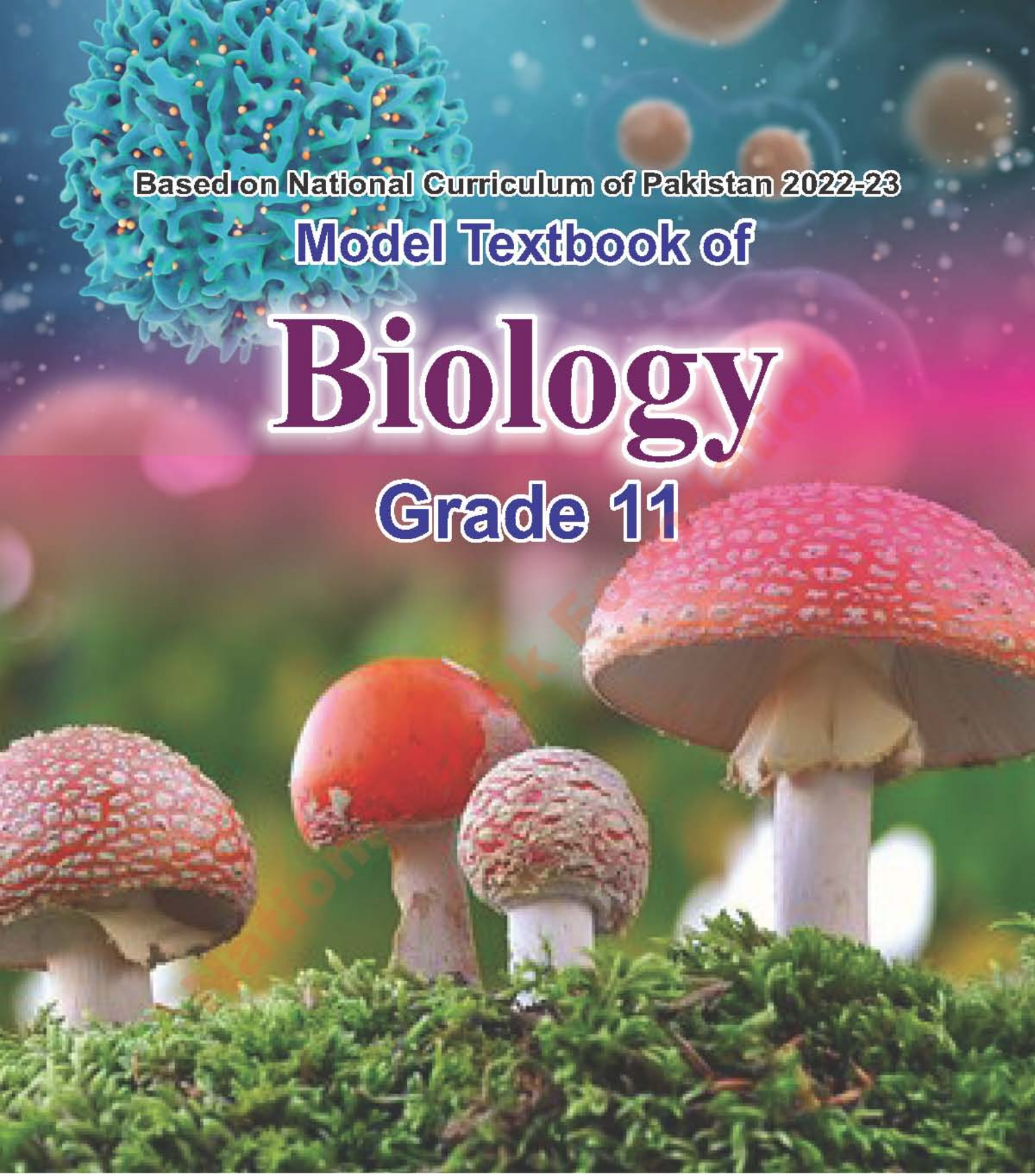
Based on National Curriculum of Pakistan 2022-23

Model Textbook of

Biology

Grade 11



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Model Textbook of **Biology**
for Grade 11

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PREFACE

This Model Textbook has been developed by NBF according to the National Curriculum of Pakistan 2022- 2023. The aim of this textbook is to enhance learning abilities through inculcation of logical thinking in learners, and to develop higher order thinking processes by systematically building upon the foundation of learning from the previous grades. A key emphasis of the present textbook is on creating real life linkages of the concepts and methods introduced. This approach was devised with the intent of enabling students to solve daily life problems as they go up the learning curve and for them to fully grasp the conceptual basis that will be built upon in subsequent grades.

After amalgamation of the efforts of experts and experienced authors, this book was reviewed and finalized after extensive reviews by professional educationists. Efforts were made to make the contents student friendly and to develop the concepts in interesting ways.

The National Book Foundation is always striving for improvement in the quality of its books. The present book features an improved design, better illustration and interesting activities relating to real life to make it attractive for young learners. However, there is always room for improvement and the suggestions and feedback of students, teachers and the community are most welcome for further enriching the subsequent editions of this book.

May Allah guide and help us (Ameen).

Dr. Raja Mazhar Hameed

Managing Director

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بِسۡمِ اللّٰہِ الرَّحۡمٰنِ الرَّحِیۡمِ

اللہ کے نام سے شروع جو بڑا مہربان، نہایت رحم والا ہے

Contents

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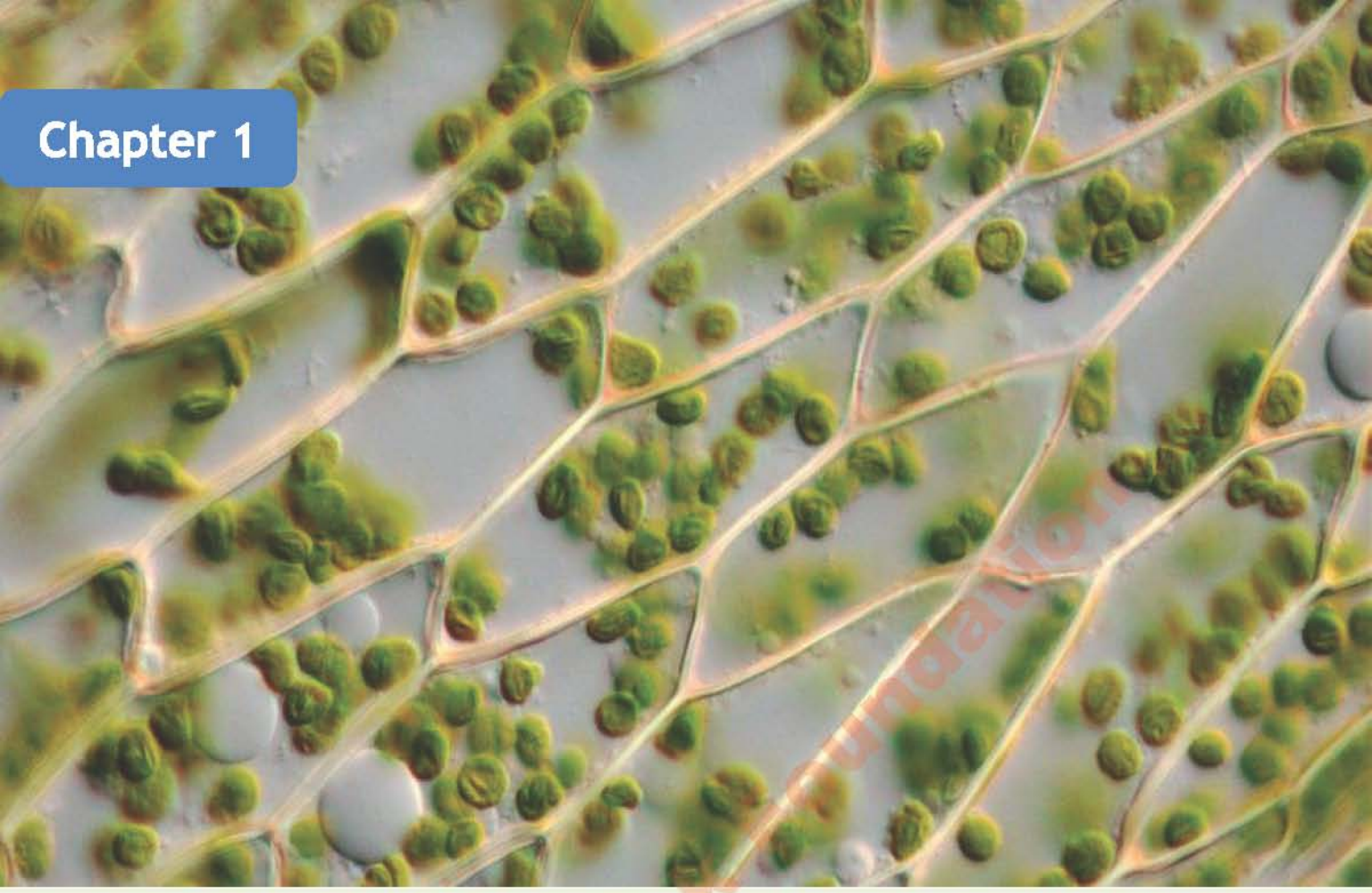

Chapter 1

CELL AND SUB-CELLULAR ORGANELLES

SLOs: After completing this lesson, the student will be able to:

1. Describe that cells are the basic unit of life with respect to seven properties of life, (Movement, Respiration, Homeostasis, Growth, Reproduction, Excretion, and Nutrition).
2. State cell theory (including how to validate it and exceptions to it.)
3. Compare and contrast the working of a light microscope and electron microscope with focus on resolution and magnification and live vs dead tissue.
4. Identify the ultra-structure of animal and plant cells.
5. Describe the structure and functions of sub-cellular organelles (cell wall, plasma membrane, endoplasmic reticulum, ribosomes, Golgi complex, vesicles, lysosomes, peroxisomes, vacuoles, mitochondria, plastids, centrioles, nucleus).
6. Explain the structure of the cell membrane and the techniques that can be used to study it.
7. Differentiate between prokaryotic and eukaryotic cells with diagrams.
8. Define cell signaling.
9. Discuss the pathway of a signal from outside the cell to the inside. (Protein signal and steroid signal).
10. Define stem cells and advantages of using stem cells.
11. Categorize different types of stem cells.
12. Evaluate the advantages and disadvantages of using induced Pluripotent Stem Cells.
13. Explain the four-membrane transport mechanism with diagrams (Simple diffusion, Facilitated diffusion, Osmosis. Active transport).
14. Compare and contrast simple and facilitated diffusion.
15. Describe endocytosis and exocytosis with diagram.
16. Explain the steps of mitosis and meiosis with diagrams.

One of the most important concepts in biology is cell. It is the basic unit of structure and function of living organisms.

1.1 CELLS – THE BASIC UNIT OF LIFE

All organisms are made of cells. There are single celled and multicellular organisms. Single celled organisms are *Amoeba*, *Paramecium*, and *Euglena* etc. More complex organisms, including plants and animals are multicellular.

The cells are defined as the structural and functional unit of living organisms. A cell is the basic structural unit of all unicellular and multicellular organisms. Cell is the smallest unit and building block of life. Cells exist in various shapes and sizes and perform a wide range of activities. Their shapes and sizes are related to the function they perform.

The basic properties of life are movement, respiration, homeostasis, growth, reproduction, excretion and nutrition. A cell does all of these basic properties of life.

1. **Movement:** Living things show movement either externally or internally.
2. **Respiration:** Living things use substances from the environment to make energy.
3. **Homeostasis:** Living things maintain a stable internal environment.
4. **Growth:** Living things can move and change shape or size.
5. **Reproduction:** Living things produce offspring, either sexually or asexually.
6. **Excretion:** Living things exhibit the removal of waste products.
7. **Nutrition:** Living things exchange materials and gases with the environment.

1.2 CELL THEORY

Robert Hooke was an English scientist. In 1665 he looked at thin pieces of cork under a simple microscope. He saw that cork was made of tiny empty spaces with walls around them. Under the microscope the cork looked like honeycomb. The honeycomb spaces reminded him of small rooms called 'cells' in a monastery. So, he called the spaces in the cork 'cells'. Now we know that what Hooke really saw was the thick, outside walls of what was once the part of cork tree.

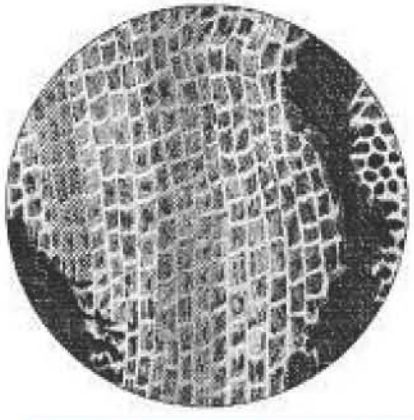

Fig.1.1 Cork cells as seen by Robert Hooke.

Schleiden was a German botanist. He examined a large variety of cells of some kind or other. In 1938 he concluded that cells are the ultimate units forming the structures of all plant tissue.

At the same time Schwann, a German zoologist studied many types of animal cells. He found that animal cells lack a cell wall. The animal cells are covered by a membrane. Thus, he recognized that apart from cell wall which was unique to plants, the inside of both plant and animal cells were identical. Schleiden and Schwann compared their findings. They combined their views and formulated the cell theory. In 1885 Rudolf Virchow (*Fir-koh*) observed that new cells develop by the division of pre-existing cells. The cell theory is a fundamental concept of biology.

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The three tenets or principle of the cell theory are:

1. The cell is the fundamental unit of structure and function in living things.
2. All organisms are composed of one or more cells.
3. Cells arise from pre-existing cells through cellular division.

The modern iteration of the cell theory consists of the following concepts:
1. Energy moves through cells.
2. DNA is transferred from cell to cell, carrying genetic information.
3. All cells share the same primary chemical makeup.

1.2.1. Validation of Cell Theory

The following experimental evidences validate the cell theory:

1. Cells removed from tissues can survive independently for short periods of time.
2. Nothing smaller than a cell has been found to be able to live independently.
3. Experiments by Francesco Redi and Louis Pasteur have demonstrated that cells cannot grow in sealed and sterile conditions.

1.2.2. Exceptions of Cell Theory

The following are the exceptions to cell theory:

1. Viruses are exception to cell theory. Viruses are considered acellular as they have no cell machinery. Viruses are alive only until they are inside their host cell. They are considered to behave dead when outside the host cell.
2. Viroid's prions also behave like viruses and are exceptions to the cell theory.
3. RBCs and sieve tube cells lack nuclei. They cannot divide to form new cells.
4. Bacteria, cyanobacteria lack well organized nucleus. Nuclear membrane, nucleolus and nucleoplasm are absent. The DNA alone forms the chromosome and lies in direct contact with cytoplasm. Basic proteins associated with nucleic acid are absent in bacteria.
5. Coenocytic hyphae of the fungus *Rhizopus* and cells of the alga *Vaucheria* are multinucleate.
6. Protozoans are not cellular. They are acellular i.e.; their body is not divided into cells.

1.3 MICROSCOPY

The discovery and study of cells progressed with the invention of microscopes. Microscopy is the technique used to view objects that cannot be seen by the naked eye.

The microscopes that you use in the laboratory are light microscopes. Light microscopes use visible light to illuminate specimens in a two-lens system. The two lenses present in a light microscope are the ocular lens in the eyepiece and the objective lens located in the revolving nosepiece. As the light microscope uses two lenses so it is also known as compound microscope. The components are shown in fig. 1.2

Working principle: The light source illuminates the object. The first image of the object is formed by objective lens. It is a real, inverted and magnified image. The image formed by the objective functions as the object for eyepiece. The eye piece produces the final, virtual and magnified image. In this way, the final image produced is inverted with respect to the object.

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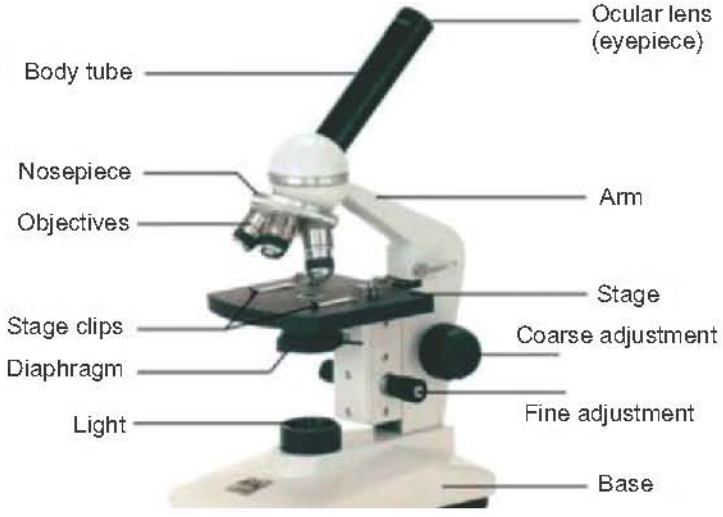

Fig.1.2 Light microscope

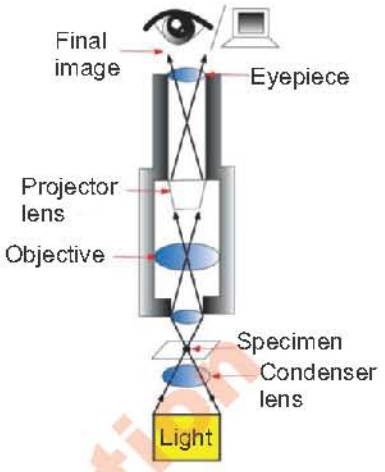

Fig. 1.3 The working principle of light microscope

Magnification: The magnification is the capacity of an optical instrument to increase the size of an object than its original size. The objects which cannot be seen by naked eye can also be observed by increasing magnification. Different lenses have different magnification powers which are represented by the letter 'X' that means that the number of times than original size. Therefore, a lens of 10X magnification can increase the size of an object of 1 µ m to 10 µ m.

The total magnification can be calculated by multiplying the objective lens value by the ocular lens value. Maximum magnification of a light microscope is 1500 X, using a 100X objective and 15X ocular lens. An electron microscope can magnify specimens between 1 and 50 million times depending on which type is used.

Resolution: It is a measure of the microscope's ability to distinguish between two points which are close together on an object. If a microscope can clearly distinguish between the two points, it has a good or high resolution. The resolution of naked eye is 0.1mm. This resolution cannot be increased by increasing magnification. The resolving power of a light microscope is 250 nm. The resolving power of an electron microscope is 0.2 nm.

Difference: The difference between magnification and resolution is that magnification is the ability to make small objects seen larger by using optical instruments e.g., microscope. Resolution is the ability to distinguish two objects from each other.

1.3.1. Electron Microscopy

Principle of Electron Microscopy: It uses the beam of accelerated electrons to visualize the specimens and has a high resolution.

The working principle of electron microscope: The beam of electrons are produced from the electron gun is focused on the specimen through two sets of condenser lens. An accelerating potential is applied between filament and anode to move the electrons downwards. The specimen to be observed is kept as thin section of 20-100 nm on specimen holder. The beam of electrons goes through specimen; the denser regions scatter more electrons on the screen than the lighter regions. The beam of electrons scattering from specimen passes through objective lens to create magnified image which is further magnified by ocular lens.

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Fig.1.4 Electron microscope.

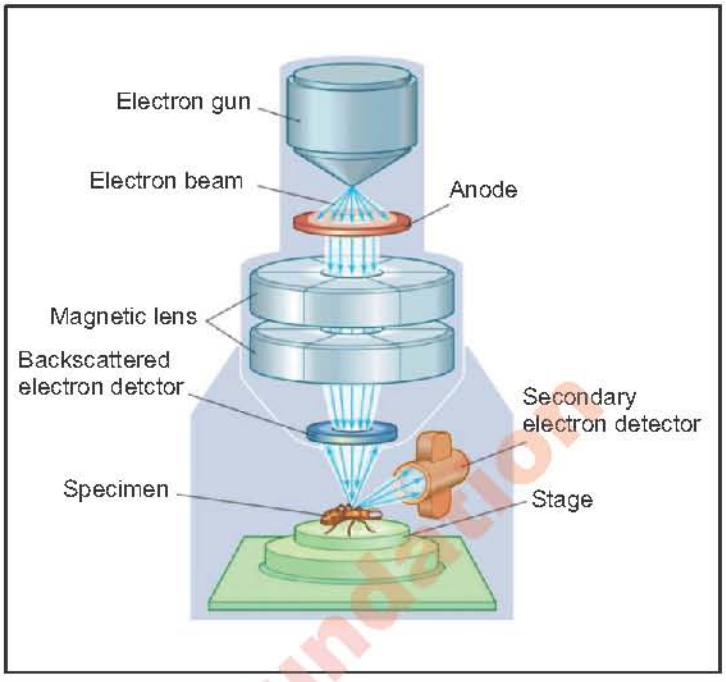

Fig.1.5 Working principle of electon microscope

Table 1.1 Comparison between Light Microscope and Electron Microscope

Light Microscope	Electron Microscope
1. It uses light (approx. 400-700 nm) as an illuminating source.	1. It uses electron beams (approx. 1 nm) as an illuminating source.
2. It has 1500 X magnification.	2. It has 1 to 50 million times, magnification depending on which type is used.
3. Through it both live and dead specimens can be seen.	3. Through it only dead and the dried specimen can be seen.
4. The formation of image depends upon the light absorption from the different zones of the specimen.	4. The formation of image depends upon the electron scattering.
5. It has low resolution than an electron microscope.	5. It has high resolution than a light microscope.
6. The useful magnification is of 500x to 1500x.	6. The direct magnification is as high as 16000x and photographic magnification is as high as 1000000x.
7. It has low resolution, then an electron microscope.	7. It has high resolution, then an light microscope.
8. Image is usually colored	8. Image is black and white
9. Vacuum is not required	9. Vacuum is essential for its operation.

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1.4 ULTRASTRUCTURE OF ANIMAL AND PLANT CELLS

The fine structure of the cell as seen with the electron microscope is known as ultrastructure.

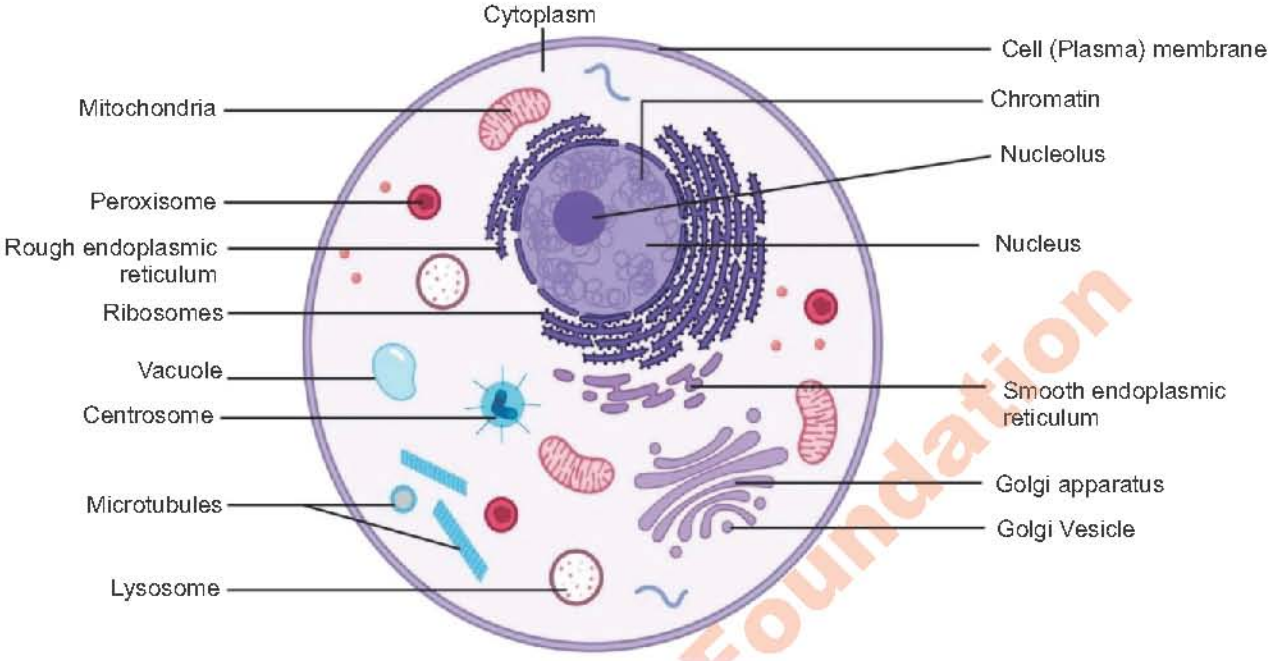

Figure 1.6 Ultrastructure of animal cell

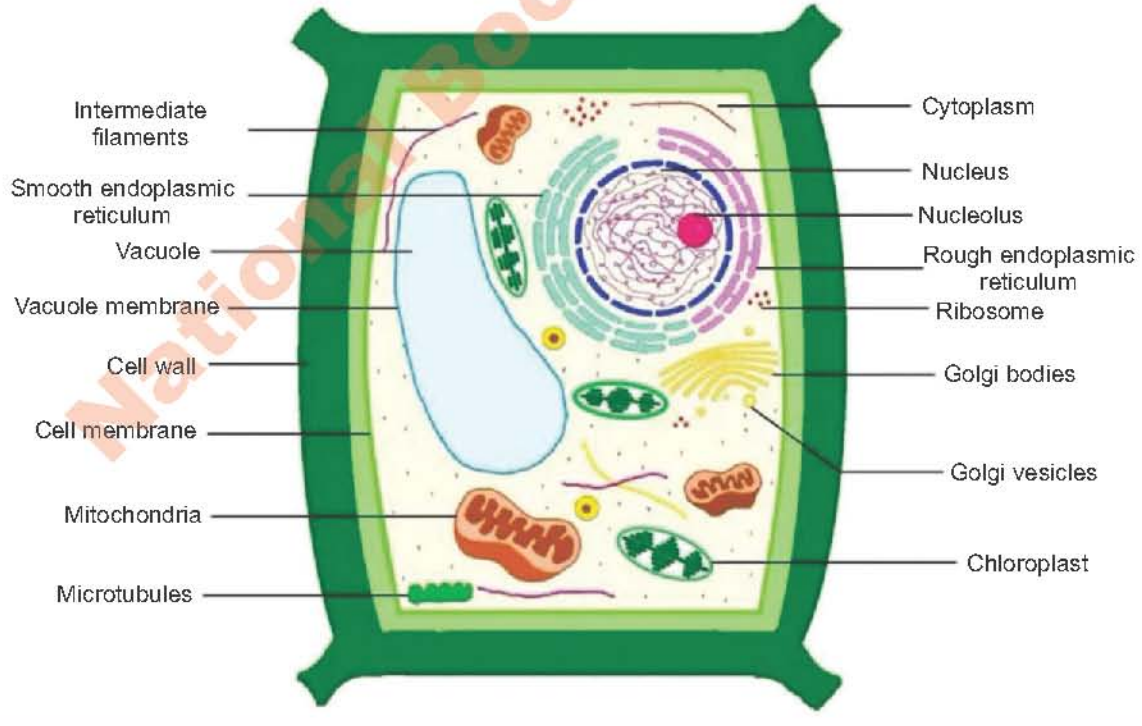

Figure 1.7 Ultrastructure of plant cell

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The Svedberg unit (Symbol S) is a measure of the sedimentation rate of a particle when centrifuged. The sedimentation rate indicates the size of the molecule, with the larger molecules having a larger sedimentation coefficient. It is dependent on the particle's volume, shape and molecular mass. If a particle is heavier with a more compact shape, its Svedberg value will be greater than lighter particles with a less compact shape. The unit is named after Swedish chemist Theodor Svedberg (1884-1971), who won the 1926 Nobel Prize in chemistry for his work on colloids and the invention of the ultracentrifuge.

There are two major types of electron microscopes. The transmission electron microscope (TEM) and the scanning electron microscope (SEM); sometimes the TEM and SEM are combined in one instrument, the scanning transmission electron microscope (STEM).The key difference between SEM imaging and TEM is that SEM produces an image by detecting secondary or backscattered electrons, whereas TEM uses transmitted electrons to form an image.

1.5 CELL WALL AND PLASMA MEMBRANE

The plasma membrane is the outer living boundary of the cell. Many cells have an extracellular component that is formed exterior to the membrane, which is called cell wall.

1.5.1 Cell Wall

Critical Thinking

Is plant cell wall permeable, semipermeable or impermeable boundary?

The cell wall is present in plant cells, prokaryotes fungi and some protists but animal cells do not have cell wall. This is probably due to their locomotor mode of life. Plant cell walls (made up of cellulose) differ in chemical composition from those of the prokaryotes (made up of peptidoglycan) and fungi (made up of chitin). We will discuss here only plant cell wall. The cell wall is an extracellular structure of the plant cells. The cell wall protects the plant cell, maintains its shape and prevents excessive uptake of water. On the level of the whole plant, the strong cell walls of specialized cells hold the plant up against the force of gravity.

The cell wall is secreted by the cell. The cell wall is porous and allows free passage of water and dissolved material. The plant cell wall consists of three main layers, primary cell wall, middle lamella and secondary cell wall.

Primary cell wall

Primary cell wall is a true wall and develops in newly growing cell i.e., during cell division. Each cell produces a primary cell wall. The primary cell wall is present inner to the middle lamella. The primary cell wall is thin and slightly flexible. The primary cell wall is composed of cellulose microfibrils (bundles of cellulose chains), running through the matrix of other

Fig. 1.8 : Crisscross arrangement of cellulose

Science Tidbits

Pectin is a polymer of around 200 galacturonic acid molecules. Majority of its carboxyl groups are methylated ($COOCH_3$). It is less hydrophilic then pectic acid but soluble in hot water. It is another major component of middle lamella but also found in primary walls.

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polysaccharides like hemicelluloses and pectin. The microfibrils show a **crisscross arrangement** in layers one above the others. This feature gives the cell great strength. The primary cell wall is adapted to growth. The wall stretches plastically i.e., irreversibly.

Secondary cell wall

Secondary cell wall is formed between the primary cell wall and plasma membrane only in sclerenchyma cells. The plant cells possessing secondary cell wall are generally dead and provide support for the plant. The secondary cell wall develops only when the cell has reached maximum size i.e., completes its growth because it is very much thick and rigid therefore it does not allow further growth. The secondary cell wall consists of cellulose, hemicelluloses, lignin, inorganic salts and waxes. Its cellulose microfibrils also show crisscross arrangement. Lignin cements and anchors cellulose microfibrils together and it is mainly responsible for rigidness. The secondary cell wall provides definite shape and mechanical support to the cell.

Science Titbits

Pectic acids are polymer of around 100 galacturonic acid molecules. These are very hydrophilic and form salts with Ca^{++} and Mg^{++} that are insoluble gels. These are major components of middle lamella but also found in primary cell walls

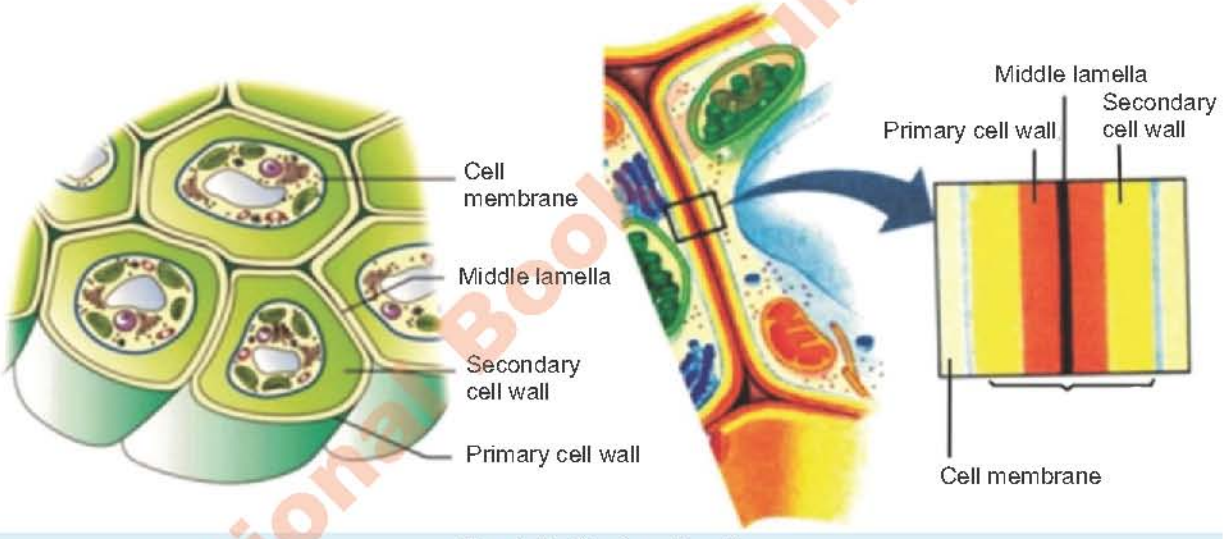

Fig. 1.9: Plant cell wall

Middle lamella

Middle lamella is present between primary cell walls of adjacent cells which holds the cells together. It is composed of sticky, gel-like magnesium and calcium salts and pectin.

1.5.2 Plasma Membrane

Plasma membrane is also called cell membrane. It is the boundary of protoplasm and most organelles. It is found in all living prokaryotic and eukaryotic cells. It controls the passage of materials into and out of the cell.

Composition of plasma membrane

Chemically plasma membrane consists of proteins 60-80%, lipids 20-40% and small quantity of carbohydrates.

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Structure of plasma membrane

Fluid mosaic model of plasma membrane: The model proposes that the membrane is a phospholipids bilayer in which protein molecules are either partially or wholly embedded. The proteins are scattered throughout the membrane in an irregular pattern just like large ice bergs float in the sea. The pattern of distribution of proteins can vary from membrane to membrane and also vary on both surfaces of membrane. The membrane is about 7 nm thick.

Science Titbits
The fluidity of membrane is dependent on its lipid components, including phospholipids, glycolipids and cholesterol.

The lipid part of plasma membrane consists of two layers (bilayer) of phospholipids which are arranged in such a way that their hydrophobic ends face each other while hydrophilic ends are appeared on the surface. The steroids, cholesterols are wedged into the phospholipid bilayer at some intervals. The plasma membrane is asymmetrical i.e., their two surface and halves are not identical.

In general most membrane proteins drift sideways in the fluid bilayer. The proteins within a membrane determine most of the functions. Carbohydrates are either attached to proteins (glycoproteins) or lipids (glycolipids) generally on the outer side of membrane. Filaments of the cytoskeleton are also present on the inner surface of the membrane. These support the plasma membrane.

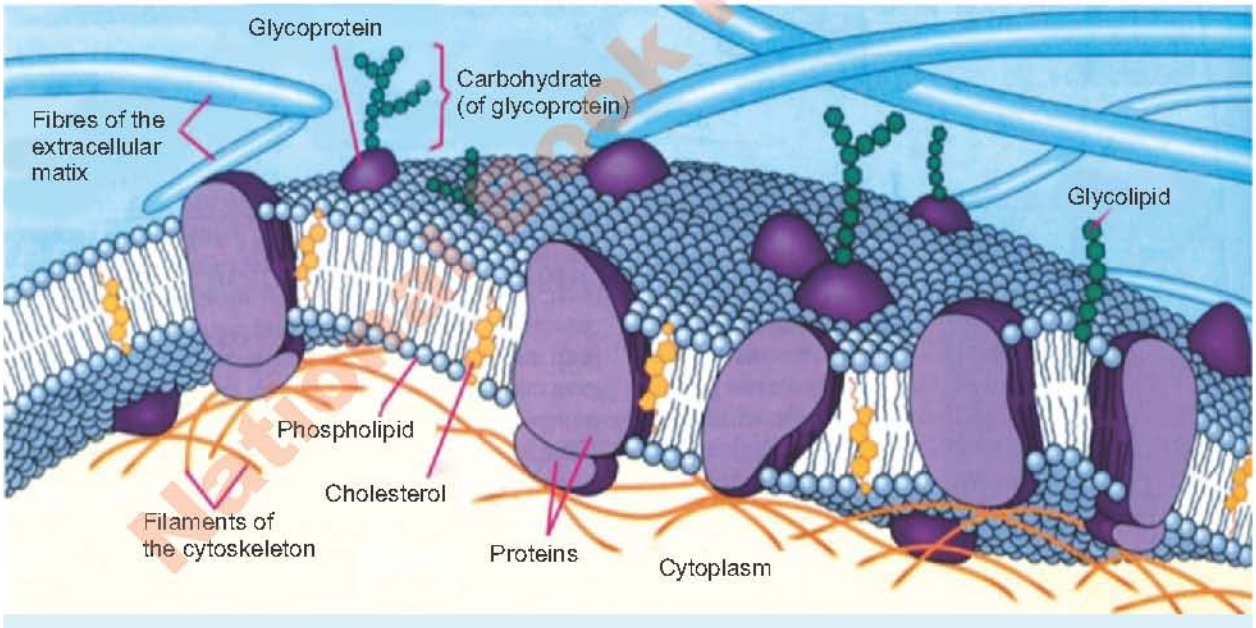

Fig. 1.10: Fluid mosaic model of plasma membrane

The lipid part of plasma membrane controls the fluidity of the membrane. When the concentration of unsaturated fatty acid in phospholipids becomes greater, the bilayer becomes more fluid like that makes cell membrane more flexible. The cholesterol helps to stabilize the lipid bilayer. It also restricts entry and exit of polar molecules and ions.

Functions of plasma membrane proteins

A great variety of proteins are found in plasma membrane which may act as transport channel or carrier, enzyme, receptors or as antigens.

Certain plasma membrane proteins are involved in the passage of molecules through the membrane. Some of those have a channel through which a substance simply can move across the membrane, other are carriers that combine with a substance and help it to move across the membrane. Some plasma membrane proteins have enzymatic functions. Some proteins in the plasma membrane are receptors that receive signals from other cells. For example, hormones circulate in the blood, but bind to specific target cells, with specific receptors. Some proteins are antigens which enable the cells to recognize other cells.

Roles of glycolipids and glycoproteins as cell surface markers

Mostly glycolipids and glycoproteins act as **cell surface markers.** They are involved in cell to cell recognition and sticking the correct cells together in tissues.

Regulation of cell's interaction with its environment by the plasma membrane

Plasma membrane regulates cell's interaction with its environment by controlling transport of material across the cell. Transport across plasma membrane occurs to maintain a suitable pH and ionic concentration within the cell for enzyme activity.

The techniques used in studying cell membrane

The biophysical imaging techniques, such as atomic force microscopy and super-resolution fluorescent microscopy, have been developed to study biological structures. Scanning electron microscopy (SEM) is one of the fundamental techniques for membrane characterization. It gives the morphology and topography data of the prepared membranes. SEM can be used to determine the pore size in the case of a porous membrane.

1.6 CELL SIGNALING

The process by which cells communicate with other cells within their body or with the external environment is called cell signaling. Or simply the transfer of information from one cell to another is called **cell signaling.**

Ligands and Receptors

The signaling **molecules** are also known as **ligands.** They initiate signaling. Different types of molecules can serve as signaling molecules. They can be proteins, lipids, amino acid metabolites, gases and many others.

The proteins that respond to ligands are called **receptors.** Signaling molecules and receptors exist in several varieties; however, a signaling molecule will bind to a specific receptor that typically binds only one type of signaling molecule.

1. **Reception:** Reception is the target cell's detection of a signal molecule coming from outside the cell. A chemical signal is detector when it binds to a receptor protein located at the cell's surface or inside the cell.

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2. **Transduction:** The conversion of a signal from outside the cell to a form that can bring about specific cellular response is called **transduction**. The binding of the signal molecule changes the receptor protein in some way initiating the process of transduction.
3. **Response:** The transduced signal finally triggers a specific cellular response such catalysis by an enzyme, rearrangement of the cytoskeleton, or activation of specific gene in the nucleus etc.

1.6.1. PATHWAY OF A SIGNAL FROM OUTSIDE TO INSIDE

The process by which a signal on a cell's surface is converted into a specific cellular response is a series of steps called a **signal transduction pathway.**

Protein Signaling

Protein and peptide hormones are water soluble. So they cannot pass through the plasma membrane of the cell. These hormones or environmental stimulus are the first messenger. They bind with their receptors on the plasma membrane of target cell, starting a series of events in the cell which generates a second messenger e.g., cAMP (Cyclic adenosine monophosphate). The second messenger then triggers various changes in the cell including activation of enzyme, gene activation etc.

Cell signaling is an important factor in life. The cells receive the signals and respond to the extracellular environment, thereby, allowing growth, development and immunity.

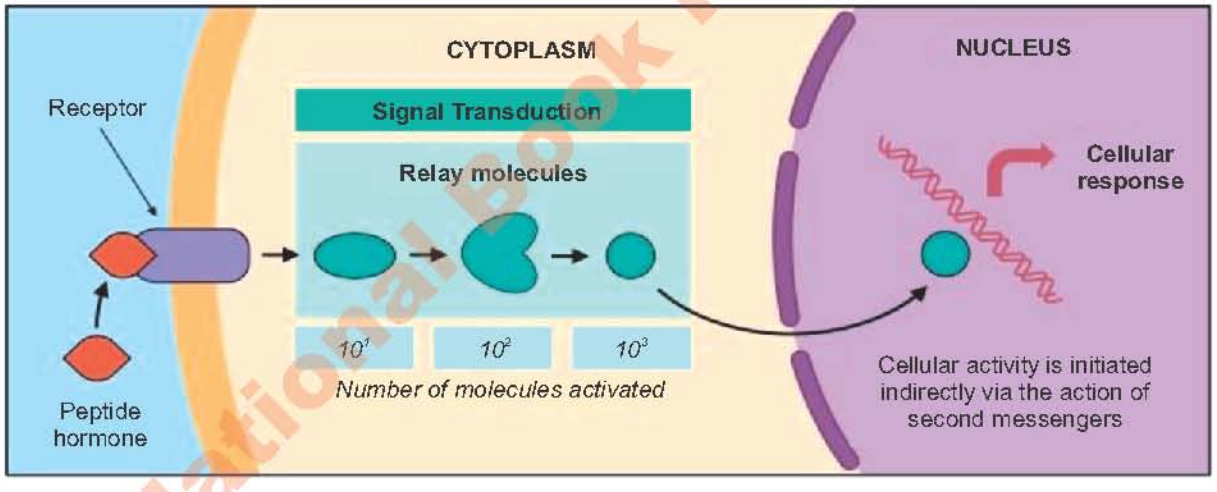

Fig.1.11 Protein signaling pathway

Steroid Signaling

Small hydrophobic ligands can directly diffuse through the plasma membrane and interact with internal receptors. Important members of this class of ligands are the steroid hormones. Steroids are lipids that have a hydrocarbon skeleton with four fused rings.

Steroid hormones are lipophilic (fat-loving). It means they can freely diffuse across the plasma membrane of a cell. They bind to receptors in either the cytoplasm or nucleus of the target cell, forming an active receptor-hormone complex. This activated complex will move into the nucleus and bind directly to DNA, acting as a transcription factor for gene expression. Estrogen, progesterone and testosterone are the examples of steroid hormones.

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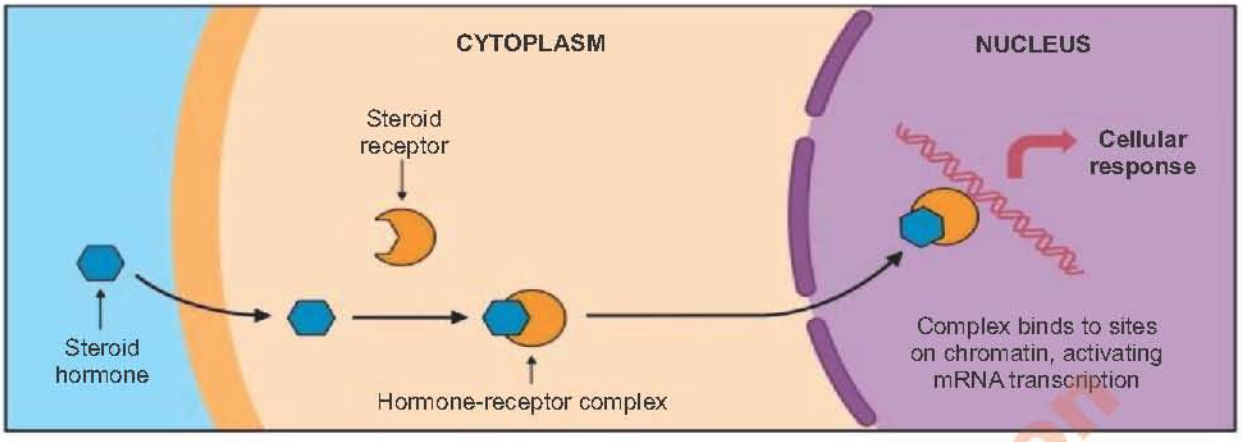

Fig.1.12 Steroid signaling pathway

1.7 MEMBRANE TRANSPORT MECHANISM

Cells need food materials, salts, water and oxygen etc. They also need to get rid of substances such as carbon dioxide. Membrane transport is a set of transport mechanisms that control the movement of solutes such as ions and small molecules through biological membrane. Membrane transport is dependent upon the permeability, solute concentration, the size and charge of the solute. There are four types of transport mechanisms in a cell. These are simple diffusion, facilitated diffusion, osmosis and active transport. Simple diffusion, facilitated diffusion and osmosis are passive transport mechanisms.

Simple diffusion

We can now define Diffusion is the 'movement of molecules from a region of their higher concentration to a region of their lower concentration, down a concentration gradient'.

> The difference in concentration between two regions in known as the concentration gradient.

The molecules can travel directly through the membrane in simple diffusion. Small molecules such as water, carbon dioxide and oxygen can pass through the cell membrane fairly easily. So diffusion equalizes the concentration of these molecules inside and outside the cell all the time. Each type of molecule moves down its own gradient independently of other molecules. For example, oxygen diffuses from the lungs into the blood while at the same time carbon dioxide diffuses in the opposite direction.

Facilitated diffusion

Most water- soluble molecules, cannot pass through the phospholipid bilayer. The molecules should be small and non-polar to traverse (travel across or through) the membrane. These molecules can diffuse across only with the help of two types of transport proteins. They are channel proteins and carrier proteins.

Channel Proteins help in the entry and exit of substances in the cell. There are

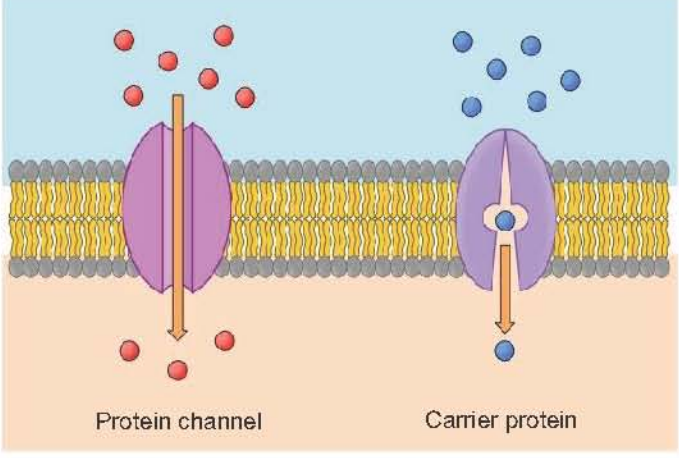

Fig. 1.13 Facilitated diffusion

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two types of channel proteins, open channel proteins, and gated channel proteins. Open channel proteins create a pore in the cell membrane and allow the charged molecules to pass through. The gated channel proteins are either closed or open and regulate the entry and exit of substances.

Carrier Proteins are present on the cell membrane. They carry the molecules, change the shape of the molecules and release the molecules to the other side. Temperature and saturation affect the carrier proteins.

Now we can define that "Facilitated diffusion is a type of diffusion in which the molecules move from the region of higher concentration to the region of lower concentration assisted by a carrier."

Osmosis

A semipermeable membrane is an impervious sheet perforated with tiny pores. The pores allow solvent molecules e.g., water to pass through by diffusion but not larger solute molecules such as sugar, salt etc. The examples are egg membrane, parchment paper, cellophane etc.

Table 1.3 Comparison between simple diffusion and facilitated diffusion.

Simple diffusion	Facilitated diffusion
1. A type of passive transport	1. A type of passive transport
2. Substances move from an area or region of higher concentration to an area or region of lower concentration	2. Substances move from an area or region of higher concentration to an area or region of lower concentration
3. Does not directly require chemical energy, e.g., ATP or GTP	3. Does not directly require chemical energy, e.g., ATP or GTP
4. Transport proteins not required	4. Transport proteins required
5. Rate is generally slower but more straightforward as it does not rely upon the binding capacity of membrane proteins with substances for transport	5. Rate is generally faster but affected by factors such as temperature and types of membrane proteins involved, and thus, may be affected by membrane protein inhibitors.
6. Small nonpolar molecules (e.g., oxygen, carbon dioxide) diffusing easily across the plasma membrane.	6. Polar molecules (e.g., glucose and amino acids), larger ions (e.g. sodium ions and chloride ions), and large nonpolar molecules (e.g. retinol) employ facilitated diffusion via membrane proteins across the plasma membrane

Now we can define that 'the process by which the molecules pass from a solution of low concentration to a solution of high concentration through a semipermeable membrane is called osmosis.'

The examples of osmosis are: Feeling thirsty after having salty food, dialysis of kidney, swelling of resins and seeds when they are soaked in water.

Osmotic pressure is the minimum pressure applied to a solution to stop the flow of solvent molecules through a semipermeable membrane. Water potential is a measure of the tendency of water to move from one place another. When a partially membrane separates two solutions of different water potential is established.

Active Transport

The movement of molecules or ions across a membrane from a region of lower concentration to a region of higher concentration, against a concentration gradient is called **active transport.** Movement is usually in one direction only.

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To achieve this movement, it needs cellular energy. There are two types of active transport, primary active transport and secondary active transport.

Primary active transport: The energy is supplied in the form of ATP, which is an energy carrier made in respiration. Without respiration, active transport is not possible. Sodium-potassium pumps is an example of this transport. Cell membrane have sodium pumps that actively pump ions out of the cell. In animal cells, the sodium pump is coupled with a potassium pump which actively moves potassium ions from outside to inside the cell. It's importance is revealed by the fact that more than a third of the ATP is used to pump sodium and potassium.

Secondary active transport: Secondary active transport uses electrochemical energy. It takes place across a biological membrane. In it a transporter protein couples the movement of an electrochemical ion (typically Na^+ or H^+) down its electrochemical gradient to the upward movement of another molecule or an ion against a concentration or electrochemical gradient.

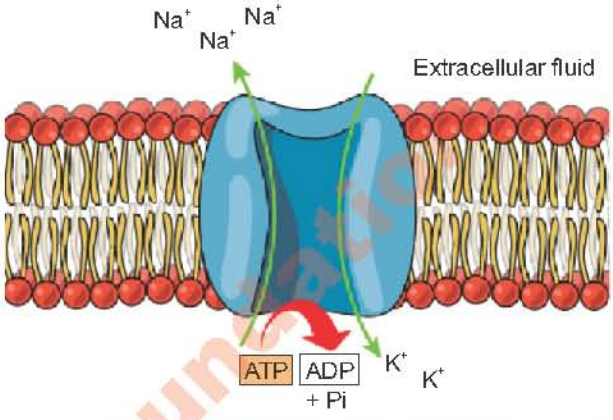

Fig.1.14 Sodium- Potassium pump.

Now we can define that "Active Transport as a process that involves the movement of molecules from a region of lower concentration to a region of higher concentration against a gradient or an obstacle with the use of external energy."

Active Transport in Plants

Active transport takes place in the root cells by absorbing water and minerals. Active transport always leads to accumulation of molecules and ions towards one side of the membrane. This mode of transportation in plants is carried out by membrane proteins and transports the substance from the lower concentration to higher concentration.

Examples of Active Transport

Some of the best examples of active transport include: (1) Phagocytosis of bacteria by macrophages. (2) Movement of Ca^{+2} ions out of cardiac muscle cells. (3) Transportation of amino acids across the intestinal lining in the human gut. (4) Secretion of proteins like enzymes, peptide hormones, and antibodies from different cells. (5) Functioning of the White Blood Cells by protecting our body by attacking diseases causing microbes and other foreign invaders.

1.8 CYTOPLASM AND ORGANELLES

The living matter of a cell is called protoplasm. In eukaryotic cells it can be divided into two parts i.e., cytoplasm and nucleus.

1.8.1 Cytoplasm

Cytoplasm is the region between nuclear membrane and plasma membrane. This is also a common component of both prokaryotic and eukaryotic cells.

It is about 90% water and forms a solution that contains all the fundamental biochemicals of life. Some of these are ions and small molecules in true solution, such as salts, sugars, amino acids, fatty acids, nucleotides, vitamins and dissolved gases. Others are large molecules, such

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as proteins, which form the colloidal solutions. **Cytosol** is known as the matrix of the cytoplasm. It surrounds the cell organelles in eukaryotes. In prokaryotes, all the metabolic reactions occur here. Cytosol is the water-soluble components of cell cytoplasm. The cytosol is a liquid matrix around the organelles. The cytosol is a complex mixture of substances dissolved in water. Although water forms the large majority of the cytosol. The term cytosol is now used to refer to the liquid phase of the cytoplasm in an intact cell. In prokaryotes, most of the chemical reactions of metabolism take place in the cytosol.

The cytoplasm acts as a site of metabolism and storehouse of a cell. The metabolic pathways generally occur in the cytosol which includes **protein synthesis, glycolysis** etc.

1.8.2 Cell Organelles

In a eukaryotic cell, the cytoplasm contains highly organized discrete structures which are specific for various cellular functions are called **cell organelles**. The cell organelles are generally enclosed by the membrane except few such as ribosome.

The organelles in the cytoplasmic matrix of a cell are: endoplasmic reticulum, ribosomes, Golgi complex, lysosomes, peroxisomes, glyoxysomes, vacuoles, mitochondria, and chloroplasts etc.

Endoplasmic reticulum

The endoplasmic reticulum (ER) is an extensive network of membrane. The word endoplasmic means 'within the cytoplasm'. The ER consists of a network of membranous tubules and sacs called **cisternae** (from Latin word cisterna, a reservoir of liquid). The ER membrane separates the internal compartment of the ER called the **ER lumen** (cavity), from the cytosol. There are two types of ER, smooth ER and rough ER. They differ in their structure and functions. Most of cells contain both types of ER. However some cells (skeletal muscle cells) have smooth ER more, where these are called **sarcoplasmic reticulum**.

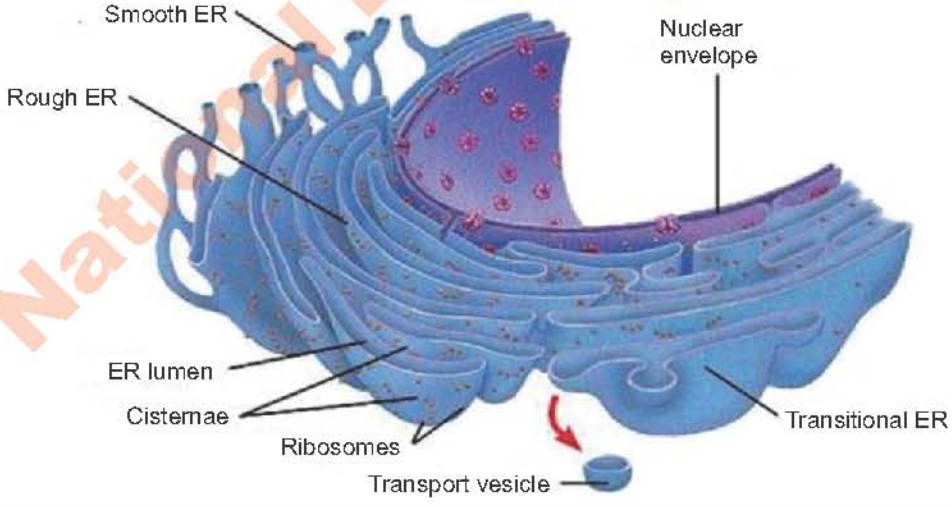

Figure 1.15 Endoplasmic reticulum

Smooth ER

The smooth ER is continuous with the rough ER. Since, ribosomes are not attached to it, therefore, it has smooth appearance. The smooth ER functions in diverse metabolic processes.

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Enzymes of the smooth ER are important to the synthesis of lipids including oil, cholesterol, phospholipids and steroids e.g., sex hormones of vertebrates and various steroid hormones of the adrenal glands. In the smooth ER, other enzymes help to detoxify drugs and poisons especially in the liver cells.

Rough ER

The rough ER has ribosomes attached to the sides facing cytoplasm and has rough appearance under electron microscope. Many types of specialized cells secrete proteins produced by ribosomes attached to the rough ER. Secretory proteins depart from the rough ER wrapped in the membranes of vesicles that bud like bubbles from specialized region called **transitional ER.** Vesicles in transit from one part of the cell to another are called **transport vesicles.** In addition to making secretory proteins, rough ER is a membrane factory for the cell, it grows in place by adding membrane proteins and phospholipids to its own membrane. The ER membrane expands and is transferred in the form of transport vesicles to other components of the endomembrane system.

Ribosomes

Ribosomes are the particles made of ribosomal RNA and protein. These organelles carryout protein synthesis.

Ribosomes were first observed using electron microscope as dense granules. Ribosomes are roughly spherical, granular, non-membranous bodies found in both eukaryotic as well as prokaryotic cells. However, eukaryotic ribosomes are larger and characterized as 80S ribosomes while the prokaryotic ribosomes are slightly smaller and are characterized as 70S ribosomes. They can be seen only under the electron microscope. They are made of almost an equal amount of RNA and protein so they are **ribonucleoprotein.** Ribosomes are formed in the nucleolus. Then these are transported to the cytoplasm through the nuclear pore.

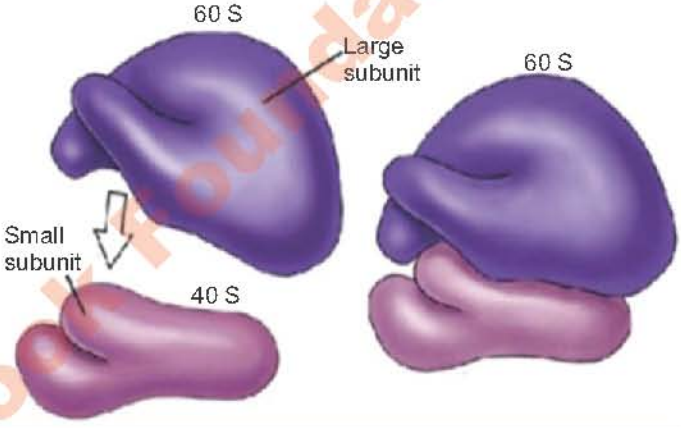

Fig.1.16: Eukaryotic 80S ribosome

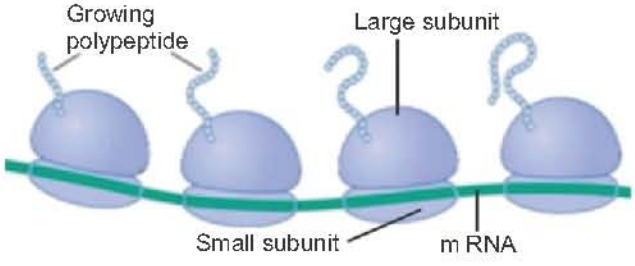

Fig.1.17: Polysome

The eukaryotic ribosomes are composed of two subunits (particles) of different sizes. The larger one is 60S particles and the smaller one is 40S particles. The two subunits on attachment form 80S particles. The attachment is controlled by presence of magnesium ions concentration or forming salt bonds between phosphate group of RNA and amino group of amino acid or both by magnesium ions and salt bonds. Both ribosomal subunits are generally attached together at the time of their function.

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The ribosomes are involved in the events of protein synthesis. Sometimes, during protein synthesis, several ribosomes are attached to one mRNA molecule. Such a chain of many ribosomes is called **polysome** or **polyribosomes**. In this way several copies of same polypeptide can be produced in very less time.

Golgi Complex

It is found in all eukaryotic cells. It was discovered by Italian biologist **Camillo Golgi** in 1898. It is also called Golgi apparatus and Golgi bodies.

Golgi complex consists of a stack of flattened, membrane bound sacs called **cisternae**, together with system of associated vesicles called **Golgi vesicles**. It is a complex system of interconnected tubules formed around the central stack. At one end of the stack new cisternae are constantly being formed by the fusion of vesicles from the smooth ER. This outer or **forming face** (cis face) is convex, while the inner end is concave and is called **maturing face** (trans face) where the cisternae breakup into vesicles again.

The most important function of Golgi complex is the processing of cell secretions. Therefore, these organelles are abundant in secretory cells. The cell secretions mainly consist of proteins. Golgi complex collects these proteins from RER through SER, modifies them to perform specific function and then exports these modified products in the form of vesicle. Certain organelles, such as lysosomes, peroxisomes and glyoxysomes also originate from Golgi complex. Golgi complex is also involved in the formation of conjugated molecules like glycoprotein, lipoprotein etc. In plant cell during cell division, Golgi complex also gives rise to vesicles which contain cell wall synthesizing materials. At cytokinesis, these Golgi vesicles are arranged on the cell equator,

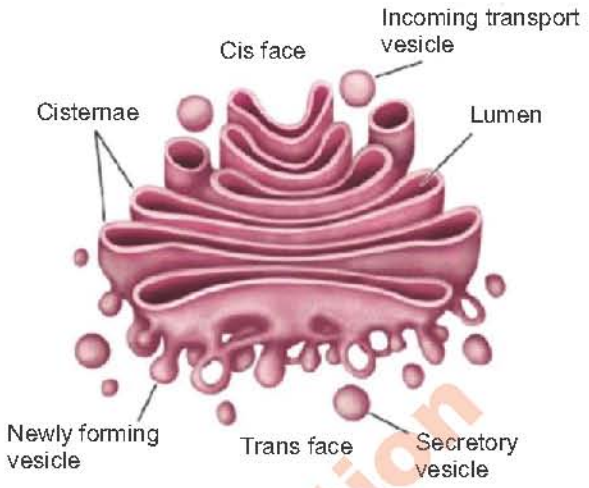

Fig. 1.18: Golgi complex

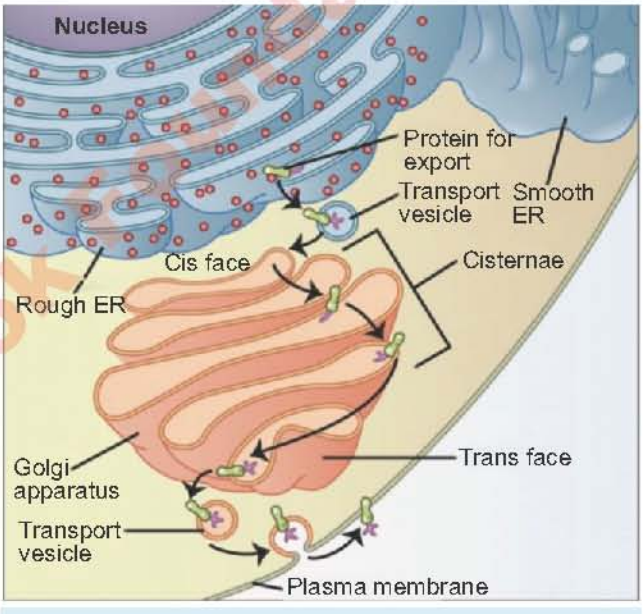

Fig. 1.19: Role of Golgi complex in a glandular cell

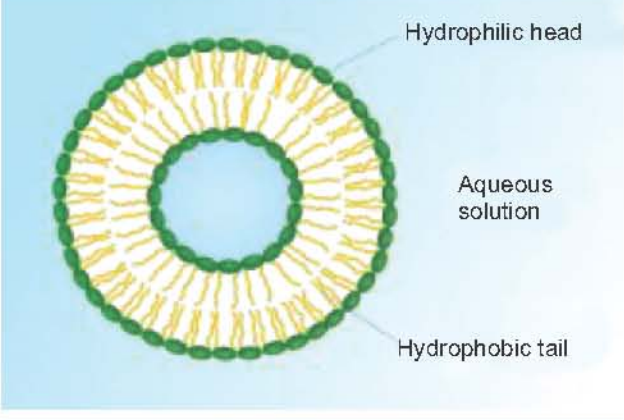

Fig 1.20. Vesicle

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fuse together and form a structure, called **phragmoplast**. Later on new cell wall is derived from this structure.

Vesicles

The word 'vesicle' derives from the Latin word *vesicula* meaning 'small bladder'. Vesicles are small cell organelles that are present in cells. These organelles are small, membrane-enclosed sacs. A vesicle is a small structure within a cell, consisting of fluid enclosed by a lipid bilayer. A vesicle consists of hydrophilic head and hydrophobic tail. Many vesicles are made in the Golgi complex and the endoplasmic reticulum or are made from parts of the cell membrane by endocytosis. Because vesicles are made of phospholipids, they can break off is and fuse with other membranous material. Examples of vesicles include secretory vesicles, transport vesicles, synaptic vesicles, lysosomes etc. Vesicles are found in bacteria, Archea, and plants as well as in animals.

Functions of vesicles is that they transport substances to and from one cell to another and also from one part of a cell to another, e.g., a protein and other molecules. The first step in vesicular transport is the formation of a vesicle by budding from the membrane. Vesicles are also involved in metabolism, buoyancy control, and enzyme storage. They can also act as chemical reaction chambers.

Lysosomes

Lyso means splitting and *soma* means body. These are single membranous, spherical vesicles. They contain digestive or hydrolytic enzymes. The lysosomal enzymes are made by the RER and then are transported to Golgi complex through SER. After modification, these enzymes are released from the *trans* face Golgi complex in the form of vesicles. Such vesicles are called lysosomes. The newly formed lysosomes before the start of their functions are usually called **primary lysosomes**. In plants and fungi, certain vacuoles carryout enzymatic hydrolysis, a function shared by lysosomes in animal cells.

Lysosomes contain about 40 different digestive enzymes. These enzymes can breakdown every major macromolecule of the cell. The contents of the **lysosome** are acidic. In order to perform its function, the lysosomes fuses with membrane bound vesicle that arises from any of these pathway's **endocytosis, phagocytosis or autophagocytosis.**

The key function of lysosomes is digestion and removal of waste. Foreign particles are pulled in to the cell through the process of **endocytosis.** The process of endocytosis happens when the cell membrane falls in on itself

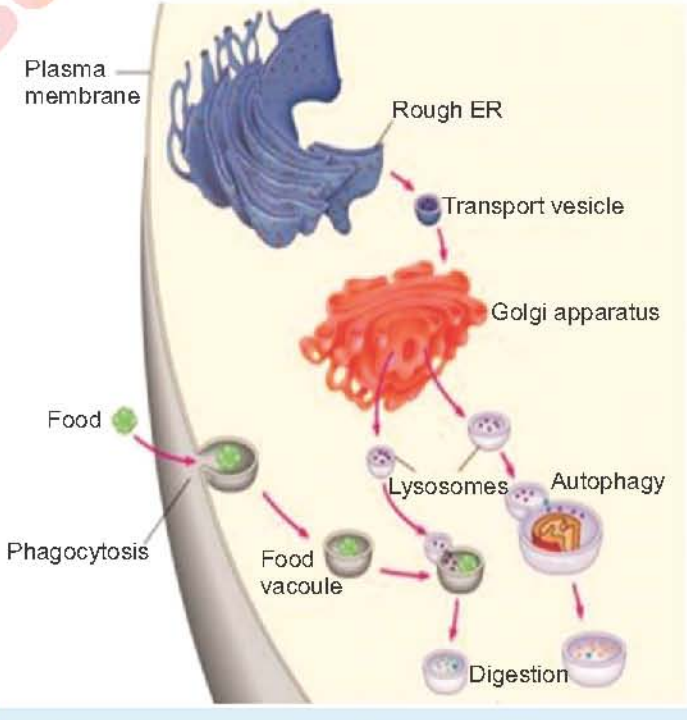

Fig. 1.21: Formation and functions of lysosomes

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(invagination), creating a vacuole or a pouch around the external contents and then bringing those contents into the cell.

The process by which unwanted structures within the cell are engulfed and digested within the lysosomes is called **autophagy**. This is self-eating process of a cell in which a lysosome begins to digest cell's own organelles. Such lysosomes are also called **autophagosomes**. This process either takes place in starvation period in order to obtain energy or it occurs in routine in order to control number of specific organelles. For example, if someone starts to perform heavy muscular exercise, the number of mitochondria begins to increase in his muscle cells, but if he leaves exercise, the number of mitochondria are again decreased by the process of autophagy.

Sometimes, especially during developmental phase, when a particular cell is required to be disintegrated, a type of cell death is committed, called **autolysis**. This is a programmed cell death in which lysosomes burst and their enzyme contents are quickly dispersed throughout the cytoplasm. In this way the cell is disintegrated into fragments which are phagocytosed by other cells. Due to this function lysosomes are also called **suicidal bags**.

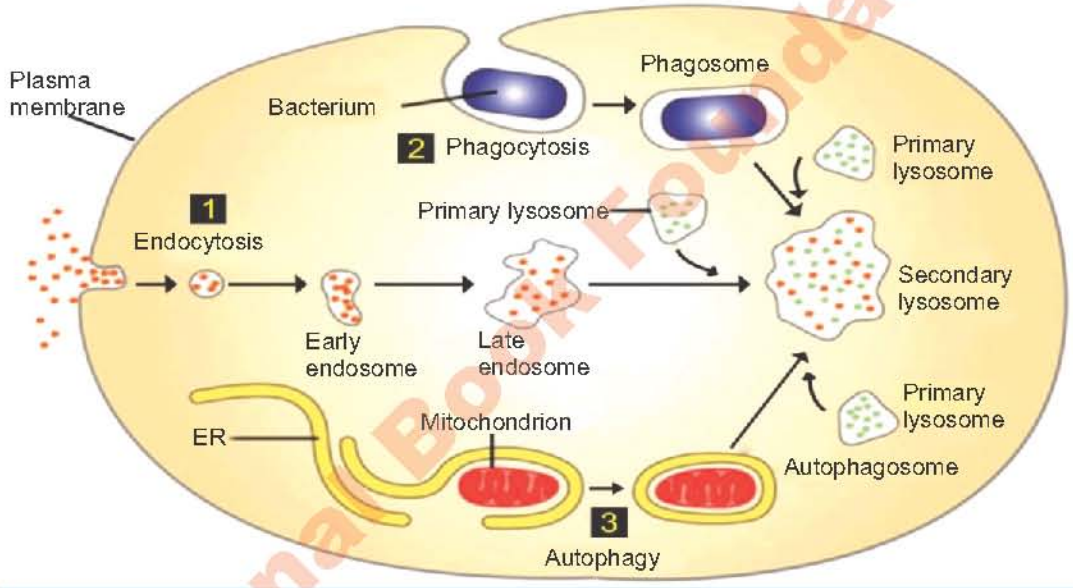

Fig. 1.22: Functions of Lysosomes

Peroxisomes and Glyoxysomes

Peroxisomes: The peroxisome is a small vesicle, single membrane bound organelle. It is an oxidative organelle that is not part of the endomembranous system. Like mitochondria and chloroplast it imports its proteins primarily from cytosol. Peroxisomes can vary in shape and size, depending on the needs of the cell they serve. They will sometimes increase in number and size if, for example, they have a lot of alcohol to break down.

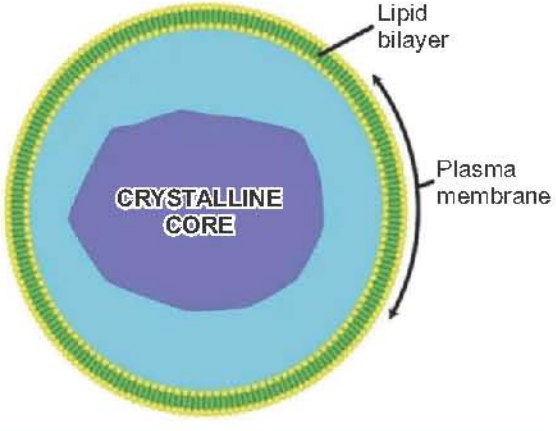

Fig. 1.23: Peroxisomes

Peroxisomes and glyoxysomes are collectively called **microbodies**. These are similar to

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lysosomes in the sense that they are single membranous, vesicular structures. They contain enzymes (although different than lysosome) and originate from Golgi complex but they are smaller than lysosome.

Peroxisomes contain some oxidative enzymes like peroxidases, catalases and glycolic acid oxidases. They are abundant in liver cells where they are specifically involved in the formation and decomposition of hydrogen peroxide so they are named **peroxisomes**. They are mainly concerned with the detoxification of alcohol. In this activity, alcohol is oxidized into hydrogen peroxide (H_2O_2) with the help of **peroxidase** enzyme. Hydrogen peroxide is itself a toxic molecule, which is immediately broken down to water and oxygen by another enzyme called **catalase**. In plant cell, peroxisomes are involved in **photorespiration**. A step of photorespiration takes place in peroxisomes in which **glycolate** is converted into **glycine** with the help of an enzyme called **glycolic acid oxidase**.

Glyoxysomes: Specialized peroxisomes are called glyoxiysomes. **Glyoxysomes** are found only at seedling stage in oil seed plants. These organelles have a number of enzymes specific for plant lipid metabolism that are not found in animal cells. The germinating seedlings convert stored fatty acids to carbohydrates. This is achieved through a metabolic pathway called **glyoxylate cycle**, the enzymes of which are located in the glyoxysomes.

Vacuoles

Vacuoles are large vesicles that originate from the endoplasmic reticulum and Golgi complex and plasma membrane. Vacuoles perform a variety of functions in different kinds of cells. In animal cells, **food vacuoles** are formed by phagocytosis. Many freshwater protists have **contractile vacuoles** that pump excess water out of the cell, thereby maintaining a suitable concentration of ions and molecules inside the cell.

In young plant cells, many small vacuoles are present which can hold reserves of important organic compounds. These vacuoles may also help in protection of plant against herbivores by storing compounds that are poisonous or unpleasant to animals. Mature plant cells generally contain a large **central vacuole** which develops by the joining of smaller vacuoles. The solution inside the central vacuole, called **cell sap**, is plant cell's main reservoir of inorganic ions, including potassium and chloride. The central vacuole plays a major role in mechanical support by maintaining turgor and also acts as a storehouse of the cell. The membrane separating the vacuole from cytoplasm is called **tonoplast**.

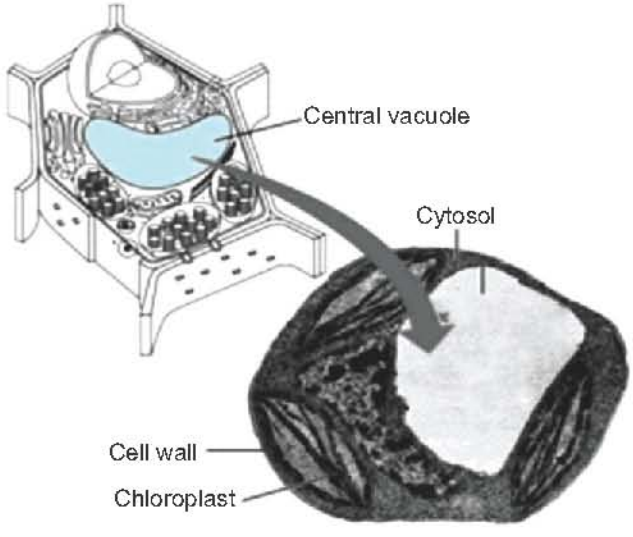

Fig. 1.24: Vacuole of a mature plant cell

Mitochondria

Mitochondria (singular: *mitochondrion*) are present in all eukaryotic cells. Some cells have a single large mitochondrion, but more often a cell has hundreds or even thousands of mitochondria; the number correlates with the cell's level of metabolic activity. For example, cells that move or contract have

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proportionally more mitochondria per volume than less active cells. Mitochondria are capable to divide themselves (self-replicating) in order to increase their number. They divide by fission.

Mitochondria are cylindrical or rod-shaped structures. They are enclosed by double membrane, the outer **membrane** and the **inner membrane**. The outer membrane is smooth and somewhat like a sieve due to presence of **porins**. These are special proteins responsible for the transport of molecules across the membrane. Porins allow free passage of various molecules into the inter-membrane space. The inner membrane is selectively permeable and folded inwards. The folds are called **cristae** which serve to increase the surface area. The inner surface of cristae has granular structures called **F0-F1 particles**. These particles are actually **ATP synthase** enzymes. In addition, several other complexes are also found in inner mitochondrial membrane, which serve as electron carriers in electron transport chain. The inner membrane divides the mitochondrion into two internal compartments. The first is the **intermembrane space**, the narrow region between the inner and outer membranes. The second compartment, the **mitochondrial matrix**, is enclosed by the inner membrane. Mitochondrial matrix is a jelly like material that contains a small circular DNA, all kinds of RNA, ribosomes (70S) and enzymes. The presence of these components indicates that mitochondria have their own genetic system. It means, the protein, which are required by mitochondria are synthesized by their own metabolic machinery.

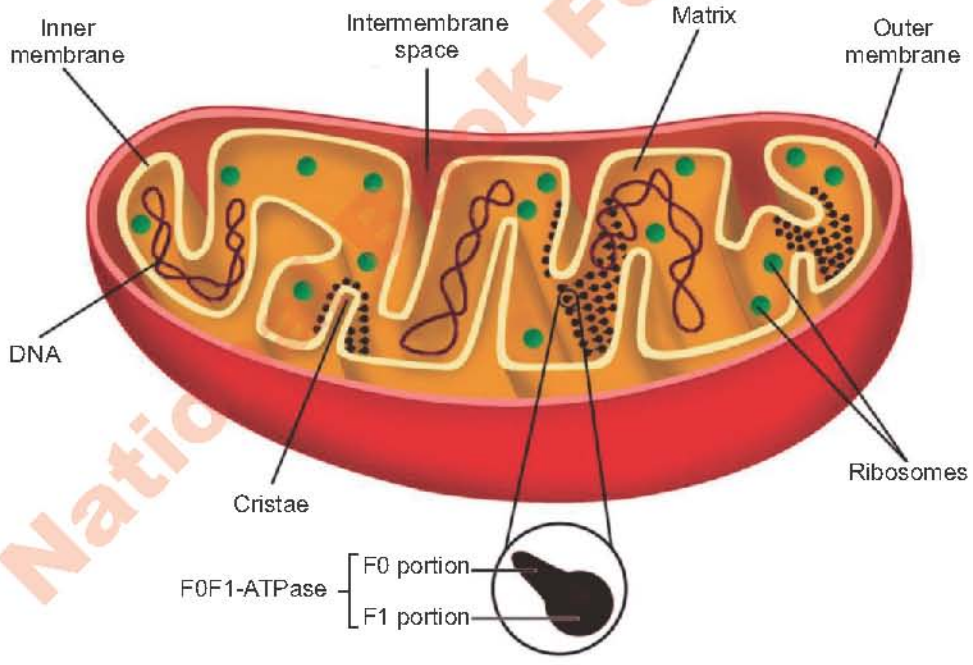

Fig. 1.25: Mitochondrion structure

Mitochondria are the sites of **cellular respiration**, the metabolic process that uses oxygen to generate ATP by extracting energy from sugars, fats, and other organic compounds. Enzymes in the matrix catalyze some of the steps of cellular respiration like Krebs cycle. Other proteins that function in ATP generation through electron transport chain are found into the inner membrane.

Mitochondria (extra reading material)

Mitochondria and chloroplasts display similarities with bacteria like both are self-replicating organelles, both have their own genetic system and metabolic machinery i.e., both has small circular DNA, all kinds of RNA and ribosomes (70S). An interesting fact about them is that they are capable to survive outside the cell in artificial medium if carefully fractionated. Based upon these observations' evolutionists believe that they were independent organisms and the early ancestor of eukaryotic cells engulfed them. Eventually, the engulfed cells formed a relationship with the host cell in which they were enclosed, becoming an *endosymbiont* (a cell living within another cell). Therefore, they are supposed as organisms within organism. Mitochondria divide and in this way their number doubles before cell division. Lysosomes regulate the number of mitochondria. Excess of mitochondria are digested by lysosomes. Because mitochondria are contained within ova (egg cells) but not within the heads of the sperm cells, all the mitochondria in a fertilized egg are derived from mother.

Plastids

On the basis of presence or absence and type of pigments, and the stage of development, plastids have been classified into proplastids, leucoplasts, chromoplasts and chloroplasts.

Proplastids are young, immature and developing plastids. They are self-replicating organelles. There are three types of plastids.

Leucoplasts are colourless, found in parenchyma cells of root, stem and seeds. **Chromoplasts** synthesize different coloured pigments other than green. **Chloroplasts** are found in green parts of the plants and act as site of photosynthesis.

Structure and functions of chloroplast

Chloroplast is a discoid structure which consists of three parts i.e., envelope, stroma and thylakoids. Each chloroplast is bounded by a smooth double membrane (envelope). The outer membrane like mitochondria contains porins and therefore is freely permeable to small molecules. The inner membrane is semipermeable and rich in protein. Between the outer and inner membrane there is intermembrane space.

The ground mass of chloroplast is called **stroma**. It is the colourless proteinaceous substance which like mitochondrial matrix also contains a small circular DNA, all kinds of RNA, ribosomes (70S) and various enzymes. The stroma contains a system of chlorophyll bearing double membrane, flattened sac-like structures called **thylakoids**. There are two types of thylakoids: smaller thylakoids and the larger thylakoids. **Smaller thylakoids** are disc like sacs which are piled over one another like stack of coins. Each stack of smaller thylakoids is called **granum** (plural: *grana*). Each granum consists of 25-50 thylakoids and there are about 40 - 60 grana found in each chloroplast. Photosynthetic pigments are also found in the membranes of smaller thylakoids. **Larger thylakoids** connect the grana with each other and are also called **intergrana**. These membranes are colourless as they do not have pigments.

Chloroplast is the site of photosynthesis in a plant cell. The first phase of photosynthesis is light dependent reaction in which sunlight is captured and transformed into ATP. This phase takes place in grana region of chloroplast. The second phase of photosynthesis is light independent reaction (dark reaction) in which CO_2 is reduced to make carbohydrates. The enzymes for this activity are found in stroma region of chloroplast.

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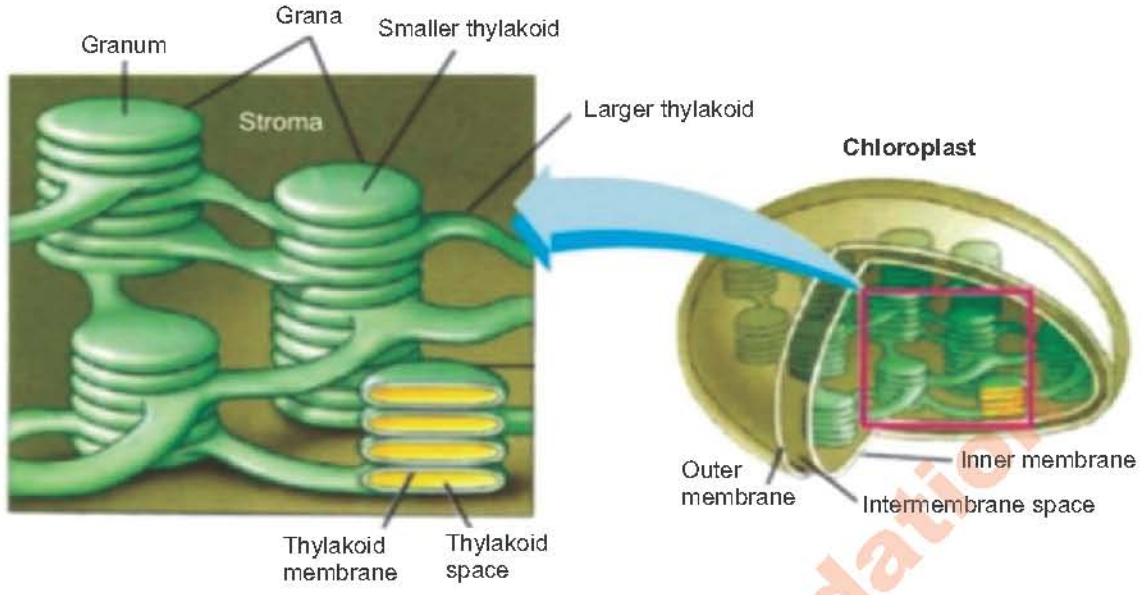

Fig. 1.26: Chloroplast

Centrioles

Centrioles are non-membranous cell organelles found mainly in animal cells. They are also found in fungi like protists such as slime molds and water molds. Centrioles are rod shaped structures and usually occur in pairs. These occur at right angle to each other near one pole of the nucleus. Each centriole is composed of nine triplets of microtubule which are circularly arranged around a central axis.

Fig. 1.27: Centrioles

Just before the cell division, the pair of centrioles duplicates and becomes two pairs which later on migrate to the opposite sides of the nucleus. Both centriole pairs give rise microtubules (spindle fibres) during cell division. The whole structure of spindle fibres is known as **mitotic apparatus** which helps in the distribution of chromosomes between the daughter cells during cell division. In addition, centrioles also give rise to **basal bodies** of cilia and flagella.

Cytoskeleton

The term cytoskeleton is generally applied to three different kinds of fibrous structures which are distributed from nucleus to the plasma membrane throughout the cytoplasm of a eukaryotic cell. These fibres include: microfilaments, microtubules, and intermediate filaments.

1.6.3 Nucleus

Nucleus is the most prominent and the most important part of a cell. In animal cells it is found in the centre (with exception of muscle fibre cells)

Science Titbits

Sieve tube cells in plants and red blood cells in human are exceptional living cells that do not possess nucleus. On the other hand some cells have more than one nuclei i.e., binucleate or dikaryotic cells (cells having two nuclei) and multinucleate or coenocytic cells (cells having many nuclei).

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but in adult plant cell it is slightly away from the centre due to the presence of a large central vacuole. A typical eukaryotic nucleus consists of nuclear envelope, nucleoplasm, nucleoli and chromatin.

Nuclear Envelope

Nuclear envelope (also called nuclear membrane) is a double membrane covering which makes the boundary of nucleus. Both membranes of nuclear envelope are separated by a fluid-filled **perinuclear space**. The membranes are composed of lipid bilayer and proteins. The outer membrane of nuclear envelope is covered with ribosomes and is connected with the membranes of ER. There are numerous pores in nuclear envelope called **nuclear pores** which are composed of a specialized transport protein called **nucleoporin**.

At the point of nuclear pore both the membranes are interconnected. These pores regulate the nucleo-cytoplasmic exchange of materials. This exchange includes RNA and ribosomal proteins moving from nucleus to the cytoplasm and proteins (such as DNA polymerase), carbohydrates, signaling and moving lipids into the nucleus. Although smaller molecules simply diffuse through the pores, larger molecules may be recognized by specific signal sequences and then be diffused with the help of nucleoporin into or out of the nucleus.

Nucleoplasm

Nucleoplasm is the transparent semifluid ground substance formed of a mixture by proteins, enzymes (DNA and RNA polymerase), free nucleotide and some metal ions (Mg) for the synthesis of DNA and RNAs. It also contains histone and non-histone protein. So the nucleoplasm is slightly different from cytoplasm.

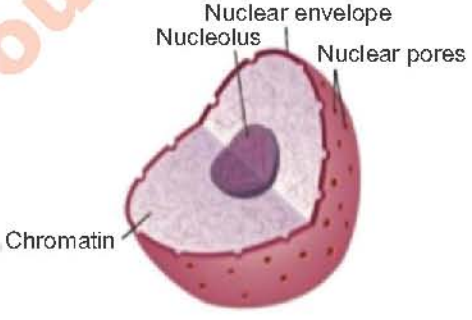

Fig. 1.28: Nucleolus

Nucleolus

Nucleolus is a non-membrane bound structure in the nucleoplasm. A cell may have one or more **nucleoli**. Nucleolus appears during interphase and disappears during cell division. A nucleolus consists of a peripheral granular area (contains ribosomal subunits) and a central fibriler area (contains rRNA and rDNA). Therefore, nucleolus is involved in the construction of ribosomes.

Chromatin and Chromosomes

Chromatin is a network of thin thread like structures made up of DNA and proteins. During cell division **chromatin** fibers begin to condense and coil up into separate structures called **chromosomes**, which are thick enough to be seen under a light microscope. A typical chromosome consists of two strands called **chromatids** which are attached with each other at a

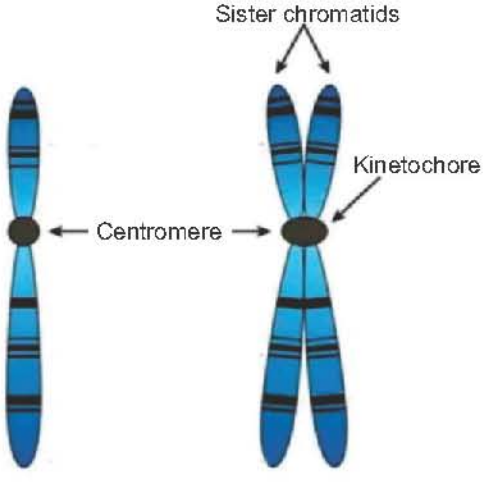

Fig 1.29. Chromosome

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point known as **centromere.** A centromere is a constriction functionally related to the movement of chromosomes during cell division. Each centromere has a complex **kinetochores** protein present on opposite sides of the constriction.

1.9 PROKARYOTIC AND EUKARYOTIC CELLS

Two kinds of structurally different cells have been evolved overtime. Prokaryotic cells include archaea, bacteria and cyanobacteria whereas all other forms of life are composed of eukaryotic cells.

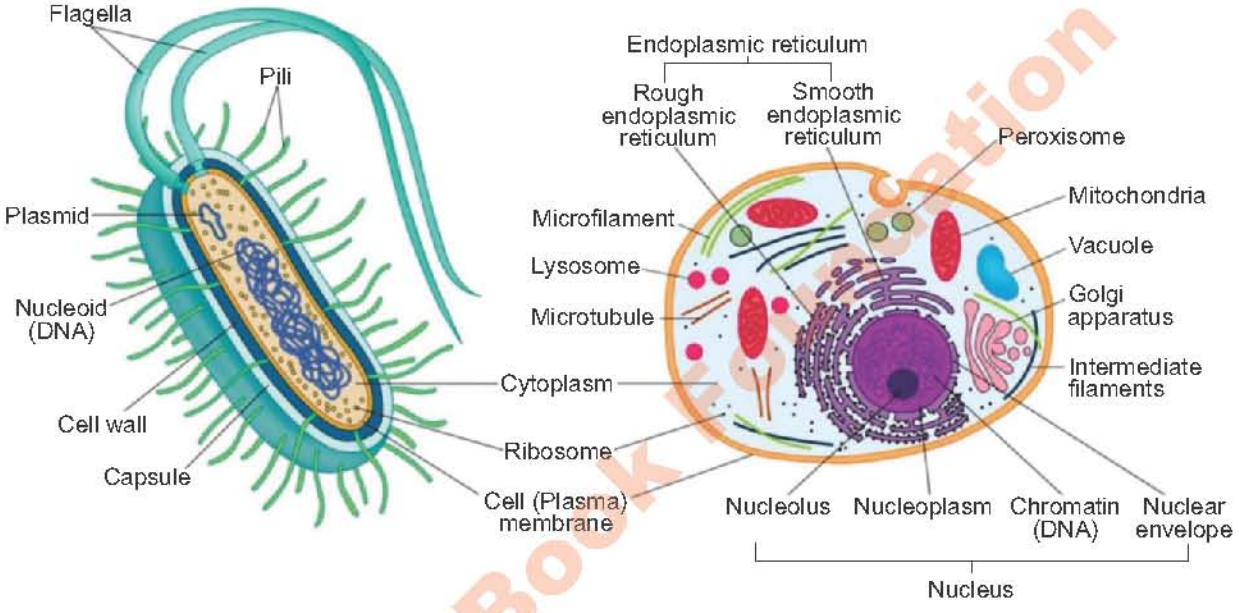

Fig 1.30. Prokaryotic and Eukaryotic Cell

Table 1.2 Differences between Prokaryotic and Eukaryotic cells

Prokaryotic cells	Eukaryotic cells,
1. Nucleus and other membrane bound organelles are absent.	1. Nucleus and other membrane bound organelles are present.
2. Prokaryotes are always unicellular.	2. Eukaryotes are unicellular and also multicellular organisms.
3. DNA is stored in the cytoplasm	3. DNA is stored within the nucleus.
4. Prokaryotic cells have one primary circular chromosome and various plasmids.	4. In eukaryotic cells DNA is stored in double stranded chromosomes.
5. Ribosomes in prokaryotic cells are 70S with 30S and 50S subunits.	5. Ribosomes in eukaryotic cells are 80 S with 40S and 60S subunits.

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6. Locomotive structures are composed of repeated flagellin, a hook and a motor complex.	6. Locomotive structures are composed of dynein and plasma membrane.
7. Prokaryotes divide by binary fission.	7. Eukaryotes divide by mitosis and meiosis.

1.10 STEM CELLS

Stem cells are cells with the potential to develop into many different types of cells in the body. Under the right conditions in the body or a laboratory, stem cells divide to form more cells called daughter cells.

> Stem cells are so named because they can either divide to produce more copies of themselves or differentiate into more specialized types of cells.

These daughter cells become either new stem cells or specialized cells (differentiation) with a more specific function, such as blood cells, nerve, cells, liver cells, heart muscle cells or bone cells. No other cell in the body has the natural ability to generate new cell types.

A stem cell can be defined as "It is a cell with the unique ability to develop into specialized cell types in the body."

Advantages of using stem cells: New cells are provided by the stem cells as they grow. They replace the specialized cells that are damaged or lost.

They can divide again and again to produce new cells. As the stem cells divide they can change into the other types of cell that make up the body.

Benefits of stem cell therapy are in the treatment of spinal cord injuries, type 1 diabetes, Parkinson's disease, Alzheimer's disease, heart disease, stroke, burns, cancer and osteoarthritis etc.

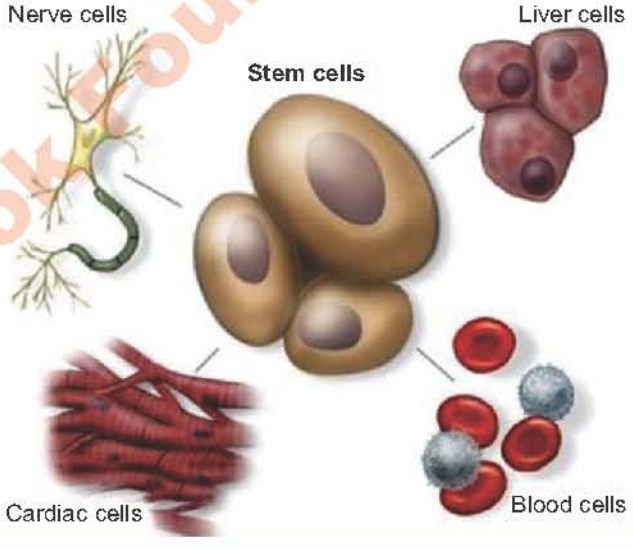

Fig. 1.31 Stem cells: The body's master cells

Different types of Stem Cells

There are several different types of stem cells, each with its own unique properties and applications in medical research and practice. Here we will discuss the three main types of stem cells: (1) Embryonic stem cells (2) Adult stem cells (3) Induced pluripotent stem cells.

1. **Adult stem cells:** These cells supply new cells as an organism grows and to replace cells that get damaged. Adult stem cells are multipotent, which means they can only change into some specific cells in the body, not any cell.
2. **Embryonic stem cells (ESCs):** These cells provide new cell for a growing embryo. They are called pluripotent, which means they can naturally produce every type of cell in the body.
3. **Induced pluripotent stem cells (iPSCs):** The stem cells that scientists make in the laboratory are called induced pluripotent stem cells. They can develop into any cell type.

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Types of stem cells

Researchers categorize stem cells, according to their potential to differentiate into other types of cells. The full classification includes:

Totipotent: These stem cells can differentiate into all possible cell types. The first few cells that appear as the zygote starts to divide are totipotent.

Pluripotent: These cells can turn into almost any cell. Cells from the early embryo are pluripotent.

Multipotent: These cells can differentiate into a closely related family of cells. Adult hematopoietic stem cells, for example, can become red and white blood cells or platelets.

Oligopotent: These can differentiate into a few different cell types. Adult lymphoid or myeloid stem cells can do this.

Unipotent: These can only produce cells of one kind, which is their own type. However, they are still stem cells because they can renew themselves. Examples include adult muscle stem cells.

Advantages of induced pluripotent stem cells

The advantages are: (1) Ability to give rise to almost any cell types desired. (2) Avoidance of ethical concerns associated with human ESCs (3) Induced pluripotent stem cells generated from the patient themselves could be used to grow new organs that would have a lower risk of being rejected.

iPSCs were first described by Shinya Yamanaka and his colleagues at Kyoto University in 2006. They were jointly awarded the 2012 Nobel Prize in Physiology or Medicine for their work.

Disadvantages of induced pluripotent stem cells

For human iPSC production, viruses were initially used as delivery vectors for the genes required for cell reprogramming.

The main issue is the use of retroviruses to generate iPSCs as they are associated with cancer. More specifically, retroviruses can insert their DNA anywhere in the genome and subsequently trigger cancer-causing gene expression. In addition, the risk of the overgrowth of the transplanted cells, or, in other words, cancer, continues to be a major hurdle.

How to generate induced pluripotent stem cells?

Signals in the body tell a cell what type of specialized cell it should be by switching some genes on and some genes off.

To generate induced pluripotent stem cells, scientists re-introduce the signals that normally tell stem cells to stay as stem cells in the early embryo. These switch off any genes that tell the cell to be specialized, and switch on genes that tell the cell to be a stem cell.

The benefits of iPSCs are catching on, which is setting the market on a course of continued expansion. BCC Research predicts the global market for induced pluripotent stem cells to grow from $2.8 billion in 2021 to $4.4 billion by 2026, at a compound annual growth rate of 9.3%.

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1.11 ENDOCYTOSIS AND EXOCYTOSIS

The **active, bulk transport** of products across the cell membrane are called endocytosis and exocytosis.

Exocytosis and endocytosis are two processes that allow large molecules, bacteria, and waste materials that cannot diffuse through the lipid bilayer to cross the cell membrane.

Endocytosis

Endocytosis is the process by which substances are **engulfed** within the cell membrane, which then forms a **vesicle** containing the ingested material.

The two main subtypes of endocytosis are Phagocytosis and Pinocytosis.

Phagocytosis

Phagocytosis is the process through phagocytes, or liver cells ingest or consume other cells or particles. A phagocyte may be Amoeba or white blood cells. Some species e.g., sponges, *Amoeba* have a way of feeding called phagocytosis. Extensions of the cytoplasm, termed **pseudopodia** ('false feet'), sense, surround and enclose the target, creating a **vacuole or phagosome** on the inside of the cell membrane. In higher animals phagocytosis is primarily a defensive mechanism to infection and antigens.

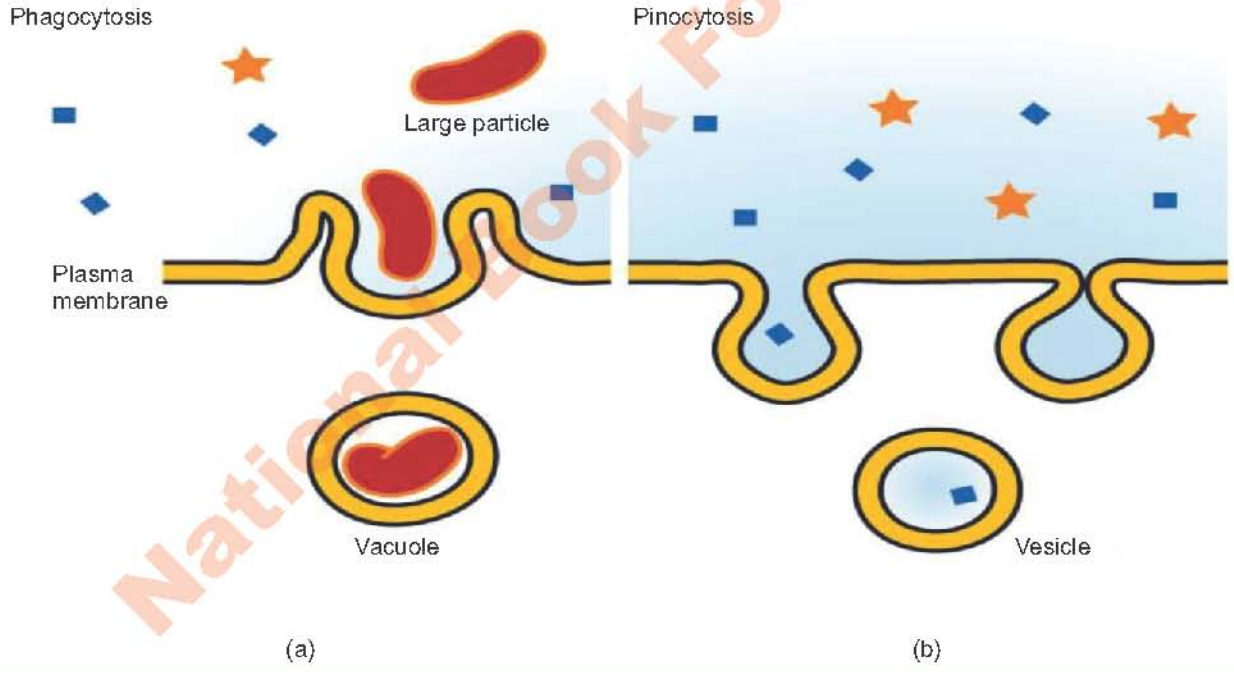

Fig. 1.32: Endocytosis

Pinocytosis

Cell drinking is also known as pinocytosis. It is widespread in plant and animal cells. Through this process cells take in nutrients such as ions, enzymes and hormones. The cell membrane invaginates before budding off to form a vesicle called **pinosome.**

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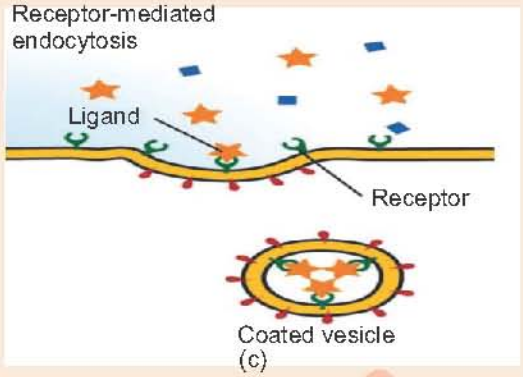
Receptor mediated endocytosis is a kind of pinocytosis. Macromolecules bind to receptors on the surface of the plasma membrane during this process.

The example of cell mediated pinocytosis is cholesterol absorption. Receptors cluster in regions termed coated pits, as they are coated with proteins causes the coated pit to invaginate and become a coated vesicle, bringing the desired ligand into the cell.



Exocytosis

Exocytosis is the process by which the transfer of material takes place from inside of the cell to the outside of the cell. It is an active transport. Vesicles are packaged within the cell and transported to the cell membrane, where their **phospholipid bilayers** fuse. This allows the contents to be released outside the cell. Exocytosis is used in many areas of the body, including neurotransmitter release at synapses or release of secretions in the sweat glands.

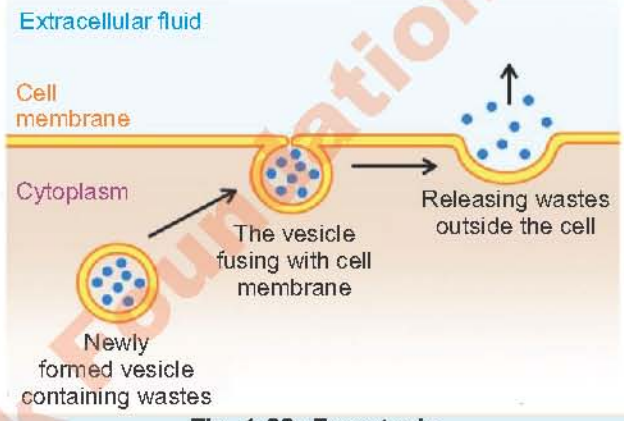


Fig. 1.33: Exocytosis

1.12 CELL DIVISION

The continuity of life is based on the reproduction of cell or cell division. The dividing cell is called parent cell and the two new cells formed are called daughter cells. The two main types of cell divisions are mitosis and meiosis. The somatic cells divide by mitosis and the germ line cells divide by meiosis.

Cell Cycle

The cell cycle is a series of stages a cell passes through, to divide and produce new cells. The process of cell division can be divided into two main phases, mitosis and cytokinesis. **Mitosis** is the process of division of the nucleus. **Cytokinesis** is the process of division of cytoplasm.

Phases of Cell Cycle

Mitosis is only a part of the cell cycle. The **mitotic (M) phase** includes both mitosis and cytokinesis. The M phase is the

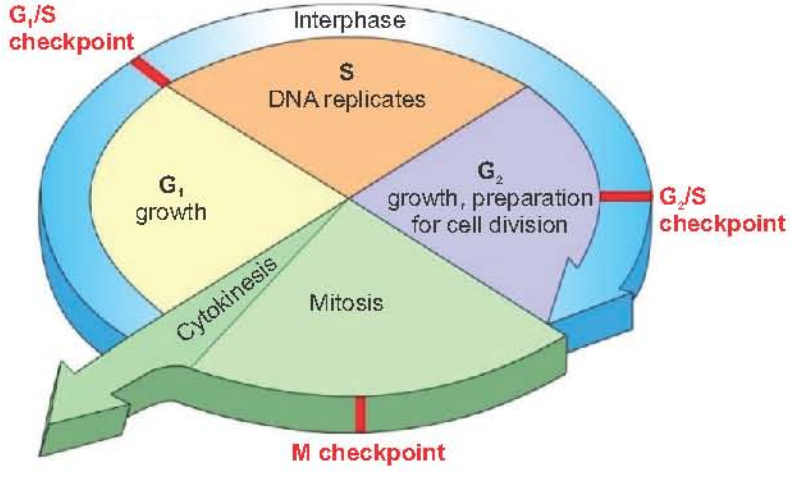


Figure 1.34 Cell cycle

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shortest part of the cell cycle. Mitotic cell division alternates with a much longer stage called **interphase**. This phase can be divided into three sub-phases: the **G_1 phase** (first gap), the **S phase** (synthesis) and the **G_2 phase** (second gap). During the sub-phases the cell grows by producing proteins and cytoplasmic organelles. Chromosomes are duplicated only during the S phase. Thus a cell grows (G^1), continues to grow as it copies its chromosomes (S), grows more as it completes preparation of cell division (G2), and divides. The daughter cells may then repeat the cycle.

Cell cycle checkpoints are control mechanisms that ensure the normal course of the eukaryotic cell cycle. In the cell cycle, there are three major checkpoints - the G1 checkpoint, the G2 checkpoint, and the spindle checkpoint. At the G1/S transition, the G1 checkpoint occurs.

MITOSIS

The word mitosis comes from the Greek word 'mitos' which means thread and refers to the threadlike appearance of chromosomes during this period. Mitosis can be defined as "The division of the cell in such a manner that the chromosomes are duplicated and distributed equally to the daughter cells".

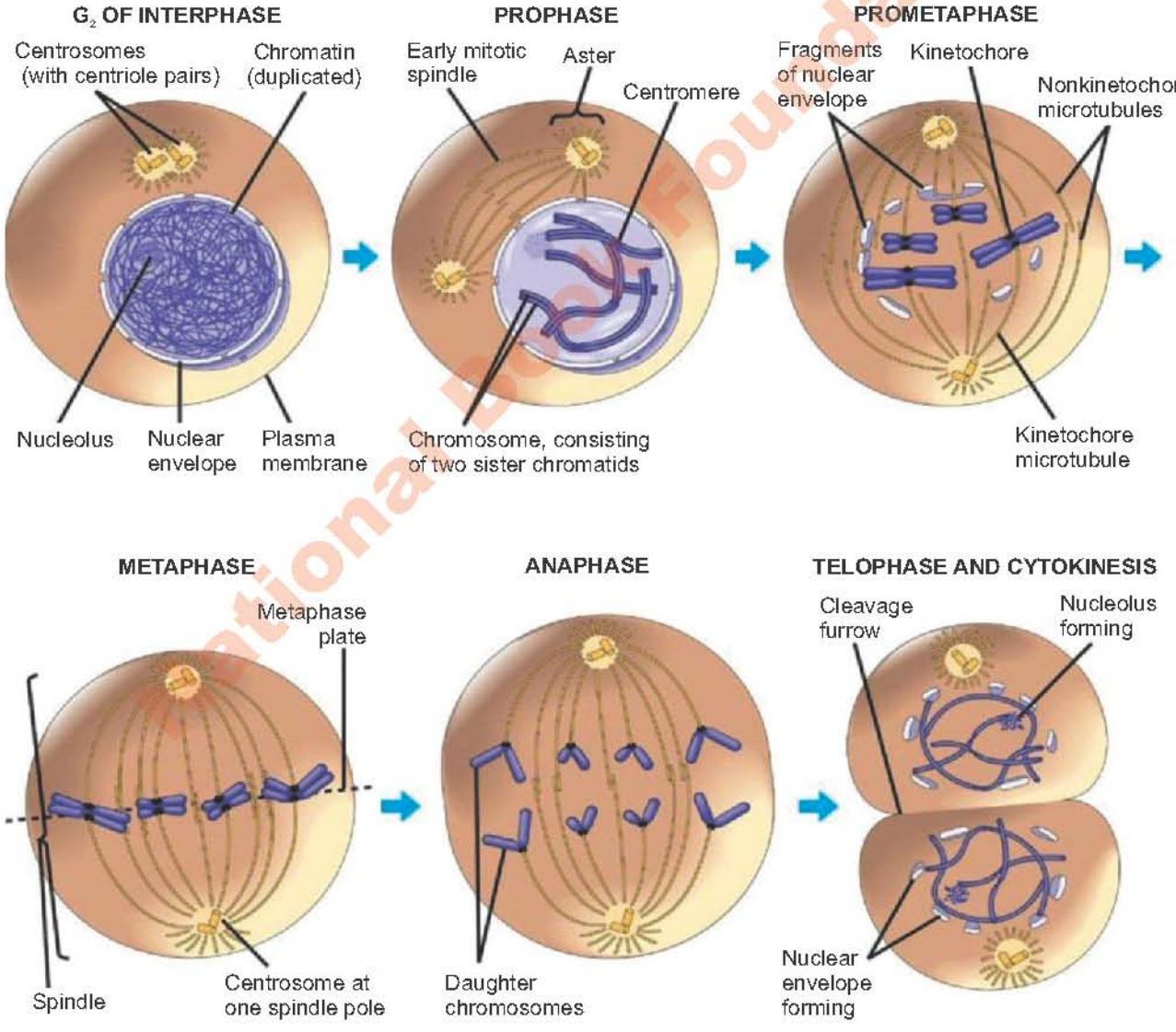

Figure 1.35 Stages of mitosis

Mitosis is a continuous process that is arbitrarily divided into five phases for the convenience of description: prophase, prometaphase, metaphase, anaphase, and telophase.

Interphase

A nuclear envelope surrounds the nucleus. The nucleus contains one or more nuclei. By the replication of a single centrosome two centrosomes are formed. Each centrosome has two centrioles. The chromosomes that duplicated during S phase, are not visible under a light microscope as they have not yet condensed.

Prophase

The chromatin fibers become more tightly coiled. They condense into discrete chromosomes which are observable with a light microscope. Each duplicated chromosome appears as two identical **sister chromatids** joined together. The nucleoli disappear. The mitotic spindle begins to form. It is composed of centrosomes and the microtubules that extends from them. The radial arrays of shorter microtubules that extends from the centrosomes are called **asters** (stars). The centrosomes move away from each other. It is due to the lengthening of the microtubules between them.

Prometaphase

During the formation of spindle, the nuclear membrane breaks. The microtubules of the spindle can now invade the nuclear area and interact with the chromosome, which have become even more condensed. Microtubules extend from each centrosome towards the middle of the cell. Each of the two chromatids of a chromosome now has a **kinetochore**. Some of the microtubules attach to the kinetochore becoming 'kinetochore microtubules'. Non kinetochore microtubules interact with those from the opposite pole of the spindle.

Metaphase

It is the longest stage of mitosis, lasting about twenty minutes. The centrosomes are now at opposite ends of the cell. The chromosomes arrange on the metaphase plate, and imaginary plane that is equidistant between two poles of the spindle. The centromeres of the chromosomes lie on the **metaphase plate**. For each chromosome, the kinetochores of the sister chromosome are attached to kinetochore microtubules coming from opposite poles. The entire apparatus is called the **spindle** because of its shape.

Anaphase

It is the shortest stage of mitosis, lasting only a few minutes. It begins when the two sister chromatids of each pair suddenly part. Each chromatid thus become a full-fledged chromosome. The two liberated chromosomes begin to start moving towards opposite ends of the cell, as their kinetochore microtubules shorten. Because these microtubules are attached at the centromere region, the chromosomes move centromere first. The cell elongates as the **nonkinetochore microtubules** lengthen. By the end of the anaphase, the two ends of the cell have equivalent and complete collection of chromosomes.

Telophase

The two daughter nuclei begin to form in the cell. Nuclear envelopes arise from the fragments of the parent cell's nuclear envelope and other portions of the endomembrane system. The chromosome becomes less condensed. Mitosis, the division of one nucleus into two genetically identical nuclei, is now complete.

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Cytokinesis

The division of the cytoplasm is usually well underway by the late telophase, so the two daughter cells appear shortly after the end of mitosis. Commonly the nuclear division is followed by separation of the cytoplasm into two parts. This separation is accomplished by pinching of the cell membrane. The pinching near the middle of an animal cell forms a **cleavage furrow.** The process of cytoplasmic division is called cytokinesis. (Gk:*cyto*; cell *kinesis*; movement). Cell organelles are distributed to the two daughter cells.

Significance of mitosis

It is important for growth and multiplication of cells. It ensures that the two daughter cells inherit the same number of chromosomes, and hence the same characteristics as the parent cells. Maintain the continuity of metabolism by transmitting to the daughter cells, exactly the same information as is coded in the DNA of the parent cell. Plays a significant role in wound healing, regeneration of damaged parts, and replacement of cells lost during normal wear and tear. May give rise to tumour or cancerous growth if the process goes out of control.

MEIOSIS

The process where a single cell divides twice to produce four cells containing half the original amount of genetic information is called meiosis. Sperms and eggs are produced by meiosis in animals. Spores are produced by meiosis in plants.

The stages of meiosis

Meiosis is a continuous process. It can be described most easily by dividing it into two arbitrary stages. The two stages of meiosis I and meiosis II.

The first Meiotic Division

It consists of: Interphase I, Prophase I, Metaphase I, Anaphase I, Telophase.

Interphase I

The DNA in the cell is copied as result two identical full sets of chromosomes are formed. There are two centrosomes outside the nucleus, each containing a pair of centrioles. During interphase, microtubules extend from these centrosomes.

Prophase I

It is lengthy process of meiosis. Each chromosome has two chromatids. Chromosomes look like interweaving threads. Due to condensation of chromatin material, chromosomes become apparent and distinct, nucleus increases in size. Initially the thin chromosomes become shorter and thicker. The chromosome number of the cell is seen. Homologous chromosomes start getting closer to each other. In the cell there are two of each type of chromosome. Identical chromosomes are called **homologous chromosoovs.** The homologous chromosomes begin to pair length wise with its homologue. The process of pairing is called **synapsis** (*si-nap-sis*). The synapsis may start from any point with the corresponding complementary DNA strand of the other.

Each pair of synapsed chromosome consists of four chromatids, two centromeres and is called a **tetrad or bivalent.** The chromatids of the homologues may cross each other and the point of crossing is X shaped figure under the light microscope. It is called **chiasma** (plural:

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chismata).Chromosome segment is exchanged between the two homologous chromosome at the chiasma and is called **crossing over.**

The paired homologous chromosomes begin to separate by repelling. The **tetrad** are more evenly distributed in the nucleus. The nucleoli disappear. The nuclear membrane is still present. The **diad** (the two chromatids attached to a single centromere is called diad) behaves as a single unit because they are held together by a common centromere, throughout the meiosis.

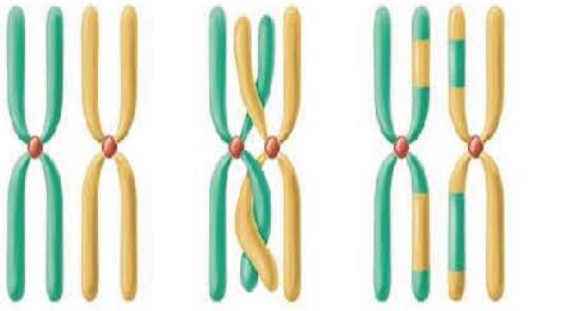

Fig. 1.36 Crossing Over

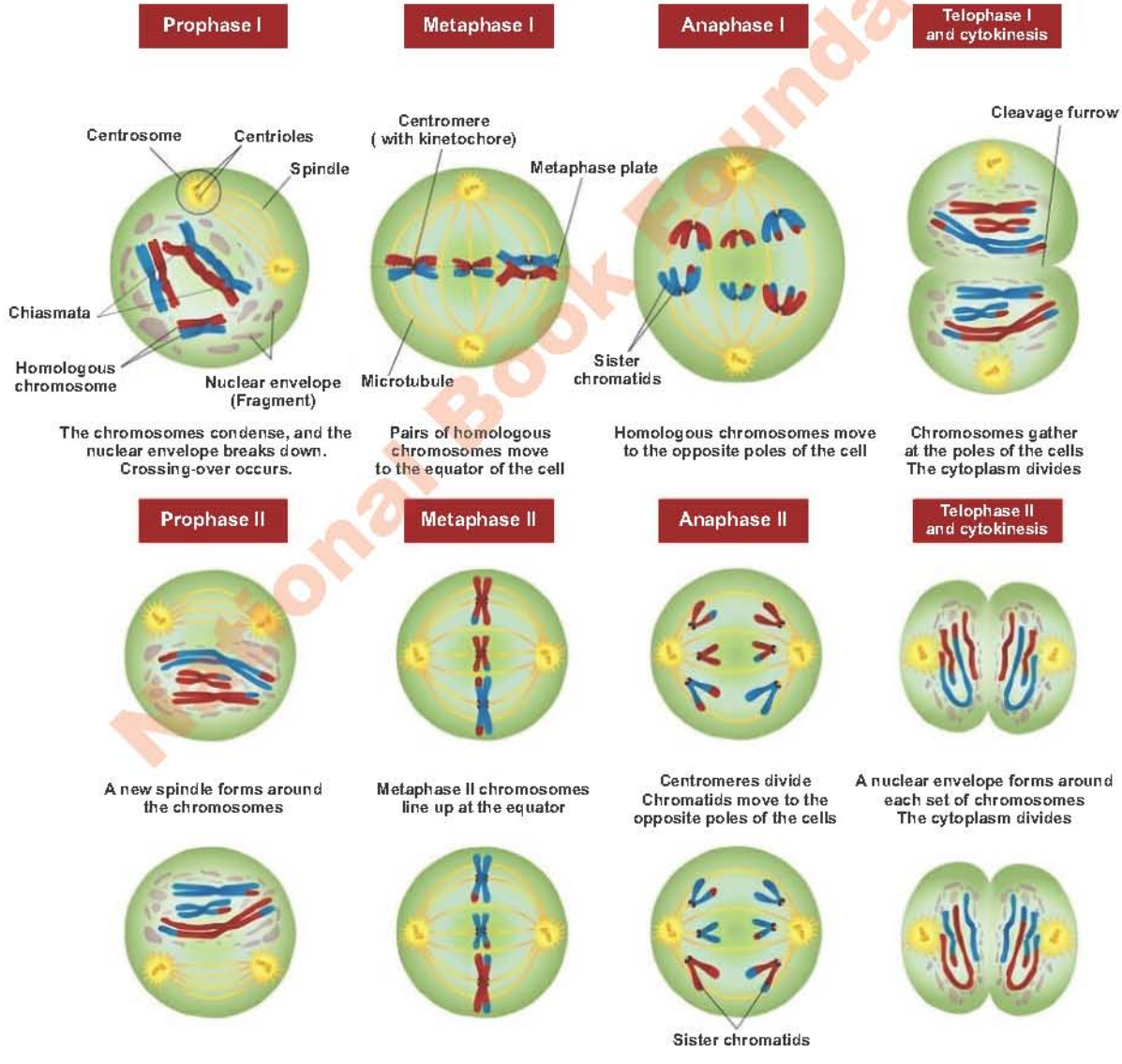

Figure 1.37. Stages of meiosis

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Metaphase I

The nuclear membrane disintegrates. The microtubules form the spindle. The chromosome line up in double row i.e., pair on the equator. Microtubules bind only to one kinetochore of each centromere. The centromere of one homologue becomes attached to microtubules extending to one pole, whereas the centromere of the other homologue becomes attached to microtubules extending to other pole.

Anaphase I

The attachment of chromosomes to the spindle is complete. The microtubules that are attached to the homologous chromosomes begin to slide over one another, as a result the spindle poles move apart. The spindle fibers become shorter as the spindle fibers are dragged to the poles. They are broken by the action of enzymes. With the shortening of the spindle fibers, the chromosomes that are attached to the spindle are also pulled towards each pole. When the shortening of the spindle fibers is completed, each pole has single set of chromosomes, consisting of one member of each homologous pair.

Telophase I and cytokinesis

The chromosomes complete their movement to the opposite poles of the cell. At each pole of the cell a full set of chromosomes gather together. A membrane forms around each set of chromosomes to create two new nuclei. The single cell through the process of cytokinesis form two separate daughter cells, which are haploid.

The Second Meiotic Division

Meiosis II is simply a mitotic division. It occurs to separate the sister chromatids as in mitosis.

Interphase II: DNA does not duplicate in interphase II. It is very brief phase. Each of the two cells resulting from meiosis I progress into meiosis II very quickly.

Prophase II: The complicated nuclear events of prophase do not take place.

Metaphase II and Anaphase II: The chromosomes line up in the same fashion as they did in metaphase I. Here again a random distribution of chromosomes take place. The **centromere** divides, as result the two **chromatids** are separated. As each chromatid is now a separate structure they are called **chromosomes.** Chromosomes are distributed in equal number, to each pole.

Telophase II: The nuclei are reconstructed in the typical manner. Each nucleus now contains haploid set of chromosomes, because DNA has duplicated only once during the cell division.

Significance of meiosis: the number of chromosomes become half in the gametes due to which the number of chromosomes remain constant from generation to generation. It provides mechanism for the transmission of genetic material from one generation to the next. It provides genetic variability. It offers a chance for the production of best adapted individual and better chance for survival in the changed environment. It provides raw material for evolution.

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EXERCISE

Section I: Multiple Choice Questions

Select the correct answer:

1. Which of the following is the major advantage of using a light microscope instead of an electron microscope?

   A) superior resolving power
   B) constant depth of focus
   C) observation of living matter
   D) use of very thin sections

2. Some cellular organelles are bound by a single membrane, while other organelles have two membranes (envelopes) around them. Which one of the following is correct?

	Single membrane	Double membranes
A)	peroxysomes, lysosome	nucleus, chloroplast
B)	chloroplast, lysosome	nucleus, peroxysomes
C)	nucleus, chloroplast	lysosome, peroxysomes
D)	nucleus, lysosome	chloroplast, peroxysomes

3. Which of the following cell structures contains the highest concentration of RNA?

   A) centriole B) lysosome C) chromosome D) nucleolus

4. A tadpole's tail is gradually broken down during metamorphosis into an adult frog. Which organelle increases in number in the cells of the tail at this time?

   A) centriole
   B) endoplasmic reticulum
   (C) Golgi complex
   (D) lysosomes

5. Which of the following organelles always contains DNA?

   A) centriole B) Golgi complex C) lysosome D) mitochondria

6. Which distinguishes a prokaryotic cell from a eukaryotic cell?

   A) prokaryotic cell have a cell wall and a nucleus
   B) prokaryotic cells have no membrane bound organelles
   C) prokaryotic cells have a centriole
   D) prokaryotic cells have no ribosomes

7. The elasticity of the plasma membrane demonstrates that it is made up in part of

   A) lipids B) nucleic acids C) carbohydrates D) proteins

8. The cell wall of plant cell is different from that of prokaryotes in:

   A) both structure and chemical composition
   B) structure only
   C) chemical composition only
   D) number of layers only

9. Which of the following are present in prokaryotic cells:

   A) chloroplast, DNA, nuclear envelope

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B) chromosomes, mitochondria, nuclear envelope

C) cytoplasm, DNA, mitochondria

D) cytoplasm, DNA, ribosome

10. Which of the following is present in all eukaryotic cells:

A) cell wall B) diploid nucleus

C) flagellum D) membrane bounded organelles

11. Which of the following would be more prominent in a secretory cell than non-secretory cell:

A) lysosome B) Golgi complex C) mitochondrion D) ribosome

12. When a glycoprotein is being synthesized for secretion from a cell, which route is it most likely to take?

A) Golgi complex → RER→ SER B) RER→ Golgi complex → SER

C) RER→ SER→ Golgi complex D) SER→ Golgi complex → RER

13. In what way do the various membranes of a eukaryotic cell differ?

A) Phospholipids are found only in a certain membrane.
B) Certain proteins are unique to each membrane.
C) Only certain membranes of the cell are selectively permeable.
D) Some membranes have hydrophobic surfaces exposed to the cytoplasm.

14. Which of the following processes includes all others.

A) osmosis
B) diffusion of a solute across a membrane
C) facilitated diffusion
D) passive transport

15. Which of the following does not occur in mitosis?

A) condensation of the chromosomes
B) replication of the DNA
C) separation of sister chromatids
D) spindle formation

16. Cell signaling is the ability of a cell to:

A) Receive signal from the environment
B) Transmit signal to other cells
C) Process signal within the cell
D) All of the above

17. Which of the following stem cells are known as true stem cells?

A) totipotent cell

B) germipotent cell

C) non-potent cells

D) pluripotent cells

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18. The fundamental property of stem cells is it does not contain any .....Structure which allows stem cells to perform specialized function.

A) nucleus B) cytoplasm C) tissue D) DNA

Section II: Short Answer Questions

1. Describe that the cells are the basic unit of life with respect to seven properties of life.
2. What is the difference between the resolution and magnification of light microscope and electron microscope?
3. Name three organelles revealed by an electron microscope.
4. Why cell wall is not present in animal cells?
5. What holds the ribosomes together in a polysome?
6. What would happen if there are no lysosomes in human cells?
7. Why lysosomes are called suicidal bags?
8. Name the structures and organelles which are common in plant cell, animal cell and a prokaryotic cell.
9. How is a chloroplast similar to a bacterium?
10. Name the organelles of eukaryotic cell and write their specific functions.
11. What are prokaryotic cells? List the structures missing in prokaryotic cells.
12. What organelles are single membrane bound, double membrane bound and lacking any membrane?
13. Compare the chemical composition of nucleoplasm with that of cytoplasm.
14. Explain that nucleoli are the areas where ribosomes are assembled.
15. Draw a labelled diagram of a section through:
    (a) mitochondrion (b) chloroplast
16. Write the difference between:
    (a) resolution and magnification
    (b) plant cell wall and bacterial cell wall
    (c) cytoplasm of eukaryotic and prokaryotic cell
    (d) rough ER and smooth ER
    (e) chromatin and chromosome
17. What are vesicles and what function they perform?
18. What are the techniques that can be used to study plasma membrane?
19. Define: cell signaling, magnification, resolution, stem cells, Pluripotent stem cells, endocytosis, phagocytosis, pinocytosis, and exocytosis.
20. What are the advantages of using stem cells?

Section III: Extensive Answer Questions

1. What are the locations, chemical compositions and significance of the following in a plant cell wall? (a) Primary cell wall (b) Secondary cell wall (c) Middle lamella.
2. Explain the (a) Chemical composition of plasma membrane (b) Role of plasma membrane in regulating cell's interactions with environment.
3. Describe the lipid composition and variety of proteins of the plasma membrane.
4. What are the functions of the plasma membrane proteins?

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5. What is the role of glycolipids and glycoproteins as the cell surface markers?
6. What is the chemical nature of cytoplasm? Explain the metabolic roles of cytoplasm.
7. Describe the structures and functions of smooth and rough endoplasmic reticulum
8. Explain the structure, chemical composition and function of ribosomes.
9. Explain the structure, and functions of Golgi complex.
10. Explain the structure, and functions of the peroxisomes and glyoxisomes in animal and plant cells.
11. Explain the formation, structure and functions of the lysosomes.
12. What are the storage diseases? Explain with reference to the malfunctioning of lysosomes.
13. Describe the external and internal structure of mitochondrion? What are the functions of these structures present in mitochondria?
14. Describe the external and internal structure of chloroplast? What are the functions of these structures present in chloroplast?
15. Compare and contrast the structure and functions of mitochondria and chloroplasts.
16. What are centrioles? Describe the structure, composition and functions of centriole.
17. What is nuclear envelope? Describe the chemical composition and structure of nuclear envelope.
18. What is the relationship of endoplasmic reticulum with Golgi complex, lysosome and plasma membrane?
19. What is cell theory? How to validate it? What are exceptions to it?
20. Compare and contrast the working of a light microscope and an electron microscope.
21. Describe the pathway of protein signal and steroid signal from outside of a cell to inside.
22. Categorize and explain different types of stem cells.
23. What are the advantages and disadvantages of using induced Pluripotent stem cells?
24. Explain the following with diagrams:
    a) Simple diffusion
    b) Facilitated diffusion
    c) Osmosis
    d) Active transport
25. Differentiate between prokaryotic and eukaryotic cells with diagram.
26. Compare and contrast simple and facilitated diffusion.
27. Explain endocytosis and exocytosis with diagram
28. Explain the stages of mitosis with diagram.
29. Explain the stages of meiosis with diagram

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Chapter 02

BIOLOGICAL MOLECULES

SLOs: After completing this lesson, the student will be able to:

1. Define biochemistry/molecular biology
2. Describe briefly the different types of bonds found in biology (hydrogen bonds, covalent bonds, interactions, ionic, hydrophobic and hydrophilic interactions etc.)
3. Distinguish carbohydrates, proteins, lipids and nucleic acids as the four fundamental kinds of biological molecules.
4. Describe and draw sketches of the condensation-synthesis and hydrolysis reactions for the making and breaking of macromolecule polymers.
5. State the properties of water (high polarity, hydrogen bonding, high specific heat, high heat of Vaporization, cohesion, hydrophobic exclusion ionization and lower density of ice) allow it to be the medium of life.
6. Define carbohydrates and classify them.
7. Compare and contrast the properties and roles of monosaccharides and write their formula
8. Compare the isomers and stereoisomers of glucose.
9. Distinguish the properties and roles of disaccharides
10. Describe glycosidic bonds in disaccharides.
11. Describe the structure properties and roles of polysaccharides starch, glycogen, cellulose and chitin.
12. Define protein, amino acid and recognized essential amino acid and structural formula of amino acid.
13. Outline the synthesis and breakage of peptide linkages.
14. Justify the significance of the sequence of amino acids through the example of sickle cell hemoglobin
15. Classify proteins as globular and fibrous proteins.
16. List the roles of structural proteins and functional proteins with 3 examples
17. Define lipids
18. Describe the properties and roles of acylglycerols, phospholipids, terpenes and waxes.
19. Illustrate the molecular structure (making and breaking) of an acylglycerol, a phospholipid and a terpene.
20. Write the chemical structure of a single phospholipid (Glycerol as a three carbon molecule, phosphate group, one unsaturated fatty acid tail and one saturated fatty acid tail). [SLO: B-11-E-19] [SLO: B-11-D-15]
21. Evaluate steroids and prostaglandins as important groups of lipids
22. Describe nucleic acids and molecular structure of nucleotides.

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23. Distinguish among the nitrogenous bases found in the nucleotides of nucleic acids.
24. Outline the examples of a mononucleotide (ATP) and a dinucleotide (NAD).
25. Illustrate the formation of phosphodiester bond
26. Explain the double helical structure of DNA as proposed by Watson and Crick.
27. Explain the general structure of RNA.
28. Distinguish in terms of functions and roles, the three types of RNA
29. Discuss the Central Dogma.
30. Define conjugated molecules and describe the roles of common conjugated molecules i.e. glycolipids, glycoproteins, lipoproteins and nucleoproteins.

You have got a very brief introduction about biological molecules in IX-X biology course. This chapter caters the detailed study of structure and roles of carbohydrates, proteins, lipids and nucleic acid as well as the importance of water and the role of conjugated molecules.

2.1 BIOCHEMISTRY/ MOLECULAR BIOLOGY

Biochemistry is the study of composition and structure of different chemical compounds found in living organisms and the chemical processes taking place within or related to living organisms.

Biochemistry is a sub-discipline of both biology and chemistry. Therefore, by using the knowledge and techniques of chemistry, biochemistry solves the problems faced by living organisms.

Sometimes, biochemistry and molecular biology are used interchangeably, but molecular biology is the specialized branch of biochemistry.

Science titbits

Study of biochemistry is greatly helpful to explore the cell biology and anatomy because all the structures of the living organisms (cells, tissues and organs etc.) have specific biochemical organization. Origin of life, evolution and variations in life forms are also discussed biochemically. Similarly, knowledge of biochemistry/molecular biology is essential for physiological studies of organisms because life processes such as photosynthesis, respiration, digestion, inheritance, muscle contraction are explained in biochemical terms.

2.2 DIFFERENT TYPES OF BONDS AND INTERACTIONS FOUND IN BIOLOGICAL MOLECULES

Various types of chemical bonds, intermolecular and intramolecular interactions play essential roles in the structural stability and functioning of biological molecules.

2.2.1 Chemical Bonds

Following are the two most prevalent types of chemical bonds found in biological molecules:

2.2.1.1 Covalent Bonds:

Covalent bond is formed by the mutual sharing of electrons between two atoms in order to get a stable electronic configuration. As biomolecules are mainly composed of non-metals like carbon, hydrogen, oxygen, sulphur, nitrogen and phosphorous thus covalent bonds are the most prevalent type of chemical bond found in them. Carbon-carbon bonds, carbon-hydrogen bonds, peptide bonds, glycosidic bonds, phosphodiester bonds, disulphide bonds, ester bonds, thioester bonds etc. are various types of covalent bonds found in carbohydrates, proteins, lipids, nucleic acids and vitamins.

2.2.1.2 Ionic Bonds

Ionic bond is formed between two atoms when one atom donates one or more electrons to become a positively charged ion while another atom gains these electrons to become a negatively charged ion.

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These oppositely charged ions are then held together by the strong electrostatic force of attraction between them.

Though ionic bonds are less common than covalent bonds in biological molecules but they still play some important roles. Salts are important part of biological systems and ionic bonding is the most common type of bonding found in them. Some of the areas where ionic bonds play important roles in the structure of biological molecules include, ionic bond between some amino acids in proteins, presence of negatively charged phosphate groups in DNA and other molecules. Ionic bonds are also present between metal ions and protein part of enzymes as well as in enzyme substrate interactions.

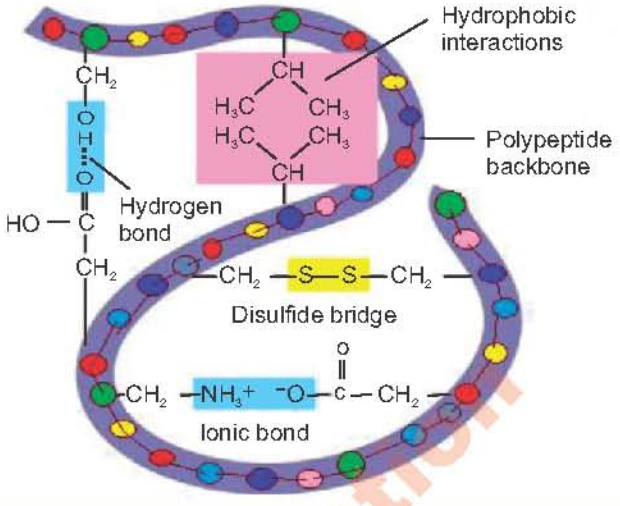

Fig: 2.1: Different types of bonds and interactions found in a protein molecule

2.2.2 Intermolecular and intramolecular interactions

Apart from chemical bonds, various types of weak interactive forces exist between molecules and between different parts of a same molecule which are known as intermolecular and intramolecular forces respectively. These interactions are of various types and various strengths and are very important in determining the physical properties of different substances. They play a crucial roles in biology, influencing the structure, function and behaviour of biological molecules and various cellular processes. Protein folding, double helical structure of DNA, structure of cell membrane, molecular recognition etc. would never be possible without these inter and intramolecular important interactions.

2.2.2.1 Hydrogen Bonding

It is a type of intermolecular force which occurs between a hydrogen atom, bonded to a highly electronegative atom i.e. oxygen, nitrogen or fluorine and a lone pair of electron present on a neighbouring electronegative atom. They may be intermolecular, i.e. between atoms of different molecules, or intramolecular, i.e. between atoms of a same molecule. Double helical structure of DNA is due to hydrogen bonds present between nitrogenous bases, i.e. three hydrogen bonds between cytosine and guanine and two hydrogen bonds between adenine and thiamine. Moreover, protein-DNA interactions and various other biological processes also involve hydrogen bonding.

2.2.2.2 Hydrophobic interactions

They are noncovalent interactions found between nonpolar molecules or nonpolar regions of molecules in the presence of water. The term "hydrophobic" literally means "afraid of water". As water is a polar molecule so the hydrophobic molecules or regions minimize their contact with water by clustering and coming together. This hydrophobic effect has several important biological implications like protein folding, membrane formation, ligand binding, protein-protein interactions etc.

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2.2.2.3 Hydrophilic interactions

The term "hydrophilic" literally means "water-loving". The molecules which show hydrophilic interactions are called as hydrophilic molecules which are found to be polar in nature, i.e. they have partially positive and partially negative regions/poles in them due to the uneven distribution of mutual shared electrons between the bonded atoms.

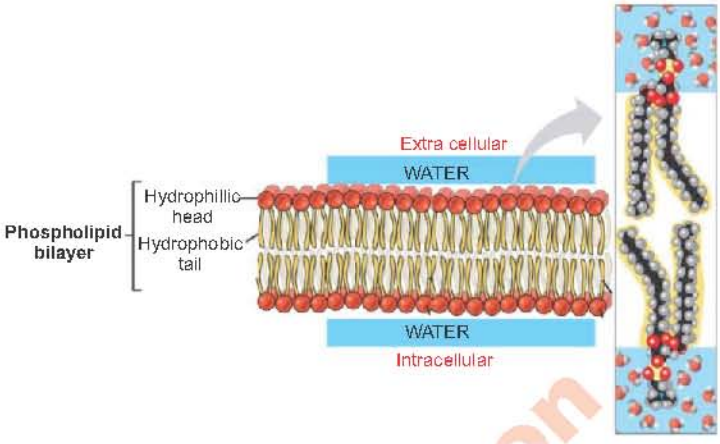


Fig: 2.2: Hydrophilic and hydrophobic interactions in cell membrane

Hydration is a hydrophilic process in which, when an ionic substance is placed in water, it dissociates into positive and negative ions, which are then surrounded by water molecules. This process helps in dissolution and stabilization of hydrophilic substances in aqueous solutions. Hydrophilic amino acid residues on the surface of proteins interacts with water and help in maintaining solubility and stability of proteins in cellular environments.

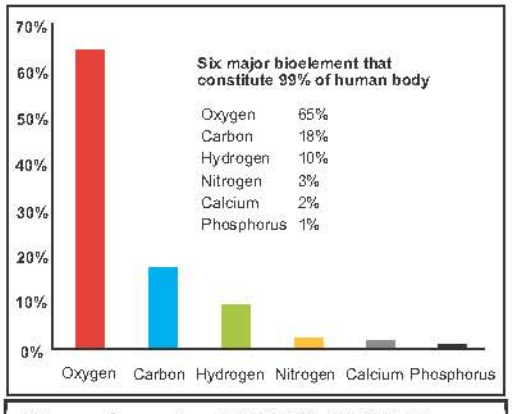
In the cell membrane phospholipids arrange in such a way that their hydrophilic heads point outward and interact with water while the hydrophobic tails are oriented towards each other and away from water. DNA and RNA have phosphate groups in their backbone, which makes them hydrophilic in nature and thus enable them to dissolve and function effectively in the aqueous environment inside the nucleoplasm and the cytoplasm. Hydrophilic active sites on various enzymes enable them to interact with water and participate in biochemical reactions and to catalyse them.

2.3 PROTOPLASM AND BIOLOGICAL MOLECULES

Living mass of the cell specifically and of the organisms generally is called protoplasm. All the biochemical reactions and activities essential for a cell to sustain take place in protoplasm. It is chiefly composed of **bioelements** and **biomolecules** which make and run the machinery of the cell i.e. cellular organelles.

2.3.1 Chemical Composition of Protoplasm

Out of 92 naturally occurring elements on earth, approximately 25 elements are found in living organisms and are called **bioelements.** However, human body is composed of only 16 of these bioelements. Only six bioelements constitute about 99% of the protoplasm so called **major** bioelements and those which are less than 1% of protoplasm are called **minor** bioelements. The bioelements combined with each other to form thousands of different biomolecules which may be inorganic (water and minerals) and organic (carbohydrates, lipids, proteins and nucleic acids).



Minor elements: K (0.35%), S (0.25%), Cl (0.15%), Na (0.15%) and Mg (0.05%).

Fig. 2.3: Proportions of various bioelements in human body

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2.3.2 Fundamental biological molecules

The four biological molecules including carbohydrates, proteins, lipids and nucleic acids are of fundamental nature in protoplasm because they are essentially present in each cell. They are found in the simplest prokaryotic cell as well as in the most advanced mammalian cell. These fundamental biomolecules are important for the maintenance of cell structure and their specific metabolic activities. **Carbohydrates** present in the cytoplasm of the cells act as fuel for the metabolic activities of the cell. **Proteins** are present in the membranes, ribosomes, cytoskeleton and enzymes of the cell. Different types of **lipids** are present in the membranes and cytoplasm of the cell. Lipids provide a reserved energy source, shape, protect and insulate the cells. The **nucleic acid** DNA is present in the chromosome. It controls the cell activity. The nucleic acid RNA is present in the nucleoplasm and cytoplasm. It takes genetic information from DNA and play role in protein synthesis. The proportions of these biomolecules in bacterial and mammalian cells are given in the table 2.1.

Table 2.1: Proportions of various biomolecules in bacterial and mammalian cells

Biomolecules	Bacterial cell	Mammalian cell
Water	70%	70%
Protein	15%	18%
Carbohydrates	3%	4%
Lipids	2%	3%
DNA	1%	0.25%
RNA	6%	1.1%
Other organic molecules (enzymes, hormones, metabolites)	2%	2%
Inorganic ions (Na^+, K^+, Ca^{++}, Mg++, Cl^-, $SO4^{--}$)	1%	1%

2.4 Condensation and Hydrolysis

A compound which has high molecular weight and is made from many repeating units is known as **macromolecule or polymer**. The individual units of a polymer are **micro-molecules** and are called **monomers**. The interconversions of these molecules are carried out by condensation and hydrolysis (Fig. 2.4).

During **condensation**, when two monomers e.g. **Alpha-Glucose** join, a hydroxyl (-OH) group is removed from one monomer (first Alpha-Glucose) and a hydrogen (-H) is removed from the other (second Alpha-Glucose) to release water and as a result a bond is formed between the monomers. The product (**Maltose**) of such reaction is called a **dimer** (Fig. 2.4).

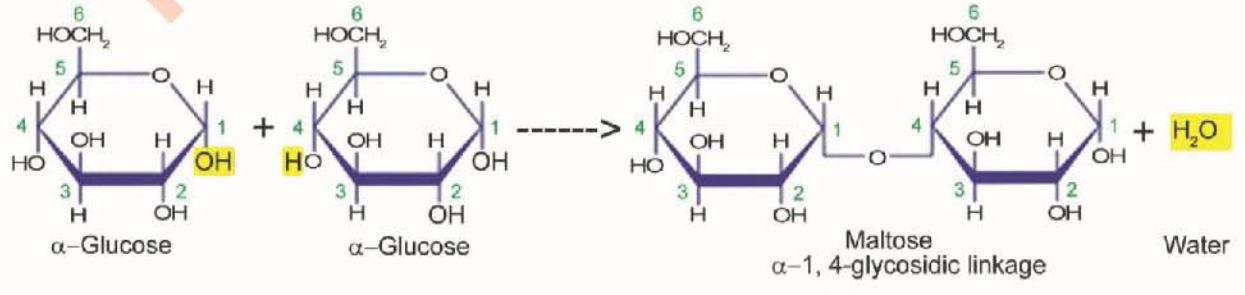

Fig: 2.4: Condensation (dehydration synthesis) reaction of A-Glucose monomers to form Maltose (dimer)

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If the same reaction is repeated several times the resulting molecule will be a **polymer.** For example, many Alpha Glucose molecules condense to form Amylose and many water molecules are released Fig.2.5 and 2.20). The number of water molecules released during condensation reaction depends upon the number of bonds formed e.g. 19 water molecules will be released when 20 monomers are condensed to form a polymer. Condensation is also called **dehydration synthesis** because water is removed (dehydration) and bond is made (synthesis). Condensation does not take place unless the proper enzyme is present and the monomers are in an activated energy- rich form.

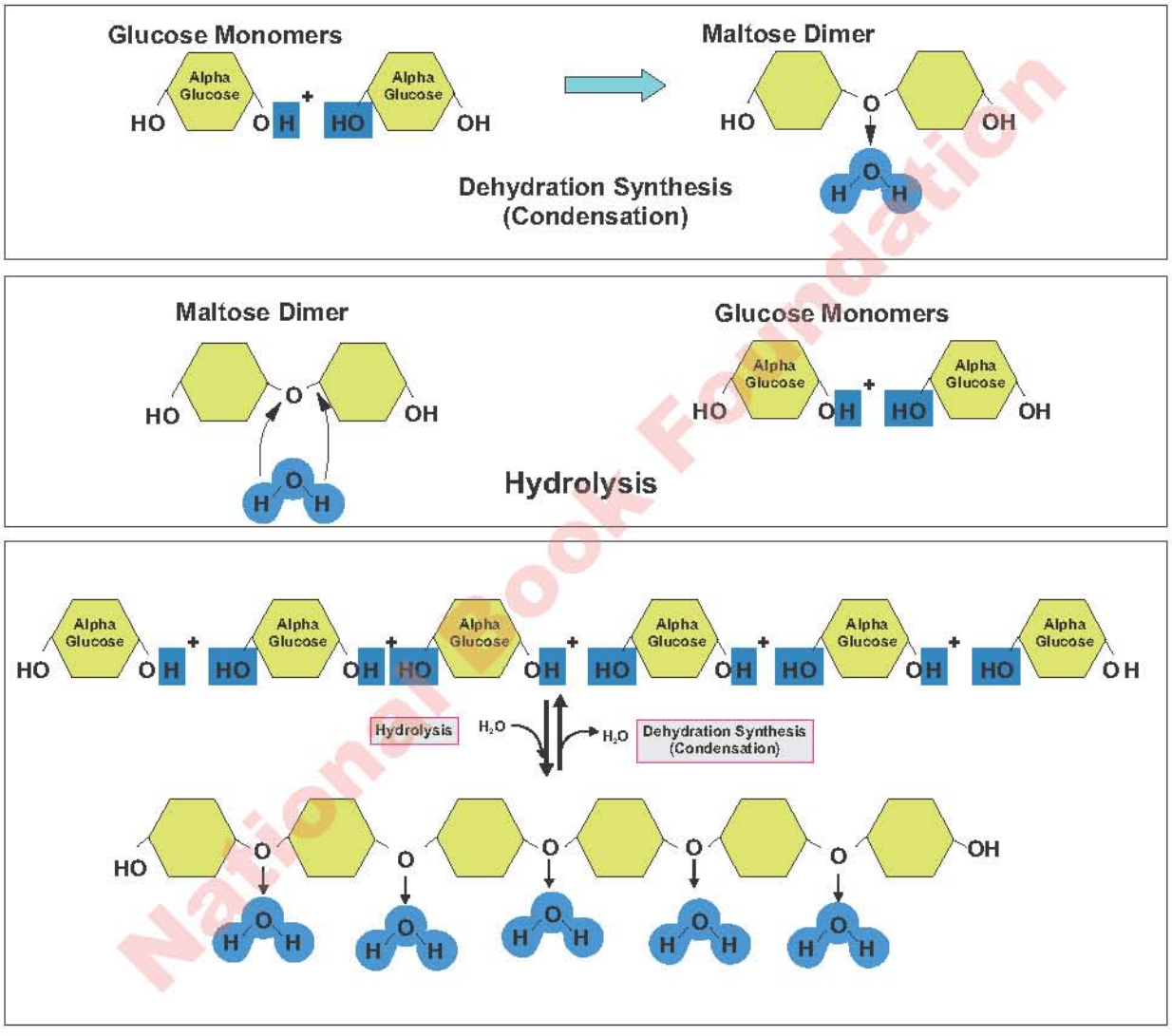

Fig. 2.5: Condensation of Alpha-Glucose monomers to form Maltose (dimer) and Amylose (polymer). Hydrolysis of Maltose (dimer) and Amylose (polymer) to Alpha-Glucose (monomers)

The **hydrolysis** is essentially the reverse of condensation i.e., the breakdown of a dimer or polymer into its monomers by the addition of water. During hydrolysis, an (-OH) group from water is attached to one monomer and (-H) is attached to the other monomer. For example, breakdown of Amylose in the presence of amylase enzyme and splitting of water molecule

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release many Alpha-Glucose monomers (Fig. 2.5 and 2.20). Actually all digestion reactions are examples of hydrolysis, which are controlled by enzymes such as carbohydrases, proteases, lipases, nucleases.

Science Titbits

Involvement of water in hydrolysis reaction should not be confused with making a solution, as water does not act as a solvent, rather just take part in a chemical reaction. Also do not assume that this breakdown releases energy, as produced when the simpler substances are oxidized in respiration. Hydration is yet another completely different process, involving the addition of water, but not breaking of bonds.

2.5 WATER AS MEDIUM OF LIFE

Water is one of the main constituents on earth. Origin, sustainability and continuity of life depends on water. More than two thirds of the earth is covered by water. Distribution of life on earth is directly proportional to the availability of life e.g. desert have few living organisms whereas rivers and oceans are full of life. Approximately 70 percent of the any organism is formed of water. Water is the most abundant component in any organism, the lowest is 20% in seeds and bones and highest is 85-90% in brain cells. Jellyfish has exceptionally large amount of water i.e., 99% making the body of jelly fish transparent.

Critical Thinking

When hydrogen gas combines with oxygen gas to form water, is the hydrogen reduced or oxidized?

2.5.1. Properties of water

The properties of water that make it the medium of life are:

1. High polarity

The bonds which are formed by the mutual sharing of electrons between two atoms are called **covalent bonds**. Normally the sharing of electrons between two atoms is fairly equal and the covalent bond is **nonpolar**. In the case of water, however the sharing of electrons between oxygen and hydrogen is not completely equal so the covalent bond is **polar**. A polar covalent bond is a chemical bond in which shared electrons are pulled closer to the more electronegative atom, making it partially negative and the other atom partially positive. Thus, in H_2O, the O atom actually has a slight negative charge and each H atom has a slight positive charge, even though H_2O as a whole is neutral. Because of its polar covalent bonds, water is a polar molecule i.e., it has a slightly negative pole and two slightly positive ones.

This is polarity of water molecules that makes it an excellent or **universal solvent** for polar substances. Ionic compound or electrolytes can be easily dissolved in water, non-polar substances having charged groups in their molecules can also be dissolved in water. Such compounds when dissolved in water, disassociates into positive and negative ions and are in more favourable state to react with other molecules and ions. This is the reason why all chemical reactions in living beings occur in aqueous medium.

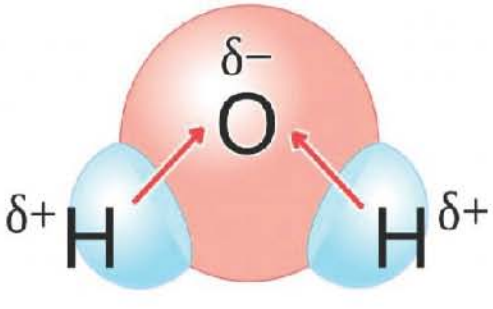

Fig: 2.6: Polarity of water molecule

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2. Hydrogen bonding

The polarity of water molecules makes them interact with each other. The charged regions on each molecule are attracted to oppositely charged regions on neighbouring molecules, forming weak bonds. Since the positively charged region in this special type of bond is always an H atom, the bond is called a **hydrogen bond.** This bond is often represented by a dotted line because a hydrogen bond is easily broken.

Because of hydrogen bonding, water is a liquid at temperatures suitable for life. The high cohesion and adhesion force of water is due to the presence of hydrogen bonds in water, which in turns makes water as transport medium.

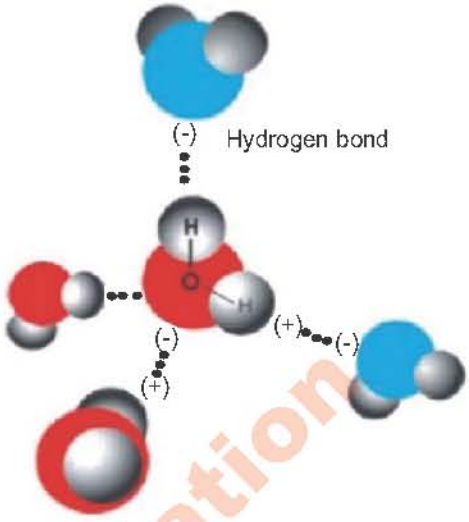

Fig: 2.7 Hydrogen bonds between water molecules

3. Cohesion and adhesion

Cohesion is the attraction among the water molecules which enables the water molecules to stick together. Water flows freely due to cohesion. Water molecules also have attraction to polar surfaces. This attraction is called **adhesion.** Both cohesion and adhesion are due to hydrogen bonds among water molecules. These properties of water enable it to circulate in living bodies and to act as transport medium.

4. High specific heat capacity

Heat capacity can be defined as the amount of heat required for minimum increase in temperature of a substance. The specific heat capacity of water can be represented as number of calories required to raise the temperature of 1g of water up to 1°C i.e., **1 Calorie (4.18 Joules).** Water has relatively a very high heat capacity than any other substance due to its hydrogen bonding, because much of the heat absorbed by water is utilized in the breakdown of hydrogen bonding therefore it does not manifest itself to raise the temperature of water. Hence, very large amount of heat can increase very little in temperature in water. Due to its high heat capacity water works as **temperature stabilizer** or regulator for organisms in the hot environment and hence protects the living material against sudden thermal changes.

5. High heat of vapourization

Heat of vapourization is the amount of heat required to convert a unit mass of a liquid into gaseous form. Heat of vapourization of water is represented as number of calories absorbed per gram vapourized. Water has high heat of vapourization i.e., **574 calories per gram.** The high heat of vapourization means that a large amount of heat can be lost with minimal loss of water from the body. This is high heat of vapourization of water that gives animals an efficient way to release excess body heat in a hot environment. When an animal sweats, body heat is used to vapourize the sweat thus cooling the animal. Due to this property of water, evaporation of only 2 ml out of one litter of water lowers the temperature of the remaining 998 ml water by 1°C.

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6. Hydrophobic exclusion

Hydrophobic exclusion can be defined as reduction of the contact area between water and hydrophobic substances which are placed in water. For example, if you place few drops of oil on the surface of a water solution, the oil drops will tend to join into a single drop. Biologically, hydrophobic exclusion plays key roles in maintaining the integrity of lipid bilayer membranes.

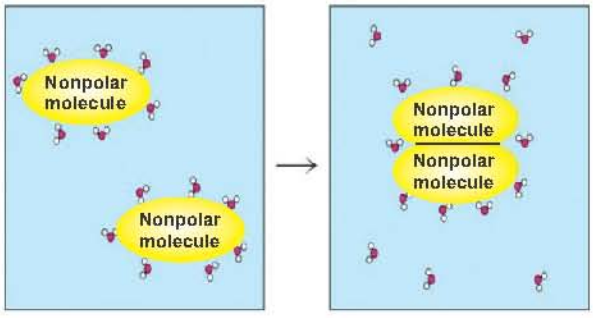

Fig: 2.8 Hydrophobic exclusion

7. Ionization

The dissociation of a molecule into ions is called **ionization**. When water molecule ionizes, it releases an equal number of positive hydrogen and negative hydroxyl ions. This reaction is reversible but equilibrium is maintained at 25°C.

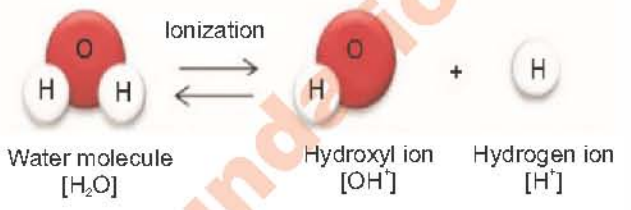

Fig: 2.9: Ionization of water

The H^+ and OH^- ions affect and take part in many of the reactions that occur in cells, e.g., it helps to maintain or change the pH of the medium.

8. Lower density of ice

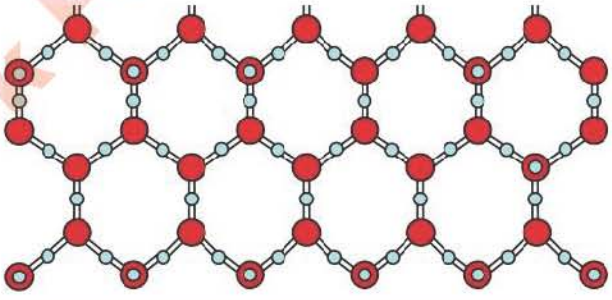

Fig: 2.10 Lattice likes arrangement of water molecules in ice

Ice floats on water. This is because ice is less dense than water. The reason is that ice has a giant structure and show maximum number of hydrogen bonding among water molecules; hence, they are arranged like a lattice. In freezing weather, ice forms on the surface of ponds and lakes forming an insulating layer above the water below. This provides a living environment for some organisms until the ice melts. Organisms can also live under the ice.

2.6 CARBOHYDRATES

Skills: Analyzing , Interpreting and Communication
Draw model diagrams to describe the hydrogen bonding.

Carbohydrates are the compounds of carbon, hydrogen and oxygen. Literally word carbohydrate means "hydrates of carbon" i.e., a carbon associated with water. According to this classic concept, carbohydrate are organic biomolecules or their derivatives in which all the carbon atoms are hydrated, having generalized formula $C_n(H_2O)_n$, where n is the number of carbon atoms and the number of water molecules. For example, glucose has molecular formula $C_6(H_2O)_6$ or $C_6H_{12}O_6$. But this formula is not exactly applicable for in some case e.g. lactic acid ($C_3H_6O_3$) and acetic acid

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($C_2H_4O_2$) are not a carbohydrates whereas rhamnose ($C_6H_{12}O_5$) and Deoxyribose ($C_5H_{10}O_4$) are carbohydrate. Hence carbohydrates are now chemically defined as:

"Organic compounds that are either polyhydroxy aldehydes or polyhydroxy ketones (monosaccharides) or their complex derivative compounds (polysaccharides) which upon hydrolysis produce either polyhydroxy aldehydes or polyhydroxy ketones or both"

2.6.1 Classification of Carbohydrates

Carbohydrates are commonly known as **sugars** or **saccharides** as most of the familiar carbohydrates are sweet in taste. Word saccharides is derived from Greek word **"sakcharon"** which means sugars. Classification of carbohydrates is based upon number of saccharide units. Carbohydrates are generally classified into three group i.e., monosaccharides, oligosaccharides and polysaccharides.

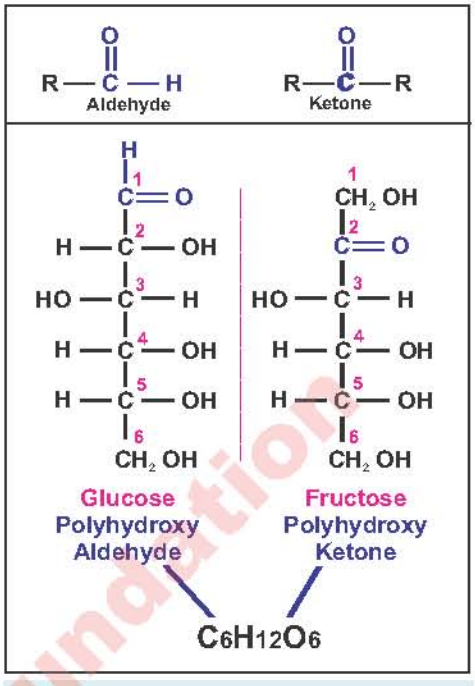

Fig: 2.11: Chemical nature of carbohydrates

Table: 2.2: Comparison of characteristics of carbohydrates

Monosaccharides	Oligosaccharides	Polysaccharides
They consist of single saccharide unit.	They are composed of 2 to 10 saccharide units.	They are composed of more than 10 saccharide units.
They are simplest carbohydrates; therefore, they cannot be further hydrolyzed.	They have less complex structure, so upon hydrolysis they yield at least 2 and maximum 10 monosaccharides.	They have highly complex structure, so upon hydrolysis they yield at least 11 monosaccharides.
They are highly soluble in water.	They are less soluble in water.	They are generally insoluble in water.
They are sweetest among all carbohydrates.	They are less sweet in taste.	They are tasteless.

2.7 MONOSACCHARIDES

Monosaccharides are true carbohydrates which are either polyhydroxy aldehydes or polyhydroxy ketones. The general molecular formula for the representation of monosaccharides is $(CH_2O)_n$ where 'n' is the number. Number of carbon atoms in monosaccharides ranges from 3 to 7. All the carbon atoms in a monosaccharide except one, have a hydroxyl group (-OH) while the remaining carbon atom is either the part of aldehyde or ketone.

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Classification of monosaccharides

Classification of monosaccharides is based upon functional group and number of carbon atoms. On the basis of functional group, the monosaccharides containing aldehyde are called **aldoses** while those containing ketone are called **ketoses**. On the other hand monosaccharides are classified into five groups based upon number of carbon atoms i.e., **trioses** (3C), **tetroses** (4C), **pentoses** (5C), **hexoses** (6C) and **heptoses** (7C).

Table: 2.3: Examples and functions of monosaccharides

Class	Formula	Aldoses	Ketoses	Function
Trioses (3C)	$C_3H_6O_3$	Glyceraldehyde	Dihydroxy acetone	Intermediates in photosynthesis and cellular respiration.
Tetroses (4C)	$C_4H_8O_4$	Erythrose	Erythrulose	Intermediates in bacterial photosynthesis.
Pentoses (5C)	$C_5H_{10}O_5$	Ribose, Deoxyribose ($C_5H_{10}O_4$)	Ribulose	Ribose and deoxyribose are components of RNA and DNA respectively. Ribulose is an intermediates in photosynthesis.
Hexoses (6C)	$C_6H_{12}O_6$	Glucose, Galactose	Fructose	Glucose is respiratory fuel (initial substrate) Fructose is an intermediate in respiration. Galactose is the component of milk sugar.
Heptoses (7C)	$C_7H_{14}O_7$	Glucoheptose	Sedoheptulose	Intermediates in photosynthesis.

Properties of monosaccharides

(1) Monosaccharides are the simplest form of carbohydrates so they cannot be hydrolysed into simpler compounds.

(2) Monosaccharides are highly soluble in water. Multiple hydroxyl groups in the structure of monosaccharides may form hydrogen bonds with the water molecules. So all the monosaccharides can easily dissolve in water.

(3) Monosaccharides are called sugars due to their sweet taste. Specifically arranged multiple hydroxyl groups in monosaccharides interact and activate the sweet taste receptors present on our tongue.

(4) Monosaccharides are called reducing sugars as they can reduce the other compounds and get themselves oxidized. Monosaccharides have a free aldehydic or ketonic group and oxygen atom of these functional groups may donate the electrons to the recipient compounds to reduce them.

(5) Monosaccharides show stereo-isomerism due to different orientation of the hydrogen and hydroxyl groups at the multiple asymmetric or chiral carbons in their structure. Asymmetric carbon is that which is attached to four different groups.

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(6) Monosaccharides with five or more carbon atoms may exist in two structural forms i.e. open-chain or acyclic structure and a ring or cyclic structure.

Chemical structures of monosaccharides

Monosaccharides are usually found in open chain structure in crystalline form but when they are dissolved in water most of them (pentoses and hexoses) are converted into ring chain structure. Two types of ring structure are found in monosaccharides i.e. furanose and pyranose.

Furanose is a five cornered ring structure with one oxygen and four carbon atoms. In furanose, C1 and C4 are attached to oxygen to form ring structure. All pentoses and ketohexoses form furanose ring structure.

Pyranose is a six cornered ring structure with one oxygen and five carbon atoms. In pyranose, C1 and C5 are attached to oxygen to form ring structure. All pentoses and ketohexoses form furanose ring structure. Only aldohexoses are converted into pyranose ring structure.

Let us understand it by taking examples of aldo hexose glucose ($C_6H_{12}O_6$) and aldo pentose ribose ($C_5H_{10}O_5$). Glucose and ribose can exist in open chain structure in dried form but it exists in ring structure in aqueous medium. When they dissolve in water, the oxygen atom from aldehyde group reacts with second last carbon i.e., C5 in case of glucose and C4 in case of ribose. In this way oxygen atom forms a link between C1 and C5 in case of glucose and between C1 and C4 in case of ribose. While the OH group of second last carbons is shifted to C1. After this modification ring structure of glucose called **glucopyranose** whereas, ring structure of ribose is called **ribofuranose**.

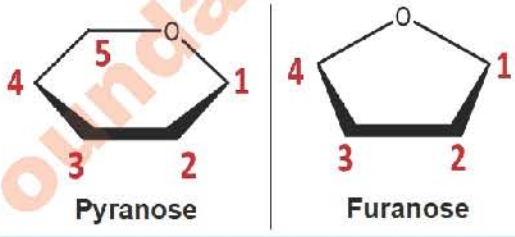

Fig: 2.12: Types of ring structure in monosaccharides

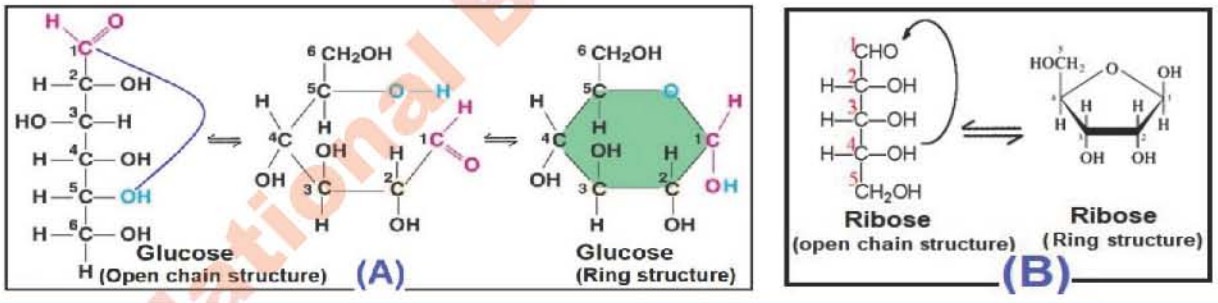

Fig: 2.13: Conversion of open chain (A) glucose into ring chain glucopyranose (B) ribose into ring chain ribofuranose

2.8 ISOMERS AND STEREOISOMERS OF GLUCOSE

Phenomenon in which compounds have the same molecular formula but they differ in structural arrangement or formula is called isomerism. For example glucose and fructose both are hexoses with same molecular formula i.e, $C_6H_{12}O_6$ but both have different structure of their functional groups. Glucose is an aldo sugar and fructose is a keto sugar so they are **functional group isomers** of each other.

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Stereoisomerism in glucose

Stereoisomers are molecules that have the same molecular formula but differ only in the relative arrangement of -H and -OH group in 3D space at the **anomeric** and **asymmetric** carbon atoms.

Anomeric carbon is the carbon in the ring structure of monosaccharides that is derived from the carbonyl carbon ($-\overset{O}{\overset{\|}{C}}-$) of ketone or aldehyde functional group of their open-chain, e.g. C-1 in the glucose and ribose.

Asymmetric carbon is that carbon which makes bonds with four different atoms or groups around it. For example, in glucose, four carbon atoms (C-2, C-3, C-4 and C-5) are asymmetric carbon atoms. Last asymmetric carbon atom or second last in carbon chain (C-5 in case of glucose) is called **penultimate carbon**. In monosaccharide the number of stereoisomers actually depends upon the number of asymmetric carbons in its structure and can be calculated by the formula 2n where n is the number of asymmetric carbon atoms so glucose has 16 stereoisomers. Stereoisomers can be classified into following groups:

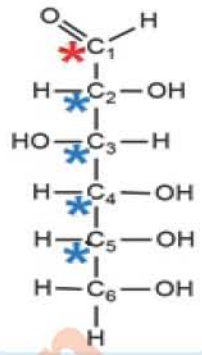

Fig: 2.14: Carbonyl carbon (red *) and Asymmetric carbon (blue *) in Glucose open chain structure

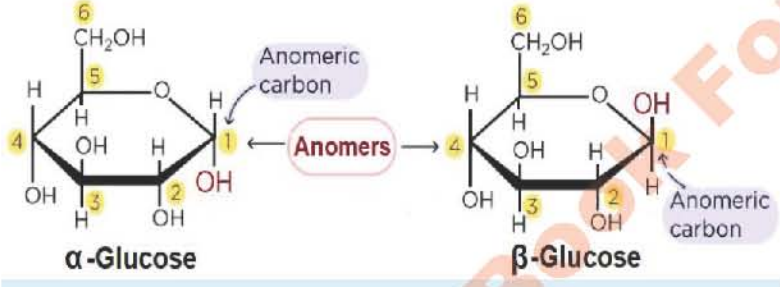

Fig: 2.15: Anomers (*α* and *β* stereoisomers) of glucose

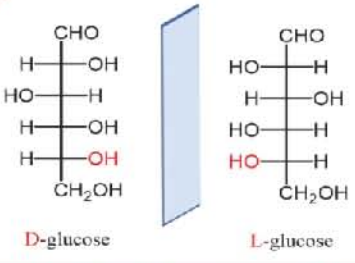

Fig: 2.16: An example of enantiomers

(i) Anomers are the stereoisomers that have different position of -H and -OH group on the C-1(anomeric carbon) of pentose (e.g. ribose) and hexose (e.g. glucose) and are called α or ß isomers. If -OH group is found downward on C-1 then it is called α sugar and if -OH is present upward on C-1 then it is known as ß sugar.

(ii) Enantiomers: Those stereoisomers which are non-superimposable mirror images of one another are called enantiomers. Example of an enantiomers is the D (Dextro) and L (Levo) isomers of glucose (figure 2.16). D isomers also called right handed form whereas in L isomers also called left handed form. In **D-glucose**, penultimate carbon (C-5) has -OH group on right side whereas in **L-glucose**, penultimate carbon has -OH group on left side. Out of 16 stereoisomers of glucose, 8 are enantiomers of other 8.

Science Titbits

Laboratory manufactured (artificial) sugars such as tagatose, sucralose etc. are left-handed forms (L-sugars), and are used as sweeteners. Whereas living organisms can exclusively use naturally occurring right handed sugars (D-sugars). Cellular proteins, receptors and enzymes are designed to react only with particular enantiomers (D-sugars). For example the enzymes in human stomach are right handed and can digest only D-sugars. Hence, right-handed human enzymes cannot metabolize left-handed sugars because their active sites cannot fit on to a left-handed substrate. So, for the left handed substrate the enzyme must be left-handed.

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(iii) Diastereoisomers: Those stereoisomers which have different position of -H and - OH groups at more than one asymmetrical carbon atoms are called diastereoisomers. Unlike an enantiomer, diastereoisomers are not mirror images of each other e.g. D-Glucose and D-Altrose are diasteriomers of each other.

(iv) Epimers: Those stereoisomers which have different arrangement of -H and -OH groups at only one asymmetrical carbon atom are called epimers e.g. D-Glucose and D- Mannose are epimers of each other.

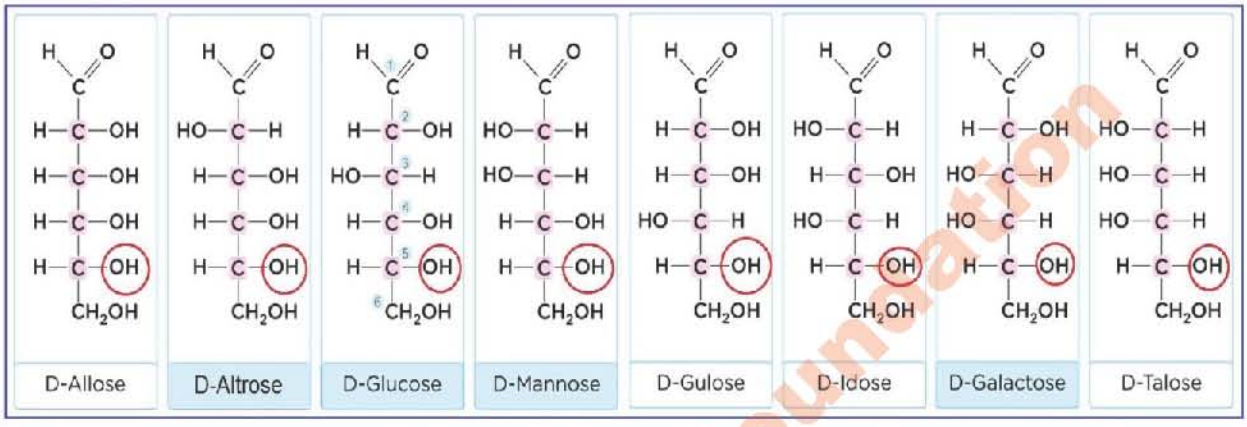

Fig: 2.17: Eight stereoisomers of D-Glucose. Encircled -OH groups at penultimate carbon indicate the D-isomers

Carbohydrates which are derivatives of monosaccharides and upon hydrolysis yield 2 to 10 saccharide units are called oligosaccharides. On the basis of number of saccharide units, the oligosaccharides are classified into **disaccharides, trisaccharides, tetrasaccharides** and so on. The most common among these are disaccharides.

2.9 PROPERTIES AND ROLES OF DISACCHARIDES

Two monosaccharides combine to form a disaccharide. It is a kind of oligosaccharides. Disaccharides are less sweet in taste and comparatively less soluble in water. These can be hydrolyzed to give monosaccharides. Disaccharides are familiar energy source for living organisms. Examples include maltose, lactose, and sucrose. The general formula of disaccharide is $C_{12}H_{22}O_{11}$. Some common disaccharides are as follows:

2.9.1 Sucrose

Sucrose is commonly known as cane sugar. It is widely used as sweetener at homes for making sweet dishes. In plants sucrose is also called **transport disaccharide** as prepared food in plants is transported in the form of sucrose. It is very soluble and can therefore be moved efficiently in high concentration in plants. It is also relatively unreactive chemically. Upon hydrolysis sucrose yields one molecule of α-glucose and one molecule of ß-fructose.

2.9.2 Maltose

Maltose is commonly known as **malt sugar.** It is an intermediate disaccharide produced during the breakdown of starch and glycogen and act as energy producing molecule. Maltose is produced in brewing industry by malting process in which barley starch is catabolized using enzyme amylase. Maltose is generally found in germinating seeds and sprouting potatoes where it act as energy source for growth and development of young seedlings.

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2.9.3 Lactose

Lactose is commonly known as **milk sugar** as it is found in milk of mammals. Ratio of lactose in in different mammals is variable e.g. 3-4% in goat milk, 4-6% in cow milk, 5-8% in human milk. It is also produced as by-product during cheese production process. Lactose is used as direct energy source by infants. Lactose act as substrate for synthesis of various macro-biomolecules which are involved in vital nervous and immunological processes.

Critical thinking?

- Why potatoes taste sweet when stored for long?
- How the initially tasteless or sour taste young fruits become sweet when ripens?

2.10 GLYCOSIDIC BONDS IN DISACCHARIDES

A glycoside is basically a ring chain hexose (6C) or pentose (5C) sugar molecule that is attached usually to another sugar molecule. Covalent chemical bonds between two glycosides that hold them together is called **glycosidic bond.**

Glycosidic bond is formed due to condensation reaction between two sugar molecules mostly hexoses. For the formation of glycosidic bond, the **H- group** from one ringed sugar interacts with the **-OH group** of another sugar and one water molecule is released. Glycosidic linkage is present in disaccharides, oligosaccharides and polysaccharides. Glycosidic bonds are named on the basis of carbon number of interacting carbons of both linked sugars. Glycosidic linkage is also named alpha or beta on the basis of alpha or beta type of first sugar involved in linkage. e.g. **α- 1,4 glycosidic linkage** is formed when first sugar is alpha, donates -OH group from its C-1 and other sugar donates H- group from its fourth carbon.

Science Titbits

Any carbohydrate which is capable of being oxidized and causes the reduction of other substances without having to be hydrolyzed first is known as **reducing sugar**, but those which are unable to be oxidized and do not reduce the other substances are known as **non-reducing sugars.** All monosaccharides and two of three types of disaccharides (maltose and lactose) have the open chemical structure needed to act as reducing agents. The third type of disaccharides, sucrose, and polysaccharides are non-reducing sugars.

2.10.1 Glycosidic linkage in sucrose

The sucrose is formed by the condensation of α-glucose and β-fructose. In this reaction, the -OH group at C-1 of α-glucose interacts with the H of -OH group at C-2 of β-fructose, releasing a water molecule forming **α-1,2-glycosidic linkage** or **α-1, β-2-glycosidic linkage.**

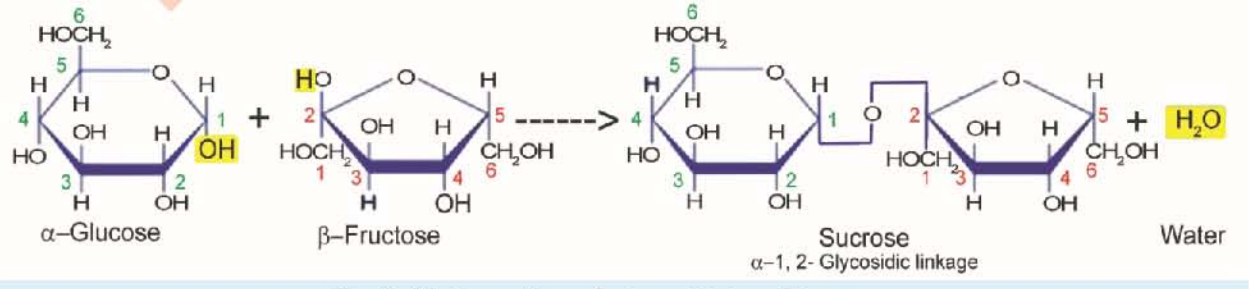

Fig: 2.18: Formation of glycosidic bond in sucrose

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2.10.2 Glycosidic linkage in maltose

Maltose is formed by the condensation of two molecules of α-glucose. In this reaction, the -OH group at C-1 of first α-glucose interacts with the H of -OH group at C-4 of other α-glucose, releasing a water molecule forming **α-1, 4-glycosidic linkage** or . **α-1, α-4-glycosidic linkage.**

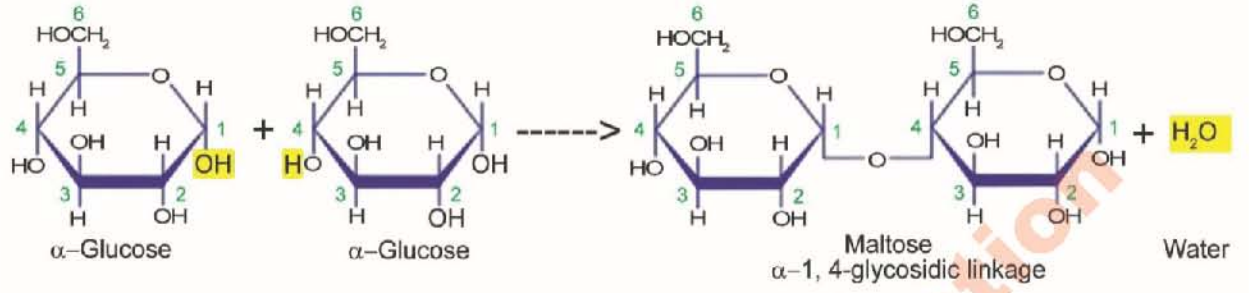

Fig: 2.19: Formation of glycosidic bond in maltose

2.10.3 Glycosidic linkage in lactose

The lactose is formed by the condensation of β-galactose and β-glucose. In this reaction, the -OH group at C-1 of β-galactose interacts with the H of -OH group at C-4 of β-glucose, releasing a water molecule forming **β-1, 4-glycosidic linkage or β-1, β-4-glycosidic linkage**

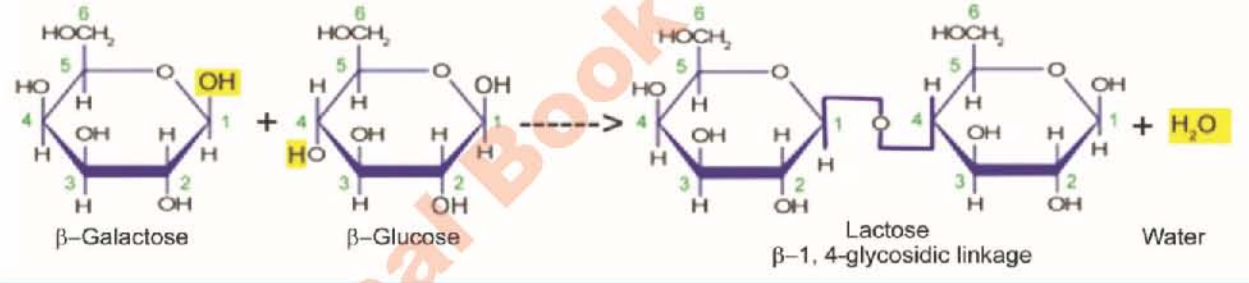

Fig: 2.20: Formation of glycosidic bond in lactose

2.11 POLYSACCHARIDES

Those carbohydrates which upon hydrolysis yield more than ten monosaccharide units are called polysaccharides. This is largest group of carbohydrates. The polysaccharides which are composed by the condensation of only one kind of monosaccharides are called **homopolysaccharides** e.g., starch, glycogen, cellulose, chitin; whereas the polysaccharide which are composed by the condensation of different kind of monosaccharides are called **heteropolysaccharides** e.g., agar, pectin, peptidoglycan. Polysaccharides function chiefly as food and energy stores, e.g., starch, glycogen, and structural material, e.g., cellulose and chitin. They are convenient storage molecule for several reasons. Their large size makes them more or less insoluble in water, so they exert no osmotic or chemical influence in the cell; they fold into compact shapes and they are easily converted to sugars by hydrolysis when required. Some common polysaccharides e.g., starch, cellulose, and chitin are being discussed here.

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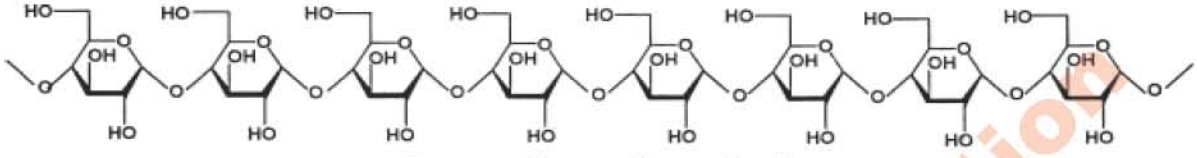
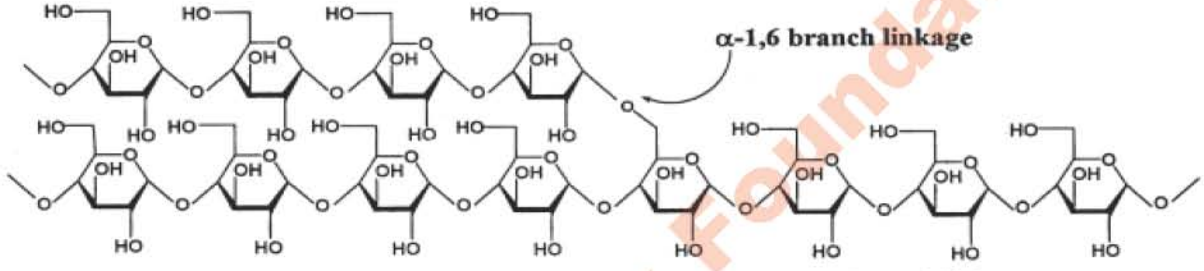
2.11.1 Structure properties and roles of starch

Starch is a homopolysaccharide which is formed by the condensation of hundreds of **α-glucose** monomers. It is storage carbohydrate of plants. It is mainly stored in root, stem and seeds. Cereal grains and potato tubers are rich sources of starch in human diet. Starch is digested in oral cavity and in small intestine by the enzyme amylase. Upon hydrolysis it yields maltose first and then maltose is further digested by maltase enzyme and yields glucoses. The presence of starch in a given sample can be confirmed by iodine test as it gives blue colour with iodine solution. There are two types of starches i.e., amylose and amylopectin.



Segment of an amylose molecule



Segment of an amylopectin molecule, showing one α-1,6 branch linkage

Fig: 2.21: Structure of starches

Amylose is un-branched i.e., a linear chain of glucoses in which glucoses are attached together by **α-1, 4-glycosidic linkages.** It is soluble in hot water only. On the other hand, **amylopectin** has branched structure i.e., a linear chain of glucoses but more chains of glucoses in the form of branches are also attached by **α-1, 6-glycosidic linkages.** It is completely insoluble in water.

2.11.2 Structure properties and roles of Glycogen

Like starch, glycogen is also a homopolysaccharide composed of multiple **α-glucose** monomers. It is storage carbohydrate of animals. It is mainly stored in liver and muscles. Therefore it is also known as **animal's starch.** The disgestion of glycogen is also quite similar to that of starch.

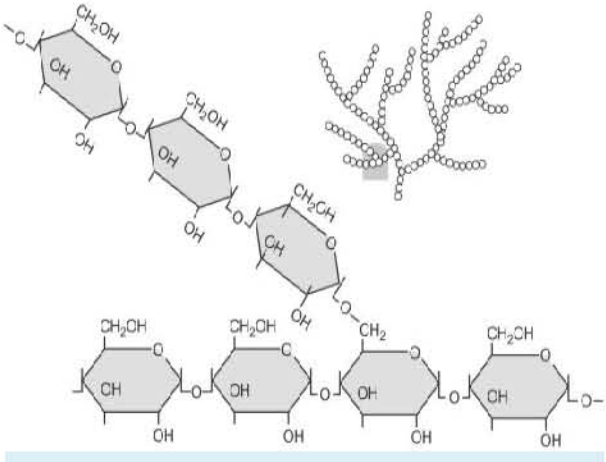


Fig: 2.22: Structure of glycogen

Science Titbits

Cellulose cannot be digested by human body but it has to be taken into diet because it works as roughage or fibre so it prevents abnormal absorption of food in intestine. However, herbivore animals have some symbiotic bacteria that secrete **cellulase** enzyme for its digestion. Upon hydrolysis it first yields a disaccharide, the **cellubiose** and then cellubiose is further digested into glucoses.

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The presence of glycogen in a given sample can also be confirmed by iodine test as it gives red colour with iodine solution. Structure of glycogen resembles with amylopectin starch but glycogen has much more branching than amylopectin.

2.11.3 Structure properties and roles of Cellulose

Cellulose is most abundant carbohydrate on earth. It is also a homopolysaccharides but unlike starch and glycogen it is formed by the condensation of hundreds of **β-glucoses**. It is structural carbohydrate of plants as it is major constituent of plant cell wall. Cotton and paper are the pure forms of cellulose. Cellulose shows no colour with iodine solution. Structure of cellulose resembles with amylose starch in such a way that it has un-branched structure but it has **β-1, 4-glycosidic linkages** between glucose residues.

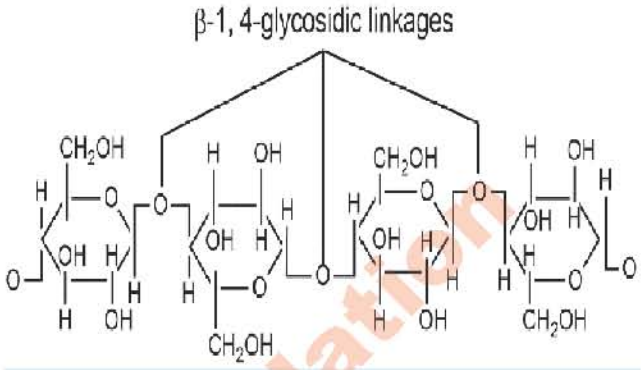

Fig: 2.23: Structure of cellulose

2.11.4 Structure properties and roles of Chitin

Chitin is the second most abundant organic molecule on earth. It is also a homopolysaccharides. It is a structural carbohydrate found in the cell walls of fungi and in the exoskeleton of arthropods. Due to the occurrence of chitin in fungal cell wall, it is also known as **fungal cellulose**. Chitin is the derivative of **N-acetyl glucosamine** monomers which is a modified form of glucose. It has an un-branched structure and its monomers are linked together by **β-1, 4-glycosidic linkages**.

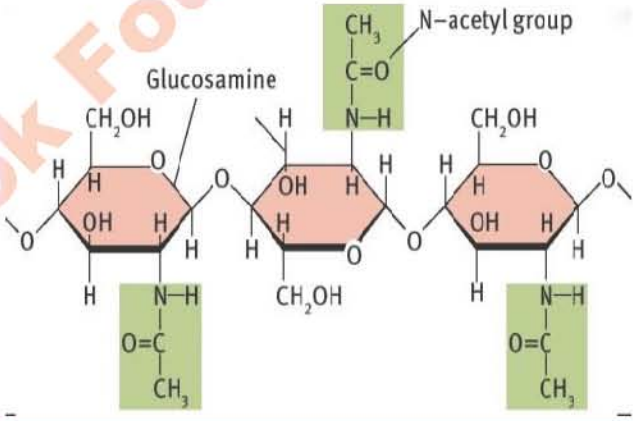

Fig: 2.24: Structure of chitin

STEAM ACTIVITY 2.1

The presence or absence of starch, glycogen, cellulose and glucose in a given sample can be confirmed by biochemical tests.

Experiment	Observations	Inference
Iodine test for Starch in solution or food material		
A. Take 5 ml water in a test tube. Add a small amount of starch in water in test tube and then boil to prepare starch solution. Add a few drops of iodine solution into the clear starch solution in the test tube.	Dark blue black coloration is produced in test tube.	Starch is confirmed in solution
B. Take food material like potato, then cut a small slice of potato and add a few drops of iodine solution on the potato slice.	Dark blue black coloration is produced on potato slice.	Starch is confirmed in food
C. Take glycogen solution in test tube and add few drops of iodine solution	Red coloration is produced in test tube.	Glycogen is present in solution

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D. Take cellulose solution in test tube and add few drops of iodine solution	No change in colour of iodine solution in test tube.	Starch and glycogen absent and Cellulose may be present
Tests for Glucose		
A. Benedict's test for glucose: Take 5 ml of Benedict's solution in a test tube, add a few drops of the test solution (glucose solution) and boil for 2-3 minutes.	A dirty green, yellow or red precipitates are formed	Glucose is present
B. Fehling's solution test: Take 3 ml of Fehling's solution in a test tube, add 3 ml of test solution (glucose solution) and heat.	Red precipitates are formed	Glucose is present
Test for disaccharide sugars		
A. Molisch's test: Take 1 ml of the test solution (sucrose), then add 4-5 drops of alcoholic alpha -Naphthol solution then slowly pour 5 ml of conc. H_2SO_4 and do not mix.	Purple ring at the junction of two solution is formed.	Disaccharide Sugar present

2.12 PROTEINS

Proteins play most of the critical roles in the cells and are required for the structure, functioning, maintenance and regulation of the body systems. Proteins are the main structural components of the cell. All proteins contain C, H, O and N, while some contains P, S. Few proteins have Fe, I and Mg incorporated into the molecule.

2.12.1 Structure of Proteins

Chemically proteins can be defined as **polymers of amino acids** or **polypeptide chains.** A protein may consist of a single polypeptide or more than one polypeptide.

2.12.2 Amino acids

Amino acids are the building blocks of proteins. There are many amino acids known to occur, but only 20 are commonly found in proteins. The amino acids are built on a common plan. Each contains a carbon atom. It is called α (alpha) carbon to this a hydrogen atom, an amino group (-NH2), a carboxyl group (-COOH) and a variable group known as -R group are attached. The R group has a different structure in each of the 20 biologically important amino acids and determines their individual chemical properties. Two simplest amino acids i.e., glycine and alanine are shown in figure 2.26.

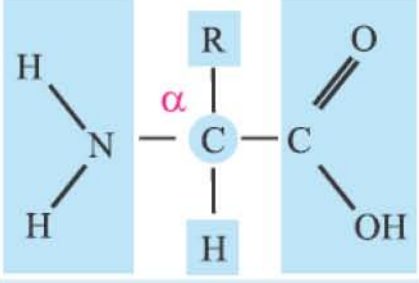

Fig: 2.25 General structure of an amino acid

2.12.3 Essential and non-essential Amino acids

Amino acids are categorized into two groups:

Essential amino acids.

The essential amino acids are those amino acids which cannot be produced by our bodies and they should essentially be acquired through our diet. They can be obtained from foods like milk, egg, fish, chicken, meat, vegetables, lentils etc. They can serve to build and repair muscle and bones act as precursor molecules for the synthesis of hormones and neurotransmitters.

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One can face the deficiency of essential amino acids and consequently suffer many health problems if not regularly taken in diet. There are nine essential amino acids which include, lysine, leucine, isoleucine, histidine, methionine, phenylalanine, threonine, valine and tryptophan.

Non-essential amino acids.

The non-essential amino acids can be synthesized in the human body modifying other amino acids and other food components. They need not to be part of our diet. Chances of their deficiency are rare but due to illness or starvation may lead to their deficiency. They are involved in removal or detoxification of toxins, essential in the RBC and WBC synthesis, improve brain and cardiac functioning. The eleven non-essential amino acids are: alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine and tyrosine.

2.13 SYNTHESIS AND BREAKAGE OF PEPTIDE LINKAGES

When two or more amino acids bind with each other to condensed molecule a bond called peptide bond is formed. On hydrolysis of proteins these peptide bonds are broken to release multiple amino acids. Peptide bonds are formed when animal and plant cells store extra amino acids as polypeptides or proteins for their structural and functional role. Each cell have this reaction of polymerization of amino acids. Peptides bonds are vital for the maintenance of primary structure of proteins.

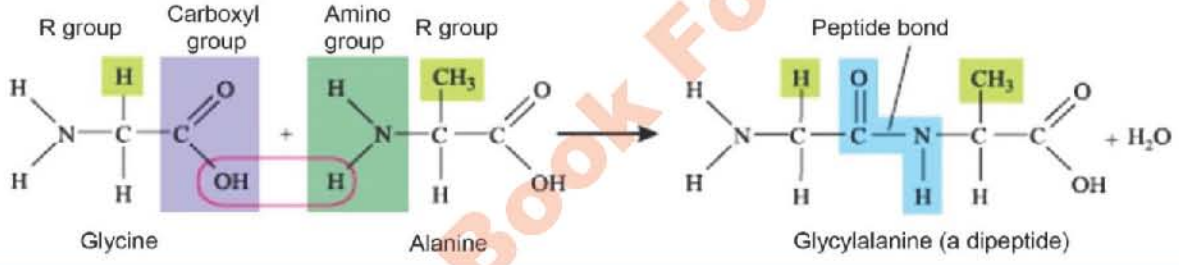

Fig: 2.26: Formation of a dipeptide and peptide bond

Dipeptides and polypeptides are formed by the condensation of amino acids on the ribosome under the instructions of mRNA which carry these coded instructions from DNA. This process is known as translation. During this process, when an amino acid reacts with another amino acid, the -OH from carboxylic acid group of one amino acid and -H from amino group of other amino acid are liberated and form a water molecule, as a result a bond is established between C of carboxylic acid group and N of amino group of two amino acids called **peptide bond.** Hence, a product of two amino acids is formed which is known as **dipeptide.**

A dipeptide has two ends; one is called amino or **-N terminal** end while other is called carboxylic acid or **-C terminal** end. A new amino acid can be added in this chain from its carboxylic acid or -C terminal end in the same way. Thus, a **tripeptide** (a product of three amino acids) is formed and another water molecule is also released. Similarly, when several amino acids are linked together by many peptide bonds, the **polypeptide** chain is formed.

Peptide bonds are broken at catabolism of proteins during digestion in gut by the action of protein and polypeptide chain digesting enzymes like pepsin, trypsin erypsin etc. Peptide bonds of proteins and polypeptides may also break at cellular level by lysosomal enzymes to release amino acids for new protein synthesis or for ATP synthesis.

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2.13.1 Structural conformations in proteins

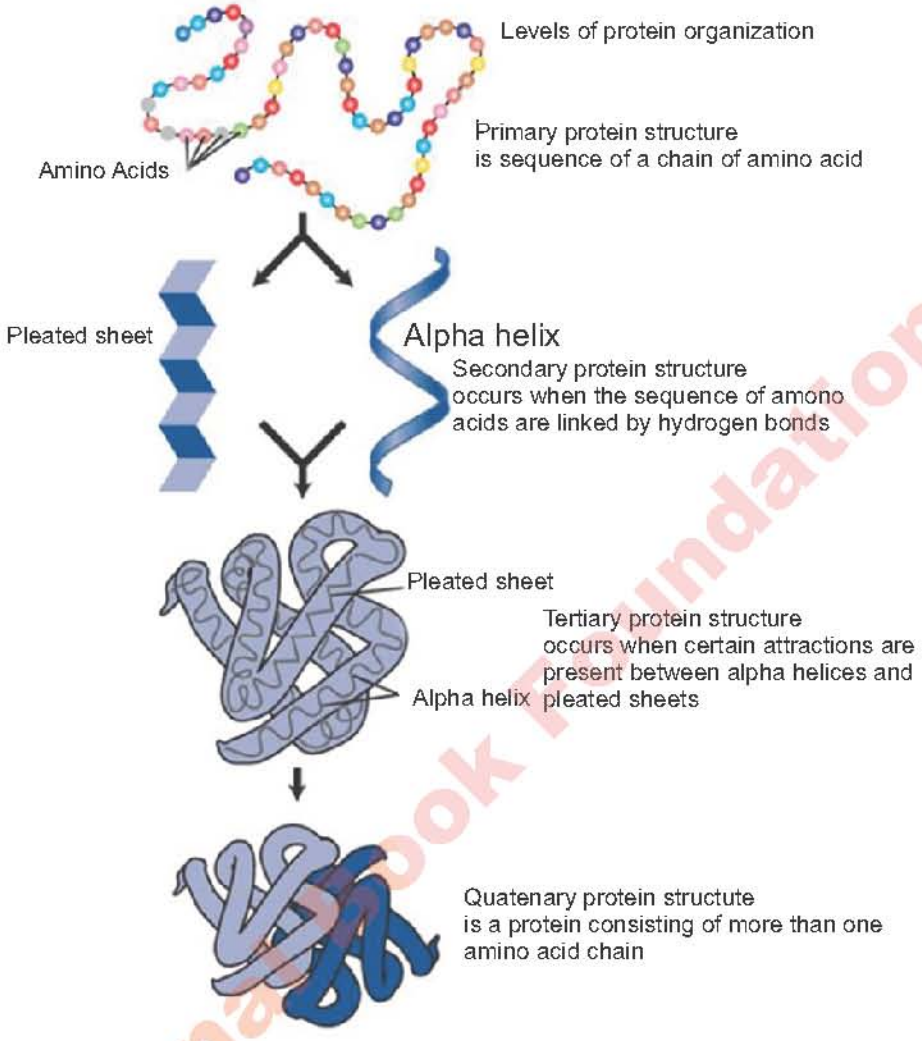

Fig: 2.27: Structural conformations in proteins

A linear polypeptide with a specific sequence and number of amino acids is called **primary structure.** It is shown by all proteins at the time of their synthesis on ribosomal surface. After synthesis a protein does not remain in its primary structure but can be changed into some other structural conformations (particular form, shape or structure).

A helical **(α-helix)** or flattened sheets **(β-pleated sheet)** like structures which are established by **H-bonding** between opposite charge bearing groups of different amino acids are called **secondary structures.** In some proteins the linear polypeptide is changed into α-helix, then α-helix fold again and again by **ionic bonds** and **disulfide bridges** to form a globular shaped structure, the **tertiary structure.**

Some proteins exist in very complex structure in which more than one globule is attached together by **hydrophobic interaction.** Such structures are called **quaternary structures.**

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2.14 SIGNIFICANCE OF AMINO ACID SEQUENCE

Sequence of amino acid in a polypeptide is a characteristic feature of primary structure of protein which is responsible for proper functioning of protein. It is determined by the sequence of nucleotide in DNA. Even due to **point mutation** (change of single or few nucleotides in DNA) the sequence of amino acid in a particular protein (polypeptide) may be disturbed which causes severe defects in the body as it happens in sickle cell anemia, a hereditary disease.

Normal red blood cells are disc-shaped and look like doughnuts without holes in the centre. They move easily through your blood vessels. Red blood cells contain an iron-rich protein called haemoglobin. This protein carries oxygen from the lungs to the rest of the body. **Normal haemoglobin (Hb^A)** contains four polypeptides i.e. two α-chains which consist of 141 amino acids each and two ß-chains which consist of 146 amino acids each.

Sickle cell anemia is a serious disorder in which the body makes sickle or crescent shaped red blood cells. Sickle cells contain abnormal hemoglobin called **sickle haemoglobin (Hb^S)**. Sickle haemoglobin causes the cells to develop a sickle, or crescent, shape. Sickle cells are stiff and sticky. They tend to block blood flow in the blood vessels of the limbs and organs. Blocked blood flow can cause pain and organ damage. Sickle cell anemia is caused by a point mutation in ß-globin gene in which only one nucleotide is replaced by another which causes a change in amino acid sequence of ß-chain of haemoglobin. Sickle cell haemoglobin (Hb^S) shows only one difference from Hb^A i.e., **glutamic acid** is replaced by **valine** at position number six in ß-chain.

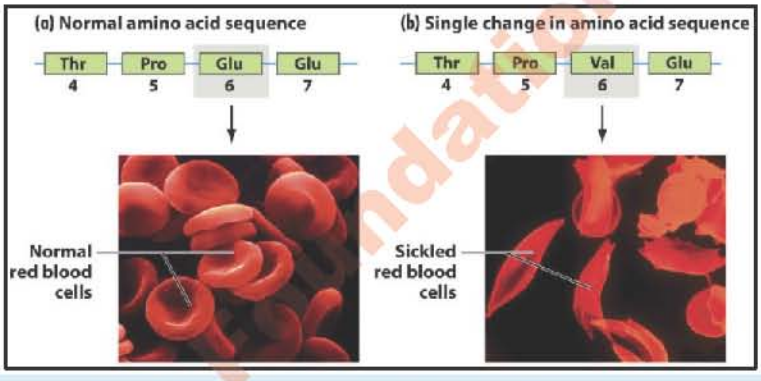

Fig: 2.28: Difference in *β*-chain of Hb^A and Hb^S

2.15 CLASSIFICATION OF PROTEINS

Based upon structure and shape proteins can be classified into two groups i.e., fibrous and globular.

Fibrous proteins

These proteins have fibre or filament like shape. Therefore, they exist in secondary structure during function. These proteins are insoluble in aqueous medium, elastic in nature and cannot be crystalized. Examples are: collagen, fibrinogen, actin, myosin and keratin.

Globular proteins

These proteins have spherical or globules like shape. Therefore, they exist in tertiary or quaternary structure during function. These proteins are soluble in aqueous medium, inelastic in nature and can be crystalized. Examples are: enzymes, hormones, antibodies, channel proteins.

2.16 ROLE OF PROTEINS

Proteins are very important molecules in our cells. They are involved in virtually all cell functions. Each protein within the body has a specific function. Some proteins are involved in support or composition of body parts i.e., structural roles, while others are involved in various physiological activities like bodily movement or in defence against germs i.e., functional roles. A list of several types of proteins and their functions is given in table 2.4 and 2.5.

Table: 2.4: List of structural proteins	
Types	**Roles of proteins**
Collagen	It establishes the matrix of bone and cartilages and components of ligaments and tendons .
Elastin	Elastin provides support for connective tissues such as tendons and ligaments.
Keratin	It strengthens protective coverings such as hair, nails, quills, feathers, horns, and beaks.
Histone	It arranges the DNA into the chromosome.

Table: 2.5 List of functional proteins	
Types	**Roles of proteins**
Enzymes	The most of enzymes are protein which control metabolism i.e., they speed up the biochemical reactions.
Hormones	Some hormones are protein in nature which are involved in the regulation of physiological activities such as regulation of glucose level, calcium level, digestion, blood pressure etc.
Antibodies	These proteins are produced by WBCs in response to antigen (a foreign particle) and provide immunity.
Haemoglobin	It is found in RBCs and is involved in the transport of oxygen mainly and carbon dioxide to some extent.
Fibrinogen	It is found in blood plasma and is involved in blood clotting process.
Ovalbumin and Casein	Ovalbumin is found in egg whites and casein is a milk-based protein. Both of them are involved in the storage of amino acids.

STEAM ACTIVITY 2.2

The presence or absence of protein in a given sample can be confirmed by biochemical tests.

Experiment	Observations	Inference
Biuret Test: Take 2 ml of test solution (Protein solution) add 2 ml of concentrated solution of NaOH and two to three drops of 1% copper sulphate solution and then shake the mixture well	Pink or Violet coloration is produced in test tube.	Protein is present in the solution
Millon's Test: Take 1 ml test solution (protein), then add 1 ml of Millon's reagent. Shake the well mixture and let is settle.	Initially White precipitate is produced which turns red if when heated	Protein is present in the solution
Ninyhdrin Test: Take 2 ml of test solution (protein), then add 3-4 drops of aqueous solution of ninhydrin reagent, boil the mixture and let it cool.	Initially White precipitate is produced which turns red if when heated	amino acids or protein is present in solution

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2.17 LIPIDS

Skills: Analyzing, Interpreting and Communication
Draw table to illustrate different structural and functional proteins with roles of each.

Lipid is the collective name for variety of organic compounds such as fats, oils, waxes and fat-like molecules (steroids) found in the body. Therefore, it is defined as a heterogeneous group of organic compounds which are insoluble in water (hydrophobic) but soluble in organic solvent such as acetone, alcohol, and ether etc. Lipids are composed of carbon, hydrogen and oxygen as carbohydrates. However, they have relatively less oxygen in proportion to carbon and hydrogen than do carbohydrates. For instance, **tristearin** is a simple lipid which shows molecular formula as $C_{57}H_{110}O_6$. Due to high contents of carbon and hydrogen, they contain double amount of energy than carbohydrates.

In general lipids are components of cell membranes (phospholipids and cholesterol), act as energy stores (triglycerides), steroid hormones and are also involved in protection, waterproofing, insulation and buoyancy.

2.18 PROPERTIES AND ROLES OF ACYLGLYCEROLS, PHOSPHOLIPIDS, TERPENES AND WAXES

2.18.1 Properties of Acyl glycerol

(1) Acylglycerols have low density than water. (2) They are insoluble in water, but they are soluble in some organic solvents like chloroform, ether and hot alcohol. (3) Acyl glycerides having saturated and long chain fatty acids have higher melting point, compare to unsaturated and short chains. For example, tristearin melts at 71°C, while triolein at −17°C. (4) Acylglycerols have great emulsifying power. (5) Hydrolysis of acylglycerols release fatty acid and glycerol when heated in an aqueous acidic medium. (6) Acylglycerols can undergo oxidation to produce odorous and flavored compounds on decayed.

2.18.2 Roles of acyl glycerides

(1) Acylglycerols make the major part of lipids in the body. (2) Triacylglycerols are the major lipids in fat de-posits and in food, (3) When high in levels, may cause various diseases such as obesity, diabetes, and hyperlipoproteinemia. (4) *Hydrogenation* of acylglycerols produce margarine and butter. Margarine lacks the vitamins that are present in butter.

2.18.3 Properties of phospholipids

(1) Phospholipids have a unique property being amphipathic molecules as they have both polar and non-polar ends. (2) They fix the proteins within the lipid bilayer cell membranes. (3) They are important components of bile and lipoproteins. (4) Properties of phospholipids

2.18.4 Functions of Phospholipids

(1) Phospholipids regulate the permeability of the membrane. (2) They are involved in the absorption of fat from the intestine. (3) They help in electron transport chain (ETC) in the mitochondria. (4) Phospholipids inhibit the accumulation of fats in the liver. (5) They transport and remove of cholesterol from the cells. (6) They are structural components of the cell membrane and are associated with proteins. (9) They act as surfactants in the respiratory

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system. (10) They help in coagulation of blood cells. (11) They synthesize different lipoproteins, prostaglandins etc.

2.18.5 Roles of Terpenes

(1) They are anti-inflammatory, analgesic (2) They are antioxidant, anticonvulsive, depressive.

(3) They are anticancer, antitumor, anti-mutagenic. (4) They are neuroprotective, anti-allergic, antibiotic. (5) They have anti-diabetic activities or role

2.18.6 Roles of Waxes

(1) Wax is effective in polishing, water proofing, anti-fouling. (2) It is used to from cosmetics and crayons, candles, car waxes. (3) Waxes are used to produce paints, inks, adhesives, and tyres.

2.19 MOLECULAR STRUCTURE OF AN ACYLGLYCEROL, A PHOSPHOLIPID AND A TERPENE

Some common lipids are acylglycerol, waxes, phospholipids, terpenes, prostaglandin and steroids.

2.19.1 Acylglycerol

The most abundant lipids in living things are acylglycerol. Chemically, acylglycerols can be defined as esters of glycerol and fatty acids. An **ester** is the compound produced as the result of a chemical reaction of an alcohol with acid and a water molecule is released such a reaction is called **esterification.**

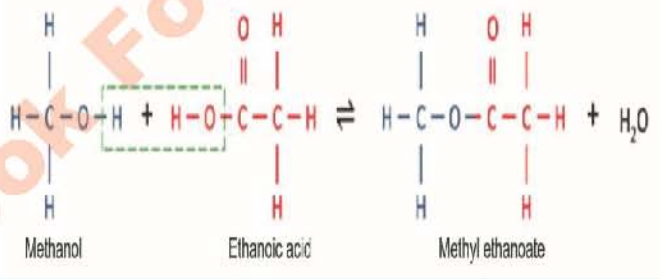

Fig: 2.29: Esterification

Glycerol is a trihydroxy alcohol which contains three carbons, each bears an OH group. A **fatty acid** is a type of organic acid containing one carboxylic acid group attached to a hydrocarbon. Fatty acids contain even number of carbons from 2 to 30. Each fatty acid is represented as R-COOH, where R is a hydrocarbon tail. When a glycerol molecule combines chemically with one fatty acid, a **monoacylglycerol** (monoglyceride) is formed. When two fatty acids combine with a glycerol a **diacylglycerol** (diglyceride) is formed and when three fatty acids combine with one glycerol molecule a **triacylglycerol** (triglyceride) is formed. Triacylglycerols are also called **neutral lipid** as all three OH groups of glycerol are occupied by fatty acids and no charge bearing OH group is left.

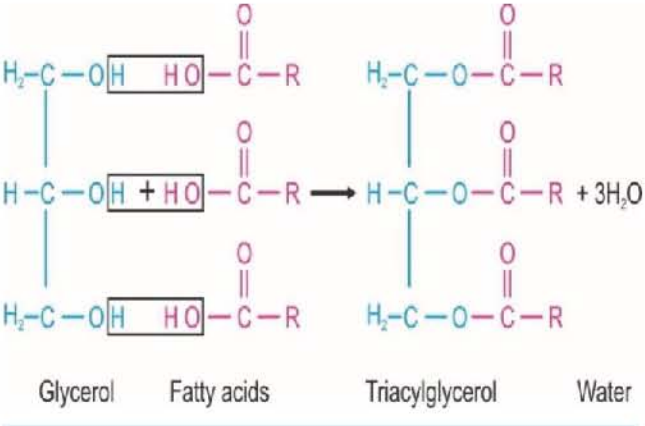

Fig: 2.30: Formation of a triacylglycerol (neutral lipid)

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Properties and types of fatty acids

About 30 different fatty acids are found. Fatty acids vary in length. Acetic acid (2C) and butyric acid (4C) are simplest fatty acid, whereas palmitic acid (16C) and stearic acid (18C) are most common fatty acids. Some properties of fatty acid are increased with an increase in number of carbon atoms, such as melting point, solubility in organic solvent and hydrophobic nature. Some common fatty acids are given in the table 2.6. Fatty acids are either saturated or unsaturated. Fatty acids in which all of the internal carbon atoms possess hydrogen side groups are said to be **saturated fatty acids** because they contain the maximum number of hydrogen atoms that are possible, e.g., palmitic acid. Saturated fatty acids tend to be solid at room temperature (higher melting point) and are more common in animal lipids (fats).

Unsaturated fatty acids have one or more pairs of carbon atoms joined by a double bond. They therefore are not fully saturated with hydrogen, e.g., oleic acid. Unsaturated fatty acids are liquid at room temperature (lower melting point) and are more common in plant lipids (oils). Triglycerides containing hydrocarbon chains melt at a low temperature. This is useful for living things.

Table: 2.6: Common types of fatty acids

Name	Typical source	No. of Carbons	Condensed Formula	Melting point (°C)
Saturated				
1. Lauric acid	Coconut oil	12	$CH_3(CH_2)_{10}COOH$	44
2. Myristic acid	Butter fat	14	$CH_3(CH_2)_{12}COOH$	58
3. Palmitic acid	Most fats and oils	16	$CH_3(CH_2)_{14}COOH$	63
4. Stearic acid	Most fats and oils	18	$CH_3(CH_2)_{16}COOH$	70
Unsaturated				
5. Oleic acid	Olive oil	18	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	4
6. Linoleic acid	Vegetable oils	18	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	-5
Linolenic acid	Soybeans and canola oils	18	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2COOH$	-11
7.Arachidonic acid	Poultry, fish egg	20	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$	-50

2.19.2 Waxes

Waxes are highly hydrophobic compounds. There are two types of waxes. **Natural waxes** are simple lipids. They are typically esters of long chain fatty acids and long chain alcohols, such as bee's wax (found in honeycomb) and cutin (on leaf surfaces of plants). These are chemically inert and resistant to atmospheric oxidation. Waxes have protective functions in plants and

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animals. **Synthetic waxes** are generally derived from petroleum or polyethylene e.g. paraffin wax which is used to make candles.

2.19.3 Phospholipids

Phospholipids are derived from **phosphatidic acid**. A phosphatidic acid molecule is most similar to diglyceride that it contains a glycerol, two fatty acids esterified with first and second OH groups of glycerol and a phosphate group esterified with third OH group of glycerol. A phospholipid is formed when phosphatidic acid combines with one of the four organic compounds such as **choline** (a nitrogenous base), **ethanolamine** (an amino alcohol), **inositol** (an amino alcohol) and **serine** (an amino acid).

Most common type of phospholipid is **phosphatidylcholine** also called **lecithin** in which choline is attached to phosphate group of phosphatidic acid. One end of the phospholipid molecule, containing the phosphate group and additional compound is hydrophilic i.e., polar and readily soluble in water. The other end, containing the fatty acid side chains, is hydrophobic i.e., non-polar and insoluble in water. These phospholipids are major constituents of lipid bilayer of cell membrane.

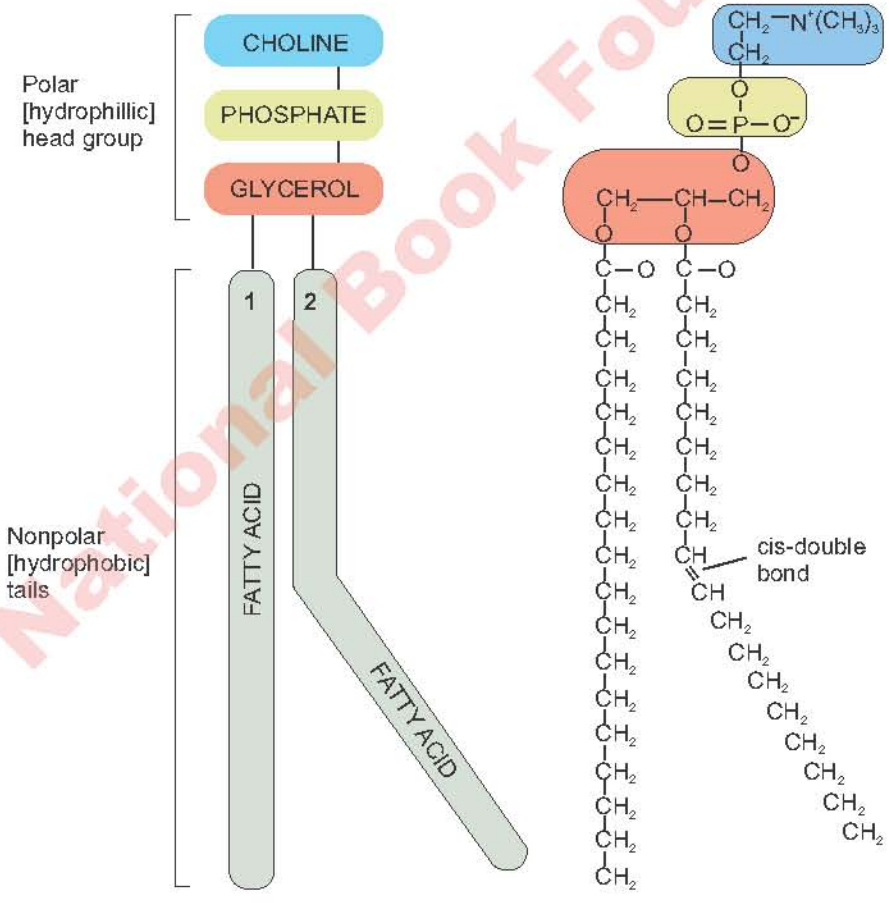

Fig. 2.31: Phosphatidylcholine (Lecithin)

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2.19.4 Terpenes

All the terpenes are synthesized from a five-carbon building block known as **isoprene unit**. This unit condenses in different ways to form many compounds. Two isoprene units form a **monoterpene** e.g., menthol, four form a **diterpene** e.g., vitamin A, phytol (chlorophyll tail) and six form a **triterpene** e.g., ambrein. Natural rubber is a **polyterpene**.

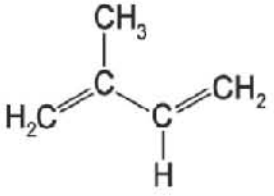

Fig. 2.32 Isoprene unit

2.20 STEROIDS AND PROSTAGLANDINS

2.20.1 Steroids

Steroids are lipids of high molecular weight which can be crystalline. A steroid nucleus consists of 17 carbon atoms arranged in four attached rings, three of the rings contain six carbon atoms, and the fourth contains five. The length and structure of the side chains that extend from these rings distinguish one steroid from other steroids. These structures are synthesized from isoprene units.

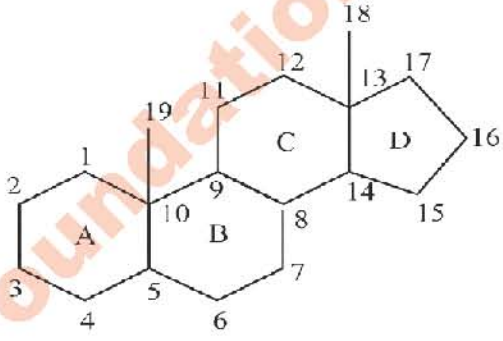

Fig. 2.33 Steroid nucleus

Cholesterol is a structural component of cell membrane. Cholesterol is the precursor of a large number of equally important steroids which include the bile acids, male sex hormone testosterone, female sex hormone progesterone and estrogen etc. Bile salts which emulsify fats and Vitamin D, which helps to regulate calcium metabolism are also steroid.

2.20.2 Prostaglandins

Prostaglandins exist in virtually every mammalian tissue, acting as local hormones. Prostaglandins are derived from **arachidonic acid**. Their functions vary widely depending on the tissue. Some reduce blood pressure, whereas others raise it. In the immune system, various prostaglandins help to induce fever and inflammation and also intensify the sensation of pain. They also help to regulate the aggregation of platelets an early step in the formation of blood clots. In fact, the ability of **aspirin** to reduce fever and decrease pain depends on the inhibition of prostaglandin synthesis.

Science, Technology and Society Connections

- Relate the role of prostaglandin in inflammation with the inhibition of prostaglandin synthesis through aspirin.

Prostaglandins play a pivotal role in inflammation a process characterized by redness (*rubor*), heat (*calor*), pain (*dolor*), and swelling (*tumor*). The changes associated with inflammation are due to dilation of local blood vessels that permits increased blood flow to the affected area. The blood vessels also become more permeable, leading to the escape of white blood cells (leukocytes) from the blood into the inflamed tissues.

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STEAM ACTIVITY 2.3

The presence or absence of lipids in a given sample can be confirmed by biochemical tests.

Experiment	Observations	Inference
A. **Spot test:** Put a drop of test solution (lipid solution) on a filter paper and allow it to dry	A clear or translucent greasy spot appears on paper	Lipid is present in the solution
B. Take a small amount of fat (butter or ghee) in a test tube and half fill the test tube with distilled water.	Fat and water remain separate	Fat is insoluble in water
C. Heat the water and fat in the test tube	Fat melts on heating but don't mix water and make clear upper layer over the lower water layer	Fat is insoluble in water and don't mix even in melted form
D. **Emulsion Test:** Take 2 ml of the test material (lipid) add 2 ml of absolute alcohol and shake well. Now add 4 ml of cold water and shake well again then allow to stand.	A cloudy white suspension (emulsion) is formed after shaking	Lipid is confirmed
E. **Sudan-III test:** Take 2 ml of the test material (lipid) add 2 ml of water, then add 4-5 drops of Sudan-III solution, shake the mixture well and let it settle.	Dark red color ring or layer formed at the surface of mixture when settles.	Lipids present in solution

2.21 NUCLEIC ACID

Nucleic acids were first reported (in 1869) by a Swiss physician when he isolated a new compound from the nuclei of pus cells (white blood cells). This compound was neither a protein nor lipid nor a carbohydrate; therefore, it was a novel type of biological molecule. He named this molecule as **nuclein**, because it was located in the nucleus. The basic structure and chemical nature of nuclein was determined (in 1920) and was renamed as **nucleic acid** because of its acidic nature.

2.21.1 Chemical Structure of Nucleic Acids

Now it has been cleared that nucleic acids are of two types i.e., **deoxyr ibo nucleic acid (DNA)** and **ribo nucleic acid (RNA)**. Both nucleic acids are linear un-branched polymers. The monomers of the nucleic acid are called **nucleotides**.

Composition of a nucleotide

Nucleotides of DNA are called **deoxyribonucleotides** and of RNA are known as **ribonucleotides**. Each nucleotide consists of pentose sugar, a phosphate and a nitrogen containing ring structure called base. The **pentose sugar** in deoxyribonucleotides is deoxyribose and in ribonucleotides is ribose. **Phosphoric acid** is a common component of both nucleotides which provides acidic properties to DNA and RNA. The nitrogen containing ring

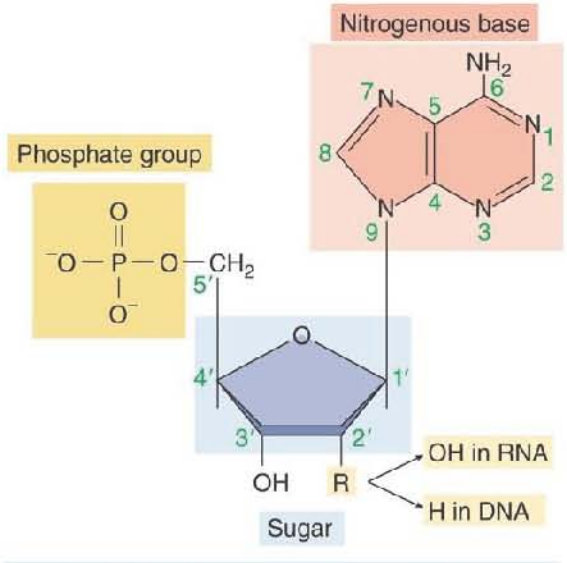

Fig. 2.34: Structure of a nucleotide

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structures are called **bases** because of unshared pair of electron on nitrogen atoms, which can thus acquire a proton.

During the formation of a nucleotide, first nitrogenous base is linked with 1′ carbon of pentose sugar. Such combination is called **nucleoside**. When a phosphoric acid is linked with 5′ carbon of pentose sugar of a nucleoside, the nucleotide is formed. A nucleotide with one phosphoric acid is called **nucleoside monophosphate** with two phosphoric acids is called **nucleoside diphosphate** and with three phosphoric acids is called **nucleoside triphosphate.**

2.22 NITROGENOUS BASES IN THE NUCLEOTIDE

There are two major classes of nitrogenous bases i.e., single ring **pyrimidine** and double ring **purines**. Pyrimidine bases are of three types i.e., cytosine (C), thymine (T) and uracil (U). Thymine is only found in DNA while the uracil is only found in RNA.

The purine bases are also of two types i.e., adenine (A) and guanine (G).

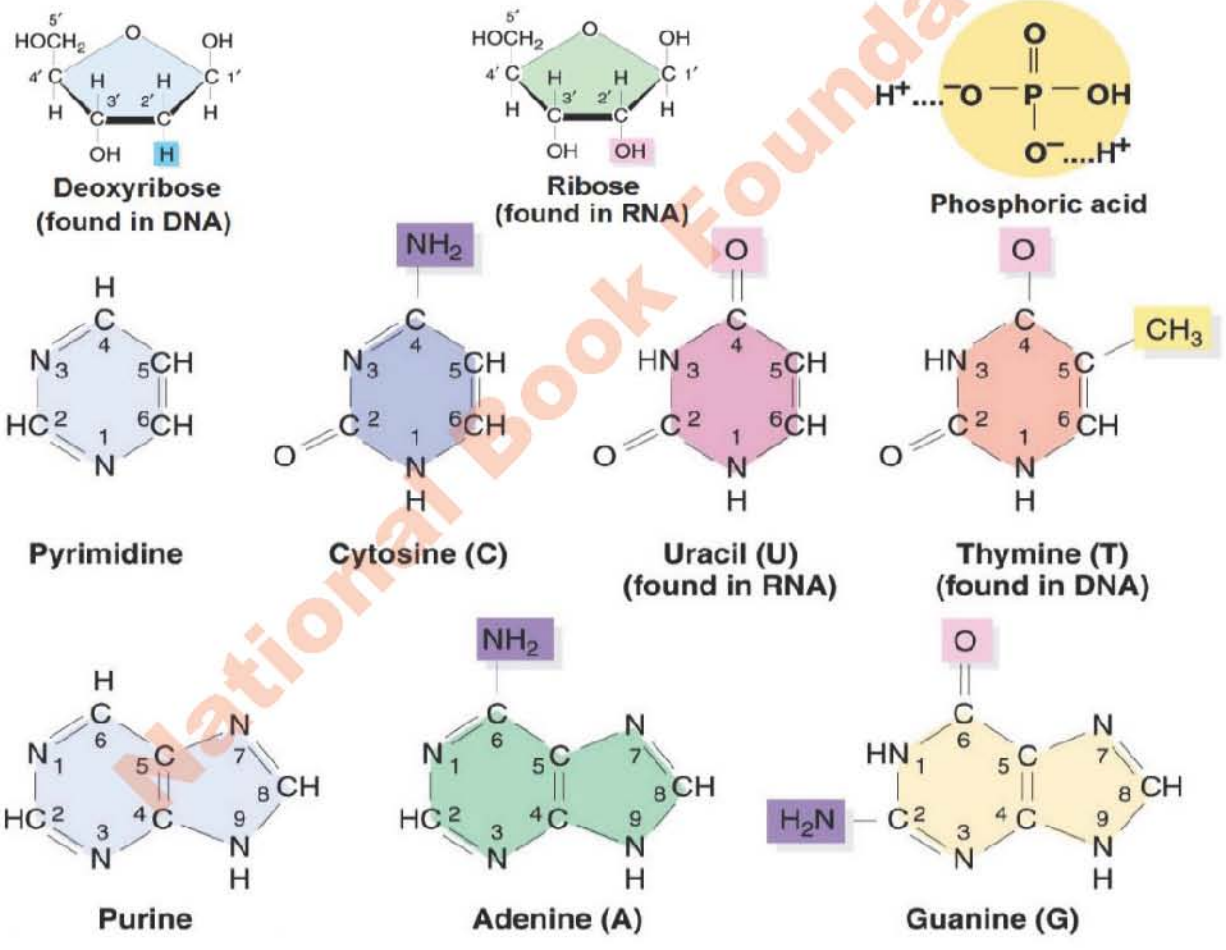

Fig. 2.35: Components of nucleotides

The nucleotides which take part in the formation of DNA or RNA must contain three phosphates but during their incorporation into DNA or RNA polymer each nucleotide losses its two terminal phosphates. Different terms used for nucleosides and nucleotides are given in the table 2.7.

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Table: 2.7: Different types of nucleosides and nucleotides of RNA and DNA				
Nitrogenos base	RNA		DNA	
	Ribonucleosides (Ribose + Base)	Ribonucleotides (Ribose+Base+ Phosphate)	Deoxyribonucleosides (Deoxyribose + Base)	Deoxyribonucleotides (Deoxyribose+Base+ Phosphate)
Adenine	Adenosine	AMP, ADP, ATP	d-Adenosine	dAMP, dADP, dATP
Guanine	Guanosine	GMP, GDP, GTP	d-Guanosine	dGMP, dGDP, dGTP
Cytosine	Cytidine	CMP, CDP, CTP	d-Cytidine	dCMP, dCDP, dCTP
Uracil/ Thymine	Uredine	UMP, UDP, UTP	d-Thymidine	dTMP, dTDP, dTTP

2.23 FORMATION OF PHOSPHODIESTER BOND (POLYMERIZATION OF NUCLEOTIDES)

Nucleotides are also joined together by a condensation reaction like other biomolecules. Unlike proteins, carbohydrates, and lipids, however, the molecule that is released is not water but pyrophosphate (two phosphate groups bound together). Mechanism of polymerization of nucleotide can be described in following steps

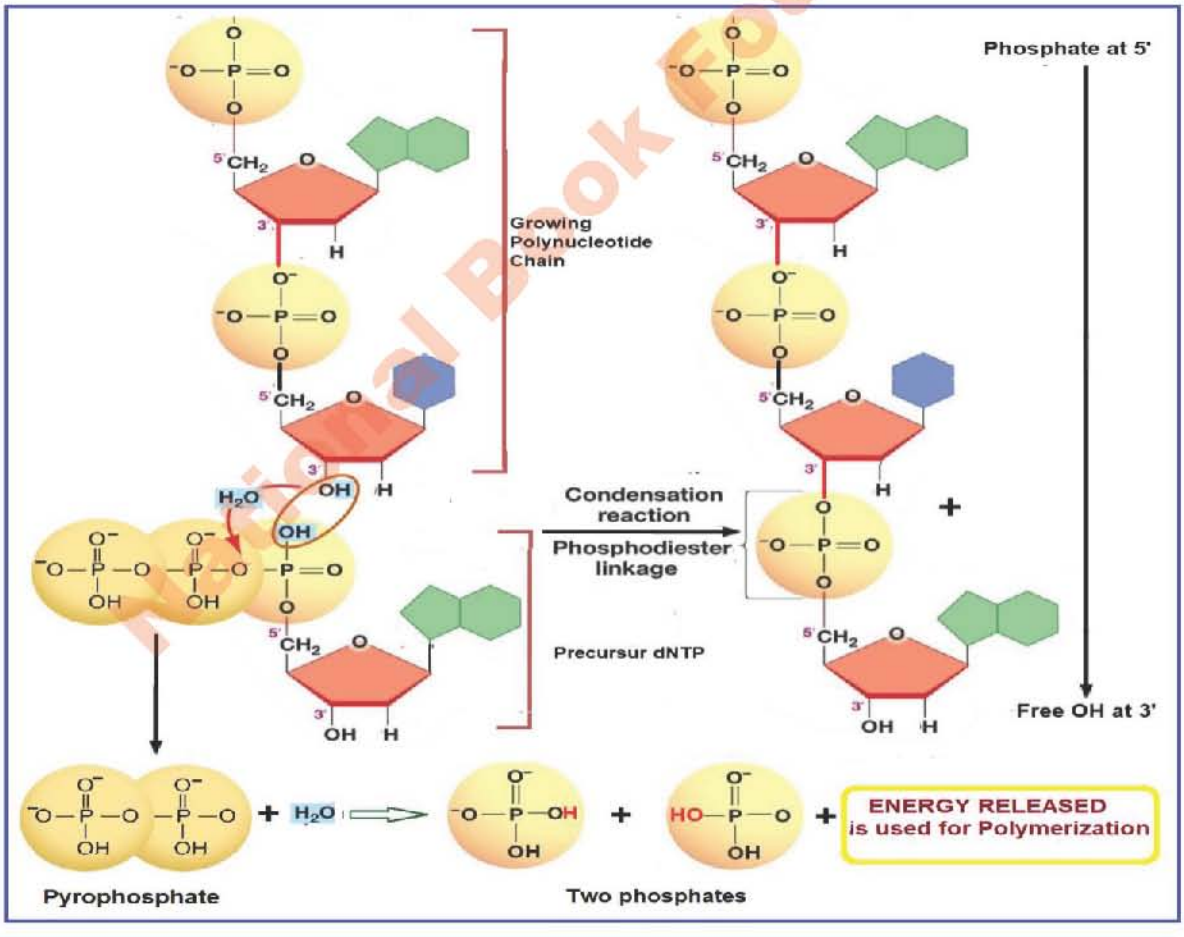

Fig. 2.36 Polymerization of nucleotides

1. The phosphate group at 5' of precursor nucleotide triphosphate (NTP) is held close to the free OH group at 3' of last nucleotide of growing polynucleotide chain to forms a phosphodiester bond between the two nucleotides.
2. Meanwhile, the bond between the alpha and beta (2nd) phosphate groups of NTP breaks and a pyrophosphate group is liberated.
3. The pyrophosphate group is hydrolysed (split by the addition of water), releasing a great deal of energy which drive the polymerisation reaction forward to completion.

In this way nucleotides begin to link by phosphodiester bonds and a polymer of nucleotides (polynucleotide) is formed. Polynucleotides have a free 5' phosphate group at one end and a free 3' hydroxyl group at the other end. By convention, these sequences are named from 5' to 3'.

2.24 Chemical Nature and Role of ATP and NAD

Adenosine triphosphate (ATP) is a mononucleotide. As shown in fig. 2.35 ATP has three parts, connected by covalent bonds: (a) adenine, a nitrogen base, (b) ribose, a five carbon sugar, (c) three phosphates. The two covalent bonds linking the three phosphates together are called **high-energy bonds**. ATP can be converted to ADP and inorganic phosphate (iP) by hydrolysis. ATP is known as the energy currency of cells.

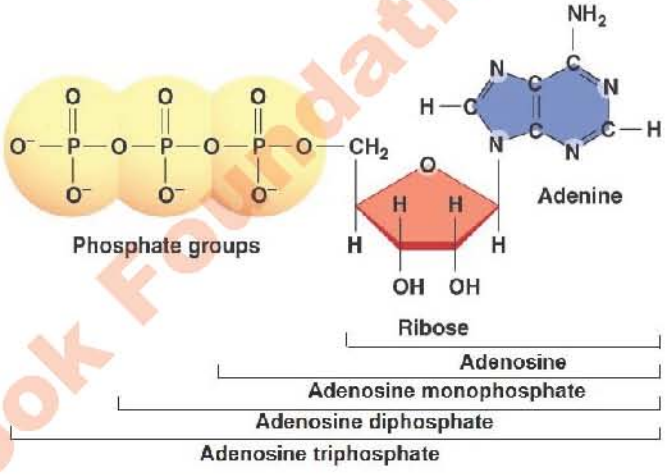

Fig. 2.37 Structure of ATP

Nicotinamide adenine dinucleotide (NAD) consists of two nucleotides. One nucleotide consists of nicotinamide, sugar and phosphate. Other nucleotide consists of adenine-sugar and phosphate. The two nucleotides are joined by their phosphate group forming a dinucleotide. NAD is a coenzyme.

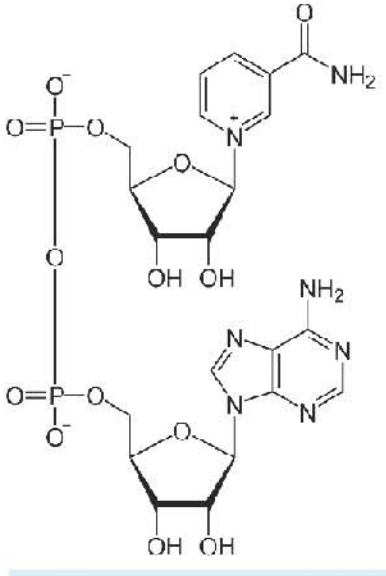

Fig. 2.38 Structure of NAD

Skills: Analyzing, Interpreting, and Communication

- Draw the Watson–Crick model of DNA
- Illustrate the formation of phosphodiester linkage.
- Elaborate the coordinated roles of all three types of RNAs in expression of genes.
- Why ATP is energy currency of cell?

Science Titbits

Watson and Crick assembled the molecular model and published their two-page article on their molecular model of DNA in the journal *"Nature"* in April 1953. Few milestones in the history of biology have as broad an impact as their double helix. They were awarded Nobel Prize in 1962 for their model of DNA.

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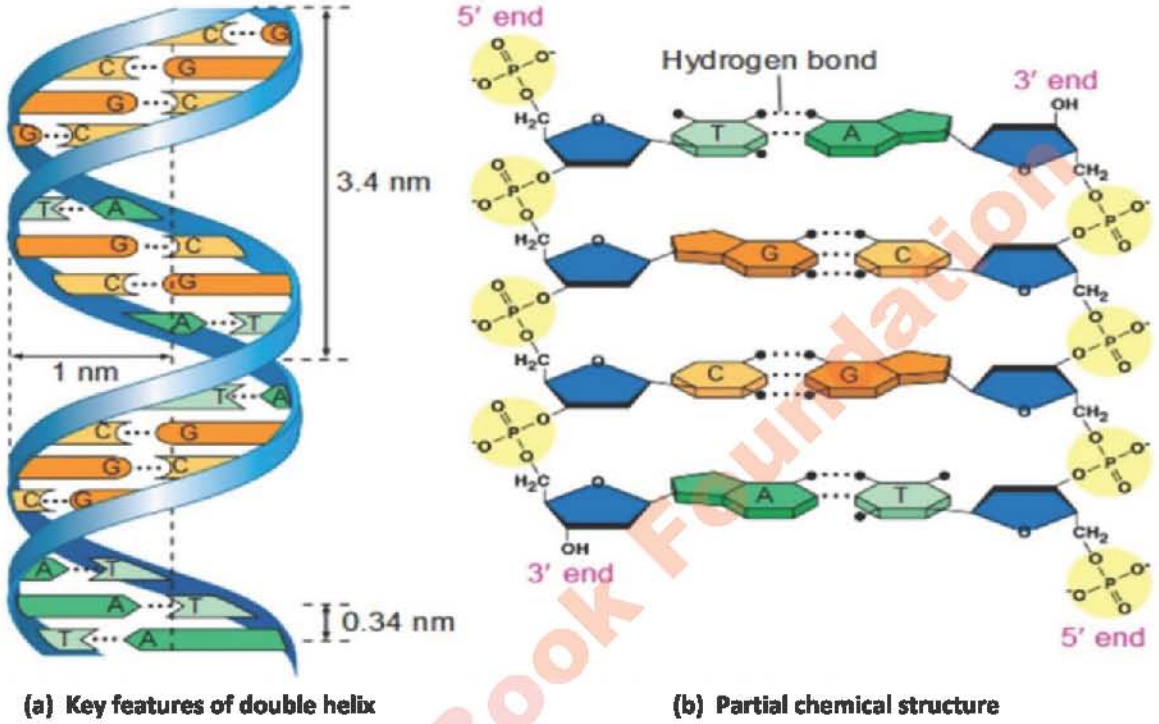
2.25 WATSON AND CRICK MODEL OF DNA

In 1951, **Erwin Chargaff** found that the nitrogenous bases in a DNA show specific ratios. He observed that amount of adenine is always equal to the amount of thymine and amount of guanine is always equal to the amount of cytosine in DNA. This implies that the total purines and total pyrimidines are in 1:1 in any DNA. This conclusion is known as **Chargaff's rule.** In



(a) Key features of double helix **(b) Partial chemical structure**

Fig. 2.39 Watson and Crick model of DNA

those days the **X-ray diffraction analysis** of DNA by **Maurice Wilkins** and **Rosalind Franklin** was published. They first time claimed that DNA is a duplex (double helix) molecule. The width of duplex is 2ηm while the length of each turn is 3.4ηm. In 1953, on the basis of these observations a graduate student **Francis Crick** and a research fellow **James Watson** of Cambridge University proposed a physical model of DNA which is now called **Watson and Crick Model of DNA.**

According to this model a DNA is made up of two polynucleotide chains which are attached together by base pairs. In order to make base pairing the two polynucleotide chains are opposite in direction i.e., one chain runs from 5′ to 3′ downward and the other c hain runs from 5′ to 3′ upward. Both chains show a constant width of 2 ηm. Therefore, both chains are supposed be antiparallel to each other. The base pairing is very specific i.e., Adenine makes the pair with Thymine and Guanine with Cytosine. The base pairs are held together by the hydrogen bond. There are three hydrogen

Science Titbits

A gene is region of DNA which is made up of nucleotides. It is the physical and functional unit of heredity. Each gene contains the information required to build specific proteins needed in an organism, such as they contain the instructions for our individual characteristics - like eye and hair colour. In order to make proteins, the gene from the DNA is copied into messenger RNA. The mRNA moves out of the nucleus and uses ribosomes to form the polypeptide that finally folds and configures to form the protein.

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bonds between Guanine and Cytosine and two hydrogen bonds between Adenine and Thymine. Each turn of the duplex consist of 10 base pairs. Both polynucleotide chains are complementary to each other. There is no restriction of the sequence of nucleotides along the length of a DNA strand. The sequence can vary in countless ways. The sequence is specific for different species, organisms and even individuals.

2.26 RIBONUCLEIC ACID (RNA)

RNA is also a polymer of nucleotides. Its detailed chemical nature has already been discussed in previous topics. Unlike DNA, the RNA is generally single stranded and does not form a double helix like DNA. However, some regions of RNA shows a secondary double stranded structure in their complementary regions. There are three major classes of RNA each with a special function in protein synthesis. These RNA are transcribed from DNA template.

2.27 ROLES AND TYPES OF RNA

2.27.1 Messenger RNA (mRNA)

mRNA consists of a single strand of variable length. Its length depends upon the size of the gene, as well as the protein for which it is taking message. For example, for a protein molecule consisting of 100 amino acids, the mRNA will have the length of 300 nucleotides. Actually every three nucleotides in mRNA encode a specific amino acid, such triplets of nucleotides along the length of mRNA are called **codons** of **genetic codes**. mRNA is about 3 to 4% of the total RNA in the cell. mRNA takes the genetic message from the nucleus to the ribosome in the cytoplasm to form particular protein. This process is known as **translation.**

2.27.2 Ribosomal RNA (rRNA)

Ribosome consists of rRNA and protein. rRNA is transcribed by the genes present on the DNA of the several chromosomes. It is called rRNA because it eventually becomes part of ribosome. The rRNA is packaged with a variety of proteins into ribosomal subunits. The base sequence of rRNA is similar from bacteria to higher plants and animals. rRNA have largest size among the RNA. Approximately, 80% of total RNA contents of a cell are rRNA. It is a part of ribosome where protein synthesis takes place. In other words rRNA provides a platform for protein synthesis.

2.27.3 Transfer RNA (tRNA)

It is the smallest of the RNA molecules and it consists of 75 to 90 nucleotides. A tRNA is a single stranded molecule but it shows a duplex appearance at its some regions where complem entary bases are bonded to one another. It shows a flat cloverleaf

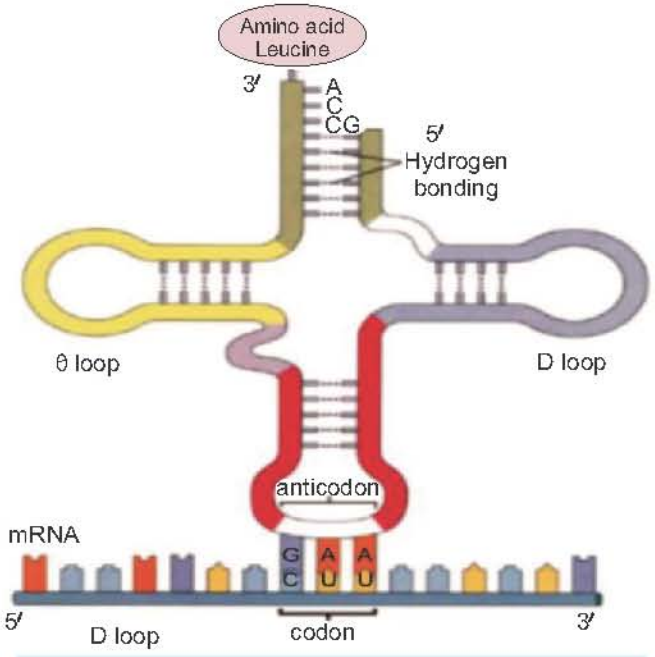

Fig: 2.40: Cloverleaf model of tRNA

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shape in two dimensional views. Its 5' end always terminates in Guanine base while the 3' end is always terminated with base sequence of CCA. Amino acid is attached to tRNA at this end. The nucleotide sequence of the rest of the molecule is variable.

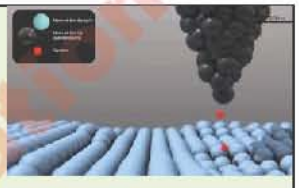
tRNA has three loops. The middle loop in all the tRNA is composed of 7 bases, the middle three of which form the **anticodon**; it is complementary to specific **codon** of mRNA. The D loop recognizes the activation enzyme. Theta (θ) loop recognizes the specific place on the ribosome for binding during protein synthesis. There is at least one tRNA molecule for each of the 20 amino acids found in proteins. Sixty tRNA have been identified. However, human cells contain about 45 different kinds of tRNA molecules, each transports a specific amino acid from cytoplasm to the surface of ribosome for protein synthesis.

Science, Technology and Society Connections

- **Correlate the scanning tunnelling microscope as the latest advancement for seeing the atoms of DNA.**

The Scanning tunneling microscope was invented in 1980. It can allow scientists to view atoms on the surface of a solid. It is a very powerful tool that can be used to resolve features less than a nanometer. The microscope's inventors, Gerd Binnig and Heinrich Rohrer were awarded Nobel Prize in Physics in 1986. Seeman's group worked on the DNA nanotechnology. They constructed molecular building blocks of DNA.

Scanning tunneling microscope



Atoms seen on the surface of a solid

2.28 CENTRAL DOGMA OF LIFE

Genes contain information needed for the synthesis of proteins molecules. The flow of biological information, from DNA to RNA and from RNA to protein is known as the **"central dogma of life"**. Protein synthesis takes place in two major steps i.e. transcription and translation. Together transcription and translation are known as **gene expression.**

Science Titbits

Why do the nucleotides in DNA have a hydrogen atom at the 2' carbon instead of the hydroxyl group in ribose? The answer is that a hydroxyl group at the 2' position can participate in a reaction that cleaves the phosphodiester bond. Thus, DNA can act as a stable long-term repository for genetic information. RNA is usually degraded within your cells in 30 minutes.

2.28.1 Transcription

Transcription is the first step of gene expression. When a new protein is needed by a cell, the two DNA strands of a gene that codes for a protein unwind from each other. A single strand of messenger RNA (mRNA) is then synthesized by pairing up complementar y mRNA nucleotides (adenine, cytosine, guanine and uracil) with the exposed DNA (non-coding) strand. After the segment is copied as mRNA, the both strands of DNA are "zipped" back together into its normal shape. Uracil is paired in mRNA as complementary to adenine instead of thymine.

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2.28.2 Translation

Translation is the second step of gene expression. The mRNA synthesized in the nucleus of the cell moves into the cytoplasm where it binds to the ribosome. The mRNA codes are translated by ribosomes with the help of tRNAs of opposite anticodon, thus synthesizing a protein with specific amino acid sequence. In this process, each set of 3 mRNA nucleotides called a codon specify an amino acid in polypeptide. Each cell has many different types of tRNAs for transferring each type of amino acid to ribosomes when needed. During protein synthesis by ribosome, amino acids are linked together by peptide bond to form a polypeptid e chain or protein.

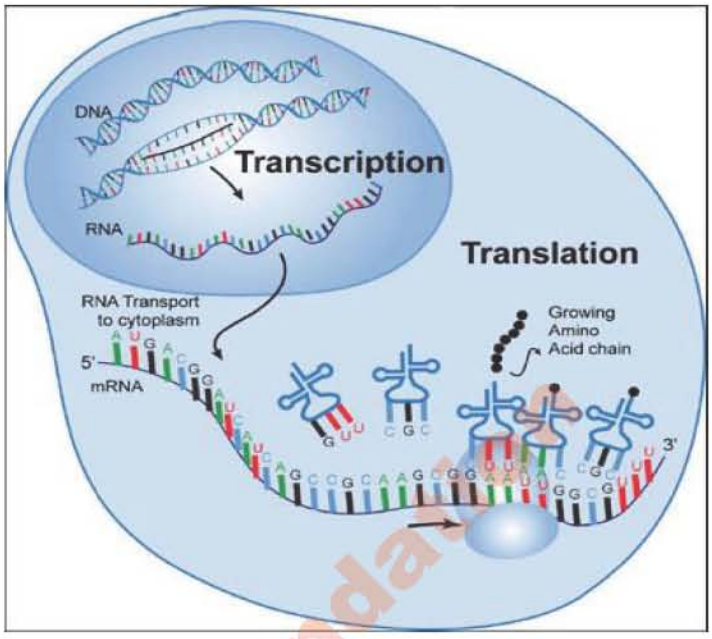

Fig: 2.41: Central Dogma of molecular biology

2.29 CONJUGATED MOLECUELS

Molecules when joined by other kinds of molecules are called conjugated molecules. The examples are glyocolipids, glycoproteins, lipoproteins and nucleoproteins.

Glycolipids are complex lipids containing one or more simple sugars in connection with long fatty acids or alcohol. Glycolipids are present in white matter of brain and myelin sheath of nerve fibres and chloroplast membrane.

Glycoproteins are formed when proteins are covalently attached to carbohydrates. Glycoproteins are widely distributed in the cells. They function as hormones, transport proteins, structured proteins and receptors. The blood group antigens contain glycoproteins, which also play an important role in blood grouping.

Lipoproteins are formed by the combination of protein with phospholipids. Phospholipid protein complexes are widely distributed in plant and animal material. They occur in milk, blood, cell nucleus, egg yolk membrane and chloroplasts of plants.

Nucleoproteins consist of simple basic protein and nucleic acid. They are found in chromosomes and ribosomes.

STEAM ACTIVITY 2.4

1. Performing Benedict's test for reducing sugars and confirmation of the presence of starch through Iodine test
2. Confirmation of the presence of proteins through Biuret test
3. Confirmation of the presence of lipids through Emulsion test
4. Demonstration of the presence of nucleic acids in biological materials e.g., onion

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Science Technology and Society Connections

- **List the career opportunities in the field of biochemistry.**

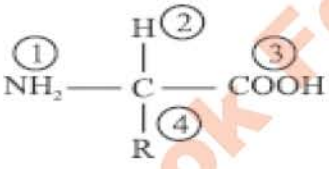
Biochemistry, the study of chemical processes that take place in living organisms, is a broad field that offers a wide range of career options. Biochemists can pursue stem cell or genetic research that has the potential to result in dramatic medical or scientific breakthroughs. Some biochemists study the body's immune response to germs and allergens or the effectiveness of drugs in treating a wide array of afflictions. Other biochemists work in the commercial food or agricultural field looking for ways to improve products and crops. The many and diverse applications of biochemistry include pharmacology, genetics, immunology, bioinformatics, environmental science, forensics, toxicological studies and food science. The career options are nearly endless, and still unfolding, as new applications for this exciting field of study continue to evolve.

EXERCISE

Section I: Multiple Choice Questions

Select the correct answer:

1. An amino acid molecule has the following structure:



Which two of the groups combine to form a peptide link between two amino acids?

A) 1 and 2 B) 1 and 3 C) 2 and 3 D) 2 and 4

2. Which class of molecule is the major component of cell membrane

A) phospholipid (B) cellulose (C) wax (D) triglyceride

3. Glycerol is the backbone molecule for

A) ATP B) terpenes C) neutral lipids D) steroids

4. A fatty acid is unsaturated if it

A) contains hydrogen B) contains double bonds

C) contains an acid group D) all of them

5. In RNA the nitrogen base that takes the place of thymine is

A) adenine B) cytosine C) guanine D) uracil

6. The ending–ose means a substance is a

A) sugar B) lipid C) protein D) nucleic acid

7. Glycolipids and lipoprotein are important components of

A) cellular membrane B) cell wall C) both of them D) none of them

8. When two amino acids are linked to form peptide linkage ..... is removed

A) hydroxyl B) water C) carbon D) nitrogen

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9. What is the theoretical number of chemically different dipeptides that may be assembled from two amino acids?

A) one B) two (C) three (D) four

10. A polar molecule is ..... in water

A) soluble (B) insoluble (C) reactive (D) innert

11. Which statement correctly describes a property of water?

A) a relatively large amount of heat is needed to increase its temperature
B) at normal room temperature, its molecules are bound together by ionic bonds
C) the highest density of water occurs below its freezing point
D) water acts as solvent for nonpolar molecules

12. Estrogen, vitamin-D and cholesterol are all examples of

A) glycolipids B) lipoproteins C) terpenes (D) steroids

13. Which term includes all others?

A) carbohydrate B) starch
C) monosaccharide D) polysaccharide

14. Choose the pair of terms that correctly completes this sentence: Nucleotide are to ------as --------are to proteins.

A) nucleic acids; amino acids B) amino acids; polypeptides
C) glycosidic linkages; polypeptide linkages D) polymers; polypeptides

15. The enantiomer of D-glucose is

A) D-galactose B) L-galactose
C) both of them D) none of them

Section II: Short Answer Questions

1. How would you describe biochemistry?
2. What are bioelements?
3. Describe the chemical composition of protoplasm.
4. What are the four fundamental kinds of biological molecules? Explain.
5. Why is the covalent bond in water polar?
6. Why water is regarded as universal solvent?
7. What is the importance of hydrogen bonding?
8. Why very large amount of heat can increase very little temperature in water?
9. How water protects living things against sudden thermal change?
10. What is the importance of high heat of vapourization of water to animals?
11. Describe classification of carbohydrates.
12. Describe the classification of monosaccharides?

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13. Describe the conversion of open chain of ribose into ring chain.
14. Draw and label the ring forms of alpha and beta glucose.
15. Justify that the laboratory-manufactured sweeteners are "left handed" sugars and cannot be metabolized by the "right handed" enzymes.
16. Illustrate the formation and breakage of (a) sucrose (b) maltose (c) lactose.
17. Draw the structural formula of amino acid.
18. Describe the synthesis of peptide bond
19. Describe the four types of structure of proteins.
20. Describe (a) globular proteins (b) fibrous proteins.
21. Describe the classification of lipids
22. What role do lipids play in living organisms?
23. Why phospholipids form a thin layer on the surface of an aqueous solution?
24. What is isoprene unit? Explain.
25. Describe a steroid nucleus.
26. How might an error in the DNA of an organism effect protein function?
27. Define gene is a sequence of nucleotides as part of DNA, which codes for the formation of a polypeptide.
28. Write the differences between:
    (a) major and minor bioelements (b)dimer and polymer (c) polar and nonpolar covalent bond (d) polyhydroxy aldehyde and polyhydroxy ketone (e)alpha and beta glucose (f)D-glucose and L-glucose (g) amylase and amylopectin (h) amylopectin and glycogen (i) primary and secondary structure of proteins (j) tertiary and quaternary structure of proteins (k) purine and pyrimidine (l) saturated and unsaturated fatty acids (m) DNA and RNA

Section III: Extensive Answer Questions

1. Describe the chemical composition of protoplasm.
2. Distinguish carbohydrates, proteins, lipids and nucleic acids as the four fundamental kinds of biological molecules.
3. Describe and draw sketches of dehydration synthesis and hydrolysis reactions for making and breaking of macromolecule polymers.
4. How the properties of water make it the cradle of life?
5. Distinguish the properties and role of monosaccharides.
6. Write the emperical formula of monosaccharides and classify them.
7. Compare the stereoisomers of glucose.
8. Distinguish the properties and role of disaccharides.
9. Describe glycoside bond in the transport of disaccharides.
10. Distinguish the properties and role of polysaccharides.
11. Describe the properties and roles of starch, glycogen, cellulose and chitin.

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12. Justify the significance of the sequence of amino acids through the example of sickle cell haemoglobin.
13. List examples and the roles of structural and functional proteins.
14. Describe the properties and roles of:

    (a) acylglycerol (b) phospholipids (c) terpenes (d) waxes

15. Evaluate the role of the following as important groups of lipids and describe their roles in living organism: (a) steroid (b) prostaglandins
16. Describe the molecular level structure of nucleotides.
17. Distinguish among the nitrogenous bases found in the nucleotides of nucleic acids.
18. Describe the structure of a mononucleotide (ATP) and a dinucleotide (NAD).
19. Explain the formation of phosphodiester bond.
20. Explain the double helical strucutre of DNA as proposed by Watson and Crick.
21. What is a gene? How gene codes for the formation of a polypeptide?
22. Explain general strucutre of RNA
23. Explain the structure and role of three types of RNA.
24. Describe the roles of the following conjugated molecules:

    (a) Glycolipids (b) glycoproteins (c) lipoproteins (d)nucleoproteins

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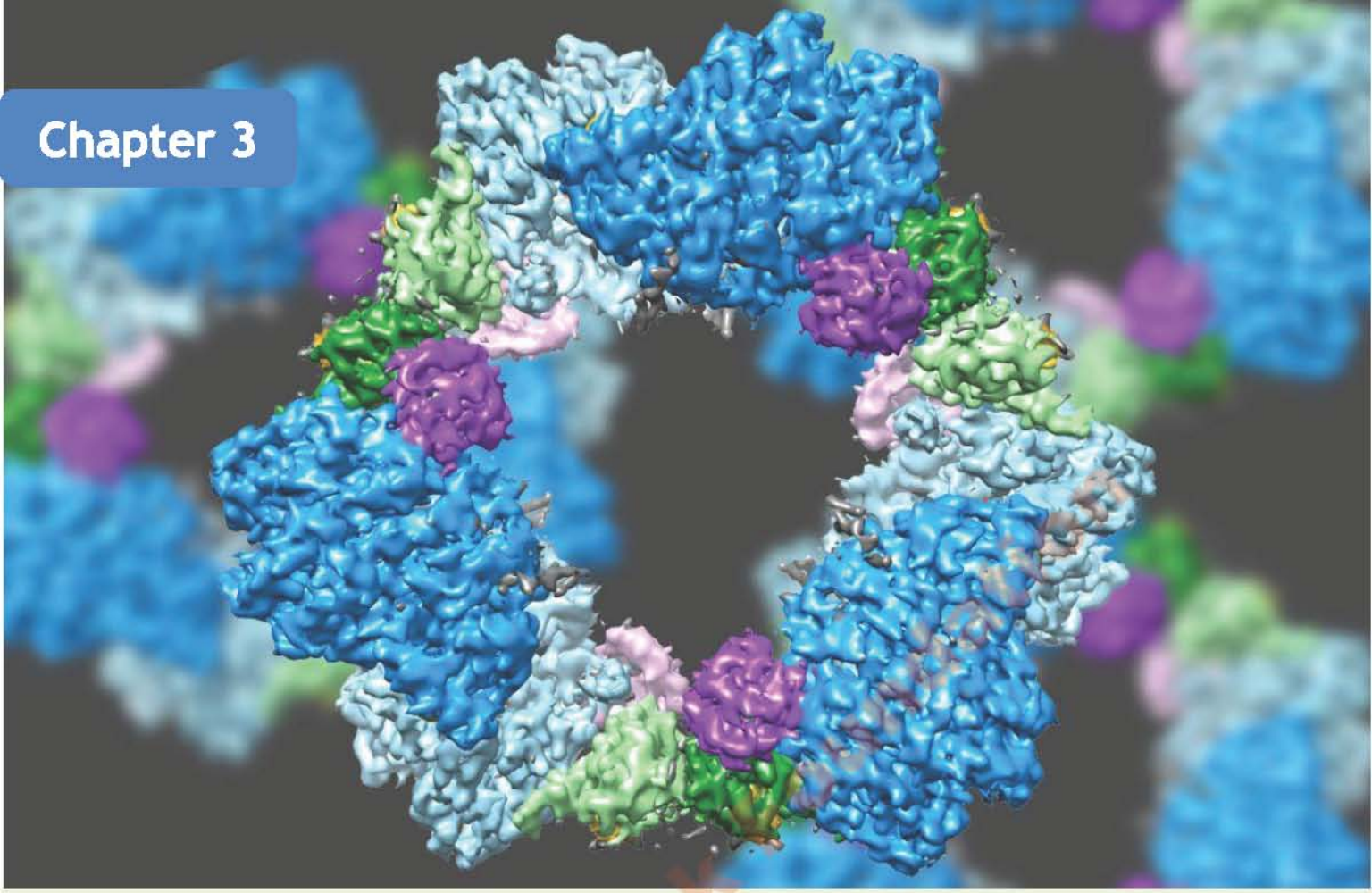

Chapter 3

ENZYMES

SLOs: After completing this lesson, the student will be able to:

1. Identify the role and component parts of the active site of an enzyme.
2. Differentiate among the three types of co-factors i.e., organic ions, prosthetic group and co-enzymes with examples.
3. Explain the mechanism of enzyme action through the induced fit model, including comparing with lock and key model.
4. Explain enzyme catalysis with examples of specific reactions.
5. Define energy of activation and discuss through graph how and enzyme speeds up a reaction by lowering the energy of activation.
6. Explain the effect of temperature on the rate of enzyme action with example of human and thermophilic bacteria.
7. Investigate the effect of pH on enzyme activity. Compare the optimum pH of different enzymes like trypsin, pepsin, and papain.
8. Demonstrate that the concentration of enzymes affects the rate of enzyme action.
9. Describe enzyme inhibition, its types and its significance with examples.
10. Name the molecules which act as inhibitors.
11. Categorize inhibitors into competitive and non-competitive inhibitors.
12. Explain feedback inhibition.
13. Classify enzymes on the basis of reactions catalyzed (oxido-reductases, transferases, isomerases and ligases.
14. Classify enzymes on the basis of substrates they use (lipases, diastase, amylase, proteases etc.

There is complete check and balance on the chemistry of cell, which is exhibited through various enzymatic reactions going on within a cell. The sum of all the chemical reactions going on in a cell is known as **metabolism**. These reactions have to be carried out very quickly so that their products can be utilized in various life activities in the cells. **Enzymes** are biological catalysts and therefore they speed up the biochemical reaction without being consumed. The enzymes exist in the cell as colloids.

Science Titbits

During the early nineteenth century, two French chemists, **Payen** and **Persoz** grounded up barley seeds in water to make a crude mixture that would digest starch. They gave the name **diastase** whatever it was that digested the starch.

We can define enzymes as 'biological polymers that catalyse biochemical reactions.'

3.1 ENZYME STRUCTURE

All the enzymes are globular proteins which are made up of one or more polypeptides. **Ribozymes** are the only which consist of RNA and are found in ribosomes. For example, peptidyl transferase is a ribozyme which forms peptide bond during protein synthesis. The enzyme consists of linear chain of amino acids, which form the three dimensional structure of an enzyme.

3.1.1 Components of an Active Site of an Enzyme

Majority of enzymes are protein in nature. The catalytic activity of an enzyme is located in its **active site.** It is a specific charge bearing, three-dimensional cavity. The substrate (the reactant which is to be converted into product) molecule is attached to the active site by non-covalent interactions like hydrogen bonding and hydrophobic interactions. Active site consists of 3-12 amino acids which may be scattered in the polypeptide but are brought together in a particular fashion due to secondary and tertiary folding of the protein molecule, e.g., the active site for aldolase consists of glycine, histidine, and alanine amino acids. An active site consists of two functional regions, i.e., binding site and catalytic site. Some amino acids have active site which makes bonds with substrate constitute the **binding site** while the other amino acids which cause conversion of substrate into product (catalysis) constitute the **catalytic site.** The shape of active site is designed according to the substrate therefore only a particular substrate can attach to the active site, however, sometime related substrate can also bind to the active site.

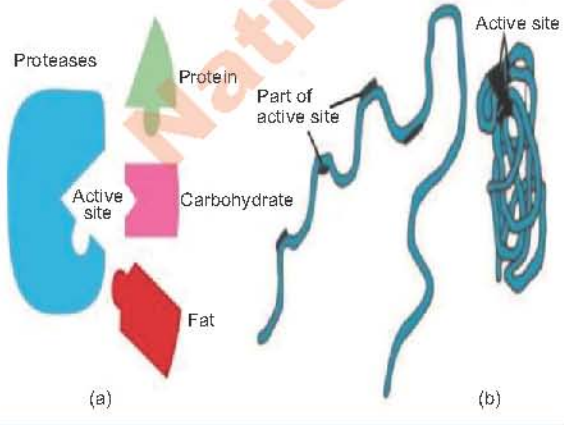

Fig: 3.1: Active site: (a) Which substrate fits the active site? (b) Grouping of amino acids of a polypeptide during the formation of tertiary

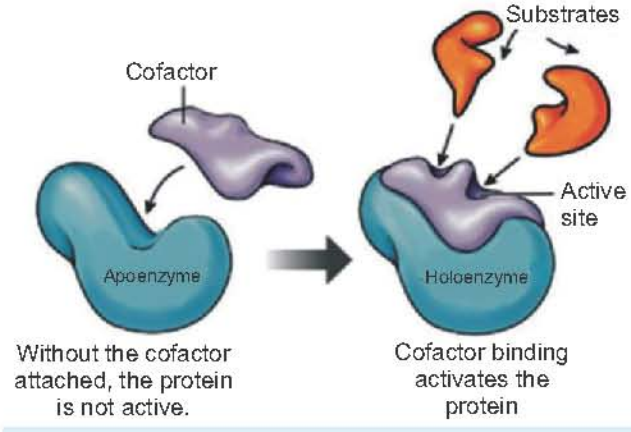

Fig. 3.2 Structure of enzyme

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Some enzymes also require a non-protein part, the **cofactor** which is not only responsible for the attachment of substrate to the active site but also participate in catalytic process. The final shape of active site is actually established after the attachment of cofactor. An enzyme which requires a cofactor becomes active only if the cofactor is combined with it. Such an active enzyme is called **holoenzyme**. If the cofactor is not available the remaining protein part of enzyme becomes catalytically inactive and is called **apoenzyme**. On the other hand, the enzymes which do not require cofactor can also show active and inactive states. Pepsin is an example of such enzyme. It is secreted by gastric gland from stomach wall in an inactive state, the **pepsinogen**. In this state, it has an additional polypeptide fragment attached to its active site which does not allow the binding of substrate, hence it remains inactive. When pepsinogen is exposed in HCl (as in stomach cavity) the additional polypeptide fragment is removed and as a result inactive (apoenzyme) pepsinogen is changed into its active (holoenzyme) form, the **pepsin**.

Science Titbits

How are enzymes formed? Enzymes are proteins, so they are formed as per message or base sequence in DNA. Enzymes are synthesized by living cells but they retain their catalytic action even when extracted from cells, i.e., they can act in vitro. These days' enzymes are also being produced by recombinant DNA technology.

3.1.2 Types of Cofactors

There are three types of cofactors: inorganic ions, organic molecules and prosthetic group.

Inorganic ions

The inorganic cofactors are different metallic ions such as Fe^{++}, Mg^{++}, Cu^{++}, Zn^{++}, etc. These are only attached to the enzymes when substrate gets bind i.e., they are detachable cofactors. Such cofactors are also called **activators**.

Glucose + ATP $\xrightarrow{\text{Hexokinase + } Mg^{+2}}$ **Glucose-6-phosphate + ADP**

Organic molecules

The organic cofactors are either co-enzymes or prosthetic groups. The **coenzymes** are the derivatives of vitamins. For example ATP, NAD^+, FAD^+ are common coenzymes. Like inorganic cofactors they are also attached to the enzymes when substrate gets bind i.e., they are also detachable cofactors.

Prosthetic group

On the other hand a **prosthetic group** is covalently bonded part of an enzyme which is permanently attached to enzyme and does not detach after the completion of reaction.

Ethyl alcohol $\xrightarrow{\text{Alcohol dehydrogenase + } NAD^+}$ **Acetaldehyde + $NADH_2$**

An iron containing porphyrin ring attached to some enzymes like cytochromes is the example of prosthetic group.

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3.2 MECHANISM OF ENZYME ACTION

The substrate first binds to the active site of the enzyme to form an **enzyme-substrate (ES) complex.** Then the substrate is converted into **product** while it is attached to the enzyme **(EP complex).** Finally the product is released, thus allowing the enzyme to start all over again.

$$\underset{\text{enzyme}}{E} + \underset{\text{substrate}}{S} \longrightarrow \underset{\text{enzyme-substrate complex}}{ES} \longrightarrow \underset{\text{enzyme-product complex}}{EP} \longrightarrow \underset{\text{enzyme}}{E} + \underset{\text{product}}{P}$$

3.2.1 Models of Enzyme Action

The mechanism of enzyme action can be explained with the help of two different models. **Emil Fischer** proposed **Lock and key model** (in 1894). According to this model, the enzyme is a lock and the substrate is a key. As the key has a similar shape to that of the keyhole of the lock, in the same manner, the substrate has a similar shape to the active site of the enzyme. The substrate binds tightly to the active site of the enzyme , just like the key into its lock. If the shape of the substrate and active site are not similar, the substrate will not be able to bind to the enzyme. An enzyme is a rigid struct ure and the shape of the active site will not change or modify during the binding process. In some books and diagrams you may find the reverse i.e. key as the enzyme and lock as the substrate.

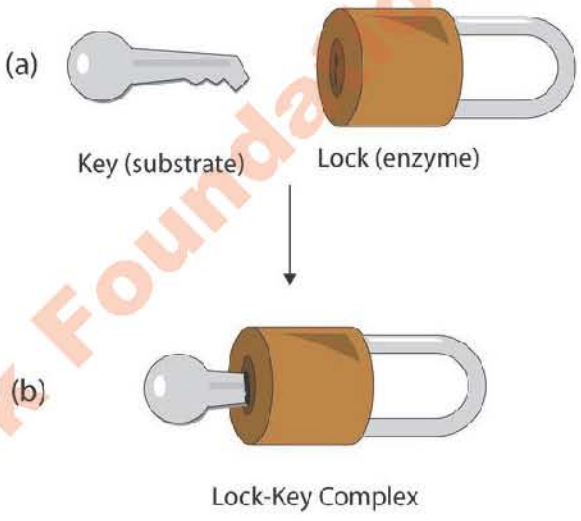

Fig 3.3: Lock (enzyme)and Key (Substrate)

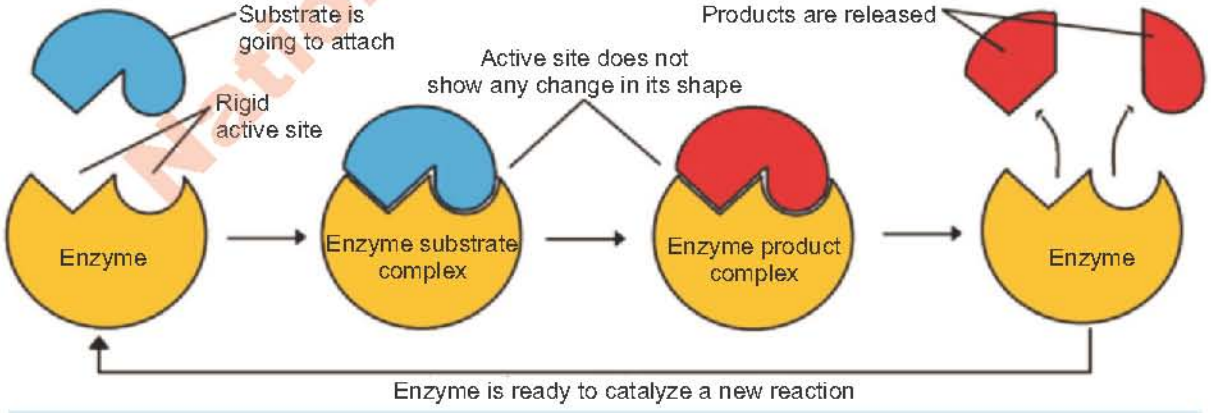

Fig: 3.4: Fischer's "Lock and Key" hypothesis of enzyme action

Actually, the notched portion of the key is equivalent to the active site on the enzyme. It reflects that enzymes are highly specific in their action and each enzyme can carry out

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only one particular reaction. The enzymes, which work according to this model, are called **non-regulatory enzymes**. However, this model is exercised by a very small number of enzymes, for example sucrase, maltase etc. The ability of enzyme to catalyze one specific reaction is perhaps its most significant property. Although, many enzymes show a broad range of specificity towards the substrate they catalyze. When one enzyme can catalyze only one substrate and essentially no others it is called **absolute specificity** e.g., urease.

$$\text{Urea + Water} \xrightarrow{\text{Urease}} \text{Ammonia + Carbon dioxide}$$

Koshland proposed **Induced fit model** (in 1959). According to this model the active site is flexible; therefore, it is modified as the substrate interacts with enzyme. The amino acids, which makeup the active site are molded into a precise shape which enables the enzyme to perform its catalytic function more effectively. The change which is induced in the shape of active site is responsible for the conversion of substrate into product. As the reaction is completed the active site regains its original shape. This is the flexibility of active site which allows more than one type of related substrates to be attached on active site and therefore, an enzyme can carry out more than one type of related reactions. The example is carbonic anhydrase which can add O_2 to haemoglobin as well as can control the formation of carbonic acid and bicarbonates in blood.

Enzymes, which follow the induced fit mechanism, are called **regulatory** or **allosteric enzymes** for example hexokinase.

$$Hb + 4O_2 \xrightarrow{\text{Carbonic anhydrase}} Hb4O_2 \text{ (Oxyhaemoglobin)}$$

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3 \xrightarrow{\text{Carbonic anhydrase}} H^+ + HCO^-$$

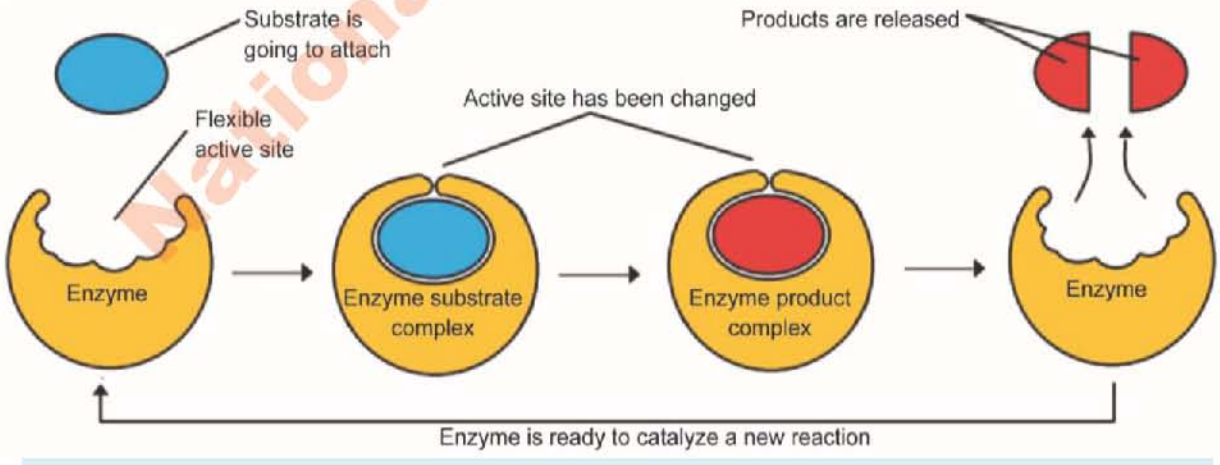

Fig: 3.5: Koshland's "Induced Fit" model of enzyme action

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Difference between induced fit and lock and key model

In the induced fit model, active site of the enzyme does not completely fit to the substrate. In the lock and key model, the active site of the enzyme is the complement of the substrate. So, it precisely fits to the substrate. In the induced fit model, the active site of the enzyme has to undergo a conformational change to improve binding. The lock and key model describes the specificity of the active site of the enzyme to a particular substrate.

3.2.2 Energy of Activation

Molecules do not react with one another unless they are activated in some way. The energy that must be added to cause molecules to react with one another is called the **energy of activation**. In non-living system, we use heat as energy of activation to increase the number of effective collision between molecules. In living systems large amount of heat cannot be used as energy of activation. Why? All living cells and organisms are mainly composed of temperature sensitive protein molecules. About 1,000 chemical reactions are being carried out in a cell at any time. Energy of activation required for such a large number of reactions cannot be provided by living system.

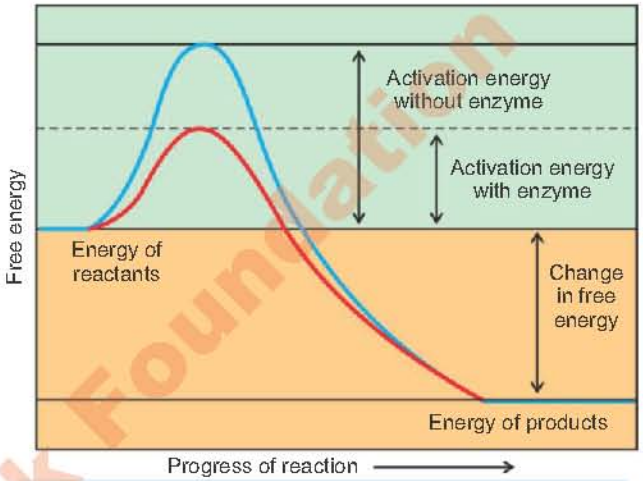

Fig: 3.6: Energy of activation: Enzymes speed the rate of chemical reactions because they lower the amount of energy required to activate the reactants and lower the need of activation energy

The living system works in isothermal condition. The excited state of molecules or reactants is achieved by biochemical process. Enzyme (E) reacts with reactant (A) to form an AE transitional complex. The energy level of AE complex reaches to the energy level of reactant B. AE complex then reacts with reactant B to form AB and enzyme (E) is released.

$$A + E \longrightarrow AE\ Complex + B \longrightarrow AB + E$$

Enzyme does decrease the energy of activation by changing energy dependent process to energy independent process. Thus the energy of activation is "energy required to break the existing bonds and begin the reaction". An enzyme greatly reduces the activation energy necessary to initiate a chemical reaction.

3.3 FACTORS AFFECTING THE RATE OF ENZYMATIC ACTION

The rate of enzymatic reaction is measured by the amount of substrate changed or amount of product formed, during a period of time. The external conditions which affect rate of enzyme reactions are: temperature, pH, concentration of enzyme and substrate concentration.

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3.3.1 Temperature

Heating increases molecular motion. Thus the molecules of the substrate and enzyme move more quickly, so probability of a reaction to occur is increased. Increasing temperature affect the rate of reaction in such a way that an increase of just 10°C in the existing temperature doubles the rate of reaction but this effect remains up to a certain limit. The temperature that promotes maximum activity is called an **optimum temperature**. If the temperature is increased above this level, then a decrease in the rate of the reaction occurs despite the increasing frequencies of collision. This is because the secondary and tertiary structures of the enzyme have been disrupted and the enzyme is said to be Denatured. The enzyme unfolds and the precise structure of the active site is gradually lost. This temperature which causes denaturation of enzyme is called **maximum temperature**. The bonds which are most sensitive to temperature change are hydrogen bonds. All human enzymes have a optimum temperature of about 37-38°C, but bacteria living in hot springs may have an optimum temperature of 70°C or higher. Such enzymes have been used in biological washing powders for high temperature washes. If temperature is reduced to near or below freezing point, enzymes are inactivated, not denatured. They will regain their catalytic influence when higher temperatures are restored. This temperature where an inactive enzyme becomes active again is called **minimum temperature.**

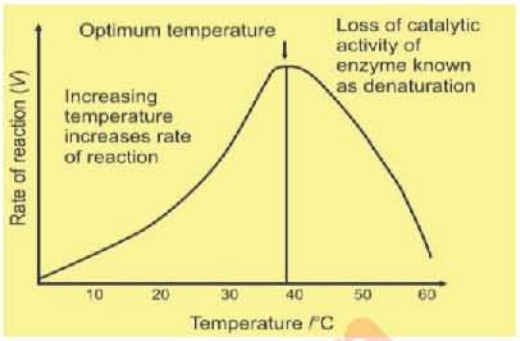

Fig: 3.7: Effect of temperature on the rate of an enzyme controlled reaction

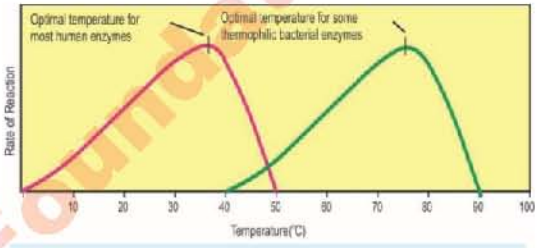

Fig: 3.8: Optimum temperature for human enzymes and thermophilic bacteria

3.3.2 pH

Every enzyme functions most effectively over a particular pH range. This narrow range of pH at which the maximum rate of reaction is achieved is called **optimum pH**. Enzyme conformation is sensitive to pH changes because pH influences the charges on the amino acid side chains that are involved in maintaining tertiary and quaternary structure of enzyme. Slight change in optimum pH of an enzyme causes ionization of amino acid of the enzyme therefore, they become inactive temporarily. On the other hand, extreme changes in optimum pH alter the ionic charge of the acidic and basic groups of enzyme and

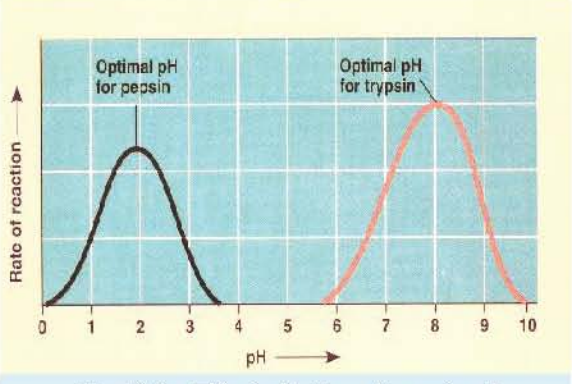

Fig: 3.9: Effect of pH on the rate of enzyme-controlled reaction

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therefore disrupts the ionic bonding (denaturation) that helps to maintain the specific shape of the enzyme.

The optimum pH values for most enzymes fall in the range of pH 6-8, but there are exceptions. Protein digesting enzyme **pepsin** is active in acidic medium at pH 2 and **trypsin** is inactive at this pH but shows maximum activity in alkaline medium at pH 8.

Critical Thinking
Industrial pollution can change the pH of a pond, lake or river to make the water more acidic. How can this affect the metabolic pathways of the plants that live in water?

Some enzymes like papain from green papaya act both in acidic and alkaline media. **Papain** is a cysteine protease acquired from the latex of the papaya plant. It has been used for protecting plants against insects. The enzyme has a high optimal temperature (65°C) and a wide pH range (5-8) for its activity.

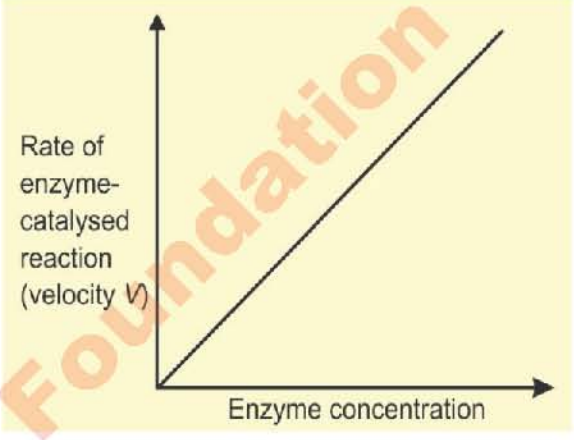

Fig: 3.10: Relationship between Enzyme concentration and the rate of an Enzyme-controlled reaction

3.3.3 Enzyme Concentration

Provided that the substrate concentration is maintained at a high level (unlimited availability), and other conditions such as pH and temperature are kept constant, the rate of reaction becomes directly proportional to the enzyme concentration. If there is only one enzyme in the system it can convert hundreds of substrates into products but it takes more time. By increasing concentration of enzyme, numbers of active sites become more available and the rate of conversion of substrate into product becomes fast. Such effect persists till the equilibrium state (when concentration of enzyme and substrate becomes equal), after that further increase in enzyme concentration will have no effect upon rate of reaction.

3.4 ENZYME INHIBITION

The phenomenon in which an enzyme fails to catalyze a reaction is called **enzyme inhibition** and the molecules which react with enzyme but are not converted into desired products are called **enzyme inhibitors**. In general, the enzyme inhibition is a normal part of the regulation of esnzyme activity within cells but sometimes when external factors cause enzyme inhibition; it may become dangerous for life. The molecules which act as inhibitors include poisons, cyanides, antibodies, anti-metabolites, penicillin, sulpha drugs etc. Inhibition may be competitive or noncompetitive.

Science Titbits
Penicillin blocks the active site of an enzyme unique to bacteria. When penicillin is taken, bacteria die but human are unaffected.

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3.4.1 Competitive Inhibition

A type of enzyme inhibition in which enzyme activity is blocked by the presence of a chemical that compete with the substrate for binding to the active site is called **competitive inhibition**. Usually, a competitive inhibitor is structurally similar to the normal substrate and so fits into the active site of the enzyme. However, it is not similar enough to substitute fully for the normal substrate in the chemical reaction and the enzyme cannot catalyze it to form reaction products.

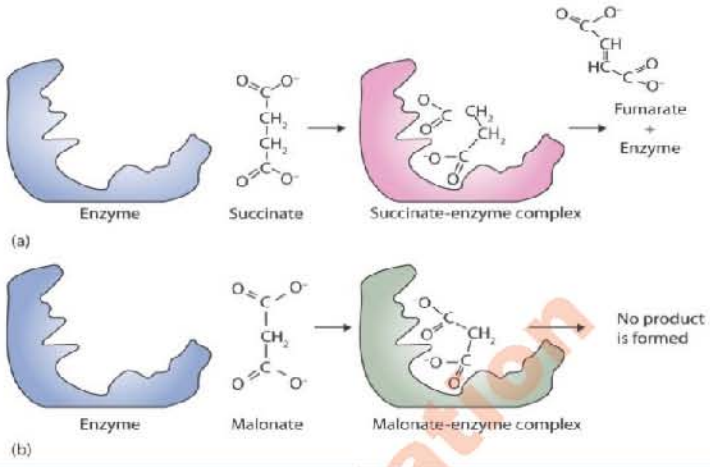

Fig: 3.11: Effect of malonate as competitive inhibitors

Competitive inhibition is usually temporary, and the inhibitor eventually leaves the enzyme hence it is also called **reversible inhibition**. This means that the level of inhibition depends on the relative concentrations of substrate and inhibitor, since they are competing for places in enzyme active sites. Therefore, if the concentration of the substrate is increased relative to the concentration of the inhibitor, the active site will usually be occupied by the substrate. An example of inhibitor is **malonate**. Succinate dehydrogenase that catalyzes the formation of fumarate from succinate is competitively inhibited by malonate.

Critical Thinking
Suggest why substrate concentration has no effect on non-competitive inhibition?

The **importance** of competitive inhibitors is: (a) It supports lock and key hypothesis. (b) It shows that substances which are similar to substrate are not acted upon by enzymes. (c) Competitive inhibitors are used as drugs in the control of bacterial pathogens. Antibiotics known as sulphonamides are used to combat bacterial infection.

3.4.2 Non-Competitive Inhibitors

In non-competitive inhibition the inhibitor molecule binds to an enzyme other than active site. The other binding site of enzyme is called **allosteric site**. The non-competitive inhibitors inactivate the enzyme temporarily (reversible inhibition) or they denature the enzyme permanently (irreversible inhibition). **Reversible non-**

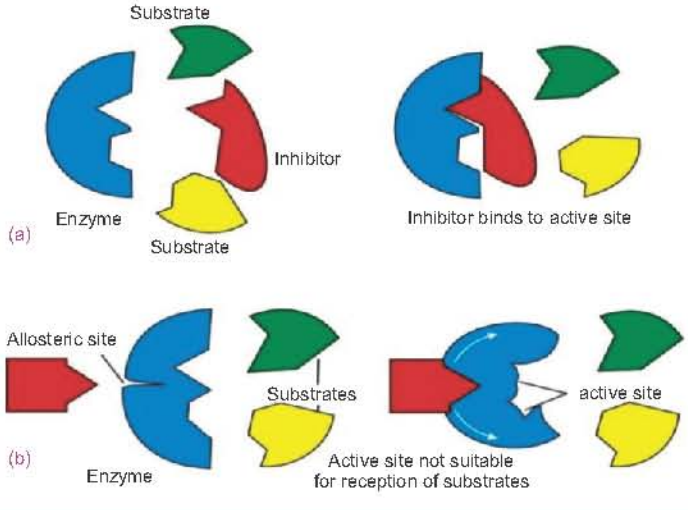

Fig: 3.12: Competitive inhibition (b) Non-competitive inhibition

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competitive enzyme inhibitors work not by preventing the formation of enzyme-substrate complexes, but by preventing the formation of enzyme-product complexes. So they prevent the substrate to be converted into product. Feedback inhibition is an example of reversible non-competitive enzyme inhibition

On the other hand, an **irreversible non-competitive** enzyme inhibitor destroys enzyme by altering its shape so that the substrate cannot bind to the active site. The examples of irreversible non-competitive inhibitors include cyanides and salts of heavy metals. **Cyanides** are potent poisons of living organism because they can kill an organism by inhibiting cytochrome oxidase essential for cellular respiration. They block the action of these enzymes by combining with iron which may be present in the prosthetic group. **Ions of heavy metals** such as mercury, silver and copper (Hg^{++}, Ag^{+}, and Cu^{++}) combine with thiol (-SH) groups in the enzyme breaking the disulphide bridges. These bridges are important in maintaining tertiary structure. When these bridges are broken, the enzyme becomes denatured and inactive.

3.4.3 Feedback Inhibition

The activity of almost every enzyme in a cell can be regulated by its product. When the activity of an enzyme is inhibited by its own product, it is called feedback inhibition. This is a type of reversible non-competitive inhibition. This phenomenon is a part of normal regulatory mechanism and usually happens during the regulation of metabolic pathways. For example, the amino acid aspartate becomes the amino acid threonine by a sequence of five enzymatic reactions. When threonine, the end product of this pathway, is present in excess, it binds to an allosteric site on enzyme 1 on this pathway and then the active site is no longer able to bind aspartate. When all the threonine is consumed in cellular events, the threonine molecule which is attached to the allosteric site is also removed; the pathway resumes its activity once again.

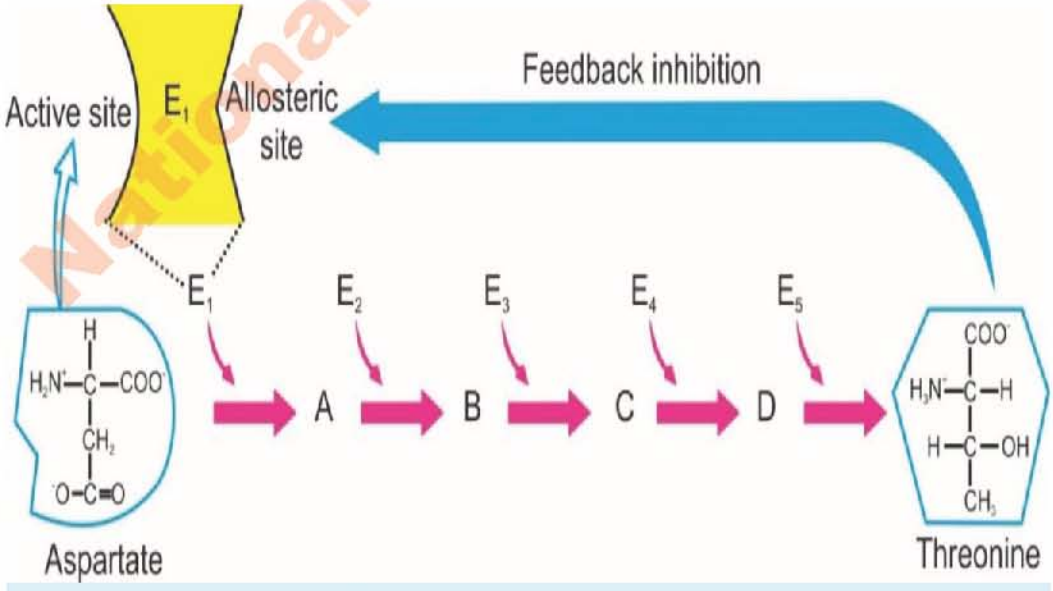

Fig: 3.13: Feedback inhibition

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The examples of Competitive inhibitors: Antibodies, antimetabolites, penicillin, iodoacetate, malonate, CoA (high conscentration) etc.

The examples of Non-competitive inhibitors: Acetaldehyde Di-Isopropyl fluorophosphate (DFP- nerve gas), mercury, silver, copper, cyanide.

3.5 CLASSIFICATION OF ENZYMES

Enzymes can be classified either on the basis of reaction types that they catalyze or on the basis of substrate which are acted upon by the enzyme.

3.5.1 Classification based upon reaction type

A systematic nomenclature and classification of enzymes based on reaction types and reaction mechanism was given by International Union of Biochemistry (in 1961).

On that basis all the enzymes have been classified into six groups:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

1- Oxidoreductases

These enzymes catalyze oxidation/reduction of their substrate and act by removing or adding electron or H^+ ions from or to the substrate. For example **cytochrome oxidase** oxidizes cytochrome.

2- Transferases

These enzymes catalyze the transfer of specific functional group other than hydrogen from one substrate to another. The chemical group transferred in the process is not in a free state, for example **hexokinase** transfers a phosphate group from ATP to glucose.

Science Titbits

How are enzymes named?

(a) Enzymes are named by adding "ase" to the name of substrate they act, e.g., proteases, lipases etc. (b) Enzymes are named according to the types of reaction they catalyse, e.g., oxidases, reductases etc. (c) Enzymes are named by taking into consideration both the substrate acted upon and the type of reaction catalysed, e.g., DNA- polymerase. (d) Some enzymes are named as per substance synthesized, e.g., rhodonase catalyses synthesis of rhodonate from hydrochloric acid and sodium thiosulphate.

3- Hydrolases

These enzymes bring about the breakdown of large complex organic molecules into smaller ones by adding water (hydrolysis) and breaking the specific covalent bonds. Examples are proteolytic enzymes which breakdown proteins into peptones and peptides such as **pepsin, renin** and **trypsin.** Other digestive enzymes that work in digestive tract are also the examples of hydrolases.

4- Lyases

These enzymes catalyze the breakdown of specific covalent bonds and removal of groups without hydrolysis. For example **histidine decarboxylase** breaks the covalent bonds between carbon atoms in histidine forming carbon dioxide and histamine.

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5- Isomerases

These enzymes bring about intra-molecular rearrangement of atoms in the molecules and thus forming one isomer from another. For example **phosphohexose isomerase** changes glucose 6- phosphate to fructose 6- phosphate.

6- Ligases (Synthetases)

These enzymes bring about joining together of two molecules. The energy is derived by hydrolysis of ATP. For example **polymerases** are responsible for linking monomers into a polymer such as DNA or RNA.

3.5.2 Classification based upon substrate

Enzymes can be classified on the basis of substrates they use. Some of the examples are: proteases, lipases, carbohydrases and nucleases.

1- Proteases

These enzymes act upon proteins. Examples are: **pepsin** and **trypsin** (both digest large polypeptides into small polypeptides or peptones), **aminopeptidases** and **carboxypeptidases** (both digest peptones into dipeptides) and **erypsin** (digest dipeptides into amino acids)

2- Lipases

These enzymes hydrolyze lipids into fatty acids and glycerols. Examples are **pancreatic lipases.**

3- Carbohydrases

These enzymes cause breakdown of carbohydrates. Examples are:

(a) **amylase** (digest starch or glycogen into maltose)

(b) **cellulase** (digest cellulose into cellubiose, a disaccharide)

(c) **maltase** (digest maltose into glucoses)

(d) **sucrase** (digest sucrose into glucose and fructose)

(e) **lactase** (digest lactose into galactose and glucose)

4- Nucleases

These are involved in the breakdown of DNA and RNA. Examples are:

(a) **RNAases** (digest RNA into ribonucleotides)

(b) **DNAases** (digest DNA into deoxyribo nucleotides).

(c) **ATPases** (cause hydrolysis of ATP in muscles etc.)

Diagnostic uses of enzymes.

(a) Aldolase: progressive muscular dystrophy, viral hepatitis and advanced cancer of the prostate
(b) Creatine Phosphokinase: damage to muscle cells.
(c) Gamma-glutamyl Transpeptidase: in assessing liver function.
(d) Lactic Dehydrogenase: in differentiating heart attack, anemia, lung injury, or liver disease.
(e) Lipase: Damage to the pancreas.

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Venoms as enzyme inhibitors

Snake venom is highly modified saliva that is produced by special glands of certain species of snakes. Snake venom is a combination of many toxins (proteins) and different enzymes, use for the purposes like increasing the prey's uptake of toxins. Snake venom inhibits cholinesterase to make the prey lose control of its muscles. Venom is an inhibitor for an essential enzyme cytochrome oxidase in the cells. There are three distinct type of venom that act on the body differently.

(1) Hemotoxic venoms act on the heart and cardiovascular system.

(2) Neurotoxic venom acts on the nervous system and brain.

(3) Cytotoxic venom has a localized action at the site of the bite. Venom occupies the active site of the enzyme or combining with the iron which may present in the prosthetic group or which may be required as an enzyme activator.

STEAM ACTIVITY 3.1

Title: "Enzyme Engineering: Creating Lactose-Free Milk with Immobilized Enzymes"

Objective

Investigate the concept of immobilized enzymes and their application in the removal of lactose from milk using lactase-embedded sodium alginate beads.

Materials Required: Lactase enzyme, Droppers or pipettes, Sodium alginate, pH meter or pH paper, Calcium chloride solution, Timer or stopwatch, Milk (containing lactose), Thermometer, Beakers and stirrers, Strainer or filter

Procedure

Begin by preparing lactase-embedded sodium alginate beads:

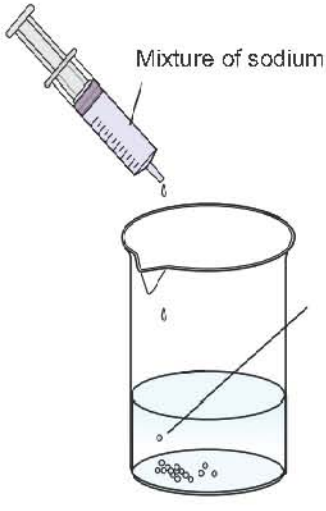
Mix lactase enzyme with sodium alginate to form a homogeneous solution.

Drop small amounts of the solution into a calcium chloride bath to create beads. Allow the beads to harden.

Rinse the beads with water to remove excess calcium chloride.

Obtain fresh milk containing lactose.

Introduce the lactase-embedded beads into the milk and stir gently.



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Monitor the reaction over time, noting any changes in lactose concentration.

Measure the pH of the milk before and after the enzymatic treatment.

Use a thermometer to monitor the temperature during the process.

Filter the milk to separate the beads from the liquid.

Test the resulting milk for lactose content using lactose detection strips or a lactose testing kit.

As an alternative, you can use Benedict's test to identify the reducing sugars, such as galactose and glucose, which are produced when lactose is broken down.

Figure 3.14 Removal of lactose

Results

Compare the lactose levels in the treated milk with the original milk.

Discussion

Discuss the immobilization process, enzyme-substrate specificity, and the advantages of using immobilized enzymes for lactose removal.

Does the processed milk contain lactase?

What potential drawbacks can there be when making lactose-free milk with non-immobilized lactase enzyme?

Explore the potential applications of immobilized enzymes in various industries, such as food and beverage.

Reflection:

This activity allows students to engage in enzyme engineering, understand the principles of immobilization of enzymes, and experience the practical application of enzymes in the food industry, addressing lactose intolerance concerns.

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EXERCISE

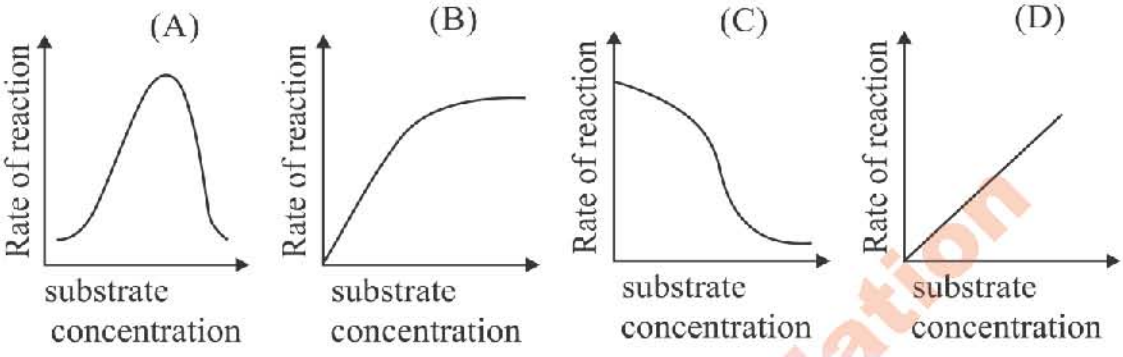
Section I: Multiple Choice Questions

Select the correct answer:

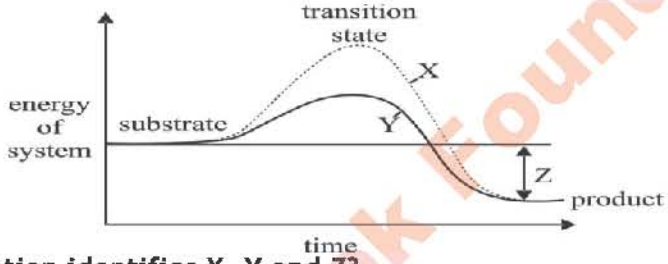
1. The catalytic activity of an enzyme is restricted to its small portion called
   - A) active site
   - B) passive site
   - C) regulation site
   - D) allosteric site
2. Which of the following has a coenzyme activity?
   - A) NAD^+
   - B) Ca^{++}
   - C) both "a" and "b"
   - D) none of them

3. Non-competitive inhibitors react with enzymes at:
   A) active site
   B) allosteric site
   C) both "a" and "b"
   D) none of them

4. Which graph shows the expected relationship between enzyme activity and substrate concentration?



5. The graph shows the effect of an enzyme on a reaction.



Which combination identifies X, Y and Z?

	X	Y	Z
A	catalyzed reaction	uncatalyzed reaction	activation energy
B	catalyzed reaction	uncatalyzed reaction	energy lost during reaction
C	uncatalyzed reaction	catalyzed reaction	energy gained by product
D	uncatalyzed reaction	catalyzed reaction	overall energy change

6. Combination of apoenzyme and coenzyme produces
   A) prosthetic group
   B) holoenzyme
   C) enzyme
   D) isoenzyme

7. The specificity of enzyme is due to their
   A) surface configuration
   B) pH
   C) hydrogen bonding
   D) high molecular weight

8. An essential feature of a competitive inhibitor is its ability to
   A) activate an operator gene
   B) combine with prosthetic group
   C) modify a substrate
   D) occupy an active site

9. The reaction rate of salivary amylase with starch decreases as the concentration of chloride ions is reduced. Which of the following describe the role of the chloride ions?
   A) allosteric inhibitors
   B) cofactors
   C) coenzyme
   D) competitive inhibitor

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10. How does an enzyme increase the rate of a reaction?
    A) by bringing the reacting molecules into precise orientation
    B) by increasing the rate of random collisions of molecules
    C) by shifting the point of equilibrium of the reaction
    D) by supplying the energy required to start the reaction
11. Many enzymes are secreted in inactive form to protect
    A) cell proteins B) mitochondria
    C) cell membrane D) cell DNA
12. Erypsin is an example of?
    A) carbohydrases B) proteases
    C) lipases D) nucleases
13. Ribozymes consist of:
    A) only protein B) protein + none protein part
    C) only RNA D) none of them
14. If an enzyme solution is saturated with substrate, the most effective way to obtain an even faster yield or products is to;
    A) Add more of the enzyme
    B) Add more substrate
    C) Add an allosteric inhibitor
    D) Add a noncompetitive inhibitor
15. If an enzyme is added to a solution where its substrate and products are in equilibrium, what would occur?
    A) Nothing the reaction would stay at equilibrium.
    B) Additional products would be formed.
    C) Additional substrate would be formed.
    D) The free energy of the system would change.
16. Enzymes
    A) Make it possible for cells to escape the need for energy.
    B) Are non-protein molecules that help coenzyme
    C) Are not affected by change of pH
    D) Lower the energy of activation
17. An allosteric site on an enzyme on an enzyme is
    A) The same as the active site
    B) Where ATP attaches and gives up its energy
    C) Often involved in feedback inhibition
    D) All of these are correct

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18. At high temperature
    A) Can affect the shape of an enzyme
    B) Lowers the energy of activation
    C) Makes cells less susceptible to diseases
    D) Both A and C are correct

19. The coenzyme is:
    A) often a metal
    B) always a protein
    C) often a vitamin
    D) always an inorganic compound

20. Blocking of enzyme action by blocking its active site is called as:
    A) feedback inhibition
    B) allosteric inhibition
    C) competitive inhibition
    D) non-competitive inhibition

Section II: Short Answer Questions

1. What are ribozymes?
2. What is the structure of enzyme?
3. Explain the enzyme pepsin which does not require cofactor.
4. What is prosthetic group? Give an example.
5. What is the mechanism of enzyme action?
6. What is the role of free energy of activation in a chemical reaction?
7. List the external conditions which affect rate of enzyme reaction.
8. Compare the optimum temperatures of enzymes of human and thermophilic bacteria.
9. Describe the range of pH at which human enzymes function.
10. What are enzyme inhibitors? Name the molecules which act as enzyme inhibitors.
11. What is the importance of competitive enzyme inhibitors?
12. Describe cyanides as irreversible non-competitive inhibitor.
13. Describe ions of heavy metals as irreversible non-competitive inhibitor.
14. Write the difference between:
    (a) binding site and catalytic site of an enzyme
    (b) apoenzyme and holoenzyme
    (c) prosthetic group and coenzyme
    (d) inorganic cofactor and organic cofactor
    (e) lock and key model and Induced fit model of enzyme action
    (f) competitive and noncompetitive enzyme inhibitors
    (g) reversible non-competitive enzyme inhibitors and irreversible non-competivie enzyme inhibitors

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Section III: Extensive Answer Questions

1. Write the properties of enzymes.
2. Explain the role and component parts of the active site of an enzyme.
3. What are cofactors? Describe the two types of cofactors by giving examples.
4. Explain the mechanism of enzyme action through induced fit model.
5. Explain the mechanism of enzyme action through lock and key model.
6. Explain how an enzyme catalyzes specific reactions.
7. Explain through graph how an enzyme speeds up reaction by lowering the energy of activation.
8. Describe the effect of temperature on the rate of enzyme action.
9. Describe how the concentration of enzyme affects the rate of enzyme action.
10. Explain the effect of substrate concentration on the rate of enzyme action.
11. Describe enzymatic inhibition, its types and its significance.
12. Explain feedback mechanism with reference to enzymes.
13. Classify enzymes on the basis of reactions catalyzed.
14. Classify enzymes on the basis of the substrate they use.

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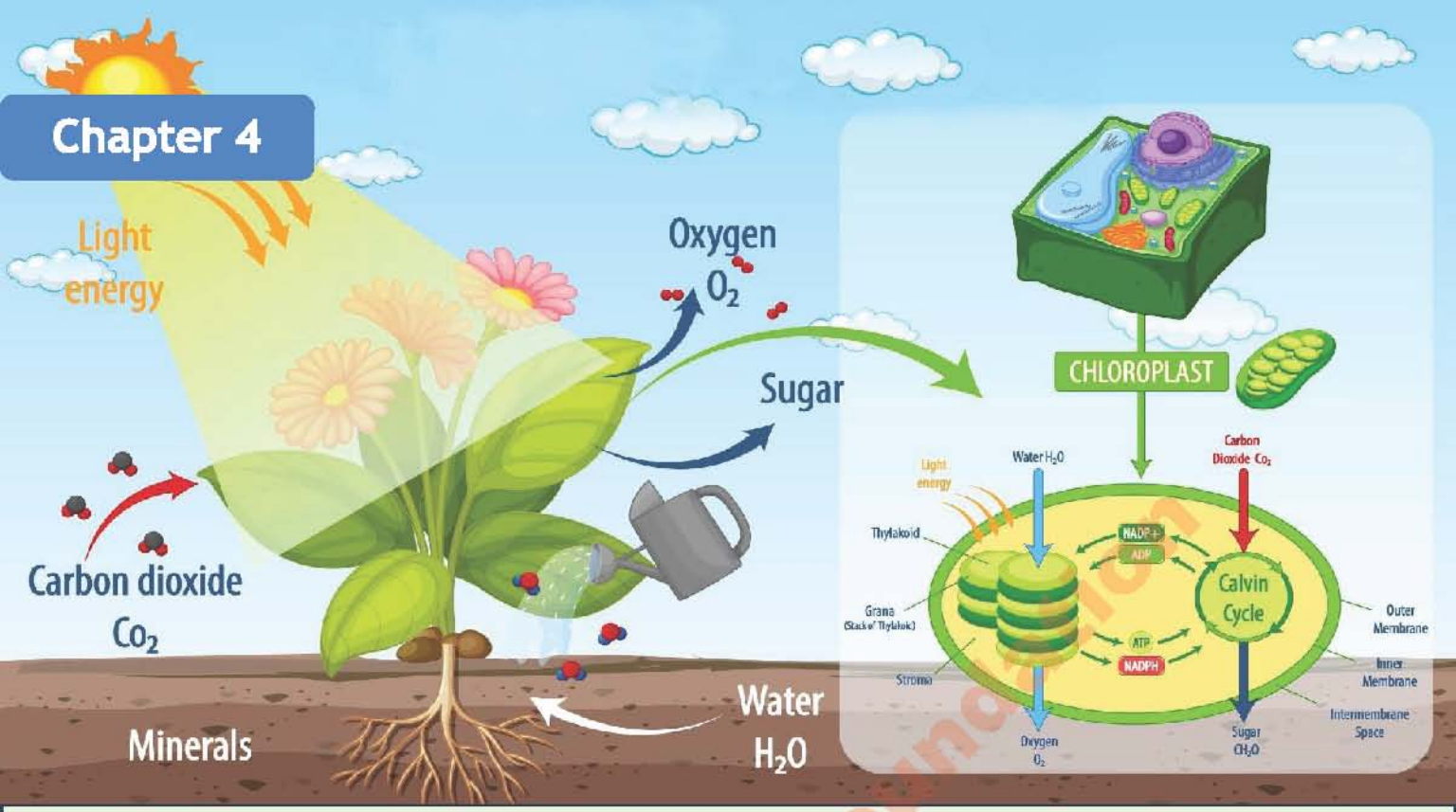


BIOENERGETICS

SLOs: After completing this lesson, the student will be able to:

1. Explain the role of light, carbon dioxide and water in photosynthesis
2. Identify the two general kinds of photosynthetic pigments (carotenoids and chlorophylls)
3. Describe the roles of photosynthetic pigments in the absorption and conversion of light energy
4. Differentiate between the absorption spectra of chlorophyll 'a' and 'b'
5. Describe the arrangement of photosynthetic pigments in the form of photosystem-I and II. [
6. Describe the events of non-cyclic photophosphorylation and cyclic photophosphorylation.
7. Explain the Calvin cycle (the regeneration of RuBP should be understood in outline only.)
8. Explain the process of anaerobic respiration in terms of glycolysis and conversion of pyruvate into lactic acid or ethanol.
9. Illustrate the links reaction as conversion of pyruvate to acetyl-CoA.
10. Outline the steps of Krebs cycle.
11. Trace the passage of electron through electron transport chain.
12. Describe chemiosmosis and relate it with electron transport chain.
13. Explain the substrate-level phosphorylation during which exergonic reactions are coupled with the synthesis of ATP.
14. Justify the importance of G3P in photosynthesis
15. Outline the formation of acetyl CoA from fats
16. Compare and contrast respiration of fats and glucose.
17. Define photorespiration
18. Outline the events occurring through photorespiration.
19. Rationalize how the disadvantageous process of photorespiration evolved.
20. Explain the effect of temperature on the oxidative activity of RuBP carboxylase.
21. Outline the process of C4 photosynthesis as an adaptation evolved in some plants to deal with the problem of photorespiration.

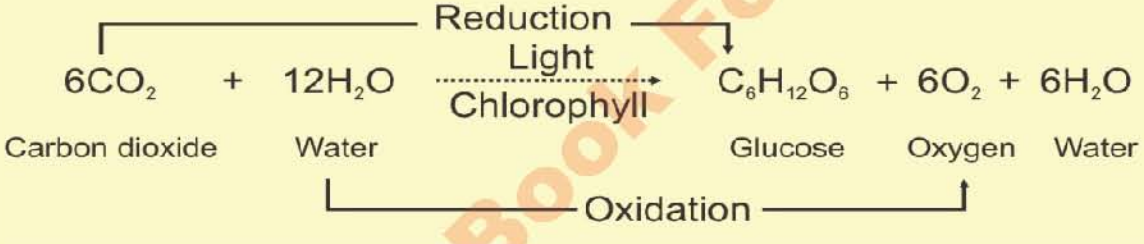
Living things cannot grow, reproduce, or exhibit any of the characteristics of life without a ready and continuous supply of energy. All metabolic reactions involve energy transformations. So the quantitative study of energy conversions and energy relationships in biological system is called **bioenergetics.**

This chapter deals with the most fundamental bioenergetics processes i.e., photosynthesis and respiration. You have already got an introduction about the major processes of bioenergetics in IX-X biology course. The detailed learning would foster the insight analysis and application of these processes in real life. This chapter also develops the basic concepts of photorespiration, the process that reduces plants productivity.

4.1 PHOTOSYNTHESIS

Chemically photosynthesis is a **"redox"** process in which CO_2 (an oxidized form of carbon) is reduced into glucose (a reduced form of carbon). Water acts as reducing agent which is oxidized into oxygen during this process. Bio-energetically photosynthesis can be defined as an energy conversion process in which energy poor molecules i.e., CO_2 and H_2O are transformed into energy rich molecule such as glucose. The extra energy is absorbed in the form of sunlight by the photosynthetic pigments.

The overall reaction of photosynthesis can be summarized as follows:



This process involves the interaction of sunlight, pigments, water and carbon dioxide.

4.1.1 Role of Light

Biological energy transformations obey the laws of thermodynamics. Sunlight is the ultimate source of energy which is continuously flowing one way from Sun to life and ecosystem. Sunlight is an electromagnetic form of energy. Light has the prime importance as light energy is converted to chemical energy and then to other forms of energy essentially required for life processes. This trapped light energy is used to convert the low energy inorganic compounds to high energy organic compounds. The full range of electromagnetic radiation in the universe is called **electromagnetic spectrum.** Visible light is only a small part of the spectrum between 380*nm*

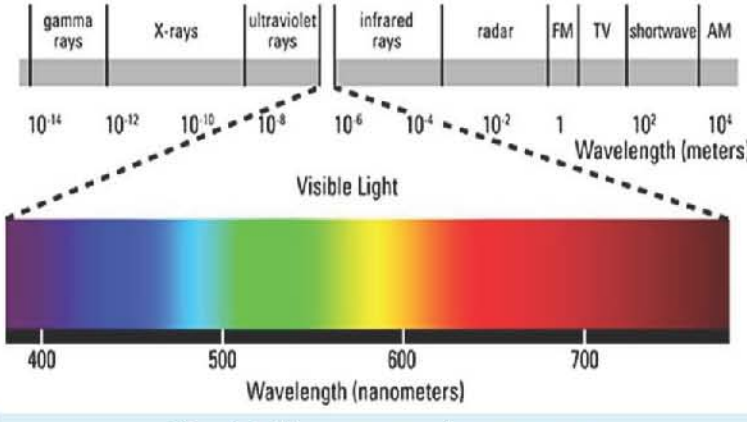


Fig. 4.1 Electromagnetic spectrum

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to 750ηm which is not only seen by naked eye but is also effective for the process of photosynthesis.

The effectiveness of a particular wavelength of light for the process of photosynthesis primarily depends upon its absorption in plant body. As different wavelengths (colours) of visible light are differently absorbed by various photosynthetic pigments, therefore, each wavelength has its own effectiveness for the process of photosynthesis. If a plant is illuminated in different colours of light one by one, the rate of photosynthesis is measured and the data obtained in this way is plotted in a graph, you will see that the rate of photosynthesis will be variable in different colours of light. Such a graph which shows the effectiveness of different wavelength of light for the process of photosynthesis is called **action spectrum**. Analysis of action spectrum indicates that blue (430ηm) and red (670ηm) wavelengths of light are the most effective for the process of photosynthesis.

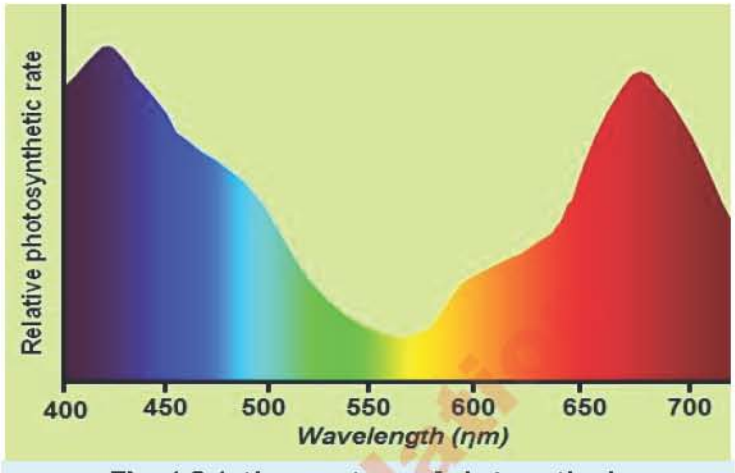

Fig. 4.2 Action spectrum of photosynthesis

4.1.2 Role of Carbon Dioxide in Photosynthesis

Carbon dioxide is one of the raw material of the photosynthetic reaction where it acts carbon source for the synthesis of organic compounds in photosynthesis. Plants are therefore known as autotrophs because they use low energy inorganic compound like CO_2 for the synthesis of high energy organic compounds by their own. Carbon dioxide is utilized in the dark or light independent reaction (Calvin cycle) of photosynthesis. Air contains about 0.03 to 0.04 percent of carbon dioxide. Land plants use this atmospheric carbon dioxide for photosynthesis. Increase in CO_2 concentration in the environment increase the rate of photosynthetic process but when CO_2 concentration rises above 1%, the rate of photosynthesis rapidly slow down due to stomatal closing. Dissolved carbon dioxide, bicarbonates and carbonates are present in water, which are used by aquatic photosynthetic organisms as carbon source.

Science Titbits

The rate of photosynthesis is directly proportional to the CO_2 consumed or O_2 released therefore; it can be measured by measuring the amount of CO_2 consumed or by measuring the amount of O_2 released during the process in a specific time.

4.1.3 Role of Water in Photosynthesis

Water is another raw materials for Photosynthesis along with CO_2. Water acts as hydrogen and electron donor in photosynthesis. During light dependent phase of photosynthesis, water molecule is broken down into $2H^+$, $2e^-$ and $_{1/2}O_2$ so this reaction is called photolysis of water. Electrons released by water molecule are used to compensate the photo excited electron lost by the chlorophyll-a molecule present in the reaction center of photosystem-II (P680). $2H^+$ ions and $2e^-$ are taken up temporarily by the $NADP^+$ to become NADPH which

are then used along to reduce CO_2 in light independent reaction. The oxygen which is produced is released in atmosphere.

This role of water in photosynthesis was first reported by **Van Niel** in 1931. He hypothesized that plants split water as a source of hydrogen, releasing oxygen as a byproduct. This observation was based on investigations of photosynthesis in bacteria that make carbohydrates from carbon dioxide, but do not release oxygen.

Neil's hypothesis was confirmed in 1940, when for the first time ^{18}O in biological research was used. In first experiment water was made of ^{18}O. The water tagged ^{18}O was added to an alga suspension. The oxygen, evolved during photosynthesis, was found to be radioactive. It was separated and identified. In another experiment carbon dioxide with tagged ^{18}O was added. The oxygen evolved contained none of the isotopes. Thus the source of evolved oxygen was proved to be water. In the following summary, red denotes labelled atoms of Oxygen ^{18}O.

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

Carbon dioxide — Water — Glucose — Water — Oxygen

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

Carbon dioxide — Water — Glucose — Water — Oxygen

4.1.4 Photosynthetic pigments and their major types

Photosynthetic organisms have different types of pigments for the absorption of light. Pigment is any substance that absorbs light energy for the use in the food synthesizing process. Algae and photosynthetic bacteria have chemically different types of pigments than plants. Pigments of bacteria are present in their cytoplasm whereas, photosynthetic pigments of plants are embedded in thylakoid membranes (grana lamellae) within their chloroplasts. Higher plants have two major group of pigments i.e., chlorophylls and carotenoids.

Chlorophyll

Chlorophylls absorb mainly violet, blue, orange and red wavelengths. Green and yellow are least absorbed and reflected. Two major types of chlorophyll are Chlorophyll-a and Chlorophyll-b. **Chlorophyll-a** is a bluish green pigment which is found in all photosynthetic organisms except photosynthetic bacteria. **Chlorophyll-b** is yellowish green pigment which is also found in all photosynthetic organisms except brown, red algae

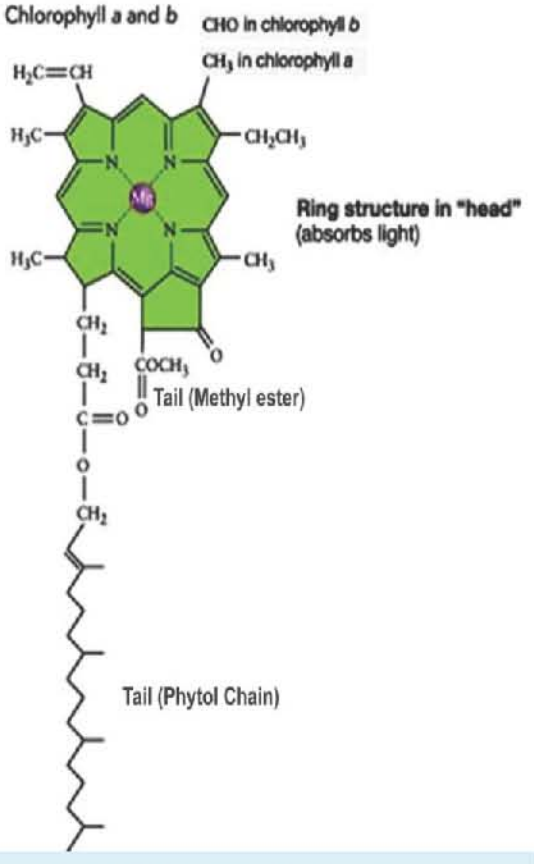

Fig 4.3: Structure of chlorophyll

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and photosynthetic bacteria. Algae also have some other form of chlorophylls i.e. **Chl-c, Chl-d** and **Chl-e** while photosynthetic bacteria have yet another type of chlorophyll i.e., **bacteriochlorophyll.**

Molecular formula of chlorophyll a and b:

Chlorophyll a = C_{55} H_{72} O_5 N_4 Mg

Chlorophyll b = C_{55} H_{70} O_6 N_4 Mg

A molecule of chlorophyll consists of a head and two tails. The head is composed of a **porphyrin ring** with Mg in the centre. The porphyrin ring further consists of four pyrrole rings (each pyrrole ring contains four carbons and one nitrogen atom). The nitrogen atoms of **pyrrole rings** interact with central Mg atom. The pyrrole rings also contain different groups around them. The only difference between chlorophyll-a and chlorophyll-b is that chlorophyll-a has methyl group ($-CH_3$) on 2nd pyrrole ring whereas, chlorophyll-b has aldehyde group (-CHO) at this point. The head of chlorophyll is hydrophilic in nature. It is exposed on the surface of thylakoid membrane. It is light absorbing part of chlorophyll.

The two side chains in the chlorophyll molecule are called tails. Side chains are phytol and methyl ester. **The chlorophyll tails** are hydrophobic in nature. They are embedded into the thylakoid membranes and serve to anchor the chlorophyll molecule in the membrane. Amount of chlorophyll in the leaves of plants is maintained for more and continuous photosynthesis so plants continuously synthesize it. The synthesis of chlorophyll in plants requires sunlight, nitrogen, Mg^{++} and warm temperatures. Therefore, leaves of the plants synthesize more chlorophyll in summer. Deficiency of chlorophyll in leaves is called chlorosis.

Critical thinking

- Why leaves turn yellow in winter and autumn?
- Why chlorosis takes place, how chlorosis differs from etiolation?

Carotenoids

Carotenoids are terpenoid lipids, which are yellow, orange, red or brown pigments. They absorb light strongly in the blue-violet range. They are seen in leaves before leaf fall, present in some flowers and fruits. The carotenoids act as accessory pigment along with chlorophyll-b as they absorb light energy and then transfer it to the chlorophyll-a. Therefore, they protect the chlorophyll-'a' from excess of light. They also attract insects, birds and other animals for pollination and dispersal.

There are two types of carotenoids: carotenes and xanthophylls. The **carotenes** are orange red pigments, composed of isoprenoid units and are found in all photosynthetic eukaryotes. The most widespread and important carotene is ß (beta) carotene. **Xanthophylls** are yellow in colour and are also composed of isoprenoid units. Lutein is widely distributed xanthophylls which is responsible for yellow colour of foliage in autumn.

4.1.5 Role of Photosynthetic Pigments

As mentioned earlier light energy is utilized by green plant cells during photosynthesis but this energy can only be used when it is absorbed. Different types of pigments absorb particular range of wavelengths of visible light spectrum. All the wavelengths which are absorbed by a specific pigment are disappeared. A particular pigment shows only those wavelengths which are reflected back. Plants appear green in colour because chlorophyll does not absorb green

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wavelength of light rather they reflect green light. Relative abilities of different pigments to absorb light can be measured by an instrument called **spectrophotometer**. Primary role of the photosynthetic pigments molecules is to absorb electromagnetic radiation and then transmit the energy of the absorbed photons to the reaction center in the photosystem.

Science Titbits

Carotenoids such as lutein, zeaxanthin and meso-zeaxanthin are also found in the macula in the center of the retina and absorb up to 90% of blue and violet light. The absorption of this intense light by these pigments protect the vital and light sensitive part of eye i.e. retina from oxidative damage.

Pigments in the photosystems are also involved in photochemical conversion of light energy to chemical energy in the photosynthetic cells. Chief component of photosystem is chlorophyll-a molecule that can absorb and then directly convert light energy to chemical energy in the form of the molecules ATP and NADPH. Whereas, other pigments like chlorophyll-b, xanthophyll and carotenes are called accessory pigments because they can only absorb light energy but not be able to convert it into chemical energy. Accessory pigments absorb different wavelengths of light spectrum other than chlorophyll-a and then transfer their absorbed light energy to chlorophyll-a for conversion to chemical energy. Thus they assist the chlorophyll-a in the reaction center to produce more chemical energy than it absorbs itself. Some carotenoids protect chlorophyll molecule from intense light as they absorb and disperse excessive light energy instead of transferring it to chlorophyll-a.

4.1.6 Absorption Spectrum

The absorption of different wavelengths of light by a particular pigment can be determined by the help of spectrophotometer. The data of spectrophotometer is represented by a graph. Such a graph which shows the relative absorption of different wavelengths of light by a particular pigment is called **absorption spectrum** of that pigment.

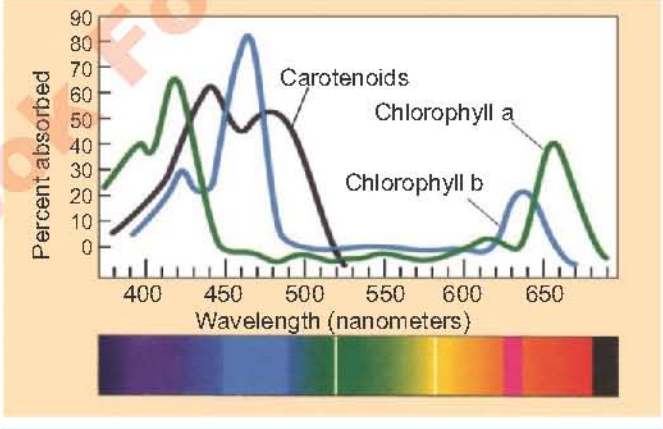

Fig: 4.4: Absorption spectra of different pigments

The absorption spectra of different pigments indicate that they absorb different wavelengths of visible light and these wavelengths are not absorbed at the same rate. The main photoreceptors are chlorophyll a and b and both show more absorption in violet blue (**400*ηm* to 470*ηm***) and orange-red (**630*ηm* to 660*ηm***) regions of the visible spectrum. On the other hand carotenoids show more absorption at 430*ηm* to 500*ηm*. Differece between the absorption spectrum of chlorphyll-a and chlorophyll-b may be relate to the differences in their molecular formulae, structural arrangements and their physical properties e.g. colour.

Critical thinking

- How does the absorption spectrum of chlorophyll-a differs from absorption spectrum of chlorophyll-b?
- How absorption spectrum of chlorophyll-a differs from action spectrum.

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4.1.7 Arrangements of Pigments (Photosystems)

For efficient absorption and utilization of light energy, the photosynthetic pigments are embedded on the surface of thylakoid membranes where they are arranged in the form of clusters. These clusters of pigment molecules are called **photosystems**. The peripheral part of photosystem is called **antenna complex** which consists of accessary pigments such as chlorophyll-b and carotenoids. The central part of photosystem is called **reaction centre** which contains only chlorophyll-a and associated proteins. Since chlorophyll-a generally has an optimal absorption wavelength of 660*ηm*, it associates with different proteins in each type of photosystem to slightly shift its optimal wavelength, producing two distinct photosystem types i.e., photosystem-I (PS-I) and photosystem-II (PS-II). The chlorophyll-a in the reaction centre of PS-I can absorb maximum 700*ηm* wavelength of light, hence called P700. Similarly, the chlorophyll-a in the reaction centre of PS-II can absorb maximum 680*ηm* wavelength of light, hence called P680. The photosystems are named for the order in which they were discovered and not for the order in which they occur in the thylakoid membrane.

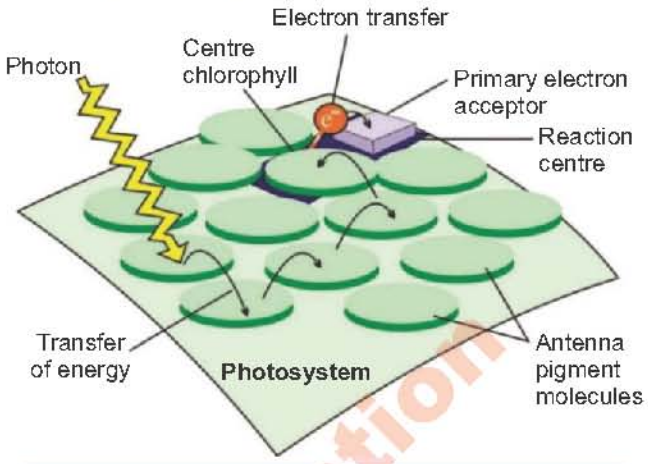

Fig: 4.5: Structure of photosystem

As chlorophyll-a can only absorb light of a narrow wavelength, it works with the pigments of antenna complex to gain energy from a larger part of the spectrum. The pigments absorb light of various wavelengths and pass on their gained energy to chlorophyll-a of the reaction centre. When the energy reaches the chlorophyll-a, its electrons become so excited due to high energy that they escape and is accepted by first molecule of a nearby electron transport chain which is even high energy and larger than chlorophyll molecule. In this way chlorophyll molecule becomes oxidized.

The electron transport system plays an important role in generation of ATP by the conversion of light energy into chemical energy.

4.1.8 Overall mechanism of photosynthesis

The process of photosynthesis has been divided into two phases. The first phase is called light dependent phase (light reaction) because it can take place only in the presence of light. The light-dependent phase occurs in the thylakoid membranes. In this phase light

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energy is used to make ATP (assimilating power) and NADPH (reducing power); whereas, water and oxygen are supposed to be input and output respectively.

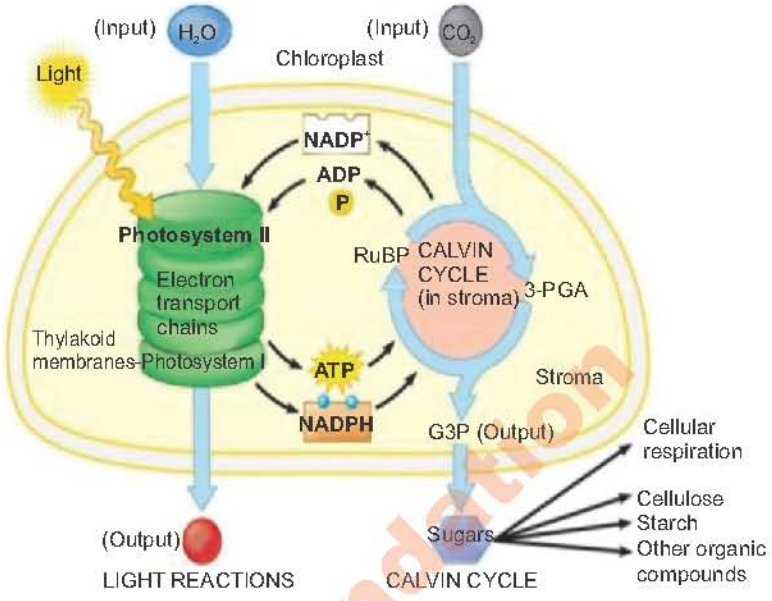

Fig: 4.6: An overview of photosynthesis

The second phase of photosynthesis is called the light independent phase (dark reaction) because it can take place whether light is present or not. This phase actually requires the products of light reaction i.e., ATP and NADPH. Since these products are available in day therefore, dark reaction also occurs in day time. In this phase CO_2 acts as input which is converted into glyceraldehyde-3-phosphate (G3P), the output of this phase. The ATP is hydrolyzed to ADP and Pi (H_3PO_4) and its energy is incorporated in this phase; whereas, NADPH provides energized electron and hydrogen for the formation of G3P, which is an energy rich molecule.

4.1.9 Light Dependent Phase (Light Reaction)

Light dependent phase of photosynthesis involves the absorption of light by the photosystems, excitation and flow of electrons through an electron transport chain, chemiosmotic synthesis of ATP, and reduction of $NADP^+$ to NADPH. The flow of excited electrons through an electron transport chain during light reaction is of two different types i.e., non-cyclic and cyclic. In non-cyclic electron flow, the excited electrons after leaving

$$ADP + Pi \longrightarrow ATP$$

a particular photosystem do not comeback; these electrons after losing their energy are incorporated into another molecule. On the other hand, in cyclic electron flow, the excited electrons after leaving a particular photosystem finally come back to their photosystem again. The most important event in light reaction is the production of ATP.

This production of ATP during light reaction is called **photophosphorylation** and the mechanism is called **chemiosmosis.** There are two types of photophosphorylation.

(a) Non-cyclic photophosphorylation

It is predominant pathway of light reaction in higher plants that occurs in routine. In this process both photosystems i.e., PS-I and PS-II are utilized and two electron transport chains are involved. When PS-II absorbs light, its excited electrons after flowing through an

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electron transport chain are transferred to PS-I. Similarly, the excited electrons which are liberated from PS-I are finally accepted by $NADP^+$. Therefore it is called non-cyclic electron flow. The events of non-cyclic photophosphorylation are continuous but here they are discussed in steps for convenience.

Absorption of light by PS-II and excitation of its electrons

When just two photons strike the antenna complex of PS-II, the two electrons become excited and begin to move along the atoms of different pigments within photosystem. Ultimately, the absorbed energy reaches the reaction centre of PS-II (P680) and causes its two electrons to be excited. These excited electrons are captured by the **primary electron acceptor** of PS-II and leave two "electron holes" in the photosystem behind making chlorophyll a strong oxidizing agent.

Photolysis of water

The electron holes of photosystem must be filled so that in the presence of water splitting enzyme reactions can proceed. When water reacts with oxidized state of chlorophyll in photosystem, it breaks up into $2H^+$ ions, $2e^-$ and $\frac{1}{2}O_2$. Since this breakdown occurs in the presence of sunlight therefore, it is termed as photolysis of water. The electrons released from water are used to fill the "electron holes" of PS-II.

$$H_2O \xrightarrow{\text{Photolysis of water}} 2H^+ + 2e^- + \tfrac{1}{2}\,O_2$$

Electron flow from PS-II to PS-I

The excited/energized electrons which have been released from PS-II and captured by primary electron acceptor now begin to flow to PS-I through an electron transport chain. The electrons move from primary electron acceptor to the **plastoquinone (PQ)**. From PQ the electrons flow through a complex of the **cytochromes (Cyt)** which consist of **Cyt-b_6** and **Cyt-f**. The cytochrome complex is not only an electron carrier but it also works as proton pump. The electron flow through the cytochrome complex stimulates it to pump the protons from stroma to the thylakoid inner space.

In this way the energy of flowing electrons is transformed into a gradient of protons (H^+) in the thylakoid inner space. The proton gradient activates an enzyme in thylakoid membrane called **ATP synthase** which not only moves the protons back into the stroma but also catalyzes a reaction in which ADP and Pi are combined to form ATP (photophosphorylation). This whole mechanism which involves flow of electron, pumping of protons and generation of ATP by thylakoid membranes is called **chemiosmosis**. This ATP, generated by light reactions will provide chemical energy for the synthesis of sugar during Calvin cycle. The energized electrons after losing their energy, move from cytochrome complex to the **plastocyanin (PC)** and finally incorporated into the PS-I

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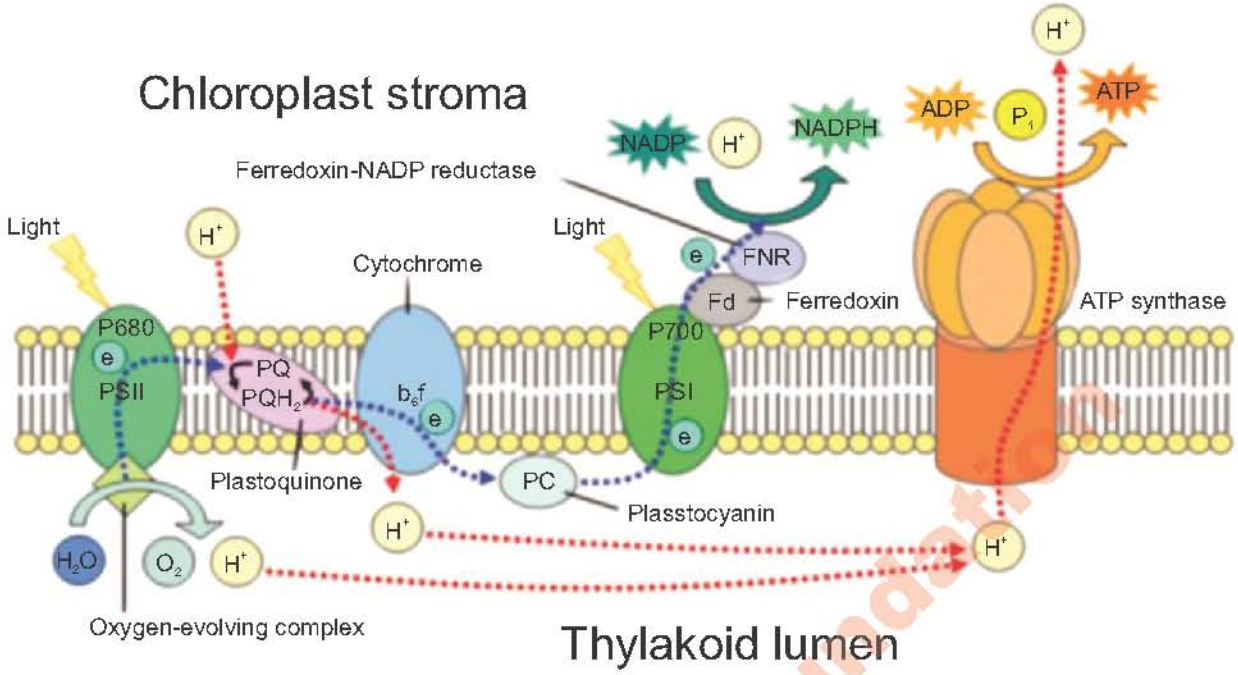


Fig: 4.7: Chemiosmotic synthesis of ATP during light reaction

Absorption of light by PS-I and excitation of its electrons

On the other hand, when P700 in the reaction centre of PS-I molecule absorbs two photon of light, electrons are boosted to a higher energy level. P700 molecule passes these excited electrons to a primary electron acceptor of PS-I, creating "electron holes". The electron holes of P700 are filled by the pair of electrons received from the P680 (photosystem II) via electron transport chain.

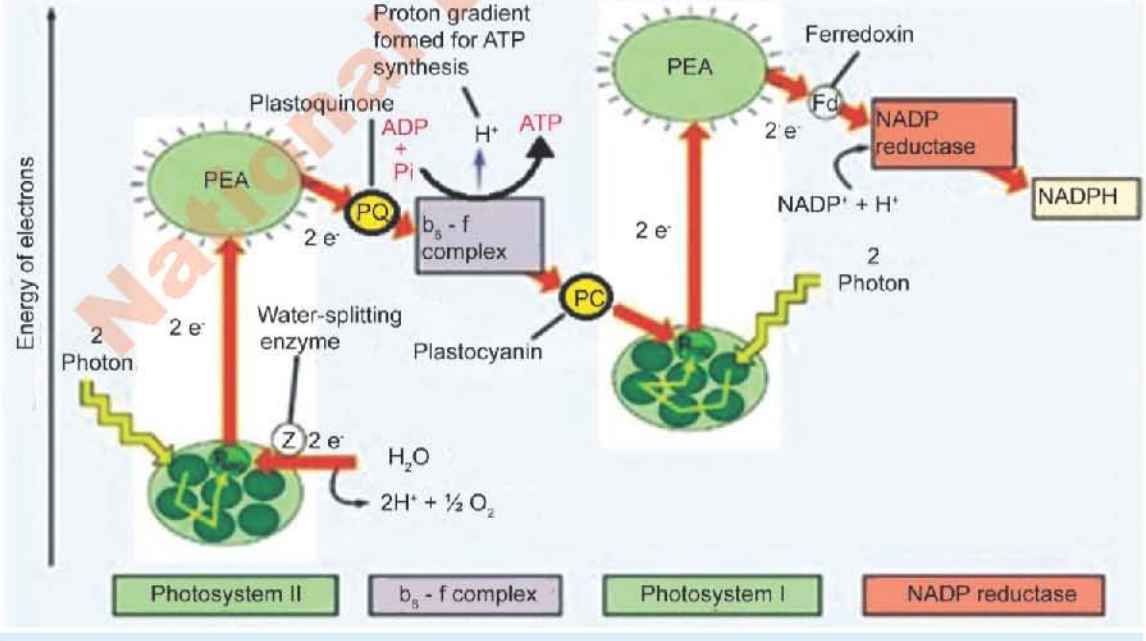


Fig: 4.8: Non-cyclic photophosphorylation (Z Scheme)

Electron flow from PS-I to NADP+

The primary electron acceptor of photosystem I passes the photoexcited electrons to a second electron transport chain. The electrons are accepted by **ferredoxin (Fd)**. It is an iron containing protein. An enzyme called **NADP reductase** (flavoprotein enzyme) transfers

$$NADP^+ + 2e^- + 2H^+ \longrightarrow NADPH + H$$

the electrons from Fd to $NADP^+$. $NADP^+$ combines with electrons and hydrogen ions to form NADPH (reduced). The NADPH will provide reducing power for the synthesis of sugar in the Calvin cycle.

The path of electron transport through the two photosystems during non-cyclic photophosphorylation is known as **Z-Scheme** due to its conceptual zigzag shape.

(b) Cyclic photophosphorylation

The rise in NADPH and deficit of ATP may stimulate a temporary shift from a non-cyclic to cyclic electron flow until ATP supply catches up the demand. In this mechanism only PS-I is utilized. It absorbs energy in the form of photons. When energy reaches the **reaction centre** of PS-I the electrons are boosted up to higher energy level. Such excited electrons are first captured by primary electron acceptor of PS-I, then they move through an electron transport chain containing ferridoxin, cytochrome b_6-f complex and plastocyanin. When electrons are passed from cytochrome b_6-f complex an ATP is generated by chemiosmosis. Finally, the electrons after losing the energy return back to P700 chlorophyll in PS-I reaction centre. There is no production of NADPH, no occurrence of photolysis of water and therefore, no release of oxygen.

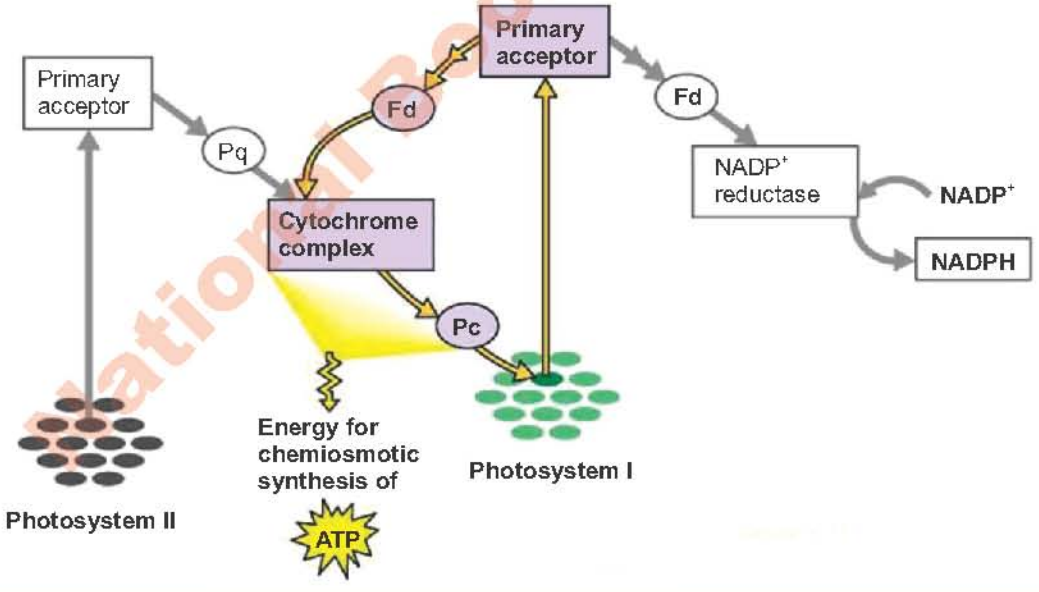


Fig: 4.9: Cyclic electron flow during photophosphorylation

4.1.10 Light Independent Phase (Dark Reaction)

The light independent phase (dark reaction) takes its name from the fact that light is not directly required for these reactions to occur. This phase requires the availability of

NADPH, ATP (the products of light reaction) and CO_2. In this phase of photosynthesis, NADPH is used to reduce carbon dioxide while ATP is used to incorporate energy. Finally, CO_2 is converted into a phosphorylated triose carbohydrate i.e., glyceraldehyde-3-phosphate (G3P) which are later on used to make glucose. Dark reaction generally involves a complicated metabolic pathway, the Calvin cycle or C3 pathway. However, in some plants, in addition to Calvin cycle another metabolic pathway is also involved, called **C4 pathway.** The plants in which only Calvin cycle occurs during dark reaction are called **C3 plants.**

Calvin cycle

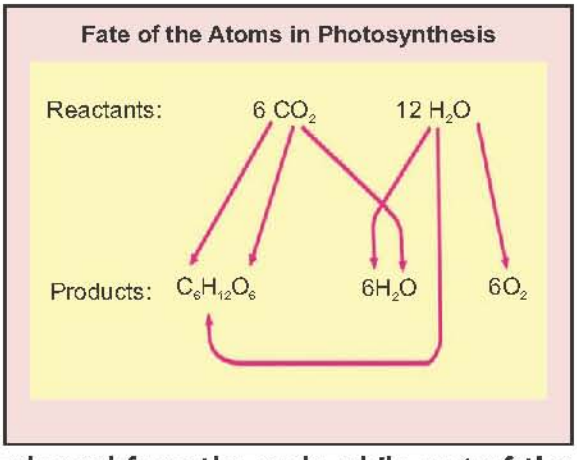
Calvin cycle term is applied to the series of metabolic reactions in which CO_2 is reduced to produce G3P. (These reactions have been explored by **Melvin Calvin** and co-workers at the University of California. Melvin Calvin won the Nobel Prize in 1961 for this work). The Calvin cycle can be divided into three phases, carbon fixation, reduction and regeneration of carbon dioxide acceptor i.e., RuBP.

Carbon fixation

One of the key substance in this process is a five carbon phosphorylating sugar called **ribulose bisphosphate (RuBP).** It is generally referred as **CO_2 acceptor** because it is capable of combining with carbon dioxide with the help of Ribulose bisphosphate (RuBP) carboxylase/oxygenase also known as **RuBisCO.** Three intermediate molecules of six carbons are formed during this reaction. These molecules are unstable and exist for such a short time that, they cannot be isolated. Each six carbon breaks down to form two molecules of 3-phosphoglycerate (3-PGA), a phosphorous containing compound with three carbon atoms. Since, the carbon of inorganic compound (CO_2) becomes the part of organic compound (RuBP) during this phase, hence, it is called **carbon fixation.** As the first stable compound in the Calvin cycle is a three carbon compound (3-PGA) that is why Calvin cycle is also known as **C3 pathway.**

Reduction

In this phase six molecules of 3-phosphoglycerate (3-PGA) react with six ATP molecules, a phosphate from each ATP is transferred to each 3-PGA. In this way, 3-PGA molecules are changed into 1,3-Bisphosphoglycerate. These molecules are then reduced by the hydrogen of NADPH and finally glyceraldehyde 3 phosphate (G3P) molecules are produced. During this reduction process a phosphate group from each 1,3-Bisphosphoglycerate molecule is also given off. There are total six molecules of G3P are produced in this phase but only one molecule is released from the cycle while rest of the five molecules are used to regenerate the CO_2 acceptor molecules in the next phase.



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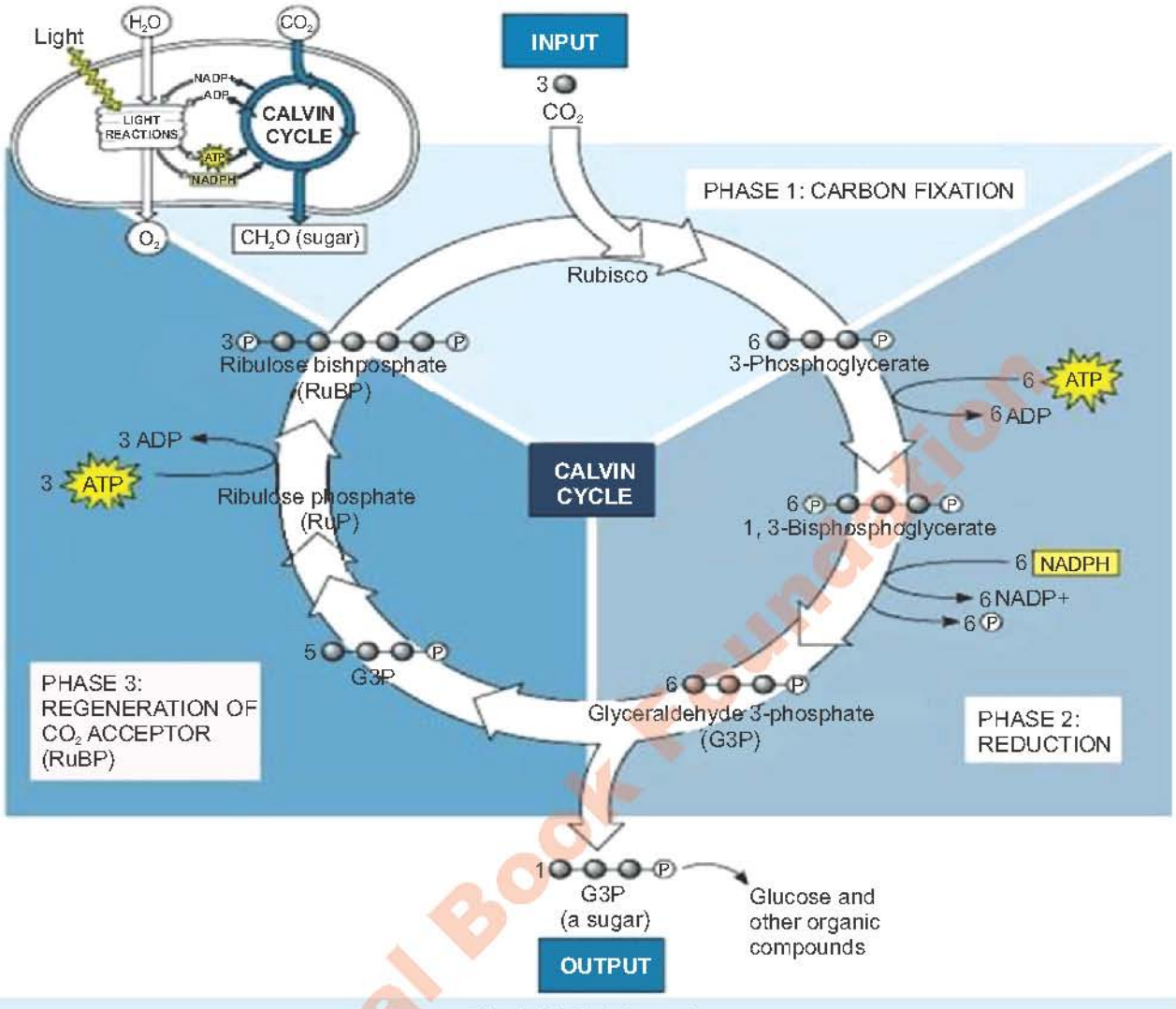

Fig 4.10: Calvin cycle

Regeneration of CO_2 acceptor

Five molecules of G3P from the previous phase are used to regenerate the RuBP (CO_2 acceptor) in this phase. These five molecules each containing three carbon atoms undergo a series of reactions in which three molecules of ribulose phosphate (RuP) each containing five carbon atoms are produced. When three molecules of RuP react with three molecules of ATP, a phosphate group from each ATP is transferred to each RuP. Ultimately RuP are converted into RuBP which again participate in the next cycle.

The whole process of Calvin cycle indicates that there are three molecules of CO_2, six molecules of NADPH (reducing power) and nine molecules of ATP (assimilating power) are used to release just one molecule of G3P form the cycle. However, in order to produce a glucose molecule, two molecules of G3P are required. The overall process of Calvin cycle can be represented as:

$$3CO_2 + 6NADPH + 9ATP \longrightarrow G3P + 3H_2O + 9ADP + 9PI + 6\ NADP^+$$

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4.2 CELLULAR RESPIRATION

In biological systems oxidation-reduction is a chemical reaction usually involves the removal of hydrogen atom from one molecule and the gain of hydrogen atom by another molecule. Cellular respiration is a series of complex oxidation-reduction reactions by which living cells obtain energy through the breakdown of organic matter.

4.2.1 Kinds of Cellular Respiration

There are two kinds of respirations: aerobic respiration and anaerobic respiration. Aerobic respiration takes place in the presence of abundant atmospheric oxygen, whereas, anaerobic respiration occurs in the absence of oxygen. The organic molecule that generally undergoes breakdown in cellular respiration in order to release energy is glucose, therefore, glucose is supposed to be **respiratory fuel**. The initial breakdown of glucose in both aerobic and anaerobic respirations is quite same, in which it is broken down into two molecules of pyruvates. This common step of aerobic and anaerobic respirations is called **glycolysis**. The pyruvates undergo in different respiratory pathways depending upon the availability of oxygen and the kind of organism. If oxygen is available, the further breakdown of pyruvates takes place aerobically and the final products are carbon dioxide and water with the release of large amount of energy i.e., 36 ATPs (in eukaryotes) or 38 ATPs (in prokaryotes). If oxygen is absent, then the pyruvates are broken down anaerobically and the final products are either lactic acid or ethanol and carbon dioxide with release of very small amount of energy i.e., just 2 ATPs.

4.2.2 Mechanism of Anaerobic Respiration

Anaerobic respiration takes place in many microorganisms (bacteria, yeast), muscle cells of vertebrates and in the cells of higher plants. Anaerobic respiration is incomplete breakdown of glucose in the absence of oxygen. It is also known as **fermentation**. There are two pathways of anaerobic respiration depending upon the nature of final products i.e., lactic acid fermentation and alcoholic fermentation.

Lactic acid fermentation

It consists of **glycolysis** followed by the **reduction** of pyruvate by NADH to lactic acid. The pathway operates anaerobically because after NADH transfers its electron to the pyruvate, it is "free" to return and pick up more electrons during the earlier reaction of glycolysis. The overall equation can be represented as:

$$C_6H_{12}O_6 + 2NAD^+ \longrightarrow 2C_3H_4O_3 + 2NADH + 2H^+$$

$$2C_3H_4O_3 + 2NADH + 2H^+ \longrightarrow 2C_3H_6O_3 + 2NAD^+$$

Lactic acid fermentation occurs in anaerobic bacteria and in the muscles of mammals as well as human during strenuous exercise when oxygen supply is exhausted. The accumulation of lactic acid causes muscles fatigue i.e., muscles become unable to contract and begin to ache.

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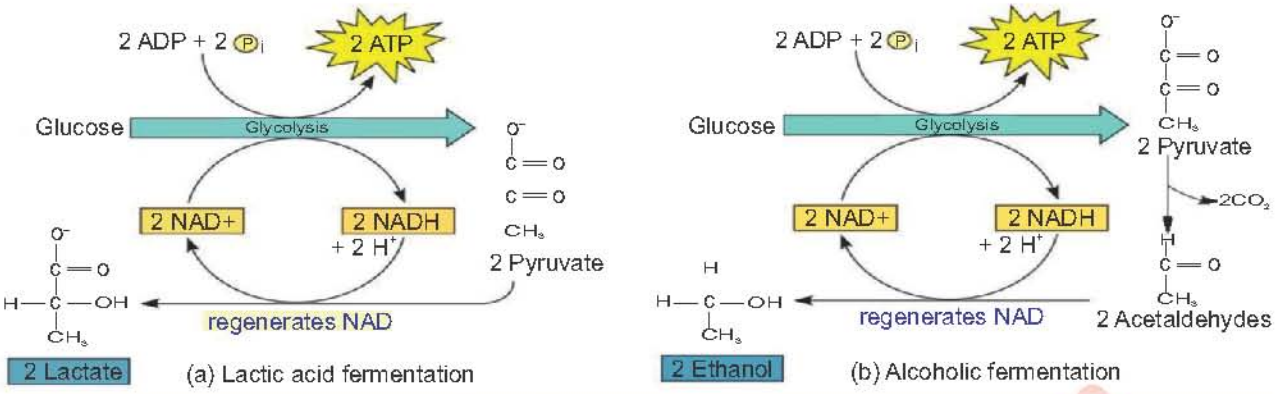

Fig: 4.11 Anaerobic respiration

Alcoholic fermentation

Alcoholic fermentation is found in yeast. It consists of **glycolysis** followed by the **decarboxylation** of pyruvate to acetaldehyde then **reduction** of acetaldehyde by NADH to ethyl alcohol or ethanol. This pathway also operates anaerobically because after NADH transfers its electron to the acetaldehyde, it is "free" to return and pick up more electrons during the earlier reaction of glycolysis. The overall equation can be represented as:

$$C_6H_{12}O_6 + 2NAD^+ \longrightarrow 2C_3H_4O_3 + 2NADH + 2H^+$$

$$2C_3H_4O_3 + 2NADH + 2H^+ \longrightarrow 2C_2H_5 - OH + 2CO_2 + 2NAD^+$$

4.2.3 Mechanism of Aerobic Respiration

Aerobic respiration is a catabolic process which involves complete oxidative breakdown of organic food (especially glucose) into carbon dioxide and water with release of great deal of energy in the form of ATPs. It is predominant respiratory pathway in most of the organisms. Aerobic respiration is completed in four phases: glycolysis, oxidation of pyruvates, Krebs cycle and respiratory electron transport chain.

Glycolysis

Glycolysis is the process of breakdown of glucose or similar hexose sugar into two molecules of pyruvates through a series of enzymatic reactions releasing some energy (as ATP) and reduced coenzymes (as NADH). It occurs in the cytoplasm. It is completed in two phases i.e., preparatory phase and oxidative phase. **Preparatory phase** is an investment phase in which two ATPs are consumed. Its end products are two molecules of G3P. On the other hand **oxidative phase** is **pay off phase** in which not only ATPs are produced through substrate level phosphorylation but it also produces NADH which upon further oxidation in respiratory electron transport chain yields more ATPs. The whole glycolysis pathway takes place in the following sub steps.

1. **Phosphorylation:** When glucose reacts with ATP in the presence of enzyme **hexokinase** or **glucokinase**, a phosphate group from ATP is transferred to glucose. In this way glucose is phosphorylated to **glucose-6-phosphate** and ATP is changed to ADP.
2. **Isomerization:** Glucose-6-phosphate is changed to its isomer **fructose-6-phosphate** with the help of enzyme **phosphohexose isomerase.**

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3. **Phosphorylation:** When fructose-6-phosphate reacts with another ATP, it is again phosphorylated to **Fructose-1, 6-bisphosphate** with the help of enzyme **phosphofructokinase** and ATP is changed to ADP.

4. **Splitting:** Now fructose-1, 6-bisphosphate splits up with the help of enzyme **aldolase** to form two molecule each of 3- carbon compounds, one is **glyceraldehyde 3-phosphate** (G3P) and other is **dihydroxyacetone 3-phosphate.**

5. **Isomerization:** The dihydroxyacetone 3-phosphate is not directly utilized further and is ultimately changed into its isomer, the **glyceraldehyde 3-phosphate** (G3P) with the help of enzyme **phosphotriose isomerase.** In this way preparatory phase is completed. Next phase of glycolysis is proceeded by two molecules of G3P, therefore, the remaining reactions occur twice.

6. **Dehydrogenation and Phosphorylation:** G3P donates hydrogen to NAD to form NADH and accepts inorganic phosphate (Pi) to form **1, 3-bisphosphoglycerate** with the help of enzyme **G3P dehydrogenase.** This step is also called oxidative phosphorylation.

7. **Formation of ATP:** The direct synthesis of ATP from organic phosphorylated substrate is called **substrate level phosphorylation.** In this step, 1, 3-bisphosphoglycerate is converted into **3-phosphoglycerate** by transferring its phosphate group to ADP and a molecule of ATP is formed. This reaction is catalyzed by enzyme **phosphoglycerate kinase.**

8. **Isomerization:** In this step position of phosphate group is changed from C3 to

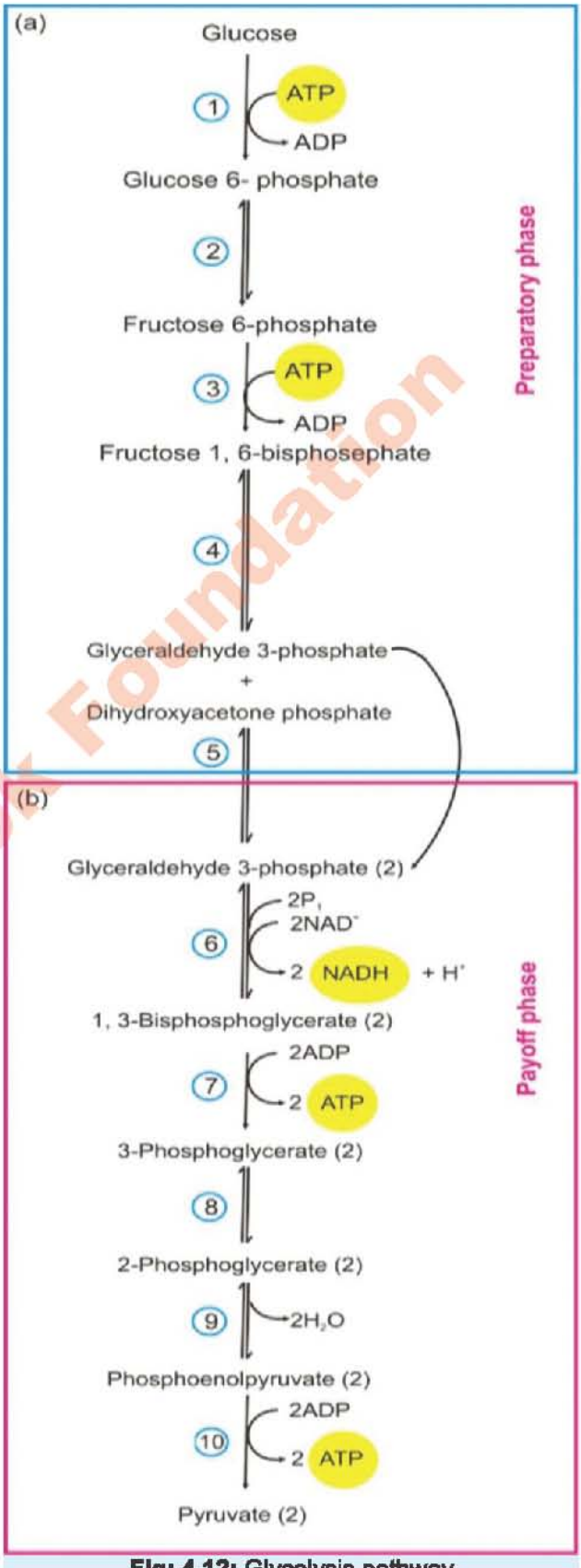

Fig: 4.12: Glycolysis pathway

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C2 of phosphoglycerate to form 2-phosphoglycerate with the help of enzyme **phosphoglycerate mutase.**

9. **Dehydration:** In this step, 2-phosphoglycerate undergoes dehydration and is converted into **phosphoenol pyruvate** (PEP) with the help of enzyme **enolase.**
10. **Formation of ATP:** Again a molecule of ATP is produced by **substrate level phosphorylation** when phosphoenol pyruvate loses phosphate group which is taken up by the ADP to form ATP in the presence of an enzyme **pyruvate kinase.** The phosphoenol pyruvate is finally converted into **pyruvate.**

4.2.4 Oxidation of Pyruvate to Acetyl-Co-A

Pyruvates are produced in cytosol. Because pyruvate is a charged molecule, it must enter the mitochondrion via active transport with the help of the transport protein **pyruvate translocase.** On entering the mitochondria, pyruvates do not directly participate in Krebs cycle but they undergo an intermediate phase, called **oxidation of pyruvate** or **link reaction** as it links the pathway of aerobic respiration that occurs outside the mitochondria with that occurs inside the mitochondria.

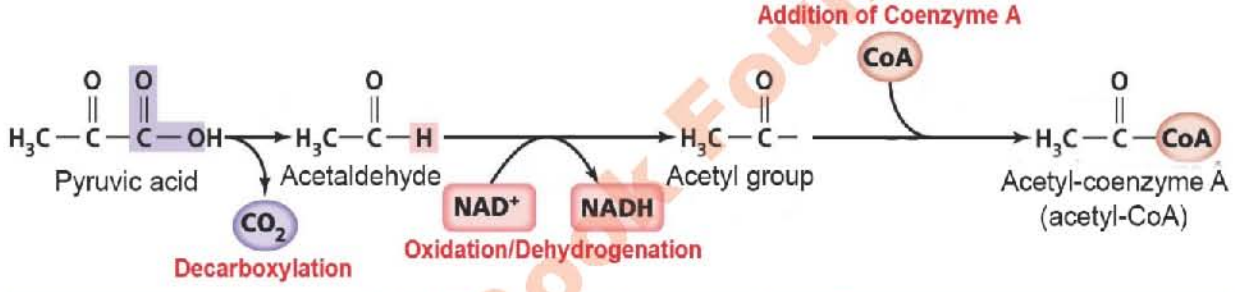

Fig: 4.13 Pathway of oxidation of pyruvate

The oxidation of pyruvate takes place in three steps. First, it undergoes **decarboxylation** in which a molecule of CO_2 is removed from pyruvate to form **acetaldehyde.** Then NAD^+ removes hydrogen from acetaldehyde. As a result of this oxidation/ dehydrogenation a 2C fragment acetyl and NADH are produced. Finally, acetyl group is combined with coenzyme-A to form **acetyl CoA.** This oxidation, decarboxylation and addition of coenzyme A is catalyzed by complex of three enzymes called **pyruvate dehydrogenase complex.**

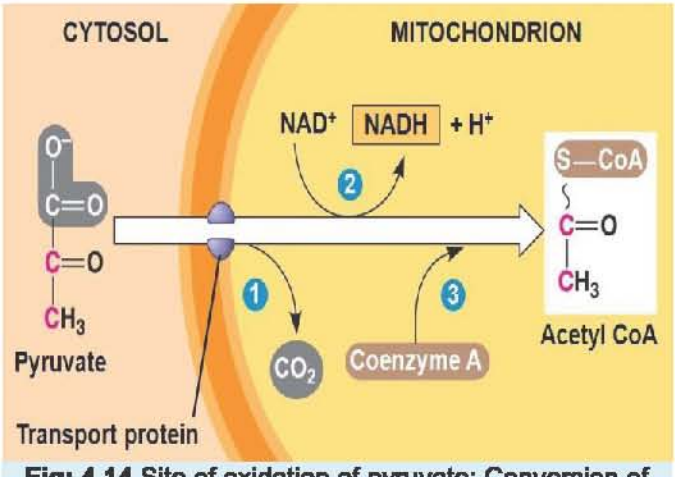

Fig: 4.14 Site of oxidation of pyruvate: Conversion of pyruvate to acetyl CoA, the junction between glycolysis and the citric acid cycle.

4.2.5 Krebs Cycle

This cycle was discovered by British scientist **Sir Hans Krebs,** therefore, called **Krebs cycle.** It is also called **Citric acid cycle** or **Tri carboxylic acid** (TCA) cycle because the first

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compound which is formed in the cycle is **citrate** (citric acid) that contains three carboxylic acid groups.

The Krebs cycle comprises following nine steps.

1. Synthesis

Acetyl CoA (2-carbon compound) and a water molecule combine with oxaloacetate (4-carbon compound) to form a 6-carbon compound called **citrate** (citric acid) with the help of enzyme **citrate synthetase.** It is the first product of Krebs cycle. CoA is liberated.

2. Dehydration

Citrate undergoes reorganization by the removal of a water molecule with the help of enzyme **aconitase.** The resulting compound **cis-aconitate** is an intermediate molecule of isomerization of citrate.

3. Hydration

With the addition of water molecule cis-aconitate is converted into **isocitrate** with the help of same enzyme **aconitase.** Actually, citrate and isocitrate are isomers of each other.

4. Oxidative decarboxylation

This is a two-step process, which involves first oxidation/ dehydrogenation of isocitrate, followed by the **decarboxylation** to form **alpha-ketoglutarate.** Both interlinked steps are catalyzed by **isocitrate dehydrogenase complex.** The hydrogen and electrons which are released from isocitrate are taken up by NAD^+ to form NADH while the carboxyl group is released in the form of CO_2.

Science Titbits:

A complex oxidation-reduction involves NAD or NADP. NAD and NADP act as intermediate in cellular reactions involving electron transfer. Many of the electrons removed from reduced carbon compounds in various enzyme-catalyzed reactions are transferred to NAD to produce NADH. When a molecule of NAD or NADP gains electrons and becomes reduced, a hydrogen ion combines with it as well. Thus the reduced form is symbolized as NADH or NADPH. In fact, another hydrogen ion becomes closely associated with each reduced molecule. Technically it is more accurate to represent the reduced form as $NADH + H^+$ or $NADPH + H^+$. For convenience, these reduced forms i.e., $NADH + H^+$ and $NADPH + H^+$ can be represented as $NADH_2$ and $NADPH_2$ respectively.

5. Oxidative decarboxylation and addition of CoA

α-Ketoglutarate again undergoes oxidative decarboxylation. The hydrogen and electrons which are released from **α-ketoglutarate** are taken up by NAD^+ to form NADH while the carboxyl group is released in the form of CO_2. Then, it combines with coenzyme A to form **succinyl CoA.** This reaction is controlled by enzyme complex **α-ketoglutarate dehydrogenase.**

6. Synthesis of substrate level ATP

Coenzyme A is removed from Succinyl CoA by the help of enzyme **succinyl thiokinase** to form **succinate.** The reaction releases sufficient energy which is used to combine GDP and Pi forming GTP. GTP reacts with ADP to form ATP while GTP is again converted into GDP. In this way a molecule of ATP is generated in this reaction.

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7. Dehydrogenation/oxidation

Succinate undergoes dehydrogenation/oxidation with the help of enzyme **succinate dehydrogenase** to form **fumarate.** The hydrogen and electrons which are released from succinate are taken up by FAD to form $FADH_2$.

8. Hydration

A molecule of water gets added to fumarate to form **malate** with the help of enzyme **fumarase.**

9. Dehydrogenation/oxidation

Malate undergoes dehydrogenation/oxidation in the presence of enzyme **malate dehydrogenase** to produce **oxaloacetate.** The hydrogen and electrons which are released from malate are taken up by NAD^+ to form NADH. Oxaloacetate picks up another molecule of acetyl CoA to repeat the cycle.

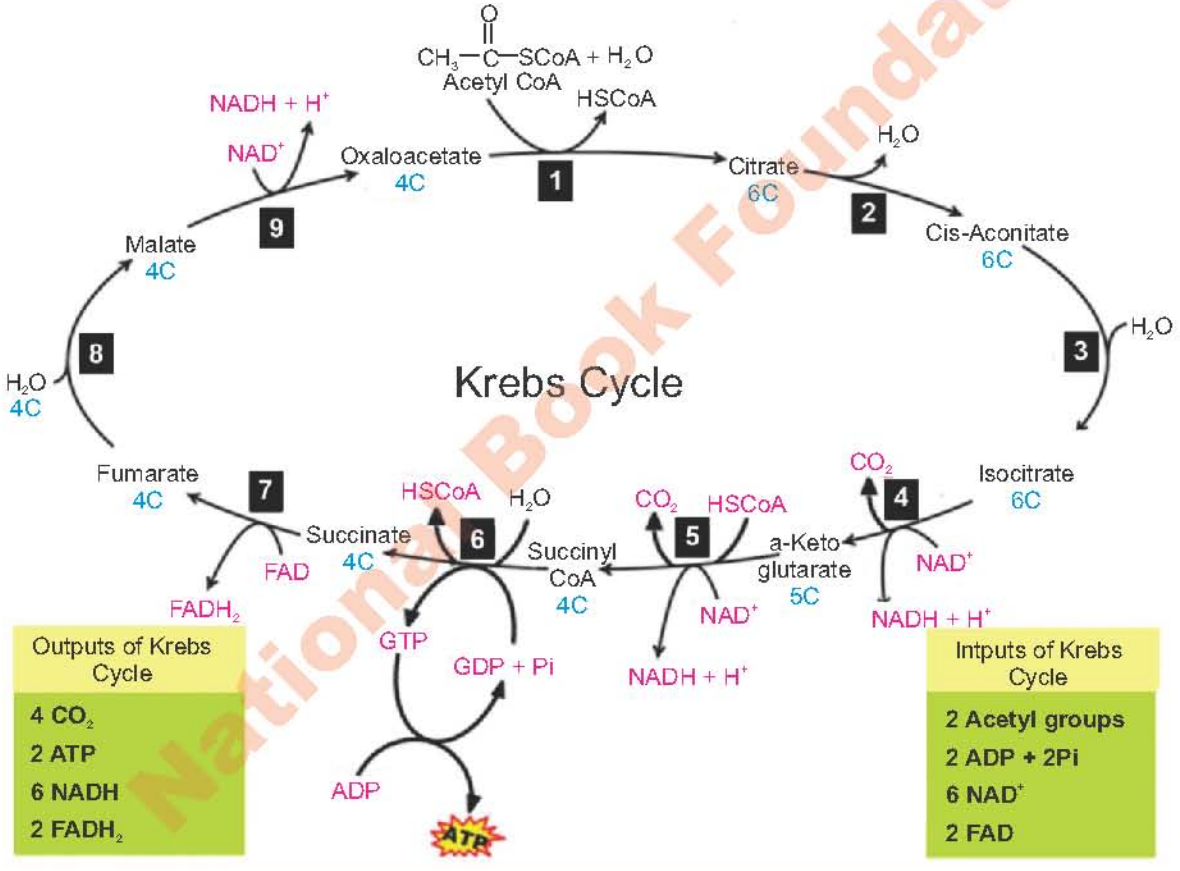

Fig: 4.15 Krebs cycle (Citric acid cycle or TCA cycle)

4.2.6 Electron Transport Chain (ETC)

After Kreb's cycle most of the energy of glucose is in the form of NADH and $FADH_2$, These two molecules enter into the electron transport chain. In this chain, the reduced NADH and $FADH_2$ are oxidized and their electrons are passed along a series of oxidation reduction reaction to the final acceptor i.e., molecular oxygen.

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Components of Respiratory electron transport chain

The components of respiratory electron transport chain include:

(1) NADH- Q reductase or NADH-dehydrogenase complex (I), further composed of FMN and Fe-S complex

(2) FADH-dehydrogenase complex (II)

(3) Coenzyme Q or Ubiquinone (mobile carrier)

(4) Cytochrome reductase complex (III) further composed of Cyto-b, Fe-S and Cyto-c_1

(5) Cytochrome-c (mobile carrier)

(6) Cytochrome oxidase complex (IV) further composed of Cyto-a, Cyto-a_3 and copper

Passage of electron flow

Passage of electrons from high energy NADH to first carrier to next is downhill journey with the release of energy at each step as the energy of the flowing electrons is used by some membrane carrier proteins to pump proton (H^+) from matrix to inter-membrane space. Accumulation of (H^+) in the inter membrane space act as proton gradient which further activate the ATP synthetase that catalyze ATP synthesis in the presence of O_2. As this ATP synthesis is due to oxidation of NADH or FADH in the presence of O_2, so ETC is also called oxidative phosphorylation.

NADH is oxidized when it reacts with **NADH- dehydrogenase complex (I).** It has two prosthetic groups FMN and Fe-S complex. Both electrons and protons pass from NADH to FMN which moves the electrons to Fe-S complex then pump the protons to intermembrane space. If $FADH_2$ is to be oxidized through ETC, it also hands over its electrons to coenzyme Q, via **FADH dehydrogenase complex (II).**

The flowing electrons from coenzyme Q are now transferred to **cytochrome reductase complex (III)** which have three components (Cyto-b, Fe-S and Cyto-c_1). This enzyme complex hands over its electron to **cytochrome** c and also pump the electrons to intermembrane space. Like co-enzyme Q, cytochrome c is also mobile carrier of electrons. Cytochrome c delivers the electrons to **cytochrome oxidase complex (IV)** which comprises of three components (Cyto-a, cyto-a_3 and copper). This enzyme complex act as both electron carrier and proton pump.

Finally, the electrons are transferred to oxygen. The oxygen is the ultimate acceptor of electrons. It becomes reactive. Each oxygen atom also picks up a pair of hydrogen ions from the aqueous solution forming water.

Energy released during downhill passage of electrons from one carrier to the next is used to pump protons (H^+) from the mitochondrial matrix to the inter membrane space. Energy released during downhill passage of electrons from one carrier to next is used by specific transmembrane protein complexes which actively pump protons (H^+) from matrix to intermembrane space. To avoid the loss of energy as unwanted heat, energy is not released in one step rather in steps. Proton pumping takes place at three specific sites of electron transport chain by action of three corresponding enzymes listed below.

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	Site-1	Site-2	Site-3
Proton Pumping sites	i) FMN ----- CoQ,	ii) Cyto-b ----- cyto-c_1	iii) Cyto-a ------ Cyto-a_3
Enzymes involved	NADH-dehydrogenase complex-I	Cytochrome reductase complex-III	Cytochrome oxidase complex-IV

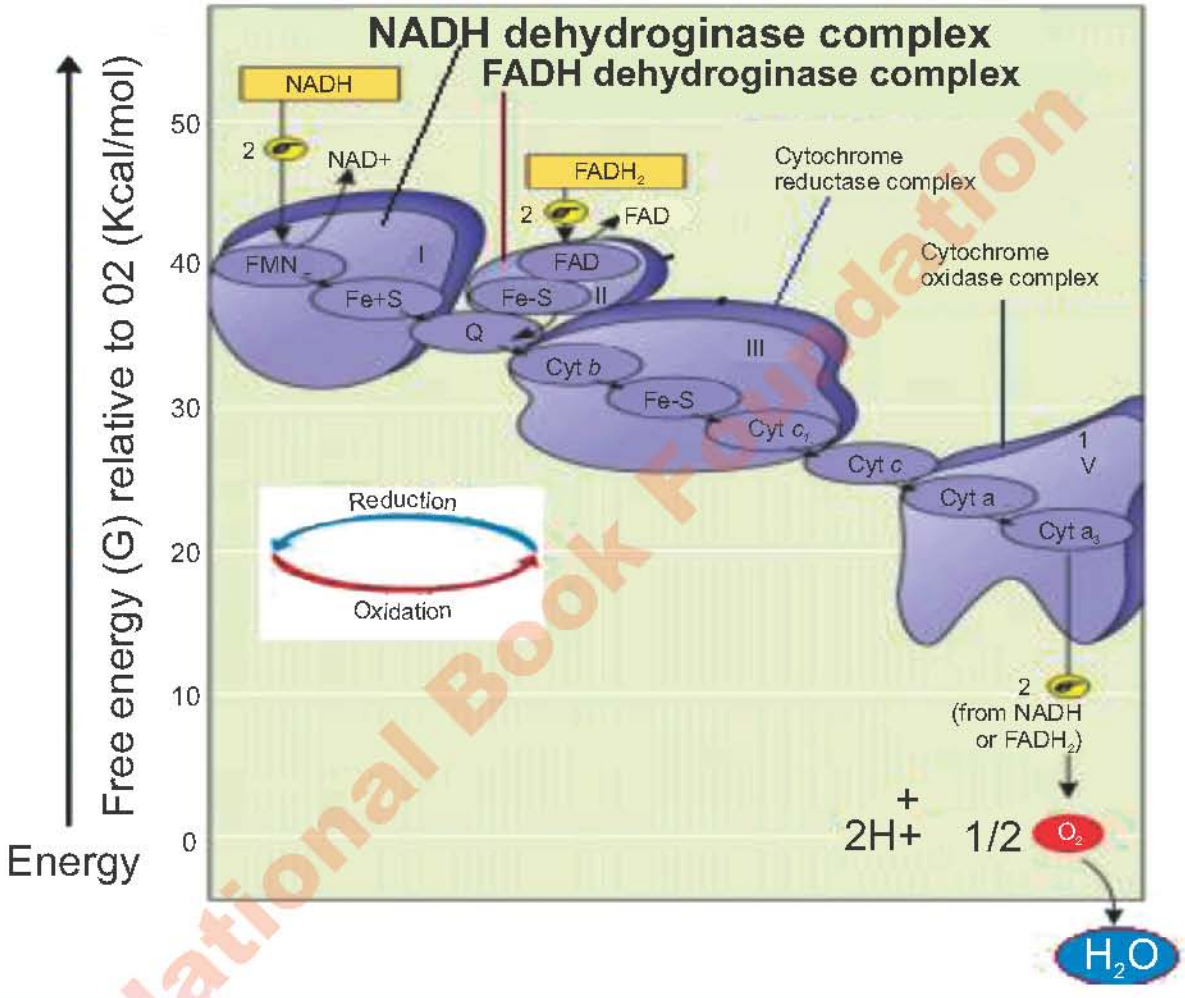

Fig: 4.16 Sequence of electron carriers in respiratory ETC

The electron transport chain makes no ATP directly. Its function is to ease the fall of electrons from food to oxygen releasing energy in manageable amounts. How does the mitochondrion couple this electron transport chain and energy to ATP synthesis? The answer is a mechanism called chemiosmosis.

Science Titbits

Ubiquinone is not a protein, but a small molecule soluble in lipids and insoluble in water Cytochromes literally means "cell colour". The reduced cytochromes are pink in colour. They are protein plus pigment molecules containing iron. They can gain or lose an electron.

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4.2.7 Chemiosmosis and Oxidative Phosphorylation

Oxidative phosphorylation is the synthesis of ATP molecules with the help of energy liberated during oxidation of reduced co-enzymes (NADH, $FADH_2$) produced in respiration. The enzyme required for this synthesis is called ATP synthetase.

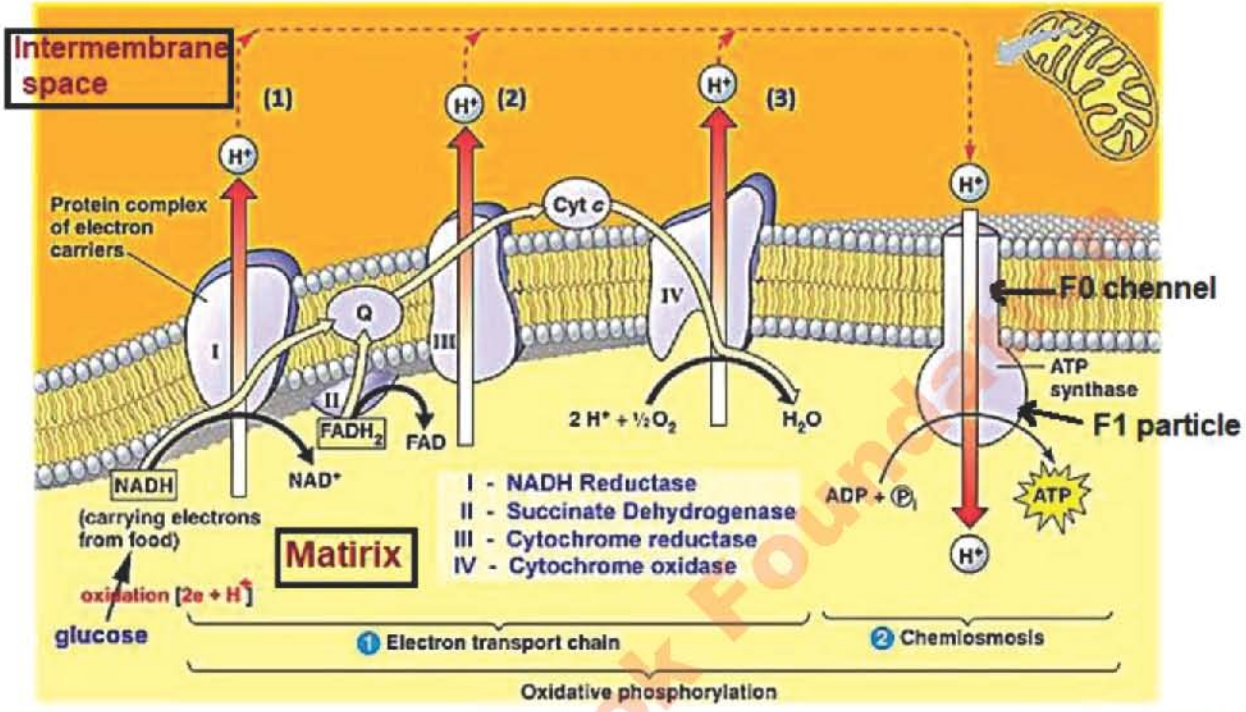

Fig: 4.17: Mechanism of chemiosmosis in respiratory electron transport chain

It is located in the inner mitochondrial membrane. It consists of two parts i.e., F_0 and F_1. F_0 is embedded in the membrane and involves in the movement of protons from inter-membrane space to mitochondrial matrix. F_1 or elementary particle is a head like part which is projected from the surface of membrane towards matrix. It catalyzes ATP synthesis by the combination of ADP and Pi. ATP-synthetase becomes active in ATP formation only when a proton gradient having higher concentration of H^+ or protons on the F_0 side as compared to F_1 side is established. The flow of protons through the F_0 channel induces F_1 particles to function as ATP-synthetase i.e., the energy of the proton gradient is used in attaching a phosphate to ADP by high energy bond. This produces ATP. Oxidation of one molecule of $NADH_2$ produces 3 ATP molecules while a similar oxidation of $FADH_2$ forms 2 ATP molecules. The theory of ATP production by this mechanism is called **chemiosmosis.**

4.2.8 Substrate Level Phosphorylation

The prime objective of cellular respiration is to generate ATPs. There are two ways to do this during aerobic respiration: chemiosmosis and substrate level phosphorylation, the former we have already discussed.

As far as substrate level phosphorylation is concerned, you are already familiar that the addition of inorganic phosphate to any organic molecule is called **phosphorylation** but, when phosphate is enzymatically transferred from an organic substrates molecule it is called **substrate level phosphorylation.** However, it accounts for only a small percentage of the ATP that a cell generates. It occurs at three occasions during aerobic respiration.

In glycolysis, substrate level phosphorylation occurs, when 1,3-bisphosphoglycerate is converted into 3-phosphoglycerate (7th reaction) and when phosphoenol pyruvate is converted into pyruvate (10th reaction). There are four ATPs produced by this mechanism during glycolysis but two of them are supposed to be consumed in preparatory phase so net product by substrate level phosphorylation is 2 ATP.

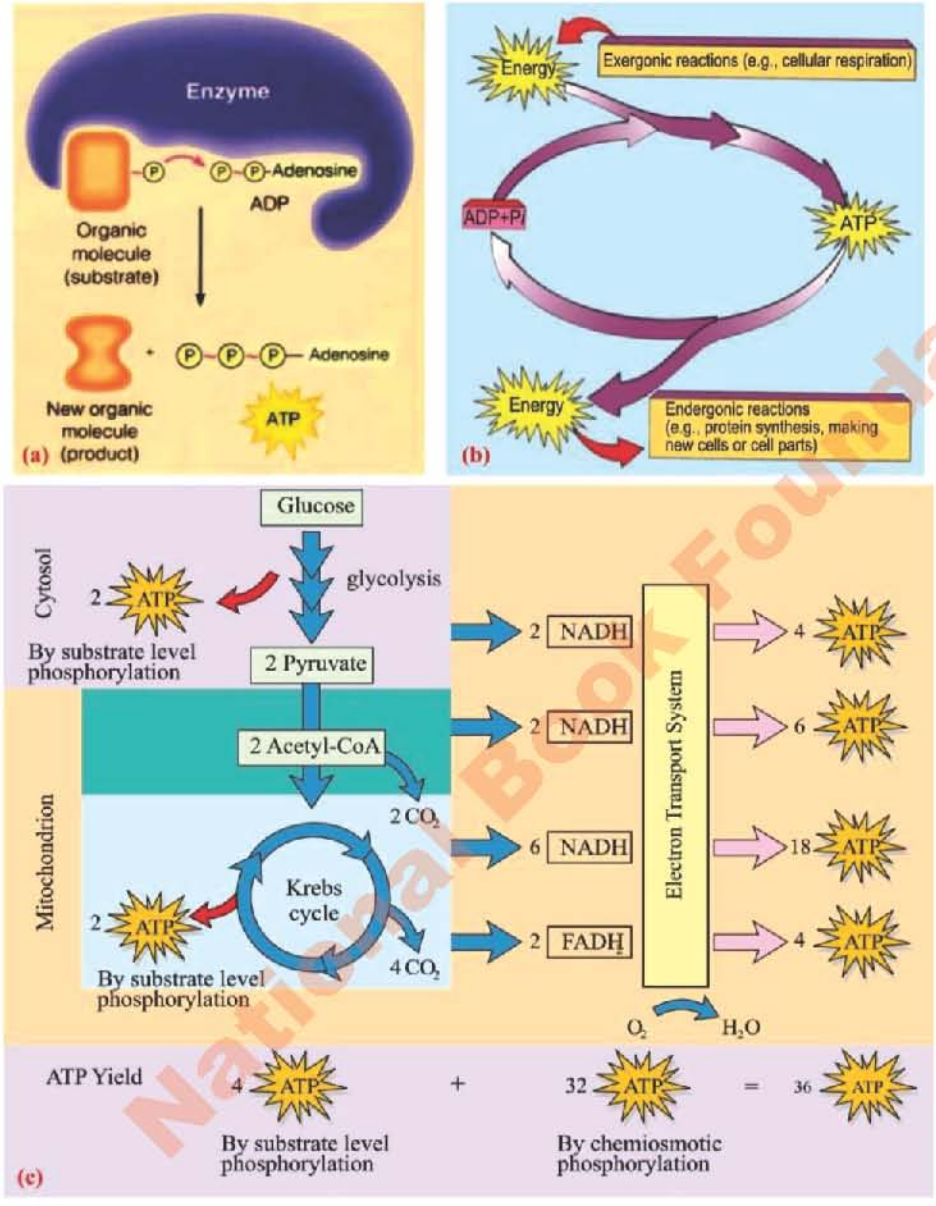

Fig: 4.18: (a) Substrate level phosphorylation. (b) Because ATP is responsible for coupling many endergonic and exergonic reactions it is an important link between anabolism and catabolism in living cells.

(c) ATP Budget in aerobic respiration

Note:
Actually, the two molecule of the NADH of glycolysis are produced in cytoplasm. These cannot be taken up by mitochondria because the mitochondrial membrane is impermeable for NADH. Therefore, at the time of their uptake only the energized electrons of NADH are transferred inside the mitochondrion by a complex mechanism. These electrons are received by two molecules of FAD^+ in the mitochondrial matrix to produce two molecule of $FADH_2$. Hence, four ATP molecules are produced instead of six. So, eukaryotes yield two less number of ATP than prokaryotes.

In Krebs cycle, substrate level phosphorylation occurs when succinyl CoA is converted into succinate. There are two molecules of ATP produced at this occasion. Since, ATP can be synthesized directly from the organic substrates of exergonic reactions (energy releasing

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reactions e.g., cellular respiration), therefore, it is said that substrate level phosphorylation couples the exergonic reactions with the synthesis of ATP. These ATP are then used to drive endergonic reactions (energy storing reaction e.g., protein synthesis). In this way, out of total 36 ATP which are produced during aerobic respiration in most of human cells, 4 ATP are the result of substrate level phosphorylation and remaining 32 ATP are produced by chemiosmosis through electron transport chain.

4.2.9 Importance of G3P

Importance of Glyceraldehyde 3-phosphate (G3P) can be summarized as follows:

i. G3P is an important intermediate of both respiration and photosynthesis.
ii. In the Calvin cycle of photosynthesis, G3P molecules are converted into glucose phosphate within the chloroplast. Glucose phosphate is then converted to glucose, fructose, sucrose, maltose and starch.
iii. In respiration, G3P appears during glycolysis pathway and act as connecting molecule between preparatory phase and payoff phase (oxidative phase). So all the energy molecule i.e. ATP or $NADPH_2$ can be produced only after the formation of G3P.
iv. G3P molecule ultimately leads to the formation of pyruvate which is the end product of glycolysis and raw material for anaerobic and aerobic respiration.
v. G3P also act as an intermediate molecule for inter-conversion of different biological molecules, ATPs and waste products.
vi. Carbohydrates in the cell may be converted to lipids and lipids to carbohydrates through the formation of G3P intermediate.
vii. Carbohydrates in the cell may be converted to amino acids and then proteins and also from proteins to carbohydrates through the formation of G3P intermediate.

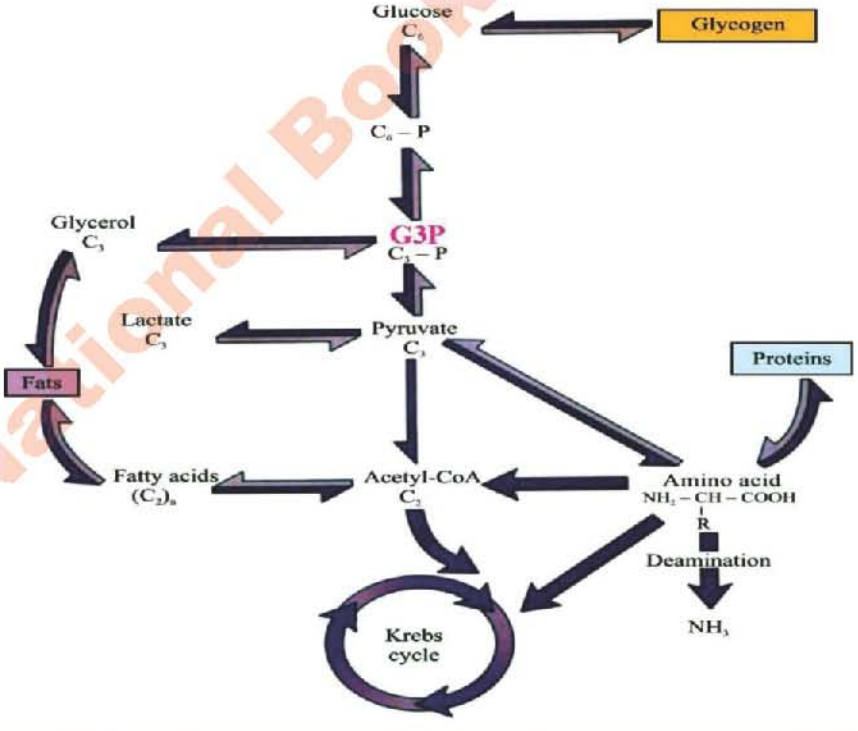

Fig: 4.19: The matabolic pool concept: When they are used as energy sources carbohydrates, fats and proteins enter degradative pathways at specific points. Degradation produces metabolites that can be used for synthesis of other biomolecules or may release of ATPs.

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4.2.10 Cellular Respiration of Fats and Proteins

When a fat is used as an energy source, it breaks down to glycerol and three fatty acids. As figure 4.20 indicates, glycerol is converted to G3P, a metabolite in glycolysis. The fatty acids are converted to acetyl-CoA, which enters the Krebs cycle. An 18-carbon fatty acid results in nine acetyl-CoA molecules.

The hydrolysis of proteins results in amino acids whose R-group size determines whether the carbon chain is oxidized in glycolysis or the Krebs cycle. The carbon chain is produced in the liver when an amino acid undergoes deamination, i.e., the removal of the amino group. The amino group becomes ammonia (NH_3), which enters the urea cycle and becomes part of urea.

4.3 PHOTORESPIRATION

The respiratory activity that occurs in green cells in the presence of light resulting in release of carbon dioxide is termed as **photorespiration**. It needs oxygen and produce CO_2 and H_2O like aerobic respiration. However ATP is not produced during photorespiration.

4.3.1 Mechanism of Photorespiration

When the CO_2 levels inside the leaf drop to around 50 ppm (part per million), ribulose bisphosphate carboxylase/oxygenase (RuBisCO) starts to combine O_2 with RuBP instead of CO_2. The net result of this is that instead of producing two 3C molecules of phosphoglycerate (PGA), only one molecule of PGA and a toxic 2C molecule called **phosphoglycolate** are produced. The plant must get rid of the phosphoglycolate. First it immediately gets rid of the phosphate group, converting the molecule to **glycolate**.

$$RuBP + O_2 \longrightarrow \text{Phosphoglycolate} + \text{Phosphoglycerate} \longrightarrow \text{Glycolate}$$

The glycolate is then transported to the peroxisome and there converted to **glyoxalate** and then **glycine**. The glycine is then transported into the mitochondria where it is converted into **serine**. The serine is then used to make other organic molecules.

$$\text{Glycolate} \longrightarrow \text{Glycine}$$

$$2\ \text{Glycine} \longrightarrow \text{Serine} + CO_2$$

4.3.2 Disadvantages and Evolution of Photorespiration

Photorespiration costs the plant energy and results in the net loss of CO_2 fixation from the plant. Thus, it reduces the rate of photosynthetic process. In most plants, photorespiration reduces the amount of carbon fixed into carbohydrate during photosynthesis by 25 percent. Photorespiration is not essential for plant. It is also observed that if photorespiration is inhibited chemically, the plant can, still grow. Furthermore, some plants are naturally resistant to photorespiration. Then why photorespiration exists? The common simple answer to this question is that the active site of RuBisCO is evolved to bind both carbon dioxide and oxygen. Originally it was not a problem as there was no oxygen in the atmosphere at the time of establishment of earth so the carbon dioxide binding activity was the only one used. The photorespiration started when the oxygen began to accumulate in the atmosphere.

Science, Technology and Society Connections

Analyze the impact of photorespiration on the agriculture yield in the tropic climates.

Photorespiration decreases net photosynthesis because a portion of CO_2 fixed in photosynthesis escapes from the leave after it is fixed. Under certain conditions, up to 5% of the photosynthetic potential is lost in photorespiratory metabolism. Thus photorespiration reduces dry matter production and agricultural yield in tropical climate.

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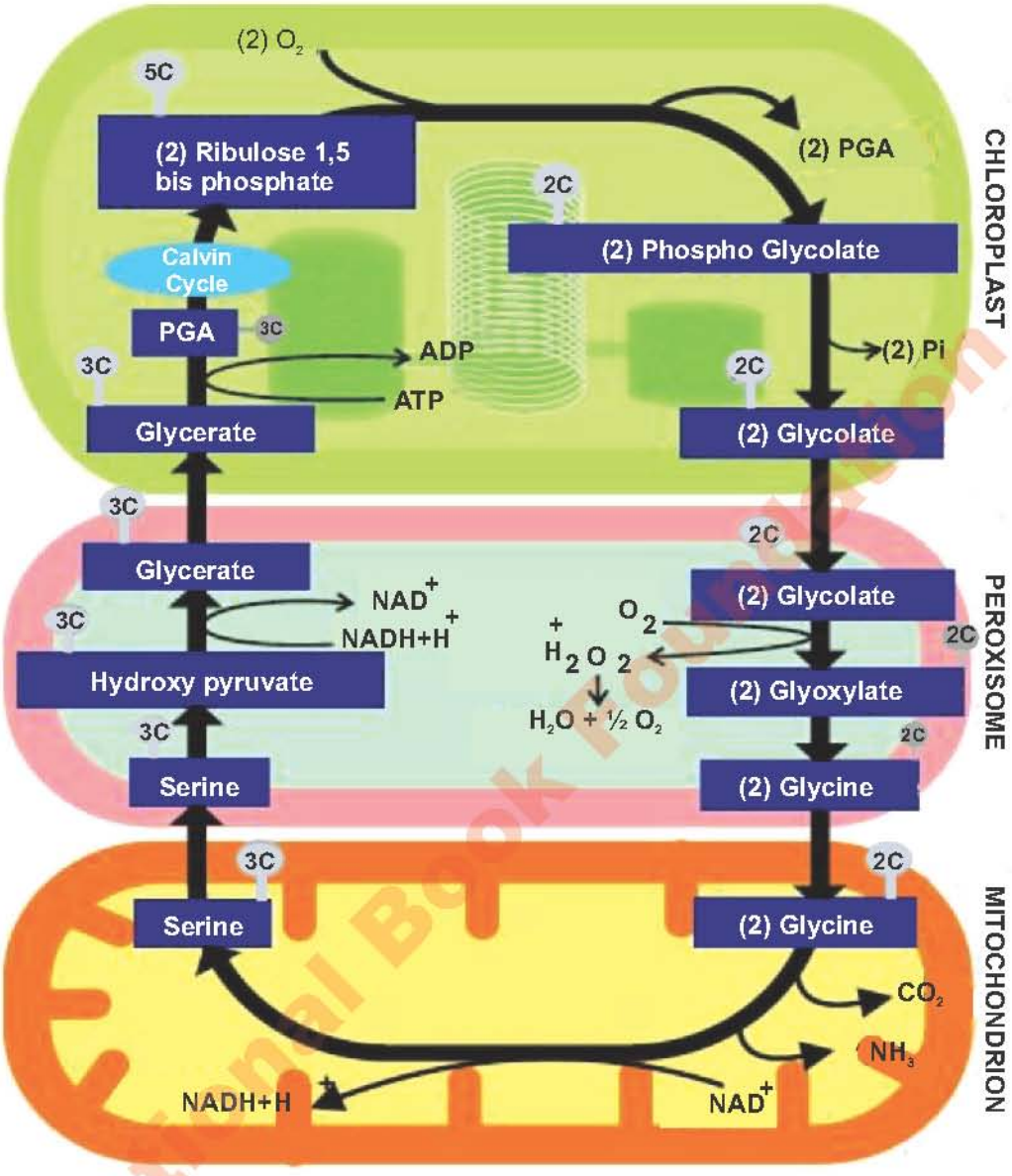

Fig: 4.20: Schematic representation of pathway involved in photorespiration in chloroplast, peroxisomes and mitochondria

4.3.3 Effect of temperature on the activities of RuBisCO

Photorespiration is related to the functioning of the enzyme ribulose bisphosphate (RuBP) **carboxylase/oxygenase.** It is often called **RuBisCO** because it can have an oxygenase activity in addition to carboxylase activity. Its activity depends upon the relative concentration of O_2 and CO_2 in leaves. Photorespiration starts when the CO_2 levels inside a leaf become low.

If the plant continues CO_2 fixation in photosynthesis when its stomata are closed, the CO_2 will be used up and the O_2 released from photosynthesis will be prevented to release out

of plant body. In this way, ratio of O_2 in the leaf will increase relative to CO_2 concentrations.

4.3.4 C4 photosynthesis: An adaptation to the problem of photorespiration

Some plants which grow in tropical climate have an adaptation to the problem of photorespiration. They have an additional metabolic pathway in light independent phase of photosynthesis beside Calvin cycle. This metabolic pathway is called Hatch-Slack cycle of C_4 pathway in which **phosphoenol pyruvate** (PEP) carboxylase is used instead of RuBisCO to fix CO_2 to phosphoenol pyruvate (a C_3 molecule), and the result is **oxaloacetate**, a C_4 molecule. It takes place in cytoplasm of mesophyll cells.

$$PEP + CO_2 \xrightarrow{\text{PEPcarboxylase}} \text{Oxaloacetate}$$

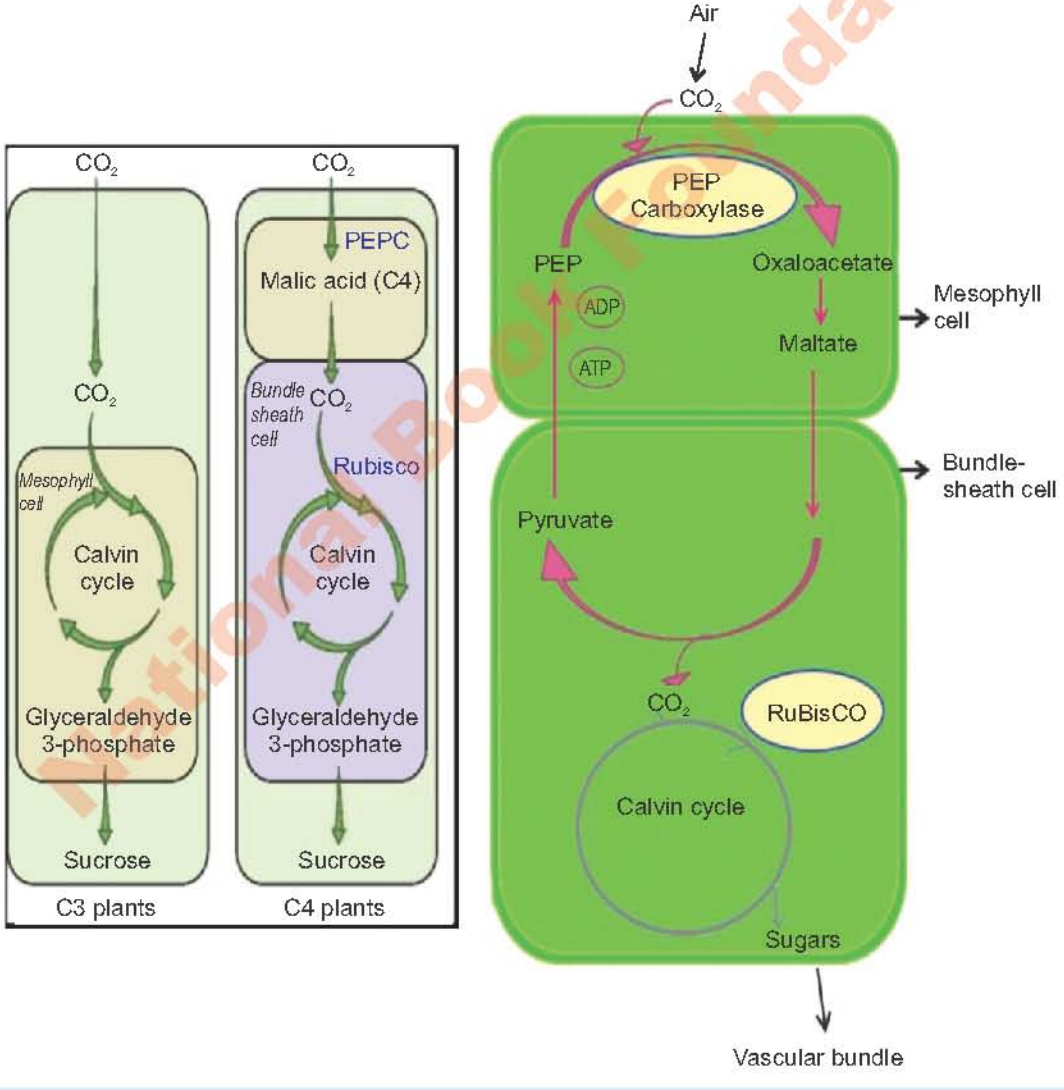

Fig. 4.21: Comparison of C3 and C4 photosynthesis and complete C_4 photosynthetic cycle

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As the first product of CO_2 fixation is a 4-carbon compound oxaloacetate, so the plants are called C_4 plants e.g., maize, sugarcane, sorghum, etc. Oxaloacetate is then transported to the chloroplasts of mesophyll cells. It is then converted to another 4-C compound, the **malate**, with the help of NADH, produced in the photochemical phase. The malate is then transported to the chloroplasts of bundle sheath cells. Here malate is converted to **pyruvate** (C_3) with the release of CO_2. Thus concentration of CO_2 increases in the bundle sheath cells. These cells contain enzymes of Calvin cycle. Because of high concentration of CO_2, RubisCO participates in Calvin cycle and not in photorespiration. Sugar formed in Calvin cycle is transported into the phloem. Pyruvate generated in the bundle sheath cells re-enters mesophyll cells and regenerates phosphoenol pyruvate (PEP) by consuming one ATP.

EXERCISE

Section I: Multiple Choice Questions

Select the correct answer:

1. Removal of the source of carbon dioxide from photosynthesizing chloroplasts results in rapid changes in the concentration of certain chemicals. Which one of the following represents the correct combination of concentration changes?

	ATP	Ribulose bishposphate	Phosphoglyceric acid (PGA)
A)	decreases	decreases	Increases
B)	decreases	increases	no change
C)	increases	increases	Decreases
D)	increases	no change	Decreases

2. What are the products of the light reactions in photosynthesis?
   A) ATP and NADP
   B) ATP, $NADPH_2$ and oxygen
   C) ATP, PGA and $NADH_2$
   D) ATP, PGA and oxygen
3. During the light dependent stage of photosynthesis, the photoactivated pigment removes an electron from the hydroxylation derived from the water molecule. The fate of the free hydroxyl radical is that it
   A) is broken down into oxygen and a free radical of hydrogen
   B) is used to raise the activation level of chlorophyll by donating a positive charge
   C) is used to produce adenosine triphosphate from adenosine diphosphate
   D) reduces carbon dioxide to sugar
4. Carbon dioxide labeled with ^{14}C has been used to identify the intermediate compounds in the Calvin cycle, the light-independent stage in photosynthesis. Which compound would be the first to contain the ^{14}C?
   A) glucose B) PGA C) RuBP D) starch
5. The rate of photosynthesis of a freshwater plant is measured using five spectral colours. Which sequence of colours would give an increasing photosynthetic response?

Smallest ⟶ Largest response					
A)	blue	green	yellow	orange	red
B)	green	yellow	orange	red	blue
C)	red	orange	yellow	green	blue
D)	yellow	green	orange	blue	red

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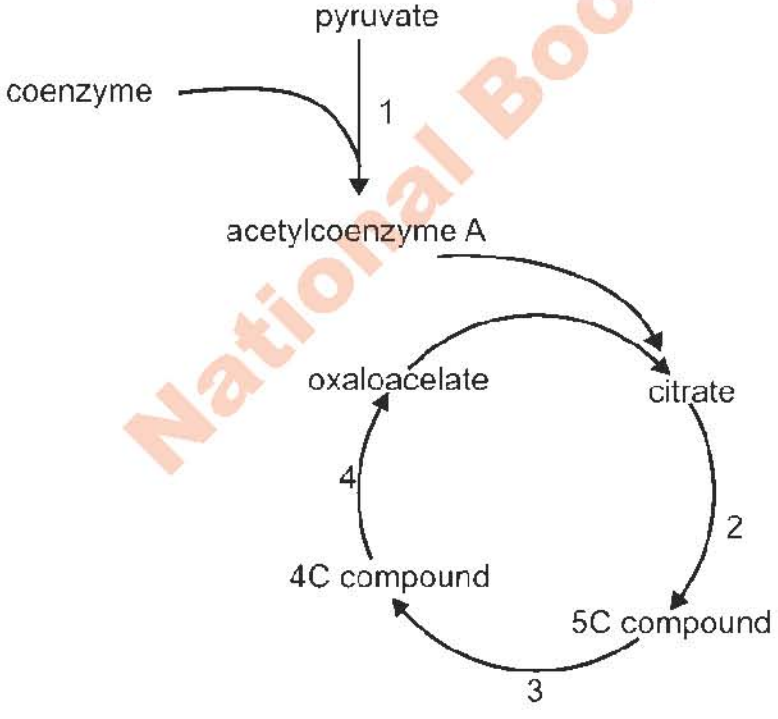
6. During dark reactions the three carbon atoms of 3-PGA are derived from
   A) RuBP only
   B) CO_2 only
   C) RuBP + CO_2
   D) RuBP + CO_2 + PEP
7. Chlorophyll is soluble in
   A) water
   B) organic solvent
   C) water and organic solvent
   D) not in any solvent
8. Photorespiration takes place only in
   A) root
   B) mitochondria
   C) green parts of the plant
   D) all cells of the plant
9. In C_4 plants, fixation of CO_2 occurs in
   A) palisade tissue
   B) cortex of stem
   C) spongy mesophyll and bundle of sheath
   D) phloem tissue
10. ATP synthesis during light reactions is
   A) oxidative
   B) photolysis
   C) substrate phosphorylation
   D) photophosphoryation
11. In C_3 plants first stable product of photosynthesis during dark reaction is
   A) PGA B) G3P C) RuBP D) oxaloacetate
12. The diagram shows the Krebs cycle. At which numbered stages does decarboxylation take place?



   A) 1 and 2
   B) 1, 2 and 3
   C) 1, 3 and 4
   D) 1, 2, 3 and 4

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Section II: Short Answer Questions

1. What is electromagnetic spectrum?
2. Explain 'action spectrum' of photosynthesis.
3. What are the types of chlorophyll?
4. What is the importance of carotene?
5. Describe 'absorption spectrum' in photosynthesis.
6. What is photosystem? Explain.
7. What is the role of carbon dioxide in photosynthesis?
8. How it was confirmed that 'plants split water as a source of hydrogen releasing hydrogen as a byproduct?
9. What is the importance of G3P?
10. What is the effect of temperature on the activities of RuBisCO?
11. What are the disadvantages of photorespiration?
12. How photorespiration evolved?
13. Write the differences between:
    (a) chlorophyll a and chlorophyll b
    (b) carotene and xanthophyll
    (c) action spectrum and absorption spectrum
    (d) absorption spectrum of chlorophyll a and b
    (e) antenna complex and reaction centre
    (f) photosystem I and photosystem II
    (g) light dependent reaction and light independent reaction of photosynthesis
    (h) oxidative phosphorylation and photophosphorylation
    (i) cyclic photophosphorylation and non-cyclic photophosphorylation
    (j) C_4 carbon fixation and C_3 carbon fixation
    (k) lactic acid fermentation and alcoholic fermentation
    (l) Calvin cycle and Krebs cycle
    (m) oxidative phosphorylation and substrate level phosphorylation

Section III: Extensive Answer Questions

1. What is photosynthesis? Explain the role of light in photosynthesis.
2. Describe the structure of chlorophyll.
3. Write a note on the photosynthetic pigment carotene.
4. Explain the arrangement of photosystems.

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5. Describe the role of water in photosynthesis.
6. Describe the mechanism of photosynthesis.
7. Explain in detail the light dependent phase of photosynthesis?
8. Explain in detail the light independent phase of photosynthesis?
9. Describe cyclic photophosphorylation.
10. Describe Calvin cycle.
11. Describe the kinds of cellular respiration.
12. Give an account of 'Glycolysis'.
13. Explain oxidation of pyruvate.
14. Explain Krebs cycle.
15. Explain electron transport chain.
16. Explain chemiosmosis and oxidative phosphorylation.
17. Describe substrate level phosphorylation.

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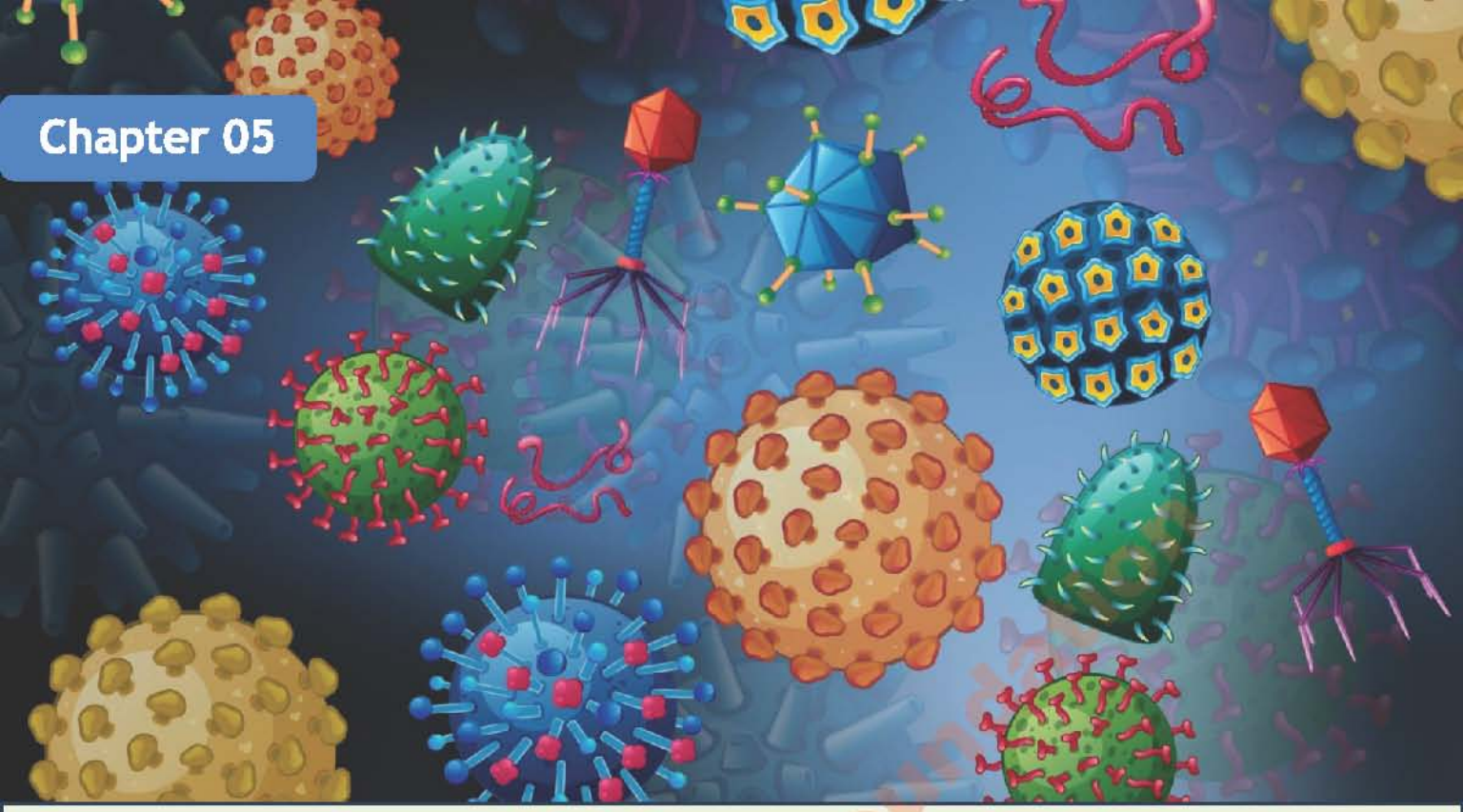


Chapter 05

ACELLULAR LIFE

SLOs: After completing this lesson, the student will be able to:

1. Justify the status of viruses among living and non-living things.
2. Trace the history of viruses since their discovery.
3. Classify viruses on the bases of their hosts and structure.
4. Describe the structure of a model bacteriophage, and HIV.
5. Justify that a virus must have a host cell to parasitize in order to complete its life cycle.
6. Explain how a virus survives inside a host cell, protected from the immune system.
7. Determine the method a virus employs to survive/ pass over unfavourable conditions when it does not have a host to complete the life cycle.
8. Describe the Lytic and Lysogenic life cycles of a virus.
9. Outline the usage of bacteriophage in genetic engineering.
10. Explain the life cycle of HIV.
11. Justify the name of the virus i.e., "Human Immunodeficiency Virus" by establishing T-helper cells as the basis of immune system.
12. Reason out the specificity of HIV on its host cells.
13. List the symptoms of AIDS.
14. Explain opportunistic diseases that may attack an AIDS victim.
15. Describe the treatments available for AIDS.
16. List some common control measures against the transmission of HIV.
17. Describe the causative agent, symptoms, treatment and prevention of the following viral diseases: hepatitis C, herpes, polio and leaf curl virus disease of cotton.
18. List the sources of transmission for each of the above-mentioned diseases.
19. Describe the structure of prions and viroids.
20. List the diseases caused by prions and viroids.
21. Interpret how viral infections cause global economic loss.
22. Describe the limitations of the vaccine for the common cold/ flu virus.

You have got a very brief introduction of biodiversity in IX-X biology course. This chapter deals with nature of virus, prions and viroids as acellular level of organization and the role they play in the economy of a country by causing preventable and fatal infectious diseases.

5.1 VIRUSES-DISCOVERY AND STRUCTURE

You must have heard about influenza, bird flu, polio, swine flu, dengue fever etc. All these and many other diseases are caused by the infectious agents called **viruses**. The viruses are pathogens, which cause diseases in animals and plants.

Viruses are not cells; they are not capable of independent replication. They cannot synthesize their own energy and proteins. They are too small to be seen under the light microscope.

5.1.1 History and Discovery of Virus

The word virus is derived from a Latin word "venom" meaning 'poison'. In past, the term virus was associated with infectious diseases which have unknown cause. The first evidence about the existence of virus came when (in 1884) **Charles Chamberland**, who worked with **Louis Pasteur**, found that the causative agents of rabies could pass through the porcelain filter (pore sizes of 100 - 1000 nm). However, such filters could be used to completely remove all bacteria or other cells known at the time from a liquid suspension.

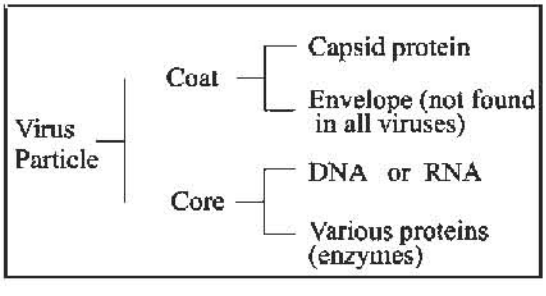
Tobacco mosaic disease was thought to be caused by bacteria. **Iwanowsky** (in 1892) extracted the juice from the leaves of tobacco having tobacco mosaic disease. In order to remove bacteria, the juice was passed through porcelain filter. He then rubbed the filtered juice on the leaves of healthy plants, expecting no disease to develop, but the healthy leaves soon showed the symptoms of the disease.

By 1900, similar disease producing substance had been discovered in both plants and animals. The name filterable viruses were given to these substances. **W. M. Stanley** (in 1935) crystallized the infectious particle, now known as tobacco mosaic virus (TMV). Subsequently many other viruses actually have been seen with the help of the electron microscope. The study of virus is called **virology**.

5.1.2 Characteristics of viruses

They show the characteristic of both living and non-living things. The **living characteristics** of viruses are: (1) Viruses occur in different varieties or strains. (2) They have their own genetic material in the form of either DNA or RNA that can undergo mutation. (3) They reproduce using the metabolic machinery of the host cell they infect. (4) They enter the cells of living organism and cause disease i.e., intracellular obligate parasite. (5) They get destroyed by ultraviolet rays.

The **non-living characteristics** of viruses are: (1) They lack cellular structure, coenzyme and enzyme system and do not have metabolic activity of their own. (2) They can be crystallized and stored in bottles. (3) They do not respire. Viruses behave as non-living, inert infectious particles outside the host.



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5.1.3 General Structure of a Virus

Viruses have a very simple structure. A complete viral particle is called **virion**. Primarily, it can be divided into two parts i.e., **core** and **coat**.

The core is inner part of virion which consists of viral genome and various proteins (enzymes). **Genome** is the genetic material, which is either DNA or RNA, which may be single stranded or double stranded. **Core proteins** include one or more enzymes that facilitate the virus in its mode of action within host body. For example; all single stranded RNA viruses have the enzyme to convert single stranded RNA genome into double stranded RNA genome. Retroviruses and hepatitis B virus contain the enzyme reverse transcriptase to convert single stranded RNA genome into double stranded DNA genome.

The **coat** is the outer covering of viral particle which consists of capsid and envelope. The **capsid** is the protective coat of protein surrounding the core. Capsid is composed of identical repeating subunits called **capsomeres**. The number of capsomeres is specific to a particular kind of virus. For example: Herpes virus has 162 capsomeres in its capsid while adenovirus that causes common cold contains 252 capsomeres in its capsid. There are two forms of symmetry in virus capsid. When the capsomeres are arranged in 20 triangles, it is called **icosahedral** (polyhedral or spherical). When the capsomeres are arranged in a hollow coil that appears rod shaped, it is called **helical**. A few viruses have an additional lipoprotein **envelope** around the capsid which is derived from the cell surface membrane of the host and also contain virally encoded proteins. The viral envelope is often covered with **glycoprotein spikes** that help them to recognize the host cell.

5.1.4 Classification of Virus

Virus classification is either based upon host organisms or on other structural characters such as morphology, genome type and mode of action in the host. The internationally agreed system of virus classification is based on the structure and composition of the virus particle (virion). In some cases, the mode of replication is also important in classification.

Classification of viruses based upon host

Viruses can be classified on the basis of their hosts e.g., bacteriophage virus, plant viruses and animal viruses.

> David Baltimore, a Nobel Prize-winning biologist, devised the Baltimore classification system, which places viruses into one of seven groups, based on their mode of replication, and genome type.

Bacteriophage virus: It attacks bacteria. It is a DNA virus with a polyhedral head and a tail.

Plant viruses: More than 2,000 types of viral plant diseases are known. Most plant viruses discovered till to date including tobacco mosaic virus (TMV), having an RNA genome. Many viruses have rod shaped capsid like TMV e.g., potato yellow dwarf virus.

Animal viruses: Animal viruses occur as parasites in animals. Viruses cause foot and mouth disease in livestock. Rous sarcoma virus causes cancer in animals. In many viral infections viruses attack and destroy certain cells in the human body causing the symptoms and diseases. Papovirus causes warts. Poxivirus causes small pox. Picornovirus causes polio, hepatitis A etc. Paramyxovirus causes measles, mumps.

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Classification of viruses based upon structure

(i) On the bases of capsid viruses are classified as:

(a) helical capsid e.g., tobacco mosaic virus.

(b) polyhedral capsid e.g., Adenoviruses.

(c) enveloped viruses e.g., Influenza viruses.

(d) complex capsid e.g., Bacteriophage.

(ii) On the bases of genomes viruses are classified as:

(a) Double-stranded (dsDNA) e.g. smallpox virus.

(b) Single -stranded DNA (ssDNA) e.g., mild rash virus.

(c) Double-stranded RNA (dsRNA) e.g., diarrhoea virus.

(d) Single-stranded RNA (ssRNA); serves as mRNA e.g., Rubella virus.

(e) ssRNA; template for mRNA synthesis e.g., Influenza virus.

(f) ssRNA; template for DNA synthesis., HIV.

5.2 PARASITIC NATURE OF VIRUS

Viruses are parasitic in nature. They are highly specific to their host. Bacteriophage infects only bacteria, the tobacco mosaic virus infects only tobacco plants and rabies virus infects only mammals. Some human viruses even specialize in a particular tissue. HIV will enter only certain types of white blood cells, the poliovirus reproduces in spinal nerve cells, and hepatitis viruses infect only liver cells. Human cold viruses infect only the cells lining the upper respiratory tract. Actually viruses have protein spikes on their surfaces which help them to attach with specific receptors on the host cells. The specificity of attachment determines the **host range** of the virus. Some viruses have a narrow range, whereas others have quite a broad range. For example, poliovirus can enter the cells of only humans and other primates whereas rabies virus can enter all mammalian cells.

5.2.1 Viruses must require host cell to complete life cycle

A virus must have a host cell to parasitize in order to complete its life cycle because viruses are obligate intracellular parasites, which means they cannot multiply outside a living cell. Viruses infect all sorts of cells, from bacterial cells to human cells. An isolated virus is unable to reproduce or do anything else except infect an appropriate host cell. Viruses lack metabolic enzymes, ribosomes, etc., for making proteins. Therefore, they need host cell to complete their life cycles

5.2.2 Survival of viruses inside a host cell

When virus infects an organism, it enters inside host cell. It hides from the immune system of host. The host immune system is unable to find the virus. Virus rapidly changes its own genetic constitution so the vaccines or antibodies of host against them become ineffective. When virus enters a cell the capsid protects the viral genome from nucleases of the host cell. Nucleases are the enzymes found in all cells. The breakdown DNA or RNA.

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5.2.3 Viruses can pass unfavourable conditions outside the host

Viruses unlike other "living" organisms do not need food to survive. They remain dormant or inactive outside the host body. Being tiny, they are not threatened by the other microorganisms. Their only concern would be the pH and temperature, as these would denature the protein. The virulence of the virus outside the host is maintained for a certain period of time and the time period depends on what virus is or pH and temperature of the medium. Viruses outside the host are non-living. It can be said that they go under dormant period as they do not have any metabolic activity outside living host, however, the genome of the virus remains viable for long time (in inactive form) outside the host.

Non-enveloped viruses can in fact survive for long periods outside the host (up to several days) whereas enveloped viruses survive for shorter time periods. This is because many enveloped viruses rely on the proteins on the surface of the membrane to attach to the host cell, this envelope is generally sensitive to degradation to sunlight and normal cleaning procedures.

5.3 BACTERIOPHAGE

The bacteriophage or simply phage is the virus that attacks upon bacteria.

5.3.1 Structure of Bacteriophage

It is generally a tadpole shaped virus. It consists of head, neck and tail. The **head** is icosahedral in shape. The inner core of head consists of a single stranded DNA genome. Below the head is narrow **neck** or collar which separates head and tail. The **tail** is a hollow tube made up of proteins through which the nucleic acid passes during infection. The tail is surrounded by a contractile sheath, which contracts during infection of the bacterium. At the end of the tail a **base plate** is present which possesses about six tail fibres around it and several **tail pins** or spikes at its lower surface. The **tail fibres** and tail pins are involved in the binding of the phage to the bacterial cell. At the bottom of core tube of tail, an enzyme, the **lysozyme** is present which is released upon contraction of tail. It digests the portion of host cell wall so that core tube can be penetrated into the host cell during infection.

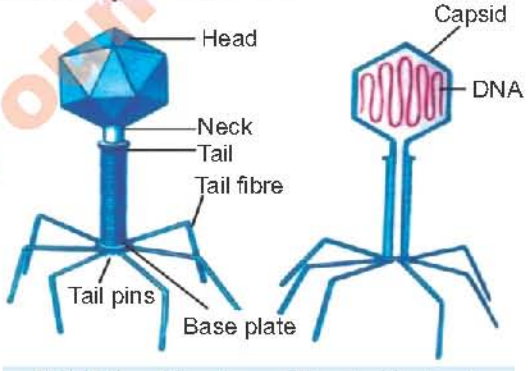

Fig. 5.1: Structure of bacteriophage

5.3.2 Life cycle of bacteriophage

Bacteriophages show two types of life cycles i.e., **Lytic cycle** and **Lysogenic cycle**. The life cycle of bacteriophage comprises two main steps i.e., infection process and replication within the host cells. The initial steps in the infection process such as adsorption, penetration and genome injection are quite similar in both cycles but mode of replication is much different in lytic cycle or lysogenic cycle.

Since the prophage contains genes, it can confer new properties to the bacteria. When a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell. This process is known as lysogenic conversion or phage conversion. *Clostridium botulinum*, a causative agent of food poisoning, makes several different toxins, 2 of which are actually encoded by prophage genomes.

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5.3.3 Infection Process

The common steps of infection process of bacteriophages to their host are as under:

Adsorption

The first step in the infection process is the adsorption of the phage to the bacterial cell. This step is mediated by the tail fibres and tail pins/spikes. Bacteriophages attach to specific receptors on the bacterial cell.

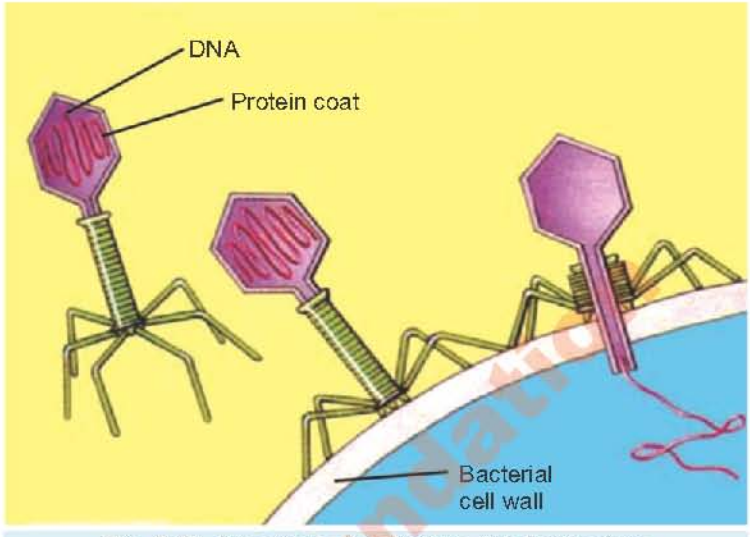

Fig. 5.2: Insertion of core into the bacterium

Penetration

The binding of the phage to the bacterium results in the contraction of the sheath and release of lysozyme that digest the portion of bacterial envelope; as a result, the hollow core tube is pushed through the bacterial envelope. This insertion of core tube is called **penetration.**

Genome injection

The penetration of core results into the injection of viral DNA in the bacterial cytoplasm whereas, the remainder of the phage remains on the outside of the bacterium.

5.3.4 Replication of Bacteriophage in Lytic Cycle

The bacteriophage that performs lytic cycle is called **lytic** or **virulent phage** because it immediately causes lysis (breakdown) of its host cell after its own multiplication. It develops **Master-Slave relationship** with the host cell because host genomic DNA is immediately disintegrated by the virally encoded DNA digesting enzyme (DNAase). Since viral DNA is already undergone certain chemical modification therefore, such enzymes do not affect it. The disintegration of host DNA enables the viral DNA to take over the control of the whole metabolic machinery of its host. In lytic cycle the subsequent steps are **synthesis of phage components, assembly, maturation, lysis** and **release.**

Soon after the disintegration of host DNA phage specified mRNAs and proteins are beginning to produce. Structural proteins (head, tail) that comprise the phage as well as the proteins needed for lysis of the bacterial cell are separately synthesized. Nucleic acid is then packaged inside the head and then tail is added to the head. The assembly of phage components into mature infective phage particle is known as maturation. Within 20 to 25 minutes, approximately 200 phage particles are produced. In lysis and release phase the bacteria begin to lyse due to the accumulation of the phage lysis protein i.e., lysozyme and intracellular phage particles are released into the medium.

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5.3.5 Replication of Bacteriophage in Lysogenic Cycle

The bacteriophages that perform lysogenic cycle are called **lysogenic** or **temperate phages.** These phages can either multiply via the lytic cycle or enter a dormant state in the cell. Such phages develop **Host-Guest relationship** because in this case the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells. This integrated state of phage DNA is termed **prophage.** This process is known as **lysogeny** and the bacteria harbouring prophage are called **lysogenic bacteria.**

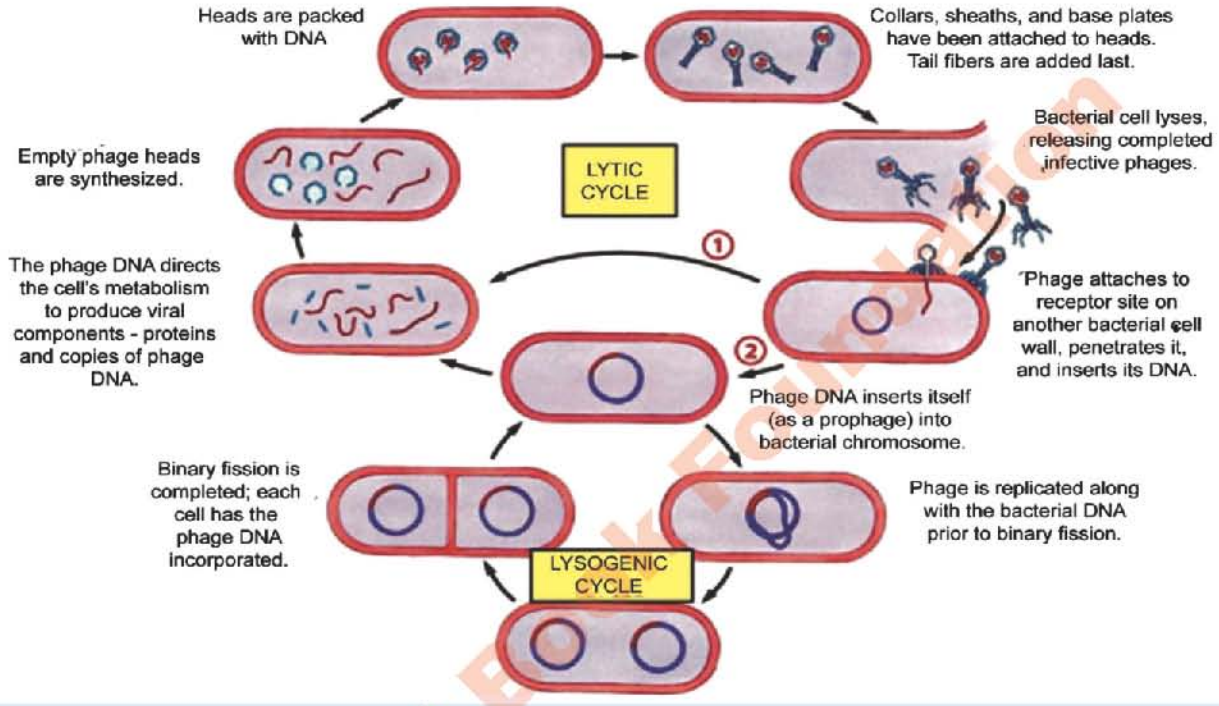

Fig: 5.3: Lytic and lysogenic life cycle of bacteriophage

The lysogenic state of a bacterium can get terminated anytime when it is exposed to adverse conditions. This process is called **induction.** Conditions that favour the termination of the lysogenic state include: desiccation, exposure to UV or ionizing radiation, exposure to mutagenic chemicals, etc. The separated phage DNA then initiates lytic cycle resulting in cell lysis and releases of phages. Such phages are then capable of infecting new susceptible cells and render them lysogenic.

5.3.6 Usage of Bacteriophages in Genetic Engineering

Genetic engineering is the field of biotechnology in which alteration in genetic material of an organism is carried out such as transfer of gene from one organism to another. Several biological tools have been used in genetic engineering to accomplish the required task. The bacteriophages have also been used in number of ways in different approaches of genetic engineering. Some of them are outlined below:

a) Beside bacterial plasmids the phage DNA has also been used as **vector** in genetic engineering techniques such as development of **genomic library** (a collection of bacteria or bacteriophage clones which contains multiple copies of all the genes of an individual's genome)

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b) **Phage therapy** is the application of genetically engineered phages that can kill pathogenic bacteria. Phage therapy has advantages over conventional antibiotic therapy. As phages are fairly narrow in their spectrum of activity, meaning that with phage treatment it is possible to kill bacterial pathogens while avoiding harming of normal bacterial flora, i.e., our good bacteria.

c) Bacteriophages have been used for many years as tools for the treatment of bacterial infections but recently a new application in the area of **antibacterial Nano medicines** has been discovered in which bacteriophages can be formulated as targeted drug-delivery vehicles.

5.4 HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome or AIDS. It was identified (in 1984) by research team from Pasture Institute in France and National Institute of Health in USA. The virus was named HIV (in 1986).

5.4.1 Structure of Human Immunodeficiency Virus

Human Immunodeficiency Virus (HIV) is a **retrovirus.** It is spherical in shape. The outer covering is a lipoprotein **envelope** which consists of two layers of lipids; different proteins are embedded in the viral envelope, forming **"spikes"** consisting of the outer **glycoprotein gp 120** and the **transmembrane gp 41.** The lipid membrane is borrowed from the host cell during the budding process (formation of new particles). gp 120 is needed to attach to the host cell, and gp 41 is critical for the cell fusion process. Beneath envelope another protein shell is present which is made up of **matrix proteins.** It lies between the envelope and capsid. The HIV capsid is somewhat conical shaped which is composed of capsomeres. The viral core contains two single strands of HIV RNA and the enzymes needed for HIV replication, such as reverse transcriptase, integrase and protease. The **reverse transcriptase** enzyme is used to convert viral RNA genome into viral DNA genome, integrase enzyme is used to incorporate viral DNA into host DNA while the protease enzyme is used to break large structural proteins into smaller units. These structural proteins are encoded by three out of the nine virus genes.

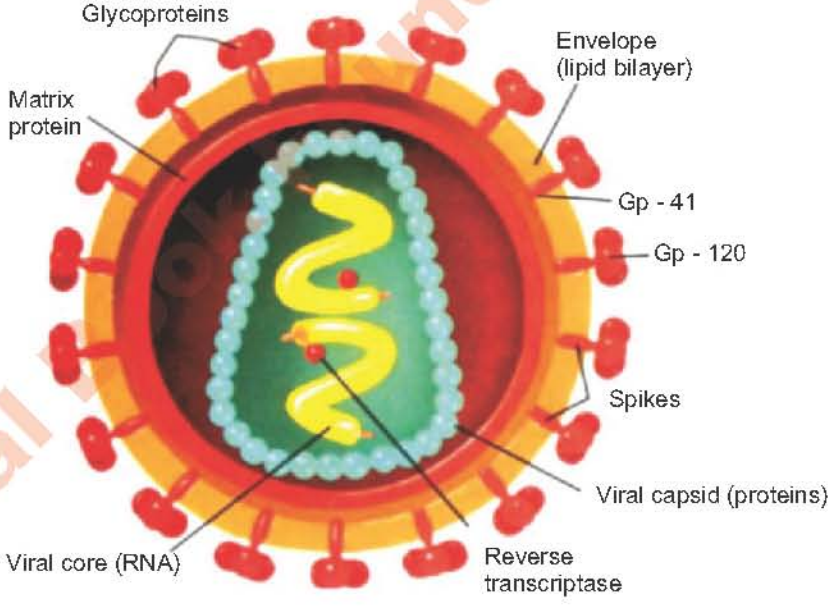

Fig: 5.4: Human immunodeficiency virus (HIV)
(cross section)

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5.4.2 Life Cycle of HIV

HIV attacks only specific cells of host called CD4 cells. Helper T cells, macrophages and certain brain cells have CD4 receptors and are called CD4 cells. CD4 receptors are recognized by HIV for attachment. As HIV only attaches with CD4 cells so it has host cell specificity.

Following steps are involved in the life cycle of HIV. (1) The initial step in the life cycle of HIV is **attachment** which is characterized by the binding of the virion gp 120 envelope proteins to the CD4 proteins (a receptor) on the surface of T cells. (2) Next the fusion of the viral envelope with the cell membrane takes place by using gp 41 and the virion enters the cell by endocytosis. Once inside the host cell, the HIV particle sheds its protective coat i.e., **uncoating** occurs. This leaves the single stranded viral RNA in the cytoplasm along with viral enzymes. (3) The enzyme called **reverse transcriptase** synthesizes a single stranded DNA complementary to virus RNA therefore, called **complementary DNA** (cDNA). (4) After reverse transcription the viral genomic RNA is disintegrated by the ribonuclease (RNAase) enzyme. (5) The single stranded cDNA is replicated to form double stranded cDNA. (6) The double stranded cDNA then integrates into the host cell DNA. Integration is mediated by a virus encoded enzyme integrase. (7) The integrated DNA is now called **provirus**. (8 and 9) Viral mRNA is transcribed from the proviral DNA by the host cell RNA polymerase. During **transcription** not only viral mRNAs for different protein are formed but viral genomic RNA is also produced. (10) The viral mRNAs are **translated** by host ribosomes into several large proteins, which are then cleaved by the virus-encoded protease to form the virion structural proteins. (11) The viral components are assembled and **mature virions** are produced.

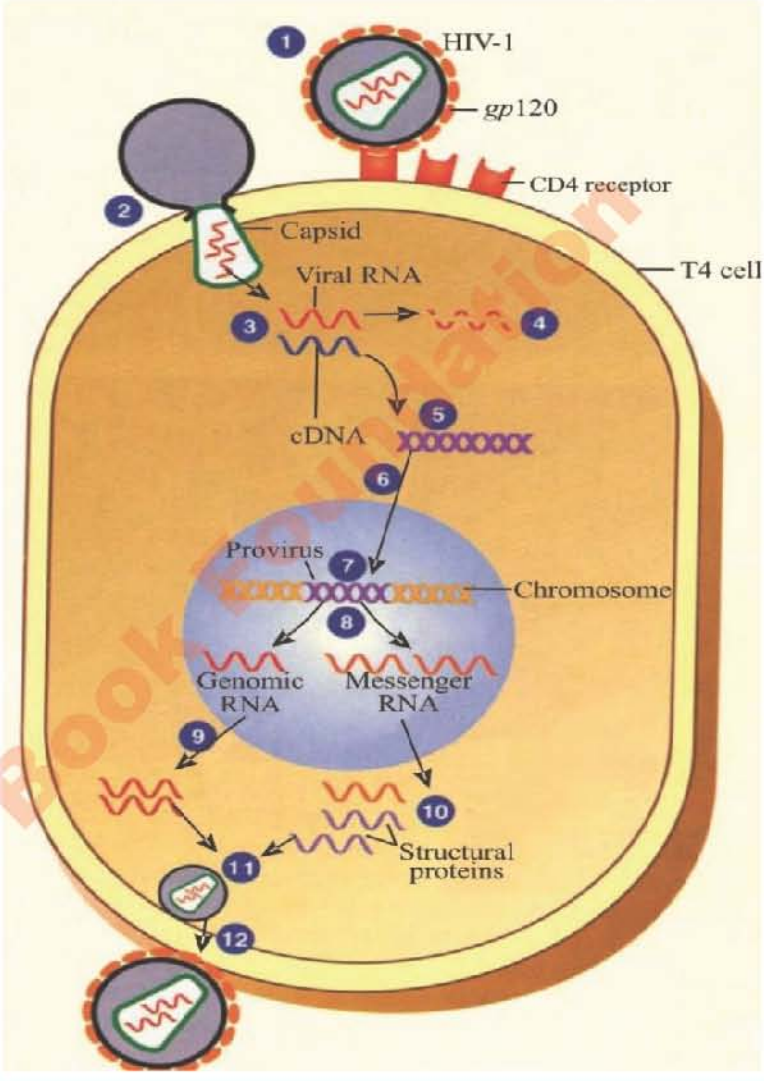

Fig: 5.5: Life cycle of HIV 1. Attachment/ adsorption. 2. Penetration. 3. Reverse transcription. 4. Breakdown of viral genomic RNA. 5. Replication. 6. Integration. 7. Provirus. 8. and 9. Transcription. 10. Biosynthesis of protein. 11. Maturation. 12. Release.

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(12) Finally, the mature virions are gradually **released by budding off** from the host cell and enclosing a portion of host cell membrane around them. In this way host cell size is decreased enough that it becomes non-functional.

HIV AIDS naming

Helper T cells are the most important cells of immune system. They help and activate other cells of immune system. Helper T cells activate B cells to secrete antibodies. They also activate cytotoxic T cells to kill virus infected and cancerous cells. Since, helper T cells regulate immunity by enhancing the response of other immune cells. The decrease in the number of helper T cells causes deficiency of the human immune system, therefore, the virus has been named Human Immunodeficiency Virus (HIV).

5.4.3 Symptoms of AIDS

An HIV infection can be divided into 3 stages: Asymptomatic carrier, AIDS Related Complex (ARC), Full Blown AIDS. In **asymptomatic carrier** symptoms that may include are fever, chills, aches (continued pain), swollen lymph glands and an itchy rash. These symptoms disappear and there are no other symptoms for nine months or longer. Although the individual exhibit no symptoms during this stage, he or she is highly infectious. The standard HIV blood test for the presence of antibody becomes positive during this stage.

The most common symptoms of **AIDS related complex** are swollen lymph glands in the neck, armpit or groin that persist for months. Other symptoms include night sweats, persistent cough, flu, and persistent diarrhoea, loss of memory and depression.

The **full blown AIDS** is the final stage. In it there is severe weight loss and weakness due to persistent diarrhoea and usually one of several opportunistic infections.

5.4.4 Opportunistic Diseases

HIV does not cause any disease nor kills any person. It only destroys T-cells of immune system. The decrease in the human immune system results in the inability of the body to fight diseases. Due to weak defence system a person suffering from AIDS is attacked by diseases called opportunistic diseases, e.g., skin cancer, fungal infection, viral infection, gastrointestinal diseases, respiratory diseases, nervous system and eye diseases.

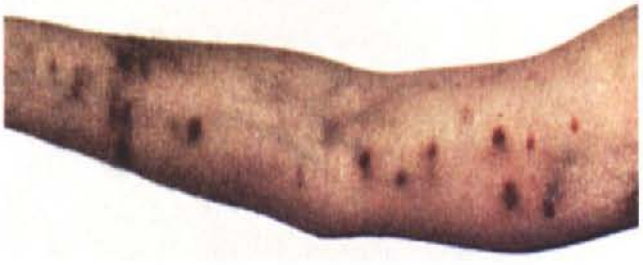

Fig. 5.6: This photograph shows the multiple wounds of the skin cancer on the arm of a patient with AIDS.

5.4.5 Treatment of AIDS

HIV is treated using a combination of medicines to fight HIV infection. This is called **antiretroviral therapy** (ART). ART is not a cure, but it can control the virus so that HIV positive person can live a longer, healthier life and reduce the risk of transmitting HIV to others. ART is a highly effective treatment for HIV infection, preventing progression of the disease in the vast majority of recipients. When ART is accessible and started early in the course of infection, the lifespan of HIV-positive people is typically very close to that of comparable HIV-negative people. But ART can have toxicities, is often costly, and requires strict daily pill taking that can

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lessen quality of life. Because of the limitations of ART, a cure for HIV infection remains a vital goal for research.

5.4.6 Control Measures Against the Transmission of HIV

AIDS can be controlled by preventing transfer of body fluid (blood, serum, semen, etc.,) from patient to unaffected person. The following behaviour of precautionary measure will prevent AIDS: (1) Do not use used syringes and needles. (2) For blood transfusion, blood must be used after proper screening for HIV. (3) Do not share toothbrushes, blades and towels with anyone. Special cares to be taken at barber's shop or hair cutting saloons, beauty salons. (4) Surgical instruments must be properly sterilized. (5) AIDS is primarily a sexually transmitted disease. Refrain from immoral sexual activities and follow Islamic teachings to pass healthy, neat and clean life. (6) Mother having HIV should not feed their babies. Shaking hands, hugging, coughing or sneezing and swimming in the same pool do not transmit HIV. One cannot get AIDS from inanimate objects such as toilets, door knobs, telephones, office machines and house hold furniture. AIDS is not transmitted by mosquitoes and other insects.

5.5 VIRAL DISEASES

In this section we will describe causative agent, symptoms, treatment, transmission and prevention of hepatitis, herpes, polio and cotton leaf curl disease.

5.5.1 Hepatitis

Hepatitis is generally characterized as inflammation of liver including other symptoms. It is generally caused by viral infection or rarely due to toxicity of drugs and certain other causes. It may present in acute (recent infection, relatively rapid onset) or chronic (slowly progressing) forms. The most common causes of viral hepatitis are Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, and Hepatitis E.

Hepatitis "A"

Cause: Hepatitis A (also called infectious hepatitis) is caused by HAV. **Transmission:** HAV is transmitted by the faecal-oral route. **Symptoms:** The typical symptoms are fever, loss of appetite, nausea, vomiting and jaundice. Dark urine, pale faeces are also seen. **Treatment:** No antiviral therapy is available. **Prevention:** Active immunization with a vaccine containing inactivated HAV is available. Observation of proper hygiene.

Hepatitis "B"

Cause: Hepatitis B (also called serum hepatitis) is caused by HBV. **Symptoms:** It is similar to hepatitis A. **Transmission:** The three main modes of transmission are via blood, sexual contact and prenatally from mother to new born. **Treatment:** Alpha interferon is effective against HBV. **Prevention:** Vaccine is highly effective in preventing hepatitis "B". All blood transfusion should be screened.

Hepatitis "C"

Cause: Hepatitis C is caused by Hepatitis C virus. **Transmission:** It is only transmitted via blood. **Symptoms:** Symptoms are just like hepatitis B. **Treatment:** A combination of alpha interferon and ribavirin is the treatment choice for chronic hepatitis C. **Prevention:** No vaccine is available. Blood transfusion should be screened as preventive measure.

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Hepatitis "D"

Cause: The only human disease known to be caused by a viroid is hepatitis D. **Transmission:** The hepatitis D viroid can only enter a human liver cell if it is enclosed in a capsid that contains a binding protein. It obtains this from the hepatitis B virus. The viroid then enters the blood stream and can be transmitted via blood or serum transfusions. **Symptoms:** As in hepatitis B but more severe. **Treatment** and **Prevention:** Same as HBV.

Hepatitis "E"

It is caused by HEV. **Transmission:** Like HAV, it is also transmitted by the faecal-oral route. **Treatment and Prevention:** There is no antiviral treatment and vaccine. Observation of proper hygiene.

5.5.2 Herpes Simplex

Herpes simplex is a superficial viral infection characterized by one or more painful, fluid-filled sores or blisters appear on the skin or epithelium of outer openings of the body. Tingling, itching, or burning may be felt on the skin before the blisters appear. Blisters break open and often ooze fluid and form a crust, before healing. The sores can last from 7 to 10 days. There are two primary types of herpes i.e., oral herpes and genital herpes.

Oral Herpes

Cause: It is caused by herpes simplex virus type-1. **Transmission:** HSV Type-1, is transmitted primarily through oral secretions (saliva) or physical contact with sores on the skin. It can also be spread by sharing objects such as toothbrushes or eating utensils. **Symptoms:** Most blisters appear on the lips or around the mouth. Sometimes blisters form on the face or on the tongue. **Treatment:** Antiviral drugs are used to treat herpes. **Prevention:** Avoid contact with affected area of the patient.

Genital Herpes

Cause: It is caused by herpes simplex virus type-2. **Transmission:** In general, a person can only get HSV type 2 infection during sexual contact with someone who has a genital HSV-2 infection. **Symptoms:** In genital herpes the sores typically occur on the penis, vagina, buttocks, or anus. **Treatment:** Antiviral drugs are used to treat Herpes. **Prevention:** Avoid contact with affected area of the patient.

5.5.3 Poliomyelitis

Cause: It is caused by polio virus which is also an enterovirus. **Transmission:** Polio virus is transmitted by the faecal oral route. **Symptoms:** It replicates in the oropharynx and intestinal tract and spread to blood and central nervous system where virus replicates in the motor neurone located in the spinal cord. Death of these cells results in paralysis of the muscles innervated by theses neuron. The motor nerve damage is permanent. **Treatment:** There is no antiviral therapy. Physiotherapy for the affected muscles is important. **Prevention:** Polio can be prevented by the killed (Salk vaccine, or injectable polio vaccine or IPV) and the live, attenuated (weakened) vaccine (sabin vaccine or, oral polio vaccine or OPV).

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5.5.4 Cotton Leaf Curl Disease

Cotton leaf curl disease (CLCuD) is a serious disease of cotton. **Cause:** Begomoviruses. **Transmission:** This disease is transmitted by the whitefly. **Symptoms:** The symptoms are initially characterized by a deep downward cupping of the youngest leaves. This is followed by development of cup-shaped, leaf-like structures. **Treatment** and **Prevention:** Control of CLCuD is mainly based on insecticide treatments against the insect vector.

Fig: 5.7: Cotton leaf curl disease

Fig. 5.8: Whitefly

5.6 ECONOMIC LOSSES DUE TO VIRAL INFECTIONS

COVID-19 is considered as the most significant threat since World War II and the greatest global health disaster of the century. The Coronavirus outbreak has caused a global economic collapse. Most countries implemented full or partial lockdown measures to slow the spread of disease. The lockdown slowed global economic activity, many companies down sized or closed down, and people lost their jobs. Service providers, manufacturers, agriculture, food industry, education, tourism, airlines, sports and entertainment sectors were adversely affected. Multiple industries were impacted by a disease outbreak, including the capital market, labour market, foreign trade, consumer spending, and production. It has also negatively affected the GDP of many countries.

The virus has claimed thousands of lives and posed considerable challenges to countries. The GDP has reduced, and this pandemic has cost the world more than 8.5 trillion dollars. The World Bank estimates that even a weak influenza pandemic, such as the H1N1 outbreak of 2009, could reduce global GDP by half, or about $300 billion.

5.7 LIMITATIONS OF FLU VACCINE

Although we can often refer to the causative agent of cold as "the cold virus" there are actually more than 200 viruses that cause cold. Developing a vaccine against the infection is not practical. In addition to the constantly evolving strains of the flu virus, our body's immune response changes over time. Taken together, those two factors essentially render the previous years' vaccinations useless against new strains.

5.8 PRIONS AND VIROIDS

The idea of an infectious agent that did not use nucleic acids and proteins together was considered impossible, but pioneering work by Nobel Prize-winning biologist **Stanley Prusiner** has convinced

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the majority of biologists that such agents do indeed exist. Prions and viroids are such agents which are acellular infectious particles like viruses but are even simpler and smaller than viruses.

5.8.1 Prions

Prions are infectious protein particles, smaller than viruses, that contain no nucleic acids (neither DNA nor RNA). Prions are much **more resistant** to inactivation by ultraviolet light and heat than are viruses. Prions are composed of a single protein. This protein is encoded by a **single cellular gene.** Prion proteins on brain cells cause clumping, memory loss, neurodegeneration and permanent damage.

Fatal neurodegenerative diseases, such as **Kuru** in humans and in cattle **mad cow disease** were shown to be transmitted by prions. Prions spread through body fluids, direct and indirect contact. There is no treatment or cure of prion diseases.

5.8.2 Viroids

Viroids are pathogens that consist of a short, circular, single-stranded RNA without a protein coat or envelope. Viroid RNA does not code for any protein. The replication mechanism involves an enzyme RNA polymerase II, which synthesizes new RNA using the viroid's RNA as template. Some viroids are ribozymes, having catalytic properties which allow self-cleavage. The only human disease known to be caused by a viroid is **hepatitis D.** Viroids cause several plant diseases, e.g., **potato spindle tuber disease, cucumber pale fruit disease,** etc. Viroids spread via mechanical damage, seed, pollen, or biological vectors.

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EXERCISE

Section I: Multiple Choice Questions

Select the correct answer:

1. Viruses are considered non-living because

   A) do not mutate
   B) they do not move
   C) cannot reproduce independently
   D) have nucleic acid

2. Which of these are found in all viruses?

   A) envelope, nucleic acid, capsid
   B) DNA, RNA and proteins
   C) proteins and nucleic acid
   D) protein, carbohydrate, lipids

3. Which step in the lytic cycle follows attachment of virus and release of DNA into the cell?

   A) production of lysosome
   B) disintegration of host DNA
   C) assemblage
   D) DNA replication

4. Which of these is a true statement?
   A) viruses carry with them their own ribosome for protein formation
   B) new viral ribosomes form after viral DNA enters the cell
   C) viruses use the host ribosomes for their own needs
   D) viruses do not need ribosomes for protein formation
5. Which part of an animal virus is not reproduced in multiple copies?
   A) envelope B) protein
   C) capsid D) ribosome
6. RNA retroviruses have a special enzyme that
   A) disintegrates host DNA B) polymerises host DNA
   C) transcribe viral RNA to DNA D) translates host DNA
7. Which of the following illness is caused by a retrovirus?
   A) typhoid B) malaria
   C) AIDS D) sleeping sickness
8. The HIV primarily infects
   A) plasma cells B) helper T cells
   C) all white blood cells D) red blood cells
9. Poliomyelitis affects
   A) motor neuron B) sensory neuron
   C) brain D) muscles
10. HIV attaches to
    A) CD4 protein B) nucleoprotein
    C) lipoprotein D) glycoprotein
11. Hepatitis D is caused by
    A) bacteria B) virus
    C) prions D) viroids

Section II: Short Answer Questions

1. What are the living and non-living characteristics of viruses?
2. Give the classification of viruses based on their hosts.
3. What are the parasitic natures of virus?
4. Justify why a virus must have a host cell to parasitize in order to complete its life cycle.
5. Explain how a virus survives inside a host cell, protected from immune system.

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6. Determine the method a virus employs to survive/ pass over unfavourable conditions when it does not have a host to complete the life cycle.
7. Justify the name of virus i.e., "Human Immunodeficiency Virus" by establishing T-helper cells as the basis of immune system.
8. Reason out the specificity of HIV on its host cells.
9. What are the symptoms of AIDS?
10. Explain opportunistic diseases that may attack an AIDS victim.
11. What are the common control measures against the transmission of HIV?
12. Describe the structure of prions and name any two diseases caused by them.
13. Describe the structure of viroids and name the diseases caused by them.
14. What do you mean by AIDS, HIV, ART, CLCuD and TMV?
15. Distinguish between:
    (a) bacteriophage and HIV virus
    (b) lytic and lysogenic cycle of bacteriophage (c) prions and viriods
    (c)

Section III: Extensive Answer Questions

1. Give the classification of viruses based upon capsid and genomes.
2. Describe the general structure of a virus.
3. Describe the structure of bacteriophage with diagram.
4. Describe the structure of human immunodeficiency virus with diagram.
5. Describe the Lytic and Lysogenic life cycles of a virus.
6. Describe the usage of bacteriophage in genetic engineering.
7. Explain the life cycle of HIV.
8. Describe the treatments available for AIDS.
9. Describe the causative agent, symptoms, transmission, treatment and prevention of the following diseases:
    (a) Hepatitis (b) Herpes (c) Polio (d) Cotton leaf curl disease

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قومی ترانہ

پاک سَرزمین شاد باد! کشورِ حسین شاد باد!
تو نِشانِ عزمِ عالی شان ارضِ پاکستان
مرکزِ یقین شاد باد!

پاک سرزمین کا نِظام قُوّتِ اُخوّتِ عوام
قوم ، مُلک ، سلطنت پائندہ تابندہ باد!
شاد باد منزلِ مُراد!

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