Biyani's Think Tank

Concept based notes

Cell Biology

[B.Sc. Biotechnology Part-I]

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Note: A feedback form is enclosed along with think tank. Kindly fill the feedback form and submit it at the time of submitting to books of library, else NOC from Library will not be given.

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Syllabus

B.Sc. Biotechnology (Part-I)

Cell Biology

Note : Question No. 1 shall consist of questions requiring short answers and shall cover entire paper. The paper is divided into four sections. Students are required to attempt five questions in all, selecting not more than one question from each section. All questions carry equal marks.

Section-A

The cell structural Organisation : Development of cell theory, Eukaryotic and Prokaryotic cells, the nucleus and cell cycle(Mitosis and meiosis). The ultra structure of the cytoplasm-the cytoskeleton, microtubules, microtubular, organelles, microfilaments, the Endomembrane system, nuclear envelop, endoplasmic reticulum & Golgi complex, membrane organelles, mitochondria, chloroplast, lysosome, peroxisomes.

Section-B

Transport Across Cell Membrane: Molecular organization of cell membrane, passive and active transport, Na-K pump, Ca^{2+} ATPase pumps, Lysosomal and Vacuolar membrane ATP dependent proton pumps, Co-trnasport into prokaryotic cells, endocytosis and exocytosis, entry of viruses and toxins into cells.

Receptors and models of extra cellular signaling: Cytosolic, nuclear and membrane bund receptors, examples of receptors, autocrine, paracrine and endocrine model of action.

Section-C

Signal Transduction: Signal amplification, different model of signal amplification, cyclic AMP, role of inositol phosphatase messenger, Biosynthesis of inositol triphosphatases, cyclic GMP and Glycoproteins in signal transduction. Calcium models of signal amplification, phosphorylation of protein kinases.

Section-D

Call Culture: Techniques of propagation of prokaryotic and eukaryotic cells, cell line, generation of cell lines, maintenance of stock cells, characterization of cells, immunochemistry, morhpological analysis techniques in cell culiure, primary cultures contamination, differentiation, three dimensional cultures.

Books :

1. Cell & Molecular Biology by De Robertis, Lea and Febiger
2. Cell & Molecular Biology by H. Baltimore, WH Freeman
3. Cell Biology by Kimball T.W.Wesley Pub.

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Section-A

The cell structural Organisation

Q.1 What is Cell Theory? Discuss its utility in the discovery of certain exceptions?

OR

Give a brief account on "Cell Theory"

Ans.

- All animals and plants consist of certain structural unit ,"Concept given by Aristotle (384-322 B.C)
- The term Cell was used by Robert Hooks in a piece of Cork (1664).
- Leuwenhoek (1674) also discovered free cells and observed organization in the cells.
- Cell is basic unit of life and known as "Cell Theory" Schleiden.
- Dutrachet gave the idea of cell theory and Schwaan clearly outlined the basic features of cell theory (1839).
- Schwan also introduced the term metabolism.
- Rudolf Virchow (1858) extended the cell theory and suggested that all living cells, arises from pre-existing living cells. Louis Pasteur support this theory.

So there are 2 main components of cell theory.

(i) All living things are composed of cells.

(ii) All cells arise from pre-existing cells.

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- In recent years, it may appear that cell is no longer a basic unit of life because life may exist without cells also.

Exceptions to Cell Theory:

- Difficulty to cell theory faced when we apply the concept of cell theory to viruses.
- Viruses defined as infectious sub cellular and ultra microscopic particles, whose transmission land replies causes reactions in the last cells.
- Viruses cannot be visible without the aid of electron microscope.
- Viruses lack the internal organization which is the characteristics of a cell.
- Due to these properties viruses do not easily fit in the definition of a cell and described as "Primitive organism that have not reached a cellular state.
- Other organism which do not fit in the definition of cell theory are :- Paramecium, a protozoan, Rhizopus, a fungus and vaucheria, an algae.

Q.2. Write the basic differences between Prokaryotic and Eukaryotic cells with some examples.

O R

Describe in brief the important features of Prokaryotic and Eukaryotic cells.

Ans. The term Prokaryotic and eukaryotic were suggested by Hans Ris in 1960s.

Prokaryotic Cells (Pro-primitive, Karyon-nuclear).

- Most primitive and "one-envelop system organized in depth.
- Cytaoplasm lacks the will defined cytoplasmic Organells such as endoplasmic reticulum, Mitochondria, Golgi apparatus, Centrioles etc.

Bacteria :-

- They are most primitive simple, unicellular, prokaryotic organism
- Occur almost everywhere.
- Size of bacteria range between 1 μm to 3 μm (smallest bacterium – Dialister pneumosintes (.15 μm) (Largest bacterium is *Spirillum volutans* (13-15 μm).
- They vary in shape and classified into Cocci, Bacilli, Spirilla and vibrios.

On the basis of structure of cell wall and stain ability with the gram stain two types of bacteria have been recognized.

(1) Gram positive bacteria.

(2) Gram negative bacteria.

- Structure details of bacterial cell, can only be seen with an electron microscope.

A typical bacteria cell has following components :

(A) Outer Covering : Outer covering comprises of following 3 layers :

(1) <u>Plasma membrane</u>

- Protoplast of bacterial cell is bound by a living plasma membrane.
- Plasma membrane comprises of lipid and protein molecules which are arranged in a fluid mosaic pattern.
- Protein molecules embedded within this lipid layer serve important functions like they act as carrier to carry on selective transport of molecules form the outer area to the cell they involve in oxidative metabolism. They act as enzyme for respiration and photosynthesis.

When plasma membrane in folds it give rise to 2 types of structure.

(i) **Mesosomes :** Increase the surface areas of plasma membrane

They are mostly seen in "Chemoautotrophic bacteria" with high rate of aerobic respiration and photosynthetic bacteria where they play a major role as the site of photosynthetic pigment.

(ii) **Chromatophores :** These are photosynthetic pigment vary in farm as vehicles, tubes, thylakoids in (BGA) and stocks.

(2) **Cellwall :** Plasma membrane is surrounded by a strong and rigid cell wall. It provides the shape and mechanical protection to the bacteria.

It differs from plant cell wall in that, it contains protein, lipids, polysaccharides but rarely cellulose. Cell wall of gram negative bacteria comprises of 2 layers :

(i) Peptidoglycan (e.g. murrain containing periplasmic space. Around, plasing membrane.

(ii) The outer layer consists of lipid bilayer with "Porin" channels. These channels allow diffusion of solutes.

Cell wall of gram positive bacteria is thicker and single layered. It contains many layers of peptidoglycans and teichoic acid.

(3) **Capsule** : Cell wall is surrounded by an additional slime layer called capsule and is secreted by plasma membrane.

It serves as protective layer against attack by phagocyte and helps in maintaining concentration of lens.

(B) **Cytoplasm :** Cytoplasm consists of water, protein, lipid carbohydrate, RNA and other molecules. Cytoplasm is divided into two types of area based on their density.

i) Dark area (2) Light Area.

In Dark area, dense area, thousand of particles called "Ribosomes" are present. They are composed of RNA + protein ;and are the sites of protein synthesis . Bacterial ribosomes are 70S type ;and consist of 2 subunits (larger 50S and smaller 30S). During, a protein synthesis, they arranged in the form of polysomes and polyribosomes. "Reserve materials" are stored in cytoplasm in the form of inclusion bodies.

Three type of reserve materials are :

i) Poly-β hyoroxybutyric acid

ii) Volutin granules

iii) Sulpher granules

C) **Nucleoids :**

Nuclear material includes a single circular and double stranded DNA molecule called as bacterial chromosome. It is usually concentrated in a clear region of cytoplasm, called "Nucleoid". It does not contain histones, protein. However, it contains small heat stable protein that may be analogous to eukaryotic histones. All 3 types of RNA are formed by the activity of a single RNA polymerase. In most of bacterial species extra chromosomal DNA is present in the form of extra chromosomal DNA molecule. Plasmid helps in production of calcium which inhibit the growth of other bacteria. Some plasmid may act as if factor which stimulate bacterial conjugation and R factor which carry genes far resistance to one or more drugs.

D) **Flagella :** Many bacteria are motile and contain one or more flagella for the locomotion. They are smaller than the eukaryotic flagella and made up of "flagillin" protein.

According to the number and arrangement of the flagella 4 types of patterns are;

i) **Monotrichous :** A single flagellum at one pale of the cell.

ii) **Lophotrchous :** Several flagella at one pale.

iii) **Amphitrichous :**One flagellum at each pale.

iv) **Peritrichous :** Flagella all over the surface of cell.

The flagellum is divided into 3 parts :

(i) Base (ii) hook (iii) Filament

Base is comprises of 4 rings. Basal body is composed of many rings in gram-ive bacteria 4 rings are present and in gram +ive 2 rings are present. These are :

M – Membrane ring

S – Super membrane ring

P – peptidoglycan ring

L – Lipopolysaccharides, arranged in inside to outside area.

Hook helps in attachment of filament to basal body. Filament presents on outer surface of bacteria and attached by hook on surface.

E) **Other Structure –** Some bacteria contain nonflagellar appendages called 'fimbriae" or pili. They enable the bacteria to stick firmly to other bacteria and to some eukaryotes. Pili helps in conjugation and are known to be code by the genes of plasmid.

Gram positive bacteria have tubular single protein unit called spinin, called spine and they help the bacteria to tolerate some environmental conditions such as salinity, pH, temperature.

Based on "nutrition' bacteria show acid diversity. Some are chemosynthetic photosynthetic, but most of them are heterotrophic. Heterotrophic bacteria are either saprophytic or parasitic. Bacteria reproduce "by asexually by binary fission and end spore foundation and sexually by conjugation.

- In binary fissions cell divides into 2 identical daughter cells.
- Conjugation is simplest form of sexual reproduction given by "Lederberg and Tatum".
- During conjugation f^+ or donor bacterium, transfer a piece of DNA (F gene) into recipient bacterium.

Examples of Prokaryotic Cells

(i) Mycoplasma/ PPLO : Unicellular, prokaryotic containing plasma membrane nucleic acid and metabolic machinery to grow in the absence of cells.

- They differ from bacteria in that they do not contain cell wall and mesosomes and are filterable through bacterial filter.
- They contain many enzymes which may be required for DNA replication.

(ii) **E.Coli :** Gram negative monotrichous, leterobophic and on pathogenic bacteria.

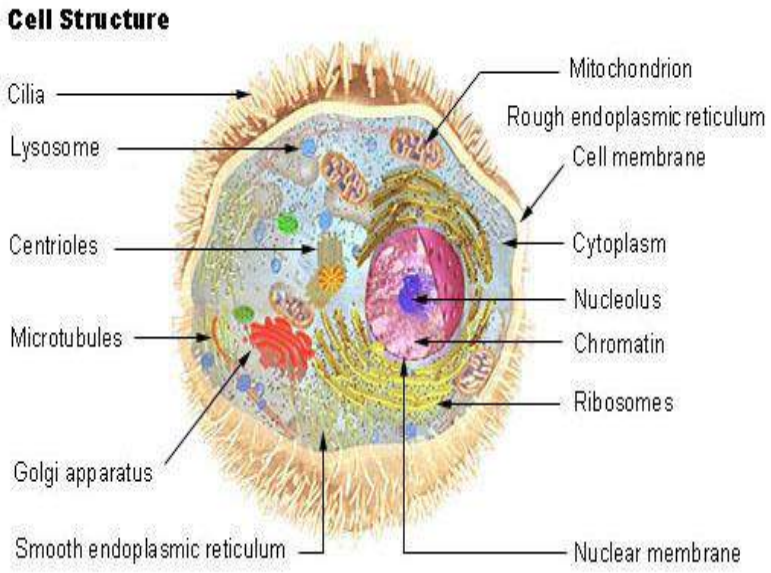
- Cytoplasm is bounded by a fluid mosaic plasma membrane (Detail note as prokaryotic cell)

(iii) **Cyanobacteria :** They are most successful and primitive organism on earth.

- They lack flagella but are capable to perform movement by rotary motion secreted through the cell surface.
- The cytoplasm is to differentiate into 2 regions. (i) outer region or chromoplasm (ii) Inner colorless region called Centroplasm
- Cyanophysin granules are located in chromaplasm Myxophysean starch and polyglucon granules are also present as storage granule.

Eukaryotic Cells

- These cells are very much larger than prokaryotic cells and essentially to envelop system.
- Eukaryotic cells are true cells and occur in the plants and in the animals.
- Shape of eukaryotic cells may be variable and fixed variable in Amoeba and fixed shape almost occures in protists plants and animals.
- Size of multi cellular organism ranges between 20-30µm
- Eukaryotic cells consist of the following components.



i) Cell wall and plasma membrane.

ii) Cytoplasm

iii) Nucleus

i). Cell wall and plasma membrane : The lead and outermost structure of plant cell is called cell wall.

- Mainly composed of all base pectin lignin and fatty substances.
- Middle lamella is present between the adjacent cells.
- Primary cell wall is composed of pectin hemicelluloses and loose network of microfibrills.
- Secondary cell wall consist of mainly of cellulose hemicelluloses and lignin.
- plasmodesmata allow communication with the other cells in a tissue.
- Cell wall is absent in animal cell and in plant cell it provides protection and mechanical support to the plant cell and prevents it from desiccation.

Plasma membrane

- Plasma membrane is a selectively permeable membrane.
- Main function of plasma membrane is to Control transportation of materials by various means such as osmosis diffusion and active transport.
- Process of transport is performed by special type of protein molecule called transport protein.
- For bulk transport plasma membrane performs exocytosis and endocytosis that utilize ATP as energy source.

ii) Cytoplasm : It is divided into following structure:-

(A) Cytosol : It is the aqueous portion of cytoplasm .

- It serves to dissolve the great variety of small molecule e.g. glucose, amino acids, nucleotides.
- Cytosol also contains fibers that provides mobility and cell shape called "Cytoskeleton".
- Cytoskeleton is divided into microtubules, active filament and intermediate filament.

(B) Cytoplasmic organelles and granules.

- Cytoplasmic granules include oil drops, secretary granules, glycogen and starch granules.
- It includes different cell organelles, are as follows :

i) Endoplasmic reticulum : It is an extensive network of membrane - limited channels.

- It divides into smooth E.R and rough E.R.

  Rough ER has attached ribosome on its outer surface, whereas smooth ER has not.
- ER is the site of biogenesis of cellular membrane.

ii) Golgi apparatus : It is a cup-shaped organelle and consist of a set of smooth cisternae and vesicles.

- Golgi consist of Cis golgi, median golgi and trans golgi.

- It performs packaging of secretary materials, processing of protein synthesis of lipids and formation of acrosome of sperm.

iii) **Lysosomes :** Cytoplasm of animal cells contains irregular shaped, membrane band structure called as Lysosomes .

It originates from gogy apparatus and contrail many hydrolytic enzymes for digestion.

- High acidic medium of lysosomes depends on ATP dependent proton-pump.

iv) **Cytoplasm vacuoles:** The cytoplasm contains numerous hollow liquid filled structure called vacude. These vacholes are bounded by amelubrane & their function is the storages of materials and maintenance of internal pressure of cell.

numerous hollow liquid filled structures called vacuoles.

v) Peroxysomes : These are tiny circular membrane bound organelle containing different enzymes which are required for detoxification activity.

- Peroxysomes are related with β-oxidation of fatty acid and in degradation of amino acid.
- In green leaves it carry out the process of photo-respiration.

vi) Glyoxysomes : These organelle develop in a germinating plant seed to utilize stored fat of seeds.

- By glyoxylate cycle emzymes of glyoxysomes are used to transform stored fat into carbohydrate.

vii) Mitochondria Mitochondria are oxygen consuming ribbon shape organelle with two unit membrane.

- In the inner membrane finger like outgrowths are present called "Cristae and contain F_1 particles that help in ATP synthesis.
- It also contains proton pumps and permeases for the transport of various molecules.

- It can synthesize protein in their own protein synthetic machinery, so they are considered as "semiautonomous organells.

viii) **Plastids :** It occur only in plant cell and are of following types.

leucoplast, amyloplast, proteinoplast, oleosomes and chromoplast.

- The green coloured chromoplast are called chloroplast and are involved in the photosynthesis of food so act like kitchen of the cell.

ix) **Ribosomes :** They are tiny dense particles that contain equal amounts of RNA and proteins .

- Ribosomes of eukaryotic cells are of 80S type and are composed of 60S and 40S subunits.

x) **Cilia and Flagella :**

- They both have similar structure but vary in size and number.
- Cilia are used for the locomotion in isolated. Cells such as protozoan (Paramecium).
- Flagella are used in the locomotion of spermatazoan and Euglena.
- Cilia and flagella both are moved by hydrolysis of ATP.

xi) **Basal bodies and centrioles :**

- They act as nucleating centre from which microtubles grow.
- The wall of centrioles has nine groups of microtubules arranged in a circle.
- At the time of all division , two pairs of centriloes are present; and form the spindle of microtubule which help in separation and Movement of chromosomes.

C) **Nucleus :** Nucleus is centrally located and spherical cellular component and consist of following 3 structures.

1) Chromatin:

- Genes are located on chromosomes which exist as chromatic network.

  The chromatin has two forms:

  1. Euchromatin 2. Heterochromatin

- Euchromatin is genetically active and takes lighter DNA stain.

Q.3 What do you understand by cytoskeleton? What are its components and how they are involved in the cell movement?

O R

Describe the structure of microtubules and its tubulin subunits. Also discuss the arrangement of microtubule in cilium and their role in movement.

O R

What are microfilament and intermediary filaments. Discuss their structure

Function and arrangement of action and myosin molecule?

O R

Write short notes on :

(i) Actin Filament (ii) Muscle protein (iii) Sarcoma (iv) Myofibrils.

Ans. Cytoskeleton consist of a network of protein filament extending throughout the cytoplasm of all eukaryotic cells.

It involves in chromosome separation and provides mechanical strength to the cell.

It provides mechanical strength to the cell. It provides machinery for contraction of muscle and enables some cells to move as spleen fibroblast & W.B.C.

Cytoskeleton filaments are divided into 3 categories.

(i) Microtubules

(ii) Intermediate filament

(iii) Actin filament .

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Structure and Organization of Actin Filaments

- Major cytoskeleton protein is actin which polymerizes to form actin filament.
- Assembly and disassembly of actin filament their cross linking into bundles and network are regulated by "actin binding protein".
- The polarity of "actin filament is important in assembly by nucleation reaction.
- Each action monomer has tight binding site that mediate head to tail interaction with other actin monomers to farm complete filament.
- Each monomer is related by 166 angle and show a double helix structure.
- Polymerization of actin filaments involves aggregate of 3 actin filaments.
- Plus end migrates faster than minus end and ATP is hydrolyzed during actin monomer assembly.
- Equilibrium of monomer is dependent on concentration.
- The reason of growing + end faster than – end is that ATP bound filament dissociate readily than ADP bound actin and this results in critical concentration of monomer needed for polymerization at two different ends. This lead to a phenomenon called "tread milling'.

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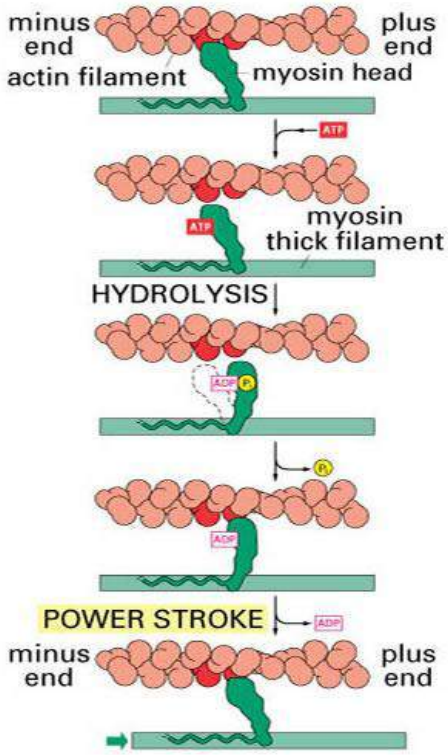

Figure 17-45 Essential Cell Biology, 2/e. (© 2004 Garland Science)

- Some drugs like "Cytocholasine" and phalloidin prevent the dissociation of actin monomer.
- Cofilin binds to minus end and enhance the rate of association.
- Profillin regulate the incorporation of action monomer

Functions of Actin filaments

- Actin usually in association with myosin are responsible for many types of cell movements.
- It is a molecular motor protein that converts chemical energy in the form of ATP to mechanical energy; thus generates force for all movements.

Muscle contraction

There are 3 different types of muscle cells in vertebrates:

i) **Skeletal muscle :** Involved in voluntary movements.

ii) **Cardiac muscle** : These muscles pump the blood from heart.

iii) **Smooth muscle :** Involuntary movements of ;organs such as stomach intestine and blood vessel.

- Cytoplasm consist of myofibrils are made up a thick filaments of myocin and then actin.
- Each myofibrils is organized as a chain of units called sarcomeres, which gives striated appearance of skeletal and cardiac muscle.
- The end of sarcomere defined by Z disc that consist of dark band (A) and light band (I). These bands corresponds to the plus or minus myosin filament.
- I band contain thin actin and A band contains thick myosin filament. Myosin and actin filament overlapped in peripheral region of 'A band', whereas middle region contain only myosin.
- Titian and nebulin involved in the regulation of muscle contraction.
- Titan acts as spring which keeps myosin centered in sarcomer and nebulin regulates the assembly of actin.
- Muscle cell contraction can be explained by "sliding filament model" propose by Huxley.
- During muscle cell contraction each sarcomere shortend, bringing Z disc closer and there is no change in width of A band.
- The molecular basis of interaction is binding of myosin to action filament and myosin function as motor that arrives filament and myosin function as motor that drives filament sliding binding of myosin hydrolyzes ATP and provides energy.
- The contraction of skeletal muscle is triggered by nerve impulses that stimulate calcium release from Sarcoplasmic reticulum that increase s the concern of calcium ions in cytosol which signals the contraction by two accessory protein both as to actin filament (i) Tropomyocin (ii) Troponin

Cell Crawling : Crawling movements of cells represents basic form of cell locomotion.

Example : Movement of Amoeba, migration of cells involved in bound healing.

Cell crawling involves a coordinated cycle of stages.

i) Extension of leading stage.

ii) Attachment of leading stage to substrate.

iii) Retraction of rear end of cell into the cell body.

Intermediate Filaments.

- Have a diameter of about 10mm which is intermediate between the diameters of 2 other filaments actin filaments and microtubules.
- They are directly involved in cell movement, in contrast to other filament.
- Based on amino acid sequences, they are classified into 6 groups.

  Acidic Keratin, basic Keratin, Vimentin, neuro filaments, Nuclear lamins, Nestin.
- Keratin have 15 types of different proteins existing in different cells.

  Example : Hard keratin present in hairs and nails.

  Soft Keratin "presenting epithetial cells.
- Vimentin exist in fibroblast smooth muscle and neuro filaments are major cytoskeleton structure
- Nuclear laminas present in all eukaryotes that forms meshwork under nuclear membrane
- All intermediary filament have a central 2 helical rod, which is flanked by N terminal and C terminal.
- Head and tail damains of this rod determines the specific function of different filament protein.

Assembly of Intermediatery Filament

- First step of filament assembly is the formation of dimmers in which the central rods are wounds around each other in a coiled structure.
- These dimers; arranged in anti parallel way to form tetramers which can assemble end to end forming proto filament.
- Final intermediary filament contains approximately 8 proto filament wound around each other in a rope like structure.
- Intermediatery filaments are non polar, filament assembly requires interaction between specific type of intermediary filament protein.
- Intermediatery filaments are non polar, filament assembly requires filaments are generally more stable than actin, filament and microtubule.
- Phosphorylation of intermediary filament proteins regulate their assembly and disassembly within the cell.

Intracellular Organization of Intermediary Filament

- Intermediatery filaments are non polar, filament assembly requires filaments forms a network in the cytoplasm of most cells, both Keratin and vimentin attach to nuclear envelop and serves in positioning the nuclear within the cell.
- It also attach within the actin filament and microtubule.
- The Keratin filament of epithetial cells are tightly anchored to the plasma membrane at two areas of specialized cell contacts called as "desmosomes" and "hemidesmosomes"
- Desmosomes are function between adjacent cell at which cell- cell contact are mediated by transmembrane protein related to cadherarins.
- Two types of intermediary filaments "desmins" and neuro filaments ;play specialized role in muscle and nerve cells.

- "Desmin" connect the individual actin-myocin assembly of muscle cells both to one another and to plasma membrane.
- "Microfilaments are major IF in most of the mature neurons. They are particularly abundant in the long, axon of motor neurons.
- Nonfunctioning of this IF results in a disease called as "Epidermolysis bullosa simplex" which develops skin blister.
- Abnormalities of neuro filament causes disease of motor neurons.

  known as "Amiotrophic lateral sclerosis: It results in accumulation of microfilament.

Microtubules

- Third principal component of cytoskeleton are rigid hollow rods, approximately 25mm in diameter.
- They are dynamic structure that determine cell shape in a variety of cell movement like cell locomotion, transplant of organelles and separation of 'chromosomes during mitosis.'

Structure and assembly of Microtubules

- Microtubules are composed of a single type of globular protein called "tubules globular protein" called Tubulin".
- Tubulin is a dimer consisting of α- and β tubulin. They polymerize

  To form microtubules.
- Protofilaments are composed of head to tail arrays of tubulin dimmers, are arranged in parallel.
- Polarity of microtubules are important in determining the directions of movement.
- To regulate polymerization X and B, tubulin bind with GTP which functions similar to the ATP bound actin filament.
- Like actin filaments microtubules undergo tread milling, a dynamic behavior in which tubulin molecules bound to GDP and continuously lost from minus end and replaced by the addition of tubulin molecules, pound to GTP to the plus end of the sane microtubule.

- GTP hydrolysis also results in 'dynamic instability' in which individual micro-tubulus alternate between cycles of growth and shrinkage.
- During mitoses, microtubules extend outward from centrosome to form mitotic spindle which is responsible for the separation of chromosome.
- Centrosome serves as initiation site for the assembly of microtubule. It can be clearly visualized in cells that have been treated to disassemble their microtubules.
- Centro some of most animal cells contain a pair of centriole, are cylindrical structure based on nine triplets of microtubules.

Reorganization of Microtubules during mitosis

Microtubules completely reorganize during mitosis

- Microtubules array present in interphase disassembles and free tubulin subunits are reassembled to form the mitotic spindle, which is responsible for the separation of daughter chromosome.
- As the cell enters in mitosis, the dynamic of microtubules assembly and disassembly also change dramatically.
- Microtubules radiating from the cenrosome are of three types.

i) Kinetochore microtubules attach to the condensed chromosomes of mitotic, cells at their centromeres, which are associated with specific ptoteins to form the kinetochore. Attachment to the kinetochore stabilizes these microtubules which plays a critical role in separation of the mitotic chromosomes.

ii) Polar microtubules are not attached to the chromosomes. Instead they are stabilized by overlapping with each other in the centre of the cell.

iii) Astral microtubules extend outward from the Centrosome to the periphery and have freely plus end. They also contribute to chromosome movement by pushing the spindle poles apart.

- Because of dynamic instability, most microtubules are disassembled with the cell
- This dynamic behavior can be modified by interaction of microtubules with other proteins.
- Microtubules associated proteins (MAPs) bind to microtubules and modify their stability.
- Interaction of microtubules to protein, allow the cell to stabilize microtubule in particular location and provides an important mechanism for determining cell shape and polarity.

Microtubules Motors and Movements

- Microtubules are responsible for a variety of movements including the intracellular transport,separation of chromosmes at mitosis and beating of Cilia.
- Movements is based on the action of motor proteins that utility energy from ATP.
- Two such types of proteins are : Kinesins and dyneins are responsible for powering the variety of movements.
- Kinesins and Dyneins move along microtubules in opposite direction – most kinesins towards the blue end and
- Dynein is thought to play a role in positioning of golgi apparatus.
- microtubules reorganize to form mitotic spindle which plays a central role in cell division by distributing the duplicated chromosomes to daughter nuclei.
- Distribution of genetic materials takes place during aphase of mitosis when sister Chromatid separates and move to opposite poles of spindles

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Q.4. Give an account of the structure of nucleus. Discuss the structure and function of different components of nucleus.

O R

Write short notes on :

(i) Nuclear envelop

(ii) Nucleolus

(iii) Nuclear Pore Complex (NPC)

(iv) Chromosomes.

Ans. Robert Brown discovered a prominent body within the cell and termed as nucleus.

- Presence of nucleus is the principle feature that distinguish eukaryotic from pro karyotic cell.
- DNA replication, transcription and RNA processing all takes place in nuclear.

A nucleus may be described as having three important parts :

(i) Nuclear membrane 'Nuclear envelop

(ii) Nucleus

(iii) Chromosome

- A fluid in which nucleolus, and chromosomes are present which is enclosed in nuclear membrane is termed as "nucleoplasm"

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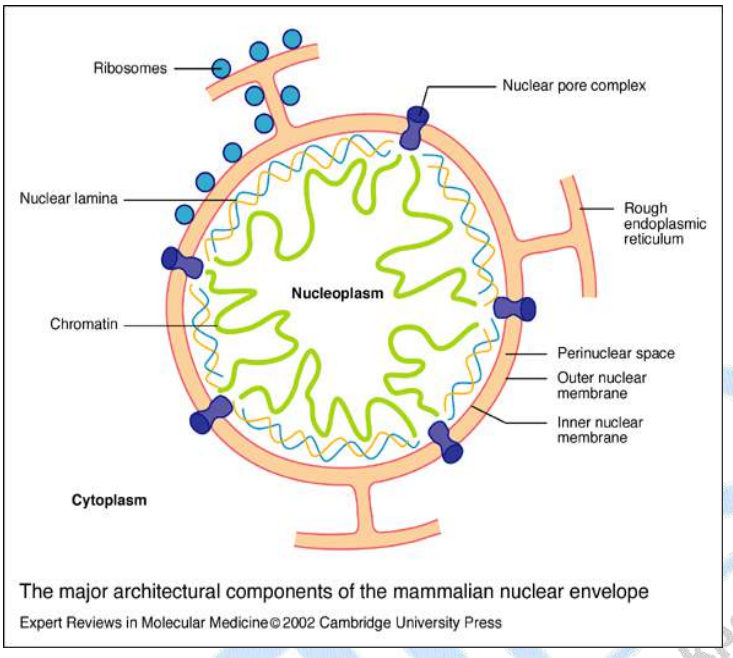

The major architectural components of the mammalian nuclear envelope

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Nuclear Envelop : Nuclear membrane acting as a barrier that prevent the free passage of molecules between the nucleus and cytoplasm. It is a complex structure consisting of two nuclear membrane.

(i) Nuclear Lamina (ii) Nuclear pore complex

Nucleus is surrounding by two concentric membrane called inner and outer nuclear membrane.

- Outer membrane continued with the endoplasmic reticulum and inner membrane carries unique protein that are specific to nuclear and line by nuclear lamina (a fibrous, meshwork)
- Space between two nuclear membrane is called as perinuclear space.
- Like other membrane, it is a "Phospholipid bilayer".
- The inner and outer nuclear membrane are joined at nucleus porecomplext. 'The Sole channel through which small polar molecules and macromolecules are able to travel through nuclear envelop.

- It is complicated structure i.e. responsible for the selective transport of protein and RNAs between the nuclear and cytoplasm
- Nuclear lamina is, composed of one or more related proteins called Lamins.
- The mammalian cells 4 types of lamins, are present AB. B and C: lamins associated with each other to form filaments.
- The first stage of association is the interaction of two lamins to form dimers in which the α- helical region of two polypeptide chain coil around to each other in a structure called coiled coil.
- After this these dimens associate with each other to form the "nuclear lamina".
- The lamins binds to inner nuclear membrane proteins. It provides a structural support to nucleus.

Nuclear Pore Complex : The diameter of nuclear pore complex is 120 nm. and made up of 100 different type of transproteins.

- These are only channels through which small polar molecules, ions and macromolecules are able to travel between nucleus and cytoplasm.
- Small molecules can pass through passive transport and large molecules can pass through active transport.
- NPC plays a fundamental role in the physiology of eukaryotic cell.
- Through these regulated channels "nuclear proteins are selectively imparted from the cytoplasm to the nuclear while RNAs are exported from the nuclear to the cytoplasm.
- NPC consist an assembly of 8 spokes arrange around a Central channel.
- The spokes are connected to the ring at the nuclear and cytoplasm rings.

2. Nucleolus : The most prominent structure with in the nuclear is the nucleolus

- In higher organism every nucleus has a spherical colloidal body called nucleolus which is associated with "nucleolar organiser chromosome"
- Nucleolus, which is not surrounded by a membrane is organized around chromosomal region that contain the genes for 5.8S, 185 and 28S r RNA.
- It is first described by "Fontana".
- It can be seen as a very conspicuous structure in the interphase.
- It disappears during mitosis and reappears at the next interphase.
- This process by which nucleolus is formed is described as "nucleogenesis".
- It sythesizes and RNA and carries genes for r-RNA.

In the nucleolus seems to proceed from center to periphery 3 distinct region are :

i) **Fibriller Center** (FC) : Where r-RNA genes of NOR are located, the transcription of r-RNA genes also takes place in this region.

ii) **Dense Fibriller Component** (DFC) : Which surround the fibriller genes and where RNA synthesis progress. The 80S ribosomal proteins also bind to the transcripts in this region.

iii) **Cortical Granular Component** : It is the inner-most region and where processing and maturation of pre ribosomal particles occurs.

Therefore, these region roles in "ribosome formation".

3. Chromosomes : (Chromatin structure) – In the nucleus chromatin, RNAase and nuclear proteins more freely in aqueous solution.

The nucleus appears to have an internal structure that organize the genetic material.

- "Strasburger" discovered these structure.
- Chromatin becomes highly condense during mitosis chromosome is normally measured at mitotic metaphase.
- Chromosome are arranged in form of chromatin in nucleus.

- Chromatin = DNA + Histone + DNA binding proteins.

Two type of chromatin are present.

(i) Euchromatin

(ii) Heterochromatin

- Nucleus contain definition of chromosome of definite size and shape.
- These chromosomes are invisible in nuclear but can be easily seen during cell division.
- Two similar chromosome are known as homologus chromosome, which come in contact at zygotene and pair length wise.
- Shape of chromosome is observed at anaphase (Centro mere determines the shape).
- There are two type of chromatin present in nuclear differentiate in mitosis.

Heterochromatin	Euchromatin
1 When chromosomes are stained with stains like feulgen at prophase, heterochromatic differentiate into dark region.	When stained with feulgen, appears in light colour.
2. Heterochromatic region constitutes three structure (i) Chromomeres (ii) Chromocenters (iii) Knobs	There is no constitution
3. There are two regions: (i) Constitutive region (ii) Facultative region	----
4. Densely organized	Loosely organized.
5. DNA replication in heterochromatin is different than that of euchromatin.	-----
6. Genes in this region are inactive.	Genes in this region are inactive (exception is drosophila land tomato)
7. Transcriptionally inactive	Transcriptionally active.

Q. The nucleus was discovered by :

(A) Robert Brown (B) Heekal

(C) Virchow (D) meischer

Ans.: (A)

Q. "Cells" derived from pre-existing cells." Concept given by :

(A) Robert Brown (B) Heekal

(C) Virchow (D) Meischer

Ans. (C)

Q. Dark region of Chromosomes are called :

(A) Euchromatin (B) Chromonema

(C) Heterochromatin (D) Chromomere

Ans. (C)

Q. Nuclear membrane is composed of :

(A) Glycoprotein (B) Phospholipid

(C) Nucleoprotease (D) Phospholipid and protein

Ans. (D)

Q.5. Give an account of structure and function of endoplasmic reticulum?

O R

Write short notes on (i) Lysosomes (ii) Golgi apparatus (iii) Centrosome (iv) Ribosome (v) Peroxisomes (vi) Polyribosomes.

Ans.

- Endoplasmic Reticulum : The ER is a network of membrane enclosed tubules; and sacs (cisternae) that extends from the nuclear membrane through out the cytoplasm.

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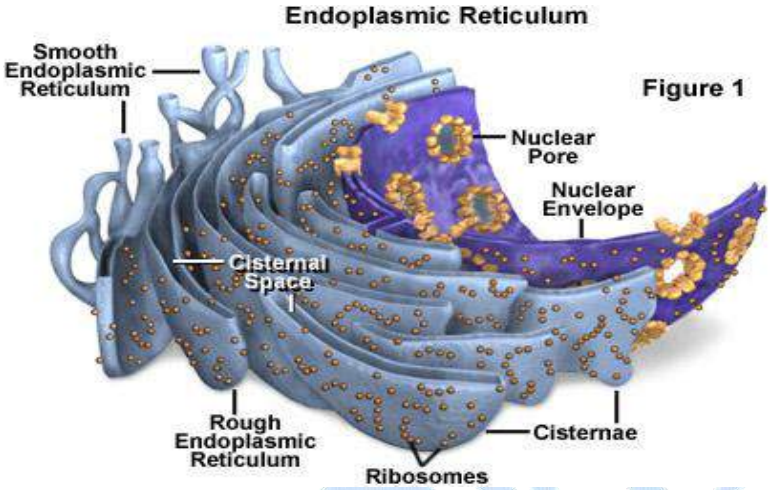
- The entire E.R is enclosed by a continuous membrane and is the largest organ of eukaryotic cell



- Two different types of ER that perform different functions within the cell.

  1. **Rough E.R** – Which is covered by ribosomes on its surface, function in protein processing.

  2. **Smooth E.R** – is not associated with ER and involved in lipid metabolism.

- E.R. consist of Network of labyrinth consisting of branching tubules and flattened sacs with variable space. These strands forms tubes and vesicles in the cytoplasm.
- Sometimes there may be chains of vehicles are found which are connected to one another by "cannaculi" or large vesicle.
- It is a hollow system connected on one side to the nuclear envelop and other side to the cell membrane.
- E.R. membrane forms a continuous sheet enclosing a single internal space or lumen, called ER. Lumen.
- It is a double membrane bound structure E.R. lumen is the site of protein sorting and processing.
- The membrane of ER carries Ribosome on its external surface.

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- Rough ER (RER) is actively engaged in protein synthesis.
- It is well developed in pancreatic and liver cell where secretary proteins are synthesized on ribosome.
- There is more lipid in smooth E.R. than in the rough E.R.
- E.R participated in many cellular structure such as :

(1) **Mechanical Supports :** E.R forms a network in cytoplasmic matrix and thus divide it into a number of chambers giving it the structure of vascular system.

(2) Exchange and translocation :

- E.R forms a surface area for exchange diffusion as well as active transport takes place through the membrane system.
- The presence of carriery and perm cases to perform these functions.
- It helps in the translocation of substances from the cytoplasm to outside the cell and vice versa.
- It also helps in the flow of RNA and nucleoprotein ;from the nuclear to the cytoplasm, which is the site of protein synthesis.

3. Synthesis of lipoprotein and glycogen : Smooth ER is engaged in synthesis of lipid in collaboration with golgy complex.

- In liver smooth ER is abundant in the hepatocytes and is involved in the production of lipoprotein particles; which carried the lipid to other cells of body.
- Smooth E.R ;is also involved in production of glycogen.
- The membrane of eukaryotic cells are composed of 3 types of lipid.

Phospholipids are synthesized on the cytosolic side of ER from water soluble precursor.

- Fatty acid are first transferred from CoA (Carrier to Glycerol – 3 – Phosphate) by membrane bound enzyme as a result phospholipids is inserted into the membrane.
- New phospholipids are added only to the cylosolic half of the ER membrane.

- To maintain its stability, sane of these newly synthesized phospholipids must be, transfer to the luminal half of the E.R membrane.
- Membrane protein called Hippase catalyzes this rapid translocation.

4. **Detoxification of drugs :** The membrane of smooth ER also contained enzyme that detoxify lipid soluble drugs.

- Cytochrome P_{450} family is an example of detoxification enzymes which render these drugs water soluble so that these may leave he cell and are excreted in the urine.

5. **Release uptake and storage of calcium :** E.R_also release calcium into the cytosol and reuptake it.

- This process helps in rapid response to extra cellular signals.
- The storage of calcium ion in lumen is facilitated by the presence of calcium binding proteins.
- Release and uptake of sarcoplasmic reticulum helps in muscle contraction.

6. **RNA localization in Ooiyte :** The vegetal end of the ooiyte contain RNA that may control "germ cell specification" and axial pattern in the developing embryo.

   The translocation of some of these m-RNA molecules is brought by a sub-domain of E.R.

7. **Protein synthesis :** Proteins are_synthesize on the surface of "rough E.R." due to presence of ribosome on its surface.

8. **Protein Secretion :** "George Palade" give the technique of protein sorting and processing.

- This process defines a pathway taken by secreted protein the Secretary pathway.

  RER – Golgy body – Secretary vessels – Cell Exterior.

- In mammalian cells most proteins are transferred into the E.R while they are being translocated on ribosome.

- In contrast , protein incorporated into the nuclear mitochondrion chloroplast are synthesized on free ribosomes and released into the cytosol, when translocation is completed.

Insertion of protein into E.R. membranes :

- Proteins destined within the lumen of E.R Golgi body lysosome are translocated across the "ER membrane and released into the lumen of ER.
- They proceed same pathway that of secretary protein.

  ER—Golgi – Plasma Membrane.
- When polypeptide chain across the membrane the signal sequence is cleared, so the amino terminus a polypeptides chain exposed to E.R. lumen.
- Translocation is halted by a "stop-transfer sequence" that close the translocation channel.

Protein folding : Proteins are Tran located across the ER as unfolded chain while translocation is still in progress. These polypeptide fold into their 3d structure within the ER, assisted by molecular chaperons that facilated the foldings of polypeptide chain.

Protein glycosylation : Proteins are also glycocylated on the specific asparginine residue within the ER while translocation is still in progress.

- Protein glycosylation : Proteins are also glycocylated on the specific asparginine residue within the ER while translocation is still in progress.
- Protein is modified further after transporting to the golgi body.

Golgi Complex :-

- First described by C. Golgi in nerve cells, also called dictyosomes.
- Golgi complex has a key position in the functioning of membrane system and provides connection between ER and per nuclear space; with the nucleus

- Performs function for transport of material from the nuclear to the cytoplasm.

Ultrastructure

- It varies in farm from a compact discrete granules to a dispersed filament.
- Pleomorphic and consist of a series of double membranes found in the form of cisternae sacs or lamellar units.
- Each golgi stack has two faces, cis face and trans face. These faces are closely connected to a network of interconnected tubules land form Cis golgi network (CGN) and trans-Golgy network (TGN).
- Isolation of Golgi complex brought by gentle homogenization followed by differential and gradient centrifugation.
- It has a lower density than E.R and mitochondria and forms a distinct band in gradient centrifugation.

Functions :

It is involved in the transport of macromolecules between the cells; within the cells and membrane trafficking.

- For export, the material is packaged into vesicle which are detached and transported across plasmamembrane via pinocytosis exocytosis.
- It includes zymogen of pancreatic cells.melanin granules compounds of thyroxin.
- It is also involved in the formation of cell plate during cell division
- Involves in intragolgi transport.

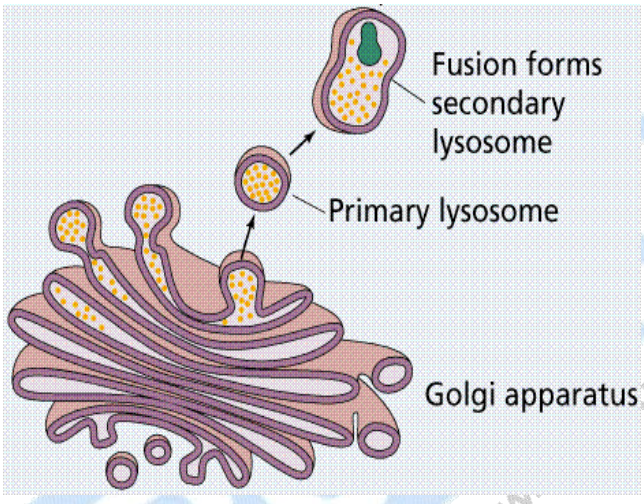
Origin : Individual stacks of cisternae may arise from pre existing stack by fragmentation.

- Cisternae membrane may arise from rough ER which changes to smooth ER and becomes the golgi cisternae.

Lysosomes :

- Lysosomes namely by De Duve.

- They contain digestive enzymes capable of lysis body.
- It's isolation is done by centrifugation after mild homogenization.
- Each lysosome contain 40 types of hydrolytic enzymes. For the activity of these enzymes lysosomes maintain a pH of about 6 in its interior. They can do little damage at higher pH.



- It also contain Trans membrane proteins, helps in transport of digestion product to the cytosol where they can be excreted by the cell.
- The proton pumps of lysosomal membrane utilize energy released due to ATP hydrolysis so maintaining the acidic nature of lumen.

Structure :

- Lysosomes appears as dense bodies ;and surrounded by a membrane.
- Their shape and density vary greatly and its interior contains granulated stroma and vesicle.
- Identification is difficult, the only criteratia is the presence of lytic enzymes.

Types of Lysosomes : Depending upon the function following types of

lysosomes are recognized :-

Primary Lysosomes : Also known as "starch granule" is a small body having enzymes synthesized by ribosomes attached to E.R.

These enzymes first enter the golgi complex where acid phosphatase reaction takes place.

Secondary Lysosome : also known as heterophagosome results from phagocytosis or pinocytosis of foreign material by the cell.

- Ingulfed material is digested by hydrolytic enzymes .

Residual bodies are formed if the digestion is incomplete. It may be due to the absence of some enzymes.

Autophagic vacuole also known as autophagosome, containing a part of the cell, itself for digestion.

- Are formed during pathologic process.
- Plant and fungal vacuoles contain hydrolytic enzymes but perform diverse function like storage, degradation.

Functions : Lysosomes are lytic in nature and are involved in digestion of intracellular/extracellular particles.

Endocytosis and digestion of macromolecules.

- Macromolecules are initially delivered into early endosome and they reach late endosome.
- Hydrolyses starts in pH of 6.0 and completed in pH of 5.0

Digestion of External Particles :

- Large particles taken into the cell by a process called phagocytosis.
- Cell ingulf the particle and forms a Sac i.e. known as **phagosome**.
- Macrophages and neutrophills are the examples of phagacytes.
- Enzymes present in lysosomes will then bring digestion

Digestion of Intracellular substances:

During this digestion, substances to be digested penetrates the cells own lysosomes, process is known as autophagy.

Cellular Digestion :

- After death of cell, liberated enzymes then digest the dead cell.

Extrcellular Digestin :

- Reverse to phagocytosis e.g. of this mechanism is the penetration of sperm to the ovum.
- It is thought that golgi apparatus involved in the formation of lysosomes.

Peroxisomes :

- Also known as "microbodies discovered by De Duve.
- Peroxisomes are ovoid granules surrounding by a single membrane.
- Their number/cell range from 70-100 as against 15-20 lysosomes / cell.
- They differ from mitchondria and chloroplast in that they do not contain DNA/ribosome.
- Peroxisomes resemble ER in being self replicating organelle without their own genome.
- Peroxisomes are synthesized outside their boundary in the cytosol because they neither have their own genome nor a protein synthesis machinery.
- Transport of peroxisomal proteins is facilitated by a specific sequence of three amino acids located near the carboxyl terminus of these proteins.
- Deficiency of this short signal sequence is called as "Zellweger Syndrome".

Functions :

Peroxisomes resembles mitochondrion in utilizing oxygen.

Mainly Peroxisomes have two functions :

(i) Lowering of the intracellular concentration of oxygen produced due to photosynthesis.

(ii) Carrying out useful oxidative reactions.

- In mitochondria these functions are coupled with ATP formation due to oxidative phospharylation.
- Peroxisomes performs some functions not taken over by mitochondria.

Removal of O_2 and production of H_2O_2

- Peroxisomes contain enzymes that use molecular O_2 to remove hydrogen from organic substances and produce H_2O_2 i.e. utilized by catalase to oxidize substances.

  $RH_2 + 0_2$ ----- $R+H_2O_2$

  (Organic Substance)

- This reaction is important in liver and kidney cells for detoxification of toxic molecules that enter the blood stream.

Breakdown of fatty acid by β-Oxidation

- Acetyl CoA produced during β-Oxidation is exported from peroxisomes to the cytosol for reuse in biosynthetic pathway.

Photorespiration and glyoxylate cycle

- In leaves peroxisomes helps in photorespiration in which O_2 is used and CO_2 liberated.
- The geminating seeds they convert fatty acids into sugars by a series of reaction known as glyoxalate cycle so they are called glyoxysomes.
- Glyoxalate cycle does not occur in animals.
- Peroxisomes are also responsible for certain disease due to deficiency of a specific matrix enzyme.
- Peroxisomes arise only from pre-existing Peroxisomes by growth and fusion.

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Endosome

- Also called receptosome.
- It is a heterogeneous structure consisting of membrane bound tubules and vesicles.
- Endosome have an acidic medium with pH ranging from 5.0-5.5 i.e. maintained through H+ pumps.
- Sometimes considered as precursors of lysosomes and differ from them only in the absence of degradative enzymes.
- Two types of Endosome are present (i) Early endosome : i.e. just beneath the plasma membrane (ii) late endosome are closer to the nucleur.
- It has been shown that early endosome may mature into late endosome which mature into lysosome

Centrosome

- Centrosome term was coined by T. Boveri.
- Present in the animal and lower plant cell.

Structures : Each Centro some contains two centrioles surrounded by pericentriolar material.

- The wall of each centrioles contains a triplet fibres arranged around a central axis.
- Triplet fibres consist of three secondary fibres
- There is evidence for the presence of DNA (RNA in centrioles)

Function : Centro some is implicated in the formation of basal bodies, spindle fibers, cilia etc. As the time of cell division approaches, the centrioles separate and more to opposite poles of the nucleus.

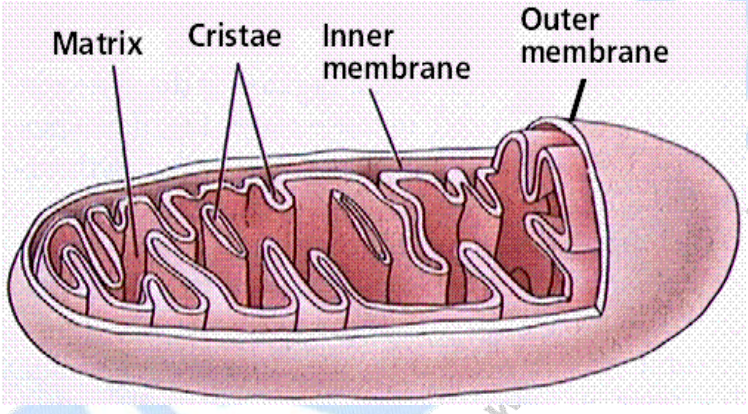
- Origin of centrosome is mainly through the replication of centrioles which are semiautonomous.

Q.7. What are the characteristic features of mitochondria that aid in their identification?

Ans. **Mitochondria**

Kolliker first recognized the structure known as Mitochondria.

- Mitochondria name given by C. Benda, mitochondria of a cell are collectively known as Chondriome.
- Can be more easily seen in cultured cell rather under in vivo condition.



Ultrastructure

- Mitochondrian has a double membrane envelop two membrances being separated by perimitochondrial space.
- Inner membrane forms mitochandrial cristae, it divides mitochondria into 2 chambers namely (i) Perimitochondrial space between outer and inner membrane. (ii) The inner chamber which is occupied by mitochondrial matrix.
- Cristae may be simple or branched forming a complex network, provides additional membrane surface.
- Particles in inner membranes are called F1 particles.
- Submitochondrial particles present in mitochondria responsible for respiratory chain phosphorylation brought about by differential centrifugation.

- Mitochondria can be easily isolated by cell fractionation brought about by differential centrifugation.
- Two miochondrial membranes Could also be separated by density gradient centrifugation.
- Mitochondria mainly consist of proteins (65-70%) and lipids (25-30%). However, DNA and RNA in small amount also present.
- Mitochondria have their own DNA t-RNA , RNA polymerase, ribosome.
- Mitochondrial DNA differs from nuclear DNA and resembles bacterial DNA.
- Mitochondrial have separate protein synthesizing machinery; therefore, considered as 'Semi-autonomous".
- Outer membrane has 40% and inner membrane has 20% lipid content.

Mitochondrial Genome :

- Can be circular or linear in shape.
- Gene content in mt DNA is relatively low, compared to Cp DNA in green plants.
- The No. of genes in mt DNA varies from 5 to 94.
- The 4 genes that are common to all mt DNAs are cab coil and rns and rnl.
- Size of mt DNA varies from 6kb. to 35 kb.

Transport of proteins into mitochondria

Few of proteins encoded by mitochondrial genome (mt- DNA).

- Generally these proteins form only subunits of protein complexes, other subunits being encoded by the nuclear genome and later transported to mitochondria.

  Transport of a protein to outer membrane or to mitochondrial matrix requires a single signal peptide, its transport to inner membrane requires second signal peptide.

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Transport of protein to mitochondrial matrix

This transportation requires :

(i) A signal peptide attached to the amino terminus of protein to be transported.

(ii) Receptor protein at outer membrane.

(iii) ATP hydrolysis to supply energy

(iv) Electrochemical gradient.

Transport of protein into the inner membrane

These proteins follow one of the two pathways

(i) They are prevented from completed the journey to the matrix.

(ii) They may reach the matrix and are then transported back across the inner membrane.

Functions: Referred to as "powerhouse" of the cell, since they produce 95% of ATP molecules in animal cell.

"This energy produced during the breakdown of food molecules that involves:

(i) EMP/Glycolysis (ii) Oxidative decarboxylation (iii) Oxidative phosphorylation.

Q.8. Discuss the structure of chloroplast giving details of structure of thylakoids. How are these organized in different plants?

OR

Discuss the chemistry of chloroplast. Do you agree that chloroplast is autonomous organelle. Why?

Ans. Chloroplast –

- Chloroplasts are discrete membrane bound structures found in eukaryotes for carrying out photosynthesis.
- Chloroplasts vary in shape, size and number in different species, but relatively constant within cells of some tissues.

- Chloroplasts are bigger in plants grown in shades than those in plants grown in sunlight.
- The chloroplasts are embedded in cytoplasm and their number per cell may be fixed or variable.
- In higher plants usually 20-40 chloroplast/cell are found.

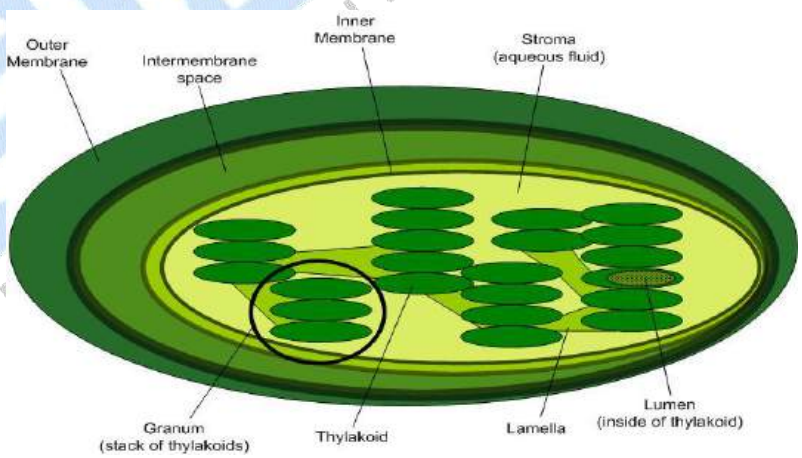
Ultrastructure

- Chloroplast is enclosed in two smooth mem branes separated from one another by peripcastidial space like per mitochondrial space.
- The internal structure of a chloroplast can be differentiated into Grana

  and Stroma where light and dark reaction is completed.
- The membrane system consist of closed thylakoids.
- Thylakoide are embedded in a proteinaceous stroma called matrix and can be of two types :

  (i) **Smaller thylacoids** are stacked over one another to give rise to grana lamellae.

  (ii) **Large thylacoids :** Connect the grana and are known as integranallamellae.



- There may be single, paired or triplet thylacoids in granum.
- It is thought that grana lamellae and stroma are similar in their origin.
- Grana lamellae contain the light absorbing pigment, inner surface of two layers is granular in organization which are called quantasome that capable of carrying photochemical reaction.
- Chloroplast are isolated by differential centrifugation after homogenization.
- Main component of chloroplast is chlorophyll, which can be found as Chl.b.

Transport of proteins into chloroplast

- For import of protein across double membrane, the chloroplast seems to employ ATP hydrolysis.
- Signal peptide on chloroplast, proteins can be recognized by receptor on chloroplast membrane.
- In first step the protein passes through chloroplast double membrane to reach stroma.
- From where in the second step they are transported to thylacoid space.
- After the protein reaches the stroma, the chloroplast signal peptide cleaved by stromal peptidase which facilitates transport to thylacoid.

Functions :

Most important function is to carry out photosynthesis; that includes :

(i) "Light reaction" which takes place in grana and involves oxidation of water.

(ii) Dark reaction "Takes place in stroma and involves use of reducing power of NADPH to change CO_2 into carbohydrates.

Autonomy of chloroplast

- Genome of chloroplast (C_PDNA) resembles to bacterial genome.

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- DNA is characterized by the presence of three regions (i) two inverted repeats (IR), (2) A short single copy sequence (SSC) (3) A long single copy sequence.
- CP contain DNA, DNA polymerase, RNA polymerase and a protein synthesizing machinery for autonomy.
- C hloroplasts contain 70S ribosome resembling of bacteria but differing from 80s ribosome found in cytoplasm.

Q.5 Who for the first time observe golgi.

(A) Golgi (B) George

(C) Hook (D) Palade

Ans. (B)

Q.6 Which enzyme is found in peroxisomes:

(A) Peroxidase (B) Stearase

(C) Hydrogenase (D) Arabinose

Ans. (A)

Q.7 The ER has the function of :

(A) Mechanical signal (B) Secretion

(C) Synthesis of lipoprotein (D) None

Ans. (A)

Q.8 Mitochondria name given by :

(A) Koliker (B) Hammerling

(C) Benda (D) Waldare

Ans. (C)

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Q.9. What is cell division? How many types of cell division occur in living organism? Define the use and biological significance of each type of cell division.

O R

What is Meosis? Describe the major features of each meotic phase? Why meosis is needed for the production of gametes.

Ans. Cell cycle can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning to the next.

It involves 3 cycles : 1. chromosome cycle; 2. cytoplasmic cycle; 3. Centrosome cycle.

Cell cycle is divided into 4 stages :

G_1, S, G_2 and M.

1. **G_1Phase :** Also called "first gap phase". It involves synthesis of RNA proteins and membrance which leads to the growth of nucleus and cytoplasm of each daughter cell towards their mature size.

- It occupies 30-50% of total time of cell cycle.
- Terminally differentiated cells that no longer divides are arrested in the G1 stage, this type of stage is called as "G_0 Phase".

2. **S.Phase :** Also called "synthetic phase"

- It involves replication of DNA and synthesis of protein. So at the end of S phase, each chromosome has two DNA molecules and a duplicate set of genes.
- It occupies 35-45% of cell cycle.

3. **G_2 Phase :** Also called "Second Gap phase"

- During this synthesize of RNA and protein continues which is required for cell growth.
- It occupies 10-20% of cell cycle.
- As the G_2 phase draws to a close, the cell enters in M phase.

- These three stages (G_1, S, G_2) are also called Interphase.

4. **M. Phase :** The mitosis : Occurs in somatic cells and it occurs for multiplication of cell no. during embryogenesis and blastogenesis of plants and animals.

- Mitosis is important for replacement of cells lost to attrition and for wound healing.
- It is divided into following stages :

i) **Prophase :** Appearance of think thread like chromosome and cell become more viscous and refractile.

- During prophase kinetochores starts depositing on the centromere of each chromosome
- In early prophase there are two pairs of centrioles each surrounded by aster which is composed of microtubules.
- Mitotic spindle contain 3 types of fibre

  (I)Polar fibres (2) Kinetechore fibres (3) Astral fibres
- During prophase; the nucleolus gradually disintegrates.

ii) **Prometaphase :** Process enables the mitotic spindle to interact with chromosomes.

- In this stage, sister Chromatids become attached by their kinetochore to opposite poles.

iii) **Metaphase :** During metaphase the chromosomes are shortest and thickest and centromere occupy the equator plane region known as "Metaphase plate".

- This stage would permit proper separation of two sister chromatid in Anaphase.

iv) **Anaphase :** It begins with the synchronans splitting of each chromosome into its sister Chromatid called daughter chromosome each with one Kinetochore.

- It involves **Anaphase A** and **Anaphase B.**

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- During Anaphase A there is polewara movements of chromated due to shortning of kinetochare microtubule.
- Anaphase B involves separation of poles accompanied the elongation of polar microtubules.

v) Telephase : The events of prophase occur in revenge sequence during this phase.

- The end of polar migration is terminated by the recognition of two nuclei and their entry into the G1 phase of interphase.
- Mitotic apparatus except centrals disappear

Cytokinesis

- During cytokinesis. the cytoplasm divides by cleavage with help of mitotic spindle.
- It begins in anaphase and continues through telephase and into interphase.
- It begins with the furrowing of plasma membrane during anaphase.
- Cleavage is accomplished by the contraction of a ring composed of actin filament. Called **"Contractil ring"**
- Actin-myocin interaction pulls the plasma membrane down into furrow.
- When cleavage ends, the contractile ring is finally dispensed with altogether ;and the plasma membrane of cleavage furrow narrow to form the 'mid body:"

Significance of mitosis

- It helps the cells in maintaining proper size.
- Mitosis provides ;the opportunity for the growth and development to organs.
- Old decaying and dead cells are replaced by help of mitosis.
- In some organisms, mitosis helps in sexual reproduction.

- Cleavage of cells, during embryogenesis and division of blastema both involves mitosis.

MEIOSIS

- Meiosis produces a total of 4 haploid cells from each original diploid cell. These haploid cells either give rise to gametes which through in vitro fertilization support sexual reproduction and a new generation of diploid organism.
- Male and female gametes are called spare and grows into multicellular haploid structure during fertilization they unit to produce zygote.
- It resembles two initatic divisions. First division is known as "Heterotypic divison". In second divison, haploid cell divides mitotically and results into four haploid cell called "homotypic divison".

Heterotypic division:

- It divides into following phases:

1.Prophase-1 : Largest prophase, includes following sub stages :

(i) Proleptotene: In this stage chromosomes are extremely thin, long, uncoiled and thread like structure.

(2) Leptotene : Chromosomes become more uncoiled and assume a long thread like shape.

The centrioles duplicates and migrates towards opposite pole.

(3) Zygotene : In this stage pairing of homologous chromosomes takes place.

"Synapsis" (Pairing of chromosome) occur at this stage.

Three types of synopsis have been recognized :

(i) Proterminal (2) Procentric (3) Localized synaptonemal complex helps in recombination or crossing over.

(4) Pachytene: In this stage pair of chromosomes become twisted around each other .

- Dyad or tetrad stage of chromosome appear in this stage.

- Crossing over takes place in this stage that involves redistribution reshuffling, which is accomplished by chiasmata formation.

(5) Diplotene : Unpairing or desynapsis is started.

- SC appears to be dissolved.

(6) Diakinesis : Bivalent chromosomes become more condensed.

- Chiasma moves from the Centromere towards the end of chromosomes, this type of movement is called as "terminalization".

2.Metaphase : Nuclear envelop disintegrates and microtubules attached between full centrioles in form of spindle.

3. Anaphase : 1. Homologues are free from each other due to shortening of chromosomal fibres.

4.Telophase

- Nucleolus reappears and two daughter chromosomes are formed.
- Both cells pass through a short resting phase of interphase where no replication occurs. So in the second prophase chromosomes are the same double-stranded structure.

Homotypic division

- It is actually a mitotic divison which divides each haploid meiotic cell into two haploid cell.
- It includes following 4 stages :

1) Prophase II : In this phase each centriole divides into two and thus 2 pairs of centrioles are formed.

- Nuclear membrane and nuclear disappear.

2) **Metaphase II :** In this phase chromosome get arranged on the equator of spindle.

- Centro mere divides into two and thus each chromosome produces two daughter chromosome microtubule or spindle are attached with the Centro mere of chromosome.

3) Anaphase II : Daughter chromosome move towards the opposite pole due to shortening of microtubule.

4) Telophase II : Chromatids migrates to the opposite poles and known as chromosome.

- ER forms nuclear envelop around the chromosome and nucleolus reappears.

Significance

- Meiosis maintains a definite and constant no. of the chromosome
- By crossing over, meiosis provides exchange a gene and cause the genetical variation among the species.

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Section-B

Transport Across Cell Membrane & Receptors, Models of Extra Cellular Signaling

Q.1. Describe the chemical constitution of plasma membrane and discuss the role of phospholipids and gycolipids in the maintenance of structure and function of plasma membrane?

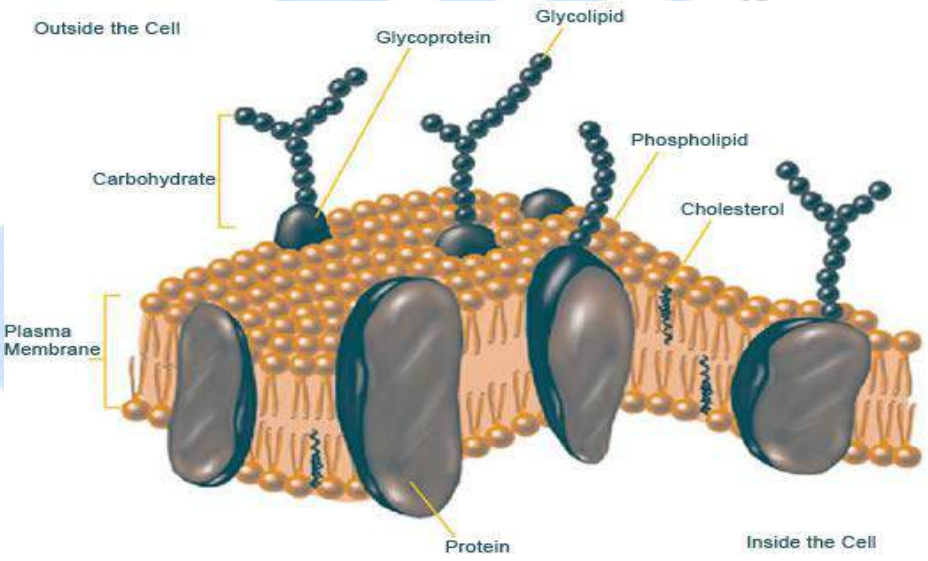
Ans. Structure and organization of plasma membrane :

- Like other cellular membranes, the plasma membrane consist of both lipids and proteins.
- Phospholipids bilayer which forms a barrier between two aqueous compartments.
- Proteins embedded in phospholipids bilayer carry out specific functions of plasma membrane, including selective transport of moleculer.
- Plasma membrane of animal cell contain 4 major phospholipids (Phophatidyl choline, Phosphatidylethanol amine. Phosphatidyl serine and sphingomyelin) which are asyminetrically distributed between the two halves of the membrane bilayer.
- PM of animal cell also contain glycol lipids and cholesterol, found exclusively in the outer layer of the PM.
- Depending on temperature cholesterol has distinct effects on membrane fluidity. At high temperature, it interferes with the movement of phosphlipid fatty acid then making outer part less fluid and reducing its permeability to small molecules.

- At low temperature cholesterol prevents membrane from freezing and maintaining membrane fluidity. Although plant and material cell lack cholesterol.
- Lipids play an important role in all signaling and uptake of extracellular molecules by endocytosis.

Membrane Proteins

- Most plasma membrane, consist of approx. 50% protein and 50% lipid since proteins are much longer than lipids. This percentage correspond about one protein molecule every 50-100 molecules of lipid.
- Singer and Nicolson proposed the **fluid mosaic model** of membrane structure which is generally accepted as basic for membrane organization.
- Singer and Nicolson distinguish two class of membrane proteins called "Peripheral" and "Integral" membrane proteins.



- "Peripheral proteins were defined as proteins that dissociates from membrane, by treatment with polar reagent such as high salt concentrated solution that do not disrupt phospholipids bilayer.

- These proteins are not inserted into the hydrophobic interior of the lipid bilayer. Instead they are directly associated with membrane through protein protein interaction.
- These interaction involve ionic bonds, which are disrupted by extreme pH or high salt.
- These proteins are not inserted into hydrophobic region of lipid layer instead associated with membrane through protein protein interaction.
- Many integral proteins are "Transmembrane proteins" which span the lipid bilayer with portion expose and on both side of membranes.
- These proteins can be visualized by freeze fracture technique. In this specimen proteins are than apparent as particles on internal faces of membrane.
- Mostly transmembrane proteins are glycoproteins with their oligosaccharides exposed on the surface of cell.
- The membranes of human erythrocytes contain about a dozen of proteins. Most of these proteins determines the cell shape.
- Major peripheral membrane proteins of RBCs includes actin and keratin.
- Two major integral membrane proteins of RBCs are glycopharin and band 3.
- Glycophorin is a small glycoprotein and crosses the membrane with a single membrane spanning of 23 amino acid.
- Within membrane dimers of band 3 from globular structure containing internal channels through which ions are able to travel across lipid bilayer.
- "Porins" – a class of proteins form channels in the outer membrane of some bacteria.
- In contrast to transmembrane proteins, avariety of proteins are anchored in plasma membrane by cavalently attached lipid.

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- Members of these proteins are inserted into outer leaflet of plasma membrane by glycosylphosphatidyl inositol (GPI) anchors.
- The extracellular portion of plasma membrane proteins, are generally glycosylated, consequently the surface of the cell is covered by a carbohydrate coat known as "Glycocalyx, formed by oligosaccharide.
- It helps in protect the cell surface serve or marker for a variety of cell –cell interaction.

Q.2. How do you distinguish passive transport from active transport? Discuss the role of Na+ K+ pump in transport across cell membrane?

OR

Write short notes on (i) Active transport (2) Passive Diffusion (iii) Na+ K+ pump.

Ans. The plasma membrane is selectively permeable to small molecules. Most biological molecules are unable to diffuse through the phospholipids bilayer. So the plasma membrane act as a barrier that blocks the free exchange of molecules between the cytoplasm and external environment.

Channel proteins and carrier proteins then mediate the selective passage of small molecules across cell membrane.

No membrane proteins are involved and direction of transport determine simply by relative concentration of inside and outside of cell.

Non selecting process by which any molecule able to dissolve in the phospholipid layer is able to cross the plasma membrane and equilibrate between the inside of outside of PM.

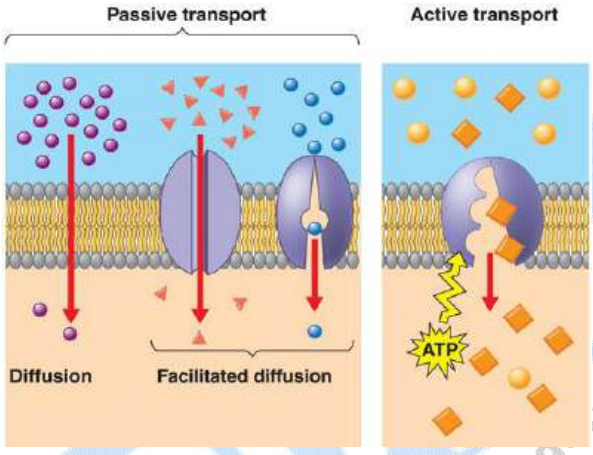
Only small molecules like gases and hydrophobic molecules are able to diffuse across plasma membrane.

Other larger uncharged polar molecules such as glucose are unable to cross plasma membrane by passive diffusion as they are charged molecules.

The passage of these molecules instead requires the activity of specific transport and channel proteins.

It differs from facilitated diffusion in that it does not require mediator proteins for transport of macromolecules across plasma membrane.

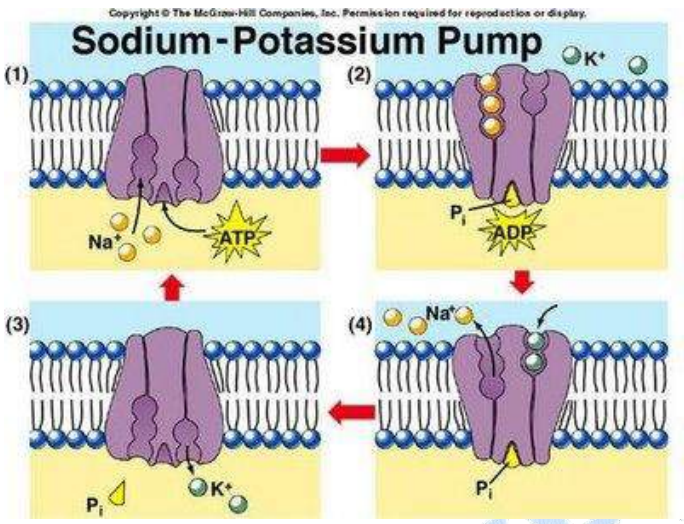
In many cases, cell must transport molecules against their concentration gradient so inactive transport energy provided by another coupled reaction is used to drive the transport of molecules in the energetically unfavourabledirection.



The ion pumps responsible for maintaining gradients of ions across plasma membranes through ATP hydrolysis.

Concentration of Na+ is approximately ten times higher outside than inside whereas K+ concentration is higher inside than outside. So these ions are maintained by the Na+ - K+ pump, which uses energy derived from ATP hydrolysis to transport Na+ and K+ against concentration, gradient.

First Na+ ions bind to high affinity sites inside the cell that stimulates hydrolysis of ATP and phosphorylation of pump inducing a conformational change that exposes the Na+ binding site to the outside of cell and reduces their affinity for Na+.



Bound Na+ is released into extracellular fluid and high affinity K+ binding sites are exposed on cell surface.

Binding of extracellular K+ to these sites then stimulates hydrolysis of phosphate group that stimulate second conformational change exposing the K+ binding sets to the cytosol and lowering their binding affinity so that K+ is released inside the cell.

Pump has 3 binding sites for Na+ and 2 for K+ so each cycle transports 3 Na+ and 2K+ at the expense of 1 molecule of ATP.

One critical role of Na+ and K+ is the propagation of electric signal in nerve and muscle Na+ gradient is also utilize to drive the active transport of other molecule and maintain osmotic balance and cell volume in animal cell.

The active transport of Ca^{+2}across plasma membrane is driven by a Ca^{+2} pump that related to the Na+ - K+ pump.

Active transport driven by the Na+ is responsible for the uptake of glucose from intestinal lumen.

The coordinate uptake of glucose and Na+ is an example of "Symport" the transport of 2 molecules in the same direction.

Facilitated diffusion of glucose is "uniport" the transport of only a single molecule.

Active transport can also take place by "antiport" in which two molecules are transported in opposite direction.

Active transport of Ca^{+2} across PM is driven by Ca^{+2} pump and powered by "Calcium ATPase" The Ca^{+2} pump transports Ca^{+2} ant of the cell. So intercellular Ca^{+2} concentration are extremely low in comparison to extracellular concentration.

This low concentration of Ca^{+2} makes the cell sensitive to small increases in intracellular Ca^{+2} makes the cell sensitive to small increases in intracellular Ca^{+2} play important role in cell signaling.

One of the best characterized ion channel is the nicotinic acetylcholine receptor of muscle cell.

Binding of acetylcholine opens a channel that is permeable to Na+ and K+. This permits the rapid influx of Na+ which depolarizes the muscle cell membrane and triggers an action potential.

The action potential then results in the opening of voltage-gated Ca^{+2} channels leading to increase in intracellular Ca^{+2} that signals contraction.

Ca^{+2} ATPase pumps maintaining a low concentration inside the cytosol.

In "erythrocytes" the Ca^{+2} pumps are located in the plasma membrane and function to transport Ca^{+2} ion out of cell and In muscle cell Ca^{+2} ion pumps are located in the membrane of ER.

Ca^{+2} – ATPase transports Ca^{+2} from cytosol to the SER for relaxation of muscle cell.

Release of Ca^{+2} from ER into cytosol causes contraction of muscle cell. It store Ca^{+2} ions by 2 types of reservoir protein :-

(i) "Calsequestrin" which tend to bind upto 43 Ca^{+2} ions with it.

(ii) "High affinity Ca^{+2} binding protein" which binds Ca^{+2} ions and reduces the concentration of Ca^{+2} ions in the co – transport of one Mg+ ion.

Q.3 Discuss the molecular mechanism of vacuolar and lysosomal transport involving Na+ pump?

Ans. Proton Pump :- Lysosomal and vacuolar membrane contains the ATP dependent proton pump that transport proton from cytosol into lumen of organelle.

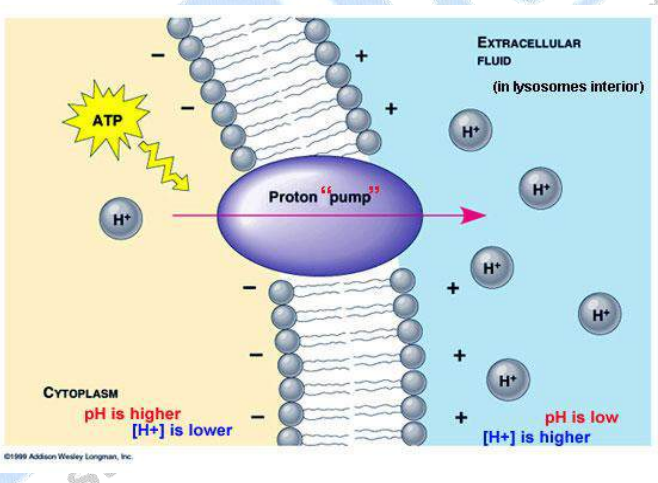
It keeps the interior membrane very acidic (pH=4.5-5.0). The pH of the cytosal is about 7.0.

Proton pumps also occur in mitochondria and chloroplast where they participate

In the generation of ATP from ADP.

Proton pumps also cause acidification of inamation stomach.

- In apical membrane of exyntic cell which secretes HCl. Are located ATP dependent protein pumps.
- Hydrolysis of ATP is coupled to the transport of H^+ions out of the cell.



- Hil thus involves 3 types of transport proteins.
  (i) Anion-exchange (2) Chloride permeases (3) ATP dependent proton (H+ pump)

Uniport, Symport and Antiport : These carrier proteins which transport a single solute from one to other side of membrane, are called uniport.

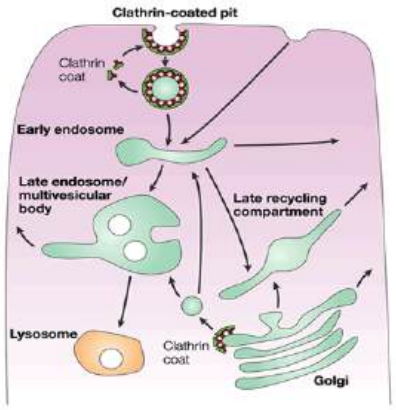
- In other transporters, transfer of one solute depends on the transfer of second solute either in same (symport) direction or in opposite (antiport) direction.

- Both symport and anti-port collectively form co-transport.
- In most animal cells must take up glucose from extra cellular fluid where concentration of glucose is high.
- In contrast, kidney cells must take up glucose from the iumen of kidney tubules where the concentration of sugar is low.
- These cells transport glucose by symport with Na+ ion whose extra cellular concentration is very high.

Q.4 How does co-transport function in different prokaryotic cells? Give an outline about Exocytosis and Endocytic pathway and discuss the role of different cell compartments in these two pathways.?

Ans. **Exocytosis**

- The fusion of vesicle to the plasma membrane is called Exocytosis. In this way cells produce and secrete most of proteoglycans of the extra cellular matrix.
- Also called "Emeiocytosis" and cell vomiting.
- In all eukaryotic cells, secretory vesicles are carrying new plasma membrane and cellular secretion such as protein, new plasma membrane and cellular secretion such as protein, lipids and carbohydrates from Golgi to the plasma membrane by exarytosis.
- The proteins to be secreted are synthesized on the RER. They pass into the lumen of ER, glycosilated and transported to the Golgi apparatus by ER derrived transport vehicles.
- In Golgi apparatus, the proteins are modified concentrated glycosidated sorted and finally packaged into vesicles that pinch off from trans Golgi tubules and migrate to plasma membrane to fuse with it and release the secretion to cells' exterior.



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- In contrast, small molecules to be secreted are actively transported from cytosol into preformed vesicle where they are complexed to specific macromolecules so that they can be stored at high concentration without generating osmotic gradient.
- During exocytosis the vesicle is incorporated into plasma membrane.
- Cells are specialized for secreting some of their products rapidly on demand concentrate and stored these products in "Secretory vesicle".
- These vesicles form by clathrin coated budding from the trans golgi network and they released their content to the cell exterior by exocytosis in response to extra cellular signal.
- Secreted product can be either a small protein or molecule.

Endocytosis

- In endacytosis small regions of plasma membrane fold inwards until it has formed new intracellular membrane limited vesicle.
- In eukaryotes, 2 types of endocytosis occur :

  (1) Pinocytosis (2) Receptor-mediated endocytosis.

(i) Pinocytosis : It is the non-specific uptake of small droplets by pinosome.

- From the inner end of each pinocytic channel small vacuoles are pinched off and these move towards the centre of the cell where they fuse with 1^0 lysosome to form food vacuoles.
- Ultimately ingested contents are digested.
- Pinocytosis which occurs at submicroscopic level is known as "micropinocytosis".
- In this process plasma membrane invaginate to form small vesicle. These vesicles are not coated by clathrin protein and they move across the cytoplasm of endothelial cell to fuse with opposite plasma membrane. This is called transcytosis.

(ii) Receptor : mediated endocytosis

- In this a specific receptor recognize an extra cellular macromolecule and bind with it substance bind with the receptor is called "Ligand". Eg.-viruses, small proteins, vita B_{12}.
- In this process following steps are there :
  - i) Interaction of legends and cell surface receptor.
  - ii) Formation of coated pits and coated vesicles.
  - iii) Fusion of endocytic vesicle
- In the cells exists a complex set of heterogeneous membrane bound vesicle, called endosome that extends from the periphery to the perinuclear region.
- Endosomes may be of two types (1) Peripheral (2) Perinuclear
- In this process, ligands are delivered to the peripheral endosomes which slowly more inward to become perinuclear endosome.
- Then these are converted into endolysosome and then lysosome due to following three activities.
  - i) Fusion of transport vesicle from golgi apparatus.
  - ii) Continuous membrane retrival.

iii) Increased acidification.

- The endosomal compartment also act as the main sorting station in the endocytic pathway.
- Acidic environment of end some cause dissociation of legends from their receptor. Such legends are destined for destruction in the lysosome.
- "Phagocytosis" is a form of endocytosis, occurs in most protozoans.
- Phagocytosis occurs very actively in granular leucocytes and in the cells of mesoblastic origin..

Q.5 What do you understand by signal simplification. Describe different modes of cell-cell signaling including autocrine, paracrine and endocrine signaling?

Ans. Different kinds of molecules transmit information between the cells of multicellular organism. These molecules act as legend that kind to receptor expressed by target cell.

- Some molecules carry signals over long distance whereas others act to convey information between neighbouring cells.
- Signaling molecular differ in their mode of action on their target cell. Some molecules are able to cross plasma membrane and bind to intracellular receptor in the cytoplasm whereas most bind to receptor expressed on the target cell surface.

Modes of cell-cell signaling

- Cell signaling can result either from direct the action of secreted signaling molecules.
- The multiple varieties of signaling by secreted molecules are divided into three general categories based on the distance over which signals are transmitted.

  1) Endocrine Signaling

  2) Paracrine Signaling

  3) Autocrine signaling.

- In endocrine signaling signaling molecules are secreted by specialized endocrine cell and secrete signaling molecules called "Hormones into the sap, which carries the signal to target cells distributed throughout the body..
- Because endocrine signaling relies on diffusion and blood flow, it is slow.
- In contrast, nerve cells can achieve much greater speed and precision.
- They can transmit information over long distance by electric impulses that travels at rate of upto 100 m/sec.
- Another difference between endocrine and synaptic signaling, is that whereas hormones are greatly diluted in the blood stream and so must be able to act a very low concentration.
- A classical example is provided by the steroid hormone estrogen, which produces by ovary and stimulate development of female reproductive system.
- In animals more than 50 different hormones are produced by endocrine glands.
- In contrast to hormones some signaling molecules act locally to affect the behavior of nearby cells.

Paracrine Signaling

In paracrine signaling, a molecule released by one cell acts on neighboring target cell.

- For paracrine signals to be delivered only to their proper target, secreted signaling molecules must not be allowed to diffuse too far. For this reason, they are rapidly taken up by neighboring target cells or destroyed by extra cellular enzyme.
- For a large complex multi cellular organism short range signaling is not sufficient on its own to co-ordinate the behavior of organism's cell.

- Specialized cells have evolved with a specific role in signaling between widely separate parts of body.
- Most sophisticated of these are nerve cells or neurons which typically extend long processes that contact target cell far away.
- When activated by signals from environment or from other nerve cell, a neuron sends electrical impulses along its axon.
- When an impulse reaches the nerve terminals at the end of axon, it stimulates the terminals to secrete a chemical signal called "Neuro-transmitter"
- The nerve terminal contacts their target cells at specialized cell junction called Chemical synopsis, which are designed to ensure that neuro-transmitter is delivered to synaptic target cell rapidly.

Autocrine Signaling

- In autocrine signaling, a cell secretes signaling molecules that can bind back to its own receptor.
- For Example : During development once a cell has been directed into a particular path of differentiation, it may begin to secrete autocrine signals that reinforce this developmental decision.
- Autocrine signaling is most effective when carried out simultaneously by neighboring cells of the same type, it may be used to encourage groups of identical cells to make the same developmental decisions..
- Thus autocrine signaling is one possible mechanism underlying the "community effect". Observed in early development where a group of identical cells can respond to a differentiation – inducing signal but a single isolated cell of same type cannot.
- "Eicosanoids" are signaling molecules that often act in an autocrine mode in mature mammals.
- They are continuously synthesized in the plasma membrane and released to the cell exterior where they are rapidly degraded by enzymes in extra cellular fluid.

- When cells are activated by tissue damage ;or by chemical signals, the rate of eicosanoid synthesis is increased.

Q.6 What are receptors? Describe different types of intracellular and membrane bound receptors?

Ans. Receptors : Different receptor hormones that are small hydrophobic molecules differ greatly to one another in both chemical structure and function. They are act by similar mechanism differing directly across plasma membrane and bind to intra cellular protein.

- Legend binding activates the receptors which then directly regulate the transcription of specific genes.
- These receptor constitute the "intracellular receptor super family."
- Steroid hormones Vita-D and molting hormones eicdiasone are all made from cholesterol, steroid sex/hormones are made in the testis and ovary and are responsible for 2^o characteristics.
- "Vita-D" is synthesized in the skin in response to sunlight, after its conversion to an active farm in the liver. It functions to regulate Ca^{+2}.
- Metabolism promoting Ca^{+2} uptakes in the gut and reducing its excretion in kidney.
- Thylacoid hormones act to increase metabolism in a wide variety of cell types while retinoias play important roles in vertebrates' development.
- The intracellular receptor far steroid thyroid and vita D all kind to specific DNA sequences adjacent to the genes that the legend regulates some (Cortico/receptor) are located in the cytosol and bound to DNA only following legend binding.
- Others (retinoid receptor) are located in the nuclear and bound to DNA even in the absence of legend.
- In either case legend binding alters the confirmation of receptor protein, while activates gene transcription.
- In many cases response takes place in two steps.

(i) The direct inactivation of the transcription of small No. of specific genes within 30 minutes is known as "Primary response".

(ii) The products of these genes then activates other genes and produce delayed "Secondary response".

Q.7 Write a short note on different receptors including some examples?

Ans. All water soluble as well as some lipid soluble bind to specific receptor proteins on the surface of target cells they influence.

- These cell surface receptor proteins act as signal transducers, they bind, to signaling lygand and convert this event into intracellular signals that alters the behavior of target cell. Examples of these receptors are :

  (i) "Ion-Channel – Linked receptor" : Also known as transmitter gated ion channels, are involved in rapid synaptic signaling between electrically excitable cells.

- This type of signaling is mediated by small no. of neurotrasmitter that open or close the 10^{th} channels formed by the protein to which they bind, changing the ion permeability of plasma membrane and so excitability of post synaptic cell.

- The ion channel linked receptor belongs to a family of Transmembrane protein.

  (2) "G-protein-Linked receptor" act indirectly to regulate the activity of a separate plasma membrane bound target protein, which can be an enzyme or idn channel.

- The interaction between the receptor and the target protein is mediated by a third protein called "dimeric GTP-binding regulatory protein" (G-Protein).

- The activation of target protein either alters the concentration of intra cellular mediators or alters the ion permeability of the plasma membrane.

- The intracellular mediator act to alter the behavior of other protein in the cell. All of G-protein receptor belong to a large super family

of humongous, seven-pass trans membrane proteins. All of G-protein receptor belong to a large super family of homologus, seven pass trans membrane proteins.

- G-protein consist of 3 subunits designated α, β and γ and called hetero dimeric G protein such as Ras protein.
- α – subunit regulate G-protein activity by binding to guanine nucleotides.
- In resting stage α – is bound to GDP in a complex with β and γ.
- Hormone binding induces a conformational change is the receptor such that the cytosolic domain of the receptor interacts with G-protein and stimulates the release of bound GDP.

(3) "Enzyme linked receptors" when activated either function as enzymes or are associated with enzymes.

- Most are single transmembrane proteins with their legend binding site outside the cell and their catalytic site inside.
- Compared with the two other classes, enzyme linked receptors are heterogeneous; although the great majority are protein kinases or are associated with protein kinases that phospharylate specific sets of proteins in the target cell. Examples :

An insulin receptor is a Tyrosine – specific protein kinase. It regulates both metabolism and gene expression :

(i) An insulin receptor binds insulin and undergoes autophospharylation on its carboxy-terminal tyr residue.

(ii) Insulin receptor phospharylates IRS-1 on its tyr residue.

(iii) SH_2 domain of Grb2 binds to (P) – Tyr of IRS-1 SOS binds to Grb2, then to Ras, causing GDP release and GTP binding to Ras.

(iv) Activated Ras binds and activates Raf-1

(v) RaS-1 phosphorylates MEK on two Serine residues, activating it. MEK phosphorylates ERK on a Thr and a Tyr residue, activating it.

(vi) ERK moves into nuclear and phosphorylates nuclear transcription factors such as EIK-1', activating them.

(vii) Phosphorylated EIk 1 joins SRF to stimulate the transcription, and translation of asetof genes needed for cell division.

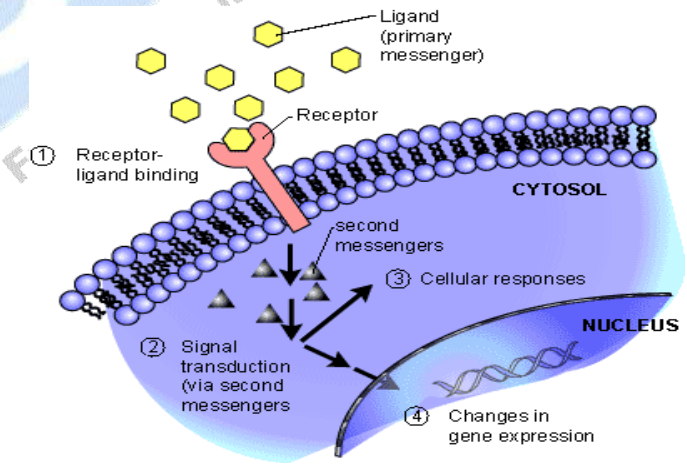
Section-C

Signal Transduction

Q.1. What is signal transduction? Describe its molecular mechanism by giving some examples of amplification?

Ans. Signal represents information. It is detected by specific receptor and converted to a cellular response, which always involves a chemical process.

- Conversion of information into chemical change is called "Signal transduction, universal property of living cell.
- The end result of a signaling pathway is the phosphorylation of target cell proteins, which changes the activity of cell.
- Signaling process used by higher eukaryotes in which they u se to communicate with one another. For example In Budding yeast S.Cerevisiae when a haploid individual is ready to mate, it secretes a peptide mating factor that signals cells of opposite mating type to stop proliferating and prepare to conjugate.



Molecular mechanism of signal transduction

- Signal transductions are specific and sensitive.
- "Specificity' is achieved by complementarity between signal and receptor molecule mediated by non-covalent weak forces.
- Multicellular organisms have additional level of specificity because the receptor will present only in certain cell types.
- Three factors responsible for sensitivity of signal transducers.

(i) High affinity of reception for signal molecule.

(ii) Co-operativity in the ligand-receptor interaction

(iii) Amplification of signal by enzyme cascades.

- Affinity between signal and receptor can be expressed as dissociation-constant (Kd).
- "Co-operativity" in receptor – legend results in large changes in receptor activated with small changes in legend concentration.

"Amplification" by enzyme "Cascades" results when an enzyme associated with a signal receptor is activated and so catalyzes the activation of many molecules of a second enzyme.

Each of second enzyme molecule activates many molecules of third enzyme and soon.

These cascades can produce amplification of several orders of magnitude within milliseconds.

When a signal is present continuously, "Desenitisation" of receptor system result when stimules falls below a certain threshold, the system again becomes sensitive.

Final feature of signal transduction is integration the ability of a system to receive multiple original and produced a unified response to the need of organism.

Following basic models are examples of signal mechanisms are :

(i) "Gated ion channels" of plasma membrane that open and close in response to binding of chemical ligends or changes in transmembrane potential.

(ii) Simplest signal transducers.

(iii) The "Acetylcholine receptor ion channel" is an example of this mechanism.

2. Receptor enzymer example – Plasma membrane receptor.
   - When one of these receptors is activated by its extra cellular legend, it catalyzes the production of an intracellular second messenger.
   - An example of this mechanism is "insulin receptor".
3. "Receptor protein", indirectly activate enzymes that generate intracellular second messengers.
4. Nuclear receptor when bind to their specific ligend, after the rate of transcription and translated into cellular protein example : Steroid receptor.
5. Receptor that lack enzymatic activity but activates cytoplasmic enzymes that act on downstream proteins either by directly converting them into gene-regulating proteins or by activating a cascade of enzyme that activates a gene regulator. Example : JAK – STAT system.

Q.2. Explain how increased CAMP in cells can activate genes?

Ans. Cyclic AMP -

C-AMP was first identified as intracellular mediator of hormone action and since has been found to act as an intracellular signaling molecule in all prokaryotic and animal cell.

- Function as an intracellular mediator, its concentration must be able to change up or down in response to extra cellular signals.
- Its concentration can change.
- five folds in seconds.

- Rapid synthesis of the molecule be balanced by rapid breakdown or removal.
- CAMP is synthesized from ATP by a plasma membrane-bound enzyme "adenyl cyclase" and it rapidly destroyed by one more CAMP phosphadiesterase which hydrolyses CAMP to 5'-AMP.
- Many extra-cellular signaling molecules alter the activity of adenylcyclase and controlling CAMP level rather than activity of phosphodiesterage.
- Different target cells respond differently to external signals that ;change intracellular CAMP level.
- All ligands that activate against cyclase.
- Different receptor for this hormone activate adrenal cyclone molecule to which they coupled by G-protein, because it is involved in enzyme activation.
- Breakdown of glycogen and most other effects of CAMP are mediated by the action of CAMP dependent protein kinases.
- Inactive farm of protein kinases A is a tetramer consisting of catalytic and two regulatory subunits.

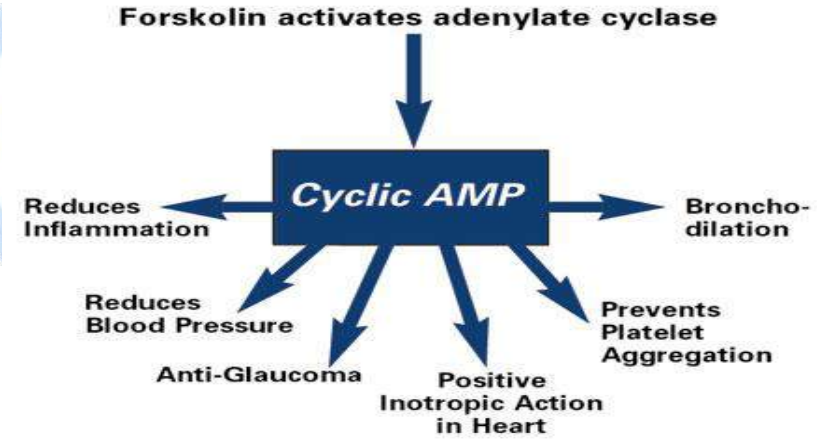


Fig. 1. Effects of increased intracellular levels of cyclic AMP (cAMP) induced by forskolin.

- CAMP binds to regulatory subunits leading to dissociation from the catalytic subunit are then enzymatic ally active and able to phosphorylates.
- In glycogen metabolism regulation protein Kphase A phosphorylates two enzymes which catalyzes the breakdown of glycogen to glucose -1-(P) by activating glycogen phospharylase.
- In this case protein kinase A phosphorylates the enzyme glycogen synthatase i.e. catalyses glycogen synthesis. In this case, phospharylation inhibits enzymatic activity.
- Elevation of CAMP and activation of protein, Kinase thus block glycogen c=synthesis and stimulate breakdown.
- In many animal cell increase in CAMP activates the transcription of target genes that contain regulatory sequence called CAMP response element.
- Sometimes CAMP function as a second messenger involved in sensing smell, when it regulates ien channels.

Q.3. Give a detail account on intracellular pathway of signal transduction and role of inositol phosphates in transduction?

O R

How does Ca^{+2} regulate many cellular responses?

Ans. G-protein linked receptor activate the signaling pathway of Inosital phospholipids.

- Phosphotidyl inositol (PI) is a minor phospholipids in cell membrane.
- By extracellular signaling molecule radioactive phosphate incorporates into PI.
- Later shown that incorporation results from the breakdown and resynthesis of inositol phospholipid.

- Two derivatives of PI, (i) PI phosphate (ii) PI biphosphate (PIP, PIP), are found to be most important in signal transduction, which are located in inner half of plasma membrane.
- The chain of events leading to PIP_2 breakdown begins with the binding of signaling molecule to a G-protein linked receptor in plasma membrane.
- An activated recept or stimulates a trimeric G. protein which activates a phospho inositide-specific phospholiphase-c called phospholipase Cβ.
- This enzyme clears PIP to generate two products; inositol triphosphate and dioxylglycerol.
- Inositol triphosphate (IP_3) produced by PIP_2 hydrolysis is a small water soluble molelcules that leaves the plasma membrane and diffuses to cytosol.
- There it releases Ca^{+2} in ER membrane.
- Channels are regulated by positive feed back in which release Ca^{+2} can bind back to channel to increase the Ca^{+2} release.

Two mechanism operate to terminate Ca^{+2} response:

(i) IP_3 is dephosphorylated by phosphatase.

(ii) Ca^{+2} that enter the cytosol is pumped out of cell.

- In most model of propagation of Ca^{+2} depend on positive feed back, whreby Ca^{+2} activates its own release.
- The models differ mainly in whether Ca^{+2} acts directly on the Ca^{+2} relese channels in ER to stimulate its own release or whether it acts indirectly by increasing the activity of phospholipids,C, so by generating surges of IP_3.. Ca^{+2} acts indirectly by increasing the activity of phospholipase C, so by generating surges of IP_3. Ca^{+2} release induces.
- Each pathway of inositol phospholipids mimicked by using a Ca^{+2} ionosphere , which allows Ca^{+2} to move into the cytosol from extra cellular fluid.

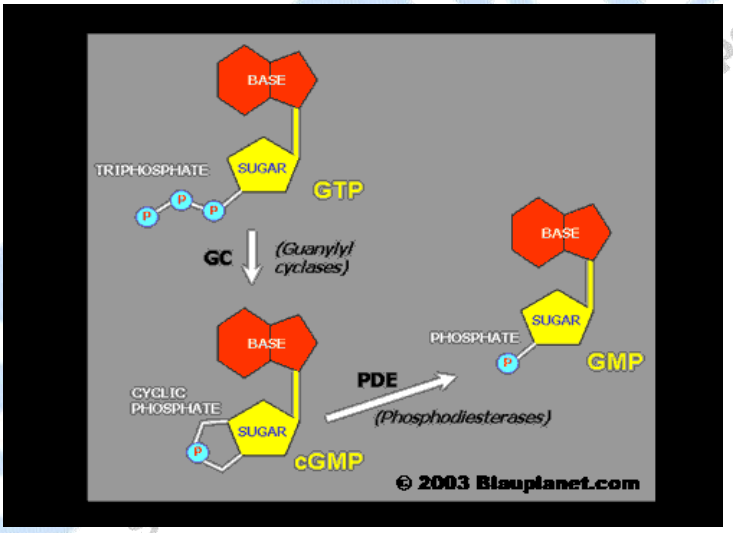
- Many of the efforts of Ca^{+2} are mediated by the Ca^{+2} – binding protein "Calmodulin" which is activated when concentration of cytosolic Ca^{+2} increases.
- Calmodulin then binds to target protein kinases that phospharylates a no. of different ion channels and transcription factor.
- In addition CaM kinase phospharylates transcription factor thus regulating gene expression (e.g. (REB).
- Thus Ca^{+2} signaling pathway functions to regulate many cellular responses.
- The entry of extracellular Ca^{+2} is important in the electrically excitable cells of nerve and muscle, in which voltage gated Ca^{+2} channels in PM are opened by membrane depolarization.
- Resulting releases of Ca^{+2} triggers the further release of Ca^{+2} from intracellular stores by activating Ca^{+2} channels.
- One effect of increasing Ca^{+2} is to trigger release of neurotransmitters so Ca^{+2} plays a critical role in converting electric to chemical signals in nervous system.

Q.4. Explain the role of CGMP in visual reception ;in the vertebrate eye?

Ans. Cyclic GMP (CGMP) is also an important second messenger in animal cells although its roles are not as clearly understood as CAMP.

- CGMP is formed from GTP by guanyl cyclases and degraded to GMP by a phosphodiesterase.
- Guanyl cyelase are activated by nitric oxide and carbon monoxide and by peptide, legands.
- Stimulation of these guanyl cyclase leads to elevated levels of CGMP – dedpendent protein kinase.
- CGMP act as to regulates ion channels.

- The best role of CGMP is the vertebrate eye, where it serves, as second messenger responsible for converting the visual signals to nerve impulses.
- The photoreceptor in rod cells of retina is a G-protein coupled receptor called "rhodopsin". It is activated as a result of absorption of light by ;the associated small molecule, which ;then isomerizes inducing a conformational change in rhodopsin protein.
- Rhodopsin then activates the G protein transducin and x-subunit of transducin stimulates the activity of CGMP phosphodiesterase, leading to decrease I n the intracellular level of CGMP.
- This change in CGMP level in retinal rod cells is translated in a nerve impulse by effect of CGMP on ion channels in the plasma membrane.



- Like CAMP, the concentration of CGMP in cells is controller by rapid synthesis (by guanyl cyclase) and rapid degradation (by CGMP phosphotriesterase).
- In this way the signal passes from the disc. Membrane to plasma membrane and a light signal is converted into an electrical one.

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Section-D

Cell Culture

Q.1. What is Primary Culture? Describe different methods of desegregation of Tissue?

Ans. A primary culture is that stage of culture following isolation of cells but before first subculture.

- There are 3 stages for consideration :

  (i) Isolation of Tissue

  (ii) Disaggregation

  (iii) Culture following seeding into culture vessel

- A primary cell culture may be obtained by allowing cells to migrate ant from fragments of tissue or by disaggregating the tissue; enzymatically or mechanically.
- Enzymes used most frequently are crude trypsin, collagenase, elastase, pronase, DNase, dispase alone or in various combination.
- Trypsin and pronse give most complete disaggregation but may damage cell.
- Collagenase and dispase give incomplete disaggregation but less harmful.
- Each tissue may require a different set of conditions.
- Embryonic tissue is preferable as it disaggregates more readily, yield more viable cells and proliferates more rapidly in primary culture than does adult tissue

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Isolation of Tissue

- Before attempting to work with human or ;animal tissue, we must be sure that our work fits within ethical rules on experimentation with animals.
- In United Kingdom, the use of embryos beyond 50% incubation is regulated.
- First sterilize the site of dissection with 70% alcohol and remove the tissue aseptically and transfer it to tissue culture laboratory.
- Transfer it into medium as soon as possible.
- Enzymatic ;disaggregation is sutiable when more tissue is available and mechanical disaggregation when large amount of soft tissue is available.
- 1^{o} explant technique was developed by Harrison.
- In this a fragment of tissue was embedded in blood plasma, mixed with embryo extract and place on a coverslip that could be examined.
- Attachment may also be promoted by treating with fibronectin.

Enzymatic disaggregation

- Intercellular matrix contain glycoproteins such as fibronectin which are protease sensitive and proteoglycans can be degraded by heparinase or hyalaronidose.
- Embryonic tissue disperses more readily; difficulty increase with obtaining viable cells with increasing age.
- Trypsin is more rapidly used in disaggregation. It is used in two opposite trends (i) Warmer trypsin (less toxic) (2) Colder trypsin (more effective).
- Cold trypsin gives a higher yield than warm trypsin.
- One of the disadvantages using trypsin to disaggregate tissue is the damage that may result from prolong exposure to the tissue.

Other Enzymatic Procedures :

Disaggregate in trypsin can be damaging or ineffective, so attempts have been made to utilize other enzymes, since the extra cellular matrix often contains collagen, particularly in connective tissue and muscle, collagenase has been the obvious choice.

Other bacterial proteases, such as pronase; and dispase have also been used.

Other Bacterial proteases, such as pronase and dispase have also been used.

The participant of carbohydrate in intra cellular adhesion has led to the use of hyaluronidase and neuraminidase in conjunction with collagenase.

Screening available samaples lis the only option if trypsin, collagenase, dispase, pronase, hyaluronicase, and DNase, alone and in combination, don't prove to be successful.

Collagenase - The technique is very simple and effective for many tissues, embryonic, adult, normal, and malignant.

Crude collag enase is often used for the purpose. More highly purified grade,s are available if non specific protenlytic activity is undesirable, but they may not be as effective as ;crude collagonase.

Disaggregaion is collalgenase has proved particularly suitable for the culture of human tumors, mouse kidney, human adult and fetal brain lung, and many other tissues, particularly epithelium.

The process is gentle and requires no mechanical agitation or special equipment.

With more than 1g of tissue, however, it becomes radius at the dissection stage and can be expensive, due to the amount of collagenase required.

Mechanical Disaggregation : There is a risk of damaging cells during enzymatic digestion. Many people use the alternative of mechanical disaggregation.

i) Collect the cells that spill out when the tissue is carefully sliced.

ii) Press the dissected tissue through a serine of seines for which the mesh is gradually reduced in size or simply pippeting it repeatedly. This procedure gives a cell suspension, more quickly than does enzymatic digestion, but may cause mechanical damage.

Only soft tissues such as spleen, embryonic liver, embryonic and adult brain and some human and animal soft tumors, respond well to this technique.

Separation of viable and non viable cells :

When an adherent primary culture is prepared from dissociated cells, nonviable cells are removed at the first change of medium.

Non viable cells may be removed from the primary disaggregate by centrifuging the cells on a mixture of FICOL and sodium metrizoate.

Q.2. What is differentiation? Give a short note on control of differentiation.

Ans. Differentiation is the process leading to the expression of phenotypic properties of the functionally mature cell INVITRO.

- Terminal differentiation "implies that a cell has progressed down to a point at which the phenotype is fully expressed and beyond which the cell cannot progress.
- Differentiation has been used to describe the loss of differentiated tissue when it becomes in alignant.
- Mainly 2 main pathways of differentiation are present in the adult.
- The renewal tissue (epidermis) a small proportion of cell give rise to cells that will proliferate and progress towards terminal differentiation losting the capacity of divide.
- In tissue that do not turn over ;rapidly, shows little proliferation when the tissue has regained the appropriate cell density by division, cell proliferation stops and differentiation is re-induced. This type of renewal is rapid because a large population of cells is recruited.

Proliferation and differentiation

- As differentiation progresses, cell division is reduced and eventually lost.

- Tumor cells sometimes break the restriction example. Melanin continues to be synthesized while the cells are proliferating.
- Synthesis of the differentiated product increases when division stops.

Introduction of Differentiation :

- 4 main parameters are following that govern the control of differentiation.

(i) Soluble Inducers

(a) Physiological inducers : It includes hormones paracrine factors that are released by one cell and influence adjacent all.

(b) Non physiological inducers :

- Many cells (e.g. myeloma, neuroblastoma) respond to DMSO by differentiating.
- Many other non physiological inducers are: HMBA, Sodium, and Butyrate and Cytotoxic drugs.
- The action of these ;compounds may be mediated by changes in membrane fluidity or by alternation in DMIT methylaatic.

(ii) Cell Interaction

(a) Homologus : Homologus cell interaction occurs at high cell density, may involve gap function communication where metabolites, 2^0 messenger Ca^{+2} or electrical charge may be communicated between cells.

- The presence of homotypic cell-cell adhesion molecule such as the CAMs or cadherins is Ca^{+2} dependent provide another mechanism, by which contacting cells may interact.

(b) Heterologus : Heterologus cell interaction between endo/ecto/mesodermally derived cells is responsible for initiating and promoting differentiation.

- During gastrulation organogenesis interaction between cells promoted differentiation.

(iii) Paracrine Growth Factors :

- Some growth-factors such as morphogenes induce tubule formation in the MDEK continuous cell line from dog kidney.
- Salivary gland epithelium produced by dermal fibroblast, influence epidermal differentiation.

(iv) Cell matrix interaction :

- Addition of liver-derived matrix material induced expression of albumin gene in hepatocytes.
- Collagen has been found to be essential for functional expression of many epithelial cells.

Differentiation and Malignancy :

- It is observed that with increasing progression of tumor indicates poorer differentiation.
- Patients with poorly differentiated tumors generally have a lower survival rate than patient with differentiated tumors.

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"Contamination"

Q.1. Describe different sources of contamination.

Ans. Sources of Contamination: Maintaining asepsis is still one of the most difficult challenges to the newcomer to tissue culture. There are several potential routes to contaminate including sterilization, poorly maintained incubators and refrigerators, faulty laminar flow hoods.

Operator technique: If reagents are sterile and equipment is in proper working order, contamination depends upon the interaction of the operators' technique with environment.

Environment: It is fairly obvious; that the environment in which tissue culture is carried out must be clean as possible land free from disturbance and through traffic.

Use and maintenance of Laminar Flow-hood: When it becomes overcrowded with bottles and equipment, the laminar flow hood is disrupted and protective layer between operator and room is lost. This leads to the entry of nonsterile air into the hood and release of bio hazardous material into the room.

Humid Incubators:

- A major source of contamination.
- High humidity is not required unless open vessels are being used, sealed, flask are better kept in dry incubator or in the hot room.
- Using permeable caps minimizes the risk of contamination, but increasing the cost.
- Copper linked incubator has reduced fungal growth, but 30-30% more expensive than conventional ones.
- So cleaning should be carried out using 10% non toxic antifungal cleaner.

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- Refrigerator and cold rooms also build up fungal. Contamination on the walls in a humid climate due to condensation that forms every time the door is opened, admitting moist air.

Types of Microbial Contamination:

- Bacteria yeast fungi and mycoplasmas appear as contaminants in tissue culture.
- If a particular type of infection recurs frequently, it may be beneficial to identify it to find its origin.

Q.2. How will you propagate a cell by subculture? Give a brief account on generation of cell line.

Ans. Subculture and propagation:

- Once a 1^{o} culture is subcultured (or passaged) it becomes known as "Cell Line".
- This term implies the presence of several cell lineage of either similar or distinct phenotypes.
- If one cell lineage is selected by cloning or by other selection technique (to have certain specific properties), this cell becomes known as "Cell Strain".
- If a cell line transforms in vitro, it gives rise to continuous cell line and strain is known as continuous cell strain.
- The first sub-culture gives rise to secondary culture the 2^{0} to a 3^{0} and so on.
- When each subculture divided the culture in half, the split ratio being 1:2.
- "Passage-number" is the number of times that the culture has been sub-cultured while the generation number is the number of doublings that the cell population has undergone.
- When split ratio is 1:2, the passage no. is equal to generation no.
- Cell lines with limited culture lifespan are known as "Finite Cell lines" and grow through a limited number of cell generations.

- Actual no. of doublings depends on species and cell lineage differences

Maintenance of Cultures:

- When a culture is initiated whether a 1^{o} culture or a subculture of a cell line, it will need a medium change periodically followed by sub-culture.
- In non proliferating cultures, the medium will still need to be changed periodically as the cells will still metabolise.
- Intervals between mediuim change and subculture vary from one cell line to another depending on the rate of growth and metabolism.
- Transformed cell that (Hela) are sub cultured once per week and medium should be changed weekly between subcultures.

Replacement of Medium:

- Four factors indicate need for the replacement of medium.

(i) **A drop in pH:** The rate iof fall and absolute level should be considered.

- Most cells stop gravity as the pH falls from pH 7.0 to pH 6.5 and start to less viability between pH 6.5 to 6.0.
- So if the medium goes from red through orange to yellow, the medium should be changed.

(ii) **Cell Concentration :** Cultures at high cell concentration exhaust the medium faster than those at lower concentration.

(iii) **Cell Type :** Normal cells stop dividing at high cell density due to cell crowding, growth factor depletion.

- Transformed cells continuous cell lines, and some embryonic cells deteriorate rapidly at high cell densities unless the medium is changed daily.

(iv) **Morphological deterioration :** This factor must be anticipated by examination with the cell line.

Holding Medium: It may be used when stimulation of mitosis is undesirable.

- It is usually a regular media with a serum concentration reduced to .5 or 2% or eliminated completely.
- For serum free media growth factors and other mitogers are anitted.

Subculture:

- The growth of cells follows a standard pattern.
- A "lag phase" is usually followed by a period of exponential phase called "Log Phase"
- When the cell concentration exceeds the capacity of the medium growth ceases; they either the culture must be divided or medium must be changed.
- Subculture usually involves removal of the medium and dissociation of the cell in monolayer with trypsin.
- Loosely adherent cells may be subcultured by shaking the bottles; collecting the cells in medium and diluting in fresh bottle.
- Monolayers can be dissociated with trypsin and other pronase dispose and collagenase pronase is most effective but cana toxic to cells, but may not give complete dissociation.
- The attachment of cells to each other is mediated by cell surface gycoproteins and Ca^2 .
- Subculture usually requires chelation of Ca^2 and degradation of intracellular matrix and cell adhesion molecule

Criteria for Subculture : Need to subculture a monolayer is determined by following criteria :

(i) **Density of culture :** Normal cells should be subcultured when they reachconflence and if left more than 24 pcs. They will withdraw from the cycle and take longer to recover when reseeded.

- Transformed cells will start to deteriorate after about two doublings and should be subcultured on reaching confluence.

(ii) Exhaustion of medium : It indicats that the medium requires replacement but if a fall in pH occurs so rapidly that the medium requires replcemn, but if a fall in pH occurs so rapidly, then subcultured may be required.

- A dropin pH accompanied by an increase in cell density.

(iii) Time since last subculture :

- If cells have not reached a high enough density by appropriate time, then increase the seeding density and if they reach confluence too soon, then reduce the seeding density.
- Once this routine is established, the growth should be consistent in duration.

(iv) Requirement for other procedure :

- Those cells have also to be subcultured that require routine propagation in order to increase the stock or to change the type of medium.
- Cells should not be subcultured while still in the lag phase and always be taken between the middle of log phase.
- The ideal method of determining the correct seeding density is to perform a growth curve at different seeding concentration.

Propagation in suspension :

- Most 1^0 and continuous cell lines grow in monolayers.
- Cells grow continuously in suspension because they are non-adhesive; or they have been kept in suspension mechanically, may be subcultured like bacteria and yeast .
- These cells have a lot of advantages e.g. trypsin treatment is not required. The whole process is quicker and scale up is easier.
- The growth cycle in these cells is similar to monolayer cells, but with a shorter lag period.
- Cells that grow in suspension can be maintained in regular culture flask.

- When the department of a suspension is increased, it requires agitation which is pest achieved with a rotating magnetic pendulum.
- Roller bottles rotating on a rack can also be used to agitate suspension cultures.

Subculture of suspension culture : The criteria for subculture are similar to those for monolayers.

(i) **Cell concentration :** Which should not exceed 10^6 /ml for most suspension growing cells

(ii) **pH –** Which is linked to cell concentration.

(iii) **Time since last subculture :** Which as far monolayers should fit a regular schedule.

(iv) **Cell production requirements :** For experimental or production purposes.

Q.3. How would you find origin of a cell by characterization?

Ans. Need for Characterization :

- When a new cell line is derived from a 1^0 culture, it is difficult to access its future value, so at that point details of its origin are required.
- Requirements for cell line characterization are :

(i) Conformation of the species of origin.

(ii) Is the cell line limited or continuous.

(iii) Identification of the lineage to which the cell belongs, and position of cells within lineage.

(iv) Conformation of the absence of cross contamination.

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Lineage or tissue Markers

(i) Cell surface antigens : These markers are useful in sorting hematopoietic cells and have also been effective in discriminating epithelium from stroma with antibodies and from cells derived from other germ layers.

(ii) Intermediate filament proteins :

- These are among the most widely used ;tissue markers.
- Glial fibrllary acidic protein (GFAP) for astrocytes and desmin for muscle cells are most specific.

(iii) Differentiated products and functions :

- Hemoglobin for erythroid cells, myosin for muscle, melanin for melanocytes and serum albumin for hepatocytes are best examples of all type markers.
- Transport of inorganic ions, water is characteristics of epithelia grown as monolayers will produce domes.

(iv) Enzymes : These parameters are their for enzymatic characterization.

- Constitutive level
- Response to inducers and repressors.
- Isonzyme polymorphism

(v) Regulation :

- Differentiation is ;an irreversible process the level of expression of many products is under the control of environmental influences, such as hormones, the matrix, adjacent cells.

Unique Markers

- Unique markers include specific chromosomal aberrations (deletions, translocations), MHC groups Ag and DNA fingerprinting.

Morphology :

- Simplest and most direct technique used to identify cells.

Fibroblastic Cell : A bipolar or multicolor migrating cell, the length of which is usually more than twice its width.

Epithelial Cell : A monolayer cell i.e. polygonal with more regular dimension and that grows in a discrete patch, along with other cells.

Microscopy : Inverted microscope is one of the most important tools in the tissue culture laboratory.

- A polychromatic blood stain provides a convenient method of preparing a stained culture.

Chromosomal Analysis : Chromosomal content is one of the most characteristic well defined criteria for identifying cell lines ;and related them to the species and sex from which they were derived.

- It can also distinguish between normal and malignant cells, since the chromosome no. is more stable in normal cells.

Chromosome Banding

- This technique was devised to enable chromosome pairs to be identified when there is little morphological differenced between them.
- For Giemsa banding, chromosomal proteins are partially digested by crude trypsin, producing a banding appearance on staining.

Chromosome analysis : For chromosomal analysis Chromosomel counting and karyotype of chromosome may be analyzed.

- Chromosomal counting done by counting the chromosome no. / spread.
- In Karyotypic analysis photographs about 100 – 20 good spreads of banded chromosome, print on high contract paper ;and cut out the chromosome and stick them down on paper.

DNA Content

- The amount of DNA/Cell is stable and is charactrisatic to the species in normal cell lines, such as human, Chick, but varies in cell lines from the mouse and from neoplasms.
- DNA can be measured by propidium iodide fluorescence with a flow cytometry.
- It is mostly useful in the characterization of transformed cells that are often anenploid and heteroploid.

DNA Fingerprinting

- DNA fingerprinting is quite stable in culture and cell line from the same origin.
- Electropharesis of DNA fragments reveals variation in length that are specific to an individual from which DNA derived.
- When this analyzed by PAM electrophoresis, it gives a specific hybridization pattern known as "DNA finger printing".

RNA and Protein : Cells can also be analyzed or recognized ;by gene expression by northern blotting using radioactive probes.

Q.4. Write short ;note on "Immuno Chemistry"

Ans. Immunochemistry is based on the detection of antigens by antibodies. Ag are large molelcule which when injected into animals activate lymphocytes to produce antibodies.

- Each antibody is composed of two identical heavy and light chains and specificity to antigenic determinants depends on amino acid sequence at binding site.
- In immunochemistry, the Ab that interacts with the tissues Ag. Is known as "Primary antibody".
- Direct detection of Ag – Ab complex can be detected by conjugation of Igh with a fluorescent due as per according to Alberts.
- In other direct method IgG was labeled with a "radioactive marker" and then revealed by radioautography.

- In other case of "enzyme" was linked to I.G. and then reacted with diaminobenziaine in the presence of H_2O_2 that can be visualized unicroscopically; other enzymes such as alkaline phosphates and B. glycosidase are being ;used.
- Another method employs antibodies coupled to ferritin which is opaque to electron.
- Indirect methods : Direct methods have low sensitivity that make difficult to detect 1^0 complex.
- In indirect case. Ag – Ab complex is amplified by the introduction of a second labled Ab.
- In this case, the reaction can be observed bu fluorescence, auto radiography, or by electron microscopy.
- In "Biotech-Avidin-Peroxidase method, the constant region of Ab (Fc) is bound to biotin and then reacted with an avialine peroxidase.
- Most commonly used enzyme is "Peroxidase" which then react with diaminobenziaine in the presence of H_2O_2. This reaction gives an electron-dense deposit that can be detected with light microscope.
- In PAP method (peroxidase-antiperoxidase) ;unlabled Ab are used and introduce a peroxidase rabbit antic peroxidase complex, which also binds to the sheep anti rabbit IgG.
- The complex is made by interacting the enzyme with the corresponding IgG made in rabbit as in indirect method peroxidase is finally detected by DAB method.
- "Protein A-Gold technique" is based on the use of colloidal gold particles, very opaque to electrons ;and are surrounded lby protein A extracted from *staphylococcus aureans*, which interacts with IgG.
- Interaction is with ;the Fc region of IgG and thus it does not affect the Ab binding site, which can react with the antigen.

- The protein A-gold complex can be used for any Ag, provided that it has interacted with the specific antibody gold partical can be easily detected with the EM.
- In "gold", Anti IgG method, colloidal gold may be used as a marker of an IgG for a tissue antigen either in direct way or by using a 2^o antibody.
- Gold particles are available for tagging Ab the gold particles are deposited at the Ag-site can be enlarged by reacting them with silver and increasing the contrast.
- By using gold particles of different sizes, associated to different IgG, it is possible to tag two more Ag. In the same tissue.
- "Monoclonal antibodies" are developed by using cell fusion bet ween lymphocytes and cells of myeloma, derived from a clone. Therefore, they are very pure and rcognized a single immunogenic determinants in the protein. This technique is used in immuno chemical methods.

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B.Sc. /M.Sc. (Part-I) Examination, 2011

(Faculty of Science)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

Paper BT- 102

CELL BIOLOGY

Year-2011

Time.: 3 Hours ***Max. Marks : 50***

Attempt Five questions in all, including Question No.1 which is compulsory, selecting ONE question from each Section. Each question carries equal 10 marks.

1. Answer in one or two lines:
   - (i) What is symport?
   - (ii) Give the names of any two prokaryotic signaling molecules.
   - (iii) What is Apoptosis?
   - (iv) What is autocrine signaling?
   - (v) Write the full form of GMP.
   - (vi) Give the name of cellular organelle where F_1 particle is located.
   - (vii) Which cellular organelle is responsible for Glycosylation of Protein?
   - (viii) In which stage does synthesis of protein for formation of spindle fibres take place?
   - (ix) What is the darkly stained part of chromosome called?
   - (x) Give the name of any one mitotic poison. 1x10=10

Section-A

2. Describe the various phases of cell cycle. What is the difference between mitosis and meiosis? 10

3. Write short notes on:
   (i) Peroxisomes
   (ii) Cell membrane
   (iii) Microtubules 3+3+4

Section-B

4. What is cell signaling? Describe the various model of cell signaling in detail. 3+7

5. Write short notes on:
   (i) ATPase pump
   (ii) Endocytosis and Exocytosis
   (iii) Virus as toxic component. 3+3+4

Section-C

6. What is signal amplification? Give the names of various models and signal amplification and describe how they work. 3+7

7. Write short notes on:
   (i) Role of phosphorylation in signal transduction.
   (ii) Cyclic GMP. 5+5

Section-D

8. What is cell line? Write down characteristic of cell line and maintenance method of cell line. 3+7

9. Write short notes on:
   (i) Techniques for prokaryotic culture
   (ii) Cell culture contamination 5+5

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B.Sc. /M.Sc. (Part-I) Examination, 2009

(Faculty of Science)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

Paper BT- 102

CELL BIOLOGY

Year-2009

Time.: 3 Hours ***Max. Marks : 50***

Attempt Five questions in all, including Question No.1 which is compulsory, selecting ONE question from each Section. Each question carries equal 10 marks.

Q.1 Write short notes on the following :

(i) Nucleolus;

(ii) Autocrine:

(iii) Cyclic GMP;

(iv) Stock cells.

Section-A

Q.2 With the help of suitable diagrams describe in detail the structure of nucleus and its components in reference to nuclear envelop karyolymph and chromatum.

Q. 3 Write short notes on:

(i) Prophase I;

(ii) Microtubules;

(iii) Lysosomes;

(iv) Cell theory.

ection-B

Q. 4 With the help of suitable examples explain nuclear and membrane bound receptors of the cells.

Q. 5 Write short notes on the following :

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(i) Ca +2 ATPase pump;

(ii) Difference between Endo and Exo cytosis;

(iii) Difference between Paracrine and Endocrine models.

Section-C

Q.6 What is signal amplification? Explain your and with different models of signal amplification.

Q. 7 Write short notes on any two of the following.

(i) Role of inositol phosphate messengers;

(ii) Biosynthesis of inositol triphosphatase;

(iii) Phosphorylation of Protein kinase;

(iv) Cyclic AMP.

Section-D

Q.8 What are the cell lines? How are the cell lines generated and how are cell stocks maintained?

Q. 9 Write notes on the following:

(i) Primary culture contamination;

(ii) Differentiation;

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