



# General Certificate of Education (Advanced Level)

# Handout for unit 02 Chemical and cellular basis of life

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# Unit 02

# Chemical and cellular basis of life

# 2.1.1. Elemental composition of living matter

There are about ninety two elements naturally occur in earth's crust. Of which, about 20-25% elements are essential to continue healthy life and reproduction. (about 25- elements are essential for humans and about 17 for plants).

Oxygen (O), Carbon (C), Hydrogen (H), and Nitrogen (N) make up 96% of living matter. Calcium (Ca), Phosphorous (P), potassium (K) and sulphur (S)- make up most of the remaining 4% of the mass of the organism.

In humans, C, H, O, N- accounts for 96.3% of the body mass and Ca, P, K, S, Na, Cl, Mg and trace elementsaccounts for the remaining 3.7%. (e.g. B (Boron), Co (Cobalt), Cu (Copper), Cr (Chromium), F (Fluorine), I (Iodine), Fe (Iron),

Mo (Molybdenum), Mn (Manganese), Se (Selenium), Si (Silicon), Sn (Tin), V (Vanadium), Zn (Zinc)

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# 2.1.2. Physical and chemical properties of water important for life

Wateris a vital inorganic molecule; life could not exist on this planet without water. It is important due to following reasons,

- 1. Vital chemical constituent of living cell
- 2. Provides a biological medium for all organisms

Most of above properties are based on the chemical structure of water molecule. Physical and chemical properties of water molecule provide the ability to render the vitality. Water molecule is a small, polar and angular molecule.



Fig 2.1: Chemical structure of the water molecule

Polarity is an uneven charge distribution within a molecule. In water molecule, oxygen atom is slightly negative and hydrogen atom is slightly positive. Weak attractions between the slightly polar hydrogen atom of one water molecuole and the slightly polar oxygen atom of adjacent water molecuoleare known as hydrogen bonds. Thesehydrogen bonds play a major role in maintaining all the properties of water.



Fig 2.2: Hydrogen bonding in water

The properties of water arise due to attractions of different water molecules. When the water is in liquid form its H bonds are very fragile. H bonds form, break and reform with great frequency.

Four major properties of water to maintain life on earth

- 1. Cohesive behavior
- 2. Ability to moderate temperature
- 3. Expansion upon freezing
- 4. Versatility as a solvent

Properties of water related to functions

#### 1. Cohesive behavior

Attraction between water molecules due to hydrogen bonding is known as cohesion. Attraction between water molecules and other substances are known as adhesion. Both of the aboveproperties of water allow it to act as a transport medium.

Due to cohesion between water molecules, water and dissolved substances such as minerals and nutrients transport through vascular tissues, xylem and phloem against gravity.

Adhesion between water molecules and cell walls also helps in conduction of water and dissolved substances.

Water has a high surface tension. This ability is given to water molecules, due to cohesion between the water molecules. Therefore, in an aquatic system, upper surface water molecules are attracted by lower surface molecules and it forms a water film. Small insects e.g. water skaters can walk on the surface of a pond.

#### 2. Ability to moderate temperature

Water can absorb or release a relatively high amount of heat energy by a slight change in its own temperature.

Due to the high specific heat, water will function as thermal buffer in living system and aquatic bodies during the temperature fluctuations on earth.

Due to the high heat of vapourization, with the minimum loss of water an organism can release much heat energy. Therefore, body surface of an organism maintained as cool surface.

e.g. Prevent from overheating.

Evaporation of sweat from human skin helps to maintain the body temperature at constant level.

Transpiration in plants keeps the plant body surface as a cool surface and prevent from becoming too warm in the sunlight.

#### 3. Expansion upon freezing

Generally, in an increase in temperature of any substances, reduces their density and on the other hand, in a decrease in temperature increases their density. When the temperature of water falls below 4 °C, it begins to freeze and forms a crystalline lattice called ice cubes. Therefore water has the maximum density at 4°C. Hence, ice floats on the surface of water bodies. It is an important property of water in polar regions, where, organisms in aquatic bodies can survive during the winter.

#### 4. Versatility as a solvent

This ability is given to water due to their polarity. Polar molecules (e.g. Glucose), non polar ionic (e.g. NaCl), both polar and ionic (e.g. lysozymes) can dissolve in water, because water molecules surround each of the solute molecules and form hydrogen bonds with them. Solubility depends on polarity and not in their ionic nature

# 2.1.3. Chemical Nature and Functions of Main Organic Compounds of Organisms

#### **Carbohydrates**

Most abundant group of organic compound on earth is carbohydrates. Major elemental composition is C, H, and O. Hydrates of carbon contain the same proportion of H: O which equals to 2:1 as in water. General formula is  $C_x(H_2O)_y$ . Three major groups of carbohydrates aremonosaccharides, disaccharides and polysaccharides.

Generally carbohydrates include sugars (monosaccharides and disaccharides) and polysaccharides.

#### Monosaccharides

The simplest form of carbohydrates having general molecular formula as  $(CH_2O)n$  are monosaccharide. Where C varies from 3-7. All monosaccharide are reducing sugars, water soluble and occur in crystalline form.

According to the number of carbon atoms, they are named as;

- 3C- Triose e.g.Glyceraldehydes (Phosphoglyceraldehyde is a derivative of Triose)
- 4C- Tetrosee.g.Erythrose(rare in nature)
- 5C- Pentosese.g. Ribose, Deoxyribose, Ribulose (RUBP is a derivative of ribulose)
- 6C- Hexoses e.g. Glucose, Fructose, Galactose

According to the type of carbonyl (Keto, aldo)group, they are classified as;

- a. Aldoses-glucose, galactose
- b. Ketoses-fructose

Aldose





Fig 2.4: Aqueous form of Glucose molecule







Fig 2.6: Aqueous form of fructose

Fig 2.5: Solid form of fructose

In aqeous media some monosaccharides are in ring form (No need to memorize the chemical structures)

#### Disaccharides

They are sugars formed by joining two monosaccharides by a glycosidic bond.





Fig 2.7: Formation of sucrose (no need to memorize the chemical structures)

Glycosidic bond is formed by removal of a water molecule from two adjacent monosaccharides by a condensation reaction. Water molecule is formed from OH group of one monosaccharide molecule and H from adjoining monosaccharide molecule.

Glucose + Glucose –	Condensation	Maltose $+$ H <sub>2</sub> 0
Glucose + fructose	<u>condensation</u>	Sucrose + $H_20$
Glucose + Galactose	Condensation	- Lactose + $H_20$

Maltose and lactose are reducing sugars and sucrose is a non reducing sugar.

#### Polysaccharides

They are macromolecules and biopolymers. Polysaccharides are made up of few hundred to a few thousand monosaccharide subunits

They arenon crystalline, water insoluble, and not considered as sugars.

Some polysaccharides act as storage components where others contribute to the structure of living organisms. Based on their function they are categorized as Storage polysaccharides and structural polysaccharides.

- 1. Storage- Starch, glycogen
- 2. Structural- Cellulose, Hemicellulose, Pectin

Based on their architecture they are categorized as

- 1. Linear forms- Cellulose, Amylose
- 2. Branched forms- Glycogen, Amylopectin, Hemicellulose

Table 2.1: Major polysaccharides, their monomer sand function

Polysaccharide	Monomer	Function
Starch	Glucose	Stored in plants
Glycogen	Glucose	Stored in animals and fungi
Cellulose	Glucose	Component of Cell wall
Inuline	Fructose	Stored in tubers of Dhalia stored in
Pectin	Galacturonic acid	Component of Middle lamella of plant cell wall
Hemicellulose	Pentose	Component of Plant cell walls
Chitin (nitrogen containing polysaccharide)	Glucosamine	Component of Fungal cell walls and exoskeleton of Arthropods

### Functions of carbohydrates

#### Monosaccharides

- Energy source
- Building blocks of disaccharides and polysaccharides (disaccharides such as maltose, sucrose and polysaccharides such as starch, glycogen)
- Components of nucleotides(DNA, RNA)

#### Disaccharides

- Storage sugar in milk- Lactose
- Translocation in phloem Sucrose
- Storage sugar in sugarcane- Sucrose

#### Polysaccharides

- a.) Storagepolysaccharides-
  - starch stores glucose as energy source in plants and chlorophytes,
  - glycogen stores glucoseas energy source in animals and fungi,
  - inulin stores fructose as energy source in Dahlia tubers
- b.) structural polysaccharides-
  - Cellulose in the cell walls of plants and chlorophytes
  - Pectin in the middle lamella of plant tissues.
  - Hemicellulose in cell walls of plants.

- Peptidoglycan in the cell walls of prokaryotes.
- Chitin in the cell walls of fungi and in exoskeleton in Arthropods.

#### **Lipids**

- Diverse group of hydrophobic molecules
- Large biological molecules but not considered as polymers or macromolecules.
- Consist of C, H, O and H:O ratio is not 2:1. Comparatively more H are present.
- Biologically important types of lipids: Fats, Phospholipids and Steroids.

#### Fats

Fats are made up of glycerol and fatty acids; Glycerol belongs to alcohol group having 3 carbons where each of them bear single hydroxyl group. Fatty acids are hydrocarbon chains with long (16-18) carbon skeleton with a carboxyl group at its one terminal.

Fatty acid molecules bind to each hydroxyl group of glycerol by ester bond. Resulting fat molecules are called as triacylglycerol.



#### Fig 2.9: Formation of Triacylglycerol

Hydrocarbon chains of fatty acids contribute to the hydrophobic nature of the fats. Based on the nature of hydrocarbon chains of fatty acids, they are categorized as

a) Saturated fats- fats made up of saturated fatty acids: fatty acids with hydrocarbons having no any double bonds. Usually animal fats come under this category. They are mostly solid at room temperature. e.g: butter

b) Unsaturated fats- fats made up of unsaturated fatty acids- fatty acids with hydrocarbons having one or more double bonds. Usually plant fats come under this category. They are mostly liquid in room temperature. e.g: Vegetable Oils. Unsaturated fats may classify based on the nature of their double bonds. a) *Cis* Unsaturated fat b) *Trans* Unsaturated fat

Consumption of excess saturated fats and trans unsaturated fats contribute arthrosclerosis.

#### Phospholipids

Phospholipids are major components of the cell membranes. They are composed of two fatty acids and one phosphate group attached to one glycerol molecule. The phosphate group gives the negative

electrical charge to the phospholipid molecule. Typically an additional polar molecule or small charged molecule is also linked to the phosphate group e.g. choline.

The two ends of the phospholipids show different behavior. The hydrocarbon tails are hydrophobic while phosphate group and its attachment (head) are hydrophilic.

**Functions of Lipids** 

- food reserve as energy source (triglycerides such as fats and oils)
- maintain the fluidity of plasma membrane (phospholipids, cholestrol)
- act as signaling molecules (eg. Hormones) that travel through the body
- found as components of animal cell membrane (cholesterol)

#### **Proteins**

Proteins are made up of amino acids. Twenty different amino acids are involved in the formation of proteins. Elemental composition is C, H,O,N and S. At the centre of the amino acid is an asymmetric carbon atom except in glycine. Each amino acid is composed of an amino group, a carboxyl group, a hydrogen atom and a variable group symbolized by R, which is an alkyl group. In the case of glycine R is replaced by H atom. The R group also called the 'side chain' differs with each amino acid where as the other groups are in the ' back bone' (including the H atom).



Amin

Annuo acids may have one or more carboxyl groups and amino groups. Amino group has alkaline nature and carboxyl group has acidic nature. When both characteristics are found in one molecule they are known as **amphoteric** molecules. Therefore, amino acids are as amphoteric.

Two Amino acids undergo condensation reactionby removing a water molecule from both and result a bond known as peptide bond;



Protein is composed of one or more polypeptide chains which are composed of amino acids.

#### Levels of protein structures

There are four levels of structure which play important roles in their functions;

- a) Primary
- b) Secondary
- c) Tertiary
- d) Quaternary

#### a) Primary structure

The unique sequence of linearly arranged amino acids linked by peptide bonds is the primary structure of proteins.

#### b). Secondary structure

The primary structure of a single polypeptide chain coils and folds, as a result of intra molecular hydrogen bonds between the oxygen atoms and the hydrogen atoms attached to the nitrogen atoms, of the same poly peptide chain backbone, to form the secondary structure, which is either  $\beta$  pleated or alpha helical.

- Alpha helix- e.g.Keratin.
- β pleated sheete.g.spider'ssilk fiber

#### b) Tertiary structure

Usually the secondary polypeptide chain bends and folds extensively forming a precise compact unique, functional and three-dimensional shape resulting from following interactions between the side chain/ R-group of amino acids;

- 1. H bonds
- 2. Disulphidebonds
- 3. Ionic bonds
- 4. Van der Waals interactions/ Hydrophobic interactions
- 5.

e.g.most of the enzymes, myoglobin, albumin

c) Quaternary structure

Aggregation of two or more polypeptide chains involve in the formation of one functional protein. Separate chains are called protein subunits which were held together by inter and intra-molecular interactions.

e.g.Haemoglobin, Collagen

#### **Denaturation of proteins**

Denaturation of protein is the loss of specific chemical three dimensional shape due to the alteration of weak chemical bonds and interactions.

Agents affecting the denaturation

- 1. High temperature and high energy radiation
- 2. Strong acids, alkaline and high concentrations of salts
- 3. Heavy metals
- 4. Organic solvents and detergents

#### **Functions of the proteins**

Table 2.2Functions of Proteins

Type of protein	Example	Functions
Catalytic protein	Pepsin, Amylase	Catalyze biochemical reaction
Structural protein	Keratin,	Prevent desiccation
	Collagen	Provide strength and support
Storage	Ovalbumin	Storage protein in egg
	Casein	Storage protein in milk
Transport	Haemoglobin	Transport O <sub>2</sub> and CO <sub>2</sub>
	Serum albumin	Transport fatty acids
Hormones	Insulin	Regulate blood glucose level
	Glucagon	
Contractile/ Motor	Actin/Myosin	Contraction of muscle fibres

Defensive	Immunoglobins	Eliminate foreign bodies

#### Nucleic acids

Nucleic acids are Polymers exist as polynucleotides made up of monomers called nucleotides. They contain C, H, O, N and P. Nucleic acidsare macromolecules, biopolymers. There are two types of Nucleic acids: DNA (Deoxyribo nucleic acids) and RNA (Ribonucleic acids).

#### Structure of nucleotides

Nucleotides have 3 components; namely pentose sugar, nitrogenous base and a phosphate group



A nucleotide without a phosphate group is called a nucleoside.e.g. Adenosine, Guanosine

#### Pentose sugar

Pentose sugars are two types; namely Deoxy ribose and ribose (in deoxyriboseone oxygen atom is less than in ribose)

#### Nitrogenous bases

There are two major groups of nitrogenous bases:

- 1. Purines- larger in size with two rings
- 2. Pyrimidines- smaller in size with a single ring

In purines there are two types; namely Adenine, Guanine. In pyrimidens there are three types, Thyamine, Uracil and Cytocine. Bases are commonly represented by letters A, G, T, U and C respectively.

#### Phosphate group

It gives the nucleic acids the acidic nature.

#### Formation of nucleic acids

Millions of nucleotides join by phospho-di-ester bond to form polynucleotide chains by condensation between the –OH group of the phosphate of one nucleotide with the –OH attached to 3<sup>rd</sup> carbon of pentose sugar of the other. These bonds results in a backbone with a repeating pattern of sugar-phosphate units. Nucleic acids are linear polymers of nucleotides. There are two kinds of nucleic acids depending on the type of the sugar molecules involved. If the sugar molecule in the nucleotide is deoxyribose, the nucleic acid is (DNA). If the pentose sugar is ribose, then the nucleic acid is RNA.

DNA contains Adenine, Thymine, Guanine and Cytosine and RNA contains Adenine, Guanine, Cytosine and Uracil.

#### Structure of DNA molecule (Watson and Crick model)

DNA molecules have twoanti-parallel polynucleotide chains that spiral around an imaginary axis, forming a double helix. The two sugar-phosphate backbones run in opposite directions from each other, and the arrangement is referred to as anti-parallel. The sugar phosphate backbones are on the outside of the helix, and the nitrogenous bases are paired in the interior of the helix. The two strands are held together by hydrogen bonds between the paired nitrogen bases.

#### Base pair rule

Always a purine base, pairs with a specific, pyrimidine base,

A=T (2 hydrogen bonds)

G≡C (3 hydrogen bonds)

Hence two chains (strands) are said to be complementary to each other. These pairs are known as complementary base pairs. In this original double helical structure, one complete turn consists of ten base pairs as shown in the diagram.

#### Functions of DNA

- Store and transmit genetic information from one generation to the next generation
- Store the genetic information for protein synthesis

#### Structure of RNA

This is normally a single strandednucleic acid composed of ribo-nucleotides containing bases, Uracil (U), Cytosine (C), Guanine (G), Adenine (A). Complementary base pairing between two RNA molecules or within the same molecule may occur in some. Complementary base pairing facilitates three dimensional shapes essential for their functioning. Adenine binds with Uracil with two hydrogen bonds and Guanine binds with Cytosine with three hydrogen bonds. There are three types of RNA present in cells,

- I. Messenger RNA (mRNA)
- II. Transfer RNA (tRNA)
- III. Ribosomal RNA (rRNA)

#### 1. Messenger RNA

Messenger RNA is a linear molecule and is the least abundant type of RNAin a cells comparatively. There are two functions;

- 1. Copies the genetic information stored in DNA molecule as a sequence of nitrogenous bases
- 2. Transports genetic information from nucleoplasam to the site of protein synthesis (ribosome) through nucleopores

#### 2. Transfer RNA (tRNA)

Smallest RNA molecule. Linear, but forms three- looped structure as shown in the diagram.



Fig 2.17: Structure of the tRNA molecule

Function - transportation of amino acids to the site of protein synthesis.

#### 3. Ribosomal RNA

It is the most abundant type of RNA. rRNA has a complex irregular structure. It provides the site where polypeptide chains are assembled.

#### **Differences between DNA and RNA**

- 1. DNA is double stranded molecule while RNA is a single stranded molecule.
- 2. DNA consists of A, T, G and Cand absence of U, while RNA consists of A,U, G and C and absence of T
- 3. Sugar molecule in RNA is ribose, while in DNA it is deoxyribose.

#### Nucleotides other than those found in nucleic acids

ATP, NAD<sup>+</sup>, NADP<sup>+</sup>, FAD and their functions

#### **Functions of ATP**

• Universal energy carrier

#### **Functions of NAD<sup>+</sup>**

- Act as a coenzyme
- Act as an electron carrier
- Function as an oxidizing agent during respiration

#### **Functions of NADP<sup>+</sup>**

- Act as coenzymes
- Act as an electron carrier
- NADP<sup>+</sup> act as a reducing agent in photosynthesis

# Functions of FAD

- Act as a coenzyme
- Act as an electron carrier

# 2.2.1. Contribution of microscope to the expansion of knowledge on cells and cellular organization

Advancement of the cytology is mostly based on the microscopy. The discovery and early study of cells progressed with the invention of microscope.

#### Light microscope

Visible light is passed through the specimen and then through glass lenses. The lenses refract the light in such a way that the image of the specimen is magnified as it is projected into the eye. The simplest microscope is a single lens.

#### The compound light microscope

Compound light microscopes are commonly used in school laboratories and it is used in medical laboratories as a diagnostic tool.

Resolution power and magnification are important parameters which can be seen in a microscope.

Magnification is ratio of an object's image size to its actual size. Usually the maximum magnification of light microscope is 1000 times the actual size of the specimen)

Resolution power is minimum distance between two points that can be distinguished as separate points (resolution power of light microscope is  $0.2\mu m$ ). It is a measure of the clarity of the image.

Magnification is limited due to the resolution.

Light from an object (specimen on the slide) passes first through objective lens. Then produce a magnified image.

Above image then acts as an object for the second lens (the eye piece lens) which further magnifies it.

The total magnification is hence the product of the magnification of each lens.

Total magnification =	Magnification of objective		Magnification of
	lens	$\times$	eyepiece

e.g.If magnification of Objective lens =  $\times 40$ , eyepiece = $\times 15$ 

Total is  $=15 \times 40 = \times 600$  time magnified

#### The Electron Microscope

The limitation imposed upon the resolution power of the light microscope by the wavelength of light. The resolution power is inversely proportional to the wavelength. Due to this, scientists considered the use of other forms of radiations with comparatively shorter wavelengths.

As a result, electron microscopes were developed. In electron microscopy, a beam of electrons is focused through the specimen or on to its surface.

This means, that in theory, the electron microscope should be able to magnify objects up to  $1 \times 10^8$  times. In practice, it magnifies just over  $5 \times 10^5$  times.

Electron microscopes have revealed many organelles and other sub cellular structures those were impossible to resolve with the light microscopes.

There are two types of electron microscopes.

- 1. Transmission electron microscopes (TEM)
- 2. Scanning electron microscopes(SEM)

#### Transmission electron microscopes

It is used to study the internal structures of cells. In this microscope, a beam of electrons is passed through a thin, especially prepared slice of material. A very thin specimen is used. Specimens stained with heavy metals which attach more to certain cellular structures than other areas. Image reflects the pattern of electrons passed through the specimen, displays on a screen. While electrons pass through the specimen, more electrons may get displayed in regions where structures were densely stained.

#### Scanning electron microscopes

In this instrument, a fine beam of electrons is reflected from the surface ofspecimen. Specimen is mostly coated with gold prior to observation. Here the specimen scatters many electrons whereas others are absorbed. This instrument is ideal to observe the surface viewinthree dimensionalappearances.

Light Microscope	Electron microscope
Glass lenses are used to focus the light rays	Powerful magnets are used to focus beam of electrons
Image is directly detected by naked eye	Not directrly detected by naked eye, micrographs are used
Living and non living objects can be observed	Only non-living objects are observed
Actual color of the object can be observed	Actual color cannot be observed. Images are developed
Dyes used to stain the object	Heavy metals are used to stain the object

Table 2.3: Differences	between light an	d electron microscope
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# Historical background of cell and the structure and functions of the subcellular units

# Cell theory

All organisms are composed of cells.

Recall the hierarchy of life, the levels of organization mentioned earlier. The basic unit which can be called "living" is the cell, which may form a single celled organism (e.g.*Chlamydomonas*, Yeast) or a multi-cellular plant or animal. The cell is the basic structural and functional unit of life.

The level of organization of matter represented by a cell shows all the characteristics of life. Any stage below level of a cell cannot be considered living, whether it is a single celled organism or multi-cellular plant or an animal.

Robert Hooke (1665) examined a cork using simple microscope and gave the term "CELL" to describe the basic units.

Anton Van Leeuwenhook (1650), a contemporary of Robert Hooke, was the first to describe and recordliving single celled organisms, *Euglena*& bacteria

Matthias Schleiden (1831), a botanist, studying plant tissues concluded that all plants are made up of cells.

Theodore Schwann a zoologist (1839) concluded that animal tissues are also made up of cells.

Rudolf Virchow (1855) showed that all cells arise from pre-existing cells by cell division,

Schleiden, Schwann and Virchow presented the 'Cell Theory' which included the following.

1.All organisms are composed of one or more cells.

2. The basic structural and functional unit of organisms is the cell.

3.All cells arise from pre-existing cells.

#### **Organization of cells**

There are two kinds of cellular organization - Prokaryotic and Eukaryotic

All cells share certain basic features. They are;

- All cells are bounded by a plasma membrane which is a selective barrier
- Within the cell have, a semifluid, jelly like substance which is called cytosol. Subcellular components are suspended within the cytosol.
- They carry DNA as genetic materials.
- Ribosomes are found in all cells

Feature	Prokaryote	Eukaryote
Organism	Bacteria, Archaebacteria	Protists,Fungi, plants, animals
Cell size	Average diameter 1-5µm	10μm-100μm diameter
Form	Mainly unicellular	Mainly multicellular (except most of protista and some fungi are unicellular)
Evolutionary origin	3.5 billion years ago	1.8 billion years ago ,evolved from prokaryotes
Cell division	Binary fission, no mitosis and meiosis	Mitosis, meiosis, or both;
Genetic material	DNA is circular and lies free in the cytoplasm. This region is called nucleoid, DNA is naked and not associated with proteins	DNA is linear and contained in a nucleus. DNA is associated with proteins
Type of ribosomes	70s ribosome (smaller)	Both 70s (Mitochondria and Chloroplast) and 80s ribosomes (larger)present (may attachto endoplasmic reticulum)
Organelles	Few organelles, none are surrounded by membrane Internal membranes scares; if present usually associated with respiration,photosynthesis and N <sub>2</sub> fixation.	Many organelles, membrane bounded organelles present. Great diversity of organelles. E.g. nucleus, mitochondria, chloroplasts bounded by two membranes. E.g. Lysosomes, Vacuole, bounded by single membrane
Cell walls	Peptidoglycan present in Bacteria and cyanobacteria, polysaccharide and protein present in Archae bacteria	Cell walls of green plants and fungi are rigid and contain polysaccharides; cellulose in plant cell walls and chitin in fungal walls (none in animal cells)
Flagella	Simple, lacking microtubules; extracellular (not enclosed by cell surface membrane) 20nm diameter	Complex, with '9+2' arrangement of microtubules; intracellular (surrounded by cell surface membrane) 200nm diameter
Respiration	Mostly by mesosomes	Mitochondria for aerobic respiration
Photosynthesis	No chloroplasts; takes place on membranes which show no stacking	Chloroplasts containing membranes which are usually stacked into lamellae or grana
Nitrogen fixation	Some have the ability	None have the ability

Table 2.4: The differences between Prokaryotic cells and Eukaryotic cells

Bacteria, Cyanobacteria and Archaea are prokaryotic cells. All the other organisms have eukaryotic cells.

Use the electron micrographs of plant cells and animal cells to study the above characteristics



Fig 2.18: Structure of an animal cell



Fig 2.19: Structure of plant cell

#### Structures and functions of organelles and other subcellular components

<u>Plasma membrane</u>Plasmamembrane is the outer limit of cytoplasm. All cellular membranes resemble the ultra structure of plasma membrane. In 1972, Singer and Nicolson put forward the fluid mosaic model ofcell membrane. It is mainly composed of;

- 1. Phospholipids (most abundant type of lipid in plasma membrane)
- 2. Protein

The Plasma membrane has the following features. It is about 7nm thick. Itismainly made up of a phospholipid bilayer. Phospholipids are amphipathic molecules. The hydrophilic heads of the phospholipids face outwards into the aqueous environment of both inside and outside of the cell.

The hydrophobic hydrocarbon tails face inwards and create a hydrophobic interior.

Plasmamembrane is compared to the fluid mosaic model. Since phospholipid molecules are moveable, they provide the fluid nature to the membrane.

Protein molecules embedded randomly contribute to its mosaic nature.

Some of the protein molecules penetrate all the way through the membrane, called transmembrane proteins and some others penetrate only part of the way into the membrane. These are called **integral proteins.** 

Most of the integral proteins are transmembrane proteins which have hydrophilic channels. These act as pores through which ions and certain polar molecules can pass.

Some proteins are not embedded in the lipid bilayer at all, and are loosely bound to the inner surface of the membrane, called **peripheral proteins.** 

Some proteins and lipids have short branching carbohydrate chains like antennae, forming **glycoprotein** and **glycolipids**, respectively.

Animal's cell membrane may contain few **cholesterol molecules** randomly integrated into the lipid bilayer.

These cholesterol molecules provide flexibility and stability to the membrane by reducing membrane fluidity at moderate temperatures and prevent membrane solidification at low temperatures.

The two sides of the membrane may differ in composition and function.



Fig 2.20: The fluid mosaic structure of the cell membrane

#### Functions

- The plasma membrane surrounds the cytoplasm of living cell physically separating the intracellular components from the extracellular environment.
- Plasma membrane is selectively permeable and able to regulate the exchange of material needed for survival.
- Proteins embedded in the plasma membrane identify the cell, enabling nearby cells to communicate with each other (involved in cell recognition).
- Some protein molecules act as receptor molecules for interacting with specific biochemical, such as hormones, neurotransmitters and immune proteins.
- Some proteins in the cell membrane attach to some cytoskeletal fibers and help to maintain the shape of the cell.
- Some proteins in the membrane act as enzymes. (e.g.Microvillus on epithelial cell lining of some parts of the gut contains digestive enzymes in their cell surface membrane.)

#### Subcellular components

There are many sub-cellular components in the cell. Some of them are organelles, which are bound by membranes and suspended in the cytosol of eukaryotic cell to perform specialized functions.

#### Nucleus

Most prominent organelle, consist most of the genes, having an average diameter of  $5\mu m$  and enclosed by a double membrane cover called nuclear envelope.

- Nuclear envelope- composed of two membranes, inner and outer membranes, separated by a space of 20-40 nm. Nuclear envelope is perforated by nuclear pores which has pore complex to regulate the entry and exit of substances. It has nuclear lamina, made up of protein filaments which line the interior side of the nuclear envelope.
- nuclear matrix made up of protein filaments and extended throughout the interior of the nucleus. Chromatin and nucleolus are embedded in the nuclear matrix.
- Nucleolus- appears as darkly stained granules with fibers adjoining part of the chromatin.
- Chromatin –appears as a diffused mass in electron micrographs of non dividing cells. It is a complex of DNA and proteins. During nuclear divisions, chromatin condenses, tightly coils and form threads, calledchromosomes. Each species has a constant number of chromosomes. (e.g. typical human cell has 46 chromosomes).

#### Functions

- Control all cellular activities.
- Synthesize DNA to produce new nuclei for cell divisions.
- SynthesizerRNAs and ribosomal subunits required for protein synthesis, through nucleolus.
- Synthesize mRNA and tRNA according to the information present on the DNA.
- Store and transport genetic information.

#### Ribosomes

These are subcellular components which carryout protein synthesis. They consist of two subunits; larger subunit and smaller subunit. They are composed of rRNA and protein. Ribosomes are found in two types; 70S and 80S. 70S ribosomes are found freely on the cytoplasam of prokaryotes, matrix of mitochondria and stroma of chloroplasts. 80S ribosomes are found only in eukaryotes. Based on the nature of presence, 80S ribosomes are categorized as two types; free ribosomes and bound ribosomes.

Free ribosomes: freely available as group in cytoplasam. Bound ribosomes are attached to the membrane surface of rough endoplasmic reticulum.

Functions

Protein synthesis

#### **Endoplasmic reticulum**

It is a network of internal membranes forming flattened or tubular sacs separating cytosol from ER lumen. It is continuous with the outer membrane of nuclear envelope. There are two types of ER; Rough ER and Smooth ER

#### **Rough ER**

Rough ER consists of flattened sacs, and ribosomes bound to surface. Proteins synthesized by ribosomes move into lumen of ER.

Functions

- Transport protein synthesized by ribosomes
- Synthesizing glycoproteins
- Produce transport vesicles
- Facilitate the growth of own membrane by adding phospholipids proteins and carbohydrates. Therefore called as membrane factory

#### Smooth ER

Smooth ER is a network of tubular sacs without ribosomes. Membrane bound enzymes are present.

#### Functions

- It synthesizes lipids including oils, steroids and phospholipids.
- Metabolism of carbohydrates.
- Produce transport vesicles to transport within cell.
- Involves in detoxification.
- Stores Ca<sup>2+</sup> ions.

#### Smooth ER



Fig 2.21: structure of the endoplasmic reticulum

#### Golgi apparatus



Golgi apparatus is a stacks of flattened sacs or Cisternae.Inner and outer surfaces can be identified as cis face and transface respectively. Cis face is located near the E.R to receive vesicles from E.R. Trans face give rise to secretory vessicles which budded off and travel other side. Golgi complex is abundant in secretory cells.

Functions

- Collecting, packaging and distribution of materials
- Manufacturing cellulose and non cellulose cell wall components such as pectin
- Produce lysosomes

Lysosomes

They are single membrane bounded vesicles contributing to digestive activity. They contain hydrolytic enzymes which catalyze breakdown of carbohydrates, proteins, lipids and nucleic acids.

#### Functions

- Digest food particles received by phagocytosis
- Transport residue material out of cell by exocytosis.
- Digest worn out organelles
- Autolysis causing cell death.

#### Peroxisome

They are single membrane bounded vesicles with oxidizing enzymes. They are present in both plants and animals. Enzymes in peroxysome catalyze the breakdown of  $H_2O_2$ .

#### Functions

- Detoxification of peroxides
- Photorespiration in plants

Specialized peroxysomes called glyoxysomes are found in fat storing tissues in plants.

Glyoxysomes converts fatty acids into sugar.

#### Mitochondria

It is one of the most common organelles in eukaryotic cells. It is an elongated organelle with two enclosing membranes. Outer membrane is smooth but the inner membrane is convoluted to form cristae. Cristae increase the surface area and they contain stalk particles. The gap/space in between inner and outer membranes of th emitochondrion is called intermembrane space. The inner most part of the organelle is known as mitochondrial matrix, which consists of 70 s ribosomes circular DNA molecule (mitochondrial DNA), phosphate granules and enzymes. The matrix carries enzymes for the reactions in Krebs cycle (in cellular respiration). Further, cristae composed of proteins and enzymes essentialforelectron transport chain and oxidative phosphorylation.

#### Functions

- Synthesize ATP in aerobic respiration
- Involve in Photorespiration

#### Chloroplast

It is a biconvex lens shaped organelle with two membraneswhich is found in plants and some protists. The outer and inner membranes aresmooth and are separated by avery narrow intermembrane space. Inside the chloroplast there is another membrane system. This membrane produces flattened and interconnected sacks called thylakoids. Thylakoids contain complexes called photosystems which are made up of photosynthetic pigments. Thylakoids stacked to form a granum. The grana are interconnected by inter granal lamellae. The fluid outside the thylakoid is stroma which contain circular DNA (chloroplast DNA), 70s ribosomes, many enzymes, starch granules and lipid droplets.

#### Functions

• Photosynthesis

#### Cytoskeleton

Cytoskeleton is the supporting structure of the cell and maintains its shape. It is more important for animal cells which lack cell walls. Cytoskeleton is made out of microtubules and protein filaments. Additionally, it is Dynamic hence, has the ability to break and reform as needed.

There are three types of components in the Cytoskeleton as follows;

- Microtubules
- Actin filaments or Microfilaments,
- Intermediate filaments

Table 2.5: Differences between Microtubules, Microfilaments and intermediate filaments

Property	Microtubules	Microfilaments	Intermediate filaments
	(Tubulin polymers)	(Actin filaments)	
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin, each strand is a polymer of actin subunitrs	Fibrous proteins supercoiled into thicker cables
Diameter	25nm with 15nm lumen	7nm	8-12 nm
Protein subunits	Tubulin	Actin	One of several different proteins (e.g. Keratin), depending on the cell type.
Main	Maintenance of cell shape	Maintenance of cell	Maintaining of cell shape
functions	Cell motility (as in cilia or flagella)	shape(tension-bearing elements)	(tension-bearing elements) Anchorage of nucleus and
	Chromosome movements	Changes in cell shape	certain other organelles.
	in cell division	Muscle contraction	Formation of nuclear
	Organelle movements	Cytoplasmic streamingin plant cells	lamina
		Cell motility (as in pseudopodia)	
		Cell division in animal cells(cleavage furrow formation)	

Functions

- Provide strength to the cytoplasm
- Anchorage organelles and cytosolic enzymes of the cell
- Movement of cytoplasm, cytoplasmic streaming, positionedorganelles and move chromosomes when necessary.
- Maintain the shape of the cell (mainly in animal cells)

#### Cilia and Flagella

Cilia and flagella share a common structure. Flagella are long elongated structures and Cilia are short cellular projections that are often organized in rows. Cilia are more numerous than flagella on the cell surface. They are made of microtubules, with a 9+2 structure (Nine doublets of microtubules are arranged in a ring, with two single microtubules in its center). They arecovered by plasma membrane and bound to a basal body which anchors thecilium or flagellum to the cell. The Basal body has 9 + 0 arrangement (no microtubules in its center)

#### Functions

- Act as locomotor appendages
- Can move fluid over the surface of the tissue
- Cilia liningin oviducts helpmove an egg toward the uterus

#### Centrioles

Centriole is made up of cylindrically arranged microtubuleswhich are non membrane bounded subcellular component present only in animal cells. Each centriole composed of nine sets of triplet microtubules arranged in a ring (9+0). A pair of centrioles which arranged perpendicular to each other are located in a region called centrosome near the nucleus.

#### Functions

• Produce aster and spindle in cell division

#### **Central Vacuole**

Central vacuole is a large structure, bound by tonoplast, filled with liquid called cell sap found in plant cells. The composition of sap differs from cytosol and it contains water, ions such as Potassium and Chloride and sometimes water soluble colored pigments such as anthocyanin.

#### Functions

- stores water and other materials such as sugars, ions and pigments.
- Maintains water balance of the cell
- Gives turgidity and support to cell.
- Produce colours in some plants with sap pigments
- Stores soluble substances needed for cellular activities.

#### Extracellular components

1. Cell wall

Cell wall is an extracellular structure of plant cells. Animal cells do not have cell walls. However, prokaryotes, fungi and some protists also have a thin and flexible cell wall. The chemical composition of the wall greatly varies from speciesto species and even from one cell type to another even in the sameplant. Nevertheless in Plants, cell wall isgenerally made up of cellulose, pectin, hemicellulose, lignin and suberin (in some plant cells only).

Plants generate two types of cell walls: primary and secondary walls. Young cells first secrete primarycell wall: it is the wall laid down during plant cell division.

Just outside the primary wall there is a thin layer (middle lamella) which is rich in sticky polysaccharidescalled pectins (magnesium and calcium pectate).Middle lamella glues adjacent cells together. Due to the deposition of hardening substances on the primary wall a secondarycell wall is generated secondarily.

Primary cell wall is permeable, relatively thin, flexible, composed mainly of cellulose fibers which are laid unevenly running through the extracellular matrix (middle lamella)Water can move freely through the free spaces of cell wall

Secondary cell wall lies between plasma membrane and primarycell wall. It contains several layers of hard materials, forming a rigid structure. In addition to cellulose, impermeable substances such as lignin and suberine are also incorporated in to the secondary wall. Lignin cement anchors cellulose fibers together providing hard and rigid matrix, giving the cell wall an extra support.

Cell wall has pits through which cytoplasm of adjoining cells join through plasmodesmata.

#### Functions

- Protection and support
- Allows development of turgidity when water enters the cell
- Prevents bursting during turgidity
- Limits and control cell growth
- Component of appoplast pathway
- Maintaining cell shape
- hold the plant up against the force of gravity

#### 2. Cell junctions

Cell junctions are structures at which neighbouring plasma membranes are joined. They are also interact and communicate via sites of direct physical contacts. There are three types of cell junctions in animal cells.

There are three types of cell junctions in animal cells

- Tight junctions connect the plasma membranes of adjacent cells tightly bound by specific proteins forming continous seals around the cells. Prevent leakages of extracellular fluids through intercellular space. e.g. skin epithelium
- Desmosomes/Anchor junctions mechanically attach the cytoskeletons of adjoining cells by intermediate filaments for strong binding.e.g. muscle tissue
- Gap junctions /Communicating junctions provide cytoplasmic channels from one cell to an adjescentcell. Gap junctions consists of special membrane proteins that surround the pore through which ions, sugars amino acids may pass. They allow signal and material exchange between adjacent cells through direct connections. e.g.heart muscles, animal embryo.

#### Plasmodesmata

- Microscopic channels which runs through plant cell walls. They are cytoplasmic living connections between cytoplasam of adjoining cells. These are membrane lined channels filled with cytoplasam.
- 3. Extracellular matrix of animal cells

Although animal cells lack cell walls they do have elaborate extracellular matrix (ECM). Main components of the ECM are glycoproteins and other carbohydrates containing molecules secreted by the cells. Most abundant glycoprotein in the ECM of most animal cell is collagen which forms strong fibres outside the cell. The collagen fibres are embedded in a network woven out of proteoglycan secreted by cells.

Functions

- Forms a protective layer over the cell surface
- Linking extra cellular matrix and cytoskeleton.
- Influences the cell behavior by Involving in the mechanical and chemical signaling.

# Cell cycle and the process of the Cell division

#### Cell cycle

The sequence of events that takes place in the cell from the end of one cell division to the end of the next cell division is referred to as cell cycle. At the end of the cell division, two genetically identical daughter cells resembling the parent cell are produced in mitosis.

# Eukaryotic cell cycle

#### Mitosis

Eukaryotic cell cycle may divided into two major phases.

- Interphase
- Mitotic phase/ M-phase

Interphase is the longer phase of cell division. It covers about 90% of the cell cycle. Interphase could be divided into three phases;

- G<sub>1</sub> phase (first gap phase)
- S phase (synthetic phase)
- G<sub>2</sub> phase (second gap phase)

#### $G_1$ phase

In this phase synthesis of proteins and production of cellular organelles leading to cell growth occur. Proteins essential for S phase are produced during this phase.

#### S phase

DNA replication occurs and synthesis of histone proteins takes place. DNA wind around histone beads and form chromatin.

#### G<sub>2</sub> phase

Cells continue to grow through protein synthesis as well as cellular organelles. Proteins essential for mitotic phase will be synthesized. Duplication of centrosomes takes place.

There are cell cycle-controlling checkpoints available at  $G_1$ ,  $G_2$  and M phases to ensure that the cell is ready for moving into upcoming phases of cell division. Some cells receive a go-head signal at the  $G_1$ check point, it will usually complete the  $G_1$ , S,  $G_2$  and M phases and divide. If it does not receive a go head signal at that point it may exit the cycle, entering into a non dividing stage called the Go phase. The most cells of the human body are actually in the Go phase. e.g. nerve cells and muscle cells.

#### Mitotic phase/ M phase

M phase covers only about 10% of cell cycle. This includes mitosis and cytokinesis.

# Mitosis

Mitosis is referred to the nuclear division which gives rise to two genetically identical daughter nuclei from a mother nucleus. This may get divided into five stages; prophase, prometaphase, metaphase, anaphase and telophase in order to ease the learning of activities of cell cycle.

#### 1. Prophase

Chromatin fibers get condensed by shortening and thickening and transformed into chromosomes. As a result chromosomes will be visible through light microscope. Nucleoli get disappeared and chromosomes appear with two sister chromatids attached at the centromere. Chromosomal arms of sister chromatids attached by special proteins called cohesion. The formation of mitotic spindles begins. Spindle includes the centrosomes, the spindle microtubules and the aster.

Centrosomes move toward opposite poles of the cell due to the lengthening of microtubules between them.

#### 2. Prometaphase

The nuclear envelope fragments. Chromosomes get even more condensed. A special protein called kinetochore attaches the sister chromatids of each chromosome at their centromere. Some of the microtubules that attach to the kinetochore of the chromosomes move the chromosomes back and forth. Microtubules which are not attached to the kinetochore interact with those from the opposite poles.

#### 3. Metaphase

Centrosomes reach the opposite poles. The chromosomes have arrived to a place called metaphase plate which is located in equal distance from each pole. The centromeres of all chromosomes are located in the metaphase plate. At the end of this phase, each chromosome of the cell get attached to the kinetochore microtubule at their centromere and aligned at the metaphase plate.

#### 4. Anaphase

Sister chromatids are separated at the centromere. Microtubules attached to kinetochore get shorten and pull sister chromatids towards the opposite poles. Cell elongates as the non kinetochore microtubules are lengthen. By the end of anaphase equal and complete set of chromosomes found at each pole of the cell.

#### 5. Telophase

Nuclear envelope reforms around each set of chromosomes at opposite poles. Nucleoli reappears. Spindle microtubules get deplolymerized. Chromosomes unwind and become less condense to form chromatin. Two genetically identical daughter nuclei are formed.

#### • Cytokinesis

The division of the cytoplasam starts at the end of the telophase. Therefore at the end of the mitosis two genetically identical daughter cells are produced.

In animal cells- a cleavage furrow forms. This produces two genetically identical daughter cells.

In plant cells- cell plate forms as a result of vesicle produced by golgi apparatus. This divides the cytoplasm in to two and generates two genetically identical daughter cells to the parent cell.

# Significances of mitosis

- 1. Maintains the genetic stability
- 2. Growth and development
- 3. Cell repair, replacement and regeneration
- 4. Asexual reproduction

### Meiosis

Sexually reproducing organisms undergo different type of cell division called meiosis.

#### Meiosis

Meiosis is a type of nuclear division which gives rise to four haploid, genetically non identical daughter nuclei, from a diploid mother nucleus.

Meiosis involves two consecutive nuclear divisions, Meiosis I and Meiosis II. Meiosis I is a reduction division and Meiosis II is similar to mitosis, each stage consists of four subphases: prophase, metaphase, anaphase, and telophase.

Before meiosis one cell is in interphase, during S phase of the interphase DNA replication occur.

#### Meiosis I

1. Prophase I

Cell enters to the prophase I from interphase. Chromosomes begin to condense. Nucleolus begins to disappear. Next the formation of zipper like structure called the synaptonemal complex by a specific proteins holds two homolg tightly together. The pairing and physical connection of homologous chromosomes is called synapsis.

During synapsis part of the DNA molecule of non-sister chromatids paired homologous chromosomes break, exchange and rejoin at corresponding point. This process is called crossing over. These points of crossing over become visible as chiasmata after the synaptonemal complex dissembles and the homologous chromosomes slightly apart from each other..

Nuclear envelop breaks. Centrosomes move towards opposite poles forming spindle in animal cells.

The kinetochore of each homologue attach to microtubule from one pole or the other. The homologous pair then moves toward the metaphase plate.

2. Metaphase I

The pair of homologous chromosomes get arranged on the metaphase plate with one chromosome of each pair faces each pole.Both chromatids of a homologue are attached to kinetochore microtubules from one pole and those of the other homolog are attached to kinetochore microtubules from the opposite pole. Homologous chromosome arrange randomly at metaphase plate.

3. Anaphase I

Kinetochore microtubules of the spindle get shorten. Homologous pair separates and one chromosome of each pair moves towards the opposite pole. Sister chromatids of each chromosome remain attached at the centromere and move as a single unit towards the same pole.

#### 4. TelophaseI

One complete haploid set of chromosomes accumulate at each pole. Nuclear envelope reforms around each set of chromosomes.Nucleoli reappear. Spindle disintegrates. Chromosomes decondensed into chromatin.Genetically non identical, haploid, two daughter nuclei are formed within one cell.

#### • Cytokinesis

Usually occurs simultaneously with telophase I. Genetically non identical, haploid, two daughter cells are formed. In animal cells, cleavage furrow is formed. In plant cells a cell plate is formed.

No DNA replication occurs between meiosisIand meiosisII.

- Meiosis II
  - **1.** Prophase II

Centrosomes start producing spindle apparatus (spindle fibers, aster centrosome). Chromatin fibers condense and produce chromosomes with two sister chromatids. Nuclearenvelope breaks down into fragments. Nucleolus disappears. During the late prophase II centromere of the chromosomes are moved to the metaphase IIplate.

#### 2. Metaphase II

All Chromosomes get attached to the microtubules at their centromere and aligned on the metaphase plate. Kinetochores of sisterchromatids are attached to microtubules extending from both poles.

Due to the crossing over in meiosisI, the two sister chromatids of each chromosome are not genetically identical.

Meiosis II usually takes place in the perpendicular direction of MeiosisI. Therefore, metaphase plate of meiosis II is perpendicular to the metaphase plate of meiosisI.

#### 3. Anaphase II

Due to the breakdown of proteins attaching sister chromatids, they are separated at centromere. As a result of shortening of microtubules, sister chromatids of each chromosome move towards opposite poles.

#### 4. TelophaseII

Nuclear envelope and nucleolus reform. Chromosomes decondense into chromatin. Spindle disassembles. Genetically non identical, haploid, four daughter nuclei are formed from one parent cell.

#### Cytokinesis

Cytokinesis occurs as in mitosis. Genetically non identical, haploid, four daughter cells are formed. These four daughter cells are not even identical to their parent cell.

Centrosomes or centrioles are not available in plant cells. However, spindle is formed during cell division from accumulated microtubule complex.

#### Significance of meiosis

- Maintain the constant number of chromosomes through generations in sexually reproducing species.
- Produce new genetic variations leading to evolution.
- Genetic variation occur due to crossing over ,recombination and independent assortment.

#### Tumor, cancer and galls

#### Tumors and cancers in animals

- Cell division is driven by external and internal factors. They may be chemical or physical factors
- Chemical factors such as growth factors, physical factors such as density dependent inhibition and anchorage dependence are needed for cell division normally.
- Normal animal cells stop deviding when they come into contact with crowded cells. this phenomena is known as density dependent inhibition.
- Most animal cells divide, if they are attached to an external surface such as the surface of a cell next to them. This is known as anchorage dependence inhibition.
- Cancer cells exhibit neither density dependent inhibition nor anchorage dependent inhibition.
- Cancer cells do not respond to normally to the body's control mechanism
- They divide excessively and invade other tissues. If unchecked they can kill the organism.
- Cancer cells do not consider the normal signals that regulate the cell cycle.
- They do not need growth factors. They may make required growth factors themselves or giving signals to continue for the cell cycle without growth factors. Another possiblility is an abnormal cell cycle control system.
- The problem begins when a single cell in a tissue undergoes transformation, the process converts a normal cell to abnormal cell.
- If the body immune system can not recognize and destroy it this leads proliferate cells and form a tumor.
- If the abnormal cells remain at the original site, the lump is called benign tumor. Most benign tumors do not cause serious problems and can be completely removed by a surgery.

- A malignant tumor becomes invasive and attack one or more organs. An individual with a malignant tumor is said to have a cancer.
- A few tumor cells may separate from the original tumor, enter blood vessels or lymph vessels and travel to other parts of the body. They may proliferate and form a new tumor.
- This spread of cancer cells to locations distant from their original site is called metastasis.

Galls in plants

- This occurs due to uncontrolled mitotic division of plant cell.
- The plant cell division is controlled by maintaining a proper balance between plant growth regulators such as auxins and cytokinins. When this balance is lost plant cells produce undifferentiated mass of cells.
- Galls are the bumbs and growths that develop on different parts of plants after being invaded by some very unique organisms.
- Galls have range of causes, including viruses, fungi, bacteria, insects and mites.
- Usually the gall causers in some way attack or penetrate the plants growing tissues and causes the host to reorganize its cells and to develop an abnormal growth.

# Metabolism

# Energy relationships in metabolic process

Sum of all biochemical reactions of living being is known as the metabolism and it consists of all catabolic and anabolic reactions.

Catabolism is breaking down of complex molecules into simple molecules by releasing free energy. Thereforeit is an exergonic reaction. Anabolism is making complex molecules from the simple molecules by absorbing free energy.Hence it is an endergonic reaction.

Biochemical reactions involved in usage of energy released by catabolic reactions in living system are called as anabolic reactions. ATP acts as the energy carrier in all living organism including the simplest bacteria. Therefore the ATP is known as the universal currency of energy transactions.

Energy can be defined as the capacity to do work. All living organisms require energy for their living process in many ways. Such processes are;

- Synthesis of substances
- Active transport across plasma membrane
- Transmission of nerve impulses
- Muscle contraction
- Beating of cilia and flagella
- Bioluminescence
- Electrical discharges.

Overall idea of the energy relations of living system on biosphere is composed of following steps.

- 1. Energy flows into biological systems from the environment through solar radiation. (Primary energy source is the Sun)
- 2. Light energy is captured in the cells having photosynthetic pigments (chlorophyll) by the process of photosynthesis and stored as chemical energy in the organic compounds such as carbohydrates
- 3. Stored energy in organic food is transformed into chemical energy in ATP by a process called cellular respiration.
- 4. The energy stored in ATP is utilized in various energy requiring processes.

#### ATP (Adenosine Tri Phosphate)

ATP is a nucleotide, consisting of,

- Ribose- sugar
- Adenine nitreogenous base
- A chain of three phosphate groups.

During the hydrolysis of ATP, ADP and Pi are produced. As a result, a very high energy is released. This is because the reactants (ATP and water) contain more energy in comparison to products (ADP and Pi). Therefore it yields energy and is an exergonic reaction.

When ATP is hydrolyzed, the free energy yield of eachof the two end phosphate groups is - 30.5kJ/mol.



Most biological reactions use the energy released during breaking of the terminal phosphate bond. ATP is mobile. Therefore it can carry energy to anywhere in the cell, for any energy consuming reaction.

ATP can be produced within living cells within a short period of time, using ADP, inorganic phosphate (Pi) and energy. Production of ATP within cells is called phosphorylation. According to the energy source phosphorylation is divided as;

i. Photophosphorylation	-synthesis of ATP using solar energy	in	
photosynthesis			
ii. Substrate phosphorylation	-synthesis of ATP using energy released by the		
	breaking down of complex molecules into simple	ones	In collular
iii. Oxidative phosphorylation	-synthesis of ATP using energy released as a resu oxidation of molecules.	ılt of	respiration

In living cells energy in ATP is transformed in to various energy forms which are used for different functions..



### Role of Enzymes in regulating metabolic reactions

An enzyme is a macromolecule, which acts as a biological catalyst. Enzymes are produced in living cells/

General characteristics of an enzyme:

- 1. Most of the enzymes are globular proteins.
- 2. Enzymes are biological catalysts. They increase the rate of reaction by lowering yhe lower the activation energy of the reaction.
- 3. Most enzymes are heat liable/ sensitive
- 4. Their presence does not alter the nature or properties of the end products of any reaction.
- 5. Enzymes are highly specific to the substrate (substrate specific)
- 6. Most enzyme catalyzed reactions are reversible.
- 7. The rate of enzyme activity is affected by pH, temperature and substrate concentrations.
- 8. They are not being used up during the reaction.
- 9. Enzymes possess active sites where the reaction takes place.
- 10. Some enzymes need non-proteinous components to catalyse the reaction which are known as cofactors.





#### The mechanisms of enzyme action

The reactant an enzyme acts on is referred to as the substrate. The enzyme binds to its substrate forming enzyme-substrate complex. While enzyme and substrate form their complex, catalytic action of the enzyme converts the substrate to the product.

Enzyme + substrate ⇔Enzyme-substrate complex⇔Enzyme + Product

The reaction catalyzed by each enzyme is very specific. The specificity of an enzyme results from its shape. The substrate binds to a specific region of the enzyme. This region is called the active site. The active site is formed by only a few amino acids. Other amino acids are needed to maintain the shape

of the enzyme molecule. The shape of the active site is complementary to the shape of the specific substrate of the enzyme, and hence important in the substrate specificity of the enzyme. The shape of the active site of an enzyme is not always fully complementary to its substrate. As enzymes are not rigid structures, the interactions between substrate and active site may slightly change the shape of the active site, so that the substrate and the active site become complementary to each other. This is called induced fit mechanism. The tight fit not only brings the substrate molecules and the active site close to each other, but also ensures the correct orientation of the molecules to help the reaction to proceed and catalyzes the conversion of substrate to product. Thereafter, the product departs from the active site of the enzyme. The enzyme is then free to take another substrate molecule into its active site.



Fig 2.30: Induced fit between an enzyme and its substrate

#### Cofactors

Non-proteinuos components which are essential for the catalytic activities of certain enzymes are called cofactors.

These cofactors bind to the enzymes in two ways. Some tightly bind and remain permanently and others loosely bind temporarily. Loosely bound cofactors are reversible under certain circumstances.

Organic cofactors are called co-enzymes. e.g. derivatives of vitamins e.g. NAD, FAD and biotin

Inorganic co-factors – e.g. Zn<sup>2+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>

#### Factors affecting the rate of enzymatic reactions

- 1. Temperature
- 2. pH
- 3. Substrate concentration
- 4. Inhibitors

#### Temperature

Increase intemperature increases molecular motion. Therefore the speed of the moving molecules of both enzymes as well as the substrate will be accelerated. This will enhance the colliding probability for both enzyme active sites and substrate molecules. More collision between the enzyme active sites and substrate molecules for the reaction to occur. This can continue up to a certain point, after which there is a rapid decline in enzyme activity. This point is referred to as optimum temperature. This may vary from organism to organism.

e.g. most of the human enzymes have optimum temperature around the body temperature (35°C-40°C). Optimum temperature of bacteria in hot springs is about 70°C.

When the temperature increases beyond the optimum temperature, the hydrogen bonds, ionic and other weak chemical bonds of enzyme active sites may be disrupted. This will result a change in the shape of the active site of enzyme which will alter the complementary nature of the active site of enzyme molecules. Therefore, the complementary binding of enzyme active sites and substrate molecules will be prevented. The above event is called as denaturation of enzyme molecules.

Therefore the rate of enzyme catalyzed reaction will start to decline when the temperature increases beyond the optimum temperature and stops completely at certain temperature, although rate of collision will keep on increasing.



#### рΗ

Enzymes function most efficiently within a certain pH range despite maintaining temperature of the environment constant.

The narrow range of pH in which a particular enzyme catalyzed reaction takes place is named as the pH range. The pH at which the highest rate of reaction occurs is the optimum pH of the enzyme. The alteration in pH above or below the optimum pH may lead to decline in enzyme activity. This is due to the alteration of chemical bonds involving in formation of enzyme substrate complex. In most enzymes optimum pH range is 6-8, but there are exceptions. Pepsin works best at pH 2 and optimum pH for Trypsin is 8.



Fig 2.32: Rates of reaction of two enzymes at various pH values

#### Substrate concentration

Increasing substrate concentration increases the probability of collision between the enzyme and substrate molecules with correct orientation. However the enzyme molecules will be saturated after a particular concentration and therefore there will not be any further increase in the rate of reaction.

#### Enzyme inhibitors

Certain molecules or ions selectively bind permanently or temporarily to the enzyme molecules and prevent them from forming enzyme-substrate complex. These substances are called inhibitors.

They are either binding reversibly with weak interactions or binding irreversibly through covalent bonds.

e.g. Irreversible inhibitors: toxins, poisons

Reversible inhibitors- drugs used against microbes

#### Competitive inhibitors

Most of these are reversible inhibitors. These chemicals resemble the shape and nature of the substrate. Therefore they compete with the substrate selectively for the active site of certain enzymes. As a result of the above, the number of active sites available for the enzymes may decline and therefore reduces the rate of enzyme catalyzed reactions.

The above situation may be reversed by increasing the substrate concentration.

#### Non-competitive inhibitors

These chemicals do not compete with substrate molecules. They interrupt enzymatic reaction by binding to a part of the enzyme other than the active site. This causes the enzyme molecule to change its shape in such a way that the active site becomes less effective for the formation of enzyme substrate complex.



Fig 2.35: noncompetitive inhibitors

#### Regulation mechanism of enzymatic activity in cells

#### Allosteric regulation of enzymes

In many cases, the molecules that naturally regulate enzyme activity in a cell behave like reversible noncompetitive inhibitors. Regulatory molecules (either activators or inhibitors) bind to specific regulatory sites elsewhere (other than the active site) of the molecule via non-covalent interactions and affect the shape and function of the enzyme. It may result in either inhibition or stimulation of an enzyme activity.

#### a.) Allosteric activation and inhibition

most enzymes regulated by allosteric regulation are made from two or more subunits. Each sub unit composed of a polypeptide chain with its own active site. The entire complex oscillates between two different shapes one catalyzing active and other inactive. In this two forms regulatory molecules bind to a regulatory site called allosteric site, often located where subunits join.

When an activator binds with this regulatory site, stabilizes the shape with functional active sites. Wheras the inhibitor binds with the regulatory site, it stabilizes the inactive form of enzyme. Subunits of enzyme arranged in a way through which they transmit the signals quickly other subunits. Through the interaction of subunits even a single activator or inhibitor molecule that bind to one regulatory site will affect the active site of all sub units. e.g. ADP function as allosteric activator bind to the enzyme and stimulates the production of ATP by catabolism. If the supply of ATP exceed demand catabolism shows down as ATP bind to the same enzyme as inhibitor.

#### b.) ccoperativity

This is another type of allosteric activation. Binding of one substrate molecule can stimulate binding or activity at other active site. Thereby increase the catalytic activity.

e.g. hemoglobin (not an enzyme) is made up of four subunits each with an O2 binding site. The binding of an one molecule of O2 to one binding site increases the affinity for O2 of the remaining binding site.

#### c.) Feed back inhibition

in feed back inhibition, a metabolic pathway is stopped by the inhibitory binding of its end product of a process to an enzyme. Thereby limit the production of more end products than required and thus wasting chemical resources.

#### Feedback inhibition

Feedback inhibition is an essential process regulates the end products produced in metabolism.

e.g. ADP function as allosteric activator and stimulates the production of ATP during the catabolism.

In case ATP supply exceeds demand, catabolism slows down as ATP molecules function as allosteric inhibitor.

Energy needed for all living processes is obtained directly from ATP. ATP is mainly produced by a process call cellular respiration, in living cells.

#### Cellular respiration and its importance

Cellular respiration is the process by which chemical energy in organic molecules such as carbohydrates is released by stepwise oxidative process, catalyzed by enzymes and made available in living cells in the form of ATP. Cellular respiration is divided as

- a) aerobic respiration
- b) anaerobic respiration

#### Aerobic respiration:-

The process of synthesize ATP from the respiratory substrates such as glucose in the presence of molecular oxygen  $(O_2)$  known as aerobic respiration. Glucose is found to be the major respiratory substrate in living cells.

The aerobic respiration of glucose molecules can be represented by the following balanced chemical equation.

 $C_6H_{12}O_6 + 6O_{2(g)} \longrightarrow 6CO_{2(g)} + 6H_2O + Energy (ATP+ heat)$ 

This process consist three main steps. They are;

- a) Glycolysis
- b) Pyruvate oxidation and citric acid cycle (Kreb's cycle)
- c) Oxydative phosphorylation (Electron transport chain)

#### Glycolysis

It takes place in the cytosol of the cell, because all enzymes that catalyze reactions of the glycolysis are found in the cytosol of the cell. This process does not depend on  $O_2$ .

During the above process a six carbon (6C) glucose molecule is broken down step by step into two three-carbon (3C) pyruvate molecules.

Two ATP molecules are used up to initiate the process.

Four hydrogen molecules and electrons released from glucose breakdown reduce two NAD<sup>+</sup> and produce two NADH.At the end of glycolysis there will be four ATP molecules produced. Since two ATP molecules were used up for the initiative step, the net yield will be two ATP molecules.

Only when O<sub>2</sub> is present, the pyruvate molecules will enter the mitochondria and further steps will take place.

#### **Oxidation of Pyruvate/ Link reaction**

These two pyruvate molecules enter mitochondrion by active transport through the membrane. In the matrix of mitochondria, Pyruvate is converted to acetyl group by releasing two  $CO_2$ molecules. Then this acetyl group combines with co-enzyme A to produce Acetyl co-A. In this reactiontwoNAD<sup>+</sup> is converted to two NADH molecules. Therefore this step can be represented as follows.

 $2C_3H_4O_3 + 2CoA + 2NAD^+$  2 Acetyl Co-A +  $2CO_2$ + 2 NADH

Oxidation of pyruvate is a linking reaction of glycolysis and citric acid cycle.

Acetyl Co-A will feed its acetyl group for citric acid cycle.

#### **Citric acid cycle**

This takes place in the matrix of mitochondria using specific enzymes. As the first product of this cyclic pathway is citric acid, it is known as citric acid cycle. The pathway was discovered by a German-British scientist Hans Krebs. Hence, it is named as Kreb's cycle. Citric acid contains three carboxylic acid groups. This cycle is also known as Tricarboxylic Acid cycle or TCA cycle. In the citric acid cycle 4 C compound oxalo acetate combines with 2 C compound acetyl Co -A to form 6 C compound, citric acid. Then citric acid undergoes a series of reactions to regenerate oxaloacetate by releasing two CO<sub>2</sub> molecules by decarboxylation reaction. One ATP molecule is produced by substrate level phosphorylation. One FADH<sub>2</sub> and three NADH will be generated as a result ofoxidation reactions. These are the products of a single acetyl group led into citric acid cycle and hence these numbers should double when the yield for a glucose molecule is considered.

#### **Electron transport chain**

This step is taken place across the inner membrane (cristae) of mitochondria. The folding of cristae increases surface area for oxidative phosphorylation. NADH and FADH<sub>2</sub> products in the early stages of aerobic respiration are oxidized by transferring electrons, through the electron transport chain andfinally to molecular oxygen (O<sub>2</sub>). The electron transport chain is located in the inner membrane of mitochondrion and composed of series of protein and non-protein molecules involving in the movement of electrons and protons across cristae. Therefore, the Molecular oxygen(O<sub>2</sub>) is the final electron acceptor in aerobic respiration. In the electron transport chain, ATP is synthesized by oxidative phosphorylation.

In this electron transport chain, energy is released progressively from NADH and FADH<sub>2</sub>and that energy is used to synthesize ATP. When one molecule of NADH is oxidized in the electron transport chain, 2.5 molecules of ATP in average are generated due to oxidative phosphorylation. When one molecule of FADH<sub>2</sub> is oxidized 1.5 molecules of ATP in averageare produced due to oxidative phosphorylation. Total number ATP that is produced in this step is 28.

This is true in the active cells such as liver cells and cardiac muscle cells but not in other cells where two ATP produced in glucolysis is used to transport 2NADH from cytosol to mitochondrial matrix. In those cells total number of ATP produced by one molecule of glucose is (32-2) = 30 ATP.

Total number of ATP molecules produced from one molecule of glucose, during aerobic respiration.

In glycolysis;

As ATP	2ATP
From 2NADH	5ATP (oxidation phosphorylation)
In pyruvate oxidation;	
From 2NADH	5ATP (oxidative phosphorylation)
In Citric acid cycle;	
As ATP	ATP (substrate level phosphorylation)
From 6NADH	15ATP (oxidative phosphyrlation)

From 2 FADH<sub>2</sub>  $\longrightarrow$  3ATP  $\therefore$  Total Number ATP = 32 ATP

#### Anaerobic respiration

Anaerobic respiration is breaking down of glucose in the absence of molecular oxygen which is regulated by enzymes of the cells occurring in cytosol. In the absence of molecular oxygen pyruvate molecules cannot be broken down further. ATP generated isutilized to fulfill energy requirements. However, NADH produced during glycolysiscannot be utilized. Therefore, since NAD<sup>+</sup> is limited it is essential for the cell to recycle NADH to enhance the availability of NAD<sup>+</sup> other than anaerobic respiration fermentation is a method of production of ATP in the absence of  $O_2$ . There are many types of fermentation, differing from end products formed by pyruvate. The two common types are;

- 1. Ethyl alcohol fermentation
- 2. Lactic acid fermentation

Ethyl alcohol fermentation

- Like in aerobic respiration, the first step of this is also Glycolysis.
- Therefore one molecule of glucose is converted to 2 molecules of pyruvate giving 2 molecules of ATP and two molecules of NADH
- Then this pyruvate involve in two steps. In the 1<sup>st</sup> step pyruvate is converted Acetyldehyde, releasing a molecule of CO<sub>2</sub>.
- in the second step acetyldehyde is reduced to ethanol using NADH that is produced in Glycolysis.
- Therefore final hydrogen acceptor in ethyl alcohol fermentation is acetylaldehyde (organic compound)
- Many bacteria carry out ethyl alcohol fermentation. The most common organism which carries out ethyl alcohol fermentation is yeast.

Lactic acid fermentation

- As in ethyl alcohol fermentation, Glycolysis takes place as the first step of lactic acid fermentation.
- Therefore one molecule of glucose produces two molecules of pyruvate, two molecules of ATP and two molecules of NADH.
- Then pyruvate is reduced directly by NADH for lactic acid as an end product with no release of CO<sub>2</sub>. Therefore final H acceptor is also organic compounds.
- Certain fungi and bacteria carryout lactic acid fermentation but the most common organisms are lactic acid bacteria involved in formation of yoghurt and curd.

#### **Respiratory quotient**

It is the ratio of  $CO_2$  evolved and the volume of  $O_2$  consumed in a given time for the respiratory substrate.

 $RQ = V_{CO2}/V_{O2}$ 

RQ of respiration of carbohydrates, fats and proteins are 1.0, 0.7 and 0.8 respectively.

Use of Proteins, Carbohydrates and Fats in respiration



# Photosynthesis as an Energy fixing Mechanism

#### Photosynthesis

Photosynthesis is a metabolic process by which light energy is trapped and converted to chemical energy. Chemical energy is stored in chemical bonds of carbohydrates, fats, oils, and proteins. All life on Earth depends on photosynthesis either directly or indirectly. Photosynthesis also occurs in algae and certain prokaryotes.

Global importance of photosynthesis;

- All life on earth depends on photosynthesis, directly or indirectly
- Fulfill both carbon and energy requirements of organisms
- Provide O<sub>2</sub> for respiration of aerobic organisms
- Maintain O<sub>2</sub> and CO<sub>2</sub> balance in the atmosphere
- Production of fossil fuel
- Maintenance of global temperature

During photosynthesis  $CO_2$  is reduced by the H of  $H_2O$  and simple sugars are made using light energy. In eukaryotic photosynthetic cells, chloroplasts are the sites of photosynthesis.

Process of photosynthesis consists of two main stages and they are integrated.

- 1. Light-dependent reaction
- 2. Calvin cycle

There are two types of photosynthetic mechanisms (path ways) based on the number of C atoms of the first stable product of the  $CO_2$  fixation.

- 1.  $C_3$  Mechanism No of C atom of the first stable compound is three
- 2.  $C_4$  Mechanism No of C atom of the first stable compound is four

Light-dependent reactions of photosynthesis take place in the membrane system of thylakoids. They are flattened fluid-filled sacs, which form stacks called grana at intervals. Chlorophylls, carotenoids and electron acceptors are located on this membrane system of thylakoids.

Stroma is a gel like structure containing soluble enzymes and other chemicals, which is the site of the Calvin cycle.

Photosynthetic pigments are substances which absorb visible light. In a leaf we see green colour because chlorophylls absorb violet, blue and red light and therefore, they transmit and reflect green colour. Different pigments absorb different wavelengths of light. In chloroplast, there are two types of chloroplast pigments such as chlorophylls and carotenoids. Chlorophyll *a* is the key light capturing pigment and they participate directly in the light reaction of photosynthesis.

According to the action spectrum, chlorophyll *a* is more effective for blue and red light. Chlorophyll *b* and caraotenoids (carotenes and xanthophylls) are effective in absorption of specific range wavelengths of corresponding to different colours.

Other important function of some carotenoids is photoprotection. Photoprotection is absorption and dissipation of excessive light energy, if not that excessive light may cause damage to the chlorophylls or interact with oxygen and form reactive oxidative molecules which are dangerous to the cell.

#### Absorption spectrum

An absorption spectrum is a graph of the relative amounts of light absorbed at different wavelengths by a pigment.

#### Action spectrum

An action spectrum is a graph showing the effectiveness of different wave lengths of light in stimulating the photosynthesis.

#### Excitation of chlorophyll by light

When a molecule of chlorophyll or other photosynthetic pigment absorbs light it becomes excited. The energy from the light is used to boost electrons to a higher level and become positively charged. The excited state is unstable and returns to their original lower energy state. The excited electrons may pass through several electron carriers until they reach the final electron acceptor.

Chlorophyll  $\xrightarrow{\text{Light energy}}$  Chlorophyll<sup>+</sup> + e<sup>-</sup>(electron) Therefore chlorophyll is oxidized and electron acceptor is reduced.

#### Photosystems

Chlorophyll molecules, other organic molecules and proteins are organized into complexes in the thylakoid membrane of chloroplasts. They are called photosystems.

A photosystem contains a reaction centre complex and light harvesting complexes. The reaction centre complex also contains a primary electron acceptor.

There are two types of photosystems found in the thylakoid membrane. They are Photosystem I (PS I) and photosystem II (PS II). In the PS I the chlorophyll *a* molecule is known as P700 since they absorb light at 700nm wave length effectively. In the PS II the reaction centre contains a chlorophyll *a* molecule which is known as P680 which absorbs light having a wavelength of 680 nm.

Light-dependent reaction /Light reaction photosynthesis

#### Linear electron flow

Light is absorbed by the photosynthetic pigments and synthesize ATP and NADPH due to the excitation of Photosystem I and Photosystem II which are embedded in the thylakoid membrane of chloroplast. The key to this energy transformation is a flow of the electron in one direction through the photosystems and other molecular components built in the thylakoid. This process is called linear electron flow.

The striking of photons of light on the pigments results in the excitation of electrons from the photosystem II to the higher energy state.

These electrons will be accepted by the primary electron acceptor of photosystem II.

Splitting of water takes place as a result of an enzyme catalyzed reaction and yields  $O_2$  (g), H+ ions and electrons.

Electrons released as a result of hydrolysis may neutralize excited photosystem II (P680).

Striking of photons of light on the pigments results in the excitation of electrons from photosystem I (P700) to the higher energy state. Excited electrons will be accepted by a primary electron acceptor of PSI.

Excited electrons of PS II at primary electron acceptor of PS II will pass through an electron transport chain to PSI and neutralize the excited PSI. The energy released due to the passage of electrons from higher energy state to lower energy result in the synthesis of ATP. This is known as photophosphorylation. Excited electrons of PSI at primary electron acceptor of PSI will pass through an electron transport chain and reduce NADP and yield NADPH. The reduction of NADP is catalyzed by an enzyme called NADP reductase.

#### **Cyclic electron flow**

This occurs in photosystem I but not in Photosystem II. Here some photoexcited electrons uses alternative cyclic pathway. This produces ATP but not NADPH and Oxygen is not released.



Fig 2.39: linear electron flow in the light reaction of photosynthesis

#### The Calvin cycle

The Calvin cycle takes place in the stroma of the chloroplast. Energy from ATP and NADPH produced by the light reaction are used to reduce CO<sub>2</sub>. The reactions are catalyzed by enzymes and their sequence was discovered by scientist Calvin. This is an anabolic reaction. The first stable product of the Calvin cycle is glyceraldehyde 3-phosphate (G3P). For the net synthesis of one molecule of G3P, the cycle must take place three times.

The Calvin cycle of photosynthesis can be described in three steps;

Carboxylation (Carbon fixation) Reduction Regeneration of carbondioxide acceptor

#### **Carbon fixation**

The  $CO_2$  acceptor is a 5 C sugar, Ribulose bisphosphate (RuBP). The addition of  $CO_2$  to a RuBP is called carboxylation. The enzyme involves in this reaction is RuBP carboxylase oxygenase or Rubisco.

The first product of RuBP carboxylation is a 6C molecule which is unstable and breaks down immediately in to two molecules of 3-phosphoglycerate (3-PGA). This is the first stable product of photosynthesis. The enzyme RuBP carboxylase oxygenase (Rubisco) is present in large amounts in the chloroplast stroma.

#### **Reduction phase**

1,3-Bisphosphoglycerate will be reduced to Glyceraldehyde 3- phosphate (G3P) through step by step. Enzyme catalyzed reactions utilizing NADPH and ATP from light reaction. G3P will act as a precursor for carbohydrate synthesis (glucose).

#### Regeneration of RuBP

RuBP is regenerated by undergoing a series of complex reactions. This process uses energy from ATP generated in light reaction.

#### Photorespiration

As its name suggests, Rubisco is capable of catalyzing two distinct reactions, acting as both a carboxylase and as an oxygenase.

In the oxygenase reaction of Rubisco uses the same substrate, RUBP, but reacts it with  $O_2$ . The reaction is catalyzed on the same active site as the carboxylation reaction. Thus  $CO_2$  and  $O_2$  are competitive substrates. Therefore  $CO_2$  inhibits the oxygenase and  $O_2$  inhibits the carboxylase reaction. The oxygenase reaction forms just one molecule of 3-PGA plus a two carbon product, 2phosphoglycolate which is of no immediate use in the Calvin cycle and in higher concentrations it is toxic for the plant. It therefore has to be processed in a metabolic pathway called photorespiration. The photorespiratory pathway involves enzymes in the chloroplasts, peroxisome and mitochondria. (detail of this pathway is not expected).

Photorespiration is not only energy demanding, but furthermore leads to a net loss of  $CO_2$ . Each time Rubisco reacts with  $O_2$  instead of  $CO_2$  the plants makes 50% less 3-PGA than it would have done if  $CO_2$  had been used. This potentially eliminates the net gain in photosynthetic carbon and loose the productivity.

The proportion of photosynthetically fixed carbon that is lost through photorespiration will principally depend on the relative rates of the carboxylase and oxygenase reactions of Rubisco. This ratio is determined by the ratio of the substrates  $CO_2$  and  $O_2$  in solution in the cell as well as the inherent specificity factor of Rubisco, that is the affinity of Rubisco for  $O_2$  is less sensitive to temperature than is the affinity for  $CO_2$ .

The (CO<sub>2</sub>) to (O2) ratio in solution is influenced by temperature. The solubility of a gas decreases with increasing temperature. As the solubility of CO<sub>2</sub> decreases more than that of O<sub>2</sub> with increasing temperature the ratio of (CO<sub>2</sub>) to (O<sub>2</sub>) decreases as the temperature rises.

These two factors result in an increase in photorespiration relative to photosynthesis so that an increasing proportion of carbon is lost as the temperature rises.

The CO<sub>2</sub> required for photosynthesis enters a leaf via stomata. However, stomata are also the main avenues of transpiration. On a hot, dry day, most plants close their stomata in order to conserves water. At the same time O<sub>2</sub> released from the light reactions begins to increase and this leads to further reduction of (CO<sub>2</sub>) to (O<sub>2</sub>) ratio in the cytosol. These conditions within the leaf favor a wasteful process photorespiration under high temperature, dryness and high light intensities.

Therefore plants developed different way to cope with this situation during the evolution that resulted a most successful solution to concentrate CO<sub>2</sub> around Rubisco provided by C4 photosynthetic pathway.

The establishment of C4 photosynthetic pathway includes several biochemical and anatomical modifications that allow plants with this pathway to concentrate  $CO_2$  at the site of Rubisco. Thereby its oxygenase reaction and the following photorespiration are largely repressed in C4 plants.

In most C4 plants the  $CO_2$  concentration mechanism is achieved by a division of labor between two distinct specialized leaf cell types, the mesophyll and the bundle sheath cells. Compared to C3 plants the bundle sheath cells of C4 plants have expanded physiological functions. This is reflected by the enlargement and higher organelle content of these cells in C4 species. For the efficient function of the C4 pathway a close contact between mesophyll and bundle sheath cells are tightly interconnected to each other by high numbers of plasmodesmata. The bundle sheath cells enclose the vascular bundles and are themselves surrounded by the mesophyll cells and this type of leaf anatomy was termed Kranz anatomy. Since Rubisco can operate under high  $CO_2$  concentrations in the bundle sheath cells, it works more efficiently than in C3 plants. Because of the  $CO_2$  concentration mechanism they can acquire enough  $CO_2$  even when keeping their stomata more closed and minimize the water loss by transpiration.

#### C<sub>4</sub>pathway of photosynthesis

In the mesophyll cells of C4 plants  $CO_2$  is converted to bicarbonate by carbonic anhydrase and initially fixed by phosphoenolpyruvate carboxylase using PEP as  $CO_2$  acceptor. The resulting oxaloacetate (OAA) is composed of four carbon atoms, which is the basis for the name of this metabolic pathway. Oxaloacetate is rapidly converted to the more stable C4 acids malate or aspertate that diffuse to the bundle sheath cells. Here,  $CO_2$  is released by decarboxylating enzymes and the released  $CO_2$  is refixed by Rubisco, which exclusively operates in the bundle sheath cells in C4 plants.

Chloroplasts found in mesophyll cells are different in anatomy in comparison to chloroplasts of bundle sheath cells.

Since chloroplasts of mesophyll cells carryout only light reaction, they are rich in grana. The grana of mesophyll chloroplasts are large and highly differentiated for light reaction. Bundle sheath chloroplasts possess a very few, less differentiated grana or grana are absent. Moreover, that PSII in the bundle sheath cells are depleted in order to lower oxygen production in these cells.

```
CO2 + H2O <u>carbonic anhydrase</u> HCO3- + H+
PEP + HCO3- PEP <u>carboxylase</u> Oxaloacetate (4C)
```

This PEP carboxylase enzyme is much more efficient than the enzyme of RUBP carboxylase for two reasons.

- 1. It reacts with bicarbonate (HCO3-) rather than with  $CO_2$ . The advantage of this is that there is a 50-fold higher concentration of HCO3- than  $CO_2$  in solution in the cytosol.
- 2. It has no affinity for  $O_2$



Fig 2.40: The C<sub>4</sub> pathway

#### Significance of the C4 pathway

- Helps plants to improve the efficiency of CO<sub>2</sub> fixation at lower CO<sub>2</sub> concentrations by preventing the gateways for photorespiration by spatially separating Rubisco.
- In hot-dry climate, it is essential for the stomata to close to prevent water loss through transpiration. This reduces CO<sub>2</sub> intake of particular plants. Therefore, plants in tropical zones or hot climate may suffer from CO<sub>2</sub> deficiency. At lower CO<sub>2</sub> concentrations, C<sub>4</sub> mechanism increases the efficiency of photosynthesis by concentrating CO<sub>2</sub> in the bundle sheath cells.

C4 plants exhibit better water-use efficiency than C3 plants because of the CO2 concentration mechanism they can acquire enough CO2 even when keeping their stomata more closed. Thus water loss by transpiration is reduced.

Since Rubisco can operate under high CO<sub>2</sub> concentrations in the bundle sheath cells, it woks more efficiently than in C3 plants, consequently C4 plants need less of this enzyme, this leads to a better nitrogen-use efficiency of C4 compared to C3 plants.

Characteristics	C <sub>3</sub> plants	C <sub>4</sub> plants
1. Representative species	Wheat, rice barley	Maize, sugarcane, grasses
2. Temperature		50% greater at 35 °C
optimum for	15-25	
photosynthesis ( <sup>0</sup> C)		
3. CO <sub>2</sub> fixation	Occurs once	Occurs twice, first in
		mesophyll cells, second in
		bundle sheath cells
4. $CO_2$ acceptor	5C, RuBP	3C, PEP mesophyll cells
		5C RuBP in bundle sheath
		cells
5. CO <sub>2</sub> fixing enzyme	Rubisco	PEP carboxylase in mesophyll
		cells which is very efficient
		Rubisco in bundle sheath
		cells, working efficiently
		under high CO <sub>2</sub> concentration
6. First product of CO2	C <sub>3</sub> compound, 3-	4C compund, oxaloacetate
fixation	phosphoglycerate (3-PGA)	(OAA)
7. Leaf anatomy	Bundle sheath cells, if	Kranz anatomy with
	present, are not green	photosynthesis occurring in
	(nonphotosynthetic),	both mesophyll cells and
	photosynthesis occurs in	bundle sheath cells
	Mesophyll cells	
8. Productivity	Yield is usually lower	Yield is usually high

Table 2.6: Comparisons of C<sub>3</sub> and C<sub>4</sub> plants

#### **Factors affecting photosynthesis**

The rate of photosynthesis is an important factor in crop production. Rate is affected by various factors.

e.g. light intensity, CO<sub>2</sub> concentration, temperature,

The photosynthesis involves a series of reactions. Therefore various factors are involved in it.

Blackman who is the scientist first proposed the idea of principal of limiting factors.

When a chemical process is affected by more than one factor, its rate is limited by the factor which is nearest its minimum value.

e.g. Light intensity

#### **Light Intensity**

The rate of photosynthesis increases linearly with increasing light intensity. Gradually the rate of increase falls off as the other factors become limiting. Very high light intensities chlorophyll may bleach and slow down photosynthesis. However, plants exposed to such conditions are usually protected by devices such as thick cuticles, hairy leaves.



Fig 2.41: Rate of photosynthesis with light intensity at different temperatures

Under normal conditions,  $CO_2$  is the major limiting factor in photosynthesis. Increase in photosynthetic rate is achieved by increasing  $CO_2$  concentration. For example some greenhouse crops such as tomatoes are grown in  $CO_2$  enriched atmosphere.