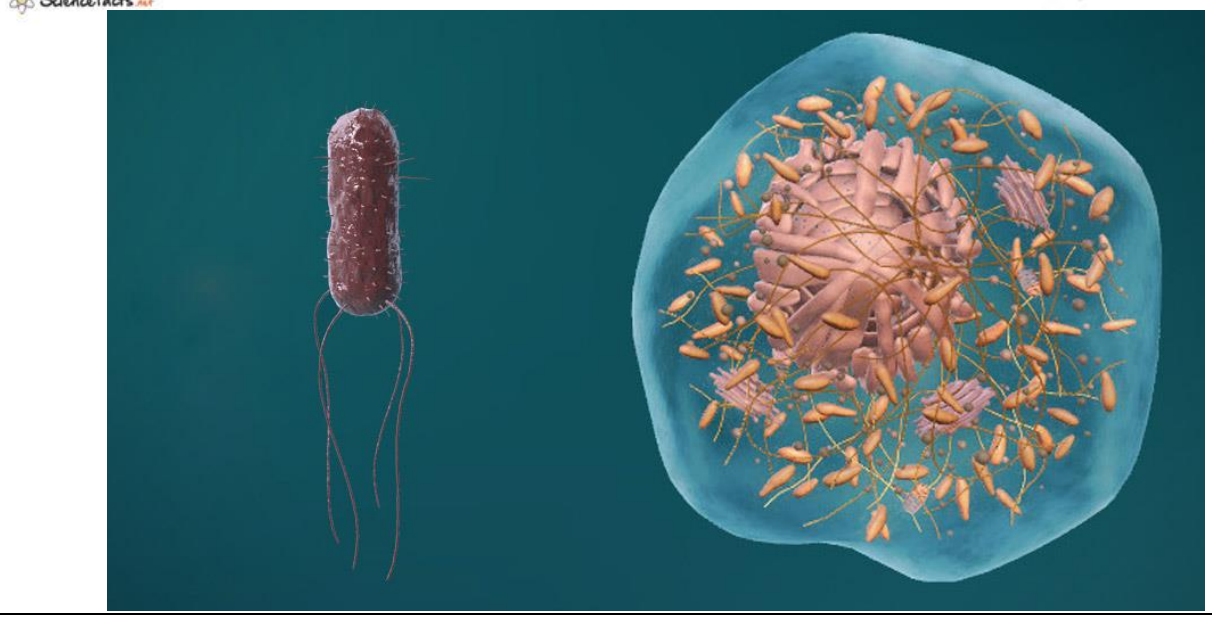
❖ Basic Properties of Cells

- Cells are Highly Complex and Organized.
- Cells Possess a Genetic Program and the Means to Use It.
- Cells Are Capable of Producing More of Themselves.
- Cells Acquire and Utilize Energy.
- Cells Carry Out a Variety of Chemical Reactions.
- Cells Engage in Mechanical Activities.
- Cells Are Able to Respond to Stimuli.
- Cells Are Capable of Self-Regulation.
- Cells Evolve.

❖ Two Fundamentally Different Classes of Cells

There are two basic classes of cells—**prokaryotic** and **eukaryotic**—distinguished by their size and the types of internal structures, or organelles, they contain.





❖ Differences between prokaryotic and eukaryotic cells

Prokaryotic cell	Eukaryotic cell
-Nucleus materials are not enclosed by nuclear membrane	-Nucleus materials are enclosed by nuclear membrane
-Contains few organelles	-Contains many organelles
-No membrane bounded organelles such as chloroplast and mitochondria	-Has membrane bounded organelles
-DNA is circular and lies free in cytoplasm	-DNA is linear and enclosed in nucleus
-No mitosis or meiosis, divide by binary fission	-Mitosis and meiosis occur
-It contains 70s ribosome (smaller)	-It contains 80s ribosome (larger)
-Mainly unicellular	-Mainly multi-cellular

Note:

In unicellular organisms a single cell, perform all the life activities and characteristics of living organisms, the cell organelles work as organs in an organism.

Cell organelles

Organelles are required for plant growth, development and function. These organelles (Figure 3) are the loci for a large number of physiological and biochemical processes. The organelle contents of plant and animal cells in common and those unique to plant cells are

depicted in Table 1. The dimensions of plant organelles are presented in Table 2. A. To enter a plant cell, molecules must traverse both the cell wall and the fluid mosaic plasmalemma (plasma membrane).

Table 1. Comparison of organelle contents of plant and animal cells.*

Organelle	Animal cell	Plant cell
Cell wall	Absent	Present
Centrioles	Present	Absent
Endoplasmic reticulum	Present	Present
Glyoxysomes	Absent	Present
Golgi apparatus	Present	Present
Microfilaments	Present	Present
Mitochondrion	Present	Present
Nucleus	Present	Present
Peroxisomes	Present	Present
Plastids	Absent	Present
Protein bodies	Absent	Present
Spindle	Present	Present
Vacuoles	Sometimes small	Present (mature cell – large central)

Table 2. Dimensions of subcellular organelles.

Organelles	Dimension
Chloroplast	4–6 µm in diameter
Golgi apparatus	Individual cisternae, 0.9 µm
	Coated vesicles 50–280 µm in diameter
Microbodies	0.1–2.0 µm in diameter
Microtubules	0.5–1.0 µm in diameter
Mitochondria	1–10 µm
Nuclear envelope pores	30–100 µm in diameter
Nucleus	5–10 µm in diameter
Peroxisome	0.2–0.7 µm
Plasmodesmata	2–40 µm in diameter
Primary wall	1–3 µm
Protein bodies	2–5 µm in diameter
Vacuoles	30–90% of cell volume

❖ Cell Specialization

Eukaryotic cells are far larger and more complex than prokaryotic cells and contain many organelles. The eukaryotic has been therefore compared to a factory. Efficiency is improved by division of labor (cell specialization), i.e., sharing out of job in such a way that each organelle has its own role involving its own specialize structure and chemistry. For-example; mitochondrion is the powerhouse of the cell providing energy in the form of ATP from the specialized reactions and respiration.

The cell as whole is in effect, divided up into compartments. This compartment is often achieved by membranes so that just as a cell surface membrane controls exchange between the cell and its environment. Each membrane bounded organelle can have its own particular unique set of chemicals and chemical reactions.

The Nature of Biological Molecules

- The bulk of an organism is water. If the water is evaporated away, most of the remaining dry weight consists of molecules containing atoms of carbon.
- The compounds produced by living organisms are called biochemicals.
- The chemistry of life centers around the chemistry of the carbon atom.
- Carbon-containing backbones may be linear, branched, or cyclic.

❖ A Classification of Biological Molecules by Function

The organic molecules commonly found within living cells can be divided into several categories based on their role in metabolism.

1. Macromolecules. The molecules that form the structure and carry out the activities of cells are huge, highly organized molecules called macromolecules, which contain anywhere from dozens to millions of carbon atoms. Macromolecules can be divided into four major categories: **proteins, nucleic acids, polysaccharides**, and certain **lipids**. The first three types are polymers composed of a large number of low-molecular-weight building blocks, or monomers. The basic structure and function of each type of macromolecule are similar in all organisms.

2. The building blocks of macromolecules. Most of the macromolecules within a cell have a short Lifetime compared with the cell itself; with the exception of the cell's DNA, they are continually broken down and replaced by new macromolecules. Consequently, most cells contain a supply (or pool) of low-molecular-weight precursors that are ready to be incorporated into macromolecules. These include sugars, which are the precursors of polysaccharides; amino acids, which are the precursors of proteins; nucleotides, which are the precursors of nucleic acids; and fatty acids, which are incorporated into lipids.

3. Metabolic intermediates (metabolites). The molecules in a cell have complex chemical structures and must be synthesized in a step-by-step sequence beginning with specific starting materials. In the cell, each series of chemical reactions is termed a metabolic pathway. The cell starts with compound A and converts it to compound B, then to compound C, and so on, until some functional end product (such as an amino acid building block of a protein) is produced. The compounds formed along the pathways leading to the end products might have no function per se and are called metabolic intermediates.

4. Molecules of miscellaneous function. This is obviously a broad category of molecules but not as large as you might expect; the vast bulk of the dry weight of a cell is made up of macromolecules and their direct precursors. The molecules of miscellaneous function include such substances as vitamins, which function primarily as adjuncts to proteins; certain steroid or amino acid hormones; molecules involved in energy storage, such as ATP; regulatory molecules such as cyclic AMP; and metabolic waste products such as urea.

The Plasma Membrane

The cell membrane or **plasma membrane** is the membrane or structure which encloses a mass of protoplasm of a cell.

The plasma membrane and all other membranes bounded organelles contain phospholipids and proteins. The lipids have hydrophilic head and hydrophobic tail which always occur in pair.

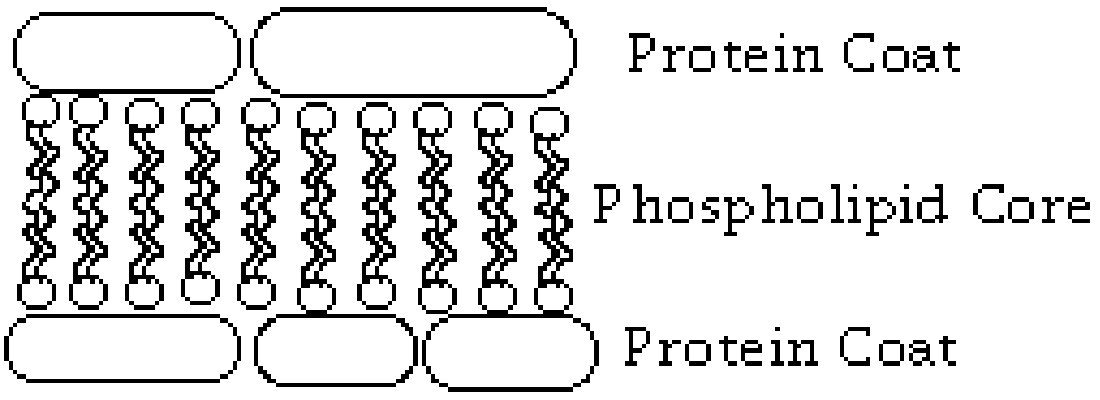
The ‘hydrophilic’ head is a polar molecule and have an affinity to water (hydrophilic i.e. water loving) and the ‘hydrophobic’ tail is non-polar and do not mix with water (hydrophobic i.e. water hating).

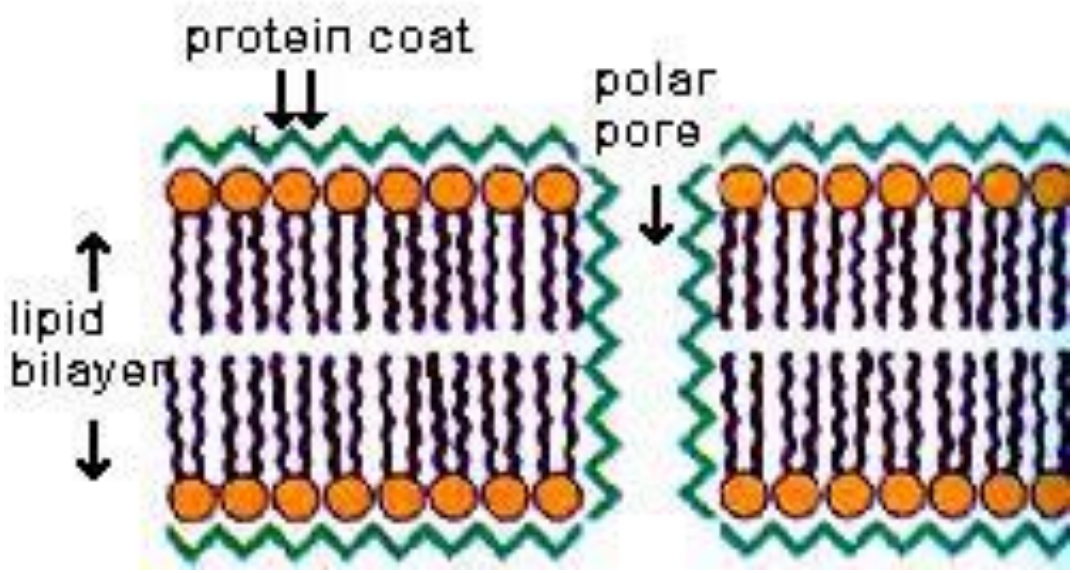
The plasma membrane has extra proteins which are special carrier molecules that act as receptors for hormones and immunological identity such as blood group antigen.

Models of plasma membrane

1- Danielli-Davson model

In 1940’s **Danielli** and **Davson** proposed that all the plasma membrane consist of lipid layer coated with protein molecules as continuous layer. These suggest the tri-laminar or having three layers. The lipid layer is a fluid medium in which the protein coated or attracted. It Called as **Sandwich model.**

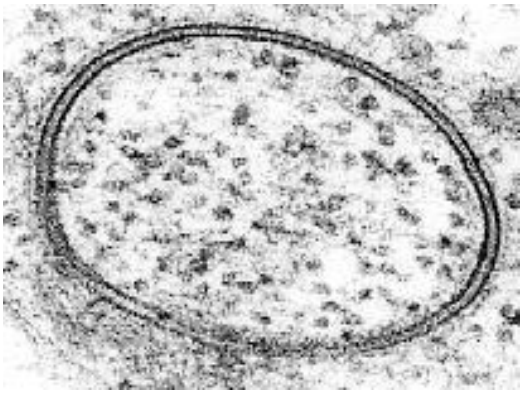




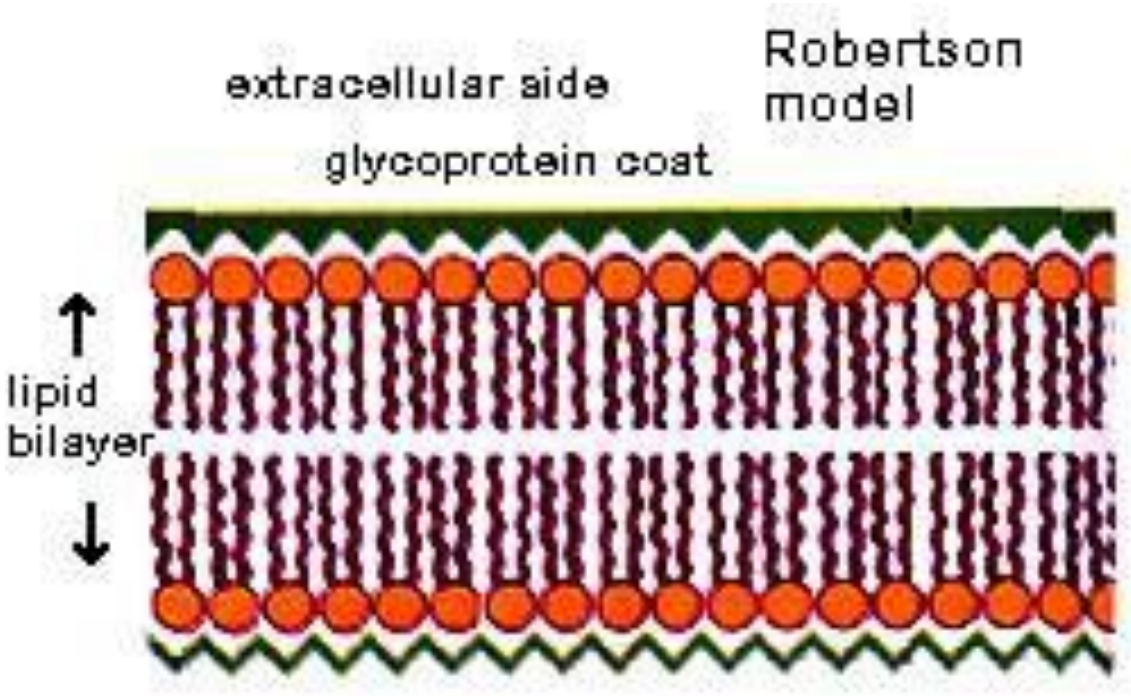
Danielli-Davson model.

2- Robertson's model (unit membrane model)

In 1965, **Robertson** noted the structure of membranes seen in the electron micrographs. He saw no spaces for pores in the electron micrographs and hypothesized that the railroad track appearance came from the binding of osmium tetroxide to proteins and polar groups of lipids. He proposed **unit membrane hypothesis**. Definite plasma membrane of 6 nm to 10 nm (10nm = 100 Å; 1 nm = 10_6mm) thickness was observed on surface of all cells, and plasma membranes of two adjacent cells were found to be separated by a space, 1-15nm wide. It was also observed that the plasma membrane of most of the cells appeared to be three layered. Two outer dense layers were about 2.0nm thick and the middle layer about 3.5nm.



A micrograph from a Transmission Electron Micrograph showing a lipid vesicle.



Robertson's model.

3- Fluid Mosaic Model

In **1972**, **Singer** and **Nicolson** put forward the "**Fluid Mosaic Model**" of membrane structure in which a mosaic protein molecules floats in a fluid lipid bilayer.

This model is proposed that membrane is made up of lipid and protein, but the protein does not form a continuous layer covering both sides of the membrane as proposed by **Danielli** and **Davson**.

In mosaic model the protein molecules are either partially (**peripheral protein**) or wholly embedded (**integral protein**). Some of these proteins that float, consist of pores that allow the passage of particular molecules or ions through the membrane. In absence of these pores, the polar molecules could be difficult to cross the membrane.

According to this model, the membrane structure is not static, the lipid molecule linked to one another only by weak bond. The structure and arrangement of membrane proteins in the fluid-mosaic model differ from

that of previous models in that they occur as a “mosaic” of discontinuous particles that penetrate the lipid sheet (**Fig.13**). Most importantly, the fluid-mosaic model presents cellular membranes as dynamic structures in which the components are mobile and capable of coming together to engage in various types of transient or semi-permanent interactions.

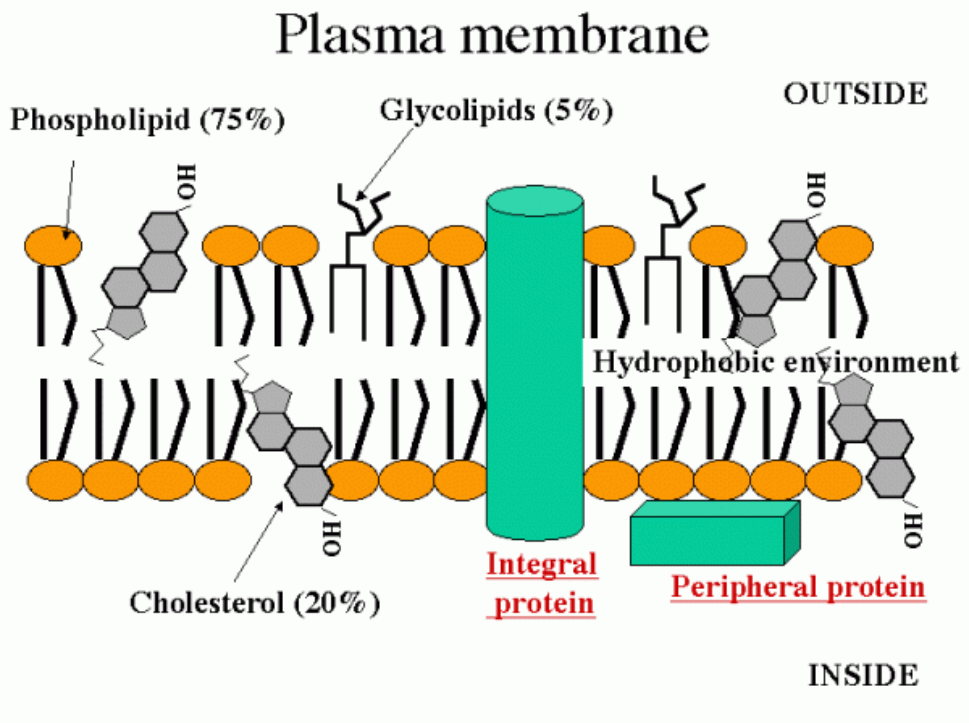


Fig.13: structure of fluid mosaic model membrane.

Chemical composition of plasma membrane

The membrane is mainly composed of lipids, proteins, and carbohydrates. Water makes about 29% of total weight. The ratio of lipid to protein in a membrane varies, depending on the type of cellular membrane. (Plasma vs. endoplasmic reticulum vs. Golgi), the type of organism (bacterium vs. plant vs. animal), and the type of cell (cartilage vs. muscle vs. liver).

1- **The lipids**:

Plasma membrane contains 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 (Phospholipids), sphingolipids and cholesterol (**Fig.14**). Glycerol and fatty acid constitute lipid molecules.

- Phospholipids (most abundant) – is the lipid which contains phosphate group.
- Sphingolipids - A less abundant class of membrane lipids, are derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain. If the substitution is a carbohydrate, the molecule is a **glycolipid**.
- Cholesterol – is close related to lipid, made up of steroid and alcohol. Cholesterol is absent from the plasma membranes of most plant and all bacterial cells.

Both phospholipids and glycolipids have polar head and non-polar tails. Cholesterol is slightly polar at one end.

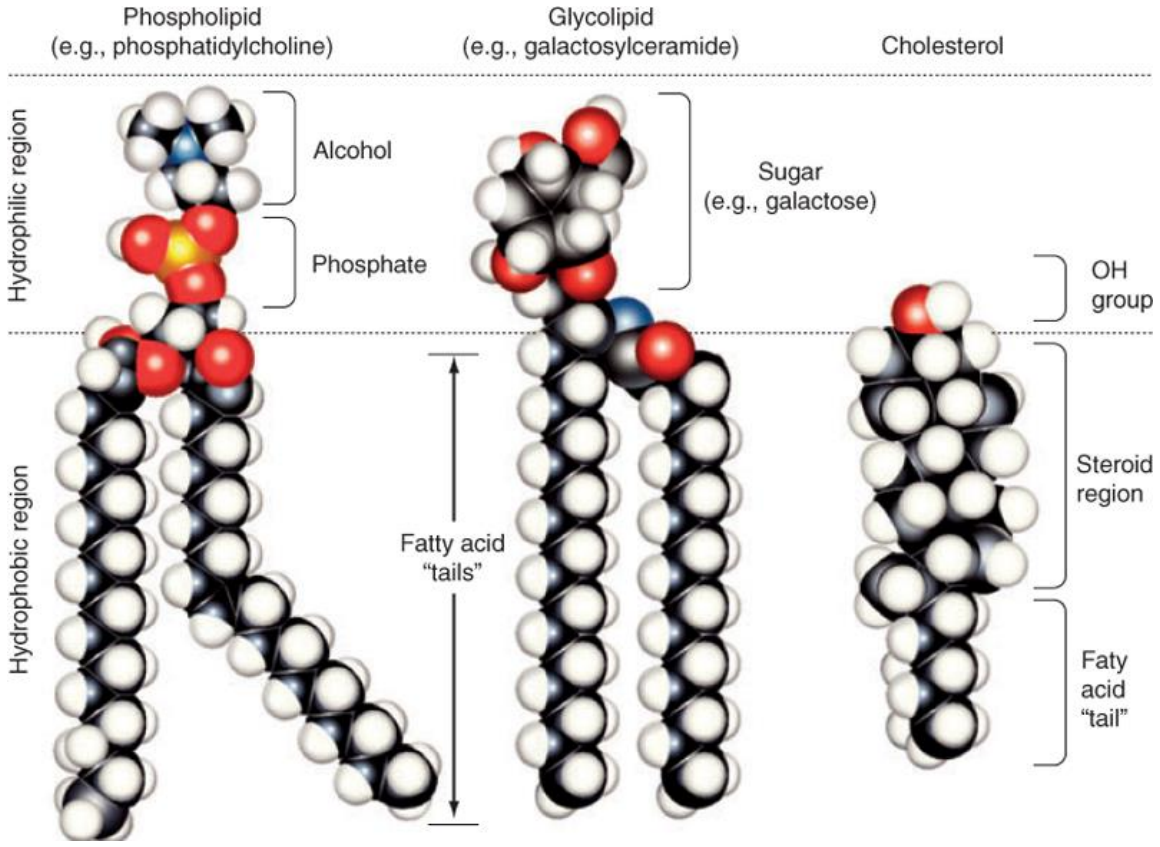


Fig.14: Types of lipids in plasma membrane.

The lipid molecules are oriented in two (bimolecular) layers with their hydrophilic polar ends directed towards protein and the hydrophobic

nonpolar ends face each other. Hydrogen bonds, ionic linkages or electrostatic forces bind the protein and lipid components.

2- **Proteins:** Plasma membrane contains about 50% protein. Amount and type are variable. Myelin cells contain about 25% proteins, internal membranes of chloroplast and mitochondria contain 50% protein. It can be grouped into three distinct classes distinguished by the intimacy of their relationship to the lipid bilayer. These are: -

I. **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.

II. **Peripheral proteins** that are located entirely outside of the lipid bilayer, on the cytoplasmic or extracellular side, yet are associated with the surface of the membrane by noncovalent bonds**.**

III. **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.

Also, according to their function, the proteins can be grouped into structural proteins, transport proteins and enzymes. Some of them act as receptors.

3- Carbohydrates

The plasma membranes of eukaryotic cells contain carbohydrates that are covalently linked to both lipid and protein components Depending on the species and cell type, the carbohydrate content of the plasma membrane ranges between 2 and 10 percent by weight. More than 90 % of the membrane’s carbohydrate is covalently linked to proteins to form

glycoproteins; the remaining carbohydrate is covalently linked to lipids to form glycolipids (**Fig.15**).

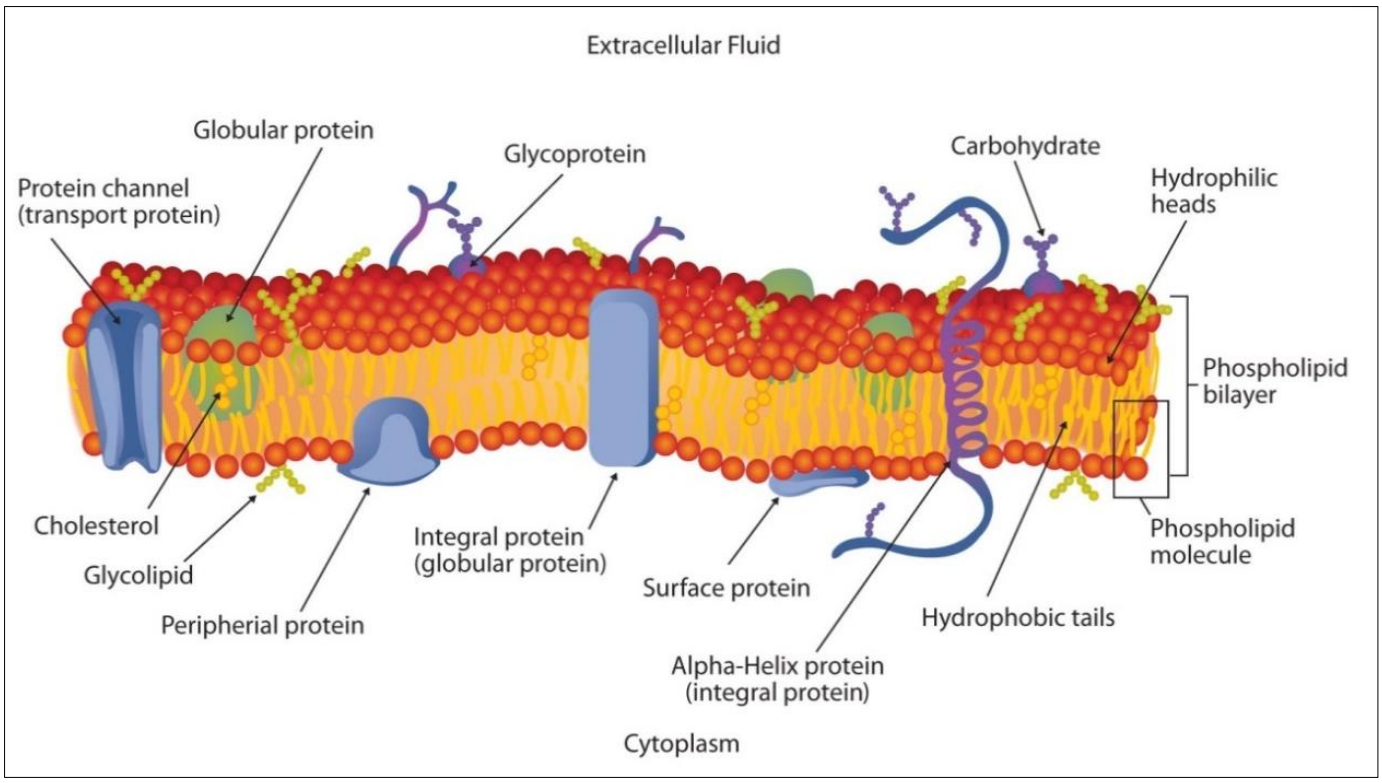


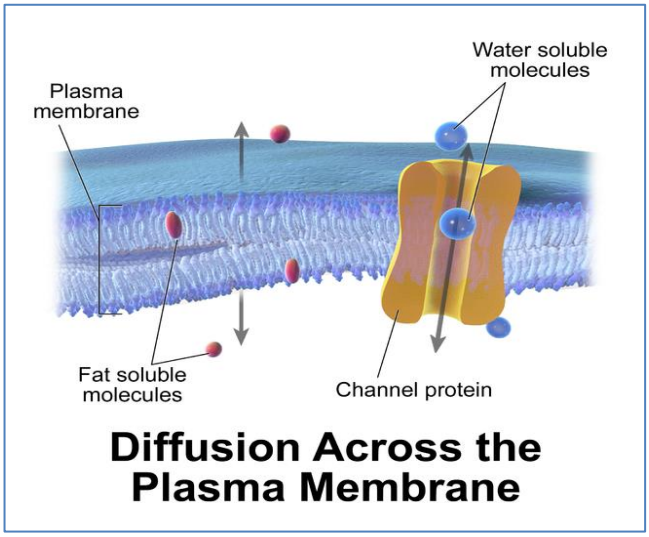
Fig.15: fluid mosaic model.

The Movement of Substances across Cell Membranes

- The cell membrane is selectively permeable and able to regulate what enters and exits the cell, thus facilitating the transport of materials needed for survival.
- The movement of substances across the membrane can be either "**passive**", occurring without the input of cellular energy, or "**active**", requiring the cell to expend energy in transporting it.
- The membrane also maintains the cell potential. The cell membrane thus works as a selective filter that allows only certain things to come inside or go outside the cell. The cell employs a number of transport mechanisms that involve biological membranes:

- **Types of cellular transport**

1. ***Passive osmosis and diffusion***: Some substances (small molecules, ions) such as carbon dioxide (CO_2) and oxygen (O_2), can move across the plasma membrane by diffusion, which is a passive transport process. Because the membrane acts as a barrier for certain molecules and ions, they can occur in different concentrations on the two sides of the membrane. Such a concentration gradient across a semipermeable membrane sets up an osmotic flow for the water.



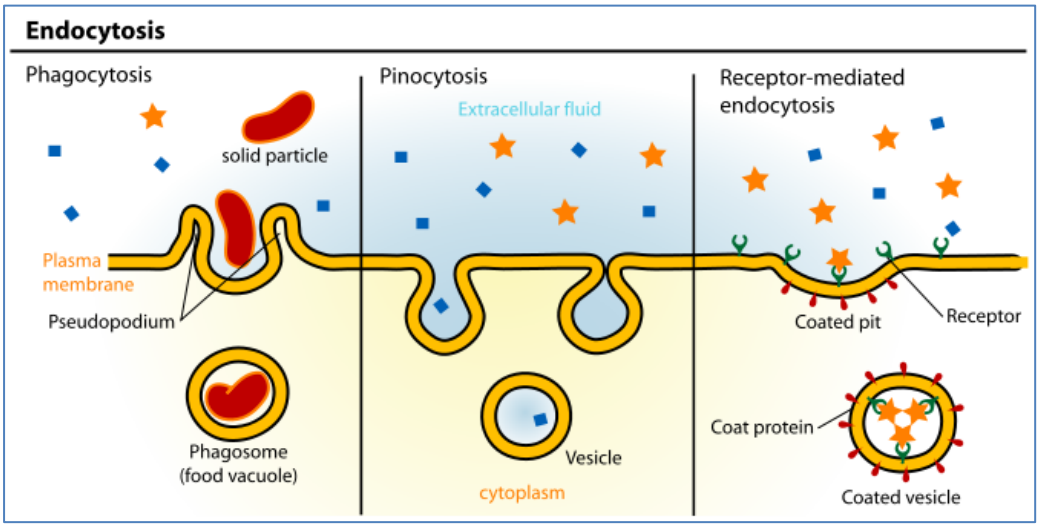
2. ***Transmembrane protein channels and transporters***

Nutrients, such as sugars or amino acids, must enter the cell, and certain products of metabolism must leave the cell. Such molecules diffuse passively through protein channels in facilitated diffusion or are pumped across the membrane by transmembrane transporters. Protein channel proteins, also called permeases, are usually quite specific, recognizing and transporting only a limited food group of chemical substances, often even only a single substance.

3. ***Endocytosis***

Endocytosis is the process in which cells absorb molecules by engulfing them. The plasma membrane creates a small deformation inward, called an invagination, in which the substance to be transported is captured. The deformation then pinches off from the

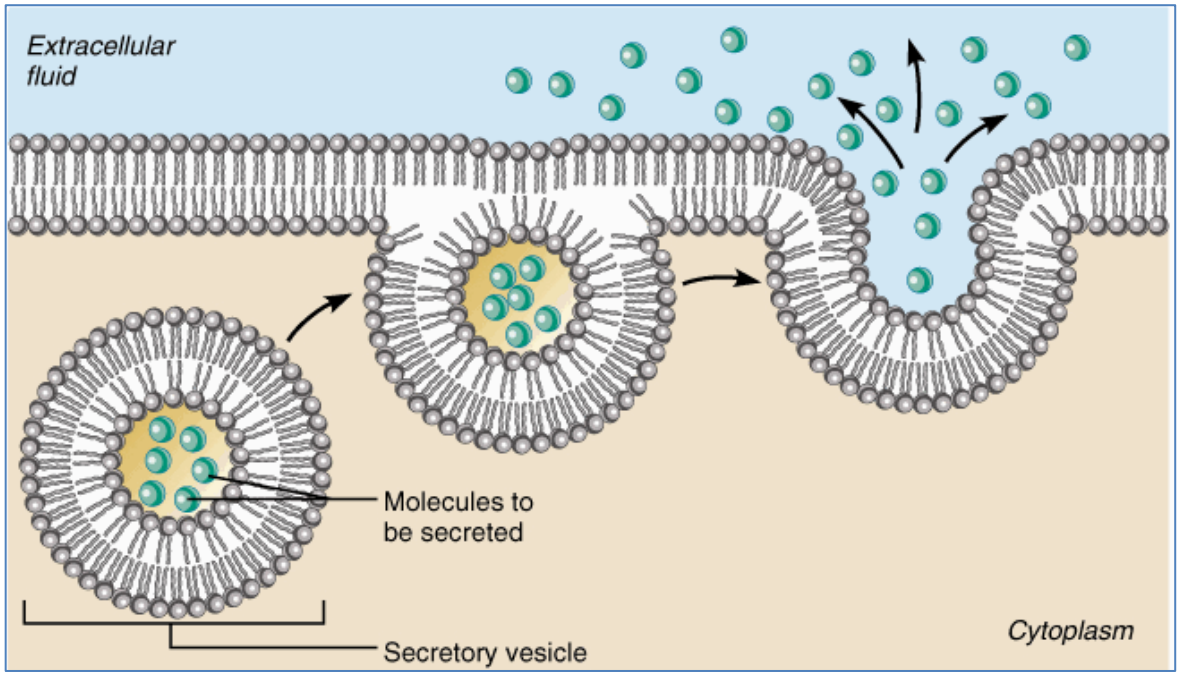
membrane on the inside of the cell, creating a vesicle containing the captured substance. Endocytosis is a pathway for internalizing solid particles ("cell eating" or phagocytosis), small molecules and ions ("cell drinking" or pinocytosis), and macromolecules. Endocytosis requires energy and is thus a form of active transport. Receptor-mediated endocytosis is a process by which cells internalize molecules (endocytosis) by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being internalized. Coat proteins of the vesicle signals proteins of specific organelles in the cell, which allow the direct transmission of specific internal molecules be delivered directly to the organelles that require them.



4. Exocytosis

Just as material can be brought into the cell by invagination and formation of a vesicle, the membrane of a vesicle can be fused with the plasma membrane, extruding its contents to the surrounding medium. This is the process of exocytosis. Exocytosis occurs in various cells to remove undigested residues of substances brought in by endocytosis, to secrete substances such as hormones and enzymes, and to transport a substance completely across a cellular barrier.

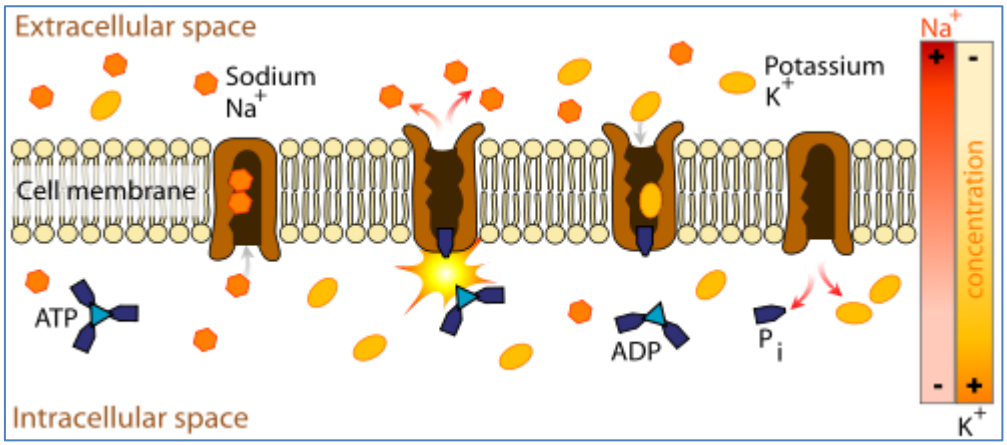
In the process of exocytosis, the undigested waste-containing food vacuole or the secretory vesicle budded from Golgi apparatus, is first moved by cytoskeleton from the interior of the cell to the surface. The vesicle membrane comes in contact with the plasma membrane. The lipid molecules of the two bilayers rearrange themselves and the two membranes are, thus, fused. A passage is formed in the fused membrane and the vesicles discharges its contents outside the cell.



5. *Active Transport*

Active transport is the movement of molecules across a cell membrane in the direction against their concentration gradient, going from a low concentration to a high concentration. Active transport is usually associated with accumulating high concentrations of molecules that the cell needs, such as ions, glucose and amino acids. If the process uses chemical energy, such as from adenosine triphosphate (ATP), it is termed primary active transport. Secondary active transport involves the use of an electrochemical gradient. Active transport uses cellular energy, unlike passive transport, which does

not use cellular energy. Active transport is a good example of a process for which cells require energy.



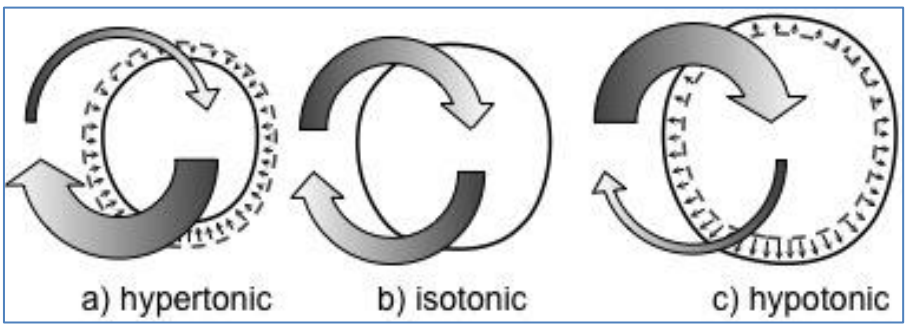
❖ Why are cells so small?

Cells are so small that you need a microscope to examine them. Why? To answer this question, we have to understand that, in order to survive, cells must constantly interact with their surrounding environment. Gases and food molecules dissolved in water must be absorbed and waste products must be eliminated. For most cells, this passage of all materials in and out of the cell must occur through the plasma membrane. Each internal region of the cell has to be served by part of the cell surface. As a cell grows bigger, its internal volume enlarges, and the cell membrane expands. Unfortunately, the volume increases more rapidly than does the surface area, and so the relative amount of surface area available to pass materials to a unit volume of the cell steadily decreases. Finally, at some point, there is just enough surface available to service all the interior; if it is to survive, the cell must stop growing. The important point is that the surface area to the volume ratio gets smaller as the cell gets larger. Thus, if the cell grows beyond a certain limit, not enough material will be able to cross the membrane fast enough to accommodate the increased cellular volume. When this happens, the cell must divide into smaller cells with

favorable surface area/volume ratios or cease to function. That is why cells are so small.

❖ Osmosis across a semi-permeable membrane

Osmosis is the diffusion of water from high concentration to low concentration. When you drink water, your cells have a lower concentration of water than the water in your digestive system. So, water flows across the cell membrane (from high concentration to low concentration) of your cells hydrating you. Thirst is our bodies way of maintaining an osmotic balance of water. In this balance, water is entering the cell at basically the same rate as it is leaving the cell, and the cell is said to be in an *isotonic* state (Fig. b). If you drink too much water, the concentration of water is much higher on the outside of your cells and enter into the cell causing it to stretch and is said to be in a *hypotonic* state (Fig. c). This is rare in humans but has occured most commonly in endurance athletes consuming more water than their body needed to maintain osmotic balance. Water can also leave the cell in greater abundances than water enters, causing it to shrink in a condition known as a *hypertonic* state (Fig. a). We see this in plants that have not received adequate watering. When this happens, water moves from high concentration on the inside of the cell to lower concentrations out of the cell. This causes the plant's cells to shrink and the plant wilts.



Functions of the Cell (Plasma) Membrane

1. It separates the contents of the cell from their external environment.
2. It controls the exchange of materials between the cell and the surrounding. e.g.: gases.
3. It acts as the site for metabolic reactions such as energy production in mitochondria and also enzymes attached to the plasma membrane.
4. Acts as a receptor site for recognizing of hormones, neurotransmitter and other chemicals.
5. The membrane protein sometimes acts as an enzyme, for-example; the microvilli on epithelial cells lining some parts of the gut contains digestive enzymes in their cell surface membrane.
6. It contains glycoprotein which acts as cell identity markers, hence enables the cell to recognize other cells and to behave in an organized way. For example, during the formation of tissue or organ in multicellular organisms.
7. It allows transportation of materials such as water, food materials and waste substances.

Cell Wall

Cell wall is the thick, rigid, non-living, semi-elastic, transparent, specialized form of protective extra-cellular matrix that present outside the plasmalemma (plasma membrane) of cells.

It found in plant cells, fungal cells, some protists and prokaryotes except a few lower plants, gametes and in animal cells. The thickness varies from 0.1 to 10/µm and xylem vessels have thickest cell wall, while thinnest cell wall found in meristematic and parenchymatous cells.

The wall formed during cell division of plants is called the **primary wall** which is later thickened to become a **secondary wall**. The primary wall consists of cellulose fibrils running through a matrix of other polysaccharides. Cellulose is a polysaccharide which has a high tensile strength which approaches that of steel.

The matrix consists of pectin and hemicellulose. Pectin are acidic and have a relatively a solubility. **The middle lamella** that hold neighboring cell walls together is composed of sticky gel-like magnesium and calcium salts of pectin.

Hemicelluloses are mixed groups of alkali soluble polysaccharides which form less organized, shorter and more branched chain like molecules.

About 60%-70% of mass of cell wall is water which can move freely through free space in the cell wall.

Wall Components – Chemistry

The main ingredient in cell walls is polysaccharides (or complex carbohydrates or complex sugars) which are built from monosaccharides (or simple sugars). Eleven different monosaccharides are common in these polysaccharides including glucose and galactose. Carbohydrates are good building blocks because they can produce a nearly infinite

variety of structures. There are a variety of other components in the wall including protein, and lignin. Let's look at these wall components in more detail:

A. Cellulose

Cellulose is the chief constituent of plant cell wall. Each cellulose chain (1 -5/µm long) 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 µm in diameter, may reach, 4/µm in length). A cotton fiber 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 (**Fig. 5**)

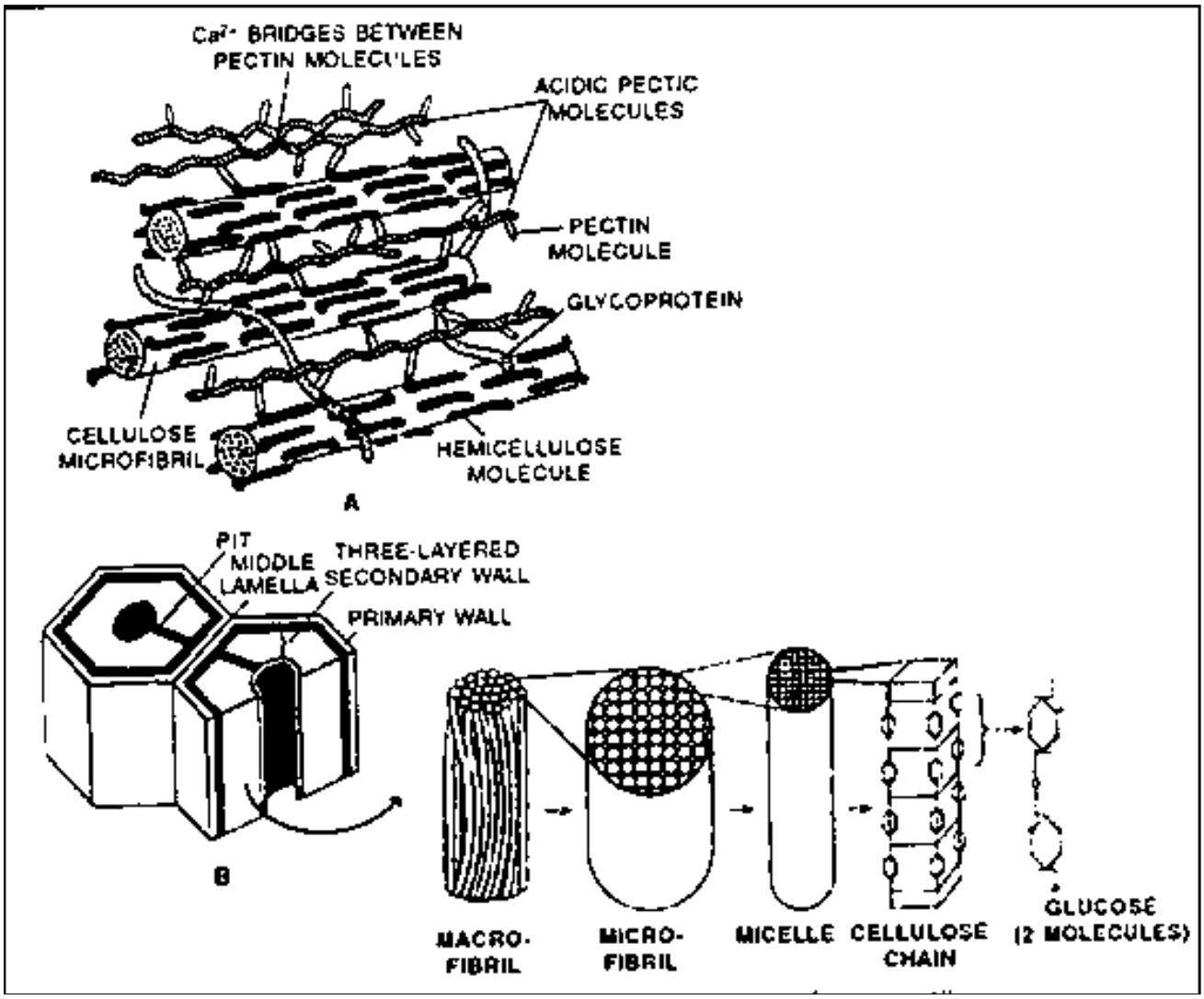


Fig.5: structure of cell wall (A) ultrastructure of primary wall (B) the detailed structure of cell wall.

Micro fibrils are synthesized on the plasma membrane by protein complexes called particle rosettes. Matrix contains a glycoprotein called expansin which causes the loosening and expansion of cell wall by the addition of cellulose molecules to the micro fibrils.

B. Cross-linking glycans (=Hemicellulose)

They are branched polysaccharides that structurally homologous to cellulose because they have a linear (straight), flat backbone composed of 1,4-linked β-D-hexosyl residues. The predominant hemicellulose in many primary walls is xyloglucan. Other hemicelluloses found in primary

and secondary walls include glucuronoxylan, arabinoxylan, glucomannan, and galactomannan. They characterized by being soluble in strong alkali.

The main feature of this group is that they don't aggregate with themselves - in other words, they don't form microfibrils. However, they form hydrogen bonds with cellulose and hence the reason they are called "***cross-linking glycans***". There may be a focused sugar at the end of the side chains which may help keep the molecules planar by interacting with other regions of the chain.

C. Pectic polysaccharides

These are extracted from the wall with hot water or dilute acid or calcium chelates (like EDTA). They are the easiest constituents to remove from the wall. They form gels (*i.e.,* used in jelly making). They are also a diverse group of polysaccharides and are particularly rich in galacturonic acid (galacturonans = pectic acids). They are polymers of primarily β 1,4 galacturonans (=polygalacturonans) are called homogalacturons (HGA) and are particularly common. These are helical in shape. Divalent cations, like calcium, also form cross-linkages to join adjacent polymers creating a gel. Pectic polysaccharides can also be cross-linked by dihydrocinnamic or diferulic acids. The HGA's (galacturonans) are initially secreted from the Golgi as methylated polymers; the methyl groups are removed by pectin methylesterase to initiate calcium binding.

Although most pectic polysaccharides are acidic, others are composed of neutral sugars including arabinans and galactans. The pectic polysaccharides serve a variety of functions including determining wall porosity, providing a charged wall surface for cell-cell adhesion - or in other words gluing cells together (i.e., middle lamella), cell-cell recognition, pathogen recognition and others.

D. Protein

Wall proteins are typically glycoproteins (polypeptide backbone with carbohydrate side chains). The proteins are particularly rich in the amino acids hydroxyproline (hydroxyproline-rich glycoprotein, HPRG), proline (proline-rich protein, PRP), and glycine (glycine-rich protein, GRP). These proteins form rods (HRGP, PRP) or beta-pleated sheets (GRP). ***Extensin*** is a well-studied HRGP. HRGP is induced by wounding and pathogen attack. The wall proteins also have a structural role since: (1) the amino acids are characteristic of other structural proteins such as collagen; and (2) to extract the protein from the wall requires destructive conditions. Protein appears to be cross-linked to pectic substances and may have sites for lignification. The proteins may serve as the scaffolding used to construct the other wall components.

Another group of wall proteins are heavily glycosylated with arabinose and galactose. These arabinogalactan proteins, or AGP's, seem to be tissue specific and may function in cell signaling. They may be important in embryogenesis and growth and guidance of the pollen tube.

E. Lignin

Polymer of phenolics, especially phenylpropanoids. Lignin is primarily a strengthening agent in the wall. It also resists fungal/pathogen attack.

F. Suberin, wax, cutin

A variety of lipids are associated with the wall for strength and waterproofing.

G. Water

The wall is largely hydrated and comprised of between 75-80% water. This is responsible for some of the wall properties. For example, hydrated walls have greater flexibility and extensibility than non-hydrated walls.

Morphology of the Cell Wall

There are three major regions of the wall:

1. **Middle lamella** - outermost layer, glue that binds adjacent cells, composed primarily of pectic polysaccharides.
2. **Primary wall** - wall deposited by cells before and during active growth. The primary wall of cultured sycamore cells is comprised of pectic polysaccharides (ca. 30%), cross-linking glycans (hemicellulose; ca 25%), cellulose (15-30%) and protein (ca. 20%). The actual content of the wall components varies with species and age. All plant cells have a middle lamella and primary wall.
3. **Secondary Wall** - some cells deposit additional layers inside the primary wall. This occurs after growth stops or when the cells begin to differentiate (specializes). The secondary wall is mainly for support and is comprised primarily of cellulose and lignin. Often can distinguish distinct layers, S1, S2 and S3 - which differ in the orientation, or direction, of the cellulose microfibrils (**Fig.6**).

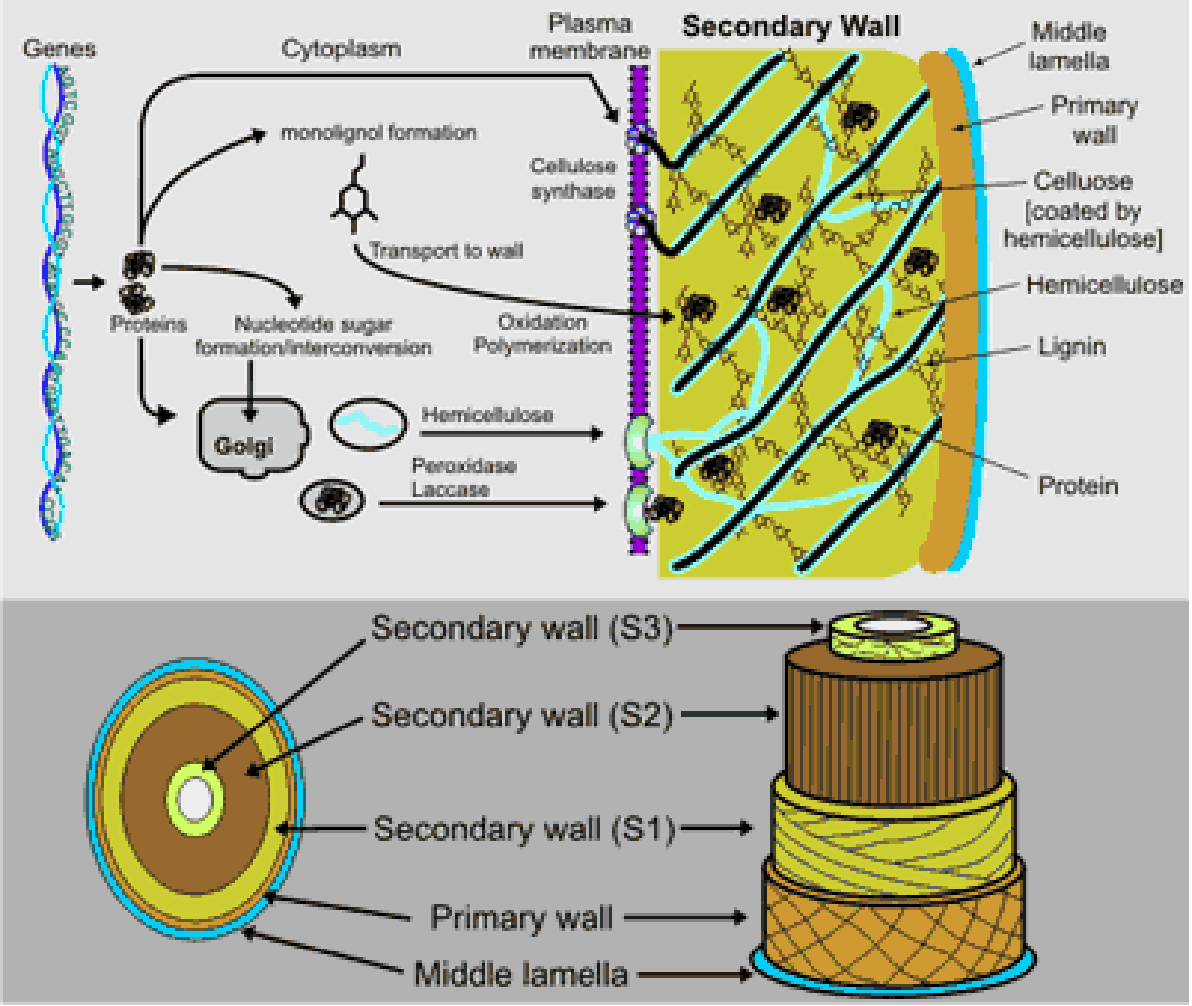


Fig.6: the structure of secondary cell wall.

The secondary walls of xylem fibers, tracheids, and sclereids are further strengthened by the incorporation of lignin.

The evolution of conducting tissues with rigid secondary cell walls was a critical adaptive event in the history of land plants, as it facilitated the transport of water and nutrients and allowed extensive upright growth. Secondary walls also have a major impact on human life, as they are a major component of wood and are a source of nutrition for livestock. In addition, secondary walls may help to reduce our dependence on petroleum, as they account for the bulk of renewable biomass that can be converted to fuel. Nevertheless, numerous technical challenges must be overcome to enable the efficient utilization of secondary walls for energy production and for agriculture.

Wall Formation

The cell wall is made during cell division when the cell plate is formed between daughter cell nuclei. The cell plate forms from a series of vesicles produced by the Golgi apparatus. The vesicles migrate along the cytoskeleton and move to the cell equator. The vesicles coalesce and dump their contents. The membranes of the vesicle become the new cell membrane. The Golgi synthesizes the non-cellulosic polysaccharides. At first, the Golgi vesicles contain mostly pectic polysaccharides that are used to build the middle lamella. As the wall is deposited, other non-cellulosic polysaccharides are made in the Golgi and transported to the growing wall.

Cellulose is made at the cell surface. The process is catalyzed by the enzyme cellulose synthase that occurs in a rosette complex in the membrane. Cellulose synthase, which is initially made in by the ribosomes (rough ER) and move from the ER → vesicles → Golgi → vesicle → cell membrane. The enzyme apparently has two catalytic sites that transfer two glucoses at a time (*i.e.*, cellobiose) from UDP-glucose to the growing cellulose chain. Sucrose may supply the glucose that binds to the UDP. Wall protein is presumably incorporated into the wall in a similar fashion.

Intracellular Communication: Pits and Plasmodesmata

With all this layering, how do the cells "talk" to one another?

- Primary cell walls have thinner areas known as **primary pit fields**.
- Pit fields contain small openings in the wall through which cytoplasmic extensions known as **plasmodesmata** (singular, plasmodesma) extend between cells.
- The plasmodesmata are bounded by plasma membrane along their length, and a single tube of endoplasmic reticulum, the **desmotubule** extends through each plasmodesma (**Fig.7**).

- When the cell constructs a secondary cell wall, it doesn't lay down secondary wall over the primary wall's pit fields.
- This creates perforations in the secondary wall called **pits**. (Sometimes a pit is formed even if there's no pit field.)
- Pits of adjacent cells are usually compressed to each other so that the two primary cell wall and middle lamella form a selectively permeable **pit membrane**.

  Pit → primary wall → middle lamella → primary wall → pit

  ...comprises the pit pair through which the cells can transmit water, nutrients, hormones, etc.

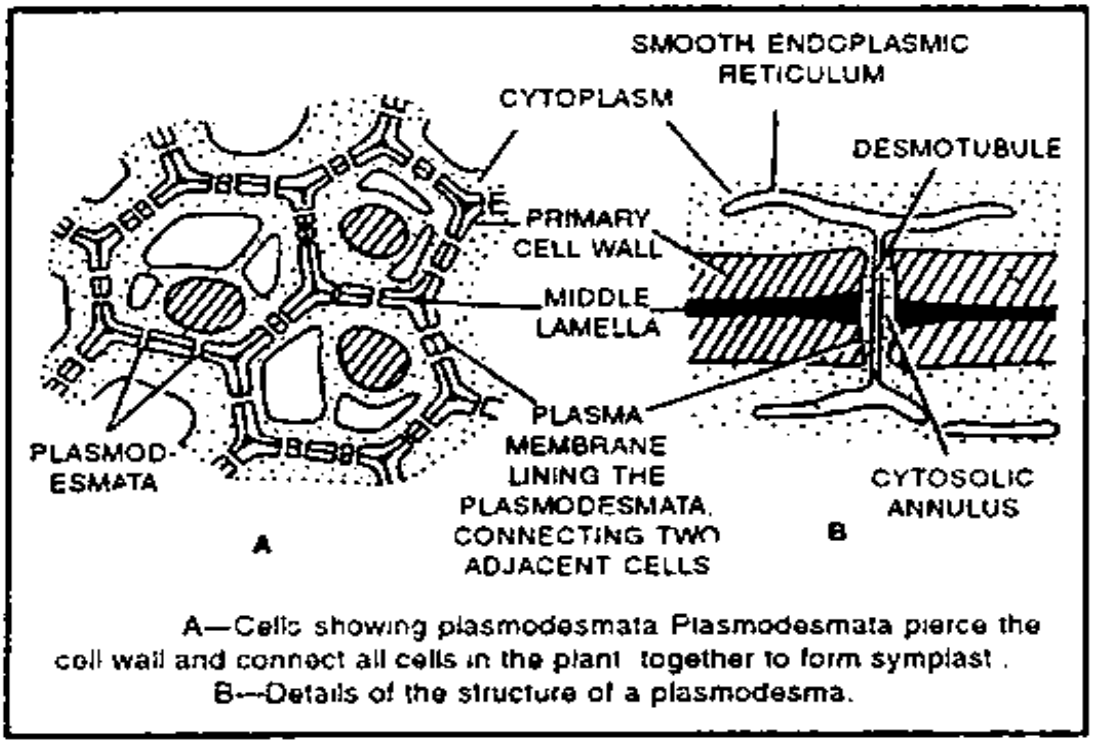


Fig.7: The structure of plasmodesmata

A pit consists of:

(i) **Pit chamber**, the actual hole within the secondary wall;

(ii) **Pit membrane**, composed of middle lamella and primary walls between two adjacent pits; and

(iii) **Pit aperture**, an opening that communicate pit chamber with the interior of the cell. Pit membrane is permeable like primary wall. Pits of adjacent cells usually occur opposite and form a pit pair. A pit present on

the free surface of cell without its corresponding partner is called blind pit (**Fig. 8, Fig.9**).

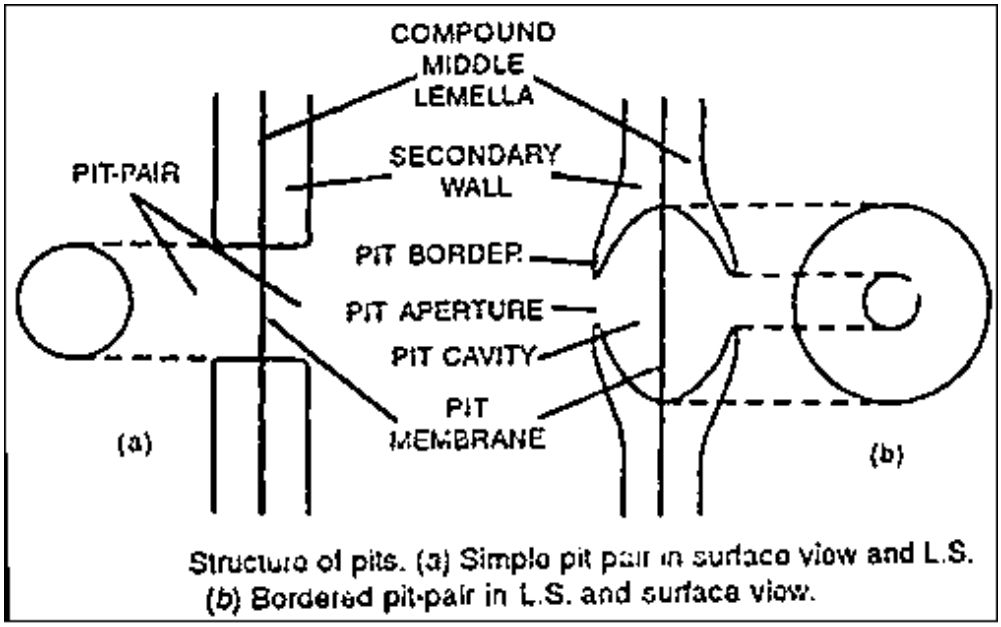


Fig.8: The structure of pits.

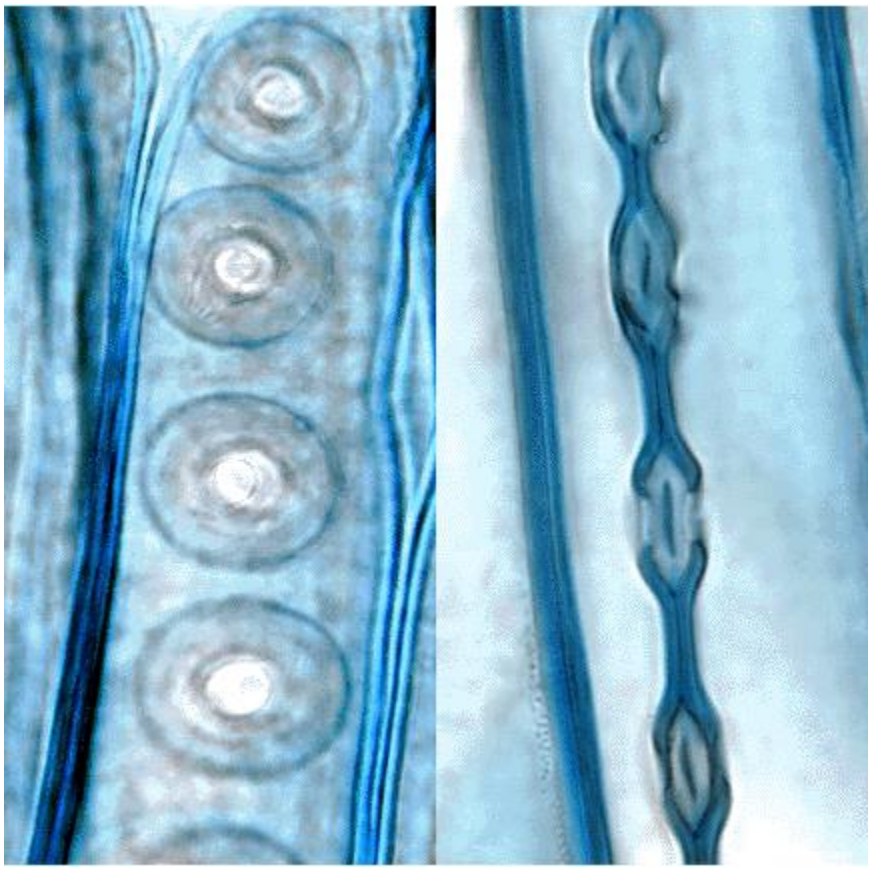


Fig.9: pits view under light microscope.

On the basis of the shape of pit chamber, pits are either simple or bordered. In **simple pit** the pit chamber has uniform width and appears

one ringed in surface view. In **bordered pit** the pit chamber is flask-shaped with narrow pit aperture and appears bordered on surface view. Sometimes the pit membrane bears a disc-like swelling called **torus**, and such pit is called **aspirated pit**.

Bacterial Cell Wall

Bacterial cell wall is made up of peptidoglycans also known as murein. The cell wall of bacteria is essential for the survival of bacteria.

Cell wall of bacteria is broadly classified into two types: Gram positive and Gram negative (**Fig.10**). The names are given to the reaction of the cells to Gram staining. This experiment is employed for the classification of bacterial species.

The Gram positive bacteria have a thick cell wall and are made up of many layers of peptidoglycan and teichoic acids.

The Gram negative bacteria have thinner cell walls, and are made up of few layers of peptidoglycans and are surrounded by a lipid membrane containing lipopolysaccharides and lipoproteins.

Fungi Cell Wall

Fungi cell wall consists of chitin and other polysaccharides (**Fig.10**). They do not have cellulose in their cell walls. Species of fungi that possess a cell wall have a plasma membrane and three layers of cell wall material surrounding it. These layers are made up of chitin, glucans and a layer of mannoproteins (mannose containing glycoproteins).

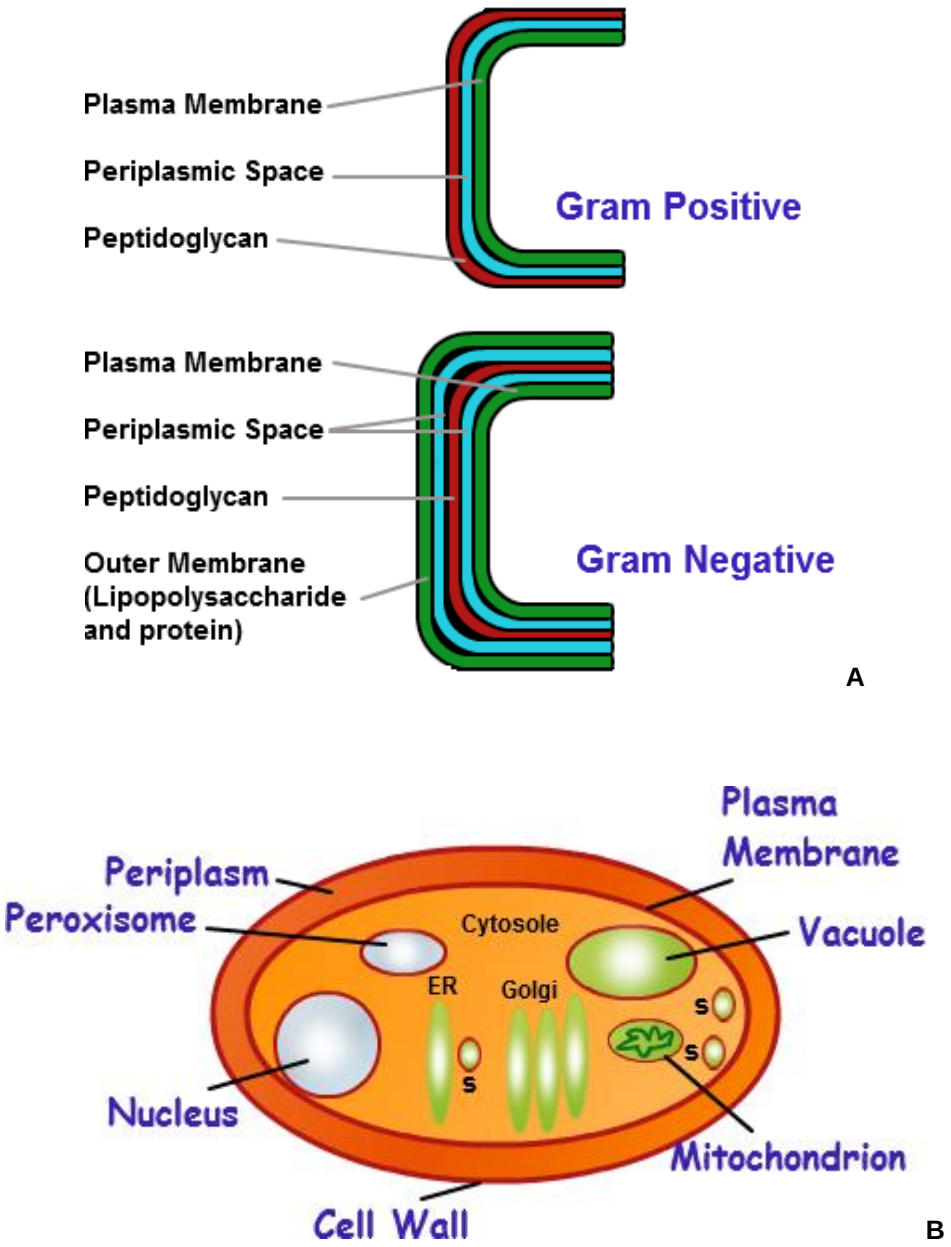


Fig.10: The structure of bacterial (a) and fungal (b) cell wall.

Functions of Cell Wall

1. It provides mechanical and skeletal support for individual cells and for the plant as a whole.
2. It allows development of turgidity when water enters the cell by osmosis since it is fairly rigid and resistant to expansion.
3. It prevents the cell from bursting when exposed to a dilute solution (hypotonic medium i.e., resists water pressure).
4. It limits and helps to control cell growth and shape since the cell's ability to stretch is determined by concentration of cellulose microfibrils.
5. It acts as a major pathway for the movement of water (**apoplast**).
6. Cell walls develop a coating of waxy cutin (cuticle) which reduces water loss and risks of infections.
7. The cell walls of root endodermal cells are impregnated with suberin that forms a banner to water movements.
8. The wall of xylem vessels and sieve tubes are adapted for a long distance translocation of materials through the cells.
9. Some cell walls are modified as food reserves as in storage of hemicellulose in some seeds.
10. Has a metabolic role (i.e., some of the proteins in the wall are enzymes for transport, secretion).
11. Recognition responses - for example: (a) the wall of roots of legumes is important in the nitrogen-fixing bacteria colonizing the root to form nodules; and (b) pollen-style interactions are mediated by wall chemistry.
12. Economic products - cell walls are important for products such as paper, wood, fiber, energy, shelter, and even roughage in our diet.

Cytoplasm

In **cell biology**, the cytoplasm is all of the material within a cell, enclosed by the cell membrane, except for the cell nucleus. The material inside the nucleus and contained within the nuclear membrane is termed the nucleoplasm. Cytoplasm is an aqueous substance containing a variety of cell organelles and other structures such as insoluble wastes and storage products.

The soluble part of the cytoplasm forms the ‘background material’ or ‘ground substances’ between the cell organelles. It contains about 90% water and forms a solution which contains all the fundamental biochemicals of life. Some of these are ions and small molecules in true solution; others are large molecules such as proteins which form colloidal solutions. Movement of calcium ions in and out of the cytoplasm is a signaling activity for metabolic processes. In plants the movements of the cytoplasm around the vacuoles, this is known as **cytoplasmic streaming**.

The three major elements of the cytoplasm are the cytosol, organelles and inclusions. **Cytosol** is the part of the cytoplasm that is not held by any of the organelles in the cell. On the other hand, cytoplasm is the part of the cell which is contained within the entire cell membrane. It is the total content within the cell membrane other than the contents of the nucleus of the cell. All the cell organelles in eukaryotic cells are contained within the cytoplasm.

Cytoplasmic inclusions are small particles of insoluble substances suspended in the cytosol. A huge range of inclusions exist in different cell types and range from crystals of calcium oxalate or silicon dioxide in plants, to granules of energy-storage materials such as starch, glycogen, or polyhydroxy butyrate.

The central, granular mass in the cytoplasm is the **endoplasm** while the surrounding lucid layer is known as the cell cortex or the **ectoplasm**.

Function of Cytoplasm

1. Cytoplasm is the site of many biochemical reactions that are vital and crucial for maintaining life.
2. The cytoplasm is the place where the cell expands and growth of the cell takes place.
3. The cytoplasm provides a medium for the organelles to remain suspended.
4. The cytoskeleton of the cytoplasm provides shape to the cell, and it also facilitates movement.
5. It also aids in the movement of the different cellular elements.
6. The enzymes in the cytoplasm metabolize the macromolecules into small parts, so that it can be easily available for the other cellular organelles like mitochondria.
7. It also transports the products of cellular respiration.
8. The cytoplasmic inclusions are non-soluble molecules, they are seen floating in the cytoplasm, and they act as stored fats and sugars that are ready for cellular respiration.
9. The cytoplasm and the proteins prevent the grouping of organelles in place due to gravity that would impede their function.

Vacuole

A vacuole is fluid filled sac bounded by a single membrane. Animal cells contain relatively small vacuoles, such as phagocytic vacuoles, food vacuoles, autophagic vacuoles and contractile vacuoles. Typically plant cells have one or two large vacuoles filled with fluid known as **cell sap** and surrounded by a membrane called **tonoplast**. The cell sap is a watery fluid containing water, sugar, organic acids, mineral salts, pigments and toxic substances (**Fig.16**).

The **tonoplast,** also called the **vacuolar membrane**, is mainly involved in regulating the movements of ions around the cell, and isolating materials that might be harmful or a threat to the cell. Transport of protons from the cytosol to the vacuole stabilizes cytoplasmic pH, while making the vacuolar interior more acidic creating a proton motive force which the cell can use to transport nutrients into or out of the vacuole. The low pH of the vacuole also allows degradative enzymes to act. Although single large vacuoles are most common, the size and number of vacuoles may vary in different tissues and stages of development. For example, developing cells in the meristems contain small provacuoles and cells of the vascular cambium have many small vacuoles in the winter and one large one in the summer.

Aside from storage, the main role of the central vacuole is to maintain turgor pressure against the cell wall. Proteins found in the tonoplast control the flow of water into and out of the vacuole through active transport, pumping potassium (K^+) ions into and out of the vacuolar interior. Due to osmosis, water will diffuse into the vacuole, placing pressure on the cell wall. If water loss leads to a significant decline in turgor pressure, the cell will plasmolyze. Turgor pressure exerted by vacuoles is also required for cellular elongation: as the cell wall is partially degraded by the action of expansions, the less rigid wall is expanded by the pressure coming from within the vacuole. Turgor

pressure exerted by the vacuole is also essential in supporting plants in an upright position.

Another function of a central vacuole is that it pushes all contents of the cell's cytoplasm against the cellular membrane, and thus keeps the chloroplasts closer to light. Most plants store chemicals in the vacuole that react with chemicals in the cytosol. If the cell is broken, for example by an herbivore, then the two chemicals can react forming toxic chemicals. In garlic, alliin and the enzyme alliinase are normally separated but form allicin if the vacuole is broken. A similar reaction is responsible for the production of syn-propanethial-S-oxide when onions are cut.

Functions of Vacuole

1. Water generally enters the concentrated cell sap by osmosis. Osmotic uptake of water is important in cell expansion during cell growth as well as in the normal water relations of plants.
2. The vacuole sometimes contains pigments in solution, e.g.: anthocyanin which are red, blue and purple and other related compounds which are yellow and ivory. They are responsible for colors in flowers, fruits, buds and leaves. They are important in attracting insects, birds and other animals for pollination and seed dispersal.
3. Plant vacuole sometimes contains hydrolytic enzymes and act as lysosomes. After cell death, the tonoplast loses its partial permeability and the enzymes escape causing autolysis.
4. Vacuoles contain waste products and certain secondary products of plants metabolism such as calcium oxalate, alkaloids and tannins which offer protection from consumption by herbivores.

5. Vacuole acts as a food storage organelle. It stores sucrose and mineral salts which can be utilized by the cytoplasm when necessary.
6. Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole.
7. In seeds, stored proteins needed for germination are kept in 'protein bodies', which are modified vacuoles.

Vacuoles also play a major role in **autophagy**, maintaining a balance between biogenesis (production) and degradation (or turnover), of many substances and cell structures in certain organisms. They also aid in the lysis and recycling of misfolded proteins that have begun to build up within the cell. **Thomas Boller** and others proposed that the vacuole participates in the destruction of invading bacteria and **Robert B Mellor** proposed organ-specific forms have a role in 'housing' symbiotic bacteria.

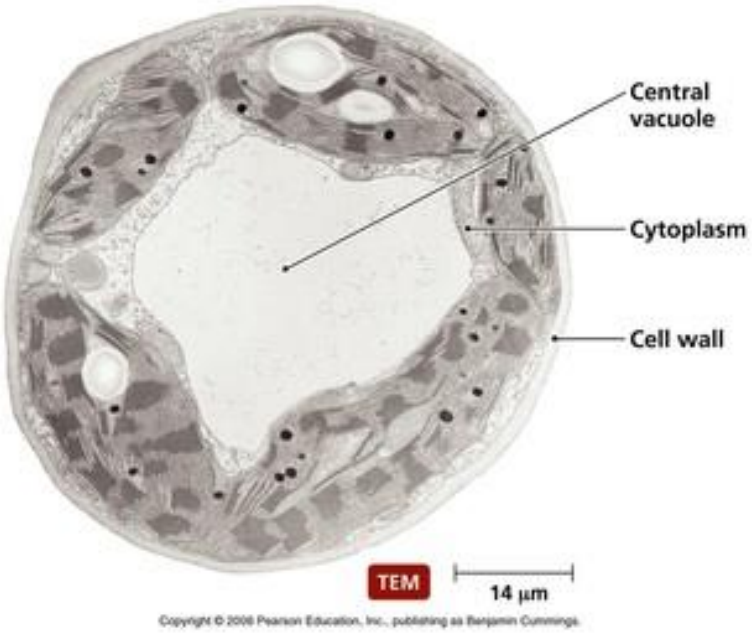


Fig.16: Photomicrograph of a plant cell showing the vacuole as seen under the TEM.

Endoplasmic Reticulum

The endoplasmic reticulum (ER) is a network of tubules and flattened sacs known as **cisternae** that serve a variety of functions in the cell.

There are two regions of the ER that differ in both structure and function (**Fig.17**). One region is called rough endoplasmic reticulum (RER) because it has ribosomes attached to the cytoplasmic side of the membrane. The other region is called smooth ER because it lacks attached ribosomes. Typically, the smooth ER is a tubule network, and the rough ER is a series of flattened sacs. The space inside of the ER is called the **lumen**. The ER is very extensive extending from the cell membrane through the cytoplasm and forming a continuous connection with the **nuclear envelope**. Since the ER is connected with the nuclear envelope, the lumen of the ER and the space inside the nuclear envelope are part of the same compartment.

Rough Endoplasmic Reticulum

The surface of the rough endoplasmic reticulum (often abbreviated RER or Rough ER) (also called ergastoplasm) is studded with protein-manufacturing ribosomes giving it a "rough" appearance (hence its name). The binding site of the ribosome on the rough endoplasmic reticulum is the **translocon**. However, the ribosomes bound to it at any one time are not a stable part of this organelle's structure as they are constantly being bound and released from the membrane. A ribosome only binds to the RER once a specific protein-nucleic acid complex forms in the cytosol. This special complex forms when a free ribosome begins translating the mRNA of a protein destined for the secretory pathway. The first 5-30 amino acids polymerized encode a signal peptide, a molecular message that is recognized and bound by a signal recognition particle (SRP). Translation pauses and the ribosome complex bind to the

RER translocon where translation continues with the nascent protein forming into the RER lumen and/or membrane.

The protein is processed in the ER lumen by an enzyme (a signal peptidase), which removes the signal peptide. Ribosomes at this point may be released back into the cytosol; however, non-translating ribosomes are also known to stay associated with translocons.

The membrane of the rough endoplasmic reticulum forms large double membrane sheets that are located near, and continuous with, the outer layer of the nuclear envelope. Although there is no continuous membrane between the endoplasmic reticulum and the **Golgi apparatus**, membrane-bound vesicles shuttle proteins between these two compartments. Vesicles are surrounded by coating proteins called COPI and COPII. COPII targets vesicles to the Golgi apparatus and COPI marks them to be brought back to the rough endoplasmic reticulum. The rough endoplasmic reticulum works in concert with the Golgi complex to target new proteins to their proper destinations.

A second method of transport out of the endoplasmic reticulum involves areas called membrane contact sites, where the membranes of the endoplasmic reticulum and other organelles are held closely together, allowing the transfer of lipids and other small molecules.

Smooth Endoplasmic Reticulum

The smooth ER has a wide range of functions including carbohydrate and lipid synthesis. It serves as a transitional area for vesicles that transport ER products to various destinations. In liver cells the smooth ER produces enzymes that help to detoxify certain compounds. In muscles the smooth ER assists in the contraction of muscle cells, and in brain cells it synthesizes male and female hormones.

In plants, rough and smooth ER are observed in differentiated cells. The sooth ER seems to be common in cells which are mainly concerned in steroid production and carbohydrate metabolism, with transport of

electrolytes as in the sieve elements and companion cells of *Cucurbit* phloem. On the other hand, the rough ER is highly developed in cells active in protein synthesis as in the cell of the glandular hairs of *Drosera* leaves.

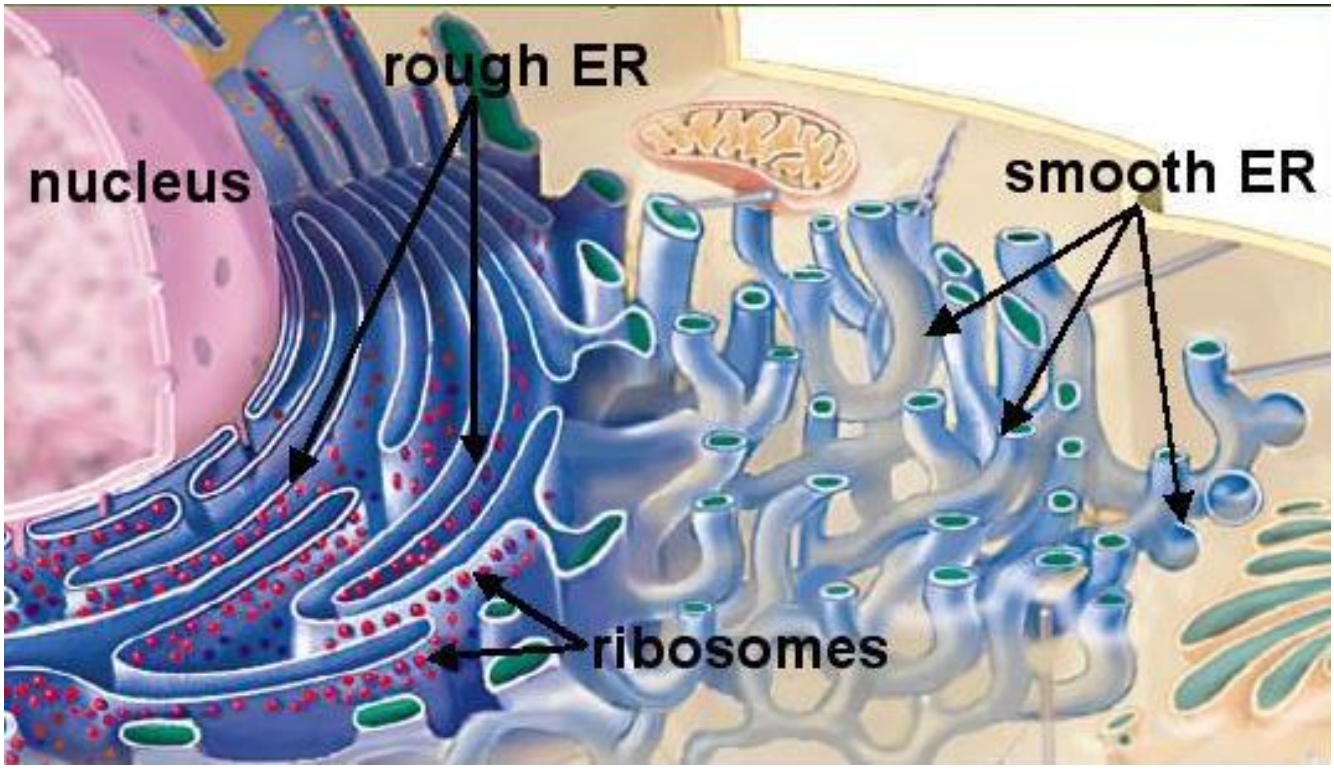


Fig.17: Rough and smooth endoplasmic reticulum.

Functions of Endoplasmic Reticulum

1. Rough endoplasmic reticulum concerned with the production and storage of protein molecules before they are used inside the cell or are secreted to the exterior.
2. They transport materials within the cell from one part to another.
3. S.E.R involved in lipids and steroid synthesis and storing.
4. The E.R provides surface or location for chemical reaction.
5. Producing and storing carbohydrates (S.E.R).

Ribosomes

Ribosomes were first observed in the mid-1950s by Romanian cell biologist **George Emil Palade** using an electron microscope as dense particles or granules for which, in 1974, he would win a Nobel Prize. The term "ribosome" was proposed by scientist **Richard B. Roberts** in 1958.

Ribosomes occur in both prokaryotic and eukaryotic cells. The ribosomes of prokaryotic cells are distinctly smaller (70's ribosomes) than those of eukaryotic cells (80's ribosomes).

The ribosome is responsible for the synthesis of proteins in cells and it serves to convert the instructions found in messenger RNA (mRNA, which itself is made from instructions in DNA) into the chains of amino-acids that make up proteins.

The ribosome is a cellular machine which is highly complex. It is made up of dozens of distinct proteins (the exact number varies a little bit between species) as well as a few specialized RNA molecules known as ribosomal RNA (rRNA).

Ribosomes are typically composed of two subunits: the **small ribosomal subunit**, which reads the RNA, and the **large subunit**, which joins amino acids to form a polypeptide chain. Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of proteins. The ribosomes and associated molecules are also known as the **translational apparatus** (**Fig.18 a&b**)**.**

Bacterial ribosomes are composed of one or two rRNA strands. Eukaryotic ribosomes contain one or three very large rRNA molecules and multiple smaller protein molecules.

Location of Ribosomes in the Cell

There are two places that ribosomes usually exist in the cell: suspended in the cytosol and bound to the endoplasmic reticulum. These ribosomes are called **free ribosomes** and **bound ribosomes**

respectively. In both cases, the ribosomes usually form aggregates called **polysomes** or **polyribosomes** during protein synthesis. Free ribosomes usually make proteins that will function in the cytosol (fluid component of the cytoplasm), while bound ribosomes usually make proteins that are exported from the cell or included in the cell's membranes. Interestingly enough, free ribosomes and bound ribosomes are interchangeable and the cell can change their numbers according to metabolic needs.

Components of Ribosomes

- Two subunits: large and small.
  - Prokaryotes: 50S + 30S = 70S
  - Eukaryotes: 60S + 40S = 80S.
- Prokaryotes: overall smaller
  - Large subunit contains two rRNAs and ~31 different proteins.
  - Small subunit contains one rRNAs and 21 different proteins.
- Eukaryotes: overall bigger
  - Large subunit contains three rRNAs and 45 proteins.
  - Small subunit consists of one rRNAs and 33 different proteins.

Synthesis of Ribosomes:

- In eukaryotes, rRNA synthesis and ribosome assembly takes place in the nucleolus.
- Before translation begins, the two ribosomal subunits exist as separate entities in the cytoplasm.
- Soon after the start of translation, they come together.

Functions of Ribosomes

1- They are the sites of polypeptide synthesis.

2- They recognize features that signal the start of translation.

3- They ensure the accurate interpretation of the genetic code by stabilizing the interaction between tRNA and the mRNA.

4- They supply the enzymatic activity that covalently links the amino acids in the polypeptide chain.

5- They facilitate the linear reading of the genetic code by sliding along the mRNA molecule.

Ribosomes: Role in translation

• The small subunit is the one that initially binds to the mRNA.

• The larger subunit provides the enzyme activity:

•Peptidyl transferase,

•catalyzes formation of peptide bonds joining amino acids

• The assembled structure of the ribosome creates three pockets for the binding of two molecules of tRNA.

•The far left pocket is the Exit site or E site

•It binds the deacylated tRNA (no amino acid attached)

• The one in the middle is referred to as the peptidyl or the P site: it binds to the tRNA holding the growing chain of polypeptide.

• The site on the right is termed the amino acyl, or the A site, it binds to the incoming tRNA molecule (**Fig. 19**).

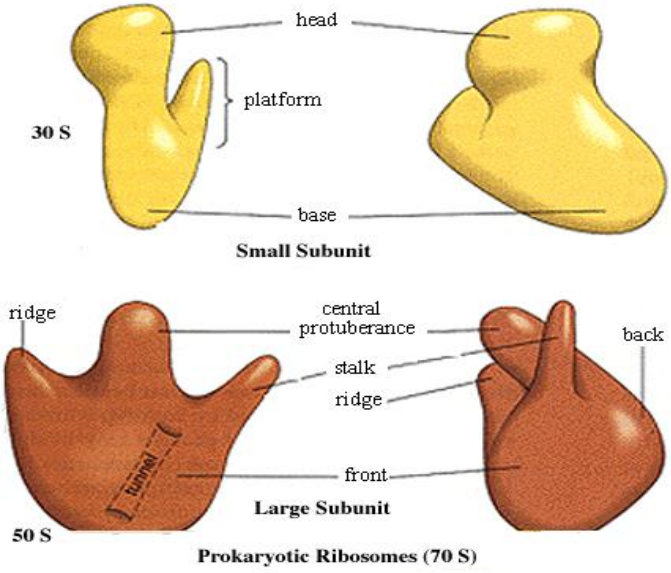


Fig.18a : Structure of ribosome.

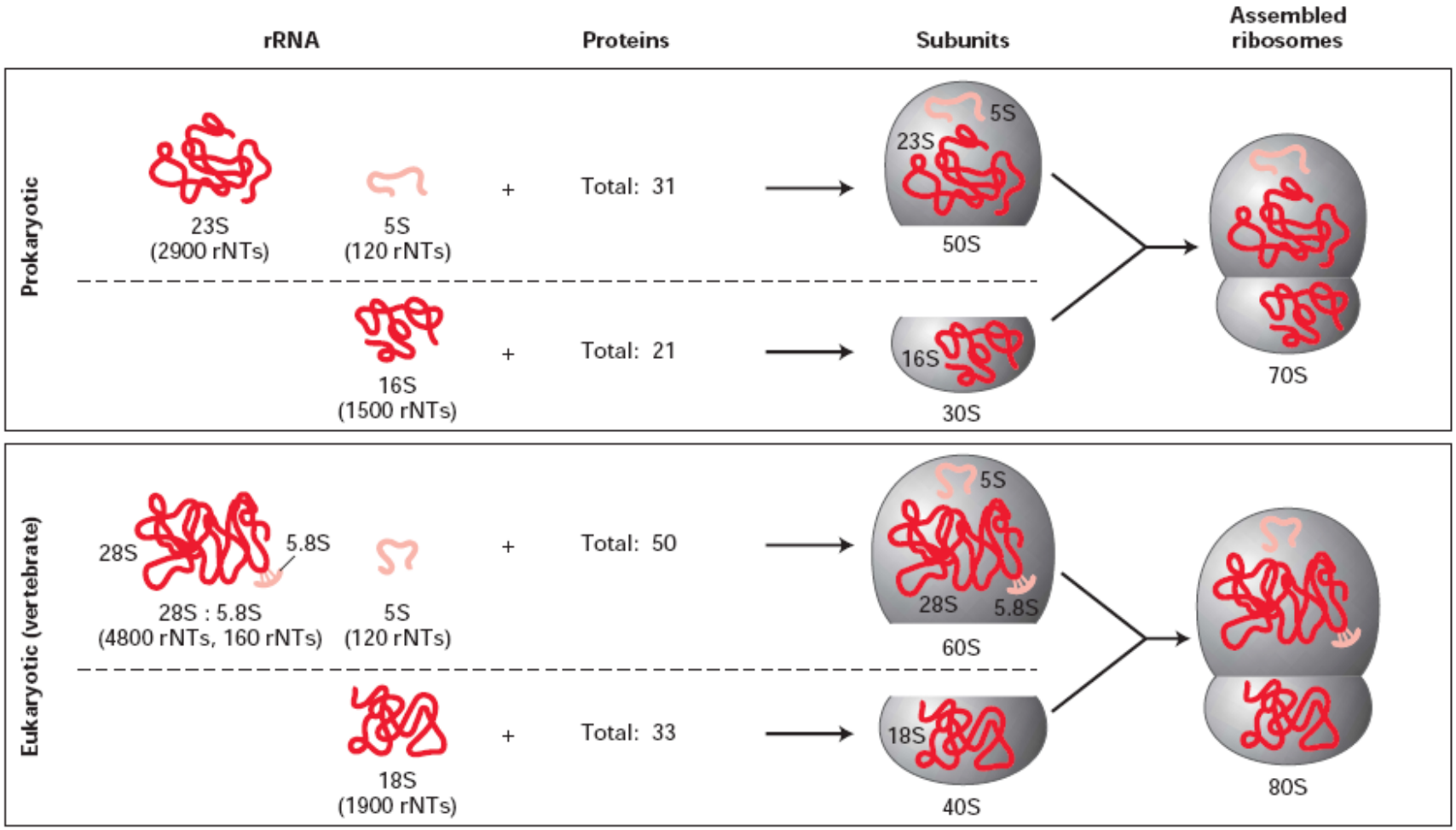


Fig.18b : A comparison of the components in the prokaryotics and eukaryotics ribosome.

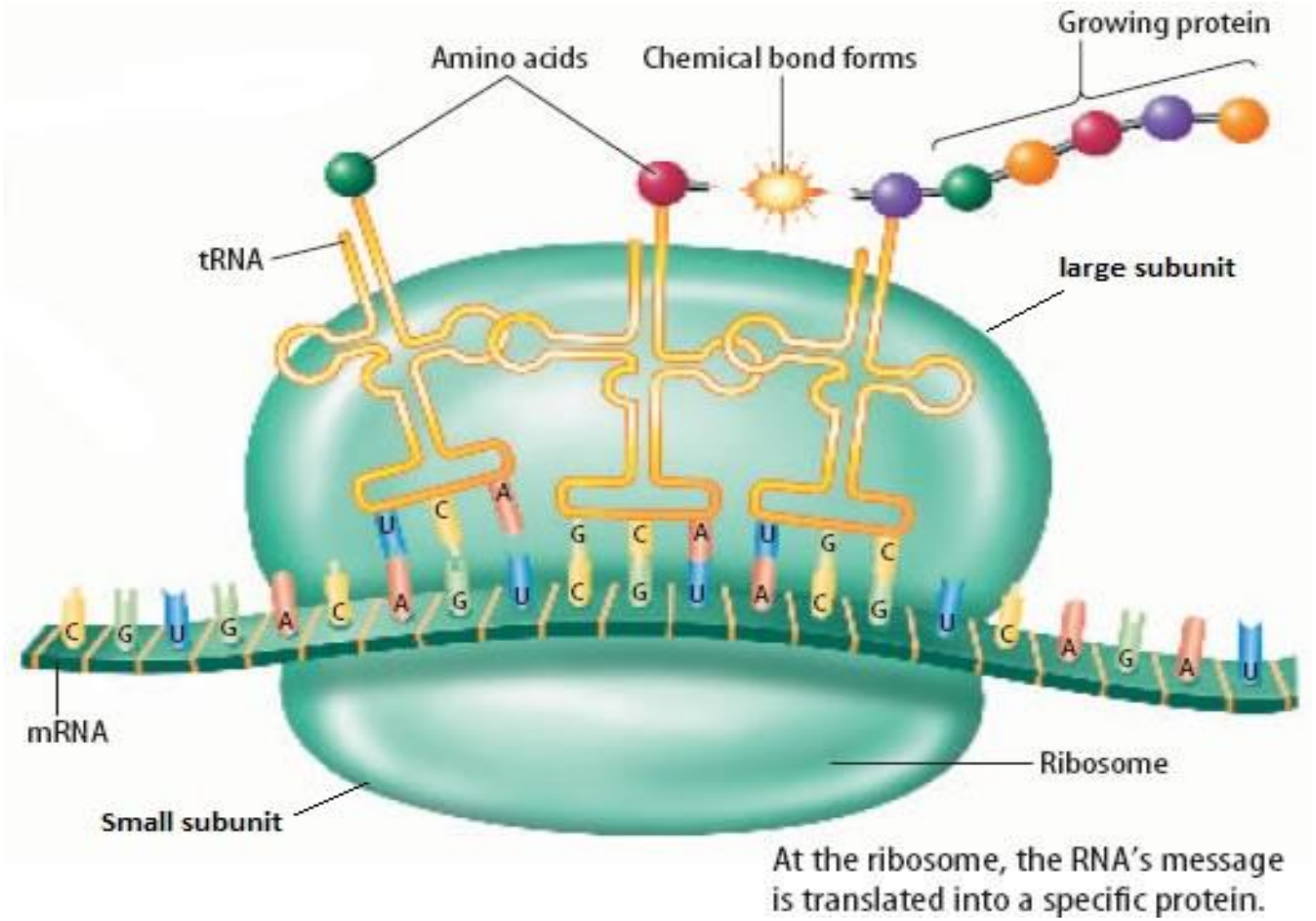


Fig.19: Protein synthesis.

Golgi apparatus

➢ Discovery

Owing to its large size and distinctive structure, the Golgi apparatus was one of the first organelles to be discovered and observed in detail. It was discovered in 1898 by Italian physician **Camillo Golgi** during an investigation of the nervous system. After first observing it under his microscope, he termed the structure the *internal reticular apparatus*. Some doubted the discovery at first, arguing that the appearance of the structure was merely an optical illusion created by the observation technique used by Golgi. With the development of modern microscopes in the 20th century, the discovery was confirmed. Early references to the Golgi referred to it by various names including the "Golgi–Holmgren apparatus", "Golgi–Holmgren ducts", and "Golgi–Kopsch apparatus". The term "Golgi apparatus" was used in 1910 and first appeared in scientific literature in 1913.

➢ Subcellular localization

Among eukaryotes, the subcellular localization of the Golgi apparatus differs. In mammals, a single Golgi apparatus complex is usually located near the cell nucleus, close to the centrosome. Tubular connections are responsible for linking the stacks together. Localization and tubular connections of the Golgi apparatus are dependent on microtubules. If microtubules are experimentally depolymerized, then the Golgi apparatus loses connections and becomes individual stacks throughout the cytoplasm. In yeast, multiple Golgi apparatuses are scattered throughout the cytoplasm (as observed in *Saccharomyces cerevisiae*). In plants, Golgi stacks are not concentrated at the centrosomal region and do not form Golgi ribbons. Organization of the plant Golgi depends on

actin cables and not microtubules. The common feature among Golgi is that they are adjacent to endoplasmic reticulum (ER) exit sites.

➢ Structure

A Golgi complex is composed of flat sacs known as **cisternae**, consists of a tubular parallel smooth membrane with membrane vesicles at their tips called **Golgi vesicles** (**Fig.20**). The sacs are stacked in a bent, semicircular shape. Each stacked grouping has a membrane that separates its insides from the cell's cytoplasm. Golgi membrane protein interactions are responsible for its unique shape. These interactions generate the force that shapes this organelle. The Golgi complex is very polar. Membranes at one end of the stack differ in both composition and in thickness from those at the other end. One end (**cis face**) acts as the "receiving" department while the other (**trans face**) acts as the "shipping" department. These two faces was known as two networks: the *cis* Golgi network (**CGN**) and the *trans* Golgi network (**TGN**). As the CGN is a collection of cisternae, originating from vesicular clusters that bud off the endoplasmic reticulum. The TGN is the final cisternal structure, from which proteins are packaged into vesicles destined to lysosomes, secretory vesicles, or the cell surface. The TGN is usually positioned adjacent to the stacks of the Golgi apparatus, but can also be separate from the stacks. The TGN may act as an early endosome in yeast and plants.

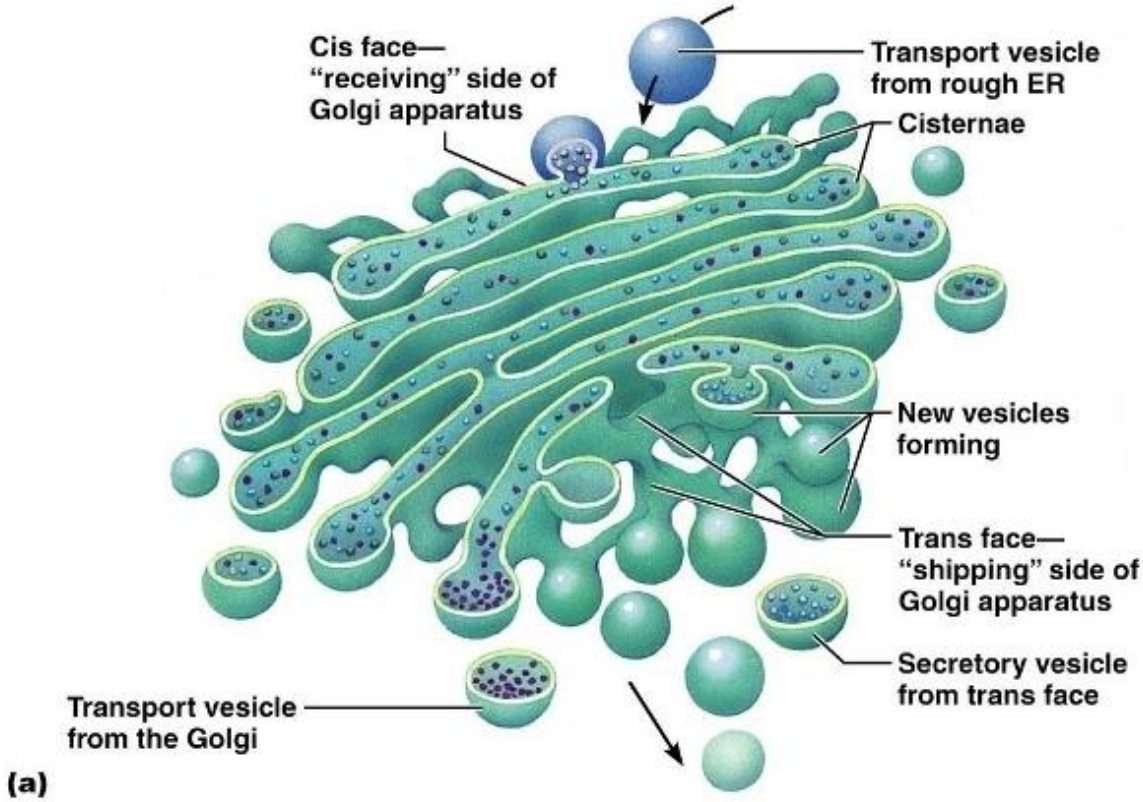


Fig.20: structure of Golgi apparatus.

➢ Golgi complex: Molecule Transport and Modification

Molecules synthesized in the ER exit via special transport vesicles which carry their contents to the Golgi complex. The vesicles fuse with Golgi cisternae releasing their contents into the internal portion of membrane. The molecules are modified as they are transported between cisternae layers. It is thought that individual sacs are not directly connected, thus the molecules move between cisternae through a sequence of budding, vesicle formation, and fusion with the next Golgi sac. Once the molecules reach the trans face of the Golgi, vesicles are formed to "ship" materials to other sites.

The Golgi complex modifies many products from the ER including proteins and phospholipids. The complex also manufactures certain biological polymers of its own. The Golgi complex contains

processing enzymes which alter molecules by adding or removing carbohydrate subunits. Once modifications have been made and molecules have been sorted, they are secreted from the Golgi via transport vesicles to their intended destinations. Some of the molecules are destined for the cell membrane where they aid in membrane repair and intercellular signaling. Other molecules are secreted to areas outside of the cell. Transport vesicles carrying these molecules fuse with the cell membrane releasing the molecules to the exterior of the cell. Still other vesicles contain enzymes that digest cellular components. These vesicle form cell structures called lysosomes. Molecules dispatched from the Golgi may also be reprocessed by the Golgi (**Fig. 21, 22**).

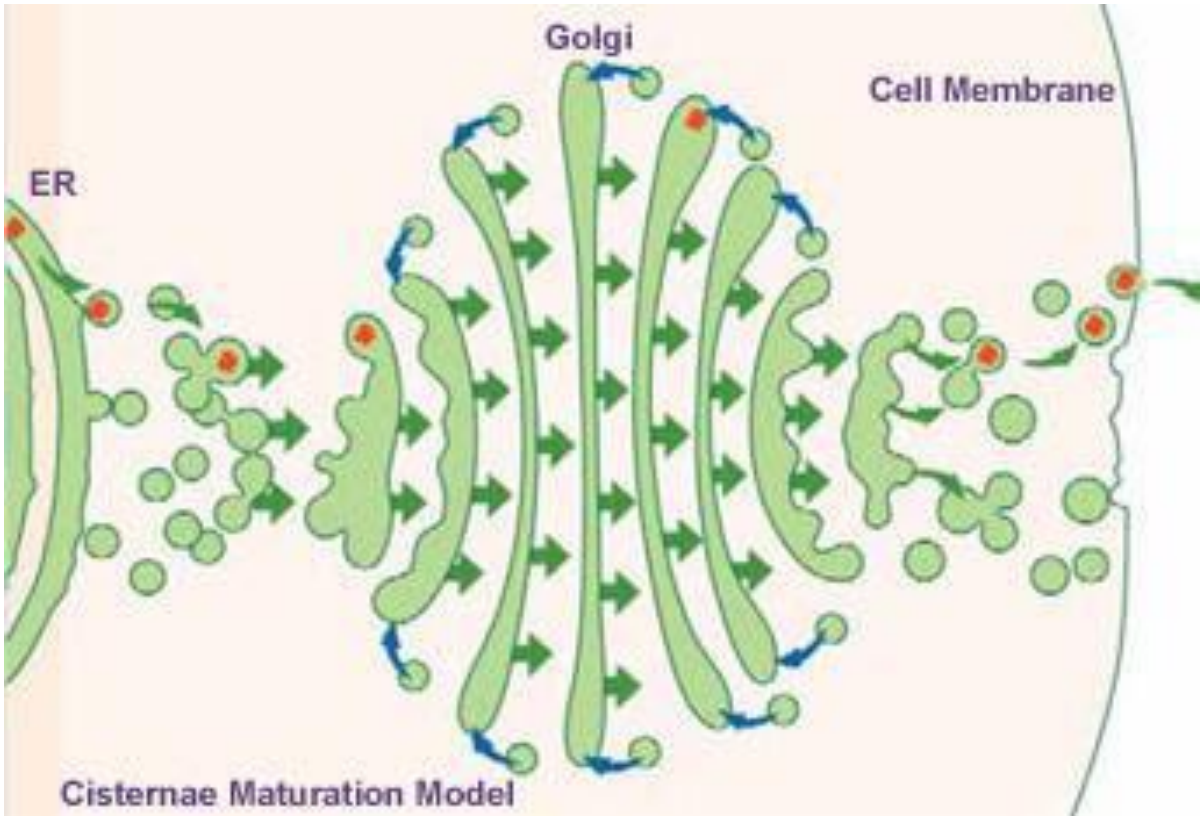


Fig.21: Diagrammatic representation of the role of Golgi apparatus in secretion.

➢ Golgi complex Assembly

The Golgi complex is capable of disassembly and reassembly. During the early stages of mitosis, the Golgi disassembles into fragments which further breakdown into vesicles. As the cell progresses through the division process, the Golgi vesicles are distributed between the two forming daughter cells by spindle microtubules. The Golgi complex reassembles in the telophase stage of mitosis. The mechanisms by which the Golgi complex assembles are not yet understood.

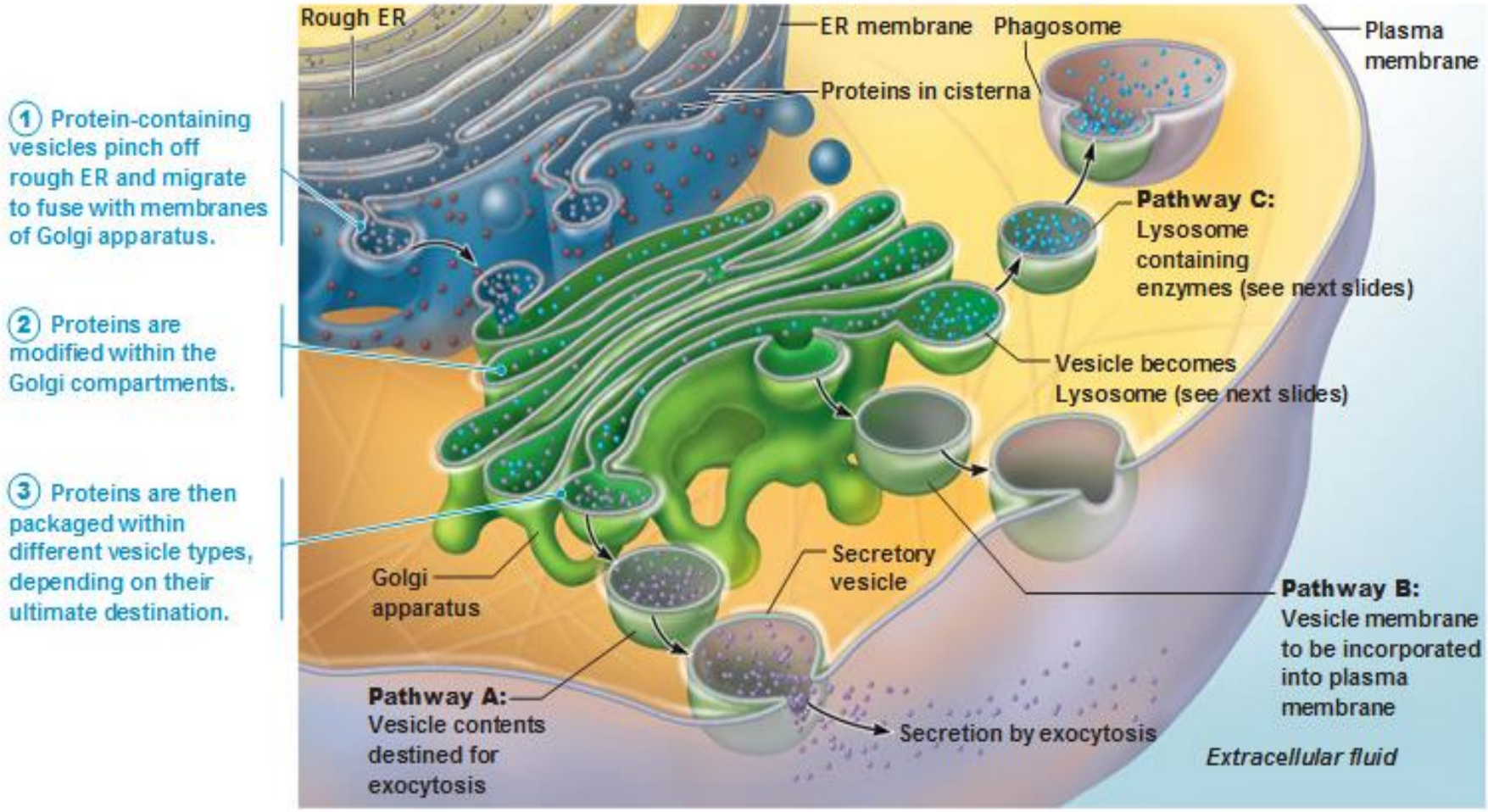


Fig.22: The sequence of events from protein synthesis on the rough ER to the final distribution of these proteins via Golgi complex.

Mitochondria

Mitochondria are the filamentous, self-duplicating, double membranous cytoplasmic organelles of eukaryotic cells which are concerned with cellular respiration.

They are the energy transducing organelle found in all aerobic eukaryotic cells. But in mature mammalian RBC mitochondria are lost secondarily. They are also absent in prokaryotic cells where mesosomes act as a substitute of mitochondria.

➢ History

-Kolliker (1850): First discovered mitochondria as granular structures in insect striated flight muscles and called as sarcosomes.

-Altmann (1894): He called them as bioblast.

-Benda (1897): First coined the term mitochondria.

-Meves (1904): First noticed the presence of mitochondria in plant cells of Nymphaea.

-Kingsbury (1912): First suggested that mitochondria are the sites for cellular respiration.

-Kennedy and Lehninger (1948- 1950): He showed that TCA cycle, oxidative phosphorylation and fatty acid oxidation took place in mitochondria.

➢ Number

The number of mitochondria varies from cell to cell; plant cells contain fewer than animal cells. The number of mitochondria in a cell is generally proportional to its energy requirement. The *Trypanosoma*, *Chlorella* and *Microsterias* contain 1 mitochondrion per cell, but the number is 25 in human sperm cell, 300-400 – in a kidney cell, 500-1000 – in a hepatic

cell, 50,000 – giant amoeba (*Chmn chaos*), 30000 -300000 – in oocytes of sea urchins and 5,00,000 -in flight muscle cell.

➢ Shape and Size:

Mitochondria vary in shape and size. Typical mitochondria are generally rod shaped, having length 1-4 /µm and breadth 0.2-1.5/µm. In some cases, these may be spherical or oval or filamentous (up to 12µ.m long). All mitochondria of a cell are collective called as **chondriome** and constitutes about 25% of the cell volume. Mitochondria appear yellowish due to riboflavin and rich in Mn. The life span of mitochondria is only 5-10 days. They are continuously produced from the pre-existing mitochondria within the cell and destroyed within the cells.

Mitochondria are the cell's power producers. They convert energy into forms that are usable by the cell. Located in the cytoplasm, they are the sites of cellular respiration which ultimately generates fuel for the cell's activities.

Mitochondria are also involved in other cell processes such as cell division and growth, as well as cell death.

➢ Ultrastructure

Each mitochondrion is bounded by a mitochondrial envelope and encloses two chambers or compartments within it.

(a) Mitochondrial envelope:

It consists to two membranes called outer membrane and inner membrane (each 60-75 A thick). Both the membranes come in contact with each other at several places called adhesion sites or contact zones (**Fig. 23, 24**). The outer membrane is smooth but porous due to the presence of integral proteins called **porins**. It contains 40% lipids and

60% proteins. The mitochondrion excluding the outer membrane is called **mitoplast**.

The inner membrane is semipermeable. It is highly convoluted to form a series of in-folding called **cristae** or mitochondrial crests. Each crista encloses intracristal spaces which is continuous with the outer chamber. The cristae greatly increase the surface areas of inner membrane. The inner membrane consists of 75% proteins and 25% lipids. It is rich in enzymes of respirators chain and a variety of transport proteins.

(b) Mitochondrial chambers:

In between two membranes a narrow space (about 6-10 nm wide, present called outer chamber or inter-membrane space. The central wider space enclosed by the inner membrane is called inner chamber or mitochondrial matrix. The outer chamber is filled with a watery fluid and contains enzymes like adenylate kinase and nucleoside di-phosphokinase.

The matrix is filled with a homogenous, granular, dense, jelly like material. It contains-circular DNAs (2-6 copies). Mitoribosomes, granules of inorganic salts, enzymes for the citric acid cycle (TCA cycle) and for the oxidation of pyruvate and fatty acids.

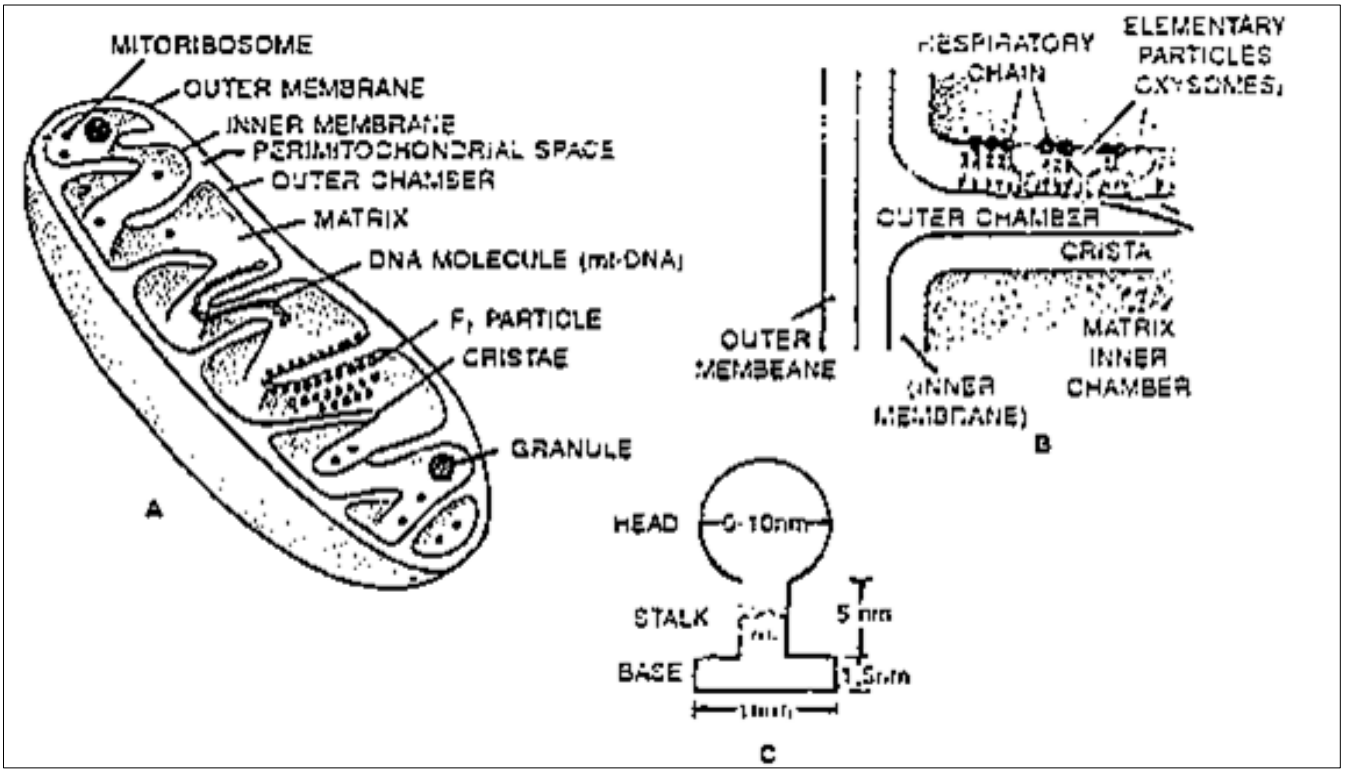


Fig. 23: Structure of a mitochondrion. (A) Mitochondrion longitudinally cut to show its internal structure, (B) inner membrane with oxysomes, (C) oxysome.

(c) Oxysomes:

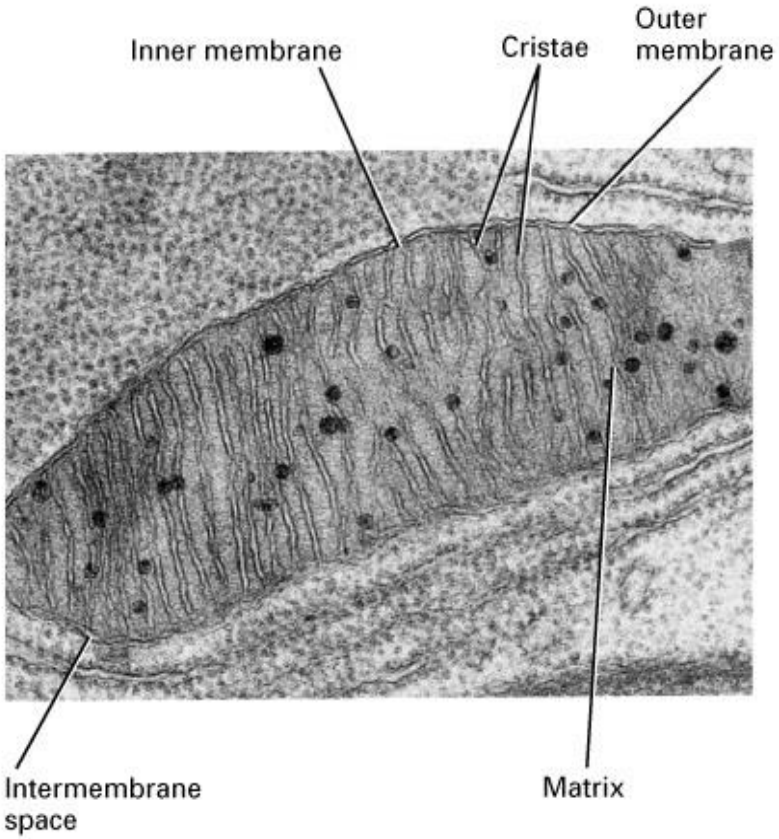
The matrix side of the inner membrane and cristae bear numerous tennis rackets like particles present called **oxysomes** (**Fig.23**). They are also known as elementary particles, Parson's particle, Fernandez-Moran particle, F_0F_1-particles, F_0F_1-ATPase, H_+ – ATPase, ATP synthetase or ATP synthase. A mitochondrion contains about 104 -105 oxysomes regularly placed at the intervals of l0 nm. Oxysomes comprise about 15% of the total inner membrane protein.

Each oxysome is a multi-polypeptide complex consists of 3 parts:

(i) Head piece or F_1 particle or soluble ATPase.

(ii) Base or F_0 subunit.

(iii) A stalk that connects F_1 subunit with the F_0 subunit.



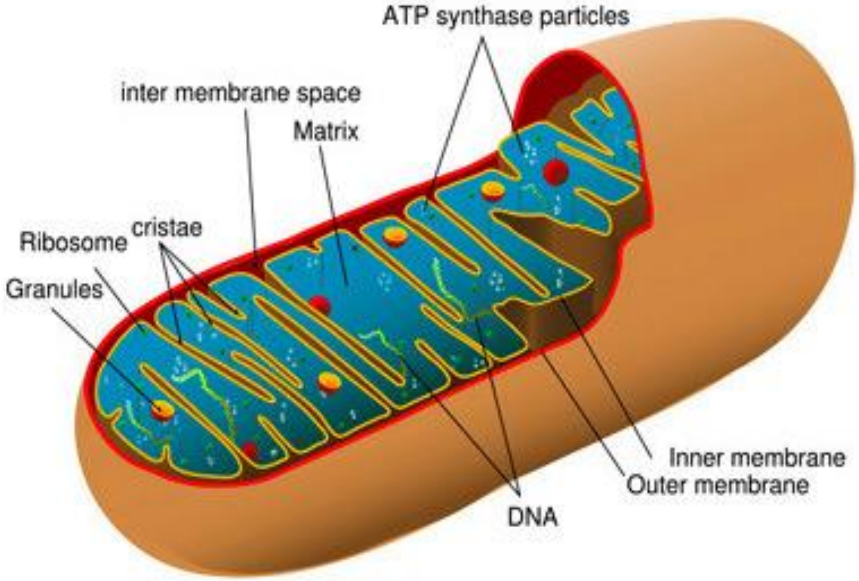


Fig. 24: Structure of mitochondria under electron microscope.

➢ Functions of Mitochondria

Mitochondria are associated with the following functions:

1- They are the main seat of cellular respiration, a process involving the release of energy from organic molecules (such as glucose) and its transfer to molecules of ATP (Adenosine triphosphate), the chief immediate source of chemical energy for all eukaryotic cells. On this account, the mitochondria are often described as the “power houses”, or “storage batteries” or “ATP mills” or “cellular furnace” of the cell. Mitochondria tend to assemble where energy is required.

2- They provide intermediates for the synthesis of important biomolecules, such as chlorophyll, cytochromes, steroids etc.

3- Some amino acids are also formed in mitochondria.

4- Mitochondria regulate the calcium ion concentration in the cell by storing and releasing Ca^{2+}as and when required. The calcium ions in turn regulate many biochemical activities in the cell.

5- They help in β oxidation of Fatty acids.

Plastids

Plastids are ovoid or spherical shaped organelles found in plant cells and in certain unicellular organism like algae. They are easily observed under light microscope. **E. Haeckel** (**1866**) introduced the term **plasmid**.

➢ Origin and Development of Plastids:

Recent studies state that all plastids arise always from pre-existing minute sub-microscopic amoeboid plastids called as **proplastids** (**Fig. 25**). The proplastid is considered as stem plastid which gives rise to either leucoplast or immature lamellar plastids, the later may form any type of plastids. The proplastids are spherical bodies of 0.5µ diameter bounded by double membranes enclosing the dense **stroma**.

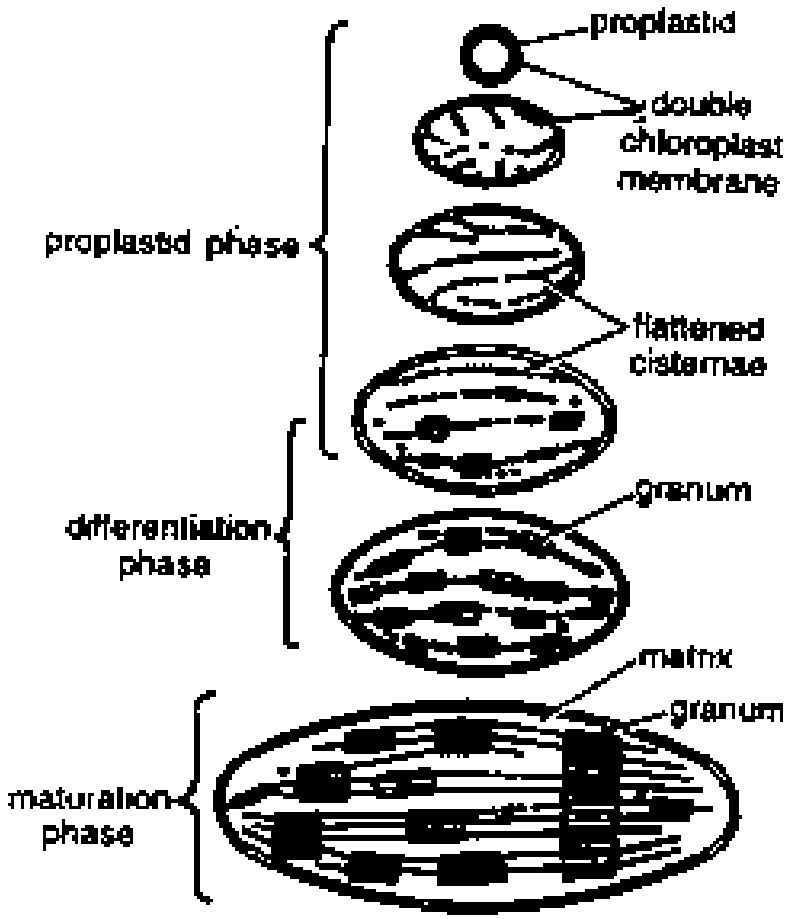


Fig. 25: Origin of chloroplast from a submicroscopic proplastid in the presence of sun light.

When light is available and the proplastid reaches a diameter of 1 μm, its inner membrane invigorates to form vesicles into the **matrix** or **stroma**, which arrange themselves parallelly in the stroma and later these vesicles fuse to form discs or **lamellae**.

These intrachloroplastic membranes are the **thylakoids** which, in certain region, pile closely to form the **grana** few thylakoids remain connected with each other by the tubules or stromal lamellae. In the mature chloroplast the thylakoids are no longer connected to the inner membrane, but the grana remain united by intergranal thylakoids.

In darkness, however, the lamellae break up into vesicles. If a plant is kept under low light intensity, the reverse sequence of changes takes place (**Fig. 26**). This process is called **etiolation**, and results in the disorganization of the membranes.

The same phenomenon occurs if the plant is grown from the very beginning in low light intensity. In this case the vesicles of the proplastid aggregate to form one or more prolamellar bodies, which can develop into grana if the plant is again exposed to light.

The plastids never arise denovo (afresh). In Monocotyledons, some of the mature plastids called, **elaioplasts**, develop from old chloroplasts. In carrot root chromoplasts become developed from **amyloplasts**. In algae and ferns the mature plastids give rise to new plastids by division. New plastids also develop by budding from the mature plastids but it occurs only in abnormal conditions, i.e., regeneration of plant from dissected leaves.

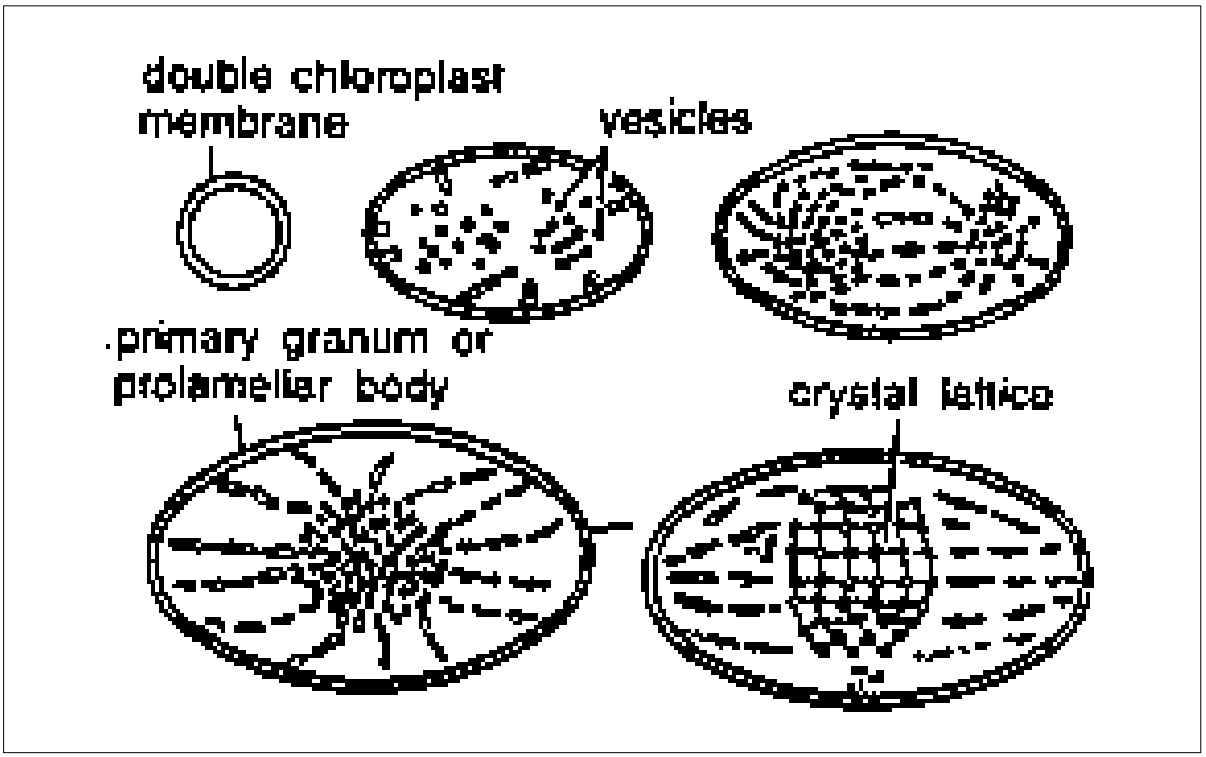


Fig. 26: Development of proplastid into chloroplast in the dark.

➢ Types

On the basis of types of pigments they contain, **Schimper** (**1883**) classified them in three types:

(i) Leucoplasts- Colourless plastids

(ii) Chromoplasts – Coloured plastids (other than green)

(iii) Chloroplasts- Green plastids

All the three types of plastids can change one form into another. Further, all plastids have a common precursor called pro-plastid. The pro-plastids are colorless undifferentiated plastids found in meristematic cells.

(i) Leucoplasts:

These are colorless, non-photosynthetic plastids found in those cells of plants which are not exposed to sunlight. They possess membranous lamellae that do not form thylakoids. They are the storage organelles and on the basis of stored food they are of three types.

1- **Amyloplasts:** They store starch, and found in underground stems (e.g. potato), cereals (e.g. rice, wheat) etc.
2- **Elaioplasts** (Lipidoplasts or oleoplasts): They store oils and found in the seeds of castor, mustard, coconut etc.
3- **Aleuroplasts** (Proteoplasts or proteinoplasts): They store proteins and found in seeds (maize)

(ii) Chromoplasts:

These are colored plastids other than green. They are non-photosynthetic which synthesize and store carotenoid pigments. They provide color to various parts of the plants which attract insects for pollination & dispersal of seeds. They also synthesize membrane lipids. During ripening of tomato and chili chloroplasts transformed into chromoplasts.

(iii) Chloroplasts (Green plastids):

The chloroplasts are green or chlorophyll containing plastids concerned with photosynthesis. The chloroplasts of algae, other than green ones (such as red and brown algae) are called **chromatophores**.

➢ Number:

A leaf mesophyll cell may contain 40-50 chloroplasts; a square millimeter of leaf contains some 500,000. The number of chloroplasts per cell in algae is usually fixed for a species. The minimum number of one chloroplast per cell is found in green alga *Ulotlirix arid* several species of *Chlamydomonas*. However, different species of a genus may have different number of chloroplasts, for example—1 in *Spirogyra indica*. 16 in *Spirogyra rectospora*. The internodal cell of the green alga *Chara* possesses several hundred chloroplasts.

➢ Shape and Orientation:

The shape of a chloroplast varies from species to species. It may be cup-shaped (e.g., *Chlamydomonas*), (e.g., *Vaucheria*), Girdle (e.g., *Ulothrix*), Stellate or Star-shaped (e.g., *Zygnema*), Reticulate or net-like (e.g., *Cladophora*, *Oedogonium*), Spiral or ribbon or scalariform (e.g., *Spirogyra*), ovoid or disc or spheroid in higher plants.

The chloroplasts are usually found with their broad surfaces parallel to the cell wall. They can reorient in the cell under the influence of light. For example, gathering along the walls parallel with the leaf surface under low or medium light intensity. Under damaging, very high light intensity, they can orient themselves perpendicular to the leaf surface.

➢ Size:

They are generally 4-10/µm in length and 2-4 nm in width. In many algae, the chloroplast may occupy almost the whole length of the cell, such as in green alga *Spirogyra*, where it may reach a length of 1 mm.

➢ Ultra-structure:

A chloroplast has three types of membranes enclosing three types of compartments (**Fig.27**). The membranes are: outer membrane, inner membrane & a system of thylakoid membranes, while the compartments are: inter-membrane space, stroma & thylakoid space.

Each chloroplast is surrounded by chloroplast envelope which consists of outer & inner membranes. Both the membranes are separated by a fluid-filled inter-membrane space of 10-20 nm width. The outer membrane is freely permeable due to the presence of porin proteins, while the inner membrane is semipermeable. Sometimes extensions of outer membrane called stromules found to connect

adjacent chloroplasts. The membranes of all plastids including chloroplast consists of entirely glycosylglycericles (=galactolipids and sulfolipids) rather than phospholipids.

The inner membrane encloses a fluid-filled space called stroma, which is analogous to the mitochondrial matrix. The stroma contains: thylakoids, various enzymes, protein synthetic machinery (i.e. 2-6 copies of circular DNAs, RNAs & 70S ribosomes), plastoglobuli, certain metallic ions (Fe, Mn, and Mg) starch grains etc. In green algae, proteinous pyrenoids present around which starch deposits in layers".

The stroma contains a membrane system which consists of many flattened, fluid-filled sacs called thylakoids or lamellae. About 2-100 thylakoids are stacked like a pile of coins forming grana. In a typical chloroplast, as many as 40-60 grana may be present. Adjacent grana are interconnected by stroma lamella or frets. The C_4 plants – maize, sugarcane- possess two type of chloroplasts (i.e. chloroplast dimorphism), agranal chloroplasts (inside bundle-sheath cells) & granal chloroplasts (inside mesophyll cells).

The thylakoid membrane system carry four protein assemblies i.e. Photosystem I (PS-I) Photosystem II (PS-ID), electrone transport system (ETS) consisting of cytochromes b and f & CF0-CF1 particles (ATP syntetase).

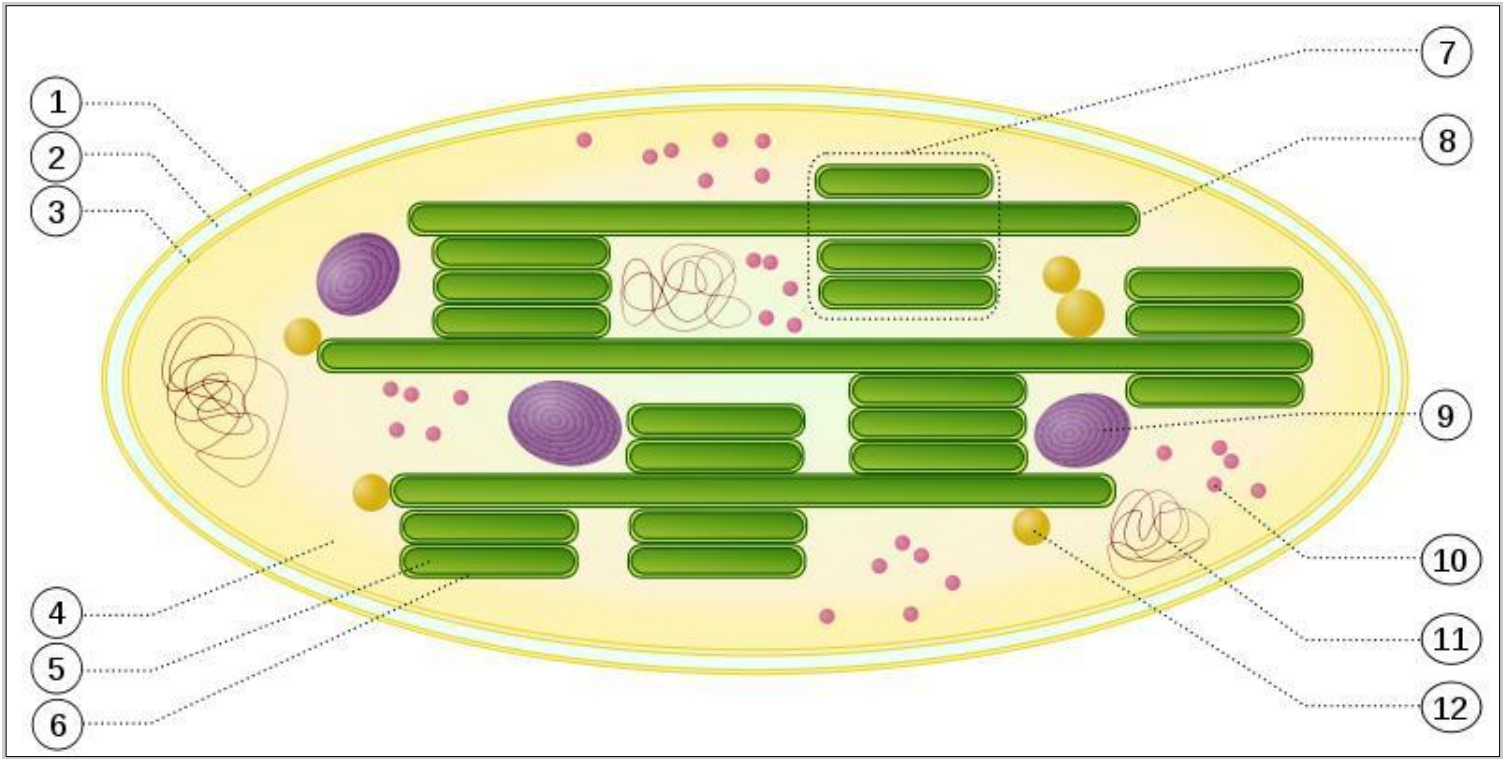


Fig.27: Chloroplast ultrastructure:

1. Outer membrane **2.** Intermembrane space **3.** Inner membrane (1+2+3: envelope) **4.** Stroma (aqueous fluid)
5. Thylakoid lumen (inside of thylakoid) **6.** Thylakoid membrane **7.** Granum (stack of thylakoids)
8. Thylakoid (lamella) **9.** Starch **10.** Ribosome **11.** Plastidial DNA **12.** Plastoglobule (drop of lipids)

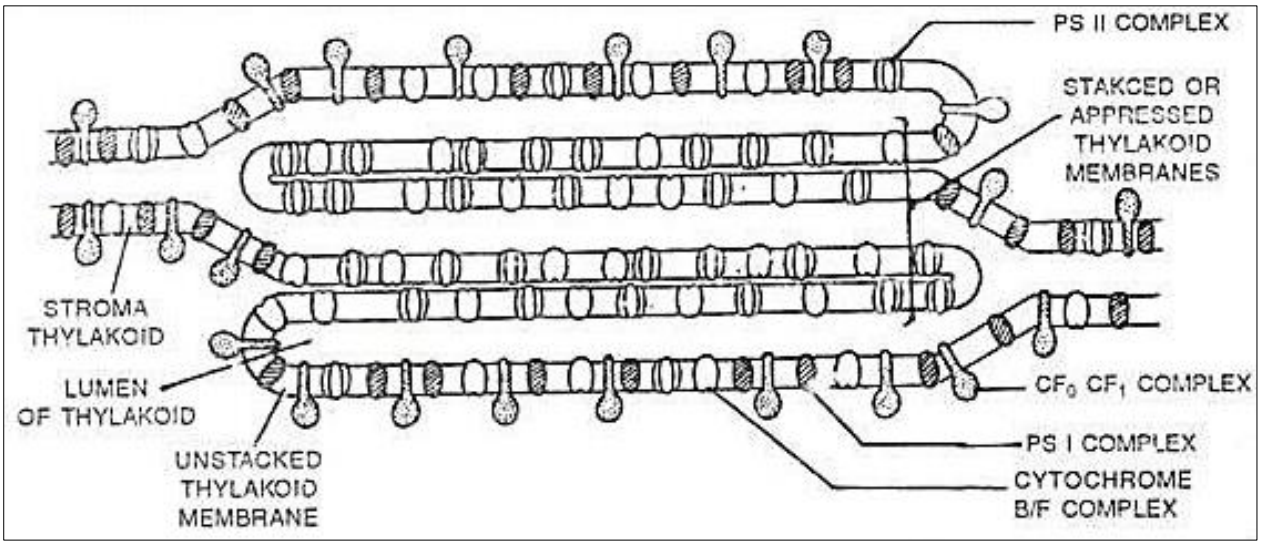


Fig.28: Distribution of protein complexes in the thylakoid membrane.

➢ Functions of Chloroplasts:

1. Chloroplasts are the site of photosynthesis. The light reactions occur in thylakoid membranes while dark reactions occur in stroma.
2. Chloroplasts convert radiant energy of sun light into chemical energy of sugars, hence called as biological transducers.
3. Chloroplasts store fat droplets in form of plastoglobuli which also contain vit-K and vit-E.
4. Synthesis of fatly acids in the chloroplast has also been reported in some plants (e.g., spinach)
5. Chloroplasts in some algae render photosensitivity because of the presence of stigma or eye spot.
6. They can be transformed into the chromoplasts which provide beautiful colors to many flowers and help in attracting insects, birds and animals for pollination and dispersal.

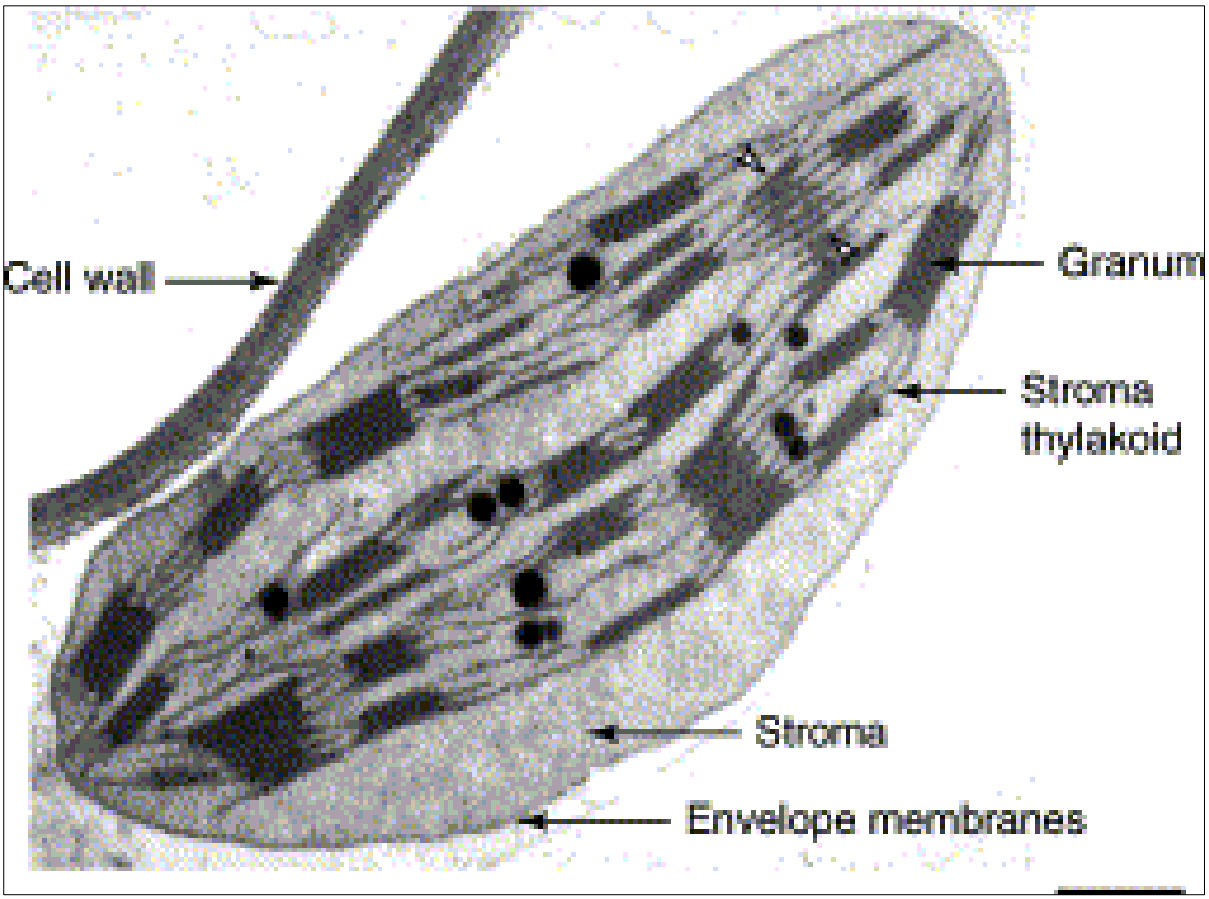


Fig.29: Chloroplast ultrastructure under electron microscope.

The Nucleus

➢ History

The nucleus was described by **Franz Bauer** in 1804 and in more detail in 1831 by Scottish botanist **Robert Brown** in a talk at the Linnean Society of London. **Brown** was studying orchids under microscope when he observed an opaque area, which he called the areola or nucleus, in the cells of the flower's outer layer. He did not suggest a potential function. In 1838, **Matthias Schleiden** proposed that the nucleus plays a role in generating cells, thus he introduced the name "Cytoblast" (cell builder). He believed that he had observed new cells assembling around "cytoblasts". **Franz Meyenwas** a strong opponent of this view, having already described cells multiplying by division and believing that many cells would have no nuclei. The idea that cells can be generated de novo, by the "cytoblast" or otherwise, contradicted work by **Robert Remak** (1852) and **Rudolf Virchow** (1855) who decisively propagated the new paradigm that cells are generated solely by cells ("Omnis cellula e cellula"). The function of the nucleus remained unclear.

Between 1877 and 1878, **Oscar Hertwig** published several studies on the fertilization of sea urchin eggs, showing that the nucleus of the sperm enters the oocyte and fuses with its nucleus. This was the first time it was suggested that an individual develops from a (single) nucleated cell. This was in contradiction to **Ernst Haeckel**'s theory that the complete phylogeny of a species would be repeated during embryonic development, including generation of the first nucleated cell from a "Monerula", a structure less mass of primordial mucus ("Urschleim"). Therefore, the necessity of the sperm nucleus for fertilization was discussed for quite some time. However, **Hertwig** confirmed his observation in other animal groups, including amphibians

and molluscs. **Eduard Strasburger** produced the same results for plants in 1884. This paved the way to assign the nucleus an important role in heredity. In 1873, **August Weismann** postulated the equivalence of the maternal and paternal germ cells for heredity. The function of the nucleus as carrier of genetic information became clear only later, after mitosis was discovered and the **Mendelian rules** were rediscovered at the beginning of the 20th century; the chromosome theory of heredity was therefore developed.

➢ Structure

The **nucleus** is a membrane-enclosed organelle found in eukaryotic cells. Eukaryotes usually have a single nucleus, but a few cell types have no nuclei, and a few others have many. In some cells , it has a relatively fixed position, usually near to center of the cell but in other it may move freely and be found almost anywhere in the cell.

Nucleus is the most important organelle in the cell and the largest one. It is enclosed by an envelope of two membranes that is perforated by nuclear pores. Like the cell membrane, the **nuclear envelope** consists of phospholipids that form a lipid bilayer. The envelope helps to maintain the shape of the nucleus and assists in regulating the flow of molecules into and out of the nucleus through **nuclear pores** (**Fig.30**).

The nuclear envelope, otherwise known as nuclear membrane, consists of two cellular membranes, an inner and an outer membrane, arranged parallel to one another and separated by 10 to 50 nanometers (nm). The nuclear envelope completely encloses the nucleus and separates the cell's genetic material from the surrounding cytoplasm, serving as a barrier to prevent macromolecules from diffusing freely between the nucleoplasm and the cytoplasm. The outer nuclear

membrane is continuous with the membrane of the rough endoplasmic reticulum (**RER**), and is similarly studded with ribosomes. The space between the membranes is called the **perinuclear space** and is continuous with the **RER** lumen.

Nuclear pores, which provide aqueous channels through the envelope, are composed of multiple proteins, collectively referred to as **nucleoporins**. The pores are about 125 million daltons in molecular weight and consist of around 50 (in yeast) to several hundred proteins (in vertebrates). The pores are 100 nm in total diameter; however, the gap through which molecules freely diffuse is only about 9 nm wide, due to the presence of regulatory systems within the center of the pore. This size selectively allows the passage of small water-soluble molecules while preventing larger molecules, such as nucleic acids and larger proteins, from inappropriately entering or exiting the nucleus. These large molecules must be actively transported into the nucleus instead.

Within the nucleus, there is a matrix called **nucleoplasm** which contains the **chromatin** and **nucleolus**.

Chromosomes are located within the nucleus. Chromosomes consist of DNA, which contains heredity information and instructions for cell growth, development, and reproduction. When a cell is "resting" i.e. not dividing, the chromosomes are organized into long entangled structures called **chromatin** and not into individual chromosomes as we typically think of them.

The chromatin materials are coiled DNA bounded by protein called **histones**. The term chromatin means colored materials. There are two types of chromatin in the nucleus, these are:

i. **Heterochromatin** – Tightly coiled and continues to stain intense.

ii. **Euchromatin** – The looser coiled and more scattered chromatin during the interphase.

The outer membrane is continuous with the endoplasmic reticulum and may be covered by ribosomes for protein synthesis.

The Nucleolus

Contained within the nucleus is a dense structure composed of RNA and proteins called the nucleolus. The nucleolus contains nucleolar organizers, which are parts of chromosomes with the genes for **ribosome synthesis** on them. The nucleolus helps to synthesize ribosomes by transcribing and assembling ribosomal RNA. A ribosome is composed of ribosomal RNA and proteins.

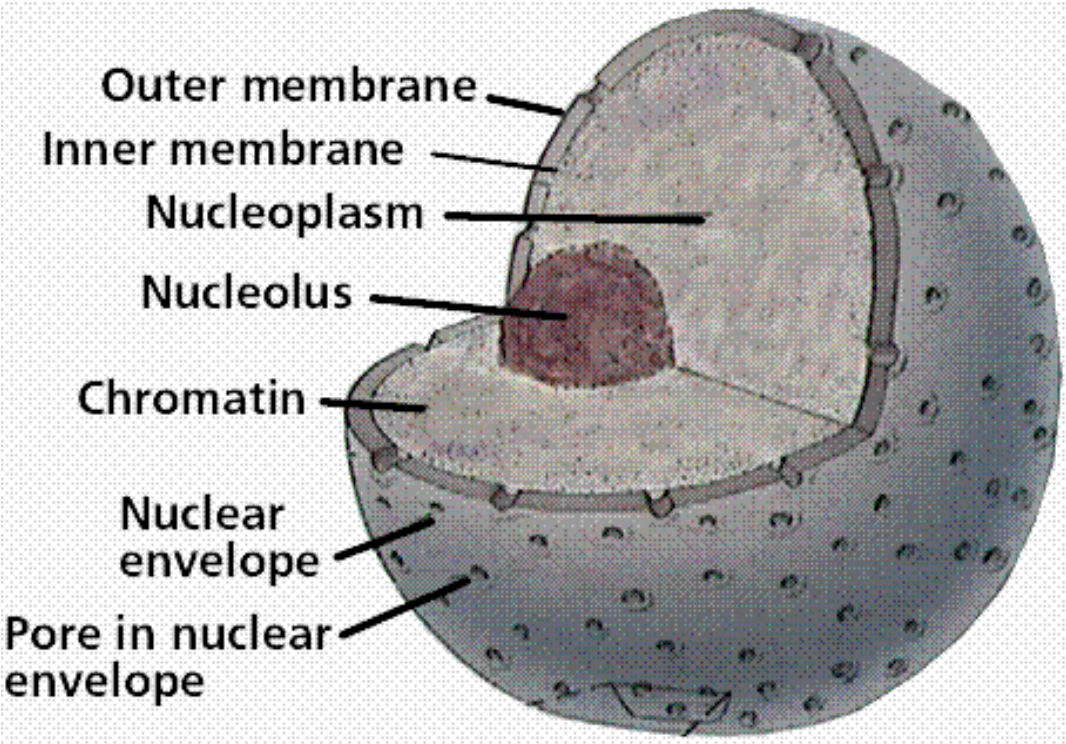


Fig. 30: structure of the nucleus.

Functions of the Nucleus

1. It contains chromosomes which have DNA (hereditary material) for the transmission of characteristics from one generation to another.
2. It controls the metabolic activities since DNA is organized into genes which control all the activities of the cell.
3. Formation of the ribosomal RNA by nucleolus.
4. Nuclear division gives rise to cell division hence reproduction.
5. It carries the instructions for synthesis of proteins in the nuclear DNA.

Lysosomes

A simple spherical sac bounded by a single membrane and contains a mixture of digestive enzymes such as protease, nuclease and lipase which break down proteins, nucleic acids and lipids respectively (**Fig.31**).

The enzymes contained within lysosomes are synthesized on rough E.R and transported to the Golgi apparatus. Golgi vesicles containing the processed enzymes later bud off to form the lysosomes.

In plant cells the large central vacuoles may act as lysosome although bodies similar to the lysosome of animal cells sometimes seen in the cytoplasm of plant cell.

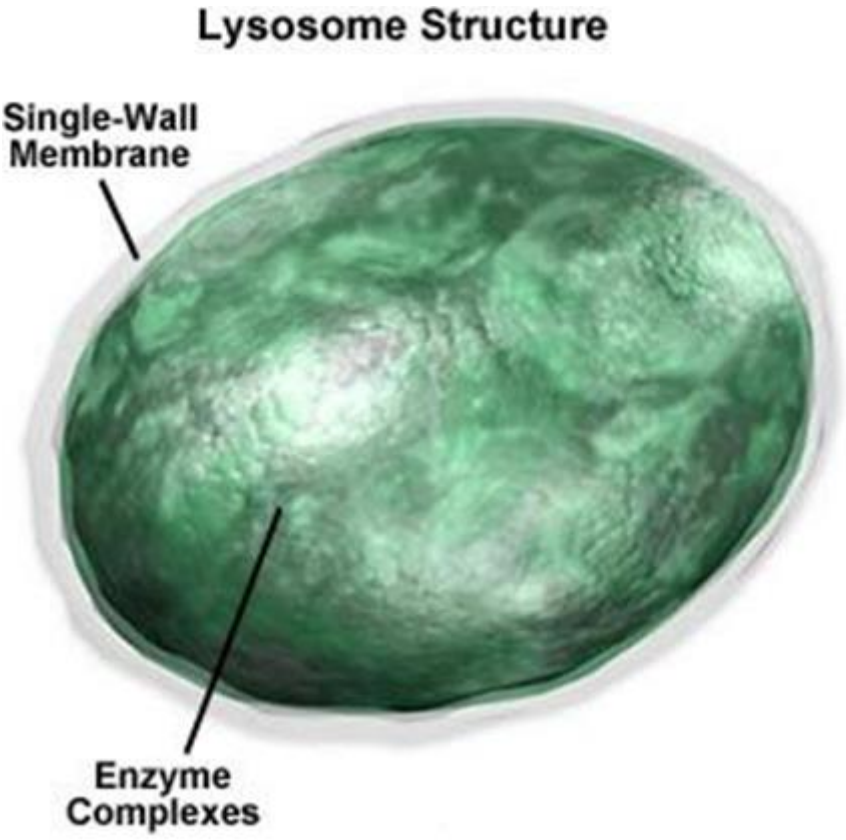


Fig.31: Structure of lysosome.

➢ Functions of Lysosome

1. Lysosomes contain digestive enzymes which are used in digestion of reductant structure or damaged macromolecule from, within or outside the cell by autolysis.
2. Lysosome destroys foreign particles such as bacteria by phagocytosis.
3. It secretes the digestive enzymes.
4. Lysosomes play part in autophagy, autolysis, endocytosis and exocytosis.

Autolysis is the self-digestion of a cell by releasing the contents of lysosome within the cell. For this reason, lysosomes sometimes called '**suicide bags**' or '**self-breaking down**'.

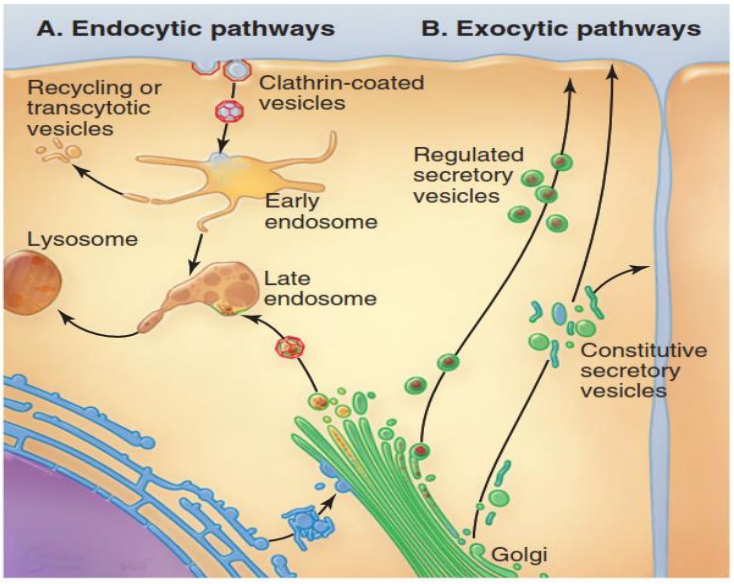
Autophagy is the process by which unwanted structures within the cell are engulfed and digested within lysosome.

Endocytosis occurs by an in folding or extension of the cell surface membrane to form vesicles or vacuoles. It is of two types, these are:

Phagocytosis – 'cell eating'. Material taken up is in solid form.

Pinocytosis – 'cell drinking'. Material taken up is in liquid form.

Exocytosis is the process in which waste materials may be removed from cells. It is the reverse of endocytosis.



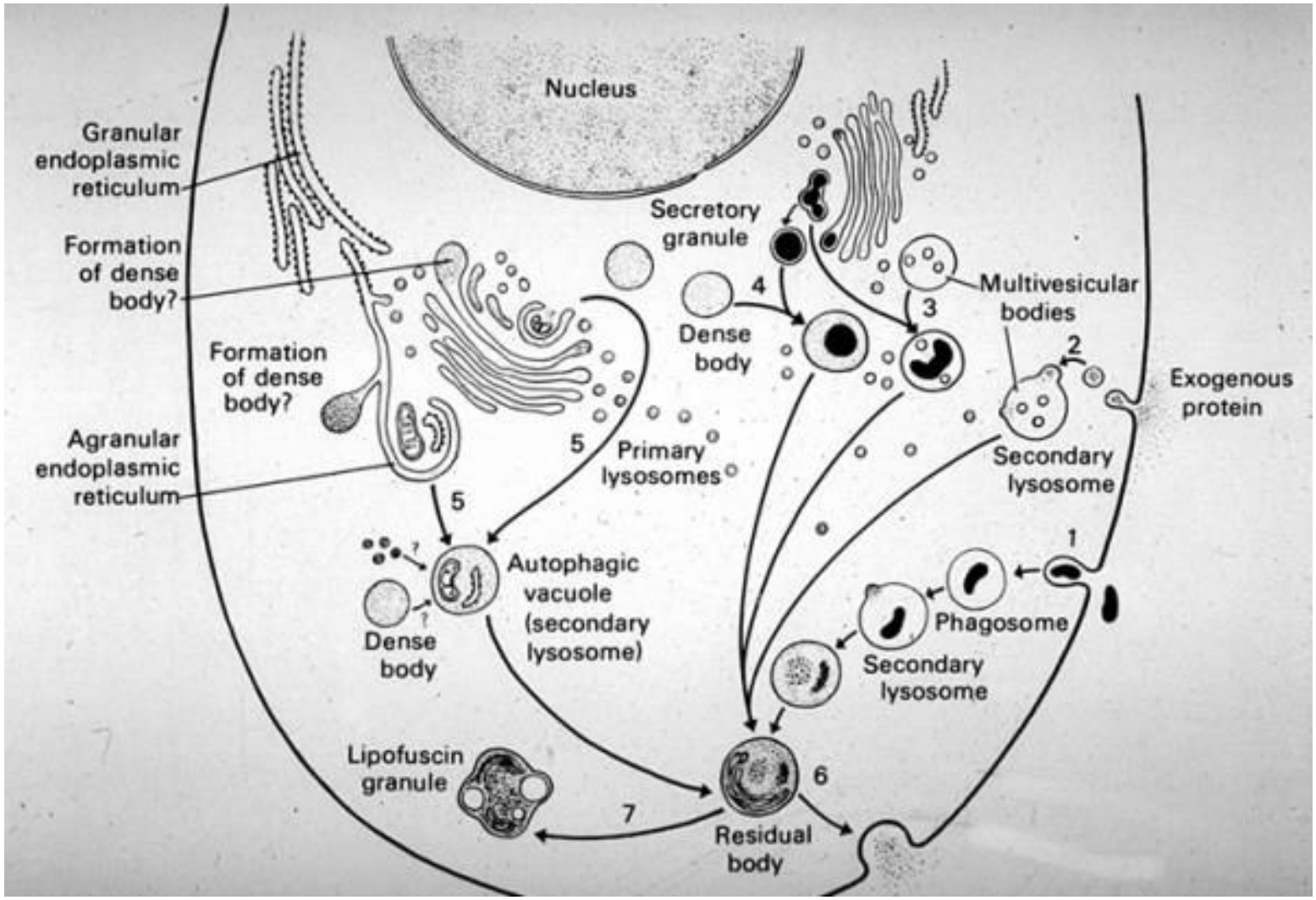


Fig.32: The three possible uses of lysosome.

Peroxisomes or Microbodies

Peroxisomes or microbodies are spherical organelles bounded by a single membrane commonly found in eukaryotic cells (**Fig.33**). They are slightly smaller than mitochondria. They are believed to derive from endoplasmic reticulum.

The peroxisomes are like the lysosomes containing the powerful enzymes but the enzymes in peroxisome are oxidative rather than digestive enzymes. e.g.: catalase which catalyzes the decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide as a byproduct of certain cell oxidation reaction is very toxic and therefore must be eliminated immediately.

In the liver cells contain large number of peroxisomes which are involved in oxidative metabolic activities. In plants peroxisomes are site of the glycolate cycle (photorespiration).

Peroxisomes reproduce by a process called peroxisomal biogenesis. They have the ability to assemble themselves. Peroxisomes have no DNA or ribosomes however, so they must take in proteins from the cytosol.

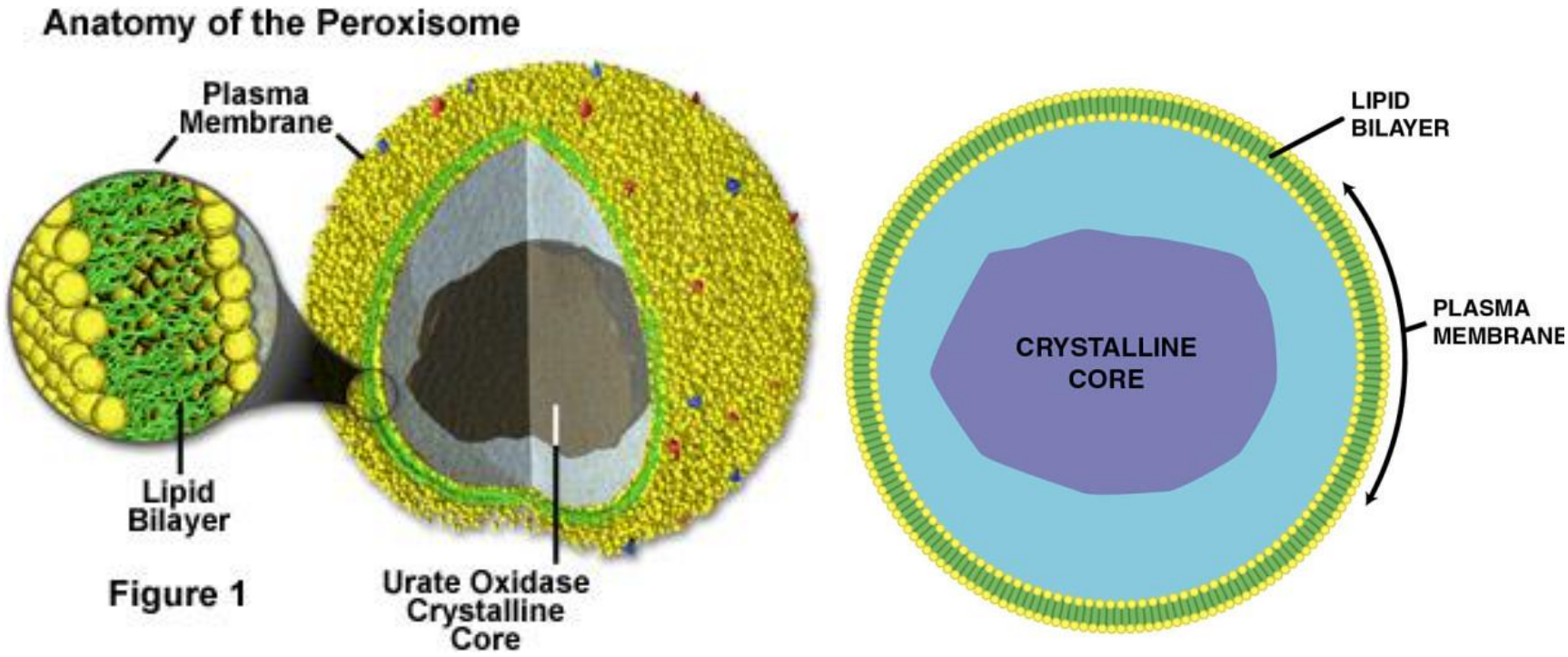


Fig.33: Structure of peroxisome.

Centrioles

Centrioles are small hollow cylinders that occur in pair in most animal cells, as it absent in a plant cell.

In centrosome (poorly defined structure which initiates the development of microtubules), the two centrioles lie right angle to each other. Each contains a 9+3 pattern of microtubule triplets, i.e.: a ring having nine sets of triplets with none in the middle (**Fig.34**).

Before an animal cell divides, the centrioles replicate, then each pair becomes part of a separate centrisome. During cell division the centrosomes move apart so that each new cell has its own centrosome. Plant cells have the equivalent of a centrosome but it does not contain centrioles.

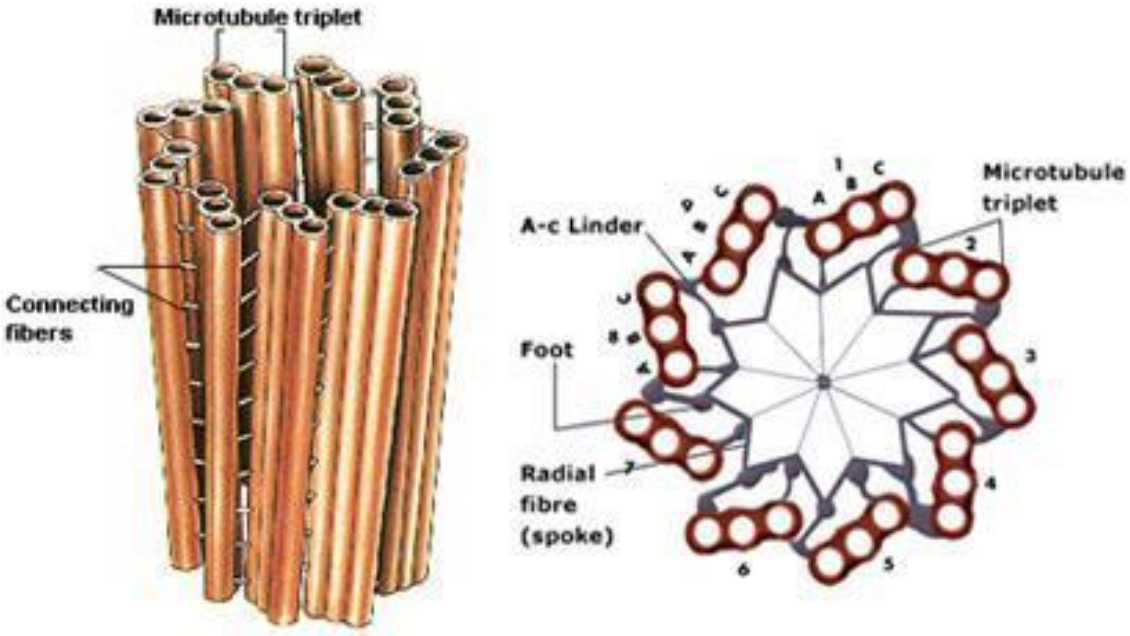


Fig.34: structure of a centriole.

The function of the centriole as microtubule organizing center is to control separation of chromatids or chromosomes by a sliding motion.

Cytoskeleton

The cytoskeleton is a network of fibers throughout the cell's cytoplasm that helps the cell maintain its shape and gives support to the cell. A variety of cellular organelles are held in place by the cytoskeleton.

➢ Cytoskeleton: Distinguishing Characteristics

The cytoskeleton is a network of interconnected filaments and tubules that extends from the nucleus to the plasma membrane in eukaryotic cells.

The cytoskeleton is composed of at least three different types of fibers: **microtubules**, **microfilaments** and **intermediate filaments**. These types are distinguished by their size with microtubules being the thickest and microfilaments being the thinnest (**Fig. 35**).

- Microtubules are hollow rods functioning primarily to help support and shape the cell and as "routes" along which organelles can move. Microtubules are typically found in all eukaryotic cells.
- Microfilaments or actin filaments are solid rods and are active in muscle contraction. Microfilaments are particularly prevalent in muscle cells but similar to microtubules, they are also typically found in all eukaryotic cells.
- Intermediate filaments can be abundant in many cells and provide support for microfilaments and microtubules by holding them in place.

In addition to providing support for the cell, the cytoskeleton is also involved in cellular motility and in moving vesicles within a cell, as well as assisting in the formation of food vacuoles in the cell.

Actin Filaments

Actin filaments or microfilaments are long extremely tin fibers that occur in bundles meshlike network. It contains two chains of globular actin monomers twisted about one another in a helical manner. It plays a structural role and involved in the movement of the cell and its organelles.

Intermediate Filaments

They are rope like assembly of fibrous polypeptides but specific types varies according to the tissue. They are intermediate in size between the actin filaments and microtubules.

Intermediate filament supports the nuclear envelope and plasma membrane and takes part in the formation of cell to cell junction.

In the skin, the intermediate filament is made up of protein keratin which gives mechanical strength to the skin cells.

Microtubules

Microtubules are straight un-branched hollow cylinders which are usually short in length. They occur I most plant and animal cells.

Microtubules are involved in the movement of cytoplasmic components within the cell. They also occur in centrioles, in the spindle, in cilia and flagella and in the basal bodies.

Microtubules are made up of proteins. They help to maintain the shape of the cell and act as routes along which organelles can move.

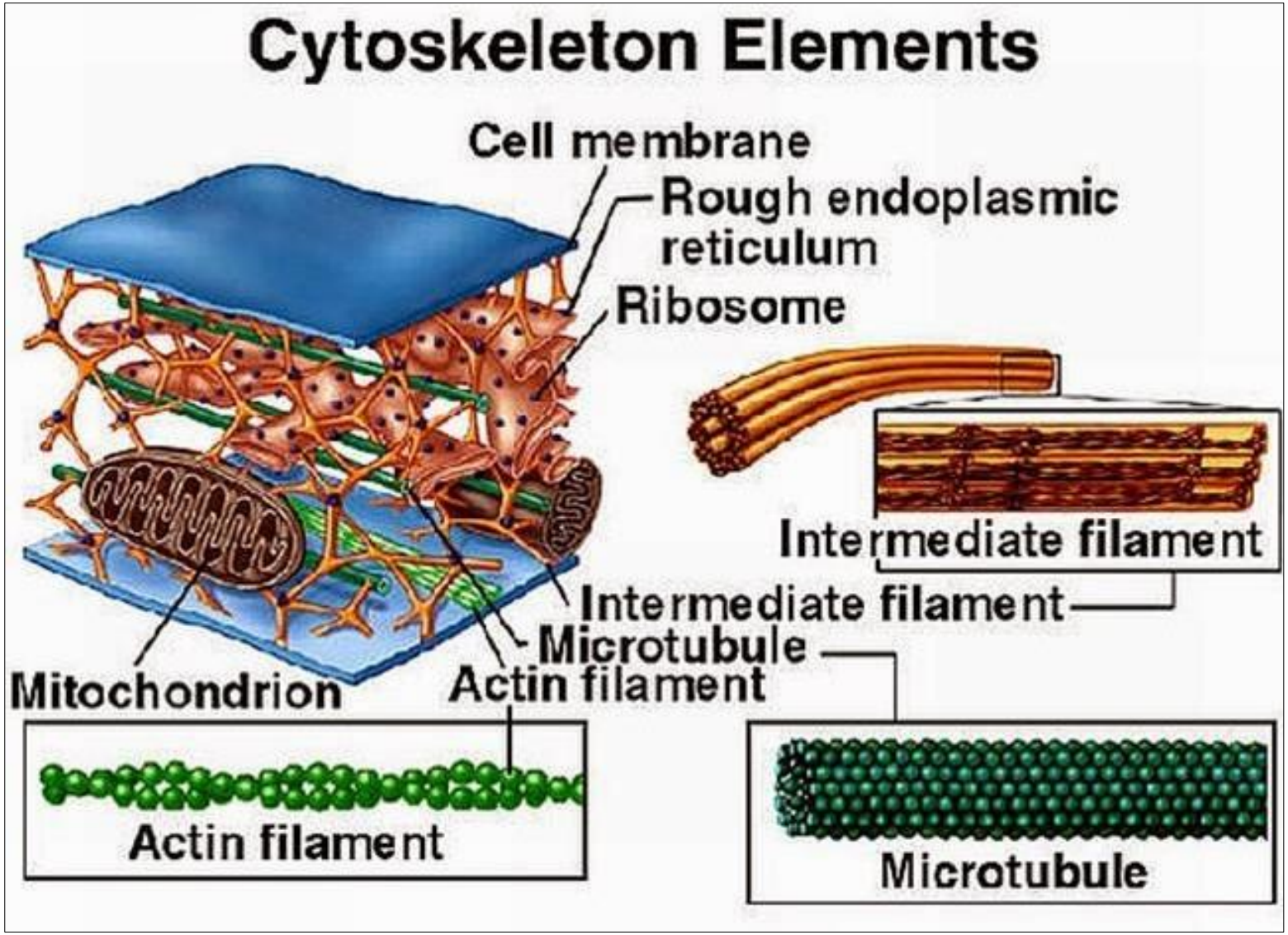


Fig.35:

Cytoskeleton elements.

Cilia and Flagella

Flagella and cilia are organelles that project from the surface of cells but are connected to a basal body just below the plasma membrane. Flagella occur singly or in small number where as cilia occur in large number on large cells and are typically shorter that flagella. Simultaneously, flagella and cilia are almost identical and both are able to move (**Fig.36**).

Flagella and cilia are enclosed to plasma membrane and internally they consist of microtubules arranged in an outer ring of nine pairs surrounding one central pair.

➢ Functions of Cilia and Flagella

1. They contain enzymes that produce energy to move a cell. e.g.: sperm or a unicellular organism such as *Chlamydomonous*.
2. They propel fluids across cells, e.g.: the ciliated cells that move mucus along the bronchial lining.
3. The energy is also used to acquire food, e.g.: feeding current generated by paramecium in its oral groove.
4. They are used to sense the environment, e.g.: sensory hair cells.

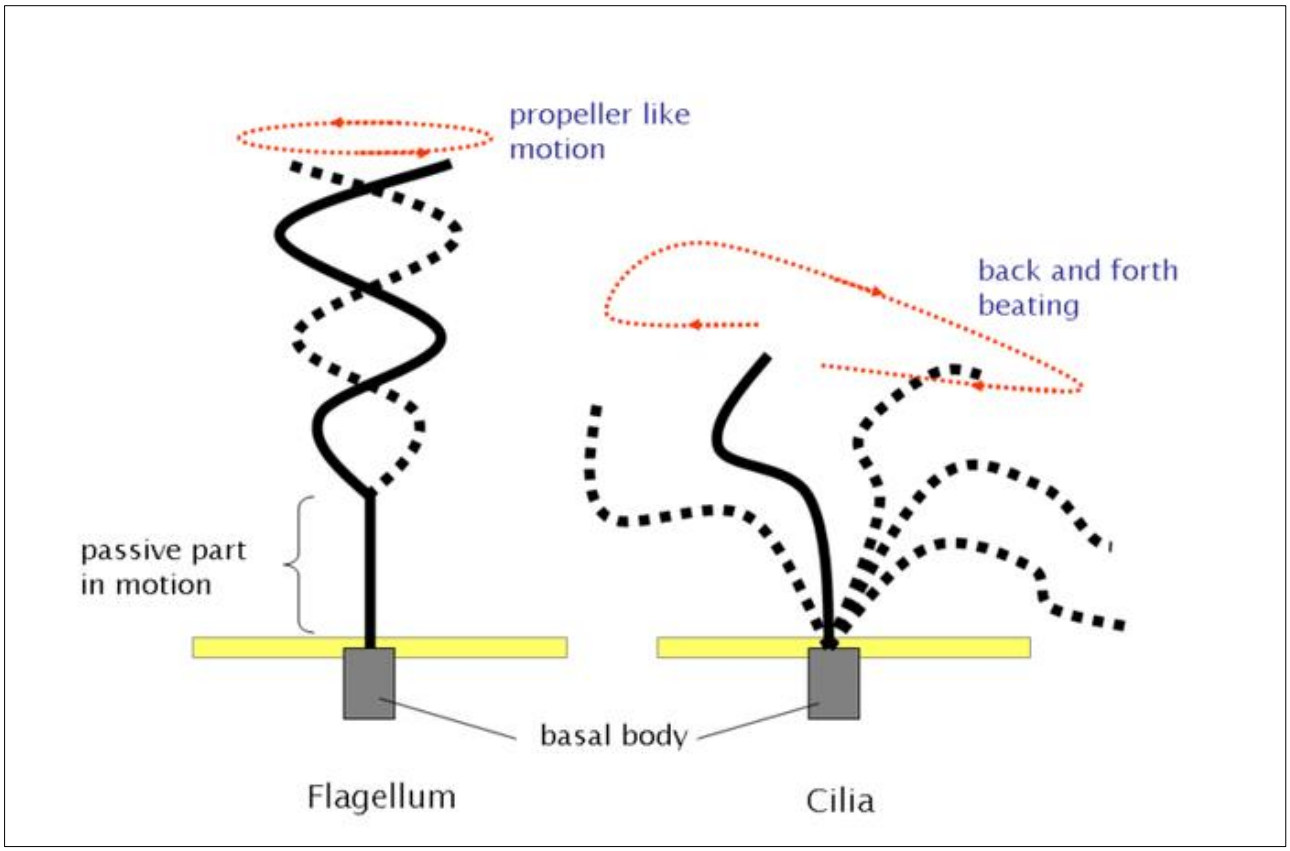


Fig.36: Cilia and flagella.

Cell division

Growth and development of every organism depends in most instances upon multiplication and enlargement of its cells. Sexually reproducing organisms also depend on cell division for gametes formation Division of nucleate cells consists of two distinct but integrated activities, nuclear division (**karyokinesis**) and cytoplasmic division (**cytokinesis**). Two types of nuclear divisions, Mitosis and Meiosis, are characteristic of most plant and animal cells. Mitosis is regularly associated with nuclear division of vegetative or somatic cells and meiosis is associated with formation of reproductive cells.

The Chromatin Material

The term chromatin received its name owing to the high tendency of the chromatin material to stain with basic dyes. The chromatin material acquires different shapes according to the stage of nuclear division in the cell. When the cell is not dividing at the interphase stage, the chromatin is diffusely distributed in the nucleus to form what is known as the chromatin reticulum. When one looks carefully at the nucleus, two types of chromatin can be observed as mention previously: (i) euchromatin, which is threadlike, delicate, and most abundant in active, transcribing cells and includes most of the active genes that are transmitted through the different generations, and (ii) heterochromatin, which is the condensed form of chromatin; it is seen as dense patches of chromatin; sometimes it lines the nuclear enveloped. Heterochromatin is considered transcriptionally inactive and genetically inert (**Fig.37**).

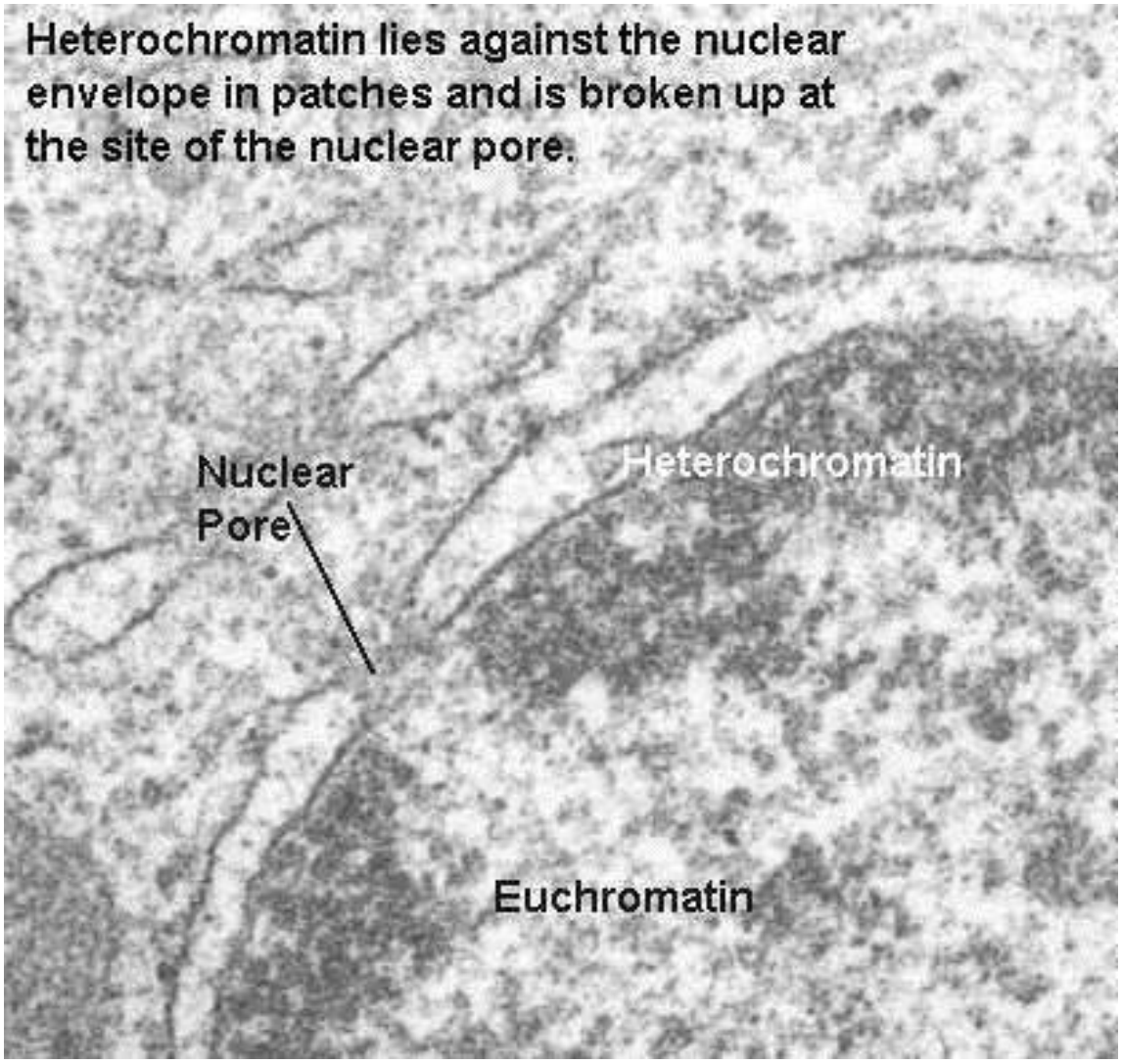


Fig.37: Photograph illustrating part of the nucleus showing euchromatin and heterochromatin as well as a pore in the nuclear envelope.

Mitosis

➢ Overview of Mitosis

1. Mitosis is characteristic cell division of all growing tissues in all eukaryotes.

2. By this process a cell with its nucleus divide precisely into two equivalent daughter cells.

3. The process of mitosis serves the duplication of genes present on the chromosomes.

4. Duplication of genes takes place before the cell division takes place i.e. in the interphase stage.

5. The duplication of the chromosomes during the interphase stage is the result of duplication of the main chemical constituent of the chromosomes, particularly the DNA.

6. During mitosis, the duplicated chromosome number of the parent cell is halved in each daughter cell that contains the same number of chromosomes so that the original diploid number is preserved.

The main stages of mitosis are similar in all plant and animal species, from the least specialized to the most highly evolved forms. Although mitosis is a continuous process it is usually divided into four stages, these are prophase, metaphase, anaphase and telophase (**Fig.38**).

Prophase

During this stage the chromosomes (chromatin fibrils) progressively shortened and thicken to form the individually recognizable, elongate, longitudinally double structures randomly arranged chromosomes in the nucleus. Each chromosome is composed of two chromatids that are closely aligned together and somewhat coiled on themselves. The tightening for these coils contributes in large part to the shortening and thickening of the chromosomes. During the prophase; nucleolus disappear and by the end of the prophase the nuclear membrane also disappears. As the prophase progresses an important and often conspicuous mitotic apparatus begins to form, this is the spindle fibers.

By the end of prophase this structure occupies a large part of the cell volume and extends nearly from one end of the cell to the other end. The spindle fibers consist of slender microtubules arranged along the long axis of the cell. As prophase progresses, the longitudinally double chromosomes move in the direction of the midplane or equator of the

developing spindle. This is a period of time often referred to as prometaphase or premetaphase. During this period the centromeres connect themselves to the spindle fibers in the equatorial region.

Metaphase

Metaphase is the stage of mitosis in which the centromeres of the longitudinally double chromosomes connected to the spindle fibres and occupy the plane or the equator of the cell. During this stage the chromatids of each chromosome are held together by chromatin fibres connecting their centromeres. Although metaphase is a physically static stage, biochemical changes may be occurring which lead, after a period of time, to sudden termination of the metaphase and the initiation of anaphase. During metaphase the chromosomes are at their shortest and thickest state and polar view may permit their counting.

Anaphase

Anaphase is the stage during which the metaphase sister chromatids are separated and passed as daughter chromosomes to the spindle poles. It begins at the moment when the centromeres of each of the sister chromatids become functionally double and ends with the arrival of daughter chromosome**s** to the poles**.** Anaphase therefore accomplishes the quantitatively equal distribution of chromosomal material to two developing daughter nuclei. The most satisfactory explanation of the mechanism of anaphase movement lies in the ability of the centromers to slide past the continuous spindle fibers, dragging their attached daughter chromosomes with them. In the meantime the equatorial region of the spindle itself begins to stretch and elongate; so that the distance between the poles increases.

Telophase

The arrival of the daughter chromosome**s** to the spindle poles marks the beginning of telophase. It is, in turn, terminated by the reorganization of two new nuclei. In general terms it is believed that during telophase, the events of prophase occur in reverse sequence. New nuclear membranes are formed from materials which may be remnants of the original membrane or derived from the endoplasmic reticulum or newly synthesized from appropriate cell component. The mitotic apparatus gradually disappears, the nucleoli are reformed and the chromosomes resume their long, slender extended form as their coils and relax to form chromatin reticulum.

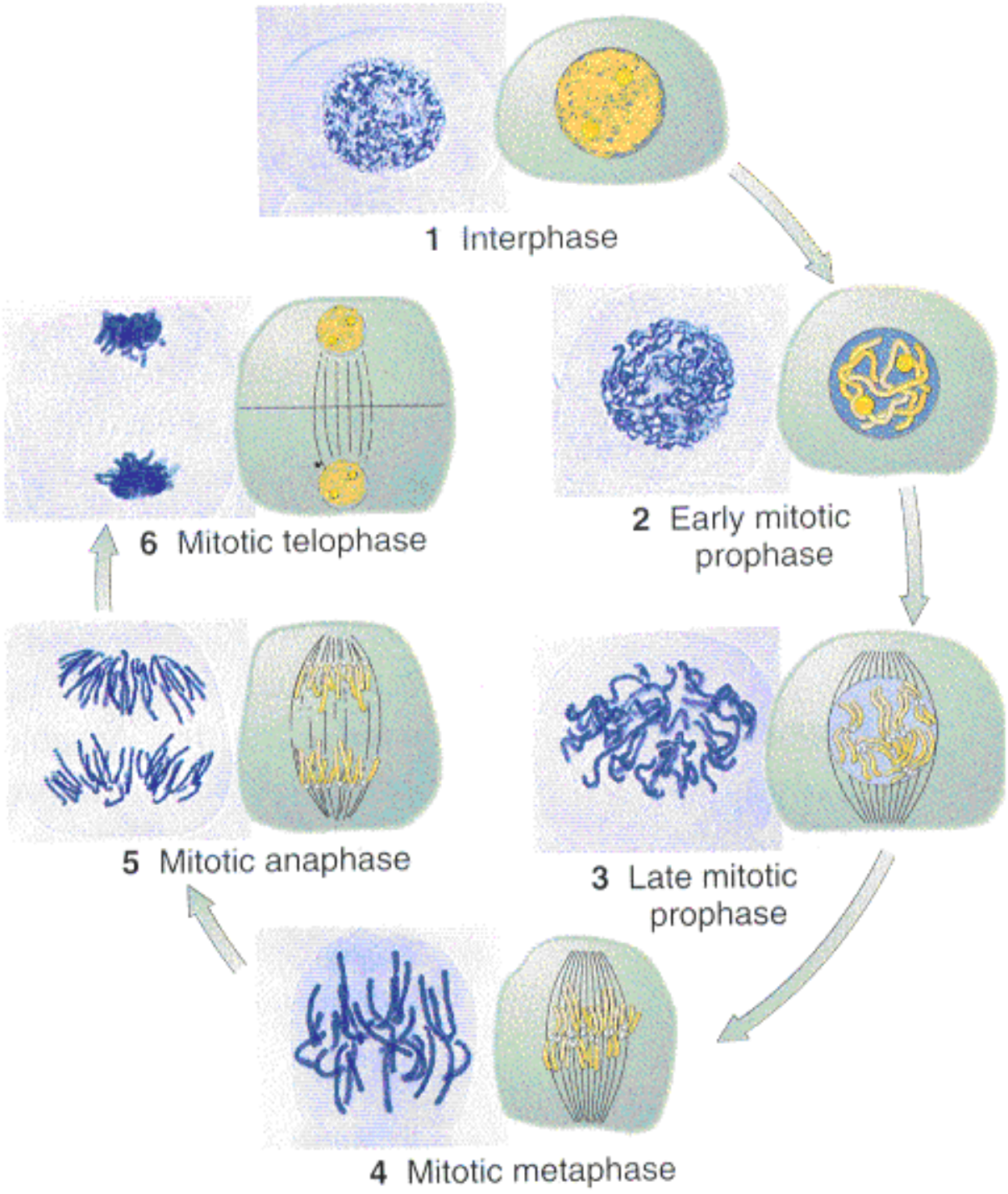


Fig.38: The stages of mitosis; photos are mitotic stages in *Lilium regale*.

Cytokinesis

Cytokinesis takes place during telophase and accomplishes by the formation of cell plate. It is formed by the formation of vesicles in the midplane of the mitotic apparatus, starting at the middle of the spindle to form a phragmoplast which gradually extend centrifugally dividing the cytoplasm into two parts before the spindle disappears. The cell plate is then converted into a middle lamella with new walls deposited between the daughter cells on each side of the middle lamella.

Mitotic cell cycle

When somatic cells are grown, they establish a repeated pattern of growth and duplication. The mitotic cycle consists of interphase, in which the chromosomes are not visible and the division stage (mitosis). Interphase is divided into three stages; the G1 stage, S-stage and G2-stage (**Fig.39**).

The G1 stage

After mitosis interphase commences with a period referred to as the first gap (G1 phase). During this stage chromosomes are fully extended and the genes are active sending messages for new enzymes to carry out the activities of the cell. G1 phase usually lasts from 10 to 24 hours, but can vary from virtual non-existence to several days. After this period DNA replication begins and the S-period starts.

The S-stage

This is a period during which DNA replication takes place and the chromosome number is doubled; it lasts 5–10 hours. During S-period not all growth stops because not all genes replicate at the same time. The protein component of the chromosomes is also duplicated so that at the

end of the S-stage each chromosome is double i.e., made of two chromatids.

The G2 stage

This is a second gap period after replication. This stage lasts of about 4 hours and the genes are again fully functional. G2**-**period is followed by the four mitotic stages; prophase, metaphase, anaphase and telophase; during which duplicated chromosomes condense and the identical halves (sister chromatids) separate equally into two daughter nuclei. Mitosis usually lasts from 1–5 hours.

Cell cycle and the DNA C-value

Following mitosis the daughter cells enter the G1 period and have a DNA content equivalent to 2C (**Fig.40**). All diploid organisms at this stage contain 2C DNA content because the two homologous chromosomes are present as single chromatids. During the S-stage the DNA is doubled and during the G2, cells contain 2 times the amount of DNA present in the original G1 cell (4C). Gametes are haploid and therefore have half the DNA content (1C). Some tissue, like liver, and many plants contains occasional cells that are polyploid and their nuclei have a correspondingly higher DNA. Each species has a characteristic content of DNA in the chromatids, which is constant and has thus been called the C-value. Eukaryotes vary greatly in DNA content but always contain much more DNA than prokaryotes.

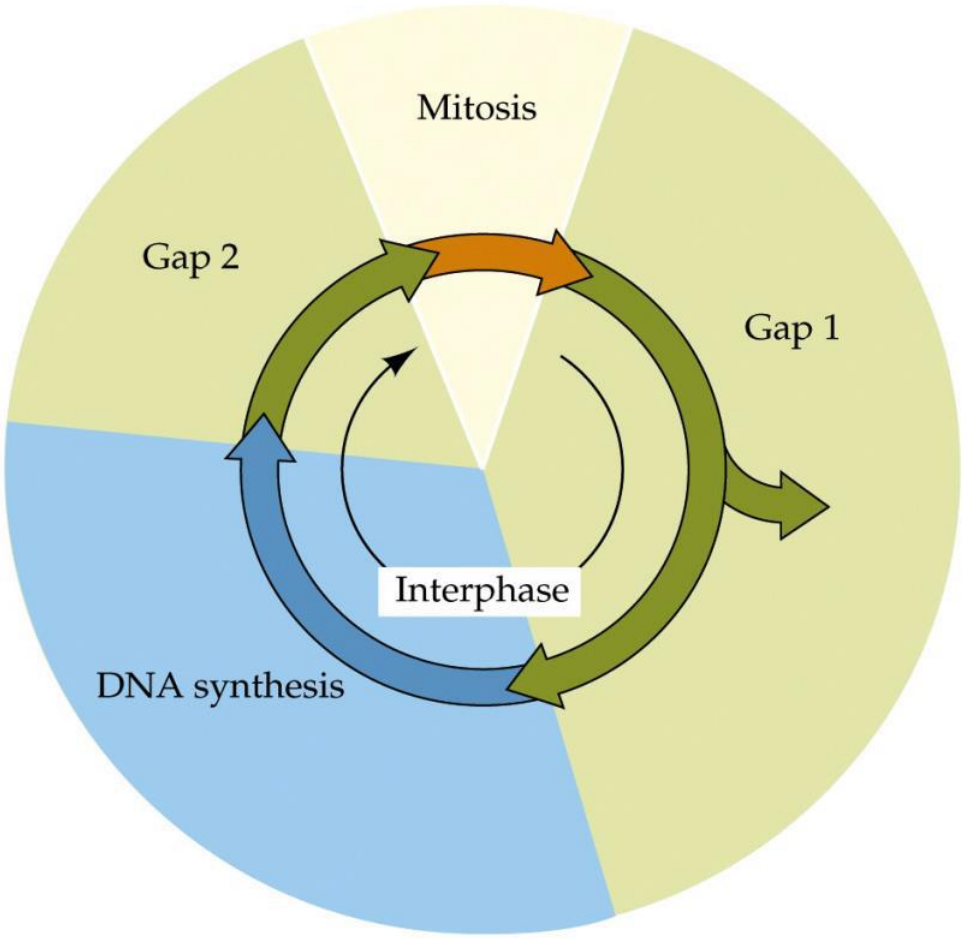


Fig.39: Diagrammatic representations of cell cycle stages.

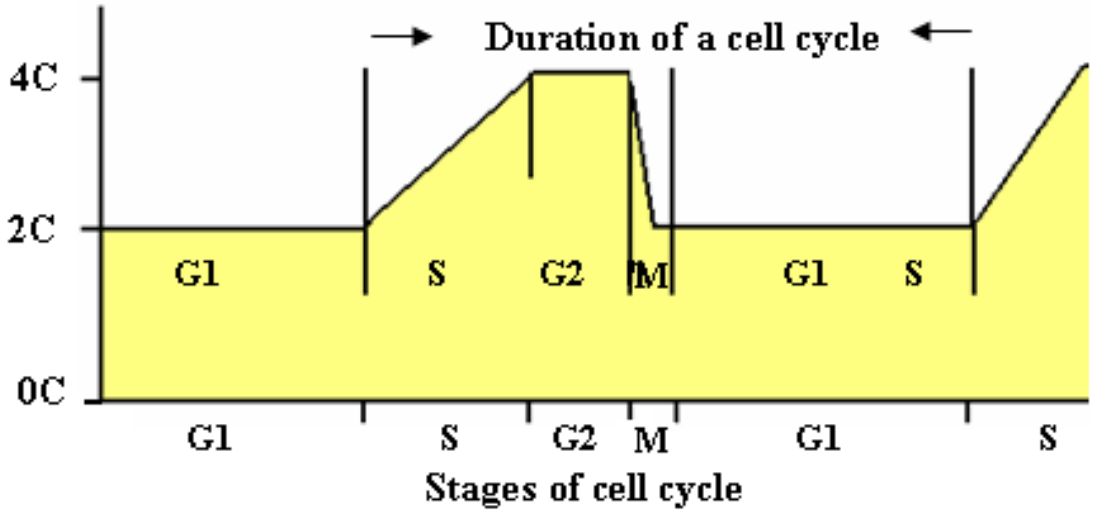


Fig.40: Diagrammatic representations of the changes in the C value during the stages of the mitotic cell cycle.

Meiosis

Studies from as long ago as last century; indicated that in the gametic union (syngamy) the chromosomes contributed by each gamete retain their separate identities in the zygote nucleus. The zygote therefore contains twice as many chromosomes as does a gamete. This fact is responsible for the occurrence in the diploid or 2*n* cells (the somatic cells) of matched pairs of homologous chromosomes.

Meiosis results in the formation of haploid gametes. During meiosis, homologous chromosomes replicate, then pair, then undergo two divisions. As a consequence, each of the four cells resulting from the two meiotic divisions receives one chromosome of each chromosome set. The two nuclear division of a normal meiosis are called meiosis I and meiosis II.

Meiosis is quite similar to mitosis. However, two cell divisions take place following one DNA replication step. Instead of having a pair of genes (as in a diploid cell), there is only one copy of each gene (a haploid cell). This one copy of genetic information is produced in gametes of either sperm or eggs. Thus, only one copy of a gene is passed on to each gamete. This process is the basis for all of **Mendel's laws**.

During meiosis the nucleus of a diploid cell actually undergoes two divisions which results in the production of four haploid daughter nuclei. As in the cell cycle the chromatin has already been doubled in the preceding interphase. Therefore two identical chromatids are attached together at the centromere.

Overview of meiosis

Prior to meiosis I, DNA replication occurs and each chromosome has two sister chromatids. During meiosis I, homologous chromosomes pair, i.e. come together and line up in synapsis. During synapsis the two sets of paired chromosomes lay alongside each other as bivalents. While paired up, the chromosomes exchange equal segments of genetic material; this is called crossing over. After crossing over occurs, sister chromatids are no longer identical. During Meiosis II, no replication of DNA occurs between meiosis I and meiosis II. During meiosis II centromeres divide and sister chromatids separate. Chromosomes in the four daughter cells have only one chromatid; the following points are the major highlights of meiosis.

1. Meiosis keeps the number of somatic chromosome constant across generations.

2. Meiosis ensures that each gamete contains only one member of each homologous pair.

3. Meiosis produces new combinations of genes through recombination of two genomes.

4. Meiosis involves two nuclear divisions; it produces four haploid daughter cells, each containing half the total number of chromosomes as the diploid parent nucleus.

5. The two phases of meiosis are designated by roman numerals; Meiosis I and Meiosis II.

Meiosis I; The first meiotic division:

In this division the chromosome number is reduced from diploid to haploid. It consists of four cytologically distinguishable stages: prophase I, metaphase I, anaphase I, and telophase I. Prophase I begins after the chromosomes have already been replicated). In prophase I the

chromosomes shorten and thicken; they pair off, crossing over occurs, the spindle apparatus forms, and the nuclear membrane and nucleolus disappear. Prophase I is divided into five stages based on the behavior of homologous pairs of chromosomes, and the occurrence of crossing-over (**Fig.41**).

Leptotene (Leptonema)

In letoptene the chromosomes begin to coil; a key event in leptotene is pairing of homologous chromosomes; that is, loose alignment of homologous region of the two chromosomes.

Zygotene (Zygonema)

During this stage the homologous chromosomes come together and become closely associated through their entire length. This process is called pairing or synapsis. The pairing is very intimate and is not only between homologous chromosomes as a whole but between strictly homologous regions of the homologous chromosomes. Synapsis is therefore important for determining the degree of resemblance between the chromosomes of organisms brought together in a hybrid combination. By the end of zygotene, synapsis is complete and in a diploid organism all the chromosomes become associated in pairs known as bivalents. There will be half as many bivalents as there were chromosomes at leptotene. Once pairing has been completed, a most significant event begins. Crossing over, the reciprocal exchange of chromosomes segments at corresponding positions along pairs of homologous chromosomes. If there are genetic differences between the homologous chromosomes, crossing-over can produce new gene combinations in a chromatid.

Pachytene (Pachynema)

This stage begins as soon as the synapsis is complete. If parts of the chromosomes are still unpaired they remain so. Pachytene is the longest stage of first prophase of meiosis. During this stage the bivalents condense by developing a complex spiral structure. They appear thick and bivalent can be recognized as two chromosomes. The nucleoli are seen at this stage attached to certain chromosomes at the nucleolar organizer region. Pachytene bivalents are theoretically four stranded i.e. they are formed of four parallel chromatids, but it is only at a latter stage that quadripartite structure is visible. The split which separates the two chromatids of each chromosome never appears until the very end of pachytene. Pachytene is terminated when the paired chromosomes start to separate.

Diplotene (Diplonema)

As soon as the paired homologous chromosomes begin to separate diplotene is said to have begun. They separate completely from one another except in some places in each bivalent where non sister chromatids are associated. Each of these configurations is known as **chiasma**. Because the sister chromatids of any chromosome do not separate latterly to any great extent the chiasma is a point of chromatin exchange that also helps to preserve the bivalent structure. **Chiasmata** are the cytological expression of genetic crossing over. During diplotene the chromosomes are still actively shortening and their coiled nature is apparent.

Diakinesis

The distinction between dipiotene and diakinesis may not be a sharp one. The transition to diakinesis involves a gradual thickening and

shortening of the chromosomes. The chiasmata rotate relative to one another through 90o from thee plane of the early diplotene bivalent. As the process of rotation is occurring there is in some plants and animals, a tendency for the chiasmata or some of them, to slip along towards the ends of the bivalents. This process of terminalization is far from universal. As a result of this process the chiasmata may actually reach the end of the bivalent. Diakinesis is also characterized by the disappearance of detachment of the nucleolus from its associated chromosomes and by the even distribution of bivalents throughout the nucleus.

Metaphase 1

After the breakdown of the nuclear membrane and the formation of the spindle fibres the bivalents attach themselves to the spindle fibres; the two centromeres of each bivalent become located on opposite sides of the equatorial plane one above and one below it. This is an essential difference between the first meiotic division and the ordinary mitosis, where the centromeres are oriented exactly on the equator, connected by spindle fibers toboth poles. The distance between homologous centromeres of each bivalent is regulated by the position of the nearest chiasma to the centromere. If there is a chiasma near the centromere on both sides they will lie near one another, one tightly below and one slightly above the equator. Like metaphase of mitosis metaphase I of meiosis is a relatively static stage. During this stage the chromosomes reach their maximum condensation.

Anaphase 1

The movement of chromosomes form the metaphase plate to the poles constitutes anaphase. A characteristic feature of anaphase 1 is that the two centromeres of the bivalent do not divide. Instead each

whole centromere moves towards the nearest pole dragging after it the two chromatids attached to it. Each anaphase group, therefore, is made of a haploid number of chromosomes instead of a diploid number of chromatids. It is by this way that a reduction in chromosome number results from the first meiotic division. The chromosomes which are separated at anaphase I are not genetically the same maternal chromosomes that came together during synapsis because they have changed segments of their length by crossing over, so that the actual chromosomes which are separated at anaphase I have new combinations of genes.

Telophase 1

The telophase of the first meiotic division does not differ essentially from the chromosomes of mitosis. However, the chromatids of each chromosome are widely separated. During telophase I, in most organisms, the nuclear membrane is reformed and a regrouping of the coiled structure of the chromosomes takes place.

Cytokinesis may or may not occur after meiosis I. In some plants such as maize the two telophase nuclei pass into a definite interchange stage (interkinesis) between the two meiotic divisions. In other organisms, e.g. *Trillium* the telophase nuclei pass directly into the prophase of the second meiotic division.

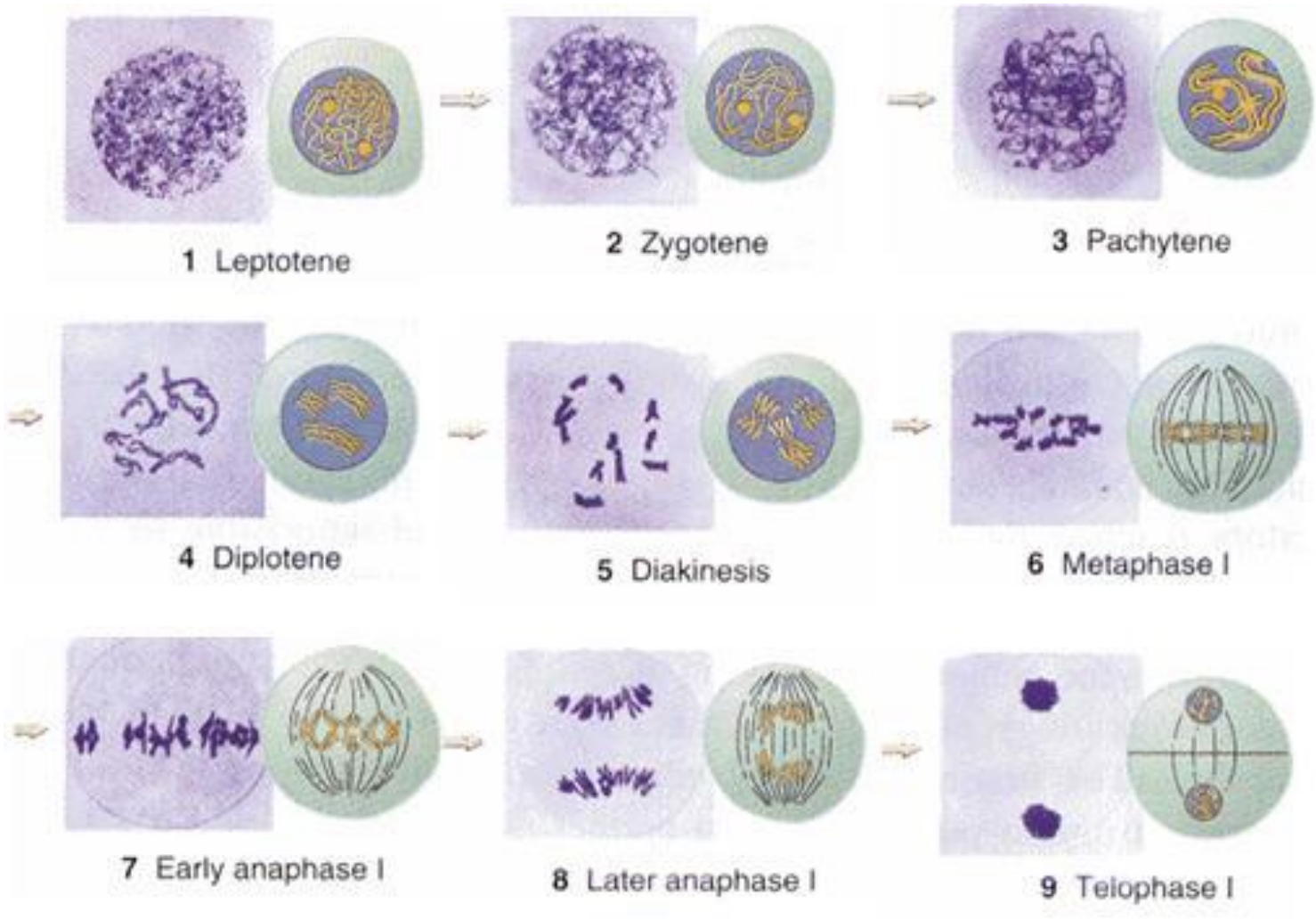


Fig.41: Photographs and diagrammatic representation illustrating the stages of Meiosis I (After Russel, 2009).

Second meiotic division (Meiosis II)

This division is essentially the same as mitosis and is comprised from the basic stages of mitotic division that may be described as follows and its stages are illustrated in **Fig.42**.

Prophase II

Nuclear envelope and nucleolus disappear and chromatin condenses into chromosomes.

Metaphase II

Spindle fibers form and attaches to centromere. Chromosomes, each consisting of two sister chromatids, line up with their centromeres on the metaphase plate.

Anaphase II:

Centromeres separate and daughter chromosomes move towards the poles of the cell.

Telophase II

Chromosomes decondense, nuclear envelope and nucleolus reform and cytokinesis produces four daughter cells.

Meiosis II is basically a mitotic division. The prophase is always short and does not involve any of the complications which occur in the prophase of the first meiotic division. The spindle fibers of the second meiotic division are rapidly formed and the metaphase stage is reached. There are four differences which distinguish the second meiotic division from a mitotic division.

1. The number of chromosomes is half the somatic number.

2. No period of DNA synthesis precedes the division.

3. The chromatids are widely separated being held together at the centromere and not throughout their length as at mitosis.

4. Each chromatid might be quite different genetically from its condition at the initiation of the meiotic process**.**

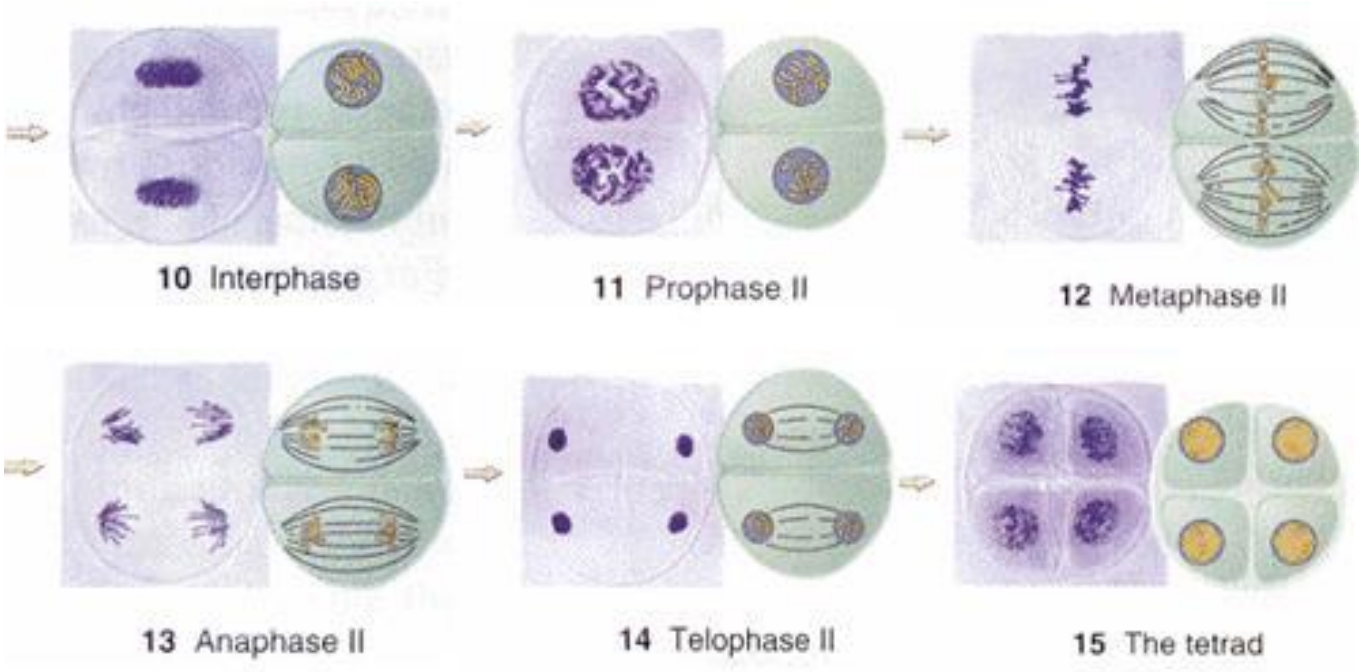


Fig.42: Photographs and diagrammatic illustrations of the stages of Meiosis II.

Cell Death

Programmed cell death is a controlled mechanism that eliminates specific cells under developmental or environmental stimuli. All organisms—from bacteria to multicellular eukaryotes—have the ability to induce Programmed cell death in selected cells. Although this process was first identified in plants, the interest in deciphering the signaling pathways leading to Programmed cell death strongly increased when evidence came to light that Programmed cell death may be involved in several human diseases. In plants, programmed cell death activation ensures the correct occurrence of growth and developmental processes, among which embryogenesis and differentiation of tracheary elements. Programmed cell death is also part of the defense responses activated by plants against environmental stresses, both abiotic and biotic.

Programmed cell death plays a fundamental role in plant life. It is involved in common and specific organ shaping and morphological adaptive responses, as well as in defense strategies activated against abiotic and biotic injuries. Programmed cell death induction and actuation show specific hallmarks in plant systems. The overview of the plant Programmed cell death process reported in this chapter highlights the existence of different subtypes and focuses on the functional role of Programmed cell death in developmental patterns and plant–environment interactions. The markers and signaling steps are not always specific for a certain subtype of Programmed cell death.

Apoptosis: Type of programmed cell death that was identified due to a particular pattern of morphologic changes but now is defined by the action of molecular pathways involving cell surface receptors or mitochondria and resulting in the activation of specialized proteases. The name comes from the Greek, referring to shedding of the petals from flowers or leaves from trees. Apoptotic death occurs in two phases. During the latent phase, the cell looks morphologically normal but is committed to death. The execution phase is characterized by a series of dramatic structural and biochemical changes that culminate in fragmentation of the cell into membrane-enclosed apoptotic bodies. Activities that cause cells to undergo apoptosis are said to be proapoptotic. Activities that protect cells from apoptosis are said to be antiapoptotic.

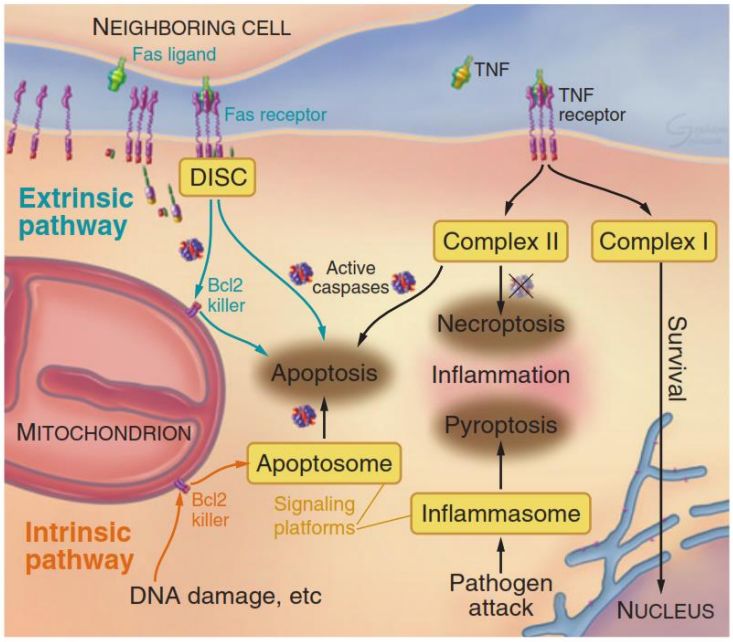
Autophagic Cell Death: It is still debated how widely Cell shrinks Chromatin condenses around nuclear periphery autophagy is used as a pathway for cell death, although Cell blebs violently Chromatin condensation continues Cell fragments into membrane-enclosed apoptotic bodies it is accepted that the pathway (which is widely assumed to be primarily a survival pathway when cells are starved for nutrients) can promote cell death during development. Autophagy may also either promote or inhibit apoptosis under specialized circumstances.

Necroptosis: Programmed necrosis that occurs when tumor necrosis factor (TNF) and certain another cell surface receptors are activated. Activation of these receptors normally leads to a proinflammatory response and cell survival but can lead to apoptosis. If certain components of the apoptotic pathway are missing, cells instead undergo necroptosis, apparently as a backup pathway.

Necrosis (Accidental Cell Death): Death that results from irreversible injury to the cell. Cell membranes swell and become permeable. Lytic enzymes destroy the cellular contents, which then leak out into the intercellular space, leading to an inflammatory response.

Programmed Cell Death: Any active cellular process that culminates in cell death. This may occur in response to developmental or environmental cues or as a response to physiological damage detected by the cell's internal surveillance networks.

Pyroptosis: Often in response to intracellular pathogens, this involves activation of caspase 1. The infected cells secrete interleukin-1β and interleukin-18, which promote an inflammatory response, and also undergo a form of cell death that resembles necrosis.



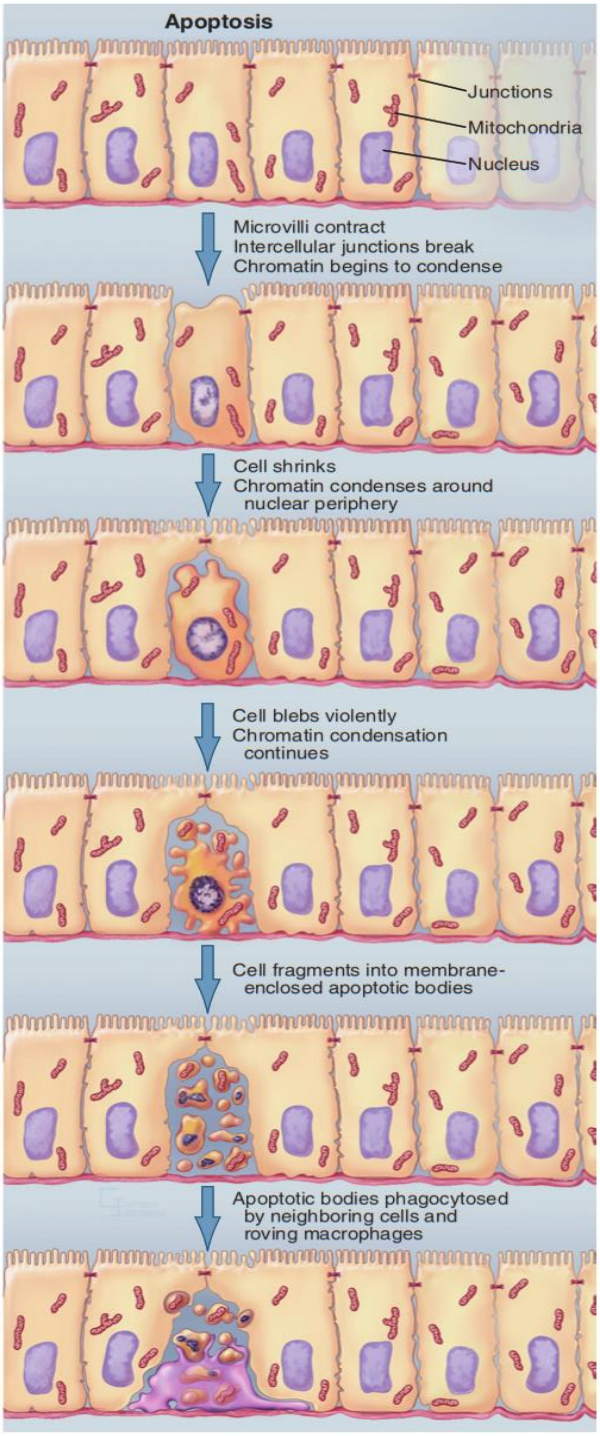


Fig. 43: Overview of programmed cell death

Cell Signaling

Individual cells, like multicellular organisms, need to sense and respond to their environment. A free-living cell—even a humble bacterium—must be able to track down nutrients, tell the difference between light and dark, and avoid poisons and predators. And if such a cell is to have any kind of “social life,” it must be able to communicate with other cells.

In a multicellular organism, things are much more complicated. Cells must interpret the multitude of signals they receive from other cells to help coordinate their behaviors. During animal development, for example, cells in the embryo exchange signals to determine which specialized role each cell will adopt, what position it will occupy in the animal, and whether it will survive, divide, or die. Later in life, a large variety of signals coordinates the animal’s growth and its day-to-day physiology and behavior. In plants as well, cells are in constant communication with one another. These cell–cell interactions allow the plant to coordinate what happens in its roots, stems, and leaves.

GENERAL PRINCIPLES OF CELL SIGNALING

Information can come in a variety of forms, and communication frequently involves converting the signals that carry that information from one form to another. When you receive a call from a friend on your mobile phone, for instance, the phone converts the radio signals, which travel through the air, into sound waves, which you hear. This process of conversion is called **signal transduction**.

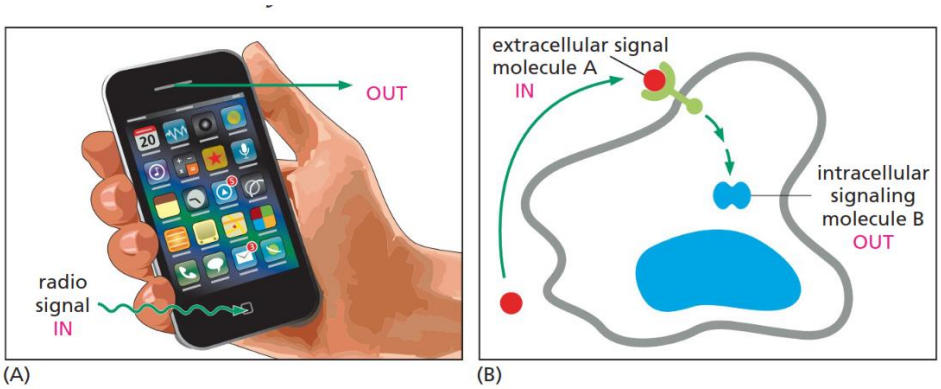


Fig. **Signal transduction** is the process whereby one type of signal is converted to another. (a) When a mobile telephone receives a radio signal, it converts it into a sound signal; when transmitting a signal, it does the reverse. (B) a target cell converts an extracellular signal molecule (molecule a) into an intracellular signaling molecule (molecule B).

The signals that pass between cells are simpler than the sorts of messages that humans ordinarily exchange. In a typical communication between cells, the signaling cell produces a particular type of extracellular signal molecule that is detected by the target cell. As in human conversation, most animal cells both send and receive signals, and they can therefore act as both signaling cells and target cells.

Target cells possess proteins called receptors that recognize and respond specifically to the signal molecule. Signal transduction begins when the receptor on a target cell receives an incoming extracellular signal and converts it to the intracellular signaling molecules that alter cell behavior. Most of this chapter is concerned with signal reception and transduction—the events that cell biologists have in mind when they refer to cell signaling. First, however, we look briefly at the different types of extracellular signals that cells send to one another.

Signals Can Act over a Long or Short Range

Cells in multicellular organisms use hundreds of kinds of extracellular signal molecules to communicate with one another. The signal molecules can be proteins, peptides, amino acids, nucleotides, steroids,

fatty acid derivatives, or even dissolved gases—but they all rely on only a handful of basic styles of communication for getting the message across. In multicellular organisms, the most “public” style of cell-to-cell communication involves broadcasting the signal throughout the whole body by secreting it into an animal’s bloodstream or a plant’s sap. Extracellular signal molecules used in this way are called hormones, and, in animals, the cells that produce hormones are called endocrine cells (Figure 16–3a). Part of the pancreas, for example, is an endocrine gland that produces several hormones—including insulin, which regulates glucose uptake in cells all over the body.

Somewhat less public is the process known as paracrine signaling. In this case, rather than entering the bloodstream, the signal molecules diffuse locally through the extracellular fluid, remaining in the neighborhood of the cell that secretes them. Thus, they act as local mediators on nearby cells (Figure 16–3B). Many of the signal molecules that regulate inflammation at the site of an infection or that control cell proliferation in a healing wound function in this way. In some cases, cells can respond to the local mediators that they themselves produce, a form of paracrine communication called autocrine signaling; cancer cells sometimes promote their own survival and proliferation in this way.

Neuronal signaling is a third form of cell communication. Like endocrine cells, nerve cells (neurons) can deliver messages over long distances. In the case of neuronal signaling, however, a message is not broadcast widely but is instead delivered quickly and specifically to individual target cells through private lines. The axon of a neuron terminates at specialized junctions (synapses) on target cells that can lie far from the neuronal cell body (Figure 16–3C). A fourth style of signal-mediated cell-to-cell communication—the most intimate and short-range

of all—does not require the release of a secreted molecule. Instead, the cells make direct physical contact through signal molecules lodged in the plasma membrane of the signaling cell and receptor proteins embedded in the plasma membrane of the target cell (Figure 16–3D).

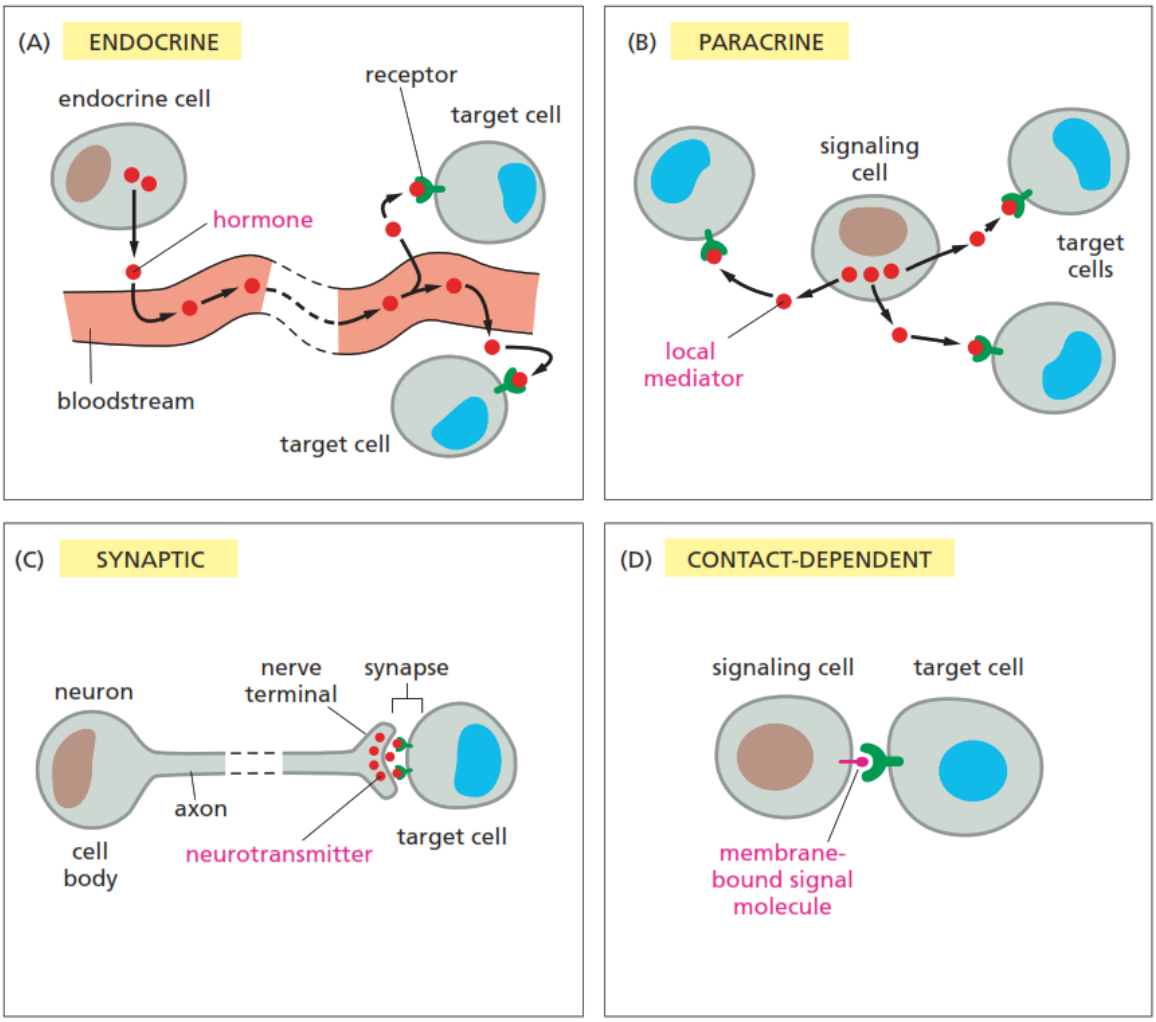


Figure 16–3 animal cells use extracellular signal molecules to communicate with one another in various ways. (a) hormones produced in endocrine glands are secreted into the bloodstream and are distributed widely throughout the body. (B) paracrine signals are released by cells into the extracellular fluid in their neighborhood and act locally. (c) Neuronal signals are transmitted electrically along a nerve cell axon. When this electrical signal reaches the nerve terminal, it causes the release of neurotransmitters onto adjacent target cells. (D) In contact-dependent signaling, a cell-surface-bound signal molecule binds to a receptor protein on an adjacent cell. Many of the same types of signal molecules are used for endocrine, paracrine, and neuronal signaling. the crucial differences lie in the speed and selectivity with which the signals are delivered to their targets.

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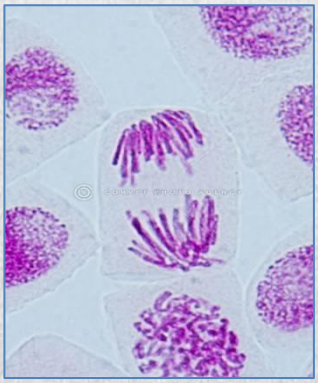
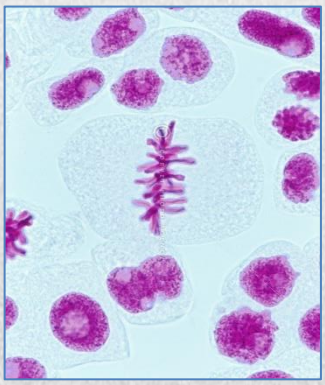
Practical Cell Biology





2nd year students

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Botany and Microbiology Department

2022-2023

Index

Laboratory Safety

Safety in the Laboratory Should always is in your mind. Throughout this manual Safety recommendations are given, below are some general consideration that anyone in a laboratory should know.

• General laboratory safety precaution.

1. Follow all instructions carefully. Use special care when you see the word CAUTION in your laboratory instructions. Follow the safety instructions given by your teacher.
2. Determine the location of Fire Extinguishers, Chemical safety showers and Eye washers, Chemical Spill Kits, and alternative exit routes for lab evacuation.
3. Remember that smoking, eating, or drinking in the lab room is totally prohibited.
4. Wear lab aprons when working with chemicals, hot material, or preserved specimens.
5. Wear safety goggles when using dangerous chemicals, hot liquids, or burners.
6. Any chemicals spilled on the hands or other parts of the skin should be washed off immediately with a plenty of running water.
7. If you have an open skin wound, be sure that it is covered with a waterproof bandage.
8. Never work alone in the laboratory.
9. Keep your work area clean & dry.
10. Turn of all electrical equipment, water, and gas when it is not in use, especially at the end of the laboratory period.
11. Tie back long hair.
12. Report all chemicals spills or fluids to your instructor immediately for proper clean up.

• Special precautions for working with heat or fire:

1. Never leave a lighted Bunsen burner of hot object unattended. When an object is removed from the heat & left to cool, it should be place where it is shielded from contact.
2. Inflammable liquid bottles should not be left open, not dispensed near a naked flame, hot electric element or electric motor.
3. Use test tube holders to handle hot laboratory equipments.
4. When you are heating something in a container such as a test tube, always point the open end of the container away from yourself & others.
5. Use only Pyrex glassware's for heating.
6. Allow hot materials to cool before moving them from your lab station.
7. Make sure that Bunsen burner hoses fit tightly.

• Special precautions for working with chemicals

1. Never taste or touch substances in the laboratory without specific instructions.
2. Never smell substances in the laboratory without specific instructions.
3. Use materials only from containers that are properly labeled.
4. Wash your hand after working with chemicals.
5. Do not add water to acid. Instead, dilute the acid by adding it to water.
6. Mix heat generating chemicals slowly.

• Special precautions for working with electrical equipment.

1. Make sure the area under & around the electrical equipment is dry.
2. Never touch electrical equipment with wet hands.
3. Make sure the area surrounding the electrical equipment is free of flammable materials.
4. Turn off all power switches before plugging an appliance into an outlet.

• **Special Precaution for working with Glassware's and other laboratory equipments.**

1. Become familiar with the names and appearance of all the laboratory equipments you will use.
2. Never use broken or chipped glassware.
3. Make sure that all glassware's are clean before you using it.
4. Do not pick up broken glass with your bare hands. Use a pan and a brush.
5. If a Mercury thermometer breaks, do not touch the mercury. Notify your teacher immediately.
6. Do not aim the mirror of your microscope directly at the sun. Direct sun light can damage the eyes.
7. Use care handling all sharp equipments, such as scalpels and dissecting needles.

• Special precautions for working with live or preserved specimens.

1. If live animals are used treat them gently. Follow instructions for their proper care.
2. Always wash your hands after working with live or preserved organisms.
3. Specimens for dissection should be properly mounted and supported. Do not try to cut a specimen while holding it in the air.
4. Do not open Petri dishes containing live cultures unless you are directed to do so.
5. Detergents (detol 5 – 10%) should be used to sterilize and clean benches, glassware and equipment.
6. Safety cabinet should be used while working with microbes.
7. Lab coats should be worn during the work in the lab.
8. Disposable items should be collected and autoclaved.

First Aid

1. Injuries: bleeding should be reduced using bandages; the wound should be cleaned with iodine alcohol mixture and wrapped with sterile bandage.
2. Acid and fire burns: body burns must be washed immediately with tap water. Eye burns must be washed using eye washer, special cream for burns can be used.
3. Poisoning: if any toxic chemical is swallowed, the mouth must be sensed with water, in case of acid, milk is drunk, in case of alkaline, diluted acetic acid (vinegar) can be used.
4. Skin contamination requires washing with water and removal of contaminated clothing, if the contaminant is insoluble in water remove with soap and water.

The Microscope

Highlights:

This Exercise focuses on how to develop a working knowledge of the Microscope and its use. Students should identify the different parts of the Microscope. List and follow recommended procedures in using and caring for the Microscope.

Material:

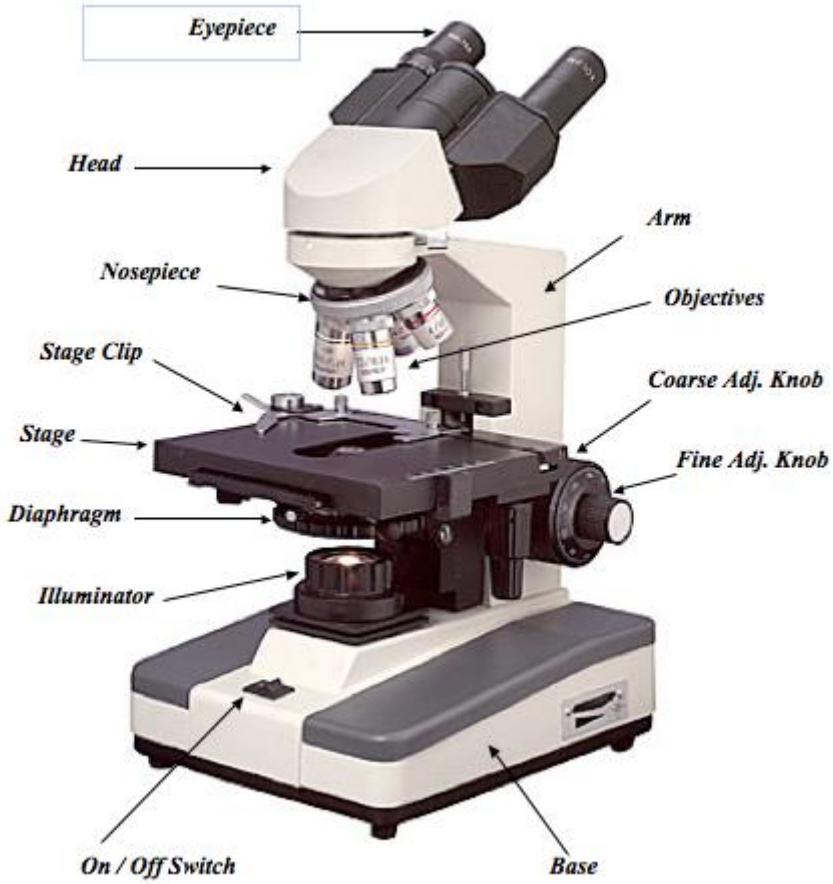
Compound Microscope
Clean Microscope Slides
Cover Slips
Lens papers
Sharp razor blades
Medicine droppers
Scissors
Distilled water
Xylene

Introduction:

Since an unaided eye cannot detect anything smaller than 0.1 mm in diameter, cells, tissues, and many small organisms are beyond our visual capability, so we need equipment to magnified objects which is too small to be seen with unaided eye. There are several types of microscopes but the only one used in this laboratory is the compound light microscope. The compound microscope (sometimes called the student microscope or light microscope); these microscopes are known as compound microscope because there are two magnifying lenses in the microscope. One magnifying lens is in the ocular or eyepiece, which further magnifies the image formed by the objective lens, and one, is in

the objective. Each contributes to the magnification of the object on the stage.

The total magnification of any set of lenses is determined by multiplying the magnification of the objective by the magnification of the ocular. The nose piece rotates the magnification of the microscope. Generally compound microscope magnifies from 40 x to 100 x.



Parts of a microscope:

The compound microscope is a delicate instrument composed of many parts that are accurately filled together in (Figure)

1. Ocular of eyepiece lens.

The ocular lens is the lens you look through, it is inserted at the top of the body tube. If your microscope has one ocular, it is a monocular microscope, if it has two, it is binocular. Its magnification is written on it.

2. Body tube.

Body tube is the optical housing for the objective lenses.

3. Objective lenses.

The objective lenses are a set of three to four lenses mounted on a rotating turret at the bottom of the body tube. The four objective lenses of your microscope and their magnifications are:

Scanning lens	4X magnification
Low power lens	10X magnification
High power lens	40-45X magnification
Oil immersion lens	100X magnification

The magnification of the objective lens is written on the lens.

Note: with the exception of the oil immersion lens all the objective lens is used dry.

The magnification of oil immersion lens requires using the lens with special immersion oil for proper resolution.

4. Stage

The horizontal surface on which the slide is placed is called the stage. It may be equipped with simple clips for holding the slide in place or with a mechanical stage, a geared device for precisely moving the slide. Two knobs, either on top of or under the stage, move the mechanical stage.

5. Condenser lens

Condenser lens system, located immediately under the stage, contains a system of lenses that focuses light on your specimen. The condenser may be raised or lowered using the condenser knob. An older microscope may have a concave mirror instead.

6. Iris diaphragm

Iris diaphragm is located below the condenser or immediately below the stage in microscopes without a condenser. It functions in regulating the light intensity passing through to the stage. More light is required at higher magnification.

7. Light source

The light source has an (ON/Off) switch & may have adjustable lamp intensities & color filters.

8. Base

Base – also called the supporting stand, rests on the bench.

9. Body Arm

The body arm is used when carrying the instrument.

10. Nose piece

Nosepiece is the mounting for the objective lenses which rotates to bring the desired objective into position.

11. Coarse adjustment

Coarse adjustment knob is a large knob located at either side of the microscope which functions in controlling the distance between the objectives and the stage. Use the coarse adjustment only with the scanning (4X) & low- power (10X) objectives. Why? So coarse adjustment is used for rapid focusing of the specimen until the specimen is roughly in focus & then left alone, in which the fine adjustment knob controls precise focusing of the object.

12. Fine adjustment

Fine adjustment is a small knob located at either side of the microscope. This is used for the control of the object, precise focusing you should use just the fine adjustment knob with the higher magnification objective lenses; Because using the coarse adjustment knob with the higher objective lenses may damage the lens &/or the slide you are observing.

Magnification:

Compound microscopes consist of two lens system: the objective lens, which magnifies & projects a “virtual image” into the body tube and the ocular lens, which magnifies the image further and projects the enlarged image into the eye.

The total magnification of a microscope is the product of the magnification of the objective and the ocular. If the objective lens has a magnification of 5X and the ocular 12X, then the image produced by these two lenses is 60 times larger than the specimen.

Microscope safety cautions:

1. Always carry the microscope in an upright position using both hands.
2. Keep the microscope away from the edge of the table.
3. Always examine a slide first with the low-or medium power objective, never use the high – power objective to view thick specimens.
4. Remove slide only after low-power objective has been rotated into viewing position, never when high – power objective is in position.
5. Keep the stage dry at all times. A wet stage will prevent the slide from being accurately positioned.
6. When returning your microscope to its proper place in the cabinet always:

• Remove the slide from mechanical stage.

• Clean all lens surface and the stage.

• Rotate the nosepiece that the scanning lens is in place.

Steps Used in viewing a slide:

1. Obtain a slide.
2. Check that the ocular and all objective lenses as well as the slide clean.
3. Use the coarse adjustment knob to obtain maximum working distance.
4. Place the slide on the stage, the slide should fit into the slide holder. Use the stage adjustment knob to move the slide over the hole in the stage.
5. Rotate the lower objective in place.
6. Use the coarse adjustment knob to obtain the minimum working distance.
7. Look through the ocular. Adjust the light with the iris diaphragm lever if necessary. Slowly turn the coarse adjustment knob until something comes into focus. Use the fine adjustment knob to sharpen the focus.
8. Using the stage adjustment knob move the slide around until you find an area you wish to examine more closely. Move the slide until the object you wish to examine is in the center of the field.
9. Rotate the high-power objective into place. Use the fine adjustment knob to sharpen the focus. Do not use the coarse adjustment knob. Adjust the light using the iris diaphragm lever if necessary.
10. Rotate the high-power object halfway to the next position, place a drop of immersion oil on the slide, and then rotate the oil immersion objective into place. The objective should be immersed in the oil on the slide. Use the fine adjustment knob to sharpen the focus. Adjust the light using the iris diaphragm lever if necessary.
11. When finished viewing the slide use the coarse adjustment knob to maximize the working distance and remove the slide from the stage. If you are finished with the microscope clean the microscope and return it to storage.

Procedure for cleaning a microscope:

1. Turn off the light.
2. Using the coarse adjustment knob to obtain maximum working distance and remove the slide from the stage.
3. Using lens paper cleans all the lenses starting with the cleanest first ocular, and objectives lens.
4. Clean any oil off of the stage using paper towels.
5. Rotate the scanning objective into place. Use the coarse adjustment knob to obtain minimum working distance.
6. Return the microscope to the appropriate storage area.

Procedure for cleaning a microscope slide:

Before placing a specimen on a slide, it must be clean, as any small foreign body might mislead the observation. If your slide is not clean, do the following:

1. Hold the slide from its ends by fingers of one hand.
2. Using a detergent liquid, rub the slide with one finger of the other hand.
3. Wash the slide under running tap water; rub again, until no trace of the detergent is left.
4. Rinse the slide with distilled water to remove the tap water.
5. Either blot dries the slide by placing it between two towel papers, or place in alcohol solution & keep until used.
6. Never touch the slide from the middle, clean slide always holds it from its ends.

Experiment No: 1

Observation of distinguishing features of prokaryotic and eukaryotic cells

Aim: To observe the characteristics of prokaryotic and eukaryotic cells.

Materials required: Slides, cover slips, stains, microscopes and sample.

Procedure:

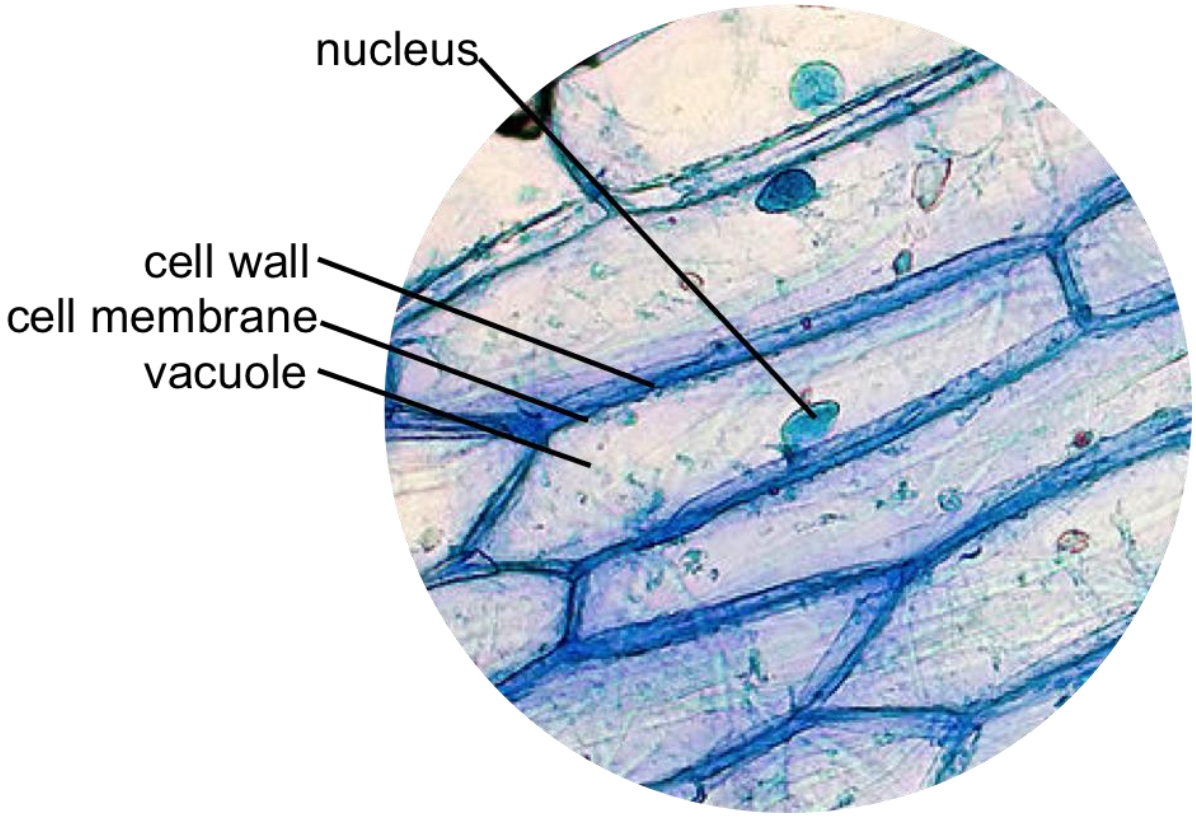
Prokaryotic sample

Prepare a smear of the bacterial suspension on a sterilized clean slide and stain by Gram staining procedure and observe under different magnification**.**

Eukaryotic sample

Peel off the epidermis of onion fleshy leaves and place on a drop of saffranin on a clean slide and observe at different magnification.

Report the differences in cell morphology between them.



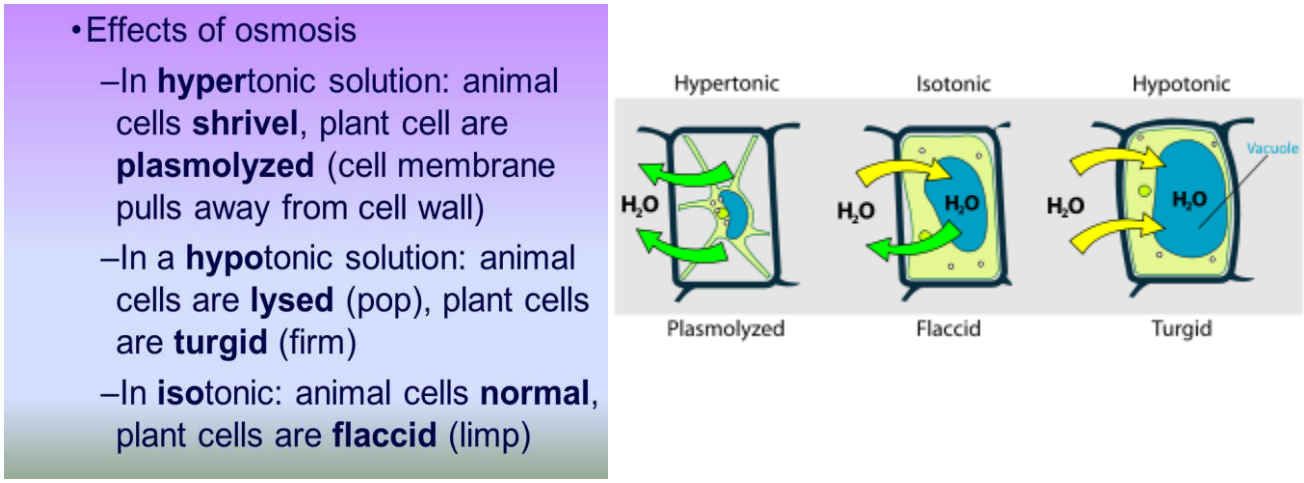
Experiment No: 2

The effect of hypertonic, hypotonic and isotonic solutions on the cell wall.

Aim: To describe experimentally the effects of hypertonic, hypotonic and isotonic solution on the cell wall.

Materials: microscope, NaCl, slides, *Ricinus Communis* petiole**,** Potato

Diagram:



Theory: When a living plant cell is placed in a salt or sugar solution that is more concentrated or stronger than the cell sap (hypertonic solution) water is lost from the cell to the stronger solution. The cytoplasm shrinks and plasmalemma gets detached from the cell wall. A cell placed in a less concentrated solution absorbs water and becomes turgid.

Materials: Distilled water, NaCl solution pond water containing spirogyra cells, microscope slide, and microscope.

Procedure: From the NaCl provided, prepare molar solutions in the following concentrations: 0.2m. 0.4m, 0.6m, 0. 8m and 0.10m. Put a drop of the greenish part of your pond water on each of the five slides provided and examine under the microscope. Once you have observed potato discs or *Ricinus Communis* petiole add each of the molar

solutions one at a time on the five (5) slides respectively. Leave for a few minutes and examine. Note which of the concentrations is hypertonic, hypotonic and isotonic. Make diagrams of the cell from each of the five (5) concentrations you observed under the microscope. Copy and complete the table below.

S/N	NaCl concentrations	Changes observed.
1	(1) 0.2m	
2	(2)0.4m	

In case of *Ricinus communis* petioles

1. In hypotonic soln. curvature of Ricinus communis petioles toward epidermis.
2. In hypertonic soln. curvature of Ricinus communis petioles toward pith.
3. In isotonic soln. there is no change.

Questions:

1. Which of the concentration of NaCl solution is hypertonic, isotonic and hypotonic to the cell plasma?

2. What are the effects of hypertonic and hypotonic solutions on the cell plasma?

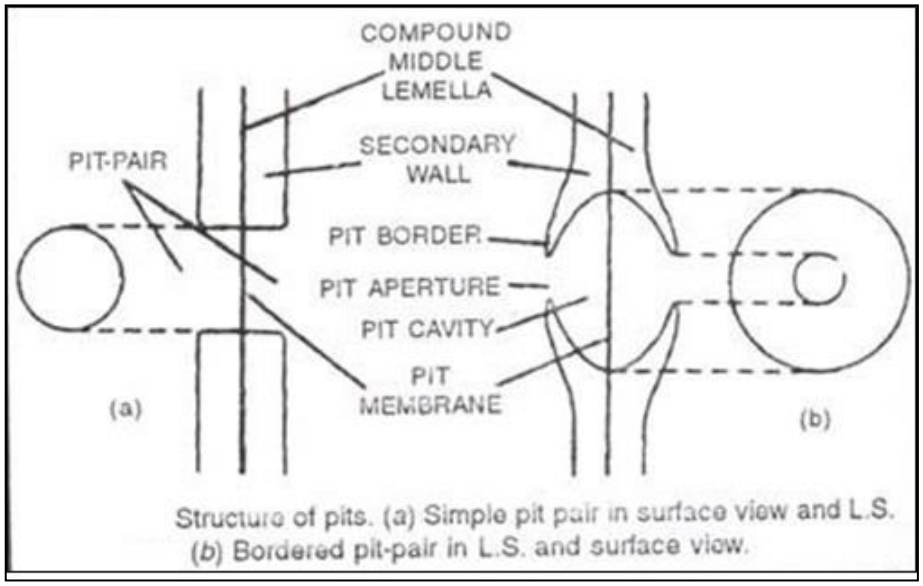
Comments:

Experiment No: 3

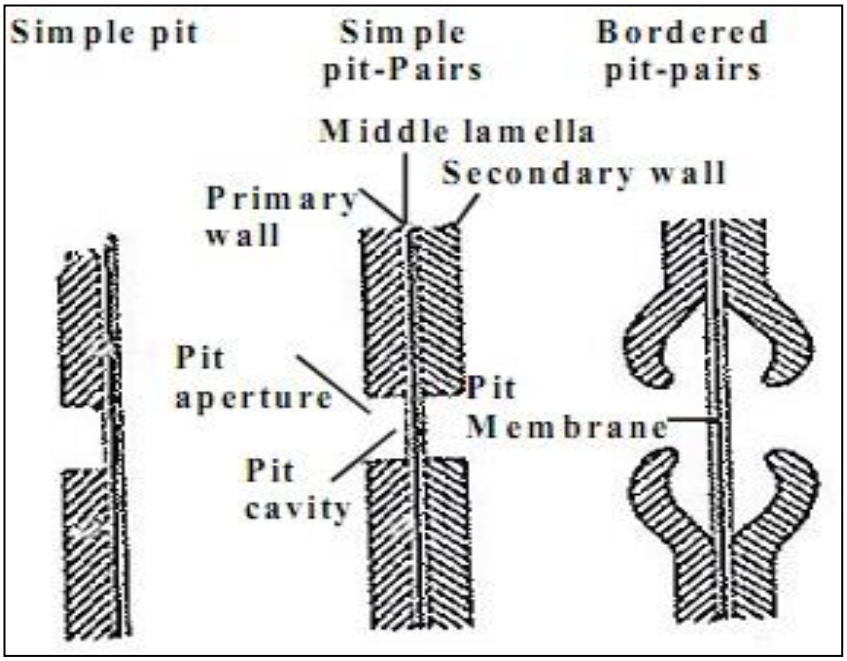
Pits of Cell Wall

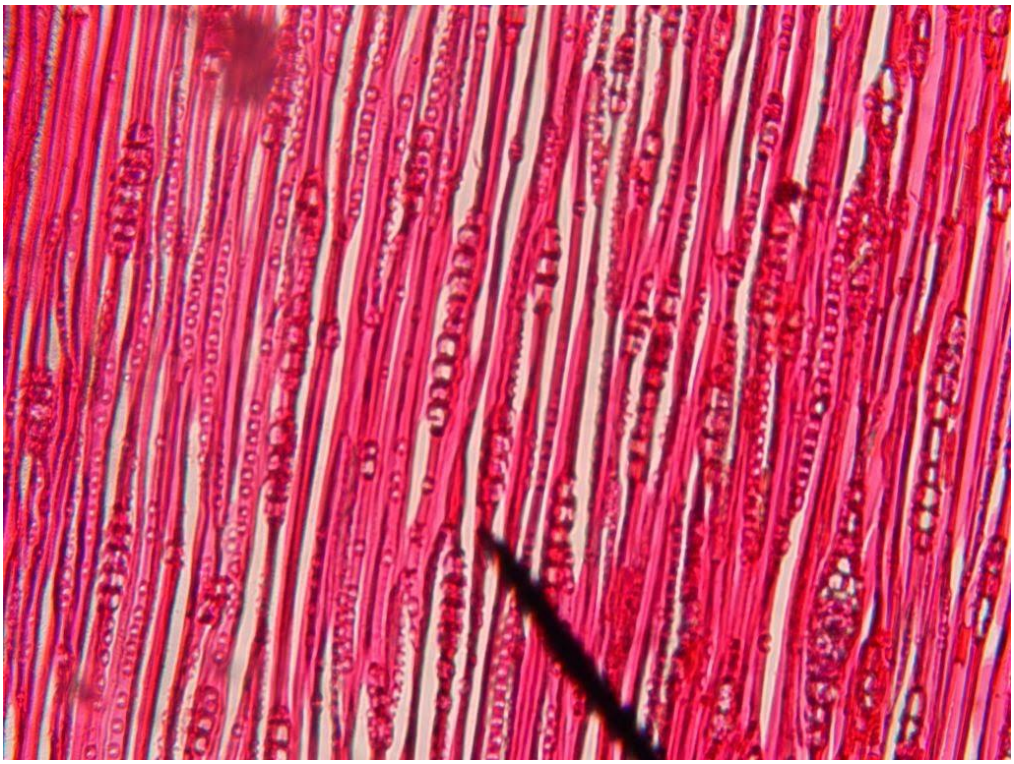
Pits are relatively thinner portions of the cell wall that adjacent cells can communicate or exchange fluid through. Pits are characteristic of cell walls with secondary layers. Generally, each pit has a complementary pit opposite of it in the neighboring cell. These complementary pits are called "pit pairs".

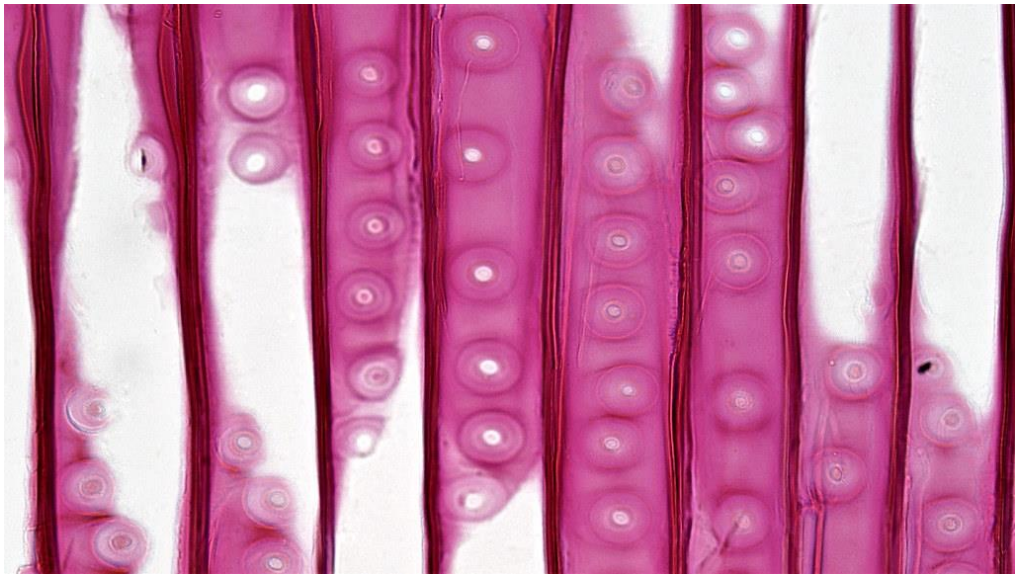
Pits are composed of three parts: the pit chamber, the pit aperture, and the pit membrane.



Structure of pits. (a) Simple pit pair in surface view and L.S. (*b*) Bordered pit-pair in L.S. and surface view.







Experiment No: 4

Living Contents of Cell: Plastid types

The three major types of plastids are:

1. **Chloroplasts** are green and serve as the sites for photosynthesis in the cells.
2. Highly pigmented plastids called **chromoplasts** give plants the colors they use to attract pollinators.
3. Non-pigmented plastids used for storing starches, lipids, and proteins are called **leucoplasts**.

Materials

Leaves of different plants, and algae, slides, coverslips, microscope, water, (leaves of spinach can also be successfully used to observe chloroplasts), etc.

Method

Separate the young leaves from the plants. Mount in water and study under the microscope.

Observations

1. Discoid or oval-flattened chloroplasts can be seen close to the cell wall.

2. Chloroplasts are green in color due to the abundance of photosynthetic pigment-the chlorophyll.

3. Other pigments present in the chloroplast include xanthophylls and carotenes.

4. Chloroplasts are the seats of photosynthesis and therefore, end product in the form of starch grains is also seen.

Study the chromoplasts

Materials

Fruits of tomato, slides, coverslips, microscope, water, etc.

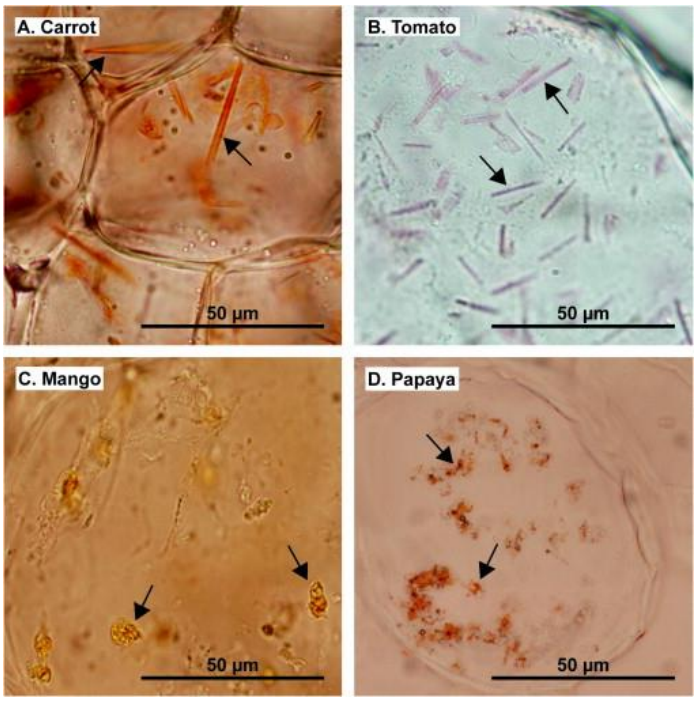
Method

Peel off a part of fruit wall with a small amount of pulp attached to it. Mount in a drop of water and observe under the microscope.

Observations

1. The cells are filled with numerous orange or red coloured chromoplasts.
2. In ripe fruits chromoplasts occur in groups.
3. Chromoplasts may be discoid or flattened. These occur close to the wall.
4. The chromoplasts have abundance of xanthophylls and carotenes and hence their colour. Chlorophyll though present is lesser in amount.

S. The major function of the chromoplasts is to protect the organ from the bright sunlight. It also helps in photosynthesis by absorbing light.



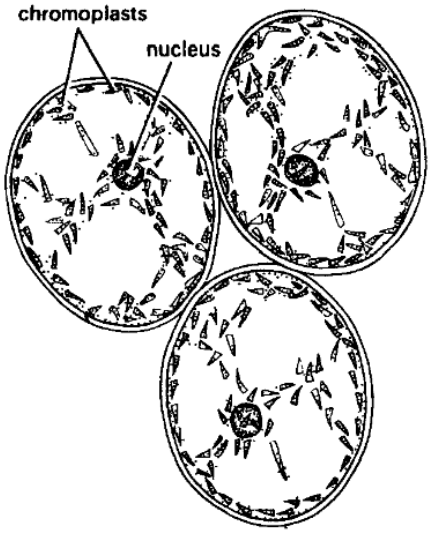


Fig. 9. Chromoplasts in the pericarp of tomato.

Study leucoplasts

Materials

Potato tuber, slides, covers lips, microscope, acid fuchsin, glycerin, water, etc.

Method

1. Cut thin sections of potato tuber.
2. Place them in a watch glass containing 1 % aqueous solution of acid fuchsin. Cover the watch glass with another but larger watch glass. Allow the sections to take stain for at least 3-4 hours.
3. 3. Wash the sections with water.
4. 4. Mount in glycerine.

Observations

1. Leucoplasts are seen as pink-colored structures amongst starch grains.
2. The shape of the leucoplasts is extremely variable.
3. It is filled with numerous starch grains.
4. Leucoplasts are the storage plastids which generally store starch.

Experiment No: 5

Non-living contents in cells

To study starch grains

Materials

Potato tuber, seeds of pea, seeds of wheat, seeds of maize, seeds of rice, slides, coverslip, microscope, Iodine solution, glycerine, etc.

Method

1. Cut a thin section of potato tuber or seed.
2. Place the section on a slide and stain it with a drop of iodine.
3. Wash the section by pouring water and draining it off. Repeat till excess stain is washed off.
4. Mount the section on another clean slide using glycerine as a mounting medium.

Observations

1. Each starch grain has a hilum which is a point of origin of starch deposition.
2. Starch is deposited in layers around hilum.
3. In the starch grains of pea, hilum is located in the centre and the layers of starch are uniformly deposited around it. These starch grains are called concentric and simple. Sometimes two or more starch grains get attached to one another. Such starch grains are called concentric and compound.
4. In the starch grains of potato, hilum is located in one corner and layers of starch are deposited centrically around it. Such starch grains are eccentric and simple. Sometimes two or more starch grains remain attached to one another. Such starch grains are called eccentric and compound.

5. The starch grains are characteristic of a particular plant and can be easily identified. The characters of starch grains of some of the common plants are listed below.

(a) Grains of wheat - simple, concentric, spherical and flattened.

(b) Grains of rice - simple, concentric, with many arms.

(c) Grains of maize - simple, concentric, angular.

(d) Seeds of pea - simple, concentric, spherical or elongated.

(e) Seeds of gram - simple, concentric. spherical or elongated.

(f) Tuber of potato - simple, eccentric, spherical or oval.

(g) Fruit of banana - simple. concentric, spherical.

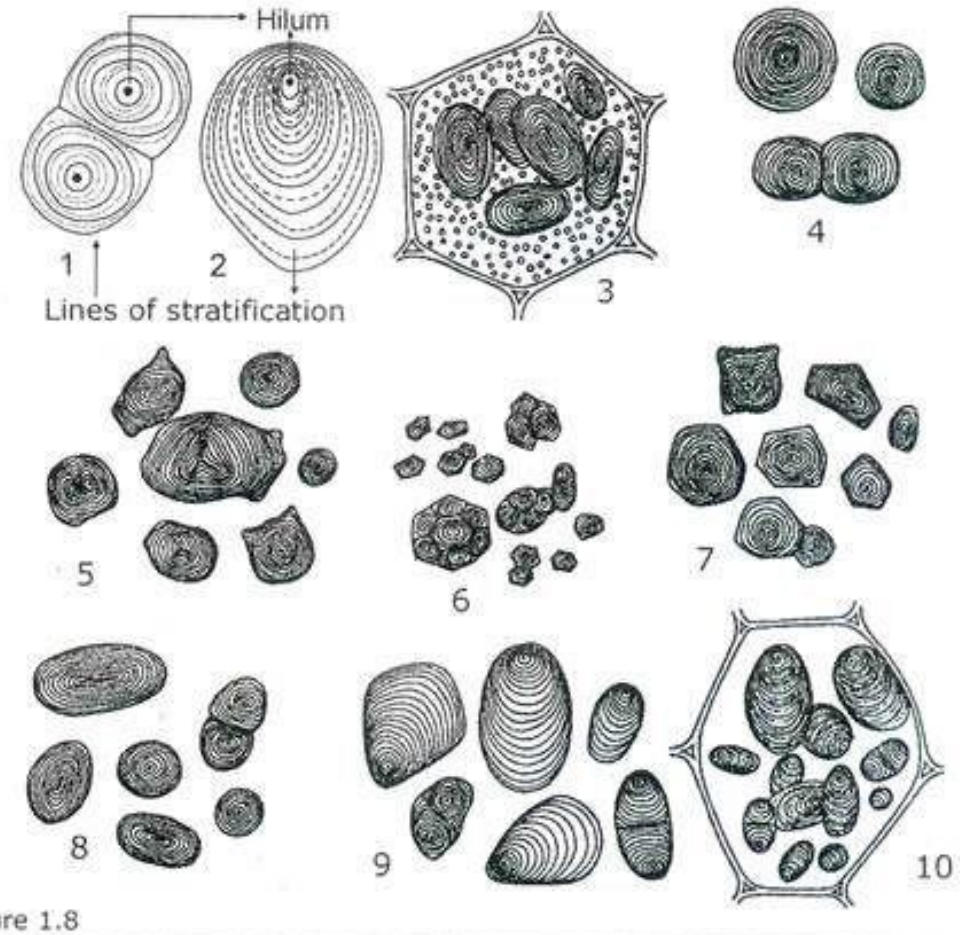
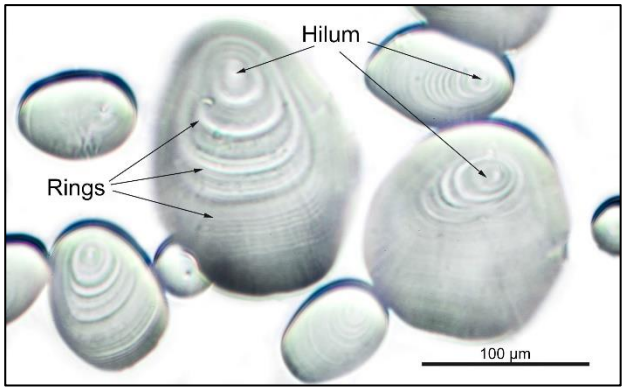
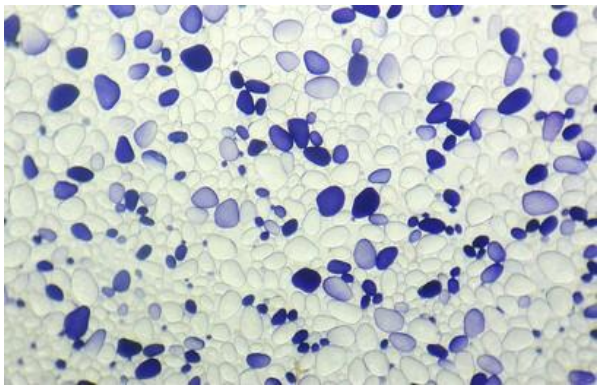


Figure 1.8

Starch grains. 1. Compound starch grain. 2. Simple starch grain. 3. From cotyledon of *Pisum* seed. 4. From flesh of *Musa*. 5. From tuberous root of *Ipomoea batatas*. 6. From endosperm of *Oryza* grain. 7. From endosperm of *Zea* grain. 8. From cotyledon of *Cicer* seed. 9. From tuber of *Solanum tuberosum*. 10. Same in situ.


Hilum
Rings
100 µm



To study Aleurone grains

Materials

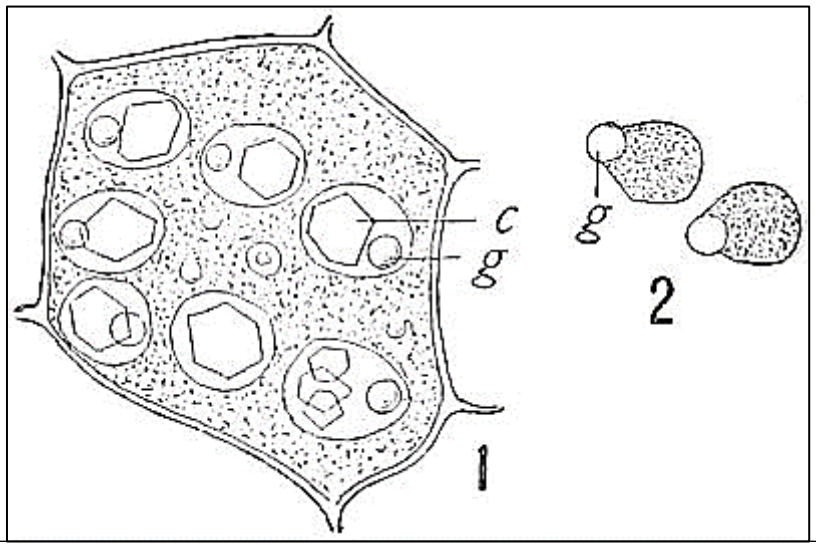
Seeds of castor, safety blade, slides, coverslips, microscope, water, glycerine, etc.

Method

1. Remove the seed coat from castor seeds.
2. Cut a thin section of the endosperm.
3. Mount the section in glycerin and observe under the microscope.

Observations

1. Each cell shows many spherical or oval aleurone grains.
2. Each aleurone grain is made of crystalloid and globoid.
3. Crystalloid is large and has many angles. It is mostly made of proteins.
4. Globoid is a small globular or oval structure. It is made of calcium or magnesium diphosphate.
5. Aleurone grains are abundant in the aleurone layer found in grains of cereals like wheat, maize, rice, etc.



1. A cell from the castor bean, as seen in water, showing roundish aleurone grains imbedded in the protoplasm. In each, one or more crystals, c, and usually a globoid, g.
2. Isolated aleurone grains of the same, as seen in olive oil.

To study Calcium Oxalate

Materials

Onion bulb, safety blade, slides, cover slips, water, glycerine, microscope, etc.

Method

1. Cut a thin section of outer scaly leaf of onion bulb.
2. Mount the section in glycerin and observe under the microscope.

Observations

1. The cells show raphides of different shapes e.g. prism-shaped, rod-like, needle-like, etc.
2. Raphides are the crystals of calcium oxalate.
3. These may be present either singly or in groups. When in groups these become star-shaped (sphaero-raphides) or form bundles.
4. Some of the plants in which raphides are found, are given below

(a) Colocasia (Eng-Taro: Hindi-Arvi, Kachalu)-petiole shows raphides, and sphaeroraphides.

(b) Amorphophallus (Eng.-Elephant-foot yam; Hindi- limikand)-needle shaped raphides in leaves.

(d) Eichhomia (Eng.-Water hyacinth; Hindi- Samundersonkh) - Raphides in the petiole.

(e) Carica papaya (Eng.-Papaya; Hindi-Papita) Raphides in the petiole.

(/) Impatiens (Eng.-Garden balsam; Hindi- Gul-mehndi) - Raphides in the leaves.

(g) Chenopodium (Eng.- Pigweed; Hindi-Bathua) Sphaeroraphides in leaf.

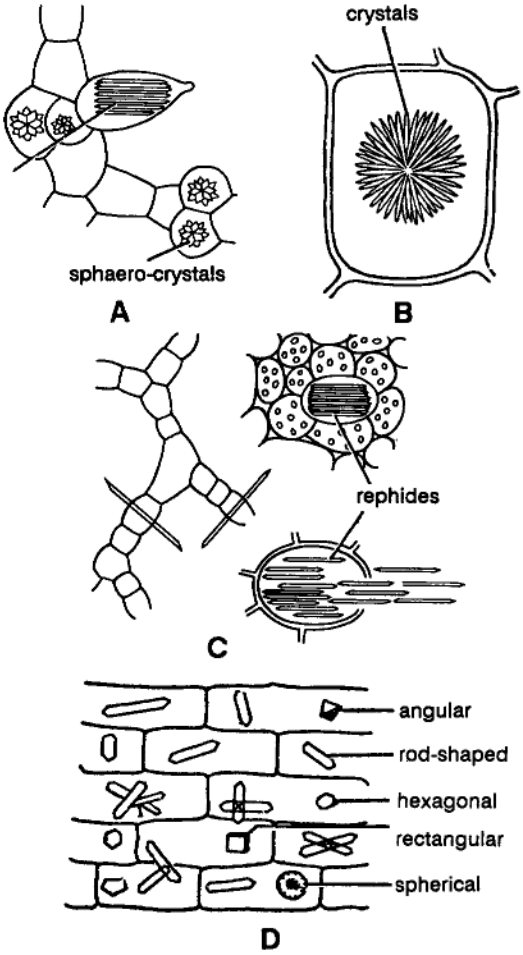
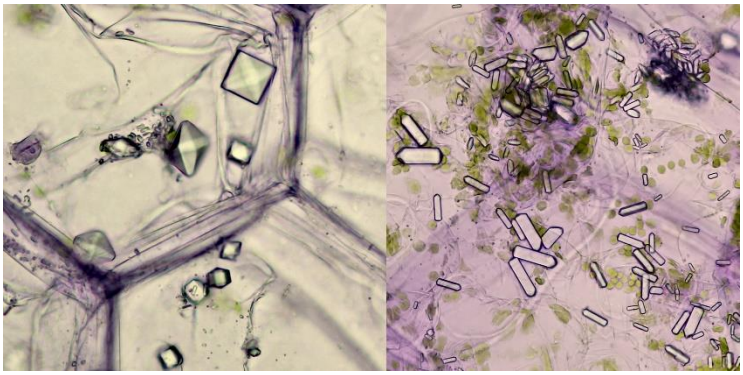
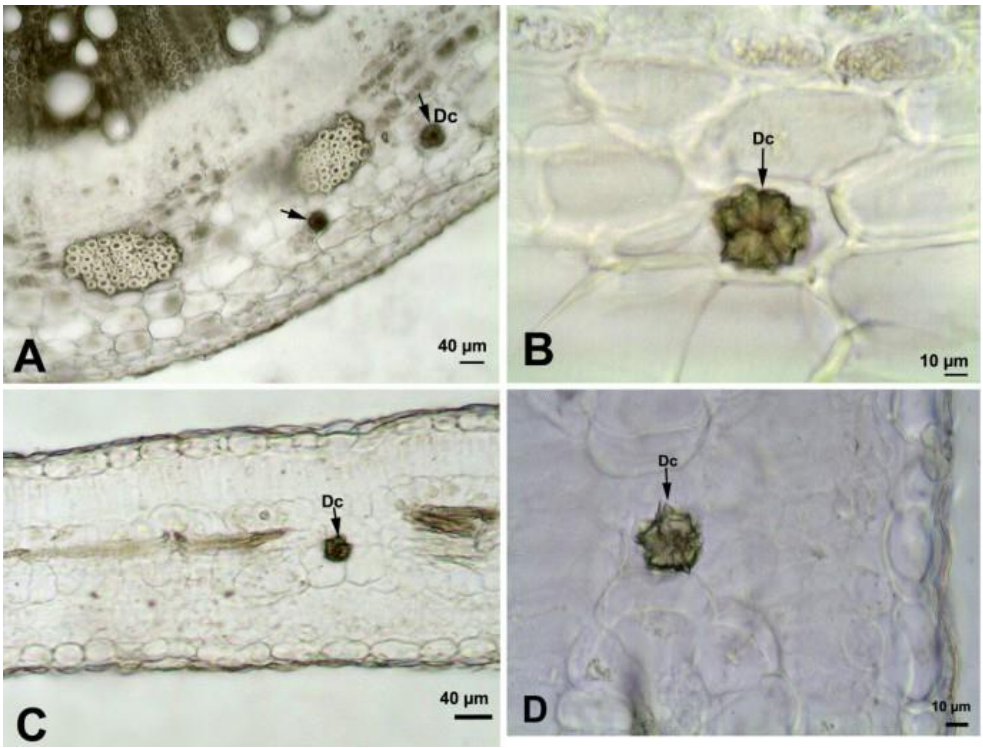


Fig. 14. Diffferent types of raphides. A. Water hyacinth, B. Balsam or Impatiens,C. Sphaeroraphides in Pistia. D. Raphides I the scales of onion.





To study Calcium Carbonate

Materials

Leaves of *Ficus elastica* (India Rubber plant), safety blade, slides, coverslips, water, microscope, safranin, etc.

Method

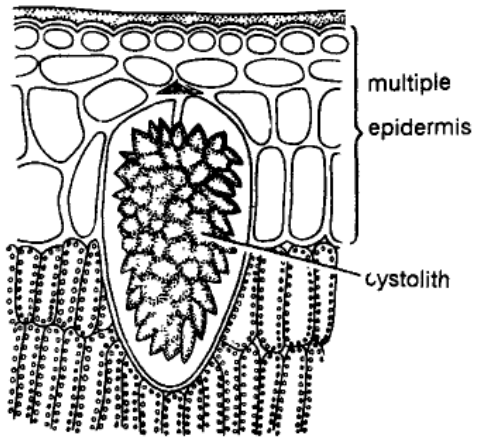
1. Cut a thin section of the leaf.
2. Stain in safranin for about 10 minutes.
3. Wash in water till proper distaining is obtained.
4. Mount in glycerin and study under the microscope.

Observations

1. The epidermis is made of many layers.
2. The cells of the innermost layer of multiple epidermis are elongated.
3. These cells show a peg-like ingrowth produced by the cell wall.
4. Many crystals of calcium carbonate are deposited on this ingrowth (stalk) to form grape -like cluster. This is known as cystolith.
5. Cystoliths are also found in (a) Leaves of Ficus benghalensis (Eng.- Banyan; Hindi-Bargad).

(b) Leaves of Momordica charantia (Eng.- Bitter gourd; Hindi.-Karela).

(c) Leaves of Ruellia tuberosa (Eng.-Meno-wecd).



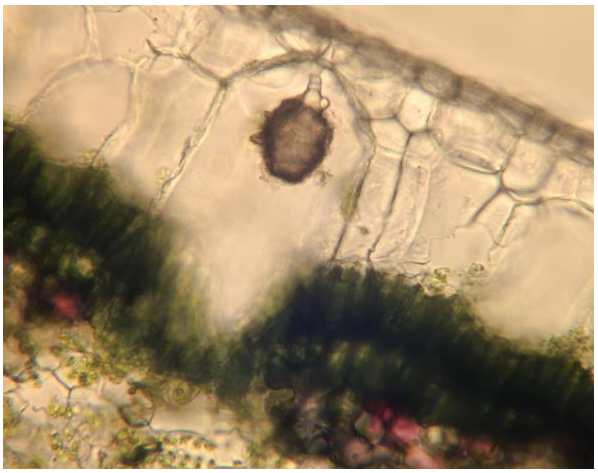


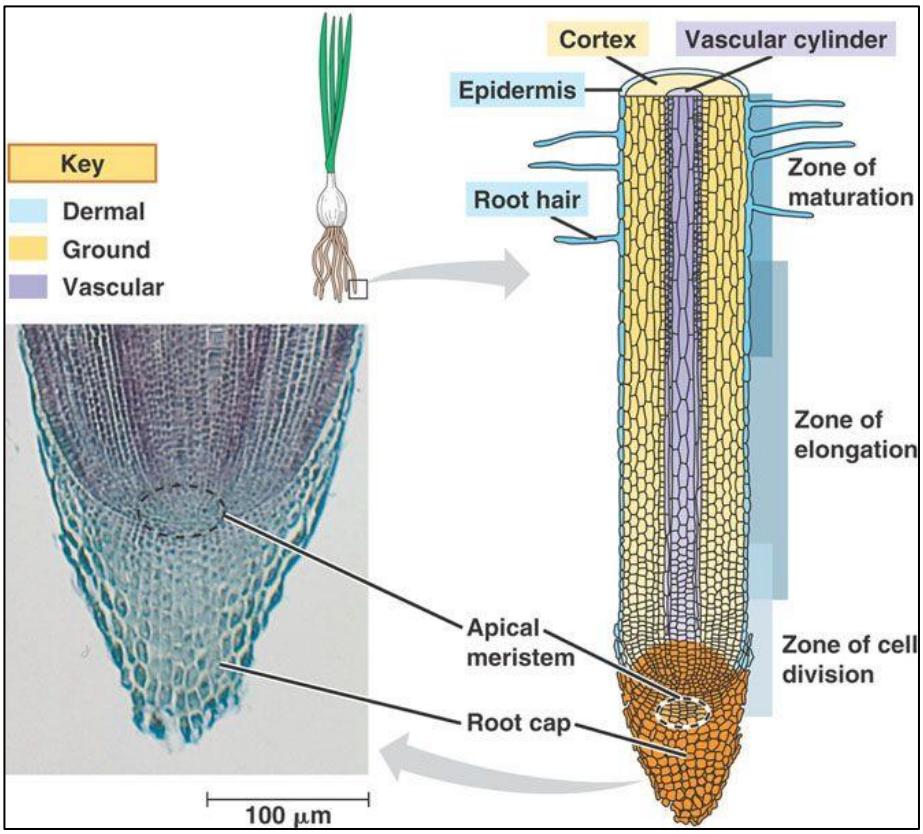
Fig. 15. Cystolith in the leaf of *Ficus elastica.*

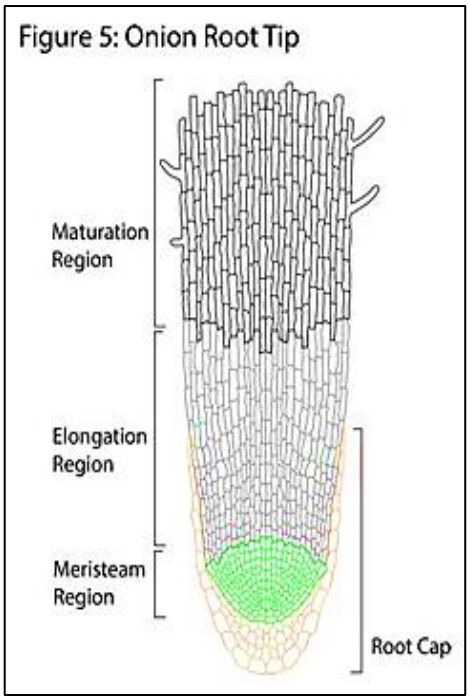
Experiment No: 6

Cell division: Mitosis

Mitosis is a type of cell division which results in the formation of two daughter cells. These cells are identical to the parent cells and have the same number of chromosomes. Mitosis occurs in vegetative cells. It can be best observed in onion root tip.

To study the mitosis by preparing squash of onion root tip





Materials and technique

Onion root tips, acetic acid, aceto-carmine, slides, cover slips, etc. Allow the onion bulbs to grow in bottles filled with water. If the lower root portion of the bulb dips in water, it quickly sends forth large number of

roots. Cut the root tips between 9 a.m. to 12 noon and fix them in Carnoy's fluid.

Procedure

The following procedure is used.

1. Place the fixed root tip in a drop of 45% acetic acid.
2. Place a cover glass over the tip and diffuse aceto-carmine.
3. Tap and apply uniform pressure over the cover glass.
4. The squash preparation is ready.

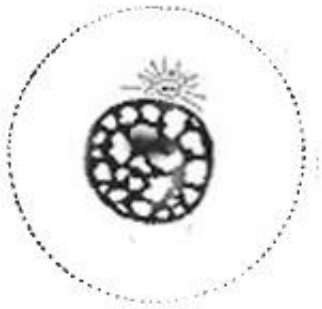
Observations

The slide shows almost all the stages of mitosis.

[I] Interphase

The following characteristics are seen:-

1. This is a stage prior to actual mitotic cycle.
2. The cell appears to be inactive or in resting stage but is metabolically the most active. DNA replication occurs during this period.
3. Nuclear membrane and nucleolus are very distinct.
4. Chromosomes are in the form of chromatin network and individual chromosomes cannot be seen separately.
5. The chromosome appears double stranded i.e., made of two chromatids.



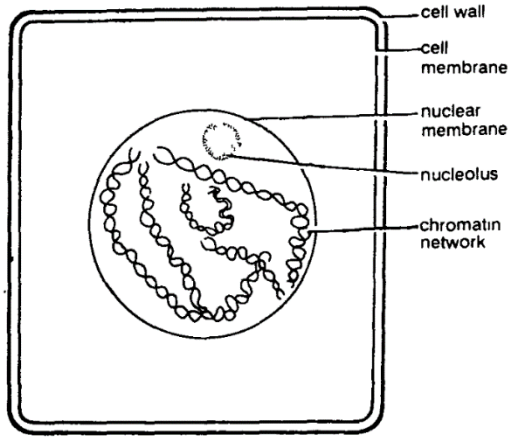


Fig. 9. **Mitosis.** Cell showing interphase.

[II] Early prophase

The following characteristics are seen-

1. This is the first stage of mitosis which is observed under the microscope.
2. Nuclear membrane appears distinct.
3. Nucleolus is also seen clearly.
4. Chromosomes become coiled and shortened and more distinct.

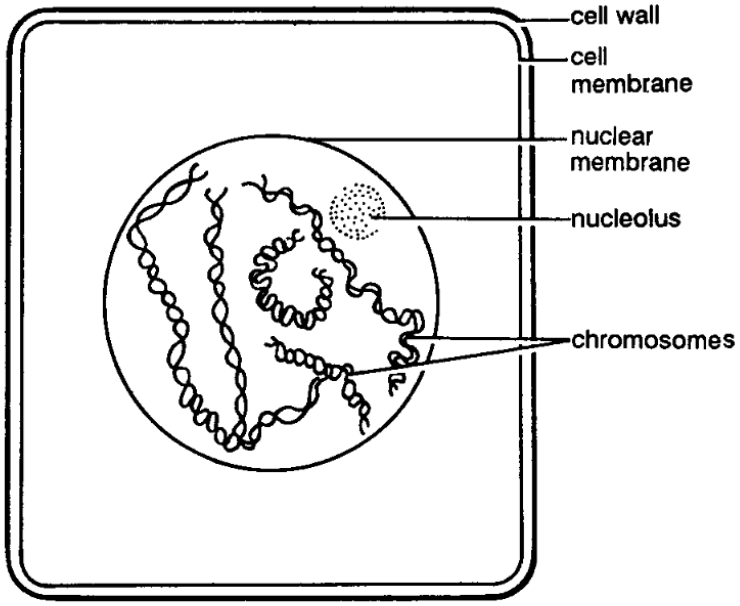


Fig. 10. **Mitosis**. Cell showing early prophase.

[III] Late prophase

The following characteristics are seen-

1. The nuclear membrane and nucleolus have partially or completely disappeared.
2. Each chromosome now begins to show chromatids, primary constriction, secondary constriction and centromeres.
3. The equatorial region appears clearly in the centre of the cell.
4. Chromosomes begin to move and gather near the equatorial plate.
5. Chromosomes are condensed and thus short and thick.
6. Spindle fibers also begin to appear.

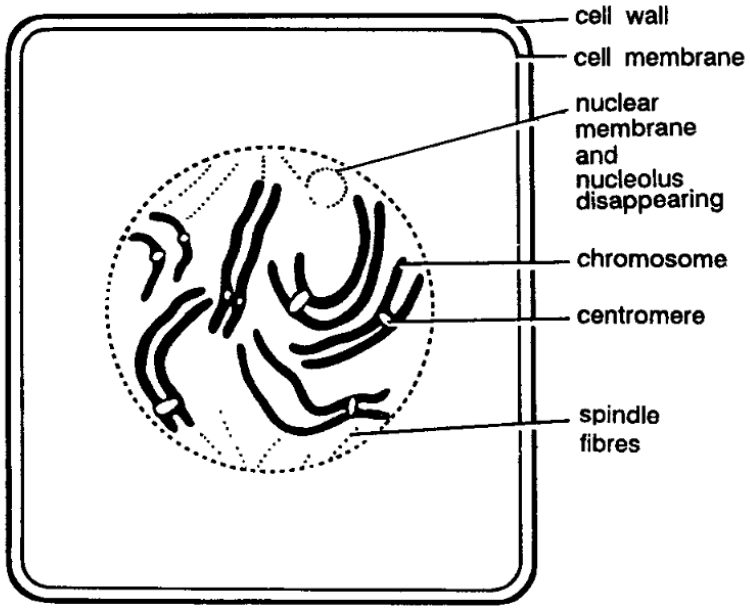


Fig. 11. **Mitosis**. Cell showing late prophase.

[IV] Metaphase

The following characteristics are seen.

1. Nuclear membrane and nucleolus are absent having disappeared.
2. Centromeres of the chromosomes are arranged on the equatorial plate, and each is attached to the spindle fibres.
3. Centrioles are absent and hence aster is not formed in plant cells. This type of mitosis is known as anastral mitosis.
4. The spindle is made of fibres only. The absence of centrioles indicates that it is a plant cell.
5. The chromosomes at metaphase are very distinct. Thus, number and morphology of chromosome is studied at this stage. Each chromosome shows two chromatids, centromere, primary constriction, euchromatic and heterochromatic regions, chromomeres, etc.

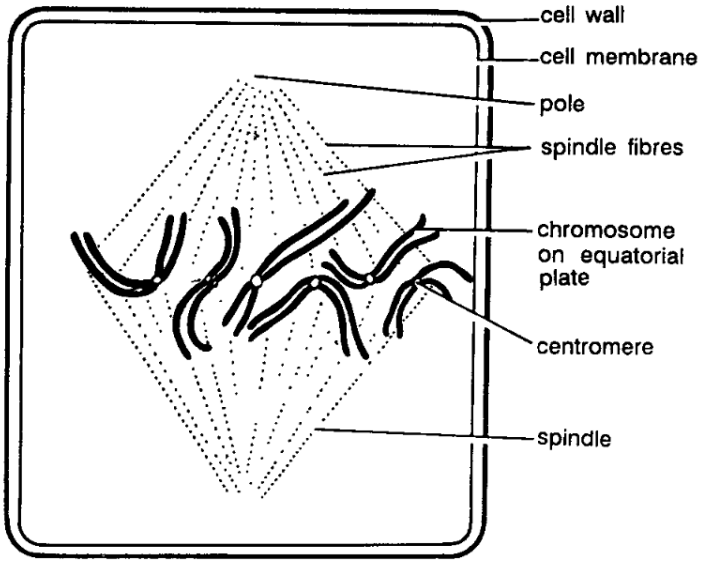


Fig. 12. **Mitosis**. Cell showing metaphase.

[V] Anaphase

The following characteristics are seen:-

1.This stage is completed in a very small period of time.

2. The centromere of each chromosome gets split into two.

3. The chromosome also gets divided into two chromatids. Each chromatid now bears one centromere each.

4. The chromosome becomes shorter and thicker.

5. The separated chromatids are now pulled towards the opposite poles due to contraction of spindle fibres.

6. During movement, each chromosome shows characteristic shape which is dependent on the position of centromere.

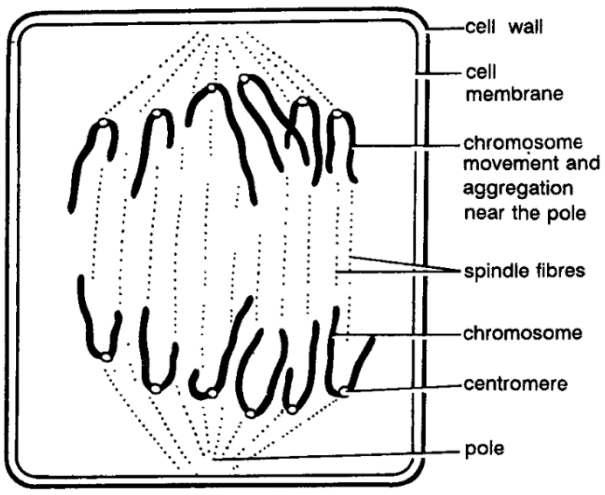


Fig. 13. **Mitosis**. Cell showing anaphase.

[VI] Telophase

The following characteristics are seen~

1. The chromosomes are present at both the poles of a parent cell.
2. The chromosomes increase in length and become thread-like. All the chromosomes together form chromatin network, and their individuality is now lost.
3. The groups of chromatin network at each are surrounded by nuclear membrane Nucleolus is also present.
4. Thus two fully formed nuclei, one at each pole are present in the parent cell.
5. Spindle fibres are absent.

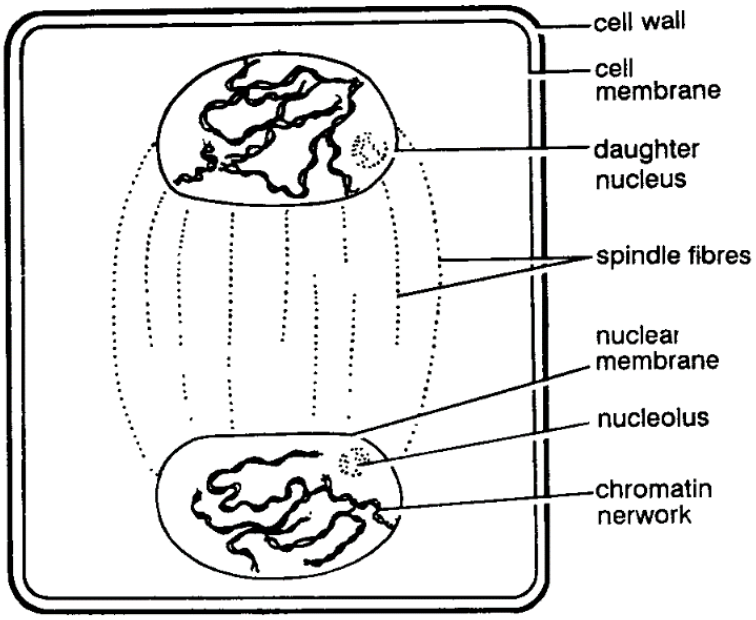


Fig. 14 **Mitosis** Cell showing telophase

[VII] Cytokinesis

The following characteristics are seen: -

1. In this stage, cytoplasm divides into two. It results in the formation of two daughter cells.
2. Division of the cytoplasm is due to formation of a cell plate in the equatorial region.

3. Cell plate formation begins in the centre of the cell and gradually progresses towards the periphery.
4. This results in the formation of two daughter cells. Organelles are also present.
5. The number of chromosomes in each daughter cell is equal to the number present in parent cell.

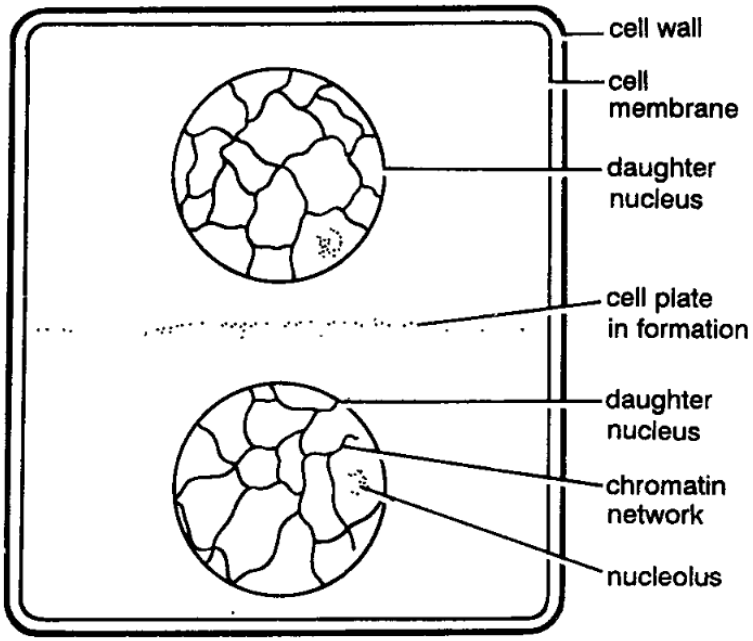
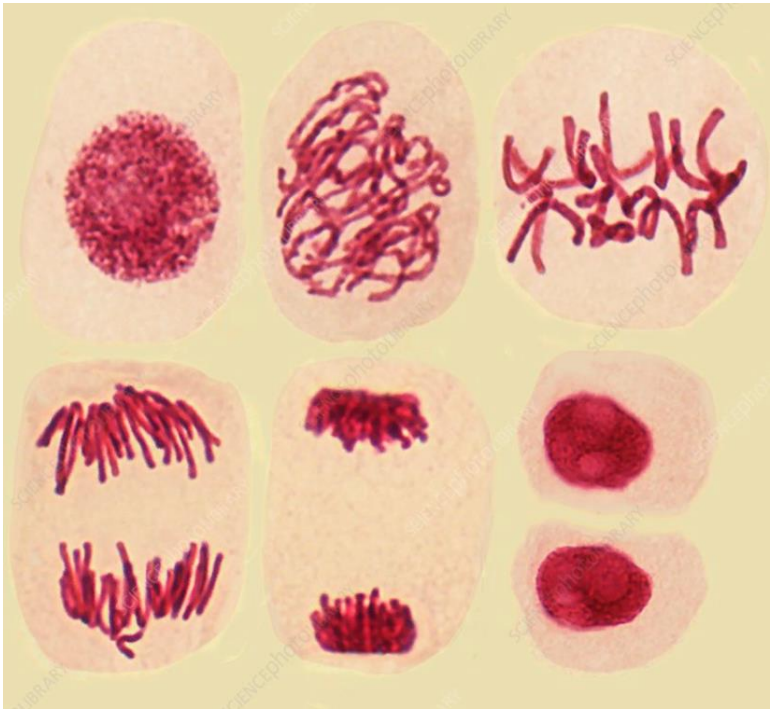
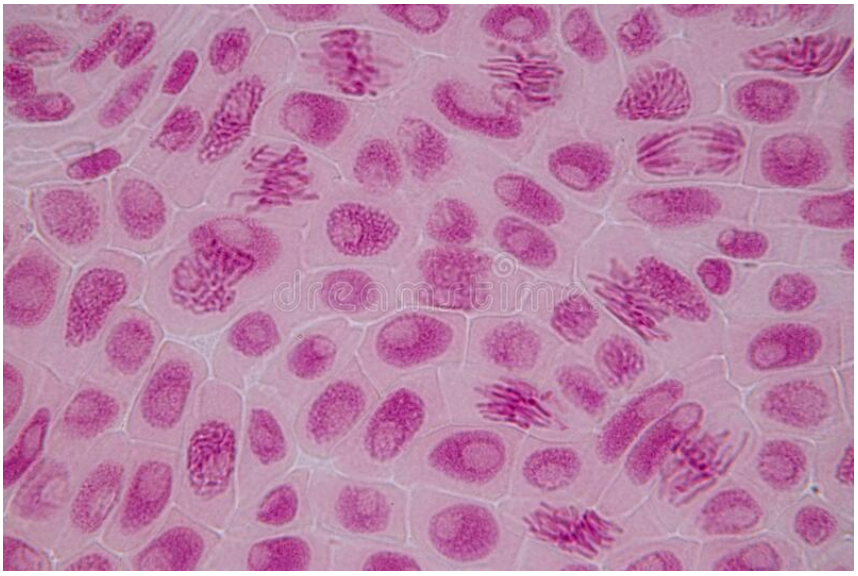
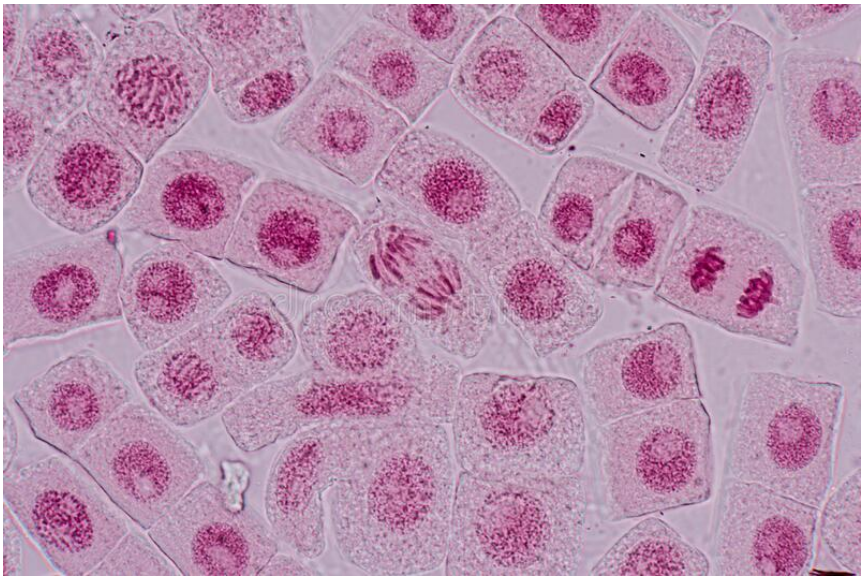
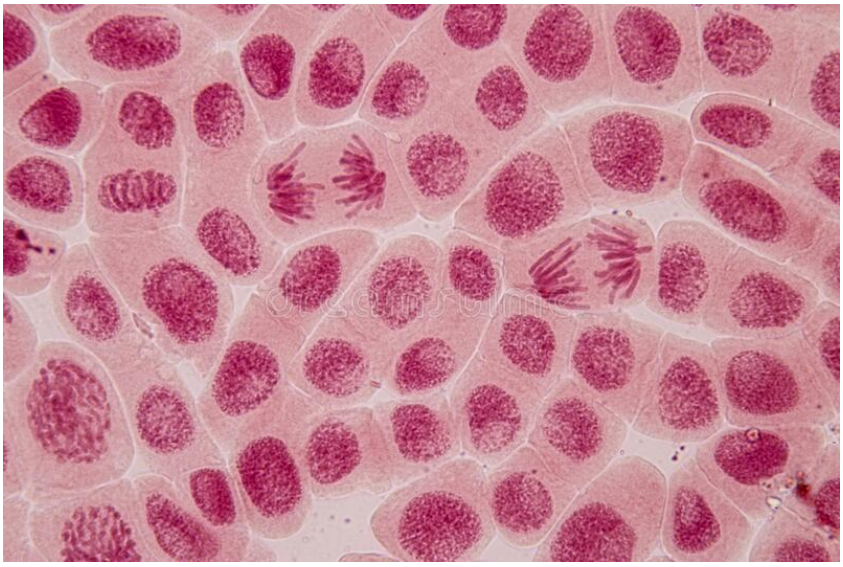


Fig. 15. **Mitosis**. Cell showing cytokinesis.









MEIOSIS

Meiosis is a cell division that is characteristic of organisms which reproduce sexually. During this division, genetic material is duplicated once, and nucleus divides twice. As a result, four daughter cells are formed. These have half the chromosomes as compared to the parent cells. Meiosis also involves crossing over, i.e. exchange of equal parts of non-sister chromatids of the homologous chromosomes. Therefore, the four daughter cells are genetically different from the parent cells. Meiosis consists of (1) Meiosis I and (2) Meiosis II.

Meiosis I involves some very characteristic and important stages such as

(1) Synapsis or pairing of homologous chromosomes,

(2) Recombination due to crossing over and

(3) Segregation of homologous chromosomes.

The stages included in Meiosis I are Prophase I, Metaphase I, Anaphase I and Telophase I. At the end of meiosis, I, two daughter cells are formed. Each cell has half the number of chromosomes compared to parent cell. Meiosis II is similar to mitosis. It results in the formation of four daughter cells, each having the same chromosome number as was present at the end of Meiosis I. Meiosis II is also sub-divided into Prophase II, Metaphase II, Anaphase II and Telophase II.

To study the meiosis by Anther preparation

Materials and Technique

Prepare a smear of young anthers of *Allium cepa* as follow: -

1. Anthers are smeared on the cover glass.

2. It is then inverted on the slide in drop of acetocarmine.

3. Cover glass is sealed with melted wax.

Observations

Following stages can be seen in different slides of Meiosis-

[I] Leptotene (Leptonema) of Prophase I

The following characteristics are seen-

1. Nuclear membrane and nucleolus are intact.
2. Chromosomes are long thread-like structures. All the chromosomes are intertwined to form chromatin network.
3. Chromosomes appear beaded due to chromomeres which are distinct at this stage.
4. All the chromosomes finally move towards one part of the nucleus. This stage is known as synizesis or bouquet formation.
5. Centrioles are not present. This indicates that it is a dividing plant cell.

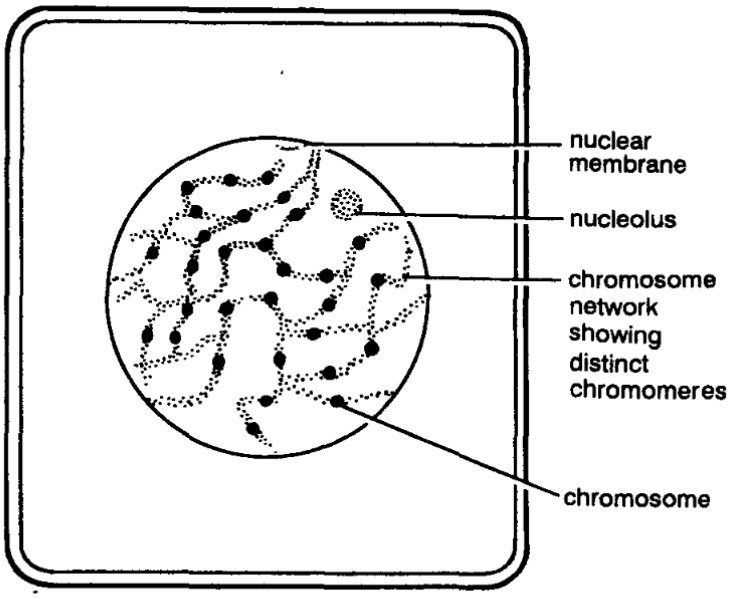


Fig. 16. **Meiosis**. Cell showing Leptotene of Prophase I.

[II] Zygotene (Zygonena) of Prophase I

The following characteristics are seen-

1. Nuclear membrane and nucleolus are still very clear.

2. The major character of this stage is synapsis pairing of homologous chromosomes.
3. Synaptonemal complex is formed as a result of synapsis. This complex is made of two lateral elements and a central region which is bisected by a narrow central component.
4. Synapsis can occur at more than one points along the length of the chromosome.
5. At each place a pair showing two chromatids is present.

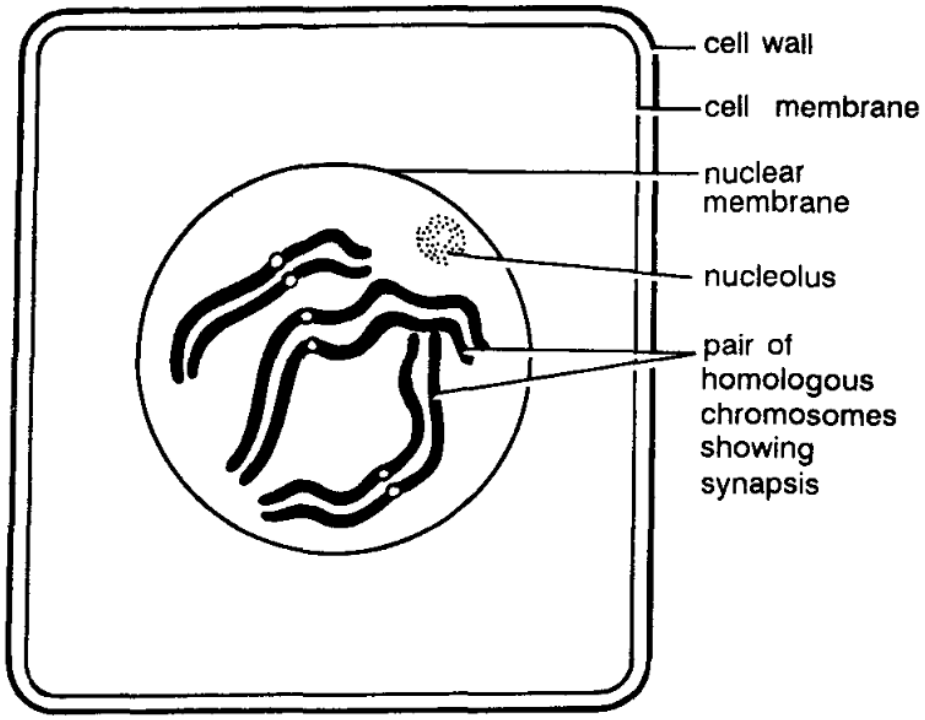


Fig. 17. **Meiosis**. Cell showing Zygotene of Prophase I.

[III] Pachytene (Pachynema) of Prophase I

The following characteristics are seen-

1. Nucleolus and nuclear membrane are distinct.
2. Chromosomes are thickened, coiled and thread-like.

3. Chromosomes are very closely coiled. Each chromosome shows its two chromatids. A pair of homologous chromosomes which is intimately coiled upon one other shows four chromatids together.
4. Pair of homologous chromosomes is called bivalent. It is made of four chromatids and hence known as tetrad.
5. The stage is characterized by crossing over. It is the exchange of equal parts of chromatids of two different but homologous chromosomes.
6. Nucleolus is distinctly attached to nucleolar organizing chromosome.
7. The length of the chromosome being more than that found at metaphase, the chromosome at this stage is also used for the study of morphology.

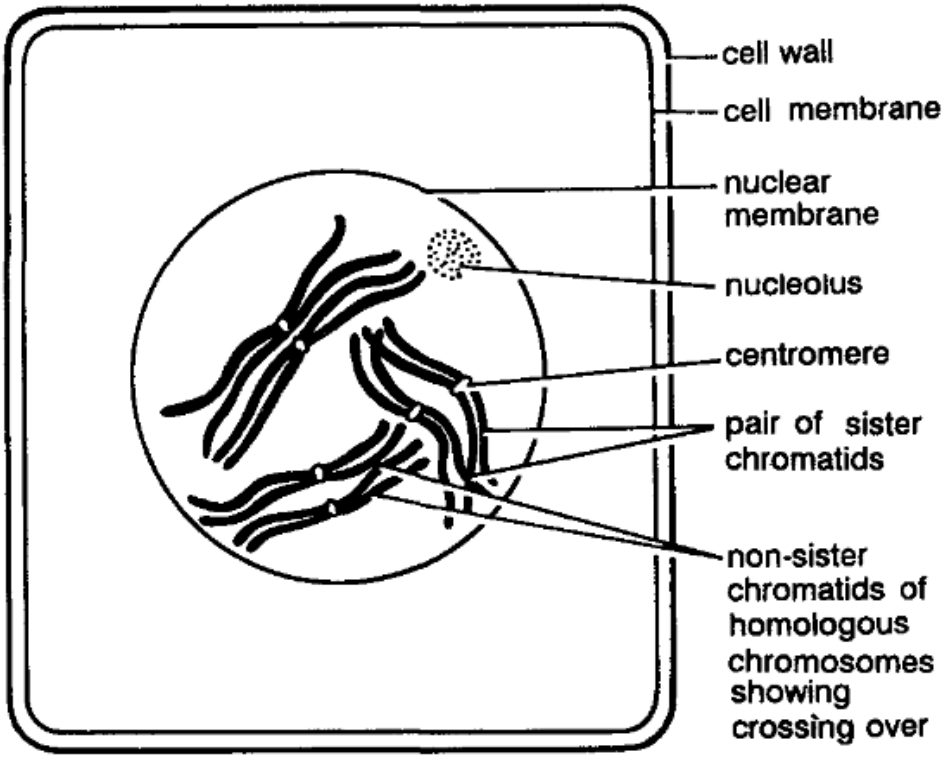


Fig. 18. **Meiosis**. Cell showing Pachytene of Prophase I

[IV] Diplotene (Diplonema) of Prophase I

The following characteristics are seen-

1. The nucleolus is disappearing while nuclear membrane is still intact.
2. The close and tight coiling of chromosomes becomes loose, and chromosomes appear more clear.
3. Homologous chromosomes still remain in contact at some points called chiasmata. These are indicators of crossing over having been completed at these points.
4. Chromosomes shorten and thicken. These become still more distinct by the end of this stage.

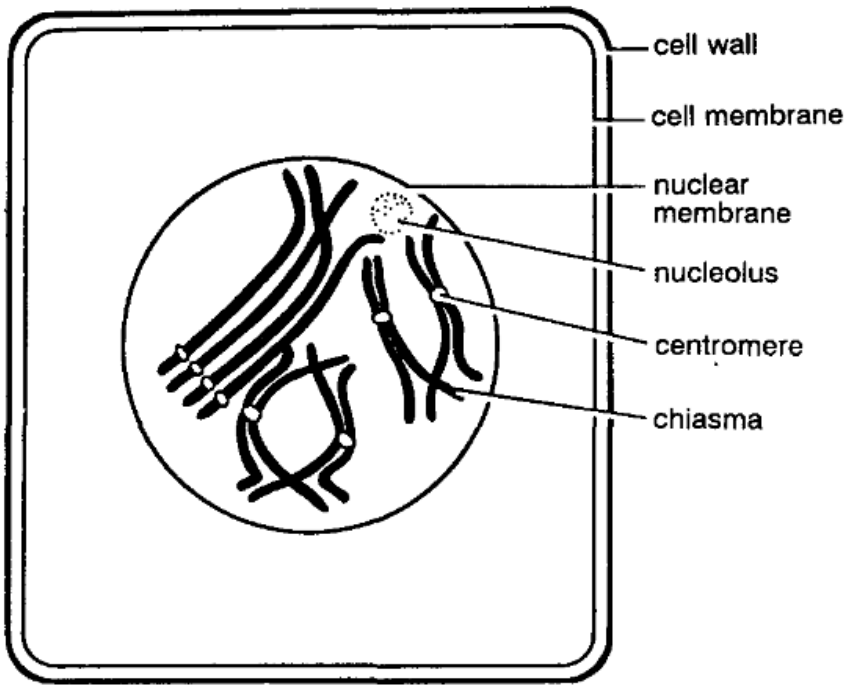


Fig. 19. **Meiosis.** Cell showing Diplotene of Prophase I.

[V] Diakinesis of Prophase I

It shows following characters.

1.Nuclear membrane and nucleolus have completely disappeared.

2. Chromatids start separating, beginning from the centromere towards the end. The chiasmata thus open. This process is known as terminalization.

3. The chromosomes appear almost circular due to continued contraction.
4. Some of the pairs of homologous chromosomes still appear joined with one another.

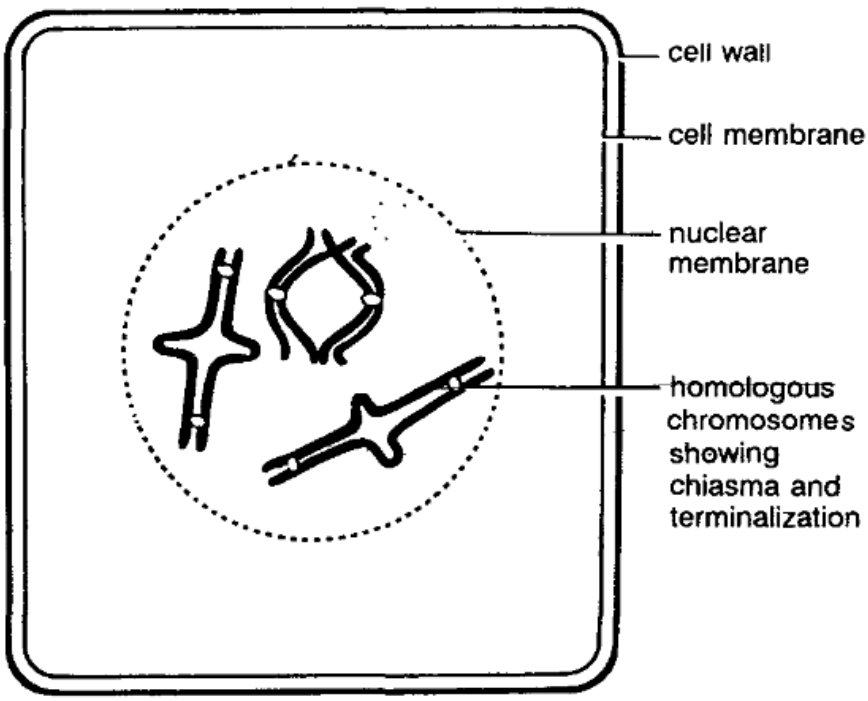


Fig. 20. **Meiosis**. Cell showing Diakinesis of Prophase I.

[VI] Metaphase I

The characters observed during Metaphase I are-

1. Nuclear membrane and nucleolus have completely disappeared.
2. Spindle formed by fibres is distinct.
3. Bivalents are arranged on the equatorial plate.
4. Each chromosome of a bivalent is attached to the spindle fibres by its centromere.
5. Centromeres are arranged on both the sides of the equatorial region, almost at equal distance.

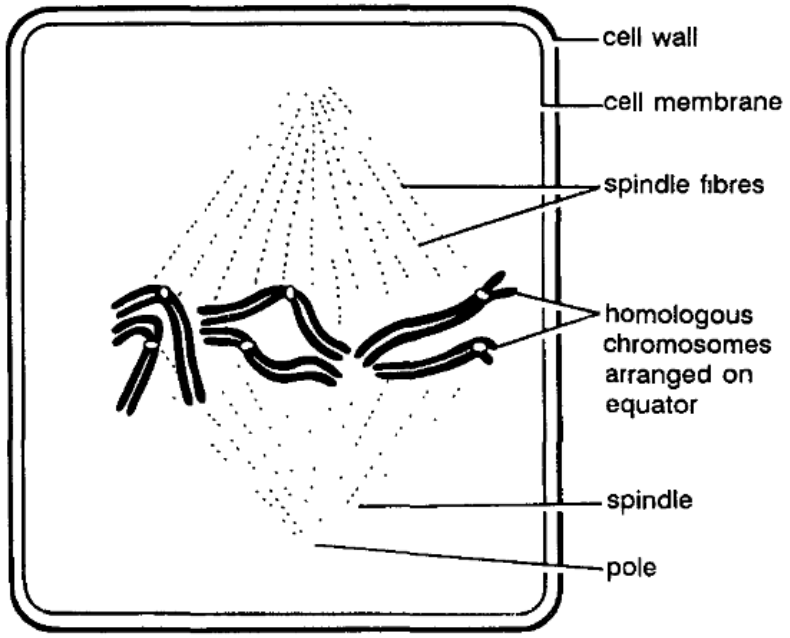


Fig. 21. **Meiosis**. Cell showing Metaphase I.

[VII] Anaphase I

The following are characteristics of this stage-

1. Nuclear membrane and nucleolus are completely absent.
2. The chromosomes separate out of the pair of homologous chromosomes.
3. Spindle fibres contract and pull the centromere along with the chromosome to opposite poles.
4. This results in two haploid sets of chromosomes, one at each pole of the cell.
5. Each chromosome shows characteristic shape during movement.

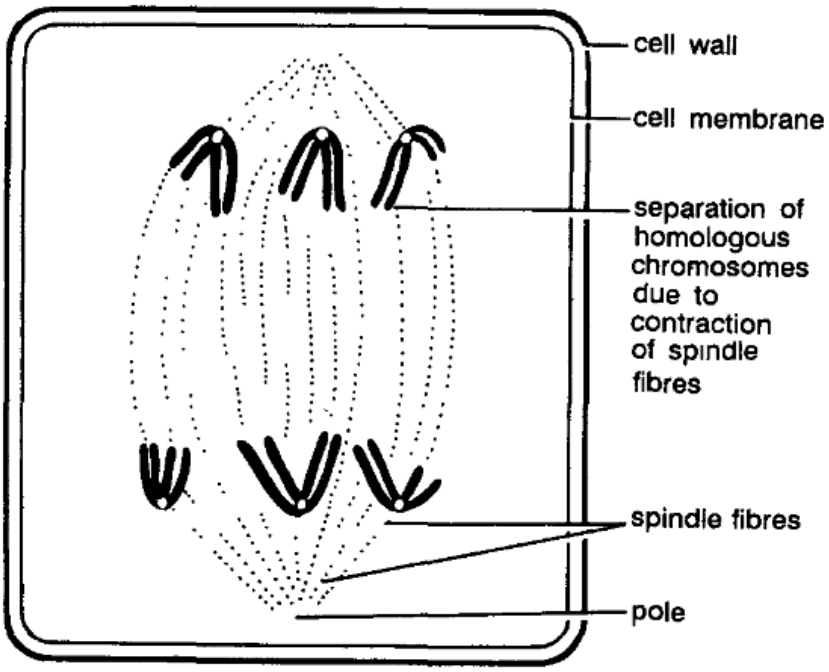


Fig. 22. **Meiosis**. Cell showing Anaphase I.

[VIII] Telophase I

The stage shows following characteristics-

1. Nuclear membrane and nucleolus have reappeared and are clearly seen.
2. There are two nuclei one each at the poles of the cell.
3. Each daughter cell has half the number of chromosomes compared to the parent cell. Chromosomes are thin and long. They are intermingled with one another to form a network.
4. Spindle fibres are totally absent.

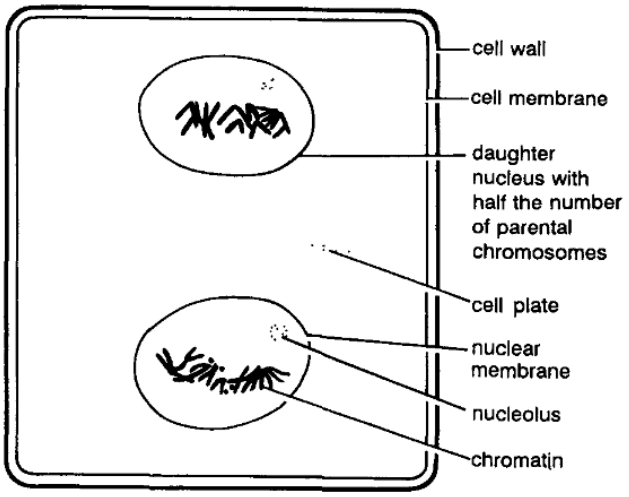


Fig. 23. **Meiosis**. Cell showing Telophase I.

[IX] Prophase II

The following characteristics are seen-

1. Nuclear membrane and nucleolus are distinct in the early stages. In late prophase, both these structures disappear gradually.
2. Chromosomes are short and thick.
3. Each chromosome is made of two chromatids bound together by a centromere.
4. The spindle fibres also begin to appear.
5. Chromosomes move towards the equatorial plate which is generally formed at right angles to the plate formed during meiosis I.

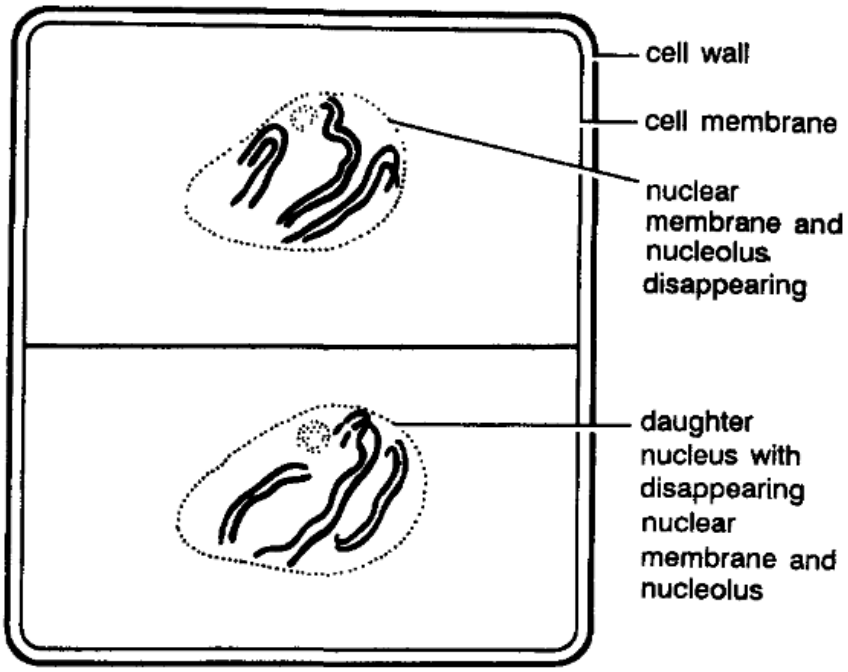


Fig. 24. **Meiosis**. Cell showing Prophase II.

[**XI Metaphase II**

It shows following characteristics-

1. Nuclear membrane and nucleolus both are absent, having disappeared.
2. Spindle fibres are formed. These are organized into a spindle.

3. Spindle fibres are joined with centromeres of the chromosomes.
4. All the chromosomes are aranged on the. equatorial plate.
5. Each chromosome is made of two chromatids held together by a centromere.

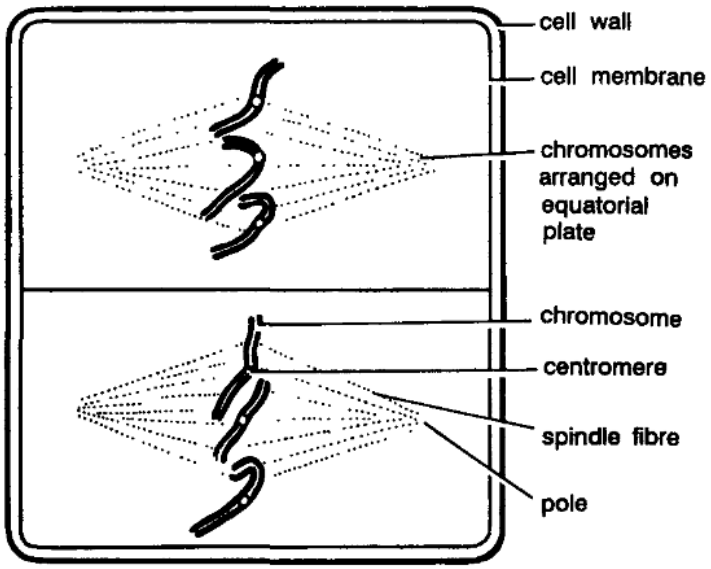


Fig. 25. **Meiosis**. Cell showing Metaphase II.

[XI] Anaphase II

This stage is characterized by the following-

1. Nuclear membrane and nucleolus are absent.
2. Centromere that holds two chromatids splits. Each chromatid now has an individual centromere.
3. Spindle fibres contract and each chromosome is now pulled to the opposite poles.
4. Chromatids (now called chromosomes) show characteristic shape during their movement.

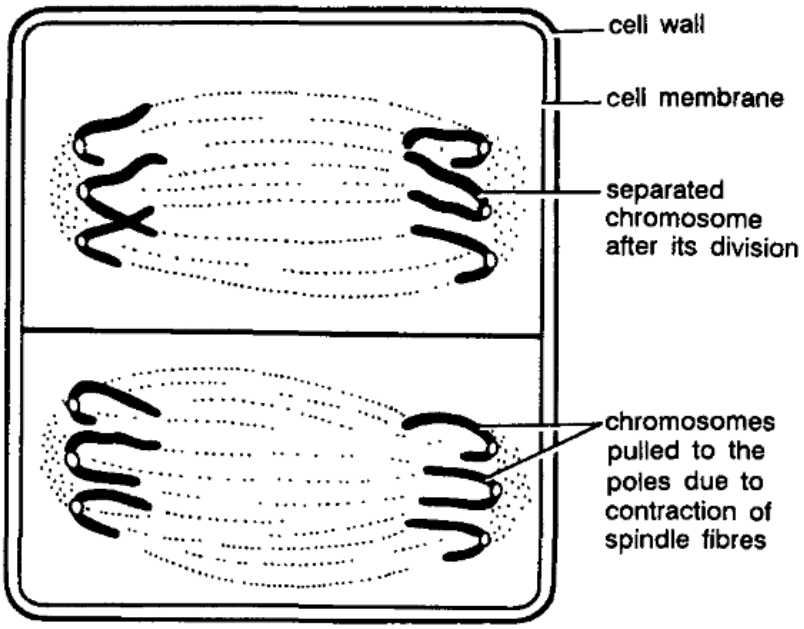


Fig. 26. **Meiosis**. Cell showing Anaphase II.

[XII] Telophase II

The following are characteristic features of this stage-

1. Chromosomes are in the form of groups at each end of the parent cell.
2. Nuclear membrane reappears and surrounds the group of chromosomes. This results in the formation of daughter nuclei at the opposite poles of the cells.
3. Spindle fibres disappear completely.

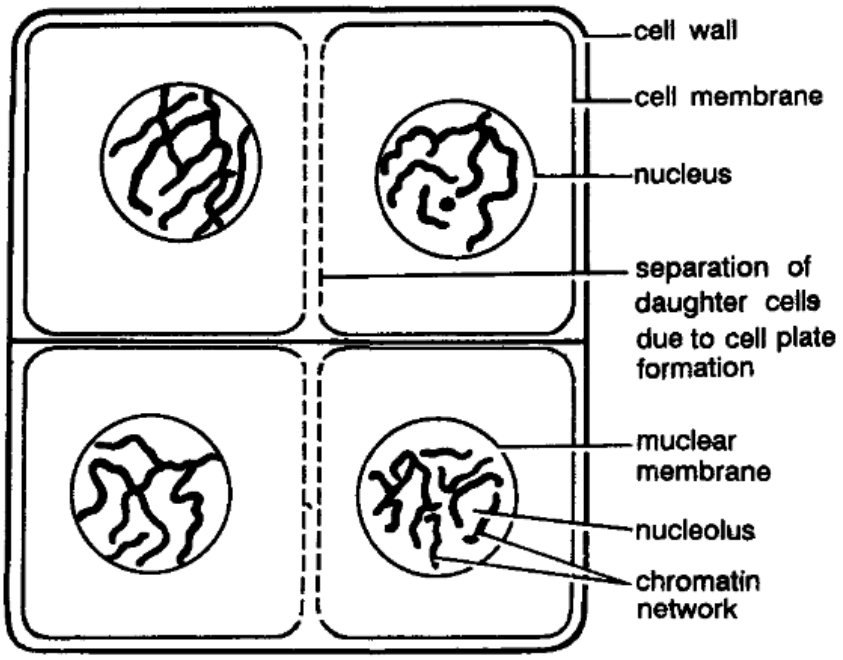
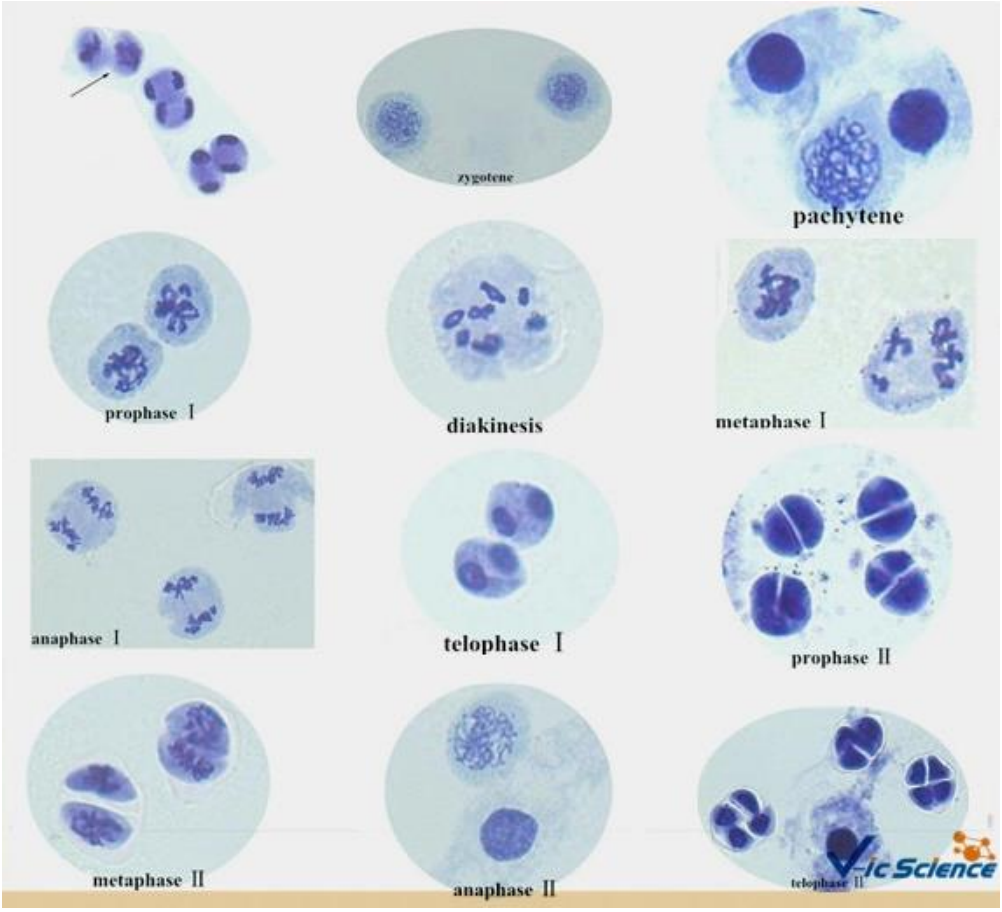
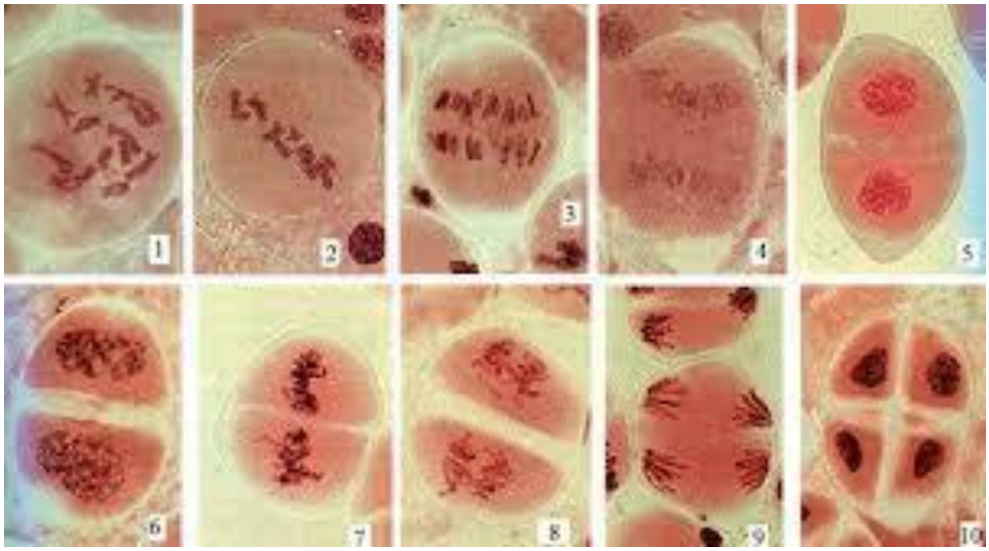


Fig. 27. **Meiosis**. Cell showing Telophase II.


zygotene
pachytene
prophase I
diakinesis
metaphase I
anaphase I
telophase I
prophase II
metaphase II
anaphase II
telophase II
Vic Science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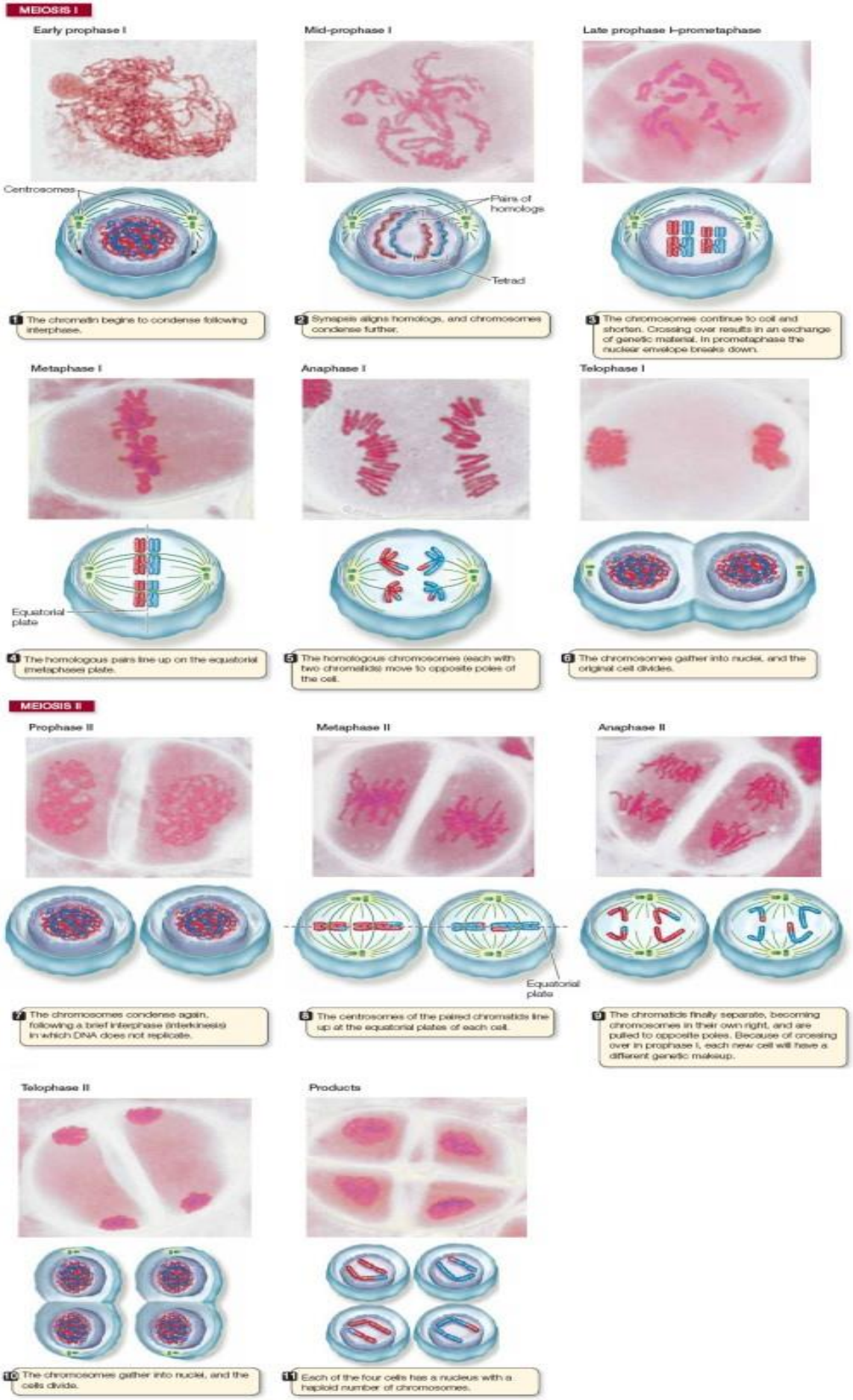
MEIOSIS I
Early prophase I
Centrosomes
1 The chromatin begins to condense following interphase.
Mid-prophase I
Pairs of homologs
Tetrad
2 Synapsis aligns homologs, and chromosomes condense further.
Late prophase I–prometaphase
3 The chromosomes continue to coil and shorten. Crossing over results in an exchange of genetic material. In prometaphase the nuclear envelope breaks down.
Metaphase I
Equatorial plate
4 The homologous pairs line up on the equatorial (metaphase) plate.
Anaphase I
5 The homologous chromosomes (each with two chromatids) move to opposite poles of the cell.
Telophase I
6 The chromosomes gather into nuclei, and the original cell divides.
MEIOSIS II
Prophase II
7 The chromosomes condense again, following a brief interphase (interkinesis) in which DNA does not replicate.
Metaphase II
Equatorial plate
8 The centromeres of the paired chromatids line up at the equatorial plates of each cell.
Anaphase II
9 The chromatids finally separate, becoming chromosomes in their own right, and are pulled to opposite poles. Because of crossing over in prophase I, each new cell will have a different genetic makeup.
Telophase II
10 The chromosomes gather into nuclei, and the cells divide.
Products
11 Each of the four cells has a nucleus with a haploid number of chromosomes.

References

- Ashok M. Bendre; Ashok Kumar (2010): A Textbook of Practical Botany Vol. II. Rakesh Kumar Rastogi for Rastogi Publications.

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- https://www.wikipedia.org/

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