BT 8305 CELL BIOLOGY

UNIT – I
CELL STRUCTURE AND FUNCTION OF THE ORGANELLES

1. Define Prokaryotic cells.

- ✶ Pro → Before & Karyon → Nucleus
- ✶ Those cells which lacks in nuclear envelope are said to be prokaryotic cells.
- ✶ The prokaryotic chromosome occupies a space in the cell called a nucleoid, and is in direct contact with the rest of the protoplasm.
- ✶ Examples: Bacteria and Blue green algae.
- ✶ From an evolutionary standpoint, prokaryotes are considered to be ancestors and eukaryotes.

2. Define Eukaryotic cells.

- ✶ Those cells which have true nucleus are said to be Eukaryotic cells.
- ✶ The word Eukaryote stands for Eu→ True and Karyon → Nucleus.
- ✶ These cells have elaborate nuclear envelope, through which the nucleo cytoplasmic interchanges takes place.
- ✶ Examples: Protozoa, other Algae & Fungi.

3. Differences between Prokaryotes & Eukaryotic Cells.

Prokaryotic cells	Eukaryotic cells
1. Nuclear envelope is absent	Nuclear envelope is present.
2. DNA is Nacked.	It is combined with proteins.
3. Chromosomes are single.	It is multiple.
4. Nucleolus is absent.	Nucleolus is present.

4. How do Eukaryotes differ basically from Prokaryotes?

Eukaryotes show some basic differences when compared to prokaryotes. These are

i) Presence of a mobile cell membrane of the rigid cell wall of prokaryotes.
ii) Presence of a membrane – bound nucleus.
iii) Presence of a photosynthetic organelle.
iv) Presence of Mitochondria &
v) A membrane – bound compartmentalized cytoplasmic interior.

5. Give some membrane – bound organelles found in Eukaryotic cells.

Nucleus, Mitochondrion, Chloroplast, Endoplasmic reticulum, Golgi apparatus, vacuoles, Lysosomes and microbodies are some of the membrane bound organelles found in eukaryotic cells.

6. How do Eukaryotic cell wall differ from Prokaryotic cell wall?

- ■ The cell wall of Eukaryotes is found to be thick, polysaccharide containing structure immediately surrounding the plasma membrane whereas the bacterial cell is enclosed within a wall that differs chemically from the cell wall of plants in that it contains protein and lipid as well as polysaccharide.
    - In Prokaryotes the cell wall is surrounded by an additional structure called a capsule. Whereas it is not so in Eukaryotes.

- In Prokaryotes peptidoglycan is the basis of the histochemical classification of bacteria, being high in the so-called "gram-positive" bacteria (eg. Bacillus subutiis) and low in the "gram negative" bacteria (eg. E. Coli & Solmonella) and it is not so in Eukaryotic cell wall.

7. Compare meiosis and Mitosis.

- Chromosome behavior
  1. Mitosis : Homologous chromosomes independent
  2. Meiosis : Homologous chromosomes pair forming bivalents until anaphase I.
- Chromosome number – reduction in meiosis
  1. Mitosis – identical daughter cells
  2. Meiosis – daughter cells haploid
- Genetic identity of progeny:
  1. Mitosis : identical daughter cells
  2. Meiosis : daughter cells have new assortment of parental chromosomes.
  3. Meiosis : Chromatids not identical, crossing over.

8. When do Meiotic errors occur?

- Nondisjunction – homologues don't separate in meiosis I
  1. Results in aneuploidy
  2. Usually embryo lethal
  3. Trisomy 21, exception leading to Downs syndrome
  4. Sex chromosomes
     1. Turner syndrome : monosomy X
     2. Klinefelter syndrome : XXY
- Translocation and deletion: transfer of a piece of one chromosome to another or loss of fragment of a chromosome.

9. Mitosis, Meiosis and Ploidy.

- Mitosis can proceed independent of ploidy of cell, homologous chromosomes behave independently.
- Meiosis can only proceed if the nucleus contains an even number of chromosomes (diploid, tetraploid).
- Trisomy 21 does not prevent meiosis.

10. Explain Asexual (vegetative) reproduction.

- A form of duplication using only mitosis.
- Example, a new plant grows out of the root or a shoot from an existing plant.
- Produces only genetically identical offspring since all divisions are by mitosis.
  1. Offspring called clones meaning that each is an exact copy of the original organism.
  2. This method of reproduction is rapid and effective allowing the spread of an organism.
  3. Since the offspring are identical, there is no mechanism for introducing diversity.

11. Explain Sexual reproduction.

- Formation of new individual by a combination of two haploid sex cells (gametes).
- Fertilization – combination of genetic information from two separate cells that have one half the original genetic information.
- Gametes for fertilization usually come from separate parents.

1. Female – produces an egg.
2. Male produces sperm

- Both gametes are haploid, with a single set of chromosomes
- The new individual is called a zygote, with two sets of chromosomes (diploid)
- Meiosis is a process to convert a diploid cell to a haploid gamete, and cause a change in the genetic information to increase diversity in the offspring.

12. Explain the chromosome characteristics in a diploid cell.

- Diploid set for humans; 2n = 46
- Autosomes; homologous chromosomes, one from each parent (humans = 2 sets of 2)
- Sex chromosomes (humans have 1 set of 2)
  1. Female-sex chromosomes are homologous (XX)
  2. Male-sex chromosomes are nonhomologous (XY)

13. Define Karyotype & ploidy.

Karyotype

- A pictorial display of metaphase chromosomes from a mitotic cell
- Homologous chromosomes – pairs.

Ploidy: Number of sets of chromosomes in a cell

- Haploid (n) – one set chromosomes
- Diploid (2n) – two sets chromosomes
- Most plant and animal adults are diploid (2n)
- Eggs and sperm are haploid (n)

14. Where are microtubules formed?

- In the cell itself, microtubules are formed in an area near the nucleus called the "aster", or the Microtubule Organizing Center (MTOC).
- Microtubules are polar with a plus end (fast growing) and a minus end (slow growing).
- Usually the minus end is the anchor point
- The plus end points peripherally, away from the cell center.

15. Dynamic instability. What is it and how does it relate to cell function?

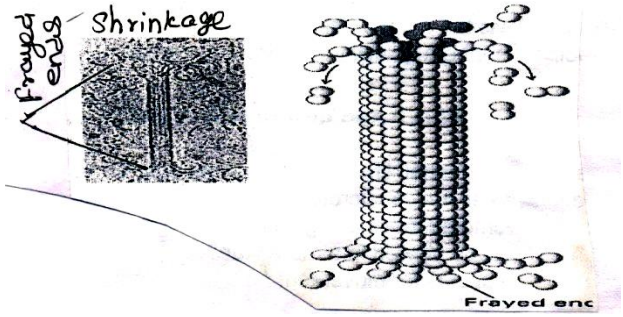
Tubulin half life is nearly a day, but half life of a microtubule may be 10 min

- Formed "on the fly" as cell needs microtubules.
- Microtubule growth promoted in a moving cell.
- Microtubule growth may be slowed in a more stable cell.
- Still need microtubules for movement of organelles inside cell.

16. GTP cap: What is it and why is it important?

- If a GTP cap is placed on the growing end (plus) of a microtubule, the tubulin molecules are added faster than the GTP can be hydrolyzed.
- Microtubule grows, but with no hydrolysis of GTP, it can't depolymerize.
- Once the GTP is hydrolyzed, the microtubule shrinks (depolymerization starts)
- Cell can cap a microtubule by attaching a structure to its plus end.

17. Is GTP hydrolysis needed for microtubule formation.



- Tests have shown that microtubules will form normally with nonhydrolyzable GTP analog molecules.

- However, they will not be able to depolymerize.

18. What does acetylated or detyrosinated tubulin signify?

- Important marker sites for polymerized, stabilized tubulin.
- Disappear when microtubules depolymerize.

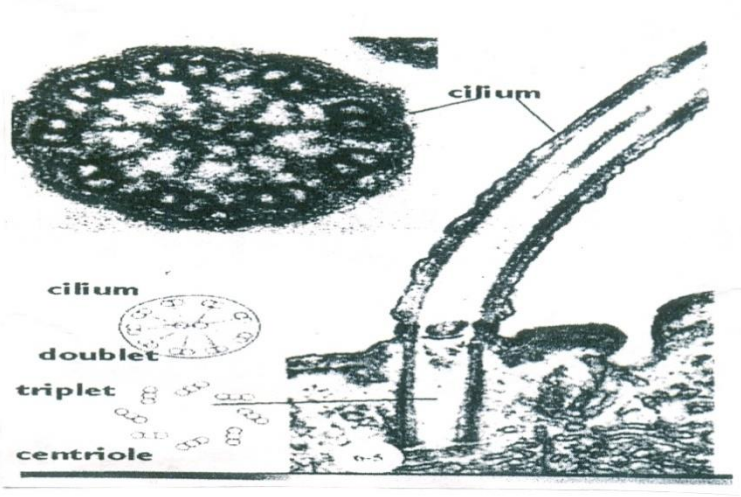
19. How would MAPs help with the functional differentiation of a cell?

- Movement of cellular processes (cell shape)
- Movement of chromosomes
- Movement of various types of vesicles and organelles to and from periphery.
- Directional movement dictated by type of MA.

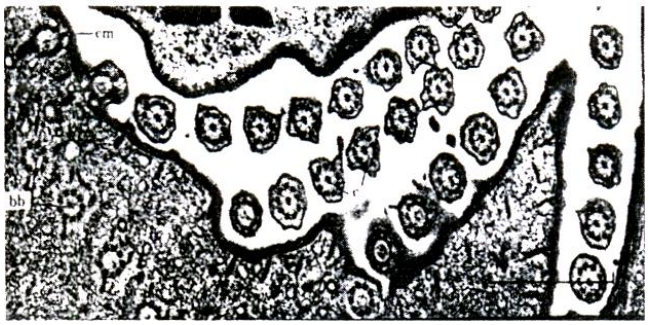
20. How do centrioles replicate?

- Autonomous, from proteins in cytoplasm.
- Form microtubule triplets
- Grow out new centrioles at right angles

21. How are cilia formed? How do they differ from flagella?



22. What is the importance of the centriole in cilium formation?



Centriole=basal body (bb) which gives rise to cilium

23. How would MAPs help with the functional differentiation of a cell?

- Movement of cellular processes (cell shape)
- Movement of chromosomes
- Movement of various types of vesicles and organelles to and from periphery
- Directional movement dictated by type of MAP.

24. Microtubules: What is the structural submit?

- Linear polymers of tubulin (globular protein)
- Organized in a linear row called a "protofilament"
- What is function?
- Tracks for organelles
- Move vesicles, granules, oganelles
- Also serve a cytoskeletal role

PART - B

1. Write an essay an structure and organization & Eukaryotic cells with suitable diagram.

Eukaryotic cells:

The composite Animal cell:

Animal cells vary considerably in size, shape, organelle composition , and physiological moles. Consequently, there is no "typical" cell that can serve as an example of all animal cells. There are, however, a number of cell structures common to the majority of animal cells that are similar or identical in organization. These structures are depicted in the composite animal cell diagrammed.

The Plasma membrane:

- ✶ The contents of the cell (cytoplasm & cytoplasmic organelles) are separated from the external surrounding by a limiting membrane, the plasma membrane (also called **cell membrane (or) plasmalemma),** which is composed of protein, lipid and carbohydrate.
- ✶ This structure regulates the passage of materials between the cell and its surroundings.
- ✶ In some tissue cells, portions of the plasma membrane are modified to form a large number & finger like projections called.

Microvilli:

- The microvilli greatly increase the surface area of the cell and provide for the increased passage of materials across the plasma membrane.

The cytoskeleton and Microtrabecular lattice:

- Radiating through the cytosol of many cells are components of the cytoskeleton & microtrabecular lattice.
- The cytoskeleton consist & arrays of thin filaments, intermediate filaments, thick filaments & microtubules.
- These structures give shape & form to the cell & are also involved in cell movement.
- The cytoskeletal elements appear to be interconnected by a network & fines thread like structures comprising what is called the microtrabecular lattice.
- This lattice also interconnects a number membranous organelles & ribosomes.

The Endoplasmic Reticulum and Ribosomes:

- Within the cytoplasm of most animal cells is an extensive network of branching and anastomosing membrane limited channels (or) cisternae collectively called the endoplasmic reticulum (ER).
- The membrane of the endoplasmic reticulum divides the cytoplasm into two phases.

→ The Lumenal (or) intracisternal phase
→ The hyalsplasmic phase (or) cytersol.

- In the cytosol are large numbers of small particles called ribosomes.

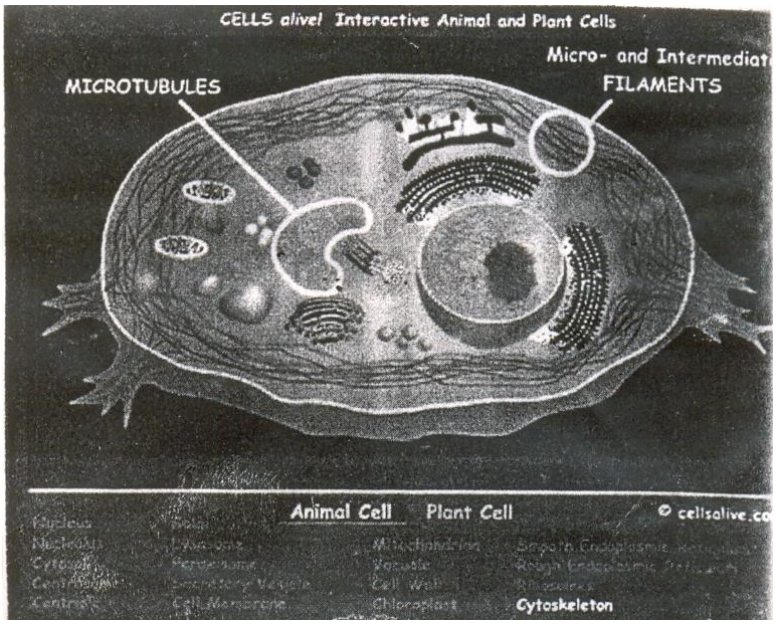
Mitochondria:

Within the cytoplasm are numerous vesicular organelles called Mitochandria.

- Each Mitochondrion is bordered by two membranes. The outer membrane is smooth, but the inner membrane displays numerous infoldings called cristae that greatly increase the surface area of the inner membrane.
- The space between neighboring cristae is called the mitochondrial matrix and often contains inclusions.
- Mitochondria are engaged in numerous metabolic functions in the cell, including the energy-producing phases of carbodydrates and fat metabolism (called) respiration), ATP systhesis and porphyrin synthesis.

The Golgi Appartus:

It is also called as Golgi complex (or) Golgi body consists of a set 2 smooth, flattened cisternae that are usually stacked together is parallel rows; in this stage, the organelle is sometimes are referred to as a dictyosome.



Lysosomes:

- ✶ Many cells contain vesicular structures that are generally smaller than Mitochondria and are called Lysosomes.
- ✶ Lysosomes are bounded at their surface by a single membrane and contain quantities of various hydrolytic enzymes capable of digesting protein, nucleic acid, polysaccharide and other materials.
- ✶ Among their various role, lysosomes take part in the intracellular digestion of particles that are ingested by the cell during endocytosis & the intracellular scarenging of worn and poorly functioning organelles.

Peroxisomes and Glyoxysomes:

- ✶ Many cells contain small number of peroxisomes and /or glyoxysomes.
- ✶ These small organelles, which are bounded by a single membrane, contain a number of enzymes whose functions are related to the metabolism of hydrogen peroxide and glyoxylic acid

The Nucleus:

- ✶ The Nucleus is a relatively large structure frequently but not always located near the centre of the cell.
- ✶ The contents of the nucleus are separated from the cytosol by two membranes that together form the nuclear envelope.
- ✶ At various position, the outer membrane the envelope fuses with the inner membrane to form pores.
- ✶ The space between the inner and outer membranes of the nuclear envelope are said to be perinuclear space.
- ✶ The Nucleus often reveals one (or) more dense, granular structures called nucleoli.

Flagella and cilia:

Many free-living cells (such as protozoa & other microorganisms) posses locomotor organelles that project from the cell surface, these are either flagella (or) cilia.

2. Describe the prokaryotic cells & its organelles with suitable diagram.

- The bacteria are structurally distinct from eukaryotic microorganisms such as protozoa and contain a number of unique cellular organelles.
- The typical bacterium is about the size of a mitochondrion of an animal (or) plant cell and in view of this small size, it is to be expected that the organelles of bacteria would be correspondingly smaller.

The Bacterial cell wall & capsule:

- The bacterial cell is enclosed within a wall that differs chemically from the cell wall & plants in that it contains protein and lipid as well as polysaccharide.
- The presence of a particular peptidoglycan is the basis of histochemical composition of bacteria, being high in the so-called "gram-positive" bacteria and low in the "gram-negative" bacteria.
- In some bacteria, the cell wall is surrounded by an additional structure called a capsule.
- The cell wall and capsule confer shape and form to the bacterium and also act as a physical barrier between the cell and its environment.
- This is important because osmotic forces usually result in a positive hydrostatic pressure inside the bacterium in the absence of a cell wall and capsule, mechanically fragile bacteria would readily rupture.

Plasma Membrane Intrusions:

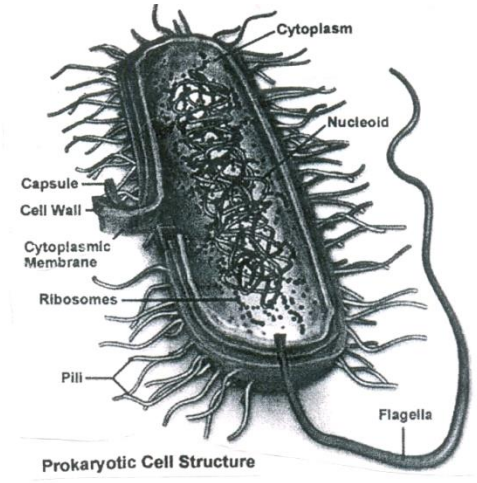
- Infoldings of the plasma membrane of gram positive bacteria give rise to structures call mesosomes (or chondrioids).
- Mesosomes are believed by many investigators to play a role in the division of the cell.
- Intrusions of the plasma membrane also form the photosynthetic organelles (chromatophores) of the photosynthetic bacteria.

Cytoplasmic Lamellae:

- In some bacteria, there is a lamellar arrangement of membranes within the cytoplasm.
- Bacteria contain large numbers of ribosomes, but most of these organelles are free in the bacterial cytosol; some ribosomes may be attached to the interior surface of the plasma membrane.
- Cytoplasmic lamellae are particularly abundant in the autotrophic bacteria, which support their growth through photosynthesis (or) similar processes.

Nucleoids:

- In bacteria the nucelear material is not separated from the cytosol by membranes as it is in eukaryotic cells.
- However, the nuclear material is usually concentrated in a specific region of the cell, referred to as a nucleoid.
- During bacterial cell division, nucleoidal DNA becomes anchored to the plasma membrane and is distributed to the daughter cells without formation of observable chromosomes.
- Nucleoli are not present in the nucleoid



Bacterial flagella:

- ✶ Many bacteria contain one (or) more flagella employed for cellular locomotion.
- ✶ These organelles arise from the cytoplasm and penetrate the plasma membrane and cell wall.
- ✶ Bacterial flagella are smaller than the flagella of animal and plant cells and are simpler in organization, containing a single filament (or) globular proteins (called Falgellin) surround by a sheath.
- ✶ Some bacteria are mutliplagellated
- ✶ Some bacteria contain non flagellar appendages called fimbrial that arise from the cytosol and project a short distance above the cell surface.
- ✶ These structures are believed to play a role in the attachment of the bacterium to a surface (or) to other bacteria.

3. Write a note on the principles of membrane organization.

Membranes have both integral and pheripheral proteins:

The idea of a lipid bilayer was first proposed by E. Gortes F. Grendel, who showed in 1925 that the phospholipids content of the erythrocyte plasma membrane is approximately the amount needed to enclose the cell with a bilayer.

- This model was suggested by H. Davson and J.F.Danielli in 1935 and was elaborated by them and others over the course of the next 30 years.
- It was suggested that the protein coats coiled take the form of B-pleated sheets.
- The Davson – Danielli model was attracts because it seemed to account for the trilaminar appearance, impermeability to ions and durability of membranes.
- The model placed all the proteins on the surface of the membrane, where they would be exposed to water and presumably would interact electrostatically with the polar head – groups of the phospholipids.
- Integral membrane proteins are much more difficult to extract.

1) Structure

→ In the fluid mosaic model, there are two classes of membrane proteins:

- Extrinsic proteins and
- Intrinsic proteins

Extrinsic proteins:

- Extrinisic (pheripheral) proteins attach to the outer and inner membrane surfaces.
- These proteins can be detached from the surface by solutions with a high ionic strength (or) by chelation of divalent cations, rather mild chemical environments often encountered during membrane isolation.
- The possibility exists that true extrinsic proteins are lost and cytoplasmic contaminants absorb during isolation.
- After detachment from the membrane, these proteins are water-soluble and free of liquid.
- Because they contain a high (over 70%) proportion of hydrophilic aminoacids, artsinic proteins probably bind to the hydrophilic regions of the lipid bilayer ionically.
- Alternatively they may bind to polar parts of intrinsic proteins.
- Three proteins, all lying on the cytoplasmic side of cellular membranes, are well- studied examples of extrinsic proteins. Spectrum in erythrocytes, clathrin in coated vesicles and the fatty acid denaturize complex containing cytochrome b_5 in endoplasmic reticulum.

L, Intrinsic proteins:

- Intrinsic (Integral) proteins are embedded in the fatty acid hydrophobic ore of the membrane.
- They can be removed from the membrane only by rather severe measures, such as detergents or organic solvents.
- Once removed, intrinsic proteins are water insoluble and must be in a non aqueous (or) detergent environment.
- The aminoacid composition of these proteins is usually high (over 40%) in hydrophobic side chains. This makes these proteins most stable in a non aqueous environment.

The amino acid sequences of the part of glycoprotien from erythrocytes a) and immunoglobulin M (b) that spans the plasma membrane.

- Each intrinisic protein has at least one domain that is composed of hydrophobic aminoacids and is embedded in the membrane lipid.
- This region adopts a typical alpha-helical confirmation. In some cases, such as cytochrom b_5 of the endoplasmic reticulum, the embedded sequence doesn't go through the membrane to the other surface.

These are termed **monotopic** proteins.

- But is most intrinsic proteins, the hydrophobic region is connected to two more hydrophilic sequences that project out from the membrane surfaces.
- Such proteins span the lipid bilayer and can be classified on the basis of how many such spanning regions the protein contains.
- **Bitopic** proteins span the membrane only once.
- Many intrinsic proteins are **polytopic,** with multiple membrane – spanning domains.
- Although most intrinisic proteins are directly embedded in the hydrophobic lipid leaflet, some lack a hydrophobic insertion sequence and are attached instead to a lipid that is the membrane anchor.
- The carboxyl terminus (aminoacid at the end of the protein that still has a free – coo^- group) covalently attaches through ethanolamine to a complex phospholipids that contains the cyclic sugar inositol.
- The later is connected to a glycerol backbone with two fatty acids, and these embed hydrophobically in the membrane. These proteins are called glycolipo- proteins.

- **Glycoprotein,** like glycolipids – occur only on the exterior – facing part of the lipid bilayer.
- Whereas animals cell membranes are relatively lightly glycolated (up to 3%) , plant plasma membranes contain about 20% sugar by weight, most of this as glycol proteins.

Mobility:

- The fluid mosaic model predicts that, because the lipid bilayer is fluid and proteins are embedded in it through noncovalent interactions, the proteins should be free to move in the membrane.
- Rotation of proteins embedded in the hydrophobic lipid is affected by the lipid's viscosity.
- Diffusion of membrane proteins occurs when they move laterally in the plane of the bilayer.
- This phenomenon has been demonstrated by both physical and biochemical techniques.

The Frye- Edition experiment showing protein diffusion in membranes

Asymmetry:

- Protein asymmetry is a major feature of unit membrane and fluid mosaic models.
- Proteolytic enzymes have also been used as impairment probes: After tan intact membrane is incubated with proteases, only those proteins facing the outside will be digested.
- The latter can be assayed by sizing the proteins on gel electrophoresis.
- Third, the ability of a membrane protein to bind to a specific maker (antibody, hormone, virus (or) enzyme substrate) can be assayed.

An organelle outside of the cell:

- Cells do not exist is isolation but are in contact either with other cells, with the environment in which an organism lives (or) both.
- For eg. A cell on the outside of human skin has one surface exposed to the outside air and its other surfaces exposed to cells adjacent.
- During the 1960s, various alternatives to the Davson – Danielli model were proposed.
- Some investigators abandoned the idea of a phospholipid bilayer and suggested instead that membrane consists of aggregates of lipid- protein complexes.
- However, in 1972, John singer and Garth Nicolson incorporated all of the available information into a model they called the **fluid –mosaic model.**
- This model which is now generally accepted, retains the idea that the phospholipids bilayer is the primary structural element of biological membranes.
- But unlike the Davson-Danielli model, it proposes that integral membrane proteins are embedded in the bilayer, in some cases only peutially, but in other cases extending all the way across.
- Peripheral proteins are attached more loosely by ionic interactions with protruding portions of integral proteins (or) with phospholipids head – groups.
- A key feature of the fluid – mosaic model is that the integral proteins are, in most cases, not linked together by protein –protein interactions.
- Integral membrane proteins move more slowly than phospholipids.

Biological membranes are Asymmetrical:

- Although phospholipids diffuse laterally is the plane of the bilayer and rotate more (or) less freely about an axis perpendicular to this plane movements from one side of the bilayer to the other are a difficult matter.
- Diffusion across the membrane, a transverse, (or) flip-flop, motion, requires getting the polar head group of the phospholipid through the hydrocarbon region in the center of the bilayer.
- Once a protein has been inserted into a meant in a particular orientation, it usually retains that orientation indefinitely.
- An important consequence of these considerations is that biological membranes are both structurally and functionally asymmetrical.
- Lipids also show asymmetrical distributions between the inner and outer leaflets of the bilayer.

4. Write an essay on differences between prokaryotes and eukaryotes.

Prokaryotes and eukaryotes are two different types of cells that make up all living things. They are very similar in that they contain many of the same parts. However, there are a few major differences between them. The evolution of prokaryotic cells preceded that of eukaryotic cells by 2 billion years. Eukaryotes contain membrane-bound nuclei and other organelles, while prokaryotes lack this membrane-bound nucleus. The reason that eukaryotes have a membrane-bound nucleus is because they have to carry out all the processes of life, which is untrue in prokaryotes. Prokaryotes rely on many cells working together to function. While eukaryotes are radically different from one another, they do have three general parts that allow them to carry out these processes of life. These are the cell membrane, the nucleus, and other organelles. The organelles are very important to the cell's functioning. The DNA of prokaryotes floats freely around the cell while the DNA of eukaryotes is held within its nucleus.

Prokaryotic cells represent the initial or primitive cell type on earth and that eukaryotic cell types evolved from them. Prokaryotic cells are less complex than eukaryotic cells at several levels. Eukaryotic cells are structurally & biochemical more complex than eukaryotic cells at several levels. Eukaryotic cells are structurally & biochemically more complex and are considered to represent a later stage of evolution. There is strong data to support the idea that Eukaryotic cell evolved from aggregates of prokaryotic cells that became interdependent upon one another and eventually merged or fused into a single larger cell. Eukaryotic cells have a high degree of organization than do prokaryotic cells, in that they contain many organelles or structures separated from the other cytoplasm components by a membrane, whereas prokaryotic cells contain no organelles.

Prokaryotes are the simpler type of cellular life form. These cells have no nucleus to contain their genetic material. The prokaryotes include the bacteria, single-celled organisms that are enclosed by a cell wall and one or two plasma membranes. The cytoplasm of a bacterial cell contains DNA (the bacterial chromosome and any episomes), RNA, and a variety of proteins. Viruses are also usually included with the prokaryotes, because they have relatively small genomes and simple construction.

Eukaryotic cells range in size between 2 and 100 micrometers and are usually much larger than prokaryotic cell, which run between 0.5 and 2 micrometers. Eukaryotes are considerably more complex, with delegation of certain cellular functions to discrete sub cellular compartments called organells. The eukaryotes include all animals and plants, as well as the fungi. Eukaryotic cells contain a true nucleus. Eukaryotic cells are surrounded by a plasma membrane. Eukaryotic cells are surrounded by a plasma membrane. In addition to this, plant

cells are surrounded by a cell wall, which provides structural support. Animal cell have no such cell wall. Prokaryotes have a cell wall composed of peptidoglycan, a single large polymer of amino acids and sugar. Many types of eukaryotic cells also have cell walls, but none made of peptidoglycan.

Surrounded the nucleus, and contained within the plasma membrane is the cytoplasm. The cytoplasm is sometimes thought to be nothing more than the liquid contents of the cell, but it is very highly organized. The cytoplasm contains a cytoskeleton, a protein scaffold made of microtubules, microfilaments, and intermediate filaments that provides shape and support to the cell and organizes the contents of the cell. Animal cell contain a pair of centrioles, which organize spindle microtubules during cell division. Plant cells do not contain centrioles; it is not known what organizes the spindle during cell division in plants.

5. Mention the Differences between prokaryotes and eukaryotes in general.

1. Nuclear body:

In eukaryotes, nuclear body is bounded by a nuclear membrane having pores connecting it with the endoplasmic reticulum. Nucleolus is present. Nuclear body is called a nucleus in eukaryotes.

In prokaryotes, nuclear body not bounded by a nuclear membrane. Nucleolus is absent. Nuclear body is called a nucleoid.

2. Cytoplasmic membrane:

In eukaryotes, Cytoplasmic membrane is a fluid phospholipid bilayer containing sterols as well as carbohydrates. These are capable for both endocytosis and exocytosis (phagocytosis and pinocytosis)

In prokaryotes, Cytoplasmic membrane is a fluid phospholipid bilayer without carbohydrates and usually lacking sterols. Many bacteria do contain sterol-like molecules called hopaloids. These are Incapable for endocytosis and exocytosis.

3. Cytoplasmic structures:

In eukaryotic cell, ribosome's composed of a 60s and 40s subunit. Internal membrane-bound organelles such as mitochondria, endoplasmic reticulum. Golgi apparatus vacuoles, and lysosomes are present. Chloroplast serves as organelles for photosynthesis. Mitotic spindle involved in mitosis is present during cell division. Cytoskeleton present.

In prokaryotic cell, ribosome's composed 70s ribosome's composed of a 50s and a 30s subunit. Internal membrane-bound organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, and lysosomes are absent. Chloroplast is absent. Photosynthesis usually takes place in infoldings or extensions derived from the cytoplasmic membrane. Absence of mitotic spindle and cytoskeleton.

4. Cell wall:

In eukaryotic cell, plant cells, algae, and fungi have cell walls, usually composed of cellulose or chitin but never containing peptidoglycan. Animal cells and protozoans lack cell walls.

In prokaryotic cell, most eubacteria have cell walls composed of peptidoglycan. The archaebacteria have cell walls composed of protein, a complex carbohydrate, or unique molecules resembling but not the same as peptidoglycan.

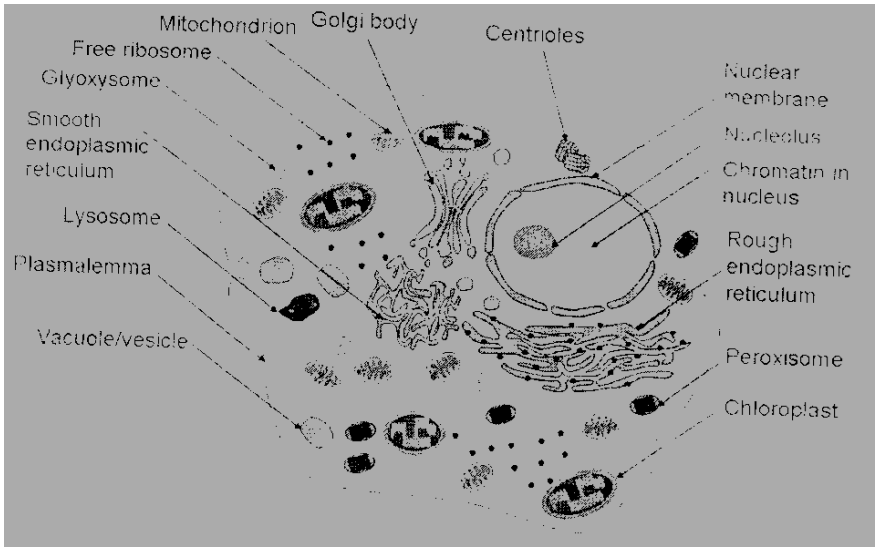
5. Locomotary organelles:

Eukaryotic cell may have flagella or cilia. Flagella and cilia are organelles involved in locomotion and in eukaryotic cells consist of a distinct arrangement of sliding microtubules surrounded by a membrane. The microtubule arrangement is referred to as a 2x9+2 arrangement.

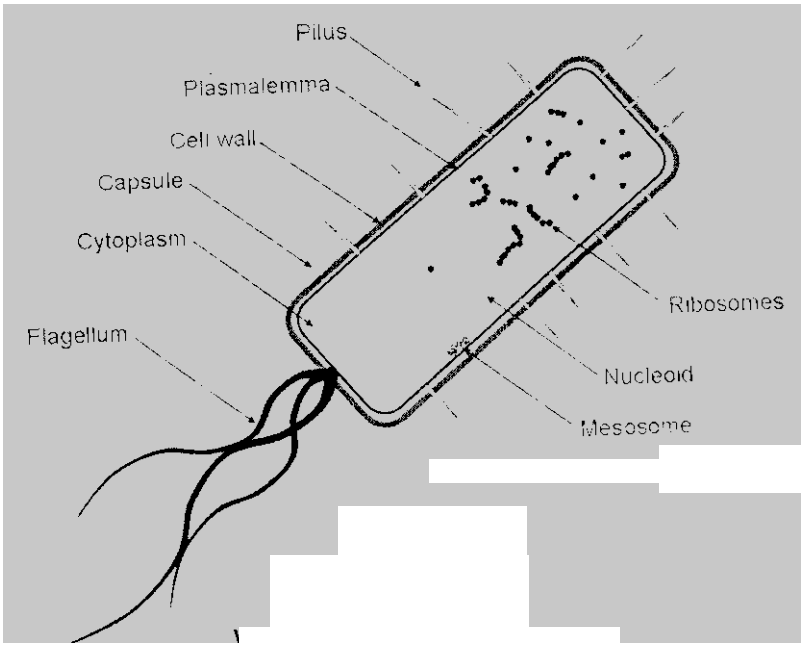
Prokaryotic cell have flagella, each composed of a single, rotating fibril and not surrounded by a membrane, No cilia.

Representative figures for prokaryotes and eukaryotes:

Eukaryotic cell:



Prokaryotic cell:



6. Draw the diagrams for the difference between prokaryotes and eukaryotes.

7. Mention the differences between prokaryotes and eukaryotes at cell cycle level.

A very important feature of cell division is the correct distribution of genetic material among daughter cells. In prokaryotes, the chromosome is simply a molecule of double stranded DNA (in bacteria, the single chromosome is a circular piece of DNA) Eukaryotic chromosomes are much more complex. Each chromosome consists of a single linear molecule of DNA complexed with specific

Proteins, forming a substance called chromatin. Eukaryotic cells contain 2 (or more) copies of each gene (with some exceptions) carried in duplicate chromosomes. During cell division the chromosomes of eukaryotic cells undergo an organized process of chromosome replication that is visible under the light microscope. This process, called mitosis insures that each daughter cell receives a complete copy of the parental genome. Prokaryotic cells usually contain only a single chromosome and, while its process of replication etc. Is also highly organized, it is not visible under the light microscopes. Eukaryotic chromosomes also have structures called telomeres. Telomeres are repetitive DNA structures at the end of the chromosomes. Prokaryotic chromosomes, because they are circular, do not have telomeres.

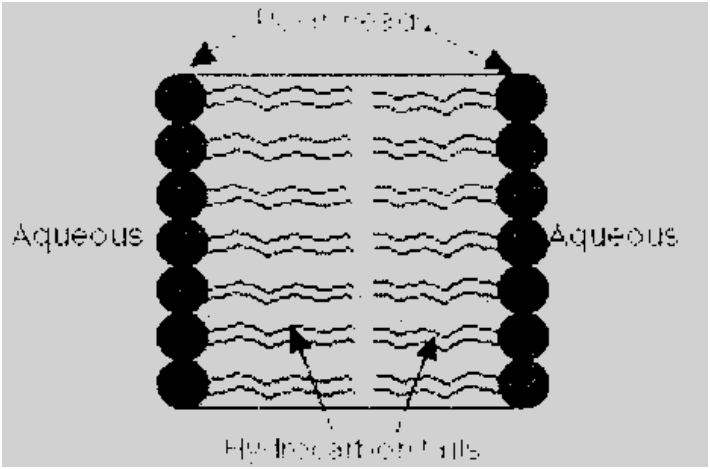
Most eukaryotic cells divide mitotically, progressing regularly the cell cycle. The cell cycle consists of a series of stage that prepare the cell for division (primarily by replicating the chromosomes). Sex cells in diploid organisms are produced through meiosis.

In prokaryotes, most cells divide by a process called binary fission where a cell divides in half forming two daughter cells, which are smaller duplicates of each other. Since prokaryotic organisms are haploid there is no occurrence of meiosis.

8. Briefly explain about the Cell Membranes.

One universal feature of all cells is an outer limiting membrane called the **plasma membrane.** In addition, all eukaryotic cells contain elaborate systems of internal membranes which set up various membrane – enclosed compartments within the cell. Cell membranes are built from **lipids** and **proteins.**

The Plasma Membrane:



The plasma membrane serves as the interface between the machinery in the interior of the cell and the extra cellular fluid **(**ECF) that bathes all cells**.**

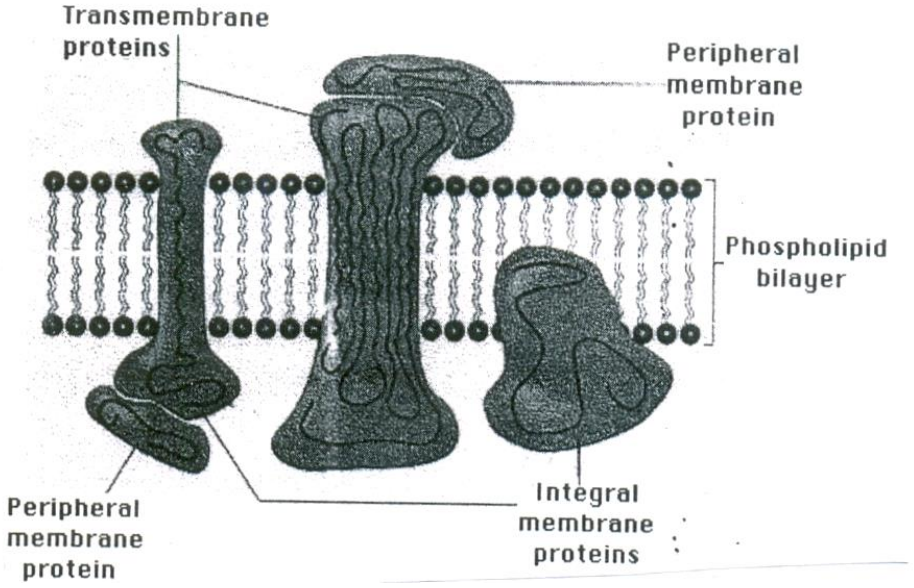
The lipids in the plasma membrane are chiefly phospholipids like phosphatidyl ethanolamine and cholesterol. Phospholipids are amphiphilic with the hydrocarbon tail of the

molecule being hydrophobic; its polar head hydrophilic. As the plasma membrane faces watery solution on both sides, its phospholipids accommodate this by forming a **phospholipid bilayer** with the hydrophobic tails facing each other.

Integral Membrane Proteins

Many of the proteins associated with the plasma membrane are tightly bound to it.

- Some are attached to lipids in the bilayer.
- In others – **trans membrane proteins** – the polypeptide chain actually traverses the lipid bilayer and another that passes through it 7 times. All G- protein- coupled receptors (e.g., receptors of peptide horomone, and odors each span the plasma membrane 7 times.



In all these cases, the portion within the lipid bilayer consists primarily of hydrophobic amino acids. These are usually arranged in an alpha helix so that the polar-C=O and –NH groups at the peptide bonds can interact with each other rather than with their hydrophobic surroundings.

Those portions of the polypeptide that project out from the bilayer tend to have a high percentage of hydrophilic amino acids. Furthermore, those that project into the aqueous surroundings of the cell are usually glycoproteins, with many hydrophilic sugar residues attached to the part of the polypeptide exposed at the surface of the cell.

Some trans membrane proteins that span the bilayer several times form a hydrophilic channel through which certain ions and molecules can enter (or leave) the cell.

Peripheral Membrane Proteins

These are more loosely associated with the membrane. They are usually attached non covalently to the protruding portions of integral membrane proteins.

Membrane proteins are often restricted in their movements.

A lipid bilayer is really film of oil. Thus we might expect that structures immersed in it would be relatively free to float about. For some membrane proteins, this is the case. For others, however, their mobility is limited:

- Some of the proteins exposed at the interior face of the plasma membrane are tethered to cytoskeletal elements like actin microfilaments.

- Some proteins are the exterior face of the plasma membrane are anchored to components of the extra cellular matrix like collagen.
- Integral membrane proteins cannot pass through the tight junctions found between some kinds of cells(e.g., epithelial cells).

9. Explain the Cytoskeletal proteins in detail.

Synopsis

- Actin filaments
- Intermediate Filaments
- Microtubules
- Microtubule motors
- The Centrosome Centrosomes and Cancer
- Centroilers
- Cilia and Flagella
- Primary Cilia

Cells contain elaborate arrays of protein fibers that serve such functions as:

- establishing cell shape
- providing mechanical strength
- locomotion
- chromosome separation in mitosis and meiosis.
- intracellular transport of organelles.

The cytoskeleton is made up of three kind of protein filaments:

- **Actin filaments (**also called **microfilaments)**
- **Intermediate filaments and**
- **Microtubules**

Actin Filaments

Monomers of the protein actin polymerize to form long, thin fibers. These are about 8 nm in diameter and, being the thinnest of the cytoskeletal filaments, are also called **microfilaments. (**In skeletal muscle fibers they are called "thin" filaments.) Some functions of action filaments:

- form a band just beneath the plasma membrane that
- provides mechanical strength to the cell
- Links trans membrane proteins (e.g., cell surface receptors) to cytoplasmic proteins
- anchors the centrosomes at opposite poles of the cell during mitosis
- pinches dividing animal cell apart during cytokinesis

- generate cytoplasmic streaming in some cells
- generate locomotion in cells such as white blood cells and the amoeba
- Interact with myosin ("thick") filaments in skeletal muscle fibers to provide the force of muscular contraction.

Intermediate Filaments

These cytoplasmic fibers average 10 nm in diameter (and thus are "intermediate" in size between actin filaments (8 nm) and microtubules (25nm) (as well as of the thick filaments of skeletal muscle fibers).

There are several types of intermediate filament, each constructed from one or more proteins characteristic of it.

- Keratins are found in epithelial cells and also form hair and nails;
- nuclear **lamins** form a meshwork that stabilizes the inner membrane of the nuclear envelope;
- **neurofilaments** strengthen the long axons of neurons;
- **vitamins** provide mechanical strength to muscle (and other) cells.

Despite their chemical diversity, intermediate filaments play similar roles in the cell: Providing a supporting framework within the cell. For example, the nucleus in epithelial cells is held within the cell by a basketlike network of intermediate filaments made of **keratins.** (photo at right)

Different kinds of epithelia use different keratins to build their intermediate filaments. Over 20 different kinds of keratins have been found, although each kind of epithelial cell may use no more than 2 of them. Up to 85% of the dry weight of squamous epithelial cells can consist of keratins.

Microtubules

Microtubules

- are straight, hollow cylinders whose wall is made up of ring of 13 "protofilaments"
- have a diameter of about 25 nm;
- are variable in length but can grow 1000 times as long as they are wide;
- are built by the assembly of dimers of **alpha tubulin** and **beta tubulin**;
- are found in both animal and plant cells
- grow at each end by the polymerization of tubulin dimers (powered by the hydrolysis of GTP), and
- shrink at each end by the release of tubulin dimers (depolymerization).

However, both processes always occur more rapidly at one end, called the **plus end.** The other, less active, end is the **minus end.**

Microtubules participate in a wide variety of cell activities. Most involve motion. The motion is provided by protein "motors" that use the energy of ATP to move along the microtubule.

Microtubule motors

There are two major groups of microtubule motors"

- **Kinesins (**most of these move toward the plus end of the microtubules and
- **dyneins (**which move towards the minus end)

Some examples:

- The rapid transport of organelles, like vesicles and mitochondria, along the axons of neurons takes place along microtubules with their plus ends pointed towards the end of the axon. The motors are kinesins.
- The migration of chromosomes in mitosis and meiosis takes place of microtubules that make up the **spindle fibers.** Both kinesins and dyneins are used as motors as we shall see below.

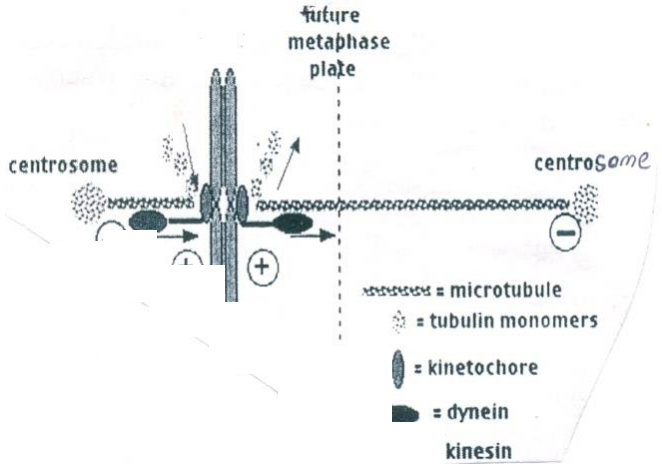
In plant cells, microtubules are created at many sites scattered through the cell. In animal cells, the microtubules originate at the **centrosome.**

The Centrosome

The centrosome is

located in the cytoplasm attached to the outside of the nucleus.

- It is duplicated during S phase of the cell cycle.
- Just before mitosis, the two centrosomes move apart until they are on opposite sides of the nucleus.
- As mitosis proceeds, microtubules grow out from each centrosome with their plus ends growing towards the metaphase plate. These clusters of microtubules are called **spindle fibers.**



The photo (courtesy of Tim Mitchison) shows microtubules growing in vitro from an isolated centrosome. The centrosome was supplied with a mixture of alpha and beta tubulin monomers. These spontaneously assembled into microtubules only in the presence of centrosomes.

Spindle fibers have three destinations:

- Some attach to one kinetochore of a **dyad** With those growing from the opposite centrosome binding to the other kinetochore of that dyad.
- Some bind to the rams of the chromosomes.
- Still others contiue growing from the two centrosomes until they extend between each other in a region of overlap.

10. Write an essay on Contractile Proteins- Actin, Myosin

Actin

Actin is one of the most condensed forms of protein, which is globular and is a monomeric subunit of microfilament. The thin filaments in actin constitute a major part of it. The formation of thin filaments involves a complex process involving the activation of **G-Actin** to ultimately form the ADP-bound **Actin**. In this case, **ATP** acts both as the activator and also the catalyser.

Actin rules over cell functions which include cell division, morphing of the shape of the cells, cell mobility and other contractile properties. It is a 42 kDa **protein** and related gene has 100 nucleotides. Functioning of **Actin** is generally hindered by introns. **Actin filaments** are linked to the membrane through **vinculin**.

Actin basic functions involve:

- ***Giving*** mechanical support to cells.
- ***Enabling*** easy movement of cellular fluids and hence **enhancing** cell mobility.
- ***Participating*** in signal transmission.
- ***Working*** upon the cytoplasm and **hardening** it.

Cellulites like yeasts possess only one **actin gene**, but higher eukaryotes have several **isoforms of actin**. Mammals possess six isoforms of **actin** which are classified as alpha, beta, or gamma depending upon their isoelectric point. Normally **alpha-actins** are found in muscular tissues whereas **beta-actins** and **gamma-actins** are found in non-muscular cells. **Alpha-Actin 1** or **ACTA 1** is one of the six identified actin isoform which are found in skeletal muscles. Actin-related myopathy may occur without any missense mutation in the ACTA1 gene, but missense mutation causes congenital disorders. **Beta-actins** or **ACTB** are categorized as one of the nonmuscular cytoskeleton actin. **Gamma-Actin 1** or **ACTG1** which exists as a component of the cytoskeleton is a cytoplasmic actin which is also dominant in nonmuscular cells. Actins are further subdivided into sub-units known as **globular actins** or **G-actins**. G-actins join to form **F-actins**which in turn give rise to the microfilaments of the cytoskeleton. **Microfilaments** are helical loops which repeat at every 37 nm. The polarity of actin is determined by aligning it with myosin. Actin in combination with **motor protein myosin** forms the **actomyosin motor** fibrils which regulates muscle contraction. Actin polymerization and depolimerisation is extremely essential for cytokinesis or cell division. Nucleating factors are necessary to catalyze **actin polymerization**.

All non-spherical prokaryotes appear to possess genes which encode homologues of actin. It helps in maintaining cellular structure and shape. Such a homologue of actin is found among bacteria which are denoted as **MreB**. It is similar to that of the arrangement at the active site of the **peptide sequence**. **MreB polymerizes** to form filaments which are structurally and characteristically similar to the **actin microfilaments**.

MYOSIN

The eukaryotes seem to contain a major amount of motor proteins. These motor proteins are found to be in great coordination with the **actin filaments**. They are known as the **Myosin**. **Myosin** can be subdivided as Myosin 1 and Myosin 2. **Myosin 1** possesses the contractile property like the actins and hence helps in muscle contraction. It also enables vesicular transportation. More of its functions are yet to be identified. **Myosin 2** contains a huge proportion of Amino acids. The structure of Myosin 2 is similar to an usual **Myosin molecule**. They are divided as the head and the tail. The head domain combines with actin in order to initiate force, whereas in Myosin 2 this head is again subdivided into two terminals. The tail domain helps in easy communication with the cargo muscles and coordinates with other **Myosin subunits**.

A human genome contains more than 40 different types of **myosin genes**. Its high viability towards the working of cargo muscles lies in the fact that the myosins have variability towards the head domain, but the head retains its original sequence. The different genes of myosin have different shapes which in turn determine the speed at which the filaments move. The tail always speeds up to catch up with the **actin filaments**. The power stroke property of the myosin which characterizes the length of it's lever arm specifies the distance moved by cargo muscles. The length of the lever arm is directly proportional to the distance traversed, which means the longer the length of the lever arms the greater the movement caused by the **cargo muscles**. The **myosins** have a number of cellular functions and they are the ultimate binding force behind active transport of proteins and vesicles in the cytoplasm.**Kinesis** and **Dyanin** are a group of related **motor proteins** and have a significant role during mitosis and myosis. A number of sicknesses are associated with the deficiencies of the myosins. Kidney troubles, teeth infection and respiratory suffocations are quite common. It also affects sense organs in certain cases and also results in deafness.

Myosins are subdivided into a number of families and chains. Among them, **myosin**

1A or **MYO1A** is a human gene. Here its terminal contains both **ATP binding site** as well as actin binding site. Its unique feature lies in brush-border which helps the head and the tail domain to interact transiently with core actin and plasma membrane respectively. Its next family is again human genome or the **myosin 3A** or **MYO3A**. They are very unconventional possessing cargo-binding site towards their tale region. It contributes to hearing in humans. **Myosin 5** which is also categorized under heavy chains usually possesses an unusually longer neck. **Myosin 6** supports organelle movement and also performs other vesicular functions. The epithelial functions are enhanced by **Myosin 7**. It is prominently expressed in a large number of mammalian tissues.

11. Briefly explain about the Extra cellular Matrix (ECM).

While it is true that all living things are made of cells, that is only part of the story. Most of the cells in multi cellular organisms are surrounded by a complex mixture of nonliving material that makes up the extra cellular matrix (ECM). In some cases, the ECM accounts for more of the organism's bulk than its cells.

- In plants, the ECM is primarily composed of cellulose.
- In arthropods and fungi, the ECM is largely composed of chitin.
- In vetrebrates, the ECM is made of a complex mixture of carbohydrates and proteins (plus minerals in the case of bone). It is the ECM of vertebrates that will be discussed on this page.

All epithelial cells as well as some other types (e.g., smooth muscle cells) are attached to a **basal lamina (**also known as the **basement membrane).**

Beneath the basal lamina lies **connective tissue.**

Connective Tissue

The cells of connective tissue are embedded in a great amount of extra cellular material. This matrix is secreted by the cells. It consists of protein fibers embedded in an amorphous mixture of huge protein – polysaccharide (" proteoglycan") molecules.

Supporting connective tissue

Gives strength, support, and protection to the parts of the body.

- Cartilage. Example: the outer ear
- Bone. The matrix of bone contains collagen fibers and mineral deposits. The most abundant mineral is calcium phosphate, although magnesium, carbonate, and fluoride ions are also present.

Binding connective tissue

It binds body parts together.

- **Tendons** connect muscle to bone. The matrix is principally collage, and the fibers are all oriented parallel to each other. Tendons are strong but not elastic.
- **Ligaments** attach one bone to another. They contain both collagen and also the protein **elastin**. Elastin permits ligaments to be stretched.

Fibrous connective tissue

It is distributed throughout the body. It serves as a packing and binding material for most of our organs. Collagen, elastin, and another proteins are found in the matrix.

Fascia is fibrous connective tissue that binds muscle together and binds the skin to the underlying structures. **Elastin** is a major protein component.

Adipose tissue is fibrous connective tissue in which the cells, called adipocytes, have become almost filled with oil.

Fibrous and binding connective tissue is derived from cells called fibroblasts, which secrete the extra cellular matrix.

The extra cellular matrix of cartilage and bone is secreted by specialized cells derived from fibroblasts:

- **Chondroblasts** for cartilage;
- **Osteoblasts** for bone.

Composition of the ECM

The ECM of vertebrates is composed of complex mixtures of

- proteins and
- proteoglycans, and
- in the case of bone, mineral deposits.

Proteins

Almost all of the proteins are **glycoproteins;** that is, have chains of carbohydrate residues attached to them. (Elastin does not.)

- A wide variety of **collagens.**
- **Laminins.** Abundant in the **basal lamina** of epithelia.
- **Fibronectin.** Binds cells to the ECM.
- **Elastins.** Provide flexibility to skin, arteries, and lungs. (These are not glycosylated.)

Proteoglycans

Proteoglycans are also glycoproteins but consist of much more carbohydrate than protein; that is, they are huge clusters of carbohydrate chains often attached to a protein backbone.

- The protein backbone of proteoglycans is synthesized, like other secreted proteins, in the endoplasmic reticulum.
- Several sugars are incorporated in proteoglycans. The most abundant one is N-acetylglucosamine (NAG) (the same monomer out of which chitin is made).
- The long chains of sugar residues are attached to serine residues in the protein backbone; that is, they are "O-linked".
    - This glycosylation occurs in the Golgi apparatus.
    - **Sulfate** groups are also added to the sugars while in the Golgi apparatus.
    - In most cases the completed molecules are then secreted by the cell.

Some examples:

- **Chondroitin sulfate**
- **Heparan sulfate**
- **Keratin sulfate**

- **Hyaluronic acid** (This one contains literally thousands of NAG residues but does not have a protein component.)

(Their presence in connective tissue like joints accounts for the popularity of N-acetyglucosamine and chondroitin sulfate as dietary supplements for arthritis sufferes.)

Proteoglycans are degraded in lysosomes. A variety of different enzymes are needed. Inherited deficiencies in any one of these produces one of some dozen different types of **mucopolyasccharidosis** (mucopolysaccharide is the earlier name for proteoglycan).

This proteoglycan differs from the others in being retained at the surface of the cell anchored in the plasma membrane as an integral trans membrane protein.

Syndecan-1 binds **chemokines** (chemotactic cytokines). When epithelia are damaged, these complexes are released and diffuse away forming a chemotactic gradient that attracts neutrophils to the site. Thus syndecan-1 plays a crucial role in inflammation.

Connecting Cells to the ECM

Most normal vertebrate cells cannot survive unless they are anchored to the extra cellular matrix. This **anchorage dependence** is often lost when a cell turns cancerous. (HeLa cells, for example, are among the few types of vertebrate cell that can be grown in liquid culture.)

View normal mouse cells anchored to the substrate and cancerous cells that are not

Cells attach to the ECM by means of transmembrane glycoproteins called **integrins.**

- The extra cellular portion of integrins binds to various types of ECM proteins:
  - ° collagens
  - ° laminins
  - ° fibronectin

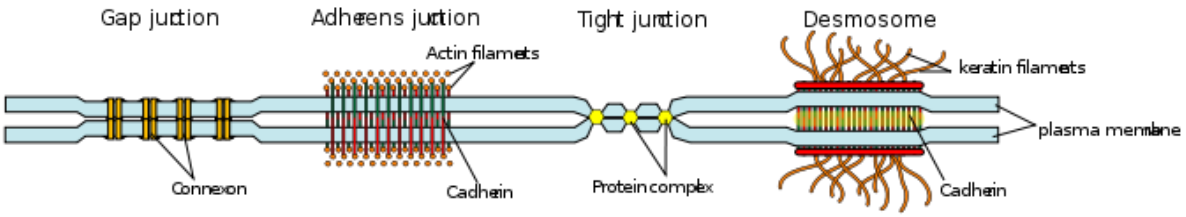
- The intracellular portion binds to the actin filaments of the cytoskeleton.

12. Write an essay on cell junction

A **cell junction** (or **intercellular bridge**) is a type of structure that exists within the tissue of some multicellular organisms, such as animals. Cell junctions consist of multiprotein complexes that provide contact between neighbouring cells or between a cell and the extracellular matrix. They also build up the paracellular barrier of epithelia and control the paracellular transport. Cell junctions are especially abundant in epithelial tissues.

Cell junctions are especially important in enabling communication between neighboring cells via specialized proteins called communicating junctions. Cell junctions are also important in reducing stress placed upon cells.

Types



Some examples of cell junctions

In vertebrates, there are three major types of cell junctions:

- Adherens junctions, desmosomes and hemidesmosomes(anchoring junctions)
- Gap junctions (communicating junction)
- Tight junctions (occluding junctions)

Invertebrates have several other types of specific junctions, for example septate junctions or the *C. elegans* apical junction.

In multicellular plants, the structural functions of cell junctions are instead provided for by cell walls. The analogues of communicative cell junctions in plants are called plasmodesmata.

Anchoring Junctions

Cells within tissues and organs must be anchored to one another and attached to components of the extracellular matrix. Cells have developed several types of junctional complexes to serve these functions, and in each case, anchoring proteins extend through the plasma membrane to link cytoskeletal proteins in one cell to cytoskeletal proteins in neighboring cells as well as to proteins in the extracellular matrix.

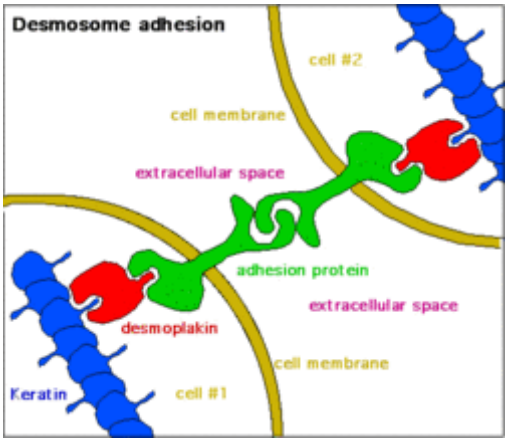
Three types of anchoring junctions are observed, and differ from one another in the cytoskeletal protein anchor as well as the transmembrane linker protein that extends through the membrane:

Junction	Cytoskeletal Anchor	Transmembrane Linker	Ties Cell To:
Desmosomes	Intermediate filaments	Cadherin	Other Cells
Hemidesmosomes	Intermediate Filaments	Integrins	EC Matrix
Adherens junctions	Actin Filaments	Cadherin/Integrins	Other Cells / the EC Matrix

Anchoring-type junctions not only hold cells together but provide tissues with structural cohesion. These junctions are most abundant in tissues that are subject to constant mechanical stress such as skin and heart.

13. Write notes on Desmosomes

DESMOSOMES



Desmosomes can be visualized as rivets through the plasma membrane of adjacent cells. Intermediate filaments composed of keratin ordesmin are attached to membrane-associated attachment proteins that form a dense plaque on the cytoplasmic face of the membrane. Cadherin molecules form the actual anchor by attaching to the cytoplasmic plaque, extending through the membrane and binding strongly to cadherins coming through the membrane of the adjacent cell.
Desmosomes are molecular complexes of cell adhesion proteins and linking proteins that attach the cell surface adhesion proteins to intracellular keratin cytoskeletal filaments.
The cell adhesion proteins of the desmosome, desmoglein and desmocollin, are members of the cadherin family of cell adhesion molecules. They are transmembrane proteins that bridge the space between adjacent epithelial cells by way of homophilic binding of their extracellular domains to other desmosomal cadherins on the adjacent cell. Both have five extracellular domains, and have calcium-binding vulvae.
The extracellular domain of the desmosome is called the extracellular core domain (ECD) or the desmoglea, and is bisected by an electron-dense midline where the desmoglein and desmocollin proteins bind to each other. These proteins can bind in a W, S, or λ manner.
On the cytoplasmic side of the plasma membrane, there are two dense structures called the outer dense plaque (ODP) and the inner dense plaque (IDP). These are spanned by the desmoplakin protein. The outer dense plaque is where the cytoplasmic domains of the cadherins attach to desmoplakin via plakoglobin and plakophilin. The Inner Dense Plaque is where desmoplakin attaches to theintermediate filaments of the cell.

14. Write short notes on tight junctions.

Tight Junctions

Found in vertebrate epithelia, tight junctions act as barriers that regulate the movement of water and solutes between epithelial layers. Tight junctions are classified as a paracellular barrier which is defined as not having directional discrimination; however movement is largely dependent upon solute size and charge. There is evidence to suggest that the structures in which solutes pass through are somewhat like pores. Physiological pH plays a part in the selectivity of solutes passing through tight junctions with most tight junctions being slightly selective for cations. Tight junctions present in different types of epithelia are selective for solutes of differing size, charge, and polarity.

Proteins

There have been approximately 40 proteins identified to be involved in tight junctions. These proteins can be classified into four major categories; scaffolding proteins, signalling proteins, regulation proteins, and transmembrane proteins.

Roles of Tight Junction Proteins

Scaffolding Proteins — organise the transmembrane proteins, couple transmembrane proteins to other cytoplasmic proteins as well as to actin filaments.

Signalling Proteins — involved in junctions assembly, barrier regulation, and gene transcription.

Regulation Proteins — regulate membrane vesicle targeting.

Transmembrane Proteins — including junctional adhesion molecule (JAM), occludin, and claudin. It is believed that claudin is the protein molecule responsible for the selective permeability between epithelial layers.

15. Give detailed account on hemidesmosomes.

HEMIDESMOSOMES

Hemidesmosomes form rivet-like links between cytoskeleton and extracellular matrix components such as the basal laminae that underlie epithelia. Like desmosomes, they tie to intermediate filaments in the cytoplasm, but in contrast to desmosomes, their transmembrane anchors are integrins rather than cadherins.

Hemidesmosomes (HD) are very small stud- or rivet-like structures on the inner basal surface of keratinocytes in the epidermis of skin. They are similar in form to desmosomes when visualized by electron microscopy. While desmosomes link two cells together, hemidesmosomes attach one cell to the extracellular matrix. Rather than using desmogleins, hemidesmosomes use desmopenetrincell adhesion proteins. Hemidesmosomes are asymmetrical and are found in epithelial cells connecting the basal face of the cell to basal lamina.

The HD comprises two rivet-like plaques (the inner and outer plaques). Together with the anchoring fibrils and anchoring filaments, these are collectively termed the HD-stable adhesion complex or HD-anchoring filament complex. Together, the HD-anchoring filament complex forms a continuous structural link between the basal keratinocyte keratin intermediate filaments and the underlying*basement membrane zone* (BMZ) and dermal components. Over the past decade, these structures have been shown to comprise a variety of some 10 or more molecular components.

An example configuration of a hemidesmosome might consist of cytosolic keratin, non-covalently bonded to a cytosolic plectin plaque, which is bonded to a single-pass transmembrane adhesion molecule such as the α6β4 integrin. The integrin might then attach to one of many multi-adhesive proteins such as laminin, resident within the extracellular matrix, thereby forming one of many potential adhesions between cell and matrix.

BT 8305 CELL BIOLOGY

UNIT II

CELL DIVISION, CANCER, APOPTOSIS AND IMMORTALIZATION OF CELLS

1. Define Meiosis .

Meiosis is a special type of cell division that occurs in sexually reproducing organisms. Meiosis reduces the chromosome number by half, enabling sexual recombination to occur. Meiosis of diploid cells produces haploid daughter cells, which may function as gametes.

2. What is the importance of check points in cell cycle of eukaryotes

Checkpoint is a critical control point in the cell cycle where stop and go-ahead signals can regulate the cell cycle Three Major checkpoints are found in the G1, G2, and M phases of the cell cycle. The G1 checkpoint - the Restriction Point. The G2 checkpoint ensures that DNA replication in S phase has been completed successfully. The metaphase checkpoint ensures that all of the chromosomes are attached to the mitotic spindle by a kinetochore.

3. What is Synapsis?

Synapsis is the pairing of two homologous chromosomes that occurs during meiosis. It allows matching-up of homologous pairs prior to their segregation, and possible chromosomal crossover between them.

4. Name the components of ECM

The ECM's main components are various glycoproteins, proteoglycans and hyaluronic acid. In most animals, the most abundant glycoproteins in the ECM are collagens. ECM also contains many other components: proteins such as fibrin, elastin, fibronectins, laminins, and nidogens, and minerals such as hydroxylapatite, or fluids such as blood plasma or serum with secreted free flowing antigens.

5. What is the significance of Prophase I?

Prophase I is the longest phase of meiosis, typically consuming 90% of the time for the two divisions. The duplicated homologous chromosomes pair, and crossing-over (the physical exchange of chromosome parts) occurs.

6. What is the role of matrix in cell growth.

Matrix stiffness (resistance to deformation), one of the many mechanical forces acting on cells, is increasingly appreciated as an important mediator of cell behavior. It regulates cell signaling broadly, with effects on growth, survival, and motility. Although the stiffness optima for different kinds of adherent cells vary widely, it is generally true that cell proliferation and differentiation increase with the stiffness of the matrix.

7. What are Desmosomes?

A **desmosome** is a cell structure specialized for cell-to-cell adhesion. A type of junctional complex, they are localized spot-like adhesions randomly arranged on the lateral sides of plasma membranes. Desmosomes help to resist shearing forces and are found in simple and stratified squamous epithelium. The intercellular space is very wide (about 30 nm). Desmosomes are also found in muscle tissue where they bind muscle cells to one another.

8. List out types of Cell Junctions?

- Adherens junctions, desmosomes and hemidesmosomes(anchoring junctions)
- Gap junctions (communicating junction)
- Tight junctions (occluding junctions)

9. What are functions of ECM?

- Mechanical support for cells and tissues
- Integrates cells into tissues

- Influences cell shape and cell movement
- Influences cell development and cell differentiation
- Coordinates cellular functions therough signaling with cellular adhesion receptors
- Reservoir for extracellular signaling molecules

10. Define Mitosis?

Mitosis is nuclear division plus cytokinesis, and produces two identical daughter cells during prophase, prometaphase, metaphase, anaphase, and telephase. Interphase is often included in discussions of mitosis, but interphase is technically not part of mitosis, but rather encompasses stages GI, S, and G2 of the cell cycle.

11. What are Chiasmata?

A chiasma (plural: **chiasmata**), in genetics, is thought to be the point where two homologous non-sister chromatids exchange genetic material during chromosomal crossover during meiosis.

12. What is the significance of meiosis?

The haploid cells resulting from **meiosis** are gametes. Each of the chromosomes in the gamete cells is a unique mixture of maternal and paternal DNA, resulting in offspring that are genetically distinct from either parent. This gives rise to genetic diversity in sexually reproducing populations.

13. Differentiate between Interphase I and Interphase II of Meiosis.

Interphase I- has both growth phase and synthesis phase
Interphase II- only growth phase, no synthesis phase

14. Define Extra-cellular matrix (ECM).

Extra cellular matrix (ECM) are a complex network of secreted proteins and carbohydrates which are fill the space between the cells. These constituents are secreted by the cells themselves and help in cells binding in tissue together. e.g. Collagen proteins & Hyaluronans.

15. What are GAP junctions?

Gap junctions are a specialized intercellular connection between a multitude of animal cell-types. They directly connect the cytoplasm of two cells, which allows various molecules, ions and electrical impulses to directly pass through a regulated gate between cells.

16. Name some adhesion proteins of Desmosomes

Desmoplakin, desmosomal cadherins, plakophillin, plakoglobin.

17. Define Cytokinesis.

Cytokinesis is the physical process of cell division, which divides the cytoplasm of a parental cell into two daughter cells. It occurs concurrently with two types of nuclear division called mitosis and meiosis, which occur in animal cells.

18. Enumerate the four major categories of Tight Junction proteins.

Transmembrane Proteins , Signalling Proteins, Regulation Proteins and Scaffolding Proteins

19. Define integrins (or) what is meant by Adhesion to matrix?

Many cells bind to components of the extracellular matrix. This cell-to-ECM adhesion is regulated by specific cell surface cellular adhesion molecules (CAM) known as integrins. The integrins transmit mechanical stimuli from the ECM to the cytoskeleton.

20. Compare meiosis and Mitosis.

- Chromosome behavior
  3. Mitosis : Homologous chromosomes independent

4. Meiosis : Homologous chromosomes pair forming bivalents until anaphase I.

- Chromosome number – reduction in meiosis
  3. Mitosis – identical daughter cells
  4. Meiosis – daughter cells haploid
- Genetic identity of progeny:
  4. Mitosis : identical daughter cells
  5. Meiosis : daughter cells have new assortment of parental chromosomes.
  6. Meiosis : Chromatids not identical, crossing over.

21. Define Karyotype.

- A pictorial display of metaphase chromosomes from a mitotic cell
- Homologous chromosomes – pairs.

22. Define ploidy.

Ploidy is the Number of sets of chromosomes in a cell . Haploid (n) – one set chromosomes , Diploid (2n) – two sets chromosomes, Most plant and animal adults are diploid (2n), Eggs and sperm are haploid (n)

23. What are Anchoring Junctions

Cells within tissues and organs must be anchored to one another and attached to components of the extracellular matrix. Anchoring proteins extend through the plasma membrane to link cytoskeletal proteins in one cell to cytoskeletal proteins in neighboring cells as well as to proteins in the extracellular matrix.

24. How do centrioles replicate?

- Autonomous, from proteins in cytoplasm.
- Form microtubule triplets
- Grow out new centrioles at right angles

25. Define Cell Junction

A **cell junction** is a type of structure that exists within the tissue of some multicellular organisms, such as animals. Cell junctions consist ofmultiprotein complexes that provide contact between neighbouring cells or between a cell and the extracellular matrix. They also build up the paracellular barrier of epithelia and control the paracellular transport. Cell junctions are especially abundant in epithelial tissues.

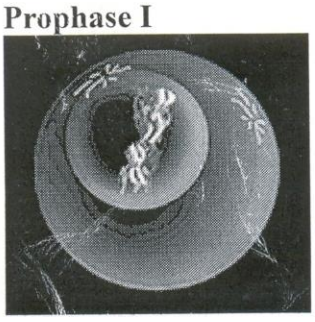
Part B

1. Explain meiosis and its phases in detail.

Meiosis I & II

In meiosis I, chromosomes in a diploid cell reseggregate, producing four haploid daughter cells. It is this step in meiosis that generates genetic diversity.

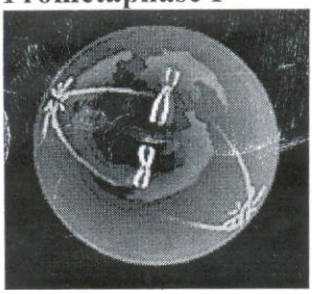
The phases of meiosis I & II



DNA replication precedes the start of meiosis I. during prophase I, homologous chromosomes

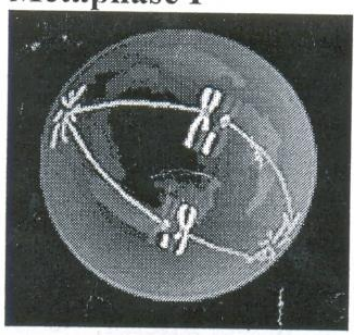
pair and form synapses, a step unique to meiosis. The paired chromosomes are called bivalents, and the formation of chiasmata caused by genetic recombination becomes apparent. Chromosomal condensation allows these to be viewed in the microscope. Note that the bivalent has two chromosomes and four chromatids, with one chromosome coming from each parent.

Prometaphase I



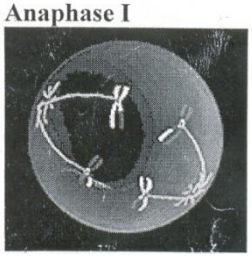
The nuclear membrane disappears. One kinetochore forms per chromosome rather than one per chromatid, and the chromosomes attached to spindle fibers begin to move.

Metaphase I

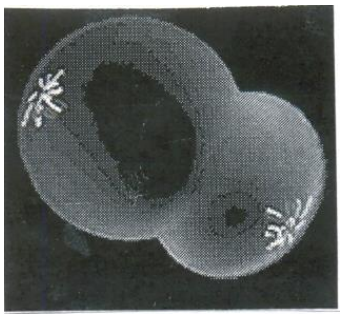


Bivalents, each composed of two chromosomes (four chromatids) align at the metaphase plate. The orientation is random, with either parental homologue on a side. This means that there is a 50-50 chance for the daughter cells to get either the mother's or father's homologue for each chromosome.

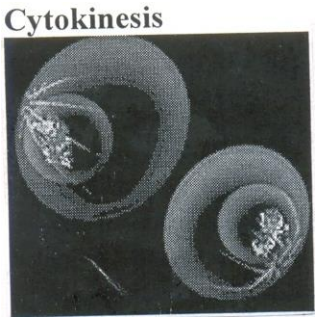
Anaphase I



Chiasmata separate. Chromosomes, each with two chromatids, move to separate poles. Each of the daughter cells is now haploid (23 chromosomes), but each chromosome has two chromatids.



Nuclear envelops may reform, or the cell may quickly start meiosis II.



Analogous to mitosis where two complete daughter cells form.

Meiosis II

Meiosis II is similar to mitosis. However, there is no "S" phase. The chromatids of each chromosome are no longer identical because of recombination. Meiosis II separates the chromatids producing two daughter cells each with 23 chromosomes (haploid), and each chromosome has only one chromatid.

2. Explain mitotic division in detail.

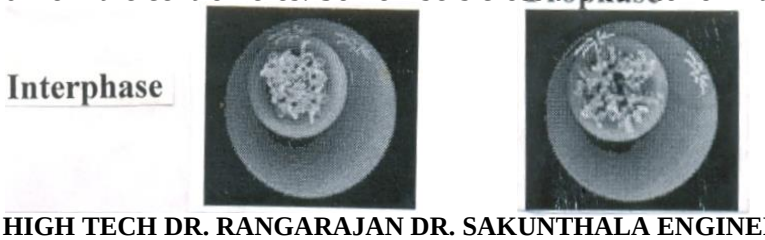
Mitosis:

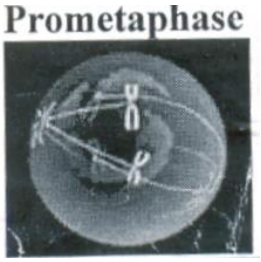
Mitosis is nuclear division plus cytokinesis, and produces two identical daughter cells during prophase, prometaphase, metaphase, anaphase, and telephase. Interphase is often included indiscussions of mitosis, but interphase is technically not part of mitosis, but rather encompasses stages GI, S, and G2 of the cell cycle.

Interphase & mitosis:

The cell is engaged in metabolic activity and performing it to prepare for mitosis (the next four phases that lead up to and include nuclear division) chromosomes are not clearly discerned in the nucleus, although a dark spot called the nucleolus may be visible. The cell may contain a pair of centrioles (or microtubule organizing centers in plants) both of which are organizational sites for microtubules.

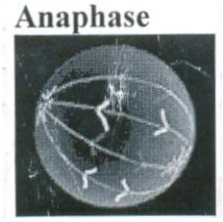
Chromatin in the nucleus begins to condense and becomes visible in the light microscope as chromosomes. The nucleolus disappears, Centrioles begin moving to opposite ends of the cell and fibers extend from the centromeres. Some fibers cross the cell to form the mitotic spindle.



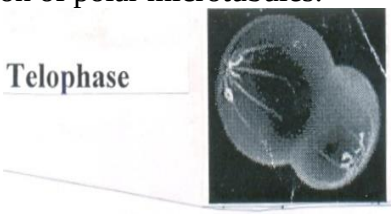


The nuclear membrane dissolves, marking-the beginning of prometaphase, proteins attack to the controversy creating the kinetochores. Microtubules attach at the kinetochores and the chromosomes being moving.

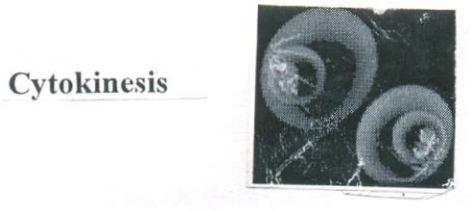
Spindle fibers align the chromosomes along the middle off the cell nucleus this is line is referred to as the metaphase plate. This organization helps to ensure that in the next phase, when the chromosomes are separated, each new nucleus will receive one copy of each chromosome.



The paired chromosomes separate at the kinetochores and move to opposite sides of the cell. Motion results from a combination of kinetochore movement along the spindle microtubules and through the physical interaction of polar microtubules.



Chromatids arrive at opposite pole of cell, and new membranes from around the daughter nuclei. The chromosomes disperse and are no longer visible under the light microscope. The chromosomes disperse and are no longer visible under the light microscope. The spindle fibers disperse, and cytokinesis or the portioning of the cell may also begin during this stage.

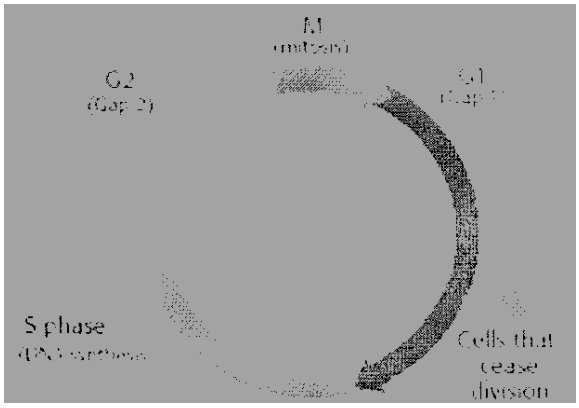


In animal cells, cytokinesis results when a fiber ring composed of a protein called action around the center of the cell contracts pinching the cell into the two daughter cells, each with one nucleus. In plant cells, the rigid wall requires that a cell plate be synthesized between the two

daughter cells.

3. Explain cell cycle and molecules that control cell cycle in detail.

The Cell Cycle



Stages of the cell cycle:

The cell cycle is an ordered set of events, culminating in ell growth and division into two daughter cells. Non-dividing cells not considered to be in the cell cycle. The stages, pictured to the left, are GI-S_G2-M. the G1 stage stands for "GAP 1". The S stage stands for "Synthesis". This is the stage when DNA replication occurs. The G2 stage stands for "GAP2". The M stage for "mitosis", and is when nuclear (chromosomes separate) and cytoplasmic (cytokinesis) division occur. Mitosis is further divided into 4 phases, which you will read about on the next page.

Regulation of the cell cycle:

How cell division (and thus tissue growth) is controlled is very complex. The following terms are some of the features that are important in regulation, and places where errors can lead to cancer. Cancer is a disease where regulation n of the cell cycle goes awry and normal cell growth and behavior is lost.

Cdk (Cyclin dependent kinase, adds phosphate to a protein), along with cyclins, are major control switches for the cell cycle, causing the cell to move from G1 to S or G2 to M.

MPF (Maturation promoting Factor) includes the Cdk and cyclins that triggers progression through the cell cycle.

P53 is a protein that functions to block the cell cycle if the DNA is damaged. If the damage is severe this protein can cause apoptosis (cell death)

1. p53 levels are increased in damaged cells. This allows time to repair DNA by blocking the cell cycle.
2. A p53 mutation is the most frequent mutation leading to cancer. An extreme case of this is Li Fraumeni syndrome, where a genetic a defect in p53 leads to a high frequency of cancer in affected individuals.

P27 is a protein that binds to cyclin and cdk blocking entry into S phase. Recent research (Nature medicine 3,152(1997)) suggests that breast cancer prognosis is determined by p27 levels. Reduced levels of p27 predict a poor outcome for breast cancer patients.

BT 8305 CELL BIOLOGY
UNIT III

TRANSPORT ACROSS CELL MEMBRANE

1. What is Passive transport

- Passive transport is a means of moving biochemicals, and other atomic or molecular substances, across membranes.
- This process does not involve chemical energy.
- Passive transport is dependent on the permeability of the cell membrane.

2. List out the four kinds of passive transport.

The four main kinds of passive transport are

Diffusion, Facilitated diffusion, Filtration and Osmosis

3. Define diffusion

- Diffusion is the net movement of material from an area of high concentration of that material to an area with lower concentration.
- The difference of concentration between the areas is often termed as concentrating gradient, and diffusion will continue until this gradient has been eliminated.

4. Explain Facilitated diffusion.

- Facilitated diffusion is movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane.
- Many large molecules, such as glucose, are insoluble in lipids and too large to fit through the membrane pores.
- Therefore, it will bind with its specific carrier proteins, and the complex will then be bonded to a receptor site and moved through the cellular membrane.

5. What is filtration process across the cell membrane?

- Filtration is movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the cardiovascular system,
- Depending on the size of the membrane pores, only solutes of a certain size may pass through its.
- For example, the membrane pores of the Bowman's capsule in the kidneys are very small, and only albumins, the smallest of the proteins, have any chance of being filtered through.
- On the other hand, the membrane pores of liver cells are extremely large, to allows a variety of solutions to pass through and be metabolized.

6. Explain osmosis.

Diffusion of fluid through a semipermeable membrane from a solution with a low solute concen tration to a solution with a higher solute concentration until there is an equal solute concentration on both sides of the membrane.

or

- Osmosis is the diffusion of a solvent across a membrane to a region of higher solute concentration. (In biological processes it is diffusion of water molecules)
- Most cell membranes are permeable to water, and since the diffusion of water plays such an important role in the biological functioning of any living being, a special term has been coined for it –osmosis.

7. What is Active transport

Active transport is the movement of molecules across a cell membrane in the direction against their concentration gradient, i.e. moving from a low concentration to a high concentration. This requires energy (uses ATP), and is known as active transport.

8. Mention the types of active transport.

- In **primary transport,** energy from hydrolysis of ATP is directly coupled to the movement of specific substance across a membrane independent of any other species.
- In **secondary active transport,** the required energy is derived from energy stored in the form of concentration gradient of the second solute was created by primary active transport, and the diffusion of the second solute across the membrane drives secondary active transport.

9. Differentiate between active and passive transport with an example.

	Active Transport	**Passive Transport**
Definition	Active Transport uses ATP to pump molecules AGAINST/UP the concentration gradient. Transport occurs from a low concentration of solute to high concentration of solute. Requires cellular energy.	Movement of molecules DOWN the concentration gradient. It goes from high to low concentration, in order to maintain equilibrium in the cells. Does not require cellular energy.
Types of Transport	Endocytosis, cell membrane/sodium-potassium pump & exocytosis	Diffusion, facilitated diffusion, and osmosis.
Types of Particles Transported	proteins, ions, large cells, complex sugars.	Anything soluble (meaning able to dissolve) in lipids, small monosaccharides, water, oxygen, carbon dioxide, sex hormones, etc.
Examples	phagocytosis, pinocytosis, sodium/potassium pump, secretion of a substance into the bloodstream (process is opposite of phagocytosis & pinocytosis)	diffusion, osmosis, and facilitated diffusion.
Importance	In eukaryotic cells, amino acids, sugars and lipids need to enter the cell by protein pumps, which require active transport.These items either cannot diffuse or diffuse too slowly for survival.	It maintains equilibrium in the cell. Wastes (carbon dioxide, water, etc.) diffuse out and are excreted; nutrients and oxygen diffuse in to be used by the cell.
Functions	Transports molecules through the cell membrane against the concentration gradient so more of the substance is inside the cell (i.e. a nutrient) or outside the cell (i.e. a waste) than normal. Disrupts equilibrium established by diffusion.	Maintains dynamic equilibrium of water, gases, nutrients, wastes, etc. between cells and extracellular fluid; allows for small nutrients and gases to enter/exit. No NET diffusion/osmosis after equilibrium is established.

10. What are Calmodulin

It is a calcium-binding messenger protein expressed in all eukaryotic cells. CaM is a multifunctional intermediate messenger protein that transduces calcium signals by binding calcium ions and then modifying its interactions with various target proteins.

11. What is Lactose permease?

- Lactose permease is a membrane protein which is a member of the major facilitator superfamily.

- Lactose permease can be classified as a symporter, which uses the gradient of H+ towards the cell to transport lactose in the same direction into the cell.

12. What is ABC superfamily?

- The *ABC (ATP-binding cassette) superfamily,* includes more than 100 different transport proteins found in organisms ranging from bacteria to humans.
- Each ABC protein is specific for a single substrate or group of related substrates including ions, sugars, peptides, polysaccharides, and even proteins.
- All ABC transport proteins share a common organization consisting of four "core" domains: two transmembrane (T) domains, forming the passageway through which transported molecules cross the membrane, and two cytosolic ATP-binding (A) domains.

13. Define a proton pump

A proton pump is an integral membrane protein that is capable of moving protons across the membrane of a cell, mitochondrion, or other subcellular compartment. In cell respiration, the pumps grab protons from the matrix, the space between the two enclosing membranes of the organelle, and release the protons within the inner membrane. The confined protons create a difference or gradient in both pH and electric charge and establish an electrochemical potential that acts as a kind of battery or reservoir of stored energy for the cell.

14. Define sodium-potassium pump.

NA^+/K^+- ATPase (also known as the **NA^+/K^+pump** or **sodium-potassium pump)** is an enzyme located in the plasma membrane (specifically an electrogenic transmembrane ATPase). It is found in the plasma membrane of virtually every human cell and is common to all cellular life. It helps maintain cell potential and regulate cellular volume.

15. Write out the Functions of Sodium-potassium pumps.

- In order to maintain the cell potential , cells must kept a low concentration of sodium ions and high levels of potassium ions within the cell (intracellular)
- Outside cells (extracellular), there are high concentrations of sodium and low concentrations of potassium's, so diffusion occurs through ion channels in the plasma membrane.
- In order to kept the appropriate concentrations, the sodium-potassium pump pumps sodium out and potassium in through active-transport

16. List out the mechanism of sodium-potassium pumps.

- The pump, with bound ATP, binds 3 intracellular Na^+ ions.
- ATP is hydrolyzed leading to phosphorylation of the pump at a highly conserved aspirate residue and subsequent release of ADP.
- A conformational change in the pump exposes the Na^+ ions to the outside. The phosphorylated form of the pump has a low affinity for Na^+ ion, so they are released.
- The pump binds 2 extracellular K^+ ions. This causes the dephosphorylation of the pump, reverting it to its previous conformational state, transporting the K^+ ions into the cells.
- The unphosphorylated form of the pump has a higher affinity for Na^+ ions than K^+ ions, so the two bound K^+ ions are released. ATP binds, and the process starts again!

17. Define Ca^{2+} ATPase pump

Ca^{2+} ATPase is a form of P-ATPase that transfers calcium after a muscle has contracted. The calcium ATPase are of two types- Plasma membrane Ca^{2+} ATPase (PMCA) and Sarcoplasmic reticulum Ca^{2+} ATPase (SERCA)

PMCA is a transport protein in the plasma membrane of cells and functions to remove **calcium** (Ca^{2+}) from the cell. PMCA function is vital for regulating the amount of Ca^{2+} within all eukaryotic cells.

SERCA is a Ca^{2+} ATPase that transfers Ca^{2+} from the cytosol of the cell to the lumen of the SR at the expense ofATP hydrolysis during muscle relaxation.

18. Define proton motive force.

The tendency of the protons (H^+) to return to the inside of the compartment is therefore quite large. It is called the proton motive force.

Or

In most cases the proton motive force is generated by an electron transport chain which acts as a proton pump, using the energy of electrons from an electron carrier (Gibbs free energy of redox reactions) to pump protons (hydrogen ions) out across the membrane, separating the charge across the membrane.

19. Write the difference between symporter and antiporter

A symporter is an integral membrane protein that is involved in movement of two or more different molecules or ions across a phospholipid membrane such as the plasma membrane in the same direction, and is, therefore, a type of cotransporter.

An antiporter is an integral membrane protein involved in secondary active transport of two or more different molecules or ions (i.e., solutes) across a phospholipid membrane such as the plasma membrane in opposite directions.

20. Define Cotransporter.

- A **cotransporter** is an integral membrane protein that is involved in secondary active transport.
- It works by binding to two molecules at a time and using the gradient of one solute's concentration to force the other molecule against its gradient.
- It is sometimes equated with symporter, but the term "cotransporter" refers both to symporters and antiporters (through not uniporters).

21. Define uniporter

A **uniporter** is an integral membrane protein that is involved in facilitated diffusion. They can be either ion channels or carrier proteins.

Uniporter carrier proteins work by binding to one molecule of substrate at a time and transporting it with its concentration gradient. Uniporter channels open in response to a stimulus and allow the free flow of specific molecules.

22. Define agonist

An agonist is a chemical that binds to a receptor and activates the receptor to produce a biological response.

23. List out the types of agonists

- **Endogenous agonist** for a particular receptor is a compound naturally produced by the body that binds to and activates that receptor. For eg, the endogenous agonist for serotonin receptors is serotonin
- A **superagonist** is a compound that is capable of producing a greater maximal response
- **Full agonists**
- **Partial agonists** (such as buspirone, aripiprazole, buprenorphine, or norclozapine) also bind and activate a given receptor
- An **inverse agonist** is an agent that binds to the same receptor binding-site as an agonist for that receptor and inhibits the constitutive activity of the receptor.

24. Define antagonist

A **receptor antagonist** is a type of receptor ligand or drug that blocks or dampens agonist-

mediated responses rather than provoking a biological response itself upon binding to a receptor.

25. Name the types of antagonists

Competitive-Competitive antagonists (also known as surmountable antagonists) reversibly bind to receptors at the same binding site (active site) as the endogenous ligand or agonist, but without activating the receptor.

Non-competitive- The term "non-competitive antagonism" (sometimes called non-surmountable antagonists) can be used to describe two distinct phenomena: one in which the antagonist binds to the active site of the receptor, and one in which the antagonist binds to an allosteric site of the receptor.

Uncompetitive- Uncompetitive antagonists differ from non-competitive antagonists in that they require receptor activation by an agonist before they can bind to a separate allosteric binding site.

Silent antagonists- Silent antagonists are competitive receptor antagonists that have zero intrinsic activity for activating a receptor.

PART – B

1. Explain active transport in detail.

Active transport (sometimes called **active uptake)** is the mediated transport of biochemical's and other atomic/molecular substances, across membranes. This process requires the expenditure of cellular energy to move molecules "uphill" against a gradient.

Process:

In this form of transport, molecules move against either an electrical or concentration gradient (collectively termed an electrochemical gradient). The active transport of small molecules or ions across a cell membrane is generally carried out by transport proteins that are found in the membrane.

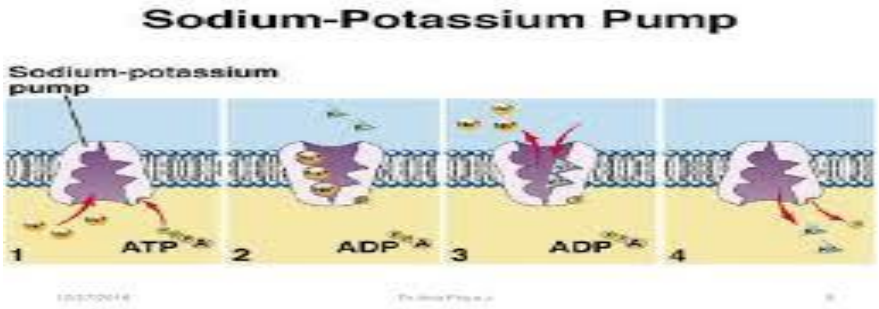
Larger molecules such as starch can also be actively transported across the cell membrane by process known as endocytosis and exocytosis.

Particle that is moved through a membrane from a region of low concentration to high is known as active transport.

TYPES:

In primary transport, energy for hydrolysis of ATP is directly coupled to the movement of a specific substance across a membrane independent of any other species.

In secondary active transport, the required energy is derived from energy stored in the form of concentration differences in a second solute. Typically, the concentration gradient of the second solute was created by primary active transport, and the diffusion of the second solute across the membrane drives secondary active transport.



Sodium-Potassium pump, an example of Primary active transport

2. Briefly explain about the sodium potassium pumps.

Na^+/K^+-ATPase (also known as the **Na^+/K^+ pump or sodium-potassium pump**) is an enzyme located in the plasma membrane. It is found in the plasma membrane of virtually every human cell and is common to all cellular lie. It helps maintain cell potential and regulate cellular volume.

Function:

In order to maintain the cell potential, cell must keep a low concentration of sodium ions and high levels of potassium ions within the cell (intracellular). Outside cells (extra cellular), there are high concentrations of sodium and low concentrations of potassium, so diffusion occurs through ion channels in the plasma membrane. In order to keep the appropriate concentrations, the sodium-potassium pumps sodium out and potassium in through active transport.

The mechanism is:

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- ATP is hydrolyzed, leading to phosphorylation of the pump at the highly conserved aspartate resisdue and subsequent release of ADP.
- A conformational change in the pump exposes the Na^+ ions to the outside. The phosphorylated form of the pump has a low affinity for Na^+ ion, so they are released.
- The pump binds 2 extra cellular K^+ ions. This cause the dephosphorylation of the pump, reverting to its previous conformation state, transportation the K^+ ions, so the two bound K^+ ions are released. ATP binds, and the process starts again!

3. Describe ca2+ATPase pumps in detail.

Small increases in the concentration of free Ca^{2+} ions in the cytosol trigger a variety of cellular responses. In order for Ca^{2+} to function in intracellular signaling, its cytosolic concentration must be kept below 0.1 – 0.2 μM.

The plasma membranes of animal, yeast, and plant cells contain Ca^{2+} ATPases that transport Ca^{2+} out of the cell against its electrochemical gradient. These P-class ion pumps help maintain the concentration of free Ca^{2+} ions in the cytosol at a low level.

In addition to a catalytic α subunit containing an ATP-binding site, as found in other P-class pumps, plasmamembrane Ca^{2+} ATPases also contain the Ca^{2+}-binding regulatory protein calmodulin.

A rise in cytosolic Ca^{2+} induces the binding of Ca^{2+} ions to calmodulin, which triggers an allosteric activation of the Ca^{2+} ATPase; as a result, the export of Ca^{2+} ions from the cell accelerates, and the original low cytosolic concentration of free Ca^{2+} is restored rapidly.

Muscle Ca^{2+} ATPase Pumps Ca^{2+} Ions from the Cytosol into the Sarcoplasmic Reticulum

Besides the plasma-membrane Ca^{2+} ATPase, muscle cells contain a second, different Ca^{2+} ATPase that transports Ca^{2+} from the cytosol into the lumen of the sarcoplasmic reticulum (SR), The SR and its calcium pump (referred to as the *muscle calcium pump*) are critical in muscle contraction and relaxation: release of Ca^{2+} ions from the SR into the muscle cytosol causes contraction, and the rapid removal of Ca^{2+} ions from the cytosol by the muscle calcium pump induces relaxation.

Each transmembrane catalytic α subunit transports two Ca^{2+} ions per ATP hydrolyzed. In the cytosol of muscle cells, the free Ca^{2+} concentration ranges from 10^{-7} M (resting cells) to more than 10^{-6} M (contracting cells), whereas the *total* Ca^{2+} concentration in the SR lumen can be as high as 10^{-2} M.

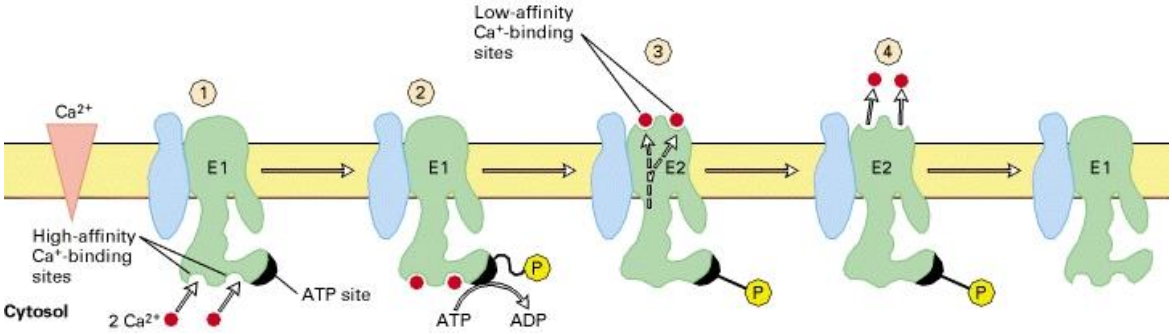
The activity of the muscle Ca^{2+} ATPase is so regulated that if the free Ca^{2+} concentration in the

cytosol becomes too high, the rate of calcium pumping increases until the cytosolic Ca^{2+} concentration is reduced to less than 1 μM. Thus in muscle cells, the calcium pump in the SR membrane can supplement the activity of the plasma-membrane pump, assuring that the cytosolic concentration of free Ca^{2+} remains below 1 μM.

The current model of the mechanism of action of the Ca^{2+} ATPase in the SR membrane is outlined in Figure.

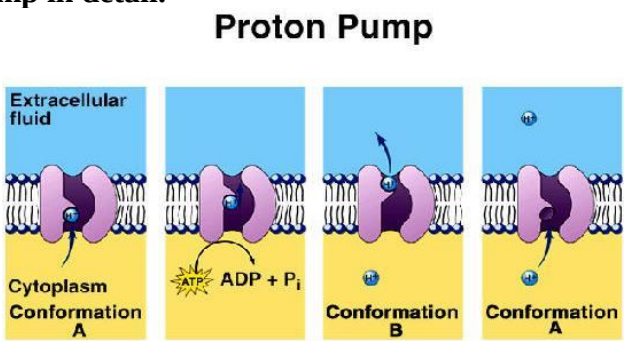
Coupling of ATP hydrolysis with ion pumping involves several steps that must occur in a defined order.

1. When the protein is in one conformation, termed *E1*, two Ca^{2+} ions bind in sequence to high-affinity sites on the cytosolic surface (step 1).
2. Then an ATP binds to its site on the cytosolic surface; in a reaction requiring that a Mg^{2+} ion be tightly complexed to the ATP, the bound ATP is hydrolyzed to ADP and the liberated phosphate is transferred to a specific aspartate residue in the protein, forming a high-energy acyl phosphate bond, denoted by E1~P (step 2).
3. The protein then changes its conformation to E2 – P, generating two lowaffinity Ca^{2+}-binding sites on the exoplasmic surface, which faces the SR lumen; this conformational change simultaneously propels the two Ca^{2+} ions through the protein to these sites (step 3)
4. and inactivates the high-affinity Ca^{2+}-binding sites on the cytosolic face. The Ca^{2+} ions then dissociate from the exoplasmic surface of the protein (step 4).
5. Following this, the aspartyl-phosphate bond in E2 – P is hydrolyzed, causing E2 to revert to E1, a change that inactivates the exoplasmic-facing Ca^{2+}-binding sites and regenerates the cytosolicfacing Ca^{2+}-binding sites (step 5).



Thus phosphorylation of the muscle calcium pump by ATP favors conversion of E1 to E2, and dephosphorylation favors the conversion of E2 to E1.

4. Explain proton pump in detail.



A proton pump is an integral membrane protein that is capable of moving protons across the

membrane of a cell, mitochondrion, or other subcellular compartment. In cell respiration, the pumps grab protons from the matrix, the space between the two enclosing membranes of the organelle, and release the protons within the inner membrane. The confined protons create a difference or gradient in both pH and electric charge and establish an electrochemical potential that acts as a reservoir of stored energy for the cell. The inner cell membrane functions in a similar way to a dam in a river. It blocks protons from drifting back into the matrix. Since the pumping action is against the gradient, it requires energy. The process is directly analogous to bicycling uphill or charging a battery (storing up potential energy). It is important to remember that the proton pump does not create energy, Instead, the gradient stores energy for the appropriate time.

5. Explain the General characteristics of ATPases and their genes in detail.

ATPases (or ATP synthases) are membrane-bound enzyme complexes ion transporters that combine the synthesis and\or hydrolysis of adenosine triphosphate (ATP) with the transport of protons across a plasma membrane. ATPases can harness the energy from an electrochemical proton gradient, using the flux of ions across the membrane via the ATPase proton channel to dirve the synthesis of ATP. Some ATPase work in reverse, using the energy from the hydrolysis of ATP to create a proton gradient. There are different types of ATPases, which can differ in function (ATP synthesis and\or hydrolsis), structure (F – V – and A-ATPases contain rotary motors) and in the type of ions they transport.

- F-ATPases (F1F0-ATPases) in mitochondria, chloroplasts and bacterial plasma membrane are the prime producers of ATP, using proton gradient generated by oxidative phosphorylation (mitochondria) or photosynthesis (chloroplasts).
- V-ATPases (V1V0-ATPases are primarily found in eukaryotic vacuoles, catalysing ATP hydrolysis to transport solutes and lower $_{p}$H in organelles.
- A-ATPases (A1A0-ATPases) are found in Archaea and function like F-A pases.
- P-ATPases (E1E2-ATPases) are found in bacteria and in eukaryotic plasma membranes and organelles, and function to transport a variety of different ions across membranes.
- E-ATPases are cell-surface enzymes that hydrolyse of NTPs, including extracellular ATP.

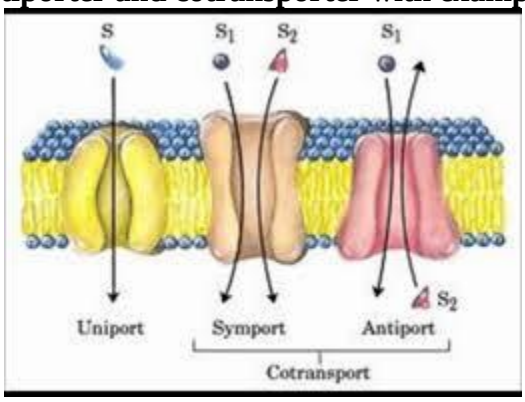
P-ATPases (sometime know as E1-E2 ATPase are found in bacteria and in a number of eukaryotic plasma membranes and organelles. P-ATPases function to transport a variety of different compounds, including ions and phospholipids, across a membrane using ATP hydrolysis for energy. There are many different classes of P-ATPases, each of which transports a specific type of ion:H+, Na+, K+, Mg2+, Ag+ and Ag2+, Zn2+, Co2+, Pb2+,Ni2+,Cd2+,Cu+ and Cu2+ P-ATPases can be composed of one or two polypetides, and can usually assume two main conformations called E1 and E2.

Genes

- - ubiquitous ATP2A1 – cardiac muscle, fast twitch 1
- ATP2A2 - cardiac muscle, slow twitch 2
- ATP2A3
- ATP2B1- plasma membrane 1
- ATP2B2- plasma membrane 2
- ATP2B2 – plasma membrane 3

- ATP2B3 - plasma membrane 4
- ATP2B4 - plasma membrane 1
- ATP2C1 – type 2C, member 1.

6. Explain symporter, antiporter and cotransporter with examples.



Symporter

A symporter is an **integral membrane protein** that is involved in **active transport** of two or more different molecules or ions across a phospholipid membrane such as the **plasma membrane** in the same direction. Typically, one molecule move along is electrochemical gradient, allowing the other to move against its electrochemical gradient.

Examples

$Na^+/K^+/2CT$ symporter in the **loop of Henle** in the **renal tubules** of the **kidney** transports 4 molecules of 3 different types; a sodium ion (Na^+), a potassium ion (K^+) and two chloride ions ($2Cl^-$).

Contransporter

A **contransporter**is an **integral membrane protein** that is involved in secondary **active transport.** It works by binding to two **molecules** at a time and using the **gradient** of one **solute's concentration** to force the other molecule against its gradient.

Symporters do not require the splitting of **ATP** because they derive the necessary energy for the movement of one molecule form the movement of the another. Overall, the movement of the two molecules still acts to increase **entropy.**

Proton-sucrose cotransporters are common in plant cell membranes. An ATP molecule in the cell phophorylates a carrier protein, causing a conformational change that shuttles a proton across the membrane. The protein binds with sucrose in the extra cellular fluid, then undergoes passive transport down its concentration gradient (i.e. up the concentration gradient of sucrose).

7. Explain passive transport in detail.

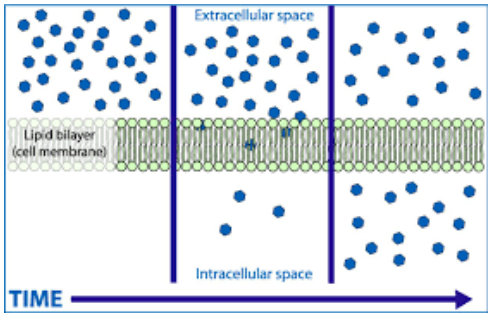
Synopsis

- **1. Diffusion**
- **2. Facilitated diffusion**
- **3. Filtration**
- **4. Osmosis**
- **5. Related materials**

Passive transport is a means of **moving biochemicals,** and other **atomic** or **molecular** substances, across **membranes**. This process does not involve **chemical energy.** Passive

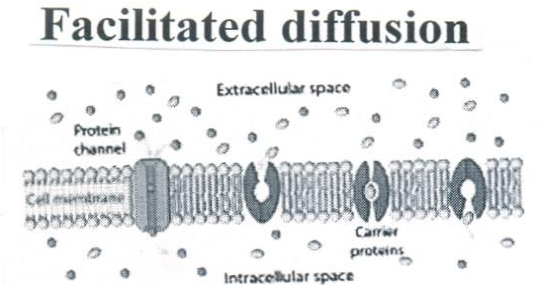
transport is dependent on the permeability of the cell membrane, which is dependent on the organization and characteristics of the membrane lipids and proteins. The four main kinds of passive transport are **diffusion, facilitated diffusion, filtration and osmosis.**

Diffusion



Passive diffusion on a cell membrane

Diffusion is the net movement of material from an area of high concentration of that material to an area with lower concentration. The difference of concentration between the two areas is often termed as the concentration gradient, and diffusion will continue until this gradients has eliminated. Since diffusion moves materials from an area of higher concentration to the lower, it is described as moving solutes" down the concentration gradient."



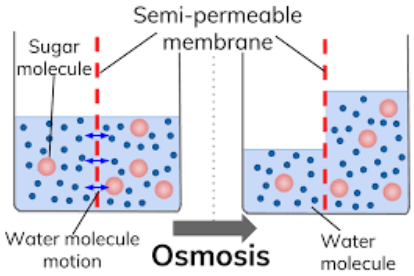
Facilitated diffusion on a cell membrane

Facilitated diffusion is movement of **molecules** across the **cell membrane** via special **transport proteins** that are embedded with in the cellular membrane. Many large molecules, such as **glucose,** are insoluble in **lipids** and too large to fit through the membrane pores. Therefore, it will bind with its specific carrier proteins, and the complex will then be bounded to a **receptor** site and moved through the cellular membrane. Bear in mind, however, that facilitated diffusion is a passive process, and the solutes still move down the **concentration gradient.** The **alveoli** are tiny grapelike sacs located at the end of the **bronchial** tubes. This is where oxygen diffuse into the alveoli and is exchanged for carbondioxide.

Filtration

Filtration is movement of water and solute molecules across the cell membrane due to hydrostatic **pressure** generated by the cardiovascular system. Depending on the size of the membrane pores, only solutes of a certain size may pass through it. For example, the membrane pores of the **Bowman's capsule** in the kidneys are very small, and only **albumins,** the smallest of the proteins, have any change of being filtered through. On the other hand, the membrane pores of **liver** cells are extremely large, to allow a variety of solutes of pass through and be metabolized.

Osmosis



Osmosis is the **diffusion** of a **solvent** across a membrane to a region of higher **solute** concentration. (In biological processes then, it is diffusion of water molecules). Most **cell membranes** are permeable to water, and since the diffusion of water plays such an important role in the biological functioning of any living being, a special term has been coined for it – osmosis.

8. What are permeases. Give example.

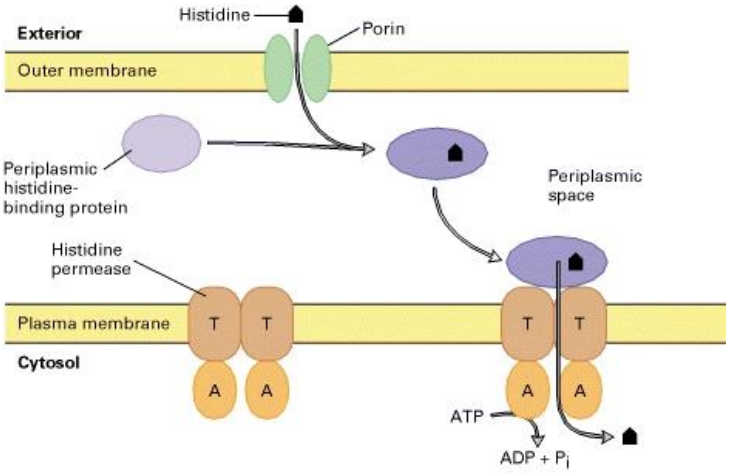
Bacterial Plasma-Membrane Permeases

The plasma membrane of many bacteria contain numerous *permeases* that belong to the ABC superfamily. These proteins use the energy released by hydrolysis of ATP to transport specific amino acids, sugars, vitamins, or even peptides into the cell. Since bacteria frequently grow in soil or pond water where the concentration of nutrients is low, these ABC transport proteins allow the cells to concentrate amino acids and other nutrients in the cell against a substantial concentration gradient. Bacterial permeases generally are *inducible;* that is, the quantity of a transport protein in the cell membrane is regulated by both the concentration of the nutrient in the medium and the metabolic needs of the cell.

In *E. coli* histidine permease, a typical bacterial ABC protein, the two transmembrane domains and two cytosolic ATP-binding domains are formed by four separate subunits.

In gram-negative bacteria such as *E. coli*, which have an outer membrane, a soluble histidine-binding protein in the periplasmic space assists in transport (Figure).

This soluble protein binds histidine tightly and directs it to the T subunits, through which histidine crosses the membrane powered by ATP hydrolysis.



(Figure :Gram-negative bacteria import many solutes by means of ABC proteins (permeases) that utilize a soluble substrate-binding protein present in the periplasmic space. Depicted here is the import of the amino acid histidine. After diffusing through porins in the outer membrane, histidine is bound by a specific periplasmic histidine-binding protein, which undergoes a

conformational change. The histidine-protein complex binds to the exoplasmic surface of a T subunit in histidine permease located in the plasma membrane. Hydrolysis of ATP bound to the A subunit then powers movement of histidine through the protein into the cytosol. The transport process does not appear to involve a phosphoprotein intermediate.)

9. Describe various types of ion channels (Ligand and voltage gated).

Ion channels are pore-forming membrane proteins whose functions include establishing a resting membrane potential, shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane, controlling the flow of ions across secretory and epithelial cells, and regulating cell volume. Ion channels are present in the membranes of all cells.

Ion channels are considered to be one of the two traditional classes of ionophoric proteins, with the other class known as ion transporters (including the sodium-potassium pump, sodium-calcium exchanger, and sodium-glucose transport proteins, amongst others)

There are two distinctive features of ion channels that differentiate them from other types of ion transporter proteins:

Ion channels may be classified by gating, i.e. what opens and closes the channels. Voltage-gated ion channels open or close depending on the voltage gradient across the plasma membrane, while ligand-gated ion channels open or close depending on binding of ligands to the channel

Voltage gated ion channel

- Voltage-gated sodium channels: This family contains at least 9 members and is largely responsible for action potential creation and propagation. The pore-forming α subunits are very large (up to 4,000 amino acids) and consist of four homologous repeat domains (I-IV) each comprising six transmembrane segments (S1-S6) for a total of 24 transmembrane segments. The members of this family also coassemble with auxiliary β subunits, each spanning the membrane once. Both α and β subunits are extensively glycosylated.
- Voltage-gated calcium channels: This family contains 10 members, though these members are known to coassemble with $\alpha_2\delta$, β, and γ subunits. These channels play an important role in both linking muscle excitation with contraction as well as neuronal excitation with transmitter release. The α subunits have an overall structural resemblance to those of the sodium channels and are equally large.
    - Voltage-gated proton channels: Voltage-gated proton channels open with depolarization, but in a strongly pH-sensitive manner. The result is that these channels open only when the electrochemical gradient is outward, such that their opening will only allow protons to leave cells. Their function thus appears to be acid extrusion from cells. Another important function occurs in phagocytes (e.g. eosinophils, neutrophils, macrophages) during the "respiratory burst." When bacteria or other microbes are engulfed by phagocytes, the enzyme NADPH oxidase assembles in the membrane and begins to produce reactive oxygen species (ROS) that help kill bacteria. NADPH oxidase is electrogenic, moving electrons across the membrane, and proton channels open to allow proton flux to balance the electron movement electrically.

Ligand-Gated Ion Channels

Many types of ion channels respond to chemical signals (ligands) rather than to changes in the membrane potential (Figure 4.4E-G).

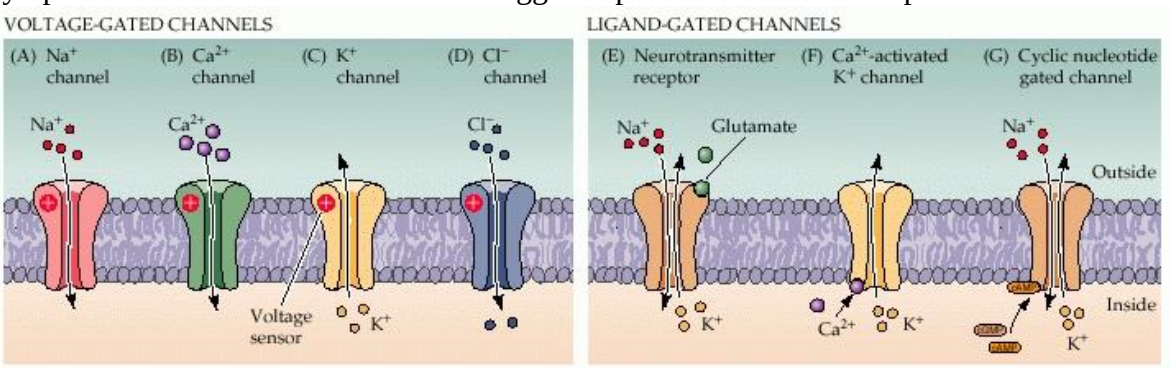
The most important of these ligand-gated ion channels in the nervous system is the class activated by binding neurotransmitters (Figure 4.4E).

These channels are essential for synaptic transmission and other forms of cell-cell signaling phenomena. Whereas the voltage-gated ion channels underlying the action potential typically

allow only one type of ion to permeate, channels activated by extracellular ligands are usually less selective, allowing two or more types of ions to pass through the channel pore.

Other ligand-gated channels are sensitive to chemical signals from within the cytoplasm of neurons. These channels have ligand-binding domains on their *intracellular* surfaces that interact with second messengers such as Ca^{2+} (Figure 4.4F) and the cyclic nucleotides cAMP and cGMP (Figure 4.4G). Such channels can be selective for specific ions such as K^+ or Cl^-, or can be permeable to all physiological cations.

The main function of these channels is to convert intracellular chemical signals into electrical information. This process is particularly important in sensory transduction, where channels gated by cyclic nucleotides convert odors and light into electrical signals. Some intracellularly activated ion channels are in the cell surface membrane, but others are in intracellular membranes such as the endoplasmic reticulum. These latter channels are selectively permeable to Ca^{2+} and regulate the release of Ca^{2+} from the lumen of the endoplasmic reticulum into the cytoplasm. The Ca^{2+} released can then trigger a spectrum of cellular responses.



Types of voltage-gated ion channels. Examples of voltage-gated channels include those selectively permeable to Na^+ (A), Ca^{2+} (B), K^+ (C), and Cl^- (D). Ligand-gated ion channels include those activated by the extracellular presence of neurotransmitters, such as glutamate (E). Other ligand-gated channels are activated by intracellular second messengers, such as Ca^{2+} (F) or the cyclic nucleotides, cAMP and cGMP (G).

10. Describe about agonists and antagonists.

AGONISTS

Receptors can be activated by either endogenous (such as hormones and neurotransmitters) or exogenous (such asdrugs) agonists, resulting in a biological response. A physiological agonist is a substance that creates the same bodily responses but does not bind to the same receptor.

An **endogenous** agonist for a particular receptor is a compound naturally produced by the body that binds to and activates that receptor. For example, the endogenous agonist for serotonin receptors is serotonin, and the endogenous agonist for dopamine receptors is dopamine.

A **superagonist** is a compound that is capable of producing a greater maximal response than the endogenous agonist for the target receptor, and thus has an efficacy of more than 100%. This does not necessarily mean that it is more potent than the endogenous agonist, but is rather a comparison of the maximum possible response that can be produced inside the cell following receptor binding.

Full agonists bind (have affinity for) and activate a receptor, producing full efficacy at that receptor. One example of a drug that acts as a full agonist is isoproterenol, which mimics the action of adrenaline at β adrenoreceptors. Another example is morphine, which mimics the

actions of endorphins at μ-opioid receptors throughout the central nervous system.

Partial agonists (such as buspirone, aripiprazole, buprenorphine, or norclozapine) also bind and activate a given receptor, but have only partial efficacy at the receptor relative to a full agonist, even at maximal receptor occupancy. Agents like buprenorphine are used to treat opiate dependence for this reason, as they produce milder effects on the opioid receptor with lower dependence and abuse potential.

An **inverse agonist** is an agent that binds to the same receptor binding-site as an agonist for that receptor and inhibits the constitutive activity of the receptor. Inverse agonistsexert the opposite pharmacological effect of a receptor agonist, not merely an absence of the agonist effect as seen with antagonist. An example is the cannabinoid inverse agonist rimonabant.

A **co-agonist** works with other co-agonists to produce the desired effect together. NMDA receptor activation requires the binding of both glutamate, glycine and D-serine co-agonists.

An irreversible agonist is a type of agonist that binds permanently to a receptor through the formation of covalent bonds. A few of these have been described.

A selective agonist is selective for a specific type of receptor. E.g. buspirone is a selective agonist for serotonin 5-HT1A.

ANTAGONIST

A **receptor antagonist** is a type of receptor ligand or drug that blocks or dampens agonist-mediated responses rather than provoking a biological response itself upon binding to a receptor. In pharmacology, **antagonists** have affinity but no efficacy for their cognate receptors, and binding will disrupt the interaction and inhibit the function of an agonist or inverse agonist at receptors. Antagonists mediate their effects by binding to the active (orthosteric = right place) site or to allosteric (= other place) sites on receptors, or they may interact at unique binding sites not normally involved in the biological regulation of the receptor's activity. Antagonist activity may be reversible or irreversible depending on the longevity of the antagonist–receptor complex, which, in turn, depends on the nature of antagonist–receptor binding. The majority of drug antagonists achieve their potency by competing with endogenous ligands or substrates at structurally defined binding sites on receptors.

Types

Competitive: Competitive antagonists (also known as surmountable antagonists) reversibly bind to receptors at the same binding site (active site) as the endogenous ligand or agonist, but without activating the receptor. Agonists and antagonists "compete" for the same binding site on the receptor. Once bound, an antagonist will block agonist binding. The level of activity of the receptor will be determined by the relative affinity of each molecule for the site and their relative concentrations. High concentrations of a competitive agonist will increase the proportion of receptors that the agonist occupies; higher concentrations of the antagonist will be required to obtain the same degree of binding site occupancy. The interleukin-1 receptor antagonist, IL-1Ra is an example of a competitive antagonist.

Non-competitive: The term "non-competitive antagonism" (sometimes called non-surmountable antagonists) can be used to describe two distinct phenomena: one in which the antagonist binds to the active site of the receptor, and one in which the antagonist binds to an allosteric site of the receptor. While the mechanism of antagonism is different in both of these phenomena, they are both called "non-competitive" because the end-results of each are functionally very similar. Non-competitive antagonists reduce the magnitude of the maximum response that can be attained by any amount of agonist. This property earns them the name "non-competitive" because their effects cannot be negated, no matter how much agonist is present.

Uncompetitive: Uncompetitive antagonists differ from non-competitive antagonists in that they

require receptor activation by an agonist before they can bind to a separate allosteric binding site. This type of antagonism produces a kinetic profile in which "the same amount of antagonist blocks higher concentrations of agonist better than lower concentrations of agonist". Memantine, used in the treatment of Alzheimer's disease, is an uncompetitive antagonist of the NMDA receptor.

Silent antagonists: Silent antagonists are competitive receptor antagonists that have zero intrinsic activity for activating a receptor. They are true antagonists, so to speak. The term was created to distinguish fully inactive antagonists from weak partial agonists or inverse agonists.

Partial agonists: Partial agonists are defined as drugs that, at a given receptor, might differ in the amplitude of the functional response that they elicit after maximal receptor occupancy. Although they are agonists, partial agonists can act as a competitive antagonist in the presence of a full agonist, as it competes with the full agonist for receptor occupancy, thereby producing a net decrease in the receptor activation as compared to that observed with the full agonist alone. Clinically, their usefulness is derived from their ability to enhance deficient systems while simultaneously blocking excessive activity. Exposing a receptor to a high level of a partial agonist will ensure that it has a constant, weak level of activity, whether its normal agonist is present at high or low levels. In addition, it has been suggested that partial agonism prevents the adaptive regulatory mechanisms that frequently develop after repeated exposure to potent full agonists or antagonists. Buprenorphine, a partial agonist of the μ-opioid receptor, binds with weak morphine-like activity and is used clinically as an analgesic in pain management and as an alternative to methadone in the treatment of opioid dependence.

Inverse agonists: An inverse agonist can have effects similar to those of an antagonist, but causes a distinct set of downstream biological responses. Constitutively active receptors that exhibit intrinsic or basal activity can have inverse agonists, which not only block the effects of binding agonists like a classical antagonist but also inhibit the basal activity of the receptor. Many drugs previously classified as antagonists are now beginning to be reclassified as inverse agonists because of the discovery of constitutive active receptors. Antihistamines, originally classified as antagonists of histamine H_1 receptors have been reclassified as inverse agonists.

BT 8305 CELL BIOLOGY

UNIT-IV

SIGNAL TRANSDUCTION

PART - A

1. Define signaling molecules

General term for any extra cellular (or) intracellular molecule involved in mediating the response of a cell to its external environment or other cells is called signaling molecules.

2. Define receptor.

- Any protein that binds specific extra cellular signaling molecules (ligand) and then initiates a cellular response.
- Receptors for steroid hormones, which diffuse across the plasma membrane, are located within the cell,
- Receptors for water – soluble hormones, peptide growth factors, and neurotransmitters are located in the plasma membrane with their ligand – binding domain exposed to the external medium.

3. Give the types of receptors.

Three types of receptors

1. Cell membrane receptors
2. Cytoplasmic receptors
3. Nucleus receptors

4. Give one example of cytosolic receptor. (AU Nov. 2017)

The class of nuclear receptors located in the cell nucleus and cytoplasm and the IP_3 receptor located on the endoplasmic reticulum.

5. Define signal transduction.

- Conversion of a signal from one physical (or) chemical from into another.
- In cell biology commonly refers to the sequential process initiated by binding of as extra cellular signal to a receptor and culminating in one (or) more specific cellular responses.

6. Give overview of extra cellular signaling.

The extra cellular signals usually involve six steps;

(i) Synthesis
(ii) Release of the signaling molecule by the signaling cell
(iii) Transport of the signal to the target cell
(iv) Detection of the signal by a specific receptor protein
(v) A change in cellular metabolism, function (or) development triggered by the receptor signal complex; and
(vi) Removed of the signal, which often terminates the cellular response.

7. Define pheromones.

- In many eukaryotic micro organisms (e.g. Yeast, slime molds, and protozoan), secreted molecules co-ordinate the aggregation of free –living cells for sexual mating or differentiation under certain environmental conditions.
- These chemicals released by one organism that can alter the behavior (or) gene expression of other organisms of the same species are called pheromones.

8. What is endocrine signaling?

- In endocrine signaling, signaling molecule, called hormones, act on target cells distant from their site of synthesis by cells of endocrine organs.
- In animals, an endocrine hormone usually is carried by the blood from its site of release to its target.

9. What is Autocrine signaling? (AU Nov. 2017)

- In Autocrine signaling, cells respond to substances that they themselves release.
- Many growth factors act in this fashion, and cultured cells often secrete growth factors that

stimulate their own growth and proliferation.

- This type of signaling is particularly common in tumor cells.

10. Define Paracrine signaling.

(Or)

Explain paracrine mode of action of a hormone (AU Nov. 2016)

- The signaling molecules released by a cell only affect target cells in close proximity to it.
- The conduction of as electric impulse from one nerve cell to another or from a nerve cell to a muscle cell (including (or) inhibiting muscle contraction) occurs via paracrine signaling.
- The role of this type of signaling mediated by neurotransmitters.

11. What is role of ligand in cell signaling?

- The cellular response to a particular extra cellular signaling molecule depends on its binding to a specific receptor protein located on the surface of a target cell (on in its nucleus (or) cytosol.
- The signaling molecule (a hormone, pheromone, (or) neurotransmitter) acts as a ligand, which binds to, (or) 'fits', a site on the receptor.
- Binding of a ligand to its receptor causes a conformational change in the receptor that initiates a sequence of reactions leading to a specific cellular response.

12. Define Second messenger Hormone.

- An intracellular signaling molecule whose concentration increases (or decrease) in response to binding of an extra cellular ligand to a cell – surface receptor.
- Examples: CAMP, Ca^{2+}, diacylglycerol (DAG), and inositol 1,4,5-triphosphate (IP_3).

13. Give the classified Based on their solubility and Receptor location in Hormones.

(i) Small lipophilic molecule that diffuse across the plasma membrane and interact with intracellular receptors.

(ii) Hydrophilic (or) lipophilic molecules that bind to cell – surface receptors

14. Give the four Major classes of cell – surface receptors.

a) G protein – Coupled receptors
b) Ion – channel receptors
c) Tyrosine kinase-linked receptors
d) Receptors with intrinsic enzymatic activity.

15. How are second messengers important? (AU Nov. 2016)

- **Second messengers** are intracellular signaling molecules released by the cell to trigger physiological changes such as proliferation, differentiation, migration, survival, and apoptosis.
- Secondary messengers are therefore one of the initiating components of intracellular signal transduction cascades.
- Examples of second messenger molecules include cyclic AMP, cyclic GMP, inositol trisphosphate, diacylglycerol, and calcium.

16. What are protein kinase and its role?

- Activation of all cell-surface receptors leads to changes in protein phosphorylation through the activation of protein kinases.
- In Some Cases kinases are part of the receptor itself, and in others they are found in the cytosol or associated with the plasma membrane.
- Animal cells contain two types of protein kinase; those directed towards tyrosine and those directed towards either serine (or) threonine.

17. What are Adapter proteins?

- Many signal – transduction pathways contain large multiprotein signaling complexes, which often are held together by adapter proteins.
- Adapter proteins do not have catalytic activity, nor do they directly activate effectors proteins.

- They contain different combinations of domains, which function as docking sites for other proteins.

18. Define specificity of receptor.

- Binding of a hormone to a receptor involves the same types of weak interactions – ionic and vander walls bonds and hydrophobic interactions – that characterize the specific binding of a substrate to an enzyme.
- The specificity of a receptor refers to its ability to distinguish closely related substances
- Eg., the insulin receptor-, binds insulin and a related hormone called insulin – like growth factor but not other peptide hormones.

19. Write the M.M. equation using Hormone binding receptors.

Hormone binding usually can be viewed as a simple reversible reaction,

R- - H → RH

Which can be described by the equation

$K_D = \frac{[R][H]}{[RH]}$ where [R] and [H] are the concentrations of free receptor and hormone (ligand), respectively, and [RH] is the concentration of the receptor – hormone complex. K_D the dissociation constant of the receptor – ligand complex, measures the affinity of the receptor for the ligand. This binding equation can be rewritten as

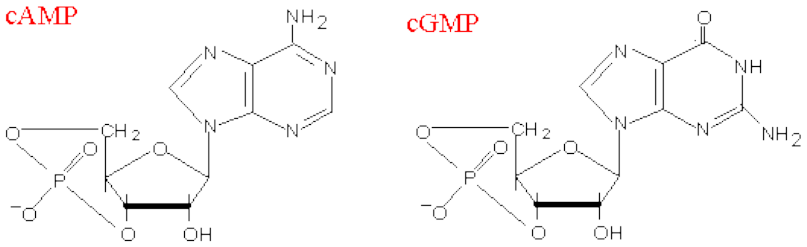
$$\frac{[RH]}{R_T} = \frac{1}{1 - H_D/[H]}$$

R_T is the sum of free and bound receptors. [R]+ [RH]

20. Define affinity labeling.

- Cell-surface hormone receptors often can be identified and followed through isolation procedures by affinity labeling.
- In this technique, cells are mixed with an excess of a radio labeled hormone to saturate the hormone – binding sites on its specific receptor.
- After unbound hormone is washed away, the mixture is treated with a chemical agent that covalently cross – links the bound labeled hormone to the receptor.

21. Give structure of CAMP and cGMP.



22. What is GT Pase Switch Proteins?

- A large group of GTP – binding proteins act as molecular switches in Signal – transduction pathways.
- These proteins are turned ‘on’ when bound to GTP and turned ‘off’ when bound to GDP.
- In the absence of a signal, the protein is bound to GDP.
- Signals activate the release of GDP, and the subsequent binding to GTP over GDP is favored by the higher concentrations of GTP in the cell.

23. Write the two classes of GTPase Switch proteins.

- There are two classes of GTPase Switch proteins, trimeric G proteins, which as noted already are

directly coupled to certain receptors, and monomeric Ras and Ras – like proteins.

- Both classes contain regions that promote the activity of specific effector proteins by direct protein – protein interactions.
- These regions are in their active conformation only when the switch protein is bound to GTP.
- G-Proteins are coupled directly to activated receptors, where as Ras is linked only indirectly via other proteins the two classes of GTP – binding proteins also are regulated in very different ways.

24. What are G-Protein coupled receptors? Give example.

- Ligand binding activates a G – protein, which in turn activates (or) inhibits an enzyme that generates a specific second messenger or modulates an ion channel, causing a change in membrane potential.
- The receptors for epinephrine, Serotonin, and glucagons are example.

25. Explain tyrosine kinases receptor.

- The receptors for insulin and many growth factors are ligand – triggered protein kinases, the ligand binds as a dimer, leading to dimerization of the receptor and activation of its kinase activity.
- These receptors often referred to as receptor Serine / threonine kinases or receptors tyrosine kinases – auto – phosphorylated residues in their own cytosolic domain and also a phosphorylate various substrate proteins.

26. Define Ras protein.

A monomeric GTP-binding protein that functions in intracellular signaling pathways and is activated by ligand binding to receptor tyrosine kinases and other cell – surface receptors.

27. What is Ligand?

Any molecule, other than as enzyme substrate, that binds tightly and specifically to a macromolecule, usually a protein, forming a macromolecule – ligand complex.

28. Define nuclear receptor.

- General term for intracellular receptor that bind lipid – soluble hormone (e.g. steroid hormone); also called steroid receptor super family.
- Following ligand binding, the hormone receptor complex translocates to the nucleus and functions as a transcription factor.

29. What is GTPase Super family?

- The group of GTP-binding proteins that cycle between an inactive state with bound GDP and an active state with bound GTP.
- These proteins – including G proteins, Ras proteins and certain polypeptide elongation factors – function as intracellular switch proteins.

PART-B

1. Explain overview of extra cellular signaling and classification of extra cellular signaling.

Communication by extra cellular signals usually involves six steps;

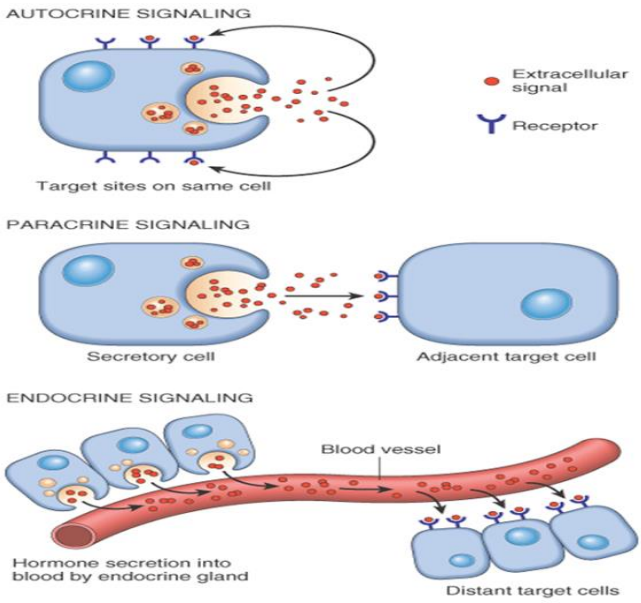
(i) synthesis
(ii) release of the signaling molecule by the signaling cell
(iii) transport of the signal to the target cell
(iv) detection of the signal by a specific receptor protein.
(v) A change in cellular metabolism, function, or development triggered by the receptor-signal complex; and
(vi) Removal of the signal, which often terminates the cellular response.

Signaling by extra cellular, secreted molecules can be classified into three types.
Endocrine, paracine, (and) autocrine – based on the distance over which the signal acts.
In addition, certain membrane – bound proteins on one cell can directly signal an adjacent cell.

- In **endocrine signaling** – signaling molecule, called hormones, act on target cells distant from their site of synthesis by cells of endocrine organs.
  - In animals, an endocrine hormone usually is carried by the blood from its site of release to its target.
- In **paracrine signaling** molecules released by a cell only affect target cells in close proximity to it. The conduction of as electric impulse from one nerve cell to another or form a nerve cell to a muscle cell occurs via paracrine signaling.

- Eg., The role of this type of signaling, mediated by neurotransmitters in transmitting nerve impulses.
- In **autocrine signaling**, cells respond to substances that they themselves release.
- Many growth factors act in this fashion, and cultured cells often secrete growth factors that stimulate their own growth and proliferation.
- This type of signaling is particularly common in tumor cells, many of which over produce and release growth factors that stimulate inappropriate, unregulated proliferation of themselves as well as adjacent nontumor cells; this process may lead to formation of tumor mass.

Some compounds can act in two or even three types of cell – to – cell signaling. Certain small amino acid derivatives, such as epinephrine, function both as neurotransmitters and as systemic hormones.



2. Explain the receptor classification Based on their solubility and location.

(Or)

Discuss in detail the different classes of receptors. (AU Nov. 2016)

Most hormones fall into two broad categories

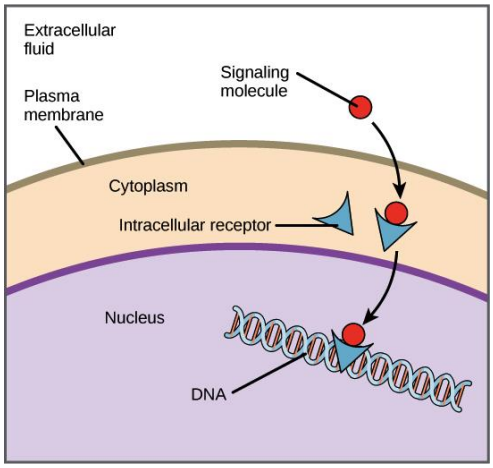
(i) Small lipophilic molecules that diffuse across the plasma membrane and interact with intracellular receptors; and

(ii) Hydrophilic (or) lipophilic molecules that bind to cell-surface receptors.

Lipophilic Hormones with Intracellular Receptors

Many lipid soluble hormones diffuse across the plasma membrane and interact with receptors in the cytosol (or) nucleus the resulting hormone. Receptor complexes bind to transcription control regions in DNA thereby affecting expression of specific genes.

(a) **Intracellular receptors** **(b) Cell surface receptors**



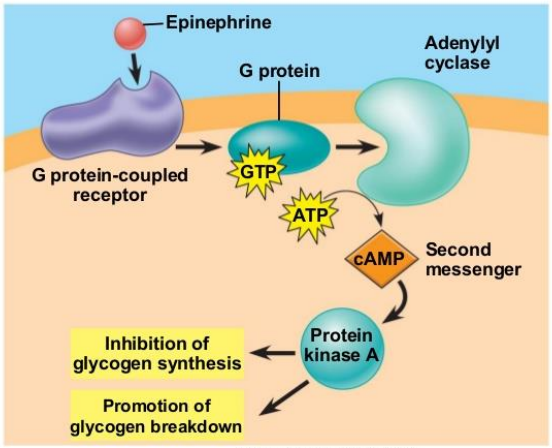


Figure 6 (b): Epinephrine Cascade

Figure some hormones bind to intracellular receptors; others, to cell-surface receptors.

a) Steroid hormones thyroxine, and retinoids being lipophilic, is transported by carrier proteins in the blood. After dissociation from these carriers, such hormones diffuse across the cell membrane and bind to specific receptors in the cytosol or nucleus. The receptor – hormone complex then acts on nuclear DNA to after transmission of specific genes.

b) Polypeptide hormones and catecholamines (e.g., epinephrine), which are water soluble, and catecholamines, which are lipophilic, all bind to cell – surface receptors. This binding triggers an increase or decreases in the cytosolic concentration of second messengers (e.g. cAMP, Ca^{2+}), activation of a protein kinase, or a change in the membrane potential.

Hormones of this type include the steroids (e.g., cortisol, progesterone, estradial and testosterone thyroxine, and retinoic acid.

This receptor – steroid complexes also may affect the stability of specific mRNAS steroids are effective for hours or days and often influence the growth and differentiation of specific tissues.

For example, estrogen and progesterone, the female sex hormones, stimulate the production of egg. White hormones in chickens and cell proliferation in the hen oviduct.

Water – soluble Hormones with cell – surface Receptors:-

Water – soluble Signaling molecules cannot diffuse across the plasma membrane, they all bind to cell – surface receptors. This large class of compounds is composed of two groups,

(i) Peptide hormones, such as insulin, growth factors, and glucagon, which range in size from a few aminoacids to protein – size compounds, and

(ii) Small charged molecules, such as epinephrine and histamine, that are derived from aminoacids and function as hormones and neurotransmitters.

Lipophilic Hormones with cell – surface Receptors:-

- The primary lipid – soluble hormones that bind to cell – surface receptors are the prostaglandins.
- There are at least 16 different prostaglandins in nine different chemical classes, designated. PGA –PGI. Prostaglandins are part of an even larger family of 20 carbons. Containing hormones called eicosanoid hormones.
- In addition to prostaglandins, they include prostacyclins, thromboxanes, and leukotrienes. Eicosonoid hormones are synthesized form a common Precursor, arachidonic acid, Arachidonic acid is generated form phospholipid and diacylglycerol.

3. Describe the cell surface Receptors and its types.

The different types of cell – surface receptors that interact with water – soluble lingands are schematically represented. Binding of ligand to some of these receptors induces second messenger formation, whereas ligand binding to others does not. For Convenience, we can sort

this receptor into four classes;

a. G-Protein – Coupled receptors:-

Ligand binding activates a G protein, which in turn activities or inhibit an enzyme that generates a specific second messenger or modulates an ion channel, causing a change in membrane potential. The receptors for epinephrine, serotonin, and glucagon.

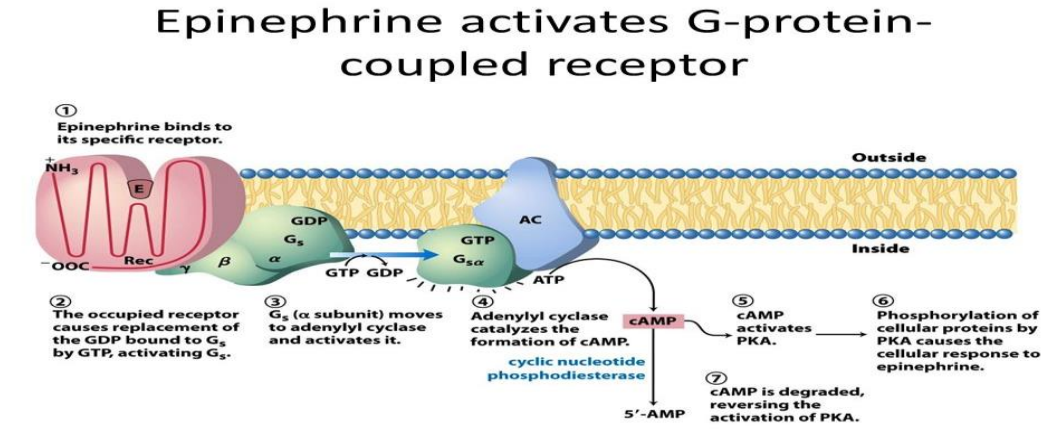
b. Ion – channel receptors:-

Ligand binding changes the conformation of the receptors so that specific ions flow through it; the resultant ions flow through it; the resultant ion movements after the electric potential across the cell membrane. The acetylcholine receptor at the nerve-muscle junction is an example.

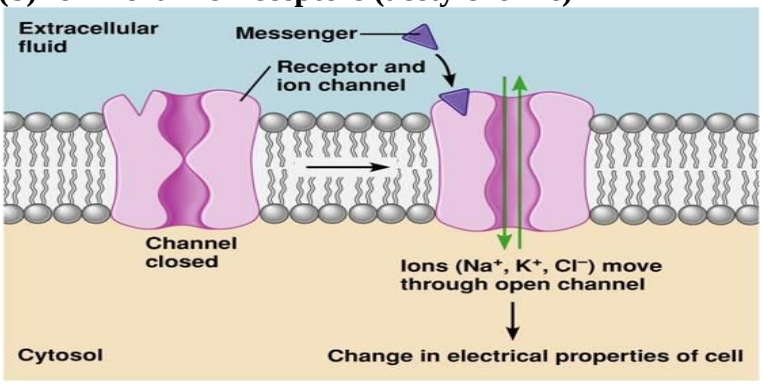
C. Tyrosine kinase – linked receptors:-

These receptors lack intrinsic catalytic activity, but ligand binding stimulates formation of a dimeric receptor which then interacts with and activities one or more cytosolic protein – tyrosine kinase. The receptors for many cytokines, the interferones, and human growth factor are of this type. This tyrosine kinase – linked receptors sometimes are referred to as the cytokine – receptor super family.

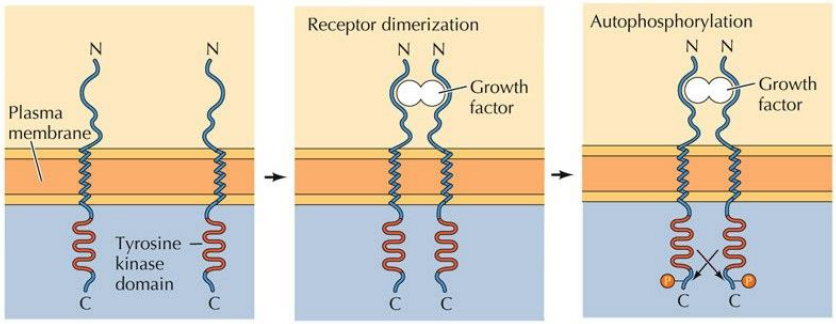
(a) G protein-coupled receptors (epinephrine, glucagons, serotonin)



(b) Ion – channel receptors (acetylcholine)



(c) Tyrosine kinase – linked receptors (erythropoietin, interferons)



(d) Receptors with intrinsic enzymatic activity

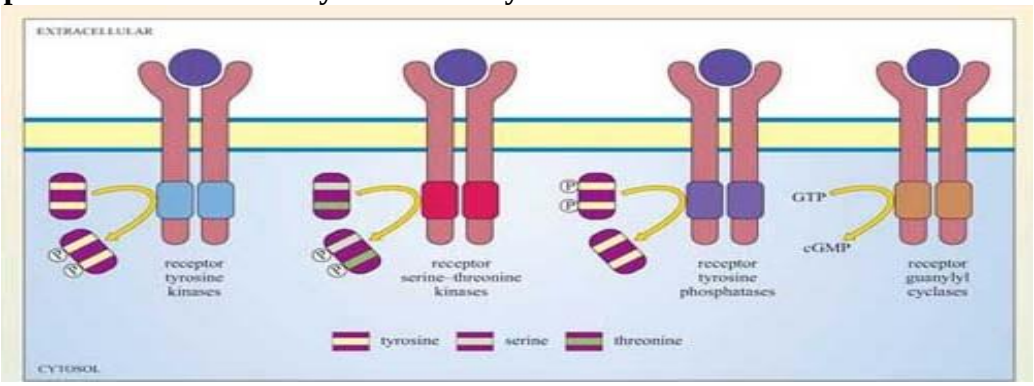


Figure Four classes of ligand – triggered cell-surface receptors. Common ligands for each receptor type are listed in parentheses

(a) G protein – linked receptors. Binding of ligand (maroon) triggers activation of a G protein, which then bind to and activates an enzyme that catalyzes synthesis of a specific second messenger

(b) ion-channel for ion flow

(c) Tyrosine kinase – linked receptors. Ligand binding causes formation of a homodimer or heterodimer, triggering the binding and activation of a cytosolic protein-tyrosine kinase. The activated kinase phosphorylatstyrosines in the receptor; substrate proteins then bind to these phosphotyrosine residues and are phosphorylated

(d) Receptors with intrinsic ligand – triggered enzymatic activity in the cytosolic domain some activated receptors are monomers with guanine cyclase activity and can generate the second messenger cGMP (left). The receptors for many growth factors have intrinsic protein –tyrosine kinase activity (right). Ligand binding to most such receptor tyrosine kinase (RTKs) cause formation of an activated homodimer, which phosphorylates several residues in its own cytosolic domain as well as certain substrate proteins.

d. Receptors with intrinsic enzymatic activity:

- Several types of receptors have intrinisic catalytic activity, which is activated by binding of ligand.
- Receptors catalyze conversion of GTP to cGMP; others act as protein phosphatases, removing phosphate groups from phosphotyrosine residues in substrate proteins, there by modifying their activity.
- The receptor for insulin and many growth factors are ligand – triggered protein kinases in most cases, the ligand binds as a dimmer, leading to dimerization of the receptor and activation of its kinase

activity.

- These receptors Often referred to as receptor serine / threonine kinases (or) receptor tyrosine kinases – autophosphorylate residues in their own cytosolic domain and also can phosphorylate various substrate proteins.

4. Describe the G protein mechanism in signaling process. (AU Nov. 2016)

The cell-surface receptors are coupled to a trimetric signal –transducting G Proteins. Ligand binding to these receptors activates their associated G proteins, which this then activates an effector enzyme to generate as intracellular second messenger.

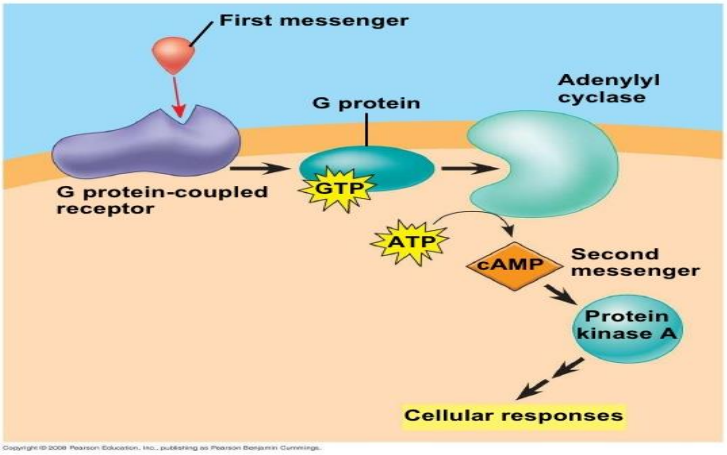
All G protein-coupled receptors (GPCRs) contain seven membrane – spanning regions with their N-terminal segment on the exoplasmic face and their C-terminal segment on the cytosolic face of the plasma membrane. This large receptor family includes light-activated receptor (rhodopsins) in the eye and literally thousand of odorant receptors in the mammalian nose, as well as numerous receptors for various hormones and neurotransmitters.

Hormone binding to the receptor initiates a series of events leading to phosphorylation of specific substrate proteins, which mediate the cellular responses such as changes in the activity of metabolic enzymes, gene expression, and cytoskeletal structures. The kinase cascade entails sequential activation of specific protein kinases induced by a signal from activated Ras protein. Second messengers (SM) play a role in some

Stimulation of β- Adrenergic Receptors Leads to a Rise in cAMP:-

- Many of the very different tissue specific response induced by binding of epinephrine to β - adrenergic receptors are mediated by a rise in the intracellular level of cAMP,
- -resulting from activation of adenylyl cyclases ,
- This converts ATP to cAMP and pyrophosphate (PPi).
- This membrane bound enzyme has two catalytic domains on the cytosolic face of the plasma membrane that can bind ATP in the cytosol.
- The link between hormone binding to as exterior domain of the receptor and activation of adenylyl cyclase is provided by Gs, which functions as a signal transducer.

Fig. 11-11



5. Elaborate the function of any nuclear receptor in relation to its ligand (AU Nov. 2017)

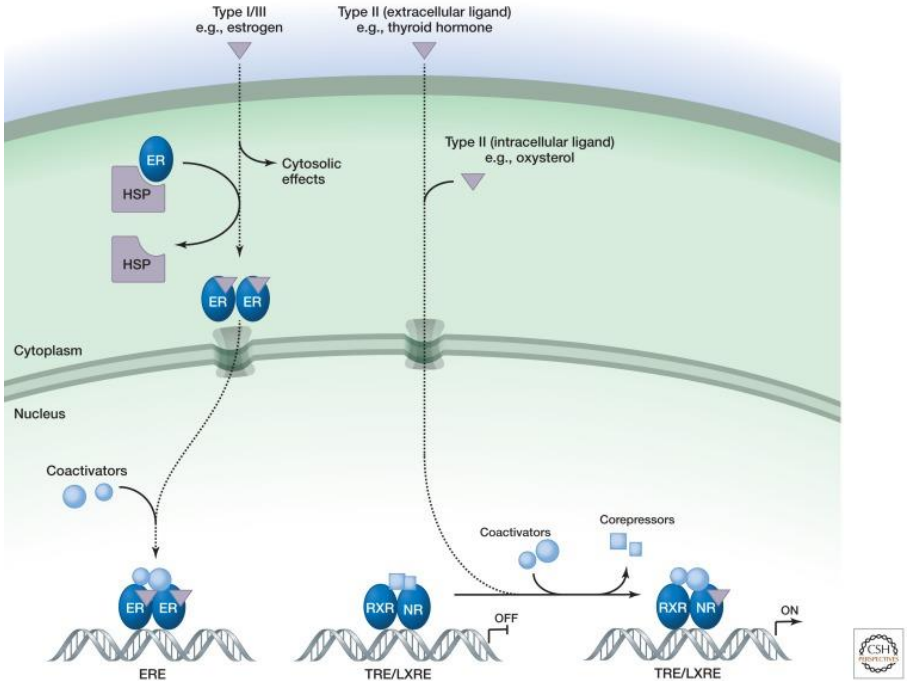
- **Nuclear receptors** are a class of proteins found within cells that are responsible for sensing steroid and thyroid hormones and certain other molecules. In response, these receptors work with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism.
- Ligands that bind to and activate nuclear receptors include lipophilic substances such as endogenous hormones, vitamins A and D, and xenobiotic endocrine disruptors.
- Small lipophilic substances such as natural hormones diffuse through the cell membrane

and bind to nuclear receptors located in the cytosol (type I NR) or nucleus (type II NR) of the cell.

- Binding causes a conformational change in the receptor which, depending on the class of receptor,
- This triggers a cascade of downstream events that direct the NRs to DNA transcription regulation sites which result in up or down-regulation of gene expression.
- They generally function as homo/heterodimers.

Type I

- Ligand binding to type I nuclear receptors in the cytosol results in the dissociation of heat shock proteins, homo-dimerization, translocation (*i.e.*, active transport) from the cytoplasm into the cell nucleus, and binding to specific sequences of DNA known as hormone response elements (HREs).
- Type I nuclear receptors bind to HREs consisting of two half-sites separated by a variable length of DNA, and the second half-site has a sequence inverted from the first (inverted repeat).
- Type I nuclear receptors include members of subfamily 3, such as the androgen receptor, estrogen receptors, glucocorticoid receptor, and progesterone receptor.



6. (a) Explain Autocrine signaling with example.

Autocrine signaling is a form of signaling in which a cell secretes a chemical messenger (called the autocrine agent) that signals the same cell.

Examples

An example of an autocrine agent is the **cytokine interleukine-1** in **monocytes.** When this is produced in response to external stimuli, it can bind to cell – surface **receptors** on the same cell that produced it.

Another example occurs in activated T cell lymphocytes, i.e. when a T cell is induced to mature by binding to a peptide:MHC complex on a professional antigen presenting cell and by

the B7:CD28 contimulatory signal. Upon activation, "low affinity" IL-2 receptors are replaced by "highly affinity" IL-2 receptors consisting ofα,β and γ chains. The cell then releases IL-2 which binds to its own new IL-2 receptors, causing self-stimulation and ultimately a monoclonal population of T cells. These T cells can then go on to perform effector functions such as microphage activation, B cell activation, and cell mediated cytoxicity.

b. Describe paracrine signaling.

Paracrine signaling is a form of cell signaling in which the target cell is close to ("para" = alongside of or next to, but this strict prefix definition is not meticulously followed here) the signal releasing cells.

The signal chemical is called the **paracrine agent.**

The distinction is sometimes made between paracrine and autocrine signaling. In both types of signaling, the signal is limited to other cells in the local area. However, paracrine signaling affects cells of a different type than the cell performing the secretion, while autocrine signaling affects cells of the same type.

Reason for degradation

Sometimes, the reason that the effects are limited to a local area is because the signal chemical is broken down too quickly to be carried to other parts of the body.

Alternatively, the signal may only reach nearby cells for one of the following reasons:

- (1) The nearby cells take up the signal at a very high rate, leaving little signal free to travel further.
- (2) The signal gets stuck in the extracelllular – matrix, or structure surroundings the signal releasing cell, and thus the signal is unable to travel far from the signal releasing cell.

Examples

Examples of paracrine signaling agents include **growthfactor** and **clotting factors.** Growth factor signaling plays an important role in many aspects of development.

In mature organisms paracrine signaling functions include responses to allergens, repairs to damaged tissue, formation of **scar tissue**, and **clotting.**

Over production of some paracrine growth factors has been linked to the development of **cancer.**

Other examples of paracrine agents are **somatostatin** and **histamine.**

7. Explain Autocrine and endocrine actions.

Some paracrine agents also have **autocrine** or **endocrine** actions as well.

For example, **testosterone** secreted form the **tests** acts in an endocrine manner to stimulate peripheral events (e.g. muscle growth), and in paracrine manner to stimulate **spermatogenesis** in the adjacent **seminiferous tubules.**

Endocrine system

The **endocrine system** is a control system of **ductless glands** that secrete **hormones** that circulate within the body via the **bloodstream** to affect cells with in specific **organs**. It is also instrumental in regulating **mood, growthand development, tissue function,** and **metabolism,** as well as sending messages and acting on them. Typical endocrine glands are **pituitary,thyroid,** and **adrenal** glands, but not **exocrine glands** such as **salivary glands**, **sweat glands** and **glands** within the **gastrointestinal tract.**

The field of **medicine** that deals with disorder of endocrine glands is **endocrinology**, a branch of the wider field of **internal medicine**.

8. Explain the role of any second messenger in Signal Transduction. (AU Nov. 2017)

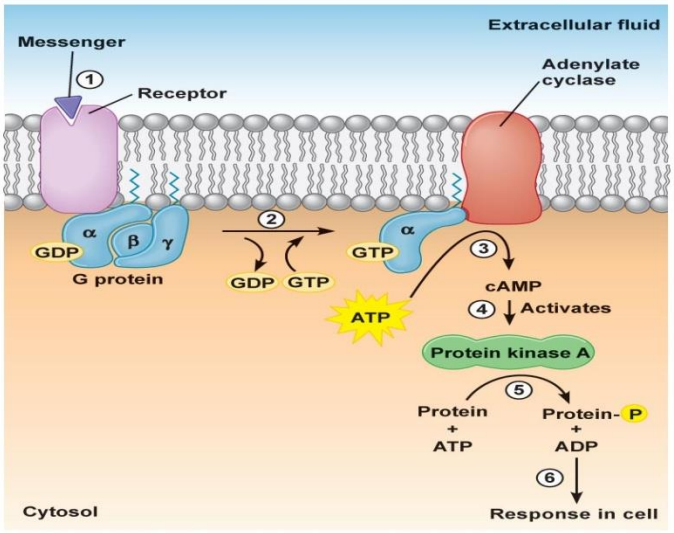
Second messengers are molecules that relay signals received at receptors on the cell surface — such as the arrival of protein hormones, growth factors, etc. — to target molecules in the cytosol and/or nucleus.

There are 3 major classes of second messengers:

1. cyclic nucleotides (e.g., **cAMP** and **cGMP**)
2. inositol trisphosphate (**IP_3**) and diacylglycerol (**DAG**)
3. calcium ions (Ca^{2+})

cAMP second messenger system:

1. Messenger binds to receptor and activates a G_s protein. (The G_i protein that inhibits adenylate cyclase is also possible in this step.)
2. □□subunit is released and activates the enzyme adenylate cyclase.
3. ATP □ cAMP by **adenylate cyclase.**
4. cAMP activates **protein kinase A (cAMP-dependent protein kinase).**
5. A protein is phosphorylated by protein kinase A
6. Phosphorylated proteins' activity is now altered. Cellular response results.



Response terminates as:

1. cAMP is degraded by **cAMP phosphodiesterase**. (The effects of caffeine are associated with its inhibition of cAMP phosphodiesterase and this results in an increase in levels of cAMP.)
2. Protein is dephosporylated by **phosphoprotein phosphatases**.

9. Explain the role of the following in Signal transduction (i) DAG AND IP_3 (ii) Ca^{++} and (iii) cGMP

(i)Inositol trisphosphate (IP_3) and diacylglycerol (DAG)

1. Messenger binds to receptor and activates G protein.
2. GTP-□□subunit is released and activates **phospholipase C.**

3. Phospholipase C catalyzes conversion of **phosphatidylinositol-4,5-biphosphate (PIP_2)** to **diacylglycerol (DAG)** and **inositol triphosphate (IP_3)** each of which serves as a second messenger:

DAG as a second messenger

4a. DAG remains in the membrane and activates **protein kinase C**.

5a. Protein kinase C catalyzes the phosphorylation of a protein.

6b. Phosphorylated protein causes an effect in the cell.

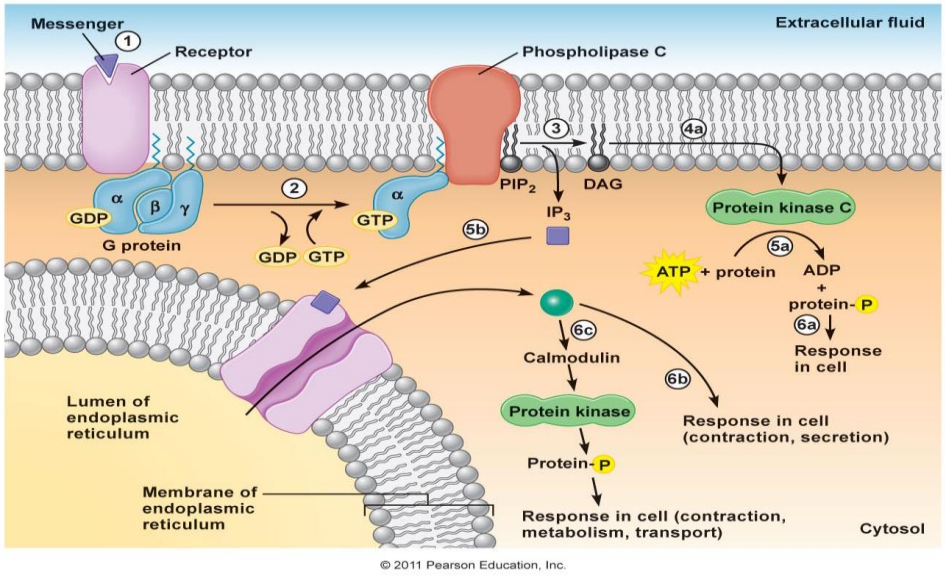
IP_3 as a second messenger (at the same time)

4b. IP_3 moves into cytosol.

5b. IP_3 triggers release of Ca^{++} from the endoplasmic reticulum.

(ii)Calcium as a second messenger

1. act on proteins directly to stimulate contraction or secretion or,
2. Binds to calmodulin to activate a protein kinase.



(iii)Cyclic GMP (cGMP)

Cyclic GMP is synthesized from the nucleotide GTP using the enzyme **guanylyl cyclase**.
Cyclic GMP serves as the second messenger for

- atrial natriuretic peptide (ANP), nitric oxide (NO), the response of the rods of the retina to light.
- Some of the effects of cGMP are mediated through **P**rotein **K**inase **G** (**PKG**) — a c**G**MP-dependent protein kinase that phosphorylates target proteins in the cell.

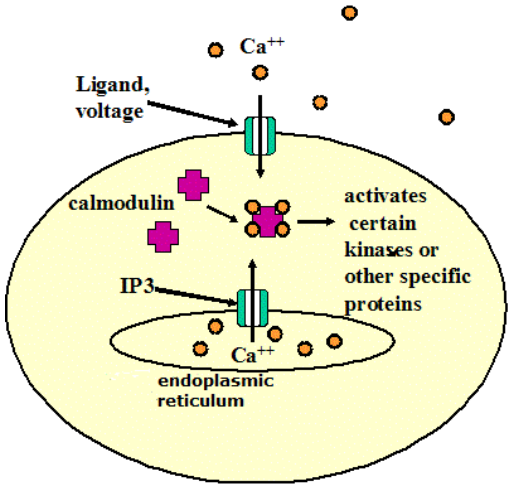
(ii)Calcium as a second messenger

Ca^{++} and Calmodulin

One of the most important **second messengers** is **Ca^{++}**.

In general, Ca^{++} enters the cytosol through gated ion channels in the plasma membrane and/or the endoplasmic reticulum. The ion channels in the plasma membrane, for example, could be voltage-gated, ligand-gated or temperature-gated. Those in the endoplasmic reticulum typically are gated by IP3.

Once in the cytosol, the Ca^{++} typically binds to a small protein, **calmodulin**. Once four Ca^{++} bind to calmodulin, it activates specific proteins inside the cell, such are certain **protein kinases**.



UNIT V
TECHNIQUES USED TO STUDY CELLS
Part A

26. Define acquisition

- The process of collecting data from samples using the flow cytometer is termed 'acquisition'.
- Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer.

27. What is Cell fractionation

Cell fractionation is where a cell are broken up and its components and organelles are separated.

28. Define flow cytometry

- Flow cytometry is an extremely powerful technology that allows the individual measurement of physical and chemical characteristics of particles as they pass one by one through a light source.
- Flow sorting is a process that allows the physical separation of a cell or particle of interest from a heterogeneous population.

29. Explain the principle of flow cytometry. (AU Nov.2016)

- The basic principle of flow cytometry is the passage of cells in single file in front of a laser so they can be detected, counted and sorted.
- Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths.
- The fluorescence can then be measured to determine the amount and type of cells present in a sample.
- Up to thousands of particles per second can be analysed as they pass through the liquid stream.

30. Enlist the steps involved in cell fractionation

- Homogenization
- Differential centrifugation
- Further separation and purification by density gradient centrifugation
- Collection of fractions
- Analysis of fractions

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31. Define Homogenization or cell disruption.

The process of breaking open cells is homogenization. A variety of different methods are available; the method chosen depends on the type of experiment and the type of sample

32. What are the methods for homogenization?

- Chemical : alkali, organic solvents, detergents
- Enzymatic : lysozyme, chitinase
- Physical : osmotic shock, freeze/thaw
- Mechanical : sonication, homogenization, French press

33. What is differential centrifugation?

- Differential centrifugation is the process where a homogenate (soup of tissue and cells)undergoes repeat centrifugations and increasing centrifugal force.
- Centrifugation is the use of increased gravity to quicken the precipitation of substances tot the bottom.

34. Write the principle of preparative ultra centrifuge.

- Sample is contained in tubes that are inserted into a ring of cylindrical holes in a metal rotor.
- Rapid rotation of the rotor generates enormous centrifugal forces, which cause particles in the sample to sediment.
- The vacuum reduces friction, preventing heating of the rotor and allowing the refrigeration system to maintain the sample at 4°C.
- When a centrifugal force is applied to an aqueous mixture, components of larger size and density will sediment faster.
- Low speed centrifugation is used to separate intact cells from medium.
- High speed centrifugation can be used to separate subcellularcomponents.

35. How will you collect fractions during cell fractionation?

Collecting Fractions-keeping samples pure and intact

I. By hand: puncture sidewall of centrifuge tube with needle and withdraw fractions through syringe
II. Machine: gradient uploader; introduces very dense, non-miscible medium into bottom of tube, pushes fractions up to be collected from top
III. If no pellet, can collect fractions through hole in bottom of tube.

36. What are methods for analyses of fractions?

Analysis of fractions-need to identify and quantify the purified fractions, so that they can be used successfully in downstream applications. The methods are:

1. Light or electron microscopy
2. Biochemical-determine presence of marker enzymes
3. Assay for a protein marker with an antibody (western)
4. Determine the protein concentration by using a spectrophotometer, e.g. Bradford assay
5. Determine specific activity (the ratio of activity of the enzyme of interest to the protein concentration

37. Give some applications of cell fractionation

- Scientists use this tool to increase their knowledge of organelle functions.
- To be able to do so they isolate organelles into pure groups, such as isolating the mitochondria or the nucleus.
- Differential Centrifugation allows us to look at each organelle within the cell.
- This helps to determine each organelles function within the cell.

38. What are the applications of flow cytometry?

- It is able to use multiparametric analysis to identify highly specific populations.
- Phenotypic characteristics can be identified by a specific antibody-antigen interaction;
- it is possible to measure the DNA content of cells, the RNA content,
- or even assess functional characteristics such as ion flux or pH or altered cell states such as apoptosis and cell death.

39. Define monoclonal antibody (MAB).

Monoclonal antibody is a single type of antibody that is produced by hybridoma cells. The AB is directed against a specific antigenic determinant (epitope)

40. List out the differences between the TEM & SEM?

TEM	SEM
1. Useful in study of detailed structure of cells.	Useful for study of three-dimensional structures.
2. The electrons will transmit through the specimen.	The electrons bombards on the surface of the specimen & scanned over the surface.
3. The finest details of structures can be seen.	3-D structures can be studied.
4. higher magnification (4,00,000 x)	Lower magnification (100,000 x)

41. Give the principle of Scanning Electron microscope (SEM)

- **Scanning Electron microscope-** The narrow electron beam rapidly moves over the surface of the specimen and the shower of secondary electrons and other types of radiation from the specimen surface.
- The secondary electrons are collected by a detector, which generates electronic signal.
- Then the signals are scanned in the manner television system to produce image.

42. Give the principle of Transmission electron microscope

- In this microscope, an electron beam from an electron gun is transmitted through an ultra-thin section of the microscopic object and the image is magnified by the electromagnetic fields.
- It is used to observe finer details of internal structures of microscopic objects like bacteria and other cells.
- The specimen to be examined is prepared as an extremely thin dry film or as an ultra-thin section on a small screen
- It is introduced into the microscope at a point between the magnetic condenser and the magnetic objective.
- The point is comparable to the stage of a light microscope.
- The magnified image may be viewed on a fluorescent screen through an airtight window or recorded on a photographic plate by an in-built camera.
- Modern variants have facility to record the photograph by digital camera.

43. List out the applications of TEM

- A Transmission Electron Microscope is ideal for a number of different fields such as life sciences, nanotechnology, medical, biological and material research, forensic analysis etc.,
- TEMs provide topographical, morphological, compositional and crystalline information.
- The images allow researchers to view samples on a molecular level, making it possible to analyze structure and texture.

44. List out the applications of SEM

- SEMs have a variety of applications in a number of scientific and industry-related fields, especially where characterizations of solid materials is beneficial.

- In addition to topographical, morphological and compositional information, a Scanning Electron Microscope can detect and analyze surface fractures, provide information in microstructures, examine surface contaminations, reveal spatial variations in chemical compositions, provide qualitative chemical analyses and identify crystalline structures.
- SEMs can be as essential research tool in fields such as life science, biology, gemology, medical and forensic science, metallurgy.
- In addition, SEMs have practical industrial and technological applications such as semiconductor inspection, production line of miniscule products and assembly of microchips for computers.

45. What is the principle of Confocal microscopy

- In confocal microscopy, a pinhole between specimen and detector is used to select information from a single focal plane, producing a sharply focussed optical slice through the specimen.
- Taking a series of optical slices from different focus levels in the specimen generates a 3D data set.

46. Write a note on the applications of confocal microscopy

- Confocal fluorescence studies provide information on the identity, size, stereo-structure, time-change, substance diffusion, and concentration of fluorescent-labeled substances.
- Intracellular or membrane-bound fluorescent dyes as well as voltage- and calcium-dependent indicators are all used to investigate the functions and activities of cells.
- In the pharmaceutical industry, confocal microscopy is now a widely applied tool for studying the cellular effects of drug candidates.
- For cellular imaging, confocal optics provide a significant improvement in spatial resolution and data quantity.
- Confocal microscopy is being applied in neurobiology for detecting microstructures and activities within neurons.
- The technique is also used in clinics for disease diagnoses, in tracing pathological changes, and in studies of angiogenesis under several conditions.
- Furthermore, researchers in genetics use confocal microscopy to trace the expression of genetically encoded fluorescent proteins.
- The field of live-cell imaging has also greatly benefited from applications of confocal microscopy.

47. What is immunostaining?

Immunostaining is a general term in biochemistry that applies to any use of an antibody-based method to detect a specific protein in a sample.

48. What are the techniques involved in immunostaining?

- Immunohistochemistry
- Flow cytometry
- Western blotting
- Enzyme-linked immunosorbent assay (ELISA) and
- Immuno-electron microscopy.

49. What is Enzyme-linked immunosorbent assay?

The enzyme-linked immunosorbent assay or ELISA is a diagnostic method for quantitatively or semi-quantitatively determining protein concentrations from blood

plasma, serum or cell/tissue extracts in a multi-well plate format (usually 96-wells per plate).

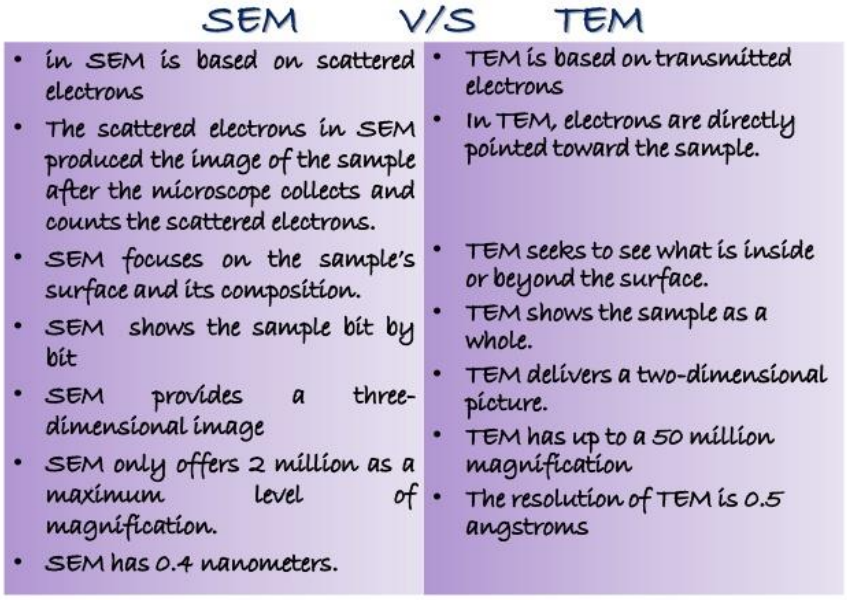
50. What are the applications of Immunostaining

- The applications of immunostaining are numerous, but are most typically used in clinical diagnostics and laboratory research.
- Clinically, IHC is used in histopathology for the diagnosis of specific types of cancers based on molecular markers.
- In laboratory science, immunostaining can be used for a variety of applications based on investigating the presence or absence of a protein, its tissue distribution, its sub-cellular localisation, and of changes in protein expression or degradation.

51. What is Western blotting

- Western blotting allows the detection of specific proteins from extracts made from cells or tissues, before or after any purification steps.
- Proteins are generally separated by size using gel electrophoresis before being transferred to a synthetic membrane via dry, semi-dry, or wet blotting methods.
- The membrane can then be probed using antibodies using methods similar to immunohistochemistry, but without a need for fixation.
- Detection is typically performed using peroxidase linked antibodies to catalyse a chemiluminescent reaction.

52. Differntiate SEM and TEM



Part B

1. Write in detail about cell fractionation technique. (AU Nov. 2016)

- Cell fractionation is where a cell is broken up and its components and organelles are separated so that scientist can observe them in isolated form.
- It can also be defined as : the separation of homogeneous sets , usually organelles, from a larger population of cells.

Cell fractionation methods

Involve the homogenization or destruction of cell boundaries by different mechanical or chemical procedures, followed by the separation of the subcellularfractions according to **mass, surface, and specific gravity.**

Steps of subcellular fractionation

1. Homogenization
2. Differential centrifugation
3. Further separation and purification by density gradient centrifugation
4. Collection of fractions
5. Analysis of fractions

1 . Homogenization

- First the cells must be broken open.
- A variety of different methods are available; the method chosen depends on the type of experiment and the type of sample (bacterial culture or mammalian tissue sample, for example).
- Detergents like SDS or Triton X disrupt the cell membrane so the contents can flow out. Subjecting the cells to ultrasound waves or sonicating them will also break them open, as will agitation in the presence of metal or glass beads.
- Blenders may work with tissue samples but will not work with bacteria or other microorganisms.

Homogenization or Cell Disruption can be done by following methods

- Chemical : alkali, organic solvents, detergents

- Enzymatic : lysozyme, chitinase
- Physical : osmotic shock, freeze/thaw
- Mechanical : sonication, homogenization, French press

Chemical Disruption

•Detergents such as TritionX-100 or NP40 can permeabilizecells by solubilizingmembranes.

•Detergents can be expensive, denature proteins, and must be removed after disruption

Sonication

A sonicator can be immersed directly into a cell suspension. The sonicator is vibrated and high frequency sound waves disrupt cells.

Homogenization

•Cells are placed in a closed vessel (usually glass). A tight fitting plunger is inserted and rotated with a downward force. Cells are disrupted as they pass between the plunger and vessel wall.

2 . Differential centrifugation

- Centrifugation is the process of isolating components of a cell.
- Differential centrifugation is the process where a homogenate (soup of tissue and cells) undergoes repeat centrifugations and increasing centrifugal force.
- Centrifugations is the use of increased gravity to quicken the precipitation of substances to the bottom.
- The centrifuge separates the cell's parts into pellet and supernatant.
- The pellets are the large cell structures that are settled at the test tube's bottom.
- The supernatant are smaller parts of the cell suspending in liquid, the supernatant is decanted and undergoes another centrifugation.
- The process is repeated and increases speed with each trial to collect successively smaller parts of a cell in pellets.

The preparative ultracentrifuge

- Sample is contained in tubes that are inserted into a ring of cylindrical holes in a metal *rotor*.
- Rapid rotation of the rotor generates enormous centrifugal forces, which cause particles in the sample to sediment.
- The vacuum reduces friction, preventing heating of the rotor and allowing the refrigeration system to maintain the sample at 4°C.
- When a centrifugal force is applied to an aqueous mixture, components of larger size and density will sediment faster
- Low speed centrifugation is used to separate intact cells from medium
- High speed centrifugation can be used to separate subcellular components

Method of Differential Centrifugation:

1. Cut tissue in an ice-cold isotonic buffer. It is cold to stop enzyme reactions, isotonic to stop osmosis and a buffer to stop pH changes.
2. Grind tissue in a blender to break open cells.
3. Filter to remove insoluble tissue
4. Centrifuge filtrate at low speeds (1000 X g for 10mins)- •This pellets the nuclei as this is the densest organelle
5. Centrifuge at medium speeds (10 000 x g for 30 mins)- •This pellets mitchondria which are the second densest organelle
6. Centrifuge at high speeds (100 000 x g for 30 mins)- •This pellets ER, golgi apparatus and other membrane fragments

7. Centrifuge at very high speeds (300 000 x g for 3hrs)- •This pellets ribosomes.

3. Buoyant density centrifugation

- The buoyant density centrifugation involves viruses with densities of 1.1-1.2 g/cm and a sucrose gradient.
- The cell suspension is added to the top of the sucrose gradient.
- In this centrifugation the densest components move fastest down the tube and stops at the sucrose density equal to its own.
- The sucrose gradient bands at the bottom contain cell components with high buoyant densities and the components at the top have low buoyant densities.

4 . Collection of fractions

Collecting Fractions-keeping samples pure and intact

1. By hand: puncture sidewall of centrifuge tube with needle and withdraw fractions through syringe
2. Machine: gradient uploader; introduces very dense, non-miscible medium into bottom of tube, pushes fractions up to be collected from top
3. If no pellet, can collect fractions through hole in bottom of tube.

5 . Analysis of fractions

Analysis of fractions-need to identify and quantify the purified fractions, so that they can be used successfully in downstream applications

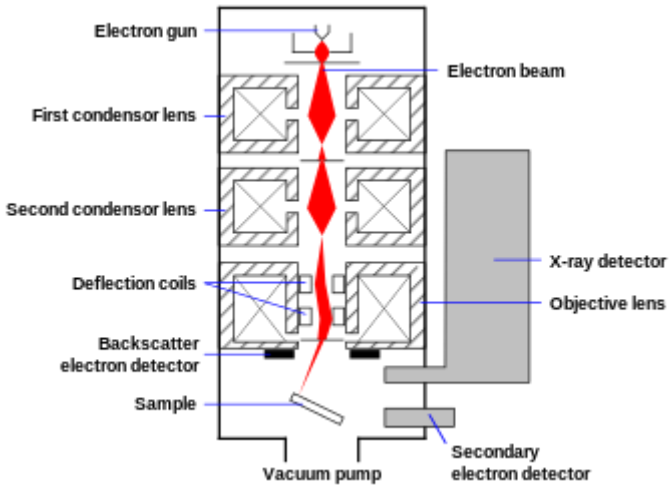
Methods:

1. Light or electron microscopy
2. Biochemical-determine presence of marker enzymes
3. Assay for a protein marker with an antibody (western)
4. Determine the protein concentration by using a spectrophotometer, e.g. Bradford assay
5. Determine specific activity (the ratio of activity of the enzyme of interest to the protein concentration.

2. Discuss in detail about morphological identification of cells using SEM and TEM

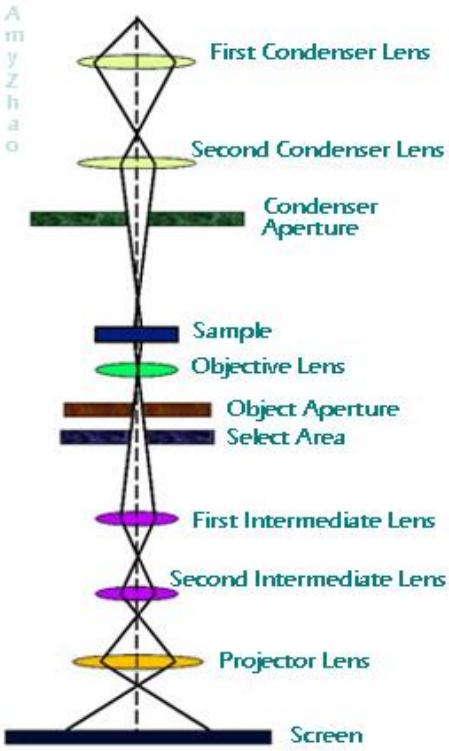
Scanning electron microscope (SEM)

- The scanning electron microscope (SEM) uses electrons to form an image.
- A beam of electrons is produced at the top of the microscope (electron gun) and follows a vertical path through the column of the microscope,
- it makes its way through electromagnetic lenses which focus and direct the beam down towards the sample.
- The beam passes through pairs of scanning coils or pairs of deflector plates in the electron column, typically in the final lens, which deflect the beam in the x and y axes so that it scans over a rectangular area of the sample surface.



- The focused beam of high-energy electrons generates a variety of signals at the surface of solid specimens.
- The signals that derive from electron-sample interactions reveal information about the sample including external morphology or surface topography, chemical composition, and others properties such as electrical conductivity.
- Different detectors collect the signals, and convert them into another signal that is sent to a viewing screen similar to the one in an ordinary television, producing an image.
- This image is then digitally captured and displayed in a computer monitor.
- Magnification in a SEM can be controlled over a range of about 10 to 500,000 times or more.
- The spatial resolution of the SEM depends on the size of the electron spot, which in turn depends on both the wavelength of the electrons and the electron-optical system which produces the scanning beam.
- Depending on the instrument, the resolution ranges between 1 and 20 nm.
- The signals result from interactions of the electron beam with the atoms at or near the surface of the sample.
- The type of signals produced by a SEM include secondary electrons, back-scattered electrons (BSE), characteristic X–rays, light (cathodoluminiscence), specimen current and transmitted electrons.
- Application Examples: Parasite study by scanning electron microscopy, Alveolar-capillary barrier, Pro-inflammatory stimuli activate dendritic cells, important players in the battle against Diseases, Intestinal epithelium, etc.

Transmission Electron Microscopy (**TEM**)



In TEM a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through.

An image is formed from the interaction of the electrons transmitted through the specimen;

The image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons.

This enables the instrument's user to examine fine detail—even as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope.

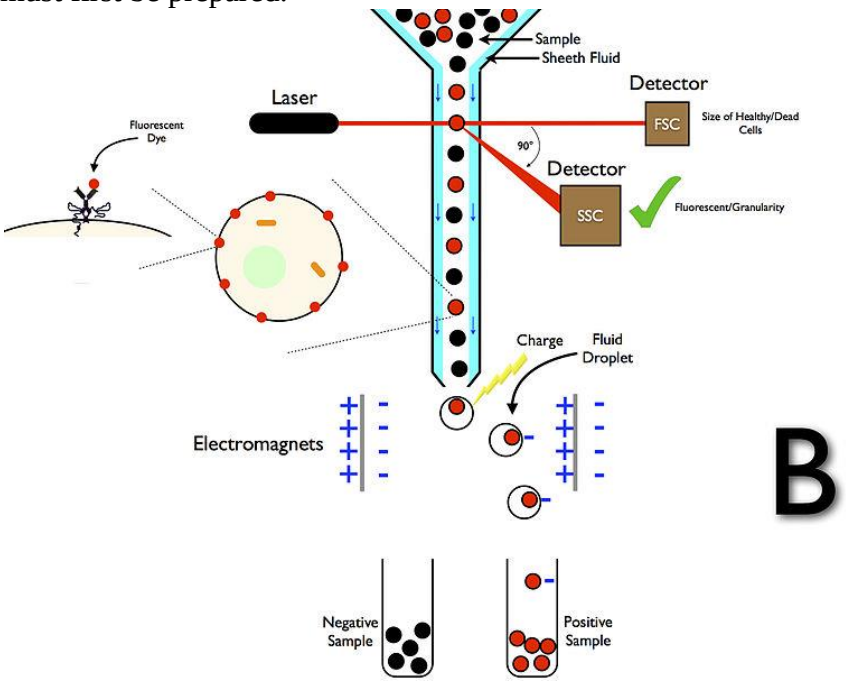
TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences.

TEMs find application in cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research.

3. Write a detailed note on Flow cytometry

- In biotechnology, **flow cytometry** is a laser-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.
- It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second.
- Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in basic research, clinical practice and clinical trials.
- Modern flow cytometers are able to analyze several thousand particles every second, in "real time," and can actively separate and isolate particles having specified properties.

- A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers "high-throughput" (for a large number of cells) automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.



A flow cytometer has five main components:

- a flow cell - liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing
- a measuring system - commonly used are measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon,krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals
- a detector and Analogue-to-Digital Conversion (ADC) system - which generates FSC and SSC as well as fluorescence signals from light into electrical signals that can be processed by a computer
- an amplification system - linear or logarithmic
- a computer for analysis of the signals.

The process of collecting data from samples using the flow cytometer is termed 'acquisition'. Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (i.e. voltage, compensation, etc.) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to ensure that parameters are set correctly.

Fluorescence Assisted Cell Sorting (FACS)

- Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry.
- It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell.

- The cell suspension is entrained in the center of a narrow, rapidly flowing stream of liquid.
- The flow is arranged so that there is a large separation between cells relative to their diameter.
- A vibrating mechanism causes the stream of cells to break into individual droplets.
- The system is adjusted so that there is a low probability of more than one cell per droplet.
- Just before the stream breaks into droplets, the flow passes through a fluorescence measuring station where the fluorescent character of interest of each cell is measured.
- An electrical charging ring is placed just at the point where the stream breaks into droplets.
- A charge is placed on the ring based on the immediately prior fluorescence intensity measurement, and the opposite charge is trapped on the droplet as it breaks from the stream.
- The charged droplets then fall through an electrostatic deflection system that diverts droplets into containers based upon their charge.
- In some systems, the charge is applied directly to the stream, and the droplet breaking off retains charge of the same sign as the stream.
- The stream is then returned to neutral after the droplet breaks off.

4. How will you localize proteins using immunostaining

Or

Expalain in detail the different immunostaining techniques. (AU Nov.2016)

Immunostaining is a general term in biochemistry that applies to any use of an antibody-based method to detect a specific protein in a sample.

Immunostaining techniques

This includes: a) Immunohistochemistry, b) Flow cytometry, c) Western blotting, d) Enzyme-linked immunosorbent assay and e) Immuno-electron microscopy

a) Immunohistochemistry

- Immunohistochemistry or IHC staining of tissue sections is the most commonly applied immunostaining technique.
- While the first cases of IHC staining used fluorescent dyes, other non-fluorescent methods using enzymes such as peroxidase and alkaline phosphatase are now used.
- These enzymes are capable of catalysing reactions that give a coloured product that is easily detectable by light microscopy.
- Alternatively, radioactive elements can be used as labels, and the immune reaction can be visualized by autoradiography.
- Tissue preparation or *fixation* is essential for the preservation of cell morphology and tissue architecture.
- Inappropriate or prolonged fixation may significantly diminish the antibody binding capability.
- One of the main difficulties with IHC staining is overcoming specific or non-specific background.
- Optimisation of fixation methods and times, pre-treatment with blocking agents, incubating antibodies with high salt, and optimising post-antibody wash buffers and wash times are all important for obtaining high quality immunostaining.

- In addition, the presence of positive and negative controls for staining is essential for determining specificity.

b) Flow cytometry

- A flow cytometer can be used for the direct analysis of cells expressing one or more specific proteins.
- Cells are immunostained in solution using methods similar to those used for immunofluorescence, and then analysed by flow cytometry.
- Flow cytometry has several advantages over IHC including: the ability to define distinct cell populations by their size and granularity;
- The capacity to gate out dead cells; improved sensitivity; and multi-colour analysis to measure several antigens simultaneously.
- Flow cytometry can be less effective at detecting extremely rare cell populations, and there is a loss of architectural relationships in the absence of a tissue section.
- Flow cytometry also has a high capital cost associated with the purchase of a flow cytometer.

c) Western blotting

- Western blotting allows the detection of specific proteins from extracts made from cells or tissues, before or after any purification steps.
- Proteins are generally separated by size using gel electrophoresis before being transferred to a synthetic membrane via dry, semi-dry, or wet blotting methods.
- The membrane can then be probed using antibodies using methods similar to immunohistochemistry, but without a need for fixation.
- Detection is typically performed using peroxidase linked antibodies to catalyse a chemiluminescent reaction.
- Western blotting is a routine molecular biology method that can be used to semi-quantitatively compare protein levels between extracts.
- The size separation prior to blotting allows the protein molecular weight to be gauged as compared with known molecular weight markers.

d) Enzyme-linked immunosorbent assay

- The enzyme-linked immunosorbent assay or ELISA is a diagnostic method for quantitatively or semi-quantitatively determining protein concentrations from blood plasma, serum or cell/tissue extracts in a multi-well plate format (usually 96-wells per plate).
- Broadly, proteins in solution are adsorbed to ELISA plates.
- Antibodies specific for the protein of interest are used to probe the plate.
- Background is minimised by optimising blocking and washing methods (as for IHC), and specificity is ensured via the presence of positive and negative controls.
- Detection methods are usually colorimetric or chemiluminescence based.

e) Immuno-electron microscopy

- Electron microscopy or EM can be used to study the detailed microarchitecture of tissues or cells.
- Immuno-EM allows the detection of specific proteins in ultrathin tissue sections.
- Antibodies labelled with heavy metal particles (e.g. gold) can be directly visualised using transmission electron microscopy.

- While powerful in detecting the sub-cellular localisation of a protein, immuno-EM can be technically challenging, expensive, and require rigorous optimisation of tissue fixation and processing methods.
- Protein biotinylation *in vivo* was proposed to alleviate the problems caused by frequent incompatibility of antibody staining with fixation protocols that better preserve cell morphology.

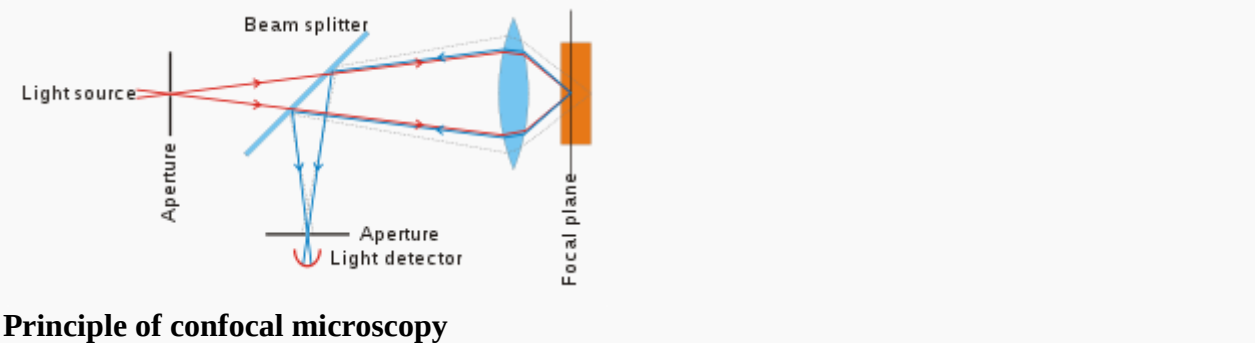
Applications of Immunostaining

- The applications of immunostaining are numerous, and typically used in *clinical diagnostics* and *laboratory research*.
- Clinically, IHC is used in histopathology for the diagnosis of specific types of cancers based on molecular markers.
- In laboratory science, immunostaining can be used for a variety of applications based on investigating the presence or absence of a protein, its tissue distribution, its sub-cellular localisation, and of changes in protein expression or degradation.

5. (i) Differentiate between SEM and TEM

SEM	TEM
Used to produce excellent images of the surfaces of cell and small organisms. Excellent for studying surface morphology of the organisms, cells or suitable material under study	Used to study the ultra structure of the cell and its components. It can see objects as small as a protein molecule or even at nano levels. Provides detail about internal composition of cells or any suitable material under study.
Electron beam scans over the surface of the sample.	Electron beam pass through the sample.
Based on scattered electrons or produces images by detecting secondary electrons which are emitted from the surface due to excitation by the primary electron beam.	Based on transmitted electrons or produces images by detecting primary electrons transmitted from the sample.
Comparatively low resolution than TEM; Resolution: 2nm(average), 0.2 nm (special)	High Resolution Resolution: 10nm(average), 0.5nm (special)
Depth of field: High	Depth of field: Moderate
Magnifying power: 1,00,000X	Magnifying power: 5,000,000X
Specimen contrast: by electron absorption	By electron scattering
Produces three- dimensional black and white images	Produces two - dimensional black and white images
Preparation technique: Easy	Skilled, very thin sample is required
Preparation thickness: variable	Very thin
Specimen mounting: Aluminium stubs	Thin films on copper grids
Field view: large	Limited

(ii) Write the principle and application of confocal microscopy



Principle of confocal microscopy

Confocal microscopy is an optical imaging technique used to increase optical resolution and contrast of a micrograph by adding a spatial pin hole placed at the confocal plane of the lens to eliminate out-of-focus light.

It enables the reconstruction of three-dimensional structures from the obtained images.

This technique has gained popularity in the scientific and industrial communities and typical applications are in life sciences, semiconductor inspection and materials science.

Basic concept

- The principle of confocal imaging was patented in 1957 by Marvin Minskyand aims to overcome some limitations of traditional wide-field fluorescence microscopes.
- In a conventional (i.e., wide-field) fluorescence microscope, the entire specimen is flooded evenly in light from a light source.
- All parts of the specimen in the optical path are excited at the same time and the resulting fluorescence is detected by the microscope's photodetector or camera including a large unfocused background part.
- In contrast, a confocal microscope uses point illumination and a pinhole in an optically conjugate plane in front of the detector to eliminate out-of-focus signal - the name "confocal" stems from this configuration.
- As only light produced by fluorescence very close to the focal plane can be detected, the image's optical resolution, particularly in the sample depth direction, is much better than that of wide-field microscopes.
- However, as much of the light from sample fluorescence is blocked at the pinhole, this increased resolution is at the cost of decreased signal intensity – so long exposures are often required.
- As only one point in the sample is illuminated at a time, 2D or 3D imaging requires scanning over a regular raster (i.e., a rectangular pattern of parallel scanning lines) in the specimen.
- The achievable thickness of the focal plane is defined mostly by the wavelength of the used light divided by the numerical aperture of the objective lens, but also by the optical properties of the specimen.
- The thin optical sectioning possible makes these types of microscopes particularly good at 3D imaging and surface profiling of samples.

Techniques used for horizontal scanning

Three types of confocal microscopes are commercially available:

- **Confocal laser scanning microscopes** use multiple mirrors (typically 2 or 3 scanning linearly along the x and the y axis) to scan the laser across the sample and "descan" the image across a fixed pinhole and detector.

- **Spinning-disk (Nipkow disk) confocal microscopes** use a series of moving pinholes on a disc to scan spot of light. Since a series of pinholes scans an area in parallel each pinhole is allowed to hover over a specific area for a longer amount of time thereby reducing the excitation energy needed to illuminate a sample when compared to laser scanning microscopes. Decreased excitation energy reduces photo-toxicity and photo-bleaching of a sample often making it the preferred system for imaging live cells or organisms.
- **Microlens enhanced or dual spinning disk confocal microscopes** work under the same principles as spinning-disk confocal microscopes except a second spinning disk containing micro-lenses is placed before the spinning disk containing the pinholes. Every pinhole has an associated micro-lens. The micro-lenses act to capture a broad band of light and focus it into each pinhole significantly increasing the amount of light directed into each pinhole and reducing the amount of light blocked by the spinning disk.
- **Programmable array microscopes (PAM)** use an electronically controlled spatial light modulator (SLM) that produces a set of moving pinholes. The SLM is a device containing an array of pixels with some property (opacity, reflectivity oroptical rotation) of the individual pixels that can be adjusted electronically. The SLM contains microelectromechanical mirrors or liquid crystal components. The image is usually acquired by a charge coupled device (CCD) camera.