



Block

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INTRODUCTION TO CELL BIOLOGY

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CELL BIOLOGY

Cell Biology is one of the core courses in Life Sciences being offered as an optional for undergraduate students at Indira Gandhi National Open University. While preparing this course, we have kept in mind that students joining our B.Sc. programme must have studied science subjects, i.e. Physics, Chemistry and Biology upto 10+2 level.

In recent years Cell Biology has made remarkable advances that have contributed to understanding of all biological and medical sciences.

In this course, the structure of procaryotic and eucaryotic cells, molecular and supramolecular organisation of cell components, chemical composition and organisation of the cells, cellular metabolism, bioenergetics etc. have been described. The entire course is divided into four blocks, each block containing five units.

Block 1 explains how cells were first formed from the simple molecules and what was the outcome of division and growth of pre-existing cells. It is considered that their potential for diversity of size, shape and function has allowed evolution to proceed in many directions. A wide range of tools and techniques used to study cells are also explained in this block. With the help of radioactive isotopes, antibodies and tissue culture techniques it was found that architecture of a cell is very complex. A short account of chemical structure of the lipids, proteins and nucleic acids will be helpful to understand the structure-function correlation in the next blocks (Blocks 2 and 3).

Cells are able to maintain their internal environment by keeping the normal constituents constant (homeostasis). The structure of cell membrane enclosing the cell, serves as a selective barrier for various transport processes, e.g. diffusion, osmosis etc. is explained in Block 2. The structure, specificity and regulation of enzyme activity which catalyses the biochemical reaction necessary for the maintenance of life are also described in this block.

The enzymatically mediated metabolic pathways by which large molecules of food are broken down or built from precursors are described in Block 3. This block also deals with the synthesis of proteins, which, though appears simple, is a very complex mechanism. For the synthesis of proteins, DNA, the hereditary material in the cells, is transcribed into RNA which is further translated into proteins (DNA → RNA → protein). It will be interesting to know how different amino acids are picked up and correctly arranged by t-RNA on a ribosome to form a polypeptide chain, which is crucial for the synthesis and function of proteins.

Block 4 explains the mechanisms by which cells communicate with each other. You will also study various types of differentiated plant and animal cells, and how the cells aggregate to form different tissues in this block. Processes of cell division, i.e. mitosis and meiosis in plant and animal cells which are of fundamental importance for growth and development are also described here.

Broad Objectives

The broad objectives of this course are to enable you to:

- describe the origin of cells, evolution of eucaryotes from procaryotes and their distinguishing characters,
- explain the principles of various tools and techniques involved in the study of the cell's ultrastructure and chemical composition,
- describe the supramolecular organisation of the cell and various membrane transport processes,
- explain the specificity and mechanism of action of enzymes, their regulation and control,
- describe pathways of the breakdown of metabolism viz. glycolysis, gluconeogenesis etc.,
- describe the transcriptional and translational processes of the cell and their differences,
- distinguish different phases of cell cycle, mitosis and meiosis,
- distinguish the salient features of cell division in plants and compare it with those found in animals,
- list the general classification of differentiated plant and animal cell types and special features of plant cells in comparison with animal cells.

BLOCK 1 INTRODUCTION TO CELL BIOLOGY

The first block of this Course is a brief description of the evolution of cell, i.e., how cells evolved during the passage of time from small precursor molecules to their present state. A short account of various tools and techniques used for studying the cell, the ultrastructure of cell, its chemical composition and the structure of macromolecules is also given. The block contains five units.

The first unit deals with the evolution of the present day cell from its components which are the building blocks for larger structures and then goes on to describe how this unit of life has become a building block for all living organisms. With the invention of microscope, it became possible to observe cells of plants and animals. By the 19th century, the cell theory became an accepted fact for understanding the organisation of living systems. Modern cell biology makes use of the methods and principles from diverse fields like biotechnology, biochemistry, biophysics and molecular genetics. This gives an interdisciplinary approach to the study of forms and functions at the cellular level.

A wide range of microscopic techniques available for studying cells are dealt with in the second unit. Living cells can be seen by using phase-contrast light microscope, while fixed dead cells can be studied under an unaided light microscope after staining with various dyes. The transmission electron microscope (TEM), (with much higher resolving power as compared to light microscope) shows the arrangement of cell membranes which can be visualised by freeze-fracture electron microscopy, while the scanning electron microscope (SEM) reveals the cell surface in three dimensions. Radioisotopes and antibodies are tools for detecting and localising specific molecules in the cell. Cell culture and cinemicrography are also described in this unit.

The third unit deals with the structural organisation of the cell, distinction between procaryotic and eucaryotic cells and also between plant and animal cells. The procaryotic cells are small and simple, consisting of ill-defined nucleus, while eucaryotic cells are characterised by the presence of well defined nucleus and many membranous compartments. The cytoplasm around the nucleus includes a number of organelles with specific structure and function: endoplasmic reticulum, mitochondria, Golgi complex, ribosomes, centriole, microfilaments and microtubules. Chloroplast and rigid cell wall, the important characteristics of plant cell, are absent in animal cells.

A proper understanding of the cell is possible if we understand the correlation between the structure i.e., the chemical composition of the various cell organelles and their functions. Unit 4 gives an account of the structure of simple organic molecules like sugars, amino acids, nucleotides and fatty acids. Common methods used for determining the concentration of macromolecules as well as methods for their isolation are described in this unit.

The last unit of the block describes the formation of macromolecules viz, proteins, nucleic acids, carbohydrates and lipids from simple molecules, such as amino acids, nucleotides, simple sugars and fatty acids. Various types of chemical bonds, which are grouped under covalent and non-covalent bonds are involved in the formation of macromolecules. Different levels of structural organisation of a protein, i.e., primary, secondary, tertiary and quaternary which finally lead to the formation of the natural shape of protein molecules are described. Elucidation of the structure and composition of DNA and its differences with RNA was a significant step towards the understanding of genetic material.

Objectives

After reading this block you will be able to:

- explain the evolution of the cell from simple molecules to the first cell, from procaryotes to eucaryotes and distinguish between a procaryote and a eucaryote, between a plant cell and an animal cell,
- explain the principle, working and use of light and electron microscopes, use of radioisotopes and antibodies for localising specific molecules in a cell and suggest the uses of cell culture and cinemicrography,

- identify the main organelles, viz. nucleus, endoplasmic reticulum, lysosome and Golgi apparatus in a eucaryotic cell in light and electron micrographs and describe their structure and function,
- describe methods of separating macromolecules on the basis of size, charge and solubility as well as monitoring of the purification processes,
- distinguish the primary, secondary, tertiary and quaternary structure of a protein and differentiate between DNA and RNA.

Study Guide

Before you begin the study of this block, we would like to advise you how best to study it. The first unit of this block deals mainly with the evolution of the cell and it also tells about structure of cell organelles, macromolecules and a few cytochemical techniques. These concepts are elaborated in the following units of this block i.e. Units 2, 3, 4 and 5. So we suggest that if you find a certain concept difficult, proceed to Unit 2, 3 and 4 etc. for further clarification. (We have given references of further units in Unit 1 wherever appropriate).

We have given figures and tables in our units to further clarify and concretise the concepts. Some remarks are given in the margin (i.e. the space left on the sides of the pages) for some more involved ideas and terms that you must have studied upto 10+2 level. These also reinforce the concepts given in the text.

We have also given many self-assessment questions (SAQs) and terminal questions in each unit to help you to grasp the ideas better. SAQs have been placed within the text after each major concept. The terminal questions are given at the end of each unit. Space is provided for answering SAQs and terminal questions. The answers of SAQs and terminal questions are given at the end of each unit. But you should solve the questions first and look for the answers later otherwise the purpose of placement of SAQs and terminal questions will be defeated.

We have explained difficult terms in the glossary given at the end of the block. While studying the unit, you may find some words difficult, for which you must consult a dictionary. Finally, if you are interested to know more about some aspects, you may refer to the books given as reference material. These books will be available in the library at your Study Centre.

UNIT 1 INTRODUCTION TO THE CELL

Structure

- 1.1 Introduction
 - Objectives
- 1.2 History of Cell Biology
- 1.3 Evolution of the Cell
 - From Molecules to the First Cell
 - Prokaryotic Structure and Diversity
 - Evolution of Photosynthesis and Aerobic Respiration
- 1.4 Prokaryotes to Eukaryotes
 - Eukaryotic Cell Organelles
 - Mitochondria and Chloroplast
 - Animal and Plant Cells
- 1.5 Summary
- 1.6 Terminal Questions
- 1.7 Answers

1.1 INTRODUCTION

This is the first unit of the block “Introduction to Cell Biology”. In this unit you will study about the evolution of the cell that involves two processes:

- i) Occurrence of genetic variations which are passed on from one generation to another; and
- ii) Selection of those genetic changes which are useful for the survival and propagation of the organism.

Discovery of the cell as a structural unit of life was possible with the development of the microscope. It is interesting to learn as to how the present day cell evolved from smaller components and then became a building block for larger structures. You will study that molecules which were capable of self-replication and carrying genetic information were evolved first. These molecules then got enclosed in a membrane, to give rise to the first ancestral cell which could propagate its own self. The development of mechanisms for photosynthesis and respiration in the early cells led to the evolution of prokaryotes (e.g. bacteria) first and then to eukaryotes (higher animals and plants). Although the basic structure of plant and animal cells is similar, they differ in certain aspects such as the absence of large vacuoles and cell wall in animal cell. You will study about the differences between plant and animal cells in this unit.

It would be easier to understand this unit if you know the basic principles of biochemistry.

Objectives

After studying this unit, you will be able to:

- outline the stages in the evolution of the cell from simple molecules,
- list the distinctive features of organelles of prokaryotic and eukaryotic cell,
- compare a plant cell with an animal cell,
- state the importance of chloroplast and mitochondria.

1.2 HISTORY OF CELL BIOLOGY

Scientific knowledge grows with the development of new tools and techniques for studying various physical and biological processes. This is true also of the origin of the concept of cell as a basic unit of life. By 1800 A.D., good microscopes were developed and consequently plants and animals were found to be made up of small units, later known as ‘cells’. However, it was only in 1838, that the cell theory was proposed. According to this theory, the cell is both the structural and the functional unit of all living organisms. Cell theory gave a great impetus to study of the cell. It was established that the cell is a mass of

protoplasm limited by a cell membrane and possessing a nucleus. Another important observation was that cells come only from pre-existing cells as a result of cell division. Details of cell division were soon worked out. It became apparent that mitosis is the formation of nuclear filaments (chromosomes) and their equal division between the daughter cells. It was also observed that organisms generally develop from the fusion of two sex cells, the spermatozoon (male sex cell) and ovum (female sex cell). In 1892, cytology (cyto = cell, logos = study) was established as a separate branch of biology. Study of cells with electron microscope gave a new dimension to the study of cell. Detailed morphological studies of the cell helped to relate the structure with function of cellular organelles.

Interaction between various branches of scientific enquiry leads to their overall growth. The interaction between cytology and genetics on one hand and between physiology and biochemistry on the other gave rise to a more comprehensive discipline, Cell Biology, which embodies all that should be known about the cell (Fig. 1.1).

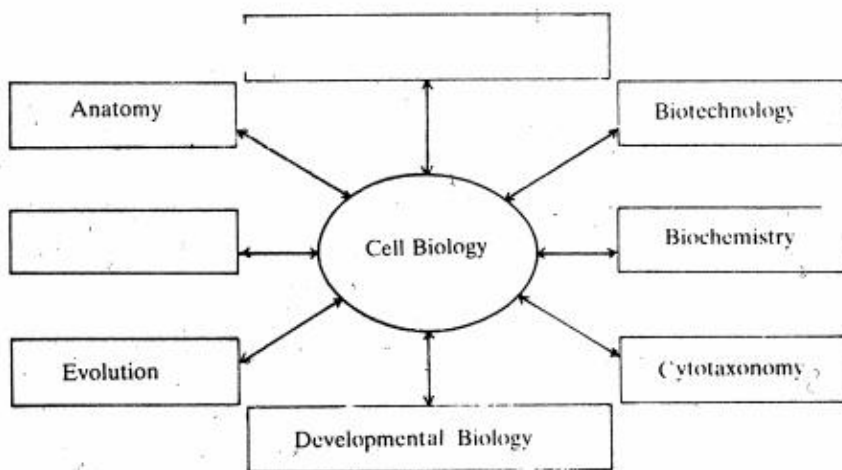


Fig. 1.1: Relationship between Cell Biology and other biological disciplines.

For example, when in 1865 Mendel put forward the fundamental laws of heredity, the cytological changes that occur during meiosis resulting in the formation of sex cells, were not known. Hence the distribution of factors, later on called as 'genes', could not be understood as proposed by Mendel. With the understanding of the processes that take place in meiosis, the importance of Mendel's work became evident. Consequently, the **chromosome theory of heredity** was finally established and genes were assigned definite places called **loci**, on the chromosomes. Later, sex determination was related to some special chromosomes. A biochemical analysis of various components of cell made a great impact on the understanding of the cellular function. Although the nucleic acids were isolated in 1869, their basic role in heredity and protein synthesis was realised only in 1950 and even later. Cell organelles like mitochondria were isolated by employing certain techniques about which you will study in detail in Unit 4. The role of mitochondria in cellular respiration was also established.

X-ray diffraction and several other physico-chemical methods helped scientists to understand the structure and function of the macromolecules in cell. Further studies on the cell revealed that cell is both a morphological and a physiological unit of the life.

SAQ 1

Fill in the blanks.

- The invention of can be considered as a landmark in the study of cell biology.
- Cell theory states that.....
- New cells arise by
- is the type of cell division that results in the equal distribution of chromosomes in daughter cells.

1.3 EVOLUTION OF THE CELL

All the living organisms are made up of cells. A living cell may be defined as a compartment enclosed by a membrane that consists of several chemical constituents and is capable of propagating itself by division. Activity of an organism is dependent on the activities of its cells, individually and collectively. Basic structures and functions are common in all cells with certain differences according to their specific needs.

As you have already studied in Unit 12 of the Foundation Course in Science and Technology, there are two stages in evolution of the cell; **chemical evolution** and **biological evolution**. Chemical evolution started with the formation of organic molecules on the earth which had no life until then. We call it **prebiotic earth**. Some of these early molecules developed the ability of producing an exact copy of their ownself by self-replication. We can mark this event as the origin of life. Later on, during the course of biological evolution, molecular aggregates organised and formed the cell which was different from the cell as it exists today. We shall trace these events which are based partly on speculation and partly on experimental evidence.

1.3.1 From Molecules to the First Cell

You may recall from Unit 12 of Foundation Course in Science and Technology that the prebiotic conditions on the earth in all probability were volcanic eruptions, lightning and heavy rains. There was no trace of free oxygen or an ozone layer in the atmosphere to absorb ultraviolet radiations from the sun. It is assumed that organic molecules would have originated in such climatic conditions. This is described as their abiotic origin. Laboratory experiments from 1953 onwards prove that organic molecules like amino acids, proteins, nucleotides, nucleic acids, lipids and ATP can be formed in similar conditions on the earth. Stanley Miller's experiment described below is one such experiment (Fig. 1.2). It is possible that such organic molecules similar to those synthesised in Miller's experiment would have got accumulated at several places in high concentration, giving rise to a kind of liquid soup, the prebiotic soup. A spherical pre-life form or **protobiont** may have been formed in such a soup.

Protobiont a spherical globule filled with large organic molecules, carrying out the functions of a living being, that ultimately evolved into a true life form, i.e. eubiont.

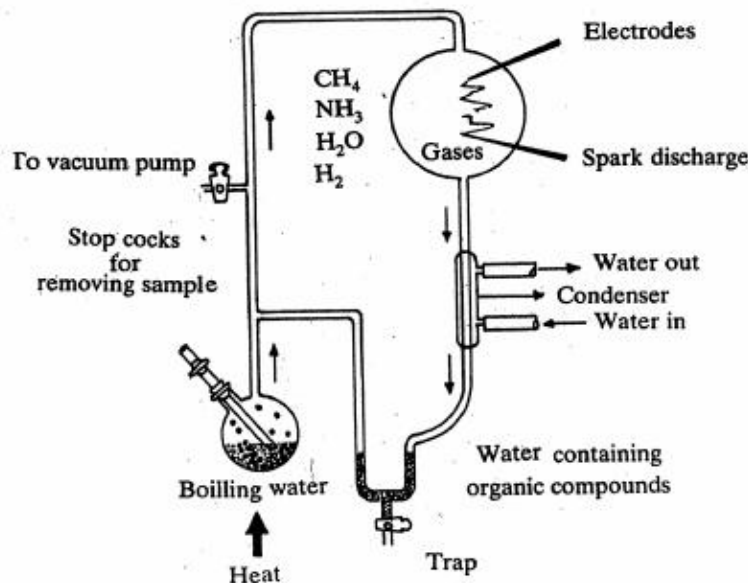


Fig. 1.2 : Apparatus used by Stanley Miller in experiments on the synthesis of organic compounds from abiotic components. In the flask, water is added and the entire apparatus is made free of air. Then hydrogen, methane and ammonia gases are introduced through a valve. When water in the flask is heated to boiling these gases circulate clockwise in the steam. Gases and steam enter the sparking chamber and are subjected to spark discharges. When the molecules move through the vicinity of water cooled condenser, condensation occurs. Gaseous substances continue to circulate in the apparatus until the experiment is concluded, which takes usually a week or so. Non-gaseous substances accumulate in the trap, and are analysed.

Scientists have three different speculations about the composition of a **protobiont**. Some consider it as an aggregate of electrically charged molecules with water around them acting as a boundary and this is called as **coacervate model** (Fig. 1.3). This model was proposed

Polymerisation : The process of linking together of many simple units (e.g. amino acids) to form larger and more complex molecules (e.g. proteins) is known as polymerisation and the product is called as polymer.

Coacervate is a fluid filled droplet formed in dilute aqueous solutions, whose boundary develops as a film of bound water molecules.

by Alexander Oparin. Sidney Fox proposed another model for protobiont formation called **protenoid microsphere model**. According to this model when water free mixtures of amino acids are heated at high temperature, they polymerise to form protein-like molecules, called **protenoids**. These can persist only if cooled quickly. According to a third model called as the **lipid bilayer model** of Richard Goldacre, an abiotically synthesised phospholipid bilayer was formed around a central space containing organic molecules. All such models are without any experimental or theoretical evidence. However, the supporters of each model try to explain how some life-processes like osmosis and budding, can be carried out by such **protobionts**.

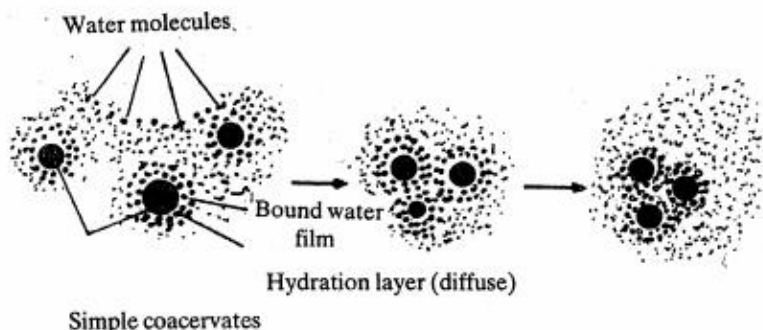


Fig. 1.3 : Coacervate model of protobiont. Water molecules become tightly bound to an electrically charged molecule to form simple coacervate droplets. Attractions between the electrically charged systems may lead to the fusion of two or more simple coacervates to form complex coacervate.

The origin of a true life form, **eubiont** from a protobiont involves the acquisition of three major features:

- 1) assembly of phospholipids and proteins into a cell membrane forms the boundary of the cell,
- 2) development of protein-directed metabolic pathways to utilise the organic molecules for energy needs, and
- 3) formation of nucleic acid molecules that are not only capable of self-replication but are also capable of storing genetic information for the synthesis of proteins.

Since we have no definite idea of the structure of a protobiont, it is difficult to speculate the manner in which a eubiont evolved from a protobiont. All the same, it is certain that a eubiont comparable to the simplest living cell today must have originated first.

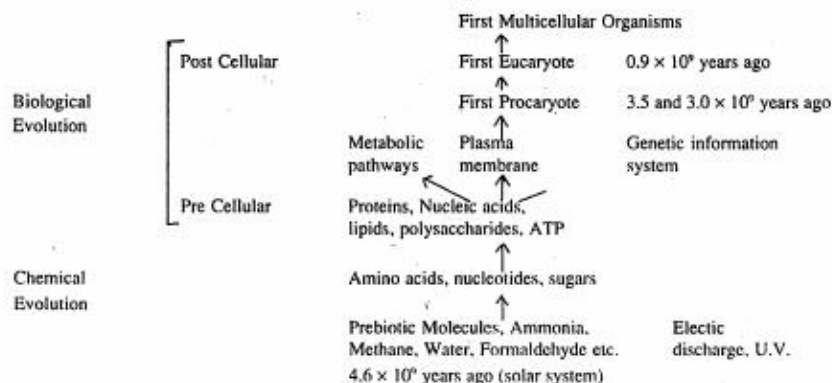
Genetic Information : Information related with the ways in which characteristics are passed on from parents to offspring.

Metabolic Pathways : Processes by which food is built up into living matter or by which living matter is broken down into simple substances.

It is quite likely that the formation of a boundary membrane would have taken place before the acquisition of a genetic information system and metabolic pathways. A separation of the cell interior from the outside, would permit sufficient interaction of molecules inside the cell.

Let us sum up in a tabular form what we have learnt so far regarding the evolution of cell.

Table 1.1: The Origin of the Cell



SAQ 2

- a) Which stage of evolution is characterised by the organisation molecular aggregates.
- b) Give the name of three protobiont models.

1.3.2 Procaryotic Structure and Diversity

The first organisms which did not have well defined nucleus, called procaryotes, (pro = earlier, caryon = nucleus) were probably **anaerobic** and **heterotrophic**. It is believed that at that time there was no free oxygen available in the atmosphere, and the autotrophs had a complex metabolism to prepare their own food. You can observe the difference between autotrophism and heterotrophism in Fig. 1.4.

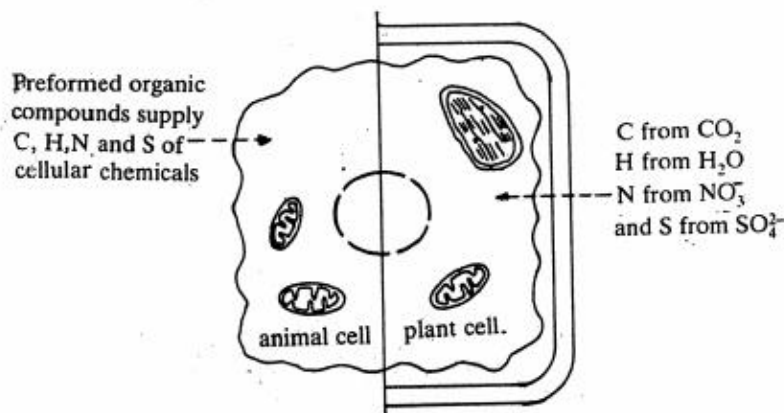


Fig. 1.4 : Sources of few main elements from which all chemicals are synthesised in autotrophic and heterotrophic organisms.

We will now discuss different types of procaryotes and their evolutionary relationship to draw an outline of organic evolution.

The 'group of procaryotes' is known as **Monera**. This group includes eubacteria, archaebacteria, green bacteria, purple bacteria, virus, prochlorophyta, cyanophyta and mycoplasma. Here we will describe the structure of two procaryotes viz. bacteria and virus.

Bacteria: Bacteria have a protective covering called 'cell wall'. Beneath this, there is a plasma membrane which encloses protoplasm of the bacterium containing various types of RNA, DNA, proteins and organic molecules. In bacteria, DNA molecule and enzymes involved in oxidation of food are found associated with the plasma membrane (Fig. 1.5).

Bacteria, though simple in their structure, are the most abundant of all the life forms on earth today. This is due to their rapid rate of multiplication and the ability to adapt to any nutrient and environment.

Viruses: Viruses are on the borderline of life between non-living and the living organisms. They have some characteristics of a living organism such as the ability to reproduce while some of non-living objects, like the absence of metabolic processes and ability to crystallise.

Viruses are very simple organisms with a circular nucleic acid molecule (DNA or RNA) surrounded by a protein coat (Fig. 1.6).

Viruses are organisms which live only as parasites and can reproduce themselves only in a host cell such as bacteria, animals or plants at the expense of the metabolic machinery of the host cell.

Anaerobic: Conditions in which oxygen is absent, reactions that proceed in the absence of oxygen or cells that function in the absence of oxygen.

Autotroph: An organism that can prepare its food on its own.

Heterotroph: An organism that cannot prepare its food on its own.

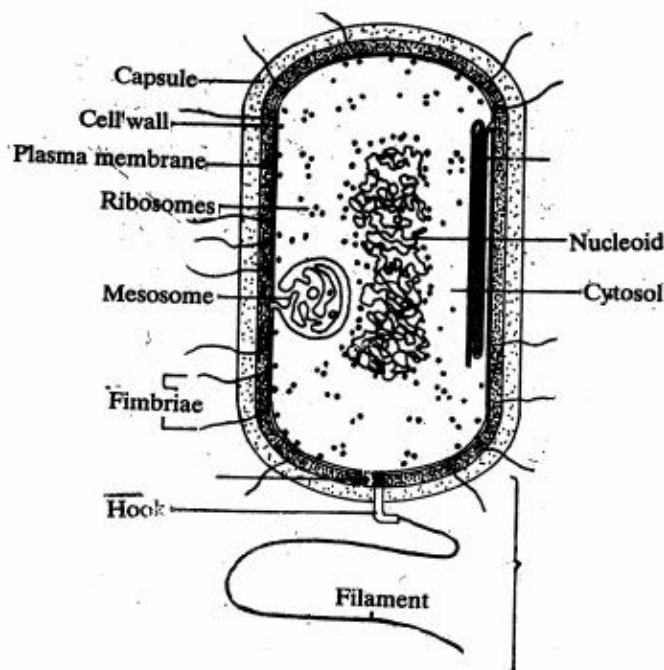


Fig. 1.5 : Structure of a bacterium. The bacterial cell is enclosed by a cell wall, which is further surrounded by capsule. Infolding of plasma membrane forms mesosomes. The nuclear material is concentrated in a specific region of the cell called nucleoid. One or more flagella may be present for locomotion. Small appendages that are called fimbriae arise from the cytosol and help the bacterium in getting attached to a surface.

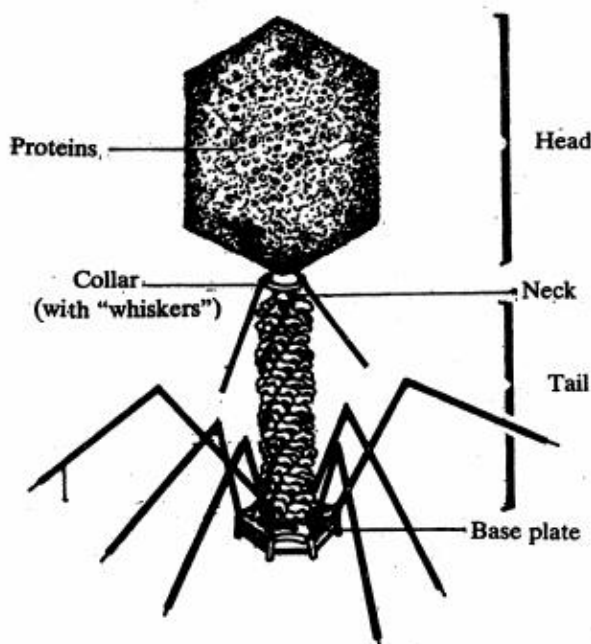
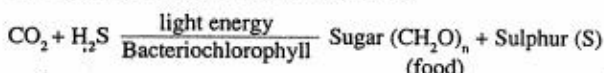


Fig. 1.6 : T₄ bacteriophage (bacteriophage is a specific type of virus which kills bacteria). Six tail fibres and collar whiskers are shown in the diagram.

The earlier living organisms must have existed independently, must have had their own metabolic machinery and capability of reproduction. It is, therefore, certain, that viruses are not in the direct line of evolution of life from non-living matter, because they are all parasites and have no metabolic machinery of their own. They are probably degenerated forms of life whose origin is a mystery.

1.3.3 Evolution of Photosynthesis and Aerobic Respiration

It is assumed that the earlier form of the bacteria utilised H_2S for the preparation of food. The following reaction shows this process



Since this reaction takes place in the presence of sunlight, it is called as photosynthesis. In this type of photosynthesis H_2S is used. Fig. 1.7 shows different types of metabolic pathways in bacteria.

Introduction to the Cell

Bacteriochlorophyll is the chlorophyll present in bacteria which is different from that found in higher plants.

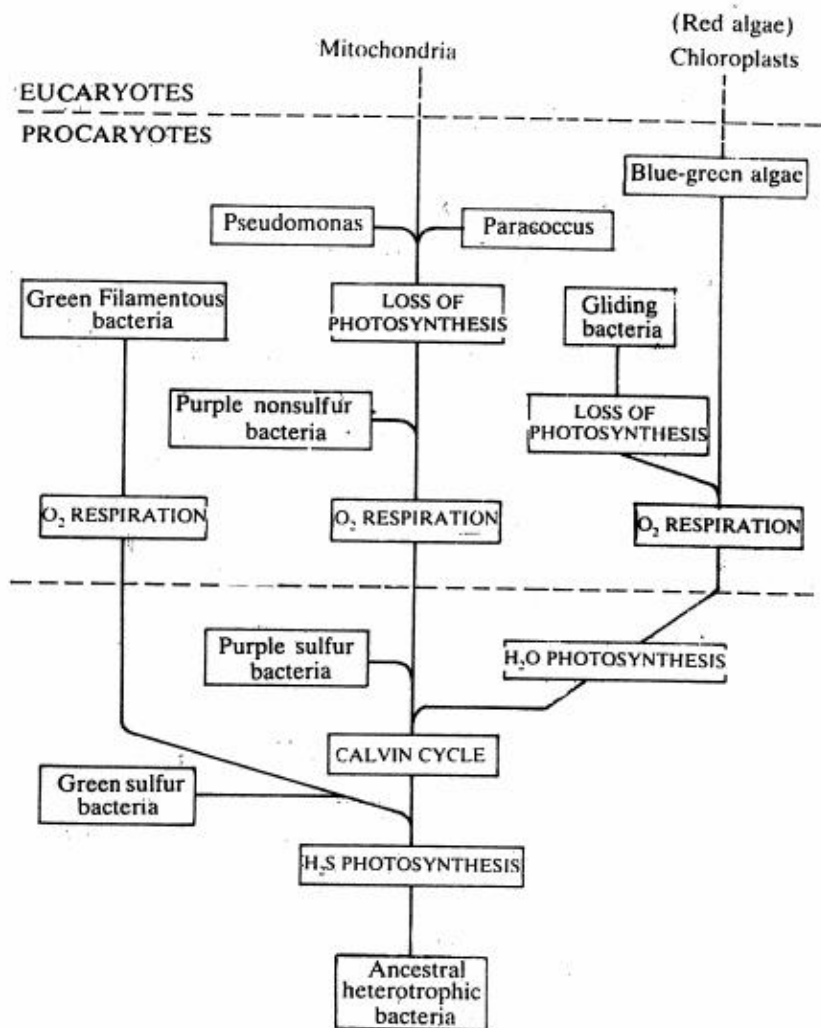
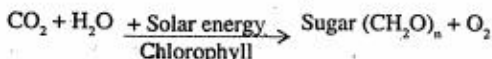


Fig. 1.7 : An evolutionary tree showing possible ways by which anaerobic and autotrophic bacteria (photosynthesizers) developed from the early anaerobic and heterotrophic bacteria. Likewise, aerobic form of bacteria also developed independently. Photosynthesis was retained in some forms of bacteria whereas it was lost in others.

Later, a more efficient photosynthetic system evolved in which H_2O instead of H_2S was used as an electron donor. This led to the release of free oxygen (O_2). This is the usual form of photosynthesis with which you are familiar.



The photosynthesis which takes place by utilising water, helped in the evolution of autotrophic mode of nutrition and release of free oxygen in the atmosphere. The release of free oxygen made possible a far more efficient method of getting energy from glucose by respiration. The free oxygen got converted into ozone (O_3) in the presence of ultraviolet radiation and formed an ozone layer in the upper atmosphere. The formation of this layer

Glycolysis: A pathway for glucose catabolism that does not require oxygen.

prevented the incoming ultraviolet rays which otherwise could have broken down the proteins, nucleic acids etc. Thus, the formation of ozone layer made existence of various forms of life on earth possible.

The free O_2 generated by photosynthesis was perhaps more like a poison to anaerobic bacteria initially. But some bacteria soon developed a metabolic system in which oxygen was used for the oxidation of food (respiration). This was a much more efficient system for the release of energy from organic molecules, compared with the earlier system of respiration, that is glycolysis. Some of these aerobic bacteria lost the ability of H_2S photosynthesis and became perhaps the ancestor of another class of organisms, that used the mitochondria for respiration. In mitochondria, the energy released by respiration is stored in the form of ATP molecules for further use.

SAQ 3

Arrange the following structures and processes in the order in which they are believed to have evolved.

- aerobic respiration
 - polymers
 - photosynthesis
 - organic monomers
 - acquisition of intracellular organelles.
-
-

1.4 PROCARYOTES TO EUCARYOTES

Procaryotes are the simple, unicellular organisms which do not have well defined nucleus, whereas eucaryotes are the complex, multicellular organisms with well defined nucleus. Procaryotes were originated about three and a half billion years ago, whereas eucaryotes originated about one and a half billion years ago. Thus, it appears that eucaryotes evolved later than the procaryotes. But in biochemical details the procaryotes and eucaryotes are very similar. Therefore, we can say that they have a common origin.

Eucaryotes, like procaryotes, have the same biochemical pathways for respiration both anaerobic and aerobic and for photosynthesis. The eucaryotes not only attained larger size and complex structure but also developed into multicellular organisms. Earlier each form of cell which was thought as an autonomous and self-sufficient unit, gradually became dependent on each other in larger organisms leading to division of labour within organisms. This type of cooperation between cells eventually led to the development of higher forms of life as they exist today.

Procaryotes and eucaryotes are compared in Table 1.2.

Table 1.2 : Comparison of Procaryotic and Eucaryotic Cell

	Procaryote (Monera)	Eucaryote
Differences		
Organisms	Monera, eubacteria, archaeobacteria, cyanobacteria (= cyanophyta = blue-green bacteria, purple bacteria).	Plants, animals, protists and fungi
Nucleus	Absent	Present
Chromosomes	Single	Multiple
DNA	Circular molecule naked (nucleoid)	Long DNA chain bound by histones
Cell Division	Binary Fission	Mitosis or meiosis
Organelle	None except ribosomes. Plasmalemma is perhaps infolded as mesosome in green bacteria and concentrically infolded in cyanobacteria.	Mitochondria, chloroplast (in plants only), Golgi bodies, lysosomes, endoplasmic reticulum, etc.
Cell wall	Peptidoglycans, Absent in mycoplasma	Cellulose (in plants only); Absent in Animals

Cytoskeleton

Absent

Present, composed of protein filaments.

Compartments in cells

Absent

Present due to membrane system

Cellular Organisation

Mainly unicellular

Mainly multicellular with differentiation

Metabolism

Anaerobic or aerobic

Aerobic

Cell size

1-10 μm linearly10-100 μm linearly

Similarities : Prokaryotes and eukaryotes have many kinds of molecules in common such as DNA, ribosomal machinery, enzymes, various similar metabolic pathways and membranes of similar organisation.

SAQ 4

- a) Name the organelle common to both prokaryotes and eukaryotes.
- b) Two figures are given below. Identify the prokaryotic and eukaryotic cell and give one important feature of identification.



- c) State one important difference between eukaryotic and prokaryotic DNA

1.4.1 Eucaryotic Cell Organelles

A eucaryotic cell has excessive foldings of intracellular membrane as compared to prokaryotic cell. The eucaryotic cell has a number of **organelles** such as endoplasmic reticulum, Golgi apparatus, nucleus, mitochondria etc. Organelles have the same relation to a cell, as organs have with an organism. The **endoplasmic reticulum** is a complex system of membranous sacs, chambers, and tubular canals. It is the site for synthesis of proteins. The **Golgi apparatus** (or complex) which is a stack of flattened sacs sorts out and processes proteins, besides, it helps in secretion. Membranes also enclose **lysosomes**, the organelles that contain enzymes necessary for degrading foreign materials thereby help in defence mechanisms. Likewise, membranes surround **peroxisomes** (microbodies) in which highly reactive hydrogen peroxide is synthesised and degraded. Peroxisomes are also the sites where a variety of biochemical reactions cause conversion of lipids into proteins and vice-versa. In plants, the membranes surround large liquid filled **vacuoles**. The remaining cytoplasm which is not bound by these organelles is referred to as the cytosol. A detailed account of these organelles is given in Unit 3.

The extensive intracellular membrane system of a eucaryotic cell is much larger in size than a prokaryotic cell. It provides enough surface area for the exchange of materials and other important cellular reactions which take place on the membrane surface.

It is assumed that membranous organelles have been formed by infolding of plasma membrane through a process called endocytosis (Fig. 1.8). In endocytosis portions of cell membrane along with the contents of the external medium invaginate and pinch off in the form of cytoplasmic vesicles. Exocytosis is just a reverse process.

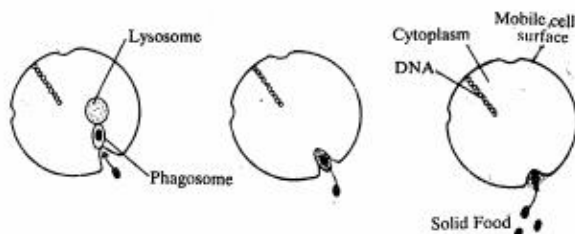


Fig. 1.8 : Showing evolution of the earliest membranous organelle, the lysosomes.

- Endocytosis was the possible mechanism for ingestion of food to prevent damage to internal cell components. Endocytotic pockets were located on the outer surface of the cell.
- In later stages of evolution, food was packaged in the membranous vesicles formed at the cell surface which also induced the digestive enzymes. The food was digested outside the cell in specialised enclosures.
- In the last stages of evolution, the digestive enzymes were packaged in lysosomes located internally and food was digested by the fusion of lysosome and phagosome vesicles, a physiological process, well known in the present day cell.

All eucaryotic cells have a cytoskeleton made up of a network of protein filaments (Fig. 1.9). This network gives the cell its shape, capacity to move and ability to transport organelles from one part of a cell to another. These protein filaments are similar in all eucaryotes. The important protein filaments are actin and myosin in muscles and tubulin in microtubules. Actin and myosin are involved in muscle contraction and in the formation of microfilaments. Microtubules are hollow and form cilia, flagella, centriole and mitotic spindle. This kind of cytoskeleton is absent in the procaryotes.

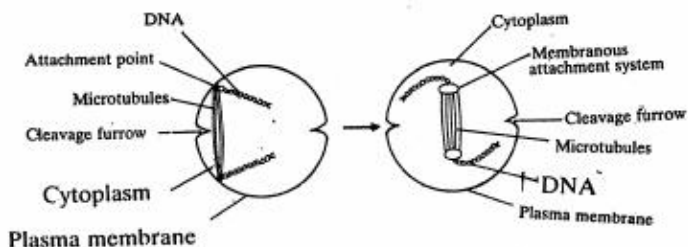


Fig. 1.9 : For the evolution of eucaryotic cell, the detachment of DNA from plasma membrane (in procaryotes DNA is attached to plasma membrane) is necessary. The orderly separation of replicated DNA molecules during cell division required system of attachment. Later, in the course of evolution, this system got detached from the mobile plasma membrane. Presence of microtubules in all eucaryotes indicates that they appeared early in eucaryotic evolution and were retained in all the later generations. Therefore, between the points of attachment, a system having tubular apparatus developed.

In procaryotes the DNA molecule lies freely in the cytoplasm attached to the cell membrane (Fig. 1.5). In eucaryotes it is enclosed inside the nucleus. This kind of formation of compartment protects DNA from many chemical changes that occur in the cytoplasm (Fig. 1.10).

The DNA found in eucaryote is not only large (human cells contain DNA thousand times larger than that in a typical bacteria) but is also packed together with histones into chromosomes. Histones are the proteins which are basic in nature, found in eucaryotes. They bind to DNA and wrap it up into compact chromosomes. Histones also control the expression of the characters called gene expression, about which you will study in detail in Units 12 and 13. Histones are important proteins which have not undergone any change during evolution and are identical in all plant and animal cells studied so far. Division of the nucleus by mitosis is another characteristic of the eucaryotes which permits proper and equal distribution of the genetic material to the daughter cells. Ribosomes which are the granular structures consisting of RNA and proteins are necessary for protein synthesis. These are the only organelles that are common to both procaryotes and eucaryotes. However, there are some minor differences between procaryotic and eucaryotic ribosomes.

1.4.2 Mitochondria and Chloroplast

Mitochondria are commonly known as the "power house" of cells. These are the distinct organelles of all eucaryotic cells, plants, fungi and some protists. In the cell, mitochondria are the sites of aerobic respiration. They are enclosed by a double membrane. The inner membrane is thrown into folds which are called as 'cristae'. Oxidative enzymes are located on these cristae. Mitochondria contain circular and naked DNA. They reproduce by fission, like bacteria. In the absence of mitochondria, eucaryotic cells would have to respire anaerobically and they will have to depend on an inefficient process, like glycolysis for their energy needs. It is interesting that mitochondria resemble an aerobic and non-photosynthetic bacterium like *Paracoccus*. In fact, in one of the eucaryotes like *Pleomyxa palustris*, there are no mitochondria. In this organism, in place of mitochondria there are aerobic bacteria which help the organism in respiration. The association of bacteria with the organism shows a kind of symbiotic (mutual advantage) relationship, wherein bacteria help the organism in respiration and in turn bacteria are benefitted by living at the expense of the organism permanently.

This relationship suggests that mitochondria are procaryotes which have entered the "eucaryotic" cell as endosymbionts, i.e., they have association inside the cell even before the entry of chloroplasts in the cell. In other words, we can say that mitochondria have evolved earlier than the chloroplasts.

Like mitochondria, chloroplasts are also distinctive organelles of eucaryotic cell. But they are found only in plant cells. Chloroplasts contain membranes forming a system of thin sacs or lamellae stacked on top of each other. These membranes contain the pigment chlorophyll whose function is to trap light energy required to make organic molecules particularly sugars. This process is called **photosynthesis**. It is worth mentioning that chloroplasts resemble the present day cyanobacteria in size and structural organisation of chlorophyll bearing membranes. Chloroplasts also contain DNA and reproduce by self-replication. In fact a number of present day eucaryotic cells do contain cyanobacteria in them (Fig. 1.11).

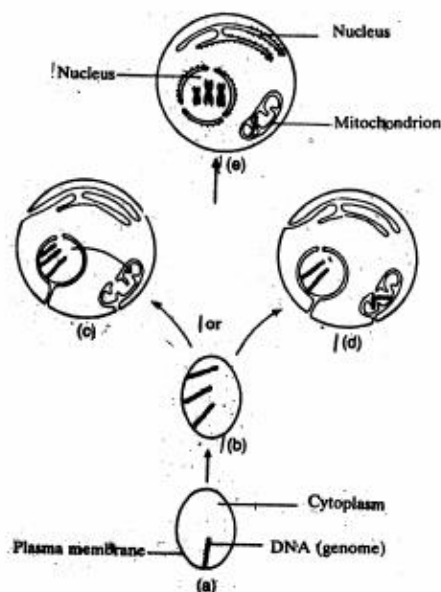


Fig. 1.11 : Evolutionary origin of mitochondria and other membranous system.

- Prokaryotic cell with one molecule of DNA (genome) is assumed to have undergone genome duplication.
- Multiple copies of genome (DNA) are formed.
- There occurs internalisation of plasma membrane and its differentiation to nuclear, endoplasmic reticulum (ER), mitochondrial and other systems in eucaryotic cells. Later on some DNA that was originally enclosed in nuclear envelope was transferred to mitochondria.
- During partition bulk of the DNA was retained in the nucleus while some of the DNA was transferred to mitochondria.
- Modern eucaryotic cells have numerous internal membrane systems in which main portion of DNA is present in nucleus whereas a small portion remains in mitochondria.

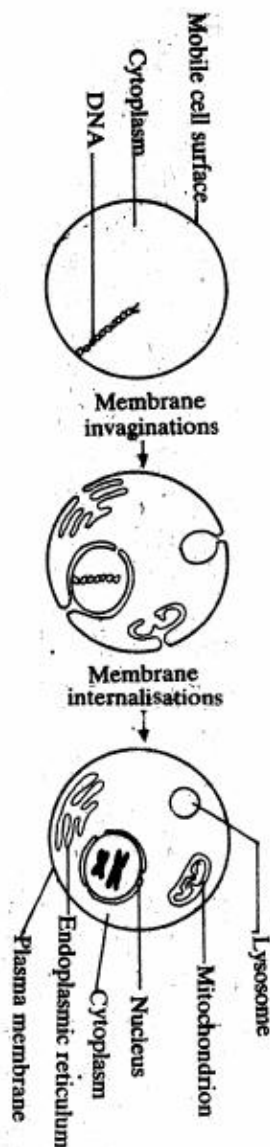


Fig. 1.10 : The process of invaginations and internalisations of membrane is proposed as an evolutionary phenomenon in which procaryotic cells with a mobile cell surface would have undergone endocytotic invaginations of certain regions of plasma membrane. These infoldings were established in eucaryotic cells as internal membrane systems independently, consequently, these remained without having any relation with plasma membrane.

It is from this symbiotic relationship that we can trace the origin of eucaryotic forms and divergence of plant and animal cells (Fig. 1.12).

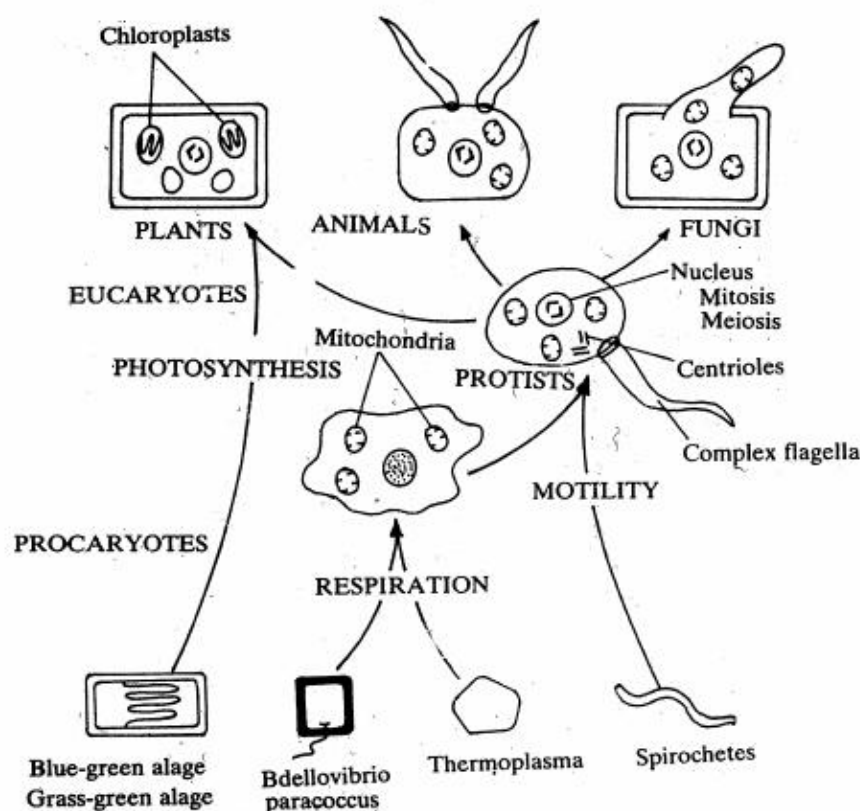


Fig. 1.12 : Schematic diagram showing the endosymbiont theory regarding origin of organelles in eucaryotes. The early procaryotic and anaerobic cell could ingest solid food through mobile cell surface. Such a cell might have engulfed respiring procaryotic cells like *Paracoccus*, leading to the development of symbiotic endosymbionts. The endosymbionts which were dependent on the host for their nutrients and protection, developed into cell organelles like mitochondria, flagella and chloroplast. These organelles were advantageous in respiration, motility and photosynthesis to host. The symbiotic relationships helped to trace the origin of eucaryotic forms and divergence of plants and animal cells.

1.4.3 Animal and Plant Cells

The internal structural organisation and information related with hereditary characters and metabolic pathways are similar in all eucaryotic cells whether they are of plants, fungi, animals or protists. On the other hand plant and animal cells differ in some respects. Cell wall in plant cells is unique. It provides mechanical support and protection. Besides, the cell wall helps to balance osmotic pressure of the cell with the surrounding medium. It consists of a network of cellulose and a gel-like matrix. Plant cells also contain pigment containing organelles called plastids such as chloroplasts (green colour), chromoplasts (red colour) and leucoplasts (no colour). Chloroplasts help the plants to synthesise their own food in presence of sunlight (autotrophs). Presence of large vacuoles is another important feature which distinguishes the plant cells from the animal cells. Endoplasmic reticulum (ER) plays a significant role in the formation of vacuoles. Glyoxysomes, the microbodies found in some plant cells, are involved in lipid metabolism. Golgi complexes found in plant cells are called as dictyosomes. The animal cells, often contain flagella and cilia for movement, and centrioles for cell division. On the other hand, these organelles are generally absent in plant cells. The difference between plant and animal cells are listed in the Table 1.3 and illustrated in the Fig. 1.13 for your ready comparison.

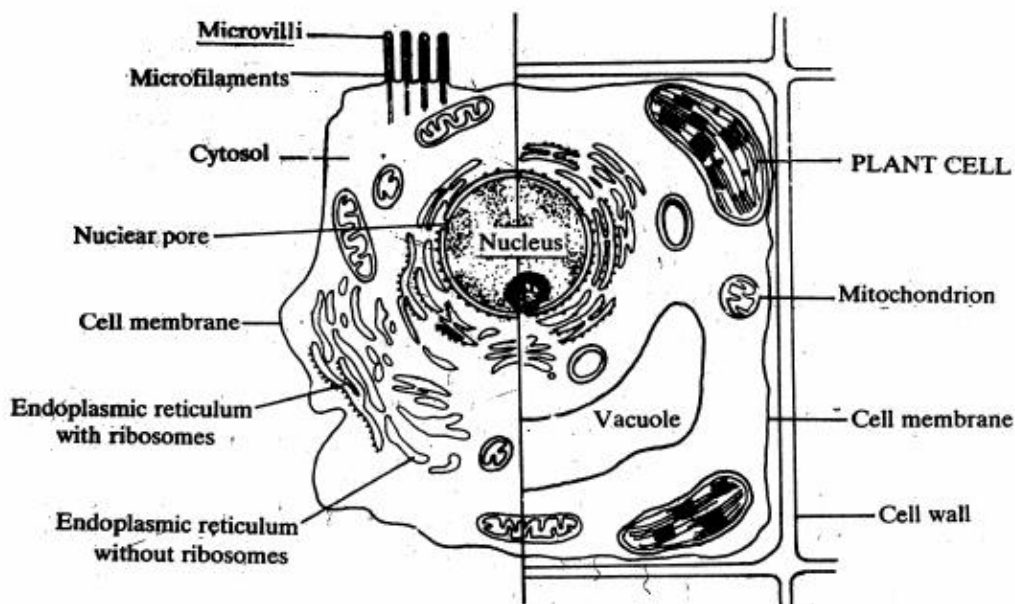


Fig. 1.13 : Generalised structure of eucaryotic cell showing the difference between plant cell and animal cell.

Table 1.3 : A Comparison of Animal and Plant Cells

	Animal Cell	Plant Cell
Exterior Structure		
Cell wall	Absent	Present (cellulose)
Cell membrane	Present	Present
Flagella	May be present	Absent except in sperm of a few species
Interior Structure		
Endoplasmic reticulum (ER)	Usually present	Usually present
Microtubules	Present	Present
Centrioles	Present	Absent
Golgi bodies	Present	Present
Organelles		
Nucleus	Present	Present
Mitochondria	Present	Present
Chloroplast	Absent	Present
Chromosomes	Multiple units, DNA associated with protein	Multiple units, DNA associated with protein
Ribosomes	Present	Present
Lysosomes	Usually present	Equivalent structures called "sphaerosomes"
Vacuoles	Absent or small	Usually a single large vacuole in mature cell

SAQ 5

Cook at the following figures and answer the questions:

Carbohydrate



- a) Name the organelles X and Y

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- b) Identify the animal and plant cell and give reasons for identification.

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- c) Which major process occurs in organelle.

.....

- d) Which major process occurs in organelle

.....

.....

1.5 SUMMARY

Let us sum up whatever we have said so far.

- Although there are different species of living organisms, there is a unique master plan of organisation at the cellular and molecular levels. The rapid progress in the field of cell and molecular biology in the present century is due to the development of microscopes and various physico-chemical methods used to analyse the cell, such as X-ray diffraction and cell fractionation.
- The evolution of life, from simple to complex, took place in two stages— chemical evolution and biological evolution. Three possible models for protobiont formation are coacervate model, proteinoid microsphere model and lipid bilayer model.

- Protobionts could become eubionts only with the development of self-replicating genetic system. Scarcity of nutrients favoured the selection of those which could obtain the nutrient from a precursor. Hence, more complex metabolic pathways such as glycolysis developed which consequently led to the development of autotrophs.
- From an evolutionary viewpoint, procaryotes are considered to be the ancestors of eucaryotes. Procaryotes and eucaryotes resemble as well as differ from one another in many respects. The complex multicellular eucaryotes like plant and animal cell differ from each other in many respects.

1.6 TERMINAL QUESTIONS

- 1) How did the primordial cell originate?
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- 2) List two changes in the environment that resulted from evolution of water splitting photosynthesis.
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.....
- 3) Name the organelle(s) responsible for the following function in a eucaryotic cell.
 - a) Production of ATP by the oxidation of food:.....
 - b) Protein synthesis:
 - c) Secretion:.....
 - d) Synthesis of food in the presence of sunlight:.....
- 4) Symbiotic relationships of mitochondria with procaryotic cells helped in tracing the evolution of eucaryotic cells. Explain it.
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1.7 ANSWERS

Self-assessment Questions

- 1) a) Microscope
b) Cell is the structural and functional unit of organisation
c) The division of pre-existing cells
d) Mitosis
- 2) a) Biological evolution
b) Coacervate model, proteinoid microsphere model, lipid bilayer model
- 3) d, b, e, c, a.
- 4) a) Ribosomes
b) (i) is eucaryotic cell, and (ii) is procaryotic cell because in (i) nucleus is well defined with nuclear membrane whereas in (ii) nucleus is not clearly demarcated.
c) In procaryote, DNA molecule is circular and naked whereas in eucaryote DNA is long chain bound by histones.

- 5) a) Organelle 'X' is mitochondria and organelle 'Y' is chloroplast.
b) (i) is plant cell because of presence of cell wall and chloroplast, and (ii) is animal cell because of their absence.
c) Respiration.
d) Photosynthesis.

Terminal Questions

- 1) The assemblage of phospholipid molecules to form membrane was the first step for the formation of primordial cell.
- 2) a) Release of free oxygen in atmosphere
b) Autotrophic mode of nutrition
- 3) a) Mitochondria
b) Ribosomes
c) Golgi complex
d) Chloroplast
- 4) Mitochondria, the power house of the cell resemble aerobic, nonphotosynthetic bacteria. It was found that some eucaryotic organisms which lacked mitochondria were in permanent symbiotic relationship with aerobic bacteria (procaryote) for respiration. Thus, symbiotic relationship suggested that mitochondria are procaryotes which entered the eucaryotic cell as endosymbionts.

UNIT 2 HOW CELLS ARE STUDIED ?

Structure

- 2.1 Introduction
 - Objectives
- 2.2 Light Microscopy
 - Cytochemistry
 - Fluorescence Microscopy
 - Phase Contrast Microscopy
- 2.3 Electron Microscopy
 - Transmission Electron Microscopy
 - Scanning Electron Microscopy
 - Freeze Fracture Electron Microscopy
- 2.4 Radioisotopes, Autoradiography and Antibodies
- 2.5 Tissue Culture and Cinemicrography
 - 2.5.1 Tissue Culture
 - 2.5.2 Cinemicrography
- 2.6 Summary
- 2.7 Terminal Questions
- 2.8 Answers

2.1 INTRODUCTION

In Unit 1, you learnt about the evolution of the cell and a historical account of the growth of cell biology. In this Unit, you will study about the various tools and techniques used to study the cell.

Cells, due to their minute size, cannot be observed by the naked eye. Lenses can magnify minute objects only upto a limited extent. Therefore, many lenses were combined together to form an instrument known as the microscope (Gr mikros—small, skopein—to see). Subsequently, many new tools and techniques were invented by cell biologists about which you will study in detail in this Unit.

We begin the Unit with light microscope (LM). Here, you will know about the difference between resolving power and magnification and various cytochemical techniques which are used to study the chemical components and structural organisation of the cell. Phase contrast microscopy; an important technique for viewing the live cells is described. You will also learn about electron microscopy (EM), the best known method for the study of the ultrastructure of the cell and an important technique called 'freeze fracturing' which is used to study the internal structure of membrane in the cell.

Use of radioisotopes, antibodies, and fluorescence microscopy for localisation of various cell components are discussed in this Unit. You will also learn about cell culture and cinemicrography; the other important techniques used to study live cells.

While going through this unit, it would be useful to recall and apply your knowledge of elementary chemistry, physics and mathematics, such as radioisotopes, refractive index and simple mathematical calculations. The study of this Unit assumes that you are familiar with the optical laws and principles on which a microscope works.

Objectives

After reading this Unit you should be able to:

- explain the working and usefulness of a light microscope and phase contrast microscope,
- list the principles and uses of fluorescence microscopy,
- suggest simple cytochemical methods to locate major cell components,
- explain the principles of transmission and scanning electron microscopy and their advantages and disadvantages as compared to light microscopy,
- explain the use of freeze fracture and cell culture techniques,

- suggest uses of cinemicrography in the study of cell,
- explain the uses of radioisotopes (with special reference to autoradiography) and antibodies for localising specific molecule in the cell.

2.2 LIGHT MICROSCOPY (LM)

A study of the structure of organisms is an essential requirement of biology. However, unaided human eye cannot see objects smaller than 0.1 mm (100 μ) in diameter. Therefore, various devices were discovered to increase his ability to observe minute structures. The knowledge of these devices is responsible for understanding structure and function of cell (Table 2.1).

Table 2.1 Some Important Landmarks in the History of Light Microscopy

Year	Nature of Discovery
1655	Small pores described in a section of cork, were named as cells by Hooke.
1674	Protozoa was discovered by Leeuwenhoek by the use of a microscope.
1835	Cell theory, i.e., nucleated cell is the unit of structure and function in all plants and animals, was proposed by Schleiden and Schwann.
1881	Use of staining techniques was introduced by Cajal and others.
1886	Lenses were made to study minute details of the structure of cell.
1898	Golgi apparatus was seen and described for the first time by Camillo Golgi by staining cells with silver nitrate.
1932	Phase contrast microscope was discovered to view live cells.
1941	Antibodies coupled to fluorescent dyes were used by Coons to detect cellular antigens.

The earliest microscopes had only one lens between the object and the eye and were, therefore, called **simple microscopes**. The modern microscopes, on the other hand have two lenses, the objective and the eye piece, in a series, to view an object. These are known as **compound microscopes** (Fig. 2.1).

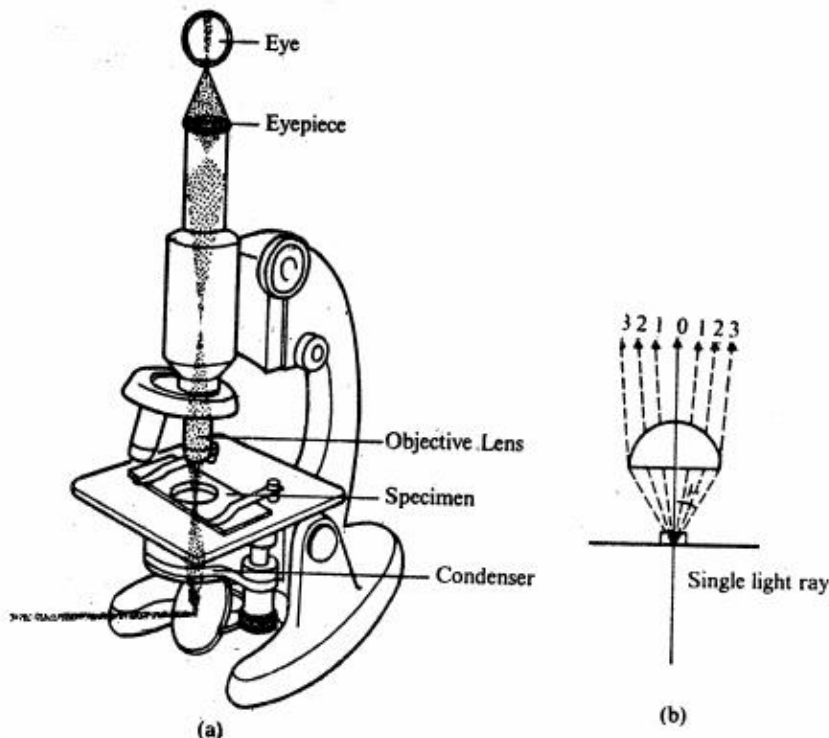


Fig. 2.1 : a) Light path in a compound microscope. The objective lens is near the specimen and the observer looks into the eye piece at the upper end of the tube of the microscope. The specimen is beyond the focal point of the objective lens.
b) A single light ray pattern.

With the help of a compound microscope one can view the objects at a very high magnification. However, the important property of a light microscope is not its magnifying power but its resolving power. Let us now explain the terms magnifying power and resolving power. The **magnifying power** of a microscope is its ability to show an enlarged or magnified view of an object to the eye. The resolving power, on the other hand, represents the ability of the microscope to show the fine details in the object to the eye (Fig. 2.2)

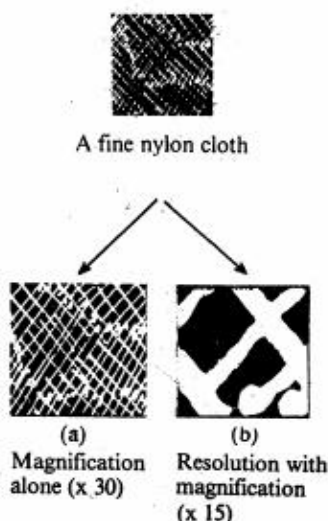


Fig. 2.2 : Magnification alone (a) the details of nylon cloth are not seen. Resolution with magnification (b) reveals that the cloth is made up of fine fibres.

When two objects are very close together, they may appear as one and when viewed through the microscope they may not appear as two separate objects. However, these two objects can be seen as two separate objects or in other words we can say that the two objects are just resolved if the microscope used is of high resolving power. The linear distance(s) between the two objects that is just resolved by the microscope is given by

$$s = \frac{0.61 \lambda}{NA}$$

.... Equation 2.1

(λ = wavelength of the light used
NA = numerical aperture of the objective of the microscope)

Numerical Aperture (NA): A measure of the resolving power of a microscope objective, equal to the product of the refractive index of the medium (n) in front of the objective and the sine of the angle ($\sin \alpha$) between the outermost ray entering the objective and the optical axis.

The minimum distance 's' is called as the '**limit of resolution**' of the microscope. Smaller the value of 's', higher is said to be the resolving power. The resolving power of a microscope depends on the numerical aperture of its objective. The greater the magnitude of NA, the smaller is the value of 's', that is, finer is the detail that can be observed.

To obtain better resolution with microscopes we often use ultraviolet light which has a smaller λ . The electron beams which behave like waves under some circumstances, have wavelengths 10^4 times shorter than visible light. This has led to the development of electron microscopes which have extremely high resolving power.

Thus, to increase the resolving power of a microscope or to diminish the distance 's' between the two points to be resolved by the microscope, we often use light which has a smaller λ and the objective whose aperture is large (see Equation 2.1).

Equation 2.1 shows that to obtain better resolution, light of smaller λ should be used e.g. ultraviolet light and even electron beams which behave like waves under some circumstances. Electron beams have wavelengths 10^5 times shorter than visible light. This has led to the development of electron microscopes which have extremely high resolving power.

Using the LM, we can study the cell by various methods. You will study about three such methods in the following sub-sections.

2.2.1 Cytochemistry

It is well known that certain stains and dyes specifically combine with certain chemical components of the cell. Cytochemical techniques take advantage of this fact and help in localisation and identification of various constituents like proteins, nucleic acids, lipids, etc. within a cell. For this a particular molecule is first immobilized and then identified by its reaction with a stain. Some molecules are also identified by their physical properties like absorption of radiation at a particular wavelength. For example, the nucleic acids absorb UV rays at 260 nm whereas the proteins absorb at 280 nm. You will learn more about the chemical components of the cell in Units 4 and 5.

The amount of the chemical component present in a cell can be quantified provided the reaction between the molecule present and the stain is strictly on 1: 1 basis, as in the case of Feulgen reaction for DNA described in Table 2.2.

A large number of dyes are in use to stain different chemical components of the cell. However, the chemical basis for the specificity of many dyes is not known, so only qualitative information can be obtained. The following table (Table 2.2) lists some of the common tests for the detection of proteins, lipids, nucleic acids and polysaccharides.

Table 2.2 : Some Reactions and Principles of Cytochemical Stains

Substance	Reaction or Test	Principle
1) Proteins	a) Diazonium reaction (staining with diazonium hydroxide)	Detects tyrosine, tryptophan and histidine groups in the protein.
	b) Fast green	Stains histone proteins.
	c) Bromophenol blue	Stains all proteins.
2) Lipids	a) Osmium tetroxide	Stains unsaturated fatty acids black.
	b) Sudan black B	Causes black colour by dissolving in lipid droplets.
3) Nucleic acids	a) Feulgen reaction	Acid hydrolysis exposes the free aldehyde groups in deoxyribose. The free aldehyde group is made to react with Schiff's reagent to produce a purple colour.
	b) Methyl green pyronin (MGP)	Methyl green stains DNA while pyronin stains RNA. Both the dyes combine with phosphoric acid.
4) Polysaccharides	Periodic acid Schiff (PAS) reaction	Periodic acid oxidises 1, 2 glycol groupings to liberate free aldehyde groups which are stained purple by Schiff's reagent.

Hydrolysis: The cleavage of a molecule into two or more molecules by the addition of a water molecule.

Glycol groupings i.e. $\begin{array}{c} \text{CH}_2\text{OH} \\ | \\ \text{CH}_2\text{OH} \end{array}$

SAQ 1

Schiff's reagent is used to localise DNA as well as polysaccharides in the cell. But in both the reactions, its mode of action is same. Explain in about 6-7 lines in the space provided.

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2.2.2 Fluorescence Microscopy

Certain compounds when exposed to short wavelength radiations, e.g., UV and X-rays, absorb and emit energy as light of a longer wavelength. This process of emitting light from a compound is known as **fluorescence** and is now commonly used in microscopy.

Compounds which naturally emit light exist in most living tissues. For example, the mitochondria of liver and kidney, collagen and chlorophyll give a strong fluorescence. Such a type of natural fluorescence is called as **autofluorescence**. Sometimes fluorescent dyes

like rhodamine, quinacrine etc. called **fluorochromes** are used to stain cell components to produce secondary fluorescence. Such important dyes are also used in the study of chromosome behaviour.

The fluorescent microscope is essentially an ordinary optical instrument. It has been modified by the incorporation of special filters. These filters allow only the required wavelength of light to pass and cause fluorescence in the specimen. The most significant application of fluorescent microscopy is in the field of immuno-fluorescent antibody labelling technique about which you will study later in this Unit.

2.2.3 Phase Contrast Microscopy

Phase contrast microscope has the resolving power similar to ordinary light microscope. However, it enables us to observe the cellular organelles in living cell. The working of this microscope is based on the principle of phase contrast.

To understand phase contrast microscopy you should know that whenever two light waves interfere with each other, the resultant light wave may have either greater or lesser amplitude depending on the phases of the light waves (Fig. 2.3).

It is a known fact that a light wave passing through a transparent object suffers a change in phase.

The living cells are heterogeneous in their cellular components having different thickness. Due to this difference, each component has different refractive index. Hence, when a light wave passes through the cell, a change in phase is produced due to differences of thickness and refractive indices of cellular components (Fig. 2.4). The phase contrast microscope exaggerates the small difference between the phases and enables one to distinguish the adjacent structures.

Phase: The fractional part of a period through which the time variable of a periodic quantity has moved, as measured at any point in time from an arbitrary time origin, usually expressed in terms of angular measure, with one period being equal to 360° .

Refractive index: The ratio of the phase velocity of light in a vacuum to that in a specified medium.

2.3 ELECTRON MICROSCOPY (EM)

In EM, the object is viewed by a beam of electrons instead of visible light. An electron beam has a shorter wavelength ($\lambda = 0.004 \text{ nm}$) compared with visible light ($\lambda = 400 \text{ nm}$). Therefore, an EM has higher resolving power than LM (Equation 2.1). Till now electron microscopy is the best available method to study biological ultrastructure (Fig. 2.5).

The resolving power of most modern EMs ranges between $100 \mu\text{m}$ to 0.1 nm . However, problems of specimen preparation, and radiation damage limit the resolution of biological objects to about 2 nm , even this is 100 times better than the resolution achieved by the best LMs. Further, the magnifying power of the EM is upto 200,000 X. The two types of EM are **transmission electron microscope (TEM)** and **scanning electron microscope (SEM)**, about which you will study in the following section.

2.3.1 Transmission Electron Microscopy

The source of illumination in a TEM is a tungsten filament that emits electrons. A vacuum is created to prevent the collision of electrons with anything else except the specimen. The electron beam is focused by different electromagnetic coils (lenses). First electrons are collected and focused in an electromagnetic 'condenser' lens and then they are collected again by an 'objective' lens. The electromagnetic objective lens produces a magnified image of the object which is received by 'ocular or projection' lens. The final image is formed either on a photographic plate or on a phosphorescent screen. The image results from differential scattering of electrons from molecular constituents of the cell.

If you compare the functioning of a TEM with a LM, you will observe that while there are many similarities between a LM and a TEM, there are also many differences (Fig. 2.6). The image formation in a LM depends on the degree of light absorption whereas in a TEM it depends on electron scattering. The lenses of LMs are glass lenses and focusing is done by moving them nearer or farther away from the specimen. In TEMs all the lenses are electromagnetic coils and focusing is done by manipulating the amount of current flowing through the magnetic coils. The final image in a LM is seen by the eye whereas in a TEM it is formed on a phosphorescent screen.

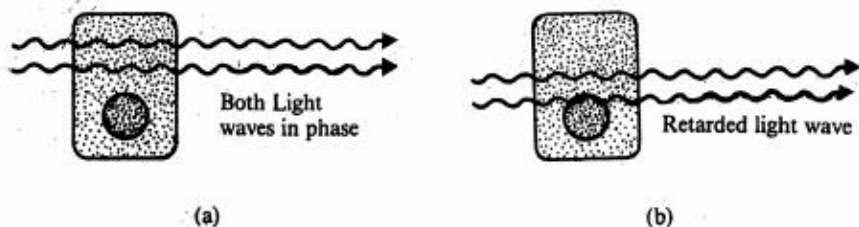


Fig. 2.3 : a) When two light waves are passing side by side, through a cell and if they have encountered the same refractive index and thickness of medium, they will emerge in phases.
b) When one light wave passes through an organelle which is not passed through by other, then it will be retarded and will emerge slightly out of phase.

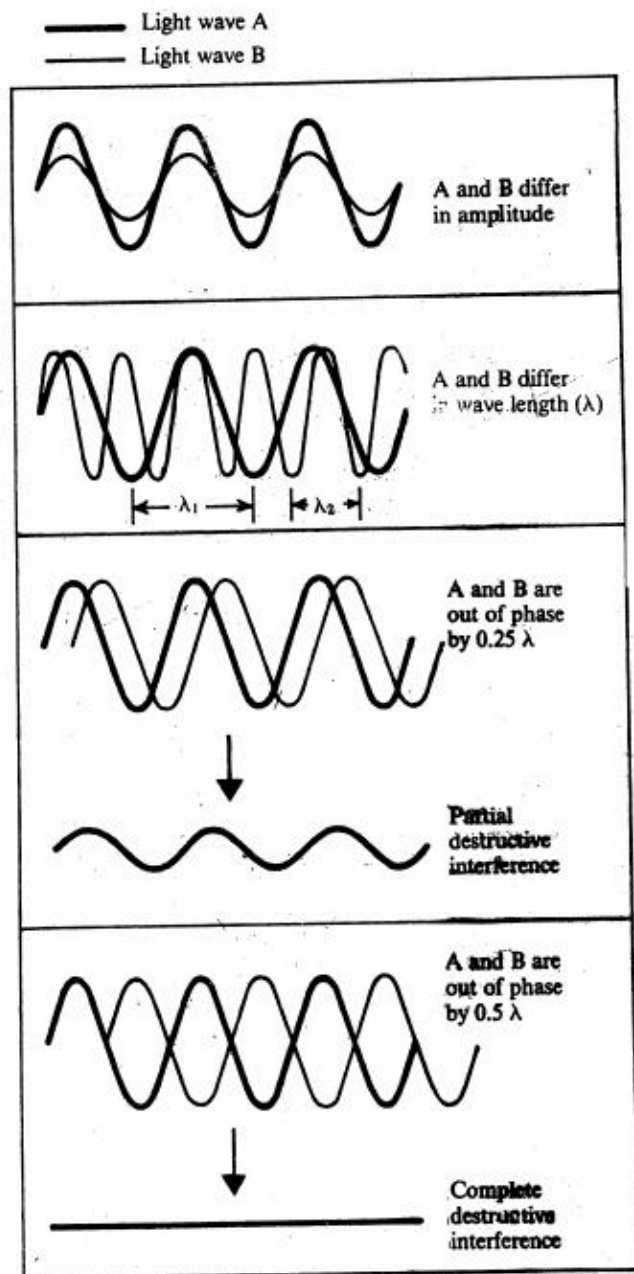


Fig. 2.4 : Properties of light waves that result in partial and complete destructive interference.

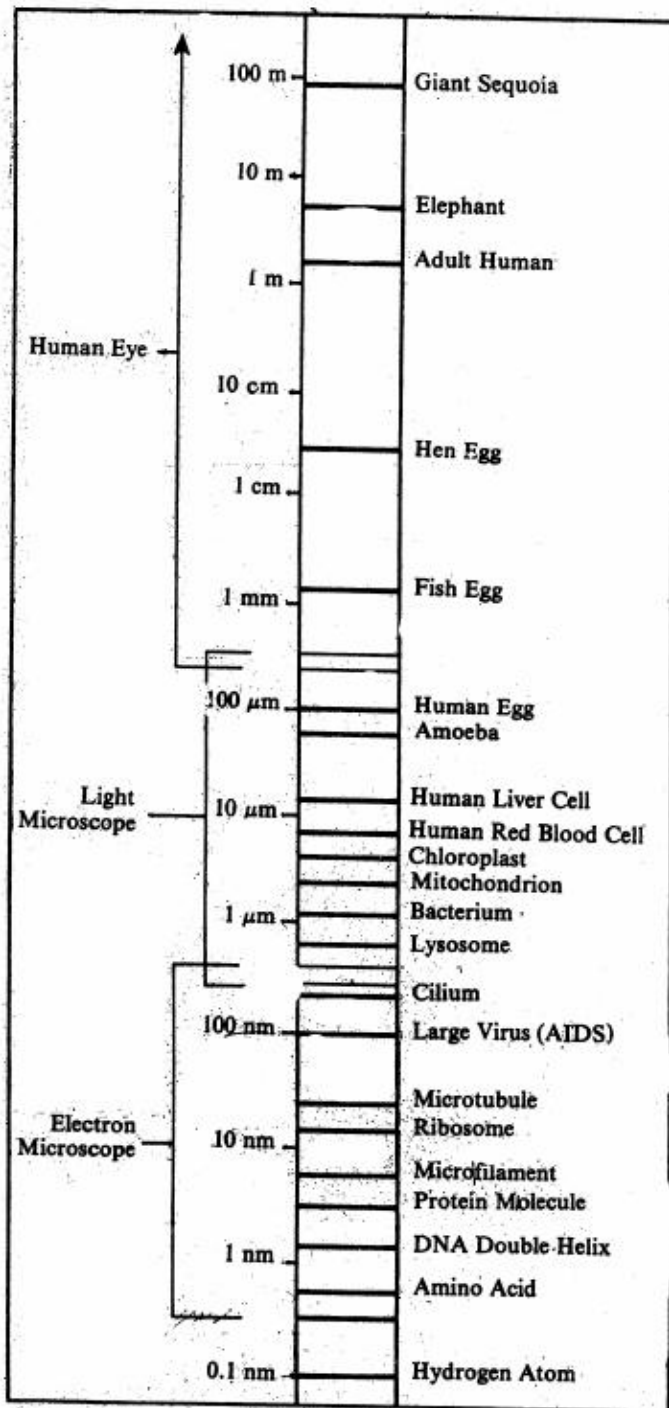


Fig. 2.5 : Logarithmic scale of microscopic dimensions. The relationship between resolving power of human eye, the light microscope and the electron microscope with sizes of various biological structures like bacteria, virus etc. is shown. Bacteria are generally 1 to 2 micrometers (μm) thick, and human cells are of larger magnitudes. The scale goes from nanometers (nm) to micrometers (μm) to millimeters (mm) to centimeters (cm) and finally meters (m)

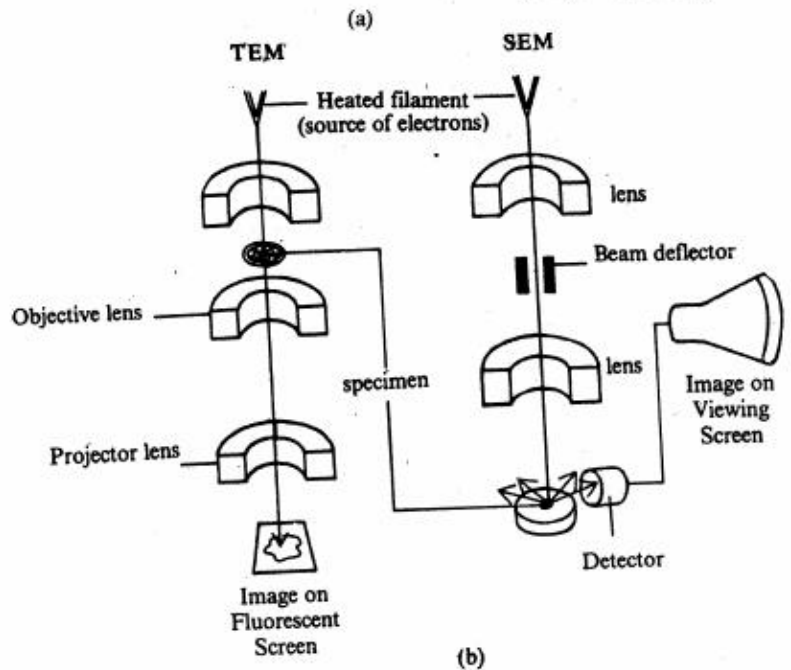
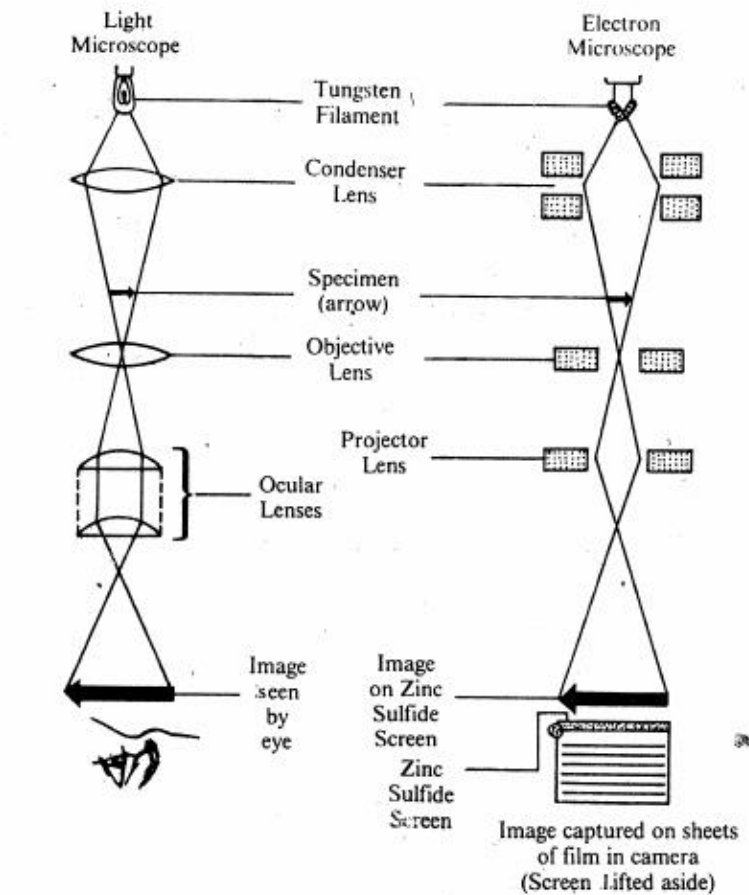


Fig. 2.6 : a) A comparison of the component parts of the light microscope and electron microscope. b) A comparison of a transmission electron microscope and a scanning electron microscope. (TEM and SEM) Both require the specimen to be placed in a vacuum. In both, the electrons are accelerated from the filament and passed through a tiny hole to form an electron beam. In both, the beam passes through different magnetic coils, but in a TEM the image is viewed on fluorescent screen whereas in a SEM, the image is viewed on viewing screen.

An EM has the major advantage of increased resolution over a LM. The applications of electron microscope to cell biology are given in table (Table 2.3). Moreover, TEM has its disadvantages which are (i) the live biological material cannot be observed since electron beams traverse in vacuum, and (ii) only very small areas of tissues can be viewed at a time. Therefore, a LM and a TEM are complementary to each other.

Table 2.3
Major Events in the Development of the Electron Microscopy

1926	Busch proved that it was possible to focus a beam of electrons with a cylindrical magnetic lens, thus laying the foundations of electron optics.
1939	Siemens produced the first commercial transmission electron microscope.
1952	Palade, Porter, and Sjostrand developed methods of fixation and thin sectioning to observe many intracellular structures.
1953	Porter and Blum developed the first widely accepted ultramicrotome.
1957	Steere developed freeze fracture techniques which were later perfected by Moore and Muhlethaler .
1965	First commercial scanning electron microscope produced.
1986	de Rosier and Klug described techniques for the reconstruction of three dimensional structures from electron micrographs.

2.3.2 Scanning Electron Microscopy (SEM)

While TEM is useful to study the internal structure of the cell, SEM is an important technique if you wish to examine the surface features of the specimen. Moreover, a specimen can be viewed at different angles. The resolving power of a SEM is lower than that of a TEM since a resolution of only about 3.0 nm can be attained by SEM.

In a SEM, a low energy beam of electrons (primary electrons) strikes the surface of a specimen. This causes the emission of the secondary electrons from the specimen which are gathered by a photomultiplier tube to form an image which is built up line by line as in the case of television. The image formed by SEM has a three-dimensional appearance. Unlike in a TEM, the electrons in a SEM do not pass through the specimen.

2.3.3 Freeze Fracture Electron Microscopy

Freeze fracture electron microscopy is a technique to reveal the surface features of hydrophobic interior of cell membranes. In this technique, the cells/tissues are first frozen in liquid freon at -130°C . The frozen block is cut with a cold knife blade along with the planes of weakness, run through each cell. Generally, these planes occur between the two layers of lipid molecules of the membrane. The plane of fracture at these places exposes the interior of a plasma membrane (Fig. 2.7).

The newly exposed faces of a plasma membrane, the exoplasmic fracture face (EF) and the protoplasmic fracture face (PF) are covered with a very thin layer of platinum by dissolving away the organic material. The freshly fractured membrane may be covered with platinum and carbon to obtain replicas (exact copies) of the surface and this is observed under a TEM.

This technique demonstrates that intercellular junctions (where cells make contact with each other) are composed of specialised membrane proteins.

SAQ 2

Which microscope (LM, TEM or SEM) will you use in the following cases. Give your answers in the space provided.

If you want to:

a) observe details of the surface of an insect's compound eye.

b) examine the patterns of capillaries in a section of skin.

c) examine a kidney cell to look at its cell membrane.

d) identify the type of flagellum of a flagellate organism.

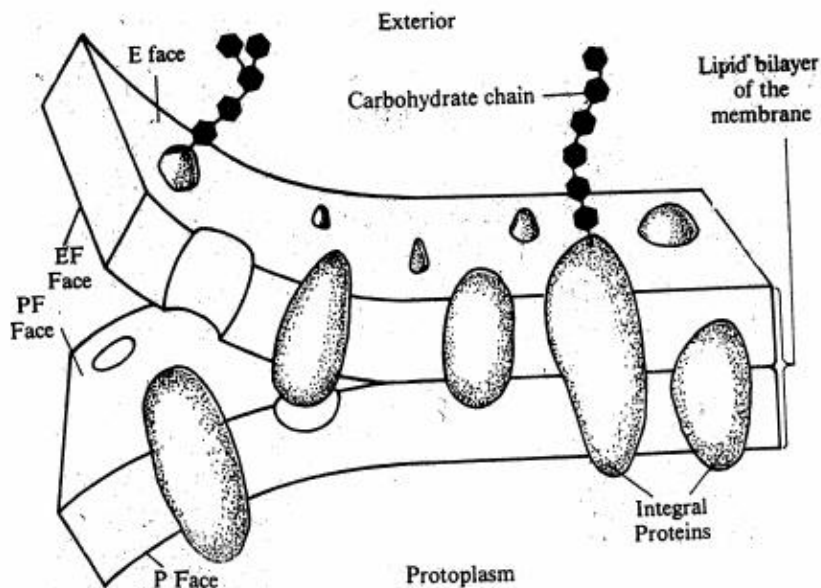


Fig. 2.7 : Tissue is fractured by using a microtome knife. When the plane of fracture intersects the cell membranes, e.g., plasmalemma, nuclear envelope, etc. the membrane is split to produce two half membranes: E (exterior) half and P (protoplasmic) half. The surface features of the fracture faces that are produced reveal the location of proteins.

2.4 RADIOISOTOPES, AUTORADIOGRAPHY AND ANTIBODIES

Isotopes: Isotopes are chemical elements that have the same atomic number (e.g. the number of protons in the nucleus of the atom) but different atomic weight (i.e. total number of protons and neutrons in the nucleus).

Radioisotopes are those forms of an element which exhibit radioactivity and so can be used as markers or tracers. For this purpose, we first introduce the radioisotope in a molecule in the cell and then detect the radioactivity with the help of certain device. For example, if we replace a suitable hydrogen atom of thymidine by ^3H , we may trace the course of thymidine in the cell. Since thymidine is a component of DNA but not of RNA, we can be sure that we are following only DNA metabolism. Similarly, to know the course of protein synthesis we may use radioactively labelled amino acids. Many of the commonly used radioactive isotopes are listed in table (Table 2.4).

Table 2.4
Some Radioisotopes Frequently Used as Tracers

Isotope	Used as a Tracer of
^2H	Virtually any organic compound
^{14}C	Virtually any organic compound
^{24}Na	Salt metabolism, exchanges across membranes
^{32}P	Nucleic acid metabolism, phospholipid metabolism
^{35}S	Protein metabolism
^{36}Cl	Salt metabolism
^{45}Ca	Salt metabolism, bone deposition
^{59}Fe	Heme synthesis

Radioisotopes are used to (a) measure the rates of metabolic turnover of a material within a cell or tissue, (b) locate the site of synthesis of molecules, (c) measure the rate of exchange of materials across cellular membranes, (d) locate a molecule in a cell. Radioisotopes are widely applied to study also the **precursor-product relationship**. Many times when a **radioactively labelled molecule (precursor)** is introduced into a system, the molecule is **chemically converted into another form (product)**. For example, when radioactive iron is introduced in blood, radioactivity can be detected in liver ferritin and haemoglobin of red blood cells. Similarly, radioactivity of ^{32}P — labelled phosphate can be detected in many phospholipids.

- i) The radioactive molecule is introduced in the cell either *in-vivo* or *in-vitro*. The cell incorporates and metabolises the radioactive molecule and after a certain time interval radioactivity can be detected and measured with the help of the special instrument routinely known as counters. The selection of instruments is based on the type and energy emitted by the radioactive substance. The most commonly used detectors are **Geiger Muller** counters commonly known as GM counters, **solid scintillation counters** and **liquid scintillation counters**. GM counters are generally used for isotopes emitting beta radiations of intermediate or high energy waves. These are generally used in tracer experiment involving ^{24}Na and ^{32}P . Liquid scintillation counters are used for isotopes emitting low energy beta radiations. These counters are used for tracing ^3H and ^{14}C . Solid scintillation counters are most commonly used for isotopes emitting gamma rays, for example ^{131}I .
- ii) Autoradiography is a modification of the radioactive tracer technique. In this technique, the radioactivity is detected by a thin film of photographic emulsion containing silver compounds which is put over a labelled cell or tissue. The rays emitted by radioisotope from the cell or tissue expose the photographic emulsion like a photographic film which is developed in a dark room. Silver grains are seen as black dots on parts of the specimen where radioisotope is accumulated.

Bioassay: A test that uses living organisms rather than chemical analysis to detect the presence of substances such as hormones, which are usually present in very small quantities and are hard to detect by chemical means but easy to detect from their effect on an organism's behaviour or physiology.

Autoradiography reveals the metabolic processes of the cell. For example, by labelling cells with ^3H thymidine, it is found that silver grains are localised in nucleus, which shows that DNA is synthesised in the nucleus (Fig. 2.8). Similarly, by labelling cells with ^3H uridine, silver grains are first seen in nucleus and then after some time these grains appear in the cytoplasm. This clearly demonstrates that RNA is initially synthesised in the nucleus but later on migrates to the cytoplasm.

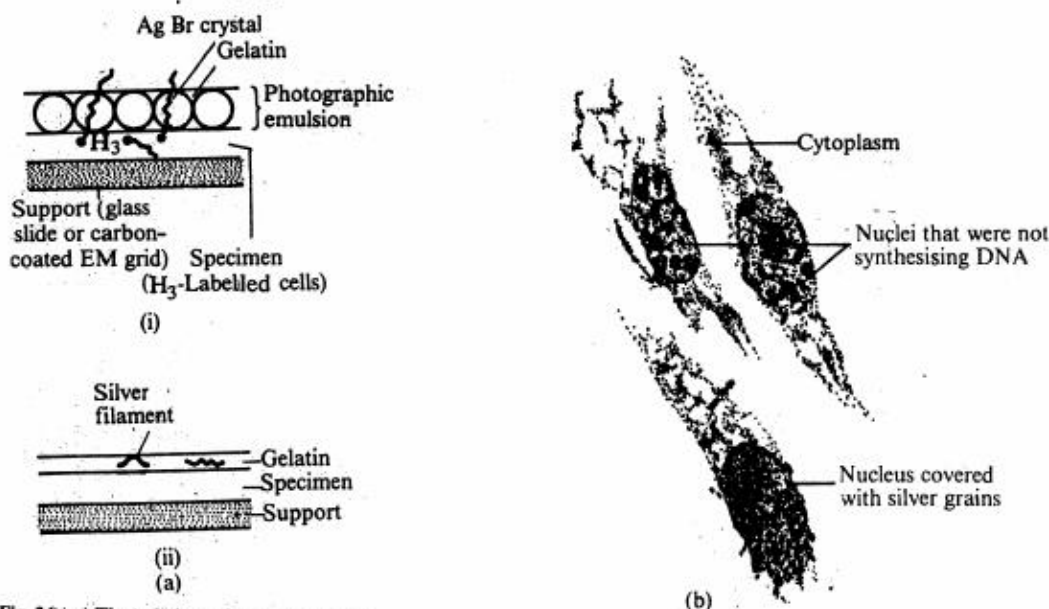


Fig. 2.8 : a) The technique of autoradiography.

Cells containing a suitable radioactive isotope after a time interval are attached to a support (glass slide in case of light microscopic autoradiography). The photographic emulsion (containing silver salts such as silver bromide) forms a layer over the cells. The regions of cells containing labelled molecules emit radioactive particles which sensitize the salts of the emulsion. The photographic emulsion is developed. The unexposed salts are dissolved and washed away. The silver grains show the location of labelled molecules in the cell.

b) An actual autoradiograph. The most commonly used tracers are ^{14}C and ^3H , as both are common constituents of organic molecules and do not involve high risk in handling by the scientists since they emit very weak beta particles.

Different radioisotopes are used for localising different macromolecules. As stated earlier, cells are labelled with ^3H thymidine for the study of DNA metabolism, ^3H uridine to study RNA metabolism, tritiated amino acids for examining the synthesis of proteins, tritiated monosaccharides such as ^3H -mannose and ^3H -fucose for examining the synthesis of polysaccharides.

Antibodies : Antibodies are proteins that bind tightly and specifically to the antigens, i.e., antibody-producing agent. Animals produce antibodies in response to an invasion by an infectious agent which acts as an antigen. Antigen causes an organism to make a larger number of different antibody proteins. Binding of an antibody with the antigen allows certain white blood cells to recognise the foreign substance and degrade it. Thus, antibody acts as a signal for the removal of infectious agents.

Antibodies

Antibodies are important tools for detecting and localising specific molecules in the cells due to their high specificity. The first requirement for this is to produce antibodies against a specific molecule. Suppose you want to produce antibodies against tubulin, the purified tubulin is first obtained from a given tissue. It is then injected into an animal such as rabbit which in response to tubulin produces antibodies. Blood sample is collected from the animal and antibodies are isolated. These antibodies are used to localise the specific molecule in the cell because they bind only to an antigen, which is a tubulin in this case. Localisation can be done by two methods: **direct** and **indirect**.

In the direct method, antibodies are first labelled with a fluorescent dye-like rhodamine for light microscopy, or with a high molecular weight, electron dense compound like ferritin for transmission electron microscopy. Fluorescent labelled antibodies are allowed to react with cells so as to form specific antigen-antibody complex. The complex is then examined under a microscope (fluorescent microscope or TEM). The specific molecules will emit light or become electron dense due to their binding with labelled antibodies (Fig. 2.9). This method is used to localise foreign bodies such as viruses, bacterial antigens, etc.

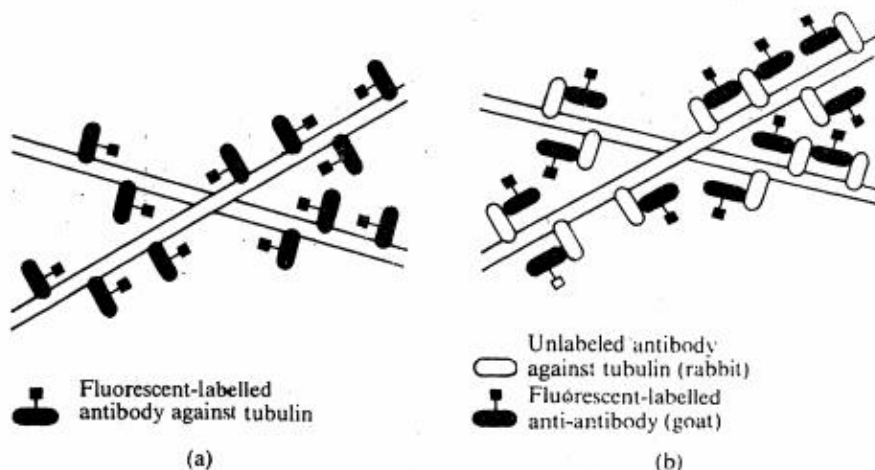


Fig. 2.9 : a) Direct fluorescent labelled antibody technique.

Tubulin antibodies attach only to microtubules, since they contain tubulin. The location of microtubules in the cell becomes evident, due to the fluorescence label of the antibody

b) Indirect fluorescent labelled antibody technique.

Two types of antibodies are used in this technique. The first one is produced in rabbit, against tubulin. It is not labelled with fluorescent material. The second antibody is produced in goat against the first type of antibody. The goat antibodies against rabbit antibodies are labelled with a fluorescent dye. The rabbit antibodies are sandwiched between antigen (tubulin) and labelled antibodies (goat antibody).

In the indirect method, two types of antibodies are used. The first antibody against tubulin is produced in the same way as described above. However, this antibody is not labelled. The second antibody is produced in another animal (it may be a goat), against the first antibody (rabbit antibodies). The second antibody binds only to the rabbit antibodies. The second antibody is labelled either with fluorescent dye or with ferritin. On reaction, the tubulin antibody (produced in rabbit) combines with the tubulin on the one hand and with the antirabbit antibody (produced in goat) on the other.

The advantage of the indirect method over the direct technique is that only one type of antibody containing a fluorescent label need be prepared. This one preparation can be used to light up any cell component that has been reacted with its homologous antibody.

SAQ 3

What is the advantage of indirect fluorescent labelled antibody technique over that of direct method? Give your answer in the space provided.

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2.5 TISSUE CULTURE AND CINEMICROGRAPHY

Tissue culture and cinemicrography are two important techniques which have helped greatly in the advancement of cell and molecular biology. Tissue culture helps in maintenance of cells alive outside the living organisms (plants and animals). Cinemicrography helps in following the events, specially slow events like cell division or cell locomotion. You will study about these two techniques in detail in the following sub-sections.

2.5.1 Tissue Culture

It is an important technique for maintaining a part or piece of animal or plant tissue alive after their removal from the organism. To maintain the living cell outside an organism, the tissue pieces or cells are kept usually in culture dishes. It is necessary to provide an environment in which cells can live and multiply over a period of time. Therefore, different media containing mixture of salts, amino acids, vitamins, growth factors etc. are used for this purpose. There are some special types of culture media such as Balanced Salt Solution (BSS), and Essential Minimal Medium (EMM).

Cultures prepared directly from the tissue of an organism are called **primary cultures**. For obtaining the primary cultures, the organ is removed in a sterile atmosphere so as to make it free from bacteria and other foreign bodies. Then it is cut into small pieces and treated with trypsin. Trypsin has the property of dissociating cell aggregates into a suspension of single cells, without affecting their viability. Cultures prepared from cells which were removed from primary cultures form **secondary cultures**. Such cells show the properties of the parent body from which they were obtained; for example, fibroblasts secrete collagen; cells derived from embryonic skeletal muscle form muscle fibres. These phenomena which otherwise cannot be studied in intact tissues are easily studied by this technique.

Some cells show prolonged growth and divide indefinitely *in vitro*. These cells are called 'variant cells'. Such cells grow or propagate in a linear order indefinitely to form a cell line. Some commonly used cell lines are BHK 21 and Human HeLa cells which are derived from fibroblast of a hamster and epithelial cells of man respectively.

Many of these cell lines were derived from tumor like growth. All these cells are capable of dividing indefinitely in tissue culture and they express at least some of the properties which are different from the cell of origin. BHK 21 cells, and HeLa cells, are capable of growing in suspension whereas other cell lines require a solid culture substratum for their growth.

Unlike variant cells, cancer cells divide indefinitely *in vivo* (within the living organism) as well as *in vitro* (in the experimental conditions). They grow rapidly compared to normal cells in a tissue culture dish.

Tissue culture is an important technique which is used to study the fundamental problems in cell biology. One of the major advancements in tissue culture is **cloning**. A clone is a population of cells derived from a single parent cell.

SAQ 4

What is the advantage of tissue culture technique? Write your answer in 2-3 lines.

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2.5.2 Cinemicrography

Earlier it was impossible to follow the events within the cell due to:

- non-visibility of cell organelles under a light microscope,
- maintenance of cells outside the body, and
- slow nature of events, like cell division or cell locomotion. It was not possible earlier to follow the events within the living cells. The first difficulty was overcome by the development of phase contrast microscopy and second was removed by the tissue culture technique. The third problem was solved with the onset of cinemicrography.

In cinemicrography the events in or about cells are continuously filmed by a camera attached to the eye piece of a microscope. The speed of exposure per unit time can be varied according to requirement. By an appropriate combination of the speed of exposure and the speed of viewing the film it is possible to see events that took 1 hour in just 1 min or 5 sec. This is very useful in making the observation on mitosis, cytokinesis, cell movement etc. On the contrary, in other cases, fast events can be slowed down for viewing, for example, the movement of cilia or flagella.

2.6 SUMMARY

To sum up, this unit dealt with the following:

- Observation of cellular structures is difficult because of their small size and lack of contrast in their components. These difficulties are overcome by the use of various tools and techniques such as light microscope and electron microscope. Cytochemistry deals with the identification and localisation of chemical components of the cell.
- Fluorescence microscopy is the technique by which components are recognised by the fluorescence they emit in the visible spectrum. Phase contrast microscopy is used for the study of living cells which are otherwise transparent to light.
- Electron microscopy (EM) is the best method for studying biological ultrastructure. The two types of EM are, transmission electron microscope (TEM) and scanning electron microscope (SEM).
- Freeze fracturing is a technique used to study the structure of membranes (plasmalemma, nuclear membrane etc.).
- Radioisotopes are used as tracers, to follow the course of a molecule or its metabolic pathways. In autoradiography substances labelled with radioisotopes are used. Like radioisotopes, antibodies are also used for localisation of macromolecules.
- Tissue culture is an important technique used to study live cells outside the parent body under suitable conditions.
- Likewise cinemicrography is another technique used to observe the flow of events within the living cells.

2.7 TERMINAL QUESTIONS

- 1) How can the resolving power of a microscope be increased? Give your answer in about 30 words.

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- 2) What are the applications of freeze fracturing? Write your answer in the space provided.

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- 3) Write two important uses of radioactive tracers in cell biology in the space given below.

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2.8 - ANSWERS

Self-assessment Questions

- 1) In the two reactions (Fehling and PAS), the first step is the exposure of free aldehyde groups of sugars (DNA, polysaccharides). This step is differently achieved in the two reactions. However the second reaction, that is, reaction of the free aldehyde group with Schiff's reagent to produce purple colour is common.
- 2) (a) SEM (b) LM (c) TEM (d) TEM
- 3) In indirect fluorescent labelled antibody technique, only one type of labelled antibody is required to detect and localise any antigen.
- 4) Tissue culture technique helps to observe cell/tissue of an organism in a suitable outside environment.

Terminal Questions

- 1) Resolving power of a microscope can be increased by using light of shorter wavelengths or increasing the numerical aperture of the objective lens (see Equation 2.1).
- 2) Freeze fracturing enables a study of the interior of cell membranes and membrane function.
- 3) Radioactive tracers are used to:
 - a) locate the site of synthesis of various molecules,
 - b) measure the rate of metabolic turnover of a material within the cell.
- 4) Primary cultures are obtained directly from an organism and secondary cultures are obtained from primary culture.

UNIT 3 FUNCTIONAL ULTRASTRUCTURE OF THE CELL

Structure

- 3.1 Introduction
 - Objectives
 - 3.2 Endoplasmic Reticulum
 - 3.3 Golgi Apparatus
 - 3.4 Lysosomes and Peroxisomes
 - 3.5 Mitochondria, Chloroplast and Nucleus
 - Mitochondria
 - Chloroplast
 - Nucleus
 - 3.6 Cytoskeletal System
 - 3.7 Summary
 - 3.8 Terminal Questions
 - 3.9 Answers
-

3.1 INTRODUCTION

In the preceding units, you have learnt about the evolution of cell and the characteristic features of procaryotic and eucaryotic cells. Besides, you have familiarised yourself with various tools and techniques to study the organisation of the cell. A cell consists of various cytoplasmic organelles. The present unit deals with the structure and functions of the organelles of eucaryotic cell, i.e. endoplasmic reticulum, Golgi apparatus, nucleus, mitochondria, chloroplast etc. Detailed functions of some of double membraned cell organelles like nucleus, ribosomes and mitochondria will be discussed later in the course.

In this unit, we shall study the structure and functions of endoplasmic reticulum, lysosomes and peroxisomes. The processes of endocytosis and exocytosis tend to delete or add to membrane components, but the surface area of the membrane remains constant through recycling of membrane components. Plastids are the important components of plant cells which contain respiratory and coloured pigments. They also act as store house of macromolecules. Cytoskeletal system of the cell constitutes microfilaments and microtubules concerned with the movement in and around the cell and keeps the cell in shape.

You have studied the basic structure of the cell in Units 1 and 2. You also know the basic differences between animal and plant cell.

Objectives

After studying this unit, you will be able to:

- identify the various organelles of a eucaryotic cell in the light micrographs and electron micrographs and state their functions,
 - describe the structure and function of cilia, flagella, smooth and rough endoplasmic reticulum, Golgi apparatus, mitochondria, chloroplast and nucleus, and
 - explain recycling of membrane components in a cell with suitable examples.
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3.2 ENDOPLASMIC RETICULUM (ER)

Eucaryotic cells have two major compartments— nucleus and cytoplasm. Cytoplasm was known to have no structure until the discovery of electron microscope. That cytoplasm is permeated by a membranous network called **endoplasmic reticulum (ER)**, was revealed only after the introduction of electron microscope. Endoplasmic reticulum is a three dimensional network of membrane channels which constitutes more than half of the total membrane of the cell. ER is a highly folded and convoluted structure and forms a single continuous sheet enclosing one continuous sac. The interior of the sac is called "**cisternal**

space" or "ER lumen". This is separated from the cytoplasm also called cytosol by a single membrane which mediates the communication between these two compartments. ER provides the cell with a compartment for storage of substances to be kept separate from cytosol. In addition, it has a key role in the biosynthesis of macromolecules.

ER is a double membraneous organelle which consists of interconnecting flattened sacs called **cisternae** or interconnected **tubules** or **vesicles**. Cisternae are temporary storage sites for nutrients (Fig. 3.1 a).

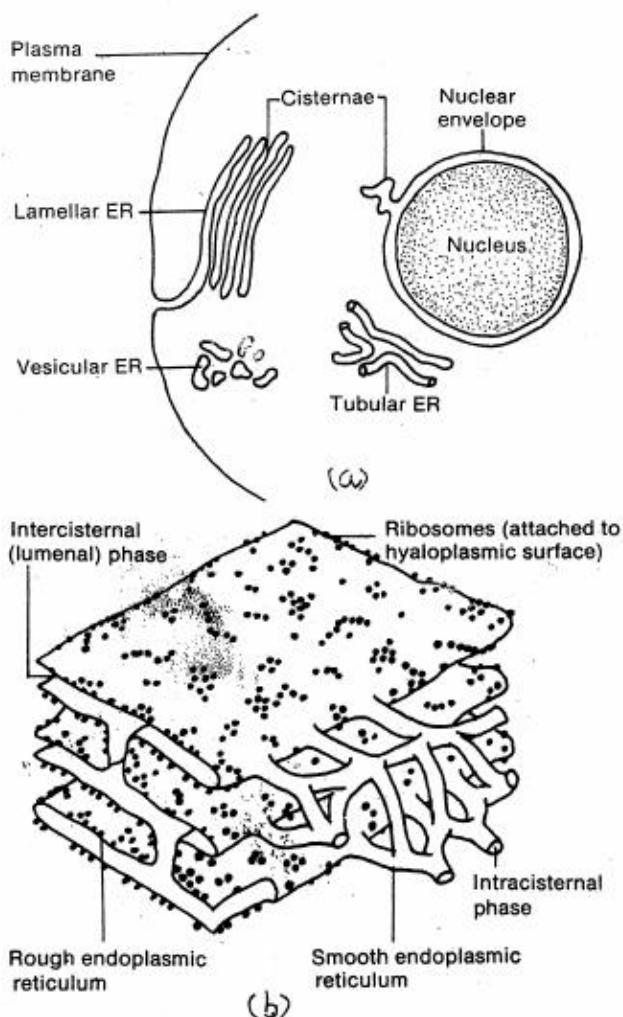


Fig. 3.1 : a) Three physical forms of ER, lamellar, vesicular and tubular. The lamellar type of ER is in the form of flattened membrane sacs, the vesicular type of ER is in the form of vesicles, and tubular type of ER is in the form of tubules.

b) Endoplasmic reticulum shows the continuity of ER types that exists between adjacent membranes and cisternae of both rough and smooth ER. Rough ER membranes are characteristically sheet like, whereas smooth ER membranes are characteristically tubular.

Rough and Smooth Endoplasmic Reticulum (RER and SER) : ER is differentiated into two regions, **granular** or **rough endoplasmic reticulum (RER)** and **agranular** or **smooth endoplasmic reticulum (SER)**. These two regions also differ considerably in shape: rough ER is organised in stacks of flattened sacs called cisternae and smooth ER consists of a mesh work of fine tubules (Fig. 3.1 b). In RER, the outer surface in the cytoplasmic site of the membrane is studded with small particles called **ribosomes** whereas in SER, the ribosomes are absent. Ribosomes are the sites of protein synthesis. Thus, it is evident that RER is more abundant in cells which are actively engaged in synthesis and export of proteins, for example, pancreatic acinar cells and plasma cells. SER is found in those cells which are specialised in lipid metabolism and which secrete steroids such as the cells of adrenal cortex, the testes and ovary. Smooth ER is also present in liver cells where it helps in detoxification of drugs and poisons.

ER (both RER and SER) performs many mechanical functions of the cell by providing mechanical support. Large surface area of ER helps in the exchange of materials across the membrane by diffusion and active transport. ER may act as a kind of circulatory system for the distribution of various substances in the cell.

SAQ 1

Which processes do you expect to occur in following cells? Write your answer in the space provided.

i) A cell in which there is an extensive network of RER.

ii) A cell in which there is a lot of SER.

3.3 GOLGI APPARATUS

Camillo Golgi in 1898 discovered a reticular structure in the cytoplasm of nerve cells with the help of metal impregnation technique using silver nitrate for which he received the Nobel Prize. This structure was named as Golgi apparatus or Golgi complex after him. Golgi complex is located near the cell nucleus.

Golgi complex is structurally similar in both plant and animal cells, but in plant cells it is more evident and is called as **dictyosomes** which are stack-like or plate-like bodies. Animal cells contain comparatively much smaller number of Golgi apparatus than plant cells.

The Golgi complex consists of (a) stack of flattened sacs or **cisternae** (b) small rounded **transport vesicles** (c) larger **vacuoles** filled with amorphous or granular material.

The cisternae, which are flattened sac like vesicles of Golgi complex, are arranged in parallel series and are separated by a space of 20-30 nm. Cisternae are gently curved so that the Golgi bodies give a bow like appearance (Fig. 3.2). The convex side facing the endoplasmic reticulum is '**forming**' or '**proximal**' face, whereas the concave side oriented towards cell surface is the '**releasing**' or '**distal**' face of the complex.

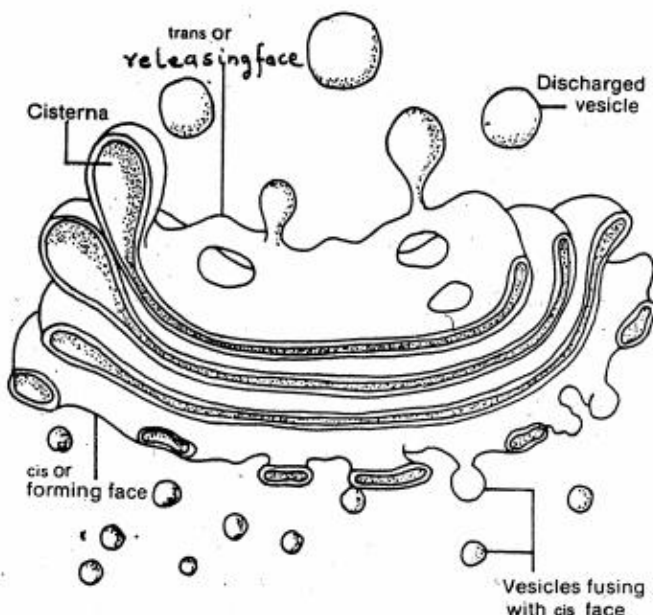
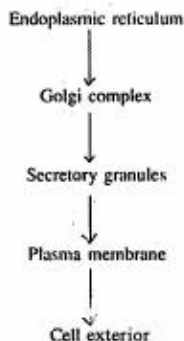


Fig. 3.2 : Golgi apparatus based on the study of electron micrograph. The cisternae exhibit a structure having two faces i.e. forming face (convex) and releasing face (concave). The forming face is generally curved and closer to the endoplasmic reticulum and the nuclear membrane. The releasing face is concave shaped and encloses a region containing large secretory vesicles.

Small vesicles called transition vesicles are frequently seen lying between rough endoplasmic reticulum and the forming face. It is thought that these vesicles are formed from the ER and migrate to Golgi, where they form new cisternae, which get fused with existing cisternal membrane, and thus the growth of the organelle occurs. New cisternae are formed at the forming face to compensate for the loss of secretory vesicles of the releasing face. The transformation of membrane from one type to another is a step which leads to the formation of a vesicle. The chemical composition of Golgi complex is intermediary between ER and the plasma membrane.

Golgi apparatus is composed of at least three types of sacks which contain different types of enzymes. The most characteristic enzymes are transferases, for example, glycosyl transferase. Besides this, acid phosphatases and other lysosomal and oxidative enzymes are also present.

Golgi apparatus performs many functions in the cell such as processing of the molecules secreted by the ER, and packaging of the molecules according to their final destination as shown below:



In plant cells, Golgi apparatus helps in the formation of cell plate and cell wall.

+SAQ 2

Golgi complex is involved in the transport and processing of many substances that are produced in ER and are discharged outside the cell. How? Write your answer in the space given below.

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Recycling of Plasma Membrane Components

During exocytosis secretory vesicles fuse with plasma membrane, adding to the membrane surface area. Yet, the surface area of plasma membrane remains constant. This is due to recycling of the membrane components. This recycling is carried out by subsequent return of the internalised membrane components and by removal of excess membrane through internalisation. For example, plasma membrane of thyroid cells can bind with ferritin, endocytosis is induced by addition of thyrotropin to the cells. Immediately after endocytosis, ferritin appears in lysosomes, and within 30 minutes it can be detected in cisternae of Golgi complex (Fig. 3.3).

Likewise, endocytosis results in the internalisation of plasma membrane. Golgi complex may reprocess and reuse the membrane components after endocytosis, and use them for secretion, lysosome formation or restoration of plasma membrane itself. That is how the Golgi apparatus functions in recycling of the membrane components.

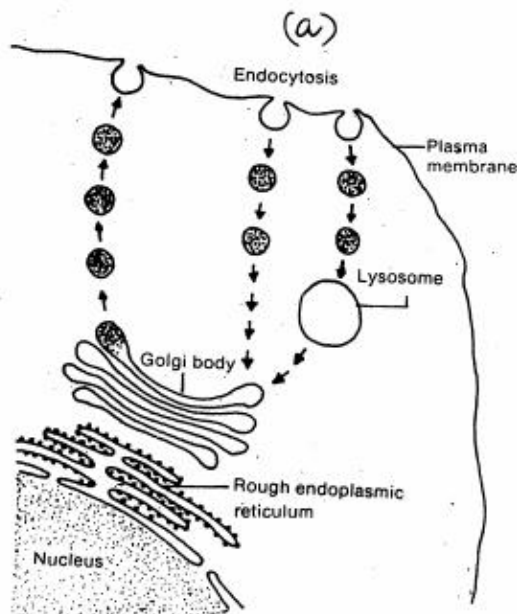
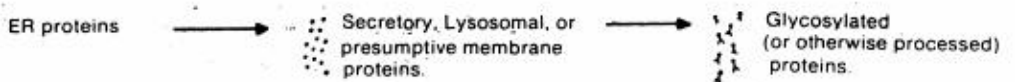
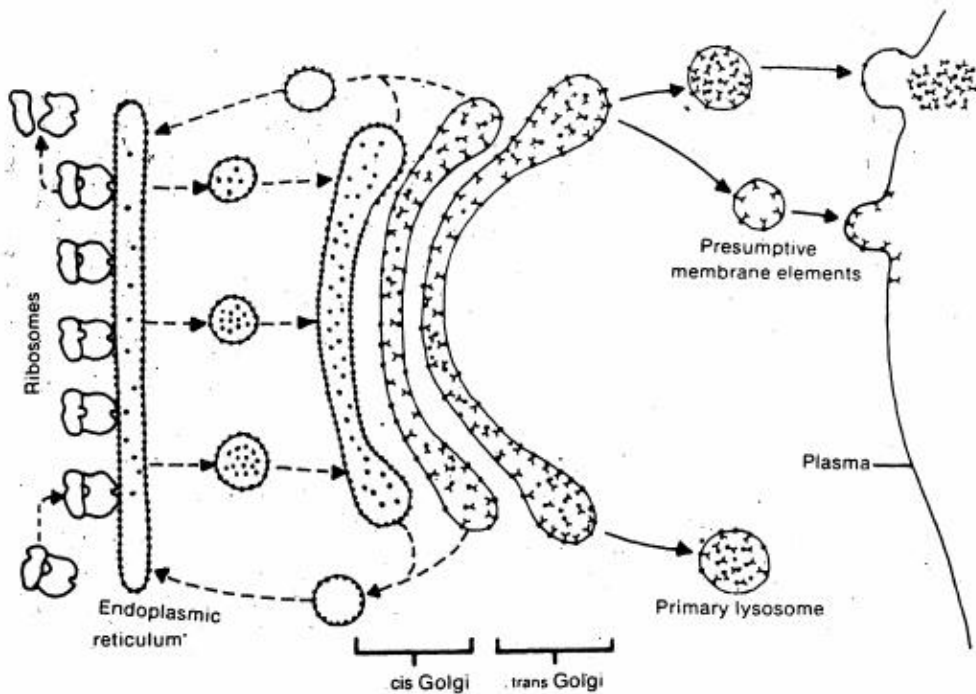
SAQ 3

A cell is very active in secretory activities. Which organelle do you expect to take this activity of the cell and why? Give one reason in the space provided.

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(b)

Fig. 3.3 : a) Secretion of proteins across membranes. Proteins from ER enter the forming face of the Golgi. Proteins from ER destined to be secreted out are successively purified and are discharged as vesicles from the distal face.
b) Role of the Golgi apparatus in recycling and reuse of plasma membrane components, which enter the cytosol during endocytosis.

3.4 LYSOSOMES AND PEROXISOMES

Lysosomes were first discovered by Christian de Duve. Unlike other organelles which were detected by microscope, these were first discovered biochemically. Later, electron microscope and biochemical methods shed more light on their nature and their participation in cell activities.

These particles were called **lysosomes** (lysis = dissolution, soma = body) due to their hydrolytic activity. A lysosome is a single membrane bound vesicle that either buds off from the releasing face of Golgi apparatus or arises directly from ER. The lysosomes are rich in **hydrolases, specially acid phosphatase**, of about 50 different types which digest all the significant groups of macro molecules. These enzymes are active at pH 5 within the organelle. The enzymes, which escape from lysosomes, become slow in their action because the pH of cytoplasm is 7.0. This large pH difference is a device to protect the cell from digestion.

Polymorphism in Lysosomes

Polymorphism, i.e. existence of a structure in more than one form, is an important feature of lysosomes. Several different forms of lysosomes have been identified within the cell as **primary lysosomes, secondary lysosomes, residual bodies** and **autophagic vacuoles** (Fig. 3.4).

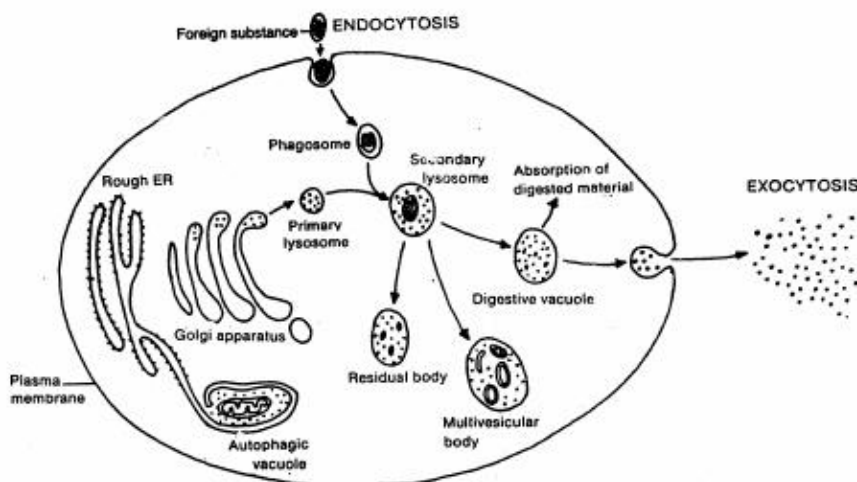


Fig. 3.4 : Figure showing the polymorphism of lysosomes. Primary lysosomes containing digestive enzymes fuse with the phagosome to form secondary lysosome. Digestion products are released by exocytosis. Primary lysosome may fuse with some organelles like mitochondria to form autophagic vacuole. Incomplete digestion of foreign substances form residual bodies.

Primary lysosomes: These are newly formed organelles. They are believed to be derived from the maturing face of Golgi complex whose digestive enzymes have not yet taken part in hydrolysis.

Secondary lysosomes are formed from fusion of primary lysosomes with the vesicles containing variety of substrates known as 'phagosome'. After fusion, their membrane undergoes a change and enzymes are activated so that the substrate is digested. After activation they may continue hydrolysis repeatedly. The secondary lysosomes which fuse with vesicles containing extracellular substrate brought into the cell by endocytosis are known as heterophagic lysosomes or heterophagosome whereas lysosomes fusing with vesicles containing particles isolated from cell's own cytoplasm like mitochondria, microbodies, and fragments of endoplasmic reticulum are known as **autophagic vacuoles**. During pathological conditions or during cell growth, autodigestion of cellular organelles is a normal event.

Residual bodies: Incomplete digestion of foreign substances leads to the formation of residual bodies. Residual bodies are huge, irregular in shape and are electron dense. In some

cells they remain for a long time and play a role in the aging process. In some other cells, the content of the residual bodies leave the cell by exocytosis.

Lysosomes are responsible for the intracellular digestion of a variety of substances such as food molecules, disease causing organisms, etc. This process is called heterophagy. Lysosomes are also responsible for digestion of cell's own cytoplasmic constituents. This process is called **autophagy**. It is important that lysosomes do not **rupture and release their contents inside living cells** as they would start digesting the cell and cell will die.

SAQ 4

Which form of lysosomes are found in abundance during pathological conditions and why?

Give your answer in about 30 words.

Endocytosis and Exocytosis

Endocytosis is the process by which substances are brought into the cell from outside by formation of membrane bound vesicles. These extra cellular substances are enclosed in a small portion of plasma membrane which invaginates and pinches off to form an intracellular vesicle. The intake of fluids by formation of vesicles is called as **pinocytosis** (pinen = drink). Pinocytosis is induced by the presence of appropriate concentrations of inducer molecules which can be proteins, amino acids or certain ions in the medium surrounding the cell (Fig. 3.5a). The ingestion of large particles by formation of larger vesicles is known as **phagocytosis** (phagein = to eat) (Fig. 3.5 b). The process of phagocytosis is associated with heterophagic activity of the lysosomes such as the destruction of worn blood cells in the liver, spleen and bone marrow. Exocytosis is the reverse sequence where the membranous intracellular vesicle fuses with plasma membrane and releases its content to the extracellular surroundings. The best understood form of exocytosis is secretion by the cell. As you have studied earlier, Golgi apparatus plays an important role in the formation of cytoplasmic intracellular vesicles. The membrane of these vesicles is incorporated into plasma membrane and the vesicle's contents are discharged to the exterior.

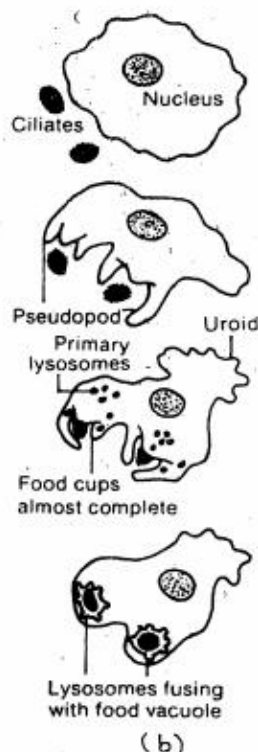
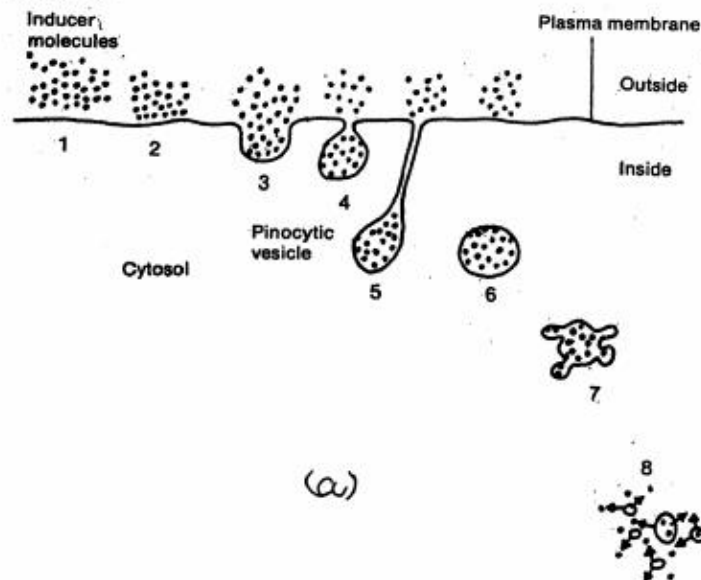


Fig. 3.5 : a) Stages of pinocytosis. 1-2 binding of inducer molecules to plasma membrane, 3-5 invagination of the membrane, 6-8 detachment from plasma membrane and fragmentation into smaller vesicles.
b) Different stages of phagocytosis. In the presence of food e.g. ciliates shown in fig (i) the cell forms pseudopodia (ii) which entrap the prey in vacuole (iii) the fusion of primary lysosomes with these vacuoles is followed by digestion of prey (iv).

Microbodies: Peroxisomes and glyoxysomes are microbodies formed from ER which superficially resemble the lysosomes. Peroxisomes are spherical bodies limited by a single membrane. The centre of peroxisomes is occupied by a fine granular core called 'nucleoid'.

Peroxisomes contain oxidising enzymes which are synthesised by ribosomes. These enzymes are uric acid oxidase, D-amino acid oxidase, hydroxyl acid oxidase which produce hydrogen peroxide on oxidation, while peroxisomal catalase, the enzyme found in lysosomes, destroys hydrogen peroxide. Since hydrogen peroxide is toxic to the cell, catalase plays a protective role by breaking down hydrogen peroxide. Hence, peroxisomes play an important role in detoxification. In green plants, peroxisomes carry out a process called photorespiration.

Glyoxysomes are a form of peroxisome which contain enzymes like isocitrate lyase, and malate synthetase which are specific to glyoxylate cycle. They also have several Krebs cycle enzymes about which you will study later. Glyoxysomes are the essential components of plant cell.

3.5 MITOCHONDRIA, CHLOROPLAST AND NUCLEUS

Mitochondria, chloroplast and nucleus are important organelles of the cell. All these organelles share a common characteristic, i.e., they are enclosed with double membranes. Mitochondria are the main energy transducing organelles in animal cells. In plant cells this function is performed by chloroplasts. Both these organelles have structural similarities.

Nucleus is the "central and commanding" organelle of the cell. It is an important distinguishing feature of prokaryotic and eukaryotic cell.

You will study about the structure and function of mitochondria, chloroplast and nucleus in the following sub-sections.

3.5.1 Mitochondria

Mitochondria, earlier called bioblasts, are found in the cytoplasm of eukaryotic cells and are characterised by specific morphological, biochemical and functional properties.

Mitochondria (mito—thread, chondrion—granules) are generally rod shaped elongated structures. They are commonly known as the "power house" of the cell, as they are the sites of ATP (energy currency) production in the cell. Mitochondria are considered to be capable of movement, changing in both shape and position within the cell.

A mitochondrion consists of two membranes (outer and inner) and two compartments (outer and inner). The outer three layered membrane is separated by a space of 6-8 nm from the inner membrane. The inner membrane has a number of infoldings which are called **cristae** or **mitochondrial crests** (Fig. 3.6). Cristae vary in number and shape. Generally they lie parallel to one another, or are stacked on top of each other forming incomplete compartments as in liver cells. The number of cristae depends on metabolic activity of the cell. Mitochondria themselves are often found concentrated in region of high metabolic activity such as cells of cardiac muscles, flight muscles of insect and bird etc.

The fluid filled space between the outer and inner membrane is called the **intermembrane space**. The space surrounded by inner membrane is called **mitochondrial matrix**. It is dense and is made-up of proteinaceous material. The matrix is generally homogeneous but sometimes, may contain a fine filamentous material or small dense granules which are the sites for binding of Mg^{2+} and Ca^{2+} ions. Matrix is continuous within the mitochondria as the cristae do not divide the inner chamber into separate sections. Matrix contains ribosomes, RNA and one or more molecules of circular DNA; an important characteristic of mitochondria. It is because of the presence of DNA, RNA and ribosomes that mitochondria are self replicating and can synthesise some of their own proteins and membrane material.

The enzymes involved in TCA cycle and fatty acid oxidation are found in the matrix. Oxidation and reduction reactions are specifically associated with the inner membrane of mitochondria which brings about the conversion of ADP to ATP.

Energy transducing: There are different sources of energy like radiant energy, chemical compounds etc. Whatever be the source, it must be converted into a useful form, i.e. adenosine triphosphate (ATP). This transformation is called as energy transformation.

Energy currency (ATP): The free energy derived from the oxidation of foodstuffs and from light is transformed into a special form before it is used for movement, active transport and biosynthesis etc. This special carrier of free energy is ATP.

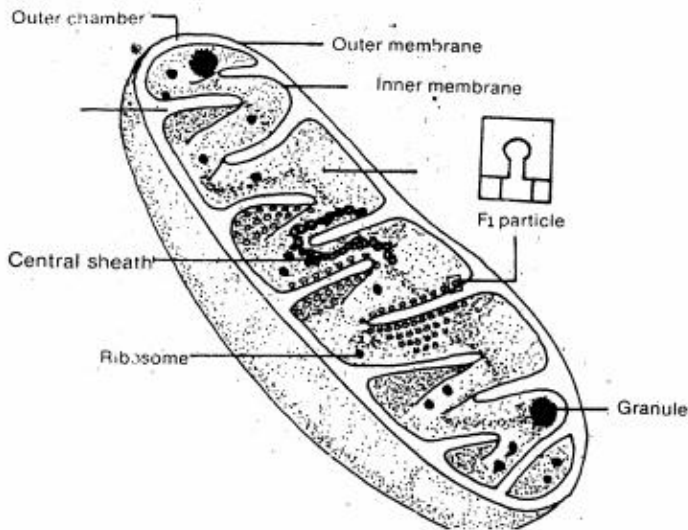


Fig. 3.6 : Three-dimensional diagram of a mitochondrion (longitudinal section). The cristae are the folds of the inner membrane. On their matrix side they have the F_1 particles. The inset shows an F_1 particle with the head piece and stalk.

Cristae appear to be covered with mushroom-like particles, called as **F_1 particles**, **elementary particles** or **oxysomes**. These particles are small stalked sphere-like structures containing an enzyme, ATPase, that is involved in oxidative phosphorylation. You will study about this in more detail in Block 3. F_1 particles cannot be seen in the intact mitochondria as they are an integral part of the inner membrane.

Since the inner membrane of the mitochondria is the site of ATP production in the cell, greater the number of cristae, the larger the surface area for ATP production.

The structure of mitochondria changes according to the **physiological** activity occurring in the organelle. If the external ADP content is low or the **respiratory chain** is inhibited, the mitochondria are seen in **orthodox state** or inactive state. In this state, matrix occupies a larger area of the mitochondria and consequently the outer chamber becomes small.

However, if ADP is added to the medium, there is a sudden contraction of the inner compartment. This is called the **condensed state**, i.e. when mitochondria are actively involved in phosphorylation and electron transport. In the condensed state, the cristae are more randomly distributed and the intermembrane space remains greatly enlarged.

3.5.2 Chloroplast

Plastids are the organelles found in plant cells only. Like mitochondria they are bounded by two membranes. Plastids self replicate as they contain their own genetic material, i.e. DNA, RNA and ribosomes. There are many types of plastids such as **chloroplasts**, **chromoplasts** and **leucoplasts** depending on the colour pigment they contain.

Chloroplasts, that are mainly found in the cells of leaves of green plants and the most common ones are biologically important plastids. Chloroplasts are limited by a smooth outer membrane which regulates the transport of materials between the cytoplasm and the interior of the organelle. The **inner membrane** runs parallel to the outer membrane and is provided with extensive foldings. The inner membrane gives rise to a series of internal parallel membraneous sheets called lamellae. Lamellae are suspended in a fluid-like matrix called **stroma**. Stroma contains about 50% of soluble protein, ribosomes, DNA and the machinery for protein synthesis (Fig. 3.7).

Most of the lamellae in the chloroplasts are organised to form sac like structures called **thylakoids**. They are flattened vesicles arranged as a membranous network within the stroma. Thylakoids may be stacked like a pile of coins forming the 'grana'.

A typical chloroplast has between 40-60 grana and each granum may have 2-100 small, flattened thylakoids. When thylakoids are unstacked they are called **stroma**. Fifty per cent.

of the total chloroplast proteins and the various components involved in the main steps of photosynthesis are present in thylakoids (Fig. 3.7).

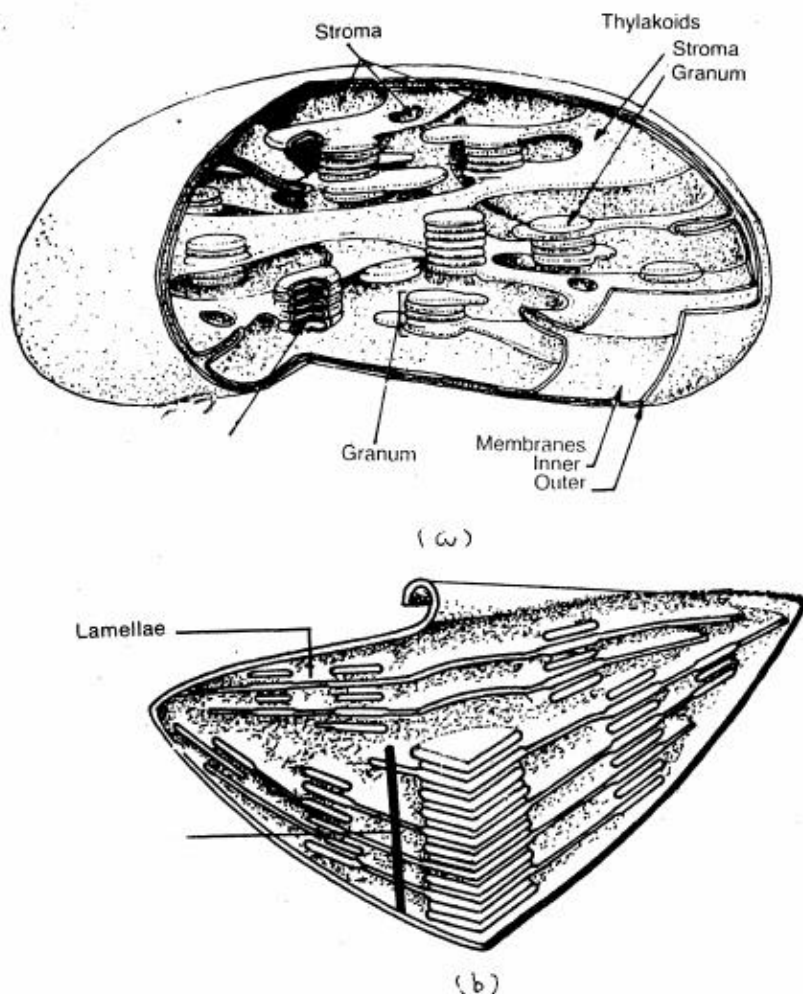


Fig. 3.7 : a) Structure of chloroplast. The inner membrane of a chloroplast is fused to form stacks called thylakoids within which photosynthesis takes place. Thylakoids are stacked on top of each other in columns called grana.

b) Cross-section of a chloroplast showing the arrangement of lamellae and grana.

The pigments, chlorophyll, carotenoids and plastiquinone are present in the thylakoid membranes involved in photosynthesis. Chlorophyll is the green coloured pigment present in chloroplasts. The function of chlorophyll is to trap the light energy required for the formation of two products: ATP and NADPH essential for the reactions involved in CO_2 -assimilation. Chromoplasts are the plastids that synthesise and store the coloured pigments like carotenoids. Carotenoids, which include carotene and xanthophyll, are responsible for the yellow, orange or red colouration in plants. They also act as the precursor of vitamin A in animal tissues. Leucoplasts are colourless plastids which act as storage organelles and are classified on the basis of the material stored, e.g. amyloplasts store carbohydrate in the form of starch.

Some Relationships between Mitochondria and Chloroplast

- Both, mitochondria and chloroplast originate and develop in the same way. They are formed by fission of pre-existing organelles, as for example, **proplastids** develop to form the mature chloroplast.
- Both are **semiautonomous organelles** as both contain DNA, RNA and ribosomes. All these components are required for protein synthesis. Mitochondrial DNA (mtDNA) present in matrix is circular in shape. DNA of chloroplast is much bigger than mtDNA. Though ribosomes are also present, but the genetic information of the chloroplasts is limited.

- c) The symbiont hypothesis suggests that there are many similarities between procaryotes and mitochondria and chloroplasts, e.g. the presence of DNA and ribosomes. It was thought that mitochondria and chloroplasts may have been procaryotic intracellular parasites which in course of time established symbiotic relationship with eucaryotic cells.

3.5.3 Nucleus

Nucleus is the dominant organelle controlling all the activities of the eucaryotic cell. As you have studied in Unit 1, the procaryotes have nuclear regions in the cytoplasm as opposed to eucaryotes, that have a prominent well defined nucleus. Some cells have more than one nucleus, as for example, rat liver cells have 2-3 nuclei per cell. There are variations in the size and shape of the nucleus. However, some cells lack nucleus at maturity such as RBCs and sieve tube cells (transport cells in vascular plants).

Nucleus was considered to have a single membrane till the use of electron microscope. Under EM, nucleus is found to be composed of two membranes known as "**nuclear envelope**". The outer and inner membranes are separated by a narrow space called the **perinuclear space**. The outer membrane remains in contact with endoplasmic reticulum and the inner membrane surrounds the nuclear contents. At certain places, the nuclear envelope is interrupted by the presence of small structures called "**pores**". The pores are enclosed by circular structures called **annuli**. The pores and annuli together constitute the **pore complex** (Fig. 3.8). Both the membranes of the envelope are in continuity around these pores. The pores help in the exchange of material between nucleoplasm (nuclear fluid) and cytoplasm. Without these pores RNA could not leave the nucleus. The nuclear envelope is a dynamic structure. It is not just a physical barrier but regulates the passage of ions and small molecules. During a cell division, the nuclear envelope disappears and reappears during nuclear reorganisation.

Nucleoplasm contains a number of structures like nucleolus, chromatin and chromatin network (chromosomes). The nucleolus is a spherical structure which is not separated from the rest of the nucleoplasm by a membrane. It is produced from and is associated with a specific nucleolar organising region (NOR) on a chromosome. In the nucleolus the rRNA is synthesised and the ribosomal subunits, rRNA and proteins are partially assembled. The nucleoli are larger and more numerous in cells that are actively involved in protein synthesis.

During the resting stage of the cell, the chromosomes are uncoiled in a loose, indistinct network called "**chromatin**". Chromosome contains DNA, RNA and protein. The types of protein present are histones and non-histones (see Unit 1).

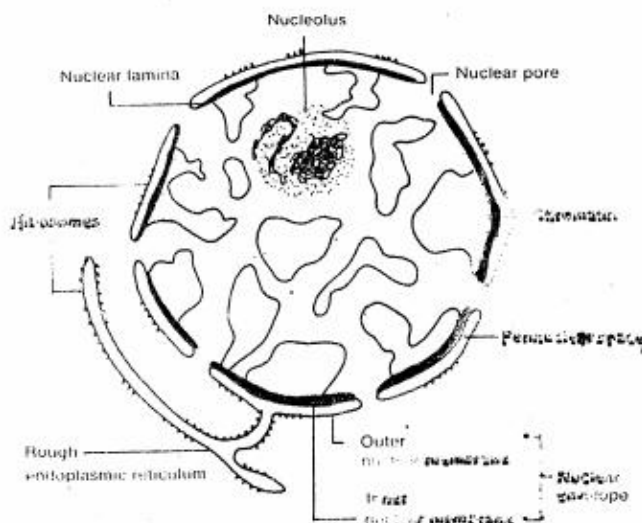


Fig. 3.8 : The nucleus is composed of a double membrane called nuclear envelope which encloses a fluid called nucleoplasm. In cross-section, the individual nuclear pores are seen to extend through the two membrane layers of the envelope.

SAQ 5

- a) In the cells of amebae a lot of energy is required for the transport of materials from the lumen of the intestine into the cells. Which organelle do you think would be very much concentrated in these cells and why? Write your answer in the space provided.
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- b) Nuclear membrane is interrupted by nuclear pores at several places. What is their significance? Write your answer in the space given below.
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3.6 CYTOSKELETAL SYSTEM

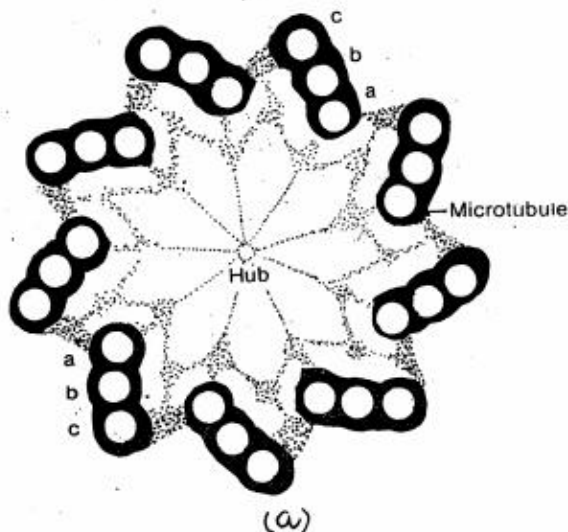
Eucaryotic cells have distinct shapes and a high degree of internal organisation. Moreover, they are capable of changing their shape, of repositioning their internal organelles and in many cases, of migrating from one place to another. These properties of shape, internal organisation and movement depend on complex networks of protein filaments in the cytoplasm that serve as the "bone and muscle" of the eucaryotic cell, which constitutes the cytoskeletal system of the cell. Cytoskeleton maintains the cell shape and helps in the cell movements.

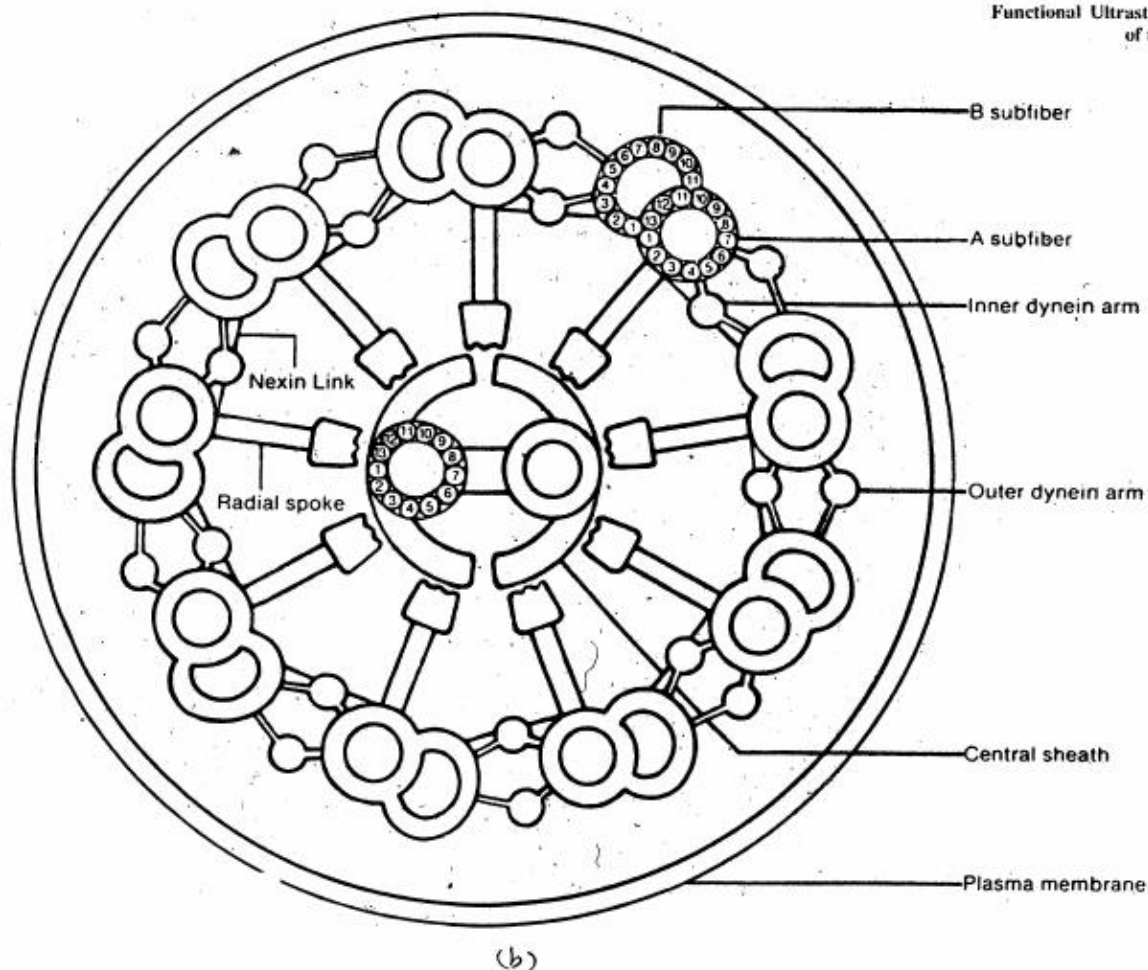
You will study about the structure and function of centriole, cilia and flagella, microtubules and microfilaments, the basic elements of cytoskeleton in this section.

Centrioles and Basal Bodies: Centrioles are usually found near the cell nucleus and occur in pairs. Nine sets of identical triplets of microtubules arranged in a radial manner is an important characteristic of centriole (Fig. 3.9). Basal bodies are structurally similar to centrioles but they produce cilia and flagella unlike centrioles.

Cilia and Flagella

Cilia and flagella are the locomotor organelles that help to propel a cell towards or away from the materials surrounding the cell. Cilia are generally shorter in size and are more in number than flagella. Flagella usually occur alone or in small groups. The structure of flagella and cilia are similar. Each contains a circle of nine pairs of microtubules with two single microtubules in the centre called as axoneme. Proteins unite the microtubules with axoneme and with each other (Fig. 3.9).





(b)

Fig. 3.9 : a) Diagram of centriole: A centriole has nine sets of identical triplets, each triplet consists of 3 microtubules, one complete (a) and two incomplete (b, c) and the triplets are arranged parallel to one another and in cross-section appear to be arranged like the vanes on a pinwheel. There is no membrane surrounding the centriole. Strands of material extend inwards from each 'a' tubule and join together at the central hub. These strands, when seen in cross-section, give the centrioles the appearance of a 'cartwheel'. b) Diagram of a cilium or flagellum seen in cross-section. Cilia and flagella contain the '9+2' arrangement of microtubules. Nine double microtubules surround the central sheath of two singlet microtubules. One microtubule of each doublet of a subfiber is composed of 13 protofilaments. The B subfiber is incomplete consisting of 11 protofilaments. Radial spokes from each subfiber A extend to the central sheath. Adjacent doublets are joined by interdoublet links extending from each A subfiber are two arms an 'outer' arm and an 'inner' arm which contain a protein dynein that can break down ATP.

Microtubules and Microfilaments

Tubules are the most prominent structural filaments. They are long, hollow cylinders which are polymers of protein tubulin. They help to maintain the cell shape and are important for intracellular movement. Microtubules form dynamic structures such as asters and spindles (related to cell division) and complex organelles such as centrioles, basal bodies, cilia and flagella. During cell division, the microtubules radiate out from the cell centre which is microtubule organising centre and is located near the nucleus. These microtubules are the aster and spindles which play an important part in equal distribution of the chromosomes to the new cells.

Microfilaments are found in close proximity of microtubules and form a network close to the cell membrane. They are made-up of actin and are in continuity with the network of the cytosol. They are the smallest of the cytoplasmic filamentous structure. They perform some cytoskeletal and contractile function and help in cellular motion.

In addition to microtubules and microfilaments, a number of cells contain a cytoskeletal component consisting of filaments of an intermediate size which help in anchorage and

3.7 SUMMARY

In this unit you have studied that:

- The cells have various dynamic cytoplasmic structures which have specific functions to perform. These cytoplasmic organelles have single or double membranous structure. Mitochondria, nucleus and plastids are double membranous while Golgi bodies, and lysosomes have a single surrounding membrane.
- The endoplasmic reticulum is double membranous cell organelle. There are two types of ER: RER and SER. RER is well developed in cells which are actively engaged in protein synthesis whereas SER is well developed in those cells which are actively involved in lipid synthesis.
- The Golgi complex (dictyosome in plants) is located between ER and plasma membrane. Each dictyosome has two faces having a proximal face near the nuclear membrane and a distal face which yields secretory vesicles.
- Lysosomes have a digestive function both in plant and animal cells. They contain hydrolases which bring about digestion of the biological substances. Lysosomes exhibit polymorphism which means occurrence of lysosomes in different forms: primary lysosomes, secondary lysosomes, autophagic vacuoles and residual bodies.
- Endocytosis is the process of taking in solid and liquid food by phagocytosis and pinocytosis respectively. Exocytosis is the opposite process through which inside content of cell is removed outside cell.
- Mitochondria, the power house of the cell are cylindrical structures having outer and inner membranes. Inner membrane is folded into a number of cristae which expose F_1 particles upon hydrolysis of membrane. Mitochondrial matrix contains DNA, ribosomes and all the enzymes which take part in Tri Carboxylic Acid cycle (TCA).
- Plastids are of various types: chloroplasts, amyloplasts, and leucoplasts. Chloroplasts are most abundant and are present in all green plants. Chloroplasts contain a matrix substance called **stroma** in which number of **lamellae** or thylakoids are suspended. The stacked **thylakoids** constitute grana.
- Nucleus is a double membranous structure. Outer membrane or envelope remains in continuation with ER whereas inner membrane encloses nucleoplasm. In nucleoplasm, the chromatin network is embedded. The nucleus contains DNA, RNA and proteins which are both acidic and basic.
- Centriole, cilia, flagella, microtubules and microfilaments constitute the cytoskeletal system. Centriole is present mostly in animal cells. The cross-section of centriole reveals nine triplets of microtubules. Each triplet having 2 complete (A and B) and 1 incomplete (C) rings. In the centre there is no microtubule, thus exhibiting 9+0 pattern. Centrioles take part in cell division. Cilia and flagella have similar structure but they differ in having centrioles with 9+2 pattern, 9 microtubules are in the form of doublets (A and B) and the central 2 microtubules. The A and B rings have dynein arm. Cilia are more in number and smaller in size than flagella. Both help in locomotion.

3.8 TERMINAL QUESTIONS

- 1) How do peroxisomes play a role in detoxification? Write your answer in 3-4 lines.

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- 2) Why are mitochondria and chloroplasts considered as semiautonomous organelles? Write your answer in about 35-40 words.

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- 3) Write two important differences between structural organisation of centriole and cilia or flagella.

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3.9 ANSWERS

Self-assessment Questions

- 1) i) Protein synthesis
ii) Lipid metabolism
- 2) This is due to location of Golgi complex between the ER and plasma membrane.
- 3) Golgi complex, as it plays an important role in secretion.
- 4) Autophagic vacuole, because during pathological conditions, autodigestion of cellular organelles, mostly mitochondria takes place to generate energy.
- 5) a) Mitochondria, because these generate energy (ATP) in the cell.
b) The pores help in the exchange of material between nucleoplasm and cytoplasm.

Terminal Questions

- 1) The enzyme catalase present in peroxisomes breaks down H_2O_2 , a toxic substance into a non-toxic substance and thereby plays an important role in detoxification.
- 2) DNA, the hereditary material, and ribosomes, the machinery for protein synthesis are present in mitochondria and chloroplasts, hence they are considered as semiautonomous organelles.
- 3) i) In centriole the microtubules have '9+0' organisation whereas in cilia/flagella, the microtubules have '9+2' organisation.
ii) In centriole, 9 microtubules are in the form of triplets whereas in cilia/flagella, 9 microtubules are in the form of doublets.

UNIT 4 MOLECULES OF THE CELL

Structure

- 4.1 Introduction
 - Objectives
- 4.2 Types of Molecules Present in a Cell
 - Water
 - Basic Organic Molecules
- 4.3 Isolation and Detection of Macromolecules
 - Monitoring the Purification Procedure
 - Specific Activity
- 4.4 Methods of Separation
 - Separation Based on Molecular Size
 - Separation Based on Solubility
 - Separation Based on Charge
 - Separation Based on Molecular Affinity
- 4.5 Summary
- 4.6 Terminal Questions
- 4.7 Answers

4.1 INTRODUCTION

So far you have learnt about the structure of the cell and the various techniques by which the cell structure is studied. You have also learnt about the function of various cellular organelles. A proper understanding of cell is possible only when we correlate the structure with chemical composition and functions of various cellular organelles. This unit will deal with the simple molecules and methods of isolating large molecules whereas the next unit will deal with the structure of large molecules, the macromolecules, present in a cell.

We begin with the description of various molecules present in a cell. Water is the most abundant of the cell molecules. The concept of its ionisation with reference to hydrogen ion concentration is discussed first. The structure of simple organic molecules like sugars, amino acids, nucleotides and lipids have been discussed along with the common methods used for their isolation and determination of concentration of macromolecules.

It is assumed that you are familiar with logarithms, capillary action, and radioactive isotopes, before studying this unit.

Objectives

After studying this unit, you will be able to:

- list different types of molecules and their numbers, present in a cell,
- state the properties of water, define and calculate pH,
- describe briefly the structure of monomeric sugars, amino acids, nucleotides and fatty acids,
- state the methods used for determination of concentration of macromolecules present in a cell,
- calculate the specific activity/concentration of a macromolecule in a biological fluid, and
- suggest methods for separating macromolecules on the basis of their size, charge, solubility and molecular affinity.

4.2 TYPES OF MOLECULES PRESENT IN A CELL

Cells in different tissues of an organism vary in their structure and function. Consequently, cells are of many types, such as muscle cells, blood cells, nerve cells in animals and meristematic cells and vascular cells in plants. Yet all the cells show considerable uniformity in their basic biochemical make-up. In other words, the chemical components of various types of cells are very similar and it is only the arrangement of components that causes a great diversity among cells, and consequently among tissues and organisms. Cells

contain various simple as well as complex molecules. Table 4.1 gives molecular composition of a cell. Let us now study some of these molecules briefly.

Table 4.1
Molecular Composition of a Cell

Type of molecules	Per cent of total mass		Number of types of molecules in a bacterium
	Bacterial cell	Mammalian cell	
Water (H ₂ O)	70	70	1
Macromolecules (proteins)	15	18	3000
RNA	6	1.1	300
DNA	1	0.25	1
Sugars and precursors	3	2.0	200
Lipids and precursors	2	5	50
Amino acids and precursors	0.4	—	100
Nucleotides and precursors	0.4	—	200
Inorganic ions (like Na ⁺ , Mg ²⁺ , Ca ²⁺ , PO ₄ ³⁻)	1	1	20
Other small molecules (like vitamins)	0.2	3	500

4.2.1 Water

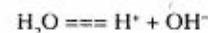
Water is the most abundant of all the molecules in a living cell and is absolutely necessary for life (Table 4.1). It is difficult to imagine the existence of any form of life in which water can be replaced by any other molecule. Uniqueness of water lies in its (i) structure, (ii) capacity as an excellent solvent, (iii) role as a medium in which all biochemical reactions occur.

Chemical reactions in the body are very sensitive to changes in the pH as most chemical reactions of life occur at neutral pH. Blood and most of the body fluids have pH values about 7.4 except for the stomach contents which have a pH value about 2.0. pH of a solution is measured in terms of its hydrogen ion (H⁺) concentration, which indicates whether a solution is acidic or basic.

The unique properties of water are due to its molecular structure. In water two hydrogen atoms are covalently held to an oxygen atom and hence dissociation of hydrogen would be very difficult. Consequently, pure water exhibits very little ionisation. (See Fig. 3.1 and 3.2 of Unit 3, Block 1 of Ecology Course.)

Ionisation of Water

In pure water, for every 550 million undissociated water molecules one molecule dissociates into one hydrogen ion (H⁺) and one hydroxyl ion (OH⁻).



The equilibrium constant between the dissociated and undissociated molecules may be represented as follows:

$$\frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} = K_{\text{eq}} \quad \dots \text{Eq. 4.1}$$

K_{eq} = equilibrium dissociation constant.

H₂O and H⁺ in brackets indicate molar concentrations.

The molar concentration of water in one liter of water is 1000/18=55.5, so that Equation 4.1 becomes

$$55.5 \times K_{\text{eq}} = [\text{H}^+][\text{OH}^-]$$

$$\text{or} \quad K_w = [\text{H}^+][\text{OH}^-] \quad \dots \text{Eq. 4.2}$$

Introduction to Cell Biology

One mole of a substance means 6.023×10^{23} molecules of that substance; mole is thus a quantity like a dozen and one mole of hydrogen weighs 21 g and 1 mole of water weighs 18 g.

$$\begin{aligned} -\log K_w &= -\log [H^+] - \log [OH^-] \\ -\log 10^{-14} &= -\log 10^{-7} - \log 10^{-7} \\ 14 &= 7+7 \\ &= 14. \end{aligned}$$

where K_w is the ionic product of water and is estimated as $=1.0 \times 10^{-14}$ at 25°C .

Since the concentration of both H^+ and OH^- is exactly equal, the concentration of each ion in pure water will be:

$$[H^+] = [OH^-] = 1 \times 10^{-7} \text{ moles/litre}$$

The pH scale is based on ionic product of water (K_w).

Taking logarithm of the quantities we get,

$$-\log K_w = -\log [H^+] - \log [OH^-] \quad \dots \text{Eq. 4.3}$$

Since $-\log K_w$ at 25°C is 14, the Equation 4.3 may be simplified as $[H^+] + [OH^-] = 14$.

pH

Acidity or basicity of a solution can be calculated in terms of its hydrogen ion concentration. Concentration of hydrogen ions in a solution is generally represented in terms of pH. pH is defined as the negative logarithm of hydrogen ion concentration. The concept of pH is very useful in understanding the change in hydrogen ion concentration in and around the cellular environment.

Let us see how we can calculate pH from hydrogen ion concentration. For example, a hydrogen ion concentration of 10^{-7} moles/litre means

$$\begin{aligned} \text{pH} &= -\log [H^+] \\ &= -\log [10^{-7}] \\ &= 7.0. \end{aligned}$$

It is important to note that pH can vary only between 0 and 14, and 7.0 is the neutral pH.

Table 4.2 gives the pH scale along with the pH of some common substances. You will learn about the importance of pH in all biochemical reactions in further units of this course.

Table 4.2
The pH Scale

	H^+ Concentration (M)	pH	OH^- Concentration (M)	Example
Acids	1.0	0	10^{-14}	1 M HCl
	10^{-1}	1	10^{-13}	Gastric juice
	10^{-2}	2	10^{-12}	Lemon juice
	10^{-3}	3	10^{-11}	Vinegar
	10^{-4}	4	10^{-10}	Tomato juice
	10^{-5}	5	10^{-9}	Black coffee
	10^{-6}	6	10^{-8}	Rain water
Neutral	10^{-7}	7	10^{-7}	Pure water
Bases	10^{-8}	8	10^{-6}	Sea water
	10^{-9}	9	10^{-5}	Baking soda
	10^{-10}	10	10^{-4}	Alkaline desert ponds
	10^{-11}	11	10^{-3}	Liquid soap
	10^{-12}	12	10^{-2}	Household ammonia (or Lime water)
	10^{-13}	13	10^{-1}	
	10^{-14}	14	1.0	1 M NaOH

SAQ 1

Calculate the pH of an aqueous solution in which 0.5 cm^3 (ml) of dilute hydrochloric acid has been added to 1 dm^3 of pure water. Assume that this dilute hydrochloric acid has the concentration of 2 mole/dm^3 and is completely ionised in solution.

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4.2.2 Basic Organic Molecules

In a living system, a large number of organic molecules are present. Diversity of organic molecules is due to the property of the carbon atom which in association with itself and with other atoms forms an unlimited number of compounds with different properties. Four major classes of organic compounds found in biological systems are: carbohydrates, proteins, nucleic acids and lipids. In this unit you will study about the monomers of these compounds in detail.

Monomers of Carbohydrates: Before you study at length about monomers of carbohydrates, you would like to know what carbohydrates are. Carbohydrates form the most abundant class of organic compounds. They are the compounds containing carbon, hydrogen and oxygen. The hydrogen and oxygen in these compounds are generally present in the ratio of two to one atom as in water.

Carbohydrates can be divided into classes based on the size of the molecules.

Monosaccharides, the basic units of carbohydrates are simple sugars having the general formula $C_nH_{2n}O_n$ where n can vary from three to eight. Depending on the number of carbon atoms, they are called as trioses (C_3), tetroses (C_4), pentoses (C_5), hexoses (C_6) and so on.

Disaccharides consist of two monosaccharide units joined together. **Oligosaccharides** include the sugars with up to 10 monosaccharide units. **Polysaccharides** are also made of monosaccharides joined together but may contain thousands of units.

Monomer: The basic subunit from which, by repetition polymers are made. For example, amino acids are the monomers of proteins, glucose is the monomer of glycogen.

$C_nH_{2n}O_n$ means that any monosaccharide molecule contains equal numbers of O and C atoms and twice as many H atoms.

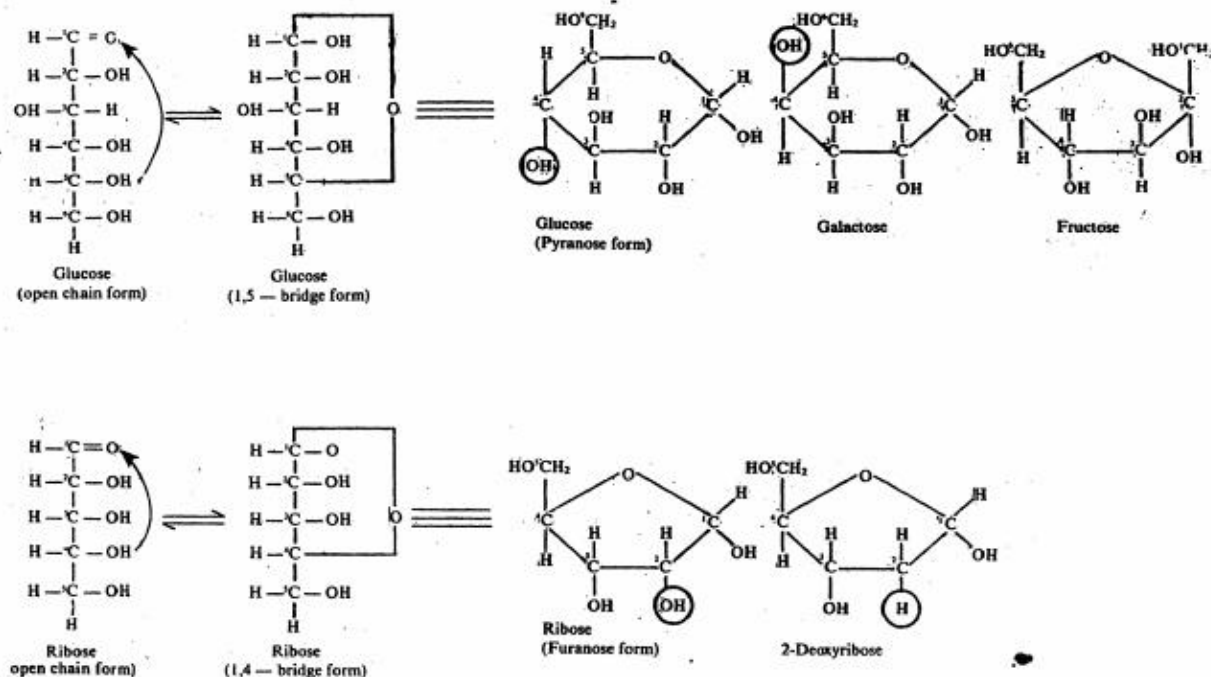


Fig. 4.1 : Structure of some monosaccharides. Monosaccharides in solutions exist in equilibrium as either an open form or a ring form. The ring form is predominant and it explains many chemical properties which are not explained by the open structure. Among hexose sugars glucose and galactose are aldoses and fructose is a ketose sugar. Pentoses are also aldoses. Fructose occurs in a furanose (4 carbon atom ring) form in sucrose but in pyranose form in free solution.

Glucose, fructose, galactose, ribose and deoxyribose are examples of monosaccharides (Fig. 4.1). Sucrose, lactose and maltose are examples of disaccharides. The table sugar or sucrose is composed of glucose and fructose, whereas milk sugar or lactose is composed of glucose and galactose. It is interesting to note that the milk sugar lactose can be digested by the

Aldoses are the monosaccharides which have aldehyde group in the 1 position — Glucose and galactose are aldoses. **Ketoses** are monosaccharides which have a ketose group in the 2 position. Fructose is a ketose.

Pyranose is six membered ring whereas **furanose** is a five membered ring.

enzymes produced in the intestine of children but in several groups of adults this enzyme is missing. The deficiency of enzyme causes intolerance of lactose and hence of milk. It is found that the percentage of lactose intolerance is more in adult populations which have not used milk and dairy products in childhood than those who have used them. Starch, cellulose and glycogen (all composed of glucose) are examples of polysaccharides about which you will study more in Unit 5

SAQ 2

Tick mark (true) the correct statement.

Glucose and galactose differ from each other in their

- | | |
|-----------------------|--------------------------|
| a) Chemical formula | ☐ |
| b) Structural formula | ☐ |
| c) Both | ☐ |
| d) None | ☐ |

Monomers of Proteins: Proteins are complex, diverse and high molecular weight polymers, found in the cell. The monomeric units of proteins are amino acids.

Amino acids join each other in a specific manner to form an unbranched, linear polymer known as polypeptide. Thousands of proteins that are present in a body are made-up of only 20 different naturally occurring amino acids (Figure 4.2). Amino acids are important not only in the synthesis of proteins but also in general metabolism.

In each amino acid, the alpha carbon atom is attached to a carboxyl group, an amino group and a side chain. Presence of both a positively and a negatively charged group in the same molecule of an amino acid is known as a "zwitterion" (German: Zwitter= hybrid, both). The variation in the different amino acids is due to presence of side chain (R) attached to the alpha carbon atom. On the basis of the side chains, i.e., polar, non-polar, uncharged polar, positively or negatively charged polar groups, amino acids are classified into four groups (Fig. 4.2).

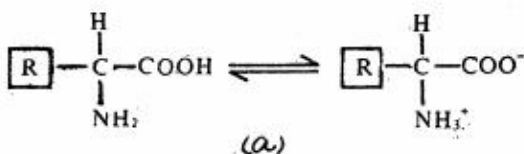
Monomers of Nucleic Acids

The nucleic acids are large complex polymers like proteins. There are two types of nucleic acids, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) found within the cell. They are made-up of four types of nucleotides. Each nucleotide is made of a sugar, a phosphate and a nitrogenous base (Table 4.3). There are only 2 kinds of sugars in nucleotides: ribose and 2-deoxyribose (Fig. 4.3). There are five types of nitrogenous bases: cytosine, thymine, uracil, adenine and guanine. The nucleotides are joined together by a specific linkage (3' → 5' phosphodiester linkage) to form a linear, unbranched chain of polynucleotides. Table 4.3 gives the components and names of some nucleic acids.

Table 4.3
Nomenclature of nucleosides and nucleotides
Base + Sugar = Nucleoside
Nucleoside + Phosphate = Nucleotide

Base	Nucleoside	Nucleotide
Purines (Pu)		
Adenine (A)	Adenosine (rA)	Adenylic acid or adenosine monophosphate (AMP)
	Deoxyadenosine (dA)	Deoxyadenylic acid or deoxyadenosine monophosphate (dAMP)
Guanine (G)	Guanosine (rG)	Guanylic acid or guanosine monophosphate (dGMP)
	Deoxyguanosine (dG)	Deoxyguanylic acid or deoxyguanosine monophosphate (dGMP)
Pyrimidines (Py)		
Cytosine (c)	Cytidine (rC)	Cytidylic acid or cytidine monophosphate (CMP)
	Deoxycytidine (dC)	Deoxycytidylic acid or deoxycytidine monophosphate (dCMP)
Thymine (T)	Thymidine (dT)	Thymidylic acid or thymidine monophosphate (TMP)
Uracil (U)	Uridine (rU)	Uridylic acid or uridine monophosphate (UMP)

- Note that the names of purine nucleosides end in -osine and the names of pyrimidine nucleosides end in -idine.
- Each nucleotide has two names.
- 'r' stands for ribose and 'd' stands for deoxyribose.



Nonpolar R group		Uncharged polar R group		Positively charged polar R group (Basic)	
Alanine		Glycine		Lysine	
Valine		Serine		Arginine	
Leucine		Threonine		Histidine	
Isoleucine		Cysteine		Negatively charged polar R group (Acidic)	
Proline		Tyrosine			
Phenylalanine		Asparagine		Aspartic acid	
Tryptophan		Glutamine		Glutamic acid	
Methionine				at pH 6.0–7.0	

(b)

Fig. 4.2 : a) Structure of a typical amino acid. The central or alpha carbon atom of each amino acid is covalently bonded to four groups i.e., hydrogen atom, an amino group, an acid (or carboxyl) group and a side chain called an R group.
b) Structure of twenty amino acids.

Monomers of Lipids

Lipids are heterogeneous groups of compounds usually formed by a combination of a fatty acid with glycerol. Fatty acids are the characteristic components of lipids present in both animal and vegetable fats such as ghee, coconut oil and mustard oil. Fatty acids are long linear chain organic compounds containing 4-24 carbon atoms. Nearly all fatty acids in

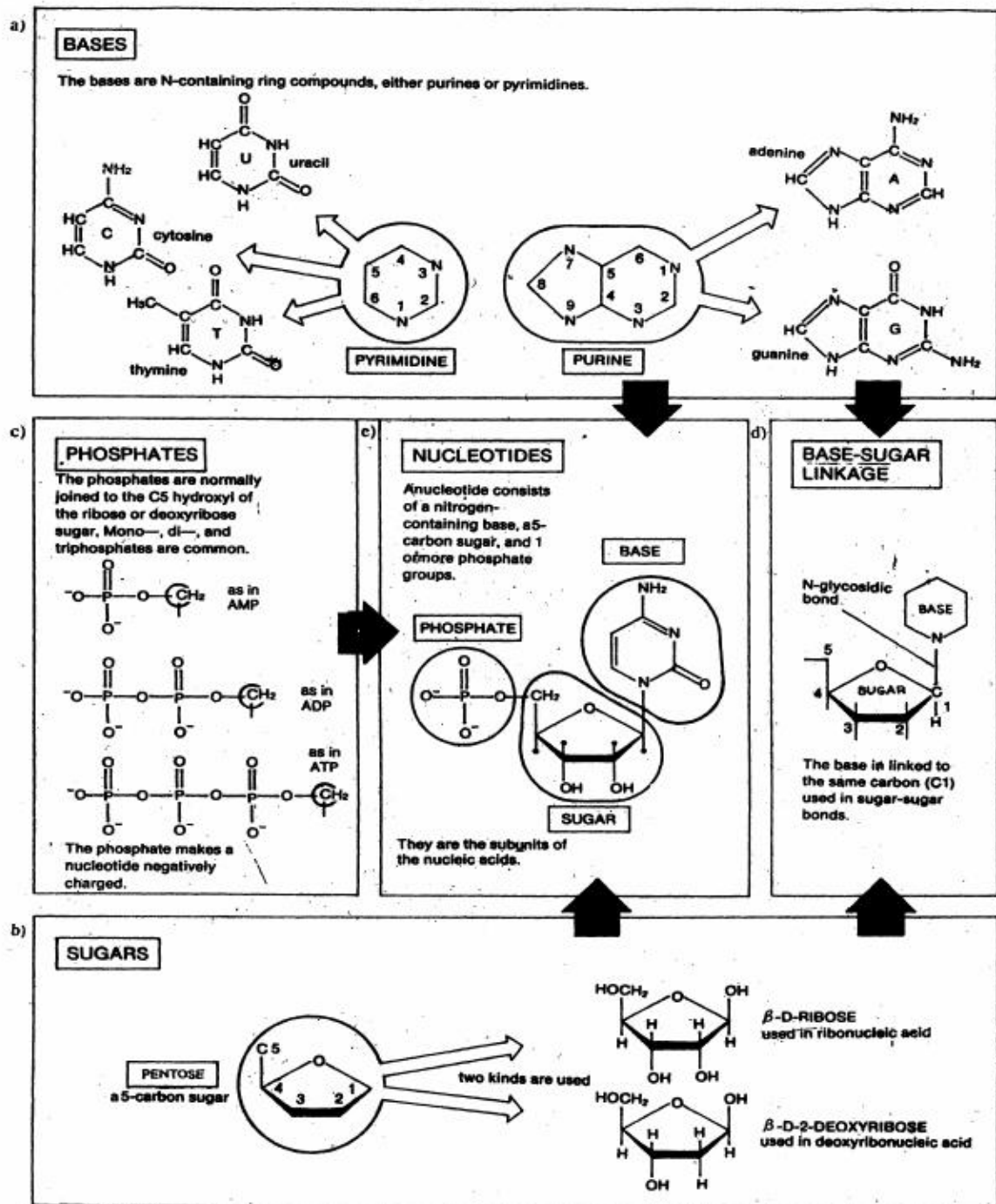


Fig. 4.3 : Components of nucleic acids.

a) Bases. The chemical compounds of the two classes of nitrogenous bases are the heterocyclic compounds, pyrimidine and purine. Pyrimidine is a 6-membered ring with nitrogen atoms at 1 and 3 position and purine is a fused ring system of nine atoms with nitrogen atoms at 1,3,7 and 9 positions. Cytosine, thymine and uracil are three pyrimidine bases. Adenine and guanine are two purine bases.

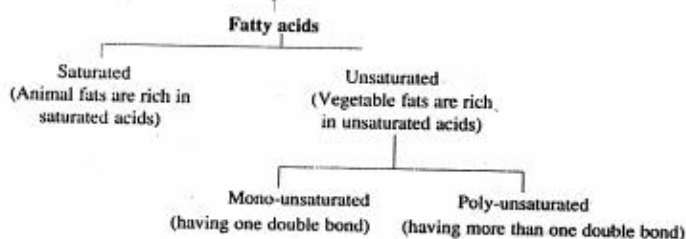
b) Sugars. Two types of pentose sugars are present in nucleic acids, D-ribose in ribonucleic acid (RNA) and 2-deoxy-D-ribose in deoxyribonucleic acid (DNA). Both sugars occur in their furanose forms in nucleotide. Note the position of H and OH at carbon atom 2 in ribose and deoxyribose sugars.

c) Phosphates. Phosphate group is attached to the hydroxyl group of carbon atom 5 of pentose sugar in a single nucleotide. Presence of this phosphate group makes a nucleotide negatively charged.

d) Nucleoside. The pentose is joined to the base by N-glycosyl bond which is formed between carbon atom 1 of the pentose and nitrogen atom 1 of pyrimidine or nitrogen atom 9 of purine.

e) Nucleotide. Esterification of phosphate group with carbon atom 5 of the pentose in a nucleoside gives rise to a nucleotide, the subunit of nucleic acids.

nature have an even number of carbon atoms, but those with 16 and 18 carbon atoms are most abundant in oil. Fatty acids have a single carboxyl group and a long, non-polar hydrocarbon chain. The presence of non-polar chain makes lipids insoluble in polar solvents such as water. The long hydrocarbon chain may be fully saturated with carbon atoms joined by single covalent bonds or it may be unsaturated with double bonds between carbon atoms. The classification of fatty acids is shown below.



Apart from mono and poly-unsaturated fatty acids, a third class of unsaturated fatty acids is **prostaglandins (PG)**. These were first discovered in seminal plasma and are physiologically very important. Cyclisation of central carbon chain of poly-unsaturated fatty acids forms a cyclopentane ring at one end of the molecule (Fig 4.4)

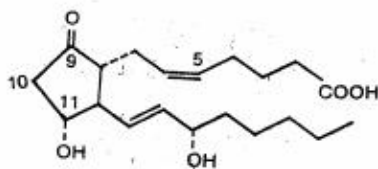


Fig. 4.4 : Prostaglandin E_2 (PGE_2). Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins designated as A, B, etc. For example, PGE_2 has a keto group in position 9.

Table 4.4 gives the source, structural formulae and names of some naturally occurring fatty acids.

Table 4.4
Some naturally occurring fatty acids
Saturated fatty acids ($C_nH_{2n-1}COOH$)

Number of carbon atoms in chain	Formula	Common name	Source
12	$CH_3-(CH_2)_{10}-COOH$	Lauric acid	Cinnamon, coconut oil
14	$CH_3-(CH_2)_{12}-COOH$	Myristic acid	Palm kernel, coconut oil
16	$CH_3-(CH_2)_{14}-COOH$	Palmitic acid	Common in all animal and plant fats
18	$CH_3-(CH_2)_{16}-COOH$	Stearic acid	and most abundant in animal fats

Unsaturated fatty acids

No. of carbon atoms in chain	Formula	Common name	Source
18	$CH_3-(CH_2)_7-CH=CH(CH_2)_7-COOH$ One double bond	Oleic acid	Found in nearly all fats
18	$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH(CH_2)_7-COOH$ Two double bonds	Linoleic acid*	Seed oils like peanut, soyabean
18	$CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-(CH_2)_4-COOH$ Three double bonds	Linolenic acid*	Found frequently with linoleic acid but particularly in linseed oil
20	$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-(CH_2)_4-COOH$ Four double bonds	Arachidonic acid*	Particularly in peanut oil

* These are called essential fatty acids, because they cannot be produced in the body in adequate amount and hence they must be provided in diet.

SAQ 3

Which organic molecules would you expect to be released on the hydrolysis of a protein and a polysaccharide?

4.3 ISOLATION AND DETECTION OF MACROMOLECULES

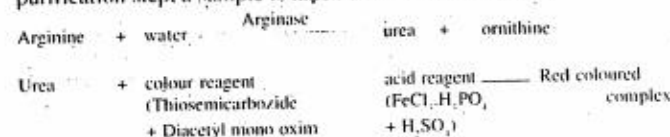
Properties of any macromolecule can be studied easily if it is in a pure form. For this we need to extract the macromolecules from cells. This is a difficult process because macromolecules occur in a mixture with other cytoplasmic extraneous matter and are susceptible to destruction and damage during extraction procedure. Therefore, to keep the macromolecules intact, the extraction should be done carefully. Purification requires constant monitoring, to ensure that after every purification step the concentration of desired macromolecule increases and that of extraneous matter decreases.

4.3.1 Monitoring the Purification Procedure

There are several methods to ensure recovery of macromolecules during purification.

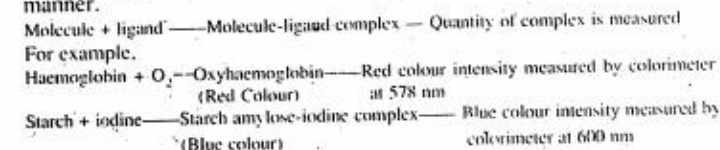
UV Absorption : Measurement of UV absorption with a spectrophotometer is useful in monitoring the purification of nucleic acids or proteins. Nucleic acids have the maximum absorbance at 260 nm (wavelength) and the proteins at 280 nm. A higher absorbance at a particular wavelength indicates a larger quantity of the specific macromolecule.

Catalytic Activity: It can be measured to monitor the purification of enzymes. For example, the enzyme arginase, which synthesises urea is monitored in this way. After each purification step, a sample is taken and treated as follows.



The colour intensity is estimated at 520 nm in a colorimeter. The amount of urea formed indicates the amount of arginase present.

Ligand Binding : It is a useful way to monitor macromolecules that have specific binding sites for other molecules. Haemoglobin and starch purification can be measured in this manner.



Radioactive isotopes: Isotopes with an unstable nucleus that emits ionising radiation, important as tracers in biology.

Isotope Tracers: They can also be used to assay the purification of macromolecules. This technique is more suited for monitoring a newly synthesised macromolecule.

First an appropriate precursor of the molecule with a radioactive isotope is incorporated in the macromolecule while it is being synthesised; for example, ^3H -thymidine for DNA and ^{125}I for thyroxine. The macromolecule, thus, becomes radioactive and can be traced due to the incorporated isotope. Finally, the level of radioactivity may be determined in the extract with the help of instruments such as Gieger-Muller counter or Scintillation counter.

4.3.2 Specific Activity

Specific activity measures the purity of the macromolecule in a mixture. Specific activity is defined as the **activity per unit mass**. Let us take an example to illustrate the concept. If a cell extract is estimated to contain 10 'units' of enzyme arginase and 50 mg of protein then the specific activity of arginase is 10 units/50 mg or 0.2 units/mg protein. After a purification step, the extract contains 5 'units' of the enzyme and 10 mg of protein, and therefore, the specific activity of enzyme is 5 units/ 10 mg or 0.5 units/mg protein.

In this example we find that during the process of purification 5 units of enzyme activity and

40 mg of protein are lost but specific activity has increased from 0.2 units/mg protein to 0.5 units/mg protein. This clearly indicates that a higher proportion of contaminant (non-arginase) proteins are removed.

4.4 METHODS OF SEPARATION

Macromolecules can be separated from a mixed molecular population based on the differences in their size, solubility, electric charge and specific binding affinity for other molecules using a variety of techniques.

4.4.1 Separation Based on Molecular Size

Several methods are used to separate macromolecules depending on their size. Proteins can be separated from small molecules and ions by **dialysis** through a semipermeable membrane. Depending upon the pore size (different membranes have different pore sizes), molecules of certain size can pass through a membrane. When the osmotic pressure responsible for passage of molecules through a semipermeable membrane is not sufficient, mechanical pressure is applied to force the molecules through. This process is known as **ultrafiltration**.

Centrifugation

It is a technique used for separation of particles like cellular organelles and macromolecules by rotation in a **centrifuge**. Separation of macromolecules is achieved by centrifugation at a very high speed, i.e. ultracentrifugation at 80,000 revolution per minute. A mixture containing particles and macromolecules is applied on the top of a salt solution in a

Supernatant: (floating above). The liquid above solid matter in the bottom of a tube, beaker etc.

Density gradient: A solution of different density gradation usually used in centrifugation to separate different kinds of cells, particles, or macromolecules.

Svedberg Unit: The unit of sedimentation equal to 10^{-11} seconds. The number of S units of a molecule or particle in a given centrifugal field is related to the weight, shape and density of the molecule or particle.

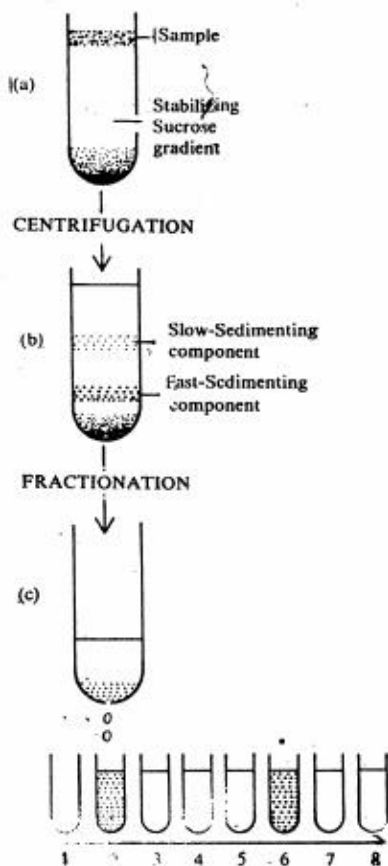


Fig. 4.5 : The fractionation of proteins by ultracentrifugation on sucrose density gradient.

a) The sample has been layered on top of the centrifuge tube containing the continuous gradient of sucrose, which increases in concentration towards the bottom.

b) The sample has been centrifuged and separated into two components which sediment at different rates.

c) A hole has been made at the bottom of the plastic centrifuge tube to collect different fractions drop by drop. Figure shows that the fraction 1 (Tube 3) is already collected and fraction 2 (Tube 7) is being collected. In other tubes only buffer solution without any protein fraction has been collected.

centrifuge tube and is subjected to centrifugation. The density gradient in the solution is created by adding the concentrated and highly soluble materials, for example, sucrose for protein and cesium chloride for nucleic acids. This gradient helps in the formation and stabilisation of distinct bands of different molecular fractions which sediment at different rates. These bands can be individually collected (Fig. 4.5). The sedimentation rate depends on the size, shape and molecular weight of the components and is called as sedimentation coefficient. It is represented by Svedberg unit or S value.

Column Chromatography

This chromatographic method is used for separating molecules on the basis of size differences (i.e. molecular weight). This technique is also popularly known as **molecular exclusion technique or gel filtration**. In this technique, a sample containing a mixture of molecules is loaded over a sieve like column packed with porous beads of specific size. The flow of molecule is retarded due to weak interactions such as van der Waals forces and hydrogen bonding with the medium. The Sephadex is an inert stable and reusable highly hydrated polysaccharide in the form of beads. Through this column, molecules move according to their molecular weight. Larger molecules are excluded first as they do not enter the beads of the gel and are localised in the solution in between the beads. Small molecules are trapped in the beads, hence they are eluted much later (Fig. 4.6).

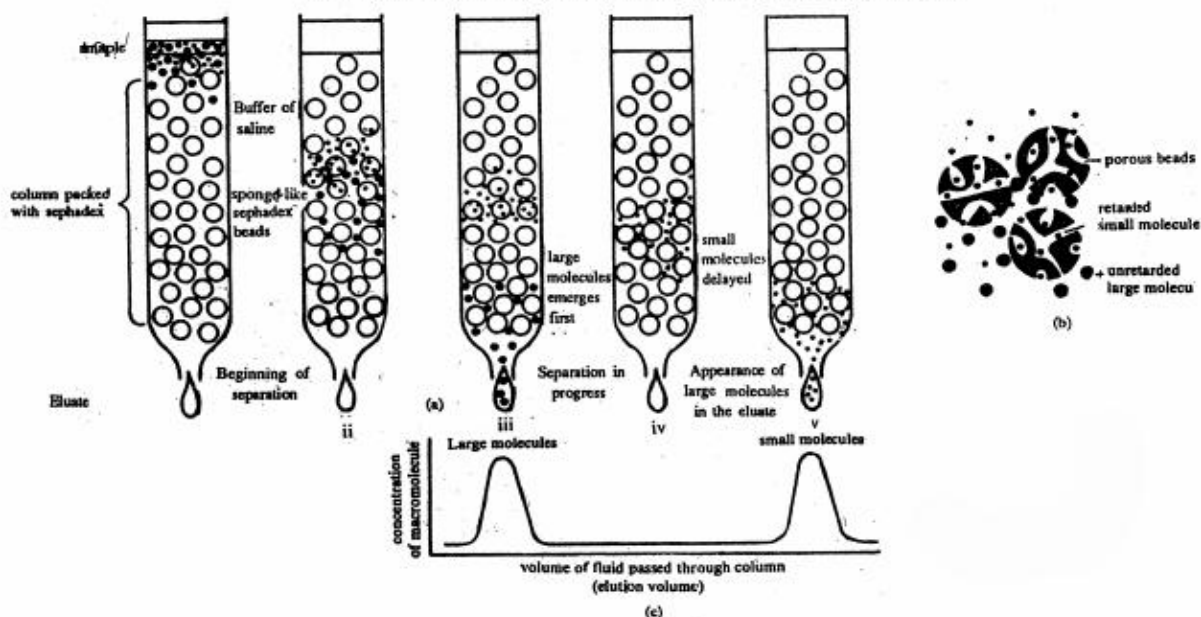


Fig. 4.6 : a) Column chromatography by molecular exclusion technique using sephadex column. (i) Glass column packed with gel particles and overlaid with sample containing molecules of various sizes (small and large dots). (ii-iii) Molecules with molecular weights in excess of the gel exclusion limit percolate between the gel particles, whereas smaller molecules may enter the gel phase. (iv-v) As a result, molecules reach the bottom of the column in order of decreasing size. b) Schematic drawing of column chromatography. c) The elution pattern as it is read on a UV spectrophotometer indicating the presence of proteins in various fractions.

SAQ 4

In the following table molecular weights of some proteins are given. Observe the data and number the sequence (like 1,2,3 etc) in which the following proteins would be eluted from Sephadex gel column. Sephadex G-200 is the generally used gel for this range of proteins. (Hint: Heavy molecular weight compounds are eluted first.)

Number	Protein	Mol weight
	Cytochrome C	13,370
	Aldolase	149,100
	Haemoglobin	64,500
	Myosin	524,800
	Catalase	221,600

SDS Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis is a technique to separate the molecules on the basis of their total charge. Such molecules when placed between two electrodes in polyacrylamide gel medium migrate to the oppositely charged electrodes. For example, in SDS polyacrylamide gel electrophoresis, a protein molecule is treated with high concentration of SDS (Sodium Dodecyl Sulfate) which dissociates the polymer into negatively charged monomers by breaking the non-covalent bonds. The amount of SDS bound and hence the negative charge on each monomer is in proportion to the size of the monomer. Consequently, in an electric field a larger protein molecule moves faster than a small protein molecule towards the anode or the positively charged electrode (Fig. 4.7).

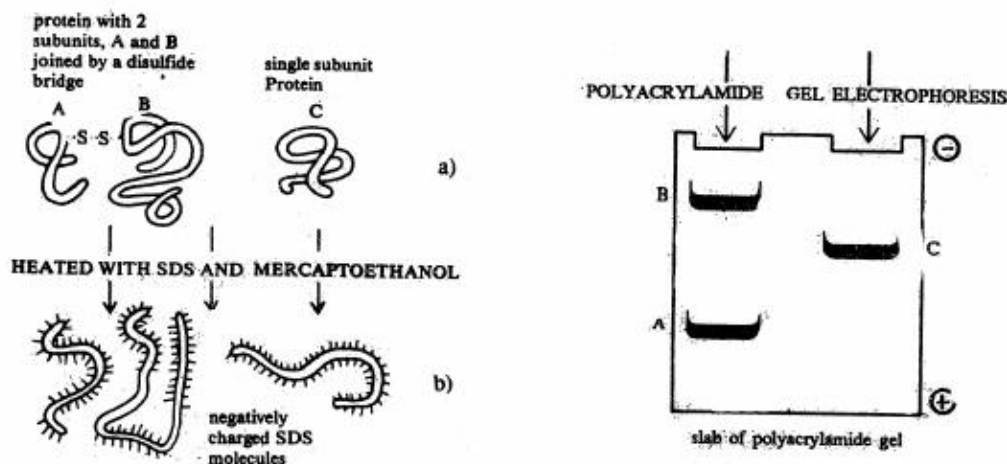


Fig. 4.7 : SDS-Polyacrylamide gel electrophoresis.

a) A polymeric protein consisting of four monomers is treated with SDS.

b) Each monomer is bound with SDS.

c) When allowed to move in an electrical field, the large monomers move faster towards the anode (positively charged electrode) as they have more SDS bound to them.

4.4.2 Separation Based on Solubility

Macromolecules can be separated and purified on the basis of their solubility which depends on pH, ionic strength, dielectric properties of the solvent and temperature.

As you have learnt in previous section, chromatography is a technique of molecular separation in which molecules migrate between a mobile (soluble) and a stationary (matrix) phase. The separation of molecules in a mixture depends upon their relative solubilities in the two phases and their binding strength with either of the phases.

Paper Chromatography is the commonly used technique to separate small molecules like sugars, amino acids, etc. A mixture of molecules is applied as a spot to a sheet of adsorbent paper, such as a filter paper. A solution containing a mixture of two solvents, such as water and alcohol is allowed to flow through the paper by capillary action. Different molecules move at different rates depending on their solubility in the solvent. The separated molecules can be seen on paper (Fig. 4.8).

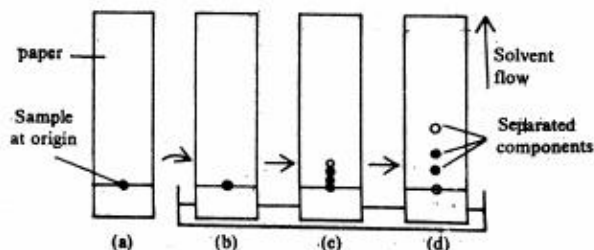


Fig. 4.8 : Paper chromatography. The sample is applied to a paper strip and allowed to dry (a). When the paper is dipped in the solvent (b), the solvent rises up due to capillary action (c). Different components move according to their relative solubility in the solvent (d).

Isoelectric Precipitation: For each protein molecule there is a pH at which it has no net electric charge and hence does not move in an electric field (Fig. 4.9). This is known as its isoelectric pH. At this pH, the protein molecule is least soluble and forms a sharp band, when a mixture of proteins in a fixed gradient is subjected to electric field. Different proteins have different isoelectric pH values.

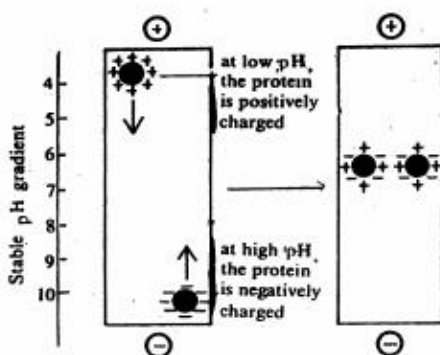


Fig. 4.9 : Principle of isoelectric precipitation and isoelectric focusing. At low pH, the protein is positively charged because excess H^+ ions uncharge the carboxyl group (COO^-) to form $COOH$ and, therefore, the amino groups (NH_3^+) give the protein a net positive charge. Similarly at high pH, the protein acquires a negative charge due to uncharged NH_2 group and negatively charged carboxyl groups (COO^-). At its isoelectric point, the negative and positive charges are equal thus the protein has no net charge.

Salt Fractionation technique is based on the fact that in a solution different proteins precipitate at different concentrations of certain salts such as ammonium sulphate. Therefore, the concentration of ammonium sulphate in a solution containing protein is gradually increased to precipitate different proteins.

4.4.3 Separation Based on Charge

Electrophoresis is the process of separation of molecules whose net charge depends upon the pH of the surrounding medium. Biological molecules having a net charge, move in an electric field. A negatively charged molecule moves towards anode and the positively charged one towards cathode. This is known as electrophoresis. A mixture of macromolecules is applied to a supporting medium such as paper or polyacrylamide gel, and an electric current is passed through the medium. Molecules separate out after an appropriate time and are detected with the help of specific staining reagents (Fig. 4.10).

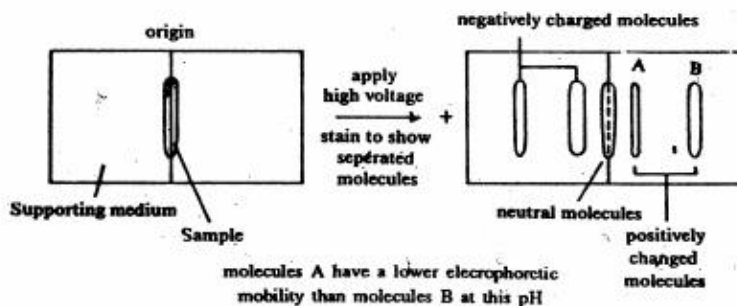


Fig. 4.10 : A sample of protein molecules is applied in the centre of the supporting medium like paper, cellulose acetate, polyacrylamide gel etc. On application of electric current, positively charged molecules move towards the cathode while negatively charged molecules move towards anode. Neutral molecules do not move at all.

Isoelectric focusing is another type of electrophoresis carried out in a pH gradient created in a column by mixing different buffers. A protein mixture is applied to a polyacrylamide gel having a pH gradient and subjected to an electric field. Each protein moves in the pH gradient till it arrives at its own isoelectric pH and stays on there forming a sharp band. The bands can be further separated, and the band containing a protein is dissolved in the appropriate buffer solution (Fig. 4.9).

4.4.4 Separation Based on Molecular Affinity

Based on molecular affinity, macromolecules can be separated by ion-exchange chromatography and affinity chromatography.

Ion-exchange Chromatography

Ion-exchange chromatography is based on the difference between the macromolecules with respect to their size and **electric charges at a given pH**. A chromatography column consists of a long column of synthetic resin comprising electrically charged molecules. Diethyl amino ethyl cellulose (DEAE-Cellulose) is positively charged (anion-exchanger) and carboxymethyl cellulose (CM-Cellulose or CMC) is a negatively charged (cation-exchanger) resin, at pH 7.0. When a protein mixture is passed through a DEAE column, the negatively charged molecules bind strongly and positively charged and uncharged molecules are eluted out (Fig. 4.11). Similarly by using CMC, positively charged protein molecules can be purified.

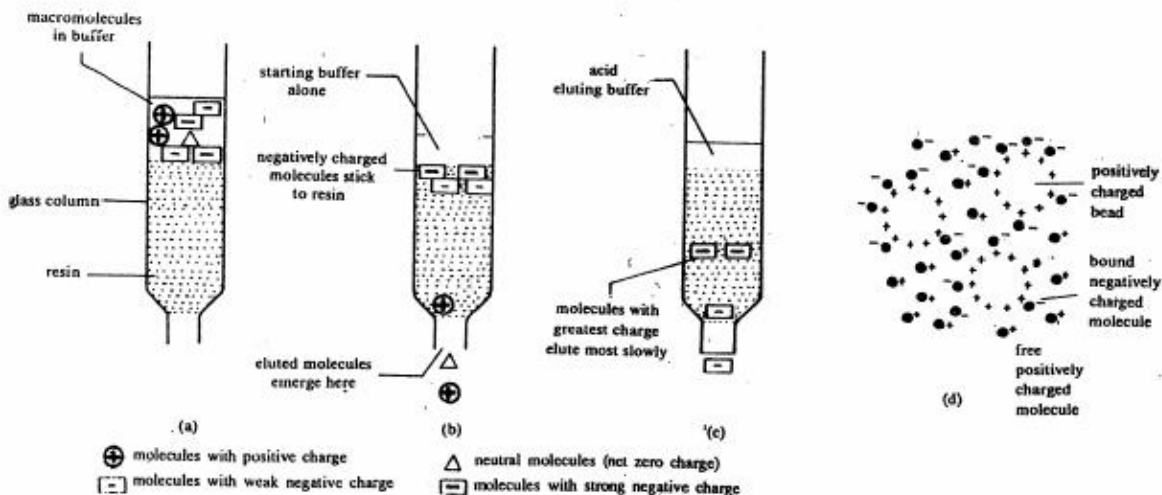


Fig. 4.11 : a) A sample is layered on a positively charged resin (resins are organic compounds prepared or synthesised by polymerisation of specific compounds such as cellulose).
b) Negatively charged molecules stick to the DEAE—cellulose resin and positively charged and neutral molecules are eluted out.
c) To get the negatively charged protein out of the column, acidic buffer (H⁺) is added which binds to negatively charged molecules and helps in their elution from the column.
d) Principle of ion-exchange chromatography.

Affinity Chromatography

Affinity Chromatography is another powerful means of purifying proteins. This technique utilises the high affinity of many proteins for specific chemical groups. It is like ion

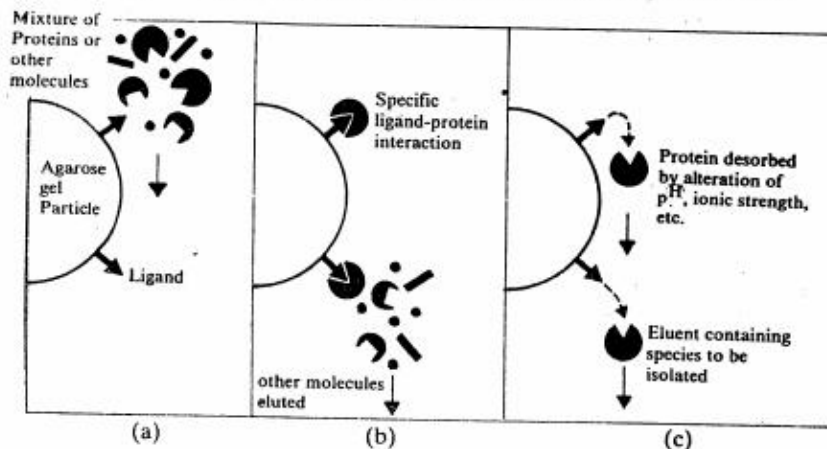


Fig. 4.12: Principle of affinity chromatography.

exchange chromatography but based on covalent bonding by appropriate chemical reaction between insoluble material (Polysaccharide—agarose beads) and specific ligand (such as an antibody molecule or a substrate of the enzyme). When a mixture of molecules is passed through such type of affinity column, those with high affinity for the ligand are retained whereas others move out. The molecules attached to the ligand are dissociated and eluted out by change in pH of the eluting solution (Fig. 4.12). Various biological molecules such as enzyme, receptor molecules are purified from their complex mixture by using this method.

SAQ 5

Protein A has a molecular weight 80,000 while protein B has 50,000. Protein A has a net charge -3 at pH 7 and 0 at pH 6. Protein B has net charge -3 both at pH 7 and pH 6. What of the following methods may be used to separate these proteins? Give reasons for the preferred method.

- Centrifugation
- Ion-exchange chromatography
- Paper chromatography
- Affinity chromatography
- Sephadex gel filtration

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4.5 SUMMARY

In this unit you have studied the

- Chemical composition of a cell in order to better understand the structure and function of the cell and its components.
- Ionisation property of water, the simplest and most abundant of cell molecules, and the method to calculate the ionic product of water (K_w) and hydrogen ion concentration (pH).
- Structure of the basic molecules found in a cell, i.e. monosaccharides, amino acids, nucleotides and simple lipids.
- Quantity of macromolecules in a sample can be estimated by various methods like UV absorption, catalytic activity, ligand binding and use of isotopic tracers.
- Macromolecules can be separated on the basis of their molecular size, solubility etc. by different techniques such as centrifugation, chromatography, electrophoresis and fractionation.

4.6 TERMINAL QUESTIONS

- Ionisation of a base produces OH^- and not H^+ ions. Why then does addition of a base such as KOH change the pH of a solution?
- Calculate the pH of an aqueous solution in which 1.5 cm^3 of calcium hydroxide $\text{Ca}(\text{OH})_2$ has been added to 2 dm^3 of pure water. Calcium hydroxide has the concentration 3 moles/dm^3 and is completely ionised in solution.
- What is the difference between a saturated and an unsaturated fatty acid?
- A cell extract contains 40 mg of protein and 200 units of an enzyme. In one experiment, after gel filtration, a particular fraction contains 3 mg of proteins and 120 units of enzyme. In the second experiment after ion-exchange chromatography, one fraction contains 20 mg of protein and 180 units of enzyme, which of the two methods is to be preferred for purification?

Self-assessment Questions

- 1) i) Since HCl is completely ionised, the concentration of H^+ ions (in HCl) will be the same as the concentration of HCl, that is, 2 moles/dm³.
 ii) Since 0.5 cm³ of the HCl is added to 1 dm³ (1000 cm³) of water, the concentration of H^+ ions will become $[H^+] = 0.5/1000 \times 2 \text{ mol/dm}^3 = 10^{-3} \text{ mol/dm}^3$.
 So $pH = -\log (10^{-3})$
 $= 3$
 iii) We have ignored the negligible contribution of 10^{-7} moles/dm³ from ionisation of water.
- 2) b)
- 3) By hydrolysis of protein and polysaccharide, amino acids and monosaccharides are released respectively.
- 4) The sequence in which proteins would be eluted from a Sephadex gel column is:

Number	Protein	Mol weight
1	Myosin	5,24,800
2	Catalase	221,600
3	Aldolase	1,49,100
4	Haemoglobin	64,500
5	Cytochrome C	13,370

- 5) Method a), b) and e) can be used to separate these proteins. Centrifugation (a) can work because proteins have different molecular masses. Ion-exchange chromatography (b) can work at pH 6 when charges are different and not at pH 7 when the charges are same. Sephadex gel filtration (e) can work because proteins have different molecular weights. Sephadex gel filtration is preferred in such a case because other two methods are more time consuming, costly and less efficient.

Paper chromatography (c) cannot work because in this problem nothing regarding solubility is given. Affinity chromatography (d) cannot work because affinity of protein for any ligand is not mentioned.

Terminal Questions

- 1) The OH^- ions produced by ionisation of a base react with free H^+ ions present in a solution. The decrease in the concentration of H^+ ions is seen as an increase in pH.
- $$KOH \longrightarrow K^+ + OH^-$$
- $$OH^- + H^+ \longrightarrow H_2O$$
- 2) $Ca(OH)_2 \rightleftharpoons Ca^{2+} + 2OH^-$
 This equation implies that 1 mol of $Ca(OH)_2$ ionises to give 2 mol of OH^- . So $[OH^-]$ from 1 dm³ of 3 mol of $Ca(OH)_2$ is 6 mol.
 Since 1.5 cm³ of $Ca(OH)_2$ has been added to 2 dm³ of pure water, the concentration of OH^- will become
 $[OH^-] = 1.5/2000 \times 6 = 4.5 \times 10^{-3} \text{ mol}$
 $pOH = -\log (4.5 \times 10^{-3})$
 $= 3 - \log 4.5$
 $= 3 - 0.6532$
 $= 2.34$
 $pH = 14 - pOH$
 $= 14 - 2.34$
 $= 11.66$
- 3) In saturated fatty acids carbon atoms are joined by single covalent bonds. In unsaturated fatty acids at least one double bond is present between the two carbon atoms.
- 4) Since specific activity measures the purity of an enzyme, we must calculate the specific activity. Specific activity in cell extract (before purification)
 $= 200 \text{ units/40 mg}$
 $= 5 \text{ units mg}^{-1}$

Specific activity after gel filtration

= 120 units/3 mg

= 40 units mg^{-1}

Specific activity after ion-exchange chromatography

= 180 units/20mg

= 9 units mg^{-1}

We see that specific activity has increased 8 times in case of gel filtration and about 2 times after ion-exchange chromatography. Hence gel filtration is the preferred method although the absolute enzyme activity recovered after ion-exchange chromatography more than that recovered after gel filtration.

UNIT 5 MACROMOLECULES OF THE CELL

Structure

- 5.1 Introduction
 - Objectives
- 5.2 Types of Bonds
- 5.3 Proteins
- 5.4 Nucleic Acids
 - Deoxyribo Nucleic Acid (DNA)
 - Ribose Nucleic Acid (RNA)
- 5.5 Carbohydrates
- 5.6 Lipids
 - Simple Lipids
 - Compound Lipids
 - Derived Lipids
- 5.7 Summary
- 5.8 Terminal Questions
- 5.9 Answers

5.1 INTRODUCTION

In Unit 4, you have learnt about the small basic molecules present in a cell-like water, amino acids, nucleotides, sugars and fatty acids and also about various techniques used for the isolation and purification of macromolecules.

In this unit you will study about large molecules present in various life forms ranging from bacteria to man. These macromolecules namely proteins, nucleic acids, lipids and carbohydrates have a common molecular organisation in different forms of life. These macromolecules of life are constantly in a state of flux. Here, you will study the various types of bonds and non-bonded interactions which help to unite the basic molecules to form these macromolecules and give them a structure and shape.

In the previous unit, you have studied about amino acids, which are the basic structural unit of proteins. Here, information is provided about how these molecules play a key role in all biological processes, and how polypeptides chains fold upon themselves and produce the specific three-dimensional structure of proteins. Proteins play an important role in carrying information from cytoplasm to nucleus, for example some promoters and repressor are proteins in nature. Proteins also mediate a wide range of other functions, such as mechanical support, transport and storage, coordinated movements, excitability, immune protection, and the control of growth and differentiation. The other important informational molecules in the cell are the nucleic acids which store, transmit and carry out the genetic information from one generation to the next. We will also discuss here about the structure and general properties of carbohydrates and lipids which are also called "non-informational molecules". Carbohydrates are the immediate source of energy, whereas lipids store energy and are the essential components of membrane structure.

Before you go in details of this unit, it is important that you must have studied Unit 4 carefully. You should also refresh your memory about the structure of an atom.

Objectives

After you have studied this unit you should be able to:

- explain different types of bonds that hold atoms and molecules together,
- identify the primary, secondary, tertiary and quaternary structures of a protein,
- distinguish between the structures of DNA and RNA,
- define polysaccharides, mucopolysaccharides, glycoproteins, proteoglycans, glycosaminoglycans, and
- list important lipids and the site of their occurrence.

5.2 TYPES OF BONDS

Macromolecules are held together by different types of chemical bonds. On the basis of energy required or released to break during bond formation or breakage, these bonds have been classified into two types, i.e., strong and weak bonds (Table 5.1). **Strong bonds** preserve the structure of macromolecules and require greater amount of energy to break. **Covalent bonds** are strong type of bonds (see Fig. 5.1(a)). Glycosidic, peptide, and nucleotide bonds are examples of covalent bonds. These are formed by sharing of electrons between two electronegative atoms. As you will proceed, you will study in detail about glycosidic, peptide and nucleotide bonds in Sections 5.3, 5.4 and 5.5. Hence, our main emphasis in this section will be on weak bonds. **Weak bonds**, called **non covalent bonds** help to carry the information and require much less energy to break. Weak bonds include hydrogen bonds, hydrophobic bonds, ionic bonds, and van der Waals forces (Table 5.1).

Table 5.1
Energies released/consumed during bond breakage/formation

Types of Bond		Bond energy kilojoules/mole
STRONG BONDS		
Covalent bonds		
Single bonds	O - H	462
	H - H	436
	S - S	426
	C - H	415
	C - O	356
	C - C	347
	C - N	292
	C = O	723
Double bonds	C = C	602
WEAK BONDS		
Ionic Bonds (In the absence of water)	NaCl	408
	NaI	304
Hydrogen Bonds		33 - 175
Hydrophobic Bonds (van der Waals attractions)		33

Hydrogen bonding is very common in biological systems. Hydrogen bonding is very important in maintaining three-dimensional structure of nucleic acids, protein molecules and specially of water (Fig 5.1b). In the absence of hydrogen bonding, water would have been in the gaseous state at room temperature and this would have made the existence of life impossible (Fig 5.1 c).

Non-polar molecules or groups tend to hold themselves together in the interior, when in water due to repulsion for water. These interactions are called **hydrophobic interactions** (hydro=water, phobia= repulsion). Dispersed oil droplets coming together in water to form a single large oil drop is a familiar example of hydrophobic interaction (Fig. 5.1 d).

Ionic or electrostatic bonds are formed by transfer of electrons between oppositely charged atoms, e.g., between Na^+ and Cl^- . Ionic bonds are very strong (like covalent bond) in the absence of water. However, they are quite weak (like hydrogen bond) in the presence of water (Fig. 5.1 e). Ionic bonds are very important in binding of enzyme and substrate.

When two atoms are not too far, i.e. distance between a pair of atoms is not more than one angstrom (10^{-10}m) they tend to interact due to the instantaneous dipoles induced. The fluctuating electrical charges set up between the nuclei and the electrons create a weak ionic interaction. This type of attraction is known as **van der Waals force** (Fig. 5.1 f). van der Waals forces break down easily when exposed to heat, because the strength of this bond is slightly more than the average molecular thermal energy at room temperature.

Dipole: A molecule carrying positive and negative charges spatially separated at opposite ends of the structure.

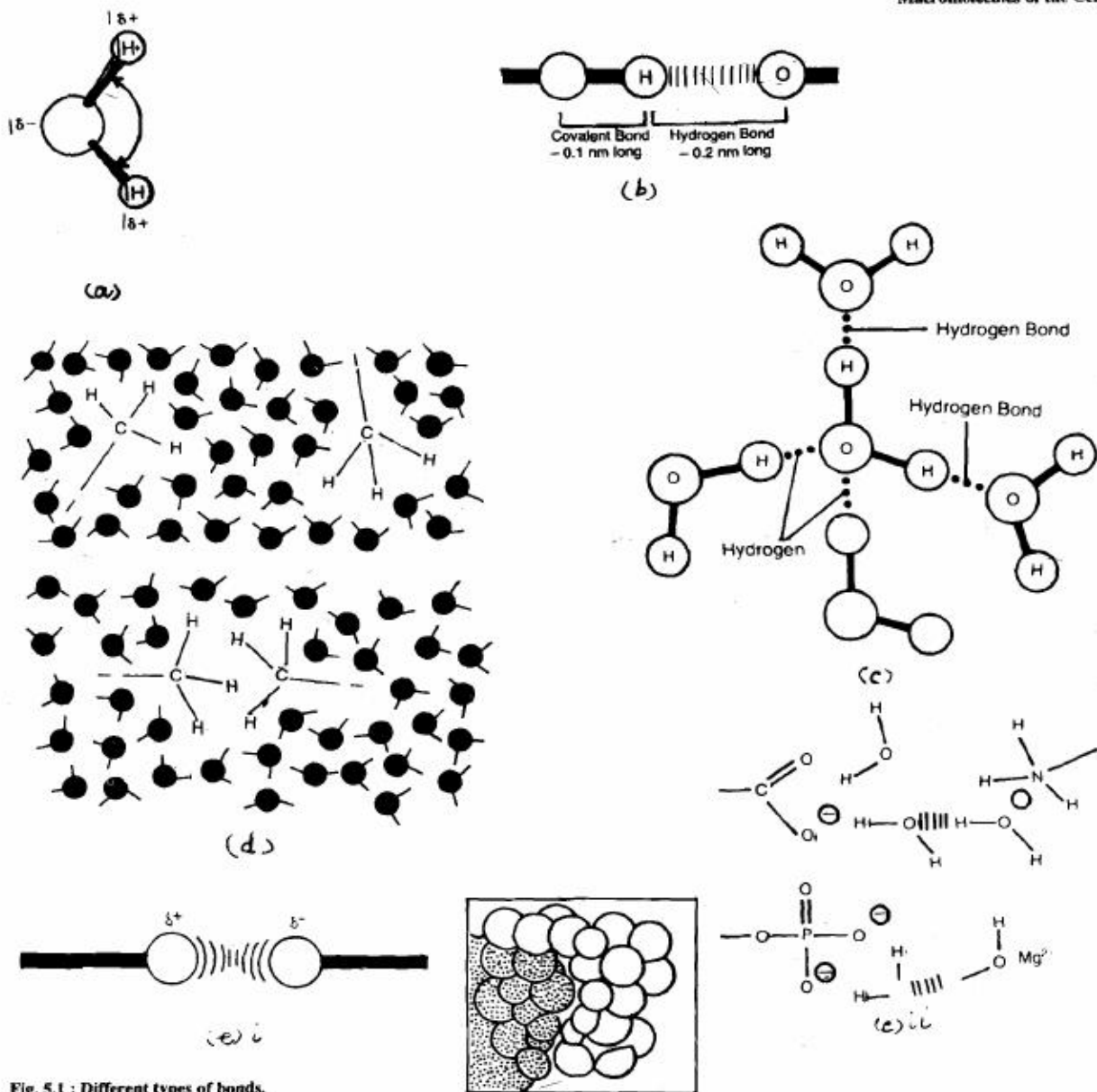


Fig. 5.1 : Different types of bonds.

a) Covalent bonds

Covalent bonds are strongest bonds formed by sharing of one or more electron pairs by the atoms. A water molecule consists of two hydrogen atoms and one oxygen atom joined by covalent bonds. The δ^+ and δ^- indicate a slight positive and negative charges on the molecules which is due to shifting of electrons to the more positively charged nucleus of oxygen.

b) Hydrogen bonds

For the formation of hydrogen bond a hydrogen atom is shared by two electronegative atoms, for example, the bond between an oxygen and nitrogen atoms and between two nitrogen atoms. Such bonds are predominantly found in proteins and nucleic acids.

c) Hydrogen bonds in water

Due to weak electrical charges, the water molecules are joined by hydrogen bonds transiently.

d) Hydrophobic bonds

A weak interacting force between water repelling, nonpolar residues and molecules, such as between fatty acid chains of membrane phospholipid or between aromatic bases in DNA.

e) Ionic bonds

These bonds are very strong in the absence of water. The force of attraction between the two charges, (+) and (-) is $F = \frac{q_1 q_2}{r^2 D}$. Where D = dielectric constant (1 for vacuum, 80 for water), r = distance between two molecules, q_1 and q_2 are the charges. Ionic bonds become weak in presence of water.

f) van der Waals force

A non specific, weak chemical interaction resulting from attractive forces produced two atoms or groups of atoms when they come near each other.

SAQ 1

Why do ionic bonds become weak in the presence of water?

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5.3 PROTEINS

Proteins are nitrogenous compounds of high molecular weight. These are the major building material of cells and take part in controlling different activities of living systems. Proteins are made-up of amino acids that are covalently linked with each other by peptide bonds. The number of amino acids in a peptide chain varies. Simple proteins consist of only amino acids, whereas complex proteins have other substances like lipids and carbohydrates also.

Proteins are classified into seven types on the basis of their biological functions (Table 5.2).

Table 5.2
Classification of Proteins according to Biological Functions

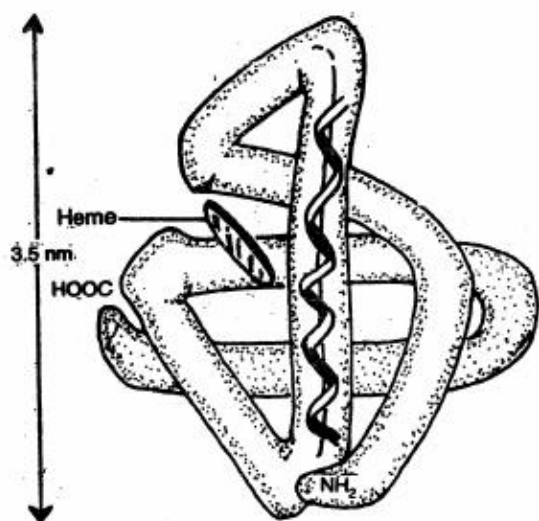
Class	Example	Functions
i) Enzymes	Ribonuclease, Trypsin	Help in catalysing chemical reactions in living cells
ii) Transport proteins	Haemoglobin, Serum albumin, Myoglobin, —Lipoprotein	Involved in the transport of molecules and ions across the membranes
iii) Nutrient and storage proteins	Gliadin (wheat), Ovalbumin (egg), Casein (milk), Ferritin	Provide the essential components by storing them in the body
iv) Contractile or motile proteins	Actin, Myosin, Tubulin, Dynein	Coordinate movements such as movements of chromosomes in mitosis
v) Structural proteins	Keratin, Fibroin, Collagen, Elastin, Proteoglycans	Essential components of various cells and tissues
vi) Defence proteins	Antibodies, Fibrinogen, Thrombin, Diphtheria toxin, Snake venoms, Ricin	Help in protection against harmful agents such as bacteria and virus
vii) Regulatory proteins	Insulin, Growth hormone, Corticotropin, Repressors	Control of growth and differentiation

The polypeptides which form proteins after being synthesised on ribosomes in linear chains are biologically inactive. But within a few seconds after their synthesis, the folding of polypeptide chain occurs in specific forms to give functionally active proteins which have many levels of organisation. These levels are expressed as primary, secondary, tertiary and quaternary structures. The **primary structure** of a protein refers to the number, type, and position of the individual amino acids in a polypeptide chain. A peptide bond is a covalent bond between the carboxyl group of one amino acid and the amino group of the adjoining amino acid. One molecule of water is removed in the formation of one peptide bond. A polypeptide chain thus will have only one free amino(-N or -NH₂) terminal and one free carboxyl (-C or -COOH) terminal (Fig. 5.2 a).

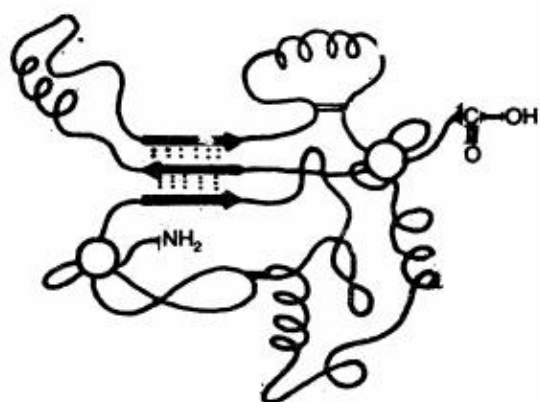
Secondary structure of a protein is determined entirely by the primary structure. The secondary structure is formed by the folding of a polypeptide chain. The folded chain is held together by hydrogen and disulphide bonds. Hydrogen-bonding interactions between peptide bonds are responsible for regular folding pattern like α -helix and β sheets. In an α -helix such as in α -keratin fibres and myoglobin the chain itself turns regularly to make a rigid cylinder. The tightly coiled polypeptide main chain forms the inner part of the rod, and the side chains extend outward in a helical array. The helix is stabilised by hydrogen bonds between residue one and five of the amino acids (Fig. 5.2 b, i & ii). Number of amino acid residues per turn comes to 3.6. In a β sheet such as in silk fibroin and parts of immunoglobulin, the polypeptide chain itself folds back and forth. In β pleated sheet, all

Secondary structure: The local structure of a polypeptide or nucleic acid chain arising from chemical bonding between the neighbouring residues to produce forms such as stem and loop of transfer RNA and α helix or beta sheet configuration in polypeptides.

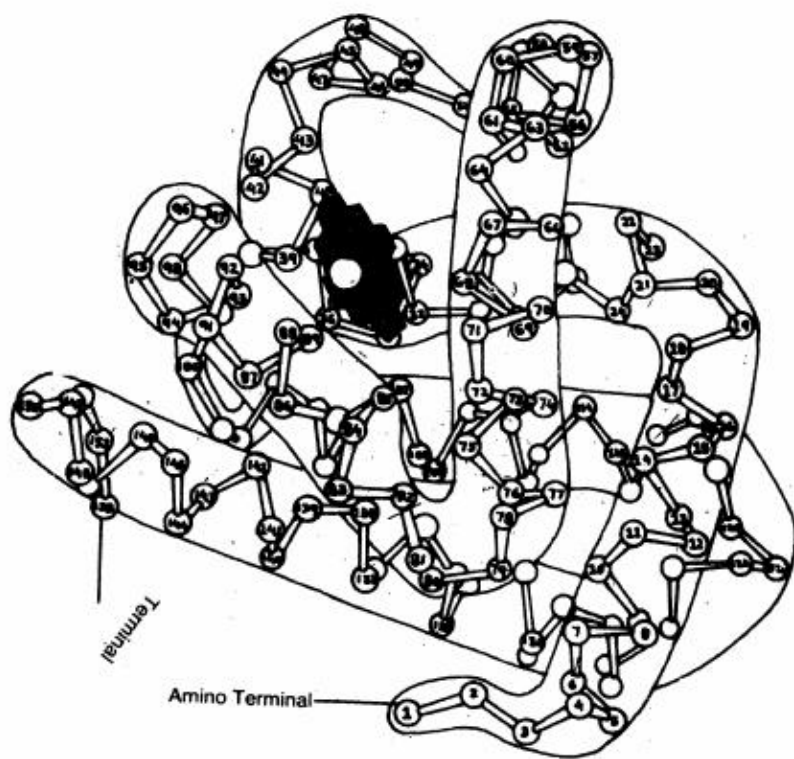
α - helix is a major secondary structure in polypeptides, characterised by regularly repeated hydrogen bonding between C = O and N - H groups in the chain.



(b) iv



(c) v



(c) ii

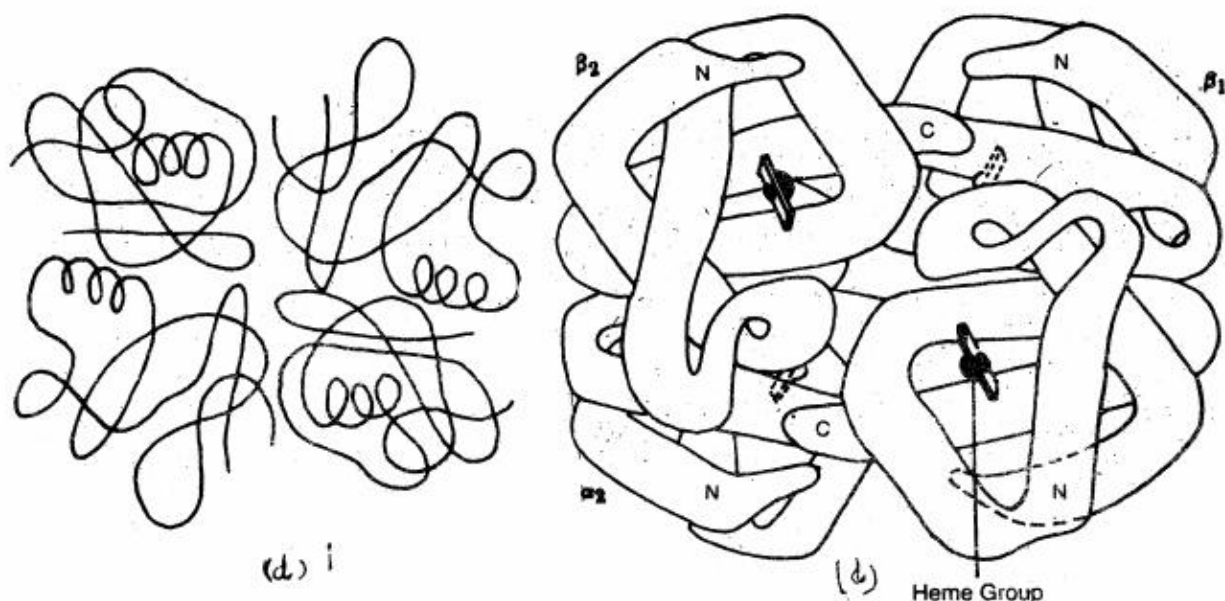


Fig. 5.2 : Different levels of protein structure

- a) Primary structure. Amino acids in the chain are linked together by peptide bonds to form linear polypeptide chains with the removal of water molecule.
- b) Secondary structure. In α -helix, the R groups of amino acids are directed radially away from the axis of helix. Every peptide bond is hydrogen bonded to a neighbour. Though the direction of helix can be right handed as well as left handed, right handed alpha helices are common. β -pleated arrangement of polypeptide are common and are present in many fibrous and most globular proteins.
- c) Tertiary structure. Myoglobin molecule has eight helical segments. The spaces between loops of the chain are filled with R groups (not shown here).
- d) Quaternary structure. The haemoglobin molecule consists of four polypeptide chains: two alpha globin and two beta globin chains. With each chain an oxygen binding heme group is attached.

Changes in pH or heating of protein molecules breaks protein polymers into monomers. The degradation results from loss of tertiary and secondary structure of monomers which leads to the loss of biological activity of the proteins. This disruption of native structure is termed as **denaturation**. Proteins regain their original structure when they return to their normal surroundings.

Diversity of protein molecules is due to the number, kinds and sequence of amino acids in a polypeptide chain. Change in amino acid sequence disrupts the structure of a protein, which thereby causes functional abnormality. For example, the change of even one amino acid residue in a chain of 146 residues of haemoglobin chain may bring about morphological and clinical changes in man causing the disease **sickle cell anemia** in which the capacity of red blood cells for oxygen uptake is impaired (Fig 5.3).

Residue: The main part of a molecule that is left after a small group has been removed in binding the molecule to another molecules.

	1	2	3	4	5	6	7	146	
Hb A	Val	His	Leu	Thr	Pro	Glu	Glu	Lys	----
	Val	His	Leu	Thr	Pro	Val	Glu	Lys	----
Hb C	Val	His	Leu	Thr	Pro	Lys	Glu	Lys	----

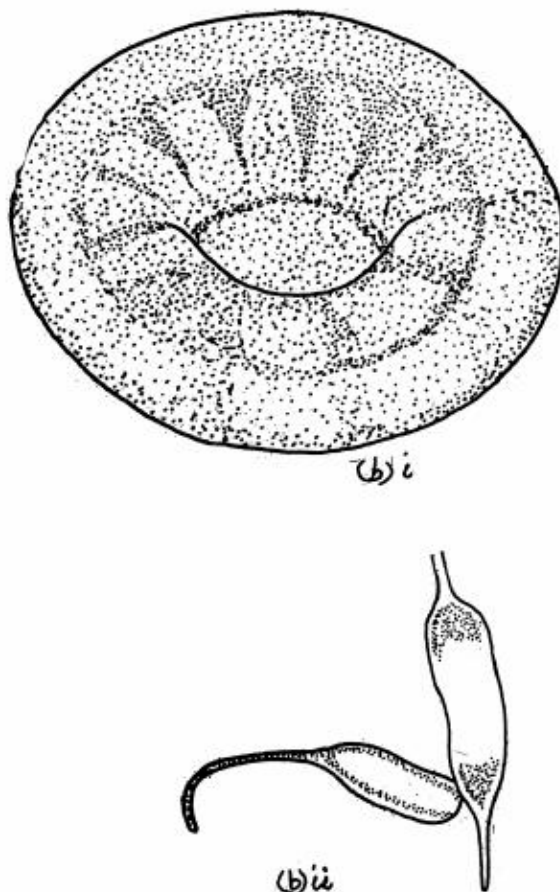


Fig. 5.3: a) In normal haemoglobin of an adult (HbA), the sixth amino acid in the beta chain from the amino terminal is glutamic acid. In haemoglobin of the sickle cell (HbS), glutamic acid is replaced by valine, but the remaining 145 amino acids are unchanged.
b) Normal human red blood cells (i) are discoidal in shape; these cells during low oxygen concentration become sickle shaped (ii) sickling of red blood cells causes the disease, known as sickle cell anaemia.

SAQ 2

Write the structure of tetrapeptide derived from glutamic acid, lysine asparagirt and serine indioating the pepptide bond clearly in the give space (see Section 4.2 of Unit stuctures of amino acids).

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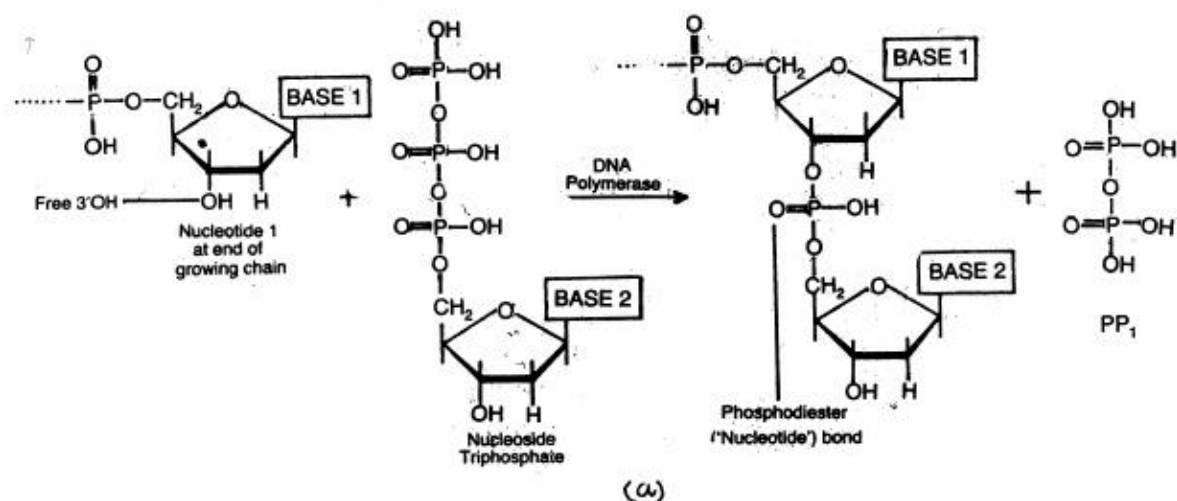
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5.4 NUCLEIC ACIDS

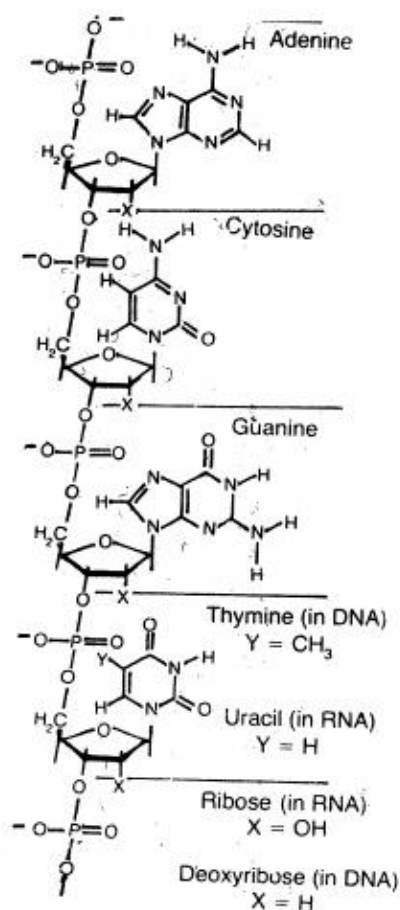
Nucleic acids are the genetic material in living organisms. Genetic information contained in each cell is encoded as polynucleotide in the form of nucleotide sequence. Information in the form of base pairing interactions of the nucleic acids is passed on from one generation to the next. There are two types of nucleic acids: deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA). In Unit 4, you have already studied about the monomers of nucleic acids, the nucleotides. We shall now discuss about the structure of nucleic acids in more detail.

5.4.1 Deoxyribo Nucleic Acid (DNA)

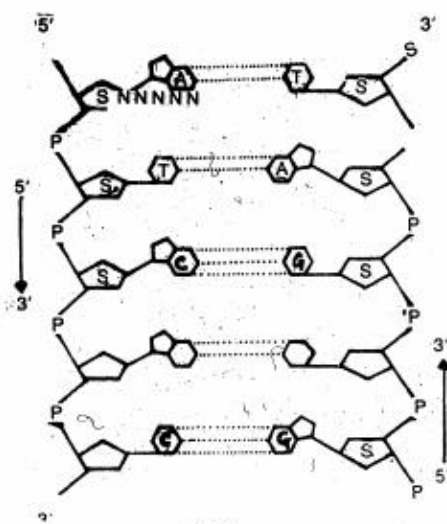
DNA is a basic molecule found in the cells. In 1953 Watson and Crick of Cambridge University proposed a molecular model for DNA for which they were awarded the Nobel Prize in 1962. They based their model on the observation of X-ray diffraction studies and on chemical analysis. The total quantity of DNA in eucaryotic cells is much higher than in procaryotic cells (see Unit 1).



(a)



(b)



(c)

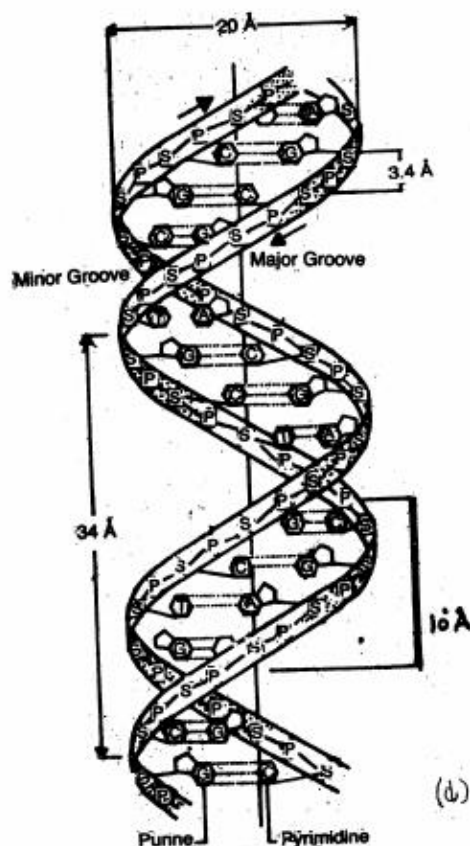


Fig. 5.4 : a) A nucleoside triphosphate. (Base 2) aligns itself to base 1 correctly. DNA polymerase enzyme links it to 3' end and releases two phosphate groups as pyrophosphates (PPi).
 b) A single nucleic acid chain showing the nucleotides. X=H in DNA (deoxyribose) and X=OH in RNA (Ribose). Y=CH₃ in DNA (Thymine) and Y=H in RNA (Uracil).
 c) Molecular structure of DNA showing the pentose sugar (S)-phosphate(P) backbone of polynucleotide chain. The two strands are complementary and antiparallel in nature. By convention the base sequence in single strand of DNA is written from 5' end on left to 3' end on the right, or a p (for phosphate) is prefixed for free 5' making its - phosphoryl end.
 d) DNA double helix showing major and minor grooves and some other molecular dimensions. The ribbons indicate phosphate deoxyribose backbones. Horizontal lines represent the base pairing. P=phosphate group, S=sugar.

The 5' carbon of the deoxyribose sugar of one nucleotide is joined to 3' carbon of deoxyribose sugar of the next nucleotide by a phosphodiester bond known as nucleotide bond or bridge (Fig 5.4 b). Phosphate group of the nucleotide can form an ester bond with both, the 3' and 5' -OH group of a pentose sugar and, therefore, the bond is known as **phosphodiester bond**.

A nucleic acid chain has one free 5' phosphoryl end and one free 3' hydroxyl end. The backbone of DNA is highly polar due to the presence of phosphate group which is acidic at the pH of the cell.

DNA molecule has two chains or strands which are known to be antiparallel as their 5'-3' polarity runs in opposite directions. Structure of a DNA molecule is helical and ladder-like, with steps made up of bases and the two sides (backbones) made up of sugar and phosphate residues (Fig. 5.4c). Nitrogenous bases of the two strands in a molecule are connected with each other by hydrogen bonds, which stabilise the DNA molecule.

The base pairing is one of the most fundamental concepts of DNA structure and function and always occurs between a purine and pyrimidine due to the geometry of the double

Antiparallel: Alignment of the two DNA strands in a helix with their 3',5' phosphodiester linkages in opposite directions.

helical structure (see below). Adenine (A) forms hydrogen bonds only with thymine (T); and guanine (G) only with cytosine (C). This is called as **complementary base pairing**. Three hydrogen bonds are formed between G and C ($G \equiv C$) but only two between A and T ($A = T$). The GC pairing is, therefore, chemically more stable than AT pairing.

SAQ 3

If one strand of double helix of DNA has a base sequence of 5-ATCGAACGT-3, what would be the base sequence of the complementary DNA strand?

(Hint: Complementary base pairing A-T and G-C)

DNA, The Double Helix

The two strands of the DNA form a helical structure, known as the **double helix**. It is important to mention here that DNA exists in five forms, i.e. A, B, C, D and E. Of these, B form is the most common about which we will discuss in detail here. It is a right handed helix and most commonly found in living system. The other forms have only minor differences. The spatial relationship between the two strands of the helix forms a major groove or wide groove and a minor groove or narrow groove (Fig. 5.4.d).

Each complete turn of helix is 3.4 nm long and contains 10 base pairs (bp). Therefore, the consecutive base pairs are 0.34 nm (3.4/10) apart and are inclined at 36° in relation to each other. The helix has a constant diameter of about 2 nm, hence, the dimensions of each base pair must be constant. Each base pair contains one two-ringed purine (1.2 nm long) and one single-ringed pyrimidine (0.8 nm long) so as to keep the dimensions of each base pair constant. A base pair formed by two purine bases or by two pyrimidine bases will, therefore, distort the helical structure.

DNA structure fulfils the basic requirement of the genetic material that means it must be copied accurately with every cell division. The accurate copying of DNA, i.e. DNA replication, is due to the complementary nature of the bases in the two strands of DNA: A pairing with T, and G pairing with C.

Complexes of DNA and Protein (Nucleosomes)

DNA in chromosomes is always associated with proteins and it coils on the protein to give rise to a highly compact tertiary structure.

A chromosome, which is a thread-like structure found in nucleus, consists of chromatin. Chromatin network contains about 60 per cent protein, 35 per cent DNA and 5 per cent RNA. On suitable treatment, chromatin can be seen under the electron microscope in its expanded form. It appears as "beads on a string" (Fig. 5.5) and these are known as **nucleosomes**. The "bead" or the nucleosome core is ellipsoidal in shape with a diameter of 11 nm, height of 6 nm and circumference of about 34 nm.

On biochemical analysis, a nucleosome is found to contain two molecules of each of the histones, H2A, H2B, H3 and H4 forming an octomeric protein disc, one molecule of histone H1 and a DNA strand of about 200 base pair long (Fig. 5.5).

DNA fragment of 140 base pairs is wrapped like a ribbon around the histone octamer. It is because this much length of DNA (67nm) may not be squashed (pressed) inside the small protein disc. Sensitivity of DNA to enzymes also supports the idea of DNA being on the outside of the protein disc of the 200 base pair DNA because the increased surface area of DNA will provide more space for enzyme reaction. About 140 base pair is sufficient to make two turns around the protein disc.

The remaining 60 base pair act as a linker or spacer DNA and joins the repeated nucleosome cores. Histone H1 is associated with DNA, at the entry and exit points of DNA from the core. It is involved in close packaging of the nucleosomes to form a 30 nm thick chromatin fibre.

5.4.2 Ribose Nucleic Acid (RNA)

As you have studied in the previous section, DNA stores the genetic information which is transferred from nucleus to cytoplasm by transcribing to the RNA molecules. These RNA molecules translate the information to synthesise proteins. The primary structure of RNA is

Double helix: Physical configuration typically adopted by the polynucleotide chains of DNA.

Transcription: Process by which an RNA molecule is synthesised on a DNA template with the aid of various enzymes.

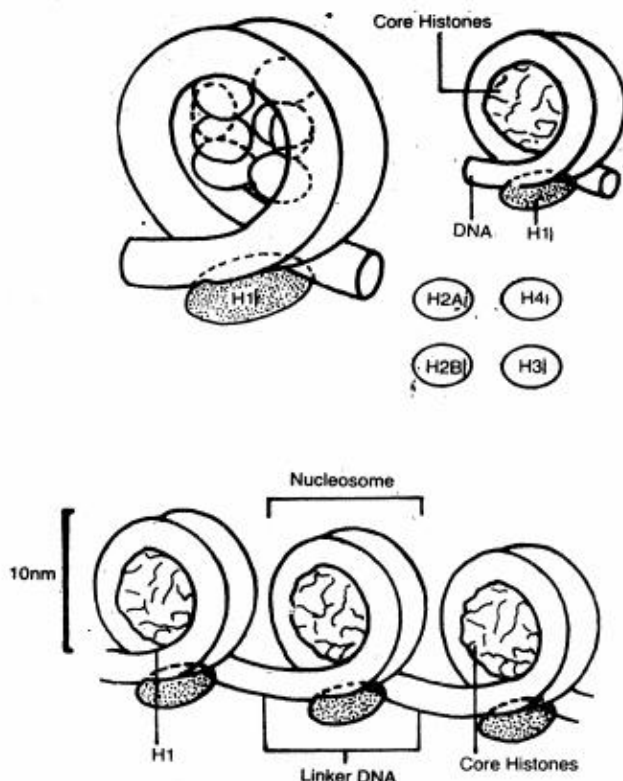


Fig. 5.5 : Chromatin appearing as beads on a string. Each nucleosome bead is connected to its neighbour by a linker DNA. Beads are released from chromatin by digestion of the linker DNA with micrococcal nuclease enzymes. Each bead has an octamer histone core around which 146 base pair DNA is wrapped.

comparable to that of DNA: a polynucleotide chain with 5'-3' sugar phosphate backbone. But it differs from DNA as the pentose sugar in RNA is ribose instead of deoxyribose. Further, thymine is substituted by uracil in RNA (Table 5.3). All RNAs exist as non-helical single stranded structure. The molecules are much shorter than DNA and the ratio of purines and pyrimidines is not 1:1. However, RNA molecules may have complementary intramolecular regions. The complementary sequences in an RNA strand may fold back upon itself to form an antiparallel duplex structure known as the hairpin loop. Such complementary sequences are palindromic in nature. Palindrome is a stretch of DNA in which the base sequences read the same from the 3' or 5' end. In these regions, hydrogen bonds may be formed between A and U, and G and C. As in the case of DNA, base pairing provides stability to the RNA also.

Translation: Process by which a protein is synthesised from amino acids according to specifications encoded in the mRNA.

Palindrome: A sequence of duplex DNA that is the same when the two strands are read in opposite direction such as Malayalam is a palindromic word with the sequence of letters on both sides of y being the same.

Table 5.3
Difference between DNA and RNA

Feature	DNA	RNA
1) Pentose sugar	Deoxyribose	Ribose
2) Nitrogenous base	Adenine, Thymine, Cytosine, Guanine	Adenine, Uracil, Cytosine, Guanine
3) Ratio of purines to pyrimidines	One	Variable
4) Secondary structure	Double stranded and helical	Single stranded and non-helical
5) Role in a eucaryotic cell	Carries genetic information	Synthesis of proteins
6) Localisation	Primarily in nucleus; also in mitochondria and chloroplast	Largely in cytoplasm, synthesised in nucleolus
7) No. of nucleotides	Contains a larger number of nucleotides, upto a few millions.	Contains much smaller number of nucleotides, upto a few thousands.

There are three major classes of ribonucleic acids (RNA) which can be distinguished structurally and functionally. These are messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). All these types of RNA are synthesised in the nucleus and are involved in protein synthesis. *

The mRNA carries the genetic message, and it is a single stranded polymer. The length of mRNA depends upon the number of polypeptides it codes for.

The tRNA molecule serves as adaptor for the translation of genetic information in the sequences of mRNA into specific amino acids. The primary structure of tRNA is a single stranded nucleotide which folds back to align with the complementary regions to form a secondary structure like a clover leaf. You will study the structure of tRNA in detail in Units 13 and 14 with reference to protein synthesis.

The rRNA is a component of ribosome. The ribosome has a nucleoprotein structure and is the site for protein synthesis.

A comparison of 'three kinds of RNA' is made in Table 5.4. You will study about all these types of RNAs in detail in Units 13 and 14.

Table 5.4
Comparison among three kinds of RNA

Property	Ribosomal RNA	Messenger RNA	Transfer RNA
Sedimentation coefficient (S units)	5-7, 18, 28	8	
Number of nucleotides	120-5500	900-12000	75-85
Molecular weight	$0.5-1.1 \times 10^6$	500,000	25,000
Unusual bases	small amount of methylated bases	small amount of unusual bases	large amount of unusual bases
Percentage of total cellular RNA	about 80	upto about 5	about 15-20
Site of synthesis	nucleolus	nucleolus	nucleolus
Role	serves as template for synthesis of ribosomal proteins	carries genetic information from chromosomal DNA to cytoplasmic ribosomes where it participates in protein synthesis.	acts as adaptor for specific amino acid attachment and transfer to mRNA template.

SAQ 4

Which of the following statements (i-iv) apply to

- DNA only
- RNA only
- Both DNA and RNA

Write your answer (s) in the space provided.

[Hint: If a particular statement applies to DNA only then the answer will be (a) and it to RNA only then the answer will be (b) and if to both DNA and RNA, then the answer will be (c)]

Statements

i) Found mainly in the nucleus.

ii) Polynucleotide chain in which sugar is ribose.

iii) Contains equal number of purine and pyrimidine bases.

iv) Contains linked nucleotides.

5.5 CARBOHYDRATES

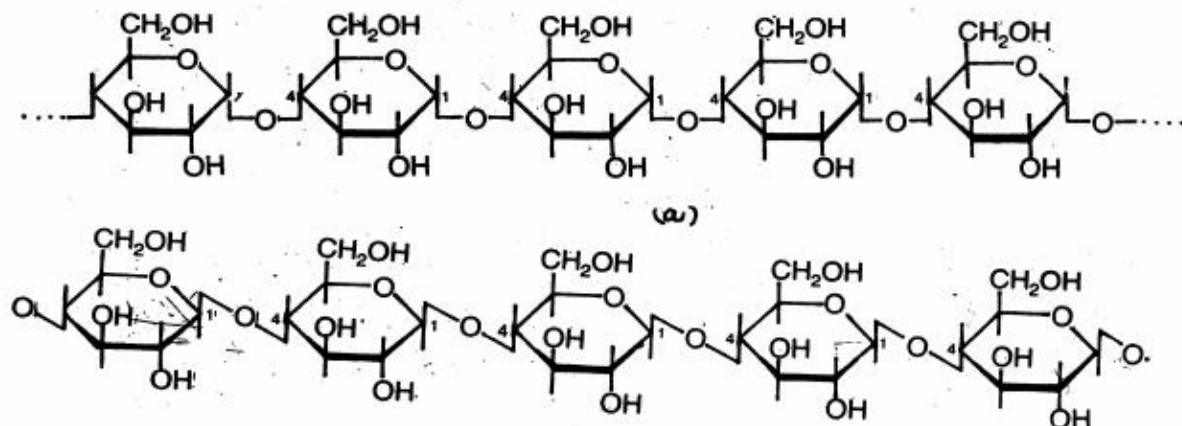
Carbohydrates, composed of carbon, hydrogen and oxygen, are the main source of cellular energy and are important structural components of cell walls and intercellular materials. Carbohydrates are classified into monosaccharides, disaccharides and polysaccharides according to the number of monomers they contain. You have studied in detail about monosaccharides and disaccharides in Unit 4, so we will discuss about the structure and function of polysaccharides in this unit.

Polysaccharides have the general formula $(C_6H_{10}O_5)_n$. They are polymers formed by condensation of large number of monomers such as glucose, fructose, etc. Major functions of polysaccharides are concerned with food storage and maintenance of cellular structure. In eucaryotes two structural polysaccharides are cellulose and chitin. Cellulose is found in plant cell wall, as well as in many algae and fungi. Chitin forms the exo-skeleton of many animals like arthropods (e.g. insects), many other invertebrates and also in many plants, e.g. fungi (Table 5.5).

Table 5.5
Some Naturally Occurring Polysaccharides

Distribution	Name	Chemical composition	Biological role
PLANTS	Cellulose	homopolymer of glucose, unbranched	the main structural component of plant cell walls
	Amylose	homopolymer of glucose, unbranched	food store : a component of starch
	Amylopectin	homopolymer of glucose, unbranched	food store : a component of starch
ANIMALS	Glycogen	homopolymer of glucose, more branched than amylopectin	food store, mainly in muscles and liver
	Chitin	unbranched non-glucose homopolymer of acetyl glucosamine and glucuronic acid	forms horny exo-skeleton in arthropods
	Chondroitin Sulphate	complex heteropolymer of galactosamine	structural component of cartilage

You have already learnt the structure of some common monosaccharides in Unit 4. Each monomeric unit is linked to the other by a glycosidic bond. The bond may be between carbon atom 1 of one monosaccharide and carbon atom 4 or 6 of the other monosaccharide. These may be α - or β -glycosidic bonds depending on the position of hydroxyl group at carbon atom -1. Amylose, cellulose and glycogen are all polymers of glucose molecules but differ in the way they are joined together (Fig. 5.6). Unlike mono and disaccharides (sugars), the polysaccharides are relatively insoluble in water and are therefore not sweet in taste.



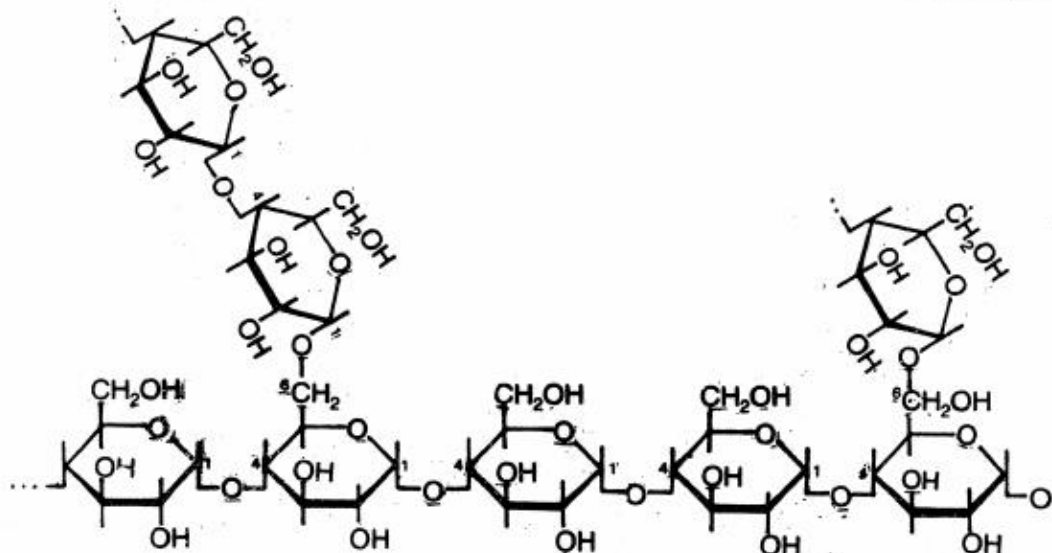


Fig. 5.6 : a) The 1-4 glycosidic bond between two glucose molecules showing a part of amylose molecule.
 b) The 1-4 glycosidic bond between two glucose molecules showing a part of a cellulose molecule.
 c) The 1-4 and 1-6 glycosidic bonds between glucose molecules which give rise to branched molecules of glycogen and amylopectin. Glycogen is highly branched, the ratio of 1,4 to 1,6 bonds being from 12 to 18, while amylopectin is less branched.

SAQ 5

Which carbon atoms of glucose are most commonly involved in glycosidic bond formation?

Write your answer in 2-3 lines.

Complex Polysaccharides

In some of the hexoses, the hydroxyl group is replaced by amino, acetylamino, carboxyl or sulphate groups to form derivatives of monosaccharides (Fig. 5.7). These derivatives of monosaccharides combine together to form complex polysaccharides. Complex polysaccharides are important in molecular organisation. They are often found in combination with proteins or lipids.

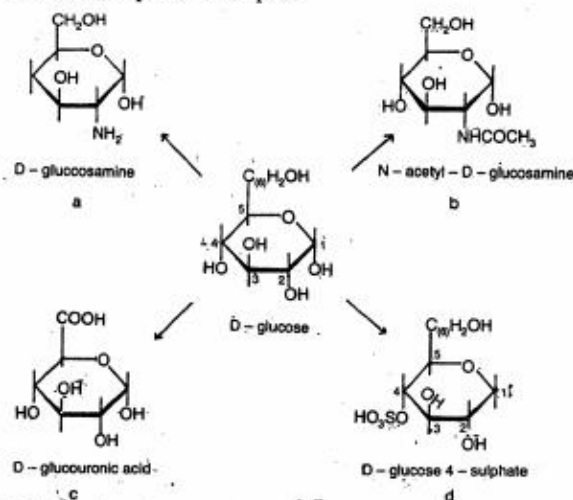


Fig. 5.7 : Derivatives of monosaccharides. The replacement of -OH group of D-glucose by -NH₂ (amino) group forms D-glucosamine (a); by -NHCOCH₃ (acetylamino) groups forms N-acetyl D-glucosamine (b); by -COOH (carboxyl) group forms D-glucuronic acid (c); and by -OSO₃H sulphate group, forms D-glucose 4-sulphate (d).

Glycosaminoglycans, Proteoglycans and glycoproteins are examples of complex polysaccharides. Glycosaminoglycans are built up of repeating disaccharides units, generally composed of an amino sugar (either glucosamine or galactosamine) and a uronic acid (e.g. glucuronic acid). Glycosaminoglycans were formerly called mucopolysaccharides. Hyaluronic acid, chondroitin sulphate and heparin are a few examples of this class. Numerous glycosaminoglycan molecules in combination with small amounts of protein form proteoglycans, formerly known as mucoprotein.

The glycoproteins, on the other hand, are protein molecules with a few short or long carbohydrate side chains. It may be noted that proteoglycans are mainly glycans whereas glycoproteins are mainly proteins.

5.6 LIPIDS

Lipids are major structural components of the cells, and are a class of chemically diverse compounds with a common property. They are all insoluble in polar solvents such as water but are soluble in non-polar organic solvents such as ether and alcohol. This is because lipids contain hydrocarbon chains which are non-polar and hydrophobic. Since they contain fatty acids (about which you have studied in detail in Section 4.2 of Unit 4) with a free carboxyl end, they can undergo saponification (soap formation). Some of the commonly known lipids are classified in three groups (Table 5.6).

Table 5.6
Classification of Lipids

Lipid group	Class name	Cellular location of compounds
Fatty acids*	Fatty acids	Cytosol, mitochondria, glyoxysome of fatty seeds
Simple lipids (Esters of fatty acids with alcohols)	Neutral fats (Triacylglycerols)	Fat storage in cytoplasm
Compound lipids (Simple lipids containing other group(s) in addition to fatty acids)	Phospholipids	Membranes
	Sphingolipids	Membranes
	Glycolipids	Membranes
Derived lipids	Steroids	Membranes
	Terpenes (Essential oils, carotenoids)	Plant cytosol and chloroplast

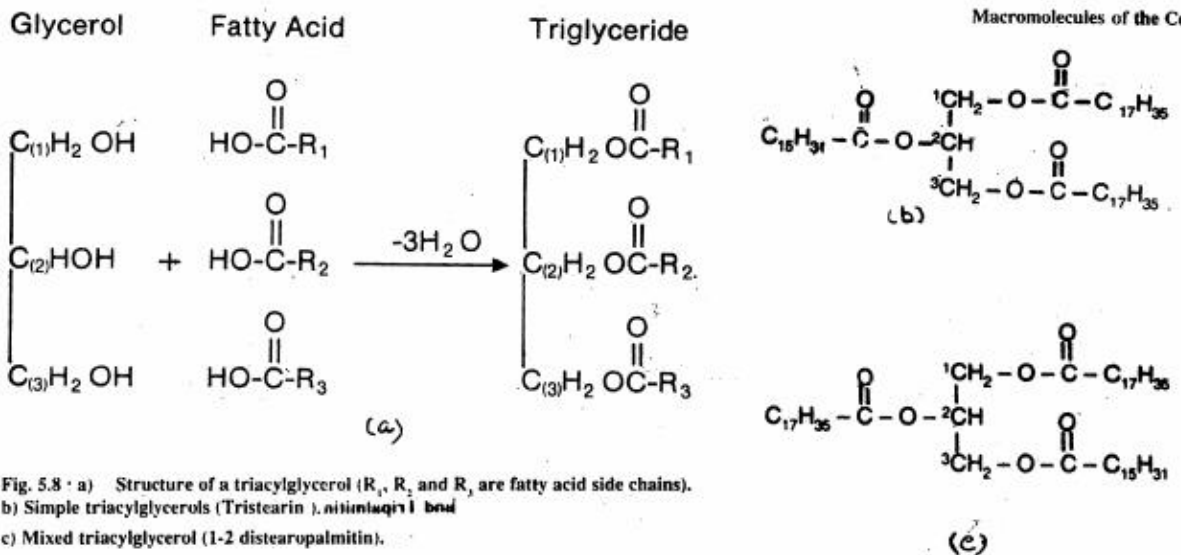
* Fatty acids as such are not lipids but they are the essential components of lipids.

5.6.1 Simple Lipids

You have already studied the structure of fatty acids in Section 4.2 of Unit 4. Simple lipids are triacylglycerols, which are formed by the combination of three fatty acid molecules with a glycerol molecule (Fig. 5.8a). Triacylglycerols are non-polar, hydrophobic molecules and since they do not contain any charged groups, they are named as neutral fats. When oxidised, these fats produce energy more than twice of that given by carbohydrates or proteins.

In simple triacylglycerols such as tristearate glycerol or tripalmityl glycerol all the three fatty acid molecules (R_1 , R_2 , R_3) are of one type (Fig. 5.8 b). But in mixed triacylglycerols such as 1,2 distearopalmitin, more than one type of fatty acid molecules are joined to the same glycerol molecule (Fig. 5.8 c).

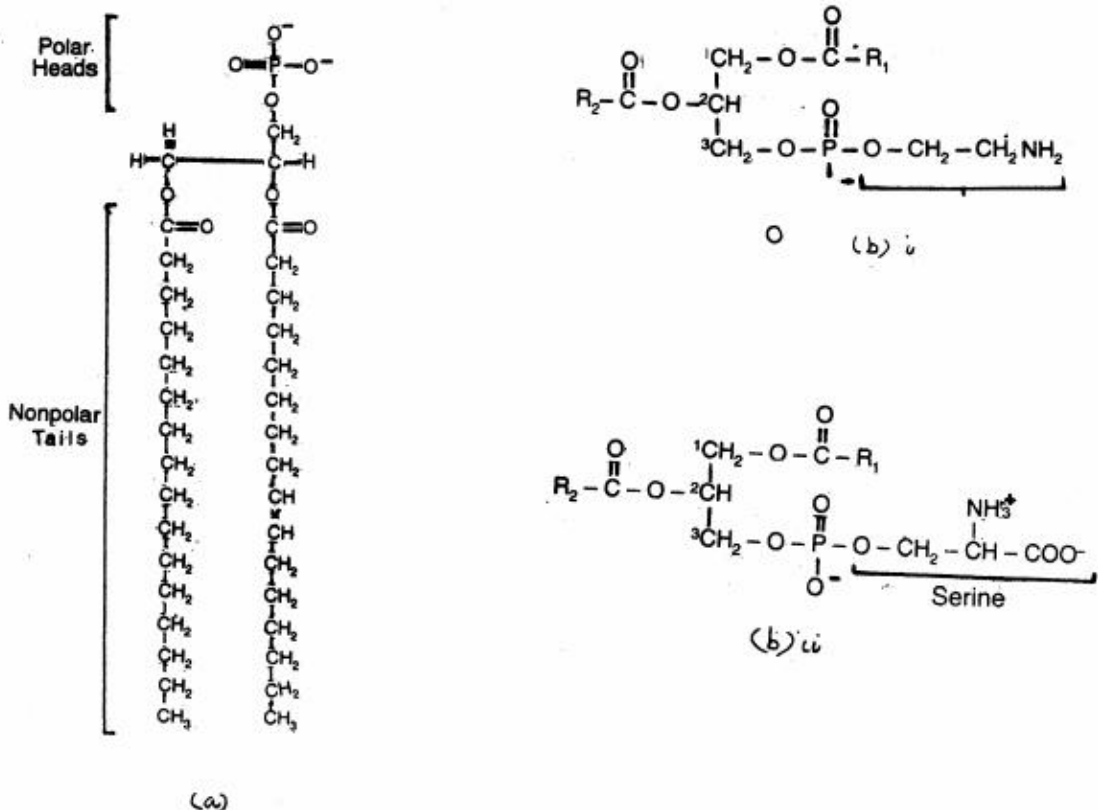
Most of the neutral fats, such as those in butter and olive oil are mixtures of simple and mixed triacylglycerols. Neutral fats containing only saturated fatty acids are solids at room temperature. Fats with largely unsaturated fatty acids like oleic and linoleic acid etc. remain liquid at room temperature and are known as oils. Oils are converted into fats by hydrogenation of the double bonds. That is how the vegetable ghee is produced. Oils, such as groundnut oil which contain unsaturated fatty acids are easily oxidised because of the presence of a large number of double bonds and hence they are spoiled easily. Saturated fats have better keeping quality.



5.6.2 Compound Lipids

These are the lipids which are composed of glycerol, fatty acids, phosphoric acid and a nitrogenous compound such as choline, serine, ethanolamine etc. Major types of compound lipids are phospholipids, glycolipids, and sphingolipids. In phospholipids, one fatty acid molecule in a triacylglycerol is substituted by a phosphoric acid molecule. Thus, all phospholipids have a hydrophobic tail consisting of two fatty acid chains and a hydrophilic head made up of negatively charged phosphoric acid residue (Fig. 5.9 a). Consequently, they are amphipathic in nature as they have both hydrophobic and hydrophilic region in the same molecule. They are the major constituents of the cell membrane.

Amphipathic: Molecules that have both hydrophilic and hydrophobic groups.



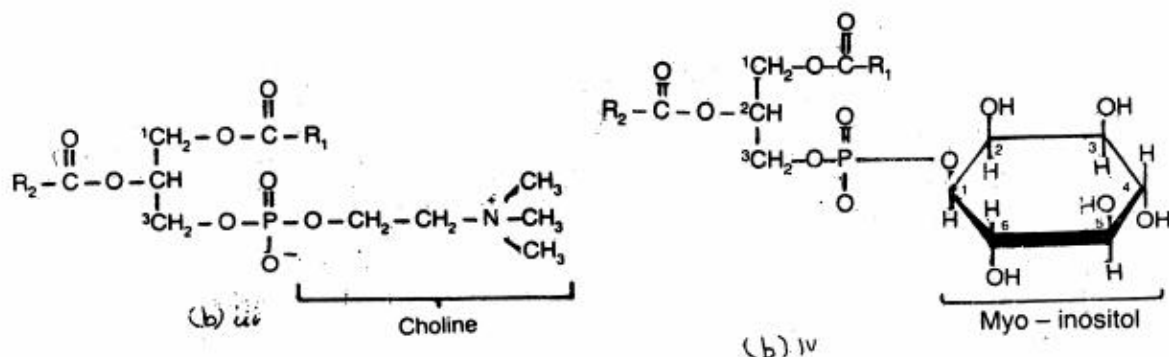


Fig. 5.9 : a) A phosphoglyceride. Two fatty acid molecules are esterified to the first and second hydroxyl groups of glycerol. Phosphoric acid is esterified to third hydroxyl group of glycerol.
b) Different types of phosphoglycerides.

The most abundant phospholipids are phosphatidylethanolamine (cephalin), phosphatidylcholine (lecithin), phosphatidylserine and phosphatidylinositol (Fig. 5.9 b). Sphingolipids and glycolipids are also amphipathic molecules and are main constituents of cell membranes. These are the main components of brain and nervous tissues.

5.6.3 Derived Lipids

Derived lipids contain no fatty acids. Therefore, they cannot be converted into soaps (non-saponifiable). These include steroids and terpenes.

All the steroids have parent nucleus called as cyclopentanoperhydrophenanthrene ring (Fig. 5.10). This structure is composed of four rings A, B, C and D which are fused with each other. A, B and C rings constitute phenanthrene ring and to them the cyclopentane ring D is attached.

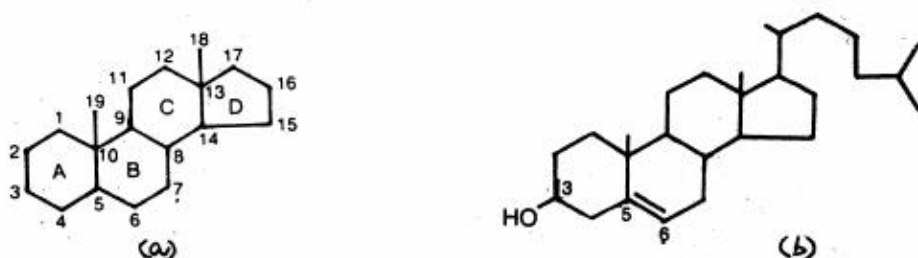


Fig. 5.10 : a) Cyclopentanoperhydrophenanthrene nucleus with numbered carbon atoms. Three cyclohexane rings (A, B and C) and a terminal cyclopentane ring (D) form the basic structure of steroid.
b) Cholesterol. The hydroxyl group is polar and rest of the molecule is non-polar in nature.

The most important members of steroids are cholesterol, bile acids and sex hormones. Cholesterol is only found in animal tissues and is the parent compound which produces ergosterol, a precursor for the formation of vitamin D (Fig. 5.10).

Terpenes are synthesised by polymerisation of the five carbon unit called isoprene unit. These units are bound in head to tail organisation. Terpenes include certain fat soluble vitamins like Vitamin A, E, K, plant pigments like carotenoids, chlorophyll and certain co-enzymes (coenzyme Q, ubiquinone). Terpenes are major components of the essential oils.

5.7 SUMMARY

In this unit, you have studied:

- Types of chemical bonds and formation of macromolecules,
- Structure of proteins which are formed from amino acids bonded together by covalent peptide bonds. Proteins have four levels of structural organisation i.e. primary, secondary, tertiary and quaternary structures. Proteins are of major importance in the living system as they are the major cellular components and are involved in several vital functions such as enzyme formation and transport of substance etc.
- Nucleic acids, the genetic material, are the linear polymers of nucleotides. Deoxyribonucleic acid (DNA) consists of two complementary strands joined by hydrogen bonds and is coiled to form a double helical structure. Ribonucleic acid (RNA) is a single stranded structure having a ribose sugar and nitrogenous base uracil in place of thymine.
- Polysaccharides may be branched or unbranched chains formed by monosaccharides joined together by glycosidic linkages. The most important storage polysaccharides are starch in plants and glycogen in animals. Cellulose is the main component of plant cell wall. Chitin forms the exo-skeleton in most invertebrates.
- Lipids are water-insoluble components of cells. Neutral fats are simple lipids and serve as storage fats. Phospholipids, sphingolipids and glycolipids are structural elements of membranes and are amphipathic in nature.
- Sex hormones and cholesterol, are some examples of steroids, which are non-saponifiable lipids. Terpenes are major components of essential oil and chlorophyll.

5.8 TERMINAL QUESTIONS

- 1) Why is the tertiary structure of a protein lost on heating to 80° whereas the primary structure remains unchanged? Give your answer in 3-4 lines.

.....

.....

.....

.....

- 2) a) How many nucleosomes would be formed by 4×10^5 bp long DNA and why?

.....

.....

- b) How many molecules each of histones H1, H2A, H2B, H3 and H4 would be required to form the nucleosomes for the above DNA?

.....

.....

- 3) In a fragment of DNA double helix there are 60 pyrimidine bases and 20 adenine bases. Calculate the number of each of the following in the fragment giving reasons.

- a) Thymine bases

.....

.....

- b) Total number of bases

.....

.....

- c) Cytosine bases

.....

.....

- d) Nucleotides
.....
.....
- e) Complementary base pairs
.....
.....
- 4) Cellulose, amylose, amylopectin and glycogen are all polymers of only one monosaccharide, i.e. glucose. Then how do they differ from each other in their structure and properties? Give four reasons.
.....
.....
.....
.....
- 5) Write the structural formula of dipalmitin stearic glycerol in the given space.
(Hint: See the structures of palmitic acid and stearic acid in Section 4.2 of Unit 4.)
.....
.....
.....

5.9 ANSWERS

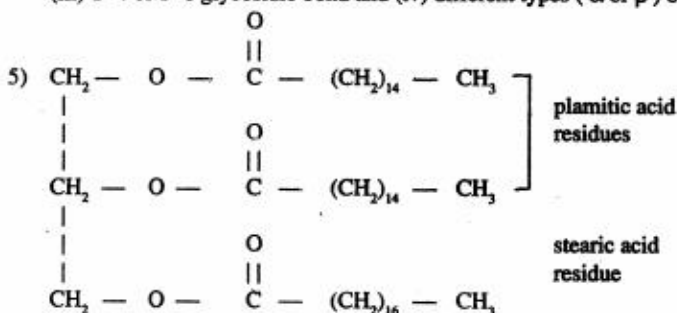
Self-assessment Questions

- 1) Ionic bonds become weak in the presence of water because water molecules prevent the interaction between ions by increasing the distance of separation between charged molecules.
- 2)
- $$\begin{array}{ccccccc}
 \text{H}_2\text{N}-\text{CH}- & \begin{array}{c} \text{O} \quad \text{H} \\ || \quad | \\ \text{C}-\text{N} \end{array} - & \text{CH}- & \begin{array}{c} \text{O} \quad \text{H} \\ || \quad | \\ \text{C}-\text{N} \end{array} - & \text{CH}- & \begin{array}{c} \text{O} \quad \text{H} \\ || \quad | \\ \text{C}-\text{N} \end{array} - & \text{CH}-\text{COOH} \\
 | & \uparrow & | & \uparrow & | & \uparrow & | \\
 \text{CH}_2 & \text{(Peptide} & \text{CH}_2 & \text{bond 2)} & \text{CH}_2 & \text{(Peptide} & \text{CH}_2\text{OH} \\
 | & \text{bond 1)} & | & & | & \text{bond 3)} & \text{(Ser)} \\
 \text{CH}_2 & & \text{CH}_2 & & \text{H}_2\text{N}-\text{C=O} & & \\
 | & & | & & \text{(Aspn)} & & \\
 \text{COOH} & & \text{CH}_2 & & & & \\
 \text{(Glu)} & & | & & & & \\
 & & \text{CH}_2 & & & & \\
 & & | & & & & \\
 & & \text{NH}_2 & & & & \\
 & & \text{(Lys)} & & & &
 \end{array}$$
- 3) 3'-TAGCTTGCA-5'
- 4) i) a
ii) b
iii) a
iv) c
- 5) Carbon atom 1 and 4 or carbon atom 1 and 6 of glucose are most often involved in glycosidic bond formation.

Terminal Questions

- 1) The tertiary structure of proteins is stabilised by weak hydrogen bonds which break at 80°, whereas the primary structure is stabilised by covalent bonds which are too strong to be affected at this temperature.

- 2) a) 2×10^3 , as 200 bp length of DNA comprises one nucleosome.
 b) 2×10^3 of H1 and 4×10^3 each of H2A, H2B, H3, and H4.
- 3) a) 20. By the base pair rule of A-T and G-C.
 b) 120. As there are 60 pyrimidine bases, there must be 60 purine bases that is 120 (60+60) bases altogether.
 c) 40. As pyrimidine bases (T+C) are 60, and thymine bases are 20, so cytosine bases are 40 (60-20).
 d) 120. As the total number of bases is 120.
 e) 60. As total number of bases is 120, the number of base pair must be half.
- 4) This is due to (i) branched or unbranched structures, (ii) sequences of different lengths (iii) 1-4 or 1-6 glycosidic bond and (iv) different types (α or β) of glucose molecules.



(The order of fatty acid residues may differ.)

GLOSSARY

affinity chromatography: a technique for separating macromolecules based on the biological affinity of the molecule for a matrix bound ligand.

autophagy: digestion of cellular material by the cell's own enzymes; part of normal regeneration and turn over of eucaryotic cellular components.

autoradiography: determining the location of radioactive tracers introduced into cell by exposure of photographic emulsions placed in contact with the cells.

basal body: an organelle located at the base of cilia and believed to be involved in the organisation of ciliary microtubules.

cell culture: a population of cells grown *in vitro*.

centromere: the primary construction of a chromosome to which the spindle fibers remain attached.

chloroplasts: membranous organelles of plant cells containing chlorophyll wherein photosynthesis occurs.

cilia (sing: cilium): locomotor organelles located at the cell surface.

cisterna (pl. cisternae): a flattened membranous sac filled with fluid.

codon: a sequence of three nucleotides of messenger RNA that codes for an amino acid or for chain termination.

complementary base pairing: hydrogen bond formation between a particular purine and a particular pyrimidine in nucleic acids, for example, guanine and cytosine and adenine and thymine.

core particle: result of partial digestion of a nucleosome leaving the histone octamer and 146 base pair of DNA.

covalent bond: bond between atoms formed by sharing of electrons.

cristae: infoldings of the mitochondrial inner membrane and the site of enzymes of oxidative phosphorylation and electron transport.

cytoskeleton: an intracellular framework composed of filaments and microtubules.

cytosol: the fluid portion of the cytoplasm in which the organelles are suspended.

deoxyribonucleic acid (DNA) : the genetic material of all cells and many viruses.

dictyosome: a stack of cisternae that forms part of the Golgi apparatus. In plant cells, the term is often used for the entire Golgi apparatus.

endocytosis: intake of solutes or particles by a cell by enclosing them in an infolding of the plasma membrane.

exocytosis: a mode of transport of substances out of the cell by enclosure in a vesicle, fusion of the vesicle with the plasma membrane and subsequent expulsion of the vesicle's contents.

ferritin: an iron-rich protein found in the liver, spleen and bone-marrow.

flagella (sing flagellum): locomotor organelles ultra structurally similar to cilia but usually longer and present in smaller numbers per cell.

fluorescent antibody technique: detection of selected antigens in cells by staining with specific antibody that has been conjugated with a fluorescent dye.

GERL: Golgi associated endoplasmic reticulum involved in the production of lysosomes.

hairpin loops: a folded region of single-stranded DNA or RNA formed by the pairing of two neighbouring complementary stretches of bases.

helix : a spiral structure having a repeating pattern described by two simultaneous operations—rotation and translation. It is the natural conformation of many biological polymers.

heterophagy : the process of forming a heterophagic vacuole.

histone: a protein component of the chromosome having a high content of basic amino acids. Eight histones comprise the core of a nucleosome.

hydrophobic bond : the associations formed by hydrophobic groups when surrounded by water.

in vitro (Latin-"in glass") : experiments carried out with isolated cells, tissues or cell free extracts.

in vivo (Latin-"in life"): experiments carried out using the intact organism.

lipid: class of organic compounds that are poorly soluble or insoluble in water but soluble in nonaqueous solvents such as ether or acetone.

lysosomes: intracellular organelles that contain a large variety of hydrolytic enzymes.

macromolecules: molecules having molecular weights in the range of few thousand to hundreds of millions of molecular weight units.

microbody: a membrane bounded cytoplasmic organelle with varied enzyme content and functions e.g. peroxisomes and glyoxysomes.

neutral fats : glycerides, fatty acid esters of glycerol, a major storage form of fats.

nucleic acid : polymer of nucleotides in an unbranched chain, DNA and RNA.

nucleoid : a region in the cytosol of procaryotic cells that contains nuclear material but is not segregated by membranes.

nucleoside: molecule containing a purine or pyrimidine linked to a pentose sugar.

nucleosomes: spherical particles seen along decondensed chromatin, each nucleosome is composed of eight histones around which there are nearly two turns of DNA.

nucleotide: a phosphorylated nucleoside.

organelle: a subcellular component, a discrete structural differentiation of the cell containing particular enzymes and performing particular functions for the whole cell; e.g. mitochondria, ribosome, etc.

peptidoglycan: macromolecule formed from both protein and carbohydrate parts, abundant in the cell walls of bacteria.

phagocytosis: a form of endocytosis in which large amounts of particulate material, even whole cells are enclosed in endocytic vesicles.

phosphodiester linkage: a covalent linkage involving esterification to phosphoric acid.

phospholipids: lipids that contain charged, hydrophilic phosphate groups; primary components of cell membranes.

photorespiration: uptake of oxygen and release of carbon dioxide by photosynthetic cells or whole plants in the light.

pinocytosis: endocytosis of small molecules in an aqueous medium.

plastid: eucaryotic cell organelle that stores pigments or carbohydrates.

polynucleotide: a linear sequence of nucleotides in which the sugar of one nucleotide is linked through a phosphate group to the sugar on the adjacent nucleotide.

procaryotes: organisms such as bacteria, blue green algae and mycoplasmas in which the nucleus is not separated from the cytoplasm by membranes.

purine: parent compound of the nitrogen containing bases adenine and guanine

pyrimidine: parent compound of the nitrogen containing bases cytosine, thymine and uracil.

radioactive isotope: isotope with an unstable nucleus that emits electrons (undergo beta decay) and are used as labels or tracers in biology.

residual bodies: secondary lysosomes containing undigested residues, membrane fragments and whorls.

ribonucleic acid (RNA): nucleic acids that function in transcription and translation. The genetic material of certain viruses.

rough ER(RER): portion of the endoplasmic reticulum bearing ribosomes.

smooth ER(SER): portion of the endoplasmic reticulum devoid of ribosomes.

steroids: compounds that are derivatives of a tetracyclic structure composed of a cyclopentane ring fused to a substituted phenanthrene nucleus.

stroma: unstructured matrix of the chloroplast in which the grana and stroma thylakoids are suspended.

template: a molecule which contains information from which other molecules can be synthesised.

thylakoid: a membranous sac present in chloroplasts that may be disc shaped (in the grana) or elongated, it is the site of reactions of the photosynthesis requiring light and CO_2 .

ultracentrifuge: centrifuge capable of producing rotor speeds up to 100,000 rpm and able to rapidly sediment tiny particles and macromolecules.

van der Waals force: a weak, attractive force between atoms, particularly important in hydrophobic bonding of amino acids in proteins.

wobble: capability of the third base in the tRNA anticodon (5' end) to form a hydrogen bond with any two or three bases at 3' end of the mRNA codon.

FURTHER READING

- 1) Avers, Charlotte J. Molecular Cell Biology, Addison-Wesley Publishing Company, Inc. USA, 1985.
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- 5) E.D.P. DeRobertis, E.M.F. DeRobertis, Essentials of Cell and Molecular Biology.
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- 7) Barry King, Cell Biology, Allen and Unwin Publishers Ltd, Inc. U.K., 1986.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-Q1

Block-1

Enrolment No.

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- 1) How many hours did you need for studying the units?

Unit Number					
No. of hours					

- 2) How many hours (approximately) did you take to do the assignments pertaining to this block?

Assignment Number		
No. of hours		

- 3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (✓) the type of difficulty and give the relevant page number in the appropriate columns.

Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

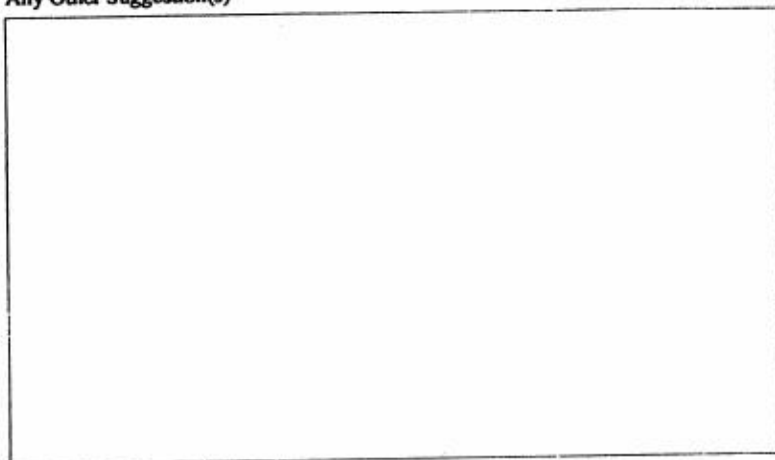
- 4) It is possible that you could not attempt some SAQs and TQs. In the following table are listed the possible difficulties. Kindly tick (✓) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

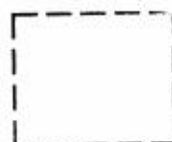
Unit No.	SAQ No.	TQ No.	Type of difficulty			
			Not clearly posed	Cannot answer on basis of information given	Answer given (at end of Unit) not clear	Answer given is not sufficient

- 5) Were all the difficult terms included in the glossary. If not, please list in the space given below.

--

6) Any Other Suggestion(s)





To,
The Course Coordinator (LSE-01; Cell Biology)
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Delhi - 110 068



Block

2

CELL MEMBRANES AND ENZYMES

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Allahabad 211002

BLOCK 2 CELL MEMBRANES AND ENZYMES

In Block 1 we had discussed the evolution of cell starting from small precursor molecules to the present day cell from its components. The study of modern cell biology has become possible with the use of various tools and techniques described in Block 1. We have tried to develop the understanding of cell by dealing with its structural and functional aspects. We will continue with the various cellular structures and their functions in this block.

We begin this block with the self-assembly of supramolecular structures. Similar or dissimilar subunits link non-covalently to form the supramolecular structures. Structures like collagen, actin and cellulose are formed by the assembly of the identical subunits, whereas structures like viruses and ribosomes are formed by the assembly of dissimilar subunits.

Cell membranes are the biological membranes formed by the assembly of lipid, protein and carbohydrate molecules. Plasma membrane encloses all living cells and is selectively permeable, meaning that some molecules move through the membrane more readily than others. Cell membranes are dealt with in Unit 6. The movement of molecules may be energy-independent, simple diffusion and facilitated diffusion along the concentration gradient or energy-dependent active transport of the solutes against the concentration gradient. Facilitated diffusion and active transport of the solutes across the membrane are mediated by specific transport protein molecules located in the cell membranes. The flow of electrons along the electron transport chain creates a proton gradient across the membrane. The ATP synthesis and active transport of specific metabolites are driven by the reverse flow of protons along the concentration gradient. We have discussed transport processes and transport molecules in Units 7 and 8.

Metabolic reactions in any organism are catalysed by the biological catalysts, i.e. enzymes. Enzymes are specific proteins which enhance the rate of specific reactions. To maintain the steady state in the organism, metabolic activities are regulated. Metabolic regulation is brought about by regulating the enzyme activity. The characteristic of enzymes and regulation of enzyme activity are discussed in Units 9 and 10. Various techniques to study biochemical processes have enriched our understanding and made it possible for us to understand the metabolic processes at all levels of organisation of life forms. An account of various techniques is given in Unit 10.

Objectives

After reading this block you should be able to :

- explain the process of self-assembly of supramolecular structures by taking the examples of collagen, actin, cellulose, virus and ribosome,
- describe the structural and molecular organisation of biological membranes and processes of transport across these membranes,
- discuss the role of transport protein molecules in mediating the facilitated diffusion and active transport across the membranes,
- explain the formation of proton gradient across the membrane and its coupling to the ATP synthesis and active transport of specific metabolites,
- discuss the characteristics of enzymes and regulation of enzyme activity, and
- describe various biochemical techniques to study the metabolic processes at various levels of organisation.

UNIT 6 SUPRAMOLECULAR STRUCTURES AND CELL MEMBRANES

Structure

- 6.1 Introduction
 - Objectives
- 6.2 Self-Assembly
- 6.3 Self-Assembling Aggregates
 - Collagen
 - Actin
 - Cellulose
- 6.4 Nucleoprotein Aggregates
 - Tobacco Mosaic Virus
 - Ribosome
- 6.5 Cell Membrane
 - Molecular Organisation of Cell Membranes
 - Structural Organisation of Cell Membranes
 - Membrane Fluidity
 - Membrane Asymmetry
- 6.6 Summary
- 6.7 Terminal Questions
- 6.8 Answers

6.1 INTRODUCTION

In the previous Block you have studied how molecules such as amino acids, sugars and nucleotides make large polymers known as macromolecules. You have also learnt that the living cell is not only a mixture of macromolecules enclosed within the plasma membrane, but in the cell, molecules and macromolecules form the supramolecular aggregates.

In this unit we will discuss the formation of supramolecular structure by the process of self-assembly. These aggregates are formed as a result of interaction(s) of the component molecules which are held together by non-covalent bonds. Assembly and disassembly of aggregates can be controlled by the low energy levels of these bonds. We will explain the self-assembly processes by giving examples of collagen, actin, cellulose, virus and ribosomes.

Later in the unit, we will discuss the supramolecular structure of the cell membrane. Cell membranes are sheet-like enclosed structures with two layers made up of lipids, proteins and carbohydrates. These structures are non-covalent assemblies that are metabolically stable and active. Various models proposed to explain the structural organisation of membranes will also be discussed briefly. The Red Blood Cell (RBC) membrane is taken as a model membrane to explain the structure of a typical biological membrane.

Before you read this unit, you should refresh your memory regarding the structures of various macromolecules such as proteins, lipids and carbohydrates.

Objectives

After reading this unit you should be able to:

- explain the process of self-assembly of macromolecules into supramolecular structures,
- list some characteristics of lipid mixtures that are important for the physical state of a bilayer,
- describe the types of membrane proteins within the lipid bilayer in cell membranes,
- explain the location and role of carbohydrates in the lipid bilayer,
- discuss the nature of the fluid mosaic model of the biological membrane.

6.2 SELF-ASSEMBLY

Supramolecular structures: The molecular and macromolecular structures interact among themselves to organize into supramolecular units. These units, in turn are parts of structures recognizable in the cell by electron microscope.

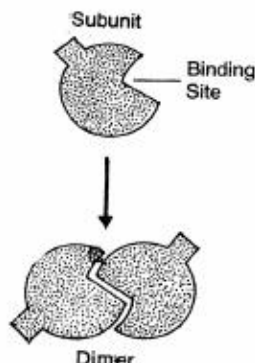


Fig 6.1: The binding site of a single type of subunit recognises itself and forms a symmetrical dimer.

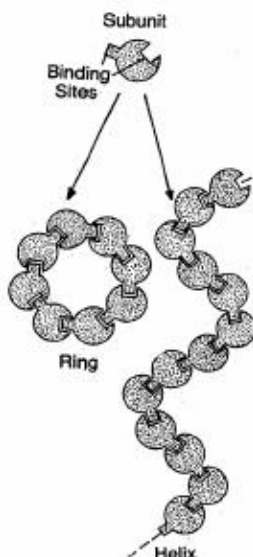


Fig 6.2: The single type of subunits having two binding sites interact with each other forming ring and helices.

Similar or dissimilar molecular constituents (monomers) of the cell having mutual binding affinity can interact among themselves and form **supramolecular structures** which are also called aggregates. The aggregates, such as filaments, ribosomes, viruses, etc., are assembled with the help of large number of non-covalent bonds joining the macromolecular monomers. You have already studied about the non-covalent bonds such as hydrogen bonds, hydrophobic bonds, ionic bonds and Van der Waals attractions in Unit 5. This process of formation of aggregates is called **self-assembly**.

Since the subunits associate through the non-covalent bonds of relatively low energy, both assembly and disassembly of aggregates can be readily controlled. Disassembly of the aggregates occurs due to the changes in physicochemical forces such as changes in pH, ionic compositions of the medium in which they assemble and thermal motions that tend to pull molecules apart. When the interacting molecular subunits are again exposed to suitable conditions, they reaggregate and regain the original form.

Three dimensional form of the interacting molecular subunits make the interactions specific so that one kind of assembly process may rule out the other possible combinations. The concept of self-assembly can be explained with the help of examples in which interacting components have mutually complementary binding sites.

Let us take an example of a single type of subunit whose binding site is complementary to a region on its own surface, i.e. the subunit has one binding site. Association of such types of subunits leads to the formation of regular aggregate structures. As you can see in the Fig. 6.1, the binding sites of the subunits recognise themselves and form a dimer. A dimer is formed by the association of two identical subunits only. Many enzymes and some proteins form such types of dimers, that act as subunit to form larger aggregate structures.

However, there are other examples where the surface of the subunit has more than one binding sites. In such cases a chain of subunits is formed which may acquire different shapes due to different orientations of binding sites. These shapes may be of closed ring or the helix forms (see Fig 6.2).

Thus the supramolecular structures formed from subunits can take the shape of fibres, sheets or globular masses depending upon the shapes of individual subunits and the location of their complementary binding surfaces. For example, the hexagonal association of the subunits can form a sheet or a tube (Fig 6.3). Formation of such structures provides additional stability because it increases the total number of bonds that can form between the subunits.

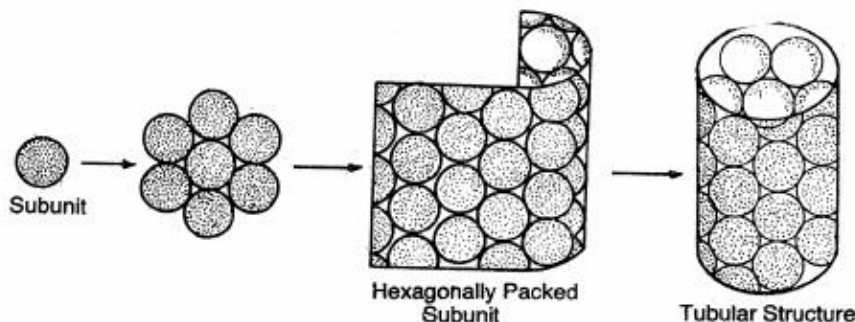


Fig. 6.3 : The sheet-like and tubular structures formed by the hexagonal packing of the subunits.

SAQ 1

Fill in the blanks with appropriate words

- Mode of the cellular structure are formed by.....of molecules
- The supraneuclear structures assemble bylinkages
- Many enzymes are assembled in the form of
- The supramolecular structures can disaggregate due to the changes in.....

6.3 SELF-ASSEMBLING AGGREGATES

As you have read above, many large cellular structures are formed by the aggregation of identical or dissimilar molecules. The supramolecular aggregates like collagen fibres and actin filaments are formed as a result of self-assembly of identical protein molecules, whereas structures like viruses and ribosomes are formed by the assembly of two dissimilar molecules, i.e. proteins and nucleic acids. You will study about these in the following subsections.

6.3.1 Collagen

We will first take collagen as an example to describe the principles involved in the formation of larger aggregates.

Collagen is a fibrous protein found in all animals except protozoa. In all vertebrates, collagen is a major component of the extracellular matrix. The molecule of collagen also called as **tropocollagen**, is about 300 nm long and 1.5 nm thick and consists of three polypeptide chains known as α -chains. These α -chains are intertwined around each other to form a left-handed, triple helical structure with three residues per turn. This helical structure gives the collagen molecule a rope like appearance (Fig. 6.4). The polypeptides are made of about 1,000 amino acid residues, of which every third molecule is glycine, about one third is proline and hydroxy-proline; and the remaining ones are other amino acids, lysine being one of them. This amino acid composition is unique to collagen. The triple helical structure of collagen is maintained by the hydrogen bonds between adjoining chains.

Extracellular matrix is an organized external structure secreted out by the cells composed of proteins and carbohydrate. These secretions form the cell wall in plant cells and play a role in cellular contacts and recognition and mechanical support in animal cell.

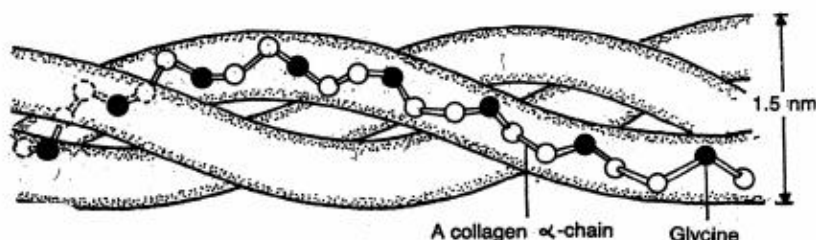


Fig 6.4: Collagen molecule. The three α -chains are wrapped around each other and form a triple stranded helical rod. Every third residue in the α -chain is glycine molecule.

So far, only seven genetically different collagen α -chains have been identified. Although more than 100 types of collagen molecules can be assembled from these seven α -chains, only a few types have been described, which are given in Table 6.1. Of the five major types of collagen molecules, type I, II and III are found in the connective tissue of the vertebrates. They get assembled into the polymers called collagen fibrils in the extracellular space. These fibrils further assemble into large bundle as collagen fibres and can be seen in a light microscope. Collagen molecules of types IV and V do not form fibrils and fibres.

Table 6.1: Types of collagen and their properties

Types	Distinctive Features	Tissue distribution
I	Low hydroxylysine low carbohydrate broad fibrils	Skin, tendon, bone, ligaments, cornea, internal organs (accounts for 90% of body collagen)
II	High hydroxylysine high carbohydrate usually thinner fibrils than type I	Cartilage, intervertebral disc, notochord, vitreous body of eye
III	High hydroxyproline low hydroxylysine low carbohydrate	Skin, blood vessels, internal organs
IV	Very high hydroxylysine high carbohydrate probably retains procollagen extension peptides	Basal laminae
V	High hydroxylysine high carbohydrate	Widespread (in small amounts)

The precursor forms of collagen α -chains are synthesised on ribosomes and move into ER lumen. These are called **pro α -chains**. Each pro α -chain has extra amino acids called extension peptides which are present at both of its amino and carboxyl terminal ends (Fig 6.5 a).

During the assembly of collagen molecules in the ER lumen, some of the proline and lysine residues are hydroxylated before the α -chains associate to form triple stranded molecules called as **procollagen**. The hydroxyl group of hydroxyproline residues form interchain hydrogen bonds which help to stabilize the triple helix. The hydroxyl group of hydroxylysine residue is important for glycosylation and cross linking of collagen molecules in the extracellular matrix. The hydroxyl groups of hydroxylysine are glycosylated by the covalent attachment of paired sugar residues (see Fig 6.5 b). The extension peptides guide the triple helix formation and are important in packaging of procollagen molecules with other matrix macromolecules in the cell prior to secretion.

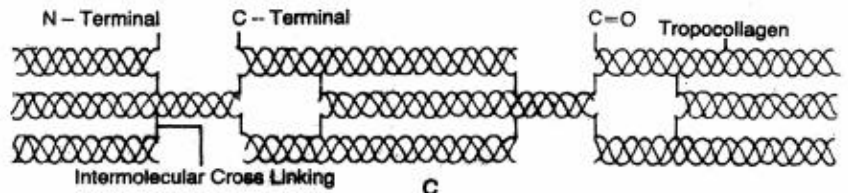
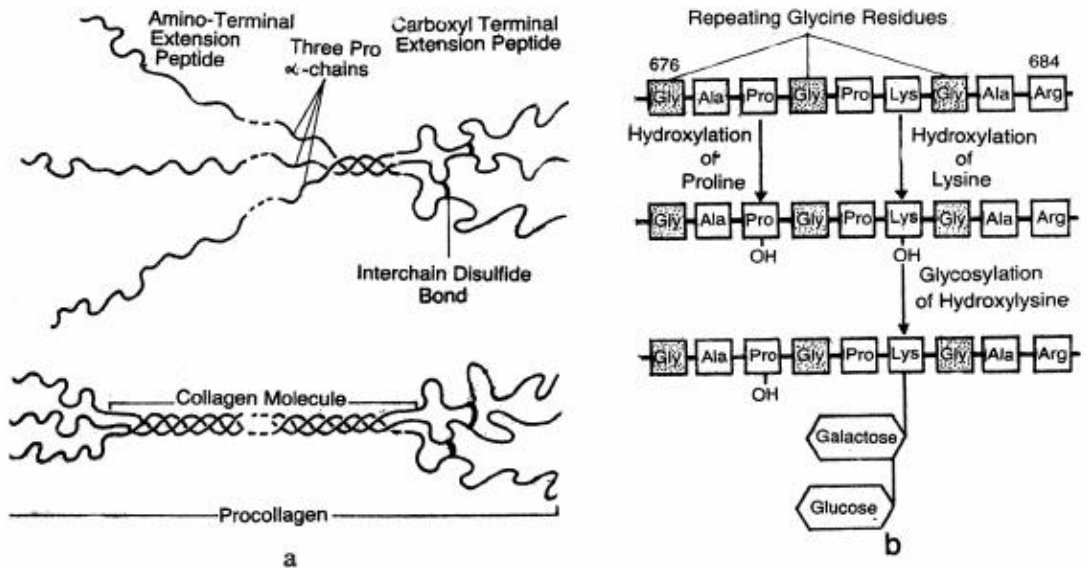


Fig 6.5: (a) Collagen α -chains are synthesised as pro α -chains with extension peptides that guide the triple helix formation. The carboxyl terminal extension peptides are linked covalently by disulphide bonds. (b) A 12 amino acid segment of a pro α -chain showing the three types of covalent modifications. Selected proline and lysine residues are hydroxylated and some of the resulting hydroxylysine residues are then glycosylated. (c) The collagen molecules also called tropocollagen, after their assembly in the cell are secreted out in the where they assemble into collagen fibrils. Collagen fibrils are bound by covalent cross linkages and form collagen fibres.

The procollagen molecules move from ER lumen to Golgi region and are secreted by exocytosis in the extracellular matrix. The extension peptides of type I, II and III procollagen molecules are cleaved by enzymes called as procollagen peptidase in the matrix, thus converting them into collagen molecules. These molecules assemble to form collagen fibrils (Fig 6.5 c). The fibrils are formed close to the cell surface and it seems likely that the cell regulates the **site and rate of fibril assembly**. The side chains of the aminoacids other than proline, hydroxyproline and glycine are responsible for fibril formation. The collagen fibrils are strengthened by the formation of cross linking covalent bonds within and between the constituent collagen molecules and assemble into collagen fibres.

Collagen fibres are intercellular component of connective tissues such as tendons and cartilage. They strengthen and help to organize the matrix and give enormous tensile strength to the tendons.

All the stages in the formation of collagen are essential to the normal development of connective tissues. Inadequacy in hydroxylation, cross linking and cleavage processes of procollagen may result in abnormal formation of connective tissues in the extracellular matrix leading to the fragility of blood vessels, skin and hypermobility of joints etc.

SAQ 2

a) Draw a diagram of collagen molecule in the space given below

b) What is the role of hydroxyproline and hydroxylysine in the formation of collagen triple helix?

6.3.2 Actin

In this subsection we will discuss another type of protein called actin to illustrate those aspects of self-assembly where the protein not only polymerises to form the aggregate but also interacts and binds with other kinds of proteins resulting in complex structures.

Actin, the solid cytoplasmic microfilament (thin filament) is found in all eucaryotic animal cells in high concentration. In the cell it exists in globular form as well as in fibrous form called G-actin and F-actin respectively. The globular actin subunit polymerises to form microfilaments of fibrous actin. These two remain almost in equilibrium in the cytoplasm, i.e. actin filaments are continuously formed and broken down in the cells. G-actin is soluble in weakly ionic solutions. The polymerisation of G-actin into insoluble F-actin is enhanced if the salt concentration is increased to a level closer to the physiological salt concentration which is 0.9%. Each filament of fibrous F-actin consists of two strands of G-actin monomers, i.e. microfilaments twisted around each other to form a double helix (Fig 6.6 a)

The two strands add to the stability and strength of the assembly, since each subunit can interact with the subunits in the opposite strand as well as with its neighbours in the same strand, i.e. four altogether. The fibrous nature of the molecules allow greater surface area for the contact with other protein molecules (Fig 6.6 b).

Actin serves various cellular functions. The cross linked bundles of actin filaments provide mechanical support for various cellular structures and extensions. In association with myosin it forms actomyosin, the contractile system responsible for various cellular movements.

Let us take an example of microvilli to understand the assembly of actin filaments in the bundle form. Microvilli are the cellular extensions that cover the exposed surfaces of many types of epithelial cells of various organs such as kidney, intestine, eye etc. The core of a microvillus contains about 40 actin filaments that run in parallel bundle along the length of

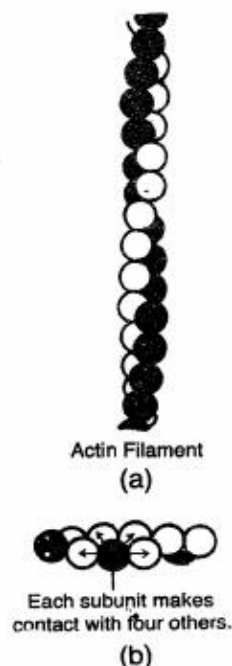


Fig 6.6 a): Actin filament is a helical structure containing double strands of globular actin subunits.

b) The globular subunit makes contact with four other subunits.

Cell Membranes and Enzymes

Myosin exists in both globular enzyme form as well as fibrous structural protein. The fibrous myosin is associated with actin in all muscle cells and probably in non-muscle cells. Myosin from smooth muscle and from striated muscle have slightly different properties.

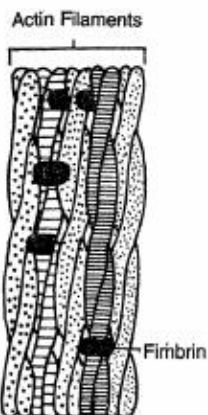


Fig 6.7: Schematic diagram of cross-linked actin filaments in the core of microvilli. The actin filaments are parallel and align longitudinally. The adjacent actin filaments are joined by cross-linking proteins.

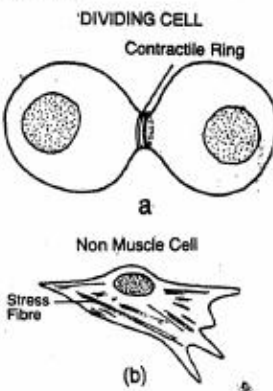


Fig 6.8 : a) Contractile ring in the dividing cell.
b) Stress fibres are the contractile bundle in the cytoplasm in the non-muscle cells.

pectins are acidic, branched polymers of various monosaccharide units and contain many negatively charged galacturonic acid residues. Hemicellulose resembles pectin in its branched polysaccharide composition but it has no net electric charge.

the microvillus. The adjacent actin filaments are bound together by cross linking protein molecules, thus making the core of the microvillus rigid. Fimbrin is one of the actin binding proteins found in cellular extensions. (Fig 6.7).

Actin polymers in association with protein myosin organise themselves into contractile filamentous polymers, controlled by a number of actin binding proteins. In non-muscle cells actin and myosin associate in bundle form. During cell division this bundle appears as a **contractile ring** just beneath the plasma membrane and leads to the separation of the daughter cells. This contractile ring is a temporary structure (Fig 6.8 a). **Stress fibres** are another type of contractile bundles of actin filament and associated proteins in the cytoplasm of non-muscle cells. These fibres can be separated from other components and contract if exposed to ATP (Fig 6.8 b).

The contractile system of skeletal muscle cells has two major types of filaments; the thick filaments that are rod like structures called myosin and the thin filaments called as fibrous actin. These filaments are arranged in parallel repeating sets. The contraction produced by the sliding of an actin filament against myosin is controlled by energy cycle, i.e. ATP hydrolysis and regulated by Ca^{2+} ions present in the cell (Fig 6.9). Hydrolysis of ATP to ADP and phosphate provides the chemical energy for the filaments to slide. Contraction is accompanied by an increase in Ca^{2+} concentration in the cell.

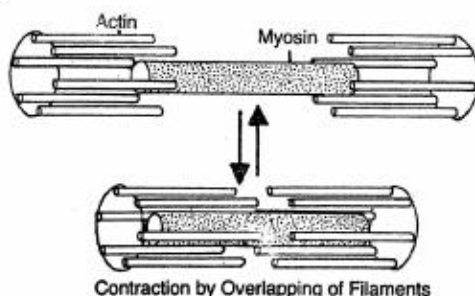


Fig 6.9: Overlapping actin and myosin filaments in the contractile system of skeletal muscle.

In smooth muscles the assembly of actin and myosin filaments is less ordered than in the skeletal muscle and the contraction depends upon the concentration of Ca^{2+} . Increased concentration of Ca^{2+} in the smooth muscles stimulates the ATPase activity, which leads to the phosphorylation of myosin. Myosin, when phosphorylated interacts with actin filaments, thereby, causing the contraction in the smooth muscle. Once dephosphorylated, myosin cannot interact with actin and the muscle relaxes.

SAQ 3

- a) Differentiate between G-actin and F-actin in the given space .

- b) Describe briefly the structure of actin filament in the space given below.

6.3.3 Cellulose

In this subsection we will discuss about the assembly of cellulose, a polysaccharide which is most abundant organic compound present on earth. Polysaccharide chains, do not fold into a globular structure like many polypeptide chains do, but some may spontaneously assemble into helical or ribbon like structures. In higher plants, for example, cellulose (polyglucose) chains assemble into ribbon like microfibrillar component of the cell wall. The cell wall is composed of cellulose fibres that are embedded in a matrix of proteins and polysaccharides and are in abundance in the higher plants. The cell wall matrix is a gel-like substance which is composed of polysaccharides, hemicellulose, pectin and small amount of proteins.

The cellulose molecule is a linear chain and is composed of several glucose molecules covalently linked by β 1-4 glycosidic bonds. You have read about glycosidic bonds in Unit 5. These bonds give the cellulose molecules a ribbon like appearance that is stabilized by intramolecular hydrogen bonding (Fig 6.10).

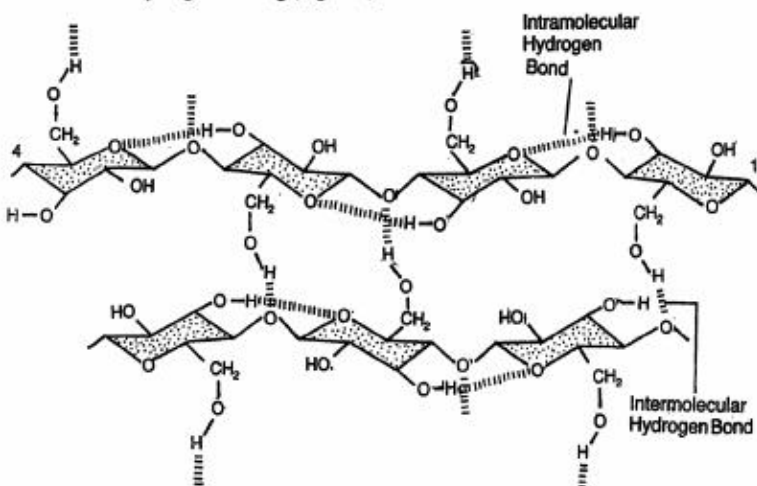


Fig 6.10: Two β 1-4 linked glucose chains of cellulose. The intermolecular hydrogen bonds crosslink adjacent chains in the microfibril.

About 60-70 cellulose chains having the same polarity are held together tightly by hydrogen bonds in parallel row, thereby, forming rodlike structures called as micelles. These micelles are packed into long aggregates called microfibrils. They in turn are clustered together as macrofibrils (see Fig 6.11). Other polysaccharides such as hemicellulose and pectin within the matrix crosslink with the cellulose fibrils. The hemicellulose molecules are linked by hydrogen bonds to the surface of cellulose fibrils and are crosslinked by pectin molecules holding the cell wall components together.

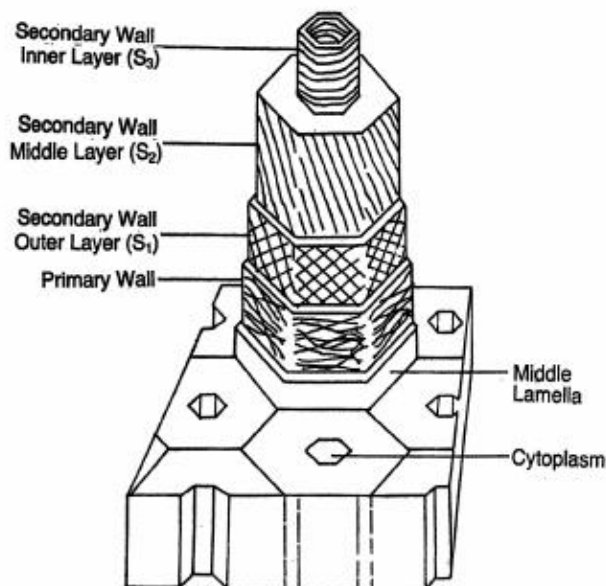


Fig 6.12 : Schematic diagram showing the primary and secondary layers of cell wall.

The cell wall is built up of a series of layers of cellulose fibrils. As plant cells grow they deposit new layers of cellulose adjacent to the plasma membrane. As shown in Fig 6.12, the oldest layers of cellulose are in the primary wall which is the outermost wall. The secondary cell wall consists of three successive layers: outer layer (S_1), middle layer (S_2) and inner layer (S_3), laid down adjacent to the plasma membranes. The cellulose fibrils are oriented in various ways in cell walls, usually more regular in secondary cell wall. In the primary wall the microfibrils are oriented transversely to the long axis but become longitudinally arranged

β 1-4 linkages cause the molecule to form straight chains. The α 1-4 linkage in polyglucose molecules such as glycogen and starch cause a turning of chain, such polyglucose molecules adopt a coiled helical conformation.

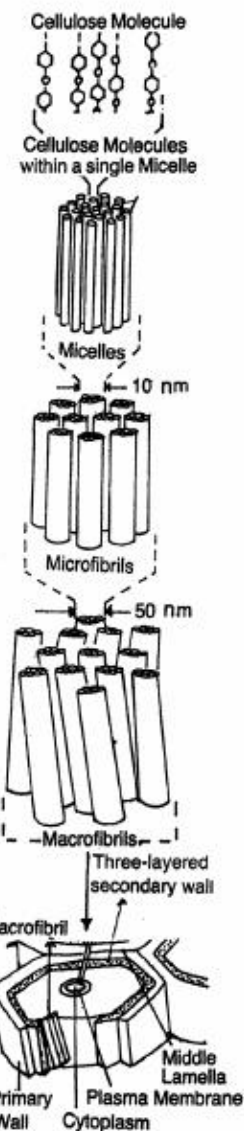


Fig 6.11 : The organisation of cellulose molecules in the plant cell wall. The linear cellulose molecules assemble into micelles, which are packed into microfibrils. Microfibrils associate to form macrofibrils, which are embedded in the cell wall matrix. Cellulose fibrils are stabilized by hydrogen bonding.

during growth of the cell. The synthesis of the cellulose molecules occur in the Golgi Complex and assembly of the fibrils occurs on the cell surface. As the main structural component of the plant, cellulose is responsible for the rigidity of the plant body.

SAQ 4

Tick mark [] the correct statements and [x] the wrong statements in the following in the given space

- i) Cellulose fibres are formed by the assembly of proteins
- ii) Micelles are the fibrils of hemicellulose
- iii) Polysaccharide in the cell wall matrix are linked by hydrogen bonds
- iv) Primary cell wall is laid down adjacent to plasma membrane
- v) In the secondary cell wall the cellulose fibrils are in three successive layers

6.4 NUCLEOPROTEIN AGGREGATES

So far we have discussed the supramolecular aggregates composed by the self-assembly of similar molecules. Now we will discuss the biological structures assembled from dissimilar molecules.

Supramolecular structures such as viruses and ribosomes are formed by the assembly of protein subunits and nucleic acids. Under appropriate conditions, the isolated subunits of these complex aggregates can spontaneously assemble into the final structure.

6.4.1 Tobacco Mosaic Virus

Viruses are noncellular organisms consisting of DNA or RNA as the genetic material and a protein coat called **capsid**. They are metabolically active only when they are in a living cell. Tobacco mosaic virus (TMV) for example, was the first macromolecular aggregate on which classic experiments and the studies of self-assembly from its component parts, have been done. TMV is cylindrical in shape and consists of a single stranded molecule of RNA forming a helical core. This RNA consists of 6,500 nucleotides. Associated with this helix are 2,130 identical protein subunits that form the protein coat.

The protein subunits bind non-covalently to each other in such a way as to form bilayer discs consisting of 34 protein subunits. Each disc is circular and interacts with RNA to form the helical structure. To start the process a disc binds to an initiator region of RNA about 1000 base from 3' end. As the RNA molecule is eleven times longer than the virus, the RNA strand assumes helical shape by the stacking of the proteins and becomes completely enclosed by the protein coat in the mature virus (Fig 6.13). The mature virus consists of 130 turns of protein subunits with 16.33 subunits per turn.

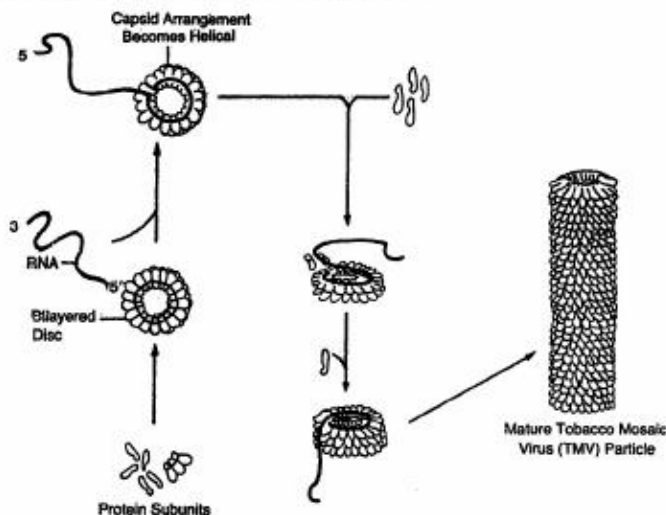


Fig 6.13 : Assembly of tobacco mosaic virus. The RNA molecule assumes a helical shape due to its association with bilayered discs of protein subunits.

The dissociated virus subunits recombine to form fully active virus particles under suitable condition. The assembly of protein subunits appears to be influenced by the RNA molecule.

6.4.2 Ribosome

Ribosome is a small spherical-shaped organelle found in both procaryotic and eucaryotic cells. Ribosomes are known to control the interaction of various forms of RNAs and synthesis of proteins. It is composed of two structurally distinct subunits: a larger subunit and a smaller subunit. Assembly of ribosomal subunits requires sequential addition of proteins to rRNA molecules. The smaller subunit consists of a single rRNA molecule and 21 to 30 protein molecules depending on the organism. These proteins are numbered as S1-S30 (S stands for small). The larger subunit contains a large rRNA molecules and one (in procaryotes) or two (in eucaryotes) small rRNA molecules and 34 to 50 protein molecules which are numbered as L1-L50 (L stands for large).

The ribosome that is studied in great details is 70S ribosome of E. Coli. The smaller (30S) subunit of E.Coli ribosome contains 21 different proteins and a single 16S rRNA molecule. The larger, 50S subunit has 34 different proteins associated with one molecule of 23S rRNA and one molecule of 5S rRNA.

We will discuss here, the assembly of E.Coli 30S subunit which has been studied in more detail. Little is known about the assembly of more complex 50S subunit of bacteria or other eucaryotic ribosomes. However, the principles involved in the assembly of ribosomal units are the same.

A functional E.Coli 30S subunit can reassemble out of a purified mixture comprising 16S rRNA and 21 proteins which is identical in structure and function to the native 30S. Thus all the information needed for assembly of this ribosomal subunit is contained in the structure and properties of the component macromolecules themselves. The assembly takes place by an ordered addition of proteins to 16S rRNA molecule in vitro preparations. Initially only 8 out of 21 ribosomal proteins bind non-covalently directly to isolated 16S rRNA molecule. This binding of such 8 **core proteins** create binding sites for all other ribosomal proteins to complete the subunit. The exact nature of other protein binding sites is not known. However, they may depend on the conformational changes either in rRNA molecule or in protein subunits (Fig 6.14). The complete ribosome complex with its two subunits is assembled outside the nucleus in the cytosol. Omission of a single protein results in a functionally defective subunit. Similarly 5S rRNA and 23S rRNA become complexed in an orderly sequence with the 34 different proteins of 50S subunit.

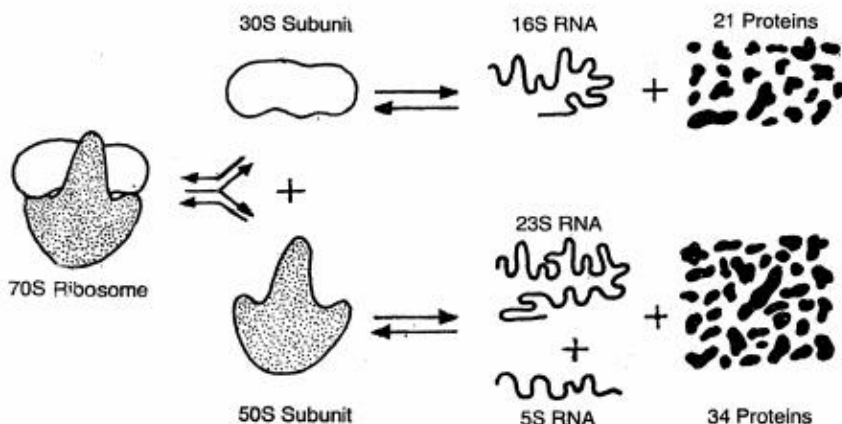


Fig 6.14: Self-assembly of two subunits of E.Coli 70S ribosome. The 50S subunit has 23S and 5S rRNAs and 30S subunit has 16S rRNA.

Try the following SAQ to test whether you have understood the self-assembly of supramolecules.

Column 1	Column 2
a) Viruses are the noncellular organism	i) has one rRNA molecule
b) Assembly of TMV involves	ii) that are active in the living cell only
c) The smaller subunit of ribosome	iii) ordered addition of proteins to RNA-molecules
d) Ribosomal assembly involves	iv) formation of bilayered disc of proteins subunits

You have studied about the self-assembly of the supramolecules. We will now discuss the basic structure of a typical membrane of the cell, which is formed as a result of self-assembly of macromolecules, i.e. lipids, proteins and carbohydrates.

6.5 CELL MEMBRANE

The biological membranes are highly organised dynamic structures and are the site of innumerable physiological and biochemical processes/reactions. They consist mainly of lipid, protein and carbohydrate. The basic framework of all the membranes is a continuous double layer of lipid molecules in which proteins are dispersed. The plasma membrane also called cell membrane, is a highly differentiated structure that encloses every cell. In Unit 3 you have read how an elaborate membrane system consisting of nuclear membrane, endoplasmic reticulum, mitochondria, chloroplasts and the Golgi apparatus has structural a functional continuity with the plasma membrane.

The plasma membrane separates the internal contents of a cell from its surroundings. It is selectively permeable and acts as a barrier by maintaining the difference in concentration of various ions and molecules between the inside and the outside of the cell. The plasma membrane also exchanges material with the extracellular environment by exocytosis and endocytosis. Adjacent cells exchange material through gap junctions in the cell membrane. Membranes surrounding specific organelles of the eucaryote cell perform specialised functions, such as oxidative phosphorylation in the mitochondrion, breakdown of macromolecules in the lysosome, photosynthesis in chloroplast and so forth.

We will discuss below the molecular and structural organization of the cell membrane along with the different models proposed to explain its structure.

The structure of plasma membranes has been studied by their isolation and purification from the cell. Although plasma membranes have been isolated from a variety of cells like liver cells, muscle cells etc., extensive studies on membranes have been carried out by using mammalian RBCs as a model. Plasma membranes are easily obtained by haemolysis of erythrocytes.

6.5.1 Molecular Organisation of Cell Membranes

All the biological membranes, i.e. plasma membrane and the internal membrane systems of eucaryotic cells have a common basic structure. The membrane assembly is composed of lipids, proteins and carbohydrates. Lipids and proteins are held together by non-covalent bonds, while carbohydrates occurring in the form of oligosaccharides, are covalently bound to some of the proteins and lipids. Various protein molecules are distributed on the surfaces of the membrane as well as embedded in the lipid bilayer forming a mosaic arrangement (see Fig 6.15)

Although all the cell membranes have basically the same structure and function, there is considerable variation in the lipid, protein and carbohydrate ratio among different cell membranes (Table 6.2) and also between the two layers of the membrane.

The erythrocytes are treated with hypotonic solution, i.e. a solution having lower osmotic pressure than blood plasma. The erythrocytes swell and burst and lose their haemoglobin content. This process is called haemolysis and the resulting membrane is called as red cell ghost.

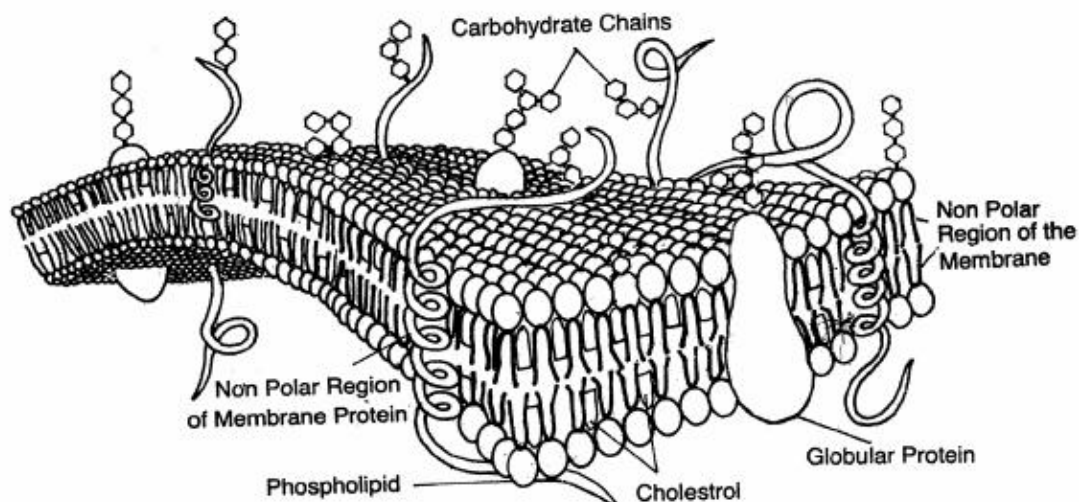


Fig 6.15 : Three dimensional diagram of a section of cell membrane. The proteins are dispersed in a mosaic arrangement in the lipid bilayer.

Table 6.2 : Protein, lipid and carbohydrate ratio in some membranes

Membrane	Protein	Lipid	Carbohydrate
Myelin	18	79	3
Plasma membrane			
Human erythrocyte	49	43	8
Mouse liver	44	52	4
Amoeba	54	42	4
<i>Halobacterium</i> purple membrane	75	25	0
Mitochondrial inner membrane	76	24	0
Chloroplast			
Spinach lamellae	70	30	0

Cell membranes are **asymmetric, dynamic and fluid** in nature. Membrane fluidity is crucial to many of its functions. Most of the lipid and protein molecules are able to move about freely within the membrane.

Let us discuss the macromolecular units of a plasma membrane in details.

Membrane lipids

The major lipid components in the cell membranes are phospholipids, cholesterol and glycolipids. You have already studied their structure in Unit 5. Cholesterol is specially abundant in plasma membrane of mammalian cells and is absent from most of the prokaryotic cells. Plant cell membranes have little or no cholesterol but large quantities of related sterols.

All the membranes regardless of the source have phospholipids, such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, sphingomyelin, and phosphatidylinositol about which you have already read in Unit 5.

You may ask, why all cell membranes are constructed as lipid bilayer. As you have already studied, all the phospholipids, are amphipathic in nature, i.e. they have a hydrophobic (water hating) part and a hydrophilic (water loving) part. These amphipathic molecules in an aqueous solution spontaneously associate in a way that their hydrophilic heads are in water while the hydrophobic tails remain away from water in contact only with each other or with any other hydrophobic material such as oil.

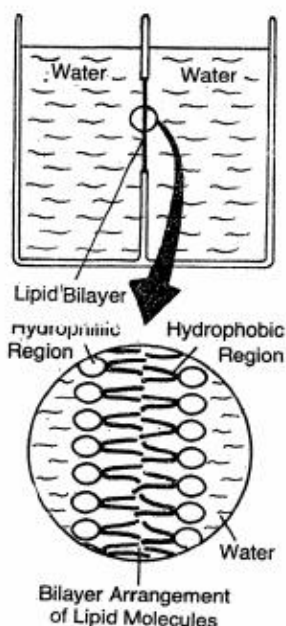


Fig 6.16 : Phospholipid bilayer formed in the aqueous environment.

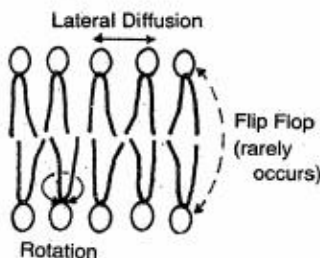


Fig 6.17 : Various types of movements of phospholipid molecules in the lipid bilayer.

Phospholipids form bilayers in an aqueous solution as their forked hydrophobic tails can fit only in bilayers forming a continuous hydrophobic interior (Fig 6.16). Hydrophobic fatty acid having the tendency to associate with each other seal the small holes or rips spontaneously thereby sealing themselves away from the aqueous environment.

Lipid molecules readily exchange places laterally with their neighbours on one side of the bilayer, but occasionally move from one monolayer to the other (Fig 6.17). This flip-flop (migration) process across the membrane occurs almost once in a fortnight, whereas lateral exchange in the same layer occurs much more frequently, about 10^7 times per second.

Membrane proteins

As you have read above, the basic structure of all biological membranes is that of a lipid bilayer. However, their specific functions are carried out largely by proteins. Accordingly, the functions of the membrane such as transport of molecules across the membrane, receiving signals from hormones and chemicals, stabilisation of cell shape etc., depend on the amount and the type of proteins present in it. The proteins are mobile laterally in the plane of the membrane and diffuse rapidly due to the fluid properties of the lipid bilayer. They form channels for the movement of ions and small molecules and serve as carrier for large molecules. Proteins also provide anchorage to the cytoskeletal components and extra cellular matrix.

Membrane proteins are also amphipathic. Their hydrophobic regions are held deep in the interior of the bilayer where they interact with the hydrophobic tails of lipid molecules. The hydrophilic regions of the protein molecules may be exposed to water on one or both the sides of the membrane.

Based on how easily they may be isolated from the membrane, proteins are classified as **peripheral or extrinsic proteins** and **integral or intrinsic proteins**. Generally, the peripheral proteins are exposed only on one side of the bilayer. They do not interact directly with the hydrophobic core of phospholipid bilayer but weakly bound to the hydrophilic regions of specific integral protein or lipid polar head groups. For example, ankyrin, a peripheral protein, is bound to integral protein band III of erythrocyte membrane. These peripheral proteins can easily be extracted from the membranes by changing the ionic composition and/or pH of the medium.

The integral proteins are usually globular and most integral proteins may extend across the bilayer. Their hydrophobic regions are held in the interior of the lipid bilayer and interact directly with the hydrophobic regions of lipid bilayer. The hydrophilic regions of most integral proteins are exposed to cytoplasm and to external aqueous phase outside the cell, i.e. on both the sides of the membrane. The proteins that extend across the membrane are called **transmembrane proteins** (see Fig. 6.18).

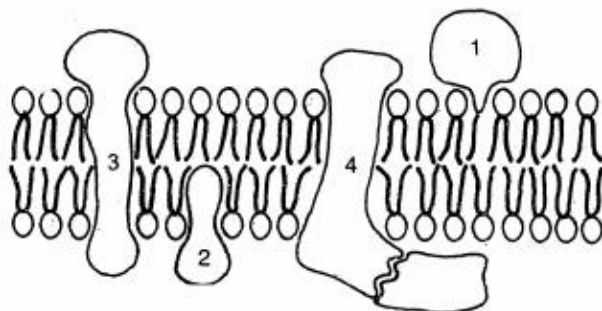


Fig 6.18: Different ways in which proteins are associated with the lipid bilayer.

- 1) peripheral proteins, 2) integral proteins, 3) transmembrane proteins and 4) proteins linked non-covalently to the other membrane proteins (peripheral proteins).

Integral proteins such as immunoglobulin molecules on the plasma membranes and many hormone receptors can be removed from the membrane only after disruption of the lipid bilayer by detergents. These proteins are soluble in detergents which disrupt the hydrophobic associations and destroy the bilayer structure. This shows that the integral proteins are held in close association with lipid bilayer.

The polypeptide chains of membrane proteins are arranged mainly as α -helices or β -sheets. Some of the proteins mainly peripheral, are bound non-covalently to the membranes, while others are covalently linked to the membrane lipids.

Spectrin, a peripheral protein, Band III, a transmembrane protein and glycophorin, an integral protein constitute 60% of the total proteins of human RBC membrane.

Membrane Carbohydrates

Carbohydrates in plasma membrane occur as glycoproteins and glycolipids. Most of the membrane carbohydrates are bound to protein molecules. A single glycoprotein may have many oligosaccharide side chains whereas a glycolipid molecule has only one. Glycoproteins are absent from all procaryotic membranes. Carbohydrate chains of all the cell membranes are located exclusively on noncytoplasmic surface, i.e. outside of the cell (Fig 6.19). In plasma membrane, the sugar residues are located at the outer surface (exterior) and are exposed towards the outer side of the cell. While in the membranes of cellular organelles, they are exposed towards the lumen of membrane-bounded compartments such as in mitochondria and chloroplast. They are absent from inner mitochondrial membrane, chloroplast lamellae and several other intracellular membranes (see Table 6.2).

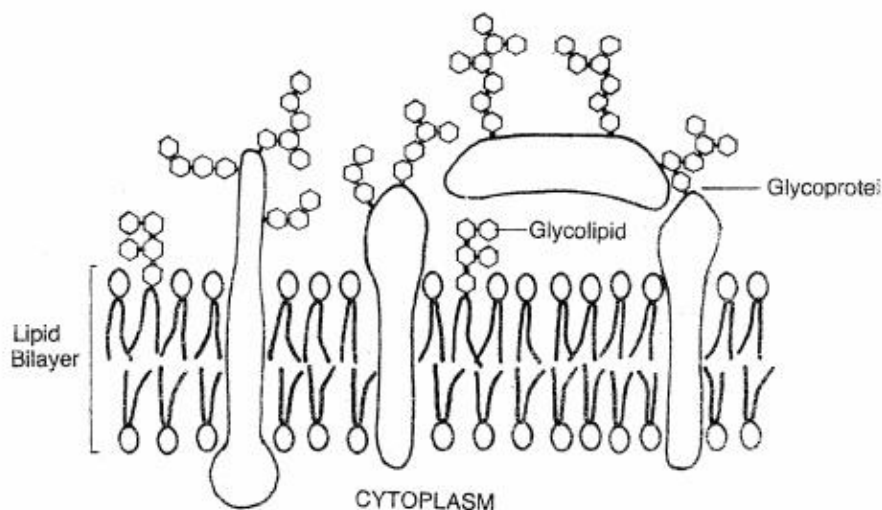


Fig 6.19: Schematic diagram of cell membrane showing oligosaccharide side chains of glycoproteins and glycolipids.

Although the functions of membrane carbohydrates are yet to be established, they seem to be involved in cell communication and recognition processes. The human ABO blood groups are characterised by different glycolipids on the surface of erythrocytes. There is an indication that structure of glycolipids changes when a cell becomes cancerous. They also help to stabilise the glycoproteins in the lipid bilayer.

Try the following SAQ and see if you have understood the molecular organisation of the biological membranes.

SAQ 6

- a) Classify the types of proteins found in cell membrane. Give your answer in the space provided

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b) Match the statements given in column 1 with those of column 2

Column 1	Column 2
a) Molecular organisation of the cell membrane []	i) Plant cell membranes have little or no cholesterol but large quantities of related sterols
b) Membrane proteins []	ii) In the internal membranes they are exposed towards the lumen of membrane found compartments such as in mitochondria.
c) Membrane carbohydrates []	iii) The cell membranes are asymmetric, dynamic and fluid in nature
d) Membrane lipids []	iv) They form channels for the movement of ions and small molecules and serve as the carrier of larger molecules

6.5.2 Structural Organisation of Cell Membranes

In the previous section you have read about the molecular organisation of the cell membranes. In this section you will study in short the different models proposed to explain the structure of plasma membranes.

In 1902, Overton postulated that the plasma membrane is composed of a thin layer of lipid molecules because only substances soluble in lipid could pass through the plasma membrane easily. Later on in 1926, Gorter and Grendell described the plasma membrane as a lipid bilayer. Membrane proteins were accounted for in the later models.

In 1935, James Danielli and Hugh Davson proposed the lipid bilayer model. According to this model, plasma membrane was composed of a lipid bilayer which was coated on both the surfaces by continuous monolayer of hydrated globular protein.

In the early 1960s David Robertson proposed a modified version of Danielli-Davson model called the **Unit membrane model** based on the information obtained by electron microscopy and functions of membranes. He proposed that the plasma layer was of uniform thickness, with two outer protein coats and the middle phospholipid layer (Fig 6.20). This unit membrane model was extended to all intracellular membranes as well.

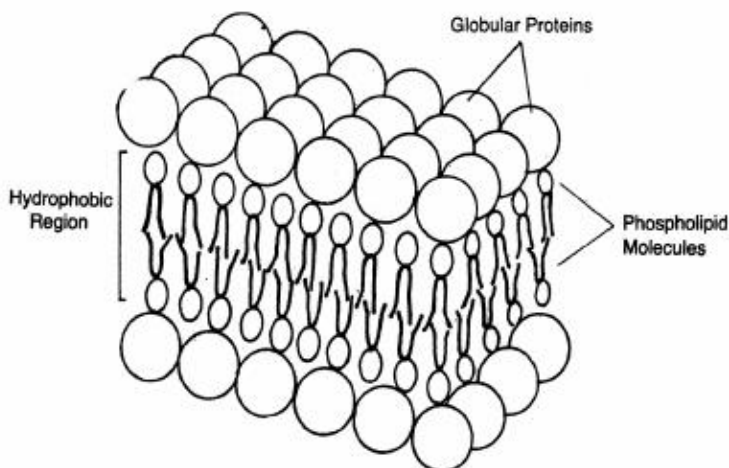


Fig 6.20: Three-layered (protein-lipid-protein) unit membrane model proposed by Davson and Danielli.

The unit membrane model could not account for proteins traversing the membrane and their mobility in the lateral plane of the bilayer.

Jon Singer and Garth Nicolson proposed a modified model of membrane known as fluid mosaic membrane model in late 1960s, according to which, the protein molecules are distributed in and on the lipid bilayer in a mosaic pattern. The Singer-Nicolson model postulates that lipid bilayer exists in a relatively fluid state, as some of the protein molecules are embedded in and float in the bilayer, while, other proteins penetrate the bilayer.

and protrude from one or both of its surfaces. Proteins and lipids in each monolayer can move laterally within the plane of lipid bilayer. Lipids rarely flip-flop from one surface to other surface across the bilayer. The lateral movements of proteins result in their aggregation into functional protein assemblies such as the formation of channel for the transport of ions and molecules.

According to Singer and Nicolson, basic framework of the membrane is same for all the membranes, but the ratio of lipid, protein and carbohydrate may vary greatly among different membranes. Predominant forces responsible for the assembly of membrane structures are ionic, hydrophilic and hydrophobic interactions. Such lipid bilayers form sealed compartments and reseal themselves when they are torn in the processes of endocytosis or exocytosis.

We will discuss membrane fluidity in the next subsection, but before we take that up, try the following SAQ.

SAQ 7

Explain briefly in the space given below, why unit membrane model was not acceptable

6.5.3 Membrane Fluidity

The fluid state of a lipid bilayer depends primarily on the types and amounts of different hydrocarbon chains of phospholipid molecules and on the cholesterol content. The lipid bilayer is in more fluid state when the hydrocarbon chains of phospholipids are not long and loosely packed. Hydrocarbon chains of shorter length interact with each other less often and unsaturated hydrocarbons are more loosely packed than saturated chains because of the bends produced at double-bond sites. A double bond lowers the melting temperature (see Fig 6.21 a).

Stearic acid, a saturated fatty acid, remains a solid till heated to 69°C, whereas oleic acid with one double bond melts at 13.4°C, and addition of one double bond lowers the melting temperature to -5°C

The fluidity of bilayer increases with the increase of temperature. As the temperature increases the fatty acyl side chains undergo a transition from an ordered or gel like state to a more fluid or mobile state. This temperature at which the structure undergoes transition from ordered to disordered state is called the **transition temperature**.

Cholesterol is another determinant of membrane fluidity. As shown in Fig. 6.21b, cholesterol prevents the hydrocarbon chains of membrane lipids from binding together, thus preventing the decrease in fluidity that would otherwise occur at low temperatures.

As the fluidity of the membrane increases, its permeability to water and other small hydrophilic molecules also increases. The lateral movement of integral proteins also increases with fluidity.

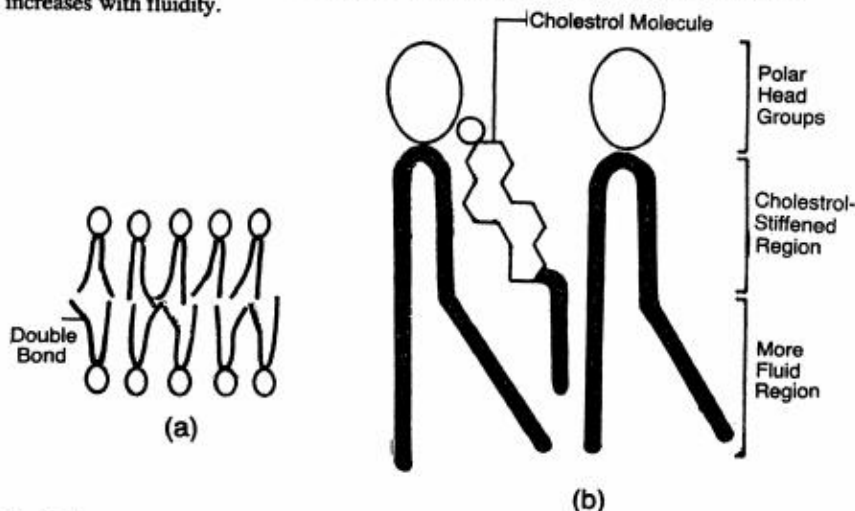


Fig 6.21: a) Unsaturated hydrocarbon chains with cis-double bond cause the fluidity of membranes. b) The interaction of cholesterol with two phospholipid molecules in the monolayer.

6.5.4 Membrane Asymmetry

One important feature of biological membranes is their asymmetry. The two monolayers of lipid bilayer are different in composition. For example in human RBC membrane, the choline containing phospholipids are found mainly in outer lipid layer, whereas phospholipids that contain a terminal primary amino groups are towards the inner lipid layer. The lipid asymmetry may help to keep the membrane proteins properly oriented in the bilayer. As you have read earlier, the membrane proteins are highly asymmetrical as their association with lipid bilayer is either peripheral or integral. The carbohydrate side chains of glycoproteins and glycolipids are found only on the outer half of the bilayer. So the distribution of different molecules such as mentioned above contribute to the asymmetry of the lipid bilayers of the membrane.

SAQ 8

Discuss asymmetry in biological membranes.

Apart from its property of selective permeability, the membrane is also the site of many functions. The protein molecules of a membrane are responsible for many of its functions and account for differing specific properties of different membranes. Various membrane proteins act as enzyme and catalyse specific reactions that occur at the membrane, aid in the transport of specific substances across the membrane, act as receptors of chemical information from surroundings and form a link between cells. You will study about these in detail in the following units.

As you have already studied about the freeze fracturing technique in Unit 2, when the cell membrane is frozen and fractured, it splits along the plane of the hydrophobic tails of lipid molecules separating the two phospholipid layers.

6.6 SUMMARY

In this unit you have studied that the:

- supramolecular structures are formed by the self-assembly of smaller subunits, which have complementary binding sites. The subunits are linked with each other non-covalently to form various supramolecular structures of different sizes and shapes,
- the fibres of the proteins, collagen and actin are formed by the assembly of similar protein molecules. Cellulose chains are formed by the polymerisation of glucose molecules whereas the structures, such as viruses and ribosomes are formed by the assembly of dissimilar molecules, i.e. proteins and nucleic acids,
- cell membranes are formed by the self-assembly of lipids, proteins and carbohydrates. The basic structure of the membrane is lipid bilayer formed due to the amphipathic nature of the lipid molecules in which proteins are dispersed and embedded to form mosaic pattern. They are present either in peripheral regions or traverse throughout the membrane and protrude on both the sides of the bilayer membrane. The carbohydrates are linked as oligosaccharide side chains to lipids and proteins on the outer surface of the bilayer,
- out of the different models proposed to explain the structure of plasma membrane the fluid mosaic model is the one that is most widely accepted. The cell membrane is fluid in nature owing to the presence of double bonds in hydrocarbon tails of lipid molecules and the interaction of cholesterol with phospholipids. The bilayer is highly asymmetrical in nature due to the uneven distributions of membrane lipids, proteins and carbohydrates on the two sides of the layers,
- the cell membrane is selectively permeable to various ions and molecules and various membrane proteins act as enzymes, and receptors of chemical information. These proteins also help in the transport across the membranes.

6.7 TERMINAL QUESTIONS

- 1) Explain briefly the concept of self-assembly.

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- 2) Discuss the assembly of bacterial ribosome very briefly.

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- 3) 'Membrane carbohydrates contribute to the asymmetry of the biological membranes.'
Justify the statement.

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- 4) Comment briefly on the fluid state of the lipid bilayer.

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- 5) Discuss the Singer-Nicolson model of the cell membrane.

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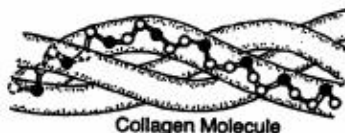
6.8 ANSWERS

Self-assessment Questions

- 1) i) Self-assembly
ii) Non-covalent
iii) Dimers
iv) Physicochemical forces

2) a)

Collagen molecule



- b) The hydroxyl groups of hydroxyproline form interchain hydrogen bonds that maintain the triple helical structure. The hydroxyl groups of hydroxylysine help in cross-linking of polypeptide chains of collagen molecules.
- 3) a) G-actin is globular form of the protein actin. It is soluble in weakly ionic solution. F-actin is the fibrous form of actin formed by the self-assembly of G-actin. F-actin is insoluble in aqueous solutions.
- b) The actin filament consists of two strands of G-actin monomers which twist around each other to form a double helix. The protein molecules of the actin filament have more extensive contact with each other due to the double helical structure of actin filament.
- 4) i) $\times$, ii) $\times$, iii) $\checkmark$, iv) $\times$, v) $\checkmark$
- 5) a) [ii], b) [iv], c) [i], d) [iii]
- 6) a) Peripheral proteins: These are generally exposed only on one side of the bilayer and can be easily extracted from the membranes.
- Integral proteins: These proteins generally extend across the bilayer and can be removed from the membrane only after total disruption of the bilayer.
- b) a [iii], b [iv], c [ii], d [i]
- 7) The unit membrane model was not accepted because it could not account for the proteins traversing the membrane and their lateral movements in the bilayer.
- 8) Asymmetry of biological membranes is caused by lipid, protein and carbohydrate contents. The two layers of lipids are different in composition. Proteins are peripheral or integral. Carbohydrates are attached to the outer layer only.

Terminal Questions

- Self-assembly is the process in which similar or dissimilar molecules associate with the help of non-covalent bonds to form supramolecular structures. The proteins like collagen and actin are formed by the assembly of similar subunits whereas structures like viruses and ribosomes are formed by the assembly of dissimilar molecular subunits.
- Bacterial ribosomes are formed by the assembly of two subunits together formed by 55 different proteins and 3 different types of rRNA. 21 protein molecules and a single rRNA form the smaller subunit and 34 protein molecules and two rRNA molecules form the larger subunit. These two subunits are complementary to each other and unite to form the ribosomal particles.
- In the cell membrane the carbohydrates occur as oligosaccharide side chains, non-covalently linked to the proteins and lipids of the outer layer of lipid bilayer. Thus the complete absence of carbohydrates on the inner layer of lipid bilayer contributes to the asymmetry of lipid bilayer.
- The fluid state of the lipid bilayer is because the hydrocarbon chains of lipid molecules are loosely packed. The hydrocarbon chains of unsaturated lipid molecules are also of shorter length and more loosely packed. Interactions of cholesterol with phospholipids in the bilayer contributes towards membrane fluidity.
- According to Singer-Nicolson model the lipid bilayer exists in a relatively fluid state where various proteins present form a mosaic arrangement. Some of the protein molecules are present only on one surface of the membrane whereas some proteins traverse across both the layers. The carbohydrates occur as oligosaccharide side chains on the outer layer of the bilayer. The basic framework for membrane structures is same for all the membrane but their lipid, protein and carbohydrate content ratio may differ.

UNIT 7 MEMBRANE TRANSPORT PROCESSES

Structure

- 7.1 Introduction
 - Objectives
 - 7.2 Basic Ideas
 - Fluxes
 - Forces
 - 7.3 Membrane Transport Processes
 - Free Diffusion
 - Osmosis : A Special Case of Diffusion
 - 7.4 Mediated Permeability
 - Facilitated Diffusion
 - Active Transport
 - Shuttle Processes
 - 7.5 Electrical Potential Across Membrane
 - 7.6 Summary
 - 7.7 Terminal Questions
 - 7.8 Answers
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7.1 INTRODUCTION

In the previous unit, you have learnt about self-assembly of macromolecules into supramolecular structures such as collagen, actin, etc. You have also studied the composition and various models of cell membrane structure.

In this unit, you will study the various membrane transport processes. In fact, every process which is a characteristic of living organisms such as growth, respiration, excretion, movement, reproduction, etc., is carried out with the help of membrane transport at some stage. Many processes such as absorption of digested food materials by the intestine, secretion of acids in the stomach, exchange of oxygen and carbon dioxide in the lungs, formation of ATP in mitochondria and chloroplasts, functions of the kidney and many other functions involve membrane transport. Likewise, many of the functional aspects of biology require a knowledge of transport processes, for example, design of heart-lung machines as well as the design of many drugs is based on transport processes.

As said above, in this unit you will learn about transport processes which take place across the membrane. The internal environment of the cell differs from that of its external environment by the presence of thin surface membrane called **cell** or **plasma membrane** that controls the entrance and exit of molecules and ions across the cell. The capacity of the plasma membrane to act as a selective barrier between the cell and the medium is called **permeability**.

The rate of movement of a substance across a membrane is called **flux**, denoted by 'J'. The flux is controlled by a number of driving forces such as difference in pressure, electrical potential and concentration. The transport of substances across the cell membrane may be **passive** through diffusion or **active** by active transport. The first law of diffusion as proposed by Fick states that the flux is directly proportional to both the area and the concentration gradient. The movement of water in response to a solute concentration gradient is called **osmosis**.

Since the membrane basically has a lipid structure, the substances which are lipid soluble and small in size can pass easily through the membrane. However, these are not the only factors that affect the permeability of the membranes. Special devices called transport systems are located in the cell membranes. These transport systems affect the permeability of the membrane for specific molecules in a very selective manner. This type of transport is **mediated** by special transport systems, and therefore, this phenomenon is called **mediated permeability**.

In this unit, we will discuss two types of mediated permeability, i.e., **facilitated diffusion** and **active transport**. You will also learn about the **shuttle process** which

helps in the transport of many molecules through the membranes. These molecules cannot pass through the membrane otherwise.

The study of this unit requires that you should have an elementary knowledge of physics, chemistry and mathematics. You should, for example, know some physical constants, conversion factors and the know-how to use logarithms for calculation.

Objectives

After reading this unit you should be able to

- explain transport phenomenon,
- describe various symbols used to denote fluxes, driving forces, permeability,
- explain the relationship of flux with driving force and permeability,
- state and explain Fick's equation,
- state the differences and similarities with regard to free diffusion, facilitated diffusion and active transport.

7.2 BASIC IDEAS

Movement of a substance from one place to another in a medium or across a membrane is controlled by certain physical processes, such as, diffusion, permeability and osmosis. To describe these processes we will use certain terms, such as flux, forces and concentration gradient. Therefore, first of all we will try to understand these basic ideas.

7.2.1 Fluxes

Flux is the amount of a substance transferred across a membrane in unit time through a unit area perpendicular to the direction of movement (Fig. 7.1 a).

Flux is generally denoted by J . If the flux is in x -direction, then the flux is denoted as J_x (Fig. 7.1 b)

The net effect of two oppositely directed unidirectional fluxes is called the net flux (symbolised as J_{net}).

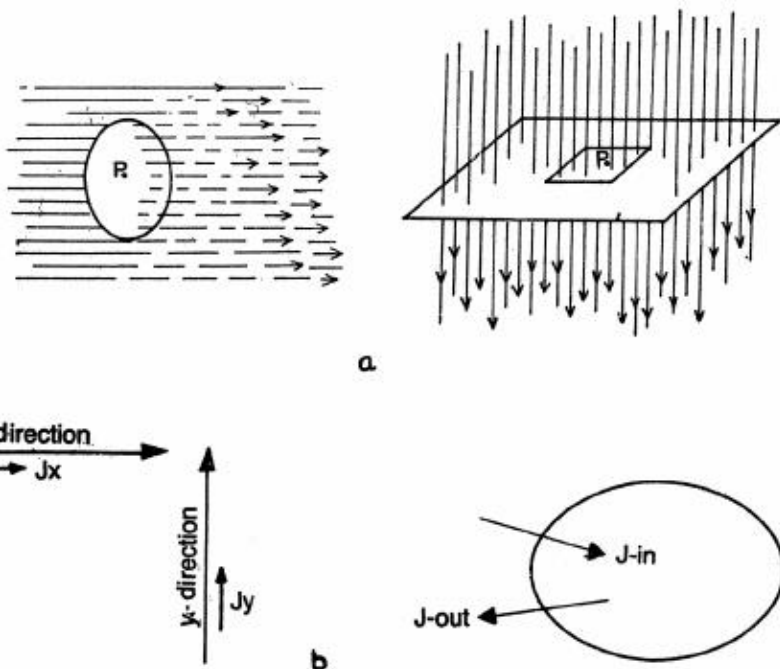
$$J_{net} = J_{ot} - J_{io}$$


Fig. 7.1 a) Flux. Arrows indicate the direction of flow of a substance (say water). To find the flux at a point P, take around P a unit area drawn perpendicular to the direction of flow. Measure the amount of the substance passing through this area in unit time (say one second).

- b) Direction of flux. Flux in the x -direction is written as J_x , in the y -direction as J_y . Flux of a substance moving inside a compartment is written as J_{in} , whereas flux outside as J_{out} .

$$\text{Flux} = \frac{\text{Amount of substance}}{\text{Area} \times \text{time}}$$

Equation 7.1

Membrane Transport Processes

If the amount of the substance is expressed in moles, the area in cm^2 and the time in sec, then the flux may be expressed as $\text{J mol cm}^{-2}\text{s}^{-1}$. In this case J denotes the mole of substance flowing through an area of 1 cm^2 in one second.

Movement of substance in a single direction is a **unidirectional flux** whereas movement in both directions is known as **bidirectional flux**.

The unidirectional flux of glucose from outside to inside is represented by J_{oi} glucose
(o = out, i = in)

7.2.2 Forces

The difference in pressure (P), electrical potential (E) and concentration (C) on the two sides of a membrane are considered to be the most important driving forces in the living systems

Concentration : Relative amounts of solute and solvent in a solution. A solution in which a large amount of solute is dissolved in per unit volume of solvent, it is said to have a high concentration of the solute.

Flux is directly proportional to the magnitude of the driving forces. Mathematically it can be stated as under.

$$J \propto \Delta P + \Delta E + \Delta C$$

Equation 7.2

{ J is the flux of any substance x,
Δ means 'the difference in' and
α means 'is proportional to' }

Relationship between forces and fluxes may be defined precisely by the following equation.

$$J \propto Y \text{ (J is flux and Y is any driving force)}$$

$$J = k \Delta Y$$

(k is the constant of proportionality)

Equation 7.3

SAQ 1

- a) What do you mean by j_{Na^+} ? Give your answer in the space provided

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- b) Calculate the flux if 5 mole of glucose is passed through an area of 0.1 cm^2 in 2 seconds in x direction (Hint : Use equation 7.1)

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7.3 MEMBRANE TRANSPORT PROCESSES

A substance can pass through a membrane by two routes. The first route is mainly used by lipophilic (lipid-loving) substances. These substances can pass through the membrane by simple diffusion. The second route is mainly used by lipophobic (lipid-hating) substances. These substances can pass through the membrane either by facilitated diffusion or active transport or the shuttle system. You will study these processes in detail in the following sub-sections.

7.3.1 Free Diffusion

Diffusion is a spontaneous process that leads to the net movement of a substance from a region of higher concentration to a region of lower concentration.

For example, if you put a few drops of a very concentrated dye solution (red or blue ink) in one end of a flat glass bowl containing water, the molecules of the dye will slowly diffuse uniformly throughout the bowl. You can watch this process by the gradual spread of the colour throughout water. The process may take several hours or even longer depending on several factors.

Solvent : Generally the substance in a solution that is present in the largest quantity. If one substance is a liquid, it is normally considered to be the solvent. When water is present, it is taken to be the solvent.

Cell Membranes and Enzymes

Solution : A homogeneous mixture of two or more different substances where one of the substances is dissolved in the other.

Solute : A substance dissolved in a solvent.

Concentration Gradient : The change of the concentration between two planes at a unit distance apart.

Diffusion Coefficient: The weight of a material, in grams, diffusing across an area of 1 square centimetre in 1 second in a unit concentration gradient, is known as diffusivity.

Diffusion of a solute takes place from a region of higher concentration of the solute to a region of lower concentration through any water (solvent) medium in almost all living systems. However, if a 'barrier' is placed between the two regions, the rate of diffusion is slowed down. The greater is the thickness of the barrier, the lower will be the rate of diffusion.

Adolph Fick in 1855 developed a quantitative relationship between the diffusive flux and its causative agent, the concentration gradient. Fick's first law states that the flux is directly proportional to both the **area** and the **concentration gradient** acting perpendicular to that area. In other words,

$$J_x = D \frac{C_2 - C_1}{x} = -D \frac{\Delta C}{\Delta x} \quad \text{...Equation 7.4}$$

Where D is the **diffusion coefficient** of the substance. The substance moves from the region of higher concentration i.e. C_1 to the region of lower concentration i.e. C_2 . Therefore, C_1 will be greater than C_2 and $C_2 - C_1$ will be negative; that means ΔC will be negative. Hence a negative sign is put before ΔC . The ratio $\Delta C / \Delta x$ is called the **concentration gradient**.

If the concentration (ΔC) is expressed in mol cm^{-3} the distance (Δx) in cm, and flux J_x in $\text{mol cm}^{-2}\text{s}^{-1}$, then the diffusion coefficient D may be expressed as cm^2s^{-1}

Fick's equation gives useful insights into various strategies by which an organism regulates the uptake or loss of a particular substance and thereby correlates structure and function. For a given substance, the diffusion coefficient depends on the nature of the solvent and the temperature.

Considering diffusion across a membrane whose thickness is not always known, one can, for convenience, merge the thickness parameter (d) into the diffusion coefficient (D) in Fick's first law (Equation 7.4)

$$\text{Diffusion Rate} = J_x = \frac{D}{d} \times (C_2 - C_1)$$

$$\text{or } J_x = P_x (C_2 - C_1) \quad \text{...Equation 7.5}$$

$P = D/d$ is called the **permeability coefficient** of the diffusing substance for the membrane. Since D has the unit cm^2/sec , $P (=D/d)$ has the unit of cm/sec , which is in fact, the unit of velocity. Thus, equation 7.5 may be written as

$$\text{Flux} = (\text{Permeability coefficient}) \times (\text{Concentration difference}) \quad \text{...Equation 7.6}$$

The diffusion rate is the amount of a substance passing through the membrane per unit time per unit area under the given concentration difference. In the above equation if $C_2 - C_1 = 1$, then $P = \text{Rate}$. In other words, permeability is the rate at which a substance diffuses down through a unit area of the membrane concentration gradient unit.

7.3.2 Osmosis: A Special Case of Diffusion

If two solutions of different concentrations are separated by a membrane, then in some cases, water tends to move through the membrane in the direction of the solution of higher concentration. This movement of water is called **osmosis**. Let us see what the essential property of the membrane in osmosis is. For osmosis to take place, the membrane should be permeable to water but not permeable to the solute. Such a membrane is said to be **semipermeable**.

Osmosis is the name given to diffusion of solvent through a semipermeable membrane from a solvent into a solution, or from a dilute to a more concentrated solution.

Let us take a particular case as shown in Figure 7.2. Compartment B has an aqueous solution of sucrose and compartment A contains just water. The liquids are separated by a semipermeable membrane which is permeable to water but not to sucrose. Now the question arises; Is the system in equilibrium? If not, then what happens? In other words does the system come to equilibrium?

Initially the system cannot be in equilibrium, because concentration in compartment B is higher than in A; remember A has just water. Hence, water tends to flow from A to B. As

Hydrostatic Pressure : The pressure at a point in a fluid at rest due to the weight of the fluid above it.

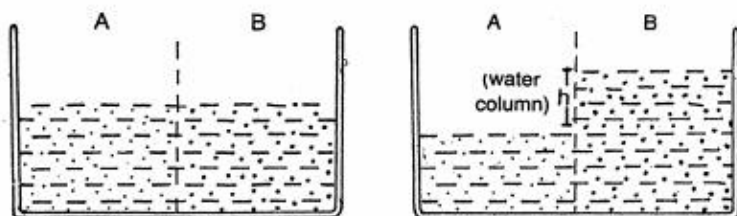


Fig 7.2 : The situation at left is not at equilibrium, water flows from compartment A to B. The situation at right is at equilibrium, the osmotic pressure is equalised by the hydrostatic pressure of water column (h) in B.

Therefore, it is clear that water in A exerts a pressure against the membrane due to the presence of the sucrose solution on the other side of the membrane. Thus pressure can be measured by the hydrostatic pressure which is applied to the solution side to establish equilibrium. This pressure is called the **osmotic pressure** (symbolised as π) of the solution. Thus, osmotic pressure of a solution may be defined as the excess pressure which must be applied to a solution to prevent the passage of water into it when it is separated from water by a semipermeable membrane. Solutions containing an equal number of molecules, irrespective of size, have equal osmotic pressures.

The osmotic pressure of a dilute solution can be calculated from van't Hoffs' equation given below

$$\pi V = nRT \quad \text{...Equation 7.7}$$

where, π = osmotic pressure of the solution

V = volume of water

n = number of moles of the solute dissolved

R = universal gas constant = $8.314 \text{ Joule K}^{-1} \text{ mole}^{-1}$

T = absolute temperature

The equation 7.7 can also be written as

$$\pi = \frac{n}{V} RT$$

$$\text{or } \pi = CRT \quad \text{...Equation 7.8}$$

where $n/V = C$, the concentration of the solute in water is expressed in mole/litre.

Osmotic pressure of a solution therefore does not depend on the nature of the solute particles. It only depends on their number which in turn is related to the **concentration and nature of the solute**.

After discussing about osmotic pressure, let us now describe the turgor pressure. As you know, plant cells have rigid walls which protect the cells from extreme swelling and bursting when the cells are in a dilute medium. In a dilute medium, cells absorb some water by osmosis. This osmotic entry of water into the cell builds up hydrostatic pressure within the cell. It is called **turgor pressure**, and the cell under this condition is said to be **turgid**. Because of the extraordinary strength of the cell wall, the cells swell only to a certain extent appreciably and do not burst even though the turgor pressure may be very large ($5 \text{ to } 15 \text{ kg cm}^{-2}$).

In plants, turgor pressure is responsible for maintaining the crispness or rigidity of most soft tissues, such as leaves, petioles, young stems, etc. When the turgor pressure is lost, these plant organs become limp and flabby, and the plants are said to **wilt**. Then normal ability of the plant cells to maintain a turgor pressure through osmotic absorption of water eliminates the need for a rigid skeleton to support the soft parts of the plants.

How a cell will behave when put in a solution depends on its tonicity. But before coming to the question of what tonicity is, let us first explain the terms: iso-osmotic, hypo-osmotic and hyper-osmotic. These terms are used to compare the osmotic pressures of two solutions. Two solutions having the same osmotic pressure are said to be **iso-osmotic** to each other. A solution having a lower osmotic pressure than the other is said to be **hypo-osmotic** whereas a solution having higher osmotic pressure than the other is called **hyper-osmotic**.

If a cell is suspended in a solution, the cell may swell or shrink or may not change its volume at all. Tonicity of a solution is defined in terms of the response of a cell to that solution. If the cell swells in a solution, the external solution is called a **hypotonic** medium. If the cell shrinks in a solution, it is a **hypertonic** medium. If the cell does not change its volume, the external solution is **isotonic**. Now, let us take an example to make it clear to you that two iso-osmotic solutions may have different tonicities for different cells. For example, 150 mM (millimolar) NaCl is iso-osmotic to 300 mM glycerol solution. If human red blood corpuscles (RBC) are suspended in a 150 mM NaCl solution, the corpuscles neither shrink nor swell. Therefore, the salt solution (150 mM NaCl) is **isotonic** for the human RBC. On the other hand, if human RBCs are suspended in 300 mM glycerol solution, they swell up and burst. This shows that 300 mM glycerol solution is **hypotonic** for human RBC. Thus, 150 mM NaCl solution, though, **iso-osmotic** to 300 mM glycerol solution; is **hypotonic** with the cell cytoplasm. Let us now see what happens to rabbit RBCs in these two solutions. Rabbit RBCs neither swell nor shrink in 150 mM NaCl or in 300 mM glycerol. Thus, both these solutions are isotonic for the rabbit RBCs.

Why is it so? This can be explained in terms of the permeability of the cell membrane to the solutes in the external medium. Human RBC membrane is permeable to glycerol, whereas rabbit RBC membrane is almost impermeable to it. When human RBC is placed in 300 mM glycerol solution, a concentration gradient of glycerol develops across the RBC membrane. Since the membrane is permeable to the glycerol, the latter diffuses into the cell and as a result the osmotic pressure of the cell cytoplasm increases. The increase in the osmotic pressure of the cell cytoplasm causes flow of water into the cell and as a result, the cell swells and eventually bursts. This process of swelling and rupturing of the cell is called **cell lysis**.

Before you proceed you may attempt the following SAQ to check your understanding about permeability of the cell membrane.

SAQ 2

Calculate the permeability of the membrane, when the concentration difference across the membrane is 0.1 moles/litre and the rate of the substance moving across the membrane is 10^{-6} mole/cm²/sec

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7.4 MEDIATED PERMEABILITY

We have seen in section 7.3 that the equation for flux (J_x) as derived from Fick's law is as given below:

$$J = P (C_1 - C_2)$$

This equation implies that if a membrane has the same value of P for two different molecules and if the concentration difference (ΔC) is also maintained the same for both types of molecules, then the flux will be the same for both types of molecules. If two molecules have a similar size, shape, structure and lipid solubility, it is expected that the membrane will have similar permeability values for both the molecules, and therefore, the flux will be similar for both molecules at a given concentration gradient. But this may not

be true in many cases. For example, two isomers, glucose and galactose, have the same size and shape and the structures are very similar to each other (Unit 4, section 4.2). The two molecules have no charge but have similar values of lipid solubility. Yet, glucose is transported much faster than galactose by human RBC. This is because the membrane of red blood corpuscles has much higher specificity for glucose than for galactose.

It is also found that the experimental value of flux is much greater than the predicted value. That is, a glucose molecule is transported across the RBC membranes much faster than expected from the simple law of diffusion.

If J_x values are calculated for different values of ΔC and a graph is plotted with J_x versus ΔC , for predicted and experimental values of permeability, we get the graph as shown in Figure 7.3.

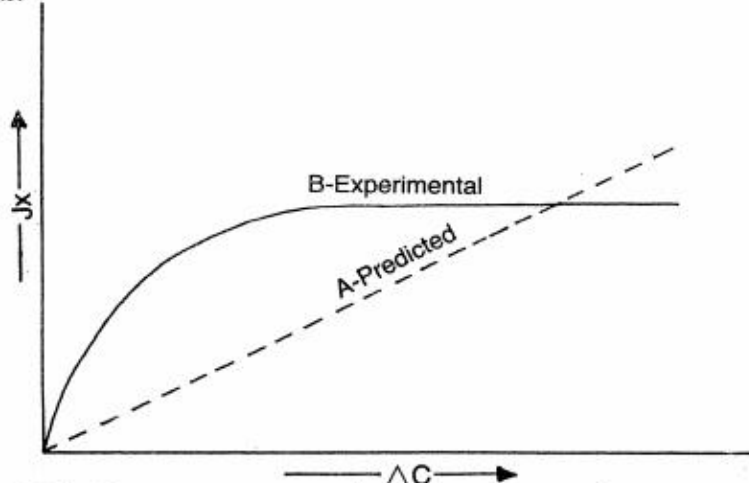


Fig. 7.3: Relationship between flux (J_x) and concentration difference (ΔC) across the membrane. For predicted value of P of glucose (marked A-predicted) a straight line is observed. For experimental values of P of glucose (marked B-experimental) it is observed that the line is not straight throughout the concentration range. At low ΔC values the line is straight, but as ΔC increases the curve deviates from linearity and becomes saturated. Any further increase in ΔC does not increase the flux.

You have read that the actual transport of glucose across RBC membranes is different from that of theoretical transport as predicted by simple diffusion. This implies that glucose transport across RBC membranes does not obey the simple law of diffusion. Then the question arises as to what is the mechanism of glucose transport. In the curve as shown in Figure 7.3, you will observe that after a certain increase of concentration difference, there is no increase in flux. This is called **saturation characteristic of the curve**. It shows that the concentration difference is not the only factor (other than P) which influences the transport; there may be another factor. This factor must have a **limited** maximum capacity in increasing the flux. When this limit is reached, the flux cannot be increased by increasing the concentration difference. You may ask what is this factor? It is assumed that there are a **limited number** of specialised molecules located in the membrane that help in transporting glucose molecules faster across the membrane. These are called **transport molecules**. Since a transport molecule can transport a glucose molecule **faster** than what is expected from the P value, the flux is higher than the theoretical value in the low range of concentration difference i.e., ΔC . As ΔC is increased, more glucose molecules are available for these transport molecules, and hence the flux increases. At a certain level of ΔC , it is possible that all the transport molecules are working at their maximum rate. Once this point is reached, the increase in glucose concentration will not further increase the flux.

Hence the curve becomes flat. Thus, the theory of transport molecule explains two important characteristics: first, why the flux is higher than that expected at low ΔC , and second, why the flux gets saturated. We have to explain another important characteristic that, why glucose, and not galactose, is transported preferentially. It is because a transport molecule is very specific in recognition to its substrate. This type of transport phenomenon is known as **mediated permeability**.

Mediated permeability has another important characteristic. It is inhibited by certain molecules, called **transport molecule inhibitors**. These inhibitor molecules bind tightly to the transport molecules. We will discuss about the inhibitor molecules in detail in the next unit.

Briefly, the mediated permeability is distinguished from simple diffusion by the following characteristics :

- 1) The transport rate (flux) and membrane permeability (P) are greater than that expected from the law of simple diffusion.
- 2) The transport rate becomes saturated at higher concentration difference (ΔC).
- 3) The transport is mediated by transport molecules located in the membranes, which are highly specific for the molecule to be transported.
- 4) The transport rate can be inhibited by inhibitors which bind tightly to the transport molecules.

There are two main types of mediated permeability: facilitated diffusion and active transport. In the following sections, we will describe these two types of mediated permeability.

7.4.1 Facilitated Diffusion

In facilitated diffusion the molecule is transported across the membrane from the higher concentration side to the lower concentration side, i.e., the driving force is concentration gradient. In this respect, facilitated diffusion is passive diffusion. But it requires transport molecules (Fig 7.4). Without the transport molecules, the membrane is practically impermeable. Hence the facilitated diffusion is a mediated permeability in which the transport molecules facilitate passive transport of specific solutes across a membrane from a higher to lower concentration gradient.

Many mammalian tissues possess facilitated diffusion system for molecules which they receive from blood plasma, e.g., sugars, and amino acids. Red blood cells have facilitated diffusion system to absorb glucose from plasma. The amino acid, phenylalanine is transported across the epithelial cells of the intestine by this process. Bacterial membrane also absorbs glucose from the outside medium by facilitated diffusion. In general, certain highly water soluble metabolites such as sugars, amino acids and glycerol are transported by facilitated diffusion.

Thus, we have learnt that there are two types of passive diffusion, one is **simple diffusion** which obeys Fick's law, and the other is **facilitated diffusion** that does not obey Fick's law. In both the cases, the direction of transport is from the high concentration to lower concentration side, and, hence no energy is required for these processes. However, while simple diffusion does not require transport molecules, facilitated diffusion is mediated by transport molecules.

7.4.2 Active Transport

Active transport process is a mediated permeability in which the molecule moves **against** the concentration gradient, that is, from the low concentration side to the high concentration side. Transport molecules are involved in active transport also because it is a mediated permeability process. Furthermore, since the molecule is transported against the concentration gradient, energy must be supplied for active transport to take place (Fig. 7.4).

As active transport can operate against a concentration gradient, the cells can accumulate substances from the environment where the concentrations of these substances are extremely low. Bacteria can remove nutrients almost completely from a culture by active transport. Even when the concentration of glucose inside the cells of a kidney is ten times more than the outside environment, the cells take up further glucose. Through this process, kidney cells reabsorb most of the salts and essentially all the amino acids and sugars in the extra-cellular fluid, in this case, urine.

Concentration gradients, especially for certain ions, are essential for the proper functioning of the cells. Active transport processes provide for the creation and maintenance of such gradients. In red blood cells at physiological temperatures, potassium ion (K^+) concentration inside is 30 times more than that in the outside environment, while sodium ion (Na^+) concentration inside the cell is 6 times less than that outside.

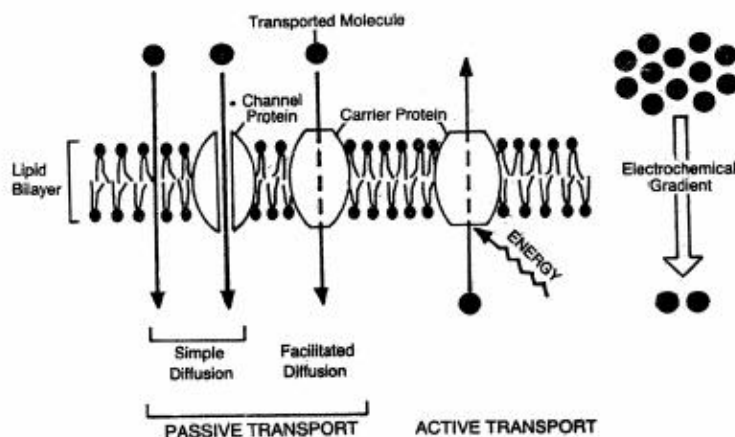


Fig. 7.4: Schematic diagram of passive transport down an electrochemical gradient and active transport against an electrochemical gradient. Whereas passive transport, either by simple diffusion or by facilitated diffusion, occurs spontaneously, active transport requires an input of metabolic energy. Although small uncharged molecules can directly cross the lipid bilayer, the transfer of charged molecules (including all small ions) occurs at a significant rate only through protein channels or carriers.

Several examples of cellular functions which operate by active transport can be cited. Muscle contraction, propagation of nerve impulse, detoxification of blood in kidney, absorption of metabolites by the intestinal mucosal cells, energy production in mitochondria and chloroplasts, flagellar movement in bacteria are some of the examples in which active transport is involved either directly or indirectly. We will discuss it in the following sections.

If there is a concentration gradient, molecules or ions must be moved from the low concentration side to the high concentration side. This does not happen spontaneously; energy must be spent for such movement to occur. The amount of energy required depends on the magnitude of the concentration gradient. The following equation is used to calculate the amount of energy required for active transport :

$$G = 2.303 RT \log \frac{C_2}{C_1}$$

Where G = amount of energy required to move one mole of the substance from low concentration (C_1) side to high concentration (C_2) side; R = universal gas constant = $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$; T = absolute temperature.
...Equation 7.9

Active transport is commonly known as pump. Active transport of Na^+ is called a Na^+ pump; active transport of H^+ is called a proton pump, and so on. Why the word 'pump' is used will be clear to you by the following analogy. When water is allowed to flow from a higher level to a lower level, it flows spontaneously, and, in fact, energy is available from this process. This energy can be utilised by coupling a turbine or some other device to the flow of water. But water does not move spontaneously from a lower level to a higher level. However, it can be moved by using a pump and, of course, one has to spend some energy to pump water from a lower to a higher level (Fig. 7.5).

Now, there is an important question. Where does the energy for active transport come from? In all living systems, hydrolysis of ATP is the most common source of energy. However, the free energy available from a concentration gradient is also used for the active transport. Hydrolysis of one mole of ATP to ADP and inorganic phosphate (Pi) produces about 8,000 calories of free energy ($\text{ATP} \longrightarrow \text{ADP} + \text{Pi} + 8000 \text{ cal.}$).

$$\begin{aligned} \text{In joules this is equal to } & 8,000 \times 4.2 \text{ (1 Cal = 4.2 J)} \\ & = 33,600 \text{ J.} \end{aligned}$$

The energy required for the active transport of Na^+ and K^+ in red blood cells comes from ATP hydrolysis which is coupled with an enzyme called Na^+/K^+ -dependent ATPase located in the

membrane. This enzyme transports both Na^+ and K^+ simultaneously in opposite directions getting the required amount of free energy from the hydrolysis of ATP

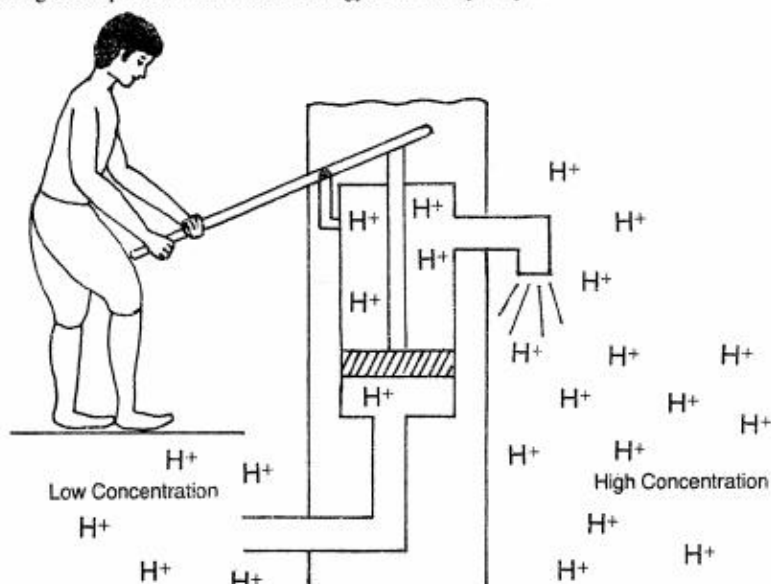


Fig. 7.5: Active transport of H^+ . Just like the ordinary hand pump the transport of H^+ ions from low concentration to high concentration requires energy. This is called proton pump.

In active transport, one important requirement of the membrane is to construct a physical system which should be located in the membrane and capable of coupling two processes. These two processes are the energy-yielding process like the ATP hydrolysis and an energy-requiring process as in the movement of the substance against the concentration gradient. An appropriate coupling is absolutely necessary to ensure that the free energy liberated from the energy-yielding reaction can be utilised to run the other metabolic reaction. The make up of the membrane which allows such coupling in any active transport process is a fascinating creation of Nature.

7.4.3 Shuttle Processes

A substance, for which the membrane is impermeable and there is no transport molecule either, is transported by an alternate method, called shuttle. In this process, the substance is converted to a second substance which is transported to the other side of the membrane by facilitated diffusion or active transport. The second substance is then converted back to the original substance on the other side of the membrane. It can be a simple shuttle involving only two molecules (X and Y in Fig 7.6) or a complex shuttle involving many other molecules and even more than one shuttle (X, Y and Z in Fig. 7.6)

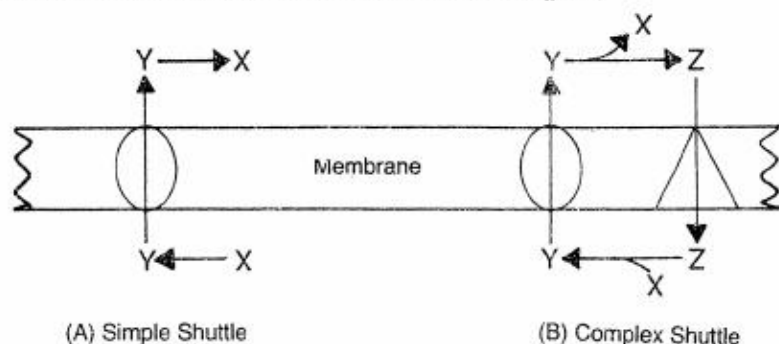


Fig 7.6 : Schematic diagram of shuttle mechanism of membrane transport. The molecule X is effectively transported from one side to another, although it is Y (in simple shuttle) or Y and Z (in complex shuttle) which are directly transported.

There are many cases of shuttle processes in living systems Malate-aspartate shuttle across the inner membrane of mitochondria is an example of a complex shuttle system about which

you will study in detail later. You may like to try the following SAQ to see if you have understood the process of diffusion across a membrane

SAQ 3

Put a cross [x] before the statement which is incorrect

Both simple diffusion and facilitated diffusion

- | | |
|--|-------|
| a) are passive diffusion | [] |
| b) transport the solutes down the concentration gradient | [] |
| c) require no energy | [] |
| d) require transport molecules | [] |

7.5 ELECTRICAL POTENTIAL ACROSS MEMBRANE

In the case of passive diffusion (simple diffusion or facilitated diffusion) of uncharged molecules, the driving force is the concentration gradient across the two sides of the membrane. But if the electrically charged ions such as Na^+ , K^+ , Cl^- , etc., are involved, then another driving force which is electrical in nature becomes operative. Let us see how this takes place.

Let us consider two compartments A and B containing equal volumes of 10 mM NaCl. The compartments are separated by a membrane which is permeable to Na^+ but not to Cl^- . Since both the compartments have solutions of equal concentrations, there is a net flux of Na^+ , even though Na^+ ions are moving freely between the compartments. There will, of course, be no movement of Cl^- between the compartments because the membrane is impermeable to Cl^- (Fig. 7.7).

If we consider the distribution of electrical charges in the two compartments, we see that there are equal number of positive and negative charges in each compartment. This keeps each compartment electrically neutral. This is called the state of electrical neutrality and each system tends to maintain this state. If the state of electrical neutrality is disturbed, then the system tends to re-establish the state of electrical neutrality.

Suppose we now increase the concentration of NaCl in the compartment A to 100 mM. There is now a concentration difference, and hence Na^+ will start diffusing from A to B. This diffusion may be simple passive diffusion or facilitated diffusion. In any case, it is the concentration difference which is the driving force. As one Na^+ goes from A to B, the state of electrical neutrality is disturbed in both the chambers. Chamber A has one negative charge (Cl^-) in excess and Chamber B has one positive charge (Na^+) in excess.

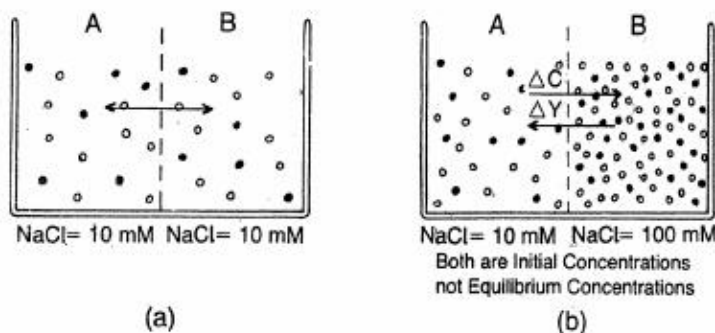


Fig 7.7: Development of electrical potential across a membrane which is permeable to Na^+ and impermeable to Cl^- .

- It is an equilibrium situation in which $C = 0$ and there is no electrical potential difference ($V = 0$) between the chambers because each chamber is in a state of electrical neutrality.
- It is also an equilibrium situation in which C and V are not zero, but equal and opposite to each other. V has been developed because the state of electrical neutrality is disturbed in the chambers.

We can, therefore, say that a negative potential has developed in A and a positive potential has developed in B. Thus, a difference in electrical potential has been developed between the two chambers. What does this potential difference do? It acts as a new driving force for the movement of Na^+ . When the next Na^+ tries to move from A to B, it will have two driving forces acting on it, one due to the concentration difference (already existing) that tends to drive the Na^+ from A to B, and the second due to an electrical potential which will also drive the Na^+ from B to A, because the excess positive charge in B repels the Na^+ and the excess negative charge in A attracts the Na^+ . At first, the driving force due to electrical potential difference, let us call it V , is weaker than the driving force due to concentration difference (ΔC), and therefore, more Na^+ will go from A to B. However, as more Na^+ cross from A to B, ΔC decreases and ΔV increases. At one point these two opposing forces will balance each other and no more movement of Na^+ will take place, although a concentration difference is still there. Thus, a state of equilibrium is reached. This type of equilibrium is called **electro-chemical equilibrium**, because one driving force is difference in concentration (ΔC), which is of chemical nature and the other driving force is of electrical nature. The electrical potential difference across the membrane is also known as the **membrane potential**.

The membrane potential developed under equilibrium conditions is measured by the following equation

$$V_{eq} = \frac{2.303 RT}{nF} \log \frac{X_{out}}{X_{in}} \quad \dots \text{Equation 7.10}$$

called as Nernst equation. Nernst equation gives valuable information on the source of membrane potential.

Where V_{eq} = membrane potential

R = universal gas constant = $8.314 \text{ J mole}^{-1} \text{ K}^{-1}$

F = Faraday = $96,500 \text{ coulombs mole}^{-1}$

n = ion valency, which is 1 for monovalent ions like K^+ , Cl^- , and 2 for divalent ions like Ca^{++}

T = temperature on kelvin scale. For instance, at 30°C , $T = 273 + 30 = 303^\circ\text{K}$

Substituting the above values for monovalent ions at temperature 303°K in the above equation we get the equation as under

$$V_{eq} = (0.060) \log \frac{X_{out}}{X_{in}}$$

Such a potential difference is also called **Nernst potential** or **equilibrium potential**.

Try the following SAQ related to development of potential across a semipermeable membrane.

SAQ 4

Suppose two chambers A and B are separated by membrane which is permeable to Cl^- but not to K^+ . Chamber A is filled with 100 mM KCl and chamber B with 10 mM KCl.

a) Do these chambers remain in a state of electrical neutrality before any movement of the ions starts? Give reason.

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b) How can the state of electrical neutrality be disturbed when diffusion starts?

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7.6 SUMMARY

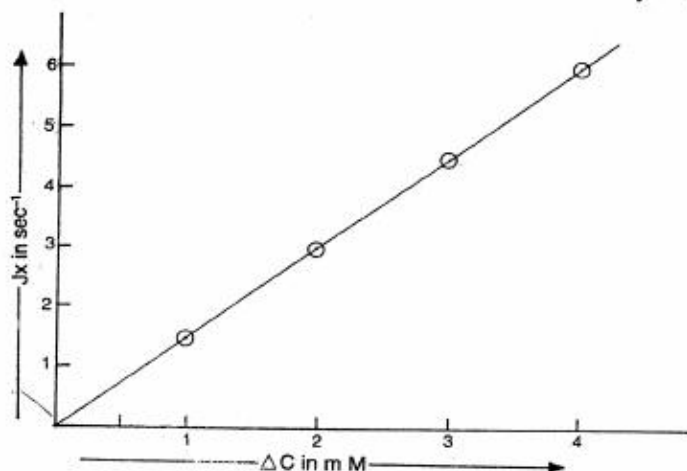
In this unit we have discussed the following aspects of membrane transport processes:

- Quantitative measurements of membrane transport indicate the rate of transport of substances across the cell membranes. Three major quantitative factors are **flux**, **driving force** and **permeability**.
- Different processes are responsible for the movement of biologically important substances across the membrane, e.g., free diffusion, facilitated diffusion, osmosis and active transport. Diffusion is a process through which atoms, ions or molecules are dispersed randomly in all directions until these particles are equally distributed in the available space. The rate of diffusion is directly proportional to the concentration gradient, and is quantitatively expressed by Fick's first law.
- Permeability is diffusion through a barrier, i.e., biological membrane. Permeability of a membrane to a substance is defined as the amount of the substance passing through the membrane in unit time under unit concentration gradient across the membrane.
- Osmosis is diffusion of water across a membrane because of the difference in concentrations of water on either side of the membrane. The driving force for water movement is the difference in concentrations of solutes on two sides of the membrane. The driving force is called osmotic pressure.
- Permeability of biological membranes for many substances is altered by transport molecules which are located in the membranes. This phenomenon is called mediated permeability.
- There are two types of mediated permeability, one is facilitated diffusion and the other is active transport. Facilitated diffusion is passive diffusion, because it occurs down the concentration gradient and hence no energy is required. Mediated permeability controls many vital functions of living organisms.
- Ions are distributed across a membrane in such a way that an electrical potential difference is developed across the membrane. This is known as a membrane potential. Under equilibrium condition the membrane potential can be calculated using Nernst equation. Membrane potential plays vital role in many biological activities such as muscle contraction and transmission of nerve impulse.

7.7 TERMINAL QUESTIONS

- What would be the osmotic pressure of one molar aluminium chloride in water at 27°C? Assume 100% ionisation.
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- The molecular weights of urea and sucrose are 60 and 342 respectively. Which one will diffuse faster in a given solvent and why?
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- 3) a) Calculate the values of flux for the concentration differences of 1mM, 2mM, 3mM and 4mM when the permeability of the membrane to the molecule is 1.5 sec^{-1} . Plot a graph with concentration difference in the x-axis and flux in the y-axis.



- b) What type of line do you get in the graph?

7.8 ANSWERS

Self-assessment Questions

- 1) a) J_{Na^+} means the unidirectional flux of sodium ions in the direction from inside to outside the cell.

$$\text{b) Flux} = \frac{\text{Amount of substance}}{\text{Area} \times \text{Time}}$$

Equation 7.1

$$= \frac{5 \text{ mole}}{0.1 \text{ cm}^2 \times 2 \text{ sec}} = 25 \text{ mole cm}^{-2} \text{ s}^{-1}$$

- 2) Flux = (Permeability) × (Concentration difference)

$$\text{Permeability} = \frac{\text{Flux}}{\text{conc. difference}}$$

$$\text{Flux} = 10^{-5} \text{ mole cm}^{-2} \text{ s}^{-1}$$

$$\text{Conc. difference} = 0.1 \text{ mole litre}^{-1} = \frac{0.1 \text{ mole}}{1000 \text{ cm}^3}$$

$$= 10^{-4} \text{ mole cm}^{-3}$$

$$\text{Permeability} = \frac{10^{-5} \text{ mole cm}^{-2} \text{ s}^{-1}}{10^{-4} \text{ mole cm}^{-3}}$$

$$= 10^{-1} \text{ cm s}^{-1}$$

- 3) d

- 4) -a) Yes, each chamber is in a state of electrical neutrality. It is because chamber A contains K^+ and Cl^- in equal concentrations, 100 mM each, chamber B contains K^+ and Cl^- in equal concentrations of 10 mM each.

- b) As the diffusion starts, it is only the Cl^- that moves from chamber A to chamber B. Consequently, the concentration of Cl^- in chamber A becomes less than 100 mM although the concentration of K^+ in A remains at 100 mM. Hence the electrical

neutrality in A is disturbed. In chamber B the concentration of Cl^- becomes more than 10 mM, while the concentration of K^+ remains at 10 mM. So the electrical neutrality is also disturbed in chamber B.

Terminal Questions

- 1) AlCl_3 ionises almost completely to



Thus the total concentration of ions is 4 molar

Osmotic pressure $\pi = CRT$

$$= (4 \text{ mole litre}^{-1}) (8.314 \text{ JK}^{-1} \text{ mole}^{-1}) (300 \text{ K})$$

$$= 9976.8 \text{ J litre}^{-1}$$

- 2) Diffusion coefficient increases with the decrease in molecular weight and flux increases with the increase in diffusion coefficient (Fick's first law). So the urea will diffuse faster than the sucrose.

- 3) a) $J_s = P \Delta C$

$$P = 1.5 \text{ sec}^{-1}$$

$$\text{when } \Delta C = 1 \text{ mM, } J_s = 1.5 \times 1 = 1.5 \text{ mM s}^{-1}$$

$$\Delta C = 2 \text{ mM, } J_s = 1.5 \times 2 = 3.0 \text{ mM s}^{-1}$$

$$\Delta C = 3 \text{ mM, } J_s = 1.5 \times 3 = 4.5 \text{ mM s}^{-1}$$

$$\Delta C = 4 \text{ mM, } J_s = 1.5 \times 4 = 6.0 \text{ mM s}^{-1}$$

- b) The graph is a straight line, i.e., the relationship between flux and concentration difference is linear.

UNIT 8 TRANSPORT MOLECULES AND PROTON PUMPS

Structure

- 8.1 Introduction
 - Objectives
- 8.2 Transport Molecules
 - Transport Molecules in Facilitated Diffusion
 - Transport Molecules in Active Transport
 - Transport Molecule Inhibitors
- 8.3 Ion Gradient and Energy Exchange
- 8.4 Proton Pumps
 - Proton Pump in Oxidative Phosphorylation
 - Proton Pump in Photosynthesis
 - Proton Pump in Bacteria
- 8.5 Summary
- 8.6 Terminal Questions
- 8.7 Answers

8.1 INTRODUCTION

You have already studied about various processes of transport across the membrane such as diffusion, osmosis and active transport in the previous unit. As you know, a membrane allows only non-polar and lipid soluble substances to diffuse freely, but it is selectively permeable to other substances such as ions, sugars, amino acids etc. This selective permeability of the membrane is mediated by specific membrane proteins called 'transport molecules'.

The nature and types of transport molecules will be discussed in this unit. Transport molecules are highly specific and increase the permeability of the membrane to various substances. Later in the unit, you will study about the transport molecules which act as pumps such as sodium-potassium pumps and proton pumps. Pumps are the transport molecules which transport the solute against their concentration gradient. The sodium-potassium pump co-transport two different molecules across the membrane. The proton pump creates an electrochemical gradient across the membrane and the gradient energy is coupled to ATP synthesis.

Before you read this unit, please recall the processes involved in transport and the concentration gradient across the membrane which have been dealt with in Unit 7.

Objectives

After reading this unit you should be able to:

- describe the various types of transport molecules such as channels, carriers, ionophores, ion gates, and their modes of functioning,
- explain the sodium gradient across the membrane for active transport of sugars and amino acids,
- explain the chemiosmotic theory of coupling,
- describe the formation of proton gradient caused by electron transfer in mitochondria, chloroplasts and bacteria.

8.2 TRANSPORT MOLECULES

Cells have evolved the means to transport molecules, macromolecules and even larger particles across their plasma membrane. You have already read about various transport

processes such as osmosis, simple diffusion, facilitated diffusion and active transport in Unit 7. Lipid soluble, non-polar substances such as fatty acids and gases, diffuse rapidly through the lipid bilayer. Polar molecules, such as sugars, amino acids and many cell metabolites permeate rapidly through the cell membrane in comparison to their slow diffusion across the artificial lipid bilayers. It is now known that specific transporting membrane proteins called **transport molecules**, mediate the movements of such solutes across the cell membranes, making them move faster. Each of these transport molecules transfers specific molecular species of the solute across the membrane. One would like to know, what these molecules are, how do they function, and why they are specific. We will discuss these aspects of transport molecules in this section. It is to be noted that this subject is still under active research, and all the necessary information is not yet known for most of the transport molecules.

Most of the transport molecules are **transmembrane proteins** and are either one protein molecule or a part of the larger protein structures that run across the bilayer. These transport molecules occur in all types of biological membranes and their function are explained by the three model systems comprising pores, mobile carriers and fixed carriers.

Pore Model : One or more than one protein molecule located in the membrane forms a channel through which the molecules to be transported are passed. Ions and water molecules move across the membrane by pore mechanism down their concentration gradient (Fig. 8.1 a).

Mobile Carrier Model : Transport molecules move within the membrane carrying molecules from one side of the membrane to the other in the direction of the concentration gradient. A transport molecule contains binding sites for binding specific molecules to be transported (Fig. 8.1 b). It is believed that these molecules facilitate the transport of sugars.

Fixed Carrier Model : The transport molecule is not mobile and stretches across the lipid bilayer. When the molecule to be transported binds to the transport molecule, a reversible conformational change takes place in the transport molecule. This change occurs in such a way that the bound molecule reaches the other side of the membrane and is released in the medium (Fig. 8.1).

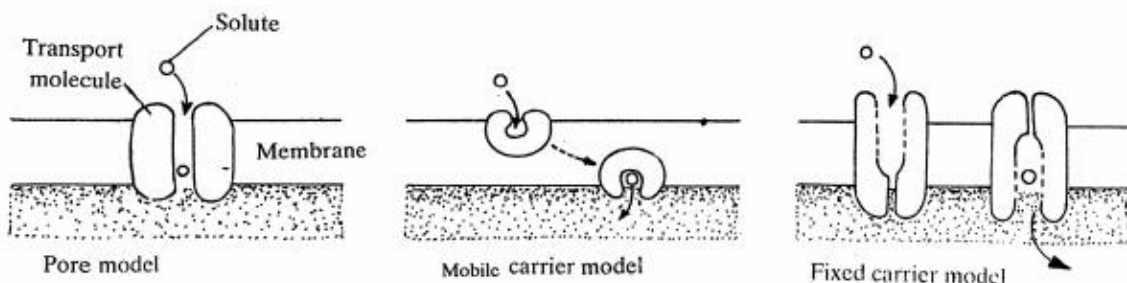


Fig. 8.1: Schematic diagram of three different models showing different of action of transport molecules.

Recent studies on membrane proteins have shown that flip flop movements of the membrane proteins across the lipid bilayer is infrequent and does not account for the observed rates of transport. That is why fixed carrier molecules (transmembrane proteins) are more probable carrier transport models.

Some transport molecules transport only one solute from one side of the membrane to the other. These transport molecules are known as **uniports**. In others, the transport of one kind of solute depends on the simultaneous transport of another kind of solute. When the flow of the solutes is in the same direction, the transport molecules are known as **symports**. If the transport of solutes is in the opposite direction, the transport molecules are known as **antiports**. Symports and antiports together form the **co-transport system** (Fig. 8.2)

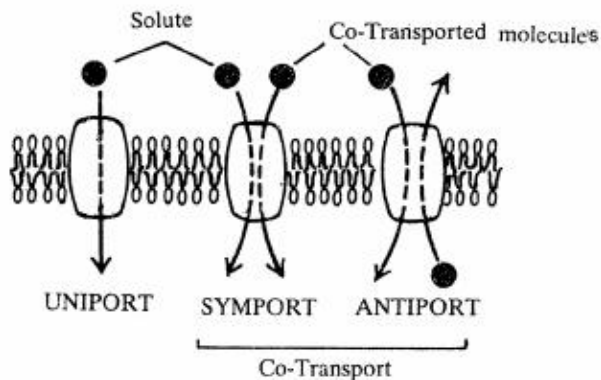


Fig. 8.2: Schematic diagram of transport molecules functioning as uniport, symport and antiport systems. Symport and antiport systems are called co-transport systems.

As mentioned earlier, all transport molecules known so far are proteins. A transport molecule may contain one subunit (one polypeptide chain) or more subunits. In general, the structure of the transport protein is such that the hydrophobic amino acid side chains are located mostly on the outer surface of the protein molecule so that it has no difficulty in getting into or staying within the hydrophobic interior of lipid bilayer. Various experiments conducted in the past indicate that some of these transport proteins form aqueous channels which permit solutes of appropriate size and charge to cross the membrane by simple diffusion. These transport molecules are called **channel proteins** and function on the basis of the pore model. Some other transport proteins, known as **carrier proteins**, on the other hand specifically bind a solute and undergo reversible changes in their conformation while transporting the solutes (Fig. 8.3). These transport proteins function on the basis of the mobile carrier model and fixed carrier model. Some specific amino acids located at a certain region of these carrier proteins form a specific site which can bind only specific solutes. This region of the transport protein is generally known as the **active site** or the **binding site**. These binding sites can be blocked by inhibitors. You will read about these later in the unit.

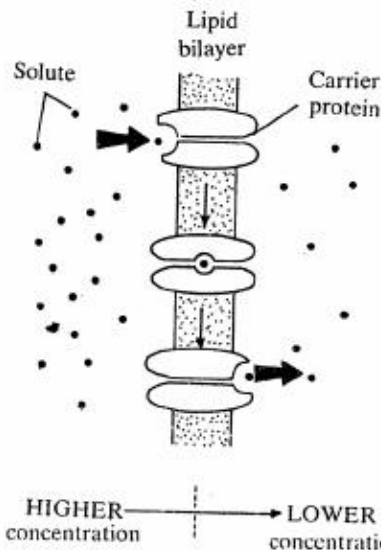


Fig. 8.3: Schematic diagram showing the carrier transport protein which undergoes conformational changes while transporting a solute across the membrane. The solute binds at the active site of the carrier protein and is transported by facilitated diffusion along the concentration gradient. After the molecule is transported the carrier protein resumes its original shape.

A particular amino acid in the binding site, or very close to the binding site, may play a crucial role in determining the specificity of binding. In that case, if that particular amino acid is removed or replaced by some other amino acid, the binding capacity may be lost. A defective transport protein of this type may be formed because of genetic disorder, and if this happens the normal transport process of a specific metabolite will be affected. Quite a few

inherited transport disorders have been detected in human beings. Several such disorders occur in bacteria through mutation.

Try the following SAQ to see if you have understood the general nature of the transport molecule.

SAQ 1

Differentiate between a channel protein and a carrier protein. Answer briefly in the space given below

.....

.....

.....

.....

8.2.1 Transport Molecules in Facilitated Diffusion

You have already learnt that facilitated diffusion is mediated by transport molecules; channels and carriers, which allow specific solutes to move across the membrane by passive transport. The driving force for the transport is the concentration gradient for uncharged molecules and electrochemical gradient for charged molecules. Simple diffusion of the solutes across the membrane does not need any type of transport molecule. Some examples of facilitated diffusion are: transport of glucose across RBC membrane, intestinal epithelial cell membrane, skeletal and cardiac muscle cell membranes and transport of CO_2 across RBC membrane. The details of how these transport proteins function at the molecular level are still unknown.

Let us discuss here one example of a channel protein that transports CO_2 across the RBC membrane by facilitated diffusion. The protein responsible for this transport is known as band-III protein (Fig. 8.4). The molecular weight of this protein is about 100,000 daltons and it contains about 800 amino acids. It has two subunits which are exposed on both sides of the membrane. From electron microscopic observation, its diameter has been estimated to be 8 nm. Each human RBC contains about one million band-III proteins.

Dalton is the unit of weight and is approximately equal to the weight of one hydrogen atom.

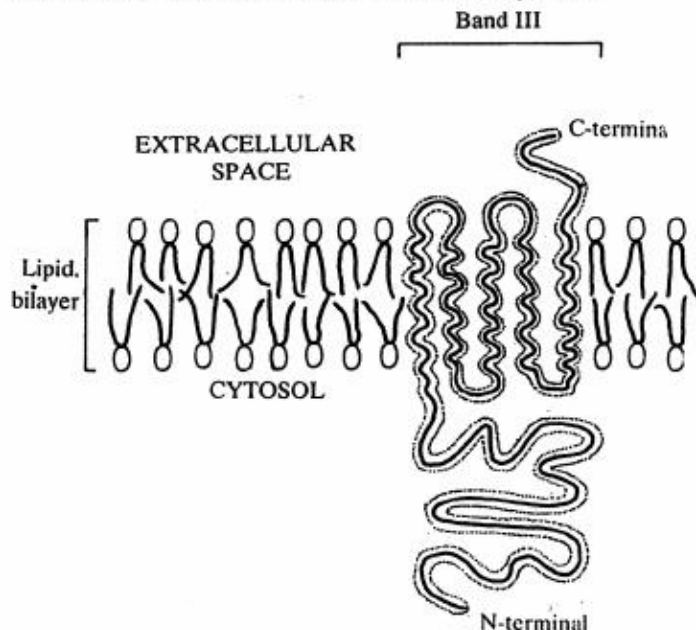


Fig. 8.4; Schematic diagram of band-III protein in the human RBC membrane. It is still unknown how exactly band-III protein is arranged in the membrane, but there is evidence that it crosses the membrane several times.

As you know the main function of red blood cells is to carry O_2 from the lungs to tissues and CO_2 from tissues to the lungs. Inside the RBC, CO_2 is converted into HCO_3^- which remains in the dissolved form. The anion HCO_3^- is at a higher concentration inside the RBC than outside, when it carries HCO_3^- from the tissues to the lungs. While the RBC

Cell Membranes and Enzymes

The gates of transport proteins open only in response to a particular stimulus such as: changes in the concentration of specific ions, ligand bindings, cell surface receptors, membrane potential etc. The gates remain closed otherwise.

moves through the lungs, band-III protein transports HCO_3^- to the outer medium through its aqueous channel by opening the 'gates' at the ends of the channel. The transport of each HCO_3^- ion is coupled with the transport of one chloride ion (Cl^-) in the opposite direction. Thus, it is an antiport system. The transport of Cl^- is also passive, because it moves down the concentration gradient; its concentration is higher outside the RBC than inside, when the RBC is in the lungs. Thus, the band-III protein creates an aqueous channel of the right size and charge to allow HCO_3^- and Cl^- ions to pass down their respective concentration gradients across the membrane. The exact nature and function of the gate is not yet certain. It is proposed that there is a small segment of the protein located at the opening of the channel which contains positive charge and acts as the 'lid' or 'gate'. When an anion (HCO_3^- or Cl^-) binds with the positive charge, the segment is displaced slightly and the 'gate' opens. Thus, it requires higher concentrations of anions (HCO_3^- and Cl^-) to open the gate. The anion binding site on the segment of band-III protein is specific for HCO_3^- and Cl^- , so that other anions cannot bind with it (Fig. 8.5). Experiments have shown that the anion binding site of band-III protein can either be on the inner cytoplasmic side or outer side of the membrane, but not on both sides simultaneously.

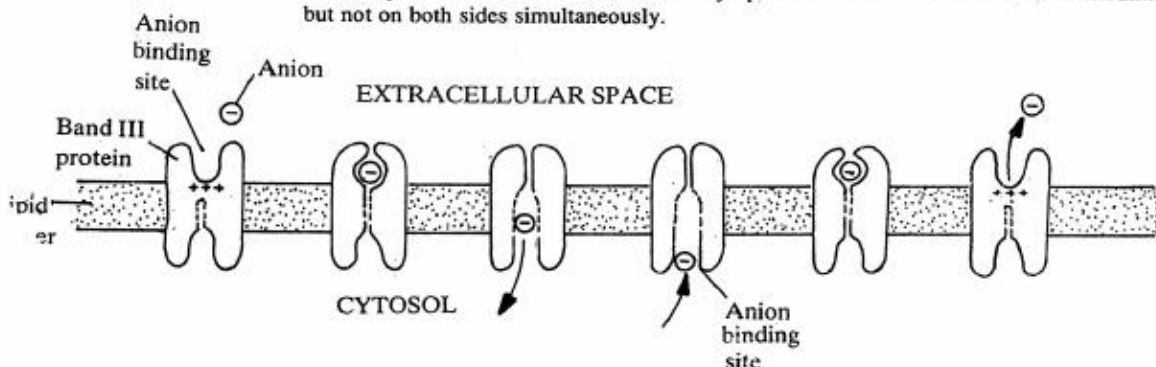


Fig. 8.5: Schematic diagram of the conformation of band-III protein during the exchange of ions across the membrane. When an anion binds at the outer face, the conformation of the protein changes and the channel opens for the anion to move across and anion binding site is created at the cytoplasmic surface (1-3). This causes another anion to bind on this site leading to the formation of original conformation which is the passage of second anion in the opposite direction. It recreates the binding site on the outer surface (4-6).

The other types of transport molecules involved in facilitated diffusion by passive transport are **ionophores** which transport the ions. We will discuss ionophores in the following text.

Ionophores are small hydrophobic protein molecules which dissolve in the lipid bilayer and increase the ion permeability of the bilayer. Most of them are synthesised by certain micro-organisms, such as bacteria and fungi, and are usually released into the surrounding medium to kill other species of bacteria and fungi. Some of the ionophores are used as antibiotics. The outside surface of the ionophores is hydrophobic, so that these can easily stay or move in the membrane environment. Ionophores are of two types—**mobile ion carriers** and **channel formers**. In both types, the ionophores shield the charge of the ion from the hydrophobic environment so that it can penetrate the membrane. Since these transport processes are not energy-dependent the net movement of ion is carried out by electrochemical gradient.

Valinomycin is an example of a mobile ion carrier ionophore which increases the permeability of a membrane to K^+ . It is a ring shaped polymer with six carbonyl groups ($\text{C}=\text{O}$) in the polar interior of the molecule. The six negatively charged oxygen atoms at the centre of the ring hold a single K^+ ion (Fig. 8.6).

The outside surface of valinomycin is hydrophobic because of the side chain of the amino acid valine. Valinomycin transports K^+ down its **electrochemical gradient** by picking up a K^+ on one side of the membrane, diffusing across the bilayer, and releasing K^+ on the other side. The transport of ionophore is temperature dependent. It is inhibited if the temperature of membrane is lowered below its freezing point.

Gramicidin is an example of a channel forming ionophore. It is an open-chain peptide of 15 amino acids all of which have hydrophobic side chains. From various experiments it appears that two gramicidin molecules associate to form a transmembrane channel which allows the

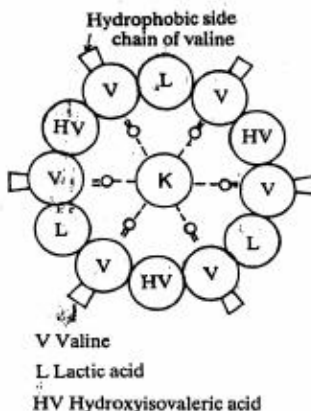


Fig. 8.6: Valinomycin molecule with a K^+ ion bound by six oxygen atoms at the centre of the ring structure.

monovalent cations to flow down their electrochemical gradients (Fig. 8.7). This channel is constantly formed and gets broken down.

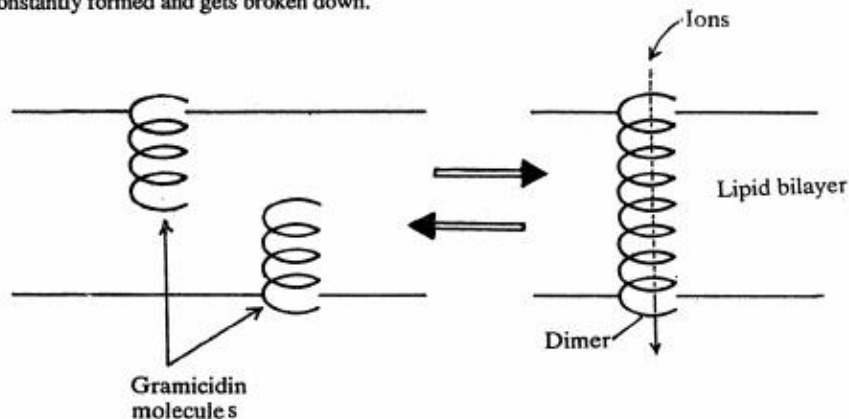


Fig. 8.7: Schematic diagram of two gramicidin molecules. The molecules are believed to diffuse and form a dimer in the membrane to form a channel for transport of ions.

Before proceeding further try the following SAQ.

SAQ 2

Tick mark (✓) the correct statements and (x) the incorrect statements in the given space

- i) Band-III protein transports HCO_3^- along with the symport of Cl^- []
- ii) Gates of the transport molecules open transiently in response to a stimulus []
- iii) Ionophores are hydrophobic, some of them are used as antibiotics []
- iv) Gramicidin is a channel forming mobile ionophore []

Till now, you have studied about the transport molecules in facilitated diffusion. We will now discuss transport molecules in active transport. You have already read about the process of active transport in Unit 7

8.2.2 Transport Molecules in Active Transport

In active transport the solute is transported across the membrane against the concentration gradient, i.e., from lower concentration region to the higher concentration region. And for this kind of transport, energy is required. Therefore, the transport molecule for active transport has an additional function of coupling the energy source with the transport process. The source of energy may be direct, i.e., by hydrolysis of ATP, or indirect, i.e., energy stored in the form of an ion gradient across the membrane. Here we will discuss one example of each type.

Active transport by sodium potassium pump

The best known example in which ATP hydrolysis is the source of energy for active transport is the **sodium potassium pump** (Na^+/K^+ pump). It should be borne in mind that when we use the word 'pump', it implies that the transport of the solute occurs against its concentration gradient. This transport system pumps Na^+ out of the cell, and K^+ into the cell. So it is an antiport system. Since Na^+ is exchanged for K^+ , this pump is called **Na^+/K^+ exchange pump**. The pump is largely responsible for generating the electrical potential across the membrane. The transporting molecule for this pump is known as **Na^+/K^+ ATPase**, which is an enzyme that hydrolyses ATP in the presence of Na^+ and K^+ to release energy.

Na^+/K^+ ATPase is an **integral membrane protein** that has been isolated and purified. Integral proteins have been discussed in Unit 6. Electron microscopic examination shows that it is a globular protein having a diameter of about 10 nm. This indicates that it runs across whole of the thickness of the membrane. The molecule is probably a tetramer having two similar, large subunits that are catalytic in nature and two smaller subunits whose function is not known.

In each red blood cell there are about 5,000 Na^+/K^+ ATPase molecules and each ATPase molecule can transport 20 Na^+ ions in one second. For every molecule of ATP hydrolysed,

three Na^+ ions are pumped out and two K^+ ions are pumped in as shown in Fig. 8.8. It is worth remembering that ATP is hydrolysed only on the inside of the cell, not outside.

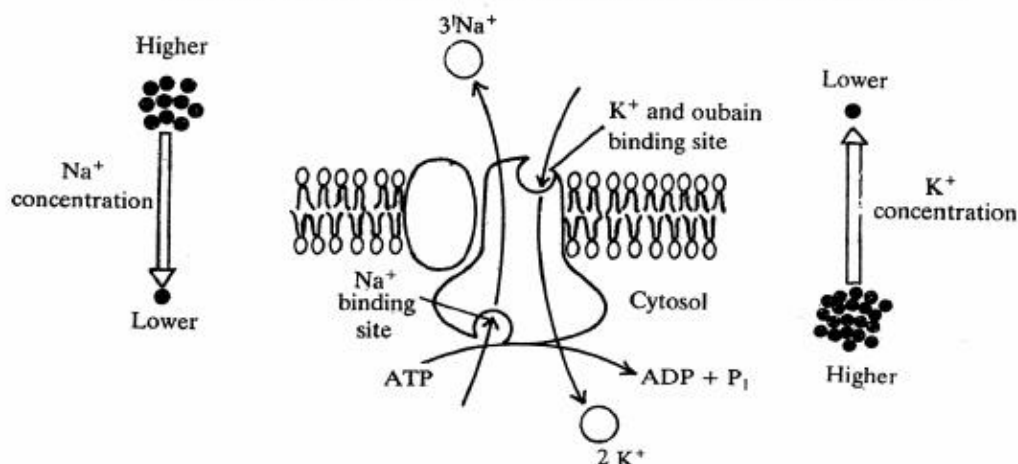


Fig.8.8: Schematic diagram of $\text{Na}^+ \text{K}^+$ ATPase actively pumping Na^+ out and K^+ into the cell against their concentration gradients. For every molecule of ATP hydrolysed, 3 Na^+ are pumped out and 2 K^+ are pumped in the cell.

Active transport by ion gradient

We have mentioned earlier that the energy required for many active transport systems can also be obtained indirectly from energy stored in the form of ion gradients. Active transport of some sugars and amino acids into animal cells is driven by the energy obtained from steep differences in Na^+ or H^+ ion concentration across the plasma membrane. All the transport molecules involved in this process function as co-transport systems; some as symports, others as antiports. Glucose absorption in the intestinal cells from the lumen of the intestine is a specific example of such a process. The glucose carrier transport molecule located in the apical membrane of the microvilli of the intestinal epithelial cells binds both glucose and Na^+ on different binding sites and transports them inside the cell. Since Na^+ moves down the electrochemical gradient, it drags glucose from the lumen to the inside of the cell even though the glucose concentration inside the cell may be higher than that in the lumen of the intestine. Na^+ ion concentration in the intestinal lumen is higher than that in the cell. This system of glucose uptake is a symport system. The greater the Na^+ gradient, the greater is the active transport of glucose into the cells. To maintain a steep Na^+ gradient, the $\text{Na}^+ \text{K}^+$ glucose symport is dependent on gradients generated by $\text{Na}^+ \text{K}^+$ pump (Fig. 8.9a).

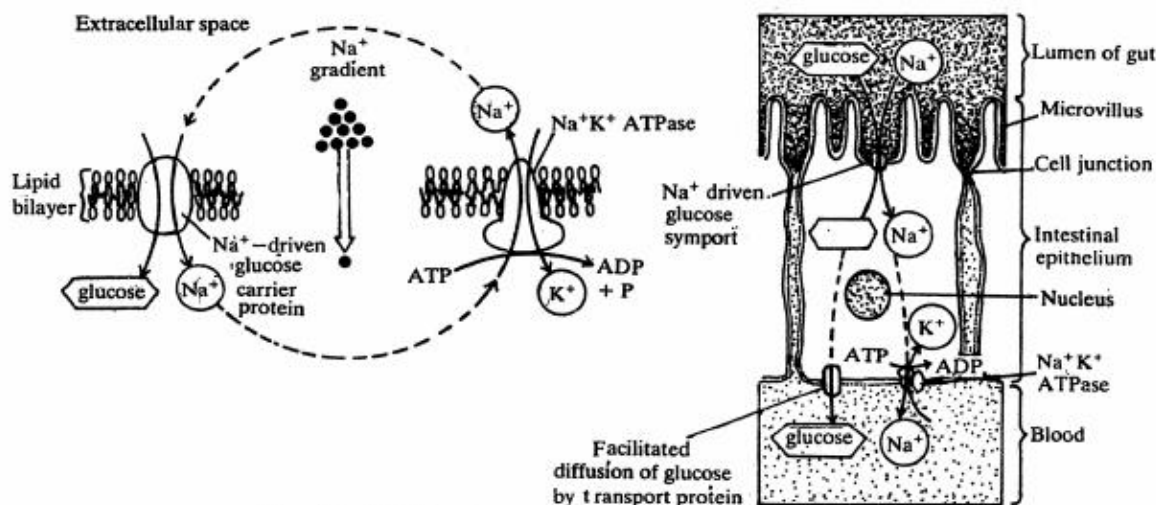


Fig. 8.9: a) The active transport of glucose is driven by a Na^+ gradient generated and maintained by a $\text{Na}^+ \text{K}^+$ ATPase pump.
b) Schematic diagram of asymmetrical distribution of transport molecules in intestinal epithelial cells. The apical membrane of the cell contains a Na^+ -linked glucose transport system. Glucose is transported out of the cell by facilitated diffusion. $\text{Na}^+ \text{K}^+$ ATPase in the basal membrane keeps the internal concentration of Na^+ low.

Different types of transport systems are distributed asymmetrically in the plasma membrane of the epithelial cells (Fig. 8.9b). The Na^+ linked glucose transport molecules are located on the lumen side of plasma membrane of intestinal epithelial cells, while $\text{Na}^+ \text{K}^+$ ATPase molecules are located on the basal and lateral surfaces of the plasma membrane. This ensures that the intracellular glucose concentration is greater than that in the blood stream, while the intracellular Na^+ concentration remains low as the excess of Na^+ is pumped out by $\text{Na}^+ \text{K}^+$ ATPase. Similar mechanisms for dissipation of ion gradient are used by various cells to transport other sugars as well as amino acids.

8.2.3 Transport Molecule Inhibitors

There are many compounds that inhibit facilitated diffusion and active transport processes by binding to the active sites of the carrier transport molecules. These are called transport molecule inhibitors. They are valuable research tools used to examine the mechanism of action of transport molecules. Many types of compounds can act as transport molecule inhibitors, for example, ouabain inhibits the $\text{Na}^+ \text{K}^+$ ATPase only if it is present on the outer side of the cell as it binds to the active site of the transport molecule located on that segment of $\text{Na}^+ \text{K}^+$ ATPase which is exposed to the outer surface of the membrane (Fig. 8.10).

One important use of inhibitors is to estimate the number of transport molecules by using radioactively labelled inhibitor molecules. For example, one radioactively labelled ouabain molecule will bind to one $\text{Na}^+ \text{K}^+$ ATPase molecule. Some of the transport molecules and their inhibitor compounds are given in Table 8.1.

Table 8.1: Some of the Transport Molecules, Their Cell Types and Their Inhibitors

Substance transported	Transport molecule and cell type	Inhibitors and their effect
Na^+	$\text{Na}^+ \text{K}^+$ ATPase in nerve cells	Ouabain inhibits $\text{Na}^+ \text{K}^+$ ATPase
Na^+	ion channels in frog skin	amiloride inhibits Na^+ channel
Glucose	epithelial cell from the intestine	phlorizin inhibits Na^+ dependent glucose uptake
Glucose	human red blood cell	phloretin inhibits Na^+ independent glucose uptake

SAQ 3

Fill in the blanks with appropriate words

- $\text{Na}^+ \text{K}^+$ ATPase functions assystem
- Energy is required for the transport.....the concentration gradient
- Na^+ linked glucose transport ison $\text{Na}^+ \text{K}^+$ pump
-proteins are responsible for Na^+ glucose symport
- The number of transport molecules can be estimated with the help of

Transport Molecules and Proton Pumps

Microvilli are thin finger like projections of the plasma membrane of animal epithelial cells, that increase the surface area for absorptive purposes

Lumen is the space within a tubular structure, or in an organelle. In mitochondrion it is called matrix space, and stroma in chloroplast

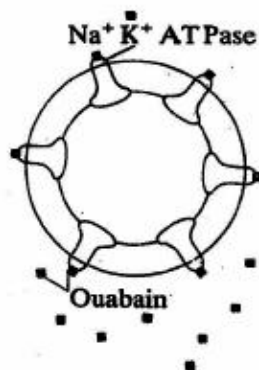


Fig. 8.10: Schematic diagram showing that each $\text{Na}^+ \text{K}^+$ ATPase transport molecule binds one ouabain molecule. The binding site is on the outer side of the cell.

8.3 ION GRADIENT AND ENERGY EXCHANGE

Although ATP is the main source of cellular energy for performing different biological functions, it is not the only source. An important alternative source exists in the form of an ion gradient across the membrane. You have read in the previous section that energy from ATP hydrolysis is used by $\text{Na}^+ \text{K}^+$ ATPase to pump Na^+ and K^+ ions against their electrochemical gradients, which further generate steep Na^+ gradient across the membrane. The steep electrochemical gradient is used for the Na^+ dependent active transport of sugars and amino acids into the cell. Thus in this process, energy stored in the chemical bonds, i.e., ATP is transformed into energy stored in the form of an ion gradient. The process of

conversion of one form of energy into another is known as **energy transduction**. As you have already read, the membrane transport system plays an important role in biological energy transduction.

In an energy transduction process, energy is exchanged between two systems or reactions: one is the reaction that gives energy, i.e. energy releasing reaction, and the other is the energy requiring reaction which consumes the energy produced by the energy releasing reactions. The **coupling of these two reactions** is extremely crucial in energy transduction. In most cases of energy transduction, an intermediate energy exchange system serves the purpose of coupling the energy releasing process and the energy requiring process. Generation and dissipation of ion gradients play the role of coupling in many biological processes. Muscle contraction, transmission of nerve impulse, ATP formation in respiration and in photosynthesis, flagellar movement in bacteria, are some of the examples. In the following section we will discuss about the energy transduction systems such as proton pumps in mitochondrial respiration, chloroplast photosynthesis, and in bacteria.

SAQ 4

In the space given below briefly explain what understand by energy transduction.

.....

.....

.....

.....

8.4 PROTON PUMPS

Although most ion transport systems in plants and animals are driven by Na^+ pump. Other pumps such as Ca^{2+} pump and H^+ pump replace Na^+ pump in many other systems. The transport of protons across the membrane is ATP-dependent and occurs in many cellular systems, for example in mitochondria, chloroplasts, lysosomes, mammalian stomach and certain bacteria. The proton pump, i.e., H^+ pump was developed in early procaryotes to expel the H^+ ions from the cell so as to maintain the internal pH.

Respiration in eucaryotic cells takes place in mitochondria whereas in procaryotic cells, such as bacteria, it occurs in the plasma membrane. During respiration ATP is synthesised by utilising the energy released by the oxidation of glucose or other metabolites. In chloroplasts, light energy is used to drive a series of oxidation-reduction reactions. In certain steps of these reactions liberated energy is coupled to synthesise ATP. The fundamental question arises as to how these two reactions, i.e., oxidation and ATP synthesis, are coupled.

Chemiosmotic theory postulates that an electrochemical proton gradient is formed across the mitochondrial inner membrane or the chloroplast thylakoid membrane during electron transport. The energy stored in this gradient is used to drive ATP synthesis during oxidative phosphorylation in mitochondria or photophosphorylation in chloroplasts.

The mechanism of coupling puzzled scientists for a long time. In 1960, Peter Mitchell, a British biochemist proposed the **chemiosmotic theory**, according to which energy is obtained from the oxidation of NADH or glucose or other organic molecules. This energy is used to transport the H^+ (proton) to the exterior medium through specific transport molecule ATPase also called **coupling factor** which is located in the membrane. The accumulation of protons outside the membrane results in the formation of a proton gradient across the membrane such as the inner membranes in mitochondria and chloroplasts and plasma membrane in bacteria. This proton gradient across the membrane causes the protons to move down their concentration gradient through a channel formed by enzyme ATPase molecule which thus acts as a **reversible coupling factor**. The free energy is released as H^+ passes into the zone of lower H^+ concentration and is utilised to make ATP from ADP and phosphate.

The proton gradient has two components: one is a chemical potential caused by the concentration difference of protons, i.e., pH difference between the inside and outside of the membrane, and the other is the electrical potential difference which arises due to the ionic nature of the proton. Mitchell proposed that the energy required to form ATP is derived from this electrochemical potential across the membrane formed due to the proton pump (Fig. 8.11).

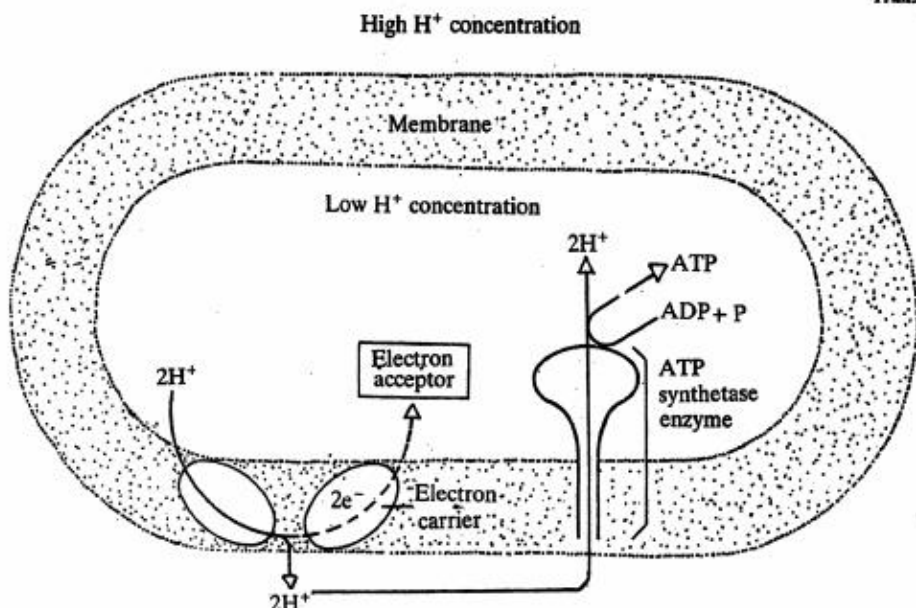


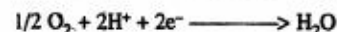
Fig. 8.11: Principles of chemiosmotic theory. The protons are translocated from inside to outside creating the electrochemical potential across the membrane. The energy obtained by the proton flow along the concentration gradient is used for ATP synthesis.

8.4.1 Proton Pump in Oxidative Phosphorylation

Oxidative phosphorylation occurs in mitochondria and enables aerobic organisms to capture a greater amount of free energy for ATP synthesis by the oxidation of organic substances, i.e., carbohydrates, fats and amino acids. This energy is obtained by a mechanism coupled with electron flow from organic substrates to oxygen along the respiratory chain, i.e., **electron transport chain** in the inner mitochondrial membrane. You have already studied the structure of mitochondria in Block 1. In oxidative phosphorylation the energy releasing reaction is the oxidation of NADH. In this process NADH is oxidized by liberating protons (H^+) and electrons (e^-), while oxygen is reduced to water by accepting protons and electrons.

Electron transport chain : A group of electron transport molecules that transfer electrons from a donor to an acceptor located in membranes.

The reactions can be written in the following way:



The electrons and protons of NADH do not react **directly** with oxygen but are carried to oxygen by many other intermediate compounds step by step from more electronegative components to more electropositive components resulting in the formation of water. These compounds are NADH dehydrogenase enzyme system, iron sulphur proteins, i.e., flavoproteins, ubiquinone, cytochrome b, cytochrome c and cytochrome a_1 , a_3 . The sequence in which electron transfer occurs through the molecules of electron transport chain is shown in Fig. 8.12. However, the details of this process are still unclear.

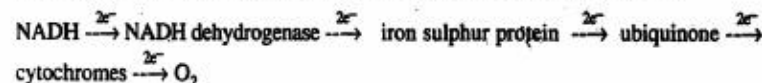


Fig. 8.12: Sequential electron transfer in oxidative phosphorylation.

You are not expected to memorise the names of all these compounds. These electron carriers are located in the inner membrane enclosing the matrix of the mitochondria. In the matrix, pyruvate is oxidized through TCA (Tricarboxylic acid) cycle producing NADH, which is a substrate for oxidative phosphorylation. You will learn more about the processes involved in oxidative phosphorylation in Block 3.

According to the chemiosmotic theory, the sequential transfer of electrons through electron carriers is accompanied by the expulsion of H^+ from the mitochondrial matrix to the

intermembrane space, i.e., space between two membranes of mitochondria. For every pair of electrons six H^+ are transported across the inner membrane. The net effect is the formation of H^+ gradient in the intermembrane space due to the oxidation of NADH which is coupled to the phosphorylation of ADP. The coupling factor involved in ATP synthesis in mitochondria is $F_0 F_1$ ATPase complex (Fig. 8.13).

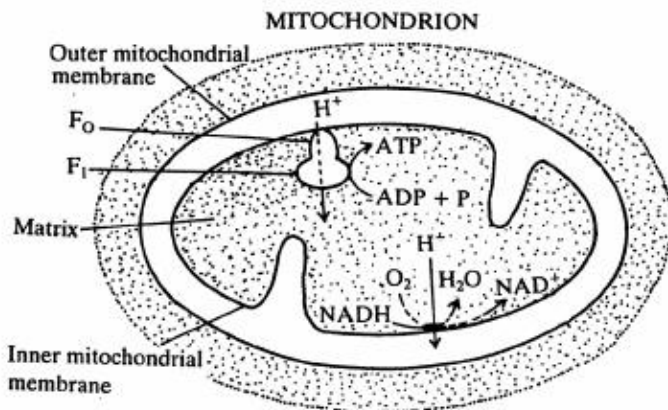
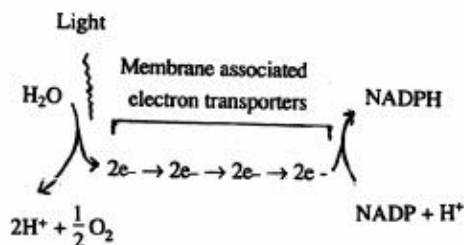


Fig. 8.13: Schematic diagram showing the proton pump in the mitochondrial inner membrane. The electrochemical gradient is formed by the translocation of H^+ across the membrane.

8.4.2 Proton Pump in Photosynthesis

In Block 1, you have read about the structure of chloroplasts which absorb light energy for photosynthesis. The absorption of light by photosynthetic pigment i.e., chlorophyll induces photosynthetic reactions resulting in the formation of ATP and NADPH which are used for production of glucose by fixing CO_2 in the cell. The formation of ATP in this manner is called photophosphorylation. No ATP formation takes place if the thylakoid sac is ruptured, but other reactions occur normally. In photosynthesis electron flow from H_2O to NADP in contrast to the mitochondrial electron transfer process where electrons flow from NADH or NADPH to oxygen. The absorbed light energy helps water molecules to break down into protons, electrons and oxygen. The electrons are transferred by a series of carriers known as electron transport chain to NADP forming the reduced NADPH molecule. The reactions can be written as :



The electron carriers include plastoquinone, cytochrome, plastocyanin, chlorophyll and iron sulphur proteins. As in the case of mitochondria, movement of electrons through carriers is coupled to the pumping of the protons (H^+) across the thylakoid membrane. However, unlike in mitochondria, in chloroplasts proton is transported from outside to the inside of the thylakoid space resulting in an accumulation of protons creating an electrochemical gradient across the thylakoid membrane (see Fig. 8.14). This electrochemical potential causes the protons to move outside the thylakoid sacs along the concentration gradient. The energy derived by this process is utilised in ATP synthesis. Thus, light energy is converted into chemical energy of proton gradient in plant cell.

As in the case of mitochondria, thylakoid membranes also contain transport molecules, called $CF_0 CF_1$ ATPase complex, which allow protons to move out of the lumen of thylakoid to stroma and utilize the energy to couple phosphorylation of ADP to form ATP (Fig. 8.14).

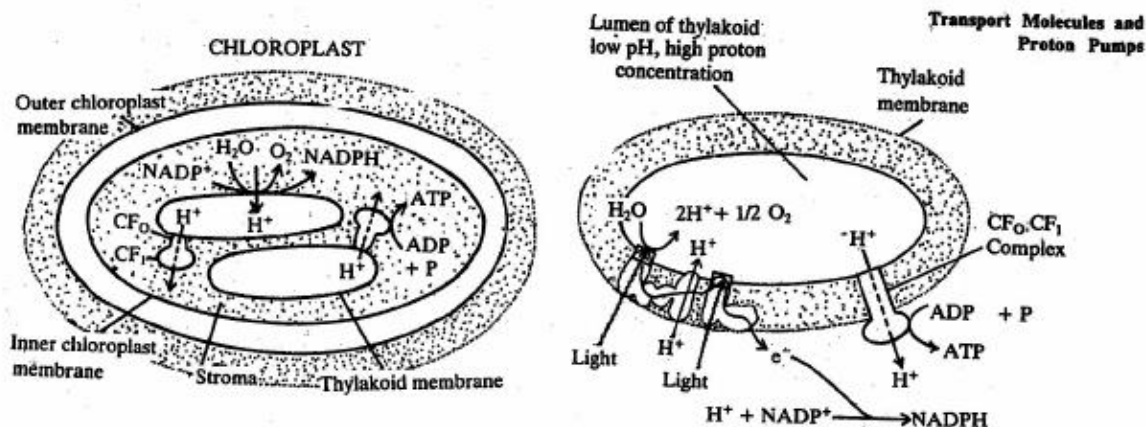


Fig. 8.14: Proton pump in chloroplasts. Movement of electrons is coupled with the pumping of protons from the exterior to the interior of thylakoid membrane. The movement of protons down their concentration gradient through CF_0CF_1 complex in the membrane is associated with ATP synthesis.

SAQ 5

Match the items in column I with those in column II

Column I		Column II
i) Peter Mitchell	[]	a) electrontransiestion H_2O NADP
ii) Oxidative phosphorylation	[]	b) chemiosmotic theory
iii) Chloroplasts	[]	c) lecttochemical potentia cross the membrane
iv) Proton gradient	[]	d) mitochondria

8.4.3 Proton Pump in Bacteria

Most bacteria are aerobic and synthesise ATP by oxidising nutrient molecule such as glucose, through the process of glycolysis and citric acid cycle. Plasma membrane of the aerobic bacteria consists of a respiratory chain similar to that of the mitochondrial inner membrane where oxygen is the final electron acceptor. In other bacteria, which are anaerobes, energy is derived by glycolysis alone and the final acceptor of electrons are molecules other than oxygen, such as nitrates, sulphates, sulphites, fumarates or carbonates. As in the case of mitochondria and chloroplast, the electron is transferred through carrier molecules such as cytochromes and iron-sulphur proteins, which are embedded in the plasma membrane of the aerobic bacteria.

In aerobic bacteria such as *E. coli* the electron transfer is coupled to the expulsion of proton to the outer side of the plasma membrane, thus forming the electrochemical potential across the membrane. The plasma membrane contains F_0F_1 - ATP synthetase molecules similar to those in mitochondria that allow the protons to move down the gradient from outside to inside the membrane and utilize the energy to synthesise ATP.

In anaerobic bacteria F_0F_1 - ATPase synthetase molecule works in reverse, i.e., it hydrolyses cytoplasmic ATP produced by glycolysis to generate energy. Since the anaerobic bacteria lack an electron transport chain, the energy released during ATP hydrolysis is coupled to export of protons from inside the cell through the F_0F_1 - ATPase synthetase. Pumping of proton forms the electrochemical gradient across the plasma membrane of the bacteria.

This electrochemical gradient is used by mitochondria and bacteria to pump other ions or molecules against their concentration gradient. In bacteria, due to the absence of Na^+K^+ Alease H^+ antiport systems are used to transport Na^+ and Ca^{++} out of the cell against a

Aerobic organisms have oxygen dependent metabolism and produce ATP by the oxidation of glucose or other food stuffs. In anaerobic organisms reactions occur in the absence of oxygen. Certain anaerobic bacteria such as salmonella species can cause poisoning in bottled or canned food.

concentration gradient. Bacteria also use the H^+ gradient to bring nutrients, such as amino acids, sugars, etc., inside the cells through H^+ symport systems (see Fig. 8.15).

a) AEROBIC CONDITIONS

(b) ANAEROBIC CONDITIONS

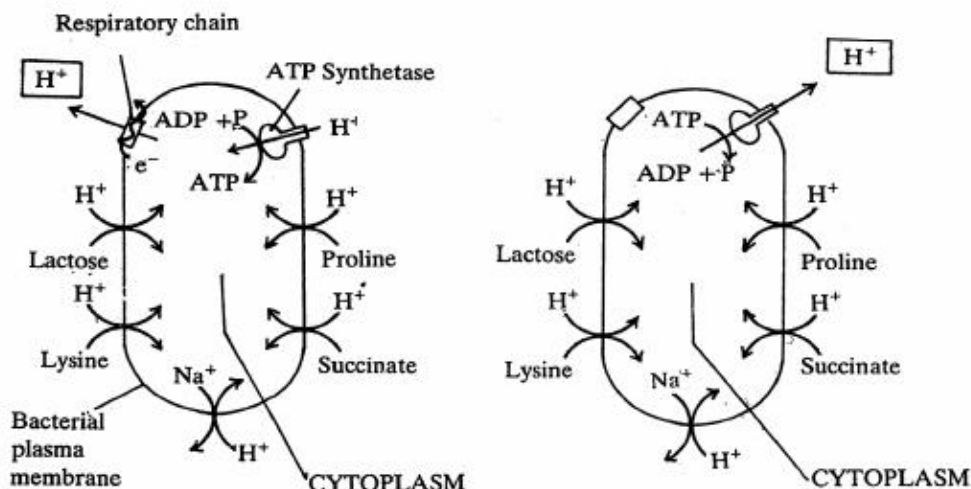


Fig. 8.15: Schematic diagram showing the formation of electrochemical potential in aerobic bacteria (a) and anaerobic bacteria (b). This proton gradient causes the antiport of Na^+ and Ca^{++} ions outside and symport of sugars and amino acids inside the bacterial cell. It pumps various nutrients into the cell and expels Na^+ out. The proton gradient is generated by the respiratory chain.

SAQ 6

Compare the formation of an electrochemical gradient in aerobic between with that in anaerobic bacteria

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With this we conclude our study of the major functions of membrane proteins, i.e., transport across the membranes. If you want to know more details about various aspects of transport processes, you may read some of the books listed at the end of this block

8.5 SUMMARY

The presence of specific transport molecules in the biological membranes make them permeable to different solutes which are otherwise insoluble in lipid bilayer. This phenomenon is known as mediated permeability. In this unit you have studied that:

- transport molecules are known to be proteins which are either small or large enough to span the whole thickness of the membrane. Transport molecules may be mobile or fixed in the membrane. In some cases it is only the specific solute that is transported, while in other cases the transport of a specific solute is associated with the transport of other solutes either in the same direction or in the opposite direction.
- $Na^+ K^+$ ATPase pumps Na^+ in one direction in exchange of K^+ in the other direction using energy from ATP hydrolysis.
- transport molecule inhibitors inhibit mediated permeability processes by binding to transport molecules. Ouabain inhibits $Na^+ K^+$ ATPase activity.
- electron transfer through electron carriers generates proton pumps in mitochondria, bacteria and chloroplasts, which are coupled to the proton transport across the membranes resulting in an electrochemical potential.

- the movement of H^+ down the concentration gradient through the coupling factor ATP synthetase leads to the formation of ATP. In anaerobic bacteria the role of ATP synthetase is reversed, i.e., it hydrolyses ATP to obtain energy

8.6 TERMINAL QUESTIONS

- Describe briefly the model systems for functioning of transport molecules.
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- Comment on the statement: Ionophores increase the ion permeability in the bilayer.
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- Explain briefly the role of the enzyme Na^+K^+ ATPase as a transport molecule.
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- Compare the proton pump in mitochondria with that in chloroplasts.
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8.7 ANSWERS

Self-assessment Questions

- Channel proteins form the aqueous channels for the transport of ions and function on the basis of the pore model. Carrier proteins bind the solutes and undergo reversible changes while transporting the solute.
- ii) and iii) are correct statements.
- i) antiport ii) against iii) dependent iv) carrier v) inhibitors.
- Energy transduction is the process of conversion of one form of energy into another. The energy flows from energy releasing reactions to energy requiring reaction. Membrane transport plays an important role in coupling these reactions.
- i) b, ii) d, iii) a, iv) c

- 6) In aerobic bacteria, the electron transfer is coupled to the proton transport across the plasma membrane. The electrochemical gradient thus formed is responsible for ATP synthesis. In aerobic bacteria, energy obtained by hydrolysis of ATP is responsible for the formation of electrochemical gradient across the membrane.

Terminal Questions

- 1) Transport molecules function on the basis of three model systems. 1) Pore model transport molecule forms the channel. 2) Carrier model transport molecules move within the membrane. 3) Fixed molecule model transport molecule runs across the bilayer and undergoes reversible conformational change.
- 2) Ionophores are the proteins that have hydrophobic exterior and dissolve in the lipid bilayer. They shield the charge of the ion from the hydrophobic environment of the bilayer so that the ions can easily move across the membrane.
- 3) $\text{Na}^+ \text{K}^+$ ATPase hydrolyses ATP to obtain energy. The enzyme uses this energy and acts as a $\text{Na}^+ \text{K}^+$ pump, pumping Na^+ out of the cell and K^+ into the cell against the concentration gradient.
- 4) Electron transfer in mitochondria occurs from NADH to H_2 whereas in chloroplasts it takes place from H_2O to NADP^+ . In mitochondria, the protons are pumped outside from the matrix whereas in chloroplast, the protons are pumped into the thylakoid space.

UNIT 9: ENZYMES : SPECIFICITY AND MECHANISM OF ACTION

Structure

- 9.1 Introduction
 - Objectives
 - 9.2 Characteristics of Enzymes
 - Activation Energy
 - Coupled Reaction
 - 9.3 Types of Enzymes.
 - 9.4 Cofactors
 - 9.5 Mechanism of Enzyme Action
 - Enzyme Specificity
 - Molecular Basis of Enzyme Action
 - 9.6 Factors Affecting the Rate of Enzyme Action
 - Effect of Temperature
 - Effect of pH
 - Effect of Enzyme Concentration
 - 9.7 Enzyme Kinetics
 - 9.8 Allosteric Enzymes
 - 9.9 Isoenzymes
 - 9.10 Inhibitors
 - 9.11 Assay of Enzyme Activity
 - 9.12 Summary
 - 9.13 Terminal Questions
 - 9.14 Answers
-

9.1 INTRODUCTION

You have studied about the structure of cell membranes and the processes of transport across these membranes in the previous units. Membrane proteins act as transporting molecules. The other important function of cellular proteins is to act as enzymes and catalyse biochemical reactions at a rate appropriate to the need of the cell.

In this unit you will study about the nature of enzymes and their functional aspects. Enzymes control the overall metabolic functions of the cell by speeding up the reaction rate. They do not affect the equilibrium constant and remain unaffected at the end of the reaction. The reactants bind to a specific site on the surface of enzyme molecule called active site. Enzymes show specificity for their substrate as well as for the reactions. Various factors such as temperature, pH, concentrations of enzymes and substrate affect the rate of enzyme-catalysed reactions. Some enzymes require additional factors for their normal activity. Allosteric enzymes have more than one active sites which may be located on the same subunit or on different subunits. Inhibitors are the substances that reduce the enzyme activity. Michaelis and Menten derived a mathematical equation to study the relationship between substrate concentration and reaction rate. The curve for enzyme kinetics showing the relationship between these two is a hyperbola. The kinetics of allosteric enzymes show a sigmoidal curve. The detection and estimation of enzymes can be done by using various techniques which can measure their ability to convert the substrate into products.

Various enzyme-catalysed reactions characterise the metabolism of the cell. Regulation of these reactions is achieved by altering the enzyme activity. In the next unit you will read about the regulation of enzyme activity. Before you read this unit, you should refresh your memory about the structure of proteins.

Objectives

After reading this unit you should be able to:

- describe the role of enzymes in lowering the activation energy and in coupled reactions.
- list the type of enzymes and describe cofactors and discuss the mechanism of enzyme action.
- discuss the effects of temperature, pH and enzyme concentration on the rate of enzyme action

- draw the curve for Michaelis-Menten equation and Lineweaver-Burk plot to show the enzyme kinetics,
- list the essential features of allosteric enzymes, isoenzymes, inhibitors and assay of enzyme activity.

9.2 CHARACTERISTICS OF ENZYMES

Enzymes act as catalysts and influence the rate of reaction in all the living organisms. In fact, essentially all chemical reactions in the living beings are catalysed by enzymes. All the enzymes known till now are proteins with a unique three dimensional structure that provides an active site for binding the other molecules, i.e. substrates to their surface. Each enzyme normally catalyses a few reactions but most often only one type of reaction. They are required in minute quantity/concentration to convert the substrates into products. The enzyme remains unaffected at the end of the reaction. The enzyme molecule is much larger than the molecule of its substrate. The molecular weights of enzymes range from thousands to millions, whereas the molecular weights of substrates are usually in few hundreds. Some enzymes are purely proteins, whereas others require non-protein assistant compounds for their catalytic activity.

9.2.1 Activation Energy

All the chemical reactions in a biological system have an energy barrier which prevents reactions from proceeding in an uncontrolled and spontaneous manner. The input of energy required to break this energy barrier or to start a reaction is called the **activation energy**. For example, a mixture of hydrogen and oxygen will not react with each other until they receive enough energy from a heat source to achieve the activation energy. You may have noticed that a tin of petrol or kerosene oil kept exposed to air at room temperature, would not catch fire unless kindled by a spark. This tiny spark is adequate to supply the activation energy for few molecules to react. The amount of energy released as a result of the conversion of the first few molecules of reactant to product is sufficient to activate other molecules. Similarly, chemical reactions taking place in living organisms also require activation energy. In biological systems, enzymes are important components which enhance the chemical reactions by lowering the activation energy and help the reactions to occur at a rate appropriate to the needs of the cell. The enzymes speed up the reactions by lowering activation energy. Enzymes combine with different types of substrates in such a way that they reduce the amount of energy required for a particular reaction (see Fig. 9.1). In the absence of enzymes in your gut, it would take many years instead of few hours for your last meal to be digested. You will read about the mechanism of enzyme actions in the following sections.

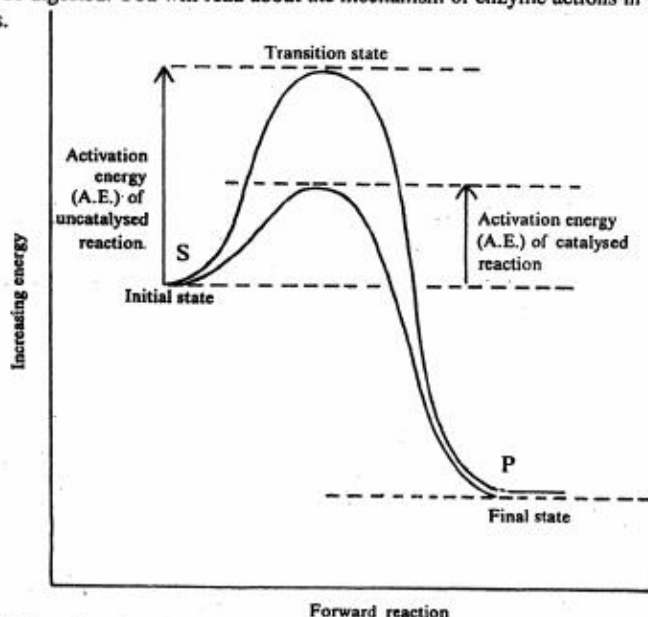


Fig. 9.1: Lowering of activation energy by an enzyme. The activation energy (A.E.) is the energy barrier which the reactants overcome as they react to form the product. The intermediate transition state of enzyme substrate complex lies at the peak of the activation energy barrier.

9.2.2 Coupled Reaction

The energy available to the cells is obtained by burning of food stuff. The breakdown of these food stuffs is carried out by series of reactions, i.e. catabolic reactions in a complex and controlled way. This energy is utilised by the cell for synthetic processes requiring energy, i.e. anabolic reactions which are endergonic in nature. Thus the catabolic reactions which are exergonic in nature and give energy, are coupled to anabolic chemical reactions. This process of coupling maintains a balance in the living systems.

The coupling of these reactions is an important characteristic of living organism and is carried out with the help of enzyme-directed reaction pathways. This coupling process can be understood by examining a simple analogy where an energy releasing catabolic chemical reaction is represented by stones falling from a hill top (see Fig. 9.2). The kinetic energy of falling stones would normally be wasted in the form of heat generated when they hit the ground (see Fig. 9.2a). A part of this kinetic energy can, however, be trapped and used to drive a paddle wheel that lifts a bucket of water, as shown in Fig. 9.2 b. So we can say that the energy producing reaction of a stone falling is directly coupled to the energy requiring reaction of lifting the bucket of water. A part of the energy of falling stones is now used in lifting a bucket of water and the stones hit the ground with less velocity. So less energy is wasted as heat. The potential energy which is stored in the form of a bucket of water can be used for other purposes such as to drive hydraulic machines (Fig. 9.2 c).

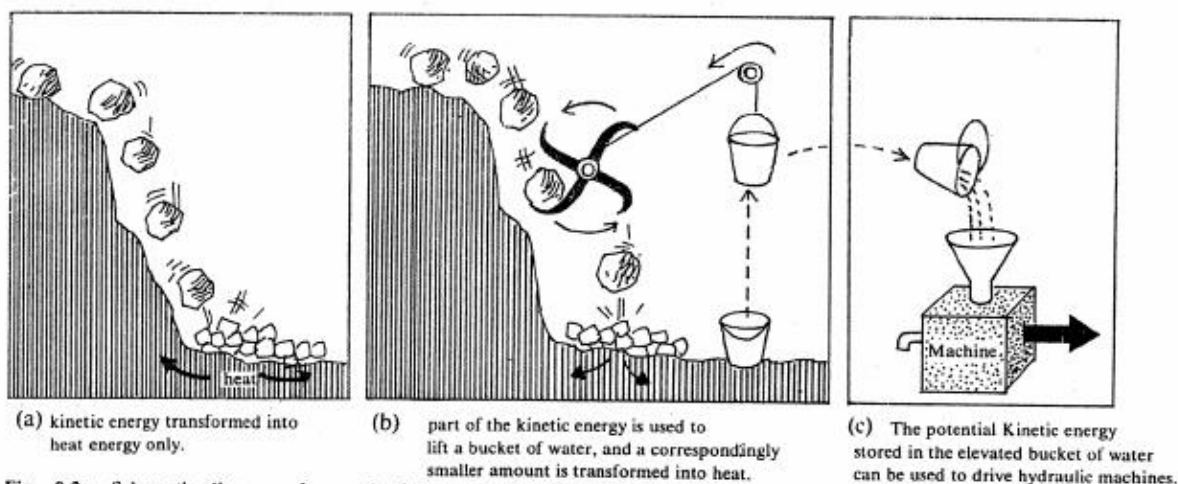


Fig. 9.2: Schematic diagram of a mechanical model indicating the coupling of reactions. Stones falling from the hill top can be compared with the oxidation of food stuffs (a). Part of the energy of falling stones is conserved in the form of lifted bucket of water which can be compared to the synthesis of ATP(b). The conserved energy can be used for energy requiring reaction such as utilisation of ATP during endergonic reactions (c).

In the living organisms, enzymes play the role of paddle wheels and generate and trap energy in the form of high energy phosphate bonds of adenosine triphosphate (ATP). The ATP thus formed is utilised as a source of energy for many energy-dependent processes and is described as an energy currency of the cell. ATP is hydrolysed in neutral aqueous solutions accompanied by release of energy. The first cleavage of ATP to produce ADP and inorganic phosphate, releases 7.3 K. cals of energy per molecule (see Fig. 9.3)

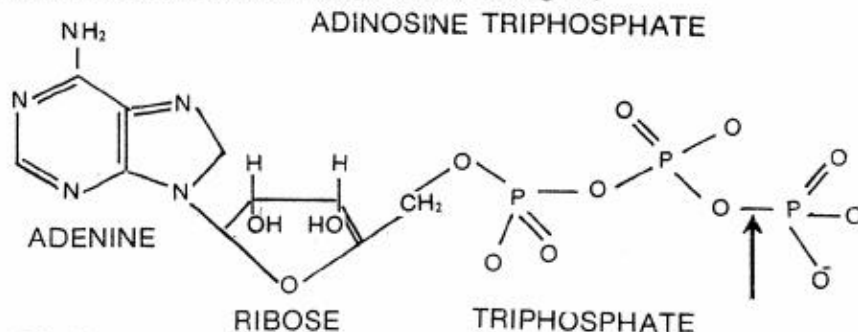


Fig. 9.3: Structural formula of ATP. The first cleavage indicated by an arrow releases 7.3 K. cals of energy per molecule.

SAQ 1

State if the following statements are True (T) or False (F).

- i) Substrate is the reactant of an enzyme catalysed reaction . []
- ii) Enzymes are proteins with an ability to catalyse specific reaction . []
- iii) All enzyme catalysed reactions produce energy for ATP synthesis . []
- iv) Exergonic and endergonic reactions are coupled in all living organisms . []
- v) All the enzyme catalysed reaction have higher activation energy . []

9.3 TYPES OF ENZYMES

The enzyme commission of the **International Union of Biochemists (IUB)** has classified enzymes into six categories on the basis of their functional specificity. Each category is further sub-divided into particular groups consisting of number of enzymes (Table 9.1). Most of the enzymes end as —'ase'. The first part of the name usually indicates the substrate on which the enzyme acts for example amylase enzyme acts on the substrate amylose or starch. A few enzymes are still called by their old names, e.g., pepsin, trypsin, chymotrypsin etc.

Table 9.1: Classification of Enzymes (IUB system)

No.	Enzyme Category	Type of reaction	Examples
1.	Oxidoreductases	Oxidation reduction reactions	<i>Oxidases</i> : transfer hydrogen to oxygen, e.g. cytochrome oxidase $\text{Cyt H}_2 + 1/2\text{O}_2 \rightleftharpoons \text{Cyt} + \text{H}_2\text{O}$ reduced oxidised cytochrome cytochrome <i>Dehydrogenases</i>: transfer hydrogen to a molecule other than oxygen. e.g. lactate dehydrogenase $\text{C}_3\text{H}_6\text{O}_3 + \text{NAD} \rightleftharpoons \text{C}_3\text{H}_4\text{O}_3 + \text{NADH}_2$ lactate pyruvate This reaction occurs in liver following heavy exercise.
2.	Transferases	Transfer a functionally important group from one molecule to another	<i>Transaminases</i> : transfer amino groups, making new amino acids from existing ones e.g. aspartate transaminase $\begin{array}{ccc} \text{aspartate} & + & \alpha\text{-ketoglutarate} \\ (\text{amino acid}) & \updownarrow & (\text{carboxylic acid}) \\ \text{glutamate} & & \text{oxaloacetate} \\ (\text{amino acid}) & & (\text{carboxylic acid}) \end{array}$ This occurs in all cells. <i>Kinases</i> : transfer phosphate, usually from ATP to another substance e.g. hexokinase $\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose-6-phosphate}$ This reaction activates glucose prior to its breakdown in respiration. Phosphorylases which add inorganic phosphate without using ATP are also in this category.
3.	Hydrolases	Split molecules in two by the action of water	All digestive enzymes fall into this category : pepsin, trypsin etc. e.g. amylase $\text{starch}_n + \text{H}_2\text{O} \rightarrow \text{starch}_{n-1} + \text{maltose}$

These reactions occur in the gut and transform large insoluble food molecules into smaller soluble ones which can be absorbed. Similar reactions also occur in the lysosomes of cells.

Phosphatases which remove phosphate groups from organic molecules by hydrolysis also fall in this category.

4. Lyases	Add or remove groups without involving water	<i>Carboxylases</i> : add CO_2 e.g. ribulose diphosphate carboxylase $\text{ribulose diphosphate} + \text{CO}_2 \rightarrow \text{phosphoglyceric acid}$ This occurs in the stroma of chloroplasts and is the principal mechanism by which atmospheric CO_2 is turned into organic material. <i>Decarboxylases</i> : remove CO_2 e.g. pyruvate decarboxylase $\text{pyruvic acid} \rightarrow \text{acetaldehyde} + \text{CO}_2$ released Intermediate step in the conversion of sugars to ethanol during alcoholic fermentation.
5. Isomerases	Convert one isomer of a compound into a different isomer by redistributing the atoms	<i>Mutases</i> e.g. phosphoglucomutase $\text{glucose-1-phosphate} \rightleftharpoons \text{glucose-6-phosphate}$ This reaction occurs at early steps in the respiration of sugars.
6. Ligases	Link together two molecules at the expense of ATP	<i>Synthetases</i> e.g. aminoacyl synthetases Join amino acids to tRNA during protein synthesis.

SAQ 2

According to the IUB system enzymes are classified into six categories. Name these categories and give one example for each

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9.4 COFACTORS

As you have read above, all the enzymes are proteinaceous in nature. Most of the enzymes consist of only polypeptide chains. Some enzymes, however, require an additional chemical component for carrying out their catalytic function. These constituents are non-protein and are called cofactors. A complete, catalytically active enzyme together with its cofactor is called a **holoenzyme**. The protein part of the holoenzyme is called **apoenzyme**. The cofactors are of three kinds; prosthetic groups, coenzymes and metal ions.

Prosthetic groups are organic compounds and are permanently bound to apoenzymes. For example, in the enzymes peroxidase and catalase, which catalyse the break down of hydrogen peroxide to water and oxygen, heme is the prosthetic group and is a permanent part of the enzyme's active site.

Coenzymes are also organic compounds, but their binding with apoenzyme is transient, and occurs only during the catalysis. The same coenzyme molecule may act as cofactor in different enzyme-catalysed reactions. Apart from helping enzymes in their catalytic activity these coenzymes also function as transient carrier, i.e. temporary acceptors for specific atoms or functional groups. Vitamins are essential components of many coenzymes. For example, the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) contain vitamin niacin and are electron carriers. Coenzyme A (CoA) contains vitamin pantothenic acid and carries (transfers) functional groups such as, an acetyl group.

Metal ions are required by certain enzymes for their catalytic activity. Metal ions such as Mg^{2+} , Mn^{2+} or Zn^{2+} bind with specific side chains at active site and at the same time with the substrate. The binding of metal ions with the substrate helps to break it down into products. For example, zinc is a cofactor for proteolytic enzyme carboxypeptidase. It binds with side chains of amino acid residues in the active site of the enzyme and with the α -carboxyl group of substrate amino acid. It is here that the peptide bonds of substrate are broken by the enzyme. Table 9.2 summarises various type of cofactors.

Table 9.2: Some cofactors and their related enzymes

Cofactor	Enzyme	Reaction
Prosthetic groups		
Heme	Catalase	$2H_2O_2 \longrightarrow 2H_2O + O_2$
Heme	Peroxidase	$2H_2O_2 \longrightarrow 2H_2O + O_2$
Coenzymes		
Flavin mononucleotide (FMN)	Some dehydrogenases	Removal of hydrogen atoms
Thiamine pyrophosphate (TPP)	Some decarboxylases	Removal of CO_2
Metal ions		
Zn^{2+}	Carboxypeptidase	Hydrolysis of proteins
Cu^{2+}	Ascorbic acid oxidase	$Ascorbate \rightleftharpoons dehydroascorbate$
Mg^{2+}	Hexokinase	$Glucose + ATP \longrightarrow glucose\ phosphate$

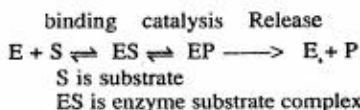
SAQ 3

Differentiate between coenzymes and prosthetic group. Answer briefly in the space provided.

9.5 MECHANISM OF ENZYME ACTION

In spite of the excellent information that has accumulated over the last several years on the physical, chemical and structural aspects of enzymes, much remains to be understood about their mechanism of action. Significant information on the mechanisms by which ribonuclease, chymotrypsin, lysozyme and some other enzyme catalysed reaction is, however, available.

The general mechanism of enzyme action can be viewed as having three stages. The enzyme first binds to the substrate followed by the formation of enzyme substrate complex (ES) which finally alters the substrate and forms the product. The events in enzyme catalysed reaction are represented by the equation.



where E is enzyme
P is product

As you know an enzyme speeds up a reaction by lowering its activation energy. Enzymes have specific regions on their surface to which the substrate binds and catalysis takes place. This region is called as the active site of the enzyme. The enzyme recognises and binds the substrate to its active site and forms an **activated enzyme substrate complex**. The enzyme acts upon the specific bonds within the substrate resulting in the formation of the product. Thus the enzymes lower the energy barrier and allow the organisms to carry out reactions faster.

An enzyme lowers the energy barrier, i.e. activation energy of both the forward and reverse reactions to the same extent. It does not alter a reaction's equilibrium state but merely increases the speed with which the reaction approaches equilibrium. Furthermore, since a catalyst such as, an enzyme is not permanently changed by participation in a reaction, it comes out exactly as it started, and is ready to catalyse the reaction again. One of the factors responsible for enzyme action is their specificity about which you will read in the following subsection.

9.5.1 Enzyme Specificity

One of the most significant features of enzymes is their specificity with respect to the nature of the reaction they catalyse and also in the actual reactant, i.e. substrate molecule. For example, proteolytic enzymes catalyse the reactions involving proteins, whereas urease and succinic dehydrogenase act exclusively on urea and succinate respectively. Enzyme specificity varies even within a particular group of enzymes. Let us take the example of proteolytic enzymes. The reaction catalysed by these enzymes is the hydrolysis of peptide bond (Fig.9.4a). Subtilisin cleaves any peptide bond joining the amino acids of the substrate, whereas enzyme thrombin catalyses the hydrolysis of peptide bonds only between arginine and glycine (Fig. 9.4 b). Still some other proteolytic enzymes have different degree of specificity, for example trypsin breaks peptide bonds only on the carboxyl side, i.e. C-terminal of amino acids lysine and arginine (Fig. 9.4 c). You have read about the N-terminal and C-terminal of amino acids in Unit 5. The specificity differences are due to the biological functions of various proteolytic enzymes. Since bacteria use any protein as a source of carbon and nitrogen, subtilisin enzyme found in them can act upon any type of proteins. Enzymes trypsin and chymotrypsin have limited specificity as they break down the proteins into 5 to 20 amino acids long fragments in the mammalian digestive system.

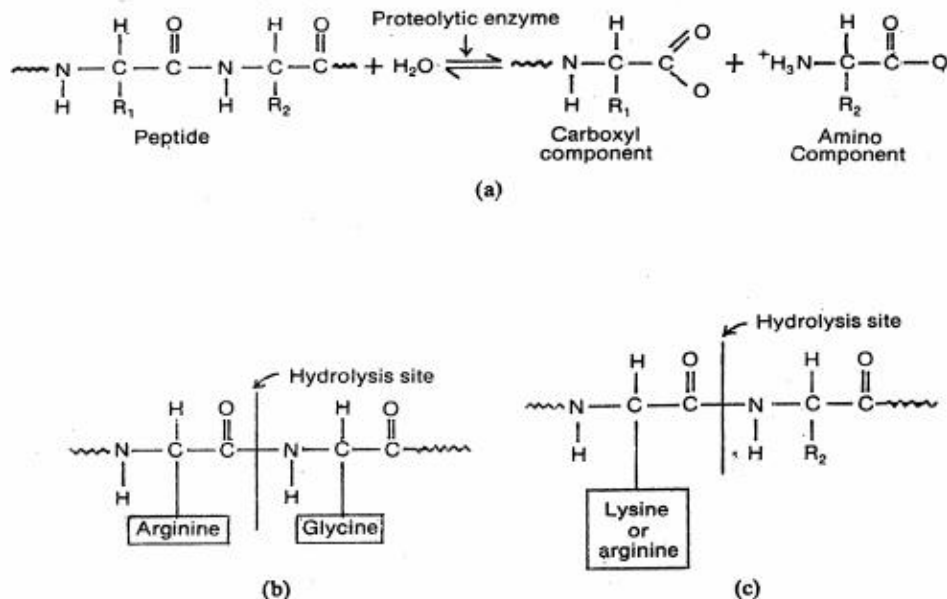


Fig. 9.4: a) Proteolytic enzymes carry out hydrolysis of peptide bonds; b) Specificity of enzyme thrombin; c) Specificity of enzyme trypsin.

Geometrical (cis-trans) isomers

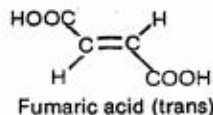
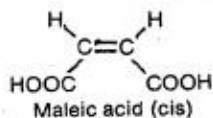


Fig 9.5: Maleic acid and fumaric acid are geometrical, cis-trans isomers.

Thrombin participates in blood clotting and shows absolute specificity as it splits only a single peptide bond in fibrinogen releasing the fibrin monomer which helps in the formation of fibrin clot. The least specific group of enzymes is hydrolases enzymes.

Some enzymes exhibit stereospecificity acting exclusively on different stereoisomers of the same compound e.g., the kidney D-amino acid-oxidase acts on D-isomers of amino acids. However, a small group of enzymes such as racemases catalyse the interconversion of D and L forms. Some enzymes exhibit geometrical specificity acting only on cis or trans isomers. Cis and trans isomers are geometrical isomers and have different arrangement of their constituent atoms and groups with respect to the double bonds joining the two carbon atoms. For example, maleic acid and fumaric acid have the same molecular formula but differ in the arrangement of the group round the C = C bond. As shown in Fig.9.5 Maleic acid, with both the carboxylic group on the same side, is the cis form of fumaric acid, the trans isomer in which these groups are on the opposite side.

In view of the specificity exhibited by enzymes in binding of the substrates, Emil Fischer in 1928 put forward the **lock and key hypothesis**. According to this hypothesis the substrate molecules fit into the active sites of the enzymes like a key fits into the lock. This results in the formation of transient ES-complexes which breaks down into enzyme and product (Fig 9.6 a). The enzyme substrate complex can be isolated from the enzymes that work slowly thus giving the direct evidence for the formation of these ES-complexes.

Although the lock and key model accounts for much of the enzyme substrate specificity, certain observations about enzyme behaviour cannot be explained by this model. For example, some times compounds other than the actual substrate bind to the enzyme and fail to form the reaction products. In the 1960's Daniel Koshland proposed the **induced-fit hypothesis** according to which the active site of the enzyme does not initially exist in a shape complementary to the substrate but is induced by the substrate to assume the complementary shape to bind the substrate molecule. Active site of an enzyme is thus regarded flexible in nature (see Fig. 9.6 b). However, a flexible active site is not necessary for all enzyme catalysed reactions as some are adequately explained by the lock and key model.

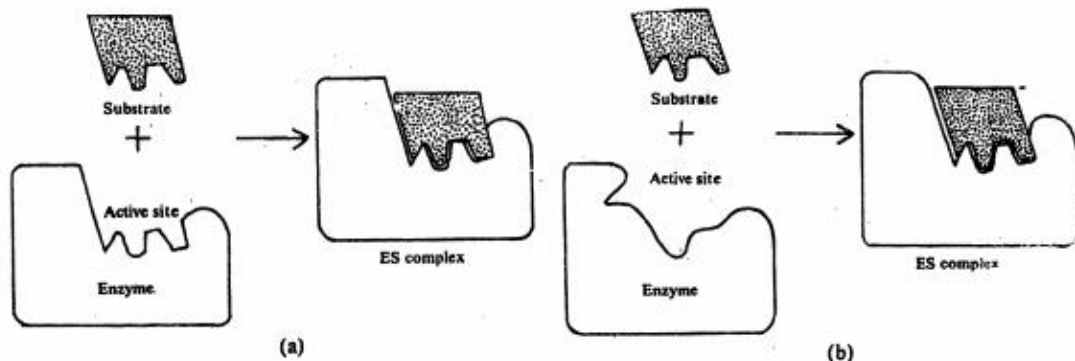


Fig. 9.6:(a) Lock and key model: the active site of the enzyme is complementary in shape to that of the substrate. Substrate fits in the active site like a key fits in the lock.
(b) Induced fit model: the enzyme changes its shape upon binding the substrate. The active site acquires complementary shape to that of substrate only after the substrate is bound.

9.5.2 Molecular Basis of Enzyme Action

The molecular basis of enzyme action depends on its three dimensional structure. The making and breaking of chemical bonds by an enzyme in a reaction are preceded by the formation of an enzyme substrate complex. As you have read earlier, the substrate is bound to a specific region of enzyme called active site. The properties and positions of side chains of the enzymes exposed at the active site determine which substrates will bind to it. The specific amino acid side chains at the active site are not necessarily close to each other in the linear protein chain. Folding of the protein chain, however, brings these groups together. For instance, negatively charged side chain groups, like those of aspartate and glutamate, can be forced together, due to the folding of the enzyme protein molecules in spite of their tendency to repulse each other. This leads to an increase in affinity for the positively charged

groups of the substrate. Similarly, some amino acid side chains forced together as a result of folding of peptide chains, may interact through hydrogen bonding, for example in trypsin a normally non-reactive serine molecule (CH_2OH) becomes highly reactive by acquiring two negative charges (Fig. 9.7).

When an enzyme is denatured, the native three dimensional structure is disrupted. Amino acid side chains of the active site unfold and separate from the site. This prevents their participation in a chemical reaction thus inactivating the enzyme. Enzyme inactivation is also caused even when only a functional side group is displaced or substituted in the enzyme.

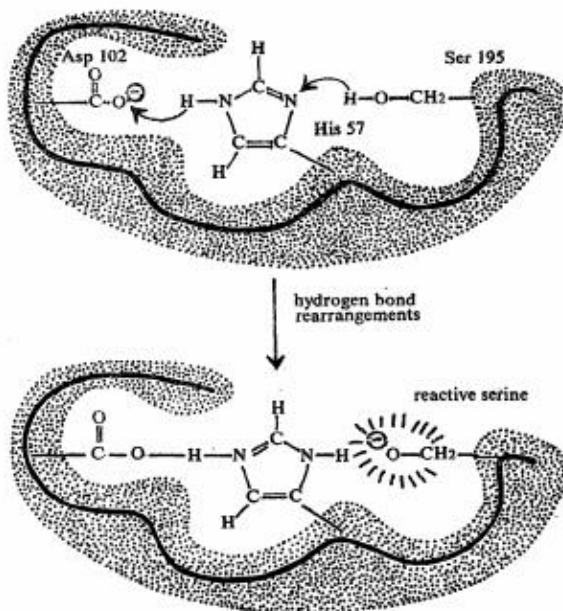


Fig. 9.7: Rearrangements of hydrogen bond makes serine molecule highly reactive in the active site of the enzyme.

Although enzymes differ widely in structure, specificity and mode of catalysis, various mechanisms operate at the active site of enzymes, each contributing to the lowering of activation energy. These mechanisms can be generalised as follows:

- The binding of reacting molecules with each other brings them in close proximity, thus increasing the chance of a reaction.
- The substrates are bound to the enzyme in such a manner that the formation of several types of temporary non-covalent bonds between them force a redistribution of charges within substrates. This redistribution imposes a strain on specific covalent bonds in the substrates resulting in the breaking of the bond.
- Hydrophobic amino acids eliminate water molecules from the active site to create a water free zone so that nonpolar groups react more easily.
- Acidic and basic amino acids in the active site of the enzyme transfer the protons to and from the reactants for catalysis of reaction. This is known as acid-base catalysis.
- Enzymes react with the substrate to form transient, high energy unstable covalently linked enzymes substrate-complex which undergoes further reactions to form products.

Although these factors are believed to contribute to the acceleration of reaction rate by various enzyme, the exact mechanism involved is yet to be known.

SAQ 4

Complete the following statements with appropriate words

- The substrate binds on theof the enzyme and forms.....
- Enzymes do not alter theof the reactions
- acts upon any peptide bond between the amino acids
- Thrombin hasspecificity

- v) According toactive site assumes the complementary shape after the.....is bound
- vi) of the polypeptide chains bring the side chains together in the active site region
- vii) amino acids create a water free zone for.....reactants.
- viii) Transfer of protons to and from the reactants iscatalysis.

9.6 FACTORS AFFECTING THE RATE OF ENZYME ACTION

Enzymes are capable of enhancing the reaction rate enormously, sometimes a million times compared to uncatalysed reactions. The rates of enzyme catalysed reactions are expressed as the "turnover number". The turnover number is the number of substrate molecules transformed into product in unit time by a single enzyme molecule or by single catalytic site, and depends upon the enzyme concentration. So if the enzyme concentration is known, the turnover number can be calculated.

$$\text{Turnover number} = \frac{\text{moles of product formed per unit time}}{\text{moles of enzymes}}$$

Turnover numbers of several enzymes are listed in Table 9.3. The highest turnover number is of carbonic anhydrase which converts 3.6×10^6 CO_2 molecules to H_2CO_3 per minute. International Union of Biochemistry has now recommended a new unit for reaction rates called Katal (Kat). One Kat is the amount of enzyme that converts one mole of substrate into product per second.

Table 9.3: Turnover Number of Some Enzymes

Enzyme	Turnover Number
Carbonic anhydrase	3.6×10^6
Acetylcholinesterase	1.5×10^6
Urease	1.0×10^6
Amylase	1.0×10^5
Lactic dehydrogenase	6.0×10^4
Chymotrypsin	6.0×10^3
Lysozyme	3.0×10^3

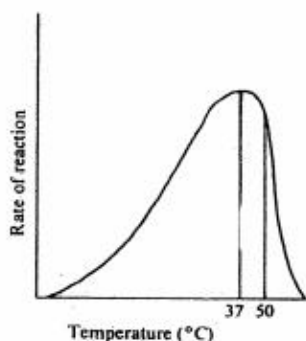


Fig. 9.8: Effect of temperature on the rate of enzyme catalysed reactions. Optimum temperature varies for different enzymes and different species.

Rates of enzyme catalysed reactions depend on several factors like temperature, pH, concentration of enzyme and substrate etc.

9.6.1 Effect of Temperature

Within certain limits temperature influences the enzyme catalysed reactions in the same way as it affects ordinary uncatalysed chemical reactions. As the temperature increases, the rate of a chemical reaction increases owing to an increase in the number of activated molecules. But when the temperature rises above a certain limit, it destroys the tertiary structure of the enzyme causing the loss of its activity. Similarly low temperature, such as freezing temperatures, generally inactivate the enzyme. It, therefore, follows the rule that for every enzyme under a given set of conditions, there is a temperature at which the activity of the enzyme is at its maximum. This is known as optimum temperature (see Fig. 9.8). Most enzymes are denatured above 50°C . Optimum temperature for mammals and birds is about $35^\circ - 40^\circ\text{C}$ whereas in plants and in other animals it is between $20^\circ - 35^\circ\text{C}$. Some procaryotes and certain fishes found in Antarctica region are exceptional in that they are adapted to life in hot spring and at freezing cold temperatures. They have optimum temperature at over 80°C and below freezing point respectively.

9.6.2 Effect of pH

The hydrogen ion concentration or the pH has a marked influence on the rate of enzyme reaction. Characteristically, each enzyme has a pH value at which the reaction rate is optimum usually within the pH range 7.0 ± 1.5 . This is known as optimum pH at which a certain enzyme causes a reaction to progress most rapidly. On each side of this pH value the reaction is decreased and at certain pH values on both sides an enzyme may be inactivated or even denatured. Therefore, while studying enzymes, buffers are used to keep the enzyme at an optimum pH.

While majority of enzymes function optimally around neutral pH values, some enzymes like pepsin in stomach has an unusually low optimum pH. This is an adaptation to the acidic conditions of stomach which has pH ranging from 1.5 to 2.5 due to the presence of HCl in the gastric juice (see Fig. 9.9). Pancreatic enzymes have their optimum pH in highly alkaline range.

Buffers are the combination of substances that resist changes in pH when acid or bases are added. It consists of a weak acid and its conjugate base or a weak base and its conjugate acid, for example acetic acid (CH_3COOH) and acetate (CH_3COO^-) can serve as a buffer when pH of the solution has to be adjusted around pH 1.

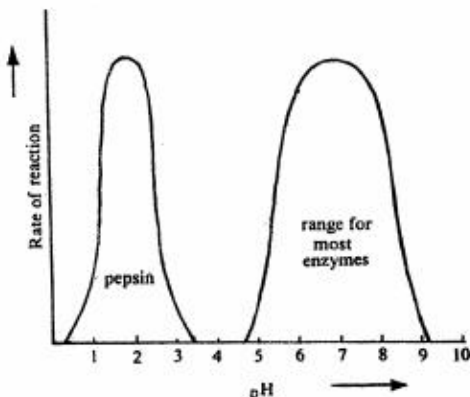


Fig. 9.9: Effect of pH on the rate of enzyme catalysed reactions. Proteolytic enzyme pepsin has low optimum pH.

9.6.3 Effect of Enzyme Concentration

As you know, Enzymes increase the reaction rate through catalysis. Under constant conditions of temperature, pH and substrate concentration, the velocity of the reaction is directly proportional to the amount of enzymes present, i.e. the reaction rate is increased with the increase in enzyme concentration. However, the equilibrium constant for the reaction is not affected by the presence of enzymes. A chemical reaction in the cell can be started or stopped by making or destroying a particular enzyme. As you have read, turnover number is a useful parameter to study the effect of enzyme concentration. Before we proceed further try the following SAQ.

SAQ 5

Match the statements in column I with those in column II

Column I	Column II
i) Temperature influences enzyme catalysed reaction []	a) It is active only in acidic medium
ii) Mammals and birds also have optimum temperature []	b) Enzyme concentration has its affect on reaction rate
iii) Enzyme pepsin acts on peptide bonds in the stomach []	c) Enzymes are inactivated at freezing temperatures
iv) Reactions rate is directly proportional to the amount of enzyme []	d) It is between 35C to 40C

Enzymes not only speed up the reactions, they also have effect on the kinetics of the reaction. Substrate concentration plays an important role in enzyme kinetics about which you will read in the following section.

9.7 ENZYME KINETICS

The concentration of substrate also influences the rate of enzyme catalysed reaction. At low substrate concentrations, the number of substrate molecules are too few to occupy all the active sites of the enzyme molecules, and the initial velocity of the reaction is directly proportional to the substrate concentration. With the increase in substrate concentration more and more active sites are occupied by many more molecules of substrate. A stage will finally be reached when the substrate concentration is high enough to occupy all the active sites of the enzymes. At this saturation point the maximum velocity of the reaction is attained and is called V_{max} . This maximal velocity is not affected by further increase in substrate concentration. The curve of the rate of enzyme reaction against substrate concentration shows the shape of a rectangular hyperbola for enzymes composed of single polypeptide chain with one active site (see Fig. 9.10). At a particular concentration of substrate where half the enzyme molecules are saturated with substrate molecules, the velocity (V) of the reaction is half the maximal velocity ($V_{max}/2$). This substrate concentration at half the maximal velocity is termed as K_m or **Michaelis constant**.

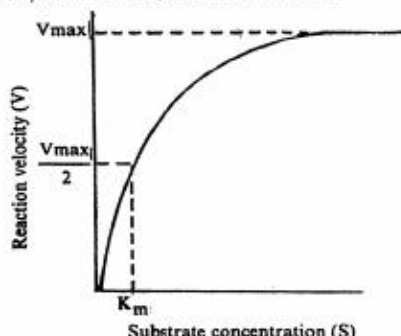
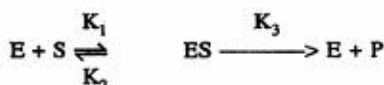


Fig. 9.10: Relationship between reaction rate (v) and substrate concentration (s). The initial velocity of the reaction increases with the substrate concentration. At high substrate concentration v becomes independent of s . Michaelis constant K_m is equal to substrate concentration when reaction rate is half its maximum value ($V_{max}/2$). All such curves are hyperbola.

In 1913 Leonard Michaelis and Maud Menten proposed a simple yet convincing model to explain the Kinetics of enzyme reaction. According to this model, enzyme (E) combines reversibly with substrate (S) to form the enzyme-substrate complex (ES). The ES subsequently breaks down to E and to product (P). This is represented by the following equation :



K_1 represents the rate constant for the formation of ES . K_2 and K_3 represent the rate constants for the dissociation of ES to $E + S$ and $E + P$ respectively.

Soon after the enzyme is mixed with substrate, the concentration of ES builds up and remains unchanged as long as S is in large excess. When the rate of formation of ES is equal to its breakdown, the ES concentration will be constant and the reaction system is said to be in a **steady state**. Putting this mathematically,

$$K_1[E][S] = K_2[ES] + K_3[EP]$$

$$\text{or } \frac{K_2 + K_3}{K_1} = \frac{[E][S]}{[ES] + [EP]}$$

At any substrate concentration some of the enzyme molecules are in the form of ES while E represents the free enzyme. Total enzyme molecules are therefore equal to free enzyme molecules plus enzyme molecules in the substrate complex form, i.e.

$$E(\text{Total}) = E + ES$$

The concentration of free enzyme or that of ES as enzyme substrate complex is difficult to determine experimentally. Equation 9.2 can, however, be, converted to a more convenient form assuming that

- at any given substrate concentration, velocity (V) of an enzyme catalysed reaction is proportional to the concentration of ES, and
- maximum velocity attainable by the enzyme at saturating substrate concentrations ($V_{\max}$) is proportional to the total enzyme concentration.

Based on these assumptions, Michaelis-Menten derived the following equation from equation 9.2.

$$V = \frac{V_{\max} [S]}{K_m + [S]}$$

K_m is the Michaelis-Menten constant and is equal to $\frac{K_2 + K_3}{K_1}$

As said earlier, K_m can be also defined as the substrate concentration where enzyme exhibits half of the maximum reaction velocity, i.e. when $S = K_m$, then $V = V_{\max}/2$. Thus K_m is a measure of the degree of binding or affinity of the enzyme for the substrate. Small values of K_m denote high affinity while larger K_m values indicate weak affinity. An enzyme has a characteristic K_m value for a given substrate.

As shown in Fig. 9.10, K_m value can be determined experimentally by determining the rates of reaction at various substrate concentrations, using a fixed amount of enzyme. However, most enzymes do not readily attain maximum velocity and continue to show small increases in velocity even at very high substrate concentration. For a more convenient calculation of K_m , Lineweaver and Burk introduced a different form of Michaelis-Menten equation by taking reciprocal of both sides of the equation 9.3

$$\frac{1}{V} = \frac{K_m}{V_{\max} [S]} + \frac{1}{V_{\max}}$$

By plotting reciprocal of velocity ($1/V$) against the reciprocal of substrate concentration ($1/S$) one gets a straight line from which K_m and $V_{\max}$ can be easily calculated (Fig. 9.11)

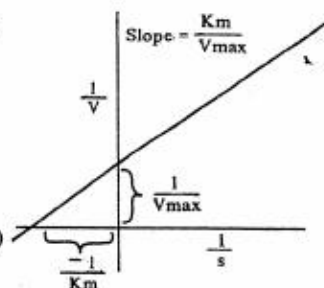


Fig. 9.11: Lineweaver-Burk plot showing the relationship between reciprocal of reaction rate ($1/V$) and reciprocal of substrate concentration ($1/S$). The plot is a straight line and holds true for enzymes obeying Michaelis-Menten relationship.

SAQ 6

Explain briefly the relationship between substrate concentration and Michaelis constant

9.8 ALLOSTERIC ENZYMES

Some enzymes may have two functionally different binding sites. One of the site, the active site, binds the substrate and catalyses the reaction. The other type of site, known as allosteric or **regulatory site** binds another molecule which is called **effector** or **modulator**. Such enzymes are known as allosteric enzymes. These enzymes are oligomeric in nature, i.e. they have more than one subunit. The active site and allosteric site may be located on the same subunit or on different subunits of the enzyme and the conformational changes caused by binding are transmitted between subunits. Effector molecules are of two types: positive effectors, i.e. **activators** that enhance enzyme activity and negative effectors or **inhibitors** which inhibit enzyme activity. Binding of effectors causes conformational changes in the allosteric enzymes which influence their catalytic activity (see Fig. 9.12). We can also say that allosteric enzyme action is regulated by effector molecules. You will read about the functions of effectors in Unit 10. These effectors molecules can bind at the same allosteric site or at different allosteric sites.

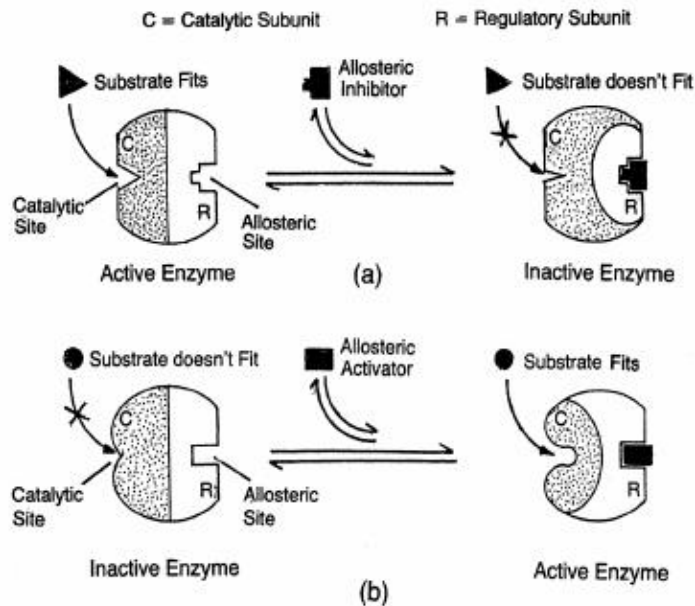


Fig. 9.12: Schematic diagram showing the functioning of allosteric enzymes.

Binding of a substrate to one active site may influence subsequent bindings to other active sites of an enzyme with more than one active sites. This behaviour of enzymes is known as **cooperativity**. When binding of the first substrate molecule promotes binding of subsequent substrate molecules, it is called **positive cooperativity**. **Negative cooperativity** is when, after the binding of the first substrate molecule subsequent substrate binding occurs less readily. Allosteric enzymes and enzymes that show cooperativity do not obey Michaelis-Menten Kinetics. Such enzymes which are composed of more than one subunit and more than one active site show a sigmoidal curve when reaction velocity is plotted against substrate concentration as shown in Fig. 9.13.

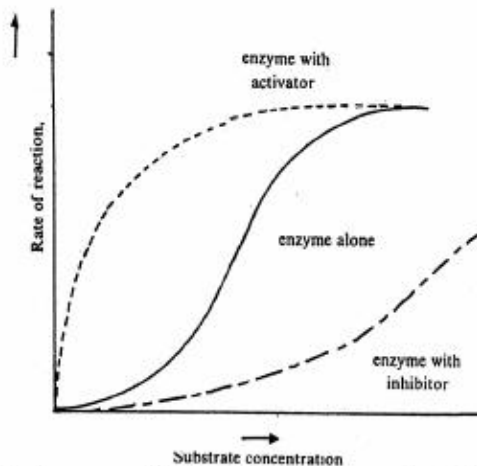


Fig. 9.13: Allosteric enzymes show sigmoidal curve when reaction rate is plotted against substrate concentration. The effects of allosteric activator and inhibitor are also shown.

Many allosteric enzymes function at the beginning of the metabolic sequence pathway and are inhibited whenever the end product of the reaction pathway accumulates. Such inhibition is described as the **feedback inhibition**. Several important metabolic pathways are regulated by feedback inhibition both in prokaryotes and eukaryotes. You will read more about it in Unit 10.

9.9 ISOENZYMES

Several enzymes exist in their multiple forms termed **isoenzymes** or **isozymes**. These isoenzymes are not isomers. All isoenzyme forms of a given enzyme catalyse the same reaction but are chemically distinct molecules. They may differ in amino acid composition, amino acid sequence, charge, molecular weight, etc. They usually differ in one or more kinetic properties such as K_m , V_{max} for their substrate.

Isoenzymes are found in all vertebrates, insects, plants and unicellular organisms. Different tissues may contain different isoenzymes which may differ in their affinity for the substrates. One of the first and best known example of universally occurring isoenzymes is lactate dehydrogenase that exists in five different forms : M_4 , M_3H , M_2H_2 , MH_3 and H_4 . Each of these isozymes is a tetramer made up of four polypeptide subunits. These subunits are of two types and occur in different combinations in the isozymes. These two types of subunits are named as M and H types depending upon their location; M in muscles and H in heart (Fig. 9.14).

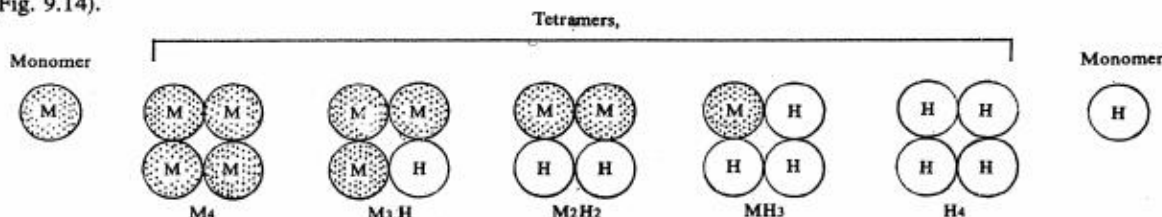


Fig. 9.14: Lactate dehydrogenase enzyme is a tetramer, has five isozymic forms. Each tetramer has M and/or H types of monomers in different numbers.

All the forms of lactate dehydrogenase isoenzymes do not necessarily occur in equal proportion in all the cells as M_4 dominates in muscles and H_4 is predominantly present in the heart. Other isoenzyme forms include oxidases, dehydrogenases. Several regulatory enzymes also exist in isoenzyme forms. The molecular difference of isoenzyme molecules is due to the difference in their locations such as malate dehydrogenase, which occurs in different forms in cytosol and in mitochondria. Analysis of isozymes is frequently used in medical diagnosis.

9.10 INHIBITORS

Enzyme activity can be inhibited by certain chemical substances called inhibitors. Enzyme inhibitors have provided information about substrate specificity of enzymes, the nature of functional groups at active site and the mechanisms of the catalytic activity. Some drugs considered to be useful in medicine function by inhibiting certain enzymes, for example, the inhibition of bacterial enzymes which affects the bacterial metabolism and, therefore, their growth and multiplication.

Enzyme inhibitors are of two types : Irreversible and reversible.

i) Irreversible inhibitors

These inhibitors bind covalently to the enzymes causing the permanent loss of their catalytic activity by altering or destroying a functional group on the enzyme molecules. Arsenic, lead, mercury and various insecticides are all irreversible inhibitors. Even at low concentration of these the enzymes are precipitated.

ii) Reversible inhibitors

These inhibitors bind non-covalently to enzymes and their effects can be reversed. Important information on the structure of the active sites of various enzymes have been obtained with the help of reversible inhibitors. The two main types of reversible inhibitors are competitive and non-competitive inhibitors.

a) **Competitive inhibitors:** Competitive inhibitors compete with the real substrate for binding to the active site of the enzyme because they resemble the substrate molecules. The catalytic ability of the enzyme is not affected by this binding but the ability of the substrate to bind to the enzyme is reduced. However, these inhibitors cannot be transformed into products. Competitive inhibition is reversed simply by increasing the substrate concentration. For example, succinate dehydrogenase, which catalyses the removal of 2 hydrogen atoms from succinate, is inhibited by malonate which resembles

succinate structurally. However, malonate is not dehydrogenated by the enzyme succinate dehydrogenase. It simply occupies the active site. The inhibition is reversed by increasing the succinate concentration.

- b) **Non-competitive inhibitors:** These inhibitors bind at a site on the enzyme other than the catalytic sites, i.e. substrate binding site, altering the conformation of the enzyme molecule and leading to the distortion and inactivation of the catalytic site. Non-competitive inhibitors bind reversibly to both the free enzyme and the ES complex to form the inactive complexes. Addition of more substrate will not restore the previous rate of reaction in contrast to competitive inhibitors. Important non-competitive inhibitors are the naturally occurring metabolic intermediates for example L - threonine dehydratase is inhibited by L - isoleucine. Competitive and non-competitive inhibitors are not necessarily harmful. Both are used extensively by cells for metabolic regulation.

SAQ 7

- a) Fill in the blanks with appropriate words
- Allosteric enzymes are.....proteins.
 - Effectors induce.....changes in allosteric enzymes.
 - Multiple forms of an enzyme are known as
 - The.....of the isoenzymes may occur in different combinations.
- b) Differentiate between competitive and non-competitive inhibitors. Answer briefly in the space provided.

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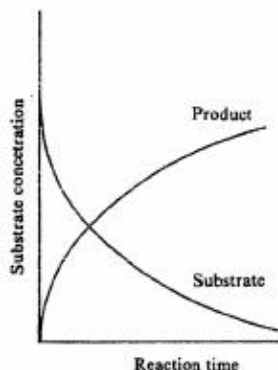


Fig. 9.15: Concentrations of Substrate and product vary during the course of enzyme-catalysed reactions. As the reaction proceeds, substrate concentration decreases and product concentration increases.

9.11 ASSAY OF ENZYME ACTIVITY

All enzymes so far known are proteins, yet the specific tests for protein can not be used for the detection and/or quantification of enzymes. Evidently such tests cannot make distinction between enzymes and non-enzymes proteins and between various enzymes. The amount of enzymes in a given solution or tissue extract can be conveniently measured or assayed quantitatively by using techniques that can measure their ability to convert the substrate into product.

One can detect the presence of an enzyme by using a specific quantification procedure either for the substrate or for product. Under optimal conditions the velocity of enzyme catalysed reaction is directly proportional to the concentration of the enzyme. One can therefore determine the concentration of enzyme from the velocity of the disappearance of the substrate or that of the formation of product under optimal conditions. As shown in Fig. 9.15 during the enzyme action the concentration of substrate decreases, while that of product increases. This quantitative estimation of enzyme activity is called as the assay of an enzyme. Activity of enzyme is represented in enzyme units.

By international convention one unit of enzyme activity is defined as that amount which transforms one micro mole substrate to product in one minute under optimal conditions. Enzyme assays are carried out at their optimal pH, temperature and with a near saturating concentration of substrates.

Concentration of different substrates or products can be determined using methods such as colorimetric, spectrophotometric, fluorometric or isotopic labelling procedures. The choice of the procedure is determined by the nature of the substrate or product. For example, spectrophotometric assays are used when either the substrate or product absorbs light of some specific wavelength. Highly sensitive assay procedures are usually based on fluorescence or radioactive measurements.

In many instances substrates are modified by introducing specific groups for the estimation of their products. Such modification of the substrate should not, however, result in decrease

in the rate of its transformation to product by the enzyme. For instance the introduction of p-nitrophenyl phosphate compound in substrate makes the substrate useful for the assay of phosphatases. The product of enzyme action is an ionised form having p-nitrophenylate ion which is of yellow colour and the intensity of colour indicates the activity of the enzyme.

We finish this unit on characteristics and mechanism of enzymes. We will continue with the regulation of enzyme activity in the next unit.

9.12 SUMMARY

In this unit you have studied that:

- enzymes are proteins that catalyse the biochemical reactions by lowering the activation energy. Enzymes increase the rate of the reactions but do not alter the equilibrium point,
- enzymes facilitate the coupling of energy releasing exergonic reactions to energy requiring endergonic reactions,
- enzymes are highly specific with respect to the nature of reaction as well as to their substrate. Enzymes substrate specificity has been explained by lock and key and induced fit hypothesis,
- reactions by the enzymes are facilitated on the basis of proximity and orientation of side chains of the substrate and enzyme, acid-base catalysis by the side chain of charged amino acids of active site and by covalent interactions between enzymes and substrates,
- most enzyme catalysed reactions follow a hyperbolic substrate saturation curve which is explained by the Michaelis-Menten equation for enzyme Kinetics. Substrate concentration at half the maximum velocity of reaction rate is called Michaelis-Menten constant, K_m ,
- changes in the concentration of enzymes, substrates, pH and temperature influence the rate of enzyme catalysed reaction,
- some enzymes require additional factors called cofactors for their catalytic activity. Prosthetic groups are organic cofactors and are permanently bound to the enzymes. Coenzymes are also organic compounds but their association is transient. Some enzymes require metal ions for their activity,
- allosteric enzymes are oligomeric and have more than one active site where the allosteric effector molecules bind and influence enzyme activity. Influence of one substrate binding on subsequent substrate bindings is known as cooperativity. The graph of kinetics of allosteric enzymes and enzyme cooperativity are sigmoidal.
- enzyme activity is also regulated by inhibitors. Inhibitor actions can be reversible or irreversible. Reversible inhibitors may be competitive or non-competitive,
- enzyme activity can be estimated by different procedures such as spectrophotometric; fluorometric and isotopic labelling methods.

9.13 TERMINAL QUESTIONS

- 1) What do you understand by activation energy?

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- 2) Discuss briefly the variations in the degree of enzyme specificity.

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- 3) Discuss briefly the Michaelis-Menten Kinetics for enzyme action.

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- 4) Explain briefly the functioning of allosteric enzymes.

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9.14 ANSWERS

Self-assessment Questions

- 1) i) T, ii) T, iii) F, iv) T, v) F
- 2) i) Oxidoreductases-Cytochrome oxidase
ii) Transferases-hexokinase
iii) Hydrolases-amylase
iv) Lyases-Decarboxylase
v) Isomerases-Phosphoglucomutase
vi) Ligases-Aminoacyl synthetases
- 3) Coenzymes are the organic cofactors which are transiently bound to enzymes for their catalytic activity.
- 4) i) active site, enzyme-substrate complex
ii) equilibrium state
iii) subtilisin
iv) absolute
v) induced fit hypothesis, substrate
vi) folding
vii) hydrophobic, non-polar
viii) acid-base
- 5) i) c, ii) d, iii) a, iv) b
- 6) Michaelis constant K_m is equal to the substrate concentration, when reaction rate is half its maximum value i.e. $\frac{V_{max}}{2}$. At this stage half the enzyme molecules are saturated with substrate molecules.
- 7) a) i) oligomeric, ii) conformational, iii) isozymes
iv) subunits
b. Competitive inhibitors resemble the substrate and compete for the active site. These do not affect the catalytic ability of an enzyme. Non-competitive inhibitors do not resemble the substrate and reduce the catalytic properties of the enzymes.
- 8) Calometric, spectrophotometric, fluorometric and isotopic procedures are used for enzyme assay. Enzyme assays are carried out at their optimum pH, temperature and saturating concentration of the substrate.

Terminal Questions

- 1) The energy required to start a reaction is called the activation energy. In biological systems activation energy is lowered with the help of enzymes.

- 2) Enzymes exhibit specificity for the substrate as well for the reactions they catalyse. There is also variation in specificity within the particular groups of enzymes. Example of proteolytic enzymes can be given.
- 3) The initial reaction rate is directly proportional to the substrate concentration. At higher substrate concentration all enzymes are saturated and maximum velocity of the reaction is achieved. The relation between substrate concentration and reaction rate is shown by an hyperbola curve. The equation derived by Michaelis-Menten for one enzyme one substrate reactions is

$$v = \frac{V_{\max} [S]}{K_m + [S]}$$

- 4) Allosteric enzymes are regulated by other molecules called as effectors. These effectors bind to the allosteric or regulatory site of the enzyme and may act as activator or inhibitor of enzyme action.

UNIT 10 ENZYMES: REGULATION AND CONTROL

Structure

- 10.1 Introduction
 - Objectives
- 10.2 Regulation of Enzyme Activity
 - Genetic Control
 - Control of Catalysis
 - Regulation by Hormones
 - Control by Calcium
 - Cell Compartmentation
- 10.3 Techniques for the Study of Metabolic Pathways
 - Studies with Whole Organisms
 - Studies with Microorganisms
 - Studies with Perfused Organs
 - Studies with Tissue Slices
 - Subcellular Fractions
 - Use of Mutants
 - Use of Isotopes
- 10.4 Summary
- 10.5 Terminal Questions
- 10.6 Answers

10.1 INTRODUCTION

In Unit 9 you have studied about the role of enzymes in the organisms, methods for detecting enzymes, specificity of the enzymes, enzyme kinetics and also about the mechanism of enzyme action.

In the previous unit you have learnt that thousands of chemical reactions are taking place in a cell involving synthesis or degradation of biomolecules. Actually, the cell synthesises or degrades more material than necessary, thereby wasting energy. Therefore, these chemical reactions occurring inside the cell must be carefully controlled in order to respond rapidly to the changing metabolic needs of the cell.

In this unit you will study how the activities of enzymes are regulated and kept under control in living organisms to maintain their internal order. We will also be dealing with various techniques to detect and measure the enzyme activities taking place in an organism.

Objectives

After reading this unit you should be able to :

- describe the need for the regulation of enzyme activity
- explain the genetic control of enzyme activity
- explain the process of feedback regulation, allosteric regulation, covalent modulation of enzyme activity, and hormonal regulation of enzyme activity
- define cell compartmentation
- outline the various techniques for the identification of enzymes involved in the metabolic pathways.

10.2 REGULATION OF ENZYME ACTIVITY

You have studied in the Block-3 of the FST course that living organisms maintain their internal order in a steady state known as homeostasis. To maintain the steady state, they utilize energy from nutrients and also free energy from the environment (e.g. plants use sun light for photosynthesis). The transformation of energy from one form to another is done

in cells, such as normal body temperature, low ionic strength, low pressure and narrow range of pH. The hundreds of enzyme catalysed reactions taking place in a living cell is collectively known as **Metabolism**. Metabolism is a highly coordinated and purposeful cell activity. It consists of many enzyme systems which cooperate and act in a consecutive linked manner, so that the product of the first enzyme catalysed reaction, becomes the substrate for the second reaction and so on. The successive transformation products in a metabolic pathway are known as **metabolites** or **metabolic intermediates**. Each of the consecutive steps in these pathways bring about a specific chemical change by the removal, transfer or addition of specific atoms, molecules or functional groups. Through step by step changes the incoming biomolecule is transformed into its metabolic end product and reconstituted. You will study various metabolic pathways in Units 11 and 12.

For continuance of life, all chemical reactions must proceed at rates in accordance with the requirements of the cell or organism. All the enzymes within the cell do not exhibit their maximum activity at all times. Some may be functioning at greatly reduced levels while others may be completely inactive when not required as the related metabolic reactions stop according to the requirement of the cell. Life processes like ATP production, synthesis of molecules and macromolecules, transport, secretion, absorption, etc., are regulated to respond to the changes in the internal as well as external environment of the cell, organ or organism. Since the metabolic reactions are enzyme catalysed, regulation is achieved by altering the enzyme activity. Regulation depends mainly on one important property of enzyme i.e. 'specificity'. If an enzyme is inactive or not available, no other enzyme can take its place and the reaction will stop. However, reactions unrelated to this particular enzyme will not be affected.

Immediate regulation of enzyme activity is achieved by changes in pH, temperature, enzyme and substrate concentration. Some enzymes are synthesised in an inactive or less active form called **proenzymes** or **zymogens**. These proenzymes or zymogens are converted to the active enzymes by removal of a specific amino acid or peptide in the protein molecule. Apart from these mechanisms mentioned above, enzyme activity is regulated by two major mechanisms. They are, **genetic control** and **control of catalysis**.

10.2.1 Genetic Control

In this type of control, the synthesis of the enzyme is regulated at the genetic level, which results in a change in the total amount of enzyme molecules. The enzymes are synthesised only when required. Their synthesis is switched on and off whenever required. You have already learnt that most of the enzymes are proteins, which are synthesised in the cell. The genetic material DNA determines the primary structure of the enzymes, i.e., the linear chain of amino acids. To synthesise a protein (enzyme) molecule, mRNA is formed which is complementary to the DNA molecule, i.e., mRNA contains all the information present in the DNA. This process is called '**Transcription**'. Later the information present in the mRNA is translated into polypeptide chains in the cytosol by a process known as '**Translation**'. The polypeptide chains thus formed later assume the native three dimensional structure with catalytic properties of the enzyme. You will study the process of protein biosynthesis later in Units 13 and 14 of this course.

Jacob and Monod, in 1961, proposed a model to explain the genetic control of enzymes in prokaryotes. The model is known as '**Operon Model**'. According to this model, an **operon** (a piece of DNA molecule) contains a **repressor gene**, a **promoter gene**, an **operator gene** and **structural genes** (Fig. 10.1 a).

Structural genes are responsible for the formation of proteins (enzymes). In order to stop the synthesis of the enzyme, the repressor gene produces (transcribes) a mRNA, which gets translated to form a repressor protein. The repressor protein binds to the operator gene and blocks the transcription of structural genes, thereby stopping the synthesis of the enzyme (Fig. 10.1 b). To induce the synthesis of the enzyme, the substrate acts as an '**inducer**'. The inducer combines with the repressor protein forming a **repressor inducer complex** that cannot bind to the operator gene. In this way, the blockage at the operator gene is removed and the structural genes again transcribe mRNA for the synthesis of enzymes (Fig. 10.1 c). Availability of the substrate induces the synthesis of the enzymes, whereas accumulation of the end product of a metabolic pathway represses the enzyme synthesis. You will study more about regulation of protein biosynthesis in Unit 14.

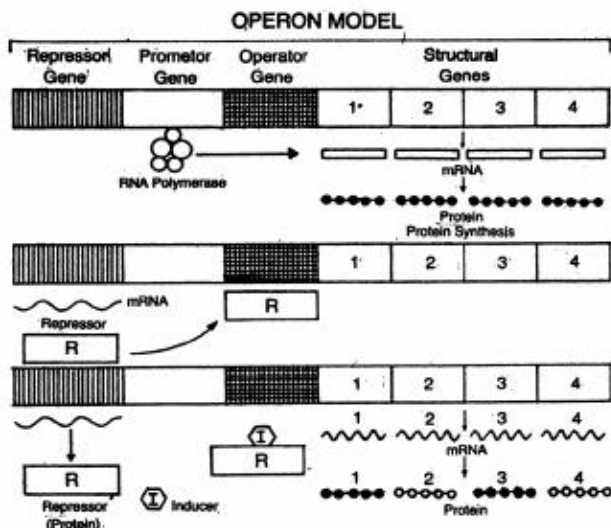
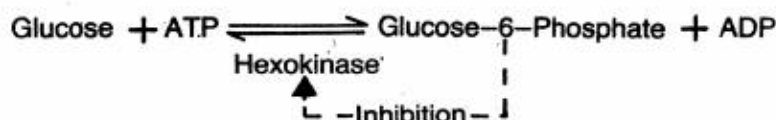


Fig. 10.1: Operon Model for the regulation of enzyme synthesis at genetic level. (RNA Polymerase = Enzyme that catalyses the transcription of mRNA)

10.2.2 Control of Catalysis

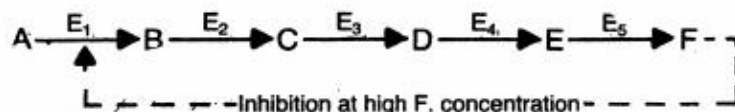
In the above subsection you have studied about the genetic control of enzyme production. Now we will study how the activity of enzymes is regulated by altering the catalytic activity of the enzyme. The mechanism of control of catalysis involves a change in enzyme activity without a change in the total amount of enzyme synthesised. Important mechanisms of this type are **feedback inhibition**, **allosteric regulation** and **covalent modulation**. Let us study in detail about these mechanisms.

i) **Feedback Regulation:** Feedback regulation means that the product of a reaction or a multienzyme reaction pathway controls its own production. Let us take the example of inhibition of the first-reaction of glycolysis. During the initial stages of glycolysis, glucose in the presence of enzyme hexokinase combines with ATP to produce glucose-6-phosphate and ADP. Increased concentration of glucose-6-phosphate inhibits the enzyme hexokinase.



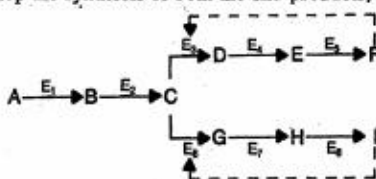
As the concentration of glucose-6-phosphate decreases, enzyme hexokinase becomes active again.

As you have read above, that metabolism involves multi-enzyme systems in which the product released by one enzyme is the substrate for the next enzyme. Often the products formed at the end of a pathway regulate the activity of the early enzymes in the metabolic pathway and affect the whole pathway. For example, in a pathway of five enzyme catalysed reactions, substrate A is converted into the end-product F. E_1 , E_2 , E_3 , E_4 , and E_5 are the enzymes involved in the pathway. Synthesis of product F can be regulated if it can combine with enzyme E_1 . In such a case, at high concentration of F, E_1 will be inhibited, whereas at low concentrations of F the metabolic pathway will proceed towards the formation of F. The first irreversible reaction in a biosynthetic pathway is called the **committed step** and final product usually inhibits the enzyme which catalyse the committed step. The process in which an intermediate metabolite or an end-product inhibits its own production is called as **feedback inhibition** or **negative feedback control**.



Some of the metabolic pathways are branched at certain points. For example, substrate A may be converted either to end-product F or end-product I. In such a case, the feedback

inhibition would occur at the branch point, i.e., end-product F will inhibit enzyme E_3 , whereas end-product I will inhibit enzyme E_6 . The inhibition of enzyme E_3 will not in any way affect the formation of end-product I and vice versa. However, feedback inhibition before the branch point will stop the synthesis of both the end-products, i.e. F and I.



An example of feedback inhibition in a pathway is the effect of cytidine triphosphate (CTP), one of the final products formed during pyrimidine synthesis, by enzyme **aspartate transcarbamoylase (ATCase)**. In the initial stage of the metabolic pathway, carbamoyl phosphate and aspartic acid interact to form carbamoyl aspartate in the presence of ATCase. This reaction is followed by four more intermediate steps, culminating in the formation of CTP. When CTP becomes higher in concentration, it inhibits the enzyme ATCase and thus the formation of carbamoyl aspartate. At low concentrations of CTP the formation of carbamoyl aspartate proceeds uninhibited (Fig. 10.2).

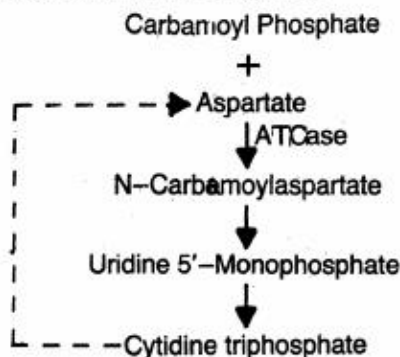


Fig. 10.2: Feedback inhibition of enzyme aspartate transcarbamoylase by the end-product cytidine triphosphate.

End-product regulation at the initial stages of a reaction pathway has its own significance. Thus the accumulation of intermediate compounds, which may not be required by the cell or organism, is avoided. Also, since all the reactions dissipate some energy into a useless form, less energy is wasted if intermediate reactions are inhibited.

ii) **Allosteric Enzyme Regulation:** As you have read above, that feedback inhibition is one of the ways in which enzyme activity can be regulated. Not only the end-products of the reaction pathways but other metabolites also may bind to the enzyme and alter the enzyme activity. The binding can occur at the **active site** or at the **regulatory site** on the enzyme surface as in the case of **allosteric enzymes**. You have read about allosteric enzymes and their mode of functioning in Unit 9. As you know, allosteric enzymes have a regulatory binding site apart from the active site. The active site binds with the substrate and converts the substrate into a product, whereas the regulatory site binds with another molecule called an **effector**, which influences the enzyme activity. It is now known that enzymes at the initial stages of metabolic pathways are usually allosteric enzymes. The regulatory sites of these enzymes are specific to the product of the metabolic pathway. As the metabolism proceeds, the concentration of the end-product increases. The end-product molecules act as negative effectors and bind to the regulatory sites of the allosteric enzyme resulting in conformational changes in the active site, thus inactivating the enzyme. The effectors bind non-covalently to the regulatory site of the enzyme. Therefore, inactivation of enzymes is reversible. Unbinding of the negative effector converts the inactive enzymes to their active form. You have read in Unit 9, that binding of a positive effector molecule to the regulatory site of the allosteric enzymes converts the inactive enzyme to its active form. This is brought about by changes in the conformational structure of the enzymes. You have already studied about the enzyme aspartate transcarbamoylase (ATCase). It is a good example of allosteric regulation. The end-product of the pathway, i.e., CTP acts as negative inhibitor. The end-product CTP binds to the allosteric site of the enzyme inhibiting the activity of the enzyme. The inhibited activity of the enzyme is again stimulated by the binding of ATP

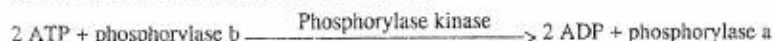
molecule at its regulatory site. Thus, ATP acts as a positive effector and it seems that both CTP and ATP compete for the same allosteric sites on the enzyme. Often in the case of positive allosteric effectors, the deficiency in the end-products of the reactions leads to accumulation of certain metabolites in the cell. These metabolites act as positive effectors and enhance the enzyme activity.

SAQ 1

Fill in the blanks with appropriate words.

- Metabolites are theproducts in a pathway.
- Regulation mainly depends upon the.....of the enzyme.
- Increased concentration of the end-product.....its own
- Inhibition.....the branching point of a pathway will stop the synthesis of all the end-products.
- Often the enzymes at the initial stages of metabolic pathways are
- Effectors bind to thesite of the allosteric enzyme.

iii) **Covalent Modulation:** Enzyme activity is also controlled by covalent modulations of the enzymes. Covalent modifications cause conformational changes in the enzymes. These conformational changes are brought about by covalent bonding of a phosphate group (**phosphorylation**) to the polypeptide chain or by removal of a small polypeptide chain by a process known as **proteolysis**. These covalent modifications stimulate the enzyme activity. For example the conversion of glycogen into glucose-1-phosphate is catalysed by an enzyme **glycogen phosphorylase**. Glycogen phosphorylase occurs in two forms: **phosphorylase a** the active form and **phosphorylase b**, the relatively inactive form. These two forms are interconvertible. Phosphorylase b is converted into active phosphorylase a by covalent binding of phosphate groups. The enzyme involved in this conversion is **phosphorylase kinase**.



The process is reversible as the phosphate groups can be removed hydrolytically (dephosphorylation) by an enzyme called **phosphorylase phosphatase**.

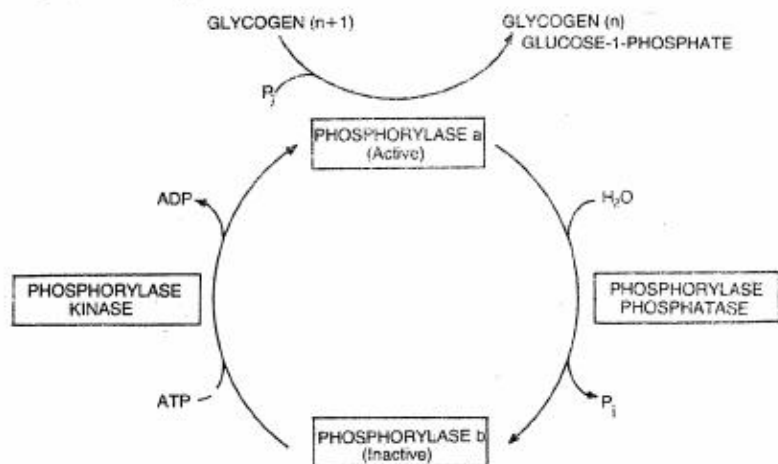
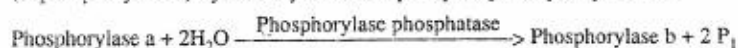


Fig 10.3: Activation and inactivation of enzyme phosphorylase.

Thus the cell regulates the rate of glycogen utilisation according to the requirement of the organism. Covalent bonds are strong and as a result covalent modifications are fairly stable.

As you have read above, in some cases covalent modifications are caused by proteolysis and are irreversible. A number of digestive enzymes including pepsin, trypsin and chymotrypsin belong to this group. These enzymes are produced in the inactive forms, known as **zymogen or proenzymes**. These inactive proenzymes are converted into active forms by the removal of a small peptide chain. For example, the digestive enzymes trypsin and pepsin are synthesised in their inactive forms **trypsinogen** and **pepsinogen** respectively and become active only when they reach the gut lumen. Pepsinogen, in the acidic gastric juice of

the stomach, hydrolyses at several points to shed a small number of peptides and becomes the active enzyme pepsin. Trypsinogen is converted to active trypsin in the duodenum of the alimentary canal. This is brought about by the removal of six amino acids from the N-terminus of the inactive enzyme. The proteolysis of trypsinogen is done by another enzyme known as **enterokinase**. Fig. 10.4 shows that the active site of the enzyme is unable to bind the substrate until certain peptides of the proenzyme are cleaved. After the cleavage of peptides, the rest of the molecule reorganises to create active site for the binding of a substrate molecule. The formation of digestive enzymes in the inactive proenzyme form is a way to protect the cells synthesising them from autodigestion. If the digestive enzymes are synthesised in their active form, autodigestion of the cells producing these enzymes would be unavoidable.

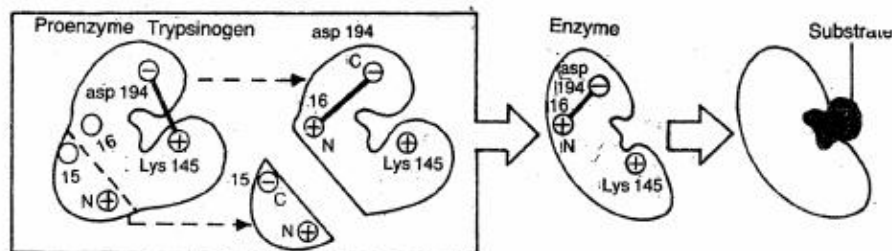


Fig. 10.4: Activation of proenzyme/trypsinogen by cleavage of peptide bonds linking few amino acids.

10.2.3 Regulation by Hormones

In the above subsections you have studied about the regulation of enzyme activity at cellular level. However, in higher organisms integrating systems like nervous and hormonal systems, exert their regulatory effect on the metabolic activities at the molecular level. In this subsection we will deal with the hormonal regulation of the metabolic activities of the cell.

Hormones are the chemical messengers which are produced and secreted by one cell or tissue and act on distant target tissues/cells by regulating the enzyme activity. For example, under conditions of stress or low blood sugar concentrations, hormone **adrenalin** stimulates the breakdown of stored glycogen into glucose. This increases the free sugar level in the blood which makes more energy available for the body to withstand the stress. If the sugar level in the blood decreases under normal condition, i.e., non-stress conditions, another hormone **glucagon** stimulates the glycogen breakdown thus restoring the normal blood sugar level. This is important as the decrease in normal sugar levels may ultimately lead to irreversible brain damage.

On the basis of their structures hormones fall under two categories: lipid derivatives (**Steroid hormones**) such as sex hormones and (**Protein hormones**) modified amino acids or small proteins such as adrenalin, glucagon and ADH. Lipid derived hormones pass through the cell membrane into the cytosol as they are relatively small and lipid soluble. In the cytoplasm these hormones combine with certain metabolites called **receptor molecules** and form a **hormone receptor complex**. This complex moves into the nucleus where it activates the related genes for enzyme synthesis. You have already studied the genetic control of enzyme activity.

Other hormones which are proteinaceous in nature are called '**primary messengers**'. They are unable to pass through the plasma membrane so they bind to the specific receptor site on the cell surface of the target cell. This binding is a signal that increases the concentration of another metabolite in the cytoplasm called '**secondary messenger**'. The secondary messenger activates the related enzyme. Fig 10.5.

The two secondary messengers known till now are cyclic adenosine monophosphate (cAMP) and Ca^{2+} . Ca^{2+} acts as secondary messenger in all living organisms whereas cAMP is restricted to animal cells and bacteria.

As shown in Fig. 10.5 the primary messenger, i.e. hormone adrenalin binds with the adrenalin receptor sites associated with enzyme **adenylate cyclase**. As a result of this binding the enzyme promotes the conversion of adenosine phosphate (ADP) into cAMP. In the cytoplasm, cAMP acts as a secondary messenger which binds to and activates enzyme protein kinase. The activated protein kinase causes the activation of phosphorylase kinase

enzyme which in turn converts the inactive form of glycogen phosphorylase into its active form. This increases the rate of glycogen breakdown into glucose. In Table 10.1 you will find some hormonal responses associated with elevation in intracellular cyclic AMP levels.

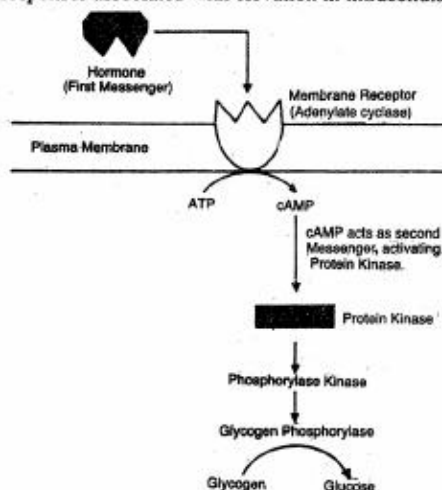


Fig. 10.5: Mechanism of action of primary messenger (hormone) and the secondary messenger (cAMP).

Table 10.1: Some Hormonal Responses Associated with Elevations in Intracellular cyclic AMP Levels

Hormone	Tissue	Response
ACTH	Adrenal	Hydrocortisone formation
TSH	Thyroid	Thyroxine formation
LH	Ovary	Progesterone formation
Vasopressin	Kidney	Water resorption
Glucagon	Adipose tissue	Lipid breakdown
Glucagon	Liver	Glycogen breakdown
Parathormone	Kidney	Phosphate excretion
Parathormone	Bone	Calcium resorption
Secretin	Intestine	Pancreatic enzyme release
Epinephrine	Cardiac muscle	Increased contractility
Epinephrine	Liver	Glycogen breakdown
Epinephrine	Erythrocyte	Increased Na permeability

SAQ 2

- a) Comment briefly on the following in the space provided:
Secondary messenger:

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- b) Tick mark () the correct statements and (x) the wrong statements in the space provided
- i) Lipid derived hormones bind to the receptosite of the cell surface ()
 - ii) Proteineceous hormones are secondary messengers ()
 - iii) Enzyme quantity is regulated at the genetic level ()
 - iv) Constitutive enzymes are always present the cell ()

10.2.4 Control by Calcium

In the previous section you have studied that protein hormones regulate enzyme activity through cAMP, the secondary messenger. Calcium also plays a fundamental role in the regulation of enzyme activity. It is also considered a secondary messenger.

In eukaryotes, calcium is involved in the regulation of processes like, contraction, secretion, endocytosis, and more general processes such as cell motility, cell growth and cell division. The enzymes are regulated by a calcium binding protein of small, lower molecular weight, that modulates the effect of cytoplasmic calcium. This protein is called **calmodulin**.

Calmodulin is present in the cytoplasm of all tissue cells except the nucleus. It is highly concentrated in the brain and probably plays an important role in the transmission of nerve impulses.

Calmodulin undergoes conformational changes after binding with calcium and alters the activities of many of its target enzymes. For example, the calcium-calmodulin complex regulates phosphodiesterase, the enzyme that hydrolyses cAMP to 5'AMP. Calcium-calmodulin complex also stimulates adenylate cyclase of the brain, Ca^{2+} ATPase of erythrocytes and phosphorylase b kinase and myosin kinase in the cell.

10.2.5 Cell Compartmentation

Till now you have studied various mechanisms by which activities of enzymes are regulated. The mechanisms you have studied so far were chemical in nature, related to the physiology of the organisms. In this sub section we will study about a mechanism in which regulation of enzyme activity is achieved by physical separation of the enzyme systems. In eukaryotes one of the regulatory mechanisms is the separation and localisation of specific groups of enzymes in membrane bound compartments in the cell. Different metabolic pathways take place in these different intracellular locations which are called organelles. For example, in both animals and plants, enzymes involved in **Kreb's cycle** are physically separated from those of glycolysis. **Kreb's cycle** is confined within the mitochondria and glycolysis takes place in the cytosol (Fig. 10.6). Prokaryotic cells contain no compartments, yet there is some degree of segregation of certain enzymes in bacteria, e.g., the enzymes participating in the biosynthesis of phospholipids are located in the cell membrane.

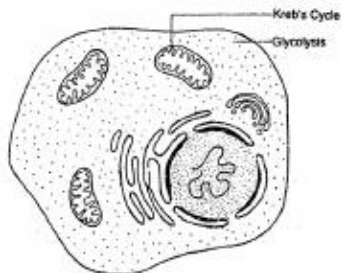


Fig. 10.6: Compartmentation in the cell. **Kreb's cycle** occurs in mitochondria and glycolysis takes place in the cytosol.

Localisation of various metabolic processes in the cytosol or in the organelles enables the regulation of these processes independent of processes proceeding elsewhere. But at the same

time translocation of metabolites across the membranes of the compartments is achieved via shuttle mechanisms which convert the metabolite into a permeable form, so that it can move cross the membrane and be transported out. Once the metabolite is outside the compartment, it is converted back to its original form. You have already read about the shuttle processes in Unit 7. Such types of interconversion, for example, interconversions of metabolites in mitochondrial and cytosolic form during Kreb's cycle and glycolysis, are carried out with the help of enzyme catalysis. As these enzymes are physically separated by the compartmental barrier, they are regulated independently.

Before we proceed further try the following SAQ and see.

SAQ 3

- a) In the space given below write very briefly about the two ways in which enzymes can be covalently modified to attain their active form

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- b) Match the items in Column I with the statements given in Column II

Column I		Column II
i) Reversible process	[]	a) various metabolic pathways are localized in the organelles
ii) Regulation in eukaryotic cells	[]	b) certain enzymes are synthesised into their inactive form
iii) Shuttle mechanism	[]	c) metabolites are transported out of the compartments by being converted into permeable form
iv) Proenzymes	[]	d) covalent modification of enzymes caused by phosphorylation

The study of enzymes in the intact animal organ, or organelles and a solution form have provided valuable information about metabolic processes. Therefore it become important to understand and appreciate the experimental approach and techniques used in the studies of biochemical processes. In the following section we will discuss various techniques used study various processes related to cell life .

10.3 TECHNIQUES FOR THE STUDY OF METABOLIC PATHWAYS

The complex biochemical processes of metabolic pathways can be studied in intact animals or in isolated components such as organs, cells and organelles. The initial biochemical researches done on humans and other organisms were performed at the level of whole organisms. Mostly the studies on respiration and nutrition were performed on whole organisms. It was realised soon that whole organisms were too complex to explain many questions. For instance, administration of glucose to a whole organism leads to its mixing with the glucose present in the body, and we cannot know what is the fate of administered glucose since both are alike in structure. Therefore, simpler *in vitro* preparations were developed in order to understand complicated biochemical processes. Various types of preparations have been summarised in decreasing order of complexity in Table 10.2. As in the case of whole animal studies, other preparations also have their own limitations. There is every possibility that *in vitro* preparations might also give incorrect results. For example, during isolation of cellular components the enzyme in the cell homogenate may partially digest the cellular components and thus wrong information may be given.

Table 10.2: Various Types of Preparation Used to Study Biochemical Processes Arranged in their Decreasing Order of Complexity.

Method	Comment
Studies at the whole animal level	This can include (1) removal of an organ (e.g. hepatectomy). (2) alterations of diet (e.g. fasting-feeding) (3) administration of a drug (e.g. phenobarbital). (4) administration of a toxin (e.g. carbon tetrachloride) (5) use of an animal with a specific disease (e.g. diabetes mellitus). (6) use of sophisticated techniques such as NMR spectroscopy and position emission tomography
Isolated perfused organ	Liver, heart, and kidney are particularly suitable.
Tissue slice	Liver slices have been specially used.
Use of whole cells	(1) Particularly applicable to blood cells, which can be purified relatively easily. (2) Use of cells in tissue culture is indispensable in many areas of biology.
Homogenate	(1) Ensures a cell-free preparation. (2) Specific compounds can be added or removed (eg. by dialysis) and their effects studied. (3) Can be subfractionated by centrifugation to yield individual cell organelles.
Isolated cell organelles	Extensively used to study the function of mitochondria, the endoplasmic reticulum, ribosomes, etc.
Subfractionation of organelles	Extensively used e.g. in studies of mitochondrial function.
Isolation and characterisation of metabolites and enzymes	A vital part of the analysis of any chemical reaction or pathway.

We will discuss various methods mentioned in Table 10.2 in the following subsections.

10.3.1 Studies with Whole Organisms

Many biochemical studies have been conducted on experimental animals like rats, mice, guinea pigs, and on plants like algae, moulds, mosses, tobacco, wheat, soyabean, pisum etc. As these organisms are small, it is less expensive to maintain them. They grow well and multiply fast under laboratory conditions. Furthermore, such organisms have rapid metabolic rate and they respond efficiently to the change in physiological conditions.

To investigate metabolic pathways of these organisms specific chemicals, like enzymes, hormones and regulating or inhibiting drugs are administered into the animals via the oral or nasal route or through injections. For plants, such chemicals are infused into the medium in which they grow. Later, the effect of these treatments is tested by analysing the blood samples of animals and sap samples of plants or by studying the histology or histochemistry of the tissues in question.

10.3.2 Studies with Microorganisms

Metabolic and nutritional studies using microorganisms such as bacteria, viruses, yeasts, moulds and bacteriophages (bacterial viruses) have been used in several areas of biochemistry and genetics. The information obtained by the studies on certain lower organisms and viruses have been useful for biochemical studies on humans. For example the studies on the regulation of enzyme and gene activities carried out on bread mould and bacteria have been extended to higher organisms. Microorganisms such as bacteria and viruses, are suitable for such studies. Because of their rapid multiplication rate and suitability for genetic studies

they are less complex in their structure having only one chromosome. The study of human cancerous cells has become possible by the information obtained from the viral genes responsible for certain types of cancer in animals. Nutritional studies have shown that microorganisms exhibit a wide range of nutrient requirements and vary from autotrophs to heterotrophs and the results have been useful in understanding the nutritional requirements of higher organisms as well.

10.3.3 Studies with Perfused Organs

In the perfusion technique, metabolism is studied by analysing the arterial blood entering into an organ and the venous blood leaving that organ. The difference between these two samples of the blood will reveal the metabolic processes taking place in the organ. Perfusion is possible in organs which have well defined arterial and venous connections like the liver, kidneys, adrenals, muscles and the brain. These organs may be retained inside the body (*in vivo*) or isolated (*in vitro*). The fluid which is drawn out for analysis is known as perfusion fluid, which is usually the animal's own blood. Artificial substitutes like Krebs's Ringer Saline, are also used as perfusion fluid. The Krebs's Ringer Saline is a solution with known quantities of substrate and other metabolites is perfused into the organ. Differences in the concentration of these substances in the arterial and the venous blood of the organ exhibit the amount of these substances used, up in the metabolic processes. The conditions of perfusion, such as infusion rate, pressure of perfusion, pH, temperature and oxygen tensions of the perfusion fluid can be modified suitably according to the need.

10.3.4 Studies with Tissue Slices

In this technique, the tissue is dissected out of the animal and frozen rapidly in order to keep the tissue alive and also to make slices of the tissue. The frozen tissue is sliced in a special instrument known as cryostat. The frozen slices of the tissue are brought to room temperature (thawing) and are incubated in the presence of oxygen (aerobic incubation) with various substrates. For example to study the activity of succinate dehydrogenase, an enzyme involved in Krebs's cycle, succinate is used as a substrate in the phosphate buffer medium along with coenzyme NAD. If the tissue slices contain active succinate dehydrogenase it converts the substrate succinate into fumarate. At the end of the incubation we find decreasing concentration of succinate and increasing concentration of fumarate. This technique was introduced by Warburg during his studies on respiration in tissues. Slices of liver tissues have been extensively used for such studies. This technique is also used to study metabolic pathways in plant tissues. For example, leaf tissues are used in studying the effect of phyto-hormone cytokinin on the phenomenon of aging in plants. By this method one can study the effect of various organic materials on the enzyme system as well as the effect of changes in the substrate concentration on enzyme kinetics. You will find that as the substrate gets utilised, the concentration of the substrate declines and that of the product rises. You have read about enzyme Kinetics in Unit 9.

10.3.5 Subcellular Fractions

You have read above in sub section 10.2 that metabolic pathways in eukaryotes are compartmented in different cellular organelles. In order to study the various metabolic pathways, it is important to isolate the cellular organelles as well as biomolecules in the pure form.

The organelles and biomolecules can be isolated from tissues or cells by centrifugation process. To isolate an organelle or biomolecule from the cell, it is necessary to disrupt the cell. The disruption of the cells is done under mild conditions (such as low pH, temperature and pressure). At extreme conditions of pH, temperature and pressure the organelles and biomolecules may lose their biological activity. Therefore, most of the isolation procedures are performed at about -4°C . At -4°C the digestive enzymes liberated during the processes become inactive and so do not act upon the other cellular components. Animal or plant tissues are disrupted by the process of homogenisation in a suitable homogenising medium. The most commonly used homogenising medium is an isotonic sucrose solution with mild conditions of pH, temperature and osmotic pressure. Sucrose is used because it does not pass through the membrane easily thus preventing internal organelles, such as mitochondria and chloroplasts from swelling. Organic solvents are used for isolation of lipids and nucleic acids.

The subcellular components of the homogenate are separated by the process of centrifugation. The process of centrifugation has been discussed in detail in Block 1. The subcellular organelles or biomolecules which differ in size, specific gravity and molecular

weight, sediment at different rates in the centrifugal field. This can be isolated from the homogenate. This process is known as **differential centrifugation**. The various biomolecules or organelles thus isolated in this way can be tested for their ability to catalyse a given metabolic activity. By using such techniques, it has been possible to know that various pathways are localised in different parts of the cell. For example, all the enzymes involved in the conversion of glucose into lactate are present in the cytosol, whereas the enzymes of the citric acid cycle are located in the mitochondria.

SAQ 4

List the various preparations to study the metabolic pathways in decreasing order of complexity and discuss briefly any one of them.

Apart from the above mentioned techniques of studying metabolism, use of mutants and isotopes have contributed a lot in biochemical studies. We will briefly discuss these in the following subsection.

10.3.6 Use of Mutants

Mutants are organisms which, due to some genetic disorder, fail to synthesise certain proteins. Study of such mutant organisms in which a given enzyme fails to be synthesised is one of the important ways of studying the metabolism. Such a type of defect often results in the accumulation and excretion of the substrate of the defective enzyme. This enables us to understand steps in the metabolic pathways. Since such genetic disorders are rare, systematic study of metabolic pathways is difficult. However, genetic defects can be produced at will in microorganisms by subjecting them to mutagenic agents, such as irradiation with x-rays or treatment with certain chemicals. These treatments can alter the structure of specific genes in the DNA and thus produce mutants. Such mutant microorganisms with their defective enzymes are powerful tools for studying metabolism.

We can study the uses of mutants by taking the example of cells of the common mold **Neurospora crassa** which can grow on a simple medium containing glucose and ammonia. However, if **Neurospora** spores are irradiated with x-rays, the mutants obtained are no longer capable of growing on a medium containing just glucose and ammonia. Such mutants will grow only if specific metabolites are added to the medium. For example, some **Neurospora** mutants will grow normally if arginine is added to the medium. In such mutants the enzyme responsible for the synthesis of arginine from ammonia is defective or lacking. Such a type of 'arginine less' mutant can be used to identify intermediates in the sequence of enzyme catalysed reactions leading to the synthesis of arginine.

10.3.7 Use of Isotopes

An isotopic form of an element is used to label a given metabolite to trace its course in a metabolic pathway. This labelling can be done either by a radioactive isotope or by a stable isotope. For example ^{14}C , the radioactive isotope of carbon atom C, is used to label the specific carbon atom in an organic compound. Although such labelled molecules are chemically the same as formal unlabelled molecules, they can be easily detected and measured due to the radioactive labelling. Isotopically-labelled compounds are used to investigate many aspects of the metabolism of proteins, carbohydrates and lipids. The principal isotopes mainly used in biochemical research are shown in Table 10.2. You have already read about the use of isotopes in Block 1.

Table 10.3: Isotopes Used in Metabolic Studies

Stable Isotopes	Radioactive Isotopes
D ²	H ³
N ¹⁵	C ¹⁴
O ¹⁸	P ³²
	S ³⁵
	Ca ³⁵
	I ¹²⁵
	I ¹³¹

SAQ 5

Fill in the blanks with appropriate words.

- are the genetically defective organisms.
- Failure of the enzyme synthesis in an organism results inandof the substrate.
- form of an element is used labelling the metabolite.
-sotope of carbon atom is used in the organic compounds.

10.4 SUMMARY

In this unit you have studied that:

- all enzyme catalysed reactions involved in the metabolic processes of the cell are controlled by regulating enzyme activity.
- the enzyme activity can be regulated by rendering the enzyme inactive or by controlling the enzyme quantity. Enzyme quantity is controlled at the genetic level. Structural genes, which code for enzyme synthesis are inactivated by the binding of repressor at the operator site of the chromosome. Feedback regulation is the process in which the end-product or an intermediate metabolite of a metabolic pathway binds with the enzyme and regulates its activity at the initial stage of the reaction pathway.
- the effector or regulator metabolite binds to the regulatory site of the allosteric enzyme producing conformational changes in the structure of the enzyme.
- covalent modifications by phosphorylation or by proteolysis cause conformational changes in the enzyme. Such types of modification convert the inactive form of enzymes into active forms. Localisation of metabolic pathways into membrane bound cellular compartments also help to regulate enzyme activity.
- enzyme activity in higher organisms is influenced by the hormones produced in their body.
- identification and estimation of various biomolecular reactions involve various techniques. Various experiments to study the metabolic pathways have been conducted on organisms at different level of organisation.
- the study of metabolic processes at whole animal levels like on humans, did not yield sufficient information because of their complexity. Simpler, *in vitro* preparations of organs, tissues, cells, organelles and biomolecules were developed to avoid the complexity of experimental procedures.

10.5 TERMINAL QUESTIONS

- 1) Explain briefly the operon model for control of enzyme quantity.

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- 2) Discuss briefly what you understand by feedback inhibition.

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- 3) Compartmentation regulates metabolic pathways. Comment briefly in the given space.

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- 4) Use of mutants is an important approach to studying metabolic pathways. Comment briefly.

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- i) i) transformation
ii) specificity
iii) inhibits, production
iv) before
v) allosteric enzymes
vi) regulatory

- 2) a) Phosphorylation: The addition of a phosphate group to an inactive form.
Proteolysis: Removal of a small peptide from the inactive enzyme converts it into active form.
- b) i) c, ii) a, iii) c, iv) b
- 3) a) Secondary messengers are the metabolites produced in the cytoplasm of the cell as a result of the binding of primary messengers i.e. hormones to the receptor site on the cell surface.
- b) i) ×, ii) ×, iii) ✓, iv) ✓
- 4) Whole organism, microorganism, perfused organs, tissue slices, subcellular fractionations. Any of these can be discussed by giving the example.
- 5) i) Mutants
ii) accumulation, excretion
iii) Isotopic
iv) Radioactive

Terminal Questions

- 1) Operon model consists of repressor gene, promoter gene, operator gene and structural genes on the operon. In the absence of an inducer, the repressor gene synthesises a repressor protein which binds with the operator thus preventing the structural genes from enzyme synthesis. Inducers otherwise bind with a repressor protein which causes structural genes to synthesise enzymes.
- 2) Feedback regulation is a process in which the product of a reaction or a pathway inhibits its own production. In the branched metabolic pathways, the end-product can inhibit its own production or the production of all the other products.
- 3) Localisation of various metabolic pathways in the membraneous compartments regulate metabolic processes. Firstly, various metabolic pathways are segregated and are carried out independently. Secondly, the translocation of metabolites from the organelle is carried out with the help of enzymes, thus providing a further step for regulation as these enzymes are physically separated.
- 4) Mutants are the organisms in which a given enzyme fails to be synthesised in its active form due to a genetic disorder. This causes the accumulation and extraction of the substrate of that enzyme which enables us to understand certain steps in metabolism.

GLOSSARY

Actin: A protein constituting the thin filaments of muscle, also found in other types of animal cells.

Activation energy: The energy barrier that blocks the tendency of a set of chemical substances to react. A reaction is speeded up if this energy barrier is surmounted by adding heat energy or if the barrier is lowered with the aid of catalysts.

Active site: The region of an enzyme surface that binds the substrate molecule and transforms it.

Active transport: Energy requiring transport of a solute across a membrane in the direction of increasing concentration.

Aerobic: Occurring in the presence of oxygen.

Amphipathic molecule: A molecule having both hydrophilic and hydrophobic regions.

Anaerobic: Occurring without the use of molecular oxygen, O_2 .

Buffer: A system capable of resisting changes in pH, consisting of a conjugate acid-base pair in which the ratio of proton acceptor to proton donor is near unity.

Capsid: The protein coat of a virus.

Catalyst: A chemical substance that accelerates a reaction without itself being consumed in the overall course of reaction. Catalysts lower the activation energy.

Cell Wall: A relatively rigid structure that encloses cells of plants, fungi, many protists and most bacteria. The cell wall gives the cell its shape.

Cellulose: A straight chain polymer of glucose molecules used by plants as a structural supporting material.

Coenzyme: A non-protein organic cofactor required for the action of certain enzymes; often contains a vitamin as a component.

Cofactor: A low molecular weight, heat stable inorganic or organic substance required for the action of an enzyme.

Collagen: A fibrous protein found extensively in bone and connective tissue of animals.

Conformation: The three-dimensional shape or form of a molecule.

Constitutive enzymes: An enzyme that is present in approximately constant amounts in a system, whether its substrate is present or absent.

Cytosol: The fluid portion of the cytoplasm excluding organelles and other solids.

Dehydrogenases: Enzymes that catalyse the removal of pairs of hydrogen atoms from their substrate.

Electron carrier: A protein, such as a flavoprotein or a cytochrome, that can gain and lose an electron reversibly and functions in the transfer of electrons.

Endergonic reaction: The reaction for which energy must be supplied.

Enzyme: A protein specialized to catalyse a specific metabolic reaction.

Exergonic reaction: A reaction in which free energy is released.

Facilitated diffusion: Passive movement through a membrane involving transport molecules; does not proceed against a concentration gradient.

Hormone: A chemical substance that is synthesised in trace amounts by an endocrine tissue and transported to other parts of the organism to regulate the functions of another tissue or organ.

Isotopes: Stable or radioactive forms of an element that differ in atomic weight but are otherwise chemically identical with the naturally abundant form of the element; used as tracers.

Isozymes: Chemically different enzyme that catalyse the same reaction.

Ligand: A molecule or ion that is bound to a protein.

Multienzyme System: A sequence of related enzymes participating in a given metabolic pathway.

Myosin: A muscle protein, the major component of the thick filaments.

Oxidation: The loss of electrons from a compound.

Plasma membrane: The membrane that surrounds the cell, regulating the entry and exit of molecules and ions. Every cell has a plasma membrane.

Prosthetic group: Non-protein part of the enzyme.

Steady state: A state of a system in which all components remain in constant concentration.

Svedberg (s): A unit of measure of the rate at which a particle sediments in a centrifugal field.

FURTHER READING

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- 3 Alberts, B: *Molecular Biology of the Cell*. Garland Publishing, Inc. 136, Madison Avenue, New York.
- 4 DeRobertis, E.D.P., DeRoberties, E.M.F : *Essentials of Cell and Molecular Biology* Eighth edition, Lea and Febiger, International Edition, Hong Kong, 1988
- 5 Phillip, S., Bianchi D.E: *Cell and Molecular Biology*, John Wiley and Sons, Inc. United States, 1987.
- 6 King, B: *Cell Biology* (Modern Views in Biology) Allen and Unwin Publishers Ltd. Inc. U.K., 1986.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE - 01

Block

Enrolment No.

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- 1) How many hours did you need for studying the units ?

Unit Number						
No. of hours						

- 2) How many hours (approximately) did you take to do the assignments pertaining to this block ?

TMA

CMA

Assignment Number		
No. of hours		

- 3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (✓) the type of difficulty and give the relevant page number in the appropriate columns.

Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

- 4) It is possible that you could not attempt some SAQs and TQs. In the following table are listed the possible difficulties. Kindly tick (✓) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	SAQ No.	TQ No.	Type of difficulty			
			Not clearly posed	Cannot answer on basis of information given	Answer given (at end of Unit) not clear	Answer given is not sufficient

- 5) Were all the difficult terms included in the glossary. If not please list in the space given below

Any other suggestion(s)

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To

The Course Co-ordinator (LSE-01, Cell
Biology) School of Sciences
Indira Gandhi National Open University
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New Delhi-110 068

NOTES



Block

3

METABOLISM AND COMMUNICATION IN CELLS

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BLOCK INTRODUCTION

So far you have studied the cell structure, including cell membranes and macromolecules present in the cell. Various techniques and tools employed for studying the cell have also been discussed. You have also studied the supramolecular structures and their assembly, the functions of biomolecules, and role of enzymes in catalysing the chemical reactions occurring in the cell.

In this block we will study metabolism; the sum of all the chemical activities of the organism, structure and function of nucleus, protein biosynthesis and how cells communicate with each other by specific signalling.

All organisms require energy to perform functions necessary to sustain life. They obtain this energy from food. The molecules of food breakdown during metabolism by a series of enzyme-catalysed reactions termed as energy releasing pathways during which the chemical bonds break and release energy. The pathways operate in cytoplasm or in specific organelles that have necessary enzymes to perform certain chemical reactions. In addition, the membranes of the organelles provide the surface upon which specific chemical reactions can occur. The process of energy release, and its utilisation is essentially same in plants, animals and microorganisms. In Unit 11 we will discuss different energy releasing pathways, their site and regulation.

Organisms also synthesise a variety of molecules and macromolecules which are mainly carbohydrates, fats, proteins and nucleic acids. The energy released from the degradation of food molecules is utilised in their synthesis. In Unit 12 on biosynthesis we discuss first the biosynthetic principles, and interrelationship of catabolic and anabolic pathways. Then we describe the synthesis and breakdown of carbohydrates namely glycogen and starch that are energy storing molecules in animals and plants respectively. We would also tell you briefly about the synthesis of fats.

In Unit 13, you will study the structure and functions of nuclear envelope and the organisation of DNA in chromosomal structure. This unit deals with the study of the central dogma regarding molecular biology, i.e. as to how the genetic information which is stored in the DNA base sequence is transcribed into RNA molecules which is further translated into protein molecules. The unit also describes the replication of DNA molecule. DNA replication is semi conservative and bidirectional and is catalysed by certain enzymes in prokaryotes and eukaryotes. In this unit you will study RNA synthesis in eukaryotes and prokaryotes and RNA processing in eukaryotes. Different types of RNAs are briefly discussed in this unit.

Unit 14 deals with the process of protein biosynthesis, in which the coded genetic information of the DNA is decoded to synthesise proteins. This unit also speaks about the processing of protein molecules that give a protein its final three dimensional functional form. You are aware that organisms maintain their internal environment in a dynamic steady state. This unit also discusses various mechanisms regulating protein biosynthesis.

In Unit 15, we have discussed chemical signalling between the cells. Cells in multicellular organisms communicate with each other. This is essential for their development, growth, division, organisation and for coordination of various cellular activities. Cells communicate directly through gap junctions or through plasma membrane bound signalling molecules. Indirect communication between the cells is through cell secretions which influence distantly located target cells. Local chemical mediators, hormones and neurotransmitters are the three types of signalling molecules. Cell surface receptors and intracellular receptors in the target cells recognise and bind the signal molecules and elicit specific responses from the target cells. Chemotaxis is the movement of cells as a result of the response due to the specific chemical agents in the medium. Target cells show various adaptations after being exposed to signal molecules for a longer period.

In the last block we will discuss cell division, cell movement and differentiated animal and plant cell types.

Objectives

After reading this block you shall be able to:

- describe major energy-releasing pathways, their site in the cell and regulation,
- explain biosynthetic principles and interrelationship of catabolic and anabolic pathways,
- describe biosynthesis of energy providing carbohydrates and fats,
- state the role of nuclear envelope in transfer of proteins and RNA molecules,
- describe DNA replication, transcription, translation and the enzymes involved in the processes,
- explain post-transcriptional and post-translational changes,
- list the strategies for chemical signalling between cells and adaptation of target cells.

UNIT 11 ENERGY RELEASING PATHWAYS

Structure

- 11.1 Introduction
 - Objectives
 - Study Guide
- 11.2 Some Basic Information
 - Functional Groups
 - Common Reactions between Functional Groups
- 11.3 Energy Releasing Pathways — An Overview
- 11.4 Glycolysis
 - Reactions of Glycolysis
 - ATP Yield of Glycolysis
- 11.5 Oxidative Decarboxylation of Pyruvate
- 11.6 Tricarboxylic Acid Cycle
- 11.7 Electron Transfer Chain and Oxidative Phosphorylation
- 11.8 Substrate Level Phosphorylation
- 11.9 Pentose Phosphate Pathway
- 11.10 Regulation
- 11.11 Degradation of Lipids and Proteins
 - Lipids
 - Proteins
- 11.12 Summary
- 11.13 Terminal Questions
- 11.14 Answers

11.1 INTRODUCTION

In the previous unit we have studied the various methods used to investigate metabolic pathways and we have also learnt that regulation is brought about by controlling the regulatory enzymes. In this unit we will learn some basic catabolic pathways and their mode of regulation which apply to most organisms.

All organisms require energy for growth, maintenance and reproduction. Cells need energy for mechanical work, for driving various chemical reactions and for transport of molecules. In certain organisms, cells require energy for special purposes, for example, light production (bioluminescence) by firefly tails, electric rays in some fish.

When plants and animals respire, oxygen oxidises the food molecules to carbon dioxide and water with the release of energy. Although this oxidation is often compared with the burning of wood because of the common end products — carbon dioxide, water and energy, but respiration is a much more complex process and occurs in a number of successive stages. Hence, besides energy, a large number of chemical intermediates are formed that are also used as building blocks in various biosynthetic processes.

Respiration requires oxygen and cannot be performed by all types of organisms. Probably, you are aware of anaerobic bacteria — that survive in the absence of oxygen. In animals not all the cells can use oxygen all the time or even some of the time. For instance, our muscle cells under resting condition utilise the machinery of respiration because plenty of oxygen is available, but under strenuous exercises such as running, the energy demand far exceeds the body's capacity to deliver oxygen to the cells. Within a short time during vigorous exercise, oxygen is depleted in the muscle cells. But still energy is produced as long as the muscles work. Then, how is the energy produced in the absence of oxygen?

Some organisms survive on materials that are not commonly considered as food. For example, silver fish eat silk and wool, white ants eat wood, fungi grow on decaying matter. Have you ever wondered how they obtain energy from these varied substances for survival. Some bacteria live on hydrogen, hydrogen sulphide.

medium containing various chemicals. How do these chemicals serve as food and provide energy? Let us find out the answer to these questions.

In this unit we will focus on the metabolism of carbohydrates, fats and proteins that release chemical energy to drive the cells for various functions. We will once again come across the undeniable unity in plants and animals at biochemical level because we will see that most of the basic biochemical pathways are the same in all living organisms. In the following unit, we will study how the energy released and intermediates produced during the breakdown of food material drive the biosynthetic reactions in the cell.

Objectives

After reading this unit you shall be able to:

- identify the following types of reactions: oxidation, reduction, esterification, phosphorylation, carboxylation, decarboxylation, hydration, hydrolysis, condensation and polymerisation
- outline the break down of carbohydrates fats and proteins.
- outline the reactions of glycolysis and account for the output of energy in the form of ATP
- outline the reactions of tricarboxylic acid (TCA) cycle and its role in energy production
- describe how glycolysis and TCA cycle are linked to each other
- describe how the products of TCA cycle enter into the electron transport chain to produce chemical energy in the form of ATP
- explain the mechanism of regulation of glycolysis, TCA cycle and electron transport chain.

Study Guide

This and the following unit requires basic knowledge of organic chemistry. Therefore, we have included the section on basic information which is about different chemical reactions. This section is optional. But we advise you to go through this section as it would help you to understand the units faster. Unit 11 is a double unit. We did not want to split it because the different process in catabolism are related.

11.2 SOME BASIC INFORMATION

The chemical reactions in the cell are governed by the simple laws of organic chemistry. Before we learn about catabolic (breakdown) pathways, it will be helpful to revise some of the basic principles involved in chemical reactions.

11.2.1 Functional Groups

In school you have learnt that organic compounds are classified according to the nature of the functional groups they contain. An atom or a group of atoms which determines the physical and chemical properties of a molecule is said to be a functional group. In Table 11.1 identify the functional groups given and write their names in column 2. If you cannot identify some, fill in column 2 with answers given below the table.

11.2.2 Common Reactions between Functional Groups

In examining the chemical reaction between compounds we should focus our attention on their functional groups, as they participate in the reaction. We are describing some common chemical reactions in Table 11.2. Example of similar biochemical reactions which occur in the cell, are illustrated for each type of reaction.

- Oxidation reaction** : Oxidation reaction can be viewed most simply as the loss of hydrogen or gain of oxygen or loss of electron(s) by the organic reactant.
- Reduction reaction** is the reverse of oxidation. It involve gain of hydrogen, or loss of oxygen or gain of electrons.

In the cell, a reduction reaction is almost always coupled to an oxidation reaction. For example, the reduction of pyruvate to lactate is coupled to the oxidation of $\text{NADH} + \text{H}^+$ which donate electron and H^+ to pyruvate. Such coupled reactions are

	Formula	Name
1	$\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}- \\ \\ \text{H} \end{array}$	
2	$\begin{array}{cc} \text{H} & \text{H} \\ & \\ \text{H}-\text{C}- & \text{C}- \\ & \\ \text{H} & \text{H} \end{array}$	
3	$\text{C}_n\text{H}_{2n+1}$ or R-	
4	$-\text{CH}_2-$	
5	$-\text{OH}$	
6	$\begin{array}{c} \\ \text{H}-\text{C}=\text{O} \end{array}$	
7	$>\text{C}=\text{O}$	
8	$\begin{array}{c} \text{CH}_3-\text{C}- \\ \\ \text{O} \end{array}$	
9	$\begin{array}{c} \text{R}-\text{C}- \\ \\ \text{O} \end{array}$	
10	$-\text{COOH}$	
11	$-\text{NH}_2$	
12	$\begin{array}{c} \text{O} \\ \\ -\text{C}-\text{NH}_2 \end{array}$	
13		
14	$\begin{array}{c} -\text{C}-\text{OR} \\ \\ \text{O} \end{array}$	
15	$\begin{array}{c} \text{OH} \\ \\ -\text{P}-\text{O}-\text{R} \\ \\ \text{O} \end{array}$	
16	$\begin{array}{c} -\text{C}-\text{S}-\text{R} \\ \\ \text{O} \end{array}$	

Answers

1. methyl, 2. ethyl, 3. alkyl, 4. methylene, 5. hydroxyl, 6. aldehyde, 7. carbonyl
 8. acetyl, 9. acyl, 10. carboxyl, 11. amino, 12. amide, 13. phenyl, 14. ester
 15. phosphate ester, 16. thioester.

iii) **Hydration reaction** occurs when, H_2O is added to a double bond, such as by the addition of a H_2O molecule to an alkene in the presence of an acid catalyst. The product of the reaction is an alcohol.

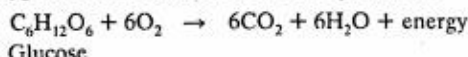
iv) **Dehydration reaction** results in the loss of water from the reactant to form a new product.

v) **Hydrolysis reaction** involves addition of elements of H_2O (H^+ and OH^- ion) resulting in breaking of the covalent bond of the molecule, thereby forming two molecules.

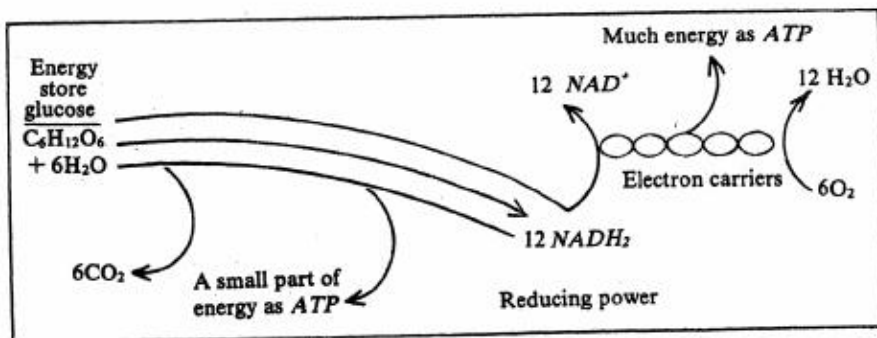
(v) Hydrolysis	$\text{CH}_3 - \text{COOR} + \text{HOH} \longrightarrow \text{CH}_3 - \text{COOH} + \text{ROH}$ ester acid alcohol $\begin{array}{ccc} \text{CH}_2 - \text{OOC} - \text{R} & & \text{CH}_2\text{OH} \\ & \xrightarrow{\text{HOH}} & \\ \text{CH} - \text{OOC} - \text{R} & & \text{CHOH} + 3 \text{R} - \text{COOH} \\ & & \\ \text{CH}_2 - \text{OOC} - \text{R} & & \text{CH}_2\text{OH} \end{array}$ triglyceride (lipid) glycerol fatty acid
(vi) Condensation esterification	$\text{R} - \text{COOH} + \text{HO} - \text{CH}_3 \rightarrow \text{R} - \text{COO} - \text{CH}_3 + \text{H}_2\text{O}$ acid methyl alcohol ester $\text{R} - \text{COOH} + \text{R}_1 - \text{SH} \rightarrow \text{R} - \overset{\text{O}}{\underset{\parallel}{\text{C}}} - \text{SR}_1 + \text{H}_2\text{O}$ acid thioalcohol thioester $\text{RCOOH} + \text{ATP} + \text{CoA} - \text{SH} \xrightarrow{\text{Mg}^{2+}} \text{R} - \overset{\text{O}}{\underset{\parallel}{\text{C}}} - \text{SCoA} + \text{AMP} + \text{P}_i + \text{P}_i$ acid Coenzyme A acyl CoA pyrophosphate $\text{HO} - \overset{\text{O}}{\underset{\parallel}{\text{P}}} - \text{OH} + \text{HOCH}_3 \longrightarrow \text{HO} - \overset{\text{O}}{\underset{\parallel}{\text{P}}} - \text{O} - \text{CH}_3 + \text{H}_2\text{O}$ Phosphoric acid methyl alcohol methyl phosphate $\text{CH}_3 - \text{CHO} + \text{CH}_3 - \text{CHO} \xrightarrow{\text{NaOH}} \text{CH}_3 - \text{CHOH} - \text{CH}_2 - \text{CHO}$ acetaldehyde acetaldehyde acetal $\text{R} - \overset{\text{O}}{\underset{\parallel}{\text{C}}} + \text{R} - \text{OH} \rightarrow \text{R} - \overset{\text{H}}{\underset{\mid}{\text{C}}} - \text{OH} + \text{ROH} \rightarrow \text{R} - \overset{\text{H}}{\underset{\mid}{\text{C}}} - \text{OR} + \text{H}_2\text{O}$ aldehyde alcohol Hemiacetal Acetal
(vii) Carboxylation	$\text{C}_2\text{H}_5\text{MgBr} + \text{CO}_2 \rightarrow \text{C}_2\text{H}_5\text{CO}_2\text{MgBr} \xrightarrow{\text{H}_2\text{O}} \text{C}_2\text{H}_5\text{COOH} + \text{MgOHBr}$ Grignard's reagent propionic acid $\text{CH}_3 - \overset{\text{O}}{\underset{\parallel}{\text{C}}} - \text{SCoA} \xrightarrow[\text{+ CO}_2]{\text{ATP} \rightarrow \text{ADP} + \text{P}_i} \text{OOC} - \text{CH}_2 - \overset{\text{O}}{\underset{\parallel}{\text{C}}} - \text{SCoA}$ acetyl CoA malonyl CoA
(viii) Decarboxylation	$\text{OOCCH}_2\text{C} - \text{COO} \longrightarrow \text{CH}_3\text{COOH} + \text{CO}_2$ malonic acid acetic acid $\begin{array}{ccc} \text{CH}_2 - \text{COO}^- & & \text{CH}_2 - \text{COO}^- \\ & \xrightarrow[\text{- CO}_2]{\text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+} & \\ \text{CH}_2 + \text{CoASH} & & \text{CH}_2 \\ & & \\ \text{O} = \text{C} - \text{COO}^- & & \text{O} = \text{C} - \text{SCoA} \end{array}$ α - ketoglutarate succinyl CoA

11.3 ENERGY RELEASING PATHWAYS — AN OVERVIEW

You know that during respiration living organisms convert glucose to carbon dioxide and water using oxygen. The overall reaction for respiration is



According to this equation, glucose is oxidised by both removal of hydrogen and addition of oxygen. But in fact, the two processes occur separately in the sense that glucose molecule does not react directly with free oxygen molecule available in the cell, as shown in the scheme below.



NAD⁺ or NADP⁺-dependent reactions involve the transfer of a hydride ion, a hydrogen nucleus with two electrons between substrate and coenzyme. Therefore, the reduced form is written as NADH + H⁺. However, for the sake of convenience it is also written as NAD and NADH₂.

This scheme shows that in the first phase, glucose is completely oxidised to CO₂, but no atmospheric oxygen is involved, and energy is released during the process. A small part of this energy is stored as ATP. The electrons and hydrogens of glucose are transferred to coenzyme NAD⁺ forming NADH₂. A large part of the energy is stored as NADH₂. In the second phase, the electrons from NADH₂ pass from one carrier to another releasing energy which is coupled with ATP formation. Finally, the electrons and H⁺ are taken up by O₂ to form H₂O. Thus, free oxygen reacts only at the last step of the overall process.

The overall scheme of the degradative or catabolic pathways operating in most organisms is shown in Fig. 11.1. It can be divided into four stages. In stage 1, polysaccharides, proteins and fats are broken down to their smaller precursor units — sugars, amino acids and fatty acids, respectively. In higher animals the initial breakdown of large molecules (1st stage) takes place in the digestive tract of animals and the products of digestion are transported to different cells. Plants do not take carbohydrates, protein and fats from outside. They make these in the plant cells, and break them when required. Thus, in plants stage 1 occurs in the cells. In stage 2, sugars and fatty acids are further degraded into pyruvate and acetyl CoA. Amino acids interact directly in stage 3. Bacteria, such as saprophytic decomposers survive on small molecules for energy, so they skip the first stage of degradation and begin from stage 2 or stage 3 according to the size of the food molecules. Pyruvate is converted to acetyl CoA which in stage 3 is completely oxidised to CO₂ via what is known as the tricarboxylic acid (TCA) cycle.

Uptil now, only a little energy is released through glycolysis in stage 2. In the TCA cycle carbon atoms of acetyl CoA are oxidised to CO₂, and the electrons and H⁺ (protons) are transferred to the carrier molecules NAD⁺ and FAD⁺, which are reduced. As a result of oxidation of acetyl CoA to CO₂ a large amount of energy is released and is stored in the reduced forms of NAD⁺ and FAD. At stage 4 of metabolism, NADH₂ and FADH₂ are oxidised and the resulting electrons hop through a series of carrier molecules. These electron carriers finally transfer electrons to oxygen thereby releasing the bulk of energy which is used for making ATP.

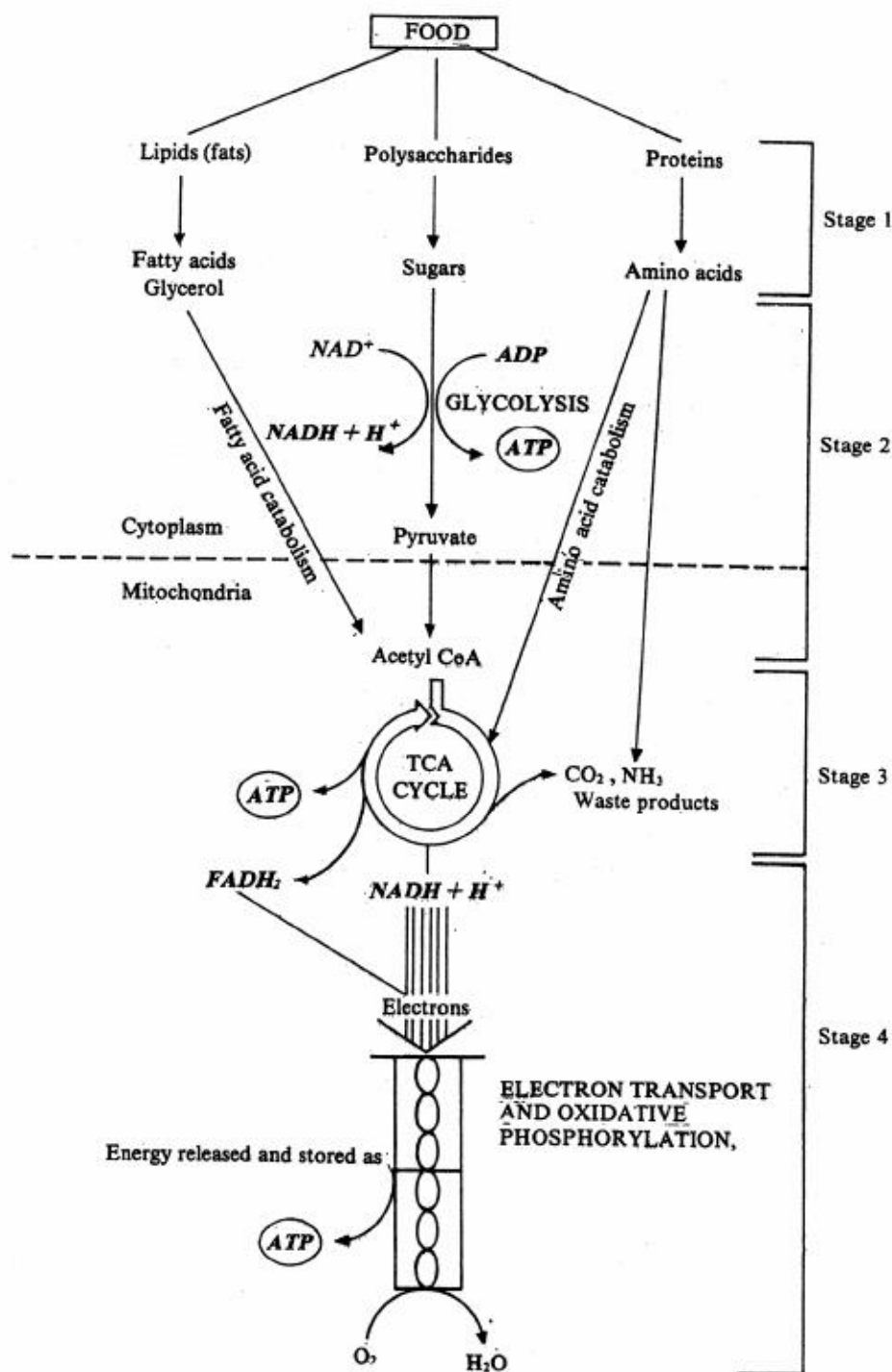


Fig. 11.1 : Four stages of metabolism for generating reducing power ($\text{NADH} + \text{H}^+$) and energy (ATP).

Before we study these stages in detail, you are requested to attempt the following SAQs.

SAQ 1

a) Match the following in the best possible way

- | | |
|--------------------------------------|---|
| i) Glycolysis | a) ATP synthesis |
| ii) TCA cycle | b) Formation of pyruvate |
| iii) Oxidative phosphorylation | c) Stage I of metabolism |
| iv) Oxidation of NADH_2 | d) Electron Transport chain |
| v) Protein breaks down to amino acid | e) Formation of NADH_2 and FADH_2 |

b) Match the following in the best possible way

- | | |
|--|-----------------------------|
| i) Polysaccharide breakdown | a) Cytoplasm |
| ii) Glycolysis | b) Mitochondria |
| iii) Formation of CO_2 | c) Electron transport chain |
| iv) Stages 3 and 4 | d) Stage I |
| v) O_2 is reduced to H_2O | e) TCA cycle |

c) i) Write the overall reaction of respiration

-
- ii) Break it into two equations, one showing the oxidation of glucose and formation of NADH_2 , the other showing the oxidation of NADH_2 and reduction of oxygen. Remember to include the energy component
-

-
- iii) Name two compounds in which the energy released on oxidation of glucose can be stored
-

- iv) The process of respiration contains many steps. At which step does molecular oxygen (also called free oxygen or atmospheric oxygen) react?
-

11.4 GLYCOLYSIS

In the metabolism of carbohydrates, polysaccharides and disaccharides are hydrolysed to different monosaccharides in stage 1. The monosaccharides, such as mannose, fructose, galactose, glucose etc. are then broken down (oxidised) to pyruvate through a series of reactions that fall under stage 2. To elaborate these reactions, we will take glucose as the starting monosaccharide because it is the main form in which cells utilise carbohydrates. The process of breakdown of glucose to pyruvate is known as glycolysis or the glycolytic pathway.

Glycolysis occurs in the cytoplasm of virtually all living cells, both in the absence and in the presence of oxygen. Since glycolysis takes place in all cells regardless of the absence of oxygen, probably it was the first energy releasing process in organism when the life evolved. The oxidative respiration in mitochondria of eukaryotic cells became possible only after molecular oxygen had accumulated in the earth's atmosphere as a result of photosynthesis by cyanobacteria.

In this section, we will learn about the reaction of glycolysis and the energy released and stored during this process.

11.4.1 Reactions of Glycolysis

The sequence of reactions of glycolysis are shown in Fig. 11.2 and a simplified scheme is illustrated in Fig. 11.3. The six-carbon glucose molecule is converted to two molecules of pyruvate in a series of ten enzyme catalysed reactions described below.

Let us study each reaction in Fig. 11.2, identify its type and fill in the spaces with the names of enzymes catalysing it. For example, the first reaction can be described as:

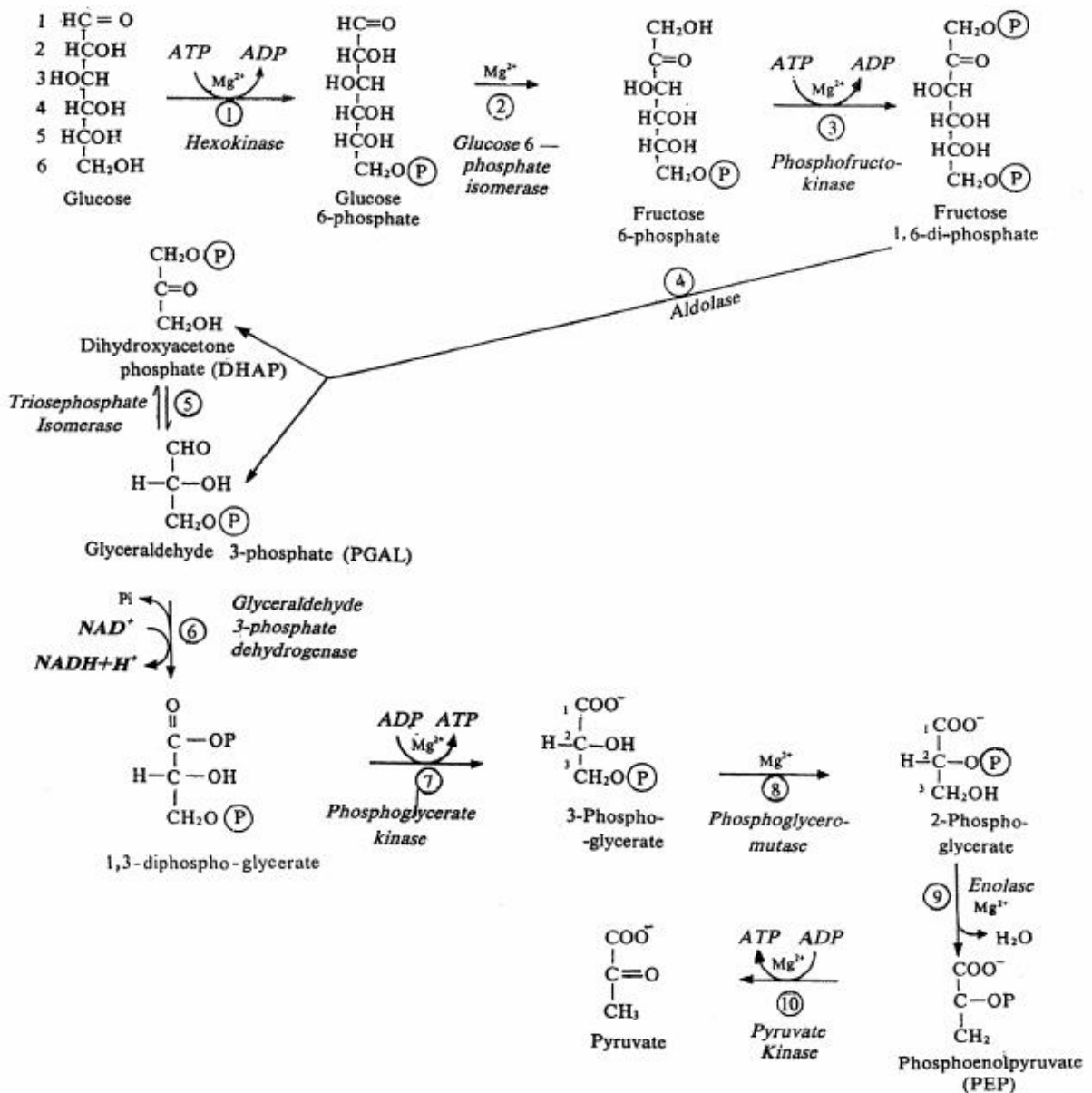


Fig. 11.2 : Reactions showing various steps in glycolysis.

- 1) The Phosphorylation of glucose by ATP at carbon-6 is catalysed by enzyme *hexokinase* to form glucose-6-phosphate (G6 - P). This type of reaction is **phosphorylation** and the name of the enzyme catalysing the reaction is *hexokinase*. Following this example, try reading the figure yourself and fill in the spaces for the remaining nine reactions of glycolysis.
- 2) Glucose-6-phosphate is..... by enzyme..... into fructose-6-phosphate (F 6-P).
- 3) A second by enzyme..... at carbon-1 forms fructose-1,6-diphosphate (F 1, 6-P).

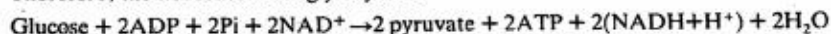
- 4) Fructose-1, 6-diphosphate is..... by enzyme into two interconvertible triosephosphates – phosphoglyceraldehyde (PGAL) and dihydroxy-acetone phosphate (DHAP).
- 5) catalysis the reversible DHAP to PGAL.
- The rest of the glycolytic pathway goes through PGAL and not through DHAP. Since PGAL is formed from DHAP in this reaction, for further reactions we will consider two molecules of PGAL, formed from one molecule of glucose.
- 6) This is an step catalysed by PGAL gives up two protons H^+ and two electrons to NAD^+ and is simultaneously phosphorylated by inorganic phosphate to form 1,3 di-phosphoglycerate, which is a high energy compound.
- 7) 1, 3 diphosphoglycerate is an unstable intermediate, hence its high-energy phosphate group is used to synthesize ATP from ADP in this reversible reaction catalysed by
- 8) Now the phosphate group its position from carbon-3 to carbon-2 catalysed by enzyme
- 9) of 2-phosphoglycerate molecules is catalysed by forming phosphoenol pyruvate (PEP) which contains a high-energy enol phosphate.
- 10) PEP is highly unstable. It readily gives up phosphate to ADP forming ATP, and the end product, pyruvate. The reaction is catalysed by

The words missing in the description of reactions are:

- 2) isomerised, *glucose-6-phosphate isomerase*, 3) phosphorylation, *phosphofructokinase*, 4) cleaved, *aldolase*, 5) *Triose phosphate isomerase*, isomerisation 6) oxidation, *glyceraldehyde 3-phosphate dehydrogenase*, 7) *phosphoglycerate kinase*, 8) shifts, *phosphoglyceromutase*, 9) Dehydration, *enolase*, 10) *pyruvate kinase*.

We can summarise the major events of glycolysis as shown in Fig 11.3. The major events are, i) glucose is phosphorylated and converted to two molecules of glyceraldehyde 3-phosphate (PGL) with the expenditure of two ATPs. ii) Four molecules of ATP are generated when two molecules of glyceraldehyde 3-phosphate are converted to pyruvate, iii) Oxidation of 2 molecules glyceraldehyde 3-phosphate reduces two molecules of NAD^+ to $NADH + H^+$

Therefore, the net reaction of glycolysis is



The overall reaction of glycolysis shows that there is a net formation of two ATP molecules as a result of one glucose molecule being oxidised to two pyruvate molecules. When glucose is completely oxidised to CO_2 and H_2O , a large amount (686 kilocalories per mole of glucose oxidised) of energy is available. When glucose is partially oxidised to pyruvate, only two ATP molecules are formed per molecule of glucose and much of the energy remains conserved in pyruvate.

What happens to pyruvate that is produced in glycolysis? The fate of pyruvate varies from cell to cell and its environment. In cells having mitochondria and adequate supply of oxygen (aerobic condition), pyruvate is further oxidised in stage 3 of metabolism (TCA cycle). In the absence of oxygen (anaerobic condition) pyruvate is reduced by NADH to either lactate or alcohol. Such anaerobic routes are called fermentation pathways. You must be familiar with the fermentation activities of yeast. In yeast, under anaerobic conditions, pyruvate is reduced to ethanol (alcohol) as shown in the following reaction.

This process of conversion of glucose to alcohol is known as **alcoholic fermentation**. In certain cells and organisms, under anaerobic condition, pyruvate is reduced to lactate and such anaerobic fermentation is known as **lactate fermentation**.

At times, our muscle cells have to work vigorously, but the rate of supply of oxygen to them cannot exceed beyond a certain limit. So all pyruvate cannot be oxidised via the TCA cycle. Instead most of it is reduced to lactate by NADH as shown below.

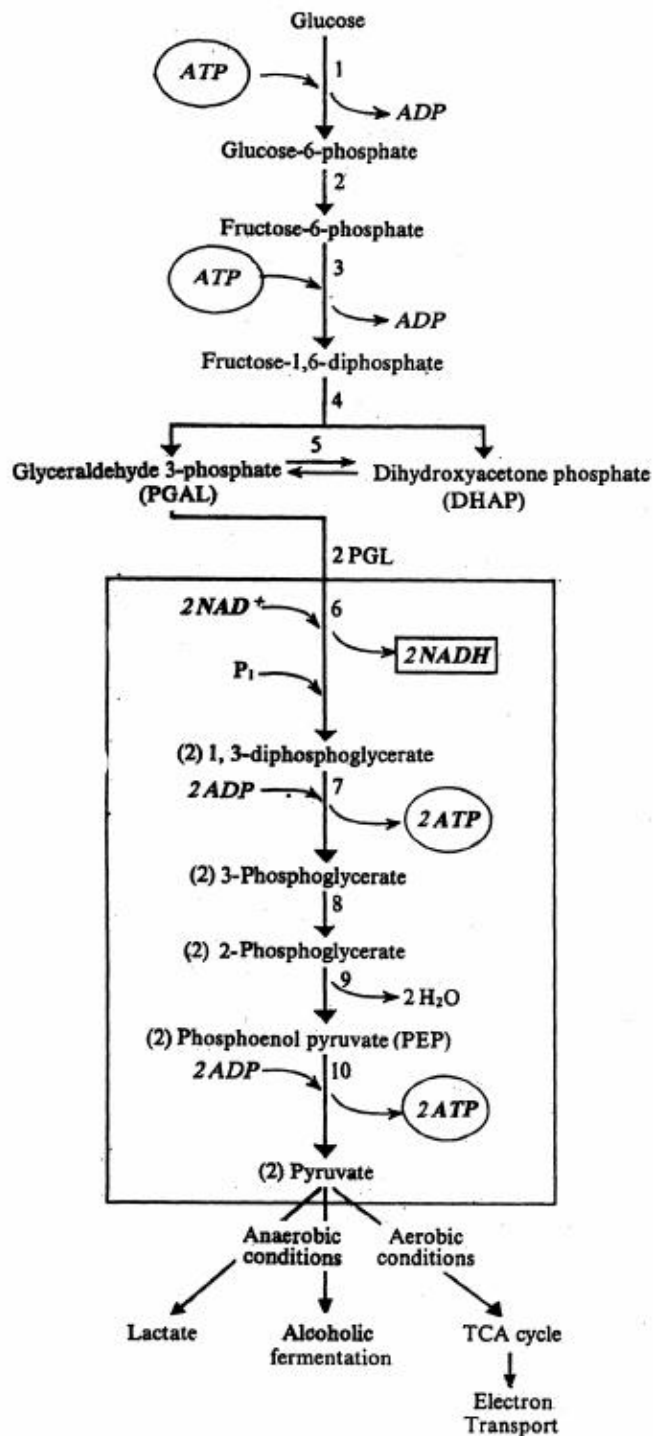
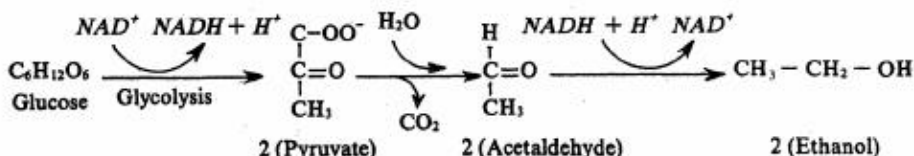
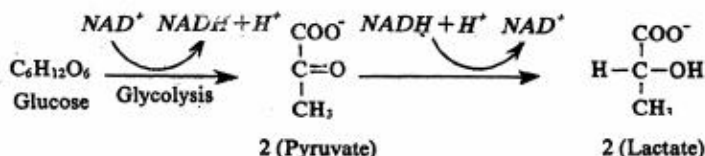


Fig 11.3 : Simplified scheme of glycolytic pathway showing utilisation and production of ATP and $\text{NADH} + \text{H}^+$.



There are many bacteria that derive energy from sugar through lactic acid fermentation under anaerobic conditions. *Lactobacilli* is such example which turns milk sour and produces curd and cheese.



Note that in fermentation (alcoholic or lactic acid), there is no net production of NADH. In reaction 6 of glycolysis NADH produced is oxidised back to NAD⁺ in the last reaction of fermentation (Table 11.3). Thus, NAD⁺ and NADH are recycled when fermentation takes place. The overall reaction of anaerobic glycolysis is

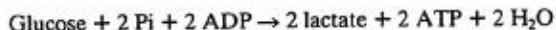


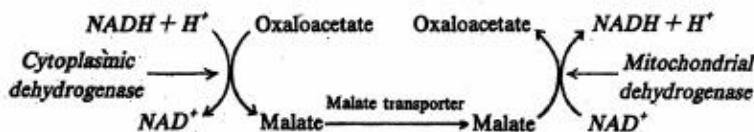
Table 11.3 : Utilisation and Production of NADH in glycolysis under Anaerobic Conditions

Reaction	NADH Changes per molecule of glucose	
	Production	Utilisation
2 × glyceraldehyde 3-phosphate → 2 × 1, 3-diphosphoglycerate	2	—
2 × pyruvate → 2 × lactate	—	2
2 × pyruvate → 2 × ethanol	—	2

Fermentation does not take place under aerobic conditions but NAD⁺ must still be regenerated from NADH, otherwise, glycolysis will come to a halt. NAD⁺ is regenerated in stage 4 of the metabolic reactions that occur under aerobic conditions. We will describe this process in a later section. However, we will learn now how NAD⁺ is regenerated in the cytoplasm under aerobic conditions.

Glycolysis takes place in the cytoplasm and the electron transport chain is located in the inner mitochondrial membrane. But the NADH molecule cannot cross the mitochondrial membrane. So it is oxidised in the cytoplasm and the two hydrogen atoms are transported to NAD⁺ molecule present inside the mitochondria. In other words, only hydrogen atoms are transported from cytoplasm to the mitochondria. The reaction involved in the transfer of hydrogen from NADH is given below.

A shuttle operates for this transfer and malate molecule is the carrier of hydrogen atoms from the cytoplasm to the mitochondrial matrix. In fact, the shuttle system is complex and involves reactions other than the formation of malate, but the net result



is the transfer of two H atoms from a molecule of NADH present in the cytoplasm to a different NAD^+ atom present in the mitochondria.

11.4.2 ATP Yield of Glycolysis

The net yield of ATP in glycolysis (oxidation of glucose to pyruvate) can be accounted as shown in the Table 11.4

Table 11.4: Utilisation and Production of ATP in Glycolysis under Anaerobic Condition

Reaction	ATP change per molecule of glucose Utilisation	Production
Glucose $\rightarrow$ Glucose 6-phosphate	1	—
Fructose 6-phosphate $\rightarrow$ Fructose 1,6 diphosphate	1	—
$2 \times$ 1,3 diphosphoglycerate $\rightarrow$ $2 \times$ 3-phosphoglycerate	—	2
$2 \times$ phosphoenolpyruvate $\rightarrow$ $2 \times$ pyruvate	—	2
Total	2	4

Therefore, the net yield is 2 ATP per molecule of glucose oxidised to pyruvate.

SAQ 2 Glycolysis occurs in

a) Choose the best answer in the following

i) The process of break down of glucose to pyruvate is known as

- a) oxidative phosphorylation
- b) Glycolysis
- c) Respiration
- d) Electron transport

ii) Glycolysis occurs in

- a) Mitochondria
- b) Digestive tract
- c) Cytoplasm
- d) Nucleus

iii) Glycolysis occurs

- a) Only in the absence of oxygen
- b) Only in the presence of oxygen
- c) Both in the absence and in the presence of oxygen
- d) Only in the presence of CO_2

iv) Which is not true for glycolysis under anaerobic condition

- a) ATP is produced
- b) ATP is produced
- c) NAD^+ is required
- d) Net NADH is produced

v) Which is true for glycolysis under aerobic condition

- a) only ATP is produced
- b) Only NADH is produced
- c) Both ATP and $NADH_2$ are produced
- d) Neither ATP nor $NADH_2$ is produced

vi) Compared to the resting state vigorously contracting muscle show

- a) Increased lactate formation
- b) Increased oxidation of pyruvate to CO_2 and water
- c) An increased $NADH/NAD^+$ ratio
- d) Increased conversion of pyruvate to acetaldehyde

b) 1) Write the overall reaction of glycolysis under aerobic condition i.e. from glucose to pyruvate

- ii) How many pyruvate and ATP molecules are formed from one molecule of glucose?
- iii) How many NAD^+ molecules are reduced per molecule of glucose?
- c) In lactic acid fermentation

- i) How many lactate molecules and ATP are formed from one glucose molecule?
- ii) How many net NAD^+ molecules are reduced?
- d) In alcohol fermentation
- i) How many ethanol ATP molecules are produced?
- ii) How many net NAD^+ molecules are reduced?

11.5 OXIDATIVE DECARBOXYLATION OF PYRUVATE

The ongoing reactions of pyruvate, under aerobic conditions occur in the mitochondria. The pyruvate molecule itself cannot enter the TCA cycle – the stage 3 of metabolism. It is oxidatively decarboxylated by a multi-enzyme complex called the **pyruvate dehydrogenase system**. Since this reaction links glycolysis with TCA cycle it is also termed as **link reaction**.

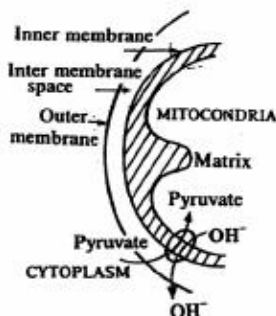
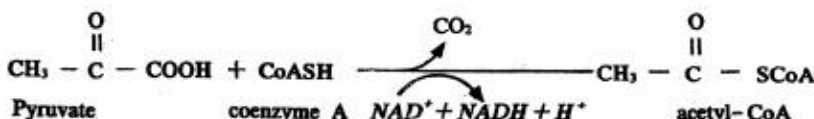


Fig. 11.4: Pyruvate-hydroxyl ion antiport system to transport pyruvate into the matrix of mitochondria.



We know that glycolysis occurs in the cytoplasm but pyruvate is metabolised aerobically in the mitochondria. How does pyruvate molecule cross the mitochondrial membrane? It is transported to the mitochondria either by simple diffusion or by the **pyruvate – hydroxyl ion antiport system**, where pyruvate is exchanged for a hydroxyl ion through conformational changes in the membrane (Fig. 11.4). The multi-enzyme complex – the **pyruvate dehydrogenase system** – is located in the mitochondrial matrix. It consists of multiple copies of three enzymes (E_1 , E_2 , E_3) each with a specific binding site for the substrate, and different cofactors (Fig. 11.5).

The advantage of such a complex system is that it retains the product of one reaction and transfers it to the active site of another enzyme thus improving the overall efficiency of the enzyme.

11.6 TRICARBOXYLIC ACID CYCLE

We have learnt that pyruvate on oxidative decarboxylation forms acetyl CoA which enters the tricarboxylic acid (TCA) cycle. Other fuel molecules such as amino acids and fatty acids can also enter the TCA cycle through acetyl CoA. In the TCA cycle the 2-carbon acetyl group of acetyl CoA is oxidised to two molecules of CO_2 and hydrogen atoms are accepted by coenzymes NAD^+ and FAD .

Let us first study broadly the reactions of TCA cycle shown in Fig. 11.6. The cycle begins with the condensation of oxaloacetate with acetyl CoA (reaction 1) and in the end (reaction 8) oxaloacetate is formed from malate. Thus oxaloacetate is used as a carrier to introduce acetyl CoA into the cycle. We will now read the reactions of the cycle step by step, the details are described below. While reading fill in the blanks with the type of reaction, and the name of the enzyme carrying the reaction. It is intended that such an exercise will help you to read other pathways from charts or figures. The blanks in reaction 1 are filled to show an example.

1) In reaction 1, acetyl CoA reacts with 4 carbon oxaloacetate to form a six carbon molecule of citrate. The enzyme is *citrate synthetase*.

2) of citric acid by the enzyme forms

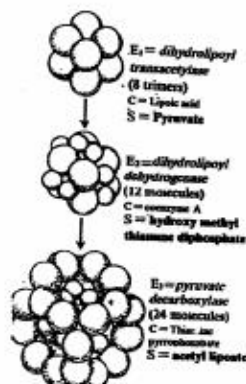


Fig. 11.5: Pyruvate dehydrogenase complex.
E = Enzyme C = cofactor
S = substrate.

3) of isocitrate by enzyme produces α -ketoglutarate.

4) α -ketoglutarate is to succinyl CoA by an enzyme the electron acceptor being NAD^+

5) The enzyme catalyses the formation of at the expense of bond.

6) Succinate is to fumarate by the enzyme The coenzyme accepts hydrogen.

7) of fumarate by the enzyme forms malate.

8) Finally oxalacetate is recovered when malate is by the enzyme

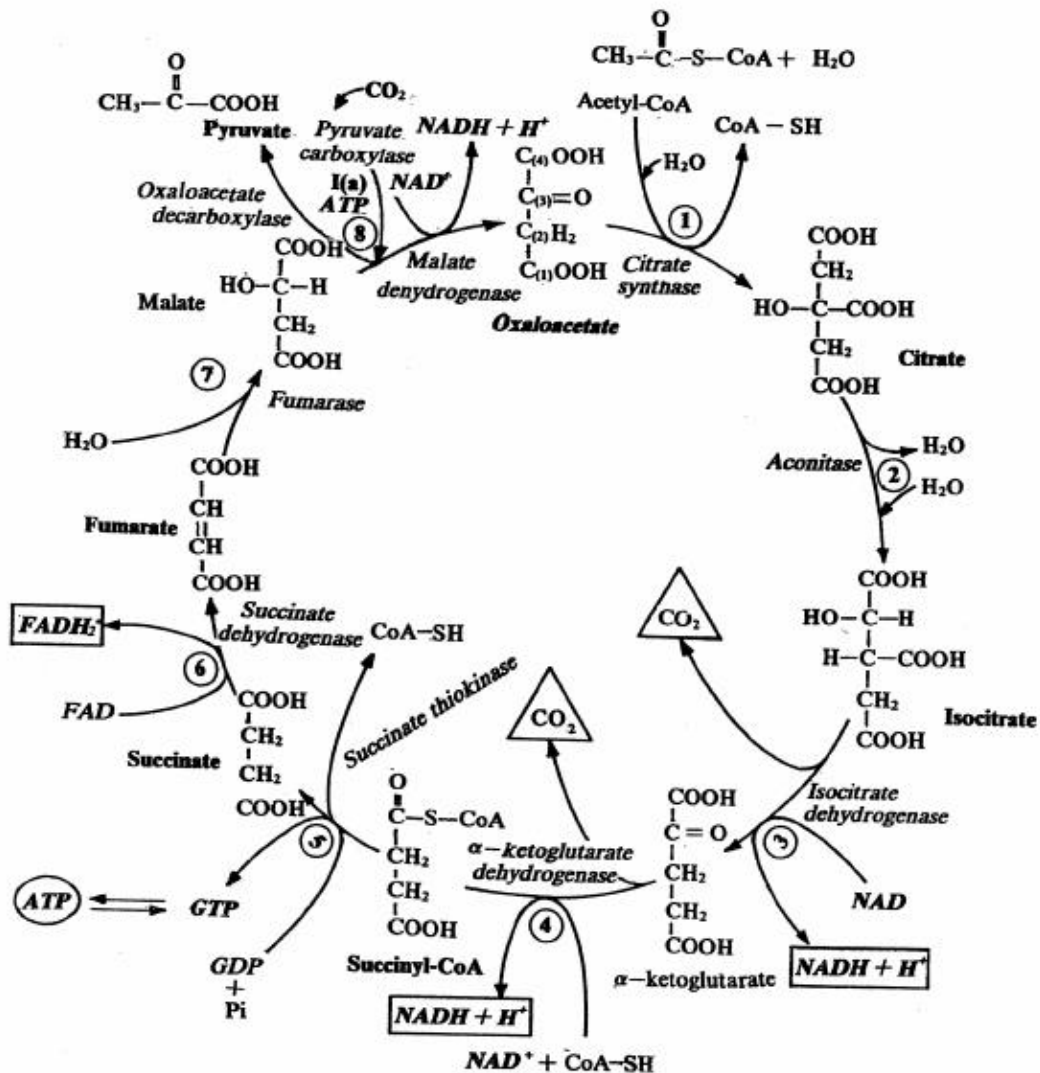


Fig. 11.6: The TCA Cycle.

Words missing in the description of reaction

2. Isomerisation, *aconitase*, isocitrate. 3. Dehydrogenation, *isocitrate dehydrogenase*, 4. oxidatively decarboxylated α -ketoglutarate dehydrogenase, NAD^+ 5. *succinate thiokinase*, GTP, thioester 6. dehydrogenated, *succinate dehydrogenase*, FAD 7. Hydration, *fumarase*, 8. dehydrogenated, *malate dehydrogenase*.

Let us now pay attention to some important steps of the cycle. The oxidative decarboxylation of isocitrate (reaction 3) and α -ketoglutarate (reaction 4) by two different dehydrogenases yields 2 NADH molecules. The CO_2 produced diffuses out from the mitochondrion and leaves the cell. In reaction 6, a molecule of FADH_2 is produced when succinate is oxidised to fumarate by *succinate dehydrogenase*. The breakdown of succinyl-CoA is linked to phosphorylation of GDP to form GTP, which can form ATP by exchange reaction.

All reactions of TCA cycle except 2 and 5, are readily reversible. Note that the two carbon atoms of acetyl CoA added in each cycle enter a different part of the citrate molecule and are not liberated immediately, as a CO_2 molecule during the first cycle. Moreover, the oxygen atoms required for oxidation of the carbon atoms of acetyl CoA are not supplied by free molecular oxygen. Instead, three molecules of water split in each cycle and their oxygen is used to make CO_2 .

TCA cycle was discovered by Sir Hans Krebs, therefore it is also called Krebs Cycle

To summarise the major events in each turn of the TCA cycle:

- one acetyl group containing two carbon atoms enters into the cycle;
- two carbon atoms are completely oxidised to two CO_2 molecules;
- three NAD^+ and one FAD are reduced; this means, four parts of hydrogen atoms are removed from the intermediates of the cycle; and
- one ATP molecule is formed via GTP.

Thus the primary role of the TCA cycle is to oxidise pyruvate, and to store the energy liberated in the form of NADH, FADH_2 and ATP. Another very important role of TCA cycle is that certain intermediates of the cycle are used for the synthesis of other molecules. For example, α -ketoglutarate, succinate and oxaloacetate are used for the synthesis of amino acids.

The second role of the TCA cycle raises an important question: when any intermediate is removed from the cycle, the concentration of each of the intermediates decreases in the cycle, and as a consequence, the rate of working of the cycle decreases. Then, how can the rate of working be increased? Well, if the concentration of the intermediates (i.e., the number of workers) is restored, the purpose will be solved. In fact, reaction 1 a (Fig. 11.5) serves this purpose. As you can see, some pyruvate molecules can be converted into oxaloacetate by this reaction.

SAQ3

- Give one example each of the following type of reaction occurring in the TCA cycle
 - hydration
 - decarboxylation
 - isomerisation
 - dehydrogenation
- In which reaction of the TCA cycle do the following events occur
 - CO_2 MOLECULES ARE RELEASED
 - GTP is formed
 - FAD is reduced
 - NAD^+ molecules are reduced
- which is not true for the TCA cycle when one acetyl group is oxidised
 - Two CO_2 Molecule are released
 - Three NAD^+ molecules and one FAD molecule are reduced
 - One CTP Molecules is formed
 - 38 ATP molecules is formed
- The rate of entry to acetyl coa into the citric acid cycle is decreased when

- i) the ratio of ATP/ADP is low
- ii) NADH is rapidly oxidised through the respiratory chain
- iii) ATP/ADP ratio is high
- iv) the ratio of NAD/NADH is high

11.7 ELECTRON TRANSFER CHAIN AND OXIDATIVE PHOSPHORYLATION

We have learnt that NADH and FADH_2 are produced in the TCA cycle when acetyl CoA is oxidised to CO_2 . NADH is also produced in glycolysis. In stage 4, of metabolism these NADH and FADH_2 molecules are re-oxidised by several electron carriers in a sequential manner. Finally, molecular oxygen is reduced to water by accepting these electrons. This electron transfer process is an energy-liberating process and is coupled to the formation of a proton gradient about which we have already learnt in Unit 8. We have also learnt in Unit 8, that the proton-gradient is further coupled to the formation of ATP from ADP and P_i . Thus the primary role of stage 4 metabolism is to reoxidise NADH and FADH_2 to NAD^+ and FAD respectively, which could be re-used in the TCA cycle and to generate ATP.

In this section we will learn about electron carriers, the mechanisms of formation of proton gradient and about the phosphorylation of ADP to P_i . The phenomenon of ATP formation is called **Oxidative Phosphorylation**, because this phosphorylation is coupled to the oxidation of NADH and FADH_2 by molecular oxygen.

The electron transfer carriers are collectively called electron transfer chain. It consists of more than 15 electron carriers embedded in the inner mitochondrial membrane. The components of the chain have been identified as three major complexes – NADH dehydrogenase complex (I), b-c, complex (II), and cytochrome oxidase complex (III). The compositions of the complexes is shown in Fig. 11.7. The complexes are linked by two mobile electron carriers ubiquinone and cytochrome c. All these carriers are protein in nature except ubiquinone (UQ also called coenzyme Q, see margin on the next page).

Flavin mononucleotide (FMN) is a cofactor of enzyme *NADH-dehydrogenase*. The cytochromes (Cyt b_1 , c, c_1 , a and a_3) are protein in nature containing an iron atom. In addition, there are about six iron-sulphur protein complexes (FeS), and two copper atoms tightly bound to the respiratory chain. For the most part, the electrons pass from one metal atom to another.

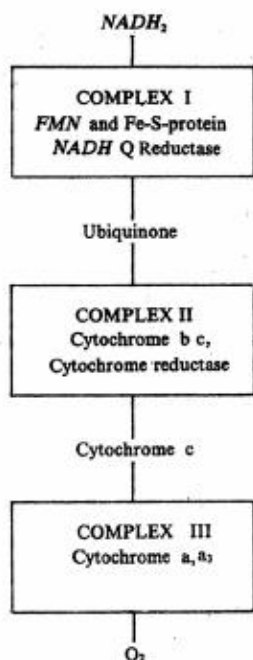


Fig. 11.7: Components of the respiratory chain of mitochondria

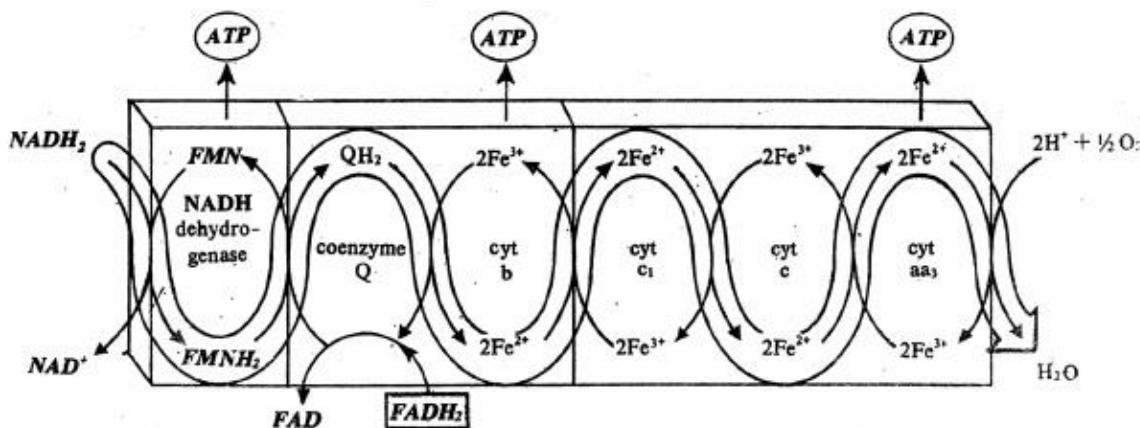


Fig 11.8 The electron transfer chain. The iron atom is either in the oxidised state (Fe^{3+}) or in the reduced state (Fe^{2+})

For instance, when the iron atom accepts an electron it is in ferrous form (Fe^{2+}) and when it gives the electron to next carrier it is in ferric (Fe^{3+}) form (Fig 11.8).

The electron transfer chain is traditionally called the electron transport chain. Since the electrons are not transported but transferred from one carrier to another it is more appropriate to call it electron transfer chain.

Thus only electrons are involved in oxidation and reduction of the cytochrome. In the cases of FMN and UQ both electrons and protons are involved in their oxidation. Fig. 11.9 shows the arrangement of three complexes, mobile electron carriers and the direction of flow of electrons. The components are arranged in such a way that if one component requires only electrons for reduction then the component next to it requires both electrons and protons for reduction. For instance, when NADH is oxidised, it liberates two electrons and two protons. The electrons are

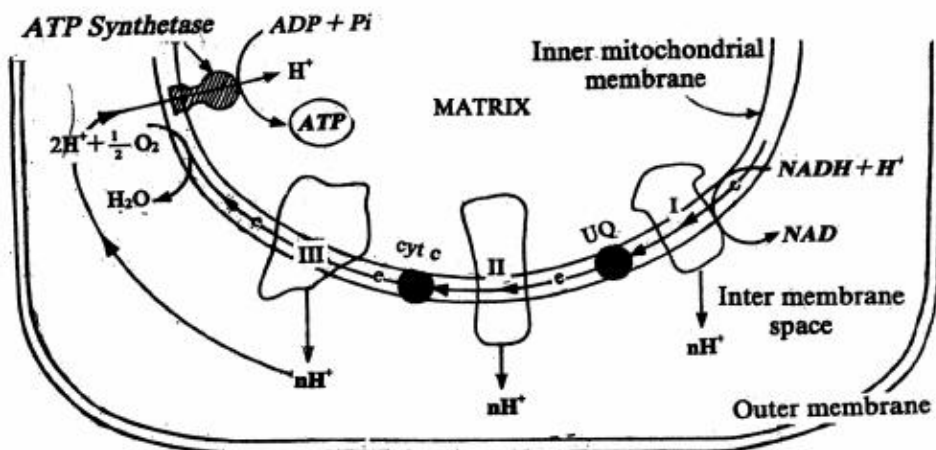
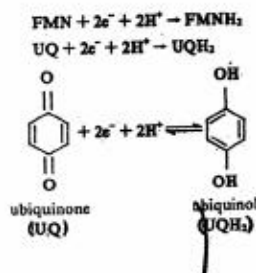


Fig. 11.9 : Schematic diagram of flow of electron through three major complexes (I, II & III) from NADH to oxygen. Cytochrome C and ubiquinone serve as mobile electron carriers. Protons pumped out across the membrane into inter membrane space during electron transfer create an electrochemical proton gradient across the inner membrane that drive H^+ back through the *ATP synthetase*

NAD is critically important for the function of all the body cells. It is synthesised in the body from vitamin B-Niacin. Therefore, Niacin deficiency in the diet has such widespread harmful effect on the body. Likewise, several other vitamins are precursors from which other coenzymes are synthesised. The study of biochemical reactions illustrates the role of vitamins in the body.



accepted by iron-sulphur protein, and thereby it is reduced (Fe^{3+} needs only electron, and not H^+ , for its reduction). What happens to the protons given up by NADH? The carriers are located in such a way inside the membrane that the protons are **liberated outside** the membrane into the inter membrane space. But when FAD gets two electrons from FeS, it needs two protons also for reduction. From where do the protons come? Again FeS and FMN are located in the matrix in such a manner, that the protons are **taken up from inside** i.e. matrix of the mitochondria. Thus we conclude two conditions are necessary for proton transport across the membrane (i) the electron carriers are arranged alternatively with respect to hydrogen (electron, proton) carrier, (ii) the carriers are located in the membrane bed in such a manner that the protons required for reduction come from the inner side (matrix of the membrane and the proton given up during oxidation are liberated outside the membrane.

Can you describe what happens when Cyt b is reduced by $FMNH_2$?

As the electron transfer continues, more and more protons are transported from inside to outside. This, a difference in proton concentration is created between inside and outside of the inner membrane. This difference in proton concentration is called **proton gradient**. The energy liberated from the electron transfer is stored in the form of a proton gradient across the membrane.

The proton gradient should persist for sometime so that its energy can be used for useful work. This means that the inner membrane should be impermeable to protons. This is indeed the case. In fact, the inner membrane is also impermeable to other small ions like K^+ and Na^+ .

How is the energy of the proton gradient utilised to form ATP from ADP? This happens through a large complex protein *ATP synthetase*, a large transmembrane protein complex. This protein is called the **coupling factor** because it couples electron transfer to oxidative phosphorylation. If ADP and P_i are available, the coupling factor allows the protons to come back into the matrix. When the protons go inside through the coupling factor, energy is liberated which is used by the coupling factor to make ATP from ADP and P_i (Fig. 11.9). The mechanism of this process is still unknown.

For every pair of electrons transferred through the chain from NADH to O_2 , three pairs of protons are transported across the membrane. For $FADH_2$ which transfers electrons to ubiquinone, two pairs of protons are transported across the membrane. In terms of ATP synthesis, for every pair of protons coming back through the coupling factor, one ATP is synthesized. Thus 3 ATPs are formed from one $NADH_2$ molecule and 2 ATP from one $FADH_2$ molecule.

Oxidative phosphorylation is inhibited by several substances such as dinitrophenol (DNP), carbon monoxide, and oligomycin. All these compounds inhibit oxidative phosphorylation (ATP synthesis by mitochondria), but their modes and sites of action are different. For example, DNP does not allow the formation of proton gradient, because in the presence of DNP, the membrane is not any more impermeable to protons. As a result, the protons which are transported outside the membrane during electron transfer immediately come back inside, and no proton gradient is formed. As a consequence, ATP is not synthesized, although electron transport is not inhibited. Such types of inhibitors are called **uncouplers** of oxidative phosphorylation.

Carbon monoxide (CO) inhibits electron transfer directly. It inhibits electron transfer at cytochrome a/a_3 . If electron transfer is inhibited, proton gradient is inhibited and, hence, ATP synthesis is inhibited. Such type of inhibitors are called **electron transfer inhibitors**.

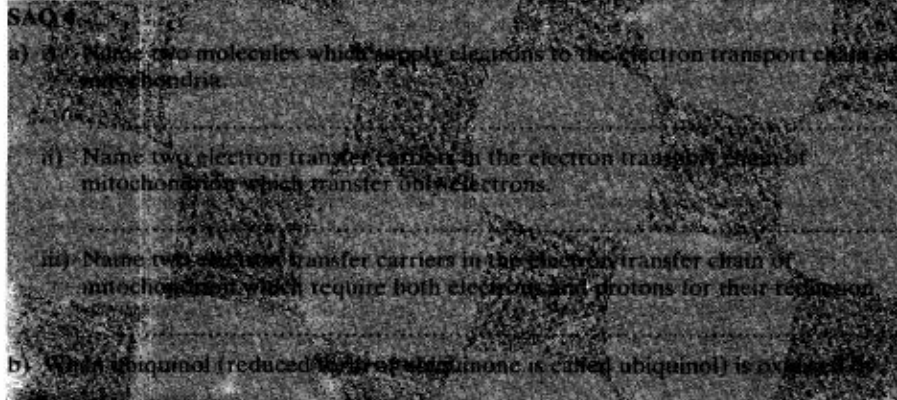
Oligomycin inhibits oxidative phosphorylation by a different mode. It binds directly to the coupling factor and thereby prevents the protons from coming back through the coupling factor. As a result ATP synthesis is inhibited although neither electron transfer nor the proton gradient is inhibited. The rate of electron transfer, however, slows down, because the proton gradient remains high and applies a back pressure on electron transfer. That is, once the proton gradient remains high, energy required to transport further proton becomes high. Such inhibitors of oxidative phosphorylation are known as **energy transfer inhibitors**.

11.8 SUBSTRATE LEVEL PHOSPHORYLATION

The phosphorylation of ADP to form ATP occurs in three different ways, each of which involves coupling. They are oxidative phosphorylation, photophosphorylation and substrate level phosphorylation. You have learnt about oxidative phosphorylation in Unit 8. Formation of ATP during photosynthesis is called photophosphorylation, which will be covered in the physiology course. In substrate level phosphorylation, ATP is formed by the enzyme-mediated transfer of a phosphate from the substrate to ADP.

You have come across two such reactions, one in glycolysis and one in the TCA cycle. Look at the glycolytic pathway. In reaction 7, the phosphate group is transferred from 1,3-diphosphoglycerate to ADP by the enzyme *phosphoglycerate kinase*.

1,3-diphosphoglycerate is a high energy compound, because a large amount of free energy is liberated when the phosphate group at position 3 is hydrolysed. A part of this energy is used to drive the phosphorylation of ADP to form ATP. In the TCA cycle, the cleavage of the high energy thioester bond of succinyl CoA (reaction 5) is catalysed by the enzyme *succinate thiokinase*. As a result a large amount of free energy is available which drives phosphorylation of GDP to form GTP.



- SAQ 4**
- Name two molecules which supply electrons to the electron transport chain of mitochondria.
 - Name two electron transfer carriers in the electron transport chain of mitochondria which transfer only electrons.
 - Name two electron transfer carriers in the electron transport chain of mitochondria which require both electrons and protons for their reduction.
 - When ubiquinol (reduced form of ubiquinone is called ubiquinol) is available

cytochrome, ubiquinol gives up electrons and protons and cytochrome accepts only the electrons. What happens to the protons given up by ubiquinol?

- c) When a proton gradient is formed in mitochondria, the pH of the matrix is (tick mark the correct statement)
- higher than the pH of the outside
 - lower than the pH of the outside
 - equal to that of the outside
- d) Give the number of
- electrons needed to reduce one O_2 molecule
 - NADH molecules oxidised to reduce one oxygen molecule
 - protons transported across the membrane when one molecule of oxygen is reduced by $FADH_2$, $NADH_2$ via ETC.
 - ATP molecules synthesised through oxidative phosphorylation per molecule of oxygen consumed if NADH is the electron donor.
- e) What is the role of the coupling factor in oxidative phosphorylation?
- f) The function of the coupling factor in mitochondria is to make ATP from ADP and P_i . Energy is required to make ATP. What is the direct source of this energy?
- g) Give one example of substrate level phosphorylation.
- h) Oxidative phosphorylation comprises three events: first electron transfer; second formation of a proton gradient; and third returning of the protons through the coupling factor with concomitant synthesis of ATP. Inhibition of any of these three events will inhibit ATP synthesis. Name three inhibitors which inhibit one each of these three events.

11.9 PENTOSE PHOSPHATE PATHWAY

Glycolysis is a major route of degradation of glucose to pyruvate. Many other routes of glucose metabolism have also been investigated in plant and animal tissue. It is beyond the scope of this unit to describe all of them. You are going to learn pentose phosphate pathway which is one of the important pathways that operates in a wide variety of organisms including animals, plants and many microorganisms. It is not an alternate pathway for obtaining energy, instead it performs a multifunctional role in the body.

The pathway is shown in Fig. 11.10. Let us first have an overview of it. We find that the pathway involves a number of reactions, and intermediates, mainly monosaccharides varying in the number of carbon atoms. Many of the reactions are reversible and operate according to the demand of the intermediates in the cell. The pathway is divided in two phases: the oxidative phase and the non-oxidative phase. In the oxidative phase, glucose-6-phosphate is oxidised to ribulose 5-phosphate, one CO_2 is released, and two molecules of NADP are reduced. In the subsequent non-oxidative phase, three molecules of ribose-5-phosphate go through several reactions producing two molecules of fructose-6-phosphate and one molecule of

glyceraldehyde 3-phosphate, both are intermediates of glycolysis. So these two end products of the pentose phosphate pathway can enter into the glycolytic pathway. Thus this pathway is basically a shunt, as it branches out from the glycolytic pathway at the point of glucose-6-phosphate and rejoins at the point of fructose-6-phosphate and glyceraldehyde-3-phosphate.

Let us now go through the sequence of reactions. Let us first study the oxidative phase

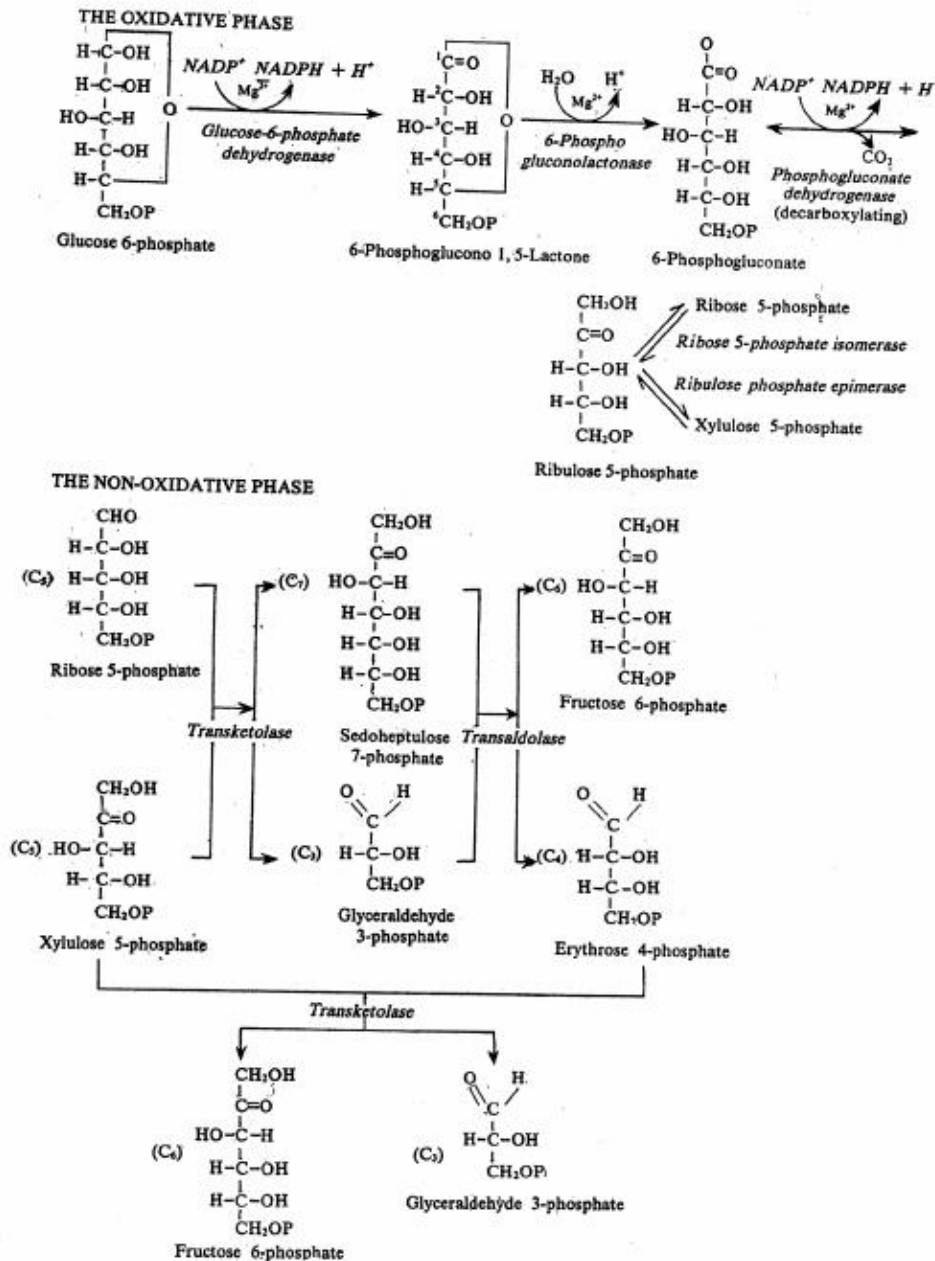


Fig. 11.10 : The pentose phosphate pathway

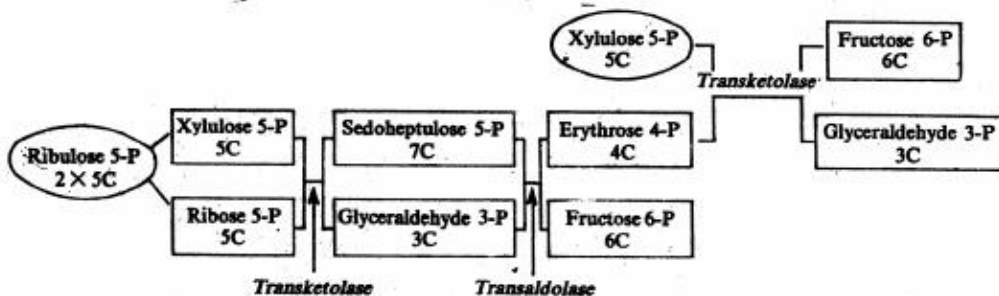
In reaction 1, glucose 6-phosphate is oxidised to 6-phosphoglucono 1, 5-lactone by removal of electrons which are accepted by NADP. Hydrolysis of the lactone to 6-phosphogluconate in reaction 2, is followed by an oxidative decarboxylation that yields ribulose 5-phosphate and a second molecule of NADPH. Enzymes of these three reactions require Mg^{2+} ions as cofactors.

In the non-oxidative phase the pathway operates according to the specific requirement of the tissue. In tissues active in nucleotide synthesis, ribulose 5-phosphate is isomerised to form ribose 5-phosphate – the sugar backbone of RNA and DNA molecules. The pathway terminates at this point. The tissues active in lipid synthesis have a great demand for NADPH than ribose 5-phosphate. In this situation, ribulose 5-phosphate goes through the non-oxidative phase.

Let us now study the non-oxidative phase. You may find the interconversion of sugars a little confusing. Try to understand the scheme given in the Fig. 11.10, in the following order:

- Isomerisation of ribulose 5-phosphate by two different enzymes forms ribose 5-phosphate and xylulose 5-phosphate (reactions 4 and 5).
- The above two 5 carbon sugars condense together by the enzyme *transketolase* to form 7 carbon sedoheptulose phosphate and 3 carbon glyceraldehyde 3-phosphate, reaction 6.
- Now 3 and 7 carbon sugars condense by another enzyme called *transaldolase* to form 6 carbon fructose 6-phosphate and 4 carbon erythrose phosphate, reaction 7.
- Another molecule of 5 carbon xylulose 5-phosphate condenses with 4 carbons erythrose phosphate and forms fructose 6-phosphate and 3 carbon glyceraldehyde 3-phosphate, reaction 8.

We can place these reactions in a simpler scheme and sum them up as shown below:



The fructose 6-phosphate may enter the glycolytic pathway or may isomerise into glucose 6-phosphate and re-enter in the oxidative phase of the cycle. The pentose phosphate pathway can, therefore, operate in a cycle. The net reaction is



We may now ask a question: what is the usefulness of the pentose phosphate pathway (PPP)? There are at least two uses. First, we have seen that NADPH, not NADH, is

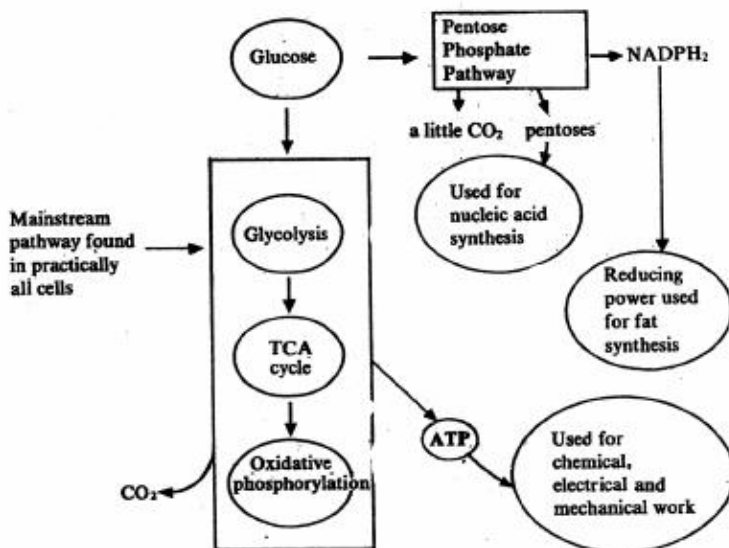


Fig. 11.11: Simplified scheme showing the role of pentose phosphate pathway

produced in PPP, which is required as reductant in fat synthesis. Second, this pathway produces several pentoses which are required for nucleic acid synthesis. This point is illustrated in Fig. 11.11. It may be mentioned that PPP is absent in the muscle cells. The reason is clear. The function of the muscle cells is primarily to carry out physical activity, not chemical activity such as synthesis of fats and nucleic acids.

SAQ5

- A) The pentose phosphate pathway is basically a shuttle of glycolytic pathway explain

.....

- b) The pentose phosphate pathway serves two useful purpose in biosynthetic pathway. what are they

.....

11.10 REGULATION

In the previous sections we have learnt about the reactions of glycolysis, the TCA cycle and oxidative phosphorylation. But we have not talked about the speed of these reactions? Do these reactions move at a constant speed all the time? No, the speed changes, either decreases or increases, depending on the need of the cells. In other words, the rates of the metabolic processes are regulated. In this section we will discuss regulation of glycolysis, TCA cycle and oxidative phosphorylation.

Glycolysis and the TCA cycle have two major roles. One is to produce ATP which is the source of energy and the other is to provide the building blocks for various biosynthetic processes of the cell. Depending upon the need of the cell the rate of glycolysis and of TCA cycle are to be regulated. Glycolytic pathway is regulated at three enzymatic reactions:

- The first reaction of glycolysis, i.e. conversion of glucose to G-6-P by the enzyme *hexokinase*;
- The third reaction of glycolysis, conversion of F-6-P to F-1,6-bisphosphate by the enzyme *phosphofructokinase*; and
- The last reaction of glycolysis, conversion of PEP to pyruvate by the enzyme *pyruvate kinase*.

How are the activities of these enzymes regulated? Some metabolites inhibit the rate of working of the enzymes, while some stimulate the rate (see Fig. 11.12). Table 11.5 shows the effect of metabolites. When the ATP level is high, glycolysis is inhibited; and when the ADP level is high, glycolysis is stimulated.

So, ATP regulates the level of two glycolytic enzymes, namely *phosphofructokinase* (abbreviated as PFK) and *pyruvate kinase*. These are the two glycolytic reactions which form ATP from ADP and Pi. Citrate, an intermediate of the TCA cycle, at high level suppresses glycolysis. Because high level of citrate means that other intermediates of the TCA cycle and of glycolysis that can be used for biosynthesis are abundant. So, additional glucose should not be degraded for this purpose. Thus the second major role of glycolysis is regulated.

Table 11.5 : Inhibitors and Stimulators of Glycolysis

Regulatory Enzyme	Inhibitors	Stimulators
<i>Hexokinase</i>	G-6-P	
<i>Phosphofructokinase</i>	ATP, NADH, Citrate	ADP, AMP
<i>Pyruvate Kinase</i>	ATP, NADH, alanine	FDP, G-6-P

Why isn't *hexokinase* inhibited by ATP or substances like citrate? Inhibition of the very first reaction of glycolysis would save some ATP molecules which are spent by *hexokinase*. But glycolysis has other roles to fulfil. One such role is to divert some G 6-p molecules toward the pentose phosphate pathway for generating NADPH and ribose-5-P which are needed for the biosynthesis of fats and nucleotides respectively. So, when the cell has abundant ATP, it is used to make some fats and nucleotides. Therefore, the first reaction continues at a normal rate even if the ATP level is high. When the need of the pentose pathway is fulfilled, G 6-P will not be used up at a high rate and then it will accumulate in the cell. But nature has created the *hexokinase* enzyme in such a way that it is inhibited by its own product G 6-P (see Fig. 11.12).

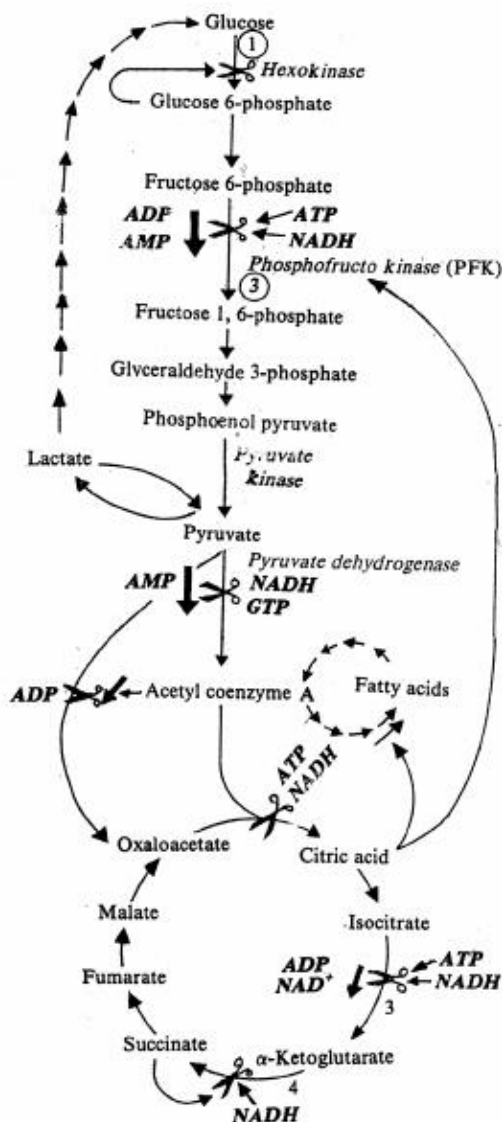


Fig. 11.12: Feedback Regulation of Glycolysis and citric acid cycle. Scissors indicate inhibition and bold arrows stimulation.

Let us now consider the regulation of the TCA cycle. The TCA cycle is regulated by three enzymes (see Fig. 11.11).

- citrate synthase* which is inhibited by ATP and NADH;
- isocitrate dehydrogenase*, which is inhibited by ATP and stimulated by ADP, and NAD⁺ and

iii) α -ketoglutarate dehydrogenase which is inhibited by succinyl CoA and NADH.

If we analyse the regulation of the TCA cycle, we come to a general conclusion that basically high levels of ATP (or ATP-to ADP ratio) and NADH, slow down the TCA cycle. Physiologically speaking, this strategy of the cell makes sense. Afterall, once glucose enters the TCA cycle via acetyl CoA, the carbon atoms of glucose are completely oxidised with the production of energy rich compounds like ATP and NADH. When these compounds are produced in abundance it is justified that they themselves inhibit the process of their production. Thus, the basic principle is maintained. Regulation works to meet the need of the cell.

Look at Fig. 11.12, citrate stimulates fatty acid synthesis for which acetyl CoA is the starting material. When the TCA cycle speeds up due to high concentration of its intermediates including citrate, and when ample ATP is present in the cell, fatty acid synthesis speeds up and the signal comes from citrate when it is at high concentration.

Before we discuss the regulation of oxidative phosphorylation, let us consider an important enzyme, *pyruvate dehydrogenase*, which catalyses the conversion of pyruvate to acetyl CoA. This step is specially important because, once pyruvate is converted to acetyl CoA, it has two main options: it can be oxidised to CO_2 via the TCA cycle, or it can be used to synthesise fats. More important is that pyruvate can still be reversed to generate glucose, while acetyl CoA can never be reversed to generate glucose (you will learn about the synthesis of glucose called gluconeogenesis in the following unit). So the cell must take a crucial decision when to convert pyruvate to acetyl CoA. Thus it strictly regulate this step. *Pyruvate dehydrogenase* is inhibited by its own products, namely, acetyl CoA, and NADH, and also by GTP. It is stimulated by AMP. Thus, the conversion of pyruvate to acetyl CoA is slowed down under a condition when the cell is rich in energy. Under this condition it is wise to convert pyruvate to glucose or to other storage carbohydrates using the available energy.

Regulation of oxidative phosphorylation is very straight forward. Low levels of ADP and Pi slow down oxidative phosphorylation, because they are the substrates of this process. Phosphorylation of ADP is very tightly coupled to the transfer of electron from NADH (or FADH_2) to O_2 . So when the rate of phosphorylation is slowed down the rate of electron transfer is bound to slow down.

In this connection the regulatory role of molecular oxygen should be emphasised. To do that let us look back into the history of science for a while. In the 19th century, when biochemical pathway including glycolysis was almost completely unknown. Louis Pasteur discovered that an organism like yeast takes up glucose from the medium at much high rate under anaerobic condition than under aerobic condition. This phenomenon is known as **Pasteur Effect**. The reason was not known to Pasteur. But we can now explain this phenomenon very easily. As you know under anaerobic condition glucose is only partially oxidised to lactate or alcohol and only two molecules of ATP are formed per glucose molecule. Under aerobic conditions glucose is completely oxidised to CO_2 and 38 ATP molecules are produced per glucose molecule. So to meet the need of ATP under anaerobic conditions, the cell must consume glucose at a much higher rate than required under aerobic conditions. This is a good and simple example of how the rate of glucose metabolism is regulated by the energy need of the cell.

SAQ6

- a) i) Name three compound which are inhibitors of the enzyme phosphofructokinase and one compound which stimulates the enzyme

.....

- ii) Explain how the inhibition of PFK by ATP help in regulating glycolysis

.....

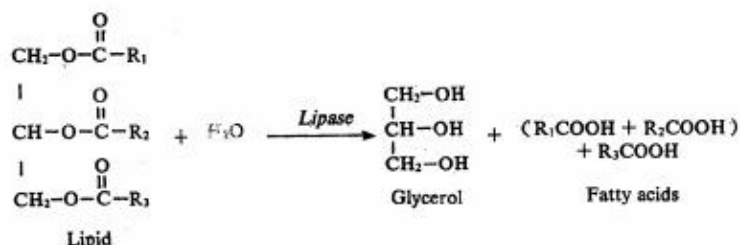
- b) i) Name three regulatory enzymes of the TCA cycle
ii) Which are the metabolites regulating the activity of these enzymes

11.11 DEGRADATION OF LIPIDS AND PROTEINS

So far we have learnt about the degradation of carbohydrates. In this section, we will study briefly how lipids and proteins breakdown and join the main scheme of metabolism to provide energy and intermediates which are required for various biosynthetic processes.

11.11.1 Lipids

Generally, neutral lipids which are triglyceride esters of fatty acids are stored in plant and animal cells. These are first hydrolysed by lipases to fatty acids and glycerol.



Glycerol is phosphorylated using ATP to glycerol 3-phosphate which is oxidised to dihydroxy acetone-phosphate. As you know the latter is an intermediate in glycolysis and thus can be metabolised through this pathway, fatty acids are step-wise oxidised and as a consequence 2 carbon fragments are successively removed as acetyl CoA (as shown in Fig. 11.13).

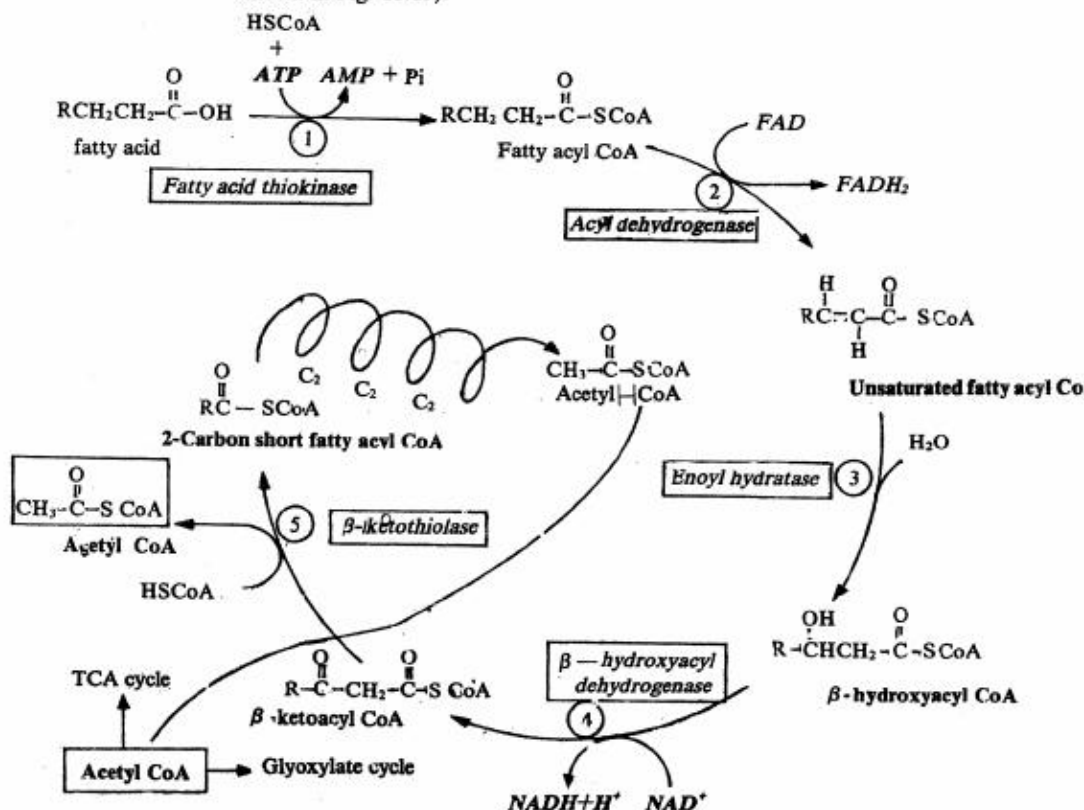


Fig 11.13: Degradation of fatty acids through β -oxidation scheme

The scheme is called β -oxidation because cleavage of fatty acid takes place between 1 and 2 carbon atoms. Let us read the reactions of the scheme and fill in the blank spaces in the sentences below describing these reactions.

1. In the first reaction fatty acids are activated to their acyl CoA derivative using coenzyme A and ATP by enzyme fatty acid This is an reaction.
2. of fatty acyl CoA by enzyme acyl results in an unsaturated acyl CoA. The electron acceptor in this reaction is
3. in the subsequent reaction by enoyl forms β -hydroxyl acyl CoA.
4. A second of the acid by NAD^+ forms β -ketoacyl CoA.
5. of the β -ketoacyl CoA by enzyme releases a molecule of acetyl CoA. The remaining fatty acyl CoA is 2 carbon less in length.

The words for the blank spaces are:

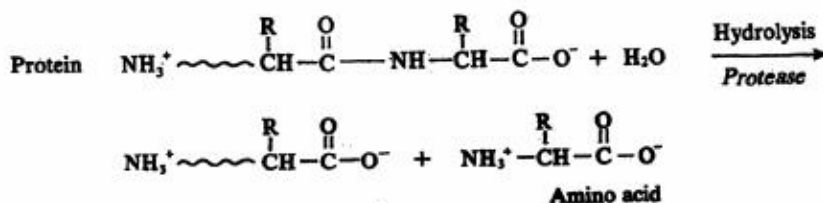
1. *thiokinase*, esterification, 2. Dehydrogenation, *dehydrogenase*, FAD,
3. Hydration, *hydratase*, 4. dehydrogenation, 5. cleavage, β -ketothiolase.

The remaining chain can undergo the same sequence of reactions from 1 to 5 again and again till all the carbons are converted to acetyl CoA. All those acetyl CoA molecules can now enter into the TCA cycle by reacting with OAA, if the latter is available in sufficient concentration.

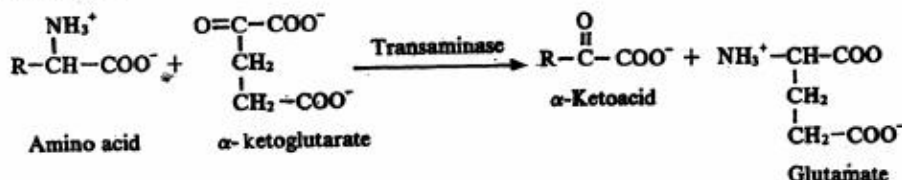
You know that a fatty acid may contain even or odd number of carbon atoms. The last fragment of odd numbered fatty acid would result in a 3-carbon fragment, namely propionyl CoA instead of acetyl CoA. Propionyl CoA is metabolised to form succinyl CoA. Succinyl CoA can enter the TCA cycle directly as an intermediate. Can you think of an advantage of metabolism of an odd-carbon fatty acid over an even-carbon fatty acid in terms of efficient running of the TCA cycle? For the TCA cycle to work continuously at a good rate, a high concentration of the intermediate should be maintained. Succinyl CoA helps in this matter, because it enters the TCA cycle as an intermediate.

11.11.2 Proteins

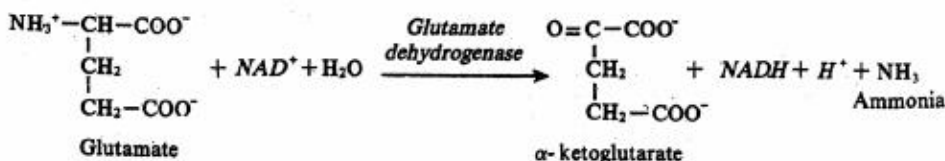
The peptide bonds of proteins are first hydrolysed by proteolytic enzymes liberating* constituent amino acids. The process is termed proteolysis. Probably you are familiar with proteolytic enzymes chymotrypsin and trypsin. They are present in our stomach and intestine which hydrolyse the proteins in our food. The hydrolysis of a peptide bond is shown below:



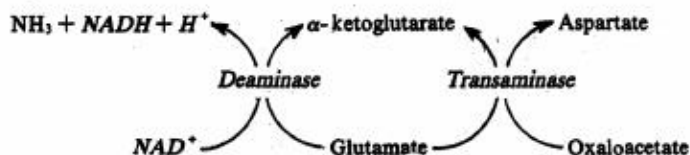
The next step is transamination, in which the amino group of an amino acid is transferred to the TCA cycle intermediate α -ketoglutarate by an enzyme called *transaminase*.



Thus by this reaction, all amino acids are converted to glutamate and α -keto-acids corresponding to amino acids. Let us now see what happens to glutamate and to α -keto-acids. In transamination amino groups of amino acids are accumulated in α -ketoglutarate as glutamate molecules hence there is no net loss of amino groups. Glutamate, in turn, releases the amino group as ammonia and regenerate α -ketoglutarate by oxidative deamination reaction. The reaction is catalysed by NAD^+ -linked enzyme *glutamate dehydrogenase*.



The two reactions — transamination and deamination can also be represented as shown below:



Thus, for each glutamate deaminated, one NADH₂ molecule is produced which can be oxidized through the electron transport chain to produce ATP. What happens to ammonia?

In mammals ammonia is excreted as urea via a pathway called urea cycle. The carbon skeleton of amino acids, i.e., the α-keto-acid can be used either for biosynthesis of carbohydrate, fats and amino acids or to form acetyl CoA which enters TCA cycle and is degraded to produce energy.

What are the last products

SAQ 7

Two ammonia molecules are excreted through each urea molecule. The C=O group in ure comes from a CO₂ molecule. Urea cycle was discovered by Hans Krebs who, as we know already also discovered the TCA cycle. The discovery of urea cycle was made in 1932 and it took about five years (1932-37) to discover TCA cycle. Krebs was awarded nobel prize for it.

- Name an intermediate of glycolysis which is also produced when glycerol is oxidised.
.....
- What are the last products of β-oxidation of both even and odd fatty acids?
.....
- How many acetyl CoA molecules will be produced from β-oxidation of a 16-carbon fatty acid molecule?
.....
- How many acetyl CoA and propionyl CoA molecules will be produced from β-oxidation of a 19-carbon fatty acid molecule?
.....
- Acetyl CoA is a product of β-oxidation of fatty acids. How is this acetyl CoA further metabolised (oxidised)?
.....
- Propionyl CoA is a product of β-oxidation of odd-carbon fatty acids. How is this propionyl CoA further metabolised (oxidised)?
.....
- Show the transaminase reaction of alanine. What is the name of α-keto-acid produced in this reaction?
.....
- How is ammonia produced from glutamate?
.....
- Ammonia is toxic for the cell. How is excreted in mammals?
.....

11.12 SUMMARY

- 1 The metabolic reactions in a cell are common organic reactions governed by the functional groups.
- 2 Cells derive energy by the oxidation of biomolecules. The break down of food molecules can be considered in four stages. In stage 1, large molecules-carbohydrates, fats and proteins break down to their precursor units monosaccharides, fatty acids, and amino acids. In stage 2 the precursor units are degraded to pyruvate via glycolysis. Pyruvate is oxidised to CO_2 via the TCA cycle in stage 3, and the energy is stored as NADH_2 and FADH_2 molecules. Electrons from these molecules pass from one carrier to another in the electron transfer chain releasing energy which is coupled to ATP formation. Finally, electrons and H^+ are taken up by atmospheric oxygen.
- 3 A variety of mechanisms control and regulate the activity of key enzymes in glycolysis and the TCA cycle in response to changing conditions in the cell.
- 4 Fatty acids are oxidised successively into acetyl CoA by β -oxidation. Acetyl CoA is further oxidised via the TCA cycle.
- 5 Amino acids are converted into their respective keto-acid by transamination reaction. Keto-acids metabolise further and enter into the TCA cycle if necessary.

11.13 TERMINAL QUESTIONS

1. Considering the whole process of glycolysis, TCA cycle and oxidative phosphorylation, make a diagram to show the total number of ATP molecules formed when one molecule of glucose is completely oxidised. Follow the steps given below to draw the diagram:
 - a) How many net ATP molecules are formed during glycolysis (glucose $\rightarrow$ 2 pyruvate)
.....
.....
 - b) How many NADH_2 molecules are formed during glycolysis and how many ATP molecules are formed when these NADH_2 molecules are oxidised through electron transfer chain.
.....
.....
 - c) When two pyruvate molecules are oxidised to acetyl CoA (link reaction) find the number of NADH_2 molecules produced and the corresponding number of ATP molecules.
.....
.....
 - d) In TCA cycle, find, the number of NADH_2 and FADH_2 and GTP molecules produced out of two acetyl CoA molecules oxidised and the corresponding number of ATP molecules.
.....
.....
2. a) In yeast, how is pyruvate metabolised in the absence of oxygen (anaerobic condition)? Write the reaction also.
.....
.....
.....
.....
.....

- b) In muscle cells, how is pyruvate metabolised under-anaerobic condition? Write the reaction also.

.....
.....
.....
.....

- c) How is pyruvate metabolised under aerobic condition?

.....
.....
.....

3. a) Mention two important roles played by TCA cycle.

.....
.....

- b) Explain how the reaction catalysed by the enzyme pyruvate carboxylase help the TCA cycle in maintaining the concentration of its intermediates.

.....
.....
.....

4. Name a TCA cycle intermediate which inhibits phosphofructokinase activity. Explain how this inhibition helps regulating glycolysis.

.....
.....

5. Make a diagramme to show the flow of major reactants in and out of mitochondria.

11.14 ANSWERS

Self-assessment Questions:

- 1 a) i) b, ii) e, iii) a, iv) d, v) c

- b) i) d, ii) a, iii) e, iv) b, v) c

- c) i) $C_6H_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 H_2O + \text{energy}$,

- ii) $C_6H_{12}O_6 + 6 H_2O + 12 NAD^+ \rightarrow 6 CO_2 + 12 NADH_2 + \text{energy (ATP)}$
 $12 NADH_2 + 6 O_2 \rightarrow 12 NAD^+ + 12 H_2O + \text{energy (ATP)}$,

- iii) ATP and $NADH_2$,

- iv) Last step (electron transport chain)

- 2 a) i) b, ii) c, iii) c, iv) d, v) c, vi) a

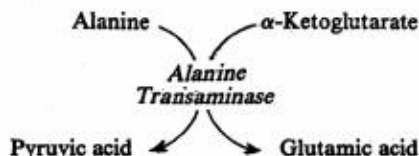
- b) i) Glucose + 2 ADP + 2 Pi + 2 NAD^+ $\rightarrow$
2 pyruvate + 2 ATP + 2 NADH + 2H⁺ + 2 H₂O

- ii) 2

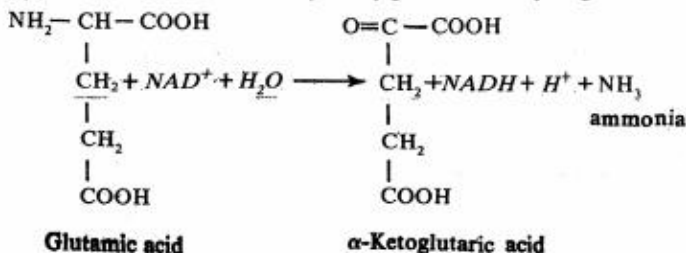
- iii) 2

- c) i) 2 each ii) None
d) i) 2 each ii) None
- 3 a) Hint: see Fig 11.6, reactions, i) 7, ii) 4, iii) 2, iv) 6
b) Hint: See Fig 11.6, reactions i) 3 and 4 ii) 5 iii) 6 iv) 3 and 4
c) iv)
d) iii)
- 4 a) i) NADH_2 and FADH_2
ii) Iron-sulphur protein (FeS) and cytochrome
iii) FMN and ubiquinone
b) The protons are liberated (or transported) outside the inner mitochondrial membrane.
c) i).
d) i) $4, 4e + 4H^+ + O_2 \rightarrow 2H_2O$
ii) $2, 2(\text{NADH} + H^+) + O_2 \rightarrow 2\text{NAD}^+ + 2H_2O$
iii) To reduce one oxygen molecule four (2 pairs) electrons are transferred from NADH_2 . For each pair of electrons transferred from NADH_2 , three pairs (i.e. six) of protons are transported. Thus total protons transported are 12. While 8 protons are transported for FADH_2 , because two pairs of protons are transported for one pair of electrons transferred from FADH_2 to O_2 .
iv) From answer iii, we have seen that twelve protons are transported under this situation. Since one ATP is synthesised when two protons are transported back, six ATP molecules will be synthesised when twelve protons are transported back.
e) The coupling factor couples proton gradient to phosphorylation of ADP. That is, coupling factor allows the protons to move inside the mitochondrial matrix and utilises the energy to form ATP.
f) The proton gradient is the direct source of energy.
g) Cleavage of thioester bond of succinyl CoA in TCA cycle.
h) Carbon monoxide, dinitrophenol, oligomycin.
- 5 a) The starting molecule of PPP is glucose-6-phosphate which is an intermediate of glycolysis. The end products of PPP are fructose-6-phosphate and glyceraldehyde-3-phosphate which are also intermediates of glycolysis. Thus, PPP branches out of glycolytic pathway and enters back into glycolytic pathway. So it is a shuttle of glycolysis.
b) One is that in PPP, NADPH is produced which is required for fat synthesis. The other is that several pentoses are formed in PPP, and these pentoses are required in nucleic acid synthesis.
- 6 a) i) Inhibitors: ATP, NADH, Citrate
Stimulator: ADP
ii) When ATP level becomes high, PFK is inhibited, i.e. glycolysis is slowed down. This makes sense, because a primary purpose of glycolysis is to produce ATP which is required for various biosynthetic and other activities. High level of ATP indicates that its demand is less; so glycolysis should slow down. High level of ATP itself does this job by inhibiting PFK activity.
b) i) Citrate, synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase
ii) Citrate synthase is inhibited by ATP. Isocitrate dehydrogenase is inhibited by ATP and stimulated by ADP, α -ketoglutarate dehydrogenase is inhibited by NADH and succinyl CoA.
- 7 a) Dihydroxy-acetone phosphate
b) acetyl CoA from even-carbon fatty acids.
acetyl CoA and propionyl CoA from odd-carbon fatty acids.
c) Eight
d) Eight acetyl CoA and one propionyl CoA molecules.

- e) Acetyl CoA enters into the TCA cycle by reacting with OAA and forming citrate. The reaction is catalyzed by the enzyme *citrate synthase*.
- f) Propionyl CoA becomes succinyl CoA which enters the TCA cycle directly as an intermediate.



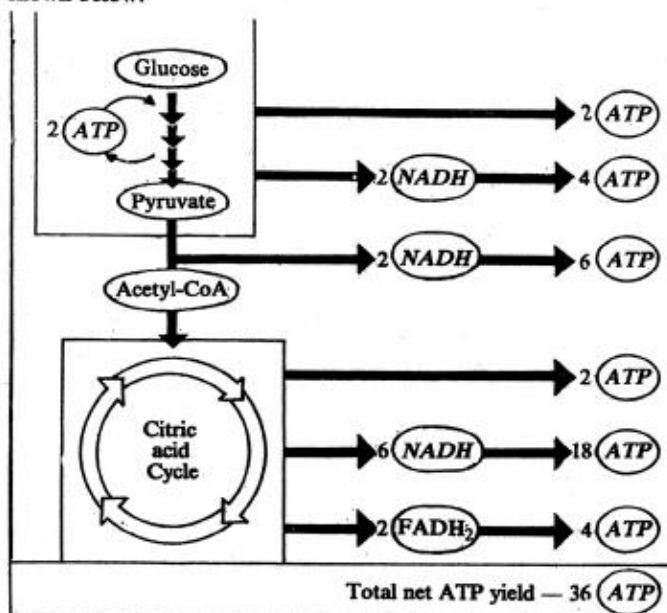
- h) By deamination reaction catalysed by glutamate dehydrogenase



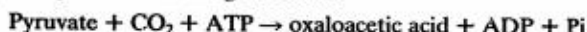
- i) In mammals ammonia is excreted as urea in urine.

Terminal Questions

- 1 The diagram can be drawn in many different ways. One representative diagram is shown below:



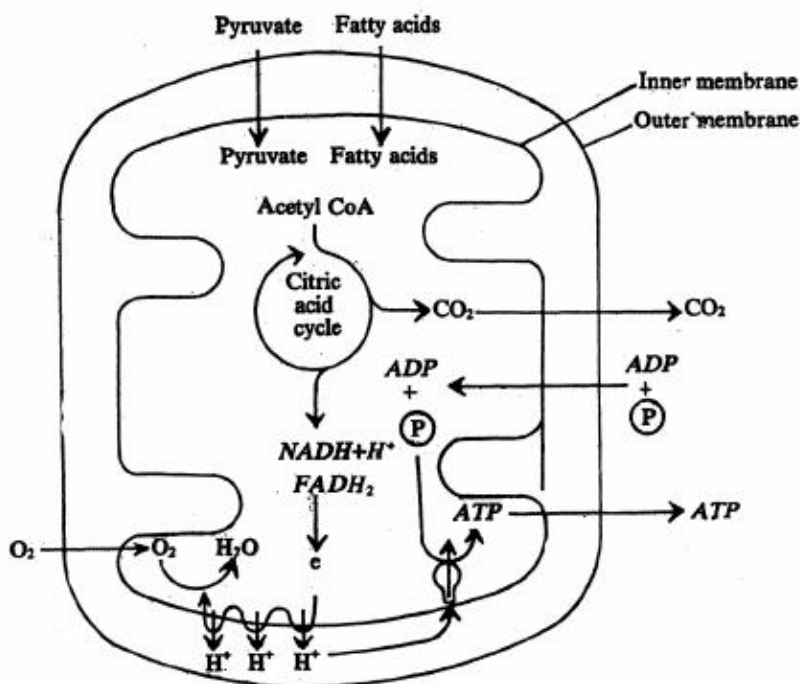
- 2 a) It is converted into alcohol. See reaction in Section 11.4
 b) It is converted to lactic acid. See reaction in Section 11.4
 c) It is metabolised through TCA cycle. (see the reaction in text).
- 3 a) (See last para of Section 11.6).
 b) catalyses the following reactions:



The product of this reaction is oxaloacetic acid (OAA) which is an intermediate of the TCA cycle.

OAA is cycled through the TCA cycle and thus the concentration of the intermediates are increased.

- 4 Citrate. The level (concentration) of a TCA cycle intermediate is controlled by two activities. One is the use of an intermediate for synthetic purpose, such as synthesis of amino acids. By this activity the concentrations of the TCA cycle intermediates fall down. The other activity is the production of oxaloacetate from pyruvate catalysed by *pyruvate carboxylase*. This activity increases the concentrations of the TCA cycle intermediates. Increase in the level of citrate indicates that the TCA cycle intermediates are at high level. Under this situation citrate inhibits the PFK activity. As a consequence pyruvate production is slowed down. This brings down the concentration of the TCA cycle intermediates to a normal (as required by the cell at that time) level.
- 5 The following Fig. shows the flow of major reactants in and out of mitochondria.



- 6 Hint: wood is a polysaccharide, wool and silk are protein in nature.

UNIT 12 BIOSYNTHESIS

Structure

- 12.1 Introduction
 - Objectives
- 12.2 Biosynthesis — An Overview
- 12.3 Carbohydrates—Interconversion in Metabolism
 - Structure of Glycogen and Starch
 - Glycogen Synthesis
 - Glycogen Degradation
 - Regulation
 - Starch Synthesis
 - Sucrose—Starch Interconversions
- 12.4 Gluconeogenesis
 - Reactions of Gluconeogenesis
 - Gluconeogenesis is Costly
- 12.5 Ketone Bodies: An Alternate Fuel Depot
- 12.6 Glyoxylate Cycle
- 12.7 Synthesis of Fats
- 12.8 Summary
- 12.9 Terminal Questions
- 12.10 Answers

12.1 INTRODUCTION

In Unit 11, we have learnt that the energy need of organisms is fulfilled by oxidative degradation (catabolic pathways) of food molecules. The main catabolic pathways are glycolysis, TCA cycle and pentose phosphate pathway. In this unit, we will study a few major biosynthetic pathways that are important in energy metabolism.

Most organisms can synthesise molecules and macromolecules that serve as the structural and functional components of the cells. These compounds belong mostly to the classes of carbohydrates, proteins, fats, nucleic acids (DNA and RNA), vitamins, hormones etc. Plants have a special ability to make many classes of molecules such as pigments, alkaloids, lignins, rubber, waxes etc., which animals cannot synthesise.

Among carbohydrates, glycogen and starch are especially important in energy metabolism. You will learn that they are synthesised when there is surplus of catabolic energy, and are degraded when the energy is in deficit. During starvation when glycogen stores are depleted, glucose is synthesised from non-carbohydrate precursors such as amino acids and lactate by an important route called gluconeogenesis.

The routes for biosynthesis of carbohydrates, fats, proteins and nucleic acids are common to most animals, plants and microorganisms. In this unit, we briefly study the biosynthesis of carbohydrates and fats. In the following two units we will study the biosynthesis of proteins and nucleic acids.

Objectives

After reading this unit you should be able to:

- schematically show the interrelationship between breakdown and biosynthetic pathways
- describe the major principles of biosynthetic pathways
- outline the major steps involved in the biosynthesis of glucose, glycogen and starch
- describe the different conditions in the organism that favour the synthesis or degradation of glycogen, starch, glucose and fatty acids and explain the mode of regulation of synthesis and degradation
- outline glyoxylate cycle and explain its significance
- describe the origin and fate of ketone bodies.

12.2 BIOSYNTHESIS—AN OVERVIEW

All biosynthetic processes have three basic requirements — energy (ATP), reducing power ($\text{NADH} + \text{H}^+$) and building blocks or their precursors. You have already learnt that these are the products of catabolism of food taken in by the organism. In fact, organisms ingest certain types of carbohydrates, fats and proteins, and break them down through catabolic pathways into smaller precursors and produce energy and reducing power. Then through biosynthesis, the organisms rebuild them into other types of carbohydrates, proteins, fats and other molecules according to their specific needs. Thus, anabolic processes are related to catabolic processes.

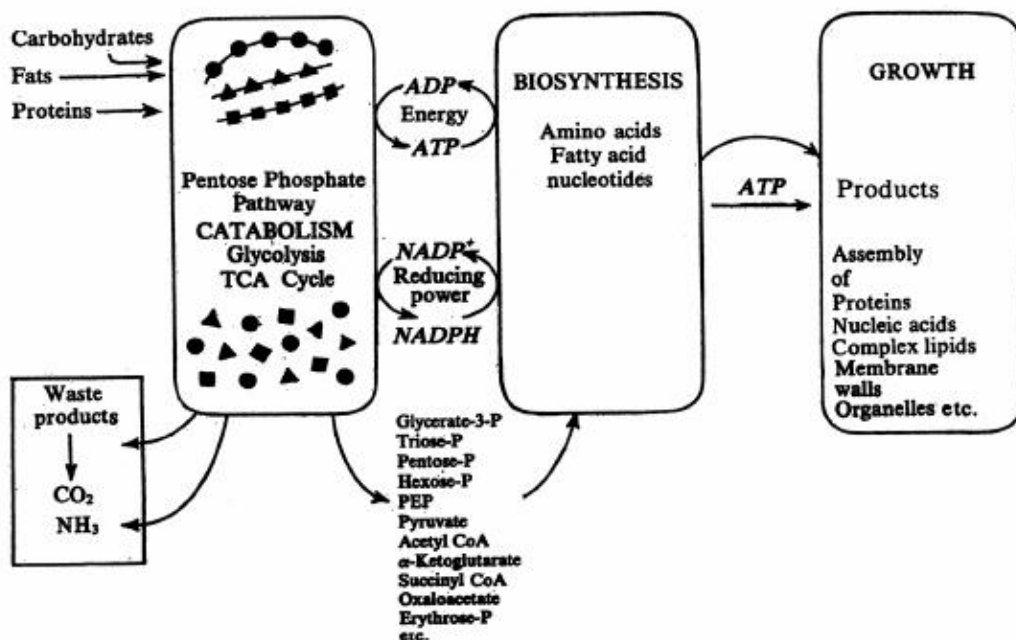


Fig. 12.1: Schematic diagram showing functional interrelationship between catabolism and biosynthesis.

Fig. 12.1 outlines the relationship between catabolic and anabolic processes. The products of catabolism ATP, NADPH and various intermediates are utilised in the biosynthesis of amino acids, fatty acids, nucleic acids, and various other molecules. Reactions involved in biosynthetic pathways are similar to catabolism in that they are also organised into sequences with specific functions. The two, however, operate in opposite directions. As shown in Fig. 12.2 the catabolic pathways show a converging pattern i.e. quite different substrates degrade to a few common products. By contrast anabolic pathways start from a few common intermediates (Fig. 12.2) and synthesise a large variety of compounds.

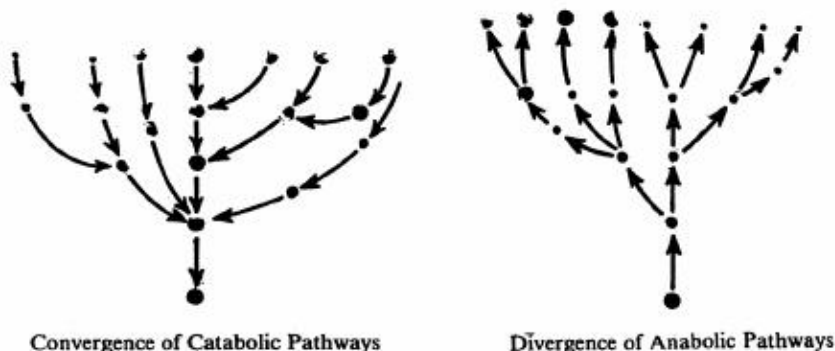


Fig. 12.2: Converging pattern of catabolic pathways and diverging pattern of anabolic pathways.

Another important feature of anabolic and catabolic pathways is that they are connected to each other through many common intermediates. Read the list of intermediates and the products shown in Fig. 12.1 (recall Unit 11, these are produced during catabolism).

In other words, the various anabolic processes are neither independent of each other nor-independent of the catabolic processes. These features of metabolism make the whole process highly complex, but it provides the system an immense scope in regulation of one pathway with respect to the other. This complexity, in fact, is beneficial because it allows the organism to utilise energy in a very economic way.

Although the whole process of metabolism is complex, it is extremely well organised and follows certain principles. It is worthwhile to know three such major principles of biosynthetic processes, before we learn about the different biosynthetic pathways and their interconnection. First, the catabolic pathways of a given molecule is not **identical** to its anabolic pathway. We have learnt that in the process of glycolysis, glucose is broken down to pyruvate through ten, enzymatic reactions. You will learn in this unit that the synthesis of glucose from pyruvate follows seven of the ten reactions in the reverse direction, but three reactions are different. You will learn that this difference provides a great deal of flexibility in regulation of both the breakdown and the synthesis of glucose.

The second general principle is that the enzyme (or enzymes) which regulates the biosynthetic pathway is **different** from the regulatory enzyme (or enzymes) of the catabolic pathway. Keeping in mind the first principle, we can understand that the second principle implies that the regulatory enzymes must be among those which are not common for both the pathways. This principle will be illustrated with examples in this unit.

The third general principle is that the two pathways (biosynthetic and degradative) are generally regulated in a **reciprocal manner**. That is, if a condition or a factor stimulates the biosynthetic pathway, the same condition or factor inhibit the degradative pathway and vice versa. A very common such factor is the ratio of ATP and ADP in the system.

SAQ1

- a) refer to 12.1 It show eleven intermediates of carbon hydrete catabolism which are used for biosynthetic purpose ,from your knowledge of unit ii identify one intermediate each for the biosynthesis of amino acid fatty acid and nucleic acid

- b) Refer to figer 12.2 one of the figer show the convergence of catabolic pathway .from your knowledge of unite ii.name three compound ,which could be metabolised to acommon product,name the common product also

- c) Give one reason why the biochemical pathway for cathabolism of a molecules among never the same as the pathway for the biosynthesis of that molecule

12.3 CARBOHYDRATE — INTERCONVERSION IN METABOLISM

All cells require a constant supply of energy for survival. But organisms can only procure food whenever it is available, at intervals. Hence, they have acquired a way to store, mobilise, degrade and continuously supply it to all cells to fulfil their energy

demand. Cells of higher animals, receive a constant supply of glucose from the blood. Liver plays an important role in regulating the glucose level in blood. It functions as a storage and distribution centre of the nutrients. Through blood, it receives the products of digestion — glucose, amino acids and other digested material absorbed by capillary beds. The nutrients are metabolised, stored and distributed to other tissues also through the liver. In addition, the liver also regulates the availability of the nutrients to all tissues.

When the concentration of glucose in blood entering the liver is high (above a normal level), liver stores glucose in the form of a polysaccharide called glycogen as dense granules in the cytoplasm of cells. When the glucose level in blood falls below the normal level, liver glycogen is degraded to glucose. The maintenance of normal levels of blood glucose within the operating range depends in large part upon the activities of two hormones, glucagon and insulin produced by the pancreas. We will describe later the role of these hormones in regulation of metabolism.

Unlike animals, plant cells make food by photosynthesis during day time. One product of photosynthesis is sucrose. Like glucose in the blood, sucrose is transported to cells of plants to fulfil their carbon and energy needs. In addition, it is also used for the synthesis of cellulose and other structural polysaccharides required for the formation of the cell wall. What is the source of energy of the plant at night when photosynthesis stops? Sucrose is not stored in the cells for the periods when photosynthesis is not operating. Instead, it is converted to starch — a storage polysaccharide of glucose in the chloroplast. Starch is structurally and functionally similar to glycogen. At night the stored starch is degraded to sucrose and is transported to all parts of the plant.

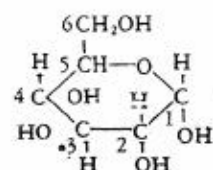
Plants also store starch in developing seeds and in vegetatively propagatory structures. Starch is utilised in high metabolic activities of germinating seeds.

Let us now start with the structures of glycogen and starch and then study their synthesis and degradation.

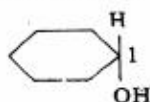
12.3.1 Structure of Glycogen and Starch

Glycogen and starch are branched-chain homopolysaccharides made up of monomeric units of glucose as shown in Figs. 12.3 and 12.4. In glycogen, glucose units are joined together in a linear way by α 1 $\rightarrow$ 4 glycosidic bonds. After 8 to 10 monomeric units the branches are formed by α 1 $\rightarrow$ 6, glycosidic linkage (see margin). In all there are about **one million** glucose units in a molecule of glycogen.

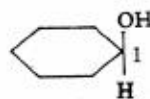
Starch is a mixture of two homopolysaccharides — amylose and amylopectin, both with glucose as monomeric units. In amylose (Fig. 12.4) about 100 to several thousand units of glucose are linked together linearly by an α 1 $\rightarrow$ 4 glycosidic linkage.



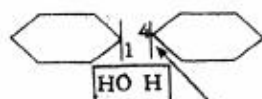
Ring form of glucose



α -form
(OH at Carbon 1 below the ring)

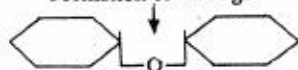


β -form
(OH at Carbon 1 above the ring)

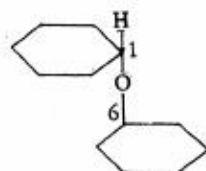


Non reducing end

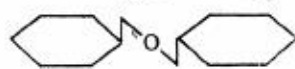
Formation of linkage



α 1 $\rightarrow$ 4 glycosidic linkage



α 1 $\rightarrow$ 6 glycosidic linkage



β 1 $\rightarrow$ 6 glycosidic linkage

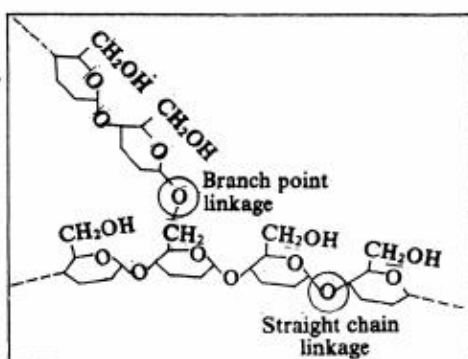
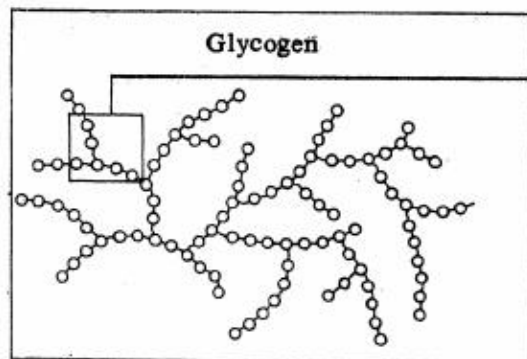


Fig. 12.3: Structure of glycogen

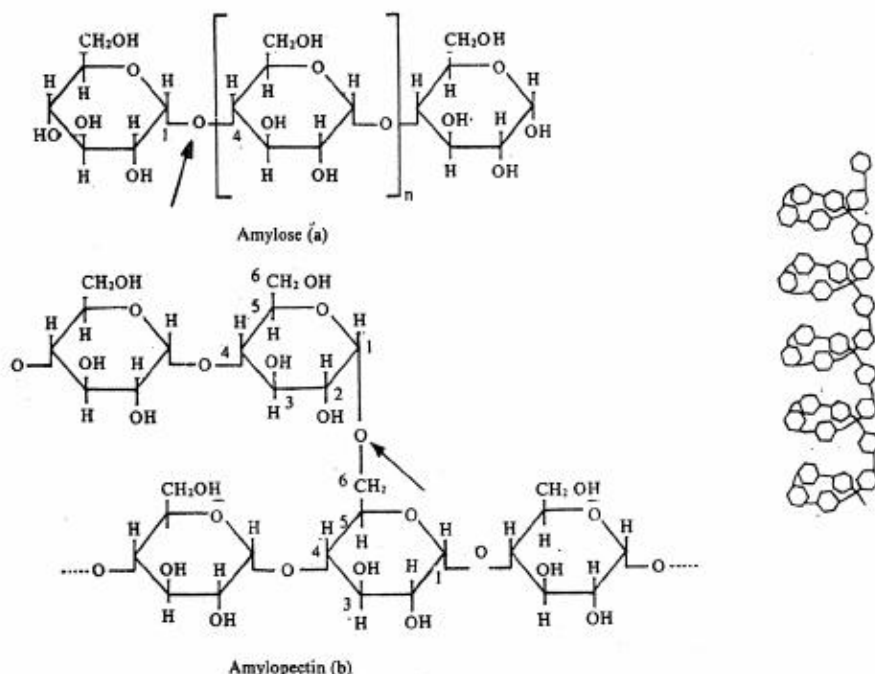


Fig. 12.4: Components of starch — a) amylose, b) amylopectin, and c) helical chain of amylose.

The amylose chain has a helical structure (Fig. 12.4 c). Amylopectin is a highly branched molecule consisting of about 50,000 monomeric units. Like glycogen, glucose molecules in amylopectin are linked linearly by α 1 $\rightarrow$ 4 glycosidic linkage, and make branch points by α 1 $\rightarrow$ 6 glycosidic linkages. But a glycogen molecule is more compact than amylopectin because in glycogen the branches are shorter (8 to 12 units of glucose), and occur at shorter intervals (8 to 10 units of glucose), whereas in amylopectin the branches are longer (12 to 25 units) and occur at longer intervals (12 to 25 glucose units). The numerous branch points in the glycogen molecule give it a spherical shape.

12.3.2 Glycogen Synthesis

Glycogen synthesis occurs in four steps: synthesis of UDP-glucose, chain initiation, chain elongation, and formation of branches.

Let us study Fig. 12.5 which shows the various reactions involved in each step of glycogen synthesis. We know that glucose is the monomeric unit in glycogen. Glucose is converted into its activated form, UDP-glucose. This conversion involves three reactions. First, free glucose is phosphorylated at the expense of an ATP molecule by enzyme *hexokinase* to glucose 6-phosphate. In the subsequent reaction, it is isomerised to glucose 1-phosphate which is then converted into UDP-glucose by *glucose 1-phosphate uridyl transferase*. The energised form, UDP-glucose, is the donor of glucose in glycogen synthesis. In the following step, UDP-glucose molecules do not polymerise with each other instead, they are sequentially added to a fragment of glycogen which is a polyglucose or a branch monomer consisting of at least four monomeric units. This fragment of glycogen is called a primer. In case of absence of glycogen fragment, a special protein serves as the acceptor of glucose units. The glucose molecule can get attached to the protein only by the amino acids containing hydroxyl groups (serine $\text{NH}_2\text{-CH}(\text{CH}_2\text{OH})\text{-COOH}$ or threonine $\text{NH}_2\text{-CH}(\text{CH}(\text{OH})\text{CH}_3)\text{-COOH}$). The addition of the initial molecule of glucose from UDP-glucose to the hydroxyl group of amino acid of the protein is called the **initiation reaction** and is carried out by the enzyme *glycogen initiator synthase*.

The elongation of the chain is a simple process. More and more molecules of glucose are sequentially transferred from UDP-glucose of the first glucose molecule (to the non-reducing end of the polyglucose) by enzyme *glycogen synthase*, resulting in a linear homopolysaccharide with α 1 $\rightarrow$ 4 glycosidic linkages.

The advantage of storing glucose in a polymeric form is that large quantities of small glucose molecules lead to high osmotic pressure within the cell. The polymeric form, glycogen, therefore is not open to the risk of membrane lysis.

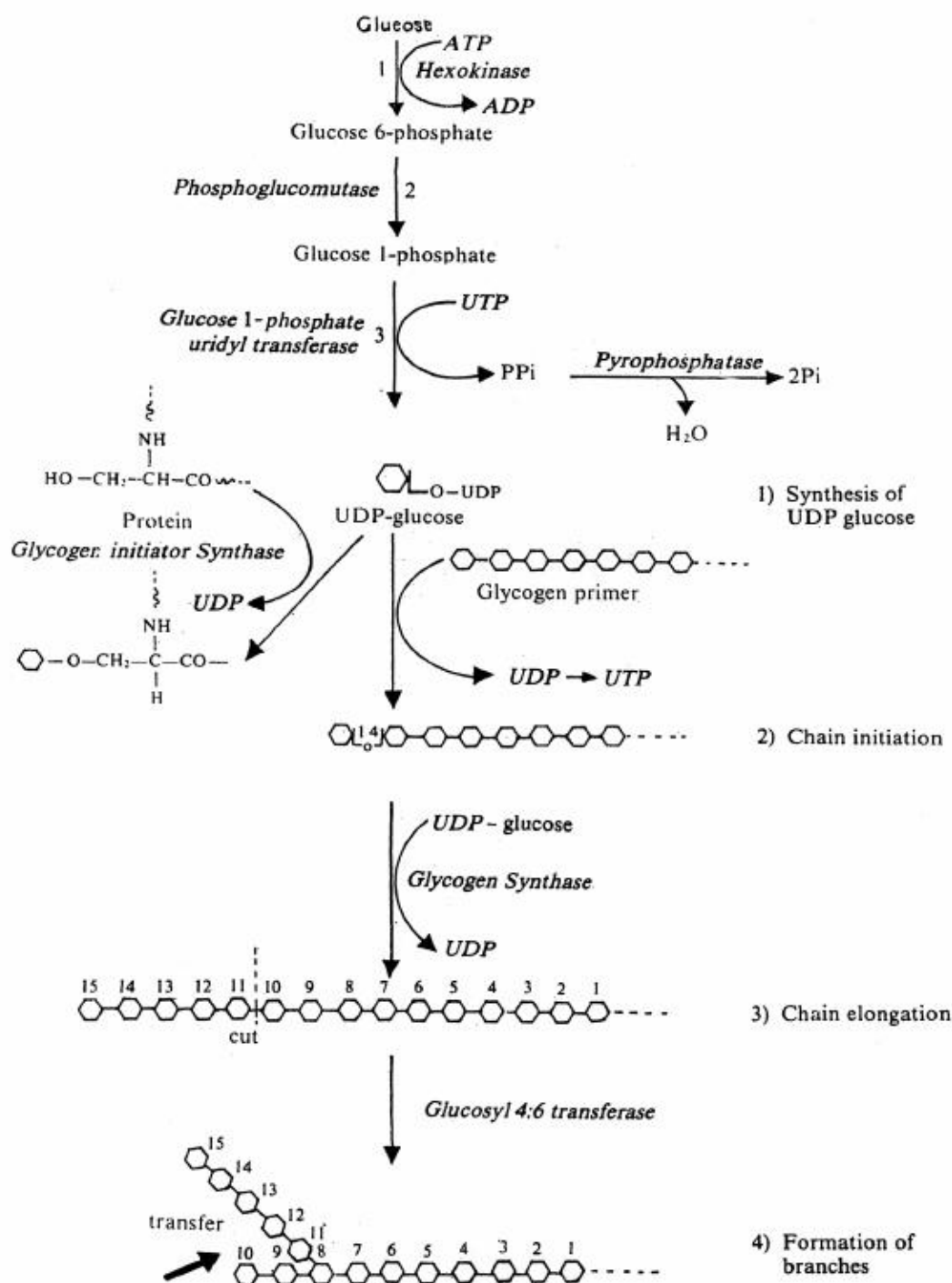


Fig. 12.5: Various steps in the synthesis of glycogen

Finally, the branch points are made through the activity of the branching enzyme namely, *glucosyl 4:6 transferase*. This enzyme has two functions. First it cuts the terminal polyglucose molecules having 6 to 7 monomeric units and then it transfers them by forming α 1 $\rightarrow$ 6 glycosidic linkage in the linear glycogen branch having at least 11 monomeric glucose units. The new ends (indicated by the bold arrow) are further elongated by *glycogen synthase* and once they reach a certain length, the polyglucose molecule is removed again and is transferred for introducing new branches. The advantage of branching is that more free ends are available for the growth of the glycogen molecule.

12.3.3 Glycogen Degradation

Let us look at Fig. 12.6 showing the degradative steps involved in the breakdown of glycogen. In the first step, enzyme *glycogen phosphorylase* sequentially cleaves

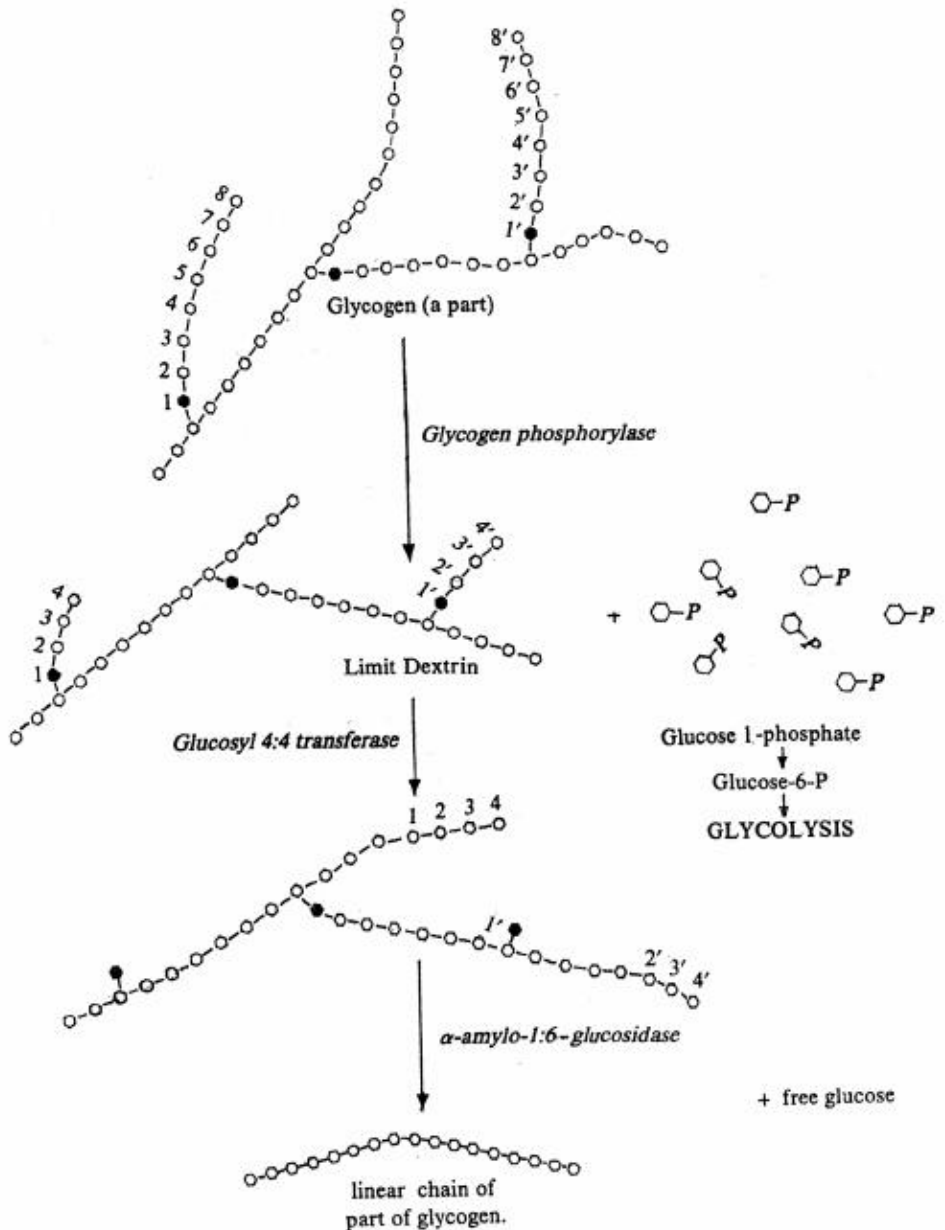


Fig. 12.6: Degradation of glycogen

monomeric units beginning from the free ends until only four glucosyl units are left on each branching chain before the branch points. In this way numerous monomeric units are released as glucose 1-phosphate. These can enter the glycolytic cycle shown in the figure. The remaining smaller molecule of glycogen is called **limit dextrin**. The second step in degradation is the preparation for delinking of α 1 $\rightarrow$ 6 linkages at the branch points. Enzyme *glucosyl (4:4) transferase* cuts the remaining chain in the form of a polyglucose molecule (3 units of glucose) but it leaves a monomeric unit at the branch point (see Fig. 12.6). In addition, this enzyme transfers these cleaved polyglucose molecules to the terminal ends of outer chains. Thus the enzyme performs two jobs — cutting the polyglucose molecule from the branches, and transferring it to the linear chain. In the third step, enzyme α -*amylase*-(1:6)-*glucosidase* breaks α 1 $\rightarrow$ 6 linkages and releases monomer units as free glucose. The remaining glycogen molecule is once again available for degradation by *glycogen phosphorylase* and the other steps of degradation are also repeated.

SAQ 2

- a) Fill in the blanks spaces with appropriate answer
- Glucose is stored in animals as
 - Glycogen is stored in the cell of.....and.....
 - Sucrose is stored in plants as
 - Starch is synthesised in
 - In human body..... processes and distributes nutrients
- b) Can you think of some of the properties of starch and glycogen which make them suitable as food reserves?

- c) What would be the effect of increase in glucose above the normal level?

- d) Fill in the blank space with appropriate words.

- The building block for glycogen is
- The glucose units in glycogen are connected by.....linkage in linear way and by.....linkage at branch points.
- The components of starch are.....and.....

- e) Match the enzymes listed in column 1 with their functions in column 2.

Column 1	Column 2
i) <i>Glycogen phosphorylase</i>	a) releases glucose phosphate from glycogen
ii) <i>Glycogen synthase</i>	b) moves three sugar residues from one chain to another
iii) <i>Glucosyl (4:6) transferase</i>	c) phosphorylates glucose to glucose 6-phosphate
iv) <i>Hexokinase</i>	d) converts glucose 1 phosphate to UDP glucose
v) <i>Glucose-1-phosphate uridylyltransferase</i>	e) transfers glucose from UDP glucose to growing glycogen chain

12.3.4 Regulation

The synthesis and breakdown of glycogen in mammals is regulated to prevent undue fluctuations in blood glucose level. This regulation is primarily controlled by the action of two pancreatic hormones, glucagon and insulin. The metabolic effects of these two hormones are opposite to each other. Increase in the circulating level of insulin results in an overall increase in glycogen synthesis; while elevated levels of glucagon cause glycogen degradation. Apart from glucagon, another hormone

epinephrine stimulates the breakdown of glycogen in muscle cells. The binding of glucagon to hepatocyte receptors or of epinephrine to the muscle cell receptors results in the cascade of regulatory interactions shown in Fig. 12.7. You know that cleavage of the glucose molecule from glycogen is catalysed by enzyme *glycogen phosphorylase*. This enzyme exists in two forms: active form a, which is phosphorylated and inactive form b, which is not phosphorylated. In other words, phosphorylation of the enzyme is necessary for its catalytic activity.

Let us now look at the cascade of events given in Fig. 12.7. The activation is signalled by hormone glucagon or epinephrine. First, glucagon activates enzyme *adenyl cyclase* which forms cyclic AMP (cAMP) (step 2). In the next step (3), cAMP activates *protein kinase*. Protein kinase has two regulatory subunits as shown in margin Figure 12.8 which block the activity of the enzyme. When cAMP binds to the regulatory units, conformational changes occur that result in the removal of blockage by the units and the enzyme becomes active. Now, the active *protein kinase* phosphorylates (step 4) the enzyme *phosphorylase kinase*. *Phosphorylase kinase* in turn activates *glycogen phosphorylase b* (step 5) by converting it into active *phosphorylase a* which cleaves glucose from glycogen (step 6).

The regulatory enzyme for the synthesis is *glycogen synthase*. The activity of this enzyme is decreased with an increase in the level of cAMP. As opposed to glucagon, insulin has a negative effect on the enzyme *adenyl cyclase*, thus inhibiting the formation of cAMP

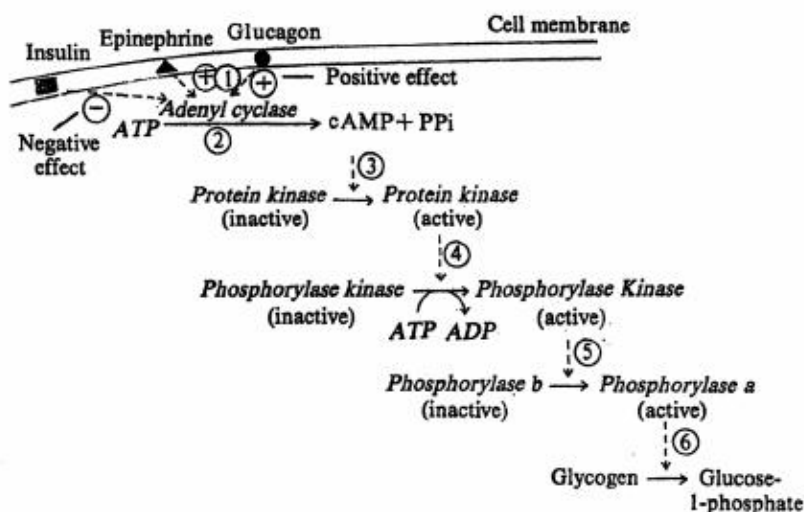


Fig. 12.7: Stimulation of glycogen breakdown by the hormone glucagon and epinephrine.

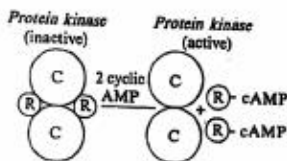


Fig. 12.8: Activation of protein kinase by cyclic AMP

12.3.5 Starch Synthesis

We know that starch is a mixture of two homopolysaccharides of glucose—amylose and amylopectin. The starch grains of most plants contain 15–25% amylose and 75–85% amylopectin. The synthesis of starch occurs in two steps. First, the linear helical molecule of amylose is formed by α 1→4 glycosidic linkages and then, on part of the molecules, α 1→6 glycosidic branches are formed resulting in the synthesis of amylopectin.

Look at Fig. 12.9 showing the synthesis of starch. The steps involved in the synthesis of this polysaccharide are similar to those of glycogen synthesis. Let us read these steps in sequence in the figure.

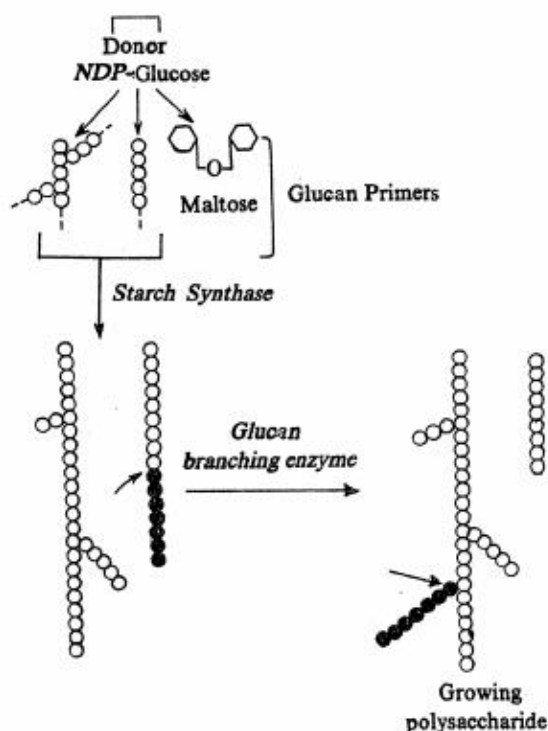


Fig. 12.9: Synthesis of Starch

The donor of glucose is generally nucleotide diphosphate glucose (NAD – UDP or ADP) which is added to a primer molecule α 1 $\rightarrow$ 4 glucan by the enzyme *starch synthase*. The size and structure of the glucan primer varies. It can range from a disaccharide maltose containing only two monomeric units of glucose to a polysaccharide amylose containing thousands of monomeric units. It can also be a small molecule of amylopectin dextrin which is similar to the limit dextrin of glycogen. Consequently, an amylose molecule is formed when the primer glucan has a linear chain but amylopectin is formed when the primer glucan has branches (dextrin).

Glucan – class of polysaccharides containing glucose units only.

The new branches are introduced in the next step in the same way as in glycogen by enzyme α 1 $\rightarrow$ 4, glucan branching enzyme. The enzyme cuts a polyglucose segment from amylose molecule and attaches it to growing α 1 $\rightarrow$ 4 glucan or to a growing amylopectin by α 1 $\rightarrow$ 6 linkage. Thus repetitive elongation in linear way forms amylose and the introduction of branching sequences ultimately builds up amylopectin molecule.

12.3.6 Sucrose—Starch Interconversion

We know that in plants sucrose is the main transporter of energy and starch is both a short-term and long-term storage keeper of energy. They are often interconverted. Sucrose is synthesised in leaves during the active period of photosynthesis and is converted into starch. In school, you may have tested starch in leaves. Starch is degraded to sucrose and transported to other tissues. Large excess of sucrose is transported to some tissues, such as grains, where it is converted into starch again and stored to fulfil future needs. On germination, the stored starch in the seeds is re-converted into sucrose to provide energy for the growth of the juvenile seedling.

Raw fruits do not taste well because they contain starch. On ripening, starch in the fruits is converted into sucrose. That is why a ripe fruit tastes sweet.

Investigations show that reactions and enzymes involved in starch-sucrose interconversion are not common to all tissues. We will study the reactions of interconversion occurring in germinating and developing seeds. Let us see Fig. 12.10a. In the developing seeds, sucrose first breaks down by the enzyme *sucrose synthase* to UDP-glucose and fructose(1). This is the reverse reaction of sucrose synthesis. In the following steps UDP-glucose changes to ADP-glucose(2) via glucose-1 phosphate (3) Finally, glucose from ADP-glucose is then incorporated into primer molecule (dextrin) by *starch synthase* (4, see also steps in Fig. 12.9).

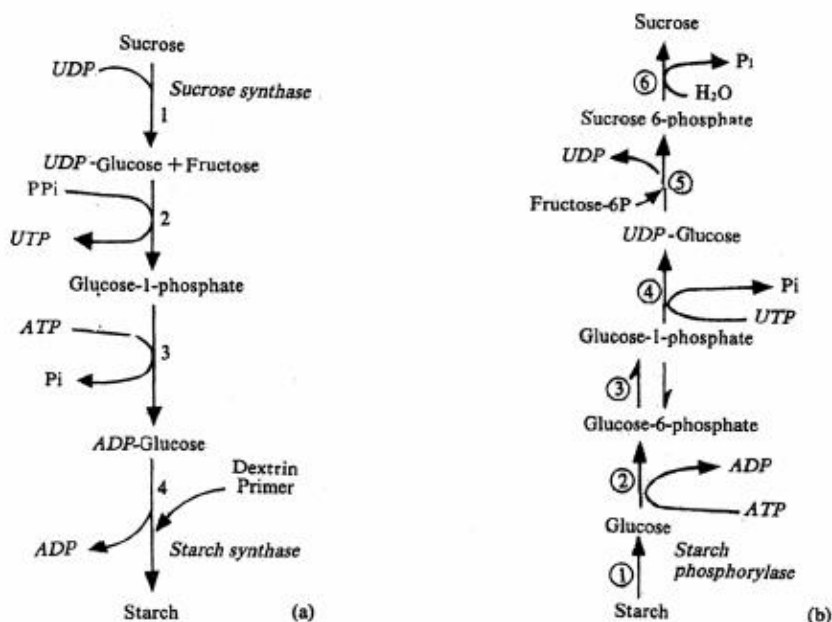
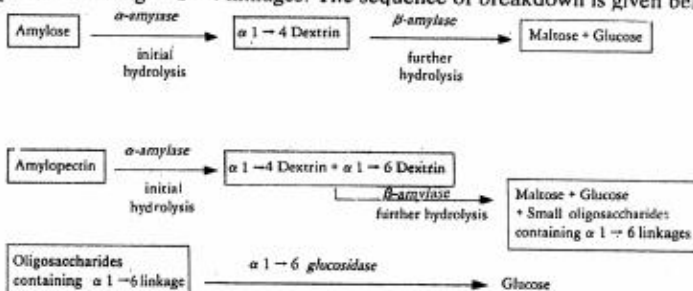


Fig. 12.10: Conversion of sucrose into starch (a) in developing seeds and conversion of starch into sucrose (b) on germination

The conversion of starch to sugar (Fig. 12.10b) is not restricted to a reversal of the reactions of its synthesis. Several types of *hydrolase* enzymes and *starch phosphorylase* can hydrolyse starch to glucose. Glucose is phosphorylated (2), isomerised (3) and converted into UDP glucose (4). Finally, UDP glucose and fructose 6-phosphate join to form sucrose. We have mentioned above that starch can be degraded to monosaccharides by *hydrolases*. Among these, α and β *amylases* are very important enzymes. α -*amylase* carries out random hydrolysis of amylose and amylopectin breaking α 1 $\rightarrow$ 4 linkages. The sequence of breakdown is given below.



α -*amylase* is present in saliva and pancreatic juice and helps in the digestion of starch. It yields a mixture of glucose and maltose. Maltose is a disaccharide of glucose.

The major end product from both molecules, amylose and amylopectin, is maltose. β -*amylase* cannot initiate the hydrolysis of starch. It works on the products formed by α -*amylase* action and degrades them to maltose. Like amylose, it cannot work on the α 1 $\rightarrow$ 6 linkages at the branch points. Debranching enzymes α 1 $\rightarrow$ 6 *glucosidase* hydrolyse the α 1 $\rightarrow$ 6 linkages at the branch point.

SAQ 3

In the first reaction of starch synthesis, a glucose molecule is attached to a primer.

- Which is the donor of this glucose molecule?
- Name the four molecules which can act as primer.
- What is the name of the enzyme catalysing this reaction?

b) In the second step of starch synthesis, branching takes place.

- What is the nature of this branch?
- Where does it come from?
- Where is this branch connected to?

c) i) From Fig. 12.10 find out which step is common to conversion of both sucrose $\rightarrow$ starch, and starch $\rightarrow$ sucrose

ii) How many ATP molecules are used up to incorporate the glucose molecule of sucrose into starch?

d) Which among the following statements are correct?

Write T for true and F for false.

- α -amylase breaks $\alpha 1 \rightarrow 4$ glycosidic linkage in amylose molecule-
- α -amylase breaks $\alpha 1 \rightarrow 6$ glycosidic linkage
- α -amylase cannot hydrolyse amylopectin
- Maltose is formed by action of α -amylase on starch

12.4 GLUCONEOGENESIS

In section 12.2.2, we have learnt that liver synthesises and stores glycogen molecules which breakdown continuously to maintain the operating levels of glucose in the blood. The glycogen store in the liver is limited and can last for 6 to 12 hours. Liver glycogen is replenished when animals take in food again. Let us now consider the situation when the glycogen store is depleted but there is no fresh intake of food. For instance, often animals, fail to procure food, people starve during famine or while fasting. You may know that the intake of food is restricted during digestive problems, after surgery or in other clinical situations. But our brain is highly dependent on glucose as a primary fuel and erythrocytes also require glucose. Then how is the concentration of blood glucose maintained in the absence of carbohydrates? Under such circumstances glucose is synthesised from the carbon skeleton of amino acids, lactate and glycerol through a pathway called **gluconeogenesis**. Generally, proteins are not used as fuel for the body but in emergency 1/3 of the body proteins can be used to produce glucose. The synthesis of glucose in human beings starts 4 to 6 hours after the last meal. It speeds up later and continues until a fresh supply of glucose from carbohydrate food is available to the liver.

You have learnt that during intense muscular activity the skeletal muscle cells accumulate lactic acid. This cannot be metabolised further and therefore diffuses into blood and is transported to the liver, where it is converted into glucose and is released back into circulation. This is a cyclic flow, as shown in Fig. 12.11, and is called the cori cycle.

12.4.1 Reactions of Gluconeogenesis

We know that the substrates for the *de novo* synthesis of glucose are lactate, pyruvate, glycerol and α -ketoacids, which join the metabolic pool of glycolytic intermediates. However, glucose is not synthesised by a simple reversal of glycolysis. This is in accordance with the organising principle of biosynthesis, mentioned in the beginning of this unit, that the pathway of synthesis of biomolecules is not identical to

The term gluconeogenesis literally means the formation of glucose from the carbon of non-carbohydrate precursors.

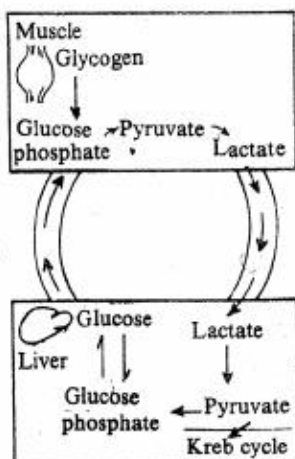


Fig. 12.11: Lactate formed by glycolysis in the muscle is transported to the liver where it is converted to glucose by gluconeogenesis. The cyclic flow is called cori cycle.

the pathway of its degradation. Instead, biosynthesis utilises one or more reactions different from the one's used in the corresponding degradation. Gluconeogenesis occurs in the cytoplasm. It utilises 7 reactions of glycolysis that are freely reversible and 3 irreversible reactions are replaced by more energetically favourable alternate routes called **bypass reactions**. Thus the two pathways are irreversible.

Fig. 12.12 shows the glycolytic pathway, the TCA cycle and the different metabolites joining these pathways at the various points. For the sake of comprehensive and integrated understanding of gluconeogenesis with other pathways, gluconeogenesis is not outlined separately. Instead, we will study it by following the glycolytic reactions backwards. We will concentrate more on the substrates and bypass reactions that are highlighted in the figure. Let us start studying the figure from the bottom beginning with pyruvate

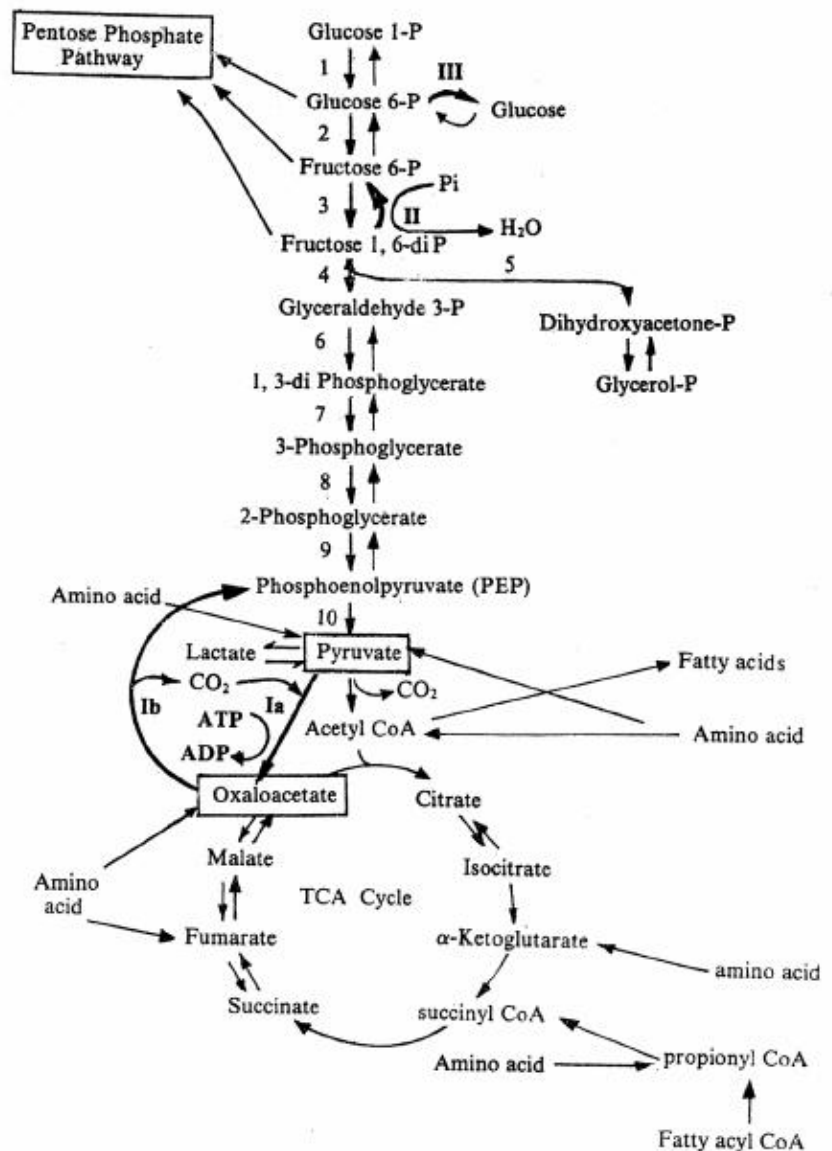


Fig. 12.12: Pathways of carbohydrate metabolism. Reactions of gluconeogenesis are highlighted.

As shown, the formation of pyruvate from phosphoenol pyruvate (PEP) is irreversible. Therefore, this "roadblock" is overcome by the bypass reactions Ia and Ib. The central compound for gluconeogenesis is oxaloacetate (OAA). It is produced by a variety of available substrates which are linked to the main pathway as indicated. Lactate and some amino acids make oxaloacetate through pyruvate. The enzymes, *lactate dehydrogenase* and *aminotransferase* convert lactate and amino acids, respectively to pyruvate, which by reaction Ia are converted into OAA. The carbon skeleton of twenty amino acids is degraded to many types of products such as pyruvate, acetyl CoA, α -keto glutarate and fumarate. Some of these may enter the mainstream at various points and are converted into oxaloacetate. Note that propionate, resulting from the breakdown of odd chain fatty acids, also forms succinate which enters the TCA cycle and finally makes oxaloacetate which can be used in gluconeogenesis. Acetyl CoA produced by the even-chain fatty acids and from some amino acids enters the TCA cycle at the beginning and results in the formation of CO_2 .

Now look at the reactions of the pathway from OAA to glucose. Once PEP is made, gluconeogenesis proceeds through the reversal of glycolytic reactions up to the formation of fructose 1,6 diphosphate. Among the 10 reactions of glycolysis 1,3 and 10 are irreversible. Can you tell why these three reactions of the downhill glycolytic sequence cannot participate in the uphill process of gluconeogenesis?

This is because they have large negative standard free energy changes and high equilibrium constants. Therefore, they are replaced by reactions I(a and b), II and III.

In the first bypass reaction Ia, pyruvate is carboxylated – to OAA by enzyme *pyruvate carboxylase*. The enzyme contains a covalently attached prosthetic group-biotin (the role of biotin in carboxylation is explained in a later section on synthesis of fats). This conversion takes place in the mitochondria. Since the subsequent reactions of gluconeogenesis occur in the cytoplasm, OAA must be transported to the cytoplasm. We know that OAA cannot cross the inner mitochondrial membrane directly. So, it is transported in an indirect fashion as shown in margin Fig. 12.13. It is first reduced to malate and then transported in exchange for phosphate by the **malate-phosphate antiport system**. In the cytoplasm, malate is reoxidised to OAA. In reaction Ib OAA, now in the cytoplasm, is decarboxylated and phosphorylated by *PEP carboxykinase*. The reaction consumes GTP for phosphorylation.

The second bypass reaction (II) is dephosphorylation of fructose 1,6-diphosphate by enzyme *fructose 1,6-diphosphate phosphatase*. In the bypass reaction (III) glucose 6-phosphate is dephosphorylated by the enzyme *glucose 6-phosphatase* to glucose. The products of essentially irreversible reactions push the equilibrium in favour of glucose synthesis.

12.4.2 Gluconeogenesis is Costly

Now we will look into the consumption of ATP and NADH in gluconeogenesis (pyruvate $\rightarrow$ glucose) and compare it with its production during glycolysis (glucose $\rightarrow$ pyruvate). Table 12.1 lists the reactions leading from pyruvate to glucose. For each

Table 12.1 : Consumption of ATP, GTP and NADH H^+ in gluconeogenesis

Substrate	Reaction	Product	ATP/GTP/NADH used/glucose molecule
Ia	$2 \times \text{pyruvate} \rightarrow 2 \times \text{oxaloacetate}$		2 ATP
Ib	$2 \times \text{oxaloacetate} \rightarrow 2 \times \text{phosphoenol pyruvate}$		2 GTP
9	$2 \times 2\text{-phosphoenolpyruvate} \rightarrow 2 \times 2\text{-phosphoglycerate}$		0
8	$2 \times 2\text{-phosphoglycerate} \rightarrow 2 \times 3\text{-phosphoglycerate}$		0
7	$2 \times 3\text{-phosphoglycerate} \rightarrow 2 \times 1,3\text{-diphosphoglycerate}$		2 ATP
6	$2 \times 1,3\text{-diphosphoglycerate} \rightarrow 2 \times \text{glyceraldehyde 3P}$		2 NADH + H^+
5	$\text{glyceraldehyde 3-P} \leftrightarrow \text{dihydroxy acetone-P}$		0
4	$\text{glyceraldehyde 3-P} + \text{dihydroxyacetone P} \rightarrow \text{Fructose 1,6-P}$		0
II	$\text{fructose 1,6-phosphate} \rightarrow \text{fructose 6-P}$		0
2	$\text{fructose 6-P} \rightarrow \text{glucose 6-P}$		0
III	$\text{glucose 6-P} \rightarrow \text{glucose}$		0

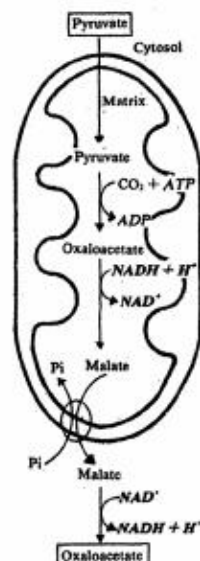
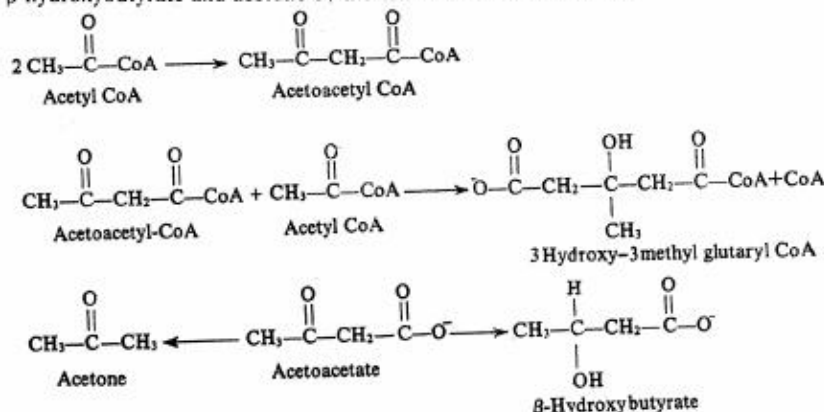


Fig. 12.13: Transport of OAA from mitochondria by malate phosphate antiport system. OAA leaves mitochondria in the form of malate which is reoxidised in the cytoplasm to OAA.

molecule of glucose produced, 6 high-energy phosphate bonds are broken. Two molecules of ATP are required in reaction 1a, 2 molecules of GTP in reaction 1b, and 2 molecules of ATP in reaction 7. In addition, two molecules of NADH are used in reaction 6. Comparison of the net reactions of gluconeogenesis and glycolysis shows that synthesis of glucose requires 4 extra high energy bonds when produced by gluconeogenesis.

12.5 KETONE BODIES : AN ALTERNATE FUEL DEPOT

We have just learnt that during starvation a variety of substrates contribute to the formation of glucose. We also know that fats (triglycerols) are the major metabolic fuel used during starvation. Triglycerols stored in adipose tissues are mobilised and free fatty acids are released in the blood. They finally reach the liver and are degraded to acetyl CoA. The production of acetyl CoA from fatty acids far exceeds its oxidation through the TCA cycle because the OAA is now utilised for gluconeogenesis and not much of it is available for the oxidation of acetyl CoA. In such conditions excess acetyl CoA is converted into acetoacetate, β -hydroxybutyrate and acetone by the reactions as shown below :



Since all the three compounds have a keto-group ($>\text{C}=\text{O}$), these compounds collectively are called ketone bodies and serve as alternate fuel for the cell and of the peripheral tissues. During prolonged fasting, even the brain can use them as an energy store instead of glucose.

SAQ 4

- Why cannot acetyl CoA be used for gluconeogenesis?
- OAA can be considered as the starting point of gluconeogenesis. But OAA itself must be produced from other sources. Name four such sources.
- Which among the following is not a precursor of gluconeogenesis?
i) Lactate ii) Acetyl CoA iii) Propionyl CoA iv) Aspartic acid
- Gluconeogenesis (pyruvate $\rightarrow$ glucose), takes place in
i) Mitochondria ii) Cytoplasm only iii) Partly in mitochondria and partly in cytoplasm iv) Neither in mitochondria nor in cytoplasm.
- How many ATPs are required to synthesise a molecule of glucose? At what point are they required (For simplicity consider $\text{GTP}=\text{ATP}$)?
- How are irreversible steps bypassed? How is it energetically possible?

12.6 GLYOXYLATE CYCLE

Most animals cannot synthesise carbohydrates from acetyl CoA, since they are unable to carry out its net conversion into either pyruvate or TCA cycle intermediates. Consequently animals are unable to convert stored fats into sugar. However, plants and micro-organisms synthesise carbohydrates from acetyl CoA via a special pathway called the glyoxylate cycle in which two molecules of acetyl CoA are converted into succinate. The glyoxylate cycle is particularly significant to the micro-organisms that survive on acetate or ethanol. Through this cycle, they can convert 2-carbon

compound (acetyl CoA) into succinate which forms glucose via gluconeogenesis. In plants, oil seeds also use this cycle to synthesise glucose during germination from stored triglycerol.

The various reactions of the cycle, their site and the formation of glucose from succinate are shown in Fig. 12.14. The cycle operates in the special organelles called **glyoxysomes** of oil-bearing cells. **Glyoxysomes** contain isoenzymes of three enzymes of the TCA cycle: *citrate synthase* (reaction 1) *aconitase hydratase* (reaction 2) and *malate dehydrogenase* (reaction 5). Two additional enzymes *isocitrate lyase* (reaction 3) and *malate synthase* (reaction 4) are special enzymes for the cycle. *Isocitrate lyase* in reaction 3 cleaves 6-carbon isocitrate to 4-carbon succinate and 2-carbon glyoxylate. Succinate leaves glyoxysomes and enters into mitochondria. Malate is formed by the condensation of a second molecule of acetyl CoA and glyoxylate by the action of the enzyme *malate synthase*. Malate, in reaction 5, regenerates oxaloacetate.

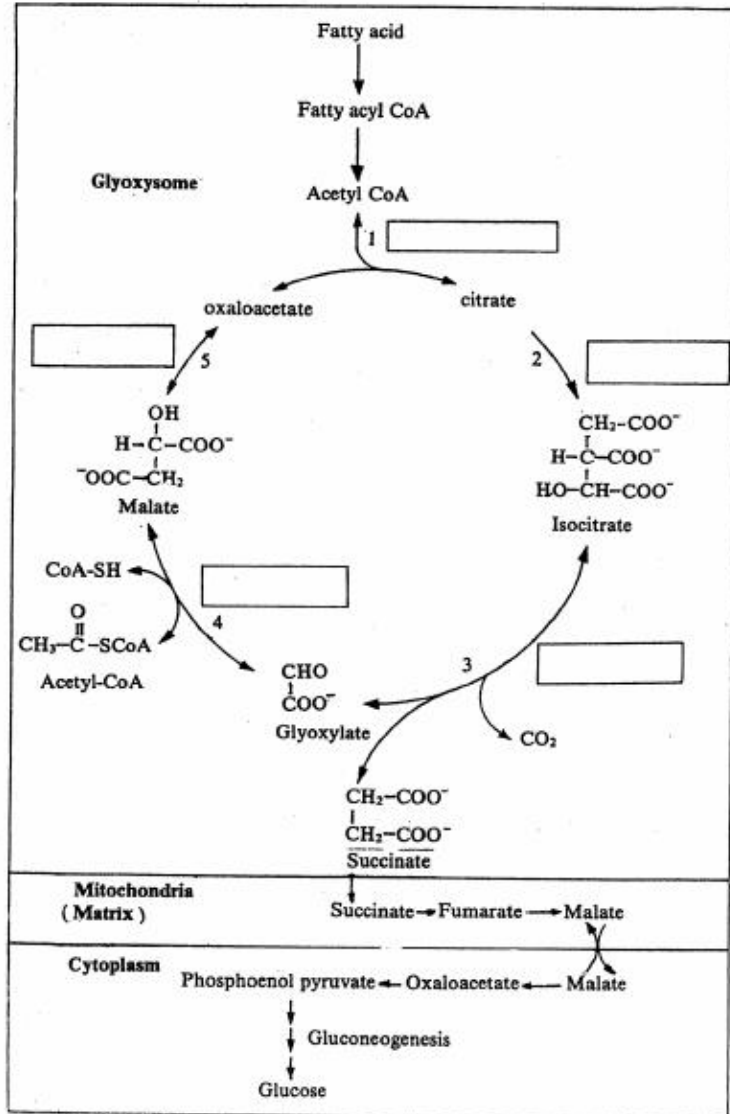


Fig. 12.14 : Glyoxylate cycle. Enzymes for reactions : 1) *citrate synthase* 2) *aconitase hydratase* 3) *isocitrate lyase* 4) *malate synthase*, and 5) *malate dehydrogenase*

Let us now concentrate on the sequence of reactions by which succinate is finally converted into glucose. As shown in Fig. 12.14, succinate in mitochondria is converted into malate via fumarate using part of the TCA cycle. Malate now moves into the cytoplasm where it is converted into phosphoenol pyruvate via OAA (see Fig. 12.12, reaction 1b). PEP forms glucose via gluconeogenesis as already described.

Now we know the cycle. Let us for practice study the reactions again and fill in the space in the given boxes-with-the names of enzymes given below Fig. 12.14.

SAQ 5

a) Answer the following questions briefly

- i) Why are animals unable to carry out carbohydrate synthesis from acetyl CoA

.....
.....
.....

- ii) Why are plants and many micro-organisms successful in synthesising carbohydrates

.....
.....

- iii) Which organelle (unique to plants) is involved in this process

- iv) What are the various compartments of a plant cell involved in synthesis of glucose from acetyl CoA

b) i) What is the glyoxylate pathway

.....
.....

- ii) How many acetyl CoA molecules are used up in one turn of the glyoxylate cycle

- iii) What is the eventual product of this cycle

.....
.....

- iv) How many high energy compound are used up in forming one molecule of this product

.....
.....

- v) In which reaction of the glyoxylate cycle is this high-energy compound used
What is this high-energy compound

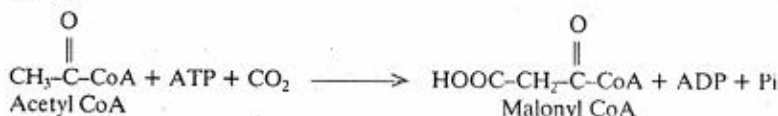
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12.7 SYNTHESIS OF FATS

We know that in higher animals fats are stored in the adipose tissue. The cells of these tissues and of many other tissues such as liver and mammary glands are specialised in fat synthesis. The synthesis occurs by a pathway which is different from that of degradation. It occurs in the cytoplasm. In plants, fatty acid synthesis occurs mainly in the chloroplast stroma. Do you remember where the degradation (β -Oxidation) of fats occurs?

When the intake of carbohydrates in food is in excess or their utilisation, they are readily converted into fats and stored. The building precursors for their synthesis are molecules of acetyl CoA. They are produced by the oxidation of pyruvate, and also by degradation of fats and proteins. The process involves a large multi-enzyme complex called *fatty acid synthase* (FAS). We do not plan to study all the reactions of fatty acid synthesis in detail but we will consider the following major steps, which are shown in Fig. 12.15. Acetyl CoA the building precursor is obtained by the degradation of carbohydrates and/or from fats and proteins.

- i) In the first reaction, acetyl CoA is carboxylated to a 3-carbon malonyl CoA. This reaction is irreversible. Once malonyl CoA is formed, it is committed to fatty acid synthesis.



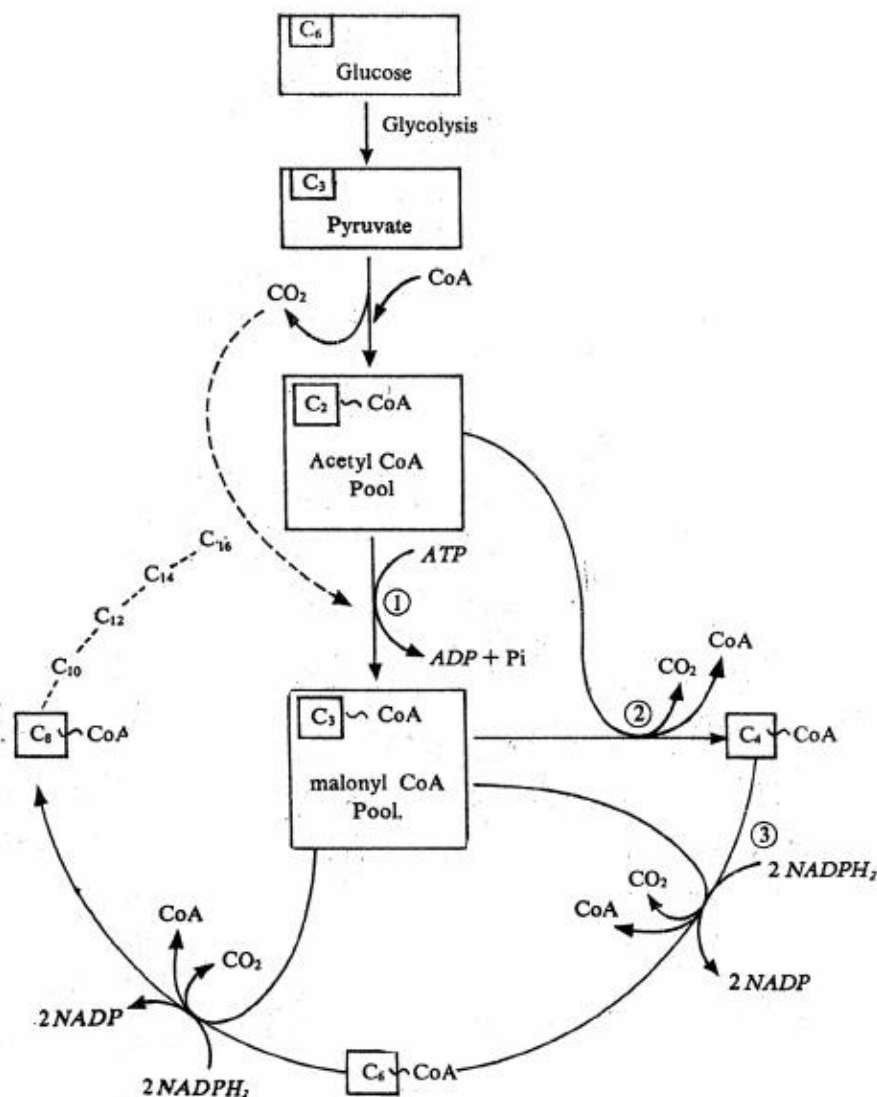
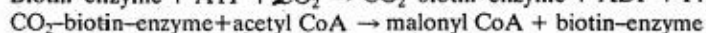
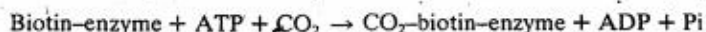


Fig. 12.15 : Synthesis of fatty acids

Can you identify the type of the above reaction? This is a carboxylation reaction catalysed by enzyme *acetyl CoA carboxylase*. Like *pyruvate carboxylase*, the enzyme has a special prosthetic group, biotin linked covalently to the protein part of enzyme. Biotin is a water-soluble vitamin. It functions as a carrier of CO₂ in carboxylation reactions. Likewise other vitamins also participate in different chemical reactions. First a CO₂-biotin-enzyme intermediate is formed by ATP as shown below. This step is in fact, CO₂ - activation step. The intermediate then transfers CO₂ to acetyl CoA.



ii) In the next step another molecule of acetyl CoA reacts with malonyl CoA to yield 4-carbon fatty acid (acetoacetyl CoA) with the release of CO₂. Decarboxylation contributes to a substantial decrease in free energy of the reaction.

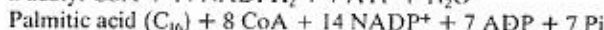


iii) The following step is the addition of two-carbon units from malonyl CoA. This is the beginning of elongation of the fatty acid chain (Fig. 12.15). This step is repetitive and each time, 2-carbon units are added to the growing chain of fatty acid, resulting in a molecule of fatty acid. This step is complex and involves a series of reactions carried out by a multi-enzyme complex— *fatty acid synthase* (FAS).



In this way all the intermediates are carried by acyl carrier proteins.

The overall reaction for the fatty acid synthesis is



We may ask why the four-carbon unit is not formed from two acetyl CoA molecules. The reason is that the equilibrium constant for the condensation of two molecules of acetyl CoA is highly unfavourable, whereas it is favourable with malonyl CoA because of its decarboxylation which contributes to decreasing free energy.

The synthesis of fatty acid occurs in the cytoplasm. What about acetyl CoA? Can you recall the link reaction? It is formed in the mitochondria and cannot pass through its membrane. How is it transported to the cytoplasm? Look at margin Fig. 12.16. Acetyl CoA condenses with OAA to form citrate which can migrate to the cytoplasm. The reverse reaction occurs in the cytoplasm. Citrate is cleaved to acetyl CoA and OAA and thus acetyl CoA is available for fatty acid synthesis in the cytoplasm.

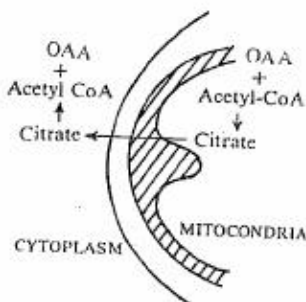


Fig. 12.16: Transport of acetyl CoA from mitochondria to cytoplasm

SAQ 6

Tick mark the correct statement(s) in the following questions.

- Synthesis of fatty acid requires
 - Only ATP
 - Only NADPH₂
 - ATP and NADPH₂
 - ATP and NADH₂
- The formation of malonyl CoA from acetyl CoA requires
 - CO₂
 - NADPH₂
 - ATP
 - biotin (as an essential coenzyme)
- The growth of fatty acid chain after the formation of acetoacetyl CoA requires
 - NADPH₂ (as reductant)
 - ATP
 - biotin as cofactor
 - Malonyl CoA
 - FADH₂
- Which among the following statements are true for fatty acid synthesis
 - It takes place in the cytoplasm
 - CO₂ is required to form malonyl CoA
 - Acetyl CoA migrates from mitochondria to the cytoplasm
 - Condensation of acetyl CoA with malonyl CoA requires NADPH₂.

12.8 SUMMARY

- The products of catabolism are used for various biosynthesis. The degradative and biosynthetic pathways utilise some common steps and are linked through certain intermediates. The biosynthetic pathways follow certain principles which are necessary for the regulation of metabolism.
- The excess of energy is stored in plants and animals in the form of polysaccharides — starch and glycogen. These polysaccharides are degraded to glucose when energy is in deficit.
- The synthesis of glycogen and starch requires high energy glucose as nucleoside glucose-NDP, which is added geneally to a smaller polymer of glucose — the prime. The two important enzymes for the synthesis are: (i) synthases which make linear

polymer; ii) *transferases* which add branches to the linear polymer. The enzyme *glycogen phosphorylase* degrades glycogen to glucose.

- The regulation of glycogen metabolism is controlled by two pancreatic hormones — insulin and glucagon — that have opposite metabolic effects. Glucagon activates *phosphorylase* enzyme leading to a breakdown of glycogen while insulin deactivates it and stimulates its synthesis.

- During starvation glucose is synthesised from non-carbohydrate precursors — amino acids, lactic acid and glycerol via gluconeogenesis. But fats which are the major store of energy in animals cannot be converted to carbohydrates; instead they make ketone bodies which serve as alternate fuel depot. Plants and micro-organisms have special pathway — the glyoxylate cycle by which fats can be converted to carbohydrates.

- Fats are synthesised from acetyl CoA by a complex process which utilises a multienzyme complex. The three carbon compound malonyl CoA is formed by carboxylation of acetyl CoA. The carbon chain of fatty acid grows by the successive addition of two carbons from malonyl CoA.

12.9 TERMINAL QUESTIONS

- 1 What is the role of UDP glucose in the synthesis of glycogen?
- 2 The activity of which of the following enzymes is not required for the release of large amount of glucose from liver glycogen?
 - a) *Glucose-6-phosphatase*
 - b) *Fructose 1,6-diphosphatase*
 - c) *Phosphoglucomutase*
 - d) *Glycogen phosphorylase*
 - e) α 1 $\rightarrow$ 6 *Glucosidase*
- 3 Which of the following statement(s) about glucose are correct?
 - a) It occurs actively in the muscle during periods of exercise
 - b) It occurs actively in the liver during periods of exercise or fasting
 - c) It occurs in the brain during periods of fasting.
- 4 From which of the following non-carbohydrate precursors glucose can be synthesised by gluconeogenesis?
 - a) Adenine
 - b) Alanine
 - c) Lactate
 - d) Palmitic acid
 - e) Glycerol
- 5 Which of the following statements about the hormonal regulation of glycogen synthesis and degradation are correct?
 - a) Insulin increases the capacity of the liver to synthesise glycogen
 - b) Insulin is secreted in response to low level of blood glucose
 - c) Glucagon and epinephrine have opposing effects on glycogen metabolism
 - d) Glucagon stimulates the breakdown of glycogen, particularly in the liver.
- 6 a) What are Ketone bodies?
 b) Under what condition Ketone bodies are formed?
 c) Which organ is the major site of their formation?
- 7 What is the role of acetyl CoA carboxylase in fatty acid synthesis?

12.10 ANSWERS

Self-assessment Questions

- 1 a) Pentose phosphate for nucleic acid, acetyl CoA for fatty acids, α -ketoglutarate for amino acid.

- b) Glucose, sucrose, fatty acids are compounds which are metabolised to a common product acetyl CoA.
 - c) It would be extremely difficult to regulate the pathway.
- 2 a) i) glycogen, ii) liver, muscle, iii) starch, iv) chloroplast, v) liver.
- b) Both are compact, easy to store, insoluble, inert. They do not interfere with metabolism (glucose, fructose and other hexoses are highly reactive). They have a negligible effect on osmotic pressure.
 - c) Excess of glucose would be potentially dangerous because it would alter the delicately balanced biochemical and osmotic properties of cells and thus of an organism.
 - d) i) glucose, ii) $\alpha 1 \rightarrow 4$ and $\alpha 1 \rightarrow 6$, iii) amylose and amylopectin
 - e) i) a, ii) e, iii) b, iv) c, v) d.
- 3 a) i) Could be NAD-glucose, but generally it is UDP-glucose or ADP-glucose.
ii) Maltose, $\alpha 1 \rightarrow 4$ linear glucan, amylose, amylopectin.
iii) Starch synthase
- b) i) It is a polyglucose molecule.
ii) It is derived by cutting amylose molecule.
iii) It is attached to an amylose molecule through $\alpha 1 \rightarrow 4$ or to an amylopectin molecule by $\alpha 1 \rightarrow 6$ linkage.
 - c) i) $\text{UDP-glucose} + \text{P}_i \rightarrow \text{glucose 1-phosphate} + \text{UTP}$
ii) One
 - d) i) true, ii) false, iii) false, iv) true
- 4 a) The reaction pyruvate to acetyl CoA is highly irreversible reaction. Moreover, there is no bypass reaction which can convert acetyl CoA to any of the intermediates of gluconeogenesis.
- b) i) Lactate which is formed during muscular activity in the muscles,
ii) Pyruvate which is formed during glycolysis,
iii) Several amino acids, which are breakdown products of proteins,
iv) Propionyl CoA which is derived from the oxidation of odd-carbon fatty acids.
 - c) Acetyl CoA
 - d) Partly in mitochondria and partly in cytoplasm.
 - e) Four ATPs from lactate to PEP, plus two ATPs from 3 PGA to DPGA. Total six ATP per glucose from lactate.
 - f) $\text{PEP} \rightarrow \text{pyruvate}$ is bypassed via oxaloacetate at the expense of 2 ATPs (or one ATP and one GTP) per lactate.
- 5 a) i) Acetyl CoA cannot be converted to carbohydrates because the reserve glycolytic reactions – conversion of pyruvate to phosphoenol pyruvate (reaction 10) and conversion of acetyl CoA to pyruvate (reaction 11) are strongly exergonic. These reactions are irreversible.
ii) Plants and micro-organisms possess an enzyme system (glyoxylate cycle) which bypasses the two irreversible reactions of glycolysis.
iii) glyoxysomes
iv) glyoxysomes, mitochondria and cytoplasm (see Fig. 12.)
- b) i) By glyoxylate cycle fats are converted to carbohydrates. It is found in plants and micro-organisms.
ii) two
iii) glucose
iv) Hint: see Table 12.1 in this cycle reaction 1 a is skipped. Four high energy compounds.
v) reaction I b uses 2 GTP and reaction 1 uses 2 ATP
- 6 a) iii)
- b) i), iii) and iv)
 - c) i) and iv)
 - d) i) and ii)

Terminal Questions

- 1 UDP-glucose, the activated form of glucose, is the glucose donor in the biosynthesis of glycogen.
UDP-glucose + glycogen $\rightarrow$ glycogen (n + 1) units
This reaction is catalysed by glycogen synthase enzyme.
- 2 b.
- 3 b.
- 4 b, c, e.
- 5 b, d.
- 6 a) Ketone bodies are, acetoacetate, hydroxybutyrate and acetone.
b) When the rate of fatty acid oxidation is high to form excess acetyl CoA which cannot enter into the TCA cycle because not sufficient amount of OAA is available, then the ketone bodies are formed from the excess acetyl CoA. This may happen when the diet is rich in fats and low in carbohydrates. This also happens in fasting or in diabetes.
c) The liver
- 7 This enzyme catalyses the first step in fatty acid synthesis
 $\text{Acetyl CoA} + \text{CO}_2 + \text{ATP} \rightarrow \text{malonyl CoA} + \text{ADP} + \text{Pi}$

UNIT 13 NUCLEUS: STRUCTURE AND FUNCTION

Structure

- 13.1 Introduction
 - Objectives
- 13.2 Nuclear Envelope and Nuclear Components
 - Nuclear Envelope
 - Other Nuclear Components
 - Role of Nuclear Envelope in Chromosome Organisation and in Transfer of Large Molecules
- 13.3 Organisation of DNA in Chromosomes
- 13.4 Replication of DNA
 - Semi-conservative Replication
 - Replication Fork
 - Enzymes Involved in DNA Replication
- 13.5 Transcription
 - RNA Synthesis in Prokaryotes
 - RNA Synthesis in Eukaryotes
 - Different Types of RNA
- 13.6 RNA Processing in Eukaryotes
 - Modification of mRNA
 - Removal of Introns
- 13.7 Summary
- 13.8 Terminal Questions
- 13.9 Answers

13.1 INTRODUCTION

In Blocks I and II, you have studied the various macromolecular components of the cell and cell organelles including nucleus. Nucleus is the dynamic control centre of eucaryotic cell for all the cellular activities such as, storage, utilisation of genetic information, reproduction, growth, development and metabolism.

In this Unit, we will discuss the various components of the nucleus. You have read about the elementary structure of DNA in Block I, here we will explain the organisation of DNA in chromosomes as well as its replication and transcription. You know that genetic information is stored in DNA. We will discuss how this information is transmitted from the nucleus to the cytoplasm by mRNA by translation into protein molecules, which are responsible for most of the cellular activities.

The actual process of translation and gene regulation will be discussed in the next Unit.

Objectives

After having read this unit you should be able to:

- apply in correct context the terms: replication fork, hnRNA, intron, exon,
- explain the role of nuclear envelope, and nuclear matrix,
- describe the current hypothesis regarding the packaging of DNA in chromosomes with reference to histones,
- describe the replication of DNA and the location of enzymes involved in DNA replication,
- distinguish between the transcription process in prokaryotes and eucaryotes,
- account for the processing of RNAs.

13.2 NUCLEAR ENVELOPE AND NUCLEAR COMPONENTS

In Block I of this course you have read about the structure of nucleus. Nucleus is composed of at least five distinct components (i) a double membraneous nuclear

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envelope, which acts as barrier between nucleus and cytoplasm, (ii) a **jelly like fluid nucleoplasm** that fills the nuclear space, (iii) **nucleolus** (iv) **chromatin fibres**, and (v) **nuclear matrix** that provides skeletal framework to the nucleus (Fig. 13.1a). Chromatin is dispersed throughout the nucleus except at the time of cell division when chromatin becomes condensed into discrete structures known as chromosomes.

We will now briefly describe the nuclear envelope, various nuclear components and their role in the following subsections.

13.2.1 Nuclear Envelope

The nuclear contents are separated from the cytoplasm by two concentric complex membranes surrounding the nucleus, together termed as **nuclear envelope**. The nuclear envelope is a double membraneous structure composed of two lipid bilayers separated by 10-30nm space known as the **perinuclear space**. Under the electron micrographs, the outer nuclear membrane is seen to be continuous with the endoplasmic reticulum membrane (ER) and like the membrane of the rough endoplasmic reticulum (RER), the outer nuclear membrane contains cytoplasmic ribosomes over its outer surface that form the sites of protein synthesis. Since the ER is in continuation with the perinuclear space, both the perinuclear space and the outer nuclear membrane become the specialised regions of the endoplasmic reticulum (Fig. 13.1a). Microtubules and microfilaments, are associated with outer surface of nuclear envelope to anchor the nucleus and maintain its proper shape. The inside of inner nuclear membrane is attached to a thin layer of fibrous proteins known as the **nuclear lamina**, which acts as a support to the nuclear envelope and nuclear pores and to which chromatin fibres are attached at multiple sites.

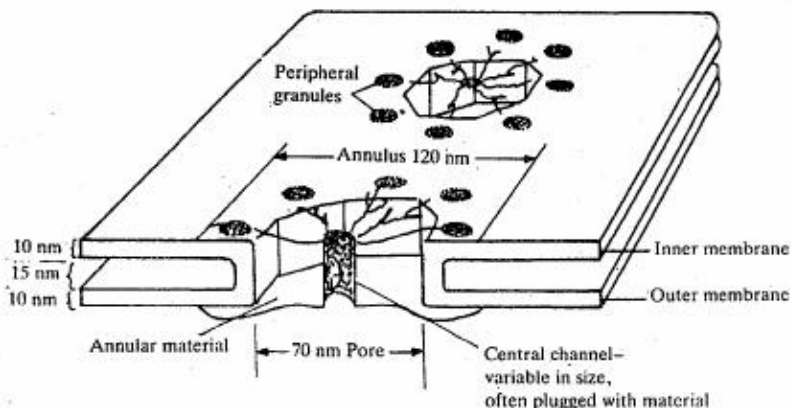
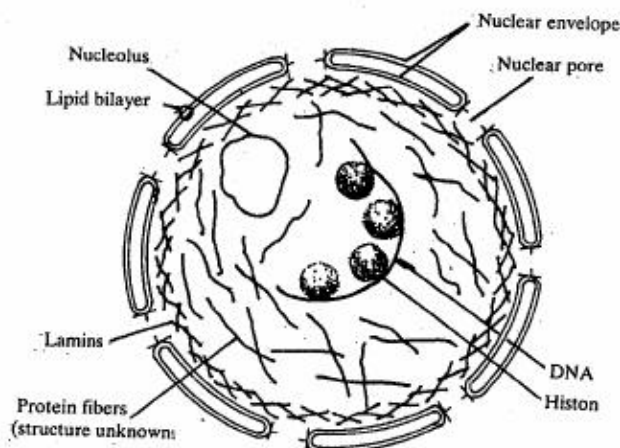


Fig 13.1(a): The eucaryotic nucleus. A generalised diagram. The double-layered envelope, nucleolus, nuclear pores, and fibrous lamins are prominent nuclear structures. The DNA forms complexes with nonfibrous proteins called histones.

Fig 13.1(b): Schematic diagram of the main elements present in a "typical" nuclear pore complex. Significant variations occur in the arrangement of the annular material.

The nuclear envelope of all eucaryotes, from yeast to man has numerous specialised structures known as nuclear pores (Fig. 13.1b). The cavity of the nuclear pores are not wide open channels, although they extend through both the membranes of the envelope, and the cavity is filled with a fibrous dense material. Under the electron microscope each pore is seen to be surrounded by a ring like structure known as annulus, composed of eight protein molecules extending on both sides of the envelope. The pore and annuli together constitute the **pore complex** that measures 50-70 nm in diameter. (Fig. 13.1b). The nuclear pores serve to transport the specific molecules between the cytoplasm and the nucleus.

In some cases, the nuclear pores are found to be highly organised in the nuclear envelope. Parallel rows of nuclear pores are observed in the elongated nucleus of a fern spore. In other cells, either concentrated clusters of nuclear pores or unusual areas free of nuclear pores have been seen in the nuclear envelope, and have some relationship with the nuclear lamina.

In the following subsection we shall study the role of the nuclear envelope in chromosome organisation as well as transport of RNA and protein. The outer membrane can form vesicles to transport the materials from one compartment to another in the cell.

13.2.2 Other Nuclear Components

Nucleoplasm

It is a jelly like fluid containing the soluble material of the nucleus. Various components of the nucleus can be isolated with dilute buffer or salt solutions such as proteins, RNA, RNA protein complexes and small molecules. Protein fraction of the nucleoplasm contains various enzymes involved in metabolic pathways, while other enzymes function in replication and transcription of DNA, and some proteins present are involved in the regulation of chromatin structure and function.

RNA and RNA protein complexes

Most of the RNA molecules in nucleoplasm are in association with proteins forming ribonucleoproteins of various sizes and types, for example some of these RNA molecules form **small nuclear RNAs**, which are involved in messenger RNA processing. These small RNAs never leave the nucleus and are destroyed inside the nucleus. The large RNA complexes pass through the nuclear pores to the cytoplasm which act as a precursor of cytoplasmic ribosome, mRNA, or mRNA precursors. Low molecular weight substances such as coenzymes, nucleosides, precursors of nucleotides, metabolic intermediates and ions are also found in the nucleoplasm. Nucleoside the nucleotide precursors are utilised in the synthesis of two nucleic acids, i.e. DNA and RNA.

13.2.3 Role of Nuclear Envelope in Chromosome Organisation and in Transfer of Large Molecules

Nuclear lamina, the fibrous protein network under the envelope, plays a major role in dissolution and reformation of the nuclear envelope during cell division. Chromatin fibres are attached to it at multiple sites. The laminal protein holds the condensed, inactive chromatin at the peripheral region of the nucleus to keep it away from the unfolded, active chromatin.

Information regarding the transport of material through the nuclear pores is obtained by the knowledge of a typical mammalian cell which contains about 3,000 to 4,000 pore complexes in its nuclear envelopes.

The nuclear envelope like other cellular membranes is also selectively permeable and capable of passive diffusion, active transport and facilitated diffusion. You have read about these transport processes in Block II. Besides the above mentioned processes, other mechanisms also operate for transporting the molecules through the envelope for example small membrane vesicles formed inside the nucleus fuse with the inner nuclear membrane discharging their contents to either the perinuclear space or to cytoplasm directly (Fig. 13.2). The protein synthesised into the outer nuclear membrane having ribosomes on its surface is discharged into the perinuclear space and into nucleus. Nuclear envelope is also involved in carrying out the function of oxidative phosphorylation and electron transport.

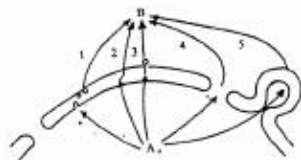


Fig. 13.2: Scheme of the possible pathways in which material is translocated from the nucleoplasm (A) to the cytoplasm (B): (1) transport by vesicles; (2) transmembrane migration or transport; (3) a combination of 2 and a subsequent vesicle pinching-off process; (4) translocation through pores; (5) extrusion of the clear material by delamination of sacs of both nuclear membranes.

Nucleolus

As you have read in the previous block, a nucleolus is generally a large spherical structure with a wide range of size, and shape and due to the large size can be seen under the light microscope. The chemical composition of the nucleolus is studied by direct chemical analysis and by cytochemical studies. Such studies have shown that nucleolus is composed of about 80% of proteins, and about 20% of a mixture of RNA and DNA. Nucleoli are formed by a special region of the chromosomes called **nucleolar organizer regions (NOR)**. The eucaryotic nucleolus is the site of ribosome synthesis and its assembly, and actively contributes to the process of protein synthesis.

SAQ 1

a) Fill in the blanks.

- Nucleus is surrounded by which is composed of membranes
- The two membranes are separated by a space called as
- Nuclear envelope is interrupted at places by which are surrounded by to from
- The anforphous fluid present in nucleus is called as
- Within the nucleus a spherical body called the is present which is formed in a special region of chromosome called

b) What are the two important functions of nuclear envelope?

13.3 ORGANISATION OF DNA IN CHROMOSOMES

In this section we would discuss the organisation of DNA in chromosomes. The procaryotes such as bacteria and blue green algae lack a well-defined genome (nucleus) and possess a single double stranded circular DNA molecule that contains all the genes and is located in a special region of their cytoplasm. In addition to the chromosomes, most bacteria contain DNA of shorter chain lengths called **plasmids** which also form the closed loops.

You have read in Block 1 about the organisation of DNA in nucleus, so we would briefly explain more about its organisation. Eucaryotes have a complex organisation of membrane bounded nucleus with multiple linear chromosomes. Each chromosome contains a single double stranded helical DNA, and a set of five types of histone proteins.

DNA, in the nucleus of eucaryotes is tightly associated with proteins forming chromatin fibres of varying sizes. These chromatin fibres are interspersed throughout the nucleus and during cell division, they are condensed to form more prominent structures known as chromosomes. Chromatin fibres contain DNA, RNA, some basic proteins called **histones** and some acidic proteins called as **non-histone** proteins. It is known that DNA and histones are always present in a fixed ratio of about 1:1. The non-histone proteins are heterogenous and vary in different tissues in quantity and type. These include RNA and DNA polymerases and regulatory proteins. Histones are small basic proteins, they have a high content (10-21%) of the basic amino acids, arginine and lysine. Being basic, and having strong positive charge at the physiological pH, histones bind tightly to DNA, which is acidic due to the presence of phosphate group in it. There are five types of histones i.e. H₂A, H₂B, H₃, H₄ and H₁. A chromatin contains almost equal number of H₂A, H₂B, H₃ and H₄ and about half the number of H₁ molecules. out of these H₂A, H₂B, H₃, and H₄ are among the most conserved proteins known during evolution and are present in all eucaryotes. For example, the sequence of histone H₃ from the rat differs from that of peas in only two amino acids, out of 102 total amino acids. Histone H₁ is not conserved between species and has tissue specific forms. It is loosely associated with chromatin and is

involved in the maintenance of a higher order of folding of chromatin. Some of the properties of histones are given in table (Table 13.1)

Table 13.1 : Some properties of Histones

Histone	Molecular Weight	Amino Acid Composition	Species Variation	Number of Molecules per 200 Base Pairs of DNA
H ₁	20,000	Lysine-rich	Wide	1
H ₂ A	13,700	Moderately lysine-rich	Fairly well-conserved	2
H ₂ B	13,700			2
H ₃	15,700			2
H ₄	11,200	Arginine-rich	Highly conserved	2

Histone Octamer: The complex containing two molecules each of H₂A, H₂B, H₃ and H₄ forming an octamer of proteins.

Nucleosomes and Chromatin Structure

You have studied about the structure of nucleosomes in Block I. We will now discuss the structure of nucleosomes and their higher organisation in detail. Electron microscopy of extended chromatin fibres reveals a "beads on a string", appearance, the 'beads' being the **nucleosomes** and the 'string' the **linker DNA**. Brief digestion with certain nucleases results in degradation of the linker DNA, liberating the nucleosomes as monomeric units. A nucleosome contains a histone octamer, DNA of 200 base pairs and one molecule of histone H₁. The nucleosome is a flat disc shaped particle 11 nm in diameter and 5-7 nm in height. The DNA makes two complete turns around each histone octamer and these two turns are sealed off by an H₁ molecule. Extensive digestion of a nucleosome by nuclease produces a nucleosome **core particle** which has a disc shaped core of an eight-histone protein molecule along with 146 nucleotide base pairs, as you can see this results from the loss of linker DNA of about 54 nucleotide bases and histone H₁.

The packing ratio is the ratio of extended length to packed length of DNA.

Histone H₁ plays an important role in packaging the nucleosomes by further folding the nucleosome's fibre into higher levels of chromatin organisation. When nucleosomes are closely condensed forming 10nm diameter filaments, the packing of DNA is about five to seven fold, i.e. five to seven times more compact than free DNA.

This is still a 1000 fold lower value than the packing ratio of DNA in metaphase, which is achieved by further folding the 10 nm diameter chromatin fibre into 30 nm diameter stage as seen in the electron micrograph. The 30 nm diameter chromatin fibre arises from the folding of the nucleosome chain into a helical **solenoidal structure** having about six nucleosomes per turn. The whole structure is stabilised by interactions between histone H₁ molecules in neighbouring nucleosomes. The H₁ molecules can interact with each other because they are located in the central hole of the solenoid. The DNA of a 30 nm solenoid has a packing ratio of about 40 fold. The DNA of metaphase chromosome, however, is packed densely between 5,000 and 10,000 fold, forming chromosomes during cell division. A schematic illustration of the various orders of chromatin organisation is shown in figure (Fig. 13.3).

Solenoid is a highly condensed spirally folded packing of DNA protein nucleosome complex.

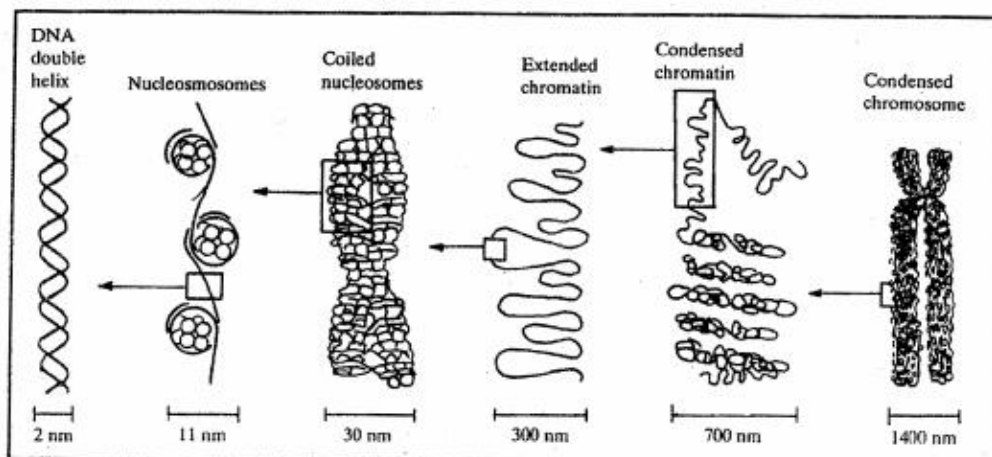


Fig. 13.3: Illustration of the levels of organisation within the eucaryotic chromosome.

The genetic material in prokaryotes is packed into one or more double stranded DNA fibres that are folded into a compact structure known as **nucleoid** that contains about 80% DNA and rest as protein and RNA. This complex is attached at one side to the membrane inside the cell. The bacterial DNA is **supercoiled** and **supertwisted** and is organised in about 45 loops radiating out from the central non-histone protein core that provides anchorage to DNA in the cell. Supercoiling and loop formation make the packaging of large DNA molecules into small structure to fit into the nucleus. The "naked" fibres of DNA can be seen when bacterial, viral or organelle chromosomes are examined under the electron microscope by staining the chromatin (Fig. 13.4).

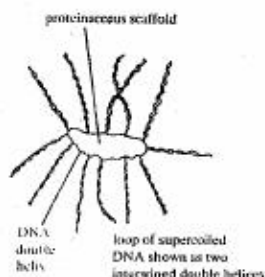


Fig. 13.4: Schematic representation of the chromosomes of *Escherichia coli*, showing only 12 of the 45 supercoiled loops.

SAQ 2

a) Which one of the following choice is best and why? Histones

i) are basic proteins rich in lysine and arginine

ii) are bound covalently to DNA

iii) have relatively high molecular weights (200,000 daltons or higher)

iv) are identical to protons

b) Fill in the blanks

i) Beads in the string appearance of chromatin represents

ii) Extensive digestion of nucleosomes by nucleases produces.....which has only 146 bp of DNA

iii) The higher levels of chromatin are maintained by histone

13.4 REPLICATION OF DNA

As a carrier of genetic information DNA has two following important functions, **heterocatalytic** and **autocatalytic**. When DNA directs the synthesis of chemical molecules other than its own, e.g., synthesis of RNA, protein etc., it is called **heterocatalytic function**. On the other hand when DNA directs its own synthesis, it is called **autocatalytic function** or **replication**.

In this section, we will only deal with autocatalytic function of DNA, i.e. DNA replication. DNA replication is carried out with high accuracy to maintain genetic stability within the organism and the species. DNA replication is a complex process and takes place in many sequential steps, that include recognition of the **point of origin**, **unwinding** of parental double helix keeping the template strand apart, **initiation** of new daughter strands, **elongation** and termination of DNA replication. Let us discuss this process in detail

13.4.1 Semi-conservative Replication

You will be curious to know what does semi-conservative replication mean and how exactly DNA replicates. You have studied in Unit 5 of Block 1 about double helical structure of DNA as proposed by Watson and Crick.

The information in the base sequence of one DNA strand is complementary to the second strand in accordance to Watson and Crick model. During replication the weak hydrogen bonds that hold the two duplex DNA strand together are broken. As a result there is a gradual separation of the two strands of the double helix in a zipper like fashion. Each strand, that separates, serves as a template for the synthesis of new complementary daughter strands. Each daughter DNA molecule in the two cells after one round of DNA replication contains one parental DNA strand and one newly formed strand. Replication of this type is termed as **semi-conservative** (Fig. 13.5)

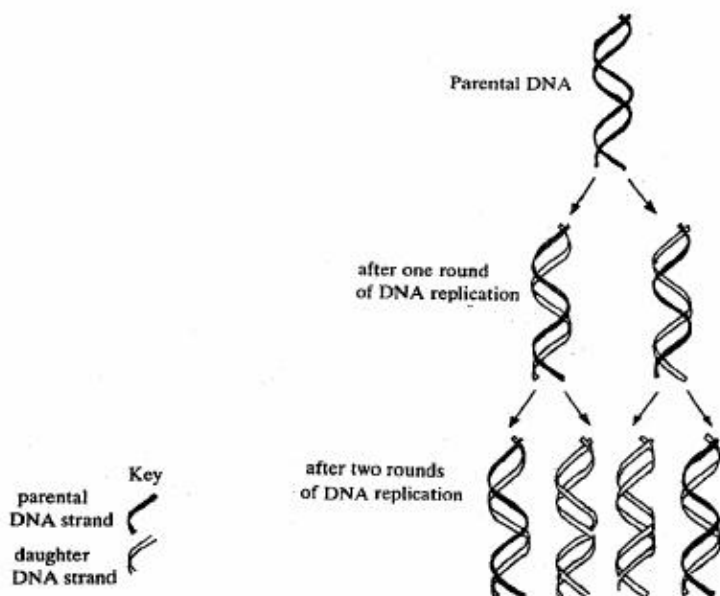
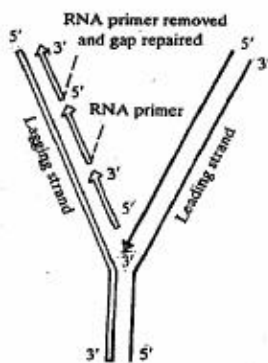


Fig. 13.5: Semi-conservative replication of DNA

13.4.2 Replication Fork

Origin of replication: A specific base sequence in DNA that is the site of initiation of replication, which proceeds in one direction or in both directions away from the site.

In the process of replication, the two individual strands of the parental double helical DNA separate in small segments, because DNA polymerase recognises only single stranded DNA as template. DNA replication in prokaryotes starts at a single specific site known as the **origin of replication fork**. But in eukaryotes replication begins at multiple sites in the DNA helix that reduces the replication time length and helps in rapid replication of long DNA molecules. The two strands separate by unwinding in the form of a 'Y', where active synthesis starts and is called as **replication fork** or **growing point**. Replication fork forms an asymmetric structure.



Replication of double stranded DNA is **bidirectional**, i.e. replication fork moves in both the directions from its **point of origin** (Fig. 13.6). The unwinding of double helix is brought about by a group of proteins that include helix destabilising (HD) proteins, and bind to the single stranded DNA. Besides HD protein, the helix unwinding proteins, called **DNA helicases**, also keep the two strands apart by binding to the single strand near the replication fork base. At replication fork, the DNA of both the new daughter helices is synthesised. Because of the antiparallel orientation of the two parent DNA strands in the DNA helix, this mechanism would require one daughter strand to grow in the 5' to 3' direction and the other in the 3' to 5' direction of parent DNA. But DNA polymerase, the major enzyme involved in DNA replication can synthesise new strands only in the 5' to 3' direction. It became difficult to explain how the two strands would transcribe differently. The solution to this problem was achieved on the basis of the following findings.

Fig. 13.6: DNA replication is continuous on one strand and discontinuous on the other. The lagging strand is made in short fragments that start with an RNA primer. The primer is removed by a repair mechanism and the gap is filled in and the molecule joined by a DNA ligase.

- One of the DNA strands is synthesised continuously towards the origin of replication fork in its 5' to 3' direction. This is called '**leading strand**' which reads the 3' to 5' direction of the parent DNA.
- Other strand which is being synthesised discontinuously away from the replication fork is called the **lagging strand**.

Since parental complementary DNA strands themselves run in opposite directions, both the new strands are being copied in 5' to 3' direction due to the specificity of DNA polymerase. Lagging strand is synthesised in small pieces (100-1000 nucleotides) which are called as **Okazaki fragments** (Reiji Okazaki discovered these fragments). These fragments are then joined together by the action of an enzyme called as DNA ligase. You will study about the enzymes of DNA replication in following subsection. But before proceeding further, you may attempt the following SAQ.

- a) During DNA replication, the sequence 5'-TpApGpAp-3' would produce which of the following complementary structures? Give reasons.

- i) 5' TpCpTpAp-3'
- ii) 5' ApTpCpTp-3'
- iii) 5' UpCpUpAp-3'
- iv) 5' GpCpGpAp-3'

- b) Give one word for the following.

- i) Short pieces of replicating DNA:
- ii) $\rightarrow \text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}$;
- iii) Replication in which half of the molecule is conserved;
- iv) The sites in DNA molecule where parental double helix is undergoing strand separation:

13.4.3 Enzymes Involved in DNA Replication

DNA replication takes place by polymerisation of about 500 nucleotides per second in bacteria and about 50 nucleotides per second in mammals. DNA polymerase requires preformed DNA for its activity. The DNA polymerase adds new nucleotides to the 3' end of one strand in sequential order, so the new strand synthesised is in the 5' $\rightarrow$ 3' direction. The replication enzymes must be both accurate and fast. Speed and accuracy are achieved by means of a **multienzyme complex** of several different proteins that constitute an elaborate "replication machine" or "replicon".

The parent DNA replication is catalysed by some twenty special enzymes and proteins, each performing a specific task in the complex process. Replication takes place in an organised and stepwise manner. Some of the enzymes involved in DNA replication are nucleases, polymerases and ligases.

(a) Helix—destabilising proteins

These proteins destabilise the parental DNA double helix by binding to separated single DNA strands, by preventing the reunion of the two separated strands of the double helix at the replication fork.

(b) Helicases

These are ATP-driven enzymes that use the energy released by ATP hydrolysis in unwinding the short segments of DNA helix just ahead of the replication fork (Fig 13.7).

(c) DNA—topoisomerases

These enzymes can change the topological form or shape of DNA molecule. A DNA topoisomerase is a reversible enzyme that first breaks the DNA chain and then binds covalently to the broken end. The break creates a transient nick in DNA helix. Nicking reaction is reversible, therefore, resealing is rapid and does not require additional energy input (Fig. 13.8).

(d) DNA polymerases

These are the major enzymes involved in DNA replication. DNA replication is a very precise and accurate process and it has been estimated that only one error is made in 10^9 - 10^{10} base pair residues. The mammalian genome of 3×10^9 DNA base pair replicates with high accuracy which is due to the fact that DNA polymerase has the ability of **proof reading** and correcting the error in addition to the polymerising activity.

Proof reading activity is carried out by DNA polymerase that can hydrolyse phosphodiester bonds in single strands if insertion of wrong nucleotide occurs at 3 terminal end of the primer strand. An incorrect base is thus removed before further synthesis of DNA occurs (Fig. 13.9)

E. coli contains three different polymerases viz. DNA polymerase I, II and III. DNA polymerase III cannot initiate the synthesis of DNA, but is mainly responsible for DNA chain elongation by adding new nucleotides to the 3' end of the primer strand.

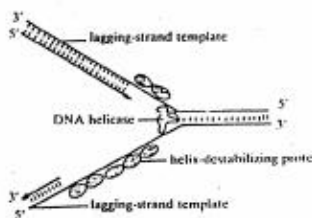


Fig. 13.7: The DNA helix ahead of a replication fork is thought to be opened at rapid rate by the combined action of a DNA helicase enzyme and helix -destabilising proteins.

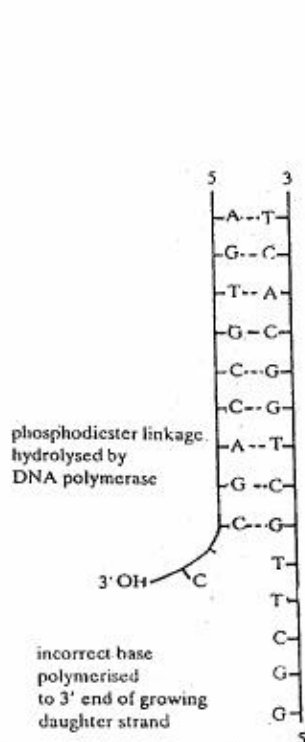


Fig. 13.9: Proof reading activity of DNA polymerase

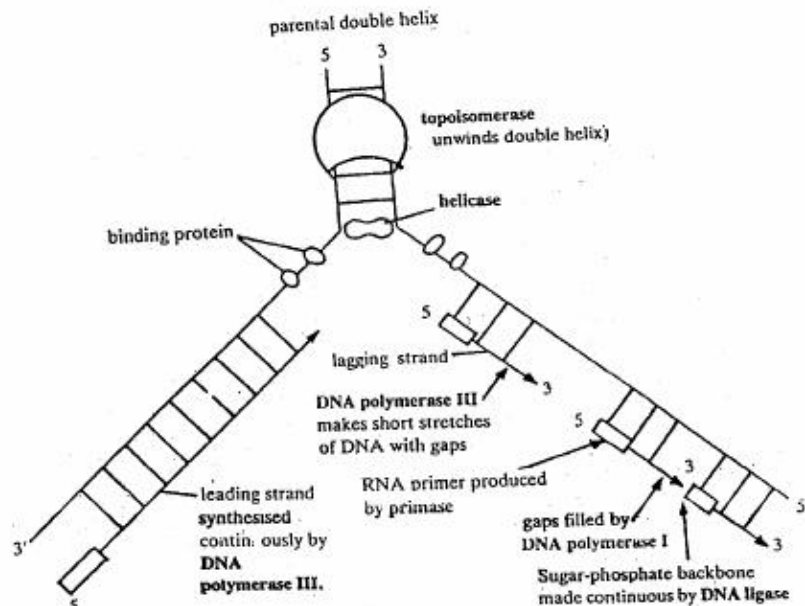


Fig. 13.8: Some of the enzymatic events at the replicating Y fork of *Escherichia coli*

DNA polymerase III functions in the form of a large complex called **holoenzyme**, that requires both a template and a primer molecule to start the synthesis. This primer is an RNA synthesised by an RNA polymerase called **primase**. This enzyme recognises some type of signal on the DNA template strand to which it binds. DNA polymerase III complex then adds this to nucleotide units at the 3' end which are RNA primer complementary to template DNA strand. The RNA primer is subsequently degraded and the gaps are later filled in by DNA polymerase I, which is involved in both replication and DNA repair process. DNA polymerase I is associated with polymerising and exonuclease activities. The exonuclease enzyme degrades nucleic acids by removal of the terminal nucleotides from either end of a strand of DNA from 5' → 3' or in 3' → 5' direction. The function of DNA polymerase II is uncertain.

DNA ligase

DNA ligase joins the DNA fragments together by catalysing the formation of a covalent phosphodiester bond between 3' end of one nucleotide chain with the 5' hydroxyl group of the newly made Okazaki fragment. Some of the enzymes involved in DNA replication and their functions in *E. coli* are summarised in the table (Table 13.2).

Table 13.2: Some of the enzymes involved in DNA replication in *Escherichia coli*

Enzyme	Function
DNA polymerase III	the major enzyme responsible for DNA replication
DNA polymerase I	fills in gaps between the fragments of DNA synthesised by DNA polymerase III
helicase	uses energy released by ATP hydrolysis to disrupt hydrogen bonds between base pairs and thereby creates single-stranded regions
DNA binding protein	stabilises single-stranded DNA, preventing re-formation of the double helix
topoisomerase	unwinds the double helix
DNA ligase	uses ATP to seal 'breaks in sugar-phosphate backbone,
RNA primase	produces short RNA sequences to prime DNA polymerase III

The replication of DNA in eucaryotes and procaryotes is basically similar with some differences as given in the following table (Table 13.3)

Table 13.3: some Differences between prokaryotic and Eukaryotic DNA Replication

	Prokaryotes	Eukaryotes
RNA primer length	— 50 nucleotides	9 nucleotides
Okazaki fragments of lagging strand	1000-2000 nucleotides	— 200 nucleotides (due to nucleosomes)
Fork movement rate	— 500 nucleotides/sec	50 nucleotides/sec
Number of origins	1	thousands, developmentally regulated
Block of reinitiation in same cycle	no	yes

Synthesis of Okazaki Fragments

In eukaryotes, the chromosomes contain an **origin site** where the replication fork starts. The origin site is a nucleotide sequence of 100-200 base pairs without which a DNA cannot be replicated. The rate of replication fork formation is reflected in the fact that over a thousand replication forks function simultaneously. Two identical DNA polymerase molecules work simultaneously at fork, one on the leading strand that continuously replicates in the $5' \rightarrow 3'$ direction towards the origin of replication site. While the other DNA polymerase works on the lagging strand by adding new nucleotide units to $3'$ end of the parent DNA, away from the base of the replication fork. The DNA helix remains opened when the DNA polymerase molecule acts on the leading strand, in coordination with **helix destabilising protein** in a DNA helicase enzyme system. The DNA polymerase molecule moves on the leading strand in a continuous way, while polymerase molecule on the lagging strand stops and restarts at intervals, to produce a series of short pieces of DNA called **Okazaki fragments**, that are about 1000 to 2000 bases long. They are joined together to form the second daughter strand by DNA ligase. (Fig. 13.10)

The nucleotide in each fragment in lagging strand is incorporated by RNA primase, and the process of addition is continued by a DNA polymerase that adds nucleotide to the $3'$ hydroxyl of the RNA primer. After the completion of the fragment, RNA moves to $5'$ end and is removed. The gap in the strand is filled with DNA and sealed. You may look back at the structure of DNA as given in figure (Fig. 13.9)

Fig. 13.10: Summary of some of the major types of proteins that act at a DNA replication fork.

SAQ 4

- a) In the following table in column I names of the enzymes involved in DNA replication are given. Complete column II by giving the functions of these enzymes

Column I

- DNA polymerase I
- DNA ligase
- DNA helicase
- DNA polymerase III
- RNA primase

Column II

- b) Which of the following statements about DNA polymerases is (are) correct? Give reasons

- DNA polymerase requires primers

- DNA polymerase can add nucleotides at both the ends by synthesis of the growing polynucleotide chain

- In addition to their polymerising activity DNA polymerases have a 3-5' exonuclease activity

- iv) DNA polymerases can synthesise DNA only in the presence of an RNA template.

13.5 TRANSCRIPTION

In the preceding sections, we discussed about replication process and the outcome of this process. We will now study about the transcription process which revolves around the central theme of transfer of genetic material and information from DNA in the nucleus to the formation of RNAs in the same location, and to the synthesis of proteins in the extranuclear cytoplasmic region.

This is called the “**central dogma**” of molecular biology

Self Replication Transcription Translation
 $\text{DNA} \longrightarrow \text{RNA} \longrightarrow \text{Protein}$

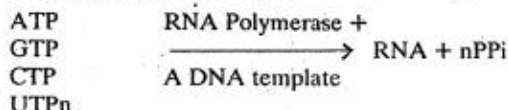
We should understand clearly about replication and transcription processes.

Transcription and **replication** processes have important differences. In replication the entire chromosome is copied to yield daughter DNA, identical to parent DNA, whereas in transcription process, only a single or a group of genes is transcribed and not the whole of DNA.

The schematic representation of the flow of information in a cell is given in following figure (Fig. 13.11).

RNA is transcribed from DNA by the catalytic activity of **DNA-dependent RNA polymerase** enzyme commonly known as **RNA polymerase**. The RNA polymerase enzyme recognises the site and the strand to be transcribed in DNA. In procaryotes there is only one type of RNA polymerase that catalyses the synthesis of all the three RNA molecules, whereas in eucaryotes there are three types of RNA polymerases. All RNA polymerases require the following prerequisite to synthesise RNAs.

- All the polymerases require a DNA template, which can either be a double or single stranded molecule, the single strand is called as **template**.
- All four energy rich ribonucleoside triphosphates (ATP, CTP, GTP and UTP) are utilised as precursors of the nucleotide units of RNA and polymerise into **DNA templates**, which greatly stimulate further polymerisation of base by RNA polymerase at 3' hydroxyl end of the existing DNA chain.



- Only one strand of a particular region of the double helix is transcribed inside the cell, hence transcription is asymmetric.

The basic mechanism of RNA synthesis is similar in procaryotes and eucaryotes with some differences. Let us first describe RNA synthesis in procaryotes.

13.5.1 RNA Synthesis in Procaryotes

In all procaryotes and eucaryotic organisms, three main classes of RNA molecules exist, i.e., mRNA, rRNA and tRNA. Each class differs from the other in size, function, location and stability. Using DNA as the template, RNA polymerase catalyses the formation of RNA from the four ribonucleotide triphosphate precursors. The DNA molecules contain two strands while most RNAs are typically single stranded. You would be inquisitive whether one or both DNA strands is copied into complementary RNA molecules. It is known that only one of the two DNA strand is copied selectively by RNA polymerase, and thus transcription is asymmetric. RNA polymerase starts transcription by recognising a specific promoter base sequence at the beginning of a gene.

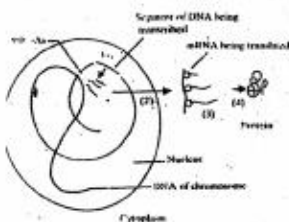


Fig.13.11: Schematic representation of the flow of information in a cell. The DNA of the chromosomes located within the nucleus contains all the genetic information. Selected sites on the DNA are transcribed.

- 1) into messenger RNAs which are transported out of the nucleus
- (2) once in the cytoplasm, the messenger RNA is translated
- (3) into a polypeptide by a ribosome that moves along its length. Following translation the polypeptide undergoes its folding to assume its native conformation

In prokaryotes, when a single mRNA codes for a single polypeptide chain, it is called **monogenic** or **monocistronic** and if it codes for two or more polypeptides, the mRNA is **polygenic** or **polycistronic**. Polygenic mRNAs may contain non-translated **spacer region** which separate the segments, coding the individual polypeptide chains.

In addition to transcription of the nucleotide sequence of RNA, the RNA polymerase in eucaryotes recognises a variety of genetic signals on the chromosomes, such as the signals for starting and stopping RNA synthesis at specific sites. *E. coli* has a single RNA polymerase that can make all the three RNAs. Purified preparations of the enzyme RNA polymerase which catalyses the transcription of DNA into RNA has two components; i) a **core enzyme** containing 4 subunits of varying molecular weights and ii) an accessory protein known as **sigma factor**. Together they constitute a complex called as **holoenzyme** (Fig. 13.12). Removal of sigma factor leads to random transcription of both the DNA strands, and RNA synthesis is not restricted to the site which is to be transcribed. This enzyme does not require a **primer** or a **helix unwinding** or **destabilising enzyme** like DNA polymerase. The RNA polymerase of *E. coli* is a large multisubunit enzyme complex mainly responsible for chain elongation of DNA, with the subunit composition shown in figure (Fig. 13.12).

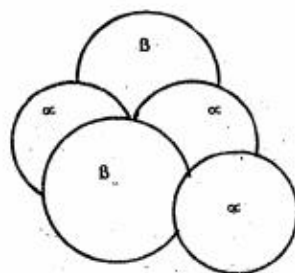


Fig. 13.12: Schematic diagram of RNA polymerase of *Escherichia coli*. The holoenzyme is composed of five subunits of four types (α , β , β'). The sigma subunit is readily dissociated forming the core enzyme.

There are three stages in the transcription process,

- binding of RNA polymerase to DNA at appropriate location called the promoter site for chain initiation
- elongation of RNA
- termination of synthesis of RNA

Let us study these steps in some detail.

Initiation

RNA polymerase, though does not require a preformed DNA strands however it cannot function until it builds to a specific initiation signal on DNA template strand, called promoter region. The RNA polymerase binds to the promoter region located towards the 5' side of DNA molecule. The polymerase unwinds the DNA helix after binding and starts copying one strand of the molecule. RNA polymerase in prokaryotes recognises characteristic nucleotide sequences in the promoter region which are about 40 bases long. They are known as **Pribnow box** and **TGTTG nucleotide base** located about 10 and 35 bases to the left of the initial base of mRNA respectively (Fig. 13.13).

Like DNA, a mRNA chain is always synthesised by the addition of nucleotides one at a time to 3' end of the growing molecule. The 5' end of a new mRNA chain always starts with a nucleotide that has a three phosphate at its 5' end and a free-OH at the 3' end.

The synthesis of new mRNA is initiated with the help of nucleotide ATP or GTP and the two nucleotides retain the triphosphates throughout the transcription.

Elongation

After the recognition of the promoter region, the RNA polymerase proceeds to synthesise RNA chain by stepwise addition of nucleotides from its 5' $\rightarrow$ 3' direction. The DNA template is copied according to the base pair rules except uracil is replaced by thymine. The DNA strand may code in stretches, i.e. the coding sequences may be interrupted by non-coding sequences. The process of elongation of mRNA continues till a terminating signal in DNA is reached. After the termination of mRNA synthesis, the sigma factor in holoenzyme dissociates and is available to bind to other free core enzyme to initiate a new round of transcription (Fig. 13.13).

Termination

Termination of RNA synthesis is brought about by another 'signal' which is called as **termination factor** or **rho factor**. This causes the release of the RNA molecule and RNA polymerase.

It is important to note that in prokaryotes, transcription is coupled to protein synthesis. As prokaryotes do not have a nuclear membrane, the ribosomes are free to attach to the mRNA molecules as they are being synthesised (Fig. 13.13).

Promoter: A specific nucleotide sequence in DNA, to which RNA polymerase binds and initiates transcription; a non-transcribed regulatory sequence upstream of the structural gene and essential for gene expression.

Pribnow box or TATA box: It is the -10 sequence of a promoter upstream of the gene, and is involved in the binding of RNA polymerase in the initiation of transcription, in prokaryotes.

rho protein is an *E. coli* gene product that binds to terminator region of an informational DNA sequence and sponsors the dissociation of RNA polymerase from DNA template thereby ending transcription of that sequence.

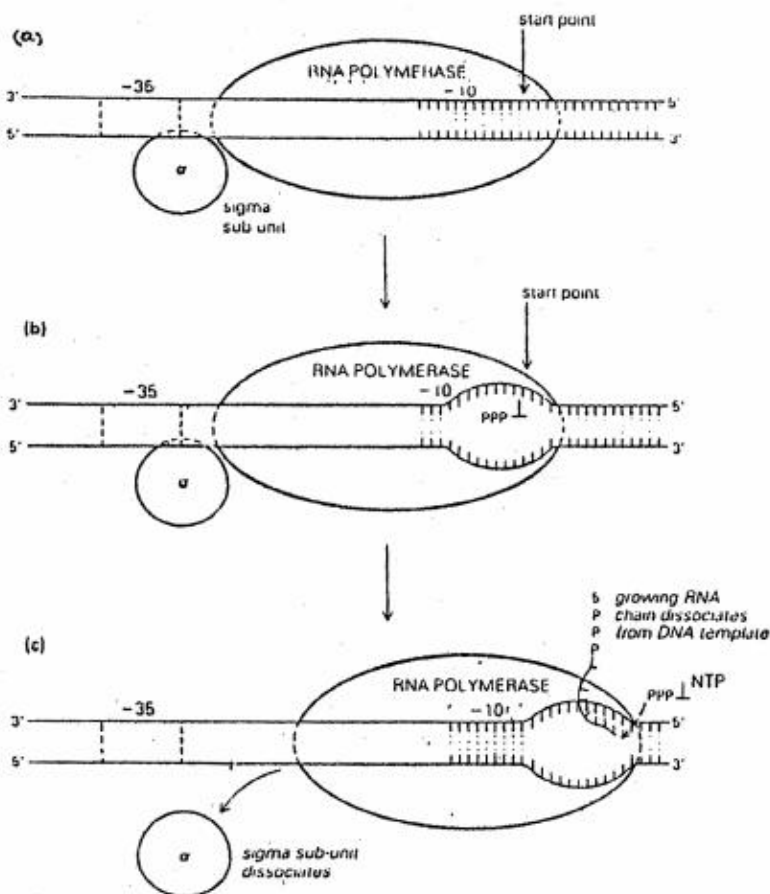


Fig 13.13: Initiation and elongation of RNA Chains by *E.coli* RNA polymerase.

- The polymerase holoenzyme binds to the promoter region of the gene. The -10 and -35 conserved sequences are important in binding. The sigma unit greatly enhances the affinity of the polymerase for specific binding to these sequences.
- The polymerase causes local 'melting' of the double helix in the region approximately -12 to +2 bases relative to the transcriptional start point. The first NTP, complementary to the base at the transcriptional start point, is then bound by the polymerase, and forms a Watson-Crick base pair as illustrated.
- RNA chain elongation commences, and as NTPs are polymerised to the 3' -OH group of the ribose of the growing chain, the transcriptional 'bubble' moves down the transcribed DNA strand in 3' $\rightarrow$ 5' direction to subunit dissociated and leaves the core enzyme to complete long chain when 10 nucleotides have been polymerised.

This is possible because both RNA synthesis and protein synthesis start at 5' end of mRNA. In *E.coli* cell about 90-95% of chromosomes code for mRNAs, and remaining code for tRNAs and rRNAs and other regulatory sequences such as spacers, and tails.

13.5.2 RNA Synthesis in Eucaryotes

Eucaryotic transcription differs in several respects from that of procaryotes. The following table (Table 13.4) shows the differences between procaryotic and eucaryotic transcription.

Table 13.4: Some Differences between Prokaryotic and Eukaryotic Transcription

Prokaryotes	Eukaryotes
1. Only one type of RNA polymerase is required for transcription	Three types of RNA polymerases are required for transcription i.e. Polymerase I, II and III.
2. Prokaryotic mRNA are polycistronic	Eukaryotic mRNAs are monocistronic which undergo special types of post transcriptional modifications
3. Transcription is coupled with translation due to lack of nuclear membrane	The nuclear membrane introduces a barrier between transcription and translation
4. The prokaryotic RNA are relatively less stable	The eukaryotic RNA are relatively more stable

Eukaryotes possess multiple forms of the RNA polymerases, localised in different part of the nucleus and activated by different ionic conditions. The genes that synthesise RNAs that form part of the Polymerase-I makes the large ribosomal RNAs in the nucleolus, polymerase II synthesises the mRNA. Polymerase III transcribes the genes coding for a variety of very small, stable RNAs — including the tRNAs and the small 5S RNA of the ribosome. The three RNA polymerases can be distinguished by their characteristics as given in table (Table 13.5).

Table 13.5: Properties of eukaryotic RNA polymerases

Polymerase type	Location	RNA transcripts	Sensitivity to the poison α -amanitin
I	nucleolus	common precursor to 18s, 5.8s and 28s rRNA	insensitive
II	nucleoplasm	mRNA precursor (heterogeneous nuclear RNA)	highly sensitive
III	nucleoplasm	tRNA precursor and 5s rRNA	moderately sensitive

As you have studied above in prokaryotic transcription, there are also specific signals in eukaryotic genes, which are responsible for the correct initiation and termination of RNA synthesis. Since RNA polymerase II makes all of the mRNA precursors and thus determines which proteins a cell will make, we shall confine our discussion mostly to the synthesis and fate of the RNA transcripts synthesised by polymerase II. The polymerase II recognition signal, has two short DNA sequences, one beginning at about 25 nucleotides upstream from the initial point of RNA synthesis called as **TATA box** or **Hegness box** and another at about 50 nucleotides further upstream known as **CAAT box**. In contrast, RNA polymerase III is unique in recognising a gene regulatory protein that binds in DNA molecule **downstream** from the point where enzyme polymerase begins its RNA synthesis (Fig. 13.14).

Upstream is the region to the left of the start point of a gene.

Downstream is the region to the right of the start point of a gene.

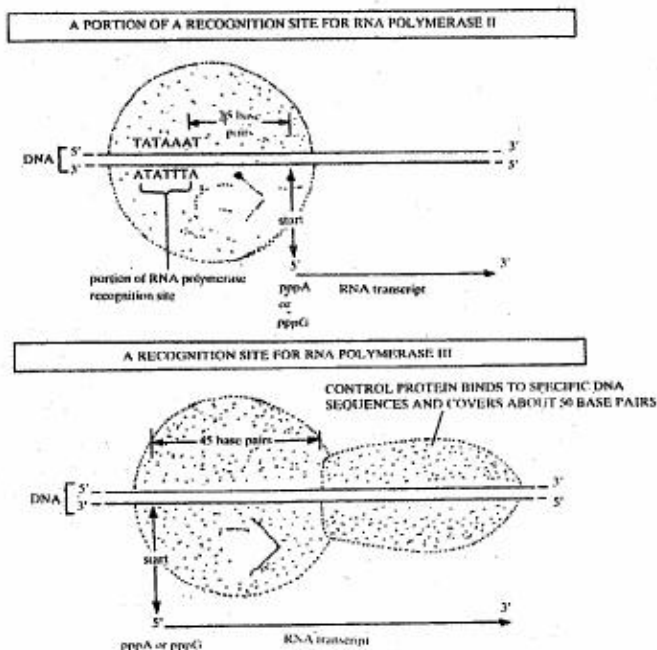


Fig. 13.14: The different types of start sites recognised by eucaryotic RNA polymerase II and III. Like bacterial RNA polymerase and RNA polymerase I, RNA synthesis begins. However, it appears that parts of the sequence recognised can extend as far as 190 nucleotides from the start of the RNA chain (only a small portion of the RNA polymerase II promotes sequence is illustrated here). In contrast RNA polymerase II is unique in recognising a gene regulatory protein (here the 5s transcription factor) that binds to a sequence downstream from the point where the polymerase begins its RNA synthesis.

SAQ 5

a) During RNA synthesis the DNA template sequence 5' TpApGpCp-3' would be transcribed to produce which of the following RNA sequences? Give reasons.

- 5'-ApTpCpGp-3'
- 5'-GpCpTpAp-3'
- 5'-CpGpTpAp-3'
- 5'-ApUpCpGp-3'

b) Which one of the following statements about RNA polymerase activity is Correct? Give reasons.

i) Transcription of DNA by RNA polymerase is a semi-conservative process because only one of the two strands of the DNA double helix is copied into RNA.

ii) Transcription of DNA by RNA polymerase requires the participation of a topoisomerase.

iii) Helix-unwinding is an important catalytic activity of one of the subunits of RNA polymerase.

13.5.3 Different Types of RNA

Cells make three main types of RNA transcripts known as *messenger RNA (mRNA)*, *ribosomal RNA (rRNA)* and *transfer RNA (tRNA)*. We will describe the structure and functions of rRNA and mRNA in this unit, the structure and function of tRNA will be explained in the next unit.

Ribosomal RNA (rRNA) — It is a cytoplasmic nucleoprotein that acts as a machine for protein synthesis on the mRNA molecule. The conversion of genetic information into protein requires the linking together of the amino acids in the correct order by peptide bonds. The classes of ribosomes found in various organisms are distinguished by their sedimentation properties, about which you have read in Block II of this course. The ribosome can reversibly dissociate into their subunits. This process is dependent on the Mg^{2+} concentration.

A striking feature of the rRNAs is that they possess a high degree of secondary structure, which arises from intermolecular hydrogen bonding between different regions of the same molecule, giving rise to double stranded stem region and single stranded loops. Such secondary structure is important for the association of rRNA with amino acids.

Messenger RNA (mRNA) — This class is the most heterogeneous in size and stability unlike rRNA and tRNA population. In most eucaryotic and procaryotic mRNAs, not all of the mRNA sequence is translated into proteins. The translated sequence is flanked on either side by non-translated sequences which may be several hundred nucleotides in length. Eucaryotic mRNAs also possess two other common features, the base at the 5' end is guanine and the 3' end of the molecule contains a sequence of 100-200 adenylic acid residues called as the **poly A tail**.

The nucleus also contains a fourth class of RNA molecules which are quite large and vary a great deal in size. These are called as **heterogeneous RNA molecules (hnRNA)**. The hnRNA are processed to generate the mRNA molecules which then enter the cytoplasm for protein synthesis.

SAQ 6

- a) Characteristics of different types of RNA are given below. Identify and write their names
- RNA which is characterised by containing a number of unusual bases, including methylated purines and pyrimidines
 - RNA that has a 3' poly (A) sequence
 - Heavy molecular weight, guanosine "cap" at its 5' end
 - RNA which has 7-methyl guanosine "cap" at its 5' end
 - RNA, which is the structural component of ribosomes
- b) Write two important differences between procaryotic and eucaryotic transcription

13.6 RNA PROCESSING IN EUKARYOTES

The RNA transcripts normally undergo further enzymatic processing before performing their normal precursor functions. Transfer and ribosomal RNAs are synthesised as long molecules which are then cleaved and modified to yield the final products. Eucaryotic mRNAs are also processed but procaryotic mRNAs are not further processed.

It must be borne in mind that the genetic information in DNA cannot be converted directly into protein synthesis, but is first transcribed into mRNA. Also that the mRNA alone cannot perform the complex function of protein synthesis, but requires other RNAs, i.e. tRNA, rRNA, 5s RNA to synthesise protein in the cell.

In eucaryotes the mRNA produced in the nucleus by the activity of enzyme polymerase II is known as the **primary transcript**. Before entering the cytoplasm, the primary transcript is extensively processed by the removal of long nucleotide sequence from the middle of the transcript molecule. Thus various types of RNAs are produced which vary from 10s to 60s containing about 800-3000 nucleotide bases. Although mRNA constitutes only about 5% of RNA in the cell, yet it is the most heterogeneous type of RNA as regards the size of the molecules. A single eucaryotic cell may have about 10^4 different mRNA molecules, each coding for one or more different polypeptide chains. Such RNAs are known as **heterogeneous nuclear RNA (hnRNA)** and most of them are processed and utilised only within the nucleus. The mRNA and other RNAs in eucaryotes are protected from degradation by modification and processing of specific groups and sequences.

There are three main types of modifications and processing of RNAs in eucaryotes. Prokaryotic mRNAs, however, are not processed like eucaryotic mRNA.

1. **5' Capping:** Addition of a methyl group (common in tRNA and rRNA) nucleotides at the terminal 5' end known as **cap** in eucaryotic mRNA.
2. **Addition on a poly 'A' tail:** A poly 'A' tail at the 3' carboxyl end, known as **tailing** is added to the mRNA.
3. **Cleavage** of long precursor RNA into smaller RNAs and removal of introns.

RNA synthesis and processing in eucaryotes and procaryotes are not the same, but differ in many respects.

13.6.1 Modification of mRNA

Capping of mRNA

In eucaryotes and procaryotes, the basic mechanisms of transcription and translation are similar but there is a basic difference between their processing regarding mRNAs. The mRNAs in bacteria are used soon after their synthesis without further processing while the mRNA of most eucaryotes known as primary transcripts is extensively modified and processed in the nucleus before entering into the cytoplasm. The methylation of terminal guanosine at 7 position at the 5' end of mRNA in the nucleus leads to formation of 5' to 5' triphosphate bridge between two ribose molecules. The methyl guanosine is joined backward to the rest of the bases of mRNA through a triphosphate linkage catalysed by nuclear enzyme **guanylyl transferase**. The cap has no free phosphates and this protects mRNAs from the attack of proteases and nucleases. This process is called as **capping** of the mRNA. Capping facilitates initiation and translation of protein synthesis. There is no cap formation in procaryotic mRNAs. (Fig. 13.15)

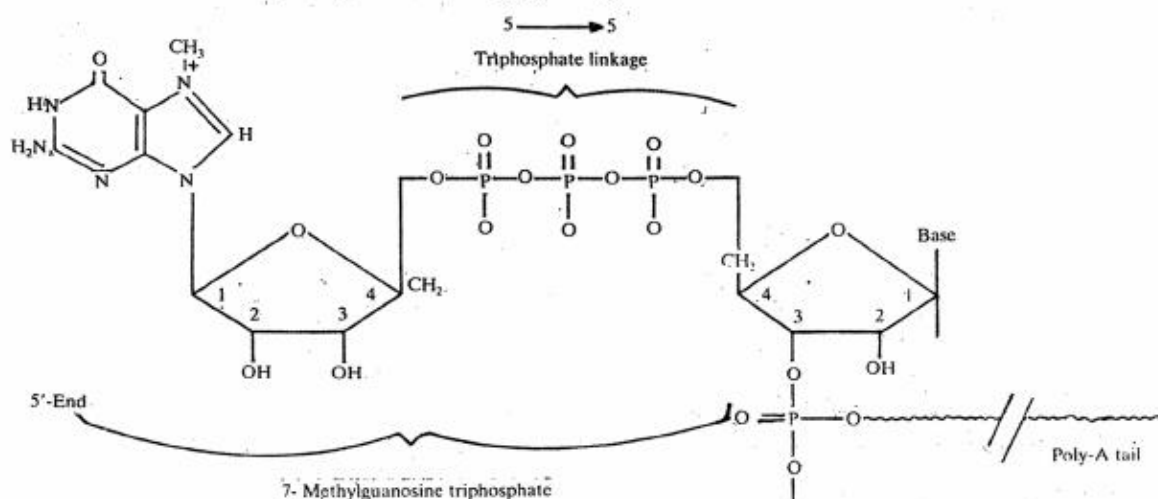


Fig. 13.15: Post-transcriptional modification of mRNA showing the 7-methylguanosine cap and poly(A) tail.

Addition of a Poly (A) Tail

Most mRNAs contain a poly 'A' segment at 3' end of the molecule. Addition of 100-200 adenine nucleotides to the 3' end of mRNA is called **poly(A)tail**. (After mRNA enters the cytoplasm, the poly A tail is gradually reduced.) The poly A tail is added in the nucleus with the help of enzyme **poly(A) polymerase** after transcription. In the nucleus poly 'A' tail protects mRNA from destruction by other enzymes and facilitates it to leave the nucleus. After mRNA enters the cytoplasm, poly(A)tail is gradually reduced (Fig. 13.16a). Bacterial mRNAs do not have a poly (A) tail.

13.6.2 Removal of Introns

In eucaryotes, many genes that code for protein synthesis contain intervening base sequences which are not transcribed. These are known as **introns**. The regions of the genes for coding protein are known as **exons** interrupted by non-coding intervening introns. To enable the RNA transcript to code for synthesis of complete protein, the intron sequences must be removed (Fig. 13.16b). This process of removal of introns is called as **RNA splicing** and by this process the long nuclear hnRNA molecule having about 50,000 nucleotides is cut shorter to about 500-3000 nucleotides. Thus the removal of intron in a mammalian cell reduces the total DNA sequences transcribed into mRNA from 7-10% to about 1-2%. Prokaryotic mRNAs are translated as soon as they start being synthesised and thus transcription and translation processes are coupled, whereas in mammalian cells most RNAs are made as inactive precursor molecules in the nucleus that have to be processed further into active RNA. The number and position of intron is not clearly understood, however, it is assumed that they contain regulatory signals and have helped in the formation of new genes during evolution. The introns in the precursors are removed in such a way, that coding segments or exons are not physically separated from each other. After removal of all introns, the mRNA molecules leave the nucleus to cytoplasm by passing through the nuclear envelope pores complex. The proteins surrounding the pore allow only the completely processed mRNA to leave the nucleus. The leftover pieces of RNA are degraded by nucleases to be used again for the synthesis of RNA in the nucleus. You would be interested to know how separated coding pieces can be assembled into correct order to form the final mRNA. The successive exons are spliced very precisely

Intron: An intervening sequence in DNA that does not transcribe.

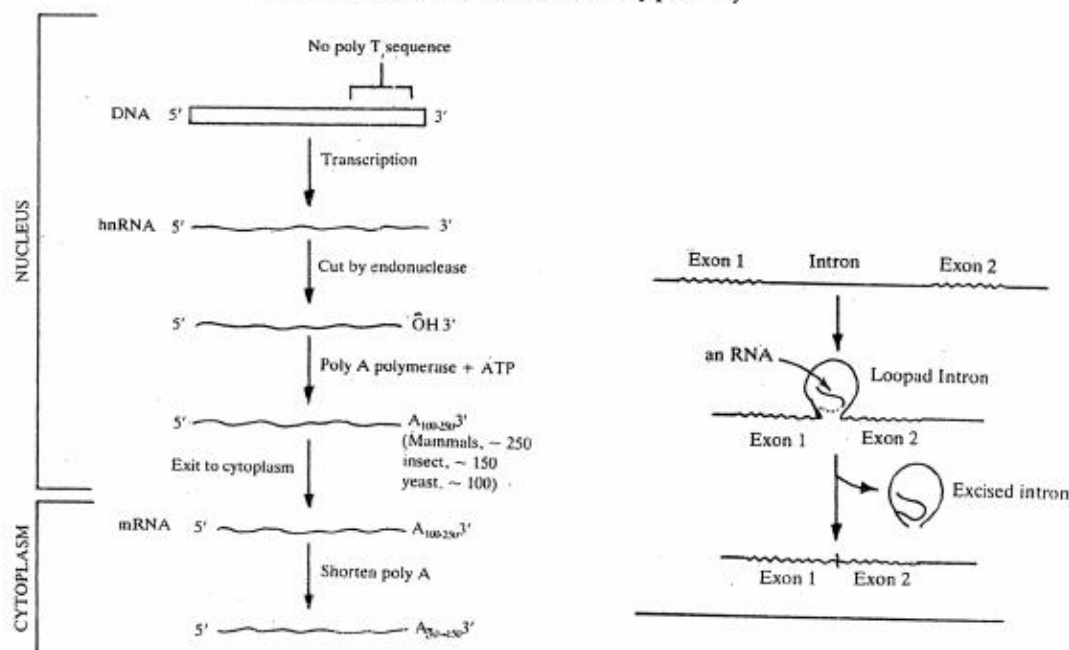


Fig 13.16(a): Poly 'A' in eucaryotic RNA. In all eucaryotic cells, from 100 to 250 adenylate residues are added to the nuclear RNA (hnRNA) that will become mRNA. This segment — the poly A — is not copied from DNA, but is added one 'A' at a time by a transferase reaction at the 3' hydroxyl of the hnRNA. The enzyme responsible for this addition is called poly 'A' polymerase, and the 3' hydroxyl end is probably created by cutting the hnRNA chain. In the cytoplasm, the poly 'A' becomes shorter as the mRNA ages. Fig 13.16 (b): Excision of introns and splicing of exons in eucaryotic RNA.

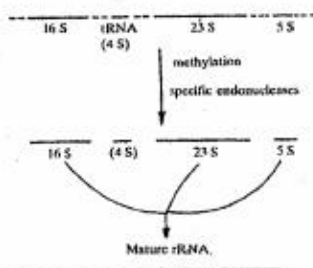


Fig. 13.17: Processing of the ribosomal RNA transcript in procaryotes.

by the help of another class of RNA molecules; the **small nuclear RNAs (SnRNAs)**. It is due to the base sequence complementarity of SnRNAs to the sequence at the ends of each intron, that the introns are looped and removed enzymatically bringing the two exons near to each other to form active complete mRNA. Error committed in processing and splicing of single nucleotide in a mRNA transcript would result in the synthesis of a defective protein molecule leading to genetic defects in metabolic processes.

The mRNAs which are transcripts of certain regions of DNA are translated into amino acids which other RNAs such as rRNAs, tRNAs, and small RNA molecules do not translate but perform other specialised functions in the cytoplasm of the cell.

The sequence of nucleotides in a mRNA is complementary to the sequence of DNA nucleotides in one strand of the double stranded DNA molecule. The strand which codes for mRNA is called **coding strand of DNA**, the other strand is called **non-coding strand**. In a double stranded DNA molecule containing many genes the coding strand may be one of the strands of DNA molecule or the other strand, and thus each strand can code for some of the genes. However, nucleotide sequence of an RNA transcript will be the same by the both coding and non-coding strands of the double helix. This, as you may infer is due to the complementarity of the double helix.

Ribosomal RNAs of both procaryotes and eucaryotes are made from longer precursors, called **preribosomal RNAs** which in procaryotes produce 16s, 23s and 5s, ribosomal RNAs. Procaryotes produce 16s, 23s rRNA by the action of nucleases (Fig. 13.17). In eucaryotes a large 45s rRNA after processing in nucleolus produces 18s and 28s rRNA.

The transfer RNAs likewise, are also processed from longer precursors by enzymatic removal of extra nucleotide sequences from the 5' and 3' ends. Sometimes, two or more different RNAs arise by enzymatic cleavage of a single long RNA precursor molecule, as there are at least 32 different RNAs. Some tRNAs, undergo attachment of trinucleotide sequence at the 3' terminal end of -C-C-A, but other tRNAs already contain this sequence. All tRNAs have characteristic modified bases such as methylated, deaminated and reduced bases. The terminal A residue at 3' end attaches specific amino acid covalently for the synthesis and addition to the growing polypeptides chain on the ribosome.

While eucaryotic transcripts have long insertions of non-coding RNA sequence as intervening sequences or as introns, there is no similar process or removal and splicing in the procaryotes, where RNA transcripts are directly translated into proteins. Procaryotic mRNA may have several coding regions each producing a specific polypeptide chain.

The proteins surrounding the pore allow only the completely processed mRNA to leave the nucleus. The leftover pieces of RNA are degraded by nucleases to be used again for the synthesis of RNA in the nucleus.

SAQ 7

What are the three main types of modifications in the processing of RNA in eucaryotes?

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Fill in the blanks.

- The non-transcribed base sequence that intervene the genes, that code for protein synthesis are called
- The mRNA produced in the nucleus by the activity of polymerase II is known as
- The process of removal of introns is called as
- The regions of the genes for coding protein are known as

13.7 SUMMARY

In this unit you have learnt that:

- The nuclear envelope is composed of two concentric membranes, i.e. the outer and the inner lipid bilayer, nuclear membrane and a perinuclear space. The nuclear envelope is interrupted at places by nuclear pores which together with annuli comprise pore complex. The nuclear envelope plays an important role in maintaining the chromosomal organisation, RNA and protein transfer into cytoplasm, and transport of various ion and molecules across the membrane.

- Chromatin is formed by a series of repeating units called nucleosomes. Each nucleosome contains a histone octamer consisting of two each of the four histones, H₂A, H₂B, H₃ and H₄ with about 200 bp of DNA coiled on the outside of the nucleosome. The packing of DNA in a chain of nucleosomes is about five to seven times more compact than free DNA and in a 30nm solenoid it is packed to about 40 fold. The higher order structure of chromatin is maintained by histone H₁.

- DNA replication is semi-conservative. In procaryotes, DNA replication is catalysed by a single DNA polymerase whereas in eucaryotes there are three types of DNA polymerases. DNA is synthesised continuously in the leading strand and discontinuously in the lagging strand. These short fragments called as Okazaki fragments are later joined by the enzyme ligase. DNA replication is bidirectional.

- RNA synthesis takes place with the help of DNA dependent RNA polymerase. In procaryotes, there is one type of RNA polymerase which synthesises polycistronic RNA and transcription is coupled to translation. The transcription process involves three stages. a) binding of RNA polymerase to promoters and chain initiation b) elongation c) termination of the synthesis of mRNA.

In eucaryotes, transcription is catalysed by three types of RNA polymerases which synthesise monocistronic messenger RNA. The precursor of mRNA are heterogeneous nuclear RNA (hnRNA) which are very large molecules. Only one DNA strand is transcribed, so transcription is asymmetric.

- RNA molecule undergoes post-transcriptional modifications called as RNA processing. RNA processing includes capping, addition of poly(A)tail and removal of non-coding regions known as introns. RNA processing occurs in eucaryotes because of the complex structure of RNA in eucaryotes.

13.8 TERMINAL QUESTIONS

1 Explain the central dogma of molecular biology.

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2 Describe the role of histone H₁.

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3 Explain the semi-conservative nature of DNA replication.

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4 Explain RNA processing in eucaryotes.

13.9 ANSWERS

Self-assessment Questions

- 1 a) i) nuclear envelope, outer, inner
ii) perinuclear space
iii) pores, annuli, pore complex
iv) nucleoplasm
v) nucleolus, nucleolar organiser region
b) i) maintaining the chromosome organisation
ii) transport of RNA and protein
- 2 a) i) Yes, because these basic amino acid give histones their positive charge at physiologic pH.
ii) No, Histones interact with the negatively charged DNA length electrostatic interactions
iii) No, Histones are relatively small proteins
iv) No, Protamines are a separate classes of small, positively charged proteins that bind to DNA.
b) i) nucleosomes
ii) nucleosome core particle
iii) H₁
- 3 a) i) 5'TpCpTpAp-3'.
Because the newly synthesised DNA strand binds to its template in an antiparallel, complementary orientation. Nucleotide sequences, however must always be written in the 5' to 3' order.
b) i) Okazaki fragments
ii) central dogma of molecular biology
iii) semi-conservative replication
iv) replication fork
- 4 a) Column I
i) DNA polymerase I
ii) DNA ligase
iii) DNA helicase
iv) DNA polymerase III
v) RNA primase
b) i) Yes, DNA polymerases cannot copy single strands of DNA rather, they require that at least a short stretch of the template be double stranded, with a free 3' - hydroxyl group on the shorter 'primer' strand.
ii) No. DNA polymerases can only add nucleotides to the 3' end of the growing chain.

Column II
fills in gaps between the fragments of DNA synthesised by DNA polymerase III
uses ATP to seal breaks in sugar phosphate backbone
uses energy released by ATP hydrolysis to disrupt hydrogen bonds between base pairs and thereby creates single stranded region.
the major enzyme responsible for DNA replication
produces short RNA sequences to prime DNA polymerase III

- iii) Yes. The 3' → 5' exonuclease activity is responsible for 'proof reading' the newly synthesised chain.
 - iv) No. Only RNA dependent DNA polymerase can synthesise DNA in the presence of an RNA template.
- 5 a) iv) The RNA sequence is transcribed antiparallel to the DNA sequence i.e. 3'-ApUpCpGp-5'. However, all nucleotide sequences must be reported in the 5' → 3' direction. Also, the RNA transcript contains uridine (U) instead of thymidine (T)
- b) Correct answer is
 - i) No. Transcription is a conservative process. However, both DNA strands can serve as template for RNA synthesis within a given region of the double helix, generally only one strand will be transcribed.
 - ii) No. RNA polymerase does not require the action of topoisomerases for transcription.
 - iii) No.
 - iv) Yes. Unlike DNA polymerase, RNA polymerase has no 'proof reading' ability.
- 6 a) a) Transfer RNA
 b) Messenger RNA
 c) Heavy nuclear RNA (hnRNA)
 d) Messenger RNA
 e) Ribosomal RNA
- b) i) In procaryotes, only one type of RNA polymerase is required for transcription whereas in eucaryotes three types of RNA polymerases, i.e. I, II and III are required.
 - ii) In procaryotes transcription is coupled with translation due to lack of nuclear membrane whereas in eucaryotes nuclear membrane introduces a barrier between transcription and translation.
- 7 a) i) Cleavage of long precursor RNA into smaller RNAs
 ii) Addition of nucleotides at the terminal 5' end known as cap and a poly A tail at the 3' carboxyl end.
 iii) Nucleotide modifications such as addition of a methyl group which is common in tRNA and rRNA.
- b) i) introns
 ii) primary transcript
 iii) RNA processing
 iv) exons

Terminal Questions

- 1 Central dogma of molecular biology states

Replication DNA $\xrightarrow{\text{Transcription}}$ RNA $\xrightarrow{\text{Translation}}$ Protein

The arrows indicate the flow of information, DNA undergoes self replication, but genetic information in DNA cannot directly be converted into protein, but is first transcribed into mRNA — thus the genetic information flows from nucleus (DNA) to cytoplasm (protein).

- 2 Histone H₁ is involved in the maintenance of higher order of folding of chromatin. It helps in packing the nucleosomes together, thereby leading to the further folding of the fibre i.e. into 30 nm, 300 nm, 700 nm and 1400 nm thick fibres.
- 3 During DNA replication, the strands separate in a 'zipper like fashion'. Each strand serves as a template for daughter strand. Thus the daughter DNA that is formed contains half new and half old DNA. Hence, it is called as semi-conservative mode of replication.
- 4 The transcripts synthesised as a result of transcription are not the final products but are modified by a series of cleavages and chemical modifications known as **processing**.

In eucaryotes the mRNA produced in the nucleus by the activity of polymerase II is known as primary transcript. From this transcript long nucleotide sequences are removed such as

- a) cleavage of long RNA
- b) addition of cap and poly 'A' tail in mRNA
- c) addition of methyl group in tRNA and rRNA.

UNIT 14 PROTEIN BIOSYNTHESIS

Structure

- 14.1 Introduction
 - Objectives
- 14.2 Protein Biosynthesis
 - Activation of Amino Acids
 - Transfer of Amino Acid to tRNA
 - Initiation of Polypeptide Chain Synthesis
 - Chain Elongation
 - Termination of Polypeptide Chain
 - Rate of Protein Synthesis
- 14.3 Processing of Protein Molecule
- 14.4 Regulation of Protein Synthesis
 - At Transcriptional Level
 - At Translational Level
 - At Post-translational Level
 - Chemical Inhibitors of Protein Synthesis
- 14.5 Summary
- 14.6 Terminal Questions
- 14.7 Answers

14.1 INTRODUCTION

In Unit 12 you have studied biosynthetic pathways such as gluconeogenesis, glycogenesis and also about the synthesis of fats from carbohydrates. In this unit we will study various steps involved in the biosynthesis of proteins. Proteins are the most important components of living organisms, about which you have already studied in Blocks I and II. Proteins are not only required as a source of energy, they are more important as enzymes, catalysing hundreds of chemical reactions in a cell. You have already read about the various aspects of enzymes and regulation of enzyme activity in Units 9 and 10.

In the previous units you have studied that organisms maintain their internal environment in a dynamic steady state. In order to maintain the dynamic steady state, a cell regulates each and every activity taking place in it. In Units 11 and 12 you have studied various mechanisms involved in the control of synthesis and degradation of biomolecules releasing energy and precursors for the synthesis of other biomolecules. In this unit we will study mechanisms that regulate protein biosynthesis.

Objectives

After studying this unit you should be able to

- define and use in correct context the terms: initiation codon, termination codon, initiation factors
- describe various steps in the synthesis of a peptide chain
- explain the processing of a protein precursor molecule
- outline the various ways in which a cell regulates protein synthesis.

14.2 PROTEIN BIOSYNTHESIS

Protein biosynthesis is the most complex of biosynthetic mechanisms requiring a large number of enzymes and other specific biomolecules. In eukaryotic cells protein biosynthesis requires the participation of over 200 different enzymes and, 70 or more kinds of tRNA and rRNAs. Thus almost 300 different macromolecules must cooperate to synthesise polypeptides. You are already aware that proteins are made up of polypeptide chains, organised into a complex three dimensional structure. Chemists can make biologically active proteins from their constituent amino acids. It takes at least 24 hours to produce, a small polypeptide chain such as ribonuclease. Living cells, however, can synthesise proteins at a rate much higher than that in the laboratory. For example only about five seconds are required for an *Escherichia coli* (*E. coli*) ribosome to make a complete polypeptide chain of 100 amino acid residues.

E. coli is a bacterium commonly found in the vertebrate intestine. It is most frequently used in the study of biochemistry and genetics.

Protein biosynthesis is under the control of genes, which contain coded instructions for the synthesis of proteins. DNA makes RNA, in accordance with the sequence of bases. RNA, in turn, makes a sequence of amino acids. The main steps in protein biosynthesis are **transcription** and **translation**.

In transcription, the information contained in DNA is transcribed into mRNA. The sequences of deoxyribonucleotides in DNA provides the template for synthesis of a complementary sequence of ribonucleotides in the RNA. In **translation**, the information in mRNA is translated into proteins. Three major types of RNA molecules are transcribed from genes in DNA, each with a particular role to play in gene expression; these molecules are mRNA, rRNA and tRNA. All three kinds of RNAs interact in the process of translating the informational DNA into proteins.

You have already studied the process of transcription in Unit 13. Now we will study how genetic information from DNA, via mRNA, is decoded to synthesise a protein by a process called **translation**.

You already know that each code is composed of three nucleotide bases known as a **triplet code** or a **codon**, that specifically codes for a single amino acid. Any alteration in the sequence of bases results in the synthesis of a wrong amino acid in the polypeptide chain. Wrong amino acid in the polypeptide chain can cause serious disorders in the organism, such as sickle cell anaemia, haemophilia, thalassaemia etc. A collection of codons comprise the genetic code, about which you will study later in this section. The codons pair with anticodons of the tRNA molecule. Each anticodon consists of three free bases. This pairing follows the A-U and G-C combination. The codon GUC pairs with the anticodon CAG of tRNA. Thus the series of codons on the mRNA determine the series of anticodons of different tRNA molecules, and hence of the amino acids. You have studied in Unit 13 that the triplets of mRNA are complementary to the series of base sequence in DNA.

So you can see that the DNA molecule ultimately determines the structure of protein molecule.

The process of translation involves the following steps:

- 1) activation of amino acids,
- 2) transfer of the activated amino acids to tRNA,
- 3) initiation of polypeptide chain synthesis,
- 4) chain elongation,
- 5) chain termination and release of the polypeptide chain, and
- 6) Folding and processing of the polypeptide chain.

Before we study the process of initiation of protein biosynthesis, let us learn about the genetic code. The genetic code is a specific sequence of bases in mRNA that leads to the synthesis of a sequence of amino acids. The individual word in the code is composed of three nucleotide bases called the codon. The sequence of codons always leads from the 5' end to its 3' end.

The pioneering work of Nirenberg and Khorana in 1960 revealed the coding pattern of all the 64 triplets. Sixty-one out of the 64 triplets code for only 20 different amino acids. There are four nucleotide bases that produce a three-base codon and there are 64 different combinations of these bases. Since there is more than one code for single amino acid, it is assumed that most amino acids are coded for by more than one codon. Such a genetic code is called a **degenerate code**, although degeneracy does not mean malfunctioning. The genetic code is associated with specific punctuation signals such as **initiation** and **termination** codons for polypeptide synthesis. Table 14.1 summarises triplet codes for 20 amino acids and for the termination signal.

Table 14.1 : The Genetic Code

5' OH terminal and First Base	Second Base				3' OH terminal Third Base
	U	C	A	G	
U	UUU = phe	UCU = ser	UAU = tyr	UGU = cys	U
	UUC = phe	UCC = ser	UAC = tyr	UGC = cys	C
	UUA = leu	UCA = ser	(UAA = STOP)	(UGA = STOP)	A
	UUG = leu	UCG = ser	(UAG = STOP)	UGG = trp	G

Sickle cell anaemia is a severe anaemia in which sickle-shaped red blood cells appear in the blood.

The patients of haemophilia bleed severely from even a slight injury, because the blood fails to clot.

Thalassaemia is a group of inherited anaemias in which there is a defect in the alpha or beta chain of haemoglobin. It is fatal in children.

5' OH terminal and		Second Base			3' OH terminal
First Base	U	C	A	G	Third Base
C	CUU = leu	CCU = pro	CAU = his	CGU = arg	U
	CUC = leu	CCC = pro	CAC = his	CGC = arg	C
	CUA = leu	CCA = pro	CAA = gln	CGA = arg	A
	CUG = leu	CCG = pro	CAG = gln	CGG = arg	G
A	AUU = ile	ACU = thr	AAU = asn	AGU = ser	U
	AUC = ile	ACC = thr	AAC = asn	AGC = ser	C
	AUA = ile	ACA = thr	AAA = lys	AGA = arg	A
	AUG = met	ACG = thr	AAG = lys	AGG = arg	G
G	GUU = val	GCU = ala	GAU = asp	GGU = gly	U
	GUC = val	GCC = ala	GAC = asp	GGC = gly	C
	GUA = val	GCA = ala	GAA = glu	GGA = gly	A
	GUG = val	GCG = ala	GAG = glu	GGG = gly	G

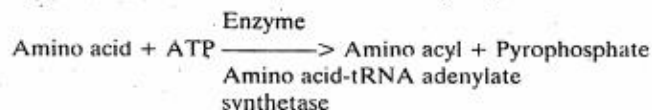
Notes: Triplets represent codons in messenger RNA.

Amino acid abbreviations:

ala = alanine	gln = glutamine	leu = leucine	ser = serine
arg = arginine	glu = glutamic acid	lys = lysine	thr = threonine
asn = asparagine	gly = glycine	met = methionine	trp = tryptophan
asp = aspartic acid	his = histidine	phe = phenylalanine	tyr = tyrosine
cys = cysteine	ile = isoleucine	pro = proline	val = valine

14.2.1 Activation of Amino Acids

You are already aware that there are at least 20 different amino acids, and these different amino acids are first activated by specific activating enzymes called **aminoacyl-tRNA synthetase** and bind to ATP to form an amino acid AMP molecule. The enzymes are also specific for the corresponding tRNA. However, some enzymes can activate more than one amino acid, for example isoleucine-tRNA synthetase, which activates isoleucine can also activate valine. A high energy acyl bond is formed between the α -phosphate of ATP and the carboxyl group of the amino acid. The β and γ -phosphates of ATP break away as inorganic pyrophosphates (PPi). The amino acyl AMP remains bound to the activating enzyme.



14.2.2 Transfer of Amino Acid to tRNA

The next step, after activation of the amino acid, is the transfer of the activated amino acids to its specific tRNA. About 100 different types of tRNA are found in each cell. The tRNA is named after the amino acid for which it is specific. For example the tRNA for the amino acid isoleucine and valine are designated as tRNA^{ileu} and tRNA^{val} respectively.

Transfer RNAs serve as adaptors in translating the language of DNA (genes) into the language of proteins. It has already been mentioned that there is at least one tRNA for each amino acid and some amino acids have two or more specific tRNAs. At least 32 tRNAs are required to recognise all the amino acid codons. However some cells possess many more than 32, for example each bacterial cell probably contains about a hundred or more different types of tRNA.

Transfer RNAs are relatively small, single-stranded molecules. In bacteria and extra mitochondrial cytosol of eukaryotes, tRNAs consist of 73 to 93 nucleotide residues having molecular weights between 24,000 and 31,000 respectively. Mitochondrial tRNAs are smaller than the tRNA present in the cytosol, and have a distinct structure.

The complete nucleotide sequence of tRNA was first worked out by Holley and his colleagues in 1965 for yeast alanine tRNA. Since then, the sequence of about 75 different tRNAs, ranging from bacteria to mammals has been established. The different tRNAs are all minor variants of the same basic structure. Several models of the secondary structure of tRNA have been proposed, and of these the **clover leaf model** of Holley is the most widely accepted.

According to the clover leaf model, the single polynucleotide chain of tRNA is folded upon itself to form a five-armed structure. As a result of the folding the 3' and the 5'

ends of the chain come close to each other. An arm consists of a stem and a loop. In the double helical stems there is internal Watson-Crick base pairing which follows the A-U and G-C combinations except for an occasional G-U base pair or a mismatch. There is no base pairing in the loops. One of the arms has a stem but not a loop and is called the **adaptor** or **acceptor arm**. The other arms are called the D arm, the anticodon arm, the variable arm and the T Ψ C arm. The variable arm may or may not have a stem (Fig. 14.1).

The **adaptor** or **acceptor arm** consists of seven base pairs and four unpaired nucleotide units. The unpaired nucleotide units include a constant 3' terminal — CCA sequence and a fourth nucleotide which is a variable purine (A or G). The amino acid molecule attaches to the — CCA sequence of 3' terminal called the amino acid binding site. The 5' end of tRNA is either guanine or cytosine.

The second arm is called the **D arm**. It consists of 15-18 nucleotides with 3-4 base pairs in the stem and 7-11 unpaired nucleotides in the loop. The loop of the D arm is called Loop I or dihydrouridine (DHU) loop or D loop. It contains two variable regions, α and β on either side of two constant guanine residues. These regions consist of 1-3 nucleotides, mostly pyrimidines with a high proportion of DHU. The synthetase site, which recognises the amino acid activating enzyme, is located on the α part of the D loop and the α part of the acceptor stem on the 5' side.

The third arm, or **anticodon arm**, consists of an anticodon stem of five base pairs and a loop, called Loop II or anticodon loop. This loop consists of seven unpaired nucleotides of which the middle three form the anticodon. The anticodon recognises the three complementary bases which constitute the codon of mRNA. On the 3' side of the anticodon is a hypermodified purine (H) while on the 5' side is U and a pyrimidine (y).

The **variable arm** (the milli loop, Loop III) is of two types. In one type, there is a loop containing 4-5 bases but no stem. In the other type, the arm consists of 13-21 nucleotide residues, and both the stem and the loop can be distinguished.

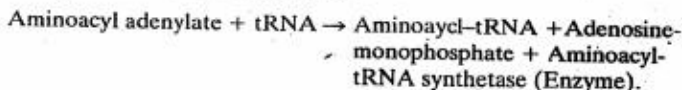
The **T Ψ C arm** consists of a stem having five base pairs and a loop of seven nucleotides. The outermost of the five pairs of the stem is C-G. The **T Ψ C loop** contains a constant T Ψ C sequence. All tRNAs have a ribosome recognition site on the T Ψ C loop consisting of the G-T Ψ C-R sequence.

A study of different tRNAs shows that the structure of the acceptor stem, the anticodon arm and the T Ψ C arm are constant. The difference in the tRNAs lie in the D arm and the variable arm.

X-ray diffraction analysis has revealed that tRNA has a tertiary structure. This structure is due to hydrogen bonds between bases, between bases and the ribose-phosphate backbone and between the ribose phosphates. Fig. 14.2 illustrates the tertiary structure of the yeast phenylalanine tRNA.

After the structure of tRNA, let us now study the next step of protein biosynthesis, the transfer of the activated amino acid to tRNA.

In this step, the aminoacyl group from the enzyme-bound aminoacyl adenylate is transferred to its corresponding specific tRNA, forming tRNA-aminoacyl complex. A high energy ester bond is formed between the carboxyl group of the amino acid and the 3'-hydroxyl group of the terminal adenosine of tRNA. You have studied above that during the activation of an amino acid, an inorganic pyrophosphate is formed. This inorganic pyrophosphate now undergoes hydrolysis to form orthophosphate by an enzyme **Pyrophosphatase**. Thus, two high energy phosphate bonds may ultimately be expended for each amino acid.



The transfer of the activated amino acid to its corresponding specific tRNA is also catalysed by amino acyl-tRNA synthetase. You have studied earlier that aminoacyl-tRNA synthetase catalyses the attachment of amino acids to their corresponding tRNAs. The enzyme aminoacyl synthetase has two active sites, one for recognising the specific amino acid and the other for recognising the specific tRNA molecule. Thus the enzyme aminoacyl-tRNA synthetase brings the amino acid

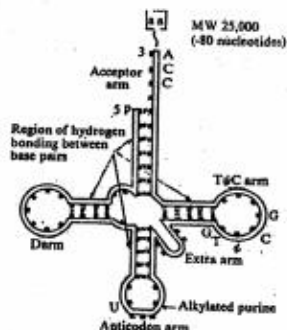


Fig. 14.1: Typical aminoacyl tRNA in which the amino acid (aa) is attached to the 3' CCA terminus

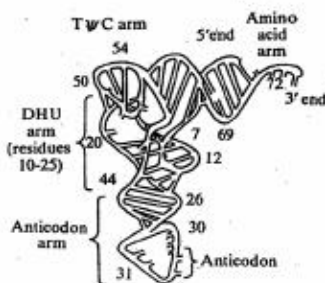
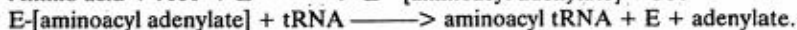
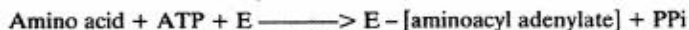


Fig. 14.2: The three-dimensional conformation of yeast phenylalanine tRNA deduced from x-ray diffraction analysis

molecule and its specific tRNA molecule together.



You are already aware that the aminoacyl-tRNA synthetases are specific for both tRNA and its corresponding amino acid. If a wrong amino acid is joined to a tRNA to form a mismatched aminoacyl-tRNA, an incorrect amino acid residue will be formed in the polypeptide chain. However, some of the aminoacyl-tRNA synthetases are capable of proof reading and correcting their own errors, like DNA polymerases about which you have studied in Unit 13.

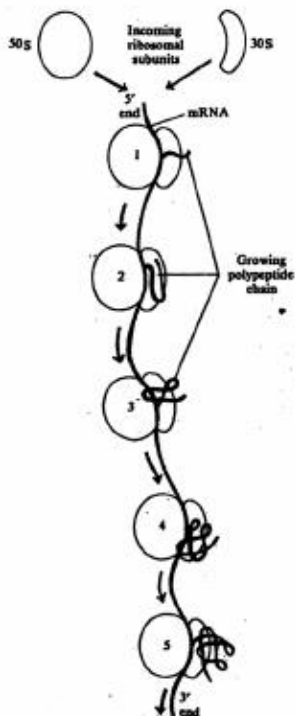


Fig. 14.3: A polyribosome. Note number of ribosomes reading an mRNA molecule simultaneously.

14.2.3 Initiation of Polypeptide Chain Synthesis

Before studying the steps involved in the initiation of polypeptide chain synthesis. Let us learn about ribosomes. You have already studied in Blocks I and II that ribosomes are cell organelles. The complex process of translation takes place on ribosomes. Therefore ribosomes are regarded as the site of protein synthesis. Several ribosomes are held along the length of a single mRNA. This structure is called a **polyribosome**. Polyribosomes greatly enhance the efficiency of mRNA by rapid translation (Fig. 14.3).

A ribosome is a spherical particle of 23 nm and is composed of a large and small subunit. Eukaryotic ribosomes sediment in sucrose gradients with a sedimentation coefficient of 80S. In the absence of Mg^{2+} , these eukaryotic ribosomes dissociate reversibly into subunits of 40S and 60S. Prokaryotic ribosomes are smaller and sediment at 70S; these have subunits of 30S and 50S. The major constituents of ribosomes are RNA and proteins, present approximately in equal amounts. Prokaryotic ribosomes contain three RNA molecules: 16S RNA present in the small subunit, and 23S and 5S in the large subunit. In eukaryotes there are four rRNAs: 18S in the small subunit and 28S, 5.8S and 5S in the large subunit.

In prokaryotes ribosomes contain 21 proteins in the small subunit and 34 in the large. In eukaryotes there are 30 proteins in the small subunit and 40 in the large subunit. There is no functional difference between the prokaryotic ribosomes and eukaryotic ribosomes; they perform the same function, in the same set of chemical reactions. The genetic code is the same for all living organisms. Eukaryotic ribosomes are able to translate bacterial mRNAs efficiently, provided that a 'cap' (30S subunit: the smaller subunit and the larger subunit of the ribosomes are also known as cap and sphere respectively) is added enzymatically (Fig. 14.4).

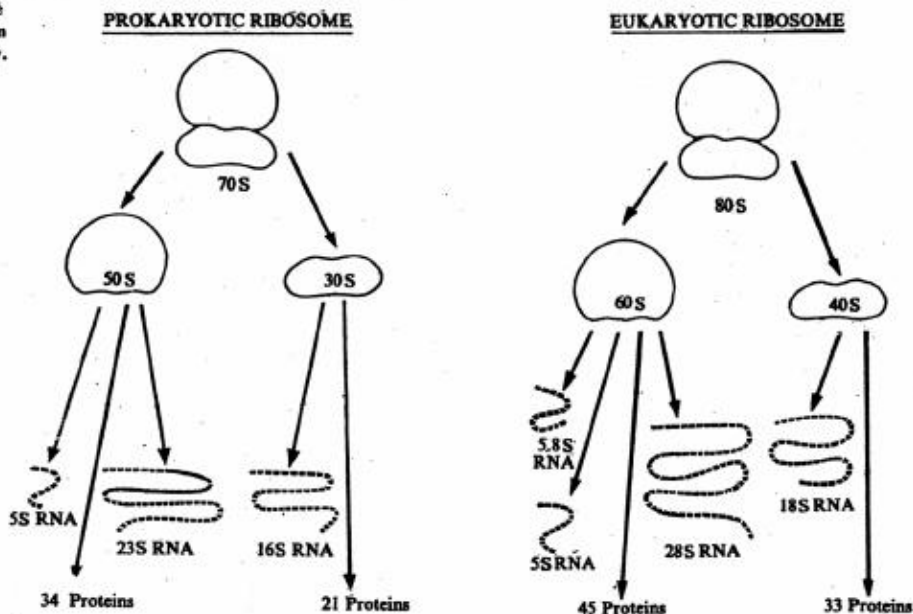


Fig. 14.4: Ribosomal Composition

After learning about the ribosomes, let us now study the initiation of the polypeptide chain. The initiation of a polypeptide chain requires,

- 1) the ribosomal subunit
- 2) mRNA coding for polypeptide
- 3) activated amino acid attached to its tRNA
- 4) an energy source (GTP and ATP) and
- 5) a set of proteins called initiation factors.

The initiation factors are called IF-1, IF-2 and IF-3 in prokaryotes and eIF-1, eIF-2, eIF-3, eIF-4A, eIF-4B, eIF-4C, eIF-4D, eIF-4E, eIF-5 and an extra ancillary protein factor (SP) in eukaryotes (e stands for eukaryotes).

The mRNA bearing the code AUG for polypeptide synthesis, binds to the smaller subunit of the ribosome, followed by the initiating amino acid attached to its tRNA, to form an **initiation complex** (70S-met-tRNA-mRNA in prokaryotes and 80S-met-tRNA-mRNA in eukaryotes)

The formation of the initiation complex takes place in three steps. In prokaryotes, at the first step, the 30S ribosomal subunit binds to the initiation factor-3 (IF-3), which prevents the 30S and 50S subunits from recombining. A segment of 16S rRNA in the 30S subunit provides a binding site for IF-3. 16S rRNA is also involved in mRNA binding. It positions the mRNA correctly at the initiation codon (AUG) for the initiation of translation.

In prokaryotes, at the second step of the initiation process, the complex of 30S subunit, IF-3 and mRNA bind with IF-2 and IF-1, and N-formylmethionine-tRNA. This step requires Mg^{2+} and GTP. IF-2 is required for the recognising and binding of N-formylmethionine-tRNA: IF-1 probably also participates, but IF-2 is the most important. You have studied that AUG is the initiation codon on mRNA. Therefore, the initiation tRNA (attached to N-formylmethionine, along with its UAC anticodon) binds to an AUG codon of mRNA. AUG and UUG rarely act as initiation codons of mRNA. (Fig 14.5a)

In the third step, the complex of 30S subunit, IF-3, mRNA, IF-2 containing bound GTP and N-formylmethionine-tRNA bind with the 50S ribosomal subunit. Simultaneously, the GTP molecule bound to IF-2 is hydrolysed to GDP and phosphate, and are set free. IF-3 and IF-2 are also released from the ribosome. Now the functional 70S ribosome is called **initiation complex** containing mRNA and the initiating N-formyl methionyl tRNA. The 70S ribosome has two binding sites for tRNA, the **aminoacyl** or **acceptor site (A-site)** and the **peptidyl** or **polymerisation site (P-site)**, also called the **donor site**. Each ribosome thus functionally accommodates two codons at a time. The P-site and the A-site are located in the 50S subunit of the ribosome. f-met-tRNA binds to the P-site. All other tRNAs first bind to the A-site and then shift to the P-site. The initiation complex is now ready for the steps of elongation (Fig. 14.5b).

In eukaryotes, the initiating amino acid for polypeptide chain synthesis is methionine. Methionine is transferred to its corresponding specific tRNA by an enzyme **methionine aminoacyl-tRNA synthetase**, forming methionine tRNA (met-tRNA). This activated methionine bound to its tRNA in the presence of GTP and the initiation factor-2 (eIF-2), forms a stable complex called **ternary complex**, which binds to the 40S subunit of the ribosome.



The mRNA next binds to the initiation complex. Four different protein factors; eIF-1, eIF-4A, eIF-4B, eIF-4E are required for the formation of the initiation complex. Later, the 60S ribosomal subunit binds to 40S **pre-initiation complex** to form an 80S initiation complex. At this stage, all the initiation factors are released and recycled.

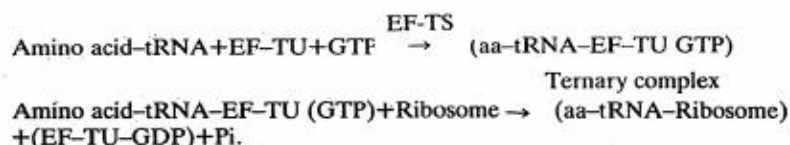
14.2.4 Chain Elongation

After the initiation, the polypeptide chain is lengthened by covalent attachment of successive amino acid units, each carried to the ribosome and put into its proper position by its corresponding tRNA (aminoacyl-tRNA is base-paired to its corresponding codon in the mRNA). Elongation involves the relative movement of

mRNA and the ribosome. The process of elongation consists of three steps and in prokaryotes the process requires,

- 1) initiation complex
- 2) the next amino acyl-tRNA, specified by the next coding triplet of mRNA,
- 3) a set of three soluble proteins of the cytosol called elongation factors, EF-TU, EF-TS, and EF-G and
- 4) GTP

You are already aware that in prokaryotes, formyl methionine is the first amino acid which binds to its corresponding specific tRNA. In the first step of the elongation process the next aminoacyl-tRNA binds to the A-site of the ribosome. This step requires GTP and elongation factors; EF-TU and EF-Ts. EF-TU is required to bring amino acyl-tRNAs to the A-site of the ribosome, whereas EF-Ts is needed to recycle EF-TU. In the presence of GTP, EF-Ts is released and then EF-TU. GTP then interacts with aminoacyl tRNA to form a ternary complex, which binds to ribosome



At this stage you will find that the first aminoacyl-tRNA^{fmet} gets attached at the P-site of the ribosome and the rest of the incoming aminoacyl-tRNA attaches at the A-site.

In the second step of chain elongation, a peptide bond is formed between the amino acids whose tRNAs are located at the A-site and P-site of the ribosome. This step occurs by the transfer of the initiating N-formyl methionine acyl group from its tRNA to the amino group of the new amino acid that has just entered the A site. This step is catalysed by peptidyl transferase and does not require any external source of energy or other factors. Now the tRNA present at the A-site contains two amino acids: the first amino acid (N-formyl methionine in prokaryotes and methionine in eukaryotes) and the second amino acid. Therefore it is called **dipeptidyl tRNA**. The empty tRNA at the P-site, whose amino acid is transferred to the tRNA at the P-site is now discharged from the P-site of the ribosome into the cytosol for recycling (Fig. 14.5c).

In the third step, after the bond formation, the ribosome moves along the mRNA strand, one codon at a time, from the initiation site (which is at or near the 5' end of mRNA) to the termination codon (which is located near the 3' end). The movement of the ribosome relative to mRNA is in the 5' → 3' direction, and is called **translocation**. As a result of the first translocation, the dipeptidyl tRNA present at the A-site shifts to the P-site. The A-site, therefore, becomes vacant to allow in the aminoacyl-tRNA. Further, at every step of translocation the new aminoacyl-tRNA occupies the A-site and forms a peptide bond with the amino acids at the P-site forming a polypeptide chain. The emptied tRNAs are discharged for recycling. The process is repeated, and elongation of the polypeptide chain is thus brought about by step-by-step addition of amino acids. Energy for translocation is supplied in the form of GTP.

In prokaryotes, translocation is brought about by **elongation factor G** (EF-G) (also called **translocase**). EF-G rapidly hydrolyses GTP to GDP + P_i. For each amino acid added to the chain, two GTPs are hydrolysed to GDP and P_i.

In eukaryotes, elongation factor, EF-2 corresponding to EF-G of prokaryotes, catalyses the transfer of peptidyl tRNA from the A-site to the P-site. GTP is hydrolysed to provide energy for translocation. When the ribosome moves about 80 nucleotides i.e. towards the 3' terminal of the mRNA, the same mRNA strand is now utilised by the second ribosome to synthesise an identical polypeptide chain.

In this way a series of ribosomes may associate with the mRNA to form a polyribosome. The number of ribosomes in a polyribosome depends upon the molecular weight of the polypeptide chain synthesised. For example, the polyribosome synthesising a haemoglobin subunit consists of about 150 amino acids and contains four to six ribosomes. The subunits of myosin are synthesised by a polyribosome of about 60 ribosomes. In eukaryotes, a mRNA strand functions simultaneously on several ribosomes. Therefore the number of mRNA required is much fewer than the number of polypeptide chains synthesised (Fig. 14.5 d.e.).

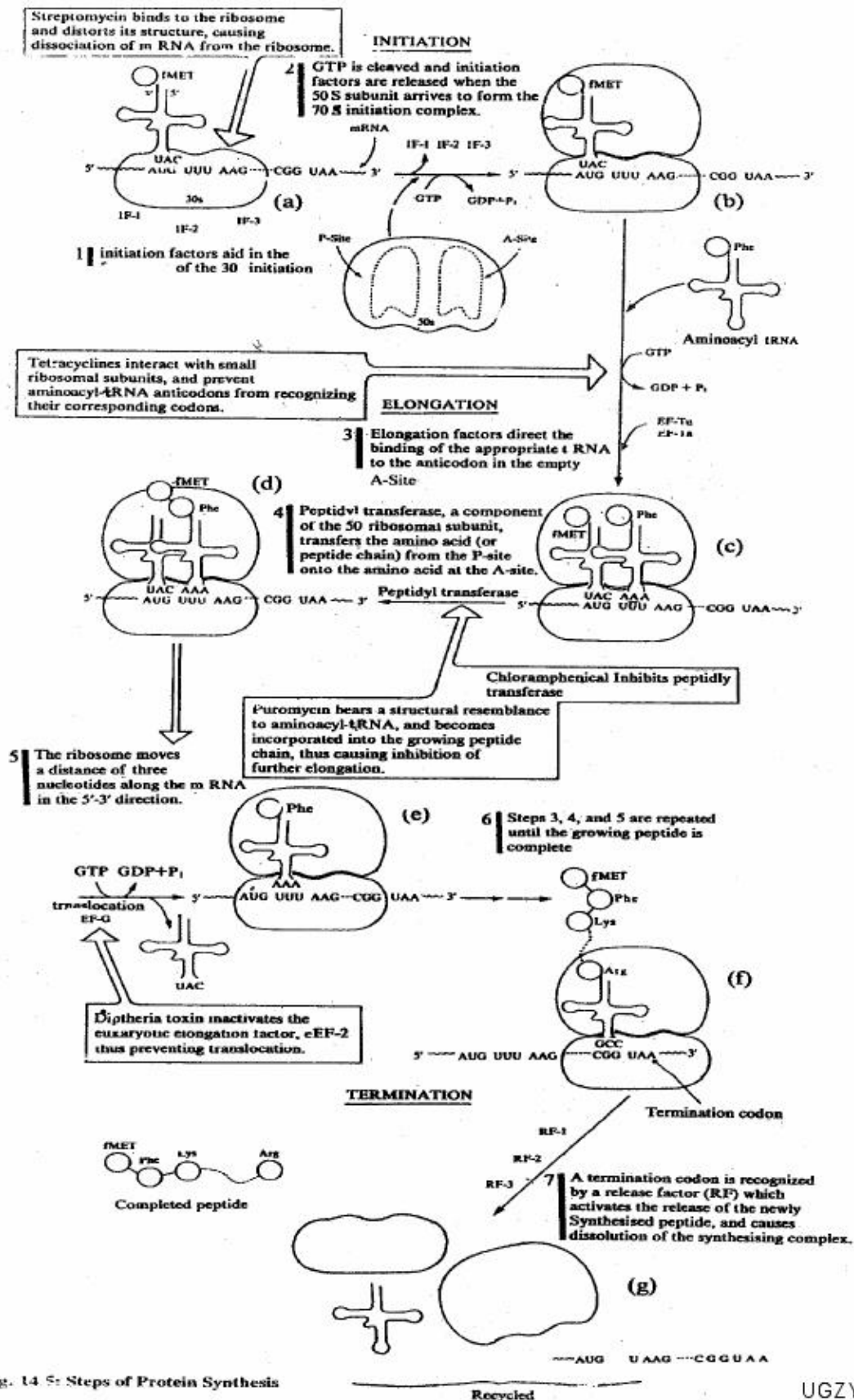


Fig. 14.5: Steps of Protein Synthesis

14.2.5 Termination of Polypeptide Chain

Till now you have studied about the synthesis of a polypeptide chain. In this section, we will study how the polypeptide chain synthesis is terminated.

Elongation of a polypeptide chain continues till the ribosome adds the last amino acid to the chain, coded by the mRNA. The termination of the polypeptide chain occurs when the 70S ribosome (in prokaryotes) carrying the peptidyl-tRNA, reaches the termination codon located at the end of the mRNA. The **termination codon** may be UAA, UAG or UGA. These are special stop signals that do not code for any amino acid and have been called '**nonsense codons**'. Ordinarily no tRNA anticodons pair with them. The termination codon need not be the last codon on the mRNA. In polygenic mRNAs, which are common in bacteria and bacteriophages, a number of initiation and termination codons are present. Each segment of the mRNA between the initiation and termination codons directs the synthesis of a separate polypeptide chain.

Chain termination leads to the release of the free polypeptide and tRNA and to the dissociation of the 70S ribosome into 50S and 30S subunits. These dissociated subunits are reutilised for the formation of new initiation complex. When a ribosome comes across a termination codon, a termination factor called '**release factor**' (RF) causes hydrolysis of the peptide bond between the completed polypeptide chain and the tRNA at the P-site. The residual tRNA is discharged from the P-site.

In prokaryotes, two different release factors (RF-1 and RF-2) with two different codon specificities have been reported. RF-1 is specific for UAA and UAG whereas RF-2 is specific for UAA and UGA termination codons. In addition to RF-1 and RF-2, another factor called RF-3 stimulates the activity of RF-1 and RF-2.

In eukaryotes, a single release factor (RF) recognises all the termination codons (UAA, UAG and UGA). The first step in chain termination is the binding of the RF to the A site containing a termination codon. This step requires GTP and activates the peptidyl transferase system. Hydrolysis of peptidyl-tRNA at the P-site results in the release of the polypeptide chain from the ribosome. GTP hydrolysis results in the dissociation of the RF from the ribosome and the released RF is recycled (Fig. 14.5 f.g).

As the synthesis of a polypeptide chain continues, it undergoes folding. Secondary bonds are formed between the folded regions. Thus the tertiary structure of the protein may be attained and the final native shape formed even before the terminal amino acids have been added. Nevertheless, proteins are known to undergo many post-translational modifications. In the next section (14.3) we will study how polypeptide chains are modified to form specific proteins.

14.2.6 Rate of Protein Synthesis

You have read in the previous section that protein synthesis is a complex process. Chemists can synthesise biologically active proteins in the laboratory, but the rate of synthesis is extremely slow with a low yield. However, in living cells proteins are synthesised at a much faster rate. For example in *E.coli*, mRNA for the enzymes controlling tryptophan biosynthesis is translated at the rate of about seven amino acids per second. Messenger RNA for haemoglobin synthesis consists of 670 nucleotides. It is translated in 35 seconds at the rate of about 4 amino acids per second.

It is evident from the above examples that the rate of protein biosynthesis varies from one individual to another. With the help of various techniques, the rate of protein synthesis can be determined; when cells are cultured without essential amino acids, glucose, or serum, there is a marked inhibition of the rate of polypeptide chain initiation due to inactivation of initiation factor IF-2. In immature red blood cells, activity of IF-2 is known to be reduced in a controlled way by phosphorylation of one of its three protein subunits. This suggests that the rate of eukaryote protein synthesis is controlled in part by specific protein kinases, by inhibiting the initiation of polypeptide chain synthesis.

The initiation factors required for protein synthesis are more numerous and complex in eukaryotes than in prokaryotes, even though they perform the same basic functions. In eukaryotes there are many regulatory proteins that control protein synthesis.

Fill in the blanks with suitable words.

- A i) The enzymes that activate binding of amino acids to their corresponding tRNA are called
- ii) In prokaryotes the P-site and the A-sites are located in the subunit of the ribosome
- During the initiation of polypeptide chain synthesis the first amino acid bound to its tRNA binds to the site of the ribosome whereas all other tRNA first bind to the site and then shift to the site
- iv) During the elongation of polypeptide chain, ribosomes move along with the mRNA, a codon at a time from the initiation site to the termination codon. This movement of the ribosome relative to mRNA in the 5' → 3' direction is called

B Explain briefly in the space given below the process of activation of amino acids.

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C What is the function of Initiation Factor-3? Explain briefly in the space given below.

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14.3 PROCESSING OF PROTEIN MOLECULE

In the previous section (14.2) you have studied how a polypeptide chain is synthesised in organisms. You have also read that a protein is not biologically active until it is in its specific three dimensional native form. The three dimensional structure of a protein is ultimately determined by its amino acid sequence. A polypeptide chain assumes its three dimensional structure either during synthesis or after synthesis. However, the newly formed polypeptide chain often does not attain its final biologically active conformation until it has been subjected to **processing** or covalent modifications, known as **post-translational modifications**. Several post-translational modifications are known to take place, depending upon the type of protein. These are:

- 1) phosphorylation
- 2) methylation
- 3) nucleotidation
- 4) attachment of carbohydrate side chain,
- 5) addition of prosthetic group, and
- 6) formation of disulphide bonds.

The modifications listed above are catalysed by specific enzymes. For example, phosphorylation of proteins is catalysed by a group of enzymes called "protein kinases". These enzymes transfer terminal phosphate of ATP to amino acid residues of the proteins. As many as 22 enzymes are known to get phosphorylated and as a result their activities are altered. In addition many structural proteins are also phosphorylated.

There is another process known as proteolysis in which some inactive enzymes are activated due to removal of a peptide fragment(s). For example, inactive trypsinogen is converted into active trypsin by removal of a peptide fragment. The other examples are:

Prothrombin → thrombin
Proalbumin → albumin, and
Proinsulin → insulin.

This proteolytic fragmentation leads to production of the final three dimensional structure of the active protein.

Another similar example is the synthesis of an inactive form of collagen that undergoes post-translational modification to become a functional collagen molecule.

The collagens are a family of highly specific, fibrous proteins, found in all multicellular organisms. They are the most abundant proteins in mammals, constituting 25% of their total protein. Collagens are the major fibrous structured elements of cartilage, tendons, skin, bone, lung and blood vessels. These are synthesised by **fibroblasts**, located in the interstitial spaces of the cells. The collagens combine with hydrated polysaccharides to form glycosaminoglycans that constitute the extracellular matrix in the multicellular organisms. Collagen fibres are embedded in the glycosaminoglycans providing strength and help to organise the matrix. Rope-like organisation of the collagens in tendons gives enormous tensile strength. Tendons consist almost entirely of collagen fibres. They usually attach muscles to the bone. In Block I and II you have already studied about the types of collagen and their properties.

In the previous sections you have studied how proteins are synthesised in the cytosol of the organisms. It is interesting to know that all the proteins are not synthesised in the cytosol. The **secretory proteins** are synthesised in the rough endoplasmic reticulum. The polypeptide chain formed is released into the lumen of the endoplasmic reticulum after their synthesis. From here, secretory proteins are transported to the Golgi complex and lysosomes. They are then secreted from the cell by a process known as exocytosis.

Blobel and his colleagues in 1975 proposed a hypothesis known as “**signal hypothesis**”, regarding the synthesis of proteins in the endoplasmic reticulum. This hypothesis was later experimentally investigated in the synthesis of immunoglobins (antibodies). It is discovered that the mRNA coding for secretory proteins contains a group of codons known as **signal codons**, located at the 3' side of the initiation codon, AUG. These signal codons are translated into **signal peptides**. These are located at the extreme amino terminus of the growing immunoglobulin polypeptide. The membrane of endoplasmic reticulum has receptors that recognise proteins containing signal peptides. As a result the ribosomes containing signal peptides are attracted towards the endoplasmic reticulum. The cells also contain a protein called **signal recognition protein**, present in the cytosol. This protein binds to the ribosome shortly after it has synthesised the **signal peptide** and halts further protein synthesis by the ribosome until it has become linked to the rough endoplasmic reticulum. The signal peptide interacts with the receptor proteins of the endoplasmic reticulum forming a tunnel in the membrane which coincides with the ribosomal tunnel. After entering the tunnel of the endoplasmic reticulum, the signal peptide is cleaved from the

Fibroblasts are flattened connective tissue cells of irregular form, believed to be responsible for the secretion of white fibres of the muscles, lamellar cells and fibrocytes.

Proteins which are secreted from the cell after their synthesis to different parts of the body are known as secretory proteins e.g. hormones secreted by pancreas, pituitary etc.

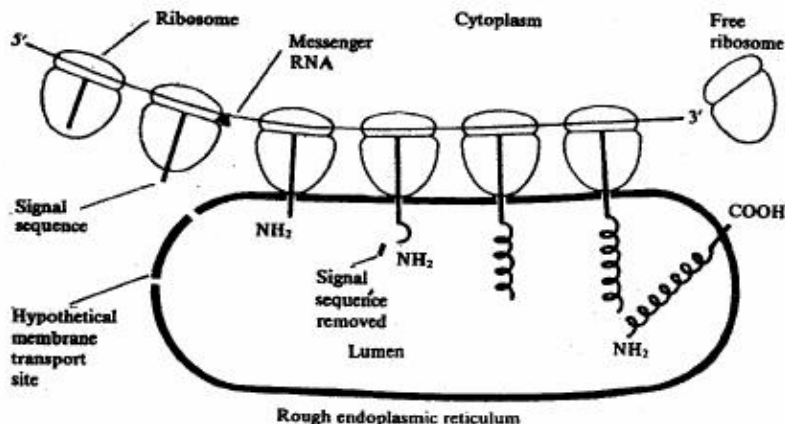


Fig. 14.6 Schematic view of the signal peptide hypothesis.

growing polypeptide by an enzyme called **signal peptidase**. After the completion of polypeptide chain synthesis, it is released into the space of the endoplasmic reticulum. The ribosome detaches from the endoplasmic reticulum and the ribosome receptor proteins diffuse into the plane of the membrane closing the tunnel (Fig. 14.6).

The signal theory also applies to the secretory proteins produced by plant cells, such as zein from corn, chymotrypsin inhibitor from tomato leaves and seed reserve protein from the common beans.

Collagens are a good example of secretory proteins. They are also synthesised in the endoplasmic reticulum. After synthesis they are released into the lumen of the endoplasmic reticulum as large precursors, called **Pro- α -Chains**. These precursors have not only the 'signal peptide' sequence as in other secretory proteins, but also other extra amino acids, called **extension peptides**, present at both amino and carboxyl ends of the polypeptide chain. In the lumen of the endoplasmic reticulum the pro- α -chains undergo post-translational modifications. Now let us study what post-translational changes a precursor collagen undergoes. At the first post-translational change, both the proline and lysine residues of the polypeptide are hydroxylated. Soon after the hydroxylation, three pro- α -chains combine to form a triple-stranded helical molecule. Each pro- α -chain combines with the other by hydrogen bonding. It has been found that the hydroxyl group of hydroxyproline forms interchain hydrogen bonds that help to stabilise the triple helix.

The second post-translational modification is the glycosylation of pro-collagen molecule. At this stage, oligosaccharide side chains of the pro-collagen molecules are covalently attached to the hydroxyl group of the hydroxylysine. The hydroxylysine residues are important as they are useful not only for glycosylation, but also for the extensive cross linking of collagen molecules that occurs in the extracellular space after its secretion.

The third post-translational modification occurs during the process of secretion. At this stage, the extension peptides of the pro-collagen molecules are removed by specific proteolytic enzymes called **pro-collagen peptidases**. This converts the pro-collagen molecule to collagen molecules. As stated earlier, this step of post-translational modification, i.e. cleavage of the extension peptides occurs after the molecules are secreted into the interstitial spaces. In the interstitial space, the collagen molecules undergo polymerisation to form collagen fibrils.

Fibroblasts modify the properties of the extracellular matrix both by synthesising different types of collagen peptides and by differentially modifying the chains to influence the type and strength of packing. Fig. 14.7 illustrates various intracellular and extracellular events involved in the formation of a collagen fibril.

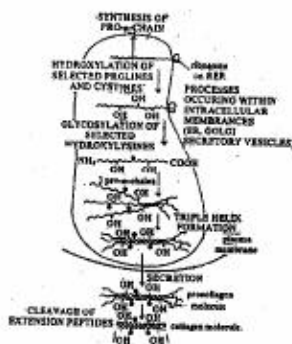


Fig. 14.7: Schematic drawing of the various intracellular and extracellular events involved in the formation of a collagen fibril.

SAQ 2

Fill in the blanks with suitable words

- Signal hypothesis was proposed by
 - Signal codons are located at the.....end of the initiation codon (AUG) on the mRNA coding for secretory proteins
- What are the several kinds of post-translational modifications that enable a protein to achieve its final biologically active conformation
 -
 -
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- What is the function of the enzyme signal peptidase? Answer briefly in the space given below

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14.4 REGULATION OF PROTEIN SYNTHESIS

In the previous section you have studied how proteins are synthesised in the organisms. You have learnt that DNA determines the primary structure of the proteins. In a DNA molecule there are varying numbers of nucleotide sequences, each of them determines a polypeptide chain. A sequence of nucleotides that specifies the amino sequence of a polypeptide chain is known as a **gene**. *E.coli* contains 2000-4000 genes that code for 3000 different proteins in different amounts.

There are two types of genes present in a DNA molecule. They are **constitutive genes** and **regulated genes**. A constitutive gene is expressed continuously, so that its product is invariably found in the cell at roughly the same concentration. Regulated genes on the other hand, do not function at all the times. Their expression is switched on and switched off as per the need of their products in the cellular or extracellular environment. The products of the constitutive genes are the enzymes involved in glucose metabolism.

Glucose metabolism is an important process for the survival of the organism. Therefore this should be continuously functional. Products of regulated genes are enzymes that metabolise less common sugars such as lactose, arabinose and galactose and also enzymes for the biosynthesis of amino acids, purines, pyrimidines and so on. Since these enzymes are not needed all the time, as the enzymes of glycolysis are synthesised according to the demand for them. The expression of regulated genes is regulated at various levels. It is regulated at the transcriptional level, the translation level and also at the post-translational level. Now we will study each of these regulatory mechanisms in detail.

14.4.1 At Transcriptional Level

You have studied in Unit 13 that synthesis of mRNA is known as **transcription**. The messenger RNA transcribed, is complementary to the DNA and it contains the DNA message for the synthesis of proteins. The process of transcription is under the control of the messenger RNA, which is transcribed only when required. Regulation of gene activity is better understood in prokaryotes than in eukaryotes.

In prokaryotes, biosynthesis of proteins is regulated at the transcriptional level by specific **controlling factors**. You have studied in Unit 10 that an operon contains a regulator gene, a promoter gene, an operator gene and structural genes. The promoter gene and the operator gene are the controlling factors. These genes respond to the environmental signals in either allow or disallow the synthesis of mRNA from the structural genes.

RNA polymerase, the enzyme that catalyses synthesis of mRNA, binds to the promoter and initiates transcription of the structural genes. Operator is the site for the binding of a regulatory protein. You have studied in Unit 10 that to stop transcription of structural genes, the regulator gene transcribes a mRNA, which is translated to form a regulatory protein. The regulatory protein binds at the operator site, blocking the movement of RNA polymerase, thereby, preventing transcription of structural genes.

The transcription which is stopped by the binding of a **regulatory protein** at the operator site, starts with the activation of molecules known as **inducer** or **effector molecules**. The effector molecules are basically the substrate of the enzymes to be coded by the structural genes. These effector molecules bind with regulator proteins thereby changing their ability to form a complex with the operator. Here, once again, transcription of structural genes begins.

One of the most intensively studied enzyme system is that of lactose metabolism. Studies have shown that in the **lactose operon** (*Lac Operon*), the substrate lactose does not function directly as the effector molecule. In the cytoplasm, lactose is acted upon by the enzyme β -galactosidase, transforming lactose into **allolactose**. Allolactose is the actual effector molecule. It binds to the repressor protein at the allosteric site. Allolactose interacts directly with the regulator protein allowing transcription of *lac* genes.

There are two types of operons found in prokaryotes: i) **inducible operons**, ii) **repressive operons**. In inducible operons transcription of structural genes do not

The *lac Operon* has three structural genes; *lac Z* or *z* gene, *lac Y* or *y* gene and *lac A* or *a* gene. *lac z* codes for the enzyme galactosidase, a lactose splitting enzyme. *lac Y* codes for β galactoside permease, an enzyme involved in concentrating lactose in the cell, *lac A* codes for β galactose acetyltransferase. This enzyme is believed to protect the cell from the toxic effects of the enzyme - galactosidase.

occur unless there are effector molecules in the cell. Operons of lactose, galactose and arabinose represent inducible operons. Their effector molecules are lactose, galactose and arabinose respectively.

On the other hand in repressible operons, full levels of transcription occur until such time as their effectors reach a critical concentration, at which point transcription is inhibited. Operons of tryptophan, histidine and arginine represent repressible operons. Their effector molecules are tryptophan, histidine and arginine respectively.

Till now you were studying the control of protein biosynthesis in prokaryotes. In eukaryotes, regulation of transcription takes place by a different mechanism. In Block I and II you have studied that in eukaryotic chromosomes, DNA is associated with proteins, called **histones**, and **non-histone chromosomal proteins**. These proteins are known to regulate the activity. Several models have been proposed to explain gene regulation by chromosomal proteins. According to the Frenster's model (1965), histones stabilise the DNA double helix by interacting with negative phosphate groups of DNA. This prevents separation of the strands and, consequently transcription. Thus histones act as general repressors of protein synthesis. According to this model, there are gene-specific **derepressor DNA** which interact with one of the non-transcribing DNA strands at a particular locus permitting the other strand to transcribe mRNA. Fig. 14.8 clearly illustrates the function of derepressor RNA.

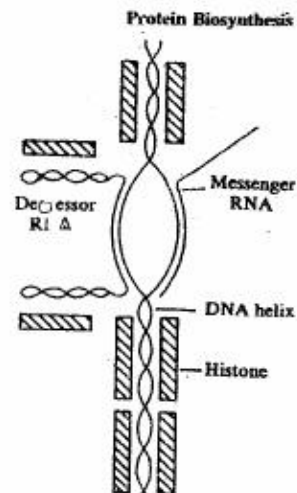


Fig. 14.8: Frenster's model of gene regulation

Paul and co-workers (1971) are of the opinion that non-histone chromosomal proteins probably act as non-specific repressors of genetic functions. They recognise certain sites of DNA and bind to it, pulling off the histone facilitating transcriptions (Fig. 14.9).

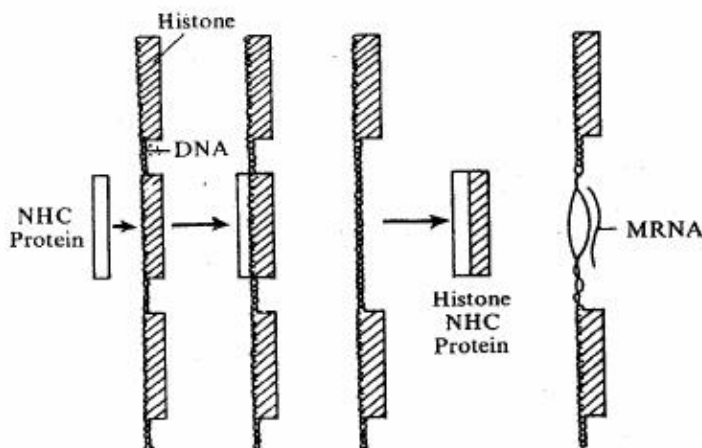


Fig. 14.9: Paul and co-worker's model of gene regulation by removal of histones.

You know that eukaryotes are multicellular organisms. They have special signalling cells that detect changes in the external as well as internal environment and send signals for the alteration of protein biosynthesis. The "signalling cells" are the **endocrine cells**. These endocrine cells secrete hormones, and they regulate gene activity. In mammals, steroid hormones enter their target cells and bind with specific receptor proteins. Binding of the receptor to the steroid changes the shape of the receptor. The steroid-receptor complex enters the nucleus and stimulates transcription of specific genes.

In eukaryotes, steroids act as effectors and their receptor proteins as regulator molecules, becoming active only when bound to the effector. In insects **ecdysone** (a steroid hormone) switches on the transcription of particular genes. Experiments have shown that the DNA of larval chromosomes uncoil within the minutes of ecdysone treatment, facilitating transcription. The uncoiled parts of the chromosome look like puffs and are known as **chromosomal puffs** or **Balbiani rings** (Fig. 14.10).

14.4.2 At Translational Level

In the previous section, control of protein biosynthesis at transcriptional level was discussed. Biosynthesis of proteins is also regulated at translational level.

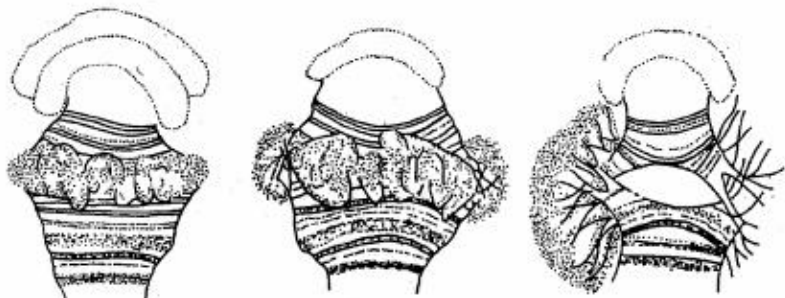


Fig. 14.10: Puffing of specific sites in giant salivary gland chromosomes. One of the three large puffs (Balbiani's rings) of a salivary gland chromosome of *Chironomus*. Three different stages of puffing are shown.

In prokaryotes, translational control is of lesser importance than transcriptional control, because their mRNAs are unstable. The life of prokaryotic mRNA is only about two minutes, therefore there is hardly any time for regulating the activity. In eukaryotes, the process of translation takes place in the cytoplasm. The mRNA transcribed are processed in the nucleus before their message is translated. Processing of mRNA is also one of the methods of regulation of gene activity. During mRNA processing, genes which are not of importance and which are not of use at the moment are spliced. You have already studied the processing of mRNA in Unit 13.

In prokaryotes genes that are transcribed last appear to be translated at a lower rate than the genes transcribed first. For example the three structural genes of the *lac* operon are transcribed roughly in ratios of 10:5:2. This is due to the polarity of the translation process, i.e. translation of the genetic code moves in the 5' to 3' direction. Therefore, genes located at the beginning of the operon will be available for translation before the genes at the end.

Translational control is also exerted by the efficiency with which the mRNA is bound to the ribosome. Different sequences in mRNA have different efficiencies of binding with ribosomes, and will be translated at different rates. The genetic code also plays a part in the translational control of some proteins, since different tRNAs occur in the cell in different quantities. Genes that cause the production of a large amount of protein products may have codons that specify the more common tRNAs. Genes that code for proteins not needed in abundance, have several codons specifying the rarer tRNAs, which would slow down the process of translation of these genes.

In eukaryotes translation of mRNA is regulated in the following three ways:

- (1) Control of the life period of the mRNA;
- (2) Control at the initiation of translation; and
- (3) By regulating the rate of overall protein synthesis.

(1) An average eukaryotic mRNA molecule has a life of about three hours, before it is degraded. However, some mRNA survive for several days during which each mRNA molecule is translated repeatedly to yield large amounts of proteins. Production of a large amount of a single type of protein by prolonging the life of an mRNA is common in highly differentiated cells. For example, cells of the chick oviduct, which make ovalbumin (for egg white) and fibroin mRNA of silk glands of the silk worm, which synthesises a single type of protein, the silk fibroin. This mode of protein production is called **gene amplification** or **translational amplification**.

(2) In some eukaryotes translation is regulated by controlling its initiation. For example, unfertilised sea urchin eggs store large quantities of mRNA. The mRNA remain inert, without being translated. Translation begins immediately after fertilisation.

(3) Translation of mRNA is also kept under control by regulating the overall rate of protein synthesis. For example production of haemoglobin is regulated in this way. Haemoglobin is synthesised in cells called reticulocytes. Reticulocytes are the penultimate cell type in the line of differentiation leading to red blood cells. In these reticulocytes, there is one molecule of hemin per globin subunit. But for optimal efficiency, globin molecule should be synthesised at twice the rate of hemin synthesis. To maintain this ratio, a two phase regulatory system operates in the cells.

- i) Hemin represses its own synthesis, and
- ii) Hemin inactivates an inhibitor of globin synthesis.

With this system, if there is more hemin than globin, hemin synthesis is shut off and globin synthesis is stimulated. On the other hand, if there is not enough hemin, globin synthesis is repressed and hemin synthesis is switched on. In this way, the amount of hemin and globin are kept in balance.

In plants, gene expression is also regulated by light. The cells of many higher plants lose their chlorophyll resulting in the loss of their green colour, when they are kept in darkness for several days. This is due to the loss of production of enzymes that catalyse chlorophyll synthesis. When such plants are exposed to light again, within few hours synthesis of photosynthetic enzymes and chlorophyll starts.

14.4.3 At Post-translational Level

In the previous sections you have studied that synthesis of proteins is regulated at transcription and at translational levels. A cell not only has an ability to stop transcription, and hence translation, it can also prevent the enzymes already translated. Control at post-translational levels is necessary to regulate the functioning of the enzymes in order to maintain the internal environment of the organisms in a dynamic steady state. In Unit 10 you have already studied various mechanisms, by which the activities of the enzymes are regulated.

14.4.4 Chemical Inhibitors of Protein Synthesis

In the previous sections you have read about regulation of protein biosynthesis at transcription, translation and at the post-translational levels. All these mechanisms are endogenous, operating in the cell itself. Protein biosynthesis can also be regulated exogenously by using certain drugs/chemicals.

Many antibiotics inhibit prokaryotic protein synthesis. This results in growth arrest or death of the bacterium. Most antibiotics do not interact with the specific proteins of eukaryotes and thus are not toxic to eukaryotes. Antibiotics are hence exploited for clinical purposes. Puromycin, an antibiotic is a structural analog of tyrosinyl-tRNA. It is incorporated in bacterial protein synthesis via the A-site on the ribosome, into the carboxyl terminal position of a peptide causing premature release of the polypeptide.

Many antibiotics are not clinically useful but have helped elucidate the role of protein synthesis in the regulation of metabolic processes, particularly enzyme induction by hormones. Table 14.2 and Fig. 14.5 summarise a few antibiotics that inhibit translation of protein biosynthesis in prokaryotes.

Table 14.2: Antibiotic Inhibitors of Translation

	Eukaryotes (Cytoplasm)	Eukaryotes (Mitochondria)	Prokaryotes
Initiation			
Aurintricarboxylic acid			
Elongation			
Amicetin	?	?	+
Anisomycin	-	?	+
Chloramphenicol	-	+	+
Cycloheximide	+	-	-
Fusidic acid	?	?	+
Lincocin	-	?	+
Puromycin	+	+	+
Sparsomycin	+	+	+
Tetracyclines	-	+	+
Termination			
Anisomycin	?	?	*
Amicetin	?	?	+
Erythromycin	-	+	+
Lincocin	?	?	*
Sparsomycin	+	+	+
Streptomycin	+	±	+

+ = inhibition; - = no inhibition; * = stimulation; ? = unknown.

SAQ 3

A Match the terms in columns A with their definition in column B.

A	B
1) Structural genes	i) are expressed continuously so their products are invariably found in the cell at roughly the same concentration. Example enzymes in glucose metabolism
2) Regulated genes	ii) do not function all the time. Their expression is switched on and switched off as per the need of their products in the cellular or extracellular environment. Example, enzymes involved in the metabolism of less common sugars amino acids and nucleic acids
3) Constitutive genes	iii) A gene encoding the amino acid sequence of a polypeptide chain

B Fill in the blanks with suitable words.

- treatment cause uncoding of the chromosomes. The uncoiled parts of the chromosome look like puffs and are known as
- Many antibiotics inhibit prokaryotic protein synthesis. This results in or..... of the bacterium.

C What is meant by translational amplification? Explain briefly in the space given below.

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14.5 SUMMARY

In this unit you have studied the various steps involved in the biosynthesis of proteins in prokaryotes and in eukaryotes. You have also studied the processing of the precursor proteins in eukaryotes and also about various methods that regulate synthesis of proteins in the organisms. We now summarise what we have learnt in this unit.

- Protein synthesis is a very complex process, involving participation of a large number of enzymes and other specific biomolecules.
- The mRNA has a large number of triplet bases, each triplet forming a codon. The codons pair with anticodons of the tRNA. This pairing follows the A-U, G-C combination.
- The translation process consists of activation of amino acids, transfer of activated amino acids to tRNA, initiation of polypeptide chain synthesis, chain elongation and chain termination.
- The rate of protein synthesis varies from one individual to another. In eukaryotes specific protein kinases control the rate of protein synthesis by inhibiting the initiation of polypeptide chain synthesis.

- Most of the proteins synthesised are not biologically active. They undergo various covalent modifications to attain biologically active forms.
- Biosynthesis of proteins is regulated at the transcriptional level, translational level and even after translation. There are some antibiotics, which inhibit prokaryotic protein synthesis.

14.6 TERMINAL QUESTIONS

- 1 What constitute an initiation complex in prokaryotes and in eukaryotes? Discuss briefly in the space given below.

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- 2 Explain the function of Release Factors RF-1, RF-2 and RF-3 in prokaryotes.

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- 3 Briefly explain the processing of the precursor polypeptides of the collagen.

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- 4 Explain briefly the role of histones in regulation of gene expression.

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List a few antibiotics that inhibit translation of protein biosynthesis in bacteria.

- 1)
- 2)
- 3)

14.7 ANSWERS

SAQs

- A. i) amino acid-tRNA-synthetase ii) 50S
 iii) P, A, P iv) Translocation
- B. For answer please see Section 4.2.1.
- C. IF-3 prevents recombining of 30S and 50S subunits of the ribosomes.

2 A. i) Blobel and his colleagues in 1975

ii) 5'

B. i) Phosphorylation

iii) Nucleotidation

ii) Methylation

iv) Attachment of carbohydrate side chain.

v) Addition of prosthetic group

vi) Formation of disulphide cross links.

C. Signal peptidase cleaves the signal peptide from the growing polypeptide chain after it enters the tunnel of the endoplasmic reticulum.

3 A

A	B
1)	iii)
2)	ii)
3)	i)

B. i) Ecdysone, Balbiani Rings

ii) growth arrest, death

C Refer to page 96 for the answer.

Terminal Questions

1 In prokaryotes, the initiation complex consists of 70S ribosome — f methionine — tRNA — mRNA. In eukaryotes it consists of 80S ribosome — methionine — tRNA — mRNA.

2 Release factors (RF) cause hydrolysis of the peptide bond between the completed polypeptide chains and tRNA. As a result, the polypeptide chain is released. There are three types of release factors; RF-1, RF-2 and RF-3. RF-1 is specific for the termination codon UAA and UAG. RF-2 is specific for UAA and UGA. RF-3 stimulates the activity of RF-1 and RF-2.

3 Refer to page 93 for the answer.

4 Refer to page 95 for the answer.

5 Refer to Table 14.2 for the answer.

UNIT 15 CHEMICAL SIGNALLING BETWEEN CELLS

Structure

- 15.1 Introduction
 - Objectives
- 15.2 Strategies of Chemical Signalling
 - Local Chemical Mediators
 - Hormones
 - Neurotransmitters
- 15.3 Solubility of Signalling Chemicals
- 15.4 Signalling Mediated by Intracellular Receptors
- 15.5 Signalling Mediated by Cell-surface Receptors
 - Cyclic AMP as Second Messenger
 - Ca^{2+} ions as Second Messenger
- 15.6 Target Cell Adaptation
- 15.7 Bacterial Chemotaxis
- 15.8 Summary
- 15.9 Terminal Questions
- 15.10 Answers

15.1 INTRODUCTION

Each cell in a multicellular organism is in constant communication with its neighbouring cells and often with cells some distance away. This ability of intercellular communication is essential for development of animal and plant cells, their organisation into tissues, control of their growth and division and coordination of diverse activities. Communication between cells may be direct or indirect. Direct communications may be cell-to-cell contact through the gap junctions or through plasma membrane bound signalling molecules. Indirect communication is through cell secretions which influence distantly located target cells.

In the previous unit, you have read about protein synthesis and its regulation at different levels. In this unit we will discuss the strategies of chemical signalling between cells, explain the role of intracellular receptors and cell surface receptors and show how target cell adaptation is reversible desensitisation to a chemical signal.

Objectives

After reading this unit you should be able to :

- list the strategies for chemical signalling between cells and relate these to the properties of the signalling molecules by giving examples,
- identify the types of signalling molecules on the basis of their solubility and their mechanism of action on the target cells,
- explain the role of intracellular receptors and cell surface receptors in the target cells,
- discuss the role of the second messengers; cyclic AMP and Ca^{2+} ions in signalling mediation,
- explain by giving examples how a target cell reversibly adapts itself to a chemical signal.

15.2 STRATEGIES OF CHEMICAL SIGNALLING

Communication between the cells is essential as it coordinates the growth, differentiation, and metabolic activities of the cells in diverse tissues and organs that make up an organism. Cells use a large variety of chemical substances and signalling mechanisms to communicate with each other.

Communication between cells may be direct or indirect. **Cell-to-cell** contact through gap junctions and **plasma membrane bound signalling molecules** that influence the **target cells** by making physical contact with these cells are some examples of **direct**

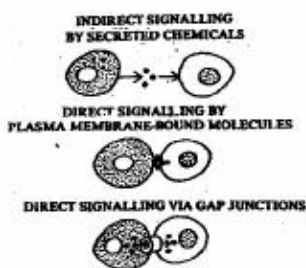


Fig. 15.1: Three different ways in which cells communicate with each other.

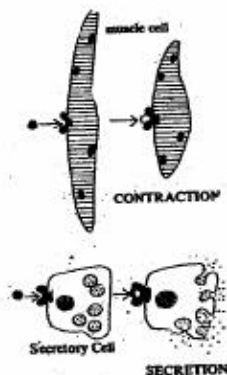


Fig. 15.2: Binding of same signal molecules to identical receptors on two different target cells can induce different responses.

communication. Indirect communication takes place through cell secretions that signal the cells some distance away to undertake some particular activity inside or at the surface of signal receiving cells also called as target cells (see Fig. 15.1).

Gap junctions are the regions of the interacting plasma membranes of the adjacent cells, aligned to form channels which permit ions and small molecules such as amino acids, vitamins, sugars and nucleotides to move freely from one interacting cell to its adjacent neighbouring cell. These are also called **communicating junctions**. We will discuss cell junctions in detail in Unit 18.

Cell communication through direct interaction of molecules bound to the plasma membrane of the cell are thought to differ from indirect communication in that the signalling molecules are membrane bound. As membrane-bound molecules cannot be easily purified, mechanism of their action is difficult to study.

Indirect communication is mediated by secreted chemical signals which interact with specific target cells nearby or at some distance away in the body. The target cells are able to respond to the signalling molecules because they have specific proteins, called **receptors** that recognise and bind the external signalling molecules. Chemicals signals influence the target cells either by initiating the synthesis of new proteins or by altering the properties or the rate of synthesis of the proteins existing already. Different target cells may respond differently to the same chemical signal. For example, acetylcholine stimulates the contraction of skeletal muscle cells but it decreases the contraction rate of heart muscle cells.

Another example is of hormone thyroxine that induces cells in the tail of a tadpole to break down by releasing lysosomal enzymes during metamorphosis. On the other hand it induces mitosis and division in particular cells in muscle and cartilage tissues as the animal changes to adult form. In some cases different target cells with even identical receptor proteins respond differently to the same chemical signals (see Fig. 15.2).

Intercellular communication is not found in plant tissues as in animals. However, plant cells are connected to each other by delicate cytoplasmic channels which pass through the cell walls, called **plasmodesmata**. These channels allow small molecules, metabolites and ions to pass from one cell to another.

Intercellular chemical signalling operates through three ways : local chemical mediators, hormones and neurotransmitters. We will discuss these in the following subsections.

15.2.1 Local Chemical Mediators

Local chemical mediators are signalling molecules that are secreted by cells into the extracellular fluid and act only on target cells in their immediate environment. It is because these molecules are either very rapidly taken up by the target cell or destroyed (see Fig. 15.3).

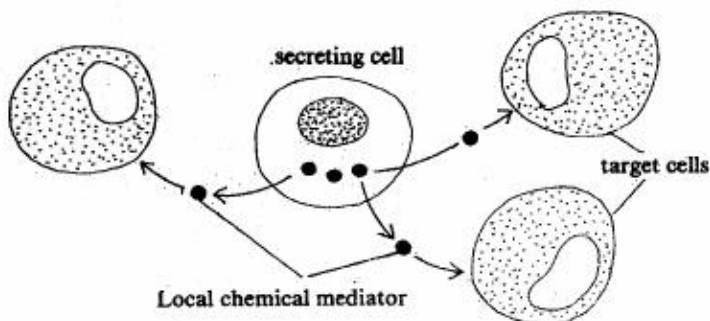


Fig. 15.3 : Chemical signalling by local chemical mediators

Histamine is a derivative of amino acid histidine. Prostaglandins are a family of fatty acids.

Histamine, prostaglandins and neuroregulators are few examples of local chemical mediators. **Histamine** is secreted by exocytosis from the **mast cells** in connective tissue. This secretion is stimulated by injury, local infection or allergic reactions.

Histamine causes local blood vessels of the affected area in the body to dilate and become more permeable. This facilitates the passage of antibodies, white blood cells etc. from the permeating blood vessels to the site of injury

Prostaglandins are important local mediators secreted by a wide variety of cells. They are continuously synthesised in membranes, released to the cell exterior and are continuously degraded. Prostaglandins are never stored. There are more than 16 prostaglandins which act locally to induce a variety of biological effects. For example, particular prostaglandins are produced in the uterus during childbirth and cause contractions of uterine smooth muscles that are required for delivery of the child. Pregnant women are advised not to use aspirin because it inhibits prostaglandin synthesis. Other types of prostaglandins may cause aggregation of platelets and inflammation.

Some local chemical mediators, also called **neuroregulators**, are released from nerve endings and diffuse locally to influence a large number of cells in the immediate environment. It is thought that such type of local chemical mediator induces sleep mechanism which requires the suppression of a large number of specific nerve cells in a particular region of the brain.

15.2.2 Hormones

Hormones are chemical messengers secreted by specialised endocrine cells into the blood, which can regulate the activity of other cells elsewhere in the body (see Fig. 15.4). You have read about hormones in Unit 10 of Block 2 also.

Some hormones induce a rapid and transient response while the response of others is slow in the onset but longer lasting thereafter. How fast a hormone acts depends on its solubility in water. **Water-soluble hormones** are broken down within minutes of entering the blood as are local chemical mediators and neurotransmitters.

Consequently, the responses are quick and of a short duration. **Water-insoluble hormones** persist in the blood stream and tissues and therefore, tend to mediate longer lasting responses. You will read about solubility of chemical signals in Section 15.3.

An example of water-soluble hormone is **insulin** secreted by beta cells of pancreas. An increase in blood-glucose level stimulates the secretion of insulin into the blood. Within minutes the resulting increase in concentration of insulin stimulates liver and muscle cells to take up more glucose and the blood-glucose level falls. Insulin secretion, and consequently the rate of glucose uptake by liver and muscle cells, returns to the previous level. In this way, a relatively constant blood-glucose level is maintained. Insulin also stimulates protein synthesis and in fat cells, the lipid synthesis.

Steroid **estradiol**, a female sex hormone is an example of water-insoluble slow-acting hormone. It is secreted in large quantities by ovarian cells at the time of puberty. Its major effects are the development and maintenance of secondary female sex characteristics, maturation and cyclic function of accessory sex organs and the development of the duct system in mammary glands.

15.2.3 Neurotransmitters

Neurotransmitters are secreted by nerve cells that form specialised junctions called chemical synapses with the target cell they influence. Neurotransmitters act only on adjoining target cells (see Fig. 15.5).

Neurotransmitters if not utilised for signalling are either rapidly destroyed by specific enzymes in the synaptic cleft or rapidly withdrawn by the nerve terminals that release them. They are different from neuroregulators in that the latter are local chemical mediators and diffuse into the extracellular fluid to influence a large number of local cells in the immediate environment whereas the former have precise target cells. That is, neurotransmitters influence only the adjacent target cell and act either by stimulation or inhibition.

Acetylcholine is a neurotransmitter secreted by the nerve endings at a neuromuscular junction, i.e. synapse formed between nerve cell and muscular cell. It is released in the synaptic cleft and stimulates the contraction of skeletal muscle cells.

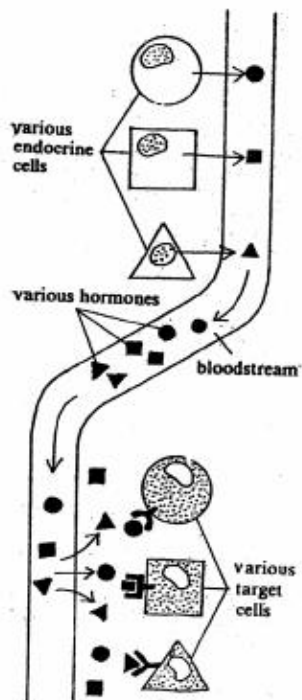


Fig. 15.4: Signalling of target cells by hormones

Synapse is the intracellular gap or junction between two nerve cells or a nerve cell and a muscle cell. The two cells do not touch each other and the gap is bridged by neurotransmitter molecules. The distance between a nerve cell and a target cell is about 10-20 nm and the gap is called synaptic cleft.

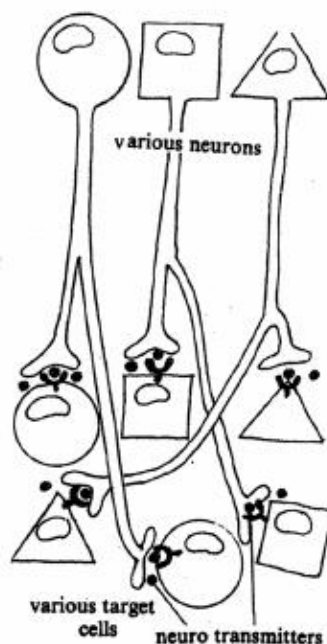


Fig 15.5 : Signalling by neurotransmitters secreted by nerve cells which act on adjoining target cells

Acetylcholine and another neurotransmitter, norepinephrine also have an excitatory effect on central nervous system and an inhibitory effect on the peripheral nervous system.

Various aminoacids, too, act as neurotransmitters, such as glycine; an inhibitory transmitter in central nervous system. Endocrine cells and nerve cells work together to coordinate various activities of the cells in higher animals. However, unlike endocrine cells, nerve cells transmit information much more rapidly as they do not depend on diffusion or blood flow to convey information at long distances. Signals are carried in the form of electrical impulses along the nerve processes and are converted into chemical signals in the form of neurotransmitters which are released at the nerve endings.

There are no specialised endocrine glands to secrete into circulatory system in plants. However, some plant cells do synthesise growth substances that influence the growth and differentiation of other parts of the plant. As mentioned earlier, in plants plasmodesmata is the communicating link between the cells.

Some examples of the three types of signalling molecules along with their site of origin and major effects are given in Table 15.1.

Table 15.1: Some Examples of Chemical Signals

	Signal Molecules	Site of Origin	Major Effects
Local Chemical Mediators	Nerve Growth factor	All tissues innervated by sympathetic nerves	Survival and growth of sensory and sympathetic neuron
	Histamine	Mast cells	Causes blood vessels to dilate and become leaky
	Prostaglandin E ₂	Many different cell types	Contraction of smooth muscle cells
	Glycine	Nerve terminals	Inhibitory transmitter in central nervous system
Neurotransmitter	Norepinephrine	Nerve terminals	Excitatory and inhibitory transmitter in central and peripheral nervous system

	Signal Molecules	Site of Origin	Major Effects
Hormones	Acetylcholine	Nerve terminals	Excitatory transmitter at neuromuscular junction; excitatory and inhibitory transmitter in central and peripheral nervous system
	Epinephrine	Adrenal Medulla	Increase in blood pressure and heart rate; increase in glucogenolysis in liver and muscle; fatty acid release from fat cells
	Thyroxine	Thyroid	Increase in metabolic activity in most cells
	Cortisol	Adrenal	Affect on metabolism of proteins, carbohydrates and lipids; suppression of inflammatory reactions
	Insulin	Beta cells of pancreas	Utilisation of carbohydrates, uptake of glucose into cells; stimulation of lipid synthesis in fat cells
	Adrenocorticotrophic hormone (ACTH)	Anterior pituitary	Stimulation of adrenal cortex to produce cortisol; fatty acid release from fat cells
	Thyroid stimulating hormone (TSH)	Anterior pituitary	Stimulation of thyroid to produce thyroxine; fatty acid release from fat cells

15.3 SOLUBILITY OF SIGNALLING CHEMICALS

Chemical signals are also classified according to their solubility in water: hydrophilic, i.e. water-soluble and hydrophobic, i.e. water-insoluble. Most hormones and local chemical mediators and all the known neurotransmitters are water-soluble. Certain hormones, such as steroids and thyroid hormones are water-insoluble. You have read earlier, that water-soluble signalling molecules mediate responses of short duration, whereas water-insoluble molecules mediate longer lasting responses.

Hydrophobic and hydrophilic signalling molecules act on their target cells by different mechanisms. Water-insoluble molecules are transported in the blood stream by binding to specific carrier proteins. When released from their carrier proteins, they pass by simple diffusion through the plasma membranes of their target cells and bind to specific receptor proteins inside the cell, i.e. intracellular receptors. On the other hand water-soluble molecules cannot pass through the plasma membrane and so bind to specific receptor proteins on the target cell surface, i.e. cell-surface receptors (see Fig. 15.6).

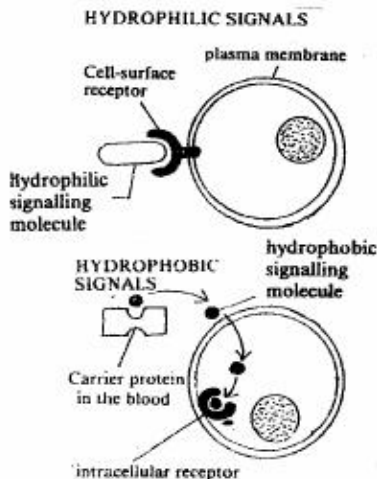


Fig. 15.6: Signalling mechanisms of hydrophilic and hydrophobic signalling molecules. Hydrophilic molecules bind to the receptors on the cell surface(a). Hydrophobic molecules are transported by binding to carrier proteins, diffuse across the plasma membrane and bind to intracellular receptors(b).

SAQ 1

- i) List three differences between hormones and neurotransmitters

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- ii) Give three examples each of local chemical mediators, neurotransmitters and hormones

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You have read above that signalling is mediated by intracellular receptors and by cell surface receptors. We will now discuss signals mediated by these receptors by taking particular examples of the signals and the responses of the target cells

15.4 SIGNALLING MEDIATED BY INTRACELLULAR RECEPTORS

You have already read that the mechanism of action of steroid and thyroid hormones is different from other signalling chemicals, in that their receptors lie inside the target cells. These molecules are water-insoluble and cross the plasma membrane by simple diffusion. In this section we will discuss signalling mediated by intracellular receptors by taking the example of steroid hormones.

Steroid hormones are synthesised from cholesterol. Being relatively small hydrophobic molecules (molecular weight around 300-dalton), steroids are able to cross the plasma membrane by simple diffusion. After entering the target cell, the steroid hormones bind tightly but reversibly with receptor proteins in the cytoplasm. There are specific receptor proteins for each steroid hormone.

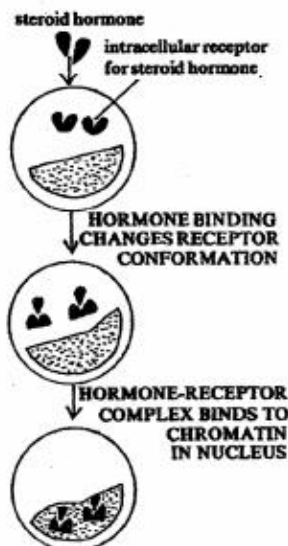


Fig 15.7: Schematic diagram showing the binding of steroid hormone with intracellular receptor proteins. Binding of hormone produces a conformational change in the receptor protein and the resulting hormone-receptor complex migrates into the nucleus and binds with chromatin.

The binding of the hormone causes reversible allosteric change in the conformation of the receptor protein that increases the ability of hormone-receptor complex to bind with chromatin in the nucleus. Since hormone-receptor complexes are able to pass through the nuclear pores, these complexes accumulate in the nucleus of the cell (see Fig. 15.7). Thyroid hormones also work in a manner similar to that of steroid hormones except that their receptors are concentrated in the nucleus even before the hormones bind to them

A receptor is a mole/molecular complex which is capable of recognising and interacting with signal molecules. After binding it is capable of changing the signal that initiates the chain of events leading to the response.

A typical target cell contains about 10,000 to 1000,000 steroid receptors, each with a high affinity for its respective hormone. When hormone levels are high, activated receptor-hormone complexes bind to chromatin in the nucleus. As hormone levels fall, hormone molecules dissociate from receptors and the freed receptors return to the cytoplasmic pool.

Some of the activated receptors that are bound to chromatin have their initial effect by regulating the transcription of specific genes into mRNA which codes for specific proteins. Subsequent synthesis of the coded proteins is the ultimate effect of the steroid hormones on target cells. But only a small number of genes in any target cell are directly influenced by steroid hormones. You have already read the process of protein synthesis in Unit 14.

In many cases response to a steroid hormone takes place in two steps — the primary response and the secondary response. The direct transcription of a few specific genes into mRNA is called **primary response**. The proteins synthesised as a result of primary response activate other genes that produce the ultimate effect on target cell which is called **secondary response**. For example, the primary response in fruit fly *Drosophila* is produced within five to ten minutes of injection of steroid hormone **ecdysone**. The hormone induces six new sites of RNA synthesis, seen as puffs in the giant chromosome of the salivary glands. This is known as a primary response, that is, the direct induction of transcription of a few specific genes. Some proteins produced as a result of this primary response induce RNA synthesis in about 100 more sites leading to the synthesis of a large group of proteins. This is the delayed response of the hormone and is known as secondary response; a major amplification of the initial hormonal effect. The response is controlled by feedback through one or more initial proteins that shut off further transcription of all primary response genes.

The same hormone-receptor complex is found to influence different sets of genes in different target cells. The role of hormones in regulation of enzyme activity by controlling its synthesis has already been discussed in Unit 10 of Block 2.

SAQ 2

Fill in the blanks.

- Steroid hormones are water
- The hormone-receptor complex enters the and bind to the
- Once bound to chromatin, the hormone-receptor complex regulate
- Six RNA synthesis sites appear as on the salivary gland chromosome of *Drosophila*
- response of the hormone is a delayed response.

Till now you have read about the mode of action of water insoluble signalling molecules on their target cells. You will now study about water soluble signalling molecules, which cannot pass through plasma membrane and signal the target cells by binding to the receptors proteins on the cell surface.

15.5 SIGNALLING MEDIATED BY CELL-SURFACE RECEPTORS

Water-soluble signalling molecules, include local chemical mediators, neurotransmitters, protein hormones and growth factors. As you have already read these molecules reversibly bind to specific receptor proteins on the surface of the

target cells. These receptors constitute less than 1 per cent of the total protein mass of the plasma membrane and are therefore difficult to isolate and study.

Many hydrophilic signalling molecules such as protein hormones and growth factors bind to the cell surface receptors and enter the target cell by receptor mediated endocytosis process. We have already discussed endocytosis in Unit 3 of Block 1. As shown in Fig. 15.8, in receptor mediated endocytosis the signal molecules bind to the receptor proteins and the signal-receptor complexes are taken into **endocytotic vesicles**, in the cell by the process of internalisation. In receptor-mediated endocytosis, the endocytotic vesicle along with extracellular molecules, normally fuse with lysosomes to form **secondary lysosomes**. To induce their effect in the target cells these hydrophilic signal molecules escape from the fused secondary lysosomal vesicle into the cytosol. Thus, these signalling molecules act directly within the target cell where they generate intracellular signals that affect the activity and behaviour of the cell. Signalling also occurs by the escape of degraded receptor molecules into the cytosol. Receptor proteins are then recycled to the plasma membrane.

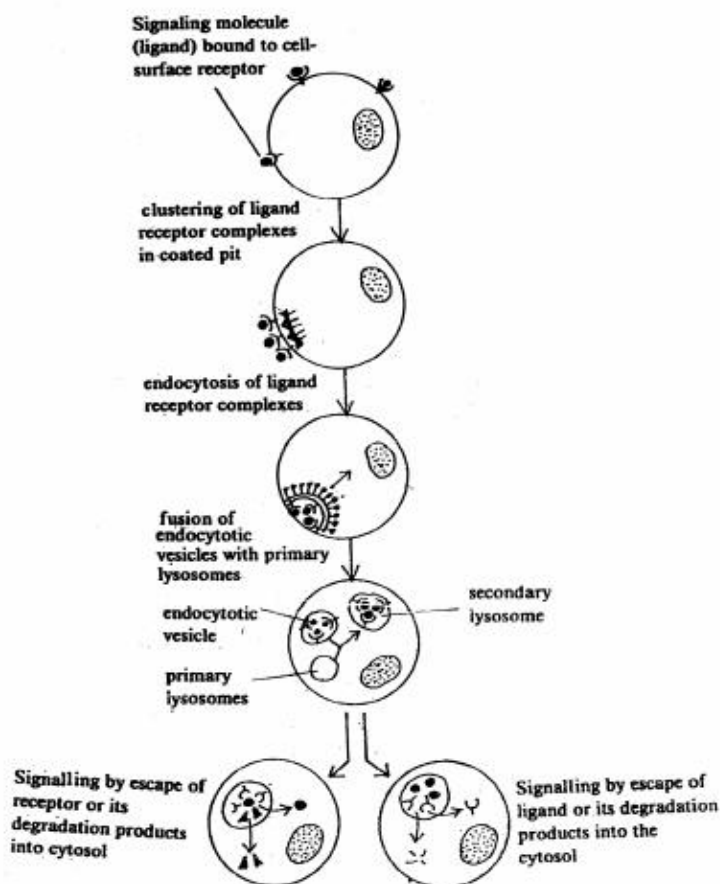


Fig 15.8: Schematic diagram showing receptor-mediated endocytosis. The endocytotic vesicles with their extracellular molecules fuse with the lysosomes. Signalling occurs by the escape of degraded signalling molecules or receptor molecules into the cytosol.

However, water-soluble signal molecules generally remain outside the cell after binding to specific cell receptors. Experiments have shown that at least some signalling molecules do not have to enter a cell to influence it. For example, the effects of insulin are exhibited as soon as an insulin molecule binds to its receptor on the target cell. Similarly, as soon as a thyroid stimulating hormone (TSH) binds with its receptor on a thyroid cell, thyroxine is secreted.

Also many hydrophilic signalling molecules induce responses that occur much too rapidly to involve receptor-mediated endocytosis. For example, mast cells secrete histamine within seconds of ligand binding to receptors while responses to some neurotransmitters occur in fractions of second. In such cases, receptors act as transmembrane transducers to generate new intracellular signals. Let us see how it happens.

When a cell-surface receptor binds to a signal molecule on the cell exterior, it undergoes a reversible conformational change. This leads to the generation of an intracellular signal that alters the behaviour of the target cell. These intracellular signalling molecules are referred to as second messengers, the first or primary messengers being the extracellular ligands themselves. You have already read about these in Unit 10 of Block 2.

Cell-surface receptors generate intracellular signals in two major ways. One way is by activating or inactivating a plasma membrane-bound enzyme. In certain cases the activated enzyme catalyses the production of a soluble intracellular mediator, i.e. the second messengers, the concentration of which serves as the signal. An important membrane bound enzyme, which is activated by many signal-receptor complexes and acts in this way is **adenylate cyclase**. This activated enzyme catalyses the synthesis of secondary messenger, cyclic AMP (cAMP) from ATP on the cytoplasmic side of the plasma membrane (see Fig. 15.9a).

However, there are some cases where an enzyme activated by an extracellular ligand directly causes phosphorylation of cellular proteins. For example, epidermal growth factor (EGF), a hormone, binds to cell-surface receptors to transfer a phosphate from ATP to a tyrosine residue on specific cellular proteins. This stimulates the target cells, i.e. the epidermal and other cells, to divide. The cell surface receptors are either protein kinases or are closely associated with them.

The second way a cell surface receptor generates signal is by opening or closing gated ion channels in the plasma membrane. You have already read about gated ion channels in Unit 8. This method generates a signal in either of the two ways: 1) it causes a small and transient flux of ions that briefly changes the voltage, i.e. electrical gradient across the plasma membrane, or 2) it causes a major flux of ions into the cytosol which in turn initiates an intracellular response. The first mechanism operates mainly in electrically active cells such as neurons where the stimulus travels in the form of electric charge along the nerve processes.

Many animal cells that are not electrically active such as secretory cells, have cell-surface receptors that are functionally linked to Ca^{2+} channels in the plasma membrane. That is, binding of ligands activates the receptors thereby opening the channels transiently and allowing Ca^{2+} to enter the cytosol, where it then functions as a second messenger (see Fig. 15.9b).

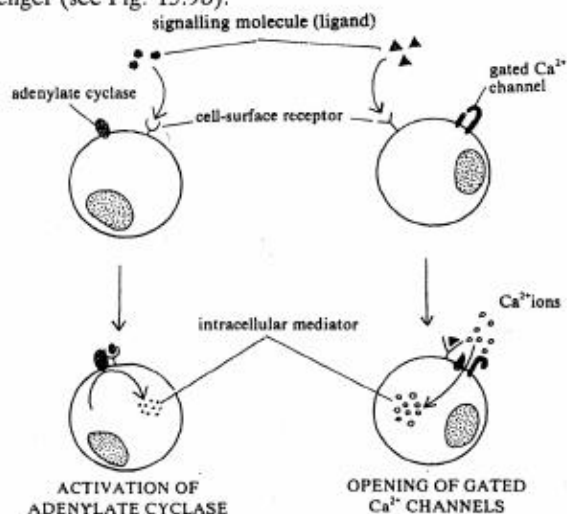


Fig. 15.9: Cell-surface receptors generate intracellular signals by two mechanisms: (a) the activation of membrane bound enzyme adenylate cyclase molecules increases the intracellular concentration of cAMP; and (b) the opening of membrane-bound gated Ca^{2+} channels allows Ca^{2+} to enter the cell.

We will discuss the second messengers; cAMP and Ca^{2+} in the target cells in the following subsections.

15.5.1 Cyclic AMP as Second Messenger

Cyclic AMP (cAMP) is a common intracellular mediator for practically all procaryotic and eucaryotic animal cells. The cAMP is synthesised from ATP by the plasma membrane bound enzyme adenylate cyclase. The effect of cAMP lasts briefly as cells change rapidly in response to the signals. But if not used it is rapidly destroyed in cells by specific enzymes called phosphodiesterases, which hydrolyse cAMP to adenosine 5'-AMP (see Fig. 15.10).

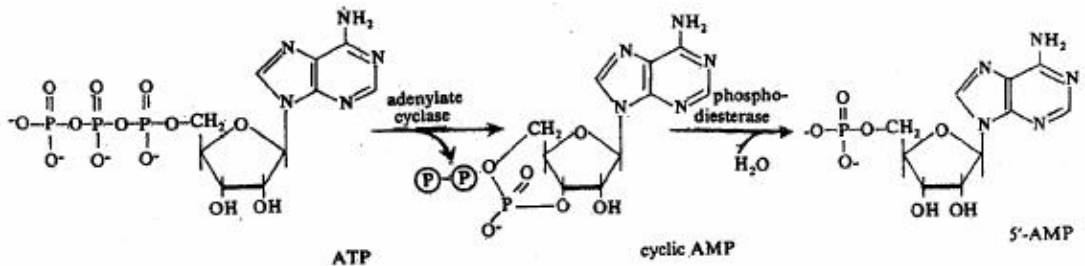


Fig. 15.10: Cyclic AMP is synthesised and degraded in the cell.

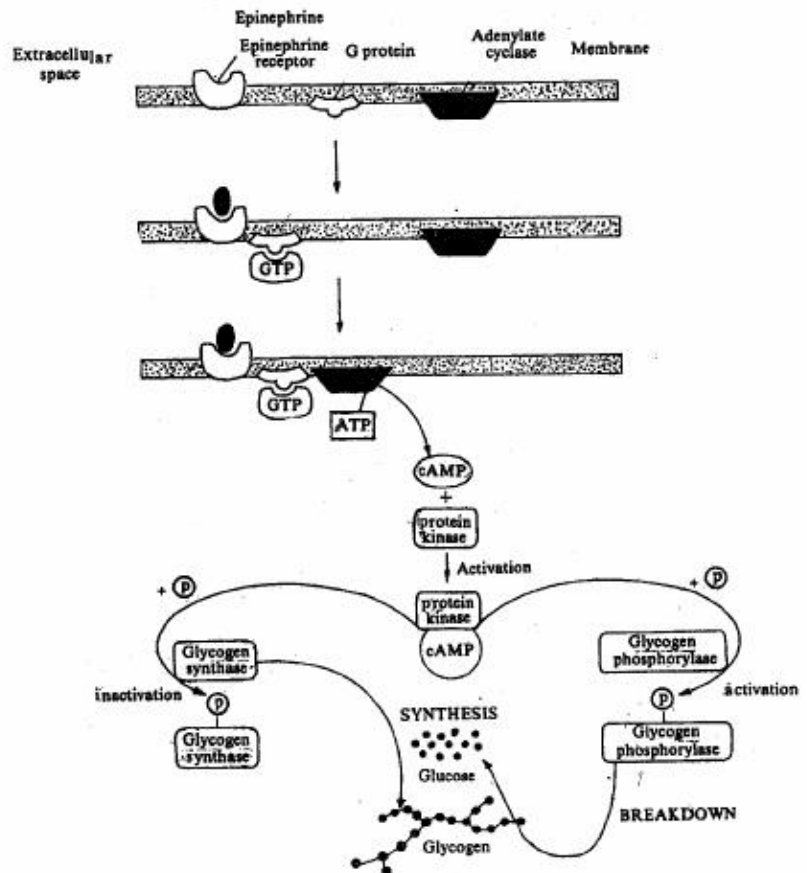


Fig. 15.11: Schematic diagram showing the influence of chemical signalling on the skeletal muscle cell. Binding of epinephrine to the receptors at cell surface influences the receptor to bind to the G protein in the membrane. The couple receptor and G-protein then bind to and activate the enzyme adenylate cyclase on the cell surface. The activated enzyme converts ATP to cyclic AMP in the cytoplasm. cAMP activates a protein kinase which stops glycogen synthesis and at the same time helps to breakdown the stored glycogen.

Activated receptors do not activate adenylate cyclase directly. Another membrane protein, G protein which binds GTP (guanosine triphosphate) on its cytoplasmic surface, couples the two together. This mechanism was suggested by the finding that hormonal activation of adenylate cyclase in disrupted cells requires GTP. Also in mutant cells which lack G-protein, activation of adenylate cyclase does not occur. For example, as shown in Fig. 15.11, activated epinephrine receptors after ligand binding do not influence adenylate cyclase directly, instead they bind to membrane bound G protein. This binding activates G protein by altering its conformation so that it can bind GTP. The coupled receptor and G protein with GTP, binds to and activates the enzyme adenylate cyclase by reversibly changing its conformation. The activated enzyme then catalyses the conversion of ATP to cyclic AMP in the cytoplasm which then activates protein kinase. You have read about the role of cAMP in glucose metabolism in Unit 10.

Through the mediation of G protein the target cells respond more quickly and specifically to the changing conditions. This is because each activated receptor is able to interact with and activate many molecules of G protein, thereby greatly amplifying extracellular signals. Thus, relatively few hormone molecules elicit a rapid and considerable response in the target cells. Formation of cAMP stops when G protein hydrolyses its bound GTP into GDP (guanosine diphosphate) and resumes its original inactive conformation. Adenylate cyclase is then released from the altered G protein and resumes its original inactive conformation, thus stopping cAMP formation.

Many hormones and local chemical mediators signal their target cells by activating adenylate cyclase. Some of such examples of hormones and their effects on the target cells are given in Table 15.2.

Table 15.2: Hormone-induced effects in some target cells mediated by cyclic AMP

Target Cells	Hormone	Response
Thyroid	thyroid-stimulating hormone (TSH)	thyroxine secretion
Adrenal cortex	adrenocorticotrophic hormone (ACTH)	cortisol secretion
Ovary	luteinizing hormone	progesterone secretion
Muscle, liver	epinephrine	glycogen breakdown
Bone	parathormone	bone reabsorption
Heart	epinephrine	increase in heart rate
Kidney	vassopressin	water reabsorption
Fat	epinephrine, ACTH, TSH, glucagon	triglyceride breakdown

Each type of animal cell responds to an increase in cAMP in its own characteristic way. Any ligand that activates adenylate cyclase in a given type of target cell usually produces the same effect. For example, at least four different hormones activate adenylate cyclase in fat cells and all of them stimulate the breakdown of triglyceride into fatty acids.

15.5.2 Ca^{2+} Ions as Second Messenger

You have read in Unit 10 of Block 2 that Ca^{2+} ions function as a second messenger for certain extracellular signalling molecules in all the organisms. You have already read above the ways in which cells utilise large Ca^{2+} gradients across their plasma membranes for transducing extracellular signals. Another important alternative mechanisms for Ca^{2+} mediated signalling is the release of Ca^{2+} from internal stores of the cells such as intracellular organelles. For example, in skeletal muscles, the activation of cell-surface acetylcholine receptors depolarises the plasma membrane which leads to the release of Ca^{2+} from the sarcoplasmic reticulum of these cells. This in turn initiates myofibril contraction (see Fig. 15.12). The exact mechanism of how activation of receptors results in opening of Ca^{2+} channels is still a mystery.

Once inside the cytoplasm of the cell Ca^{2+} acts as secondary messenger by interacting

G protein is GTPase molecule that is bound to the cytoplasmic side of the membrane and functions as a communicator between receptor and membrane bound enzyme adenylate cyclase. It is called G protein because it binds non-covalently to guanosine phosphates — GTP and GDP. It is composed of α , β and γ peptide chains. The α -subunit binds GTP, GDP, receptor and the enzyme. It self inactivates by hydrolysing GTP into GDP.

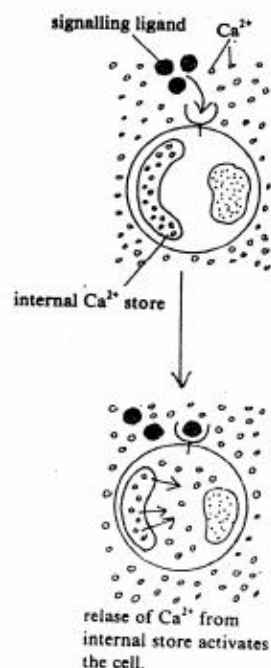


Fig. 15.12 : Schematic diagram showing the activated receptors helping to release Ca^{2+} from internal stores. In muscle cell this store is sarcoplasmic reticulum

with Ca^{2+} binding molecules. As you have read in Unit 10, one particular Ca^{2+} binding protein is **calmodulin**, present in every plant and animal cell that has been studied. Calmodulin has four Ca^{2+} binding sites and its conformation is altered in different ways depending on the number of occupied binding sites. Thus, as shown in Fig. 15.13, calmodulin is allosterically activated by Ca^{2+} bindings. This activated form of the protein mediates intracellular response by regulating the activation of certain cellular enzyme proteins, such as cyclic nucleotide phosphodiesterase, Ca^{2+} ATPase, phosphorylase, kinase, some forms of adenylate cyclase and myosin light chain kinase in muscle and non-muscle cells. You need not memorise the names of these enzymes. The response of the target cell to an increased Ca^{2+} concentration in cytosol depends on which type of calmodulin binding target proteins are present in the cell. Since calmodulin can exist in different conformations depending on the number of bound Ca^{2+} , it is possible that these different conformations interact with different target proteins.

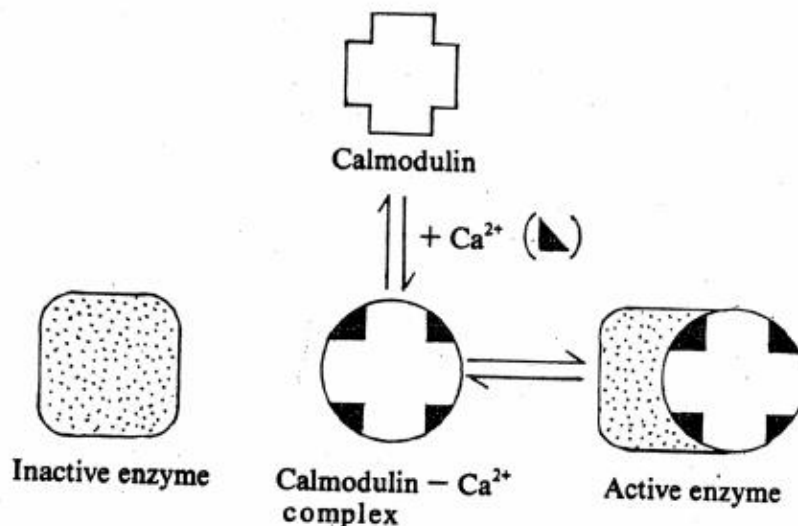


Fig. 15.13: Schematic diagram showing the allosteric activation of calmodulin by Ca^{2+} binding. The activated Ca^{2+} calmodulin complex mediates the intracellular response by activating the target proteins in the cell.

The increase in the concentration of free Ca^{2+} in the cytosol during cell signalling is always transient, since the gated Ca^{2+} channels open transiently. As such, the concentration of free Ca^{2+} in the cytoplasm is much lower than the extracellular Ca^{2+} level, so there is steep Ca^{2+} concentration gradient across the plasma membrane. Because of this, Ca^{2+} continues to move into the cell. When additional Ca^{2+} floods into the cell in response to the extracellular signals, it becomes important for the cell to pump out or sequester Ca^{2+} into intracellular organelles such as mitochondria. This is to maintain the steep gradient across the membrane for continued Ca^{2+} influx as shown in Fig. 15.14. This is achieved by a plasma membrane bound Ca^{2+} ATPase that uses the energy of ATP hydrolysis to pump Ca^{2+} out of the cell. In some cells Ca^{2+} is also actively expelled by other plasma membrane bound active transport pumps such as the Na^+ – driven antiports which couple the efflux of Ca^{2+} to the influx of Na^+ . Ca^{2+} are also sequestered into internal stores, i.e. various cellular organelles. Membrane bound Ca^{2+} ATPases enable the endoplasmic reticulum, especially the sarcoplasmic reticulum of muscle cells, to take up large amounts of Ca^{2+} from the cytosol against a steep concentration gradient. Mitochondria use the electrochemical gradient across the inner mitochondrial membrane generated during oxidative phosphorylation, to drive the uptake of Ca^{2+} from the cytosol. In addition to the various membrane transport proteins that actively pump Ca^{2+} out of the cytosol, a variety of Ca^{2+} binding molecules contribute to the removal of free Ca^{2+} from the cytosol. These include binding with small molecules such as phosphates forming insoluble precipitates as well as with macromolecules such as Ca^{2+} binding proteins.

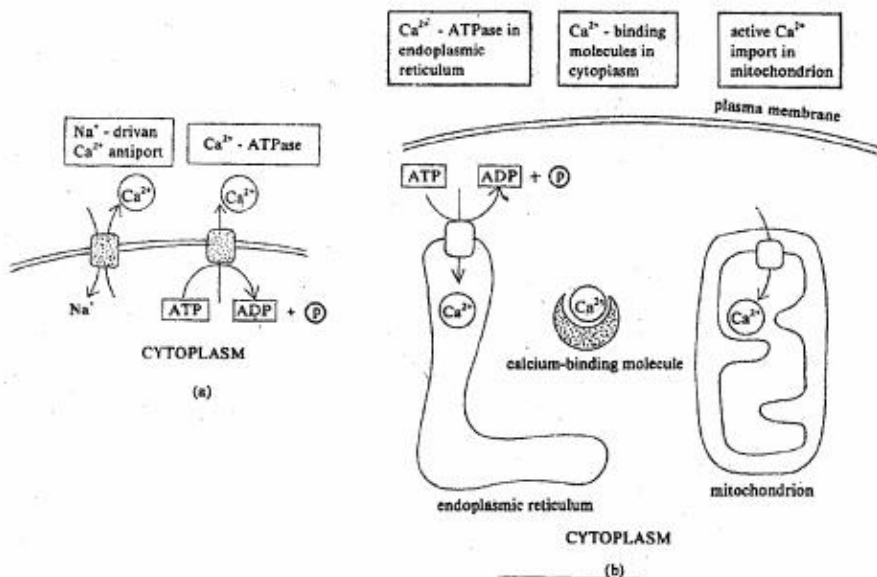


Fig. 15.14: Schematic diagram showing ways in which cells maintain a low concentration of free Ca^{2+} in the cytosol. Ca^{2+} is actively pumped out of the cytosol to the cell exterior as well as into intracellular, membrane-enclosed organelles such as the endoplasmic reticulum and mitochondria. In addition, various molecules in the cell bind free Ca^{2+} .

Before you proceed further, try the following SAQ to see whether you have understood the above text

SAQ 3

(a) Tick mark (✓) the correct word.

- Cell- surface receptors convert an extracellular signal into an extracellular/intracellular response.
- A signalling ligand is a first/second messenger.
- During receptor mediated endocytosis the signal acts from outside/inside of the target cell.
- A signal-receptor complex stimulates intracellular signalling molecules that are referred to as first/second messenger.

(b) Fill in the blanks with appropriate words.

- A denylate cyclase catalyses the synthesis ofform ATP on the cytoplasmic side of the plasma membrane.
- is required to couple receptor activation to adenylyate cyclase activation in target cell.
- In skeletal muscle cells, the activation of acetylcholine receptors depolarises the plasma membrane leading to the release of
- Colmodulin is.....by Ca^{2+} binding for mediation of response.

Till now you have read about the action of chemical signals on target cells. We will now discuss the adaptation of target cells to the ligands.

15.6 TARGET CELL ADAPTATION

Target cells exposed to a signalling ligand for a prolonged time often lose their ability to respond to that ligand. This process is called **adaptation** or **desensitisation** and is reversible. It makes cells especially sensitive to the changes in the concentration of a chemical signal rather than to the absolute or fixed concentration of the signal. Desensitisation of the target cell may result from a decrease in the number of specific cell-surface receptor proteins or from the inactivation of such receptors. In some

other cases, it is due to changes in the proteins involved in transducing the signal after the receptor is activated. Ligand binding to the receptor molecule is primary and the most important step in chemical signalling. Desensitisation at this level of signalling, affects all the subsequent events and decreases the influence of the signals on the target cell. In this section we will deal with three aspects of target cell adaptation at the ligand binding stage. These three ways have been summarised in Fig. 15.15.

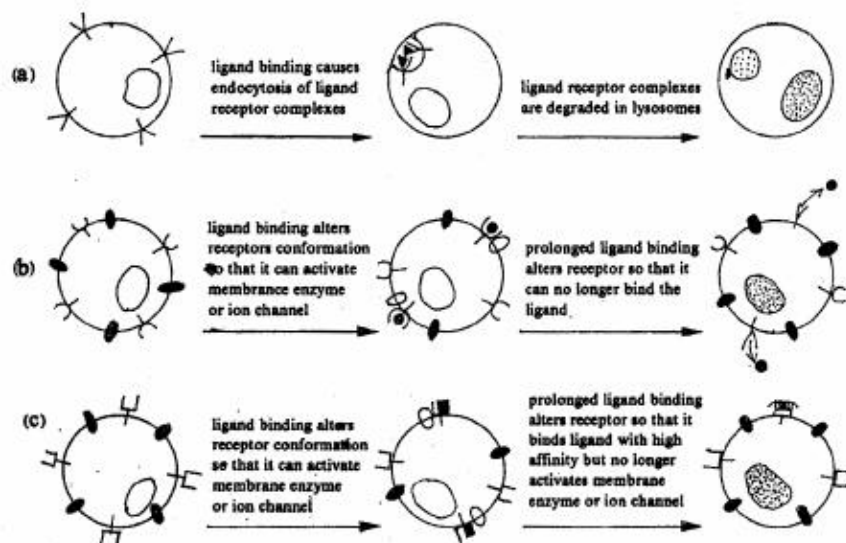


Fig. 15.15: Three different ways in which receptors can be inactivated by high concentrations of a ligand. Receptor mediated endocytosis of ligand receptor complexes, which are degraded in lysosomes, causes the slow replacement of receptors to the cell surface (a); prolonged ligand binding alters the conformation of the receptor so that it can no longer bind to the ligand (b); prolonged ligand binding alters receptors in such a way that it increases its affinity with the ligand but does not activate membrane enzyme or ion channel (c).

You have already read earlier that some hydrophilic protein hormones and growth factors, after binding to cell-surface receptors on the target cells, are often ingested by receptor-mediated endocytosis. The endocytotic vesicles deliver their contents to lysosomes where the ligand; and often the receptor to which it is bound, are degraded by hydrolytic enzymes. Receptor degradation and its replacement to the cell surface takes place continuously. But by inducing endocytosis, the rate of receptor degradation is increased so that at high ligand concentrations, the number of receptors available for ligand binding at cell surface decreases. The result is a decrease in the sensitivity of the target cell to the ligand (see Fig. 15.15a). This type of target cell desensitisation is known as **receptor-down regulation**.

A second type of cell surface receptor regulation occurs in response to unusually high concentrations of small signalling ligands such as epinephrine and acetylcholine. Instead of inducing receptor mediated endocytosis, these ligands reversibly inactivate the receptors. For example, frog red blood cells, exposed to high concentration of epinephrine, gradually lose their sensitivity to that substance as well as their ability to bind to it. It is likely that the binding of epinephrine induces a prolonged but reversible change in the conformation of the receptor protein that prevents the protein from binding the other ligands again for a long period after the ligand dissociates (see Fig. 15.15b).

In the third type of ligand binding adaptation the reversible inactivation of cell surface receptors does not always involve the loss of the receptors' ability to bind the ligand. For example, prolonged binding of acetylcholine to skeletal muscle cell receptors at a neuromuscular junction induces the receptors to adopt an inactive conformation. These are able to bind acetylcholine with an even higher affinity than normal receptors. However, such signal-receptor complexes become inactive and are unable to activate membrane enzyme or open ion channels in the plasma membrane of muscle cells and therefore cannot stimulate muscle contraction (see Fig. 15.15c).

Not all target cell adaptation is due to ligand-induced receptor's degradation or inactivation. In morphine addicts, for example, target cells in the brain become desensitised to morphine and yet have normal levels of functional cell surface morphine receptors. This explains why morphine addicts should take higher doses of morphine than a normal person.

Morphine receptors are functionally coupled to adenylate cyclase molecules by means of GTP-binding proteins, much as epinephrine receptors are coupled to adenylate cyclase in muscle cells. In contrast, to epinephrine receptors on muscle cells, however, morphine-induced receptor activation leads to the inactivation of adenylate cyclase, thereby causing a decrease in intracellular cyclic AMP levels.

15.7 BACTERIAL CHEMOTAXIS

The mechanisms involved in chemical signalling between cells in multicellular animals may have evolved from mechanisms used by unicellular organisms for responding to chemical changes in their environment.

Some of the best studied reactions of unicellular organisms to extracellular signals are **chemotactic responses** in which cell movement is oriented towards or away from a specific chemical substance present in the environment. This simple but intelligent behaviour is referred to as **chemotaxis**. Movements towards a chemical substance are called positive chemotaxis and away from a particular substance are called negative chemotaxis. Prokaryotic chemotaxis has been well studied in bacteria. Bacteria swim by means of flagella. When viewed by high speed cinematography, bacterial flagella appear to rotate in a screw like fashion. The flagella can rotate in either clockwise or counterclockwise direction. The direction of the rotation of the flagella and the ability of the cell to reverse the direction of flagellar rotation is important for the movement of the cell. Observations of bacteria indicate that they generally move in a straight line for a few seconds and then undergo a type of random tumbling motion which orients them in a new randomly chosen direction. The movement of a bacterium in a straight line occurs during counterclockwise rotation of its flagella, while tumbling motion results from a clockwise rotation. When the flagella rotate in the counterclockwise direction, they remain associated with one another in a bundle and thereby act, in a concerted manner as if they were a single multistranded organelle (see Fig. 15.6a). However, when rotating in the clockwise direction, the flagella are separated from one another and each acts independently, producing the tumbling behaviour (see Fig. 15.6b).

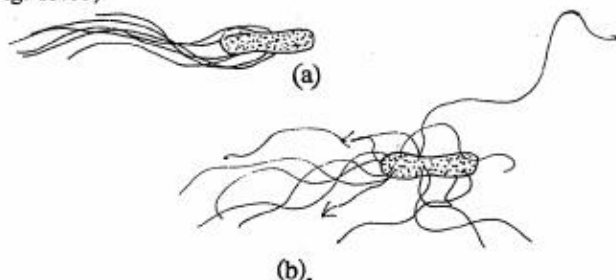


Fig. 15.16: Schematic diagram showing positions of flagella of bacterial cell during its movements. They are drawn into single bundle by counterclockwise rotation and produce smooth motion (a); clockwise rotation causes the flagella to fly apart and produces tumbling (b).

If a bacterium is exposed to a chemical that acts as an attractant e.g., glucose, the rotation of flagella will be in counterclockwise manner, as long as the cell continues to swim smoothly towards higher concentration of the substance. In contrast, in presence of repellent chemical, clockwise rotation of bacterial flagella will be initiated causing the bacterium to tumble until it moves off in a direction away from the source of the substance.

Such changes in the motion of bacterial cells can be studied in the laboratory by addition or removal of a chemical to the culture medium. Adding an attractant suppresses tumbling but on the continued presence of the attractant tumbling frequency returns to normal. This process of desensitisation or adaptation is specific for a particular type of attractant. Sudden removal of the attractant enhances tumbling until the bacterium adapts to the new level again.

Methylation is the process of
addition of methyl group by the
donor to the acceptor molecule.

The presence of chemical substances in the environment is detected by several types of periplasmic receptors, (found between outer membrane and the plasma membrane). Once a chemical substance present in the medium binds to these receptor, it influences the flagellar rotation. In bacteria, transmembrane proteins become methylated during chemotactic signals and are known as **methyl-accepting chemotaxis proteins (MCPs)**. Each MCP is activated by binding to its own set of periplasmic receptor proteins. When a bacterium is exposed to a chemotactic attractant, the binding of the attractant induces a conformational change in the periplasmic receptor protein causing the latter to bind to, and thereby activate the appropriate MCP. The resulting activation has two effects: 1) excitation occurs because the activated MCP generates an intracellular signal that causes the flagella to rotate counter-clockwise resulting in continuous smooth swimming and a suppression of tumbling; 2) adaptation occurs because the activated MCP can now be methylated by enzymes in the cytoplasm reversing its activation. Therefore covalent methylation of membrane proteins is responsible for adaptation in bacteria. When methylation is blocked by mutation, adaptation does not occur and exposure of the mutant bacteria to an attractant results in the suppression of tumbling for days instead of for minutes.

Chemotaxis also takes place in higher organisms. For example, sperm cells of certain ferns and invertebrate animals are guided towards the surface of an egg by chemotactic responses. Similarly phagocytic white blood cells (leucocytes) are guided by chemotaxis towards the invading bacteria at the site of inflammation. However, unlike bacteria and sperm cells, white blood cells crawl by the use of pseudopodia that are formed at the front end of the cell in the direction of the source of attractant. As in case of prokaryotes, binding of attractants to the membrane receptors influence the chemotactic responses in these cells.

SAQ 4

Explain briefly in the given space, the three way in which ligand binding causes adaptation in target cell

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15.8 SUMMARY

In this unit you have studied the following:

- In a multicellular organism, cells communicate with each other through signalling molecules. Signalling molecules are of three types; local chemical mediators, hormones and neurotransmitters. Steroid hormones are lipid soluble and bind specifically and reversibly to specific receptor carrier proteins in the blood for transport to the distantly located target cells where they bind to specific intracellular receptor proteins in the cytosol. By complexing with the hormone, the receptor acquires an affinity for chromatin that causes it to accumulate in the nucleus. There, the hormone-receptor complex binds to chromatin and regulates the transcription of specific genes.
- Cell surface receptor proteins activated by extracellular hydrophilic signalling ligands such as local chemical mediators, neurotransmitters and water-soluble hormones generate intracellular signals by altering the activity of a plasma membrane-bound enzyme, or by altering the permeability of ion channels in the plasma membrane.
- Many cell surface receptors are functionally-linked to the membrane bound enzyme adenylate cyclase. The activation of other types of cell surface receptors opens membrane bound Ca^{2+} channels. This results in a rise in intracellular Ca^{2+} concentration. The signal receptor complex gets coupled with G-protein in the membrane so as to activate the enzyme or open the Ca^{2+} channel

- Target cell adaptation is reversible desensitisation of the cell so that it is not able to respond efficiently to the external signal. Ligand binding with the receptor brings out this desensitisation of target cell in various ways.
- Bacteria are able to respond to changes in the concentration of ligands, i.e. attractants and repellents. Bacterial chemotaxis is the best understood example of adaptation.

15.9 TERMINAL QUESTIONS

1 What are the three ways of intercellular chemical signalling?

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2 Give an outline of the mechanism of steroid hormone action. Answer very briefly in the space given below.

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3 Describe briefly in the given space how the changes in concentration of Ca^{2+} are brought about in a skeletal muscle cell.

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4 Explain briefly what is chemotaxis.

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15.10 ANSWERS

Self-assessment Questions

- | Hormones | Neurotransmitters |
|--|---|
| 1 i) 1) Secreted by specialised endocrine cells. | Secreted by nerve endings. |
| 2) Secreted into and transported by blood. | Secreted into extracellular fluid at synapse. |
| 3) Target cells situated some distance away in the body. | Target cells adjoining the site of secretion. |
| 4) Act at a slower rate than neurotransmitters. | Transmitted in fraction of seconds. |
| ii) Local chemical mediators: histamine, prostaglandins, growth factors. | |
| Neurotransmitters: acetylcholine, norepinephrine, glycine. | |
| Hormones: insulin, thyroxine, thyroid stimulating hormone. | |
| 2 i) insoluble | |
| ii) nucleus, chromatin | |
| iii) the transcription of specific genes | |
| iv) primary response | |
| v) secondary | |
| 3 a) i) intracellular | |
| ii) first | |
| iii) inside | |
| iv) second | |
| b) i) cyclic AMP | |
| ii) G protein | |
| iii) calcium ions | |
| iv) allosterically activated | |
| 4 Target cell adaptation by ligand binding is brought about by various ways; ligand binding can inactivate a cell surface receptor by its internalisation and degradation, or by causing the receptor to adopt reversible inactive conformation. An inactivated receptor either does not bind with a signal molecule or attains high affinity for the signal. In either case it cannot activate the enzymes or open the ion channel. | |

Terminal Questions

- The three ways in which intercellular chemical signalling occurs are through; gap junctions between the adjacent cell, plasma membrane bound signalling molecules and cell secretions. Signalling through gap junctions and plasma membrane bound molecules are direct communications between the cells whereas, cell secretions give signals to the distantly located cells and are called indirect communication between the cells.
- Signalling by steroid hormones is indirect communication. Steroids being water-insoluble pass through the plasma membrane by simple diffusion. Inside the cytosol, steroids bind with intracellular receptors. These hormone receptor complexes move into the nucleus and elicit the response of target cell by regulating protein synthesis.
- In skeletal muscles the binding of ligand activates the cell surface acetylcholine receptors which depolarises the plasma membrane. This leads to the release of Ca^{2+} from internal stores, i.e. sarcoplasmic reticulum, thereby increasing the concentration of Ca^{2+} in the cytosol. This increased Ca^{2+} concentration initiates myofibril contraction.
- Chemotaxis is the intelligent behaviour of bacteria by which these unicellular organisms respond to the extracellular signals by moving towards or away from specific chemicals. Most bacteria swim towards high concentrations of nutrients (attractants) and away from high concentrations of repellents.

anticodon: a sequence of three bases on the tRNA that pair with the bases in the corresponding codon on the mRNA.

chemotaxis: sensing of and movements towards or away from a specific chemical agent by cells.

chromatid: one of the two halves of a replicated chromosome.

chromatin: a filamentous complex of DNA, protein and RNA that constitute eucaryotic chromosome.

chromosome puff (Balbiani rings): a swollen region of a giant chromosome; the swelling reflects a high degree of transcriptional activity.

codon: a sequence of three bases in a messenger RNA molecule, that represents a particular amino acid.

ecdysone: a hormone that stimulates the moulting process in insects.

electrically active cells: cells which are stimulated by electric charge, e.g. muscle cells and nerve cells. Nerve cells also carry the nerve impulse in the form of electric charge.

fibroblast: connective tissue cells that secrete intercellular substances.

gene: a unit of inheritance that occupies a particular site or locus, on the chromosomes and encodes the structure of a polypeptide chain on RNA molecule or regulates the activity and behaviour of a structural gene, and that can mutate to one or more alternative allelic form.

gene amplification: repeated replication of some genes producing many copies and other genes do not replicate in the same cell.

genetic code: the set of triplet code words in DNA and RNA coding for twenty amino acids used in protein synthesis, along with **start** and **stop** codons that start and punctuate the genetic message.

genome: all the genes present in the chromosome or set of chromosomes that specify all the inherited traits of a cell or individual.

initiation complex: an aggregate of mRNA, small ribosomal subunit, and initiator aminoacyl tRNA that marks the start of polypeptide transcription from encoded genetic information.

initiator aminoacyl — tRNA: it is the met-tRNA or fmet-tRNA that binds to the initiation codon AUG in mRNA and starts translation at the ribosome synthesising polypeptide.

initiation factors: these are the small molecules that bind to the small ribosomal subunit of the initiation complex and helps in the initiation of polypeptide synthesis during translation.

N-formylmethionine: the formylated derivative of methionine that serves as the initiating amino acid in polypeptide synthesis.

nonsense codon: the codon which do not specify any amino acid.

operator site: the site on DNA to which repressor molecules are bound, inhibiting the synthesis of mRNA by the gene in the adjacent operon; adjacent to the structural genes in the operon.

operon: a collection of genes consisting of a promoter, an operator, and one or more structural genes that function coordinately in bacterial gene expression.

post-translational modification: enzymatic processing of a polypeptide chain after its translation from its mRNA.

receptor-mediated endocytosis: intake of ligands bound to specific receptors of a coated pit region in plasma membrane and the subsequent pinching off of a coated vesicle containing ligand-receptor complexes for processing inside the cell.

RNA polymerase: enzymes that catalyse the synthesis of RNA molecules from ribonucleotide 5'-triphosphate using a strand of DNA or RNA as a template during transcription.

sequestered: to be taken away, e.g. taken from the cytosol into the internal organelles.

sigma factor: a polypeptide subunit of E.coli RNA polymerase which is required to

initiate transcription correctly and is released afterwards leaving the core enzyme of the holoenzyme to carry out RNA polymerisation.

signal hypothesis: it assumes that growing polypeptide chains of structural proteins have an N-terminal signal peptide sequence that indicates that it is to be translocated across the ER membrane or bacterial plasma membrane discharging polypeptide chain into ER lumen or out of the ER of bacterial cell.

structural genes: a gene coding for the structure of a protein.

template: a molecule which contains information from which other molecules can be synthesised.

Further Reading

- 1 Conn, Eric E, and Stumpf P.K., *Outlines of Biochemistry 4th edition* New Delhi, Wiley Eastern, 1988 7613
- 2 Sheeler Phillip, Bianchi Donald E; *Cell and Molecular Biology*, John Wiley and Sons, Inc. United States, 1987
- 3 Champe Pamela C., Harvey Richard A.; *Biochemistry*, J.B. Lippincott Company, Philadelphia, 1987

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-01
Block-3

Enrolment No.

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- 1) How many hours did you need for studying the units?

Unit Number					
No. of hours					

- 2) How many hours (approximately) did you take to do the assignments pertaining to this block?

Assignment Number		
No. of hours		

- 3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (✓) the type of difficulty and give the relevant page number in the appropriate columns.

Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

- 4) It is possible that you could not attempt some SAQs and TQs. In the following table are listed the possible difficulties. Kindly tick (✓) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	SAQ No.	TQ No.	Type of difficulty			
			Not clearly posed	Cannot answer on basis of information given	Answer given (at end of Unit) not clear	Answer given is not sufficient

- 5) Were all the difficult terms included in the glossary. If not, please list in the space given below.

6) Any other suggestion(s)

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Indira Gandhi National Open University
Maidan Garhi
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Cell Biology Course in Life Sciences

Block

4

CELL DIVISION, CELL MOVEMENT AND DIFFERENTIATED CELL TYPES

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UNIT 17

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By Dr. Arun Kumar Gupta, Registrar, U.P.R.T. Open University, Allahabad (July, 2018)

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UNIT 4 CELL DIVISION, CELL MOVEMENT AND DIFFERENTIATED CELL TYPES

There are five units in this block. Unit 16, the first unit in this block, deals with cell cycle which is a complex series of stages by which cellular material is divided equally between two daughter cells. The unit describes the process of mitosis that splits a chromosome into two daughter cells and maintains chromosomal continuity and diploid number. The central role that spindle microtubules play in chromosomal movement towards the poles during cell division is also discussed here.

Unit 17 deals with the behaviour of chromosomes during the process of meiosis that leads to distribution of haploid number of chromosomes in the daughter cells and recombination of genetic material among the homologous chromosomes. The unit discusses the molecular mechanism involved in the process of cytokinesis in both animal as well as plant cells.

Unit 18 describes how specific cells in multicellular organisms recognise each other and aggregate into co-operative assemblies called tissues. Adjacent cells are joined and interact with each other with the help of cellular junction—the region of interacting adjacent plasma membranes. You will read here about various free living cells and certain types of cells which perform movement with the help of different cellular processes in multicellular animals. In this unit, cell adhesions and cellular movements are discussed at length. Extra cellular matrix which is formed as a result of cellular secretion is dealt with in this unit.

Animal tissues are taken up in Unit 19. Broadly, four types of tissues, viz. epithelial, connective, nervous and muscular tissues are described in this unit. You will read about the origin, structure and functions of these tissues. Besides, this unit deals with the structure and origin of mesenchyme cells.

Unit 20 deals with plant cells which are not as specialised as animal cells. Plant cells differ in having a rigid cell wall made up of cellulose and the ability to synthesise their food with the help of plastids in the presence of sunlight. In this unit, we discuss about plants that are composed of individual cells that are organised into groups of cells called tissues. Parenchyme cells are least specialised living cells and most ubiquitous. You will read that collenchyme gives mechanical and tensile strength, whereas sclerenchyma provides mechanical strength to young stems and to those of climbers and twiners. Special types of sclerenchymatous cells are known as sclereids.

Objectives

After having read this block you should be able to:

- explain the cell cycle and its control by DNA,
- describe the different phases of mitosis and the role of spindle microtubules,
- define mitosis and explain chromosomal behaviour during this process,
- discuss the molecular mechanism involved in the process of genetic recombination
- explain the process of cellular recognition and formation of cooperative cellular assemblies,
- appreciate the importance of locomotory apparatus in cellular movements,
- discuss the formation of extracellular matrix and list its main components,
- explain the structure and functions of epithelial, muscular, connective and nervous tissues,
- describe the differentiation process and origin of mesenchyma,
- explain the difference between plant and animal cells,
- list various types of plant tissues and their functions.

UNIT 16 CELL CYCLE AND MITOSIS

Structure

- 16.1 Introduction
 - Objectives
- 16.2 Cell Cycle
 - Interphase—The G₁, S and G₂ Phases
 - G₁—The most Variable Period of the Cell Cycle
 - Events Occur at Defined Stages of Cell Cycle
 - Determination of Cell Cycle Times
- 16.3 Regulation of Cell Division
 - Control of the Cell Cycle in Dividing Cells
 - Factors that Stimulate Cell Division
- 16.4 Mitosis
 - Chromosomal Events
 - Role of Spindle
 - Chromosomal Movements
- 16.5 Summary
- 16.6 Terminal Questions
- 16.7 Answers

16.1 INTRODUCTION

In the previous block you have studied about the structure of nucleus and the various processes occurring within it. Nucleus contains chromosomes which contain DNA and proteins. You have studied how DNA and proteins form complexes and how DNA ultimately synthesises protein by an intermediate RNA. Also, you have read about replication and the flow of informations from nucleus to cytoplasm, for the genetic continuity in Block 3.

In higher eucaryotes life starts with the formation of a fertilized egg cell of approximately 100 μm in diameter leading to the formation of trillions of cells with a greater mass. As more and more cells are added as a result of successive divisions the developing embryos undergo a process of differentiation and adult structures are organised. During this period, the rate of cell division and the number of dividing cells change so that there are fewer cells dividing in a fully formed organism than during the course of development. In an adult organism there are tissues where no cells are dividing and are mitotically inactive, while there are other tissues wherein cells die and are replaced by new cells. There are also some tissues like bone marrow or germinal tissues or meristematic regions in plants where continuous cell division takes place. The cells divide by two specific methods known as 'mitosis' and 'meiosis'. In this section we shall describe the events associated with the process of cell division known as mitosis. You will read about meiosis in the next unit.

Objectives

After reading this unit you should be able to:

- recall the rate of observed cell division in some cell types and relate it to generation/cell/cycle time
- define and use in correct context and terms 'cell cycle'/'generation' time, mitotic index, S, G₁, G₀, G₂
- state the four factors that are considered important in controlling the cell division
- outline methods to determine different phases in a cell cycle
- explain in brief as to how the cell cycle control is studied
- summarise the logic for the minimum cell cycle time
- distinguish the different phases of mitosis and draw diagrams of the same
- list the functions of spindle
- give causes for chromosomal movements during mitosis.

16.2 CELL CYCLE

A growing cell undergoes a cell cycle that comprises essentially two periods:

- (1) interphase i.e. the period of non-apparent division and (2) the period of division

(Fig. 16.1). In eucaryotes, division generally takes place by mitosis or meiosis. For many years, cytologists were concerned mainly with the period of division in which dramatic changes visible under the light microscope could be observed, and interphase was considered a "resting" phase. Cells, however, spend most of their life span in interphase. The interphase is a period of intense biosynthetic activity in which the cell doubles in size and duplicates precisely its chromosome complement. Some differentiated cell types divide rarely such as red blood cells, skeletal muscle cells and nerve cells and you will be amazed to know that the mammal nerve cells do not divide at all after birth. Thus, for a human neuron the interphase period lasts the entire life time of a person.

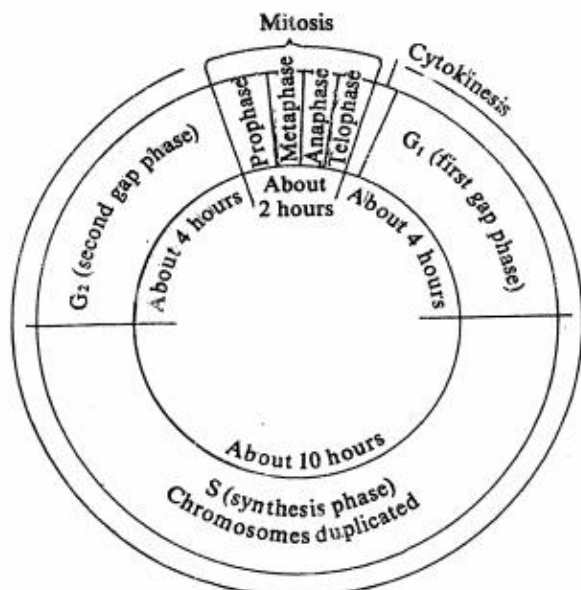


Fig. 16.1 The cell cycle. Time intervals are relative; actual time varies with the cell type and species

Therefore, the cell cycle represents a complex series of stage by which cellular material is divided equally between daughter cells. Cell division is only the final phase. Before, the cell divides by mitosis, its main molecular components have already been duplicated. In this respect, cell division can be considered as the final separation of the already duplicated molecular units.

16.2.1 Interphase—The G₁, S and G₂ Phases

The life of the cell is mostly spent in interphase, a stage between the two successive cell divisions. The introduction of cytochemical methods such as the feulgen stain, followed by cytophotometric quantitative assay and radioautography, for the first time, suggested that the doubling of DNA takes place during interphase. These studies demonstrate that the synthesis occurs only in a restricted portion of the interphase stage, the so called S-period or synthetic period which is preceded and followed by two "gap" periods in G₁ and G₂. There is no DNA synthesis between G₁ and G₂ phase of cell cycle which is divided into four successive stages: S is the period of DNA synthesis and G₂, the interval between the end of DNA synthesis and the start of mitosis.

During G₂ a cell contains twice the amount of DNA present in the original diploid cell. Following mitosis the daughter cells again enter the G₁ period and have a equivalent DNA content.

16.2.2 G₁—The Most Variable Period of the Cell Cycle

G₁ is the time interval between mitosis and the beginning of S-phase, which is associated with the growth and increased activity of enzymes specific for DNA synthesis. The duration of the cell cycle varies greatly from one cell to another. For mammalian cell growing in culture with a generation time of 16 hours, the different periods would be as follows: G₁=5 hours, S=7 hours, G₂=3 hours and mitosis=1 hour. Generally, the S, G₂ and mitotic periods are relatively constant in the cells of

same organism. The G₁ period is the most variable in cell life. Depending on the physiological condition of the cells it may last days, months or years.

The regulation of the duration of the cell cycle occurs primarily by arresting it at a specific point of G₁ and the cell in the arrested condition is said to be in the G₀ state. In the G₀ state the cell may be considered to be withdrawn from the cell cycle (Fig. 16.1). When conditions change and growth is resumed the cell reenters the G₁ period.

Eucaryotic chromosomes undergo condensation-decondensation process during interphase (Fig. 16.2).

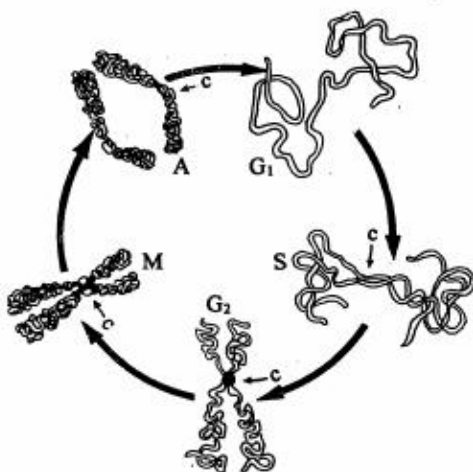


Fig. 16.2 The condensation-decondensation cycle of chromosomes. G₁ chromosomes are completely dispersed; S, duplication occurs; and G₂ condensation starts. At metaphase, M and anaphase, A, the condensation is maximal and the two centromeres are clearly visible.

The DNA of prokaryotes is not processed in this way and this forms a major difference between eucaryotes and prokaryotes. The DNA of prokaryotes is replicated continuously. Most protein and RNA synthesis occurs through the interphase.

16.2.3 Events Occur at Defined Stages of the Cell Cycle

If the biochemical events that occur at defined stages of the cell cycle (Fig. 16.3), the most noticeable one is DNA synthesis. S-phase cells contain a factor that induces DNA synthesis. This has been demonstrated by cell fusion experiments, in which one set of DNA replication in G₁ nuclei can be accelerated by fusion with S-phase cells. It is important to note here that G₂ nuclei do not respond to this factor, indicating that some mechanism must exist to block reinitiation of DNA synthesis within the same cell cycle. Evidence for a factor that includes DNA synthesis comes from multi-nucleated cells, such as the plasmodium of the slime mold *Physarum polycephalum*, in which all the nuclei start DNA synthesis simultaneously.

S-phase lasts for several hours, during which many units of replication are sequentially activated. In all cells, the more condensed, heterochromatic regions of the chromosomes replicate late during S-phase. The centromeric heterochromatin which contains satellite DNAs, replicates later than the rest of the chromosome. Similarly, the X-chromosome that becomes inactivated in mammalian females is heterochromatic and late replicating, whereas the one that remains activated is euchromatic and replicates earlier, about which you have read in Unit 13. Molecular events linked to the cell cycle, including the synthesis of histones during S-phase; the decrease in protein synthesis that occurs during mitosis; the decrease in cAMP levels during mitosis, phosphorylation of histones (especially H1) during chromatin condensation and many other events.

The most important point in the regulation of the cell cycle occurs in the G₁ phase, during which it must decide whether the cell will start a new cell cycle or becomes arrested in the G₀ state. Once this G₁ checkpoint has been passed, the cell goes on to complete a new cycle.

Fig. 16.3 Molecular events during the cell cycle

How does one determine the length of each phase of the cell cycle? First it is necessary to establish the length of the total cycle, which can easily be done in a homogeneous population of cultured cells by periodically counting the number of cells present under a microscope and reecording the number of hours required for the total cell number to double. Alternatively, the total cell mass can be monitored. Once this interval is known the length of the S-phase can be estimated by adding titrated thymidine to the tissue culture medium for a brief period that is much shorter than the S-phase itself (typically 30 minutes or less). The cells are then preserved for autoradiography and the fraction of the cells that have incorporated the radioisotope into their DNA is determined by counting the fraction of cell with exposed silver grains over their nucleus. As explained in Figure 16.4, this fraction multiplied by the total cell cycle time is roughly equal to the average length of the S-phase in the population.

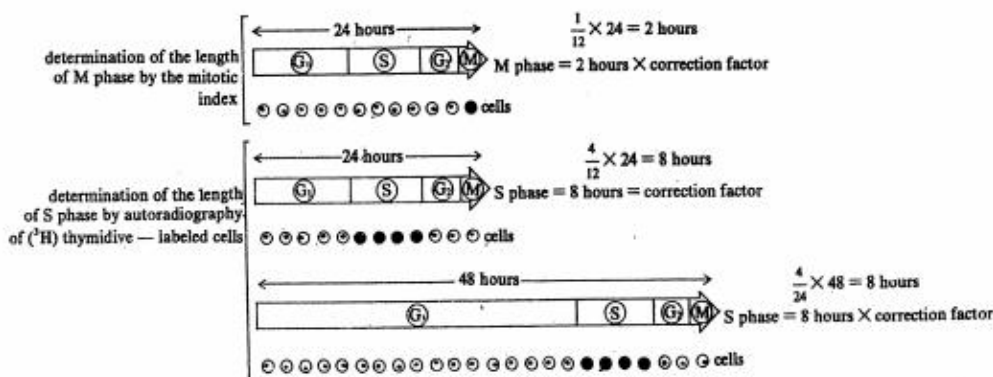


Fig. 16.4 Illustration of the general principle that the length of each phase of the cell cycle is approximately equal to the fraction of the cells in that phase at any instant, multiplied by the total cell cycle time. This calculation is based on the assumption that all the cells in the population are growing at the same rate. The indicated 'correction factors' range from 0.7 for early G1 cells to 1.4 for mitotic cells with an intermediate value for S-phase cells. A correction factor is needed because there are always more young cells than old cells in a continuously dividing population. The exact age distribution is given by the equation $y=2^{x-1}$ whereby varying from 2 to 1 is the relative number of cells at cell cycle age x (where x varies from 0 for the earliest G1 cells to 1 for the latest M-phase cells).

The length of M-phase can be determined in an analogous fashion by scanning the cell population by light microscopy and determining the fraction of cells containing condensed chromosomes at any one time since the last cell division. For example, a single old cell becomes two young cells as soon as it divides, and there will be twice as many cells in early G₁ as in late M-phase.

Cell cycle analysis has been made much easier by the use of a fluorescence-activated cell analyser. With the help of this analyser one can rapidly determine the relative fluorescence of a large number of cells, and their relative amounts of DNA. Those cells with the least amount of DNA are in G₁ phase, those with double this amount are either in G₂ or M, while cells in S have intermediate amounts (shaded area in Figure 16.5).

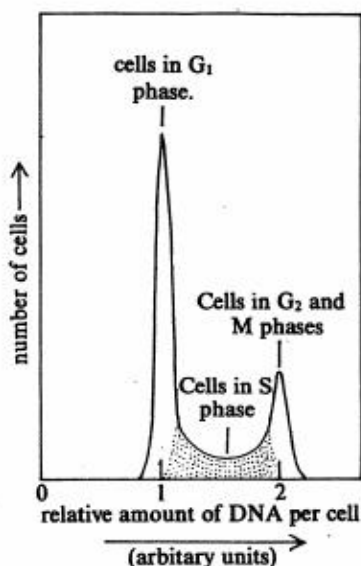


Fig. 16.5 Typical results obtained for a growing cell population when the DNA content of its individual cells is determined with a fluorescence analyser. DNA amounts are given on arbitrary units and are determined by the amount of fluorescent dye bound. The fact that the number of cells in G₁ is much greater than the number of cells in G₂ and M indicates that the G₁ phase is longer than G₂ phase in this population.

The length of G₁, and G₂ plus M and S-phase of the cell cycle can be readily calculated (Fig. 16.4).

SAQ 1

State in the boxes provided, whether the following statement regarding cell cycle are true or false. In the case of a false answer, write the correct answer also in the given space

- i) The interphase is a period of intense biosynthetic activity in which the cell doubles in size and duplicates precisely its chromosome complement ()
-
-
-
- ii) During G₂ a cell contains four times (4C) the amount of DNA present in () the original diploid cell
-
-

- iii) The DNA synthesis does not occur in a restricted portion of the interphase the so called S-period ()
- iv) Tissues such as nerve cells or skeletal muscles do not normally divide or they rarely divide ()
- v) During cell division eucaryotic chromosomes do not undergo condensation decondensation whereas the DNA of procaryotes is cycled in this way ()
- vi) Cells with the least amount of DNA are in G₁ and those with double this amount are either in G₂ or M, whereas cells in S-phase have intermediate amount ()

16.3 REGULATION OF CELL DIVISION

You have studied till now the cell cycle and cell division. The manner in which mitosis is regulated is yet to be understood. However, cell division regulation encompasses two fundamental questions: firstly, by what mechanism the cell cycle in dividing stage is controlled in cells, and secondly by which mechanisms external agents trigger the onset of cell division in cells that are not actively dividing.

16.3.1 Control of the Cell Cycle in Dividing Cells

As pointed out in the earlier section cells generally shorten or lengthen the times between successive cell divisions by shortening or lengthening the time spent in G₁ stage. The regulation of the eucaryotic cell cycle is intimately associated with events during the G₁ phase. Direct evidence for the importance of G₁ in regulating the cell cycle has come from studies that limit cell growth, such as lack of nutrients or appropriate growth factors. Under such conditions, cells stop growing at specific point in the cell cycle situated near the end of G₁ called the restriction point, and then enter into a special phase called G₀, where they may exist for long periods outside the normal cycle of cell division.

The cell fusion experiments reveal that cytoplasmic factors present in S-phase cells are capable of triggering the premature entrance of G₁ nuclei into S-phase. Such observations suggest that the production of specific substances during G₁ is responsible for triggering the entry into S-phase.

Yeast cells have helped us in understanding molecular event that trigger the transition from G₁ to S-phase. Yeasts are powerful tool for investigating the cell cycle because of the ease of production of genetic mutants which can be characterised. Such studies have shown that a small group of genes controlling an event occur in G₁, called start, this represents the point when cells become committed to entering the mitotic cell cycle. Cells enter into a programme leading to S-phase, mitosis and cell division. The genes found to be essential for the start junction, codes for a protein resembling a protein kinase (an enzyme catalysing the phosphorylation of other proteins).

In bacterial cells commitment to the cell division cycle requires the production of DNA binding proteins that destabilise the double helix and allowing the enzymes involved in replication to have direct access to the DNA. Proteins capable of destabilising the DNA double helix are involved in the initiation of DNA replication in eucaryotic cells about which you have studied in Block 3. The mechanism responsible for unpacking this nucleosomal organisation is not known in detail, but it is thought that phosphorylation of histone molecules by protein kinase occurs during S-phase. Histone phosphorylation might be an essential requirement for the entrance of the cell into S-phase.

Although G1 phase appears to be the main site of regulation of the cell cycle in eucaryotes, some cells stop their cycle in G2. Such G2-arrested cells are capable of quickly initiating mitosis in response to an appropriate stimulus, and therefore occur in places such as skin where rapid cell division may be required in response to an injury. In contrast to G2-arrested cells, cells in either G0 or G1 require many hours to enter mitosis because of the necessity of first passing through the remainder of G1, S, and G2. The completion of the cycle from G1 to G2 varies from 8-20 hours in actively growing animal and plant cells.

16.3.2 Factors that Stimulate Cell Division

In addition to the importance of metabolic events occurring within the cell, external conditions also exert controls over the process of cell division. In procaryotic cells the major controlling factor is the presence of sufficient nutrients in the external environment. As long as adequate nutrients are available, cell division generally continues. In eucaryotic cells, which are typically multicellular organisms, division of each cell must be carefully regulated to suit the needs of the organism as a whole. A variety of external factors are involved in coordinating the division of such cells. Certain steroid hormones such as testosterone and estrogens have been found to stimulate the growth and division of specific types of animal cells; while hormones like auxins, cytokinins, and gibberellins stimulate the division of plant cells. Although the preceding types of hormones act by entering directly into their target cells, other growth promoting factors have been identified which bind to specific receptors in the plasma membrane. Well known examples of such growth factors include epidermal growth factor, platelet-derived growth factor, nerve growth factor, thymosin and erythropoietin. All these proteins stimulate the proliferation of a specific group of target cells.

Although the mechanism by which such molecules stimulate cell division is not completely understood, progress has been made in unravelling some of the early steps involved. One of the more thoroughly investigated examples involves the response of cultured cells to the addition of epidermal growth factor (EGF), a polypeptide of approximately 6400 molecular weight. Experiments with radioactive EGF have revealed that this polypeptide first binds to specific membrane receptors located on the surface of the cell being stimulated to divide. These receptors which are initially distributed randomly over the cell surface, become clustered into patches after binding to EGF and are subsequently taken into the cell by endocytosis.

One of the first biochemical changes observed in cells treated with EGF is an increased rate of phosphorylation of tyrosine residues located in certain intracellular proteins as well as the EGF receptor itself. The enzymatic activity responsible for catalysing this tyrosine-specific phosphorylation reaction resides in a portion of the EGF receptor that faces the interior. In other words the EGF receptor, a trans-membrane protein, binds to EGF molecules at the outer surface of the plasma membrane and it functions as a tyrosine-specific protein kinase at the inner surface of the plasma membrane (Fig. 16.6). Binding of EGF to the membrane receptor causes its protein kinase to become activated. The ability of certain tumor viruses to transform normal cells into cancer cells also involves the production of a tyrosine specific protein kinase.

The above information indicates that the interaction of external factors with the plasma membrane can trigger intracellular changes by altering the activity of an enzyme localised within the plasma membrane. The binding of any protein hormones to the plasma membrane triggers activation of the membrane-bound enzyme adenylate cyclase which catalyses the formation of 3', 5'-cyclic adenosine monophosphate (cyclic AMP). Intracellular concentration of cyclic AMP has been found to change during the normal cell cycle as well as in response to agents that stimulate or inhibit cell division. Intracellular calcium can also trigger the onset of cell division with the help of protein calmodulin.

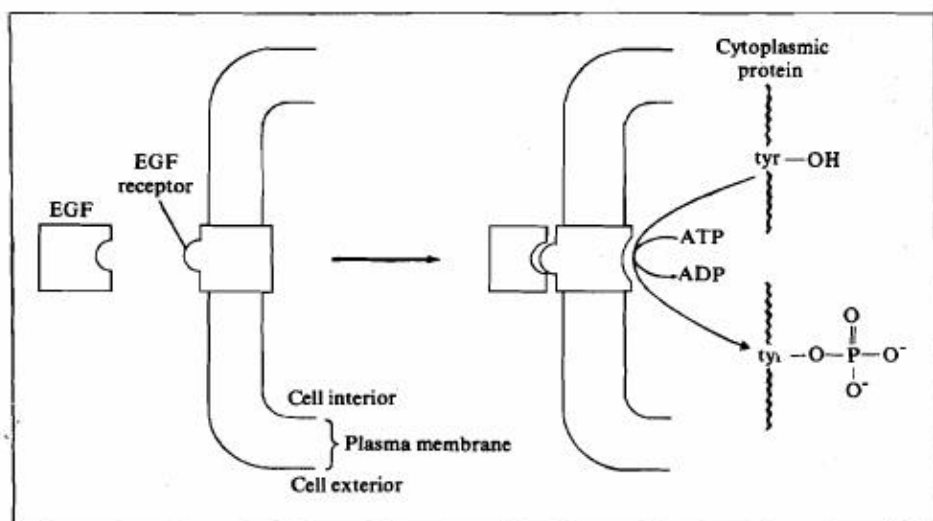


Fig. 16.6 Summary of the mechanism by which epidermal growth factor (EGF) influences protein phosphorylation. Binding of EGF influences protein phosphorylation. Binding of EGF to the exterior surface of its plasma membrane receptor cause a conformational change in the receptor that permits it to function as a tyrosine-specific protein kinase at the cytoplasmic surface of the plasma membrane. This enzymatic activity in turn catalyses the phosphorylation of the amino acid tyrosine (tyr) in specific cytoplasmic proteins.

EXQ 2

In the set of statements given below, tick mark the correct answer and cross the wrong ones.

- a) The study on yeast cells has indicated that
- the alternative for the molecular event does not trigger the transition from G1 to S-phase.
 - the alternative for the molecular event triggers the transition from G1 to S-phase.
 - the alternative for the molecular event reverses transition from G1 to S-phase.
- b) The cell fusion experiments reveal that
- cytoplasmic factors present in S-phase cells are capable of triggering the entry of G1 nuclei into S-phase.
 - the non-availability of cytoplasmic factors in S-phase cells are capable of triggering the entry of G1 nuclei into S-phase.
 - cytoplasmic factors present in S-phase cells are capable of triggering the immediate entry of G1 nuclei into S-phase.
- c) In prokaryotic cells
- the main controlling factor appears to be the presence of sufficient nutrients in the external environment.
 - the main controlling factor appears to be the absence of sufficient nutrients in the external environment.
 - the main controlling factor is due to the absence of certain growth factors in the external environment.
- d) Studies have confirmed that
- binding of many protein hormones to the plasma membrane does not trigger activation of the membrane-bound enzyme adenylate cyclase that catalyses the formation of 3',5' cyclic adenosine monophosphate.
 - binding of many protein hormones to the plasma membrane bound enzyme adenylate cyclase that inhibits the catalysis of the formation of 3',5',5'-cyclic adenosine monophosphate (cyclic AMP).
 - binding of many protein hormones to the plasma membrane triggers activation of the membrane-bound enzyme adenylate cyclase that brings about catalysis of adenosine monophosphate.

Mitosis is a complex process which splits the chromosomes equally into two daughter cells. It involves maintenance of chromosomal continuity and diploid number. The continuity of the chromosomal set is maintained by a special type of cell division which is called mitosis. At the time of cell division the nucleus becomes completely reorganised. In a somatic cell the nucleus divides by mitosis in such a way that each of two daughter cells receives exactly the same number and kind of chromosomes that the parent cell had.

16.4.1 Chromosomal Events

Figure 16.7 represents two pairs of homologous chromosome in a diploid nucleus. Each chromosome duplicates itself sometime during interphase before the visible mitotic process begins. Although mitosis accounts only for a short period of time relative to the total time between cell division, the remaining portion of the cell cycle is termed interphase. The nucleolus is the only structure that is visible under the light microscope.

I) Interphase

During interphase, chromosomes are present in a different network of chromatin that is not visible under the light microscope as an individual i.e. DNA-protein complexes called chromatin are dispersed throughout the nucleoplasm. The events during mitosis that follow unfolding are conventionally divided into four sub-stages: prophase, metaphase, anaphase and telophase (Figure 16.7).

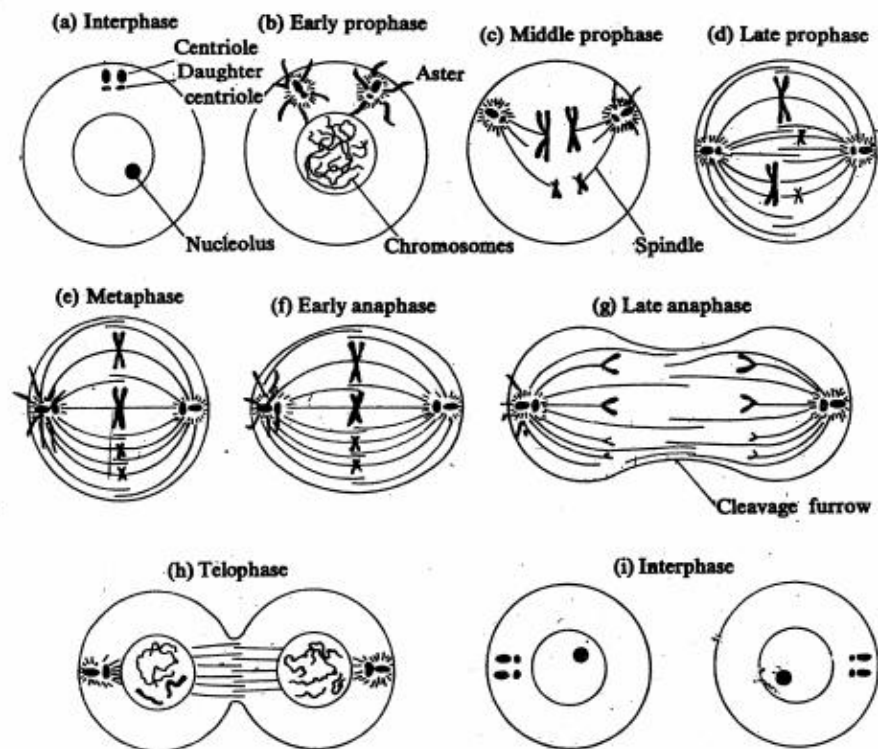


Fig 16.7. The stages of mitosis and cytokinesis in an animal cell

Prophase marks the beginning of the cell cycle in which condensation of chromatin fibres occurs in discrete and visibly separate chromosomes. During prophase, each chromosome consists of two identical units termed as sister chromatids each of which contains identical daughter DNA molecules that were produced in the S-phase. As prophase advances the chromatids become more condensed, owing to the packing of the nucleoprotein fibres. Sister chromatids are held together at their centromeres or constricted regions about which you have read in 10+2 course. At this time, the small cylindrical centrioles begin to play a key role. As the centrioles move apart, they generate fibrous microtubules which radiate from them in all

directions. Centrioles, which are themselves formed of microtubules, duplicate during interphase by forming daughter centrioles at right angles to their own positions. Some of these microtubules connect the centrioles with the kinetochores, granular regions that are attached to the centromeres of the chromatids. Other microtubules form a network that links the centrioles as they move apart. These microtubules, together with associated fibres and proteins, are called the spindle. The regions surrounding the centrioles, from which the microtubules radiate, are termed the poles or polar regions of the cell. At the end of prophase the nuclear membrane and nucleolus disappear, and each chromatid remains attached to spindle microtubule at its centromere.

II) Early Prophase

The centrioles begin moving towards opposite poles of the cell; the chromosomes can be seen as long threads, and the nucleus is dispersing and becoming less distinct.

III) Middle Prophase

Chromosomes condensation is completed; each chromosome is composed of two chromatids held together at their centromeres. Each chromatid contains one of the two newly replicated daughter DNA molecules. The microtubular spindle begins to radiate from the regions just adjacent to the centrioles, which are moving close to their poles.

IV) Late Prophase

The centrioles reach the poles, and some spindle fibres extend from pole to the centre or equator of the cell. Other spindle fibres extend from the poles to the chromatids and attach to the kinetochores, which are near the centromeres of the chromosomes. The nuclear membrane begins to disperse and disappear, and the nucleolus is not visible.

During metaphase, the condensed sister chromatids which are connected to each pole of the cell by the microtubules attached to their kinetochores, and migrate to the equatorial plane of the cell. The microtubules attached to the chromosomes appear to play a role in orienting them. It is the time when chromosomes appear thick and discrete and can easily be identified, and counted and photographed for studies of chromosomal abnormalities.

V) Metaphase

The chromosomes move towards the equator of the cell, where they become aligned in the equatorial plane. The sister chromatids have not yet separated.

VI) Early Anaphase

The two daughter chromatids separate. Each contains a centromere that is linked by a spindle fibre to one pole. Led by the centromere, each chromosome begins to move towards the pole to which it is attached. Simultaneously, the cell elongates as do the pole-to-pole spindles.

VII) Late Anaphase

Each set of chromosomes (the daughter chromatids) moves closer to its pole and cytokinesis begins as the cleavage furrow starts in animal cells (Fig. 16.8).

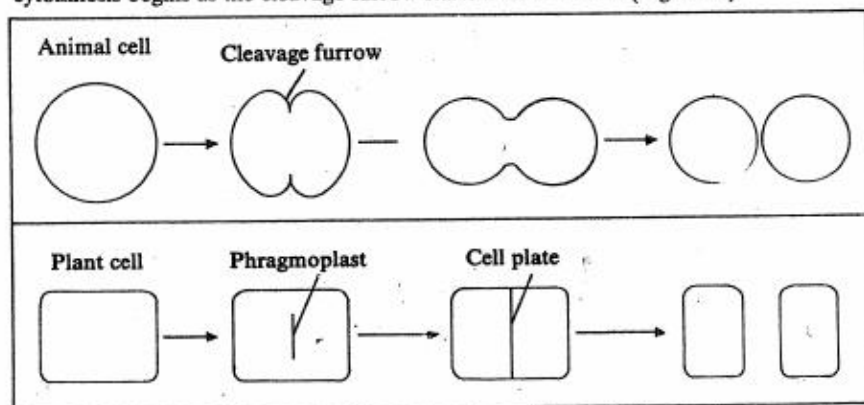


Fig. 16.8 Diagram summarizing the difference between the mechanism of cytokinesis in animal and plant cells. In animal cells invagination of the plasma membrane proceeds from the periphery of cell towards the interior; in plant cells the phragmoplast begins to form in the centre of the cell and then expands towards the periphery.

Anaphase is marked by the separation of the two sister chromatids at their centromeres. This happens simultaneously to the entire set of chromosomes. The members of each chromatid pair now exist as independent chromosomes, that migrate to opposite poles of the cell, with each daughter cell having the same chromosome complement. The movement towards the two poles is due to a shortening of the microtubules attached to the kinetochores.

VIII) Telophase

It is the final stage of mitosis and reaches in interphase stage. New nuclear membrane is formed around each daughter nucleus and when the chromosomes uncoil and become less distinct, the nucleolus becomes visible again. Throughout mitosis the "daughter" centriole at each pole has continued to grow until it is fully formed. At telophase the duplicate of each of the original centrioles is completed and each of the two centrioles at each pole begins to generate a new daughter centriole at right angles to it.

When cytokinesis is nearly complete, and the spindle disappears as the microtubules and other fibres depolymerise, the nucleoli reappear.

16.4.2 Role of Spindle

In most eucaryotic cells, chromosome movement is dependent on the presence of the mitotic spindle. This spindle apparatus contains at least three different types of microtubules (1) astral microtubules, which radiate from the centrioles that are only found in animal cells, (2) kinetochore microtubules, which attach to the chromosomal kinetochores, and (3) inter polar microtubules, which span the area between the two spindle poles and account for the bulk of spindle's structure (Fig. 16.9).

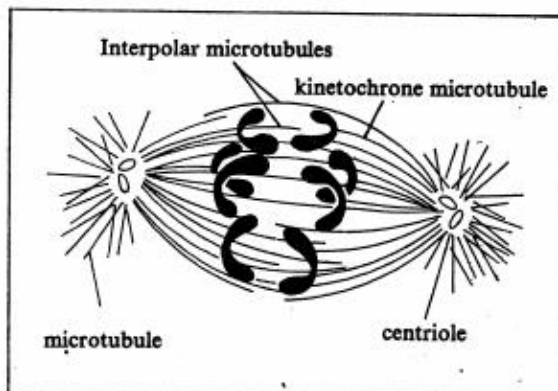


Fig. 16.9 Diagrammatic model of the spindle apparatus illustrating the three types of spindle microtubules

The assembly of spindle is regulated by changes in the equilibrium between the polymerised and unpolymerised states of tubulin. It was found that exposing cells to low temperature, high pressure, or the drug colchicine causes breakdown of the mitotic spindle and the cessation of mitosis. However, the spindle spontaneously reforms, as soon as the temperature is raised, the pressure lowered or colchicine removed. This rapid reappearance of the spindle occurs even when inhibitors of protein synthesis are present, indicating that the assembly of spindle is not dependent on the synthesis of new spindle proteins. Calcium ions play an important role in the process of microtubule assembly/disassembly during normal mitosis. Micro injection of Ca^{++} into metaphase cells has been found to hasten the onset of anaphase while conditions that lower the cytoplasmic Ca^{++} concentration show the progression into anaphase. The mitotic spindle has been found to contain large amounts of calmodulin, a calcium binding protein that mediates the regulatory effects of calcium ions upon a variety of cellular processes.

Centrioles usually replicate during the S-phase of the cell cycle yielding two pairs of centrioles that migrate to opposite sides of the nuclear envelope at the time of prophase. Careful replication is not a true division but rather a sequence of events in which each pre-existing centriole induces the formation of a new centriole.

At the beginning of prophase, the two pairs of centrioles and their associated asters begin to separate and move towards opposite sides of the cell. At the same time, spindle microtubules start forming between the centriole pairs. By the time centriole

have reached opposite sides of the cells, the spindle microtubules are almost completely assembled. Microtubules exhibit an intrinsic polarity, one end functioning in tubulin assembly and the other functioning in disassembly. Centrioles themselves are not directly involved in generating the mitotic spindle.

16.4.3 Chromosomal Movements

Several theories have been proposed to explain the mechanism of the movement of the two sets of chromosomes to opposite poles of the spindle during anaphase. Two distinct types of movements occur at this time: migration of the chromosomes towards the poles and movement of the two poles away from one another. Several observations suggest that the spindle microtubules play a central role in these two types of movements. The kinetochore microtubules are the only external structures that appear to be attached to the moving chromosomes. The kinetochore region of the chromosome leads the way during chromosome movement, suggesting that the chromosome is being pulled towards the pole by the microtubules attached to the kinetochore. The chromosome movements can be inhibited by exposing cells to microtubule disrupting agents such as colchicine.

A general description of three types of interactions for chromosome movements is presented below:

i) Microtubule-Microtubule Interactions

You also know that ciliary and flagellar movements are based on the active sliding movement that is driven by the transient formation and breakage of bonds between adjacent microtubules. The reaction involves the ATP hydrolysing protein, dynein. The chromosome separation during mitosis is driven by the two sets of forces: a dynein-based, ATP-requiring process of microtubule sliding that pushes the spindle poles apart, and a separate ATP independent mechanism for pulling the chromosomes towards the pole.

ii) Microtubule Polymerisation/Depolymerisation

Although the microtubule sliding plays a role in chromosome movement, other factors also play a role. First the overall length of the interpolar microtubule increases as the two poles of the spindle separate from one another, and secondly the individual kinetochore microtubules shorten as the chromosomes move towards the pole. These changes suggest that interpolar microtubules are being lengthened by the addition of tubulin subunits to their polymerising ends, while the kinetochore microtubules are being shortened by the loss of tubulin subunits from their depolymerising ends.

iii) Interaction of Microtubules with other Components

A number of proteins other than tubulin have recently been detected within the mitotic spindle. The components other than microtubules may provide the driving force for chromosome movement. Proteins like actin and myosin which have been detected in close association with the spindle apparatus suggest their possible role in chromosome movement. The role of actin and myosin in other types of motility is well established. However, their role in chromosome movement has not been established as yet.

SAQ 3

Give short answer to the questions given below

- i) What signals the beginning of the cell division ?

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- ii) In which stage does nuclear fragments help to form the new nuclear membrane ?

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iii) What regulates the assembly of spindle?

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iv) What indicates the chromosomes being pulled towards pole by the microtubule?

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16.5 SUMMARY

Let us summarise what we have learnt so far.

- A growing cell consists of two essential periods, interphase i.e. the period of non-apparent division and the period of division. The cell cycle is the complex series of stages by which cellular material is divided equally between two daughter cells.
- Doubling of DNA takes place during interphase and the synthesis of DNA occurs only in restricted portions of the interphase called S-period followed by the "gap" periods of interphase (G1 and G2) in which there is no DNA synthesis. S-phase cells contain a factor which induces DNA synthesis and this phase lasts for several hours. The regulation of the cell cycle occurs in the G1 phase, during which it has to decide whether the cell will start a new cell cycle or become arrested in the G0 state. Once this G1 check point has been passed, the cell goes on to complete a new cycle.
- Mitosis is a complex process that splits chromosomes equally into two daughter cells and maintains chromosomal continuity and diploid number. The stage of mitosis comprises interphase, early prophase, middle prophase, late prophase, metaphase, early anaphase, late anaphase, telophase and cytokinesis.
- Assembly of spindle is regulated by the polymerised and unpolymerised states of tubulin. During interphase, centrioles are surrounded by the scattered cluster of short microtubules. At the beginning of prophase, the two pairs of centrioles and their associated aster begin to separate and move towards opposite poles of the cell. By the time, centrioles have reached opposite sides of the cell, the spindle microtubules almost completely disappear. Microtubules exhibit an intrinsic polarity, one end functioning in tubulin assembly and the other functioning in disassembly.
- Two distinct types of movements occur during anaphase i.e. migration of the chromosomes towards the poles and the movement of the two poles away from one another. Spindle microtubules play a central role in these two types of movements.

16.6 TERMINAL QUESTIONS

1) What are cell cycle and period of intense biosynthetic activity?

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- 2) Describe the factors that regulate cell division.

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- 3) State the role played by spindle in mitotic cell division.

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16.7 ANSWERS

Self-assessment Questions

- 1) (i) True (ii) False (iii) False (iv) True (v) False (vi) False (vii) True
- 2) a) (i) Correct, b) (ii) Correct, c) (i) Correct, d) (iii) Correct
- 3) i) The beginning of the cell division is signalled by the appearance of chromosomes as thin threads inside the nucleus.
ii) During telophase nuclear fragments begin to form nuclear membrane.
iii) Assembly of spindle is regulated by changes in equilibrium between the polymerised and unpolymerised states of tubulin.
iv) Kinetochore microtubules are the external structures attached to the moving chromosomes and the chromosomes movements can be inhibited by exposing cells to microtubule disrupting agents such as colchicine.

Terminal Questions

- 1) A growing cell undergoes a cell cycle which comprises essentially two periods (1) the period of non-apparent division and (2) the period of division. The interphase is a period of intense biosynthetic activity in which the cell doubles in size and duplicates precisely its chromosome complement.
- 2) Certain direct evidences indicate that conditions such as lack of nutrients or appropriate growth factors limit cell growth. Cytoplasmic factors present in S-phase are known to be capable of triggering the premature entry of G1 nuclei into S-phase.
- 3) In eucaryotic cells, chromosome movement is dependent on the presence of the mitotic spindle. Spindle formation is regulated by changes in the equilibrium between the polymerised and unpolymerised states of tubulin. Several observations suggest that the spindle microtubules play a central role in the movement of the two sets of chromosomes to opposite poles of the spindle during anaphase.

UNIT 17 MEIOSIS AND CELL DIVISION

Structure

- 17.1 Introduction
 - Objectives
- 17.2 Meiosis
 - Chromosomal Events
- 17.3 Genetic Recombination
 - Molecular Theory of Recombination
 - How does Meiosis Differ from Mitosis
- 17.4 Cytokinesis
 - Cytokinesis in Animal Cells
 - Cytokinesis in Plant Cells
 - Distribution of Cytoplasmic Components
- 17.5 Summary
- 17.6 Terminal Questions
- 17.7 Answers

17.1 INTRODUCTION

In the previous unit (16) you have studied about mitosis, cell cycle and factors that determine and control cell cycle and cell division. You have also read about the chromosomal events taking place during mitosis. Mitosis gives rise to daughter cells, each of them receiving exactly the same **number** and **kind** of chromosomes that the parent cell had. During the late 1800s, it became apparent that another, modified form of cell division must occur in the life cycle of sexually reproducing organisms in order to ensure the constancy of the chromosome number in the successive generations. The term meiosis is taken from the Greek word **meioun**, which means 'to diminish'. This unit deals with the process of meiosis.

In addition to reducing the chromosome number of the daughter cells, meiosis also brings about **recombination**, a mechanism by which segments of chromosomes are physically exchanged to mix the gene sequence into new combinations. Recombination provides a variety of genetic types to meet the demands of a changing environment. Here you will study chromosomal behaviour during meiosis, genetic recombination and also about division of the cytoplasm, i.e., **cytokinesis**.

Objectives

After reading this unit you shall be able to:

- draw diagrams to show chromosomal behaviour in various phases of meiotic prophase
- explain the molecular theory of genetic recombination
- list the chromosomal and genetic differences in daughter cells produced by mitosis and meiosis
- give distinctive features of cytokinesis in plant cells compared with those in the animal cells.

17.2 MEIOSIS

You must be aware that organisms reproduce basically in two different ways:

i) **Asexual** reproduction, and ii) **Sexual** reproduction. In asexual reproduction, a single parent usually splits, buds, or fragments to give rise to two or more individuals. The individuals produced by the ways mentioned above is through the process of mitosis, and therefore have the same number of chromosomes as that of their parent. They also have identical genes and therefore inherit traits similar to those of their parent organism.

In contrast, sexual reproduction involves two parents, each of which contributes a specialised sex cell, or **gamete**. In the case of animals and plants, the male gamete is called **sperm** and the female gamete is called an **ovum** or **egg**. At the time of

fertilisation the male and the female gametes fuse to form a **zygote**. The zygote divides through a series of mitotic divisions to give rise to a new individual.

You must be aware that the number of chromosomes carried by the cells in an individual, and all the individuals of a species is constant. Chromosomes normally exist in pairs; there are two of each kind in the somatic (body) cells of higher plants and animals. Thus, the 46 chromosomes in human cells constitute 23 different pairs. The member of a pair, called **homologous chromosomes** are similar in size, in shape, and in the position of their centromere. A set containing two of each kind of chromosome is said to have the **diploid** number or $2n$.

You have learnt above that, at the time of fertilisation male and the female gamete fuse to form a zygote. Let us consider that in an organism four is the diploid number of chromosomes and the gametes too carry four chromosomes. In this case, when the gametes fuse to form a zygote, the chromosomal number in the zygote will be 8 ($4+4=8$) and all the cells derived from the zygote would contain 8 chromosomes. Thus chromosome number would increase at the turn of each generation by the square of number of chromosomes of the previous generation. This would consequently lead to an untenable situation. There would remain no significance to the number of chromosomes an organism carries. But in nature this is not so. We see that chromosome number is constant no matter how many generations succeed one another. The constancy of the chromosomal number in successive generations of sexually reproducing organisms is ensured by the process of **meiosis**. Gametes have only one homologue of each homologous pair and thus have the **haploid** or n number of chromosomes. At the time of fertilisation when the sperm and egg fuse, each gamete contributes its haploid number of chromosomes; the diploid number is thereby restored in the fertilised egg (zygote). When the zygote divides by mitosis to form the first two cells of the embryo, each cell receives a diploid set of chromosomes; thus most body cells are diploid. Now we will study about the behaviour of chromosomes during meiosis.

17.2.1 Chromosomal Events

Meiosis consists of a series of nuclear and cell divisions, designated as the first and second meiotic divisions, or simply **meiosis I** and **meiosis II**. Each of these includes prophase, metaphase, anaphase and telophase. During the first meiotic division, the members of each of the homologous chromosomes separate and are distributed into separate cells. In the second meiotic division, the chromatids that make up each chromosome separate and are distributed to the daughter cells. In this way the number of chromosomes is reduced by half, which gives rise to four haploid cells at the end of meiosis. The meiotic division takes place at the end of the G₂ phase of the interphase, as in the case of mitotic cell division.

The essential processes that take place during meiosis are:

- pairing (synapsis) of the homologous chromosomes
- formation of chiasmata, and
- segregation of homologous chromosomes.

From the morphological point of view the prophase of the first meiotic division is a long process during which homologous chromosomes pair closely and interchange hereditary material. For convenience the first meiotic prophase is divided into the following five stages:

- Leptonema (Leptonema)
- Zygotene (Zygonema)
- Pachytene (Pachynema)
- Diplotene (Diplonema)
- Diakinesis

However these stages are not sharply demarcated. Now we will study about each of these stages in detail.

Leptonema: Leptonema stage begins when each chromosome is first seen to have condensed from its interphase conformation to produce a long thread with a proteinaceous central axis. Each chromosome is attached at both of its end to the nuclear envelope via a specialised structure called an **attachment plate**. Although each chromosome has replicated and consists of two **sister chromatids**, these chromatids are closely opposed and therefore appears to be single (Fig. 17.1A).

Zygotene: Leptotene is considered to end and the zygotene stage begins as soon as intimate pairing between the two homologous chromosomes is initiated by the process called **synapsis** or zygotene pairing. The homologous chromosomes which pair are coming from different sexes (father and mother). Synapsis often starts when the homologous ends of the two chromosomes are brought together on the nuclear envelope and continues inwards in a zipper-like manner from both ends, aligning the two homologous chromosomes side by side (Fig. 17.1B). The pairing is completed in three different ways, as follows:

- i) **Proterminal Pairing:** The two homologous chromosomes start pairing at the ends or terminals, which gradually progress towards the centromere.
- ii) **Procentric Pairing:** The pairing starts at the centromere and proceeds towards the end.
- iii) **Random or Intermediate Pairing:** The pairing may be at many points towards the ends.

As a result of synapsis each gene is thus thought to be brought into close contact with its homologous gene on the opposite chromosome. The two homologous chromosomes are brought together to form a characteristic ladder-like structure, called **synaptonemal complex**. Each homologous chromosome pair in meiotic prophase I consists of two closely opposed sister chromatids, thus each pair containing four replicas known as **tetrads**.

Pachytene: As soon as the synapsis is complete all along the chromosome, the cells are said to have entered the pachytene stage of prophase, where they may remain for days. At this stage large **recombination nodules** appear at intervals on the synaptonemal complex. These recombination nodules mediate for chromosomal changes. The **non-sister chromatids** (chromatids of different chromosomes) twist around each other. This process is called **crossing over** (Fig. 17.1C).

Diplotene: The beginning of diplotene stage is marked by the beginning of separation of non-sister chromatids and the tight pairing is relaxed. This process is known as **desynapsis**. The separation of homologous chromosomes is however not completed. They remain attached at one or more points where crossing over has occurred. These points of attachment are called **chiasmata**. In oocytes, diplotene can last for months or years, since it is at this stage that the chromosomes decondense and engage in RNA synthesis. In some cases the chromosomes expand enormously, producing the **lampbrush chromosomes**, found in amphibians and some other organisms (Fig. 17.1D).

Diakinesis: Diplotene stage merges into diakinesis. At this stage RNA synthesis stops and the chromosomes condense, thicken, and become detached from the nuclear envelope. Each bivalent is clearly seen to contain four separate chromatids, with each pair of sister chromatids attached at their centromeres, whereas non-sister chromatids that crossed over are linked by chiasmata (Fig. 17.1E).

Prometaphase I: During this phase the bounding membrane of centrosome degenerates, leaving behind two centrioles along with astral rays. Then both of them move apart and take their final position at opposite poles of the cell. Each centriole is provided with its own astral rays. Nuclear membrane also breaks down and results in the formation of spindle fibres.

Metaphase I: During metaphase-I, the bivalent chromosomes arrange themselves in the plane of the equator forming equatorial plate. The centromere of each chromosome is directed towards the opposite poles and the arm of chromosomes is faced towards the equatorial plate (Fig. 17.1F).

Anaphase I: All the bivalent chromosomes repel each other towards opposite poles. Thus each pole receives half the number of haploid set of the chromosomes. Thus, actual reduction occurs at this stage. The movements of chromosomes is brought by the spindle fibres, which is similar to the mitotic movements (Fig. 17.1G).

Telophase I: At this stage, the nuclear membranes are formed by the endoplasmic reticulum around the groups of chromosomes which disappear with the appearance of nucleolus or nucleoli (Fig. 17.1H).

(A) The Five Stages of Meiotic Prophase I

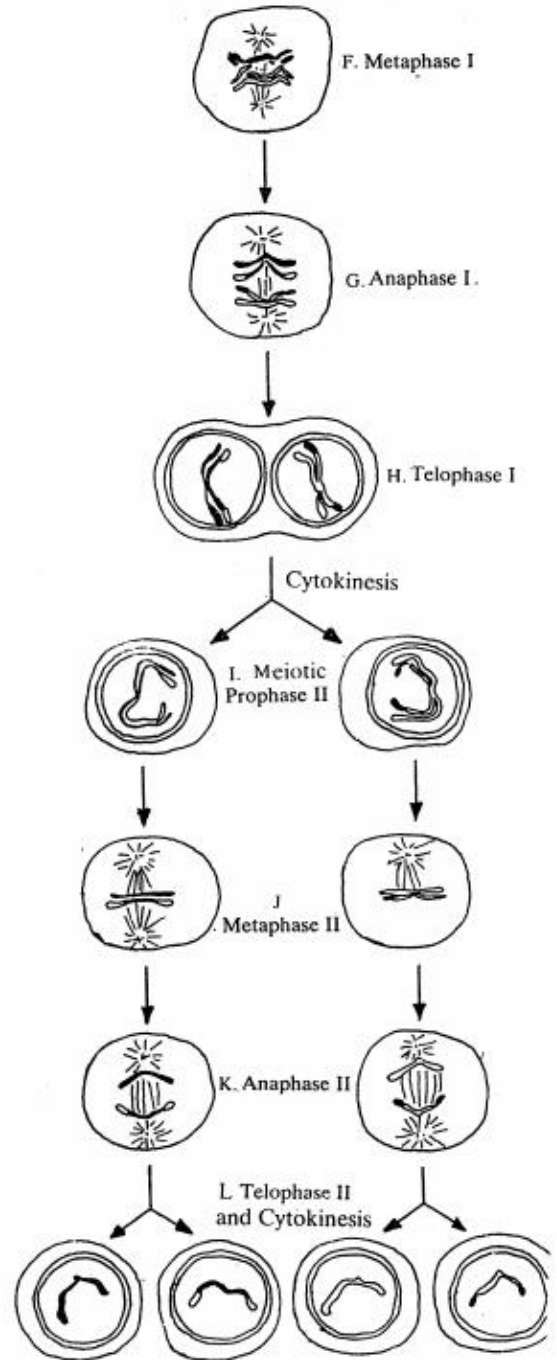
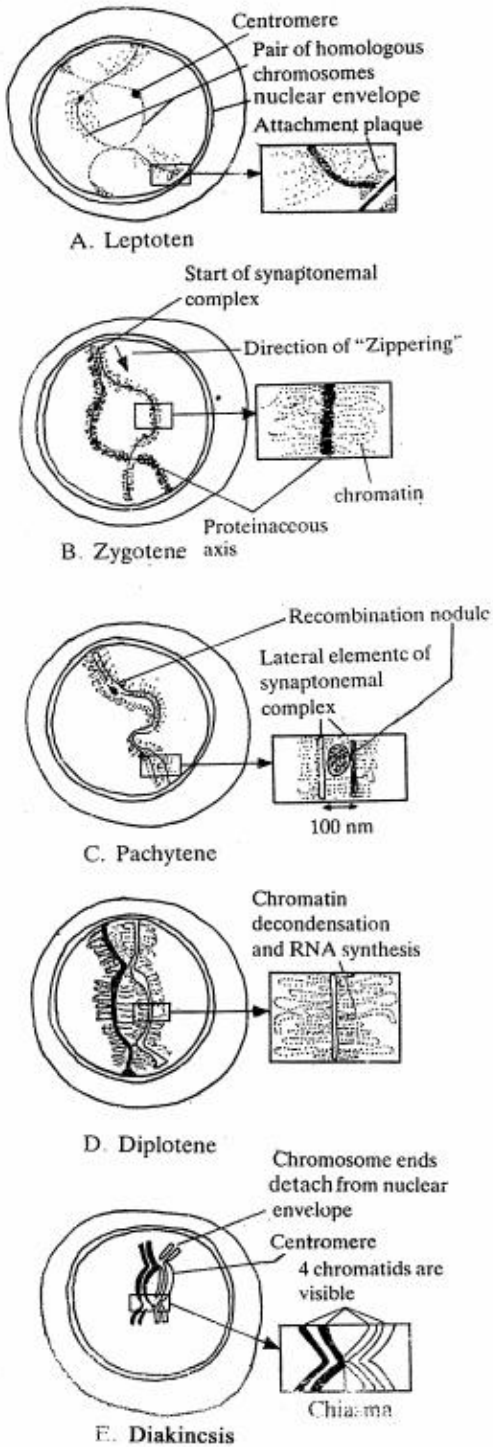


Fig. 17.5 stages of Meiosis

Intrameiotic Interphase: This is the stage between the telophase of the first meiotic division and the prophase of the second meiotic division. In the previous unit you have studied that replication of chromosomal DNA takes place during this phase. But in meiosis, no replication of DNA takes place during the interphase.

The Second Meiotic Division: The second meiotic division is essentially similar to mitosis. It divides each haploid cell into two daughter haploid cells. Like mitotic division it can be studied under four phases:

Prophase II: Prophase II does not show the complex nuclear behaviour of prophase I. The chromatids of prophase II have widely separated rims, and in this respect differ from the chromatids of the mitosis, which are close together. Spindle formation takes place in prophase II as in mitosis, and the nuclear membrane disappears (Fig. 17.1I).

Metaphase II: The chromosomes become oriented on the equatorial plate and have the same relationship to the spindle as in mitosis (Fig. 17.1J).

Anaphase II: The centromeres divide and the two chromatids of each chromosome separate and move to the poles. After separation, the chromatids are called chromosomes (Fig. 17.1K).

Telophase II: At this stage reconstruction of the nuclei takes place as in mitosis. The nucleus, centriole and the chromosomes return to the interphase condition. Each nucleus contains the haploid number of chromosomes (Fig. 17.1L).

In animal oocytes, anaphase II and telophase II are again asymmetrical. A **second polar body** is formed. The first polar body may also undergo a meiosis II to give rise to two haploid daughter polar bodies. Thus at the end of meiosis II we find 4 haploid cells, three polar bodies and the **ovum** (Fig. 17.2).

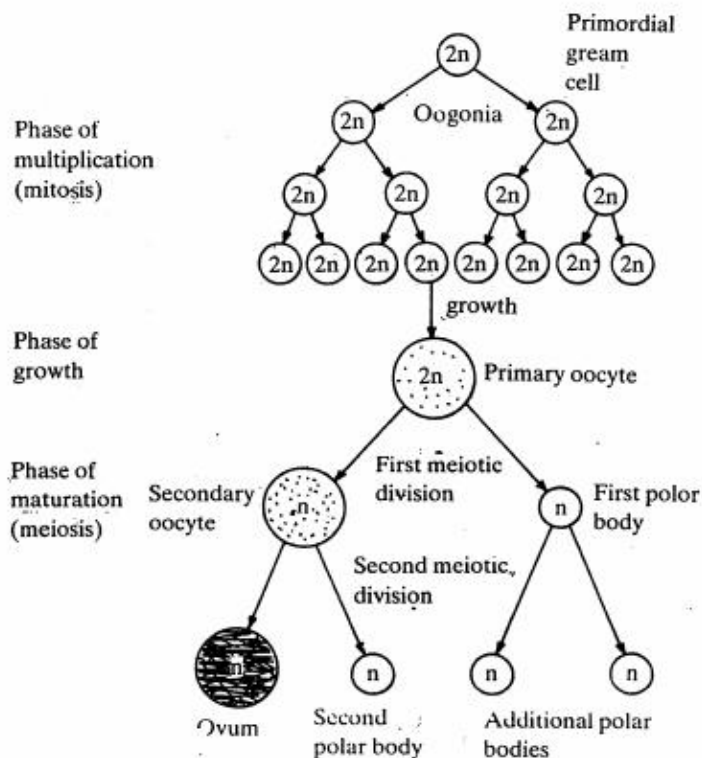


Fig. 17.2 Meiotic Division in Animal Oocyte

SAQ 1

Fill in the blanks with suitable words

- a) Meiosis is a special type of cell division in which the chromosomal number is reduced to.....of the original number.

- b) The essential processes that take place during meiosis are.....of homologous chromosomes, formation of..... andof nonhomologous chromosomes
- c) Crossing over takes place during.....stage of the first meiotic prophase
- d) is the point of attachment of non-sister chromatids during crossing over
- e) In amphibians chromosomes expand enormously during diplotene stage producing the

17.3 GENETIC RECOMBINATION

In the previous section you have learnt that at the end of the meiosis, four haploid daughter cells are produced from one diploid germ cell, maintaining constancy of chromosome number in the species. In addition to maintaining the constancy of chromosomal number in the species, meiosis also brings about genetic variability in the population.

Genetic variability is brought about by genetic recombination. You have learnt in the previous section that the genetic recombination takes place by the process of crossing over during pachytene. In this process the precise pairing of the homologous chromosomes plays a very important role and this is brought about by the development of synaptonemal complex. Synaptonemal complex provides the structural framework necessary for recombination events. The actual recombination is mediated by recombination nodules, which are either spherical, ellipsoidal, or bar-like protein-containing assemblies with a diameter of about 90 nm. These nodules sit at intervals on the synaptonemal complex, placed like basketballs on a ladder between the two homologous chromatids (Fig. 17.3). There are about as many recombination nodules as crossover events. Since recombination nodules initiate the process of crossing over, it implies that recombination nodules must be extremely efficient in causing the chromatids on opposite homologues to recombine.

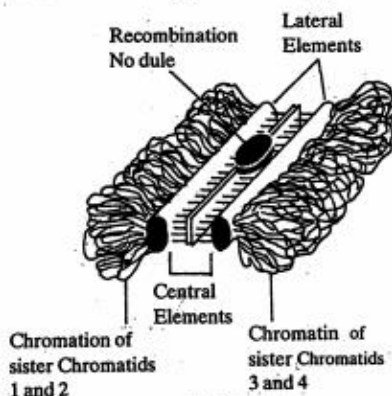


Fig. 17.3 Synaptonemal Complex

Recombination of genetic material results from physical exchanges between homologues, which involve a process of breakage and reunion of chromosome segments. Figure 17.4 clearly illustrates the process of recombination of genetic material between the homologous chromosomes (Fig. 17.4).

You can see in Fig. 17.4 that a homologous pair of chromosomes contain the genes A, a, and B, b, at different sites on the chromosome arm (Fig. 17.4A). One member of the pair has the alleles A and B at these locations. As a result of replication of premeiotic interphase, both chromatids of the chromosomes will have the A and B alleles at these sites. The opposite member of the homologous pair has the alleles a and b at the corresponding locations. At this stage, these chromosomes are identical in sequence of chromosomes of the same homologous pair in any cell of the organism. During prophase I of meiosis, the homologous pair closely. If recombination occurs between these genes (Fig. 17.4B), two of the four chromatids

at the close of meiosis will contain the new combinations A-b and a-B (Fig. 17.4C). As a result, the products of recombination consists of two chromatids of unchanged sequence (the parentals) and two with altered sequence (the recombinants). In the subsequent section we will study about the molecular mechanism involved in the process of recombination.

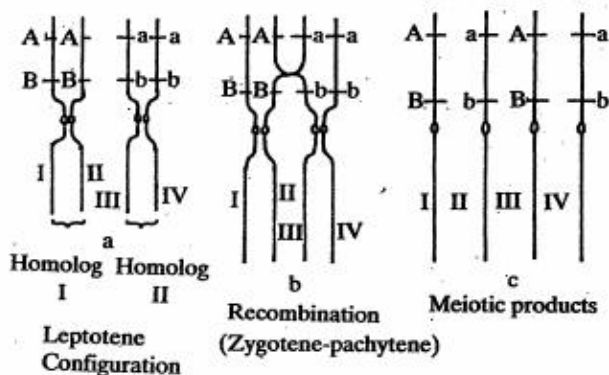


Fig. 17.4 The effect of recombination in mixing the alleles of the chromatids entering meiosis into the new combinations.

17.3.1 Molecular Theory of Recombination

Whitehouse (1963), Holliday (1964), Meselson (1964) and others proposed models to explain recombination of genetic material at the molecular level. However, all of these models share several basic assumptions about the molecular events taking place in the breakage and exchange mechanisms.

As per the molecular model, two of the four chromatids, or two DNA molecules of opposite parentage approach and pair in such a way that homologues are opposite to each other (Fig. 17.5A). In the subsequent step, openings are made (by DNA endonuclease enzyme) in each of the nucleotide chains of the two DNA molecules (Fig. 17.5B). These openings are called **nicks**.

Following the openings of the nicks, the two DNA molecules unwind to expose four single-stranded DNA (Fig. 17.5C). Unwinding is promoted by DNA-binding proteins that stabilise and protect DNA in the single-stranded form, and possibly by unwinding enzymes and DNA gyrases. These enzymes are identical to those involved in DNA replication.

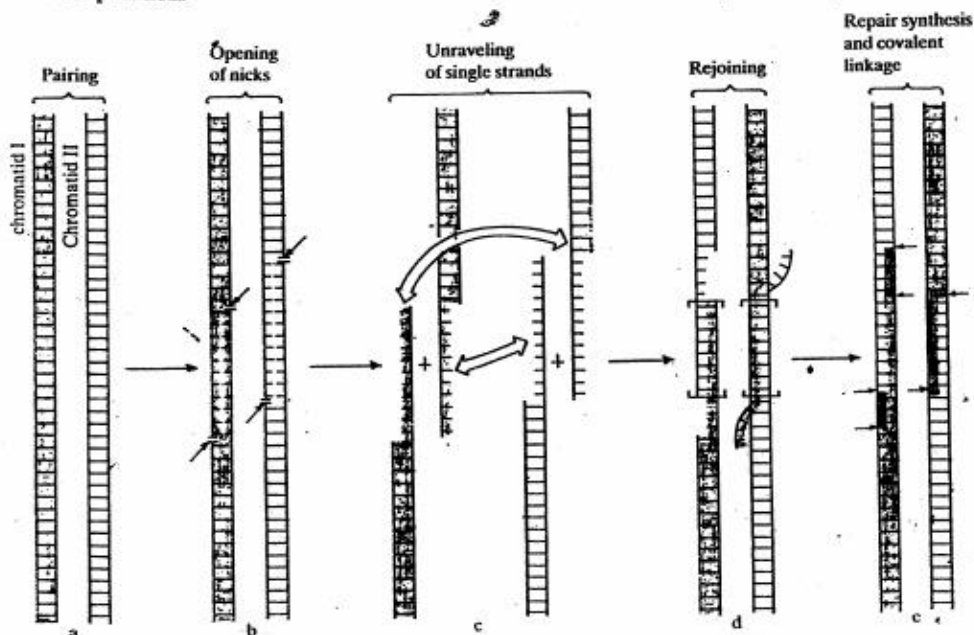


Fig. 17.5 A simplified model for the molecular steps in recombination by breakage and exchange.

While the segments are unwound, one or more of the exposed single-stranded ends may be "nibbled" or shortened by DNA exonuclease. The complementary nucleotide chains from opposite chromatids then pair by alignment of complementary bases and wind into double helices producing hybrid DNA (Fig. 17.5D). The hybrid DNA may not be completely complementary and may contain sequences originating from opposite alleles. At the ends of the hybrid regions, gaps or overlaps may be produced because of inexact location of the single-strand nicks and because of nibbling. The overlaps are then excised by DNA exonuclease, and the gaps filled in by DNA polymerases. Finally, the remaining nicks in the recombined DNA molecule are covalently linked by the enzymes DNA ligase (Fig. 17.5E). Table 16.3 summarises the enzymes involved in the process of recombination.

Table 17.1 Enzymes and Factors Active in Recombination

Enzymes or Factors	Possible Role in Recombination
DNA endonuclease	Opening initial nicks in the homologous DNA molecules.
DNA-binding proteins, unwinding enzymes, DNA gyrase	Unwinding and stabilising single nucleotide chains after nicking; Promoting invasion of homologous helix.
DNA exonuclease	Nibbling ends of exposed single nucleotide chains.
DNA polymerase	Gap filling after formation of hybrid helix.
DNA ligase	Sealing nicks after gap filling.

Experimental results from the variety of sources indirectly support the molecular model for recombination and indicate that the proposed mechanism, or one very much like it, probably operates in the breakage and exchange of chromosome segments during meiotic prophase I. All of these enzymes required including DNA endonuclease, DNA exonuclease, binding proteins, unwinding enzymes and DNA gyrases, and DNA polymerases and ligases are known and well characterised. There is also evidence from the work of Stern and Hotta (1973) that these enzymes increase in activity during the recombination stage.

17.3.2 How does Meiosis Differ from Mitosis?

The events of meiosis are similar to the events of mitosis, but there are several important differences. Let us study these differences following Table 17.2.

Table 17.2 Comparison of Mitosis and Meiosis

Mitosis	Meiosis
1. The cell divides only once.	1. There are two cell divisions, the first and the second meiotic divisions.
2. Mitosis takes place in the somatic cells of the body.	2. Meiosis takes place in the germ cells.
3. Occurs in both sexually as well as asexually reproducing organisms.	3. Occurs only in sexually reproducing organisms.
4. DNA replication takes place during interphase I.	4. DNA replication takes place during interphase I but not interphase II.
5. The DNA replicates once for one cell division.	5. The DNA replicates once for two cell divisions.
6. The duration of prophase is short, usually of a few hours.	6. Prophase is comparatively longer and may take days.
7. Prophase is comparatively simple.	7. Prophase is complicated and is divided into leptotene, zygotene, pachytene, diplotene and diakinesis.
8. The cell divides only once and the chromosomes also divide only once.	8. There are two cell divisions but the chromosomes divide only once.
9. There is no synapsis.	9. Synapsis of homologous chromosomes takes place during prophase.
10. The two chromatids of a chromosome do not exchange segments during prophase.	10. Chromatids of two homologous chromosomes exchange segments during crossing-over.
11. Each chromosome consists of two chromatids united by a centromere.	11. The two homologous chromosomes form bivalents or tetrads. Each bivalent has four chromatids and two centromeres.
12. The arms of the prophase chromatids are close to one another.	12. The arms of the chromatids are separated widely in prophase II.

Mitosis	Meiosis
13. Chromosomes are already duplicated at the beginning of prophase.	13. When prophase I commences the chromosomes appear single (although DNA replication has taken place in interphase I).
14. In the metaphasic plate all the centromeres line up in the same plane.	14. In metaphase I the centromeres are lined up in two planes which are parallel to one other.
15. The metaphasic plate is made up of chromosome pairs.	15. The metaphasic plate is made up of paired chromosome pairs.
16. Division of centromeres takes place during anaphase.	16. There is no centromeric division during anaphase I. Centromeres divide only during anaphase II.
17. The chromosomes separate simultaneously during anaphase.	17. Short chromosomes separate early; separation of long chromosomes is delayed.
18. Spindle fibres disappear completely in telophase.	18. Spindle fibres do not disappear completely during telophase I.
19. Nucleoli reappear at telophase	19. Nucleoli do not reappear in telophase I.
20. The chromosome number remains constant at the end of mitosis.	20. The chromosomal number is reduced from the diploid to the haploid.
21. The genetic constitution of the daughter cells is identical to that of parent cells	21. The genetic constitution of the daughter cells differs from that of the parent cell. The chromosomes of daughter cells usually contain a mixture of maternal and paternal genes.

SAQ 2

Explain in just one line the role of the enzymes listed below, in the process of recombination

- DNA Endonuclease.....
- DNA gyrase.....
- DNA exonuclease.....
- DNA polymerase.....
- DNA ligase.....

17.4 CYTOKINESIS

In Unit 16 and the earlier sections of this unit you have been studying the chromosomal events taking place during mitosis and meiosis. In this section we will study about division of the cytoplasm, cytokinesis in both animals and plants.

The nuclear division and cytokinesis usually occur together; however, this is not always the case. For example in *Drosophila* eggs, early development consists of many successive and synchronous nuclear divisions in the fertilised egg without accompanying cytokinesis. This creates a single cytoplasmic mass with several thousand nuclei. Occasionally even in animal cells cytokinesis may fail to occur, creating binucleated cells.

Cytoplasmic division in most cells begins during late anaphase and continues during telophase, when the daughter nuclei have reached the G1 stage. Division of the cells occurs in a plane at the right angle to the long axis of the spindle, midway between the separated groups of daughter chromosomes. The process of cytokinesis in animal cells differs from the process in plant cells. The two mechanisms of cytokinesis are described in the following sub-sections.

17.4.1 Cytokinesis in Animal Cells

Cytokinesis in animal cells is a contractile process. The contractile mechanism is

contained in a **contractile ring** located just inside the plasma membrane. The ring forms around the circumference of the cell at the edge of the metaphase plate. The contractile ring consists of a bundle of microfilaments of actin and myosin. An interaction between actin and myosin, accompanied by hydrolysis of ATP to ADP and Pi generates a contractile force that draws the contractile ring inwards, forming a furrow in the cell surface and pinching the cell in two. To accomplish cytokinesis the contractile ring must be attached to the inside of the plasma membrane. As the ring contracts, actin and myosin are released from the ring. The contractile ring reduces the connection between the two daughter cells to a narrow bridge that contains a compressed bundle of microtubules of the spindle. This compact bundle, called the **mid-body**, persists as the last remnant of the spindle (Fig. 17.6) and then disappears as the two cells finally break apart from each other. In animal cells cytokinesis takes place within a few minutes. In amoeba, *Acanthamoeba*, the contractile ring contracts extremely rapidly, and all of cytokinesis takes place in 40 to 50 seconds.

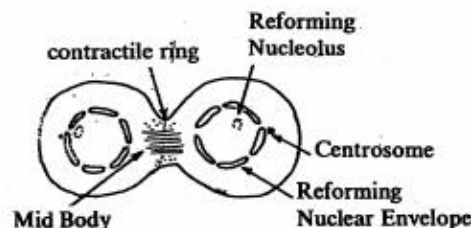


Fig. 17.6 Cytokinesis in Animal Cell

17.4.2 Cytokinesis in Plant Cells

Plant cells do not divide by means of contractile ring. Instead they assemble plasma membranes and cell walls between the two daughter cells in the plane of the metaphase plate. When the daughter chromosomes have left the metaphase plate as a result of their anaphase movements, membranous vesicles derived from the golgi complex accumulate in the plane of the metaphase plate. The vesicles are drawn into the plane from both sides of the spindle by the action of microtubules. The small vesicles fuse to form a large, flat vesicle that grows perpendicular to the spindle until it reaches the plasma membrane. The accumulated vesicular material is called the **phragmoplast**. By their fusion the vesicles of the phragmoplast form a double plasma membrane. The two plasma membranes fuse peripherally with the main plasma membrane of the cell, thereby forming two separate daughter cells. Therefore, a double cell wall is formed between the two plasma membranes by the daughter cells. The new cell wall, at first very thin and highly flexible, is gradually thickened by the secretion of additional layers of wall material. Narrow openings called **plasmodesmata** persist in the new cell wall and remain as direct cytoplasmic connections between the daughter cells. When the plasma membrane and new cell wall between the daughter cells are fully formed, the partition at the middle region is termed the **cell plate**. Complete formation of the cell plate may take from 30 minutes to 2 hours in different species. Figure 17.7 illustrates the process of cytokinesis in a higher plant cell with a rigid cell wall (Fig. 17.7).

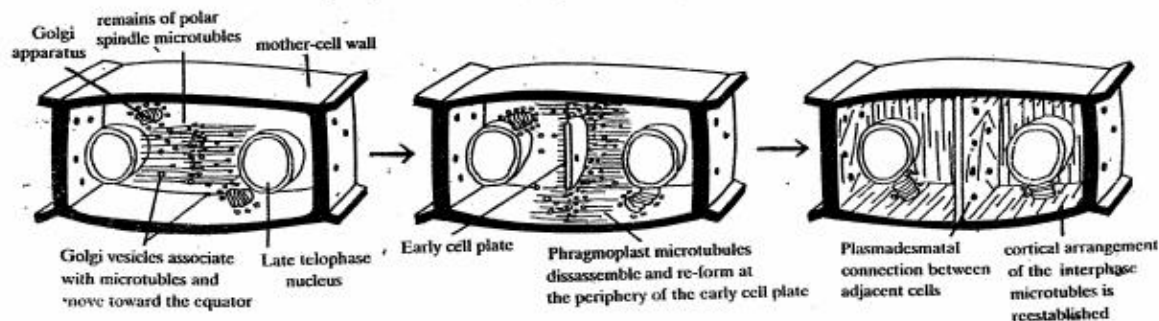


Fig. 17.7 Cytokinesis in Plant Cell

17.4.3 Distribution of Cytoplasmic Components

In the earlier sections you had studied about the events of nuclear division and cytoplasmic division. In this sub-section we will study about the distribution of cytoplasmic components during cytokinesis.

In most instances cytokinesis divides a cell into roughly equal-sized daughters. Exceptions occur in early cleavage stages of embryos of many species, where cytokinesis produces large and small daughter cells in precisely defined patterns. Even in the usual case of equal division, cytokinesis often does not divide a cell into two exactly equal parts. One cell receives slightly more of the cytoplasm than the other. This unequal distribution of the cytoplasm is not a matter of major consequence since most cytoplasmic organelles like mitochondria and ribosomes are present in many copies and each cell always receives some of these. Failure of a daughter cell to receive at least one mitochondrion (and at least one chloroplast for plant cell) is likely to be lethal. Since DNA-containing organelles form by fission of pre-existing organelles, they cannot be formed in the cell. However a golgi complex or a contractile vacuole can be formed inside the cell.

SAQ 3

Fill in the blanks with suitable words.

- Cytokinesis in animal cell is a.....process. An interaction between and.....accompanied by hydrolysis of.....generates contractile force that draws the contractile ring involves, forming a furrow in the cell surface pinching the cell in two.
- During cytokinesis in plant cells.....is formed by the fusion of vesicular material derived from the....., which later fuses with the plasma membrane of the cell, forming two separate daughter cells.

17.5. SUMMARY

In this unit you have studied that:

- Meiosis is a special type of division that occurs during the formation of gametes in animals and spores in plants. It involves two successive cell division (meiosis I and meiosis II), during which the chromosome number is reduced to one-half of its original number.
- The prophase of the first meiotic cell division is a long process during which homologous chromosomes pair and interchange genetic material. During anaphase of the first meiotic division, two chromatids of each homologous chromosomes separate, and one is distributed in each daughter cell. When zygote is formed, one member of each homologous pair is contributed by one parent and the other member by the other parent. Therefore, each parent contributes half of the chromosomes of the zygote.
- Recombination involves physical exchange of chromosome segments between the homologues. The sites of breakage and exchange of chromatids become visible as chiasmata.
- Recombination occurs within the framework of the synaptonemal complex in which DNA molecules from opposite homologues loop out and undergo close molecular pairing. The breakage and exchange of chromatids involves enzymatic steps, these include (1) nicking by endonucleases; (2) unwinding promoted by binding proteins, unwinding enzymes, and DNA gyrases; (3) nibbling of unwound chains, catalysed by exonucleases; (4) rewinding, possibly promoted by DNA gyrases; (5) gap filling and chain extension by DNA polymerases; and (6) nick sealing by DNA ligases.
- Cytokinesis in animal cells is a contractile process. Microfilaments of actin and myosin form the contractile ring. An interaction between actin and myosin accompanied by hydrolysis of ATP generates contractile force that draws the contractile ring inwards, forming a furrow in the cell surface, pinching the cell in two.
- Cytokinesis in plants involves accumulation of vesicular material in the plane of the metaphase plate, perpendicular to the spindle. Fusion of vesicles form phragmoplast which fuses with the main plasma membrane of the cell thereby forming two separate cells

17.6 TERMINAL QUESTIONS

- 1) Explain diagrammatically the behaviour of chromosomes during various phases of meiotic prophase I.

a. Leptotene b. Zygotene c. Pachytene d. Diplotene e. Diakinesis

- 2) Explain briefly in the space given below the molecular mechanism involved in the genetic recombination

.....

.....

.....

.....

- 3) List five chromosomal and genetic differences in daughter cells produced by mitosis and meiosis.

.....

.....

.....

.....

- 4) Explain briefly in the space given below, the process of cytokinesis in animal and plant cells.

.....

.....

.....

.....

17.7 ANSWERS

Self-assessment Questions

- 1 a) one-half.
b) pairing, chiasmata, segregation
c) Pachytene
d) chiasmata
e) Lampbrush chromosomes
- 2 Please refer Table. 17.1
- 3 a) contractile, actin, myosin, ATP
b) Phragmoplast, Golgi complex.

Terminal Questions

- 1) Please refer Fig. 17.1A, 17.1B, 17.1C, 17.1D, 17.1E and 17.1F.
- 2) Please refer sub-section 17.3.1
- 3) Please refer Table 17.2.
- 4) Please refer Section 17.4.

UNIT 18 CELL ADHESION, CELL MOVEMENT AND EXTRACELLULAR MATRIX

Structure

- 18.1 Introduction
 - Objectives
- 18.2 Intercellular Recognition and Cell Aggregation
 - Aggregation of Slime Mold Amoebae
 - Species-Specific Aggregation
- 18.3 Cell Junctions
 - Desmosomes
 - Tight Junctions
 - Gap Junctions
 - Plasmodesmata
- 18.4 Cell Movements
 - Pseudopodia
 - Lamellipodia
 - Filopodia
 - Ciliary and Flagellar Movements
 - Phagocytosis
- 18.5 Extracellular Matrix
 - Proteins and Glycosaminoglycans
 - Basal Lamina
- 18.6 Summary
- 18.7 Terminal Questions
- 18.8 Answers

18.1 INTRODUCTION

You have read in previous units, how cells divide by mitosis and meiosis to produce daughter cells. Specific cells formed by cell division recognise each other and adhere together with the help of specific cell surface molecules and organise into co-operative assemblies called tissues. These tissues in multicellular animals organise to form organs. Cells join and interact with each other with the help of various types of cell junctions. These junctions are located in the regions of interacting plasma membranes and underlying cytoplasmic regions. In this unit we have discussed how cells recognise each other and also the types and formation of the cellular junctions. Free living cells and certain cells in multicellular organisms are motile. The cellular locomotion occurs with the help of various cellular processes which are discussed in this unit. Locomotory apparatus of the cells consists of actin filaments and the variety of actin-binding proteins which are associated with the plasma membrane of these cells.

Later in the unit, we have dealt with the extracellular matrix. Extracellular matrix is secreted by the cells it surrounds. The matrix is composed of various types of macromolecules such as proteins and glycosaminoglycans. The composition of these macromolecules differ from tissue to tissue on the basis of various functions performed by these tissues. Extracellular matrix perform various functions, such as giving strength and elasticity to the tissues, cell migration, cell differentiation, developmental processes, filtration of the cells and solutes etc. In the next unit we will discuss various types of animal tissues. Before you read this unit, you should refresh your memory with the structures and assemblies of collagen fibres and actin filaments about which you have already read in Unit 6 of Block 2.

Objectives

After studying this unit you will be able to:

- explain how cells recognise and adhere to each other with the help of specific cell surface molecules to form co-operative assemblies,
- enlist various types of cell junctions and describe their structure, and functions,

- discuss the cell movements and explain the role of various cellular processes and locomotory apparatus in these movements, and
- list the main components of the extracellular matrix and discuss their synthesis and organisation.

18.2 INTERCELLULAR RECOGNITION AND CELL AGGREGATION

Cells in multicellular organisms are specialised and organised into co-operative assemblies called tissues. Different tissues in multicellular animals combine to form larger functional units called organs. Cells in tissues and organs are held together by extracellular matrix and cell junctions. There are two ways in which specialised cells associate with each other to form a tissue. The first and the simplest way of tissue formation is that the progeny of the founder cells are prevented from wandering away by extracellular matrix macromolecules such as basal lamina and by formation of specialised cell junctions (see Fig. 18.1). It is in this way that the cells in epithelial cell sheets remain together. Formation, folding and differentiation of such cell sheets are very much a part of animal development.

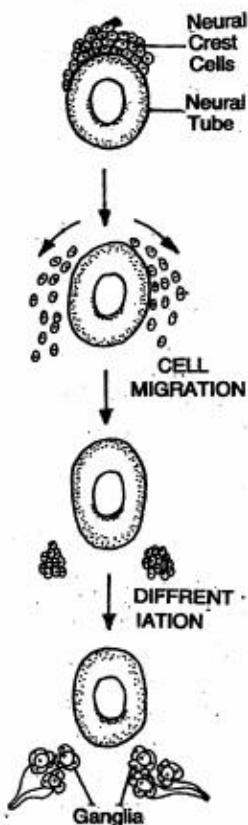


Fig. 18.2 The second and the complex way by which cells assemble to form tissues. In vertebrate embryos neural crest cells migrate from the upper surface of the neural tube to form various nervous tissues such as peripheral ganglia.

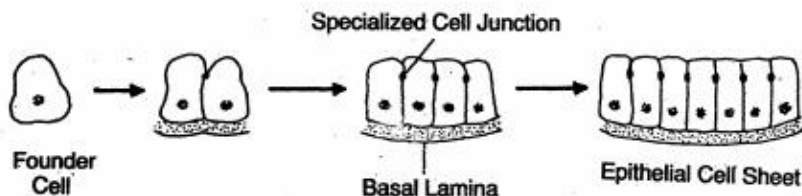


Fig. 18.1 The simplest way of associations of cells into tissues. The progeny of the founder cells forms the epithelial sheet with the help of basal lamina and intercellular junctions.

The second way is comparatively complex that involves migration of the cells to new locations where they assemble with local or other migrant cells into tissues. For example, as shown in Fig. 18.2, the cells of neural crest of vertebral embryonic neural tube migrate to different regions and assemble into various tissues such as ganglia of peripheral nervous systems.

18.2.1 Aggregation of Slime Mold Amoebae

Relatively less is known about the mechanisms of intercellular recognition and aggregation, which result into complex tissues and organs. However, understanding of these mechanisms have been derived from the studies of simple eucaryotic organisms such as slime molds and sponges. We will discuss here the example of slime mold amoeba *Dictyostelium discoideum* which can be studied in vitro as the phenomenon proceeds quite normally in a culture dish. These amoebae live as independent cell until their food supply is exhausted. Starving cells stop dividing and began to aggregate in response to cyclic-AMP which is synthesised and secreted by individual cells and act as a chemotactic signal. You have already read about chemotaxis in Unit 15 of cell Biology course (Block 3). In each multicellular aggregate the cells adhere to each other by means of specific cell surface molecules to form a tiny vertical worm like structure. This aggregate may form a stationary pseudoplasmodium or a slug that is later attached to a surface and stops moving. In both the cases, cells differentiate and migrate to form a stalked fruiting body in which spores are produced, 30 hours after the beginning of the aggregation. As shown in Fig. 18.3 the cells in front of the slug form the stalk, those in the middle region migrate up word and differentiate into spores, while the cells at the tail end form the foot plate. The direction of the migration and the ultimate differentiation of cells in fruiting body are under CAMP control, but the exact nature of this control is uncertain. Both the stalk cells and spore cells are eivered with extracellular matrix in the form of cellulose warts and in

the end all cells except spore cells die. Under favourable conditions spores germinate into free living amoeboid cells that start the cycle again.

In the initial stages of aggregations, starved cells synthesise and secrete cAMP and attract other starved amoebae to join them to form the aggregate. These new starved cells again synthesise cAMP and attract new starved cells and this process goes on. Thus, the aggregate grows at a constant rate as cyclic AMP flows from cell to cell causing more distant amoebae to move in. In addition to serving as an attractant, cAMP binds to specific receptors on the cell surface of starved amoebae and induces the formation of pseudopod at the point of binding. The pseudopod, thus formed adheres to the surface with cAMP source and pulls the cell along in the same direction (see Fig. 18.4).

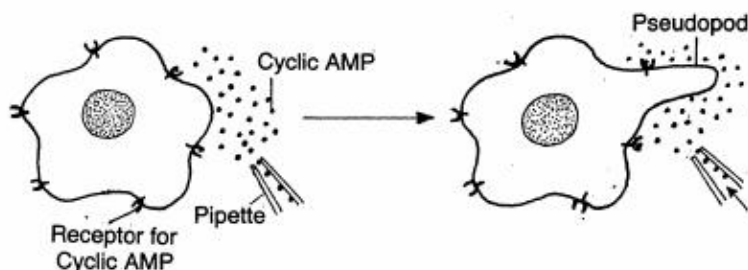


Fig. 18.4 Schematic diagram showing the binding of cAMP on the specific receptors on the surface of starved amoeba which induces the formation of pseudopod at that point.

In the starved amoeboid cells in response to new conditions many genes are activated to synthesise many new molecules. Some of these molecules are synthesised on the cell surface. Cells adhere to each other tightly, end to end due to binding between these cell surface molecules. Some of the cell surface molecules are glycoproteins. Studies have shown that antibodies directed against these cell surface glycoproteins inhibit the aggregation.

18.2.2 Species-Specific Aggregation

Experimental studies of tissue and organ formation in multicellular animals are conducted with dissociated cells. Sponges are the simplest multicellular animals consisting of only few kinds of cells. These can be dissociated into single cells mechanically by pressing through a mesh or by incubating in sea water devoid of Ca^{2+} and Mg^{2+} . These dissociated cells when mixed, reaggregate and reorder themselves to form a normal sponge. When the dissociated cells from two species of sponge of different colour were mixed together, the cells adhered to each other in a **species-specific manner** to form aggregates of each colour separately (see Fig. 18.5).

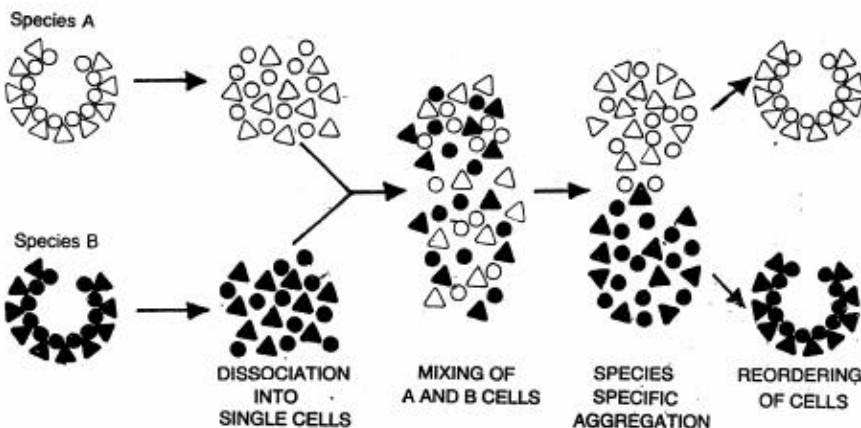


Fig. 18.5 Species-specific aggregation of sponge cells. Dissociated cells of two differently coloured sponge species when mixed with each other, form aggregates of each colour independently.

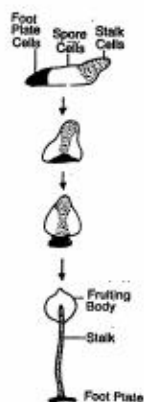


Fig. 18.3 Schematic diagram showing the cell migration forming the fruiting body in the multicellular aggregate of *Dictyostelium discoideum* amoebae.

The species-specific aggregation is mediated by extracellular **aggregation factor** which is a huge particle with diameter over 100 nm and molecular weight of about 20 millions. It is believed to be a complex of proteoglycan and/or glycoprotein molecules. The specific factor binds to the specific surface protein receptors also called base plates of the sponge cells of same species only. Aggregation factors help to link sponge cells by binding first to cell surface receptors and then to each other. Binding of factors to each other occurs only when Ca^{2+} is present (see Fig. 18.6).

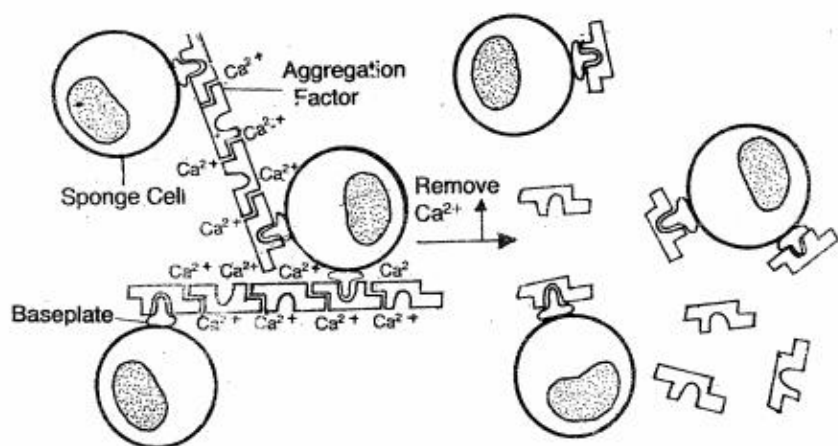


Fig. 18.6 Schematic diagram shows that aggregation factors bind to the surface receptors of sponge cells independently of Ca^{2+} , but cross link by binding to each other in presence of Ca^{2+} to form aggregates.

Cells in young embryos or in tissue culture are separated by subjecting these to the digestion with dilute trypsin solution for a brief period.

Another example of specific aggregation is of **tissue specific aggregation**. When the dissociated cells from two different vertebrate embryonic tissues are mixed together they form mix aggregates initially. Eventually they are separated into groups each containing cells from their original tissues. Dissociated cells from frog embryos can reaggregate in such a way that the cells separate to form ectodermal, mesodermal and endodermal layers of the cells.

SAQ 1

Fill in the blanks with the appropriate words.

- acts as a chemotactic signal for the aggregation of slime mold.
- Cells in slime mold aggregate, differentiate and migrate to form,
- Species-specific aggregation in sponges occurs only in the presence of
- Sponge cells are crosslinked by..... which bind to cell surface receptors and to each other.

Till now you have read about the cell recognition and aggregation with the help of cell surface binding molecules. We will now discuss binding of cells lying adjacent to each other with the help of cell junctions which are the specialised areas on the cell surface.

18.3 CELL JUNCTIONS

Cell junctions are the regions where interacting plasma membrane of the adjacent cells are highly specialised. Often these junctions include underlying cytoplasm and the intervening intercellular spaces as well. Cell junctions are grouped into three functional categories: 1) **adhering junctions**, which hold the cells together mechanically; 2) **impermeable junctions**, which bind and seal the adjacent cells in such a way that molecules cannot pass through the space between these cells; 3) **communicating junctions**, which allow ions and small molecules to pass from one interacting cell to another. **Desmosomes** are the main type of adhering junctions. Tight junctions and gap junctions are the examples of impermeable junctions and the communicating junctions respectively. We will discuss each of these examples in the following Subsubsections.

18.3.1 Desmosomes

Desmosomes are cell junctions which mechanically hold the cells together enabling them to function as structural units. They are most abundant in tissues that are subjected to severe mechanical stress, such as in case of cardiac muscle and uterine cervix. Desmosomes are of three different forms; spot desmosomes, hemidesmosomes and belt desmosomes. All these occur in most epithelial cells.

Spot desmosomes are button like point of contact that hold the adjacent cell together. As shown in Fig. 18.7 at the point of contact a disc of dense fibrous material called a plaque occurs just underneath the plasma membrane in both the cells. **Keratin filaments**, also called tonofilaments, either terminate at or are inserted into each plaque and extend across the interior of each cell. Separating the two plasma membranes at the spot desmosome there is an intercellular space which is filled with moderately dense **intercellular filamentous material**. Therefore, the keratin filament networks inside the adjacent cells in the epithelial sheet are connected indirectly through these junctions with the help of intercellular filamentous materials, and form a continuous net work of fibres across the entire sheet of cells. Such a fibrous, framework provides the tissue considerable mechanical stability. The plaques, keratin filaments and intercellular filaments in whole or in parts are formed of proteins. Intercellular filaments are probably glycoproteins.

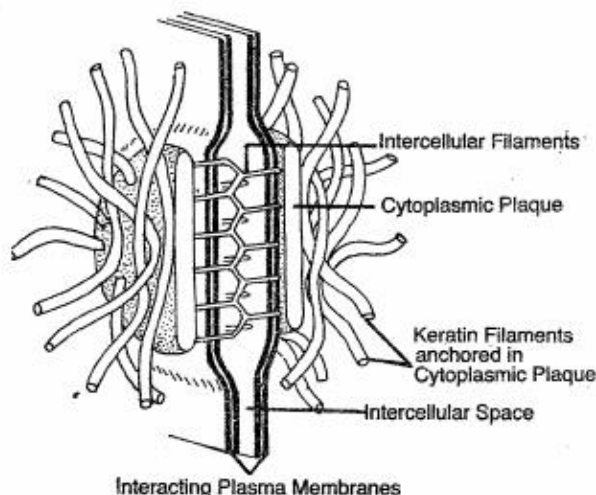


Fig. 18.7 Schematic diagram of spot desmosome showing cytoplasmic plaque and keratin filaments in adjoining cells, which are connected with the help of intercellular filaments filling the intercellular space.

Hemidesmosomes resemble spot desmosomes but instead of joining adjacent cell membranes together, they join the basal surface of epithelial cells with the underlying basal lamina. Basal lamina is a specialised extracellular matrix composed of secretions of epithelial cells. So hemidesmosomes have a cytoplasmic plaque on only one side of the junction. You will read about basal lamina later in this unit.

Hemidesmosomes also develop on the inside surface of plasma membrane in cells grown in tissue culture and help bind the cells to the culture vessels in which they are grown.

Belt desmosomes form a continuous band or belt around each of the interacting cells in an epithelial sheet, near the apical end of the cells (see Fig. 18.8a). The bands in the adjacent cells face each other and are separated by intercellular space which is filled with fine filamentous materials made up of glycoproteins. These intercellular filaments hold the interacting cell membranes together. In each cell, belt desmosomes are composed of contractile bundles of actin filaments that run along the cytoplasmic surface of the membrane. Contraction of these actin filaments causes the narrowing of apical ends of epithelial cells. These filaments mediate the cell movement and change in shape during morphogenesis in animals. For example, as shown in Fig. 18.8b, the sheet of cells forming the wall of bird oviduct develops knob like bulges during embryogenesis. This is because the bundles of actin filaments present across the inner ends of the cells of the bulge region contract in such a way that produces cell narrowing and consequent bulge development.

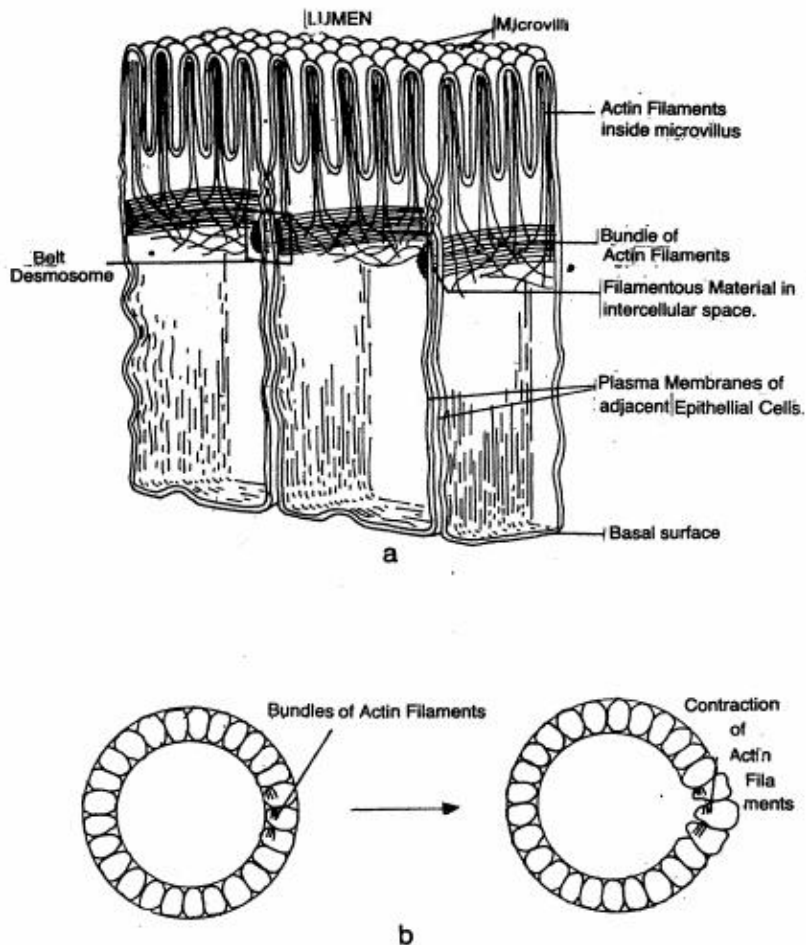


Fig. 18.8 a) Schematic drawing of belt desmosomes between epithelial cells of small intestine, forming a continuous band around each interacting cell. These are composed of bundles of actin filaments just underneath the plasma membrane encircling the cells. These bundles interact with actin filaments inside each microvillus. Intercellular space is filled with filamentous material; b) Bulge in the oviduct sheet of bird embryo caused by narrowing of cells in the inner region. The encircling bands of actin filaments located at the inner end of each cell contract to produce these narrowings.

18.3.2 Tight Junctions

Tight junctions are impermeable junctions that seal the interacting plasma membrane of adjacent cells and obstruct the passage of intercellular fluid. In such junctions the membranes of adjacent cells are so close that the intercellular space is excluded. As shown in Fig. 18.9a, in tight junctions the interacting plasma membrane are held together by transmembrane particles made up of proteins. These particles form the **sealing strands** and create a permeability barrier to the passage of solutes across the epithelial sheets. The sealing strands form a belt like structure that encircles each cell in the sheet by anastomosing at various places (Fig. 18.9 b). More impermeable junctions have more sealing strands per unit area and junctions through which molecules leak out have relatively fewer sealing strands.

Tight junctions are found only in vertebrate animals and play a crucial role in maintaining the selective barrier function of cell sheets. For example, in the epithelial sheet that lines the lumen (inner cavity) of the small intestines, tight junctions prevent the gut content from spilling back into the tissue. At the same time nutrients in the gut are pumped in by the cells and transferred across the cell sheet into extracellular fluid on the other side, from where they are absorbed into the blood. Since tight junctions can be disrupted by proteolytic enzymes or by agents that chelate Ca^{2+} or Mg^{2+} , it can be said that both proteins and cations are required to maintain these junctions.

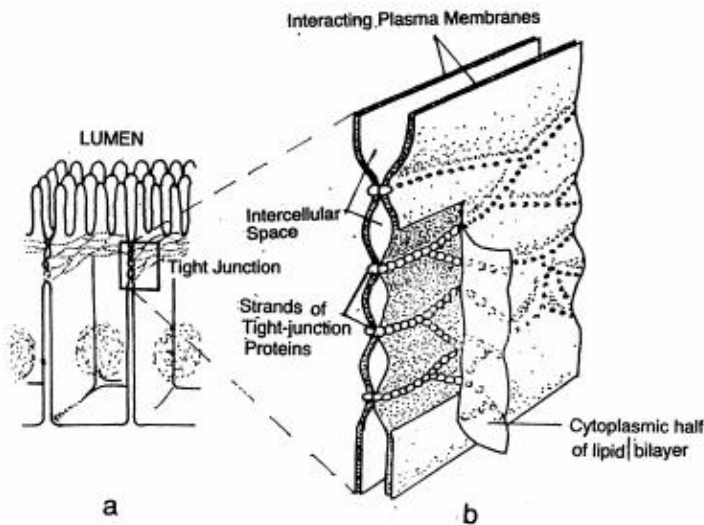


Fig. 18.9 Schematic diagram of tight junctions between epithelial cells creating the permeability barrier (a). Transmembrane protein particles seal the junction area of the interacting plasma membrane and encircle around each cell in the sheet (b).

In invertebrates, instead of tight junctions, **septate junctions** seal the cells to form sheets. Unlike in tight junctions, proteins found in septate junctions are arranged in regular and parallel rows which interact across the intercellular space. These junctional proteins themselves form a seal without actually bringing the two adjacent plasma membranes into direct contact. However, like in the tight junctions septal proteins completely encircle each of the interacting cell.

18.3.3 Gap Junctions

Gap junctions are the most common type of cell junctions widely present in all animals. Such junctions are communicating junctions as they allow small water soluble molecules and ions to pass directly from one cell to another. As shown in Fig. 18.10, gap junctions are formed in the adjacent plasma membranes of the two cells. These are the narrow continuous aqueous channels of diameter 1.5 nm connecting two cell interiors. These channels are constructed from protein molecules that extend out from the plasma membrane and are called **connexons**. Each of the two interacting cells contributes enough protein to form one connexon which forms half the length of aqueous channel. Each connexon is composed of six subunits, each made up of protein called **connexin**.

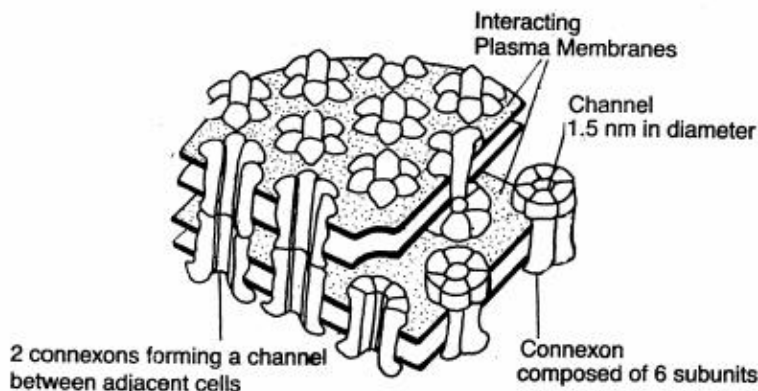


Fig. 18.10 schematic diagram showing the gap junctions formed as narrow channels in two adjacent plasma membranes. These channels are constructed by protein assemblies called connexon each of which is made up of six protein subunits called connexin. Two connexons join across the intercellular gap to form a channel.

Gap junctions allow only molecules smaller than 1000 to 1500 dalton such as inorganic ions, sugars, aminoacids, nucleotides, vitamins etc. to pass between the cells. Whereas larger molecules such as proteins, nucleic acids, polysaccharides etc. cannot pass through them.

Gap junctions mediate metabolic co-operation between coupled cells by giving and receiving metabolites and ions. Thus the gap junctions couple the cells both metabolically and electrically. The harmonious and co-ordinated activities of cells in the tissues are also the outcome of cell-to-cell communications via gap junctions. It has been shown that communication across gap junction is essential during embryogenesis. Injecting antibodies against gap junction proteins into a cell of an early (8-16 cell stage) embryo of *xenopus* leads to very abnormal development.

18.3.4 Plasmodesmata

With the exception of few specialised cell types, all cells in higher plant tissues are connected with their neighbouring cells by fine cytoplasmic channels, each of which is called a **plasmodesma** (plural is **plasmodesmata**). These channels pass through the intervening cell walls. As shown in Fig. 18.11a, plasma membrane of one cell is continuous with that of its neighbouring cell at each plasmodesma.

Plasmodesma is a cylindrical membrane lined channel with a diameter of 20 nm to 40 nm. As shown in Fig. 18.11b, a narrow cylindrical structure called **desmotubule** runs from cell to cell through the centre of plasmodesma. Electron micrograph shows that desmotubule is continuous with endoplasmic reticulum membranes in each of the connected cells. The area between the outside of desmotubule and the inner face of cylindrical plasma membrane is called **annulus of cytosol**, which is often constricted at each end of the plasmodesma. These constrictions at both ends are important because they regulate the flow of the molecules through annulus that joins the cytosol of the cells.

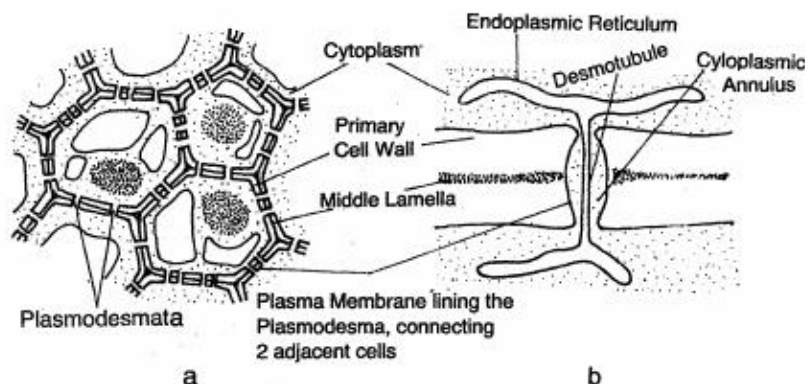


Fig. 18.11 Various plant cells are interconnected with the help of cytoplasmic channels called plasmodesmata (a). Plasmodesma is lined by connecting plasma membranes and contains a fine tubular structure called desmotubule which is in continuation with ER membranes of connecting cells (b).

The transfer of ions and molecules occurs rapidly between cells through the cytoplasmic strands in plasmodesmata. These are especially frequent in walls of columns of cells that are towards the sites of intense secretion such as in nectar secreting glands. Therefore, it can be said that plasmodesmata mediate the communication and transport between adjacent plant cells much as the gap junctions do in animal cells. After having read about cell recognition and cellular junctions, you will study about modes of movements of cells. But before that try the following SAQ.

SAQ 2

Name three categories of cell junctions by giving one example of each and state their functions. Answer in the space provided below.

.....

.....

18.4 CELL MOVEMENTS

Most of the free cells exhibit a marked motile behaviour. Embryonic cells undergo extensive displacement before acquiring their definite locations. In the adult body there are several kinds of free cells such as macrophages which retain and exhibit motility. Typical adult tissue cells are not motile. However, if isolated and placed in vitro as single cells they show motility. It seems therefore that motility is an inherent property of cells, varying in degrees, depending on their environment.

Various types of the cellular processes which help in cellular locomotion are pseudopodia, lamellipodia, filopodia. We will discuss these in following subsections.

18.4.1 Pseudopodia

Free living amoeba and various types of leucocytes exhibit a characteristic locomotion by forming pseudopodia. Such type of locomotion is called **amoeboid movement**. Large fresh water amoeba are suitable for study of locomotion using a light microscope. Amoeba moves by extending and retracting cellular processes called pseudopodia that act like little legs. Cytoplasm of amoeba has two regions; **endoplasm** sol the central fluid core also called **plasma sol**, and a surrounding transparent, thick gel like **ectoplasm** also called **plasma gel**. As a pseudopod extends from the cell, plasma sol streams into the extension and changes into gel (gelation at the tip). At the trailing end the gel becomes sol (solation), and flows towards the pseudopod. It is postulated that contraction of gelated ectoplasm at trailing or posterior end pushes the endoplasm forward creating a strong **cytoplasmic stream** that causes each pseudopod to extend. This theory of amoeboid movement is known as **gel-sol theory** as it is based on the interchange of cytoplasmic contents alternatively into sol and gel (see Fig. 18.12).

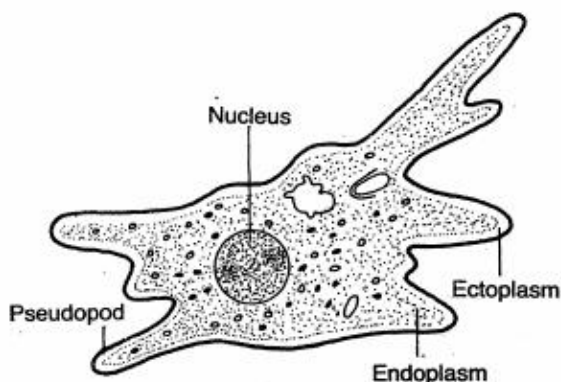


Fig. 18.12 Locomotion in amoeba occurs by formation of pseudopodia.

There is evidence to show that cytoplasmic streaming is actin based movement and sol to gel transformation is accompanied by polymerisation of actin into microfilaments. In Unit 6 of Block 2 you have read about actin and its polymerisation. As shown in Fig. 18.13, contraction of gelated ectoplasm is mediated by non-muscle myosin, two molecules of which bind with two actin filaments and produce the sliding which brings about the amoeboid movement. This interaction between myosin and actin filaments is Ca^{2+} dependent. The energy obtained by hydrolysis of ATP is used for contraction and movement of amoeba.

Myosin Molecule align with polarity of actin can bind to actin filament & move

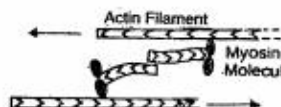


Fig. 18.13 Schematic diagram showing the sliding of two actin filaments with the help of non-muscle myosin molecules.

18.4.2 Lamellipodia

The locomotion of most vertebrate cells are different from amoeboid movement. Most of the information of motility in vitro of vertebrate cells is derived from the study of fibroblasts, the connective tissue cells that readily grow and migrate in the culture. As a fibroblast moves, it continuously forms thin sheet like extensions called **lamellipodia**, from its leading edge. Some of these podia adhere to the substratum, but many are carried back in a sweeping wave like motion to the upper surface of the cell. This motion is referred to as **ruffling**. Small hair like extensions called **microspikes** may extend from a lamellipodium which too are carried back on the surface of ruffles (see Fig. 18.14). Ruffling occurs only when cell has a free upper surface as in tissue culture and is due to the flow of surface membrane and/or intracellular movements responsible for cell locomotion.

Microspikes are the hair like extensions on the cell surface. Each is about 0–1 μm wide and 5 μm long.

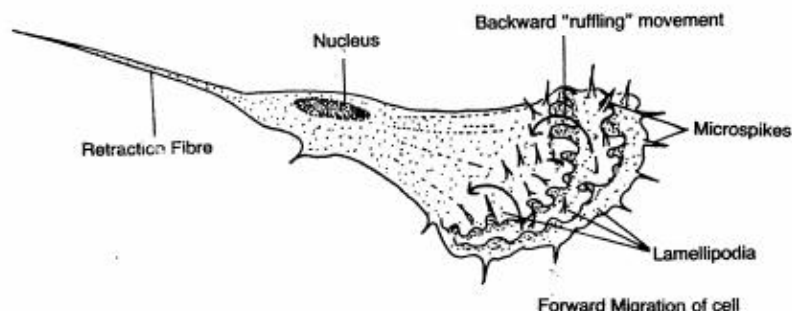


Fig. 18.14 Schematic diagram showing the movement of fibroblasts along the surface of tissue culture

As shown in the Fig. 18.14, the ruffling is occurring at the front of the cell, whereas its rear end remains stuck to the culture dish and forms a long **retraction fibre** as the cell moves forward. The fibre breaks suddenly with a jerk and retracts into the body of the cell. So the moving cells always adhere to the substratum and move at a rate of 40 μm per hour. The contractile apparatus of the fibroblasts consists of actin filament bundles and actin binding proteins in the stretched part of the cell. Although the exact mechanism of the movement of fibroblasts is still not understood, it is possible the locomotion with pseudopodia and lamellipodia have fundamentally similar mechanism.

When a moving fibroblast encounters another cell in the culture the ruffling activity is paralysed and locomotion stops. This tendency of fibroblasts to stop locomotion on coming in contact with another cell is called **contact inhibition** of locomotion.

18.4.3 Filopodia

Filopodia are the long hair like cytoplasmic extensions (microspikes of up to 50 μm in length) formed at the tip of cells or cellular processes and are used for locomotion by certain cells. These extensions are motile structures that are formed and retracted with great speed. We will study these by taking the example of embryonic nerve cells.

The embryonic nerve cells in the tissue culture send out processes called neurites. The growing tip of these processes, also called **growth cone**, develop many long microspike extensions, i.e. filopodia. As some of these extensions retract back into growth cone, others extend out, waving about and touching down and adhering to the substratum (see Fig. 18.15). The formation and retraction of filopodia is possibly controlled by polymerisation and depolymerisation of actin filaments.

18.4.4 Ciliary and Flagellar Movements

Cilia and flagella are small hair like structures extending from the cell surface, found in most animal species and some lower plants. In the cells, cilia are short and numerous whereas flagella may be more than one. Both have the same ultrastructure about which you have already read in Unit 3 of Block 1. One of their functions is to help the cells in their movement through the fluid. For example, ciliary movements propel paramecium, and flagellar movement causes sperms to swim.

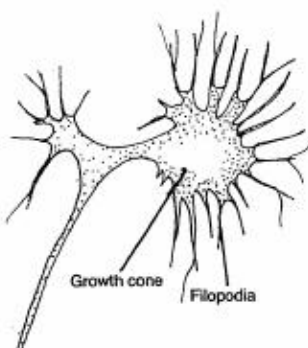


Fig. 18.15 Schematic diagram showing the two growth cones at the end of neurite with many filopodia. Filopodia are responsible for the movement of neurite and not the cell body.

The movement of cilia is whip like and involves ciliary bending in cycles consisting of two strokes: 1) forward active power stroke in which cilium is fully extended and exerts its maximum force against the surrounding fluid, driving it over the cell surface; 2) return recovery stroke in which cilium returns to its original position by unrolling movement. Cycles of alternating power stroke and return stroke result in the ciliary movements which propel the cell to move in the fluid. Each cycle requires 0.1 second to 0.2 second (see Fig. 18.16a) to be completed.

The flagellum acts like a propeller which undergoes undulations. The undulatory waves which pass from the base to the tip of flagellum propel the animal in opposite directions. Whereas waves of undulations that pass from tip to base pull the organism (see Fig. 18.16b).

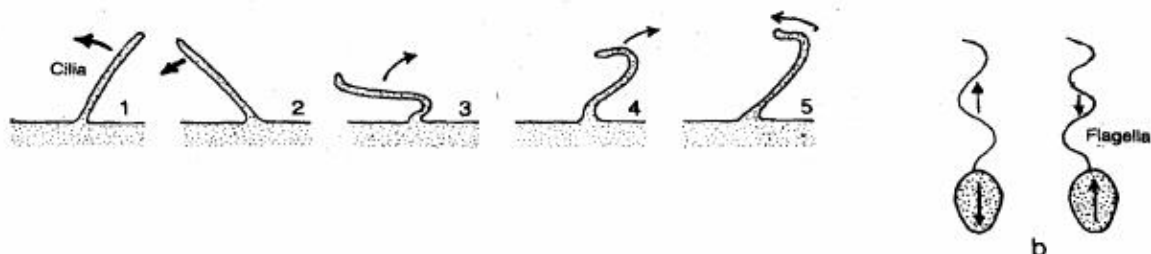


Fig. 18.16 (a) Schematic diagram of the movement of cilium showing power stroke (1,2) and recovery strokes (3,4,5); (b) the undulatory waves of flagellum.

The basis of the ciliary and flagellar movements are same. Both movements occur as a result of sliding of microtubules. You should not confuse flagella of eucaryotes with those of bacteria which are quite different. We have discussed bacterial movements in Unit 15 of Block 3.

18.4.5 Phagocytosis

You have already read in Unit 3 of Block 1 that the process of intake of substances from the surroundings into the cell by the formation of plasma membrane bound vesicles is called **endocytosis**. The intake of fluid or dissolved material is called **pinocytosis** and the intake of particulate material is called **phagocytosis**. Most kinds of cells continually take in solutes and fluids through the process of pinocytosis for which the term endocytosis is also used. Phagocytosis in protozoa is carried out routinely for the intake of food. However, in multicellular animals phagocytosis is carried out by specialised cells called as phagocytes such as macrophages and polymorphonuclear leucocytes, two kinds of white blood cells in mammals. These are scavenger cells which defend us against infections by engulfing the invading microorganisms.

Phagocytes have specialised surface receptors, to which the particles to be phagocytosed first bind. In response to this stimulus the phagocyte extends pseudopods around the particle, engulfing and enclosing it in the cytoplasm in a vacuole, called phagosome (Fig. 18.17).

In the cell the phagocytic vacuoles fuse with primary lysosomes to form secondary lysosomes, where the ingested material is degraded. Substances which cannot be digested remain in secondary lysosomes forming residual bodies.

In macrophages and polymorphonuclear leucocytes phagocytosis is a highly selective process. Not all the particles that bind to the surface receptors of phagocytes are ingested. Only the infectious microorganisms and cells with altered and damaged surfaces are recognised and engulfed by the phagocytes. One of the important receptors of these phagocytes are those which recognise molecules of antibody.

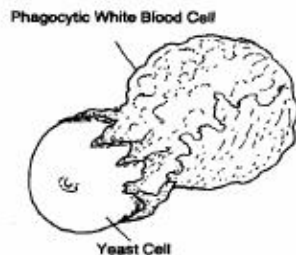


Fig. 18.17 Schematic diagram showing a polymorphonuclear leucocyte phagocytosing a yeast cell.

Antibodies bind to the surface of microorganism to form a coat around it. Both macrophages and polymorphonuclear leucocytes recognise the coated particle with the help of specific receptors and engulf it. Little is known about the molecular mechanisms involved in the process of phagocytosis. There is evidence that actin, myosin and some of their accessory proteins are involved.

So far you have studied various cellular processes involved in the cellular locomotion. We will now discuss the extracellular matrix which is a meshwork formed by cellular secretions. But before that try the following SAQ to see that you have understood the preceding text.

SAQ 3

- a) Differentiate between pseudopodia and lamellipodia in the space given below.

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- b) Fill in the blanks with appropriate words.

- i) The long hair-like cellular extensions are called
- ii) Growing tip of neurites of embryonic nerve cells is called
- iii) The stroke in which cilium is fully extended and exerts maximal force is
- iv) Phagocytes have to bind the particles to be phagocytosed.

18.5 EXTRACELLULAR MATRIX

Most cells in multicellular organisms secrete proteins and polysaccharides in the extracellular space where these secretions interact to form organised meshwork called **extracellular matrix**. In plants the extracellular materials form the rigid cell walls whereas in animals they form highly specialised structures such as cartilage, tendons, bones, chitinous coverings of crustaceans and insects, shells of molluscs etc. Apart from holding cells, tissues and organs together, extracellular matrix provides protection, support and elasticity to the various structures. Matrix also influences the development, polarity and behaviour of the cells it remains in contact with. In this section we will discuss the extracellular matrix in animals only. Plant cell wall will be dealt within Unit 20. In animals, cells such as fibroblasts, macrophages and mast cells which secrete the macromolecules of the extracellular matrix are distributed in the matrix. The matrix together with these cells are called **connective tissues** (see Fig. 18.18). The fibroblasts in the connective tissues of bones are called osteoblasts whereas in cartilage as chondroblasts. The extracellular matrix consists of proteins and polysaccharide glycosaminoglycans which are discussed in the following subsections.

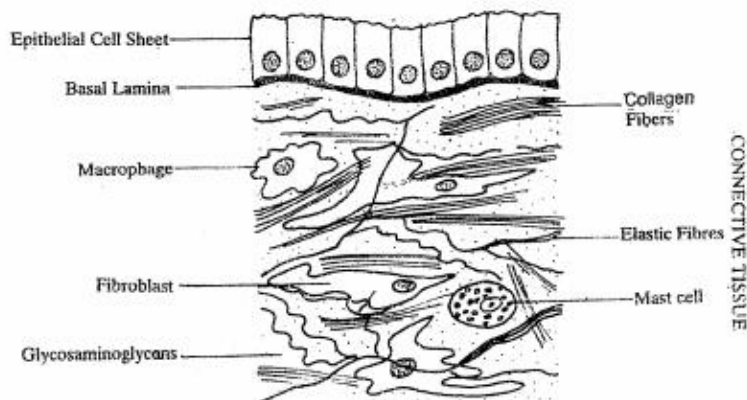


Fig. 18.18 Schematic diagram of the connective tissue underlying a cellular sheet.

18.5.1 Proteins and Glycosaminoglycans

Extracellular matrix in animals is known to contain fibre forming proteins; collagen, elastin, fibronectin, chondronectin and laminin; and polysaccharide glycosaminoglycans (GAGs). Glycosaminoglycans are usually covalently linked to large core protein of about 2,50,000 molecular weight to form proteoglycans. We will now discuss these macromolecular components of the matrix.

Collagens are the major proteins found in the matrix. The collagen fibres are embedded in highly hydrated gel like network formed of glycosaminoglycans chains and proteoglycans. These fibres strengthen and organise the matrix. Collagen molecule is a triple helical structure composed of three α -chain polypeptides. In the matrix it interacts with glycosaminoglycans and proteoglycans. You have already read in Unit 6 of Block 2 about the structure and assembly of collagen fibres.

In tissues like skin, lungs, blood vessels etc. extensive network of elastic fibres is present in the matrix enabling them to recoil after transient stretch. Elastic fibres are made up of **elastin**, a protein of 70,000 molecular weight. Elastin, like collagen is rich in proline and glycine molecules but it contains little hydroxyproline and no hydroxylysine. Little is known about elastin synthesis and processing. Elastin molecules are secreted into extracellular space by exocytosis where they form filaments and sheets in which elastin molecules crosslink with each other to produce extensive network. Elastin fibres also contain microfibrils of an unnamed glycoprotein which are distributed on their surfaces. Unlike most other proteins the polypeptide chains of elastin remain unfolded as random coils, thus allowing the network to stretch and recoil like a rubberband (Fig. 18.19). The long, inelastic collagen fibres interwoven with elastin fibres limit the stretching, thereby, preventing the tissues from tearing.

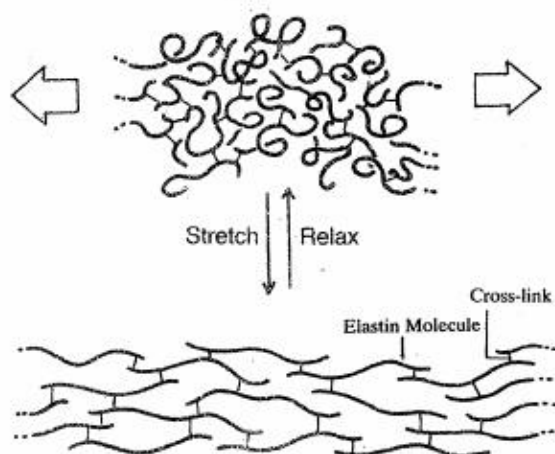


Fig. 18.19 Elastin molecules with various random coil conformation join covalently to form extensive cross linked network. The entire network can stretch and recoil like a rubberband because of the random coiling of molecules.

Fibronectin is a fibre forming glycoprotein composed of two subunits of 2,20,000 daltons each, interconnected by disulphide bonds. It has about 5% carbohydrate by weight. Fibronectin exists in two forms: one form, called cellular fibronectin exists as large aggregates in the extracellular matrix; another form, called plasma fibronectin is found in blood and other body fluids. Both forms of fibronectin are able to bind to fibrin and aid in blood clotting. Cellular fibronectin in the matrix aids in cell adhesion through indirect binding with intracellular actin filaments across the plasma membrane. Since fibronectin has been found in high concentrations in regions of cell migration during development, this glycoprotein is thought to influence cell migration and tissue development in orderly processes and tumor development in unrestrained cell behaviour.

Chondronectin, the other fibre forming glycoprotein is restricted in its distribution. It is responsible for binding of chondrocytes to collagen fibres in cartilage matrix.

Laminin is another glycoprotein which binds epithelial cells to collagen in the basal lamina about which we will discuss in the next section.

Glycosaminoglycans (GAGs) are, long unbranched polysaccharide chains consisting of repeating disaccharide units. These are called glycosaminoglycans because one of the two monosaccharide residues in repeating disaccharide is always an aminosugar which may be N-acetylglucosamine or N-acetylgalactosamine. Glycosaminoglycans are highly negatively charged due to the presence of sulphate or carboxyl groups or both on many of the sugar residues (see Fig. 18.20), GAGs are hydrophilic and attract large amount of water to form hydrated gels even at very low concentrations.

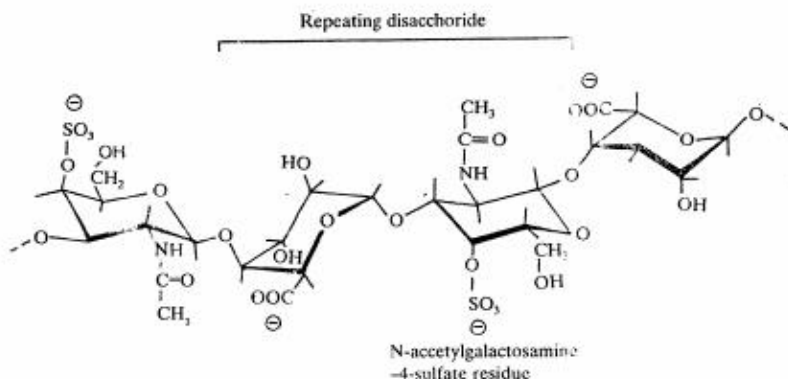


Fig. 18.20 Repeating disaccharide units of glycosaminoglycan chain, High negative charges along the chain are due to the presence of carboxyl and sulphate groups

Extracellular matrix consists of seven types of glycosaminoglycans which are distinguished by their sugar residues, types of linkage between these residues, and number and location of sulphate groups. These are hyaluronic acid, chondroitin 4-sulphates, chondroitin 6-sulphate, dermatan sulphate, heparan sulphate, heparin and keratan sulphate. **Hyaluronic acid** also called hyaluronate is the only group in which none of the sugars is sulphated. Hyaluronic acid is important in aiding cell migration in the tissues during development or wound repair by making their matrix highly hydrated as this attracts water. Cell migration stops when these molecules are destroyed by the enzyme **hyaluronidase**.

All other GAGs are covalently linked to serine residues to form proteoglycans, before being secreted by exocytosis in the extracellular matrix. These proteoglycans can differ in protein content, molecular size and number and type of GAG chains per molecule.

Due to this structural heterogeneity proteoglycan molecules possibly form gels of varying pore size, charge and density, thus functioning as sieves to regulate the movements of molecules and cells according to their size and/or charge, in the matrix. For example in kidney glomerulus matrix functions like this for filtration of molecules from blood into urine. The exact manner in which glycosaminoglycans and proteoglycans are organised in the extracellular matrix is still unknown. Some of these molecules can bind in specific ways to each other as well as to other components of the matrix such as collagen, elastin and fibronectin

18.5.2 Basal Lamina

Basal Laminae are thin layers of specialised extracellular matrix. These layers are found underneath all epithelial cell sheets and tubes, surround individual muscle cells, fat cells and Schwann cells, thus separating these sheets and cells from underlying or surrounding connective tissue. In some cases, such as kidney glomerulus and lung alveolus, basal lamina lies between two different cell layers thus functioning as highly selective filter (see Fig. 18.21).

Basal Lamina is synthesised by the cells that rest on it. The major component of all the basal laminae is type IV collagen. As you have read in Unit 6, pre- α -chain of type basal laminae is type IV collagen. As you have read in Unit 6, pro- α chains of secretion out of the cell. Other components of laminae are proteoglycans fibronectin and a large glycoprotein laminin. Laminin consists of two subunits of 2,20,000 and 4,40,000 molecular weight and they are bound to each other by disulphide bonds. Basal

Schwann cells wrap around peripheral nerve fibres to form myelin.

lamina also contains other proteins which are yet to be identified. The detailed molecular organisation of lamina is unknown. Although evidences show that laminin and proteoglycans are found along the inner and outer surfaces of lamina with collagen molecules lying in the middle. The precise composition varies from tissue to tissue and also from region to region within same lamina.

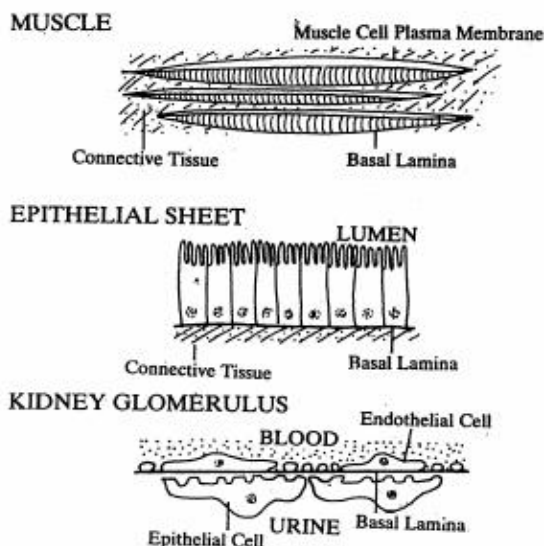


Fig. 18.21 Schematic diagram showing three distributions of basal laminae: (a) surrounding the cell, (b) underlying epithelial cell sheets, (c) interposed between two cell sheets.

Basal lamina performs diverse functions. In kidney and lungs it acts as a semipermeable filter. It also acts as a selective cellular barrier, e.g. beneath epithelial cells it prevents fibroblasts in the underlying connective tissue from making contacts with epithelial cells, but allows macrophages, lymphocytes or nerve processes to pass through it. It is likely that basal lamina also plays an important role in tissue regeneration after injury, induces cell differentiation, influences cell metabolism and organises proteins in adjacent plasma membrane.

SAQ 4

Match the items in column 1 with those of column 2.

Write the answers in the box provided

Column 1	Column 2
i) Collagen fibre []	a) Polypeptide chains remain unfolded as random coil.
ii) Elastin filaments []	b) The polysaccharide chains are highly negatively charged.
iii) Glycosaminoglycans []	c) Polysaccharide chains are not sulfated.
iv) Hyaluronic acid []	d) In basal lamina pro α -chains have long extension peptide.

Thus we see that relative amounts of different types of macromolecules in the matrix and their organisation give rise to diversity of forms each highly adapted to the functional requirement of the particular tissue. The matrix can become calcified to form hard structures of bone and teeth, or assume almost crystalline form for the transparent matrix of the cornea, the internal covering of the eye. With this we end our discussion about cell adhesion, cell movements and extracellular movement. In the next unit we will discuss about various types of animal tissues.

18.6 SUMMARY

In this unit you have studied that:

- Cells in multicellular organisms recognise each other and aggregate into co-operative assemblies with the help of specific cell surface molecules and this aggregation can be species-specific or tissue-specific.
- Adjacent cells are joined and interact with each other with the help of various types of cellular junctions which are the regions of adjacent interacting plasma membranes.
- Free living unicellular organisms and certain cells in multicellular animals move with the help of various types of cellular processes and locomotory apparatus.
- Extracellular matrix in the tissues is formed by the cellular secretion and consists of mainly proteins and glycosaminoglycans.

18.7 TERMINAL QUESTIONS

- 1) Discuss briefly the role of cAMP in aggregation of *Dictyostelium discoideum* in the space given below.

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- 2) Make comparison between tight junctions and gap junctions. Answer briefly in the given space.

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- 3) Explain briefly the process of phagocytosis in macrophages in the space provided.

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- 4) Enlist the major components of extracellular matrix and discuss briefly glycosaminoglycans.

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Self-assessment Questions

- i) Cyclic AMP
 - ii) stalked fruiting body
 - iii) Ca^{2+}
 - iv) aggregation factor.
- 2
- i) Adhering junctions; they mechanically hold the cells together, e.g. desmosomes.
 - ii) Impermeable junctions; they seal the interacting plasma membrane and make the passage impermeable to molecules, e.g. tight junctions.
 - iii) Communicating junctions; they allow molecules to pass directly from one cell to another, e.g. gap junctions.
- 3
- a) Pseudopodia are small cellular projections and means of locomotion in unicellular organisms and various leucocytes. Lamellipodia are sheet like cellular extensions and are means of locomotion in fibroblasts of vertebrates.
 - b)
 - i) filopodia
 - ii) growth cone
 - iii) power stroke
 - iv) surface receptors
- 4
- i) d, ii) a, iii) b, iv) c,

Terminal Questions

- 1) Cells of slime mold *Dictyostelium discoideum* synthesise and secrete cAMP which acts as chemotactic signal. The starving cells aggregate in response to this signal by means of specific cell surface molecules which further secrete cAMP attracting other starving cells and so on. Thus the pulse of cAMP is formed for cellular aggregation.
- 2) Tight junctions seal the interacting plasma membrane of adjacent cells which are held together by transmembrane protein strands. Gap junctions are the narrow channels called connexon formed in the adjacent plasma membranes connecting the two cell interiors. The channel is formed by six subunits of connexin proteins.
- 3) Macrophages engulf the microorganisms and worn out cells by phagocytosis. The antibody molecules bind to the surface of microorganisms and form a coat. The antibody receptors on surfaces of macrophages recognise the coated particle and engulf it by forming pseudopodia.
- 4) The major components of extracellular matrix are proteins collagen, elastin, fibronectin, chondronectin, laminin and glycosaminoglycans. Glycosaminoglycans are long unbranched polysaccharide chains with repeating disaccharide units, each with one amino sugar. These polysaccharide chains are highly negatively charged because of the presence of sulphate or carboxyl groups on sugar residue.

UNIT 19 DIFFERENTIATED ANIMAL CELL TYPES

Structure

- 19.1 Introduction
 - Objectives
- 19.2 Differentiated Cell Types—A General Classification
- 19.3 Muscle Tissue
 - Skeletal Muscle
 - Smooth Muscle
 - Cardiac Muscle
- 19.4 Nervous Tissue
- 19.5 Connective Tissue
 - Bone
 - Cartilage
 - Blood
- 19.6 Epithelial Tissue
 - Types of Epithelial Cells
 - Functions of Epithelial Tissue
 - Origin of Epithelial Tissue
- 19.7 Mesenchyme
- 19.8 Summary
- 19.9 Terminal Questions
- 19.10 Answers

19.1 INTRODUCTION

In Block 1 of this course, you have studied about the ultrastructure of the generalised cell and the differences between plant and animal cell. In this unit you will study about differentiated animal cell types and in the next unit you will study about differentiated plant cell types.

In this unit, you will study about the differentiation process. Differentiation produces cells which have different structures and functions. The cells with similar structure and function are grouped together to form tissues. As it is difficult to study and describe a cell in isolation, we will generally discuss the structure of tissue. You will study the further details of differentiation of cells at embryonic stage in Developmental Biology course.

The four major types of animal tissues are **epithelial, nervous, muscular** and **connective**. You will study in detail about the structure and function of the cells comprising these tissues. The details of how a particular tissue 'works' will be dealt in Animal Physiology course. A short account of mesenchyme and epithelial tissue is also given.

Objectives

After studying this unit you shall be able to:

- explain differentiation process
- compare and contrast the structures of striated, smooth and cardiac muscle
- explain the functions of three types of muscles with special reference to the muscular contraction
- describe the structure, types and functions of neuron
- classify connective tissues and describe the structure and function of cells of blood, bone and cartilage
- identify different types of blood cells (granular and agranular leucocytes erythrocytes and platelets), bone cells and cartilage cells
- list various types of epithelial tissue and explain their structure and function
- describe the structure of mesenchyme.

19.2 DIFFERENTIATED CELL TYPES—A GENERAL CLASSIFICATION

There are various ways of classifying tissues based on their function. We can distinguish four primary kinds of tissues in vertebrates:

- Muscular or contractile tissue which consists of muscle cells,
- Nervous or sensory tissue which consists of nerve cells or neurons,
- Epithelial or covering tissue which consists of epithelial cells,
- Connective or supporting tissue. Blood, lymph, cartilage and bone are the examples of this tissue. All these tissues consist of specialised cells, the structures of which you will study in this unit.

To start with, all cells at the embryonic stage are similar, but as the organism grows, organised patterns of cell differentiation occur and patterns of different tissues develop. Most animal cells do not have rigid walls, and cytoplasmic bridges are unusual. Instead, the cells are bound together by adhesions between their plasma membranes or by a relatively loose meshwork of large extracellular organic molecules. Differentiation of cells is believed to take place by specific changes in the cytoplasmic components of cells at the molecular level. These changes are under genetic control. Certain genes are switched on and switched off according to the specific requirement of a particular cell type. The underlying mechanisms of cell differentiation are still not completely known. However, there is a wonderfully organised division of labour in the cell types which collectively operate in the form of tissues in multicellular organisms to perform activities such as movement, sensation, communication and social organisation. This enables them to compete, propagate and survive in several complex situations that are created in the external and internal environment of an organism. Fig. 19.1 shows how cells of similar types form the four main categories of tissues.

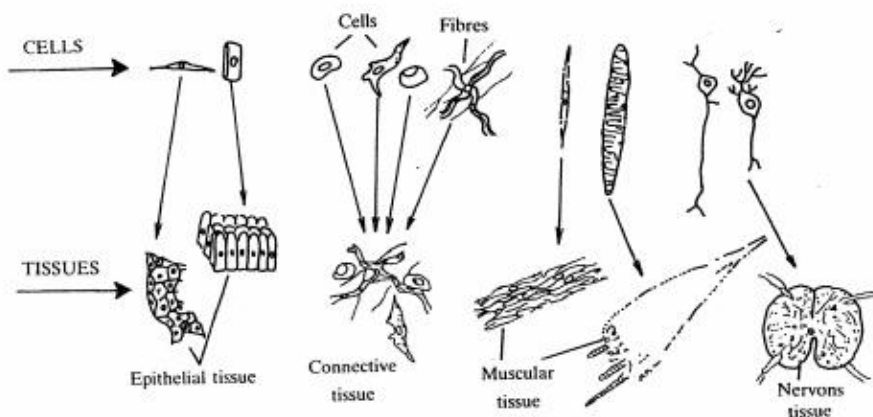


Fig. 19.1 How cells of similar types form tissues.

Now let us have a closer look at each cell type and try to understand their structural and functional relationships. In order to understand the various cell types, it will be easier for starting from tissues because a single cell cannot work independently.

19.3 MUSCLE TISSUE

In higher organisms, the movement of the body and its parts is due to the activity of muscle cells. Muscle cells contract to produce mechanical force. There are three main types of muscle cells, which constitute 60-75% of the total body tissue weight—

a) Skeletal b) Smooth and c) Cardiac. Muscle cells are generally arranged in lengthwise bundles or sheets with some connective tissue, blood vessels and nerves

The skeletal muscles are striated muscles and are called voluntary muscles. These usually move the bones and form the bulk of the body which we call flesh. The smooth muscles are called involuntary or unspecialized muscles. These contract and expand in

the internal organs such as the stomach, blood vessels and urinary bladder. The **cardiac muscles** are those which move the heart. The movement of the body or its parts is due to the activity of the muscle cells. The functions of various systems such as digestion, reproduction and excretion are also due to the movement of muscles of which they are made.

19.3.1 Skeletal Muscle

This type of muscle produces movements of the limbs, trunk, face, jaws, eyeballs and all the movements associated with bones. Skeletal muscles are attached to the bones and help in the movement of the skeleton and body as a whole. It is the most abundant tissue in vertebrates and comprises about 40% of the total body weight. Anatomically, a skeletal muscle is composed of a number of cylindrical muscle fibres which form the basic unit of the muscle. Each muscle fibre is a long cylindrical, multinucleate cell ranging from 0.01 to 0.1 mm in diameter. It has an outer covering of a membrane called **sarcolemma** beneath which are located numerous nuclei scattered throughout the fibre. A muscle fibre consists of a semifluid cytoplasm termed as **sarcoplasm** containing numerous longitudinal **myofibrils** which range from 10-100 μm in diameter. The myofibrils are the actual contractile elements which run all along the length of the fibre. The striated appearance of the muscle is due to the presence of dark and light bands in the myofibrils. The light bands are called I bands and the dark bands are A bands. Each light band is divided in the middle by a z line or Krause's membrane which goes across the whole fibre to join the sarcolemma at the edges. The z line functions to carry the signal for contraction. The area between two z lines is called **sarcomere**.

Now look at Fig 19.2. This is at a magnification which shows you the banded nature at the muscle and the scattered nuclei in the sarcoplasm. You can also make out how the muscle fibres are organised to form a muscle.

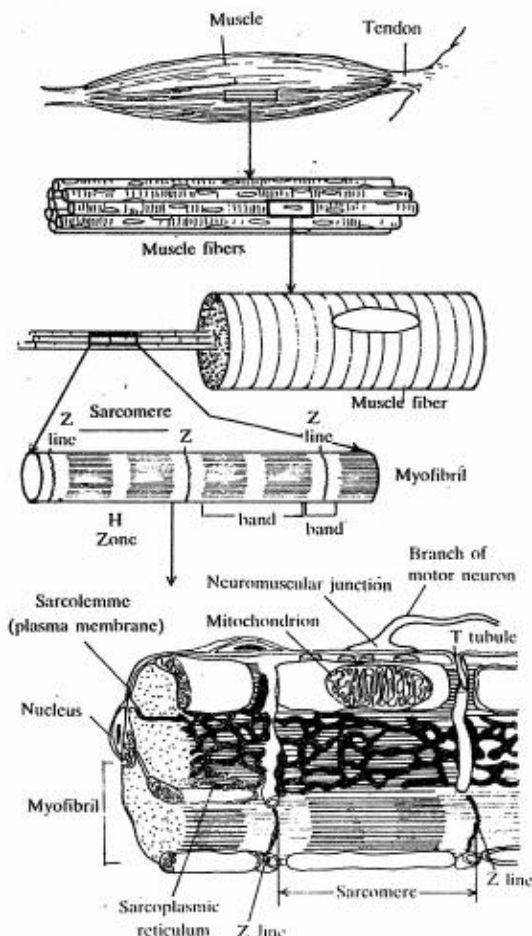


Fig. 19.2 a) Structure of skeletal muscle. A muscle is composed of numerous muscle fibres bound together by connective **tissboe**) When viewed under the light microscope each fibre can be seen to contain numerous longitudinal fibrils (myofibrils) and to display a characteristic pattern of striations.

Fig. 19.3 shows the ultrastructure of muscle fibres and the surface view of a sarcomere. A sarcomere is composed of thread like myofilaments which are of two types—primary and secondary. The former are thicker than the latter. The thicker filaments or the primary filaments are made up of protein **myosin** and are present only in the A band. The thinner or secondary filaments are made of the protein called **actin** and are located in the I band and also extend at both ends of the A band. Therefore, the centre of the A band has only primary or thick filaments and appears as a less dense H band.

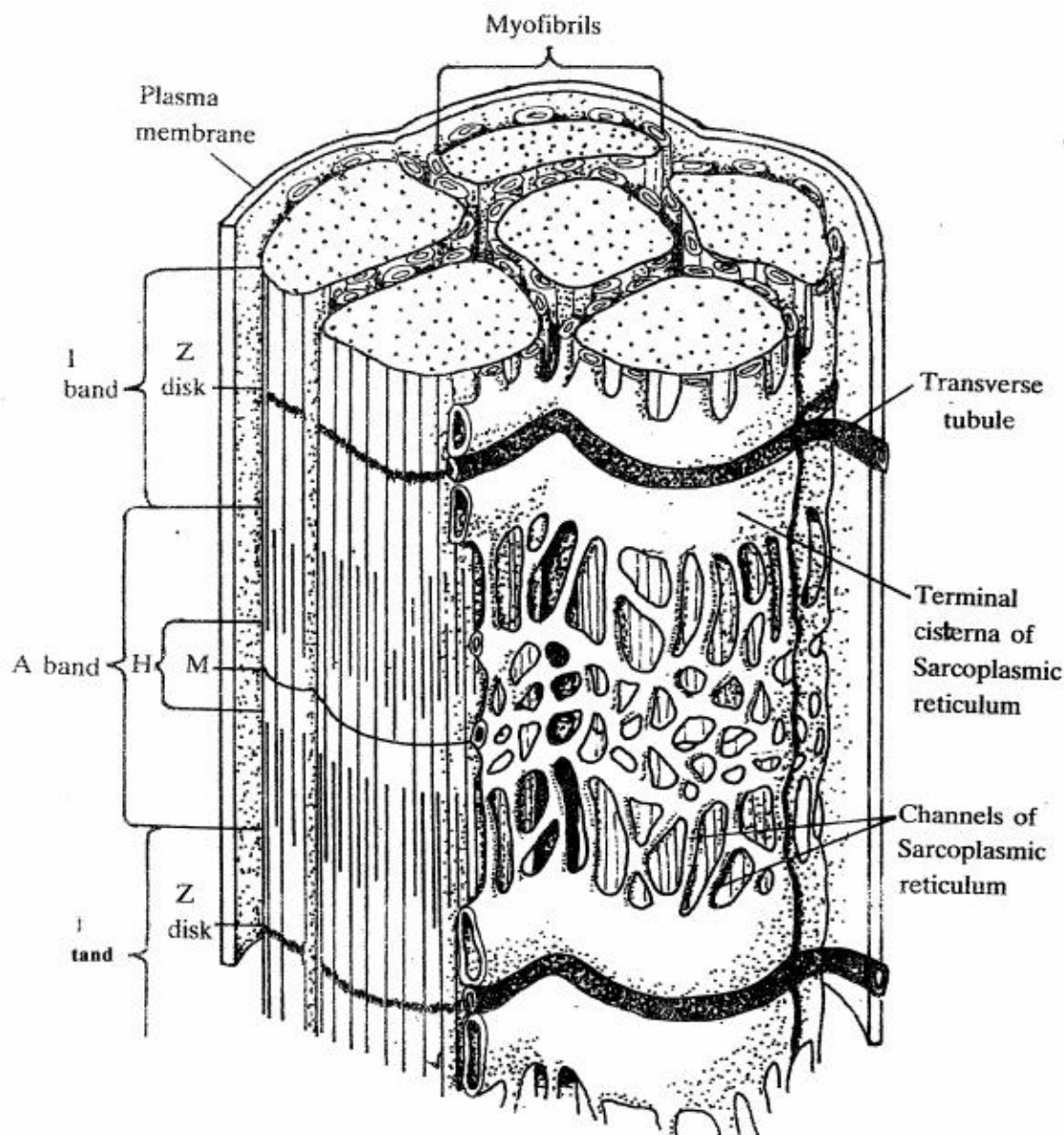


Fig- 19.3 Three-dimensional diagram of part of a frog striated muscle fibre as seen by electron microscopy demonstrating the various bands and sarcoplasmic reticulum and T tubules. Note that in mammalian muscle there are two triads per sarcomere, located at A-I junctions in the frog, triads lie at z lines, as shown here.

During contraction the length of A band remains the same but I and H bands **disappear** which results in the shortening of the sarcomere. The shortening of the

sarcomere appears to be the result of the secondary filaments sliding inwardly and joining together as shown in Fig. 19.4. This has been confirmed by taking electron micrographs of contracted as well as stretched muscle fibres. The high energy bond molecule-ATP is formed in the myofibrils and supplies the energy required for the contraction and relaxation of the myofibrils.

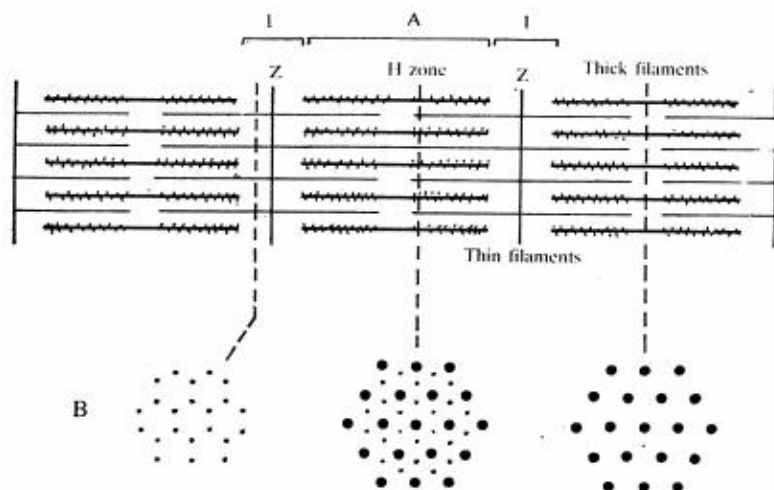


Fig. 19.4 The sliding hypothesis proposes that when a muscle shortens, the thick and thin filaments slide in between one another as shown here. Although this has never been seen taking place, it is supported by electron microscopy and other evidence. Note that when contracted the dark bands should remain the same length as the light bands and H zones should shorten. It is in fact the case.

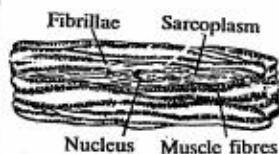


Fig. 19.5 A bundle of unstripped muscle fibres

19.3.2 Smooth Muscle

These muscles form muscle layers on the walls of the digestive tract and various other internal organs. The walls of arteries and veins are also lined with smooth muscles. The cells are uninucleate elongated narrow, spindle shaped, tapering at both ends with the nucleus in the middle. Look at Fig. 19.5, where you can see fine thread like fibres running longitudinally—these are the myofibrils. The contraction and relaxation of these myofibrils are under the control of the autonomic nervous system. The fibres interlace to form sheets of muscle tissue rather than bundles. The contractions of these muscles are slow and sustained and these muscle fibres do not respond immediately to external stimuli. In blood vessels they help in the flow of blood and in the eye they help in changing the shape of the pupil.

19.3.3 Cardiac Muscle

The muscles of the heart consist of fibres which are branched and the branches join to form a network. The structure of these muscles show characteristics that are intermediate between the striped and unstripped muscles. A look at Fig. 19.6 will show you that this type of muscle has dark and thick discs in between the fibres in addition to being banded. These discs are called **intercalated discs**. These muscles are also involuntary like the smooth muscles and the fibres are uninucleate. Each fibre has an outer membrane, the sarcolemma. The sarcoplasm has longitudinally running myofibrils. The cardiac muscles contract rhythmically and the contraction of the heart is initiated within the muscles and not by an external nerve impulse. Thus the heart beat is **myogenic** and not **neurogenic**. Another interesting feature of the heart muscles is that these muscles are immune to fatigue.



Fig. 19.6 Cardiac muscle fibres

The characteristics of skeletal, smooth and cardiac muscles are summarised in Table 19.1.

Table 19.1 Types of Muscle Tissues

	Skeletal	Smooth	Cardiac
Location	Attached to skeleton	Walls of stomach, intestine, etc.	Walls of heart
Type of control	Voluntary	Involuntary	Involuntary
Shape of fibres	Elongated, cylindrical, blunt ends	Elongated, spindle-shaped, pointed ends	Elongated cylindrical fibres that branch and fuse
Striations	Present	Absent	Present
Number of nuclei per fibre	Many	One	One or two
Position of nuclei	Peripheral	Central	Central
Speed of contraction	Most rapid	Slowest	Intermediate
Ability to remain contracted	Least	Greatest	Intermediate

SAQ 1

- a) Outline two similarities between smooth and cardiac muscles

.....

.....

- b) State two important similarities regarding the shape of fibres of skeletal muscle and cardiac muscle

.....

.....

SAQ 2

Give one word for the following

- i) Chief protein components of myofibrils

.....

- ii) Membrane covering the muscle fibre

.....

- iii) Semifluid cytoplasm of the muscle fibre

.....

- iv) Area between two z lines

.....

- v) Dark and thick discs in between the fibres of cardiac muscle

.....

19.4 NERVOUS TISSUE

Nervous tissue is composed of **neurons** or **nerve cells** which are the functional units of nervous tissue. Neurons are specialised for conducting electro-chemical nerve impulses. Neurons are supported and nourished by another type of cells called as **glial cells**. Certain neurons receive signals from the external or internal environment and transmit them to the spinal cord and brain, other nerve cells process and store the information. This is the cellular basis for the complex functions of consciousness, memory, thought and directive movement.

Having known the functional importance of the neurons, let us now discuss their basic structure and characteristics in order **to understand** their functioning.

Nerve Cell or Neuron

As already mentioned, a neuron is a functional unit of the nervous system. The structure of a typical neuron is shown in Fig. 19.7. A typical neuron is a flattened cell consisting of cell body called cyton or soma from which arise the fibres. The cytoplasm contains granules which are made of RNA. These are called Nissl granules.

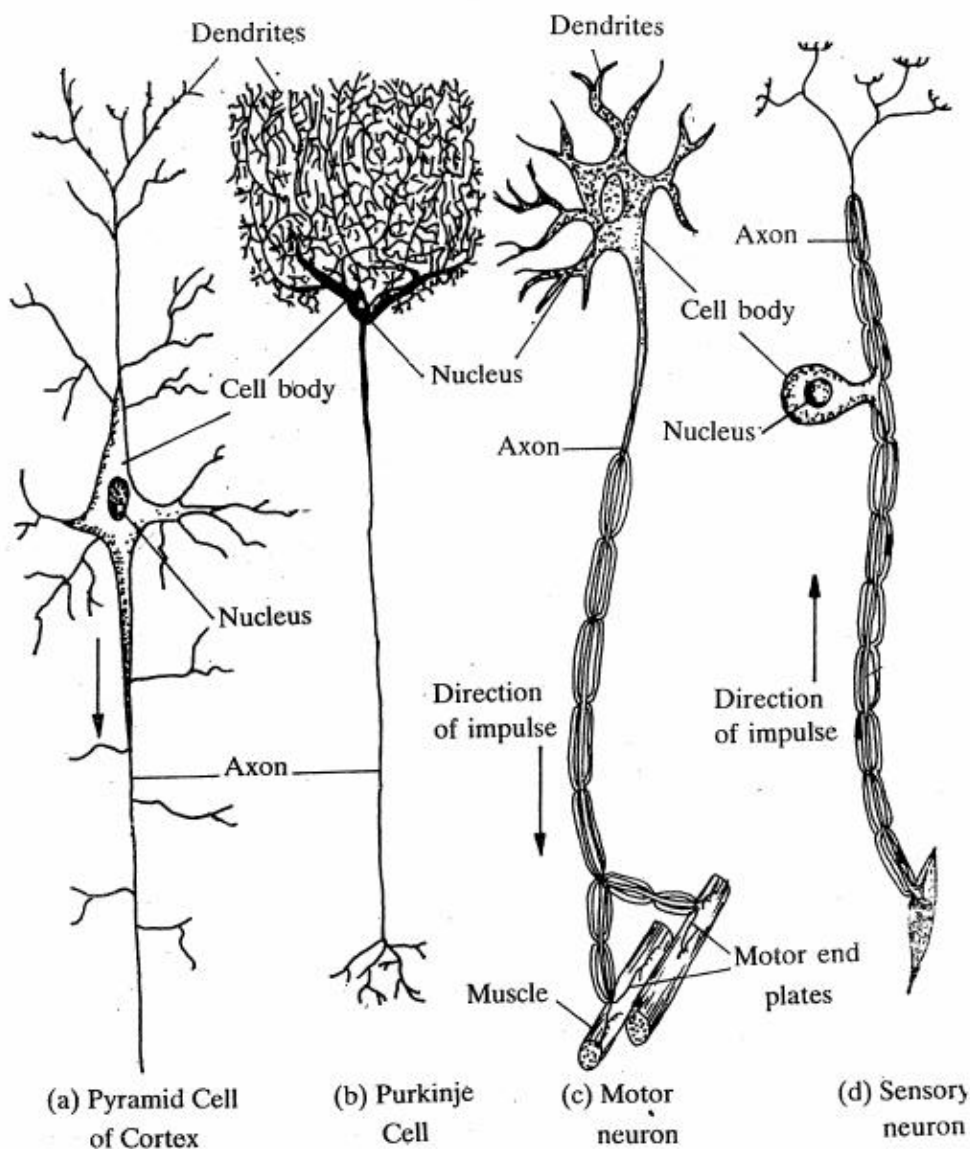


Fig. 19.7 a) Nerve fibre, b) Nerve cells connect with one another at synapses. Synaptic knobs on the surface of a vertebrate motor nerve cell. A single neuron may be covered by as many as 50,000 synaptic knobs derived from many different neighbouring neurons.

Dendrons and Axons

These are two types of processes which arise from the cyton. The processes through which impulses are received are called **dendrons**. These further branch into minute processes called **dendrites**. The impulses received by the dendrites and dendrons are transmitted to the next neuron by a long process called **axon**, which ends in branches known as axon endings. Main axon fibres give off the collateral axon fibres. Nerve impulses are transmitted from the axon endings at one neuron to the dendrites of another neuron. There is a minute microscopic gap between two neurons. The linking up of two neurons is not by physical connection but by chemical action. Such a

linking of neurons is called a **synapse**. The axon ending as revealed by the electron microscope has minute terminal ends or buttons (Fig. 19.7b) which secrete acetylcholine. This causes the impulse to reach the dendrites of the next nerve cell where a fresh impulse is formed. The secretion of acetylcholine is stopped by an enzyme, called **cholinesterase**, after it has set up a fresh impulse in the next neuron. The axon is enclosed within a sheath of fatty substance known as **myelin sheath** which in turn is covered by a thin covering called **neurilemma**. This covering sheath is formed of sheath cells, or **Schwann cells**, having flattened nuclei embedded in the cytoplasm at intervals. Schwann cells are responsible for the secretion of the myelin sheath. The myelin sheath is not continuous. It is interrupted at regular intervals by constrictions called **Nodes of Ranvier**. At these nodes, the layer of cytoplasm just below the neurilemma comes into direct contact with the axon cylinder. The part of the nerve between two adjacent nodes is called **internode**. Nerve fibres having a myelin sheath are called **medullated fibres**.

Neurons exist in a bewildering variety of shapes and sizes but all share certain common features. They can be classified on the basis of the number of cytoplasmic processes extending from the cell body. Fig. 19.8 shows you the various types of neurons. A neuron with a single process arising from the cytoplasmic process is called **unipolar**. The process has several branches, some of which function as dendrites, others being axonal. Unipolar neurons are rare in the adult vertebrates. **Bipolar neurons** are those which have a single axon and a single dendrite usually located at opposite poles of a spindle shaped soma, i.e. it has two processes arising from the cell body. **Multipolar neurons** have several dendrites at one end and an axon on the other. This is the most common and typical type of neuron. Functionally, neurons can be classified into four major types:

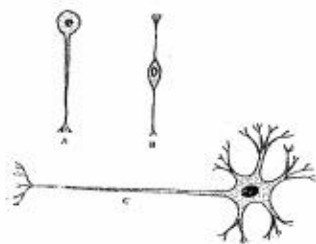


Fig. 19.8 Diagram to show basic morphological features of the types of neuron: A unipolar, B bipolar, C multipolar.

- 1) **Motor neurons** which convey information from the central nervous system to the effector organs, such as muscles, glands etc.
- 2) **Sensory neurons** which transmit sensory information from the peripheral parts of the body to the central nervous system.
- 3) **Interneurons** which lie between motor (efferent) and sensory (afferent) neurons and transmit signals in several directions, by modifying them in a way best suited to the needs of the animal. The motor (efferent) neurons have larger axons than the dendrites. In sensory (afferent) neurons the dendrites are relatively larger.
- 4) **Neurosecretory neurons** are those neurons that are specialised for the production of hormones.

So you have seen that neurons or nerve cells are specialised for performing the function of communication. The brain and spinal cord are composed of a network of neurons structurally supported by neuroglial cells. The axon conducts electrical signals away from the cell body. These signals are produced by a flow of ions across the nerve cell membrane. **Schwann cells** wrap around the axon to form a multilayered membrane sheath. At synapses, signals pass from one neuron to another or from a neuron to a muscle cell.

SAQ 3

- a) In column I some terms are given and in column II, the description of these terms are given Match column I and II.

Column I	Column II
i) Axon	A) Cell body of the neuron
ii) Cyton	B) Small process arising from cyton
iii) Dendrites	C) Long process arising from neuron
iv) Node of Ranvier	D) Enclosing sheath of axon
v) Myelin Sheath	E) Constrictions of Myelin sheath
vi) Nissl granule	F) Granules present in cyton

- b) Fill in the blanks.
- i)neurons have a single axon and a single dendrite located at opposite poles.
 - ii) The neurons which transmit sensory information from the peripheral parts of the body to the central nervous system are.....neurons.
 - iii)neurons are specialised for the production of hormones.

19.5 CONNECTIVE TISSUE

The main function of **connective tissues** is to join together the different tissues of the body. Connective tissues also support the body and its structures and protect underlying organs. In addition, almost every organ in the body has a supporting framework of connective tissue, called **stroma**. The epithelial components of the organ are supported and cushioned by the stroma.

There are many kinds of connective tissues and many systems for classifying them. Some of the main types of connective tissues are 1) loose connective tissue, 2) dense connective tissue, 3) elastic connective tissue, 4) reticular connective tissue, 5) adipose tissue, 6) cartilage, 7) bone, 8) blood, lymph, and tissues that produce blood cells. These tissues vary widely in the details of their structure and in the specific functions they perform. In this section we will describe about bone, cartilage and blood which are specialised forms of connective tissue, in detail.

Connective tissues contain relatively few cells embedded in an extensive **intercellular substance** consisting of threadlike microscopic **fibres** scattered throughout a matrix secreted by the cells. The **matrix** is a thin gel composed of polysaccharides. The cells of different kinds of connective tissues differ in their shape and structure and in the kind of matrix they secrete. The nature and function of each kind of connective tissue are determined in part by the structure and properties of the intercellular substance. Thus, to some extent, connective tissue cells perform their respective functions indirectly by secreting the matrix, which provides the connection and support.

19.5.1 Bone

Bone is a solid hard tissue which constitutes most of the skeleton of higher vertebrates. The bone cells are known as **osteocytes** which are embedded in a solid matrix. The deposition of carbonates and phosphates of calcium makes the matrix very firm. About 30-40 per cent by weight of the living bone is made of organic matrix. The bone is surrounded by a fibrous membrane, the **periosteum**. The central part of the bone has a cavity which is filled with soft fatty tissue, the **marrow**. When the marrow contains vascular sinuses it is called **red bone marrow**. The blood corpuscles are formed by the marrow. The coating of the bone is lined with a delicate membrane called the **endosteum**. The bony substance is arranged in concentric layers called the bone **lamellae** that surround the marrow cavity. Numerous irregular cell spaces—the lacunae are scattered in the lamellae. Osteocytes or the bone cells are found in the lacunae. Neighbouring lamellae are connected with each other by means of a delicate branching of canals called 'canaliculi' (Fig. 19.9 a & b).

Classification of Bones

Two kinds of bones are distinguishable on the basis of structure:

- a) **Compact or periosteal bone:** This type of bone is hard and dense without any space (Fig. 19.10a). The shaft of long bones is of this type.

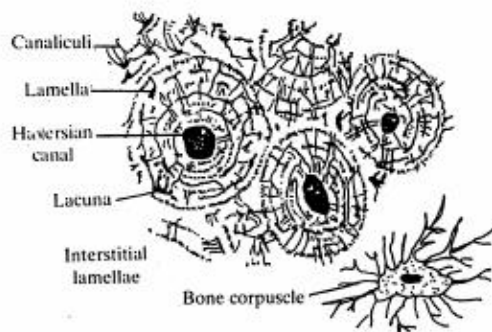
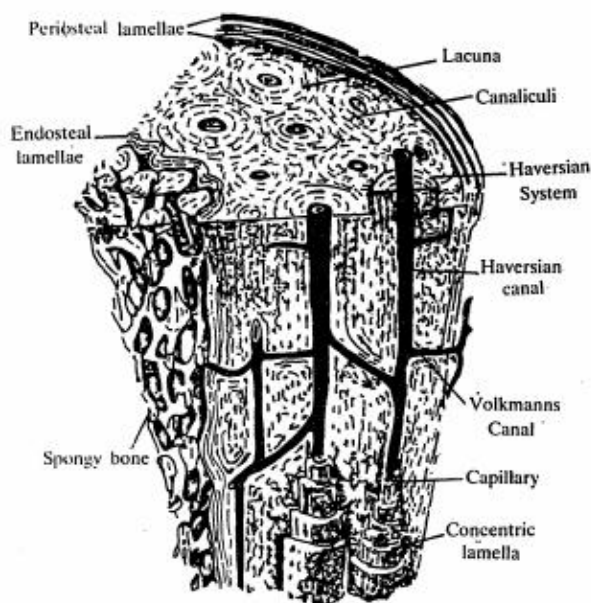


Fig. 19.9 a) A diagrammatic representation of a small portion of compact bone, b) Haversian systems in a transverse section of dried material: a bone corpuscle is shown on one side.

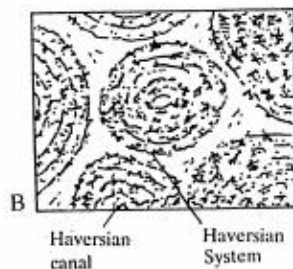
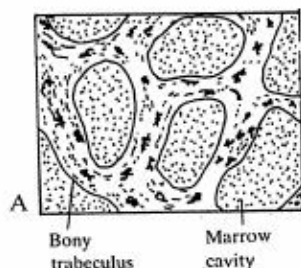


Fig. 19.10 Structure of spongy (a) and compact bone (b)

- b) **Spongy or cancellous bone:** This type of bone consists of small pieces, joined together irregularly, where the marrow is contained in between the pieces (Fig. 19.10b). Such bones constitute the broad end of long bones and the centre of vertebrae. Spongy bones form the inner surface of long bones.

On the basis of their formation, two kinds of bones can be distinguished:

- a) **Replacing bones or cartilage bones:** They are initially laid down in cartilage, which is later replaced by bone substance. All the bones of the body except the skull and part of the pectoral girdle are formed this way.

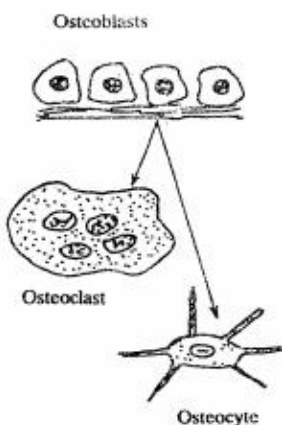


Fig. 19.11 Types of bone cell

- b) **Dermal or membrane bones:** These bones arise directly by the ossification of the connective tissue cells of the dermis e.g. most part of the skull and part of the pectoral girdle. These two types of bones are structurally similar, once they are formed. All the three types of bone cells are derived from the mesenchyme, about which you will study later in Section 19.9. The process of bone formation is called ossification.

Types of Bone Cells

If you examine a piece of bone you will find that there are three kinds of bone cells in it, namely, i) osteoblasts, ii) osteocytes, and iii) osteoclasts (Fig.19.11).

- Osteoblasts:** These are the cells which form the bone by secreting the fibres and inorganic salts. These are found only in places where new bone is being formed. The nuclei of the cells are situated away from the side where the bone is being formed. Their cytoplasm is basophilic. By the deposition of salts and fibres they themselves get transformed into osteocytes.
- Osteocytes:** These are transformed osteoblast cells which have branching cytoplasmic processes. These cells are found in the lacunae of fully formed bones, with their protoplasmic processes extending into the canaliculi. Osteocytes are considered to play an active role in releasing calcium from bone to blood.
- Osteoclasts:** These are multinucleated cells much larger than osteocytes. They are associated with the destruction of bone and are found near the inner surface of the endosteum. If we compare the femur bone of an infant with that of an adult, the marrow cavity of the adult femur is much bigger—the widening of the marrow cavity is due to osteoclast activity. A bone grows in size by the destruction of bone from inside and deposition of one from outside. In growing persons, the amount of destruction is less than deposition. In bones of old persons destruction is slightly more than deposition, that is why the bones of an old person are more fragile.

19.5.2 Cartilage

Cartilage is a tissue with clear matrix of dense and elastic nature in which cartilage cells or **chondroblasts** are embedded in irregularly scattered fluid-filled spaces called **lacunae**. The matrix is made up of **chondrin** in which there is a delicate network of collagen fibres. The chondroblasts secrete chondrin and often lie in pairs. Each chondroblast is a subspherical corpuscle of protoplasm with a round nucleus. The surface is covered by a layer of cells, the **perichondrium** which has a good blood supply from which nutritive material diffuses into the cartilage. For growth, new layers are added to the outside just internal to the perichondrium.

Below the perichondrium new chondroblasts are added by a process of active division. In a transverse section of cartilage we can see that the chondroblasts are abundant and close to each other near the perichondrium (Fig.19.12).

Types of Cartilage

Structurally four main types of cartilage can be distinguished

- 1) Hyaline cartilage
- 2) Fibrous cartilage
- 3) Calcified cartilage
- 4) Elastic cartilage

1) **Hyaline Cartilage:** This type of cartilage has a transparent and homogeneous matrix as seen in the ends of limb bones and several other organs of the body.

2) **Fibrous Cartilage:** In this type of cartilage the matrix is not as abundant as in hyaline cartilage. A large number of elastic collagen fibres are present in the matrix. The chondroblasts are found in lacunae. This type of cartilage is found in the intervertebral disc of mammals.

3) **Calcified Cartilage:** In this type of cartilage the matrix contains deposits of lime salt as seen in the heads of humerus and femur bones.

4) **Elastic Cartilage:** This is basically the same as hyaline cartilage and the elasticity is due to the yellow elastic fibres present in the matrix. Elastic cartilage is found in the pinnae (external ear) of mammals.

By now you must have learnt that both bone as well as cartilage share a common feature i.e. a matrix with cells and fibres that form part of the connective tissue known as skeletal connective tissue. In the foetus, most of the skeleton is laid down as hyaline cartilage which is subsequently replaced by bone. But the typical hyaline cartilage persists throughout the life of an organism i.e. the cartilage of the nose and trachea.

19.5.3 Blood

Blood is a fluid connective tissue consisting of plasma—the extra cellular liquid matrix, and blood corpuscles floating in it. Ordinarily, in connective tissue the matrix is secreted by the cells themselves, but in blood the matrix (plasma) is not wholly produced by the blood corpuscles. The other difference between blood and other connective tissues is that the former does not contain fibres in its matrix. In spite of these differences, the blood is regarded as a connective tissue, because the blood vessels and blood arise from the same stock, the mesoderm, which gives rise to the connective tissue in the embryo, and there is a constant interchange of plasma and cells between the connective tissue and the blood.

Plasma

Plasma is a pale yellow homogeneous fluid and forms 55-60 per cent of the total volume of blood. It is composed of roughly 91 per cent water which is the basic solvent of plasma and 9 per cent solid materials out of which about 7 per cent are proteins. Some of important plasma proteins are globulins, albumins and fibrinogen.

Plasma proteins have many important functions. They help in maintaining osmotic equilibrium of blood, and are essential in immunological reactions of the body. The protein deficiency of food is compensated to a certain extent by utilising the plasma proteins. Plasma fibrinogen is essential in clotting of blood. The cells suspended in plasma are red blood cells or **erythrocytes**, white blood cells or **leucocytes** and **platelets** or **thrombocytes** (Fig.19.13).

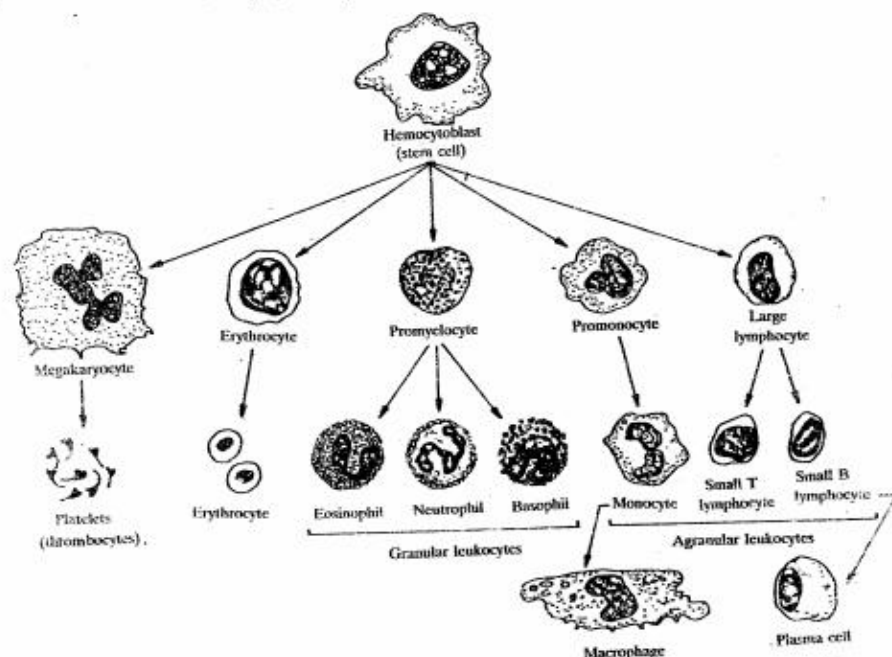


Fig. 19.13 A composite picture of blood cells from several preparations stained with Leishmann's stain. X C 1900.

Red Blood Cells (RBCs)

They are very small, biconcave and disc-shaped cells surrounded by a thin membrane. There are roughly four million cells per cubic millimetre in number. The

number is slightly lower in women than in men. The RBCs are made up of stroma of protoplasm permeated with haemoglobin which constitutes about 90 per cent of the weight of the corpuscles. There are approximately 280 million molecules of haemoglobin per erythrocyte. Nucleus is lacking in RBC. However, they arise from normally nucleated connective tissue cells of the bone marrow.

Let us now discuss some interesting features of red blood cells. The RBCs are produced by the liver, spleen and thymus in the embryonic period. After birth they are formed in the bone marrow. From the age of about 20 when the bones stop growing, most red blood cells are produced in the bone marrow of the ribs and the vertebrae.

Erythrocytes have a life span of about 120 days after which they are broken down in the liver forming bile pigments (bilirubin). Approximately 2.5 million cells are destroyed per second and almost the same number are produced each second. The primary function of the red cells is to assist in respiration. This function is possible due to the presence of the protein haemoglobin.

The principal functions of haemoglobin are transportation of oxygen and CO_2 and prevention of acidity in the blood. pH of blood is $7.3 \pm .1$. In a healthy normal male, the haemoglobin content ranges from 12.5 gm/100 ml to 14.5 gm/100 ml and in healthy normal female this content ranges from 10 gm/100 ml to 12 gm/100 ml. As mentioned earlier, haemoglobin is always bound to RBC. If this was free from red cells, circulation of blood would have been an impossibility owing to high viscosity.

White Blood Cells (WBCs)

They are slightly larger than red cells. They are colourless or transparent. They are amoeboid in shape and can move about with pseudopodia. They may possess two or more nuclei. The average count of leucocytes in human blood varies from 5 to 10 thousand cells per cubic millimetre. There are five main classes of white blood cells varying concentrations of which are described as follows:

1) Neutrophils	59.0	} Granulocytes
2) Eosinophils	2.5	
3) Basophils	0.5	
4) Lymphocytes	34.0	} Agranulocytes
5) Monocytes	4.5	

The first three are collectively called **granulocytes** since they have a granular appearance. Granulocytes live for about 10 days, lymphocytes for about 2-3 days and monocytes appear to live relatively longer. They can be distinguished on the basis of staining reactions and their names have been derived accordingly.

Neutrophils are those leucocytes which get stained with neutral dyes. They are phagocytic in function and engulf bacteria and other extraneous substances. They move with pseudopodia and get attracted to the site of infection or injury.

Eosinophils are stainable with acidic dyes and show amoeboid movements. Their percentage increases when parasites invade the body. **Basophils** are stainable with basic dyes and contain heparin in the cytoplasmic granules suggesting their anticoagulant function. There is no evidence to prove their phagocytic function. These are the smallest of leucocytes.

Lymphocytes: These have large nuclei that are pear-shaped. They are produced scattered throughout the body and do not show any movements. Lymphocytes contain antibodies and carry on immunological reactions.

Monocytes: They are the largest of white cells varying in diameter from 16 to 22 μm . They have a horse-shoe shaped or indented nucleus. They are phagocytic in function. With the help of pseudopodia they destroy harmful germs that enter the body.

The platelets: These are also called **thrombocytes**. They are minute cells, 2-3 μm in diameter, irregular in shape and very fragile containing distinct granules but no nucleus. In blood the number of platelets ranges from 2,00,000 to 3,00,000 per cubic mm of blood. Their number varies. It increases generally after exercise and haemorrhage. They have two main functions. i) they take active part in coagulation of blood and ii) they are believed to possess adhesive qualities which enables them to stick to foreign surfaces and patch up holes, in the capillary walls.

Table 19.2 Specialised Types of Connective Tissue

Types of Tissue	Main Location	Functions	Description & Comments
Cartilage	Supporting skeleton in sharks, rays, and some other vertebrates; forms ends of bones; supporting rings in walls of some respiratory tubes; tip of nose; external ear	Flexible support and reduction of friction in bearing surfaces	Cells (chondrocytes) separated from one another by the gristly intercellular substance; occupy little spaces in it
Bone	Forms skeletal structure in most vertebrates	Support, protection of internal organs; calcium reservoir; skeletal muscle attach to bones	Osteocytes located in lacunae; compact bone, lacunae arranged in concentric circles about haversian canals
Blood	Within heart and blood vessels of circulatory system	Transports oxygen, nutrients, wastes, and other materials	Consists of cells dispersed in a fluid intercellular substance

SAQ 4

- a) What are the three important types of cells in bone?

- b) Write four main types of cartilage.

- c) What are the five main classes of leucocytes?

19.6 EPITHELIAL TISSUE

In this section we will study the structure and function of epithelial cells which constitute various types of covering tissue found in animals.

Epithelial cells form coherent sheets of tissues called epithelium which cover the internal or external surface of the body such as the lining of the digestive tract, lungs, blood vessels or surface of the skin.

The individual cells of the epithelium are firmly attached to each other and rest on a basement membrane. The outer surface is generally free and exposed to air or fluid. The main function of the epithelium is protective. It provides a continuous protective barrier between the cells and the external medium.

19.6.1 Types of Epithelial Cells

Based on the height of the cells there are three main types of epithelium. The least specialised is the **cuboidal epithelium** whose cells are cubical in shape, that is, they are as long as they are wide. The **squamous epithelium** has cells that are much broader than long and have the appearance of thin flat plates. **Columnar epithelium** has cells that are much longer than wide and appear as rectangles set on end.

Epithelial tissue may be only one cell thick in which case it is called **simple epithelium** or it may be two or more cells thick in which case it is called **stratified epithelium**. The latter occurs when there is constant wear and tear and the cells of the upper layer need rapid replacement e.g. the outside surface of the land animals. The stratified epithelium consists of several layers of cells of which the lowermost layer next to the basement membrane is the germinative layer and is always active in producing cells which renew the overlying layers. This is called stratum mainphigil in the epidermis of skin. The cells of the outermost layer are dead and friction at the surface causes these dead cells to be sloughed off so that the deeper layers come to the position of the layer removed. Pseudostratified epithelium appears stratified

but is never beyond one or two layers. This is present where considerable stretching of the surface is needed. Now look at Fig. 19.14 and try to find the difference between the three main types of epithelial tissues.

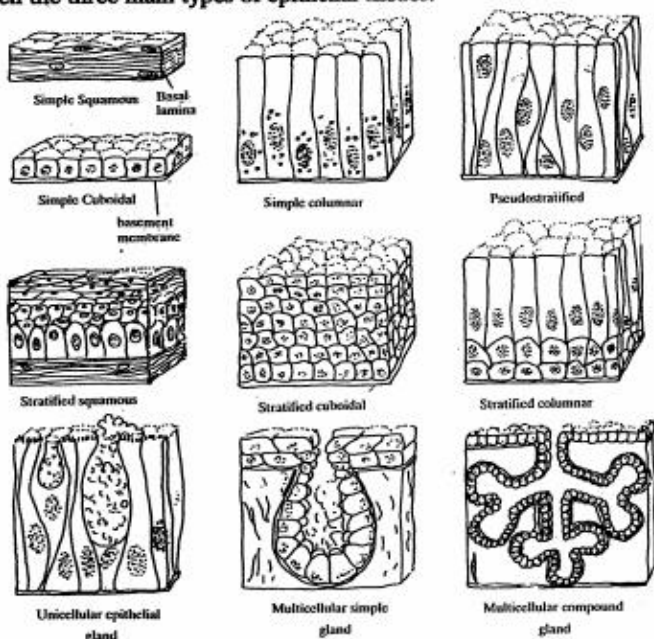


Fig. 19.14 Epithelial tissues

Let us summarise in a tabular form what we have said so far in following table (Table 19.3).

Table 19.3 Epithelial Tissues

Type of Tissue	Main Locations	Functions	Description and Comments
Simple squamous epithelium	Air sacs of lungs, lining of blood vessels	Passage of materials where little or no protection is needed and where diffusion is major form of transport	Cells are flat and arranged as single layers
Simple cuboidal epithelium	Lining of Kidney tubules, gland ducts	Secretion and absorption	Single layer of cells; from the side each cell looks like short cylinder; sometimes have microvilli for absorption
Simple columnar epithelium	Lining of much of digestive tract, upper part of respiratory tract	Secretion, especially mucus; absorption, protection, movement of mucous layer	Single layer of columnar cells, often with nuclei located in the base of each cell almost in row; sometimes with enclosed secretory vesicles (goblet cells), highly developed golgi complex, and cilia.
Stratified squamous epithelium	Skin, mouth lining, vaginal lining	Protection only; little or no absorption or transit of materials; outer layer continuously sloughed off and replaced from below	Several layers of cells, with only the lower ones columnar and metabolically active. Division of lower cells causes older ones to be pushed upward toward surface
Pseudostratified epithelium	Some respiratory passages, ducts of many glands, sometimes ciliated	Secretion, protection, movement of mucus	Comparable in many ways to columnar epithelium, except that not all cells are of the same height. Thus, though all cells contact the same basement membrane, the tissue appears stratified. Nuclei not in line. Ciliated, mucus-secreting, or with microvilli

Ciliated Epithelium

In this type, the surface of the epithelial cells bears numerous cilia which beat rhythmically and collectively to cause movement by bending and straightening alternatively. Ciliated epithelium may cover the outer surface of an animal to enable it to move. When present internally, it generally lines a cavity and causes a current in a fluid. Ciliated epithelium is found only in moist surroundings and almost invariably one can find mucous secreting cells in between ciliated cells. The cells of the ciliated epithelium may be cuboidal, columnar or of any other shape (Fig.19.15).

Glandular Epithelium

This is the second type of modified epithelium wherein the glandular epithelial cells secrete certain substances useful to the body such as mucous or gastric juice. The epithelial surface may be flat but it is usually folded or pitted so as to increase the secretory surface. There are two main types of glandular epithelium a) simple glands b) compound glands. (See Fig.19.14 for differentiation in simple and compound glands.)

In simple glands the surface of the epithelium is increased by a simple pit-like depression whose mouth serves to discharge a secretion on the free surface. The glandular epithelium lining the pit consists of a single layer of short columnar or cuboidal cells with granular cytoplasm.

In compound glands, the depression is not a simple pit but is subdivided or branched, often in a complicated manner. These branches are hollow or tubular lined by short columnar cells.

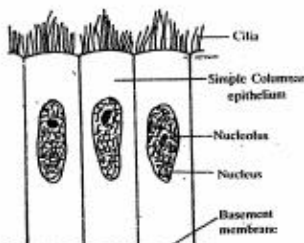


Fig. 19.15 Ciliated epithelium

Sensory Epithelium

In this type the cells have one or two stiff processes of varying shape and size, each cell being connected to a sensory nerve fibre. Such epithelia occur in the retina, taste buds, the nasal chamber and the inner ear. Fig.19.16 illustrates this modified form of epithelium known as neuroepithelium.

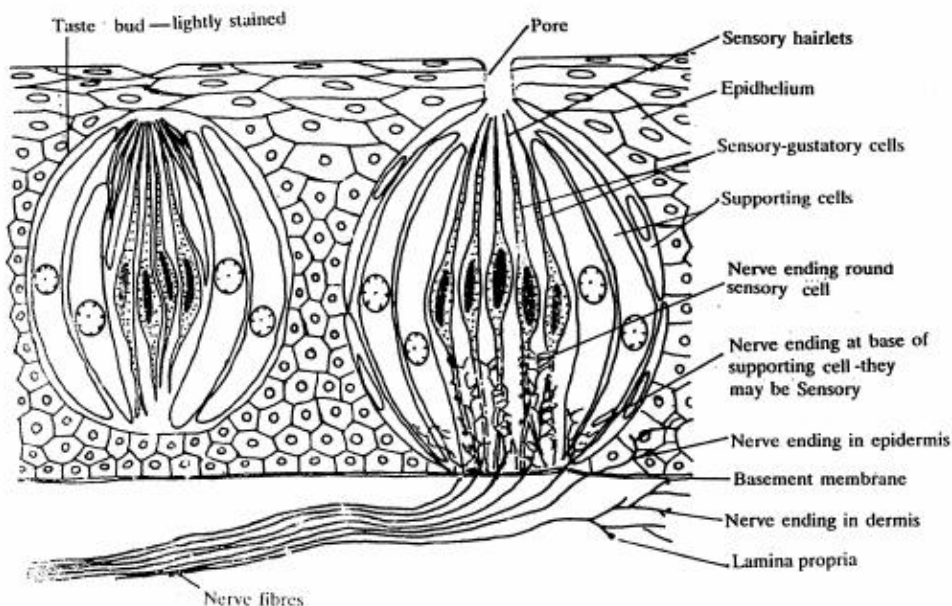


Fig. 19.16 Taste bud from human tongue

19.6.2 Functions of Epithelial Tissue

Let us now discuss the functions of the epithelial cells depending upon the structure of the cells that constitute the epithelial tissue. The following are the various functions that the epithelial cells can perform.

- i) **Protection:** As you already know the epithelial tissue covers other tissues e.g. epidermis, skin, mouth lining, enamel of tooth etc., so the first and foremost function of the epithelium is protection.
- ii) **Secretion:** The glandular epithelium secretes enzymes, mucous, watery fluids and other substances, e.g. gastric glands, liver, etc.
- iii) **Excretion:** Epithelial cells also form glands such as kidney tubules and sweat glands which excrete unwanted materials from the body.
- iv) **Absorption:** Epithelial cells at certain locations of the body absorb substances for example, the columnar epithelial cells absorb nutrients from the intestine.
- v) **Sensation:** Some epithelial cells get modified to form neuroepithelial cells to receive sensation, for example, the cells of the taste buds of the tongue receive sensation of taste, and the hair cells of the internal ear receive the sensation of sound.

19.6.3 Origin of Epithelial Tissue

You have studied in your 10+2 level course that, depending upon their location in the body, various types of epithelial tissues are derived from different embryonic zones, for example, the epidermis of the skin, epithelial lining of the mouth, sweat and salivary glands arise from the ectoderm of the embryo.

The epithelium of certain parts of the urino-genital tract and the endothelium arise from the mesenchyme and mesoderm of the embryo. The structure and function of the mesenchyme will be described in the next section.

The epithelium of the digestive tract from the pharynx downwards, the epithelium of the respiratory tract from larynx downwards and cells of the thyroid gland are some examples of the epithelium derived from the endoderm of the embryo.

SAQ 5

- a) Which type of epithelium will be surrounding the following

i) Air sacs of lung, lining of blood vessels

ii) Lining of kidney tubules

iii) Lining of digestive tract

iv) Skin, mouth lining

v) Ducts of many glands, some respiratory passages

- b) What are the three important functions of epithelial tissue?

19.7 MESENCHYME

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As already mentioned earlier in Section 19.5 this is a type of embryonic connective tissue. It is quite different from adult connective tissue. In the early embryo, besides the three embryonic layers i.e. ectoderm, endoderm and mesoderm, there are loose cells floating in a fluid between the ectoderm and endoderm. These cells constitute the mesenchyme. They are derived from the mesoderm. A small proportion of mesoderm cells give rise to epithelia and the remaining cells that make up the bulk of

the mesoderm get converted into mesenchyme. Each mesenchymal cell has a big nucleus, scanty cytoplasm and a few cytoplasmic thread-like processes that radiate from the cell and give it a star-shaped appearance. The cytoplasmic processes establish contact with those of neighbouring cells and form a network. The spaces of the network are filled with a jelly-like intercellular substance. Fig. 19.17 shows you how the mesenchyme appears. Mesenchymal cells have the capacity to form various types of cells and fibres of adult connective tissue. Some mesenchymal cells show amoeboid movement and are called wandering cells. As the development of the embryo progresses, the mesenchyme gets completely converted into several different kinds of cells which in turn give rise to various tissues (Fig. 19.18). A look at the figure will show you that chondroblasts arise from mesenchymal cells. These form cartilage, and also osteoblasts which have a similar origin and you know that these form bone. As development of the embryo advances, some mesenchymal cells remain dormant in the periosteum. These dormant cells change to chondroblasts and osteoblasts to form cartilage or bone respectively as the occasion arises. Mesenchymal cells also give rise to myoblasts which form muscle, while lymphoblasts and haemocytoblasts form the various types of blood cells. Endothelial cells also arise from mesenchyme from which blood vessels and primitive heart tubes are formed.

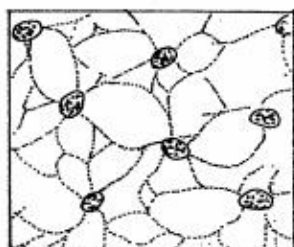


Fig. 19.17 Mesenchymal cells

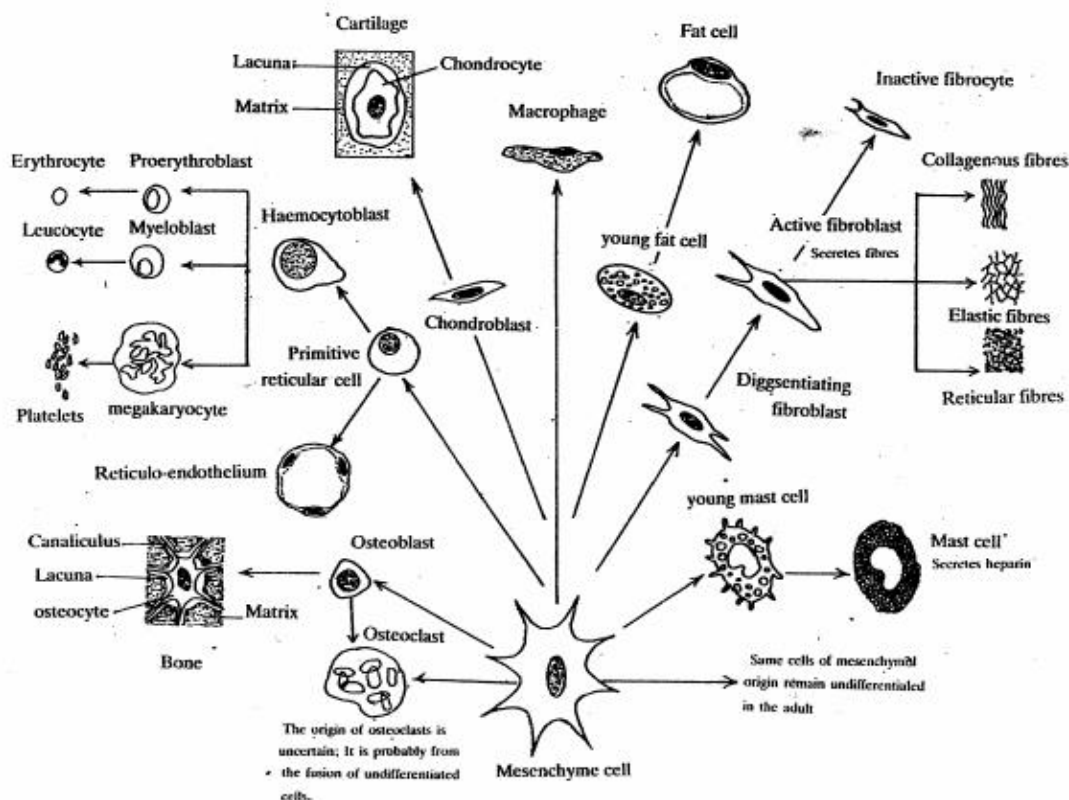


Fig. 19.18 Differentiation of mesenchymal cells

After differentiation of the tissues, as described above, a considerable mass of mesenchymal cells is still left and this gives rise to the cells and fibres of the connective tissue. Some mesenchymal cells differentiate into fibroblasts which are stellate in shape. These have the ability to form **collagen fibres** which are components of ordinary connective tissue.

Mesenchyme therefore, is an embryonic connective tissue whose cells have the capacity to form the various cells and fibres of adult tissues.

19.8 SUMMARY

Let us summarise what we have studied so far.

- The somatic cells of multicellular animals get differentiated by as yet, not fully known mechanisms to form tissues in which cells manifest marked division of labour. All the cell types have morphological and physiological characteristics to suit their functions individually as well as collectively at different levels of organisation such as cellular, intercellular tissue, organ and finally organismal level in a perfectly co-ordinated manner.
- There are four major types of tissues in vertebrates i.e. epithelial tissue, connective tissue, muscular tissue and nervous tissue.
- The muscular tissue forms the largest bulk of the animal body. Three types of muscular tissues are striated or skeletal, smooth and cardiac. Striated and smooth muscular tissues are voluntary whereas cardiac muscles are involuntary. The most abundant type of muscle cells are the striated muscle fibres. The movements of the animal body are caused by the contraction and expansion of the muscle cells.
- The nervous tissue helps the animal to respond to external stimuli. The functional unit of the central and peripheral nervous system is the neuron which is among the largest of cell types found in animal tissues.
- A neuron is structurally composed of dendron, dendrites and axon. Depending on the number of dendrites and axon they are classified as unipolar, bipolar and multipolar neurons.
- Connective tissue performs the function of supporting the animal body. Connective tissue basically consists of matrix, fibres and cells. Three major types of connective tissue are blood, cartilage and bone. In contrast to cartilage and bone, blood is a fluid connective tissue. Blood consists of three types of cells, erythrocytes, leucocytes and platelets. Cartilage consists of chondrocytes, and bone consists of osteocytes, osteoblasts and osteoclasts.
- Epithelial tissues cover the internal or external surface of the body and its organs in the form of sheets of cells without intercellular spaces. The major functions of epithelial tissue are protection, absorption and secretion. Depending on the shape they may be classified as squamous, cuboidal and pseudostratified. Depending on the number of layers it may be classified as simple and stratified.
- Mesenchyme is an embryonic connective tissue which gives rise to cells of all types of adult connective tissue.

19.9 TERMINAL QUESTIONS

- 1) Explain the contraction mechanism of skeletal muscle.

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- 2) Complete the following table:

Connective Tissue	Location	Functions	Brief Description
Blood			
Bone			
Cartilage			

- 3) Explain the structure of mesenchyme.

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.....

- 4) Describe the structure of simple squamous, cuboidal and columnar epithelium.

19.10 ANSWERS

Self-assessment Questions

- 1 a) i) In both the muscles, nucleus is present centrally.
ii) Both are involuntary muscles.
b) i) The fibres are elongated.
ii) They are cylindrical in shape.
- 2 i) Myosin
ii) Sarcolemma
iii) Sarcoplasm
iv) Sarcomere
v) Intercalated discs
- 3 a) i) C ii) A iii) B iv) E v) D vi) F
b) i) Bipolar
ii) Sensory
iii) Neurosecretory
- 4 a) Osteoblasts, osteocytes, osteoclasts
b) Hyaline, Elastic, Fibrous, Calcified
c) Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes
- 5 a) i) Simple squamous epithelium
ii) Simple cuboidal epithelium
iii) Simple columnar epithelium
iv) Stratified squamous epithelium
v) Pseudostratified epithelium
b) Protection
Absorption
Secretion

Terminal Questions

- 1) A muscle fibre consists of sarcoplasm which contains numerous myofibrils. The myofibrils are the actual contractile elements. The striated appearance of skeletal muscle is due to the presence of A (dark) and I (light) bands. The A or thick band is made up of protein, actin. During contraction, the length of A band remains the same but I and H bands disappear which results in shortening of sarcomere. The shortening of the sarcomere is the result of secondary filaments sliding inwardly.

2) Connective Tissue	Locations	Functions	Brief Description
Blood	Heart, blood vessels	Transports oxygen, nutrients, wastes	Consists of cells dispersed in a fluid, intercellular substance
Bone	Forms skeletal structure in most vertebrates	Support, protection calcium reservoir	Osteocytes, lacunae arranged in concentric circles
Cartilage	Supporting skeleton in sharks, rays, forms ends of bones, supporting rings in walls of respiratory tubes	Flexible support and reduction of friction	Chondrocytes separated from one another by intercellular substances

- 3) The loose cells floating in a fluid between the ectoderm and endoderm constitute the mesenchyme. Each mesenchymal cell has a big nucleus, cytoplasm and a few thread like processes which make contact with the neighbouring cells. Mesenchymal cells have the capacity to form various types of cells and fibres of adult connective tissue.
- 4) In cuboidal cells, cells are cubical in shape. In squamous epithelium, the cells are much broader than long and in columnar epithelium, the cells are much longer than wide and appear as rectangles.

UNIT 20 DIFFERENTIATED PLANT CELL AND TISSUE TYPES

Structure

- 20.1 Introduction
 - Objectives
- 20.2 Differences between Plant and Animal Cells
- 20.3 The Plant Cell Wall
- 20.4 Different Cell and Tissue Types
 - Parenchyma
 - Collenchyma
 - Sclerenchyma
 - Xylem
- 20.5 Phloem Dermal Tissue System
 - Epidermis
 - Periderm
 - Plasmodesmata
- 20.6 Plant Cell Interior
 - Plastids
 - Vacuoles
- 20.6 Cell Division and Cell Growth
 - Meristems and their Distribution
 - Plane of Cell Division
 - Cell Growth
- 20.8 Summary
- 20.9 Terminal Questions
- 20.10 Answers

20.1 INTRODUCTION

Differentiated cells are the product of the process by which cells achieve stable differences and persist through many generations, for example a cell committed to become skin cell will keratinize and die. During differentiation, cells develop difference in structure and all the functions necessary to sustain the organism. The basis of cell types and their function such as formation of simple and complex tissues depends upon the number and nature of the cell types. In the previous unit you have learnt about the various animal cell types and tissues and how they perform their functions. Now we shall study the differentiated plant cell types that are produced during growth of cells. A tissue is defined as a group of cells with common origin, development, structure and functions. They are basically formed to share the various functions, thus affecting division of labour.

Differentiation of plant cells forming specific tissues is greatly influenced by the environmental and the genetic factors. However, non-genetic factors such as hormones affecting plant growth and differentiation play an important role and produce some specific types of growth responses throughout its life time. Growth is a complex process that involves, an increase in cell number with corresponding increase in size. Plants being immobile, are greatly affected by their outside environs, for example in many plants, flowering is controlled by differences in length of light and dark periods during the 24 h cycle, that are affected by the change of seasons. The external factors control the growth of plants and in some cases even gene expression.

Much of the knowledge and understanding of plant cell tissue and differentiation has come from tissue culture studies. By 1950 plant cells from different sources could be successfully cultured. A plant physiologist, F.C. Steward, at Cornell University in 1958 was able to produce an entire carrot plant from a single cell of carrot root, that also showed that each differentiated cell has all the genes to produce an entire plant.

Tissue System	Tissue	Cell Types	Major Functions
Vascular	Xylem	Vessel members	Conduction of water and mineral nutrients, support
		Tracheids	Conduction of water and mineral nutrients, support
		Sclerenchyma cells Fibres, sclereids	Support, protection
		Xylem parenchyma cells Laticifers	Storage, short-distance transport Secretion, storage of secondary compounds
		Resin and gum duct epithelial cells	Secretion
	Phloem	Sieve tube members and companion cells	Conduction of sugars, organic nitrogen compounds, and hormones in angiosperms
		Sieve cells, albuminous cells	Conduction of sugars, organic nitrogen compounds, and hormones in gymnosperms and lower vascular plants (ferns and their allies)
		Sclerenchyma cells Fibres, sclereids	Support, protection
		Phloem parenchyma cells Laticifers	Storage, short-distance transport Secretion, storage of secondary compounds
		Resin and gum duct epithelial cells	Secretion

20.3 THE PLANT CELL WALL

Higher plants are composed of cells that are cemented together in fixed positions by cell walls which surround them. Many of the unique features of plants are related to the presence of the cell wall. The plant cell wall is a specialised form of extra cellular matrix that is closely associated with the external surface of the plant cell membrane. Most animal cells have extra cellular matrix components on their surface known as cell coat or glycocalyx. The outer coat of plant cell, i.e. cell wall is much thicker, stronger, and more rigid structure. In evolving rigid cell wall varying from 0.1 nm to several micrometers in thickness, plants have lost the ability to move about and therefore do not develop tissues such as muscles, bones, or nervous systems present in animals.

The composition and appearance of cell walls differ according to cell types and their functions, but the basic structure of cell walls is consistent. The fibres of the polysaccharide cellulose are embedded in a highly cross-linked matrix of polysaccharides, such as pectins and hemicelluloses (Fig. 20.1a-b).

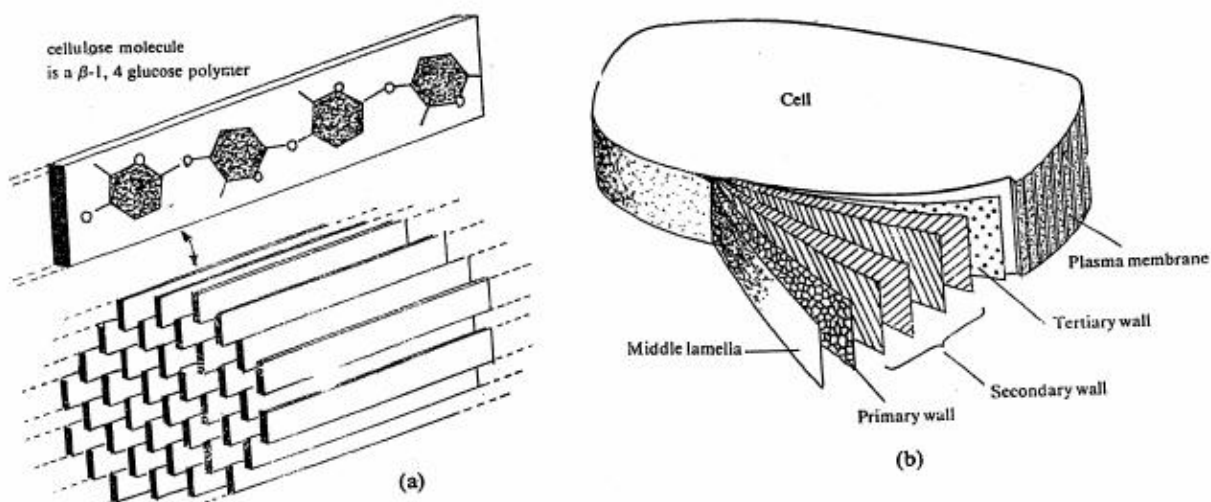


Fig. 20.1 a) Cellulose molecule is a 1,4 glucose polymer, b) Organisation of a generalised plant cell wall.

This constitutes the primary cell wall which has great tensile strength and is permeable only to relatively small molecules. If a plant cell without a wall is kept in water it will take up water by osmosis and swell and burst (Fig. 20.2).

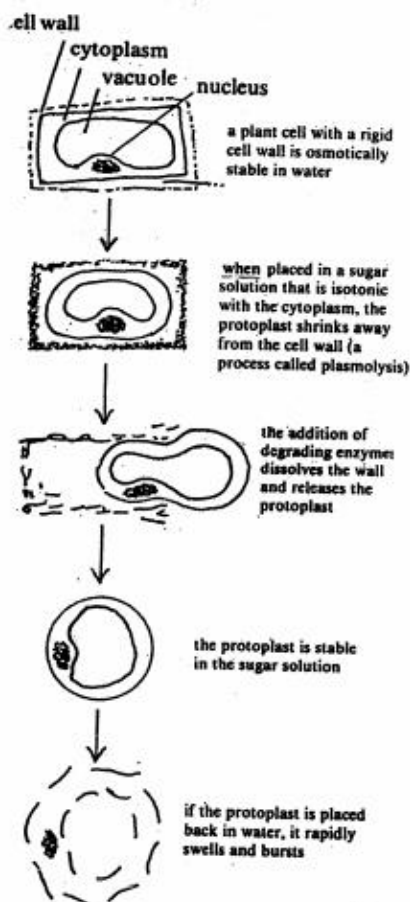


Fig. 20.2 A plant cell without its cell wall is osmotically unstable and will burst if placed in water. Inside its rigid cell wall, however, it can swell only as far as the wall will allow. The pressure developed by the cell pressing against the wall keeps the cell turgid, and the cell wall prevents it from bursting.

The advantage of a cell wall is that when the protoplast swells, it presses against the cell-wall creating a pressure known as turgor pressure. You can compare this situation with the bicycle tube pressing against a tyre. During the formation of specialised cell types, for example, in xylem and phloem the cell wall is modified by special types of thickenings. There can also be addition of one or more new layers (the secondary cell walls) and some regions may be selectively removed as in the formation of a conducting tube from a long row of cylindrical cells. The cell wall is thus modified during the differentiation of specialised cell types which are related to their functional activities. These cell types will be discussed in Section 20.3.

In a multicellular plant, the newly formed cells are small in relation to their final size. The cell wall of young growing cells is thin and semirigid and comprises the **primary cell wall**. The primary cell wall is the first zone to appear and consists of loosely organised cellulose network. The fully grown cells may either retain the primary wall structure with some more thickening, or new multilayered structure is superimposed on the inner surface of primary wall called as **secondary cell wall** which serves to reinforce the primary cell wall. The secondary cell wall after cessation of growth gets localised between the primary wall and the plasma membrane.

Each layer of the secondary cell wall is formed of cellulose fibres arranged in parallel rows at an angle with the other fibre bundles. The fibres are generally made of cellulose, the most abundant macromolecule on earth. You have read about the structure and assembly of cellulose fibrils in Unit 6. The other types of

polysaccharides are **hemicellulose**, **pectin** and **lignin** (Fig. 20.3 a, b). The fibres of matrix molecules are cross linked by covalent bonds and weak interactions forming a very complex structure which makes it difficult to obtain a complete picture of all the molecules and their cross links in any one cell wall. In some plants there is another innermost layer of the cell wall, which is relatively thin and lacks cellulose microfibrils and is called **tertiary wall**.

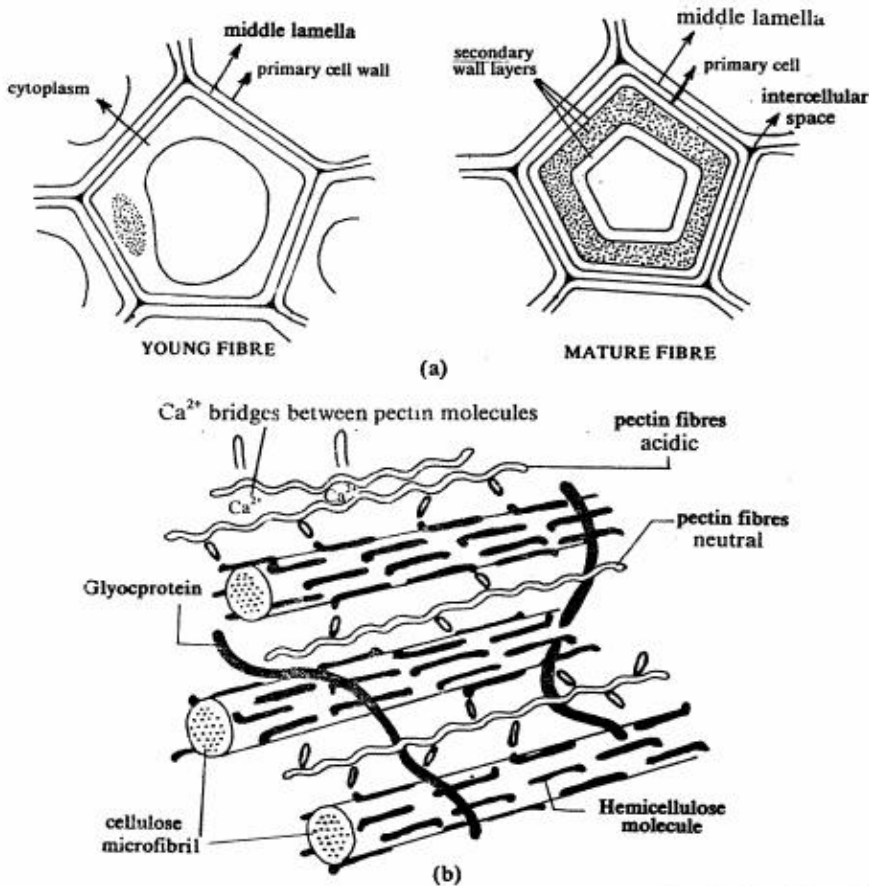


Fig. 20.3 a) Secondary cell wall deposition shown schematically in a cross-section of a fibre cell. In this case, three new cell wall layers have been laid down within the primary cell wall. Because the net orientation of the cellulose microfibrils is different in each layer, a strong plywood like effect is created. In many mature fibres, as shown here, the cell inside the wall dies. (b) Cellulose fibrils are arranged in parallel rows and cross linked with other polysaccharides.

At the molecular level, intermolecular hydrogen bonds tightly cross link adjacent chains of hemicellulose molecules outside a cellulose microfibril. Pectins are heterogeneous branched and a highly hydrated polysaccharides. Pectin is particularly abundant in the **middle lamella**, the region that serves to cement the cell walls of adjacent cells together. Air gaps between cells are formed due to ruptures in this layer. In addition to the three classes of polysaccharides, the primary cell wall also contains small amount of proteins in the form of glycoproteins. Since it is difficult to extract glycoprotein without destroying the structure of the cell wall, it is believed that all the four types of molecules are tightly integrated to form the complex hetero charide matrix of the cell wall. Since the matrix of cell wall is a highly hydrated polysaccharide gel (primary cell wall being 60 per cent water by weight) water, gases, and small water soluble molecules penetrate more rapidly through the cell wall than the plasma membrane.

Parenchyma cells have primary walls only. This is sometimes deposited in successive layers with different orientation and composition. The new polymers that are deposited in the secondary cell wall are less hydrated thus making it more dense than the primary wall. The wall of the mature or differentiated plant cell is suited to the function that the cell performs. This is achieved by controlled deposition of new wall material and removal of old wall material, in accordance with the type of function.

Though most of the cell wall matrix components comprising polysaccharides are produced in the golgi complex, cellulose is synthesised on the exterior surface of the cell with the help of enzyme complexes which become active only after they have been exposed to the extracellular environment. Newly formed cellulose chains spontaneously assemble into microfibrils which become integrated into the general fabric of the cell wall. Their orientation determines the shape of the cells. The orientation of microtubules lying just inside the plasma membrane is quite similar. The ability of cortical microtubules to influence the orientation of newly formed cellulose microfibrils suggests that these microtubules are directly or indirectly connected to extra-cellular components across the plasma membrane. The molecular basis of this connection, however, is not known. The parallel orientation of microtubules and microfibrils is visible both in the primary as well as secondary cell wall deposition.

You have read about the structure of golgi complex in Block 1. In plant cells each golgi stack is separate rather than connected as in animal cells. Golgi vesicles deliver cell wall material to specific regions of the plasma membrane. The microtubules derived from the endoplasmic reticulum direct the golgi vesicles in the specific regions. During the formation of the new primary cell wall after mitosis, a set of microtubules remain between the two daughter nuclei parallel to the spindle axis. At the end of **telophase**, a set of microtubules remain between the two daughter nuclei parallel to the spindle axis and form a **phragmoplast** in the plane of the former spindle equator. Golgi derived vesicles containing cell wall precursors especially pectin are guided inward along these oriented microtubules until they reach the central disc, where they fuse with each other to form the cell plate which rapidly extends sideways by further addition of vesicles and microtubules until it reaches and fuses with the natural cell wall.

Associated with the vesicles of the cell plate are elements of the endoplasmic reticulum, which often remain trapped across the cell plate. These subsequently are transformed into plasmodesmata. The complex pores that traverse the mature cell wall, interconnect the cytoplasm of all the cells in the plant with the help of these plasmodesmata.

SAQ 1

Fill in the blanks with appropriate words.

- In plant cells are the organs for manufacturing the food.
- Cell wall in the plants is specialised
- Air gaps between the cells are formed due to ruptures in
- Microtubules influence the of cellulose microfibrils.

20.4 DIFFERENT CELL AND TISSUE TYPES

In order to illustrate the variety of cell types that a cell wall make, we shall now deal with the different cell types. They are all easily distinguished by the shape and structure of their cell wall. All these cell types arise from cells with a primary cell wall in the process of cell growth. There are two types of growths in the plants, **primary** and **secondary growth**. Primary growth is found in all plants and accompanied by plant length while the secondary growth consists of an increase in girth or width of the wood plants only.

There are two types of tissues you have read about so far. The first is simple tissue or a homogeneous tissue having only one type of cells. Another complex type of tissue is heterogeneous and comprises more than one type of cells functioning as a unit. Parenchyma, collenchyma and sclerenchyma are simple tissues. We will discuss these in the following sub-sections.

20.4.1 Parenchyma

As already mentioned earlier these types of cells are the least specialised ones. Most parts of the plant body are composed of this tissue. It is, therefore, called ground tissue. Parenchyma are living, isodiametric, oval, round, polygonal or elongated cells

with thin walls and vacuolated protoplasm and have intercellular spaces (Fig. 20.4a). Parenchyma cells that contain chloroplasts, as in the mesophyll of leaves are called chlorenchyma. These cells help in photosynthesis. In many aquatic plants and in the petioles of banana leaves, the parenchyma cells assume a star-like appearance and have a lot of air cavities between them. Such a tissue is called **aerenchyma** (Fig. 20.4b). Parenchymatous cells perform the most important vital functions in plants such as preparation of food (photosynthesis) and storage of nutritive material such as starch, proteins, mineral and water. The process of respiration is carried out by parenchymatous cells. Parenchyma cells are therefore concerned with several physiological functions of the cells. **Phylogenetically** parenchyma is considered to be a primitive tissue system, since the lowest multicellular plants consist of parenchyma only. **Ontogenetically** too, they are primitive since they closely resemble meristematic cells. The other important feature of parenchyma cells is that they retain the ability to divide and even mature and can be induced to divide under special cultural conditions. Each parenchymatous cell is enclosed by a cell wall that provides structural support to the plant. These cells have a primary cell wall and during maturation many cells deposit additional cell wall between the primary wall and plasma membrane, that reinforces the primary wall. But typical parenchyma cells have primary walls only.

Phylogeny: The complete evolutionary history of a group of organisms.

Ontogeny: The complete developmental history of the individual organism.

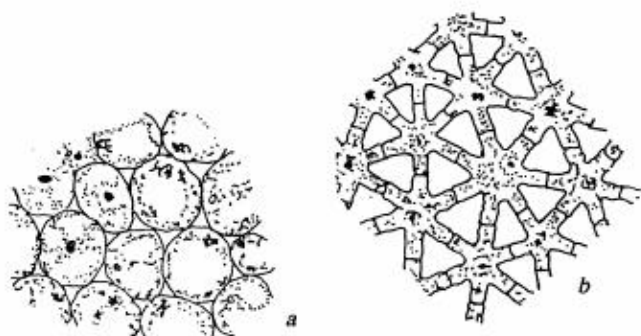


Fig 20.4 a) Parenchyma; b) Aerenchyma

20.4.2 Collenchyma

They are also one of the **simple** plant tissues that provide structural support the plants. It is also a **living tissue** whose cell walls are greatly thickened especially at the corners where cells **meet**. The corners are thickened by cellulose. The cells may be round, oval, or **angular**. They may or may not contain chloroplasts. No intercellular spaces are found **between them** (Fig 20.5a, b). The surface of herbaceous parts such as midrib of leaves, **petioles**, young shoots, especially in dicotyledons is formed by this tissue. The cells of **collenchyma** have the primary walls that are unevenly thickened forming **riuges** at the angles (as in gourds). The chief function of collenchyma is to **give mechanical** strength and flexibility to the young stems especially those of **climbers and twidders** to facilitate bending when a strong

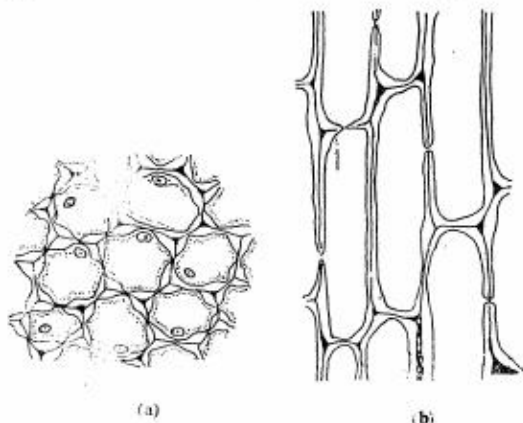


Fig. 20.5 Collenchyma in T.S (a) and L.S (b)

breeze is blowing. Collenchyma is, therefore, a typically juvenile supporting tissue and being extensible, it readily adapts itself to rapid elongation of the stem.

20.4.3 Sclerenchyma

This tissue is specially structured to give strength to the parts of the plant that have stopped growing in length. Sclerenchyma cells have both primary and secondary cell walls. It is a dead mechanical tissue consisting of very long narrow, thick walled and lignified cells with pointed ends and is called fibres. The fibres have simple often oblique pits in their walls (Fig. 20.6a, b, c). There are no intercellular spaces among the cells and cells have no protoplast. This tissue is found in the **pericycle** of dicot stems or **hypodermis** of monocot stems. It is also found in the cortex of many stems in the form of isolated strands. The main function of sclerenchyma is to impart rigidity and hardness to the plant. Special types of sclerenchyma e.g. **sclereids** or **stone cells** are also developed in certain plants to impart mechanical strength. They may occur in the cortex, pith, phloem, hard seeds, nuts, stony fruits and in the leaf and stem of many dicotyledons and gymnosperms. When you eat a pear or a guava, you experience a gritty feeling in your mouth while chewing. This is because of the presence of stone cells in the pulp of these fruits.

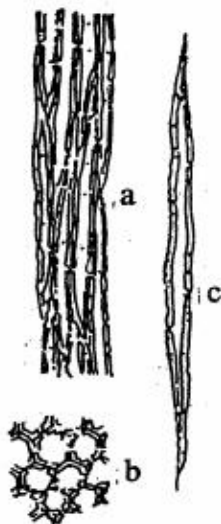


Fig. 20.6 Sclerenchyma. In L.S. a) and T.S. b). c) is a fibre in L.S.

There are four types of **sclereids** based on their shape. **Brachysclereids** are more or less isodiametric usually found in phloem, cortex, bark of stem, flesh of fruits intermingled with soft parenchyma, e.g. pears (Fig. 20.7a). **Macrosclereids** are rod shaped. Such sclereids often form a continuous layer in the testa of seeds e.g. in the seed coats of legumes (Fig. 20.7b). **Osteosclereids** are bone or spool-shaped sclereids, the ends of which are enlarged, lobed and sometimes even somewhat branched found in the seed coats (Fig. 20.7c). **Asterosclereids** are variously branched sclereids that are often star-shaped. Such sclereids are found in leaves of *Nymphaea* plant (Fig. 20.7d).

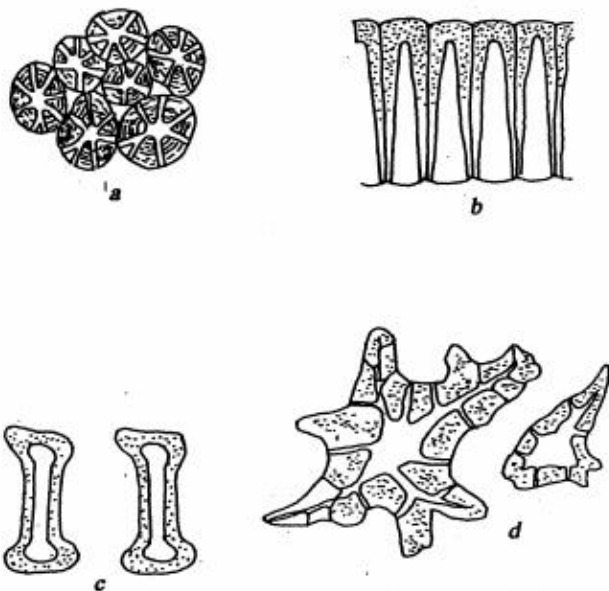


Fig. 20.7 Types of sclereids. a) Brachysclereids, b) Macrosclereids c) Osteosclereids, d) Asterosclereids

Now let us study complex tissues system. **Xylem** and **phloem** are the best examples of complex tissues. They also are called **conducting** or **vascular tissues**. Since they are helpful in the transport of water, salts and food material to the various parts of the plant body. **Phloem** is main tissue for conduction of food material (also see Fig. 20.11).

20.4.4 Xylem

Xylem is the principle water-conducting tissue which carries mineral solution from roots to upper organs. It is a complex tissue having four different types of cells:

- a) Tracheids
- b) Tracheae or Vessels
- c) Wood Parenchyma
- d) Wood Fibres

Tracheids are composed of elongated cells with tapering ends. They are angular in cross-section and have thick, lignified walls. Tracheids are dead empty cells with annular (ring-like), spiral (spring-like), scalariform (ladder-like), reticulate (net-like) or pitted secondary walls (Fig 20.8a-e). **Tracheids** occur alone in the wood of ferns and gymnosperms, whereas in the wood of angiosperms they are associated with the vessels. Tracheids serve a mechanical function due to their hard and thick walls, but their main function is conduction of water from the root to the leaf.



Fig. 20.8 Types of tracheids. a) Annular, b) Spiral, c) Scalariform, d) Reticulate, e) Pitted.

Tracheae or Vessels

They are cylindrical tube like structures formed of a row of cells joined end to end in which the transverse partition walls are perforated. These walls are thickened in the same way as tracheids and according to the mode of thickening the vessels have received their names such as annular, spiral, scalariform, reticulate and pitted (Fig. 20.9a-e). The main difference between vessels and tracheids is that the latter have non-perforated end walls. Tracheae are the main channels of conduction of water in angiosperms.

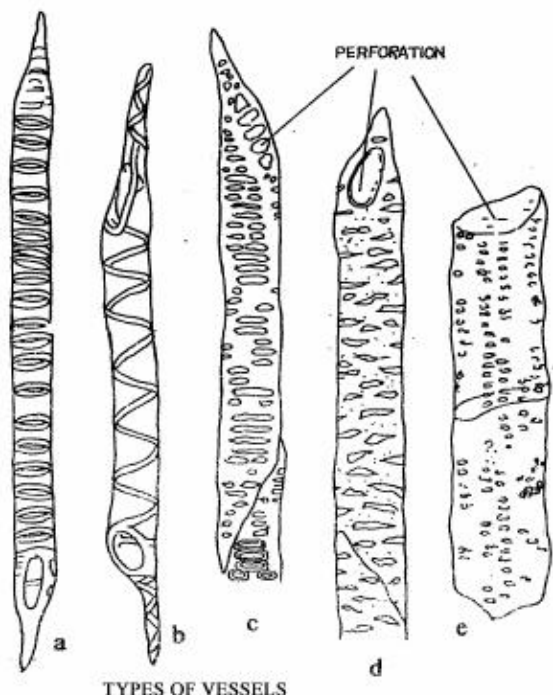


Fig. 20.9 a-e) Types of vessels

The central cavity is called lumen. If the lumen is wide it is called **metaxylem** and if the lumen is narrow it is called **protoxylem**. Generally **protoxylem** is the term given to the primary xylem elements that develop early. Those completed later are called metaxylem (also see Fig. 20.11). Depending upon the position of protoxylem, xylem is of three types:

metaxylem. Depending upon the position of protoxylem, xylem is of three types:

- If the protoxylem is towards the centre the xylem is said to be **endarch** e.g. xylem of a dicot stem.
- If the protoxylem is towards the outside, it is **exarch** as in roots.
- If the protoxylem is at the centre, flanked by metaxylem on either side, it is called **mesarch**, e.g. in *Cycas rachis*.

Wood Parenchyma

Parenchyma cells are of frequent occurrence in xylem and are known as wood parenchyma. This is a living tissue associated with dead tracheids and vessels. When secondary xylem is developed, the walls of the parenchyma may become lignified. In the secondary xylem the wood parenchyma cells are elongated into rays (secondary medullary rays) for radial conduction. They also serve for food storage. Now look at Fig. 20.10 and you will see the secondary medullary rays in the first and second annual ring.

Wood Fibres

These are sclerenchymatous cells found both in the primary as well as secondary xylem. Wood fibres add to mechanical strength of xylem and of the plant body as a whole. We have thus seen that xylem is a complex tissue consisting of living as well as dead cells performing a dual function. It provides mechanical strength and helps in water conduction (Fig. 20.10).

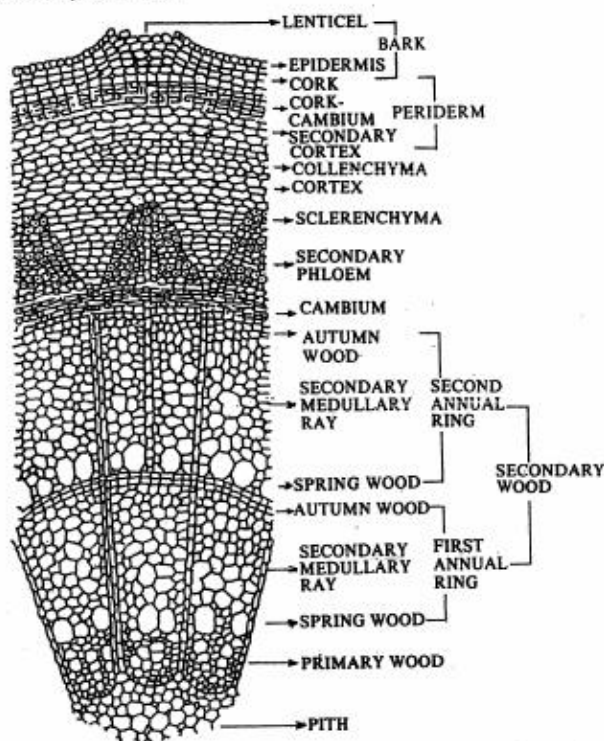


Fig. 20.10 T.S. of two year old dicot. Stem showing primary and secondary tissues with secondary medullary rays.

20.3.5 Phloem

The phloem is a complex tissue that conducts prepared food materials from the leaf to the storage organs and growing regions. It consists of the following in the flowering

a) Sieve Tubes b) Companion Cells c) Phloem Parenchyma d) Bast Fibres

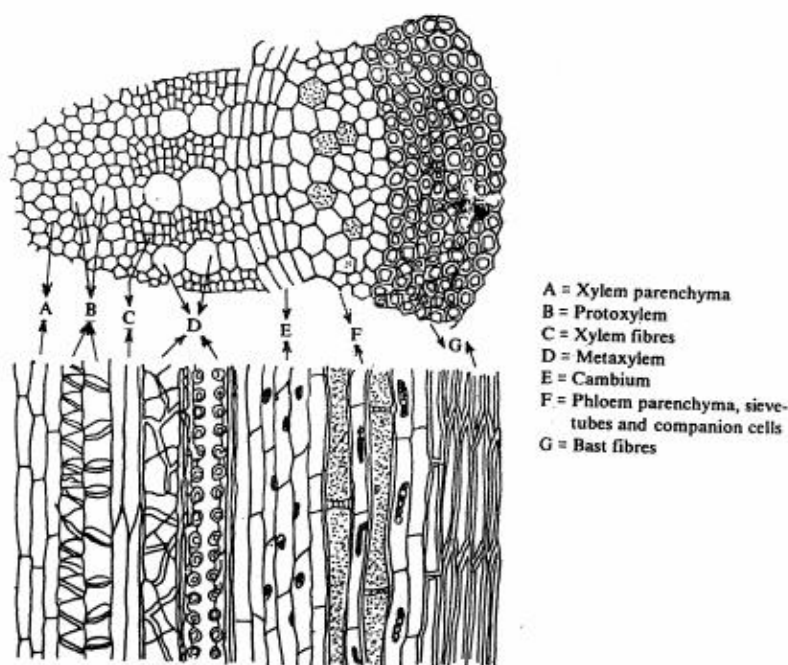


Fig. 20.11 T.S. and L.S. of a dicot vascular bundle showing the various cell types of xylem and phloem.

Sieve Tubes

These are tube-like elongated cells placed end to end in verticle rows; their walls are thin and made of cellulose. Their transverse partition walls are perforated by a number of pores which look very much like a sieve and hence are called sieve plates which may be horizontal or oblique. Sometimes sieve plates may be formed on the side walls as well. Through the pores the sieve tubes conduct prepared food i.e. protein and carbohydrates from the leaves to the storage organs in the downward direction, and later from the storage organs to the growing regions in the upward direction. At the end of the growing season, the sieve plate is covered by a deposit of a colourless shining substance in the form of a pad called **callus** which is composed of a specific carbohydrate called **callose**. In winter the callus completely clogs the pores but in spring it gets dissolved. In old sieve tubes, however, the callus forms a permanent deposit. The sieve tube contains no nucleus at maturity but has a lining layer of cytoplasm which is continuous through the pores (Fig 20.12) and lives only for less than a year. But there are a few exceptions such as palm trees that maintain their sieve tube members alive for about 100 years.

Companion Cells

These are elongated thin walled cells associated with sieve tubes and are connected with it by pores. There are generally one or two companion cells associated with each sieve tube. The companion cells are alive cells having a nucleus and other organelles and contain proteins, so they are also called protein cells. They are present only in angiosperms and assist the sieve tubes in the conduction of food (Fig.20.12).

Phloem Parenchyma

In higher plants except monocotyledons, there are always some parenchymatous cells associated with the phloem. They help in the storage and conduction of food materials (Fig.20.12).

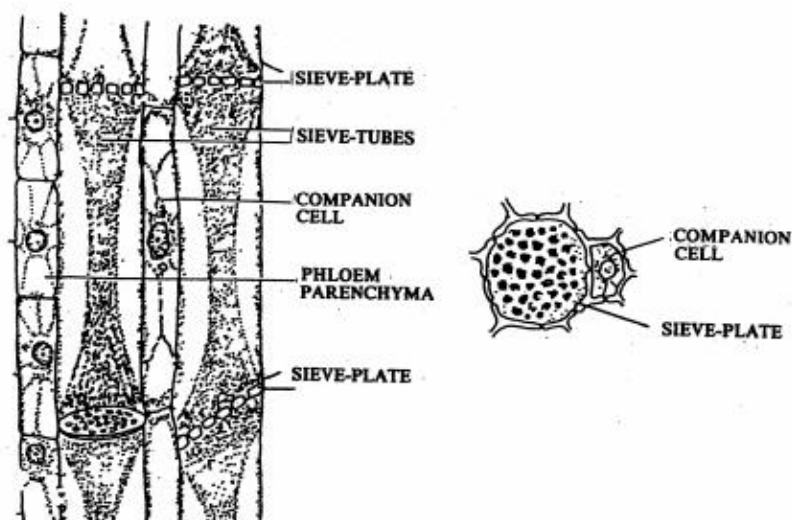


Fig. 20.12 a) L.S. of Sieve tubes and companion cells, b)
T.S. of Sieve tubes and companion cells.

Bast Fibres or Hard Bast

Sclerenchymatous fibres occurring in the phloem or bast are known as bast fibres and are meant to provide mechanical support. Like the xylem, the phloem is also classified into two categories namely **primary phloem** and **secondary phloem**. The primary phloem, like the primary xylem is of two types, **protophloem** and **metaphloem**. The metaphloem is retained for a longer time in the plant body than the protophloem. In plants showing secondary growth, the metaphloem becomes inactive after the formation of the secondary phloem, and may be either partially crushed or completely obliterated.

By now you must have understood how the xylem and phloem are organised and are suited to perform the dual function of conduction and mechanical support. Water and minerals travel up the stem in tracheids and vessels. The food manufactured in the green parts of the plant body by the process of photosynthesis, moves both up and down in the stem through the sieve tubes. A summary of plant cell types is shown in Fig.20.13.

Cell type		Function	Location
Parenchyma		Secretion storage Photosynthesis	Throughout the plant body
Collenchyma		Support	Just under stem epidermis along leaf veins
Sclerenchyma		Support	Throughout the plant body common in stems and certain leaves

Cell Type		Function	Location
Tracheids		Conduction of water and minerals Also provide support	Xylem
Vessel elements		Conduction of water and minerals Also provide support	Xylem
Sieve tube members		Conduction of food	Phloem
Companion cells		Aids sieve tube members in food conduction	Phloem

Fig. 20.13 A summary of plant cell types.

SAQ 2

Match the items in column I with those in column II

Column I	Column II
i) Parenchyma	a) Dead mechanical tissue and impart rigidity and hardness to the plants
ii) Collenchyma	b) Complex tissue with four different kind of cells and also consist of living as well as dead cells performing dual function
iii) Sclerenchyma	c) The chief function is to give mechanical strength and flexibility to the young stems
iv) Xylem	d) The cell are isodiametric with than walls and vaciolated protoplasm
v) Phloem	e) Conduct food material to storage organs and growing region

20.5 DERMAL TISSUE SYSTEM

The dermal system provides a protective covering to the plant body, and consists of two types of tissues—epidermis and peridermis (see Fig. 20.10). We will discuss these in the following sub-sections.

20.5.1 Epidermis

It is a complex tissue having many types of cells mostly parenchyma and covers the plant's parts during primary growth. The outside wall during the growth becomes thicker compared to inside part to give protection to the plant. The epidermal parenchyma are devoid of chloroplasts, but allow the light energy to penetrate into interior of the tissue of stem and leaf for photosynthesis. The epidermal cells protect plant against evaporation by secreting a waxy layer on the outer walls, known as cuticle. The root epidermis does not synthesise cuticle as it should allow water to enter it from the soil. Epidermis in root has evolved root hair known as trichomes which are extension of epidermal cells that increase the surface area of the root for absorption. Trichomes present on the aerial parts of desert plants cool the internal tissues by reflecting light away from the plants, and thus decrease water loss. The cuticle also functions as a barrier against gases. The exchange of gases such as carbon dioxide, oxygen and water vapour takes place through stomatal pores, about which you have read at 10+2 stage. Hypodermis consists of one to several layers of thick walled cells and occurs just below the dermis.

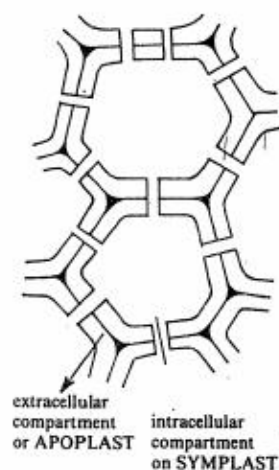


Fig. 20.14 Highly schematic diagram of a group of plant cells interconnected by plasmodesmata that are lined by plasma membrane. This membrane divides the plant into an extracellular space (apoplast) and an intracellular space (symplast). For clarity, no organelles are shown.

20.5.2 Periderm

It is a complex tissue found in woody plant and a substitute of epidermis which gets broken as the plant expands, and grows in girth. Periderm constitutes the outer bark of the stem and root. It is continuously formed by **lateral meristem** located within the cork cambium which are rectangular. Cork cambium cells divide to produce cork to the outside and cork parenchyma towards inside of the periderm. The cork cells die at the maturity and their walls are heavily coated with a water proof material called suberin that reduces water loss and protects the dermis beneath the bark, and are environment, for example in many plants, flowering is controlled by differences in length of light periods during the day, and dark periods in night that occur with the change of seasons. The environmental factors control the growth of plants and in some cases even gene expression.

20.5.3 Plasmodesmata

Plant cells are separated from each other by rigid, thick cell walls that contain channels, which are used to connect the cytoplasm of neighbouring cells. Plasma membrane of one cell is continuous with its neighbouring cell through plasmodesmata. In the absence of a nervous system in plants the problem of intercellular communication has been solved by the evolution of these fine cytoplasmic channels of plasmodesmata connecting the neighbouring cells (Fig. 20.14). A plasmodesma is a roughly cylindrical membrane-bound channel having a diameter of 20-40 nm. Plasmodesmata in most plants under an electron microscope shows another structure known as **desmotubule** that runs through the middle of most plasmodesmata as a narrower cylindrical structure. You have read about the structure and function of plasmodesmata in Unit 18.

SAQ 3

Tick mark (✓) the correct word/s in the following sentences:

- Epidermal parenchyma are full/devoid of chloroplasts.
- Cuticle is the waxy layer on outer/inner walls.
- Presence of trichomes/stomata helps the desert plants to remain cool.
- Cork cambium/parenchyma cells function as store.
- Epidermis/periderm constitute the bark of stem.

20.6 PLANT CELL INTERIOR

As mentioned earlier, plants have certain special features which distinguish them from animal cells, among these, plastids, and cell walls are of major importance in the evolutionary diversification of the plant and animal world. Another very important feature that plant cells possess is the presence of vacuoles which play a significant role in osmotic regulations of plant cells as well as in the collection and storage of a wide range of substances some of which constitute the excretory substances in the cells.

Let us now discuss these characteristic components of the plant cell.

20.6.1 Plastids

Plastids are a family of cell organelles that are closely related to each other and are double membranous structures. They develop from precursor **proplastids** which are distributed throughout the cell. Depending upon the function they develop into various types of plastids. In darkness they develop into etioplasts and contain a yellow precursor of chlorophyll called protochlorophyll. If exposed to sunlight, the etioplasts develop into **chloroplast** which are most common types. They occur in a variety of shapes and sizes such as ribbon like found in the cells of green algae *Spirogyra*, star-shaped chloroplast in another green algae such as *Zygnema*. A unicellular algae may have only one to two chloroplast, whereas a leaf of higher plant may have 20-100 chloroplasts. You have read about the structure of chloroplast in previous blocks.

Plastids containing carotenoid pigments impart yellow-orange-red colour to petals and fruits in many species, are called as **chromoplasts**. **Leucoplasts** are not pigmented hence are colourless, found in roots and tubers where they store starch. All

plastids contain DNA and produce new plastids. Plastids of one form or another are present in all living plant cells and all cells inherit their plastids from parent gametes. In some cases only the egg cell carries the plastids and are maternally inherited.

20.5.2 Vacuoles

In young dividing cells there are many small vesicles which fuse to form one or more large vesicle called **vacuoles** (Fig. 20.15). The vacuoles are separated from the cytoplasm by a single vacuolar membrane called the **tonoplast**.

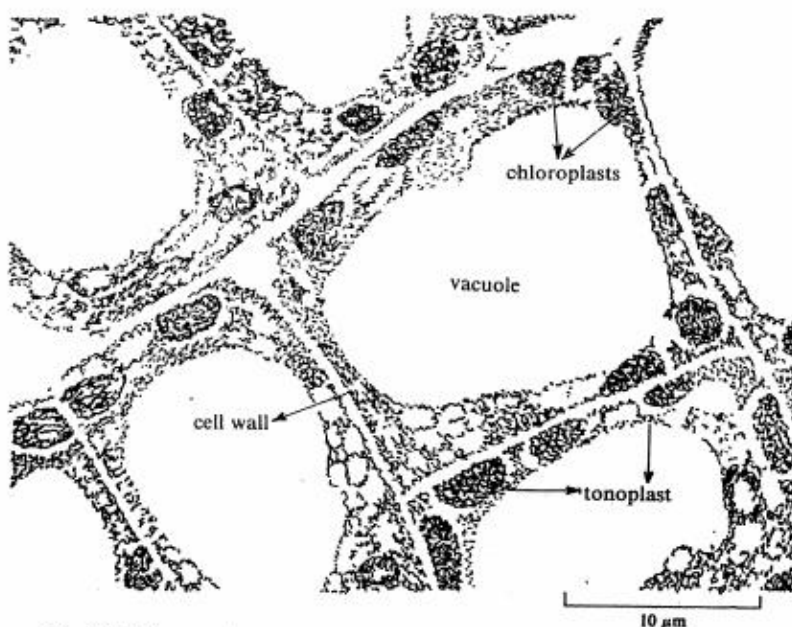


Fig. 20.15 Electron micrograph of cells in a young tobacco leaf. The cytoplasm in these highly vacuolated cells is confined to thin layer containing numerous chloroplasts pressed against the cell wall.

A vacuole generally occupies around 50 per cent of the cell volume but the range of variation may be from 5 per cent to 95 per cent depending upon the cell type. The functions carried out by lysosomes in animal cells are performed by large vacuoles in plants. They are similar in structure and function like plasma membranes, and they are filled with cell sap which is acidic in nature and plays a role in maintaining pressures within the cell. Plant cells use their vacuoles for transporting and storing nutrients such as salts, sugar, organic acid, small quantities of soluble proteins, pigments and waste products. The vacuoles regulate the flow of water and solute of the cells such as osmotic regulation, and help in digestion and recycling of certain materials within the cell, and help in breakdown of organelles such as mitochondria, and plastids. The water soluble pigments called the anthocyanin pigments, which are found in vacuoles, impart colour to the flower petals to attract insects for pollination. Some vacuoles participate in defence mechanisms. Plants cannot move to avoid being eaten by herbivores, so sometimes toxic metabolites are stored as a means of defence that are released from vacuoles of the plants when they are eaten or being damaged. There are various view points regarding the origin of the vacuoles that include their origin from either pre-existing vacuoles, from golgi vesicles or by dialation of cisternic vesicles from ER.

The substances of the vacuoles differ in quality as well as in quantity from those of the cytoplasm. The plasma membrane and tonoplast have different permeability properties. The permeability of these two membranes is regulated by **turgor pressure**. The mechanical stability provided by the combination of a cell wall and turgor pressure allows plant cells to grow to a relatively large size so that they generally occupy a much larger volume than animal cells and majority of them accumulate water in their vacuoles with the cytoplasm generally forming a thin peripheral layer (Fig. 20.16). You have thus seen that the vacuole is a remarkably versatile organelle of the plant cell interior and the plant cells use their vacuoles in a variety of ways of which space filling and storage are the most prominent ones.

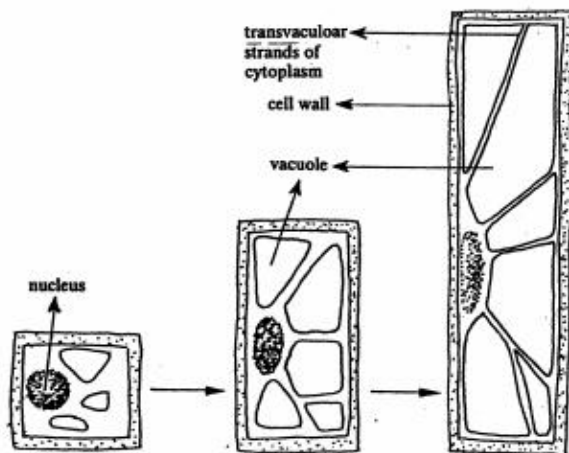


Fig. 20.16 Schematic diagram showing how a large increase in cell volume can be obtained with no increase in cytoplasmic volume. Turgor-driven cell expansion accompanies the uptake of water into an expanding vacuole. The cytoplasm is eventually confined to a thin peripheral layer interconnected by transvacuolar strands of cytoplasm that emanate from the region of the nucleus.

SAQ 4

List the three components of plant cell in the space provided below

.....

.....

20.7 CELL DIVISION AND CELL GROWTH

Because plant cells have rigid cell walls and cannot move about from the place of their origin, they generally follow a very precise pattern of cell division followed by selected cell growth. Plants differ from animals in their growth pattern. When an animal grows, all parts of its body grow according to its genetic constitution, however, plant growth is localised into **meristematic** parts which are composed of unspecialised cells and divide by mitosis. The differentiation is carried out by cells that are genetically identical and develop different structures and functions. Plants living in unusual environments evolve specialised adaptation mechanism suited for that environment. Microtubules not only play an important role in the mitotic spindle formation and cell plate formation but also are involved in orienting cellulose microfibrils in the cell wall. They also influence the site and plane of cell division and cell elongation (Fig. 20.17). Thus, the cytoplasmic division or cytokinesis in plant cells

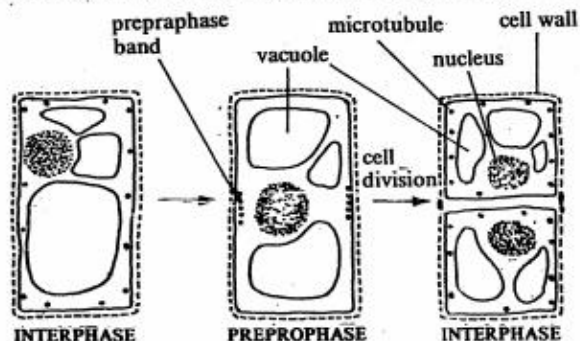


Fig. 20.17 Schematic diagram showing the arrangement of the cortical array of microtubules during plant cell division in interphase, cortical microtubules are distributed along the length of the cell wall. During preprophase, however, they congregate in a discrete band circling the cell. The preprophase band of microtubules accurately predicts where the new cell wall will join the old one when the cell later divides.

is markedly different from that in animal cells. For the organised growth of a plant it is required that cells divide in selected sites and particular plane and at a particular time to provide the orderly distribution of cells. Chromosomes and various cytoplasmic organelles for example, chloroplast and mitochondria undergo multiplication just before cytokinesis.

These organelles are formed by the division of pre-existing chloroplast or mitochondria. As they are present in multiple copies they are equally distributed between the daughter cells at cytokinesis. The importance of such a control is expressed in asymmetric division that commonly creates two daughter cells of unequal size accompanied with non-uniform distribution of organelles.

20.7.1 Meristems and their Distribution

A meristem is a tissue that is in a continuous state of division and retains its ability to divide. During the growth of the plant, cell division is continued entirely to specialised regions called the meristems (Meristos—divide). There are usually two types of growth in plants which are as follows:

- 1) One is primary growth which is due to **apical meristems** which are present at the tip of growing shoots and roots. They give rise to primary permanent tissues. The plant body increases in length due to primary growth.
- 2) The other is called as secondary growth which is due to **lateral meristems** that are situated around the side of stems and give rise to the secondary permanent tissues which bring about increase in girth of the plant. This increase is brought about by a special type of cell; the cambium which gives rise to secondary phloem and xylem. Cells of plant meristem are small, contain abundant cytoplasm and a distinct nucleus and have no intercellular spaces. They undergo divisions and produce new tissues by cell differentiation. Most cells produced in the root tip meristem go through three distinct phases of development: i) division, ii) growth and iii) differentiation which gives rise to the characteristic architecture of a root tip. There might be a certain amount of overlapping, but is not difficult to distinguish three distinct zones in a root tip—a zone of cell division which brings about increase in cell numbers, a zone of cell elongation which accounts for the growth in length of the root and a zone of cell differentiation wherein further enlargement and modification of cells into different cell types takes place. Three main types of tissues: the epidermal, cortical and the vascular tissues are differentiated (Fig 20.18). When the differentiation process is completed some of the cell types created remain alive for example, phloem cells and parenchyma while others such as the xylem vessels and sclerenchyma die.

20.7.2 Plane of Cell Division

You have already learnt about the different types of cell divisions and their significance in earlier units. We will now study about cell growth and how the different planes of cell division are determined. Although nuclear and cytoplasmic divisions are considered to be linked, they are quite separable events. Cytokinesis usually starts in **anaphase** continuing through **telophase** and into the following interphase period. The mitotic spindle plays an important role in determining the location and timing of cytokinesis.

When a cell becomes committed to divide in a particular plane, about 100 microtubules aggregate into a narrow band. This band passes right around the cell just beneath the plasma membrane. This happens just before the onset of prophase and is called the **preprophase band** which disappears at the onset of division. At telophase the cell plate forms precisely at the same zone that was formerly occupied by the preprophase band. The preprophase band therefore defines the plane of division of the cell (Fig. 20.19). This is perhaps necessary because spatial reorganisation after division is precluded by the cell walls. Thus, irrespective of whether the division is symmetrical or asymmetrical, transverse, longitudinal or tangential, the plant cell specifies the plane of division before it enters mitosis.

20.6.3 Cell Growth

Cell growth is a very well controlled and directed phenomenon of cell expansion which plays a major role in determining the final form of a plant. Turgor is the driving force for cell growth. It exerts a uniform outward pressure which is caused by the osmotic imbalance between its intracellular and extracellular fluids.

Differentiated Plant Cell Types

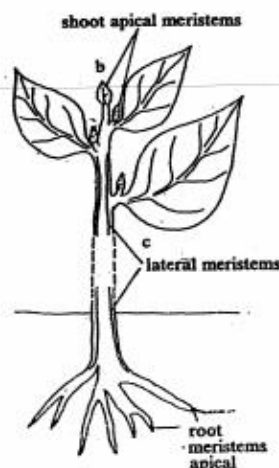


Fig. 20.18 Schematic diagram of a higher plant, showing the locations of the main meristematic regions, the areas of most rapid cell division. The activity of the shoot and root apical (a and b) meristems is responsible for increasing the length, while the activity of the lateral meristem (c) is responsible for increasing the girth of the various parts of the plant.

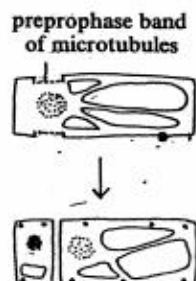


Fig. 20.19 Schematic drawing of an elongated epidermal cell from a root, showing how the plane of asymmetric cell division is predicted by the preprophase band of microtubules. This epidermal cell divides asymmetrically to form a large daughter cell that will continue as an epidermal cell, and a small daughter cell that will become a root hair cell.

One of the most common cell shapes in plants is cylindrical, i.e. plant cells are usually longer than broad (Fig. 20.20). Since cellulose microfibrils in the cell wall are not stretchable, they must slide past each other to allow the cell to grow. In spherical cells the arrangement of the microfibrils tends to be random. However, cell growth can occur only if the turgor pressure in the cell exceeds the local tensile strength of the wall. Therefore, the plant cell can use two different strategies to grow i.e. by increase in turgor, and by weakening of the cell wall in local areas. Plants cells can weaken their walls by local secretion of protons (H^+) into the walls. To facilitate wall growth other complex changes occur that are not yet completely known. Cell growth therefore, is the result of carefully controlled external as well as internal factors. Plant hormones which can readily penetrate cell wall constitute part of the internal factors that regulate cell growth.

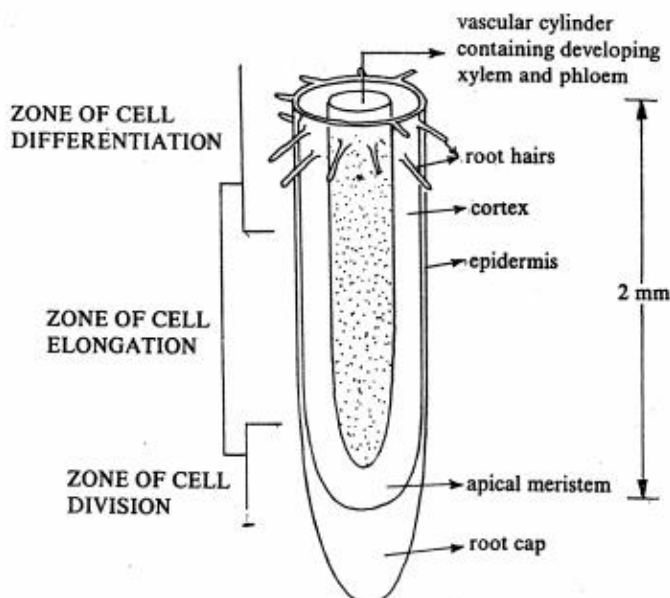


Fig. 20.20 The organisation of the final 2 mm of a growing root tip. The approximate zones in which cells can be found dividing, elongating and differentiating are indicated.

SAQ 5

Fill in the blanks with appropriate words.

- The growth in plants is localised into.....parts
- Site and plane of cell division and cell elongation is influenced by
- Primary and secondary growth in plants are due to.....andmeristems respectively
- Preprophase band defines the.....of cell division

20.8 SUMMARY

In this unit you have studied that:

- Plant cells are not as highly specialised as animal cells. Most of the differences between plants and animals are based on the presence of cell wall and the ability to carry on photosynthesis.
- Plant body is composed of a variety of cell types which are distinguishable on the basis of structure and function. Parenchyma cells are living and least specialised and the most ubiquitous type of cells. Parenchyma cells that contain chloroplasts and carry out photosynthesis are called chlorenchyma. Collenchyma is also a living tissue where cells are thickened especially at the corners; their chief function is to give mechanical and tensile strength to living parts of the body. Sclerenchyma give strength to parts of plants that have stopped growing; the cells are dead, thick wall and have no intercellular spaces. Special types of sclerenchyma are known as sclereids. They are of various shapes.

- Cells that constitute a homogeneous tissue are called simple tissue. A complex tissue is heterogeneous and comprises more than one type of cells functioning as a unit. Xylem and phloem are complex tissues and their main function is conduction of water and food materials in the plant body in addition to providing mechanical support.
- Since cell walls are rigid they pose problems for communication and interaction between cells. This is taken care of by plasmodesmata which pass through the pores of the cell wall and bring about cytoplasmic continuity between neighbouring cells.
- In addition to plastids and cell walls, vacuoles play a significant role in the plant body. They regulate osmosis and also collect and store a wide range of cell inclusions containing nutrients, metabolites and waste products.
- Cell wall formation is initiated at the end of telophase. Golgi-derived vesicles and microtubules fuse with each other at the equatorial region of the spindle and form the cell plate. A meristem is a tissue that is in a continuous state of division; cell division in plants is confined to specialised regions.
- There are 2 types of meristems: Apical meristems are present at the tip of growing shoots and roots. They give rise to primary permanent tissues and enhance the length of the plant body. Lateral meristems are situated along the side of the stem and give rise to secondary permanent tissues and enhance the width of the plant. Cell growth is the result of several interrelated internal as well as external factors. These include the influence of turgor, proton and hormone concentration along with some complex changes that are not yet clearly understood.

20.9 TERMINAL QUESTIONS

- 1) Differentiate very briefly between primary and secondary cell walls. Write your answer in the space provided below.

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- 2) Name four types of sclereids and discuss briefly about these in the space given below.

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- 3) Differentiate very briefly between xylem and phloem.

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- 4) Discuss briefly the functions of vacuoles in plant cells.

20.10 ANSWERS

Self-assessment Questions

- 1) i) plastids ii) extracellular matrix iii) middle lamella iv) orientation
- 2) i) d ii) c iii) a iv) b v) e
- 3) i) devoid ii) outer iii) trichomes iv) parenchyma v) periderm
- 4) The three characteristic components of the plant cell are plastids, chromoplasts and vacuoles.
- 5) i) meristematic ii) microtubules iii) apical, lateral iv) plane

Terminal Questions

- 1) Primary cell walls are thin walls of the growing cells and comprise loosely organised cellulose network. Secondary cell walls are thick walls of grown cells, deposited between primary wall and plasma membrane. These consist of cellulose fibres arranged in parallel bundles.
- 2) **Brachysclereids**—isodiametric, found in phloem, cortex, bark of stem, flesh of fruits intermingled with soft parenchyma.
Macrosclereids—rod shaped, form a continuous layer in the testa of seeds.
Osteosclereids—bone or spool shaped with enlarged, lobbed and branched ends found in seed coats.
Asterosclereids—star-shaped, branched and found in Nymphaea plant.
- 3) Xylem is composed of tracheids, tracheae, wood parenchyma and wood fibres. It provides mechanical strength and helps in water conduction. Phloem is composed of sieve tubes, companion cells, phloem parenchyma and bast fibres in flowering plants. It conducts prepared food materials from leaf to the storage organs and growing regions.
- 4) Vacuoles in the plant cell perform various functions carried out by lysosomes in animal cell. In the cells they also transport and store nutrients, metabolites and waste products. They regulate the flow of water and solutes of the cell and also participate in defence mechanism.

Glossary

Amoeboid movement: a mode of cellular motility characterised by crawling a solid substratum using pseudopod surface extensions that are formed in response to an internal flow of cytoplasm.

Auxins: auxins are synthesized by living organisms from aminoacids, that mainly occur in apical meristems buds, young leaves and other active young parts of the plants.

Bivalent: a synapsed pair of homologous chromosomes typical of prophase I and metaphase I of meiosis.

Cell cycle: the sequence of events between one mitotic division and another in a eucaryotic cell, consisting of the G₁, S and G₂ phases of interphase and the M phase of mitosis proper.

Cell division: formation of two daughter cells from a parent cell by enclosure of the individual nuclei or nucleoids in separate cell compartments.

Cell plate: the new boundary that separates daughter nuclei into individual plant cells and consists of plasma membranes and new primary walls that become continuous with the pre-existing membranes and cell wall of the original plant cell.

Cell surface: the cell covering, consisting of the exoplasmic layer of the membrane and any external coats covering this membrane surface.

Cell wall: the rigid or semi-rigid covering secreted by the living protoplast of plant, algal, fungal, and procaryotic cells.

Centriole: a cellular organelle at micro-tubular organisation occurring only in animal cells. It migrates to opposite poles of the cell during mitosis and meiosis and thus ensuring through spindle apparatus the separation of each replicated chromosome and equal partition of the chromosome among the daughter cells.

Chiasmata: a site of exchange between two chromatids of a bivalent, visible as a crossover point in the bivalent during diplotene, diakinesis, and metaphase of meiosis I.

Chromatid: one of the two identical longitudinal halves of a chromosome which shares a common centromere with a sister chromatid, results from the replication of chromosome during a nuclear division.

Chromatin: the deoxyribonucleoprotein material of the chromosome.

Communicating junction: sites on adjacent plasma membranes that allow ions and small molecules to move freely from one interacting cell to its joined neighbour cell and through the inter-cellular space.

Connexon: a component of the gap junction between adjacent cells, composed of six molecules of the protein connexin.

Crossing over: physical exchange of homologous chromosomes or DNA segments leading to recombination of linked genes.

Cytokinesis: cell division; any process that separates daughter cells through the enclosure of individual nuclei in individual cells.

Diakinesis: the last event of prophase I in meiosis.

Differentiation: as the various cells mature, they become large and then differentiate i.e. they assume different form adapted to specific functions such as support, secretion, conduction etc. If differentiation did not occur, the result would be a shapeless structure with no distinct tissues and co-ordination.

Diploid: an individual or cell having two complete sets of chromosomes.

Duplication: the occurrence of the segment twice in the same chromosome or in the same complement.

Ectoplasm: outermost gel like layer of cytoplasm in amoeba and certain other simple organisms.

Endoplasm: more fluid inner region of cytoplasm in amoeba and other simple organisms.

Fertilisation: the fusion of egg and sperm or other pairs of gametes to produce the zygote that initiates new generation.

Fibroblast: a type of cell of connective tissue in animals.

Furrowing: a cleavage process of cell division in animals.

G 1 phase: the portion of a cell cycle that begins after mitosis (M phase) and terminates when DNA replication begins (S phase); early interphase.

Interphase: the interval between mitotic or meiotic divisions of the eucaryotic nucleus.

Laminin: a fibre forming glycoprotein in the underlying basal lamina that serves as a binding agent for epithelial cells to collagen.

Meiosis: the reduction division of the nucleus in a sexual organism, which produces daughter nuclei having half the number of chromosomes and half the DNA content of the original meiotic nucleus. The process consists of two successive divisions and

results in the production of a quartate of gametes or sexual spores, only one of which may be functional in females.

Mid body: dense materials and remnants of spindle fibres in the fusion between cleaving cells; formed during cytokinesis and disappears shortly before cell division is complete.

Mitosis: nuclear division in which replication of chromosomes is followed by separation of products of replication and their incorporation into two daughter nuclei. Daughter nuclei are normally identical with each other and with the original parent nucleus in both kind and number of chromosomes.

Prophase: the first stage of mitosis or meiosis, occurring after DNA replication and before chromosome alignment on the equatorial plane of the spindle.

Recombination: the combination of linked alleles in the progeny that differs from the parental sets because of crossing over between linked genes; the process or occurrence of new allelic combination of linked genes in viruses, cells, or individuals.

Recombination nodule: a large, protein-containing structure that is believed to mediate the process of recombination and can be observed by electron microscopy within the 100 nm-wide space of the synaptonemal complex between paired chromosomes at pachytene.

Synaptonemal complex: a structural component that is situated between paired chromosomes in pachytene of meiotic prophase I and holds the chromosomes in register during crossing over.

FURTHER READINGS

1. Albert L. Lehninger: Principles of Biochemistry, Worth Publishers, Inc. New York, 1984.
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6. King. B: Cell Biology, (Modern Views in Biology) Allen and Unwin Publishers Ltd. Inc. U.K. 1986.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-01

Block

Enrolment No.

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- 1) How many hours did you need for studying the units?

Unit Number					
No. of hours					

- 2) How many hours (approximately) did you take to do the assignments pertaining to this block?

TMA

CMA

Assignment Number		
No. of hours		

- 3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (✓) the type of difficulty and give the relevant page number in the appropriate columns.

Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

- 4) It is possible that you could not attempt some SAQs and TQs.

In the following table are listed the possible difficulties. Kindly tick (✓) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	SAQ No.	TQ No.	Type of difficulties			
			Not clearly posed	Cannot answer on the basis of information given	Answer given (at end of Unit) not clear.	Answer given is not sufficient

- 5) Were all the difficult terms included in the glossary. If not please list in the space given below.

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6) Any other Suggestion(s)

To
The Course Coordinator (LSE-01, Cell Biology)
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