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SYLLABUS

CELL BIOLOGY AND GENETICS
SC-111

CHAPTER – I :

Structure of plasma membrane and cell wall; structure and function of cell organelle : nucleus, golgi bodies, ER, ribosomes and peroxisomes; general characters of chromosomes; mitosis and meiosis

CHAPTER – II :

DNA the genetic material; Mendelism; linkage analysis; allelic and non-allelic inetractions.

CHAPTER – III :

Gene expression, structure of gene; regulation of protein synthesis; structure of proteins; mutations; extra nuclear genome.

UNIT

1

STRUCTURE OF PLASMA MEMBRANE

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know the structure of plasma membrane and its functions.

1·0. INTRODUCTION

All living cells are surrounded by thin membrane. **Purkinji** (1840) used the term **protoplasm** for what is contained inside a cell. **Nageli** and **Cramer** (1855) called the outermost layer of protoplasm as **cell membrane**. **Plowe** (1931) used the term **plasmalemma**. However, the term **plasma membrane** is generally preferred to other terms.

1·1. CHEMICAL COMPOSITION

Chemically, the plasma membrane is made up of 60% proteins, 40% lipids and a small percentage of carbohydrates (by dry weight). The membranes contain three different kinds of proteins—the structural proteins, enzymes and carrier proteins. The structural proteins form the internal part of the cell membrane. The plasma membrane consists largely of structural proteins. These proteins have little catalytic property and are extremely lipophilic. The enzymes are catalytic proteins and are associated with endoplasmic reticulum, mitochondria and plasma membrane. The carrier proteins are associated with transport across membranes. Based on their location in the biological membranes, the proteins are differentiated into two groups (**Singler**, 1971) :

(i) Peripheral proteins or extrinsic proteins. These are present on the outermost and inner surface of lipid bilayer. These do not interact with its hydrophobic core of phospholipid membrane directly. These are loosely

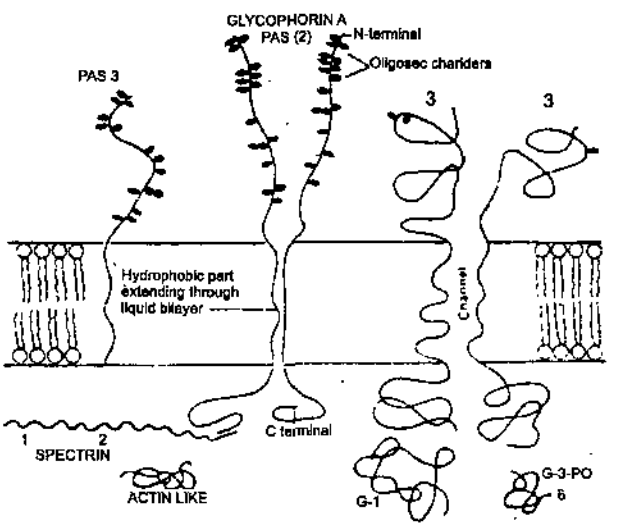


Fig. 1. Plasma membrane. Major polypeptides of R.B.C. membrane.

bound to the surface of lipid bilayer either indirectly by interactions with integral proteins or directly by interactions with polar heads of phospholipid molecules (Fig. 1).

Peripheral protein constitute about 20–30 percent of the total membrane proteins for example—spectrin (Fig. 2) cytochromic, etc. These proteins can be separated by mild treatment such as concentrated salt solution or by some chelating agents. These proteins are attached to lipid layer either through covalent linkage with fatty acid chain or through an oligosaccharide to a phospholipid.

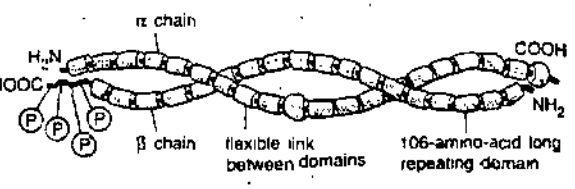


Fig. 2. Plasma membrane. Structure of spectrin molecule.

(ii) Integral proteins or instrinsic proteins. These proteins penetrate the lipid layer partially or wholly. Their polar heads protude from the surface of lipid bilayer and nonpolar regions are embedded in it. These integral proteins remain embedded in the bilayer by three basic types of interactions (1) ionic (2) hydrophobic (3) specific interactions with defined regions of lipids, glycolipids, oligosaccharides. Integral proteins represent more than 70 percent of the two types of proteins. Usually these are insoluble in aqueous solutions. The organic solvents are required to separate them from the membrane. Some examples of integral proteins are glycophorin in mammalian RBCs and bacterial rhodopsin in the membrane of photosynthetic bacteria.

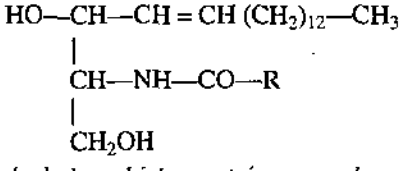
The integral proteins occur in the following five forms :

(1) Large globular integral protein molecules project beyond lipid bilayer on both the sides called transmembrane or channel proteins. *These proteins have central hydrophobic region with a* hydrophilic or polar region at either end that projects out of the lipid bilayer. Their hydrophobic regions have non-polar or hydrophobic amino acids on their surface. The hydrophobic surface forms *hydrophobic bonds with the fatty acid tails of the phospholipids. It is found that transmembrane* protein chain crossing the lipid bilayer is mainly formed of α-helix form of protein. The transmembranes are of two types–single pass and multipass proteins.

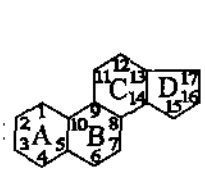
(2) *Small integral protein molecules partially penetrate the lipid bilayer and are exposed only* on one surface.

(3) Glycoproteins are attached to the integral protein molecules that project on the outer surface of plasma membrane.

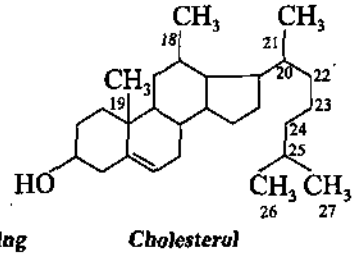
The lipids of plasma membrane are mainly of three types—**phospholipids, glycolipids** and **sterols**. The phospholipids are found in higher cencentration in the cellular and subcellular membranes of all living organisms. The phospholipids contain a phosphate moeity in the hydrophilic head. The phospholipids can be distinguished to **glycerolphospholipids** and **sphingophospholipids.** Glycolipids contain one or more monosaccharide residues and are based on **4-sphingenine** (sphingosine) or **ceramide.** A ceramide is a sphingosine to which a fatty acid is linked by an **amide bond**. A glycolipid may be **cerebroside** or **gangilioside.**



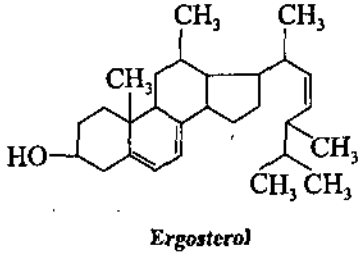
Sterols are steroid alcohols which contain a cyclopentanoperhydrophenanthene ring. **Cholesterol** is the most abundant sterol in animal tissues. It is usually a minor component in cell membranes of higher plants. **Phytosterols** (plant sterols) are the major sterol components of plant cell membranes such as stigmasterol. **Ergosterol** is a major sterol of the membranes of eukaryotic microorganisms.



Cyclopentanophenanthremic ring



Cholesterol



Ergosterol

1·2. MEMBRANE MODELS

The existence of the plasma membrane of the cell was difficult to prove by direct examination before 1930's because of technological limitations. Thus most of the experimental approaches have been provided by only indirect evidences of the existence of such a membrane around the cells.

In an attempt to explain the physical and biological features of cell membranes two main types of hypotheses have been proposed, the **bilayer** models and the **micellar** or **subunit models.** In the bilayer models the proteins and lipids constituting the membrane are believed to occur in layers. In the micellar model the membrane is believed to consist of a number of similar units. Several theories and hypothessis have been put forth, but the most accepted theory is **fluid-mosaic model theory** which explains the structure of the plasma membrane.

1. Bilayer Models

(A) Existence of lipid in plasma membrane.

(I) **Overton** (1895) observed that substances soluble in organic solvents entered the cell more rapidly than the substances soluble in water. On this basis he concluded that the plasma membrane is made of a thin layered lipid. It concludes that the peculiar osmotic properties of living protoplasts are due to a selective solubility mechanism. He also speculated that this layer might contain cholesterol, lecithin and fatty oils.

(II) **Hober** (1910) and **Fricke** (1925) found that the intact cells had low electrical conductivity indicating the presence of a lipid bilayer around it.

(III) Later on it was found by **Dawson** and **Belkin** (1929) that when a drop of oil is placed on cell surface it spreads and adheres to the cell surface. If a lipid containing hydrophilic groups such as the carboxyl groups of fatty acids or the phosphate groups of phospholipids is dissolved in a highly volatile solvent (*e.g.*, Benzene) and several drops of it are then carefully applied to the surface of the water the lipid spreads out to form a thin molecule–thick or monomolecular film. This suggests the existence of lipid layer in membrane.

(IV) In 1917 **Langmuir** carried out an experiment in a shallow trough filled with water on which he spread lipid substance to make a mono molecular film. He used a barrier to push the trough to compress the film. He carried out this experiment for measuring the specific minimum surface area occupied by a mono molecular layer of lipid and the force necessary to compress all the lipid molecules into this area.

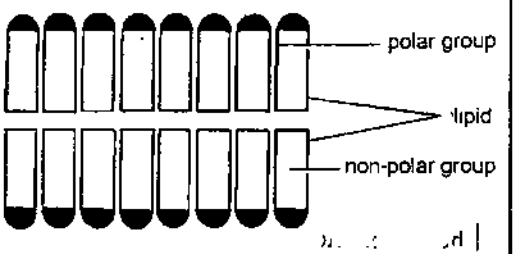


Fig. 3. Plasma membrane. The lipid membrane of Gorter and Grendel (1925).

(V) In 1925, **Gorter** and **Grendel** extracted the lipids from erythrocyte ghosts of a vareity of mammals and spread them out on monolayers in the Langmuir trough. These investigations suggested that the cell membrane was formed of phospholipids arranged to make bimolecular lipid sheet. The polar groups of lipid molecules of one layer were directed outward and those of the other layer were directed towards cell cytoplasm (Fig. 3).

(B) Protein-lipid-protein (Sandwich Models)—(I) **Harvey** and **Cole** (1931) and **Danielle** and **Harney** (1935) by studying the surface tension of cells suggested the presence of proteins in the plasma membrane in addition to lipids.

(II) Danielli-Davson Model—In 1935, **Danielli** and **Davson** proposed a model, called **Sandwich model** for membrane structure. In their lipoprotein model of the cell membrane they explained that the plasma membrane is formed of bimolecular layer of

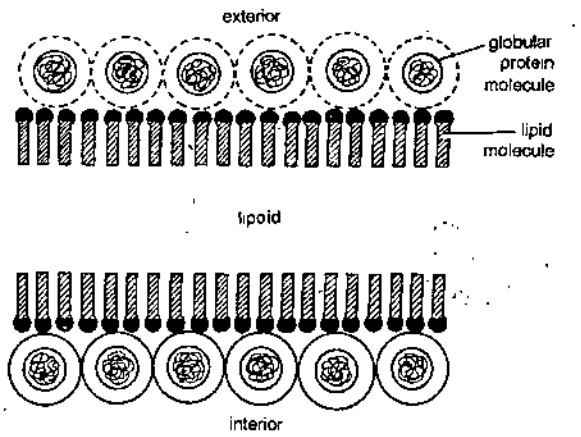


Fig. 4. Plasma membrane. The danielli-Davson model (1935) of membrane structure. The bimolecular layer of lipid molecules is of undefined thickness and is covered on each side by a continuous layer of globular proteins.

phospholipid sandwiched between two layers of proteins (hydrated globular proteins). The bimolecular lipid layer was similar to that proposed by **Gorter** and **Grandel** (Fig. 3) They predicted the lipid bilayer to be about 6·0 nm in thickness, and each of the protein layers of about 1·0 nm thickness, giving a total thickness of about 8·0 nm (Fg. 4).

Danielli-Davson model

The Danielli-Davson model got support from electron microscopic studies which showed that membrane consisted of two dark layers (electron dense granular protein layers), both separated by a lighter area in between (the central clear area of lipid bilayer). The total thickness observed was about 7·5 nm.

The lipid in sandwich model is phospholipid with its nonpolar groups facing each other and polar groups face proteins on either side.

Danielli (1938) had suggested many modifications in the sandwich model with regard to association of lipids and proteins. He suggested that proteins are of two types-globular proteins are present on the outer surface and the tangentially arranged proteins are in contact of the lipid.

Davson and **Danielli** (1943) considered the proteins to be in the form of folded β chains. The membranes have protein lined polar pores of about 7Å in diameter. The pores help in transport of small ions and water moelcules (Fig. 5A). In another modification, the proteins were considered to be in ∝ form present on both sides of lipid layer (Fig. 5B), or proteins are considered asymmetrical, with folded β chain on one side and globular proteins on the other side (Fig. 5C). Other modifications of the basic model have globular proteins on both surfaces (Fig. 5 D) and with folded proteins on both surfaces having helical proteins extending into the pores (Fig. 5E).

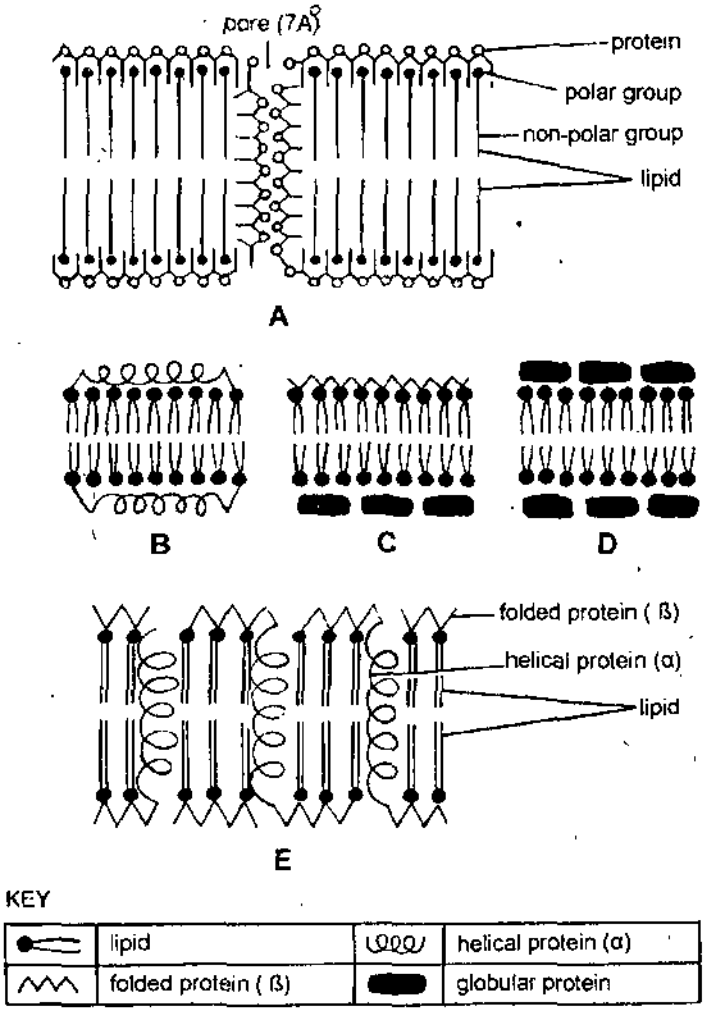


Fig. 5. (A–E). Plasma membrane. (A) The cell membrane with protein lined pores (Danielli, 1954), (B–E) Other postulated arrangement of proteins.

(III) **Hilleir** and **Hoffman** (1963) proposed that the plasma membrane consists of a mosaic of globular subunits or micelles of lipid molecules thus called **micelle models.** In each subunit hydrophilic polar ends of its lipid molecules are directed towards the priphery of the subunit. The globular protein forms a mono layer on either side of the lipid micelles. The space between globular micelles represents pore bounded partly by the polar groups of micelles and partly by polar groups of associated proteins. **Sjostrand** (1963) and **Fernadez-Moren** (1962) have supported the micelle structure.

(IV) **The Unit Membrane Model**—In 1960 **Robertson** examined the structure of different membranes of the cell. Using evidence from various electron micrographs he proposed his **unit membrane model.** Robertson described that the membrane is a **trilaminar structure** consisting of two parallel outer dense osmophilic layers of 2·0—2·5 nm (20–25 Å) of proteins : and a middle light coloured osmophobic layer of 30–35 Å thickness corresponding to the hydrocarbon chains of the lipids. Thus the unit membrane is 75 Å thick. The basic unit membrane structure was considered

to be general for a wide variety of plants and animal cells (Fig. 6). **Robertson** demonstrated that all biomembranes present inside the cells and around various cell organelles like ER, Golgi apparatus, lysosomes, mitochondria, chloroplasts and nuclear membrane etc. are unit membrane structures.

Robertson's unit membrane concept was like that of **Danielli-Davson** model with some differences. In **Danielli** model the protein structure is asymmetrical. The unit membrane like trilaminar artificial membranes has been produced using bimolecular lipid layer with protein absorbed on both sides. However, later studies have shown that all cell membranes are not the unit membranes 75Å in thickness but there can be variation in thickness and structure.

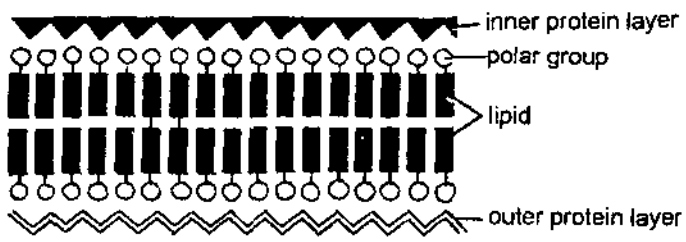


Fig. 6. Plasma membrane. The unit membrane.

(V) **Kavanau's lipid pillar model.** This is a modification of the **Danielli-Davson** model. The lipid layer in this model exists in two forms. Under certain conditions it is in the form of pillars while in others in the form of flattened discs. The spaces between pillars function as pores for transport.

The membrane with pillar structure is thicker than the membrane with the disc structure. The surface of pillar lipids is formed of the polar head lipids and interior is formed of the non-polar tails of the phospholipids. Protein layers are present on both sides of the membrane.

2. Models in which the proteins are considered to penetrate the lipid layer.

(I) **Bensons Model.** According to these types of models the proteins are not present as surface layer over lipids but the proteins penetrate inside the lipid layers. These models can explain the low surface tension properties of membranes. In 1966 **Benson** proposed a model on the basis of a study of chloroplast membranes. He said that proteins are not found on the outside of the lipid layer as in Danielli-Davson model, but are considered to partially or fully penetrate the lipid layer. He found that the membrane lipids and proteins have a hydrophobic association. The lipid tails bound by hydrophobic regions are complementary with hydrophoic regions of the interior proteins. The charged polarheads of phospholipids are on the surface of membrane and are capable of binding ions.

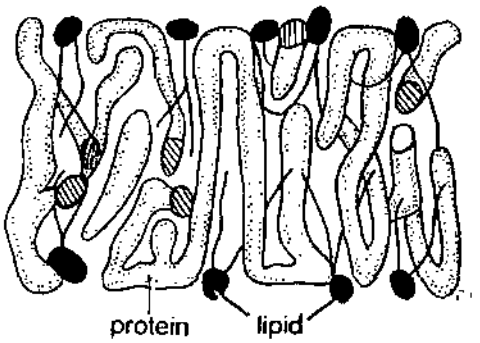


Fig. 7. Plasma membrane. Benson's model (1966). Protein within lipid bilayer.

(II) **Lenard and Singer model. Singer** (1966) proposed a model of plasma membrane. According to them the arrangement of one-third to one-fourth of the proteins is in **helical conformation** while the rest of the proteins form **random coils** in the membrane.

(III) **Mosaic-membrane Model. Baum** 1967, put forth the mosaic membrane concept. He considered that the back bone of the membrane consists of cuboidal units. Each unit of protein is toast shaped, and is covered on both sides and along the edges by phospholipids. The heads of the phospholipids stick out while their hydrophobic tails form complexes with hydrophobic surfaces of the protein. In mitochondrial membrane the stalk and head are attached to the backbone.

(IV) **Fluid Mosaic Model. S.J. Singer** and **G. L. Nicolson** (1972) suggested the widely accepted **fluid mosaic model** of biological membranes. According to this model the biological membranes are considered to be the semifluid structures so that the lipids as well as intrinsic proteins are able to make movement within the bilayer. This model assumes that there is a continuous bilayer of phospholipid molecules in which are embeded globular proteins. Proteins are present in mosaic pattern. The globular proteins of the membrane are considered to be of two different types. Proteins found attached at the polar surface of the lipid called **extrinsic** (peripheral) proteins while other proteins found either partially penetrating the bilayer or spaning the membrane entirely to stick out on both sides called (transmembrane) **integral proteins.** Further, the peripheral proteins and those parts of the integral proteins that stick on the outer surface (*i.e.*, ectoproteins) frequently contain chains of sugar or oligosaccharides likewise, some lipids of outer surface are glycolipids (Fig. 8 A, B).

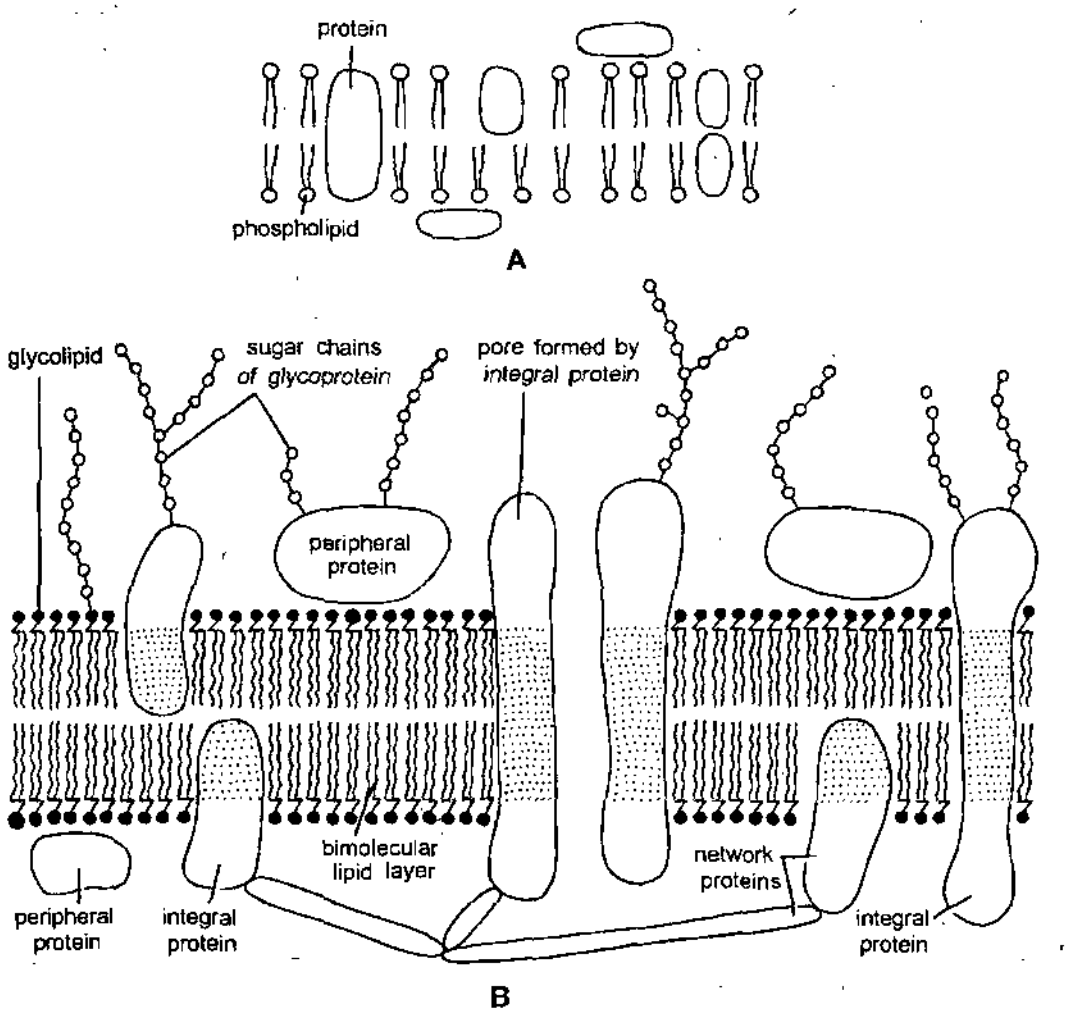


Fig. 8. (A, B). Plasma membrane. Fluid mosaic model of the plasma membrane. (A) Simplistic view, (B) complex view.

Experiments on the viscosity of membrane suggest that it is of a fluid consistency rather like the oil, and that there is a considerable side ways movement of the lipid and protein molecules within it. Becasue of the fluidity and the mosaic arrangement of protein molecules, this model of membrane structure is known as the **"fluid mosaic model".**

The concept of fluidity in the model assumes that lipids, proteins and oligosaccharides in membranes are held in their position by means of noncovalent interactions. It has been observed that the components can be dispressed by solvents without breaking bonds between them.

3. Micellar Models

According to **Fernadez-Moraur** (1962) and **Sjostrand** (1963) the membrane consists of **elementary particles** or **globular units.** These globules are 40-70Å in diameter and repeating units which are closely packed together. All biological membranes do not have globular structure and both unit membranes and globular structures coexist. The globular forms and unit membranes are interconvertible in certain environmental or metabalic conditions (Fig. 9).

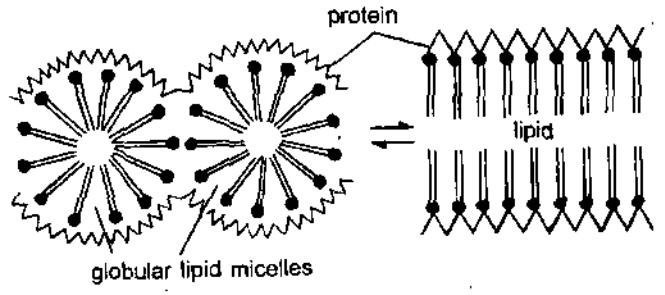


Fig. 9. Plasma membrane. Transformation between micellar and bilayer states of the cell membrane.

Green (1970) considered that the cell membranes consist of **repeating units** and there are two types of membranes : **monopartite** and **multipartite.** The monopartite membranes have repeating units without any projection e.g., the outer membrane of mitochandria and the plasma membrane of erythrocytes. The multipartites have repeating units with projections (Fig. 10).

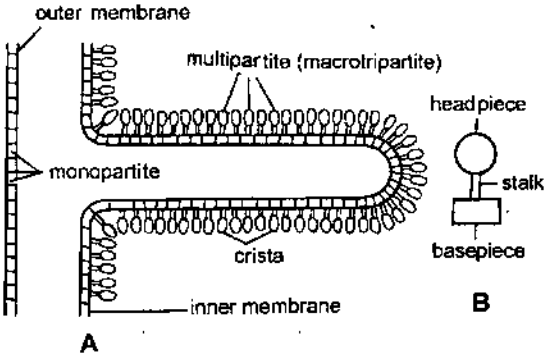


Fig. 10. Plasma membrane. Green's repeating unit model
(A) Crista of mitocohondrion. (B) Multipartite repeating unit.

The repeating units consist of a **basepiece, stalk,** and a **headspace.** The monopartite units correspond to the basepieces of multipartite units. The multipartite units may be **macrotripartate** and **microtripartite.** The macrotripartile units are found in membranes performing electron transfer and ATP synthesis e.g., inner membrane of mitochondria. In microtripartite units the headspace and stalk are more firmly attached to the basepiece than in macrotripartite units.

1.3. FUNCTIONS OF PLASMA MEMBRANE

The plasma membrane or cell membrane performs the following functions :

(I) Regulation of the passage of material. The plasma membrane is selectively permeable. It facilitates the entrance of required nutrients into the cells and permits the exit of nitrogenous wastes. It prevents the exit of substances from the cell. Thus cell membrane regulates the passage of materials inside and outside the cell. It plays important role in transport of small ions and molecules of various substances.

Transport of substances across the plasma membrane into cell can be achieved by the following methods :

(A) **Transport of water**	by	1. Osmosis
(B) **Transport of ions and molecules**	by	2. Passive transport (i) Simple diffusion (ii) Facilitated diffusion (iii) Dialysis
		3. Active transport
(C) **Transport of large molecules**		1. (Pinocytosis) Endocytosis
(D) **Transport of solid particles**		2. Phagocytosis and exocytosis.

Transport of the substances across the plasma membrane depends upon the permeability of the plasma membrane. Plasma membrane acts as a semi-permeable barrier between the cells and extracellular environment. This permeability is of highly selective type, it ensures that essential molecules like glucose. amino acids and lipids can readily enter the cell. The selective permeability of the plasma membrane allows the cells to maintain a constant internal environment (homeostasis). Organelles within the cell often have a different internal environment from that of surrounding cytosol and the organelle membrane maintains this difference. For example, in lysosomes the concentration of proteins (x) is 100 to 1000 times that of the cytosol. This gradient is maintained solely by the lysosomal membrane. So transport across the membrane occurs in several ways in the phospholipid bilayer or with the help of specific integral membrane protein called **transport protein, the permeases.**

(A) Transport of Water :

(1) Osmosis. Osmosis can be defined as **"the diffusion of water or solvent through a semipermeable membrane from a region of low solute concentration to a region of high solute concentration."** The plasma membrane is permeable to water molecules and movement of water molecules through the plasma membrane occurs due to differences in the concentration of the solute

on its either side. This process of movement of water molcules is known as **osmosis.** The process in which the water molecules enter into the cell is known as **endosmosis.** In endosmosis water moves from outer higher water concentration to the region of inner lower water concentration. Reverse of this process, which involves the exit of the water molecule from the cell is known as **exosmosis.**

The phenomenon of osmosis is of vital importance to the plants as the mineral salts and water are absorbed by this process *i.e.*, due to endosmosis and exosmosis water molecules come in or go out of the cell. The amount of water inside the cell causes a pressure known as **hydrostatic pressure.** The hydrostatic pressure which is caused by the osmosis is known as **osmotic pressure**. The plasma membrane maintains a balance between the osmotic pressure of the intracellular and intercellular fluids. The osmotic pressure and turgidity developed by osmosis are necessary for the cells to exhibit various processes such as growth, cell division and other metabolic activities.

(B) Transport of ions and molecules :

The transpart of ions and molecules is of two types :

(1) Passive Transport.

(2) Active Transport.

Passive transport. It is a type of diffusion in which transport of molecules and ions takes place along electro-chemical or concentration gradient without consuming any energy. This can be described as the transport of material "downhill". Passive transport is of following three types :

(i) Simple diffusion. In simple diffusion the transport across the membrane is without any help of permeases (transport proteins) *i.e.*, molecules of gases such as oxygen and carbon dioxide and small molecules *e.g.*, ethanol, enter the cell by crossing the plasma membrane without any permeases. In simple diffusion, a small molecule in aqueous solution dissolves into phospholipid bilayer, crosses it and then dissolves into the aqueous solution on the opposite side. The relative rate of diffusion of the molecule across the phospholipid bilayer will be proportional to the concentration gradient across the membrane (Fig. 11).

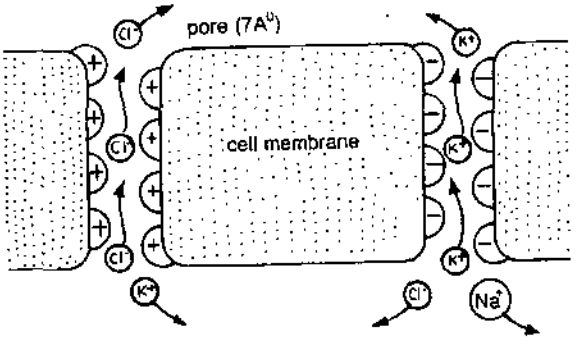


Fig. 11. Plasma membrane. Passage of substance through pores in a membrane.

(ii) Facilitated diffusion. This is a specific type of passive transport in which various polar *molecules such as ions, sugars, amino acids, nucleotides and many* cell metabolites cross the membrane rapidly because of permeases. Permeases are special membrane proteins also called membrane transport proteins. They occur in many forms in all types of biological membranes. There are two major classes of membrane transport proteins : **carrier proteins** and **channel proteins,** Carrier Protein Transporters bind to specific solute to be transported and undergo a series of conformational changes in order to transfer bound solute across the membrane. Channel proteins need not bind to the solute. They form hydrophillic pores that extend across the lipid bilayer. When these pores are open, they allow specific solutes (usually inorganic ions of appropriate size and change) to pass through them and thereby cross the membrane (Fig. 12).

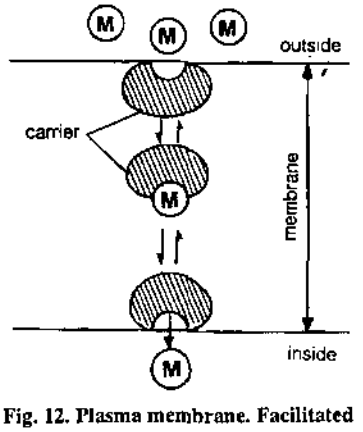


Fig. 12. Plasma membrane. Facilitated diffusion. M-metabolite.

The rate of transports of the molecules across the membrane is far greater than would be expected from simple diffusion. There is a maximum rate of transport *i.e.*, when the concentration gradient of molecules across the membrane is low, an increase in concentration gradient results in a corresponding increase in the rate of transport. This process is specific; each facilitated diffusion protein (channel protein) transports only a single species of ion molecule.

(C) Transport of Large Molecules :

Endocytosis. An important activity of the plasma membrane of certain cell types is endocytosis. Endocytosis is a process by which material is transported into the cells by formation

of vesicles. In endocytosis, small regions of the plasma membrane fold inward or invaginate, until it has formed new intracellular vesicles. In eukaryotes the following two types of endocytosis can occur : **pinocytosis** and **receptor-mediated endocytosis.**

(I) Pinocytosis (*Gr. Pinein*, to drink). The pinocytosis is the process of ingestion of fluid materials by the cell through plasma membrane. This process was first observed by **Edward** in *Amoeba* and by **Lewis** (1931) in the cultured cells. **Lewis** was the first to observe pinocytois in living cells in culture. He described the uptake of fluids by an active movement of undulating membrane formed at the periphery of the cell (Fig. 13). The substances of high molecular weight such as proteins and amino acids which can not enter cell through plasma membrane by simple osmosis, are ingested by the cell by pinocytosis.

The light microscopy has shown that in *Amoeba* tiny pinocytic channels are continually being formed at the cell surface by invagination of the plasma membrane. From the inner end of each channel small vacuoles or pinosomes are pinched off, these move towards the centre of the cell, where they fuse with primary lysosomes to form food vacuoles.

Pinocytosis has been observed at the subcellular or sub-microscopic level. It is known as **micropinocytosis**. In such type of pinocytosis, the plasma membrane invaginates to form small vesicles or pinosomes about 650 Å in diameter. These vesicles pinch off from the plasma membrane and they move across the cytoplasm to fuse with opposite plasma membrane discharging their contents. The micropinocytosis has been observed in endothelial cells, schwann cells of nerve ganglia, macrophages, muscle cells and reticular cells.

Rhopheocytosis. In rhophaeocytosis small quantities of cytoplasm together with their inclusions are transferred from one cell to the other. It was first demonstrated by **M. Bessess** in bone marrow tissue. During maturation of RBCs the ferretin granules along with small amount of cytoplasm are transferred from reticuloendothelial cells to erythroblad. This passes into the cytosol and is utilized in the synthesis of haemoglobin.

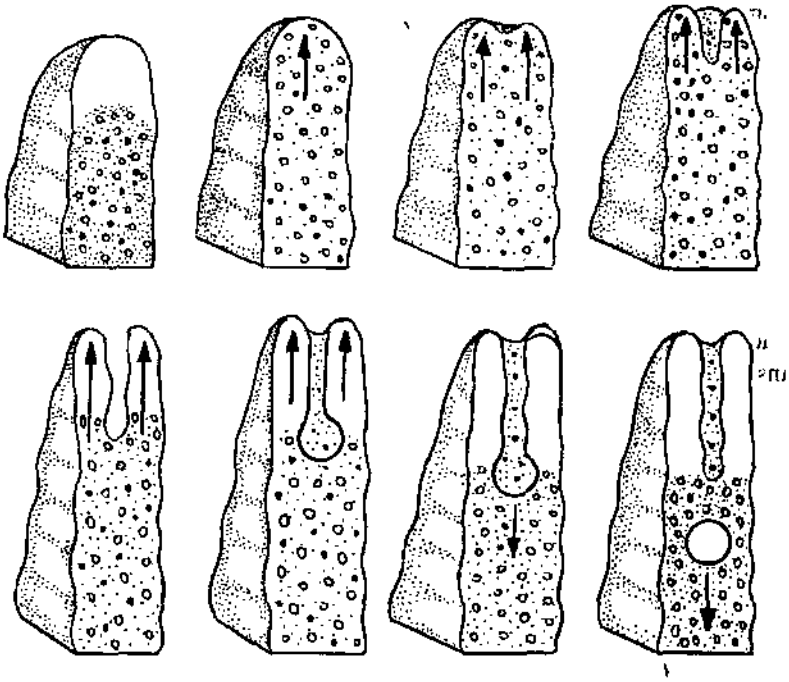


Fig. 13. Pharma membrane. Pinocytosis.

(II) Receptor-mediated endocytosis : In this type of endocytosis, a specific receptor on the surface of the plasma membrane recognizes an extra-cellular macromolecule and binds with it. The substance bound with the receptor is called the ligand. The whole process of receptor-mediated endocytosis, includes the following steps.

1. First macromolecules or ligands bind to the specific receptor sites on the plasma membrane. Small proteins like insulin, immunoglobulin lipoprotein, oligosaccharide, vitamins B_{12}, Viruses are some examples of ligands. About more than 25 different types of receptors involved in receptor-mediated endocytosis have been identified. These receptors are transmembrane proteins and contain specific binding sites.

2. Next step is the formation of coat-pits. Coat pits are depression of plasma membrane. The ligand bound receptor diffuse into these coated pitcs. These coated pits invaginates into the cell and pinch off to form the coated-vesicles. The coat of coated pits and coated vesicles is made up of protein called **Clathrin**, these vesicles are called Clathrin coated vesicles.

3. Once a coated vesicle is formed, the clathrin and associated proteins dissociate from the vesicle and return to the plasma membrane to form a new coat-pit. After shedding their clathrin-coats these endocytic vesicles deliver their contents to endosomes.

Endosomes. In the cells exists a complex set of heterogeneous membrane bound tubes and vesicles, called **endosome**, which extends from the periphery of the cell to the perinuclear region, where it lies quite close to Golgi apparatus. Endosomes lack in degradative enzymes.

Thus, via receptor-mediated coated-vesicles the ligands are delivered to the peripheral endosomes which slowly move inward to become perinuclear endosomes. These perinuclear endosomes are converted into endolysosomes and then into lysosomes. In most animal cells uptake of cholesterol occurs due to this receptor-mediated endocytosis.

(D) Transport of Solid Particles :

Solid particles or macromolecules are secreted out from the cell by exocytosis and are ingested into the cell from outside through phagocytosis.

(I) Exocytosis. It is also called **emeiocytosis** or **cell-vomiting.** In all eukaryotic cells, secretory vesicles carry new plasma membrane and cellular secretions such as proteins, lipids and carbohydrates from Golgi apparatus to plasma membrane or to cell exterior by the process of exocytosis. Exocytosis also serves as a means of extruding undigested material from the cell. Exocytosis is a process which is essentially the reverse of endocytosis. The membrane of a cell vesicle fuses with the plasma membrane and releases its contents outside the cell.

In pancreatic cells, the vacuoles containing digestive enzymes move from the interior of the cytoplasm towards the surface. Then they fuse with plasma membrane and discharge their contents to the exterior.

(II) Phagocytosis (*Gr Phagein*, to eat, *Kytes*, cell). The process of engulfing large size particles of solid food or foreign particles by the cell through the plasma membrane is known as **phagocytosis.** This process was first described by Russia scientist **Methnikoff** in 1893. for the description of phagocytosis **Methnikoff** got Nobel Prize of Physiology and Medicine in (1980).

Phagocytosis is found in many protozoans, (Fig. 14) where it works for the nutrition of the cell. In the metazoa it is a method of defence against foreign bodies like bacteria, dust, viruses, parasitic intestinal cell, debris and colloids etc. In mammals leucocytes of blood, histocytes of

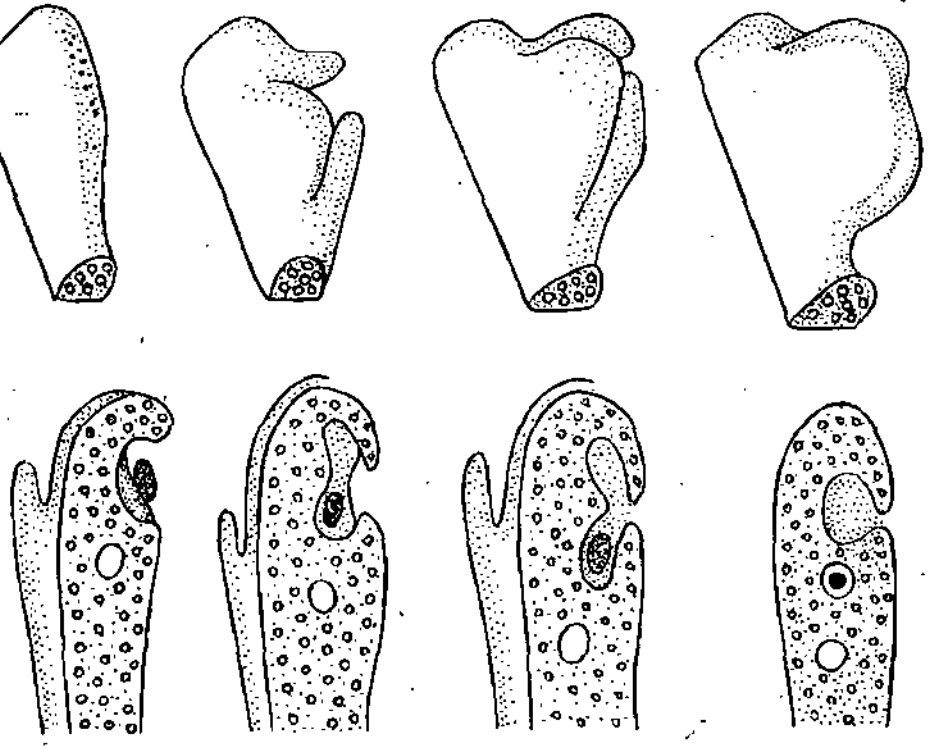


Fig. 14. Plasma membrane. Phagocytosis.

connective tissue, the reticular cells of haemophoetic tissue (bone maronss lymph nodes and spleen) and endothelial cells lining the capillar sinusoids of liver, adrenal gland etc. exhibit phagocytosis.

In phagocytosis the plasma membrane expands along the surface of the particle (bound to the specific receptors on the cell surface) and eventually engulfs it. It thus, pinches off from the cell membrane forming phagocytic vesicle known as **phagosome**. Phagosome is typically 1 to 2 μm or larger in diameter, much larger than those formed during pinocytosis and receptor-mediated endocytosis. The phagosome is transported deep into the cytoplasm where it joins the lysosomes forming **phgolysosomes.** The enzymes of lysosomes dissolve and digest the contents of the phagosomes and the residue is ejected out of the plasma membrane. The digestion is also known as **ephagy.**

Phagocytosis is of two types :

(i) Ultraphagocytosis or colloidopexy. In this type of phagocytosis plasma membrane ingests small colloidal particles. This phenomenon is exhibited by leucocytes and macrophagic cells of mammals.

(ii) Chromopexy. When the cell ingests colloidal chromogen particles phagocytically, the process is known as **chromopexy**. It is seen in some mesoblastic cells of mammals.

• STUDENT ACTIVITY

1. Define plasma membrane. Give its functions.

2. Give the fluid mosaic model of plasma membrane.

• SUMMARY

- The outermost layer of protoplasm has been termed as cell membrane or plasma membrane. Chemically, it is composed of 60% proteins and 40% lipids. The percentage of carbohydrates ranges from 1–10 on the basis of location. The proteins are differentiated into two groups : extrinsic and intrinsic. The lipids are of three types namely phospholipids, glycolipids and sterols. Three different types of membrae models are proposed : bilayer models, micellar models and the models in which the proteins are considered to penetrate the lipid layer. Main functions of the plasma membrane are transport of water, ions, molecules and solid particles.

1.6. TEST YOURSELF

1. Name the outermost layer of protoplasm.
2. What is the important difference between the active and passive modes of membrane transport?
3. Who coined the term plasmalemma ?

4. Who proposed the first bilayer lipid model of plasma membrane ?
5. Name the method in which transport of water across the plasma membrane into the cell takes place.
6. Name the proces in which the plasma membrane of the plant cell shrinks away from the cell wall.
7. Write the main function of plasma membrane.

• ANSWERS

1. Plasma membrane or cell membrane or plasmalemma.
2. Active transport requires metabolic energy while passive transport requires concentration *gradient.*
3. **Plowe** 4. **Overton** 5. **Osmosis** 6. **Plasmolysis**
7. Regulate the flow of materials into and outside the cell.

❑❑❑

UNIT

2

CELL WALL

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know structure, chemical composition, utra structure, function and formation of cell wall.

2·0. INTRODUCTION

The plant cell is always surrounded by a cell wall and this feature distinguishes them from animal cells. Animal cells lack cell wall and their cells are delimited by a delicate plasmamembrane. The cell wall is made up of nonliving matter secreted by the protoplasm. The cell wall can be thin or thick, smooth or sculptured, its structure and thickness varies in different tissues of the plants. It is relatively very thin in parenchymatous tissue and is probably thickest in the xylem vessels.

Cellulose, hemicellulose, pectin and proteins are the main constituents of the cell wall in most of the cells of plants. It may be impregnated with lignin, suberin, waxes, salts and fatty acids. The bacterial cell wall is made up of protein-lipid-polysaccharide complexes. The chief polysaccharides of bacterial cells are formed of two amino sugars : N-acetyle glucosamine and N-acetyl nuramic acids, while in fungi the cell wall is formed of chitin.

2·1. STRUCTURE OF PLANT CELL WALL :

Plant cell wall can be distinguished in the following layers :

(i) Primary cell wall

(ii) Secondary cell wall

(iii) Tertiary cell wall and middle lamella

(i) Primary cell wall. The first deposited cell wall is known as primary cell wall. It is formed during the early stages of growth and development. The primary cell wall is comparatively thin permeable and anisotropic. It is found in all plant cells and is 1 to 3 μm thick. The primary cell wall is elastic, and undergoes extension with the growth of the cell. Primary cell wall is chiefly composed of cellulose, hemicellulose and pectic compounds. In many roots, fleshy stems, fruits

and leaves the cells contain only the primary cell wall and the middle lammella. Certain epidermal cells of the leaf and the stem also possess cutin and waxes, which make the primary cell wall permeable. The primary cell wall of yeast and fungi is composed of chitin.

(ii) Secondary cell wall. *It is laid down on* the primary cell wall when the cell stops to elongate and the changes appear to be irreversible. It is found only in certain mature and highly specialized cells. It is about 5 to 10 μm thick. The secondary cell wall is thick and permeable. The secondary cell wall consists of three concentric layers S_1, S_2 and S_3, which occur one after the other (Fig. 1). These layers are also known as outer layer, *the middle layer and* the inner layer. The secondary wall consists of cellulose, hemicellulose and polysaccharides. The macrofibrils of the cellulose are compactly arranged and between these sometimes lignin occurs as an inter fibrillar material. In some algae there are many fibrils of xylan and mannan.

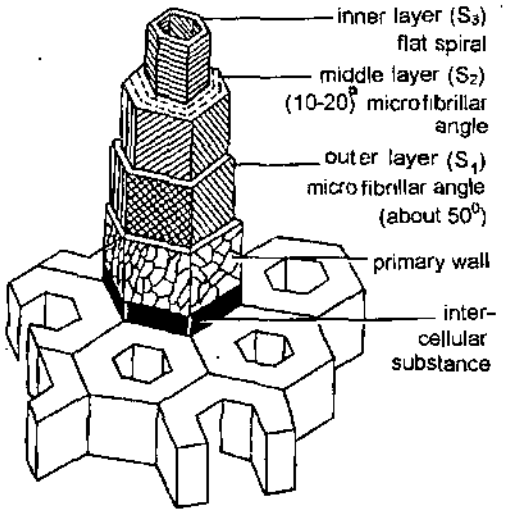


Fig. 1. Cell wall. Structure of a cell wall showing middle lamella, primary cell wall and three regions (S_1, S_2 and S_3) of the secondary cell wall.

(iii) Tertiary cell wall. In some plant cells, there occurs another cell wall beneath the secondary cell wall which is known as tertiary cell wall. The tertiary cell wall differs from the *primary and secondary cell wall in its morphology, chemistry* and staining properties. Besides the cellulose, the tertiary cell wall is composed of another chemical substance **xylan.** Tertiary cell wall is found in xylem trachieds of Gymnosperms.

Bailey (1934) first described difference between primary and secondary walls. The middle lamella and primary wall are associated with living cytoplasm and can increase in surface area as well as in thickness. Their process of increase is called **interssupception**. The secondary wall is nonliving, it can increase in thickness but not in surface area by **apposition.** The process of secondary wall formation is irreversible.

Middle Lamella :

Middle lamella is formed between adjacent cells during cell division and is shared by the adjoining cells. It consists of a comparatively thin layer of intracellular material. It is a viscous and jelly like substance and functions as a cementing material between the primary cell walls. The middle lamella is mainly composed of the chitin, pectin, lignin some proteins and calcium or magnesium pectate.

2.2. ORGANIZATION OF CELL WALL

The cell wall is composed of macrofibrils of about 0.5 μm in thickness. The macrofibrills are made of a bundle of microfibrils, the diameter of a microfibril is about 250 Å. The microfibril consits of a bundle of elementary fibrils or **micelles**, each of about 100 Å in diameter. The fibrils are made of cellulose which is a polymer of glucose attached by β 1–4 bonds.

According to arrangement of the fibrils, the primary wall has **dispersed texture** which can be **fibroid**, **tubular** and **foliate**. The fibroid is scattered arrangement. It is more on longitudinal plane and less in transverse. The tubular is more on transverse plane and less in longitudinal. The foliate is random type arrangement. The secondary wall has **parallel** texture which can be fibrillar, annular and helical. The fibrillar is parallel along the cells axes while annular is perpendicular to cell axis. There is a composite capillary system in cell wall. The space between two elementary fibrils is < 10 Å while the space between two macrofibrils is < 100 Å.

2.3. CHEMICAL COMPOSITION

Plant cell wall consists of carbohydrates (polysaccharides), lipids, proteins and mineral deposits. All these chemicals exhibit distinct staining properties. The polysaccharides of cell wall are cellulose, hemicellulose pectin compounds and lignins.

Cellulose. The structure of the cell wall is mainly based on cellulose. Cellulose is a linear, unbranched polysaccharide, consisting of straight chains of glucose units linked by β 1-4 bonds. The cell wall is made of macrofibrils of 0·5 μm in width. These macrofibrils consist of a bundle of microfibrils of about 250 Å diameter. The microfibril is visible under electron microscope. It is ribbon like flat fiber consisting of a bundle of micelles or elementary fibrils each about 100 Å in diameter. The micelles contain about 100 cellulose chains. Each cellulose molecule is made up of 40 to 70 glucan chains. In the primary cell wall the macrofibrils are arranged at random. In the secondary cell wall macrofibrils are closely packed and are arranged parallel to one another. Cellulose is synthesized by all plants and microbiral cells.

Hemicelluose. Hemicelluloses are short, branched hetropolymers of various kinds of monosaccharides such as arabinase, xylose, mannose, galactose, glucose and uronic acid.

Pectin. The pectin is water soluble, hetrogeneous branched polysaccharide. The pectin has negative charge because of the presence of D-galactouronic acid. This negative charge makes pectin highly hydrated. Other polysaccharides like homopolysaccharide of mannose are found in the cell wall of yeast fungi and bacteria. During the later stages of growth large amount of lignin is laid down in plant cells, and the walls harden into wood. Lignin gives rigidity and strength to the cell wall. Lignin is present in all the three layers of the cell wall. The process of lignification starts with the primary cell wall and then extends to the middle lamella and the secondary cell wall.

Lipids. Lipids like suberin, cutin and waxes are also found as structural components of the cell wall. A **suberin** lamella is found in the suberized cells of cork tissue. It is a water resistant substance, comprising of fatty acids. It is separated from the contents of the cell by a cellulose wall. **Cutin** is also a biological plastic substance made up of fatty acids. A layer of cutin is found on the surface of epidermal cells. Cutin along with cellulose layer forms the cuticular membrane. **Sporopollenin** is a lipid substance. It forms a protective layer over pollen grains.

Minerals like pectates, carbonates and silicates of calcium and magnesium also occur in cuticle of some plants. In cruciferous and curcurbitaceous plants calcium deposits are found in the cell wall and silicates are found in the cell wall of Poaceae.

Glycoproteins. They are present upto 20% in primary cell wall. The glycoproteins are known to act like glue to increase the strength of the wall.

2·4. ULTRA STRUCTURE

Electron microscopy has shown that the cell wall consists of two main parts : The **matrix** and **fibril.**

I. The matrix. It is the ground substance in which microfibrils of cellulose are embedded. It is amorphous and is deposited with hemicellulose, pectin and proteins.

The matrix mainly consists of polysaccharide substance. The substance present in matrix varies with growth phase of the plant. In earlier stages pectic substances are dominant and in later stages xylans and hemicellulose are more prominent.

II. Fibrils. The fibrils are made of cellulose. Cellulose molecules are polymers of disaccharide cellobiose having approximately 3000 glucose units arranged in a straight chain. The cellulose molecules of the primary wall are found arranged in bundles called **macrofibrils**. Macrofibrils are made of thread like structures called **microfibrils**. In the primary cell wall these fibers and matrix molecules are cross-linked by a combination of covalent bonds and non covalent bonds to form a highly complex strucutre whose composition is generally cell-specific. In this complex, hemicellulose molecules are linked by hydrogen bond to the surface of the cellulose microfibrils. Pectin molecules and glycoproteins are tightly woven into the texture of the wall.

The secondary wall is also made of cellulose macrofibrils. These macrofibirls are arranged in the same fashion but the cellulose microfibrils of macrofibrils are more compactly arranged and the inter fibrillar material is mainly lignin. The microfibrils are oriented in a parallel fashion and are compactly arranged.

Thickenings of the Cell Wall

During maturation phase the cell may undergo secondary thickening by the deposition of cellulose, pectin, lignin suberin etc. However, some cells *e.g.*, parenchyma cells remain unthickened. The thickening material is secreted by the protoplasm and is laid down in a more or less uniform manner giving the wall a stratified appearance.

Different patterns of thickning may be of the following types : (1) Annular, (2) Spiral, (3) Scalariform, (4) Reticulate, (5) Pitted. Pits are also of two types : simple and bordered pits (Fig. 2).

The pits always occur in pairs, one on either side of the middle lamella. The wall separating the two paired pits is known as **closing membrane.**

In annular type lignin is deposited in the form of rings that are placed one above the other. In spiral form the thickening takes the form of spiral band. In scalariform the thickening matter lignin is deposited in the form of transverse rods or rungs of a ladder and the whole structure gives appearance of ladder. In reticulate type thickening takes the form of network.

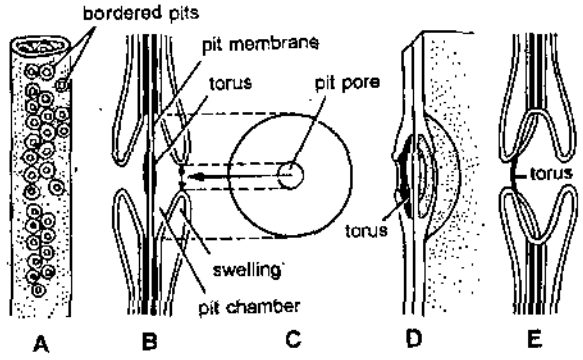


Fig. 2. Simple pits and bordered pits in cell wall.

2·5. PLASMODESMATA

Every living cell in a higher plant is connected to adjacent living cells by fine cytoplasmic bridges, called ***plasmodesmata*** (Fig. 3). The cytoplasm of one cell passes through the pores in intervening cell walls. A plasmodesma is about 20 to 40 nm is diameter. Each plasmodesma is lined

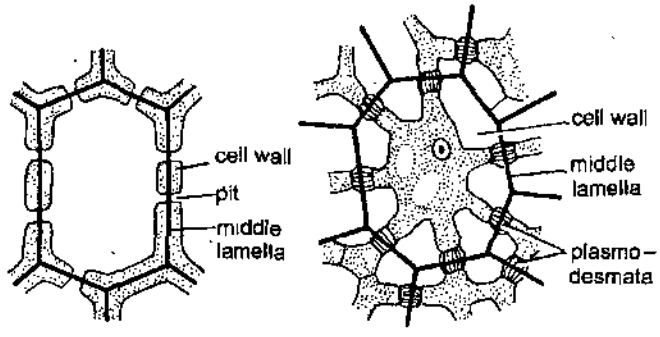


Fig. 3. Plasmodesmata

with plasma membrane common to two connected cells It usually also contains a fine tubular structure—the **desmotubule**, derived from smooth endoplasmic reticulum. Between the outerside of the desmotubule and the inner face of the cylindrical channel formed by plasmamembrane is an annulus through which small molecules can pass from cell to cell. Plasmodesmata are normally created in all new cell walls. They were first observed by **Strasburger** in *1901*.

2·6. FUNCTIONS OF THE CELL WALL

The chief function of cell wall in plant cells is that it provides mechanical strength to the plant cells. Cell wall acts like a skeletal framework of plants.

Plant cell wall is fully permeable to solvents and solutes. This is because the matrix is filled with minute water-filled channels through which free diffusion of water and water soluble substances such as gases, sugars, salts, harmones etc. takes place.

Lignification and suberization (deposition of lignin and suberin in primary cell wall respectively) makes plant cell impermeable to water. The lignified tissue provides the mechanical strength to the plant cell due to its lingo-cellulose composition. The suberization is found in the walls of cork cells. Suberin is impermeable to water and checks evaporation also. Many enzymatic activities are also known to occur within the cell wall.

2·7. FORMATION OF CELL WALL

The formation of cell wall starts immediately after nuclear division. First the matrix of the cell wall is formed. The cell wall matrix components are transported via vesicles derived from Golgi apparatus and secreted by exocytosis at the plasma membrane. At the end of mitosis granules or vesicles arising from the Golgi apparatus arrange themselves on the equator of the cell. Golgi derived vesicles containing cell wall precursars, especially pectin are guided to the equator along with

oriented microtubules of phragmoplast, where they fuse with one another to form the cell plate. The cell plate at this stage comprises of following structure : (i) central middle lamella. During the development of the cell wall the middle lamella is formed first, (2) Primary cell wall on both sides of middle lamella (3) plasma membrane enclosing living cytoplasm of each daughter cell.

The cell plate grows in thickness by addition of new material from the Golgi apparatus. Ultimately the cell plate fuses with the mother cell wall to create two separate daughter cells.

The enzymes needed to form a typical primary wall are found in endoplasmic reticulum and Golgi apparatus and some enzymes are also found in cell wall itself.

The fibrils of cellulose in most plants are synthesized on the external surface of the cell by a plasma membrane bound enzyme complex, called **cellulose synthetase.** After synthesis α cellulose chains spontaneously assemble into **microfibrils** that form a layer on the surface of the plasma membrane. Since the cellulose is synthesized at the plasma membrane, each new wall lamella forms internally to the last formed lamella. The cell wall therefore, consists of concentrically arranged lamellae with the oldest on the outerside and youngest inside. The primary cell wall consists of these interveined fibers of cellulose. The secondary cell wall consists of up to three layers of parallel fibers of cellulose. The direction of fibre shifts through 120° in each layer thus the orientation of the fibre is same in every fourth layer.

• STUDENT ACTIVITY

1. Describe the chemical composition of cell wall.

2. Describe the functions of cell wall.

• SUMMARY

- In Plants the cells are held by middle layer. Each plant cell is surrounded by a cell wall. The cell wall is distinguishable into three layers, primary, secondary and tertriary. The cell wall is made up of cellulose, hemicellulose, proteins and lipids. Ultrastructure of cell wall shows that it consists of two main parts : matrix and fibrils. Formation of cell wall starts immediately after cell division. Cell wall provides mechanical strength to plant cells. It also acts as a skeletal frame work of plants.

• TEST YOURSELF

1. Which layer is nearer to plsma membrane in plants ?
2. Name the most abundant water insoluble polysaccharide of a plant cell.
3. Name the chemical substance which is most abundantly presnet in the middle lamella.
4. What is the chemical composition of bacterial cell wall ?
5. Name the lipid which forms the protective layer over pollen grain.

• ANSWERS

1. Secondary cell wall **2.** Cellulose **3.** Pectin
4. Two amino sugars namely *N*-acetyl glucosamine and *N*-acetyl muramic acid
5. Sporopollenin.

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UNIT

3

STRUCTURE AND FUNCTIONS OF CELL ORGANELLE : NUCLEUS

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know the structure and functions of nucleus.

3·0. INTRODUCTION

Nucleus is the most conspicuous body within the protoplast of a stained cell. Nucleus was first observed by **Robert Brown** (1833) in the cells of orchids. He pointed out the nucleus as a normal and characteristic constituent of cells. In all **eukaryotes** *i.e.*, all higher plants and animals, the nucleus is bounded by a definite nuclear membrane, and such organisms are called **eukaryotes.** In **bacteria, blue green algae** and in some **dinoflagellates**, the nuclei are not bounded by nuclear membrane and such organisms are called **prokaryotes.** The nuclear material in prokaryotes is called **nucleoid.**

3·1. SHAPE

The shape of nucleus may be related to the shape of the cell or may be different. In spheroid, cuboid and polyhedral cells, the nucleus is generally spherical (Fig. 1 E). In cylindrical, prismatic and fusiform cells, nulceus is elliptical. In flattened squamous epithetial cells, it is discoid (Fig. 1 D). The

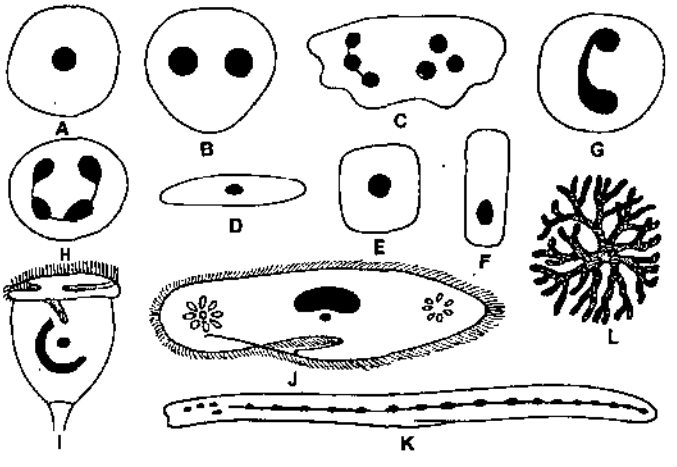


Fig. 1 (A–K). Nucleus : Different numbers and shapes of nuclei : A. Mononucleate cell, B. Binucleate cell, C. Polynucleate cell, D. Disc shaped nucleus, E. Spherical nucleus, F. Oval nucleus, G. Bilobed nucleus, H. Multilobed nucleus, I. C-shaped nucleus of *Vorticella*, J. Pyriform nucleus of *Paramecium*, K. Moniliform nucleus of *Spirostomum*, L. Labyrinthine nucleus of *Platyphylax*.

nucleus is horse–shoe shaped or multilobed in leucocytes, C shaped in *Vorticella* (Fig. 1I), pyriform in *Paramecium* (Fig. J), moniliform in *Spirstomum* (Fig. 1K) and in glandular cells of many insects it is branched. In Spermatozoa the nucleus may be ellipsoid, pyriform and lanceolate (Fig. 1 A–L).

3.2. SIZE

The size of nucleus is variable but in general it is directly proportional to cytoplasm. According to **R. Hertwig** *it is numerically expressed as nucleoplasmic index (NP) :*

$$NP = \frac{V_n}{V_c - V_n}$$

(NP = nucleoplasmic index, V_n = nuclear volume, V_c = volume of the cell)

All somatic nuclei have specific size that depends upon the contents of DNA and proteins. The nucleus size is also related to its functional activity during interphase **Boveri** (1905) observed that nucleus size was proportional to ploidy levels. The size of nucleus increases from haploids to *diploids and to tetraploid cells.*

3.3. NUMBER

Most of the cells of plants and animals contain one nucleus and are called **mononucleate** (Fig. 1A). Some cells of cartilage and liver have two nuclei and are called **binucleate** (Fig. 1B). Cells with many nuclei are called **polynucleate** (Fig. 1C), the nuclei can be upto 100 in osteoclasts of bone marrow. In *Syncitia* the nuclei can be several hundred *e.g.*, in striated muscle cells and in siphonaceous algae.

3.4. STRUCTURE AND FUNCTIONS OF NUCLEUS

Ultrastructure

In general the following structures are distinguishable in the interphase nucleus :

(i) Nuclear membrane (ii) Nucleoplasm
(iii) Chromatin (iv) Nucleolus.

(i) Nuclear Membrane

The interphase and prophase nuclei remain surrounded by **nuclear membrane** or **karyotheca**. The nuclear membrane is two layered consisting of the outer and inner nuclear membranes. Each nuclear membrane is similar to cell membrane and is trilaminar unit structure, 75 Å in thickness. The two membranes are separated from each other by a space called **perinuclear space** or **perinuclear cisterna**. It varies from 100–700 Å in width but usually it is 200 Å in width. The perinuclear space is filled with fluid similar to that of endoplasmic reticulum cisternae. Antibodies, lipid droplets and crystalline deposits are observed in perinuclear space.

The outer nuclear membrane may be continuous with the endoplasmic reticulum at various points. The outer membrane has ribosomes attached on it. Some areas are without ribosomes and appear to take part in vesicle formation or blebbing. In some animal cells a 300Å thick fibrous layer is seen next to inner membrane and it is called **fibrous lamina**. The nuclear membrane helps in nucleo–cytoplasmic exchanges and separates nucleoplasm from cytoplasm.

The nuclear membranes are discontinuous and these are perforated by many **nuclear pores**. The pores are 400–1000 Å wide, circular or octagonal in shape. The inner and outer membranes are continuous at the margin of pores. The nuclear pores were first demonstrated by **Callan** and **Tomlin** (1950) in amphibian oocytes. They thought that pores are present only in the outer membrane. **Afzelius** (1955) described a diaphragm extending across the nuclear pore (Fig. 2). The pores can be randomly distributed, arranged in rows or clustered.

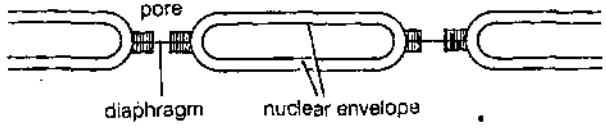


Fig. 2. Nucleus : Structure of nuclear pore (Afzelius, 1955)

Annuli in the nuclear pore were first observed by **Callan** and **Tomlin** (1951) but were considered as artifacts, **Afzelius** (1955) described annuli as cylinders of non-membranous materials attached to the rim of nuclear pore. Annuli appear as electron dense ring within the pore. The annulus

consists of eight subunits arranged radially around the periphery of pore. The subunits have been described differently. These are considered as **microcylinders** by **Witschnitzer** (1958), **Vivier** (1967), **filaments** by **Maul** (1971), **stacks of disc** by **Harris** (1974), **series of spheres** by **Norrevang** (1965) and **ovoids** by **Robert** and **Northcote** (1970). The structure of nuclear pore has been described mainly according to the following three theories :

1. Annulus as cylinder fitting into the nuclear pore : According to **Witschnitzer** (1958) annulus is a non-membranous hollow cylinder fitting into the nuclear pore. The nuclear pore is a cavity of 500 Å diameter through which the substances pass. The wall of cylinder is made of eight microtubules or microcylinders each of 200 Å diameter (Fig. 3 A, B).

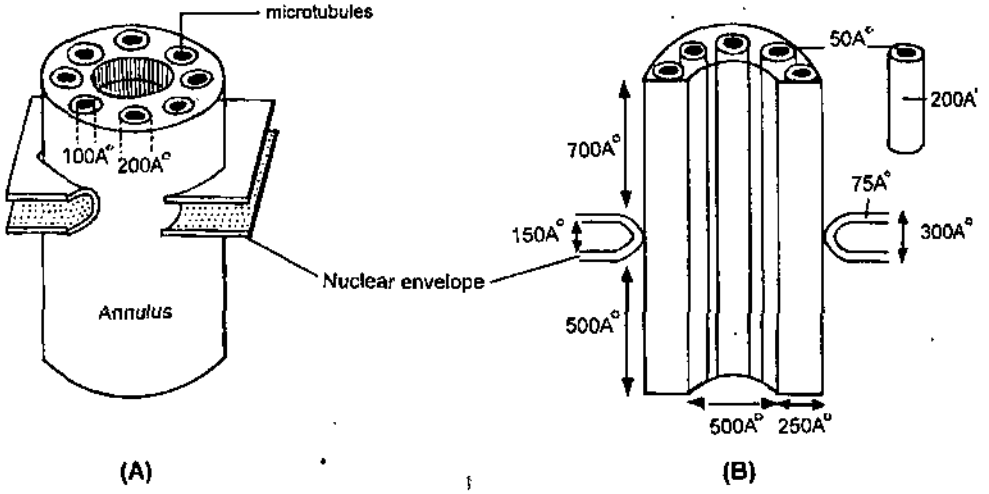


Fig. 3. Nucleus : Cylindrical model of the nuclear pore and annulus (Witschnitzer, 1958)

According to **Vivier** (1961) a **central microtube** of 150–180 Å in diameter is present in the cylinder. This tube is attached to the inner wall of cylinder by fibrous struts. The microtubules in cylinder wall are 120–150 Å in diameter. (Fig. 4).

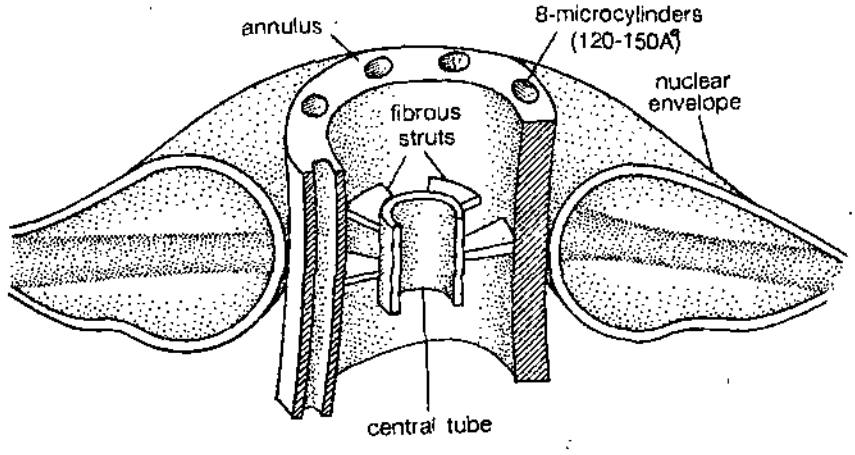


Fig. 4. Nucleus : The pore complex (Vivier, 1961).

2. Annular material extending laterally and the pore margin. According to **Gall** (1956) and **Rebhum** (1956) the annulus is made of 8–10 granules. According to **Franke** (1974) the annular material is present not only within the nuclear pore but also extends largely beyond the pore margin. On the inner and outer margins of the pore are eight evenly spaced granules of 150 Å diameter. Inside pore there is a central granule. Fine fibrils of 30 Å diameter extend from central granule to peripheral granules making **cartwheel** like structure. (Fig. 5A, B).

According to **Franke** (1974), the inner annulus and outer annulus are made up of **annular granules** of 100–250 Å diameter. The finger like projection of 100 Å called **peripheral granule** is attached to pore wall in equatorial plane. Between annular granules and peripheral granule a material forms a cuff over the periphery of the pore. Inside pore is a **central granule** 40–350 Å in diameter. The **inner pore filaments** of 25–50 Å connect central granule and annular granules. The **equatorial**

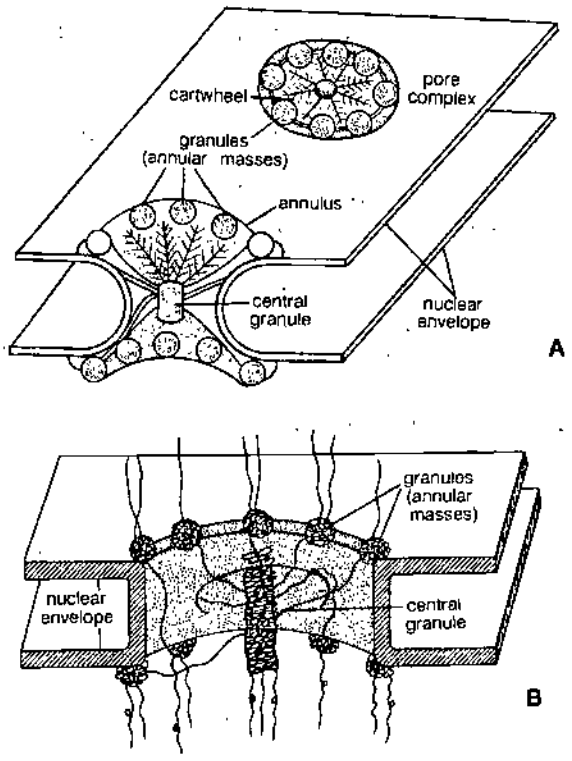


Fig. 5. (A, B). Nucleus : (A) The pore complex, (B) Pore complex in fibrillar form.

filaments connect central granule and pore interior. Axial filaments of 30–60 Å are attached to and are continuous with the annular granules and the central granules. (Fig. 6).

3. Kessel (1969) described pores as octagonal : The annular material of the pore is an amorphous matrix. The granules of 40–70 Å and filaments of 30 Å diameter are present in matrix. The central region of pore contains high concentration of granules in closed configuration. The central portion when clear represents open configuration.

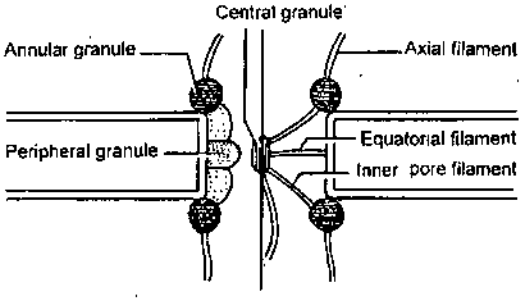


Fig. 6. Nucleus : The pore complex (Franke, 1974)

4. The Fibrous Lamina : Harris and **James** (1952) reported the presence of fibrous material on inner surface of the nuclear envelope in *Amoeba proteus*. It makes hexagonal compartments and it is called **honey-comb layer** or **fibrous lamina**. The fibrous lamina may be present in many types of cells. The functions of fibrous lamina are not clear. It can contribute to annular material or may be supporting structure of nuclear envelope. The fibrous lamina is made of mainly acid proteins. (Fig. 7)

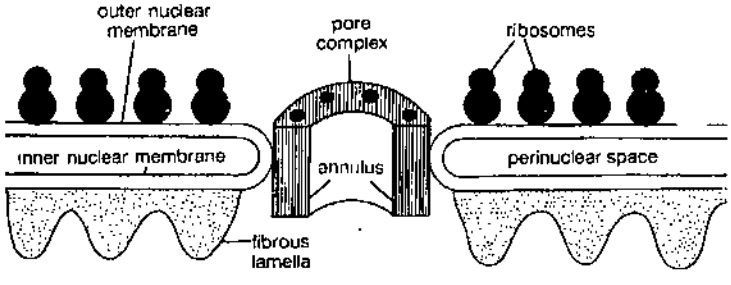


Fig. 7. Nucleus : Nuclear membrane with pore complex, ribosomes and fibrous lamina

(i) Chemical composition of nuclear membrane : The nuclear membranes like unit membranes are made of 60–75% proteins and 15–35% lipids. The sugars are associated with membranes in low quantities. The nuclear membranes exhibit ATPase activities in higher concentration in nuclear pores. Many transport enzymes are also associated with nuclear membranes.

Functions of Nuclear Membrane

1. Protection of genetic material : The nuclear membrane separates genetic material from cytoplasm. Genetic material is saved against the mutagenic effects of cytoplasmic enzymes.

2. Nucleocytoplasmic exchange : During transcription all RNA are synthesized in nucleus, these are transported across nuclear envelope before translation takes place in cytoplasm.

Similarly inorganic ions, organic molecules and water are transported across membrane by exchange across nuclear envelope, by blebbing, by active transport or by exchange through *endoplasmic reticulum.*

3. Attachment of nuclear components : The nuclear membrane may have attachment of chromosomes by centromeres or telomeres. The lateral components of the synaptonemal complex remain attached to inner nuclear membrane during meiosis.

4. Attachment of structural elements of cytoplasm : The nuclear membrane provides surface for attachment of structural elements of cytoplasm *e.g.*, microfilaments, microtubules and *myofilaments.* The filaments attached to nuclear membranes also connect to cell organelles like mitochondria.

5. Electron transport systems : Nuclear membranes have electron transport activity as that of endoplasmic reticulum.

6. Protein synthesis : Since ribosomes are present on the outer membrane of nucleus, it may have protein synthesis function like that of endoplasmic reticulum.

7. Origin of other cell membranes : The nuclear membrane and endoplasmic reticulum are source of other cell membranes. The vesicles given off by nuclear membrane can contribute to formation of endoplasmic reticulum, golgi complex and plasmamembrane.

(ii) Nucleoplasm

The nucleus is filled with dense but clear mass of protoplasm called **nucleoplasm, karyolymph** or **nuclear sap.** The nucleoplasm is acidophilic, this gives nucleus the turgescene and transparency. When acted upon by certain fixatives the protein of nucleoplasm forms artificial fibrillar structure called **linin.**

(iii) Chromatin

The fine network of beaded fibres with coarse granules is called **chromatin network.** During cell division it becomes more clear and visible as definite number of individual chromosomes. The choromatin network is made of strongly stainable chemical substance called **chromatin** or **nuclein.** The coarse granules studded on chromatin network are called **chromocentres** or **karyosomes.** These are more condensed regions of chromatin called heterochromatin. Some of the chromocentres are associated with nucleolus.

(iv) Nucleolus

The nucleus contains some definite number of spherical bodies called nucleoli. These are highly refractile, dense, acidophilic and are made of ribonucleo-proteins. The nucleolus was first described by **Fontana** in 1874. Nucleolus is spherical structure inside nucleus, it can be peripheral or central in position. Nucleolus is associated with nucleolar organizer region of chromosomes. It has been found that this region contains cistron for ribosomal RNA and this may be a place of synthesis. During interphase the nucleolus remains attached with nucleolar organizer and remains in close contact with DNA and histone of the organiser. Nucleoli may be of two kinds—**plasmasomes** and **karyosomes**. Plasmasomes are true nucleoli which get stained with acidic dyes and disappear during cell division, karyosomes are false nucleoli which are stained with basic dyes and are flakes of chromatin.

The number of nucleoli can be one to many in a cell. For example, liver and lymphocytes have two nucleoli in each cell and polyploids may have many nucleoli.

Structure of Nucleolus

In 1951 **Estable** and **Sotelo** studied structure of nucleolus using special silver impregnation technique. According to them nucleolus consists of two distinct regions :

(a) The pars amorpha—*It is structureless, homogeneous matrix but under high magnification* it appears to be a mass of filaments about 50 Å in thickness.

(b) The nucleolonema—It is a fibrillar component which appears as coiled thread.

The nucleolus ultrastructure was first given by **Borysko** and **Bary** (1951) and **Bernard** (1952). They confirmed presence of homogeneous matrix and fibrillar component in nucleolus. Later **Gonzales Ramiry** (1961) and **Izard** and **Bernhard** described nucleolonema as spongy network *instead of a continuous filament. The ultrastructural studies of nucleolus by* **Day** *(1968),* **Bush** *and* **Smetana** (1970) have recognized four components in structure of nucleolus :

(i) Pars amorpha—This homogeneous matrix contains scattered granules and fibres.

(ii) Chromatin—The chromatin contains abundant DNA which serves as template for

synthesis of RNA. The **perinucleolar chromatin** surrounds nucleolus like a shell. These can form thick continuous wall or may be thin with holes. From perinuclear chromatin, septa like trabeculae project inside nucleolus and these are called **intranucleolar chromatin** (Fig. 8).

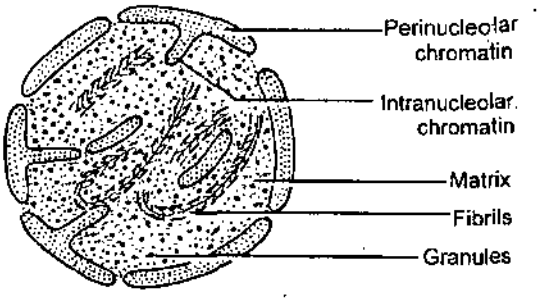


Fig. 8. Nucleus : Ultrastructure of nucleolus

(iii) Fibrils—These are 80–100 Å in diameter, they contain RNA and may be precursor of the granules.

(iv) Granules—The granules are present in pars amorpha. The granules are 150–200 Å in diameter, they contain proteins and RNA in ratio of 2 : 1. The granules are thus called ribonucleic protein or RNP. These granules are precursor of ribosomes as they have base composition and staining properties like cytoplasmic ribosomes. **Brinstiel** (1963) called them **nucleolar ribosomes**.

At high magnification the granules appear to be vesicles with light central core and dense peripheral structure. These are connected together by thin filaments forming structure **primary nucleolonema**. The primary structure is folded to make **secondary nucleolonema**.

The nucleolonema may consist of either fibrils or ganules or both. The granular and fibrillar components may be separated in nucleolus or may be mixed together to form fibrillo granular structure.

Functions of Nucleolus

(1) Ribosome formation. According to **Perry** (1964) nucleolus is the site of assembling or manufacture of ribsomes. The nucleolus organiser sends message for the assembly of ribosomes and the ribsomes are synthesized in nucleolus. The DNA functions as template for 45 S rRNA. Half of the 45 S rRNA is broken down to nucleotide level and half is broken down to form 28 S and 18 S rRNA.

Inside nucleolus the 28 S rRNA combines with proteins made in cytoplasm to make 60 S ribosomes and 18 S rRNA combines with proteins to make 40 S subunits of ribosomes. The ribosomes come out of the nucleolus and the nucleus through pores in the nuclear membrane.

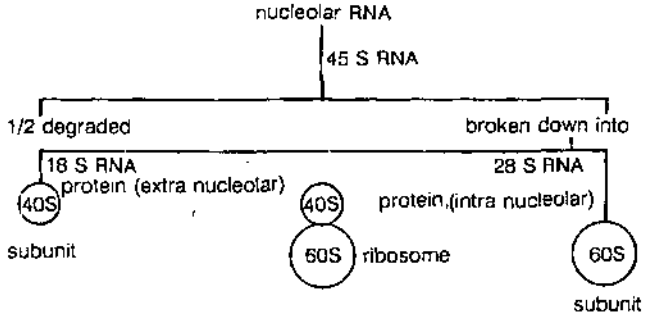


Fig. 9. Nucleus : Ribosome formation

(2) Protein formation. Maggio (1960) suggeted that protein synthesis takes place in the nucleolus. However, later studies suggest that protein synthesis takes place in cytoplasm. **Brustiel** and **Slaman** (1964) suggested that histone proteins are manufactured in nucleolus.

(3) RNA synthesis. The nucleolus is active site of RNA synthesis and major part of cellular RNA is synthesized here. The chromatin in nucleolus contains DNA for coding rRNA. The fibrils represent the origin of rRNA and granule, the next stage is precursors of ribosomes.

$$\underset{\text{DNA}}{\text{Chromatin}} \longrightarrow \underset{\text{(RNA)}}{\text{Fibrils}} \longrightarrow \underset{\text{RNA}}{\text{Granules}} \longrightarrow \text{Ribosomes}$$

(4) The disappearance of nucleolus during cell division suggests its role. It passes genetic information and materials from nucleus to cytoplasm. Experimentally it has been observed that if labelled precursor of RNA is supplied to nucleolus, it first appears in nucleus and then in cytoplasm.

Functions of Nucleus

J. Hammerling (1934), a German biologist demonstrated that the characters of the cell and ultimately the characters of the individual are determined by nucleus. This controlling activity of the characters by nucleus takes place due to the presence of chromosomes in it.

The Nuclear membrane functions as a diffusion barrier to small cations and anions due to its electrochemical property. The transport of macromolecules and ribonucleoproteins into the cytoplasm takes place through the nuclear membrane.

The nucleus plays important role in protein synthesis and the cells lacking nucleus show little or no protein synthesis.

3·5. NUCLEO–CYTOPLASMIC RELATIONSHIP

A German biologist **J. Hammerling** (1934) performed an experiment to demonstrate that nucleus controls all the activities of cells and not the cytoplasm. The nucleus determines the character of cell and ultimately the character of the individual. He conducted experiment on a green alga *Acetabularia*, its thallus is made of single cell and is divided into cap, stalk and rhizoids. The two species of *Acetabularia*, *A. crenulata* and *A. mediterranea* differ in shape of their cap. In *A. crenulata* the cap is made of loose rays and in *A. mediterranea* the cap is umbrella type. In both species a single nucleus is located in rhizoid at the bottom of stalk. If the cap of the alga is cut, it develops again as it has power of regeneration.

If the caps of both the species are cut and stalk of one species is grafted on rhizoid of the other species, the shape of the new cap developed will be of *Acetabularia* species type which contributed the nucleus and not the cytoplasm of stalk. The nulceus of *A. crenulata* will develop cap of *crenulata* type even if the stalk is of *mediterranea* type. Similarly, the nucleus of *A. mediterranea* will develop the cap of *mediterranea* type. If both nuclei are present in grafted stalk, the cap will be of intermediate type. Hence it can be concluded that nucleus controls the activities of cell and not cytoplasm.

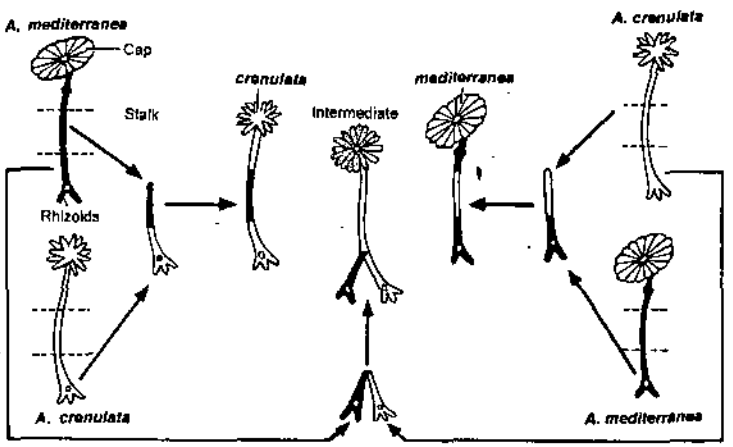


Fig. 10. Nucleus : Hammerling's experiment on *Actabularia* to show

3·6. DIFFERENCE BETWEEN PROKARYOTIC AND EUKARYOTIC CELL

S.N.	Character	Prokaryotic Cell	Eukaryotic Cell
1.	Well organised nucleus	absent	present
2.	Nuclear membrane	absent	present
3.	DNA, histone association	not associated	associated
4.	Chromosome formation	absent	present
5.	ER, Golgi bodies, chloroplast mitochondria and lysosomes	absent	present
6.	Chemical nature of cell wall	Innermost layer made up of mucopeptide	Made up of cellulose or hemicellulose or fungal cellulose or chitin.
7.	Structure of flagella	Do not show 9 + 2 pattern	show 9 + 2 pattern
8.	Cyloplasmic streaming	absent	May occur
9.	Respiratory enzymes	Localized on the plasma membrane	Localized in mitochordrion
10.	Thylakoids	present in cytoplasm	present inside chloroplast
11.	Examples	Bacteria, Cyanobacteria (blue green algae)	All other groups

• STUDENT ACTIVITY

1. What is nucleoplasmic index ?

2. Give structure of nuclear pore.

• SUMMARY

- The nucleus was first discovered by **Robert Brown** in 1833. Depending upon the presence of well organised nucleus, plants have been divided into two categories, namely prokaryotes and eukaryotes. Shape of the nucleus may be spherical, elliptical, discoid, horse-shoe shaped, pyriform or moniliform. The size of the nucleus is variable but in general it is directly proportional to cytoplasm. Mostly the cells are uninucleate. Ultrastructure of nucleus shows that it is surrounded by double layered nuclear membrane. It is discontinous due to many nuclear pores. Some of the important functions of the nuclear membrane are to protect the nuclear material from cytoplasmic enzymes and in nucleo cytoplasmic exchange. The nuclear membrane encloses dense but clear mass of protoplasm called nucleoplasm. A nucleus may contain one or more nucleoli. Each nucleolus contains four components : pars amorpha, chromation, fibrils and granules. Main functions of the nucleous are RNA synthesis, ribosome and protein formation. However, the characters of the cell and ultimately the characters of the individual are determined by the nucleus.

• TEST YOURSELF

1. Who first of all demonstrated that nucleus plays a determinative role in a cell and organisms?
2. Name the algae in which the role of nucleus in controling the form of a plant was demonstrated.
3. In prokaryotic cell, where are the respiratory enzymes located ?
4. What is size of the nuclear pore ?
5. What is the chemical nature of nucleolus?

• ANSWERS

1. **Hammerling (1934)** 2. *Acetabularia* 3. Plasma membrane
4. 300 – 1000Å 5. Composed of RNA and protein

❑❑❑

UNIT

4

GOLGI COMPLEX

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know the structure of golgi complex and its functions.

4.0. INTRODUCTION

Golgi complex also called **Golgi apparatus;** a differentiated or specialized part of internal membrane system is found in the cells of both plants and animals. It is mainly associated with all secretions. It plays a key role in sorting out cell's protein and membrane constituents and in directing them to their proper destination. In 1890, Italian scientist **Camillo Golgi** found that the nerve cells of cerebral cortex of brain of barn owl contained an internal reticular network which stains black with silver nitrate. He called this structure ***apparato reticulare interno*** (internal reticular apparatus). This structure had, however, been discovered earlier by la **Valetle St. George** (1865, 1867), **Plattner** (1885) and **Hermann** (1891). **Holngren** (1900) described golgi apparatus as a clear system of canals and named it **trophospangium. S. R. Cajal**, a contemporary of **Golgi** called golgi apparatus as the **Golgi Holmgren canals. Camillo Golgi** and **S. R. Cajal** were jointly awarded the Nobel Prize in year 1906 for discovery of Golgi apparatus. **Perroncito** (1910) termed the division of Golgi apparatus as **dictyokinesis** and the divided smaller units were called **dictyosomes. R. Cajal** (1914) studied Golgi apparatus in different cell types and studied functional aspects of apparatus using experimental methods. **Gatenby** (1917) demonstrated the similarity between the Golgi apparatus and the dictyosomes of invertebrates. **Nassonov** (1924) and **Bowen** (1929) established the relation of Golgi apparatus to the process of secretion. The electron microscopic studies in early 1950s clearly confirmed the existence of Golgi apparatus. **Baker** (1951, 1953) gave Golgi apparatus a new name **lipochondria,** because of their presumed high lipid contents. **Sjorstrand** (1956) earlier proposed the term **r cytomembranes** for the Golgi apparatus but later preferred the term Golgi membranes. The term Golgi apparatus is more prevalent than any other name. The Golgi apparatus of the plants and

lower invertebrates is usually referred as **Golgi body** or **dictyosome**. **Perner** (1956) observed Golgi apparatus in plants and these were termed as **dictyosomes.**

4·1. OCCURRENCE

Golgi apparatus is found in all eukaryotic cells but is absent in prokaryotes and a few eukaryotic cells like certain fungi, sperm cells of bryophytes and pteridophytes, cells of mature sieve tubes of plants and mature sperms and red blood cells of animals.

4·2. NUMBER

Their number also varies per cell from a single in some algae to several hundred *e.g.,* corn root tissue and algal rhizoids. Their number also varies from animal to animal and from cell to cell. A *Paramoeba* species has two large Golgi apparatus, while in the *Amoeba stereomyxa* there are many Golgi apparatus. Cells with dispersed Golgi apparatus may have hundreds scattered throughout the cytoplasm of the cell.

4·3. SHAPE AND POSITION

The shape of the Golgi apparatus is variable in different types of somatic cells of animals. The shape may also vary in the same cells at different functional stages. The shape of Golgi apparatus is generally constant in a cell, the shape generally varies from a compact mass to a dispersed filamentous network.

The position of the Golgi apparatus is also variable. In higher plants golgi bodies are found scattered throughout the cytoplasm. However, in animals Golgi apparatus is a localized organelle such as in cells of ectodermic origin it is polarized between the nucleus and the periphery. In secretory (exocrine) cells it lies between the nucleus and the secretory pole. In endocrine secretory cells the position is variable. In the nerve cells it occupies perinuclear position.

4·4. SIZE

The size of Golgi apparatus is linked with the functional state of the cell. The Golgi apparatus enlarges during hyperfunctions and becomes reduced during hypofunction. The Golgi complexes are generally large in nerve and gland cells and small in muscle cells.

4·5. STRUCTURE

The Golgi complex is morphologically very much similar in both plant and animal cells. It consists of a collection of flattened, membrane-bounded **cisterna** and thus resembles a stack of plates. Many small vesicles are also found associated wtih the Golgi complex (Fig. 1 A, B, 2). The simplest

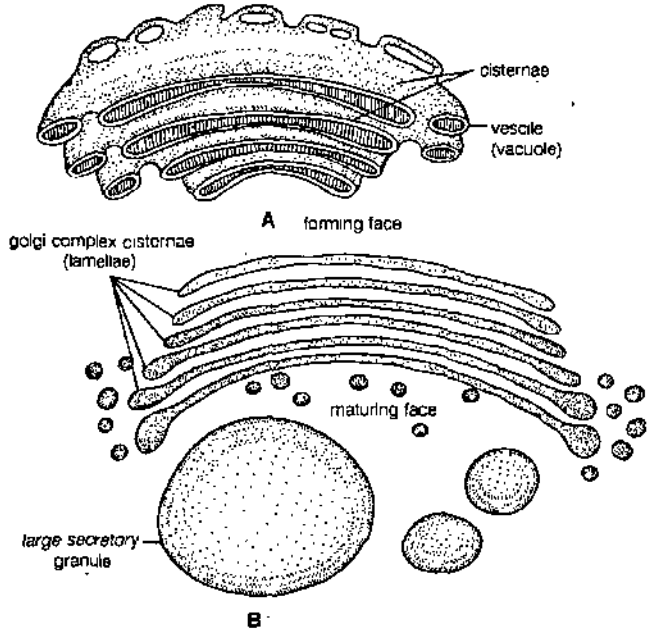


Fig. 1. (A, B). The Golgi complex (A) Steroscopic view, (B) Section.

unit of the Golgi apparatus is the cisterna. A group of cisterna is called the dictyosome and a group of dictyosomes makes up the cell's Golgi apparatus.

Typically, Golgi apparatus is a complex of interconnecting **tubules, vesicles** and **cisternae** (Fig. 2). In general, three mebranous components are recognized under the electron microscope : (1) flattened sacs or cisternae (ii) small tubules or vesicles (iii) large vacuoles.

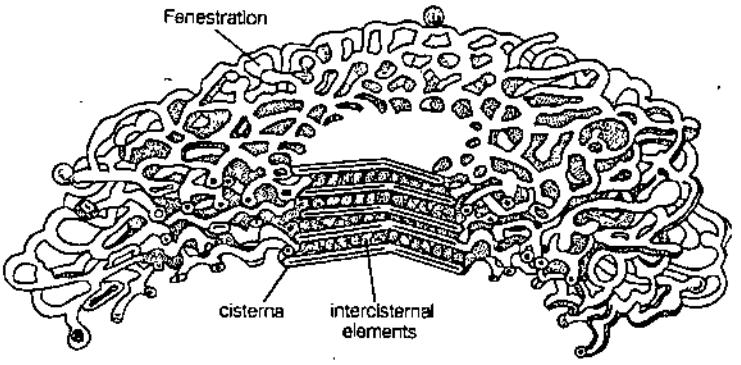


Fig. 2. Plant dictyosome. Note the extensive fenestration of cisternae and intercisternal elements between the cisternae.

A. Flattened sacs (*i.e.*, cisternae)

These are tubular or flattened fluid filled sacs held in parallel bundles or stacks one above the other. In each stack, cisternae are separated by space of about 20-30 nm. Each of these stacks usually consists of 3-8 cisternae in animal cells and 20-30 cisternae in plant cells. Each cisternae is bounded by a smooth unit membrane (7.5 nm), having a lumen varying in width from about 500 to 1000 nm.

Each Golgi stack has two distinct faces : a cis face (or entry face) and transface (or exit face). Cis face lies close to the nuclear membrane or the endoplasmic reticulum. This ER lacks bound ribosomes and is called "Transitional Endoplasmic Reticulum." Cis face of Golgi cisternae is also called forming face. Trans face or distal face of stack is towards the plasma mebrane and is associated with special GERH (Golgi + smooth ER + ribosomal) and secretory vesicles and vacuoles. The transface is also called **maturing face**.

B. Tubules

Both cis and trans face of golgi apparatus are closely connected to special compartments, which are composed of a network of inter-connected tubular and cisternal structures. These are called the **cis Golgi network** (also called the intermediary or saluge compartment) and the **trans Golgi compartment,** respectively, so it is a complex array of associated vesicle and anastomosing tubules surround the dictyosome and radiate from it. In fact, the periphernal arc of dictyosome is fenestrated (lace like) structure.

C. Vesicles

The vesicles are of three types :

(i) Transitional vesicles. These are small, spherical structures about 400–800° in diameter. These lie between endoplasmic reticulum of cis face of golgi apparatus. These are formed as blebs from the transitional ER to miagrate and coverage to cis face of Golgi, where they coalesce to form new cisterne.

(ii) Secretory vesicles. They often occur between maturing face (transface) of Golgi and plasma membrane. These are pinched off from the ends of Golgi cisternal and give fenestrated appearance. These contain secretory products of Golgi apparatus and are finally, converted into zymogen granules or lysosomes.

(iii) Clathrin-coated vesicles. These are spherical protuberances, about 50 µm in diameter and with a rough surface. They are found at the periphery of the organelle. Morphologically different from secretory vesicles, they play a role in intra cellular traffic of membranes and of secretory products *i.e.*, between ER and Golgi apparatus, between GELR region and the endosomal and lysosomal compartments.

4·6. CHEMICAL COMPOSITION

The Golgi membranes are rich in the folllowing chemcials :

1. Lipids : Golgi apparatus of rat liver contains 60% lipid material. The Golgi apparatus of animals contains phospholipids in the form of phosphatidyl choline, whereas plant cells contain phosphatidic acid and phosphatidyl glycerol. Stain osmium tetraoxide (O_5O_4) adhere well to lipids, especialy phospholipids and unsaturated fats.

2. Carbohydrates : Both plant and animal cells have some common carbohydrates like glucosaine, galactose, glucose, mannose and fructose. Plant cells lack sialic acid, but it occurs in high

concentration in rat liver. Some carbohydrates like xylose and arabinose are present only in plant cells. Stain phosphotunozstic acid ($H_3PO_4 . 12WO_3 . 24H_2O$) has special affinity for polysaccharides and protein contents of the membrane.

3. Enzymes : Golgi complex from different plant and animal cells show variation in the protein and enzyme content. Enzyme glycosyl transferase and thiamine pyrophosphatase can be localized in the trans cisternae of Golgi apparatus. Transferase enzymes are found to be located in the membrane of Golgi and not in the lumen of cisternae.

5.7. ORIGIN OF GOLGI APPARATUS

The Golgi complex is active organelle. It is constantly being formed, changed, disintegrated and reformed. It has been variously described as being formed from endoplasmic reticulum, plasma membrane, nuclear envelope and pre existing Golgi apparatus (Fig. 3).

1. From endoplasmic reticulum

This theory was proposed by **Essner Novicoff** (1962), **Beams** and **Kessel** (1968). They proposed that the Golgi cisternae arise from SER which, in turn, have originated from the RER by the loss of ribosomes. The forming face Golgi saccules are formed by fusion of ER derived vesicles, while maturing face saccules "are invovled" in the vesicle formation and disappear. Thus Golgi saccules are contantly and rapidly renewed (Fig. 3).

2. From Nuclear envelope

Bouch (1965) described the origin of Golgi from outer membrane of nuclear envelope in brown algae (Fig. 3). Vesicles are pinched off from outer nuclear membrane which fuse to form cisternae on the forming face of dictyosomes.

3. From plasmalemma

Danial (19654) described the origin of Golgi cisternae from plasmalemma in amoeba. The vesicles are formed by pinocytosis and phagocytosis from plasmalemma which, in turn, form Golgi complex (Fig. 3).

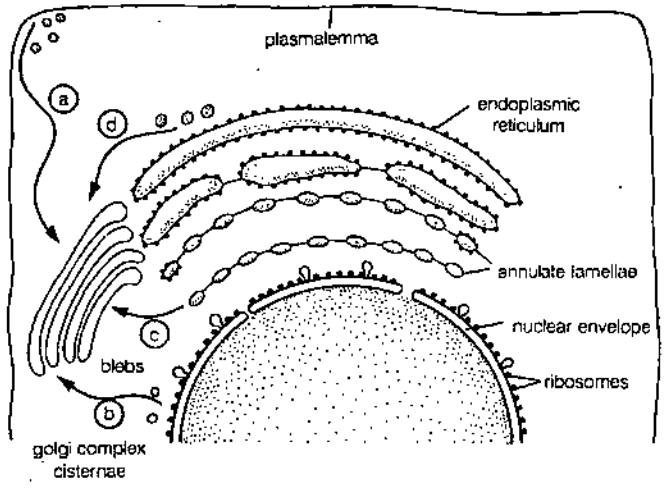


Fig. 3. The Golgi complex. Different views regarding the formation of the Golgi complex. (a) From the plasmaemma, (b) From the nuclear envelope, (c) From the annulate lamellae, (d) From the endoplasmic reticulum.

4. From annulate lamellae

Ward (1965) studied the oocyte naturation in frogs and described that residues derived from the annulate lamellae fuse and give rise to Golgi membranes. It has also been reported that the stacks of cisternae may arise from the pre-existing stacks by fragmentation or division.

4.8. FUNCTIONS

The Golgi apparatus performs many different functions in a variety of cells. However, all the functions are associated with secretion activity of the Golgi apparatus.

Golgi apparatus plays a key role in sorting of many of cell proteins and membrane constituents and in directing them to their proper destinations. To perform this function proper compartmentalizaiton is found in Golgi apparatus of the plantas well as animal cell. Proteins exported from the ER enter the first of the Golgi processing compartment (compartment continuous with the cis Golgi network); then they move to the next compartment — the medial compartment (consisting of the central cisternae of the stack) and finally to the trans compartment.

The lumen of the trans compartment is thought to be continuous with trans Golgi network, where proteins are seggregated into different transport vesicles and dispatched to their final destinations —the plasma membrane, lysosomes or secretory vesicles. Modification of secretory proteins is completed in trans compartment like formation of glycoprotein, glycosphingolipids etc. For this enzymes, (Niz glycosyl transferase) found in Golgi apparatus add a signal or tag of carbohydrate or phosphate residues to certain protein to direct them to their proper sites in the cells.

This compartmentalization shows a kind of division of labour between cis and trans region. Some proteins are sorted out and some of them are returned back possibly by coated vesicles.

Thus, Golgi apparatus is a centre of reception finishing packaging and dispatch for a variety of materials in animals and plant cells.

In many cells the protein released from them is combined with carbohydrates to produce complex carbohydrates like glycoproteins, mucopolysaccharides, glycogen and glycolipids. Addition of carbohydrate to protein is called **Glycosylation**, it begins in ER and is completed in Golgi apparatus *e.g.*, thyroglopalin synthesis by the thyroid follicle in rat shows that incorporation of the sugar mannose in the protein takes place in the ER, while galactose and fructose are incorporated in the Golgi complex.

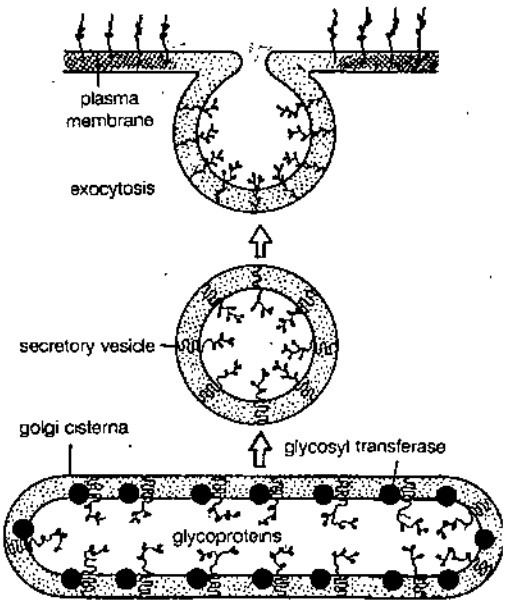


Fig. 4. The Golgi complex. Formation of a secretory vesicle from the Golgi complex cisterna and its subsequent fusion with the plasma mebrane (exocytosis). The glycoproteins remains attached to the membranes.

- The Golgi apparatus appears to be involved in the addition of sulphate to the carbohydrate moiety of the glycoproteins. In cartilage cells, mucopolysaccharide as well as glycoproteins are synthesized in the Golgi complex. Addition of sulphate requires two separate enzymes. Sulphate is first activated by ATP then activated sulphate is tranferred to the acceptor by sulphotransferences *e.g.*, in chicken ehondrocytes.
- Secretory granules originating from the Golgi apparatus fuse with the plasma membrane during exocytosis (Fig. 4). The membrane of the granules becomes incorporated into the plasma membrane and thus contributes to the renewal of the membrane constituents. The Golgi complex plays an important part in the synthesis of the carbohydrate components of the plasma membrane. In plant cells the plasma membranes of the cells resulting from cell division are contributed by the Golgi complex.
- In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls, such as formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin etc. During cytokinesis of mitosis or meiosis, the vesicle originating from the periphery of Golgi apparatus, coalesce in the phragmoplast area to from a semi solid layer, called cell plate. The unit membrane of Golgi vesicles fuses during cell plate formation and becomes part of plasma membrane of daughter cells.
- The electron microcopic and auto radiographic studies of **Stein** and **Stein** (1967) clearly demonstrated the role of Golgi apparatus in lipid secretion. The overall role of the Golgi complex in lipid metabolism appears to be the concentration and modification of scretory material. The Golgi apparatus also provides a membrane for the envelopment of lipid so that it can be released from the cell.
- **Novikoff** *et.al.* (1961-64) have proposed that the primary lysosomes are formed by the Golgi apparatus. The ribosomes synthesize lysosomal hydrolases which enter in ER and forever to Golgi complex in the form of small vesicles. The cisternal, a Golgi apparatus, in turn, forms vesicles by blebbing. Then primary vesicles fuse with pinocyte vesicle and form secondary lysosomes. Lysosomes can also arise directly from the ER without the Golgi apparatus taking any part *e.g.*, in liver cells and in neurons.

Besides these secretion golgi complex also envelopes in packaging and exocytosis of the following materials.

- Secretion of zymogen of exocrine pancreatic cells.
- Mucous secretion by goplet cells of intestine.
- Lactoprotein (Casein secretion by man mammary gland cells).
- Secretion of tropocollagen and collagen.
- Formation of melanin granules and other pigments, and
- Formation of yolk and vitellin membrane of growing primary oocytes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

• STUDENT ACTIVITY

1. Describe the functions of Golgi complex.

2. Describe the structure of Golgi complex.

• SUMMARY

- The Golgi complex (GC) was first described by **C. Golgi** in 1898. Golgi complex is found in all eukaryotic cells but is absent in prokaryotes and a few eukaryotic cells. Their number also varies from cell to cell. Ultra structure shows that it contains three elements — Cisternae, tubules and vesicles. The Golgi are lipoproteinaceous structures. The main enzymes are thiamine pyrophosphatase and transferase. The Golgi is concerned with general secretion of metabolites. It is also concerned with the secretion of proteins and polysaccharides. In all probability it is originated from the endoplasmic reticulum.

• TEST YOURSELF

1. What is the main function of Golgi complex ?
2. Who observed Golgi apparatus in plant cells and termed as dictyosomes ?
3. Name the main enzymes of Golgi complex.
4. According to majority of workers Golgi complex is originated from which cell organelle ?
5. What is the number of cisternae in plant cell ?

• ANSWERS

1. Cell secretion **2. Perner** **3.** Thiamine pyrophosphotransferase
4. Endoplasmic reticulum **5.** 20–30.

UNIT

5

ENDOPLASMIC RETICULUM

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know the structure of endoplasmic reticulum, its origin and functions.

5.0. INTRODUCTION

The eukaryotic cells have high degree of ultrastructural organization, the enzymes, RNA, DNA and other solutes are present in the cell in organized membrane systems. In all eukaryotic cells, the cytoplasm is divided into multiple interconnected compartments by an elaborate system of membranes. These mebranes are generally referred to as the **cytoplasmic membrane system.** This system was initially named as **cytoplasmic vascular system** by **Roberts Nowinski** and **Saez** (1970) and **De Robertis** (1975). Its main components are : (1) ER (2) Golgi complex (3) Lysosomes (4) Peroxisomes (5) Nuclear envelope.

The endoplasmic reticulum is the main component of the cytoplasimc vacuolar system and is also called **endomembrane system.** Endoplasmic reticulum was first observed by light microscope as filmentous structure in cytoplasm with basophilic staining property and was termed as **eragastoplasm**. This first electron microscopic study showed a lace like arrangement of tubules that did not reach the periphery of the cell, hence named as **endoplasmic reticulum** by **Porter, Claude** and **Pullam** in 1945. The term endoplasmic reticulum (ER) was first used by **Forter** and **Kallman** (1952). **Watson** (1955) demonstrated the continuity between endoplasmic reticulum and the outer nuculear membrane. **Porter** and **Machado** (1960) considered endoplasmic reticulum as an extension of the nuclear membrane.

5.1. OCCURRENCE

Although Endoplasmic reticulum occurs in all eukaryotic cells except erythrocytes of mammals, yet the occurrence of ER varies from cell to cell. The endoplasmic reticulum is absent in prokaryotic cells. It is found in small and undifferentiated form in eggs and embryonic cells and some

times these cells lack ER. The spermatocytes have poorly developed ER: The ER of muscle cells is called **sacroplasmic reticulum**. The cells engaged in the synthesis of protein such as cells of pancreas, plasma cells, goblet cells and cells of some endocrino gland are found to contain **Rough endoplasmic reticulum**. The adipose tissues, adrenocortical cells, interstitial cells of testes, cells of corpus luten of ovaries, sepacious gland and cutinal pigment cells contain only SER *i.e.*, **smooth endoplasmic reticulum.**

5.2. TYPES OF ENDOPLASMIC RETICULUM

George Palade (1945) discovered that endoplasmic reticulum has two morphological different forms, differentiated by the presence or absence of ribosomes on the surface of ER. : (A) the "rough" or "granular" endoplasmic reticulum RER and (B) the "smooth" or "agranular" endoplasmic reticulum (SER).

(A) Smooth Endosplasmic Reticulum (SER)

The smooth endoplasmic reticulum or agranular type forms a continuous system with rough endoplasmic reticulum but differs only in having smooth membrane structure. The SER is usually tubular and cisternae are rare. The SER is less stable than the RER and vasiculates easily. The SER and RER are interconvertible structures. Their relative presence depends upon the metabolic state of the cell.

The SER type of endoplasmic reticulum occurs motly in those cells, which are invovled in the transport and mmetabolism of lipids and glycogen. It is found in cells that synthesize steroid hormones from choleterol. The SER is generally found in adipose cells, interstitial cells of testes, glycogen storing, cells of the liver, conduction fibres of heart, adreno cortical cell and leucocyte etc. The muscle cells are also rich in SER. The pigmented retinal cells in frog have small tubules stacked over one another and are called **myeloid bodies.**

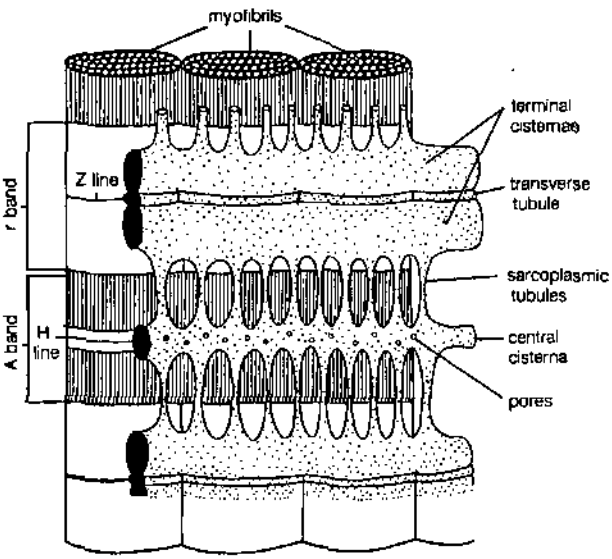


Fig. 1. The sarcoplasmic reticulum.

Many glycosomes (contain enzymes) involved in the synthesis of glycogen attached to the membrane of SER have been observed by electron microscopy in liver and conduction fibre of heart.

The modified form of SER is the **sarcoplasmic reticulum** found in striated mucles. This is delicate structure surrounding the mycofibrils. The longitudinal tubules merge to form terminal cisternae (fig. 1) The terminal cisterna from each sarcomere together with small transverse tubule between them form a **triad** which lies over I band. Along the H band level is a **central cisterna** which has pores. The central cisterna is formed by confluences of the longitudinal sarcoplasmic tubules. In some muscles the triad lies over the junction of A and I bands, thus there are two triads per sarcomere.

(B) Rough Endoplasmic Reticulum (RER)

The rough endoplasmic reticulum or granular forms have membranes covered with ribosomes which are associated with protein synthesis. The ribosomes are associated to it through a glycoprotein called **ribophorin.** The RER consists of stacks of large flattened cisternae which are interconnected. The stacks are connected laterally to SER which are tubular. The RER is found abundanlty in those cells which are active in protein syntheis such as pancreatic cells, plasma cells, goblet cells and liver cells. The region of the matrix containing granular type (RER) of endoplasmic reticulum takes basophilic stain and is named **ergastroplasm.**

5.3. ANNULATE LAMELLAE

The annulate lamellae have been observed in oocytes and spermoatocytes of the vertebrates and in some somatic cells of invertebrates and vertebrates. They are more commonly found in embryonic and foetal cells of high metabolic activity. The annulate lamellae were first described by

MC Culloch in 1952. The annulate lamellae consist of double membraned sheets of which 2-12 lamelale are arranged in stacks. Like the nuclear envelope they have pores with annual masses and central granules. It has been suggested that the outer membrane of the nuclear envelope is involved in the formation of annulate lamellae. This membrane forms finger like processes which are pinched into the cytoplasm to form **blebs** or **vesicles** and this process is called blebbing. The rows of such blebs migrate towards periphery of the cell and fuse to form **cisternae**. The matrix material gets associated with the pores of each cisternae to from **annuli** (Fig. 2). They ultimately form **annulate lamella.**

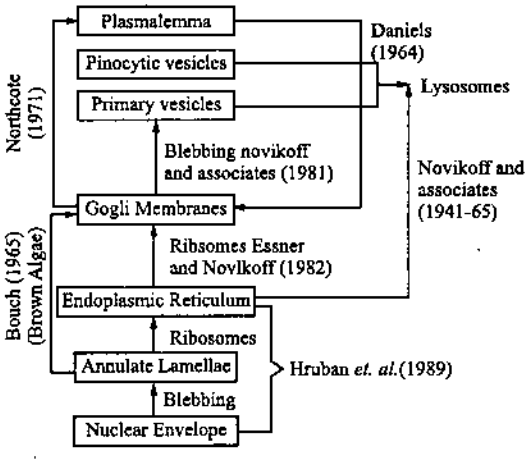


Fig. 2. Endoplasmic reticulum. Interrelationships of different cell membranes.

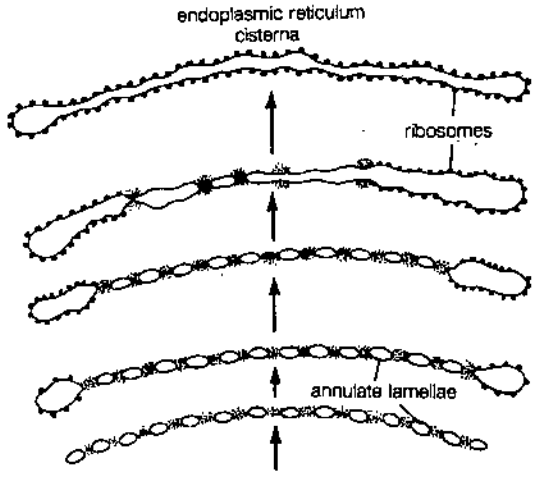


Fig. 3. Endoplasmic reticulum. Formation of cisterna of endoplasmic reticulum from anulate lamella.

According to **Hruban** (1965) the annulate lamellae represent an intermediate stage in the formation fo ER. The annulate lamellae lose their pores and acquire ribosomes on surface (Fig. 3). It is supported by the fact that the ribosomes are frequently found attached to annulate lamellae. There is continuity between the annulate lamellae and ER. The annulate lamellae are transitory cyloplasmic organalle and increase cytoplasmic membrane surface for greater metabolic activity.

5·4. MORPHOLOGY

Morphologically, the ER may occur in the following three forms :

(1) Lamellar form or cisternae, (2) Vesicles, (3) Tubules.

I. Cisternae : These are elongated, flattened and usually unbranched tubular vesicles arranged in parallel rows. These form succesive layers around the nucleus. These are about 40 to 50 µm in diameter. REK usually exists as cisternae, and is found in the cells, active in protein synthesis such as cells of pancreas, brain and liver.

II. Vesicles : These are rounded, spherical or ovoid in shape. These are membrane bound vacuolar structures measuring from 25 to 500 µm in diametre. Vesicles are common in most cells but especially abundant in smooth endoplasmic reticulum.

III. Tubules : The tubules are small, smooth walled branched tubular spaces having a diametre from 50 to 190 µm. These tubules form the reticular system along with the cisternae and vesicles. Tubular form of ER is often found in SER. It is associated with membrane movement, fission and fusion between membranes of cytocavity network.

The entire system of endomembrane represents a barrier (**Jhorpe,** 1984) separating cytoplasmic compartments. The membranes of the compartments exhibit the two faces of each membrane (1) cytoplames face (towards cytosol), (2) lumiminal face (facing the lumen of tubules).

5·5. ISOLATION AND CHEMICAL COMPOSITION

The ER, is isolated from the cells or tissues by homogenization followed by the differential **centrifugation**. ER isolated by this procedure was in the form of released fragments of the ER and these small closed vesicles of ER are called microsomes. The term microsome was coined by **Claude** in 1940, and the relation between microsomes and ER in the intact cells was established by **Palade** and **Sickereitz** in 1956. These microsomes are used to study the biochemistry and function of ER.

Microsomes derived from rough ER are shedded with ribosomes and are called **rough microsomes.** The ribosomes are always found on the outside surface, so that the exterior of the microsome is biochemically equivalent to the luminal space of the ER. Because they can be readily purified in functional form, rough microsomes are especially useful for studying the many processes carried out by RER. These rough microsomes represent small authentic versions of the RER, still capable of protein synthesis, protein glycosylation and lipid synthesis.

Homogenate also contains smooth or agranular microsomes which lack attached ribosomes. They may be derived in part from fragments of smooth ER., plasma membrane, Golgi apparatus, endosomes and mitochondria. Thus, while the rough microsommes are derived from RER, the origin of smooth microsome cannot be so easily assigned.

The membranes of microsomes in liver cells are composed of a molecular layer of phospholipid (30–40%) by weight) with 60 to 70% floating proteins. The phospholipids are mainly in form of **lecithin** and **cephalins**. The membrane of microsomes is found rich in phosphotidyl choline and poorer in sphingomyelin (**Jhorpe**, 1984). The SER contains more lipids relative to proteins than in RER. The RER also contains less sphigomeylin and cholesterol.

5.6. ENZYMES OF ER MEMBRANES

The membranes of ER are found to contain many kinds of enzymes involved in biosynthesis of phospholipids, ascorbic acid, steroids and hexose metabolism etc. The following enzymes are found associated with ER membranes :

1. Stearases
2. NADH cytocrome reductase
3. NADH diphosphatase
4. Glucose–6–phosphatase
5. Mg^{++} activated ATpase

Functions of enzymes :

(i) Synthesis of glycerides e.g., triglycerides, phospholipids, glycolipids and plasmalogens.
(ii) Synthesis of fatty acids
(iii) Synthesis of steroid, such as cholesterol.
(iv) Synthesis of L-ascorbic acid
(v) Aromatization and hydroxylation
(vi) Aromatic hydroxylation, side chain oxidation
(vii) Metabolism of plasmalogens
(viii) Dephosphorylation of UDP glucose
(ix) UDP-uronic acid metabolism

5.7. ORIGIN OF ER

The origin of ER is not definitely known. They are selfreplicating organelles without their own genome. The assumed common methods of origin are :

1. From nuclear membrane. Since the membrane of endoplasmic reticulum resembles the nuclear membrane, it is presumed that ER develops from the nuclear membrane by **evagination.** This is further supported by the fact that nuclear envelope is formed from the vesicles of ER at telophase stage of the division.

2. Multistep mechanism. It is now belived that the membranes are synthesized in the RER. Through a progressive series of changes, these are transformed into the membranes of SER and Golgi apparatus. The process by which a membrane is modified chemically and structurally is called membrane differentiation. It is suggested that the synthesis of membrane follows the directions RER to SER.

5.8. FUNCTIONS OF ENDOPLASMIC RETICULUM

The ER acts as secretory, storage, circulatory and nervous system of the cells. It performs the following important functions :

1. The vacuolar system ER provides mechanical spport to the colloidal matrix of cytoplasm.
2. Endoplasmic membrane contains many enzymes which perform various synthetic and metabolic activities.
3. ER provides increased surface area for various enzymatic reactions.

4. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membrane of ER. Like plasma embrane, the ER membrane has permeases and carriers.

5. The ER found in muscles is found to conduct intra-cellular impulses.

6. The ER acts as an intracellular circulatory system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows :

Granular ER →SER →Golgi apparatus →lysosomes →transport vesicles or secretory granules.

Export of RNA and nucleoprotein from nucleus to cytoplasm may also occur by this type of flow.

7. The ER membrane for the new nuclear envelope after each nuclear division.

Functions of Smooth Endoplasmic Reticulum

(1) Biosynthesis of lipid bilayers of cellular membranes. The ER membrane produces nearly all the lipids required in the biosynthesis of cellular membranes. The major phospholipid formed is **Phosphatidylcholine** (Lecithin). It is made of base choline, two fatty acids and glycerol phosphate. All these lipids are formed in the cytosolic half of ER and are organised into monolayer. A translocated enzyme, flippase catalyses the flipping or transfer of phospholipid molecules from this monolayer to luminal side which then organizes to form luminal half of lipid bilayer.

(2) Sterol metabolism. SER contains several enzymes that catalyze the synthesis of cholestrol, which is also a precursor of two types of compounds — steroid hormone and bile tissues.

(a) Cholestrol. Cholestrol is one of the most important lipids of biosystems. It is precursor of many steroid hormones. The site of synthesis of cholesterol is SER. The studies of radioactive –labelled acetate show that microsomes are the main sites of cholesterol synthesis.

Cholesterol is synthesized from the acetate and its entire biosynthesis involves about 20 steps. Each step is catalyzed by an enzyme. Out of these 20 enzymes, 11 enzymes are bounded to SER, rest nine are the soluble enzymes located in cytosol and mitochondria. SER is involved both in synthesis and storage of cholesterol.

(b) Steroid hormones. Steroid hormones are synthesized in the cells of various organs such as the cortex of adrenal gland, the ovaries, the testes and the placenta. Cholesterol is the precursor for both types of sex hormones **estrogen** and **testosterone.**

SER is involved in the synthesis of steroid hormones. The enzymes catalyzing the biosynthesis of androgens have been found located in SER. There is strong relation between the amount of SER in cells and the capacity to synthesize steroid hormones. Very well developed SER have been demonstrated in the interstitial cells of the testes in guinea pigs **Christensen,** (1965) and inadrenal cortex of the rat **Rhodin** (1971).

(c) Bile acid synthesis. It is also synthesized from cholesterol. For its synthesis, cholesterol is first converted into 7 α-hydroxyl cholesterol, with the help of enzyme cholesterol 7α-hydroxylase, which is then converted into bile acid with the help of hydroxylase enzyme. The later reaction requires NADPH and molecular oxygen and depends on the enzymes of electron transport chains of SER, cytochrome P-450 and NADPH-cytochrome-C-reductase.

(3) Newly synthesized phospholipids are rapidly transferred to other cellular membranes. Transfer is carried out with the help of phospholipid exchange proteins, which are found in the cytosol.

(4) Glycogenolysis. SER is found related to glycogenolysis or break down of glycogen. In fasted animals it has been observed that the residual glycogen remains associated with SER. When feeding is resumed, there is an increase in SER.

The enzyme **glucose-6-phosphatase** required for breakdown of glycogen (glycogenolysis) has been reported in the ER of rat liver cells. It was also suggested that SER plays role in synthesis of glycogen (glycogeneis). Since the enzyme UDPG glycogen-transferase responsible for glycogen synthesis is not associated with ER but is bound to glycogen suggests that ER may be involved in glycogenolysis but not glycogenesis.

Blood glucose homeostasis. An enzyme, called glucose-6-phosphatase exists as an integral protein of the mebrane of SER (*e.g.,* liver cell). This enzyme catalyzes the release of free glucose molecule in the lumen of SER. Thus, this process operates to maintain homeostatic levels of glucose in the blood for the maintenance of functions of red blood cells and nerve tisues.

(5) Detoxification. The SER membranes contain enzymes with detoxification properties. SER contains enzymes that catalyze series of reactions to detoxify both lipid soluble drugs and various harmful compounds produced by metabolism. The most extensively studied of the detoxification reactions are catalyzed by the **cytochrome P-450** family of enzymes. They make them more

hydrophilic to leave the cell and be excreted in the urine. When large quantities of harmful substances enter the biosystems, SER increases its surface area and more detoxification enzymes are synthesized. As these harmful substances are detoxified and excreted, the excess SER is romoved by lysosome dependent autophagocytosis.

(6) Lipid Synthesis and Storage. Autoradiographic studies of **Stein** and **Stein** (1967) suggested that the ER was the stie of triglyceride formation. When fasted and ethanol treated rats were injected with radioactive H^3-palmitate and H_3 glycerol, the labelled substances were seen in SER and RER **Claude** (1970) suggested that triglyceride may be synthesized in SER and the phospholipids may be synthesized in RER. The SER membranes are also involved in the formation of lipoprotein complexes. The ER is also associated with the storage of lipids and have general role in the intracellular transport of lipids.

Functions of Rough Endoplasmic Reticulum

The ribosomes that synthesize proteins are directly attached to the ER membrane. These membrane bound ribosomes coat the surface of ER, termed as **rough endoplasmic reticulum**. The membrane bound ribosomes are attached with specific binding sites or receptos of RER membrane by their large 60 S subunit. The receptor protein with bound ribosomes can float laterally like other membrane proteins and may facilitate the formation of polysome and probably translation which requires that mRNA and ribosomes move with respect to each other.

The ER captures selected proteins from the cytosol :

(1) Transmembrane proteins, which are only partly translocated across the ER membrane and become ebedded in it.

(2) Water-soluble proteins, which are fully translocated across the ER membrane and are released into the ER lumen from ribosomes. These soluble proteins are destined to other organelle or for secretion.

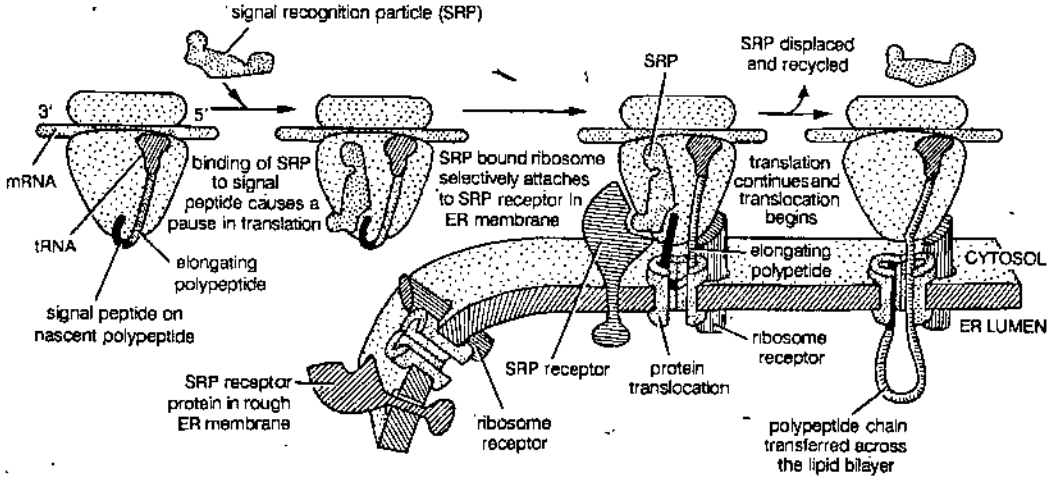


Fig. 4. Endoplasmic reticulum. The role of ER signal peptide, SRP and SRP receptor in directing ribosome to ER membrane.

In mammalian cells the import of proteins into ER begins before the polypeptide chain is completely synthesized *i.e.,* it occurs co-translationally. This distinguishes the process from the import of proteins into mitochondria, chloroplasts, nuclei and peroxisomes, which is post translational.

Membrane bound and free ribosomes are structurally and functionally identical. They differ only in the proteins they are making at any given time. Ribosomes which have fate to get attached with ER make protein with an ER **signal peptide**, which directs the ribosome to the ER. So, instead of passing into cytoplasm these proteins pass into cisternae of the RER and are, thus protected from **protease** enzyme of cytoplasm. Since many ribosomes can bind to single mRNA molecule, a **polyribosome** is usually formed (Fig. 4).

It is estimated that about 40 AA residues long segment at one end of the nascent protein remains protected inside the tunnel of ribosomes and rest of the chain remains into ER cisternae during translation. Only a small segment is exposed to the cytoplasm at any one time. As soon as the growing polypeptide chain reaches the cisternae, it folds into secondary and tertiary structures.

Signal Hypothesis

Signal perptides or the signal peptide strategy of protein import were first discovered in 1971 by **Blobel** and **Sabatini** in secretory proteins as a first step towards their eventual discharge from the cell. This also explains that how the ribosomes which are meant for the biosynthesis of secretory proteins for lysosomes and the membrane formation get specifically attached to RER membranes.

In-vitro experiments demonstrated the presence of amino-terminal leader peptide sequence in protein. Signal hypothesis explains that the **leader** serves as a **signal peptide** that directs the secreted protein to the membrane and it is then cleaned off by a **signal peptidase** in the ER membrane before the polypeptide chain in ER including soluble proteins and membrane proteins.

Later on, it is found that ER signal peptide is guided to the ER membrane by at least two components : a **signal recognition particle** (SRP), which cycles between the ER membranes and the cytosol and binds to the signal peptide, and **SRP receptor** (docking protein), in the ER membrane. ERP and SRP receptor and present in all eucaryotic cells and probably in prokaryotic cell as well (Fig. 5).

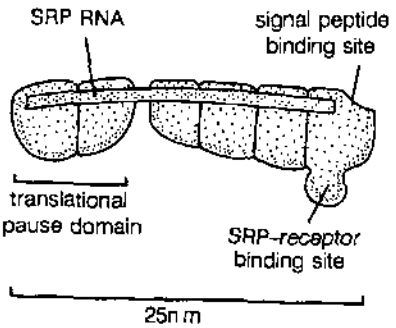


Fig. 5. Endoplasmic reticulum. Structure of signal recognition particle (SRP) consisting of six protein subunits and one molecule of SRP RNA.

This SRP binds to the ER signal peptide and causes a pause in protein synthesis. After this ribosome binds to the ER membrane. Once formed, the SRP ribosome complex binds to the SRP receptory (integral protein on the cytosolic surface). The SRP is then released and the translocation apparatus transfer the growing polypeptide chain across the membrane. SRP release occur only after the ribosome has become properly engaged with the translocation apparatus of the ER membrane.

Translocated polypeptide chains fold and assemble in the lumen of the RER. These polypeptide chains are folded by the ER resident proteins such as **Protein disulfide isomerease** (PDI), which catalyses the oxidation of free sulfhydryl (5 + 1) groups to form disulfide (5 – 1) bonds. Another ER resisdent protein known as binding protein (Bip) (structurally related to hsp 70 S proteins). BiP hydrolyses ATP to provide the energy for its role in protein folding.

Protein glycosylation. The covalent addition of sugars to proteins is one of the major biosynthetic functions of ER. Most of the soluble and membrane bound proteins that are made in the ER and which are destined for transport to Golgi apparatus, lysosomes, plasma membrane are glycoproteins. In contrast very few poteins in the cytosol are glycosylated and if then only by a single N-acetyloglucoamine group to serine or threonine residue of the protein.

The process of protein glycosylation in RER is one of the most well understood cell biological phenomena. During this process a single species of oligosaccharides (compresis of N-acetyl glucosomine, mannose and glucose, containing a total of 14 sugar residues) is transferred to proteins in the ER. It is always transferred to NH_2 group on chi side chain of an asparagine residue of protein, that is why it is called N-linked or asparagine linked oligosaccharide. The transfer is catalysed by a membrane bound enzyme, an **ologosaccharyl transferase.** This precursor oligosaccharide is held in the ER membrane by a special lipid molecule dolicol, and transferred to the target in a single enzymatic step imediately after that amino acids (Asparagine) emerge in the ER lumen. The oligosaccharide is linked to the dolicol by a high-energy **pyrophosphate bond**. (it provides activation energy for the glycosylation reaction).

Structure and Function of Other Organelles

While still in RER lumen, three glucose residues and one mannose residue are quickly removed from the oligosaccharide of most glycoproteins, this is called "trimming" or "processing" of oligosaccharides.

• STUDENT ACTIVITY

1. Describe functions of ER.

2. How does class SER differ from RER

• SUMMARY

- The endoplasmic reticulum is a membrane bound cytoplasmic organelle. It is found in all plant and animal cells except mature red blood cells and prokaryotes. The ER is of two types—smooth and rough. Chemically it is made up of 50–70% proteins and 30–50% lipids. Morphologically, the ER may occur in three forms—Cisternae, Vesicles and Tubules. The ER forms an endoskeleton of the cell, provides surface area for exchanges and distributes metabolites. It participates in membrane flow. The RER participates in protein synthesis and synthesis of phospholipids. It is also involved in cholestrol synthesis and break down of glycogen. The membranes of ER are found to contain many kinds of enzymes. The annulate lamellae are thought to give rise to ER.

• TEST YOURSELF

1. Who coined the term endoplasmic reticulum ?
2. Name the site of cholestrol synthesis.
3. Who first observed the annulate lamellae ?
4. Name the well developed ER in the cells actively engaged in protein synthesis.
5. What is the diameter of veiscles ?

• ANSWERS

1. Porter **2.** ER **3. Mc Culloch** (1952) **4.** RER
5. 25 to 500 µm.

❑❑❑

UNIT

6

RIBOSOMES

STRUCTURE

LEARNING OBJECTIVES

By studying this chapter you will be able to know the structure of ribosomes, its biogenesis and functions.

6·0. INTRODUCTION

Claude (1943) separated a fraction by ultra centrifugation and named it as **microsomes**. It was rich in ribosomes. **Robinson** and **Brown** (1953) while studying the bean root under electron microscope also noted the presence of ribosomes. **Palade** (1955) is generally credited with the discovery of ribosomes in animal cells, hence they are also called **Palade granules**.

The ribosomes are found in all prokaryotic and eukaryotic cells except in mature mammalian erythrocytes. The ribosomes are many in all cells but their number increases sharply in cells engaged in protein synthesis, *e.g.,* in liver and pancreatic cells. The ribosomes are found either in **free state** in cytoplasm or attached to the outer membrane of endoplasmic reticulum. The ER without ribosomes are called **smooth endoplasmic reticulum** or SER and the ER attached with ribosomes are called rough endoplasmic reticulum or RER of **ergastoplasm** or **alpha cytomembrane** (**Sjostsand,** 1956).

6·1. TYPES OF RIBOSOMES

There are two types of ribosomes 70S and 80S. S stands for **Svedberg constant.** It is sedimentation coefficient which is a measurement to show how fast a cell organelle sediments in an ultracentrifuge.

70S Ribosomes

The 70S ribosomes are relatively smaller and are found in prokaryotes. The sedimentation coefficient of prokaryotic ribosomes varies from 64S to 72S, the average being considered as 70S. The 70S ribosomes consist of a large 50S subunit and a small 30S subunit. The ribosomes have two unequal subunits, the larger unit is almost double the size of smaller subunit.

There are about 15000 ribosomes in a growing *E coli.* The *E coli* ribosomes can be taken as an example of prokaryotic ribosomes structure. The X ray diffraction studies reveal their dimension as $170 \times 230 \times 250$Å. (**Vasiliev**, 1971). The 30S subunits are ellipsoid with dimension

$80 \times 100 \times 190$Å The 30S subunits are ellipsoids with dimension $80 \times 100 \times 190$Å and the 50S subunits are $160 \times 200 \times 230$Å in dimension.

Ribosomal Proteins

The 30S subunits contain about 41 different proteins called S1–S21. The 50S subunit contains 34 proteins called L1–L34. S stands for small and L stands for large protein nomenclature. All the ribosomes were supposed to consist of 55 different proteins. Later the protein S20 was found to be identical to L26. L8 was shown to be the aggregate of proteins L7, L10 and L12. Thus prokaryotic ribosomes consist of 53 different proteins (**Wool**, 1979, Fig. 1). Some of ribosomal proteins are called **primary binding proteins** which are attached to the specific regions of rRNA and these, in turn, give attachment to other proteins.

Ribosomal Nucleic Acids

The nucleic acid in the ribosomes is in the form of rRNA and it remains in between the subunits in a highly folded manner with attached proteins. The three types of rRNA found in

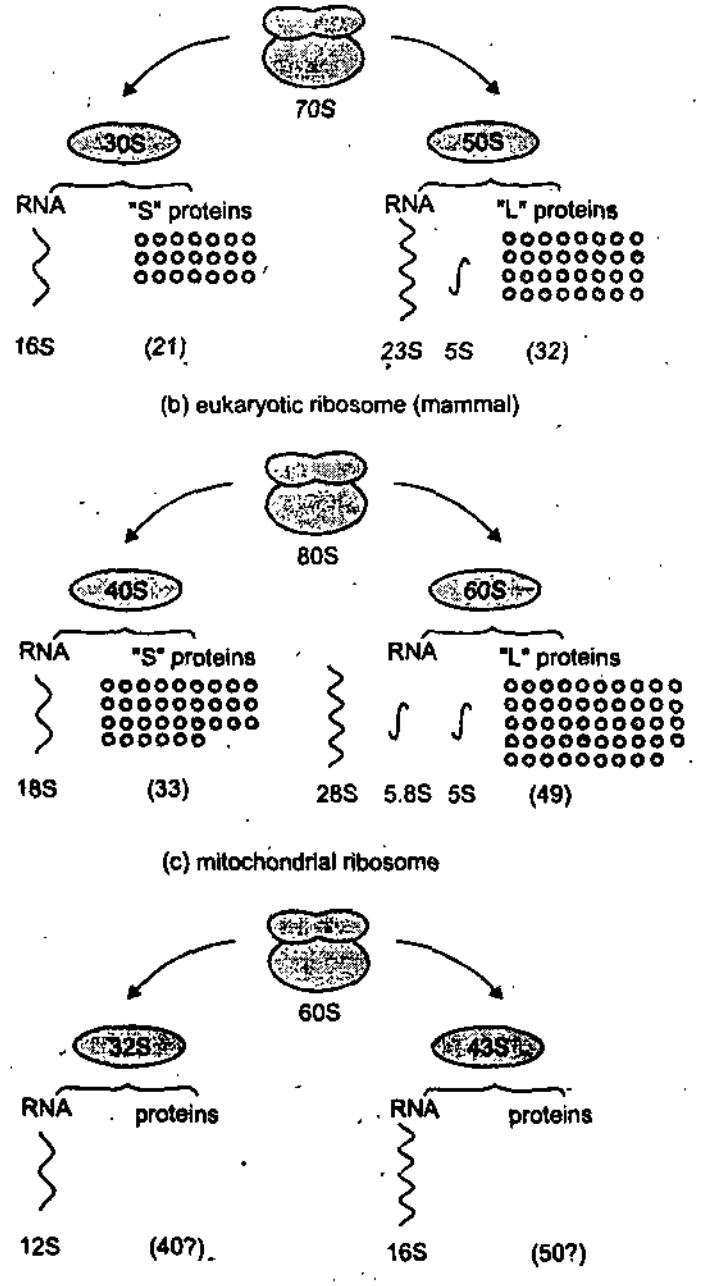


Fig. 1. Ribosomes. Diagram of ribosomal components in (a) prokaryotes, (b) eukaryotes, and (c) mitochondria. So far, all of the RNA components and most of the protein components in prokaryotes have been found in a frequency of about one copy each per ribosome. However, a few of the protein components are not present in all of the ribosomes, but may perhaps become attached to them for specific protein synthesizing functions.

prokaryotic ribosomes are 5S, 16S and 23S. The 50S subunit contains 5S and 23S rRNA with molecular weight of 40,000 and 1,100,000 respectively. **Sanger** in 1968 studied the structure of 5S RNA, it contains 120 nucleotides. The 23S RNA is the largest and contains about 2904 nucleotides. The 30S subunit contains 16S ribosomal RNA which has molecular weight of about 5,50,000. It contains 1600 nucleotides coiled in a complex secondary structure organised into 5 regions, each with a distinct role.

The RNA and proteins are inertwined in two subunits of ribosomes. The 70S *E. coli* ribosomes contain 63% RNA and 37% protein *i.e.,* a ratio of 2 : 1. The two subunits of ribosomes are held together by magnesium ions. There are two critical levels of Mg^{++} concentration. Below the **first critical level** 0.5 micromolecule of Mg^{++} per phorphorus two subunits of ribosomes separate. The dissociation of two subunits is reversible. Thus the two subunits can reassociate in increased Mg^{++} level. The lowering of Mg^{++} below **secondary critical level** (0.3 micromol of Mg^{++}) results in breaking up of the subunits. Ca^{++} Mn^{++} and Co^{++} play stabilizing role in ribosome structure.

The 30S and 50S subunits have different binding properties. The 30S subunits bind to m RNA to form 30S-m RNA complex and then 50S unit attaches to this complex. The 50S subunit can not attach to mRNA if 30S subunit is not present. The segment of m RNA binding to 30S subunit is about 27 nucleotides in length.

80S Ribosomes

The 80S ribosomes are found in eukaryotic cells. The sedimentation constant is 79S–80S in fungi and 80S in mammals. The 80S ribosome consists of 60S larger subunit and 40S smaller subunit. In rat liver, ribosome 40s is a curved, flattened, ellipsoid consisting of a 'heat' and 'body'. Its dimensions are $230 \times 140 \times 115$Å. It contains one molecule of 18S RNA and 33 proteins. The 60S subunit contains one molecule each of 5S, 5.8S and 28S RNA and 49 proteins. In 80S ribosome the first critical level of Mg^{++} is 0.3 to 0.1 micromoles of Mg^{++} per micromole of phosphorus. The second critical level is 0.15 micromoles of Mg^{++} per micromole of phosphorus.

Generally 80S ribosomes of plants contain 16–18S RNA in 40S subunit and 25S RNA, 5S RNA and 5.8 RNA in 60S subunit. The 80S ribosomes of animals contain 18S RNA in 40S subunit and 28–29S RNA, 5S RNA and 5.8S RNA in 60S unit.

55S Ribosomes

The organelles like mitochondria, chloroplast which have protein synthetic machinery also contain ribosomes. These ribosomes were earlier considered to be 70S type as in prokaryotes. These ribosomes are now found to be 55S type which have larger subunit of 40S type and the smaller subunit of 30S type. The 40S subunit contains 16–17S and 5S RNA and 30S subunit contains 12–13 S RNA.

6.2 DIFFERENCE BETWEEN 70S AND 80S RIBOSOMES

Presence	70S Ribosomes	80S Ribosomes
	In prokaryotes	In eukaryotes
Sedimentation	64S–72S (average 69S)	79–85S in fungi, 80S in mammals
Coefficient size	Relatively smaller	Relatively larger
Molecular weight	3 million (3×10^4)	4–5 million ($4–5 \times 10^4$)
Subunits	Small 30S and large 50S	Small 40S and large 60S
RNA	3 molecules of RNA : 16S RNA in 30S subunit, 23S and 5S RNA in 50S subunit.	4 molecules of RNA : 16S-18S RNA in 40S subunit. 25S-29S, 5.8S and 5S RNA in 60S subunit.
MW of RNA	16S RNA–5550,000 23S RNA–1,100,000 5S RNA–40,000	18S RNA–700,000 28S RNA–1,700,000 8.8 RNA–51,000 5S RNA–39,000
No. of proteins	21 (S1–S21) is small subunit 34(S1–L34) in large subunit Total in prokaryotes : 50–60 proteins.	33 in small subunit 49 in large subunit, Total in eukaryotes : 70S–80 proteins.

Average MW of proteins	18,000	21,000
No. of amino acids	~ 8,000	~ 16,000
RNA – protein ratio	2 : 1	1 : 1
Ist critical Mg^{++} level	0.5 micromoles of Mg^{++} per micromole of phosphorus	0.3–0.1 micromoles of Mg^{++} per micromole of phosphorus
2nd critical Mg^{++} level	0.3 micromoles of Mg^{++} per micromole of P.	0.15 micromoles of Mg^{++} per micromole of P.

6.3. POLYRIBOSOMES

During protein synthesis ribosomes form a group or cluster called polyribosomes, polysomes or ergosomes. In polyribosome the ribosomes may be arranged in a linear manner or may form a rosette cluster (Fig. 2).

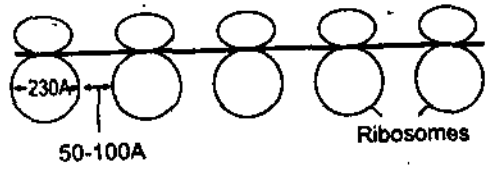


Fig. 2. Ribosomes. A polyribosome consisting of five ribosomes.

The two 70S ribosomes form diamers of 100S and the 80S ribosomes form diamers of 120S (Fig. 3).

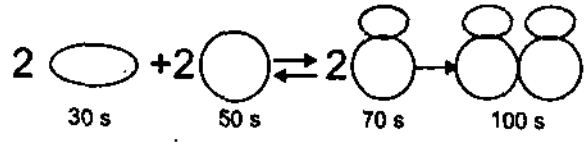


Fig. 3. Ribosomes. Formation of 100S dimer from two 70S ribosomes.

Polyribosomes are formed with increase in Mg^{++}, the 70 S ribosomes form diamers of 100S when Mg^{++} concentration if increased about ten times. The number of ribosomes in a polyribosome depends on the length of the m *RNA molecule that takes part in protein synthesis.* The 200S polyribosome for haemoglobin synthesis has 5–6 ribosomes. The 600S polyribosomes of polio virus 16–18 ribosomes in a cluster.

The dissociation and reassociation of ribosomal subunits takes place during protein synthesis. The 30S subunit first makes complex with m RNA and then 50S unit gets attached to the 30S-m RNA complex. When amino acids form peptide bonds, the ribosome unit separates and gets detached from one end of polyribosome. The association of ribosomes was experimentally shown in *E coli.* The ribosomes were made heavy by isotopes and were mixed with light ribosomes. When protein

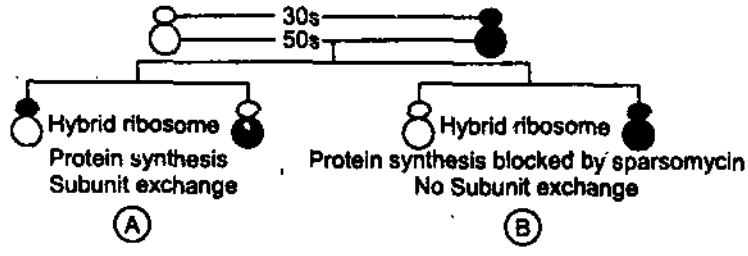


Fig. 4. Ribosomes. Experiment with 'light' and 'heavy' *E. coli* ribosomes, (A) subunit exchange takes place during protein synthesis and (B) when protein synthesis is blocked by sparsomycin there is no subunit exchange.

synthesis takes place some hybrid ribosomes the protein synthesis was blocked by sparsomycin, the hybrid ribosomes were not formed. This evidence indciates that exchange of subunits takes place during protein synthesis.

6.4. FUNCTIONS OF RIBOSOMES

The ribosomes form polyribosome clusters and take active part in protein synthesis. The interaction of the t-RNA amino acid complex with m RNA *i.e.,* the translation of genetic code is coordinated by the ribosomes. The ribosomes also provide protective function. The m RNA strand which remains between the two subunits is protected from the action of nucleases. The newly synthesized polypeptide chain passing through channel between the subunits is protected from proteinase enzymes.

6.5. BIOGENESIS OF RIBOSOMES

The origin of ribosomes has been studied in eukaryotes. Different ribosomal proteins are synthesized in the cytoplasm and the subunits are processed in the nucleolus. Thus the nucleolus plays pivotal role in the biogenesis of ribosomes.

• STUDENT ACTIVITY

1. Describe the functions of ribosomes.

2. Describe the structure of ribosomes.

• SUMMARY

- The ribosomes are small (150 to 200A° in diameter), dense rounded particles. These are found in all prokaryotic and eukaryotic cells. These are composed of RNA and proteins. In prokaryotes ribosomes are found freely distributed in the cytoplasm. However, in eukaryotic cells they are found freely distributed in cytoplasm as well as attached to the endoplasmic reticulum. All ribosomes contain a large and a small unit. Magnesium ions play important role in maintaining its structure. Prokaryotic ribosomes contain a larger 50S subunit and a smaller, 30S subunit. Chemically ribosomes are composed of RNA and proteins. The ribosomes are concerned with protein synthesis. During protein synthesis ribosomes form a group of cluster called polyribosomes. The ribosomes probably originate from the nucleolus.

• TEST YOURSELF

1. Who is generally credited with the discovery of ribosomes.
2. What type of ribosomes are found in eukaryotic cells ?
3. What are the subunits of 70S ribosomes ?
4. What is the main function of ribosomes ?
5. Which are the smallest cell organelles ?

• ANSWERS

1. Palade (1963) **2.** 80S **3.** Small 30S and large 50S **4.** Protein synthesis **5.** *Ribosomes.*

UNIT

7

PEROXISOMES

STRUCTURE

LEARNING OBJECTIVES

By studying this chapter you will be able to know the types of peroxisomes in plants, their structure and biogenesis.

7.0. INTRODUCTION

In mid 1950s electron microscopic studies have shown small structure or bodies in the cells. On morphological ground these have been termed as **microbodies.** The microbodies are of two types **peroxisomes** and **glyoxysomes.** The peroxisomes were first described by **Mollenhauer Moore** and **Kelly** in 1966. The term **peroxisomes** was coined by **C. de Duve** and **P. Baudhuin** (1966) for the microbodies of mammalion system who studied their structure and function. These microbodies were given name **peroxisomes** due to their richness in peroxidases, catalase, D amino acid oxidase and urate oxidase those peroxisomes which contain the enzymes of glycolate cycle (*i.e.,* glycolate metabolism) are called glyoxysomes.

7.1. TYPES OF PEROXISOMES IN PLANTS

In plants four types of peroxisomes are found :

(i) leaf type peroxisomes (ii) glyoxysomes (iii) peroxisomes for ureide metabolism and (iv) unspecialized peroxisomes.

Leaf peroxisomes were first isolated from spinach leaf homogenate by **Tolbert** *et. al.* (1968). Peroxisomes without nucleoid are called **microperoxisomes.** Peroxisomes are found in liver, kidney and all photosythetic cells of higher plants in leaf tissue, in coleoptiles and hypocotyle, in ripening pear fruits, euglenophyta, brown algae, fungi, liverworts, mosses, ferns and in protozoa.

7.2. STRUCTURE

The name peroxisome was applied to the microbodies because this organelle is specifically found involved in the formation and decomposition of hydrogenperoxide.

Peroxisomes are ovoid granules surrounded by a single membrane and they contain a fine, granular substance. In quantitative study of rat liver cells the average diameter of peroxisomes was found to be 0.6 to 0.7 μm. The number of peroxisomes per cell varied between 70 and 100, whereas 15 to 20 peroxisomes were found per liver cell.

Peroxisomes have a single limiting unit membrane of lipid and protein molecules which enclose their granular matrix. In some cases (*e.g.*, infestacoid grasses) the matrix contains numerous threads or fibrils, while in others they are found to contain either amorphous nucleoid or a dense inner core which in many species shows a regular crystalloid structure *e.g.*, in tobacco leaf cell. (**Newcomb** and **Frduick** (1971).

The peroxisomes contain catalases and oxidases. The oxidases include urate oxidase, D-amino acid oxidase and L-α-hydroxylic acid oxidase, which produce hydrogen peroxidase. Enzyme catalase destroys H_2O_2. Enzyme calatase acts as a "Safety valve" to deal with peroxides that are harmful to the cell.

7.3. BIOGENESIS OF PEROXISOMES

It was earlier considered that peroxisomes were formed as dilatations or "buds" from the ER, which became swollen and filled with electron dense material. However, most peroxisomes exist without connections to the ER.

There is now evidence suggesting that the new peroxisomes always arise from pre-existing ones. These are self replicating organelles without their own genome. All their proteins both structural and enzymatic are synthesized in the cytosol on free ribosoes and are then imported, post translationally, into these organelles.

It is assumed that the peroxisome as a whole grows slowly and is destroyed, probably by autophagy, with a life span of 5 to 6 days. These studies have led to a mixed model of peroxisome biogenesis in which : (1) their membrane proteins are mainly synthesized on membrane bound ribosomes (2) the peroxisomal enzymes are formed in the cytosol on free ribosomes and are translocated into these organelle.

7.4. FUNCTIONS

Peroxisomes function like mitochondria in utilization of oxygen. Hence, peroxisomes were considered to be a vestige of an ancient organelle which carried out oxygen metabolism in primitive cells lacking mitochondria. According to this hypothesis peroxisomes must have performed these two functions in the past. (a) lowering of intracellular concentration of oxygen produced by photosynthesis which otherwise would have become toxic (b) carrying out required oxidative reactions. After the development of mitochondria, peroxisome become less used. Peroxisome still perfer some functions not performed by mitochondria. Some comon functions of peroxisomes are :

(A) Hydrogen peroxidase metabolism

Peroxisomes contain enzyme that removes hydrogen from organic substances using molecular oxygen. The peroxisomes oxidize a variety of substrates in a two step reaction :

In the **first step** substrate like uric acid, amino acid. lactic acids are oxidized by molecular oxygen to form H_2O_2. This oxidation is catalyzed by enzyme **oxidases** *e.g.*, D-amino acid oxidase and urate oxidase.

$$\underset{\text{organic substance}}{RH_2} + \underset{\text{molecular oxygen}}{O_2} \rightarrow \underset{\text{hydrogen peroxide}}{R + H_2O_2}$$

$$\left.\begin{array}{l}\text{Uric acid} + O_2 + \text{Urate oxidase}\\ \text{Amino acids} + O_2 + \text{D-amino oxidase}\\ \text{Lactic acid} + O_2 + \alpha\text{-hydroxy acid oxidase}\end{array}\right\} \longrightarrow 2H_2O_2$$

In **second step** substances like ethyl alcohol, methyl alcohol, nitrites, formic acid or another molecule of H_2O_2 are oxidized by the H_2O_2 formed in the first step, the catalyzing enzyme being catalases. As hydrogenperoxide is destructive to cells have it is necessary to remove it for proper functioning of the cell.

$$H_2O_2 + RH_2 \xrightarrow[\text{catalase}]{} R' + 2H_2O$$

$$\left.\begin{array}{l}H_2O_2 + \text{Ethyl alcohol}\\ H_2O_2 + \text{Methyl alcohol}\\ H_2O_2 + \text{nitrites}\\ H_2O_2 + \text{Formic acid}\\ H_2O_2 + H_2O_2\end{array}\right\} \text{Peroxisoms catalase} \longrightarrow 2H_2O$$

The entire reaction may be summarized thus

$$O_2 \xrightarrow[RH_2 \rightarrow R]{\text{Oxidase}} H_2O_2 \xrightarrow[R'H_2 \rightarrow R']{\text{Catalases}} 2H_2O$$

This type of oxidative reaction is particularly important in liver and kidney cells, whose peroxisomes detoxify various toxic molecules that enter the blood stream. Almost half of alcohol one drinks is oxidized to acetaldehyde in this way.

(B) Glycolate cycle

Peroxisomes of plants contain enzymes of glycolate path way, as glycolate oxidase, glutamate glyocylate, serine glyocylate, hydroxy pyruvate, malic dehydrogenase and asparate- α-ketogulatarate amino transferase. Besides these they also contain FAD, NAD and NADP co-enzymes. The glycolate cycle is thought to bring about the formation of the amino acids glycine and serine in a sequence of reactions which involve chloroplast, peroxisomes, mitochondria and cytosol. (**Tolbert** 1971).

Photorespiration. In green leaves, there are peroxisomes called leaf type peroxisomes, which carry out a process called photorespiration. Photorespiration is a process by which certain plants release CO_2 in light. In photorespiration, glycolic acid or glycolate, a two carbon byproduct of photosynthesis is released from chloroplast and is oxidized into glycoxylate and H_2O_2 by oxidase enzyme **glycolic acid oxidase.** Later on glycoxylate is oxidized to CO_2 and formate. The entire process has involvement of three basic organelles : **chloroplast, peroxisomes** and **mitochondria.**

(C) β-oxidation.

Peroxisomes contain enzymes of β oxidation for the metabolism of fatty acids. They are capable of oxidizing fatty acyl-CoA to acetyl CoA using molecular oxygen and NADP as electron acceptor. The peroxisomal β-oxidation cycle involves severai enzymes (*i.e.,* acyl-CoA oxidase, enoyl CoA hydratase, 3-hydroxyacyl CoA and 3-keto acyt CoA thiolase). All these enzymes serve to activate and oxidize fatty acids and produce —CoA. In these functions peroxisomes play a role in thermogenesis and the concentration of brown fat, which is abundant in hibernating animals. In animals β-oxidation occurs both in mitochondria and peroxisomes while in plant cells and yeast it occurs only in peroxisomes.

(D) Peroxisomes and Diseases

Proxisomes are responsible for two types of diseases. (a) diseases due to defficiency of single specific matrix enzyme.*g.,* adrenolmkodeutrophy ALD and (b) diseases due to defects in formation of peroxisomes *e.g.,* **Zellweger syndrome.**

• STUDENT ACTIVITY

1. Describe structure of peroxisomes

2. Describe functions of peroxisomes.

• SUMMARY

- Peroxisomes are enzyme containing microbodies in cytoplasm of cells bounded by a single membrane. They are ovate in shape. They contain the catalases and oxidases. The oxidases include urate oxidase, D-amino acid oxidase and L-α-hydroxylic acid oxidase which produces H_2O_2. The catalase destroys it because it is toxic. The glyoxysomes show glycolate cycle. These peroxisomes are associated with photorespiration in C_3 plants. The entire process involves chloroplast, peroxisomes and mitochondria which lie close to each other. Peroxisomes also contain enzymes for β-oxidation. The peroxisomes, in all probability, originate from the ER or pre-existing ones.

• TEST YOURSELF

1. Which cell organelle is essential for photorespiration ?
2. Which peroxisomal enzymes destroy the H_2O_2 ?
3. Name the set of organelles associated with photorespiration.
4. What are glyoxysomes ?
5. Which cell organelle participates in β- oxidation.

• ANSWERS

1. Peroxisome **2.** Catalases. **3.** Chloroplast, peroxisomes and mitochondria
4. Peroxismes having enzymes of glycolate metabolism **5.** Peroxisomes.

❑❑❑

UNIT

8

GENERAL CHARACTERS OF CHROMOSOMES

STRUCTURE

LEARNING OBJECTIVES

By studying this chapter you will be able to know the number, size, shape, structure and chemical composition of chromosomes.

8·0. INTRODUCTION

Chromosomes are thread like structures present in the nucleus which become visible during cell division, **mitosis** and **meiosis**. The chromosomes were first discovered by **Hofmeister,** 1848 in the pollengrains of *Tradescantia* but the credit of their discovery is generally given to **E. Strasburger** (1875). They were named by **Waldeyer** in 1888. The term chromosome means coloured bodies, (chroma means colour, somes means bodies), as these have strong affinity for basic dyes like aniline. The chromosomes are not visible in undividing nucleus due to high water content but become visible during cell division.

Diploid organisms have two chromosomes of each type, which are called **homologous chromosomes**, of which one has maternal origin and one has paternal origin. In diploid cell there are two **sex chromosomes** XX or XY and all other chromosomes leaving sex chromosomes are called **autosomes.** The sex chromosomes are also called **heterosomes.** The nuclei of all cells in each individual have definite number of chromosomes, of definite size and shape.

8·1. NUMBER OF CHROMOSOMES

The number of chromosome is characteristic of a species *i.e.*, all individuals of a species have the same number of chromosomes. Closely related species have similar chromosome numbers. The minimum chromosome number (2n = 4) is found in *Haplopappus gracilis* (Asteraceae), *Mesotoma*

(flatworm) and *Ophryotrocha puerilis* (polychaete). In *Ascaris megalocephala* one choromosome is reported but it is compound chromosome consisting of many chromosomes. The maximum number of chromosomes are found in pteridophytes *i.e.*, more than 1200 in *Ophioglossum reticulatum*. In animals highest number of approximates 2n = 1600 chromosomes was reported in *Aulocantha* by **Belar** (1926).

8·2. SIZE OF CHROMOSOMES

The size of chromosomes is determined from metaphase configuration. The size is relatively constant within species although there can be some variation in size of chromosomes in different tissues of the same organism. Chromosome size may vary greatly in closely related genera. The size of chromosome in *Trillium* can be many times larger than in closely related genus *Medeola*. Similarly, size of chromosome can be different in different species of the same genus. The size of chromosomes may vary in different regions or at different stages of life in some organisms.

The size of chromosome is, however, not related to the number of genes it contains. In *Drosophila* in somatic cells Y chromosome is much larger than X chromosome but Y chromosome is made of heterochromatin that does not contain genes. The chromosomes are generally small in fungi and these can be as short as 0·25 μm in length. Among higher plants, monocots generally have larger chromosomes than dicots. In *Trillium* the chromosomes are as large as 30 μ.

In **symmetrical karyotypes** all the chromosomes of a cell do not have much size variation while in the **asymmetrical karyotypes** the size difference of all chromosomes is large.

8·3. SHAPE OF CHROMOSOMES

The shape of chromosomes is observed during anaphasic movement and the shape depends upon position and type of centromere. The chromosomes on the basis of position of centromere can be classified into four types (Fig. 1A, D):

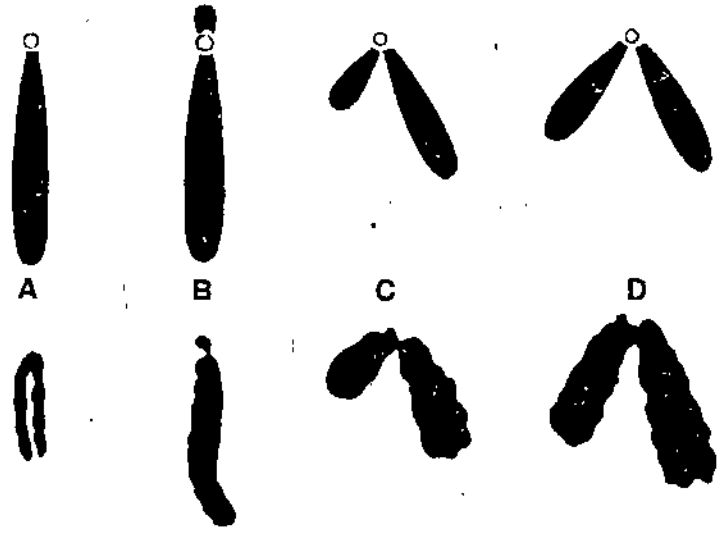


Fig. 1. (A–D) Chromosome organisation : Shapes of chromosomes

(i) Telocentric : In telocentric chromosomes the centromere is present on proximal end or at tip of the chromosome. The chromosomes make rod shapes during anaphasic movement. Telocentric chromosomes are rare. Telocentric chromosomes are reported in some plants, protozoa and some mammals (Fig. 1A).

(ii) Acrocèntric : A centromere is present near the end of the chromosome, these chromosomes have two arms, the short arm is extremely short and can be even imperceptible. The acrocentric chromosome appears as rod shaped during anaphasic movement (Fig. 1B).

(iii) Submetacentric : The centromere is situated at some distance away from the centre of chromosomes. The two arms of chromosomes are unequal. These chromosomes make L shape during movement (Fig. 1C).

(iv) Metacentric : The centromere lies in the centre of chromosomes. The chromosomes have equal or almost equal arms. These chromosomes make V-shape figures during movement (Fig. 1D).

8.4. KARYOTYPE

The karyotype is a set of chromosome in an individual or species. Following characteristics are used to identify individual chromosomes :

(i) Total length of chromosomes

(ii) Centromeric index (*ci*) $ci = \frac{\text{short arm length}}{\text{total chromosome ratio}}$

(iii) Proportion of the arms–a ratio between the long and short arm of the chromosomes.

(iv) The position of the secondary constriction and nucleolar organizers.

(v) Sub-division of the chromosomes into euchromatic and heterochromatic region.

In karyotype of man 23-pairs of chromosomes are found (Fig. 2). When homologous pairs of chromosomes are arranged in series of decreasing length the arrangement is called as **idiogram.** If there is small difference in size of chromosomes and most of the chromosomes are metacentric, the

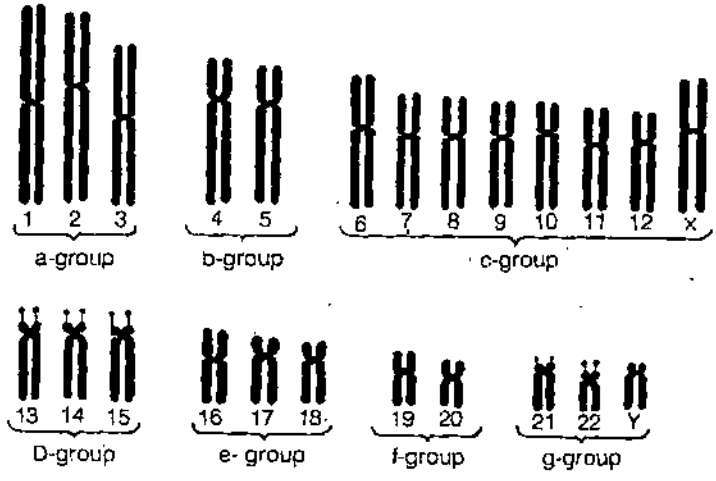


Fig. 2. Chromosome organization : Idiogram of human male karyotype

karyoype is called **symmetric.** If size differencs are large and many chromosomes are not metacentric the karyotype is called **asymmetric.** The asymmetric one is considered more advanced than symmetric.

8.5. STRUCTURE OF CHROMOSOME

The study of chromosomes indicated that chromosomes consist of a fibrillar structure **chromonema** lying in proteinaceous **matrix** and surrounded by membrane **pellicle.** Electron microscopic studies indicate that definite membrane pellicle surrounding the chromosome may not be present. The structure of chromosome consists of the following parts :

The Chromonemata

The metaphasic chromosomes appear to consist of two subunits called **chromatids**. Within chromatids are coiled filamentous structures. These structures were first observed by **Baranetzyky** in 1880 and were called **chromonema** (pl. chromonemata) by **Vejdovsky** in 1912. The chromonema forms gene bearing part of chromosome and also contains non-genic material to maintain integrity. The number of threads within the chromonema may be two, four or more. The two or more chromonemal threads may form two types of coils. The **paranemic** coils have freely separable units and the **plectonemic** coils have intertwined subunits. When plectonemic coils are stretched a **relational coil** is formed between the two chromonemata.

The Matrix

The achromatic material surrounding the chromonemata is called **matrix.** It is bounded at its outer limits by a **sheath** or **pellicle.** The presence of matrix is denied by some cytologists like **Darlington** but many evidences confirm their presence at certain stages. According to cytochemists like **Schrader** (1953) matrix is main mass of chromosome and is Feulgen positive. When it is removed by enzymatic reactions a, Feulgen-negative strand is left which is called **residual chromosome.** The matrix may function as insulating sheath for the genes during cell division.

The Chromomeres

The chromomeres were first described by **Balbiani** in 1876 and by **Pfizer** in 1881. These are morphologically distinct bead like regions on chromosomes which are constant in size and position. These are observed during early miotic prophase specially in zygotene. These structures are supposed to be the gene bearing part of chromosome.

The Centromere

The constricted region of chromosome is called the **centromere, kinetochore** or **primary constriction** (Fig. 3).

The centromere is indispensible part of chromosome, the chromosmes lacking a centromere fail to orient properly on metaphasic plate, lag at anapahse and get eliminated later. The location of centromere is defnite on a chromosome.

The shape of chromosomes during an anaphasic movement is decided by the position of centromere. As discussed earlier the chromosomes can be telocentric, acrocentric, submetacentric and metacentric depending upon the position of centromere (Fig. 1A–D).

The centromere is active during cell division, it may be locus of genes for mitotic and meiotic activity. The centromeres are also supposed to be at least partially responsible for the formation of chromosomal fibres in the spindle. The centromere is functionally divided in the longitudinal axes of the chromosomes at the beginning of anaphase and its poleward movement is governed by its attachment to the spindle. If chromomeres split at right angle to long axis, the two genetically similar arms remain attached to divided centromere and these structures are called **isochromosomes.**

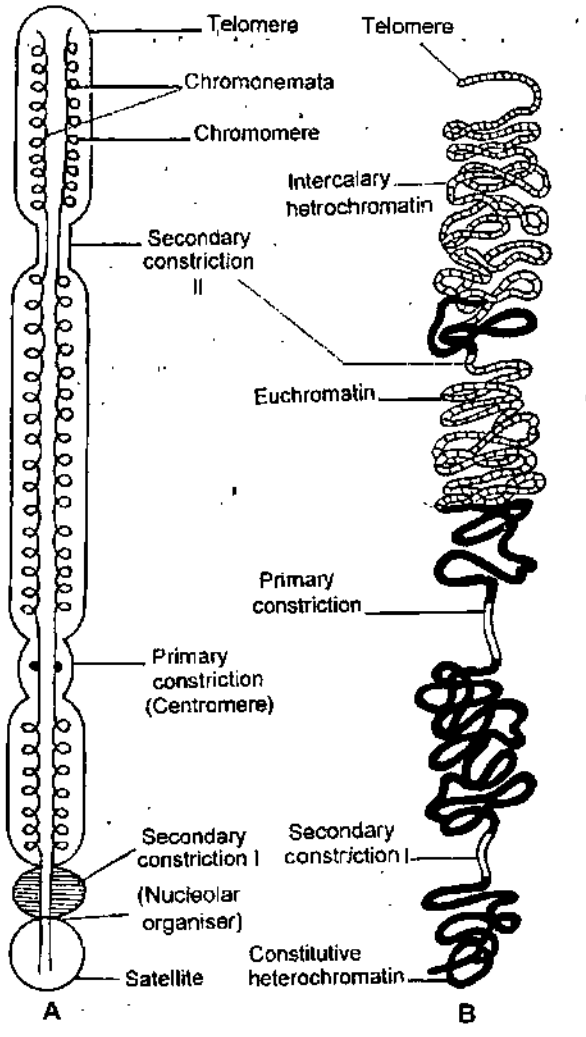


Fig. 3. Chromosome organization : Structure of a typical chromosome.

The chromosomes generally have one centromere *i.e.*, the chromosomes are **monocentric**. Sometimes two centromeres are present as a result of abnormal pairing and breaking of chromosomes, such chromosomes are called **dicentric**. When no centromere is present in chromosome due to abnormal breaking the chromosomes are called **acentric**. If centromere is not located in one position and it lies diffused along the length of chromosome, such chromosomes are called **polycentric chromosomes**.

Polycentric Chromosomes : Schrader (1935, 1953) described **diffused centromere** in hemipteran and heteropteran insects. In these chromosomes the centromere is not localized at one place. The diffused or polycentric chromosomes are also found in germ line of *Ascaris megalocephala* and in some plants. According to **Vaarama** the diffused centromere represents a primitive condition from which the localized forms are developed.

Secondary Constrictions

The constrictions other than the primary constrictions are called secondary constrictions. The secondary constrictions are regarded as **secondary constriction II** and **secondary constriction I** or **nucleolar organizer** (Fig. 3). As secondary constrictions are constant in their position and extent, these are useful in identifying particular chromosome in a set. The secondary contrictions may be short or long. The secondary constrictions can be differentiated from primary constriction by the absence of marked angular deviations of the chromosomal segments.

Nucleolar organizer : In each diploid set of chromosomes, two homologous chromosomes have nucleolar organizer or secondary constriction I. The nucleolar organizer is required for the formation of nucleolus during post mitotic reconstruction phase. The part of the chromosome beyond nucleolar organizer is called **satellite.** The satellites are round, elongated-structure separated from rest of the chromosome by delicate chromatin filament. The chromosomes having satellite are called **SAT chromosomes.** The prefix SAT stands for "Sine acid thymonucleinico". The nucleolar organizers have been observed in many plants and animals. One or both the nucleolar organizers may be found associated with nucleolus. The nucleolar organizers may be the locus of genes concerned with the formation of 28 S ribosonal RNA.

Telomeres : The term telomere was given by **Muller** (1938). The tip of the chromosomes at either end are called **telomeres.** The telomere is different in structure and composition from the rest of the chromosomes. The telomeres prevent the ends of the chromosomes from striking together. The loss of telomere imparts instability to the chromosomes.

The broken ends of chromosomes are in unsaturated state, they have tendency to fuse with other broken segments but presence of telomere prevents fusion of chromosomes. The telomeres have tendency for attraction to the nuclear membrane in vicinity of the centromere. This results in formation of **"bouquet stage"**– polarization of chromosomes.

8.6. MODELS OF CHROMOSOME STRUCTURE

The chromosomes are made of DNA and proteins. The problem, however, remains that how long DNA is arranged in short structure of proteins. A large number of models are proposed to explain the chromosome structure. The different models can broadly be classified into two types:

(A) Multistrand models : The multiple strand models are based on the fact that many strands of fibrils have been observed in chromosome structure. For example, simple multistrand model (**Steffensen**, 1952), Ris multistrand model (**Ris**, 1966).

(B) Single-strand model : The single strand models are supported by X–ray studies, by stretching of lampbrush chromosome by enzyme action and by labelling of duplicating chromosome.

Some examples are—Taylors side chain model (**Taylor**, 1957), Freese-Taylor model (**Freese** and **Taylor** 1958–59), Folded fibre model (**Du Praw**, 1966).

The most accepted recent model is **Nucleosome** structure of chromosomes.

Multistrand Models

(i) Simple multistrand model : **Steffensen** (1952, 1961) proposed that the chromosomes consist of 64 double helices of DNA, arranged in parallel manner. These helices are twisted together like the strands of a rope. This model does not explain the easy unwinding of DNA during replication.

(ii) Ris multistrand model : According to **Ris** model the 20 Å wide DNA helix is associated with histone protein to form 40 Å DNA-histone nucleoprotein fibril. The two 40 Å nucleoprotein fibrils make 100 Å thick structure called **elementary chromosome fibril**. The two 100 Å elementary fibrils twist around to form 200 Å fibril. This way each 200 Å fibril contains four parallel DNA-histone double helices. The 200 Å fibrils are associated to make chromatid of chromosome.

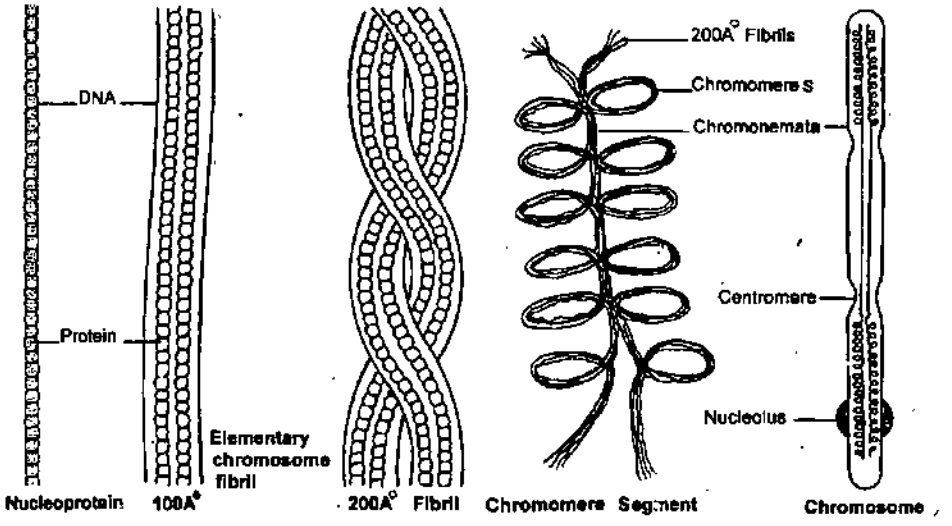


Fig. 4. Chromosome organization : Ris multistrand model

Single Strand Models

(i) Taylor's side chain model or centripede model : According to **Taylor** (1957) the chromosomes consist of a long protein back bone from which many DNA coils branch off as the legs of a centripede. He explained that the protein back bone is two layered and those layers can easily separate during replication. Each protein layer will have one strand of each DNA helix. The complimentary strands will be synthesized to make new chromosome. However, this model does not explain the linear arrangement of genes on chromosomes.

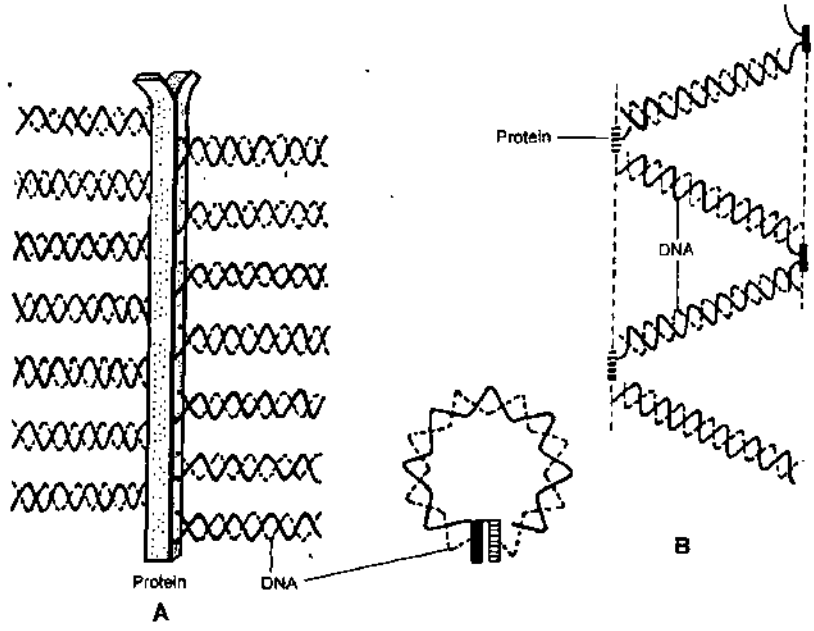


Fig. 5 (A, B). Chromosome organiation : (A) Taylor's centripede model (B) The Freese-Taylor's model

(ii) The Freese-Taylor's model : This model was proposed by **Taylor** (1959) based on suggestions of **Freese** (1958). According to this model there are two protein spines instead of one. The DNA helix stretch between them like the steps of a ladder. The DNA molecules are kept in position by **protein linkers** when the linkers are closely apposed, they form the axis of chromosomes and the DNA would be in form of lateral loops. This model is better than Taylor's earlier model as it explains the linear sequence of the genes.

(iii) Du Praw's Folded-fibre model

According to **E.J. Du Praw** (1965) the 20 Å DNA double helix, 56 μ long is spirally packed in protein to form **fibril**. This fibril is coiled to form a fibre 10–100 Å in diameter and 7–8 μ in length, such fibres are called **Type A fibre**. The DNA is packed inside the Type A fibre in packing ratio 6 : 1 (the packing of ratio is the ratio of extended length/packed length).

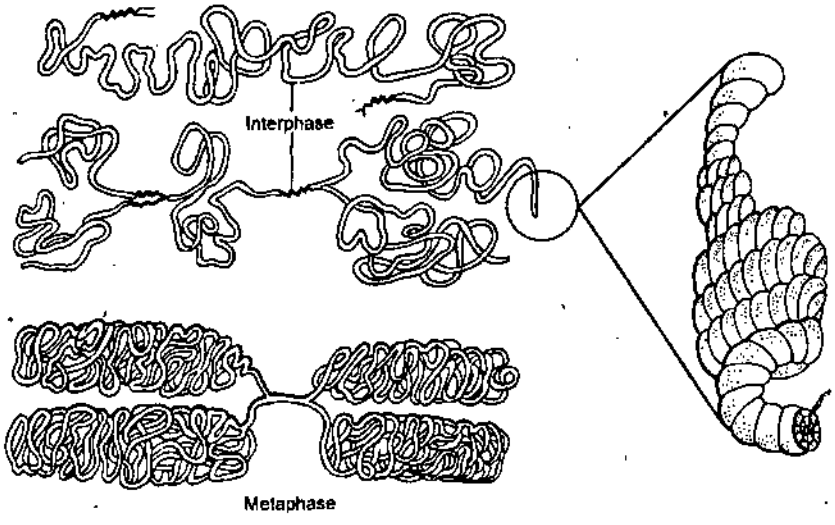


Fig. 6. Chromosome organisation : Du Praw's folded-fibre model of chromosome structure

The Type–A fibre is further coiled to make Type–B fibre of 200–250 Å diameter, the packing ratio in type B fibre is 10 : 1. The ultimate packing ratio in Type 'B' fibre is 56 : 1. The Type–B fibres are further extensively folded to form the chromatid.

Nucleosome Model of Chromosome Structure

The most accepted, latest proposed model of chromosome structure is **nucleosome** model. The chromosomes of eukaryotes are made up of nucleoprotein material called **chromatin**.

The **nucleoproteins** are made of nucleic acid–DNA and **histone proteins**. In certain types of sperms the protamines are present instead of histones. **Woodcock** (1973) on the basis of electron microscopic studies suggested that chromatin appears to have "strings on beads structure" and is made of a number of repeating units.

R.D. Kornberg and **J.O. Thomas** in 1974 proposed **Nucleosome model** of chromosome structure. The histone proteins are in form of octamer consisting of 2 molecules each of H2A, H2B, H_3 and H_4 (Fig. 7A). This octamer makes a core particle. In an octamer of 8 histone molecules the

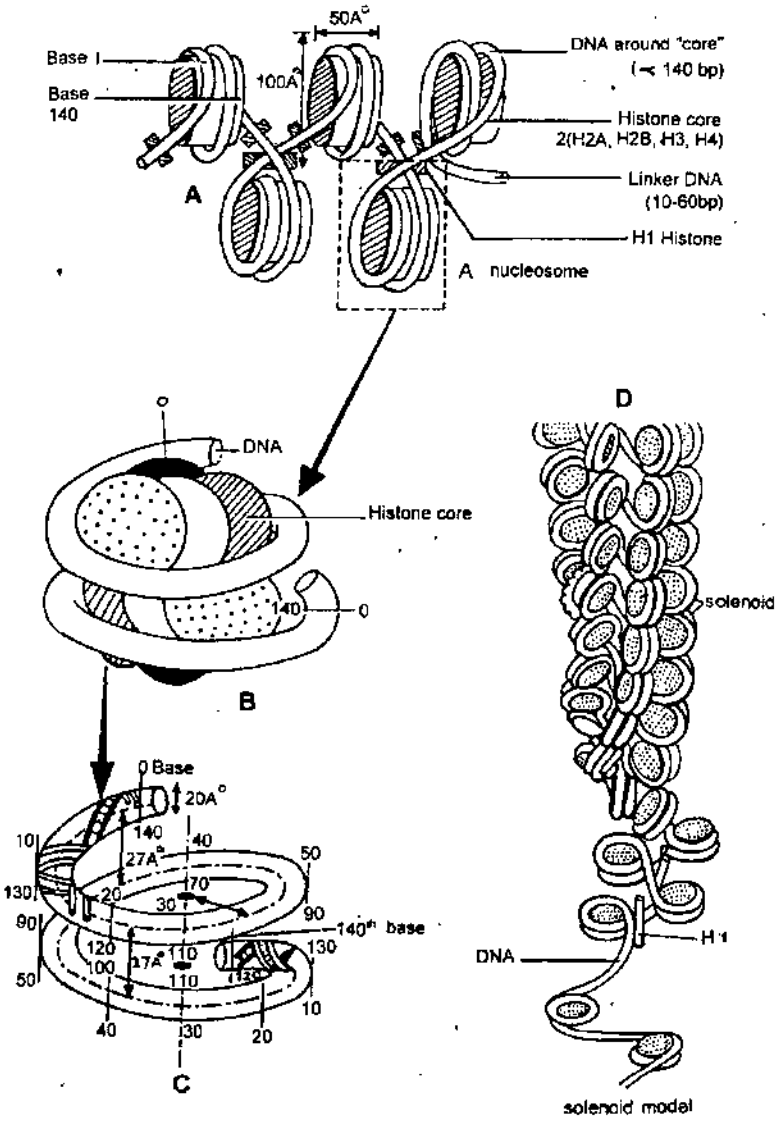


Fig. 7 (A–D). Chromosome organization : (A) Nucleosome structure of chromosome, (B) A nucleosome in detail, (C) DNA superhelix in nucleosome core, (D) Solenoid structure of a chromosome

two flat tetramers of H2a, H2b, H_3 and H_4 are stacked one on the top of the other, it is 50–55 Å high and of about 110 Å diameter. The double helical DNA is coiled over core particle as 'string over the beads. Each DNA coil makes about 1.75 turns over core particle and this length contains about 140 base pairs. Such repeating units were called **nucleosome** by **Oudet** (1975). The nuclesome units are linked to each other with **linker DNA** and $\mathbf{H_1}$. The length of linker DNA is 15–100 base pairs depending upon cell type.

Nucleosome = 140 base pairs + 2 mols. each of H2A, H2B, H_3 and H_4.

The string of nucleosome is then coiled into a **solenoid** of about 300 Å diameter. The solenoid is further coiled into **super-solenoid** structure with a diameter of 4000 Å. This super solenoidal structure makes the **unit fibre**. The solenoid model for the superstructure in chromatin was proposed by **Finch** and **Clug** (1976) (Fig. 7B–D).

8.7. CHEMICAL COMPOSITION

The chromosomes or chromatin material is made of DNA, RNA, histone and non-histone proteins. Calcium is also present in some chromosomes.

DNA is the main genetic material and is measured in units of picogram (10^{-12} gm = 1 picogram). Histone proteins are of five types *i.e.*, H_1, H2A, H2B, H_3 and H_4. The histones are non-genetic but have structural and regulatory role. They repress the activity of genes. The non-histone, proteins are of many types. In an organism they can be 12–20 types. The non-histone proteins may vary in tissues of the same organism. The non-histone proteins regulate the activity of specific genes.

8.8. SPECIAL TYPES OF CHROMOSOMES

Giant Chromosomes

The giant chrmosomes are of two types :

(1) Salivary gland chromosomes or Polytene chromosomes.

(2) Lampbrush chromosomes.

(1) Salivary Gland Chromosomes or Polytene Chromosomes

The salivary gland chromosomes were first observed by **E.G. Balbiani** in 1981. These are commonly found in salivary glands, malpighian tubules, fat bodies, ovarian nurse cells and gut epithelium in *Diptera*. The cell and nucleus containing these chromosomes are large. These chromosomes may be 200-300 times larger than chromosomes of other somatic cells in salivary glands of *Drosophila*. These chromosomes are easily visible in dissecting microscopes. These chromosomes remain paired in somatic cells. The giant chromosomes have distinct pattern of bands and interbands. The bands often form reversible chromosome puffs or **Balbiani rings** due to differential gene activation (Fig. 8 A–C).

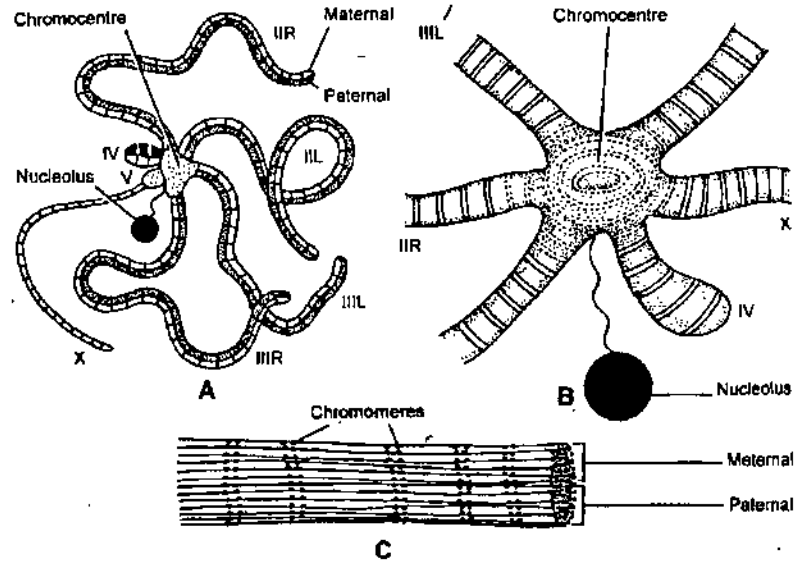


Fig. 8 (A–C). Chromosome organization : Salivary gland chromosomes : (A) from male. J nucleus, (B) Enlarged view of central part of (Fig.8A), (C) Detail of a part of chromosome.

In giant chromosomes there are many layers of chromonemata. These bundles are formed due to endoduplication of chromatids. The number of chromonemata per chromsome may be upto 2000. Hence, these are also called **polytenes.**

In *Drosophila* the giant chromosome is made of five long and one short strand arising from central **chromocentre**. One long strand is X-chromosome and other strands are arms of II and III chromosomes. The centromeres of all chromosomes fuse to make **chromocentre**. In male *Drosophila*, the Y chromosome remains attached to chromocentre.

(2) Lampbrush Chromosomes

The lampbrush chromosome was first described by **Flemming** in 1882. These are mainly found in oocyte nuclei of many vertebrates and invertebrates. They have the characteristic looped structure like lampbrush. These are large in size and may be upto 1000 μ. The loops project in pair. The number of loops can be 1–9 from each chromomere (Fig. 9).

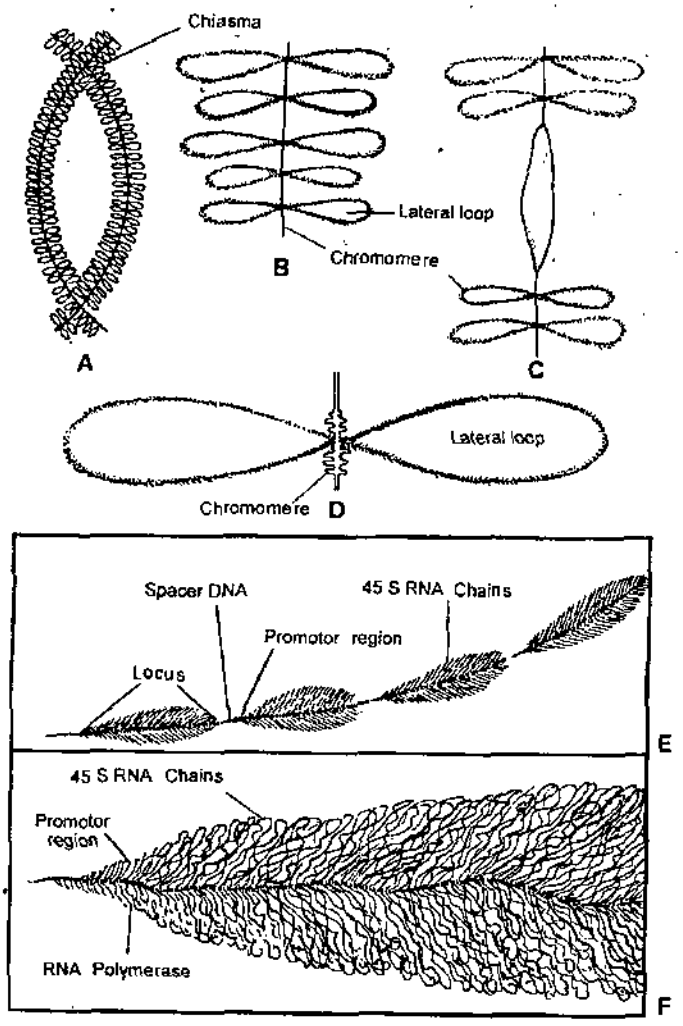


Fig. 9 (A–F). Chromsome organization : Lambrush Chromosomes. (A) Meiotic chromosome with the chiasmata; (B) Part of Chromosome enlarged showing lateral loops, (C) Loops continuous with the axis of chromosme, (D) A lateral loop showing transcription; (E) Tandem series of genes; (F) A gene locus magnified.

Each chromosome of homologous pair is made of two chromatids. The axial filament is made of DNA. The loops are lateral extension of axial filament. The loop matrix is made of RNA and proteins. These loops are site of RNA synthesis and axial filaments do not synthesize RNA.

8.9. EUCHROMATIN AND HETEROCHROMATIN

The chromosomes are made up of two types of chromatin material— **euchromatin** and **heterochromatin**. Various segments of chromosomes show difference in condensation or thickening during different stages of cell cycle. The difference in thickening and staining is called **heteropycnosis.** In heteropycnosis the over condensation is called **positive** and under condensation is called **negative.**

The chromatin material showing heteropycnosis at any stage is called **heterochromatin** and the regions which do not show heteropycnosis are called **euchromatin** (Fig. 9). In 1928 **Heitz** defined heterochromatin as the chromosomal regions that remain condensed during interphase forming the so called chromocentres of false nucleoli. The other part of chromosomal substance uncoils and swells during interphase and it is called euchromatin.

The difference between heterochromatin and euchromatin can be summarised as follows:

• The heterochromatin stains deeply and the euchromatin is comparatively lightly stained. Heterochromatin is found in condensed regions of chromosomes, it shows more coiling of chromatin material. Euchromatin is found in diffused regions and shows less coiling.

• Heterochromatin is considered to be genetically inert, it was considered to be devoid of genes. But heterochromatin contains relatively few genes, the X chromosome of *Orthoptera* and Y chromosome of *D. melanogaster* carry some genes. According to **Pontecarvo** (1944) the heterochromatin may be involved either in chromosome metabolism, or in the modification of process governed by euchromatic genes.

• Heterochromatin is late replicating. It replicates at the end of S-phase. Euchromatin replicates at the begining of S-phase.

• Heterochromatin is more labile than euchromatin. It is affected by temperature, and age of S-phase.

• Heterochromatin segments contain relatively less genes. The genes are inert or active for short period. Euchromatin *region contains active genes.*

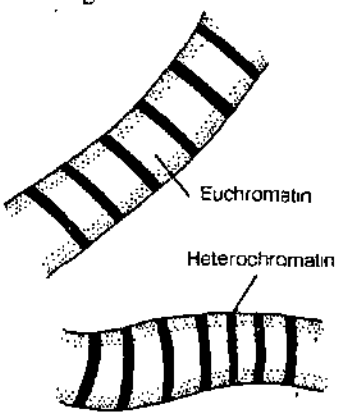


Fig. 10. Chromosome organization : Euchromation and heterochromatin.

• The DNA of heterochromatin does not synthesize mRNA for protein synthesis. The DNA of euchromatin synthesizes mRNA during interphase.

• The crossover frequency is less in heterochromatin than in euchromatin.

• The heterochromatin region is made of structures **chromomeres, chromocentres** and **knobs**.

• The heterochromatin can be **constitutive** or **facultative**. The constitutive heterochromatin shows heteropycnosis (condensation) in all cell types. The facultative heterochromatin shows heteropyknosis ony in some special cells at some particular stage of life cycle.

8.10. FUNCTIONS OF CHROMOSOMES

1. The Chromosomes are the bearers of the hereditary characters.
2. They govern the biochemistry of an organism through transcription and translation, and through this the phasic as well as controlled development of an organism takes place.
3. Changes in the number or structure of chromosomes, and gene mutations produce variations in the characters.
4. The nucleolar organizer region forms the nucleus after cell division.

• STUDENT ACTIVITY

1. What is the difference between heterochromatin and euchromatin ?

2. Describe the structure of chromosome.

• SUMMARY

- Chromosomes can be defined as gene containing structures in the nucleus. The chromosomes were first discovered by Hofmeister (1848) but the credit of their discovery is given to **Srasburger** (1875). The minimum number of chromosomes is recorded in plants ($n = 2$; *Haplopappus gracilis*) and maximum number in pteriodophytes (*Ophioglossum reticulatum*). The chromosomes may be as small as 0.25 μm or as large as 30 μ. On the basis of the position of the centromere we differentiate four types of chromosomes namely telocentric, acrocentric, submetacentric and metacentric. A set of chromosome in an individiual or species is called karyotype. A chromosome contains chromatids, chromonema, matrix, chromomeres, centromere, kinetochroes, telomere, satellite, secondary constrictions and nucleolar organizer. The chromosomes are made up of DNA, RNA, histone and non-histore proteins. Two types of chromosomal models have been proposed : multistrand model and single strand model. Giant chromosomes like salivary gland chromsomes and lambrush chromosomes are also found. The chromosomes are the bearers of heridity traits and responsible for controlling the physology of organisms.

• TEST YOURSELF

1. Who proposed the folded fiber model for chromosomes ?
2. Define Karyotype.
3. Name the arrangement when homologous pairs of chromosomes are arranged in series of decreasing length.
4. Name the shape of the chromosome when the centromere lies in the centre of the chromsome.
5. Name any giant chromsome.

• ANSWERS

1. Du Praw (1965) **2.** A set of chromosomes in an individual or species
3. Idiogram **4.** Metacentric **5.** Lampbrush Chromsome.

❑❑❑

UNIT 9

MITOSIS

STRUCTURE

- Introduction
- Cell cycle
- Mitosis Phase and Prophase
- Metaphase
- Anaphase
- Telophase
- Cytokinesis
- Significance of Mitosis
- Student Activity
  - Summary
  - Test Yourself
  - Answers

LEARNING OBJECTIVES

By studying this chapter you will be able to know the Cell Cycle, the different phases in the milotic cell division and significance of mitosis.

9.0. INTRODUCTION

The growth and development of an organism from zygote to mature individual takes place by cell division and cell enlargement. The cell enlargement can take place only within limits. The cell enlargemnet beyond limit could result in death of cell, hence cell division is primary necessity for maintenance of life. The cell division is required not only for the growth of individual, it is also required for replacement of cells. There are two kinds of cell division required to maintain life —**mitosis** and **meiosis. The proces of cell division whereby the chromosomes are duplicated and distributed equally between the daughter cells is called mitosis.** The term mitosis was introduced by **Flemming** (1882). In vascular plants, the best part for studying mitosis is the root tip. They are fixed at a proper time in Carnoy's fluid (3 parts ethanol + 1 part glacial acetic acid) and then stored in 70% ethanol, squash preparations are made from fixed material.

9.1. CELL CYCLE (interphase + mitosis)

In continuously dividing cell, a period of interphase and a mitosis phase make cell cycle. The cell cycle may be divided into following four phases, their relative periods may vary from organism to organism and from tissue to tissue (Fig. 1).

Interphase	G_1	Pre DNA synthesis phase	12 hours
	S	DNA synthesis phase	6-8 hours
	G_2	Post DNA synthesis phas	3-4 hours
Mitosis	M	Mitotic phase	1 hour

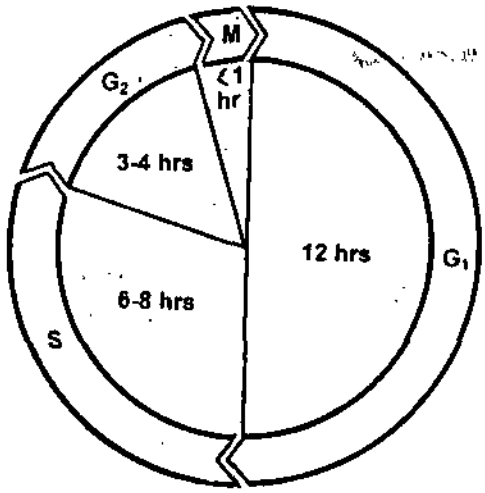


Fig. 1. Cell Division. Relative period of different phases in a cell cycle of 24 hours.

Interphase : The interphase is also called "**resting stage**" but it is a period of great activity (Fig. 2). During this period the cell prepares itself for further process of cell division. The main three activities during interphase are :

(i) DNA replication and synthesis of basic nuclear proteins— the histones;

(ii) The synthesis of energy rich compounds which provides energy for mitosis, synthesis of proteins for spindle formation;

(iii) The centriole divided into three phases G_1, S and G_2.

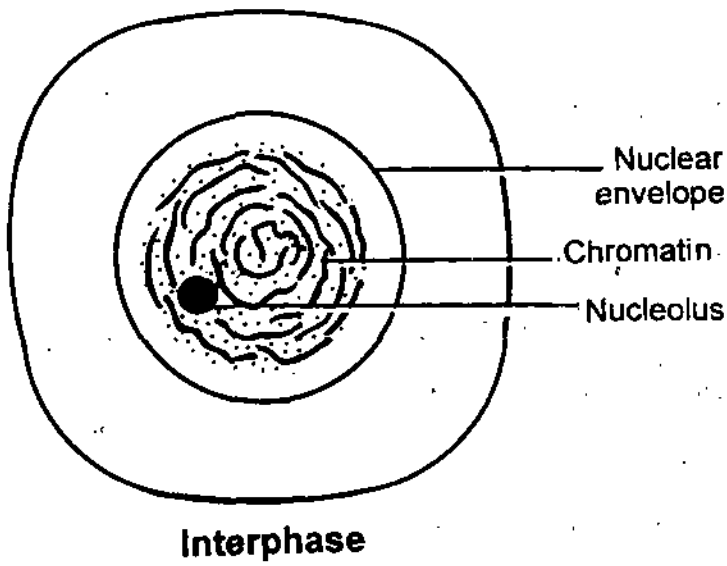


Fig. 2. Cell Division. Interphase

G_1 Phase : The post mitotic gap phase I—The G_1 phase takes place at the end of one cell division. During this period there is synthesis of RNA and proteins but there is no synthesis of DNA.

***S* Phase** : Synthesis phase —During S phase DNA is synthesised from purine and pyrimidine nucleotides. The contents of DNA get doubled.

G2 Phase : Premitotic gap phase I — Synthesis of RNA and protein takes place.

The time taken for the S phase, G_2 and mitosis is nearly equal. The duration of G_1 is usually greater, the cells which divide less frequently have larger G_1 phase while the frequently dividing cells have shorter G_1 phase. The DNA synthesis takes place only in S phase, duplication of chromosome thus takes place in S phase. Once a cell has entered the S phase and DNA replication takes place, the cell is committed itself to division.

9.2. MITOSIS PHASE AND PROPHASE

The cell division is a continuous process and it is artifically divided into different phases for the convenience of study only. The mitotic cycle is divided into phases — **prophase, metaphase, anaphase and telophase** (Fig. 9). After division of the nucleus, *i.e.*, **Karyokinesis** the division of cytoplasm by cytokinesis takes place.

Prophase is the longest part of mitotic cycle. During prophase the cell becomes spheroid with increase in viscosity and refractivity. In early prophase the nuclear membrane and nucleus are present,

the chromosomes are thin, uncoiled and filamentous structures. With the progress of prophase the chromosomes become thick, condensed and coiled structures. The chromosomes may contract to 1/25 of their original length. The double nature of chromosome becomes visible as the longitudinal splitting of chromosome takes place and the two chromatids remain attached by centromere, the chromosomes at this stage are called **dyad** (Fig. 3). The chromosomes which were evenly distributed in early prophase move towards nuclear membrane leaving a clear central area.

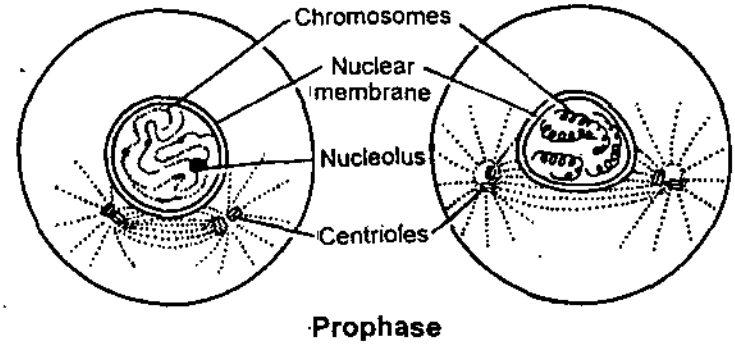


Fig. 3. Cell Division. Prophase.

The centrioles which had undergone duplication in interphase begin to move towards opposite poles. These movements take place due to growth of spindle fibre between them. According to **Taylor** in newts the centrioles move at a velocity of one micron per minute. In cells which do not have centrioles, the mitosis is called **acentric**. When centriole is present and takes part in division the mitosis is called **centric.** The centric mitosis can be **astral** and **anastral.** Cell divisions in which aster is formed around centriole the mitosis is astral, *e.g.*, in animals and lower plants. When there is no aster around centriole the mitosis is anastral, *e.g.*, in higher plants. The asters are cytoplasmic in origin.

The spindle formation starts between the two poles. The spindles are microtubules of about 250Å diameter. The asters, centrioles and the spindle fibres make the **mitotic apparatus** or **achroamtic figure.** The late prophase (Fig. 4) or prometaphase is characterised by disappearance of the nuclear membrane. After disappearance of nuclear membrane there remains no differentiation between cytoplasm and nucleoplasm. Such mitosis is called **extranuclear** or **eumitosis.** In many protoza and some animal cells the nuclear membrane does not disappear and mitosis takes place within nuclear membrane and such mitosis is called **premitosis** or **intranuclear mitosis**. When nuclear membrane dissolves, a fluid area is formed in the centre of the cell. The chromosomes move freely in this area when they move towards equator.

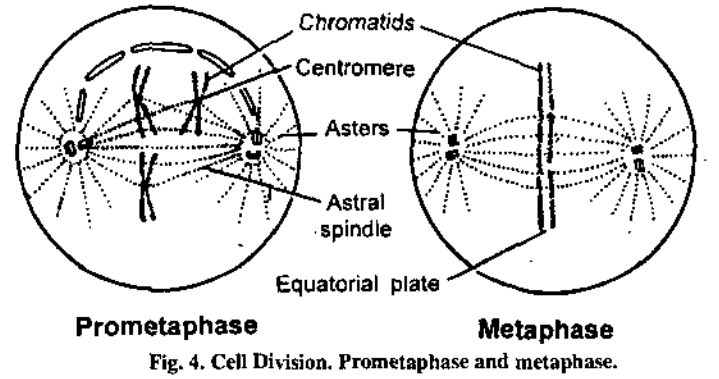


Fig. 4. Cell Division. Prometaphase and metaphase.

9.3. METAPHASE

At metaphase the spindle fibres appear. The spindle fibres get attached to the chromosome at centromere. It is experimentally proved that spindle fibres are definite structures. According to **Taylor** when a microdissection needle is inserted into a cell it moves freely in longitudinal direction but meets resistance when moved transversely, this indicates the presence of spindle fibres in longitudinal direction. The spindle fibres are of two types — **continuous fibres** and **chromosomal fibres.** The continuous fibres extend from pole to pole, they form the pushing body in animal cells (Fig. 5 A-C) The chromosomal filres extend from the pole to the centromere of the chromosomes.

The spindles can be **direct** and **indirect fig. (B, C).** In direct type the chromosomal fibres connect the chromosome directly with the pole. In the indirect type the chromosomes are connected with the continuous fibres.

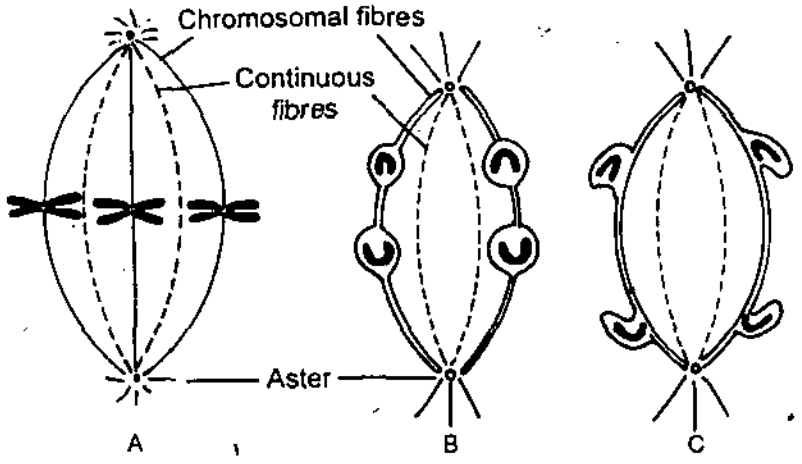


Fig. 5. (A–C). Cell Division (A) metaphasic spindle. (B) Direct spindle, (C) Indirect spindle.

During metaphase the chromosomes attached to spindle fibers, show active movement towards equator plate. The chromosomes can arrange on equotrial plate or metaphasic plate in such a way that the centrioles lie on equator plate and arms also lie on equator plate or only the centromere lies on equator plate and the arms are free in cytoplasm (fig. 4). Smaller chromsomes are generally arranged in the centre of the cell while largwer chromosomes remain on periphery.

9.4. ANAPHASE

The chromosomes are arranged on the equator for a short period only. During anaphase the chromosomes split at centromeres. The sister centromeres separate from each other and the two sister chromatids separate as **daughter chromosomes** (Fig. 6). The daughter chromosomes from each pair move towards the opposite poles. Many theories have been put forward for the movement of chromosomes, *e.g.*, pulling by contraction of the spindle fibres, pushing by expansion of fibres, a combiantion of contraction and expansion, viscosity changes in cell, electrostatic forces, diffusion currents, viscous streaming of protoplasm and hydrodynamic forces.

The most accepted view for the movement of chromosomes is that spindle fibres attached to the centromeres condense and pull the chromosomes to the poles. Simultaneously, there is expansion of spindle fibres between the two poles and the poles are pushed apart. The evidence for pulling of chromosome is that dragged chromosomes become V-shaped with their arms trailing. The centromeres are essential for chromosome movement as chromosomes without centromeres fail to move to the poles. The evidence against the contraction of spindle fibres is that on contraction the spindle should become thicker. This thickening has not been observed. Secondly, in cells when spindle fibres are cut by micro needles, the movement of chromosomes still takes place. It is still believed that the chromosomes are pulled by spindle fibres, the spindle fibres may not become thicker on contraction because they dissolve progressively in the region of centrioles. Shortening of spindle fibres is associated with slow removal of protein monomers from the polar region and the elongation of continuous fibres results from addition of new monomers.

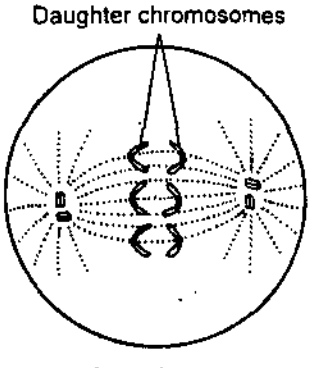


Fig. 6. Cell Division. Anaphase.

All the chromosomes of a cell move simultaneusly towards the poles except the sex chromosomes which are a little slower in movement. There is no relation btween the rate of movement and the size of the chromosomes. The chromosomes move at a deaccelerating rate and never at accelerating rate and the velocity of chromosomal movement ranges from 0.2 µm per minute to about 5 µm per minute.

9.5. TELOPHASE

The telophase changes are associated with the restoration of interphase conditions. The chromosomes reach the poles. The nuclear membrane reappears around each set of chromosomes. The nucleolus reappears. The chromosomes uncoil, become loose fibre-like structure. The chromosomes cannot be stained at this stage. The gel of the spindle reverts to the sol state and the spindle disappears. The division of nucleus called **karyokinesis** takes place and this is followed by the division of the cytoplasm called **cytokinesis** (Fig. 7).

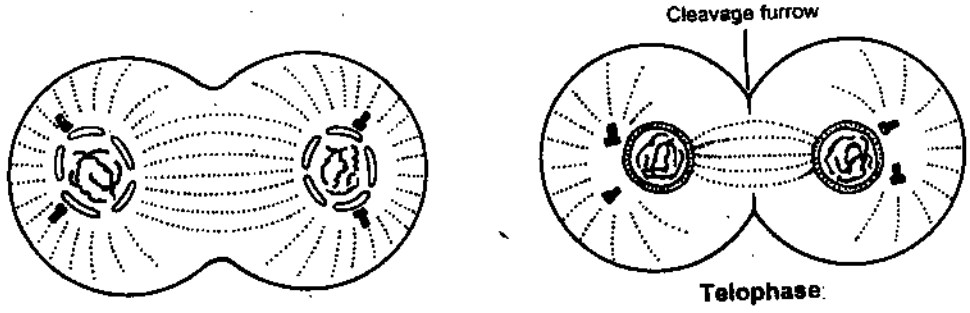


Fig. 7. Cell Division. Telophase

9.6. CYTOKINESIS

Cytokinesis takes place by two methods (Fig. 8 A, B) :

(i) Cell furrow formation : In animal cells the cell wall is absent and the outer layer is more flexible. The cleavage furrow formation starts in early part of telophase, the furrow formation takes place from periphery to the centre at the region of equator. With the dissolution of spindle fibres the furrow progresses inwardly and ultimately joins in the centre to separate the two daughter cells.

(ii) Cell plate formation : In plants the cytokinesis takes place by cell plate formation. The cell plate formation starts in the centre and gradually advances towards the cell wall. The cell plate initially is the middle lamella of the many layered cell wall. After the cell plate formation, the primary walls are deposited on either side. The thick secondary cell walls of cellulose are laid down in later stages.

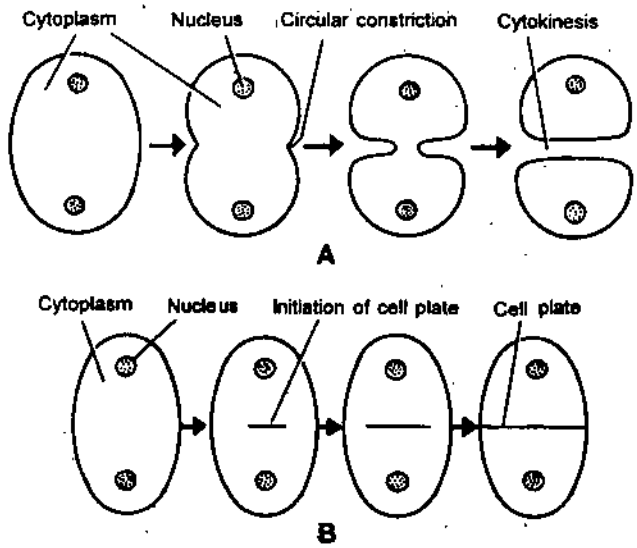


Fig. 8. (A, B). Cell Division. Two methods of cytokinesis (A) By cell furrow; (B) By formation of cell plate.

9.7. SIGNIFICANCE OF MITOSIS

1. Mitosis is responsible for equal distribution of chromosomes in all cells of the body. After mitosis two daughter cells are formed in which number of chromosomes are equal to the parent cell. The zygote cell divides by mitosis to make individuals with all cells in the body having same chromosome number.

2. The smaller cells have greater amount of surface area available as compared to larger cells. As the cell increases in size, the available surface area in relation to increased volume becomes less. The mitosis divides the larger cell and helps in restoring the surface volume ratio of the cell.

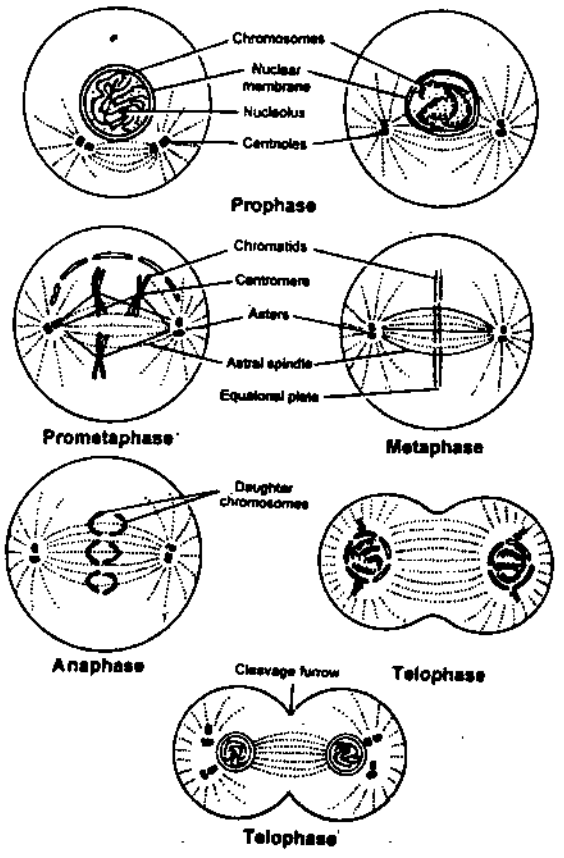


Fig. 9. Cell Division. Stages of mitosis.

3. The growth in all individuals takes place by mitosis. A cell cannot grow in size to a large extent without disturbing the ratio between nucleus and the cytoplasm. After a particular size the cell divides by mitosis to maintain the nucleoplasmic index.

4. The replacement of cells in the injured parts, in upper layer of epidermis, in the lining of the gut, and the replacement of red blood corpuscles takes place by mitosis only.

• STUDENT ACTIVITY

1. Describe Cell Cycle

2. With the help of suitable diagrams describe the metaphase stage of mitosis.

• SUMMARY

• The cell division is of two types — mitosis and meiosis. In a continuously dividing cell, the period of interphase and mitosis phase makes cell cycle. The inerphase is di-stinguished into G_1 –phase (pre DNA synthesis phase), S-phase (DNA synthesis phase) and G_2–phase (post DNA synthesis phase). The mitotic phase comprises prophase, metaphase, anaphase and telophase. The prophase is characterised by separation, spiralization and condensation of chromosomes, longitudinal splitting of chromosomes except the centromere, disppearance of nuclear membrane and nucleous. During metaphase the chromosomes are attached to spindle fibres and show active movement towards equator plate. During anaphase the chromosomes split at centromeres. The chromatids now move to opposite poles. At telophase, the nuclear membrane and the nucleolus re-appear and the nucleus organises. The cytokinesis may occur either by furrowing or by cell plate formation. After mitosis two daughter cells are formed which have the number of chromosomes equal to parent cells. Mitosis is responsible for equal distribution of chromosomes in all cells of the body.

• TEST YOURSELF

1. What plant material is best suited for studying mitosis in class room ?
2. What is the sequence of cell cycle ?
3. In which stage of cell division is DNA content doubled ?
4. At what stage in mitosis, the nuclear membrane, nucleolus re-appear and organise ?
5. Who introduced the term mitosis ?

• ANSWERS

1. Root tips **2.** G_1 S, G_2M **3.** Interphase **4.** Telophase
5. Flemming (1882).

❑❑❑

UNIT

10

MEIOSIS

STRUCTURE

LEARNING OBJECTIVES

By studying this chapter you will be able to know the meiosis and its significance.

10·0. INTRODUCTION

The chromosome number is constant for a particular species. In sexually reproducing organisms male and female gametes fuse to form zygote. If gametes also have the same chromosome number as the other cells of the indvidual, after fertilization the zygote formed will have chromosome number double of that species. In next generation the gametes will have double number and zygote will have chromosome number four times of the normal number and this will create instability. The chromosome number is characteristic of a species and remains constant because the diploid organisms form haploid gametes by meiosis and the diploid number in zygote is restored by the process of fertilization. **Meiosis** can be defined as **a type of cell division in which the chromosomes are first duplicated and then divided equally into four daughter cells, *i.e.*, the daughter cells have chromosome number half of the parent cell, hence it is also called reduction division. Farmer** and **Moore** (1905) coined the term maiosis which was later on changed to **meiosis.**

10·1. TYPES OF MEIOSIS

Depending upon the stage at which meiosis takes place. It can be of three types — (i) zygotic or initial meiosis, (ii) gametic or terminal meiosis and (iii) sporic or indeterminate meiosis.

(i) Zygotic or initial meiosis : In lower plants, *e.g.*, in *Chlamydomonas*, and in some animals the individuals and the main part of life cycle is haploid, the diploid generation is represented only

by the zygote. The zygote divides by meiosis to form haploid spores. The haploid spores develop into haploid individuals (Fig. 1).

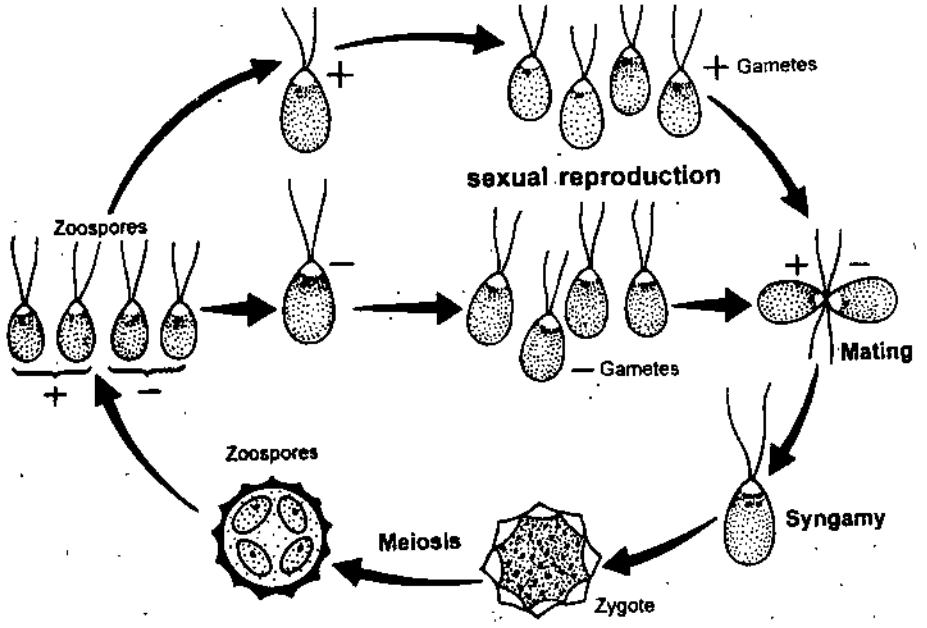


Fig. 1. Cell Divsion. Zygotic meiosis.

(ii) Gametic or terminal meiosis : In higher plants and animals the individual and the dominant phase of life cycle is diploid and reduction division or meiosis takes place only at the time of gamete formation (Fig. 2). The diploid reproductive mother cells divide by meiosis to make haploid male and female gametes. The gametes fertilize and the diploid condition is restored in zygotes. The zygotes develop into diploid individual.

(iii) Sporic or indeterminate meiosis : In bryophytes the thallus is haploid which bears male and female sex organs. The gametes are formed without reduction division which fuse to form diploid zygote. The diploid zygote develops in a dependent sporophyte. In the diploid sporophyte meiosis takes place at some indeterminate point between the zygote and the gamete formation. After meiosis haploid megaspores and microspores are formed which develop into male and female gametophytes (Fig. 3).

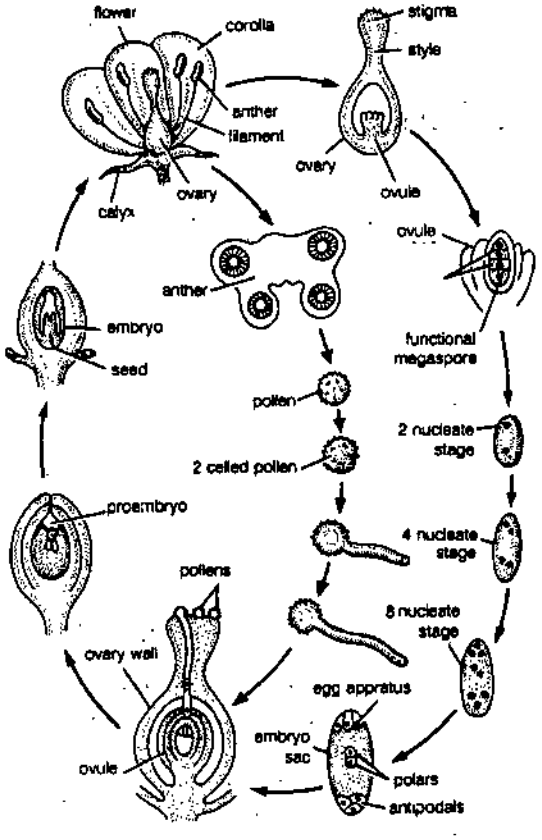


Fig. 2. Cell Division. Diagram showing gametic meiosis.

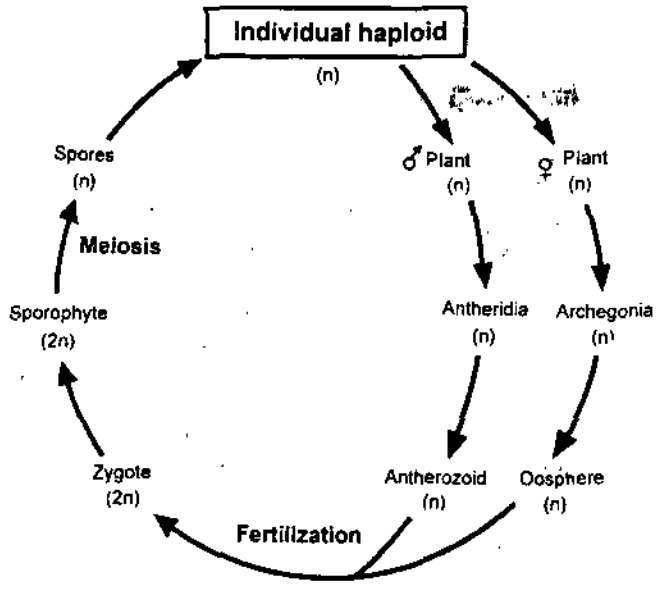


Fig. 3. Cell Division. Alternation of generation in Bryophytes.

10.2. STAGES OF MEIOSIS

Meiosis consists of two successive divisions—The first meiotic division and the second meiotic division. The first meiotic division results in reduction of chromosome number (heterotypic division) and the second meiotic division is equational division like mitosis (homeotypic division). Meiosis results in formation of four haploid daughter cells. The interphase precedes meiosis as in mitosis.

The division of meiosis for convenience of study can be divided into the following substages (Fig. 16)

Interphase

G_1

S

G_2— Very short or absent

First Meiotic Division

Prophase I —— { Leptotene, Zygotene, Pachytene, Diploten, Diakinesis

Metaphase I

Anaphase I

Telephone I

Interkinesis

Prophase II

Methaphase II

Anaphase II

Telophase II

Cytokinesis

Interphase : The interphase has been divided into G_1, S, and G_2 phases as in mitosis. In meiosis the G_2 phase is either very short or completely absent.

G_1 phase : It is post mitotic gap phase I. During this period the synthesis of RNA, proteins and energy rich compounds takes place. The cell prepares itself for division.

S phaseor : DNA synthesis phase : DNA synthesis takes place during S phase. The synthesis of DNA is semiconservative, the DNA unwinds, the two strands separate and the complementary strands are synthesised on template strands. After S phase the meiosis may start direct or very short G_2 phase takes place.

10.3. HETEROTYPIC DIVISION : PROPHASE- I

Among all the stages of meiosis the prophase I is more complex and is of the largest duration. The prophase of meiosis is very much different from prophase of mitosis. The prophase I has been divided into the following substages :

(i) Leptotene or Leptonema (*Lepto* — slender, *nema* — threads)

The beginning of meiotic prophase is marked by increase in nuclear volume. In the beginning of leptotene the chromosomes are thin and appear as large thread like structures (Fig. 4). The chromosomes gradually become more distinct, a number of bead like structures called **chromomers** are seen on chromosomes. The leptotene chromosomes are irregularly distributed or may be polarised towards the centrioles forming "bouquet" (Fig. 5). The electron microscopic studies have shown that bouquet formation appears because a group of chromosomes are attached close together on nuclear membrane. In plant cells the chromosomes may form a tangle of threads called **synizetic knot** on one side of nucleus.

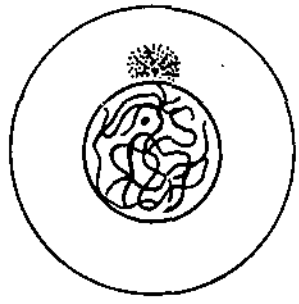


Fig. 4. Cell Division. Leptotene

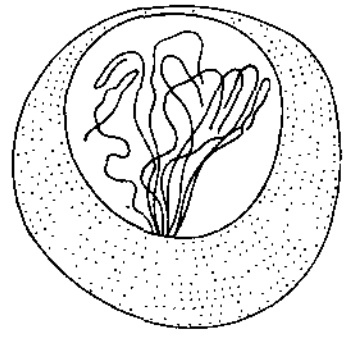


Fig. 5. Cell Division. Leptotene chromosomes arranged in a 'bouquet'

In maize the nucleolus increases in size during leptotene due to synthesis of RNA and proteins. In some cases there may be premeiotic pairing of chromosomes. The homologous chromosomes pair, the pairing is exact from chromomere to chromomere but there is no synaptonemal complex formation.

Zygotene or Zygonema (pairing-threads)

During zygotene the chromosomes condense and become shorter and thicker. The zygotene is characterised by the pairing of chromosomes (Fig. 6). In homologous chromosomes one chromosome is paternal and the other chromosome is maternal. The pairing called **synapsis,** or **syndesis** or **synizesis,** is exact and takes place from chromomere to chromomere. The pairing may begin at the centromere and proceed towards the ends called **procentric pairing** or the pairing may begin at the ends and proceed towards the centromeres. The pairing takes place between homologous segments even if they are present in non-homologous chromosomes due to translocations. The pairing always

Fig. 6. Cell Division. Zygotene

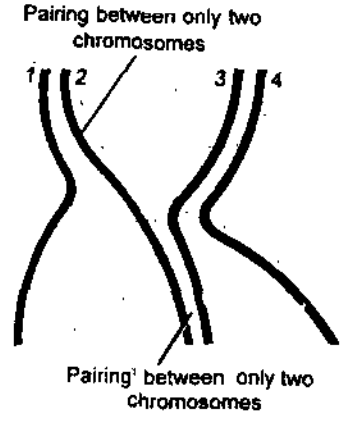


Fig. 7 Cell Division. Meiotic pairing among four homologous chromosomes.

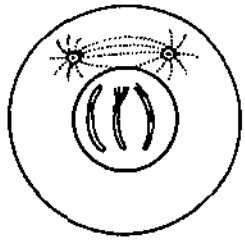
takes place between two chromosomes only in one region. For example in autotetraploids there are four homologous chromosomes in a cell but in one region the pairing is only between two chromosomes. (Fig. 7).

The pairing sometimes takes place between non-homologous chromosomes, it is called **pseudosynapsis**. The synaptonemal complex formation takes place during zygotene.

Many factors may be responsible for meiotic pairing. According to **Darlington's precocity theory** (1930) meiosis is a precocious mitosis—the chromosomes enter prophase even before duplication of chromosome is complete, this is responsible for pairing of chromosomes. According to **Stern** and **Hotta** (1969) the chromosome replication in premeiotic S phase is not complete, about 0.3% DNA replicates during zygotene. This significant DNA is responsible for chromosome pairing.

Pachytene or Pachynema (*Pachy* — thick, *nema* — threads)

After the zygotene stage when pairing is over and synaptonemal complex is formed there is beginning of pachytene. Pachytene is sometimes of the longest duration of all prophase stages. During pachytene the chromosomes are further shortened, coiled into thick structures (Fig. 8). Each homologous pair is called **bivalent**. The number of bivalents is half the diploid number of chromosomes. Since the double nature of chromosome becomes distinct and each chromosome is divided into two chromatids attached at centromere. The bivalents thus have four chromatids and the chromosomes are called **tetrads**. Each bivalent consists of two homologous chromosomes twisted about each other in **relational coiling**.



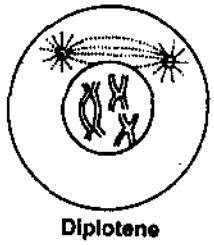
Pachytene

Fig. 8. Cell Division. Pachytene.

Pachytene is characterised by crossing over which takes place between the non-sister chromatids in homologous pair. The chromatid is the unit of crossing over. The non-sister chromatids of homologous pair undergo one or more transverse breaks at the same level. The break is followed by interchange and fusion of segments between the two homologous chromosomes. *Thus each bivalent has* two chromatids as non-cross overs and the two chromatids as cross overs.

The electron microscopic studies have confirmed that zygotene and pachytene bivalents are organsied into structures called synaptonemal complexes. The complex consists of a **central element** and two **lateral elements**. The lateral elements and central elements are connected by a series of lateral loops called L–C fibres. The loops fuse in the middle to form central elements. The lateral elements are attached at both ends to the nuclear membrane. The synaptonemal complex is the proteinaceous structure and is involved in chiasmata formation.

Diplotene or Diplonema (*Diplo* — double, *nema* — threads). At diplotene stage more thickening and shortening of the chromosomes takes place. The homologous chromosomes start separating from each other. The separating chromosomes are held together at certain point of contact called **chiasmata** (singular chiasma) (Fig. 9). The chiasmata represent the place of crossing over, *i.e.*, where breaks and fusion had occurred.



Diplotene
prophase-I

Fig. 9. Cell Division. Diplotene.

The number of chiasmata per bivalent is normally dependent on the length of the chromosome and species. The shorter chromosomes have fewer chiasmata and the larger chromosomes have more. The number of chiasmata is generally not more than four but the maximum reported number is 13 or 14. The number of *chiasata is variable in same bivalent* which can be two in one cell, three or four in other cells, rarely the chiasma is one. Chiasmata are generally found in meiosis of all eukaryotes however, achiasmatic meiosis has been reported in males of higher Diptera *e.g.*, *Drosophila*, scorpion fly, grasshopper and scorpions.

Terminalisation : *The chiasma at the end of chromosome is called* ***terminal*** *chiasma and* the chiasmata all along the length of chromosomes in between are called **interstitial** chiasma. During deplotene when separation of homologous chromosomes takes place, the termial chiasma slips off the end of the chromosome and the next interstitial chiasma becomes the terminal chiasma. This process of separation is called terminalisation. As this process continues the number of interstitial chiasmata becomes less. According to **Darlington** the electrostatic force is responsible for terminalisation and according to **Swanson** (1957) the despiralisation of chromosomes is responsible for separation. When terminalization is over, the homologous chromosomes remain in

contact through the terminal chiasma (Fig. 10). The frequency of chiasmata is the average number of chiasmata in bivalents of a nucleus.

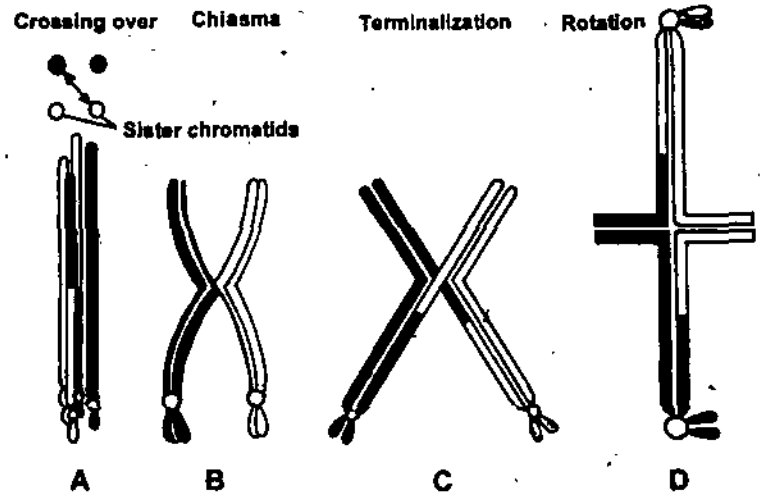


Fig. 10. Cell Division. Crossing over, chiasma formation, terminalization and rotation.

$$F = \frac{\text{The number of chiasmata}}{\text{Total number of bivalents of a nucleus}}$$

The terminalisation coefficient (T) is the measurement of degree of terminalisation

$$T = \frac{\text{Number of terminal chiasmata}}{\text{Total number of bivalents of a nucleus}}$$

Rotation : The rotation is due to forces of repulsion which also cause terminalisation. If there is a single chiasma the arms of the bivalent rotate through 180° and form a cross, with two chiasmata a loop is formed .

The synaptonemal complexes disappear during diplotene but sometimes the short segments may persist (a) near the ends of bivalents where lateral elements are attached to the nuclear membrane (b) at the site of chiasmata formation.

Diakinesis : Diakinesis is not much differentiated from diplotene, only that the chromosomes become more contracted in diakinesis. The bivalents become more evenly distributed towards periphery. The homologous remain in contact with each other by their terminal chiasmata. The nucleolus and the nuclear membrane gradually disappear in the end.

10·4. METAPHASE I

The nuclear membrane disappears. The chromosomes become most condensed. The spindle formation takes place. The spindle fibres get attached to the homologous pairs of chromosomes on their centromeres. The pairs move to the equator plates, (Fig. 11) their movements is called **congression**.

The chromosomes get arranged on equator plate in such a way that the two centromeres of each bivalent are on opposite sides of the equatorial plate. Thus the arrangement of chromosomes on equator in meiosis is different from their arrangement in mitosis. The chromosomes then start separation as active repulsion arises between homologous centromeres.

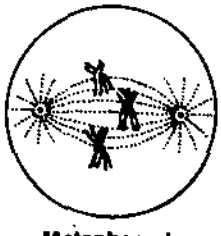


Fig. 11. Cell division.

10·5. ANAPHASE I

During anaphase the movement of chromosomes of a bivalent from equatorial plates to the poles takes place (Fig. 12). In meiotic anaphase I the splitting of centromere does not take place, hence the two sister chromatids without separation reach the same pole. This causes reduction of chromosome number, each pole receives chromosomes half of the diploid number hence, meiosis is called reductional or disjunctional

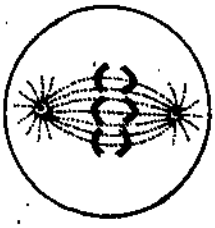


Fig. 12. Cell division.

division. The meiotic anaphase I is different from mitotic anaphase because in mitotic anaphase the centromeres split longitudinally and the sister chromatids separate and reach the opposite poles hence, mitosis can be called **equational division**.

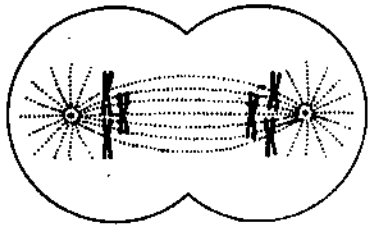
All the chromosomes in anaphase do not separate simultaneously, the shorter chromosomes separate quickly, the separation of larger chromosoms takes more time as they have interstitial chiasmata. Because crossing over takes place in meiotic prophase the composition of the separating homologous chromosomes is different from that of chromosomes undergoing synapsis. In homologous pair a bivalent has one chromatid of non-crossover type and the other chromatid of crossover type having both maternal and paternal characters.

10·6. TELOPHASE I

During telophase I the chromosomes reach the poles. The chromosomes remain condensed for short time, then they become uncondensed, elongated structure. The nuclear membrane reappears but nucleoli are not formed. Unlike meiosis the spindle fibres do not disappear completely.

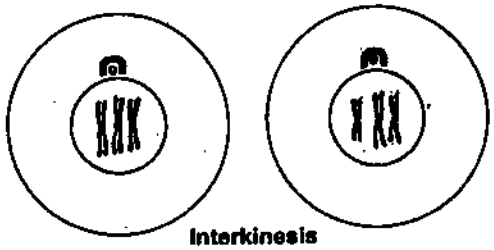
After telophase the two nuclei may be separated by cytokinesis and a dyad is formed. Such division is called **successive division**. In some cases the cytokinesis takes place at the end of second meiotic division and four daughter cells are formed. This type of division is called **simultaneous.**

The first meiotic division may be followed by a short **interphase** or interkinesis before the start of second meiotic division (Fig. 13, 14). In some cases the telophse I and interkinesis are absent, the chromosomes after anaphse I directly pass into metaphse of meiosis II.



Telophase-I

Fig. 13. Cell Division.



Interkinesis

Fig. 14 Cell Division.

10·7. HOMEOTYPIC DIVISION : PROPHASE II

The second meiotic division is very much similar to mitosis and is often called **meiotic mitosis.** The interkinesis before second meiotic division is like interphse before meiosis but during this peirod there is no synthesis of DNA (Fig. 15). The prophase II of meiosis is not as complex as prophase I of meiosis. The chromosomes become shorter and condensed, they are already double structure, each chromosome consists of two chromatids attached by centromere. The nuclear membrane disappears and the spindle formation takes place at this satge.

10·8. METAPHASE II

As in mitosis the chromosomes attached by spindle fibres move to equator. The chromosomes lie on equator in such a way taht the centromeres remain on equator and the chromatids are free to move.

10·9. ANAPHASE II

The centromeres of the chromosomes split longitudinally, the two sister chroamtids get separated as distinct chromosomes. The chromosomes after splitting move to different poles.

10·10. TELOPHASE II

The chromosomes after reaching pole again become uncondensed, thin and elongated structures. The nuclear membrane and nucleoli reappear. The spindle fibres get dissolved and after cytokinesis four daughter cells are formed. In each cell the chromosome number is half of the diploid cell. Thus, reduction takes place during meiosis (Fig. 15, 16).

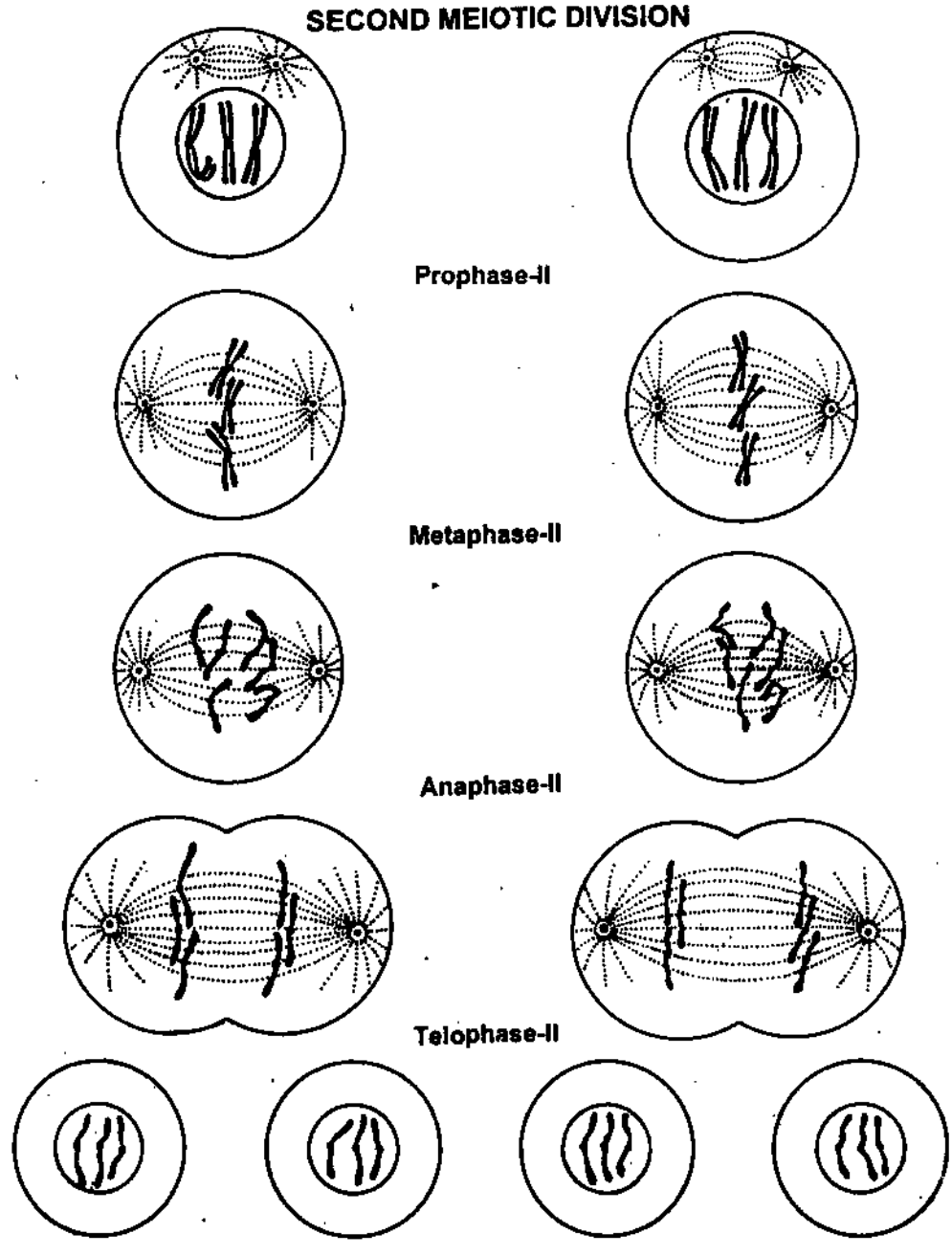


Fig. 15. Cell Division. The second meiotic division.

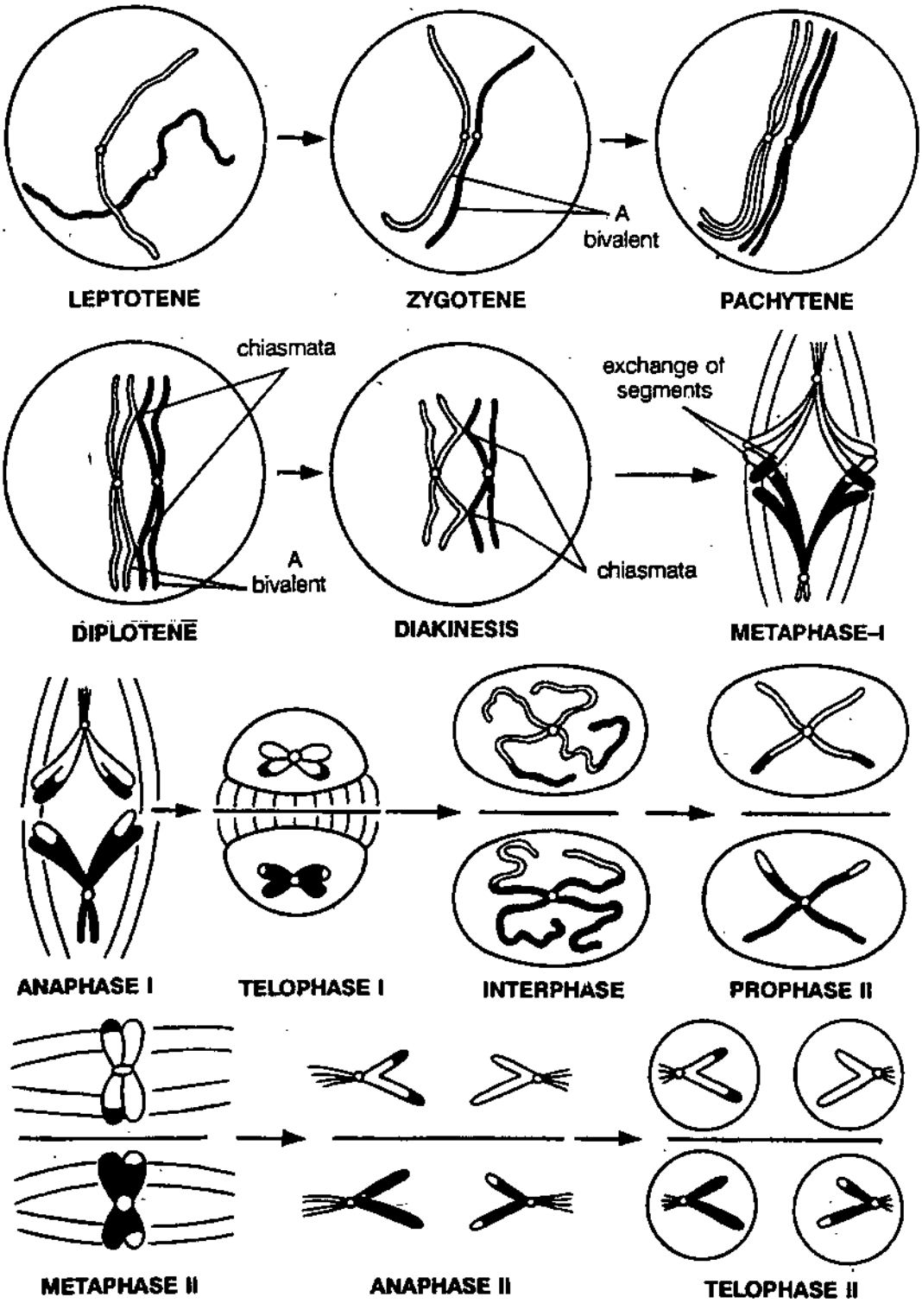


Fig. 16. Cell Division. Diagrammatic meiotic division.

10.11. DIFFERENCE BETWEEN MITOSIS AND MEIOSIS

Mitosis	Meiosis
1. Mitosis takes place in somatic cells of body.	Meiosis takes place in the germinal cells of body.
2. Mitosis takes place as simple division in one cycle. It results in formation of two cells.	Miiosis takes place in two parts, i.e., Meiosis I and Meiosis II. It results in the formation of four cells.
3. DNA replication takes place during interphse before mitotic cycle.	DNA replication takes place during interphase.
4. During interphse G_2 phase is normally present.	During interphase G_2 phase is either very short or absent.
Prophase	
5. The duration of prophase is short, it may last a few hours.	The duration of prophase is comparatively longer, it may take days.
6. Prophase is comparatively simple.	Prophase is complicated and is divided into leptotene, zygotene, pachytene, diplotene and diakinesis.
7. The cell divides only once and the chromosomes also divide only once.	There are two cell divisions but the chromosomes divide only once.
8. There is no synapsis during zygotene.	Synapsis of homologous chromosomes takes place during zygotene.
9. The two chromatids of a chromosome do not exchange segments during prophase.	Chromatids of two homologous chromsomes exchange segments during crossing over.
10. Each chromosome consists of two chromatids united by a centromere.	The two homologous chromosomes form bivalent or tetrads. Each bivalent has four chromatids and two centromeres.
11. The arms of the prophase chromatids are close to one another.	The arms of the chromatids are separated widely in prophase II.
12. Chromosomes are already duplicated at the beginning of prophase.	When prophase I commences the chromosomes appear single, (although DNA replication has taken place in interphase I).
Metaphase	
13. In the metaphasic plate all the centromeres line up in the same plane.	In metaphase I the centromeres are lined up in two planes which are parallel to each other.
14. The metaphasic plate is made up of chromosome pairs.	The metaphasic plate is made of paired chromosome pairs.
Anaphase	
15. Division of the centromeres takes place during anaphase.	There is no centromeric division during anaphase I. Centromeres divide only during anaphase II.
16. The chromosomes separate simultaneously during anaphase.	Short chromsomes separate early, separation of long chromsomes is delayed.
Telophase	
17. Spindle fibres disappear completely in telophase.	Spindle fibres do not disappear completely during telophase I.
18. Nucleoli reappear in telophase.	Nucleoli do not reappear in telophase I.
Significance	
19. The chromosome number remains constant at the end of mitosis.	The chromosomal number is reduced from the diploid to the haploid.
20. The genetic constitution of the daughter cells is identical to that of parent cells.	The genetic constitution of the daughter cells differs from that of the parent cell. the chromosomes of daughter cells usually contain a mixture of maternal and paternal genes.
21. It has no bearing on the occurrence of variation on evolutionary process.	It is responsible for occurrence of variation in nature and continuation of evolutionary process.

• STUDENT ACTIVITY

1. Define meiosis. Describe the heterotypic division in meiosis.

2. Write the differences between mitosis and meiosis.

• SUMMARY

- The term maiosis was coined by **Farmer** and **Moore** (1905). It is a special type of division which occurs in eukaryotic cells at the time of production of reproductive cells. The overall process of meiosis involves two separate cell divisions occurring in sequence. The first meiotic division (heterotypic division) reduces the chromosome number from diploid to haploid, while the second meiotic division (homeotypic division) separates the two paired chromatids that comprise each chromosome. Preceding meiosis there is an interphase just like the one found in mitosis, consisting of G_1 phase, S-phase and G_2 phase. However, in meiosis G_2 phase is very short or almost absent. The heterotypic division is distinguishable into prophase I (comprising leptotene, zygotene, pachyene, diplotene and diakinesis), metaphase I, anaphase I and telophase I. At leptotene, the chromosomes appear as long beaded threads. Longitudinal splitting of chromosomes except centromere takes place at this stage. The zygotene is characterised by pairing of homologus chromosomes (synapsis). A synaptonemal complex appears at this stage between homologous chromosomes. At pachytene, the bivalents (paired chromosomes) appear as tetrads and crossing over takes place. After exchanging chromosomal pieces, the chromatids separate at diplotene. As a result chiasmata appear which may be

intercalary or terminal. At the same time, a process known as terminalization causes the chiasmata to migrate towards the ends of the chromosomes. This may be followed by rotation. At diakinesis nucleolus, nuclear membrane gradually disappears. Homologous pair of chromosomes becomes attached to spindle fibres and migrates to the equational plate (congression). During anaphase I the movement of chromosomes of a bivalent from equatorial plate to the poles takes place. The splitting of centromere does not take place, hence the two sister chromatids without separation reach the same pole. This causes reduction of chromosome number. The nuclear membrane reappears but nucleoli are not formed at telophase I. Then it passes into a period of short interphase or interkinesis. The nuclear membrane again disappears at prophase II. Chromosomes attached by spindle fibres move to equator at metaphase II. The centromere split longitudinally at anaphase II and the two sister chromatids get separated as distinct chromosomes. The chromosomes after splitting move to different poles. The nucleolus and nuclear membrane appear at telophase II and the nucleus passes into interphase condition. Meiosis is necessary because the formation of reproductive cells is accompained by a reduction in chromosome number from a diploid state to the haploid state. Meiosis is also associated with an exchange of genetic information between the two sets of chromosomes, which creates individual chromosomes that have a new combination of parental and maternal genetic information.

• TEST YOURSELF

1. Who coined the term meiosis ?
2. Which is the best plant part to study the meiosis ?
3. What is the characteristic feature of zygotene ?
4. At what stage do the bivalents (paired homologues) appear as tetrads ?
5. At what stage does synaptonemal complex (S.C.) appear between homologous chromosomes?
6. At which stage of meiosis does crossing over take place ?

• ANSWERS

1. Farmer and **Moore** (1905) **2.** Anther
3. Pairing of homologous chromosomes (synapsis)
4. Pachytene **5.** Zygotene **6.** Pachytene

❑❑❑

UNIT

11

DNA : THE GENETIC MATERIAL

STRUCTURE

LEARNING OBJECTIVES

By Studying this chapter you will be able to know the structure, replication, identification and significance of DNA.

11.0. INTRODUCTION

DNA is the genetic material in all eukaryotes, most of the bacteria and viruses. In eukaryotes DNA is mostly found in nucleus and it is also reported in chloroplast and mitochondria.

The DNA is double stranded structure but it is single stranded in $\phi \times 174$. In eukaryotes DNA is combined with proteins to make nucleoprotein structure called **chromosomes**. In prokaryotes DNA is not associated with proteins. The chloroplastic or mitochondrial DNA is more like the one in prokaryotes than that of eukaryotes.

11.1. CHEMICAL STRUCTURE

The DNA is a polymer, the monomeric unit of which is a nucleotide *i.e.*, DNA is **polynucleotide**, the basic unit of which is **nucleotide**. The components of DNA are **bases, sugar and phosphate**. The base and sugar make **nucleoside**. The nucleoside and phosphate make **nucleotide**

Nucleoside = Base + Sugar

Nucleotide = Base + Sugar + phosphate.
(Nucleoside)

BASES

The nitrogenous organic bases in DNA are of four types which can be grouped in two classes:

(i) Pyrimidines

The pyrimidine bases consist of a six membered ring like benzene. It has N at 1 and 3 positions. The pyrimidine bases of DNA are **thymine** and **cytosine**. In RNA **uracil** is present instead of thymine (Fig. 1B).

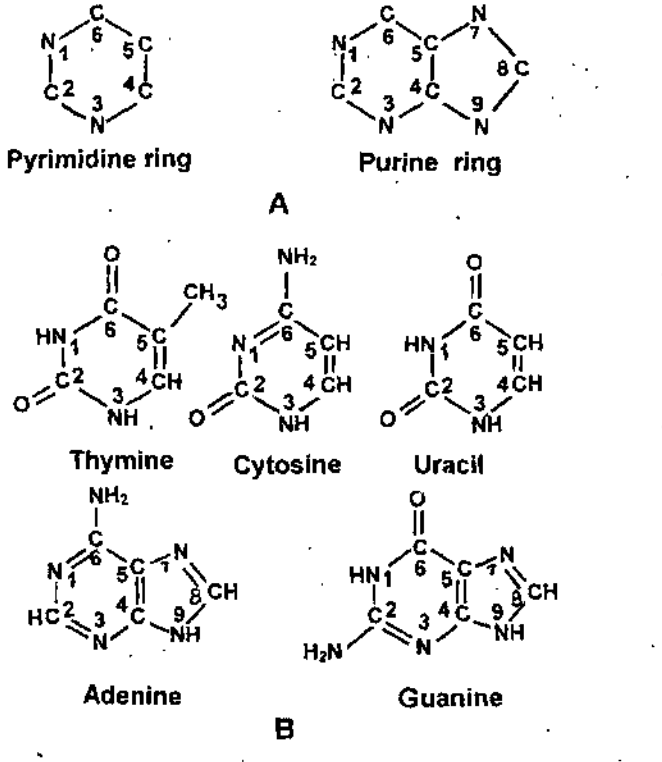


Fig. 1. DNA : (A) Pyrimidine and purine ring (B) Bases of nucleic acids.

(ii) Purines

The purine bases are made of purine rings. The purine ring is made of 6 membered pyrimidine ring attached to 5 membered imidazole ring. The purine bases are similar both in RNA and DNA. These bases are **adenine** and **guanine** (Fig. 1B).

SUGAR

The sugar in DNA and RNA is pentose. The five C pentose ring of sugar ribose, has 4 carbons in the ring and the fifth C is outisde the ring. There are three OH groups in position 1′, 3′ and 5′. Hydrogen atoms are attached to C atoms 1′, 2′, 3′ and 4′. The sugar is **D-ribose** in RNA and **deoxy D-ribose** in DNA. In **deoxyribose** sugar the 2nd C of ribose sugar is without oxygen.

NUCLEOSIDES

In nucleosides the organic bases are linked to pentose sugar. The C_1 of sugar is attached to N at position 3 of pyrimidines, and N at 9 position in purines. The nucleosides of DNA (deoxyribosides) are called **deoxyadenosine, deoxyguanosine** (Fig. 3B), **deoxycytidine** (Fig. 3B) and **deoxythymidine** (Fig. 3B). The nucleosides of RNA are called **adenosine, guanosine, cytidine** and **uridine** (Fig. 3B).

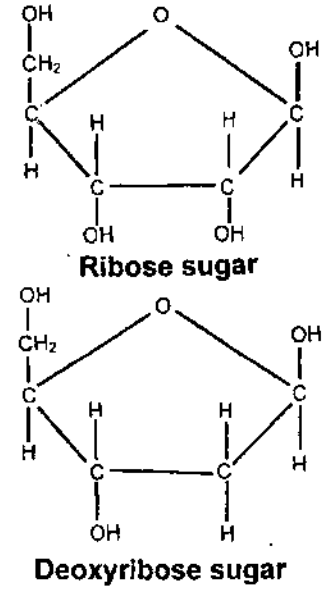


Fig. 2. DNA : Fibose and deoxyribose sugars.

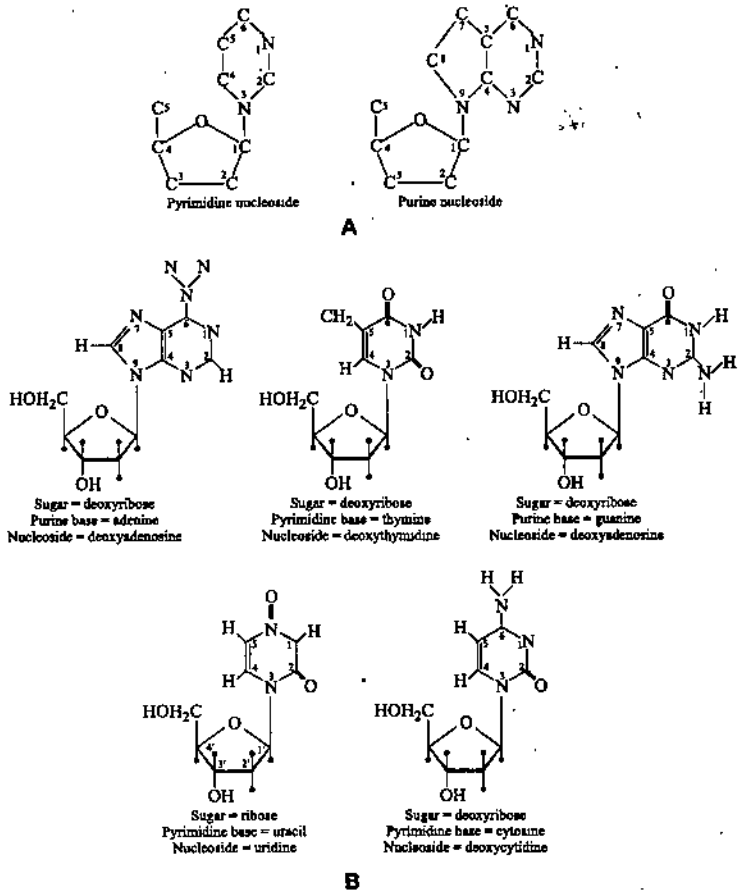


Fig. 3. (A, B). DNA : (A) The nucleosides, (B) Structure of different nucleosides.

NUCLEOTIDES

The nucleotide is formed by attachment of phosphoric acid to a nucleoside. The phosphoric acid is attached to sugar molecule at carbon no. 3 or at C no. 5. These nucleotides are called 3′ p5′ OH nucleotide and 5′ p3′ OH nucleotide respectively.

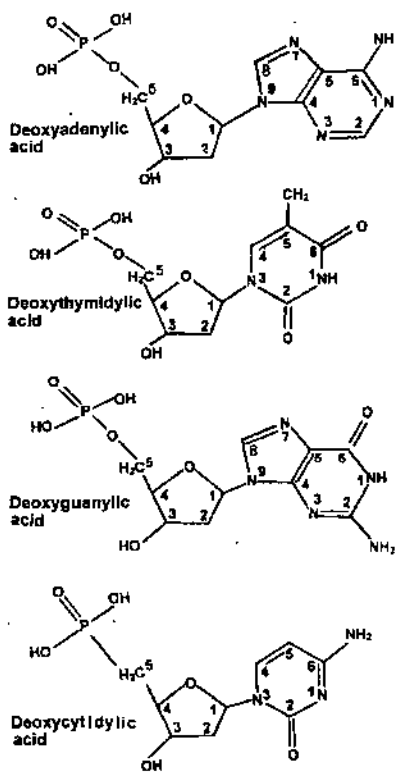


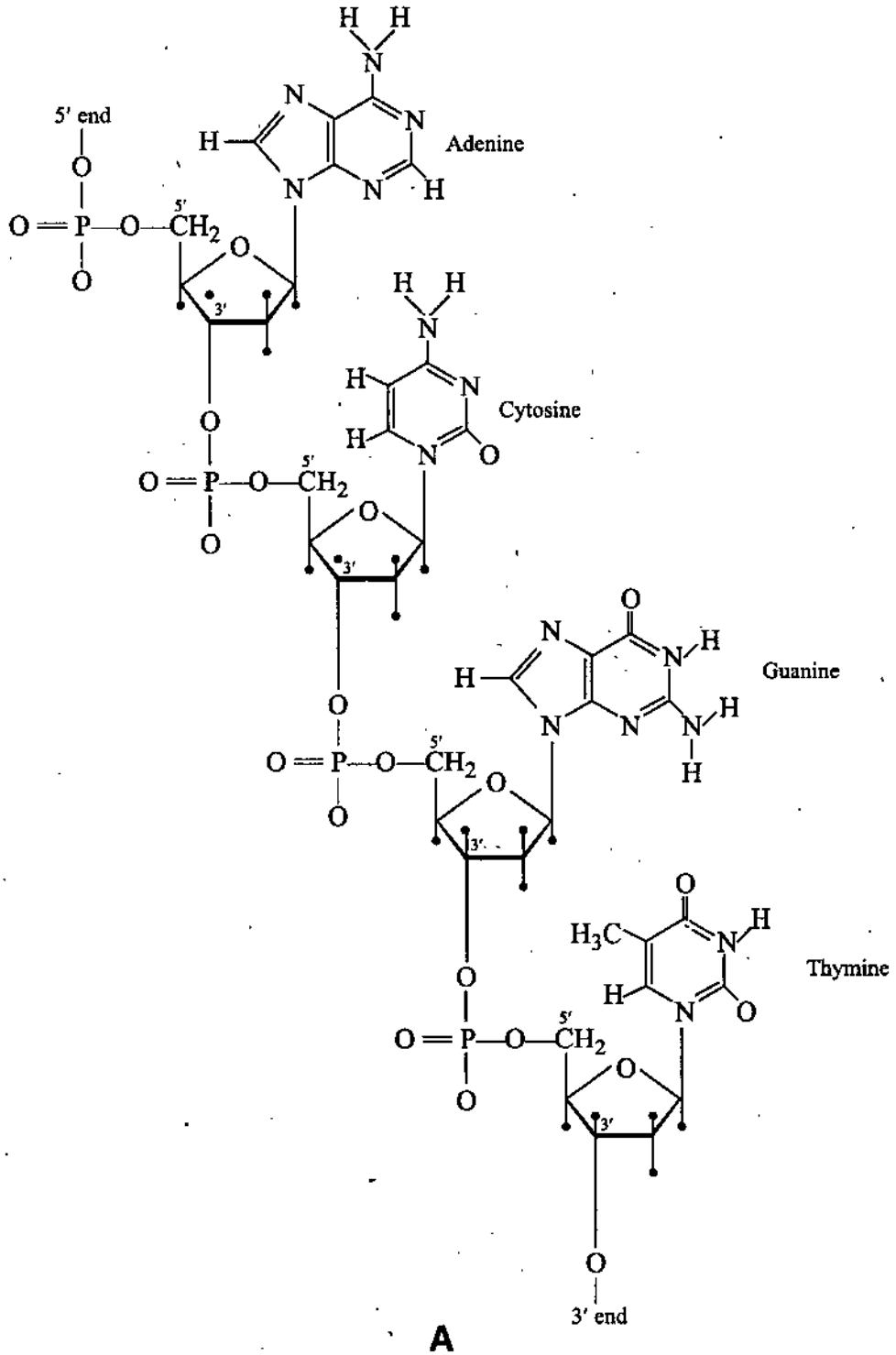
Fig. 4. (A, B). DNA : (A) Nucleotide structure, (B) Structure of Differnt nucleotides found in DNA.

The nucleotides of DNA are called **deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid** and **deoxythymidylic acid.**

The nucleotides of RNA are called **adenylic acid, guanylic acid, cytidylic acid** and **uridylic acid**.

Polynucleotides

The nucleotides are attached to each other by formation of **phophodiester bond**. Many nucleotides unite to make **polynucleotide chain**. In DNA the two polynucleotides chains are present from $C^3 \rightarrow C^5$ and $C^5 \rightarrow C^3$ direction. The deoxyribonucleotides and their ability to form polynucleotides were discovered by **Levene** in 1931.



A

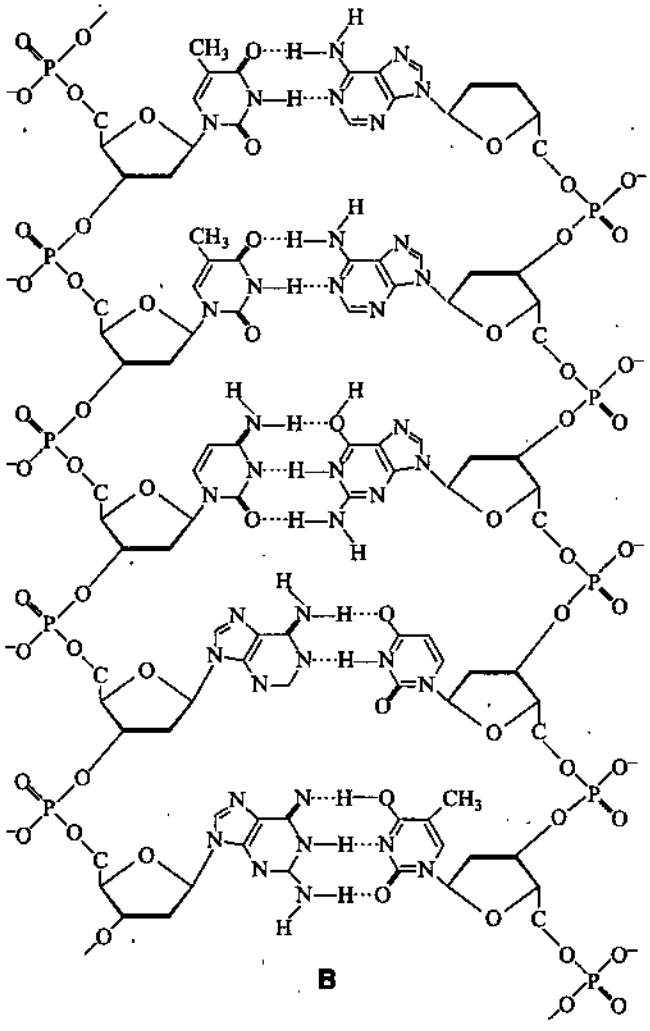


Fig. 5. (A, B). DNA : (A) Polynucleotide Chain, (B) Base Pairing.

BASE PAIRING

The DNA molecule consists of two helically twisted strands connected together by steps of hydrogen bonds.

11·2. DNA DOUBLE HELIX STRUCTURE/ MOLECULAR STRUCTURE OF DNA

Charagaff in 1940 made chemical analysis of DNA and found that :

(1) As a rule the number of pyrimidine bases (C + T) is equal to number of purine bases (A + G).

(2) The number of adenine bases is always equal to number of guanine bases and similarly cytosines are equal to thymine.

$$A = G$$
$$C = T.$$

(3) The ratio of A + T/C + G is rarely 1. It varies from 0·4 – 1·9. The low ratio is generally found in lower plants and animals and high ratio in higher plants and animals.

Wilkins, Franklin and **Astbury** studied purified DNA by X-ray crystallography. They demonstrated DNA to be multistranded with diameter of about 22 Å, with groups at a distance of 3·4 Å in the fibre. On the basis of chemical analysis and X-ray crystallographic studies, **Watson** and **Crick** in 1953 proposed **double helix** model of DNA for which they were awarded Nobel Prize in 1962.

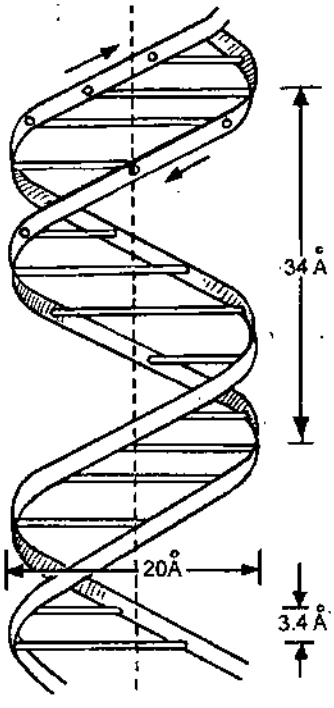


Fig. 6. DNA : he double helix model.

The main features of the model are :

1. DNA helix is made of two polynucleotide strands, which are spirally twisted and the diameter of the helix is 20 Å.

2. The two strands of DNA are antiparallel to each other *i.e.*, the 5′ end of one strand faces 3′ end of the other strand.

3. Each polynucleotide strand is made of many nucleotides attached by phophodiester bonds. The sugars and phophates are attached alternately to make backbone of DNA.

4. The bases are in a plane at right angle to long axis.

5. The two strands are complimentary. The adenine of one strand is always attached to thymine of the other strand. Similarly, cytosine is always attached to guanine.

Hence A and T, C and G are called complementary bases.

6. There are two hydrogen bonds between A = T and three hydrogen bonds between C ≡ G.

7. The DNA double helix is right handed.

The distance of one twist or pitch of helix is 34 Å. Since one twist has 10 base pairs, the distance between two base pairs is 3·4 Å.

11·3. SIGNIFICANCE OF DNA

1. DNA is the genetic material. The characters are transferred from one generation to the other by DNA which makes the genes.

2. The base sequences on DNA make genetic code. This decides the formation of all proteins and enzymes. Hence, DNA controls all the metabolic reactions in organism.

1·4. OTHER FORMS OF DNA

The structure of DNA helix given by **Watson** and **Crick** (1953) is right handed and this form of DNA is called as **B form**. The B form X-ray diffraction pattern is obtained at humidity more *than 66% and in presence of excess of salt. The pitch of helix* is 34 Å with 10 base pairs in one twist. The rise of the helix per base pair is 3·37 Å

A Form DNA (A-DNA)

The A form of DNA is the dehydrated form. It is right handed with pitch 28·15 Å. There are 11 base pairs in one twist. The base pairs are considerably tilted from the axis of the helix.

The tilt of the base pairs is 20·2 Å. Because of this displacement the depth of the deep groove is increased and the depth of shallow groove decreased. The rise of helix per base pair is 2·56 Å.

C Form DNA (C-DNA)

The reduction of hydration of the B form below 66%, with excess of salts results in C-form DNA. The pitch of C form DNA is 31 Å, there are $9\frac{1}{3}$ base pairs per turn. The axis of the helix is located in or near the minor groove. The tilt of the base pair is 7·8 Å. The rise of helix per base pair is 3·32 Å.

D Form DNA (D-DNA)

This DNA has 8 base pairs per turn of helix. The base pairs are displaced backwards relative to the axis of helix. The tilt of the base pair is 16·7 Å. The rise of helix per base pair is 3·03 Å.

Table 1. Characteristics of different forms of DNA

	A form	B form	C form	D from
Abbreviation	A-DNA	B-DNA	C-DNA	D-DNA
Base pairs per turn of the helix	11	10	$9\frac{1}{3}$	8
Axial rise (h)	2.5 A	3.37A	3.32A	3.03A
Tilt of base pairs (γ)	20.2°	6.3°	– 7.8°	– 16.7°
Pitch of the hleix	28.15A	34A	31A	...
Sugar puckering	3′-endo	3′-exo	2′-endo	3′-exo

Z DNA

Rich and coworkers at MIT, USA have studied structure of DNA fragment by atomic resolution. This form of DNA is obtained by synthesizing $(C - G)_3$ molecules. It is also detached in living cells using certain antibodies. This is called as **Z-DNA** or **Z form** because sugar and phosphate backbone follows **zig-zag path.** This DNA has certain similarities and differences with B-DNA. It is double helical but with **left handed coiling.** The two strands are antiparallel with A = T, G ≡ C pairing but repeating units are dinucleotide. The pitch of the helix is 45 Å and there are 12 base pairs per turn. The diameter of the helix is 18 Å.

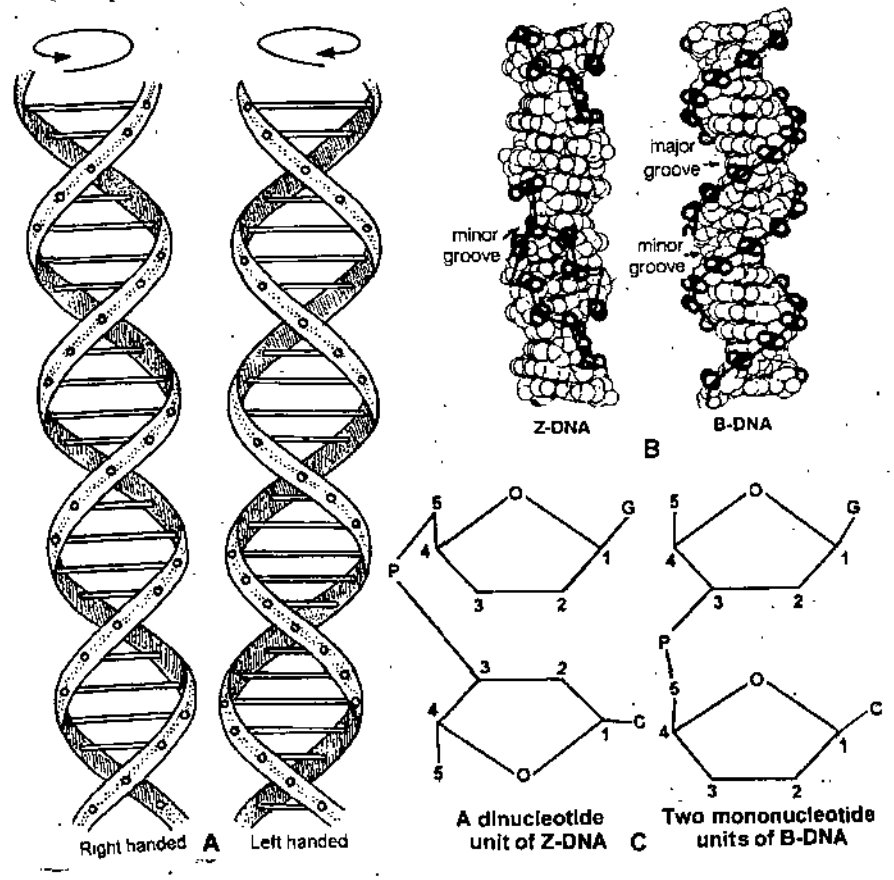


Fig. 7 (A–C). DNA. (A) Helice showing right hand sens & as found in B-DNA and other helic showing left handed sense as found in z-DNA, (B) Side view of z-DNA and B-DNA showing the path of phosphate residues which are quite regular and smooth in B-DNA, (C) Orientation of adjacent sugar residues in Z-DNA and B-DNA.

R. L. Model of DNA

The 'B' form DNA helical structure of DNA as given by **Watson** does not explain unwinding of DNA in easy manner. A new model called **RL helix model** has been independently proposed by **V. Sasisekhran** of Indian Institute of Science Banglore, India and **G.A. Rodley's** group in New Zealand.

According to the model DNA duplex is formed of altenating right and left handed helices arranged side by side. The repeating unit of helix is 10 base pairs. Each DNA strand has 5 base pairs in the right handed helix alternating with 5 base pair in the left handed helix.

Palindromic DNA

The term **palindromic DNA** was first used by **Wilson** and **Thomas** (1974). A **palindrome** is a sentence that reads the same message forwards and backwards *e.g.*, "madam i m adam". The palindromic sequences are present in DNA of several eukaryotes. In palindromic DNA sequence the nucleotides of one strand going in one direction are the same as the nucleotides of the other strand going in the other direction *e.g.*,

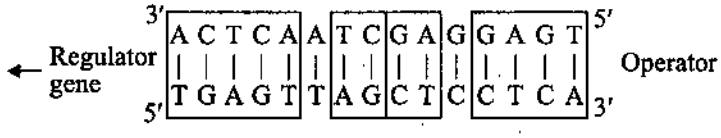
—A—A—T—T—G—C—A—A—T—T—
—T—T—A—A—C—G—T—T—A—A—

Thus DNA contains regions with an **axis of two fold** rotational symmetry which reads the same in both directions. The sequence given above would transcribe the same RNA that RNA polymerase transcribes from either side.

The palindromic regions may be relatively short consisting of 3–10 bases or may be long consisting of hundreds of base pairs. The eukaryotic DNA contains many large palindromic regions consisting of hundred to several thousand base pairs. The prokaryotic DNA usually contains small palindromic sequence *e.g.*, the recognition sites for the *lac* repressor, the CAP-protein and restriction nucleases of bacteria.

The two regions containing the inverted sequences may be very close to each other or may be separated by spacer regions more than a thousand bases long.

The *lac* promoter gene of *E. coli* contains a palindromic sequence as given below in (Fig. 8)



A

Enzyme RI′ from E. coli (EcoRI)′

↓

3′—Y—Y—T—A—R—R—5′

5′—R—R—A—T—Y—Y—3′

Enzyme I from Haemophilus parainfluenzae (Hpa I)

↓

3′—N—C—A—A—T—T—G—N—5′

5′—N—G—T—T—A—A—C—N—3′

B

Fig. 8. (A, B) DNA : (A) Part of the nucleotide sequence of the *lac* promoter gene of *E. coli* showing palindromic sequences. (B) Sequence recognized by restriction enzymes. Cleavage points indicated by arrows R = purine, Y = pyrimidine.

The *lac* operator of *E. coli* is protected from pancreatic deoxyribonuclease digestion by the *lac* repressor protein. The protected region contains 27 base pairs of which 16 are palindromic.

Functions of palindromic DNA. The exact significance of palindromic DNA is not known, although several functions have been suggested. Short palindromes may be functional as **recognition sites** of DNA for proteins which also have a two-fold rotational symmetry, e.g., *lac* repressor protein, CRP protein and many bacterial **restriction enzymes**. Binding of proteins to DNA is due to interaction between specific regions of the DNA and protein. Restriction enzymes of bacteria serve a protective function against foreign DNA by destroying it. These enzymes are specific for palindromic sequences in double stranded DNA, which is cleaved only at few locations (Fig. 8B).

Palindromes also provide **structural strength** to the transcribed RNA by hydrogen bonding in the hairpin loops. When the palindrome sequences are not perfectly symmetrical, imperfect loops may result.

Protein-DNA interation might take place through the cruciform structure, although this view has been challenged. The cruciform structures are also suggested to be involved in **genetic recombination.**

Long palindromic DNA molecular form in some low eukaryotes has been shown to contain genes coding for ribosomal RNA. Extranuclear DNA palindromes may have arisen by the process of copying of rRNA genes during gene amplification.

REPETITIVE AND SATELLITE DNA

It was discovered in 1964 that large part of mouse DNA consisted of multiple copies of same sequence of base pairs. This DNA was called as **repetitive DNA**. In higher organisms the repetitive DNA can be 5 percent to 90 percent of the total DNA. The repetitive DNA generally does not carry any genetic information and therefore dose not form genes but this plays structural or regulatory roles.

When DNA is subjected to heat, the two strands of DNA separate *i.e.*, DNA is denatured. If the solution of single stranded DNA thus obtained is cooled slowly the two strands reassociate to form active double helical DNA *i.e.*, DNA is renatured.

The bacterial DNA with small genome size is renatured easily but most vertebrate DNAs do not reassociate easily. This happens because the degraded DNA molecules form heterogenous populations and the vertebrate DNA has large molecular weight. **Britten** (1968) demonstrated that

vertebrate DNAs reassociate easily if these are broken into small pieces. This is evidence to the hypothesis that certain short sequences of bases are repeated hundreds of times in DNA and this DNA is called as **repetitive DNA**.

Table 2. Satellite DNAs of *Drosophila virilis* (Gall *et. al.*; 1947).

Repetitive DNA	Bouyant density	Repeat sequence
Satellite DNA I	1.692	5′—ACAAACT—3′ 3′—TGTTTGA—5′
Satellite DNA II	1.688	5′—ATAAACT—3′ 3′—TATTTGA—5′
Satellite DNA III	1.671	5′—ACAAATT—3′ 3′—TGTTTAA—5′

When prepared for ultracentrifugation, DNA is fragmented into smaller pieces. Some of these pieces will contain mostly repetitive DNA. If the base composition of repetitive DNA differs appreciably from the average base composition of the rest of the DNA, it will form a **satellite band** in the ultracentrifuge tube distinct from main DNA band. Thus, repetitive DNA can also be identified as **satellite DNA.**

In *Drosophila melanogaster* major part of DNA forms a band in the ultracentrifuge tube at a position corresponding to 42 percent G+C. It has satellite DNA which is very much similar to that in mouse in the following respects :

(i) it involves about the same proportion of the DNA *i.e.*, about 10%

(ii) it has the same base ratio *i.e.*, about 32% G+C and more of A+T.

(iii) it is composed of many copies of a few sequences.

(iv) it is located near the centromere of all chromosomes.

In *Drosophila* about 25% of the DNA is repetitive type. In *Drosophila virilis*, in addition to main DNA there are three other highly repetitive DNAs, making up 25 percent, 8 percent and 8 percent of the nuclear DNA. (Table 2). Certain crabs contain a satellite DNA, "natural poly (dA + dT)" which comprises about 30 percent of the total nuclear DNA. In mouse 10% of the DNA is highly repetitive and is half reassociated in a few second, about 20% is moderately repetitive and reassociates at an intermediate rate. Mouse DNA is composed of 2×10^7 copies of a sequence 8 to 13 base pairs long, one of its compliments, contains a repeat sequence of 5′ TTTTTC 3′. All eukaryotes except perhaps yeast contain repetitive DNA.

11.5. SINGLE STRANDED DNA :

The DNA of most of the organisms is double stranded helix. The single stranded DNA is present in the bacteriophage virus $\phi \times 174$ and in some other bacterial viruses (Fig. 9). The single stranded DNA from $\phi \times 174$ was isolated by **Sinsheimer** in 1959. The single stranded DNA differs from doube stranded DNA in following respects :

(i) UV absorption of double stranded DNA remains constant from 0–80°C and then rises rapidly while UV absorption of single stranded DNA increases steadly from 20°C to 90°C.

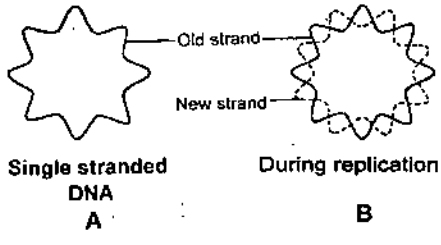


Fig. 9. (A, B). DNA : (A) Single stranded DNA, (B) During replication.

(ii) The double stranded DNA is resistant to the action of formaldehyde and the single stranded DNA is non-resistant as the reactive sites are exposed.

(iii) In double stranded DNA the ratio of A : T and G : C is 1 : 1 and in single stranded DNA of $\phi \times 174$ the ratio of A : T : G : C is 1 : 1·33 : 0·98 : 0·75.

(iv) The double stranded DNA is linear while single stranded DNA is circular.

(v) During replication the single strand makes the complimentary strand and becomes double stranded before separation.

11.6. DNA REPLICATION

All cells of a living organism possess similar quantities and quality of DNA because exact replica of the hereditary material DNA is transmitted to progeny of cells. DNA is capable of making exact replica of itself in a very organised and orderly fashion, this process is called as **DNA replication.**

In eukaryotes DNA is found in chromosomes, when the chromosomes double during interphase of mitosis, it can be considered the period of DNA replication. When chromosomes divide during cell division the daughter chromosomes have identical DNA helix. Since in a living organism all cells are derived from zygote by mitosis, all resultant cells have identical DNA and consequently identical genes. The synthesis of DNA on DNA template is **DNA directed DNA** synthesis. In viruses where RNA is the genetic material, **RNA directed DNA synthesis** can take place by process of **reverse transcription.**

Delbruck suggested that Watson and Crick model for DNA can be theoretically duplicated in three manners *i.e.*, conservative, semi–conservative and dispersive manner. Of these alternatives the semiconservative model was also suggested by **Watson** and **Crick.**

(i) According to the conservative method the old double stranded molecule is conserved as such and a new copy is synthesized from the parent old molecule. Hence the old parent helix remains unchanged (Fig. 10A).

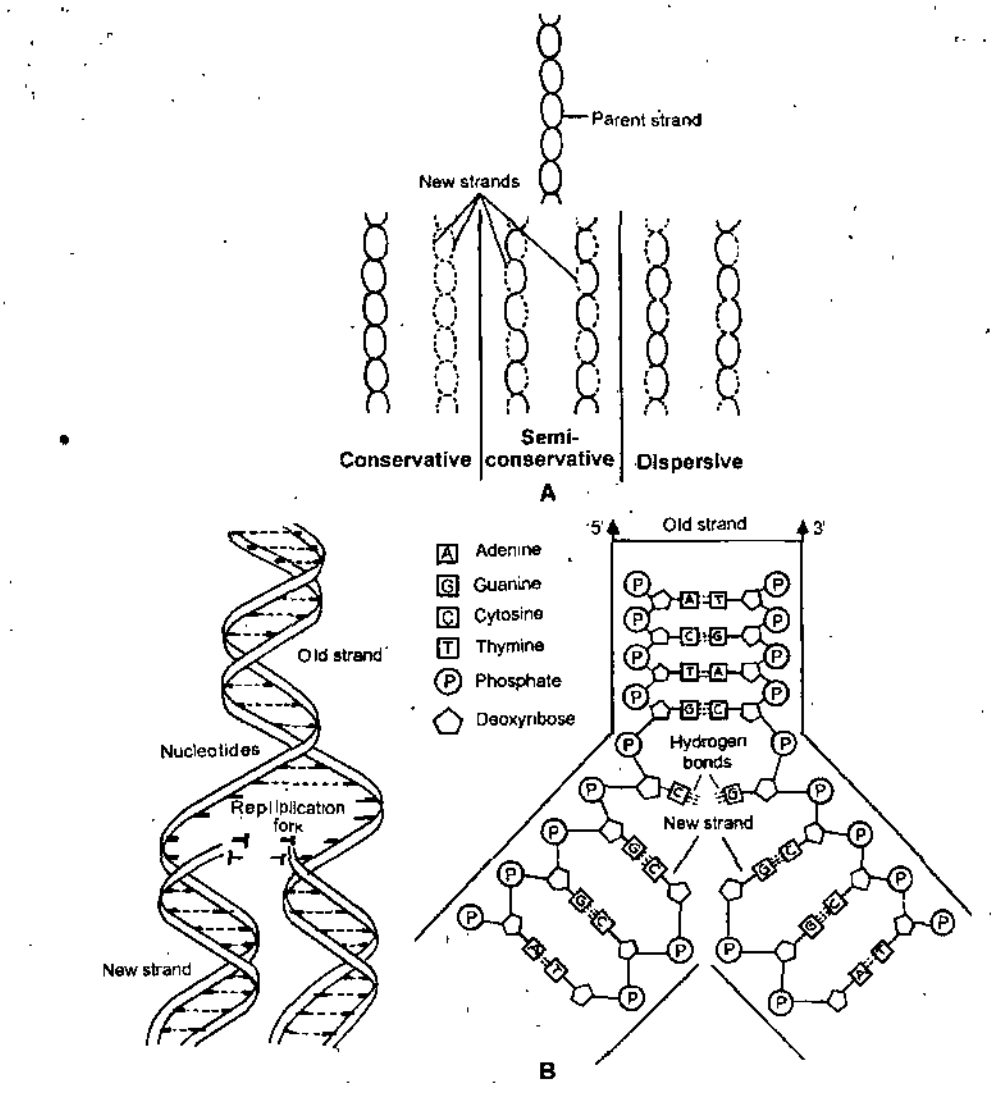


Fig. 10 (A, B). DNA : Modes of replication. Three theoretically possible modes of replication (A) Conservative, semi–conservative and dispersive, (B) Replication of DNA.

(ii) According to the dispersive method of replication the parent DNA double helix disintegrates into many fragments, each fragment replicates. The fragments reunite to make two double helices. The two double helices have patchwork of old and new pieces (Fig. 10 A).

(iii) According to semi-conservative method proposed by **Watson** and **Crick** the two strands separate from each other, synthesize their complementary strand and form two helices of DNA. Both of new helices have one parental strand and one newly synthesized strand. Thus during replication one parental strand is conserved in each helix (Fig. 10 B).

Experimental Evidences for Semi-conservative DNA Replication

There are sufficient experimental evidences to prove that DNA replicates by semi-conservative method. These are :

(1) Meselson and Stahl's experiment : M. Meselson and **F.W. Stahl** (1958) successfully demonstrated the semiconservative **nature** of DNA replication.

Meselson and **Stahl** (1958) allowed *Escherichia coli* to grow on medium containing N^{15}, the heavy isotope of Nitrogen for 14 generations. As a result all N^{14} DNA of *E. coli* was replaced by N^{15}. This DNA was also subjected to a cesium chloride density gradient. On centrifugation the substance finds place on matching density level on cesium chloride gradient. The N^{15} DNA band indicated higher density of DNA.

These N^{15} labelled *E. coli* were suddenly transferred to normal N^{14} nitrogen containing medium. After first generation the DNA was extracted and found to be hybrid of N^{14} and N^{15}. This first generation hybrid DNA was homogeneous and made band at density between N^{15} and N^{14} DNA. This clearly indicated that replication is semi-conservative.

In each DNA one parental N^{15} strand was conserved and the other N^{14} strand was synthesized from the medium. After the second generation two kinds of DNA were formed, the normal N^{14} type and the hybrid N^{14} and N^{15} type. These two bands were formed on N^{14} and $N^{14} + N^{15}$ region in density gradient. After third generation 3/4 of DNA was N^{14} type and 1/4th was hybrid $N^{14} + N^{15}$ type.

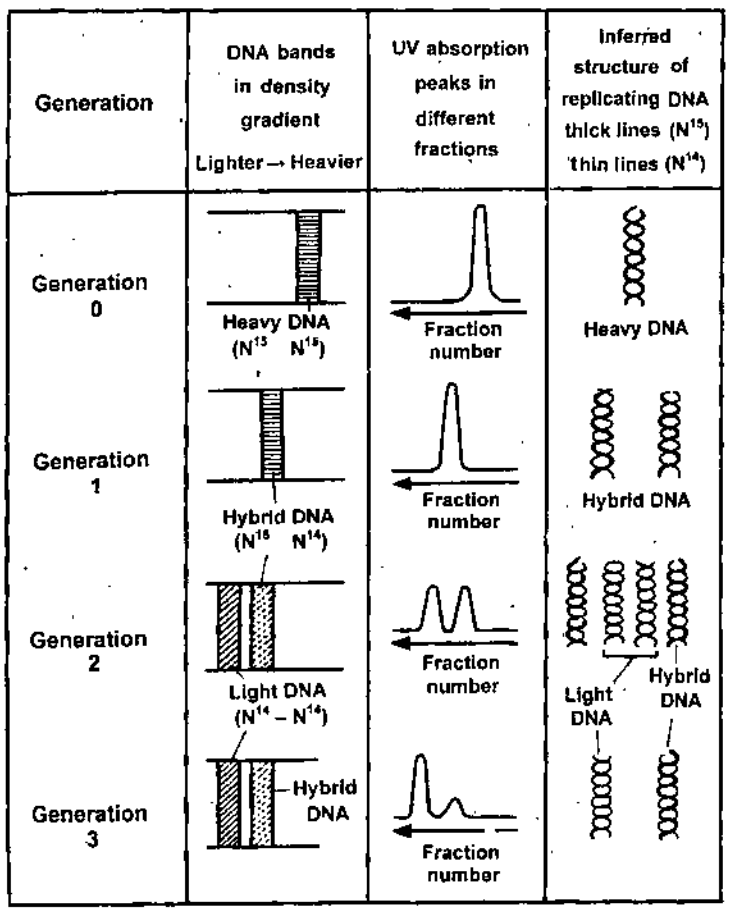


Fig. 11. DNA : Experiment of Meselson and Stahe showing semi-conservative replication.

This result on cesium chloride gradient clearly showed that the DNA replication is semi–conservative.

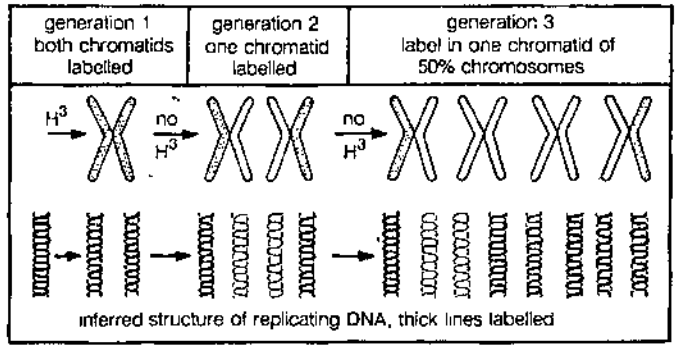


Fig. 12. DNA : Taylor's experiment on *Vicia faba* root tips using autoradiography technique.

(2) Taylor's Experiment : J. Herbert Taylor (1957) and his associates performed autoradiography experiment on root tips of *Vicia faba*. They tracked the synthesis of DNA with the help of tritiated thymidine in duplicating chromosomes in root tip cells. The plant roots were kept submerged in a solution containing the H^3 thymidine. The uptake of the labelled base the tritiated thymidine by successive generations of root cell was demonstrated by the technique of autoradiography.

Taylor autoradiographed root cell preparations :

(i) before treatment with hot or labelled thymidine

(ii) after one round of cell division in thymidine, and

(iii) after a second round of division in cold or unlabelled thymidine.

The first set of observation was controlled and did not show the presence of H^3 in chromosome. One round of replication in labelled thymidine clearly demonstrated that both daughter chromosomes or chromatids were equally labelled. (**Taylor** demonstrated that each chromatid is a bipartite structure, each half of which acts as a template during replication and consequently the replication of DNA is semi-conservative).

After first round of multiplication in normal thymidine only one of the chromatids is labelled and the other remains unlabelled. When these chromatids only half of which are labelled, undergo a second replication in normal thymidine medium, one daughter cell will receive totally unlabelled DNA while the other will receive one of the labellled DNA's After each cell division the original labelled strand will be seen in progressively less number of cells.

Though **Taylor's** experiment was the first quantitative demonstration of possible semi-conservative manner of DNA replication, there is great disparity in DNA and chromosome multiplication. Taylor's experiment demonstrated the semi–conservative mode of replication of chomosome in higher plants, this may not be parallel to semi–conservative replication of DNA. As the size of DNA molecules and the macroscopic chromosomes are different and the organization of DNA in chromosomes is much more complex than considered by **Taylor.**

(3) Cairn's Experiment : J. Cairns (1963) used autoradiography technique to demonstrate the semi–conservative mechanism of DNA replication. The *E. coli* cells where treated with radioactive tritiated thymidine. Tritiated thymidine contains heavy isotope of hydrogen H_3. The thymidine selectively labels only DNA and not RNA. The tritiated thymidine replaces nromal thymidine in DNA. The *E. coli* cells were broken down to release bacterial chromosomes on slides. These slides when covered with photographic emulsion and stored in dark get exposed due to particles emitted by tritiated thymidine. The exposed slides can be photographed to show the presence of labelled DNA. The θ shape figures obtained during replication cycle show that when labelled DNA is supplied with thymidine the density of particles is low in freshly replicating DNA. The particle density is higher in the labelled part when lighter DNA synthesizes new strand on supply of tritiated thymidine. The autographic study of DNA strands in cell cycles demonstrate that the replication of DNA is semi-conservative.

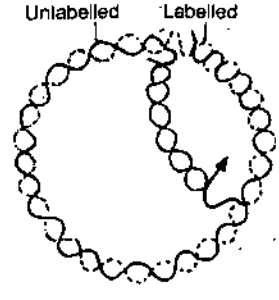


Fig. 13. DNA. Cairns autoradiography experiment.

11·7. MECHANISM OF DNA REPLICATION

The **Watson** and **Crick** model for DNA can be theoretically replicated in conservative, semi-conservative and random manner. It has been proved with evidences that the mode of replication is **semi-conservative**.

During semi-conservative replication the two strands of DNA separate, each strand serves as a template and synthesizes a complimentary strand and as a result two double stranded molecules identical to parental DNA are formed.

The process of replication requires the products of following genes :

Gene	Functions
dna A	initiation of DNA synthesis
dna B	continuation of DNA synthesis
dna C–D	initiation of DNA synthesis
dna F	continuation of DNA synthesis
dna G	primary (DNA polymerase)
dna E	polymerase III (DNA polymerase)
dna Z	polymerase III (DNA polymerase)
Pol A	polymerase I
Pol B	polymerase II

Before a cycle of DNA replication begins the *E. coli* chromosome attaches to an infolding of the cell membrane called the mesosome. It has been suggested that the replication enzyme complex *e.g.*, Pol II and Pol III are associated with cell membrane at the point of attachment.

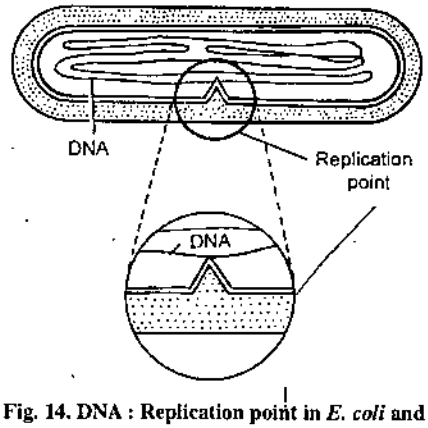


Fig. 14. DNA : Replication point in *E. coli* and enlargment of replication point.

The process of replication takes place in the following stages :

(1) Replication origin — The replication starts at a specific point in the circular chromosome called as **replication origin gene** or **origin**. In *E. coli* the origin of replication is at about 82·5 minutes on the chromosome map which is divided in 100 minutes (Fig. 15).

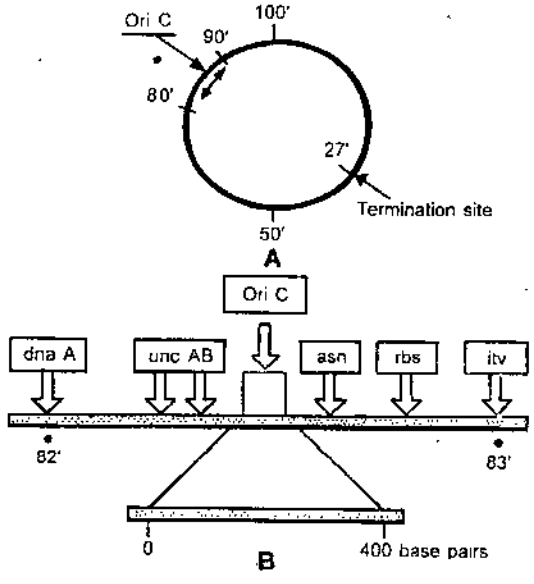


Fig. 15. DNA : The origin of replication in *E. coli*.

(2) Incision : The DNA of chromosomes has many replicating units or **replicons.** In the replication an **endonuclease** called **incision enzyme** or **swivelase** nicks one of the strands so that duplex can untwist its spherical turns as well as helical turns.

(3) Unwinding of DNA helix : The **unwinding** of DNA double helix or **denaturation** is assisted by a protein called **unwinding protein** or **DNA binding protein.** This protein preferentially

binds to single strand of DNA at a point of replication, this protein prevents the reunion of parental strands.

The region of double helix where inciusion and unwinding take place makes Y shaped **replication fork**. There are about 200 molecules of unwinding protein attached to replication fork and the binding of proteins to DNA is co-operative. The binding of each moelcule of proteins enhances the binding of next molecule.

The DNA unwinding protein is a tetramer, the molecular weight of each subunit being 18500–22000. Each protein molecule has a binding site of about 10 nucleotides. Thus 200 molecules of proteins can separate about $200 \times 10 = 2000$ base pairs in a replication fork. But 2000 base pairs do not open at the same time. DNA polymerases II and III do not continue synthesis when DNA template strand is longer than 50 nucleotides. The addition of unwinding proteins overcomes the inhibition of synthesis in case of polymerase II but not in polymerase III. The addition of protein factors I and II can overcome inhibition of synthesis in case of polymerase III.

Superhelix Relaxing Proteins

As the replication fork moves down, the untwisting in circular DNA imposes a strain on the rest of DNA. The strain can be relieved to some extent by super twisting in the unreplicated part of DNA.

The super twisting has to be removed for continuation of synthesis. The strain imposed by unwinding is relieved by action of the **superhelix relaxing** proteins. These proteins are enzymes *that belong to* ***topoisomerases.*** *This topoisomerase enzyme is known by names* **swivelase, relaxing enzyme, nick closing enzyme** and **omega protein** (ω). This protein has **nicking-closing enzyme activity.**

In DNA replication topoisomerase relaxes the DNA by removing the progressively generated supercoils. It cuts one of the strands of DNA and joins the cut ends again after one free end has swivelled around the uncut strand and relaxed the DNA.

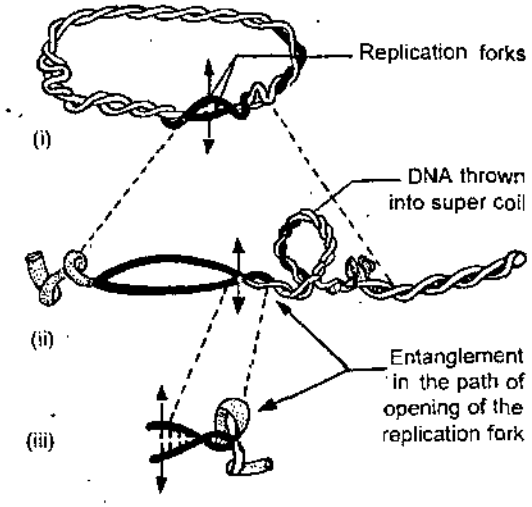


Fig. 16. DNA : Supercolling of DNA hinders the opening of the replication fork.

(4) Template DNA : The two strands of double stranded DNA after unwinding become separate and each strand functions as a **DNA template.** The template DNA is the master copy on which many copies of complimentary copies of DNA are synthesized. *In vitro* synthesis of DNA can take place without template DNA but there is a time lag of some hours before the synthesis begins. The synthesis of DNA begins immediately when DNA template is present. Single stranded DNA *e.g.*, of bacteriophage $\phi \times 174$ directly starts synthesis as it does not require unwinding. In double stranded DNA unwinding is required prior to DNA synthesis.

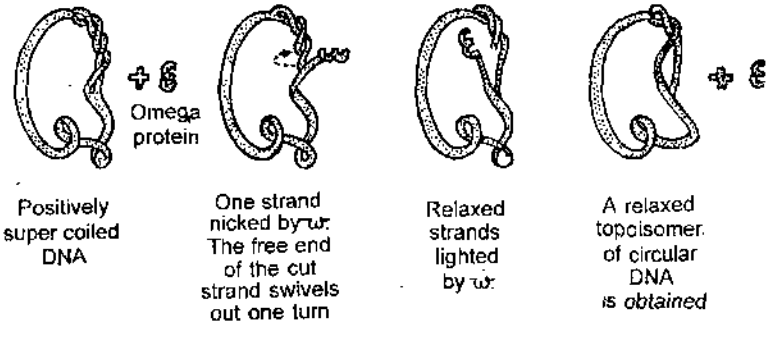


Fig. 17. DNA : Action of topoisomerase.

(5) Formation of RNA Primer : It was demonstrated in the laboratories of **Kornberg** and **Wickner** that initiation of DNA synthesis requires an **RNA primer** and DNA polymerase cannot

initiate DNA chains *de novo*. Newly synthesized DNA was seen to have a short stretch of RNA attached to its one end.

When unwinding occurs at **origin** a **DNA dependent RNA polymerase** inserts itself and binds to single stranded DNA. This enzyme called as **primase** synthesizes a short segment of RNA in 5′–3′ direction. This short RNA is called the **primer** as it "primes" the start of new DNA strand.

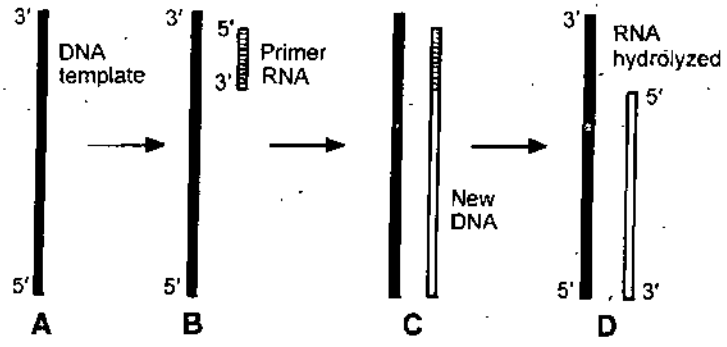


Fig. 18. DNA : Initiation of DNA synthesis (A) DNA Template, (B) Primer RNA synthesiszed on DNA Templete, (C) New DNA synthesized on primers, (D) RNA hydrolyszed.

The RNA primer provides a free 3′ OH end for the start of DNA synthesis. The formation of RNA primer also helps in "edit" of synthesis process, it makes possible to remove a wrong terminal base in the primer and DNA pol can start correct synthesis (Fig. 18 A–D). It has been suggested that laying down of first few nucleotides during DNA, synthesis is more subject to errors than addition of further nucleotides in chain. The formation of RNA primer prior to DNA segment which is removed by polymerase I later thus helps in elimination of any error.

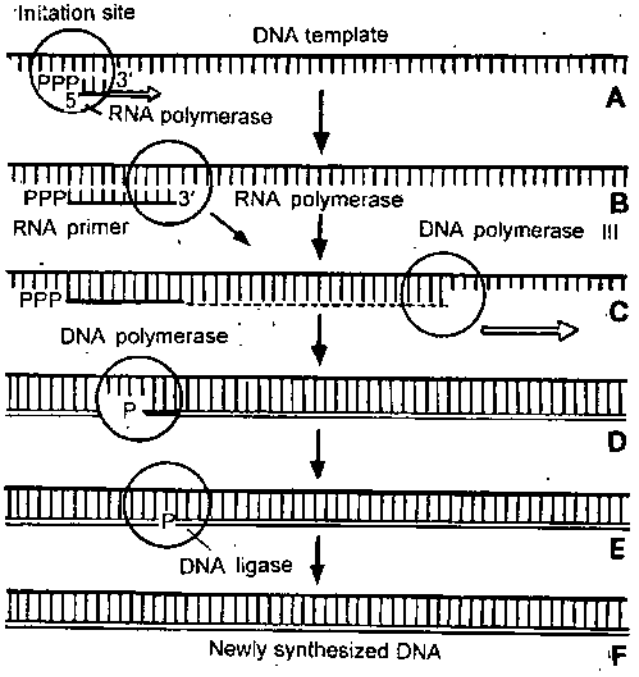


Fig. 19. (A–F). DNA : The role of DNA polymersase in DNA replication.

(6) Chain Elongation : The synthesis of new DNA strand takes place by addition of DNA nucleotide to the 3′–OH group of the last ribonucleotide of the RNA primer. The DNA synthesis takes place in the 5′–3′ direction and is catalized by the enzyme **DNA polymerase III.** A phosphodiester bond links the last nucleotide of RNA with the incoming nucleotide of DNA and similarly many nucleotides of DNA are linked to the chain (Fig. 20). The DNA polymerase III can add thousands of nucleotides without detaching from DNA template.

Sve Wickner has suggested that three protein factors EF I, EF III and protein product of the gene DNA Z are required for binding of Pol III to the primed DNA template. This mechanims takes place as follows : (Fig. 20) the DNA Z protein, and DNA EF III form complex, the complex

interacts with DNA EF I in presence of ATP, binding the EF I to the DNA template at the site of the free end of the primer. DNA Pol III then attaches to the site of DNA primer EP I complex and starts synthesis of DNA strand.

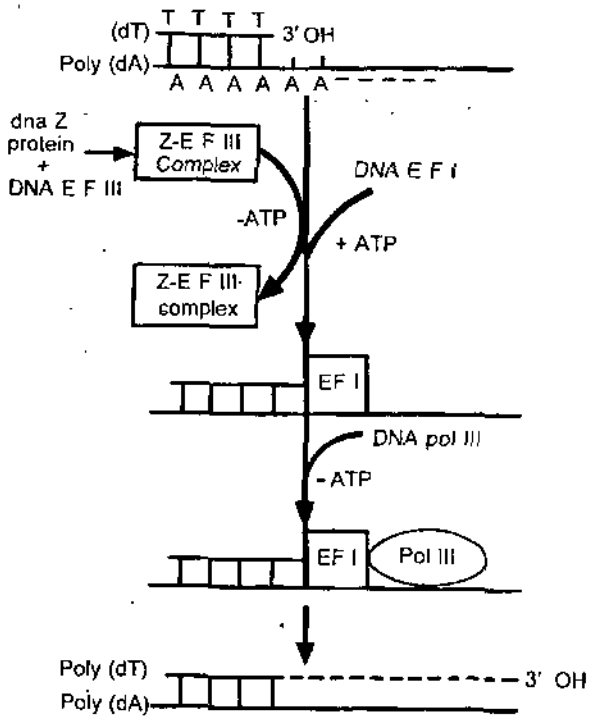


Fig. 20. DNA Structure. A scheme for the steps in chain elongation.

11·8. OKAZAKI FRAGMENTS

The DNA synthesis is brought about by the movement of the replication fork. The replication of the two parallel strands takes place simultaneously. But the DNA polymerase can function only in one direction *i.e.*, 5′–3′ direction of the new strand. As the complimentary strands of DNA are antiparallel the question arises "how can DNA polymerase learn about simultaneous replication of the two strands ? *i.e.*, How can enzyme move forward on one DNA strand and backward on the other" ?

This problem was solved when **Reiji Okazaki** (1968) observed that early in DNA replication smaller DNA fragments were formed and at later periods progressively larger DNA were formed. He suggested that on one strand *i.e.*, of 3–5′ direction the synthesis is continuous and on the complimentary 5′–3′ strand the synthesis is discontinuous. The small fragments formed on discontinuous strand are called **Okazaki fragments**. It takes slightly longer time for the strand with **Okazaki** fragments to complete synthesis, hence it is called **lagging strand** and the continuous strand is called **leading strand**.

It is also suggested that there are two molecules of DNA Pol III in the replication fork and both face the same way. One molecule catalyses the synthesis on continuous strand and the other catalyses on the lagging strand. **Okazaki** also suggested that both strands may replicate discontinuously forming short segments.

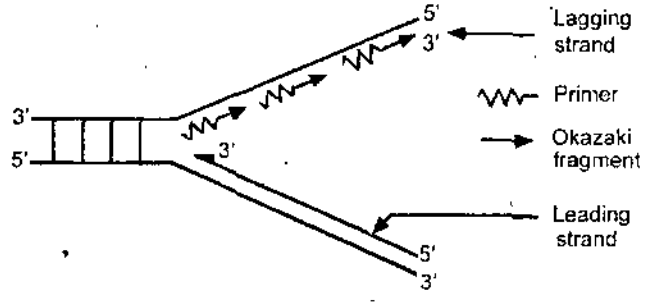


Fig. 21. DNA : Okazaki's scheme for discontinuous chain elongation.

If both strands are synthesized discontinuously, the fragments formed in the same direction of the movement of the fork would be larger than the segments on the opposite side.

All Okazaki fragments are initiated with RNA primers. This RNA is later degraded by the 5′–3′ exonuclease activity of **DNA polymerase I.** The gap is filled in by new nucleotides DNA synthesized by the catalytic activity of DNA polymerase I. The Okazaki fragments are later joined by the polynucleotide **ligase enzyme** to make continuous strand of DNA. The process of formation of continuous strand from small Okazaki fragments is called **maturation.**

Chain Termination : The DNA synthesis is automatically terminated when the replication forks reach the ends in linear molecule. In circular molecule it terminates when the two forks meet at a point 180° from the origin. The presence of a definite termination site has been demonstrated in *E. coli.*

Replication Direction

The replication of DNA may be **unidirectional** or **bidirectional.** The replication in one direction with reference to point of origin is called unidirectional and in both directions it is called bidirectional.

In unidirectional replication, the replication fork appears to be Y shaped (Fig. 22). The unidirectional replication has been reported in *E. coli* bacteriophages P_2 and 186 and in mitochondrial DNA.

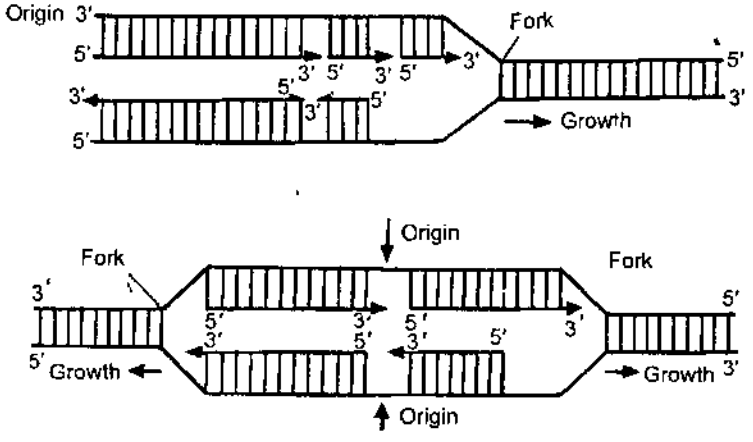


Fig. 22. DNA : Unidirectional and bidirectional replication.

In bidirectional replication the replication fork appears to be 'bubble' or 'eye' shaped (Fig. 22). The advantage of bidirectional replication is that origin is completely duplicated before the replication is complete and this permits control of replication at the site of origin. The bidiectional replication is more common and has been reported in *Bacillus subtilis, Salmonella typhomurium, E. coli, E. coli* phages and in mammalian cells.

Post Termination Events — The post termination events take place after the termination of chain elongation. These events are different in the case of circular and linear DNA. In circular DNA, the two rings obtained after one strand of replication remain interlocked with each other. These are separated by the opening of one of the rings by enzymatic cleavage. The nicking occurs at a site rich in complimentary, redundant base pairs, so that, until the cut ends are rejoined, the complementary base pairs would keep the cut ends annealed. This method prevents the conformational change in chromosomes.

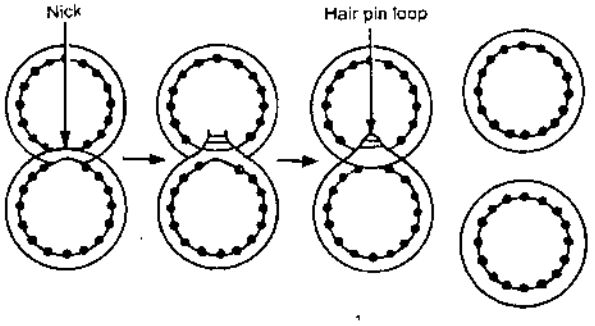


Fig. 23. DNA structure. Post termination events in a circular DNA molecule.

According to **Cavalier Smith**, the palindromic end of the extended strand folds back into a hair pin loop and is ligased to the 5′ end of the shorter complementary strand.

11.9. IDENTIFICATION OF GENETIC MATERIAL

Mendel discovered that characters are transferred from one generation to the other by **particulate factors.** These factors were later given name **genes. Sutton** made observation that genes are located on the chromosomes. The chromosomes consist of nucleic acid DNA and proteins. Hence, the genetic material or genes must be made either of proteins or nucleic acid.

PROPERTIES OF GENETIC MATERIAL

Whatever may be the chemical nature of genetic material, proteins or nucleic acids, the genetic material must have these properties:

1. This should replicate or multiply accurately, and this should be distributed uniformly in all daugther cells and from generation to generation.

2. This should be susceptible to occasional changes or errors in low frequency due to mutation, which increase genetic variation in population.

3. The genes should be able to express themselves by having control on development process of character they govern.

Experimental Evidences for Nature of Genetic Material

The proteins are highly specific, synthesized in living organisms, occasional changes take place in their structure. The proteins control many metabolic reactions as enzymes. These properties of proteins make a possible suggestion that the proteins could be the genetic material.

The following experiments prove that **nucleic acids DNA or RNA make the genetic material and not the proteins.** These experiments are :

(i) Transformation experiment

(ii) Bacteriophage infection experiment

(iii) Experiment with TMV

(i) Transformation experiment. The transformation experiment was conducted by **Frederick Griffith** (1928). He used bacteria *Diplococcus pneumoniae* to cause **pneumonia**

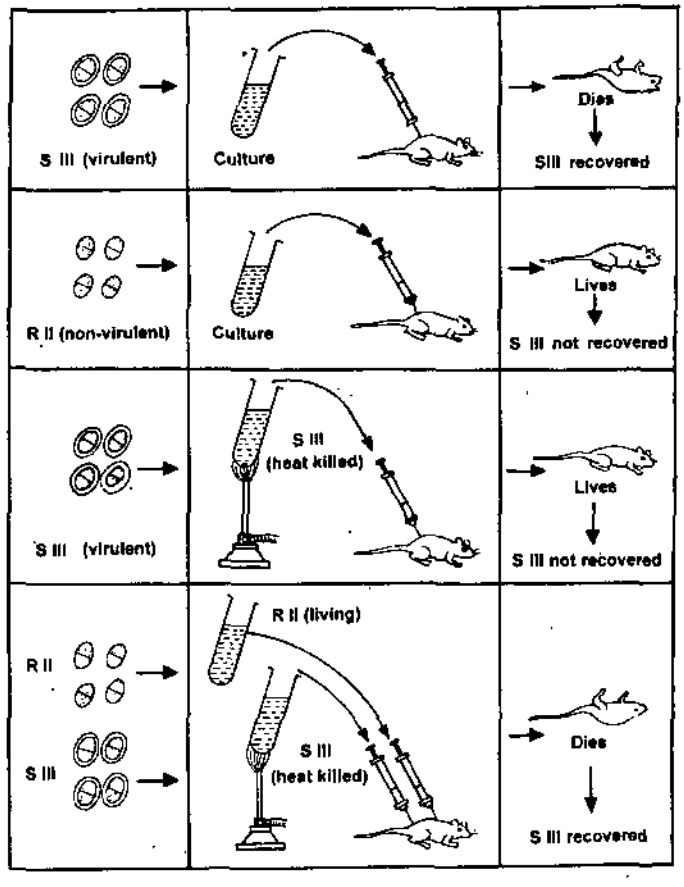


Fig. 24. DNA : Griffith's experiment.

infection in mice. *Diplococcus* has two strains **S III** and **R II.** The S III strain has polysaccharide capsule outside, it forms smooth colonies in culture, S III causes pneumonia in mice and is called **virulent.** R II strain is without capsule, forms rough colony in culture. R II does not cause pneumonia and is called **avirulent** type. When S III heat killed is injected into mice it does not cause pneumonia, and S III could not be recovered from dead mice : But when S III heat killed was given with R II the mice die due to pneumonia, S III and R II could be recovered from dead mice. **Griffith** concluded that something from the heat killed S III transformed the R II strain to S III and this phenomenon was called **transformation.** The substance from S III which changes R II was called **transforming principle.**

O. T. Avery, C. M. MacLeod and **M. McCarthy** in 1944 repeated the Griffith experiment. They found that the polysaccharide capsule in S III strain is responsible for virulence. To identify the **transforming principle** they mixed R II strains with DNA extract from S III. When DNA extract of S III was treated with RNAse and proteinase, it had the capacity to change R II. And when DNA extract was treated with DNAse, it lost the capacity of transformation. Hence it was concluded that DNA or nucleic acid carries the genetic information and not the proteins.

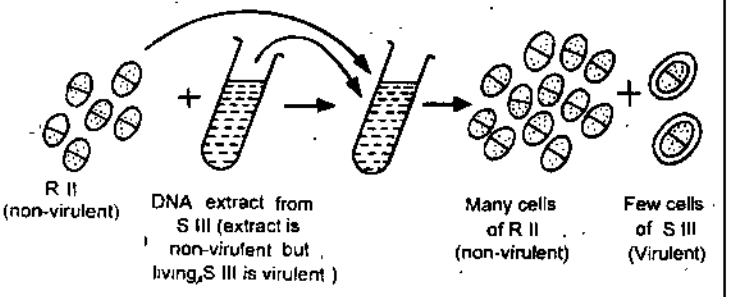
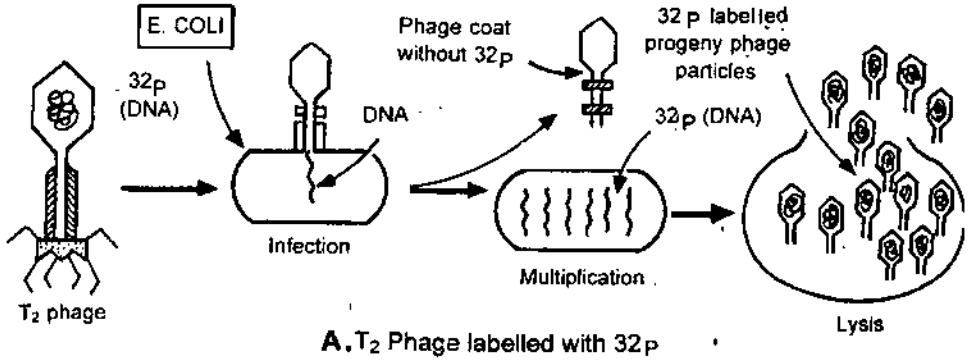
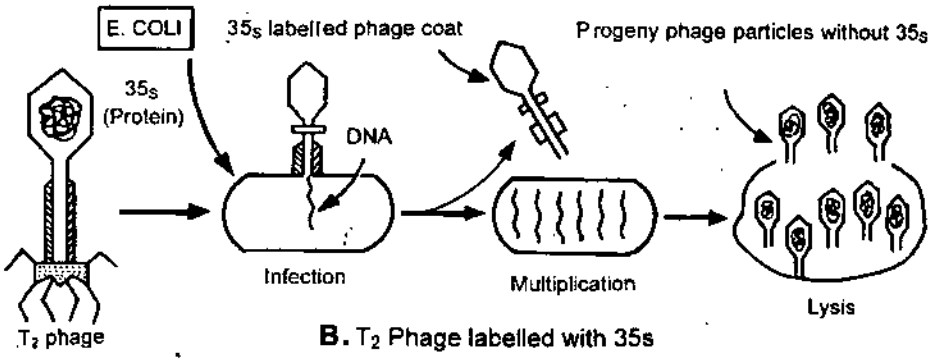


Fig. 25. DNA : Transformation experiment of Avery MacLeod and McCarthy.

(ii) Bacteriophage infection experiment. A. D. Hershy and **M. J. Chase** conducted an experiment to clearly show that DNA is the genetic material. The bacteriophage T_2 consists of protein coat and DNA core (Fig. 26). The phage particles were made radioactive by growing infected bacteria on medium containing P^{32}. The protein coat did not contain phosphorus, hence these bacteriophage had **DNA core labelled** with radioactive P^{32}. Similarly bacteriophage grown on S^{35} medium had only **protein coat labelled** as DNA and did not contain sulphur.



A. T_2 Phage labelled with 32_P



B. T_2 Phage labelled with 35s

Fig. 26. DNA : Hershey and Chass experiment
(A) T_2 phage labelled with P^{32} (B) T_2 phage labelled with S^{35}.

Hershey and **Chase** used both kinds of labelled phages to infect bacteria. The infected bacteria were gently agitated in blender to separate the empty phage particles called **ghosts,** remaining outside bacteria as only DNA core enters the host cell. It was found that phage progeny carried only P^{32} and no traces of S^{35}. This experiment clearly showed that only DNA of phage enters the bacterial cell and the protein coat is left outside. **Hence DNA of T_2 is transmitted from one generation to the next and protein is not transmitted. The DNA is the genetic material and not proteins.**

(iii) Experiment with TMV. : **H. Fracnkel-Conrat** and **Singer** in 1957 demonstrated that RNA functions as genetic material in virus like TMV where DNA is absent. **Tobacco Mosaic Virus,** TMV consists of RNA surrounded by hollow protein structure. The technique was developed to separate RNA and proteins of TMV chemically and under appropriate conditions the proteins and RNA can reassociate to make active TMV. The TMV whole, and only RNA were found capable of causing mosaic infection on tabacco leaves while protein alone could not cause infection. It proved that **only RNA of TMV is capable of causing disease due to virus multiplication hence RNA is the genetic material.** (Fig. 27).

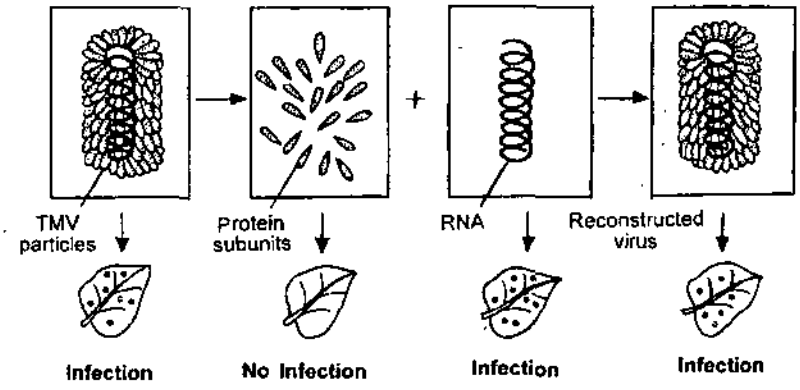


Fig. 27. DNA : Experiment of Fracnkel-conrat.

In another experiment hybrid DNA was used. Type A TMV was taken from tabacco and Type B from *Plantago lanceolata* also called as Holmes rib grass virus or HRV.

Fracnkel constructed two types of hybrid virus :

1. RNA from type A and protein from type B.
2. RNA from type B and protein from type A.

It was found that first type of hybrid with type A RNA caused TMV or A type infection and *virus progeny were of type A only. When second type hybrids with type B RNA were used the* infection was like that of type B and progeny was only of 'Type B'. This clearly demonstrated **that only RNA and not the proteins in TMV has capacity to produce disease and is the genetic material.** (Fig. 28).

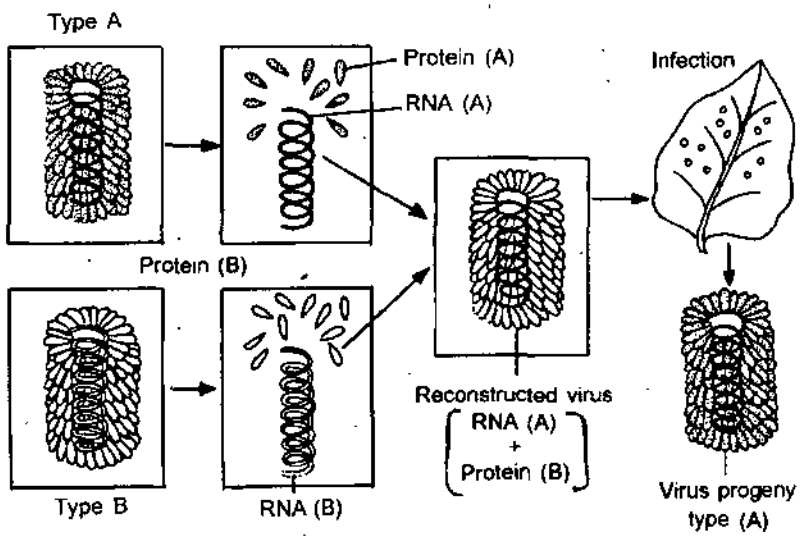


Fig. 28. DNA : Reconstruction of virus.

11·10. GENETIC CODE

For understanding the concept of genetic code, it is desirable to understand the relationship between genes and proteins. The proteins are highly specific polypeptide structures consisting of definite sequence of amino acids. The sequence of amino acids in a polypeptide chain is under control of genes. The gene is a segment of DNA strand consisting of a definite sequence of nucleotides. The information of DNA is transcribed in form of m RNA during transcription, the m RNA, in turn, translates a polypeptide chain. According to **sequence hypothesis** m RNA acts as an intermediate in converging information from the sequence of nucleotides in DNA to the sequence of amino acids.

The proteins are made of 20 types of amino acids hence 20 amino acids are the alphabets of protein language. Similarly, DNA and RNA are made of 4 types of bases, thus the bases are the alphabets of the language of DNA. The **codes** formed by the bases of DNA which give specific information for amino acid sequence in protein structure are called **genetic code.**

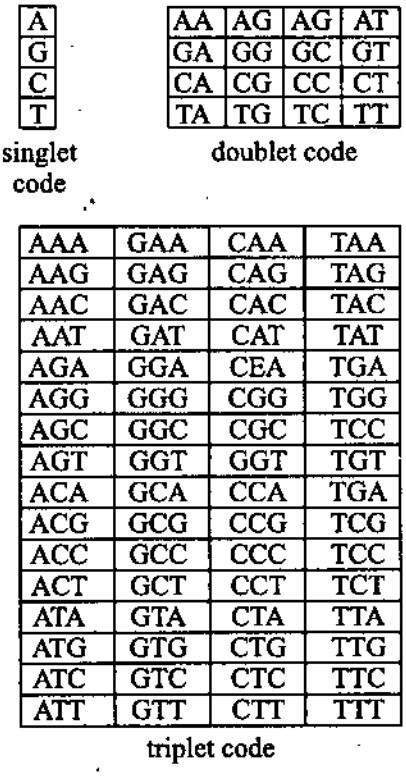


Fig. 29. DNA : A single code, a doublet code and a triplet code.

Properties of Genetic Code

The genetic code has the following properties which have been proved by experimental evidences :

(i) the code is triplet
(ii) the code is degenerate
(iii) the code is non-overlapping
(iv) the code is comma-less
(v) the code is non–ambiguous
(vi) the code is universal
(vii) the code has polarity
(viii) Codons and anticodons
(ix) Chain initiation condons
(x) Termination codons.

(i) The code is triplet. The DNA or RNA is made of 4 types of bases A, G, C, T (Fig. 29, 30) or U and proteins are made of 20 types of amino acids which are coded by DNA. If one base

First letter	*Second letter* U	*Second letter* C	*Second letter* A	*Second letter* G	*Third letter*
U	UUU, UUC Phe; UUA, UUG Phe	UCU, UCC, UCA, UCG Ser	UAU, UAC Try; UAA†, UAG†	UGU, UGC Cys; UGA**, UGG Tryp	U C A G
C	CUU, CUC, CUA, CUG Leu	CCU, CCC, CCA, CCG Pro	CCU, CCC His; CCA, CCG Glun	CCU, CCC, CCA, CCG Arp	U C A G
A	AUU, AUC, AUA Ilue; AUG*	ACU, ACC, ACA, ACG Thr	ACU, ACC AspN; ACA, ACG Lys	AGU, AGC Ser; AGA, AGG Arg	U C A G
G	GUU, GUC, GUA, GUG* Ilue	GGU, GCC, GCA, GCG Ala	GGU, GCC Asp; GCA, GCG Glu	GGU, GGC, GGA, GGG Gly	U C A G

*Chain initiation codons; †, **Chain termination codons

Fig. 30. DNA : Genetic code dictionary.

makes code for one amino acid only 4 amino acids can be coded hence the singlet codon will be insufficient to code 20 amino acids. The two bases codon or a doublet can make $4 \times 4 = 16$ codons. The doublet codons will also be insufficient to code 20 amino acids. The three base codon or a

triplet codon can make $4 \times 4 \times 4 = 64$ codons. The triplet codons will be sufficient to code 20 amino acids. The **triplet** or three letter code was first suggested by physicist **Gamow** in 1954. The experimental evidence proves that the code is a triplet.

(ii) The code is degenerate. The codes are specific for every amino acid if there is only one relationship between codes and aminoacids, out of 64 codons only 20 codons will be used. 44 codons would be useless or extra. The codon in such case will be called non-degenerate. But it has been observed that no codon is useless or extra, 61 out of 64 codons specify amino acids. This is possible **when one amino acid is coded by more than one codon,** this property of genetic code shows that code is **degenerate.**

(iii) The code is non-overlapping. The DNA molecule is a long chain of nucleotides. The bases form genetic code in non–overlapping manner. One base is used only once for a codon and it can not be part of more than one codon. A sequence of 6 bases thus will code only 2 amino acids. This property is called non-overlapping nature of codons. If codes were overlapping a base can be part of more than one codon and 6 bases will code utpo 4 amino acids (Fig. 31).

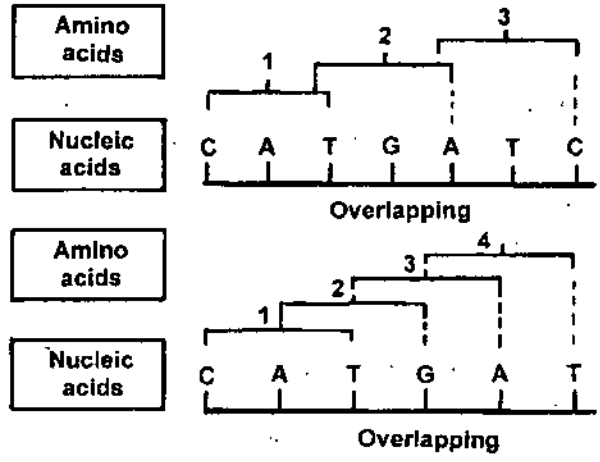


Fig. 31. DNA : Ovwerlapping of codons.

Experimental evidences indicate that the code is **non-overalpping.** The mutational change in one base would affect only one word in the non-overlapping code and it can affect three words in case of overlapping code. In TMV mutation of one base of the nucleic acid into another results in the alteration of only single amino acid. The studies on normal and sickle cell haemoglobin show that single mutational change results in the substitution of only one amino acid.

However, the presence of overlapping genes has been shown in $\phi \times 174$ (**Barrel** *et. al.* 1976, **Sanger** *et. al.* 1977).

(iv) The code is comma-less. The comma-less code property means that no base is extra between codons. No punctuation is required between two codons. After one amino acid is coded the second amino acid will be automatically coded by next three letters. In comma-less codons the genetic code reads in an uninterrupted manner from one end of the nucleic acid chain to the other (Fig. 32).

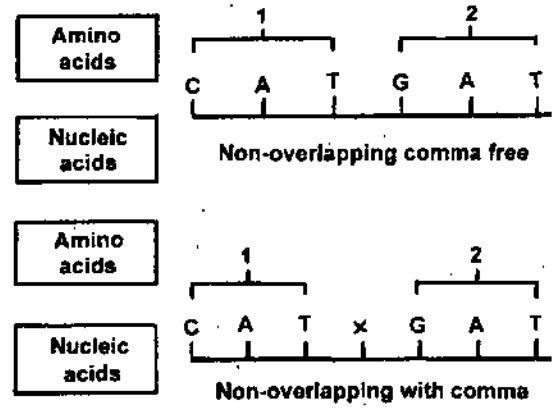


Fig. 32. DNA : Genetic code without comma and with comma.

(v) The code is non-ambiguous. The meaning of every codon is always specific and same wherever it is found *i.e.*, code is non-ambiguous. A genetic codon will always code same amino acid. An amino acid can be coded by more than one codon but one codon can never code more than one amino acid *e.g.*,

AAA will always code for amino acid **lysine**
CCC will always code for amino acid **proline**
GGG will always code for amino acid **glycine**
UUU will always code for amino acid **phenylalanin**

(vi) Code is universal. The property of universatility of codon means that same codons are present in all organisms be it microbes, plants, animals or man *e.g.*, AAA will code for lysine in plants, man, animals as well as in microbes. The universality of the code was demonstrated by **Marshall, Casky** and **Nirenberg** (1967), they found that the genetic codes of *E. coli*. (bacterium), *Xenopus laevis* (amphibian) and guinea pig (mammal) were similar.

Codons	UUG	AUC	GUC	UCG	CCA	ACA	AGG	
→	Leu	Ile	Val	Ser	Pro	Thr	Arg	
	Val	Leu	Leu	Ala	Thr	Thr	Gly	←

(vii) The code has polarity. The synthesis of protein takes place under the direct control of DNA through RNA. The synthesis of specific protein takes place as messages of synthesis are always read between point of initiation and termination in a fixed direction *i.e.*, the codes are polar. For example, in the given sequence from left to right the first codon, UUG, would code leucine. If read from right to left the codon would become GUU and would code valine. The sequence of amino acids constituting the protein would undergo a drastic change if the code is read in the opposite direction. It is provided that the message in mRNA is read in the 5′ → 3′ direction. The polypeptide chain is synthesized in the N →C direction, *i.e.*, from N terminal to the C terminal.

(viii) Codons and Anticodons : During translation the codons of mRNA pair with complementary anticodons of tRNA. Since mRNA is read in a polar manner in the 5′ → 3′ direction the codons are also written in the 5′ → 3′ direction. Thus, the codon AUG is written as 5′AUG3′. In such a configuration the first bases of both codon and anticodon would be the ones at the 5′ end and third bases at the 3′ end.

Base		1	2	3	
Codon (mRNA)	5′	A	U	G	3′
Anticodon (tRNA)	3′	U	A	C	5′
Base number		3	2	1	

Often, however, the anticodon is written in the 3′ → 5′ direction so as to bring about an easier correlation between the bases of the codon and anticodon. Thus the anticodon for AUG is written as 3′ UAC 5′ or, more simply, UAC. Where the first letter in the codon is at the 5′ end and the first letter of the anticodon at the 3′ end.

(ix) Chain Initiation Codons : The first amino acid in the synthesis of most of the chains is methionine (eukaryotes) or N-formylmethionine (prokaryotes). Methionyl or N-formyl methionyl-tRNA specifically binds to initiation sites containing the AUG codon. AUG codon in therefore, called the initiation codon. Sometimes, GUG also functions as the initiation codon in protein synthesis. Normally GUG codes for valine. In the phage MS2, GUG is the initiation codon for the A protein. GUG has been found to initiate protein synthesis when the normal AUG codon is lost by deletion. However, initiation by GUG is less efficient since it has a lower affinity for fMet-tRNA.

Both AUG and GUG codons show ambiguity in one sense, that each of them codes for two different amino acids. When these two codons are at **initation positions** of mRNA they code for **N-formyl methionine**. In **internal positions** AUG codes for **methionine** and GUG for **valine.**

(x) Termination Codons. Three of the 64 codons do not specify any amino acid and were hence called **nonsense codons**. These codons are UAG (*amber*), UAA (*ochre*) and UGA (*opal* or *umber*). Since they bring about termination of polypeptide chain synthesis they are also called **termination codons**. UAG was the first termination codon to be discovered. It was named 'amber' after a graduate student named Bernstein (the German for 'amber') who helped in the discovery of a class of mutations. Apparently to give uniformity the other two termination codons were also named after colours.

Termination codons do not code for any amino acids and hence cause termination and release of polypeptide chains. Apparently no tRNA species has anticodons complementary to the termination codons. There are mRNAs with single termination codons and also mRNAs with two successive termination codons (*e.g.*, MS2 coat protein mRNA). Termination codons are not read by any tRNA molecules but by proteins called **release factors**. In **prokaryotes** there are three release factors RF-1, RF-2 and RF-3, RF-1 recognizes UAA and UAG, while RF-2, recognizes UAA and UGA. RF-3 stimulates RF-1 and RF-2. In **eukaryotes** a single release factor (RF) recognizes all three termination codons.

11.11. WOBBLE HYPOTHESIS

The **Wobble hypothesis** was proposed by **F.H.C. Crick** in 1965. According to this hypothesis only the first two positions of a triplet codon on mRNA have a precise pairing with the base of the tRNA anticodon. The pairing of the third position bases of the codon may be ambiguous and varies according to the nucleotide present in this position.

Thus a single tRNA type is able to recognize two or more codons differing only in the third base. This kind of wobbling allows economy of the number of tRNA molecules.

The evidence for wobble hypothesis comes from the fact that there are 61 codons which specify for amino acids, then the cell should contain 61 different t RNA moelcules, each with different anticodon. However, the number of tRNA molecule types discovered is much less than 61. The anticodon UCG of serine tRNA recognises two codons AGC and AGU. The bonding between UCG and AGU follows usual Watson-Crick pairing. In UCG and AGU pairing the bonding takes place between G and U, this is different from Watson-Crick pairing and is called as **Wobble pairing.**

The amino acid leucine is coded by six codons UUA, UUG, CUU, CUC, CUA and CUG. The first two letters of two codons are UU and of four codons are CU. Since the first two letters cannot have wobble pairing at least two different types of t RNA are required for leucine. In four codons with CU, two codons CUU and CUC have pyrimidine as the third base and the codons CUA and CUG have purine as the third base. Hence two different types of t RNA are required by these pairs as the purine of the anticodon can only pair with pyrimidine and *vice-versa*.

11.12. GENETIC CODE OF MITOCHONDRIA

The genetic codes are universal in nature. The genetic code has remained constant since the time it was fixed and is believed that the genetic code does not undergo any kind of evolution. However, the genetic code of mitochondria has been found different from genetic code of nuclear DNA.

In nuclear genes UGA is termination codon but in yeast mitochondria UGA codes for tryptophan. Hence a different genetic code may exist in mitochondria but mitochondria of all organisms may not have same genetic codons. In maize mitochondrion UGA codon is not present and tryptophan is coded by CGG and UGG. In nuclear genes CGG codes for arginene. In nuclear genes AUA codes for isoleucine while it codes for methionine in human mitochondria.

• STUDENT ACTIVITY

1. Give an experiment as evidence to semi-conservative mechanism of DNA replication.

2. Give properties of double helical DNA structure.

3. Write short note on nucleotides.

• SUMMARY

- On the basic of Griffith's bacterial transformation experiment, conducted on *Pneumococcus pneumoniae*, **Avery** *et.al.*, concluded that the genetic material is DNA. **Hershey** and **Chase** concluded that DNA is the genetic material. The DNA is made up of nitrogen bases, sugars and phosphate. The nitrogen bases are of two types : pyrimidines and purines. The sugar in DNA is pentose. The attachement of phosphate generally occurs at 5′ or 3′ carbon. A nitrogen base combines with a sugar to form a nucleoside, which combines with phosphate group to form nucleotide. The nucleosides of DNA are called deoxyadenosine, deoxycytidine, deoxyguanosine and deoxythmidine. The nucleotides of DNA are called deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid and deoxythymidylic acid. The phosphate group of one nucleotide is linked to the hydroxyl group of sugar of adjacent nucleotide by a phosphodiester bond, thus forming a polynucleotide. So, the DNA is a polymer, the monomeric unit of which is a nucleotide *i.e.*, DNA is polynucleotide. **Watson** and **Circk** (1953) proposed double helical model of DNA. **Wilkins** *et. al.* by X-ray crystallogrophy gave many details of DNA molecule. The DNA exists in atleast four forms mainly A, B, C, and D. **Meselson** and **Stahl** (1958) confirmed that replication of DNA in *E. coli* is semiconservative. The proteins are made of 20 types of aminoacids. Similarly DNA and RNA are made of four types of bases, thus the bases are the alphabets of the language of DNA. The codes formed by the bases of DNA which give specific information for amino acid sequence in protein structure are called genetic code.

• TEST YOURSELF

1. Name the organism in which DNA is single stranded.
2. Name the organisms in which RNA is double stranded.
3. Give names of pyrimidine bases of DNA.
4. Give names of purine bases of DNA.
5. Describe the role of tRNA in translation.
6. What is Central Dogma ?
7. What is reverse transcritpion ?
8. What is the role of rho factor ?
9. What is the role of sigma factor ?
10. What function does amino acyl tRNA synthetase perform ?
11. What functions does transfer factor (TF1) perform ?
12. What are release factors ?
13. Which are terminating codons ?
14. What are codons of chain initiation ?

15. Who gave the double helix model of DNA structure ?
16. What are Okazaki fragments ?
17. What is the diameter of DNA helix ?
18. DNA polymerase I was discovered by...

ANSWERS

1. Parvo ...
5. ...

homozygous recessive. The individuals having identical phenotype may or may not have the same genotype, *e.g.* Tall plants can be *TT* and *it*. Indivdiuals having similar genotype will have same phenotype in comparable situation.

Dominant and recessive

In a hybrid both alleles of a gene are present but phenotypically only one allelic form is expressed and the other remains hidden. The character or allele which is expressed in heterozygous form is called **dominant** and the charcter or allele which is not **expressed** is called recessive. *e.g.*, *Tt* plants are tall, hence the tall character and *T* allele are dominant, dwarf character and *t* allele are recessive.

The characters are now preferably expressed by mutant forms hence the tall plants are denoted by sign *DD* or *Dd* and homozygous recessives are represented by *dd*.

Reciprocal Crosses

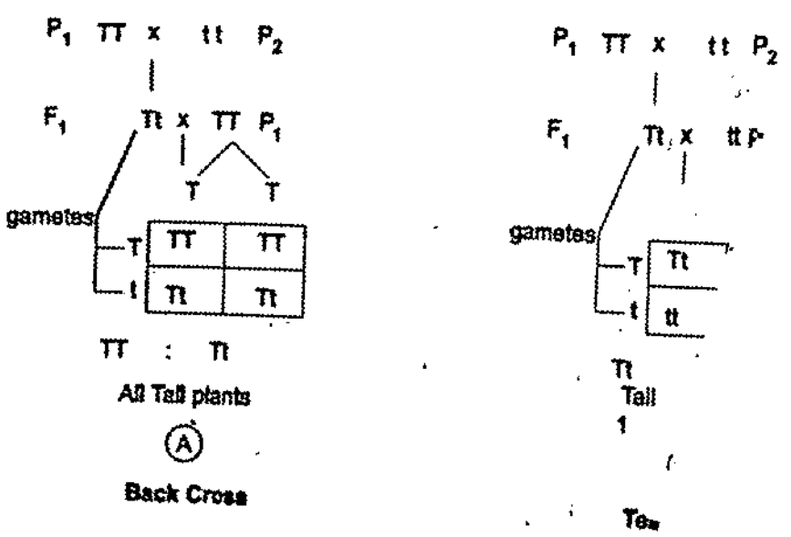
The reciprocal crosses are the set of two crosses made in such a way that parents with one character are taken as male in one cross and female in the other cross. For example, if in the first cross 'A' is used as female parent, in second cross A will be used as male parent.

The reciprocal crosses are made to see if the concerned character is located on sex chromo or autosomes. If in such cross the results are similar in I and II cross, the character will be loca autosomes.

Back cross and test cross

When any cross is performed the F_1 individuals are normally selfed to produce F_2 ge The F_1 can also be crossed with parents P_1 or P_2. Such crosses are called **back crosses.**

If F_1 is crossed with homozygous dominant parent, the F_2 progency obtained has all T phenotype, *e.g.* (A).



If F_1 is crossed with homozygous recessive parent the F_2 progency h and genotypes. Such back cross with homozygous recessive parent is call is performed to know the homozygosity or heterozgosity of the parent in

Monohybrid, dihybrid and trihybrid crosses

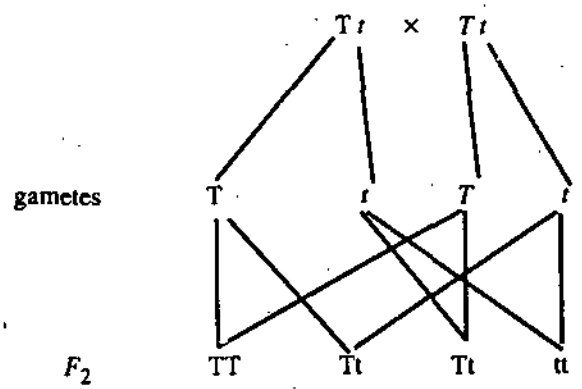
In monohybrid cross, the cross is made between parents for a gene. In this case the phenotype ratio obtained in F_2 is 3 : 1 and gen

Phenotypic ratio

Tall × Dwarf
↓
F_1 Tall × Tall
↓
F_2 Tall : Dwarf
3 : 1

Genotypic ratio

TT × *tt*
↓



1 : 2 : 1

Dihybrid cross

In dihybrid cross the cross is made between parents for two characters of for two genes. If assortment of the two genes is independent the F_2 progeny will have phenotypic ratio 9 : 3 : 3 : 1.

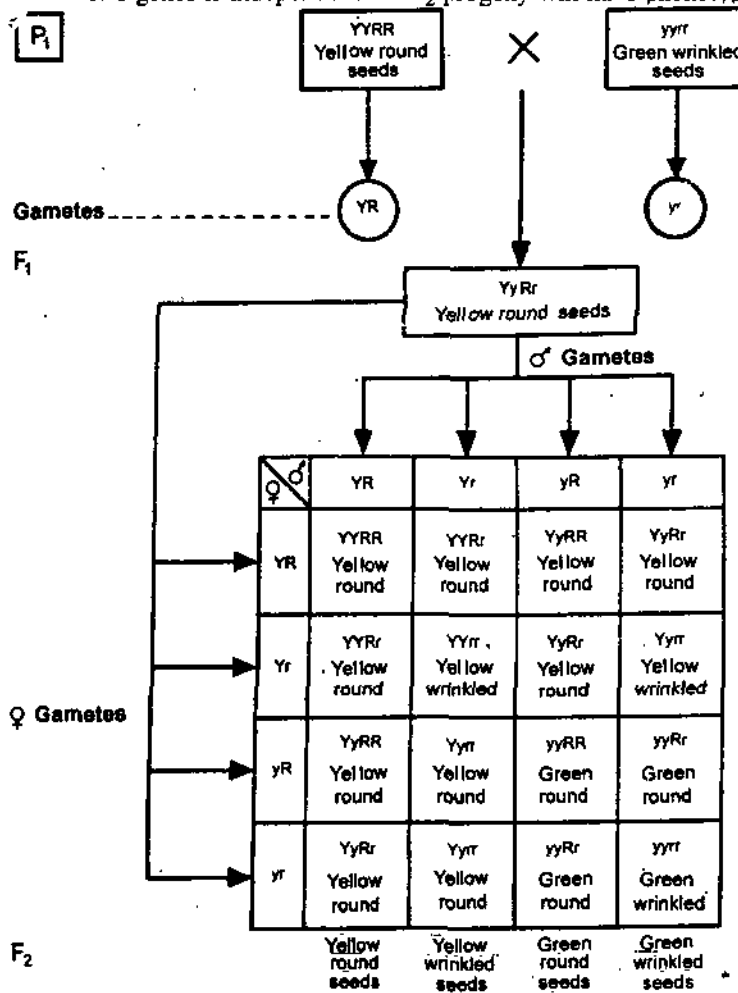
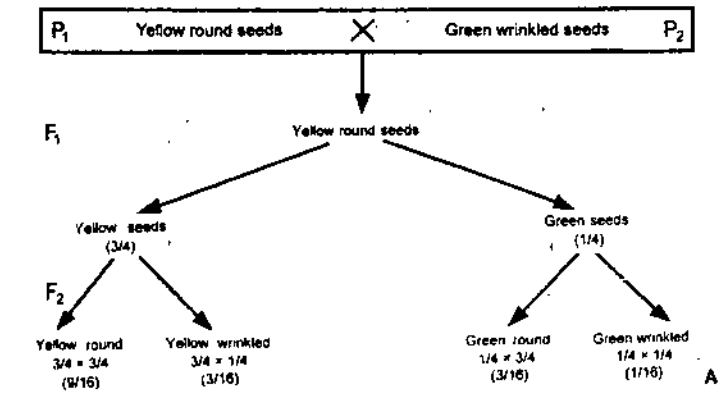


Fig. 1. Genetic inheritance checkerboard showing Dihybrid cross involving characters for seed shape and seed colour.

For example, if a dihybrid cross between plants homozygous dominant for round and yellow seed is performed with homozygous recessive green and round seed plants the results as shown in fig. 1 will be obtained. The result of F_2 progeny can be shown as in checker board. (Fig. 1).

The results of phenotypic ratio and genotypic ratio in F_2 generation can be easily and in a better way be obtained by Fork line method.



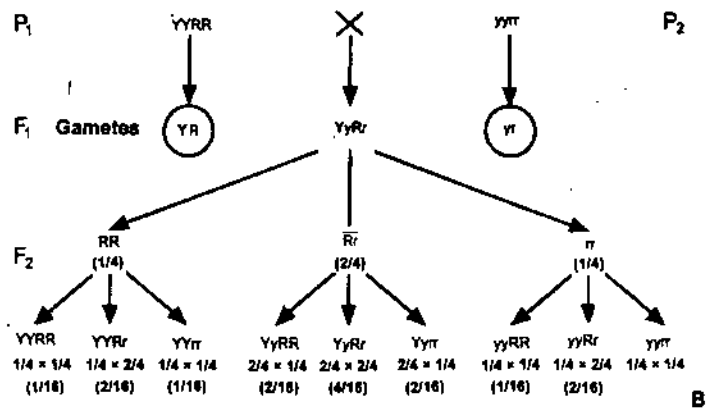


Fig. 2. (A, B). Genetic inheritance. (A) Derivation of the phenotypes in a dihybrid cross using forked line method, (B) Derivation of the genotypes in a dihybrid cross using forked line method.

1·1. GREGOR JOHANN MENDEL (1822-1884)

Mendel was born on 22nd July 1822 in a village in Germany. His father was a peasant, so **Mendel** became interested in plant hybridization. He was educated in a monastery in **Brunn in Austria** (now in Czechoslovakia). In 1847 he became the priest in Brunn. In 1851 he studied science and mathematics at University of Vienna. In 1854 he started teaching in the school of monastery. During 1856-1864, he performed hybridization experiments on Pea plants. He published the results of his experiments as **"Experiments in Plant Hybrdization"** in Brunn Natural History Society, proceedings. His results were not given any attention at that time because :

(i) **Darwin's** publication of **"Origin of Species"** in 1859, was considered controversial, it attracted interests of all scientists.

(ii) **Mendel** published his reuslts in little known journal.

(iii) His results were not well understood at that time.

(iv) **Mendel** analysed his results mathematically which attracted little interest.

Mendel worked in the same moanstery till his death in 1884. MENDEL'S laws were rediscovered in 1900 independently by a Dutch biologist **Hugo de Varies,** a German botanist **Carl Correns** and Austrian botanist **Erich van Tschermark.** Mendel's laws are since considered very important as there explain nature of inheritance and Mendel is regarded as **"Father of Genetics."**

2. Pea Plant : As Experimental Material

(A) Mendel selected pea plant *Pisum sativum* as experimental material, because pea plant had the following advantages :

(i) Pea plant has **many varieties with clearly distinguishable alternate traits** or characters such as tall and dwarf plants, yellow or green seeds, etc.

(ii) Pea plants are mainly **self-pollinated.** The plants have pure lines due to self fertilization and plants are pure for the character they are used.

(iii) Pea plants can be easily made **cross pollinating** by checking self pollination. The **stamens** (9) + 1 are in fused structure and **can be easily removed.** These emasculated plants can be fertilized with pollens of desired male plants.

(iv) Pea plants are **annuals, easy to grow** and large number of offsprings are obtained in each generation.

(B) Mendel selected the following **seven pairs** of characters in pea plants for his experiments (fig. 3) :

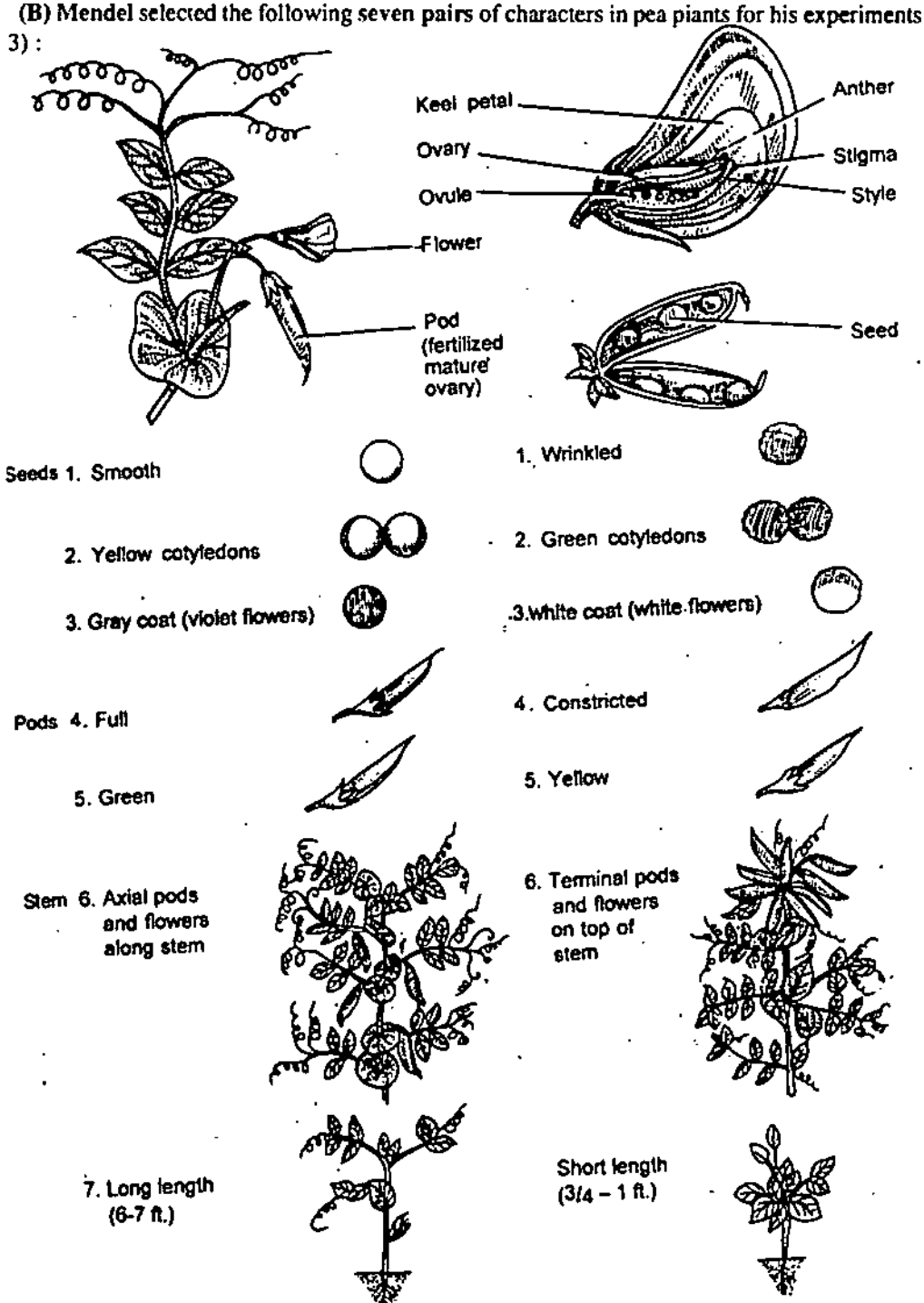
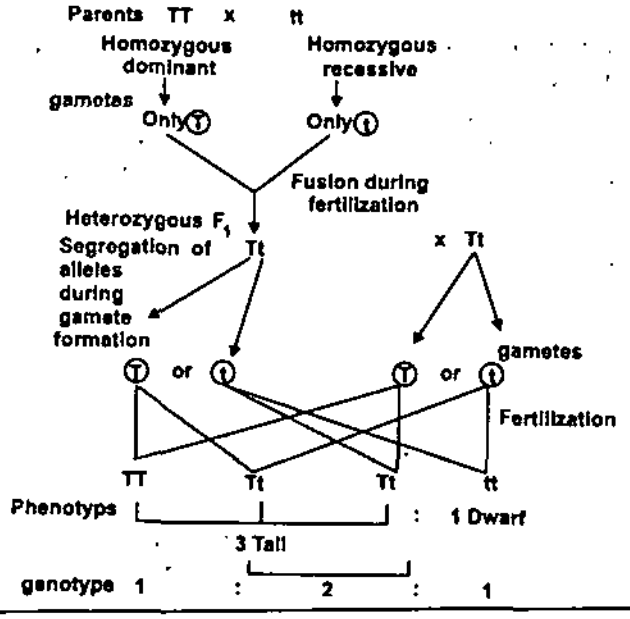


Fig. 3. Genetic Inheritance, seven characteristics in pea that were used by Mendel in his experiments.



12.6. PRINCIPLE OF INDEPENDENT ASSORTMENT (Mendel's Second Law)

"According to Principle of independent Assortment, if we consider the simultaneous inheritance of two or more characters or genes, their distribution in gametes and in subsequent generation is independent of each other."

Seed colour : yellow round 9, green round 3 } = 12

Seed shape : yellow wrinkled 3, green wrinkled 1 } = 4

Round : Wrinked	Yellow : green
12 : 4 = 3 : 1	12 : 4 = 3 : 1

In this experiment Mendel considered one chracter at one time and he could explain the segregation of characters in F_2 generation in the ratio of 3 : 1.

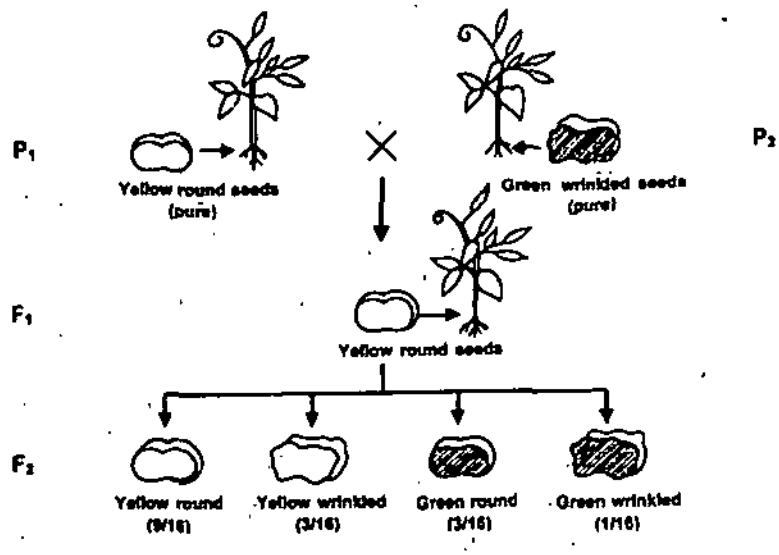
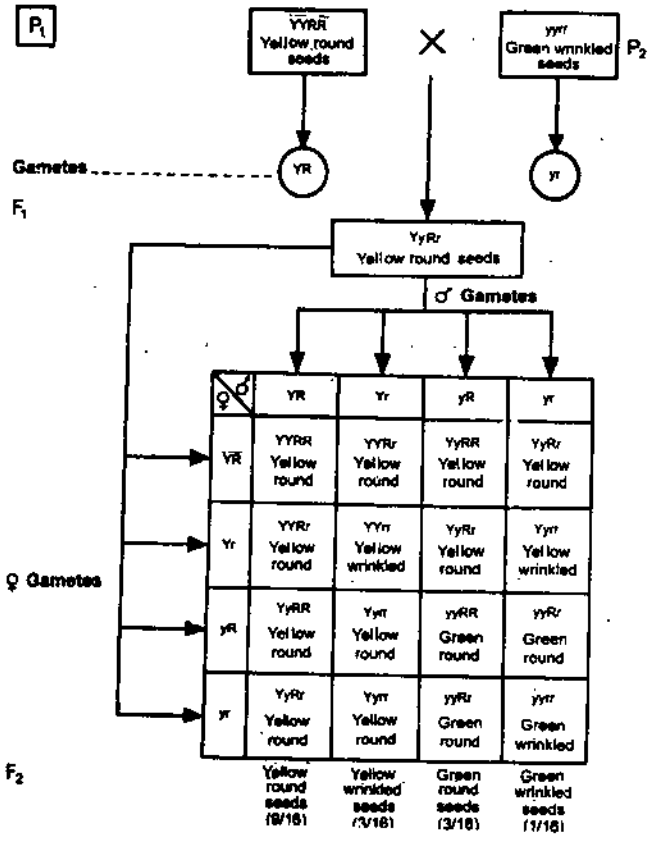


Fig. 4. Genetic Inheritance. Independent assortment of seed shape and seed colour in dihybrid cross.

After this **Mendel** tried to find out when two or more characters are considered together whether the two characters segregate independently or they influence each other.

To explain this we can take the example of a dihybrid cross for seed colour and seed shape. In pea plants the seed colour yellow (*Y*) is dominant over green (*y*), the seed shape round (*R*) is dominant over wrinkled (*r*). When homozygous double dominant parent *YYRR* (yellow and round seeds) are crossed with double recessive *yyrr*. (green and wrinkled seed) plants, F_1 and *YyRr* with yellow and round seeds (fig. 5).

Dihybrid Cross : (i) Checkerboard Method.



(ii) Forked line method

In F_1 allels segregate to make *YR*, *Yr*, *yR*, *yr* in male and female gametes. Using either checkeboard or forked line method we find the phenotype ratio of 9 : 3 : 3 : 1 in F_2 of dihybrid cross. The seed colours. Yellow and green appear in ratio of 3 : 1 and the seed shape round and wrinkled also appears in ratio of 3 : 1 as in monohybrid cross. Hence, the segregation of one trait like seed colour is independent of segregation of seed shape. This is known as the **"Principle of Independent Assortment."**

Mendel is rightly regarded as **Father of Genetics** as he was the first person to scientifically explain the mechanism of inheritance. His law of segregation can be considered most important as it explains the formation of gametes with pure alleles and appearance of traits in subsequent generation. **Mendel** also disproved the earlier concept of **blending Inheritance.** He proved that inheritance is particulater in nature of **factors** are transferred from parents to offsprings. These factors are now recognized as **genes** located on **chromosomes.**

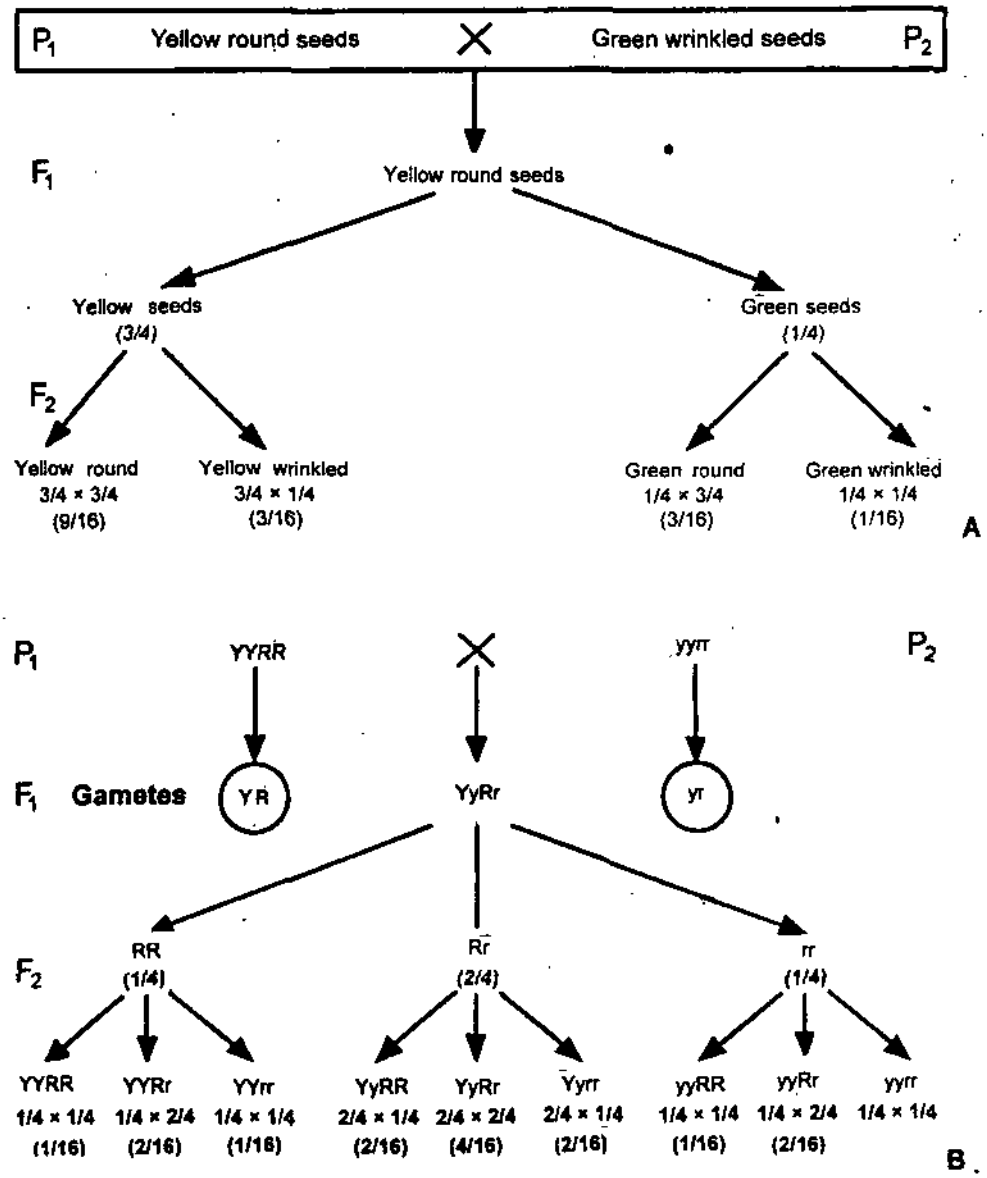


Fig. 5. Genetic inheritance. Derivation of the genotypes in a dihybrid cross using forked line method.

12.7. DEVIATION FROM MENDELIAN INHERITANCE

Mendel's laws hold true in large number of inheritance patterns of plants and animals. Later studies of inhertance in plants and animals show many variations from Mendelian ratio. There are many reasons for much variations. Some examples are :

1. In **Mendel's** experiments one trait or factor was found completely dominant over its other form. Many cases are known where one factor (gene) is not completely dominant over the other *i.e.*, **Incomplete Dominance**, or the two factors are equally domainant *i.e.*, **codominance.**

2. The characters selected by Mendel in his experiments were all controlled by separate genes. But sometimes one chracter is controlled by two or more genes and the phenotype is the result of **Interaction of genes.**

3. The genes control the phenotype through developmental process or metabolic reactions. Some genes also affect viability of the indivdiual besides the expression of phenotype. *i.e.*, **Lethality.**

4. In examples selected by **Mendel**, each factor has only two forms : the dominant and recessive, but genes sometimes have more than two alternate forms. (**multiple alleles**).

5. In Mendelian inheritance the two or more genes assort independently, but sometimes the genes may be linked together to givce ratios other than Mendel's ratios (**linkage**).

12.8. INCOMPLETE DOMINANCE

In **Mendel's** experiment all the seven characters were controlled by genes, in which one allele was completely dominant over the other allele. Later on it was found that in Four-O-clock plant (*Mirabilis jalapa*), R allele is responsible for red flowers and *r* allele makes flowers white. When homozygous dominant red flower plant *RR* is crossed with homozygous recessive white flower plant *rr*, the heterozygous F_1, *Rr* bears pink flowers. This is because *R* allele is not completely dominant over *r* allele. In F_2 *RR* (Red) : *Rr* (Pink) and *rr* (white) appear in 1 : 2 : 1 ratio. The pink flowers in F_1 are not due to blending of characters, because red flowers again appear in F_2 by selfing of pink flower F_1 plants. This phenomenon is called as **incomplete dominance or partial dominance**. (Fig. 6).

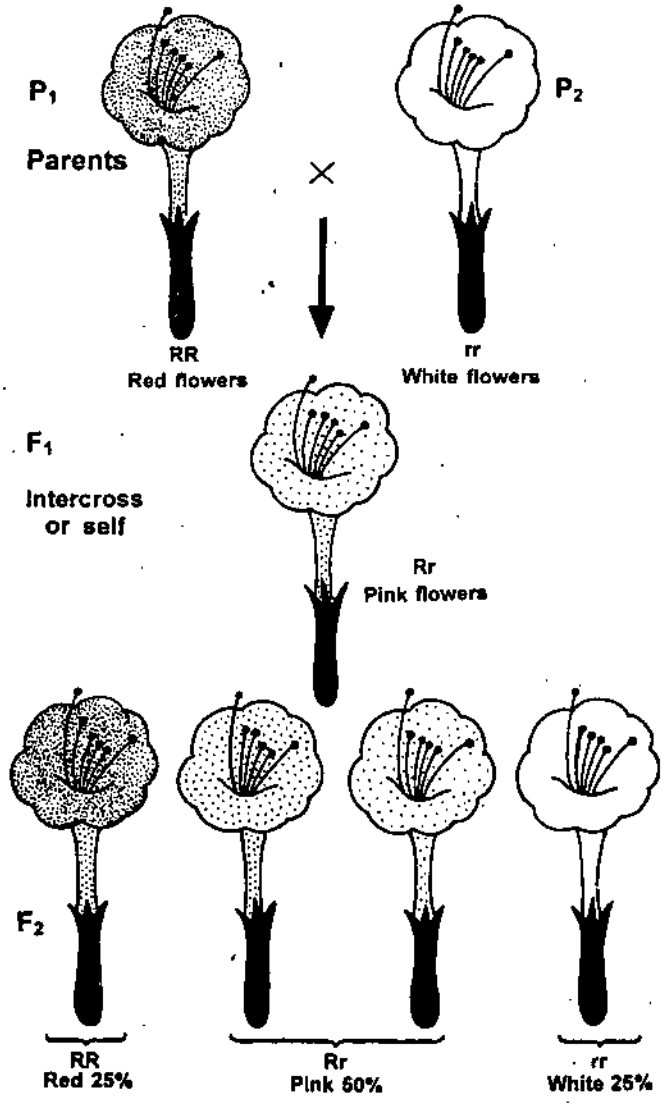


Fig. 6. Genetical inheritance of flower colour in *Mirabilis jalapa* showing incomplete dominance.

Similarly, in Snapdragon (*Antirrhinum majus*) broad leaves (*B*) are incompletely dominant over narrow leaves (*b*) and red flowers (*R*) are incompletely dominant over white flowers (*r*). A dihybrid cross between homozygous dominant plant *BBRR* (Broad leaves Red flowers) with homozygous recessive *bbrr* (Narrow leaves, white flowers) results in F_1 plants *BbRr* (Intermediate leaves and Pink flowers). On selfing F_1, following nine phenotypes corresponding to nine geneotypes are found. (Fig. 7).

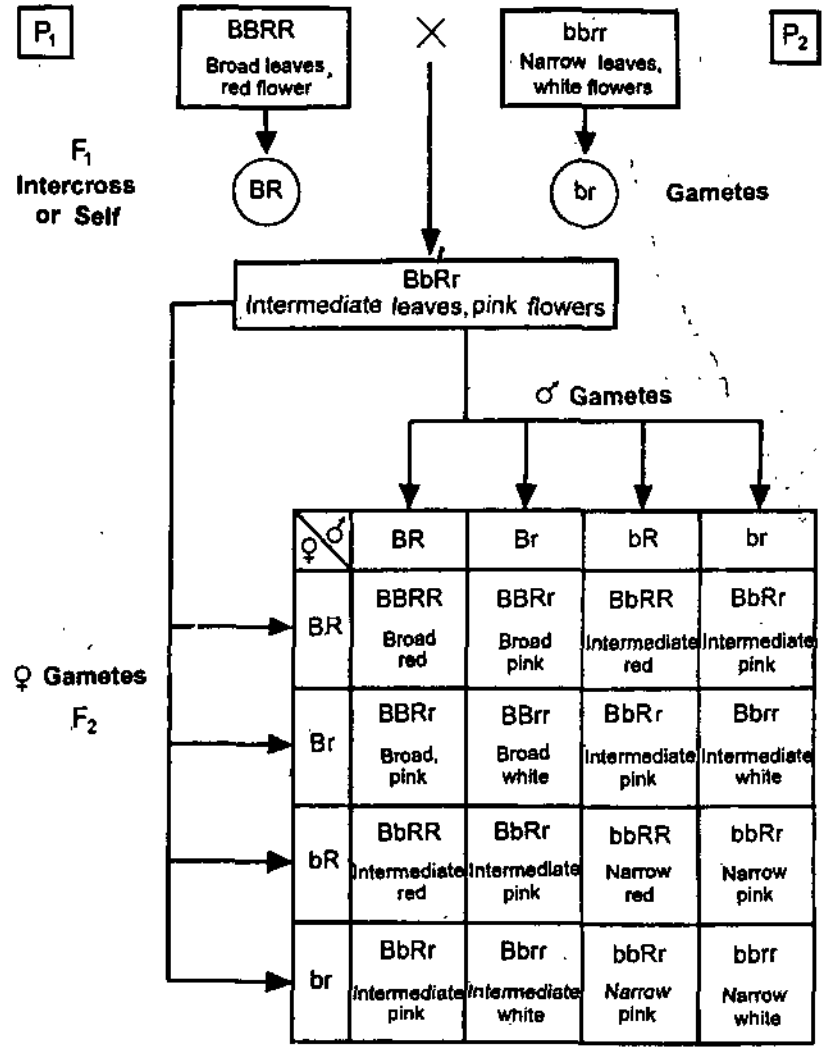


Fig. 7. Genetical inheritance. A dihybrid cross in sanapdragon showing incomplete dominance.

Co-dominance : When both the alleles of a gene express themselves in hetherozygous condition, the phenomenon is called **co-dominance.** In co-dominance the heterozygous individuals express the phenotype of both the alleles in pure form. For example, in cattle, when black coat cattle are crossed with white coat cattle, the F_1 heterozygous cattle are roan coats. The cattle have distinct black and white patches but hair of intermediate type do not occur. In man blood group antigens are good example of co-dominance.

12.9. MULTIPLE ALLELES

Most of the genes have two alternate forms of alleles and generally one form of allele is dominant over the other form. Some genes have more than two allelic forms which may have different phenotypic expressions. These alleles are called as multiple alleles. When some alleles express themselves within same phenotypic range these are called **isoalleles**. The isoalleles. The isoalleles can be **wild** or **normal isoalleles** and **mutat isolleles**. In Man, blood groups are examples of multiple allelism.

The blood group gene has three allelic forms $I^A I^B$ and *i*. I^A and I^B are responsible for synthesis of glycoprotein (antigen) *A* and *B* coating over red cells. The three alleles form six types of genotypes, which form four blood group types, *A, B, AB* and *O*.

Genotypes	Phenotypes
$I^A I^A, I^A i$	*A*
$I^B I^B, I^B i$	*B*
$I^A I^B$	*AB*
ii	*O*

The various genotypes, their phenotypes and the compatible blood groups for transfusion in the case of human ABO blood group system.

Blood group	Genotype	Antigen present on the surface of RBC's	Antibody present in blood serum	Compatible transfusion from the blood groups
A	$I^A I^A$ $I^A i$	A (galactosamine)	Anti-B	A and O
B	$I^B I^B$ $I^B i$	B (galactose)	Anti-A	B and O
AB	$I^A I^B$	A and B	Nil	A, B, Ab and O
O	*ii*	Nil	Anti-A and Anti-B	O

- Incompatible blood transfusion : In group A, from groups B and AB; in group B, from groups A and AB; in group AB, from none; in group O, from groups A, B and AB.
- In the determination of compatibility for blood transfusion, Rh blood is also important.
- ABO blood group system is also involved in histocompatibility, *i.e.*, rejection of tissue transplants.
- In fact, the number of blood groups in this system is more than the four listed above; as this locus at least five different alleles are known.

While I^A and I^B are dominant over *i* separately, I^A and I^B are codominant. Hence $I^A I^B$ genotype has blood group *AB* type. Other common examples of multiple alleles are : skin colour in rodents, eye colour in *Drosophila*. Self-sterility in *Nicotiana*, MN blood groups in man.

• STUDENT ACTIVITY

1. What is law of segregation ?

2. When pink flowered plants were crossed, they produced white and red flowered plants. Give explanation for this phenomenon.

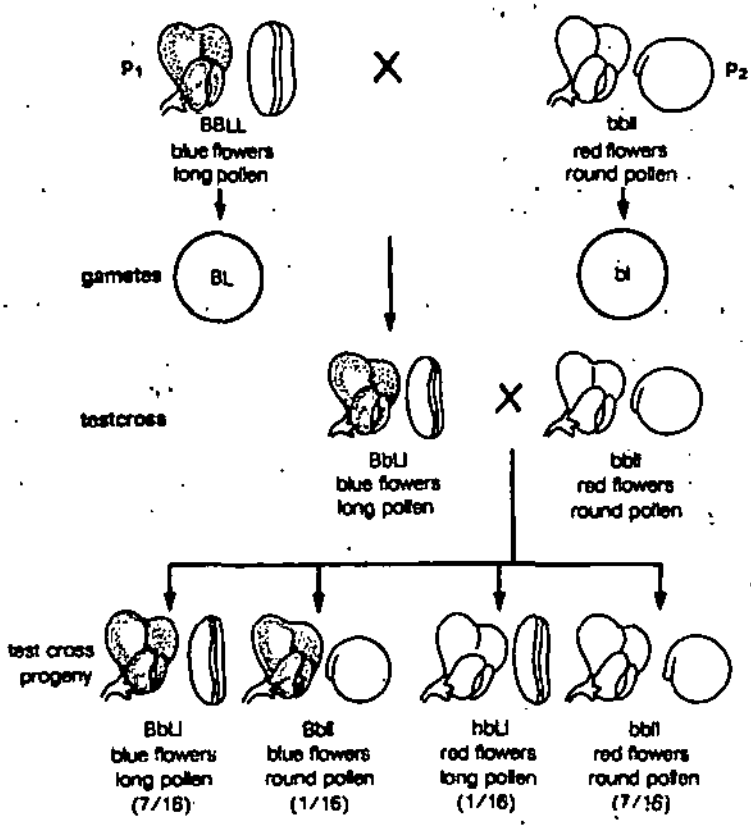


Fig. 1. Linkage. Ratios obtained by Bateson in sweet pea when two characters, blue flowers and long pollens, were present in coupling phase.

In this cross 7 : 1 : 1 : 7 ratio was obtained as the dominant alleles have strong tendency to stay together and similarly the recessive alleles tend to stay together. This phenomenon was called **gametic coupling by Bateson.**

When Bateson crossed plants having blue flowers and round *BBll* with plants having red flowers and round pollens *bbLL*, in F_1 plants *BbLl* were obtained. These blue flower long pollens F_1 plants were crossed with *bbll* homozygous recessive plants, in F_2 the 1 : 7 : 7 : 1 ratio (Fig. 2) was obtained instead of 1 : 1 : 1 : 1

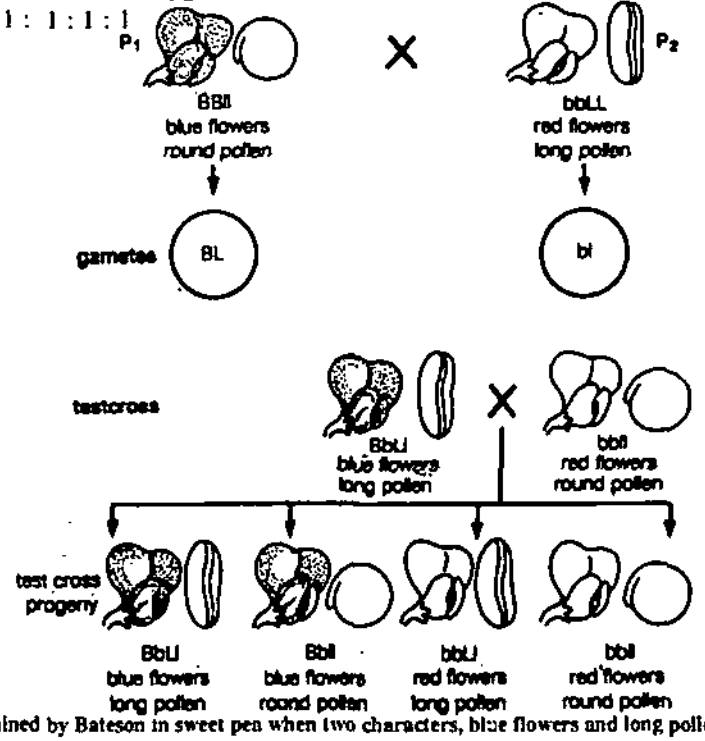


Fig. 2. Linkage. Ratios obtained by Bateson in sweet pea when two characters, blue flowers and long pollens, were present in repusion phase.

In this case the two dominant alleles or the two recessive alleles come from different parents, the two dominant alleles try to remain separate. Similarly, the two recessive alleles try to remain separate and this phenomenon was called **repulsion** by **Bateson.**

13·2. Morgan's theory

T. H. Morgan 1910 demonstrated that white eye (*w*) gene inheritance pattern can easily be explained by assuming that *w* is located on *X* chromsome, *i.e.*, it was sex linked. Later on he studied inheritance of several sex linked genes. **Morgan** proposed following conclusions from his studies:

(i) genes are arranged in linear fashion in chromosomes.

(ii) genes located in the same chromosome tend to stay together during inheritance and this phenomeon is called **linkage.**

(iii) The strength of linkage between two genes is inversely related to the distance between them in the chromosome. The greater the distance, the lower will be the linkage strength. The linkage is broken by crossing over. The percentage of crossing over will be more if the distance between genes is more and the linkage will be broken easily.

(iv) Coupling and repulsion phases are two aspects of the same phenomenon called linkage. In coupling phase the two genes are located on the same chromsome and in repulsion phase the two genes are located on different but homologous chromsomes.

Linkage in a two point test cross

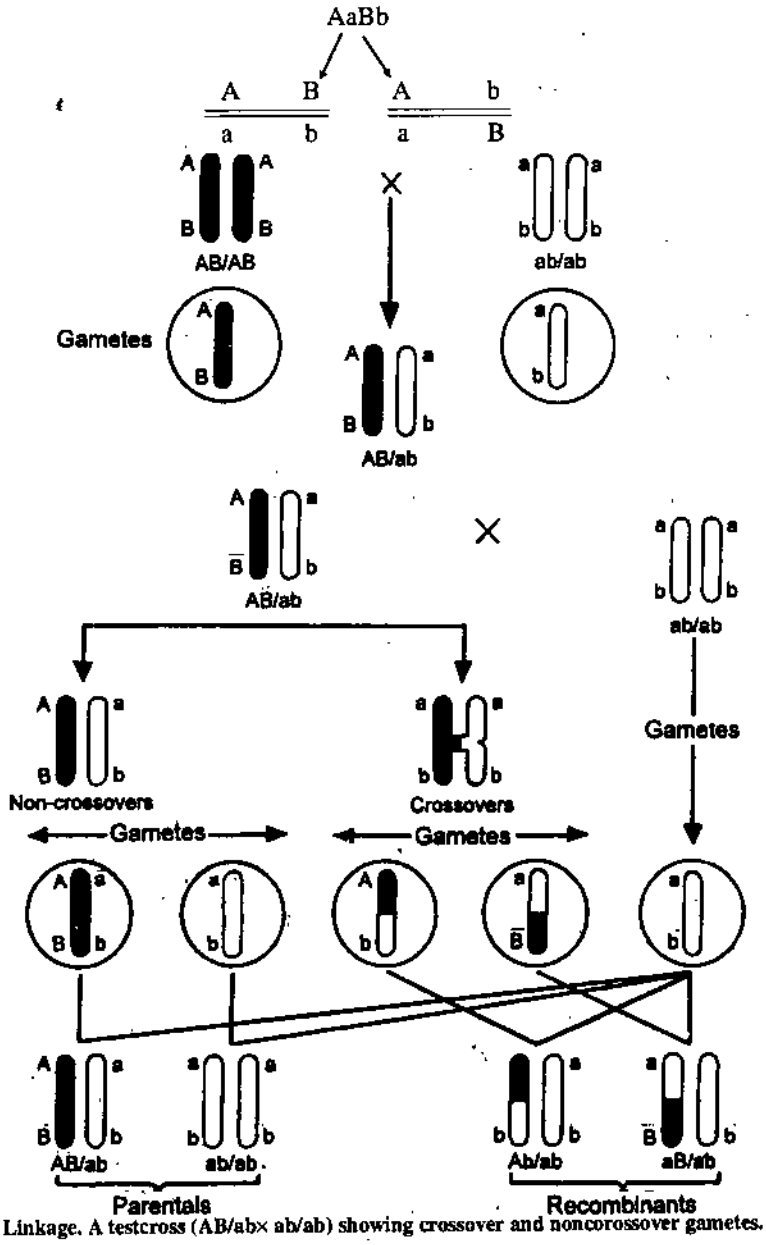


Fig. 3. Linkage. A testcross (AB/ab× ab/ab) showing crossover and noncorossover gametes.

If we take example of a two point cross, we can understand linkage as follows. In the example the two genes are *A* and *B* and their respective recessive alleles are *a* and *b*. When a cross of $AB/AB \times ab/ab$ is made in F_1 dihybrid *AB/ab* would be produced. *AB/ab* is then crossed with double recessive *ab/ab* for test cross.

The **cis form** *AB/ab* form gametes *AB* and *ab* non-crossing over and *Ab*, *aB* by crossing over (fig. 3).

The **non-cross overs** in such cases vary froms 50%-100% and the cross overs vary from 0-50% and will never exceed 50% (Fig. 4).

In transform

Linkage Groups : It has been observed in many organisms, that the number of linkage groups is equal to the halpoid number of chromsomes. For example, in *Drosophila* the halpoid number is 4 and the number of linkage groups is also 4. In pea, *Fisum sativum*, $n = 7$ and linkage groups are 7, in maize $n = 10$ and linkage groups are 10. Sometimes there can be some variations, for example in the mice the haploid chromosome number *n* is 20 while the number of linkage groups is 19.

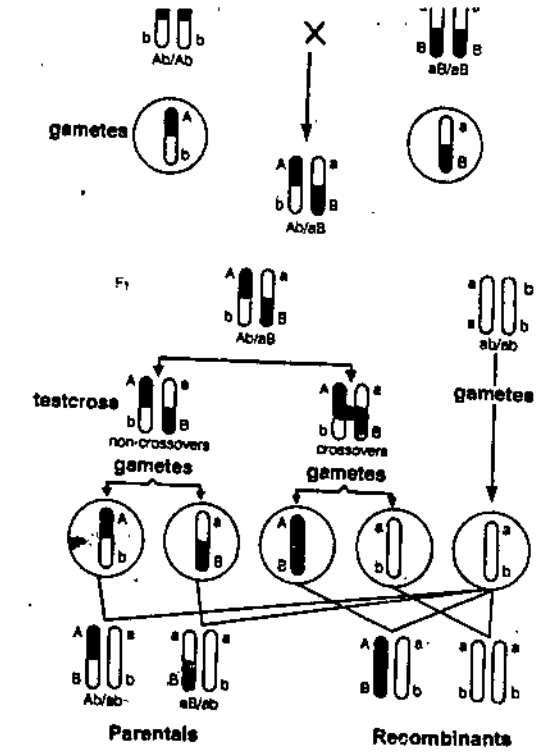


Fig. 4. Linkage. Results obtained by Hutchinson in maize, when characters coloured seeds and full seeds were taken in repulsion phase.

13.3. TYPES OF LINKAGE

The linkage can be classified as **complete linkage** and **incomplete linkage.**

(i) Complete Linkage : When two or more characters consistently appear together through two or more generations without deviation they can be regarded as complete linkage. The complete linkage is of rare occurrence. It has been observed in male *Drosophila*. When males heterozygous for two linkage autosomal genes, *AB/ab* are mated with

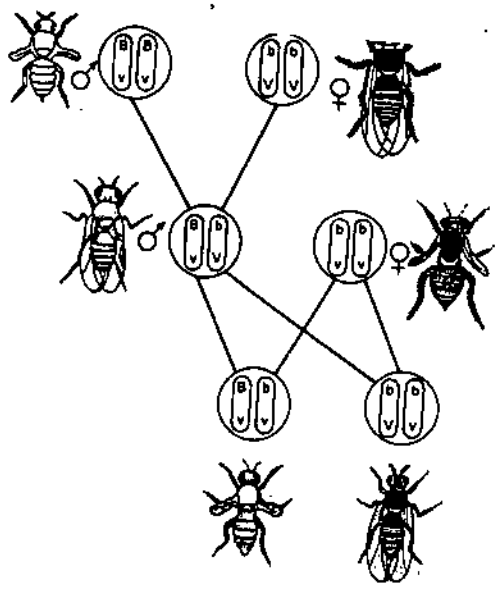


Fig. 5. Linkage. Cross in *Dropsophila* showing complete linkage

double recessive homozygous females, *ab/ab* only the two parental character combinations *AB/ab* and *ab/ab* are recovered in this progeny and there is complete absence of the recombinant types (Fig. 5). Then complete linkage occurs due to the absence of crossing over in male *Drosophila*.

Sometimes some genes are so closely linked that they may show a very low frequency of recombination and such genes are called **tightly linked.**

(ii) Incomplete Linkage : In most of the cases except in male *Drosophila* as explained earlier, linkage as a rule is incomplete, *i.e.*, the linkage is mostly incomplete. In incomplete linkage in F_1 indivdiuals, new combinations of parental characteristics are found because of recombination.

Linkage Strength : According to **Morgan** the strength of linkage is inversely proportional to the distance between the genes. If the distance between genes is more, the linkage will be weaker and if the genes are closer the linkage will be stronger. The linkage between the genes is borken by crossing over between them and if the genes are at greater distance the frequency of crossing over or the frequency of recombination increases. The frequency of crossing over or recombination percentage can be used as measurement of distance between the genes. 1% of recombinant frequency is considered as one **morgan unit** or **centimorgan**.

An example of a test cross in maize is used to work out the recombination frequencies. **Hutchinson** used the two characters, endosperm shape and colour of aleurone in maize, which were located on the chromosome IX. The coloured aleurone *C* is dominant over colourless aleurone and the full endosperm *S* is dominant over shrunken endorperm *s*. In coupling phase when parents *CS/CS* and *cs/cs* were crossed in F_1 *CS/cs* offsprings were obtined. For test cross F_1 *CS/cs* offspring were obtained. For test cross F_1 *CS/cs* were crossed with homozygous recessive parent *cs/cs*.

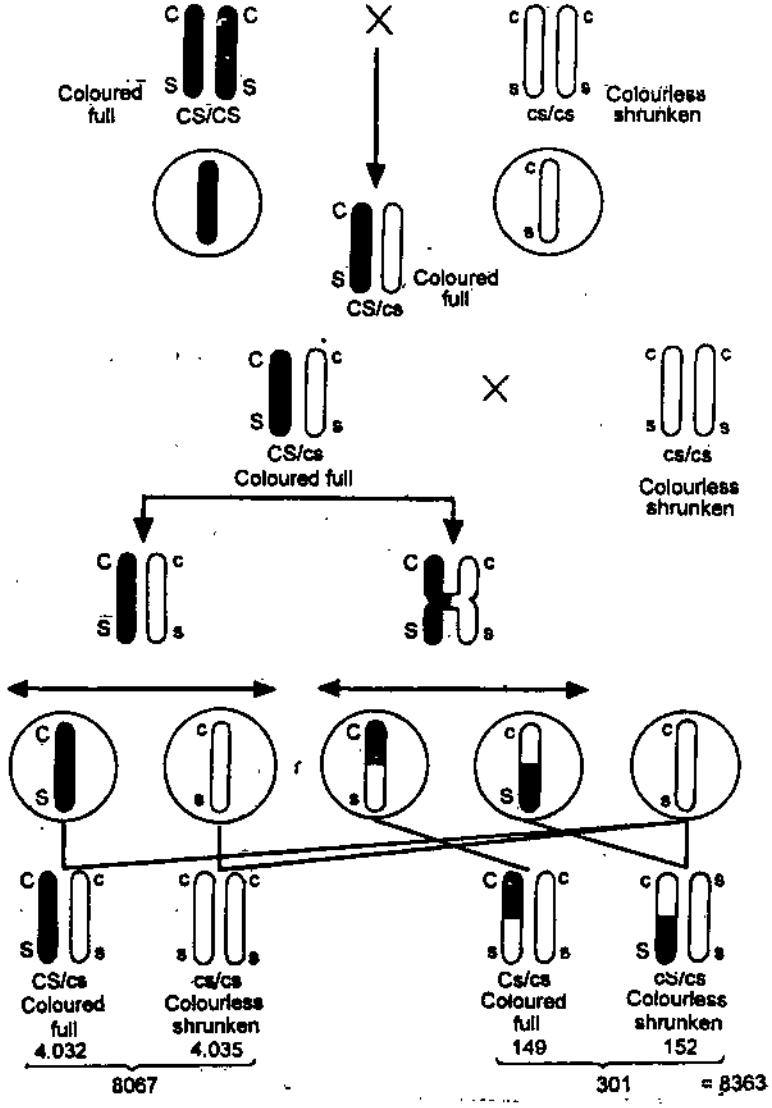


Fig. 6. Linkage. Results obtained by Hutchinson in Maize, where characters were coloured seeds.

In F_2 parental types coloured full and colourless shrunken were 4032 + 4035 = 8067. The recombinants coloured shurnken and colourless full were 149 + 152 = 301. The total number of offsprings was 8067 + 301 = 8368.

Thus, the recombination frequency in this case was $\frac{301}{8368} \times 100 = 3{\cdot}6\%$.

For transphase the cross was made betwen parents *CS/Cs* and *cS/cS*. In F_1 *Cs/cS* offspring were obtained. The F_1 *Cs/cS* were crossed with homozygous recessive *cs/cs* for test cross (Fig.7).

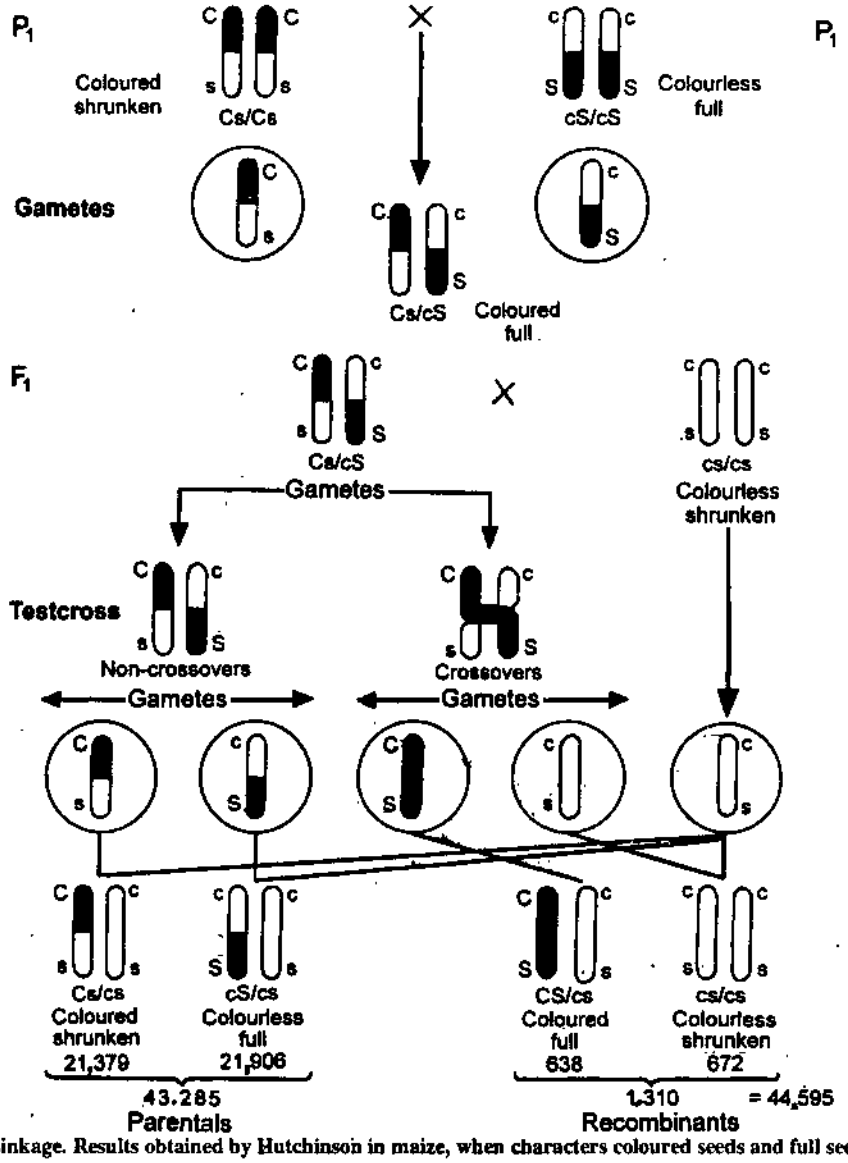


Fig. 7. Linkage. Results obtained by Hutchinson in maize, when characters coloured seeds and full seeds were taken in repulsion phase.

In F_2 the parentals or non-recombinants coloured shrunken and colourless full 21379 + 21906 = 42385 and recombinants coloured full and colourelss shrnken 638 + 672 = 1310 were obtained. The total number of offsprings was 43285 + 1310 = 44595. The recombination frequency in transphase was $\frac{1310}{44595} \times 100 = 2{\cdot}49\ \%$.

13·4. CROSSING OVER AND LINKAGE MAPS

The exchange of material between homologous chromosomes is called crossing over. This term was coined by **Morgan** and **Cattell** (1912).

Three Point Cross

A three point test cross example can be used to work out the relative distance between the genes and to find out the linear sequence of the genes on the chromosome. For example, three genes

A, B and *C* are present on one chromosome, then their possible linear order can be *A – B – C, A – C – B* or *B – A – C*. This suggests that the gene in the centre can be *B, C* or *A*.

We can make a test cross presuming that sequence of the gene is *A – B – C*. A cross is made betwee parents *ABC/abc* and *abc/abc*. In F_1 *ABC/abc* offsprings are obtained which are test crossed with homozygous pecessive *abc/abc* parents (Fig. 8).

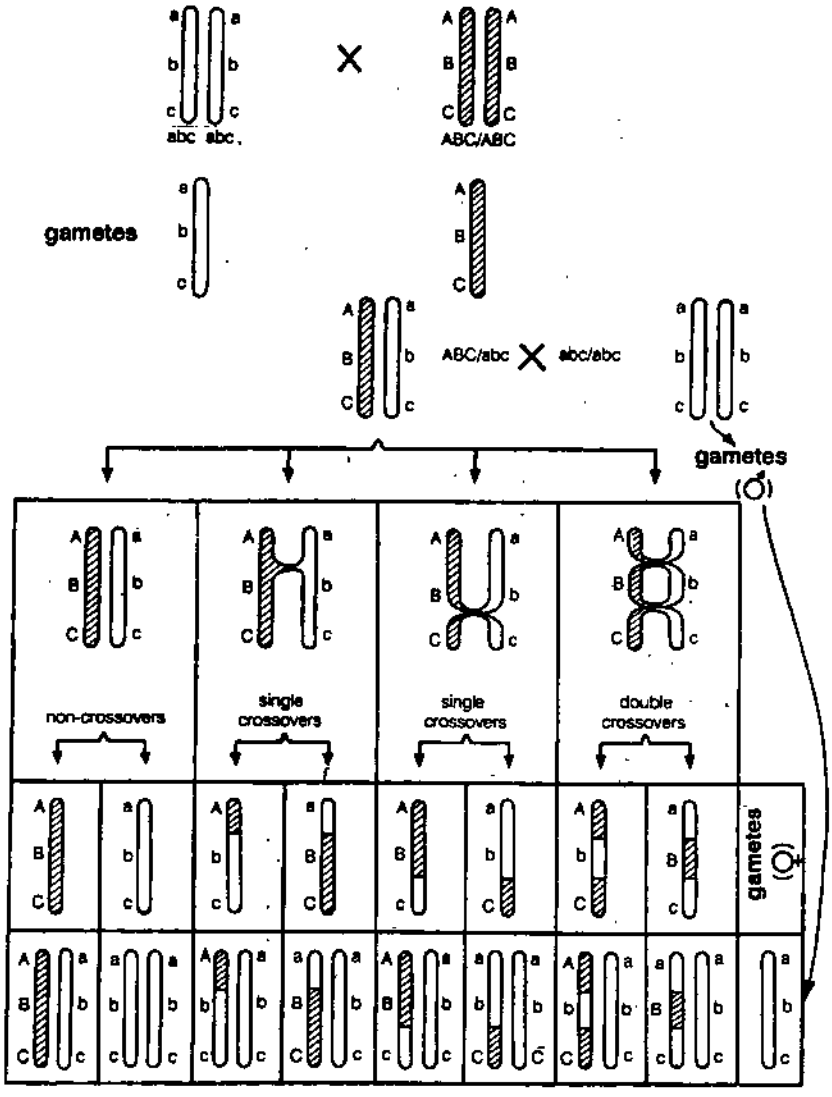


Fig. 8. Linkage. A three-point testcross involving three hypothetical genes A, B and C.

13.5. CYTOLOGICAL BASIS OF CROSSING OVER

The genes present in a chromosome can be linked. In the test cross progeny, the lack of independent assortment shows linkage and the presence of recombinants shows crossing over. The crossing over is the exchange of the concerned segments between the homologous chromosomes. Since the homologous chromosomes exchange reciprocal parts, there is no morphological difference in the resulting chromosomes. Special experiments were designed by **C. Stern** in *Drosophila* and by **Creighton** and **Mc Clintock** in maize to demonstrate that crossing over involves the actual exchange of chromosome segments.

Stern's Experiment in *Drosophila.*

In this experiment **C. Stern** used *Drosophila* flies in which female *Drosophila* had a part of the *Y* chromosomes attached to one of the two *X* chromosomes. The other *X* chromosome of this female fly was shorter than normal. Hence both the *X* chromosoms were identifiable. These two *X* chromosomes in the female flies could be distinguished from each other as well as from normal *X* chromosomes. The short *X* chromosome of female fly had the recessive gene car (carnation eye

colour) and the dominant gene *B* (Bar eye shape). The other *X* chromosome with attached *Y* part had the dominant gene Car^+ (wild type allele of car^+ expressed as dull red eye) and the recessive gene *B* + (wild type allele of *B* producing normal oval shape, Fig. 9).

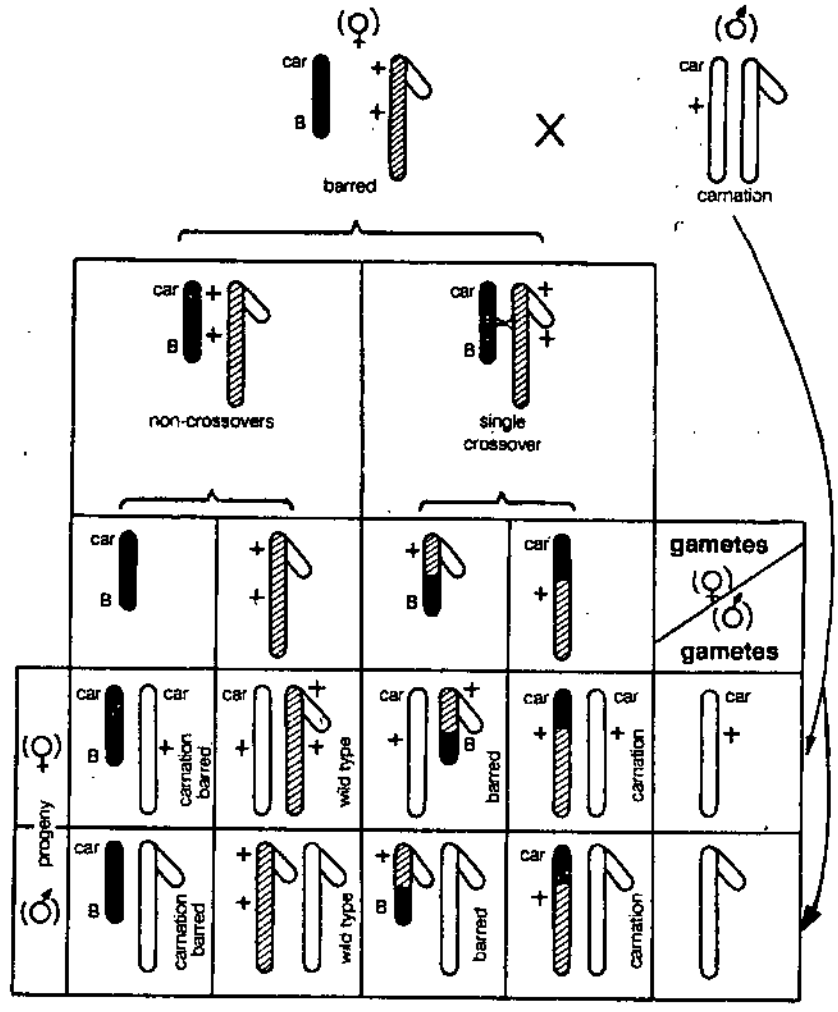


Fig. 9. Linkage. Stern's experiment showing cytological crossing over.

Stern test crossed this female to a car *B* + male. In such a cross if no crossing-over takes place between the two genes, the two types of gametes car *B* and ++ will be produced from the female flies. And if crossing-over takes place the two additional types of gametes Car + and + *B* would be formed. Due to fertilization of two types of non-cross-over and the two types of crossover gametes by the male gamete carrying *X* chromosomes Car +, four types of females are formed. Four kinds of male flies would be formed due to fertilization by male gamete carrying *Y* chromosome.

The crossover flies on the basis of phenotypes, *i.e.*, carnation with normal eye shape and barred with normal eye colour were studied cytologically. It was observed that carnation flies had normal chromsomes and not the short X chromosomes. The barred flies had a short X chromosome with segment of *Y* chromosome. This observation provided evidence of crossing over.

Stern concluded that

(i) during meiosis, there is exchange of precisely homologous chromatid segments between homologous chromosomes *i.e.*, crossing over takes place.

(ii) Crossing over is responsible for recombination between linked genes.

Creighton and Mc Clintock's Experiment in Corn

Creighton and **Mc Clintock** used a maize plant in which one chromosome 9 was normal in morphology and had the recessive gene *c* (colourless, aleurone) and the dominant gene *Wx* (non waxy endosperm). Thus, the chromosome 9 had a knob and was involved in a an unequal reciprocal translocation with chromosome 8 it had the domainant gene *C* (coloured aleurone) and the recessive

gene wx (waxy endosperm). Thus, the chromosome 9 was a hetermorphic pair and was heterozygous for the genes *C* and wx linked in repulsion phase cWx/Cwx. This plant was test crossed with the double recessive stain cwx/cwx having normal chromosomes.

If the region of chromosome between knob and *c* gene is considered as region I and that between *C* and *Wx* as region II then there would be formation of two types of non-crossover gametes. *cWx* and *Cwx* and six types of cross over-gametes would be formed (Fig. 10).

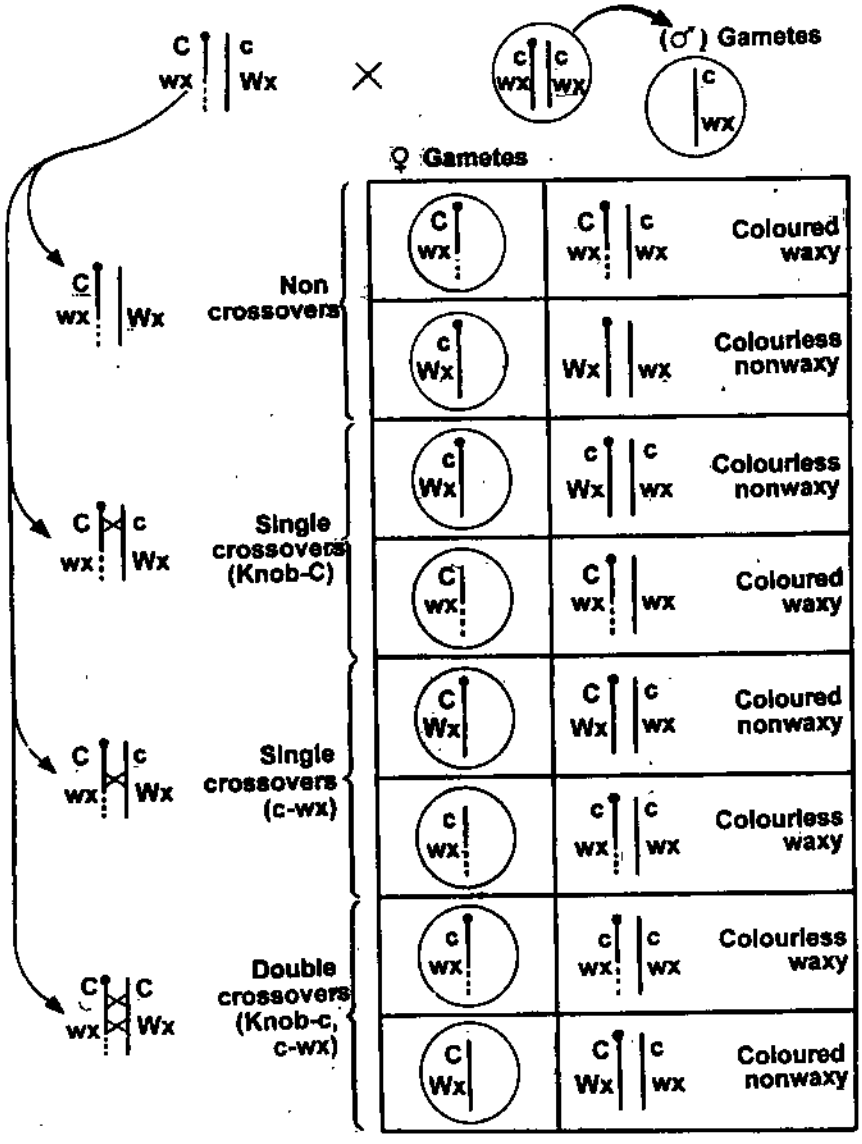


Fig. 10. Linkage. Creighton and McClintock's experiment in corn to domonstrate cytological crossing over.

The progeny can be classified into 8 types based on the phenotype and cytological observations. The following observations suggested that the exchange of chromosome segment takes place during crossing over :

(i) association of knob in the chromosomes with phenotypes colourless seed *C* and non-waxy endosperm Wx indicated crossing over betwen the knob and *C* because in the parent these were located on knobless chromosomes;

(ii) At meiotic metaphase I, the ptresence of a ring of four chromosomes without a knob suggested the actual exchange of chromosome segments;

(iii) there were no quadrivalents and only 10 bivalents were observed. The presence of the knob in one of these bivalents can be treated as an evidence for cytological crossing over, since the knob was originally associated with the translocation.

• STUDENT ACTIVITY

1. Describe the types of linkage.

2. Give cytological evidences of crossing over.

• SUMMARTY

• **Bateson and Punnett** observed that (i) in dihybrid cross when F_1 offspring was test crossed, the F_2 offsprings appeared with the ratio of 7 : 1 : 1 : 7, (ii) when the two alleles come from different parents, they enter different gametes and remain apart (repulsion), and (iii) when the two alleles come from same parent, they tended to enter the same gamete and remain together (coupling). The two phenomena *i.e.*, coupling and replusion jointly constitute phenomenon known as linkage. The genes present on a chromosome constitute one linkage group. Thus, there are as many linkage groups in an organism as the haploid number of chromosomes. The linkage may be complete or incomplete. The incomplete linkage is due to the phenomenon of crossing over. The exchange of chromatids between homologous chromsomes is calleed crossing over. The recombination frequencies are directly proportional to the distance between genes and these values can be used for preparation of linkage maps.

• TEST YOURSELF

1. Name the two aspects which phenomenon of linkage comprises.
2. Name the phenomenon which permits the exchange of chromosome segments.
3. At what stage of meiosis crossing over occurs ?
4. Who coined the term crossing over ?
5. What term is used for the degree of intensity with which two independent genes are linked with each other ?

• ANSWERS

1. Coupling, repulsion **2.** Crossing over **3.** Pachytene stage
4. Morgan and Cattell **5.** Linked value.

UNIT

14

ALLELIC AND NON-ALLELIC INTERACTIONS

STRUCTURE

- Introduction
- Two gene pairs affecting same character
- Complementary genes
- Epistasis
- Duplicating genes
- Lethality
- Student Activity
  - Summary
  - Test Yourself
  - Answers

LEARNING OBJECTIVES

By studying this chapter you will be able to know the allelic and non-allelic interactions.

14·0. INTRODUCTION

Allele is a Greek word which means belonging to one another. Alleles are the alternating form of the same gene. Generally, there are two alternate forms of a gene, hence, a gene generally has two alleles. The two alleles of a gene are located on two separate chromosomes of a homologous pair on the same locus. According to Mendelian genetics the characters are controlled by genes or factors. When we consider the inheritance of two genes together, the two genes show indepdent assortment. One character is normally controlled by one gene but sometimes a single character may be controlled by more than one, *i.e.*, two or more genes. In such cases the phenotype will be the result of **interaction of two genes.**

For example, if a chracter is controlled by two genes *A* and *B*, the phenotype will depend upon the interaction of *A* and *B* genes. If *A* gene is responsible for phenotype *A*, *B* gene is responsible for phenotype *B*, when both *A* and *B* genes are present, the phenotype may be *C i.e.*, different from both *A* and *B*.

If *A* and *B* are two genes controlling the same character and they are dominant over their respective alleles *a* and *b*, then the interaction between these two genes will depend upon :

(i) the presence of both dominant alleles *A* and *B*;

(ii) the presence of only *A* allele;

(iii) the presence of only *B* allele;

(iv) the absence of both dominant alleles *A* and *B*.

Sometimes interaction between more than two genes takes place as the character is controlled by more than two genes.

Abbreviated dihybrid genotypic ratio : When two gene pairs, each having two alleles, *i.e.*, *A a* and *B b* assort independently, the cross between heterozygotes for both pairs will give the following genotypes in F_2 generation :

Table 2 : Abbreviated genetypes.

Genotypes	Ratio	Abbreviated genotypes	Ratio
AABB	1	AB	9
AaBB	2		
AABb	2		
AaBb	4		
AAbb	1	Ab	3
Aabb	2		
aaBB	1	aB	3
aaBb	2		
aabb	1	ab	1

Abbreviated genotype 9*AB* : 3*Ab* : 3*aB* : 1*ab*.

The above abbreviated genotype can be used to interpret the interaction between genes *A* and *B* as discussed earlier.

(i) In this ratio 9*AB* means those indivdiuals which have both *A* and *B* either in homozygous or in heterozygous condition (1*AABB*, 2*AaBB*, 2*AABb* and 4*AaBb*).

(ii) 3*Ab* are those indivdiuals in which *A* is present in homozygous or heterozygous condition but *A* is absent (1*AAbb*, 2*Aabb*).

(iii) 3*aB* are individuals in which *B* is present in homozygous or heterozygous condition but *B* is absent (1*aaBB*, 2*aaBb*).

(iv) 1*aabb* indivdiual has absence of both *A* and *B* dominant forms.

Mendel believed that one factor (or gene) produced one character (allelic interaction) but production of many characters can not be explained on the basis. **Bateson** proposed factor hypothesis to explain the production and inheritance of such characters. According to this hypothesis :

(a) Several non-allelic gene pairs may interact to produce a character (gene interaction), and

(b) A single gene many participate in the production of several characters (Pleiotrophy).

However, characters like combs in fowls, fowls colour in pea, coat colour in mice and feather colour in fowls, show clear differences. Such characters are called qualitative characters. Many of these characters are produced by interaction of several genes.

Some examples of interaction between two or several genes can be as follows :

1. Two gene pairs affecting same character

In poultry the two gene pairs *R* and *P* control the shape of the comb. The *R* gene gives rise to rose comb and is dominant over non-rose type. The *P* gene gives rise to **pea** comb and is dominant over non-pea type. When both *R* and *P* are present together, a new phenotype **walnut** appears. the absence of both *R* and *P*, *i.e.*, rrpp gives rise to **single** type comb. (Fig. 1).

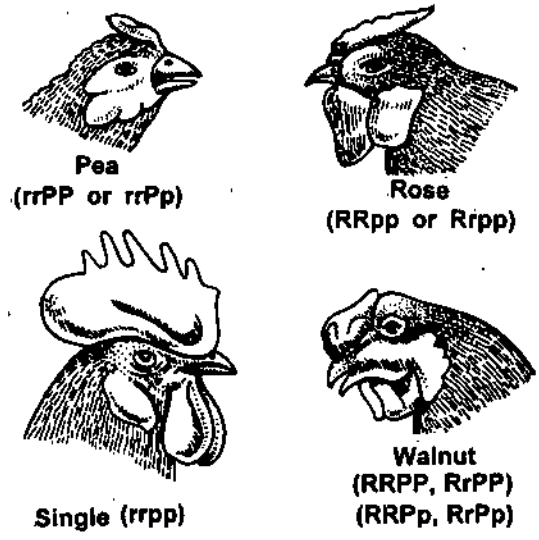


Fig. 1. Allelic and non allelic interaction. Differnt comb shapes in poultry and their genotypes.

When **W. Bateson** and **R.C. Punnett** crossed the rose comb birds homozygous dominant for *R* with pea comb birds homozygous dominant for *P*, in F_1 the heterozygous birds *RrPp* were obtained. These birds had walnut comb due to presence of both *R* and *P* (Fig. 2).

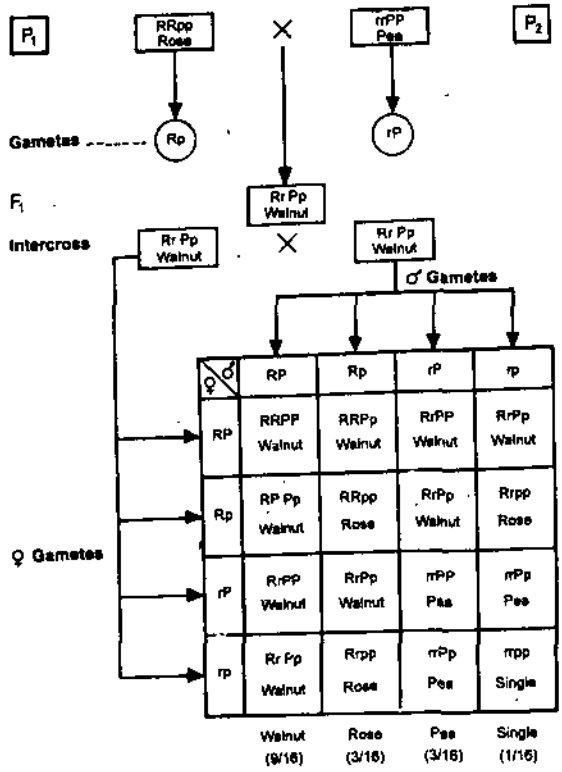


Fig. 2. Allelic and non-allelic interaction. A cross between rose and pea comb characters in poultry.

In this case there is complete dominance at both gene pairs, new phenotype results from interaction between dominant alles and also new phenotype results from interaction between both homozygous recessive alleles.

This ratio of 9 : 3 : 3 : 1 is different from normal dihybrid ratio of 9 : 3 : 3 : 1 because in simple dihybrid cross it represents the assortment of two different characters while in this case this result is for one character, *i.e.*, shape of comb in poultry.

14.2. COMPLIMENTARY GENES

In interaction of genes when the two genes control the same character and are complimenatry to each other, the presence of both genes is required for expression of a phenotype. In absence of either of the domainant alleles that phenotype will not appear.

W. Bateson and R.C. **Punnett** found that in *Lathyrus odoratus* the flower colour is controlled by two genes *C* and *P*. When both *C* and *P* alleles are present, the flower colour is purple. The *C* allele alone and *P* allele alone make the flowers white and in the absence of both dominant alleles *C* and *P* flowers are white.

When white flowered plant homozygous dominant for *C* (*CCpp*) are crossed with white flowered plant homogyzous dominant for *P* (*ccPP*), in F_1 heterozygous purple *CcPp* flower plants were obtained. When F_1 plants are selfed in F_2 the following results were obtained (Fig. 3).

The purple and white flowered plants were obtained in ratio of 9 : 7. Similar results will be obtained when homozygous dominant (*CCPP*) purple flower plants are crossed with homozygous recessive (*ccpp*) white flower plants.

The interaction is based upon the fact that genes as such do not carry character with them, instead the genes control the metabolic activities and other reactions in plants which are expressed as phenotypes. In this example both *C* and *P* are required for the development of purple pigment, the absence of any of these genes will result in lack of purple pigment. Since both *C* and *P* are required for the development of purple pigment, these genes are **complimentary.**

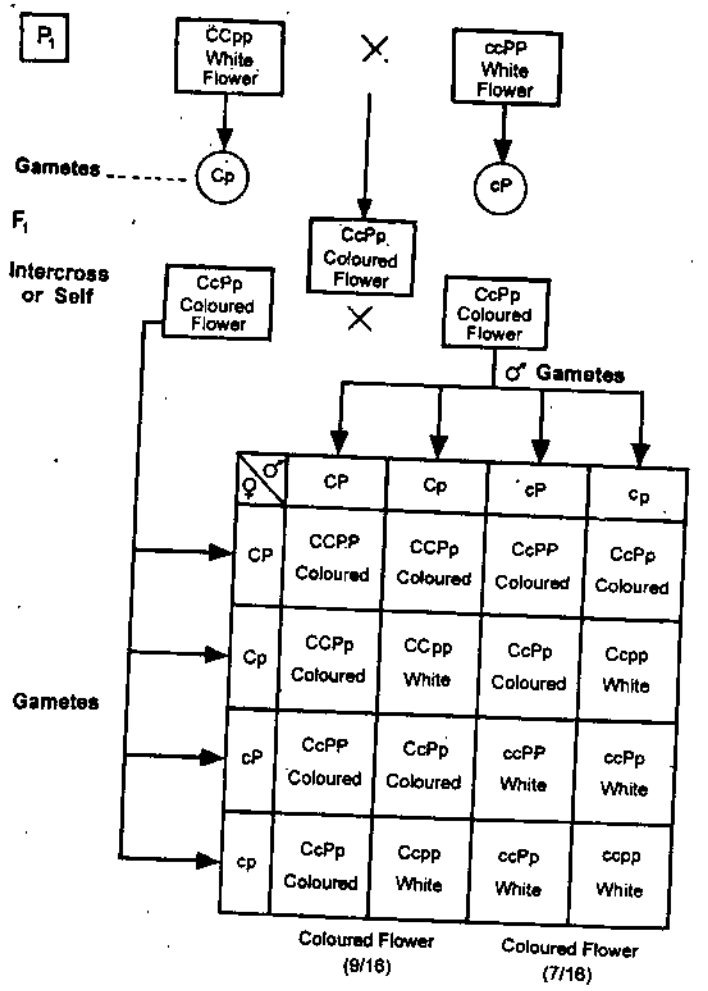


Fig. 3. Allelic and non-allelic interactions. Cross between white flowered varieties of sweet pea showing complimentary interaction.

14.3. EPISTASIS

In epistasis interaction one gene hides effect of the other gene that is one gene does not permit the other gene to express its phenotype. Epistasis is different from simple dominance. The dominance works at intragenic level while epistasis works at intergenic level.

If a character is controlled by two genes *A* and *B* geneh ides the effect of *B* gene then the gene hiding the effect, *e.g.*, A will be called as **epistatic gene** and the gene whose effect is suppressed will be called as **hypostatic gene**. The epistasis can be of following types :

(i) Dominant epistasis : The dominant allele hides the effect of other gene *e.g.*, *A* gene hides the effect of *B* gene.

(ii) Recessive epistasis : The recessive allele hides the effect of other genes, *e.g.*, *aa* hides the effect of *B*.

(iii) Mutual epistasis : In this both genes hide the effect of each other, *e.g.*, the dominat alleles *A* and *B* can be mutually epistatic to each other.

It was observed that in mice the body colour is controlled by two genes *A* and *C*. The gene *A* is hypostatic to recessive allele *cc*. The *A* gene is responsible for agouti colour in mice. It is a wild trait with characteristic bending of hair. Since gene *A* is hypostatic to *cc* the dominant allele *A* can not form agouti in presence of *cc* and such mice will be albino. *C* gene is responsible for coloured mice in absence of *A* gene. The *A* in presence of *C* will give agouti mice as *A* is not affected by *C*.

When coloured mice homozygous for *C* gene *CCaa* are crossed with albino mice *ccAA*, in F_1 agouti mice *CcAa* are obtained, on selfing of F_1 agouti mice. Agouti, coloured and albino are obtained in ratio of 9 : 3 : 4. (Fig. 4).

In this case *ccaa* mice are albino becasue of absence of both dominant alleles and *ccAA* are albino because *cc* hides the effect of dominant allele *A*.

In summer squash the fruit colour is controlled by two genes *A* and *B*. The gene *A* hides the effect of *B* gene *i.e.*, *A* gene is epistatic and *B* gene is hypostatic. The *A* gene is responsible for

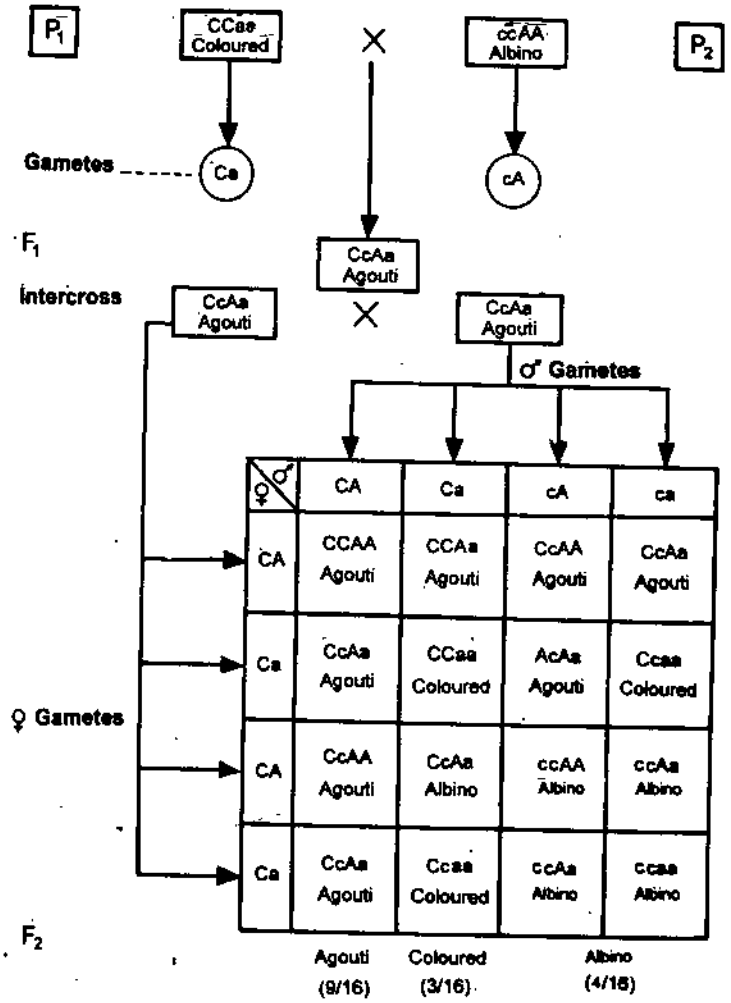


Fig. 4. Allelic and non-allelic interactions. A cross between coloured and albino mice showing epistasis.

white fruits and the *B* gene is responsible for yellow fruits in the absence of *A*. The *B* dominant allele in presence of a dominant allele *A* gives white fruit. The fruits are green in absence of both the dominant alleles.

When white fruit plants homozygous for *A* and crossed with yellow fruit plant homozygous for *B*, in F_1 heterozygous, white fruit plants were obtained. On selfing of F_1 the white, yellow and green fruit plants were obtained in ratio of 12 : 3 : 1.

		AAbb	×	*aaBB*		
		White fruit		White fruit		
		↓				
F_1	White fruit	*AaBb*	×	*AaBb*		
		↓				
F_2		9*AB* : 3*Ab*	:	3*aB*	:	1*ab*
		White White		Yellow		Green
		12	:	3	:	1

14.4. DUPLICATING GENES

In dupliating interaction when a character is controlled by the two genes, both the genes have the same phenotypic effect.

G. H. Shull observed that in Shepherd's purse plant (*Capsella*) the fruit shape is controlled by two genes *A* and *B*. The dominant allele *A* is responsible for the triangular capasules, the dominant allele *B* is also responsible for triangular capsules. Hence *A* and *B* both genes have duplicating action. The presence of both dominant alleles, *A* and *B* also makes triangular capasules. When triangular capsule plants homozygous for *AA* were crossed with triangular fruit plants homozygous

for B, in F_1 heterozygous triangular pod plants were obtained. On selfing of these F_1 heterozygous plants in F_2 the triangular and top shaped plants were obtained in ratio of 15 : 1 (Fig. 5).

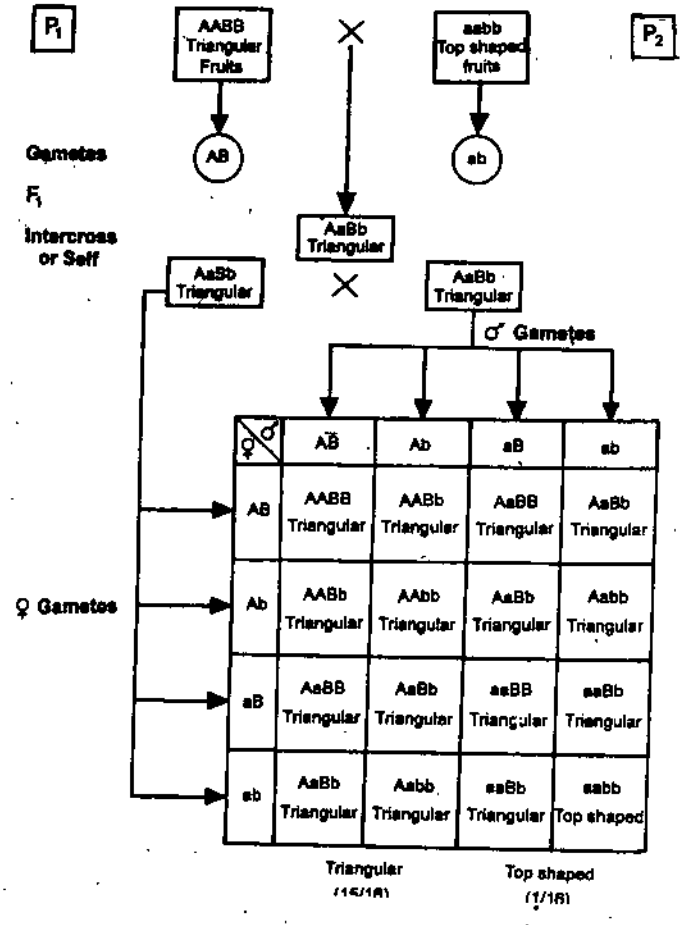


Fig. 5. Allelic and non allelic interactions. A cross between two varities of *Capsello*. One having triangular and the other having top shaped capsules.

In summer squash plant the fruit shape is controlled by two genes A and B. The gene A is responsible for spherical shape and the B gene is also responsible for spherical shape. When both dominant allels A and B are present the fruits are disc shape and in absece of both dominant alleles the fruits are long shape. If spherical fruit plants homozygous for A are crossed with spherical fruit plants homozygous for 'B", in F_1 heterozygous disc shape fruit plants were obtained. On selfing of F_1 plants disc, spherical and long shape fruit plants were obtained in ratio of 9 : 6 : 1.

$$\underset{\text{Spherical}}{AAbb} \times \underset{\text{Spherical}}{aaBB}$$

$$\downarrow$$

$$\text{Disc} \qquad AaBb \times AaBb$$

$$\downarrow$$

$$\underset{\text{Disc}}{9AB} : \underset{\text{Spherical}}{3Ab} : \underset{\text{Spherical}}{3ab} : \underset{\text{Long}}{1ab}$$

$$9 : 6 : 1$$

The interaction of genes can be of many types and depending upon the type of interaction the basic dihybrid ratio of 9 : 3 : 3 : 1 can be modified to 7 : 6 : 3, 9 : 7, 9 : 3 : 4, 9 : 6 : 1, 11 : 5, 12 : 3 : 1, 13 : 3 and 15 : 1, 1 : 4 : 6 : 4 : 1.

14.5. LETHALITY

Genes are considered to be the units of heredity which transmit the charcters from one generation to the other. Genes in true sense do not carry character with them, but genes control the various development processes or metabolism in plants and animals which are manifested as phenotypes.

There are some genes which control certain phenotypes and at the same time affect the viability of the indivdiuals. There are some genes which do not have any effect in phenotype but affect the viability of the individual. On the basis of effect of gene on survival of individuals the genes can be of following types : (i) lethal (ii) semilethal (iii) subvital (iv) vital (v) supervital.

The genes which cause the death of indivdiuals are called as lethal genes. The lethal genes on the basis of their dominance relationship can be of the following types :

(i) Recessive lethals
(ii) Dominant lethals
(iii) Conditional lethals
(iv) Balanced lethals
(v) Gametic lethals

Recessive lethals : Most of the lethal genes are **recessive lethals** as their lethal effect is expressed only when they are in homozygous state and the survival of heterozygotes is unaffected. The recessive lethals besides affecting viability of the indivdiual also affect some phenotypic traits. For their phenotypic affect these genes are dominant and the phenotypic affect is expressed in their heterozygous state.

In 1905 a French scientist **L. Cuenot** discovered a recessive lethal in mice which also affects the coat colour. He found that in mice coat colour is controlled by a gene *Y*, it makes yellow body colour, its recesive allele *y* in homozygous form produces brown coat colour. Yellow mice were always found in heterozygous *Yy* condition of homologous *YY* mice were never obtained. Later **Castle** and **Little** suggested that dominant allele *Y* is a recessive lethal, all homozygous *YY* embryos die in early development stages (Fig. 6).

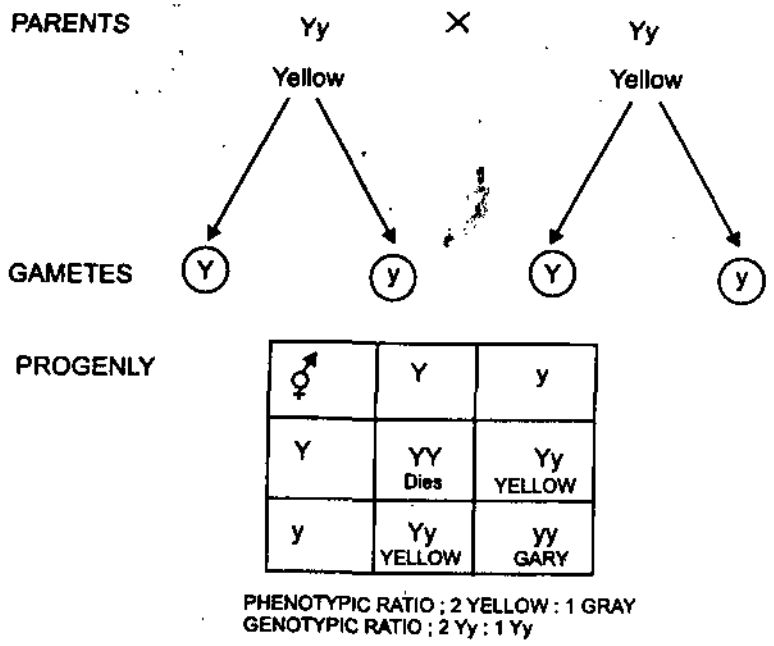


Fig. 6. Genetic inheritance of coat colour in mice showing lethality. Mice with yellow coat colour are always heterozygous.

When heterozygous *Yy* yellow mice were crossed in the first generation 25% or one foruth *YY* die due to recessive lethality, 50% or one half *Yy* yellow mice and 25% or one fourth *yy* brown mice were obtained. Thus instead of phenotypic, *radio of* 3 : 1 in monohybrid cross, the ratio 2 : 1 was obtained.

Similarly, **E. Baur** in 1907 found that when in *Antirrhinum* of Snapdragon yellow leaved dominant genotype *aurea* were crossed, in next generation heterozygous *aurea* and homozygous green plants were obtained. *Aurea* homozygotes lack the ability to make chlorophyll and died during germination or in seedling stage. Thus in *Antirrhinum* aurea gene is dominant for phenotype but recessive for lethality.

There are many examples of such recessive lethals, *e.g.*, dexter gene is cattle, creeper gene in chickens, in *Drosophila* genes for Curly wings, *Cy*, plum eyes Pm, stubble bristles and in man genes for infantile amauroic idiocy. Thallasemia major. *Achrondroplastic dwarfness and Xendoerma pigmentosum.*

Dominant lethals : The lethal genes which reduce viability in the homozygous state as well as in heterozygous state are called **dominant lethals** *e.g.*, *epiloia* gene in man. This gene causes abnormal skin growth, multiple tumors and severe mental defects. These heterozygotes die even before reaching adulthood. The dominant lethals, therefore, cannot be maintained in population and the domiant lethals have to be produced in every generation through mutation.

Conditional lethals : Some lethal genes required specific conditions to manifest their lethal effects otherwise these genes allow normal development and survivial. Such lethal genes are called conditional lethals. Some mutants of *Drosophila, Neurospora*, barley and maize show **temperature sensitive mutations** as they require specific temperature to show lethal effect. For example, the kidney eyed mutant gene of the wasp *Bracon hebetor* allows normal development at lower temperature but is lethal at 30°C or more. A chlorophyll mutant of barely allows normal chlorophyll development at a temp. of 19°C or more but at temperature bellow 8°C it produces albino seelings.

Some temprature sensitive mutants can show their effect only during a specific short period of development. In *Drosophila* a temperature sensitive mutant is lethal when larvae in the 3rd inster and exposed to high temperature. The *Drosoplila* earlier to this stage or in adult conditions are not affected by these lethal genes.

The Conditional lethals may required conditions other than temperature to be lethal, *e.g.*; some genes require light or nutritional conditions to be lethal.

Balanced lethals : In balanced lethal systems the homozygous dominant and homozygous recessive both are lethals and such individuals die. The whole population thus consists of heterozygous individuals when hetherozygous indivdiuals are crossed in F_1 only heterozygous indivdiuals survive as both homozygous dominant as well as as recessive die. The balanced lethal systems are found in *Drosophila*, mice and *Oenothera*.

$Aa \times Aa$

↓

gametes	A	a
A	*AA* Dies.	*Aa*
a	*Aa*	*aa* Dies

Balanced lethal system.

Gametic lethals : Some genes are responsible for the inviability of gametes or these gametes are incapable of fertilization. These genes are called **gametic lethals**. Due to gametic lethals there is **significant** departure from the typical segregation ratio. Hence, this phenomenon is called as **segregation distortion** or **meiotic drive**. Certain 'sex ratio' males of *Drosophila pseudoobscura* produce half the amount of sperm as compared with normal males. When these males are crossed with normal females, all their progeny are females. This happens because the sperms produced by these males have only *X*-chromosomes and *Y*-chromosome sperms are non-functional.

• STUDENT ACTIVITY

1. Lethality.

2. What is interaction of genes ? How is the dihybrid ratio of 9 : 3 : 3 : 1 can be modified in each of these reactions ?

• SUMMARY

- The genes present on different chromosomes interact with each other. The shape of combs in fowl is determined by two pairs of genes, *R* and *P*. The *R* gene gives rise to rose comb and is dominant over non-rose type. The *P* gene gives rise to *P* comb and is dominant over non-pea type. When both *R* and *P* are present together, a new phenotype walnut appears, the absence of both *R* and *P*, *i.e.*, *rrpp* gives rise to single type of comb. In *Lathyrus odoratus* a gene c compliments the expression of colour gene *P*. This is called as complimentary gene. The F_2 ratio of the cross between purple and white flower is 9 purple : 7 white. When one gene influences the expression of another gene, the pheomenon is called as epistasis. Different types of epistatis behaviourc have been obsérved in different cases. In duplicating interaction a character is controlled by the two genes, both the genes have the same phenotypic effect. The fruit shape in *Capsella* is controlled by duplicate genes and here the F_2 ratio is 15 triangular : top shaped. The genes which cause the death of indivdiuals are called lethal genes.

• TEST YOURSELF

1. How many pairs of gene determine the combs in fowls ?
2. What term is given to an interaction between non-alletic genes in which one allele at a particular locus prevents the expression of an allele at another locus but not vice-versa.
3. Name the gene which causes the death of indivdiuals.
4. What is the ratio of recessive epistasis ?
5. In *Lathyrus* the dominant gene *P* is responsible for corolla colour and the dominant gene C compliments the expression of gene *P*. If a cross is made between two genotypes *ppCc* and *Ppcc*. What will be the percentage of coloured offsprings ?

• ANSWERS

1. Two **2.** Epistasis **3.** Lethal gene **4.** 9 : 3 : 4
5. 25.

UNIT

15

GENE EXPRESSION

STRUCTURE

LEARNING OBJECTIVES

By studying this chapter you will be able to know the chemical nature, location, fine structure and diferent types of genes.

15.0. INTRODUCTION

The concept of gene has undergone many transformations from the premendelian days to the present time. First **Charles Naudin**, a botanist, postulated the existence of **'essences'** in gametes. The essences were considered to be responsible for the characteristics of the species. **Mendel**, on the basis of his experiment, proposed that 'factors' were responsible for transmission of characters. The factors were discrete bodies which segregated in offspring and remained independent and intact even in those offsprings where the trait was not visible. The factors did not mix and maintained their discrete particulate nature through generations. The factors alternate forms were manifested as dominant and recessive traits. A similar idea was put forward by **Darwin** as **pangenesis** and by **Hugo de Vries** as **Intercellular pangenesis**. The term **gene** was coined by **Johannsen** in 1903 for the hereditary factors of **Mendel.**

15.1. CLASSICAL CONCEPT OF GENE

Weismann 1887 suggested that vital units were arranged on chromosomes and were distributed to succeeding generations. **T.H. Morgan's** group at Columbia University, New York demonstrated the association of inherited traits with the presence and segregation of particular chromosome. The cytological observations of chromosomes were published by **Morgan** in 1915 in the book **"The Mechanism of Mendelian Heredity"**. The chromosome theory of heredity was

later updated in terms of the gene and was published in the book **"The Theory of Gene"** in 1926. On the basis of the theory of gene proposed by **Morgan,** following conclusions can be drawn as the classical concept of gene :

1. Both male and female parents contribute equally to the inheritance of characters to next generation, *i.e.,* the inheritance is biparental.

2. Genes occur in pair of alternate states called alleles, each member of a pair is transmitted to separate gamete. Random matings between gametes occur which reassociate two alleles in the zygote cell.

3. Several genes are present in each chromosome and the genes have definite fixed position in a particular chromosome, the change in locus can be brought about by mutation.

5. Genes are arranged in a linear sequence on chromosome.

15·2. THE CHEMICAL NATURE OF GENE

Chromosomes are the physical bearers of heredity as the genes are located in chromosomes. The chromosomes are made up of proteins and nucleic acid. Either proteins or nucleic acid could have been the genetic material. The proteins were considered to be the likely genetic material. The complexity of protein structure and the potential for infinite variations in protein structure were strong indication that proteins could be the genetic material. However, the transformation of T_{20} bacteriophage by **Griffith** in 1928 nad later by **Hershey** and **Chase** in 1952 proved beyond doubt that the genetic material was chemically DNA and not proteins.

Chromosomes consist of DNA, proteins and a varying amount of RNA. The genes are not made of proteins as the proteins associated with sperm heads differ in composition from histones of other tissues. The RNA also can not be the genetic material as sperms contain very little amount of RNA. Thus DNA must be the genetic material.

The DNA content of all cells of a species of an organism is constant. the DNA contents are halved in the gametes. The cells of similar hereditary origin have DNA of similar composition, *i.e.,* the relative amount of the nucleotides is constant. Gené mutations are very much correlated to the alteration of DNA structure. All these evidences indicate that the genes are made of DNA. The double helix model of DNA proposed by **Watson** and **Crick** in 1953 illustrated that different regions of the DNA could be delineated as discrete genes.

15·3. THE LOCATION OF GENES

It was suggested by **Belling** in 1928 that chromomeres which appear as series of granules on chromosomes might be the genes. The later studies showed that chromomeres are the sites of genes. The electron microscopic studies suggest that the chromosomes consist of **axial filaments** from which arise **chromatin fibre loops.** The density of loops in more is the chromomeric part of the chromosome. According to **Callon** and **Llyod** (1960) each loop of lampbrush chromosome is related with the activity of gene. The loop may possibly contain several identical **"slave genes"** controlled by a **'master gene'** (Fig. 1) **Thomas** (1969) also suggested that each chromomere represents a functional gene, *i.e.,* a master gene wtih many slave genes.

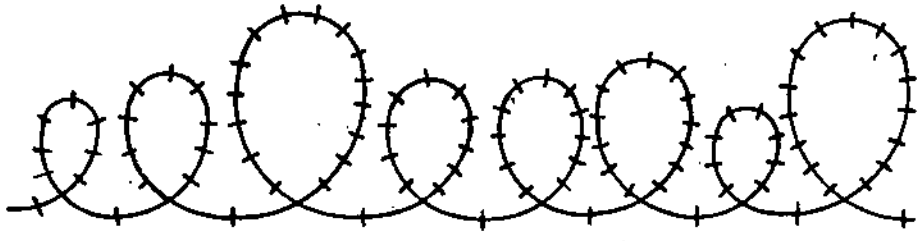


Fig. 1. Gene expression. the 'master gene' and 'slave gene' concept (Callon and Llyod).

Beerman's (1967) studies on mutation suggest that in polytene chromosomes the dark bands represent genes. However, **Watson** (1970) suggested that the interband region of polytene chromosomes also contains genes. There is one-to-one relationship between chromomeres and genes, usually the number of genes is much more than the number of chromomeres. **Coming** (1972) suggested that chromomeres may represent families of related genes.

The genome and the plasmon : The total hereditary material may be divided into **genome** and the **plasmon**. The **genome** can be expressed as the total sum of genes present on haploid set of chromosomes. The term plasmon was coined by **Fritz van Wettstein in** 1924. The plasmon is the total heriditary material outside the chromosomes. **Darlington** in 1939 introduced the term **plasmagene** which is the smallest heritable unit of the plasmon. The plasmagenes are known by different names depending upon their location in cell organelle, for example the plasmagenes of plastids are called **plastogenes,** of kinetosomes are **kinetogenes,** of mitochondria are **chondriogenes** and those of centrioles are called **centriogenes.**

15.4. FINE STRUCTURE OF A GENE

Earlier the genes were considered to be the structural and functional unit of heredity. The genes are now considered as a unit of function (cistron), a unit of recombination (recon) and as a unit of mutation (muton).

Cistron : The **one gene-one enzyme** hypothesis was first proposed by **Garrod** in 1908. **Beadle** and **Tatum** in 1941 clearly demonstrated one gene-one enzyme hypothesis. The gene was considered to be the unit for coding the synthesis of single enzyme. The gene was thus identified by its product enzyme.

Since one gene forms a mesenger RNA, the hypothesis can also be called as **one gene-one messenger RNA**. This concept can also be expressed as one gene-one protein, since the mRNA molecule serves for coding of a protein. Some enzymes or proteins consist of more than one polypeptide chain, hence, the hypothesis can be stated as **one gene-one polypeptide chain.**

The functional unit of gene has been called cistron by **Benzer.** The cistron can be defined as the segment of DNA which represents the unit of function, it consists of linear sequence of nucleotides which controls some cellular functions. In most of the cases the cistron is equivalent to the gene. In *E.coli* the cistron may contain about 1500 base pairs and in some cases there may be as many as 30000 nucleotide pairs in a cistron. The number of nucleotides in a cistron is equal to

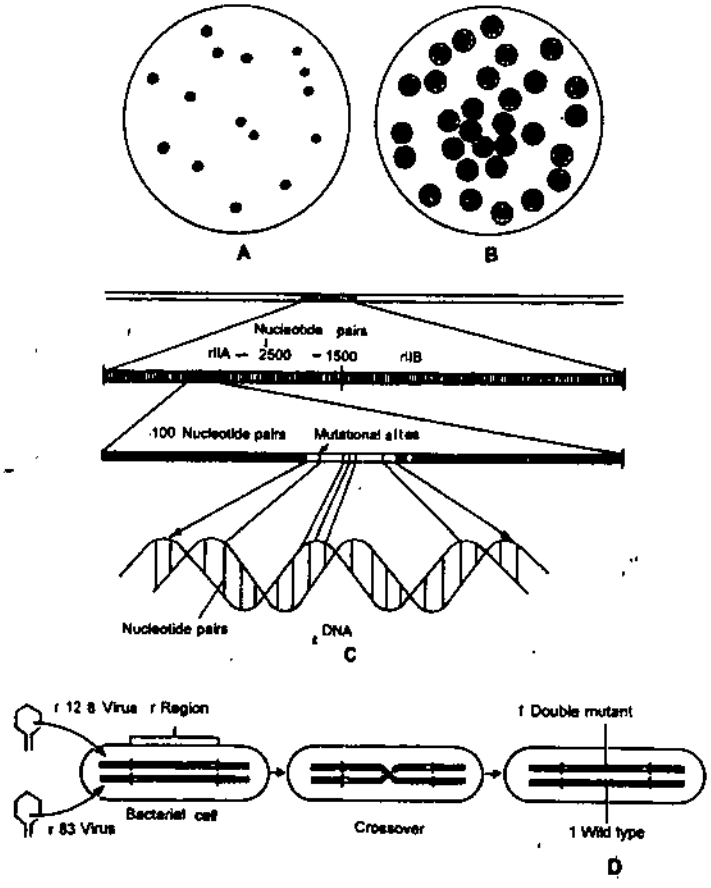


Fig. 2 (A-D) Gene expression. Normal smooth plaques of the T_4 bacteriophase, (B) The rough-edged plaques of the mutant T_4r (C) the r_{11} region of the T_4 bacteriphage (virus) and its relationship with the structure of DNA, (D) Demonstration of the fact that crossing over takes place within the gene.

three times the number of amino acids in polypeptide chain, it synthesizes. The cistron starts with an initiating codon and ends with a termination codon. Each cistron is responsible for coding one messenger RNA molecule which later on forms a polypeptide chain. Cistron occupies much greater chromosomal length than mutons and recons. It has been observed that hundreds of units of mutation (mutons) and recombinations (recons) can exist within one cistron.

Recon : Recon is the smallest unit of DNA capable of recombination. Earlier the gene was considered to be shortest segment of chromosome which could be separated from its adjacent segment by crossing over.

The crossing over was considered to be **intergenic** phenomenon. Crossing over was supposed to occur between the genes and not within the gene. The gene was thus indivisible unit of chromosome controlling a phenotypic character. The recombination experiments in viral DNA strands have shown that crossing over can take place within the genes, *i.e.,* crossing over is **intragenic** or **interallelic** phenomenon. The **recon** is the smallest unit capable of recombining genetically. Structurally the recon may consist of one or two pairs of nucleotides.

Benzer 1955 demonstrated that in T_4 bacteriophage the crossing over takes place within the gene. The T_4 bacteriophages when cultured on agar plates where the normal wild types form smoth colonies.

The mutant rII bacteriophages form larger, rough edged plaques. **Benzer** found that two adjacent genes rII and rIIB were responsible for the rough edged plaques. The gene mapping studies have shown that in rIIA gene there are at least 500 mutational sites where crossing over could take place. When crossing over occurs within the gene, the mating of two rII mutants can form normal wild type. **Benzer** found that the lowest frequency of crossing over was 0.2% and the crossing over can occur between the two adjacent nucleotides of DNA

Muton : The shortest chromosomal unit capable of undergoing mutation was called **muton** by **Benzer.** The unit of mutation was earlier considered to be the gene. Later studies *e.g.,* haemoglobin studies have shown that mutation can take place in a pair of nucleotides. Hence, the muton consists of one or many pairs of nucleotides within the DNA molecules.

The cistron, recon and muton are the functional, recombination and mutational units of DNA sequence or a gene, cistron is the largest unit while recon and muton can consist of one or two pairs of nucleotides.

15·5. CLASSIFICATION OF GENES

Muller (1932) divided the genes into five categories on the basis of relation between a particular gene and standard gene.

(i) Hypomorphs : These genes have the same effect as the standard gene but their expression is less effective. The hypomorph genes can cause deficiency and pseudodominance can be expressed.

(ii) Amorphs : These genes have very little effect as compared to the standard genes. The addition of such genes has hardly any effect on phenotypes.

(iii) Hypermorphs : These genes have greater effect than the standard genes.

(iv) Antimorphs : These genes have effect opposite to that of the standard genes. The standard genes have normal expression and the addition of antimorphs will give abnormal phenotypes.

(v) Neomorphs : these genes are reverse of amorphs. Their effect can not be detected. When added these genes give results different from the standard genes.

15·6. NUMBER OF GENES

The number of genes present is an organism has relationship to its complexity. Viruses require few genes as they can produce messenger RNA but depend upon host cell for tRNA, rRNA and enzymes. The bacteriophages R17 and QB have single stranded RNA as genetic material which cotnains only three genes. Of these one codes for A protein, the second for coat protein and the third for one of four subunits of replicas. SV40 viruses have 5–10 genes and their DNA is 1.7 micron in length. Virus φ× 174 has single stranded DNA which codes for 9 proteins. Bacterial virus λ has about 40 genes and T_4 bacteriophages have about 100 DNA which codes for 9 proteins. Bacterial virus λ has about 40 genes and T_4 bacteriophages have about 100 or more genes. Generally, the number of genes in viruses ranges from 3 to 250.

In bacterium *E.coli* the chromosome is about 1 mm long and it contains about 4000 genes and 4,00,000 base pairs. In *Drosophila melanogaster* the estimated number of genes is 5000 on the basis of observation of bands in salivary gland chromosomes. However, according to **Gower** and

Gay in *Drosphila* there are about 10,000 to 15,000 genes. The normal diploid human cell contains about 5 picogram (5×10^{-12}) of DNA having (5×10^{9}) base pairs. If the average gene contains 1000 base pairs the number of genes in haploid human cell would be about 5 million, but this number in actual condition is quite less as all DNA is not used for coding proteins.

Number of nucleotides in average gene: It is possible to find out the number of nucleotides per gene, if we know the molecular weight of the gene, as one nucleotide pair has molecular weight of about 600. In T_4 bacteriophage the chromosomal molecular weight is 120 million and there are 100 genes. Thus the molecular weight of the gene is 1.2 million. Since the molecular weight of a nucleotide is 600, there should be 2,000 base pairs per gene.

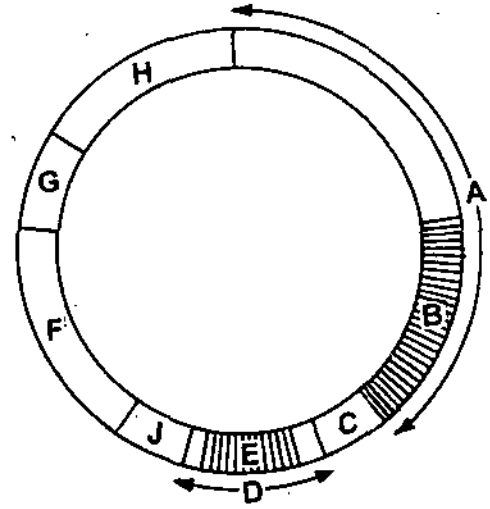


Fig. 3. Gene expression. Genetic map of the bacteriophage X174. Note the overlapping of gene E over gene D and of gene B over gene A.

15·7. OVERLAPPING GENES

The synthesis of proteins is under direct control of DNA, according to dogma of the genetic code one gene is responsible for coding of one protein or polypeptide. The hypothesis "One gene-one protein/enzyme was proposed by **Beadle** and **Tatum** in 1940. **Barrel** (1976), first gave evidence that suggests the possibility of overlapping of genes in bacterial virus $\phi \times 174$. **Barrel** showed that a gene can be read or translated in two different ways to form two different proteins. Genetic mapping has shown that bacteriophase $\phi \times 174$ has 9 genes $A - B - C - D - E - J - F - G - H$ which code for 9 proteins (Fig. 3).

The functions of these genes are :

Gene A — DNA replication

Gene B and D — assembly of phage particles

Gene C — function not known

Genes, F, G and H – for structural capsid protein.

According to characteristics of genetic code each amino acid is coded by a triplet codon. In almost all organisms the total length of DNA molecule required for coding protein exceeds the total length of protein product. However, in $\phi \times 174$ the total length of protein coded is more than the length of DNA coding it. **Sanger** (1976) found that $\phi \times 174$ DNA contains 5386 nucleotides. As each codon is a triplet, these nucleotides can code a maximum of about 1800 amino acids having a total weight of about 2,00,000 daltons. But the proteins coded by these genes have molecular weight of about 2,50,000 daltons. This phage DNA whch has coding capacity of about 5–6 average size proteins actually codes for 9 proteins. The comparison of the DNA base sequence with the amino acid sequence revealed that in two cases the same gene coded for two different proteins. According to **Sanger** the gene B (360 nucleotides) was completely contained within gene A (1536 nucleotides). The gene E (273 nucleotides) was contained in gene D (1456 nucleotides). The protein synthesized by gene B was completely different from protein synthesized by gene A. Similarly, the proteins of genes D and E were different. The difference in amino acid sequence was due to difference in reading frame of these genes.

The gene *D* had its 'stop' codon or terminating codon overlapping the start codon or initiating codon of gene *J* (Fig. 4).

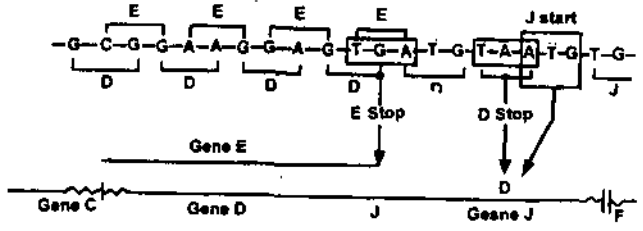


Fig. 4. Gene epxression. Overlapping and included genes.

Similarly, in bacteriophage G_4 the gene *B* is completely contained within gene *A* and gene *E* is completely within gene *D*. Gene *K* is made up of the last 86 nucleotides of gene *A* and the first 89 nucleotides of gene *C*. The significance of overlapping gene is economy of DNA in prokaryotes. The short DNA of prokaryotes can code for all needed proteins because of overlapping genes (Fig. 5).

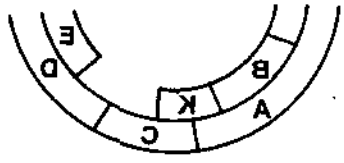


Fig. 5. Gene expression. A part of the genetic map of the virus G4 showing overlapping genes.

15·8. SPLIT GENES

According to characteristics of genetic code, the code is comma-less, *i.e.*, a gene coding the synthesis of a polypeptide has continuous sequence of nucleotides. The study of 'hexon' gene of adenovirus and the ovalbumin gene of chickens showed that DNA sequence coding for a polypeptide is not continuous.

Hexcon Gene : The DNA of adenovirus is double stranded and coded for about 20 proteins, eight of these genes transcribe mRNA late in virus life and are called "late genes". These eight late genes transcribe a single RNA which is later cut into eight mRNAs. The total length of the eight mRNA's is less than the length of the original transcript. When the hexan gene of these eight genes was paired with its mRNA, the DNA formed three loops which represent the non-transcribed region of DNA. Thus the hexon mRNA was transcribed by four different regions and the four segments were then joined together. Hence, the hexon gene is a split gene in which there are untranscribed parts along with transcribed parts.

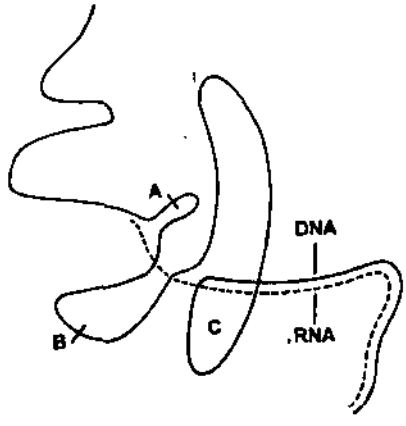


Fig. 6. Gene expression. The hexon gene of adenovirus with its transcribed mRNA. The loops (A, B, C) represent regions of DNA which do not transcribe RNA.

15·9. OVALBUMIN GENE

P. Chambon's group (France) and **B.W.O' Malley's** group (USA) showed that the ovalbumin gene of chicken is a split gene. The gene is not continuous and is made of segments scattered in the chromosome. The gene has many silent zones which are not transcribed into polypeptide chain. The ovalbumin gene transcribes precursor RNA (*p*RNA) which contains intervening sequences not

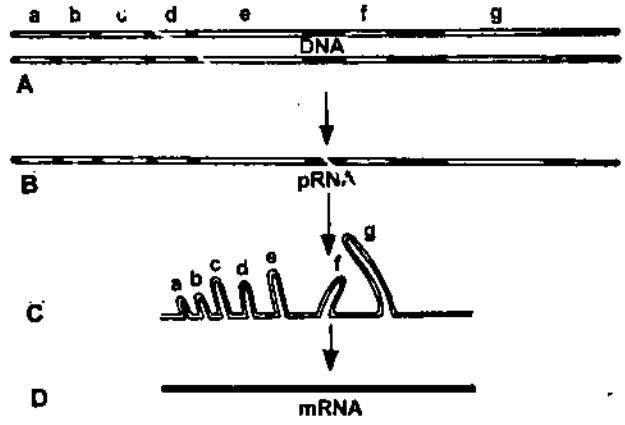


Fig. 7. Gene expresion. Transcription and process in mRNA of the ovalbumin gene. (A) Ovalbumin gene with coding sequences (dark) and noncoding sequences (light), (B) precursor RNA (pRNA) transcribed by DNA (C) Noncoding sequence of pRNA loop out, (D) mRNA after excision of loops.

found in *m*RNA. The precursor RNA loops out these intevening sequences which are excised to form in *m*RNA (Fig. 7).

The other examples of split genes are β-globin genes of mice and rabbits. *t*RNA genes of yeast and ribosomal gene of *Drosophila.* The discovery of split genes is against the concept of colinearity of genes.

15.10. JUMPING GENES

According to Classical Theory of Genes, a gene has a definite site in the chromosome and remains intact until mutation or recombination takes place. Now there are reports that the genes can change site even without mutation and recombination. Such genes are called jumping genes. Both lower and higher organisms have gene jumping with frequencies as high as 10^2. **Mc Clintock** (1951) showed the presence of jumping genes in maize. *e.g.*,'Dissociation' Ds and 'Activator' Ac gene. The Ds gene is active only in the presence of Ac and can physically shift sites within and between the chromosome with considerable phenotypic effect. **Mc Clintock** called these genes '**controlling elements**' as these genes Ds and Ac regulate the activity of other genes at the DNA on chromosomal level and can cause mutation.

15.11. CHEATING GENES/SELFISH GENES

It is common observation that every gene has equal chance of being transmitted to the progeny and to form new gene combinations. These genes are **faithful genes** as these are inherited freely and fairly to the next generation. Recently some **selfish genes** are separated which help in perpetuating themselves in population by tricking in reproduction process to favour themselves. Such genes are called **cheating genes**. These genes ensure their own inheritance at the cost of other genes linked with them. These genes are reported to be present in corn, tobacco, grasshoppers, mosquitoes, mice and *Drosophila.*

In Drosophila males have *XY* chromosomes and females have *XX*. The female produces all identied gametes with *X* chromosomes. The male produces 50% gametes with *X* chromosome and 50% gametes with *Y* chromosome. On crossing the progeny is expected to have 50% males and 50% females. But *Y* chromosome has sometimes cheating genes, when only *Y* chromosomes are inherited the entire progeny will be male and no females.

• STUDENT ACTIVITY

1. Describe the modern concept of the structure of gene in brief.

2. Describe how the genes can be classified.

• SUMMARY

The term gene was coined by **Johannsen** (1903). Genes are arranged in a linear sequence on chromosomes. Genes are made of DNA. Earlier the genes were considered to be the structural and functional unit of heredity but now-a-days genes are considered as a unit of function (cistron), a unit of recombination (recon) and a unit of mutation (muton). **Muller** (1932) divided the genes into five categories on the basis of relation between a particular gene and standard gene. These categories are : hypomorphs, amorphs, hypermorphs, antimorphs and neomorphs. The number of genes varies from organism to organism. The number of genes in virus ranges from 3 to 250. However, in bacteria the number is about 4000.

• TEST YOURSELF

1. Name the heredity material present outside Chromosome.
2. Name the smallet subunit of gene capable of replication.
3. What is the name of the smallest unit of DNA capable of recombination ?
4. Name the genes which accomodate large amount of extra DNA.
5. Who first of all suggested one gene-one enzyme hypothesis ?

• ANSWERS

1. Plasmon **2.** Replicon **3.** Recon **4.** Split-genes **5. Garrod.**

❑❑❑

UNIT

16

REGULATION OF PROTEIN SYNTHESIS

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know operon model, structure and classification of proteins.

16.0. INTRODUCTION

According to Central Dogma the flow of genetic information takes place from DNA to RNA to proteins. The proteins and enzymes that control all growth and metabolic process are thus synthesized under control of DNA or the genes. The genetic make up of all the cells in any animal and plant body is always identical *i.e.,* all the genes are present in all cells. Different genes in an organism control synthesis of different proteins. The requirement of a particular protein or polypeptide is different at different times at different places and in different amounts. For example, the enzymes required for germination of seeds will not be required at the time of flowering, the proteins required at the site of photosynthesis will not be required in roots and the enzymes required for digestion of food vary in amount depending upon the nature and amount of the substrate. Thus regulation of protein synthesis is also required for various metabolic activities which occur only at specific times and places in a cellular organism.

A basic question arises as to how the expression of genetic information is regulated in the cells. Since every cell contains identical set of genes, there must exist a control mechanism which would permit some desired genes to function at a particular time while the activity of the other genes must be completely checked. Hence at any time certain genes become active while others remain inactive *i.e.,* the genes are "switched on" and "switched off". This process is called differential gene action. A variety of mechanisms have been discovered for regulation of protein coding action at both transouptional and translation levels in prokaryotes and enukaryotes. The regulation at the transcriptional level involves the regulation of DNA to yield mRNA coding for a specific protein and the regulation at the translational level involves the control of enzyme synthesis. A precise, meticulous regulation of gene expression makes organisms capable of rapid adaptation to varying nutritional and environmental conditions.

16·1. REGULATION IN PROKARYOTES

Inducible and repressible systems :

In *E.Coli* the enzyme β galactosidase is required for hydrolysis of lactose into glucose and galactose.

$$\text{Lactose} \xrightarrow{\beta \text{ galactosidase}} \text{glucose + galactose}$$

The enzyme β galactosidase is either completely absent or present in traces when lactose, the substrate is not supplied in the system. The concentration of the enzyme can quickly increase a thousand times or more when the substrate is present in the medium. The quantity of the enzyme again drops down as quickly as the substrate is removed. The enzymes, whose synthesis canbe induced by addition of the substrate are called **inducible enzymes** and the genetic mechanim of control of synthesis of inducible enzyme is known a **inducible system**. (Fig. 1). The substrate whose addition induces the synthesis of enzyme is called **inducer**. In inducible systems the genes for synthesis are kept shut due to formation of **repressors.** The inducer makes active repressor inactive to start synthesis.

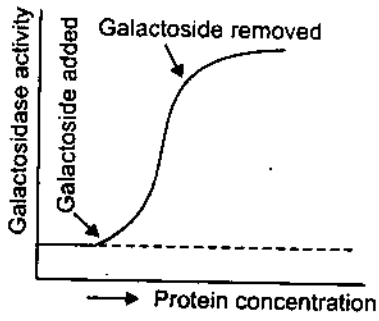


Fig. 1. Regulation of protein synthesis. Inducible System-β-galactosidase activity after addition of Lactose.

Active repressor + inducer = inactive repressor

The synthesis of catabolic enzymes is mainly regulated by inducible system. The cell synthesizes such enzymes only when the enzymes are required for the initialization of the substrate *i.e.*, the catabolic enzymes that the cell is potentially able to produce are not synthesized until they are required.

Many enzymes are synthesized continuously without the requirement of an inducer. In such system the addition of end product of biosynthetic pathway stops the synthesis of the enzyme. The enzymes whose synthesis can be stopped by addition of end product are called repressible enzymes and the genetic mechanism of their regulation of synthesis is called repressible system. For example, in *E. Coli* the synthesis of all enzymes required for synthesis of amino acids takes place but the addition of end product like histidine will stop the synthesis of histidine synthesis enzymes (Fig. 2).

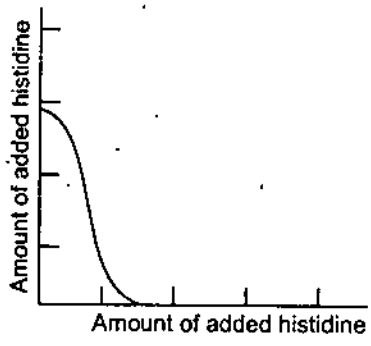


Fig. 2. Regulation of protein synthesis. Gene expression. Repressible system of histidine synthesis enzyme.

In repressible systems the end prioduct functions as corepressor which makes inactive repressior active to top the synthesis of the enzyme.

Inactive repressor + corepressor = active repressor

The synthesis of anabolic enzymes is mainly regulated by repressible systems. For metabolic economy when acumulation of end product takes place in reasonable amount, all enzymes required for biosynthetic pathway are not produced.

16·2. THE OPERON MODEL

Francois Jacob and **Jacques Monod** (1961) studied the induction of β galactosidase enzyme in *E.coli* and proposed a model for molecular and genetic relationship between enzyme induction and enzyme repression. Their model is popularly known as **Operon model.** For their significant contribution **Jacob** and **Monod** were awarded the Nobel prize in Medicine in 1965. In operon model the **operon** represents a unit of co-ordinated control of protein synthesis. The operon consists of a regulator gene, promoter gene, operator gene, structural gene repressor, corepresor and inducer (Fig. 3).

The Structural gene : The structural genes direct by synthesize proteins through messenger RNA and determine the sequence of amino acids in polypeptide chains. The structural genes synthesize mRNA under the operational control of operon gene. the number of structural genes in any regulatory system is equal to the number of proteins or polypeptide chains produced in that

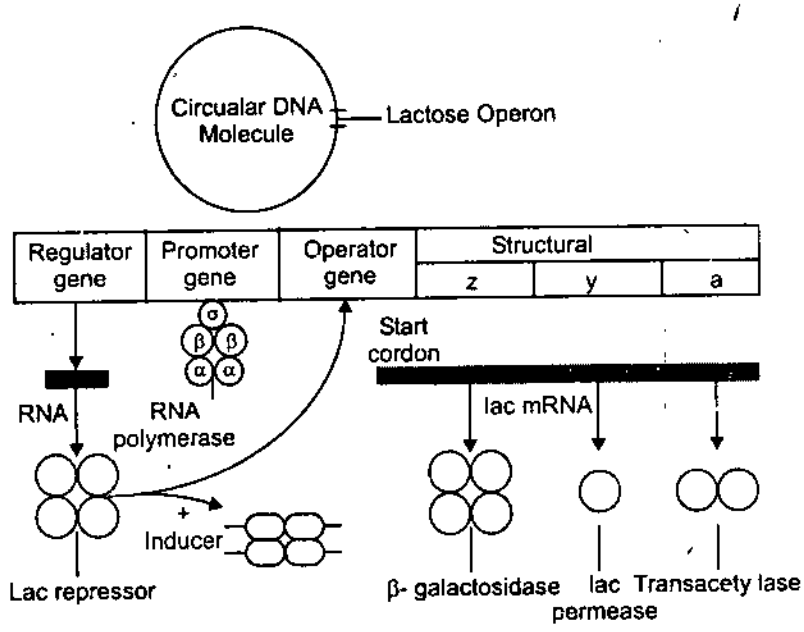


Fig. 3. Regulation of protein synthesis. The Lac operon in *E. coli*

system *e.g.*, the lac operon has three structural genes. Each structural gene may be controlled independently and transcribe a separate mRNA, or all the structural genes of an operon may form one long polycistronic mRNA molecule.

The lactose or lac operon of *E. coli* has been studied in detail and has been variously modified since its original version. The lac operon has three structural genes *z, y* and *a* which transcribe one long polycistronic mRNA molecule (Fig. 3) (i) The gene *z* consisting of 3510bp, codes for the enzyme β **galactosidase,** which is active as a tetramer and splits lactose into glucose and galactose (ii) The gene *y* consisting of 780bp codes for β galactose permease, it is a single unit membrane bound protein which facilitates the entry of lactose into the cell. (iii) The gene *a* consisting of 825 bp. codes for enzyme β galactose tranacetylaes which is active as a diamer and transfers in acetyl group from acetyl CO to β galactosides. The initiation codon of structural gene *z* is TAC corresponding to AUG of mRNA and it is located 10 base pairs away from the last base of operator gene.

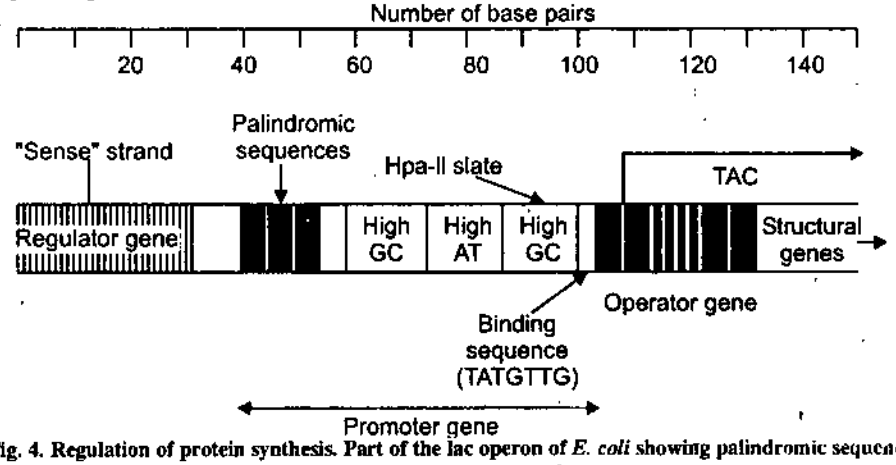


Fig. 4. Regulation of protein synthesis. Part of the lac operon of *E. coli* showing palindromic sequences and initiating codon TAC.

The Operator gene : The operator gene controls the activity of the structural genes. the operator gene is located close enough to the structural gene for β galactosidae, it is adjacent to the first structural gene. The lac operator of *E.coli* is made of a sequence of 35 nucleotide pairs. The base pairs in *O* gene have palindromic sequences (Fig. 4).

The operator gene *O* controls the activity of structural gene as it functions on the site at which the repressor molecule can bind. The operator is recognized by repressor protein which binds at operator on *O* site, forming an **operator-repressor** complex.

The formation of operator repressor complex physically prevents RNA polymerase from forming the initiation complex. The presence of a complimantary lac operator sequence at the

beginning of lac mRNA suggests that the transcription of mRNA begins in the operator gene rather than at the polymerase binding site of the promoter gene.

The Promoter Gene : The promoter consists of short sequence of bases generally less than 100 nucleotides. The promoter gene is continuous with operator gene. The genetic and biochemical studies have confirmed that it is located between the regulator gene *i* and the operator gene *O* of the lac operon. It is believed that promoter gene is the site of start of action of repression. The DNA dependent RNA polymerase recognizes P gene, it binds to and moves from promoter or P gene. When repressor binds at operator and makes operator-repressor complex the movement of RNA polymerase is blocked.

There are two proteins involved in the regulation of lac operon — (i) lac repressor and (ii) catabolite gene activator (iga) protein also known as cyclic AMP protein (CAP) or cyctic AMP receptor protein (CRP). The lac repressor exercises negative control as it blocks the movement of RNA polymerase.

The CRP excercises a positive control. The CRP site binds the CRP protein which is essential for the binding of the enzyme RNA polymerase to the promoter. In *E.Coli,* the CRP is activated by cyclic AMP molecules. The CRP combines with cyclic adenosine monophosphate (cAMP) to form a CRP-cAMP complex). This complex binds to the promoter. The CRP has strong affinity for DNA which further increases after the attachement to the promoter and thus increases the transcription and protein synthesis.

According to the model proposed by **Pribnow** (1975), the promoter gene has three elements (Fig. 5) : (i) Recognition site (ii) binding sequence and (iii) mRNA initiation site.

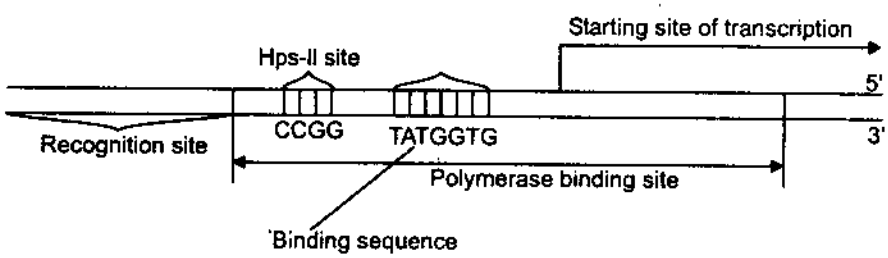


Fig. 5. Regulation of protein synthesis. Promoter gene in lac operon of *E. coli.*

The **recognition site** is outside the polymerase binding site and this is the region of DNA protected against the action of DNAse. The **RNA polymerase first binds** to DNA by forming a complex with the recognition sequence. It then binds with the binding sequence to form the pre-initiation complex.

The binding site consists of a sequence of seven bases-5' TATPATG. These are present in constant location in the protected fragments. These seven bases are found to be almost constant in bacteria and phage.

mRNA initiation site : The start of mRNA transcription takes place on one of the two bases near the binding sequence. In lac operon there is overlapping of promoter and operator sites. A part of the operator sequence appears in the polymerase binding site sequences.

The Regulator gene : In lac operon the regulator gene controls the synthesis of protein by forming an active repressor. In inducible systems like lac operon the active repressor has an affinity for the operator gene. In the absence of the inducer lactose the active lac repressor protein binds to the operator gene and blocks the parth of RNA polymerase. The structural genes then are unable to transcribe mRNA and protein synthesis does not take place. The inducer lactose when present makes the active repressor complex. The repressor subunit has one binding site for inducer. The repressor undergoes a comformational change and becomes inactive. The inactive repressor can not bind to the operator gene and the structural genes can synthesize proteins.

In repressible systems the regulator gene forms the inactive repressor aporepressor. The inactive repressor protein can not block the operator site and the structural genes synthesize proteins. The repressor is activated in the presence of a compressor and the repressor-corepressor complex blocks the operator gene, the structure genes, then cannot synthesize proteins.

Mutation in the operon

When mutation takes place in regulator gene lac i^c, the mutant i^c forms non-functional repressor protein with changed amino acid sequence. The non-functional repressor can not bind at O site hence

the uncontrolled synthesis of enzyme takes place. Such mutants in which the synthesis of enzyme takes place regardless of need are called constitutive strains.

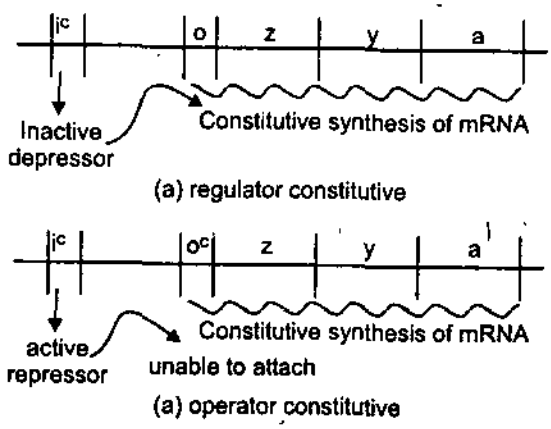


Fig. 6. Regulation of protein synthesis. Constitutive strains in lac operon of *E.coli* (A) Regular constitutive (B) Operator constitutive

The constitutive strains are also obtained by mutation in the operator gene. If the operator gene O gets mutated to *O*, the active repressor can not block the mutated operator site and synthesis of enzyme takes place irrespective of the need. Hence the mutant lac operons can be : (i) regulator constitutive (ii) operator constitutive (Fig. 6).

The synthesis of protein on the enzyme always takes place irrespective of need, both in the regulator constitutive and operator constitutive strains. But these strains differ in their dominant-recesive relationships.

In heterozygotes of regulator constitutive strain one chromosome carries the normal *i* gene and the other carries mutant i^c gene. The repressor formed by *i* gene is active and can block the O sites of both chromosomes hence *i* can be considered dominant over i^c (Fig. 7).

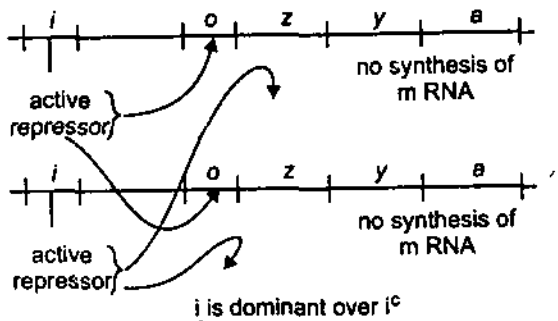


Fig. 7. Regulation of protein synthesis. Constitutive strain i dominant over i^c

In heterozygotes of operator gene, the active repressor formed by *i* gene can block the normal O site but can not block the mutant O^c site. The constitutive synthesis of enzyme takes place in such case. Hence O^c can be considered dominant over O (Fig. 8).

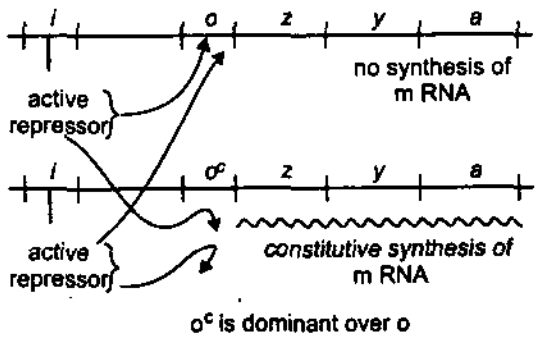


Fig. 8. Regulation of protein synthesis. Constitutive Strain O^c dominant over O.

16.3. STRUCTURE OF PROTEINS

The proteins are polypeptide chains of high molecular weight, the single unit of which is amino acid. The amino acids are attached by peptide (—CO—NH) bonds. The two amino acids condense to make dipeptide, three make tripeptide. The peptide chain upto 10 residues (amino acids) is called oligopeptide. The peptide chain with more than 10 amino acids is called polypeptide chain. Some chains are very large called macropeptides.

The proteins generally have more than 100 amino acids in peptide chain. The shortest protein is insulin with amino acids 51 in number. The short peptide chains are metabolically active and sometimes function as antibiotics for example Penicillin G is tripeptide with composition valine, cysteine and phenyl alanin. Alkaloids are oligopeptides and are used medicinally. Insulin is derived from proinsulin. The proinsulin has 81 amino acids. The insulin has two peptide chains α and β with amino acids 21 and 30 in number. These chains are attached by di-sulphide bonds.

The proteins are complex polypeptide chains. Some proteins are linear *e.g.*, membrane proteins. Some proteins are coiled *e.g.*, silk, wool or hair fibres. Most of the proteins are globular with 3 dimensional structure *e.g.*, all enzymes.

The structure of proteins can be studied at 4 levels of organisation. These are :

(a) Primary structure (b) Secondary structure

(c) Tertiary structure (d) Quaternary structure

(a) Primary structure : The primary structure deals with number, sequence and types of amino acids. There are 20 types of L-amino acids, which make various types of proteins. This is because every protein is highly specific in number, sequence and types of amino acids.

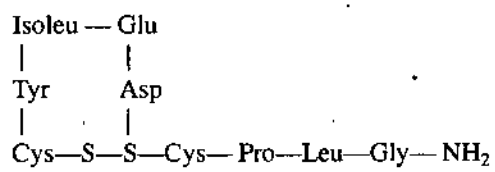
The polypeptide chain has fixed number of amino acids. Any change in number changes the type of protein e.g., the insulin has two chains α and β with amino acids 21 and 30 in number.

The amino acids are arranged in definite sequence in every polypeptide chain. This sequence is determined by genetic code of DNA. Any change causes abnormality in protein structure.

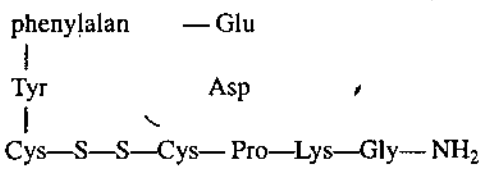
The polypeptide chain always has same specific composition. The change of amino acid changes the type of protein *e.g.*,

The enzyme oxytocin and vasopressin has the following amino acid composition.

(i) Oxytocin :



(ii) Vasopresin :



The oxytocin and vasopresin differ only in 2 amino acids but the enzymes have very much different properties. Oxytocin is concerned with contraction of smooth muscles and vasopresin is related to regulation of blood pressure.

- The R.B.C. protein has the following composition

  – Val —hist — Leu— Thr — Pro — Glt — Glt — Lys
- The sickle cell R.B.C. has composition

  – Val —hist — Leu — Thr — Pro —Val — Glt — Lys
- Hence every polypeptide chain is highly specific for number sequence and composition of amino acids
- The sequence of amino acid is determined from N terminal to C terminal.
- The N terminal is on left and C terminal is on right side of the polypeptide chain.
- The analysis of amino acid is performed by the following methods :

(i) Sanger's method : di nitro flurobenzene is used to separate one amino acid every time from N terminal.

(ii) Dansyl chloride method

(iii) Hydrazine method

(iv) Use of carboxypeptidase and endopeptidase enzymes : The carboxypeptidase and endopeptidase have property of hydrolysing only one amino acid at one time. These can be used for C terminal and N terminal analysis.

(b) Secondary structure : The secondary structure was first studied by **Linus Pauling** and **R.B. Corey.** The secondary structure of protein was studied using the technique of X-ray crystallography. The secondary structure deals with coiling of polypeptide chain on its own axis.

The long polypeptide chain is coiled at specific places. The structure of coil is definite. The coiling can be α type and β type.

(i) α coiling :

According to **Pauling,** the secondary structure has the following characteristics (Fig. 9) :

(a) The amino acid residues are 3.6 in one oil.

(b) The pitch of coil is 5.4Å.

(c) The distance between residues is 1.47Å.

(d) The structure is stabilised by hydrogen bonds.

(e) The side chains project out of the coil and often make bonds between themselves.

(f) The coiling of chain can be right handed or left handed.

These coils are called α coils because α carbon is free to move inside coil. α coils are very common in all proteins.

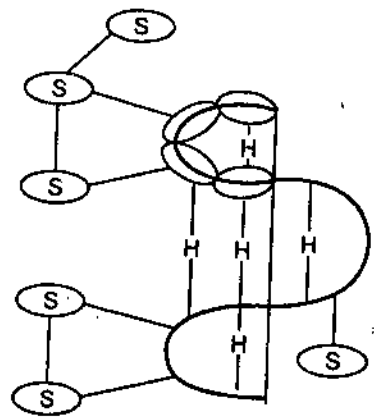


Fig. 9. Regulation of protein synthesis. α coiling in secondary structure of Protein.

(ii) β coiling : The β coils are found in silk fibre, hair and wool. The β coils are made of more than one polypeptide chain attached to each other by H bonds. The two chains can be parallel or antiparallel..

The antiparallel chains have more H bonds. The β chains are pleated structure (foil like structures).

(c) Tertiary structure : The tertiary structure is the study of dimensional structure of protein molecule. Every polypeptide chain has fixed spatial arrangement. All the tabular proteins exist in 3D structure. The 3D structures are established by many forces. These can be :

(i) Disulphide bond : The disulphide bonds are formed between sulphur containing amino acids *e.g.,* cystejne and cysteine are joint by di-sulphide bond to make cystine. The disulphide bonds are also formed in Histidine and Asparagine.

(ii) Hydrogen bond : H bonds are formed between oxygen of carboxyl and nitrogen of amino groups. This is also formed between carboxyl and OH groups of amino acids. Hydrogen bonds are weak bonds but the large number of hydrogen bonds can stabilize secondary and tertiary structures.

(iii)Ionic bonds : The ionic bonds are formed between charged amino acids. The similar charges repel each other and opposite charges attract. This results in accumulation of many groups together. The Aspartic, Glutamic, lysine, Arginine are responsible for ionic bonds.

(iv) Hydrophobic and hydrophillic interactions : The amino acids which are non-polar *e.g.,* glycine, alanine, valine are hydrophobic. The hydrophobic groups collect to make water free environment.It is called hydrophobic interaction (Fig. 10). In water free environment polar groups collect to make a force called hydrophylic interaction.

All these forces make specific organization for any protein. When proteins are subjected to high temperature, acidity or alkalinity the bonds are disrupted. The protein becomes denatured.

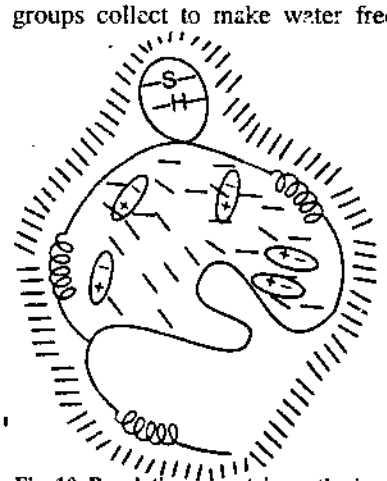


Fig. 10. Regulation of protein synthesis. Tertiary structure of protein showing S-Bonds, H-Bonds, hydrophobic and hydrophylic interactions.

(d) Quaternary structures : The quaternary structure deals with the study of sub-units. Some proteins are complex. They have more than one polypeptide chain. These chains are associated in definite fashion. Such complex proteins are called oligomeric proteins. Every sub-unit is called protomer. The protomers can be all similar. Such proteins are homoquaternary proteins *e.g.,* LDH (Lactic acid dehydrogenase). The protomers can be different in 1 protein *e.g.,* Haemoglobin it has 4 protomers, 2α and 2β. Such proteins are hetero-quarternary.

16.4. CLASSIFICATION OF PROTEINS

The proteins are classified on the basis of structure, composition and solubility.

(a) On the basis of structure : The proteins can be globular and fibrillar on the basis of form.

(i) Globular proteins : The globular proteins are mostly enzymes, protein hormones are oxygen carriers. The globular proteins have axial ratio 3-4. These proteins are spherical or globular in shape. These are highly soluble and have specific functions.

(ii) Fibrillar protein or fibre proteins : These proteins are mainly structural proteins. The axial ratio is more than 10. The fibre proteins are flexible, elastic and mostly insoluble. Some fibre proteins are collagens. These are mesenchymal in origin.

Collagens : Collagens are white proteins forming connective tissues like cartilage. These are insoluble in water, with dilute acids, alkalies, collegens form gelatin. Collagnes are rich in hydroxy proteines and poor in sulphur amino acids.

(2) Elastin : Elastin is mesenchymal in origin. If forms yellow elasted tissues like ligaments and blood vessels. The elastins do not form gelatins.

(3) Keratin : It is ectodermal in origin. It forms epithelial tissue like skin, hair feathers and nails.

(4) Fibroin : Thee are fibres of silk consiting of mainly glycine, alanine and serine.

(b) On the basis of composition and solubility : The proteins are classified according to solubility in following groups.

(1) Simple proteins (2) Conjugated proteins (3) Derived proteins

• STUDENT ACTIVITY

1. Describe the operon model in brief.

2. Describe the structure of proteins.

• SUMMARY

- **Jacob** and **Monod**, 1961 proposed a scheme for induction and repression of enzyme synthesis popularly known as operon model. For their excellent work, they were awarded Nobel prize in 1969. The operon consists of a regulator gene, promoter gene, operator gene, structural gene repressor, corepressor and inducer. The structural genes directly synthesize proteins through messenger RNA and determine the sequence of amino acids in polypeptide chain. The operator gene controls the activity of the structural genes. The promoter consists of short sequence of bases generally less than nucleotides. The proteins are complex polypeptide chains. The structure of proteins can be divided into four levels of organisation. These are : primary, secondary, tertiary and quaternary structures.

• TEST YOURSELF

1. Who proposed the operon model of gene regulation and organization in prokaryotes ?
2. Name the gene which controls the activity of structural genes.
3. Name the substrate whose addition induces the synthesis of enzymes.
4. How many amino acids do the proteins generally have in the polypeptide chain ?
5. In which year **Jacob** and **Monod** were awarded Nobel prize for proposing the operon model ?

• ANSWERS

1. Jacob and **Monod 2.** Operator gene **3.** Inducer **4.** More than 100 **5.** 1965.

❑❑❑

UNIT

17

MUTATION

STRUCTURE

LEARNING OBJECTIVES

By studying this chapter you will be able to know about mutations, their kinds and applications.

17·0. INTRODUCTION

The transfer of genetic information from generation to generation is precise and faithful *i.e.*, the genetic information is passed on from one generation to the next without alterations. But sometimes "mistakes" or changes in genetic material occur both in replication and distribution of genetic material. Such **sudden heritable** changes in the genetic material are called **mutation.** The term mutation refers both to the change in the genetic material and to the process by which the changes occur. Genotypic changes include changes in chromosome number (**euploidy** and **aneuploidy**), changes in the structure of chromosomes **(chromosome aberrations)** and changes in individual genes. The word mutation these days is referred to change in the sttructure of gene called as **gene mutation** or **point mutation** which may arise due to change in base sequence of gene. The mutational change in phenotype is sudden and heritable. The individual showing these changes is called **mustant**, the industrial exhibiting altered phenotype is called **variant** and the allele producing changed phenotype is called **mutant allele.**

Many mutations involve changes in single base pair, the substitution of one base-pair for another, or the duplication or deletion of single base-pair. Such mutations are called **point** or **gene mutation.** A gene mutation is abrupt inheritable qualitative or quantitiative change in the genetic material of an organism. Since in most organisms genes are segments of DNA molecule, so a mutation can be regarded as a change in the DNA sequence which is expressed as in the change of sequence of corresponding RNA or protein molecules.

Mutations occur in random manner, this means that they are not directed according to the requirements of the organism, these can be induced artificially also. A unicellular organism is more subjected to environmental effects since it is at the same time a somatic and a germ cell. In multicellular organisms the germ cells are distinct cells, and are relatively protected from the environmental effects.

17·1. HISTORICAL BACKGROUND

Hugo de Vries (1900) first, used the term "mutation" to describe the heritable phenotypic changes of the **evening primerose** (*Oenothera lamarkiana*). Many mutations, described by **Hugo de Varies** in *Oenothera lamarkiana*, are now known to be due to certain numerical and structural changes in the chromosomes (*e.g.*, gigas mutant). The earliest record of a sudden heritable change *i.e.*, mutation dates back to 1791, when an English farmer **Seth Wright** observed a male lamb with very short legs among the normal legged population. This lamb was used to develop dominant short legged trait. The short legged breed was called as **"Ancon breed."** The first scientific study of mutation started in 1910 by **Morgan** on fruitfly, *Drosophila melanogaster*. He reported white eyed male individuals among red eyed male individuals. After the discovery of white eyed mutant, a thorough search for mutant was made by **Morgan** and his co-worker in *Drosophila* and about 500 different mutations were observed by geneticists all over the world. Later on several cases of mutation have been reported in a variety of micro-organisms, *e.g.*, bacteriophages, bacteria (*E. coli*), *Neurospora*, in plants like pea, snapdragon, maize etc. and animals such as mice, poultry, man etc.

17·2. KINDS OF MUTATIONS

The mutations can be classified on the basis of many criteria such as (1) type of cells (2) type of amino acid replacement.

1. Classification of Mutation according to types of cells.

Mutation may occur in any cell and at any state in the cell cycle *i.e.*, either at **somatic state** or **germinal state.**

(i) Somatic mutation. If the mutation occurs in a somatic cell *i.e.*, in cells other than reproductive cells, which can prduce cells like themselves but not the whole organism is called **somatic mutation**. If a somatic mutation occurs early during embryonic life, the mutant cells may constitue a large proportion of body cells and the animal body may be a mosaic for different types of cells. When a somatic mutation occurs in axiallary bud it is called budsport or bud mutation.

Some examples of somatic mutation are : In man several fatal diseases such as **paraoxysmal nacturnal haemoglobinura, circumscribed neurofibroma, unilateral retinoblastoma** and **heterochromia of the iris** are caused by somatic mutation. Somatic mutations have also been reported in *Oenothera lamarkiana.*

(ii) Gametic or germinal mutations. The gametic mutations are those mutations which occur in gamete cells or germ cells, *e.g.*, **sperma andona.** Such mutations are transferred to next generation and are of immense genetical significance. If the mutation arises in a gamete then only a single member of the progeny is likely to have the mutant gene. If a mutation occurs in gonidial cells, then several gametes may receive the mutant gene. The gametic mutations only form the raw material for the natural selection.

The earliest recorded dominant germinal mutation was that observed by **Seth Wright** in 1791. **Seth** noticed a lamb with exceptionally short legs in his flock of sheep. Since the short legged sheep could not cross the low stone fence and damage the crop fields, he produced a flock of sheeps, each of which having short legs by employing artificial breeding techniques. The short legged breed of sheep was known as **Ancon breed.** The mutation that gave rise to the short legged was the germinal type, because the cell carrying the mutant allele has the capacity to reproduce the entire organism. Germinal mutations have since been described in a variety of animals and plants.

2. Classification of Mutation according to consequent change in Amino acid sequence.

(i) Mis sense mutation. In mis sense mutation there is replacement of one amino acid in a polypeptide chain by another. These mutations change the message of codon, *i.e.*, as a result of mutation one base of a codon may be substituted by another base. The changed codon may then code for different amino acid. A mis sense mutation can be caused by substitution, deletion or insertion. About half of the known human haemoglobin has amino acid substitution involving single base transversion. For example, the codon AAA codes for aminoacid lysine but the substitution of A by G makes codons. GAA which codes for Glutamic acids, thus the single base substitution may change the nature of protein.

(ii) Nonsense mutations. They are also called chain termination mutations. Any mutation resulting in the alteration of a codon specifying an amino acid to a termination codon (UAG, UGA and UAA) is called a nonsense mutation. For example, the codon UAC codes for amino acid tyrosine,

the substituion of C by G makes codon UAG which is termination codon. These will terminate protein synthesis at the place of substituion only.

A nonsense mutation brings about termination of polypeptide synthesis at that undesired point. As a result the polypeptide chain synthesized remains incomplete. Such chains are likely to be biologically inactive. Nonsense mutations bring about a drastic change in the enzyme synthesis and cause deleterious effect on the phenotype. The polypetide chains are synthesized in 5 → 3 direction. The nonsense mutation near 5′ end will make very short biologically inaclive chain and the nonsense mutation near the 3′ end can make almost perfect polypeptide chain with normal biological activity.

(iii) Mutation in termination codon. Sometimes mutation can convert a termination codon to a sense codon specifying some amino acid. For example, a mutation (U → C) converts the termination codon UAA to CAA in α chain of human haemoglobin (141 amino acid residues long) to the codon for glutamine. In such case synthesis continues beyond the normal termination point, producing a polypeptide chain containing 172 amino acids.

(iv) Silent mutation. In some cases the cahnge in nucleotide and consequently in codon takes place due to mutation but the result of mutation is not expressed or the mutation is not significant, such mutations are called silent mutations. There can be many reasons for the mutations to be silent.

3. Classification of Mutation according to the Direction

According to their mode of direction following types of mutation have been recognized :

(i) Forward Mutation. In an organism when mutations create a change from wild type to abnormal phenotype, then this type of mutations are known as **forward mutations.**

(ii) Reverse or back mutations. Sometimes mutation occur in such a fashion that restore the original "wild type" phenotype. This is referred as back-mutations, reverse mutation, or reversion These may be of following types :

(A) True reversion. There is a reversal of the original genetic change. The mutation C → A would change the codon GCU(alanine) to GAU (aspartate). This may result in the formation of inactive enzyme. In a true reversion the reverse mutation from A → C would restore the codon for alanine GAU → GCU. Such mutations are called reverse or back mutations.

(B) Mutation suppresor. When a mutation occurs at a diffeent site from the site where already primary mutations occurred and that mutated gene reverses the effects of primarily mutated gene, then such mutation are called **mutation suppressors**. They may be of following types : **Extragenic** and **Intragenic suppresson.**

Extragenic suppresor : If the deleterious effects of a mutation in a gene are overcome by a mutation in another gene, the process is called **extragenic** or **intergenic** suppression. In extragenic mutation the interacting mutational events take place in two separate genes. These two genes may be located on different chromosomes even.

Intragenic suppression : In intragenic suppression the mutation in a gene is suppressed by another mutation in the same gene. In this kind of mutation the effects of previous mutation in a gene are removed or reduced by another mutation in the same gene. The first and the second mutation take place in same cestron. The intragenic nutation can be **intra codon suppression** or **reading frame mutation**.

4. Classification of mutation according to the origin

According to the mode of origin, following two kinds of mutation have been recogzined :

(1) Spontaneous mutation—The spontaneous mutations ocurr in nature without an apparent or known cause. These mutations occur suddenly in the nature and have been reported in many organisms such as, *Oenothera*, maize, bread molds, bacteria viruses, *Drosophila*, mice and man etc. The spontaneous mutations may arise due to :

(i) errors during DNA replication

(ii) tautomerism

(iii) mutagenic effects of the natural environment of organism.

Tautomerism. The ability of a molecule to exist in more than one chemical form is called tautomerism. **Watson** and **Crick** described the double-helical structure of DNA and faithful transmission of genetic information from generation to generation, and also proposed a mechanism to explain spontaneous mutations. They pointed out that the structure of the bases of DNA are not static. Hydrogen atoms can move from one position in a purine or pyrimidine to another position. Thus all the four common bases of DNA (adenine, guanine, cytosine and thymine) have unusual tautomeric forms, which are, however, rare. Normally in DNA double helix adenine always pairs with thymine and guanine always pairs with cytosine. During tautomeric shift the more stable **keto**

forms of thymine and guanine and **amino** forms of adenine and cytosine may infrequently undergo less stable **enol** and **imino** forms, respectively.

The bases would be expected to exist in their less stable tautomeric forms for only very short period of time. However, if a base existed in the rare form at the moment that it was being replicated or being incorporated into a nascent DNA chain, a mutation might result. When the bases are present in their rare imino or enol states, they can form **adenine-cytosine** and **guanine-thymine** base-pairs. The net effect of such an event, and subsequent replication required to seggregate the "mismatched" base-pairs, is an AT to GC or a GC to AT base-pair substitution. Many changes do not produce mutational effects as these bases may be located in noncritical part of the polypeptide chain.

When heritable alternations occur in a very small segment of DNA molecule *i.e.*, a single nucleotide or nucleotide pair, then this type of mutations are called "point mutation". The point mutation or mutations resulting from tautormeric shift may be of following types :

(A) Transition. When a purine base (*e.g.*, adenine) of a triplet codon is substituted by another purine base (*e.g.*, guanine) or a pyrimidine (*e.g.*, thymine) is replaced by another pyrimidine (*e.g.* cytosine) in the complementary strand, then such kind of substituion is called **transition.** Transitions are the most common type of mutation. Four different transitions are possible.

(B) Transversion. Base-pair substitutions involving the substition of a purine for a pyrimidine and vice versa are called **transversions.** The existance of transversion mutation was first of all postualted by **E. Fruse** in 1959. Eight different transversions are possible. Each base pair can undergo one type of transition and two types of transversions. The transition can cause nonsense mutations but the changes of misssense mutations are always greater.

(C) Inversion. During inversion the segment of DNA is removed and then inserted in reverse direction. The changed DNA gives wrong information and mutational change occurs.

(D) Frame shift mutations. *The mutations which arise from the insertion or deletion of* individual nucleotides are called **frame shift mutations.**

These are called frameshift mutations because there is shift in the reading frame forward or backward by one or more nucleotides. The addition or deletion of one or two nucleotides results in different sequences of codons which code different aminoacids and nonfunctional or malfunctional proteins are formed. If reading frame shifts by three nucleotides, the resulting protein may be normal except that it may lack one amino acid or one amino acid will be extra. As translation takes place in 5′ → 3 direction, a change near 3′ end of polynuclotide chain will have protein allered in terminal part and such proteins may be functional.

Deletion mutation. The point mutation caused due to loss or deletion of some portion (single nucleotide pair) in triplet codon of a gene is called deletion mutation. They have been frequently reported in some bacteriophages.

The deletion of even single base will change the frame of message. The altered sequences after point of deletion completely change the code and drastic mutations occur. For example :

Original reading frame

ATC TCA ATC TCA ATC TCA

↓

delection of T

Altered reading frame

ATC CAA TCT CAA TCT CA

Insertion or addition mutation. The mutations which occur due to addition of one or more extra nucleotides to a gene are called insertion mutations. The addition of one or more bases changes the reading frame as in case of deletion. For example, The insertion mutation can be artificially induced by certain chemical substances also.

The addition of one or more bases changes the reading frome as in case of deletion. For example:

Original reading frame

ATC TCA ATC TCA ATC TCA

↑

delection of C

Altered reading frame

ATC TCC AAT CTC AAT CTC A

2. Induced mutation. Besides nautrally occuring spontaneous mutations, the mutations can be induced artificially in the living organisms by exposing them to abnormal environment such as

radiations, certain physical conditions (like temperature) and chemicals. The agents causing mutation are called **mutagens** which can be divided into two groups-physical mutagens and chemical mutagens.

(i) Physical mutagens. *Among the physical mutagens radiations are the most important* mutagens. These radiations can be divided into **ionizing radiation** and **nonionizing radiations**. The ionizing radiations may be X-rays, gamma rays and cosmic rays; alpha and beta rays; electrons, neutrons, protons and other fast moving particles. The non-ionizing radiations are ultraviolet rays and visible light. Both types of radiation induce mutation.

(A) Ionizing radiation. The ionizing radiations commonly used are X-rays, gamma rays, β-rays and neutrons. The effect of mutation depends upon its wavelength, in general the shorter the wavelength the greater will be the energy of radiation and the penetrance will be high. The ionizing radiations having lower wavelength have high penetrance. Such radiations are normally given as short base and for hand material like seeds. *X-rays were first used by* **H.J. Muller** *(1927) for causing* mutations in *Drosophila* and **L.J. Stadler** (1920) used X-rays on barby and maize plants.

Ionizing radiation such as X-rays (about 0·1 and 1 nm) are high energy rays, they can penetrate the living tissues. In the process of penetrating matter, these high energy rays collide with atoms and dissipate their energy in part through the ejection of electrons from the outer shell of atoms. The loss of these electrons leaves atoms no longer neutral but make them positively charged. The positively charged atom is called **ion.** These ions, in turn collide with other molecules causing the release of further electrons. The net result is that a "CON" of ions is formed along the track of each high-energy ray as it passes through matter or living tissue. Molecules containing atoms in ionic forms are chemically more reactive than those containing atoms in their normal stable states. To achieve their stable configuration ions undergo many chemical reactions and during these chemical reactions ionizing radiation is supposed to cause mutation.

Ionizing radiations like X-rays bring about mutations by breaking the phosphate ester linkage in DNA causing chromosomal mutation such as break, deletion, addition, inversion and translocation. The breaks may occur at one or more points on one or the both stands. The breaks in both strands of double stranded DNA may be lethal. Gamma rays like X-rays have high penetrance, the gamma rays radiations can be given to seedlings or whole plants in gamma gardems for long duration but at a slow rate.

(B) Non-ionizing radiation. The UV rays are non-ionizing radiations which may cause mutation. The UV rays have lower energy and penetrate only the surface layer of cells in higher plants and animals and do not induce ionization. The UV rays were first used to cause mutation in *Drosophila* eggs by **Altenburg** in 1930. In plants the pollens are generally given UV treatement *for mutation since pollens have germinal nucleus. The seeds are not* treated by UV rays due to their lower penetrance. UV rays dissipate their energy to atoms that they encounter, moving the electrons in the outer orbitals to higher energy levels, a state referred to as excitation. Molecules containing atoms in excited state are chemically more reactive than those containing atoms in their normal stable states. The increased reactivity of atoms present in DNA molecules is the basis of mutagenic effects. The maximum absorption of UV rays by DNA is at a wavelength of 2,600 Å. At this wavelength UV rays are absorbed by purines and pyrimidines. *In vitro* studies show that the pyrimidines (especially thymine) absorb strongly at 2,600 Å, as a result, become very reactive.

The two major products of UV absorption by pyrimidines appear to be : (1) Pyrimidine hydrates (2) Pyrimidine dimers. *The pyrimidine hydrates are formed as UV radiations cause addition* of water molecules to pyrimidines in both DNA and RNA. The water molecule is added across the C5-C6 double bond to make pyrimidine hydrates (Fig. 1).

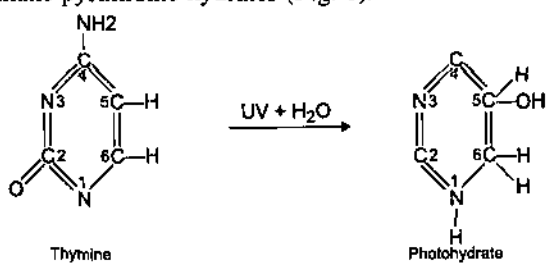


Fig. 1. Mutation. Formation of photohydrate by ultravoilet radiation.

The main mutagenic effect of UV rays appears to be due to formation of **thymine dimers**. The association of two thymine residues by formation of a chemical bond makes thymine diamer

(fig. 1). Thymine appears to cause mutations in two ways. (1) Dimers apparently perturb the DNA replication (2) Occasional errors are made during the cell process for the repair of "damaged" DNA, such as DNA containing thymine dimers.

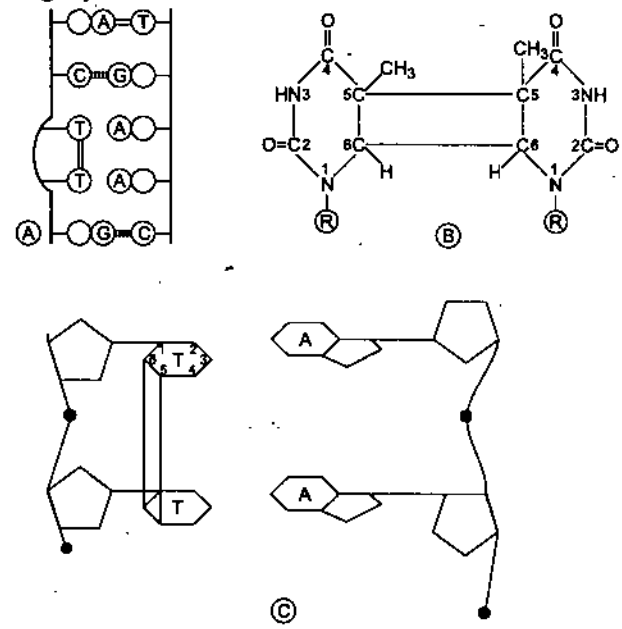


Fig. 2. Mutation. Thymine dimer formed as a result of exposure of DNA to ultraviolet radiation. (A) Distortions of DNA by thymine dimer, (B) Molecular structure of a thymine dimer, (C) Linking of two adjacent thymine residues to form a dimer.

The pyrimidine dimers can also be formed between adjacent strands. The pyrimidine dimers cannot fit into DNA double helical structure and cause distortions. The distortions in DNA by thymidine dimers can be corrected by repair mechanism. If the damage is not repaired the replication is blocked causing mutation or lethal effects.

(ii) Temperature as mutagen. It is reported that the rate of mutation is increased due to increase in temperature. The temperature shocks can be mutagenic. Temperature affects the thermal stability of DNA and the rate of reaction of other substances with DNA. It is possible that an increase of 10°C temperature may increase the mutation rate two or three times.

(iii) Chemical mutagen. Many chemical substances have been found to increase the mutability of genes. The ability of **mustard gas** as a mutagen was first demonstrated by **Auerbach and Robson** in 1947. They experimented mustard gas male *Drosophila melanogaster*. Mustard gas comes under the group of alkylating agents.

Chemical mutagens can be divided into two classes :

(A) Those that are mutagenic to both replicating and non-replicating DNA, such as the **alkylating agents** and **nitrous acid.**

(B) Those that are mutagenic only to replicating DNA, such as **acridine dye** and **base analogues.**

Chemical mutagens can also be classified as the bases of the their mode in which they cause mutation. *e.g.*,

1. Base analogues : which get incorporated into DNA in place of normal bases.
2. Mutagens modifiying purines and pyrimidines and agents labilizing bases.
3. Mutagens causing distortions of DNA.

(A) Chemicals mutagenic to both replicating and non replicating DNA are :

Nitrous oxide (HNO_2) : It is very potent mutagen, that acts by oxidative deamination of the bases that contain amino groups–adenine, guanine and cytosine. When purines or pyrimidines containing the amino group are treated with HNO_2, the amino group ($—NH_2$) is replaced by the hydroxyl group (– OH). The order of frequency of deamination is adenine, cytosine and guanine.

Deamination of adenine results in the formation of **hypoxanthine** (Fig. 3). Hypoxanthine for pairing behaves like guanine and hence pairs with cytosine. Cytosine is converted to uracil by deamination. The hydrogen bonding properties of uracil are similar to those of thymine, that is why it pairs with adenine instead of guanine. Deamination of guanine produces xanthine, but xanthine base-pairs with cytosine just like guanine.

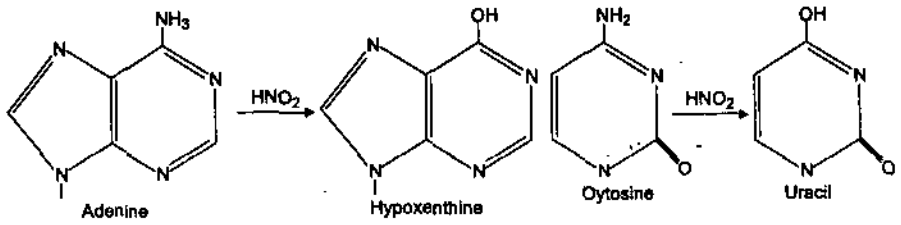


Fig. 3. Mutation. Deamination by nitrous acid.

Since the deamination of adenine leads to AT → GC transition and the deamination of cytosine results in GC → AT transition, nitrous acid induces transition in both directions, AT ↔ GC.

Alkylating agents : are the most widely used mutagenic reagents. They include : **nitrogen and sulphur mustard. Methyl methane sulphonate and ethyl methane sulphonate** (MMS and EMS) **Dimethyl sulphate** (DMS), **ethyl ethane sulphonate** (EES), **nitrogenanidin** and many others. *Mustard gases are highley mutagenic and have delayed effect, i.e.,* if the treatement is given in first generation the mutations could occur in second or third geerations.

One major mechanism of mutagenesis is by alkylating agents involves the transfer of methyl or ethyl groups to the bases such that their base pairing potentials are altered and transition results. The main chemical reaction of these agents is alkylation at the N-7 position of guanine or at the N-3 position of adenine residues. Alkylation increases the probability of ionization and introducing pairing error. The base-sugar linkage undergoes hydrolysis and releases the base from the DNA molecule. This creates gap in one chain, which may be filled with a wrong base, thus, producing mutation. The gap may also produce a deletion, causing mutation.

Ethyl methane sulphonate (EMS) particularly removes guanine from the DNA strand (Fig. 4). The DNA strand without gap will form normal DNA. In the strand with gap any base A.T. C or G may be inserted across gap. During replication the base complimentary to inserted base is intrioduced. If the base insertion is correct the normal DNA is formed. The incorrect base inserton may cause **transition** or **transversion.**

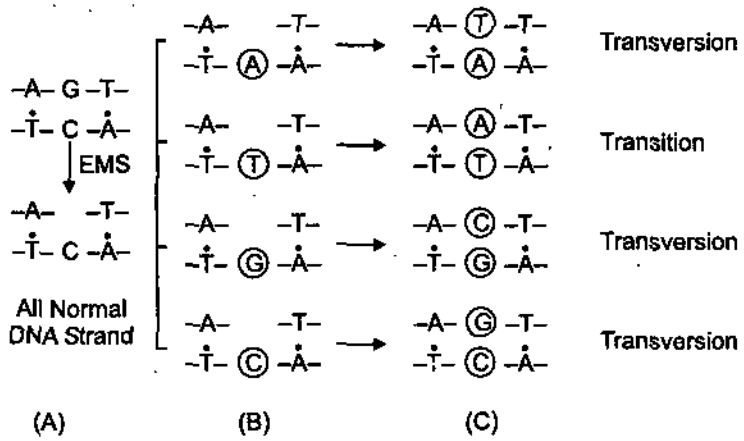


Fig. 4. (A–C). Mutations. Effect of the alkylating agent ethyl methane sulphonate (EMS) on DNA. (A) EMS removes G and creates a gap in DNA, (B) Ist replication. A/T/G/C Inserted across the gap, (C) 2nd replication. Complementary base fills the gap.

Hydroxylating agents :The hydroxylating agent **hydroxylamine,** NH_2OH in contrast to many of the alkylating agents, has a very specific mutagenic effect. It reacts with cytosine and guanine residues and brings about transition and mispairing. It deaminates cytosine to a base which pairs with adenine instead of guanine. Thus C-G pairing is changed to A-T pairing.

B. Chemicals that are mutagenic to replicating DNA :

Acridine dye : The flourescent acridine dyes such as **proflavine** and **acridine orange**, and a whole series of compounds called **ICR-170, ICR 191,** and so on are very powerful mutagens that induce frame shift mutations. The ICR compounds have acridine moities with various side chains, often alkylating agents.

The acridine are planer (flat) molecules, like the purine bases, and can be intercalated between the bases of the DNA helix. In doing so, they increase the rigidity and alter the conformation of the double helix, possibly causing slight *"kinks" in the molecule.* When DNA molecules containing intercalated acridines replicate, addition and deletion from one to a few base-pairs occurs.

(i) Intercalation resulting in insertion of base. Intercalation of the acridine molecule between two bases of the tempelate strand results in the lengthening of the DNA molecule. During replication a base (X′) is inserted at random opposite the acridine molecule in the new chain. In the next replication a complementary base (X) will pair with the newly **inserted base**. Thus the new DNA has an **additional base pair** (Fig. 5).

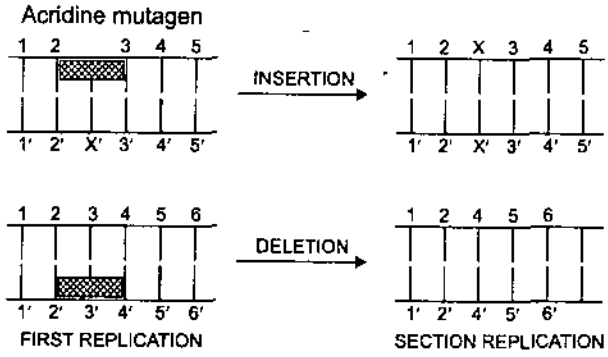


Fig. 5. Mutations. Mutagenic action of acridine dyes.

(ii) Intercalation resulting in deeletion of base. The acridine molecule may be inserted in the new chain during synthesis. This blocks the base in the template strand and does not permit any base to pair with it. The chain produced is thus deficient in one base, and in the next replication produces DNA with a deficient base pair (Fig. 5).

Base analogues. A chemical substance resembling a base is called a base analogue. The base analogue may be incorporated into newly synthesized DNA instead of a normal base. These base analogues although sufficiently similar to the normal bases of DNA are yet sufficiently different. Thus they incease the frequency of mispairing and can cause mutation.

The two most important and commonly used base analogues are **5-bromouracil** and **2-amino purine, 5-bromouracil** (5BU) or its nuleoside 5-bromodeoxyuridine (5-BUDR) in its usual (Keto) form is a structural analgoue of thymine (5-methyl uracil) and it can substitute for thymine. In its more stable ketoform, 5-bromouracil pairs with adenine. After a tautomeric shift to its enol form, 5-bromouracil pairs with guanine. In its rare enol form 5-bromouracil causes a GC-AT transition and if bromouracil is in keto form, then it will cause AT-GC transition. Thus, 5-Bromo uracil induces transition in both directions AT ↔ GC.

2 Aminopurine. (2-AP) is another analogue which can be read as either **adenine** or **guanine.** It normally pairs with **thymine** but can also form a single hydrogen bond with **cytosine.** It can therefore produce A–T → G–C transitions. It is less effective as mutagens than 2-BU and 5-BU.

Inhibition of precursors of nucleic acids

There are some mutagens which interfere with the synthesis of nitrogenous bases of nucleic acid such as purines or pyrimidines. For example, **azaserine** inhibits purine synthesis and **urethane** is an inhibitor of pyrimidine synthesis.

17.3. DETECTION OF MUATIONS

The mutations induced by the physical or chemical mutagens can be **lethal** or **visible mutations.** These mutations could be located on sex chromosomes or autosomes. There are different methods used for detection of mutation on sex chromosomes and autosomes.

(a) Detection of sex linked lethal mutations

In *Drosophila*, **Muller** used **CLB method** and **Muller-5 method** for detection of sex-linked lethal mutation.

CLB Method : In the method CLB flies were used. The CLB stock carries (i) C-an inversion in heterozygous state which functions as **cross-over suppressor,** (ii) L-a recessive lethal on X chromosome in heterozygous state (iii) a dominant marker B for Barred eye due to gene duplication.

The CLB female flies carried these three features on one X chromosome, and the other X chromosome was normal. The male flies were irradiated with X rays for induction of mutations.

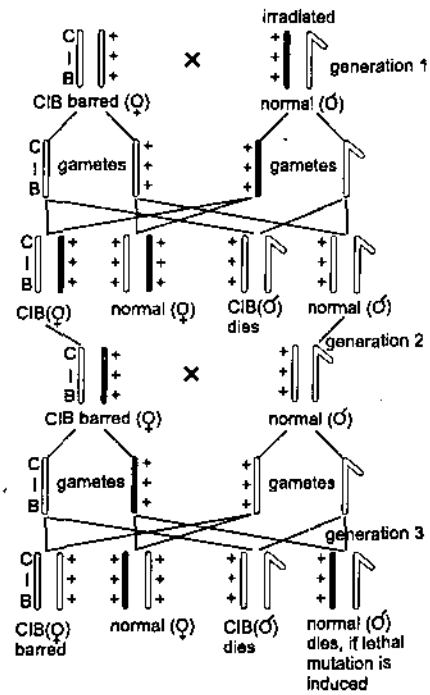


Fig. 6. Mutations. Muller's CIB method for detection of sex linked lethal mutations in *Drosophila*.

In this method the irradiated male flies were crossed with normal heterozygous CLB females. In F_1 the male progency with CLB X chromosome died due to lethal effect. The remaining F_1 males were wild type. The CLB female flies of F_1 progeny could be detected by barred eye phenotype (Fig. 6). The F_1 CLB females were crossed to normal males of F_1. In F_2 generation 50% of males receiving CLB and X chromosome die. The rest 50% males of F_2 generation may carry induced mutation. If the induced mutation was lethal these males also died otherwise these 50% of males would survive. These males of F_2 progency were used to detect the induced lethal mutations.

Muller-5 method

In this method Muller-5. *Drosophila* flies were used. These flies carried two marker genes, domainant 'Bar' barred eye and recessive 'apr' apricot. The muller 5 flies also carry a cross over suppressor C but lack lethal gene. When muller-5 barred-apricat females were crossed with X-ray irradiated moles in F_1 barred heterozygous females and muller-5 barred, apricot males were obtained. When F_1 flies were crossed in F_2 50% males were muller-5 type and 50% were wild type. If the induced mutation in X chromosome is lethal no wild type male would appear in F_2 (Fig. 7.)

The relationship between frequency of lethal mutations and increasing dose of irradiation was found to be linear. Such studies showed that increasing rate of lethal mutation was found in order of (α) alpha particles, (β) beta rays, (γ) gamma rays and X-rays, *i.e.*, α particles gave lowest frequency and X-rays were the most mutagentic (Fig. 8).

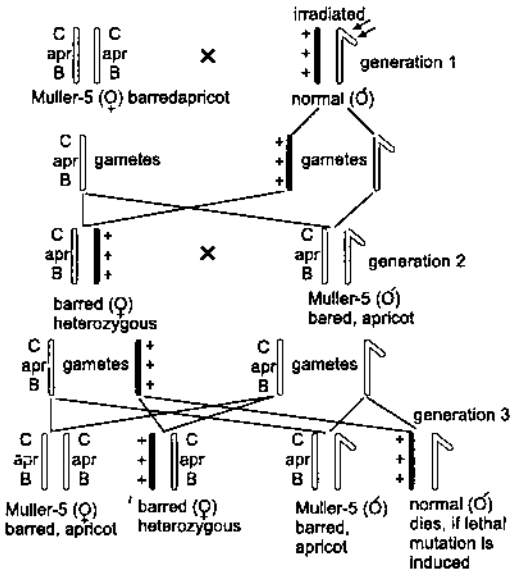


Fig. 7. Mutation. Muller-5 method for detection of sex linked mutations of *Drosophila*.

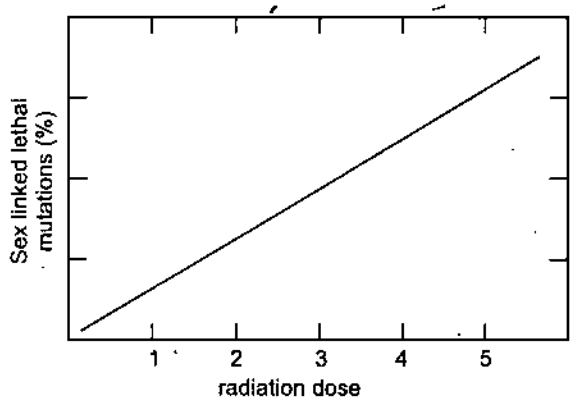


Fig. 8. Mutation. A linear relationship between the frequency of sex linked lethal mutations and the dose to radiation of kilo Rotengens (kR).

Detection of sex linked visible mutation

Attached X method : In this method attached X females XXY were crossed with irradiated males. In F_1 female obtained were superfemales XXX and XXY normal females. The male XY will express any recessive visible mutation. This method is better because the X chromosome of parent male passes to F_1 male direct and mutation could be detected in F_1 generation itself.

Detection of autosomal mutations

For detection of autosomal mutations in Drosophila balanced lethal stocks were used. In *Drosophila* this stock carried the dormant gene Cy for only wings, L for lobed eyes on the chromosome and P_m for plum eyes on the other chromosome. These Cy L/Pm were crossed to irradiated flies (fig. 9). The curly lobe flies CyL from F_1 were backcrossed to only lobe plum CyL/pM flies and in F_2 curly lobe heterozygotes male and female flies were obtained. When such CyL heterozygotes were crossed among the themselves in F_3 CyL/CyL, CyL/++ and ++/++ were supposed to occur in ratio of 1 : 2 : 1. The homozygous CyL/CyL being lethal the phenotypic ratio of curly lobe and wild ++/++ is 2 : 1. Hence if the induced mutation is lethal only. Cy L/++ curly

lobe flies would occur and if the mutation is not lethal ++/++ would express autosomal recessive mutation (Fig. 10).

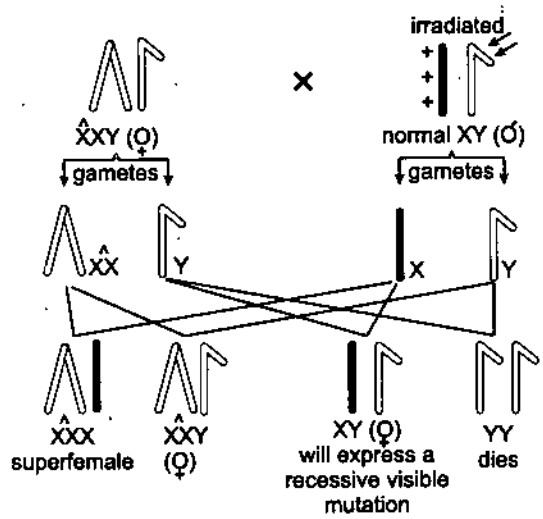


Fig. 9. Mutation. Attached X-method for detection of sex linked visible mutations.

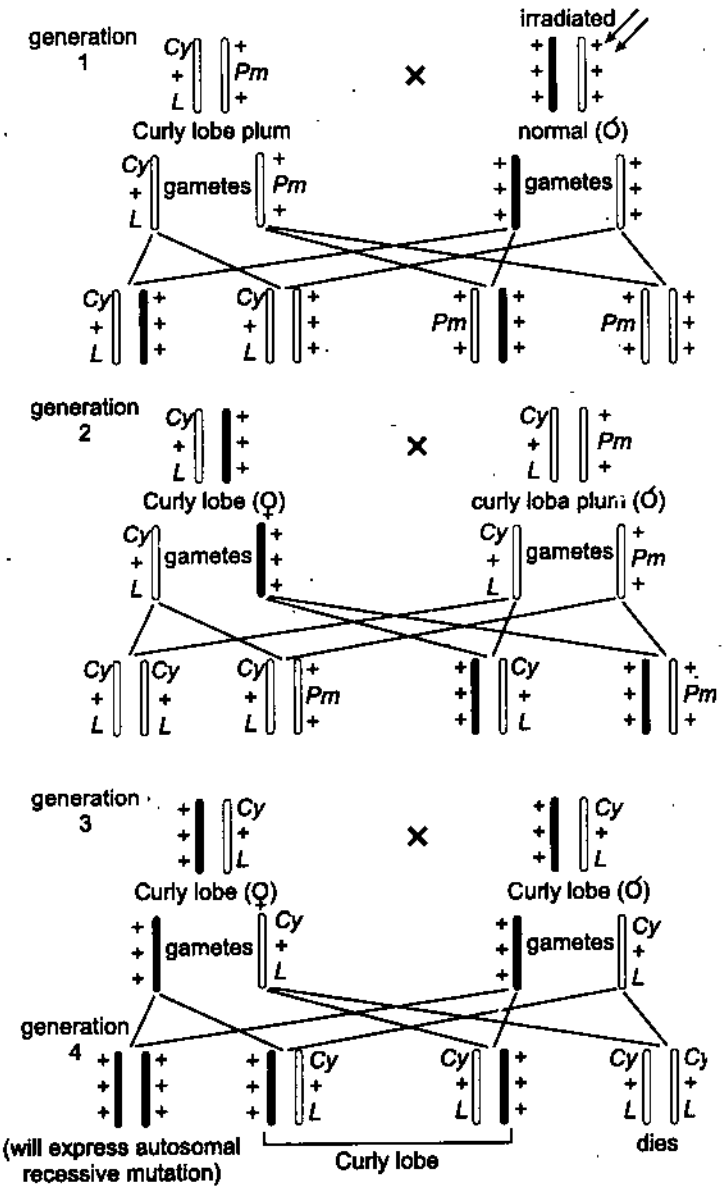


Fig. 10. Mutation. Detection of autosomal mutation in *Drosophila*.

Detection of mutation in plants

The techniques for detection of mutations are not very well developed for plants.

Stadlers Method : L. J. Stadler studied frequency of spontaneous mutations in maize endosperm characters. He selected the maize stock homozygous for several dominant genes and treated with a mutagen. These M_1 plants (treated with mutagen) were crossed with the strains having the recessive alleles of same genes called tester strain. The treated plants were taken as female plants due to partial male sterility of mutagen treated plants.

In the progency of this cross the number of seeds showing recessive character represented mutation in female gametes: The frequency of mutation for a gene is calculated as follows :

Mutation frequency (%) for a gene

$$= \frac{\text{Number of plants with recessive form of the trait governed by a gane}}{\text{Total number of plants in progeny}}$$

The plants having recessive form the trait receive one recessive allele from the tester parent with recessive traits and the other recessive allele is due to mutation of the dominant allele in M_1 plant.

Mutation at unspecified loci :

When the plant species do not have any marker stock the detection of mutation involves following steps. The seeds of the plant variety are treated with mutagen and grown to obtain the M_1 generation.

The M_1 plants are selfed to avoid outcrossing due to partial male sterlity in M_1 plants. The seeds obtained represent the M_2 generation.

M_2 plants are grown and segregation in M_2 families in studied. The plants having mutant traits are scored. The mutation frequency is calculated as follows :

$$\text{Mutation frequency (\%)} = \frac{\text{Number of plants having mutation in } M_2 \times 100}{\text{Total number of plants in } M_2}$$

17.4. APPLICATION OF MUTATIONS

Although mutations are necessary to provide genetic variability required for the evolutionary adaptation of species, the mutations are normally harmful and recessive. These mutations are not economically significant. Some mutations are beneficial and are used for crop improvement. Nearly less than one in 1000 mutations is found to be useful. Mutations in both qualitative and quantitative traits have been used to develop about three hundred varieties of crops. Some examples of useful mutant characters are :

Wheat : branched ears, awned spikelets, lodging resistance, amber seed colour, high protein and lysine contents.

Paddy : Lodging resistance, increased protein and lysine content, reduction of duration of crop, high yielding reimei variety was developed by gamma radiation. High yielding **japonica** strain unsuitable for Indian climate was transformed into "indica' strain.

Barley : Mutant high yielding varties **erectoides** and **eceriferum** developed, resistace to smut, stiff straw, hull less seeds and increased protein contents.

Castor : Amina variety developed has reduced crop duration from 270 days to 110 days.

Pepermint-Todd's Mitchan variety developed which is disease resistant and has desisred gualitis of oil.

Penicillin : The mutant strains capable of giving high yield of penicillin.

Besides these large number of fruits and ornamental varities are developed by mutation. The mutations are also used for identification of genes, fine structure analysis of gene, and to workout the biosynthetic and biodegradation pathways.

Beneficial Mutations

Plant breeders have reported induced mutants in wheat, barley, oats, soyabeans, tomatoes and fruit trees that may improve presently cultivated strains. Wheat mutants, have been obtained that provide increased yield, high protein and lysine content, lodging resistance, amber seed colour and awned spikelet. **Dr. M. S. Swaminathan,** utilized amber mutation of Mexican wheat variety and developed a new variety of wheat, called Sharbati Sonora, while working at Indian agriculture Research Institute.

Barley mutants, for example, have been obtained that provide increased yield, resistance to smut, stiff straw, increased protein contents and hull-less seeds. The induced mutation considerably improved the yield of penicillin by the mold *Penicillium.*

Phenotypic Effects of Mutation :

Mutations normally cause some deleterious phenotypic change and some mutations alter the phenotype of an organism so slightly that they can be detected only by special techniques. The mutual genes are called **isoallels.** Some mutations result in total loss of gene product activity and if such mutation occurs in essential genes, death of the organism is possible. Such mutations are caleld **lethal mutations.** Lethal mutations can be classified as **lethal, subvitals** and **supervitals.** Subvital mutations reduce the chances of survival of the organism and in case of supervital, they cause the improvement of biological fitness under certain conditions.

Mutation may be either recessive or dominant. The mutations which have dominant phenotypic expression are called dominant mutations. The disease **aniridia** (absence of iris of eyes) in man occurs due to dominant mutations. Recessive mutations will be recognized only when present in the homogyzous conditon. The phenotypic effects of mutation of recessive gene is seen only after one or more generations, when the mutant gene is able to recombine with another similar recessive gene.

17.5. RATE OF MUTATION

The frequency with which genes mutate spontaneously is called **mutation rate.** The rate of spontaneous mutation for most of the genes is very low, it ranges between 10^{-7} to 10^{-4}. Spontaneous forward mutation frequencies for various genes of phages and bacteria range from about 10^{-7} to 10^{-8} detectable mutations per nucleotide pair per generation. For eukaryotes, estimates of forward mutation rates range from about 10^{-9} to 10^{-7} detectable mutations per nucleotide per generation. Treatment with mutagenic agents can increase mutation frequencies by orders of magnitude. The mutation frequency per gene in bacteria and viruses, for example, can easily be increased to over 1 percent by treatment with potent chemcial mutagens.

The rate of spontaneous mutations varies considerably from one gene to an other. Mutation occurs much more frequently in certain regions of the gene than in others. The favoured regions are called **hot spots.** The genes which show relatively higher mutational rates are called unstable genes e.g., yellow body locus of *Drosophila.* The genes which have very low mutational frequency are called **stable genes** e.g., waxy locus of maize. The mutation rate is influenced by various factors such as genetic control of mutation rate by some mutator genes (in *Drosophila*), viral control of mutation rate in maize, *Drosophila* etc. and environmental factors such as temperature, certain radiations and chemicals.

• STUDENT ACTIVITY

1. Write short note on spontaneous mutations.

2. Write the applications of mutations.

• SUMMARY

- The term mutation ws introduced by **Hugo de Vries** on the basis of his studies on evening primrose. At molecular level, mutation is change in the constitution of DNA. Any substance or physical influence that brings about mutation is called as mutagen or mutagenic agent. Mutations are classified according to types of cells, consequent changes in amino acid sequence, direction, and origin. Various physical agents and chemicals are capable of causing mutations. The mutations induced by physical or chemical mutagens can be lethal or visible mutations. Mutations are the sole materials for evolution. Mutations are normally harmful and recessive but some mutations are beneficial and are used for crop improvement.

• TEST YOURSELF

1. Give the name of one non-inoizing radiation used as mutagen.
2. Give names of any two chemical mutagens.
3. Name the substance which can substitute bases in DNA strand.
4. Write the term which is used for the substitution of one purine by pyrimidine or one pyrimidine by purine base.
5. Who coined the term mutation ?

• ANSWERS

1. X-rays **2.** EMS, MMS **3.** Base analogues **4.** Trannsversion
5. Hugo de Vries.

❑❑❑

UNIT

18

EXTRANUCLEAR GENOME

STRUCTURE

LEARNING OBJECTIVES

By reading this chapter you will be able to know about the mitochondrial DNA, plastid DNA and plasmids.

18·0. INTRODUCTION

DNA and RNA are found not only in nuclei but in other organelles of the cell as well. Extra chromosomal DNA occurs in the form of plasmid, as DNA molecules in organelle such as mitochondria and chloroplasts. Extrachromosomal DNA is not segregated equitably in daughter cells.

Their traits do now show identical patterns of transmittance in reciprocal crosses. The function, organisation, distribution and recombination of extranuclear DNA have been studied in detail in recent years. Their inheritance is often called as **extranuclear, extra chromosomal, maternal or cytoplasmic inheritance.**

Extra chromosomal traits are often inherited only from the maternal side as the amount of cytoplasm contributed by egg cell is far greater than male gamete.

18·1. MITOCHONDRIAL DNA

The mitochondrial DNA or *mit DNA* is double stranded circular structure. These molecules are naked and are not complexed with histones. These are located inside the inner membrane, attached to it at one point. The length of *mit* DNA varies considerably from 5μm in higher animals to 20 μm in fungi and 30 μm in peas. In higher organisms it has a molecular weight of 9 to 10×10^6 and contains about 14000 base pairs.

A mitochondrion typically contains 1–3 nucleoids and each nucleoid can contain more than one circular molecule of DNA. A mitochondrion can contain two to eight circular molecles of DNA but the average number is four to five. The total *mit* DNA is only a fraction of the totai nuclear DNA. In mouse fibroblast cells about 250 mitochondria are present, the DNA of which represents only 0.15% of the nuclear DNA.

The mitochondrial DNA is generally circular. Some circular *mit* DNA is found in yeast (Fig. 1) and molds but there are other microorganisms such as protozoans *Tetrahymena* and *Paramecium* which have usually rod shaped *mit* DNA with higher molecular weight.

Difference between mit DNA and nuclear DNA : The *mit* DNA and nuclear DNA differ in their buoyant density value, G–C content and melting temperature.

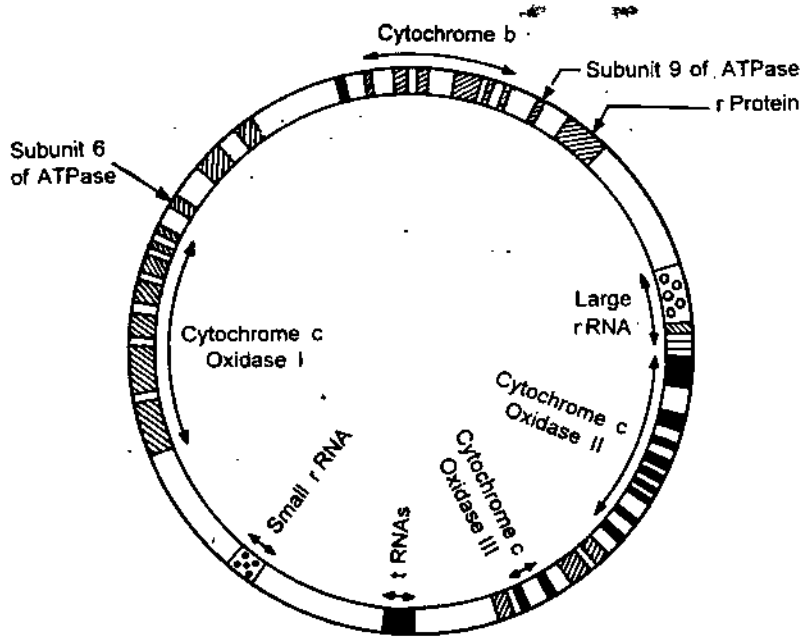


Fig. 1. Extranuclear genome. Maps of yeast *mit* DNA

- Buoyant density value (*P*) for yeast *mit* DNA is 1683 and that of nuclear DNA is 1699.
- The *mit* DNA of yeast has a G–C content of 18-20% and nuclear DNA of mammals has G–C content of 40%.
- There are several *A–T* rich stretches in yeast *mit* DNA. There are no homologies between the sequence of *mit.* DNA and nuclear DNA.

Functions of mit. DNA

The functions performed by *mit.* **DNA or mitochondrial genes** can be of two types :

(i) Those involved in the production of some of the enzymes and proteins of respiratory activities and (ii) those which provide resistance to certain antibacterial drugs.

(A) Genes for respiratory functions

(i) 'Petite' mutations of yeast

B. Ephrussi found certain mutations in yeast colonies, which were small, grew slowly and had impaired respiratory apparatus. These petite mutants can survive by switching to anaerobic respiration where aerobic respiratory machinery of mitochondria is not required. The slow growth of petite is largely due to the absence of respiratory enzymes cytochromes *a*, a_3, *b* and c and a deficiency in some dehydrogenases.

The petites were found to be of following types : (i) Due to mutation in nuclear gene and (ii) Due to mutation in mitochondrial genes. The nuclear gene mutants showed Mendelian patterns of segregation and were named **segregational** mutants.

The petites due to mitochondrial genomes were called as **neutral** and **suppressive** mutants. The neutral mutations are due to loss of rho (ρ) gene and suppressive mutations are the result of mutation in the rho (P) gene.

(ii) *Mit* mutation of Yeast : Mutation in genes coding for membrane proteins especially those affecting phosphorylations are called **mit mutations**. The two regions, mutations in which alter cyt *b* proteins are the cob and box sites in the *mit* DNA. Cob mutations affect cytochrome *b* and box interferes with both cytochrome b cytochrome *c* oxidase.

(iii) *Syn* mutation of yeast : ***Syn*** mutations in yeast mitochondrial genes affect mitochondrial tRNA and rRNA and this impairs the protein synthesizing ability of mitochondria.

(iv) "Poky" Mutation of *Neurospora* : Poky mutants in Neurospora were first reported by **M.B. Mitchell** and **H.K. Mitchell** in 1952. The poky slow growing strain failed to show segregation when crossed with wild type. This trait is not linked to any nuclear chromosome and it is apparently due to maternally transmitted mutant, non-nuclear gene. The poky strains have morphologically abnormal mitochondria in which the structural protein is altered due to mutation. They have no

cytochromes *a* and *b* but make excess of cyt ochrome *c*. They have atleast four defective mit tRNAs and they lack the small sub-unit of the mitochondrial ribosome. when hyphae of wild type and poky type fuse, the fused hyphae are wild type at first but later become poky, with the nuclear genotype having no effect. This suggests that selection favours the non-mendelian poky mutant. Another maternally transmitted mutant in *Neurospora* has defective respiratory metabolism even though all normal respiratory enzymes are present. These strains are due to defective mitochondrial structural proteins.

(B) Genes for Drug Resistance : *mit* like plasmids have been found to carry genes that provide resistance to certain drugs. Drug resistance genes have been identified in the mitochondria of yeast, *Aspergillus, Podospora, Paramaecium* and in some mammals.

Besides these the evidences confirm that (i) mitochondria DNA codes for components needed for translation within the mitochondrion. (ii) Mitochondrial DNA undergoes breakage and non-breakage recombinations.

18.2. PLASTID DNA

Many eukaryotic plant cells contain membrane bound cytoplasmic bodies called plastids. Chloroplasts are the plastids which contain the green pigment chlorophyll and the machinery required for the process of photosynthesis. Chloroplasts, like mitochondria, possess small circular DNA molecules (Fig. 2) which encode some of the proteins used in the process of photosynthesis and the rRNA, tRNA and ribosomal proteins required for protein synthesis.

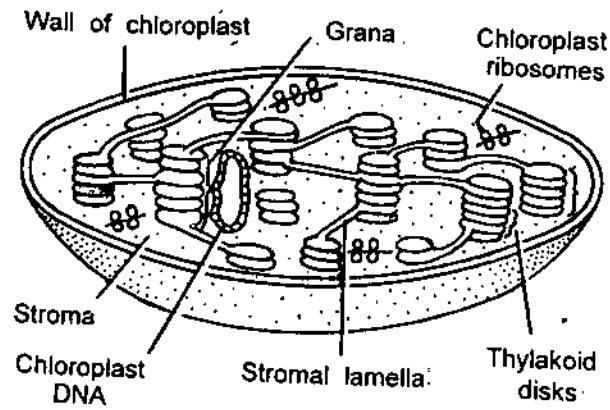


Fig. 2. Extranuclear genome. The diagrammatic structure of a chloroplast.

- The first intact chloroplast DNA called chl DNA was isolated from *Euglena* by **Manning** *et.al.* in 1971. The length of this DNA was 44.5 μm, the normal range of chi DNA is 37-44 μm.
- Chl DNA has molecular weight greater than 10^7 and has more than 15,000 base pairs. Chl DNA is not complexed with histone and contains little or no 5 methylcytosine.
- The evidence that chl DNA is self replicating includes the finding that chl DNA replicates several times faster than nuclear DNA and that the DNA content of an immature *Euglena* plastid doubles when it becomes chloroplast.
- Chl DNA replicates semi-conservatively.
- Chl DNA is highly redundant and this can be derived from the fact that the loss in a single generation of upto 80 percent of chl DNA is reversible. The fast renaturation of denatured chl DNA indicates the existence of 20 copies of a major component.

***Chlamydomonas* Chl DNA :** Circular genetic map of nine non-mendelian genes location on chl DNA ha been obtained by using recombination frequencies (Fig. 3).

Chl DNA of *Chlamydomonas* is non-conserved, that is, it is not distintegrated and dispersed during mito and meiotic divisions. It has lower G + C content than cellular DNA.

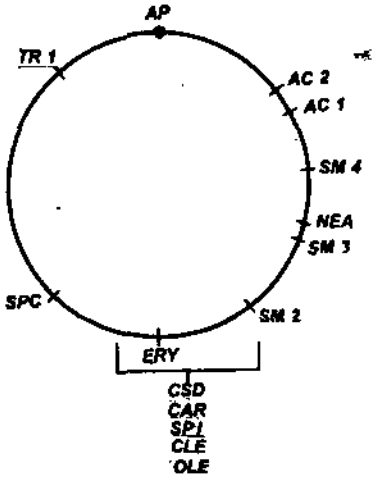


Fig. 3. Extranuclear genome. Circular genetic map of non-mendelian genes in *Chlaymydomonas* based on recombination frequencies. *ap*, attachement point; ac2 and *ac1*, acetate requirement; sm2 high-level streptomycine resistance; ery, erythromycin resistance; *csd*, conditional streptomycin dependence; car, carboymycin resistance; spi, spiramycin resistance; cle, cleasine resistance; ole oleandomycin resistance; *spc*, spectinomycin resistance; and *tr1*, temperature sensitivity

18.3. PLASMIDS

The term **plasmid** was given by **Lederberg** in 1952 to describe extrachromosomal hereditary units. Generally, bacteria contain one or more small **circular double stranded DNA molecules** besides the main chromosomal DNA. Such **extrachromosomal DNA** are called **plasmids**.

The plasmids can exist in two forms :

(a) Single copy plasmids : These plasmids occur as one plasmid DNA per host genome.

(b) Multi copy plasmids : These plasmids occur as many copies per genome, their number can be upto 1000 per cell.

A plasmid is **autonomous**, it is independent of the chromosomal DNA for its replication. A plasmid carries a small number of genes which may not be essential for the life of bacterium but may have other functions in bacterium.

A plasmid always possesses at least two sets of genes (i) those needed for replication (ii) those specifying a property of incompatability for similar plasmids.

Besides genes for replication and incompatability plasmids may also have the following types of genes :

(i) transfer genes;

(ii) genes for some special characteristic such as resistance to drugs, antibiotics and bacteriotoxins, production of colicins; production of specific enzymes

On the basis of special functions of genes the plasmids can be **R-plasmids, Col plasmids, F-plasmids. Col Plasmids** carry genes which code for bacteriocidal proteins. **F-plasmid** is a double stranded circular DNA carrying three sets of genes. One set is involved in the process of conjugation.

Plasmids as Vector in Moleculer Cloning : It is now possible to transplant genes from any organism into microorganisms where they will produce clones of genes by endoreplication and cell division. The multicopy plasmids are used as cloning vectors.

Plasmid to be useful as vector should possess the following properties (Fig. 4).

(i) it must contains gene for replication of DNA. The replication gene region must not contain the cutting site for restriction enzyme to be used;

(ii) it must contain a gene to be used as marker for selection of plasmid in transformed cell. It can be gene specific for resistance to an antibiotic;

(iii) it must contain a marker gene which possesses within it a cutting site for restriction enzyme. Cutting plasmids at this site by restriction enzyme will inactivate this gene. The absence of character of this gene is used to distinguish hybrid plasmids from non-hybrid plasmids;

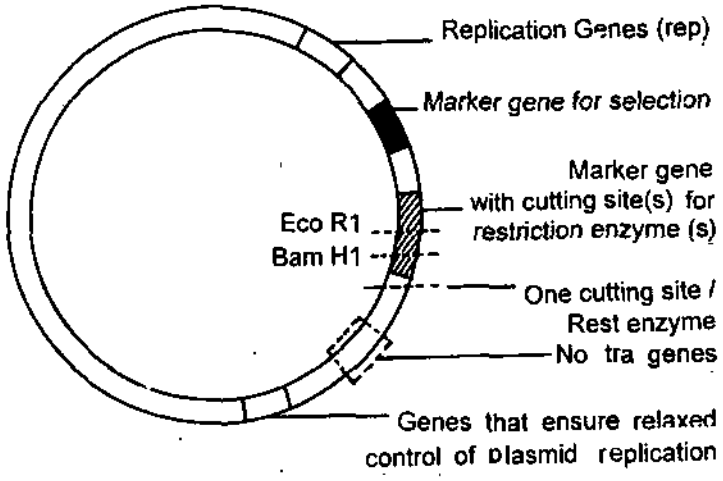


Fig. 4. Extranuclear genome. Essential features of a plasmid vector

(iv) A plasmid to be used as vector must not be self-transmissible by the process of conjugation;

(v) A plasmid which can be multiplied into large number of copies per cell *i.e.*, a plasmid under **relaxed replication** control is preferred to a plasmid which in under **stringent control**; (Fig. 5).

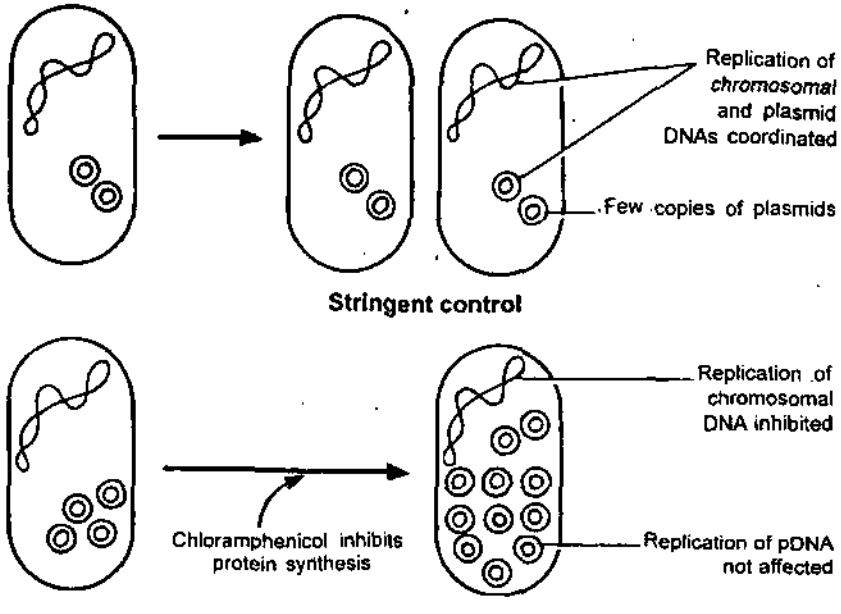


Fig. 5. Extranuclear genome. Plasmids with stringent and relaxed control

(iv) Engineered plasmid vectors : The naturally occurring plasmids do not possess all characteristics required to make them ideal vector. Hence, naturally occuring plasmids are tailored to make suitable vectors.

The first engineered plasmid vector is pSC 101. The pSC 101 was derived from *E.coli* plasmid R6-5 which contains a rep function and gene for tetracycline resistance. The rep region of R 6-5 was tailored to make the smaller pSC 01. The pSC 101 was further modified by the association of kanamycin resistance gene.

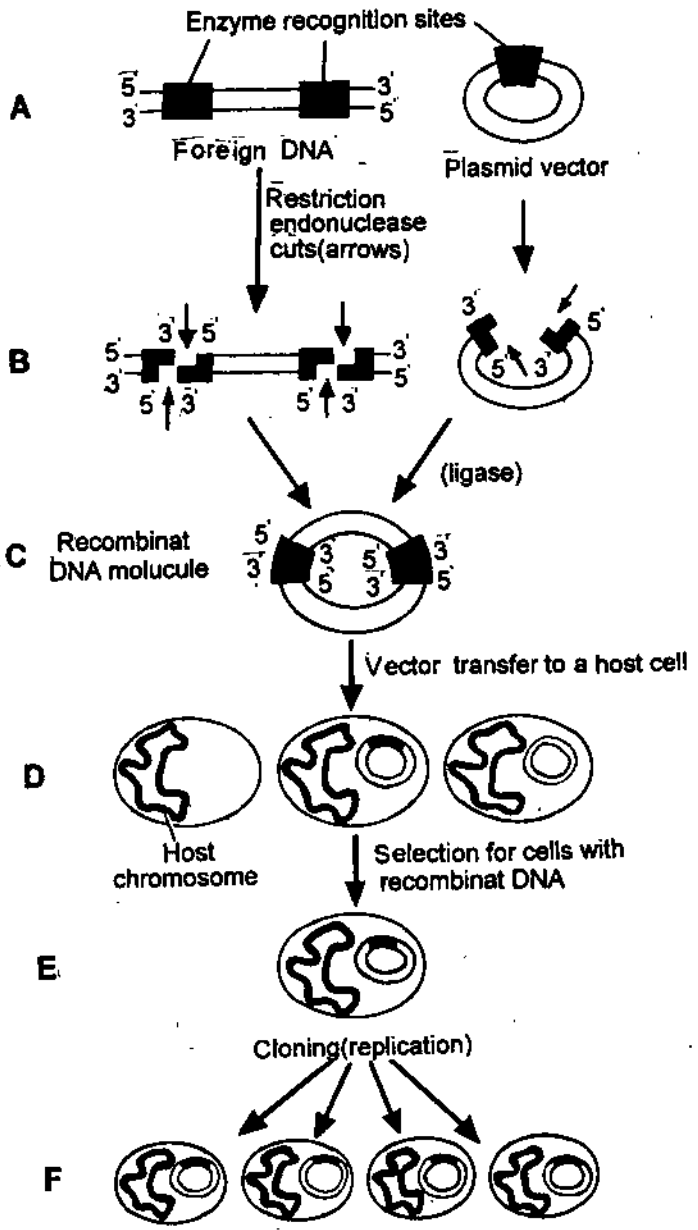


Fig. 6. Extranuclear genomes. A general scheme for constructing a clone of recombinant DNA molecules using plasmids and restriction endonuclease enzymes. (A) a "foreign" DNA molecule and a plasmid vector are selected, both carrying recognition sites that can be cleaved by the same restriction endonuclease. (B) Cleavage produces one or more fragments of the foreign DNA, and opens the plasmid vector. (C) The cuts produced by the restriction enzyme are staggered so that complementary base pairing can occur between single -stranded ("sticky") ends of the foreign DNA fragments and of the opened plasmid DNA. A DNA ligase enzyme then covalently bonds the two DNAs into a recombinant DNA molecule. (To prevent the plasmid vector form resealing its own sticky ends before it is joined to the foreign DNA, it is often treated with an alkaline phosphatase which inhibits ligation by removing the phosphate at each open5' end of the vector. The donor DNA molecule, however, is not treated that way, and therefore allows bonding between its 5' –P ends and the 3'–OH ends of the vector. Apparently, once these bonds are formed, extra concentrations of DNA ligase can cause bonding between the paired 5'–OH and 3'–OH ends.) (D) the plasmid carrying the foreign DNA is inserted into a host cell. (E) Each such isolate is then further propagated thereby generating a clone carrying the particular foreign DNA fragment that was incorporated in step (C).

• STUDENT ACTIVITY

1. Define mitrochondical DNA.

2.. What is plastid DNA ?

• SUMMARY

- Extra chromosomal DNA occurs in the form of plasmid, as DNA molecules in organelle such as mitochondria and chloroplast. The term plasmid was given by **Lederberg** in 1952 to describe extrachromosomal hereditary units. The mitochondrial DNA is double stranded circular structure. Chloroplasts, like mitochondria possess small circular DNA molecules which replicate semi-conservately. Plasmids are involved in the production of some of the enzymes, proteins of respiratory activities and provide resistance to certain antibacterial drugs.

• TEST YOURSELF

1. Who first of all reported the poky mutations of *Neurospora* ?
2. What is the term given to small piece of autonomous, extrachromosomal, circular DNA found in some bacterial cells.
3. What is the mode of replication of chl DNA.
4. Name the type of plasmids on the basis of functions genes.
5. Extrachromosomal or hereditary units function either independently or in collaboration with nuclear genetic system. What it is called ?

• ANSWERS

1. M.B. Mitchell and H. K. Mitchell **2.** Plasmid **3.** Semi-conservatively

4. *R-plasmids, col plasmids, F-plasmids*

5. Extra chromosomal or organellar or cytoplasmic inheritance.

❑❑❑

2. Give properties of double helical DNA structure.

3. Write short note on nucleotides.

• SUMMARY

- On the basic of Griffith's bacterial transformation experiment, conducted on *Pneumococcus pneumoniae*, **Avery** *et.al.,* concluded that the genetic material is DNA. **Hershey** and **Chase** concluded that DNA is the genetic material. The DNA is made up of nitrogen bases, sugars and phosphate. The nitrogen bases are of two types : pyrimidines and purines. The sugar in DNA is pentose. The attachement of phosphate generally occurs at 5′ or 3′ carbon. A nitrogen base combines with a sugar to form a nucleoside, which combines with phosphate group to form nucleotide. The nucleosides of DNA are called deoxyadenosine, deoxycytidine, deoxyguanosine and deoxythmidine. The nucleotides of DNA are called deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid and deoxythymidylic acid. The phosphate group of one nucleotide is linked to the hydroxyl group of sugar of adjacent nucleotide by a phosphodiester bond, thus forming a polynucleotide. So, the DNA is a polymer, the monomeric unit of which is a nucleotide *i.e.*, DNA is polynucleotide. **Watson** and **Circk** (1953) proposed double helical model of DNA. **Wilkins** *et. al.* by X-ray crystallogrophy gave many details of DNA molecule. The DNA exists in atleast four forms mainly A, B, C, and D. **Meselson** and **Stahl** (1958) confirmed that replication of DNA in *E. coli* is semiconservative. The proteins are made of 20 types of aminoacids. Similarly DNA and RNA are made of four types of bases, thus the bases are the alphabets of the language of DNA. The codes formed by the bases of DNA which give specific information for amino acid sequence in protein structure are called genetic code.

• TEST YOURSELF

1. Name the organism in which DNA is single stranded.
2. Name the organisms in which RNA is double stranded.
3. Give names of pyrimidine bases of DNA.
4. Give names of purine bases of DNA.
5. Describe the role of tRNA in translation.
6. What is Central Dogma ?
7. What is reverse transcritpion ?
8. What is the role of rho factor ?
9. What is the role of sigma factor ?
10. What function does amino acyl tRNA synthetase perform ?
11. What functions does transfer factor (TF1) perform ?
12. What are release factors ?
13. Which are terminating codons ?
14. What are codons of chain initiation ?

15. Who gave the double helix model of DNA structure ?
16. What are Okazaki fragments ?
17. What is the diameter of DNA helix ?
18. DNA polymerase I was discovered by which scientist.

• ANSWERS

1. Parvo virus 2. Reo virus 3. Thymine, uracil 4. Adenine, Guanine
5. Identifies an amino acid in the cytoplasm
6. Flow of information from DNA → RNA → Proteins
7. Enzyme that can transcribe RNA into DNA 8. Dependent termination
9. Start signals on DNA molecules and directs RNA polymerase in selecting the initiation sites
10. Enzyme responsible for attaching the correct amino acid to tRNA molecule
11. Transfers AA-*tRA* to *A site*
12. A protein that terminates translation to messenger RNA (mRNA) during protein synthesis and releases the completed polypeptide chain from the ribosome
13. UAG, UAA and UGA 14. AUG 15. **Watson** and **Crick** (1953)
16. DNA fragments formed in DNA replication 17. 20 Å 18. **Homberg.**

UNIT

12

MENDELISM

STRUCTURE

LEARNING OBJECTIVES

By studying the chapter you will be able to understand the principle of segregation and principle of independent assortment.

12.0. INTRODUCTION

In order to study genetic inheritance or Mendelian inheritance or genetics as such, it is desirable to understand the terminology used in the study of genetics.

Heredity and Variation

The transmission of characters or traits from one generation to another is called heredity. The characters from one generation to the other are passed through genes. It is because of heredity that offsprings resemble their parents.

The offsprings are never exactly identical to parents and the siblings of one generation also show some variations in their characters. **The differences shown by individuals of the same species and also by their offsprings are called variations.**

Genetics is the science of study of heredity and variations. The word genetics has been derived from the word **gene.** The term genetics was coined by **William Bateson** in 1906. The factors or units which transfer characters from one generation to the other are called genes. The genes were considered to be the structural and functional units of heredity. But modern studies have proved that genes can be further divided into units like-**cistron, recon** and **muton**, hence, genes are no longer structural units.

Allelomorphs or Alleles

Allele is the abbreviated form of the term allelomorph. Allele is a Greek word which means belonging to one another. Alleles are the alternating form of the same gene. Generally, there are two alternate forms of a gene. Hence, a gene generally has two alleles. For example, gene for the height of plant has two allelic forms *T* and *t*. The two alleles in a plant can be *TT*, *Tt*, *tt*. The individuals having both alleles identical are called **homozygoous,** *e.g.*, *TT* is homozygous dominant and *tt* is

homozygous recessive. The individuals having identical phenotype may or may not have the same genotype, *e.g.* Tall plants can be *TT* and *it.* Indivdiuals having similar genotype will have same phenotype in comparable situation.

Dominant and recessive

In a hybrid both alleles of a gene are present but phenotypically only one allelic form is expressed and the other remains hidden. The character or allele which is expressed in heterozygous form is called **dominant** and the charcter or allele which is not **expressed** is called recessive. *e.g.*, *Tt* plants are tall, hence the tall character and *T* allele are dominant, dwarf character and *t* allele are recessive.

The characters are now preferably expressed by mutant forms hence the tall plants are denoted by sign *DD* or *Dd* and homozygous recessives are represented by *dd*.

Reciprocal Crosses

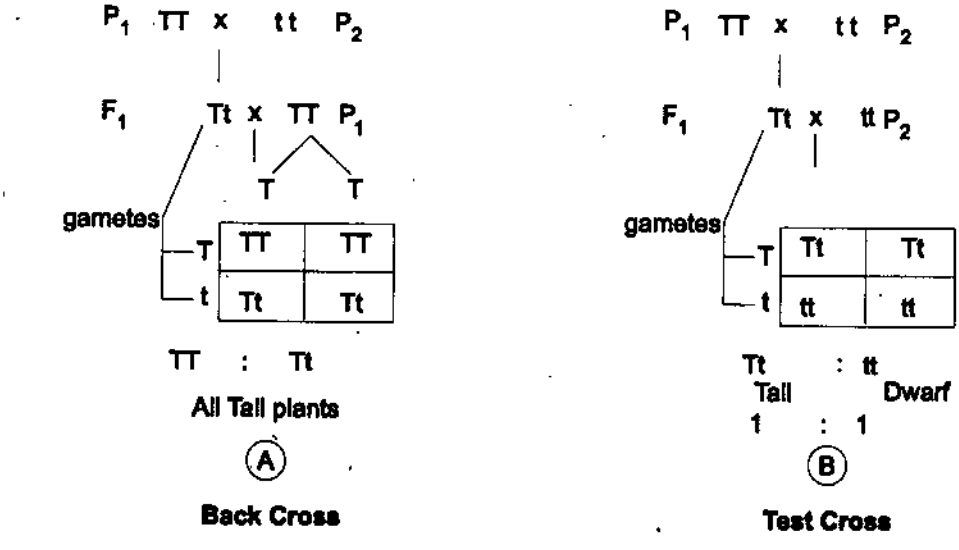
The reciprocal crosses are the set of two crosses made in such a way that parents with one character are taken as male in one cross and female in the other cross. For example, if in the first cross 'A' is used as female parent, in second cross A will be used as male parent.

The reciprocal crosses are made to see if the concerned character is located on sex chromosome or autosomes. If in such cross the results are similar in I and II cross, the character will be located on autosomes.

Back cross and test cross

When any cross is performed the F_1 individuals are normally selfed to produce F_2 generation. The F_1 can also be crossed with parents P_1 or P_2. Such crosses are called **back crosses.**

If F_1 is crossed with homozygous dominant parent, the F_2 progency obtained has all domainant phenotype, *e.g.* (A).



If F_1 is crossed with homozygous recessive parent the F_2 progency has 1 : 1 ratio of phenotypes and genotypes. Such back cross with homozygous recessive parent is called **test cross.** The test cross is performed to know the homozygosity or heterozgosity of the parent individual.

Monohybrid, dihybrid and trihybrid crosses

In monohybrid cross, the cross is made between parents for a single character or a single gene. In this case the phenotype ratio obtained in F_2 is 3 : 1 and genotype ratio in F_2 is 1 : 2 : 1.