Chemical and cellular basis of life

Elemental composition of living matter

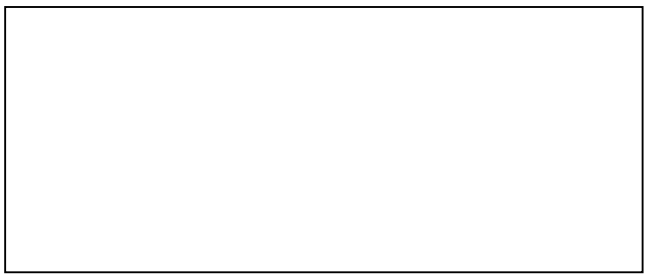
- There are about ninety two elements naturally occur in earth's crust.
- Of which, about 20-25% elements are essential **to continue healthy life and reproduction**.
- The essential elements are similar among organisms , but there is some variation.
- Example-about 25- elements are essential for humans and about 17 for plants.
- Oxygen (O), Carbon (C), Hydrogen (H), and Nitrogen (N) make up 96% of living matter.(including water)
- 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 [including water]
- Ca, P, K, S, Na, Cl, Mg and trace elements account 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).
- The elements that are needed in large amounts and make up 0.01% or more of the dry mass of the body are called macro elements.
- The elements that are needed in small amounts and found less than 0.01% of dry mass of an organism are called trace elements.

Element	Symbol	Percentage in Body
Oxygen	O	65.0
Carbon	C	18.6
Hydrogen	H	9.5
Nitrogen	N	3.2
Calcium	Ca	1.5
Phosphorous	P	1.0
Potassium	K	0.4
Sulfur	S	0.3
Sodium	Na	0.2
Chlorine	Cl	0.2
Magnesium	Mg	0.1
Trace elements include Boron, Chromium, Cobalt, Copper, Fluorine, Iodine, Iron, Manganese, Molybdenum, Selenium, Silicon, Tin, Vanadium and Zinc	B, Cr, Co, Cu, F, I, Fe, Mn, Mo, Se, Si, Sn, V, Zn	Less than 1.0

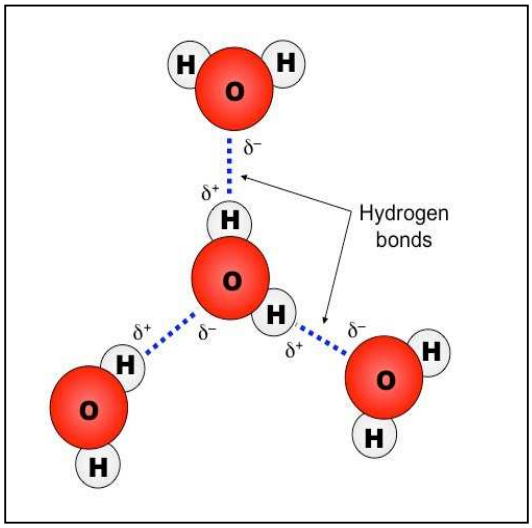
Physical and chemical properties of water important for life

- Water is 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.
- Consists of one oxygen atom and 2 hydrogen atoms.

- The 2 hydrogen atoms are joined to the oxygen atom by two covalent bonds.
- Oxygen is more electronegative than hydrogen.(the attraction of a particular atom for the electrons of a covalent bond is called electronegativity.)
- Because the electrons have a negative charge and the shared electrons are pulled toward oxygen atom in a water molecule , the oxygen atom has a partial negative charge indicated by δ^- and each hydrogen atom has a partial positive charge indicated by δ^+
- 1.The unequal sharing of electrons
- 2. Water molecule is shaped like a wide V with its 2 hydrogen atoms joined to the oxygen atom by single and polar covalent bonds
- Make it a polar molecule, meaning that its' overall charge is unevenly distributed.



- Weak attractions between the slightly polar hydrogen atom of one water molecule and the slightly polar oxygen atom of adjacent water molecules are known as hydrogen bonds.
- These hydrogen bonds play a major role in maintaining all the properties of water.



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 above properties 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 upwards by offsetting gravitational pull.
- 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

2 properties of water are important- 1. High specific heat 2. High heat of vaporization

- 1. 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, aquatic bodies and air during the temperature fluctuations on earth and maintain a temperature suitable for life.
- 2. Due to the high heat of vaporization, 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.
- On a global scale this property helps moderate Earth's climate. A considerable amount of solar heat absorbed by tropical seas is consumed during the evaporation of surface water. Then as moist tropical air circulates poleward it releases heat and condenses forming rain.

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 fl oats 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.

Chemical Nature and Functions of Main Organic Compounds of Organisms

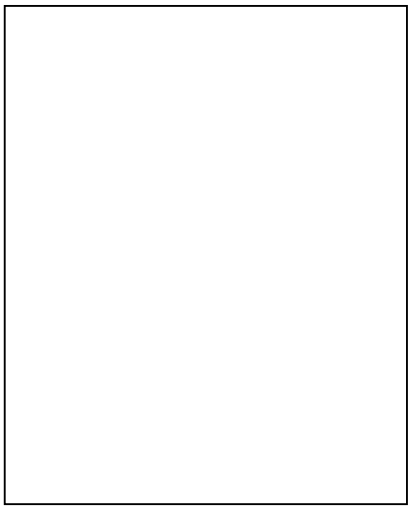
Carbohydrates

- 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 are monosaccharides, disaccharides and polysaccharides.
- Generally carbohydrates include sugars (monosaccharides and disaccharides) and polysaccharides.

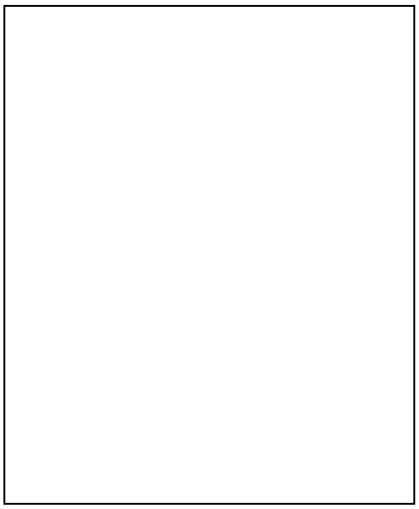
Monosaccharides

- 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;
    1. 3C- Triose e.g. Glyceraldehydes (Phosphoglyceraldehyde is a derivative of Triose)
    2. 4C- Tetroses.g. Erythrose (rare in nature)
    3. 5C- Pentoses.g. Ribose, Deoxyribose, Ribulose (RUBP is a derivative of ribulose)
    4. 6C- Hexoses e.g. Glucose, Fructose, Galactose
- According to the type of carbonyl (Keto, aldo)group, they are classifi ed as;
    a. Aldoses-glucose, galactose
    b. Ketoses-fructose

Aldose

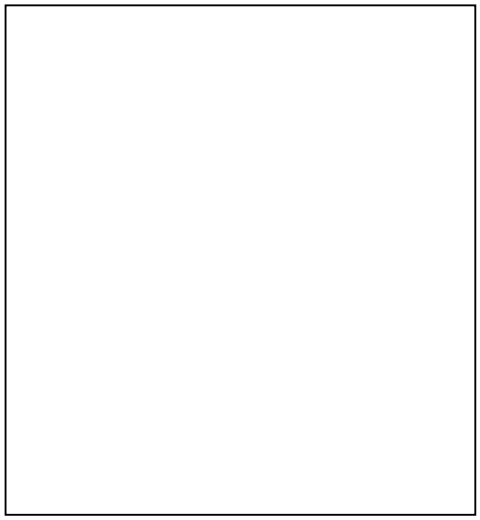


Solid form of Glucose

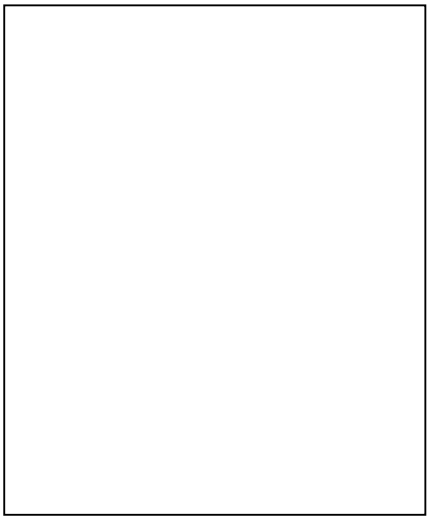


Aqueous form of Glucose

Ketose



Solid form of fructose

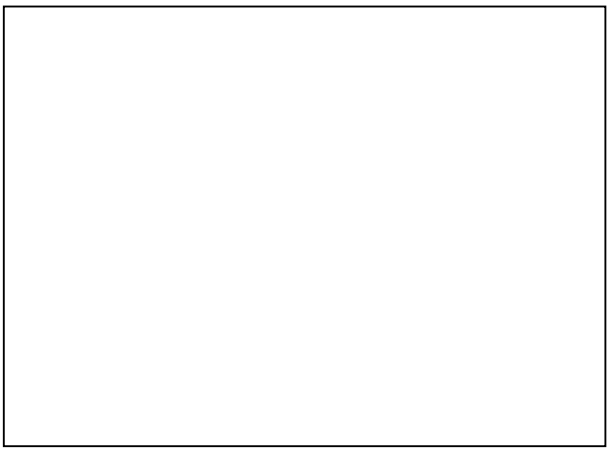


Aqueous form of fructose

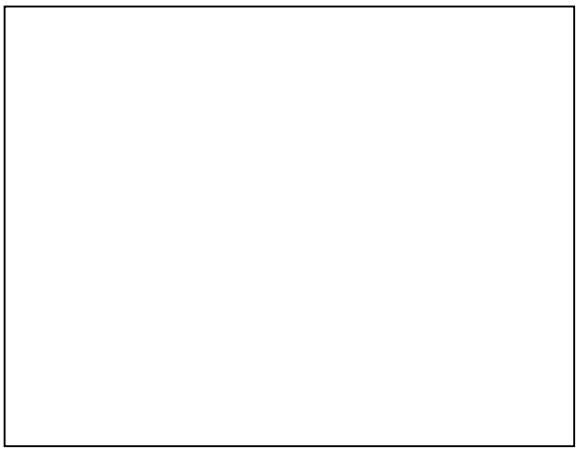
- In aqueous media some monosaccharides are in ring form

Disaccharides

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



Formation of sucrose



Formation of maltose

- 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 $\longrightarrow$ Maltose + H_20

Glucose + fructose $\longrightarrow$ Sucrose + H_20

Glucose + Galactose $\longrightarrow$ 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 are non 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.
    - ✓ Storage- Starch, Glycogen
    - ✓ Structural- Cellulose, Hemicellulose, Pectin
- Based on their architecture they are categorized as
    - ✓ Linear forms- Cellulose, Amylose
    - ✓ Branched forms- Glycogen, Amylopectin, Hemicellulose

Polysaccharide	Monomer	Functions
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
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.) Storage polysaccharides-

- starch stores glucose as energy source in plants and chlorophytes
- glycogen stores glucose as energy source in animals and fungi
- Inulin stores fructose as energy source in Dahlia tubers

b.) structural polysaccharides-

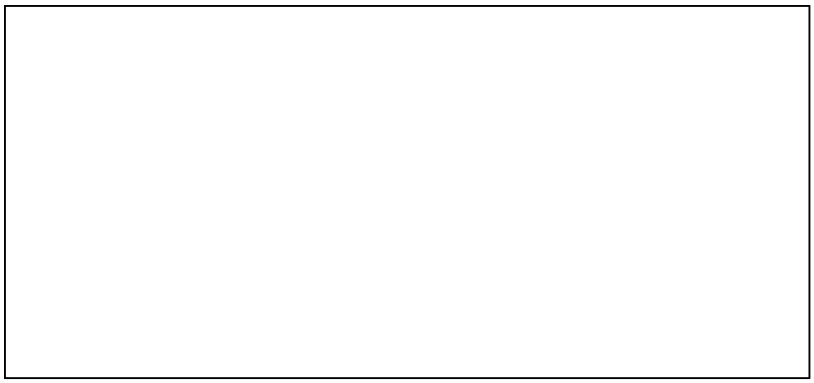
- Cellulose in the cell walls of plants and chlorophytes
- Pectin in the middle lamella of plant tissues.
- Hemicellulose in cell walls of plants.
- Peptidoglycan in the cell walls of prokaryotes.
- Chitin in the cell walls of fungi and in exoskeleton in Arthropods.

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.



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

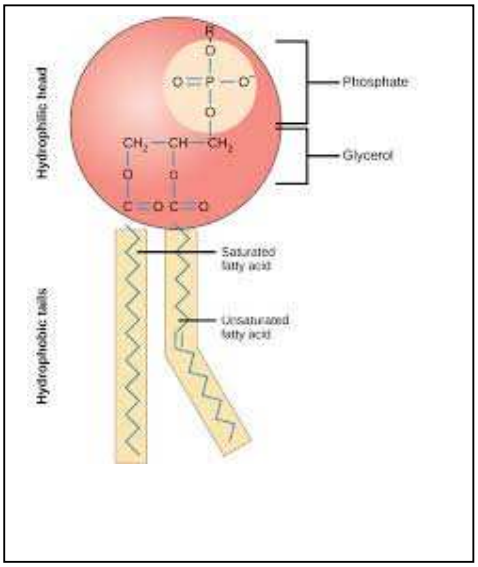
a) Saturated fats- fats are made up of saturated fatty acids:
   - Fatty acids with hydrocarbons having no any double bonds.
   - Usually animal fats come under this category.
   - They are mostly solid at room temperature.
     e.g: butter

b) Unsaturated fats- fats are 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.



structure of the phospholipid molecule

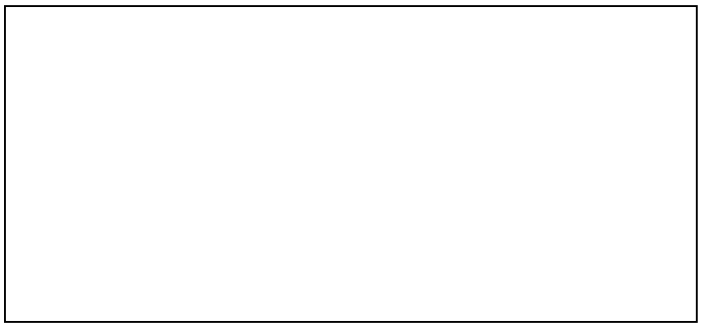
Functions of Lipids

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

Protein

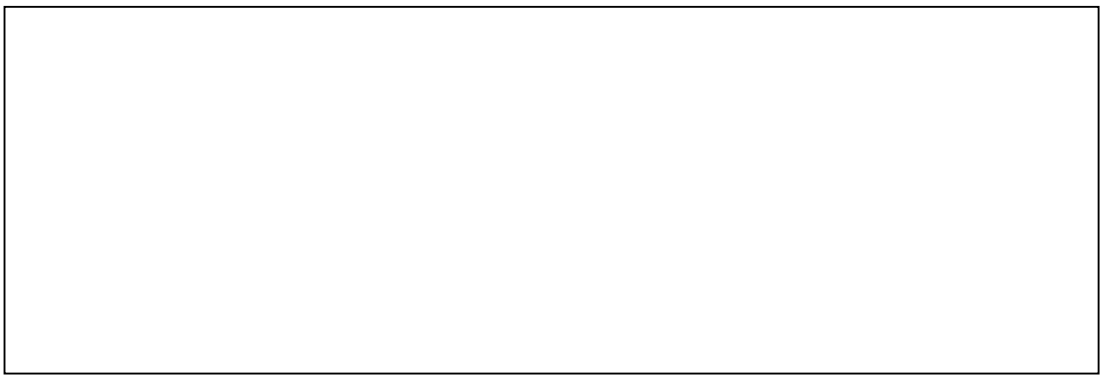
- Proteins are made up of amino acids.
- Twenty different amino acids are involved in the formation of proteins.
- Elemental composition is C, H, O, N and S.
- At the centre of the amino acid is an asymmetric carbon atom except in glycine.

- 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).



Structure of an Amino acid molecule

- Amino 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 amphoteric.
- Two Amino acids undergo condensation reaction by removing a water molecule from both and result a bond known as peptide bond;



Formation of 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 β pleated or alpha helical.
  1. Alpha helix e.g.Keratin.
  2. β pleated sheet e.g.spider's silk fiber

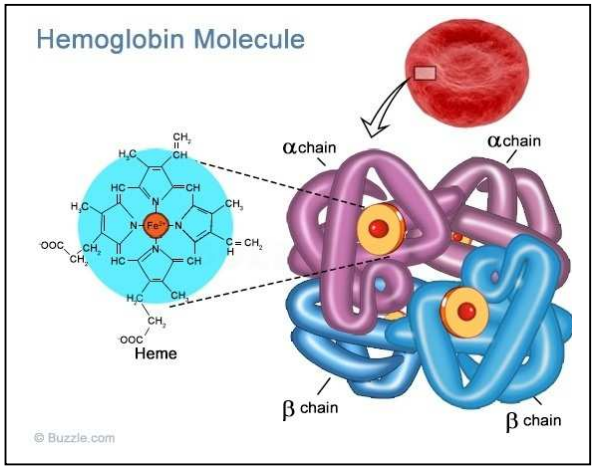
beta pleated sheet and alpha helix of secondary structures of proteins

c)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;
- H bonds
- Disulphide bonds
- Ionic bonds
- Van der Waals interactions/ Hydrophobic interactions
  e.g.most of the enzymes, myoglobin, albumin

d) Quaternary structure

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



structure of the hemoglobin molecule

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

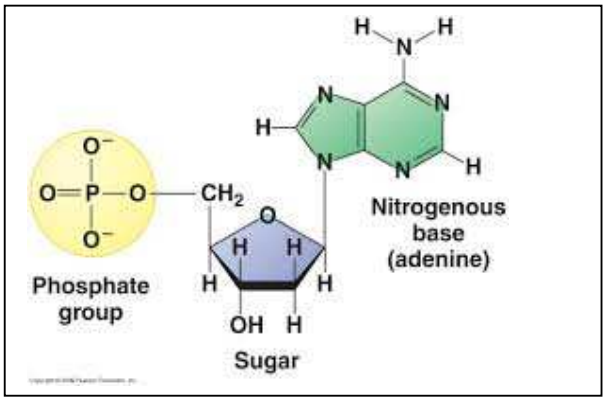
Type of protein	Example	Functions
Catalytic protein	Pepsin, Amylase	Catalyze bio – chemical reaction
Structural protein	Keratin	Prevent desiccation
	Collagen	Provide strength and support
Storage	Ovalbumin	Storage protein in egg
	Casein	Storage protein in milk
Transport	Haemoglobin	Transport O_2 and CO_2
	Serum albumin	Transport fatty acids
Hormones	Insulin	Regulate blood glucose level
	Glucagon	
Contractile/Motor	Actin/Myosin	Contraction of muscle fibers
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 acids are macromolecules, biopolymers.
- There are two types of Nucleic acids:
  1. DNA (Deoxyribo nucleic acids)
  2. RNA (Ribonucleic acids)

Structure of nucleotides

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



Structure of nucleotides

- 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 deoxyribose one 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^{rd} 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.

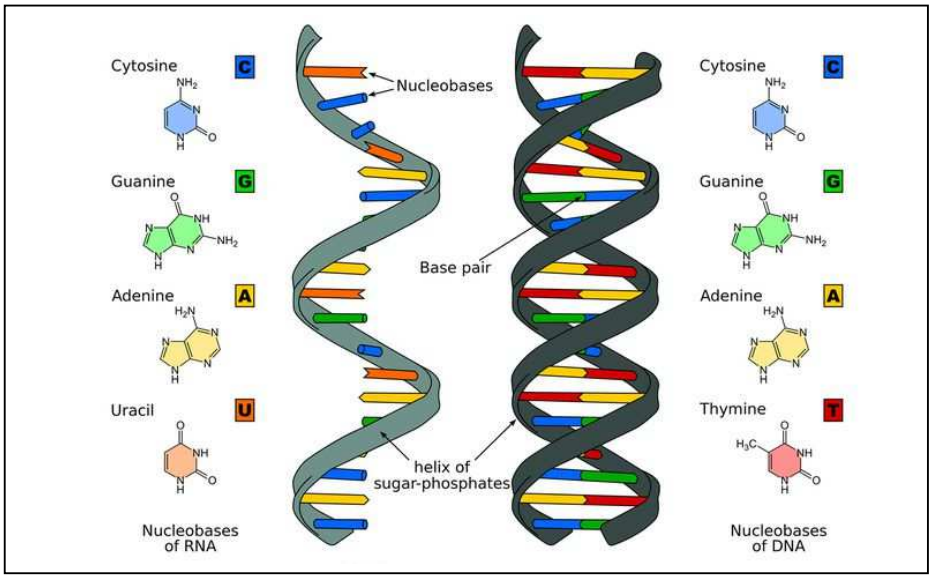
Structure of DNA molecule (Watson and Crick model)

- DNA molecules have two anti-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.



The structure of the RNA and DNA molecules

Functions of DNA

1. Store and transmit genetic information from one generation to the next generation
2. Store the genetic information for protein synthesis

Structure of RNA

- This is normally a single stranded nucleic 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,
  1. Messenger RNA (m -RNA)
  2. Transfer RNA (t -RNA)
  3. Ribosomal RNA (r -RNA)

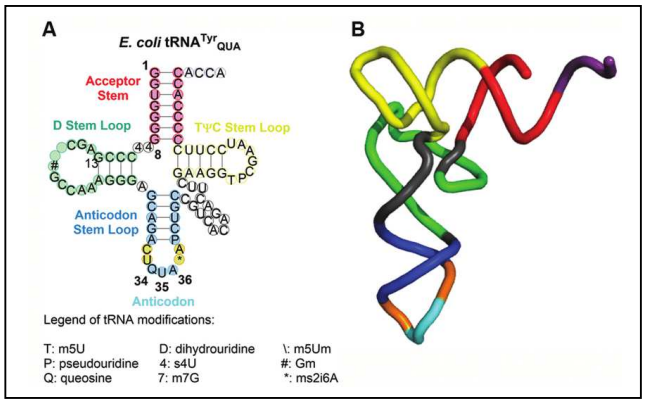
1. Messenger RNA (m –RNA)

- Messenger RNA is a linear molecule and is the least abundant type of RNA in 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 (t -RNA)

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



Structure of the t -RNA molecule

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

3.Ribosomal RNA (r –RNA)

- It is the most abundant type of RNA. r-RNA 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 C and 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

Nucleotide	Functions
ATP	❖ Universal energy carrier
NAD^+	❖ Act as a co- enzyme ❖ Act as an electron carrier ❖ Function as an oxidizing agent during respiration
$NADP^+$	❖ Act as co- enzymes ❖ Act as an electron carrier ❖ Act as a reducing agent in photosynthesis
FAD	❖ Act as a co- enzyme ❖ Act as an electron carrier

Competency 2.2.0 : **How cells and tissues contribute to the functioning of Organisms**

Competency level 2.2.1 **The contribution of microscopes to the expansion of knowledge on cells and cellular organization**

Light microscopy

- Basic principle - Uses visible light for illuminating the object and pass through glass lenses to form a magnified image of the object which focus/project into the observer's eye. The image of the specimen can be even projected onto photograph film or onto a video screen.
- In simple light microscope , a single lens is used for both magnification and focusing.
- In compound light microscope two lenses are used ; ocular / eye piece closer to the eye and the objective lens closer to the object.
- Usually objective lenses of various magnifying powers are mounted on a revolving turret/ rotating nose piece at the lower end of the body tube/ barrel
- The object supported by a glass slide under the objective lens, is illuminated by light beneath it.
- In ordinary microscope light is reflected on the object by a mirror having concave and plane surfaces.

- In some microscopes a third lens called condenser lens is located between the object and the light source and serve to focus the light on the object.
- Iris diaphragm located in the path of light below the condenser to control the amount of light if needed

Parts of the compound light microscope	**Function**
Substage lamp/ mirror	Illumination of the specimen
Condenser lens	Focus light on to the specimen
Iris diaphragm	Regulate the amount of light
Stage with a hole in the centre	Secures the glass slide on which the specimen is mounted
Objective lens	Magnification and resolution of the image
Revolving nose piece	Mounting 3 or 4 objective lenses to select as needed
Ocular lens	Magnification and focusing the image
Body tube	Passing light from the objective to eye piece
Coarse and fine adjustment knobs	To change the specimen- objective distance to focus the objective lens on the specimen. Coarse –For initial focusing specially in viewing specimen under low power. Fine - fine focusing specially viewing specimen under high power.

Proper use of light microscope

1. Rotate the nose piece until the low power objective is in line with the body tube in a well illuminated place
2. place the microscope slide so that the specimen to be viewed is directly below the low power objective lens and right side up. It should be centred over the hole in the stage.

3. Look through the ocular lens with both eyes open
4. Adjust the mirror and iris diaphragm to get sufficient light rays to brighten the field.
5. rotate the coarse adjustment knob until the specimen comes into focus.
6. To view with high power , eyes should be taken away from the ocular first and rotate the nose piece until the high power objective is in line with the body tube.
7. rotate the coarse adjustment clockwise until it is very close to the specimen.
8. look through the ocular and rotate the fine adjustment knob counter clockwise and fine focus the image.

Functions of microscopes-

- There are 2 important parameters of microscopes
  1- magnification 2- resolution

Magnification

The ratio of size of optical image to the real size of the object being viewed.

- Magnification of a compound light microscope is the product of the magnification of the objective lens and that of eye piece.
- Eg If the specimen is viewed with a 10x objective and 10x ocular , the total magnification is 100x

Which provides the largest field of view, the low or high power objective ?

- In theory it might seem possible to magnify an object indefinitely by means of glass lenses in series. But in practice this would produce a large but a blurred image.
- The limited resolution is imposed by the wave length of light and it means that little can be gained by magnifying an object more than x 1000 times.
- So the maximum useful magnification of a light microscope is x1000 times.

Resolution- measure of the clarity of the image

Resolving power is the minimum distance by two points must be separated in order for them to be seen as two distinct points rather than a single fused image.

- The limit of resolution of any optical instrument is given by the following relationships

$$\text{Resolution} = \frac{\text{Wave length } [\lambda]}{\text{Numerical aperture } [n\sin\alpha]}$$

λ = wave length of the radiation used to form the image .Resolution is inversely proportional to the wave length.

n = refractive index of the material between the specimen and the objective lens

[air or water]

Sin α = sin of semi angle of aperture. nsinα is often called the numerical aperture.

- Higher the magnifying power of the objective lens greater the resolving power of the microscope . [due to the increased angle of aperture]
- The eye piece lens can only magnify the image formed by the objective lens**. It cannot improve upon it's resolution.**
- By increasing the refractive index of the medium , higher resolution can be obtained.
- In practice cedar wood oil can be used which has the same refractive index as the glass ,to fill the space between the specimen and the oil immersion objective lens.
- It has a refractive index of 1.6 which has a big improvement over air / water (n=1)

Which combination of lenses will give a higher resolution?

A- eye piece 5x and objective 40x
B- eye piece 10x and objective 20x
A- eye piece 10x objective 40x
B- eye piece 10x oil immersion objective 40x.
1. the resolving power of healthy human eye is 0.1 mm/ 100µm
2. the resolving power of light microscope is 0.2 µm
3. the practical limit of resolving power of electron microscope is 2 nm

Q1 -The resolution power of L.M is ---------- times greater than the unaided healthy human eye.

Q2 - The resolution power of E.M is ---------- times greater than the light microscope.

Electron microscope

- Much greater resolution has been obtained with the transmission electron microscope developed in 1930s.

1. The electron microscopy uses a **beam of electrons** in place of light with much shorter wave length to achieve high resolution.
   Resolution is inversely related to the wavelength of the radiation used for imaging.
   A beam of electrons is accelerated from a heated metal wire by **high voltage**[20,000-100.000 or more] The wave length of electrons depend on the magnitude of the voltage.
2. **Electromagnetic coils / lenses** are used to control and focus the beam of electrons on the specimen and to produce a magnified image.
   The electrons are scattered by a specimen placed in the path of the beam.
   The electrons that manage to pass through the specimen are focused by an objective coil and a final magnified image is produced by the projector lens
3. The final image is viewed directly on the **fluorescent screen or is recorded on photographic film to produce electron micrograph.**
4. The specimen must be extremely thin [10nm - 100 nm] If the sections are not **ultra thin** all the electrons will be scattered and a uniform dark image will be produced.
5. Since electrons are even scattered by gas molecules the electrons must travel through a **high vacuum** , therefore the **sample must be completely dry and living cells which are wet cannot be viewed in E.M**

6.There is a standard procedure for the preparation of specimen
fixation→dehydration→sectioning→mounting→staining→viewing

	Light microscope	Transmission electron microscope
Radiation source	Light	Electrons
Lenses	Glass	Powerful electromagnets
Maximum useful magnification	1000x	500,000x
Resolution	0.2µm	2nm
Specimen	Living or non living Thin[used razor blades or microtome] Wet / dehydrated Placed under normal conditions	Non living Ultra thin [used glass or diamond knives] Dehydrated. Placed in a vaccum
Stains	Coloured dyes	Heavy metals / salts of lead ,uranium, Osmium
Image	Can be directly viewed with eyes [retina is sensitive to light] Natural colors can be seen and a colored image after staining.	Has to be taken on to a fluorescent screen or on a photographic plate as a micrograph. Black and white

Scanning electron microscope

- This was introduced in 1960's. The electron beam does not pass through the specimen , but it scans over the surface of the specimen .
- Secondary electrons are emitted by the specimen which are detected by a device that translates the pattern of electrons into an electronic signal to a video screen.
- Samples to be examined are usually coated with a thin film of gold to enhance the efficiency of production of secondary electrons.
- The resolution of SEM is 5-20 nm.

Advantage of using SEM

1. Surface topography can be viewed.
2. 3D appearance of whole cells / organs/ organisms can be examined due to great depth of field.

The problem with these forms of electron microscope is complex preparation techniques coupled with the need for a high vacuum means that not only must the materials being observed be dehydrated and therefore dead , it is frequently distorted.(distortion in architecture – artefacts)

Comparison of TEM and SEM-

similarities-

1. non living object
2. source of radiation- electrons
3. medium- high vacuum
4. lenses- electromagnetic lenses
5. magnification and focusing – by the current in the lens coil

Scanning electron microscope	Transmission electron microscope
Electron beam scans over the surface of sample	Electron beam pass through the sample
Image is formed by scattered electrons or secondary electrons emitted from the surface due to excitation by the primary electron beam	Based on transmitted electrons
Suitable to study entire cell, small organisms or parts of organisms	Ultra thin sections of cells
Used to study shapes and surface structures/ topography of specimen	Ultra structure of cells/ cellular components
3-D image	2-D image of the plane of section
Low resolution than TEM	High resolution

Disadvantages of electron microscopes

1. Expensive.
2. very large and must be operated in special rooms
3. preparation of material requires expertise and time
4. preparation of material may distort it.
5. natural colours cannot be observed.
6. living cells cannot be observed.

Which type of microscope would you use to study

1. the changes in the shape of a living white blood cell.
2. the details of surface texture of a hair
3. the detailed structure of an organelle.

Because most biological structures are colorless and transparent staining is used to obtain contrast between different structures in light microscopy.

Stain	Final colour	Suitable for
Eosin	Red/pink	Cytoplasm
Haemotoxylene Methylene blue	blue	Nucleus Nucleus, bacterial cell wall
Safranine	red	Nucleus, lignified cell walls, bacterial cell wall
Aniline sulphate	yellow	Lignified cell walls.
Feulgen's stain	Red/ purple	DNA/ chromosomes

Observation of cells in an onion peel-

1. make thin epidermal peels of onion and place in water in a watch glass
2. transfer onion peel into a drop of water on the center of a clean glass slide by using a fine paint brush
3. Hold the coverslip at the edge of the water drop with the help of a mounting needle and gently lower the coverslip onto the drop of water ensuring no air bubbles are trapped under the coverslip.
4. Place the slide on the stage of the microscope and move the low power objective into position.
5. Adjust the mirror to get optimum illumination
6. Use coarse focus knob to get the image as clear as possible.

Historical background of the cell and analyses the structure and functions of the sub cellular units

Cell theory

- All organisms are composed of cells.
- 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 cork tissue using compound light microscope and gave the term "CELL" to describe the basic units, he observed which are really dead cells.
- **Antoni Van Leeuwenhook (1650),**
  a contemporary of Robert Hooke, was the first to describe and record living 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 include 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

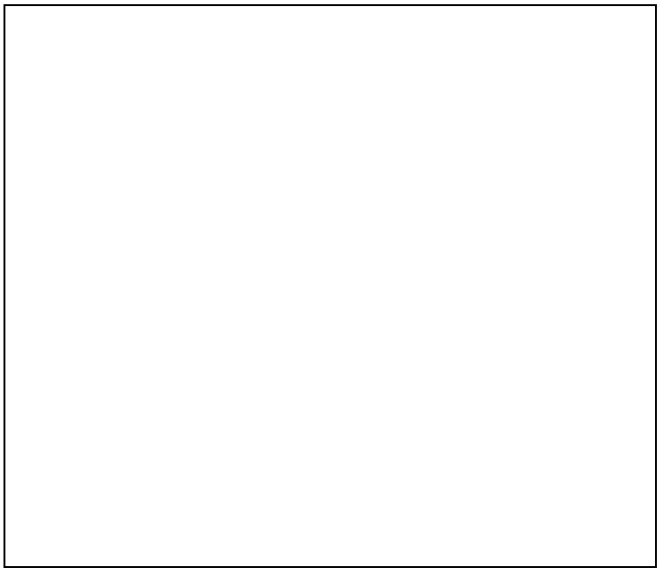
- There are two kinds of cellular organization - Prokaryotic and Eukaryotic
- All cells share certain basic features. They are;
  1. All cells are bounded by a plasma membrane which is a selective barrier
  2. Within the cell have, a semi fluid, jelly like substance which is called cytosol. Subcellular components are suspended within the cytosol.
  3. They carry DNA as genetic materials.
  4. Ribosomes are found in all cells.
- In addition to the plasma membrane a eukaryotic cell has extensive internal membranes that divide the cell into compartments. They are called organelles.
- The cell's compartments provide different local environments that support specific metabolic functions , so incompatible processes can occur simultaneously in a single cell.
- The membranes participate directly in cell metabolism as many enzymes are built right into the membrane
- **Therefore the compartmentalization of eukaryotic cells contribute to**
  1. increase the efficiency of metabolic functions due to the larger surface area provided by membranes.

2. Division of labor, each organelle type is involved in its specific metabolic function which can function independently and simultaneously.

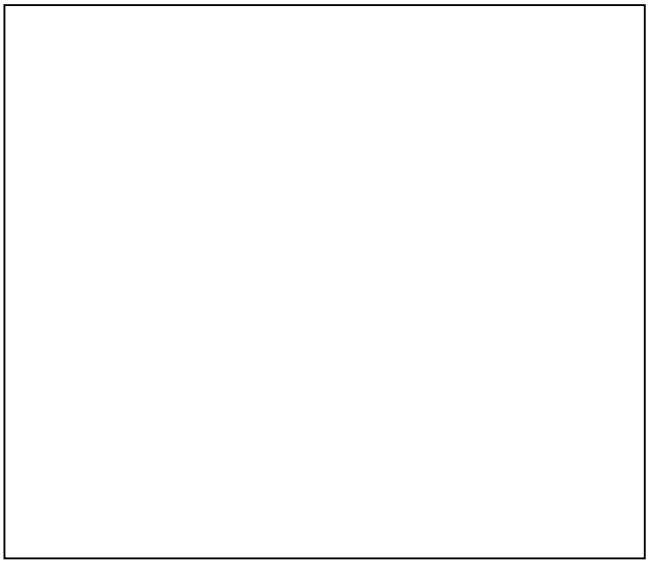
General structure of prokaryotic cell

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 histone proteins in bacteria	DNA is linear and contained in a nucleus. DNA is associated with histone proteins
Type of ribosomes	70s ribosome (smaller)	Both 70s (Mitochondria and Chloroplast) and 80s ribosomes (larger) present (may attach to endoplasmic reticulum)
Organelles	Few organelles, none are surrounded by membrane Internal membranes scarce; if present usually associated with respiration, photosynthesis and N_2 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 Archaebacteria	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

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



Structure of an animal cell



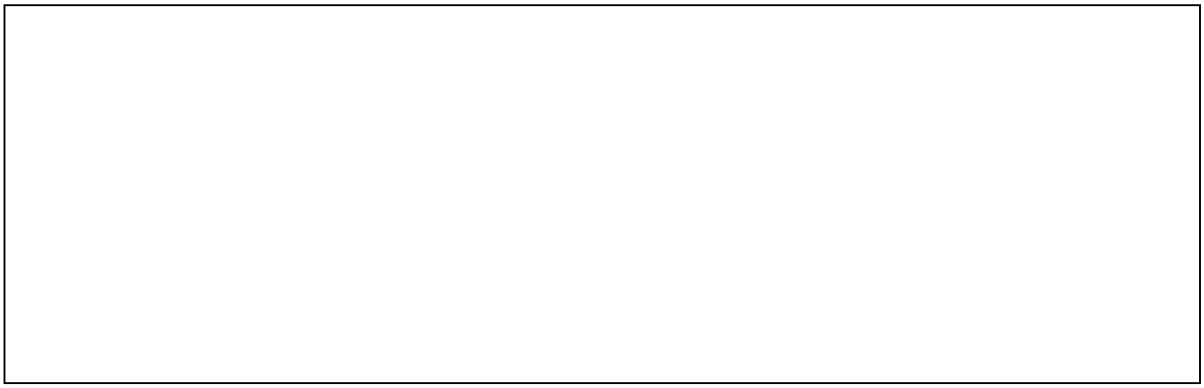
Structure of plant cell

Structures and functions of organelles and other subcellular components

Plasma membrane:

- 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 of cell 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.
- It is mainly 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.
- A variety of protein molecules embedded randomly contribute to its mosaic nature.
- According to the organization of proteins-
- A- Integral B- peripheral proteins can be identified.

- Integral proteins are found within the membrane, penetrating the hydrophobic interior of the phospholipid bilayer.
- Majority of the integral protein molecules penetrate all the way through the membrane, called **transmembrane proteins** and some others penetrate only part of the way into the membrane.
- Some transmembrane proteins have hydrophilic channels./ channels lined by hydrophilic parts of proteins
- 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 surface of the membrane, called **peripheral proteins**.
- Some proteins and lipids have short branching carbohydrate chains like antennae, attached covalently forming **glycoprotein** and **glycolipids,** respectively.
- They are found attached to the outer surface and project out of the cell.
- 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 high temperatures and prevent membrane solidification at low temperatures.
- The two sides of the membrane may differ in composition and function.



Structure of the plasma membrane

Functions

1. The plasma membrane surrounds the cytoplasm of living cell physically separating the intracellular components from the extracellular environment.
2. Plasma membrane is selectively permeable and able to regulate the exchange of material needed for survival.
3. Proteins embedded in the plasma membrane identify the cell, enabling nearby cells to communicate with each other (involved in cell recognition).
4. Some protein molecules act as receptor molecules for interacting with specific biochemical, such as hormones, neurotransmitters and immune proteins.
5. Some proteins in the cell membrane attach to some cytoskeletal fibers and help to maintain the shape of the cell.
6. 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.)

Write the functions of different chemical components of the plasma membrane.

Phospholipids-

Cholesterol-

Proteins-

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 fi laments which line the interior side of the nuclear envelope.
- **Nuclear matrix –**
  - ✓ It is 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, called chromosomes.
  - ✓ Each species has a constant number of chromosomes. (e.g. typical human cell has 46 chromosomes).

Functions

1. Control all cellular activities.
2. Synthesize DNA to produce new nuclei for cell divisions.
3. Synthesize r-RNAs and ribosomal subunits required for protein synthesis, through nucleolus.
4. Synthesize mRNA and t-RNA according to the information present on the DNA.
5. 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 r-RNA 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 are freely available as group in cytoplasam.
- Bound ribosomes are attached to the membrane surface of rough endoplasmic reticulum and outer membrane of nuclear envelope

Functions

1. 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

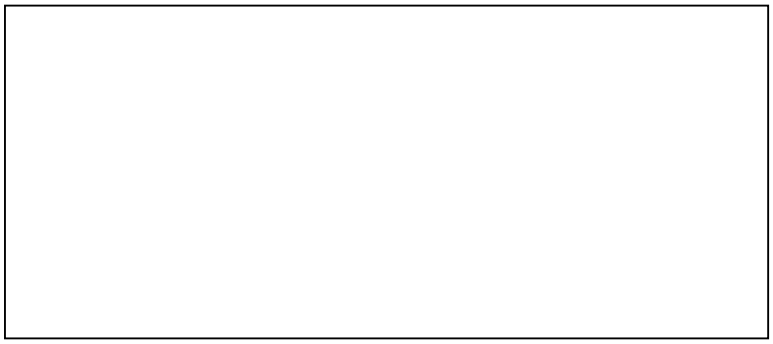
1. Transport protein synthesized by ribosomes
2. Synthesis of glycoproteins
3. Produce transport vesicles
4. Facilitate the growth of own membrane by adding phospholipids and proteins . Contribute to form components of endomembrane system by expanding and producing transport vesicles. Therefore called as membrane factory.

Smooth ER

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

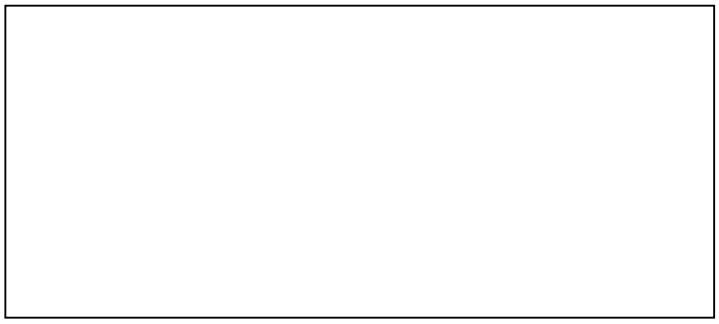
Functions

1. It synthesizes lipids including oils, steroids and phospholipids.
2. Metabolism of carbohydrates.
3. Produce transport vesicles to transport within cell.
4. Involves in detoxification.
5. Stores Ca^{2+} ions.



structure of the endoplasmic reticulum

Golgi apparatus



Structure of the golgi apparatus

- Golgi apparatus is a stack of flattened sacs or Cisternae.
- Inner and outer surfaces can be identified as cis face and trans face respectively.
- Cis face is located near the E.R to receive transport vesicles from E.R.
- Trans face give rise to secretory vesicles which are budded off and travel to other locations.
- Golgi complex is well developed in secretory cells.

Functions

1. Collecting, packaging and distribution of materials
2. Manufacturing pectins and certain other non cellulose cell wall components of plants
3. 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

1. Digest food particles received by phagocytosis.
2. Transport residue material out of cell by exocytosis.
3. Digest worn out organelles.
4. 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.
- Specialized peroxisomes called glyoxysomes are found in fat storing tissues in plants.
- Glyoxysomes converts fatty acids into sugar.

Functions

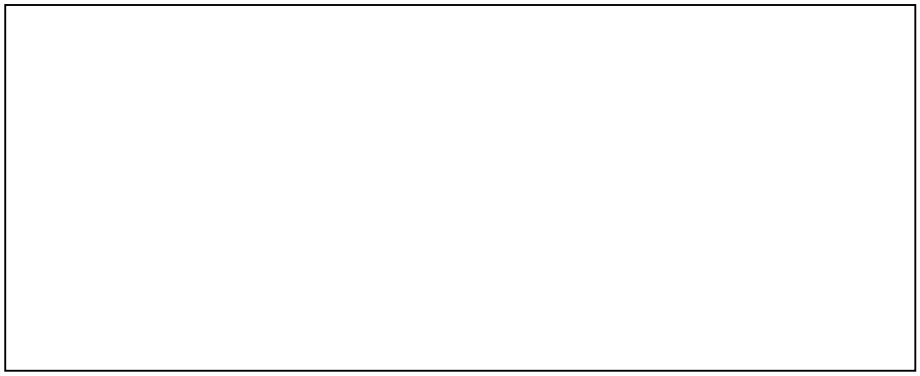
1. Detoxification of peroxides.
2. Photorespiration in plants.

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 the mitochondrion 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 essential for electron transport chain and oxidative phosphorylation.

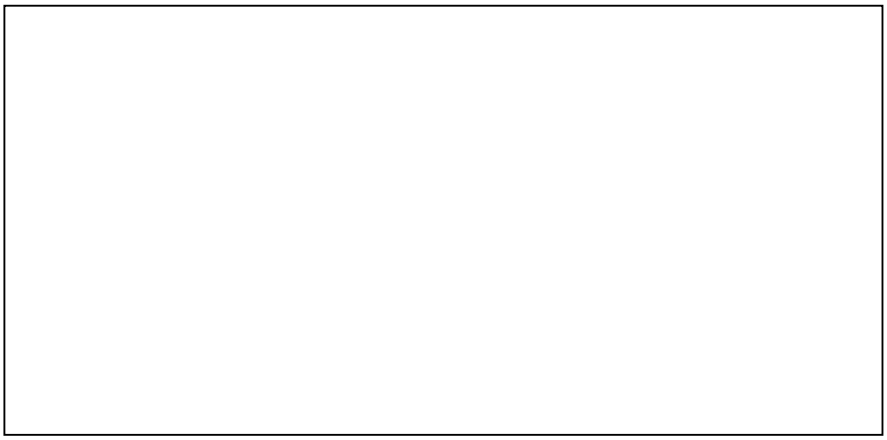
Functions

1. Synthesize ATP in aerobic respiration.
2. Involve in Photorespiration.



Chloroplast

- It is usually a biconvex lens shaped organelle with two membranes which is found in plants and some protists.
- The outer and inner membranes are smooth and are separated by a very 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 grana. 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.



Structure of the chloroplast

Functions

1. 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 fi laments.
- 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 Micro filaments
    - ✓ Intermediate fi laments
    - ✓

Property	Microtubules (Tubulin polymers)	Micro filaments (Actin filaments)	Intermediate 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
Protein subunits	Tubulin	Actin	One of several different proteins (e.g. Keratin), depending on the cell type.
Main functions	Maintenance of cell shape Cell motility (as in cilia or fl agella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tensionbearing elements) Changes in cell shape Muscle contraction Cytoplasmic streamingin plant cells Cell motility (as in pseudopodia) Cell division in animal cells (cleavage furrow formation)	Maintaining of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles.Formation of nuclear lamina

Functions

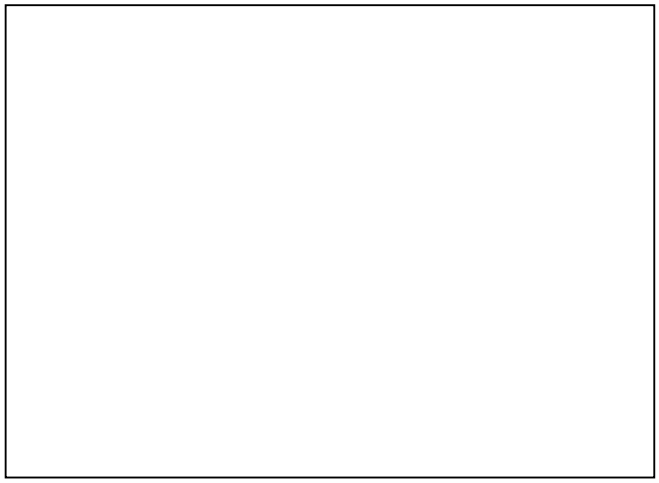
1. Provide strength to the cytoplasm
2. Anchorage organelles and cytosolic enzymes of the cell
3. Movement of cytoplasm, cytoplasmic streaming, positioned organelles and move chromosomes when necessary.
4. Maintain the shape of the cell (mainly in animal cells)

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 are covered by plasma membrane and bound to a basal body which anchors the cilium or flagellum to the cell.
- The Basal body has 9 + 0 arrangement of microtubules (no microtubules in its center)

Functions

1. Act as locomotor appendages
2. Can move fluid over the surface of the tissue
3. Cilia lining in oviducts help move an egg toward the uterus



Structure of the Cilium

Centrioles

- Centriole is made up of cylindrically arranged microtubules which 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

1. 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

1. Stores water and other materials such as sugars, ions and pigments.
2. Maintains water balance of the cell.
3. Gives turgidity and support to cell.
4. Produce colours in some plants with sap pigments.
5. 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 species to species and even from one cell type to another even in the same plant.
- Nevertheless in Plants, cell wall is generally made up of cellulose, pectin, hemicelluloses.
- lignin and suberin (in some plant cells only).
- Plants generate two types of cell walls: primary and secondary walls.
- Young cells first secrete primary cell 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 polysaccharides called pectins (magnesium and calcium pectate).
- Middle lamella glues adjacent cells together.
- Due to the deposition of hardening substances on the primary wall a secondary cell wall is generated secondarily.
- Primary cell wall is permeable, relatively thin, flexible, composed mainly of cellulose fibrill 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 primary cell wall.
- It contains several layers of hard materials, forming a rigid structure.
- In addition to cellulose, impermeable substances such as lignin and suberin are also incorporated in to the secondary wall.
- Lignin cement anchors cellulose fibrils 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

1. Protection and support
2. Allows development of turgidity when water enters the cell
3. Prevents bursting during turgidity
4. Limits and control cell growth
5. Component of appoplast pathway
6. Maintaining cell shape
7. hold the plant up against the force of gravity

2. Cell junctions

- Cell junctions are structures at which neighboring plasma membranes are joined.
- They also interact and communicate via sites of direct physical contacts.

Functions

1. Connects the internal chemical environment of adjacent cells.
2. Cell junctions are structures at which cytoplasm of adjoining cells are joined.

- There are three types of cell junctions in animal cells

1. Tight junctions –
   - ✓ Connect the plasma membranes of adjacent cells tightly bound by specific proteins forming continuous seals around the cells.
   - ✓ Prevent leakages of extracellular fluids through intercellular space. e.g. skin epithelium
2. Desmosomes/Anchor junctions –
   - ✓ Mechanically attach the cytoskeletons of adjoining cells by intermediate filaments for strong binding.e.g. muscle tissue
3. Gap junctions /Communicating junctions –
   - ✓ Provide cytoplasmic channels from one cell to an adjacent cell.
   - ✓ Gap junctions are 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 run through plant cell walls.
- They are cytoplasmic living connections between cytoplasm of adjoining cells.
- These are membrane lined channels filled with cytoplasm.

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

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

The cell cycle and the process of cell division

- 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

- Eukaryotic cell cycle may divided into two major phases.
  1. Interphase
  2. 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;
  1. G_1 phase (first gap phase)
  2. S phase (synthetic phase)
  3. G_2 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_2 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.

Signifi cances of mitosis

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



The phases of mitotic cell cycle

Meiosis

- Sexually reproducing organisms undergo different type of cell division called 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 sub-phases: 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 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 homolog 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. **Telophase I**
   - ❖ 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 meiosis I and meiosis II

Meiosis II

1. **Prophase II**
   - ❖ Centrosomes start producing spindle apparatus (spindle fibers, aster centrosome).
   - ❖ Chromatin fibers condense and produce chromosomes with two sister chromatids.

- ❖ Nuclear envelope breaks down into fragments.
- ❖ Nucleolus disappears.
- ❖ During the late prophase II centromere of the chromosomes are moved to the metaphase II plate.

2. Metaphase II

- ❖ All Chromosomes get attached to the microtubules at their centromere and aligned on the metaphase plate.
- ❖ Kinetochores of sister chromatids are attached to microtubules extending from both poles.
- ❖ Due to the crossing over in meiosis I, the two sister chromatids of each chromosome are not genetically identical.
- ❖ Meiosis II usually takes place in the perpendicular direction of Meiosis I.
- ❖ Therefore, metaphase plate of meiosis II is perpendicular to the metaphase plate of meiosis I.

3. Anaphase II

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

4. Telophase II

- ❖ 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.

The phases of meiosis

Significance of meiosis

1. Maintains the constant number of chromosomes through generations in sexually reproducing species.
2. Produce new genetic variations leading to evolution.
3. Genetic variation occurs due to crossing over, recombination and independent assortment.

Tumor, cancer and galls

- Cell division is driven by external and internal factors. They may be chemical or physical factors.
- 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 cell cycle without growth factors.
- Another possibly 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 cannot recognize and destroy it, it may leads to proliferation of cells and formation of 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.

The energy relationships in metabolic processes

- Sum of all biochemical reactions of a 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.
- Therefore it 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.
  - ✓ Energy flows into biological systems from the environment through solar radiation. (Primary energy source is the Sun)
  - ✓ 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.
  - ✓ Captured energy in organic food is transformed into chemical energy in ATP by a process called cellular respiration.
  - ✓ The energy stored in ATP is utilized in various energy requiring processes.

ATP (Adenosine Tri Phosphate)

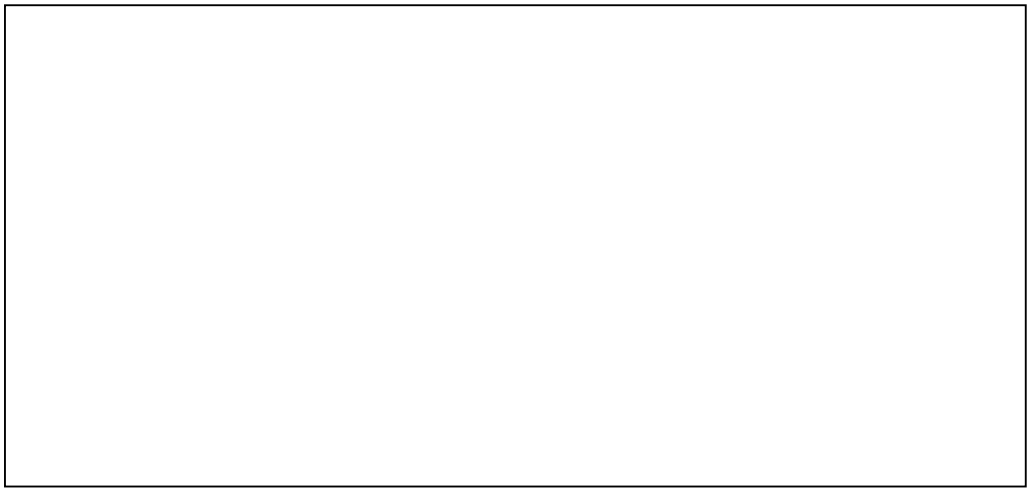
- ATP is a nucleotide, consisting of,
  1. Ribose- sugar
  2. Adenine - nitrogenous base
  3. A chain of three phosphate groups.
- During the hydrolysis of ATP, ADP and Pi are produced.
- As a result, a very high energy is released.
- This is because the reactants (ATP and water) contain more energy 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 each of the two end phosphate groups is -30.5kJmol^{-1}.

Chemical structure of ATP molecule

- 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.
  iii. Oxidative phosphorylation – synthesis of ATP using energy released as a result of oxidation of molecules.
- In living cells energy in ATP is transformed in to various energy forms which are used for different functions.

The role of Enzymes in regulating metabolic reactions

- An enzyme is a macromolecule, produced in living cells which acts as a biological catalyst, to speed up a bio-chemical reaction , without being consumed by the reaction.
- They increase the rate of the reaction by lowering the activation energy of the reaction at a given temperature.
- General characteristics of an enzyme:
  1. Most of the enzymes are globular proteins.
  2. Enzymes are biological catalysts. They lower the activation energy of the reaction they catalyze (increases the rate of 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-protein components/ helpers to catalyse the reaction which are known as cofactors.



The relationship between activation energy and the enzyme

The mechanisms of enzyme action

- The reactant and enzyme acts on is referred to as the substrate.
- The enzymes are larger molecules than their substrates
- 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, which is a pocket or groove like portion on the surface of the enzyme molecule.
- 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 fi t 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 as it does not match with the active site The enzyme is then free to take another substrate molecule into its active site.

Cofactors

- **Non-protein 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.
    1. Organic cofactors are called co-enzymes.
       e.g. derivatives of vitamins e.g. NAD+, FAD and biotin
    2. Inorganic co-factors – e.g. Zn^{2+}, Fe^{2+}, Cu^{2+}

Factors affecting the rate of enzymatic reactions

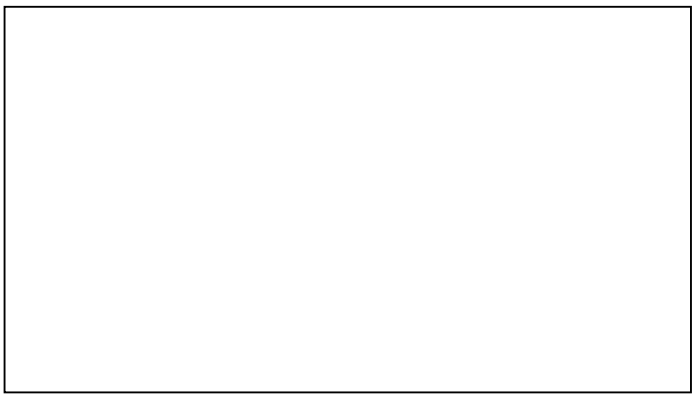
1. Temperature
2. pH
3. Substrate concentration
4. Enzyme concentration
5. Inhibitors

Temperature

- Increase in temperature 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 generate greater chances for the reaction to occur. For a rise of every 10^0C, the rate is doubled.

- This can continue up to a certain point, increasing the rate ,after which there is a rapid decline in enzyme activity and rate of the reaction.

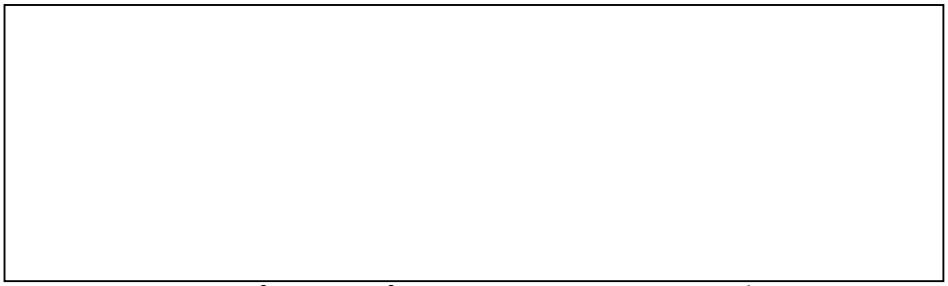
- This point is referred to as optimum temperature.
- This may vary from organism to organism.
  e.g. most of the human enzymes have optimum temperature around the body temperature (35˚C-40˚C). Optimum temperature of bacteria in hot springs is about 70˚C.
- When the temperature increases beyond the optimum 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.



The graph of Rate of reaction (V) vs Temperature(T)

pH

- 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.



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 beyond this concentration.

Enzyme concentration

- Provided that the substrate concentration is maintained at a higher level and other conditions are kept at constant and optimum levels , the rate of reaction is directly proportional to the enzyme concentration.

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;

1. Irreversible inhibitors: toxins, poisons ,Example Sarin; nerve poison and competitive; Penicillin irreversible and competitve

2.Reversible inhibitors- drugs used against microbes Example - Sulfonamides

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.
- The inhibitors block the substrates from entering the active sites
- 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.
  e.g. Protease inhibitor drugs against HIV.

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.

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 non-competitive 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 is 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 these 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, the enzyme stabilizes the shape with functional active sites.
- Whereas 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 to other subunits.
- Through the interaction of subunits even a single activator or inhibitor molecule that bind to one regulatory site will affect the active sites of all sub units.
  e.g. ADP function as an allosteric activator which bind to the enzyme and stimulates the production of ATP by catabolism. If the supply of ATP exceeds the demand catabolism slows down as ATP bind to the same enzyme as an inhibitor.

b.) Cooperativity

- This is another type of allosteric activation.
- Binding of one substrate molecule can stimulate binding or activity at other active sites.
- Thereby increase the catalytic activity.
  e.g. hemoglobin (not an enzyme) is made up of four subunits each with an O_2 binding site. The binding of one molecule of O_2 to one binding site increases the affinity for O_2 of the remaining binding sites of hemoglobin

c.) Feedback inhibition

- In feedback inhibition, a metabolic pathway is stopped by the inhibitory binding of its end product of the process to an enzyme that acts early in the pathway.
- **Advantage**- limits the production of more end products than required and thus preventing wasting chemical resources.
- Feedback inhibition is an essential process regulates the end products produced in metabolism.
  e.g. 1
    - ADP function as an allosteric activator and stimulates the production of ATP during the catabolism.
    - In case ATP supply exceeds the demand, catabolism slows down as ATP molecules function as an allosteric inhibitor.
    - Energy needed for all living processes is obtained directly from ATP.
    - ATP is mainly produced by a process called cellular respiration, in living cells.
    - Eg-2 Production of the amino acid isoleucine from the amino acid threonine is a metabolic pathway involving 5 enzymes. If isoleucine accumulates it binds with an allosteric site of the enzyme 1 making the active site of the enzyme unable to bind with substrate threonine and halting the metabolic pathway.

Experiment to demonstrate enzyme activity and how temperature affects the rate of enzyme activity using starch-amylase reaction

1. Measure definite volumes of 5 ml of Amylase and 10 ml of 1% starch solution into separate test tubes.
2. Allow the solutions to attain the same temperature in water bath.
3. Mix up the solutions and start the stop watch while keeping them in the same water bath at a required temperature.
4. Test a drop of reaction mixture with a drop of iodine solution on a white porcelain tile at 2 minutes intervals.
5. Note the time taken-t minutes, for the initial blue-black color/ blue-violet color to disappear and to remain the same color of Iodine/ yellowish brown
6. Repeat the above procedure at different temperatures as 5^0C, room temperature, 40^0C, 60^0C.
7. Plot a graph of rate of reaction vs temperature.
8. Rate of reaction can be taken as 1/t.

Photosynthesis as an energy fixing mechanism

Photosynthesis

- Photosynthesis is a metabolic process by which light energy trapped by photosynthetic pigments is converted to chemical energy stored as chemical bonds of organic molecules such as carbohydrates, fats, oils, and proteins using inorganic raw materials like CO_2 and water.
- All life on Earth depends on photosynthesis either directly or indirectly.
- Photosynthesis also occurs in algae and certain prokaryotes.
- Global importance of photosynthesis;
  1. All life on earth depends on photosynthesis, directly or indirectly
  2. Fulfill both carbon and energy requirements of organisms
  3. Provide O_2 for respiration of aerobic organisms
  4. Maintain O_2 and CO_2 balance in the atmosphere
  5. Production of fossil fuel
  6. 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 of chloroplasts
- 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 ground substance surrounding thylakoids 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.

Photosynthetic pigments.

- Photosynthetic pigments are substances, which absorb certain wavelengths of visible light very strongly to drive the process of photosynthesis.
- They reflect the other wavelengths of visible light so that they display the color of the reflected light.
- The key light capturing pigment that participates directly in the light reactions is chlorophyll a in plants.
- Chlorophyll b and carotenoids are considered as accessory pigments in plants.
- Chlorophyll a is present in all photosynthetic organisms except photosynthetic bacteria.

- According to the action spectrum, chlorophyll a is more effective for blue and red light.
- Chlorophyll b and carotenoids (carotenes and xanthophylls) are effective in absorption of specific range wavelengths of corresponding to different colours. Chlorophylls strongly absorb violet, blue and red light therefore the reflected green gives the color to the pigment.
- carotenoids absorb light in blue and violet range and reflect yellow,orange, red ranges giving the same colors, by those pigments.

Function of chlorophyll a/ primary pigment.

- It is directly involved in the light reaction by providing photo-excited electrons, which have the potential energy to synthesize ATP and NADPH.

Functions of Accessory Pigments.

- 1-Broaden the range of wavelength of visible light to harvest energy and transfer to Chl a in the reaction center complex.
- 2-Photo-protection of chlorophyll a.
- Protect the plant from harmful and reactive free radicals formed from the UV and other radiation.

- **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.

Absorption spectrum

Action spectrum

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

Action spectrum

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.

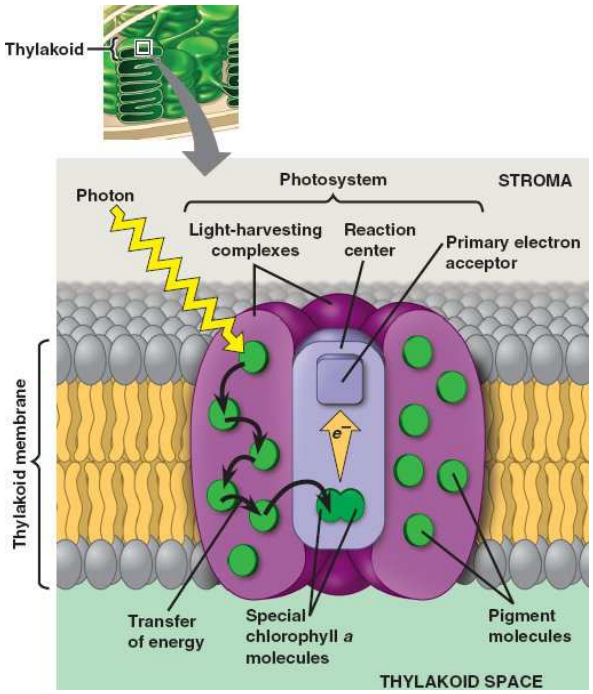
$$\text{Chlorophyll} \longrightarrow \text{Chlorophyll}^{+} + e^{-} \text{ (electron)}$$

- 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 surrounded by several light harvesting complexes.
- Reaction center complex is an organized association of proteins holding a special pair of 'chlorophyll a' molecules and a primary electron acceptor.
- Light harvesting complexes are made of large number of variety photosynthetic pigment molecules bound to proteins. The photosynthetic pigment molecules found in these light harvesting complexes are chlorophyll a, b and carotenoids.
- The 2 photosystems differ mainly by the reaction center chlorophyll a molecules, the PS1 contains 'chlorophyll a' known as P_{700} and the PSII contains P_{680}

  'chlorophyll a' because they are best at absorbing light at those wave lengths 700nm and 680nm respectively. (The photosystems are named in order of their discovery but not in the order of participation, because PS11 function first in the light dependent reaction.)



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 in blue-violet and red range on the pigments of light harvesting complex and transferring the excitation energy to special chlorophyll a-p680 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. Chlorophyll a → Chloropyll a^+ + e
- Electrons released as a result of hydrolysis neutralize p680 of excited photosystem II (P680).
- Striking of photons of light of blue-violet and red range on the pigments of light harvesting complexes of photo system 1 and transferring the excitation energy to P700 chlorophyll a at the reaction center results in the excitation of electrons from photosystem I (P700) to the higher energy state.
- Photo excited electrons will be accepted by a primary electron acceptor of PSI.and making P700 oxidized. $P700^+$
- Excited electrons at primary electron acceptor of PS II will pass through an electron transport chain to PS I and neutralize the excited P700 of PS I.

- The energy released due to the passage of photoexcited electrons(exergonic fall of electrons) from higher energy state(PSII) to lower energy(PSI) is coupled to the synthesis of ATP, using ADP and Pi is known as photophosphorylation.
- Excited electrons at primary electron acceptor of PSI will pass through second 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 are released.

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_2.
- 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;
    1. Carboxylation (Carbon fixation)
    2. Reduction
    3. Regeneration of carbondioxide acceptor

1. 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.

2. 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).

3. 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, 2-phosphoglycolate 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.
- 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_2 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_2 released from the light reactions begins to increase and this leads to further reduction of (CO_2) to (O_2) 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_2 around Rubisco provided by C_4 photosynthetic pathway.
- The establishment of C_4 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 C_4 plants.
- In most C_4 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 C_3 plants the bundle sheath cells of C_4 plants have expanded physiological functions.
- This is reflected by the enlargement and higher organelle content of these cells in C_4 species.
- For the efficient function of the C_4 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 C_3 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_4 pathway of photosynthesis

- In the mesophyll cells of C_4 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 C_4 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 C_4 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.

$$CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} HCO_3^- + H^+$$

$$PEP + HCO_3^- \quad \text{PEP carboxylase} \quad \text{Oxaloacetate (4C)}$$

- This PEP carboxylase enzyme is much more efficient than the enzyme of RUBP carboxylase for two reasons.
    1. It reacts with bicarbonate (HCO_3^-) rather than with CO_2 The advantage of this is that there is a 50-fold higher concentration of HCO_3^- than CO_2 in solution in the cytosol.
    2. It has no affinity for O_2

Significance of the C4 pathway

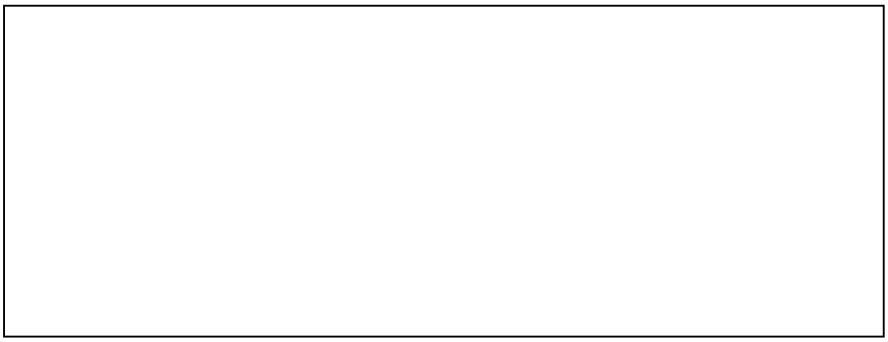
1. Helps plants to improve the efficiency of CO_2 fixation at lower CO_2 concentrations by preventing the gateways for photorespiration by spatially separating Rubisco.
2. In hot-dry climate, it is essential for the stomata to close to prevent water loss through transpiration. This reduces CO_2 intake of particular plants. Therefore, plants in tropical zones or hot climate may suffer from CO_2 deficiency. At lower CO_2 concentrations, C_4 mechanism increases the efficiency of photosynthesis by concentrating CO_2 in the bundle sheath cells.
3. C_4 plants exhibit better water-use efficiency than C_3 plants because of the CO_2 concentration mechanism they can acquire enough CO_2 even when keeping their stomata more closed. Thus water loss by transpiration is reduced.
4. Since Rubisco can operate under high CO_2 concentrations in the bundle sheath cells, it woks more efficiently than in C_3 plants, consequently C_4 plants need less of this enzyme, this leads to a better nitrogen-use efficiency of C_4 compared to C_3 plants.

Characteristics	C_3 plants	C_4 plants
Representative species	Wheat, rice barley	Maize, sugarcane, grasses
Temperature optimum for photosynthesis (^0C)	15-25	50% greater at 35 ˚C
CO_2 fixation	Occurs once	Occurs twice, first in mesophyll cells, second in bundle sheath cells
CO_2 acceptor	5C, RuBP	3C, PEP mesophyll cells 5C RuBP in bundle sheath cells
CO_2 fixing enzyme	Rubisco	PEP carboxylase in mesophyll cells which is very efficient Rubisco in bundle sheath cells, working efficiently under high CO_2 concentration
First product of CO_2 fixation	C3 acid, 3- phosphoglycerate (3-PGA)	4C acid, oxaloacetate (OAA)
Leaf anatomy	Bundle sheath cells, if present, are not green (non photosynthetic), photosynthesis occurs in Mesophyll cells	Kranz anatomy with photosynthesis occurring in both mesophyll cells and bundle sheath cells
Productivity	Yield is usually lower	Yield is usually high

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_2 concentration, temperature, water, pollutants and inhibitors
- 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.
- 

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.

Cellular respiration as a process of obtaining energy

- 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 synthesis of ATP from the respiratory substrates such as glucose in the presence of molecular oxygen (O_2), and using oxygen as a reactant for complete oxidation of respiratory substrate is 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.

$$C_6H_{12}O_6 + 6O_2(g) \longrightarrow 6CO_2(g) + 6H_2O + \text{Energy (ATP+ heat)}$$

- This process consists of three main steps. They are;
  a) Glycolysis
  b) Pyruvate oxidation and citric acid cycle (Krebs cycle)
  c) oxidative 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, in order to make glucose chemically reactive.
- Four hydrogen ions and electrons released from glucose breakdown, reduce two NAD^+ and produce two NADH.
- At the end of glycolysis there will be four ATP molecules produced by substrate level phosphorylation.
- Since two ATP molecules were used up for the initiative step, the net yield will be two ATP molecules.
- Only when O_2 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, the 2 Pyruvate undergoes a decarboxylation and oxidation reactions and is converted to 2x acetyl groups by releasing two CO_2 molecules and 2x NADH molecules.

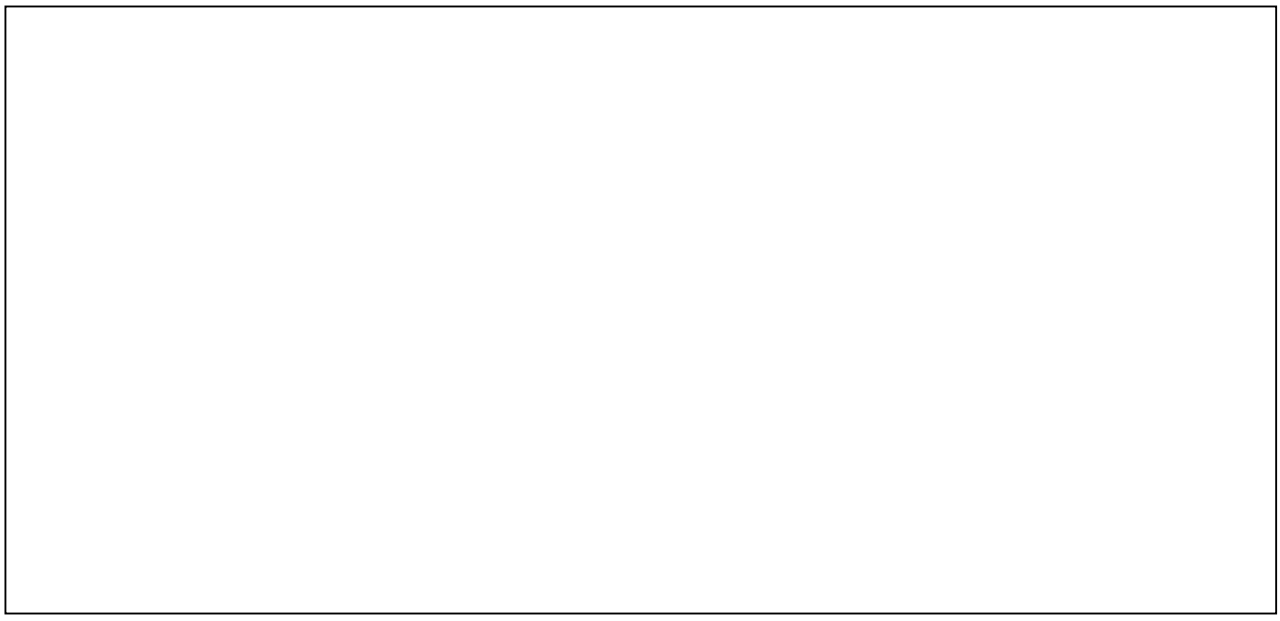
- Then this acetyl group combines with co-enzyme A to produce Acetyl co-A.
- In this reaction two NAD^+ is reduced to two NADH molecules, because NAD+ functions as oxidizing agent. Therefore this step can be represented as follows.

$$2\ C_3H_4O_3 + 2\ \text{Co-A} + 2\ NAD^+ \longrightarrow 2\ \text{Acetyl Co-A} + 2\ CO_2 + 2\ NADH$$

- 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 Krebs 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 4C compound oxalo acetate combines with 2C 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_2 molecules by decarboxylation reactions.
- One ATP molecule is produced by substrate level phosphorylation.
- One $FADH_2$ and three NADH will be generated as a result of electron yeilding oxidation 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.



Krebs cycle

Electron transport chain

- This step takes place across the inner membrane (cristae) of mitochondria.
- The folding of inner membrane to make cristae increases surface area for oxidative phosphorylation.

- NADH and $FADH_2$, products in the early stages of aerobic respiration are oxidized by transferring electrons, through the electron transport chain and finally to molecular oxygen (O_2).
- 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_2)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 $FADH_2$ 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 $FADH_2$ is oxidized 1.5 molecules of ATP in average are produced due to oxidative phosphorylation.
- Total number of 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 glycolysis 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 $\longrightarrow$ 2ATP
  From 2NADH $\longrightarrow$ 5ATP (oxidative phosphorylation)
  In pyruvate oxidation;
  From 2NADH $\longrightarrow$ 5ATP (oxidative phosphorylation)
  In Citric acid cycle;
  As ATP $\longrightarrow$ ATP (substrate level phosphorylation)
  From 6NADH $\longrightarrow$ 15ATP (oxidative phosphorylation)
  From 2 $FADH_2$ $\longrightarrow$ 3ATP

❖ Total Number of 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 is utilized to fulfill energy requirements.
- However, NADH produced during glycolysis cannot be utilized.
- Therefore, since NAD^+ is limited it is essential for the cell to recycle NADH to enhance the availability of NAD^+ 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 1st step pyruvate is converted in to Acetyldehyde, releasing a molecule of CO_2.
- 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 acetyldehyde (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 1st 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_2.
- Therefore final H acceptor is also organic compound/ pyruvate
- Certain fungi and bacteria carryout lactic acid fermentation but the most common organisms are lactic acid bacteria involved in formation of yoghurt and curd.

Make comparisons of aerobic respiration and fermentation in cells

Make a comparison between lactic acid fermentation and ethanol fermentation.

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 CO_2/ V O_2 .
- RQ of respiration of carbohydrates, fats and proteins are 1.0, 0.7 and 0.8 respectively.

Use of proteins, carbohydrates, fats in respiration.

How Proteins and Lipids are used in Aerobic Respiration.

- When fats are used as respiratory substrate's it is first hydrolyzed to fatty acid and glycerol.
- Glycerol is converted to glyceraldehyde- 3 phosphates in the cytoplasm and enter glycolysis pathway.
- The fatty acids are oxidized to 2 carbon parts forming acetyl co-A and enter TCA cycle.
- This process is called β-oxidation and takes place in the matrix of mitochondria.
- The number of acetyl co-A produced from a fatty acid is dependent on the length of the hydrocarbon chain.
- The acetyl CO-A molecules produced as a result enter Krebs cycle resulting generation of NADH and $FADH_2$.
- Due to the high no.of Krebs cycle and high amount of NADH and $FADH_2$ produced the amount of oxygen used for oxidation and the no.of ATP produced is extremely high with regards to lipids.
- When proteins are used as respiratory substrate they are 1sthyrolized to amino acids, the amino acids are converted to ammonia and carboxylic acid through deamination.
- The resulting carboxylic acids enter the respiratory pathway/Krebs cycle in particular at various points, depending on their chemical nature.

Bio synthesis/ metabolic pathway.

- In addition to calories food must also provide the carbon skeletons that cells require to make their own molecules.

Ex; the end products of digestion of proteins, which are amino acids, can be used to synthesis the cells protein.

- However compounds formed as an intermediate of glycolysis and citric acid cycle can be diverted in anabolic pathway as precursors of important organic molecules.

Ex;humans can make about 10 amino acids in protein by modifying the intermediate products of citric acid cycle.

- Such amino acids are called non-essential amino acids.

- The intermediate of Krebs cycle and glycolysis can be used to synthesis several other organic molecules such as,

- Glucose from pyruvate.
- Fatty acids from acetyl CO-A

- Therefore Krebs cycle can be considered as the very important metabolic center because it can furnish with several compounds that can be used to synthesis several important molecules such as fatty acids and amino acids.

Determination of Rate of Respiration of Germinating Seeds.

- Two parameters can be used,
    a. The volume of oxygen used by unit mass of tissue per unit time.
    b. The volume of CO_2 released by unit mass of tissue by unit time.
- Germinating seeds provide a suitable tissue to determine the rate of respiration because,
    a. It's highly metabolically active and the rate of respiration is high.
    b. It is a non-photosynthetic tissue therefore the amount of O_2 used and CO_2 evolved can only be related to the respiratory process.

1st Experiment

1. Introduce a known mass of germinating green grams seeds around 25g(weight) into the flask of the respirometer.
   Purpose of weighing the sample is to quantify the measurement.

2. Insert an ignition tube containing KOH into the flask and close the mouth tightly with a stopper and apply vasline, allow the mouth to make it airtight.
3. A U- shape glass tube containing coloured water is also fixed to the stopper.

4. Make the water levels in the right and the left arms equal and close the tap.
5. Mark the water level in the left arm.
6. Temperature must be maintained constant by immersing the flask in the water bath.
7. Let the seeds respire for a known time period (t)(about 2 hours)
8. Mark the water level in the left arm of the U- tube and determine the length of the water column risen in the left arm (h cm)
9. This is the volume change due to absorption of oxygen by the germinating seeds over time t, in respirometer, therefore the volume of oxygen absorbed in time 't' by a seed sample weighing "w grams=$h_1$cm". Therefore the rate of respiration of same sample is equal to h_1/t therefore the rate of respiration of unit mass of tissue is "h_1/wxt ($cmgh^{-1}$)

2nd Experiment

Volume of CO_2 released per unit mass per unit time can be taken as a parameter.

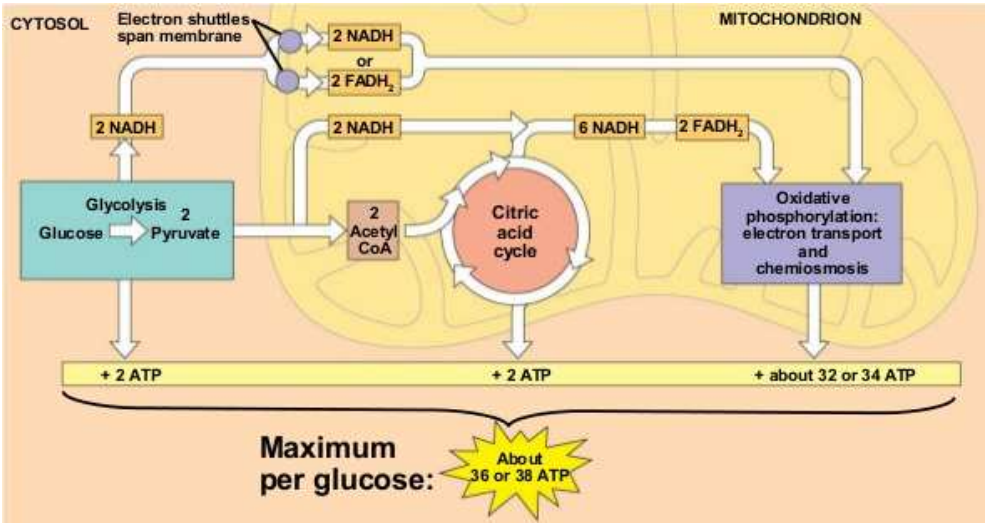
1. Do the same experiment as explained above.
2. Remove the KOH tube and close the mouth tightly.
3. Make the water levels equal in the 2 arms of U- tube and close the tap, mark the water level in the left arm and the let the seeds respire for the same time "t" and note the water level in the left arm.
4. If the water level rises by a distance of "h_2 cm" the volume of CO_2 is equal to h_1-h_2(volume change with the KOH tube – volume change without KOH)
5. If the water level remains unchanged volume of CO_2 released is equal to volume of O_2 absorbed.
6. If the water level goes down by "h_2 cm" in the left arm the volume CO_2released = h_1+h_2 therefore there are 3 possible volumes for CO_2 released such as, h_1-h_2/h_1/ h_1+h_2, therefore rate of respiration also can be expressed as follows,

Respiratory Quotient.

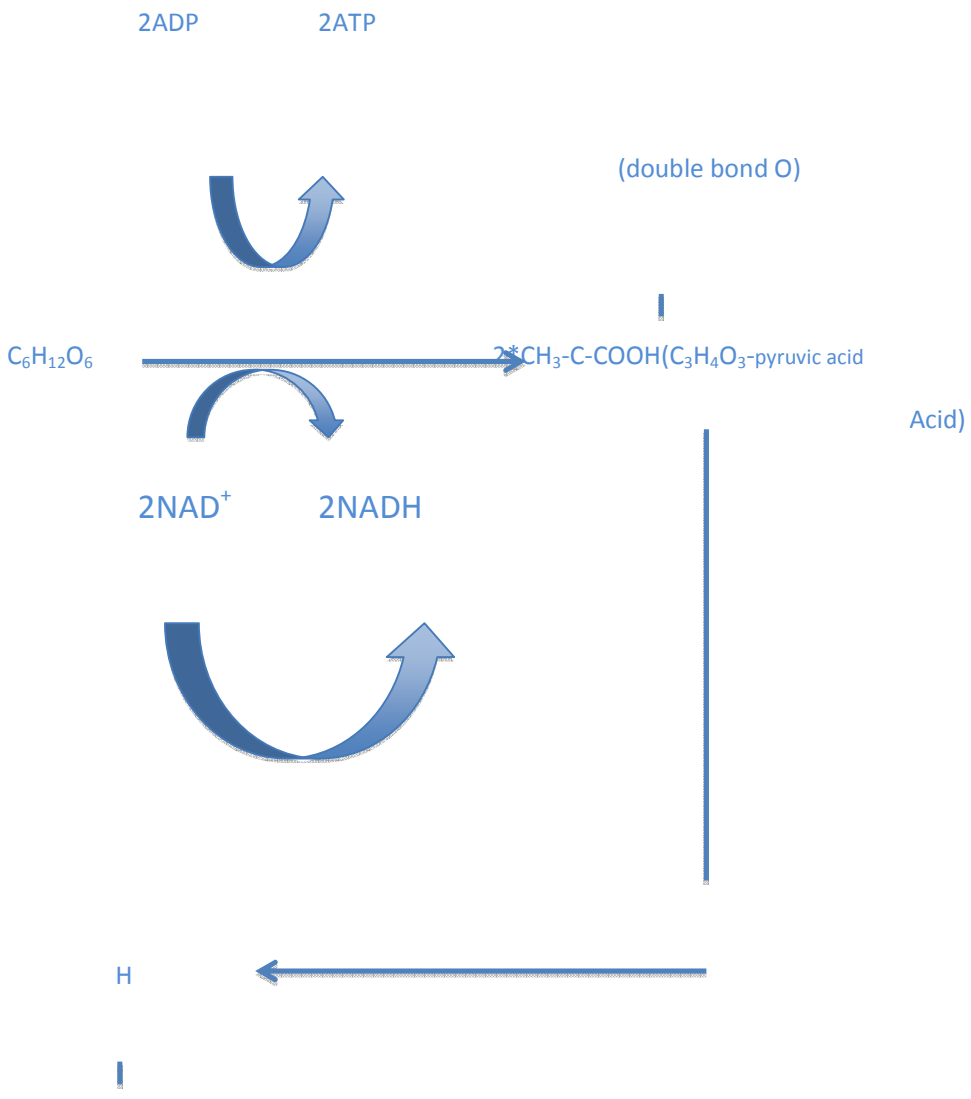
- It is the ratio of volume of CO_2 evolved to the volume of O_2 consumed for a given time by a respiratory substrate.
- RQ=vCO_2/vO_2
- RQ value of a respiratory substrate like glucose,

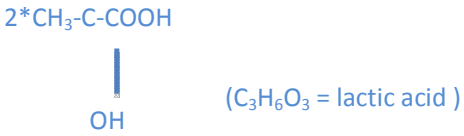
Substrate	RQ value
Carbohydrate	1
Carbohydrates by aerobic respiration and followed by aerobic respiration in plants	>1
Proteins	0.8
Lipids	0.7

Picture of Mitochondria undergoing Aerobic Respiration.



Lactic Acid Fermentation.




2*CH3-C-COOH
OH
(C3H6O3 = lactic acid)