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SYLLABUS

B. Sc. (Part III) Zoology

PAPER – II : CYTOGENETICS AND MOLECULAR BIOLOGY

(SC-123)

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UNIT

1

INTRODUCTION

STRUCTURE

- Cell Biology.
- History of Celll Biology.
- Cell Theory
- Exception of Cell Theory
- Protoplasm Theory
- Organismal Theory
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- History of cell biology and cell theory, Exception to cell theory, Proplasm theory and Organismal theory.

One of the most fundamental and obvious statements is that the cell is the microscopic structural and functional unit of the living organisms. There are many cell types among fungi, protozoans and higher plants and animals. They differ in size, form and function, degree of specialization and average generation time. At the ultrastructural level, all the cells are similar and tedious. The same basic structures : nuclei, cytoplasmic matrix or cytosol, plastids, mitochondria, endoplasmic reticulum, Golgi apparatus, plasma membrane etc., all appear with predictable regularity. Such a similarity can also be observed at the molecular level; all cell parts are made of highly organized groups of a few types of molecules, *i.e.*, carbohydrates, proteins, lipids and nucleic acids etc.

• 1.1. CELL BIOLOGY

Definition

The biological science that deals with the study of structure, function, molecular organization, growth, reproduction and genetics of the cells is called **cytology** (Gr., *kytos* = hollow vessel of cell; *logous* — to discourse) or **cell biology**. Much of the cell biology is devoted to the study of structures and functions of specialized cells and the results of these studies are used to formulate the generalization applied to almost all cells. It provides the basic understanding of how a particular cell type carries out its specific functions.

Cell biology has been studied under three headings : **Classical cytology** deals with only light microscopically visible structure of the cell; **cell physiology** deals with bio-chemistry, biophysics and functions of the cell and **cell biology** interpreted the cell in terms of micro-molecules such as nucleic acids and proteins. Now, cytology and cell biology are used as synonyms (**Novikoff** and **Holtzmann**, 1970).

• 1.2. HISTORY OF CELL BIOLOGY

Aristotle (384–322 B.C.) and **Paracelsus** (Ancient Greek Philosophers) concluded that — "*all animals and plants are constituted of a few elements, which are repeated in each of them*". They were referring to the macroscopic structures of an organism, such as roots, leaves and flowers common to all different plants or segments and organs that

are repeated in the animal kingdom. Later. after the invention of magnifying lenses, **Da Vinci** (1485) recommended the use of lenses in seeing the small objects. In 1558. **Conrad Gesner** (1516–1565) a Swiss biologist published results of his studies on the structure of a group of protists, called foraminifera. Further growth and development of cell biology is intimately associated with the development of optical lenses and to the combination of these lenses in the construction of the compound microscopes (Gr., *mikros* = small; *skopein* = to see). The first useful compound microscope was invented in 1590 by **Francis Janssen** and **Zacharias Janssen.** Their microscope had two lenses and total magnifying power was between 10X and 30X. Such microscopes were called flea glasses, because they were used to examine small whole organisms, such as fleas and other insects. In 1610, an Italian Galileo Galilei (1564–1642) invented a simple microscope having only one magnifying lens and it was used to study the arrangement of facets in the compound eye of insects. **Marcello Malpighi** (1628–1694) an Italian first used the microscope to examine thin slices of animal tissues from brain, liver, kidney, spleen, lungs and tongue. He also studied plant tissues and suggested that they were composed of structural units called **utricles. Robert Hooke** (1635–1703) an English micropist coined the term cell (L., *Cella* = hollow space) in 1665. He examined a thin slice cut from a piece of dried cork under the compound microscope, which was built by him. He described cork as a honey comb of chambers or **cells.**

Dutch microcopist, **Anton van Leeuwenhoek** (1632–1723) had succeeded in greatly improving the art of polishing lenses of short focal length. He later builds up numerous microscopes, some with magnifications approaching 300X. He was the first to observe living free-living cells. In 1675, he described microscopic organisms collected in tubes inserted into the soil during rainfall. His sketches included numerous bacteria (bacilli, cocci, spirilla and other Monera), protozoa, rotifers and Hydra. He was also the first to describe the sperm cells of human beings, dogs, rabbits, frogs, fish and insects. He also observed the movement of blood cells of mammals, birds, amphibians and fish. He noted that those of fish and amphibians were oval in shape and contained a central body the nucleus, while those of human and other mammals were round. He also observed the striated muscles. His observations were recorded in a series of reports that he sent during 1675 and 1683 to the Royal Society of London.

Nehemiah Grew (1641–1721) an English plant microanatomist published accounts of the microscopic examinations of sections through flowers, roots and stems of plants that clearly indicated that he recognized the cellular nature of plant tissues.

Mirbel in 1807 stated that all plant tissues were composed of cells. **Rene Dutrochet** (1776–1827) concluded in 1824 that all animal and plant tissues were aggregates of globular cells.

Robert Brown (1773–1858) an English botanist discovered and named the nucleus in cells of epidermis, stigmas and pollen grains of the plant *Tradescantia* in 1831. He established that the nucleus was the fundamental and constant component of cells.

• 1.3. CELL THEORY

In 1838, **Mathias Jacob Schleiden** (1804–1881) put forth the idea that cells were the units of structure in the plants. In 1839, **Theodor Schwann** (1810–1882), German zoologist applied Schleiden's thesis to the animals. Both of them, thus, postulated that cell is the basic unit of structure and function in all life. This biological generalization is known as **cell theory** or **cell doctrine.** But this generalizaton was infact based on the works of their predecesors, such as **Oken** (1805), **Mirbel** (1807), **Lamarck** (1809), **Dutrochet** (1824), **Turpin** (1826) etc. However, Schleiden was the first to describe the nucleoli. Schwann studied both plant and animal tissues and his work with connective tissues, such as bone and cartilage led him to modify the cell theory that — living things are composed of both cells and the products (secretions) of the cells. He also introduced the term metabolism to describe the activities of the cells.

K. Nageli (1817–1891) showed in 1846 that plant cells arise from the division of pre-existing cells. **Rudolf Virchow** (1821–1902) a German pathologist confirmed the **Nageli's** principle of cellular basis of life's continuity. He stated that the cells arise only

from pre-existing cells (*Omnis cellula e cellula* – every cell from a cell). Virchow, thus, established the significance of cell division in the production of organisms.

Louis Pasteur *(1822–1895) in 1865, in France gave experimental evidence to* support **Virchow's** extension of cell theory. Now the cell theory states that :

1. All living organisms (animals, plants and microbes) are made of one or more cells and cell products.

2. All metabolic reactions in unicellular and multicellular organisms take place in cells.

3. Cells originate only from other cells. No cell can originate spontaneously or *de novo, but* comes into being only by division and duplication of already existing cells.

4. The smallest clearly defined unit of life is the cell.

Kolliker applied the cell theory to embryology. He demonstrated that the organisms developed from the fusion of two cells – spermatozoon and ovum. In recent years a large number of sub-cellular structures, like ribosomes, lysosomes, mitochondria, chloroplasts etc., have also been discovered and studied in detail.

• 1.4. EXCEPTIONS TO CELL THEORY

There are certain living organisms, which do not have true cells. All kinds of true cells have three basic characteristics :

1. A set of genes which regulate cellular activities and make new cells.

2. A limiting plasma membrane that permits controlled exchange of matter and energy with the external world.

3. A metabolic machinery for sustaining life activities like growth, reproduction and repair of parts.

Viruses do not fit in these parameters of a true cell. They lack a plasma membrane and metabolic machinery for energy production and for the synthesis of proteins.

But viruses have the following features :

1. They have a definite genetically determined macromolecular organisation.

2. A genetic or hereditary material in the form of either DNA or RNA.

3 A capacity of auto-reproduction.

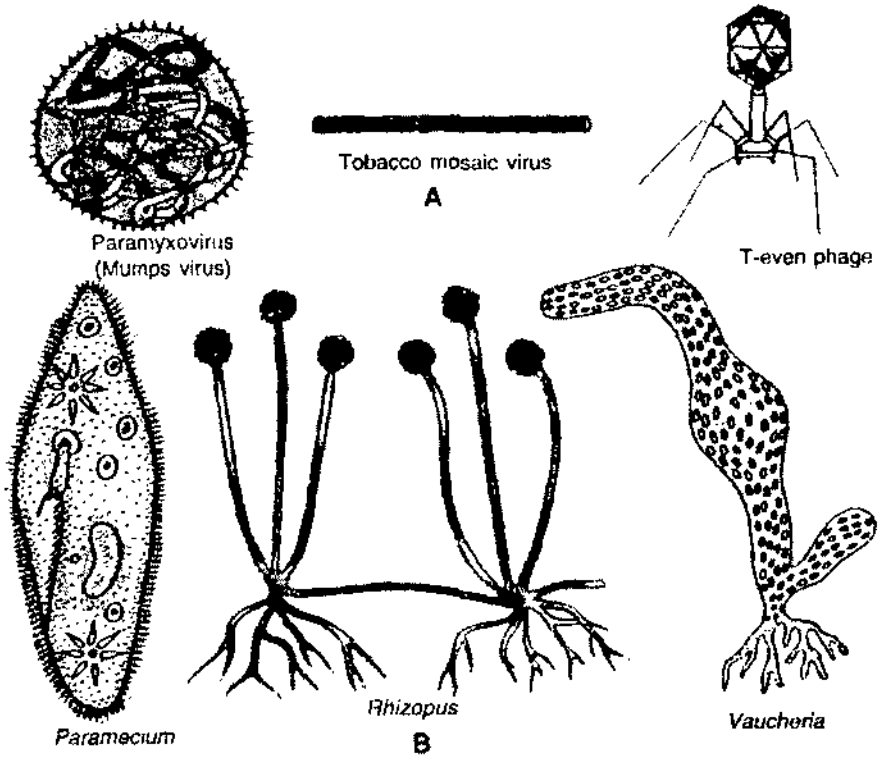


Fig. 1. Organisms which do not fit into cell theory. Three types of viruses. B–Three organisms of cellular organization.

4. A capacity of mutation in their genetic substance.

Viruses can reproduce only inside the host cells, which may be animal, plant or bacteria. They use their own genetic programme for reproduction, but rely on the raw materials, like amino acids and nucleotides and biosynthetic machinery of the host cells (*i.e.,* ribosomes, tRNA, enzymes) or their mulitplication. Thus, virus is an infectious, subcellular and ultramicroscopic particle. It is an obligate cellular parasite and a potential pathogen whose reproduction or replication in the host cell and transmission by infection causes characteristic reaction in the host cells. Outside the host cells, viruses are like non-living inert particles like the salt or sugar. They can be purified, crystallized and placed into jars on a shelf for years. **Alberts *et. al.,*** 1989 described viruses as naked genes that had somehow acquired the ability to move from one cell to another or they are cellular forms that have degenerated through parasitism, or they are primitive organisms that have not reached a cellular state.

Paramecium, Rhizopus (fungus) and *Vaucheria* (alga) also do not fit into the scope of cell theory. All of these organisms have undivided mass of protoplasm, which lacks cell-like organization and has more than one nucleus.

• 1.5. PROTOPLASM THEORY

In 1835, **Felix Dujardin** termed the jelly-like material within protozoans as **sarcode**. In 1835, **H. von Mohl** (1805–1875) described cell division. In 1839, **J. E. Purkinje** (1789–1869) coined the term **protoplasm** to describe the cell contents. In 1840, **Von Mohl** applied the name protoplasm to the contents of embryonic cells of the plants. In 1861, **Max Schultze** established similarity between sarcode end protoplasm of animal and plant cells. Protoplasm theory was given by **O. Hertwig** in 1892.

Protoplasm theory holds that all living matter from which animals and plants are formed is the **protoplasm.** The cell is an accumulation of living protoplasm limited in space by an outer membrane and possesses a nucleus. The protoplasm filled in the nucleus is called **nucleoplasm** and that found between nucleus and plasma membrane is **cytoplasm.**

• 1.6. ORGANISMAL THEORY

The body of all multicellular organisms is a continuous mass of protoplasm, which remains divided into small centres, the cells, for various biologcal activities. These differ from the unicellular protozoa only in size and degree of dfferentiation of the protoplasm. The differentiation is the separation of protoplasm into subordinate semi-independent compartments, called the cells. Even embryological development of a multicellular individual includes only growth and progressive internal differentiation of small single protoplasmic egg. The position of viruses also could not ascertain the organismal theory.

Table. 1. Units of measurement used in cell biology

LENGTH				
Metre (m)	**Millimetre (mm)**	**Micrometre or micron (mm)**	**Millimicron or Nanometre (nm or mμ)**	**Angstrom Å**
1	1,000 (1×10^{3})	1,000,000 (1×10^{6})	1,000,000,000 (1×10^{9})	1×10^{10}
0.001	1	1,000	1,000,000	1×10^{7}
0.000001	0.001	1	1,000	1×10^{4}
1×10^{-9}	1×10^{-6}	0.001	1	10
1×10^{-10}	1×10^{-7}	1×10^{-4}	0.1	1

• STUDENT ACTIVITY

1. What is cell theory ? Describe the cell theory and explain the exceptions to cell theory.

• TEST YOURSELF

1. Write short notes on :
   (i) Protoplasm theory
   (ii) Organismal theory
   (iii) Cell theory.

UNIT

2

CELL THEORY, STRUCTURE OF PROKARYOTIC AND EUKARYOTIC CELLS

STRUCTURE

- Cell theory given by various biologists.
- Structure of Prokaryotic cell.
- Structure of Eukaryotic cell.
- Comparison between prokaryotic and eukaryotic cells.
- Comparison between plant and animal cells.
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- The cell viruses, Cell theory, Structure of prokaryotic cell, Structure of eukaryotic cell, Comparison between prokaryotic and eukaryotic cells, Differences between animal and plant cells.

• 2.1. CELL

The cell is the basic unit structure of all living organisms. Within a semipermeable membrane, cell contains a complete set of different kinds of units, which are necessary for its own growth and reproduction from simple nutrients. Cell has been defined by different biologists in a different manners. **A. G. Loewy** and **P. Siekevitz** in 1963 defined cell as a unit of biologcal activity delimited by a semipermeable membrane and is capable of self reproduction in a medium free of other living systems. **Wilson** and **Morrison** in 1966 defined the cell as an integrated and continuously changing system. **John Paul** in 1970 defined the cell as the simplest integrated organization in living systems, capable of independent survival. All these defintions have not included the viruses. Virus is neither a cell nor an organism, but it contains a core of DNA or RNA, enclosed within a mantle of protein. Virus is quite inert in the free state and becomes activated when it infects a living host cell. In this process only the nucleic acid core enters the host cell. The nucleic acid is the genetic substance and it takes over the metabolic activity of the host cell and utilizes the cell machinery for the formation of more viruses, finally killing the host cell. Thus, viruses are cellular parasites, which can not undergo reproduction by themselves.

• 2.2. VIRUSES

(L., *Venoum* or poisonous fluid)

Viruses are very small submicroscopic structures, which have no cellular organization. They possess their own genetic material and have their own characteristic mode of inheritance. They multiply only within some host cells.

Virus's size varies in beween 30 to 300 nm and hence they can be observed under electron microscope. An infectious virus, called **virion** is formed of a core of DNA or RNA wrapped in a protein coat, called **capsid.** The capsid consists of numerous **capsomeres,** each has a few **structural units** or **monomers.** Each structural unit (capsomere) is formed of one or more polypeptide chains. Capsomeres are also of different shapes — hollow prisms, hexagonal, pentagonal, lobular etc.

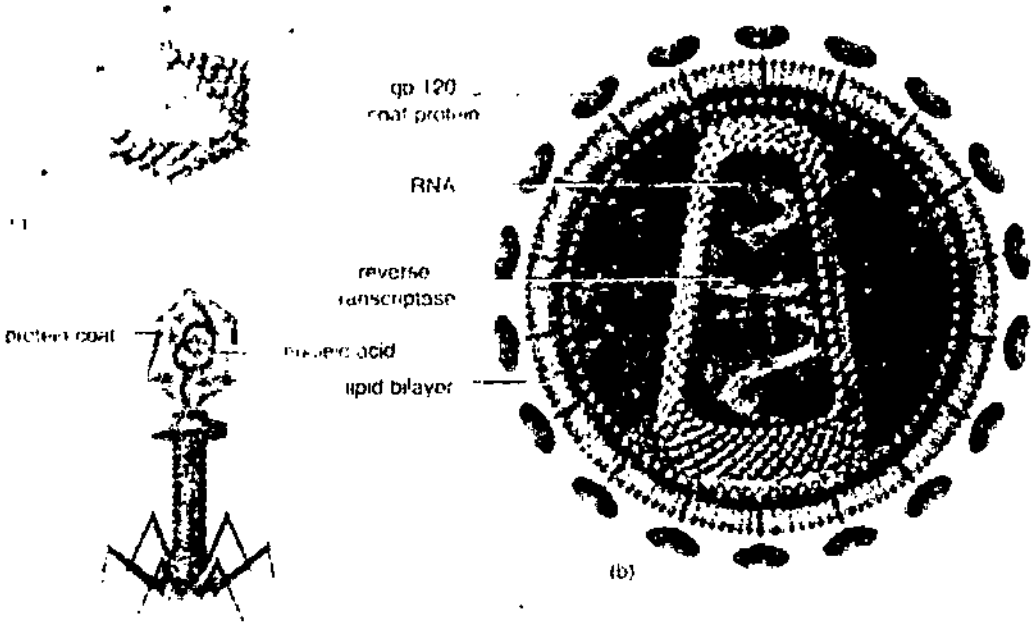


Fig. 1. Virus diveristy (a) Adenovirus, (b) HIV (c) T-even bacteriophage.

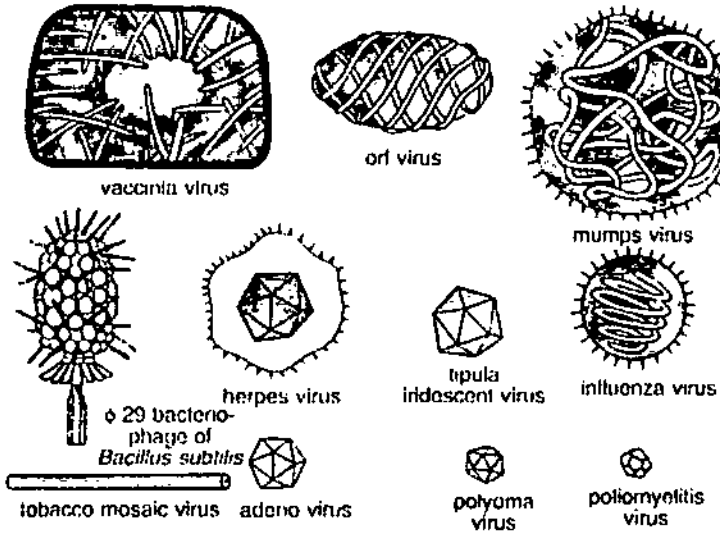


Fig. 2. Different kinds of viruses

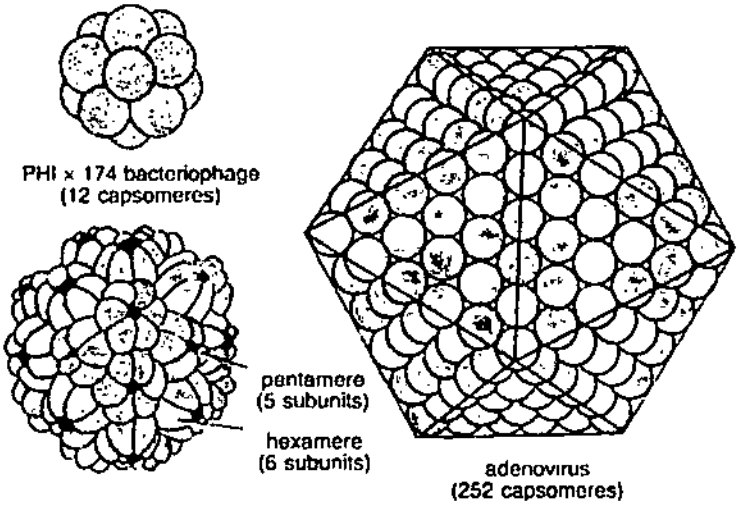


Fig. 3. Certain polyhedral (icosahedral) viruses

Viruses do not give rise directly to new viruses. Viruses use the biosynthetic machinery of their host cell to make virus specific proteins and nucleic acids, according to viral genetic information. Eventually virus particles are assembled from newly made molecules in the host cell and are released when the host cell bursts. Later they again initiate new cycles of infection in other host cells. Thus, viruses borrow metabolism and a shelter membrane from their host, and provide their own genetic instructions, which ensure continuity of their species from generation to generation.

Viruses that parasitize the bacterial cells are called **bacteriophages** or simply **phages.** T-even bacteriophages infect the colon bacillus, *Escherichia coli* and are also known as **coliphages.** T_4 bacteriophage is a large-sized tadpole-shaped complex virus. Its capsid has a 20-sided head, a short neck with **collar** bearing whiskers and a long helical **tail.** Tail is made up of a thick and hollow mid-piece, a hexagonal basal or end plate to which are attached six spikes and six long tail fibres.

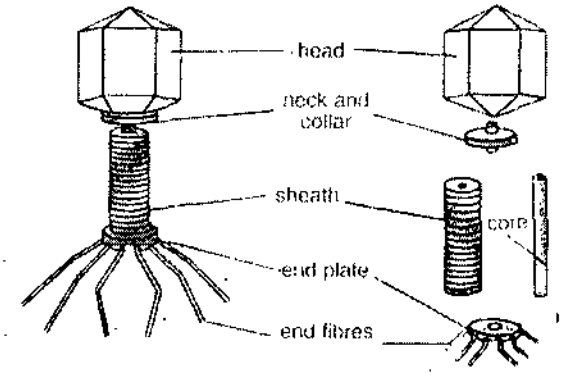


Fig. 4. A bacteriophage

• 2.3. CELL THEORY

A single cell constitutes an entire organism, as in protozoa or many cells grouped and differentiated into tissues and organs to form a multicellular organism. The cell is thus, a fundamental structural and functional unit of living organisms, just as the atom is the fundamental unit in chemical structures. If cellular organization is destroyed by mechanical or other means, cellular function is destroyed. Cell becomes disorganized and dies.

All living organisms are composed of cells and cell products. Cell is a mass of protoplasm limited in space by a cell membrane and possessing a nucleus (**Schwann, 1839**, and **Schleiden, 1838**). The protoplasm surrounding the nucleus became known as **cytoplasm** and protoplasm of the nucleus is called **karyoplasm. Virchow** (1855) expressed the cell theory as *Omnis cellulae e cellula*, *i.e.*, all cells arise from preexisting cells. Cells ensure continuity between one generation and another by the mechanism of mitosis (**Flemming**, 1880) and the precise partitioning of the chromosomes (**Waldeyer**, 1890).

Hertwig, (1875) discovered that the development of an embryo starts with the fusion of two nuclei, one coming from an egg and the other from a sperm cell introduced during fertilization. It was also established that egg and sperm cells (gametes) are formed by a reductional division, that was later called **meiosis**, by which number of chromosomes of a species remains constant from one generation to another. All the above discoveries led to the modern version of cell theory, which states that :

1. Cells are the morphological and physiological units of all living organisms.
2. Properties of a given organism depend on those of its individual cells.
3. Cells originate from other cells, and continuity is maintained through genetic material (chromosomes and their genes).
4. Smallest unit of life is the cell.

• 2.4. STRUCTURE OF PROKARYOTIC CELL

Cells are identified being or one or two recognizable types, prokaryotic or eukaryotic. **Monera**, *i.e.*, bacteria and blue-green algae are prokaryotic cells, while all the other kingdoms consist of organisms made up of eukaryotic cells. The main difference between these two cell types is that the prokaryotic cells lack a nuclear envelope. Prokaryotic chromosome occupies a space in the cell, called a **nucleoid**, which is in direct contact with the rest of protoplasm.

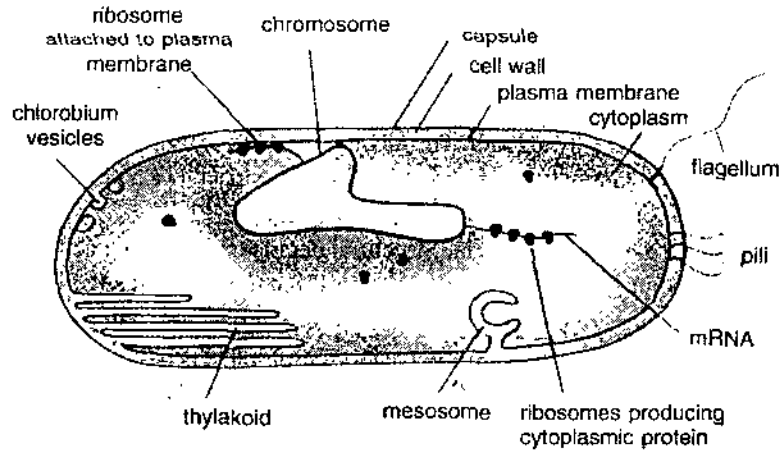


Fig. 5. Structures seen in the prokaryotic (bacterial) cell.

Eukaryotic cells have a true nucleus with an elaborate nuclear envelope, through which the nucleocytoplasmic interchanges take place.

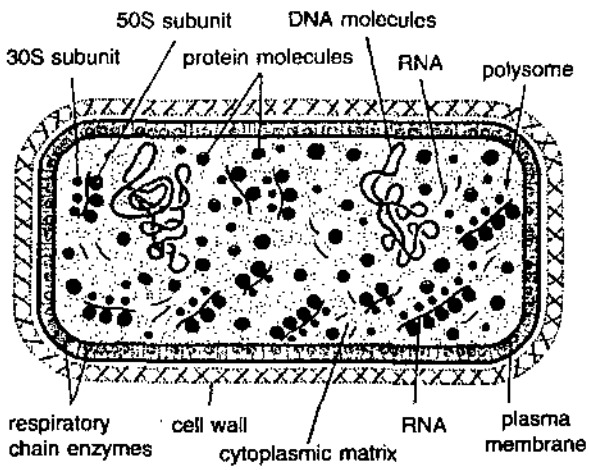


Fig. 6. Prokaryotic cell of *E. coli* (bacteria)

Prokaryotes are considered to be ancestors of eukaryotes. The most studied prokaryote cell is of bacteria, *Escherichia coli*. The bacterium is surrounded by two definite membranes separated by the **periplasmatic space.** The outer membrane is rigid and serves for mechanical protection and is, called **cell wall.** It contains polysaccharide, lipid and protein molecules. **Porin** is the most abundant polypeptide that is made of six to eight subunits that extend in the full thickness of the outer membrane. Periplasmatic space contains polysaccharides associated with proteins, *i.e.*, proteoglycans, or mucoproteins, forming a gel.

The inner membrane called **plasma membrane,** is a lipoprotein structure, that forms a molecular barrier with the surrounding medium. Plasma membrane controls the entrance and exit of small molecules and ions and thus, establishes a carefully regulated internal milieu. Plasma membrane contains enzymes involved in the oxidation of metabolites (*i.e.*, respiratory chain), as well as photosystems used in photosynthesis. Between the inner and outer membranes are present localized regions of adhesion or junctions. These adhesion zones contain receptors (*i.e.*, recognition sites, for bacteriophages and for the

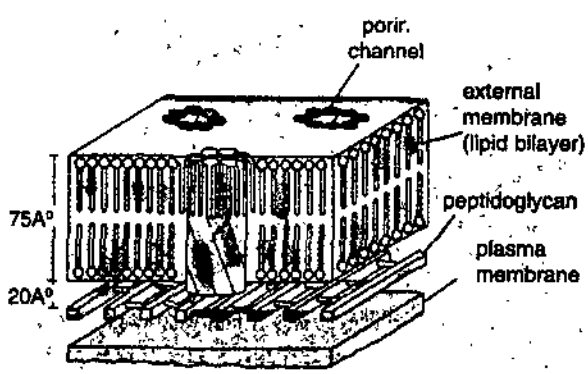


Fig. 7. Ultra-structure of cell wall.

attachment of flagella and pili. These regions are probably also the sites through which lipid and proteins are exported, across the inner membrane, to the periplasmatic space and the surrounding medium.

Bacterial chromosome is a single circular molecule of naked DNA tightly coiled within the nucleoid. This is a lighter region of protoplasm. The DNA of *E. coli* is about 1 μm long when uncoiled, contains all the genetic information of the organism. The single chromosome is circular and at one point it is attached to the plasma membrane.

In addition to the circular chromosome, certain bacteria contain a small extra chromosomal circular DNA, called a **plasmid.** A plasmid may confer resistance to one or more antibiotics upon the bacterial cell. Plasmids can be separated and reincorporated; **genes** (specific pieces of DNA) can be inserted into plasmids, which are then transplanted into bacteria.

Surrounding the DNA, in the dark region of protoplasm are 20,000 to 30,000 particles, about 25 nm in diameter, called **ribosomes.** Ribosomes are composed of ribonucleic acid (RNA) and proteins. Ribosomes consist of a large and a small subunit

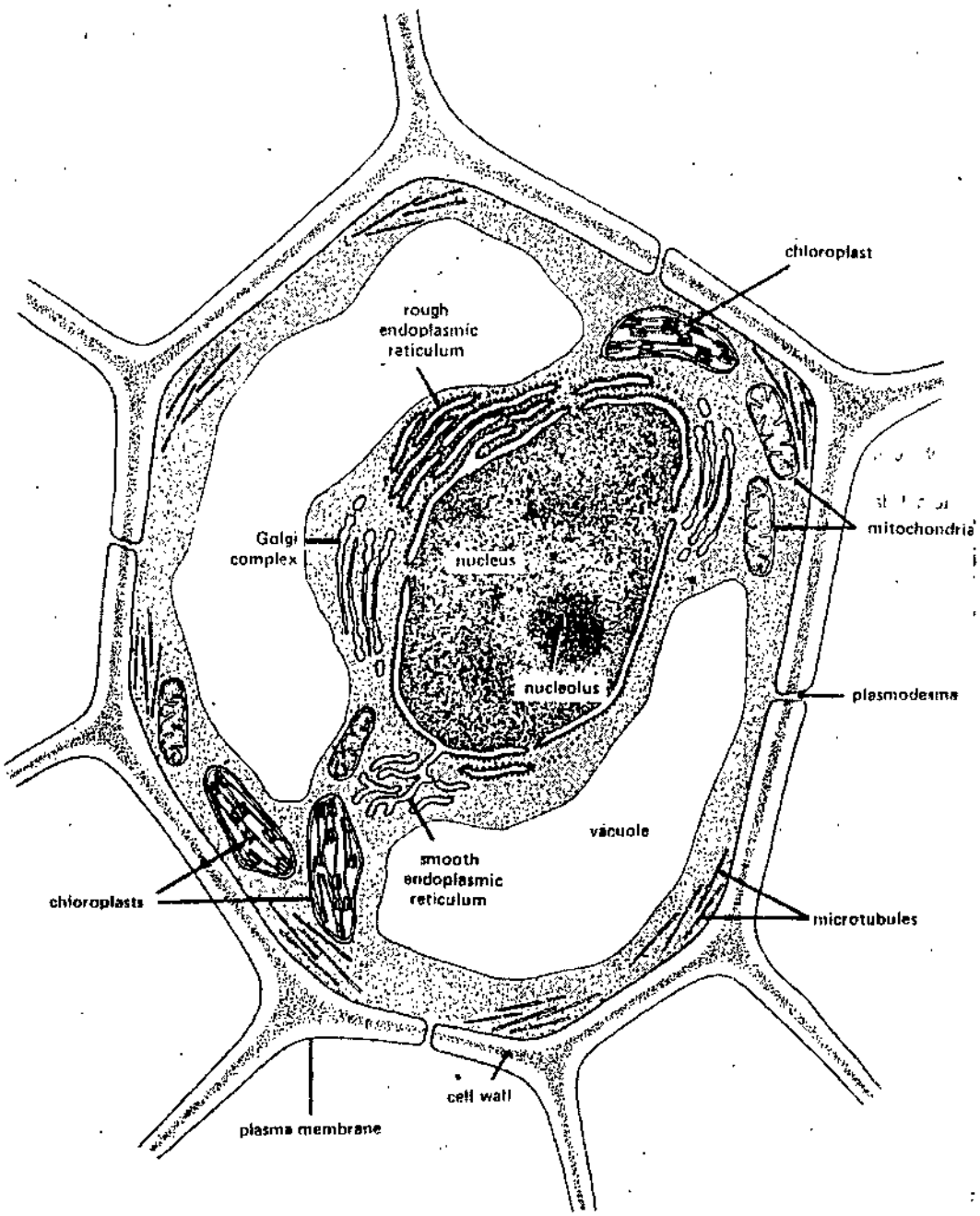


Fig. 8. Ultra structure of plant cell.

and exist in groups, called **polyribosomes** or **polysomes.** The remainder of the cell is filled, with water, various RNAs, protein molecules and various smaller molecules.

Certain motile bacteria have hair-like processes of variable length, called flagella, used for locomotion. Each flagellum is made of a single fibril. (In eukaryotic cells, cilia and flagella contain several microtubules).

• 2.5. STRUCTURE OF EUKARYOTIC CELL

Eukaryotic cell consists of a cell wall and plasma membrane, cytoplasm and nucleus.

Cell wall and Plasma Membrane

Most plant cells have an outer dead and rigid cell wall. It is composed of carbohydrates (cellulose, pectin, hemicellulose, lignin) and certain fatty substances, like waxes. Between the adjacent cells is found the **middle lamella,** which is pectin-rich cementing substance. The cell wall formed immediately after the cell division is the **primary cell wall.** It is composed of pectin, hemicellulose and loose network of cellulose microfibrils. In xylem and phloem cells, a **secondary cell wall** is formed on the inner side of primary cell wall. It is composed of cellulose, hemicellulose and lignin. In many plant cells, tunnels run through the cell wall, called **plasmodesmata.** These allow communication with the neighbouring cells. Cell wall provides protection and mechanical support to the plant cells.

Plasma membrane is found in all animal cells, and in plant cells on the inner side of cell wall. Plasma membrane is a very thin and delicate living membrane. This is also called **plasmalemma** or **cell membrane.** Plasma membrane is a three – layered structure with a translucent layer sandwiched between two dark layers. Plasma membrane is selectively permeable membrane, *i.e.*, it controls selectively the entrance

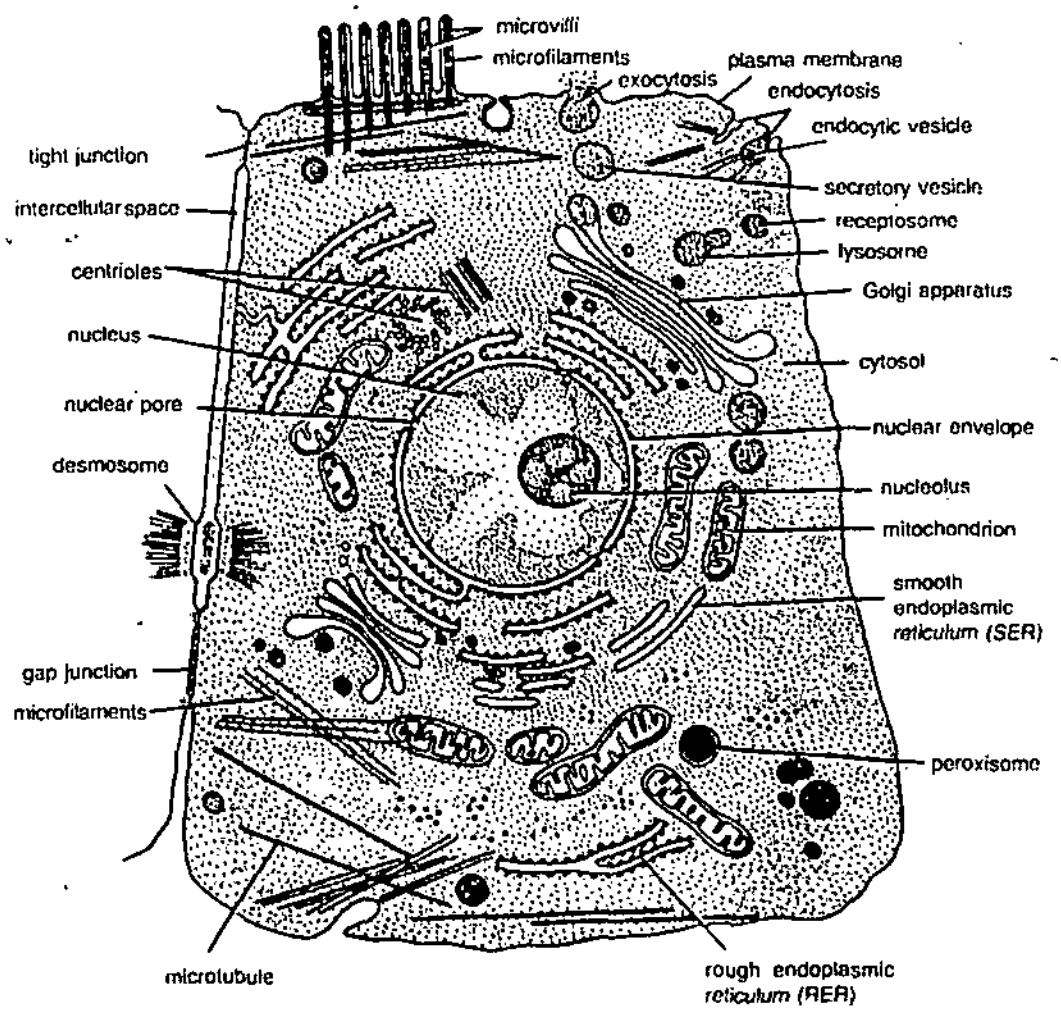


Fig. 8. Ultra-structure of animal cell.

and exit of materials. Water, oxygen, carbon dioxide, ethanol, ions, glucose, etc. are transported across the membrane by osmosis, diffusion and active transport.

Plasma membrane is also found around certain cell organelles like chloroplast, mitochondria, endoplasmic reticulum, and lysosomes.

Cytoplasm : Beneath the plasma membrane is found the cytoplasm, distinguished into cytosol, cytoplasmic structures and nucleus.

Cytosol : This is an organic colloidal fluids called **matrix** or **cytosol.** Cytosol is the aqueous portion of cytoplasm and of the nucleoplasm. Cytosol contains the soluble proteins and enzymes involved in glycolysis and activation of amino acids for protein synthesis. In many types of cells, it is differentiated into an outer viscous, clear and rigid layer, the **ectoplasm** or **cell cortex** and inner granular and less viscous **endoplasm.**

Cytosol of cells also contains fibres, termed **cytoskeleton.** Fibres are of three types : **microtubules** formed of tubulin protein; **microfilaments** are thinnest formed of actin protein and **intermediate filaments** (desmin filaments, keratin filaments, neurofilaments, vitamentin and glial filaments.

Cytoplasmic structures

Certain non-living and living structures are found suspended in the cytoplasm. Non-living structures are, called **inclusions** or **paraplasm**, and the living structures are membrane bound and are called **organelles** or **organoids.**

Cytoplasmic inclusions are oil drops, triacylglcerols, yolk granules or deutoplasm, secretory granules, glycogen granules and starch granules found in plant cells. **Cytoplasmic organelles** are **mitochondria** in which ATP molecules are generated, **plastids** in which formation and storage of carbohydrates takes place, rough **endoplasmic reticulum** in which protein synthesis takes place, **smooth endoplasmic reticulum** in whih synthesis of lipid and hormone occurs, **Golgi apparatus** for secretion, **lysosomes** for degradation of macromolecules, **nucleus** for the regulation of cellular activities and **centrosomes** for organization of spindle apparatus, etc.

• COMPARISON OF PROKARYOTIC AND EUKARYOTIC CELLS

Characteristics	Prokaryotic cells	Eukaryotic cells
Cell wall	Present in most but not in all cells.	Present in plant and fungal cells.
Plasma membrane	Present beneath cell wall.	Present
Nucleus	Absent	Present
Genetic material (chromosomes)	Circular or linear, double-stranded DNA	Linear double-stranded DNA
Nucleoli	Absent	Present
Plastids	Commonly present	Present only in plant cells.
Mitochondria	Absent	Present
Endoplasmic reticulum	Absent	Present
Lysosomes	Absent	Present
Chloroplasts	Absent	Present only in plant cells.
Ribosomes	Present (70S)	Present (80S)
Vacuoles	Absent	Present in plant cells.
Centrioles	Absent	Present, absent in higher plants.
Microtubules	Absent.	Present
Flagellae	Simple structure composed of flagellin protein.	Complex 9 + 2 structure of tubulin and other protein

• COMPARISON BETWEEN ANIMAL CELL AND PLANT CELL

Animal Cell	Plant Cell
Cell wall absent.	Cell wall present.
Plasma membrane present.	Plasma membrane present beneath the cell wall.
Plastids are present only in *Euglena* (protozoan).	Plastids are present.
Golgi apparatus single.	Golgi apparatus many and simple, called dictyosomes.
Centrosome and centrioles present.	Centrosome and centrioles absent.
Vacuoles many and small.	Most mature cells have large central vacuole filled with sap.
Mitochondria, ribosomes, endoplasmic reticulum, lysosomes, present.	All these cellular organelles present.

• SUMMARY

- Cell is a mass of protoplasm limited in space by a cell membrane, and possessing a nucleus.
- Viruses are submicroscopic biological structure lacking cellular organization. They possess their own genetic material.
- Protoplasm surrounding the nucleus is called cytoplasm and protoplasm of the nucleus is known as karyoplasm.
- **Virchow** expressed the cell theory as *Omnis cellulae e cellula*, *i.e.*, all cells arise from pre-existing cells.
- Cells are the morphological and physiological units of all living organisms.
- Prokaryotic cells are found in bacteria and blue-green algae, and have no definite nucleus. Their genetic material is naked, not bounded by nuclear membrane.
- Eukaryotic cells are bounded by outer cell wall (in plants) and plasma membrane in animal cells. In plant cells, plasma membrane is found on the inner side of the cell wall. Eukaryotic cells have a definite nucleus bounded by nuclear membrane.

• QUESTIONS

1. Describe the structure of prokaryotic cell.

2. Give an account of the structure of a typical animal cell.

3. Give a comparative account of prokaryotic and eukaryotic cells.

• VERY SHORT ANSWER QUESTIONS

1. **Who gave the aphorism, *"Omnis cellulae e cellula"* ?**
   Ans. This aphorism was given by Virchow in 1855.
2. **What is the meaning of *Omnis cellulae e cellula* ?**
   Ans. All cells arise from pre-existing cells.
3. **In which cell type definite nucleus is found ?**
   Ans. Definite nucleus is found in eukaryotic cells.
4. **In which organisms prokaryotic cells are present.**
   Ans. Bacterial blue-green algae and mycoplasms are prokaryotic cells. Small bacteria are called mycoplasmas which produce infectious diseases in animals including human beings and which can be cultured *in vitro* like any bacteria ranging from 0.25 to 0.1 um.
5. **What are viruses ?**
   Ans. Viruses are not true cells. Viruses are dependent on the host cells for reproduction and are considered obligatory parasites. Viruses may contain either DNA or RNA and they rely on the biosynthetic machinery of host to produce capsid proteins from their genetic information.
6. **What are viroids ?**
   Ans. Viroids are even simpler organisms than viruses. They are infectious agents that attack plant cells and consist of a single RNA molecule that is not covered by a capsid of protein.

UNIT

3

STRUCTURE AND FUNCTION OF CELL MEMBRANE

STRUCTURE

- Structure of plasma membrane
- Molecular organization of cell membrane.
- Composition of cell membrane.
- Cell permeability.
- Active transport.
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Cell membranes structure, its chemical composition, structure and functions of Cell Membrane: Permeability, Osmosis, facilitated diffusion and active transport.

• 3.1. CELL MEMBRANE

The cell or plasma membrane controls the entrance and exit of molecules and ions. The plasma membrane regulates this exchange between the cell and the medium is called **permeability.** Most plant cells have a thick cellulose wall that covers and protects the true plasma membrane. Most animal cells are surrounded by a **cell coat** or **external laminae** of cement-like substance. Plasma membrane is found in both prokaryotic and eukaryotic cells. Plasma membrane is an ultra-thin, elastic, living, dynamic and selective transport barrier. It is composed of lipids (phospholipids and cholesterol), proteins and carbohydrates. Plasma membrane produces difference in ion concentration between the interior and exterior of the cell. The name **cell membrane** was given by **C. Nageli** and **C. Cramer** (1855) and the term **plasmalemma** had been given by **J.Q. Plowe** (1931).

Plasma membranes are more easily isolated from erythrocytes by hemolysis. When red blood cells are treated with hypotonic solutions, they due to endosmosis swell and then lose haemoglobin. Th resulting membrane is called **red cell ghost.**

• 3.2. CHEMICAL COMPOSITION OF CELL MEMBRANE

Cell membrane contains proteins, lipids and carbohydrates in different ratios. In human red blood cells, plasma membrane contains 52 percent proteins, 40 percent lipids and 8 percent carbohydrates. In nerve cells, protein (myelin) is 18 percent, lipids are 79 percent and carbohydrates 8 percent.

Lipids are phospholipids, sphingolipids, glycolipids and cholesterol (sterols). All of these are amphipathic molecules, having both hydrophilic and hydrophobic domains (teritory). Cholesterol is not found in plasma membrane of prokaryotic cells.

Phospholipids may be acidic phospholipids (20%) such as sphingomyelin, neutral phospholipids, (80%) such as phosphatidyl choline, phosphatidyl-serine etc. Cordiolipin is found only in inner mitochondrial membrane.

Proteins : Plasma membrane contains about 50 percent protein. Proteins are of two main types : integral or intrinsic proteins and extrinsic or peripheral proteins. **Intrinsic proteins** are associated firmly with the membrane, while the **extrinsic proteins** have a weak association and are bound to lipids of membrane by electrostatic

interaction. On the basis of their functions, plasma memberane proteins are classified into three types : structural proteins, enzymes and transport proteins (carriers or permeases). **Structural proteins** are extremely lipophilic and form the main bulk, *i.e.*, backbone of the plasma membrane. **Enzymes** are ecto- or endo-enzymes and are of 30 types. **Transport proteins** transport specific substances across the cell membranes. All types of oligosaccharides of plasma membrane are formed of six principal sugars, all of which are glucose derivatives : D-galactose, D-mannose, L-fucose, sialic acid etc.

Carbohydrates : These are present as short, unbranched or branched chains of sugars like oligosaccnarides, attached either to exterior ectoproteins forming glycoproteins or to the polar ends of phospholipids at the external surface of the plasma membrane forming glycolipids. Carbohydrate is not found on the inner surface of plasma membrane.

Table 1. Some important enzymes present in the plasma membrane.

1.	Acetyl phosphatase	11.	Cholesterol esterase
2.	Acetyl cholinesterase (Ectoenzyme of erythrocytes)	12.	Guanylase cyclase
3.	Acid phosphatase	13.	Monoglyceride lipase
4.	Adenosine triphosphatase	14.	NAD-ase (Ectoenzyme of erythrocyte)
5.	Mg^{2+} ATPase (Endoenzyme of erythrocyte)	15.	Protein kinase (Endoenzyme of erythrocyte)
6.	Na^{+}-K^{+} ATPase Ectoenzyme of erythrocyte)	16.	Phospholipase A
7.	Adenylate cyclase (Endoenzyme of erythrocyte)	17.	Lactase
8.	RNAase	18.	Maltase
9.	Alkaline phosphatase	19.	Sialidases
10	Aminopeptidase	20.	UDP glycosidase

3.3. STRUCTURE OF PLASMA MEMBRANE

1. **Danielli** and **Davson** (1935) proposed a sandwich model for membrane structure. In it a lipid bilayer was coated on its either side with hydrated proteins (globular proteins). Mutual attraction between the hydrocarbon chains of lipids and electrostatic forces between protein and head of the lipid molecules maintain the stability of the membrane. Lipid bilayer is about 6 nm thick and each of protein layers is about 1.0 nm thick, having a total thickness of the membrane about 8.0 nm.

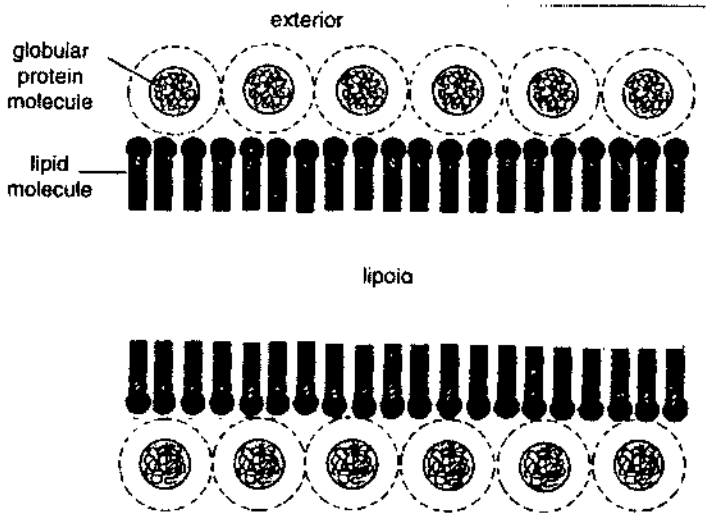


Fig. 1. Danielli-Davson model of membrane structure.

2. **Robertson unit membrane model of cell membrane.** Unit membrane hypothesis of **Robertson** (1960) states that cell membranes have trilaminar structure. Clear central layer corresponds to the hydrocarbon chains of lipids and dense surrounding layers to the proteins on both sides. But unit membrane model is somewhat artifactual, perhaps signifying only the appearance of three-layered structure after preparation for electron microscopy.

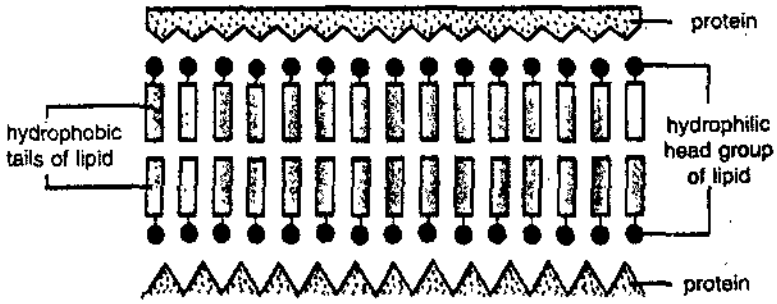


Fig. 2. Robertson model of unit membrane structure.

3. **Fluid mosaic model of S.J. Singer and G.L. Nicolson :** Fluid mosaic model has been widely accepted. According to this model, plasma membrane contains a bimolecular lipid layer, both surfaces of which are interrupted by protein molecules. Proteins occur in the form of globular molecules and they are found here and there in a mosaic pattern. The extrinsic proteins are attached at the polar surface of the lipid, while integral proteins either partially penetrate the bilayer or span the membrane entirely to stick out on both sides, called **transmembrance proteins.** Peripheral proteins and those parts of the integral proteins that stick on the outer surface frequently contain chains of sugar of oligosaccharides, *i.e.*, glycoproteins. Some lipids of outer surface are glycolipids.

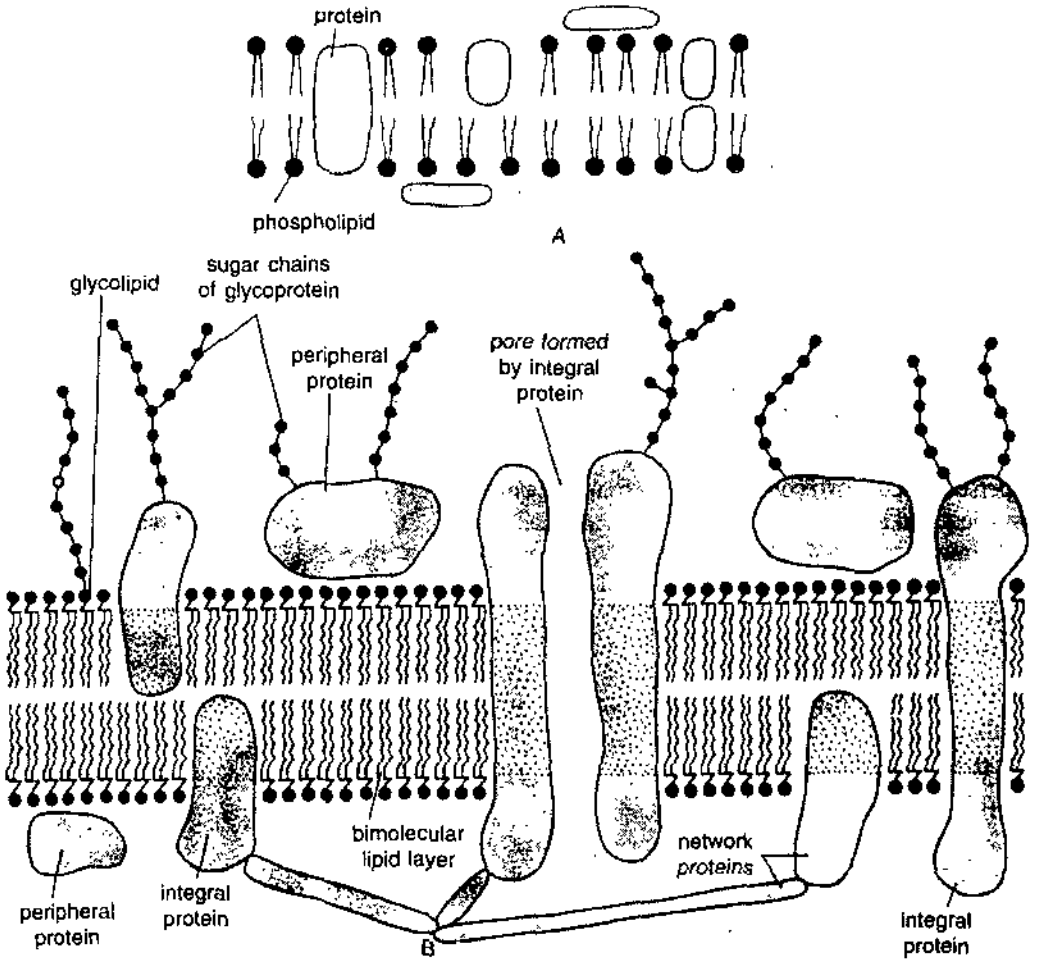


Fig. 3. Fluid mosaic model of plasma membrane. A. Simple view. B. Complex view.

Fluid mosaic membrane is of a fluid consistency and there is a considerable sideways movement of lipid and protein molecules within it.

Functions of Plasma Membrane

Plasma membrane is a thin barrier between intra-cellular fluid (cytoplasm) and extra-cellular fluid in which cell lies. In case of Protozoa (unicellular organisms) extracellular fluid is fresh water or marine water, while in multicellular organisms extracellular fluid is interstitial fluid, blood or lymph. Plasma membrane performs various important physiological functions as given below :

1. Permeability :

Permeability is fundamental to the functioning of living cell and to the maintenance of intracellular physiological conditions. This function determines, which substances can enter the cell, which may be necessary to maintain its vital processes and the synthesis of living substances. It also regulates the out flow of excretory material and water from the cell. Plasma membrane establishes a net difference between the intracellular fluid and extracellular fluid in which the cell is bathed. The cell membrane maintains a balance between the osmotic pressure of the intracellular fluid and that of the interstitial (extracellular) fluid.

Permeability may be **passive** if it obeys only physical laws. Its example is **diffusion.** If a concentrated solution of a soluble substance (for example sugar) is placed in water, there will be a movement of the solute along the concentration gradient, *i.e.,* from the region of high concentration to that of low concentration. If a plasma membrane is interposed, the diffusion process is greatly modified and the membrane acts as a **barrier** to the passage of water soluble molecules.

Overton demonstrated that substances that dissolve in lipids pass more easily into the cell. In diffusion or passive transport an ion or molecule crossing a membrane moves down its concentration gradient. In all cells there is a difference in ionic concentration with the extracellular medium, an electrical potential exists across the membrane. The electrical potential depends on an unequal distribuion of the ions on both sides of the membrane.

No metabolic energy is consumed in **passive diffusion.**

2. Osmosis : Plasma membrane is permeable to water molecules. The movement of water molecules through the plasma membrane takes place due to the difference in the concentration of the solute on both the sides. The process by which water molecules pass through the membrane from a region of higher concentration to the region of low concentration is called **osmosis.** It is of two types :

(i) Endosmosis : In it the water molecules enter into the cell.

(ii) Exosmosis : In it the water molecules pass out from the cell.

Plasmolysis occurs in plant cells. In excessive exosmosis in plant cells, cytoplasm along with plasma membrane shrinks away from the cell wall.

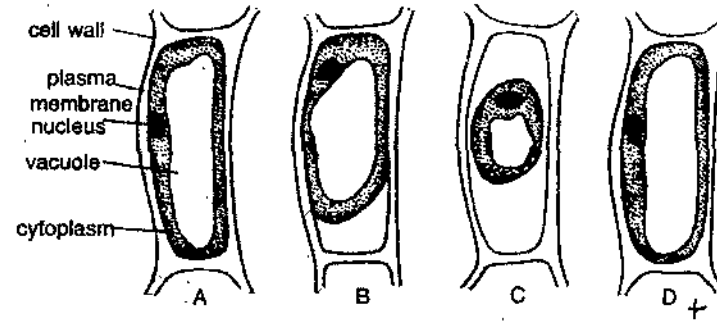
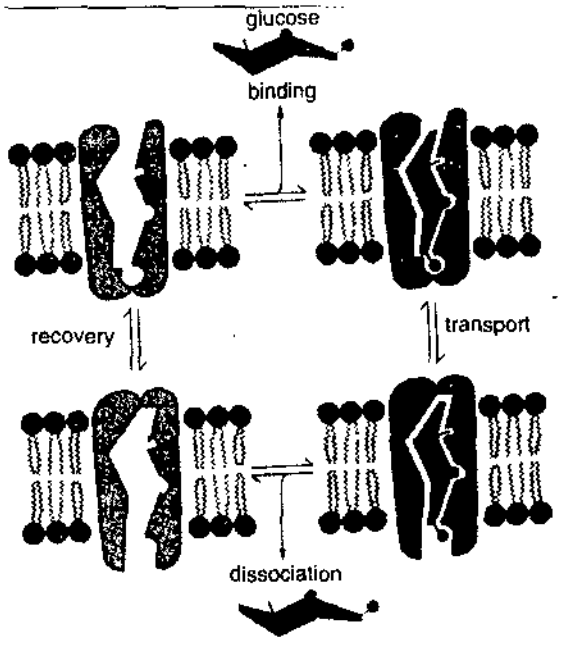


Fig. 4. Plasmolysis in plant cell.

It occurs when cells are kept in hypertonic solution, *i.e.,* extracellular fluid concentration is higher than that of intracellular fluid. The water inside the cell develops a pressure, called hydrostatic pressure and the **hydrostatic pressure**

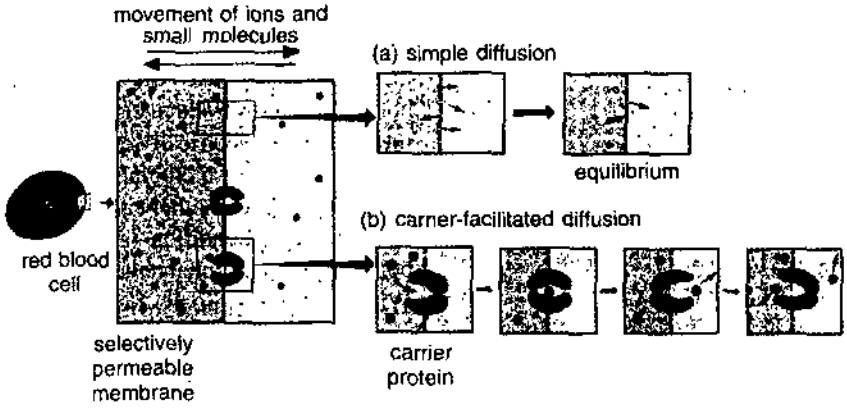
caused by osmosis is, called **osmotic pressure.** Plasma membrane maintains a balance between osmotic pressure of intracellular and intercellular fluid.



Facilitated diffusion.

3. Facilitated diffusion. In facilitated diffusion ions or molecules pass through the membrane rapidly with the help of specific permeases present in the membrane, which help in their crossing. It does not require the metabolic energy and it takes place only in the direction of a concentration gradient. Facilitated diffusion also involves the movement of molecules from higher concentration to lower concentration. Their passage is mediated by proteins that make the transported molecules to pass the membrane without directly interacting with its hydrophobic interior. Thus, polar and charged molecules like carbohydrates, amino acids, nucleosides and ions pass through the plasma membrane.

Carrier proteins and **channel proteins** mediate facilitated diffusion. **Carrier proteins** bind specific molecules to be transported on one side of the membrane and



Diffusion in the cell.

Fig. 5. Diffusion in the cell. A–Simple diffusion. B–Carrier facilitated diffusion.

then undergo conformational change (change of shape), which allows the molecule to pass through the membrane and is released on the inner side. Carrier proteins allow the diffusion of sugars, amino acids and nucleosides across the plasma membranes of most cells.

Channel proteins simply form open pores in the membrane through which small molecules of appropriate size and charge pass freely through the plasma membrane. Channel proteins, **porins** allow the free passage of ions and small polar molecules through outer membranes of bacteria. These porins form open aqueous channels through which ions and small molecules pass freely.

• 3.4. ACTIVE TRANSPORT

Active transport employs specific transport proteins, the **pumps,** which use metabolic energy, ATP, to move ions or molecules against their concentration gradient.

When an ion is transported against an electrochemical gradient, extra consumption of oxygen is required. For example, in invertebrates and vertebrates, concentration of

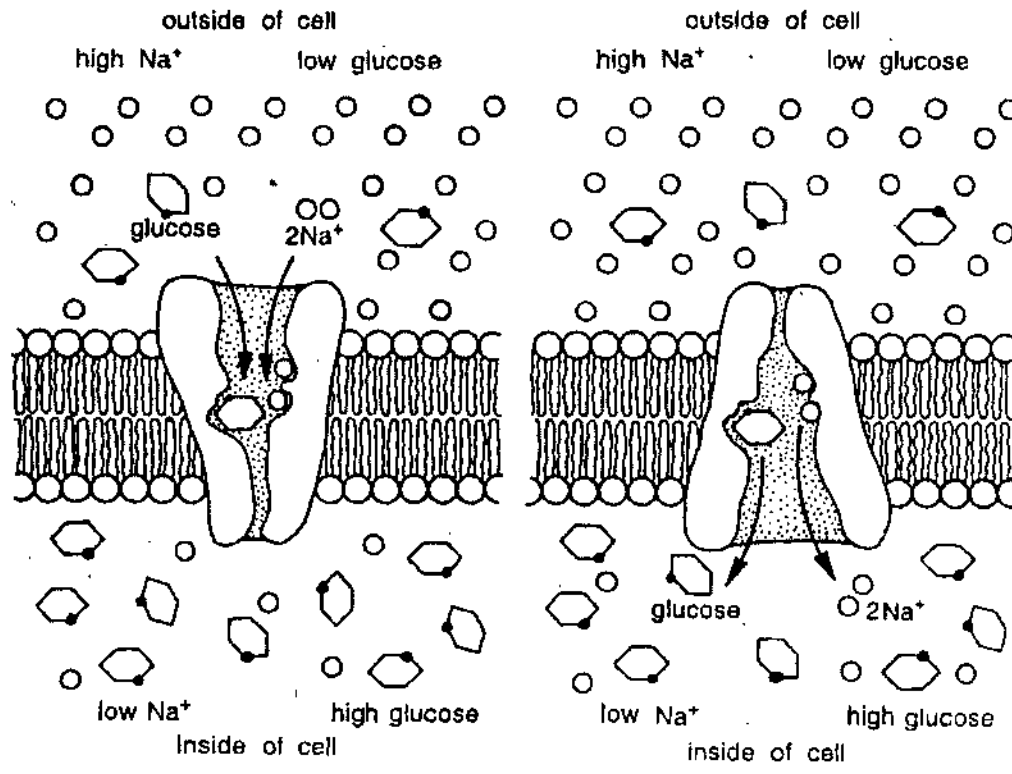


Fig. 6. Active transport of ions across plasma mmbrane (Na^+ .... K^+ pump).

sodium ions is about 10 to 20 times higher in blood than within the cell, while concentration of potassium ions is generally 20 to 40 times higher inside the cell. Low concentration of sodium inside the cell is maintained by the **sodium-potassium pump** or **Na^+K^+ATPase.** Pumps are of different types for different types of ions or molecules, such as calcium pump, proton pump, etc. Active transport is directly related to cell respiration.

Active transport may be defined as the movement of ions or molecules through the cell membrane against the prevailing concentration with the help of energy. The energy is derived from ATP hydrolysis. First Na+ ions bind to high affinity sites inside the cell. This binding stimulates hydrolysis of ATP and phosphorylation of the pump, inducing a conformational change that exposes Na^+ binding sites to the outside of the cell and reduces their affinity for Na^+. Consequently bound Na^+ is released into the extracellular fluids. At the same time, high affinity K^+ binding sites are exposed on the cell surface. The binding of extracellular K^+ to their sites stimulates hydrolysis of phosphate group bound to the pump and thus, induces a second conformational change, exposing K^+ binding sites to cytosol and lower thier binding affinity so that K^+ is released inside the cell. There are three binding sites for Na^+ and two for K^+, so each cycle transports three Na and two K^+ ions across the plasma membrane at the expense of one molecule of ATP.

Na^+ ___ K^+ ATPase is found associated with plasma membrane that actively transports Na^+ and K^+. Hydrolysis of ATP and transport of Na^+ and K^+ is closely linked, and ATP is not hydrolysed unless Na^+ and K^+ are transported. Thus, hydrolysis of ATP by $Na^+ - K^+$ ATPase is linked to the transport of K^+ into the cell and Na^+ out of the cell. Na^+ and K^+ should be located in aqueous medium for transportation.

5. Phagocytosis : Ingestion of solid particles by the cell through plasma membrane is, called phagocytosis. *Amoeba* catches its prey by this process. In this process, pseudopodia of *Amoeba* surround the food particles and ingest it directly. Leucocytes of blood ingest bacteria, cell debris, etc. by this process. First the particle to be ingested becomes absorbed at the membrane surface and later on is taken into cytoplasm by infolding of plasma membrane. The ingested particles form vacuoles, called **phagosomes,** which are later digested by lysosomal activity.

6. Pinocytosis : Liquid substances are incorporated into the cell by plasma membrane. Proteins of high molecular weight, such as ribonuclease enter the cell through plasma membrane. Molecules or particles become absorbed at the surface of plasma membrane, infolding of which occurs, called **pinosome** or **pinocytic vesicle,** which is then released into the cytoplasm.

• SUMMARY

- Cell membrane controls the entrance and exit of molecules or ions.
- Plasma membrane is the outermost layer or coat of the cell, surrounding cytoplasm in animal cells and in plant cells it is found beneath the cell coat.
- Plasma membrane is very thin, elastic, living, dynamic and selectively permeable.
- Cell membrane is composed of lipids, proteins and carbohydrates. Lipids are phospholipids, sphingolipids, glycolipids and cholesterol. Cholesterol is not found in cell membranes of prokaryotic cells. Proteins are of two types : Integral or intrinsic and extrinsic or peripheral. Carbohydrates are present as short unbranched or branched chains of sugars like oligosaccharides, either attached to outer ectoproteins or to polar ends of phospholipids at the external surface of membrane. Carbohydrates are not found on inner surface of membrane.
- Fluid mosaic model of S.J. Singer and G.L. Nicolson is the most universally accepted model of plasma membrane. Membrane contains a bimolecular lipid layer, both surfaces of which are interrupted by protein molecules. Membrane is of a fluid consistency and there is a considerable sideways movement of lipid and protein molecules within it.
- Plasma membrane is a thin barrier between intracellular fluid and extracellular fluid in which cell bathes.
- Plasma membrane allows the movement of small ions and molecules of various substances. This nature of membrane is called permeability. It is selectively permeable membrane because it allows only certain selected ions and small molecules to pass through it.
- Transport across the membrane may be passive-diffusion of ion or molecule across the membrane against concentration gradient. In passive transport energy is not required.
- ***Osmosis :*** *Water molecules pass through the membrane from a region of higher* concentration to the region of lower concentration, this is called Osmosis.
- **Facilitated diffusion :** In this process ions or molecules pass through the membrane with the help of specific permeases. It also needs energy. *It also occurs against the* concentration gradient.
- **Active transport :** In it metabolic energy is utilized. Movement of ions and molecules against their concentration gradient is aided by metabolic energy. It's example is $Na^+ \leftrightarrow K^+$ pump or Na^+ K^+ ATPase, Calcium ATPase, Proton pump, etc.
- **Phagocytosis.** Ingestion of solid food particles by the cell through plasma membrane.
- **Pinocytosis :** It is cell drinking. Intake of small droplets of extracellular fluid by pinosomes *is pinocytosis.*

• STUDENT ACTIVITY

1. Describe various models of plasma membrane, and explain which model is dynamic.

2. Describe fluid mosaic model of plasma membrane.

3. Describe the various functions of cell membrane.

• VERY SHORT ANSWER QUESTIONS

1. **Define red cell ghost.**

   Ans. Isolated plasma membrane of red blood corpuscles is called red cell ghost.

2. **Describe the structure of plasma membrane.**

   Ans. Plasma membrane is formed of bilayer of lipids into which protein molecules are embedded in a kind of mosaic arrangement.

3. **What is Na^+ __ K^+ ATPase.**

   Ans. It is an ion pump or cation exchange pump driven by energy of one ATP molecule to export three Na ions outside the cell in exchange of two K^+ ions inside the cell.

4. **Define phagocytosis.**

   Ans. It is a process of ingestion of large solid food particles by the cell through plasma membrane.

5. **Define pinocytosis.**

   Ans. Intake of tiny droplets of extracellular fluid by pinosomes.

6. **Define endocytosis.**

   Ans. Small regions of plasma membrane fold inwards (invaginate) to form an intracellular membrane bound vesicle within the cell.

UNIT

4

STRUCTURE OF NUCLEUS

STRUCTURE

LEARNING OBJECTIVES

After going through this unit you will learn :

- Nucleus, its structure, number, shape and size and its contents, ultrastructure of nucleus, chromatin fibres, ultrastructure of nucleolus.

• 4.1. NUCLEUS

The heart of the cell is nucleus in which is found the DNA. Nucleus controls different metabolic activities of the cell as well as hereditary activities. Nuclei were first discovered and named by **Robert Brown** (1833) in the plant cells. Nucleoli were first noted by **Pontana** (1781) and described by **M.J. Schleiden** (1838). **Bowman** (1840) coined the term nucleolus. Term chromatin for chromosomal meshwork was given by **W. Flemming** (1879). Terms cytoplasm and nucleoplasm were given by **Strasburger** in 1882. **O. Hertwig** in 1893 demonstrated the presence of delimiting membrane around the nucleus. **Hammerling** in 1955 by his grafting experiments on *Acetabularia* confirmed the role of nucleus in heredity.

• 4.2. OCCURRENCE

Nucleus is found in all the eukaryotic cells of animals and plants, but erythrocytes of mammals have no nucleus, whereas prokaryotic cells (bacteria) do not have true nucleus. DNA is single, circular and large body found within the cytoplasm. Nucleus is usually found in the centre of the cell.

• 4.3. NUMBER, SHAPE AND SIZE

The cells usually contain a single nucleus, but number of nuclei varies from cell to cell. According to the number of nuclei, cells are of the following types :

1. *Mononucleate* Cells : Mostly animal and plant cells have single nucleus, such cells are called mononucleate cells.

2. Binucleate Cells : Cells having two nuclei are called binucleate cells, for example *Paramecium* (Protozoa), cells of liver and cartilage.

3. Polynucleate Cells : Cells having more than two nuclei, from three to 100, are called polynucleate cells. In animals, such cells are called syncycial cells and in plants these are called coenocytes. **Osteoblast cells** of bone marrow and striated muscle fibres are examples of syncytial cells. Example of coenocytic plant cell is *Vaucheria* (siphonal algae).

Shape of the nucleus is normally related with the shape of the cell. Spheroidal nuclei are found in spheroidal, cuboidal or polyhedral cells. Elipsoidal nuclei are found in prismatic or fusiform cells. Discoidal nuclei are found in squamous epithelial cells. Irregular-shaped nuclei are found in leucocytes (white blood corpuscles), glandular cells or some insects, etc.

Size of the nuclei varies from about 3 μm to 25 μm in diameter. Size of the nucleus is related with the number of chromosomes present within the nucleus.

• 4.4. ULTRASTRUCTURE OF THE NUCLEUS

The following structures are generally recognized in the interphase nucleus :

1. A nuclear envelope composed of two membranes and perforated at intervals by the nuclear pores.
2. Nucleoplasm or nuclear sap that fills most of the nuclear space.
3. Chromatin fibres.
4. Nucleolus

1. Nuclear Envelope : The nuclear envelope is a special perinuclear cisterna with an inner and outer membranes enclosing a lumen and traversed by pores. Nuclear envelope consists of two concentric membranes separated by a **perinuclear space** 10 to 15 nm in width. These membranes have a bilayer structure similar to that of other biological membranes, having ribosomes only on the outer surface. Lipid constitution of these membranes is similar to that of the rough endoplasmic reticulum and direct continuities with rough endoplasmic reticulum can frequently be observed branching from the nuclear membrane.

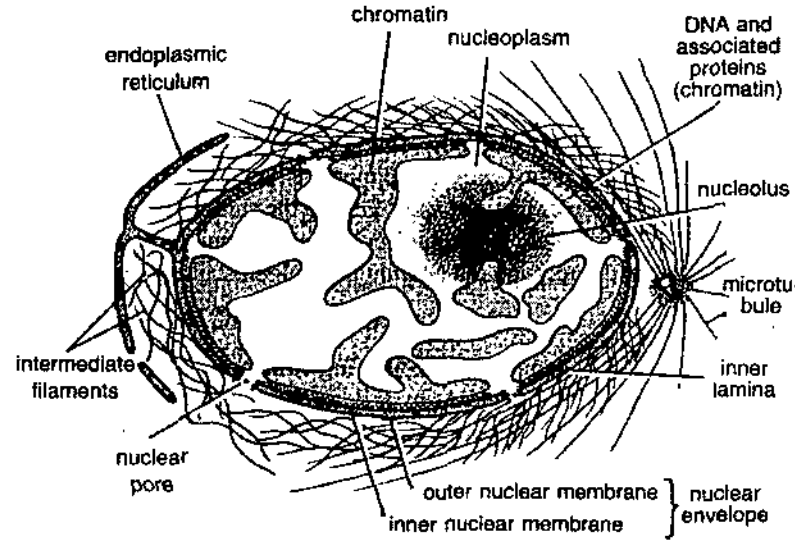


Fig. 1. Section of a typical cell nucleus.

The nuclear envelope at certain points is interrupted by **pores.** Around the margins of these nuclear pores, both membranes are in continuity.

2, Structure of Nuclear pore : The nuclear pores are large. 80 nm in diameter. Nuclear pores are not wide-open channels and they are occluded by an electron dense material. The pores are enclosed by circular annuli. The pores and annuli are together, called as **pore complex.** Pore complex consists of two rings or annuli with an inside diameter of 80 nm, large particles that form a central plug and eight radial spokes that extend from plug to the rings. Sometimes, but not always, particles attached to the cytoplasmic side of the

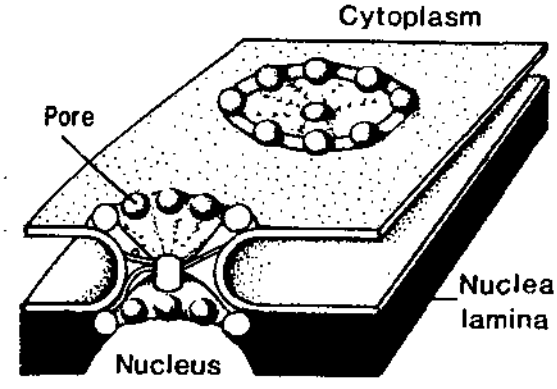


Fig. 2. Nuclear pore envelope, consists of two lipid bilayers occupied by pore complexes. On the inner side, a nuclear lamina covering the membrane, except the nuclear pores. Lamina proteins bind chromatin, which thus becomes attached to the nuclear envelope.

ring that are also octagonally arranged. These particles might be inactive ribosomes attached to the periphery of the pore complex.

The number of pores in the nuclear membrane seems to correlate with the transcriptional activity of the cell. In frog *Xenopus (laevis),* oocytes have 60 pores, whereas mature erythrocytes have only three pores. In the nuclei of mammals, the nuclear pores are about 5 to 15 percent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the nuclear pores may be 20 to 36% of the surface area of the nuclear membrane.

Two proteins have been found associated to the nuclear envelope.. One is an integral membrane protein, a glycoprotein that may anchor the annuli to the lipid bilayers. The second protein located on the cytoplasmic side of the electron-dense material that occludes the nuclear pores. It may be involved in the transport of materials through the nuclear pore.

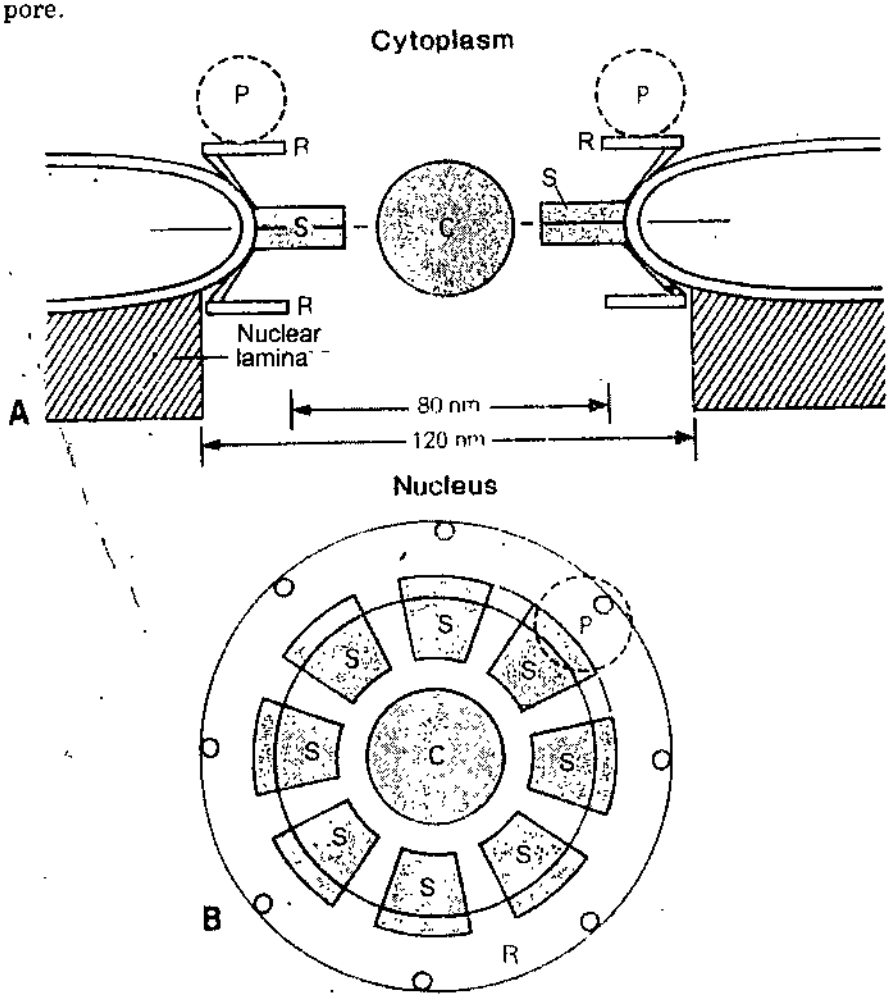


Fig. 3. Diagram of the pore complex. A. Cross section, B = Top view. R = Two peripheral rings, S = Central spokes, C=central plug, P = cytoplasmic particles.

Nuclear Lamina : In most eukaryotic nuclei, a proteinaceous layer 50 to 80 nm thick separates the peripheral heterochromatin from the inner nuclear membrane. Chromatin binds strongly to the inert part of the nuclear lamina.

The materials exchanged between nuclear and cytoplasm must traverse the nuclear pore complexes. This exchange is very selective and allows passage of only certain molecules, of either low or very high molecular weight. One of the main functions of nuclear envelope is to prevent the entrance of active ribosomes into the nucleus. Ribosomes and other cytoplasmic components are selectively excluded from the nucleus.

Nucleus Proteins Accumulate Selectively in the Nucleus : The cell nucleus contains a specific protein, such as RNA polymerase, DNA polymerase and histones. All these proteins are synthesized in the cytoplasm and transported into the nucleus.

Nucleoplasm

Within the nucleus is filled a transparent, semisolid, granular and slightly acidophilic **nucleoplasm** or nuclear sap (**karyolymph**). The chromatin threads and nucleolus are found suspended in the nucleoplasm. Nucleoplasm is composed of mainly the nucleoproteins, and other inorganic and organic substances, like nucleic acids, proteins, enzymes and minerals.

1. Nucleic acids of the nucleoplasm are DNA and RNA. Both may occur in the macromolecular state or in the form of their monomer nucleotides.

2. Proteins. Proteins of the nucleoplasm are of complex type. Nucleoproteins are of two types :

(i) **Basic proteins.** They take basic stain and the most important basic proteins of the nucleus are **nucleoprotamines** and **nucleohistones. Nucleoprotamines** are simple and basic proteins of very low molecular weight. Arginine is the most abundant amino acid of this protein. Protamines usually remain bounded with DNA molecules by salt linkage. Protamines are found in the spermatozoa of certain fishes.

Nucleohistones have high molecular weight. Histones are composed of basic amino acids like arginine, lysine and histidine. Histone proteins are associated with DNA by ionic bonds and they are found in the nuclei of most organisms.

(ii) **Acidic or non-histone proteins.** These are found in the nucleoplasm or in the chromatin. The most abundant acidic proteins of euchromatin are phosphoproteins.

3. Enzymes. Nucleoplasm contains many enzymes, which are essental for the synthesis of DNA and RNA. The most important nuclear enzymes are DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine diaminase, nucleoside phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceralydehyde dehydrogenase, and pyruvate kinase. ATP and acetyl CoA are also present in nucleoplasm.

4. Lipids. These are found in a very small amount.

5. Minerals. Several inorganic compounds like phosphorus, potassium, sodium, calcium and magnesium are also found in the nucleoplasm. These are more abundant in chromatin in comparison to nucleoplasm.

• 4.5. CHROMATIN FIBRES

Nucleoplasm contains many thread-like, coiled and much elongated **chromatin fibres,** which take basic stains like basic fuchsin. These fibres are observed in the interphase nucleus. During cell division chromatin fibres become thick ribbon-like, known as **chromosomes.** Chemically, chromatin consists of DNA and **proteins.** Small quantity of RNA may also be present. Most of the protein of chromatin is **histone,** non-histone proteins are also present. The fibres of chromatin are twisted, anastomosed and uniformly distributed in the nucleoplasm. Chromatin material is of two types, *i.e.,* heterochromatin and euchromatin.

Heterochromatin is darkly stained, condensed region of chromatin. Heterochromatin occurs around the nucleolus and at the periphery. It contains comparatively small amount of DNA and large amount of RNA.

Euchromatin is lightly stained and diffused region of the chromatin. It contains large amount of DNA.

Nucleolus

Most cells contain in their nuclei one or more spherical, colloidal acidophilic nucleoli. Nucleolus is not found in cells of bacteria and yeast. The size of nucleolus is related with the synthetic activity of the cell. Cells with little or no synthetic activities (sperm cells, muscle cells, blastomeres, etc.) contain smaller or no nucleoli, while oocytes, neurons and secretory cells, which synthesize proteins etc., contain large-sized nucleoli. Nucleolus is often associated with nucleolar organizer (NO), which represents the secondary constriction or the nucleolar organizing chromosomes.

Nucleolus is not bounded by any limiting membrane. Calcium ions are supposed to maintain its intact organization. Nucleolus contains DNA of nucleolar organizer, four types of rRNAs, 70 types of ribosomal proteins, RNA binding proteins and RNA splicing nucleoproteins. Nucleolus also contains phospholipids, ortho-phosphates, Ca^{2+} ions, some enzymes, like acid phosphatase, nucleoside phosphorylase and DNA synthesizing enzymes, etc.

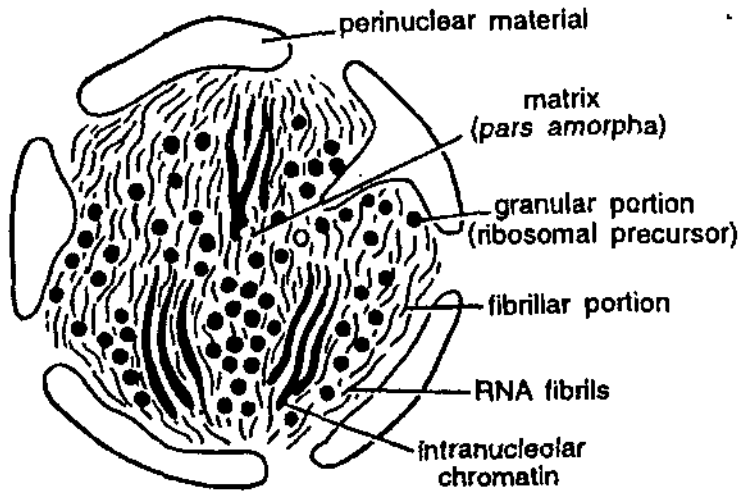


Fig. 4. Ultrastructure of nucleolus.

Ultra-structure of Nucleolus : The electron microscopy of nucleolus shows two regions in most nucleoli : a **granular zone** and a **fibrillar zone. Granular zone** consists of granules 15 to 20 nm in diameter and occupies the peripheral region of the nucleolus. *The* ***fibrillar zone*** *consists of fine fibres 5 to 10 nm in diameter* and is located in a most central region of the nucleolus. The fibrillar area contains rRNA genes that uncoil from the chromosome and penetrate the nucleolus, together with nascent RNA molecules attached to it. The granular area represents ribosome precursor particles and various stages of assembly and processing. Once the ribosomal subunits are mature, they are released from the nucleolus and exit into the cytoplasm through nuclear pores. The nucleolus is the cellular site at which all the ribosomal components are assembled together into ribosomal subunits. The genes coding for 5S rRNA are not located in the nucleolus. The 5S genes are present in multiple copies, which are located in the tips (telomeres) of most chromosomes.

Direct evidence of the function of the nucleolus as a ribosome factor, was obtained with the discovery of an anucleolate mutant of *Xenopus laevis* which can not synthesize 18S, 5.8S and 28S rRNAs.

• SUMMARY

- Nucleus is the heart of the cell, in which is found the DNA.
- Nucleus controls all the metabolic activities of the cell and heredity.
- Nucleus is found in all eukaryotic cells, but prokaryotic cells lack definite nucleus.
- *Interphase nucleus contains outer nuclear envelope composed of two membranes* perforated at intervals by the nuclear pores, nucleoplasm filling the nuclear space, chromatin fibres and nucleolus.
- Both the membranes of the nuclear envelope are joined with each other around the margins of the nuclear pores. Nuclear pores are enclosed by circular annuli. Pores and annuli are together *called pore complex.*
- Materials transported from the nucleus into cytoplasm pass through nuclear pores.
- Nucleoplasm or nuclear sap is found in the nucleus.
- Nucleoplasm contains thread-like, coiled and elongated chromatin fibres.
- *Cells contain one or more* spherical, colloidal acidophilic nucleoli.
- Nucleolus is not found in bacteria and yeast.

• STUDENT ACTIVITY

1. Describe the nuclear envelope and structure of the pores.

2. Describe the structure of nucleus.

3. Describe the structure and function of the nucleolus.

• VERY SHORT ANSWER QUESTIONS

1. **Who discovered the nucleus in plants ?**
   Ans. Robert Brown in 1831.
2. **What is the composition of chromatin ?**
   Ans. Chromatin is a viscous, gelatinous substance and contains DNA, RNA, histones and non-histone proteins.
3. **Who named the term chromatin for chromosomal meshwork ?**
   Ans. W. Flemming in 1882.
4. **Name the type of cells in which definite nucleus is not found ?**
   Ans. Prokaryotic cells (bacteria, blue-green algae) have no definite nucleus.
5. **Which type of cells have more than two nuclei ?**
   Ans. Polynucleate cells. In animals these are called syncytial cells and in plants coenocytes.
6. **Where are nuclear pores found ?**
   Ans. Nuclear pores are found in nuclear envelope.
7. **What is pore complex ?**
   Ans. Pores and circular annuli found in the nuclear envelope together are called pore complex.
8. **What is nuclear lamina ?**

Ans. It is a proteinaceous layer about 50-80 nm thick that separates the chromatin from the inner membrane of nuclear envelope.

9. **What are the two types of chromatin material ?**

   Ans. Heterochromatin (darkly stained, condensed region of chromatin) and lightly stained diffused region of chromatin is euchromatin.

10. **What is the function of nucleolus ?**

    Ans. Nucleolus is a ribosome factory – 18S, 5.8S and 28S rRNAs are synthesized in the nucleolus, 5S rRNA is synthesized on the chromosomes outside the nucleolus and 70S ribosomal proteins are synthesized in the cytoplasm. All these components migrate to the nucleolus, where they are assembled into ribosomal subunits and transported to the cytoplasm.

11. **Name the two regions of the nucleolus.**

    Ans. Outer granular region surrounded by the associated chromatin and central region is fibrillar or nucleolonema.

UNIT

5

STRUCTURE AND FUNCTIONS OF ENDOPLASMIC RETICULUM, GOLGI COMPLEX, RIBOSOMES AND LYSOSOMES

STRUCTURE

- Structure of Endosplasmic reticulum : Tubular, Vesicular and Cisternae
- Types of Endoplasmic reticulum : Smooth and rough.
- Functions of Endoplasmic reticulum : Smooth and rough
- Structure of Golgi complex : Cisternae, tubules and vesicles
- Functions of Golgi complex
- Types of ribosomes and Structure of ribosomes
- Function of ribosomes
- Structure of lysosomes
- Kinds of lysosomes : Primary lysosomes, heterophagosomes, autophagosomes and residual bodies
- Functions of lysosomes
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Structure and functions of endoplasmic reticulum; Golgi complex, Ribosomes and Lysosomes.

5.1. STRUCTURE AND FUNCTIONS OF ENDOPLASMIC RETICULUM

STRUCTURE AND FUNCTIONS OF ENDOSPLASMIC RETICULUM (ER)

Endoplasmic reticulum, Golgi complex, ribosomes and lysosomes, present in cytoplasm, called cytoplasmic organelles are membrane-bound living structures and perform biosynthetic and metabolic activities.

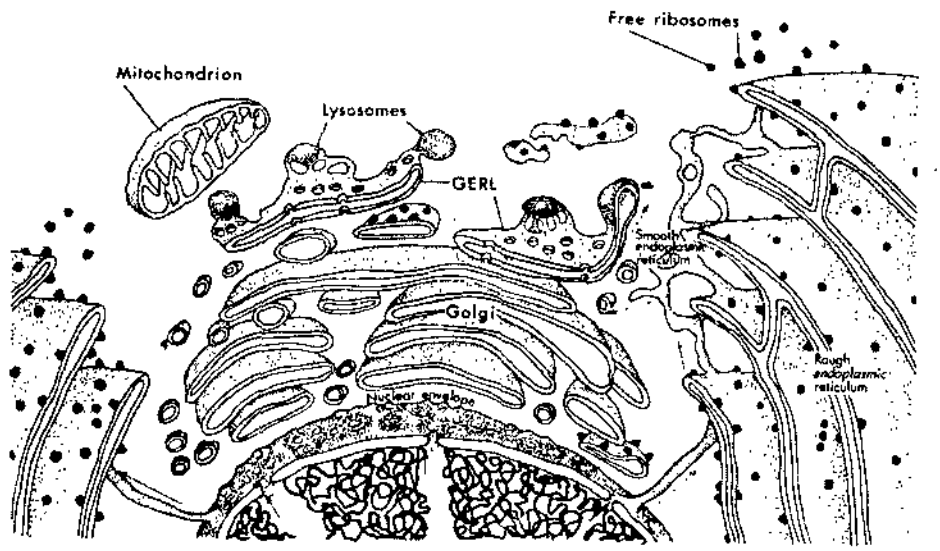


Fig. 1. Three dimensional diagram of the endomembrane system of the cell.

In both plant and animal cells, the cytoplasm is permeted by a complex system of membrane-bound **tubules, vesicles** and **cisternae** (flattened sacs). These at many points intercommunicate with each other. The endoplasmic reticulum is a vast network that subdivides the cytoplasm into two main compartments : one enclosed within the membranes, while the other situated outside, called the **cytoplasmic matrix** or **cytosol.**

The existence of ER was discovered in 1945 by electron microscopy. ER is a lace-like arrangement of tubules that did not reach the periphery of the cell, hence the term "endoplasmic".

One of the main functions of the endomembrane system is segregation, within the lumen of proteins that are synthesized by ribosomes. Many of these proteins are then processed and chanelled for export.

• 5.2. MORPHOLOGY OF ENDOPLASMIC RETICULUM

The ER is also called the **cytoplasmic vacuolar system.** ER on the presence or absence of ribosomes on the outer (cytoplasmic surface) is of two types : rough or granular ER (RER) and smooth or agranular ER (SER).

Smooth endoplasmic reticulum has smooth walls due to the absence of ribosomes. This type of ER is found in those cells in which metabolism of lipids including steroids and glycogen takes place. SER is generally found in adipose cells, interstitial cells, liver cells storing glycogen, heart fibres, leucocytes and spermatocytes. SER in striated muscle cells is termed as **sarcoplasmic reticulum. Myeloid bodies** are tightly packed vesicles and tubules in pigmented retinal cells.

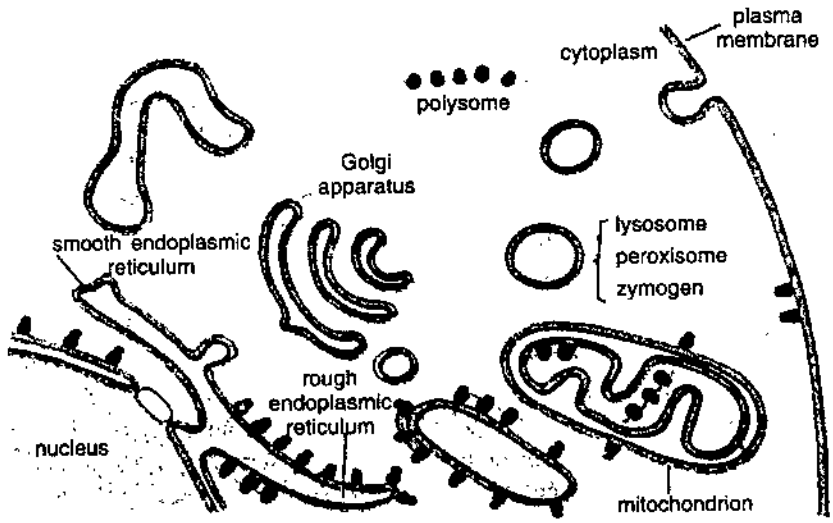


Fig. 2. Two faces of membranes of endomembrane system. Thick lines shows luminal faces and thin lines for cytoplasmic faces in each endomembrane system

In liver cells rich in glycogen, tubular network in the cytoplasmic matrix is visible in the form of dense particles, called **glycosomes** (50-200 nm in diameter). SER forms a continuous system with RER, but it has a different morphology.

Rough endoplasmic reticulum is found in cells, which are active in protein synthesis, like pancreatic cells, plasma cells, goblet cells and liver cells. RER can be stained by basophilic stain and are named **ergastoplasm,** basophilic bodies, chromophilic substances or Nissl bodies.

Annulate lamellae or pores are found in ER of invertebrates, ovocytes and spermatocytes of vertebrates. These annuli resemble with the pores or annuli of nuclear membrane. Evagination from the nuclear envelope forms the annulate lamellae of ER to which are associated the ribosomes.

GERL (Golgi, ER, Lysosome) refers to a special region of endomembrane system, which is more related to the Golgi complex, which forms the lysosomes. Each

endomembrane system has two faces : **cytoplasmic face** and **luminal face** or extracellular face. The luminal face borders the cavities of RER and SER and Golgi bodies.

The **size of ER** varies considerably in different cell types and is related to their functions. It is often small and relatively undeveloped in eggs and in embryonic cells, but increases in size and complexity with differentiation of cells. Simple SER are found in cells engaged in lipid metabolism, such as adipose, brown fat and adrenocortical cells. ER is poorly developed or is non-existent in reticulocytes, which produce only proteins that are retained in the cytosol (haemoglobin).

Endoplasmic recitulum is found in three forms : Lamellar or cisternae (closed fluid-filled sac), vesicle and tubular or tubules.

1. Cisternae : These are long, flattened sac-like unbranchd tubules whose diameter is 40 to 50 μm. These are arranged in parallel bundles. Rough ER are usually found in the form of cisternae. These are found in those cells. which are synthetically active, *e.g.*, cells of pancreas, brain and notochord.

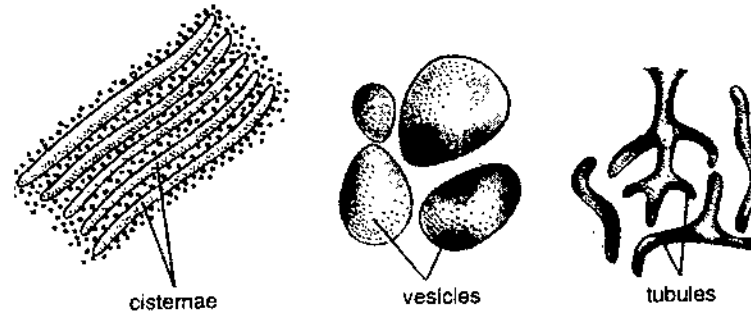


Fig. 3. Various forms of endoplasmic reticulum.

2. Vesicles : These are oval vacuolar structures whose diameter is 25 to 500 μm. These are abundant in SER and are found isolated in cytoplasm. These are abundant in pancreatic cells. Tightly packed vesicles are found in pigmented retinal cells as **myeloid bodies.**

3. Tubules : These are branched reticular system found along with cisternae and vesicles in all the cells. Their diameter is 50 to 100 μm. These are found in SER. Tubules are found in those cells, which are active in synthesis of cholesterol, glycerides etc. These are also found in epithetical cells of retina involved in metabolism of vitamin A. In striated muscle cells, ER is arranged in a network of tubules called **sarcoplasmic reticulum.**

• 5.3. FUNCTIONS OF ENDOPLASMIC RETICULUM (ER)

A large portion of the ER is associated with the ribosomes, and thus, it plays a fundamental role in the storage and processing of proteins that are destined for export from the cell (cell secretion). ER represents a fundamental part of the endomembrane system, which divides the cytoplasm into two definite compartments. Furthermore, these membranes contain many enzyme systems that are able to carry out various functions, including biogenesis of some of the membrane components.

1. Membrane Biogenesis : ER membrane may develop by evagination from the nuclear envelope. At telophase the nuclear envelope is reformed by vesicles of ER. In the period of rapid growth of ER, the incorporation into proteins and lipids is greater in rough than in smooth type. Thus, synthesis of membrane follows the direction RER —> SER.

Current concepts of membrane biogenesis generally assume a multi-step mechanism : First synthesis of a basic membrane of lipids and intrinsic proteins and thereafter, the addition, in a sequential manner, of other constituents, such as enzymes, specific sugars or lipids.

Lipids and proteins may be added to the various cellular membranes independent of each other. Thus, the growth of the membrane results from the insertion of individual lipid and protein molecules.

SER contains the main phospholipid synthesizing enzymes.

Insertion of proteins into ER membrane is independent of that of lipids. Most proteins are formed on membrane-bound ribosomes. Some proteins of ER are formed by free ribosomes in the cytosol and then inserted into the membrane. NADH-cytochrome-b_5 reductase is synthesized in cytosol and becomes incorporated in various parts of endomembrane system (*i.e.*, RER and SER, Golgi complex, and in outer mitochondrial membranes).

2. ER acts as a circulatory system for intracellular circulation of various substances. Membrane flow is an important mechanism for carrying particles, molecules and ions into and out of the cells. Export of RNA and nucleoproteins from the nucleus to the cytoplasm occurs through ER.

Membrane of ER at body temperature is highly dynamic and also fluid. Bound ribosomes are mobile on the membrane. Transfer of secretory proteins is accompanied by a flow of newly synthesized membrane proteins that are incorporated into RER.

3. Transport of ions and small molecules across ER membrane.

ER along with cytoplasmic matrix participates in many of the mechanical functions of the cell.

ER provides mechanical support for the colloidal structure of the cytoplasm.

Sarcoplasmic reticulum (SR) is a specialized form of ER found in striated muscle fibres F, is considered as an intracellular conducting system.

4. Functions of SER : The upper given functions are applied to both parts of ER. The following are the functions of SER.

(i) Detoxification : Drugs such as Phenobarbitol administered to an animal, result in increased activity of enzymes related to detoxification and hypertrophy of SER. Administered steroid hormones also produce detoxification. Detoxifying enzymes are aryl hydroxylases (benzopyrene). The effect of these drugs is to produce a true induction of the enzymes of ER.

(ii) Synthesis of lipids : Phospholipid biosynthesis is largely confined to the membranes of SER.

(iii) Glycogenolysis : In fasting animals, the residual glycogen remains associated with the tubules and vesicles of ER. When feeding is started, there is an increase in SER, which maintains its association with the accumulating glycogen in the form of glycosomes. Thus, SER is related to glycogenolysis.

In prenatal liver cells, just before birth, the amount of glycogen increases and then decreases simultaneously with an increased amount of glucose-6-phosphatase. This depletion of glycogen is accompanied by an increase in SER.

5. Exchange of molecules by process of osmosis, diffusion and active transport takes place through the membranes of ER. ER membrane contains permeases and carriers like plasma membrane.

6. ER membranes form the new nuclear envelope after divisions.

• 5.4. GOLGI COMPLEX

Golgi complex or Golgi apparatus is a differentiated portion of the endomembrane system. It is found in both animal and plant cells. Golgi complex is spatially and temporally related to ER on one side and by way of secretory vesicles may fuse with specific portions of the plasma membrane. It works as an intermediary in secretory processes.

Camillo Golgi in 1898 discovered a reticular structure in the cytoplasm of nerve cells by means of silver staining.

• 5.5. STRUCTURE OF THE GOLGI COMPLEX

Golgi complex is not found in prokaryotic cells and certain fungi, sperm cells of bryophytes and pteridophytes, sieve tubes of plants, mature sperms and red blood cells of animals.

Golgi complex in animal cells is located in between the nucleus and the periphery. Golgi complex is a complex array of interconnecting membranous flattened sacs or cisternae, clusters of tubules and vesicles of about 60 nm and large vacuoles filled with an amorphous or granular content.

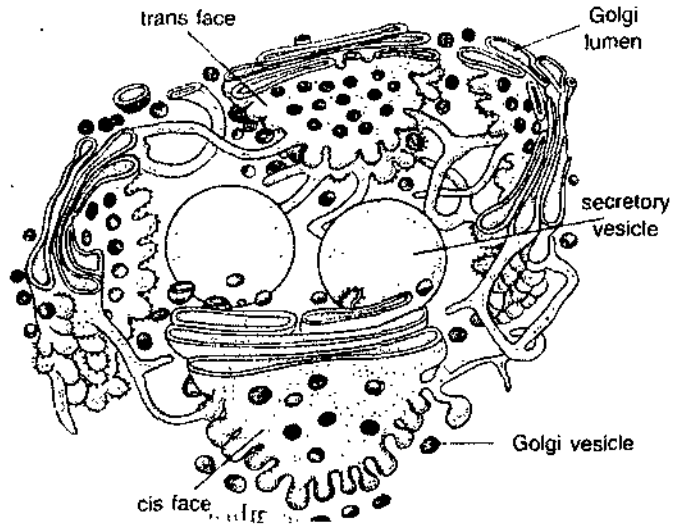


Fig. 4. Structure of Golgi complx : (Three dimensional structure)

In plant cells, it has been collectively, called the **dictyosome** and may consist of flattened disc-shaped cisternae and associated secretory vesicles.

1. Cisternae or Flattened sacs : Golgi cisternae are arranged in parallel bundles or stacks and are separated by a space of 20 to 30 nm, which may contain rod-like fibres. Cisternae are flattened, plate-like or saucer-like closed compartments held in parallel bundles one above the other. Often cisternae are arranged concentrically with a convex and concave face. In most animal and plant cells these may be three to seven. In certain algae there may be as many as 10 or 20 cisternae.

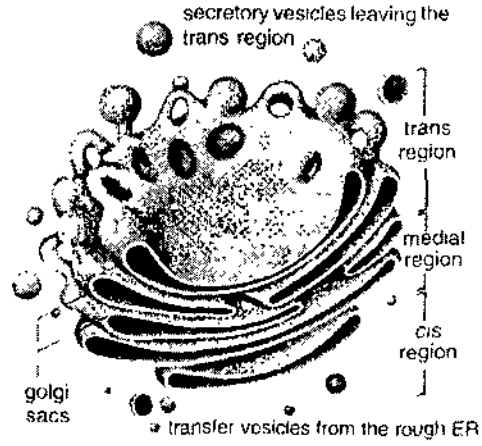


Fig. 5. Cisternae are flattered closed compartments.

Each bundle of cisternae forming a dictyosome is a polarized structure having a **proximal** or **forming face** (*cis-face*) generally convex and closer to the nuclear envelope or ER, and a **distal** or **maturing, face** (*trans-face*) of concave shape. Cis-face is characterized by the presence of small transition vesicles or tubules that converge upon the Golgi cisternae, forming a kind of fenestrated plate. These cisternae vesicles are formed as blebs from ER and migrate to the Golgi, where by coalescence, they form new cisternae.

Associated with the trans or maturing face there is often a sacular structure rich in acid phosphatase and has been, called **GERL**. It indicates that it has been interpreted as a region of SER, near the Golgi, which is involved in the production of **lysosomes.** Most recent work relates the GERL to **Golgi condensing vacuoles** or **presecretory granules.**

2. Tubules : A complex array of associated vesicles and anastomosing tubules having diameter of 30 to 50 nm surrounds the dictyosome and radiate from this. The peripheral area of dictyosome is fenestrated.

Fig. 6. Secretory vesicles leaving the trans region.

3. Vesicles : Vesicles of 60 nm diameter are of three types :

(i) Transition vesicles : These are small and are supposed to form as blebs from the ER to migrate and converge to *cis-face* of Golgi where they coalesce forming new cisternae.

(ii) Secretory vesicles : These are discharged from the margins of cisternae of Golgi found between the maturing face of Golgi and plasma membrane.

(iii) Clathrin-coated vesicles : These are spherical outgrowths having about 50 μm diameter and with a rough surface. These are found at the ends of tubules or cisternae. These are morphologically distinct from the secretory vesicles. These are known to play a role in intracellular traffic of membranes and secretory products, *i.e.*, between ER and Golgi, and between GERL region and the endosomal and lysosomal compartments.

5.6. FUNCTIONS OF THE GOLGI COMPLEX

Golgi complex represents a special membranous compartment interposed between ER and extracellular space. Through this compartment there is a continuous traffic of substances, which may have been synthesized elsewhere, but which are modified and are transformed while transported.

Most of the cytoplasmic membranes of eukaryotic cells arise from RER (except inner membranes of mitochondria and chloroplasts). Due to this mechanism, occurs loss of attached ribosomes to form SER, pinching of vesicles, which fuse with the cis-face of Golgi complex, modification of proteins within the Golgi and production of secretory vesicles from the trans-face of Golgi cisternae. After this process of membrane flow and differentiation, vesicles finally fuse with the plasma membrane.

Golgi complex plays a major role in the glycosidation of lipids and proteins to produce glycosphingolipids and glycoproteins.

Golgi complex also appears to be involved in the addition of sulphate to the carbohydrate moiety of glycoproteins. In cartilage cells, mucopolysaccharides as well as glycoproteins are synthesized in the Golgi complex.

Golgi complex plays a central role in the biosynthesis of gangliosides and other glycosphingolipids.

Secretion : ER and Golgi complex are directly involved in the synthesis, transport, and release of macromolecules from the cell. Cell secretion is not confined to animal cells alone, plant cells secrete polysaccharide and proteins to make their cell walls. The enzymes present in lysosomes are produced by a kind of secretory process.

Golgi membranes are involved in the formation of the primary lysosomes. This process have the sequence-synthesis, aggregation, transport and packaging of the enzymes (proteins).

In the maturing face of the Golgi is the GERL region, in which acid phosphatase (lysosomal enzyme) is present. This region has been involved in the formation of primary lysosomes.

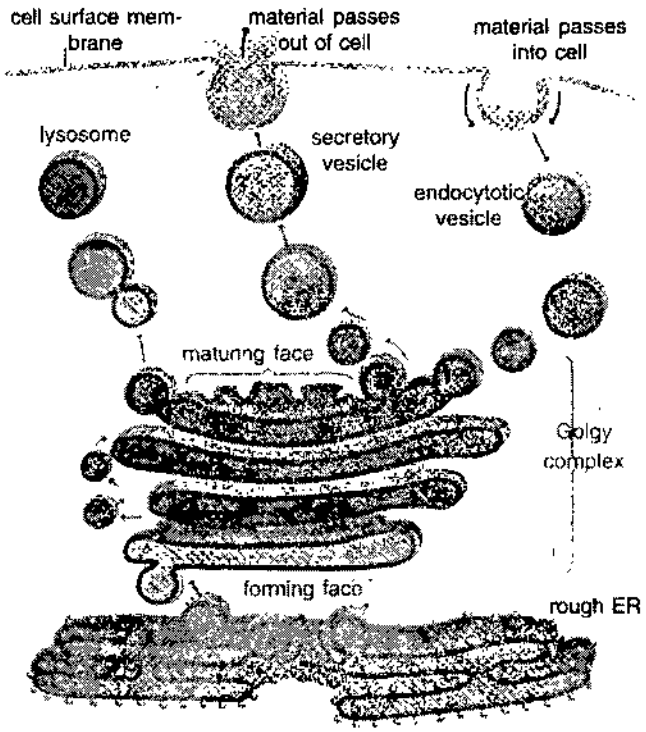


Fig. 7. Golgi complex with vesicle (Electron microscope)

According to **Novikoff** the GERL of the Golgi complex appears to be involved in the formation of melanin granules, in the processing and packaging of secretory material in endo- and exo-crine cells and in lipid metabolism.

Acrosome Formation. Golgi complex forms the acrosome of sperm during sperm maturation.

• 5.7. RIBOSOMES

Ribosomes were first observed by **Palade** in electron microscope as dense particles or granules. They were shown to contain approximately equal amount of RNA and protein. Ribosomes are found in all cells and these are involved in protein synthesis. An *E. coli* cell contains 10,000 ribosomes and these represent 25% of the total mass of the bacterial cell. Mammalian cultured cells contain 10 million ribosomes per cell and is about double as large as a prokaryotic ribosome. **Albert Claude** showd that cytoplasmic RNA was included in tiny particles of ribonucleoprotein, which were later called ribosomes.

STRUCTURE OF RIBOSOME

Ribosome is a spheroidal particle of 23 nm and is composed of large and a small subunits. **Eukaryotic ribosomes** sedimentation coefficient is 80S, and in the absence of Mg++ these ribosomes dissociate reversibly into subunits of 40S and 60S. **Prokaryotic ribosomes** are smaller and their sedimentation coefficient is 70S. Their subunits are 30S and 50S. Ribosomes are also found in the mitochondria and chloroplast of eukaryotic cells, but they are always smaller than the 80S cytoplasmic ribosomes and are comparable to prokaryotic ribosomes in size and sensitivity to antibiotics. Their sedimentation values vary in different phyla.

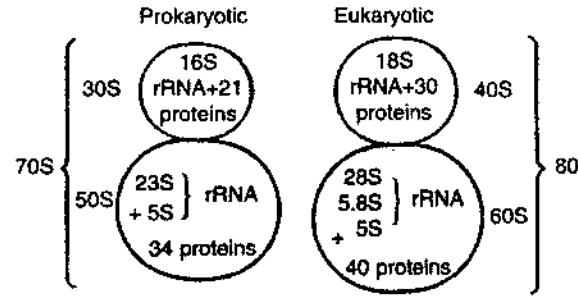
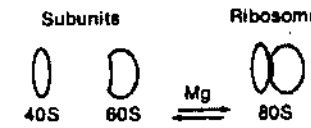


Fig. 8. Component of prokaryotic (70S) and eukaryotic (80S) ribosomal subunits.



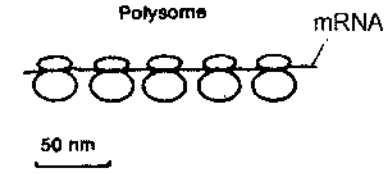


Fig. 9. Subunit structure of ribosome and influence of Mg. A polyribosome is formed by five ribosomes. mRNA filament unites the ribosomes

Polyribosomes : During protein synthesis several ribosomes become attached to one mRNA molecule, forming a **polyribosome** or **polysome.** In this way a single mRNA molecule can be translated by several ribosomes at the same time. The mRNA is located in the gap between the two ribosomal subunits, as a result of which the ribosome protects a stretch of 25 nucleotides of mRNA from degradation by ribonuclease. The nascent peptide chain grows through a channel (groove) in the large subunit. Ribosomes protect a segment of 30 to 40 amino acids from degradation by proteolytic enzymes.

The major constituents of ribosomes are RNA and proteins present in about equal amount. Ribosomes are strongly negative and bind cations and basic dyes. Ribosomal RNA (rRNA) are more than 80% of the RNA present in cells.

Prokaryotic ribosomes contain three RNA molecules : 16S rRNA in the small subunit and 23S and 5S in the large subunit.

Eukaryotic ribosomes contain four rRNAs : 18S in the small subunit, and 28S, 5.8S and 5S in the large subunit. The 28S, 5.8S and 18S rRNAs are synthesized in the nucleolus by cleavage of a single precursor RNA, while 5S rRNA is synthesized outside the nucleolus. Eukaryotic ribosomes and rRNAs are much larger than their counterparts of prokaryotes.

Ribosomal Proteins : The *E. coli* ribosome contains 21 proteins in the small subunit and 34 in the large. All the proteins are different with the exception of one that is present in both subunits (S20 and L26). Therefore the total number of proteins in one ribosome is 54. Most of them are rich in basic amino acids.

Ribosomal proteins can be dissociated from the ribosomes and then added back to reconstitute active ribosomes.

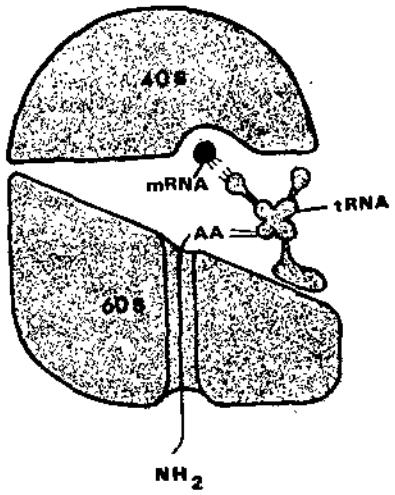


Fig. 10. A ribosome have two subunits and *probable position of mRNA and tRNA.* The nascent polypeptide chain passed through a tunnel within the subunit.

Ultrastructure of Ribosomes

This has been studied more intensively in prokaryotes. Ultrastructure of 70S ribosome is more complex. In it, RNA and proteins are intertwined and arranged in a complex manner in the two subunits. Ribosomes are strongly negative and bind cations and basic stains.

70S Prokaryotic Ribosomes : According to **Stoffer** and **Wittmann's** model the 30S ribosomal subunit has an elongated, slightly bent prolate shape (egg-shaped). It is a *bipartite structure.* A transverse cleft divides the 30S subunit into two parts : a smaller head and larger body. While 50S subunit in frontal view appears bilaterally symmetrical and shows three protuberances arising from a rounded base. The central protuberance is the most prominent. 50S subunit can be compared with an arm chair, having a rounded base forming a vaulted (curved) seat, the central protuberance forming the base and lateral protuberances the arms of a chair. A tunnel is formed between the cleft (hollow) of the small subunit and vaulted seat of the large subunit.

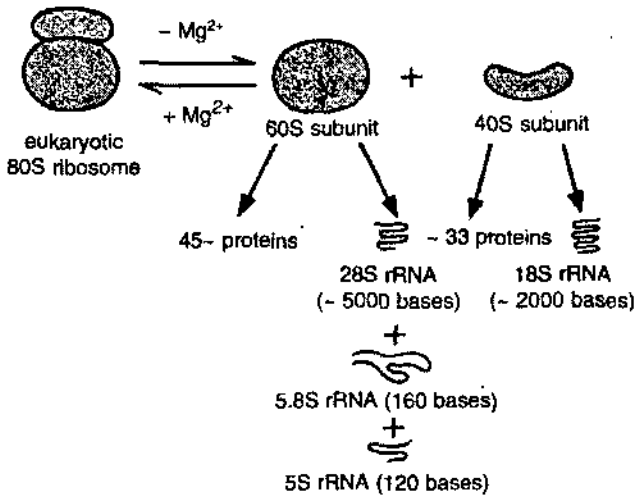


Fig. 11. RNA and protein components of eukaryotic ribosomes.

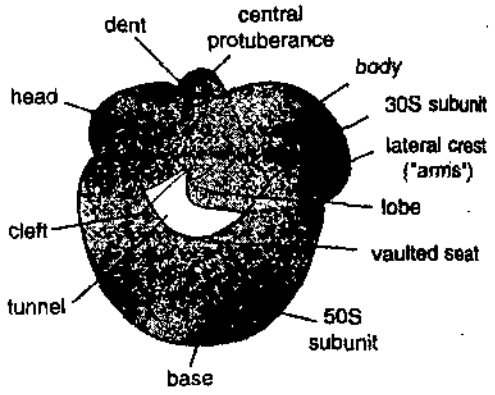


Fig. 12. Model of 70 stribosome.

Functions of Ribosomes

Ribosomes play a significant role in protein synthesis. When not engaged in protein synthesis, most ribosomal subunits exist as a cytoplasmic pool of free, exchangeable subunits. The ribosomes are separated into subunits at the end of protein synthesis by a **dissociation factor** that *binds to the 30S subunit.* The dissociation factor is an initiation factor (IF3), which is also required for the binding of mRNA to the 30S subunit.

5.8. LYSOSOMES

Lysosomes are tiny membrane bound vesicles whose main function is intracellular or extracellular digestion. They contain many hydrolytic enzymes, which remain active under acidic conditions **C. de Duve** in 1955 named the particles having centrifugal properties and somewhat intermediate between mitochondria and microsomes as **lysosomes** (Gr., *lysis* = dissolution and *soma* = body). He found them to have a high content of acid phosphatase and other hydrolytic enzymes. At present some 50 lysosomal hydrolases are known, which are able to digest most of the biological substances. Lysosomes have been found in animal and plant cells and in protozoa. In bacteria, lysosomes are not found. Periplasmatic space found between plasma membrane and cell wall may play a role similar to that of lysosomes. The enzymes are enclosed by a membrane and are not available to the substrate. The membrane is resistant to the enzymes that it encloses, and the entire process of digestion is carried out within the lysosome. In this way, it protects the rest of the cell from the destructive effect of the enzymes and its stability is of fundamental importance to the normal function of the cell. Most of the lysosomal enzymes act in an acid medium. Acidification depends on an ATP-dependent **proton pump,** present in the membrane of the lysosome, by which H^+ is accumulated.

Structure of Lysosomes

Lysosomes are rounded vacuolar structures filled with dense material and are bounded by single unit membrane. Their size is 0.2 to 0.5 μm. Lysosomes are polymorphic. They contain acid hydrolases. Latency of the lysosomal enzymes is due to the presence of the membrane.

• 5.9. KINDS OF LYSOSOMES (POLYMORPHISM IN LYSOSOMES)

Lysosomes are polymorphic, particularly regarding the size of the particle and the irregularities of its internal structure. Lysosomes are extremely dynamic. Within the cell, lysosomes are surrounded by **multivesicular bodies,** smooth and coated vesicles and dense bodies. These were first named **pericanalicular dense bodies,** because of their location along the bile canaliculi in liver cells.

Polymorphism is the result of the association of primary lysosomes with the different materials that are phagocytized by the cell.

At present four types of lysosomes are recognized, of which only the first is the **primary lysosome,** the other three may be grouped together as **secondary lysosomes.**

1. Primary lysosome (*i.e.*, storage granule) is a small body whose enzymatic content is synthesized by ribosomes and accumulated in the endoplasmic reticulum (ER). From lysosome the enzymes penetrate into Golgi region, in which first acid phosphatase reaction takes place. Primary lysosome may contain only one type of enzyme or another. Only in the secondary lysosome full complement of acid hydrolases is present.

2. Heterophagosome or digestive vacuole results from the phagocytosis or pinocytosis of foreign material by the cell. This body, which contains the engulfed material within a membrane, shows a positive phosphatase reaction, which may be due to the association with primary lysosome. The engulfed material is progressively digested by the hydrolytic enzymes, which have been incorporated into the

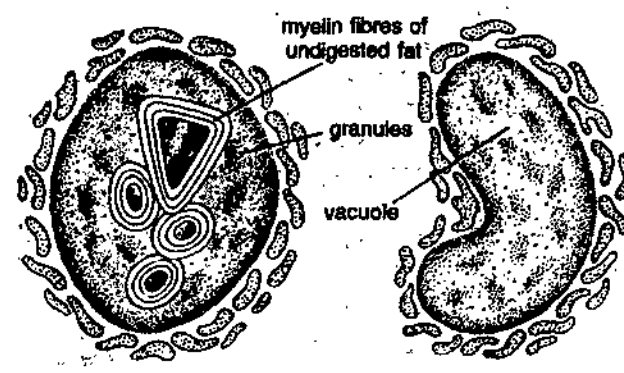


Fig. 13. Lysosomes of kidney cells of rat, showing presence of residues.

lysosome. Under ideal conditions, digestion leads to products of low molecular weight that pass through the lysosomal membrane and are incorporated into the cell to be used again in many metabolic path ways.

3. Residual bodies are formed if the digestion is incomplete. In *Amoeba* and other protozoa, these residual bodies are eliminated by **defecation.** In other cells they may remain for a long time and load the cell. For example, pigment inclusions found in nerve cells of old animals may be the result of this type of process.

4. Autophagic vacuole, cytolysosome or autophagosome is a spherical body found in normal cell, in which lysosome contains a part of the cell in the process of digestion, *e.g.*, a mitochondrion or portions of ER.

Lysosomes regularly engulf bits of cytosol, which is degraded by the process of **microautophagy.** During starvation the liver cells show numerous autophagic vacuoles; mitochondrial remnants also can be found in some of these.

Functions of Lysosomes

Within secondary lysosome the ingested materials (or those resulting from autophagy) are digested by many hydrolases present in the lysosome. The pH of secondary lysosomes and majority of these enzymes both have acidic pH. Digestion of proteins may end at the level of dipeptide, which can pass through the membrane and further degraded into amino acids.

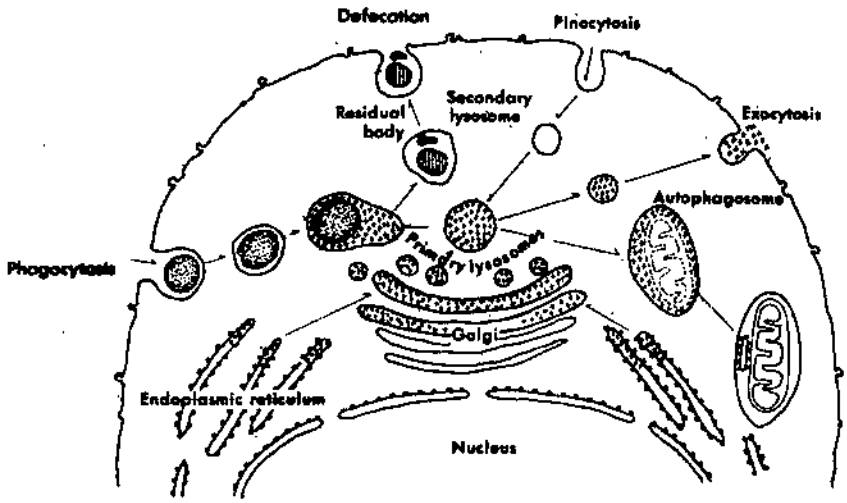


Fig. 14. Diagram showing dynamic aspects of the lysosome system. Relationships between processes of phagocytosis, pinocytosis, exocytosis and autophagy.

Carbohydrates are usually hydrolyzed to monosaccharides, which are easily released. Cellobiose, inulin or dextran (Disaccharides or polysaccharides) are not digested and remain within lysosome.

Autophagy of lysosomes : Mitochondria and other cellular components are constantly removed from the cell by the lysosomes. Cytoplasmic organelles become surrounded by membranes of SER, then lysosomal enzymes are discharged into autophagic vacuoles and organelles are digested.

Autolysis : Digestion of various organelles of the cells by lysosomes is, called **autolysis** or **cellular autophagy.** When a cell dies, lysosome membrane ruptures and enzymes are liberated, which digest the dead cells. During metamorphosis of amphibians and tunicate tadpoles, embryonic tissues like gills, fins, tail etc., are also digested by the lysosomal enzymes.

Lysosomes in human diseases : In certain pathological conditions, such as rheumatoid arthritis, silicosis and asbestosis and gout (in which crystals of urate accumulate in joints), release of lysosomal enzymes from the macrophages and acute inflammation of the tissues lead to an increase in collagen synthesis (fibrosis).

An acute release of lysosomal enzymes occurs in states of anoxia, acidosis and shock results in increased amount of enzymes in the blood.

Membrane bound organelles containing digestive enzymes have been identified in plant cells. In corn seedlings large vacuoles of parenchymatous cells show some characteristic lysosomal enzymes, *e.g.*, protease, carboxypeptidase, DNAse, RNAse, β-amylase or α-glucosidase. Lysosomes in plants may be involved in intracellular and extracellular digestion and also in the process of development.

• SUMMARY

- **Endoplasmic reticulum** : Endoplasmic reticulum is a vast network that subdivides the cytoplasm into two compartments : one enclosed within the membrane and the other situated outside called cytoplasmic matrix.
- Cytoplasm is permeated by a complex system of membrane bound tubules, vesicles and cisternae (flattened sacs).
- Proteins synthesized by ribosomes are segregated within the lumen of endo-membrane system.
- ER on the presence or absence of ribosomes on the cytoplasmic surface is of two types : Smooth ER and rough or granular ER.
- SER is found in those cells in which metabolism of lipids takes place.
- SER is found in adipose cells, interstitial cells, liver cells, heart fibres etc.
- Sarcoplasmic reticulum is SER of striated muscle fibres.
- Vesicles and tubules in pigmented cells of retina are called myeloid bodies.
- **Glycosomes** are dense particles found in liver cells rich in glycogen.
- **Rough endoplasmic reticulum** is found in those cells which are active in protein synthesis like pancreatic cells, goblet cells, plasma cells and liver cells.
- Annulate lamellae or pores are like pores and annuli of nuclear envelope to which are attached ribosomes.
- Various forms of ER are cisternae (long, flattend sacs) arranged parallely in bundles, oval vacuolar vesicles and branched reticular system of tubules.
- Cisternae are found in actively synthesizing protein cells found in the form of RER. Vesicles and tubules are found in abundance in SER.
- Tubules are found in those cells which are active in synthesis of cholesterol and glycosides etc.
- ER associated with ribosomes plays a role in storage and processing of proteins. ER membranes contain many enzymes to carry various functions including biogenesis of some membrane compartments.
- ER membranes may develop by evagination from the nuclear envelope.
- Nuclear envelope is formed by vesicles of ER at telophase stage of cell division. Synthesis of ER membrane follows the direction of RER —> SER.
- In the biogenesis of ER membrane the following sequence takes place : first synthesis of basic membrane of lipids and intrinsic proteins and later addition of enzymes, specific sugars or lipids etc.
- Lipids and proteins are added to various cellular membranes independently of each other.
- Some ER membrane proteins are formed on membrane bound ribosomes and some are formed by free cytosols ribosomes and then inserted into the membrane.
- ER acts as a circulatory system for intracellular circulation of various substances.
- ER provides mechanical support for the cytoplasmic structure.
- SER plays a role in detoxification and hypertrophy of SER.
- Synthesis of lipids (phospholipid) occurs on SER membranes.
- SER is related to glycogenolysis.
- **Golgi Complex**
- It is found in both animal and plant cells.
- Golgi complex is related to ER.
- Golgi complex is not found in prokaryotic cells, certain fungi and mature sperms and red blood cells of animals.

- Golgi complex is an array (arrangement) of interconnecting membranous flattened sacs (cisternae), tubules and vesicles.
- In plant cells it has been called dictyosomes.
- Cisternae are arranged in parallel bundles having a convex and a concave surface. Convex face is called forming face or cis-face and concave face is called trans-face or maturing face.
- Cis-face has small transition vesicles or tubules converging upon the cisternae.
- GERL is a saccular structure associated with the trans-face. It is rich in acid phosphatase.
- Vesicles of 60 nm diameter and tubules of 30 to 50 nm diameter surround the Golgi complex cisternae.
- Golgi complex remains busy in trafficking of substances synthesized elsewhere but modified and transformed while transported.
- Golgi complex plays a role to produce glycosphingolipids and glycoproteins from lipids and proteins.
- In cartilage cells, mucopolysaccharides and glycoproteins are synthesized in Golgi complex.
- Golgi complex and ER are involved in the synthesis, transport and release of macromolecules from the cell.
- **Ribosomes**
- Ribosomes contain about equal amount of RNA and protein.
- Ribosome is a spheroidal particle of 23 nm. It is composed of a large and a small subunit.
- Eukaryotic ribosome is of 80S and prokaryotic is of 70S, "S" is sedimentation coefficient.
- 80S ribosome has 40S and 60S subunits.
- 70S ribosome has 30S and 50S subunits.
- During protein synthesis, several ribosomes are attached to one mRNA molecule to form polyribosome or polysome.
- mRNA is located in the gap between two ribosomal subunits.
- Peptide chain grows through a groove in the large subunit.
- Prokaryotic ribosomes contain 16SrRNA in small subunit and 23S and 5S in the large subunit.
- Eukaryotic ribosomes contain 18S rRNA in the small subunit and 28S, 5.8S and 5S in the large subunit.
- The 28S, 5.8S and 18S rRNA are synthesized in the nucleolus, while 5S rRNA is synthesized outside the nucleolus, in the cytoplasm.
- Ribosomes synthesize proteins.
- **Lysosomes**
- Lysosomes are tiny membrane bound vesicles. Their function is intracellular and extracellular digestion. They contain hydrolytic enzymes. Lysosomes are found both in animal and plant cells.
- Lysosomes are spherical vacuolar structures filled with dense material and are bounded by single unit membrane.
- Lysosomes are polymorphic.
- Lysosomes are of four types : Primary lysosomes or storage granules, heterophagosome or digestive vacuole, residual bodies and autophagic vacuole or autophagosome. Ingested materials are digested within secondary lysosome (other than primary lysosome) by hydrolytic enzymes whose pH is acidic. Proteins are digested at the level of dipeptides and carbohydrates into monosaccharides. Cellular organelles surrounded by ER membranes are digested by lysosomal enzymes.

• STUDENT ACTIVITY

1. Give an account of ultra-structure of endoplasmic reticulum and describe its functions in brief.

2. Describe the ultra-structure, types and functions of ER.

3. Describe the structure and functions of ribosomes.

4. Describe electron microscopic structure of ribosome. Explain their role in protein synthesis.

5. Give an account of the structure and functions of Golgi complex.

6. What is Golgi complex ? Mention its functions.

7. What are lysosomes ? Describe their structure and functions.

8. Lysosomes are said to be the suicidal bags in the cells. Explain it. Give the structure and functions of lysosomes.

• VERY SHORT ANSWER QUESTIONS

1. **Define endoplasmic reticulum.**
   Ans. ER is a membrane-bound system of cisternae, vesicles and tubules. These do not reach the periphery of the cell.
2. **What are the two forms of ER ?**
   Ans. ER are of two types : smooth endoplasmic reticulum and rough endoplasmic reticulum. Ribosomes are found attached to the outer membrane of RER.
3. **What is the name of ER found in striated muscle fibres ?**
   Ans. Sarcoplasmic reticulum is the name of SER found in striated muscle fibres.
4. **What are myeloid bodies ?**
   Ans. Myeloid bodies are highly packed vesicles and tubules found in pigmented retinal cells.
5. **In which type of cell RER is found ?**
   Ans. Cells busy in protein synthesis contain RER, *e.g.*, cells of pancreas, plasma cells, goblet and liver cells etc.
6. **Write the names of various forms of endoplasmic reticulum ?**
   Ans. ER is found in the cytoplasm in the form of cisternae or flattened sacs, vesicles and tubules.
7. **Which types of cellsare possessed by smooth ER ?**
   Ans. Those cells which are engaged in lipid metabolism such as adipose, brown fat and adrenocortical cells. In reticulocytes ER is poorly developed or non-existent.
8. **What is the main characteristic of RER ?**
   Ans. Main characteristic of RER is the presence of attached ribosomes on the outer surface.
9. **Who first observed the ribosomes as dense particles or grains ?**
   Ans. G.E. Palade first observed ribosomes in cells.

10. **What are two subunits of ribosomes ?**

    Ans. A large and a small eukaryotic ribosome has two subunits of 40S and 60S and prokaryotic ribosome of 70S has 30S and 50S.

11. **What is polysome ?**

    Ans. A number of ribosomes attached to one mRNA forms a polyribosome (polysome).

12. **What are major constituents of ribosomes ?**

    Ans. RNA and proteins in equal amounts are the major contituents of ribosomes.

13. **Who discovered the Golgi complex ?**

    Ans. Camillo Golgi in 1898 discovered it in nerve cells of barn owl.

14. **By which name it is known in plant cells ?**

    Ans. In plants its name is dictyosome.

15. **In which cells Golgi complex is not found ?**

    Ans. Prokaryotic cells, mature vertebrates sperms, red blood cells, and sieve tube cells in plants do not contain it.

16. **Which cell organelle forms the sperm's acrosome ?**

    Ans. Golgi complex.

17. **Who gave the name lysosome ?**

    Ans. C. de Duve in 1955.

18. **What is the main function of lysosome ?**

    Ans. Intracellular or extracellular digestion of food; digestion of parts of cells by autophagy and breakdown of extracellular material by the release of enzymes into the surrounding medium.

19. **How is the cell protected from the destructive effect of lysosomal enzyme ?**

    Ans. Lyosomal membrane does not allow the enzymes to go out of the lysosome.

20. **Name the different types of lysosomes.**

    Ans. Primary lysosomes, heterophagosome (digestive vacuole), residual bodies and autophagosome or cytolysosome.

21. **How are residual bodies formed ?**

    Ans. Lysosomes having undigested material or debris are called residual bodies.

22. **Name the animal cells in which lysosomes are not found.**

    Ans. Red blood corpuscles.

23. **Why are lysosomes called the suicidal bags in the cells.**

    Ans. In pathological conditions its membrane becomes more labile and permits the exit of the enzymes with catastrophic consequences to the cell.

UNIT

6

CELL CYCLE

STRUCTURE

- Cell Cycle
- Three phases of cell cycle
- Growing cells undergo cell cycle
- 4 phases of cell cycle
- G_1, S, G_2 and M phase
- G_1 is the longest phase – Resting phase
- S is synthetic phase
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Cell cycle : Chromosome, Cytoplasmic and Centrosome cycles, Phases of cell cycle, DNA synthesis.

• 6.1. CELL CYCLE

The ability to reproduce is the fundamental property of cells. An adult person is formed by 10^{14} cells and all are derived from a single cell, *i.e.*, the fertilized egg. Cell reproduction is regulated with perfect correctness so that the production of new cells make up exactly the loss of cells in adults. An increase in cell number due to cell multiplication (division) is called multiplication growth. All cells are produced by division of pre-existing cell. Cell division is essential for the continuity of life.

Cell cycle can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next. The cell cycle includes three phases : chromosome cycle, cytoplasmic cycle and centrosome cycle.

Chromosome cycle : In it DNA synthesis takes place, each double helical DNA molecule is replicated into two similar DNA molecules. After replication of DNA molecule, mitosis (nuclear division) follows, in which duplicated copies of DNA (genome) are finally separated.

Cytoplasmic cycle : In it cell growth is followed by cytoplasmic division (cytokinesis). During cell growth, various components of cell (*e.g.*, endomembranous structures, RNA and proteins) become double in number and during cytokinesis, cells divided into two equal halves. Generally nuclear division (karyokinesis) is followed by cytoplasmic division. Some times cytoplasmic division does not follow the nuclear division, resulting into the multinucleate cell. Its example is cleavage of egg in *Drosophila.*

Centrosome cycle : Centrosome is also inherited and duplicated exactly in two, which form two poles of the mitotic spindle. This cycle is required for the completion of above two cycles.

A growing cell always undergoes a cell cycle. It is comprised of two periods : interphase (period of non-apparent division) and **period of division.** In eukaryotes, division takes place by mitosis or meiosis (reduction division of chromosomes). **Interphase** is the first period that is considered as **resting phase.** Cells spend most of their life span in interphase. It is a period of intense biosynthetic activity in which the

cell doubles in size and chromosomes precisely duplicates. In mammals, nerve cells do not divide after birth. Thus, human nervous interphase period lasts the entire life time.

In cell cycle, cellular material is divided equally between daughter cells. Cell division is the final phase in which change at molecular level takes place and before the cell divides (by mitosis or meiosis) main molecular components have been duplicated).

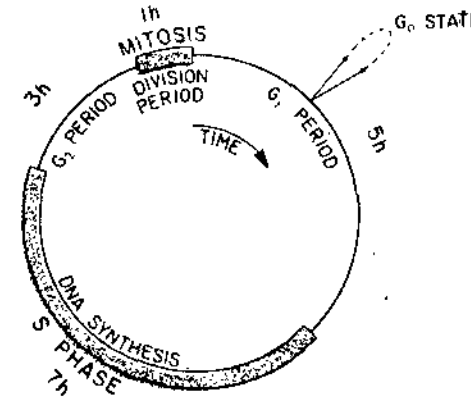


Fig. 1. Cell cycle

Howard and **Pele** divide the cell cycle into four successive intervals : G_1, S, G_2 and mitosis (cell division).

G_1 is the period between the end of mitosis and the start of DNA synthesis.

S is the period of DNA synthesis.

G_2 is the interval between the end of DNA synthesis and start of mitosis. During G_2 a cell contains double the amount of DNA present in the original diploid cell (2C). After mitosis the daughter cells, again enter G1 period having DNA content equivalent to 2C.

G_1 phase : It is the most variable period of the cell cycle. A mammalian having a generation time of 16 hours, the different periods would be : G_1 = 5 hours, S = 7 hours, G_2 = 3 hours and mitosis = 1 hour. Generally S and G_2 and mitotic periods are relatively constant in the cells of the same organism. G1 period is the most variable in length. Those tissues that normally did not divide, *e.g.*, nerve cells or skeletal muscle or that divide rarely, *e.g.*, lymphocytes of blood contain the amount of DNA present in the G_1 period.

The regulation of the duration of cell cycle occurs primarily by arresting it at G1. The cell in arrested condition is said to be in G_0 state. This cell is considered to be withdrawn from the cell cycle. When conditions change and growth is resumed, cell again enters the G_1 period.

Chromosomes during G_1, S and G_2 phases : Eukaryotic chromosomes undergo condensation – decondensation cycles at cell division. DNA of prokaryotes is never cycled in this way. During interphase, the chromosomes are decondensed and are not visible under microscope. RNA synthesis occurs throughout interphase, but it stops during mitosis.

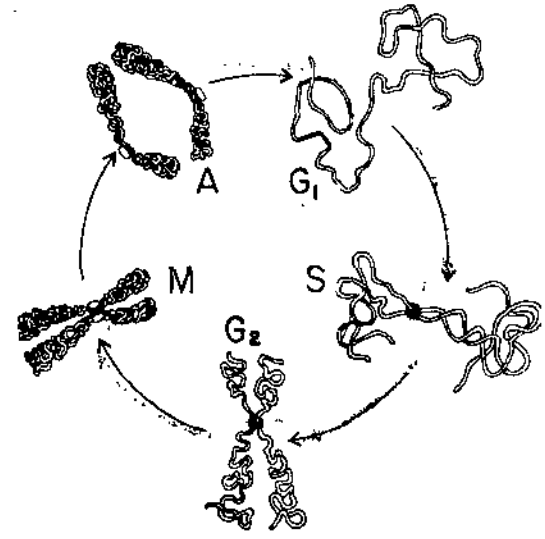


Fig. 2. Condensation decondensation cycle of chromosomes.

DNA synthesis : S phase cells contain a factor that induces DNA synthesis, S phase lasts for several hours, during which many units of replication are sequentially activated. In all cells, more condensed, heterochromatic regions of the chromosomes replicate late during S phase. Centromeric heterochromatin, which contains satellite DNAs replicates later than the rest of the chromosome, while euchromatic replicates earlier. The molecular events are linked to the cell cycle, including synthesis of histones during S phase; decrease in protein synthesis that occurs during mitosis; decrease in cAMP levels during mitosis; phosphorylation of histones (especially HI) during chromatin condensation, etc.

Cell proliferation in the organism is controlled by a number of specific growth factors (nerve growth factor, epidermal growth factor, fibroblast growth factor, platelet derived

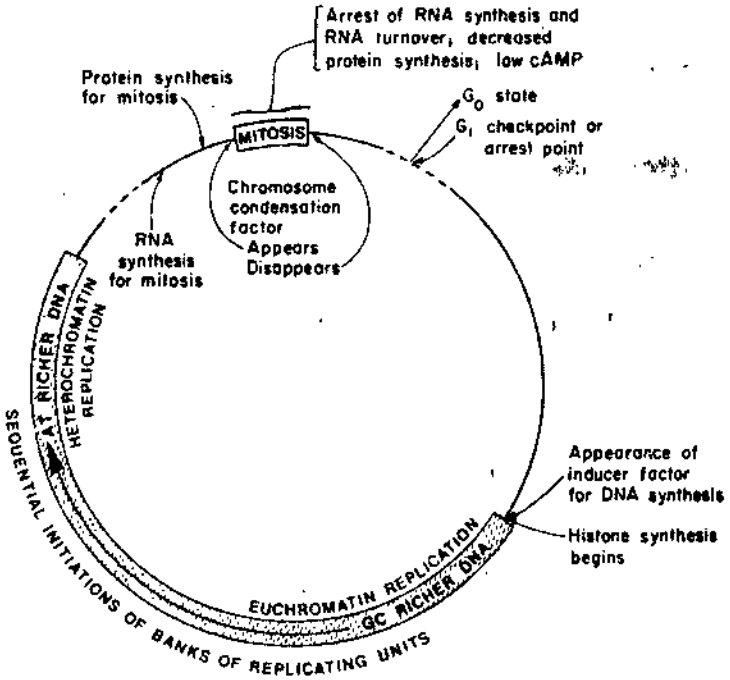


Fig. 3. Molecular events during cell cycle.

growth factor and lymphokines) that can induce cell proliferation at extremely small concentrations and in a tissue-specific way. In foetal development these factors play an important role.

• SUMMARY

- The cell spends most of its life time in interphase. In interphase period cell doubles in size and replicates its DNA.
- Cell cycle can be divided into G_1, S, G_2 and mitosis.
- G_1 is the time gap between the end of mitosis and the start of DNA synthesis. S is the period of DNA synthesis.
- G_2 is the time gap between the end of DNA synthesis and the beginning of mitosis.
- G_1 is the most variable period, depending on the physiological conditions of the cell.
- G_1 may last for days, months or years.
- At G_1 cells stop proliferation.
- G_0 state is that when cell withdraws from the cell cycle.
- During mitosis RNA synthesis stops in the condensed chromosomes and rate of protein synthesis decreases.
- During S phase heterochromatin replicates lately and sometimes only a part of chromosome is late replicating, for example, centromeric heterochromatin, which contains satellite DNA.
- Cytoplasm may play an important role in timing the cell cycle.
- Cell proliferation is controlled by a large number of specific growth factors.

• STUDENT ACTIVITY

1. Define the term cell cycle. Name the stages of cell cycle. Which is the longest stage ?

• VERY SHORT ANSWER QUESTIONS

1. **What is interphase ?**
   Ans. It is the period of non-apparent division. It is a resting phase and a period of intense biosynthetic activity. DNA duplicates.
2. **Which body cells do not divide after birth ?**
   Ans. Nerve cells-neurons.
3. **In which period of interphase does DNA replicate ?**
   Ans. Synthetic period (S period).
4. **Who divided the cell cycle into 4 periods ?**
   Ans. Howard and Pele divided the cell cycle into G_1, S, G_2 and mitosis.
5. **What is G_1 period ?**
   Ans. Period between the end of mitosis and start of DNA synthesis.
6. **Name the period in which DNA doubles ?**
   Ans. G_2 period. DNA replicates in S period and becomes double (4C).
7. **Write the proper sequence of cell cycle.**
   Ans. G_1, S, G_2 and M.

UNIT

7

CHROMOSOMES : SRUCTURE AND TYPES, SPECIAL KINDS OF CHROMOSOMES

STRUCTURE

- Chromosomes : Introduction, Autosomes and Sex Chromosomes.
- Structure of Chromosomes : Its various parts : Chromatid, Chromonema, Chromomeres, Centromeres and Kinetochore, Telomere, Secondary Constriction, Nucleolar Organizers, Satellite.
- Types of Chromosomes : Shape of Chromosome on the basis of position of centromere in the chromosome
- Types of Chromatin on the basis of staining properties : Euchromatin and heterochromatin.
- Special Kinds of chromosomes : Polytene chromosome or Salivary gland chromosome and Lampbrush chromosome
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Chromosomes : Autosomes and sex chromosomes. Structure of chromosome, types of chromosome, Chromatin, Ultrastructure of chromatin, Special kinds of chromosomes.

• 7.1. CHROMOSOMES : INTRODUCTION, AUTOSOMES AND SEX CHROMOSOMES

Introduction

Chromosomes are found in the nuclei of animal and plant cells. Chromosomes observed in plant nuclei by **Karl Nageli** in 1842. **E. Strasburger** in 1875 discovered thread-like structures, which appeared during cell division. The term chromatin, thread-like material of the nucleus was given by **Walter Flemming** in 1878. Chromosomes number for each species is constant (**Benden** and **Bovery** (1997)). The term chromosomes was given to darkly stained bodies of nucleus by **W. Waldeyer** in 1888. **W.S. Sutton** and **T. Boveri** (1902) suggested that chromosomes acted as messengers of heredity. In 1924 **Robert Feulgen** showed by his Feulgen reaction (colour test) for DNA, that chromosomes contain DNA. Presence of RNA in chromosomes was demonstrated by **Brachet** in 1942. **Mirsky** and **Pollister** in 1946 showed the association of proteins with chromosomal materials.

Chromosome number

The chromosome number is constant for a particular species. In body (somatic) cells of most organisms **two haploid sets** of chromosomes are found, while in gametes (sperms and ova) **haploid set** (half number) of chromosomes is found. Haploid set of chromosomes is also known as **genome.** Cells containing two haploid sets of chromosomes are called **diploid cells.** The number of chromosomes in each body or somatic cell remains the same in all members of a given species. The lowest number of chromosomes is found in *Ascaris megalocephalus univalens,* only two chromosomes in somatic cells. In *Aulacantha* (Protozoa), chromosomes number (diploid) is about 1600. Chromosome numbers in various animals are : Pigeon 80, rabbit 44, Gorilla 48, man 46, *Hydra* 32, xoundworm 24, housefly 12, mosquito 6, and frog 26. Haploid (gametic) chromosomes number is represented by "**n**" (haploid), while somatic chromosome number is written by "**2n**" (diploids).

Autosomes and Sex Chromosomes

In a diploid cell there are two chromosomes of each kind, these chromosomes are, called **homologous chromosomes.** Male and female of each organism, there are paired sex chromosomes besides paired somatic chromosomes. For example, in human beings there are 23 pairs (*i.e.*, 46) of homologous (similar) chromosomes. In women, these are 44 **autosomes** (non-sex chromosomes) and are a pair of morphologically similar (**homomorphic**) sex chromosomes represented by XX. In man, there are 44 autosomes and one pair morphologically dissimilar (**heteromorphic**) sex chromosomes, *i.e.*, one **X** chromosome and one **Y** chromosome. The number of chromosomes was given by **Tizo** and **Levan** (1956).

7.2. STRUCTURE OF CHROMOSOME

Size. The size of chromosomes varies in different organisms. Organisms having less number of chromosomes, the chromosome size is comparatively larger than those organisms having large number of chromosomes. Monocotyledon plants contain large sized chromosomes than the dicotyledon plants. The plants generally contain larger-sized chromosomes in comparison of animals. The chromosomes in a cell are not of equal size. Largest chromosomes are found in certain vertebrate oocytes and are, called **lampbrush chromosomes,** and **polytene chromosomes** of certain dipteran insects.

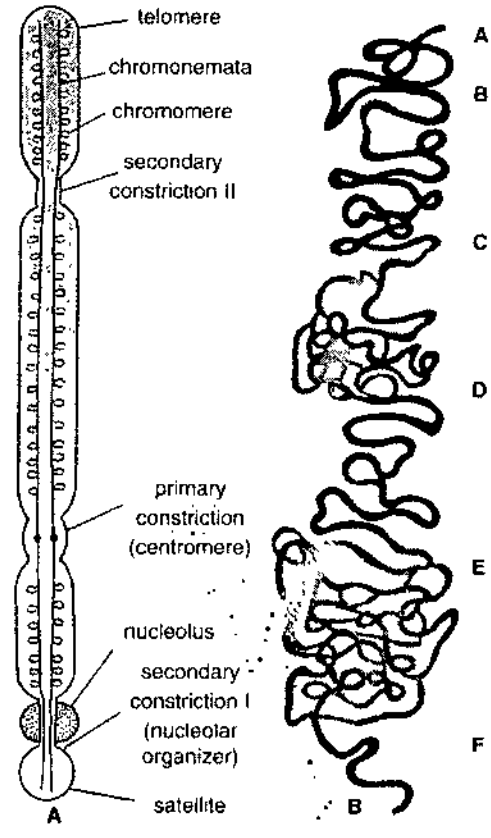


Fig. 1. A–Structure of a typical chromosome; B–Model of constitutive heterochromatin in a mammalian metaphase chromosome. A–Constiutive heterohomain; B–Secondary constriction; or nucleolar organizer, C–Primary constriction or cenromere; D–Euchromatin; E–Secondary constriction. II–possible site of 5S rRNA cistrons; F–Telomere

Shape. The shape of chromosomes in interphase (resting phase) stage is thin, coiled, elastic and contractile thread-like structures (chromatin thread). In metaphase and anaphase stage, they become thick and filamentous. Each chromosome, along its length has a clear zone, called **centromere (kinetocore).** The centromere divides each chromosome into two equal or unequal parts, called **chromosome arms.** The position of centromeres varies in chromosomes, due to which their shapes also vary.

At the time of cell division, **chromatin** becomes condensed into the chromosome. Chromosome's structure can be studied during metaphase and anaphase, because maximum contraction occurs at this stage. They are best studied in squash preparations. For this fragments of tissues (onion root tips) are stained with orcein or Giemsa stains and these are squashed between two slides (or pressed between slide and cover slip) by gentle pressure. Sometimes hypotonic solutions are used before squashing to produce swelling of nucleus and a better separation of the chromosomes.

Structure

1. Chromatid : At metaphase, each chromosome consists of two symmetrical structures, called the **chromatids.** Each chromatid contains a single linear **DNA molecule.** The chromatids are attached to each other by the **centromere** and become

separated at the start of anaphase, when sister chromatids migrate to opposite poles. Metaphase chromosomes have two chromatids, while anaphase chromosomes have only one chromatid.

2. Chromonema : During prophase (and sometimes during interphase) chromosomal material becomes visible as very thin filaments, which are called **chromonemata,** which represent chromatids in early stages of condensation. Chromatid and chromonema are two names for the same structure : a single linear DNA molecule with its associated proteins.

3. Chromomeres : These are bead-like accumulations of chromatin, visible along chromosomes. Chromomeres are obvious in polytene chromosomes, where they become aligned side by side constituting the chromosome bands. At metaphase, the chromosome is tightly coiled and chromomeres are not visible.

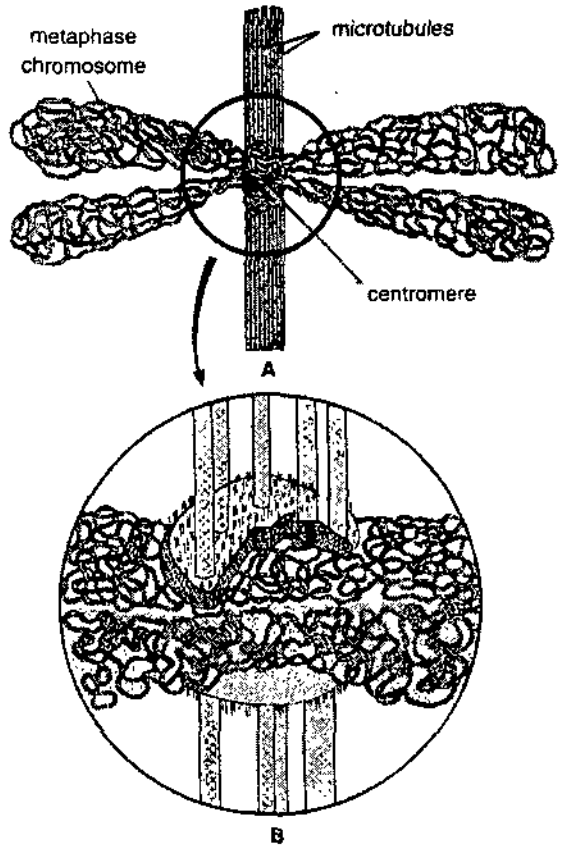


Fig. 2. A–diagram of metaphase chromosome showing folded-fibre structure and kinetochore with implanted microtubules. B–Diagram showing condensed electron-dense layer and the fibrillar material forming "corona" of the kinetochore. Several micro- tubules of the spindle penetrating the kinetochore.

4. Centromere or Kinetochore : The centromere lies within thinner segment of the chromosome, called the **primary constriction.** This region of the chromosome is attached to the mitotic spindle. The regions flanking the centromere frequently contain highly repetitive DNA and may stain with basic dyes (heterochromatin). Centromeres contain specific DNA sequences with special proteins bound to them, forming a disc-shaped structure. To this disc microtubules bind and is called **kinetochore.**

Kinetochore shows a trilaminar structure : a dense outer proteinaceous layer, middle layer of low density and a dense inner layer tightly bound to the centromeric DNA. About 4 to 40 microtubules become attached to the kinetochore and provide the force for chromosomal movement during mitosis. Function of kinetochore is to provide a centre of assembly for microtubules.

The majority of chromosomes have only one kinetochore (**monocentric chromosomes**). Some species have diffuse kinetochores with microtubules attached along the length of the chromosome (**holocentric chromosomes**). In some, chromosomes may break and fuse with other ones, producing chromosomes without kinetochores (**acentric**). Some chromosomes have two kinetochores (dicentric). Acentric and dicentric chromosomes appear due to X-ray etc., producing such abnormalities. Both types of errors are unstable : One because it can not attach to the mitotic spindle and remains in the cytoplasm and the other because the two centromeres tend to migrate to opposite poles, thus leading to chromosomal fragmentation.

5. Telomere (Tips of the chromosomes). Telomeres contain the ends of long linear DNA molecule contained in each chromatid. They have an unusual structure. When chromosomes are broken by X-rays, free ends without telomeres become **sticky** and fuse with other broken chromosomes. They do not fuse with a normal telomere.

6. Secondary Constrictions : These constrictions are constant in their position and extent and are useful in identifying particular chromosomes in a set. Secondary constrictions are distinguished from the primary constriction by the absence of angular deviations of chromosomal segments during anaphase.

7. Nucleolar Organizers : These areas are certain secondary constrictions that contain the genes coding for 18S and 28S ribosomal RNA and that induce the formation of nucleoli. The secondary constriction may arise because rRNA genes are transcribed very actively, interfering with chromosomal condensation. In man, nucleolar organizers are located in secondary constrictions of chromosomes 13, 14, 15, 21 and 22, all of which are acrocentric and have satellites.

8. Satellites : In certain chromosomes is present the satellite. This is a rounded body separated from rest of the chromosome by a secondary constriction. The satellite and the secondary constriction are constant in shape and size for each particular chromosome. The chromosomes with satellite are called **sat-chromosomes.**

• 7.3. TYPES OF CHROMOSOMES

On the basis of the shape of the chromosomes, which is determined by the position of the centromere, the chromosomes are of four types (Fig. 3) :

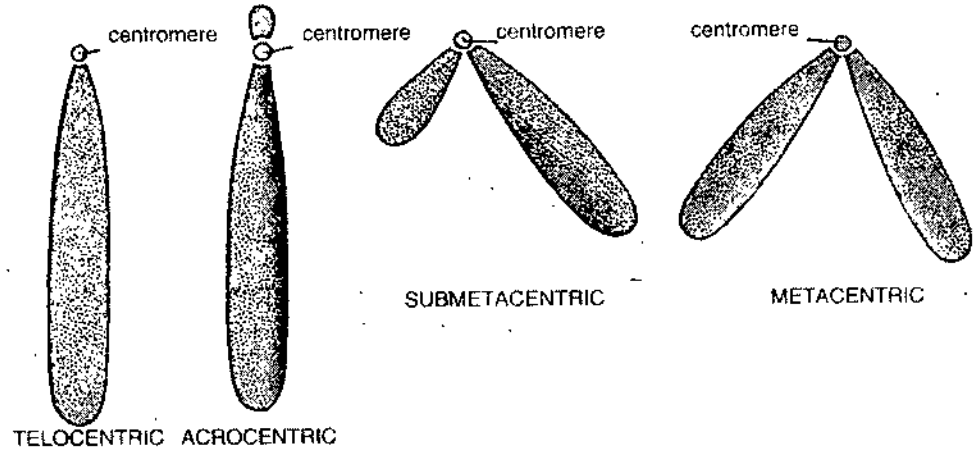


Fig. 3. Four different types of chromosomes on the basis of position of centromeres

1. Telocentric chromosome : Centromere is located at one end of the chromosome. Chromosme is rod-shaped.

2. Acrocentric chromosome : Such chromosome has a very small or imperceptible short arm and another arm is very long. This type of chromosome is also rod-shaped *e.g.,* locusts..

3. Submetacentric chromosome : Such chromosomes have arms of unequal length. They are j or L-shapd. Centromere lies near the centre or at medium portion of chromosome.

4. Metacentric chromosome : This type of chromosome contains equal or almost equal arms. During anaphase movements, chromosome bends at the centromere and becomes V-shaped, *e.g.,* amphibian chromosomes.

Chromosomes material

The material of chromosome is the chromatin.

Chromatin : The chromatin of interphase nucleus is of two types, on the basis of staining properties :

1. Euchromatin : Partially condensed parts of chromosomes that stain lightly are called **euchromatic chromatin.** It contains structural genes, which replicate and transcribe during G_1 and S phase of interphase. The euchromatin is genetically active chromatin and plays a role in phenotype expression. In it DNA is found packed in 3 to 8 nm fibre.

2. Heterochromatin : Chromatin of this region remains in the condensed state and is darkly stained. **Heitz** (1928) defined heterochromatin as those regions of the chromosome that remain condensed during interphase and early prophase and form the so called **chromocenters.** This region contains repetitive DNA sequences and contains very few structural genes (genes that encode proteins). It replicates late, *i.e.* when bulk of DNA has already been replicated, and this is not transcribed. In heterochromatin DNA is tightly packed in 30 nm fibre.

Heterochromatin is of two types :

(i) Constitutive heterochromatin : This type of heterochromatin remains permanently condensed in all types of cells. This is the most common type of heterochromatin. Most chromosomes contain large blocks of heterochromatin flanking the centromeres. This type of heterochromatin contains highly repeated DNA sequences, called satellite DNA, which might have a structural role in chromosomes. The condensed chromatin is inactive in RNA synthesis. Genes present in this region are not expressed.

(ii) Facultative heterochromatin : This is condensed only in certain cell types or at special stages of development. Frequently, in facultative heterochromatin the chromosome of the pair becomes either totally or partially heterochromatic, *e.g.*, X-chromosomes of the mammalian female, one of which is active and remains euchromatic, while the other is inactive and forms **sex chromatin** or **Barr** body at interphase.

Function of Chromosomes : The chromosomes carry the genetic information from one cell generation to another. DNA is the permanent component of chromosome structure, and is the sole genetic material of eukaryotes.

• 7.4. CHEMICAL COMPOSITION

Chromatin contains DNA, RNA and protein. Protein of chromatin is of two types : histone and non-histone.

1. DNA. DNA is the most important chemical component of chromatin. It plays the main role in heredity. The nuclei contain a constant amount of DNA (all cells in an organism contain the same amount of DNA). Gametes contain the half amount of DNA in comparison to other body cells.

Eukaryotes vary greatly in DNA content, but always contain much more DNA than prokaryotes. Lower eukaryotes like nematodes have less DNA, but 20 times more than *E. coli*. Vertebrates have greater DNA content (about 700 times more than *E. coli*). This is called **C-value paradox (Gall**, 1981).

2. Histones. Histones are basic proteins. They contain amino acids arginine and lysine. Histones bind tightly to DNA which is an acid. In eukaryotic chromosomes, histones are of five types : H1, H2A, H2B, H3 and H4.

Histone H1 is the least rigidly conserved histone protein. It contains 210 to 220 amino acids. H1 is present only per 200 base pairs of DNA (other four types of histones represent twice) and is loosely associated with DNA. H1 histone is absent in yeast.

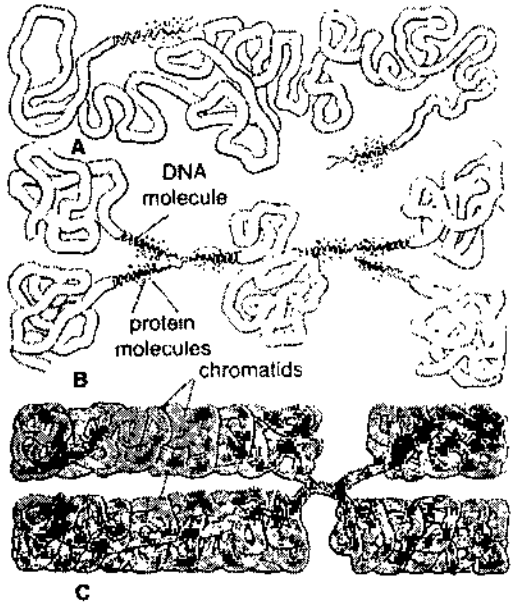
3. Non-histone. Number of non-histone proteins can vary from 12 to 20. Non-histone proteins differ between different tissues of the same organism. They regulate the activity of specific genes.

• 7.5. ULTRASTRUCTURE OF CHROMATIN

Chromosomes have very fine fibrils having a thickness of 2 nm to 4 nm. DNA is 2 nm wide and hence a single fibril corresponds to a single DNA molecule.

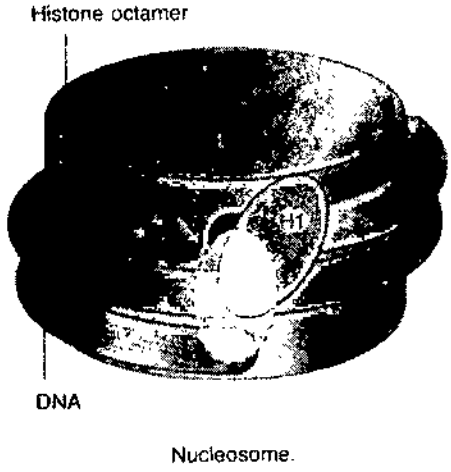
Single-stranded and Multi-stranded hypothesis. Lateral multiplication of chromonemata leads to multiple or multi-stranded chromosomes. **Tandem duplication** of DNA or chromonemata where lengthwise duplication is responsible for chromatin differences. This tandem duplication will retain the single stranded feature of chromosomes. Multistranded condition is demonstrated in a number of plants like *vicia faba* and animals like dipteran salivary gland chromosomes. Each chromosome is formed of a single linear DNA molecule in yeast, *Saccharomyces cerevisiae* (pulsed gel electrophoresis).

Folde fibre model : If a single chromatid has a single long DNA molecule, then DNA should be present in a coiled or folded manner. Folded-fibre model was proposed by **E. J. Dupraw** in 1965. According to him, the bulk of the chromosome is composed of a tightly folded fibre, which has a rather homogeneous diameter of 200 to 300 Å. The folded-fibre is supposed to contain the DNA histone helix or 30Å diameter in a supercoiled condition.



Nucleosome Model : It is universally accepted model of chromatn proposed by **R. D. Kornberg** in 1974. This model was confirmed by **P. Oudet** *et. al.*, in 1975. In eukaryotes, DNA is tightly bound to an equal mass of histones, which serve to form a repeated arrangement of DNA protein particles, called **nucleosomes.** Nucleosomes are the fundamental packing unit particles of the chromatin and give a chromatin a beads on-a-string appearance in electron micrograph.

The nucleosome beads can be removed from long DNA string by digestion with enzymes that degrade DNA, like micrococcal nuclease (bacterial enzyme). The DNA between the nucleosome beads is degraded, and the rest is protected from digestion and as double-stranded DNA fragemets 146 nucleotide pairs long is bound to a specific complex of 8 nucleosome histones (histone octamer).



Fg. 4. Dupraw's folded-fibre model of chromatin in interphase (A and B) and in metaphase (C).

Each nucleosome is a disc-shaped particle with about 11 nm diameter and 5.7 nm in height containing two copies of each 4 nucleosome histones — H2A, H2B, H3 and H4. This histone octamer forms a protein core, *i.e.*, a core of histone tetramer H3 and H4 and a polar region of 2 H2A and H2B, around which the double-stranded DNA helix is wound $1\frac{3}{4}$ times containing 146 base pairs.

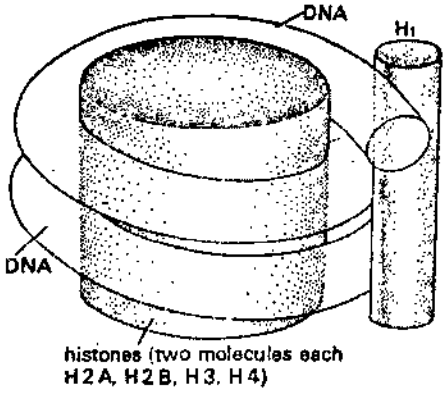


Fig. 5. Model of nucleosome. Histone H1 is attached at the entry and exit of two turns of 140 base pairs long DNA.

In undigested chromatin, DNA extends as a continuous thread from nucleosome to nucleosome. Each nucleosome bead is separated from the other by a region of **linker DNA,** which is generally 54 base pair long and contains a single H1 histone protein molecule. Generally, DNA makes two complex turns around the histone octamers and these two turns of 200 bp length are sealed off by H1 molecules.

• 7.6. SPECIAL KINDS OF CHROMOSOMES (GIANT CHROMOSOMES)

Special kinds or giant chromosomes are the polytene (salivary gland chromosomes) and lampbrush chromosomes.

1. Polytene chromosome : E.G. Balbiani in 1881 observed peculiar chromosomes in the nuclei of salivary glands of *Chironomus* (midge) belonging to Diptera order of class insecta. These were long and sausage-shaped having swellings and transverse

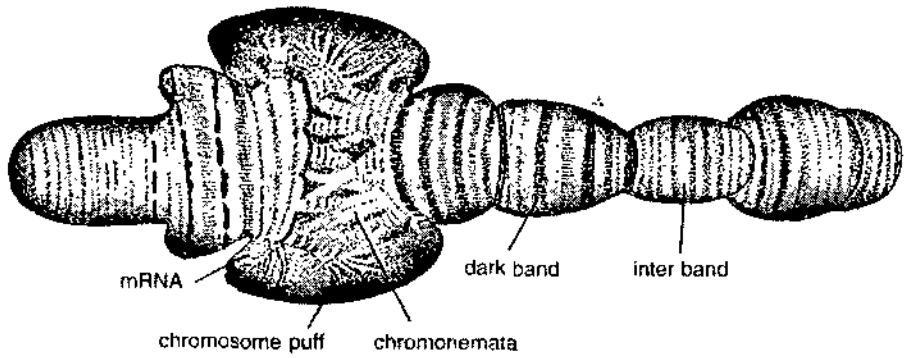


Fig. 6. A polytene chromosome of an insect showing bands and interbands, and a puff or Balbiani ring.

bands. He could not recognize them as chromosomes. **Theophilus Painter, Ernst Heitz** and **H. Bauer** in 1933 discovered them in *Drosophila* and recognized them as chromosomes. Since these chromosomes were discovered by them in salivary gland cells, hence they were named **salivary gland chromosomes.. Kollar** named them **polytene chromosomes** due to presence of many chromonemata or DNA in them. Thus, some cells of larvae of the order Diptera, class Insecta like *Drosophila*, mosquitoes and *Chironomus* (midge) are very large having high DNA. Such cells do not undergo metamorphosis and die during metamorphosis. Such polytenic cells are mostly present in the salivary gland, Malpighian tubules, gut, foot pads, fat bodies, etc. Polyteny is achieved by replication of the DNA several times without nuclear division, and the resulting daughter chromatids do not separate and remain aligned side by side.

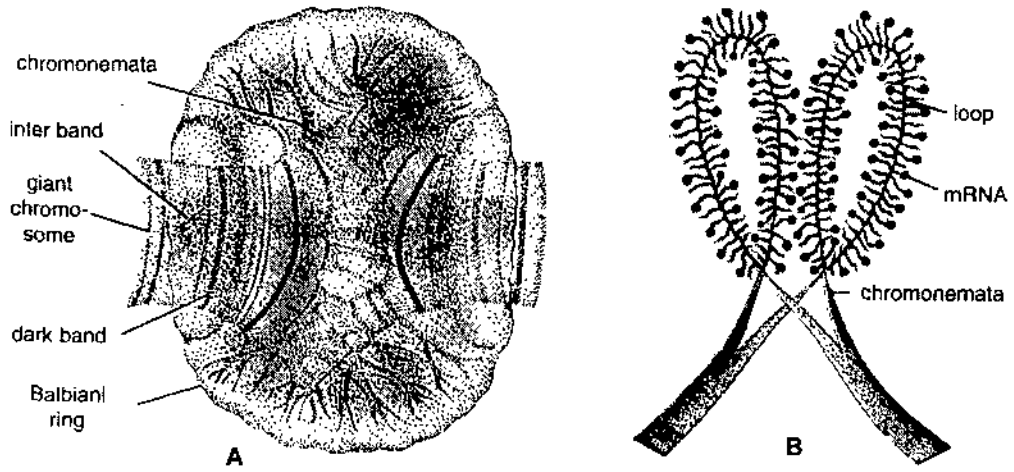


Fig. 7. A. Balbiani ring of polytene chromosome. B. Individual loops in chromosome puffs. Tiny fibrils of RNA and proteins attached to the loop.

A polytene chromosome of *Drosophila* salivary glands has about 1000 DNA molecules arranged side by side, which arise from 10 rounds of DNA replication. *Chironomus* has 16,000 DNA molecules in one polytene chromosome.

In polytene cells, the chromosomes are visible during interphase, and the chromosomes (regions in which chromatin is more tightly coiled) atternate with regions where DNA fibres are folded more loosely. In polytene chromosomes a series of **dark bands** alternate with clear zones, called **interbands.** There are about 5000 bands in

the *Drosophila* chromosome. Polytene chromosomes of maternal and paternal homologues remain associated side by side, called somatic pairing.

Puffs and Balbiani rings : Puffs in polytene chromosomes are uncoiled local regions. Puffs are regions of active RNA synthesis (transcription). In the puff individual fibres remain continuous across the puff and they become extended as short lateral loops.

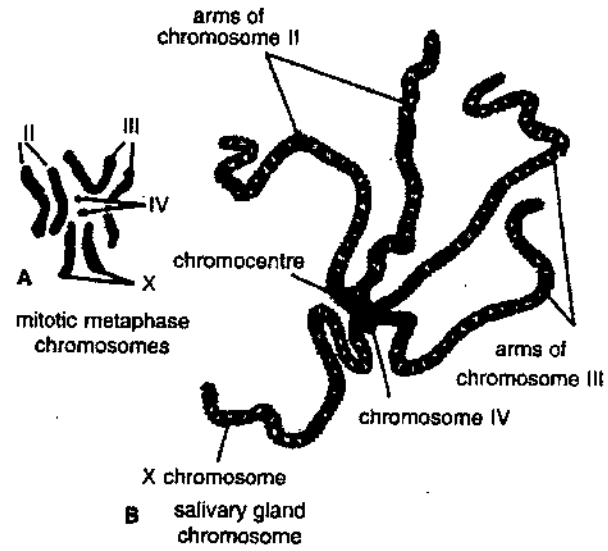


Fig. 8. Polytene chromosomes of *Drosophila* A. Polytene chromosomes of female *Drosophila*. B. An enlarged IVth chromosome.

During development these bands or interbands of the chromosomes exhibit puffs or swellings. A puff can be considered a band in which DNA unfolds into open loops due to intense gene (DNA) transcription. In salivary glands the appearance of some puffs has been correlated with the production of specific proteins secreted in large amounts in the larval saliva.

Puffing is a cyclic and reversible phenomenon. At definite times, and in different tissues of the larvae, puffs may appear, grow and disappear. Chromosomal RNA differs from the nuclear and cytoplasmic RNA. The RNA of puffs is also not similar, it differs from each other in chemical composition. Some regions show larger puffs than others. These larger puffs are called **Balbiani rings,** which are formed by lateral stretching of loops caused by chromonemata. These loops of chromonemata make up **Balbiani rings** and they give up the chromosome a fuzzy outlook. Balbiani rings are rich in DNA and mRNA.

Function of polytene chromosome is to carry genes, which control physiology of an organism.

Chromosomes also help in protein synthesis indirectly.

Lampbrush Chromosomes

Lampbrush chromosomes were first observed by **Flemming** (1882). **Ruckert** in 1892 described it in shark oocytes. He coined the name because chromosomes look-like the brushes used to clean the chimneys of oil lamps **Gall** and **Callan** gave their function.

Lambrush chromosomes occur at the diplotene stage of meiotic prophase in oocytes of all animal species, and in the giant nucleus of unicellular algae *Acetabularia.* Lampbrush chromosomes have many fine lateral projections showing hairy appearance. They best appeared in salamander oocytes because they have a high DNA content.

Lampbrush chromosomes are found in meiotic prophase, hence they are present in the form of bivalents in which the maternal and paternal chromosomes are held together by chiasmata at the sites where crossing over has previously taken place. Each

bivalent has four chromatids, two in each homologue. The axis of each homologue consists of a row of granules or chromomeres from which lateral loops extend. The loops are symmetrical, each chromosome having two loops, one for each chromatid.

Each loop appears at a constant position in the chromosome. There are about 10,000 loops per chromosome set. Each loop has an axis formed by a single DNA molecule (a single thread of DNA runs through each chromatid unineme view of chromosome structure) that is unfolded from the chromosome as a result of intense RNA synthesis. About 5 to 10 percent DNA is in the lateral loops and the rest is tightly condensed in the chromomeres of the chromosome axis, which are transcriptionally inactive.

The loop is covered by a matrix consisting of RNA transcripts with hn-RNA binding proteins attached to them. Ribonucleoprotein matrix is thicker at one end of the loop than the other end. RNA synthesis starts at the thinner end of the loop and progresses toward the thicker end. Nascent ribonucleoprotein chains are attached perpendicularly to the DNA axis.

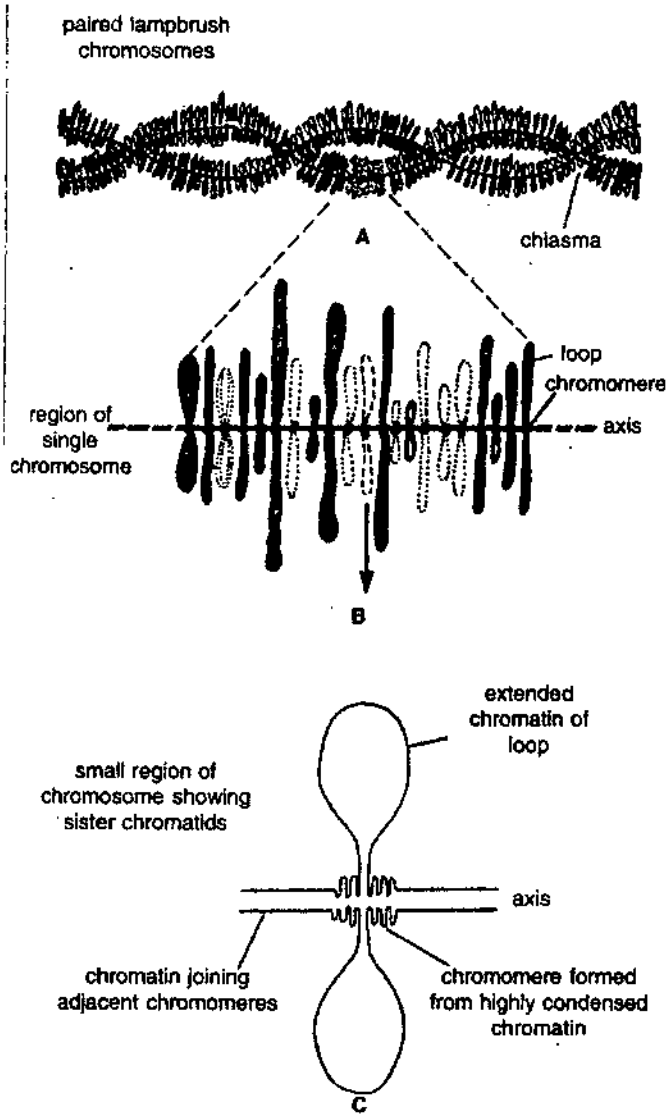


Fig. 9. Lamphrush chromosome sructure, A–Bivalent or paired homologous chromosomes in pairing showing chiasmata, B–A part of the homologue showing paired loops given out by two chromatids; C–Single pair of loop.

• SUMMARY

- Morphology of chromosomes can be best studied during metaphase and anaphase.
- Chromosomes are of four types : telocentric, acrocentric, submetacentric and metacentric, depending on the position of the centromere.
- Centromere is placed at the primary constriction. Chromosome has secondary constriction., telomere, satellite and nucleolar organizer. Each chromosome has two chromatids united at the centromere.
- Each chromatid contains a single linear DNA molecule with its associated proteins (unineme theory of chromosome).
- Both chromatids of a chromosome contain identical DNA molecules.
- Mitotic chromosomes are made of chromatin fibres of 30 nm diameter. They remain in condensed form in metaphase stage.
- Structural non-histone proteins could be involved in the coiling of chromatin fibers.

- Some regions of chromosome remain condensed during interphase and are stained differentially by basic dyes. These heterochromatic regions are late replicating and generally inert. Heterochromatin are of two types – Constitutive and facultative.
- Constitutive heterochromatin remains condensed in all types of cells and is related to satellite DNAs.
- Facultative heterochromatin is condensed in certain cell types or at special stages of development.
- Chromatin contains DNA, RNA and protein. Protein is of two types : histones and non-histones. DNA controls the heredity. Nuclei contain a constant amount of DNA. All the cells in an organism contain same DNA content (2C) if they are diploid. Gametes are haploid, they contain half the DNA (1C). Histones are basic proteins enriched in arginine and lysine. Histones bind tightly to DNA which is an acid. In eukaryotic chromosomes, histones are of five types : H1, H2A, H2B, H3 and H4. Nucleosomes are repeating arrangement of DNA-protein particles. Each nucleosome is a disc-shaped particle and contains two copies of each of 4 nucleosome histones — H2A, H2B, H3 and H4. A histone octamer forms a protein core-histone tetramer H3, H4 and 2H2A and H2B around which double stranded DNA helix is wound $1\frac{3}{4}$ times containing 146 base pairs.
- Polytene chromosomes are found in the salivary gland cells of dipterans (flies, mosquitoes, midges) larvae. Polytene chromosomes are large and have large DNA content.
- Polytene chromosomes have alternate dark and clear interbands. Such chromosomes have puffs (local enlargements) where DNA undergoes intense gene transcription.
- Lampbrush chromosomes are found at diplotene stage of meiotic prophase of all animal species.
- In it highly condensed chromomomeres form the chromosome axis, from which loops of DNA extend laterally due to intense RNA synthesis.
- Its each loop has an axis formed by a single DNA molecule covered by a matrix of nascent RNA with hnRNA binding proteins attached to it.

• STUDENT ACTIVITY

1. Give an account of the morphology, and ultrastructure of the chromosomes.

2. Write an account of the structure and functions of chromosomes.

3. Write an account of specific kinds of chromosomes.

• VERY SHORT ANSWER TYPE QUESTIONS

1. **How many types of chromosomes are found on the basis of position of centromere?**

   Ans. Four types : metacentric, submetacentric, acrocentric and telocentric.

2. **What are chromomeres ?**

   Ans. These are bead-like accumulations of chromatin material.

3. **What is centromere (kinetochore) ?**

   Ans. Centromere lies within a thinner segment of chromosome, primary constriction.

4. **What is chromonema (singular chromonemata) ?**

   Ans. Chromosome during prophase is visible as a very thin filament called chromonema.

5. **Define secondary organizers.**

   Ans. Secondary constrictions in the chromosome contain genes that code for 18S and 28S ribosomal RNA and that induce the formation of nucleoli.

6. **What are statellite in the chromosomes ?**

   Ans. In certain chromosomes is present a rounded body separated from the rest of the chromosome by a secondary constriction.

7. **Explain heterochromatin.**

   Ans. This is the darkly stained region of chromosome.

8. **Name the animal in which polytene chromosomes are found.**

   Ans. In the salivary gland cells of *Chironomus, Drosophila* and mosquitoes.

9. **What are chromosome puffs or Balbiani rings in a polytene chromosome ?**

   Ans. In the polytene chromosome swellings of bands are the puffs (Balbiani rings). Here DNA unfolds into open loops due to intense gene transcription.

10. **In which organ lampbrush chromosomes are found ?**

    Ans. Lampbrush chromosomes are found at diplotene stage of meiotic prophase in primary oocytes of invertebrates and vertebrates.

11. **Who first observed lampbrush chromosomes ?**

    Ans. Flemming in 1882 observed them in shark oocytes and Ruckert (1892) described them in detail.

12. **Who discovered the polytene chromosomes ?**

    Ans. E.G. Balbiani in 1881 observed them in the nuclei of salivary gland cells of dipteran midge, *Chironomus*. These were rediscovered by Theaphilus, Painter, Ernst Heitz and H. bauer in 1933 in *Drosophila*.

UNIT

8

MENDELISM (MENDELIAN INHERITANCE)

STRUCTURE

- Genetics
- Gregor Johann Mendel : Father of Genetics
- Mendelian inheritance (Mendelism)
- Early work of Mendel. Selection of garden pea for his experiments
- Results of monohybrid and dihybrid crosses
- Law of dominance, law of segregation and law of independent assortment postulated by Mendel
- Postulates of Mendel
- Basic terms of genetics
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Mendelian inheritance, Reasons for selecting pea plants, Theory of inheritance and laws governing inheritance, Results of monohybrid and dihybrid cross, variations in Mendels laws and some basic terms.

• 8.1. MENDELISM (MENDELIAN INHERITANCE)

Genetics is the science of **heredity** and **variations.** The word "Genetics" means "to become" or "to grow into". **Heredity** is transmission of characteristics from one generation to the next generation of organisms. In sexually reproducing organisms offsprings resemble their parents as well as differ in some characters. The resemblances of offsprings to parents and each other are due to **heredity** or **inheritance.** The difference between offspring and parents and among siblings is due to **variations.**

The word "Genetics" was used for the first time by **William Bateson** in 1855. The word "gene" was used by **Johannsen** (1909). **Gregor Johann Mendel** (1822-1884) is known as father of genetics because he laid the foundation of the science of genetics. Laws of genetics were published in 1866 by **Gregor Johann Mendel,** but remained unnoticed till 1900. In 1900 Mendel's laws were rediscovered by **de Vries, Correns** and **Tschermak** independently.

• 8.2. BRIEF HISTORY

Gregor J. Mendel (1866) : Published laws of inheritance.

Schneider (1873) : First account of Mitosis.

Walter Flemming (1879-1892) : Gave details of mitosis and coined the term "mitosis".

O. Hertwig, Benden, Boveri (1870 to 1880) : Discovered fertilization in animals and plants and observed haploid condition of gametes.

Boveri (1887) : Described meiosis in egg of Ascaris.

Brauer (1893) : Studied meiosis in sperm of *Ascaris.*

Waldeyer (1888) : Coined the term chromosome.

Correns, Tschermak and Hugo de Vries (1900) : Rediscovered Mendel's laws independently.

C.E. McClung (1902) : Discovered sex chromosomes and gave "Chromosomal theory of sex determination".

Bateson and **Punnett** (1906) : Observed linkage.

Nilsson and **Ehle** (1908) : Described quantitative genes.

Johannsen (1909) : Coined the words gene, genotype and phenotype.

T.H. Morgan (1910) : Discovered that sex-linked inheritance and genes are situated on chromosomes.

H.J. Muller (1927) : Induction of artificial mutation by x-rays.

S. Stern (1931) : Cytological proof of crossing over.

G. W. Beadle and **F.L. Tatum** (1941) : Gave one gene one enzyme theory by studies on *Neurospora*.

O.T. Avery, C.M. McLeod and M. McCarthy (1944) : DNA is the heredity material.

E. Chargaff (1950) : Number of adenine = thymine and guanine = cytosine.

J.D. Watson and **F.H.C. Crick** (1953) : Proposed double helical structure of DNA.

S. Ochoa and **A. Korenberg** (1959) : *In vitro* synthesis of DNA and RNA.

M.W. Nirenberg, J.H. Matthaei and **F.H. Crick** (1961) : Worked on genetic code.

F. Jacob and **J. Monod** (1961) : Operon concept of gene regulation.

H.G. Khorana, R.H. Holley and **M.W. Nirenberg** (1968) : Prepared complete genetic code dictionary.

Knippers, A. Korenberg (1970) : Identified DNA polymerase II in *E. coli*.

H.G. Khorana and **K.L. Agarwal** (1971) : Artificial synthesis of gene.

Early Life and Work of Mendel

Johann Mendel was born in a peasant family, in 1822.

Gregor Johann Mendel was the pioneer of classical genetics. The early training of Mendel was in mathematics and physics. During his tenure in monastery, Mendel took keen interest in natural sciences and started experiments on garden pea in 1856.

Mendel selected seven pairs of contrasting characters in pea olants for his experiments.

Reason for Selecting Pea Plants

Mendel selected garden pea as his experimental material after carefully considering many points :

1. In peas many observable contrasting varieties were available like axial and terminal position of flower, yellow seed coat and green seed coat etc.

2. The structure of pea flower is such that it can be self pollinated (self fertilized) as well as cross pollinated (cross fertilization).

3. The life cycle of pea plant is completed in one year only.

4. In small space large number of plants can be grown.

Mendel studied 7 pairs of contrasting characters in *Pisum sativum* during his experiments.

Dominant characters	Recessive characters
Yellow seed coat	Green seed coat
Round seed coat	Wrinkled seed coat
Grey cotyledon (purple flower)	Green cotyledon (white flower)
Axial position of flower	Terminal position of flower
Green coloured pods	Yellow coloured pods
Inflated pods	Constricted pods
Tall plants	Dwarf plants.

First he obtained true breeding forms of all fourteen characters by continuous self pollination for several generations. These true breeding forms formed the **parent generation** (P). Offsprings of first generation were called F_1 or **first filial** (son) generation; next generation was called F_2 generation and so on.

During second stage of experiments, Mendel cross pollinated plants with alternate forms of various traits. In second stage of experiments, hybrids of F_1 were allowed to self pollinate. Mendel also raised F_2, F_3, F_4 etc. generations.

Mendel conducted his breeding experiments over a period of eight years and then formulated laws of genetics. Mendel kept careful record of each experiment, counted the number of each type of contrasting forms in every generation and carried out statistical analysis of the results obtained.

Reason of Success

1. Mendel concentrated on only one pair of contrasting characters at a time and kept meticulous record for several generations.

2. Mendel counted offsprings of all results and did statistical analysis of the data.

3. Mendel selected pea plants, which has many contrasting characters.

4. First of all, Mendel established the fact that all these characters were true breeding.

5. Mendel was lucky in selecting pea plant and all characters he selected were situated on separate **chromosomes** or **genes** and were also situated far enough from each other to show any linkage (except genes for plant height and pod shape, which are located on same chromosome and show linkage).

• 8.3. MENDELIAN INHERITANCE (MENDELISM)

Mendel experimented with garden pea, *Pisum sativum*. Mendel precisely counted and noted results of each experiment and used probability and reasoning in interpreting the results of his experiments. Results of Mendel's experiments were published in 1866, in a paper entitled "experiments in Plant Hybridization", in the proceedings of the **Brunn Natural History Society.**

During initial stage of experiments, Mendel concentrated on a **single character (Monohybrid cross).** After completing experiments of monohybrid crosses, Mendel studied inheritence of **two characters (Dihybrid cross)** and followed the same procedure.

Mendel had no idea of physical or chemical nature of gene. He observed the gene only through its effects on the organism containing it. Mendel's results were published in the form of postulates. **Correns,** one of the rediscoverers of Mendel, presented findings of Mendel in the form of **three laws :**

A. Results of Monohybrid Cross

Mendel crossed tall and dwarf plants of garden pea. In first generation, all progeny was of tall plants. He repeated this experiment with all 7 pairs of contrasting characters and found that always only one character of parents appears in the first generation. On this basis he formulated **law of dominance.**

When individuals of F_1 generation were allowed self-fertilization, in the F_2 generation dominant and recessive characters appeared in the ratio of 3 : 1. Here when tall plants of F_1 generation were allowed self-pollination then both tall and dwarf plants appeared in F2 generation in a definite ratio, *i.e.,* 3 tall plants : 1 dwarf plant. To be exact, Mendel counted total 1064 plants in F_2 generation, out of those, 787 were tall plants and 277 were dwarf plants.

To prevent self fertilization in experimental plants, anthers were removed from those chosen to be seed parents for next generation. Pollen from designated pollen parents were transferred at appropriate time to the stigma of seed parent flower. Seeds were allowed to mature on the plants and then collected. Results of seed characters such as colour or shape of seeds were immediately visible but plant height and flower position are such characters, which are visible only when these seeds were sown and grown.

There are two methods for using symbols : For dominant character capital letter is used and for recessive character small letter is used. For example, T for tall plant and t

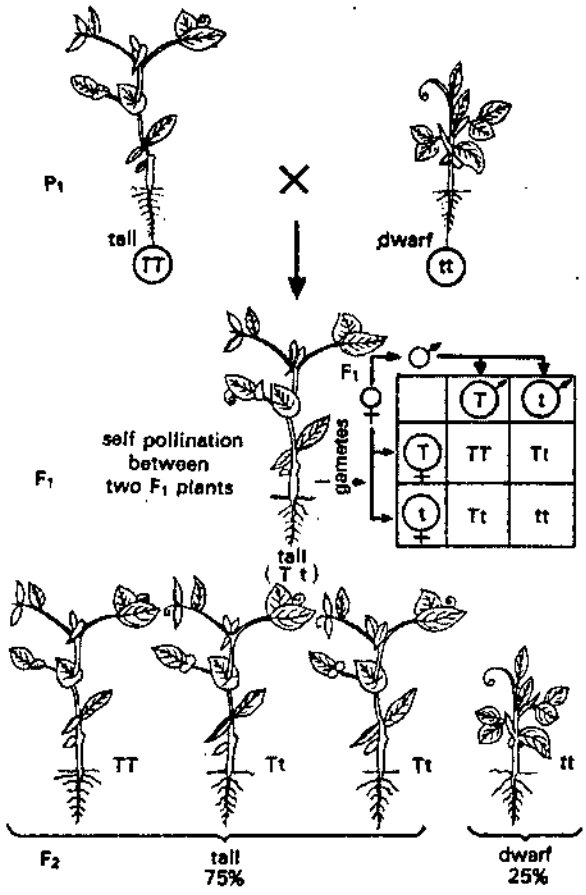


Fig. 1. A cross among tall and dwarf plants to show dominance.

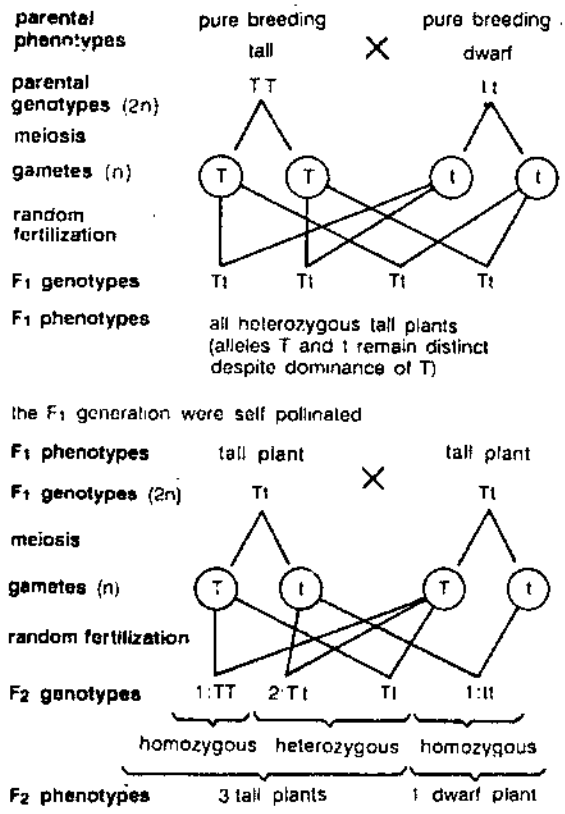


Fig. 2. Mendel's monohybrid cross showing segregation in F_2 generation.

for dwarf plant. Modern genetists consider that wild type alleles are found in nature in abundance, and its alternate form appeared by mutation.

1. Law of Dominance : When a cross is made between two plants or animals having a set of contrasting characters, in first generation (F_1) only one character appears. The character which appears in the F_1 generation is **dominant** and the one which remains hidden is **recessive.**

2. Law of Segregation : The law of segregation states that alleles pair separate or segregate at the time of gamete formation and paired condition of alleles is restored at the time of fertilization. So each gamete is pure of a particular character.

Law of segregation is also known as **law of purity of gametes.** It states that heterozygotes (hybrids) of F_1 generation have two contrasting characters (allelomorphs) of dominant and recessive nature. These alleles remain together for a long time but do not contaminate or mix with each other and segregate at the time of gametogenesis. Thus, each gamete receives only one allele of a character, either dominant or recessive. **Example :** Mendel crossed a homozygous red flowered (**RR**) pea plant with a homozygous white flowered (**rr**) pea plant. They both produced gametes of **R** and **R** and **r**. The gametes of both united to form a hybrid having alleles **Rr** both for redness and whiteness. The allele **R** for red colour partially expresses in F_1, while allele **r** for white colour remains recessive (latent). These two alleles remain together for long time, but they do not effect each other. The gametes **R** and **r** are of equal number. In F_1 generation hybrids were found to be pink or purple flowered, thus showing **incomplete dominance** of red colour over white colour. When F_1 hybrids were self fertilized, these gametes united in three combinations : **RR, Rr** and **rr** to produce three types of plants in F_2 generation : 75% plants had coloured flowers and 25% had white colour. The reappearance of white colour in F_2 generation shows the process of segregation.

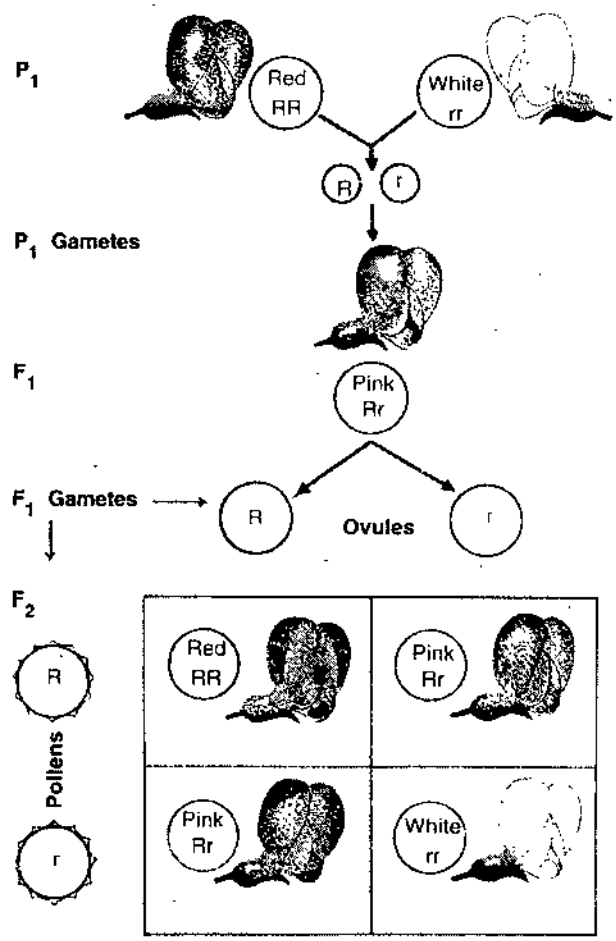


Fig. 3. A cross between a red flowered and a white flowered pea plant showing incomplete dominance.

B. Results of Dihybrid Cross

The law of independent assortment is based on dihybrid crosses (when two contrasting characters are taken into consideration).

3. Law of Independent Assortment : When two or more pairs of contrasting characters are crossed, in the F_1 generation, only dominant characters appear. During self-fertilization of F_1 generation, in the F_2 generation along with two parental combinations, two new combinations also appear.

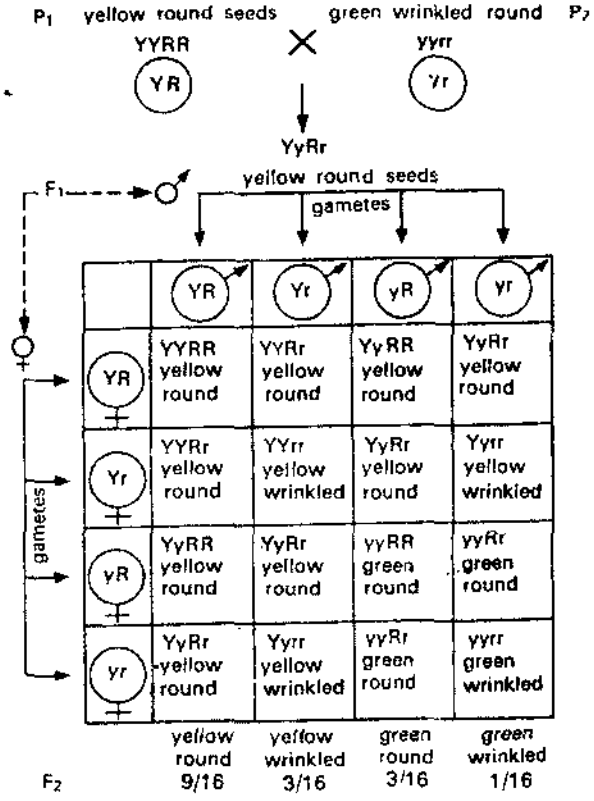


Fig. 4. Mendel's dihybrid cross showing Law of Independent assortment.

According to the law of independent assortment, if we consider the inheritance of two or more pairs of contrasting characters, at the time of gamete formation, each character segregates or separates into gametes, independent of each other.

Exception of Law of Independent Assortment : Law of independent Assortment is true only for those traits where genes are present on different chromosomes.

• 8.4. POSTULATES OF MENDEL

In the modern text books, Mendel's work is given in the form of four postulates :

1. Unit factors in Pairs : Genetic characters are controlled by unit factors that exist in pairs in individual organisms.

2. Dominance and Recessiveness : When two unlike unit factors responsible for a single character are present in a single individual, one unit factor is dominant over other, which is said to be **recessive**.

3. Segregation : During the formation of gametes, the paired unit factors separate randomly so that each gamete receives one or the other with equal likelihood.

4. Independent Assortment : During gamete formation, segregating pairs of unit factors assort independently of each other.

Molecular Basis of Mendelian Characters

In cotyledons of pea a **starch branching enzyme (SBE I)** is present which is responsible for the formation of starch from simple sugars.

In normal plants **gene R** codes for **SBE I** (round seed coat).

The mutant form of this **gene r** forms an aberrant form of SBE I.

Aberrant form of SBE I is unable to synthesize starch.

Failure of starch formation results in the increase of free sugars in the cells of cotyledons. Presence of large number of free sugars leads to higher water content and larger cell volume in early stages of seed development.

On maturation seeds loose this water content that causes shrinkage in volume, hence **wrinkled seed coat.**

Simple Mathematical Rules for Calculating number of Gametes and Genotypes

Number of heterozygous gene pairs	Number of different types of gametes formed	Number of differnt genotypes produced	Number of different phenotypes produced
n	2^n	3^n	2^n
1. Aa, Aabb, aabbCc	2	3	2
2. AaBb, AaBb, cc, aabbCc	4	9	4
3. AaBbCc, AaBbCcDD	8	27	8
4. AaBbCcDd, AaBbCcDdEE	16	81	16

This number of phenotypes is applicable for simple dominant and recessive genes.

• 8.5. VARIATIONS IN MENDEL'S LAWS

1. Incomplete Dominance. In incomplete dominance, sometimes in a heterozygote dominant allele does not mask the phenotypic expression of the recessive allele and there appears an intermediate phenotype in the heterozygote. This is called **incompelete dominance.** Its example is when a red flowered plant (RR) is crossed with white flower pea plant (rr), the F_1 hybrid pea plants are found to have pink flowers. It shows that gene for red colour could not completely dominate the gene for white colour as shown in the figure. 3.

Another example is of Andalusian fowl. In Andalusian fowl when a cross is made between black and white splashed plumage, the hybrids show blue plumage.

2. Co-dominance. In co-dominance both alleles of dominance and recessive characters express themselves in F_1 generation. Its example is coat colour in cattle. When a cattle of red coat (CC RR) is crossed with cattle of white coat (CC WW), F_1 heterozygote or hybrid is found to have (CRCW) roan coat. That is red and white coloured hairs are found in patches, but no intermediate red and white coloured hair appeared (Fig. 5).

Additional Information

Unit factor (gene of today) is unit of heredity that determines a biological character of an organism.

Mendel's laws of heredity are actually behaviour of chromosomes at the time of meiosis.

As **homologous chromosome pairs** separate at the time of gamete formation and go to different gametes, the alleles present in chromosomes are also carried to different gametes. Similarly after segregation, each chromosome of a homologous pair has equal chance to enter any gamete, thus giving all possible combinations and recombinations.

Emasculation is removal of anthers in bisexual flowers to prevent self-fertilization.

The term **"pure line"** was introduced by **Johannsen.** A pure line is a plant or animal, that is, genetically pure for a particular character and will give rise to same character after self-fertilization or inter-breeding.

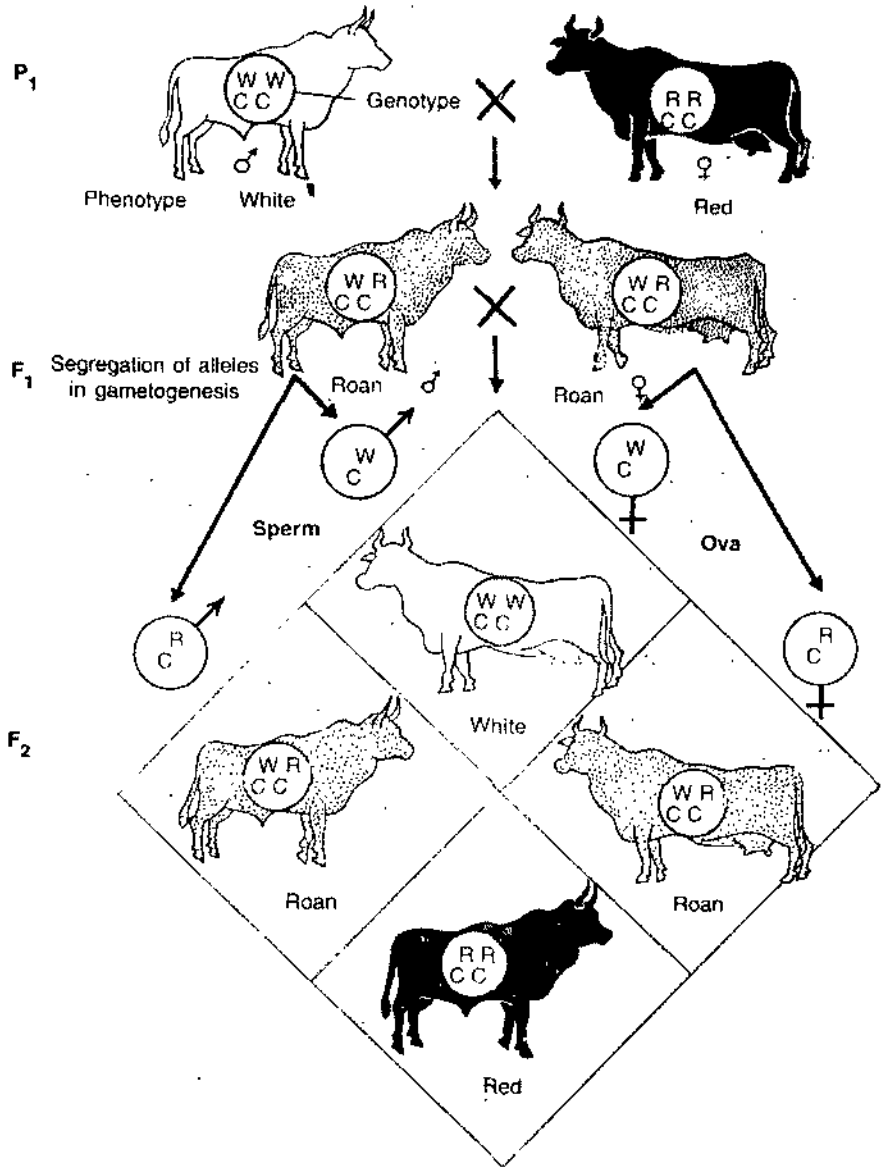


Fig. 5. Monohybrid cross of white coated and red coated cattle showing dominance.

All seven characters in pea studied by Mendel were not situated on separate chromosomes. The genes for plant height and pod shape are located on the same chromosome and show linkage.

Results of these crosses were not reported by Mendel.

The important phenomenon not observed by Mendel was linkage.

Dominant traits can appear in homozygous as well as heterozygous condition but recessive traits appear only in homozygous condition.

• 8.6. SOME BASIC TERMS

Alleles : Various forms of a gene are called **alleles** or **allelomorphs.**

The term 'allelomorph' was given by **Bateson.**

Homozygous : Homozygous is the condition when a pair of alleles has same genes *e.g.*, AA or aa condition. Homozygous condition was called **'pure'** by Mendel.

Heterozygous : Heterozygous is the condition when a pair of alleles are different *e.g.*, Aa.

Heterozygous condition is also called **hybrid.**

Phenotype : Phenotype is expression of a gene *i.e.*, external characters *e.g.*, skin colour, flower colour, shape of seed etc.

Genotype : Genetic information of an organism is, called genotype, *e.g.*, genotype of a plant is **Tt** and its phenotype is **tall stem.**

Dominant : The character which is expressed both in homozygous and heterozygous state is called **dominant,** *e.g.*, Tallness – a plant with genotype TT is tall and another plant with genotype Tt is also tall.

Recessive : The character which is expressed only in homozygous state is called recessive, *e.g.*, a plant will be dwarf only with genotype tt.

F_1 or First Filial Generation : The first generation of offspring after making a cross between two selected parents is called F_1 or first filial generation, *e.g.*, Aa is F_1 of AA × aa.

F_2 or Second Filial Generation : The offsprings of interbreeding of F_1 generation are called F_2 generation.

Test Cross : In a test cross the offspring with unknown dominant phenotype is crossed with a homozygous recessive individual.

In a test cross, in F_1 generation there are two possibilities.

(i) If all individuals are of dominant phenotype then unknown sample was **homozygous.**

(ii) If individuals are of dominant and recessive phenotype in ratio of 1 : 1 then unknown sample was **heterozygous.**

In a dihybrid test cross a ratio of 1 : 1 : 1 : 1 is obtained in heterozygous individuals.

Back Cross : When F1 offspring is crossed with any of the parental genotype, the cross is called **back cross.** Back cross is used for obtaining pure lines of a usefull trait.

Back cross was devised by Mendel for obtaining pure lines for his experimental plants.

• SUMMARY

- **Genetics** is a science of heredity and variation.
- **Heredity** is transmission of characters from one generation to the next generation.
- Resemblance of offsprings to parents and also to each other is due to heredity.
- **Variations** are differences between offsprings and parents.
- **Father of Genetics** is Gregor Johann Mendel. Mendel selected garden pea plants for his experiments.
- Mendel selected 7 pairs of contrasting characters in *Pisum sativum* (garden pea)
- Word **P** is used for parent plants, F_1 for first generation and F_2 for second generation offsprings and so on.
- All characteristics of an organism are located on **genes** in pairs present in chromosomes, arranged in a linear fashion.
- In monohybrid cross, a single contrasting character is selected, and in dihybrid cross two contrasting characters of an organism are selected.
- In F_1 generation, after a cross (self-fertilization) only dominant character appears and recessive character remains hidden. But in F_2 generation both dominant and recessive characters appear.
- **Law of dominance :** A cross between two plants or animals having contrasting characters, in F_1 generation dominant character appears and the other character which remains hidden is recessive.
- **Law of segregation :** Alleles pair of a gene segregate at the time of gamete formation, but paired condition of alleles is restored at the time of fertilization.
- **Law of independent assortment :** When two or more pairs of contrasting characters are crossed, in F_1 generation only dominant characters appear. During self-fertilization of F_1 generation, in F_2 generation along with two parental combinations two new combinations also appear.

• STUDENT ACTIVITY

1. What do you understand by Mendel's laws of Inheritance ?

2. Describe monohybrid and dihybrid crosses as explained by Mndel.

• VERY SHORT ANSWER QUESTIONS

1. **What are seven pairs of contrasting characters of Mendel.**

   Ans. Yellow and green seed coat
   Round and wrinkled seed coat
   Purple and white flowers.
   Axial and terminal flowers.
   Green coloured and yellow coloured pods.
   Inflated and constricted pods.
   Tall and dwarf plants.

2. **Write about genotype and phenotype.**

   Ans. Genetic expression of an organism is called genotype.
   Phenotype is the physical or external observable expression of an organism.

3. **Define allele.**

   Ans. Alternative forms of the same gene which determine contrasing characters, *e.g., T and t, (tall and dwarf).*

4. **Define dominant and recessive terms.**

   Ans. Character which is expressed both in homozygous and heterozygous state is called **dominant,** *e.g.,* tallness of plant TT and Tt.

   Recessive : Character that is expressed only in homozygous state is called recessive, *e.g.,* tt (dwarfness).

5. **Define test cross.**

   Ans. A cross between the recessive parent and an individual of F_1 generation, *e.g.,* P (tt) × Tt (of F_1).

6. **Explain law of dominance.**

   Ans. When a cross is made between two organisms having a set of contrasting characters, in first generation (F_1) only one character appears, which is called ***dominant.*** *The other character remains hidden is called* ***recessive.***

7. **Define law of segregation.**

   Ans. F_1 plant having a set of contrasting characters obtained by a cross between two organisms having a set of contrasting characters, when their F_2 generation was raised, the pair of parental characters separate, *i.e.,* one factor is present in each gamete. This became known as law of segregation.

8. ***Explain law of independent assortment.***

   Ans. In a cross between two organisms having two contrasting characters, during gamete formation segregating pairs of unit characters assort independently of each other.

9. **Define back cross.**

   Ans. A cross between two plants (parental) results into F_1 hybrids. An F_1 hybrid when crossed with any one of the two parents is called back cross.

10. **What is emasculation ?**

    Ans. Removal of anthers from one flower before cross pollination between two flowers.

11. **Which plant Mendel selected for his experiment ?**

    Ans. Garden pea (*Pisum sativum*).

12. **Define heredity.**

    Ans. Transmission of characters from parents to the offspring is called heredity.

13. **Who is known as father of Genetics ?**

    Ans. Gregor Johann Mendel.

14. **Diploids, where both the alleles are identical are called .....**

    Ans. Homozygous (TT or tt).

15. **If both the alleles of a diploid are different. What are they called ?**

    Ans. Heterozygous (Tt).

UNIT

9

CYTOPLASMIC (EXTRANUCLEAR) INHERITANCE

STRUCTURE

- Introduction of cytoplasmic inheritance
- Maternal effects caused by egg cytoplasm
- Inheritance caused by cytoplasmic organelles besides nuclear inheritance
- Inheritance by chloroplast DNA in four O'clock plant
- Inheritance by Mitochondrial DNA in yeast.
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Cytoplasmic inheritance, Chloroplast inheritance, Mitochondrial inheritance, Maternal inheritance, Shell coiling in Limnaea.

9.1. INTRODUCTION OF CYTOPLASMIC INHERITANCE

DNA is the basic genetic material and all cellular DNA is localized in the chromosomes. The mode of inheritance of a particular character is tied to the behaviour of chromosomes. DNA alone does not initiate development of biological characteristics in the absence of all other cellular components. Raw DNA does not produce an organism alone. It depends upon an already existing medium in order to function. Genotypic effects may be considerably modified by environment. In the cell, the source of environment is the cytoplasm surrounding the nucleus. The effects produced by the egg cytoplasm are called as **maternal effects.** It was found by **Caspari** in flour moth, *Ephestia kuhniella.* Maternal effects arise from egg cytoplasm that has been modified chromosomally transmitted genes. In the maternal effect, phenotypic changes appear because of differences in egg cytoplasm rather than differences in sex chromosomes and often, not always, affect both male and female offsprings equally.

Some cytoplasmic factors show independent transmission and are considered as genetic units fully equal to those in the chromosomes, *i.e.*, genes. Since these are found outside the nuclear chromosomes, hence these are, called cytoplasmic or extranuclear or extrachromosomal genetic factors. These have been called **plasmagenes, plasmons, plasmids** and **cytogenes** etc.

When crosses are made between organisms carrying traits caused by cytoplasmic factors, the traits are not transmitted according to expected mendelian ratios. This departure is due to difference in the results of reciprocal crosses (crosses between two hermaphrodite or bisexual organisms)., The difference caused by extranuclear factors do not usually disappear after one generation, but persist as long as the extranuclear factors do not usually disappear after one generation, but persist as long as the extranuclear factor can perpetuate itself.

Chloroplast Inheritance in Variegated four O'clock plant

First example of cytoplasmic genes inherited solely through maternal parent was reported by **Correns** in 1909. He found that a certain variety of *Mirabilis jalapa* (four-O-clock'-plant), had branches, which, produce either green, white or mixed green-white (variegated) leaves. In crosses between flowers of these branches the offsprings are all green, if the maternal parent flower is from a green branch. Such offspring remains green throughout later generations as long as the maternal plant is green.

Similarly as long as the maternal plant is from a white branch, the offsprings are all white. However, pure white plants die due to absence of photosynthesis.

If maternal parent is mixed green and white (variegated), then the offsprings are similarly variegated. Thus, the phenotype depends upon a factor present within the maternal cytoplasm, which is probably self perpetuating (everlasting).

In *Mirabilis*, the cytoplasmic factor is probably the chloroplast organelle containing the green chlorophyll.

From the results of Corens' experiments and also from others, it is likely that there is genetic continuity between chloroplasts, as that found between nuclei. For this, evidences have been observed in *Micromonas* (an algae) in which chloroplast divides. In this alga, only one chloroplast and one mitochondrion is present.

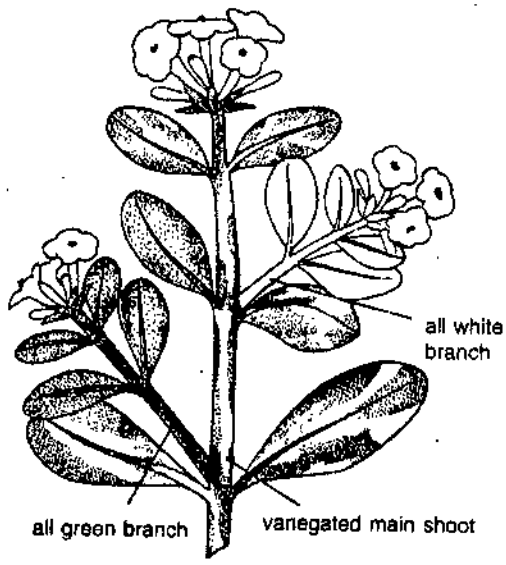


Fig. 1. Leaf variegation in *Mirabilis jalapa*, (four-o'clock plant). Flowers may form on any branch (variegated, green, or white).

Another example is of an abnormal form of *Spirogyra* in which one mutant chloroplast having no pyrenoid body and one normal chloroplast is found. It was observed that chloroplast divide due to which each daughter cell received both the kinds of chloroplasts. Also, when chloroplasts are shown to be absent in a particular plant cell, no new chloroplasts are formed. It indicates that their existence depends upon pre-existing chloroplasts. Chloroplasts have their own DNA is a fact that strongly supports the idea that some changes in chloroplast structure can be perpetuated, like the changes governed by nuclear DNA. However, since chloroplasts are found only in the cytoplasm, hence their transmission to new generations takes place mainly through the maternal gamete (*i.e.*,, ovum).

These results do not mean that nuclear genetic material (*i.e.*, chromosomes) has no effect on chloroplast production. The interrelationship that must occur between all phenotypes shuts out any opinion that any single part of an organism develops in complete isolation from the effect of nuclear genes. Investigations of **Baur** showed that green and white leaved factors segregate according to Mendelian pattern, *i.e.*, self fertilized F_1 heterozygotes produce F2 plants in a ratio of 3 green : 1 white. **Von Wettstein** observed the same in barley chloroplast development that may be affected by a large number of nuclear genes. **Levine, Goodenough** and others have identified a wide arrangement of nuclear genes that block specific steps in photosynthesis.

According to the present concepts, it is likely that the pre-plastid functions as the physical link between chloroplast generations.

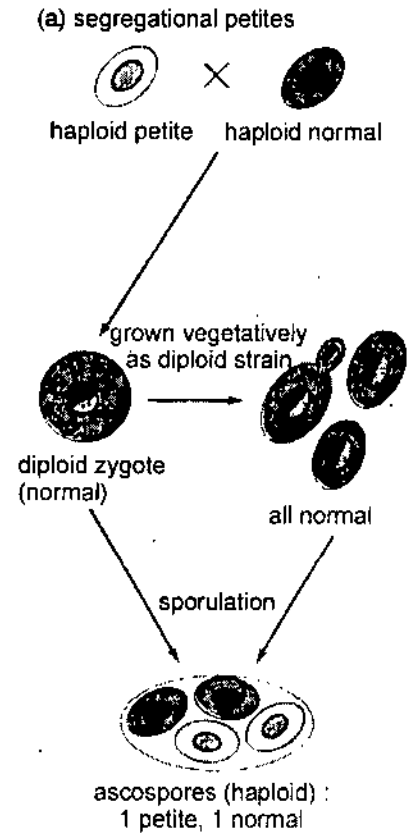


Fig. 2. Result of cross between segregational petites and a neutral petite.

• 9.2. EXTRANUCLEAR INHERITANCE BY MITOCHONDRIA

Cytoplasmic DNA is also found in mitochondria of plant and animal cells, and mitochondria is like kinetoplasts (basal flagellar organs) of some protozoan parasites.

Most eukaryotic cells are aerobic, dependent upon oxygen for respiration, but some eukaryotes such as yeast survive by metabolizing carbohydrates both aerobically and anaerobically (fermentation). Due to absence of oxygen, yeast cells grow slowly. Mutations stimulate such deprivations of oxygen and cause defects in ability to utilize oxygen and thus, produce small colonies called **petites.**

Strains of mutant **petites** were first discovered by **Ephrussi** and collaborators in baker's yeast *Saccharomyces cerevisiae.* They lack components (enzymes) necessary for respiratory activity, *e.g.,* cytochromes b, C_1 and cytochrome oxidase a, a_3. These are normally associated with the inner membranes of mitochondria. These deficiencies prevent growth on carbon source and also prevent **petites** from producing spores – a process that is dependent upon oxygen respiration. Thus, **petite** diploid cells can not sporulate, nor a mating between two haploid **petites** can produce a zygote that will produce spores. However, petites can be maintained indefinitely in the vegetative state, either as diploid or haploid, and can be mated with normal yeast cells. In such matings, three varieties of petites can be classified :

1. Segregational (nuclear) petites : Such petites, when crossed to wild type (normal), produced haploid spores, which segregate in the ratio of 1 petite : 1 normal. This is ordinary nuclear Mendelian inheritance

2. Neutral petites : Only wild type (normal) **ascospores** and colonies arise from mating between such **petites** and normal yeast strains. In further generations, petite characteristic never reappears and has been physically lost. This behaviour can not be explained on the basis of nuclear genes. Such petites are produced by an extrasomal particle, *i.e.,* mitochondria.

3. Suppressive petites : Such **petites** suppress normal respiratory behaviour in crosses with normal strains. As much as 99% of the diploid cells derived from a zygote consist of normal **petites.** When suppressive X normal zygotes are induced to sporulate in a special environment, most of them give rise to ascospores in which all four spores are **petites.** The suppressive **petite** factor acts as a **dominant,** but ratios among diploid strains produced are not Mendelian and vary from 99% to 1 percent **petites.**

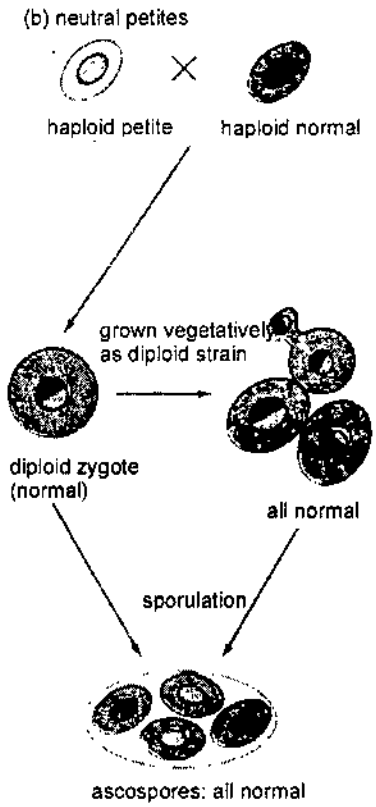


Fig. 3. Cross between neutral petite and normal haploid yeast.

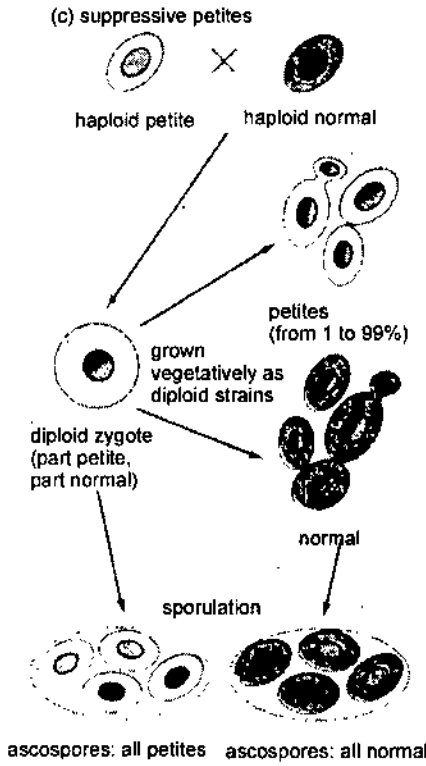


Fig. 4. Cross between suppressive petite with normal strain.

Thus, there are two distinct genetic causes for respiratory deficiency in yeast : nuclear and extranuclear. On this basis, a **neutral petite,** which is cytoplasmically affected, has nuclear genes for normally functioning mitochondria.

A cross between a segmentational **petite** and a neutral **petite** should produce a zygote that can utilize the normal nuclear genes from the neutral **petite** and respire normally. Here diploid zygotes function normally and are of normal size. When such diploid cells are induced to sporulate, the **petite** character reappears (in the ratios 1 : 1 Mendelian segregation). The neutral **petite** strains carry the normal nuclear genes for respiratory enzymes, which are not found in segregational **petite.** The cytoplasmic factor causing neutral **petite** character that appears is independent of nuclear control.

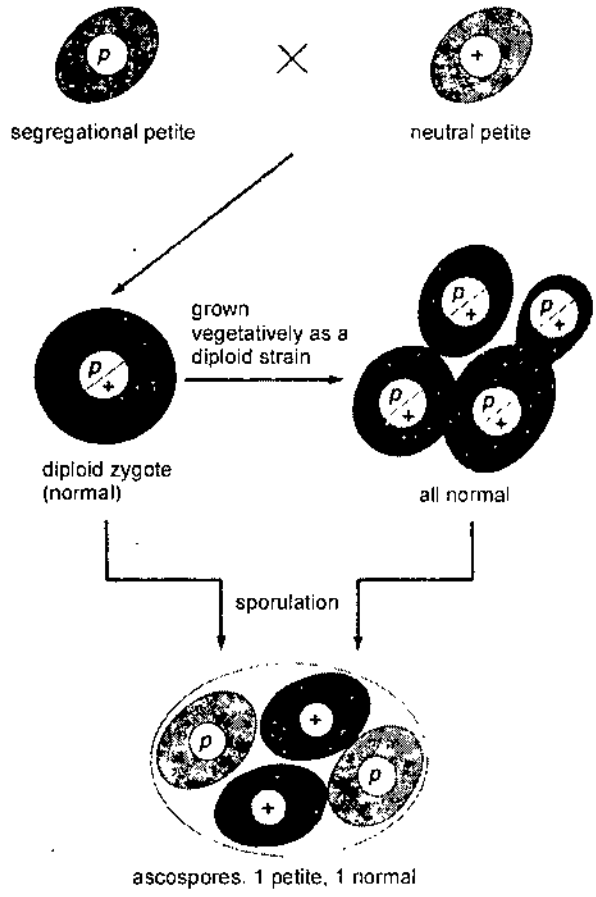


Fig. 5. Cross between a segregational petite and a neutral petite.

Neutral petites are easily produced by subjecting normal strains to low doses of dyes **acriflavines** and **ethidium bromide.** These produce high frequency of **petites,** which indicates the occurrence and transmission of extranuclear changes.

It is known that mitochondria have their own DNA and cellular division of mitochondria has also been observed in algae *Micromonas* and *Nitella*. The cellular continuity of mitochondria and mitochondrial DNA explains the cytoplasmic continuity of some of the **petite** strains.

• 9.3. EXTRA-NUCLEAR INHERITANCE IN EUKARYOTES

Maternal Inheritance

1. Eye pigmentation in flour moth. A clear example of maternal effect was found by **Caspari** in the flour moth, *Ephestia kuhniella*. This moth has dark-brown eyes and various pigmented larval parts, that owe part of their colour to the presence of a pigment precursor, **kynurenine,** produced by the dominant gene **A.** When the organism is homozygous for a recessive allele of this gene, **a**, kynurenine is absent, causing red eye colour and a lack of larval pigmentation.

In a normal expected case, when a male heterozygous for **Aa** fertilizes an **aa** recessive female, their offspring will appear in the genotypic ratio 1 Aa : 1 aa. Phenotypically the larvae will consequently be in the ratio 1 pigmented : 1 non-pigmented, and will then mature into dark-brown and red-eyed offsprings, respectively.

When the reciprocal cross is made, Aa female × aa male. In this case all early larvae appear pigmented, as though kynurenine were present in all offsprings. However, when the larvae mature, only half of them are dark brown-eyed, and the other half are red-eyed. This is based on the egg cytoplasm contributed by the heterozygous mother. That is, all eggs of the dark brown-eyed female contain kynurenine no matter what

their genotype and therefore begin development as pigmented larvae. However, half the larvae are aa and unable to synthesize further kynurenine. Such larvae as a result develop into red-eyed adults as the initial kynurenine is expended.

This represents a maternal effect transmitted through the egg cytoplasm for only one generation, since in the next generation new egg cytoplasm will be formed according to the pattern of the new maternal genotype.

Such phenotypic expressions of maternal genes (genotype) may be short-lived or may persist throughout the life span of the individual. The substances that produce the maternal effects in the progeny are found to be transcriptional products *i.e.*., mRNA, rRNA and tRNA, of maternal genes which have been produced during oogenesis and exist in the ooplasm of unfertilized eggs in the form of inactive protein coated and late translating mRNA molecules (**informosomes**) or inactivated rRNA and tRNA. These transcriptional products of maternal genes produce their phenotypic effects during early cleavage and blastulation when there occur little or no transcription because, maternal and paternal genes of zygote remain engaged in mitotic duplication of DNA.

2. Shell coiling in *Limnaea* : In a species of the snail *Limnaea*, the maternal effect does not diminish during development and lasts throughout life in the adult. In *Limnaea* the direction of coiling is determined by a single pair of genes. Snails can coil to the left (**sinistral**) or right (**dextral**). The allele for dextral coiling **R** is dominant over that of sinistral coiling **r**. In crosses between these snails the direction of coiling of the offsprings is always determined by the genotype of the maternal snail. When this is

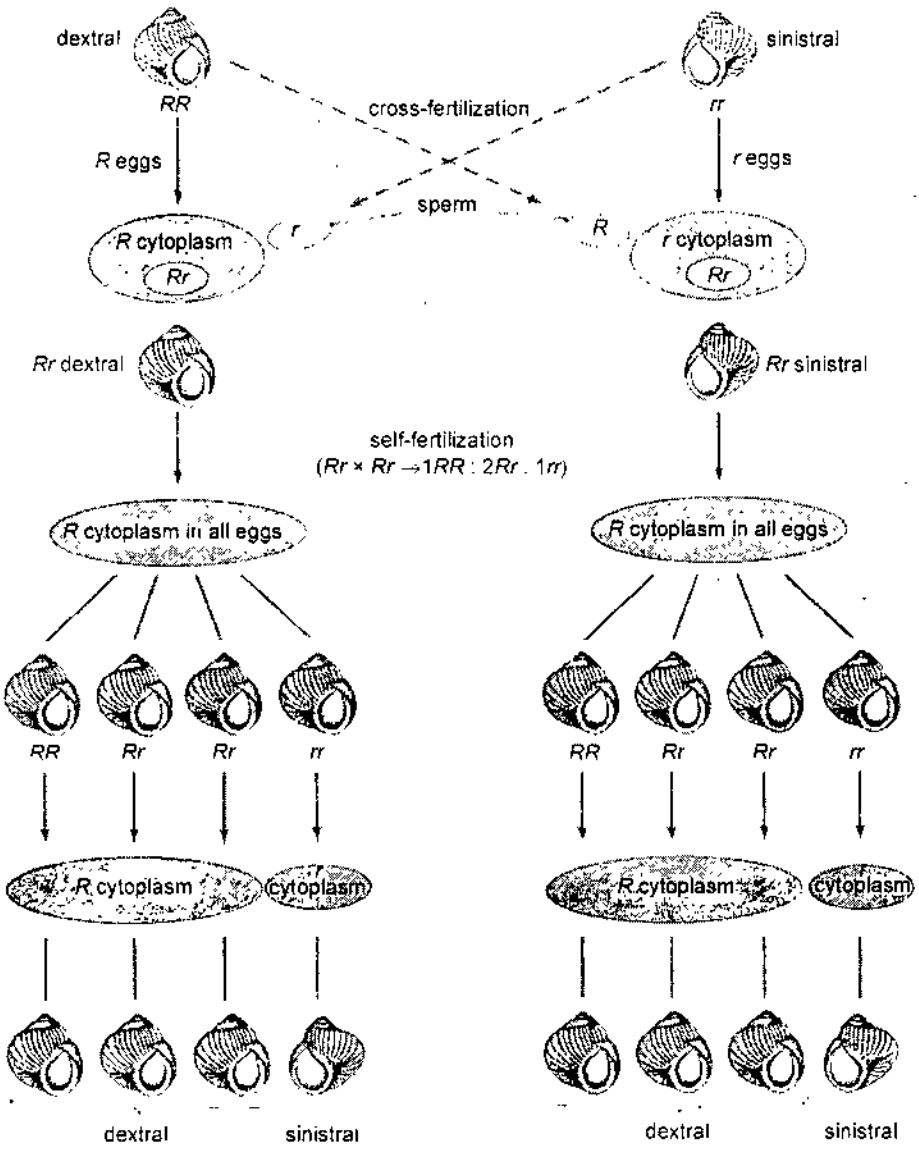


Fig. 6. Maternal effect in direction of coiling of the shell in *Limnaea*.

completely different from the genotype of the zygote. Thus, a self-fertilized dextrally coiled heterozygote **Rr** will always produce eggs that develop into dextrally coiled offspring even though some fertilized eggs are homozygous recessive **rr** and should be sinistrally coiled. Similarly, the products of a self-fertilized sinistrally coiled heterozygote (heterozygous offsprng of an **rr** mother) wll be dextrally coiled even though some eggs are again homozygous **rr.** These maternal effects last only one generation. In the next generation sinistrally coiled offsprings are produced by the homozygous **rr** maternal parents even though they themselves are dextrally coiled.

Shell coiling appears to lie in the direction of the cleavage pattern initiated by the first few cell divisions of the fertilized egg (**Conklin**). Apparently the direction of displacement, the right or left, is determined by the maternal genotype. This case also represents a maternal effect transmitted through the egg cytoplasm for only one generation. Because, in the next generation new egg cytoplasm will be formed according to the pattern of the new genotype.

• SUMMARY

- DNA is the basic genetic material found in nuclear chromosomes.
- DNA is also located in certain cytoplasmic organelles like plastids and mitochondria.
- Effect produced by the egg cytoplasm is called maternal effect.
- Maternal effect was found by Caspari in flour moth *Ephestia Kuhniella*. Such effects were produced by egg cytoplasm.
- Cytoplasmic factors show independent transmission and are called genetic units. These are found outside the nucleus and hence are called cytoplasmic or extranuclear genetic factors.
- Traits developed by cytoplasmic factors do not follow mendelian ratios.
- Chloroplast (plastid) inheritance was observed by Correns (1909) in *Mirabilis jalapa* (Four O'clock plant).
- Four O'clock plants have leaves, green, white and variegated.
- Divisions in chloroplast have been observed in *Micromonas* (algae) and *Spirogyra*.
- Cytoplasmic inheritance is also found by mitochondria which also contain DNA. This type of inheritance has been observed in yeast which respire arobically and anaerobically.
- **Petites** are small colonies of yeast which are formed due to mutation.
- Mutant yeasts have been discovered in *Saccharomyces* (baker's yeast)
- In certain cases certain characteristic phenotypic traits of progeny are expressions of maternal parents (cytoplasm of egg). It persists only for one generation transmitted through the egg cytoplasm.

• STUDENT ACTIVITY

1. Discuss the role of chloroplast or mitochondria in the cytoplasmic inheritance.

2. A four-O'clock plant has green, white and mixed branches. What kinds of progeny are to be formed from the crosses : green female × white male; white female × green male; and variegated female × green male.

• VERY SHORT ANSWER QUESTIONS

1. **Define maternal effects.**

   Ans. The effects produced by the egg cytoplasm are called maternal effects.

2. **In which organisms maternal effect was observed ?**

   Ans. Casper observed the maternal effects in flour moth, *Ephestia*.

3. **In which plant was the chloroplast inheritance observed ?**

   Ans. It was observed in four-O'clock plant, *Mirabilis jalapa*, which contains green, white and variegated branches.

4. **In which organism was the mitochondrial inheritance observed ?**

   Ans. It was observed in yeast. *Saccharomyces* (baker's yeast).

5. **Write the genetic causes for respiratory deficiency in yeast.**

   Ans. Respiratory deficiency is caused due to nuclear and extranuclear factors (DNA).

6. **A snail had a dextral coiling upon self-fertilization, it produces the progeny having sinistral coiling. Explain.**

   Ans. RR for dextral coiling is dominant and rr for sinistral coiling is recessive.

$$Rr \times Rr \xrightarrow[\text{fertilization}]{\text{self}} 1\,RR : 2\,Rr : 1\,rr$$

   R cytoplasm in all eggs, hence coiling in progeny will be dextral.

UNIT

10

DNA AS GENETIC MATERIAL

STRUCTURE

- DNA is found in chromosomes located in the nucleus of a cell. Chromosomes contain proteins and nucleic acids, i.e., DNA and RNA.
- DNA is the genetic material in most organisms.
- RNA is the genetic material in some viruses.
- Transformation experiments of Frederick Griffith (1928) worked on pneumonia causing bacterium (*Diplococcus pneumoniae*) grown on agar (nutrient) and found two strains of bacterium : smooth (S) virulent forms, and rough avirulant forms.
- Blender experiment on *E. coli* phage T2 confirmed the genetic nature of DNA.
- Chromosomes in higher organisms contain DNA.
- Amount of DNA in different cells
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- DNA as a genetic material. Transformation Experiments, Blender Experiment, Bacterial conjugation and other evidences.

• 10.1. DNA AS A GENETIC MATERIAL

Chromosomes are the organs of heredity and form a link between generations. They carry linearly arranged genes, which are very minute in structure. Chromosomes contain proteins and nucleic acids, *i.e.,* DNA and RNA. **A. Mirsky** and **H. Ris** had found that all cells of an organism contain the same amount of DNA, whereas different cell types have quite different amounts and kinds of proteins. It shows that DNA is the genetic material. Around 1953, it was universally accepted that DNA is the genetic substance (*i.e.,* chemical of which genes are composed) of most microorganisms and higher organisms. In some viruses, the genetic material is RNA. DNA is the genetic material of most organisms has been supported by the following experiments :

1. Transformation Experiments

Frederick Griffith (1928) faced a phenomenon, called Genetic transformation. Colonies of virulent strain (pathogenic) of pneumonia causing bacterium (*Diplococcus pneumoniae*) grown on agar have a **smooth** (S) glistening appearance. It was due to the presence of a specific type polysaccharide capsule. Polysaccharide was polymer of glucose and glucoronic acid. Avirulent (non-pathogenic) strains have no such capsule and they produce dull and **rough (R)** colonies. This trait is known to be genetically determined.

Both S and R forms are found in several types and are designated SI, SII, SIII, etc, and RI, RII and RIII, etc., respectively. These subtypes of S and R bacteria are different from each other on the basis of antigens produced. The type of antigen produced is genetically determined. Sometimes S-forms mutate to R forms, but this change was not found reversible.

Griffith injected live R II pneumococci in laboratory mouse. This mouse did not suffer from illness since RII pneuococci was non-pathogenic.

But when mouse was injected the virulent strain S III pneumococci, it died. And when he injected heat killed S III pneumococci in mice, they did not suffer from pneumonia. When Griffith injected the mixture of living avirulent R II and heat killed SIII virulent in mice, unexpected symptoms of pneumonia appeared and high mortality took place in mice. He postmortemed the dead mice, which showed that their heart blood had both RII and SIII pneumococci. He concluded that presence of heat killed SIII bacteria might have transformed the living RII bacteria. RII bacteria might have restored the capacity of capsule formation, which they had lost earlier by gene mutation. This was called **Griffith effect** or **bacterial transformation.**

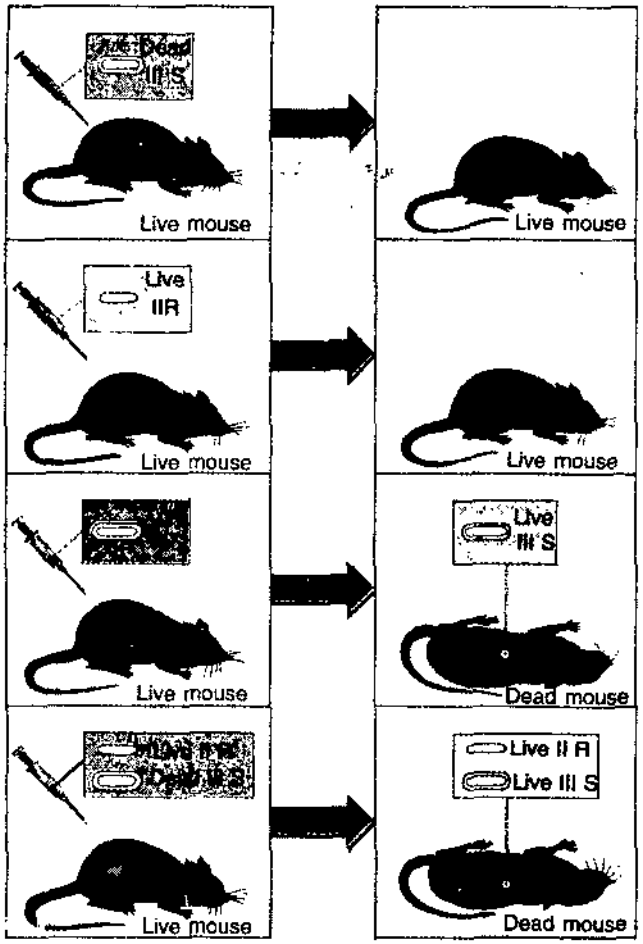


Fig. 1. Experiment of Griffrith showing principle of transformation.

Oswald Avery, Colin MacLeod and **Maclyn McCarty** in 1944 identified the cause of bacterial transformation. They partially purified the transforming substance from the cell free extract of S-III bacteria and demonstrated that it was DNA.

2. Blender Experiment of *E. coli* phase T_2

Blender performed an experiment with *E. coli* phage T2 and confirmed the genetic nature of DNA. **Alfred Hershey** and **Martha Chase** (1952) also demonstrated that DNA injected by a phage particle into a bacterium contains all the information required to synthesize progeny phage particles. A single particle of phase T2 consists of DNA encased in a protein shell. The DNA of the phage particle is the only phosphorus containing substance. The proteins of the shell containing amino acids methionine and cysteine have sulphur atoms. Phage DNA was made radio-active by growing infected bacteria on a medium containing radioactive phosphate ($^{32}PO_4$). Phage proteins do not contain phosphorus, only DNA was labelled. In the same way phage proteins were labelled with $^{35}SO_4$. Since DNA does not have sulphur, only protein was labelled with 35 sulphur. Such differential labelling enabled the workers to distinguish between DNA and proteins of phage without performing any chemical tests. Hershey and Chase later allowed both kinds of labelled phage particles to infect *E. coli* bacteria. The infected

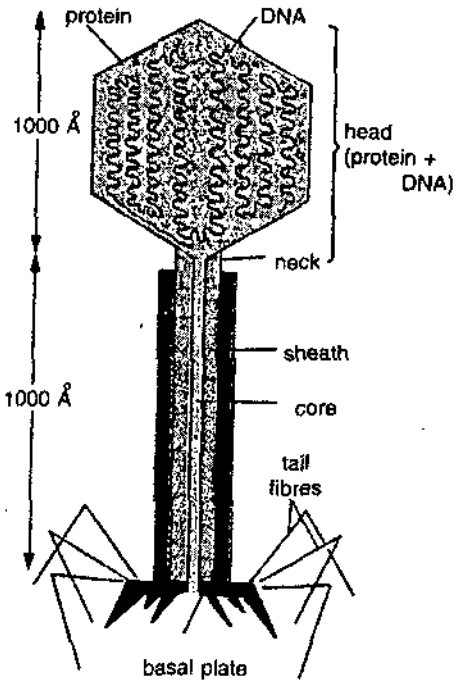


Fig. 2. Structure of T_2 backteriophage.

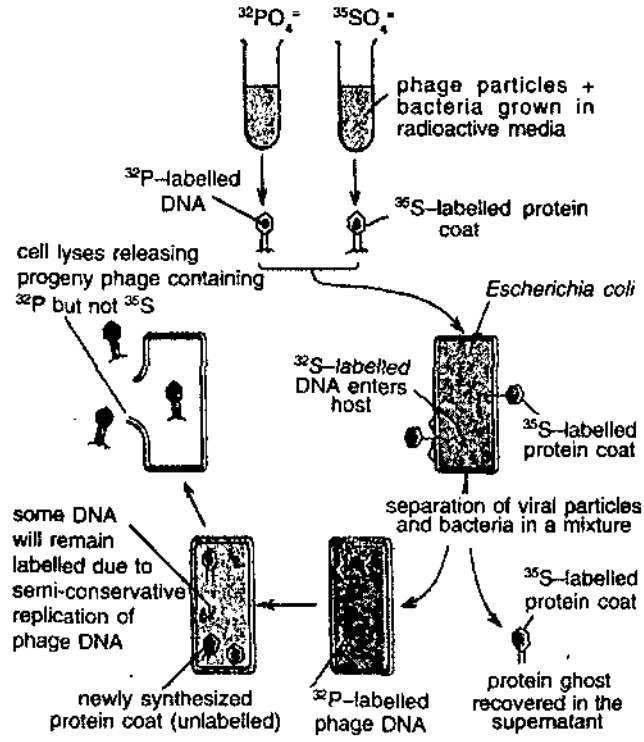


Fig. 3. Experiment of Hershey and Chase in which DNA of T_2 phage is labelled with ^{32}P and protein is labeled with ^{35}S.

bacteria were immediately agitated in a waring blender. They found that only radioactive 32 phosphorus was associated with bacterial cells and 35 sulphur was found only in surrounding medium. When phage progeny was studied for radioactivity, it was found that the phage progeny carried labelled only with 32 P and was not labelled with 35S. This indicates that only DNA is injected into bacterial cell and not protein. The empty protein coat (called ghost) is left outside. Thus, this experiment proved that DNA is the genetic material in DNA bacteriophage, because it carries all the genetic information of new phage particles. DNA is also the genetic material of higher organisms. Feulgen techniques have shown that DNA is solely found in chromosomes. It is one of the major component of chromosomes.

Quantitative measurements of amount of DNA in different cells have shown the correlation between the amount of DNA and the number of chromosomes set (ploidy). Diploid cells contain double DNA in comparison to haploid cells of the same species.

3. Bacterial Conjugation.

The phenomenon of conjugation in bacteria also provided an evidence for DNA as genetic material. **Laderberg** and **Tatum** in 1946 found that when an F^+ (male) *E. coli* cell was conjugated with an F^- (female) *E. coli*, uni-directional transfer of F^+ factor of male cell to F^- or female cell took place. Thus, the female (F^-) was converted into a F^+ (male) strain. The F^+ factor was found to be a fragment of DNA molecule, which is found in the cytoplasm of bacterial cell.

Other Evidences for DNA as the Genetic Material

The DNA is the genetic material of higher organisms has also been supported by the following facts :

1. Feulgen techniques had shown that DNA remains restricted to the chromosomes. It is one of the major component of chromosomes.

2. The amount of DNA in different cells has shown that there is a co-relation between the amount of DNA and the number of chromosome sets (ploidy). Diploid cells contain double amount of DNA in comparison of haploid cells of the same species.

3. The diploid amount of DNA is constant within a species, but it varies from one species to another.

• SUMMARY

- Chromosomes are the organs of heredity. Chromosomes have linearly arranged genes. Chromosomes contain proteins and DNA (nucleic acids).
- Nucleic acids (DNA) carry genetic informations (tetranucleotide hypothesis). Cells of the organism contain the same amount of DNA, while different cell types contain different amounts and kinds of protein. Its constancy favoured that DNA is the genetic material.
- DNA is the genetic substance of most microorganisms and higher organisms was universally accepted.
- DNA as the genetic material had been supported by the following experiments :
- 1. Transformation of bacteria. Experiment conducted by Griffith (1928) on *Diplococcus pneumoniae* injected in laboratory mice.
- 2. Blender experiment with *E. coli* phage T_2.
- DNA entirely remains restricted to the chromosomes. It is one of the major component of chromosomes.

• STUDENT ACTIVITY

1. Describe any experiment which demonstrates that DNA is the genetic material.

• VERY SHORT ANSWER QUESTIONS

1. **Name the scientist who gave the phenomenon of genetic transformation.**
   Ans. Frederick Griffith (1928).
2. **Name the organism on which Griffith worked.**
   Ans. Pneumonia causing bacterium *Diplococcus pneumoniae.*
3. **Write the names of two strains developed when *D. pheumoniae* is grown on agar.**
   Ans. Virulent smooth (S) type with capsule and avirulent R type without capsule.
4. **What is Griffith effect or bacterial transformation ?**
   Ans. Griffith injected the mixture of living avirulent R II and heat-killed S III virulent in mice, pneumonia appeared and mortality took place in mice. Griffith showed in dead mice that their heart blood has both R II and S III pneumococci. He concluded that presence of S III bacteria might have transformed the living R II bacteria. R II bacteria have restored the capacity of capsule formation, which they had lost earlier by gene mutation. This was called **Griffith effect.**
5. **On which organism Blender performed the experiment for proving DNA as genetic material ?**
   Ans. He performed experiment on *E. coli* phage T_2.

UNIT

11

DNA REPLICATION

STRUCTURE

- DNA replication is autocatalytic function : It directs the synthesis of DNA itself.
- Watson and Crick's model for DNA replication
- DNA replication is semiconservative in *E. coli.* Semiconservative replication of DNA in eukaryotes.
- Semi-discontinuous DNA replication : Okazaki fragments.
- Unidirectional and Bidirectional DNA replication.
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- Replication and synthesis of DNA. Semiconservative DNA replication. Conservative DNA replication. Semidiscontinuous DNA replication. Unidirectional DNA replication. Enzymes of DNA replication.

• 11.1. REPLICATION AND SYNTHESIS OF DNA

The mechanism of DNA replication was proposed by **Watson** and **Crick.** According to Watson and Crick, DNA molecule is two-stranded and coiled like a rope

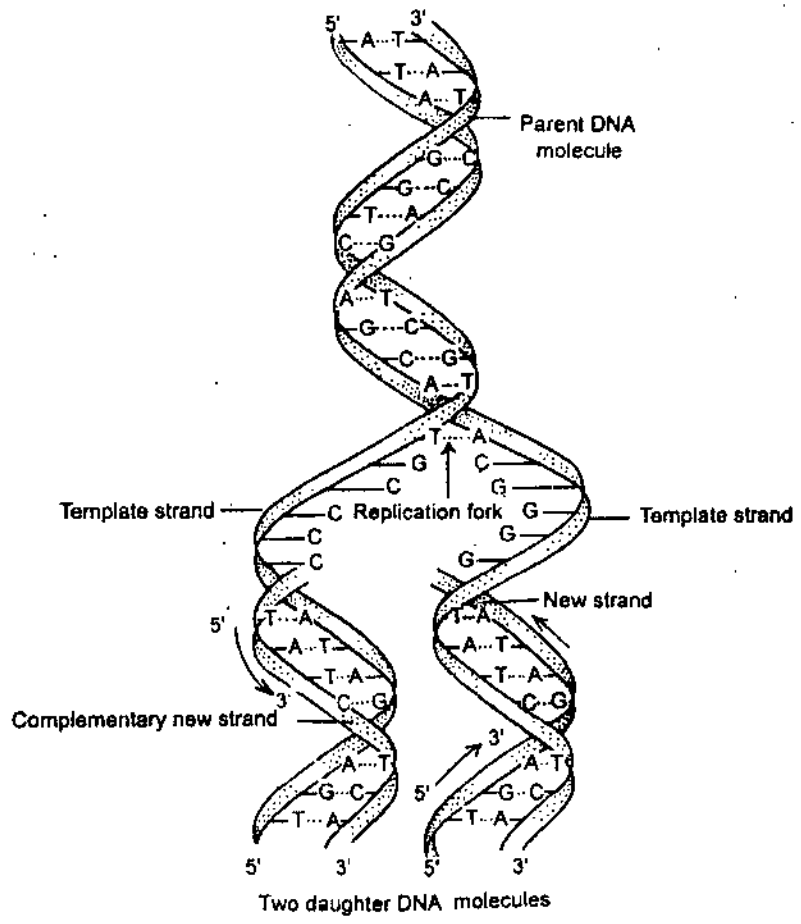


Fig. 1. Two daughter DNA molecules.

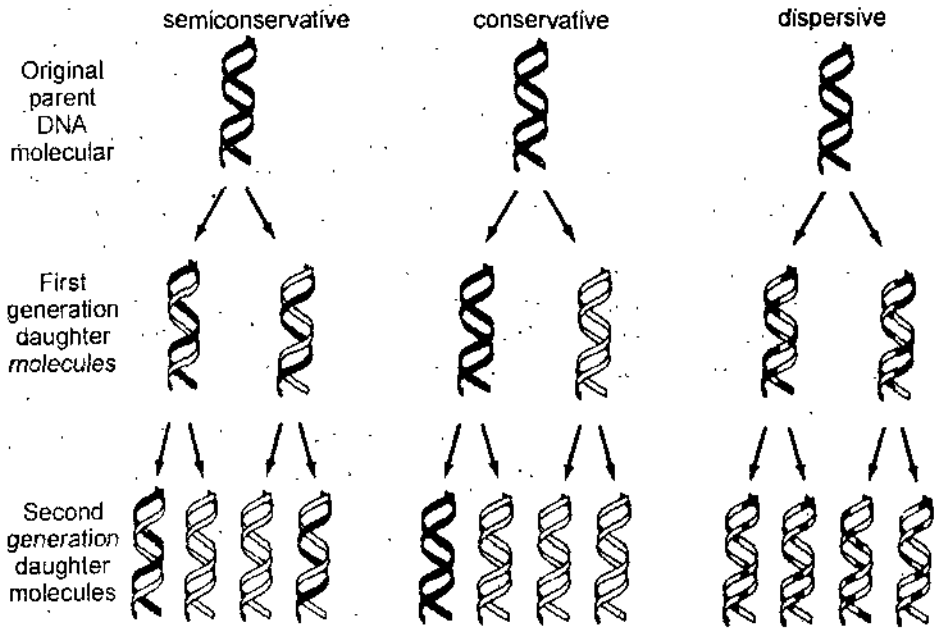


Fig. 2. Three modes of replication of DNA molecule, beginning with a double-stranded helix.

(plectonomic). The coiling is helical, in the fashion of circular staircase that always maintains the same diameter and the same width of the steps, with a connecting railing on either side. The railing or backbone of a strand is composed of the phosphate-sugar linkages, which are continuously repeated without change.

According to Watson and Crick, each single strand is a ***template*** or ***mold*** for its complement, and a new helix has one old strand and one that is newly synthesized. This type of replication is called **semiconservative.** In the conservative type of replication, two new strands are synthesized in the form of a double helix, while the old double helix remains unchanged.

• 11.2. WATSON AND CRICK'S MODEL FOR DNA REPLICATION

In a DNA molecule, the base pairing is specific, and the sequence of base along one chain automatically determines the base sequence along the other chain. Thus, each chain of the double helix serves as **template** for the synthesis of the other chain.

Watson and Crick proposed that in the replication of DNA the disruption of hydrogen bonds takes place and then rotation and separation of two polynucleotide strands occurs. Each purine and pyrimidine base of each polynucleotide strand attracts a complementary free nucleotide available for polymerization in cell and holds it in place by means of the specific hydrogen bonds. Once placed on the parent template chain, the nucleotides are sewn together by formation of phosphate diester bonds, which link adjacent deoxyribose residues forming a new polynucleotide molecule. In this way two double helical molecules are formed, which are identical (Fig. 2 and 4).

• 11.3. SEMICONSERVATIVE DNA REPLICATION IN *E. COLI*

According to Watson and Crick, as the DNA replication is started, the two original polynucleotide strands of the helix unwind locally and thus, each strand serves as a template for a new strand. Both duplexes resulted from replication should be **hybrid** in nature, *i.e.*, each contains one old strand derived from the original molecule and a new strand formed due to replication process. Since each of the two helices conserves one of the parent polynucleotide strands, the process is called **semiconservative** (Fig. 2 and 4).

Fig. 2 shows that two hybrid duplexes further replicate themselves. Four duplexes are thus, formed, two of which contain a single strand derived from the original chromosome and two of which contain totally new DNA strands.

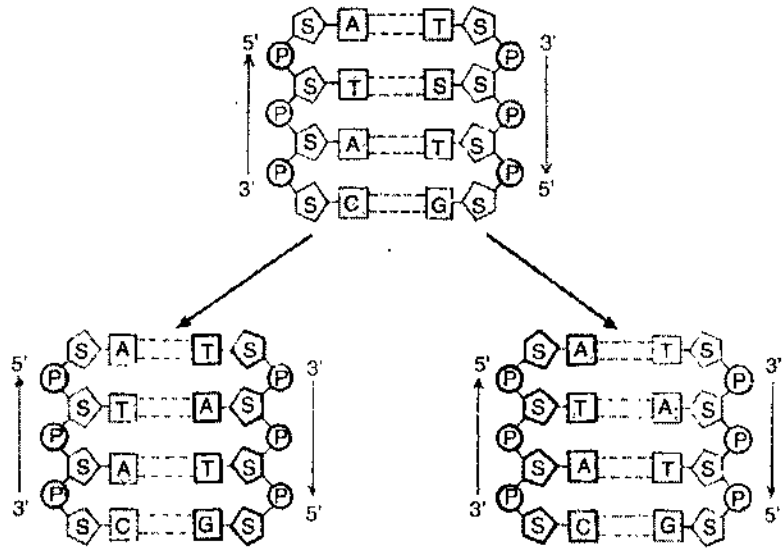


Fig. 3. Semiconservative model of DNA replication.

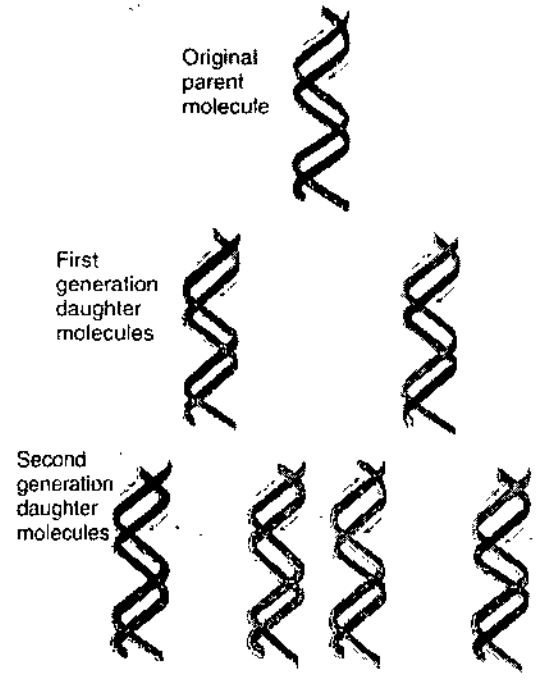


Fig. 4. Semiconservative model of DNA replication.

• 11.4. CONSERVATIVE DNA REPLICATION

In conservative method, the doubl- stranded molecule is conserved as such and the two new DNA molecules are synthesized from the old molecule.

Dispersive Replication

In this method the old DNA molecule disintegrates in the process of replication and two new molecules are synthesized. Intermixing of pieces of parent strands with newly synthesized pieces takes place. Thus, two new double helices are formed (Fig. 2).

Meselson and Stahl's Experiment Showing Semiconservative Method of DNA Replication :

M. Meselson and **F. W. Stahl** (1958) proved the semiconservative method of DNA replication. The technique used by Meselson and Stahl was that of *density-gradient equilibrium centrifugation.* They dissolved the DNA in a solution of **cesium chloride** and then rotated at high velocity in a centrifuge, thereby allowing the DNA to form a

band at its specific density on the gradient produced by cesium chloride molecules. They labelled the DNA of *Escherichia coli* bacteria with heavy nitrogen, ^{15}N, by growing them on an ^{15}N-conaining medium that was the sole source of nitrogen for many generations. The DNA extracts of such cells gave a characteristic ultra-violet light pattern showing a dark band near one end of the centrifuge tube. When these labelled ^{15}N cells were grown on nonlabelled ^{14}N media for one cell generation, the DNA was extracted and shown to consist of hybrid DNA double-helical molecules, each carrying both ^{14}N and ^{15}N at the same time, *i.e.*, DNA had not replicated conservatively to yield one parental ^{15}N double helix and one unlabelled ^{14}N newly synthesized progeny double helix.

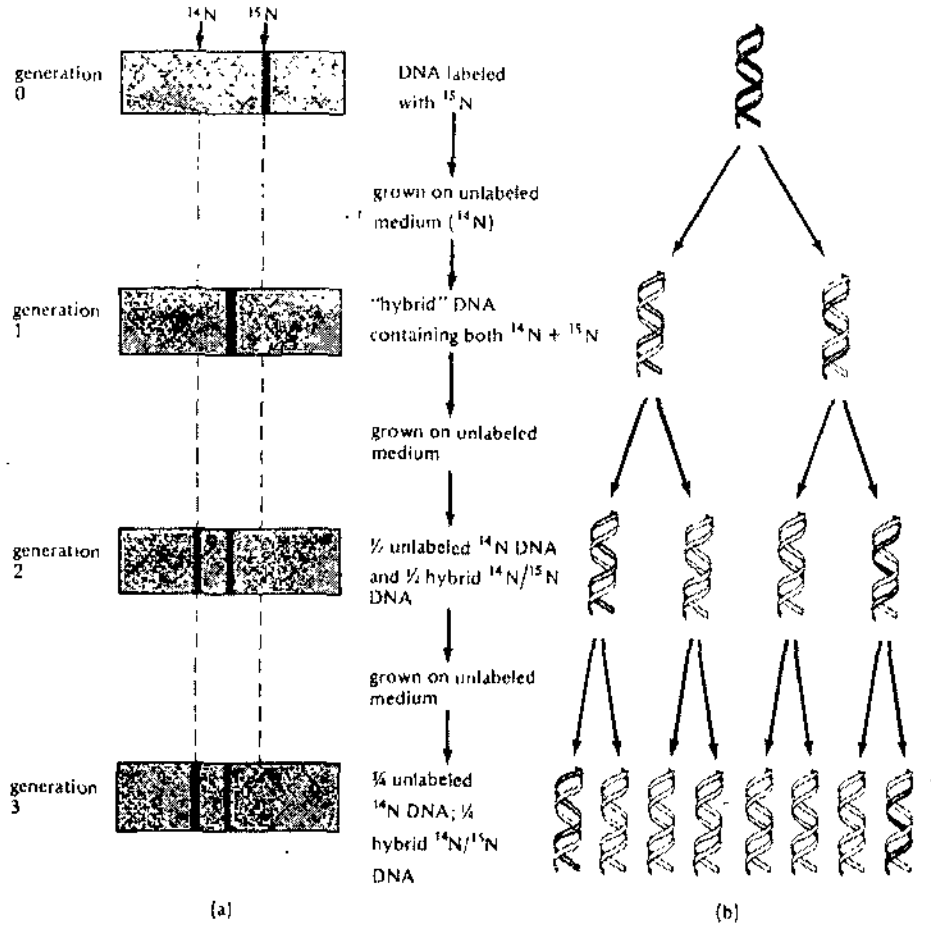


Fig. 5. Meselson and Stahl experiment. (Results left side and interpretation right state).

Since conservative replication was excluded, now they were to distinguish between semiconsrvative and dispersive replication. The cells were now allowed to grow on a ^{14}N medium for an additional cell generation. Analysis of the extracted DNA showed that unlabelled DNA was now formed in amounts equal to the partially labelled hybrid DNA. Furthermore, although some hybrid DNA still remained, additional generations of growth on unlabelled media gave a relative increase in the amount of unlabelled DNA. Since unlabelled DNA was always formed despite the presence of labelled hybrid DNA, duplication did not involve random dispersive labelling of all newly formed DNA, showing that the DNA must replicate semiconservatively.

Cairns Experiments with Radioactively labelled *E. coli.*

Cairns experiments with radioactively labelled *E. coli* chromosomes. *E. coli* chromosome is circular and is also double stranded structure. Cairns showed that the two circular component strands separate during replication and each strand duplicates individually. This duplication produces a **Y-type** of joint, while the remainder of the chromsome is still double-stranded indicating that unwinding of the two complimentary DNA strands is not completed before replication begins, but proceeds

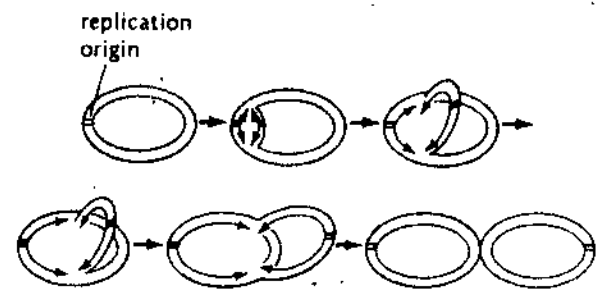


Fig. 6. An *E. coli* chromosome schematic model of its semiconservative mode of duplication.

simultaneously with the replication. These findings have led to the general acceptance of the semiconservative theory of DNA replication.

Biological Reactions

The synthesis of any substance needs the presence of the compounds, called **substrates** in cells that enter into the synthesis as well as source of energy for occurring that synthesis. In the cell the fuel for many reactions is **adenosine triphosphate** (ATP), which can release energy by losing one or two of its three phosphate groups. The cell also maintains a large complement of different enzymes. Each enzyme is specific for a particular kind of reaction or synthesis. Some enzymes aid in the removal of hydrogen atoms (**dehydrogenases**) and others aid in the addition of units to a multiunit structure, called **polymerases.** Thus, a cellular (*in vitro*, in life) reaction usually requires the presence of substrates, enzyme, ATP, and in many cases metallic ions, *e.g.*, magnesium, to activate the enzyme.

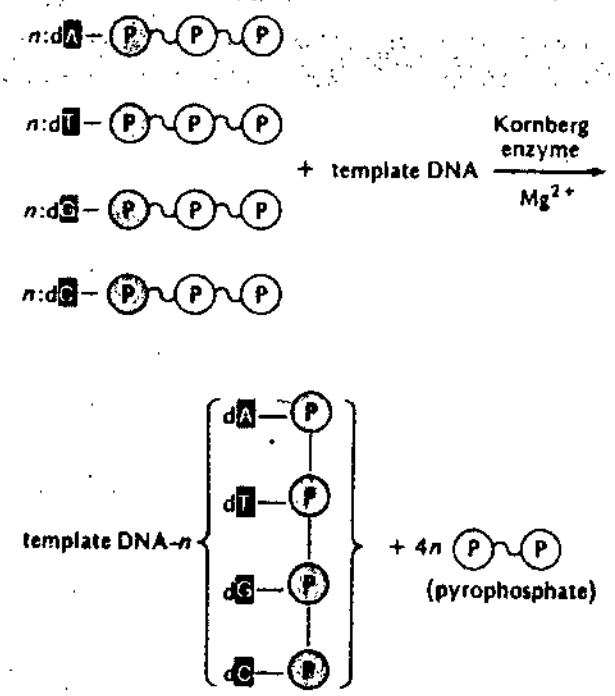


Fig. 7. Reaction system for the synthesis of DNA *in vitro* using multiple numbers (n) of the four different deoxyribonucleotide triphosphates and template DNA.

DNA Synthesis

In 1957 **Kornberg** and co-workers isolated a **polymerase** enzyme from *Escherichia coli* bacteria that could be used *in vitro* synthesis of DNA. The reaction mixture contained the polymerase enzyme (**polymerase** I), four different kinds of **deoxyribonucleotides** in the form of **triphosphates, magnesium ions** ($MgCl_2$), and the presence of some previously formed DNA, called **template DNA.** Once all necessary materials were present, DNA production took place 20 or more times greater than the amount of initial DNA (Fig. 7).

Further evidence by Kornberg and others indicated that the enzyme acts by hooking together the free added nucleotide units into a DNA strand. Using different phosphodiesterase enzymes that could break the phosphate-sugar ester bonds of the DNA chain, they showed that the new DNA molecule grown by the addition of nucleotides to the hydroxyl group at number 3 carbon (3′) position of the deoxyribose sugar at one end of a chain.

• 11.5. EVIDENCES FOR SEMI-CONSERVATIVE REPLICATION OF DNA OR CHROMOSOME IN EUKARYOTES

J. H. Taylor and **P. Woods** in 1957 used the technique of radiography and light microscopy in dividing root tip cells of bean, *Vicia faba* to provide evidence in support of semi-conservative method of DNA replication. After incorporation of tritiated thymidine, root tips were transferred to unlabelled culture medium. Colchicine was also added to the medium to prevent anaphase separation of sister chromatids. In the first generation of duplication both chromatids were labelled. (One DNA double helix in each chromatid and only one of the two strands labelled). In the second cycle of duplication, in the unlabelled medium, in each chromosome, one of the two strands was found to be labelled. This showed the semi-conservative method of replication.

Semi-discontinuous DNA Replication

Experimental evidences had also suggested that DNA synthesis is continuous on one strand, 3′ to 5′ strand, called **leading strand** and discontinuous on the other strand, *i.e.*, 5′ to 3′ strand, called **lagging strand.** Because DNA synthesis always preceeds in 5′ to 3′ direction, hence on the lagging strand, synthesis takes place dis-conti-

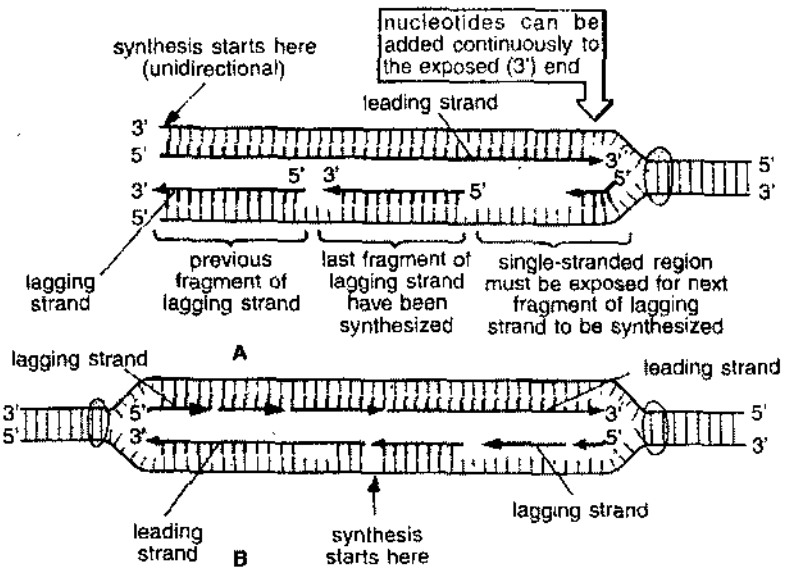


Fig. 8. A. Unidirectional DNA replication. B. Bidirectional DNA replication.

nuously in pieces, whcih are called **Okazaki fragments. R. Okazaki** in 1968 discovered this type of DNA synthesis. Later on, these pieces were used with the help of **ligase enzyme** to form an intact lagging strand. Such type of replication where leading strand is synthesized continuously and lagging strand is synthesized discontinuously *is, called* ***semi-discontinuous replication.***

Uni-directional Replication

In this type of DNA replication, DNA synthesis starts at a fixed point on the chromosome and proceeds in one direction, replication of mitochondrial DNA (Fig. 8).

Bi-directional Replication

In many cases, DNA replication is bidirectional, *i.e.*, proceeds in both directions from the origin of replication (Fig. 8).

• 11.6. ENZYMES OF DNA REPLICATION

There are three kinds of nuclear enzymes, which act on DNA. These are nuclease, polymerase and ligases.

1. Nuclease. Nuclease enzymes hydrolyze (break down) a poly-nucleotide chain into its component nucleotides. A polynucleotide is held together by 3′ and 5′ phosphodiester bonds. A nuclease enzyme attacks either 3′ or 5′ end of this linkage. Nuclease enzymes are of two kinds : exonuclease and endonuclease enzymes.

(i) Exonuclease enzymes attack on a free end of a polynucleotide. Exonuclease either attacks at a free 3′-OH end of a polynucleotide and progressively cleaves the bonds of the 3′-OH side of phosphodiester back bone or if attacks at a free 5′-P end and

digest the polynucleotide in a 5 → 3 direction. In both cases enzyme moves along the chain in stepwise manner liberating single nucleoside monophosphate molecules and finally digest the entire polymer.

(ii) Endonuclease also attacks one of the two sides of phosphodiester linkages, but they attack those bonds that are found wthin the interior of a polynucleotide chain. In case of viral DNA whose polynucleotide chain is single stranded, it cuts the chain into two pieces. In case of prokaryotic and eukaryotic DNA, a single cut by the endonuclease creates a nick in the helix. Helix remains in one piece, but it possesses a gap that contains two free ends, which can serve as substrates for exonucleases.

2. Polymerase or replicase enzyme catalyses the formation of a polymer. Polymerase enzymes bring about the synthesis of one polynucleotide chain that is a copy of another. A replicase enzyme brings about DNA replication. Polymerization always proceeds in a 5′→ 3′ direction and nucleotide at 3′ end is recently added to the chain. In *E. coli*, DNA polymerases are of three kinds : polymerase I and II are for DNA repair and polymerase III is for DNA replication. In eukaryotes, five types of polymerases are found : DNA polymerase alpha (α), polymerase beta (β), polymerase gamma (γ), polymerase delta (δ) and polymerase epsilon (ε).

3. DNA ligases catalyses, phosphodiester bond formation between free 3′-OH and free 5′-P groups of a notch (nick) of DNA created by endonuclease enzyme, thereby restoring an intact DNA duplex.

• SUMMARY

- Mechanism of DNA replication was given by Watson and Crick.
- DNA molecule is double stranded and each strand is a template (mold) for its complement.
- A new helix has one old strand and the other is newly synthesized. This type of replication is called semiconservative.
- Each chain of the double helix serves as a template for the synthesis of the other chain.
- In the replication of DNA disruption of hydrogen bonds takes place and then separation of two polynucleotide strands takes place. Two bases of each strand attract a complementary free nucleotide and hold it in place by means of hydrogen bonds, and then free nucleotides are sewen together by formation of phosphate digested bonds linking them together to form a new polynucleotide chain.
- Since each of the two helices conserves one of the parent polynucleotide strands, the process is called **semiconservative.**
- In **conservative method** of replication, new DNA molecule is synthesized from the old one.
- In **dispersive method** of DNA replication, old DNA molecule disintegrate and two new molecules are synthesized in which intermixing of pieces of parent strands takes place.

• STUDENT ACTIVITY

1. Describe in detail the DNA replication.

• VERY SHORT ANSWER QUESTIONS

1. **Who proposed the mechanism of DNA replication ?**
   Ans. Watson and Crick.
2. **What is the characteristic of a newly synthesized semiconservative DNA helix.**
   Ans. A new DNA helix has the old strand and the other is newly synthesized.
3. **Write about dispersive method of DNA replication.**
   Ans. Old DNA molecule breaks up into pieces and a new DNA molecule its formed after linking the pieces of old molecule.

4. **Write the name of scientist who proved semiconservative method of DNA replication.**

   Ans. M. Meselson and F.W. Stahl in 1958 proved the semiconservative method of DNA replication.

5. **What is the position of two strands before replication ?**

   Ans. Two strands must be in 3′-OH end before replication.

6. **Who performed experiment to prove semiconservative replication of DNA?**

   Ans. Meselson and Stahl used technique of density gradient equilibrium configuration to prove DNA replication by semiconservative method.

7. **In which direction does the growth of new strands proceed ?**

   Ans. In 5′ → 3′ direction.

8. **In which phase of division DNA replication occurs ?**

   Ans. In S-phase of interphase of cell division.

UNIT

12

SYNTHESIS OF RNA TRANSCRIPTION

STRUCTURE

- RNA Transcription
- Central dogma : Biosynthesis of proteins is under control of DNA and where DNA is absent, it is under control of genetic RNA.
- Protein synthesis is controlled by DNA while undergoing self-replication.
- It also controls synthesis of non-genetic RNA.
- RNA controls synthesis of specific proteins.
- Flow of information from DNA to mRNA — and then protein.
- **Transcription :** Transfer of genetic information from DNA to mRNA.
- In bacteria; a single RNA polymerase species undertakes synthesis of mRNA, rRNA and tRNA.
- In Eukaryotes three RNA polymerases take part in the synthesis of RNAs.
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :
- RNA Transcription, process of RNA synthesis, Trancription in Prokaryotes and Eukaryotes.

• 12.1. RNA TRANSCRIPTION

In 1960 an enzyme responsible for the transcription of DNA into RNA was isolated independently by **Weiss. Hurwitz** and **Stevens.** This enzyme **RNA polymerase** functions only in the presence of DNA as a template and hooks together ribonucleotides that have been added in the form of triphosphates (Fig. 1). In these reactions template DNA is not affected. It remains fully biologically active. The template DNA acts as the form upon which complimentary RNA is made or RNA is transcribed from DNA templates. Whether one or both strands in a double helix serve as a template for RNA transcription was addressed in an experiment by **Marmur** and his associates. They used bacteriphage SP8 in which the two strands in its DNA double helix differ from each other both in composition and in molecular weight. Heat treatment to break the hydrogen bonding between complimentary strands of this DNA produces two distinct kinds of strands that can be separated in a cesium chloride density gradient, one **light** and one **heavy.** When SP8 was permitted to infect its bacterial host, *B. subtilis and the* RNA produced by this infection labelled wth **tritiated uridine,** this RNA was found to hybridize only with **heavy** DNA strand of SP8 and never with the light one.

This experiment thus, demonstrated that RNA *in vivo* is copied only from one strand of DNA called the **sense strand.** However, transcription is not confined to only one of the two complimentary strands that run continuously through the entire chromosome. In many of the organisms, one of the two strands serves as the **sense strand** for transcription in some regions of the DNA double helix, and the complementary DNA strand serves this purpose in other regions very rarely and only in special regions of certain viruses, RNA transcribed from both paired DNA strands of a single region.

• 12.1. TRANSCRIPTION

Transcription is the transfer of genetic information from DNA to mRNA. **In bacteria,** synthesis of all RNAs (mRNA, rRNA and tRNA) takes place with the help of a single RNA polymerase species. **In eukaryotes,** three RNA polymerases take part in the synthesis of RNAs. **RNA polymerase I** or **A** is located in the nucleolus and takes part in the synthesis of rRNA. **RNA polymerase II or B** is present in nucleoplasm and it is responsible for the synthesis of HnRNA (heterogeneous nuclear RNA). It is precursor of mRNA in eukaryotes. **RNA polymerase III or C** is also found in the nucleoplasm. It takes part in the synthesis of 5SrRNA and tRNA etc. In eukaryotes, RNA polymerases are also found in mitochondria and plastids.

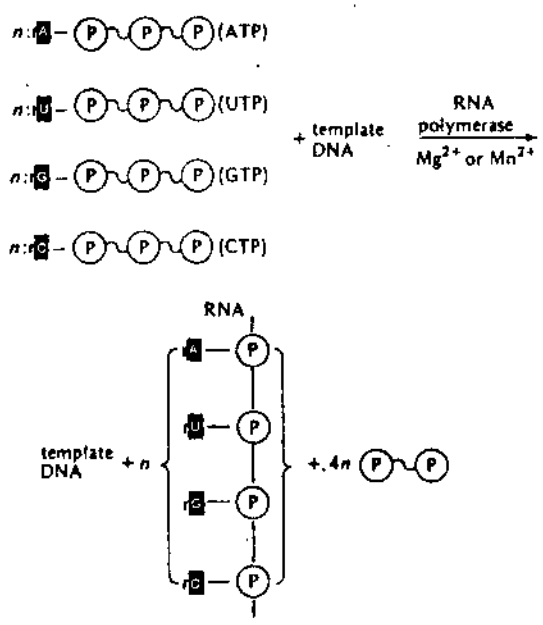


Fig. 1. Polymerization of four kinds of ribonucleotides into an RNA chain using RNA polymerase and DNA template.

RNA polymerase only functions in the presence of DNA as a template and hooks together ribonucleotides that have been added in the form of triphosphates. In these reactions the DNA template is not affected, but remains fully biologically active.

For example, when DNA from *D. pneumoniae* is used as template in such an RNA polymerase reaction, it can be extracted and has retained its full transforming ability.

In replication and transcription, chain elongation is similar, nucleotides are added one by one in 5′ → 3′ direction. In replication, both DNA strands are formed, while in transcription one particular gene or group of genes is transcribed at any one time, producing one to numerous number of copies. Some parts of DNA are never transcribed. In transcription, RNA strand is made up of ribonucleotides. Transcription needs no primer, while replication requires primer for copying both strands.

• 12.3. PROCESS OF RNA SYNTHESIS

1. Building blocks of RNA are four ribonucleotide 5-triphosphates. These are ATP, GTP, CTP and UTP. In polymerization, ribonucleotides are added one-by-one. The 3′–OH group of one nucleotide reacts with 5′–triphosphate of next nucleotide and pyrophosphate (PP) is released. In DNA replication, DNA polymerization is similar to that given above.

2. The sequence of bases of RNA is determined by the base sequence of DNA template strand. The base sequence of RNA is complementary to the template strand of DNA. Thymine is replaced by uracil in RNA.

```
3′  C  T  A  G  T  G  A  C  G  T  A   5′ Strand template
    |  |  |  |  |  |  |  |  |  |  |
5′  G  A  U  C  A  C  U  G  C  A  U   3′ RNA strand
```

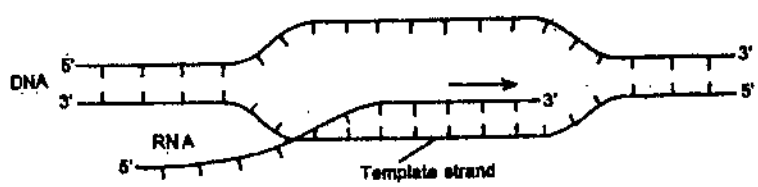


Fig. 2. Transcription of RNA from one strand of DNA template.

3. Only one strand out of two DNA strands acts as a template for RNA synthesis. The RNA formed is antiparallel to the DNA template.

4. RNA polymerase (enzyme) which synthesizes RNA strand does not need a primer and can initiate transcription *de novo*.

RNA synthesis has four stages :

(i) Binding of RNA polymerase to the template at a promoter site on DNA.

(ii) Initiation of new strand.

(iii) Elongation

(iv) Termination and release

• 12.4. TRANSCRIPTION IN PROKARYOTES

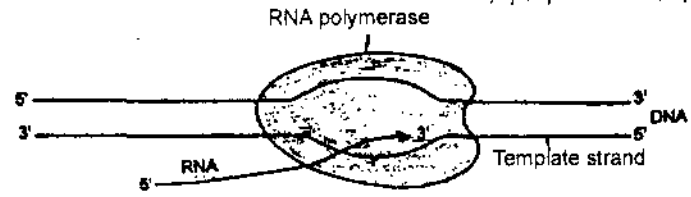
Length of mRNA chain depends upon the length of polypeptide chain for which it codes. The mRNA in prokaryotes is generally polycistronic. which codes for several proteins, which represent proteins of a single metabolic pathway.

RNA Polymerase Enzyme

RNA polymerase enzyme requires a DNA template. It requires four ribonucleotide-triphosphates, which are ATP, GTP, UTP and CTP. Each nucleotide in the newly formed RNA strand is selected on the basis of Watson-Crick base pairing rule.

In prokaryotes all types of RNAs are transcribed by the same RNA polymerase. When it binds DNA, it covers many bases of DNA simultaneously.

RNA polymerase consists of subunits — 2α subunits, β, β' and σ, (sigma factor).



Complete enzyme is called **holoenzyme.** The enzyme without sigma factor is called **core enzyme.**

Promoter : There is a definite sequence of bases on DNA, called **promoter** to which RNA polymerase binds. RNA polymerase recognizes this promoter and then locally unwinds the DNA strands in order to gain access to the bases, which are to be copied. In prokaryotes, the promoter is a sequence of six bases which is generally TATAAT or its slight variant. This sequence is called **Pribnow box.** It lies before the first base to be transcribed and is also called **10 region.** This region is called **upstream region** and is denoted by a minus sign (–).

Pribnow box orients DNA polymerase in such a way that transcription proceeds in 5′, → 3′ direction. On the left of pribnow are present several promoters, which is a – 35 squence. It is another six base sequence TTGACA. RNA polymerase enzyme covers – 35 sequence, pribnow box and transcriptional site.

The *open-promoter* complex is a highly stable complex and is the active intermediate in chain initiation. In this complex a local unwinding (melting) of the DNA helix occurs starting about ten base pairs from the left end of the pribnow box and extending to the end of the position of the first transcribed base. This melting is necessary for pairing of the incoming ribonucleotides. The base composition of the sequence of pribnow box makes the DNA strand open to denaturation. RNA polymerase induces this conformational change.

Once an open-promoter complex is formed, RNA polymerase is ready to initiate RNA synthesis. RNA polymerase contains two nucleotide binding sites, called **initiation site,** and the **elongation site.** Initiation site binds only purine triphosphates, namely ATP and CTP; and one of these, usually ATP, is the first nucleotide in the growing RNA chain. Thus, the first DNA base transcribed is usually thymine (T). The initiation nucleoside triphosphate binds to the enzyme in the open-promoter complex and forms a

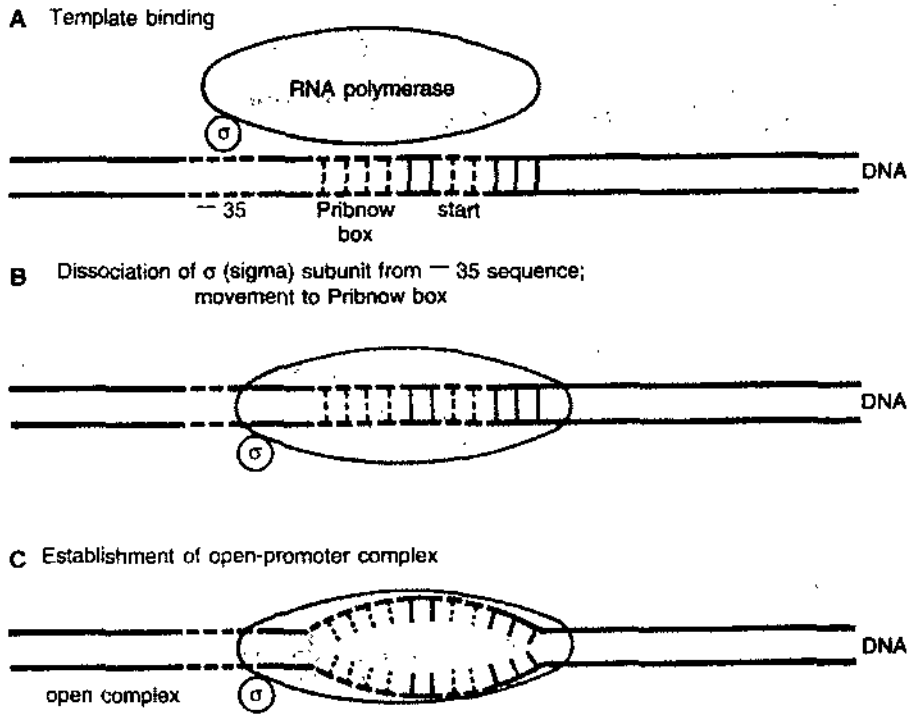


Fig. 3. A model for the binding of RNA polymerase to a promoter to form an open-promoter complex. PB = Pribnow box.

hydrogen bond and the complementary DNA base. The elongation site is then filled with a nucleoside triphosphate, which is selected strictly by its ability to form a hydrogen bond with the next base in the DNA strand. The two nucleotides are then joined together, the first base is released from the initiation site, and initiation is completed. The dinucleotide remains hydrogen bonded to the DNA. The elongation phase begins when the **polymerase** releases the base and then moves along the DNA chain.

I. RNA polymerase binds to promoter, slides into place, and forms an open complex. ATP in initiation site binds to T on coding strand.

II. A NTP is added to elongation site and is covalently linked to the A.

III. RNA polymerase moves to the next DNA base. The initiating dinucleotide is released. A NTP enters the elongation site and is covalenty linked to the dinucleotide. Then movement of RNA polymerase continues.

Fig. 4. Method of initiation of transcription.

Elongation : After initiation, elongation of RNA chain occurs. New ribonucleotides are added one by one, it is called **polymerization.** During elongation, RNA polymerase copies DNA sequence accurately, this is called **processivity.** Elongation of RNA proceeds only in 5′ → 3′ direction like DNA replication.

During elongation, the RNA strand base pairs temporarily with DNA template to form a short RNA-DNA hybrid double helix. Later RNA separates and two strands of DNA again form duplex structure. DNA strands unwinds over a short distance to enable RNA polyemrase to synthesize RNA strand complementary to one of DNA strands.

A wave of unwinding is generated by RNA polymerase. In this way DNA is unwound ahead and rewound behind as RNA is transcribed. RNA polymerase also performs proof reading functions as transcription makes some mistakes, one mistake in about 1000 nucleotides added.

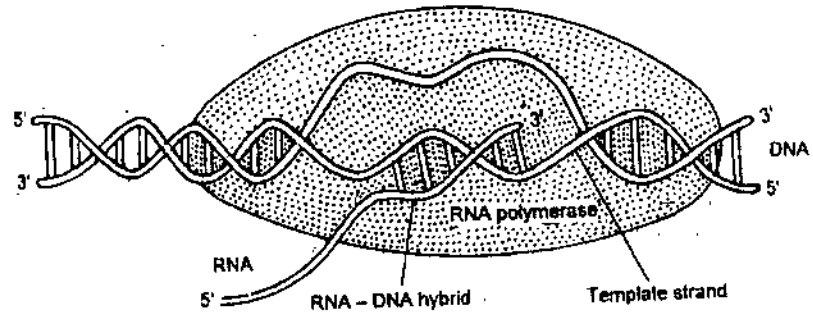


Fig. 5. Direction of transcription of RNA.

Termination and Release : Termination of RNA synthesis occurs at specific bases sequences in the DNA molecule. Twenty termination sequences have so far been determined. After RNA synthesis is completed, RNA polymerase reaches a stop signal or termination factor on DNA. In *E. coli* there are two types of termination signals, Rho independent and Rho-dependent.

Rho-independent termination is also called **intrinsic termination.** Near 3′ end of newly synthesized RNA, there is a sequence of inverted repeats of 20 nucleotides long, which form a self complementary hairpin or stem structure. At the end of this hairpin structure there is a sequence of uracil bases, which are transcribed from adenine bases on the template strand. This A-U hybrid region has weakest bands and is therefore unstable and leads to the termination and dissociation of newly synthesized RNA molecule. This is self-terminating and depends upon DNA base sequence.

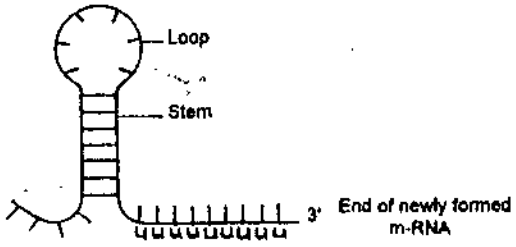


Fig. 6. Inverted repeats forming stem loop structure and terminal U-sequence at 3′-terminus of-mRNA molecule.

Rho-dependent termination is dependent on a protein factor, called **Rho factor**. Here Rho-protein has an ATP-dependent RNA-DNA helicase activity that causes release of newly synthesized RNA molecule.

In prokaryotes, transcription and translation go on simultaneously, because they have no nuclear membrane. As synthesis of RNA is in progress, ribosomes bind to the free 5′ end of mRNA and start protein synthesis (translation). This is called coupled transcription and translation. Transcription, translation and degradation of mRNA occur in 5′ → 3′ direction.

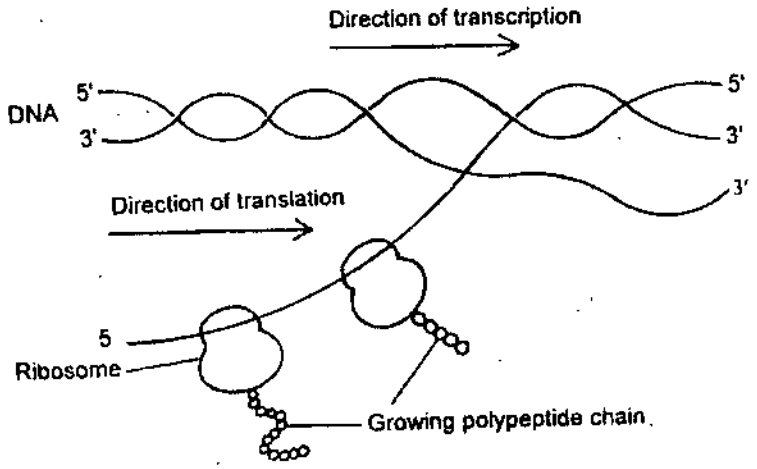


Fig. 7. Coupled transcription and translation in prokaryotes.

• TRANSCRIPTION IN EUKARYOTES

The basic features of transcription and structure of mRNA in eukaryotes are similar to those of bacteria. But there are certain differences between prokaryotes and eukaryotes. In eukaryotes, the mRNAs are generally monocistronic as compared to polycistronic mRNA of prokaryotes.

RNA Polymerase of Eukaryotes : There are three kinds of RNA polymerase in eukaryotes. They are RNA polymerase 1, RNA polymerase II and RNA polymerase III.

RNA polymerase I transcribes only rRNAs *i.e.,* 5.8S, 18S and 28S, rRNAs. The RNA polymerase II transcribes all mRNAs. The RNA polymerase III transcribes tRNA and 5S rRNA.

Promoters for RNA Polymerase II : The promoters for RNA polymerase II in eukaryotes are more complex. One promoter consists of a sequence of bases which lie – 25 bases upstream of transcription start site. It consists of a sequence of seven bases TATAAAT called **TATA box.** It is also called **Hogness box.** It can be compared to Pribnow box of prokaryotes. Only TATA box is present in almost all the eukaryotes. In many cases another sequence is also present which lies – 75 base region, called **CAAT box.** It has a sequence GGTCAATCT. Transcription start site lies in the initiator sequence where DNA is unwound.

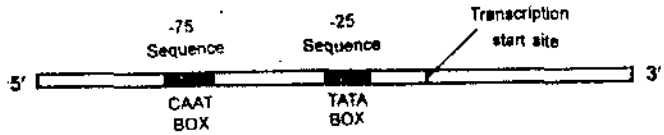


Fig. 8. Promoters in eukaryotes.

Various transcription factors bind to TATA box and other sequences. RNA polymerase does not bind to these promoter sequences directly, but to these factors as eukaryotic DNA is in the form of chromatin.

Post Synthesis Processing of RNA : Newly synthesized RNA is called **primary transcript** or **precursor RNA** (pre-RNA). These RNA molecules undergo extensive changes, called processing to form mature RNA molecules which take part in protein synthesis. The most complex and characteristic feature of pre-mRNA is the presence of non-coding regions called **introns** present in between coding regions, called **exons.** These non-coding regions, or **introns,** are removed and discarded before the protein synthesis can take place. They are removed by a process, called **splicing.** This is essential to get a correct sequence of amino acids of a polypeptide.

Almost all kinds of RNA molecules undergo post synthesis processing. Prokaryotic *mRNA is generally not processed. Eukaryotic mRNA undergoes maximum processing.* Both prokaryotic and eukaryotic tRNAs and rRNAs undergo extensive processing.

2006 Nobel Prize : Roger Kornberg of America won Nobel Prize for Chemistry for describing the molecular basis of transcription in eukaryotic cells, how the information in the genes is copied and transferred.

His father Arthur Kornberg had won Nobel Prize in medicine in 1959 also for genetic work.

• SUMMARY

- RNA is synthesized from a portion of one strand of DNA which acts as a template.
- One gene or group of genes are transcribed at any one time producing one to many numerous copies.
- *Polymerization is similar in replication and transcription.*
- Ribonucleotides of RNA are ATP, GTP, CTP and UTP.
- Thymine of DNA is replaced by uracil in RNA.
- RNA strand formed is complementary and antiparallel to DNA template strand.
- Transcription does not require primer.
- In prokaryotes a single RNA polymerase synthesizes all types of RNAs.

- There is a definite sequence of bases on DNA called promoter on which DNA polymerase binds.
- In prokaryotes a sequence of six bases called pribnow box lies 5 to 10 bases before the transcriptional start site. Another sequence called – 35 sequence may be present.
- RNA polymerase binds these promoter sites and unwinds the DNA molecule for copying the portion of DNA template.
- In bacteria two types of termination signals-Rho-independent and Rho-dependent are present.
- In eukaryotes basic steps of transcription are similar to prokaryotes. In eukaryotes three kinds of RNA polymerase are present.
- RNA polymerase I transcribes ony rRNAs (5.8S, 18S and 28S rRNAs).
- RNA polymerase II transcribes all mRNAs.
- RNA polymerase III transcribes tRNA and 5S rRNA.
- Promoters for RNA polymerase II are – 25 sequence called TATA box and – 25 sequence called CAAT box.

• STUDENT ACTIVITY

1. Write down about transcription and process of RNA synthesis.

2. Discuss the process of RNA transcription in prokaryotes.

3. Write down about RNA transcription in Eukaryotes.

• VERY SHORT ANSWER QUESTIONS

1. **Define transcription.**
   Ans. Transfer of genetic information from DNA to mRNA is called transcription.

2. **How many RNA polymerases take part in transcription of RNA in prokaryotes and eukaryotes ?**

   Ans. One RNA polymerase in prokaryotes and three RNA polymerases in eukaryotes.

3. **How may building blocks of RNA are present ?**

   Ans. Four – ATP, GTP, CTP and UTP.

4. **In which direction is RNA strand synthesized ?**

   Ans. In 5′ → 3′ direction.

5. **As compared to DNA template strand RNA strand is copied in which direction ?**

   Ans. In 3′ → 5′ direction.

6. **Write the name of promoter on DNA formed of six bases.**

   Ans. Pribnow that is formed of six bases TATAAT.

7. **What is TATA box ?**

   Ans. TATA box has a sequence of seven bases TATAAAT. This is promoters for RNA polymerase II. There is nother sequence lying – 75 base region in eukaryotes called CAAT box. It has a sequence GGTCAATCT.

UNIT

13

SYNTHESIS OF PROTEINS : TRANSLATION

STRUCTURE

- Translation involves three processes : initiation, elongation and termination.
- **Initiation :** Binding of a specific initiator methionyl tRNA and mRNA to small unit of ribosome.
- In prokaryotes, binding of three initiation factors to 28S ribosomal subunit.
  - Summary
  - Student Activity
  - Test Yourself

LEARNING OBJECTIVES

After going through this unit you will learn :

- The synthesis of proteins and the process of translation. Process of translation and Elongation

• 13.1. TRANSLATION

Activation of amino acids : First event is the selection of the correct amino acid by **aminoacyl-tRNA synthase** for incorporation into an **aminoacyl-tRNA.** The second event occurs in ribosome and involves the selection of correct tRNA in response to an mRNA codon.

For each amino acid in cytoplasm there is a specific **aminoacyl-tRNA synthetase** that activates the carboxyl group of that amino acid for covalent binding with adenylic acid residue of the **CCA** end of the tRNA. The first step of this reaction requires ATP, which reacts with free amino acids producing **aminoacyl adenylate** and **pyrophosphate.**

$$\underset{\text{(amino acid)}}{\text{AA}} + \text{ATP} \xleftrightarrow[\text{synthetase}]{\text{Aminoacyl}} \underset{\substack{\text{(AA~ AMP-amino}\\ \text{acyl adenosine}\\ \text{monophosphate)}}}{\text{AA} \sim \text{AMP}} + \underset{\text{(Pyrophosphate)}}{\text{PP}}$$

Transfer of amino acid to tRNA : The **AA~AMP** intermediate, thus formed remains bound to the enzyme until it collides with the proper tRNA and thus, the formation of **AA~tRNA** complex occurs :

$$\text{AA} \sim \text{AMP} + \text{tRNA} \xleftrightarrow[\text{synthetase}]{\text{Aminoacyl}} \text{AA} \sim \text{tRNA} + \text{AMP}$$

To perform these two functions, the activating enzyme has two sites : one to recognize the amino acid and another to recognize the specific tRNA.

The bond between amino acid and **CCA** end of tRNA is a high-energy one which stores the energy from the ATP used in the first reaction. The energy, thus stored is used in the ribosome to derive the formation of the peptide bond.

The **aminoacyl adenosine monophosphate complex** (AA~AMP) remains bound to the enzyme with the liberation of **pyrophosphate** (PP).

Process of Translation

Translation is divided into three stages : **initiation, elongation** and **termination.**

Initiaion : In both prokaryotes and eukaryotes, in initiation binding of a specific initiator **methionyl tRNA** and **mRNA** to the small ribosomal subunit occurs. The large ribosomal subunit then joins the complex, forming a functional ribosome on which elongation of polypeptide chain proceeds.

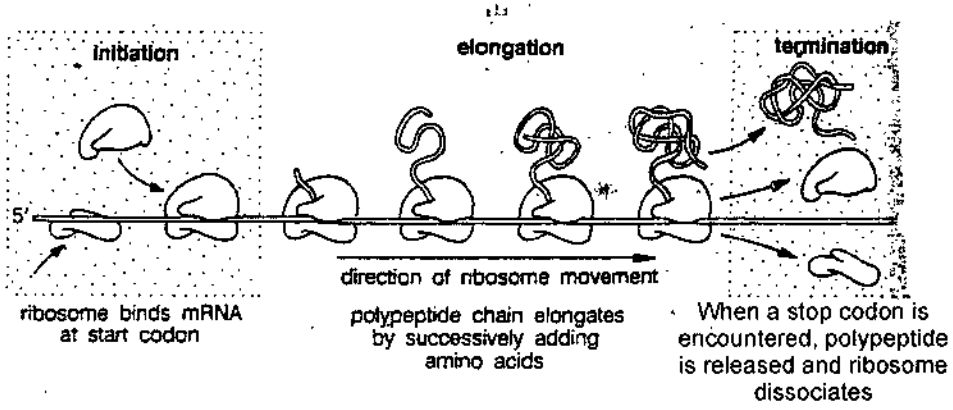


Fig. 1. Process of translation.

In bacteria (prokaryotes), the first translation step is binding of three initiation factors (IF-I, IF-2 and IF-3) to 30S ribosomal subunit. The mRNA and initiator N-formylmethionyl tRNA then join the complex, with IF-2 (which is bound to GTP) specifically recognizing the initiator tRNA. IF-3 is then released, allowing a 50S ribosomal subunit to associate with the complex. This association triggers the hydrolysis of GTP bound to IF-2, which leads to release IF-1 and IF-2 (bound to GDP). Its result is the formation of a 70S initiation complex (with mRNA and initiator tRNA bound to ribosome) that is ready to begin **peptide formation** during elongation state of translation.

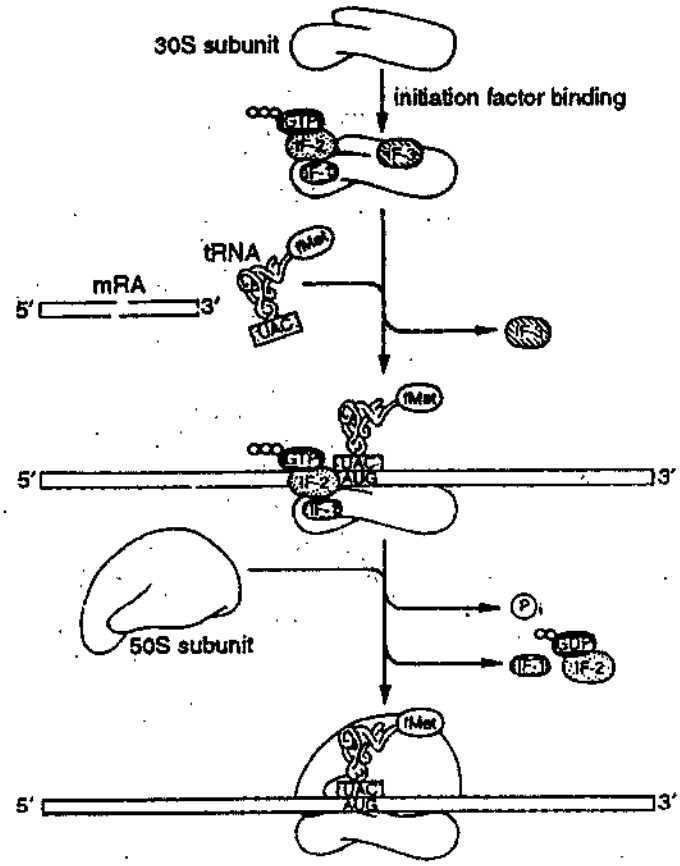


Fig. 2. Initiation of translation in bacteria.

In eukaryotes, initiation is more complicated and requires atleast ten proteins (each consisting of multiple polypeptide chains), which are designated elFs (eukaryotic initiation factors). The factors elF-I, elF-1A and 3lF-3 bind to 40S ribosomal subunit and elf-2 (in a complex with GTP) associates with the initiator methionyl tRNA.

The mRNA is recognized and brought to ribosome by elF-4 group of factors. The 5′ cap of mRNA is recognized by elF-4E. Another factor, elF-G, binds to both elF-4E and to a protein (poly-A binding protein or PABP) associated with the poly-A tail at 3′ end of

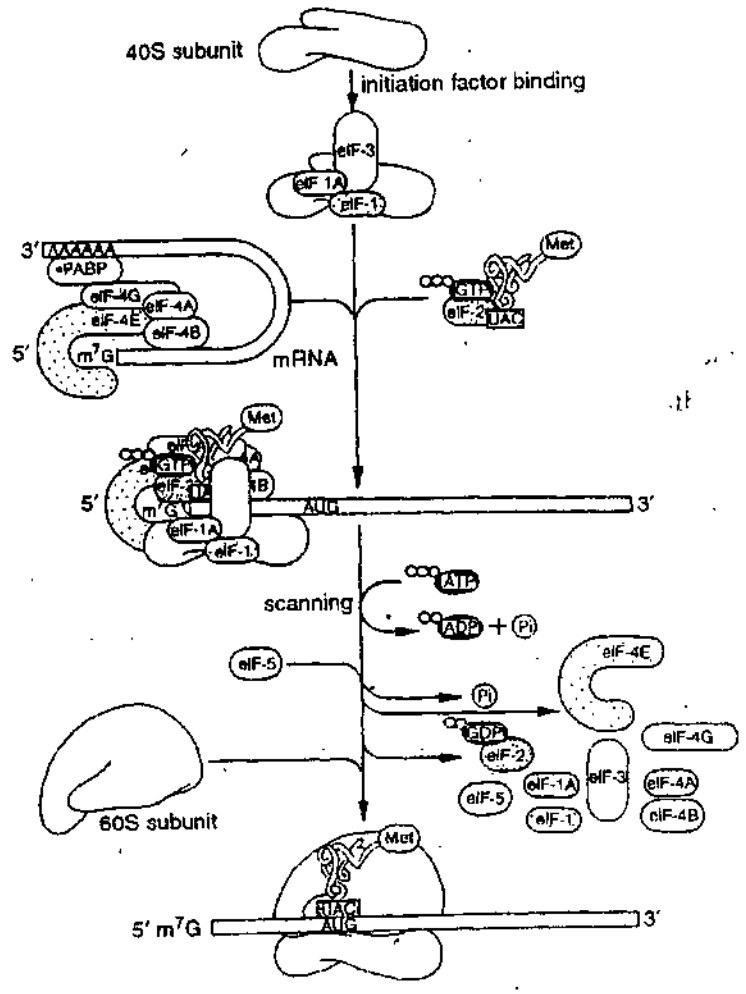


Fig. 3. Initiation of translation in eukaryotic cells.

mRNA. Thus, eukaryotic initiation factors recognize both the 5′ and 3′ end of mRNAs. This accounts for the stimulatory effect of polyadenylation on translation. The initiation factors elF-4E and elF-4G, in association with elF-4A and elF-4B, then bring the mRNA to 40S ribosomal subunit with elF-4G interacting with elF-3. The 40S ribosomal subunit, in association with bound mehionyl tRNA and elFs then scans mRNA to identify **AUG initiation codon.** When AUG codon is reached elF-5 triggers the hydrolysis of GTP bound to elF-2. Initiation factors (including elF-2 bound to GDP) are then released, and a 60S subunit binds to 40S subunit to form 80S initiation complex of eukaryotic cells.

After initiation complex is formed, translaion proceeds by elongation of polypeptide chain. The mechanism of elongation in prokaryotes and eukaryotes is very similar. The ribosome has three sites for tRNA binding designate the **P (peptidyl), A (aminoacyl)** and **E (exit)** sites. The initiator methionyl tRNA is bound at the **P** site.

• 13.2. ELONGATION

The first step in elongation is the binding of the next aminoacyl tRNA to the **A site** by pairing with the second codon of mRNA. The aminoacyl tRNA is carried to the ribosome by an **elongation factor** (EF-Tu in prokaryotes, eEF-1α in eukaryotes) which is complexed to GTP. The GTP is hydrolyzed to GDP as the correct aminoacyl tRNA is inserted into the **A** site of the ribosome and elongation factor bound to GTP is released.

Once EF-Tu or eEF-1α has left the ribosome, a peptide bond can be formed between the initiator methionyl tRNA at the **P site** and the second aminoacyl tRNA at the **A site.** this reaction is catalyzed by the large ribosomal subunit, with the rRNA playing a critical role. The result is the transfer of methionine to the aminoacyl tRNA at **A site** of

the ribosome, forming a **peptidyl tRNA** at this position and leaving the uncharged initiator tRNA at the **P site.**

The next step in elongation is **translocation,** which requires another elongation factor (**EF-G in prokaryotes,** and **eEF-2 in eukaryotes**) and is again coupled to GTP hydrolysis. During translocation, the ribosome moves three nucleotides along the mRNA, positioning the next codon in an empty **A site.** This step translocates the peptidyl tRNA from **A site** to the **P site**, and the uncharged tRNA from **P site** to **E site.** The ribosome is then left with a peptidyl tRNA bound at P site and an empty A site. The binding of a new aminoacyl tRNA to the **A site** then induces the release of the uncharged tRNA from **E site,** leaving the ribosome ready for insertion of the next amino acid in the growing polypeptide chain.

As elongation continues, EF-Tu or eEF-1α that is released from the ribosome bound to GDP must be reconverted to its GTP form. This conversion needs a third elongation facor, **EF-Ts** (eEF-1βγ in eukaryotes), which binds to EF-Tu/GDP complex and promotes the exchange of bound GDP for GTP. This exchange results in the regeneration of EF-Tu/GTP, which is now ready to carry a new aminoacyl tRNA to the **A site** of the ribosome, beginning a new cycle of elongation.

Elongation of the polypeptide chain continues until a **stop codon** (UAA, UAG or UGA) is translocated into the **A site** of the ribosome. Cells do not contain tRNAs with anticodons complementary to these termination signals. Instead they have **release factors** that recognize the signals and terminate protein synthesis. **Prokaryotic cells** possess **two release factors** that recognize termination codons : **RF-I** recognizes UAA or UAG, and **RF-2** recognizes UAA or UGA.

In **eukaryotic cells** a single release factor (**eRF-I**) recognizes all three termination codons. Both prokaryotic and eukaryotic cells also contain relase factors (RF-3 and eRF-3, respectively) that do not recognize specific termination codons, but act together with RF-I (or eRF-1) and RF-2. The release factors bind to termination codon at the **A** site and stimulate hydrolysis of the bond between the tRNA and the polypeptide chain at the **P** site, resulting in release of the completed polypeptide from the ribosome. The RNA is then released, and the ribosomal subunits and the mRNA template dissociate.

Messenger RNAs can be translated simultaneiously by several ribosomes in both prokaryotic and eukaryotic cells. Once one ribosome has moved away fromthe initiation site, another can bind to the mRNA and begin synthesis of a new polypeptide chain.

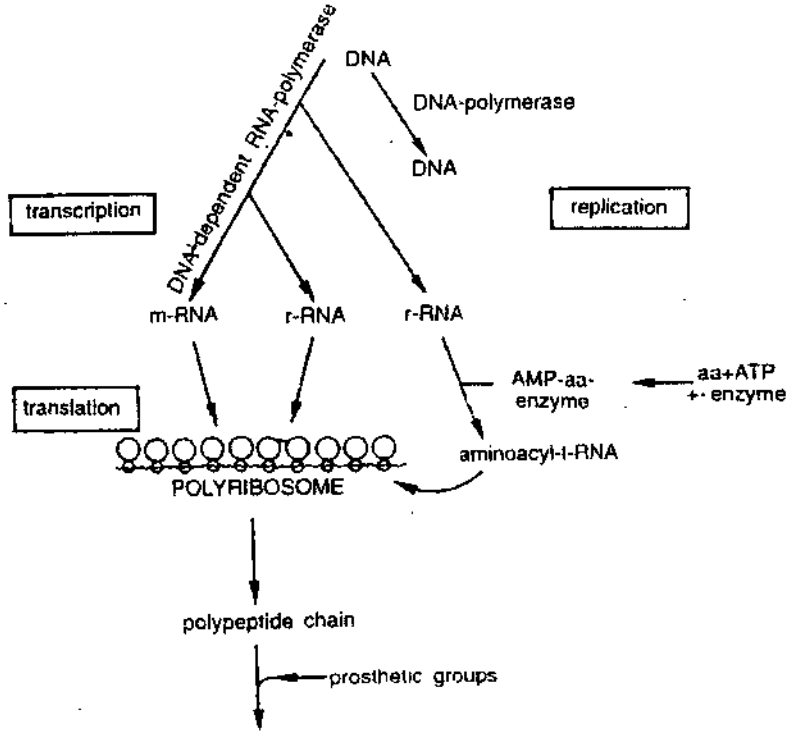


Fig. 4. Overall scheme of DNA, RNA and protein synthesis.

Thus, mRNAs are usually translated by a series of ribosomes, spaced at intervals of about 100 to 200 nucleotides. The group of ribosomes bound to an mRNA molecule is called a **polyribosome** or **polysome.** Each ribosome within the group functions independently to synthesize a separate polypeptide chain.

Table : Protein factors in *E. coli* protein synthesis

Phase	Factor	Function
1. Initiation	IF_1	Dissociation of ribosomal subunits.
	IF_1, IF_2, IF_3 } GTP	Binding of mRNA and initiator tRNA to 30S subunit.
2. Elongation	Tu, Ts } T + GTP	Binding of aminoacyl tRNA. Tu-GTP complex to ribosome.
	Peptidyl transferase	Peptidyl transfer from peptidyl-tRNA to aminoacyl-tRNA
	G + GTP	Translocation of peptidyl-tRNA; release of free tRNA
3. Termination	R_1, R_2	Release of protein at UAA, UAG or UGA codons.

• SUMMARY

- Initiation, elongation and termination are three stages of translation. In prokaryotes, RNA transcription and translation (protein synthesis) take place in the cytoplasm because nucleus is absent.
- In eukaryotes, RNA synthesis (transcription) takes place in the nucleus and translation takes place in the cytoplasm.
- mRNA synthesized in the nucleus is transported to cytoplasm through nucleopores.
- Amino acids are attached to tRNA before formation of polypeptides. tRNA has three nucleotides long codon which recognizes 3 nucleotide long codon on mRNA.
- Ribosome is ribonucleoprotein particle having many proteins and enzymes needed for protein synthesis.
- Ribosome brings together a single mRNA molecule, and tRNA with amino acids in a proper orientaion so that base sequence of mRNA is translated into amino acid sequence of polypeptides.
- In *E. coli* 30S + 50 S $\underset{\text{Low Mg. concentration}}{\overset{\text{High Mg. concentration}}{\rightleftharpoons}}$ 70S ribosome. Direction of translation – A protein mole has – NH_2 end and – COOH end. Synthesis begins at – NH_2 and ends at COOH end. mRNA is translated in 5′ → 3′ direction from amino to carboxyl end.
- **Initiation :** 30S subunit, mRNA and charged tRNA combine to from **pre-initiation complex.** It needs 3 initiation factors IF1, IF2 and IF3 along with GTP. then 50S ribosome subunit joins 30S to form 70S initiation complex.
- Protein coding region on mRNA is called **open reading frame** which has a start codon on 5′–AUG–3′ and a stop codon in the end.
- **Polycistronic RNA** contains many open reading frames and thus encode multiple polypeptides in prokaryotes.
- Near 5′ end of mRNA lies start codon AUG (rarely GUG) in prokaryotes and eukaryotes.
- Ribosome binding site in prokaryotes lies near 5′ end of mRNA upstream of AUG codon.
- Between 5′ end AUG codon is a sequence of 20-30 bases. Of these there is a sequence 5′–AGGAGGU–3′. This sequence is called **Shine-Dalgarno sequence.** It lies-4 to 7 bases upstream of AUG codon. It is the ribosome binding site. There are two tRNA binding sites on ribosome covering 30 and 50 subunits. First is called **P site** and second is **A site** (aminoacyl site). Initiator tRNA enters **P-site** and other tRNAs enter **A-site.**

- Every amino acid has a separate tRNA. In prokaryotes first amino acid starting protein is formyl methionine (f Met). AUG start codon on mRNA is incorporated with f Met.
- tRNA carrying formyl methionine is called $tRNA^{fMet}$. First initiator charged aminoacyl tRNA is fMet-tRNA f^{Met}.
- fMet-tRA^{fMet} occupies p site on ribosome.
- **Activation of amino acid :** $\underset{\text{amino acids}}{AA} + ATP \xrightarrow{\text{AA-RNA synthase}} \underset{\text{enzyme complex}}{\xleftarrow{(AA \sim AMP)}} \text{enzyme complex} + PP.$
- **Transfer of amino acids to tRNA :** (AA ~ AMP) enzyme complex + tRNA ⟶ AA – tRNA + AMP + enzyme.
- AA-tRNA is fMet-$tRNA^{fMet}$ (amino acid formyl methionine bound to tRNA). It fixes to P site on ribosome . After this second AA–tRNA attaches itself to A site on ribosome. In this way polypeptide chain elongates.
- **Elongation of polypeptide chain :** It needs elongation factors-, Tu and G.
- EF-TU forms complex with AA_2 – tRNA + GTP complex and brings it to A site of ribosome. Now GTP hydrolysed to GDP and EF – TU is released from ribosome.
- EF-TU-GTP complex is regenerated with the help of another factor Ts.
- Peptide bond is formed between free – COOH of first amino acid and free – NH_2 of second amino acid at A-site with the help of an enzyme peptidyl transferase. Now tRNA at P-site becomes uncharged and tRNA at A-site carries a small protein chain of two amino acids occurring in 50S subunit.
- **Translocation :** The peptidyl-tRNA carrying two amino acids present at A site is now translocated to P site. This is called translocation. EF-G factor controls translocation. Factor G is called translocase. Translocation involves movement of ribosome along mRNA towards its 3′ end from first to second codon. This movement shifts peptidyl tRNA from A to P site.
- E site (exit site) is present on 50S ribosome. At each step of movement a new amino acid is added to the polypeptide chain. After each elongation, ribosome moves by one codon in 5′ → 3′ direction.
- **Chain termination :** Termination codon (stop codon) on mRNA causes polypeptide chain to be terminated. Stop codons are UAA, UGA and UAG. RF1, RF2 and RF3 factors help in termination of polypeptide chain.
- **In eukaryotes** initiation amino acid is methionine.
- tRNA binds methionine to any of Shine-Dalgarno sequence in mRNA to function as ribosome binding site.
- Between 5′ end and AUG codon of mRNA is a sequence of bases called cap.
- There are ten initiation factors–eIF. Two elongation factors eEF1 and eEF2. One release factor eRF.

• STUDENT ACTIVITY

1. Describe translation in prokaryotes.

2. Write down the differences in translation in prokaryotes and eukaryotes.

• VERY SHORT ANSWER QUESTIONS

1. **Where does translation take place in prokaryotes ?**
   Ans. Since nucleus is absent, it occurs in the cytoplasm.
2. **In eukaryotes, where translation occur ?**
   Ans. Within the cytoplasm but transcription occurs within the nucleus.
3. **Write the location of synthesis of mRNA in eukaryotes.**
   Ans. It takes place in the nucleus.
4. **In which direction does protein synthesis take place ?**
   Ans. In 5′ → 3′ direction.
5. **What is Shine Dalgarno site ?**
   Ans. This is a sequence of bases (AGGAGGU) present upstream of AUG codon on mRNA.
6. **Write the first amino acid of polypepide chain in prokaryotes.**
   Ans. Formyl methionine.
7. **Where was first AA-tRNA carrying methionine bind on ribosome.**
   Ans. At P site of ribosome.
8. **What activates the amino acid in the begining of translocation ?**
   Ans. ATP : ATP reacts wih free amino acids producing aminoacyl adenylate and pyrophosphate.
9. **Write about initiation process of translation.**
   Ans. Initiator methionyl tRNA and mRNA bind to small ribosomal unit.
10. **Write what are the three initiation factors in bacteria ?**
    Ans. IF1, IF2 and IF3 bind to 30S ribosomal subunit.