Module1-Lecture 1

Prokaryotic and Eukaryotic cells

To venture into biology lets start with the cell!!!

In this chapter we will learn about what is a cell and further explore what a prokaryotic and eukaryotic cell is.

The cell was first seen by Robert Hooke in 1665 using a primitive, compound microscope. He observed very thin slices of cork and saw a multitude of tiny structures that he resembled to walled compartments of a monk. Hence, named them cells. Hooke's description of these cells was published in Micrographia. The cell is smallest unit of a living system and fall in the microscopic range of 1 to 100 µm. They attain various shapes and sizes to attain variety of functions. The understanding of cell is necessary to understand the structure and function of a living organism. One of most important characteristics of cell is ability to divide. The existence of a cell indicates that it has evolved from an already existing cell and further it can give rise to a new cell. This was first stated by Theodor Schwann. Pioneering work by Theodor Schwann, Matthias Jakob Schleiden on cells, gave birth to the cell theory. Their theory states:

1. All living things are made of cells.
2. Cells are the basic building units of life.
3. New cells are created by old cells dividing into two.

In 1855, Rudolf Virchow added another point to the theory and concluded that all cells come from pre-existing cells, thus completing the classical cell theory. The cell theory holds true for all living things, no matter how big or small, or how simple or complex. Viruses are exception to the cell theory. Cells are common to all living beings, and provide information about all forms of life. Because all cells come from existing cells, scientists can study cells to learn about growth, reproduction, and all other functions that

living things perform. By learning about cells and how they function, we can learn about all types of living things.

Classification of cells:

All living organisms (bacteria, blue green algae, plants and animals) have cellular organization and may contain one or many cells. The organisms with only one cell in their body are called unicellular organisms (bacteria, blue green algae, some algae, Protozoa, etc.). The organisms having many cells in their body are called multicellular organisms (fungi, most plants and animals). Any living organism may contain only one type of cell either **A.** Prokaryotic cells; **B.** Eukaryotic cells. The terms prokaryotic and eukaryotic were suggested by Hans Ris in the 1960's. This classification is based on their complexcity. Further based on the kingdom into which they may fall i.e the plant or the animal kingdom, plant and animal cells bear many differences. These will be studied in detail in the upcoming sections.

Prokaryotic cells

Prokaryote means before nucleus in Greek. They include all cells which lack nucleus and other membrane bound organelles. Mycoplasma, virus, bacteria and cyanobacteria or blue-green algae are prokaryotes.

Most prokaryotes range between 1 µm to 10 µm, but they can vary in size from 0.2 µm to 750 µm (*Thiomargarita namibiensis*). They belong to two taxonomic domains which are the bacteria and the archaea. Most prokaryotes are unicellular, exceptions being myxobacteria which have multicellular stages in their life cycles. They are membrane bound mostly unicellular organisms lacking any internal membrane bound organelles. A typical prokaryotic cell is schematically illustrated in Figure 1. Though prokaryotes lack cell organelles they harbor few internal structures, such as the cytoskeletons, ribosomes, which translate mRNA to proteins. Membranous organelles are known in some groups of prokaryotes, such as vacuoles or membrane systems devoted to special metabolic properties, e.g., photosynthesis or chemolithotrophy. In addition, some species also contain protein-enclosed microcompartments, which have distinct physiological roles (carboxysomes or gas vacuoles).

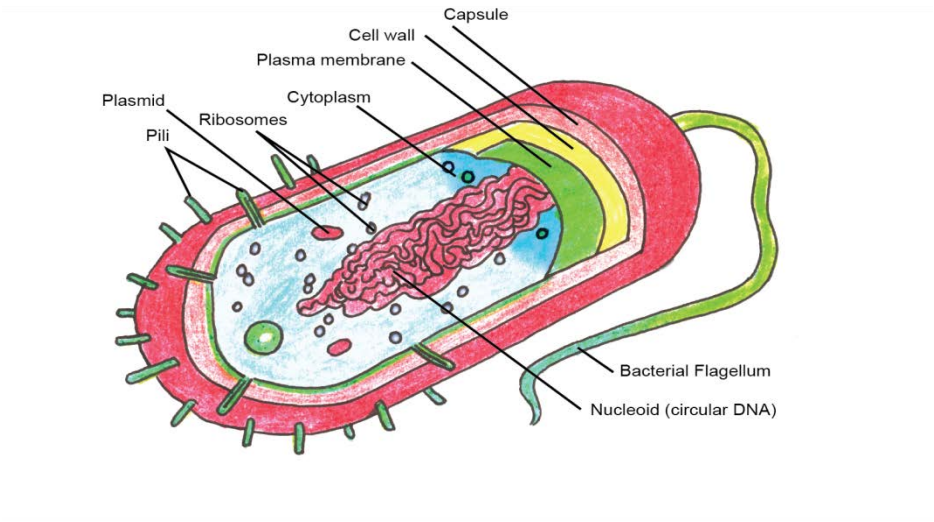


Figure 1: Schematic diagram of a prokaryotic cell

The individual structures depicted in Figure 1 are as follows and details will be discussed in forthcoming chapters:

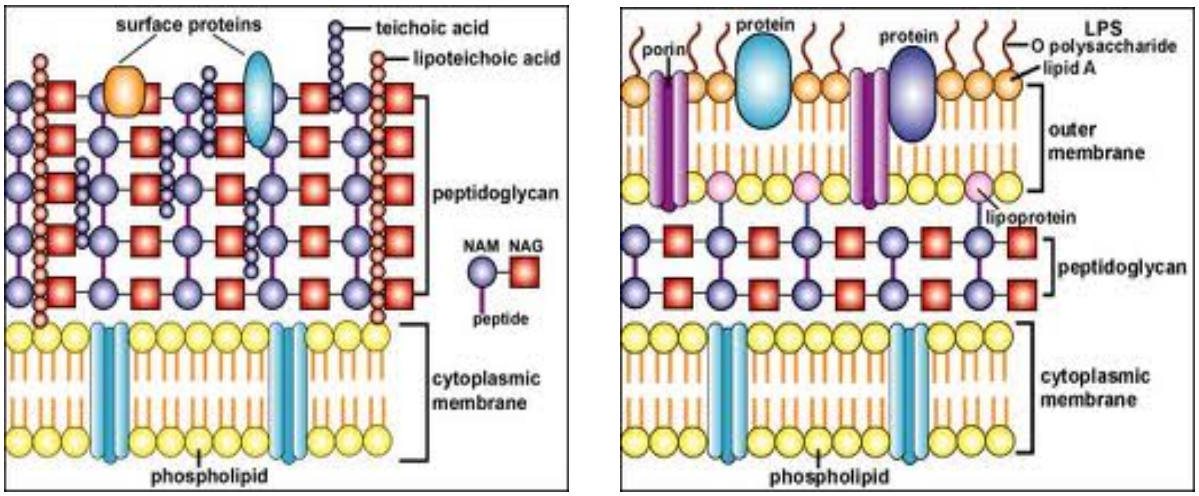
Flagella: It is a long, whip-like protrusion found in most prokaryotes that aids in cellular locomotion. Besides its main function of locomotion it also often functions as a sensory organelle, being sensitive to chemicals and temperatures outside the cell.

Capsule: The capsule is found in some bacterial cells, this additional outer covering protects the cell when it is engulfed by phagocytes and by viruses, assists in retaining moisture, and helps the cell adhere to surfaces and nutrients. The capsule is found most commonly among Gram-negative bacteria. *Escherichia coli*, *Klebsiella pneumoniae* *Haemophilus influenzae*, *Pseudomonas aeruginosa* *and* *Salmonella* are some examples Gram-negative bacteria possessing capsules. Whereas examples of Gram positive bacteria are *Bacillus megaterium*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*.

Cell wall: Cell wall is the outermost layer of most cells that protects the bacterial cell and gives it shape. One exception is Mycoplasma which lacks cell wall. Bacterial cell walls are made of peptidoglycan which is made from polysaccharide chains cross-linked by unusual peptides containing D-amino acids. Bacterial cell walls are different from the cell walls of plants and fungi which are made of cellulose and chitin, respectively. The cell wall of bacteria is also distinct from that of Archaea, which do not contain peptidoglycan.

The cell wall is essential to the survival of many bacteria. The antibiotic penicillin is able to kill bacteria by preventing the cross-linking of peptidoglycan and this causes the cell wall to weaken and lyse. Lysozyme enzyme can also damage bacterial cell walls.

There are broadly speaking two different types of cell wall in bacteria, called Gram-positive and Gram-negative (Figure 2). The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in structure can produce differences in property as antibiotic susceptibility. For example vancomycin can kill only Gram-positive bacteria and is ineffective against Gram-negative pathogens, such as *Pseudomonas aeruginosa* or *Haemophilus influenzae*.



A: Gram positive cell wall **B: Gram negative cell wall**

Figure 2: A: Gram positive bacterial cell wall B: gram negative bacterial cell wall

Cell membrane: Cell membrane surrounds the cell's cytoplasm and regulates the flow of substances in and out of the cell. It will be discussed in detail in one of the coming chapters.

Cytoplasm: The cytoplasm of a cell is a fluid in nature that fills the cell and is composed mainly of 80% water that also contains enzymes, salts, cell organelles, and various organic molecules. The details will be discussed in forthcoming chapter.

Ribosomes: Ribosomes are the organelles of the cell responsible for protein synthesis. Details of ribosomes will be explained in coming chapter.

Nucleiod Region: The nucleoid region is possessed by a prokaryotic bacterial cell. It is the area of the cytoplasm that contains the bacterial DNA molecule.

Plasmids: The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952. A plasmid is a DNA molecule (mostly in bacteria) that is separate from, and can replicate independently of, the chromosomal DNA. They are double-stranded and circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms. Their sizes vary from 1 to over 1,000 kbp. The number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances and it is represented by the copy number. Plasmids can be considered mobile because they are often associated with conjugation, a mechanism of horizontal gene transfer. Plasmids that can coexist within a bacterium are said to be compatible. Plasmids which cannot coexist are said to be incompatible and after a few generations are lost from the cell. Plasmids that encode their own transfer between bacteria are termed conjugative. Non-conjugative plasmids do not have these transfer genes but can be carried along by conjugative plasmids via a mobilisation site. Functionally they carry genes that code for a wide range of metabolic activities, enabling their host bacteria to degrade pollutant compounds, and produce antibacterial proteins. They can also harbour genes for virulence that help to increase pathogenicity of bacteria causing diseases such as plague, dysentery, anthrax and tetanus. They are also

responsible for the spread of antibiotic resistance genes that ultimately have an impact on the treatment of diseases. Plasmids are classified into the following types.

1. Fertility F-plasmids- These plasmids contain tra genes and are capable of conjugation.

2. Resistance (R) plasmids: They contain genes that can build a resistance against antibiotics or toxins and help bacteria produce pili.

3. Col plasmids: They contain genes that code for bacteriocins, proteins that can kill other bacteria.

4. Degradative plasmids: Degradative plasmids enable the metabolism of unusual substances, e.g. toluene and salicylic acid.

5. Virulence plasmids: These plasmids enable the bacterium to become pathogenic.

The other types of plasmids are:

1. *Yeast integrative plasmid (YIp)*: yeast vectors that rely on integration into the host chromosome for survival and replication.

2. *Yeast Replicative Plasmid (YRp):* which transport a sequence of chromosomal DNA that includes an origin of replication. These plasmids are less stable, as they can *get lost* during the budding.

Pili: Pili are hair-like structures on the surface of the cell that help attach to other bacterial cells. Shorter pili called fimbriae help bacteria attach to various surfaces. A pilus is typically 6 to 7 nm in diameter. The types of pili are Conjugative pili and Type IV pili. Conjugative pili allow the transfer of DNA between bacteria, in the process of bacterial conjugation. Some pili, called type IV pili, generate motile forces.

Morphology of prokaryotic cells

Prokaryotic cells have various shapes; the four basic shapes are (Figure 3):

- Cocci - spherical
- Bacilli - rod-shaped
- Spirochaete - spiral-shaped
- Vibrio - comma-shaped

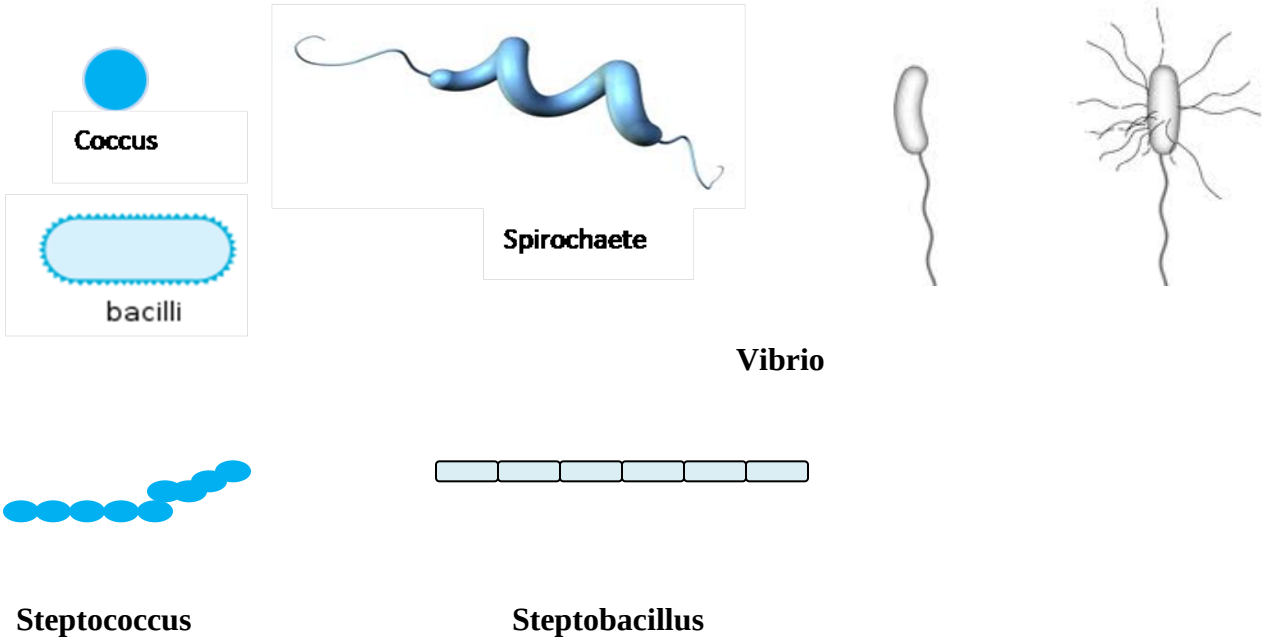


Figure 3: Morphology of prokaryotic cells

Milieu

Prokaryotes live in nearly all environments on Earth. Some archaea and bacteria thrive in extreme conditions, such as high temperatures (thermophiles) or high salinity (halophiles). Organisms such as these are referred to as extremophiles. Many archaea grow as plankton in the oceans. Symbiotic prokaryotes live in or on the bodies of other organisms, including humans.

Sociability

Prokaryotes are believed to be strictly unicellular though most can form stable aggregated communities in a stabilizing polymer matrix called “biofilms”. Cells in biofilms often show distinct patterns of gene expression (phenotypic differentiation) in time and space. Also, as with multicellular eukaryotes, these changes in expression appear as a result of quorum sensing or cell to cell signal transduction. Bacterial biofilms are often made up of approximately dome-shaped masses of bacteria and matrix separated by “voids” through which the medium (water) may flow relatively uninhibited and such system are termed as

microcolonies. The microcolonies may join together above the substratum to form a continuous layer, closing the network of channels separating microcolonies. Bacterial biofilms may be 100 times more resistant to antibiotics than free-living unicells and may be difficult to remove from surfaces once they have colonized them. Other aspects of bacterial cooperation like bacterial conjugation and quorum-sensing-mediated pathogenicity provide additional challenges to researchers and medical professionals seeking to treat the associated diseases.

Colony of bacteria

Most bacteria represent themselves in colonies. By colony we mean individual organisms of the same species living closely together in mutualism. All species in a colony are genetically equivalent. The shape of the colony can be circular and irregular. Bacterial colonies are frequently shiny and smooth in appearance. In microbiology, colony-forming unit (CFU) is a measure of viable bacteria in such colonies. If a bacterial cell like *Escherichia coli* divides every 20 minutes then after 30 cell divisions there will be 2^{30} or 1048576 cells in a colony.

Reproduction

Bacteria and archaea reproduce through asexual reproduction known as binary fission. Binary fission is an asexual mode of reproduction. During binary fission, the genomic DNA undergoes replication and the original cell is divided into two identical cells. Due to binary fission, all organisms in a colony are genetically equivalent (Figure 4). The process begins with DNA replication followed by DNA segregation, division site selection, invagination of the cell envelope and synthesis of new cell wall which are tightly controlled by cellular proteins. A key component of this division is the protein FtsZ which assemble into a ring-like structure at the center of a cell. Other components of the division apparatus then assemble at the FtsZ ring. This machinery is positioned so that division splits the cytoplasm and does not damage DNA in the process. As division occurs, the cytoplasm is cleaved in two, and new cell wall is synthesized.

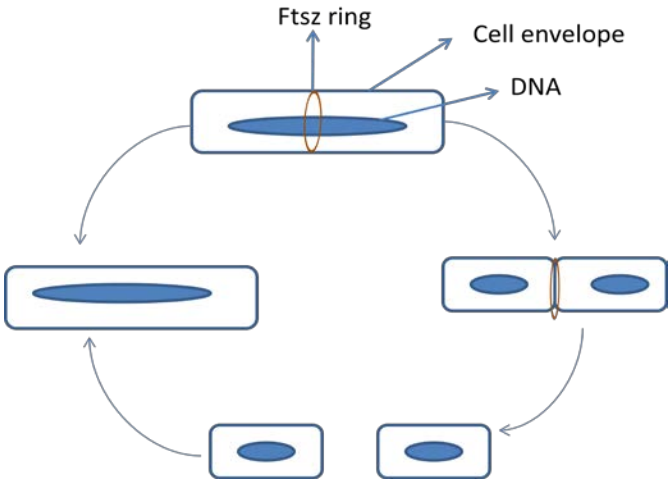


Figure 4: Binary fission in prokaryotes

Products/Application

Prokaryotes help manufacture yogurt, cheese, sour cream, antibiotics etc. They are the store house of many industrially important enzymes such as lipases, proteases, amylases which find use in detergent, paper and leather industries.

Eukaryote

A eukaryotic cell consists of membrane bound organelles. They belong to the taxa Eukaryota. All species of large complex organisms are eukaryotes, including animals, plants and fungi and most species of protist microorganisms. Eukaryotes appear to be monophyletic (organisms that form a clade) and make up one of the three domains of life. The two other domains, Bacteria and Archaea, are prokaryotes and have none of the above features. Eukaryotes represent a tiny minority of all living things; even in a human body there are 10 times more microbes than human cells. However, due to their much larger size their collective worldwide biomass is estimated at about equal to that of prokaryotes. Unlike prokaryotes, eukaryotic genome is enclosed in the nucleus surrounded by the nuclear membrane. Other then the nucleus many membrane bound organelles dwell in their cell cytoplasm. Cell division involves separating of the genome which is in the form of tightly packed condensed structure known as the chromosomes, through movements directed by the cytoskeleton.

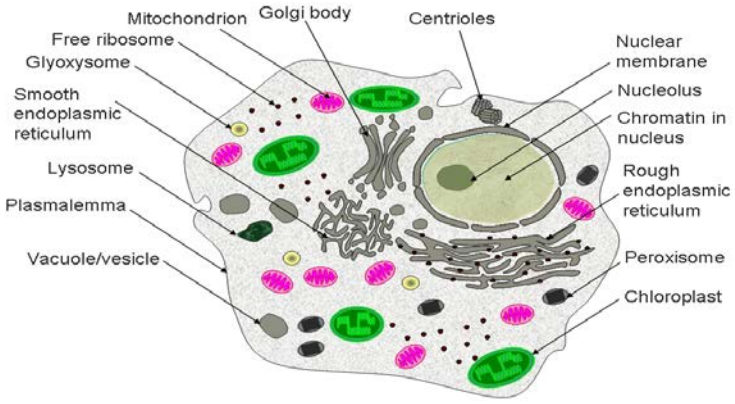


Figure 5 Eukaryotic cell:

Classification

The eukaryotes are composed of four kingdoms:

- Kingdom Protista
- Kingdom Fungi
- Kingdom Plantae
- Kingdom Animalia

Cell features

Eukaryotic cells are much larger than prokaryotic cells. Range between 10 to 100 micrometers. They have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of microtubules, microfilaments, and intermediate filaments, which play an important role in defining the cell's organization and shape. Eukaryotic DNA is divided into several linear bundles called chromosomes, which are separated by a microtubular spindle during nuclear division.

Internal membrane

Eukaryote cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system involved in various functions. Simple compartments, called vesicles or vacuoles, can form by budding off other membranes. Many cells ingest food and other materials through a process of endocytosis, where the outer membrane invaginates and then pinches off to form a vesicle. It is probable that most other membrane-bound organelles are ultimately derived from such vesicles. The nucleus is surrounded by a double membrane (commonly referred to as a nuclear envelope), with pores that allow material to move in and out. Various tube and sheet like extensions of the nuclear membrane form what is called the endoplasmic reticulum or ER, which is involved in protein transport and maturation. It includes the rough ER where ribosomes are attached to synthesize proteins, which enter the interior space or lumen. Subsequently, they generally enter vesicles, which bud off from the smooth ER. In most eukaryotes, these protein-carrying vesicles are released and further modified in stacks of

flattened vesicles, called golgi bodies or dictyosomes. Vesicles may be specialized for various purposes. For instance, lysosomes contain enzymes that break down the contents of food vacuoles, and peroxisomes are used to break down peroxide, which is toxic otherwise. Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole, which primarily maintains its osmotic pressure. The individual cell organelles will be discussed in detail in the upcoming chapters.

Reproduction:

Nuclear division is often coordinated with cell division. This generally takes place by mitosis, a process that allows each daughter nucleus to receive one copy of each chromosome. In most eukaryotes, there is also a process of sexual reproduction, typically involving an alternation between haploid generations, wherein only one copy of each chromosome is present, and diploid generations, wherein two are present, occurring through nuclear fusion (syngamy) and meiosis. There is considerable variation in this pattern.

Association/hierarchy: In the plant and animal kingdom cells associate to form tissue, tissue to organs which finally makes the whole organism.

Prokaryotes versus Eukaryotes:

The difference between prokaryotes and Eukaryotes are detailed below. Eukaryotes have a smaller surface area to volume ratio than prokaryotes, and thus have lower metabolic rates and longer generation times. In some multicellular organisms, cells specialized for metabolism will have enlarged surface area, such as intestinal vili.

Table 1: Difference between prokaryotes and eukaryotes:

Characteristic	**Prokaryotes**	**Eukaryotes**
Size of cell	Typically 0.2-2.0 m m in diameter	Typically 10-100 m m in diameter
Nucleus	No nuclear membrane or nucleoli (nucleoid)	True nucleus, consisting of nuclear membrane & nucleoli
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria & chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoplasm	No cytosketeton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA) arrangement	Single circular chromosome; lacks histones	Multiple linear chromosomes with histones
Cell division	Binary fission	Mitosis
Sexual reproduction	No meiosis; transfer of DNA fragments only (conjugation)	Involves Meiosis

Phytoplanktons and zooplanktons:

Phytoplankton are photosynthesizing microscopic organisms that inhabit the upper sunlit layer of almost all oceans and bodies of fresh water and obtain their energy through photosynthesis. Interestingly Phytoplankton account for half of all photosynthetic activity on Earth. Some phytoplankton are bacteria, some are protists, and most are single-celled plants. Among the common kinds are cyanobacteria, silica-encased diatoms, dinoflagellates, green algae, and chalk-coated coccolithophores. Phytoplankton growth depends on the availability of carbon dioxide, sunlight, and nutrients. Phytoplankton require nutrients such as nitrate, phosphate, silicate, and calcium at various levels depending on the species. Some phytoplankton can fix nitrogen and can grow in areas where nitrate concentrations are low. They also require trace amounts of iron which limits phytoplankton growth in large areas of the ocean because iron concentrations are very low.

Zooplankton is a group of small protozoans and large metazoans. It includes holoplanktonic organisms whose complete life cycle lies within the plankton, as well as meroplanktonic organisms that spend part of their lives in the plankton before graduating to either the nekton or a sessile, benthic existence. Although zooplankton is primarily transported by ambient water currents, many have locomotion, used to avoid predators (as in diel vertical migration) or to increase prey encounter rate.

Module 1- Lecture 2

Plant and animal cells

In this chapter we will learn how similar and different are plant and animal cells.

Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. Their distinctive features include the following organelles:

1. Vacuole: It is present at the centre and is water-filled volume enclosed by a membrane known as the tonoplast. The function is to maintain the cell's turgor, pressure by controlling movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

2. Cell Wall: It is the extracellular structure surrounding plasma membrane. The cell wall is composed of cellulose, hemicellulose, pectin and in many cases lignin, is secreted by the protoplast on the outside of the cell membrane. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan. An important function of the cell wall is that it controls turgity. The cell wall is divided into the primary cell wall and the secondary cell wall. The Primary cell wall: extremely elastic and the secondary cell wall forms around primary cell wall after growth are complete.

3. Plasmodesmata: Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

4. Plastids: The plastids are chloroplasts, which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis. A typical plant cell (e.g., in the palisade layer of a leaf) might contain as many as 50 chloroplasts. The other plastids are amyloplasts specialized for starch storage, elaioplasts specialized for fat storage, and chromoplasts specialized for synthesis and storage of pigments. As in mitochondria, which have a genome encoding 37 genes, plastids have their own genomes of about 100–120 unique genes and, it is presumed, arose as prokaryotic endosymbionts living in the cells of an early eukaryotic ancestor of the land plants and algae.

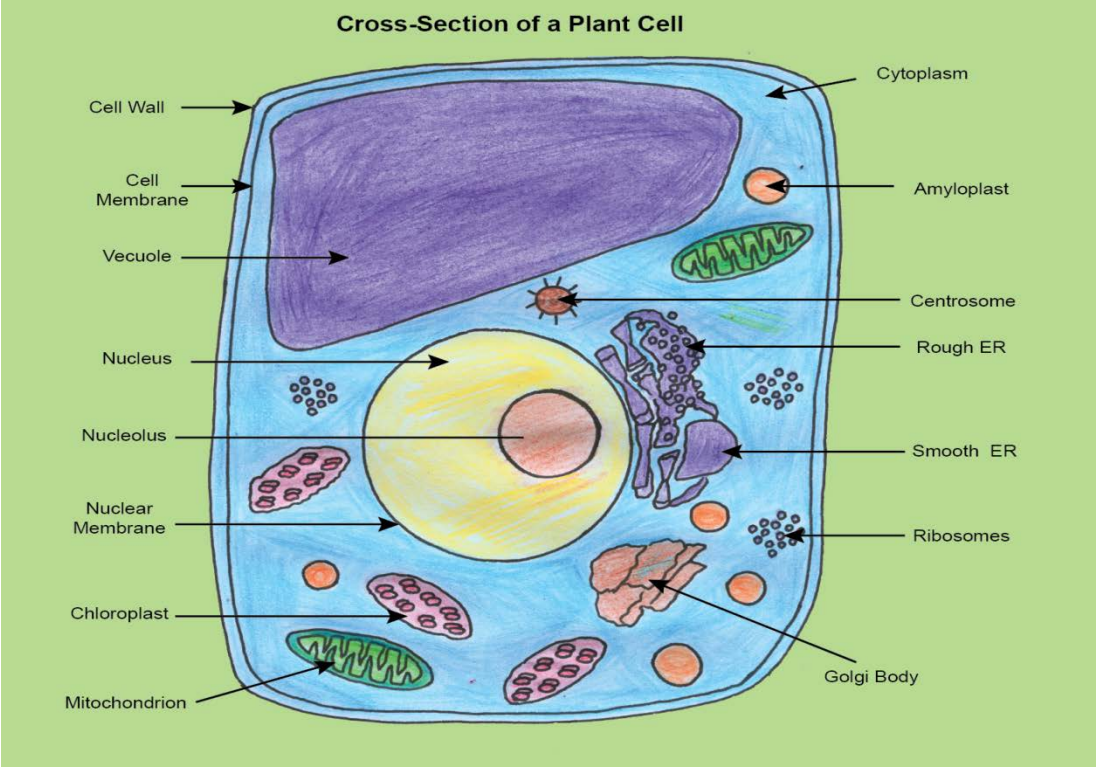


Figure 1: Schematic representation of a plant cell.

Plant cell types

Parenchyma cells: These are living cells that have diverse functions ranging from storage and support to photosynthesis and phloem loading (transfer cells). Apart from the xylem and phloem in its vascular bundles, leaves are composed mainly of parenchyma cells. Some parenchyma cells, as in the epidermis, are specialized for light penetration and focusing or regulation of gas exchange, but others are among the least specialized cells in plant tissue, and may remain totipotent, capable of dividing to produce new populations of undifferentiated cells, throughout their lives. Parenchyma cells have thin, permeable primary walls enabling the transport of small molecules between them, and their cytoplasm is responsible for a wide range of biochemical functions such as nectar secretion, or the manufacture of secondary products that discourage herbivory. Parenchyma cells that contain many chloroplasts and are concerned primarily with photosynthesis are called chlorenchyma cells. Others, such as the majority of the parenchyma cells in potato tubers and the seed cotyledons of legumes, have a storage function (Figure 2a).

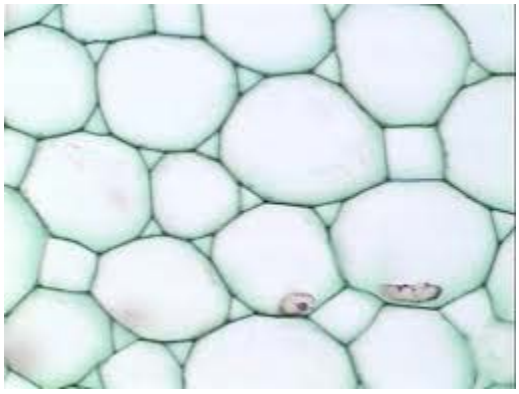


Figure 2a: Parenchyma cells which have thin primary cell wall.

Collenchyma cells: Collenchyma cells (Figure 2b) are alive at maturity and have only a primary wall. These cells mature from meristem derivatives that initially resemble parenchyma, but differences quickly become apparent. Plastids do not develop, and the secretory apparatus (ER and Golgi) proliferates to secrete additional primary wall. The wall is most commonly thickest at the corners, where three or more cells come in contact, and thinnest where only two cells come in contact, though other arrangements of the wall thickening are possible. Pectin and hemicellulose are the dominant constituents of collenchyma cell walls of dicotyledon angiosperms, which may contain as little as 20% of cellulose in *Petasites*. Collenchyma cells are typically quite elongated, and may divide transversely to give a septate appearance. The role of this cell type is to support the plant in axes still growing in length, and to confer flexibility and tensile strength on tissues. The primary wall lacks lignin that would make it tough and rigid, so this cell type provides what could be called plastic support – support that can hold a young stem or petiole into the air, but in cells that can be stretched as the cells around them elongate. Stretchable support (without elastic snap-back) is a good way to describe what collenchyma does. Parts of the strings in celery are collenchymas (Figure 2b).

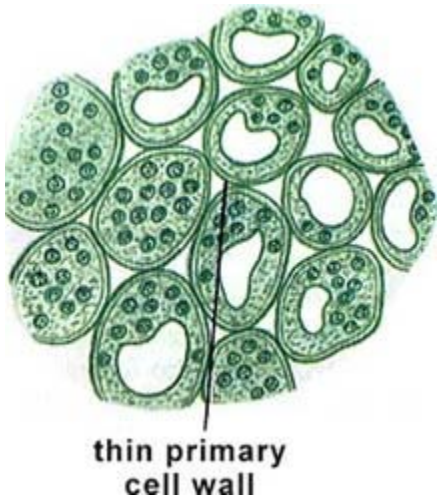


Figure 2b: Typical collenchyma cell.

Sclerenchyma cells: Sclerenchyma cells (from the Greek **skleros**, *hard*) are hard and tough cells with a function in mechanical support. They are of two broad types – sclereids or stone cells and fibres. The cells develop an extensive secondary cell wall that is laid down on the inside of the primary cell wall. The secondary wall is impregnated with lignin, making it hard and impermeable to water. Thus, these cells cannot survive for long' as they cannot exchange sufficient material to maintain active metabolism. Sclerenchyma cells are typically dead at functional maturity, and the cytoplasm is missing, leaving an empty central cavity.

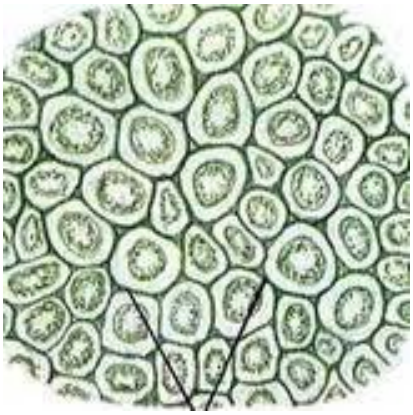


Figure 2c: Sclerenchyma cells with irregularly thickened cell wall.

Animal cells:

An animal cell is a form of eukaryotic cell that makes up many tissues in animals. Figure 7 depicts a typical animal cell. The animal cell is distinct from other eukaryotes, most notably plant cells, as they lack cell walls and chloroplasts, and they have smaller vacuoles. Due to the lack of a rigid cell wall, animal cells can adopt a variety of shapes, and a phagocytic cell can even engulf other structures. There are many different cell types. For instance, there are pproximately 210 distinct cell types in the adult human body.

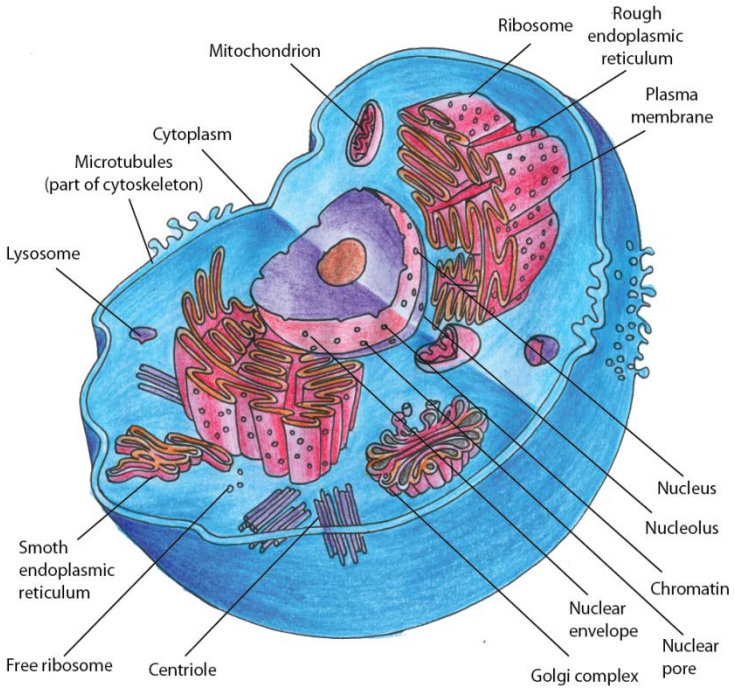


Figure 3: Schematic representation of a typical animal cell.

Cell organelles in animal cell:

Cell membrane: Plasma membrane is the thin layer of protein and fat that surrounds the cell, but is inside the cell wall. The cell membrane is semipermeable, allowing selective substances to pass into the cell and blocking others.

Nucleus: They are spherical body containing many organelles, including the nucleolus. The nucleus controls many of the functions of the cell (by controlling protein synthesis) and contains DNA (in chromosomes). The nucleus is surrounded by the nuclear membrane and possesses the nucleolus which is an organelle within the nucleus - it is where ribosomal RNA is produced.

Golgi apparatus: It is a flattened, layered, sac-like organelle involved in packaging proteins and carbohydrates into membrane-bound vesicles for export from the cell.

Ribosome and Endoplasmic reticulum: Ribosomes are small organelles composed of RNA-rich cytoplasmic granules that are sites of protein synthesis and Endoplasmic reticulum are the sites of protein maturation and they can be divided into the following types:

a. Rough endoplasmic reticulum: These are a vast system of interconnected, membranous, infolded and convoluted sacks that are located in the cell's cytoplasm (the ER is continuous with the outer nuclear membrane). Rough ER is covered with ribosomes that give it a rough appearance. Rough ER transport materials through the cell and produces proteins in sacks called cisternae (which are sent to the Golgi body, or inserted into the cell membrane).

b. Smooth endoplasmic reticulum: These are a vast system of interconnected, membranous, infolded and convoluted tubes that are located in the cell's cytoplasm (the ER is continuous with the outer nuclear membrane). The space within the ER is called the ER lumen. Smooth ER transport materials through the cell. It contains enzymes and produces and digests lipids (fats) and membrane proteins; smooth ER buds off from rough ER, moving the newly-made proteins and lipids to the Golgi body and membranes.

Mitochondria: These are spherical to rod-shaped organelles with a double membrane. The inner membrane is infolded many times, forming a series of projections (called cristae). The mitochondrion converts the energy stored in glucose into ATP (adenosine triphosphate) for the cell.

Lysosome: Lysosomes are cellular organelles that contain the hydrolase enzymes which breaks down waste materials and cellular debris. They can be described as the stomach of the cell. They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles.Lysosomes digest excess or worn-out organelles, food particles, and engulf viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at the 4.5 pH they require. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are created by the addition of hydrolytic enzymes to early endosomes from the Golgi apparatus.

Centrosome: They are small body located near the nucleus and has a dense center and radiating tubules. The centrosomes are the destination where microtubules are made. During mitosis, the centrosome divides and the two parts move to opposite sides of the dividing cell. Unlike the centrosomes in animal cells, plant cell centrosomes do not have centrioles.

Peroxisome

Peroxisomes are organelles that contain oxidative enzymes, such as D-amino acid oxidase, ureate oxidase, and catalase. They may resemble a lysosome, however, they are not formed in the Golgi complex. Peroxisomes are distinguished by a crystalline structure inside a sac which also contains amorphous gray material. They are self replicating, like the mitochondria. Components accumulate at a given site and they can be assembled into a peroxisome. Peroxisomes function to rid the body of toxic substances like hydrogen peroxide, or other metabolites. They are a major site of oxygen utilization and are numerous in the liver where toxic byproducts accumulate.

Vacuoles and vesicles

Vacuoles are single-membrane organelles that are essentially part of the outside that is located within the cell. The single membrane is known in plant cells as a tonoplast. Many organisms will use vacuoles as storage areas. Vesicles are much smaller than vacuoles and function in transporting materials both within and to the outside of the cell.

Table 1: Differences between Animal and Plant cell

S.No	Animal cell	Plant cell
1.	Animal cells are generally small in size.	Plant cells are larger than animal cells.
2.	Cell wall is absent.	The plasma membrane of plant cells is surrounded by a rigid cell wall of cellulose.
3.	Except the protozoan *Euglena* no animal cell possesses plastids.	Plastids are present.
4.	Vacuoles in animal cells are many and small.	Most mature plant cells have a large central sap vacuole.
5.	Animal cells have a single highly complex Golgi	Plant cells have many simpler units of and prominent Golgi apparatus. apparatus, called dictyosomes.
6.	Animal cells have centrosome and centrioles.	Plant cells lack centrosome and centrioles.

Some Typical cells:

Cyanobacteria: Cyanobacteria are aquatic and photosynthetic. They are quite small and usually unicellular, though they often grow in colonies large enough to see.

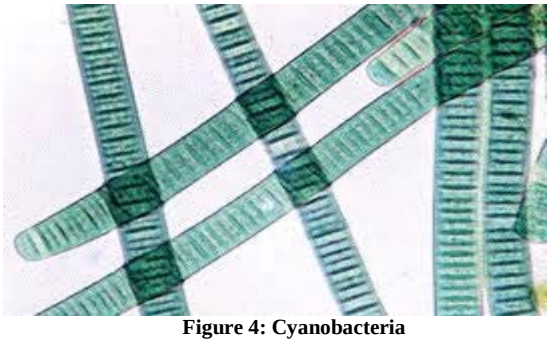


Figure 4: Cyanobacteria

Virus: A virus is a small infectious agent that can replicate only inside the living cells of organisms. Viruses infect all types of organisms, from animals and plants to bacteria and archaea. Their genetic material is DNA or RNA.

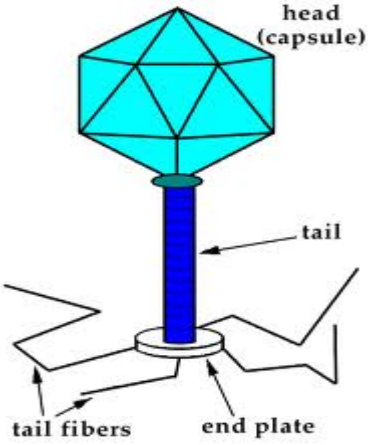


Figure 5: Virus

Red Blood Cells: Red blood cells are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O_2) to the body. They lack organelles like nuleus and mitochondria unlike typical eukaryotic cells.

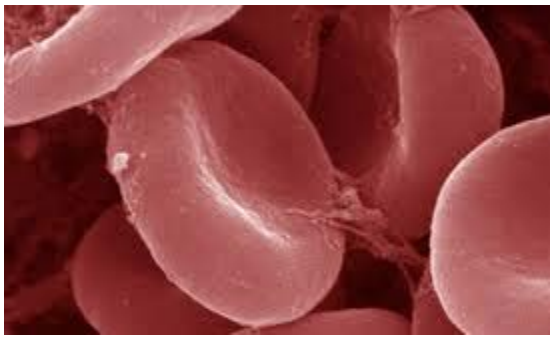


Figure 6: Red blood cell.

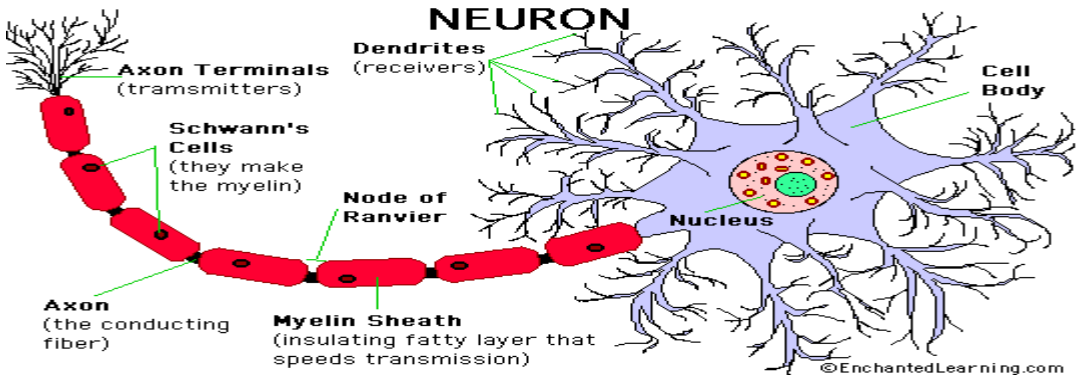


Figure 7: Nerve cell

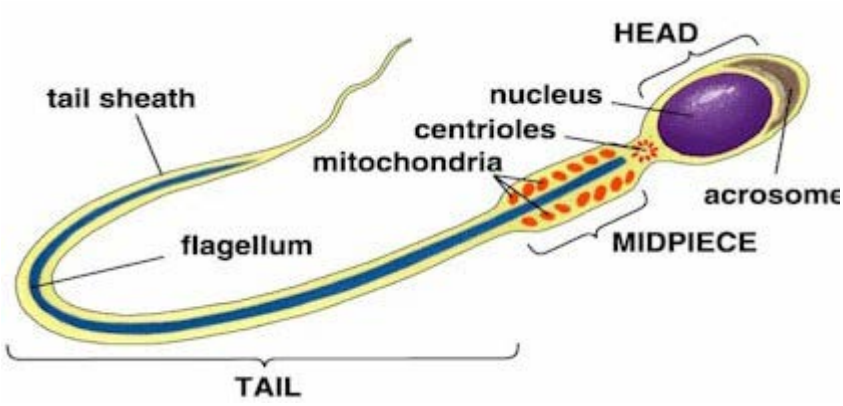


Figure 8: Human sperm cell

Interesting Facts:

1. There are anywhere from 75 to 100 trillion cells in the human body.
2. There are more bacterial cells in the body than human cells.
3. *Thiomargarita namibiensis* is the largest bacterium ever discovered, found in the ocean sediments of the continental shelf of Namibia and can be seen through the naked eye.
4. An unfertilized Ostrich egg is the largest single cell.
5. The smallest cell is a type of bacteria known as mycoplasma. Its diameter is 0.001 mm.
6. The Longest Cell in your body is the motor neuron cell, which is located in the spinal cord, near the central nervous system.

Questions

Multiple choices (Tick the correct answer)

Q1. Prokaryotic organisms have the following structures:

a. Ribosomes, cell membrane, cell wall, surface layer, cilia.
b. Genome, ribosomes, cell wall, surface layer, cilia.
c. Genome, ribosomes, cell membrane, cell wall, surface layer.

Q2. Gram stain is performed on the **_____** of the cell:

a. Cell membrane
b. Genome
c. Cell wall
d. Ribosomes

Q3. Which of the following is false about prokaryotes:

a. They consist of bacteria and archaea
b. Most are unicellular
c. They have no cell nucleus
d. Cell division occurs by mitosis and meiosis

Q4. Eukaryotic cells do not have:

a. A double stranded DNA, enclosed within a nuclear membrane
b. Nucleoli for production and maturation of ribosomes
c. Binary fission reproduction
d. Cell division by mitosis, reproduction by "meiosis".

Q5. What controls most of the cell processes and contains the hereditary information of DNA.

a. Mitochondria.
b. Chloroplast.
c. Nucleus.
d. Nucleolus.

Descriptive:

Q1. What organelles are specific to a plant cell?
Q2. Draw the schematic diagram of a plant and animal cell with proper labeling.
Q3. How do prokaryotes and eukaryotes reproduce?
Q4. Name the components of chloroplast which are involved in photosynthesis.
Q5. What are grana and stroma?

Q6. Describe plastids.
Q7. How did the prokaryotes and eukaryotes evolve?
Q8. Name the important structure missing in Prokaryotes.
Q9. Find out the industrial applications of prokaryotes and list them.
Q10. Name the common organelles found in both plant and prokaryotic cells.

Module 1 Lecture 3

Principles of membrane organization, membrane proteins

Introduction

All living cells possess a cell membrane. These membranes serve to contain and protect cell components from the surroundings as well as regulate the transport of material into and out of the cell. Cell membranes are the selectively permeable lipid bilayers inclusive of membrane proteins which delimits all prokaryotic and eukaryotic cells. In prokaryotes and plants, the plasma membrane is an inner layer of protection bounded to the inner side of a rigid cell wall. Eukaryotes lack this external layer of protection or the cell wall. In eukaryotes the membrane also forms boundary of cell organelles. The cell membrane has been given different specific names based on their lipid and protein composition such as “sarcolemma” in myocytes and “oolemma” in oocytes. The plasma membrane is just 5-10nm wide thus cannot be detected under the light microscope. It can only be observed under the Transmission electron microscope as a trilaminar structure which is a layer of hydrophobic tails of phospholipids sandwiched between two layers of hydrophillic heads.

Functions

Functionally membranes take part in several cellular activities covering motility, energy transduction in lower unicellular organisms to immunorecognition in higher eukaryotes. The most valuable function is segregation of the cell into compartments. This functional diversity is due to the variability in lipid and protein composition of the membranes. The various functions can be summarized as given below.

1. Diffusion: Diffusion of small molecules such as carbon dioxide, oxygen (O_2), and water happens by passive transport.

2. Osmosis: Cell membrane is semipermeable thus it sets up an osmotic flow for solvent such as water, which can be transported across the membrane by osmosis.

3. Mediated Transport: Nutrients are moved across the membrane by special proteins called transport proteins or permeases which are quite specific, recognizing and transporting only a limited group of chemical substances, often even only a single substance.

4. Endocytosis: Endocytosis is the process in which cells absorb molecules by engulfing them small molecules and ions and macromolecules through active transport which requires ATP.

5. Exocytosis: The plasma membrane can extrude its contents to the surrounding medium to remove undigested residues of substances brought in by endocytosis, to secrete substances such as hormones and enzymes, and to transport a substance completely across a cellular barrier.

6. Cell adhesion.

7. Cell signaling.

Theories:

Quincke first perceived the lipid nature of the cell membranes and proposed it to be less than 100 nm thick. With time many researchers have proposed models for cell membrane.

In 1935, Danielli and Davson, proposed a model, called sandwich model, for membrane structure in which a lipid bilayer was coated on its either side with hydrated proteins (globular proteins). Mutual attraction between the hydrocarbonchains of the lipids and electrostatic forces between the protein and the "head" of the lipid molecules, were thought to maintain the stability of the membrane. From the speed at which various molecules penetrate the membrane, they predicted the lipid bilayer to be about 6.0 nm in thickness, and each of the protein layer of about 1.0 nm thickness, giving a total thickness of about 8.0 nm. The Danielli-Davson model got support from electron microscopy. Electron micrographs of the plasma membrane showed that it consists of two dark layers (electron dense granular protein layers), both separated by a lighter area in between (the central clear area of lipid bilayer). The total thickness of the membranes too turned out to be about 7.5 nm.

Currently, the most accepted model for cell membrane is fluid mosaic model proposed by S.J.Singer and G.L.Nicolson (1972). According to this model, the plasma membrane contains a bimolecular lipid layer, both surfaces of which are interrupted by protein molecules. Proteins occur in the form of globular molecules and they are dotted about here and there in a mosaic pattern **(see Figure 1)**. Some proteins are attached at the polar surface of the lipid (i.e., the extrinsic proteins); while others (i.e., integral proteins) either

partially penetrate the bilayer or span the membrane entirely to stick out on both sides (called transmembrane proteins). Further, the peripheral proteins and those parts of the integral proteins that stick on the outer surface (i.e., ectoproteins) frequently contain chains of sugar or oligosaccharides (i.e., they are glycoproteins). Likewise, some lipids of outer surface are glycolipids.

The fluid-mosaic membrane is thought to be a far less rigid than was originally supposed.

In fact, experiments on its viscosity suggest that it is of a fluid consistency rather like the oil, and that there is a considerable sideways movement of the lipid and protein molecules within it. On account of its fluidity and the mosaic arrangement of protein molecules, this model of membrane structure is known as the “fluid mosaic model” (i.e., it describes both properties and organization of the membrane). The fluid mosaic model is found to be applied to all biological membranes in general, and it is seen as a dynamic, ever-changing structure. The proteins are present not to give it strength, but to serve as enzymes catalysing chemical reactions within the membrane and as pumps moving things across it.

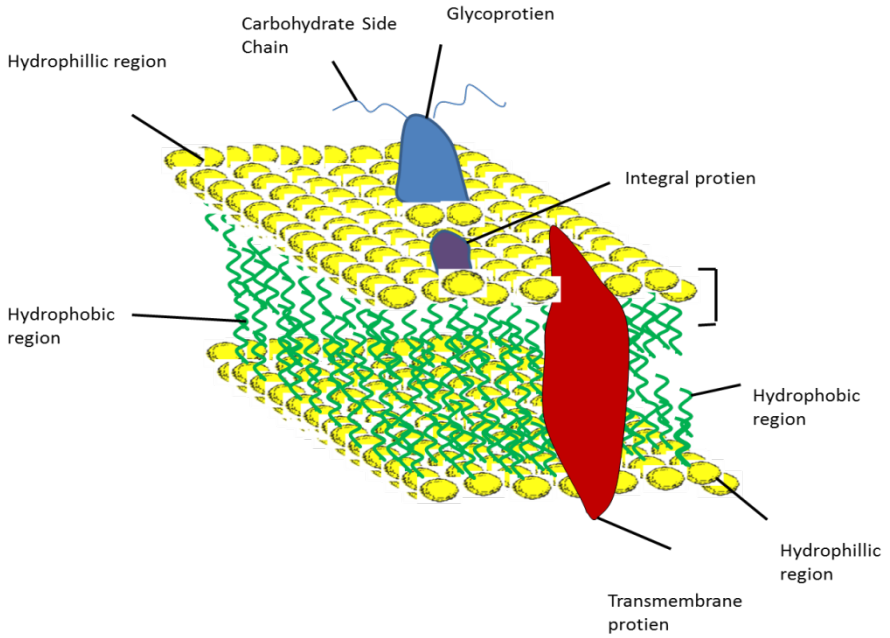


Figure 1: The architecture of the cell membrane

Biochemistry of the cell membrane

Membrane lipids

The cell membrane lipids are highly complex comprising of

- Phospholipids,
- Glycolipids,
- Cholesterols.

The major membrane phospholipids and glycolipids are phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns) and phosphatidylserine (PtdSer) (Figure 2, Table 1). Eukaryotic membrane lipids are glycerophospholipids, sphingolipids, and sterols. Sphingolipids (SPs) and sterols enable eukaryotic cellular membranes with the property of vesicular trafficking important for the establishment and maintenance of distinct organelles. Mammalian cell membranes contain cholesterol which imparts stiffening and strengthening effect on the membrane, along with glycerophospholipids and sphingolipids. The head group of glycerophospholipids can vary, the fatty acids can differ in length (16- and 18-carbon fatty acids are the most common) Fatty acids can be saturated or unsaturated with the double bonds always in *cis* configuration in the later. The unsaturated fatty acids prevent tight packing of the fatty acid chains leading to lowering of melting temperature and increase in membrane fluidity. Also, the sphingolipids have the combinatorial propensity to create diversity by different ceramide backbones. Lipid molecules are free exhibit lateral diffusion along the layer in which they are present. However, the exchange of phospholipid molecules between intracellular and extracellular leaflets of the bilayer is a very slow process. The lipid composition, cellular architecture and
function of cell membrane from unicellular bacteria to yeast and higher eukaryotes is presented in Table 2.

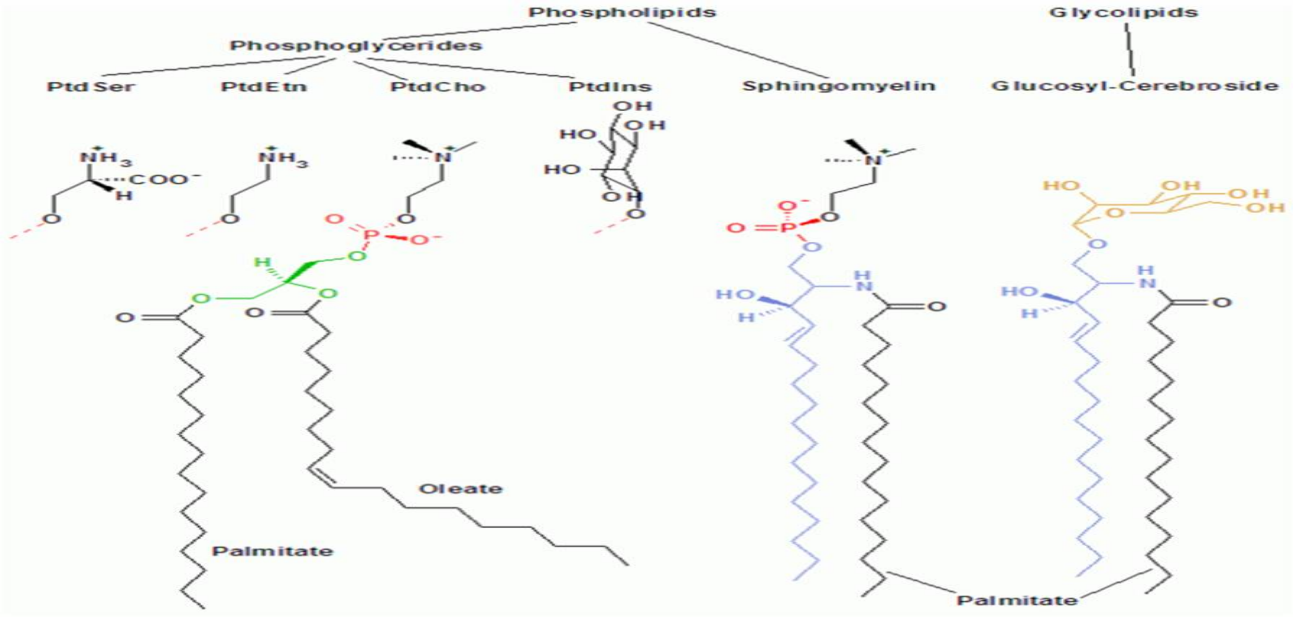


Figure 2 The major membrane phospholipids and glycolipids. The figure has been adapted from the "Membrane Organization and Lipid Rafts" by Kai Simons and Julio L. Sampaio, 2011, Cold Spring Harbor Laboratory Press.

Table 1 The composition of different membrane lipids

Type	Composition	Example/ Remarks
Phosphoglycerides	esters of phosphoric acid and a trifunctional alcohol-glycerol	Phosphatidate four common substituents for phosphatidate; Serine, ethanolamine, choline and inositol.
Sphingolipids	Phosphoglycerides where glycerol is substituted with sphingosine.	Sphingomyelin, Glycosphingolipid Found in particularly nerve cells and brain tissues

Table 2 The cellular architecture and function of cell membrane

Organism	Lipid composition	Membrane properties	Functionalities
Bacteria	Phosphatidylethanolamine and Phosphatidylglycerol	Robust Different shapes	Membrane protein incorporation
Yeast	Sphingolipids, Glycerophospholipids and Sterols	Robust Different shapes Complex organelle morphology	Membrane protein incorporation Membrane budding Vesicular trafficking
Higher Eukaryotes	Glycerophospholipids, sterols, and tissue-specific Sphingolipids	Robust Different shapes Complex organelle morphology Complex and specific cellular architecture	Membrane protein incorporation Membrane budding Vesicular trafficking Specific functions depending on the cell type

Role of Lipid Molecules in Maintaining Fluid Property of Membrane

Types of movements of lipid molecules.

In lipid monolayer flip-flop or transbilayer movement occurs once a month for any individual lipid molecule. However, in membranes where lipids are actively synthesized, such as smooth ER, there is a rapid flip-flop of specific lipid molecules across the bilayer and there are present certain membrane-bound enzymes, called phospholipid translocators like flippases to catalyze this activity. The other movement is lateral diffusion. Individual lipid molecules rotate very rapidly about their long axes and their hydrocarbon chains are flexible, the greatest degree of flexion occurring near the centre of the bilayer and the smallest adjacent to the polar head groups.

Role of unsaturated fats in increasing membrane fluidity.

A synthetic bilayer made from a single type of phospholipid changes from a liquid state to a rigid crystalline state at a characteristic freezing point. This change of state is called a phase transition

and the temperature at which it occurs becomes lower if the hydrocarbon chains are short or have double bonds. Double bonds in unsaturated hydrocarbon chains tend to increase the fluidity of a phospholipid bilayer by making it more difficult to pack the chains together. Thus, to maintain fluidity of the membrane, cells of organisms living at low temperatures have high proportions of unsaturated fatty acids in their membranes, than do cells at higher temperatures.

Role of cholesterol in maintaining fluidity of membrane

Eukaryotic plasma membranes are found to contain a large amount of cholesterol; up to one molecule for every phospholipid molecule. Cholesterol inhibits phase transition by preventing hydrocarbon chains from coming together and crystallizing. Cholesterol also tends to decrease the permeability of lipid bilayers to small water-soluble molecules and is thought to enhance both the flexibility and the mechanical stability of the bilayer.

Membrane proteins

In addition to the lipid bilayer, the cell membrane also contains a number of proteins. 35% of the genes in any genome encode membrane proteins, and many other proteins spend part of their lifetime bound to membranes. The amount of protein differs between species and according to function, however the typical amount in a cell membrane is 50%. Membrane proteins are free to move within the lipid bilayer as a result of its fluidity. Although this is true for most proteins, they can also be confined to certain areas of the bilayer with enzymes.

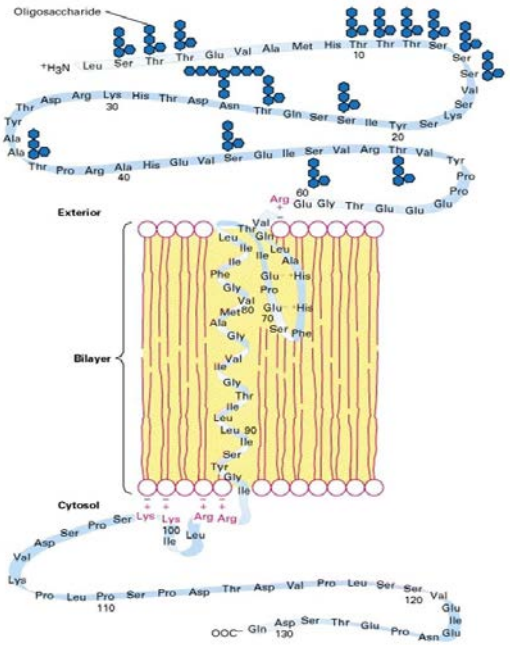
They can be classified into

- Integral (intrinsic)
- Peripheral (extrinsic)

which is based on the nature of the membrane-protein interactions (Figure 3). Integral proteins have one or more segments that are embedded in the phospholipid bilayer from four to several hundred residues long, extending into the aqueous medium on each side of the bilayer. The transmembrane embedded in the hydrophobic core of the bilayer are α

helices or multiple β strands interacting with the lipid bilayer with hydrophobic and ionic interactions. An example is Glycophorin which is a major erythrocyte membrane protein and bacteriorhodopsin, a protein found in a photosynthetic bacterium (Figure 3a, 3b). Glycophorin is a homodimer containing α helix in coiled-coiled conformation, composed of uncharged amino acids. Few positively charged amino acids (lysine and arginine) prevent it from slipping across the membrane by interacting with negatively charged phospholipid head groups. Most of these charged residues are adjacent to the cytosolic face of the lipid bilayer. Bacteriorhodopsins have serpentine membrane spanning domain. Other examples of seven-spanning membrane proteins include the opsins (eye proteins that absorb light), cell-surface receptors for many hormones, and receptors for odorous molecules. Some integral proteins are anchored to the exoplasmic face of the plasma membrane by a complex glycosylated phospholipid that is linked to the C-terminus. A common example of this type of anchor is glycosylphosphatidylinositol, which contains two fatty acyl groups, *N*-acetylglucosamine, mannose, and inositol for example alkaline phosphatase. Whereas some are attached by a hydrocarbon moiety covalently attached to a cysteine near the C-terminus. The most common anchors are prenyl, farnesyl, and geranylgeranyl groups.

Peripheral membrane proteins do not interact with the hydrophobic core and are bound to the membrane indirectly by interactions with integral membrane proteins or directly by interactions with lipid polar head groups. Peripheral proteins localized to the cytosolic face of the plasma membrane include the cytoskeletal proteins spectrin and actin in erythrocytes and the enzyme protein kinase C involved in cell signaling. An important group of peripheral membrane proteins are water-soluble enzymes that associate with the polar head groups of membrane phospholipids.



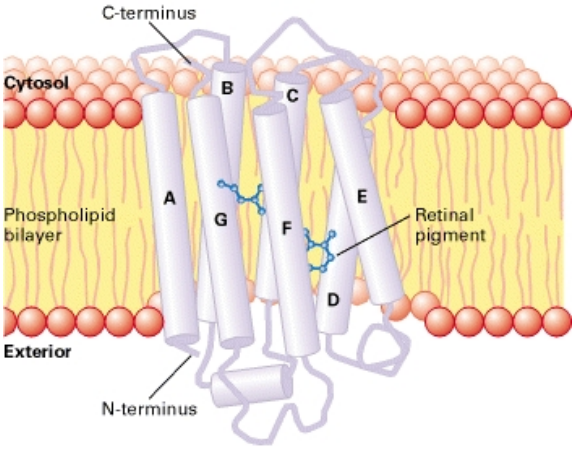
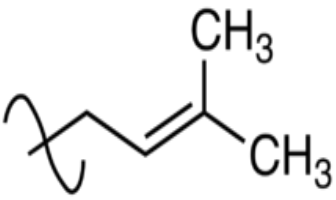
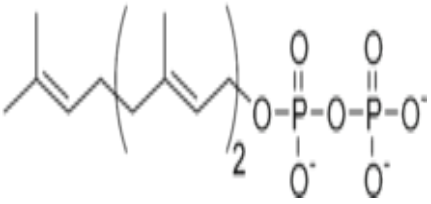


Figure 3a, 3b. Figure 3a This protein is a homodimer, but only one of its polypeptide chain is shown. Residues 62–95 are buried in the membrane, with the sequence from position 73 through 95 forming an α helix. The ionic interactions shown between positively charged arginine and lysine residues and negatively charged phospholipid head groups in the cytosolic and exoplasmic faces of the membrane are hypothetical. Both the amino-terminal segment of the molecule, located outside the cell, and the carboxy-terminal segment, located inside the cell, are rich in charged residues and polar uncharged residues, making these domains water-soluble. Note the numerous carbohydrate residues attached to amino acids in the exoplasmic domain. Adapted from V. T. Marchesi, H. Furthmayr, and M. Tomita, 1976, *Ann. Rev. Biochem.* 45:667.

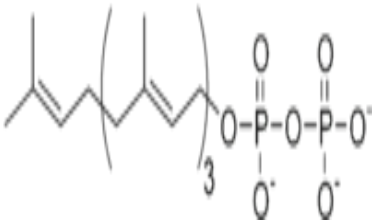
Fig 3b The seven membrane-spanning α helices are labeled A–G. The retinal pigment is covalently attached to lysine 216 in helix G. The approximate position of the protein in the phospholipid bilayer is indicated. Adapted from R. Henderson et al., 1990, *J. Mol. Biol.* 213:899.



group
Prenyl group



Farnesyl pyrophosphate



Geranylgeranyl pyrophosphate

Figure 4: Anchor moieties of integral membrane proteins.

Membrane Lipid Rafts

The plasma membrane is made of a combination of glycosphingolipids and protein receptors organized in glycolipoprotein microdomains termed lipid rafts which are 10–200 nm in size. In addition to an external cell membrane (called the plasma membrane) eukaryotic cells also contain internal membranes that form the boundaries of organelles such as mitochondria, chloroplasts, peroxisomes, and lysosomes. Functional specialization in the course of evolution has been closely linked to the formation of such compartments. Lipid rafts is the principle of membrane sub compartmentalization. The concept stresses on the fact that lipid bilayer is not a structurally passive solvent but possesses lateral segregation potential. The lipids in these assemblies are enriched in saturated and longer hydrocarbon chains and hydroxylated ceramide backbones. The types of lipid rafts are given in Table 3.

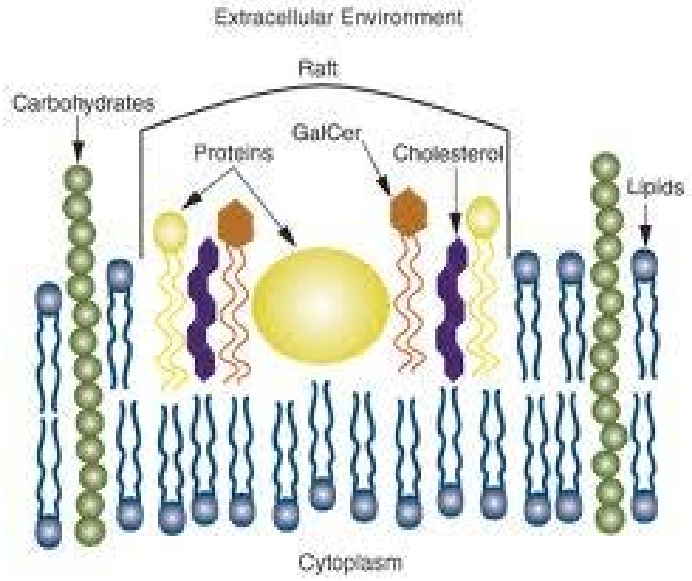


Figure 5: Lipid raft

The difference between lipid rafts and the plasma membranes is their lipid composition because lipid rafts are enriched in sphingolipids such as sphingomyelin, which is typically elevated by 50% compared to the plasma membrane. There are two types of lipid rafts i.e., planar lipid rafts) and caveolae. Planar rafts are continuous with the plane of the plasma membrane and contain flotillin proteins. Caveolae are flask shaped invaginations formed by polymerization of caveolin proteins. Both types are enriched in cholesterol and sphingolipids. Flotillin and caveolins recruit signaling molecules into lipid rafts, thus playing an important role in neurotransmitter signal transductions. It has been proposed that these microdomains spatially organize signaling molecules to promote kinetically favorable interactions which are necessary for signal transduction. These microdomains can also separate signaling molecules, inhibiting interactions and dampening signaling responses.

One of the most important properties of lipid rafts is that they can include or exclude proteins to variable extents. Proteins with raft affinity include glycosylphosphatidylinositol (GPI)-anchored proteins. One subset of lipid rafts is found in cell surface invaginations called caveolae (Table 3). Caveolae are formed from lipid rafts by polymerization of caveolins — hairpin-like palmitoylated integral membrane proteins that tightly bind cholesterol.

Table 3 Types of lipid Rafts

Raft type	Constituent	Function	References
Caveolae	Cholesterol, glycoshingolipid, Arachidonic acid, Plasmenylethanolamine, Caveolin1 and 2, hetero- trimeric G-proteins and monomeric G-proteins, EGF & PDGF receptors, Fyn, GPI-linked enzymes, integrins. Flotillin	Presumed to be signalling centres and perhaps regions of cholesterol import	Pike et al, 2002.
Glycosphingolipid enriched	Cholesterol, glycoshingolipid, low in PI and other anionic phospholipids	Signalling?	Simons 2000
PIP2 enriched	PIP2, MARKS, CAP, GAP-43	Signalling, Structural.	Laux et al, 2000

Rafts in signal transduction

The most important role of rafts at the cell surface may be their function in signal transduction. They form platforms for receptors which are activated on ligand binding. If receptor activation takes place in a lipid raft, the signaling complex is protected from non-raft enzymes such as membrane phosphatases that otherwise could affect the signaling process. In general, raft binding recruits proteins to a new micro-environment, where the phosphorylation state can be modified by local kinases and phosphatases, resulting in downstream signaling. Examples of raft signaling are Immunoglobulin E signaling, T-cell antigen receptor signaling, GDNF signaling, Ras signaling, Hedgehog signaling.

Models for signal initiation in rafts

A common theme of signal transduction is that individual rafts cluster together to connect raft proteins and interacting proteins into a signalling complex**.** Receptors have at least three different options in rafts for signal transduction (Figure 6). First, receptors could be activated through ligand binding (Figure 6). Second, individual receptors possessing weak raft affinity can oligomerize on ligand binding (Figure 6). Last, crosslinking proteins can be recruited to bind to proteins in other rafts (Figure 6). The formation of clustered rafts would lead to amplification of signal. The interactions that drive raft assembly are dynamic and reversible. Raft clusters can be also be disassembled by removal of raft components from the cell surface by endocytosis. The coalescence of individual rafts to form raft clusters has been observed when crosslinking raft components with antibodies. The movement and behavior of the raft clusters can also be influenced by interaction with cytoskeletal elements and second messengers, which help organize actin assemblies on the cytoplasmic surface of the rafts.

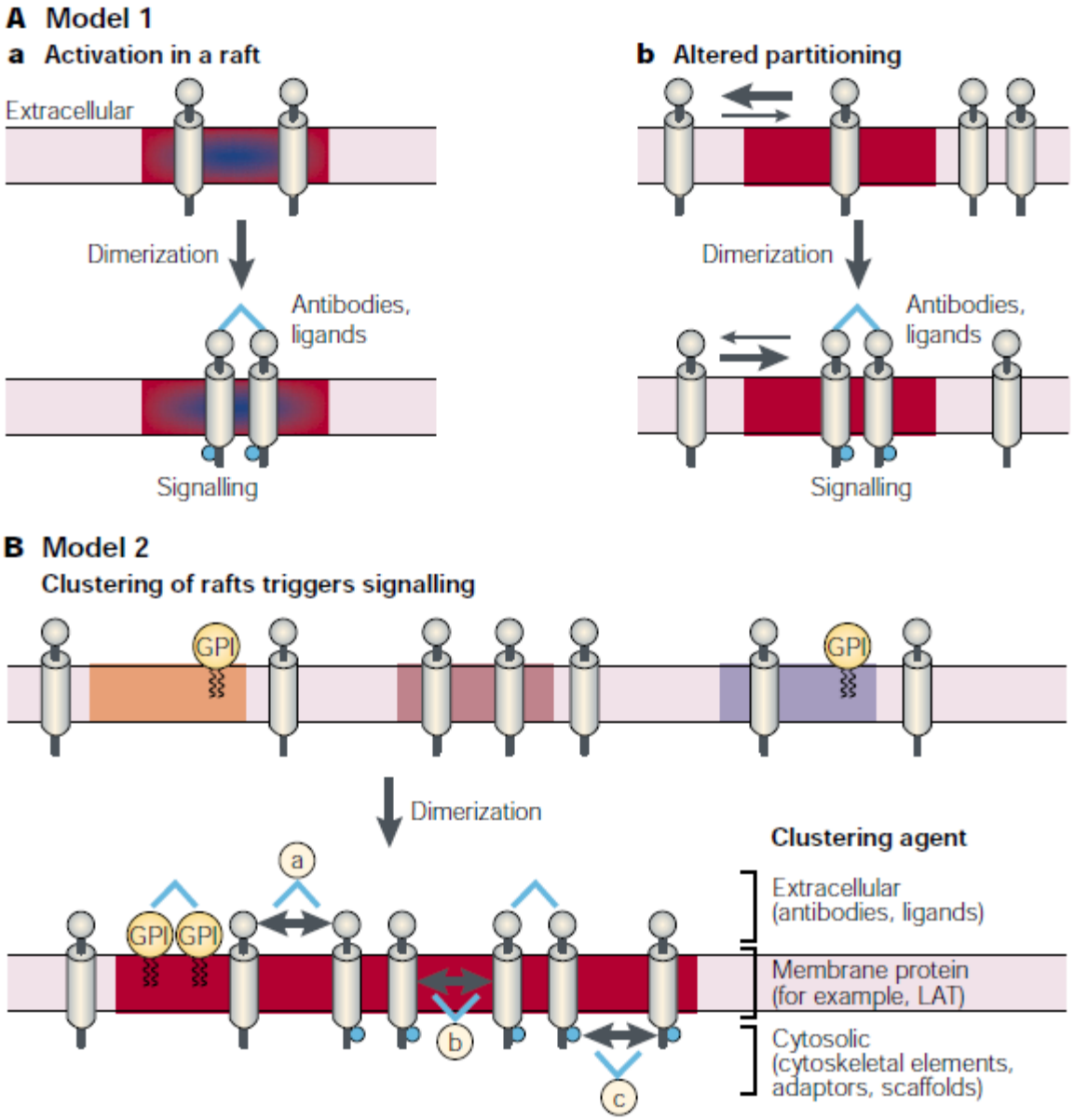


Figure 6: Models of how signalling could be initiated through rafts. A. In these models, signalling occurs in either single rafts (Model 1) or clustered rafts (Model 2). Following dimerization the protein becomes phosphorylated in rafts. B. In the second model we assume that there are several rafts in the membrane, which differ in protein composition (shown in orange, purple or blue). Clustering would coalesce rafts (red), so that they would now contain a new mixture of molecules, such as crosslinkers and enzymes. Clustering could occur either extracellularly, within the membrane, or in the cytosol (a–c, respectively). Raft clustering could also occur through GPIanchored proteins (yellow), either as a primary or co-stimulatory response. Notably, models 1 and 2 are not mutually exclusive. For instance, extracellular signals could increase a protein's raft affinity (for example, similar to the effect of single versus dual acylation) therefore drawing more of the protein into the raft where it can be activated and recruit other proteins, such as LAT, which would crosslink several rafts. Printed with permission from Simons K Sampaio J L. Membrane Organization and Lipid Rafts. Cold Spring Harbor Perspective Biology. 2011.

Interesting Facts

1. Cells spend a lot of energy trying to maintain their membranes.

2. Eukaryotic animal cells are generally thought to have descended from prokaryotes that lost their cell walls.

3. Acidity (pH) in cells of baker's yeast, *Saccharomyces cerevisiae*, regulate the synthesis of cell membranes by controlling the production of enzymes that synthesize membranes. (Universiteit van Amsterdam (UVA), 2010).

4. Cell membrane associated diseases are Alzheimer's, Hyaline Membrane Disease and Cystic fibrosis.

5. The oxidative stress caused by Alzheimer's disease in the brain results in phospholipid alterations.

6. The conductance of biological membranes is high, the reason is that there are all kinds of ion channels and other pores penetrating the membrane and allowing additional currents to flow. It is these currents that make cells behave in complex and interesting ways.

Questions:

Q 1. A protein in the phospholipid bilayer binds with an ion, and then changes shape so that the ion, can move into the cell, is an example of?

a. osmosis

b. facilitated diffusion

c. endocytosis

d. active transport

Q 2. How is phospholipid bilayer formed?

Q3. Suppose Red blood cells are broken due to snale venom which has three enzymes: phospholipase, which degrades phospholipids; neuraminidase, which removes cell surface carbohydrates; and protease which degrades proteins. Which of these enzymes do you think was responsible for his near fatal red blood cell hemolysis? Why?

a. The neuraminidase lysis the carbohydrate-rich membrane, leading to cell breakage.

b. The protease would degrade transmembrane proteins leading to cell lysis.

c. The phospholipase would degrade the phospholipids, the component of a membrane creating a barrier.

Q3. Lipid bilayer is formed when phospholipids are placed into an aqueous solution. What is the driving force causing this ordered arrangement?

a. The phospholipids are very ordered in water, and gain freedom of movement by forming a bilayer.
b. Water, when associated with lipids, is forced into an ordered arrangement with fewer hydrogen bonds.
c. Phospholipids have a strong affinity for other phospholipids, leading to self assembly.

Q4. Which component of a cell membrane forms receptor in cell to cell signaling?

a. lipids
b. proteins
c. carbohydrates
d. cholesterol

Q5. The major driving force for the formation of a lipid bilayer is _____; once formed the membrane is further stabilized by _____.

a. Electrostatic attractions between phospholipid head groups; hydrophobic forces and hydrogen bonds.
b. Hydrophobic forces on the phospholipid fatty acid carbon chains; hydrogen bonds, electrostatic attractions, and van der Waals contacts.
c. Repulsion between negative charges of phospholipid fatty acids; hydrogen bonds and van der Waals contacts.
d. van der Waals contacts between phospholipid charged groups; hydrophobic forces, hydrogen bonding and electrostatic attractions.
e. electrostatic attractions, hydrogen bonds, and van der Waals contacts; covalent bonds.

Q6. Phospholipids are _____.

a. Amphipathic.
b. Electrostatic.
c. Polar.
d. Non-polar.
e. Ionic.

Q7. Explain Fluid Mosaic Model and enumerate the functions of membrane proteins and membrane lipids.

Q8. What are the compositions of membrane lipids? How do they differ in prokaryotes and eukaryotes?

Q9. Write briefly about membrame proteins.

Q10. What are lipid rafts? Enumerate its structure and functions.

Q11. What are the different types of lipid rafts known? Write briefly about the signal initiation steps in the lipid rafts.

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Module 1 Lecture 4

Cytoskeletal elements and architecture

The existence of an organized fibrous array or cytoskeleton in the structure of the protoplasm was postulated in 1928 by Koltzoff. The cytoskeleton can be defined as a cytoplasmic system of fibers which is critical to cell motility. It is dynamic three-dimensional scaffolding contained within a cell's cytoplasm and is made of protein. The ability of eukaryotic cells to adopt a variety of shapes and to carry out coordinated and directed movements depends on the cytoskeleton. The cytoskeleton was known to be unique to eukaryotic cells. Recent research has found cytoskeletal elements in bacteria showing that it has evolved early in evolution. Several proteins that are involved in cell division, cell structure and DNA partitioning have been found to form highly dynamic ring structures or helical filaments underneath the cell membrane or throughout the length of the bacterial cells. The cytoskeleton can also be referred to as cytomusculature, because, it is directly involved in movements such as crawling of cells on a substratum, muscle contraction and the various changes in the shape of a developing vertebrate embryo; it also provides the machinery for cyclosis in cytoplasm. The main proteins that are present in the cytoskeleton are tubulin (in the microtubules), actin, myosin, tropomyosin and other (in the microfilaments) and keratins, vimentin, desmin, lamin and others (in intermediate filaments). Tubulin and actin are globular proteins, while subunits of intermediate filaments are fibrous proteins. The use of high-voltage electron microscopy on whole cells has helped to demonstrate that there is a highly structured, three-dimensional lattice in the ground cytoplasm. Figure 1 gives an overview of the cytoskeletal system. The primary types of fibers comprising the cytoskeleton are:

- Microfilaments
- Intermediate filaments
- Microtubules

They are classified based on their size, function and distribution within the cell. The differences among the three cytoskeletal elements is given in Table 1 and are individually explained in the following subsections.

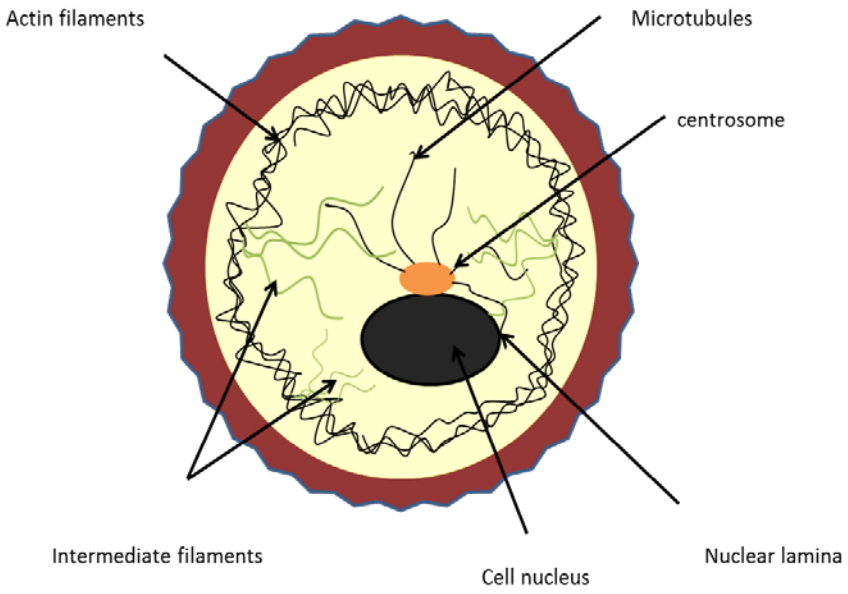

Figure 1: The cytoskeletal system

Table 1: Differences among cytoskeletal elements

Microfilaments	Intermediate filaments	Microtubules
Depolymerize into their soluble subunits	Extremely stable	Depolymerize into their soluble subunits
7 nm in diameter	10 nm in diameter	24 nm in diameter
Beaded structure	α-helical rods that assemble into ropelike filaments	Hollow tubules
Require nucleotide hydrolysis for polymerization G-actin	Subunits do not require nucleotide hydrolysis for polymerization	Require nucleotide hydrolysis for polymerization of αβ-tubulin

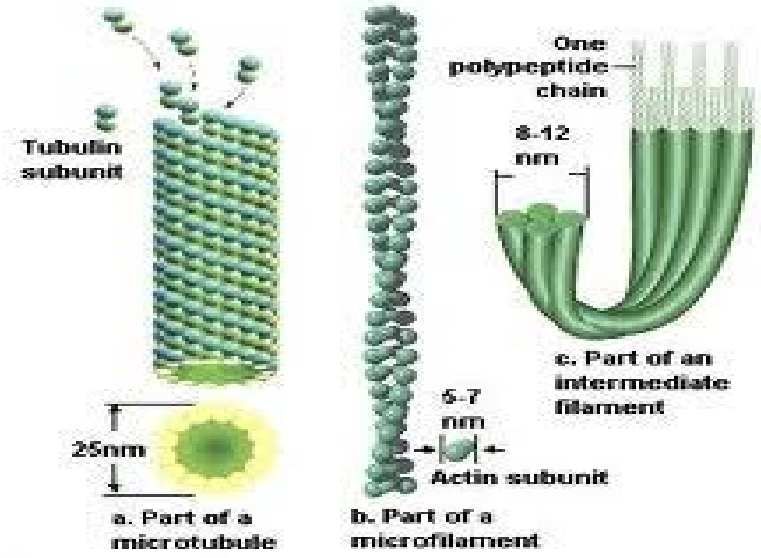

Figure 2: The difference among the various cytoskeletal systems

a. Microfilaments

Structure

Microfilaments are involved in cell locomotion. Microfilaments also extend into cell processes, especially where there is movement. Thus, they are found in the microvilli of the brush border of intestinal epitheliun and in cell types where amoeboid movement and cytoplasmic streaming are prominent. Microfilaments are powered by actin cytoskeleton which is a medium sized protein of 375 amino acid residues which is encoded by a highly conserved gene family. Actin proteins are localized in cytoplasm, nucleus and in the muscles. However the richest area of actin filaments in a cell lies in a narrow zone just beneath the plasma membrane known as the cortex. Actin protein is structurally globular composed of G-actin and F-actin; which in turn is a linear chain of G-actin subunits. Each actin molecule contains an Mg^{2+} ion cofactor bound ATP or ADP. Thus there are four states of actin: ATP–G-actin, ADP–G-actin, ATP–F-actin, and ADP–F-actin. The assembly of G-actin into F-actin is accompanied by the hydrolysis of ATP to ADP and P_i. In F-filament all actin moieties point toward the same filament end. ATP-binding cleft of an actin subunit is exposed to the surrounding solution. Finally actin filaments form bundles and networks which provide a framework that supports the plasma membrane.

Structurally, bundles differ from networks mainly in the organization of actin filaments. In bundles the actin filaments are closely packed in parallel arrays, whereas in a network the actin filaments crisscross, often at right angles, and are loosely packed. Cells contain two types of actin networks. One type remain associated with the plasma membrane and is planar, the other type is present within the cell and gives the cytosol its gel-like properties. Filaments are connected through a cross-linking protein having two actin-binding sites, one site for each filament. The length and flexibility of this cross-linking protein critically determine whether bundles or networks are formed. Short cross-linking proteins hold actin filaments close together, forcing the filaments into the parallel alignment characteristic of bundles (Figure 3). In contrast, long, flexible cross-linking proteins are able to adapt to any arrangement of actin filaments and tether orthogonally oriented actin filaments in networks as given in Figure 3. Again membrane microfilament binding proteins join membrane to the cytoskeleton framework. The simplest connections entail binding of integral membrane proteins directly to actin filaments.

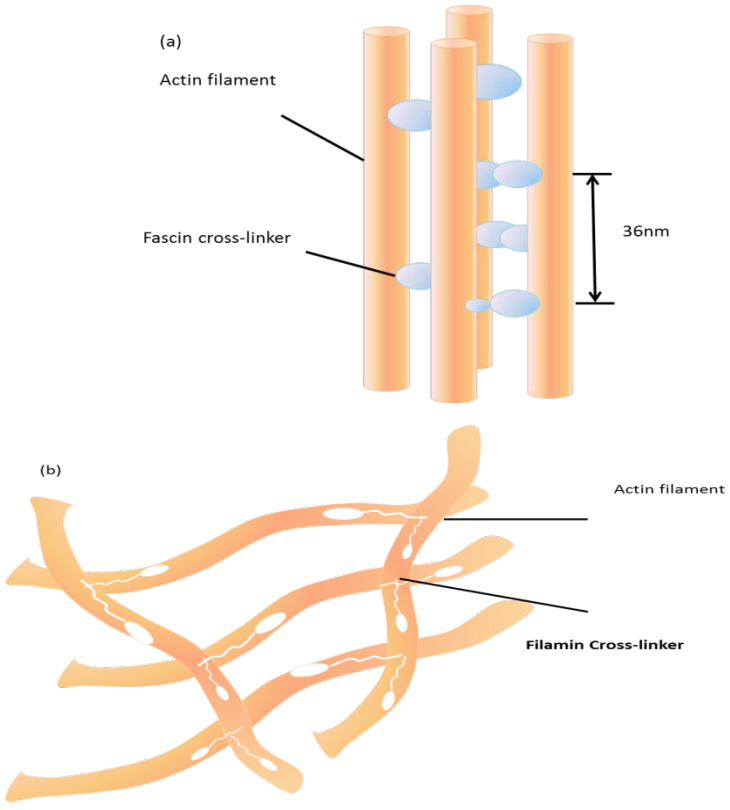

Figure 3: Actin cross-linking proteins bridging pairs of actin filaments

Function

1. An important function of actin microfilament is that it can produce movement in the absence of motor proteins. At the cell membrane microfilament assembly protrudes the membrane forward producing the ruffling membranes in actively moving cells.
2. Microfilaments can also play a passive structural role by providing the internal stiffening rods in microvilli, maintaining cell shape, and anchoring cytoskeletal proteins.

b. Intermediate filaments

Intermediate filaments (IFs) are tough, durable protein fibres in the cytoplasm of most higher eukaryotic cells typically between 8 nm to 10 nm in diameter. They are particularly prominent where cells are subjected to mechanical stress, such as in epithelia, where they are linked from cell to cell at desmosomal junctions, along the length of axons, and throughout the cytoplasm of smooth muscle cells. Intermediate filaments are typically organized in the cytosol as an extended system that stretches from the nuclear envelope to the plasma membrane. Some intermediate filaments run parallel to the cell surface, while others traverse the cytosol. They also form the nuclear lamina. In cross-section, intermediate filaments have a tubular appearance. Each tubule appears to be made up of 4 or 5 protofilaments arranged in parallel fashion (Figure 2). IFs are composed of polypeptides of a surprisingly wide range of sizes (from about 40,000 to 130,000 daltons). Protein subunits from the family of α-helical proteins make the intermediate filaments and these protein subunits can be divided into six major classes which are widely divergent in sequence and vary greatly in molecular weight (Table 2).

Table 2: Classes of proteins making the intermediate filaments. Students need not have to remember the mass (MW). The values just indicates the molecular mass range of different proteins

IF protein	MW (10^{-3})	Tissue distribution
Type I		
Acidic keratins	40-57	Epithelia
Type II		
Basic keratins	53-67	Epithelia
Type III		
Vimentin	57	Mesenchyme
Desmin	53	Muscle
Glial fibrillay acidic protein	50	Glial cells and astrocytes
Peripherin	57	Neurons
Type IV		
NF-L	62	Mature neurons
NF-M	102	Mature neurons
NF-H	110	Mature neurons
Internexins	66	Developing central nervous system
Non standard type IV		
Filensin	83	Lens fibre cells
Phakinin	45	
Type V		Cell nucleus
Lamin A	70	
Lamin B	67	
Lamin C	67	

The keratins are the most diverse classes of IF proteins and can be divided into two groups: keratins specific for tough epithelial tissues, which give rise to nails, hair, and wool and cytokeratins which are more generally found in the epithelia that line internal body cavities. Each type of epithelium always expresses a characteristic combination of type I and type II keratins which associate in a 1:1 ratio to form heterodimers, which assemble into heteropolymeric keratin filaments. Apart from keratins most widely distributed of all IF class III proteins is vimentin, which is typically expressed in

leukocytes, blood vessel endothelial cells, some epithelial cells, and mesenchymal cells such as fibroblasts. Vimentin filaments help support cellular membranes. Vimentin networks also may help keep the nucleus and other organelles in a defined place within the cell. Vimentin is also frequently associated with microtubules. Neurofilaments which are type IV proteins make the core of neuronal axons. Each of which is a heteropolymer composed of three type IV polypeptides which differ greatly in molecular weight. In contrast to microtubules, which direct the elongation of an axon, neurofilaments are responsible for the radial growth of an axon and thus determine axonal diameter. The diameter of an axon is directly related to the speed at which it conducts impulses. The influence of the number of neurofilaments on impulse conduction is highlighted by a mutation in quails named quiver, which blocks the assembly of neurofilaments. As a result, the velocity of nerve conduction is severely reduced. Lamins which are type V proteins are found exclusively in the nucleus. Of the three nuclear lamins, two are alternatively spliced products encoded by a common gene, while the third is encoded by a separate gene. The nuclear lamins form a fibrous network that supports the nuclear membrane.

Structure

Intermediate filament proteins are 10 nm in diameter, a central α-helical conserved core flanked by globular N- and C-terminal domains which vary in different IF proteins. The core helical domain is conserved among all IF proteins. It consists of four α-helices separated by three spacer regions. The polypeptide chains are parallel in a dimer. A pair of dimers associate laterally into a tetramer. Tetramers bind end to end, forming protofilaments $2-3$ nm thick, which pair together into protofibrils. Finally, four protofibrils form a single intermediate filament that is 10 nm in diameter. IFs do not have a polarity like an actin filament or a microtubule. The N-terminal domain plays an important role in assembly of most intermediate filaments. The C-terminal domain affects the stability of the filament. An IF filament can be a homo- or a heteropolymer whose formation is dependent on the spacer sequences. Proteins cross-link intermediate filaments with one another, forming a bundle (a tonofilament) or a network, and with

other cell structures, including the plasma membrane. The structure of and formation intermediate filament has been illustrated in Figure 4 and 5.

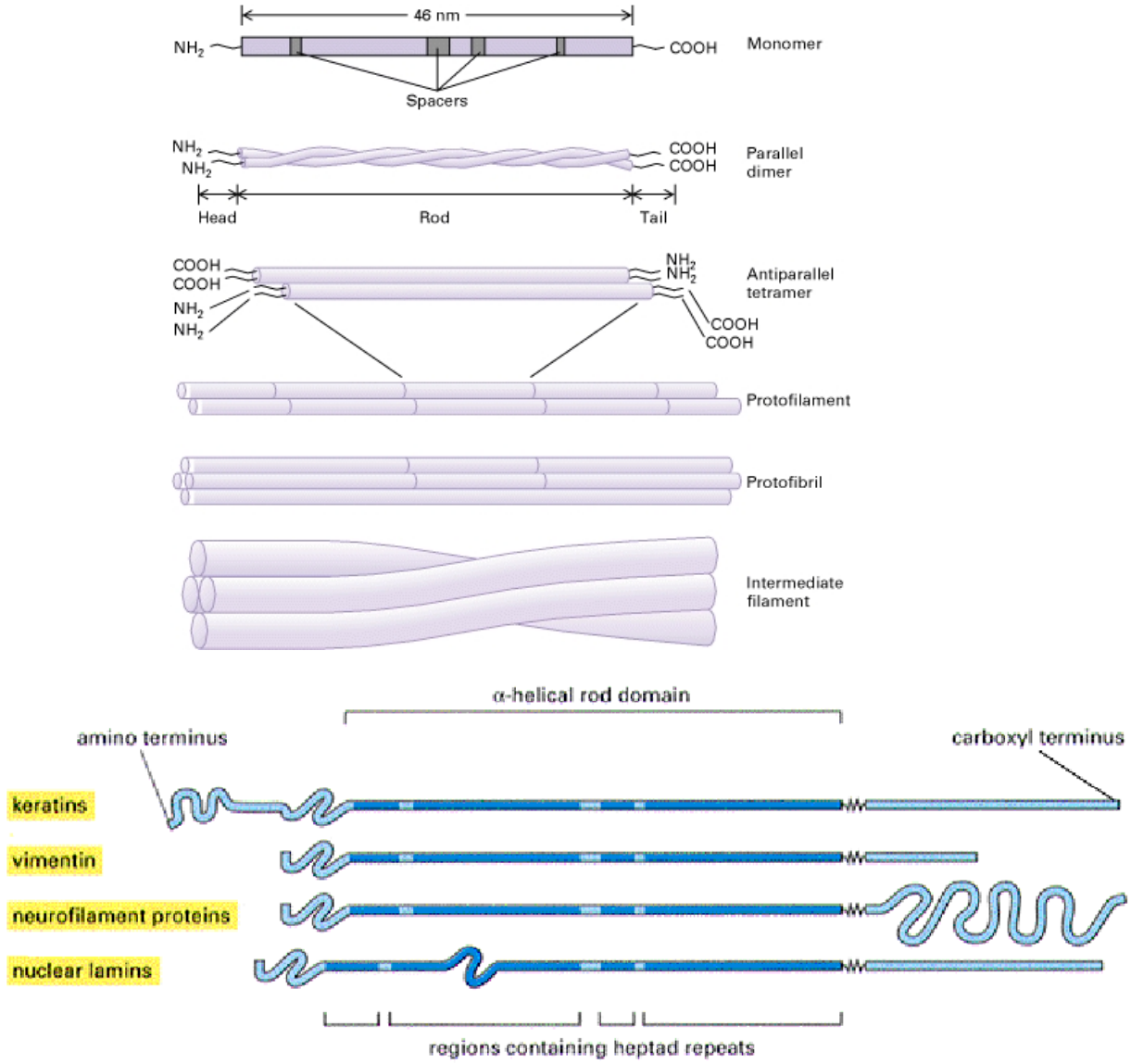

Figure 4 Monomer of Intermediate filaments. The above figure is from Alberts et al, Molecular Biology of the Cell, Garland Publishing, NY, 1996.

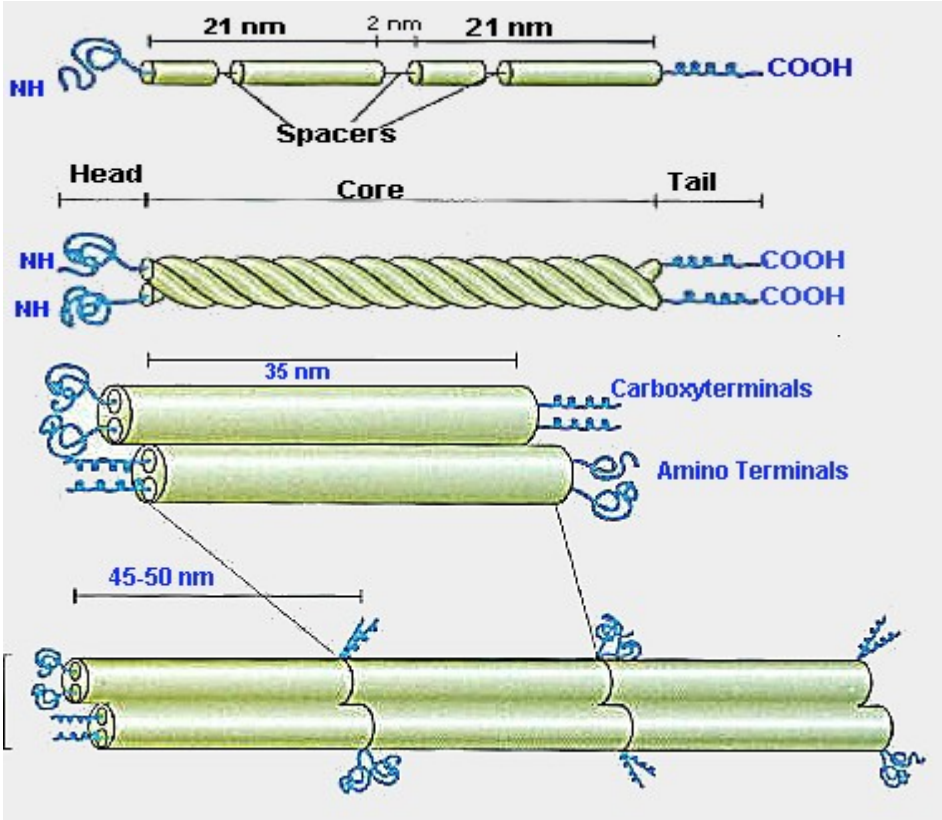

Figure 5: Formation of Intermediate filaments. The rods coil around another filament like a rope to form a dimer. The N and C terminals of each filament are aligned. Some Intermediate filaments form homodimers; other form heterodimers. These dimers then form staggered tetramers that line up head-tail. Note that the carboxy and amino terminals project from this protofilament. This tetramer is considered the basic subunit of the intermediate filament.

Function

1. The main function of Intermediate filament is mechanical support. The best example is the nuclear lamina along the inner surface of the nuclear membrane. IFs in epithelia form a transcellular network that resists external forces. The neurofilaments in the nerve cell axons resist stresses caused by the motion of the animal, which would otherwise break these long, thin cylinders of cytoplasm. Desmin filaments provide mechanical support for the sarcomeres in muscle cells, and vimentin filaments surround and probably support the large fat droplets in the fat cells.

2. They form an internal framework that helps support the shape of the cell. In vitro binding experiments suggest that at the plasma membrane, vimentin filaments bind two proteins: ankyrin, the actinbinding protein associated with the Na^+/K^+ ATPase in nonerythroid cells, and plectin.

c. Microtubules

Microtubules were fisrt of all observed in the axoplasm of the myelinated nerve fibres by Robertis and Franchi (1953). In the plant cells they were first described in detail by Ledbetter and Porter (1963). A microtubule is a polymer of globular tubulin subunits, which are arranged in a cylindrical tube measuring 24 nm in diameter which is more than twice the width of an intermediate filament and three times the width of a microfilament (Figure 6). Microtubules are also much stiffer than either microfilaments or intermediate filaments because of their tubelike construction. The building block of a microtubule is the tubulin subunit, a heterodimer of α- and β-tubulin. Both of these 55,000-MW monomers are found in all eukaryotes, and their sequences are highly conserved. Although a third tubulin, γ-tubulin, is not part of the tubulin subunit, it probably nucleates the polymerization of subunits to form αβ-microtubules. The interactions holding α-tubulin and β-tubulin in a heterodimeric complex and are strong enough ensuring rare dissociation of a tubulin subunit under normal conditions. Each tubulin subunit binds two molecules of GTP. One GTP-binding site is located in α-tubuli and binds GTP irreversibly and does not hydrolyze it, whereas the second site, located on β-tubulin, binds GTP reversibly and hydrolyzes it to GDP.

In a microtubule, lateral and longitudinal interactions between the tubulin subunits are responsible for maintaining the tubular form. Longitudinal contacts between the ends of adjacent subunits link the subunits head to tail into a linear protofilament. Within each protofilament, the dimeric subunits repeat every 8 nm. Polarity of microtubule arises from the head-to-tail arrangement of the α- and β-tubulin dimers in a protofilament. Because all protofilaments in a microtubule have the same orientation, one end of a microtubule is ringed by α-tubulin, while the opposite end is ringed by β-tubulin. Microtubule-assembly experiments discussed later show that microtubules, like actin microfilaments, have a (+) and a (−) end, which differ in their rates of assembly.

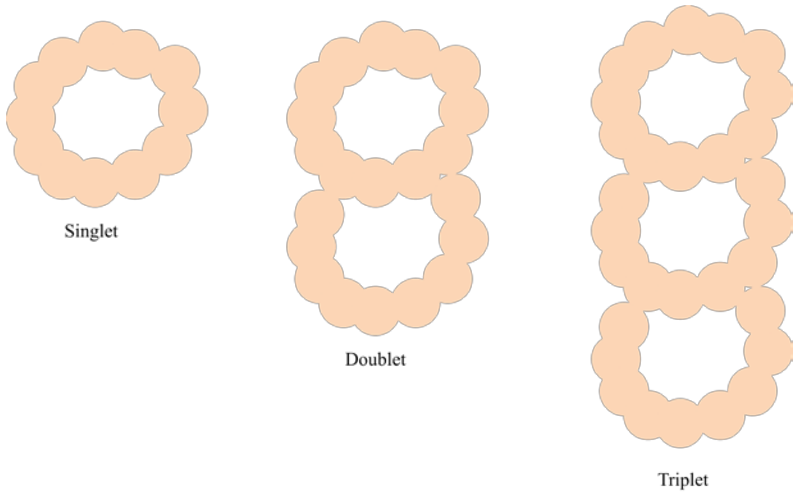

Figure 6: In cross section, a typical microtubule, a singlet, is a simple tube built from 13 protofilaments. In a doublet microtubule, an additional set of 10 protofilaments forms a second tubule (B) by fusing to the wall of a singlet (A) microtubule. Attachment of another 10 protofilaments to the B tubule of a doublet microtubule creates a C tubule and a triplet structure.

Every microtubule in a cell is a simple tube or a singlet microtubule, built from 13 protofilaments. In addition to the simple singlet structure, doublet or triplet microtubules are found in specialized structures such as cilia and flagella (doublet microtubules) and centrioles and basal bodies (triplet microtubules). Each of these contains one complete 13-protofilament microtubule (the A tubule) and one or two additional tubules (B and C) consisting of 10 protofilaments.

Functions

1. Mechanical function: The shape of the cell (red blood cells of non-mammalian vertebrates) and cells such as axons and dendrites of neurons, microvilli, etc., have been correlated to the orientation and distribution of microtubules.

2. Morphogenesis: During cell differentiation, the mechanical function of microtubules is used to

determine the shape of the developing cells. The enormous elongation in the nucleus of the spermatid during spermiogenesis is accompanied by the production of an orderly array of microtubules that are wrapped around the nucleus in a double helical arrangement. Similarly, the elongation of the cells during induction of the lens placode in the eye is also accompanied by the appearance of numerous microtubules.

3. Cellular polarity and motility: The determination of the intrinsic polarity of certain cells is governed by the microtubules. Directional gliding of cultured cells is depended on the microtubules.

4. Contraction: Microtubules play a role in the contraction of the spindle and movement of
chromosomes and centrioles as well as in ciliary and flagellar motion.

5. Circulation and transport: Microtubules are involved in the transport of macromol-ecules, granules and vesicles within the cell. The protozoan *Actinosphaerium* (Heliozoa) sends out long, thin pseudopodia within which cytoplasmic particles migrate back and forth. These pseudopodia contain as many as 500 microtubules disposed in a helical configuration.

6. The Microtubule Organizing Centre (MTOC) is the major organizing structure in a cell and helps determine the organization of microtubule-associated structures and organelles (e.g., mitochondria, the Golgi complex, and the endoplasmic reticulum). In a nonpolarized animal cell such as a fibroblast, an MTOC is perinuclear and strikingly at the center of the cell. Because microtubules assemble from the MTOC, microtubule polarity becomes fixed in a characteristic orientation. In most animal cells, for instance, the (−) ends of microtubules are closest to the MTOC or basal body (Figure 7). During mitosis, the centrosome duplicates and migrates to new positions flanking the nucleus. There the centrosome becomes the organizing center for microtubules forming the mitotic apparatus, which will separate the chromosomes into the daughter cells during mitosis.

7. The microtubules in the axon of a nerve cell are all oriented in the same direction and help stabilize the long process of nerve conduction (Figure 7).

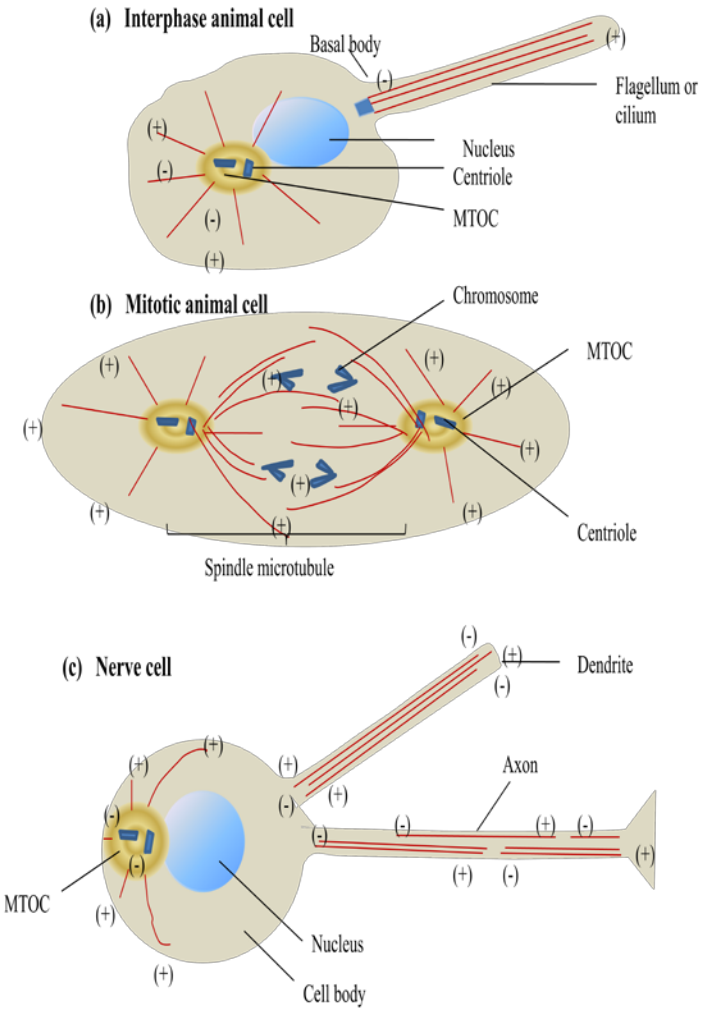

Figure 7: (a) In interphase animal cells, the (−) ends of most microtubules are proximal to the MTOC. Similarly, the microtubules in flagella and cilia have their (−) ends continuous with the basal body, which acts as the MTOC in these structures. (b) As cells enter mitosis, the microtubule network rearranges, forming a mitotic spindle. The (−) ends of all spindle microtubules point toward one of the two MTOCs, or poles, as they are called in mitotic cells. (c) In nerve cells, the (−) ends of axonal microtubules are oriented toward the base of the axon. However, dendritic microtubules have mixed polarities.

Cytoplasmic microtrabecular system (lattice)

Keith Porter proposed a fourth eukaryotic cytoskeletal element which is called the microtrabeculae based on images obtained from high-voltage electron microscopy of whole cells in the 1970s. The images showed short, filamentous structures of unknown molecular composition associated with known cytoplasmic structures. Porter proposed that this microtrabecular structure represented a novel filamentous network distinct from microtubules, filamentous actin, or intermediate filaments. It is now generally accepted that microtrabeculae are nothing more than an artifact of certain types of fixation treatment, although it is yet to fully understand the complexity of the cell's cytoskeleton.

These are 2-3nm in diameter and 300nm long forming link with all elements within the cell.

Prokaryotic cytoskeletal system

Like eukaryotes cytoskeletal elements are also characteristics of prokaryotes. Bacteria generally employ the tubulin ortholog FtsZ instead of tubulin of eukaryotes for cell division. Tubulin in eukaryotes form microtubules that provide cellular tracks for organelle transport and that form the mitotic spindle apparatus, among other functions. Some plasmids also encode a partitioning system that involves an actin-like protein ParM. Filaments of ParM exhibit dynamic instability, and may partition plasmid DNA into the dividing daughter cells by a mechanism analogous to that used by microtubules during eukaryotic mitosis. Two bacterial genes MreB and Mbl code for actin like proteins which form filamentous helical structures underneath the cell membrane, MreB filaments control the width of the cell, whereas Mbl filaments control the longitudinal axis of the cell. Recent research has showed that *Caulobacter crescentus* cells are vibrio-shaped, due to the action of CreS protein which is a homolog of eukaryotic proteins that form intermediate filaments.

Interesting Facts

1. Cytoskeleton is involved in cell division cycle of mitosis and meiosis which can be visualized by confocal fluorescence micrograph.

2. Cytoskeleton in orientation of cell division in contact guided cells like Single human skin fibroblasts and the skin keratinocyte.

3. Microtubule dynamics can also be altered by drugs. For example, the taxane drug used in the treatment of cancer, blocks dynamic instability by stabilizing GDP-bound tubulin in the microtubule. Nocodazole and Colchicine have the opposite effect, blocking the polymerization of tubulin into microtubules.

4. Neurodegenerative diseases like Alzheimer's disease, are associated with dysfunction of cytoskeletal components that influence vesicular biogenesis, vesicle/organelle trafficking and synaptic signaling.

Questions:

Q1. Do all cells possess cytoskeleton?

Q2. Where are microfilaments, microtubules and intermediate filaments located in a cell?

Q3. Differentiate among the structure and functions of microfilaments, microtubules and intermediate filaments.

Q4. What are cytoplasmic microtrabecular system?

Q5. Write about the different types of microtubules.

Q6. Describe the prokaryotic cytoskeletal system.

Q7. Name the different proteins that make up intermediate filaments.

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Module 1 Lecture 5

The present lecture discusses about structure and function of cytoplasm, nucleus and mitochondria

Structure and function of cytoplasm

Cytoplasm was discovered in 1835 and no single scientist can be credited for discovering cytoplasm the discovery was possible due to contribution of several scientists. It is worth mentioning that the discovery of different organelles in the cytoplasm was attributed to different scientist. The cytoplasm is the part of the cell outside the largest organelle, the nucleus. Cytoplasm appears as thick, gel-like semitransparent fluid that is found in both plant and animal cell. It is bounded by the plasma membrane, and contains many organelles in a eukaryotic cell (cell containing membrane bounded nucleus). The constituent parts of cytoplasm are cytosol, organelles and cytoplasmic inclusions. The cytosol, the aqueous part of the cytoplasm outside all of the organelles, also contains its own distinctive proteins.

Cytosol

Cytosol is the part of the cytoplasm that is not occupied by any organelle. It accounts for almost 70% of the total cell volume. Cytosol (cytoplasmic matrix) like many colloidal systems, shows the property of phase reversal. Under the natural conditions, the phase reversal of the cytosol (cytoplasmic matrix) depends on various physiological, mechanical and biochemical activities of the cell. It is a gelatinous substance consisting mainly of cytoskeleton filaments, organic molecules, salt and water. Chemically, the cytoplasmic matrix is composed of many chemical elements in the form of atoms, ions and molecules. Of the 92 naturally occurring elements, approximately 46 are found in the cytosol (cytoplasmic matrix). Twenty four of these are essential elements, while others are present in cytosol only because they exist in the environment with which the organism interacts. Of the 24 essential elements, six play especially important roles in living systems. These major elements are carbon (C, 20 per cent), hydrogen (H, 10 per cent), nitrogen (N, 3 per cent), oxygen (O, 62 per cent), phosphorus (P, 1.14 per cent) and sulphur (S,0.14 per cent). Most organic molecules are built with these six elements. Another five essential elements found in less abundance in living systems are calcium

(Ca, 2.5 per cent), potassium (K, 0.11 per cent), sodium (Na, 0.10 per cent), chlorine (Cl, 0.16 per cent) and magnesium (Mg, 0.07 per cent).Several other elements, called trace elements, are also found in minute amounts in animal and plant cell cytosol. These are iron (Fe, 0.10 per cent), iodine (I, 0.014 per cent), molybdenum (Mo), manganese (Mn), Cobalt (Co), zinc (Zn), selenium (Se), copper (Cu), chromium (Cr), tin (Sn), vanadium (V), silicon (Si), nickel (Ni), fluorine (F) and boron (B).

The cytoplasmic matrix consists of various kinds of ions. The ions are important in maintaining

osmotic pressure and acid-base balance in the cells. Retention of ions in the matrix produces an increase in osmotic pressure and, thus, the entrance of water in the cell. The concentration of various ions in the intracellular fluid (matrix) differs from that in the interstitial fluid. For example, in the cell K^+ and Mg^{++} can be high, and Na^+ and Cl^-high outside the cell. In muscle and nerve cells a high order of difference exists between intracellular K^+ and extracellular Na^+. Free calcium ions (Ca^{++}) may occur in cells or circulating blood. Silicon ions occur in the epithelium cells of grasses.

Chemical compounds present in cytosol are conventionally divided into two groups: organic and inorganic. Organic compounds form 30 per cent of a cell, rest are the inorganic substances such as water and other substances. The inorganic compounds are those compounds which normally found in the bulk of the physical, non-living universe, such as elements, metals, non-metals, and their compounds such as water, salts and variety of electrolytes and non-electrolytes. In the previous section, we have discussed a lot about the inorganic substances except the water which will be discussed in the following paragraph. The main organic compounds of the matrix are the carbohydrates, lipids, proteins, vitamins, hormones and nucleotides.

Properties of cytoplasmic matrix

The most of the physical properties of the matrix are due to its colloidal nature. The cytosol shows Tyndal effect (light scattering by particle in colloidal solution) and Brownian motion (random moving of particles). Due to the phase reversal property of the cytoplasmic matrix, the intracellular streaming or movement of the matrix takes place and is known as the cyclosis. The cyclosis usually occurs in the sol-phase of the matrix and is effected by the hydrostatic pressure, temperature, pH, viscosity, etc. Cyclosis has been observed in most animal and plant cells. The amoeboid movement depends directly on the cyclosis. The amoeboid movement occurs in the protozoans, leucocytes, epithelia, mesenchymal and other cells. Due to cyclosis matrix moves these pseudopodia and this causes forward motion of the cell. The cytoplasmic matrix being a liquid possesses the property of surface tension. The proteins and lipids of matrix have less surface tension, therefore, occur at the surface and form the membrane, while the chemical substances such as NaCl have high surface tension, therefore, occur in deeper part of the matrix. Besides surface tension and adsorption, the matrix possesses other mechanical properties, *e.g.*, elasticity, contractility, rigidity and viscosity which provide to the matrix many physiological utilities. The colloidal system due to its stable phase gives polarity of the cell matrix which cannot be altered by centrifugation of other mechanical means. The matrix has a definite pH value and it does not tolerate significant variations in its pH. Yet various metabolic activities produce small amount of excess acids or bases which is maintained by certain chemical compounds as carbonate-bicarbonate buffers. The matrix is a living substance and possesses various biological properties as irritability, conductivity, movement, metabolic activity, growth and reproduction.

Organelles

Cytoplasm contains all the organelles like nucleus, mitochondria, endoplasmic reticulum, lysosomes and Golgi apparatus. Besides, it also contains chloroplast in plant cells. Each organelle is bounded by a lipid membrane, and has specific functions.

Cytoplasmic inclusions

Some insoluble suspended substances found in cytosol. They are basically granules of starch and glycogen, and they can store energy. Besides, crystals of some minerals and lipid droplets can also be found in cytoplasm. Lipid droplets act as storage site of fatty acid and steroids.

Functions of Cytoplasm

Cytoplasm is the site of many vital biochemical reactions crucial for maintaining life.

1. It is the place where cell expansion and growth take place.
2. It provides a medium in which the organelles can remain suspended.
3. Besides, cytoskeleton found in cytoplasm gives the shape to the cell, and facilitates its movement.
4. It also assists the movement of different elements found within the cell. The enzymes found in the cytoplasm breaks down the macromolecules into small parts so that it can be easily used by the other organelles like mitochondria. For example, mitochondria cannot use glucose present in the cell, unless it is broken down by the enzymes into pyruvate. They act as catalysts in glycolysis, as well as in the synthesis of fatty acid, sugar and amino acid.
5. Cell reproduction, protein synthesis, anaerobic glycolysis, cytokinesis are some other vital functions that are carried out in cytoplasm. However, the smooth operation of all these functions depend on the existence of cytoplasm, as it provides the medium for carrying out these vital processes.

Cell Organelles

Nucleus

Nucleus means kernel and was the first organelle to be discovered. It was discovered and named by Robert Brown in 1833 in the plant cells and is recognized as a constant feature of all animal and plant cells. Certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. It is the largest cellular organelle in eukaryotes. Prokaryotic cells lack nucleus and is complemented by nucleoid. In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers (μm), occupying about 10% of the total cell volume. The contents of the nucleus are DNA genome, RNA synthetic apparatus, and a fibrous matrix. It is

surrounded by two membranes, each one a phospholipid bilayer containing many different types of proteins. The inner nuclear membrane defines the nucleus itself. In most cells, the outer nuclear membrane is continuous with the rough endoplasmic reticulum, and the space between the inner and outer nuclear membranes is continuous with the lumen of the rough endoplasmic reticulum. The two nuclear membranes appear to fuse at nuclear pores, the ringlike complexes composed of specific membrane proteins through which material moves between the nucleus and the cytosol. It contains cell's genetic material, organized as multiple long linear DNA molecules in complex with histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function is to maintain the integrity of the genes that controls the activities of the cell by regulating gene expression. The schematic presentation of nucleus is in Figure 1.

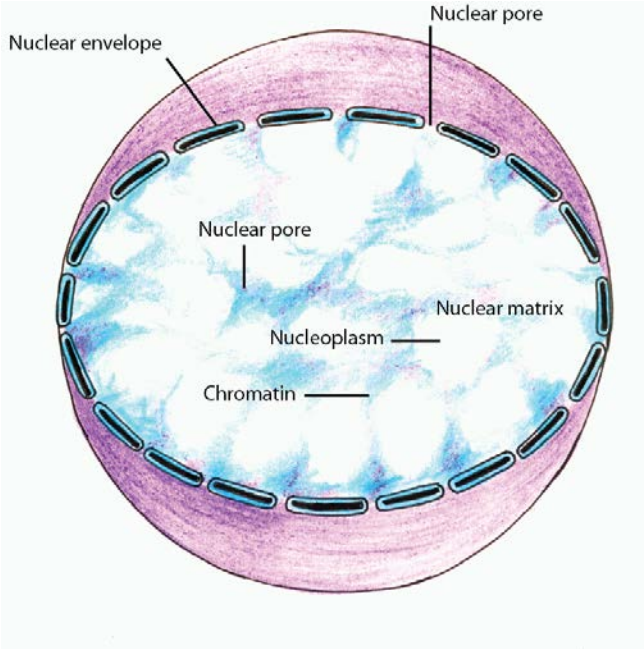

Figure 1: The schematic representation of nucleus.

In a growing or differentiating cell, the nucleus is metabolically active, replicating DNA and synthesizing rRNA, tRNA, and mRNA. Within the nucleus mRNA binds to specific proteins, forming ribonucleoprotein particles. Most of the cell's ribosomal RNA is synthesized in the nucleolus, a subcompartment of the nucleus that is not bounded by a phospholipid membrane. Some ribosomal proteins are added to ribosomal RNAs within the nucleolus as well. The finished or partly finished ribosomal subunits, as well as

tRNAs and mRNA-containing particles, pass through a nuclear pore into the cytosol for use in protein synthesis. In a nucleus that is not dividing, the chromosomes are dispersed and not dense enough to be observed in the light microscope. Only during cell division are individual chromosomes visible by light microscopy. In the electron microscope, the nonnucleolar regions of the nucleus, called the nucleoplasm, can be seen to have dark and light staining areas. The dark areas, which are often closely associated with the nuclear membrane, contain condensed concentrated DNA, called heterochromatin. Fibrous proteins called lamins form a two-dimensional network along the inner surface of the inner membrane, giving it shape and apparently binding DNA to it. The breakdown of this network occurs early in cell division.

Cell Nucleus: Ultrastructure

The structure of a cell nucleus consists of a nuclear membrane (nuclear envelope), nucleoplasm, nucleolus, and chromosomes. Nucleoplasm, also known as karyoplasm, is the matrix present inside the nucleus. Following section discusses in brief about the several parts of a cell nucleus.

a. Nuclear Membrane

It is a double-membrane structure each 5–10 nm thick . Numerous pores occur in the envelope, allowing RNA and other chemicals to pass, but not the DNA. Because the nuclear membrane is impermeable to most molecules, nuclear pores are required to allow movement of molecules across the envelope. These pores cross both of the membranes, providing a channel that allows free movement of small molecules and ions. The movement of larger molecules such as protein requires active transport regulated by carrier proteins. Figure 2 illustrates the nuclear membrane. The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei.

The spherical inner nuclearmembrane contains specific proteins that act as binding sites for the supporting fibrous sheath ofintermediate filaments (IF), called nuclear lamina. Nuclear lamina has contact with the chromatin (or chromosomes) and nuclear RNAs. The inner nuclear membrane is surrounded by the outer nuclear membrane, which closely resembles the membrane of the endoplasmic reticulum, that is continuous with it. Like the membrane of the rough ER, the outer surface of outer nuclear membrane is generally

studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into space between the inner and outer nuclear membrane, called perinuclear space. The perinuclear space is a 10 to 50 nm wide fluid-filled compartment which is continuous with the ER lumen and may contain fibres, crystalline deposits, lipid droplets or electrondense material. Nuclear pores and nucleocytoplasmic traffic. The nuclear envelope in all eukaryotic forms, from yeasts to

humans, is perforated by nuclear pores which have the following structure and function:

Structure of nuclear pores: Nuclear pores appear circular in surface view and have a diameter between 10nm to 100 nm. Previously it was believed that a diaphragm made of amorphous to fibrillar material extends across each pore limiting free transfer of material. Such a diaphragm called annulus has been observed in animal cells, but lack in plant cells. Recent electron microscopic studies have revealed that a nuclear pore has far more complex structure, so it is called nuclear pore complex with an estimated molecular weight of 50 to 100 million daltons. Negative staining techniques have demonstrated that pore complexes have an eight-fold or octagonal symmetry.

Nuclear Pore density: In nuclei of mammals it has been calculated that nuclear pores account for 5 to 15 per cent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the surface occupied by the nuclear pores may be as high as 20 to 36 per cent.

Arrangement of nuclear pores on nuclear envelope: In somatic cells, the nuclear pores are

evenly or randomly distributed over the surface of nuclear envelope. However, pore arrangement in other cell types is not random but rather range from rows (spores of *Eqisetum*) to Clusters (oocytes of *Xenopus laevis*) to hexagonal (Malpighian tubules of leaf hoppers) packing order.

Nucleo-cytoplasmic traffic: Quite evidently there is considerable trafficking across the nuclear envelope during interphase. Ions, nucleotides and structural, catalytic and regulatory proteins are imported from the cytosol (cytoplasmic matrix); mRNA, tRNA are exported to the cytosol (cytoplasmic matrix). However, one of the main functions of the nuclear envelope is to prevent the entrance of active ribosomes into the nucleus.

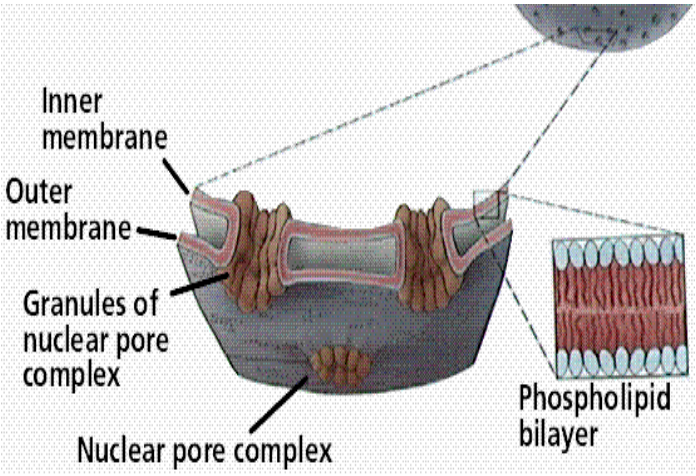

Figure 2: An illustration of the nuclear membrane

Nucleoplasm:

The space between the nuclear envelope and the nucleolus is filled by a transparent, semi-solid,

granular and slightly acidophilic ground substance or the matrix known as the nuclear sap or

nucleoplasm or karyolymph. The nuclear components such as the chromatin threads and the

nucleolus remain suspended in the nucleoplasm which is composed mainly of nucleoproteins

but it also contains other inorganic and organic substances, namely nucleic acids, proteins, enzymes and minerals. The most common nucleic acids of the nucleoplasm are the DNA and RNA. The nucleoplasm contains many types of complex proteins

categorized into: (i) Basic proteins. The proteins which take basic stain are known as the basic proteins. The most important basic proteins of the nucleus are nucleoprotamines and the nucleohistones. (ii) Non-histone or Acidic proteins. The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the

euchromatin (a type of chromatin) are the phosphoproteins. The nucleoplasm contains many enzymes which are

necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins. The most important nuclear enzymes are the DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine diaminase, nucleoside phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceraldehyde dehydrogenase and pyruvate kinase. The nucleoplasm also contains certain cofactors and coenzymes such as ATP and acetyl CoA. The nucleoplasm has small lipid content. The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium. The chromatin comparatively contains large amount of

these minerals than the nucleoplasm.

The nucleoplasm contains many thread-like, coiled and much elongated structures which take readily the basic stains such as the basic fuchsin. These thread-like structures are known as the chromatin (*chrome*=colour) substance or chromatin fibres. Chromosome will be discussed in detail in the next module.

Nucleolus:

Most cells contain in their nuclei one or more prominent spherical colloidal acidophilic bodies,

called nucleoli. However, cells of bacteria and yeast lack nucleolus. The nucleolus is mainly involved in the assembly of ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate mRNA. Some of the eukaryotic organisms have nucleus that contains up to four nucleoli. The nucleolus plays an indirect role in protein synthesis by producing ribosomes. Nucleolus disappears when a cell undergoes division and is reformed after the completion of cell-division. The size of the nucleolus is found to be related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities, sperm cells, blastomeres, muscle cell, etc., are found to contain smaller or no nucleoli, while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large-sized nucleoli. The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or

four. A nucleolus is often associated with the nucleolar organizer (NO) which represents the secondary constriction of the nucleolar organizing chromosomes, and are 10 in number in human beings. Nucleolar organizer consists of the genes for 18S, 5.8S and 28S rRNAs. The genes for fourth type of r RNA, *i.e.,* 5S rRNA occur outside the nucleolar organizer. Nucleolus is not bounded by any limiting membrane; calcium ions are supposed to maintain its intact organization. Nucleolus also contains some enzymes such as acid phosphatase, nucleoside phosphorylase and NAD^+ synthesizing enzymes for the synthesis of some coenzymes, nucleotides and ribosomal RNA. RNA methylase enzyme which transfers methyl groups to the nitrogen bases occurs in the nucleolus of some cells. Functionally nucleolus is the site where biogenesis of ribosomal subunits (40S and 60S) takes place. In it three types of rRNAs, namely 18S, 5.8S and 28S rRNAs, are transcribed as parts of a much longer precursor molecule (45S transcript) which undergoes processing (RNA splicing) by the help of two types of proteins such as nucleolin and U3 sn RNP (U3 is a 250 nucleotide containing RNA, sn RNP represents small nuclear ribonucleoprotein). The 5S r RNA is transcribed on the chromosome existing outside the nucleolus and the 70S types of ribosomal proteins are synthesized in the cytoplasm. All of these components of the ribosomes migrate to the nucleolus, where they are assembled into two types of ribosomal subunits which are transported back to the cytoplasm. The smaller (40S) ribosomal subunits are formed and migrate to the cytoplasm much earlier than larger (60S) ribosomal subunits; therefore, nucleolus contains many more incomplete 60S ribosomal subunits than the 40S ribosomal subunits. Such a time lag in the migration of 60S and 40S ribosomal subunits, prevents functional ribosomes from gaining access to the incompletely processed heterogeneous RNA (hn RNA; the precursor of m RNA) molecule inside the nucleus.

Functions of the nucleus

Speaking about the functions of a cell nucleus, it controls the hereditary characteristics of an organism. This organelle is also responsible for the protein synthesis, cell division, growth, and differentiation. Some important functions carried out by a cell nucleus are:

1. Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatins.
2. Storage of proteins and RNA (ribonucleic acid) in the nucleolus.
3. Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for the protein synthesis.
4. Exchange of hereditary molecules (DNA and RNA) between the nucleus and rest of the cell.
5. During the cell division, chromatins are arranged into chromosomes in the nucleus.
6. Production of ribosomes (protein factories) in the nucleolus.
7. Selective transportation of regulatory factors and energy molecules through nuclear pores.

As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell. Overall, the cell nucleus stores all the chromosomal DNA of an organism.

Mitochondria

Structure and Function

The mitochondria were first observed by Kolliker in 1850 as granular structures in the striated muscles. Mitochondria are called the 'powerhouse of the cell'. They are intracellular organelles found in almost all eukaryotic cells having bilayered membranes. Most eukaryotic cells contain many mitochondria, which occupy up to 25 percent of the volume of the cytoplasm. These crucial organelles, the main sites of ATP production during aerobic metabolism, are generally exceeded in size only by the nucleus, vacuoles, and chloroplasts. They are responsible for aerobic metabolism through oxidative phosphorylation, which leads to energy production in the form of adenosine triphosphate (ATP). Mitochondria contain a number of enzymes and proteins that help in processing carbohydrates and fats obtained from food we eat to release energy. Each human cell contains on average hundreds to thousands of mitochondria. The exception is mature red blood cells, which rely exclusively on anaerobic metabolism and contain no mitochondria. Figure 3 gives the schematic representation of a typical mitochondria.

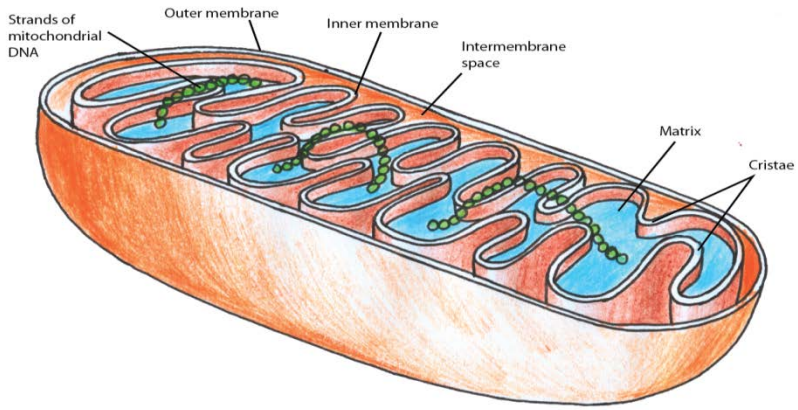

Figure 3: Schematic representation of mitochondria

Localisation:

Mitochondria are present in all eukaryotic cells. They move autonomously in the cytoplasm, so they generally have uniform distribution in the cytoplasm, but in many cells their distribution is restricted. The distribution and number of mitochondria can be correlated with type of function the cell performs. Typically mitochondria with many cristae are associated with mechanical and osmotic work situations, where there are sustained demands for ATP *e.g*., between muscle fibres, in the basal infolding of kidney tubule cells, and in a portion of inner segment of rod and cone cells of retina. Myocardial muscle cells have numerous large mitochondria called sarcosomes that reflect the great amount of work done by these cells. Often mitochondria occur in greater concentrations at work sites, for example, in the oocyte of *Thyone briaeus*, rows of mitochondria are closely associated with RER membranes, where ATP is required for protein biosynthesis. Mitochondria are particularly numerous in regions where ATP-driven osmotic work occurs, *e.g.,* brush border of kidney proximal tubules, the infolding of the plasma membrane of dogfish salt glands and Malpighian tubules of insects, the contractile vacuoles of some protozoans as Paramecium. Non-myelinated axons contain many mitochondria that are poor ATP factories, since each has only single cristae. In this case, there is a great requirement for monoamine oxidase, an enzyme present in outer mitochondrial membrane that oxidatively deaminates monoamines including neurotransmitters (acetylcholine).

Orientation:

The mitochondria have definite orientation. For example, in cylindrical cells the mitochondria

usually remain orientated in basal apical direction and lie parallel to the main axis. In leucocytes, the mitochondria remain arranged radially with respect to the centrioles. As they move about in the mitochondria form long moving filaments or chains, while in others they remain fixed in one position where they provide ATP directly to a site of high ATP utilization, *e.g.,* they are packed between adjacent myofibrils in a cardiac muscle cell or wrapped tightly around the flagellum of sperm.

Morphology:

Number: The number of mitochondria in a cell depends on the type and functional state of the

cell. It varies from cell to cell and from species to species. Certain cells contain exceptionally large number of the mitochondria, for example the *Amoeba*, *Chaos chaos* contain 50,000; eggs of sea urchin contain 140,000 to 150,000 and oocytes of amphibians contain 300,000 mitochondria. Liver cells of rat contain only 500 to 1600 mitochondria. The cells of green plants contain less number of mitochondria in comparison to animal cells. Some algal cells may contain only one mitochondrion.

Shape: The mitochondria may be filamentous or granular in shape and may change from one form to another depending upon the physiological conditions of the cells. Thus, they may be of club, racket, vesicular, ring or round-shape. Mitochondria are granular in primary spermatocyte or rat, or club-shaped in liver cells. Time-lapse picturisation of living cells shows that mitochondria are remarkably mobile and plastic organelles, constantly changing their shape. They sometimes fuse with one another and then separate again. For example, in certain euglenoid cells, the mitochondria fuse into a reticulate structure during the day and dissociate during darkness. Similar changes have been reported in yeast species, apparently in response to culture conditions.

Size: Normally mitochondria vary in size from 0.5 μm to 2.0 μm and, therefore, are not distinctly visible under the light microscope. Sometimes their length may reach up to 7 μm.

Structure: Each mitochondrion is bound by two highly specialized membranes that play a crucial role in its activities. Each of the mitochondrial membrane is 6 nm in thickness and fluidmosaic in ultrastructure. The membranes are made up of phospholipids and proteins. The space in between the two membranes is called the inter-membrane space which has the same composition as the cytoplasm of the cell. Inner and the outer membrane is separated by a 6–8 nm wide space.

Outer Membrane

The two membranes that bound a mitochondrion differ in composition and function. The outer membrane, composed of about half lipid and half protein, contains porins that render the membrane permeable to molecules having molecular weights as high as 10,000 dalton. In this respect, the outer membrane of mitochondria is similar to the outer membrane of gram-negative bacteria. The outer membrane is smooth unlike the inner membrane and has almost the same amount of phospholipids as proteins. It has a large number of special proteins called porins that allow molecules of 5000 daltons or less in weight to pass through it. It is completely permeable to nutrient molecules, ions, ATP and ADP molecules.

Inner Membrane

The inner membrane is much less permeable, than the outer membrane. It has about 20 percent lipid and 80 percent protein. The surface area of the inner membrane is greatly increased by a large number of infoldings, or finger like projections called cristae, that protrude into the matrix, or central space, increasing the surface area for the complexes. It contains the complexes of the electron transport chain and the ATP synthetase complex, they also serve to separate the matrix from the space that will contain the hydrogen ions, allowing the gradient needed to drive the pump. It is permeable only to oxygen, carbon dioxide and water and is made up of a large number of proteins that play an important role in producing ATP, and also helps in regulating transfer of metabolites across the membrane. In general, the cristae of plant mitochondria are tubular, while those of animal mitochondria are lamellar or plate-like. Some mitochondria, particularly those from heart, kidney and skeletal muscles have more extensive cristae arrangements than liver mitochondria. In comparison to these, other mitochondria (from fibroblasts, nerve axons and most plant tissues) have relatively few cristae.

Attached to matrix face of inner mitochondrial membrane are repeated units of stalked particles, called elementary particles, inner membrane subunits or oxysomes. They are also identified as F1 particles or F0-F1 particles and are meant for ATP synthesis (phosphorylation)

and also for ATP oxidation (acting as ATP synthetase and ATPase). F0-F1 particles are regularly spaced at intervals of 10 nm on the inner surface of inner mitochondrial membrane. According to some estimates, there are 104 to 105 elementary particles per mitochondrion. When the mitochondrial cristae are disrupted by sonic vibrations or by detergent action, they produce submitochondrial vesicles of inverted orientation. In these vesicles, F0-F1 particles are seen attached on their outer surface. These submitochondrial vesicles are able to per- form respiratory chain phosphorylation. However, in the absence of F0-F1 particles, these vesicles lose their capacity of phosphorylation as shown by resolution (removal by urea or trypsin treatment) and reconstitution of these particles.

Matrix

The matrix is a complex mixture of enzymes that are important for the synthesis of ATP molecules, special mitochondrial ribosomes, tRNAs and the mitochondrial DNA. Besides these, it has oxygen, carbon dioxide and other recyclable intermediates.

Chemical composition

Mitochondria are found to contain 65 to 70 per cent proteins, 25 to 30 per cent lipids, 0.5 per cent RNA and small amount of the DNA. The lipid contents of the mitochondria is around 90 per cent phospholipids (lecithin and cephalin), 5 per cent or less cholesterol and 5 per cent free fatty acids and triglycerides. The inner membrane is rich in one type of phospholipid, called cardiolipin which makes this membrane impermeable to a variety of ions and small molecules (Na^+, K^+, Cl^-, NAD^+,AMP, GTP, CoA and so on). The outer mitochondrial membrane has typical ratio of 50 per cent proteins and 50 per cent phospholipids of ‘unit membrane’. However, it contains more unsaturated fatty acids and less cholesterol. It has been estimated that in the mitochondria of liver 67 per cent of the total mitochondrial protein is located in the matrix, 21 per cent is located in the inner membrane, 6 per cent is situated in the outer membrane and 6 per cent is found in the outer chamber. Each of these four mitochondrial regions contains a special set of proteins that mediate distinct functions. Besides Porin, enzymes of outer membrane consists of, other proteins involved in mitochondrial lipid synthesis and those enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix. Certain

important enzymes of this membrane are monoamine oxidase, rotenone-insensitive NADH-cytochrome-C-reductase, kynurenine hydroxyalase, and fatty acid CoA ligase. Enzymes of intermembrane space contains several enzymes that use the ATP molecules passing out of the matrix to phosphorylate other nucleotides. The main enzymes of this part are adenylate kinase and nucleoside diphosphokinase. Enzymes of inner membrane contains proteins with three types of functions: 1. Those that carry out the oxidation reactions of the respiratory chain; 2. an enzyme complex, called ATP synthetase that makes ATP in matrix ; and 3. specific transport proteins The significant enzymes of inner membrane are enzymes of electron transport pathways, namely nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), diphosphopyridine nucleotide (DPN) dehydrogenase, four cytochromes (Cyt. b, Cyt. c, Cyt.c1, Cyt. a and Cyt. a3), ubiquinone or coenzyme Q10, non-heme copper and iron, ATP synthetase, succinate dehydrogenase; β-hydroxybutyrate dehydrogenase; carnitive fatty acid acyl transferase. Enzymes of mitochondrial matrix contains various enzymes, including those required for the oxidation of pyruvate and fatty acids and for the citric acid cycle. The matrix also contains several identical copies of the mitochondrial DNA, special 55S mitochondrial ribosomes, tRNAs and various enzymes required for the expression of mitochondrial genes. Thus, the mitochondrial matrix contains malate dehydrogenase, isocitrate dehydrogenase, fumarase, aconitase, citrate synthetase, ⟨-keto acid dehydrogenase, β-oxidation enzymes.

Viewing mitochondria

Mitochondria can be isolated by cell fractionation brought about by differential centrifugation. Homogeneous fractions of mitochondria can be obtained from liver, skeletal muscle, heart, and some other tissues. They can be observed easily in cells cultured *in vitro*, particularly under darkfield illumination and phase contrast microscope. Janus green stains living mitochondria greenish blue due to its action with cytochrome oxidase system present in the mitochondria. This system maintains the vital dye in its oxidized state. In the surrounding cytoplasm the stain is reduced to a colourless base. Fluorescent dyes (rhodamine 123), which are more sensitive, have been used in isolated mitochondria and intact cultured cells. Such stains are more suitable for *in situ* metabolic

studies of mitochondria. Different parts of mitochondria have distinct marker enzymes for histochemical markings, such as cytochrome oxidase for inner membrane, monoamine oxidase for outer membrane, malate dehydrogenase for matrix and adenylate kinase for outer chamber.

Function of mitochondria

1. The most important function of the mitochondria is to produce energy. The food that we eat is broken into simpler molecules like carbohydrates, fats, etc., in our bodies. These are sent to the mitochondrion where they are further processed to produce charged molecules that combine with oxygen and produce ATP molecules. This entire process is known as oxidative phosphorylation.
2. It is important to maintain proper concentration of calcium ions within the various compartments of the cell. Mitochondria help the cells to achieve this goal by serving as storage tanks of calcium ions.
3. Mitochondria help in the building of certain parts of the blood, and hormones like testosterone and estrogen.
4. Mitochondria in the liver cells have enzymes that detoxify ammonia.

Although most of the genetic material of a cell is contained within the nucleus, the mitochondria have their own DNA. They have their own machinery for protein synthesis and reproduce by the process of fission like bacteria do. Due to their independence from the nuclear DNA and similarities with bacteria, it is believed that mitochondria have originated from bacteria by endosymbiosis.

Interesting Facts

- The endosymbiotic relationship of mitochondria with their host cells was popularized by Lynn Margulis.
- Mitochondria and chloroplast follow maternal inheritance.
    - Some of the diseases caused by defective mitochondria are: Diabetes mellitus and deafness (DAD), Leber's hereditary optic neuropathy and Leigh syndrome.
- A few groups of unicellular eukaryotes lack mitochondria: the microsporidians, metamonads, and archamoebae.

Questions

Q1. What controls most of the cell processes and contains the hereditary information of DNA.

A. Mitochondria

B. Chloroplast

C. Nucleus

D. Nucleolus

Q.2 What regulates what enters and leaves the cell and provides protection and support?

A. Nucleus

B. Ribosomes

C. Cell Wall

D. Cell Membrane

Q3. The best choice for a microscope would be to see chromosomes during cell division.

A. light microscope, because of its resolving power.

B. transmission electron microscope, because of its magnifying power.

C. scanning electron microscope, because the specimen is alive.

D. transmission electron microscope, because of its great resolving power.

E. light microscope, because the specimen is alive.

Q4. Illustrate the structure and function of nucleus.

Q5. What is nucleolus and what is its role in a cell.

Q6. Describe cytoplasmic inclusions.

Q7. Write about the properties of cytosol.

Q8. What is the nucleus made of?

Q9. How would mutational inactivation of the nuclear export signal of a protein that normally shuttles back and forth between the nucleus and cytoplasm affect its subcellular distribution?

Lecture 6

In previous lecture we had discussion about few cell organelles like mitochondria, nucleous etc. During current lecture, we will have discussion about few other cell organelles. The present lecture discusses about ribosome, endoplasmic reticulum, golgi bodies and lysosomes.

Ribosomes

Ribosomes are the protein synthesis units of a cell described by G.E. Palade in 1952. They are complex of ribosomal RNA and various proteins. Their presence in both free and endoplasmis reticulum membrane attached form (rough endoplasmic reticulum) was confirmed by Palade and Siekevitz by the electron microscopy. We will have discussion about endoplasmic reticulum in this lecture after discussion about ribosome. Ribosomes are small, dense, rounded and granular particles of the ribonucleoprotein. As mentioned, they occur either freely in the matrix of mitochondria, chloroplast and cytoplasm or remain attached with the membranes of the endoplasmic reticulum. They occur in most prokaryotic and eukaryotic cells and provide a scaffold for the ordered interaction of all the molecules involved in protein synthesis. They are the most abundant RNA-protein complex in the cell, which directs elongation of a polypeptide at a rate of three to five amino acids added per second. Small proteins of 100–200 amino acids are therefore made in a minute or less. On the other hand, it takes 2–3 hours to make the largest known protein, titin, which is found in muscle and contains about 30,000 amino acid residues.

Occurrence and distribution:

The ribosomes occur in both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often occur freely in the cytoplasm or sometimes as polyribosome. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum. The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissues, embryonic nerve cells and cancerous cells contain large number of ribosomes which often occur freely in the cytoplasmic matrix. Cells like the erythroblasts, developing muscle cells, skin and hair which synthesize specific proteins for the intracellular utilization and storage contain also contain large number of free ribosomes. In cells with active protein synthesis, the ribosomes remain attached with the membranes of the endoplasmic reticulum. Examples

are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells, or the submaxillary gland, thyroid cells and mammary gland cells.

Types of ribosomes:

Ribosomes are classified into two types based on their sedimentation coefficient, 70S and 80S. S stands for Svedberg unit and related to sedimentation rate (sedimentation depends on mass and size). Thus, the value before S indicates size of ribosome.

70S Ribosomes: Prokaryotes have 70S ribosemes. The 70S ribosomes are comparatively smaller in size and have sedimentation coefficient 70S with molecular weight 2.7×10^6 daltons. Electron microscopy measures the dimension of the 70S ribosomes as$170 \times 170 \times 200$ A^o. They occur in the prokaryotic cells of the blue green algae and bacteria and also in mitochondria and chloroplasts of eukaryotic cells.

80S Ribosomes: Eukaryotes have 80S ribosomes. The 80S ribosomes have sedimentation coefficient of 80S and molecular weight 40×10^6 daltons. The 80S ribosomes occur in eukaryotic cells of the plants and animals. The ribosomes of mitochondria and chloroplasts are always smaller than 80S cytoplasmic ribosomes and are comparable to prokaryotic ribosomes in both size and sensitivity to antibiotics. However their sedimentation values vary in different phyla, 77S in mitochondria of fungi, 60S in mitochondria of mammals and 60S in mitochondria of animals.

Number of ribosomes:

An *E. coli* cell contains 10,000 ribosomes, forming 25 per cent of the total mass of the bacterial

cell. Whereas, mammalian cultured cells contain 10 million ribosomes per cell.

Chemical composition:

The ribosomes are chemically composed of RNA and proteins as their major constituents; both occurring approximately in equal proportions in smaller as well as larger subunit. The 70S ribosomes contain more RNA (60 to 40%) than the proteins (36 to 37%). The ribosomes of *E. coli* contain 63% rRNA and 37% protein. While the 80S ribosomes contain less RNA (40 to 44%) than the proteins (60 to 56%), yeast ribosomes have 40 to 44% RNA and 60 to 56% proteins; ribosomes of pea seedling contain 40% RNA and 60% proteins. There is no lipid content in ribosomes.

Ribosomal RNAs:

RNA constitutes about 60 percent of the mass of a ribosome. The 70S ribosomes contain three types of rRNA, *viz*., 23S rRNA, 16S rRNA, 5S rRNA. The 23S and 5S rRNA occur in the larger 50S ribosomal subunit, while the 16S rRNA occurs in the smaller 30S ribosomal subunit. Assuming an average molecular weight for one nucleotide to be 330 daltons, one can calculate the total number of each type of rRNA. Thus, the 23S rRNA consists of 3300 nucleotides, 16S rRNA contains 1650 nucleotides and 5S rRNA includes 120 nucleotides in it. The 80S ribosomes contain four types of rRNA, 28S rRNA (or 25-26 rRNA in plants, fungi and protozoa), 18S rRNA, 5S rRNA and 5.8S rRNA. The 28S, 5S and 5.8S rRNAs occur in the larger 60S ribosomal subnit, while the 18S rRNA occurs in the smaller 40S ribosomal subunit. About 60 per cent of the rRNA is helical (*i.e*., double stranded) and contains paired bases. These double stranded regions are due to hairpin loops between complimentary regions of the linear molecule.

The 28S rRNA has the molecular weight 1.6×10^6 daltons and its molecule is double stranded

and having nitrogen bases in pairs. The 18S rRNA has the molecular weight 0.6x106 daltons and

consists of 2100 nucleotides. The 18S and 28S ribosomal RNA contain a characteristic number of methyl groups, mostly as 2'-O-methyl ribose. The molecule of 5S rRNA has a clover leaf shape and a length equal to 120 nucleotides. The 5.8S rRNA is intimately associated with the 28S rRNA molecule and has, therefore, been referred to as 28S-associated ribosomal RNA (28S-A rRNA). The 55S ribosomes of mammalian mitochondria lack 5S rRNA but contain 21S and 12S rRNAs. The 21S rRNA occurs in larger or 35S ribosomal subunits, while 12S rRNA occur in smaller or 25S ribosomal subunit. It is thought that each ribosomal subunit contains a highly folded ribonucleic acid filament to which the various proteins adhere. But as the ribosomes easily bind the basic dyes so it is concluded that RNA is exposed at the surface of the ribosomal subunits, and the protein is assumed to be in the interior in relation to non-helical part of the RNA.

Ribosomal Proteins:

A ribosome is composed of three (in bacteria) or four (in eukaryotes) different rRNA molecules and as many as 83 proteins, organized into a large subunit and a small subunit. The primary structure of several of these proteins has been elucidated. Most of the recent knowledge about the structure of ribosomal proteins has been achieved by dissociation of ribosomal subunits into their component rRNA and protein molecules. When both 50S and 30S ribosomal subunits are dissociated by centrifuging both of them in a gradient of 5 M cesium chloride, then there are two inactive core particles (40S and 23S, respectively) which contain the RNA and some proteins called core proteins (CP) at the same time several other proteins—the so-called split proteins (SP) are released from each particle (Fig. 14.3). There are SP50 and SP30 proteins which may reconstitute the functional ribosomal subunit when added to their corresponding core. Some of the split proteins are apparently specific for each ribosomal subunit. The split proteins have been further fractionated and divided into acidic (A) and basic (B) proteins. According to Nomura (1968, 1973) and Garett and Wittmann (1973) each 70S ribosome of *E. coli* is composed of about 55 ribosomal proteins. Out of these 55 proteins, about 21 different molecules have been isolated from the 30S ribosomal subunit, and some 32 to 34 proteins from the 50S ribosomal subunit. Similar organization of ribosomal proteins and RNA is found in 80S Ribosomes. Different rRNA molecules evidently play a central role in the catalytic activities of ribosomes in the process of protein synthesis.

Metallic Ions:

The most important low molecular weight components of ribosomes are the divalent metallic ions such as Mg++, Ca++ and Mn++.

Structure

The ribosomes are oblate spheroid structures of 150 to 250A^{o} in diameter. Each ribosome is porous, hydrated and composed of two subunits. One ribosomal subunit is large in size and has a domelike shape, while the other ribosomal subunit is smaller in size, occurring above the larger subunit and forming a cap-like structure. The small ribosomal subunit contains a single rRNA molecule, referred to as small *rRNA.* The large subunit contains a molecule of large *rRNA* and one molecule of 5S rRNA, plus an additional molecule of 5.8S rRNA in vertebrates. The lengths of the rRNA molecules, the quantity of proteins in

each subunit, and consequently the sizes of the subunits differ in bacterial and eukaryotic cells. The assembled ribosome is 70S in bacteria and 80S in vertebrates. There are great structural and functional similarities between ribosomes from all species which is another reflection of the common evolutionary origin of the most basic constituents of living cells.

The 70S ribosome consists of two subunits, 50S and 30S. The 50S ribosomal subunit is larger in size and has the size of 160 A^o to 180 A^o. The 30S ribosomal subunit is smaller in size and occurs above the 50S subunit like a cap. The 80S ribosome also consists of two subunits, 60S and 40S. The 60S ribosomal subunit is dome-shaped and larger in size. In the ribosomes which remain attached with the membranes of endoplasmic reticulum and nucleus, the 60S subunit remains attached with the membranes. The 40S ribosomal subunit is smaller in size and occurs above the 60s subunit forming a cap-like structure. Both the subunits remain separated by a narrow cleft. The two ribosomal subunits remain united with each other due to high concentration of the Mg++ (.001M) ions. When the concentration of Mg++ions reduces in the matrix, both ribosomal subunits get separated. Actually in bacterial cells the two subunits are found to occur freely in the cytoplasm and they unite only during the process of protein synthesis. At high concentration of Mg++ ions in the

matrix, the two ribosomes (monosomes) become associated with each other and known as the

dimer. Further, during protein synthesis many ribosomes are aggregated due to common messenger RNA and form the polyribosomes or polysomes.

The actual three-dimensional structures of bacterial rRNAs from *Thermus thermopolis* recently have been determined by x-ray crystallography of the 70S ribosome. The multiple, much smaller ribosomal proteins for the most part are associated with the surface of the rRNAs. During translation, a ribosome moves along an mRNA chain, interacting with various protein factors and tRNAs and very likely undergoing large conformational changes **(see Figure 2)**. Despite the complexity of the ribosome, great progress has been made in determining the overall structure of bacterial ribosomes and in identifying various reactive sites. X-ray crystallographic studies on the *T. thermophilus* 70S ribosome, for instance, not only have revealed the dimensions and overall shape of

the ribosomal subunits but also have localized the positions of tRNAs bound to the ribosome during elongation of a growing protein chain. In addition, powerful chemical techniques such as footprinting, have been used to identify specific nucleotide sequences in rRNAs that bind to protein or another RNA. Figure 1 illustrates the ribosomes.

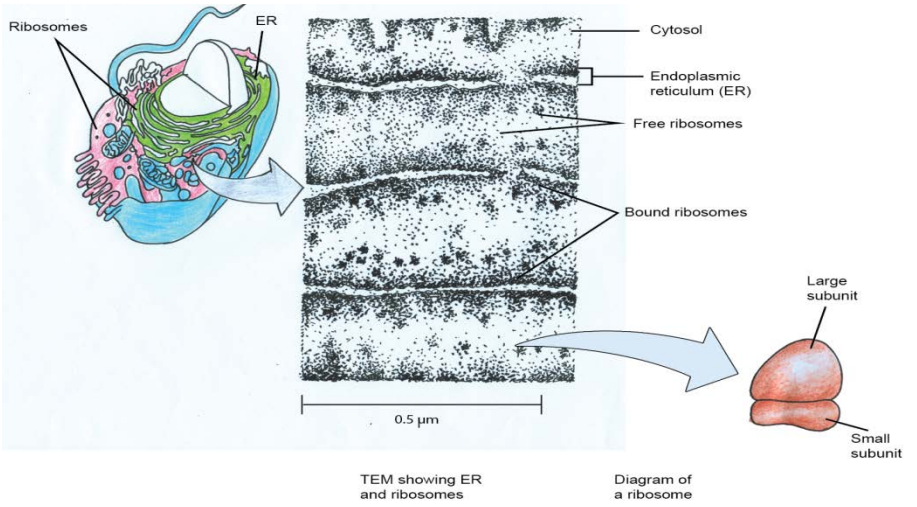

Figure 1: Schematic representation of the ribosome.

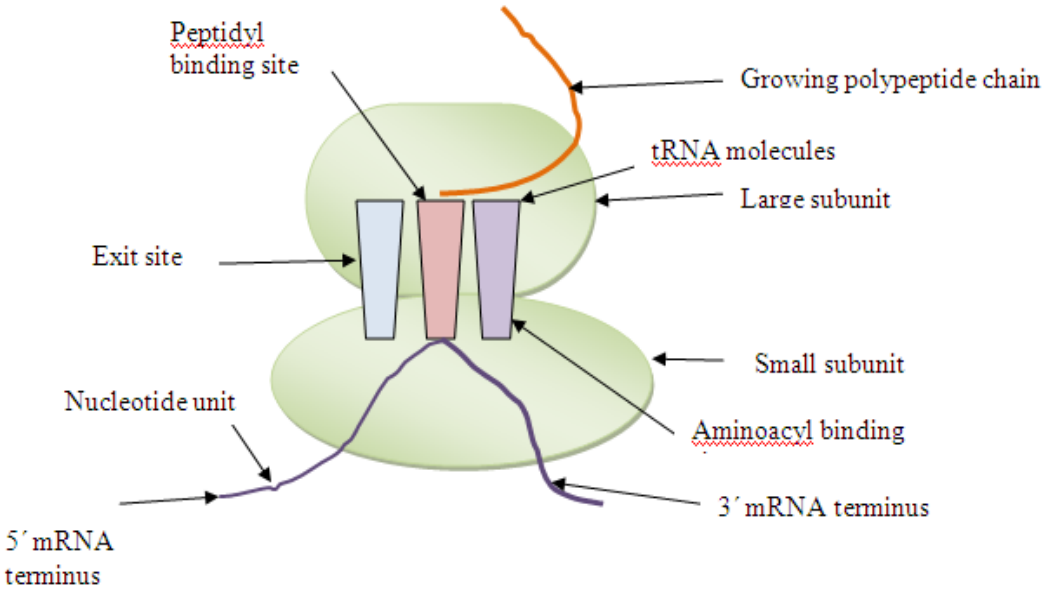

Figure 2: The detailed structure of a ribosome involved in protein synthesis. The figure is not upto the scale of ribosome.

Endoplasmic reticulum:

Endoplasmic reticulum is a network of interconnected internal membranes generally, the largest membrane in a eukaryotic cell—an extensive network of closed, flattened membrane-bounded sacs called cisternae (Figure 3). The name "endoplasmic reticulum" was coined in 1953 by Porter, who had observed it in electron micrographs of liver cells. The endoplasmic reticulum has a number of functions in the cell but is particularly important in the synthesis of lipids, membrane proteins, and secreted proteins.

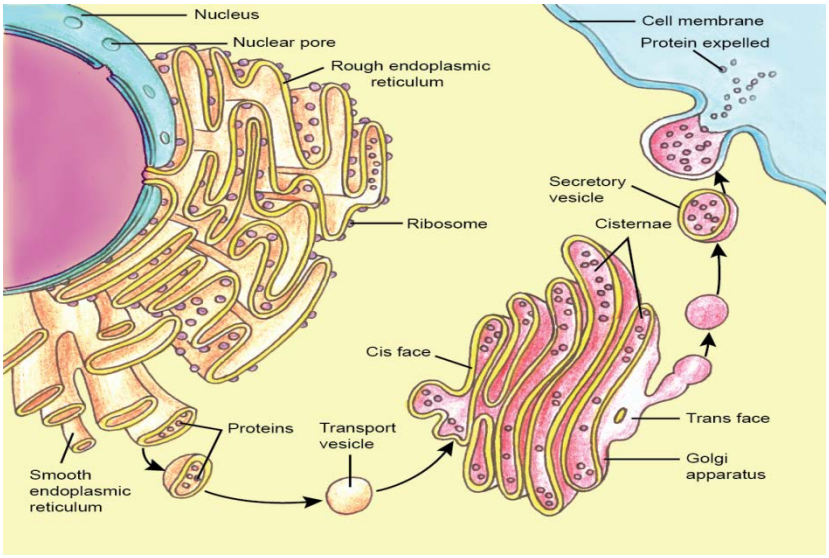

Figure 3. The Endoplasmic reticulum.

Occurrence:

The occurrence of the endoplasmic reticulum is in eukaryotic cells with variation in its position from cell to cell. The erythrocytes (RBC), egg and embryonic cells lack in endoplasmic reticulum. ER is poorly developed in certain cells as the RBC which produces only proteins to be retained in the cytoplasmic matrix (haemoglobin), although the cell may contain many ribosomes). The spermatocytes also have poorly developed endoplasmic reticulum.

Morphology:

The endoplasmic reticulum occurs in three forms: 1. Lamellar form or cisternae which is a closed, fluid-filled sac, vesicle or cavity is called cisternae; 2. vesicular form or vesicle and 3. tubular form or tubules.

1. Cisternae: The cisternae are long, flattened, sac-like, unbranched tubules having diameter of 40 to 50 μm. They remain arranged parallely in bundles or stakes. RER mostly exists as cisternae which occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.

2. Vesicles: The vesicles are oval, membrane-bound vacuolar structures having diameter of 25 to 500 μm. They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER.

3. Tubules: The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 μm and occur almost in all the cells. Tubular form of ER is often found in SER and is dynamic in nature, *i.e.*, it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

Ultrastructure:

The cavities of cisternae, vesicles and tubules of the endoplasmic reticulum are bounded by a

thin membrane of 50 to 60 Aº thickness. The membrane of endoplasmic reticulum is fluid-mosaic like the unit membrane of the plasma membrane, nucleus, Golgi apparatus. The membrane of endoplasmic reticulum remains continuous with the membranes of plasma membrane, nuclear membrane and Golgi apparatus. The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products. Palade in the year 1956 has observed secretory granules in the cavity of endoplasmic reticulum amking it rough in appearance. Sometimes, the cavity of RER is very narrow with two membranes closely apposed and is much distended in certain cells which are actively engaged in protein synthesis (acinar cells, plasma cells and goblet cells). The membranes of the endoplasmic reticulum contains many kinds of enzymes which are needed for various important synthetic activities. Some of the most common enzymes are found to have different transverse distribution in the ER membranes. The most important

enzymes are the stearases, NADH-cytochrome C reductase, NADH diaphorase, glucose-6-phosphotase and Mg++ activated ATPase. Certain enzymes of the endoplasmic reticulum such as nucleotide diphosphate are involved in the biosynthesis of phospholipid, ascorbic acid, glucuronide, steroids and hexose metabolism.

Types of endoplasmic reticulum:

Agranular or smooth endoplasmic reticulum:

ER with no studded ribosomes makes it smooth in appearance. The adipose tissues, brown fat cells and adrenocortical cells, interstitial cells of testes and cells of corpus luteum of ovaries, sebaceous cells and retinal pigment cells contain only smooth endoplasmic reticulum (SER). The synthesis of fatty acids and phospholipids takes place in the smooth ER. It is abundant in hepatocytes. Enzymes in the smooth ER of the liver modify or detoxify hydrophobic chemicals such as pesticides and carcinogens by chemically converting them into more water-soluble, conjugated products that can be excreted from the body. High doses of such compounds result in a large proliferation of the smooth ER in liver cells.

Granular or rough endoplasmic reticulum:

Ribosomes bound to the endoplasmic reticulum make it appear rough. The rough ER synthesizes certain membrane and organelle proteins and virtually all proteins to be secreted from the cell. A ribosome that fabricates such a protein is bound to the rough ER by the nascent polypeptide chain of the protein. As the growing polypeptide emerges from the ribosome, it passes through the rough ER membrane, with the help of specific proteins in the membrane. Newly made membrane proteins remain associated with the rough ER membrane, and proteins to be secreted accumulate in the lumen of the organelle. All eukaryotic cells contain a discernible amount of rough ER because it is needed for the synthesis of plasma membrane proteins and proteins of the extracellular matrix. Rough ER is particularly abundant in specialized cells that produce an abundance of specific proteins to be secreted. The cells of those organs which are actively engaged in the synthesis of proteins such as acinar cells of pancreas, plasma cells, goblet cells and cells of some endocrine glands are found to contain rough endoplasmic reticulum (RER) which is highly developed.

Rough endoplasmic reticulum and protein secretion:

George Palade and his colleagues in the 1960s were the first to demonstrate the role of endoplasmic reticulum in protein secretion. The defined pathway taken by secreted protein is: Rough ER - Golgi - secretory vesicles- cell exterior. The entrance of proteins into the ER represents a major branch point for the traffic of proteins within eukaryotic cells. In mammalian cells most proteins are transferred into the ER while they are being translated on membrane bound ribosomes. Proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a signal sequence (short stretch of hydrophobic amino acid residues) at the amino terminus of the growing polypeptide chain. The signal sequence is K/HDEL which is Lys/His-Asp-Glu-Leu. This signal peptide is recognized by a signal recognition particle consisting of six polypeptides and srpRNA. The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane. Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel or translocon with the aid of GTP. Transfer of the ribosome mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the ER membrane as translation proceeds. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

Smooth endoplasmic reticulum and lipid synthesis:

Hydrophobic lipids are synthesized in the ER and then they are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins. Phospholipids are synthesized in the cytosolic side of the ER membrane from water-soluble cytosolic precursors. Other lipids that are synthesized in the ER are cholesterol and ceramide which is further converted to either glycolipids or sphingomyelin in the golgi apparatus. Smooth ER are also the site for the synthesis of the steroid hormones from cholesterol. Thus steroid producing cells in the testis and ovaries are abundant in smooth ER.

Common functions of SER and RER:

1. The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic martix.

2. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of endoplasmic reticulum. The ER membrane has permeases and carriers.

3. The endoplasmic membranes contain many enzymes which perform various synthetic and

metabolic activities and provides increased surface for various enzymatic reactions.

4. The endoplasmic reticulum acts as an intracellular circulatory or transporting system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows: Granular ER– agranular ER – Golgi membrane–lysosomes, transport vesicles or secretory granules. Membrane flow may also be an important mechanism for carrying particles, molecules and ions into and out of the cells. Export of RNA and nucleoproteins from nucleus to cytoplasm may also occur by this type of flow.

5. The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibres.

6. The sarcoplasmic reticulum plays a role in releasing calcium when the muscle is stimulated and actively transporting calcium back into the sarcoplasmic reticulum when the stimulation stops and the muscle must be relaxed.

Lysosomes:

C. de Duve, in 1955, named these organelles as 'lysosomes'. Lysosomes is an organelle which provides an excellent example of the ability of intracellular membranes to form closed compartments in which the composition of the lumen (the aqueous interior of the compartment) differs substantially from that of the surrounding cytosol. Found exclusively in animal cells, lysosomes are responsible for degrading certain components that have become obsolete for the cell or organism. Lysosomes are often budded from the membrane of the Golgi apparatus, but in some cases they develop gradually from late endosomes, which are vesicles that carry materials brought into the cell by a process known as endocytosis. The biogenesis of the lysosomes requires the synthesis of specialized lysosomal hydrolases and membrane proteins. Both classes of proteins are synthesized in the ER and transported through the Golgi apparatus, then transported from the trans Golgi network to an intermediate compartment (an endolysosome) by means of transport vesicles (which are coated by clathrin protein).

Occurrence:

The lysosomes occur in most animal and few plant cells. They are absent in bacteria and mature mammalian erythrocytes. Few lysosomes occur in muscle cells or in acinar cells of the pancreas. Leucocytes, especially granulocytes are a particularly rich source of lysosomes. Their lysosomes are so large-sized that they can be observed under the light microscope. They are also numerous in epithelial cells of absorptive, secretory and excretory organs (intestine, liver, and kidney). They occur in abundance in the epithelial cells of lungs and uterus. Phagocytic cells and cells of reticuloendothelial system (bone marrow, spleen and liver) are also rich in lysosomes.

Structure:

The lysosomes are round vacuolar structures bounded by single unit membrane. Their shape and density vary greatly. Lysosomes are 0.2 to 0.5μm in size. Since, size and shape of lysosomes vary from cell to cell and time to time (they are polymorphic), their identification becomes difficult.

Isolation and chemical composition:

Lysosomes are very delicate and fragile organelles. Lysosomal fractions have been isolated by

sucrose-density centrifugation (Isopycnic centrifugation) after mild methods of homogenization.

The location of the lysosomes in the cell can also be pinpointed by various histochemical or cytochemical methods. For example, lysosomes give a positive test for acid Schiff reaction.

Certain lysosomal enzymes are good histochemical markers. For example, acid phosphatase is the principal enzyme which is used as a marker for the lysosomes by the use of Gomori'staining technique. Specific stains are also used for other lysosomal enzymes such as B- glucuronidase,

aryl sulphatatase, N-acetyl-B-glucosaminidase and 5-bromo-4-chloroindolacetate esterase. A lysosome may contain up to 40 types of hydrolytic enzymes. They include proteases (cathepsin for protein digestion), nucleases, glycosidases (for digestion of polysaccharides and glycosides), lipases, phospholipases, phosphatases and sulphatases. All lysosomal enzymes are acid hydrolases, optimally active at the pH5. The membrane of the lysosome normally keeps the enzymes latent and out of the cytoplasmic matrix or cytosol (pH is ~7.2), but the acid dependency of lysosomal enzymes protects the contents of the cytosol (cytoplasmic matrix) against any damage even if leakage of lysosomal enzymes occur. The latency of the lysosomal enzymes is due to the presence of the membrane which is resistant to the enzymes that it encloses. Most probably this is due to the fact that most lysosomal hydrolases are membrane-bound, which may prevent the active centres of enzymes to gain access to

susceptible groups in the membrane.

Lysosomal Membrane:

The lysosomal membrane is slightly thicker than that of mitochondria. It contains substantial amounts of carbohydrate material, particularly sialic acid. In fact, most lysosomal membrane proteins are unusually highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration. Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue. The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H+ inside the organelle. Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

Functions:

1. Lysosomes serve as digestion compartments for cellular materials that have exceeded their lifetime or are otherwise no longer useful by autophagy. When a cell dies, the lysosome membrane ruptures and enzymes are liberated. These enzymes digest the dead cells. In the process of metamorphosis of amphibians and tunicates many embryonic tissues,

e.g., gills, fins, tail, etc., are digested by the lysosomes and utilized by the other cells.

2. Lysosomes break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds, which are then transferred back into the cytoplasm as new cell-building materials. To accomplish the tasks associated with digestion, the lysosomes utilize about 40 different types of hydrolytic enzymes, all of which are manufactured in the endoplasmic reticulum and modified in the Golgi apparatus.

3. Digestion of large extracellular particles: The lysosomes digest the food contents of the phagosomes or pinosomes. The lysosomes of leucocytes enable the latter to devour the foreign

proteins, bacteria and viruses.

4. Extracellular digestion: The lysosomes of certain cells such as sperms discharge their enzymes outside the cell during the process of fertilization. The lysosomal enzymes digest the limiting membranes of the ovum and form penetra path in ovum for the sperms. Acid hydrolases are released from osteoclasts and break down bone for the reabsorption; these cells also secrete lactic acid which makes the local pH enough for optimal enzyme activity. Likewise, preceding ossification (bone formation), fibroblasts release cathepsin D enzyme to break down the connective tissue.

The Golgi Complex: Processes and Sorts Secreted and Membrane Proteins

The golgi complex was discovered by Camillo Golgi during an investigation of the nervous system and he named it the "internal reticular apparatus". Functionally it is also known as the post office of the cell. Certain important cellular functions such as biosynthesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of exocytotic (secretory) vesicles and differentiation of cellular membranes, occurs in the Golgi complex or Golgi apparatus located in the cytoplasm of animal and plant cells.

Occurrence:

The Golgi apparatus occurs in all eukaryotic cells. The exceptions are the prokaryotic cells (mycoplasmas, bacteria and blue green algae) and eukaryotic cells of certain fungi, sperm cells of bryophytes and pteridiophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals. Their number per plant cell can vary from several hundred as in tissues of corn root and algal rhizoids (*i.e.*, more than 25,000 in algal rhizoids, Sievers,1965), to a single organelle in some algae. In higher plants, Golgi apparatuses are particularly common in secretory cells and in young rapidly growing cells. In animal cells, there usually occurs a single Golgi apparatus, however, its number may vary from animal to animal and from cell to cell. *Paramoeba* species has two golgi apparatuses and nerve cells, liver cells and chordate oocytes have multiple golgi apparatuses, there being about 50 of them in the liver cells.

Morphology

The Golgi apparatus is morphologically very similar in both plant and animal cells. However, it is extremely pleomorphic: in some cell types it appears compact and limited, in others spread out and reticular (net-like). Its shape and form may vary depending on cell type. It appears as a complex array of interconnecting tubules, vesicles and cisternae. There has been much debate concerning the terminology of the Golgi's parts. The simplest unit of the Golgi apparatus is the cisterna. This is a membrane bound space in which various materials and secretions may accumulate. Numerous cisternae are associated with each other and appear in a stack-like (lamellar) aggregation. A group of these cisternae is called the dictyosome, and a group of dictyosomes makes up the cell's

Golgi apparatus. All dictyosomes of a cell have a common function. The detailed structure of three basic components of the Golgi apparatus are as follows:

1. Flattened Sac or Cisternae

Cisternae of the golgi apparatus are about 1 μm in diameter, flattened, plate-like or saucer-like closed compartments which are held in parallel bundles or stacks one above the other. In each stack, cisternae are separated by a space of 20 to 30 nm which may contain rod-like elements or fibres. Each stack of cisternae forms a dictyosome which may contain 5 to 6 Golgi cisternae in animal cells or 20 or more cisternae in plant cells. Each cisterna is bounded by a smooth unit membrane (7.5 nm thick), having a lumen varying in width from about 500 to 1000 nm. Polarity. The margins of each cisterna are gently curved so that the entire dictyosome of Golgi apparatus takes on a bow-like appearance. The cisternae at the convex end of the dictyosome comprise proximal, forming or cis-face and the cisternae at the concave end of the dictyosome comprise the distal, maturing or trans-face. The forming or cis face of Golgi is located next to either the nucleus or a specialized portion of rough ER that lacks bound ribosomes and is called “transitional” ER. Trans face of Golgi is located near the plasma membrane. This polarization is called cis-trans axis of the Golgi apparatus.

2. Tubules

A complex array of associated vesicles and tubules (30 to 50 nm diameter) surround the dictyosome and radiate from it. The peripheral area of dictyosome is fenestrated or lace-like in structure.

3. Vesicles

The vesicles are 60 nm in diameter and are of three types : (i) Transitional vesicles are small membrane limited vesicles which are form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they coalasce to form new cisternae.

(ii) Secretory vesicles are varied-sized membrane-limited vesicles which discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.

(iii) Clathrin-coated vesicles are spherical protuberances, about 50 μm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and are morphologically quite distinct from the secretory vesicles. The

clathrin-coated vesicles are known to play a role in intra-cellular traffic of membranes and of secretory products.

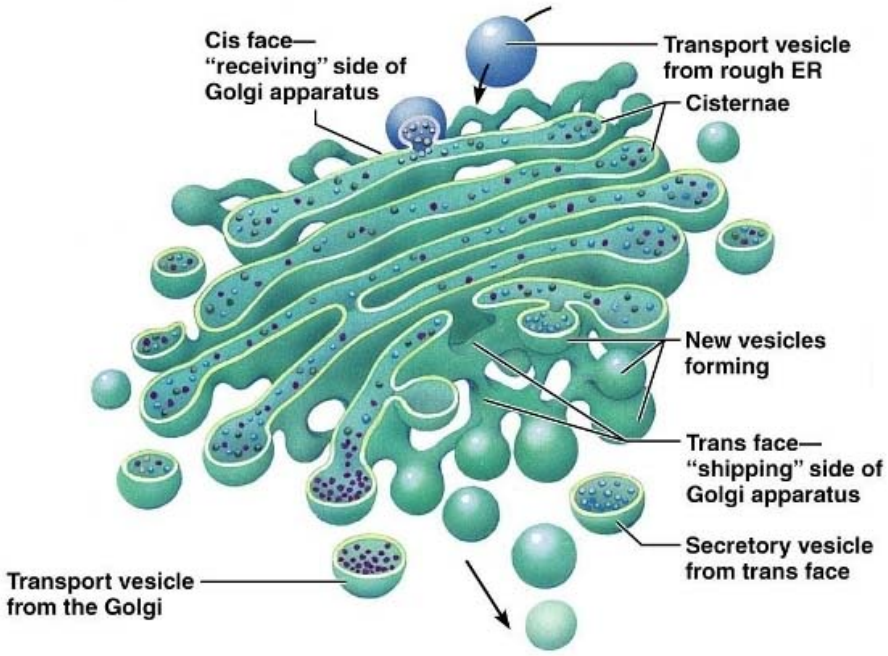

Figure 5: The Golgi complex.

Functions:

1. Modifying, sorting, and packaging of macromolecules for cell secretion: The golgi complex is involved in the transport of lipids around the cell, and the creation of lysosomes. Proteins are modified by enzymes in cisternae by glycosylation and phosphorylation by identifying the signal sequence of the protein in question. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes. One molecule that is phosphorylated in the Golgi is Apolipoprotein, which forms a molecule known as VLDL that is a constituent of blood serum. The phosphorylation of these molecules is important to help aid in their sorting for secretion into the blood serum.

2. Proteoglycans and carbohydrate synthesis: This includes the production of glycosaminoglycans (GAGs), long unbranched polysaccharides which the Golgi then attaches to a protein synthesised in the endoplasmic reticulum to form proteoglycans.

3. Golgi Functions in Animals:

In animals, Golgi apparatus is involved in the packaging and exocytosis of the following: Zymogen of exocrine pancreatic cells; Mucus (a glycoprotein) secretion by goblet cells of intestine; Lactoprotein (casein) secretion by mammary gland cells (Merocrine secretion); Secretion of compounds (thyroglobulins) of thyroxine hormone by thyroid cells; Secretion of tropocollagen and collagen; Formation of melanin granules and other pigments; and Formation of yolk and vitelline membrane of growing primary oocytes. It is also involved in the formation of certain cellular organelles such as plasma membrane, lysosomes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

4. Golgi Functions in Plants:

In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls (formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin). During cytokinesis of mitosis or meiosis, the vesicles originating from the periphery of Golgi apparatus, coalesce in the phragmoplast area to form a semisolid layer, called cell plate. The unit membrane of Golgi vesicles fuses during cell plate formation and becomes part of plasma membrane of daughter

Interesting Facts:

- George Palade, a Romanian-born naturalized American and cell biologist, was the first to describe free ribosomes.
- An example of an animal cell with many Golgi bodies is an epithelial cell that secretes mucus.
- The cell wall of plant cells is exported to the outside of the membrane by Golgi bodies.

Questions

Q1. Proteins synthesized by the rough ER are

A) for internal storage

B) to build more membranes in the cell

C) to digest food in lysosomes

D) for internal regulation

E) exported from the cell

Q2. Glycoproteins and glycolipids assembled in Golgi bodies are packaged for distribution in

A) cisternae

B) lysosomes

C) peroxisomes

D) liposomes

E) glyoxysomes

Q3. The rough ER is so named because it has an abundance of _____ on it.

A) mitochondria

B) lysosomes

C) Golgi bodies

D) ribosomes

E) vesicles

Q4. Clusters of rRNA where ribosomes are assembled are called

A) nuclei

B) cisternae

C) nucleoli

D) Golgi complexes

E) centrioles

Q5. The smooth ER is especially abundant in cells that synthesize extensive amounts of

A) toxins

B) proteins

C) enzymes

D) lipids

E) nucleic acids

Q6. Enzymes embedded in the membrane of the smooth ER

A) synthesize lipids

B) may be used for detoxification

C) synthesize carbohydrates

D) mostly are active only when associated with a membrane

E) all of the above

Q7. The Golgi apparatus is involved in

A) transporting proteins that are to be released from the cell

B) packaging proteins into vesicles

C) altering or modifying proteins

D) producing lysosomes

E) all of the above

Q8. Ribosomes are found

A) only in the nucleus

B) in the cytoplasm

C) attached to the smooth endoplasmic reticulum

D) only in eukaryotic cells

E) both b and c

Q9. Is protein synthesis effected by the cell growth temperature?

Q10. How does protein enter the Endoplasmic reticulum?

Q11. Why is a Ribosome Important? How do ribosomes differ in prokaryotic and eukaryotic cells?

Q12. What Diseases Affect Ribosomes?

Q13. Ribosomes ate present in mitochondria. True/False.

Q14. How do the golgi bodies and lysosomes work together?

Q15. What is the function of smooth and rough endoplasmic reticulum?

Q16. How does the cell make golgi apparatus and endoplasmic reticulum?

Q17. What is the structure and function of a lysosome?

Q18. How do lysosomes and vesicles assist each other by working together?

Q19. Do plant cells have lysosomes?

Q20. What is endocytosis?

Q21. What happens if a cell does not produce the enzymes that lysosomes need in order to function?

Q22. What is the role of the endoplasmic reticulum as a site of protein folding?

Further readings

Fabene PF, Bentivoglio M (October 1998). "1898–1998: Camillo Golgi and "the Golgi": one hundred years of terminological clones". *Brain Res. Bull.* 47 (3): 195–8.

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Module 1 Lecture 7

The present lecture details few other cell organelles like Peroxisomes, chloroplast and vacuoles.

Peroxisomes:

All animal cells (except erythrocytes) and most plant cells contain peroxisomes. They are present in all photosynthetic cells of higher plants in etiolated leaf tissue, in coleoptiles and hypocotyls, in tobacco stem and callus, in ripening pear fruits and also in Euglenophyta, Protozoa, brown algae, fungi, liverworts, mosses and ferns. Peroxisomes contain several oxidases.

Structure:

Peroxisomes are variable in size and shape, but usually appear circular in cross section having

diameter between 0.2 and 1.5μm. They have a single limiting unit membrane of lipid and protein molecules, which encloses their granular matrix. Like mitochondria and chloroplasts, they acquire their proteins by selective import from the cytosol. Peroxisomes resemble the Endoplasmic reticulum by being self-replicating, membrane-enclosed organelle that exists without a genome of its own.

Peroxisomes are unusually diverse organelles, and even in the various cell types of a single organism they may contain different sets of enzymes. They can also adapt remarkably to changing conditions. Yeast cells grown on sugar, for example, have small peroxisomes. But when some yeasts are grown on methanol, they develop large peroxisomes that oxidize methanol; and when grown on fatty acids, they develop large peroxisomes that break down fatty acids to acetyl CoA by β oxidation. Peroxisomes are also important in plants. Two different types have been studied extensively. One type is present in leaves, where it catalyzes the oxidation of a side product of the crucial reaction that fixes CO_2 in carbohydrate. This process is called photorespiration because it uses up O_2 and liberates CO_2. The other type of peroxisome is present in germinating seeds, where it has an essential role in converting the fatty acids stored in seed lipids into the sugars needed for the growth of the young plant. Because this conversion of fats to sugars is accomplished by a series of reactions known as the glyoxylate cycle, these peroxisomes are also called glyoxysomes. In the glyoxylate cycle, two molecules of

acetyl CoA produced by fatty acid breakdown in the peroxisome are used to make succinic acid, which then leaves the peroxisome and is converted into glucose. The glyoxylate cycle does not occur in animal cells, and animals are therefore unable to convert the fatty acids in fats into carbohydrates. Glyoxysomes occur in the cells of yeast, Neurospora, and oil rich seeds of many higher plants. They resemble with peroxisomes in morphological details, except that, their crystalloid core consists of dense rods of 6.0 μm diameter.

Chemical composition:

Internally peroxisomes contain several oxidases like catalase and urate oxidase-enzymes that use molecular oxygen to oxidize organic substances, in the process forming hydrogen peroxide (H_2O_2), a corrosive substance. Catalase is present in large amounts and degrades hydrogen peroxide to yield water and oxygen.

A specific sequence of three amino acids located at the C-terminus of many peroxisomal proteins functions as an import signal. Other peroxisomal proteins contain a signal sequence near the N terminus. If either of these sequences is experimentally attached to a cytosolic protein, the protein is imported into peroxisomes. The import process is yet to be understood completely, although it is known to involve soluble receptor proteins in the cytosol that recognize the targeting signals, as well as docking proteins on the cytosolic surface of the peroxisome. At least 23 distinct proteins, called peroxins, participate as components in the process, which is driven by ATP hydrolysis. Oligomeric proteins do not have to unfold to be imported into peroxisomes, indicating that the mechanism is distinct from that used by mitochondria and chloroplasts and at least one soluble import receptor, the peroxin Pex5, accompanies its cargo all the way into peroxisomes and, after cargo release, cycles back out into the cytosol. These aspects of peroxisomal protein import resemble protein transport into the nucleus.

Formation of peroxisomes:

Most peroxisomal membrane proteins are made in the cytosol and then insert into the membrane of pre-existing peroxisomes. Thus, new peroxisomes are thought to arise from pre-existing ones, by organelle growth and fission **(Figure 1)**.

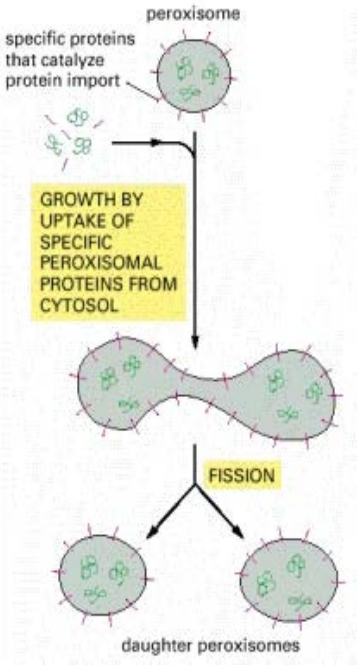

Figure 1 Production of new peroxisomes. The figure has been printed with permission from Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.

Functions:

1. Hydrogen peroxide metabolism and detoxification: Peroxisomes are so-called, because they usually contain one or more enzymes (D-amino acid oxidase and urate oxidase) that use molecular oxygen to remove hydrogen atoms from specific organic substrates (R) in an oxidative reaction that produces hydrogen peroxide (H_2O_2): RH_2+O_2 ––––– $R + H_2O_2$

This type of oxidative reaction is particularly important in liver and kidney cells, whose peroxisomes detoxify various toxic molecules that enter the blood stream. Almost half of alcohol one drinks is oxidized to acetaldehyde in this way. However, when excess H_2O_2 accumulates in the cell, catalase converts H_2O_2 to H_2O : $2H_2O_2$ ––––––––– $2H_2O + O_2$

Catalase also utilizes the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substrates like phenols, formic acid, formaldehyde, and alcohol. This type

of oxidative reaction occurs in liver and kidney cells, where the peroxisomes detoxify various toxic molecules that enter the bloodstream.

2. Photorespiration: In green leaves, there are peroxisomes that carry out a process called photorespiration which is a light-stimulated production of CO_2 that is different from the generation of CO_2 by mitochondria in the dark. In photorespiration, glycolic acid a two-carbon product of photosynthesis is released from chloroplasts and oxidized into glyoxylate and H_2O_2 by a peroxisomal enzyme called glycolic acid oxidase. Later on, glyoxylate is oxidized into CO_2 and formate:

$CH_2OH.\ COOH + O_2 \text{ −−−−−−−−−−} CHO – COOH + H_2O_2$

$CHO — COOH + H_2O_2 \text{ −−−−−−−−} HCOOH + CO_2 + H_2O$

3. Fatty acid oxidation: A major function of the oxidative reactions performed in peroxisomes is the breakdown of fatty acid molecules. In mammalian cells, β oxidation occurs in both mitochondria and peroxisomes; in yeast and plant cells, however, this essential reaction occurs exclusively in peroxisomes. Peroxisomal oxidation of fatty acids yield acetyl groups and is not linked to ATP formation. The energy released during peroxisomal oxidation is converted into heat, and the acetyl groups are transported into the cytosol, where they are used in the synthesis of cholesterol and other metabolites. In most eukaryotic cells, the peroxisome is the principal organelle in which fatty acids are oxidized, thereby generating precursors for important biosynthetic pathways. In contrast with the oxidation of fatty acids in mitochondria, which produces CO_2 and is coupled to the generation of ATP, peroxisomal oxidation of fatty acids yield acetyl groups and is not linked to ATP formation. The energy released during peroxisomal oxidation is converted into heat, and the acetyl groups are transported into the cytosol, where they are used in the synthesis of cholesterol and other metabolites.

4. Formation of plasmalogens: An essential biosynthetic function of animal peroxisomes is to catalyze the first reactions in the formation of plasmalogens, which are the most abundant class of phospholipids in myelin **(Figure 2)**. Deficiency of plasmalogens causes profound abnormalities in the myelination of nerve cells, which is one reason why many peroxisomal disorders lead to neurological disease.

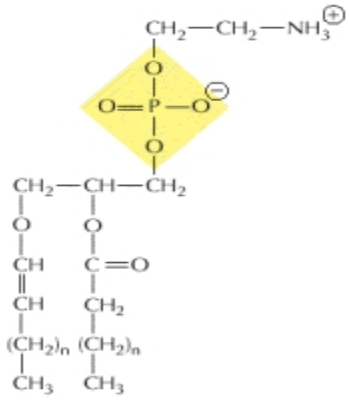

Figure 2: The structure of plasmalogen. The figure has been printed with permission from Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.

Peroxisome and diseases:

In most eukaryotic cells, the peroxisome is the principal organelle in which fatty acids are oxidized, thereby generating precursors for important biosynthetic pathways. In the human genetic disease X-linked adrenoleukodystrophy *(ADL)*, peroxisomal oxidation of very long chain fatty acids is defective. The *ADL*gene encodes the peroxisomal membrane protein that transports into peroxisomes an enzyme required for the oxidation of these fatty acids. Persons with the severe form of ADL are unaffected until midchildhood, when severe neurological disorders appear, followed by death within a few years.

Zellweger syndrome is an inherited human disease, in which a defect in importing proteins into peroxisomes leads to a severe peroxisomal deficiency. These individuals, whose cells contain “empty” peroxisomes, have severe abnormalities in their brain, liver, and kidneys, and they die soon after birth. One form of this disease has been shown to be due to a mutation in the gene encoding a peroxisomal integral membrane protein, the peroxin Pex2, involved in protein import. A milder inherited peroxisomal disease is caused by a defective receptor for the N-terminal import signal.

Plastids:

Plant cells are readily distinguished from animal cells by the presence of two types of membrane-bounded compartments– vacuoles and plastids.

Types of plastids:

The term 'plastid' is derived from the Greek word "*plastikas*" (formed or moulded) and was

used by A.F.W. Schimper in 1885. Schimper classified the plastids into following types according to their structure, pigments and the functions:

1. Leucoplasts

The leucoplasts (*leuco* = white; *plast* = living) are the colourless plastids which are found

in embryonic and germ cells. They are also found in meristematic cells and in those regions of the plant which do not receive light. Plastids located in the cotyledons and the primordium of the stem are colourless (leucoplastes) but eventually become filled with chlorophyll and transform into chloroplasts. True leucoplasts occur in fully differentiated cells such as epidermal and internal plant tissues. True leucoplasts do not contain thylakoids and even ribosomes. They store the food materials as carbohydrates, lipids and proteins and accordingly are of following types:

(i) Amyloplasts. The amyloplasts (*amyl*=starch; *plast*=living) are those leucoplasts which synthesize and store the starch. The amyloplasts occur in those cells which store the starch. The outer membrane of the amyloplst encloses the stroma and contains one to eight starch granules. Starch granules of amyloplasts are typically composed of concentric layers of starch.

(ii) Elaioplasts. The elaioplasts store the lipids (oils) and occur in seeds of monocotyledons and

dicotyledons. They also include sterol-rich sterinochloroplast.

(iii) Proteinoplasts. The proteinoplasts are the protein storing plastids which mostly occur in

seeds and contain few thylakoids.

2. Chromoplasts

The chromoplasts (*chroma*=colour; *plast*=living) are the coloured plastids containing carotenoids and other pigments. They impart colour (yellow, orange and red) to certain portions of plants such as flower petals (daffodils, rose), fruits (tomatoes) and some roots (carrots). Chromoplast structure is quite diverse; they may be round, ellipsoidal, or even needle-shaped, and the carotenoids that they contain may be localized in droplets or in crystalline structures. In general, chromoplasts have a reduced chlorophyll content and are, thus, less active photosynthetically. The red colour of ripe tomatoes is the result of chromoplasts that contain the red pigment lycopene which is a member of carotenoid family. Chromoplasts of blue-green algae or cyanobacteria contain various pigments such as phycoerythrin, phycocyanin, chlorophyll a and carotenoids.

Chromoplasts are of following two types:

(i) Phaeoplast. The phaeoplast (*phaeo*=dark or brown; *plast*=living) contains the pigment fucoxanthin which absorbs the light. The phaeoplasts occur in the diatoms, dinoflagellates and brown algae.

(ii) Rhodoplast. The rhodoplast (*rhode*= red; *plast*=living) contains the pigment phaeoerythrin which absorbs the light. The rhodoplasts occur in the red algae.

3. Chloroplasts

The chloroplast (*chlor*=green; *plast*=living) is most widely occurring chromoplast of the plants. It occurs mostly in the green algae and higher plants. The chloroplast contains the pigment chlorophyll a and chlorophyll b and DNA and RNA.

Chloroplasts:

Chloroplasts were described as early as seventeenth century by Nehemiah Grew and Antonie van Leeuwenhoek.

Distribution:

The chloroplasts remain distributed homogeneously in the cytoplasm of plant cells. But in certain

cells, the chloroplasts become concentrated around the nucleus or just beneath the plasma membrane. The chloroplasts have a definite orientation in the cell cytoplasm. Chloroplasts are motile organelles, and show passive and active movements.

Morphology:

Shape: Higher plant chloroplasts are generally biconvex or plano-convex. However, in different plant cells, chloroplasts may have various shapes, *viz*., filamentous, saucer-shaped, spheroid, ovoid, discoid or club-shaped. They are vesicular and have a colourless centre.

Size: The size of the chloroplasts varies from species to species. They generally measure 2–3μm in thickness and 5–10μm in diameter (*Chlamydomonas*). The chloroplasts of polyploid plant cells are comparatively larger than those of the diploid counterparts. Generally, chloroplasts of plants grown in the shade are larger and contain more chlorophyll than those of plants grown in sunlight.

Number: The number of the chloroplasts varies from cell to cell and from species to species and is related with the physiological state of the cell, but it usually remains constant for a particular plant cell. Algae usually have a single huge chloroplast. The cells of the higher plants have 20 to 40 chloroplasts. According to a calculation, the leaf of *Ricinus communis* contains about 400,000 chloroplasts per square millimeter of surface area. The chloroplasts are composed of the carbohydrates, lipids, proteins, chlorophyll, carotenoids (carotene and xanthophylls), DNA, RNA and certain enzymes and coenzymes. The chloroplasts also contain some metallic atoms as Fe, Cu, Mn and Zn. Chloroplasts have very low percentage of carbohydrate. They contain 20–30 per cent lipids on dry weight basis. The most common alcohols of the lipids are the choline, inositol, glycerol, ethanolamine. The proteins constitute 35 to 55 per cent of the chloroplast. Chlorophyll is the green pigment of the chloroplasts. It is an asymmetrical molecule which has hydrophilic head of four rings of the pyrols and hydrophobic tail of phytol. Chemically the chlorophyll is a porphyrin like the animal pigment haemoglobin and cytochromes except besides the iron (Fe), it contains Mg atom in between the rings of the pyrols which remain connected with each other by the methyl groups. The chlorophyll consists of 75 per cent chlorophyll *a* and 25 per cent chlorophyll *b*.

The carotenoids are carotenes and xanthophylls, both of which are related to vitamin A. The carotenes have hydrophobic chains of unsaturated hydrocarbons in their molecules. The xanthophylls contain many hydroxy groups in their molecules. Chloroplast have their own genetic material which is circular like that of bacterial chromosome.

Isolation:

Chloroplasts are routinely isolated from plant tissues by differential centrifugation following the
disruption of the cells.

Ultrastructure:

Chloroplast comprises of three main components:

1. Envelope

The entire chloroplast is bounded by a double unit membrane. Across this double membrane envelope occurs exchange of molecules between chloroplast and cytosol. Isolated membranes of envelope of chloroplast lack chlorophyll pigment and cytochromes but have a yellow colour due to the presence of small amounts of carotenoids. They contain only 1 to 2 per cent of the total protein of the chloroplast.

2. Stroma

The matrix or stroma fills most of the volume of the chloroplasts and is a kind of gel-fluid phase
that surrounds the thylakoids (grana). It contains about 50 per cent of the proteins of the chloroplast, most of which are soluble type. The stroma also contains ribosomes and DNA molecules both of which are involved in the synthesis of some of the structural proteins of the chloroplast. The stroma is the place where CO_2 fixation occurs and where the synthesis of sugars, starch, fatty acids and some proteins takes place.

3. Thylakoids

The thylakoids (thylakoid = sac-like) consists of flattened and closed vesicles arranged as a membranous network. The outer surface of the thylakoid is in contact with the stroma, and its inner surface encloses an intrathylakoid space. Thylakoids get stacked forming grana. There may be 40 to 80 grana in the matrix of a chloroplast. The number of thylakoids per granum may vary from 1 to 50 or more. For example, there may be single thylakoid (red alga), paired thylakoids (Chrysophyta), triple thylakoids and multiple thylakoids (green algae and higher plants).

Like the mitochondria, the chloroplasts have their own DNA, RNAs and protein synthetic machinery and are semiautonomous in nature. Chloroplasts are the largest and the most prominent organelles in the cells of plants and green algae. Chloroplasts and mitochondria have other features in common: both often migrate from place to place within cells, and they contain their own DNA, which encodes some of the key organellar proteins. Though most of the proteins in each organelle are encoded by nuclear DNA and are synthesized in the cytosol, the proteins encoded by mitochondrial or chloroplast DNA is synthesized on ribosomes within the organelles.

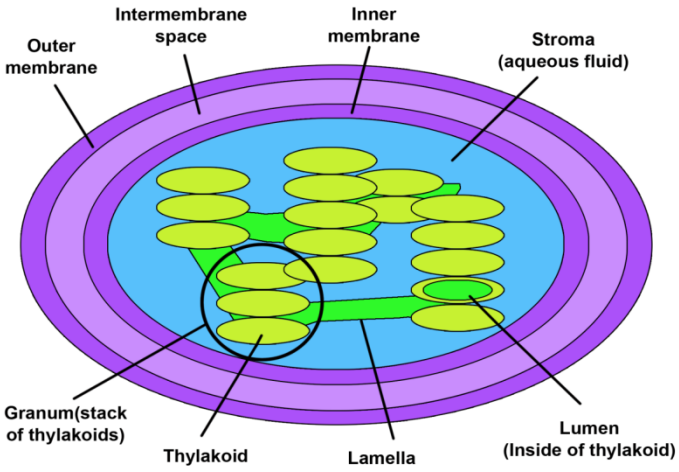

Figure 3: Structure of chloroplast.

Chloroplasts have a highly permeable outer membrane; a much less permeable inner membrane, in which membrane transport proteins are embedded; and a narrow intermembrane space in between. Together, these membranes form the chloroplast envelope **(Figure 3)**. The inner membrane surrounds a large space called the stroma, and contains many metabolic enzymes.

The electron-transport chains, photosynthetic light-capturing systems, and ATP synthase are all contained in the thylakoid membrane, a third distinct membrane that forms a set of flattened disclike sacs, the thylakoids **(Figure 4)**. The lumen of each thylakoid is connected with the lumen of other thylakoids, defining a third internal compartment called the thylakoid space, which is separated by the thylakoid membrane from the stroma that surrounds it.

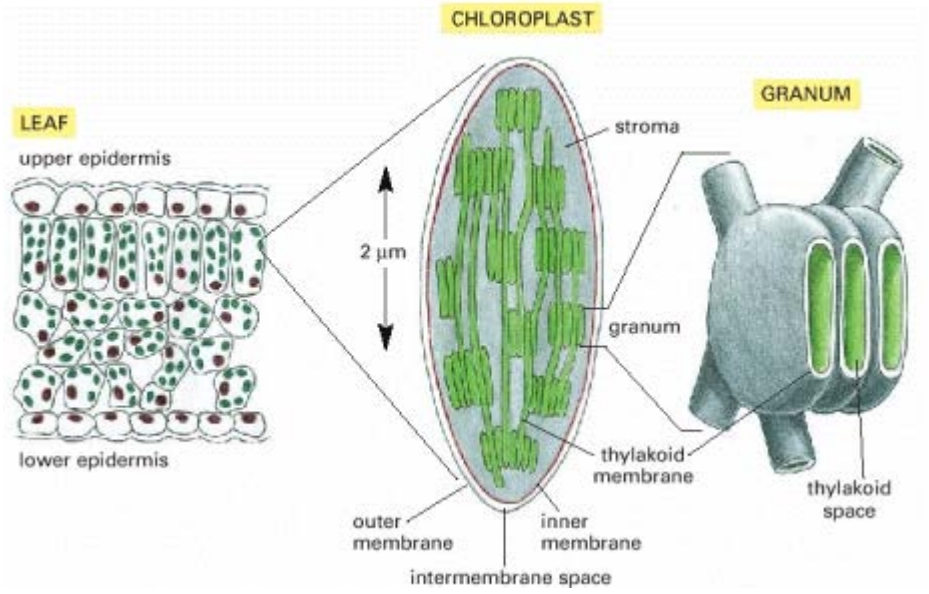

Figure 4. The structure of chloroplast and thylakoid. The figure has been printed with permission from Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.

Photosynthesis

The many reactions that occur during photosynthesis in plants can be grouped into two broad categories:

1. Electron-transfer reactions or the light reactions: In the choloroplast, energy derived from sunlight energizes an electron of chlorophyll, enabling the electron to move along an electron-transport chain in the thylakoid membrane in much the same way that an electron moves along the respiratory chain in mitochondria. The chlorophyll obtains its electrons from water (H_2O), producing O_2 as a by-product. During the electron-transport process, H^+ is pumped across the thylakoid membrane, and the resulting electrochemical proton gradient drives the synthesis of ATP in the stroma. As the final step in this series of reactions, high-energy electrons are loaded onto $NADP^+$, converting it to NADPH. All of these reactions are confined to the chloroplast.

2. Carbon-fixation reactions or the dark reactions wherein the ATP and the NADPH produced by the photosynthetic electron-transfer reactions serve as the source of energy and reducing power, respectively, to drive the conversion of CO_2 to carbohydrate. The carbon-fixation reactions, which begin in the chloroplast stroma and continue in the cytosol, produce sucrose and many other organic molecules in the leaves of the plant. The sucrose is exported to other tissues as a source of both organic molecules and energy for growth.

Thus, the formation of ATP, NADPH, and O_2 and the conversion of CO_2 to carbohydrate are separate processes, although elaborate feedback mechanisms interconnect the two. Several of the chloroplast enzymes required for carbon fixation, for example, are inactivated in the dark and reactivated by light-stimulated electron-transport processes.

The chloroplast genome

It is believed that evolved from bacteria that were engulfed by nucleated ancestral cells and this theory is known as the endosymbiotic theory. All angiosperms and land plants have chloroplast DNAs (cp DNA) which range in size from 120-160 kb. They are circular possessing very few repeat elements and other short sequences of less than 100 bp. The notable exception is a large inverted repeat (10-76 kb) section, which when present, always contains the rRNA genes. For the majority of species, this repeat region is 22-26 kb in size. More than 20 chloroplast genomes have now been sequenced. The genomes of even distantly related plants are nearly identical, and even those of green algae are closely related.

Plant Vacuoles:

The most conspicuous compartment in most plant cells is a very large, fluid-filled vesicle called

a vacuole. There may be several vacuoles in a single cell, each separated from the cytoplasm by a single unit membrane, called the tonoplast. Generally vacuoles occupy more than 30 per cent of the cell volume; but this may vary from 5 per cent to 90 per cent, depending on the cell type. Plant cell vacuoles are widely diverse in form, size, content, and functional dynamics, and a single cell may contain more than one kind of vacuole. Most plant cells contain at least one membrane limited internal vacuole. The

number and size of vacuoles depend on both the type of cell and its stage of development; a single vacuole may occupy as much as 80 percent of a mature plant cell. They are lytic compartments, function as reservoirs for ions and metabolites, including pigments, and are crucial to processes of detoxification and general cell homeostasis. They are involved in cellular responses to environmental and biotic factors that provoke stress. A variety of transport proteins in the vacuolar membrane allow plant cells to accumulate and store water, ions, and nutrients (sucrose, amino acids) within vacuoles. Like a lysosome, the lumen of a vacuole contains a battery of degradative enzymes and has an acidic pH, which is maintained by similar transport proteins in the vacuolar membrane. Plant vacuoles may also have a degradative function similar to that of lysosomes in animal cells. Similar storage vacuoles are found in green algae and many microorganisms such as fungi. Like most cellular membranes, the vacuolar membrane is permeable to water but is poorly permeable to the small molecules stored within it. Because the solute concentration is much higher in the vacuole lumen than in the cytosol or extracellular fluids, water tends to move by osmotic flow into vacuoles, just as it moves into cells placed in a hypotonic medium. This influx of water causes both the vacuole to expand and water to move into the cell, creating hydrostatic pressure, or turgor, inside the cell. This pressure is balanced by the mechanical resistance of the cellulose-containing cell walls that surround plant cells. Most plant cells have a turgor of 5–20 atmospheres (atm); their cell walls must be strong enough to react to this pressure in a controlled way. Unlike animal cells, plant cells can elongate extremely rapidly, at rates of 20–75 µm/h. This elongation, which usually accompanies plant growth, occurs when a segment of the somewhat elastic cell wall stretches under the pressure created by water taken into the vacuole.

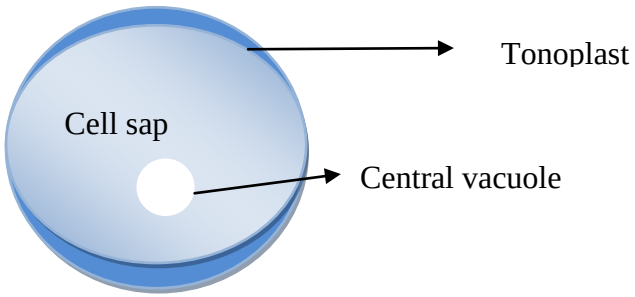

Figure 5: Plant cell central vacuole.

The central vacuole in plant cells (Figure 5) is bounded by a membrane termed the tonoplast which is an important constituent of the plant endomembrane system. This vacuole develops as the cell matures by fusion of smaller vacuoles derived from the endoplasmic reticulum and Golgi apparatus. Functionally it is highly selective in transporting materials through its membrane. The cell sap inside the vacuole differs from the cytoplasm.

Functions:

1. Vacuoles often store the pigments that give flowers their colors, which aid them in the attraction of bees and other pollinators.
2. It can also be comprised of plant wastes that while developing seed cells use the central vacuole as a repository for protein storage.
3. The central vacuole also is responsible for salts, minerals, nutrients, proteins and pigments storage which in turn helps in plant growth, and plays an important structural role for the plant.
4. Vacuoles are also important for maintaining turgor pressure which controls the rigidity of the cell. Due to the process of osmosis when a plant receives large amounts of water, the central vacuoles of the cell swell as the liquid enters within them, increasing turgor pressure, which helps maintain the structural integrity of the plant, along with the support from the cell wall. In the absence of enough water, however, central vacuoles shrink and turgor pressure is reduced, compromising the plant's rigidity and wilting takes place.
5. Plant vacuoles are also important for their role in molecular degradation and storage. Sometimes these functions are carried out by different vacuoles in the same cell, one serving as a compartment for breaking down materials (similar to the lysosomes found in animal cells), and another storing nutrients, waste products, or other substances. Several of the materials commonly stored in plant vacuoles have been found to be useful for humans, such as opium, rubber, and garlic flavoring, and are frequently harvested.
6. Sometimes Vacuoles contain molecules that are poisonous, odoriferous, or unpalatable to various insects and animals.

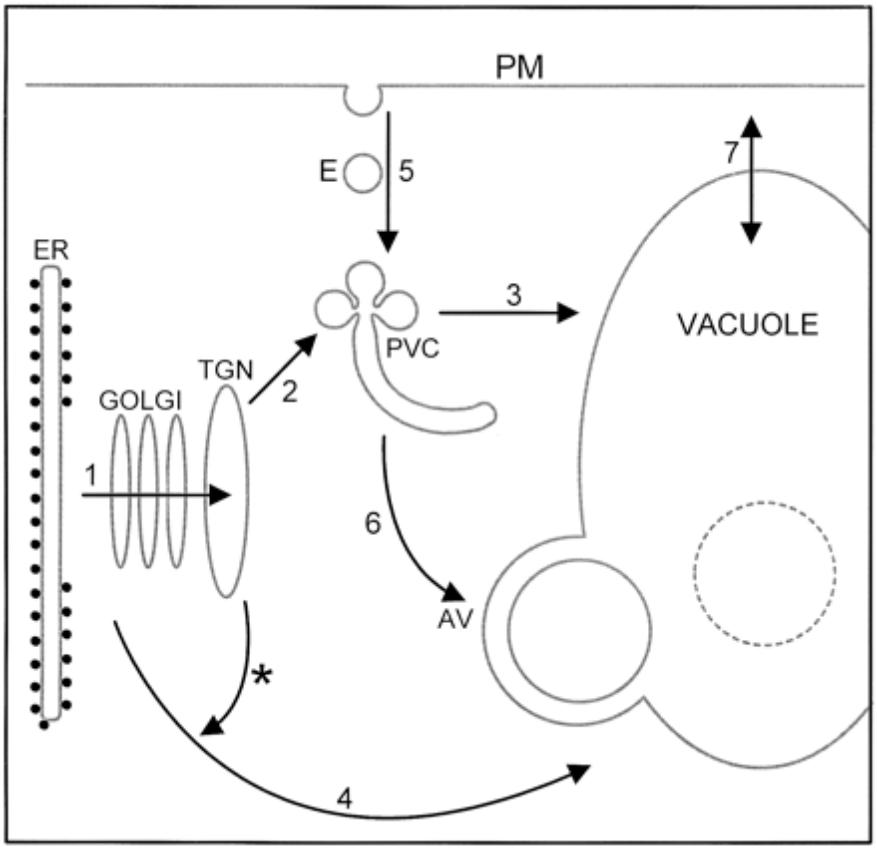

Figure 6: Vascular transport pathway. This Figure has been reprinted with permission from Plant Vacuoles by Francis Martya (1999), Plant Cell, Vol. 11, 587-600.

Proteins destined for degradation are delivered to the vacuole via the secretory pathway, which includes the biosynthetic, autophagic, and endocytotic transport routes **(Figure 6**).

Interesting Facts:

- The large central vacuoles often found in plant cells enable them to attain a large size without accumulating the bulk that would make metabolism difficult.
- The importance of peroxisomes for human health is highlighted by the number of peroxisomal disorders (PDs).
- In addition to the synthesis of food, chloroplasts are also the site of production of plant fats and oils.
- It has been found that following the infection of a plant with the tobacco mosaic virus (TMV), the viral helicase protein and a chloroplast protein form a complex that is recognized by a plant immune receptor.
- Chloroplast can be used to derive recombinant human vaccine.

Questions:

Q1. What are peroxisomes? Name the important function of peroxisomes.

Q2. What is the difference between vacuoles of plant and animal cells?

Q3. How are fatty acids degraded in peroxisomes?

Q5.Name an essential function of peroxisome whose abnormality affects nerve cells.

Q6.What is the role of specific signal sequences in perixisomal proteins?

Q7. Illustrate the structure and function of chloroplast?

Q8. Write about chloroplast genome?

Q9. What is the function of plant vacuoles.

Q12. Name a disease caused due to peroxisomal disorder.

Further readings

1. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.
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3. Deng, X. W., R.A. Wing, W. Gruissem. *1989*. The chloroplast genome exists in multimeric forms. *Proc Natl Acad Sci* USA *86:4156*-4160.
4. Davidson M W (1995-2012). Molecular expressions. http://micro.magnet.fsu.edu/index.html.
5. Martya F (1999). Plant Vacuoles. Plant Cell 11:587-600.

Module 1 Lecture 8

During the current lecture we shall discuss about Extracellular matrix and their role in cell signaling and adhesion

Extracellular matrix

Animal cells are surrounded by extracellular matrix beyond the immediate vicinity of their plasma membrane, filling spaces between cells and adhering cells together. Extracellular matrices are of various types consisting of secreted proteins and polysaccharides and are most abundant in connective tissues. One of the examples of extracellular matrix is the basal laminae. It is a continuous sheet of 50 to 200 nm thickness and on top of which a thin layer of epithelial cells rest. Such basal laminae surround muscle cells, adipose cells, and peripheral nerves. The differences between various types of extracellular matrices result from both quantitative variations in the types or amounts of these different constituents and from modifications in their organization. The three major components of extracellular matrix are matrix proteins, matrix polysaccharides and the matrix adhesion proteins. The major components of the extracellular matrix have been illustrated in Figure 1.

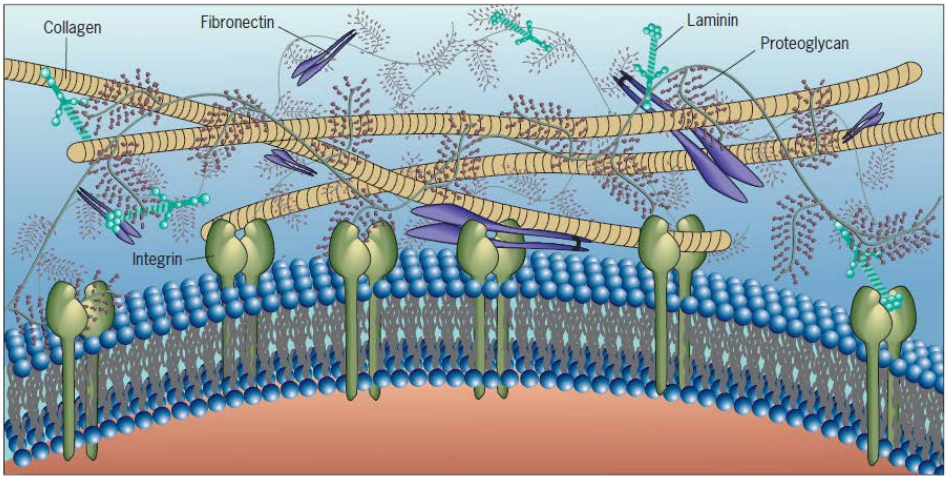


Figure 1: An overview of the extracellular matrix molecular organization. The proteins; fibronectin, collagen, and laminin contain binding sites for one another, as well as binding sites for receptors like integrins that are located at the cell surface. The proteoglycans are huge protein polysaccharide complexes that occupy much of the volume of the extracellular space. This figure has been adapted from Cell and Molecular Biology Concepts and Experiments by Karp, 2010.

Structural proteins of Matrix

Matrix proteins are fibrous in nature. The major structural protein is collagen whose secondary structure is a triple helix. The collagens belong to large family of proteins and are characterized by the formation of triple helices in which three polypeptide chains are wound tightly around one another in a ropelike manner. The different collagen polypeptides can assemble into 42 different trimers. The triple helix domains of the collagens consist of repeats of the amino acid sequence Gly-X-Y. The most abundant type is collagen type I and is one of the fibril forming collagens that are the basic structural components of connective tissues **(Figure 2)**. Elastin is another matrix protein, which gives elasticity to tissues, allowing them to stretch when needed and then return to their original state. They are present in blood vessels, the lungs, in skin, and the ligaments. Elastins are synthesized by fibroblasts and smooth muscle cells.

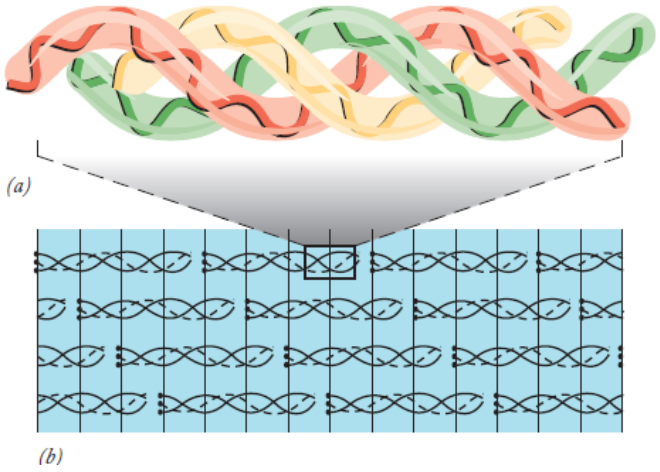


Figure 2: The structure of collagen I. (*a*) The monomer of collagen. (*b*) Collagen I molecules become aligned in and a bundle of collagen I molecules, such as that shown here, form a collagen fibril.

This figure has been adapted from Cell and Molecular Biology Concepts and Experiments by Karp, 2010.

Polysaccharides of Matrix

The structural proteins of the extracellular matrix are rooted in polysaccharides called glycosaminoglycans (GAGs). One sugar of the disaccharide is either N-acetylglucosamine or N-acetylgalactosamine and the second is usually glucuronic acid or iduronic acid. They can also be sulfated like the chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate. These polysaccharides are highly negative in charge and bind positively charged ions and water molecules to form hydrated gels. The function of such gels is to provide support to the matrix. Hyaluronan is the only GAG that occurs as a single long polysaccharide chain. GAGs also attach with proteins through Serine residues and are known as proteoglycans. A number of proteoglycans interact with hyaluronan to form large complexes in the extracellular matrix e.g., aggrecan which is the major protein of the cartilage. Proteoglycans also interact with collagen and other matrix proteins to form gel-like networks in which the fibrous structural proteins of the extracellular matrix remain rooted.

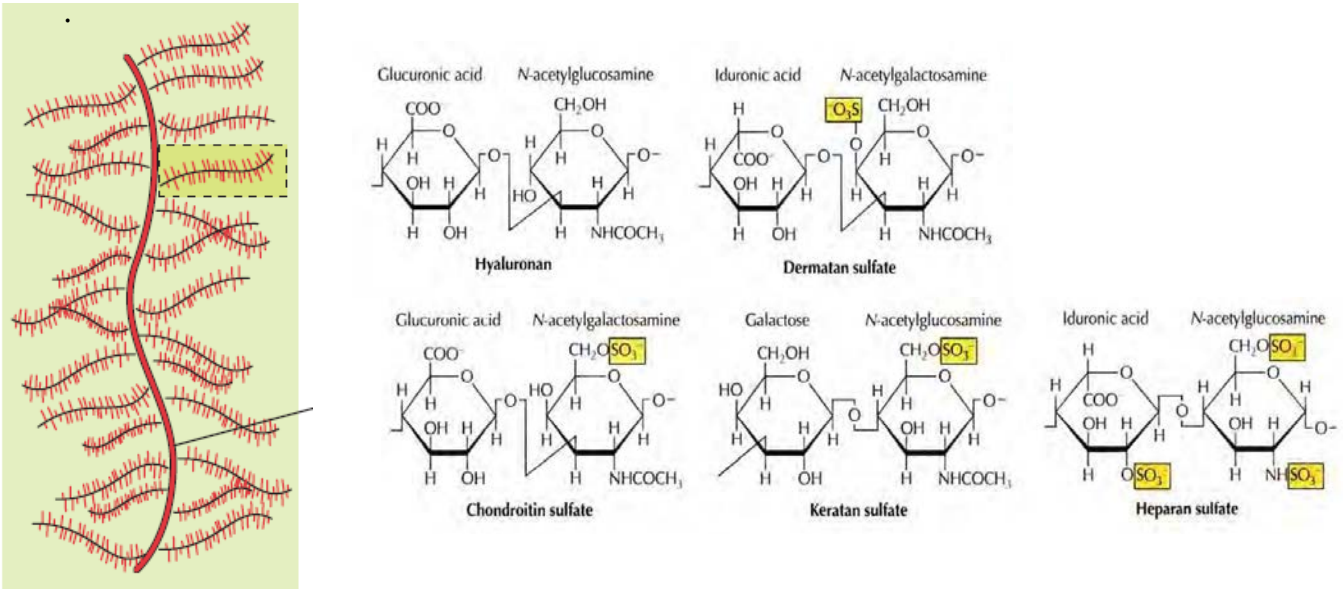


Figure 3: Proteoglycan complex with the major types of matrix glucosaminoglycans. Glycosaminoglycans consist of repeating disaccharide units. With the exception of hyaluronan, the sugars frequently contain sulfate.

Adhesion proteins of Matrix

Matrix adhesion proteins are accountable for connecting the components of the matrix to one another and to the surfaces of cells. They act together with collagen and proteoglycans to direct matrix organization and bind to integrins. The first of its kind is fibronectin, which is the main adhesion protein of connective tissues. Fibronectin is a glycoprotein with two polypeptide chains, of 2500 amino acids. Additionally fibronectin possess binding sites for both collagen and GAGs thus crosslinking these matrix. A specific site on the fibronectin molecule is responsible for recognizing cell surface receptors like integrins attaching of cells to the extracellular matrix. Prototype of adhesion proteins belong to the laminin family with the property of self assembly into mesh like networks.

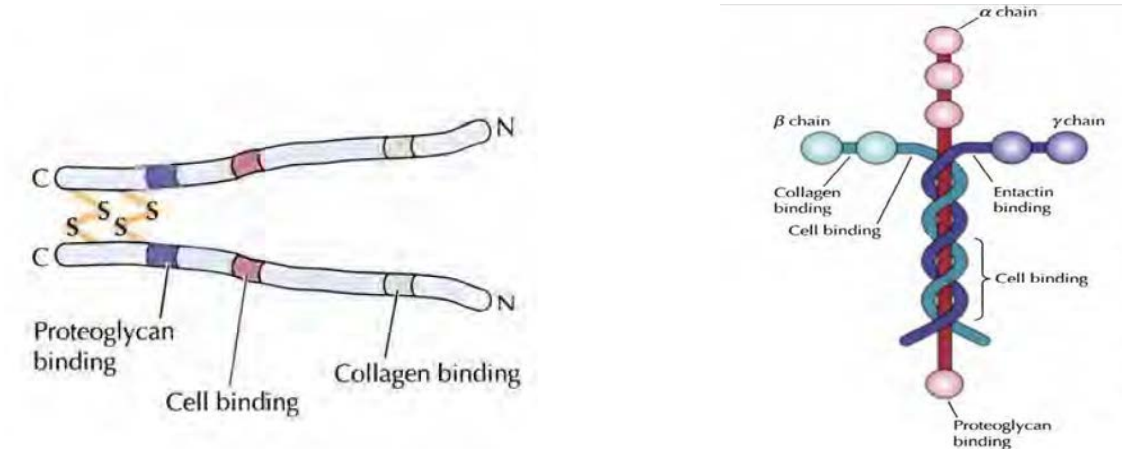


Figure 4: An illustration of matrix associated proteins. A. Fibronectin. B. Laminin

Cell matrix interaction

Cells remain attached to the extracellular matrix through the aid of cell surface receptors such as integrins. The integrins belong to the family of transmembrane proteins consisting of one α and one β subunits. The integrins bind to short amino acid sequences present in multiple components of the extracellular matrix, including collagen, fibronectin, and laminin. In addition to attaching cells to the extracellular matrix the integrins also provide anchors for the cytoskeleton resulting in stability of the cell matrix junctions. Integrins interact with the cytoskeleton at two junctions of the extracellular matrix known as the focal adhesions and hemidesmosomes. Focal adhesions attach a variety of cells, including fibroblasts, to the extracellular matrix and hemidesmosomes mediate epithelial cell attachments at with a specific integrin (Figure 5).

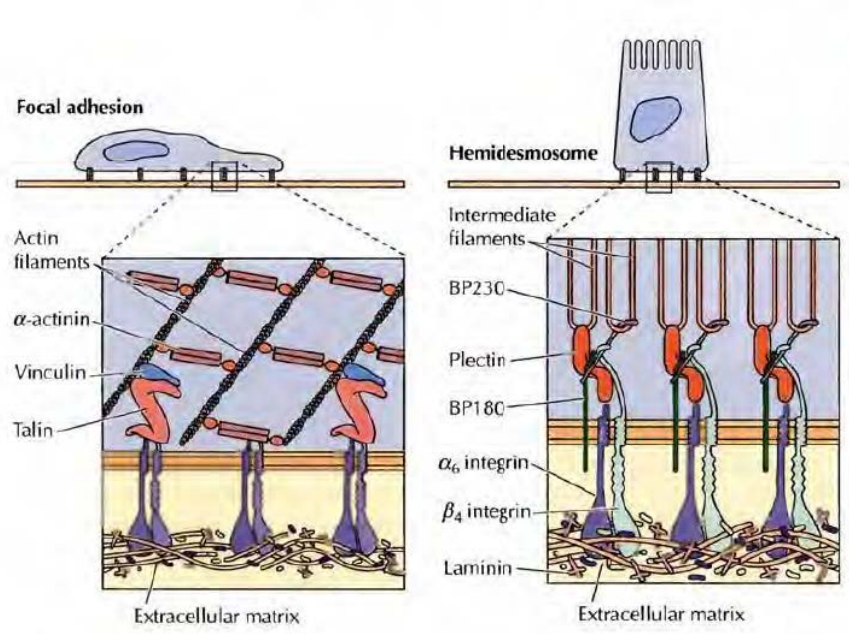


Figure 5: Cell-matrix junctions mediated by integrins. Integrins mediate two types of stable junctions the focal adhesions where bundles of actin filaments are anchored to integrins through associations with a number of other proteins, including α-actinin, talin, and vinculin. In hemidesmosomes, integrin links the basal lamina to intermediate filaments via plectin and BP230. BP180 functions in hemidesmosome assembly and stability. This figure has been printed with permission from The figure has been adapted from "The Cell, A Molecular Approach" by Geoffrey M. Cooper, 4th Ed. 2007.

Cell-matrix interaction is a step wise process and occurs through recruitment of specific junctional molecules. Focal adhesions develop from a small cluster of integrins, termed focal complexes, by the sequential recruitment of talin, vinculin, and α-actinin. This follows recruitment of formin, which initiates actin bundle formation. Myosin II then comes leads the development of tension at the point of adhesion resulting in cell signaling.

Cell to cell integration

Direct interactions between cells, as well as between cells and the extracellular matrix, are critical to the development and function of multicellular organisms. Some cell-cell interactions are transient, such as the interactions between cells of the immune system and the interactions that direct white blood cells to sites of tissue inflammation. In other cases, stable cell-cell junctions play a key role in the organization of cells in tissues. For example, several different types of stable cell-cell junctions are critical to the maintenance and function of epithelial cell sheets. Plant cells also associate with their neighbors not only by interactions between their cell walls, but also by specialized junctions between their plasma membranes called plasmodesmata.

Cell adhesion proteins

Cell-cell adhesion is a selective process, such that cells adhere only to other cells of specific types. This is accomplished with the aid of the selectin and integrin proteins. The selectins mediate the initial adhesion this is followed by the formation of more stable adhesions, in which integrins on the surface of leukocytes bind to intercellular adhesion molecules (ICAMs), which are members of the Ig superfamily expressed on the surface of endothelial cells. The fourth group of cell adhesion molecules, are the cadherins. They are not only involved in selective adhesion between embryonic cells but are also primarily responsible for the formation of stable junctions between cells in tissues. The cell-cell interactions mediated by the selectins, integrins, and members of the Ig superfamily are transient adhesions in which the cytoskeletons of adjacent cells are not

linked to one another. Stable adhesion junctions involving the cytoskeletons of adjacent cells are instead mediated by the cadherins.

Adhesion between plant cells is mediated by their cell walls rather than by transmembrane proteins. In particular, a specialized pectin-rich region of the cell wall called the middle lamella acts as a glue to hold adjacent cells together. Because of the rigidity of plant cell walls, stable associations between plant cells do not require the formation of cytoskeletal links, such as those provided by the desmosomes and adherens junctions of animal cells.

Interesting Facts

- Extracellular matrix cells have been found to cause regrowth and healing of tissue.
- Several diseases, including osteogenesis imperfecta, the Ehlers-Danlos Syndromes, the Marfan syndrome and the chondrodysplasias, have been attributed to mutations in collagens I, III, II or other structural glycoproteins of the Extra Cellular Matrix.

Q1. How is the extracellular matrix organized?

Q2. Describe in details the various components of the extra cellular matrix.

Q3. What are cadherins?

Q4. Write briefly on how cell to cell interaction possible in plants.

Q5. What are cell adhesion proteins and what are their functions ?

Q6. **Cell-cell adhesion is a selective process**

A. True

B. False

Q7. **Adhesion between plant cells is mediated by proteins**

A. True

B. False

Module 1 Lecture 9

Cell locomotion (amoeboid, flagella, cillar)

Cell Movement

Cell movement; is both internal, referred to as cytoplasmic streaming, and external, referred to as motility. Internal movements of organelles are governed by actin filaments and other components of the cytoskeleton. These filaments make an area in which organelles such as chloroplasts can move. Internal movement is known as cytoplasmic streaming. External movement of cells is determined by special organelles for locomotion. These could be pseudopodia, cilia and flagella.

Elements of cell movement

Cell movement is brought about by the cytoskeleton which is a network of connected filaments and tubules. It extends from the nucleus to the plasma membrane. Electron microscopic studies showed the presence of an organized cytoplasm. Immunofluorescence microscopy identifies protein fibers as a major part of this cellular feature. The cytoskeleton components maintain cell shape and allow the cell and its organelles to move. The cytoskeleton is composed of actin and microtubules. Actin filaments are thoroughly described in later lectures. In short, they are long, thin fibers approximately seven nm in diameter. These filaments occur in bundles or meshlike networks. These filaments are polar, meaning there are differences between the ends of the strand. An actin filament consists of two chains of globular actin monomers twisted to form a helix. Actin filaments play a structural role, forming a dense complex web just under the plasma membrane. Actin filaments in microvilli of intestinal cells act to shorten the cell and thus to pull it out of the intestinal lumen. Likewise, the filaments can extend the cell into intestine when food is to be absorbed. In plant cells, actin filaments form tracts along which chloroplasts circulate. Actin filaments move by interacting with myosin. The myosin combines with and splits ATP, thus binding to actin and changing the configuration to pull the actin filament forward. Similar action accounts for pinching off cells during cell division and for amoeboid movement.

Other components are the intermediate filaments which are between eight and eleven nm in diameter. They are between actin filaments and microtubules in size. The intermediate fibers are rope-like assemblies of fibrous polypeptides. Some of them support the nuclear envelope, while others support the plasma membrane, form cell-to-cell junctions. Similarly, microtubules are small hollow cylinders (25 nm in diameter and from 200 nm-25 µm in length). These microtubules are composed of a globular protein tubulin. Assembly brings the two types of tubulin (alpha and beta) together as dimers, which arrange themselves in rows.

Cilia and Flagella

Cilia and flagella are micro tubular projections of the plasma membrane responsible for movement of a variety of eukaryotic cells. Many bacteria also have flagella, but these prokaryotic flagella are quite different from those of eukaryotes. Bacterial flagella are protein filaments projecting from the cell surface, rather than projections of the plasma membrane supported by microtubules. Cilia are short, usually numerous, hairlike projections that can move in an undulating fashion (e.g., the protzoan Paramecium, the cells lining the human upper respiratory tract). Flagella are longer, usually fewer in number, projections that move in whip-like fashion (e.g., sperm cells). Cilia and flagella grow by the addition of tubulin dimers to their tips.

Eukaryotic cilia and flagella are very similar structures, each with a diameter of approximately 0.25 pm. Many cells are covered by numerous cilia, which are about 10 pm in length. Cilia beat in a coordinated back-and-forth motion. For example, the cilia of some protozoans (such as Paramecium) are responsible both for cell motility and for sweeping food organisms over the cell surface and into the oral cavity. In animals, an important function of cilia is to move fluid or mucus over the surface of epithelial cell sheets. A good example is provided by the ciliated cells lining the respiratory tract, which clear mucus and dust from the respiratory passages. Flagella differ from cilia in their length (they can be as long as 200 pm) and in their wavelike pattern of beating. Cells usually have only one or two flagella, which are responsible for the locomotion of a variety of protozoans and of sperm.

Occurrence:

The flagella occur in the protozoans of the class Flagellata, choanocyte cells of the sponges, spermatozoa of the Metazoa and among plants in the algae and gamete cells. The cilia occur in the protozoans of the class Ciliata and members of other classes and ciliated epithelium of the Metazoa. The cilia may occur on external body surface and may help in the locomotion of such animals as the larvae of certain Platyhelminthes, Nemertines, Echinodermata, Mollusca and Annelida. The cilia may line the internal cavities or passages of the metazoan bodies as air passage of the respiratory system and reproductive tracts. The nematode worms and arthropods
have no cilia. Except for sperm, the cilia in mammalian systems are not organelles of locomotion. But their effect is the same, that is, to move the environment with respect to the cell surface.

Arrangement:

Different species of bacteria have different numbers and arrangements of flagella. Monotrichous bacteria have a single flagellum. Lophotrichous bacteria have multiple flagella located at the same spot on the bacteria's surfaces. Amphitrichous bacteria have a single flagellum on each of two opposite ends. Peritrichous bacteria have flagella projecting in all directions.

Structure

Cilia and flagella is made of the axoneme **(Figure 1)** which is composed of microtubules and their associated proteins. The microtubules are arranged in a characteristic "9 + 2" pattern in which a central pair of microtubules is surrounded by nine outer microtubule doublets (Figure 1). The two fused microtubules of each outer doublet are distinct: One (called the A tubule) is a complete microtubule consisting of 13 protofilaments; the other (the B tubule) is incomplete, containing only 10 or 11 protofilaments fused to the A tubule. The outer microtubule doublets are connected to the central pair by radial spokes and to each other by links of a protein called nexin. In addition, two arms of dynein are attached to each A tubule. It is the motor activity of these axonemal dyneins that drives the beating of cilia and flagella. The minus ends of the microtubules of cilia and flagella are anchored in a basal body, which is similar in structure to a centriole and contains nine triplets of microtubules. Basal bodies thus serve to

initiate the growth of axonemal microtubules as well as anchoring cilia and flagella to the surface of the cell.

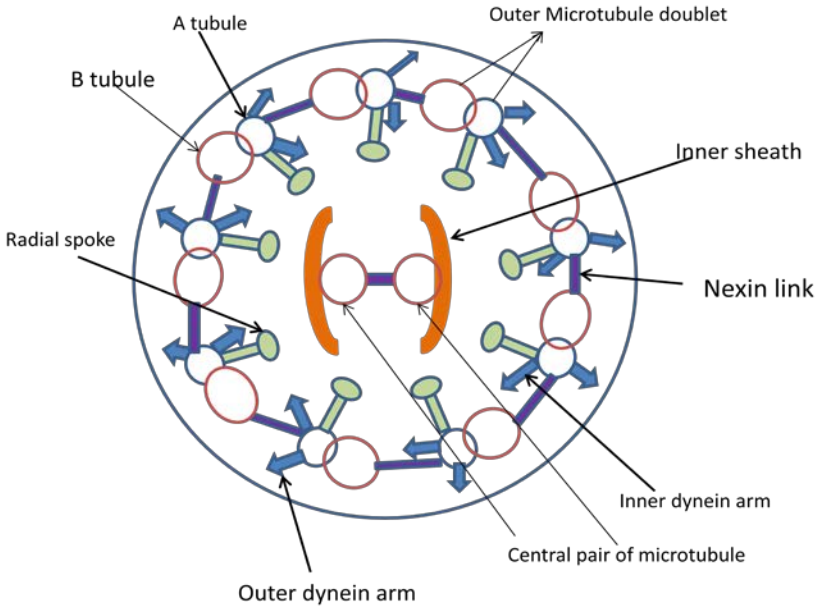


Figure 1: Structure of axoneme of cilia and flagella

Movement:

Generally speaking flagella work as whips pulling (as in *Chlamydomonas* or *Halosphaera*) or pushing (dinoflagellates, a group of single-celled Protista) the organism through the water. Cilia work like oars on a viking longship (*Paramecium* has 17,000 such oars covering its outer surface). Figure 1 illustrates the movement of cilia and flagella. More precisely the movements of cilia and flagella result from the sliding of outer microtubule doublets relative to one another, powered by the motor activity of the axonemal dyneins. The dynein bases bind to the A tubules while the dynein head groups bind to the B tubules of adjacent doublets. Movement of the dynein head groups in the minus end direction then causes the A tubule of one doublet to slide toward the basal end of the adjacent B tubule. Because the microtubule doublets in an axoneme are connected by nexin links, the sliding of one doublet along another causes them to bend, forming the basis of the beating movements of cilia and flagella. It is apparent, however, that the activities of dynein molecules in different regions of the axoneme must be carefully regulated to produce the coordinated beating of cilia and the wavelike oscillations of flagella-a process about which little is currently understood. Another important thing is

that counterclockwise rotation of monotrichous polar flagella pushes the cell forward with the flagella trailing behind, much like a corkscrew moving inside cork. Indeed water in the microscopic scale is highly viscous, very different from our daily experience of water. The flagella are left-handed helices, and bundle and rotate together only when rotating counterclockwise. When some of the rotors reverse direction, the flagella unwind and the cell starts "tumbling" (see Figure 2).

The beating of cilia or flagella is caused by the intraciliary excitation which is followed by the interciliary conduction. Recent studies have shown that cytoplasm is necessary for the ciliary movements. The ATP provides necessary amount of energy for the motion of the cilia and flagella. Four types of ciliary movements have been recognized which are as follows :

1. The pendulus ciliary movement: The pendulus type of ciliary movement is carried out in asingle plane. It occurs in the ciliated protozoans which have rigid cilia.

2. The unciform ciliary movement: The unciform (hook-like) ciliary movement occurs commonly in the metazoan cells.

3. The infundibuliform ciliary movement: The infundibuliform ciliary movement occurs due to the rotary movement of the cilium and flagellum.

4. The undulant movement: The undulant movement is the characteristic of the flagellum. In undulant movement the waves of the contraction proceed from the site of implantation and pass to the border.

Each beat of cilium or flagellum involves the same pattern of microtubule movement. Each cilium moves with a whip-like motion and its beat may be divided into two phases:

1. The fast effective stroke (or forward active stroke or power stroke) in which the cilium is fully extended and beating against the surrounding liquid.

2. The slow recovery stroke, in which the cilium returns to its original position with an unrolling movement that minimizes viscous drag.

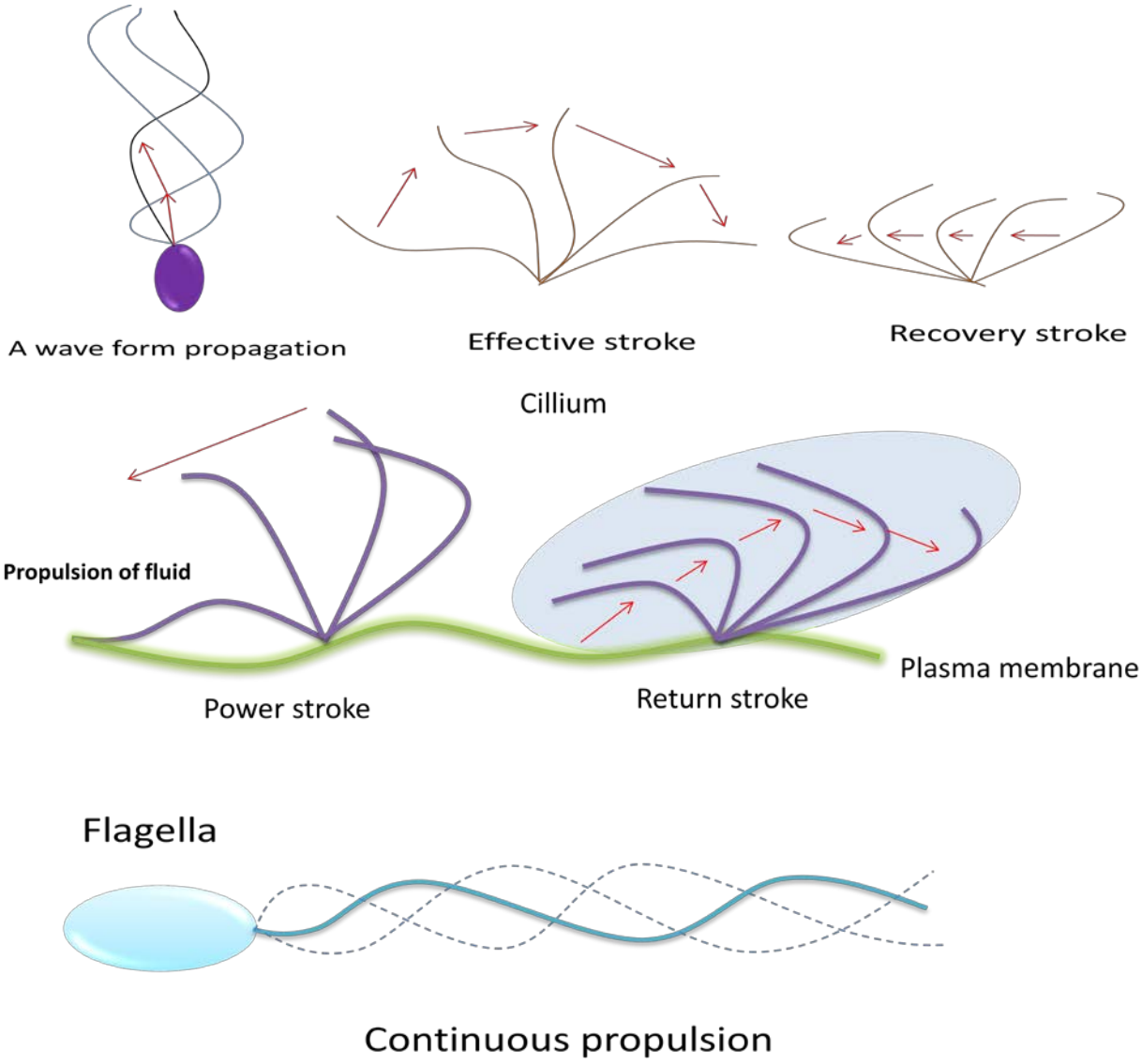


Figure 2: Ciliary anf flagellar movement

The mechanism of force and movement (bending) by the flagellum has recently been studied extensively. It is well established now that the ciliary movement is generated by the microtubules and the associated structures of the flagellum. It was shown that the cell free flagella can be caused to move by adding an energy source such as ATP. Even broken pieces of cilia or isolated axoneme itself continue to beat, suggesting the role of microtubules in the movement. The contractile axostyle of some microorganisms such as *Metamonadida*. Bending force is produced by the sliding of microtubules.

Recent experimental work on ciliary motion has shown notable similarities with the sliding mechanism involved in the interaction of actin and myosin in muscle. The dynein arms attached to subfibre A have been compared with the cross bridges of myosin and it has been postulated that they form intermittent attachments, by which one doublet (N1) is able to push the adjacent one (N1 + 1) toward the tip of the axoneme. Under normal conditions, the attachment of subfibre A of N to subfibre B of N + 1 by dynein arms is not observed in an intact cilium. Only when the ciliary membrane is extracted with a detergent, the axoneme enters in a state of rigor in which the attachment is produced. Addition of ATP to axonemes in the state of rigor restores motility and causes release of the dynein arm. In this mechano-chemical cycle, the next step would be reextension of the dynein arm and its rebinding at an angle, with a new, more proximal site on subfibre B. This step involves the hydrolysis of ATP to ADP + Pi. In the last step, the arm returns to the rigor position and displacement of the doublets results. Force is generated when dynein arms move. The movement of sliding is converted to bending by virtue of radial spokes that bridge each other doublet to the inner pair of microtubules **(**Figure 3).

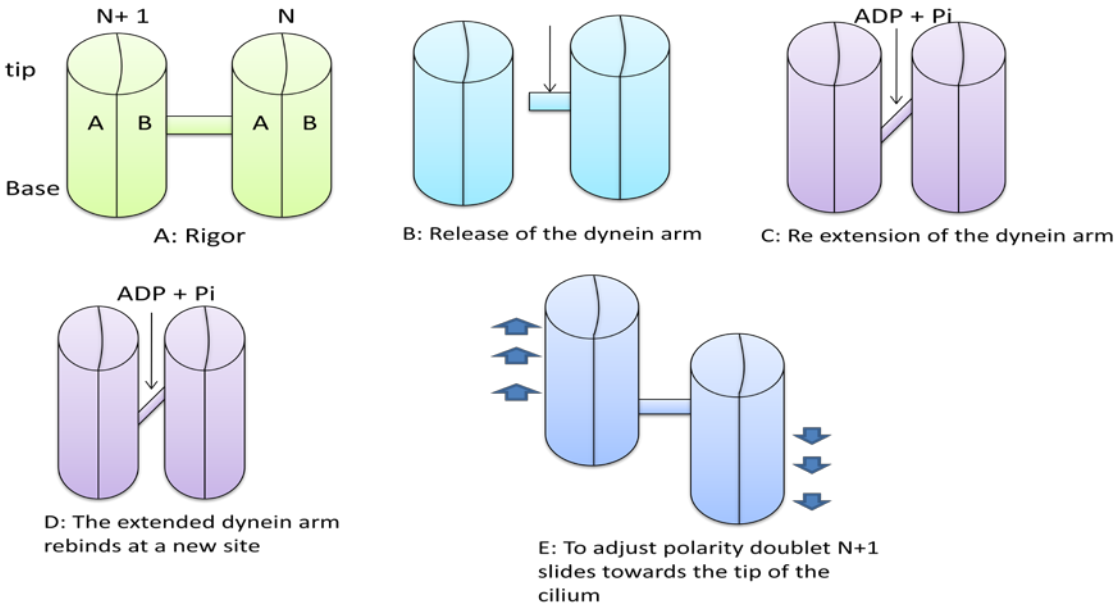


Figure 3: Schematic representation of the mechanochemical cycle involved in sliding of filament in ciliary movement.

The overall structure of bacterial flagella

The bacterial flagellum (Figure 4) is made up of the protein flagellin. Its shape is a 20 nanometer thick hollow tube. It is helical and has a sharp bend just outside the outer membrane which is called the hook. It allows the axis of the helix to point directly away from the cell. A shaft runs between the hook and the basal body, passing through protein rings in the cell's membrane that act as bearings. Gram-positive organisms have 2 of these basal body rings, one in the peptidoglycan layer and one in the plasma membrane. Gram-negative organisms have 4 such rings: the L ring associates with the lipopolysaccharides, the P ring associates with peptidoglycan layer, the M ring is embedded in the plasma membrane, and the S ring is directly attached to the plasma membrane. The filament ends with a capping protein. The bacterial flagellum is driven by a rotary engine (the Mot complex) made up of protein, located at the flagellum's anchor point on the inner cell membrane. The engine is powered by proton motive force, i.e., by the flow of protons (hydrogen ions) across the bacterial cell membrane due to a concentration gradient set up by the cell's metabolism. The rotor transports protons across the membrane, and is turned in the process.

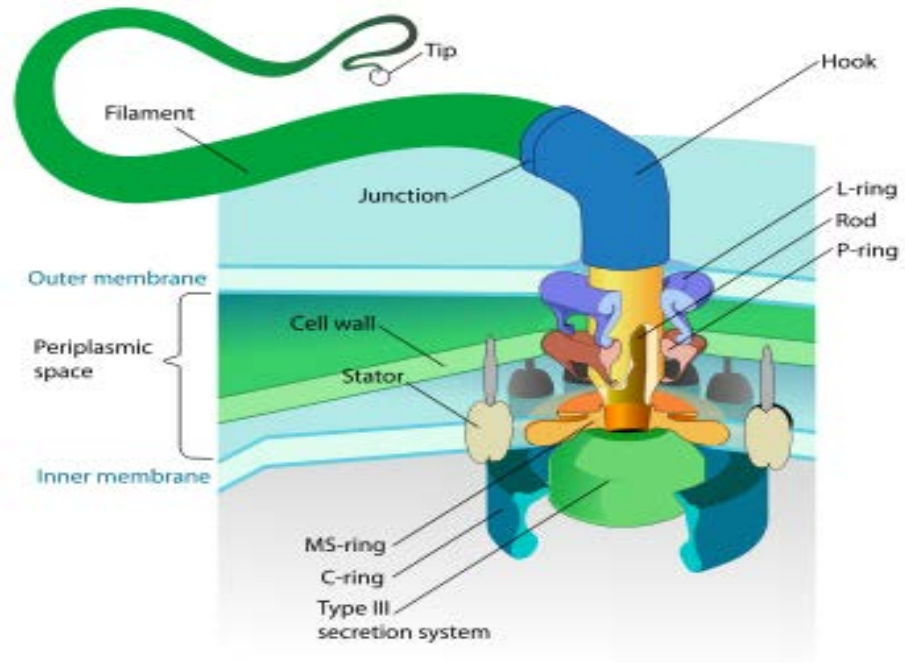


Figure 4: Flagellum of gram negative bacteria

During flagellar assembly, components of the flagellum pass through the hollow cores of the basal body and the nascent filament. During assembly, protein components are added at the flagellar tip rather than at the base. In vitro, flagellar filaments assemble spontaneously in a solution containing purified flagellin as the sole protein.

The flagellar filament is the long helical screw that propels the bacterium when rotated by the motor, through the hook. In most bacteria that have been studied, including the Gram negative *Escherichia coli*, *Salmonella typhimurium*, *Caulobacter crescentus*, and *Vibrio alginolyticus*, the filament is made up of eleven protofilaments approximately parallel to the filament axis. Each protofilament is a series of tandem protein chains. However in *Campylobacter jejuni*, there are seven protofilaments. The basal body has several traits in common with some types of secretory pores, such as the hollow rod-like "plug" in their centers extending out through the plasma membrane. Given the structural similarities between bacterial flagella and bacterial secretory systems, it is thought that bacterial flagella may have evolved from the type three secretion system; however, it is not known for certain whether these pores are derived from the bacterial flagella or the bacterial secretory system.

Other Functions:

1. The ciliary or flagellar movement provides the locomotion to the cell or organism.
2. The cilia create food currents in lower aquatic animals.
3. In the respiratory tract, the ciliary movements help in the elimination of the solid particles
from it.
4. The eggs of amphibians and mammals are driven out from the oviduct by the aid of vibratile
cilia of the latter.

Thus, the cilia and flagella serve many physiological processes of the cell, such as locomotion,
alimentation, circulation, respiration, excretion and perception of sense.

Amoeboid movement

Amoeboid movement is a type of movement accomplished by protrusion of cytoplasm of the cell involving the formation of pseudopodia. The cytoplasm slides and forms a pseudopodium in front to move the cell forward. This type of movement has been linked to changes in action potential; the exact mechanism is still unknown. This type of movement is observed in amoeboids, slime molds and some protozoans, as well as some cells in humans such as leukocytes. Sarcomas, or cancers arising from connective tissue cells, are particularly adept at amoeboid movement, thus leading to their high rate of metastasis. Locomotion of amoeba occurs due the sol-gel conversion of the cytoplasm within its cell. The ectoplasm is called the plasma gel and the endoplasm the plasma sol. The conversion of the endoplasm to ecto and vice versa is called sol-gel conversion.

Pseudopodia

All cells do not use cilia or flagella are for movement. Some, such as *Amoeba*, Chaos (*Pelomyxa*) and human leukocytes (white blood cells), employ pseudopodia to move the cell. Unlike cilia and flagella, pseudopodia are not structures, but rather are associated with actin near the moving edge of the cell. They are temporary projections of eukaryotic cells. Pseudopodia extend and contract by the reversible assembly of actin subunits into microfilaments. Filaments near the cell's end interact with myosin which causes contraction. The pseudopodium extends itself until the actin reassembles itself into a network. This is how amoebas move, as well as some cells found in animals, such as white blood cells.

Pseudopods can be classified into several types:

1. Lobopodia is bulbous, short and blunt in form as in *Amoebozoa*. These finger-like, tubular pseudopodia contain both ectoplasm and endoplasm.

2. Filopodia is more slender and filiform with pointed ends, consisting mainly of ectoplasm. These formations are supported by microfilaments as in *Euglypha*.

3. Reticulopodia is complex formations where individual pseudopods are blended together and form irregular nets. The primary function of reticulopodia, also known as myxopodia, is the ingestion of food, and the secondary function is locomotion.

4. Axopodia are thin pseudopods of complex arrays of microtubules enveloped by cytoplasm. They are mostly responsible for phagocytosis by rapidly retracting in response to physical contacts.

Interesting Facts:

- The first detailed chemical analysis of the protein components of the cilia of Tetrahymena pyriformis was conducted by I. R. Gibbons in 1963.
- In Chlamydomonas several mutational defects have been studied in the axoneme of flagellum which may lead to paralysis of the flagellar function.
- The cilia are modified into a variety of structures such as the rods and cones of the retina, crown cell of saccus vasculosus of third ventricle of fishes, primitive sensory cells of the pineal eye and cnidocil of the nematocysts of the coelenterates.

Questions

1. Organelles found outside a eukaryotic cell and usually involved in movement of the cell or movement of substances past the cell are called

A. cilia and flagella

B. Cell walls and plasmodesmata

C. Nucleus and nucleolus

D. cytoplasm and endoplasm

2. A scraping of material from a person's tooth revealed many bacteria found on the tooth surface. Such bacteria remain attached to the tooth surface by structures called

A. pili

B. anchoring junctions

C. mitochondria

D. flagella

3. A slippery outer covering in some bacteria that protects them from phagocytosis by host cells is

A. capsule

B. cell wall

C. flagellum

D. peptidoglycan

4. When flagella are distributed all around a bacterial cell, the arrangement is called

A. polar

B. random

C. peritrichous

D. encapsulated

5. Bacteria may be propelled by

A. rotating thread-like flagellum

B. cilia

C. undulating 9+2 type flagellum

D. gel-sol changes in the cytoplasm

E. an undulating thread-like flagellum

6. The microtubules of cilia and flagella are organized in a characteristic 9 + 2 pattern, and they slide past one another.

A. True

B. False

7. Bacterial flagella propel the cell by using

A. a whipping-like motion

B. two flagella that move in opposite directions, like a flutter kick

C. a rotating motion

D. a flicking motion

E. none of the above

8. Which characteristic do eukaryotic and prokaryotic flagella have in common?

A. chemical composition

B. structure

C. location in the cell

D. function

E. source of energy

9. Differentiate between cilia and flagella. Describe the structure of the axoneme.

10. Describe the types of pseudopodia with their functions.

11. What are protofilaments?

12. Describe the structure of bacterial flagellum.

Module 1 Lecture 10

After studying all about cell lets study how cells give rise to a new cell. During the current lecture we will be discussing types of cell division and its various phases.

Cell division and its significance:

Continuity of life depends on cell division. All cells are produced by divisions of pre-existing cell (Please recall our discussion about the cell theory in our first lecture). A cell born after a division, proceeds to grow by macromolecular synthesis, and divides after reaching a species-determined division size. Growth of a cell is an increase in size or mass which is an irreversible process that occurs at all organizational levels.

Cell cycle:

Cell cycle can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next division. Cells have the property of division and multiplication and consist of three major phases namely mitosis (M phase) or the nuclear division, cytokinesis or the division of the cell and interphase where replication of genetic material occurs. The M phase lasts only for an hour in a period of 24 hour required for a eukaryotic cell to divide. The interphase can be further divided into G1 (gap phase 1), S (synthesis) and G2 (gap phase 2) phases (Figure 1). This division of interphase into three separate phases based on the timing of DNA synthesis was first proposed in 1953 by Alma Howard and Stephen Pelc of Hammersmith Hospital, London, based on their experiments on plant meristem cells. Cell cycles can range in length from as short as 30 minutes in a cleaving frog embryo, whose cell cycles lack both G1 and G2 phases, to several months in slowly growing tissues, such as the mammalian liver. Cells that are no longer capable of division, whether temporarily or permanently, remain in G0 phase. A cell must receive a growth-promoting signal to proceed from the quiescent stage or G0 into G1 phase and thus reenter the cell cycle.

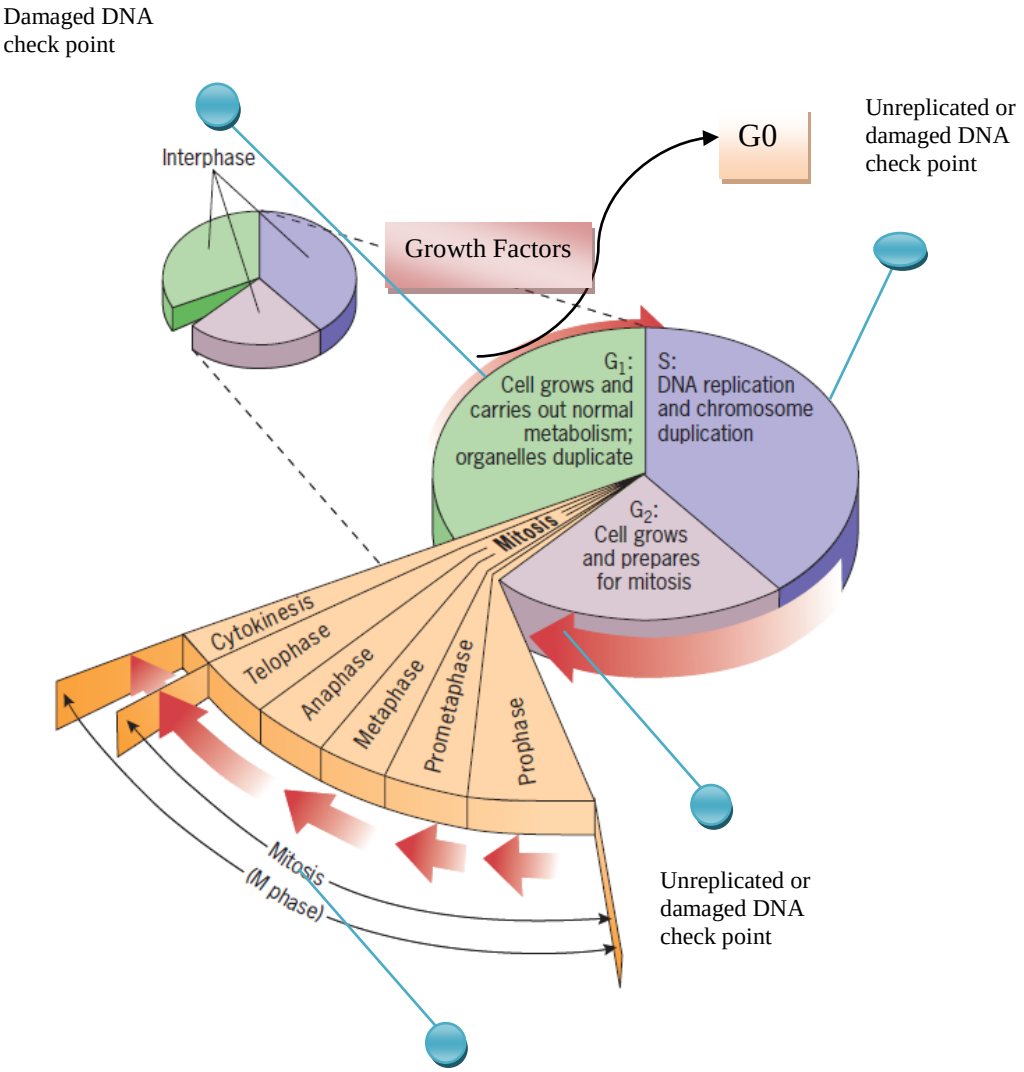


Figure 1: An overview of the cell cycle.

This figure has been adapted with permission from Cell and Molecular Biology Concepts and Experiments by Karp, 2010.

Interphase: During interphase the chromosomes are not visible with a light microscope when the cell is not undergoing mitosis. The genetic material (DNA) in the chromosomes is replicated during the period of interphase to carry out mitosis and is called S phase (S stands for *synthesis* of DNA). DNA replication is accompanied by chromosome duplication. Before and after S, there are two periods, called G1 and G2, respectively, in which DNA replication does not take place. The order of cell cycle events is G1 → S → G2 → M and then followed by cytokinesis. The G1 phase, S phase and G2 phase together form the interphase.

Events of Interphase: The interphase is characterized by the following features: The nuclear envelope remains intact. The chromosomes occur in the form of diffused, long, coiled and indistinctly visible chromatin fibres. The DNA amount becomes double. Due to accumulation of ribosomal RNA (rRNA) and ribosomal proteins in the nucleolus, the size of the latter is greatly increased. In animal cells, a daughter pair of centrioles originates near the already existing centriole and, thus, an interphase cell has two pairs of centrioles. In animal cells, net membrane biosynthesis increases just before cell division (mitosis). This extra membrane is stored as blebs on the surface of the cells about to divide. Events in interphase takes place in three distinct phases.

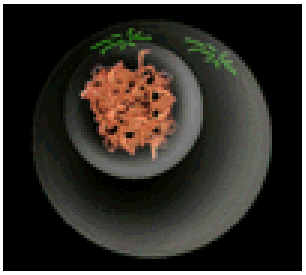


Fig.2: Interphase

G1 Phase: After the M phase of previous cell cycle, the daughter cells begin G1 of interphase of new cell cycle. G1 is a resting phase. It is also called first gap phase, as no DNA synthesis takes place during this stage. It is also known as the first growth phase, since it involves synthesis of RNA, proteins and membranes which leads to the growth of nucleus and cytoplasm of each daughter cell towards their enhancing size. During G1 phase, chromatin is fully extended and not distinguishable as discrete chromosomes with the light microscope. Thus, it involves transcription of three types of RNAs, namely

rRNA, tRNA and mRNA; rRNA synthesis is indicated by the appearance of nucleolus in the interphase (G1 phase) nucleus. Proteins synthesized during G1 phase (a) regulatory proteins which control various events of mitosis (b) enzymes (DNA polymerase) necessary for DNA synthesis of the next stage and (c) tubulin and other mitotic apparatus proteins. G1 phase is most variable as to duration it either occupies 30 to 50 per cent of the total time of the cell cycle. *Terminally differentiated somatic cells (end cells such as neurons and striated muscle cells) that no longer divide, are arrested usually in the G1 stage, such a type of G1 phase is called G0 phase.*

S phase: During the S phase or synthetic phase of interphase, replication of DNA and synthesis of histone proteins occur. New histones are required in massive amounts immediately at the beginning of the S period of DNA synthesis to provide the new DNA with nucleosomes. At the end of S phase, each chromosome has two DNA molecules and a duplicate set of genes. S phase occupies roughly 35 to 45 per cent time of the cell cycle.

G2 phase: This is a second gap or growth phase or resting phase of interphase. During G2 phase, synthesis of RNA and proteins continues which is required for cell growth. It may occupy 10 to 20 per cent time of cell cycle. As the G2 phase draws to a close, the cell enters the M phase.

Dividing phase: There are two types of cell division possible. Mitosis and meosis. The mitosis (Gr., *mitos*=thread) occurs in the somatic cells and it is meant for the multiplication of cell number during embryogenesis and blastogenesis of plants and animals. Fundamentally, it remains related with the growth of an individual from zygote to adult stage. Mitosis starts at the culmination point of interphase (G2 phase). It is a short period of chromosome condensation, segregation and cytoplasmic division. Mitosis is important for growth of organism, replacement of cells lost to natural friction or attrition, wear and tear and for wound healing. Hence, mitosis is remarkably similar in all animals and plants. It is a smooth continuous process and is divided into different stages or phases.

Mitosis

Mitosis is a process of cell division in which each of two identical daughter cells receives a diploid complements of chromosomes same as the diploid complement of the parent cell. It is usually followed by cytokinesis in which the cell itself divides to yield two identical daughter cells.

The basics in mitosis include:

1. Each chromosome is present as a duplicated structure at the beginning of nuclear division (2n).

2. Each chromosome divides longitudinally into identical halves and become separated from each other.

3. The separated chromosome halves move in opposite directions, and each becomes included in one of the two daughter nuclei that are formed.

Mitosis is divided into four stages: prophase, metaphase, anaphase and telophase. The stages have the following characteristics:

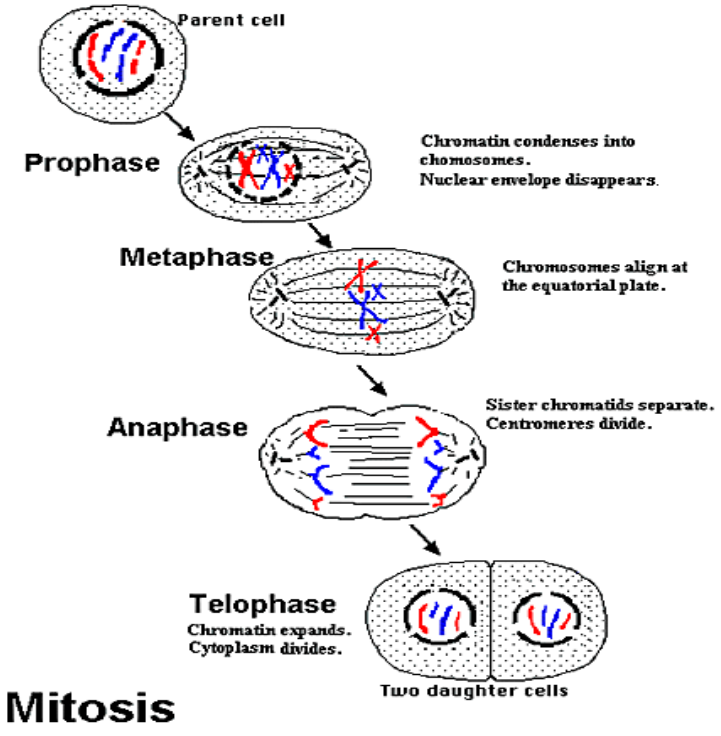


Fig.3: Mitosis cell cycle

1. **Prophase:**

The chromosomes are in the form of extended filaments and cannot be seen with a light microscope as discrete bodies except for the presence of one or more dark bodies (i.e. nucleoli) in the interphase stage. The beginning of prophase is marked by the condensation of chromosomes to form visibly distinct, thin threads within the nucleus. Each chromosome is already longitudinally double, consisting of two closely associated subunits called chromatids which are held together by centromere. Each pair of chromatids is the product of the duplication of one chromosome in the S period of interphase. As prophase progresses, the chromosomes become shorter and thicker as a result of intricate coiling. At the end of prophase, the nucleoli disappear and the nuclear envelope, a membrane surrounding the nucleus, abruptly disintegrates.

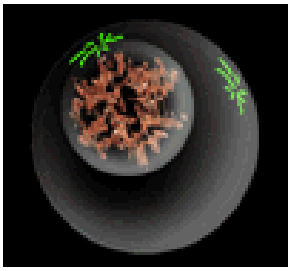


Fig.4: Prophase

2. **Metaphase:**

At the beginning of metaphase, the mitotic spindle forms which are a bipolar structure and consist of fiber-like bundles of microtubules that extend through the cell between the poles of the spindle. Each chromosome attached to several spindle fibers in the region of the centromere. The structure associated with the centromere to which the spindle fibers attach is known as the kinetochore. After the chromosomes are attached to spindle fibers, they move towards the center of the cell until all the kinetochores lie on an imaginary plane equidistant from the spindle poles. This imaginary plane is called the metaphase plate. Hence the chromosomes reach their maximum contraction and are easiest to count and examine for differences in morphology. The signal for chromosome alignment comes from the kinetochore, and the chemical nature of the signal seems to be the dephosphorylation of certain kinetochore-associated proteins. The role of the kinetochore is demonstrated by the finding that metaphase is not delayed by an unattached chromosome whose kinetochore has been destroyed by a focused laser beam. The role of

dephosphorylation is demonstrated through the use of an antibody that reacts specifically with some kinetochore proteins only when they are phosphorylated. Unattached kinetochores combine strongly with the antibody, but attachment to the spindle weakens the reaction. In chromosomes that have been surgically detached from the spindle, the antibody reaction with the kinetochore reappears. Through the signaling mechanism, when all of the kinetochores are under tension and aligned on the metaphase plate, the metaphase checkpoint is passed and the cell continues the process of division.

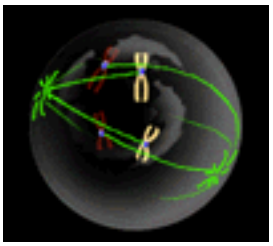


Fig.5: Prometaphase

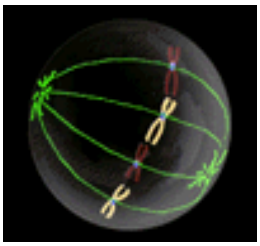


Fig. 6: Metaphase

3. Anaphase:

In anaphase, the centromeres divide longitudinally, and the two sister chromatids of each chromosome move toward opposite poles of the spindle. Once the centromere divide, each sister chromatid is treated as a separate chromosome. Chromosome movement results from progressive shortening of the spindle fibers attached to the centromeres, which pulls the chromosomes in opposite directions toward the poles. At the completion of anaphase, the chromosomes lie in two groups near opposite poles of the spindle. Each group contains the same number of chromosomes that was present in the original interphase nucleus.

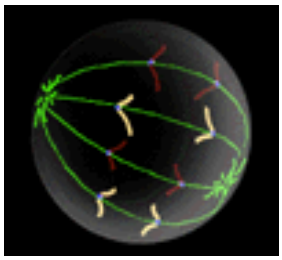


Fig.7: Anaphase

4. Telophase:

In telophase, a nuclear envelope forms around each group of chromosomes, nucleoli are formed, and the spindle disappears. The chromosomes undergo a reversal of condensation until and unless they are no longer visible as discrete entities. The two daughter nuclei slowly goes to interphase stage the cytoplasm of the cell divides into two by means of a gradually deepening furrow around the periphery.

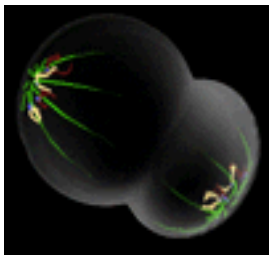


Fig.8: Telophase

5. Cytokinesis:

The chromosomes moved close to the spindle pole regions, and the spindle mid-zone begins to clear. In this middle region of the spindle, a thin line of vesicles begins to accumulate. This vesicle aggregation is an indication to the formation of a new cell wall that will be situated midway along the length of the original cell and hence form boundary between the newly separating daughter cells.

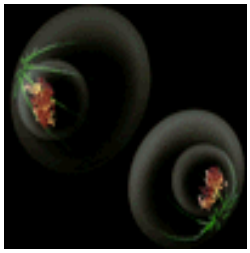


Fig.9: Cytokinesis

Interesting Facts:

- The drug Colchicine arrests cell cycle proression.
- A disregulation of the cell cycle components may lead to tumor formation.
- Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, serum starvation and treatment with thymidine or aphidicolin halt the cell in the G1 phase.
- Some organisms can regenerate body parts by mitosis. For example, starfish regenerate lost arms through mitosis.
- Some organisms produce genetically similar offspring through asexual reproduction. For example, the hydra.
- Although errors in mitosis are rare, the process may go wrong, especially during early cellular divisions in the zygote.
- Endomitosis is a variant of mitosis without nuclear or cellular division, resulting in cells with many copies of the same chromosome occupying a single nucleus.

Questions:

Q1. If a person dies from ruptured aorta and is found to have a history of such deaths in family. The gene for what protein is likely to be mutated in this patient?

A. fibronectin
B. heparin
C. proteoglycan aggregate
D. fibrillin

Q2. When a benign adenoma becomes a metastatic adenocarcinoma, which group of molecules are almost certainly degraded by the tumor cells?

A. collagen type I, II and III
B. fibronectin and b2 integrins
C. type IV collagen and laminin
D. elastin, type IX collagen, and selectins

Q3. Chromosomes are duplicated during which phase of the cell cycle?

A. G1 phase
B. G2 phase
C. S phase
D. metaphase
E. prophase

Q4. If a cell is in G2 then-------

A. it has twice the amount of DNA present in a telophase nucleus.
B. it has visibly distinct chromosomes.
C. it lacks a visible nuclear membrane.
D. it is in mitosis.
E. it is in cytokinesis.

Q5. The _____ is responsible for the separation of the chromosomes during _____ of mitosis.

A. cell wall; anaphase
B. flagellum; metaphase
C. mitotic spindle; anaphase
D. kinetochore; prophase
E. centromere; telophase

Q6. The ____ surrounds the cell like a belt, preventing the passage of substances between the cells.

A. gap junction
B. desmosome
C. hemidesmosome
D. tight junction

Q7. During which stage does DNA replication occur?

A. Prophase.
B. Anaphase.
C. Metaphase.
D. None of those above.

Q8. Which of the following is NOT correct?

A. Mitosis is produces genetically identical cells.
B. Cytokinesis is a part of mitosis
C. Metaphase occurs before anaphase.
D. All somatic cells are produced by mitosis.

Q9. Match the terms with the appropriate stages in the answer: Migration, Shortening and Thickening, Cytokinesis, Prophase.

A. Telophase, Anaphase, Prophase, centrioles forming.
B. Anaphase, Prophase, Metaphase, microtubules.
C. Anaphase, Prophase, Telophase, centrioles forming.

D. Metaphase, Anaphase, Telophase, microtubules.

Further reading:

1. Alberts B, Johnson A, Lewis J, et al. 2008. Molecular Biology of the Cell (5th ed.). Garland Science. USA.
2. Karp G. 2010. Cell and Molecular Biology: Concepts and Experiments, John Wiley & Sons, Inc. USA.
3. Cooper G M, Hausman R E. 2007. The Cell: A Molecular Approach (4th ed.). ASM Press,Washington, D.C.

Module 1 Lecture 11

Meiosis

Meiosis

In the last chapter you studied about mitosis as cell division. Meiosis is the second type of cell division occurring in the gametic cells. Meiosis was first described by the German biologist Oscar Hertwig in 1876 in the sea urchin egg. Meiosis is the process of cell division that occurs only in the germ cells of eukaryotes unlike mitosis which takes place in the somatic cells. Unlike mitosis meiosis is only initiated once in the life cycle of eukaryotes **(John 1990)**. The cells produced by meiosis are known as gametes or spores. Meiosis leads to reduction of chromosome number, of a diploid cell (2n) to half (n). Meiosis begins with one diploid cell containing two copies of each chromosome and ultimately produces four haploid cells containing one copy of each chromosome which have undergone recombination, giving rise to genetic diversity in the offspring. High order transcriptional and translational control of genes known as "meiome" controls the events of meiosis **(Snustad 2008)**.

Cell cycle and Meiosis

The preparatory steps that lead up to meiosis are identical in pattern to mitosis and occurs in the interphase of the mitotic cell cycle. Interphase is followed by meiosis I and then meiosis II.

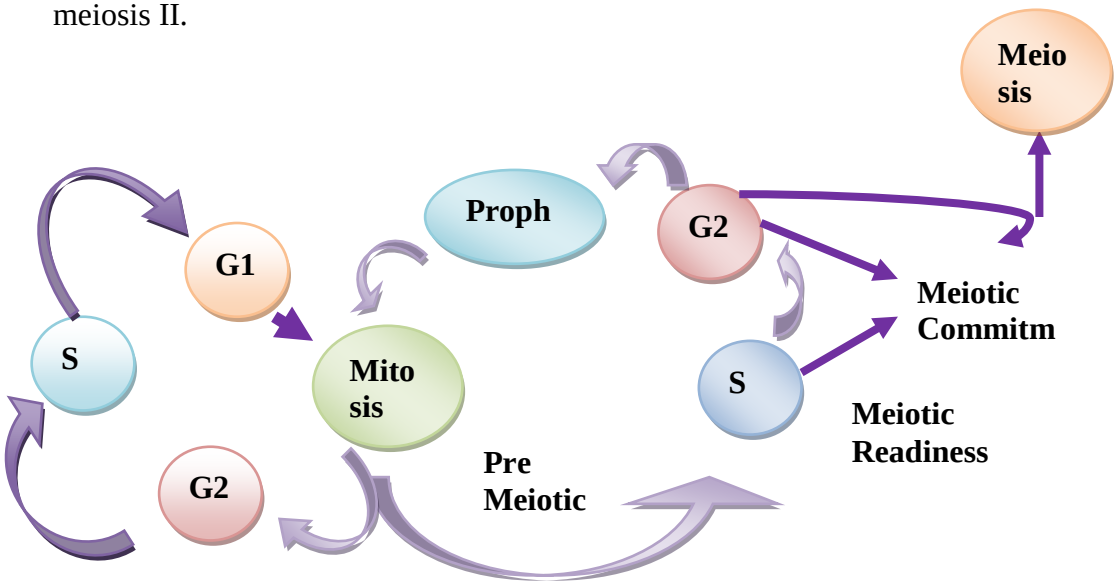


Fig 1: Position of meiosis in the Cell cycle.

Stages of meiosis

Meiosis can be separated into two phases which are meiosis I and meiosis II and they can be further subdivided into numerous phases which have particular identifiable features. They have been broadly described in the following sections.

Meiosis I

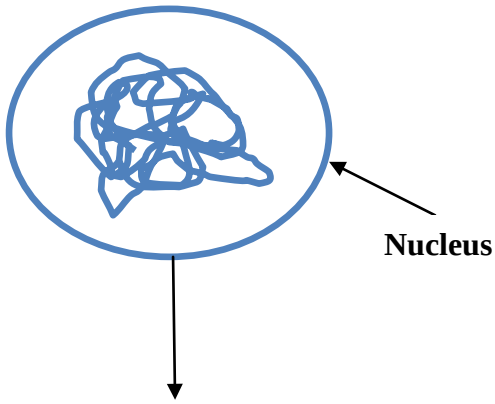
In meiosis I, chromosomes in a diploid cell segregate, producing four haploid cells generating genetic diversity. The stages of meiosis I are:

A. Prophase I

During this phase DNA is exchanged between homologous chromosomes or sister chromatids in a process called homologous recombination. The replicated chromosomes are called bivalents and have two chromosomes and four chromatids, with one chromosome coming from each parent. This phase can be further subdivided into Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis. The different stages have been pictorially presented in the following section.

1. Leptotene

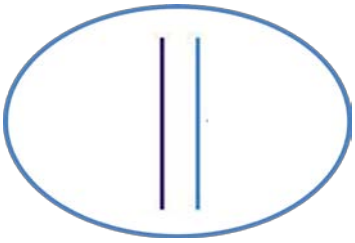
It is a very short duration stage and progressive condensation of chromosomes takes place. In this stage the chromosomes are first observed as thin threads and are said to be in a diffused state. The sister chromatids are tightly packed and indistinguishable from one another.

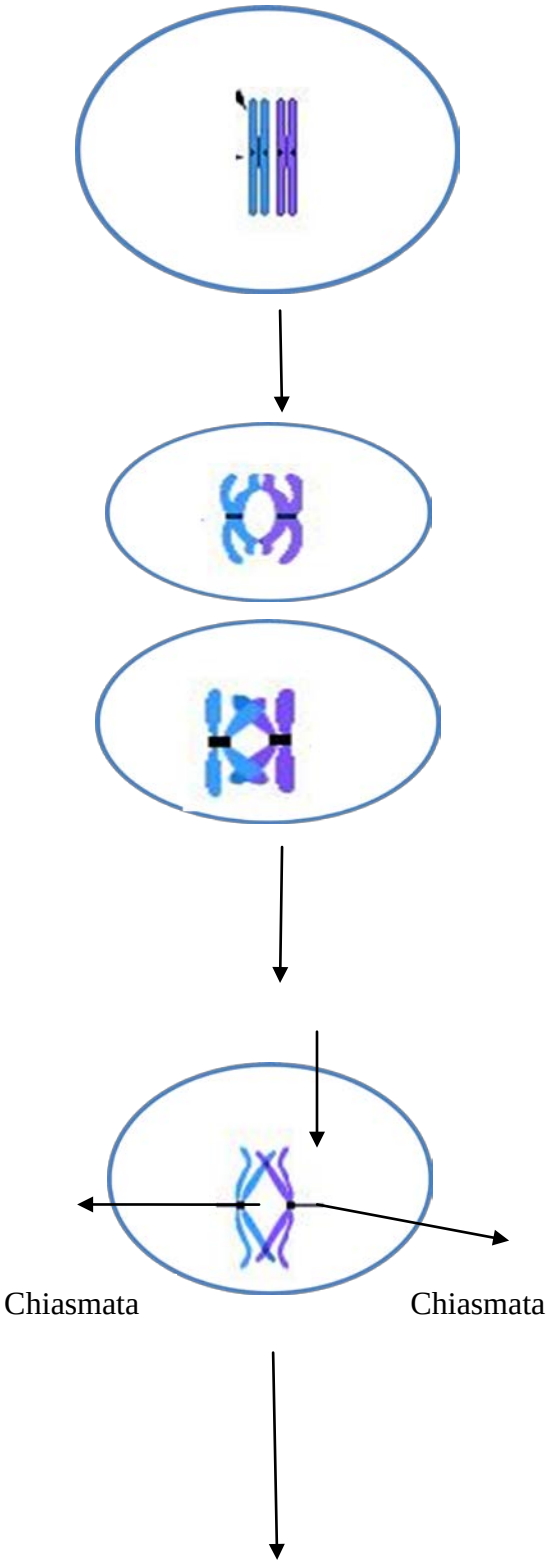


2. Zygotene

Chromosome duplication occurs and the homologous chromosomes pair up with each other.

Purple and blue represent homologous duplicated chromosomes.





3. Pachytene

Chromosomal crossover (crossing over) occurs by chiasma formation between homologous chromosomes. Nonsister chromatids of homologous chromosomes may exchange segments over regions of homology by a process called recombination. The region where crossing over occurs is known as chiasmata.

4. Diplotene

Homologous chromosomes separate from one another a little but remain attached at the chiasmata.

5. Diakinesis

Chromosomes condense further during the diakinesis stage. This is the first point in meiosis where the four parts of the tetrads are actually visible. Sites of crossing over

entangle together, effectively overlapping, making chiasmata clearly visible. The rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.

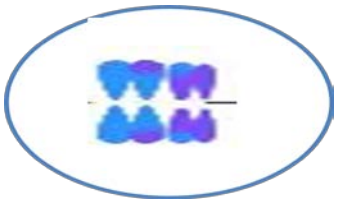


Figure 2: Stages of Meiosis I

Metaphase I

Homologous pairs move together along the metaphase plate: As kinetochore microtubules from both centrioles attach to their respective kinetochores, the homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent along the metaphase plate, with respect to the orientation of the other bivalents along the same equatorial line (see Fig 3).

Anaphase I

Homologous chromosomes are pulled apart by shortening of spindle fibres, each chromosome still containing a pair of sister chromatids. The cell then elongates in preparation for division down the center (see Fig 3).

Anaphase I

Chromosomes are at two different poles in the cell and the nuclear envelopes may reform, or the cell may quickly start meiosis II. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids (see Fig 3).

Telophase I

The two daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The spindle networks disappear, and a new nuclear membrane forms. The chromosomes decondensation occurs and finally cytokinesis pinches the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells.

Meiosis II

Meiosis II is the second stage of the meiotic process. The overall process is similar to mitosis. The end result is production of four haploid cells. The four main steps of Meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II (see Fig 3).

Prophase II

In prophase II the nucleoli and nuclear envelope disappear. Centrioles move to opposite poles and arrange spindle fibers for the second meiotic division (see Fig 3).

Metaphase II

In metaphase II, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes (centrioles) at each pole. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate (see Fig 3).

Anaphase II

This is followed by anaphase II, where the centromeres are cleaved, allowing microtubules attached to the kinetochores to pull the sister chromatids apart. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles (see Fig 3).

Telophase II

The process ends with telophase II, which is similar to telophase I, and is marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes. Meiosis is now complete and ends up with four new daughter cells (see Fig 3).

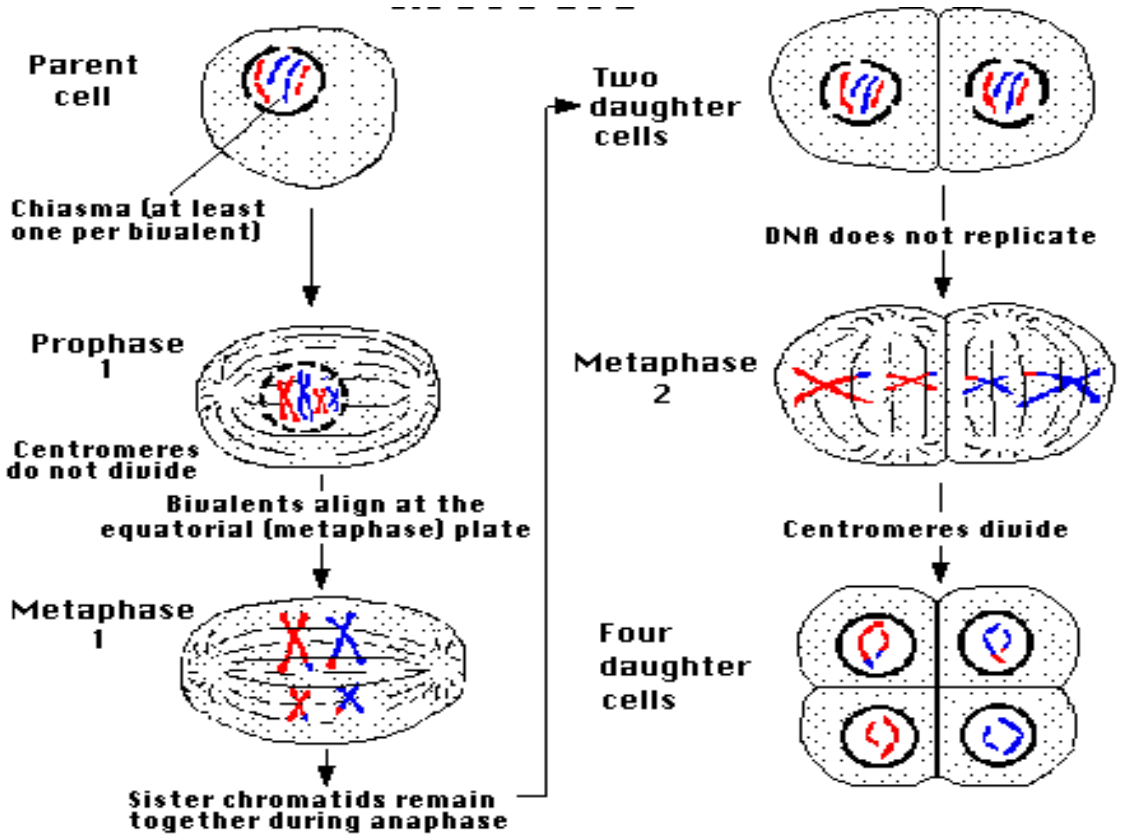


Figure 3: Events in meiosis I and II

The difference between male and female meiosis

There are mainly three differences between male and female meiosis

1. Male meiosis creates sperm, while female meiosis creates eggs.

2. Male meiosis takes place in the testicles, while female meiosis takes place in the ovaries.

3. A male will generally have one X and one Y sex chromosome, while a female have two X chromosomes, however only one of the two is active and the other is known as a barr body . During meiosis I, the sex chromosomes separate and enter different sperm or egg cells (gametes). Males will end up with one half X sperm and the other half Y sperm, while females will all have X eggs because they had no Y chromosome in the first place. There are more subtle differences though. At the end of meiosis I females have two daughter cells and meiosis II only occurs if and when fertilization occurs by a sperm cell. At that time both daughter cells divide to form 4 cells and of the 4 cells formed, 3 are discarded as polar bodies and the 4th cell having an enhanced cytoplasmic component combines its nuclear component with the sperm cell's nuclear component and crossing

over occurs to form the embryo which then begins to divide via mitosis to become two cells, then four and so on.

Interesting Facts:

- Meiosis was discovered and described for the first time in sea urchin eggs in 1876 by the German biologist Oscar Hertwig.
- Saccharomyces cerevisiae reproduces mitotically (asexually) as diploid cells when nutrients are abundant, but switches to meiosis (sexual reproduction) under starvation condition.
- Abnormalities in meiosis in human causes the following diseases.
    - Down Syndrome - trisomy of chromosome 21.
    - Patau Syndrome - trisomy of chromosome 13.
    - Edward Syndrome - trisomy of chromosome 18.
    - Klinefelter Syndrome - extra X chromosomes in males - i.e. XXY, XXXY, XXXXY, etc.
    - Turner Syndrome - lacking of one X chromosome in females - i.e. X0.
    - Triple X syndrome - an extra X chromosome in females.
    - XYY Syndrome - an extra Y chromosome in males.

Questions:

Q1. A muscle cell of a mouse contains 22 chromosomes. Based on this information, how many

chromosomes are there in the following types of mouse cells?

A. Daughter muscle cell formed from mitosis

B. Egg cell

C. Fertilized egg cell

Q2. A nuclear envelope forms around each set of chromosomes and cytokinesis occurs, producing four daughter cells, each with a haploid set of chromosomes.

A. prophase I

B. metaphase I

C. anaphase I

D. telophase I

E. prophase II

F. metaphase II

G. anaphase II

H. telophase II

I. cytokinesis

Q3. If a diploid cell entering meiosis has 6 chromosome pairs, what is the number of possible chromosome combinations in the haploid nuclei?

Q4. What is the difference between metaphase I and metaphase II?

Q5. How are haploid cells different from diploid cells in humans?

Q6. What are homologous chromosomes?

Q7. Do homologous chromosomes have identical genes? Explain

Q8. List the events that occur in prophase I.

Q9. What are the mechanisms by which genetic variation is produced by meiosis?

Mod 1 Lecture 12 Cell cycle regulation

After studying mitosis and meiosis it is important to know how are cell cycles regulated. The present chapter talks about the cell cycle regulatory methods.

Cell cycle regulation:

Cell cycle is a highly regulated and coordinated process mediated by extracellular signals from the environment, as well as by internal signals. In most cells, this coordination between different phases of the cell cycle is dependent on a series of cell cycle checkpoints that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed. The major cell cycle regulatory check point occurs late in G1 and controls progression from G1 to S. Other check points function to ensure complete genome transmittance to daughter cells. DNA damage checkpoints in G1, S, and G2 lead to cell cycle arrest in response to damaged or unreplicated DNA. Another checkpoint, called the spindle assembly checkpoint, arrests mitosis if the chromosomes are not properly aligned on the mitotic spindle (Figure 1).

To restrict DNA replication once per cell cycle the G2 checkpoint ensures that the genome is replicated only once per cell cycle and that incompletely replicated DNA is not distributed to daughter cells. The molecular mechanism underlying this involves the action of the MCM (minichromosome maintenance complex) helicase that bind to replication origins together with the origin recognition complex (ORC) proteins. The MCM proteins are allowed to bind to replication origins during G1, leading to DNA replication when the cell enters S phase. After initiation the MCM proteins are dissociated from the origin, so replication cannot initiate again until next cell cycle. The association of MCM proteins with DNA during the S, G2 and M phases of the cell cycle is blocked by activity of the protein kinases that regulate cell cycle progression.

The cell cycle itself is under genetic control and the mechanisms of control are identical in all eukaryotes. There are two critical transitions: from G1 into S and from G2 into M. The G1/S and G2/M transitions are called "checkpoints" because the transitions are delayed unless key processes have been completed. For example, at the G1/S checkpoint, either sufficient time must have elapsed since the preceding mitosis or the cells have attained sufficient size for DNA replication to be initiated. Similarly, the G2/M

checkpoint requires that DNA replication and repair of any DNA damage be completed for the M phase to commence.

Both control points are regulated in a similar fashion and use a specialized protein kinase called the p34 kinase subunit that regulates the activity of target proteins by phosphorylation and regulates cellular processes also. To become activated, this p34 polypeptide subunit combines with several other polypeptide chains called cyclins. At the G1/S control point, one set of cyclins combines with the p34 subunit to yield the active kinase which triggers DNA replication and other events of the S period. Similarly, at the G2/M control point, a second set of cyclins combines with the p34 subunit to yield the active kinase which initiates condensation of the chromosomes, breakdown of the nuclear envelope, and reorganization of the cytoskeleton in preparation for cytokinesis.

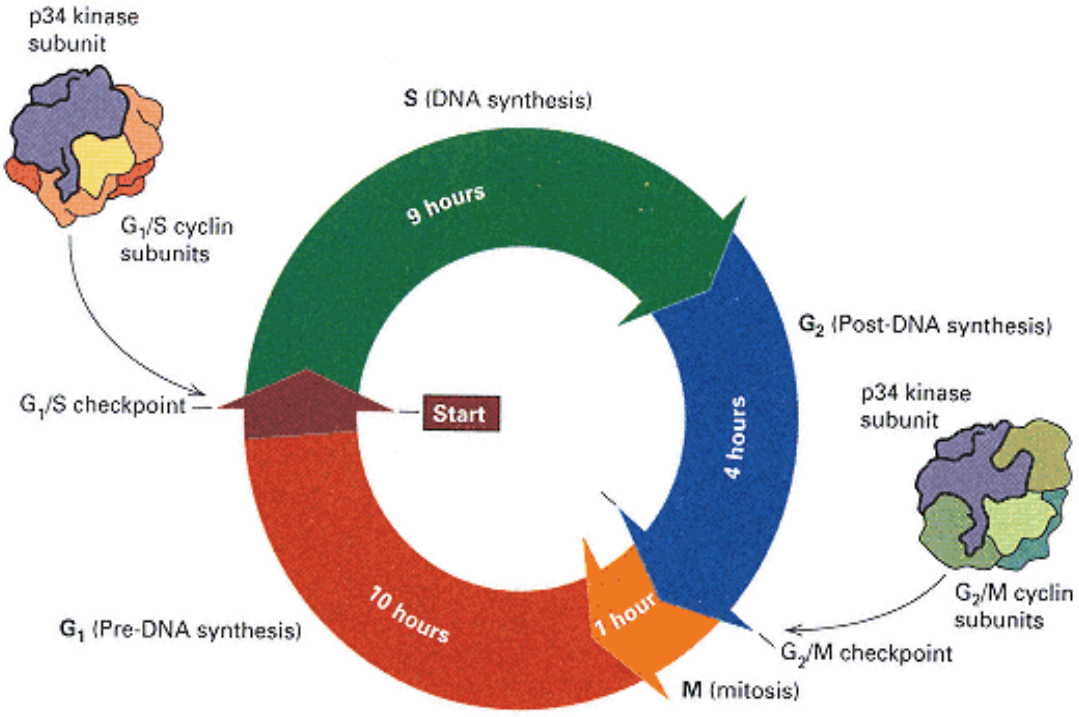


Figure 1: The cell cycle of a typical mammalian cell growing in tissue culture with a generation time of 24 hours. The critical control points for the G_1S and G_2M transitions are governed by a p34 kinase that is activated by stage-specific cyclins and that regulates the activity of its target proteins through phosphorylation.

Cell cycle regulatory elements

Cyclin dependent kinases (Cdks) are the central components that coordinate activities throughout the cell cycle whose activities in turn are regulated by cyclin binding. The cyclin-Cdk complex causes phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown and other events that occur at the onset of mitosis. Cyclins can be divided into four classes.

1. G1/S cyclin: They activate Cdks in late G1 and their level fall in S phase.
2. S cyclin: They stimulate DNA replication and their level remains high until mitosis.
3. M cyclin: Activate Cdks that stimulate entry into mitosis at the G2/M checkpoint.
4. G1 cyclins: Governs the activities of G1/S cyclins.

The cyclin protein not only activates Cdks but directs them to specific target proteins phosphorylating a different set of proteins. The different cyclin and Cdks of vertebrates has been presented in Table 1.

Table 1: The major cyclins and Cdks

Cyclin-Cdk complex	Vertebrates	
	Cyclin	Cdk partner
G1-Cdk	D	Cdk4, Cdk6
G1/S	E	Cdk2
S	A	Cdk2
M	B	Cdk1

Full activation of cyclin-Cdk complex occurs when Cdk-activating kinase phosphorylates an amino acid residue near the active site of Cdks. Furthermore Cdk activity peaks and falls during cell cycle and this process is controlled by Cdk-Inhibitory proteins (CKI) like p27 which inactivates cyclin A-Cdk2 complex. The structural basis of Cdk activation is illustrated in Figure 2. In inactive state without bound cyclin the active site is blocked by a protein region known as the T-loop. Cyclin binding causes T-loop to move out and its phosphorylation by CAK.

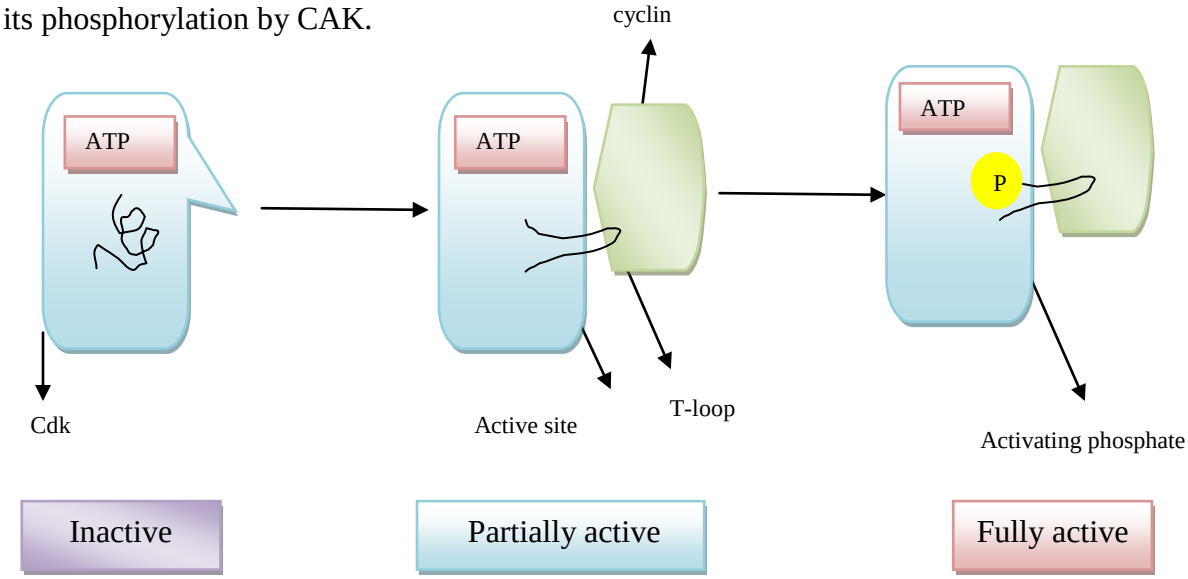


Figure 2: The structural basis of Cdk activation.

Other than phosphorylation/dephosphorylation, protein degradation also controls cell cycle progression. During the metaphase to anaphase transition the key regulator which is the anaphase promoting complex (APC) catalyses ubiquitinylation and proteosomal destruction of S and M cyclins. Destroying these cyclins inactivates most Cdks in the cell. Another ubiquitin ligase called SCF ubiquitinylates certain CKIs in late G1phase controlling activation of S-Cdks and thus DNA replication. APC activity is in turn regulated by subunits which are Cdc20 during anaphase or Cdh1 during early G. An overview of cell cycle control system is illustrated in **Figure 3.**

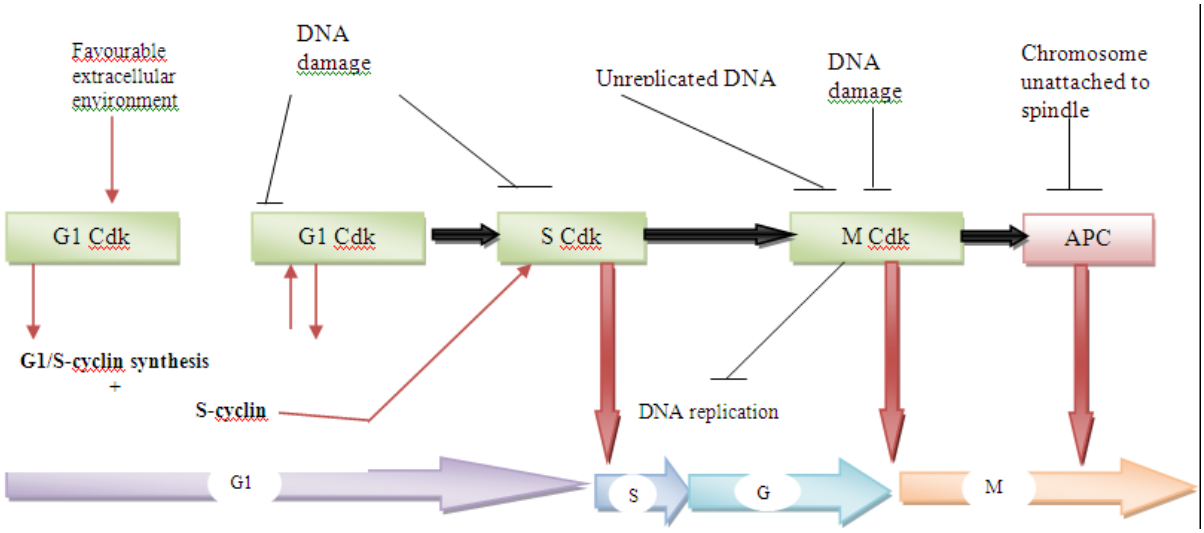


Figure 3: An overview of the cell cycle control system. Activation of G1-Cdk is stimulated through various external and internal signals. This in turn activates genes encoding G1/S and S cyclins. G1/S Cdk results in wave of S-Cdk activity which initiates chromosome replication in S-phase and contributes to some early events in mitosis. M-Cdk activity then triggers progression through G2/M checkpoint. APC with its activator Cdc20 triggers metaphase to anaphase transition. Further multiple mechanisms suppress Cdk activity after mitosis resulting in stable G1 period. This figure has been adapted from "Molecular Biology of the Cell" by Alberts B et al., 2008 Vth edition, Garland Science, USA.

Events of cell cycle in S-Phase

1. DNA replication starts at origins of replication and cell cycle ensures that replication occurs once per cell cycle.

2. In late mitosis and early G1 complex of proteins known as prereplicative complex (pre-RC) assemble at origin of replication. S-Cdk activity leads to the assembly of pre initiation complex.

3. After initiation pre-RC is dismantled and cannot be reassembled until the following G1. Assembly of pre-RC is stimulated by APC thus ensuring pre-RC assembly only at late mitosis and early G1 when Cdk activity is low and APC activity is high. The events of cell cycle during S-phase has been schematically represented in Figure 4.

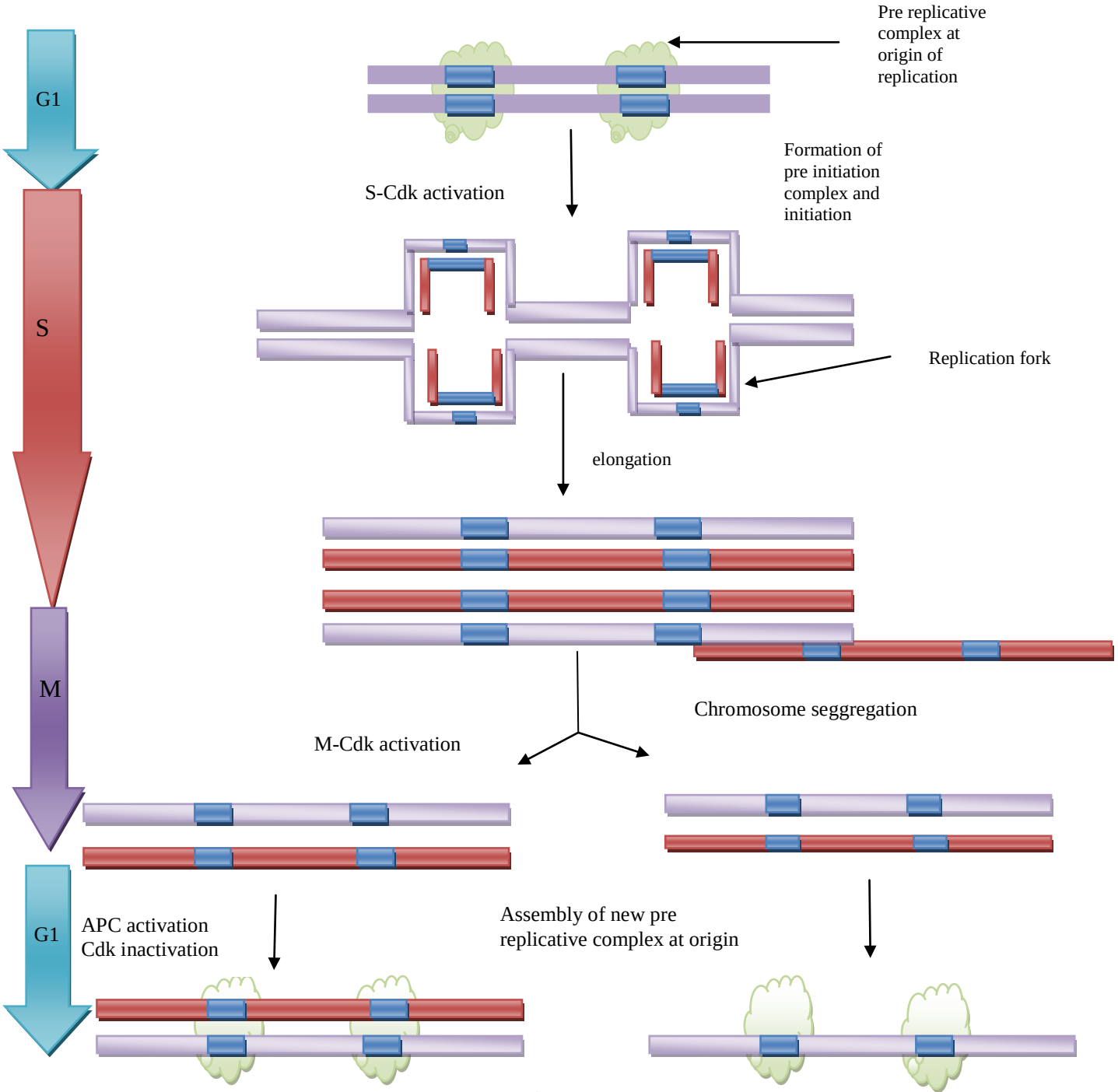


Figure 4: Cell cycle control of chromosome duplication.

Proteins involved in the initiation of DNA replication

Many proteins play part in initiation of DNA replication. The events are summarized in the following text and Figure 5.

1. A large multiprotein complex (origin recognition complex/ORC), binds to the replication origin throughout the cell cycle.

2. In late mitosis and early G1, proteins Cdc6 and Cdt1 bind to the ORC at origin and load a group of six related proteins called the Mcm proteins. This protein complexes leads to origin of replication.

3. The six Mcm proteins form a ring around the DNA and serves as the major DNA helicase causing unwinding of DNA when DNA synthesis begins and replication forks move out of the origin.

4. The activation of S-Cdk in late G1 causes assembly of several other protein complexes at the origin causing formation of large pre-initiation complex that unwinds the helix and begins DNA synthesis.

5. Parallel action of S-Cdk triggers the disassembly of some pre-RC components at the origin. Cdk's phosphorylates both the ORC and Cdc6.

6. Inactivation of APC in late G1 occurs and in turn turns off pre-RC assembly. In late mitosis and early G1 the APC triggers the destruction of a protein called geminin that binds and inhibits the Cdt1 protein.

7. S and M-Cdk activity along with low activity of APC block pre-RC formation at S-phase and thereafter.

8. After the end of mitosis APC activation leads to the inactivation of Cdks and destruction of geminin. Pre-RC components are dephosphorylated and Cdt1 is activated leading to pre-RC assembly to prepare the cell for the next S-phase.

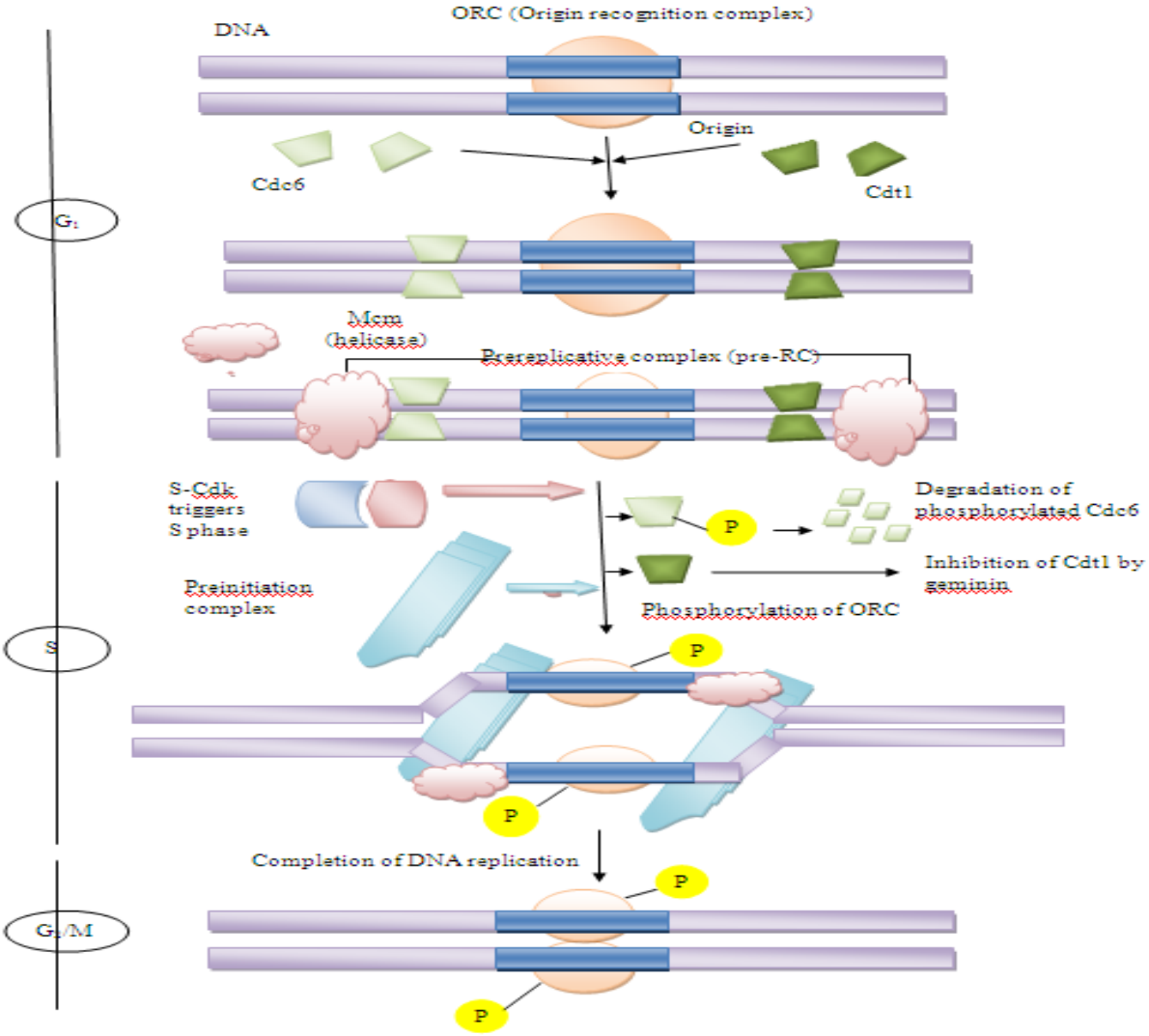


Figure 5: Control of the initiation of DNA replication.

How cell division is blocked by DNA damage?

When DNA is damaged for example by X-rays, protein kinases are activated and recruited to the site of damage. They in turn initiate a signaling cascade that causes arrest of the cell cycle. The first kinase at the site of damage is either ATM (Ataxia telangiectasia mutated) or ATR (Ataxia telangiectasia and Rad3 related) which recruits Chk1 and Chk 2 kinases at the same site. These kinases cause phosphorylation of the gene regulatory protein p53. Phosphorylation of p53 blocks Mdm2. Mdm2 is responsible for p53 ubiquitinylation and its proteosomal degradation. Thus blocking Mdm2 keeps p53 activity intact causing high level p53 accumulation. p53 then leads to transcription of

CKI protein p21. The p21 binds and inactivates G1/S-Cdk and S-Cdk arresting the cell cycle at G1.

Interesting facts:

- Two families of genes, the cip/kip family (CDK interacting protein/Kinase inhibitory protein) and the INK4a/ARF (Inhibitor of Kinase 4/Alternative Reading Frame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.
- Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.
- A semi-autonomous transcriptional network acts in concert with the CDK-cyclin machinery to regulate the cell cycle.

Further reading:

4. Alberts B, Johnson A, Lewis J, et al. 2008. Molecular Biology of the Cell (5th ed.). Garland Science. USA.
5. Karp G. 2010. Cell and Molecular Biology: Concepts and Experiments, John Wiley & Sons, Inc. USA.
6. Cooper G M, Hausman R E. 2007. The Cell: A Molecular Approach (4th ed.). ASM Press,Washington, D.C.

Questions:

Q1. The role of 'cyclin' in the regulation of the cell cycle would be best compared to:

A. a digital watch that produces a precisely timed signal every few microseconds.
B. a row of dominoes, that all fall sequentially after the first one is flipped.
C. a light switch that alternates between on and off states.
D. the accumulation of sand in an hourglass.

Q2. All of the following statements correctly describe M-Cdk, EXCEPT:

A. M-Cdk causes the cell to enter S phase and begin DNA replication.
B. M-Cdk has two subunits, a protein kinase and a cyclin-type protein.
C. M-Cdk only becomes active during M-phase.
D. M-Cdk triggers many events by phosphorylating other proteins.

Q3. Enumerate the cell cycle check points. Why does the cell enter the G0 phase.

Q4. Cyclins are targeted for destruction through ubiquination. Descibe the process.How are Cyclin dependent kinases (CDks) activated?

Q5. Different cyclin-Cdks are responsible for triggering different stages of the cell cycle. Elaborate.

Q6. Are the genes that code of checkpoints most likely to be protooncogenes or tumor suppressor genes? Explain.

Q7. What happens to the cell cycle when DNA is damaged?

Module 2- Chromosome structure and organisation

This module deals with the genetic material of the cell, its structure, with details of the human chromosome and the giant chromosomes.

Module 2 Lecture 1

Genetic material in a cell: All cells have the capability to give rise to new cells and the encoded information in a living cell is passed from one generation to another. The information encoding material is the genetic or hereditary material of the cell.

Prokaryotic genetic material:

The prokaryotic (bacterial) genetic material is usually concentrated in a specific clear region of the cytoplasm called nucleiod. The bacterial chromosome is a single, circular, double stranded DNA molecule mostly attached to the plasma membrane at one point. It does not contain any histone protein. *Escherichia coli* DNA is circular molecule 4.6 million base pairs in length, containing 4288 annotated protein-coding genes (organized into 2584 operons), seven ribosomal RNA (rRNA) operons, and 86 transfer RNA (tRNA) genes. Certain bacteria like the *Borrelia burgdorferi* possess array of linear chromosome like eukaryotes.

Besides the chromosomal DNA many bacteria may also carry extra chromosomal genetic elements in the form of small, circular and closed DNA molecules, called plasmids. They generally remain floated in the cytoplasm and bear different genes based on which they have been studied. Some of the different types of plasmids are F plasmids, R plasmids, virulent plasmids, metabolic plasmids etc. Figure 1 depicts a bacterial chromosome and plasmid.

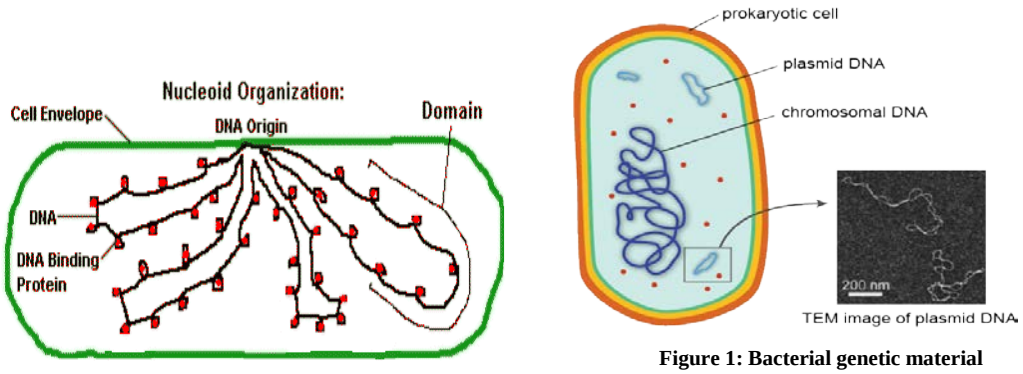


Figure 1: Bacterial genetic material

Virus genetic material:

The chromosomal material of viruses is DNA or RNA which adopts different structures. It is circular when packaged inside the virus particle.

Eukaryotic genetic material:

A Eukaryotic cell has genetic material in the form of genomic DNA enclosed within the nucleus. Genes or the hereditary units are located on the chromosomes which exist as chromatin network in the non dividing cell/interphase. This will be discussed in detail in the coming sections.

Chromosome:

German biologist Walter Flemming in the early 1880s revealed that during cell division the nuclear material organize themselves into visible thread like structures which were named as chromosomes which stains deep with basic dyes. The term chromosome was coined by W. Waldeyer in 1888. Chrome is coloured and soma is body, hence they mean "colored bodies" and can be defined as higher order organized arrangement of DNA and proteins. It contains many genes or the hereditary units, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve in packaging the DNA and control its functions. Chromosomes vary both in number and structure among organisms (Table 1) and the number of chromosomes is characteristic of every species. Benden and Bovery in 1887 reported that the number of chromosomes in each species is constant. W.S. Sutton and T. Boveri in 1902 suggested that chromosomes are the physical structures which acted as messengers of heredity.

Chromosomes are tightly coiled DNA around basic histone proteins, which help in the tight packing of DNA. During interphase, the DNA is not tightly coiled into chromosomes, but exists as chromatin. The structure of a chromosome is given in Figure 2. In eukaryotes to fit the entire length of DNA in the nucleus it undergoes condensation and the degree to which DNA is condensed is expressed as its packing ratio which is the length of DNA divided by the length into which it is packaged into chromatin along with proteins.

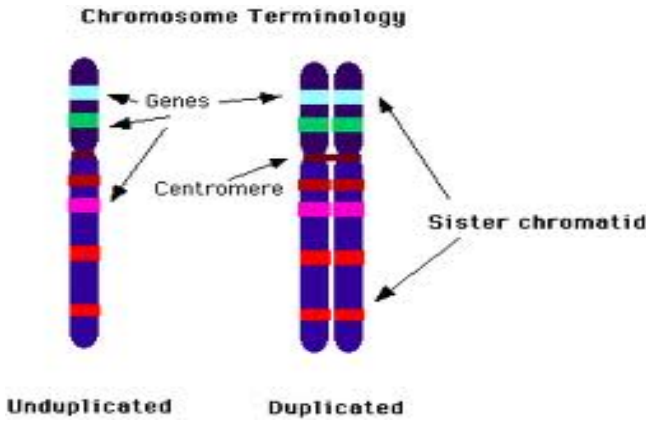


Figure 2: Eukaryotic chromosome

The shortest human chromosome contains 4.6 x 10^7 bp of DNA. This is equivalent to 14,000 µm of extended DNA. In its most condensed state during mitosis, the chromosome is about 2 µm long. This gives a packing ratio of 7000 (14,000/2). The DNA is packaged stepwise into the higher order chromatin structure and this is known as "hierarchies of chromosomal organization". The level of DNA packaging is schematically represented in Table 2.

Chromosome number:

There are normally two copies of each chromosome present in every somatic cell. The number of unique chromosomes (N) in such a cell is known as its haploid number, and the total number of chromosomes (2N) is its diploid number. The suffix 'ploid' refers to chromosome 'sets'. The haploid set of the chromosome is also known as the genome. Structurally, eukaryotes possess large linear chromosomes unlike prokaryotes which have circular chromosomes. In Eukaryotes other than the nucleus chromosomes are present in mitochondria and chloroplast too. The number of chromosomes in each somatic cell is same for all members of a given species. The organism with lowest number of chromosome is the nematode, *Ascaris megalocephalusunivalens* which has only two chromosomes in the somatic cells (2n=2).

Table 1: Number of chromosomes in different organisms

Organism	No. of chromosomes
Arabidopsis thaliana (diploid)	10
Maize (diploid)	20
Wheat (hexaploid)	42
Common fruit fly (diploid)	8
Earthworm (diploid)	36
Mouse (diploid)	40
Human (diploid)	46
Elephants (diploid)	56
Donkey (diploid)	62
Dog (diploid)	78
Gold Fish (diploid)	100-104
Tobacco(tetraloid)	48
Oat (hexaploid)	42

Autosomes and sex chromosomes:

In a diploid cell, there are two of each kind of chromosome (termed homologus chromosomes) except the sex chromosomes. In humans one of the sex has two of the same kind of sex chromosomes and the other has one of each kind. In humans there are 23 pairs of homologous chromosomes (2n=46). The human female has 44 non sex chromosomes, termed autosomes and one pair of homomorphic sex chromosomes given the designation XX. The human male has 44 autosomes and one pair of heteromorphic sex chromosomes, one X and one Y chromosome.

Morphology:

Size: The size of chromosome is normally measured at mitotic metaphase and may be as short as 0.25μm in fungi and birds to as long as 30 μm in some plants such as Trillium. However, most mitotic chromosome falls in the range of 3μm in Drosophila to 5μm in man and 8-12μm in maize. The monocots contain large sized chromosomes as compared to dicots. Organisms with less number of chromosomes contain comparatively large sized chromosomes. The chromosomes in set vary in size.

Shape: The shape of the chromosome changes from phase to phase in the continuous process of cell growth and cell division. During the resting/interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread like stainable structures, the chromatin threads. In the metaphase and the anaphase, the chromosome becomes thick and filamentous. Each chromosome contains a clear zone, known as centromere or kinetochore, along their length. The centromere divides the chromosome into two parts and each part is called chromosome arm. The position of centromere varies from chromosome to chromosome providing it a different shape. They could be telocentric (centromere on the proximal end of the chromosome), acrocentric (centromere at one end giving it a very short and another long arm), submetacentric (J or L shaped chromosome with the centromere near the centre), metacentric (v shaped with centromere at the centre).

Structure of Chromosome: A chromosome at mitotic metaphase consists of two symmetrical structures called chromatids. Each chromatid contains a single DNA molecule and both chromatids are attached to each other by centromere and become separated at the beginning of anaphase. The chromomeres are bead like accumulations of chromatin material that are sometimes visible along interphase chromosomes. The chromomere bearing chromatin has an appearance of a necklace in which several beads occur on a string. Chromomeres are regions of tightly folded DNA and become especially prominent in polytene chromosomes. Centromere in a chromosome contain specific DNA sequences with special proteins bound to them, forming a disc shaped structure, called kinetochore. In electron microscope the kinetochore appears as a plate or cup like disc, 0.20-0.25 nm, in diameter situated upon the primary constriction or centromere. The chromosomes of most organisms contain only one centromere and are known as

monocentric chromosomes. Some species have diffused centromeres, with microtubules attached along the length of the chromosomes and are termed holocentric chromosomes. Chromosomes of *Ascaris megalocephala* are examples of diffused centromeric chromosomes. Telomere is the chromosomal ends which prevents other chromosomal segments to be fused with it. Besides the primary constrictions or centromeres, chromosomes also posses secondary constriction at any point of the chromosome and are constant in their position and extent. These constrictions are helpful in identifying particular chromosomes in a set. Chromosomes also contain nucleolar organizers which are certain secondary constrictions that contain the genes coding for 5.8S, 18S and 28S ribosomal RNA and induce the formation of nucleoli. Sometimes the chromosomes bear round, elongated or knob like appendages known as satellites. The satellite remains connected with the rest of the chromosomes by a thin chromatin filament.

Chromatin:

Chemical composition of chromatin

Chromatin consists of DNA, RNA and protein. The protein of chromatin could be of two types: histones and non histones.

DNA: DNA is the most important chemical component of chromatin, since it plays central role of controlling heredity and is most conveniently measured in picograms. In addition to describing the genome of an organism by its number of chromosomes, it is also described by the amount of DNA in a haploid cell. This is usually expressed as the amount of DNA per haploid cell (usually expressed as picograms) or the number of kilobases per haploid cell and is called the C value. This is constant for all cells of a species. For diploid cells it is 2C. Extending the C value we reach the C-value paradox. One immediate feature of eukaryotic organisms highlights a specific anomaly that was detected early in molecular research. Even though eukaryotic organisms appear to have 2-10 times as many genes as prokaryotes, they have many orders of magnitude more DNA in the cell. Furthermore, the amount of DNA per genome is correlated not with the presumed evolutionary complexity of a species. This is stated as the C value paradox: the amount of DNA in the haploid cell of an organism is not related to its evolutionary complexity. Lower eukaryotes in general have less DNA, such as nematode

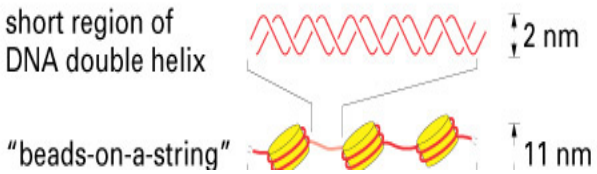
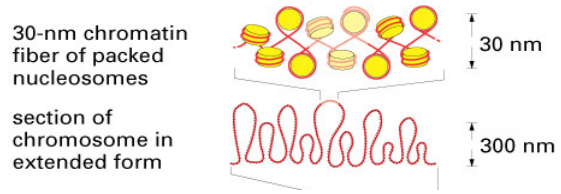
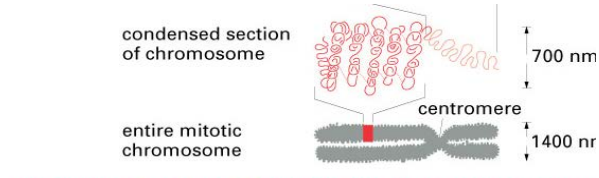
Caenorhabditis elegans which has 20 times more DNA than *E. coli*. Vertebrates have greaer DNA content about 3pg, in general about 700 times more than *E. coli*. Salamander *Amphiuma* has a very high DNA content of about 84pg. Man has about 3pg of DNA per haploid genome.

Histones: Histones are basic proteins as they are enriched with basic proteins arginine and lysine. At physiological pH they are cationic and can interact with anionic nucleic acids. They form a highly condensed structure. The histones are of five types called H1, H2A H2B, H3, and H4-which are very similar among different species of eukaryotes and have been highly conserved during evolution. H1 is the least conserved among all and is also loosely bound with DNA. H1 histone is absent in *Sacharomyces cerevisiae*.

Non-histones: In addition to histones the chromatin comprise of many different types of non-histone proteins, which are involved in a range of activities, including DNA replication and gene expression. They display more diversity or are not conserved. They may also differ between different tissues of same organism.

Roger Kornberg in 1974 described the basic structural unit of chromatin which is called the nucleosome. The structural organization of nucleosome to chromosome is explained in Table 2.

Table 2: The hierarchies of chromosomal organization

Levels	Summary	Schematic representation
The first level of packing	Winding of DNA around a protein core to produce a "bead-like" structure called a **nucleosome**. This gives a packing ratio of about 6. This structure is invariant in both the euchromatin and heterochromatin of all chromosomes. The protein core is composed of 8 histone proteins, two each of H2A, H2B, H3 and H4. Histone H1 forms the linker between to nucleosomes. 146 bp of DNA is wrapped around each nucleosome.	
The second level of packing	Coiling of beads in a helical structure called the **30 nm fiber** that is found in both interphase chromatin and mitotic chromosomes. This structure increases the packing ratio to about 40. This appears to be a solenoid structure with about 6 nucleosomes per turn. This gives a packing ratio of 40, which means that every 1 µm along the axis contains 40 µm of DNA. The stability of this structure requires the presence of the last member of the histone gene family, histone H1. Because experiments that strip H1 from chromatin maintain the nucleosome, but not the 30 nm structure, it was concluded that H1 is important for the stabilization of the 30 nm structure.	
The final level	The fiber is organized in loops, scaffolds and domains that give a final packing ratio of about 1000 in interphase chromosomes and about 10,000 in mitotic chromosomes. The final level of packaging is characterized by the 700 nm structure seen in the metaphase chromosome. The condensed piece of chromatin has a characteristic scaffolding structure that can be detected in metaphase chromosomes. This appears to be the result of extensive looping of the DNA in the chromosome.	

Euchromatin: The lightly-stained regions in chromosome when stained with basic dyes are called euchromatin and contain single-copy of genetically-active DNA. The extent of chromatin condensation varies during the life cycle of the cell and plays an important role in regulating gene expression. In the interphase of cell cycle the chromatin are decondensed and known as euchromatin leading to gene transcription and DNA replication.

Heterochromatin: The word heterochromatin was coined by Emil Heitz based on cytological observations. They are highly condensed and ordered areas in nucleosomal arrays. About 10% of interphase chromatin is called heterochromatin and is in a very highly condensed state that resembles the chromatin of cells undergoing mitosis . They contain a high density of repetitive DNA found at centromeres and telomeres form heterochromatin. Heterochromatin are of two types, the constitutive and facultative heterochromatin. The regions that remain condensed throughout the cell cycle are called constitutive heterochromatin whereas the regions where heterochromatin condensation state can change are known as facultative. Constitutive heterochromatin is found in the region that flanks the telomeres and centromere of each chromosome and in the distal arm of the Y chromosome in mammals. Constitutive heterochromatin possesses very few genes and they also lead to transcriptional inactivation of nearby genes. This phenomenon of gene silencing is known as “position effect”. Constitutive heterochromatin also inhibits genetic recombination between homologous repetitive sequences circumventing DNA duplications and deletion. Whereas facultative heterochromatin is chromatin that has been specifically inactivated during certain phases of an organism’s life or in certain types of differentiated cells. Dosage compensation of X-chromosome or X-chromosome inactivation in mammals is an example of such heterochromatin (Karp 2010). Heterochromatin spreads from a specific nucleation site, causing silencing of most of the X chromosome, thereby regulating gene dosage.

Centromeres: Centromeres are those condensed regions within the chromosome that are responsible for the accurate segregation of the replicated chromosome during mitosis and meiosis. When chromosomes are stained they typically show a dark-stained region that is the centromere. The actual location where the attachments of spindle fibres occur is called the kinetochore and is composed of both DNA and protein. The DNA sequence within these regions is called *CEN* DNA. Because *CEN* DNA can be moved from one chromosome to another and still provide the chromosome with the ability to segregate, these sequences must not provide any other function. Typically CEN DNA is about 120 base pairs long and consists of several sub-domains, CDE-I, CDE-II and CDE-III (Figure 3). Mutations in the first two sub-domains have no effect upon segregation, but a point mutation in the CDE-III sub-domain completely eliminates the ability of the centromere to function during chromosome segregation. Therefore CDE-III must be actively involved in the binding of the spindle fibers to the centromere. The protein component of the kinetochore is only now being characterized. A complex of three proteins called Cbf-III binds to normal CDE-III regions but cannot bind to a CDE-III region with a point mutation that prevents mitotic segregation. Furthermore, mutants of the genes encoding the Cbf-III proteins also eliminates the ability for chromosomes to segregate during mitosis. Additional analyses of the DNA and protein components of the centromere are necessary to fully understand the mechanics of chromosome segregation.

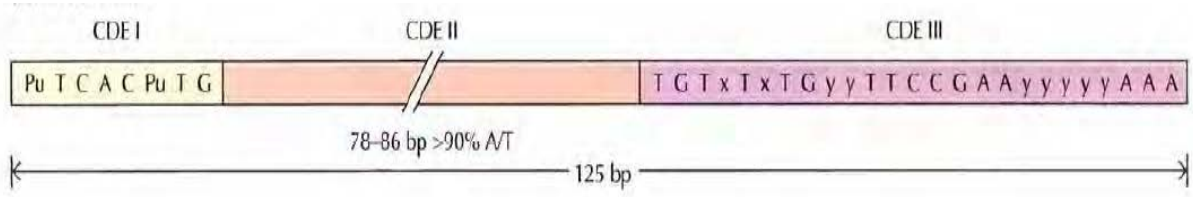


Figure 3: The *S. cerevisiae* centrosome. The *S. cerevisae* centromere (CEN) sequences consist of two short conserved sequences (CDE I and CDE Ill) separated by 78 to 86 base pairs (bp) of ATrich DNA (CDE II). The sequences shown are consensus sequences derived from analysis of the centromere sequences of individual yeast chromosomes. Pu = A or G; x =A or T; y = any base. The figure has been adapted from "The Cell, A Molecular Approach" by Geoffrey M. Cooper, 4th Ed. 2007.

Telomeres: Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome. McClintock recognized their special features when she noticed, that if two chromosomes were broken in a cell, the ends were sticky and end of one could attach to the other and vice versa. However she never observed the attachment of the broken end to the end of an unbroken chromosome suggesting that the end of chromosomes have unique features. Telomere sequences remain conserved throughout vertebrates and they form caps that protect the chromosomes from nucleases and other destabilizing influences; and they prevent the ends of chromosomes from fusing with one another.The telomeric DNA contains direct tandemly repeated sequences of the form $(T/A)_xG_y$ where x is between 1 and 4 and y is greater than 1. Human telomeres contain the sequence TTAGGG repeated from about 500 to 5000 times. Certain bacteria possess telomeres in their linear genetic material which are of two types; one of the types is called a hairpin telomere. As its name implies, the telomeres bend around from the end of one DNA strand to the end of the complimentary strand. The other type of telomere is known as an invertron telomere. This type acts to allow an overlap between the ends of the complimentary DNA strands.

Telomere replication: Telomere replication is an important aspect in DNA replication. The primary difficulty with telomeres is the replication of the lagging strand. Because DNA synthesis requires a RNA template (that provides the free 3'-OH group) to prime DNA replication, and this template is eventually degraded, a short single-stranded region would be left at the end of the chromosome. This region would be susceptible to enzymes that degrade single-stranded DNA. The result would be that the length of the chromosome would be shortened after each division. This is known as the end replication problem which is not observed. The action of the telomerase enzymes ensure that the ends of the lagging strands are replicated correctly. Telomerase was discovered in 1984 by Elizabeth Blackburn and Carol Greider of the University of California, Berkeley. It is a reverse transcriptase that synthesizes DNA using an RNA template. Unlike most reverse transcriptases, the enzyme itself contains the RNA that serves as its template, i.e., telomerase can add new repeat units to the 3’ end of the overhanging strand. A well-studied system involves the *Tetrahymena* protozoa organism. The telomeres of this

organism end in the sequence 5'-TTGGGG-3'. The telomerase adds a series of 5'-TTGGGG-3' repeats to the ends of the lagging strand. A hairpin occurs when unusual base pairs between guanine residues in the repeat form. Next the RNA primer is removed, and the 5' end of the lagging strand can be used for DNA synthesis. Ligation occurs between the finished lagging strand and the hairpin. Finally, the hairpin is removed at the 5'-TTGGGG-3' repeat. The replication of telomere has been presented in Figure 4.

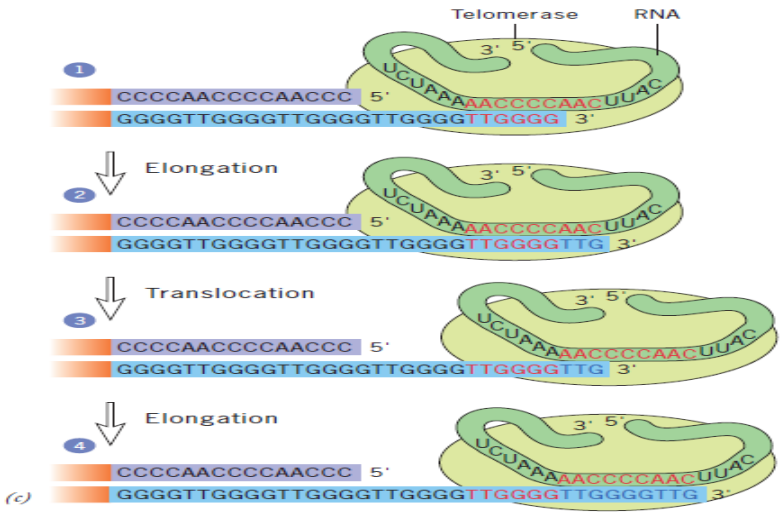


Figure 4: Telomerase replication. Telomerase contains an RNA primer that is complementary to the end of the G-rich strand, which extends past the C-rich strand. The telomerase RNA binds to the protruding end of the G-rich strand in step 1 and then serves as a template for the addition of nucleotides onto the 3' terminus of the strand in step 2. After a segment of DNA is synthesized, the telomerase RNA slides to the new end of the strand being elongated in step 3 and serves as the template for the incorporation of additional nucleotides in step 4. The gap in the complementary strand is filled by the replication enzymes polymerase α-primase. This figure has been adapted from Cell and Molecular Biology Concepts and Experiments by Karp, 2010.

Telomerase activity is retained in germ cells and zygote and somatic cells after few cell division cycles do not show such activities because otherwise they would divide indefinitely and lead to cancer. Thus telomeres shrink causing chromosome shortening to a critical point when the cell ceases to grow and divide. An inherited disease called the Werner's syndrome that causes patients to age much more rapidly than normal is characterized by abnormal telomere maintenance.

Module 2 Lecture 2

Human Cromosome: The human genome is 3 x 10^9 base pairs of DNA and the smallest human chromosome is several times larger than the entire yeast genome; and the extended length of DNA that makes up the human genome is about 1 m long. The human genome is distributed among 24 chromosomes (22 autosomes and the 2 sex chromosomes), each containing between 45 and 280 Mb of DNA **(Figure 1)**. The sex chromosomes are denoted by X and Y and they contain genes which determine the sex of an individual i.e., XX for female and XY for male. The rest are known as autosomes. The haploid human genome contains about 23,000 protein-coding genes, which are far fewer than had been expected before sequencing. In fact, only about 1.5% of the genome codes for proteins, while the rest consists of non-coding genes, regulatory sequences, introns, and noncoding DNA. Chromosomes are stained with A-T (G bands) and G-C (R bands) base pair specific dyes **(Figure 1)**. When they are stained, the mitotic chromosomes have a banded structure that unambiguously identifies each chromosome of a karyotype. Each band contains millions of DNA nucleotide pairs which do not correspond to any functional structure. G-banding is obtained with Giemsa stain yielding a series of lightly and darkly stained bands. The dark regions tend to be heterochromatic and AT rich. The light regions tend to be euchromatic and GC rich. R-banding is the reverse of G-banding where the dark regions are euchromatic and the bright regions are heterochromatic.

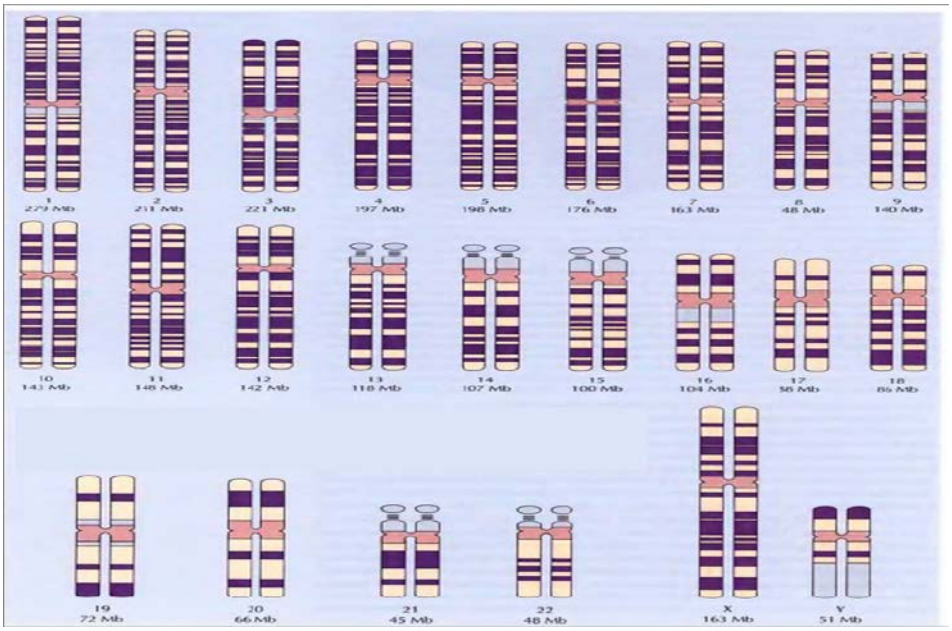


Figure 1: Human metaphase chromosome showing the banding pattern obtained after cytogenetic staining. This figure has been adapted from "The Cell, A Molecular Approach" by Geoffrey M. Cooper, 4th Ed. 2007.

Types of human chromosomes

There are four types of chromosomes based upon the position of the centromere in humans **(Figure 2)**.

1) **Metacentric**: In this type of chromosome the centromere occurs in the centre and all the four chromatids are of equal length.

2) **Submetacentric**: In this type of chromosome the centromere is a little away from the centre and therefore chromatids of one side are slightly longer than the other side.

3) **Acrocentric**: In this type of chromosome the centromere is located closer to one end of chromatid therefore the chromatids on opposite side are very long. A small round structure, attached by a very thin thread is observed on the side of shorter chromatid. The small round structure that is a part of the chromatid is termed as satellite. The thin strands at the satellite region are termed as Nucleolar Organiser Region.

4) **Telocentric**: In this type of chromosome the centromere is placed at one end of the chromatid and hence only one arm. Such telocentric chromosomes are not seen in human cells.

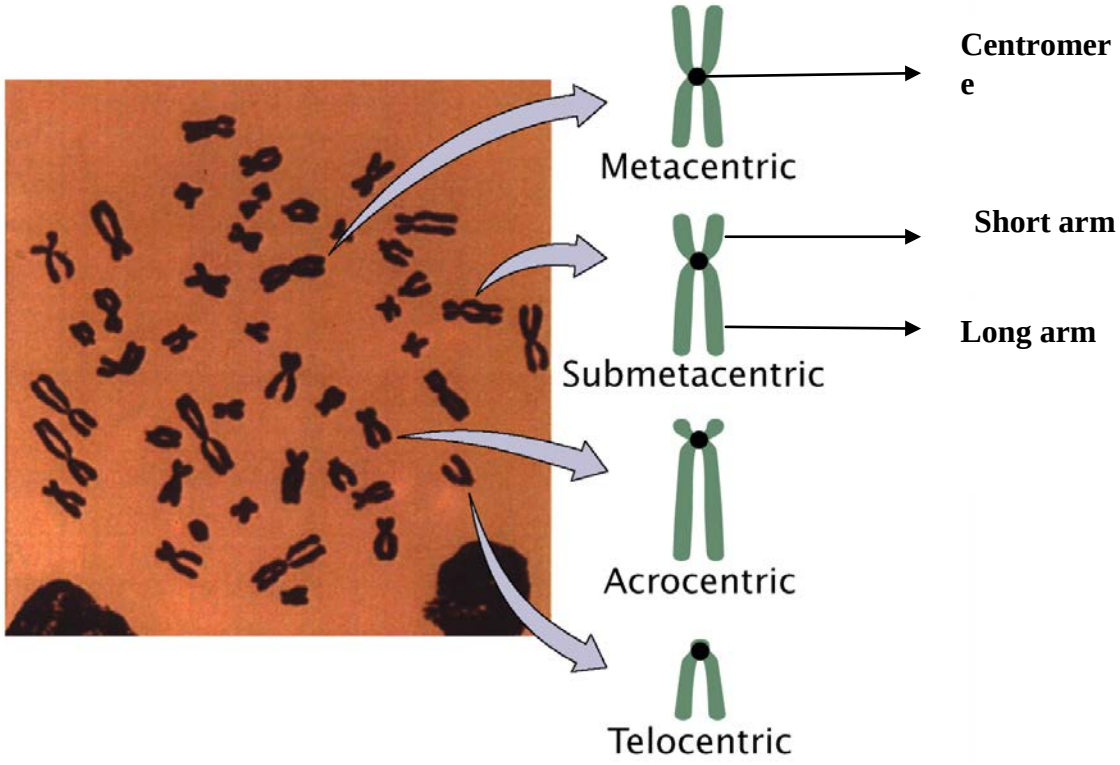


Figure 2: Types of human chromosomes. This figure has been adapted from the "Genetics" by Freeman and company. 2nd Ed, 2005.

Human Chromosome Karyotype

Eukaryotic species have several chromosomes and are detected only during mitosis or meiosis. They are best observed during the metaphase stage of cell division as they are found in the most condensed state. Thus each eukaryotic species is characterized by a **karyotype** which is the numerical description (number and size) of chromosomes in the normal diploid cell. For example, the *Homo sapiens* possess 46 chromosome i,e., 23 pairs **(Figure 3)**. The karyotype is important because genetic research can correlate changes in the karyotype with changes in the phenotype of the individual. For example, Down's syndrome is caused by duplication of the human chromosome number 21. Insertions, deletions and changes in chromosome number can be detected by the skilled cytogeneticist, but correlating these with specific phenotypes is difficult.

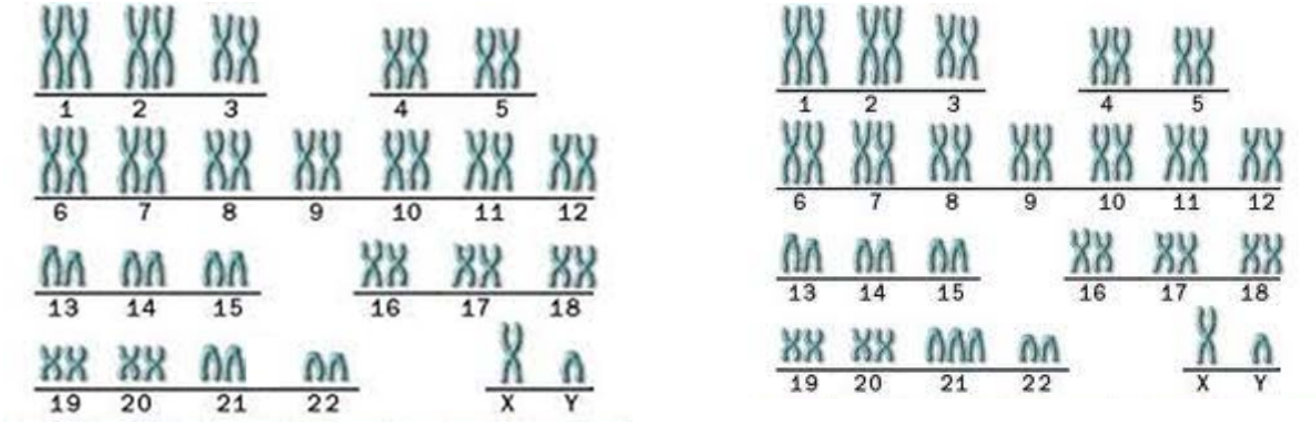


Figure 3: The normal human karyotype (left) and human karyotype in Down's syndrome (Right).

Module 2 Lecture 3

Giant chromosomes:

Some cells at certain particular stage of their life cycle contain large nuclei with giant or large sized chromosomes. Polytene and lampbrush chromosomes are examples of giant chromosomes.

Polytene Chromosome

Giant chromosomes were first time observed by E.G. Balbiani in the year 1881 in nuclei of certain secretory cells (salivary glands) of Chironomas larvae (Diptera). However he could not conclude them to be chromosomes. They were conclusively reported for the first time in insect cells (*Drosophila*) by Theophilus Painter of the University of Texas in the year 1933. Since they were discovered in the salivary glands of insects they were termed as salivary gland chromosomes. The anme polytene chromosome was proposed by Kollar due to the occurrence of many chromonemata (DNA) in them. Cells in the larval salivary gland of *Drosophila,* mosquito *and Chironema* contain chromosomes with high DNA content. However they may also occur in malphigian tubules, rectum, gut, foot pads, fat bodies, ovarian nurse cells etc. Polyteney of giant chromosomes happens by replication of the chromosomal DNA several times without nuclear division (endomitosis) and the resulting daughter chromatids do not separate but remain aligned side by side. During endomitosis the nuclear envelope does not rupture and no spindle formation takes place. The polytene chromosomes are visible during interphase and prophase of mitosis.

They are about 100 times thicker contain 1000 to 2000 chromosomes, than the chromosomes found in most other cells of the organism. When stained and viewed under compound microscope at 40X magnification they display about 5000 bands. In them the chromomere or the more tightly coiled regions alternate with regions where the DNA fibres are folded loosely. A series of dark transverse bands alternates with clear zones of inter bands. Such individual bands can be correlated with particular genes (Figure 1). About 85% of the DNA in polytene chromosomes is in bands and rest 15% is in inter bands. The cross banding pattern of each polytene chromosome is a constant characteristic within a species and helps in chromosome mapping during cytogenetic studies. In *Drosophila melanogaster* there are about 5000 bands and 5000 interbands per genome. These chromosomes are not inert cellular objects but dynamic structures in

which certain regions become "puffed out" due to active DNA transcription at particular stages of development. These chromosome puffs are also termed Balbiani rings. Puffs may apprear and disappear depending on the production of specific proteins which needs to be secreted in large amounts in the larval saliva. Another peculiarity of the polytene chromosomes is that the paternal and maternal chromosomes remain associated side by side and the phenomenon is termed somatic pairing.

Both polyteney and polyploidy have excess DNA per nucleus, but in the later the new chromosomes are separate from each other. A polytene chromosome of *Drosophila* salivary glands has about 100 DNA molecules which are arranged side by side and which arise from 10 rounds of DNA replication (2^{10}=1024). *Chironimus* has 16000 DNA molecules in their polytene chromosomes.

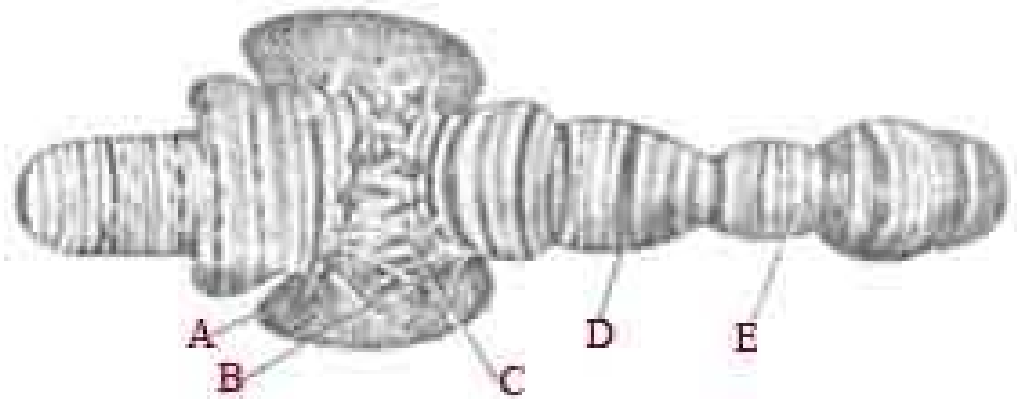


Figure 1: The structure of Drosophilla polytene chromosome. A: mRNA; B-Chromosome puff; C: Chromonemata; D: Dark band; E: Interband. The figure has been adapted from the site http://www.microbiologyprocedure.com/genetics/chromosomes/special-types-of-chromosomes.htm.

Lampbrush chromosome

Lampbrush chromosomes were first observed by Flemming in 1882 in sections of Salamander oocytes and later described by Ruckert in the year 1892. They appeared like brushes used for cleaning lamps, hence the name lampbrush chromosome. They are transitory structures and can be observed during the diplotene stage of prophase I in meiosis in the oocytes of all animal species both vertebrates and invertebrates. They have been described in Sepia (Mollusca), Echinaster (Echinodermata) and in several species of insects, shark, amphibians, reptiles, birds and mammals (humans). Lampbrush chromosomes have also been found in spermatocytes of several species, giant nucleus of Acetabularia and even in plants. Generally they are smaller in invertebrates than vertebrates. They are observed in oocytes because oocytes are high in DNA content.

Lampbrush chromosomes are functional for studying chromosome organization and genome function during meiotic prophase. Additionally lampbrush chromosomes are widely used for construction of detail cytological maps of individual chromosomes.

They are of exceptionally large sizes and present in bivalent form. They are formed due to the active synthesis of mRNA molecules for future use by the egg cells, when no synthesis of mRNA molecule is possible during the mitotic cell division. Lampbrush chromosomes are clearly visible in the light microscope they are organized into a series of chromomeres with large chromatin symmetrical loops extending laterally **(Figure 2)**. Each loop appears at a constant position in the chromosome (10,000 loops per chromosome set or haploid set). Each loop has an axis made up of DNA unfolded from the chromosome and is transcriptionally highly active. Wherein several transcription units with polarized RNP-matrix coats the DNA axis of the loop. The majority of the DNA, however, is not in loops but remains highly condensed in the chromomeres on the axis and lacks expression of genes.

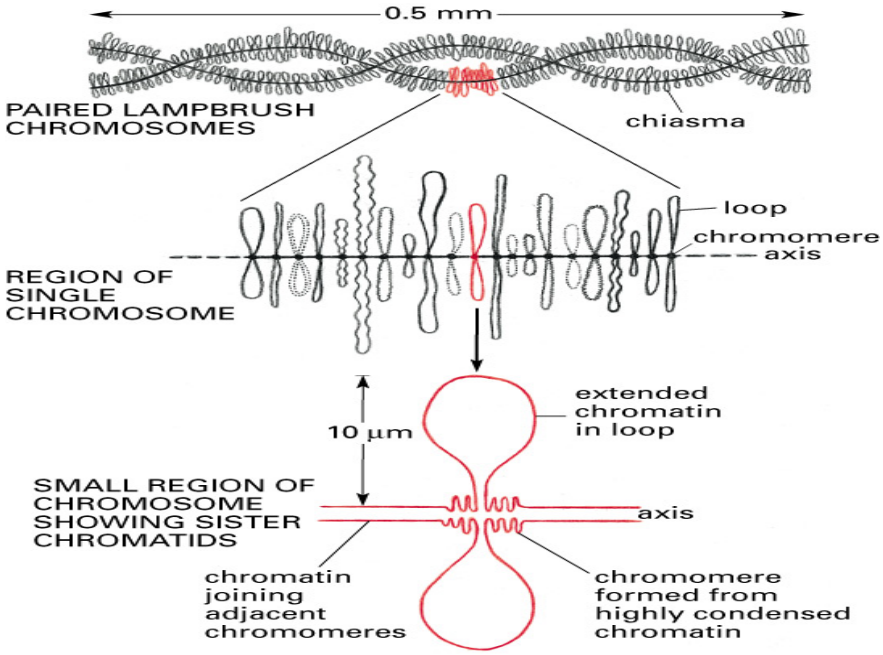


Figure 2: Lampbrush chromosome. This figure has been adapted from the molecular biology of the cell, by Bruce Alberts, 4th Ed. 2008.

The loops perform intense transcription of heterogenous RNA (precursors of mRNA molecules for ribosomal and histone proteins). Thus each lateral loop is covered by an assymetrical matrix of RNA transcripts; thicker at one end of the loop than other. The number of pairs of loops gradually increases during meiosis till it reaches maximum at diplotene. This stage may persist for months or years as oocytes build up supply of mRNA required for further development. As meiosis proceeds further number of loops gradually decrease and loops ultimately disappear due to reabsorption into the chromosome or disintegration.

Certain hypothesis regarding loops are that they may be static or dynamic with new loop material spinning out of one side of a chromosome and returning to a condensed on the other side. This is called spinning out or retraction hypothesis. This hypothesis has been rejected recently through DNA-RNA hybridization studies. The other hypothesis is known as the Master and Slave hypothesis which suggested that each loop pairs and thus chromomere is associated with the activity of many copies of specific genes. There is a master copy at each chromomere and information is transferred to the slave copies which are matched against it to ensure that all are identical. The master copy does not take part in RNA synthesis, but the slave copy is involved in transcription. Large number of duplicate genes ensures higher level of transcription.

Interesting Facts

- If unfolded the DNA in each cell's nucleus would be 2 meters long. Humans have an estimated 100 trillion cells. In other words, if the all the DNA from every cell in a person's body were patched up together they would form a strand of 200 billion kilometers, or more than 1,000 times the distance between Earth and the Sun.
- Genes for the same feature appear in the same locus (place) on each matching pair of chromosomes in every human body cell.
- The 23rd chromosome pair in humans decides what sex you are, and the sex chromosomes are called X and Y.
- In some rare cases people are born with one extra chromosome. Those born with three chromosome 21 have Down's syndrome.
- It takes about 8 hours for one of your cells to completely copy its DNA.

- Human beings share 7% of genes with *E. coli* bacterium, 21% with worms, 90% with mice and 98% with chimpanzees.

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Questions:

1. The products of mitosis are _____.

A. one nucleus containing twice as much DNA as the parent nucleus

B. four genetically identical nuclei

C. four nuclei containing half as much DNA as the parent nucleus

D. two genetically identical nuclei

E. two genetically identical cells

2. Genetically diverse offspring result from _____.

A. binary fission

B. mitosis

C. sexual reproduction

D. cytokinesis

E. cloning

3. How many chromosomes do humans have in their body cells?

A. 48
B. 46
C. 50

4. Which answer is in order from SMALLEST to BIGGEST?

A. gene, chromosome, cell

B. chromosome, gene, cell

C. nucleus, gene, chromosome

5. Sizes of genomes of free-living organisms have been found to range from approximately

A. 2-200 Mbp
B. 0.5-1,000 Mbp
C. 100-2,000,000 Mbp
D. 1,000-1,000,000,000 Mbp
E. 0.5-200,000 Mbp
[1 Mbp = 1 million bp]

6. Most sequences in the human genome belong to

A. Genes

B. Pseudogenes

C. Gene fragments

D. Interspersed repeats

E. Tandem repeats

7. Which of the following genomes is richest in interspersed repeat sequences?

A. *Drosophila* genome

B. Human genome

C. Maize genome

D. *Saccharomyces* genome

E. *E. coli* genome

8. A nucleosome consists of

A. Chromatin and nucleotides

B. Chromatin and histones

C. DNA and chromatin

D. DNA and histones

E. Nucleoids and histone

9. Centromers contain

A. Repeated DNA

B. Chromatids

C. Telomeres

D. Proteins

E. Microtubules

F. Genes

10. Gene density can be high

A. in telomeres

B. anywhere on the chromosomes

C. in centromeres

D. in metaphase chromosomes

E. in anaphase chromosomes

Q11. When can we see chromosomes easily?

Q13. How does dense packing of DNA in chromosome prevent gene expression?

Q14. Illustrate the hierarchy of DNA condensation into chromosomes.

Q15. Differentiate between prokaryotic and eukaryotic genome.

Q16. What are lampbrush and polytene chromosomes and where are they observed?

Q17. What is karyotype? What will happen to human Karyotype in Down Syndrome?

Q18. What is C-value paradox?

Q19. How are telomeres replicated?

Q20. Describe the types of chromosomes.

Q21. What is centromeric DNA?

Q22. What is chromatin? Differentiate between heterochromatin and Euchromatin.

Q23. How polytene chromosomes are formed?

Q24. What will be the result of defective telomere replication?

Module 3 lecture 1

Transport across cell membrane

All cells are generally separated from their surrounding environment by plasma membrane. In addition, the eukaryotic cells are compartmentalized by intracellular membranes that form the boundaries and internal structures of various organelles.Thesebiological membranes are semi-permeable in nature that is their permeability properties ensure that the specific molecules and ions readily enter the cell and the waste products leave the cell. These movements of solutes into the cell are mediated through the action of specific transport proteinsthat arepresent on the cell membrane. Such proteins are therefore required for movements of ions, such as Na^+, K^+, $Ca2^+$, and Cl^-, as well as metabolites such as pyruvate, amino acids, sugars, and nucleotides, and even water. Transport proteins are also responsible for biological electrochemical phenomena such as neurotransmission.

Cell membranearchitecture in transport across cell membrane:

The cell membrane plays an important role in transport of molecules. Because it acts as a semi-permeable barrier, allowing specific molecules to cross while fencing the majority of organically produced chemicals inside the cell. Electron microscopic examinations of cell membranes reveal the development of the lipid bilayer model (fluid-mosaic model). The model consists ofphospholipid, which has a polar (hydrophilic) head and two non-polar (hydrophobic) tails. These phospholipids are aligned tail to tail so the non-polar areas form a hydrophobic region between the hydrophilic heads on the inner and outer surfaces of the membrane.

Permeability of molecules across phospholipid bilayer:

Most of the molecule will diffuse across a protein-free lipid bilayer down its concentration gradient, if provided enough time. The diffusion rate is the function of the size of the molecule and its relative solubility in oil. In general, the smaller the molecule and the more soluble in oil (the more hydrophobic or non-polar), the more rapidly it will diffuse across a cell membrane. Small non-polar molecules, such as O_2 and CO_2, readily

dissolve in cell membrane and therefore diffuse rapidly across them whereas small uncharged polarmolecules, such as water or urea, also diffuse across a bilayer, but much more slowly but ethanol diffuses readily. Conclusively it can be said that lipid bilayers are highly impermeable to charged molecules (ions) by considering its size also because the charge and high degree of hydration of such molecules prevents them from entering the hydrocarbon phase of the bilayer. Thus, these bilayers are 10^9 times more permeable to water than to even such small ions as Na^+ or K^+(**M. Lodish et al., 2003**).

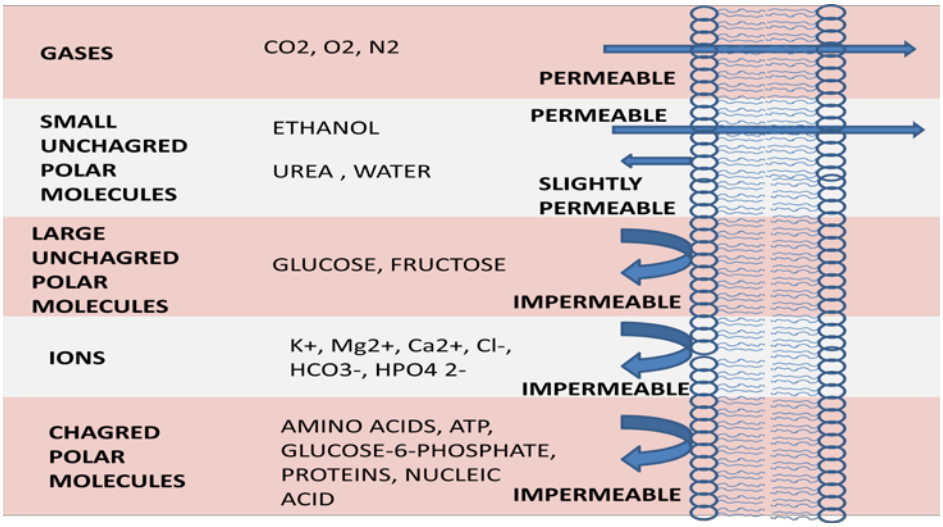

Figure 1:Relative permeability of a pure phospholipid bilayer to various molecules. A bilayer is permeable to small hydrophobic molecules and small uncharged polar molecules, slightly permeable to water and urea, and essentially impermeable to ions and to large polar molecules.

Thermodynamics of transport :

The diffusion of a substance A, across the two sides of a membrane thermodynamically resembles a chemical equilibration.

A(*out*) → A(*in*)

In the following sections, the free energy of a soluteA, varies with its concentration:

$$\overline{G}_A - \overline{G}_A^{o\prime} = RT \ln[A]$$

$$\bar{G}_A = \bar{G}_A^{o,} = RTin\,(A)$$

where

$G_A$is the chemical potential(partial molar free energy) of A (the bar indicates quantity per mole)

$G°`_A$ is the chemical potential of its standard state.

Thus, a difference arises in the concentrations of the substance on two sides of a membrane and generates a chemical potential difference:

$$\Delta\bar{G}_A = \bar{G}_A(in) - \bar{G}_A(out) = RTin\left(\frac{[A]_{in}}{[A]_{out}}\right)$$

If the concentration of A outside the membrane is greater than that inside, ΔG_A for the transfer of A from outside to inside will be negative and the spontaneous net flow of A will be inward. Conversely, if [A] is greater inside than outside, ΔG_A is positive and an inward net flow of A can occur only if an exergonic process, such as ATP hydrolysis, is coupled to it to make the overall free energy change.

The transmembrane movement of ions also depends in charge differences across the membrane, thereby generating an electrical potential difference which is given by:

ΔA=A(in) – A(out),

whereΔAis termed the membrane potential.Consequently, if A is ionic, must be amended to include the electricalwork required to transfer a mole of A across the membrane from outside to inside:

$$\Delta\bar{G}_A = RTin\left(\frac{[A]_{in}}{[A]_{out}}\right) + Z_A\,°\mathrm{F}\,\Delta A$$

$$\Delta\overline{G_A} = RT\ln\left(\frac{[A]_{in}}{[A]_{out}}\right) + Z_A\mathcal{F}\Delta\Psi$$

where

Z_A is the ionic charge of A

F, the Faraday constant, is the charge of a mole of electrons (96,485 C /mol; C is the symbol for coulomb)

G_A is now termed the electrochemical potentialof A.

The membrane potentials of living cells are commonly as high as 100 mV (note that 1 V = 1 J /C).

Types of transport process:

Two types of transport processoccuracross the membrane.

1. Non-mediated transport
2. Mediated transport

Non-mediated transport occurs through the simple diffusion process andthe driving force for the transport of a substance through a medium depends on its chemical potential gradient. Whereasmediated transportrequires specific carrier proteins. Thus, the substance diffuses in the direction that eliminates its concentration gradient; at a rate proportional to the magnitude of this gradient and also depends on its solubility in the membrane's non-polar core. Mediated transport is classified into two categories depending on the thermodynamics of the system:

1. Passive-mediated transport, or **facilitated diffusion:**In this typeof process a specific molecule flows from high concentration to low concentration.

2. Active transport: In this typeof process a specific molecule is transported from low concentration to high concentration, that is, against its concentration gradient. Such an endergonic process must be coupled to a sufficiently exergonic process to make it favorable (ΔG <0).

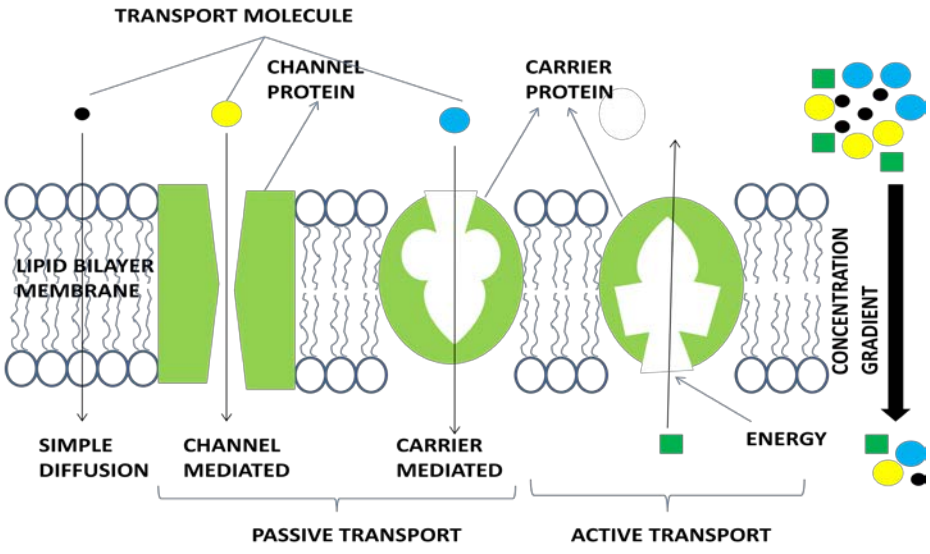

Figure 2: Mediated transport. (A) Passive transport and (B) Active transport

Passive mediated transport:

Substances that are too large or polar diffuse across the lipid bilayer on their own through membrane proteins called carriers, permeases, channels and transporters. Unlike active transport, this process does not involve chemical energy. So the passive mediated transport is totally dependent upon the permeability nature of cell membrane, which in turn, is function of organization and characteristics of membrane lipids and proteins.

Types of passive transport:

1. **Diffusion:**

The process of the net movement of solutes from a region of high concentration to a region of low concentration is known as diffusion. The differences of concentration between the two regions are termed as concentration gradient and the diffusion continues till the gradient has been vanished. Diffusion occurs down the concentration gradient.

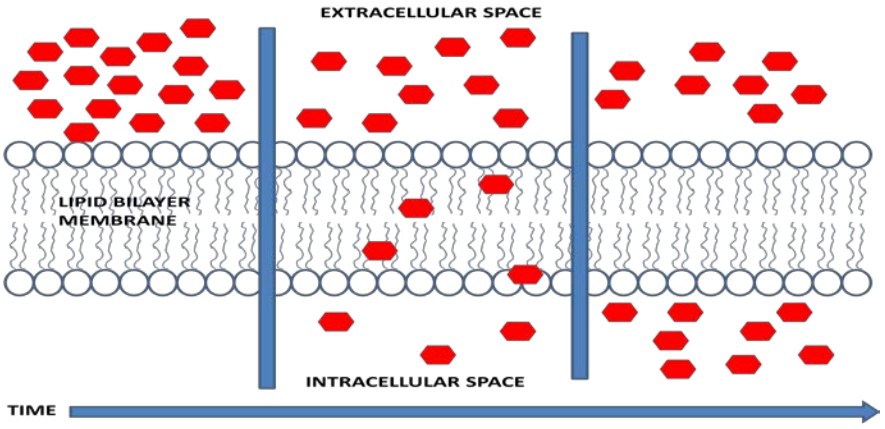

Figure 3: Diffusion.Extracellular space contains high concentration of solutes than intracellular space and hence the solutes move from extracellular space to intracellular space till there is no concentration gradient between the spaces.

2. Facilitated diffusion :

The process of the movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane is known as facilitated diffusion or called carrier-mediated diffusion. Many large molecules, such as glucose, are insoluble in lipids and too large to fit into the porins, therefore, it will bind with its specific carrier proteins, and the complex will then be bonded to a receptor site and moved through the cellular membrane.

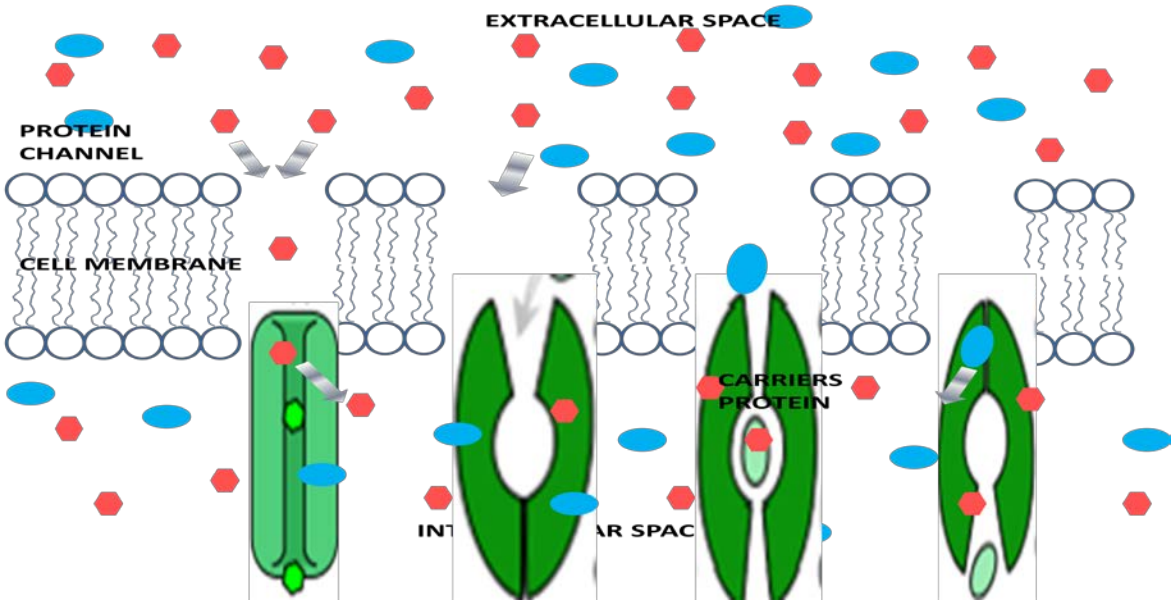

Figure 4: Facilitated transport.Movement of the solutes from extracellular space to intracellular space via carrier proteins and down its concentration gradient.

3. Filtration:

Filtration is the process of the movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the system. Depending on the size of the membrane pores, only solutes of a certain size may pass through it. The membrane pores of the Bowman's capsule in the kidneys are very small, and only albumins (smallest of the proteins)can filter through. On the other hand, the membrane pores of liver cells are extremely large, to allow a variety of solutes to pass through and be metabolized.

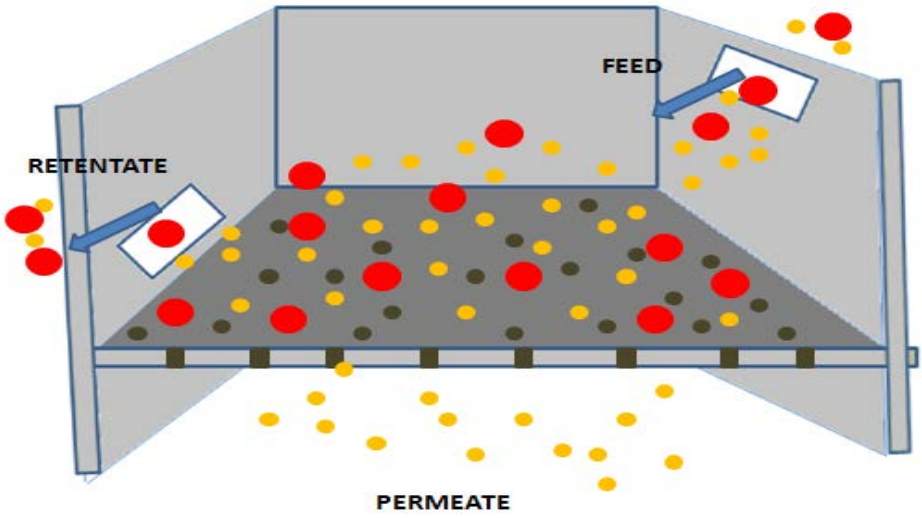

Figure 5: Filtration

4. Osmosis:

Osmosis is the type of diffusion of water molecules across a semi- permeable membrane, from a solution of high water potential to a region of low water potential. A cell with a less negative water potential will draw in water but this depends on other factors as well such as solute potential (pressure in the cell e.g. solute molecules) and pressure potential (external pressure e.g. cell wall).

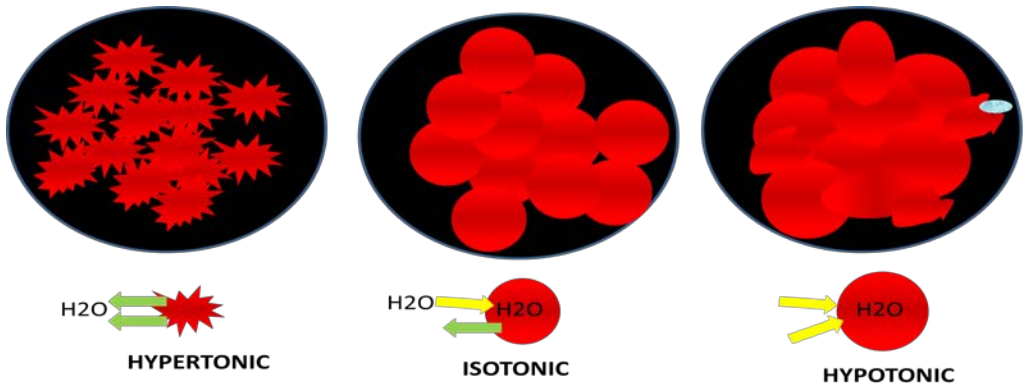

Figure 6: Osmosis.(A) In hypertonic solution, there are more solute molecules outside the cell, which causes the water to be sucked in that direction which leads to the shrinkage of cells. (B) In isotonic solution, there is equal concentration of solute on both sides, henceforth the water with move back in forth. (C) In hypotonic solution, there are less solute molecules outside the cell, since salt sucks and water will move inside the cell. The cell will gain water and grow larger, and finally burst.

Active transport:

Active transport is the movement of a substance against its concentration gradient (i.e. from low to high concentration). It is an endergonic process that, in most cases, is coupled to the hydrolysis of ATP.

Types of active transport:

1. **Primary active transport:**Primary active transport, also called direct active transport, directly uses energy to transport molecules across a membrane.
   Example:Sodium-potassium pump, which helps to maintain the cell potential.

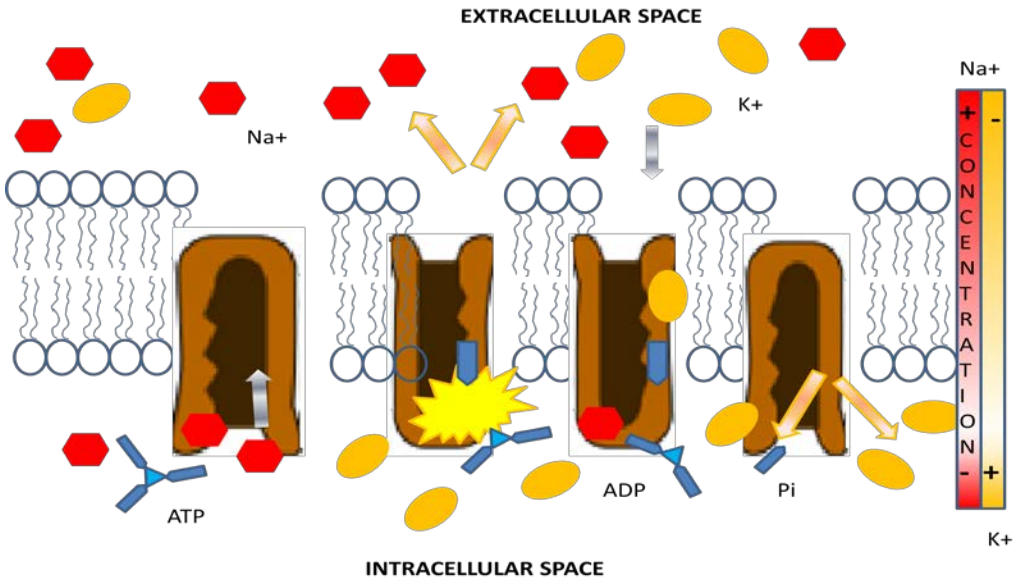

Figure 7: Primary active transport.The action of the sodium-potassium pump is an example of primary active transport.

2. **Secondary active transport:**Secondary active transport or co-transport, also uses energy to transport molecules across a membrane; however, in contrast to primary active transport, there is no direct coupling of ATP; instead, the electrochemical potential difference created by pumping ions out of the cell is instrumental.

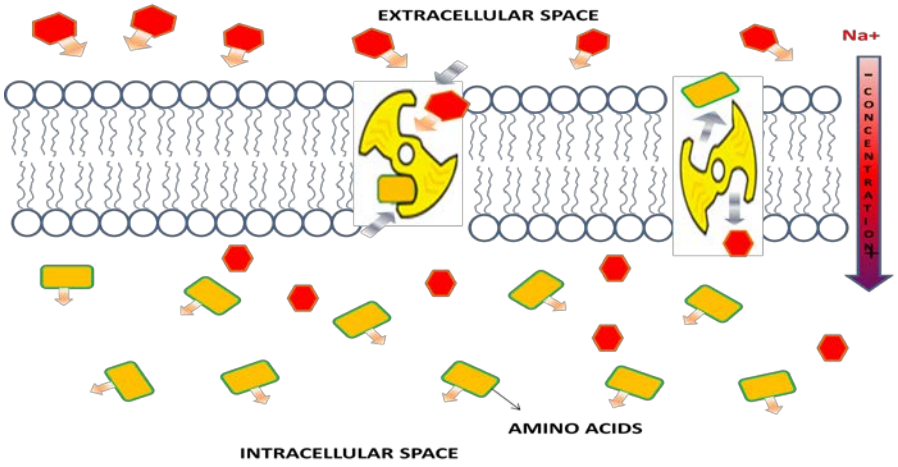

Figure 8: Secondary active transport

The two main forms of active transport are antiport and symport.

(a) Antiport:

In antiport two species of ion or solutes are pumped in opposite directions across a membrane. One of these species is allowed to flow from high to low concentration which yields the entropic energy to drive the transport of the other solute from a low concentration region to a high one.Example: the sodium-calcium exchanger or antiporter, which allows three sodium ions into the cell to transport one calcium out.

(b) Symport:

Symport uses the downhill movement of one solute species from high to low concentration to move another molecule uphill from low concentration to high concentration (against its electrochemical gradient).

Example: glucose symporterSGLT1, which co-transports one glucose (or galactose) molecule into the cell for every two sodium ions it imports into the cell.

Examples:

(A) (Na^+–K^+)–ATPase

(Na^+ - K^+)–ATPaseactive transport system is commonly found in the plasma membranes of higher eukaryotes, which was first characterized by Jens Skou. This transmembrane protein consists of two types of subunits: a 110-kD non-glycosylated α- subunit that contains the enzyme's catalytic activity and ion-binding sites, and a 55-kD glycoprotein β-subunit of unknown function. Sequence analysis suggests that the α- subunit has eight transmembrane α-helical segments and two large cytoplasmic domains. The β- subunit has a single transmembrane helix and a large extracellular domain. The protein may function as an (αβ)2 tetramer *in vivo*.

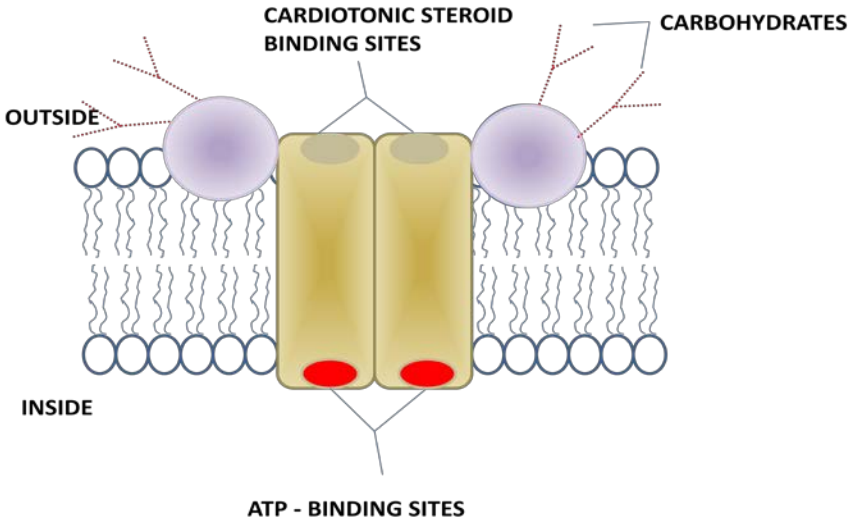

Figure 9: (Na^+ - K^+) – ATPase.This diagram shows the transporter's dimeric structure and its orientation in the plasma membrane. Cardiotonic steroids bind to the external surface of the transporter, thereby inhibiting transport.

The (Na^+ – K^+)–ATPase is also called as the (Na^+ – K^+) pumpbecause it pumps 3 Na^+ out of and 2 K^+ into the cell in presence of hydrolysis of intracellular ATP. The overall stoichiometry of the reaction is:

$3\ Na^+(in) + 2\ K^+(out) + ATP + H_2O \rightarrow 3\ Na^+(out) + 2\ K^+(in) + ADP + Pi$

(B) Ion Gradient–Driven Active Transport

For example, cells of the intestinal epithelium take up dietary glucose by Na^+ -dependentsymport. This process is an example of secondary active transport because Na^+ gradient in these cells is maintained by the (Na^+–K^+)–ATPase.The Na^+ –glucose transport system concentrates glucose inside the cell. Glucose is then transported into the capillaries through a passive-mediated glucose uniport (which resembles GLUT1).

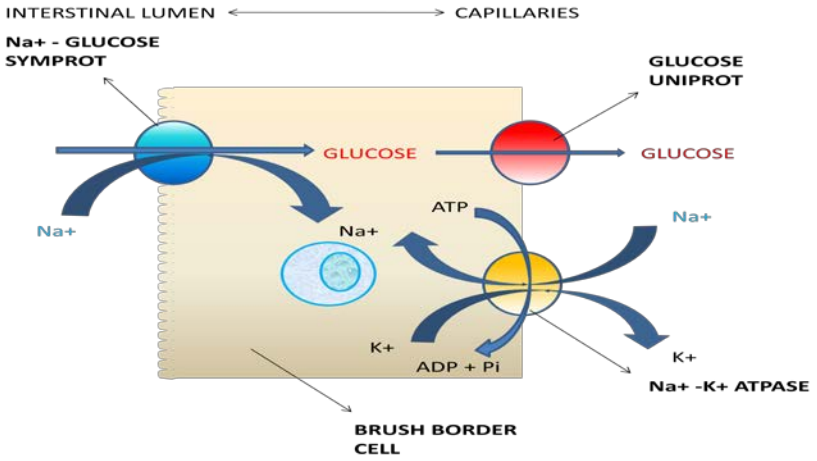

Figure 10: Glucose transport across Interstinal epithelium.The brushlike villi lining the small interstine greatly increases the surface area (a), thereby facilating the absorption of the nutrients. The brush border cells from which the villi are formed (b) concentrate glucose from the interstinal lumen in symport to Na^+ (c), a process that is driven by (Na^+ - K^+) – ATPase, which is located on the capillary side of the cell and functions to maintain a low internal [Na^+]. The glucose is exported to the bloodstream via a passive-mediated uniprot system similar to GLUT1.

Differentiating mediated and non-mediated transport:

Glucose and many other compounds can enter cells by a non-mediated pathway; that is, they slowly diffuse into cells at a rate proportional to their membrane solubility and their concentrations on either side of the membrane. The flux(rate of transport per unit area) of asubstance across the membrane increases with the magnitude of its concentration gradient. If glucose moves across a membrane by means of a transport protein, its flux is no longer linear.

This is one of four characteristics that distinguish mediated from non-mediated transport:

1. **Speed and specificity**-The solubilities of the chemically similar sugars D-glucose and D-mannitol in a synthetic lipid bilayer are similar. However, the rate at which glucose moves through the erythrocyte membrane is four orders of magnitude faster than that of D-mannitol. The erythrocyte membrane therefore contains a system that transports glucose and that can distinguish D-glucose from D-mannitol.
2. **Saturation**-The rate of glucose transport into an erythrocyte does not increase infinitely as the external glucose concentration increases. Such an observation is evidence that a specific number of sites on the membrane are involved in the transport of glucose; which becomes saturatedat high [glucose] and the plot of glucose flux versus [glucose] is hyperbolic. The non-mediated glucose flux increases linearly with [glucose].

3.**Competition**-The curve is shifted to the right in the presence of a substance that competes with glucose for binding to the transporter; for example, 6-*O*benzyl-D-galactose.Competition is not a feature of non-mediated transport, since no transport protein is involved.

4.**Inactivation**-Reagents that chemically modify proteins and hence may affect their functions may inhibit the rapid, saturatable flux of glucose into the erythrocyte.

Interesting facts:

- The binding of the neurotransmitter acetylcholine at certain synapses opens channels that admit Na+ and initiate a nerve impulse or muscle contraction.
- Sound waves bending the cilia-like projections on the hair cells of the inner ear open up ion channels leading to the creation of nerve impulses that the brain interprets as sound.
- Mechanical deformation of the cells of stretch receptors opens ion channels leading to the creation of nerve impulses.
- The crucial roles of the Na+/K+ ATPase are reflected in the fact that almost one-third of all the energy generated by the mitochondria in animal cells is used just to run this pump.

- ABC transporters must have evolved early in the history of life. The ATP-binding domains in archaea, eubacteria, and eukaryotes all share a homologous structure, the ATP-binding "cassette".

Questions:

1. **Carrier molecules that bring materials into cells are**
   a. Lipids
   b. Proteins
   c. Glycogen
   d. Phospholipid
2. **Arrange the following compounds in order of increasing membrane permeability: N_2, water, glucose and RNA.**
   a. RNA>glucose>water>N_2
   b. N_2>water>glucose>RNA
   c. Water>N_2>glucose>RNA
   d. N_2>water>RNA>glucose
3. **The rate of diffusion across the cell membrane is affected by the**
   a. temperature and pinocytosis.
   b. temperature and size of the molecule.
   c. membrane structure and phagocytosis.
   d. shape of glycolipids and glycoproteins.
4. **How many of the following factors would affect the permeability of the cell membrane? • Size of molecules • Lipid solubility of molecules •Presence of transport channels • Presence of ATP inside the cell.**
   a. One.
   b. Two.
   c. Three.
   d. Four.

5. **Which of the following aids the movement of glucose across a cell membrane?**
   a. Protein.
   b. Phosphate.
   c. Glycolipid.
   d. Cholesterol.
6. **In the parietal cells of the stomach, the uptake of chloride ions is coupled to the transport of bicarbonate ions out of the cell. This type of active transport system is called,**
   a. Uniprot
   b. Symprot
   c. Antiprot
7. **Which of the following conditions is required for diffusion to occur?**
   a. ATP energy.
   b. A living cell.
   c. A concentration difference.
   d. A selectively-permeable membrane.
8. **Frog eggs placed in an isotonic solution will**
   a. burst.
   b. shrink.
   c. remain the same.
   d. increase in volume.
9. **When put in a hypotonic environment, an animal cell will**
   a. swell.
   b. shrink.
   c. secrete enzymes.
   d. remain unchanged.
10. **Which of the following conditions would cause red blood cells to burst?**
    a. pH of 7.5.
    b. Temperature of 3°C.
    c. Being placed in distilled water.
    d. Being placed in an 11% salt solution.

11. In an experiment, frog's eggs were placed in a salt solution. After several hours their mass increased significantly. We can therefore conclude that, compared to the frog's eggs, the solution was

a. isotonic.
b. saturated.
c. hypotonic.
d. hypertonic.

12. Which of the following moves material against a concentration gradient?

a. osmosis
b. diffusion
c. active transport
d. facilitated transport

13. Which of the following processes moves molecules using cellular energy?

a. Osmosis.
b. Diffusion.
c. Pinocytosis.
d. Facilitated transport.

14. Which of the following processes would be directly affected by a lack of cellular ATP?

a. Osmosis.
b. Diffusion.
c. Active transport.
d. Facilitated transport.

15. Which of the following will be affected directly if the mitochondria in a cell are not functioning properly?

a. Absorption of alcohol by the cell.
b. The movement of water into and out of the cell.
c. The movement of oxygen across the cell membrane.
d. The movement of sugar from a low to a high concentration.

16. The cell process which uses ATP to bring substances into the cell is

a. Osmosis.

b. Diffusion.

c. Active transport.

d. Facilitated transport.

17. A bacterium is living in a pond where the concentration of sodium ions is 0.005mM. This ion is found in the bacterial cytoplasm at a concentration 0.10 mM. Therefore the sodium ion is probably entering by:

a. Simple diffusion

b. Facilitated diffusion

c. Passive transport

d. Active transport

18. What are the two factors that are responsible for diffusion rate?

19. What are the membrane potentials of living cells?

20. How the opening and closing of ion channels occur in a cell?

21. Explain glucose transporter or GLUT1 with a diagram.

22. What are the different types of mediated transport depending on the thermodynamics of the system?

23. How the mediated transport can be differentiated from non-mediated transport. Explain with a graph.

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Module 3 Lecture 2
Membrane transport facilitators

Membrane transport is assisted by various facilitators to ease their job. We will study a few of them in detail.

Permeases

Permeases are a class of membrane transport proteins which facilitate the diffusion of a specific molecule by passive mediated transport. These are divided into following types:

1. Lactose permease: It is a transmembrane protein that consists of N- and C- terminal domains, each consisting of six membrane-spanning alpha helices in a symmetrical fashion. These two domains are well separated and are joined by a single stretch of polypeptide. There are six side chains amino acids that play an important role in the active transport of lactose through the protein. Some of the examples are: Glutamic Acid 126, Arginine 144, and Glutamic Acid 269 plays role in substrate binding activities where as Arginine 302, Histidine 322, and Glutamic Acid 325 plays a significant role in proton translocation throughout the transport process. These side chains, make up the active site of the protein and found within the large internal hydrophilic cavity of the lactose permease where the substrate is received for transport and it is the location from which it is sent into the cell.

It is an active co-transport that facilitates the passage of lactose across the phospholipid bi-layer of the cell membrane by using the inwardly directed H^+ electrochemical gradient as its driving force. The proton gradient is metabolically generated through oxidative metabolism. The electrochemical potential gradient created by both these systems is used mainly to drive the synthesis of ATP. As a result, the lactose is accompanied from the periplasam to the cytoplasm of the cell by an H^+ proton.

Lactose permease has two major conformational states:

1. E-1, which has a low-affinity lactose-binding site facing the interior of the cell.

2. E-2, which has a high-affinity lactose-binding site facing the exterior of the cell.

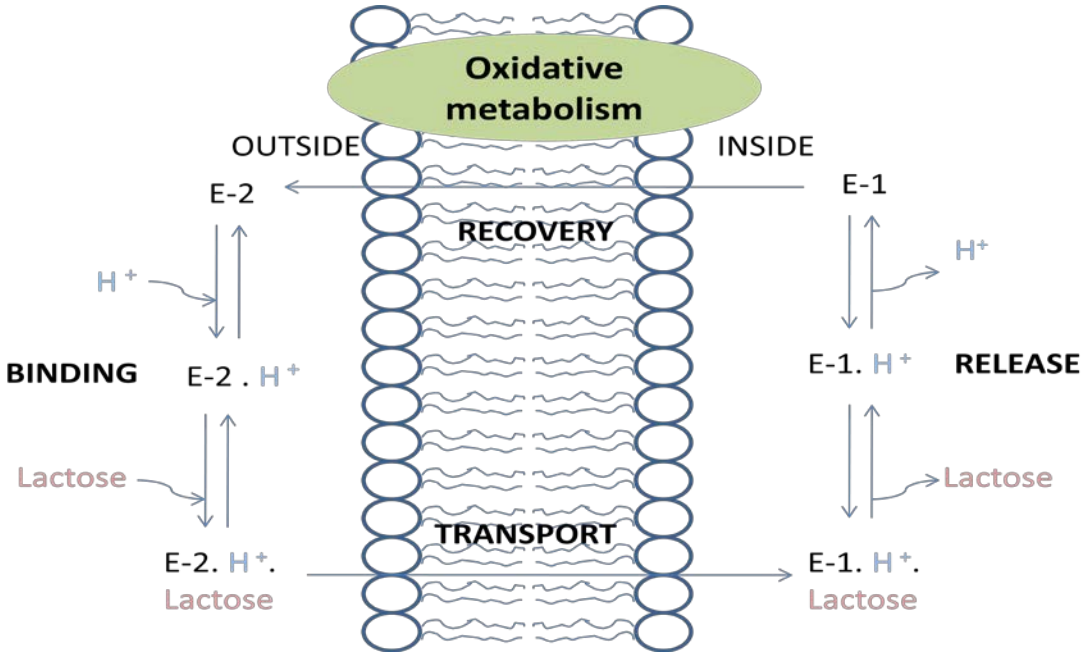


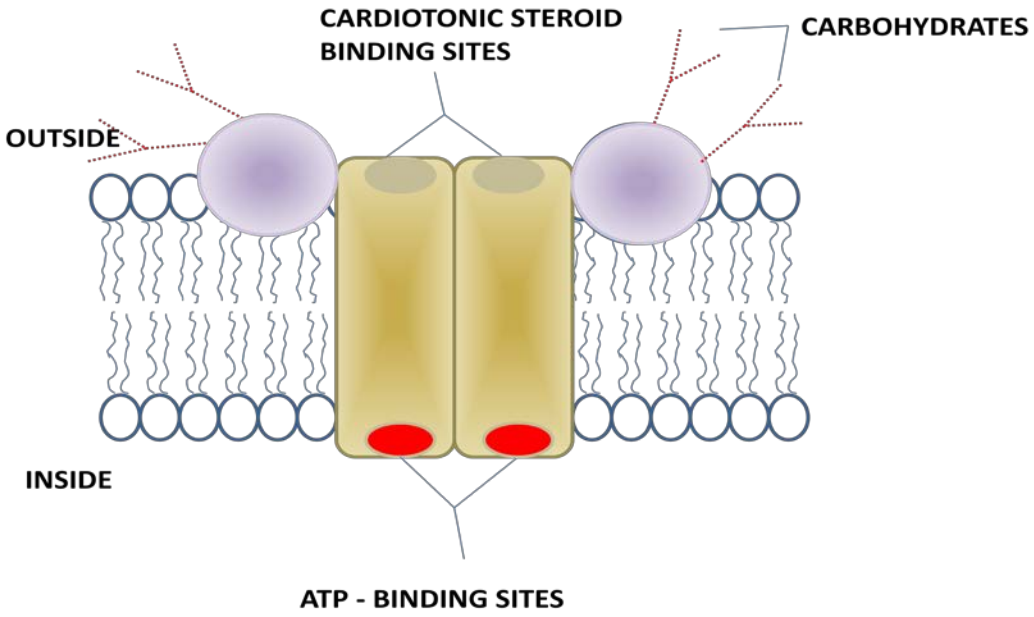
Figure 1: Schematic diagram for the cotransport of H^+ and lactose by lactose permease in *E.Coli*. H^+ binds first to E-2 outside the cell, followed by lactose. They are sequentially released from E-1 inside the cell. E-2 must bind to lactose and H^+ in order to change the conformation to E-1, thereby cotransporting these substances in the cell. E-1 changes the conformation to E-2 when neither lactose nor H^+ is bound, thus completing the transport cycle.

2. β-galactoside permease is a membrane-bound transport protein that facilitates the uptake of β-galactosides across the cell. The common example is melibiose carrier protein from *Klebsiella pneumonia*, which is capable of using hydrogen and lithium cations as coupling cations for cotransport, depending on the particular sugar transported (H^+-melibiose, Li^+-lactose).

3. Amino acid permeases are integral membrane proteins involved in the transport of amino acids into the cell. One of the examples of amino acid permease is histidine permease which is a bacterial ABC protein in *E.coli* and located in the periplasmic space of cell. Histidine binding protein binds histidine tightly and directs it to T sub-units of permease, through which hisitidine crosses the plasma membrane along with ATP hydrolysis.

Na^+/K^+ ATPase :

In mammalian cells, the Na^+ and K^+ gradients are the two major components of the electrochemical gradient across the plasma membrane. The cells maintain a lower intracellular Na^+ concentration and higher intracellular K^+ concentration with relative to extracellular space. Hence, for the generation and maintenance of the electrochemical gradients for Na^+ and K^+, it requires Na^+/K^+ ATPase, which is an ion pump that couples ATP hydrolysis to cation transport. It also helps to set the negative resting membrane potential, which regulates the osmotic pressure to avoid cell lysis. The Na^+/K^+ ATPase belong to P-class ATPase which is commonly found in the plasma membranes of higher eukaryotes. This transmembrane protein consists of two types of subunits: a 110-kD non-glycosylated α- subunit that contains the enzyme's catalytic activity and binding sites for ATP, Na^+ and K^+ ions, and a 55-kD glycoprotein β-subunit of unknown function. The smaller β-subunit has one transmembrane domain that stabilizes the α-subunit and is important in membrane insertion. The α- subunit has eight transmembrane α-helical segments and two large cytoplasmic domains and the β- subunit has a single transmembrane helix and a large extracellular domain. The protein may function as an (αβ)2 tetramer *in vivo*.



Figur 2: Na^+/K^+ ATPase. The diagram shows the transporter's putative dimeric structure and its orientation in the plasma membrane. Cardiotonic steroids bind to the external surface of the transporter, thereby inhibiting transport.

The Na^+/K^+ ATPase is also called as the Na^+/K^+ pump because it pumps 3 Na^+ out of and 2 K^+ in both direction across the membrane in presence of hydrolysis of ATP. The overall reaction is:

3 Na^+(*in*) + 2 K^+(*out*) + ATP + H2O →3 Na^+(*out*) + 2 K^+(*in*) + ADP + P*i*

The important feature to the Na^+/K^+ ATPase is the phosphorylation of a specific Asp residue of the transport protein which phosphorylates only in the presence of Na^+, whereas the resulting aspartyl phosphate residue is subject to hydrolysis only in the presence of K^+. Hence it has two conformations named E1 and E2. The protein appears to operate in the following (explained in figure 4):

1. The protein in the *E*1 state has three high-affinities Na^+ binding sites and two low-affinity K^+ binding sites accessible to the cytosolic surface of the protein. Hence E1 binds three Na^+ ions inside the cell and then binds ATP to yield an *E*1 .ATP.3 Na^+ complex.
2. ATP hydrolysis produces ADP and a "high-energy" aspartyl phosphate intermediate *E*1-P.3 Na^+.
3. This "high-energy" intermediate relaxes to its "low-energy" conformation, *E*1~P.3 Na^+, and releases its bound Na^+ outside the cell.
4. *E*2-P binds two K^+ ions from outside the cell to form an *E*2-P.2 K^+ complex.
5. The phosphate group is hydrolyzed, yielding *E*2 .2 K^+.
6. *E*2 .2 K^+ changes conformation, releases its two K^+ ions inside the cell, and replaces them with three Na^+ ions, thereby completing the transport cycle.

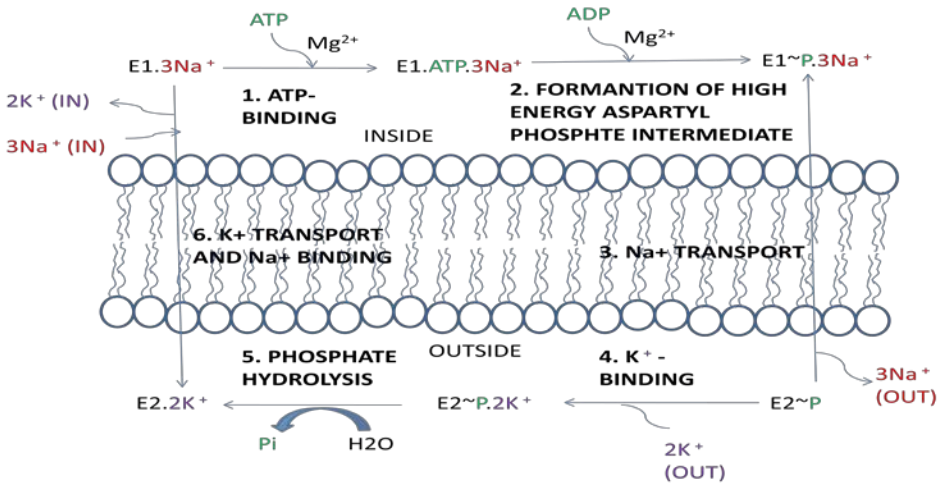


Figure 3: Scheme for the transport of Na^+ and K^+ by the Na^+/K^+ ATPase.

These are mostly target of a large number of toxins and important drug target. Some of the examples are: the naturally occurring steroids called cardiac glycoside such as ouabain and digitalis, inhibit ion transport by Na^+/K^+ ATPase by binding reversibly to the extracellular side of pump which in turn inhibit ATP hydrolysis and ion transport. Other toxins like palytoxin from marine corals are also specific inhibitor. They block the ATPase in an open state, allowing ions to flow down their concentration gradient, which destroys electrochemical gradient.

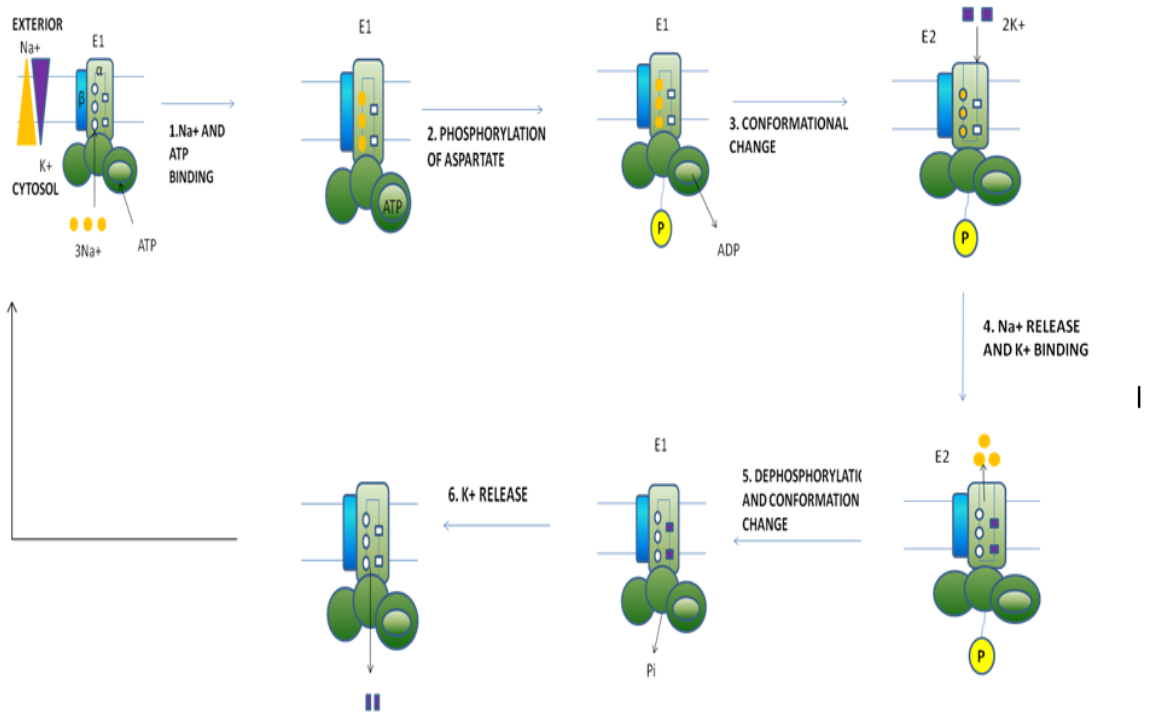


Figure 4: Operational model of the Na^+/K^+ ATPase in the plasma membrane. Only one of the two catalytic α subunits of this P-class pump is depicted. It is not known whether just one or both subunits in a single ATPase molecule transport ions. Ion pumping by the Na^+/K^+ ATPase involves phosphorylation, dephosphorylation, and conformational change. In this case, hydrolysis of the E2–P intermediate powers the E2 → E1 conformational change and concomitant transport of two ions (K^+) inward. Na^+ ions are indicated by red circles; K^+ ions, by purple squares; high-energy acyl phosphate bond, by ~P; low-energy phosphoester bond, by –P.

Ca^{2+} ATPase

Eukaryotic cells maintain a low concentration of free Ca^{2+} in the cytosol (10^{-7} M) whereas the extracellular concentration is very high on the opposite face (10^{-3} M). Henceforth, a small influx of Ca^{2+} significantly increases the concentration of free Ca^{2+} in the cytosol and the flow of Ca^{2+} down its steep concentration gradient in response to the extracellular signals is one of the means of transmitting these signals rapidly across the plasma membrane. Hence cells maintain a steep Ca^{2+} gradient across the plasma membrane. The $Ca2^{+}$ ATPases are commonly found in muscle cells and neurons. The skeletal muscle have specialized structure of large intracellular Ca^{2+} stores called sarcoendoplasmic recticulum which controls Ca^{2+} uptake and release throughout the cell volume. These are mainly responsible for Ca^{2+} extrusion from cytosol in muscle cells which is required to stop muscle contraction and to initiate relaxation.

Ca^{2+} transporters are the common example of P-type transport ATPase. It is also known as Ca^{2+} pump or Ca^{2+} ATPase or SERCA pump (Sacroendoplasmic recticulum Ca^{2+} ATPase). These transporters actively pump Ca^{2+} out of the cell and helps in maintaining the gradient. The structure of Ca^{2+} pump has an asymmetrical arrangement of transmembrane and cytosolic domains that undergo movements during Ca^{2+} transport. It contains 10 tranmembrane α-helices and two cytoplasmic loops between the transmembrane α-helices. The transmembrane α-helices form Ca^{2+} binding site which binds two Ca^{2+} ions from cytosol. And the two cytoplasmic loops form three separate domains: nucleotide binding domains that binds ATP, actuator domain that contains catalytic phoshorylation site and P domain which is important for transmission of conformational changes between cytosolic and transmembrane domains. In unphosphorylated state, the two helices are disturbed and form a cavity for binding of two Ca^{2+} ions from the cytosolic side of the membrane. ATP also binds to a binding site on the same side of the membrane and the subsequent transfer of the terminal phosphate group of ATP to an aspartic acid of an adjacent domain lead to a drastic rearrangement of the transmembrane helices. This rearrangement disturbs the Ca^{2+} binding site and releases Ca^{2+} ions on the other side of the membrane that is into the lumen of SR. With respect to figure 5 and 6, the mechanism of the Ca^{2+} ATPase in the SR membrane can be understood clearly through following steps:

1. The protein in E1 conformation has two high affinity binding sites for Ca^{2+} ions accessible from the cytosolic side and ATP binds to a side on cytosolic surface.
2. In the presence of Mg^{2+}, the bound form of ATP is hydrolyzed to ADP and phosphate. Later the liberated phosphate is transferred to a specific aspartate residue in the protein, forming the high-energy acyl phosphate bond denoted by E1 ~ P.
3. Then the protein undergoes a conformational change and generates E2, which has two low-affinity Ca^{2+} binding sites accessible to the SR lumen.
4. The free energy of E1 ~ P is greater than E2-P, and this reduction in free energy leads to the E1 → E2 conformational change. Simultaneously, the Ca^{2+} ions also dissociate from the low-affinity sites to enter the SR lumen, following which the aspartyl-phosphate bond is hydrolyzed.
5. Dephosphorylation then again leads to the E2 → E1 conformational change, and E1 is ready to transport two more Ca^{2+} ions.

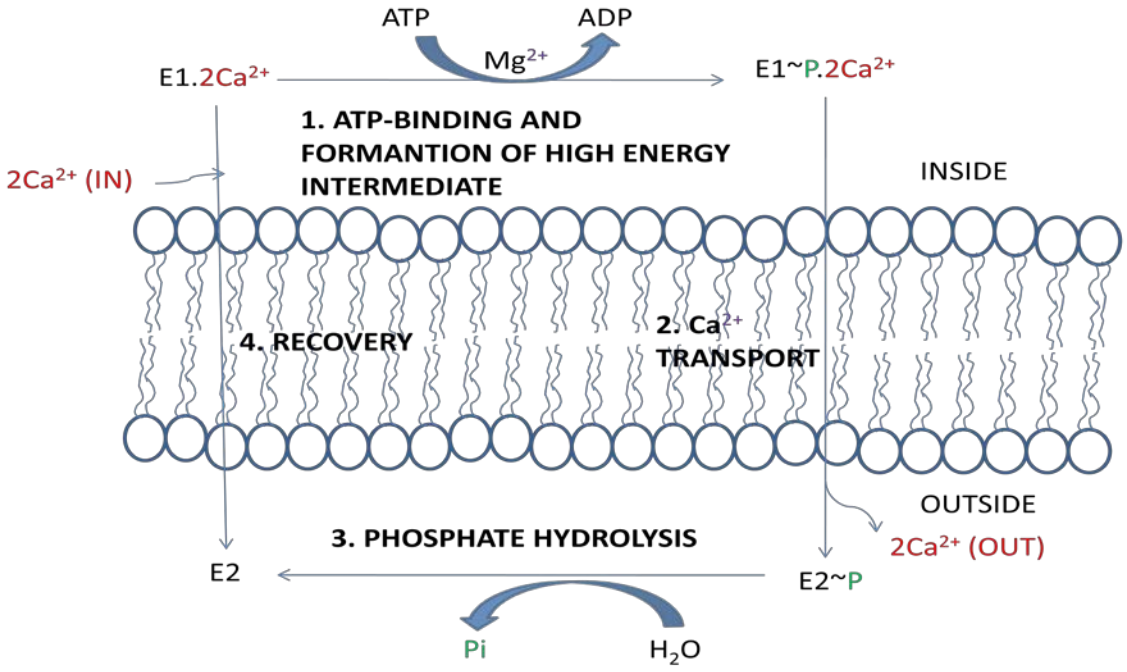


Figure 5: Scheme for the active transport of Ca^{2+} by the Ca^{2+} ATPase. Here (*in*) refers to the cytosol and (*out*) refers to the outside of the cell for plasma membrane Ca^{2+} ATPase or the lumen of the endoplasmic reticulum (or sarcoplasmic reticulum) for the Ca^{2+} ATPase of that membrane.

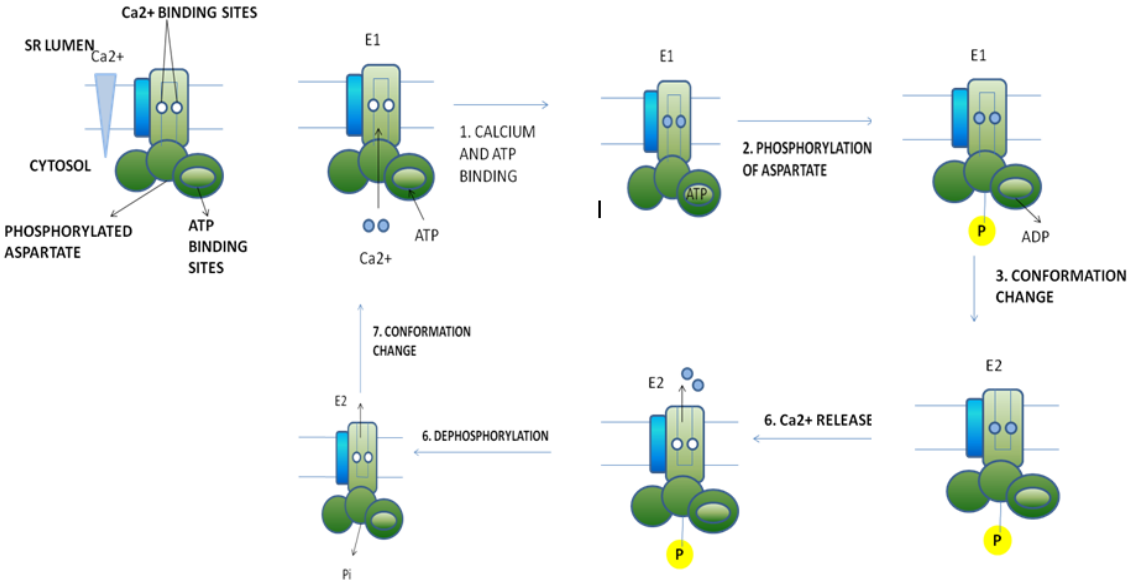


Figure 6: Operational model of the Ca^{2+} ATPase in the SR membrane of skeletal muscle cells. Only one of the two catalytic α subunits of this P-class pump is depicted. E1 and E2 are alternative conformations of the protein in which the Ca^{2+} binding sites are accessible to the cytosolic and exoplasmic faces, respectively. An ordered sequence of steps (1 – 6), as diagrammed here, is essential for coupling ATP hydrolysis and the transport of Ca^{2+} ions across the membrane. In the figure, ~P indicates a high-energy acyl phosphate bond; –P indicates a low-energy phosphoester bond.

Interesting facts:

- The X-ray crystal structure of lactose permease was first solved in 2003 by J. Abramson et al.
- Ouabain is a cardiac glycoside toxin. Potent inhibitors that bind to potassium binding sites. In the presence of Ouabain, Na^+/K^+ ATPase cannot return to its resting state.
- One major type of gradient linked active permeases is the sodium-glucose symport carrier.

Questions:

1. **Which of the following uses energy to transport molecules or ions against their concentration gradient?**

a. Voltage-gated Na+ channel
b. Acetylcholine receptor
c. Glucose transporter
d. ATP-ADP transporter
e. Na+/K+-ATPase

2. **A membrane-spanning transporter protein that is also characterized as a "symporter" would be involved in which one of the following transport processes?**

a. Simple transport (e.g., lactose via Lac permease)
b. Simultaneous transport of one type of molecule into the cell and a different molecule out of the cell (e.g., Na+ "pump" to move Na+ out of the cell)
c. Transport of potassium ions into the cell without any other ion or molecule being transported in any direction
d. Unidirectional transport into the cell of only one type of molecule (found in very low concentration in the periplasm) using the ATP-driven ABC translocation system

3. **The sodium-potassium pump passes**

a. more Na+ out than K+ in
b. K+ out and Na+ in on a one-for-one basis
c. Na+ out and K+ in on a one-for-one basis
d. K+ and Na+ in the same direction

4. **The sodium-potassium pump moves sodium and potassium ions against the concentration gradient.**

a. True
b. False

5. **The Na^+-K^+ pump consumes a third of the total ATP supply of a typical animal cell and is responsible for maintaining the high concentration of K^+ inside cells, for controlling cell volume, and for driving the uptake of sugars and amino acids in the intestine and kidneys.**
   a. True
   b. False

6. **The energy needed to power the sodium-potassium pump is provided by the**

a. Binding of ATP to the pump
b. Transport of ATP by the pump.
c. Splitting of ATP.
d. Formation of ATP.

7. **Which of the following moves Ca^{2+} back into the tubules of the SR after a contraction?**

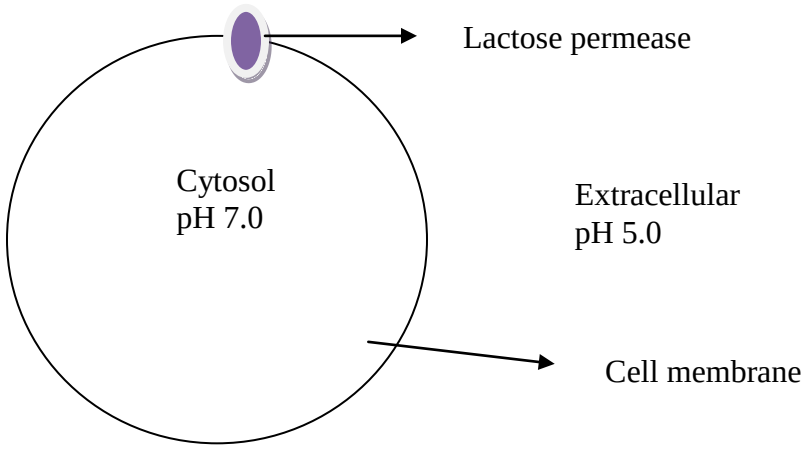
a. The ATP-dependent H^+ pump
b. The ATP-dependent myosin pump
c. Simple diffusion
d. The ATP-dependent Na^+/K^+ pump
e. The ATP-dependent Na^+/K^+ pump
f. The ATP-dependent calcium pump

8. **SERCS pumps actively transport calcium:**

a. From ER to cytosol
b. From cytosol to ER
c. From extracellular space to the cytosol
d. From the cytosol to the extracellular space
e. From the mitochondria to the cytosol

9. **In each cycle, the Na^+-K^+ pump transfers ____K^+ ions in the cell and ____ Na^+ out of the cell.**

10. **Bacterial lactose permease is a symporter of lactose and H+. When the lactose concentrations in the cytosol and in the extracellular space are identical but the pH's in the two locations are different as indicated below, which direction would lactose be transported? Explain briefly why you think that way.**



11. **Why the sodium-potassium transport mechanism is called a pump?**
12. **Explain Na^+/K^+ pump with a schematic diagram.**
13. **Explain Ca^{2+} pump with a schematic diagram**
14. **What are the types of permeases?**

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Module 3 Lecture 3

Lysosome and vacuolar membrane

In earlier lecture we have studied about plasma membrane. However some cell organelles have depending on the function which they perform have modified membranes. We will study the membrane of a lysosome and vacuoles in detail in this lecture.

Lysosomes:

Lysosomes are central, acidic and membrane bound organelles that contain hydrolase enzyme for the breakdown of all types of biological polymers- proteins, nucleic acids, carbohydrates and lipids. They are mostly found in animal cells, while in yeast and plants, it acts as lytic vacuoles. It is enclosed by membrane known as lysosomal membrane that maintains the digestive enzyme at pH 4.5. Figure 1 shows the structure of lysosome.

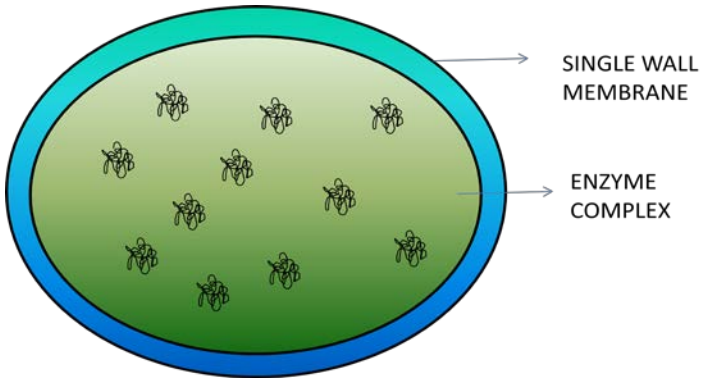


Figure 1: Lysosome

Functions of lysosomes:

- Maintains pH by pumping protons from cytosol across the membrane via proton pumps and chloride ion channels.
- Protects the cytosol and rest of the cells from degradative enzymes within the lysosome.
- Acts as digestive system of the cell, serving both to degrade material taken up from the outside of the cell and to digest obsolete components of cell itself.

- Sequestration of lysosomal enzymes.
- Mediation of fusion events between lysosomes and other organelles.
- Transport of degradation products to the cytoplasm

Lysosomal Membrane: To perform its function with efficacy the lysosomal membrane needs some additional features in its membrane. It is slightly thicker than that of the plasma membrane. It contains substantial amounts of carbohydrate component, particularly sialic acid. In fact, most lysosomal membrane proteins are highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration. Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue. The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H+ inside the organelle. Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

Lysosomal membrane composition:

The V-class H^+ ATPase pump is generally present in lysosomal membrane. This class of ATPase pump only transports H^+ ions. Its main function is to acidify the lumen of the organelles. The proton gradient between the lysosomal lumen (pH ≈4.5–5.0) and the cytosol (pH ≈7.0) depends on ATP production by the cell.

These V-class proton pumps contain two domains: a cytosolic hydrophilic domain (V_1) and a transmembrane domain (V_0) with multiple subunits in each domain. Binding and hydrolysis of ATP by the B subunits in V_1 provides the energy for pumping of H^+ ions

through the proton-conducting channel formed by the c and a subunits in V_0. These V-class proton pumps are not phosphorylated and dephosphorylated during proton transport. Figure 2 depicts a V-class proton pump.

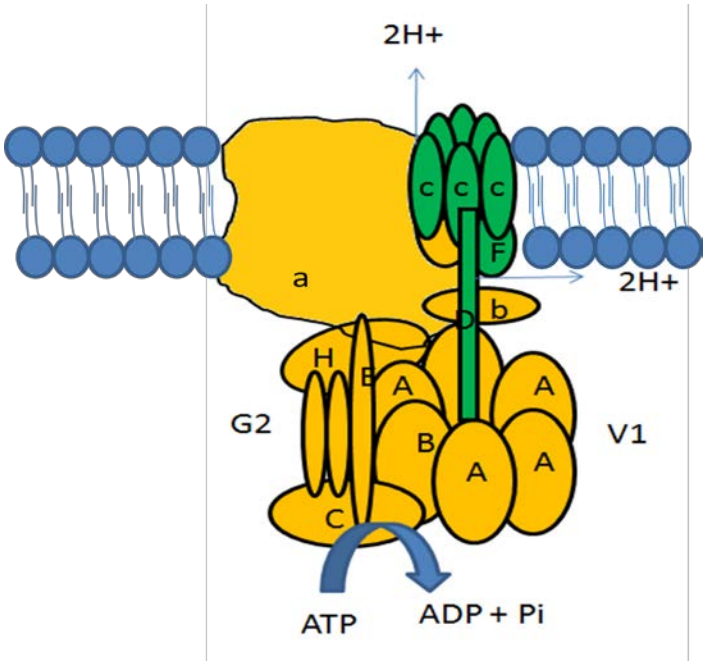
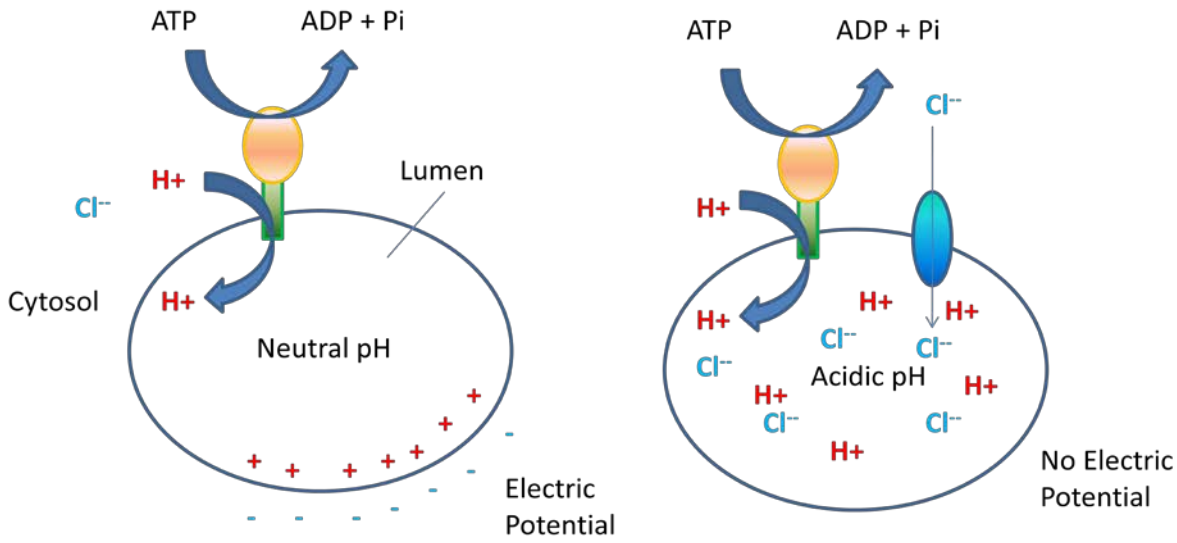


Figure 2: V-class proton pump

These protons cannot acidify by themselves because a net movement of electric charge occurs. Only a few protons build up positive H^+ ions on exoplasmic face (inside) and for each H^+ pumped across, a negative ion will be left behind on cytosolic face, building negative charged ions. These oppositely charged ions attract each other on opposite faces of the membrane, generating a charge separation, or electric potential, across the membrane. If more protons are pumped, the excess positive ions on exoplasmic face repels other H^+ ions and prevents pumping of extra proton long before a significant transmembrane H^+ concentration gradient had been established .



(a) **(b)**

Figure 3: Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change occurs in the intraluminal pH. (b) If the organelle membrane also contains Cl^- channels, anions passively follow the pumped protons, resulting in an accumulation of H^+ ions (low luminal pH) but no electric potential across the membrane.

Lysosomal membrane proteins:

Lysosomes are formed by the fusion of transport vesicles budded from Golgi network with endosomes, which contain molecules taken up at the cell surface. And its membrane proteins are usually highly glycosylated proteins decorating the luminal surface of lysosomal membranes. They are most often known as lysosomal associated membrane proteins (LAMP). LAMP-1, LAMP-2 and LIMP-2 are the most abundant components of this membrane. And mainly involved in transport of newly synthesized hydrolases to the lysosome (lysosomal integral membrane protein 2 (LIMP2)) and across the lysosomal membrane (the V-type H^+-ATPase complex and chloride channel protein 7 (CLC7).

Vacuolar membrane:

Vacuoles are the membrane bound sac within the cytoplasm which are filled with water containing organic and inorganic molecules including enzymes and mostly present in plants, fungi and some animals. This vacuole slowly develops as the cell matures by fusion of smaller vacuoles (vesicles) derived from the endoplasmic reticulum and Golgi apparatus.

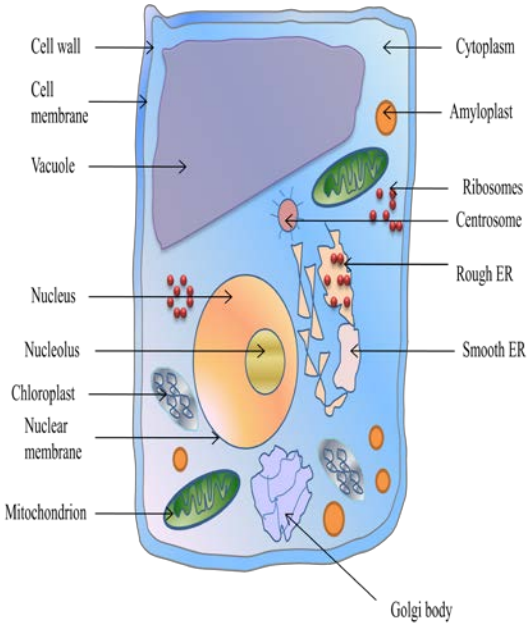


Figure 4: Plant cell structure

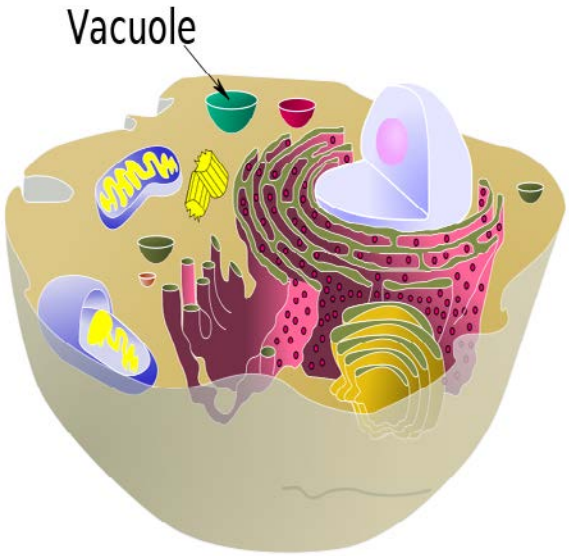


Figure 5: Animal cell structure

Function of vacuoles:

- Acts as storage organelles and contains water and small molecules. Stores salts, minerals, nutrients, proteins, pigments, helps in plant growth, and plays an important structural role for the plant.
- Maintains internal hydrostatic pressure or turgor pressure within the cell
- Maintains an acidic internal pH
- Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole. Also maintains turgor pressure against the cell wall. Because of osmosis, water diffuses into the vacuole, and exerting pressure on the cell wall. And water loss leads to shrinkage of the cell. Hence turgor pressure needs to be maintained. Turgor pressure also dictates the rigidity of the cell and is associated with the difference between the osmotic pressure inside and outside of the cell.
- In seeds, stored proteins needed for germination are kept in protein bodies, which are modified vacuole.
- Regulating the movements of ions around the cell.
- Transports proton from cytosol to vacuole and hence stabilizes cytoplasmic pH making the vacuolar interior most acidic by creating a proton motive force which in turn used for the transport of nutrients into and out of the cell and allows degradative enzymes to act.
- Vacuoles also often store the pigments that give certain flowers their colors, which aid them in the attraction of bees and other pollinators, but also can release molecules that are poisonous, odoriferous, or unpalatable to various insects and animals, thus discouraging them from consuming the plant.

Plant vacuoles:

Most of the plant cell contains large, single central vacuoles and can occupy at least 30% to 80% of the cell. Generally vacuole is surrounded by membrane known as tonoplast, or vacuolar membrane. It separates the vacuolar contents from cell's cytoplasm and an important and highly integrated component of the plant internal membrane network (endomembrane) system. The vacuole solution (also known as cell sap) differs markedly from that of the surrounding cytoplasm.

Vacuolar membrane:

The V-class H+ ATPase pump is present in vacuolar membrane. More details of V-class H+ ATPase pump is described earlier (Figure 2 and Figure 3).

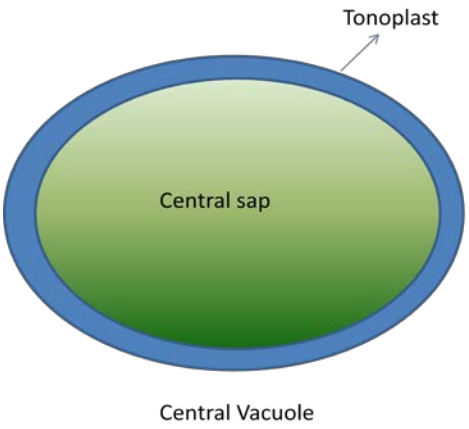


Figure 6: Plant cell vacuole

Other transport proteins present in vacuolar membrane:

1. Proton Pump:

Proton pumps play a central role in the function of the tonoplast by generating a transmembrane H+ electrochemical gradient which can be utilized to drive the transport of solutes. The tonoplast contains different proton pumps, an ATPase and a PPase. V-ATPases (vacuolar-type) are present on different membranes of eukaryotic cells and is constituted of 13 subunits whereas tonoplast PPase is also an integral entity of the tonoplast and consists of one 80 kDa protein.

2. Aquaporins:

Major intrinsic membrane proteins (MIPs), are very small hydrophobic proteins abundantly present in membranes. But these MIPs form water channels. Later α-TIP (tonoplast intrinsic protein) which is a member of MIPs was described and found abundantly. Another major membrane protein of the central vacuole is the γ-TIP (observed in radish). Both TIPs have been shown to act as water channels. α-TIP is associated with the storage vacuole while the γ-TIP is localized on the lytic vacuole. Interestingly, α-TIP has to be phosphorylated in order to exhibit water channel activity.

3. ABC transpoters:

Another class of transporters are ABC type transporters, which are directly energized by MgATP and do not depend on the electrochemical force. Their substrates are organic anions formed by conjugation, e.g. to glutathione.

Examples of solute transport across vacuolar membrane in plant cells:

Transport of products of primary metabolites:

The various types of Primary metabolites could be:

1. Carbohydrates: Sucrose uptake occurs by facilitated diffusion in leaf vacuoles. Later it was also observed that active transport of sucrose takes place for vacuoles isolated from sugar cane cell cultures, which accumulates sucrose at concentrations comparable to those in the stalk tissue and tomato fruit vacuoles. Furthermore, it was also found that sucrose transport was stimulated by MgATP and to occur via a sucrose/H+ antiport in red beet. Larger carbohydrates such as stachyose, which is present in large quantities in *Stachys sieboldi*, may also be accumulated in the vacuole by proton antiport mechanisms. Many sugar alcohols also found in plants accumulate within the vacuoles. Transport of sorbitol across the tonoplast appears to be ATP-dependent in case of immature apple fruit tissue. Transport experiments suggest that mannitol crosses the tonoplast by facilitated diffusion.

2. Amino acid:

The first amino acid transport system was observed in barley plants. These are carriers or channles which are modulated by free ATP (but not by MgATP) which induces inward as well as outward fluxes of all amino acids tested.

3. Organic acids:

With context to organic acids, malate transport across the vacuolar membrane has been studied most intensively. This is due to the central role of malate in plant metabolism. The uptake of maltate is mainly governed by the electrical component of the electrochemical potential generated by the proton pumps. This channel also mediates uptake of succinate, fumarate, and oxaloacetate. The malate channel is not affected by cytosolic Ca2+ or ATP and it is a 32 kDa subunit protein. Citrate crosses the tonoplast using the same transporter as malate.

4. Inorganic anions:

The H+ pumps generate a positive potential inside the vacuole, which is the driving force for anion movements. Anion-dependent dissipation of a proton-pump generated by anions revealed that NO_3^- permeates more rapidly than Cl^- and $SO4^-$ whereas HPO_4^{2-} crossed the tonoplast considerably slowly.

Chloride:

An ATP-dependent Cl^- uptake was studied in barley mesophyll vacuoles. Later a vacuolar Cl^- channel (VCL) was identified in *Vicia faba* guard cells which is activated by a calcium dependent protein kinase (CDPK) in the presence of ATP and $Ca2^+$ and, to a weaker extend (22%), by protein kinase A. The VCL channel was activated at physiological potentials enabling Cl^- uptake into vacuoles.

Nitrate:

Amongst the anions it exhibits the highest permeability through the vacuolar membrane. It was concluded in one of the experiment that a membrane potential driven nitrate transporter, a NO_3^-/H^+ antiporter is present on the tonoplast.

Sulphate:

Using tonoplast vesicles, it has been shown that $SO4^{2-}$ and HPO_4^{2-} anions cross the tonoplast slowly as compared to NO_3^- or Cl^-. It has been found that SO_4^{2-} uptake is stimulated by Mg^+-ATP.

Phosphate:

Pi starvation leads to an efflux of Pi from the vacuole. It has been shown that Pi concentrations in the cytosol are maintained at a constant level in *Acer pseudoplatanus* cells using ^{31}P NMR.

5. Inorganic cation:

The membrane potential of the cytosol with respect to the vacuole is negative (20–40 mV). This implies that cations are excluded from the vacuole unless transport is coupled to an energy-dependent uptake mechanism.

Potassium:

Several channels exhibiting potassium permeability have been described. The first channel demonstrated for vacuolar membrane was called SV (slow activating vacuolar) channel. This channel is a slow activated channel and is associated with Ca^{2+} and calmodulin-induced K^+ and Ca^{2+} fluxes. These channels have been reported for the permeability of Na^+ if Ca^{2+} concentrations are increased by a signal. Secondly, FV (fast vacuolar) channel activates instantaneously in response to voltage changes. These channels may allow the release of K^+ at low Ca^{2+} concentrations. Thirdly, the vacuolar K^+ (VK) channel is activated instantaneously but it can be distinguished from the FV channel. It is voltage independent and fully activated at low cytosolic pH.

A K^+/H^+ antiport mechanism has been also reported for tonoplast enriched fractions from zucchini, *Brassica napus* hypocotyls, and *Atriplex*.

Sodium:

Na^+ accumulation is accompanied by vacuolar alkalinization in barley roots. This was established by using NMR spectroscopy.

Calcium:

Calcium plays a central role in signal transduction and higher concentrations are observed in apoplast and within the vacuole. An energized, highly specific calcium uptake by the vacuole is, therefore, a prerequisite for maintaining a low cytosolic calcium concentration. P-type Ca-ATPases have been identified at the plasma membrane, the ER, and the vacuolar membrane. A Ca^{2+} pump called a Ca^{2+}/H^+ antiporter has been demonstrated in vacuolar membrane fractions. This antiporter exhibits a far lower affinity

than the Ca^{2+}-ATPase. Also, a vacuolar voltage gated Ca^{2+} channel (VVCa) has been reported which is activated on membrane hyperpolarization.

Magnesium:

The presence of a Mg^{2+}/H^{+} antiporter has been described for the vacuole-like lutoids of *Hevea brasiliensis* and tonoplast vesicles isolated from maize roots.

Heavy metals:

Plants need some heavy metals such as Cu^{2+} or Zn^{2+} as micronutrients. Therefore, they need to be transported and a large portion of the heavy metals absorbed by the cell is usually concentrated within the vacuole. A vacuolar Cd^{2+}/H^{+} antiport activity has been demonstrated. However, it is known that plants form chelates with heavy metals by synthesizing phytochelatins (PCs), and these PCs can be transported into vacuoles of *Schizosaccharomyces* as apoPC or as PC-Cd complexes by ABC transporters. Vacuoles of higher plants are also known to transport phytochelatins.

Transport of products of the secondary metabolites: Involvement of secondary energized transporters and directly energized, ABC-type transporters

Plants synthesize an huge number of secondary metabolites and many of these have been found to be exclusively localized in the vacuole. The electrochemical gradient established by the two vacuolar proton pumps is used by the secondary energized transporters as a source of energy. It was demonstrated that the ΔpH was essential for the uptake of a number of phenolics, such as esculin, *o*-coumaric acid glucoside, apigenin- 7-(6-*O*-malonyl) glucoside, and anthocyanins from carrot. Recently it became evident that in addition to transporters depending on the proton motive force, directly energized transporters are also present on the vacuolar membrane. The first demonstration for a directly activated transport of solutes into the vacuole was provided for glutathione conjugates. Flavonoid glucuronides, a secondary plant compounds in rye vacuoles are transported by directly energized transport processes. Furthermore, studies with lucifer yellow, a sulfonated compound also indicates that sulfonated and sulfated secondary compounds cross the tonoplast by direct energization.

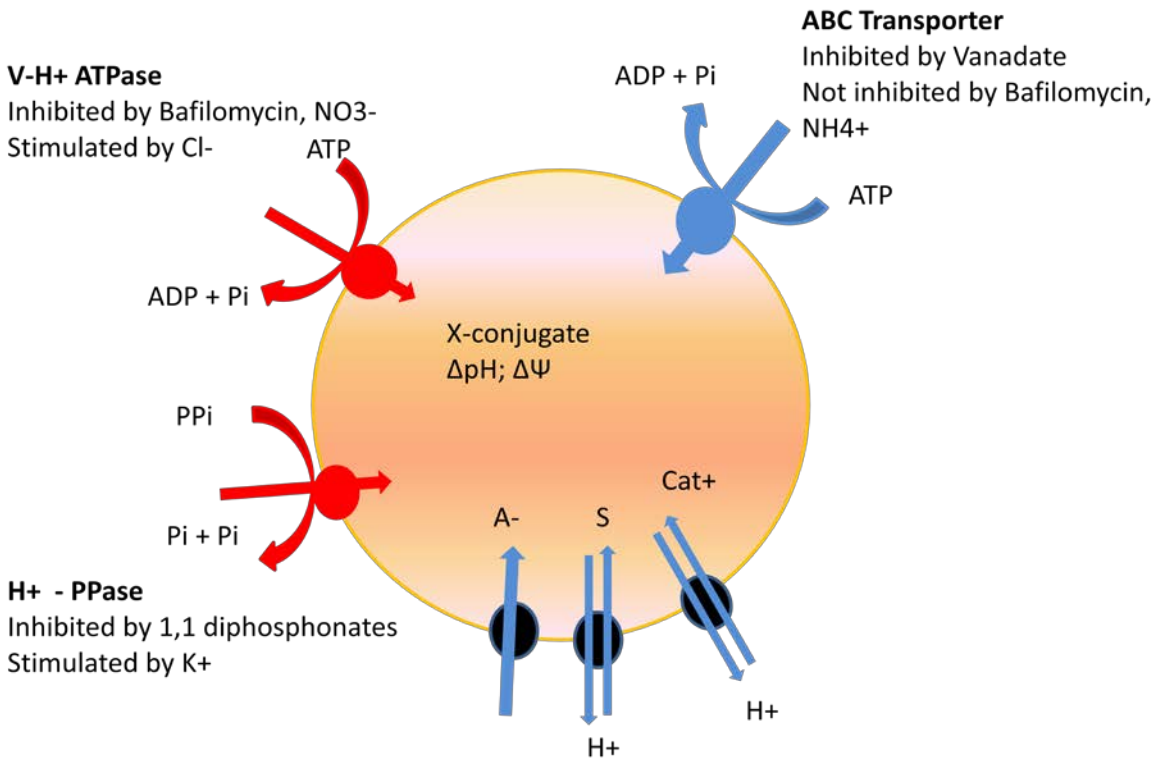


Figure 7: Proton pumps establishing a electrochemical gradient (red), secondary energized uptake mechanisms (green), and directly energized, ABC-type transporters (blue) of the plant vacuole. S, neutral solute; A–, anion; cat+, cation; X-conjugate, conjugate of a compound X (secondary metabolite or xenobiotic) with a hydrophilic compound such as glucose, glutathione, an amino acid, malonate, or sulphate.

Interesting facts:

- During endocytosis, these intra-lysosomal membranes are formed and prepared for digestion by a lipid-sorting process during which their cholesterol content decreases and the concentration of the negatively charged bis(monoacylglycero)phosphate increases.
- Lysosomal enzyme disorders contribute to several human diseases, either due to genetic defects in its enzyme expression or the escape of lysosomal enzymes (lysozymes) into extralysosomal medium.
- Permeabilization of lysosome, has been shown to initiate a cell death pathway or apoptosis.

Questions

1. Which pump is present in lysosomal membrane?
    a. P-class pump
    b. ABC transporter
    c. V-class pump
    d. F-class pump
2. The pH of the lysosomal compartment is
    a. 4
    b. 4.6
    c. 5
    d. 5.6
3. Which of the following correctly matches an organelle with its function?
    a. mitochondrion….photosynthesis
    b. Nucleus….cellular respiration
    c. Ribosome….manufacture of lipids
    d. Lysosome….movement
    e. Central vacuole….storage
4. Lysosomes are reservoirs of
    a. Hydrolytic enzymes
    b. Fat
    c. Secretory glycoproteins
    d. RNA
5. A function of lysosomes is
    a. Syntheisis
    b. Hydrolysis
    c. Replication
    d. Respiration

6. For digestion to occur in a vacuole, the vacuole must first fuse with
    a. Nucleus
    b. Ribosome
    c. Lysosome
    d. Golgi bodies
7. Lysosomes can be expected to be present in large numbers in cells which
    a. Have cilia.
    b. Produce centrioles.
    c. Are actively dividing.
    d. Carry out phagocytosis.
8. For digestion to occur in a vacuole, the vacuole must first fuse with
    a. Nucleus
    b. Ribosome
    c. Lysosome
    d. Golgi body
9. The proton gradient between the lysosomal lumen (pH ≈4.5–5.0) and the cytosol (pH ≈7.0) depends on ATP production by the cell.
    a. True
    b. False
10. What is the function of permanent vacuole?
    a. Supports and protects the cell
    b. Controls what enters and leaves the cell
    c. Controls the cell
    d. Stores water and mineral ions
    e. Stores water and mineral ions
11. Vacuole is surrounded by membrane called

a. Tonoplast
b. Chloroplast
c. Plasma membrane

18. What are the most abundant components of lysosomal membrane?
19. Write the composition and functions of vacuolar membrane.

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Module 3 Lecture 4
ATP dependent proton pumps

Proton pump

The proton pump is a transmembrane protein that is capable of transport of protons across the cell membrane, mitochondria and other cell organelle.

ATP dependent proton pumps

ATP dependent proton pumps or transport ATPase are the pumps that transport H^+ ions against their concentration gradients. These pumps are transmembrane proteins with one or more binding sites for ATP located on the cytosolic face of the membrane and these proteins are called ATPases. They normally do not hydrolyze ATP into ADP and Pi unless H^+ ions are simultaneously transported. Because of this tight coupling between ATP hydrolysis and transport, the energy stored in the phosphoanhydride bond is not dissipated but rather used to move ions or other molecules uphill against an electrochemical gradient.

ATP dependent proton pumps can be categorized into different classes. Generally, ATP dependent proton pumps are divided into 4 classes:

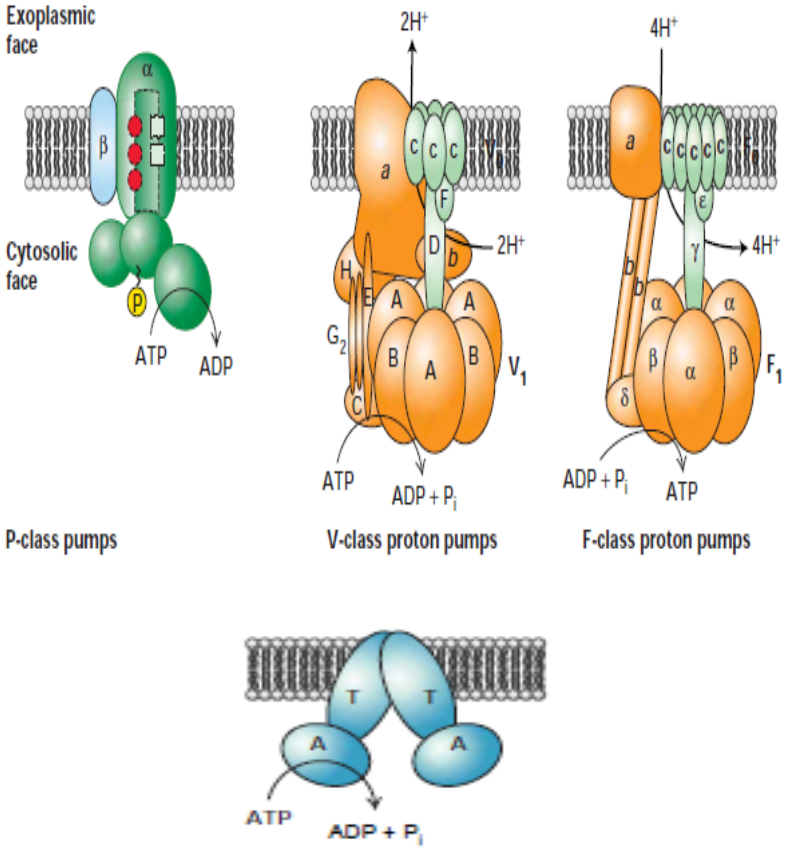

Figure 1: Different types of ATP dependent proton pumps

1. **P-class ion pumps:**

These are multipass transmembrane proteins having two identical catalytic α-subunits that contain an ATP binding site. Some have two smaller β-subunits that usually have regulatory functions. During the transport process or pumping cycle at least one of the α-subunit must be phosphorylated and the H^+ ions are thought to move through the phosphorylated subunit. This class includes many ion pumps that are responsible for setting up and maintaining gradients of Na^+, K^+, H^+ and $Ca2^+$ across the cell membrane.

a) The common P-type pump is mostly found in parietal cells of the mammalian stomach which transport protons (H^+ ions) out of cell and K^+ ions into the cell and is mainly responsible for the acidification of the stomach contents. The pump is known as H^+/K^+ ATPase. It is a heterodimeric protein. The H^+/K^+ ATPase transports one H+ from the cytoplasm of the parietal cell in exchange for one K^+ retrieved from the gastric lumen. As an ion pump the H^+/K^+ ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transferred from ATP to the H^+/K^+ ATPase during the transport cycle.

b) Another example of P-type pump is Na^+/K^+ ATPase in the plasma membrane, which generates low cytosolic Na^+ and high cytosolic K^+ concentration which is typical of animal cells (discussed in earlier lecture).

c) Certain Ca^{2+} ATPase pump Ca^{2+} ions out of the cytosol into the external medium while others pump Ca2+ from the cytosol into the endoplasmic recticulum or into the specialized sarcoplasmic reticulum, which is more common in muscle cells (discussed in earlier lecture).

2. **F-class ion pumps:**

The F class ion pumps contain different transmembrane and cytosolic subunits. They are known for only transport of protons, in a process that does not involve phosphoprotein intermediate. They generally behave as reverse proton pump by synthesizing ATP from ADP and Pi by movement of protons from the exoplasmic to the cytosolic face of the membrane down the proton electrochemical gradient. Therefore, these pumps are also known as ATP synthases or F_0F_1 complex. F-class ion pump is most common in bacteria, yeast and animal mitochondria and also in chloroplast.

The F_0F_1 complex is a multi-protein having two components F_0 and F_1. Both are multimeric proteins. The F_0 component contains three integral membrane proteins named a, b and c. The a and two b subunits are linked tightly but not to the donut- shaped ring of c subunits. And the F_1 component is water soluble complex of five distinct polypeptides with the composition $\alpha 3\beta 3\gamma\delta\varepsilon$. The lower part of the F_1 γ subunit is a coil which fits into the centre of the c-subunit ring of F_0 and appears rigidly attached to it. The F_1 ε subunit is rigidly attached to γ and also forms rigid contacts with c subunits. The F_1 and subunits associate in alternating order to form a hexamer αβαβαβ. The F_1 δ subunit is permanently linked to one of the F_1 subunits and also to the b subunit of F_0. Thus the F_0 a and b subunits and the δ subunit and (αβ)3 hexamer of the F_1 complex form a rigid structure anchored in the membrane. The rodlike b subunits form a stator that prevents the (αβ)3 hexamer from moving while it rests on the γ subunit.

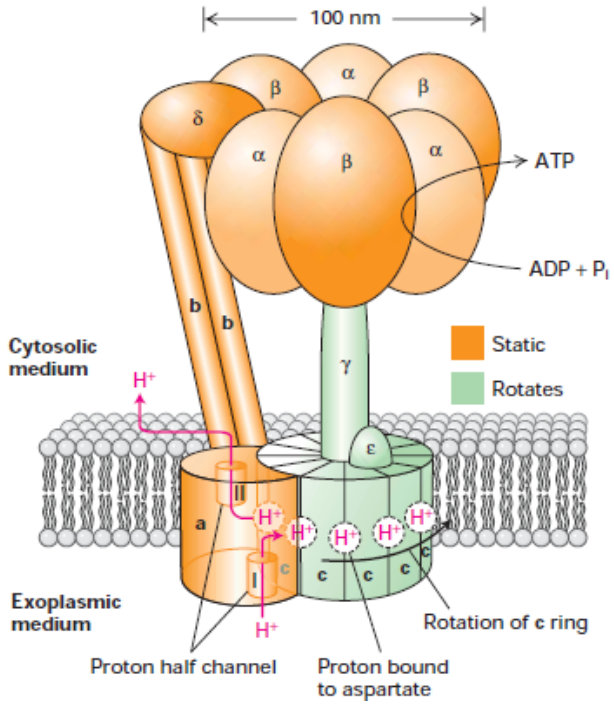

Figure 2: Model of the structure and function of ATP synthase (the F_0F_1 complex) in the bacterial plasma membrane.

The F_0 portion is built of three integral membrane proteins: one copy of a, two copies of b, and on average 10 copies of c arranged in a ring in the plane of the membrane. Two proton half-channels lie at the interface between the a subunit and the c ring. Half-channel I allows protons to move one at a time from the exoplasmic medium and bind to aspartate-61 in the center of a c subunit near the middle of the membrane. Half-channel II (after rotation of the c ring) permits protons to dissociate from the aspartate and move into the cytosolic medium.

3. V-class ion pumps:

It is almost similar to F-class ion pumps in structure and function. But none of their subunits are related to each other. F-class pumps operate in reverse direction to F-class. These pumps generally function to maintain low pH of plant vacuoles and lysosome and other acidic vesicles in animal cells by pumping protons from cytosolic to exoplasmic face (inside) of membrane against the proton electrochemical gradient. The acidification between the lysosomal lumen and cytosol lumen can be maintained by production of ATP by cells.

These V-class proton pumps contain two domains: a cytosolic hydrophilic domain (V_1) and a transmembrane domain (V_0) with multiple subunits in each domain. Binding and hydrolysis of ATP by the B subunits in V_1 provide the energy for pumping of H^+ ions through the proton-conducting channel formed by the c and a subunits in V_0. These V-class proton pumps are not phosphorylated and dephosphorylated during proton transport.

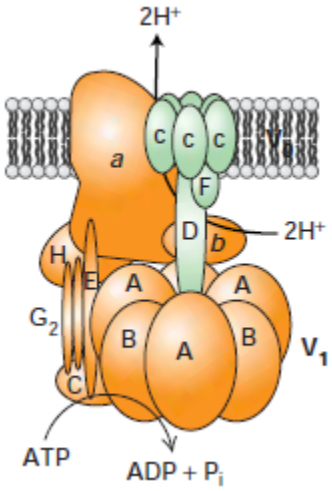

Figure 3: V-class proton pump

These protons cannot acidify by themselves because a net movement of electric charge occurs. Only a few protons build up positive H^+ ions on exoplasmic face (inside) and for each H^+ pumped across, a negative ion will be left behind on cytosolic face, building negatively charged ions. These oppositely charged ions attract each other on opposite faces of the membrane, generating a charge separation, or electric potential, across the membrane. If more protons pumped, the excess positive ions on exoplasmic face repels other H^+ ions and prevents pumping of extra proton long before a significant transmembrane H^+ concentration gradient had been established. If the organelle lumen or

the extracellular space has to be acidified, the net movements of protons must be accompanied either by movement of equal number of anion eg Cl^- in same direction or my movement of different cation in the opposite direction. The first process occurs in lysosomes and plant vacuoles whose membrane contains V-class H^+ ATPase and anion channels for Cl^- movement. And the second process is observed in the lining of the stomach which contains a H^+/K^+ ATPase and pumps one H^+ outward and one K^+ inward.

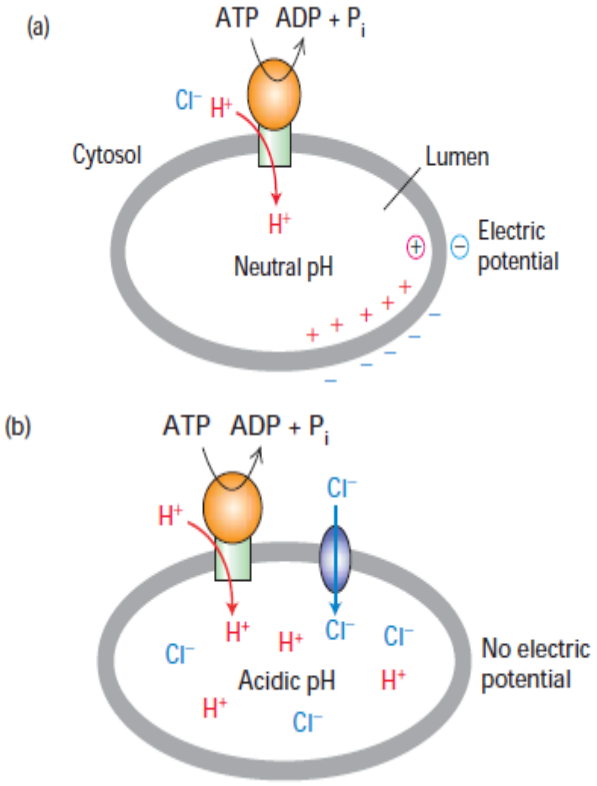

Figure 4: Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change in the intraluminal pH. (b) If the organelle membrane also contains Cl^- channels, anions passively follow the pumped protons, resulting in an accumulation of H^+ ions (low luminal pH) but no electric potential across the membrane.

4. ABC (ATP binding cassettes) superfamily:

The final class of ATP-powered pumps is a large family of multiple membranes. This class includes several hundred different transport proteins found in all organisms ranging from bacteria to mammals. Each ABC protein is specific for single substrate or group of related substrate, which may be ions, sugars, amino acids, phospholipids, cholesterol, peptides, polysaccharides or proteins. All ABC transport protein share a structural organization consisting of four core domains: two transmembarne (T) domains, forming

the passageway through which transported molecules cross the membrane and two cytosolic ATP-binding (A) domains. The core domains are generally present in separate polypeptides which are more common in bacterial cell. In others, the core domains are fused into one or two multidomain polypeptides. ATP binding leads to dimerization of two ATP-binding domains and ATP hydrolysis leads to their dissociation. Theses structural changes in the cytosolic domains are thought to be transmitted to the transmembrane segments, driving cycles of conformational changes that alternately expose substrate-binding sites on one side of the membrane and then on the other. In this way, ABC transporters use ABC binding and hydrolysis to transport small molecules across the bilayer. Some common example of ABC transporters are found in bacterial plasma membranes which contain amino acid, sugar and peptide transporters. These cells use H^+ gradient across the membrane to pump variety of nutrients into the cell. It is also present is mammalian plasma membrane that contains transporters of phospholipids, small lipophillic drugs, cholesterol and other small molecules. One example of eukaryotic ABC transporters is multidrug resistance (MDR) protein which has the ability to pump hydrophobic drugs out of the cytosol. Overexpression of these MDR protein in human cancer cells, make the cells resistant to variety of chemically unrelated cytototoxic drugs.

Interesting facts:

- Valinomycin is a carrier for potassium.
- Lactose permease has been crystallized with thiodigalactoside (TDG), an analog of lactose.
- Adenine nucleotide translocase (ADP/ATP exchanger), which catalyzes 1:1 exchange of ADP for ATP across the inner mitochondrial membrane.
- The reaction mechanism for a P-class ion pump involves transient covalent modification of the enzyme.
- Gramicidin is an example of a channel. It is an unusual peptide, with alternating D and L amino acids. In lipid bilayer membranes, gramicidin dimerizes and folds as a right handed β-helix. The dimer just spans the bilayer.

Questions

1. The functional mechanism of P-class ion pumps is ..... by the ATP.
2. V-class pumps pumps exclusively .....
3. Substance concentration + electric potential = ... which determines the energetically favorable direction of transport a charged molecule across a membrane.
4. Differentiate among Transporters, pumps and channels.
5. Is calcium pump and ATP dependent proton pump are same?
6. Describe ABC (ATP binding cassettes) superfamily.
7. Differentiate between V class proton pump and P-class ion pumps.
8. What are F-class ion pumps? How do they differ from the other classes of ion pumps?
9. What is the main function of a V-class proton pump?
10. Give atleast three examples of ATP-binding cassettes.
11. Give a brief overview of the structural organization of the ABC transport proteins.

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Module 3 Lecture 5
Cotransport: Symport, Antiport

Transporters:

Transporters (also known as carriers) are the membrane proteins that transport a wide variety of ions and molecules across the lipid bilayer membrane.

Cotransporters:

Cotransporters are proteins that transport two different solutes such as glucose and amino acids simultaneously across the cell membrane against a concentration gradient. It mediates coupled reactions in which an energetically unfavorable reaction (uphill movement of molecules) is coupled to an energetically favorable reaction. Unlike ATPase pump, it uses the energy stored in electrochemical gradient. This is called secondary mediated active transport (discussed in earlier lecture). An important feature is that neither molecule can move alone; movement of both molecules together is obligatory, or coupled. One of the common example is the energetically movement of Na^+ ions into the cell across the plasma membrane driven both by its concentration gradient and by the transmembrane voltage gradient, which can be coupled to movement of the transported molecule (glucose) against its concentration gradient.

How cotransportersare differentiated from uniporters?

Both transporters share some common feature with respect tostructural similarities, operation at equivalent rates, and undergo cyclical conformational changes during transport of their substrates. They differ in that uniporters can only accelerate thermodynamically favourable transport down a concentration gradient, whereas cotransporters can harness the energy of a coupled favourable reaction to actively transport molecules against a concentration gradient.

Types of cotransports:

On the basis of movement of solutes, cotranporters can be divided into following categories:

1. **Symport:**When the transported molecule and cotransported ion move in the same direction, the process is called symport.
2. **Antiport:**When the transported molecule and cotransported ion move in the opposite direction, the process is called antiport.

Both the above mentioned cotranporter move one solute against its transmembrane concentration gradient. This movement is powered by coupling to the movement of second solute down its transmembrane concentration gradient.

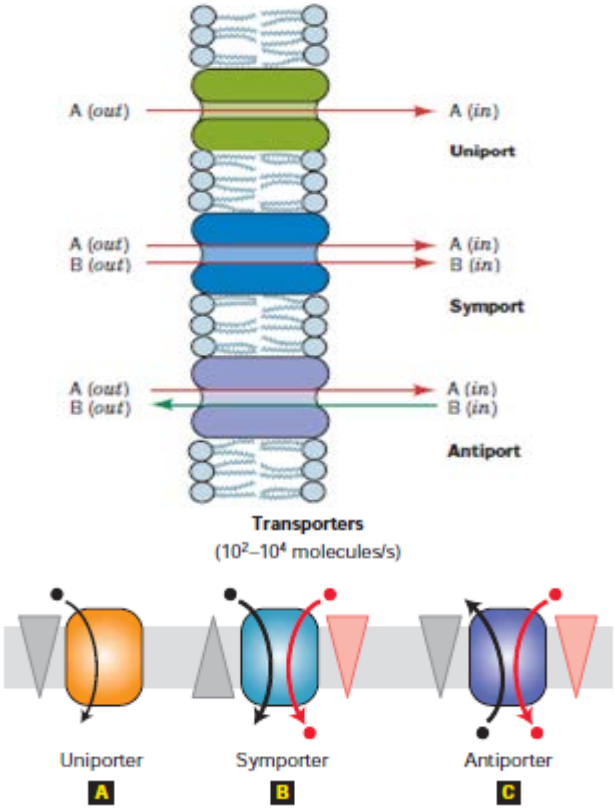

Figure 1: Transporters, which fall into three groups, facilitate movement of specific small molecules or ions.(A) Uniporters transport a single type of molecule down its concentration gradient. Cotransport proteins (symporters (B), and antiporters (C)) catalyze the movement of one molecule againstits concentration gradient (black circles), driven by movement of one or more ions down an electrochemical gradient (red circles). Differences in the mechanisms of transport by these three major classes of proteins account for their varying rates of solute movement.

On the basis of movement of ions, cotransporters can also be categorized into:

1. Cationcotransporter:Example of cation transporter is Na^+/H^+antiporter, which exports H^+ from cells coupled to the energetically favorable import of Na^+.
2. Anion cotransporter:Example of anion transporter is exchange of Cl^- and HCO_3^- across the plasma membrane.

Some common examples of cotranporter are:

1. **Oligosaccharide/H^+ symporter:**They are also known as LacYsymporter. It is most common in bacterial cell which has lactose permeaseLacY and functions as symporter. It uses the free energy released from translocation of H^+ down its electrochemical gradient to drive the accumulation of nutrients such as lactose against its concentration gradient. The H^+ gradient across the cytoplasmic membrane is established by the respiratory chain and by the action of F_1F_0-ATPase, which couples ATP hydrolysis to the export of protons from the cell. For LacY, the stoichiometry of lactose and H^+ translocation is 1:1, with both substances movement in the same direction. Thus, the lactose gradient can drive the uphill translocation of protons and generate an inward or outward H^+ gradient, depending on the direction of the lactose concentration gradient.

   Structure of Lac Y symporter: Structurally theLacYsymporter contains 12 transmembarne helices which are connected by hydrophilic loops and cytoplasmic N- and C- termini. There are two domains of 6 transmembranesegments each, forming a symmetrical structure. The hydrophilic cavity which lies in the centre of lipid bilayer forms the substrate binding site. This substrate binding site is accessible from either the intracellular or extracellular side of the membrane but never to both sides simultaneously. Protonation and binding of lactose in the outward-facing conformation induces a conformation change, resulting in inward-facing conformation. This structural arrangement involves binding of both substrates initially and allows for coupled and then simultaneous transport. Release of lactose and protons into the cell then induces a transition back to the outward-facing conformation. Hence it lowers the energy barrier between inward and outward-facing conformation and facilitates interconversion.

2. **Glycerol-3-phosphate transport (GlpT):**It is an antiporter that accumulates glycerol-3-phosphate into the cell for energy production and phospholipid synthesis. GlpT is an organic phosphate/inorganic phosphate exchange which is driven by Pi gradient. Similar to LacY, it has also symmetrical N- and C- terminal domains, each consisting of 6 transmembrane segments surrounding the substrate translocation pathway. It also works as same mechanism asLacY but glycerol-3-phosphate binds

and phosphate is released in the outward conformation and opposite occurs in the inward conformation.

3. **Na^+ linked symporter:**This symporter imports amino acid and glucose into the animal cells against the concentration gradient. Anexample is GLUT protein which imports glucose from the blood downs its concentration gradient. On the other hand, certain cells such as those lining the small intestine and kidney tubules, import glucose from intestinal lumen or forming urine against a large concentration gradient. Such cells utilize two Na^+/one glucose symporter, a protein that couples to import one glucose to import two Na^+. This symporter contains 14 transmembrane α helices with both its N- and C- termini extending in the cytosol. The N-terminal portion of the protein, including helices 1–9, is required to couple Na^+ binding and influx to the transport of glucose against a concentration gradient.

The following steps occur for transport of Na^+ and glucose:

1. Simultaneous binding of Na^+ and glucose to the conformation with outward-facing binding sites
2. A second conformation generates with inward facing side
3. Dissociation of Na^+ and glucose into the cytosol
4. The protein reverts back to original outward-facing conformation, ready to transport the next substrate

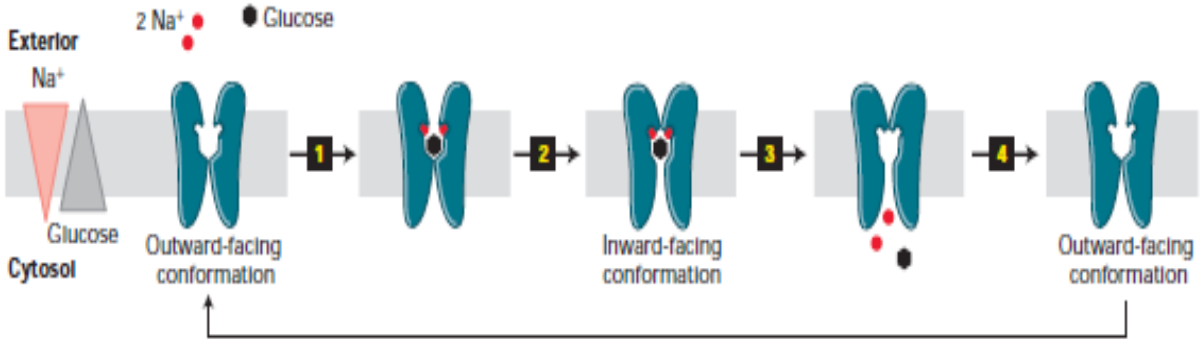

Figure 2: Operational model for the two-Na^+/one glucose symporter.

The overall reaction is:

$2Na^+$ (out) + Glucose (out) ↔ $2Na^+$(in) + Glucose (in)

The free energy change for the symport transport of two Na^+ and one glucose is the sum of the free energy changes generated by the glucose concentration gradient (1 molecule transported), the Na^+ concentration gradient (2 Na^+ ions transported), and the membrane potential(generated by two Na^+tranported):

$$\Delta G = RT\ in\ \frac{[glucose_{in}]}{[glucose_{out}]} + 2RT\ in\ \frac{[Na^+_{in}]}{[Na^+_{out}]} + 2FE$$

When ΔG=0 and the free energy released by movement of Na^+ into cells down its electrochemical gradient has a free energychange Δ*G* of about -3 kcal per mole of Na^+ transported.Thus the Δ*G* for transport of two moles of Na^+inward is about -6 kcal. By substituting in above equation, the ratio of glucose (in)/glucose (out) =30,000. Thus if 2 moles of Na^+ inward then it generates an intracellular concentration of glucose of 30,000 times more than extracellular glucose. Thus if only one Na^+ ion were imported per glucose molecule, then the available energy could generate a glucose concentration gradient (inside- outside) of only about 170-fold. Thus by coupling the transport of two Na^+ ions to the transport of one glucose, the two-Na^+/one-glucose symporter permits cells to accumulate a very high concentration of glucose relative to the external concentration.

4. **Na^+ linked antiporter:**A cotranporter, $3Na^+/Ca2^+$antiporter in cardiac muscle cell maintain a low concentration of Ca^{2+} in cytosol. The reaction for this cation transporter is:

$3Na^+$ (out) + Ca^{2+}(in) ↔ $3Na^+$ (in) + Ca^{2+}(out)

The movement of three Na^+ ions is required to power the export of one Ca^{2+} ion from the cytosol with a [Ca^{2+}] of ≈2 * 10^{-7} M to the extracellular medium with a [Ca^{2+}] of 2 * 10^{-3} M, a gradient of some 10,000-fold form. By lowering cytosolic Ca^{2+}, operation of the Na^+/Ca^{2+}antiporter reduces the strength of heart muscle contraction.

Function of cotransporter:

1. **Regulation of cytosolic pH:**

The anaerobic metabolism of glucose yields lactic acid whereas the aerobic metabolism yields CO_2, which reacts with water to form carbonic acid (H_2CO_3). This weak acid dissociates yielding H^+ ion or proton. If these excess protons werenot removed from cells, then the cytosolic pH would drop and will be unfavourable to cellular fractions. Hencecotransports are required to remove excess of protons. One is Na^+HCO3^-/Cl^- antiport imports one Na^+ down its concentration gradient together with one $HCO3^-$ in exchange for export of one Cl^- against its concentration gradient. The enzyme named carbonic anhydrase catalyzes dissociation of imported HCO_3^- ions into CO_2 and OH^- by the reaction:

$HCO^{3-} \leftrightarrow CO_2 + OH^-$

$OH^- + H^+ \rightarrow H_2O$

Then CO_2 diffuses out of the cell and OH^- ions combine with intracellular protons, forming water. Thus the overall action of this transport is to consume cytosolic H^+ ions, thereby raising cytosolic pH.

Secondly Na^+/H^+antiporter plays an important role in raising cytosolic pH which couples entry of one Na^+into the cell down its concentration gradient to export of one H^+ ion.

Thirdly, anion antiporterthatcatalyzes the one-for-one exchange of HCO_3^- and Cl^- across the plasma membrane. At high pH, this Cl^-/HCO_3^-antiporterexports HCO_3^- in exchange for Cl^-, thus lowering the cytosolic pH. The import of Cl^- down its concentration gradient (Cl^-(medium) >Cl^-(cytosol)) powers the reaction.

The activity of all these antiports depends upon pH.The two antiportersthat operate to increase cytosolic pH are activated when the pH of the cytosol falls. Similarly, a rise in pH above 7.2 stimulates the Cl^-/HCO_3^-antiporter, leading to a more rapid export of HCO_3^- and decrease in the cytosolic pH. In this manner the cytosolic pH of growing cells is maintained very close to pH 7.4.

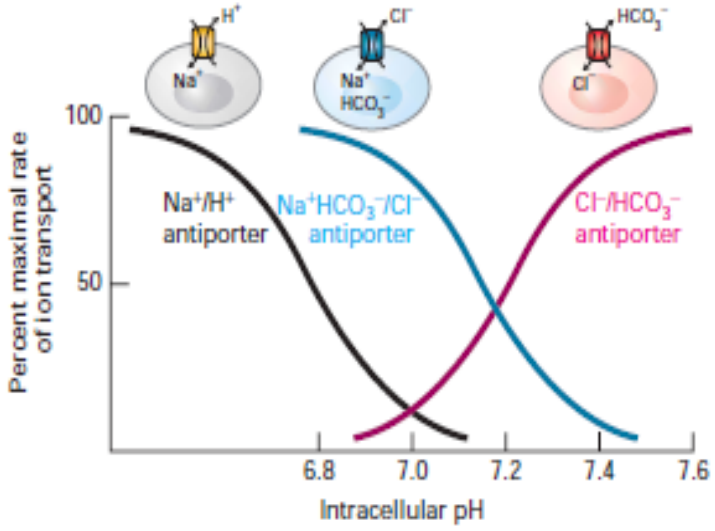

Figure 3: The activity of membrane transport proteins that regulate the cytosolic pH of mammalian cells changes with pH.Direction of ion transport is indicated above the curve for each protein

2. Accumulation of metabolites and ions inplant vacuoles:

The lumen of plant vacuoles is much more acidic(pH 3 to 6) than is the cytosol (pH 7.5). The vacuolarmembrane contains Cl^- and NO_3^- channels that transportthese anions from the cytosol into the vacuole against their concentration gradients and is drivenby the inside-positive potential generated by the H^+ pumps.

One more example is proton/sucrose antiporterin the vacuolar membrane that accumulate sucrose in plant vacuoles. During photosynthesis, sucrose is generated and stored in vacuole. But during night these stored sucrose moves into the cytoplasm and is metabolized to CO_2 and H_2O with generation of ATP from ADP and Pi. The inward movement of sucrose is governed by movement of H^+ which is favoured by its concentration gradient(lumen cytosol) andby the cytosolic-negative potential across the vacuolar membrane.

Uptake of Ca^{2+} and Na^{+} into the vacuolefrom the cytosol against their concentration gradients issimilarly mediated by proton antiporters.

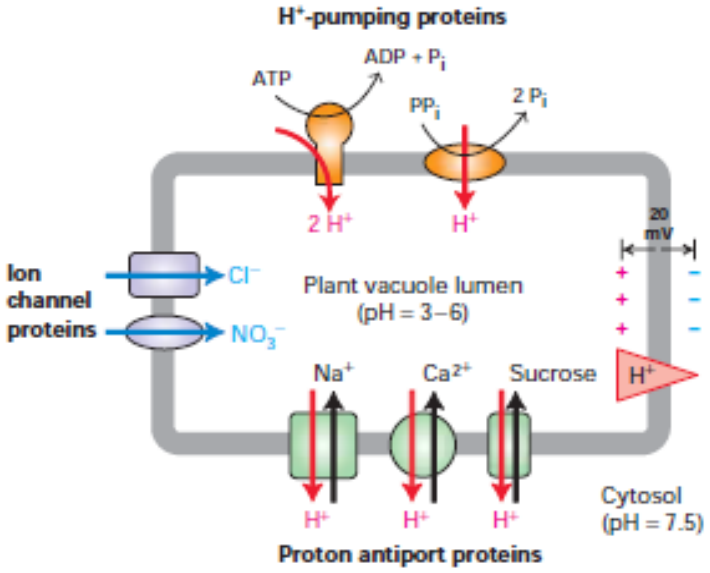

Figure 4: Accumulation of ions and sucrose by the plant vacuole.The vacuolar membrane contains two types of proton pumps (orange): a V-class H^+ ATPase (*left*) and a pyrophosphate-hydrolyzing proton pump (*right*) that differs from all other ion-transport proteins and probably is unique to plants. These pumps generate a low luminal pH as well as an inside positive electric potential across the vacuolar membrane owing to the inward pumping of H^+ ions. The inside-positive potential powers the movement of Cl^- and $NO3^-$ from the cytosol through separate channel proteins (purple). Proton antiporters (green), powered by the H+ gradient, accumulate Na^+, $Ca2^+$, and sucroseinside the vacuole.

Interesting facts:

- Na+/K+/2Cl- symporter in the loop of Henle in the renal tubules of the kidney transports 4 molecules of 3 different types; a sodium ion (Na+), a potassium ion (K+) and two chloride ions (2Cl-).
- In the roots of plants, the H+/K+ symporters are only one member of a group of several symporters/antiporters that specifically allow only one charged hydrogen ion (more commonly known as a proton) and one charged K+ ion. This group of carriers all contribute to modulate the chemiosmotic potential inside the cell.

Questions:

1. An example of cation transporter is ..... and an example of anion transporter is .....
2. The activity of antiports depends upon .....
3. Glycerol-3-phosphate transport is
   a. symport
   b.uniport
   c. antiport
   d. ATP dependent transport.
4. What are transporters and cotransporters?
5. Differentiate between symport and antiport.
6. Describe Na+ linked antiporters.
7. What are uniports?
8. Describe the mechanism of accumulation of metabolites and ions in plant vacuoles.
9. What are the functions of cotransporters?
10. What are LacYsymporters?Describe their structure.

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Module 3 Lecture 6

Transport in prokaryotic cells

Transport in prokaryotic cells: The transport system of a cell depends upon the substrate requirements of the cell, the bioavailability of the substrate and the environmental conditions. It also depends on the metabolic features and physiological state of the organism. Prokaryotic cells have simpler structure and mostly are unicellular. Hence their transport system is different from higher eukaryotes. Here we will study the transport in prokaryotic cells with respect to bacteria.

Membranes in bacteria: Membranes play a major role in transport. The different types of membrane found in bacteria are:

1. **Cytoplasmic membrane, in all bacteria**

The inner membrane of a cell is different from outer membrane of a cell. And the space between these membranes is called periplasam. The membrane is symmetrical, with an equal distribution of lipids (exclusively phospholipids, mainly phosphatidylethanolamine, phosphatidylglycerol and cardiolipin) among the inner and outer surface. Some of the functions associated with cytoplasmic membrane which has role in transport mechanism of cell are:

- Osmotic and permeability barrier
- Presence of transport system for various solutes
- Synthesis of membrane lipids
- Assembly and synthesis of extracytoplasmic proteins
- Coordination of DNA replication and segregation with septum formation and cell division
- Energy generation functions such as electron transport system, establishment of photon motive force and transmembrane ATP-synthesizing ATPase

2. Outer membrane, mostly in gram negative bacteria

The outer membrane is highly asymmetrical, with the inner leaflet, oriented to the periplasam. The outer leaflet, facing the external medium contains lipopolysaccharides (LPS) constituting of three parts: lipid A as anchor, the core oligosaccharide functioning as spacer element and an O-specific polysaccharide consisting of oligosaccharide repeating unit. Proteins are the integral components or associated with OM. Some of the functions associated with OM are:

- Involved in transport mechanism.
- Contribution of membrane integrity
- Serves as anchor for flagellae, fimbriae and pili. Hence important for locomotion, cell-cell interaction, adhesion to surfaces and formation of biofilms.
- LPS are major antigenic determinants, preventing entry of cell-damaging components and serve as receptor for a number of bacteriophages.

3. Cell walls of gram positive bacteria

The cell walls of gram positive bacteria are devoid of outer membrane but possess a thick murein layer. In Gram-positive bacteria, teichoic acids are covalently linked to peptidoglycan. Teichoic acids are polyol phosphate polymers with a strong charge. They are strongly antigenic and absent in Gram-negative bacteria. In some species, teichuronic acids are found as lipoteichonic acids which are composed of glycerol teichoic acid linked to glycolipid. Additional wall components can be polysaccharides, lipids and proteins.

4. Membrane that forms envelope in mycobacteria

Membrane that forms envelope in mycobacteria is characterized by their low permeability, which contributes resistance of the microbes to therapeutic agents. It contains two special features: an outer lipid barrier based on a monomer of mycolic acids and a capsule-like coat of polysaccharide and protein. The cell wall contains a large amount of C_{60}-C_{90} fatty acids, mycolic acids that are covalently linked to arabinogalactan.

Transport process:

Transport process can be divided into four classes on the basis of driving forces and modes of energy coupling (Milton H. Saier et al., 2000):

1. **Passive diffusion:**

The passive diffusion occurs along the concentration gradient and without the use of metabolic energy. Some solutes pass the permeability barrier of a lipid bilayer by passive diffusion. This is valid for small apolar molecules and small slightly polar but uncharged molecules like water and dissolved gases. Some other solutes are also transported via channels or channel type proteins to overcome in a diffusion-controlled movement.

2. **Primary active transport:**

Primary active transport is characterized by coupling translocation of solute directly to a chemical or photochemical reaction. Primary source includes pyrophosphate bond hydrolysis, methyl transfer and decarboxylation. Transport of Na^+ and K^+ by carrier protein, Na^+ - K^+ ATPase, is the most common example of primary active transport.

3. **Secondary active transport:**

In secondary active transport the translocation step across the membrane is coupled to the electrochemical potential of a given solute. The solute chemical potential created by primary active transport systems is the driving force, which allows an uphill transport of another solute, against its own concentration gradient. The uptake can be mediated as uniprot, symport and antiport. A common example of secondary active transport is the symport of Na^+ and glucose. The transmembrane protein Na^+ - glucose transporter, acts as a carrier, allows Na^+ and glucose to enter the cell together. The Na^+ flow down their concentration gradient while the glucose molecules are transported against their concentration gradient into the cell. Later the Na^+ is pumped back out of the cell by the Na^+ -K^+ ATPase.

4. Phosphophenolpyruvate: sugar phosphotransferase system (Pts):

Pts translocation process is exclusive to bacterial species which phosphorylates its carbohydrate substrates during transport.

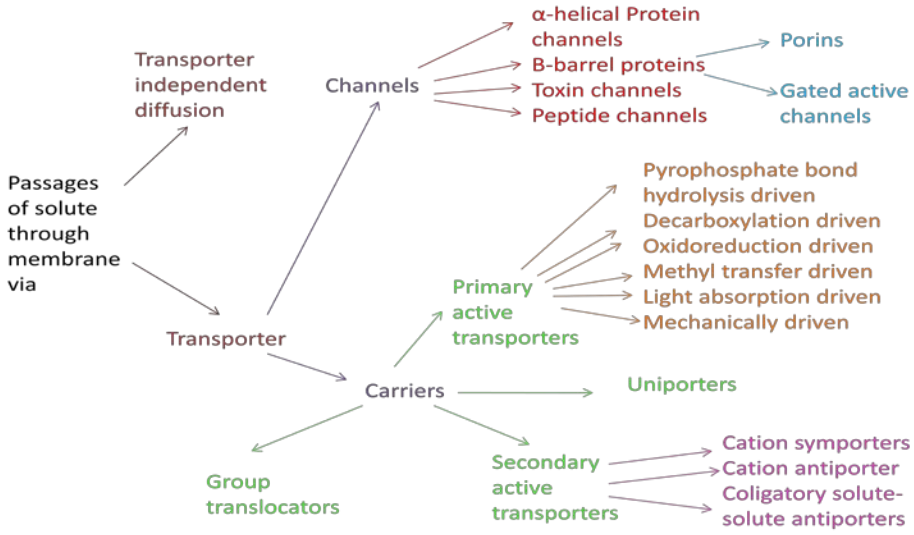

Figure 1: Classification of major types of transport mechanism across biological membranes based on function and phylogeny

The major transport mechanism based on the mode of transport, energy coupling mechanism and substrate specificity and protein phylogenetic grouping that reflects structure, function and its mechanism are:

1. Transport independent diffusion

   Gases (such as O_2 and CO_2); hydrophobic molecules (such as benzene) and small polar but uncharged molecules (such as H_2O and ethanol) are able to diffuse across the plasma membrane.

2. Transport dependent diffusion

This transport takes allows polar and charged molecules such as carbohydrates, amino acids, nucleotides and ions, to cross the plasma membrane.

a. Channels

Some examples are voltage gated channels which open in response to change in electric potentials; others called ligand gated channels open in response to the binding of the ligand.

(i) α-helical protein channel

(ii) β-barrel proteins

(iii)Toxin channels

(iv) Peptide channels

b. Carriers

The common example is the movement of glucose mediated by carrier protein called glucose transporter (GLUT).

(i) Primary active transport: Mechanically driven, Light absorption driven, Methyl transfer driven, Oxidoreduction driven, Decarboxylation driven, Pyrophosphate bond hydrolysis driven

(ii) Uniporters

(iii) Secondary active transport: Cation symporters, Cation antiporters, Solute solute antiporters

(iv) Group translocators

Some examples of transporter in bacteria can be studied with the following examples:

1. Phosphate transport:

Two major phosphate transport systems are involved in bacteria:

a. Low affinity Pit (phosphate inorganic transport) system

b. High affinity Pst (phosphate specific transport) system

Pit consists of a single trans-membrane protein and is constitutively expressed secondary transporter. This system is characterized by uptake of phosphate which is in the form of a neutral metal phosphate complex and is in symport with a proton. This transport of phosphate is achieved by binding and dissociation of the neutral metal phosphate complex and H^+ on the outer and inner surface of the trans-membrane protein carrier protein. Pit is reversible and therefore allows both import and export of divalent ions and phosphate. Also it has a relatively low specificity for both phosphate and arsenate (toxic analogue of phosphate).

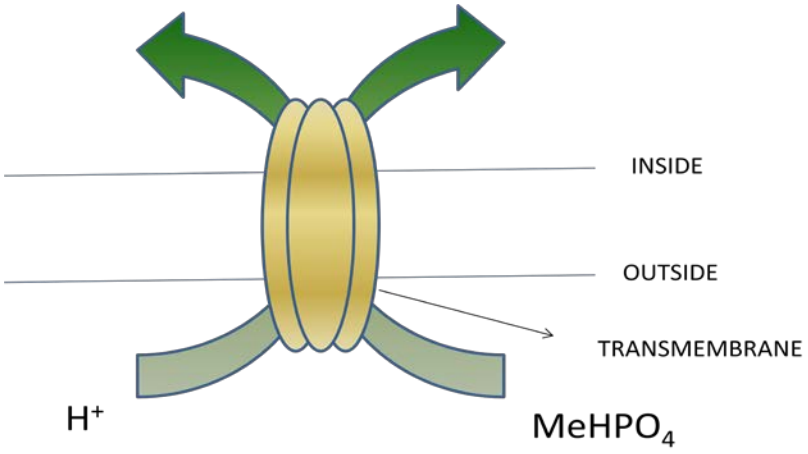

Figure 2: Phosphate transport by low affinity pit

In contrast, protein specific transport (Pst) is a periplasmic protein-dependent transporter. It consists of four subunits: a phosphate-binding protein located in the periplasmic space, two cytoplasmic associated proteins that contain six membrane spanning helices and a dimeric ATP binding protein. It operates as a primary transport mechanism i.e. unidirectional phosphate transport is coupled to a chemical reaction**.** Phosphate is transported in the form of $H_2PO_4^-$ and HPO_4^{2} in Pst system and has a relatively high substrate affinity.

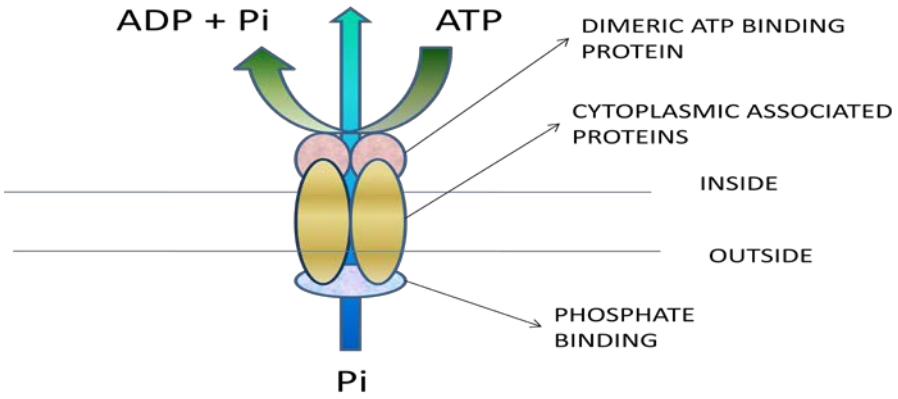

Figure 3: Phosphate transport by ATP dependent high affinity Pst system

Besides, phosphate also enters the cell in the form of esters such as *sn*-glycerol-3-phosphate, glucose-6-phosphate or mannose-6-phosphate. Other organic phosphate compounds may diffuse through the outer membrane before hydrolysis in the periplasm by phosphatases - allowing transport of Pi into the cytoplasm. Pi linked antiport systems of *sn*-glycerol-3-phosphate (GlpT) and glucose-6-phosphate (UhpT) mediate the translocation of organo-phosphate compounds across the cell membrane. Phosphate is also accepted as an analogue of organo-phosphate by these exchange systems; the affinity for phosphate is lower than for the organo-phosphate. PhoE pores are formed in *E. coli* cell membranes during phosphate limitation and have a preference for anions such as phosphate and phosphate-containing nutrients, facilitating the unspecific entry of phosphate into the cytoplasm by diffusion.

2. Arsenic transport:

It was studied that auqaporins facilitate the diffusion of metalloids such as arsenic (As) and antimony (Sb). The trivalent forms of these metalloids are structurally similar to glycerol at neutral pH and hence enter cells through aquaporins.

3. Magnesium transport:

Transport of Mg^{2+} into the cell is problematic, in spite of their largest hydrated radius, smallest ionic radius, and highest charge density. Transport systems for Mg^{2+} have been characterized well in *Salmonella typhimurium*. The CorA transport system is expressed constitutively and is the major Mg^{2+} transporter in Eubacteria and Archaea. It consists of three transmembrane domains, a large periplasmic domain, and no sequence homology to other proteins. The MgtE Mg^{2+} transporter also lacks sequence homology to other proteins, and it is unclear if Mg^{2+} transport is its primary function. The MgtA and MgtB Mg^{2+} transporters have sequence homology to P-type ATPases and closely related to the mammalian Ca^{2+} ATPases than to the prokaryotic P-type ATPases. Both transporters mediate Mg^{2+} influx with, rather than against its electrochemical gradient. Unlike CorA and MgtE, the MgtA and MgtC/MgtB loci are regulated, being induced by the two-component regulatory system PhoP/PhoQ. PhoQ is an Mg^{2+} membrane sensor kinase that phosphorylates the transcription factor PhoP under Mg^{2+} - limiting conditions. This factor then induces transcription of MgtA and MgtCB.

4. In hyperthermophillic Archaea, only tranporters of ABC type are useful in uptake of carbohydrates (e.g. glucose, cellobiose, maltotriose, arabinose, trahalose). This reflects an adaptation to the extreme habit, enabling organisms to acquire all available sugars very effectively.

Interesting facts:

- Transport system of a cell depends upon the substrate requirements of the cell, the bioavailability of the substrate, environmental conditions and membrane permeability.
- Phosphate can be transported either by low affinity pit or ATP dependent high affinity Pst system.
- In spite of largest hydrated radius, smallest ionic radius, and highest charge density of Mg^{2+}, its transport into the cell is problematic.
- Only tranporters of ABC type are useful in uptake of carbohydrates in hyperthermophillic Archaea.

Questions:

1. Transport of solutes across cells depends upon:
   a. Substrate requirements of the cell and bioavailability of the substrate.
   b. Environmental conditions and membrane permeability.
   c. Metabolic features and physiological state of the organism.
   d. All of the above.
2. The type of transport without any energy input in the cell is called:
   a. Passive transport
   b. Active transport
   c. Osmosis
   d. Plasymolysis
   e. Turgor pressure
3. Which of the following pieces of evidence would suggest that a substance entered a cell via active transport as opposed to passive transport?
   a. The substance moved from a high concentration to a low concentration.
   b. ATP was required for transport.
   c. The substance moved across the membrane via a carrier protein.
   d. None of the above.

4. What are the functions associated with cytoplasmic membrane which has role in transport mechanism of cell?
5. What are the composition of outer membrane and its functions that has role in transport mechanism of cell?
6. What is the classification of transport mechanism in the cells? Explain with example.
7. Explain the transport mechanism of phosphate in the cell.

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Module 3 Lecture 7

Endocytosis and Exocytosis

Endocytosis: Endocytosis is the process by which cells absorb larger molecules and particles from the surrounding by engulfing them. It is used by most of the cells because large and polar molecules cannot cross the plasma membrane. The material to be internalized is surrounded by plasma membrane, which then buds off inside the cell to form vesicles containing ingested material.

The endocytosis pathway is divided into 4 categories:

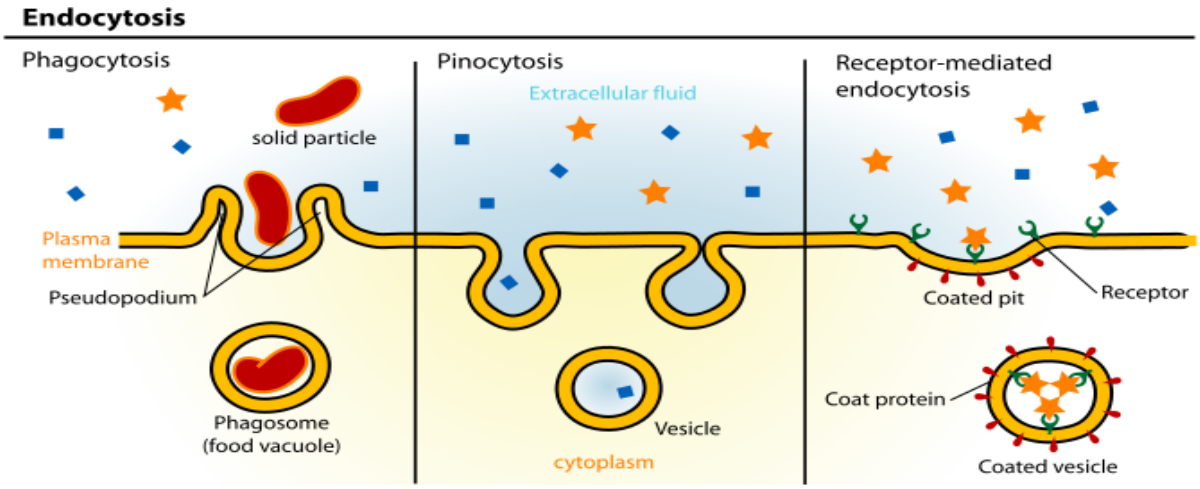

Figure 1: Types of endocytosis process (4th pathway is not shown in above figure)

1. **Phagocytosis:** Phagocytosis is the process by which certain living cells called phagocytes engulf larger solid particles such as bacteria, debris or intact cells. Certain unicellular organisms, such as the protists, use this particular process as means of feeding. It provides them part or all of their nourishment. This mode of nutrition is known as phagotrophic nutrition. In amoeba, phagocytosis takes place by engulfing the nutrient with the help of pseudopods, that are present all over the cell, whereas, in ciliates, a specialized groove or chamber, known as the cytostome, is present, where the process takes place.

When the solid particle binds to the receptor on the surface of the phagocytic cell such as amoeba, then the pseudopodia extends and later surrounds the particle as shown in figure

2. Then their membrane fuses to form a large intracellular vesicle called phagosome. These phagosomes fuse with the lysosome, forming phagolysosomes in which ingested material is digested by the action of lysosomal enzymes. During its maturation, some of the internalized membrane is recycled to plasma membrane by receptor mediated endocytosis.

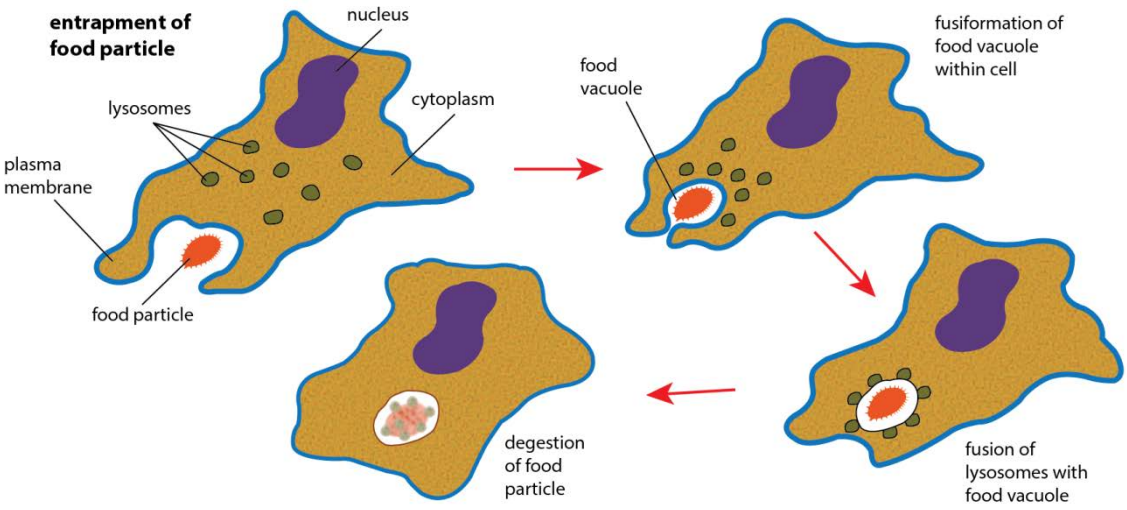

Figure 2: Example of phagocytic process for entrapment of food particle

Draw the above figure

The various phases of phagocytosis in amoeba for food capturing are:

- Adherence of the macromolecules to the receptor on the phagocytic cell
- Extension of pseudopodia and ingestion of microbe by phagocytic cell
- Formation of phagosome by the fusion of surrounding membrane
- Fusion of phagosome and lysosome to form phagolysosome
- Digestion of the ingested macromolecules by the acid hydrolytic enzymes in the lysosome
- Formation of residual body coating indigestible material
- Discharge of waste materials

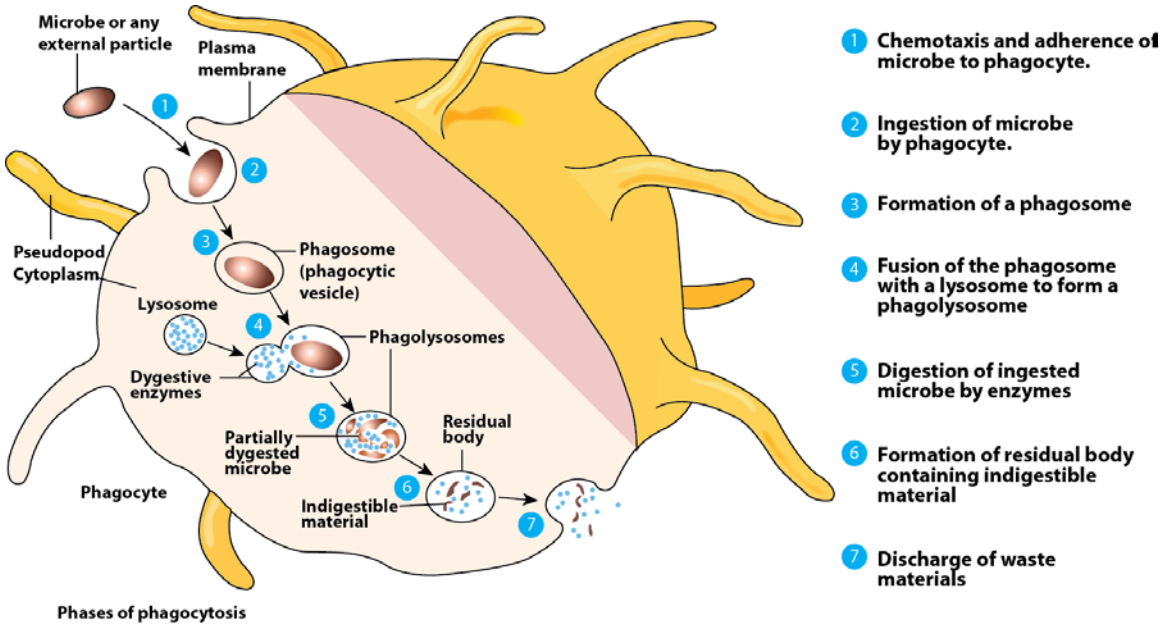

Figure 3: Phases of phagocytosis process Draw this figure

Other examples of phagocytosis include some immune system cells, that engulf and kill certain harmful, infectious micro-organisms and other unwanted foreign materials which in turn provides defence against invading micro-organism and eliminate damaged cells from the body. There are two types of phagocytes (WBC) in mammals: Macrophages and Neutrophils. These WBC acts as defence system by eliminating micro-organisms from infected tissues. In these cells, the engulfment of foreign material is facilitated by actin-myosin contractile system. It allows the cell membrane to expand in order to engulf the particle and then contract immediately, ingesting it. Macrophages also remove dead cells.

Steps of phagocytosis in the immune system:

The WBC cells are activated in the presence of certain bacterial cells, inflammatory cells or other foreign bodies. It includes the following steps:

- Phagocytes get activated by the presence of certain particles around them. As soon as they detect a foreign particle, the phagocytes produce surface glycoprotein receptors which increase their ability to adhere to the surface of the particle.
- The phagocyte slowly attaches to the surface of the foreign particle. The cell membrane of the phagocyte begins to expand and forms a cone around the foreign particle.

- The cell membrane surrounds the foreign particle to create a vacuole, known as phagosome or food vacuole. The phagosome is then passed into the cell for absorption.
- The lysosomes break the food vacuole or phagosome, into its component materials. The essential nutrients, if any, are absorbed in the cell, and the rest is expelled as waste matter. In case of the immune system, the cell creates a peroxisome, a special structure that helps the body to get rid of the toxins.

2. **Clathrin-mediated endocytosis:** Clathrin-mediated endocytosis is also known as receptor mediated endocytosis. It is the process of internalizing molecules into the cell by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being internalized (**Jackson et al.**).

Phases of clathrin-mediated endocytosis:

- Macromolecules (as ligands) bind to the specific cell surface receptors
- Then the receptors are concentrated in specialized regions of plasma membrane and clathrin and adaptor protein binds to these receptor forming clathrin-coated pits
- These pits bud from the membrane and form clathrin-coated vesicles containing receptors, proteins and ligands
- Then these vesicles fuse with early endosomes, in which the contents are sorted for the transport to lysosomes and receptors and proteins are recycled to plasma membrane

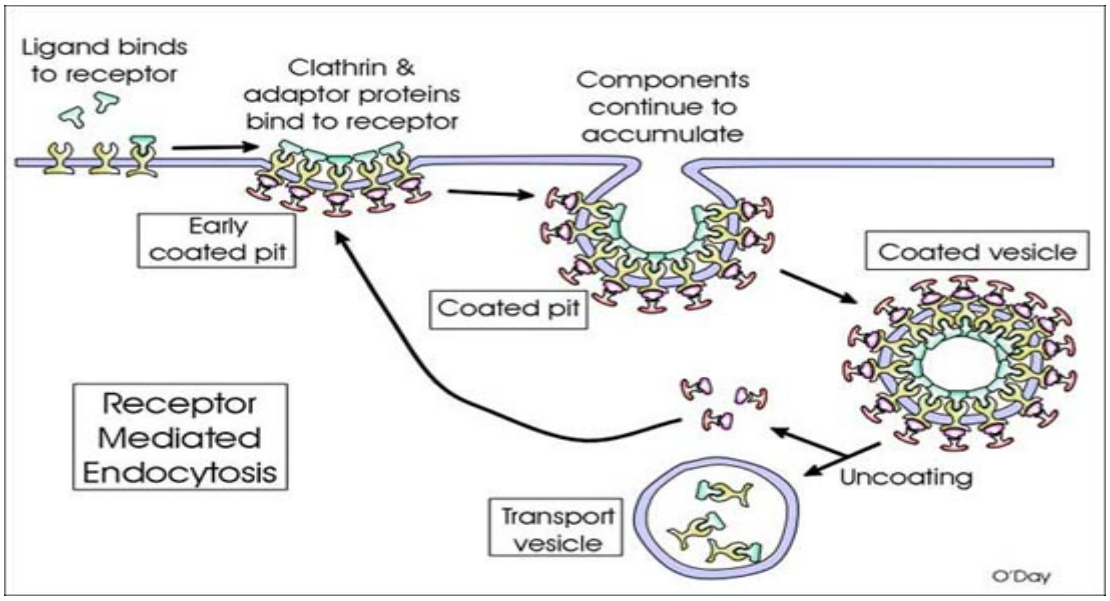

Figure 4: Phases of clathrin-mediated endocytosis

The example for clathrin mediated endocytosis is uptake of cholesterol by the mammalian cells. Here cholesterol is transported through the blood stream in the form of lipoprotein or LDL. The LDL particle consists of phospholipid bilayer, esterified and non-esterified cholesterol and Apo B protein as shown in figure 4. It was first demonstrated by Michael Brown and Joseph Goldstein in which uptake of LDL requires the binding of LDL particle to the specific cell receptor. Later it was found that it is concentrated in clathrin coated pits and internalized by endocytosis. Then receptor is recycled to plasma membrane and LDL is transported to lysosome and cholesterol is released for use by the cell.

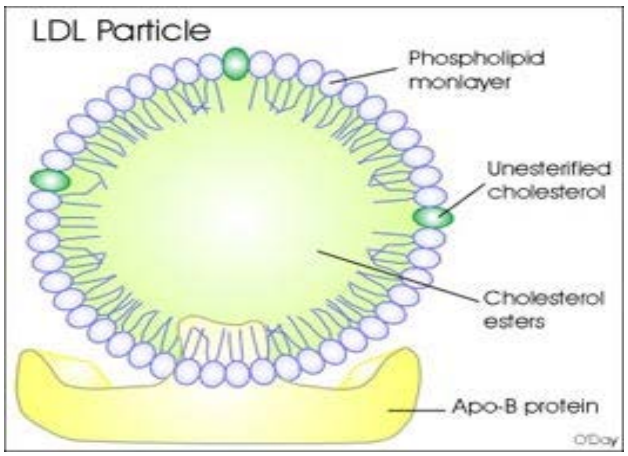

Figure 5: LDL particle or low density lipoprotein

Phases for receptor mediated endocytosis for cholesterol uptake involves:

- Receptor Binding & its activation: Here LDL receptor binds to Apo-B protein on the LDL particle
- Coated Pit Formation: Clathrin forms cage around forming endosome
- Clathrin-Coated Vesicle Budding
- Uncoating of the Vesicle
- Early Endosome associates with other vesicles
- Formation of CURL (Compartment for Uncoupling of Ligand and Receptor) or Late Endosome
- Recycling of the Receptor to the cell surface
- Fusion of Transport Vesicle with Lysosome
- Digestion of the LDL to Release Cholesterol

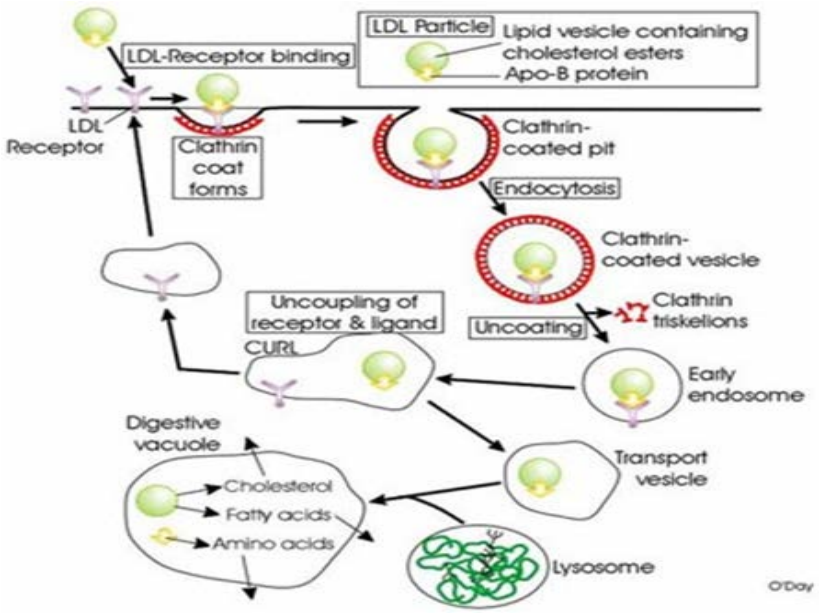

Figure 6: Receptor mediated endocytosis: Cholestrol uptake

In patients suffering from familial hypercholesterolemia, having high levels of cholesterol in serum and hence suffer from heart attacks early in life. Because these patients are unable to internalize LDL from the extracellular fluids, result in high accumulation of cholesterol. Normal individual possess LDL for transport of cholesterol but familial hypercholesterolemia results from inherited mutation in LDL receptor. These mutations can happen in two ways.

Either the patients simply fail to bind with LDL, demonstrating that a specific cell surface receptor is required for uptake of cholesterol. Or the patients are able to bind with LDL but are unable to internalize it. Because they are unable to concentrate in coated pits, demonstrating that coated pits in receptor plays an important role for cholesterol uptake. This mutation lies in the cytoplasmic tail of the receptor and can be subtle as the change of a single tyrosine to cysteine.

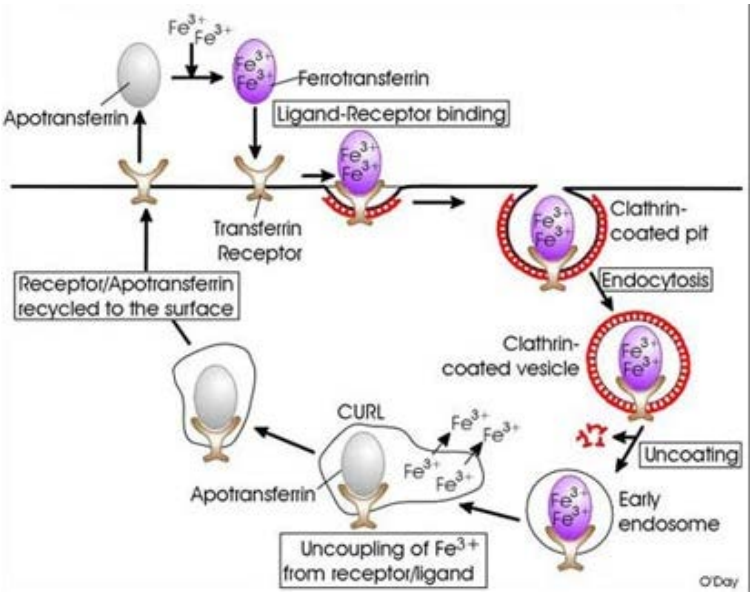

Figure 7: Example for receptor mediated endocytosis for ion uptake

3. Caveolae:

Caveolae is a pathway which is independent of clathrin- endocytosis process and involves in the uptake of molecules in small invaginations of the plasma membrane (50 to 80 mm diameter). These are enriched in lipid rafts of cholesterol, phospholipid and sphingolipids and possess a distinct coat formed by a protein called caveolin **(**cholesterol binding protein**)**. It is abundant in smooth muscle, type I pneumocytes, fibroblasts, adipocytes, and endothelial cells (**Parkar et al., 2009**).

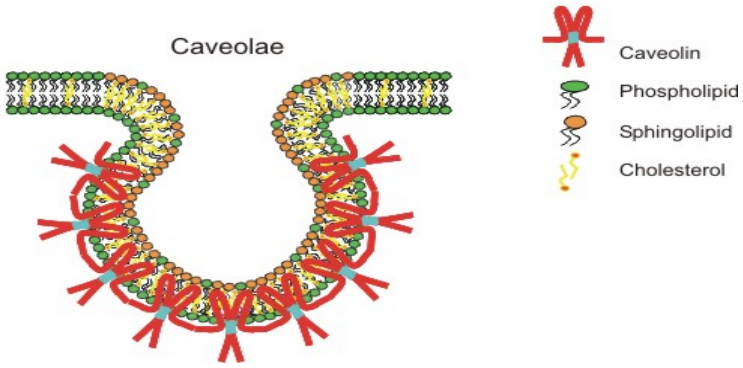

Figure 8: Structure of caveolae

Cells mostly use caveolae for the selective uptake of molecules as small as folate to full size proteins such as albumin and alkaline phosphatase. Many studies have shown that caveolae-mediated uptake of materials is not limited to macromolecules. In certain cell-types, viruses as simian virus 40 and even entire bacteria as some specific strains of *E. Coli* are engulfed and transferred to intracellular compartments in a caveolae-dependant fashion.

4. Macropinocytosis: The process of uptake of fluids in large vesicles (0.15 to 5 µm in diameter) is called macropinocytosis.

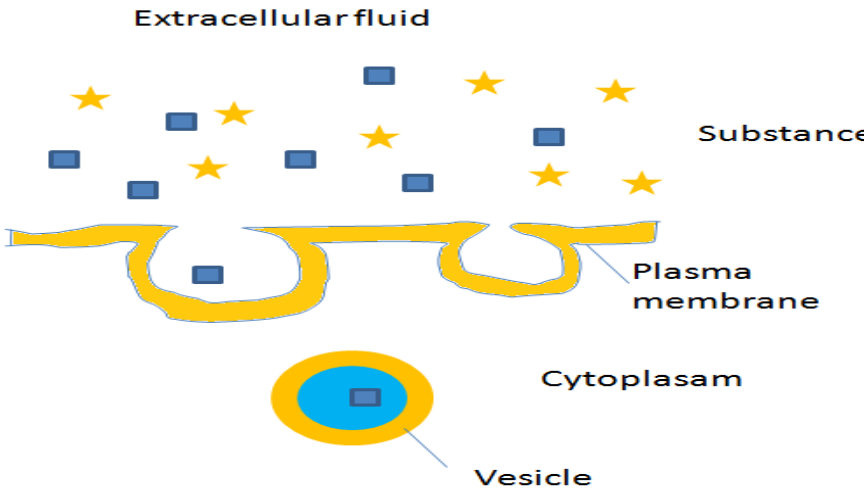

Figure 9: Diagram depicting Pinocytosis

Macropinocytosis is a different phenomenon from phagocytosis.

Macropinocytosis can uphold for particle uptake and involves the uptake of large amounts of fluid and solutes which is non- specific in nature. The receptors that trigger macropinocytosis have other physiological roles and are present on many cell types. These include growth factor receptors that activate common signalling pathways and involve global activation of the actin cytoskeleton resulting in plasma membrane ruffling and the formation of lamellipodia or blebs over the entire surface of the cell. In contrast, phagocytosis is particle-driven, and it depends on receptor interactions over the entire surface of the ingested particle. The receptors that trigger phagocytosis are usually specialized for interaction with the surface components of relevant cargo particles and actin modifications are localized to the phagocytic cup that forms around the particle.

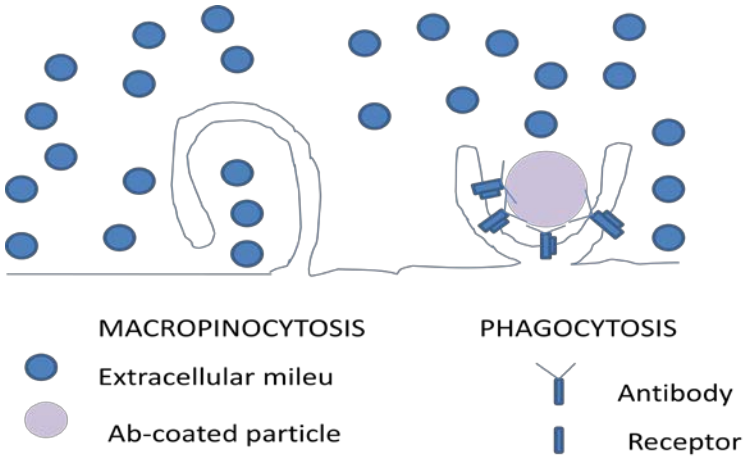

Figure 10: Differences between macropinocytosis and phagocytosis

Protein Trafficking in Endocytosis:

After internalization, clathrin-coated vesicles rapidly shed their coats and fuse with early endosomes which maintain an acidic internal pH and are located in periphery of the cell. This acidic pH leads to the dissociation of many ligands from receptors within early endosome compartment and hence serves as sorting compartment, from which molecules taken up by endocytosis are either recycled to the plasma membrane or transported to lysosomes for degradation. Later, ligands and membrane proteins for degradation are transported to late endosomes which are mediated by movement of large endocytic

carrier vesicles along microtubules. The late endosomes are more acidic than early endosomes and fuse with transport vesicles carrying hydrolases from Golgi apparatus. Late endosomes mature into lysosomes when they acquire a full complement of lysosomal enzymes and become acidic. Hence the endocytosed materials are degraded by action of acid hydrolases (**Cooper et al., 2000)**.

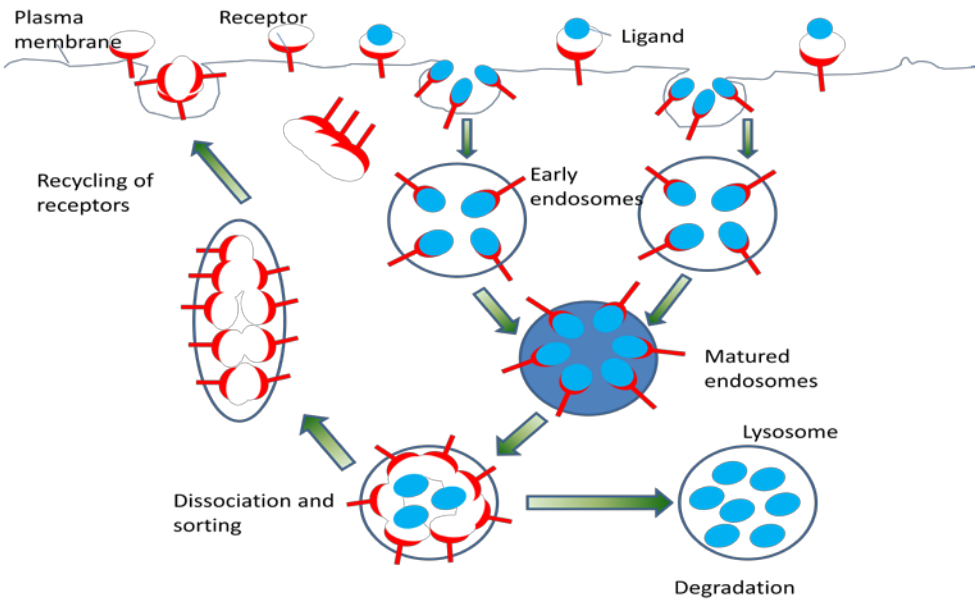

Figure 11: Protein trafficking during endocytosis. The figure shows the recycling of the plasma membrane and degradation of ligands and other membrane proteins in lysosome

The most common example for protein trafficking is recycling of synaptic vesicles. As when an action potential arrives at the terminal of most neuron signals, the fusion of synaptic vesicles with the plasma membrane releases neurotransmitter that carry signal to post synaptic cells. The empty synaptic vesicles are then recovered by plasma membrane in clathrin-coated vesicles, which fuse with early endosomes. The synaptic vesicles are then regenerated directly by budding from endosomes. They accumulate a new supply of neurotransmitter and recycle to the plasma membrane and ready for next cycle of synaptic transmission.

Transcytosis:

The process of transfer of internalized receptor across the cell to opposite domain of the plasma membrane is called transcytosis. It occurs in polarized cells or epithelial cells mostly. It is used for protein sorting and also a mean for transport of macromolecules across the epithelial cell sheets.

Example: Transport of Abs from blood to other secreted fluids. The Abs bind to the receptor on basolateral surface and then transcytosed along with their receptors to apical surface. The receptors are then cleaved and release Abs into extracellular secretions.

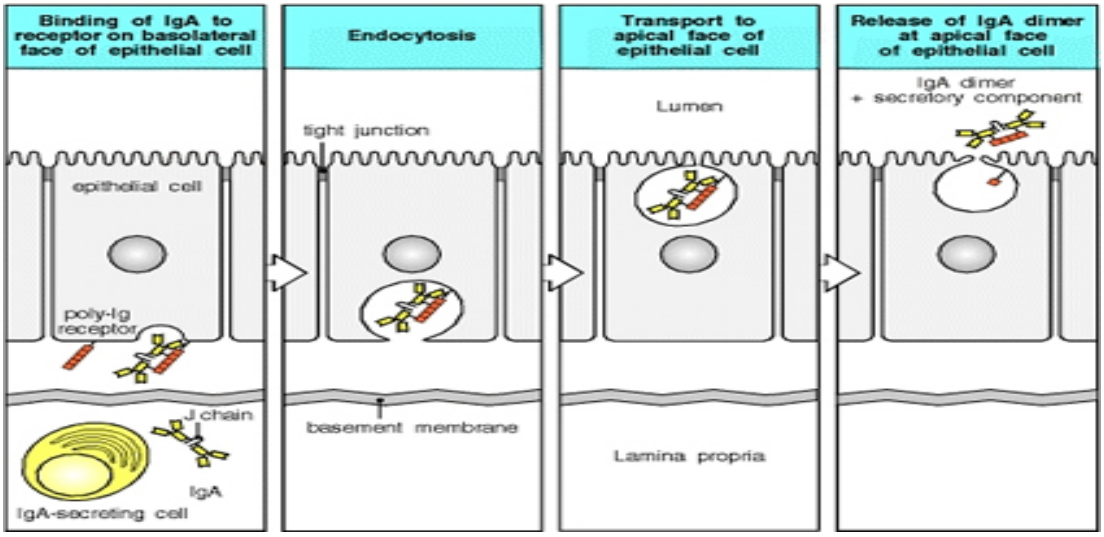

Figure 12: Process of transcytosis of IgA, an immunoglobulin, within an epithelial cell.

Exocytosis:

The process by which the cells direct the contents of secretory vesicles out of the cell membrane is known as exocytosis. These vesicles contain soluble proteins to be secreted to the extracellular environment, as well as membrane proteins and lipids that are sent to become components of the cell membrane. It is the final step in the secretory pathway that typically begins in the endoplasmic reticulum (ER), passes through the Golgi apparatus, and ends at the outside of the cell. Some of the examples include secretion of proteins like enzymes, peptide hormones and antibodies from cells and release of neurotransmitter from presynaptic neurons.

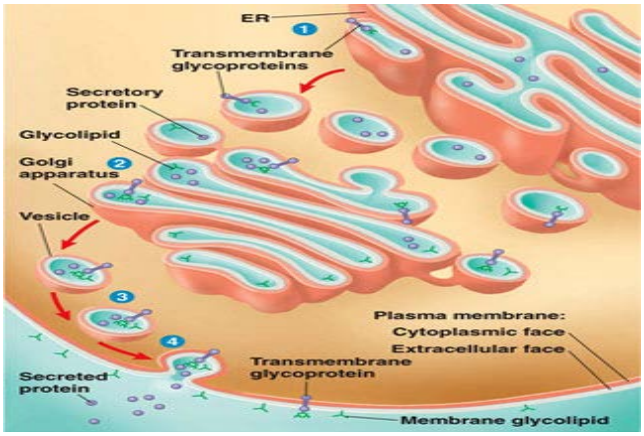

Figure 13: Diagram depicting Exocytosis process

Types of exocytosis: Exocytosis are of two types. Constitutive exocytosis and Regulated exocytosis

1. **Constitutive exocytosis: S**ecretory materials are continuously released without requirement of any specific kind of signal.
2. **Regulated exocytosis:** Regulated exocytosis requires an external signal, a specific sorting signal on the vesicles for release of components. It contains a class of secretory vesicles that fuse with plasma membrane following cell activation in presence of signal. Examples of regulated exocytosis are secretion of neurotransmitter, hormones and many other molecules

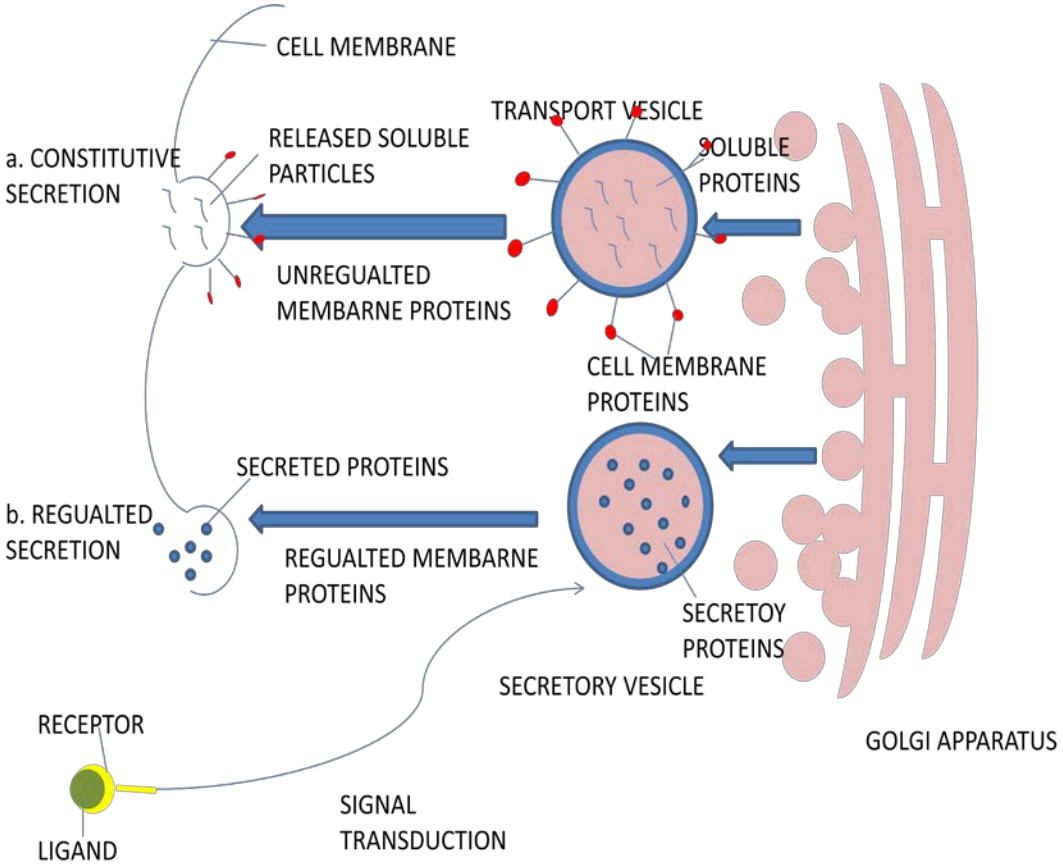

Figure 14: Types of exocytosis. (a) Constitutive secretion and (b) Regulated secretion

Steps in exocytosis:

Vesicles are used to transport the proteins from the Golgi apparatus to the cell surface area using motor proteins and a cytoskeletal track to get closer to cell membrane. Once these vesicles reach their targets, they come into contact with tethering factors that can restrain them. Then the process of **vesicle tethering** distinguishes between the initial, loose tethering of vesicles from the more stable, packing interactions. Tethering involves links over distances of more than about half the diameter of a vesicle from a given membrane surface (>25 nm). The process of holding two membranes within a bilayer's distance of one another (<5-10 nm) is called **vesicle docking.** Stable docking indicates the molecular interactions underlying the close and tight association of a vesicle with its target may include the molecular rearrangements and ATP-dependent protein and lipid modifications, needed to trigger bilayer fusion called **vesicle priming.** It is mostly takes place before exocytosis and used in regulated secretion type of exocytosis but not used in constitutive secretion. It is followed by **vesicle fusion** which includes merging of the vesicle membrane with the target and hence there is release of large biomolecules in the extracellular space with the help of some protein complex.

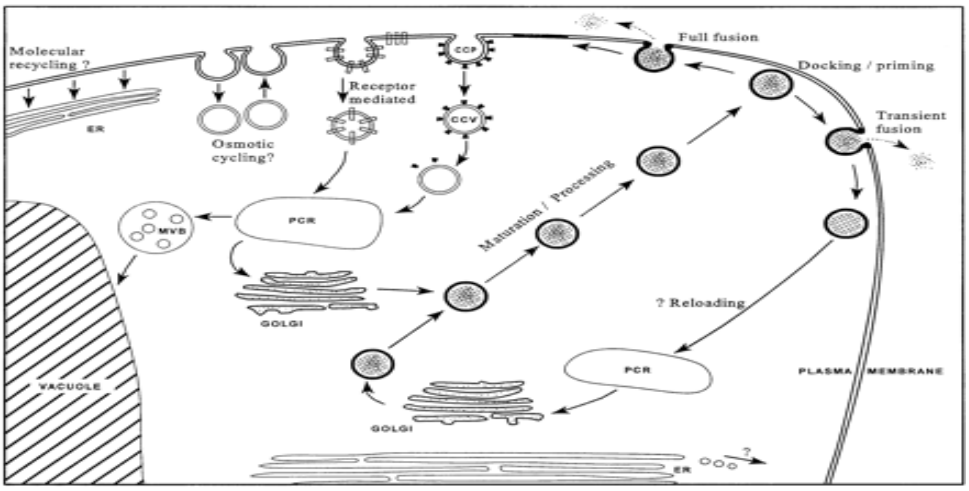

Figure 15: Sequence of Exocytosis and Endocytosis: Transport, Docking, Fusion, Content Release, and Recycling.

Interesting facts:

- Many cells in the body use exocytosis to release enzymes or other proteins that act in other areas of the body like secretion of the hormones glucagon and insulin, or to release molecules that help cells communicate with one another more directly through the products that they secrete like neurotransmitters.

- The immune system also uses exocytosis to communicate information between cells.

- Both endocytosis and exocytosis involve active transport, that is, energy must be expended to move particles against the concentration gradient.

- Both endocytosis and exocytosis involve the formation of vesicles: endocytosis forms them in order to take particles into the cell via the cell membrane and involves a reduction in cell membrane area, as part of the membrane is pinched off to form a vesicle; exocytosis forms them in order to expel things from the cell via the cell membrane and results in an increase in cell membrane, as the vesicle wall joins that of the cell membrane and is incorporated into it. Thus, the two processes also serve to balance each other.

- The vesicle fusion is driven by SNARE proteins process of merging the vesicle membrane with the target one resulting in release of large biomolecules in the extracellular space.

- Ca2+ might control the exocytotic/endocytotic balance in plants.

- Synaptotagmin I (syt1) is required for normal rates of synaptic vesicle endo- and exocytosis.

Questions:

1. The transport method of neurotransmitters between nerve cells is:
   a. Exocytosis
   b. Passive transport
   c. Receptor-mediated endocytosis
   d. Active transport
2. Which of the following membrane activities does NOT require the expenditure of energy by the cell?
   a. Active transport
   b. Osmosis
   c. Endocytosis
   d. Exocytosis
   e. Synthesis of more membrane
3. The membrane transport mechanism used when an amoeba engulfs a bacterial cell is called:
   a. Carrier-mediated facilitated diffusion
   b. Exocytosis
   c. Phagocytosis
   d. Pinocytosis
   e. Sodium-potassium pump
4. A person has a genetic disease that prevents the phospholipids in the plasma membrane of the white blood cells from freely fusing with the other membranes within the cell. How would this disease affect phagocytosis?
   a. Lysosomes would not be formed
   b. Facilitated diffusion would not occur
   c. Lysosomes would be formed lacking hydrolytic enzymes
   d. The phagocytic vacuole would not fuse with the lysosome
   e. Endocytosis would not occur

5. Pinocytosis:
    a. Is engulfment of large particles by the cell
    b. Occurs in protozoans and algae but not in more complex organisms
    c. Involves the specific binding of molecules to receptors on the cell surface
    d. Is the nonspecific uptake of fluids by pinching inward of the plasma membrane
    e. Is movement of molecules against the concentration gradient through a permeable membrane
6. Receptor-mediated endocytosis:
    a. Is a passive process
    b. Involves only membrane transport proteins
    c. Brings about the selective uptake of materials by enclosing them in membranous vesicles
    d. Does not require energy
    e. Is most likely to be found in cells that release large amounts of hormones
7. Exocytosis is a process by which cells
    a. Pass substances out of the cell in vesicle
    b. Pass substances out of the cell through the membrane by osmosis
    c. release substances directly into the extracellular fluid through a pore
    d. Release substances directly into the extracellular fluid through a pit
    e. Identify substances in the environment
8. A cell engaged in phagocytosis must be
    a. Engulfing a live organism
    b. Acquiring a liquid
    c. Engulfing a dead organism
    d. Transporting bulk dissolved nutrients
    e. Transporting bulk solid material

9. Coated pits are required for which type of membrane transport?
    a. Receptor-mediated endocytosis
    b. Phagocytosis
    c. Pinocytosis
    d. Exocytosis
10. Certain white blood cells are designed to engulf and destroy microbes such as bacteria. The process used to achieve this would be:
    a. Phagocytosis
    b. Receptor-mediated endocytosis
    c. Pinocytosis
    d. Exocytosis
11. What is the primary factor that determines if endocytosis or exocytosis would be needed to move a substance across the cell membrane as opposed to diffusion or osmosis?
    a. the chemical properties of the molecules being moved
    b. the size of the molecules being moved
    c. the differences in concentration of the substance inside and outside the cell
    d. whether water is being moved across the membrane
12. In endocytosis, a transport vesicle is derived from _____.
    a. plasma membrane
    b. ribosomes
    c. Golgi complex
    d. Lysosomes
13. Low density lipoproteins (LDL's or bad cholesterol) are taken up "in-bulk" into the cytoplasm of a cell. This process is an example of
    a. Endocytosis
    b. Exocytosis
    c. Molecular transport
    d. Oosmosis
    e. Diffusion

14. How the phagocytosis takes place in the immune system of cells?
15. Describe the uptake of cholesterol by the mammalian cells using receptor-mediated endocytosis.
16. What is protein trafficking? Explain with an example.
17. What is transcytosis? Explain with an example.
18. What are the two types of exocytosis? Explain with a schematic diagram.

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Module 3 Lecture 8

Entry of virus in cells:

Introduction:

The interactions between viruses and cells are complex, in spite of their simple structure and components. Viruses utilize a number of cellular processes which contains cellular proteins to enter into the cells. Some viruses are able to cross plasma membrane into cytosol by endocytic uptake, vesicular transport via cytoplasm and transport to the endosomes and other intercellular organelles. These processes are associated with clathrin-mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, or other mechanisms. Generally viruses are attached to the cell surface of proteins, carbohydrates and lipids. The interaction of viruses receptors are specific and require at least 3 point for interaction, which results in activation of cellular signalling pathways. The viruses enter the cell by endocytic mechanism. After the viruses go in the lumen of endosomes or the endoplasmic reticulum, they obtain signals which are in the form of being exposed to low pH, proteolytic cleavage, and the initiation of viral proteins, which results in modifications in the viral proteins, and then they are able to penetrate the vacuolar membrane. After they penetrate the vacuolar membrane, they pass the viral genome, the caspid, or the viral particle that is kept together into the cytosol. Afterwards, the majority of RNA viruses replicate at a variety of positions within the cytosol. In contrast, most DNA viruses continue through their passage towards the nucleus.

Viral entry into the cell:

Viruses enter the cell, which is covered by a phospholipid bilayer and acts as a cells natural barrier to its surrounding. The process by which this barrier is crossed depends upon the type of virus. There are 4 types of viral entry into the cell:

1. Attachment or Viral Adsorption: The viral receptors attached to the complementary receptors on the cell membrane.
2. Membrane Fusion: The cell membrane is punctured and later attached with the unfolding viral envelope.
3. Entry via Pore formation: An opening is established for the entry of viral particles.

Viral Penetration: The viral capsid or genome is injected into the host cells cytoplasm directly.**Entry through endocytosis:**

The type of entry through endocytosis is carried out by bacteriophages infection that form coliphages T4 and T2 and are hypodermic syringes with a tail that is capable of contracting. Endocytic vesicles carry viruses from the outer edges to the perninuclear area of the host cell, where the conditions for infection are changed and the distance is minimized towards the nucleus. Also the maturation of endosomes has slowly changing conditions, like lowering of pH or the switching of a redox environment, which enables viruses to detect their position within a cell and the passage and allows the endosomes to utilize this information to put a time of penetration and uncoating.

Viruses as Endocytic Carriers:

This type of viral entry is mostly observed in animal viruses. When these viruses attach to cells, they do not become disfigured. Rather the plasma membrane changes shape according to the shape of the virus. The outer layer of viruses is coverer proteins that attaches to receptors and formed an icosaherdral grid or as spike glycoprotein that span the entire viral envelope. The single interactions with the receptors are weak, but interaction with many different receptors increases the activity and results the binding to cells almost impossible to reverse. Lipid raft functions to control the signalling, fluidity and receptor functions on the membrane. These are rich in cholesterol and sphingolipids.

Viruses that use the Caveolar/Raft-dependent pathways form primary endocytic vesicles are dependent on cholesterol, lipid rafts, and complex signalling pathways.

Attachment Factors and Receptors:

Some viruses bind to defined endocytic receptor, like transferrin and low-density lipoprotein receptors, but viruses attach to the majority of other molecules like carbohydrate moieties with different functions like cell to cell recognition, ion transportation and attachment to the extracellular matrix. The difference between attachment factors and virus receptor is that the attachment factor simply attached to the viruses and hence focused on the top area of the cell, while virus receptors modify the viruses, promote cellular signalling or activate penetration. The situation of attachment receptor is common in influenza and polyomaviruses, where the attachment is very specific and deals with lectin domains. The other example is HIV-1 because two receptors Adenoviruses 2 and 5 are necessary to promote conformational modifications to assist fusion and encourage endocytosis.

Entry by macropinocytosis:

Viruses belonging to vaccinia, adeno, picorna and other virus families enter by macropinocytosis, an endocytic mechanism involved in fluid uptake. The virus particles first activate signalling pathways that trigger actin-mediated membrane ruffling and blebbing. This is followed by the formation of large vacuoles (macropinosomes) at the plasma membrane, internalization of virus particles and penetration by the viruses or their capsids into the cytosol through the limiting membrane of the macropinosomes.

Examples for entry of viruses into the cells on the basis of types of viruses:

1. Bacterial virus entry:

Bacteriophages attack bacterial cells and inject their genomes through specific receptor sites which include lipopolysaccharides, cell wall proteins, teichoic acid or flagellar or pilus proteins and also contain specific attachment proteins on the bacteria. The phage tail fibres are the attachment sites and bind reversibly to lipopolysaccharides and outer membrane protein OmpC. And then the base plate then settles down onto the surface and binds tightly to it which results in conformational changes in the short tail fibres, which then later contracts, pushing the tail core through the cell wall, in an ATP-driven process and is aided by a lysozyme activity associated with the tip of the tail tube.

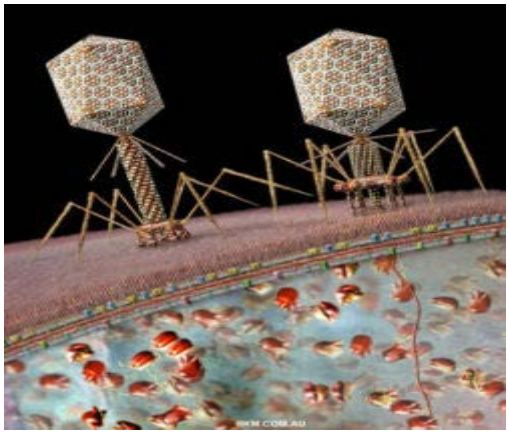

Figure 1: Enterobacteria phage T4 or viruses with 34-170 kbp dsDNA genomes, isometric heads and contractile tails - infects the gram-negative bacterium E coli

Phage lambda or enterobacteria phage λ is a tailed phage with an isometric head which attaches to the maltose receptor on the surface of the *E.coli* cell via the J protein in the tail tip. Although the tail is non-contractile, a DNA injection mechanism allows entry of DNA into the cell, via a sugar transport protein (PtsG) in the inner membrane, leaving the capsid behind.

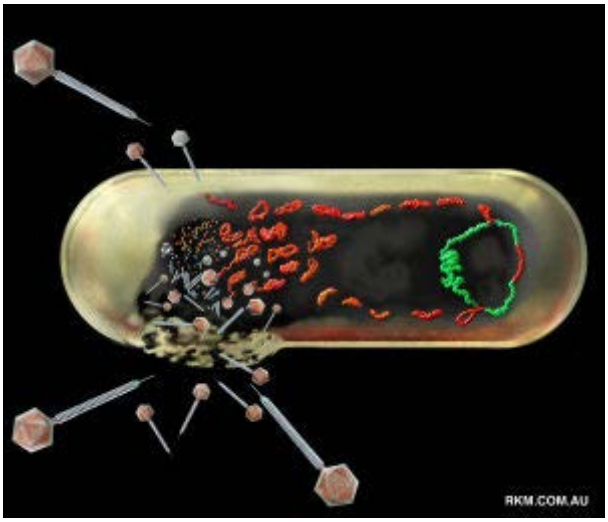

Figure 2: Lambda phage infecting *E.coli*

MS2 phage is an isometric single-stranded RNA-containing virus which infects *E.coli*. And the phage attaches to the F pili of *E.coli* via its single attachment or a protein. The A protein is covalently linked to the 5'-end of the genomic RNA; binding pilin causes cleavage of protein and releases it from the capsid. Thus, when the pilus is retracted into the cell, protein and RNA are pulled with it, leaving the empty capsid outside.

2. Animal cell entry:

Animal viruses enter into the cell by direct cell membrane fusion and entry via endocytotic or other vesicle. Direct membrane fusion involves direct attachment to the cell surface via **binding to a specific receptor.** Here the virion membrane fuses with the cell membrane, and the virion nucleoprotein complex is delivered into the cell cytoplasm directly. An example is HIV entry process. Here the virion attachment protein **gp120** attaches initially to the **CD4** protein on a helper T-cell. The gp120 then undergoes conformational change due to binding and binds the **accessory receptor CCR-5,** a **chemokine**. **gp41**, a cleavage product of a **gp160** precursor, and a part of the **"spike protein"** of the viral membrane is then able to bind into the cell membrane, via a

hydrophobic domain. A condensation of the gp41 structure results in formation of a "6 helix bundle" and causes close juxtaposition of cell and viral membranes, which promotes membrane fusion and nucleoprotein entry into the cell.

Fusion Protein mode of entry: All enveloped viruses follow fusion mode of entry, whether they fuse with the cell membrane directly or with the membrane via an internalised vesicle. This is mediated by three identified classes of envelope glycoproteins:

Class I fusion proteins

These fusion proteins are most common in retroviruses, myxoviruses, coronaviruses and paramyxoviruses. The "spikes" are composed of three identical protein subunits, largely alpha-helical in structure and assembled as trimers which are later on cleaved into two pieces. The carboxy-terminus of one piece is anchored to the viral membrane and the new amino terminus has a characteristic stretch of 20 hydrophobic amino acids.

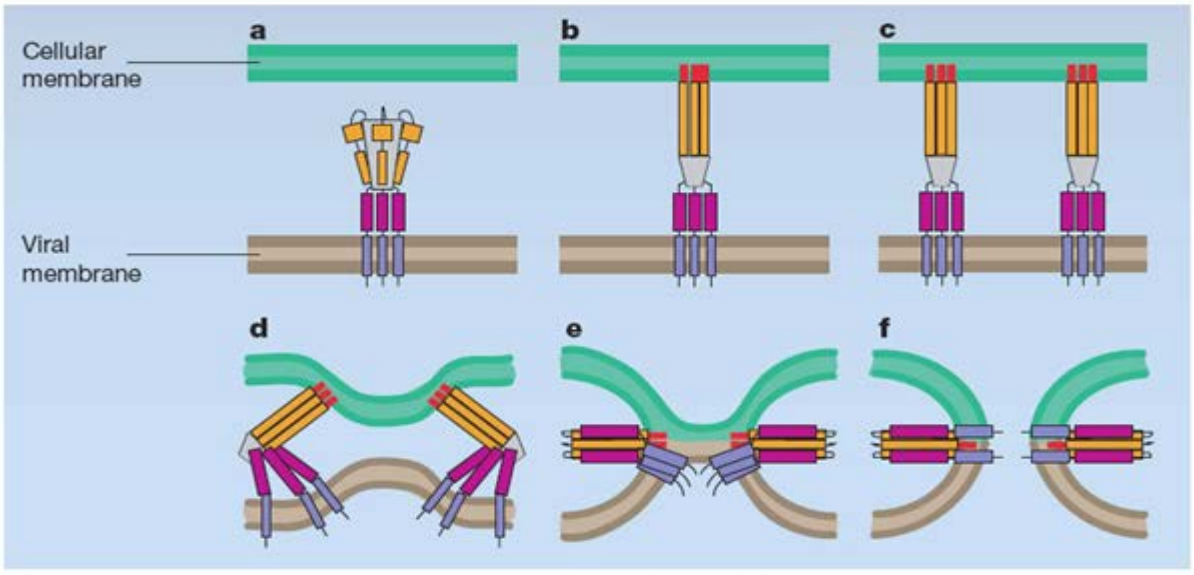

Figure 3: Proposed mechanism for membrane fusion by class I fusion proteins. (a)The metastable conformation of a trimeric fusion protein: with helical domain in orange, helical domain B in pink and the transmembrane domain in purple. (b)After binding to a receptor on the cellular membrane or an exposure to low pH found in intracellular compartment, the protein forms an extended conformation and hydrophobic fusion peptide (red) inserts into the target membrane. (c)Several trimers thought to be involved in this mechanism. (d)Protein refolding begins. The free energy thereby released causes the membrane to bend towards each other. (e)Formation of restricted hemifusion stalk allows the lipids in the outer leaflets of membrane to mix. (f)Protein refolding completes, forming the most stable form of fusion protein with fusion peptide and transmembrane domain anti-parallel to each other but in the same membrane.

Class II fusion proteins

They are found in dengue, tick-borne encephalitis, yellow fever and other flaviviruses, and Semliki Forest virus. This class of fusion proteins have a β-sheet-type structure and are not cleaved during biosynthesis. The proteins have three principal domains: Domain I begin at the amino terminus, domain II contains the internal fusion loop and domain III is at the carboxy terminus.

The dimeric protein binds to few cellular receptors for virus internalisation. The acidic pH inside endosomes causes domain II to swing upward, allowing monomers to rearrange laterally. The fusion loop inserts into the host-cell membrane, enabling trimer formation of the viral glycoprotein. Domain III shifts and rotates to create contacts, bending the membrane. The formation of further contacts leads to unrestricted hemifusion and the most stable form of the protein.

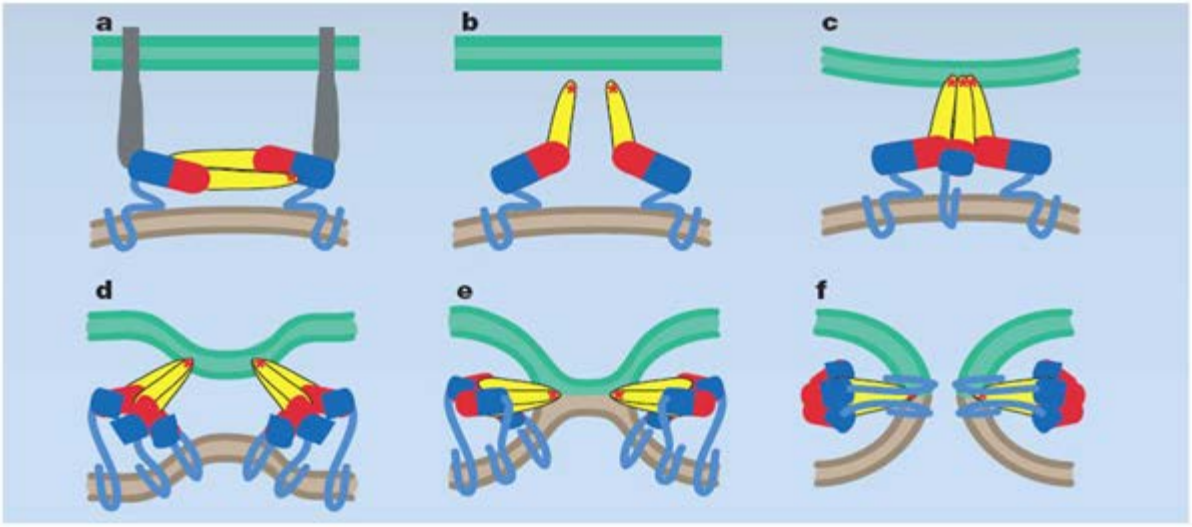

Figure 4: Proposed mechanism for fusion by class II proteins. (a)The dimeric E protein binds to a cellular receptor (grey) and the virus is internalized to endosomes. Membrane fusion release the virus into the cell body, takes place within endosomes. Domain I is in red, domain II in yellow and domain III in light and dark blue. (b)The acididc pH inside the endosomes cause domain II to swing upward and permit E monomers to rearrange laterally. (c)The fusion loop (red dot) inserts into the outer leaftlet of the host-cell membrane, enabling trimer formation. (d)The formation of trimer contacts extends from the top of the molecule. Domain III shifts and rotates to create contacts, bending the membrane. (e)The formation of further contacts leads to unrestricted hemifusion. (f)The final most stable form of the protein.

Class III fusion proteins

These proteins are characteristic of rhabdoviruses and vesicular stomatitis viruses. They form trimers of hairpins as a fusion structure by combining two structural elements. The post-fusion trimer has a central α-helical trimeric core; however, the fusion domains have two fusion loops at the tip of an elongated β-sheet. Most **non-enveloped viruses**, such as dsDNA adenoviruses and ssRNA picornaviruses, enter cells via vesicles. The former appear to enter via an endocytotic vesicle. In later, the capsid becomes rearranged as receptor binding induced structural transitions, whereby the VP4 internal protein is externalised and the virion surface becomes more lipophilic, and interacts with a vesicle membrane to form a pore so as to allow exit of the RNA into the cytoplasm.

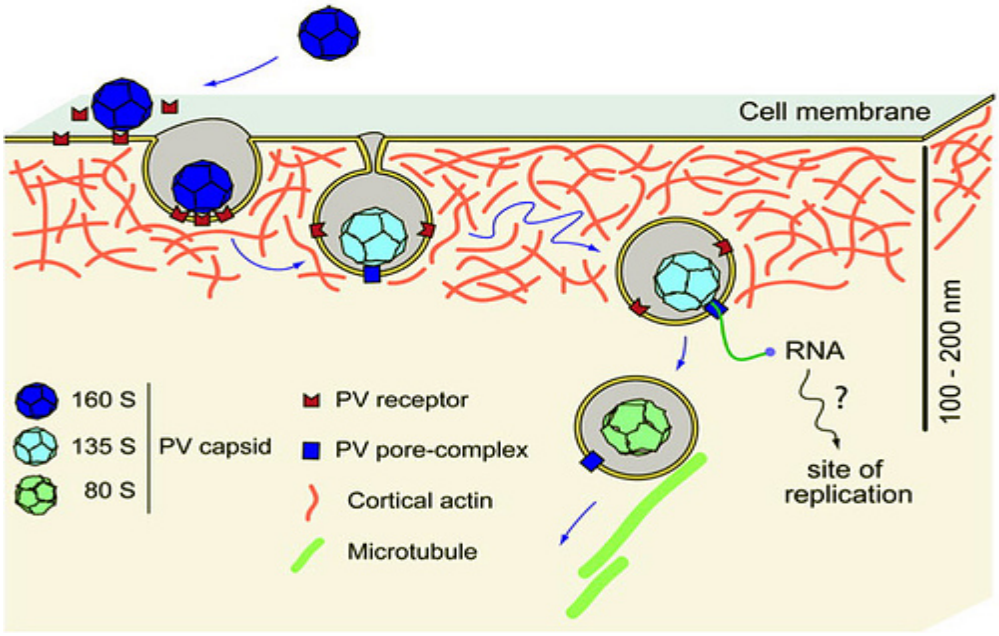

Figure 5: Poliovirus entry into live cells

Nuclear targeting:

A final result in the infection is that DNA genomes end up in the nucleus except for Poxviridae, Phycoviridae and some Baculoviridae and that RNA genomes end up in the cytoplasm except for myxoviruses. These are the sites where the respective viruses may be expected to replicate their genomes.

Adenoviruses are transported by means of the hexon protein of the partially degraded naked capsid which is released into the cytoplasm. This reaches a nuclear pore and allows escape of viral DNA plus certain viral polypeptides into the nucleus.
Parvoviruses enter host cells by receptor-mediated endocytosis, escape from endosomal vesicles to the cytoplasm, and then replicate their DNA in the nucleus.
Herpesviruses generally enter by fusion with the cell membrane by a process that involves several envelope glycoproteins acting together and the core particles migrate to nuclear pores, and release DNA there.
Poxviruses have "intracellular" single-enveloped and "extracellular" double-enveloped forms which enter by **direct cell fusion** (pH-independent) or **lysosomal vesicle fusion** (pH-dependent). Once core virions are in the cytoplasm, they uncoat further to expose a nucleoprotein complex which is first transcriptionally and later, replicationally, active.

3. Plant cell entry:

Every cell in plant is separated from every other cell by thick cell walls, whose dimensions are far larger than the size of the average virion. So plant cells are inaccessible to viruses. These plant cells interconnect only via specific discontinuities in the cellulose walls. These act as gated intercellular channel, which limit the passage of both molecules and virions between cells. Plant viruses possess ssRNA + ve **sense, non-enveloped** and **do not specifically interact with host cell membranes.** The mechanisms employed to enter cells appear to be passive carriage through breaches in the cell wall, followed by cell-to-cell spread in a plant by means of specifically-evolved "movement" functions, and then spread via conductive tissue as whole virions.

The mode of transmission of viruses affects their concentration and localisation in plants. For example, mechanically transmitted viruses (eg: bromoviruses, tobamoviruses) tend to reach very high concentrations in most tissues by non-specific means. Whereas viruses which are introduced into plants via insect vectors with piercing mouthparts tend to be limited in their multiplication to phloem elements, which are preferred target tissues for insect feeding. Consequently, these viruses (eg: luteoviruses, geminiviruses) reach only very low concentrations in whole plants.

Entry of virus on the basis of morphological structure:

The naked virus enters either via translocation (i.e. crosses cell membrane intact directly); genome injection (attachment to the cell surface and releases its genome which penetrates the cytoplasm via a pore in the plasma membrane) or receptor mediated endocytosis. Whereas the enveloped virus enters the cell via receptor mediated endocytosis or membrane fusion.

Interesting facts:

- Some viruses are able to cross plasma membrane into cytosol by endocytic uptake, vesicular transport via cytoplasm, and transport to the endosomes and other intercellular organelles. These processes are associated with clathrin-mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, or other mechanisms.
- HIV-1 uses two receptors Adenoviruses 2 and 5 are necessary to promote conformational modifications to promote the fusion and encourage endocytosis.
- The bacteriophage tail fibres are the attachment sites and bind reversibly to lipopolysaccharides and outer membrane protein OmpC.
- Phage lambda is a tailed phage which attaches to the maltose receptor on the surface of the *E.coli* cell via the J protein in the tail tip.
- Animal viruses enter into the cell by direct cell membrane fusion and entry via endocytotic or other vesicle.
- Neuclear targeting is the result of final destination in the infection of viruses. In some, DNA genomes end up in the nucleus except for Poxviridae, Phycoviridae

and some Baculoviridae and that RNA genomes end up in the cytoplasm except for myxoviruses.

- The mechanisms employed to enter virus into the plant cells appear to be passive carriage through breaches in the cell wall, followed by cell-to-cell spread in a plant by means of specifically-evolved "movement" functions, and then spread via conductive tissue as whole virions.
- Entry of the dengue virus to mammalian cells can occur via receptor-mediated endocytosis in clathrin coated pits.

Questions:

1. How do viruses gain entry to a host cell?
    a. by dissolving a piece of the host cell membrane
    b. by binding to a receptor site on the host cell
    c. by binding to an antibody site on the host cells
    d. all of the above
2. How the dengue viruses enter the cells?
    a. Receptor-mediated endocytosis
    b. Binding to the cell surface receptor
    c. Phagocytosis
    d. Pinocytosis
3. Which receptor plays an important role for the entry of retoviruses?
    a. Glycoproteins
    b. Integrins
    c. Hexon
    d. Chemokine
4. Entry of HIV is an example of
    a. Macropinocytosis
    b. Endocytosis
    c. Attachment factors and receptors
    d. Vesicles

5. Describe the different modes of entry of viruses across the plasma membrane of the cells. Explain with an example.
6. What is fusion protein mode of entry?
7. What is nuclear targeting? Explain with an example.

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Module 3 Lecture 9

Entry of toxins into the cells

Toxins are poisonous substances produced by certain microbes. And the ability to produce toxins by which many pathogens produce disease is known as “toxigenesis”. Toxins may be carried far from the site of invasion by the blood or lymph. Various toxins may cause fever, cardiovascular disturbances, diarrhea, and shock. They can inhibit protein synthesis, destroy blood vessels, and disrupt the nervous system. Most of the toxins are bacterial origin whereas some toxins are also produced by some fungi as a competitive resource. The toxins, named mycotoxins, deter other organisms from consuming the food colonised by the fungi.

In response to the presence of a toxin, the body produces antibodies called antitoxins, which will combine with the toxin and make it harmless. The active toxins are treated by heat or expose to chemicals such as formaldehyde. This makes them harmless but still able to trigger the immune response that causes the production of antibodies. The inactivated toxins are called toxoids, and are used for vaccinations. Diphtheria and tetanus vaccines are prepared this way.

Bacterial Toxins:

There are two main types of bacterial toxins, lipopolysaccharides, which are associated with the cell wall of Gram-negative bacteria, and proteins, which are released from bacterial cells and may act at tissue sites removed from the site of bacterial growth. The cell-associated toxins are referred to as endotoxins and the extracellular diffusible toxins that are secreted by bacteria are referred to as exotoxins. However, in some cases, exotoxins are only released by lysis of the bacterial cell. Exotoxins are usually proteins, minimally polypeptides that act enzymatically or through direct action with host cells and stimulate a variety of host responses.

The production of the toxin is specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin). Usually, virulent strains of the bacterium produce the toxin while nonvirulent strains do not, and the toxin is the major determinant of virulence (e.g. tetanus and diphtheria).

Usually the site of damage caused by a toxin indicates the location for activity of that toxin. Some protein toxins have very specific cytotoxic activity. For example, tetanus and botulinum toxins attack only neurons. But some toxins (as produced by staphylococci, streptococci, clostridia, etc.) have fairly broad cytotoxic activity and cause nonspecific death of various types of cells or damage to tissues, eventually resulting in necrosis. Bacterial protein toxins are strongly antigenic. Protein exotoxins are inherently unstable. In time they lose their toxic properties but retain their antigenic ones.

A plus B Subunit Arrangement

Many toxins act intracellularly and consist of two components: subunit A which is responsible for the enzymatic activity of the toxin and subunit B is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

Pore forming toxins:

Lipids are hydrophobic molecules which are essential constituents of membranes in the cells, whereas bacterial toxins are mainly hydrophilic proteins. All bacterial toxins interact first with their target cells by recognizing a surface receptor, which is either a lipid or a lipid derivative, or another compound but in a lipid environment. When bound to the receptor, some toxins act locally at the cell membrane, triggering pore formation across the lipid bilayer to release cell nutrients or kill target by disturbing their membrane. In contrast, other toxins enter cells and modify an intracellular target. These active toxins are trapped into endocytotic vesicles and follow different steps to access into the cytosol. One of the example is Staphylococcus which secretes pore forming toxins, to alter the host cells and trigger a release of nutrients useful for their growth.

Pore forming toxins use two mechanisms to form pores in the cell membrane, according to the structural domain building the channel:

- Insertion of amphipathic α-helices or α-PFT

Represented by colicins and staphylococcus δ-toxins

- Insertion of amphopathic β-hairpins organized in β-barrels or β-PFT

These are hydrophilic proteins rich in β-sheets and most of the bacterial PFT belong to this class. β-PFTs bind to a cell surface receptor, oligomerize and one or two β-hairpins of individual monomers associate into a β-barrel structure which inserts into the lipid bilayer and creates a channel.

Example: β-PFTs forming large pores: cholesterol dependent cytolysins (CDCs)

CDCs, such as *Clostridium perfringens* PFO (perfringolysin), recognize cholesterol as a receptor. The molecule is rich in β-sheets and is hydrophilic.

PFO pore formation includes the binding of water-soluble PFO monomers to cholesterol in lipid bilayers, which is mediated by the short hydrophobic undecapeptide loop at the tip of the domain 4. Domains 1, 2, and 4 fit into L-shaped forming a cylindrical structure. Interaction of domain 4 with cholesterol induces a conformational change of domains 3, which are rotated from domains 2 and form a belt in the outside face of the cylinder which promotes in the exposure of hydrophobic residues and the insertion of a transmembrane β-barrel into the lipid bilayer. And hence larger pores are formed which contain 35-50 monomers.

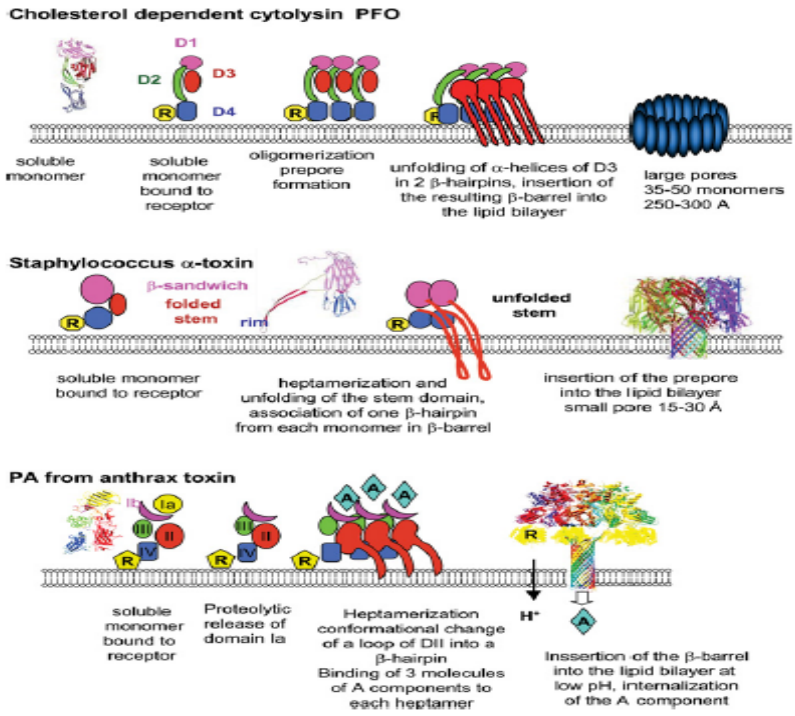

Figure 1: Pore formation of three β-PFTs. Pore formation can occur in the following ways: large pores induced by PFO, an example of a CDC; small pores resulting from heptamerization of *Staphylococcus* α-toxin; and pores formed by the binding component of anthrax toxin (PA) through endosome membrane, permitting the internalization of the corresponding enzymatic components. All the three types of β-PFTs show a common mode of β-barrel formation and subsequent insertion into lipid bilayers. Please draw this figure

Receptor mediated endocytosis:

The bacterial toxin consist of two components: one component (subunit A) which is responsible for the enzymatic activity of the toxin and the other component (subunit B) which is concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane. The enzymatic component is not active until it is released from the native (A+B) toxin. Isolated A subunits are enzymatically active but lack binding and cell entry capability. Isolated B subunits may bind to target cells (and even block the binding of the native toxin), but they are nontoxic.

There are two mechanisms of toxin entry into target cells:

1. Direct entry:

The B subunit of the native (A+B) toxin binds to a specific receptor on the target cell and induces the formation of a pore in the membrane through which the A subunit is transferred into the cell cytoplasm.

2. Receptor mediated endocytosis:

Here the native toxin binds to the target cell and the A+B structure is taken into the cell by the process of receptor-mediated endocytosis. The toxin is further internalized in the cell in a membrane-enclosed vesicle called an endosome. H^+ ions enter the endosome lowering the internal pH which causes the A+B subunits to separate. The B subunit affects the release of the A subunit from the endosome so that it will reach its target in the cell cytoplasm. The B subunit remains in the endosome and is recycled to the cell surface.

In both cases, a large protein molecule must insert into and cross a membrane lipid bilayer, either the cell membrane or the endosome membrane. This activity is based in the ability of most A+B or A/B toxins, or their B components, to insert into artificial lipid bilayers, creating ion permeable pathways.

Example: Diphtheria Toxin

The diphtheria toxin is produced by *Corynebacterium diphtheriae*. It is a bacterial exotoxin of the A/B prototype. It has two parts: subunit A, contains the enzymatic activity for inhibition of elongation factor-2 involved in host protein synthesis and subunit B, is responsible for binding to the membrane of a susceptible host cell. The B subunit possesses a region T (translocation) domain which inserts into the endosome membrane thus releasing the enzymatic component into the cytoplasm. In vitro, the native toxin is produced in an inactive form which is activated by the proteolytic enzyme

trypsin in the presence of thiol. The diphtheria toxin enters its target cells by either direct entry or receptor mediated endocytosis.

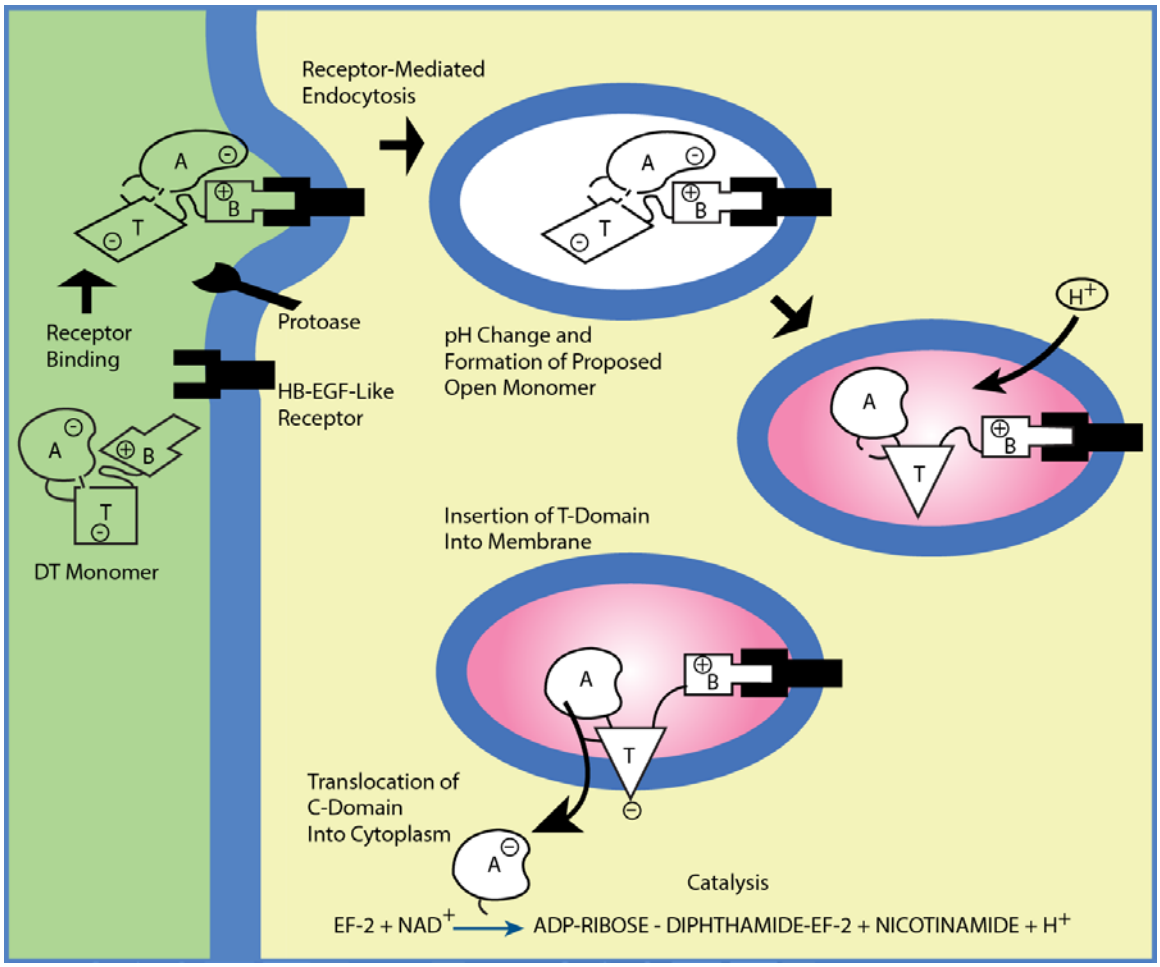

Figure 2: Entry and activity of diphtheria toxin (Dtx) in susceptible cells. The B domain of the toxin binds to a cognate receptor on a susceptible cell. The toxin is taken up in an endosome by receptor mediated encocytosis. Acidification of the endocytic vesicle allows unfolding of the A and B chains exposing the hydrophobic T domain of the toxin. The T domain inserts into the endosome membrane translocating the A fragment into the cytoplasm where it regains its enzymatic configuration. The enzymatic A component utilizes NAD as a substrate. It catalyzes the attachment of the ADP-ribose portion of NAD to elongation factor (EF-2) which inactivates its function in protein synthesis. Redraw this figure

Clathrin independent endocytosis of ricin and Shoga toxin:

Plant toxin ricin binds to both glycolipids and glycoproteins with terminal galactose all over the cell surface and is therefore localized to all types of membrane invaginations and the toxin is internalized by all endocytic mechanisms. Ricin has been localized in clathrin-coated pits, but is still endocytosed when this pathway is blocked.

Clathrin-independent endocytosis is different from uptake by caveolae and macropinocytosis. For instance, clathrin-independent endocytosis occurs on the apical side of polarized cells, whereas caveolae are localized in the basolateral domain. Clathrin-independent endocytosis of ricin occurs when uptake from caveolae and clathrin-dependent endocytosis are inhibited by extraction of membrane cholesterol. Removal of cholesterol leads to the disappearance of caveolar and inhibits formation of invaginated clathrin-coated pits (**Sandvig et al.,2000).**

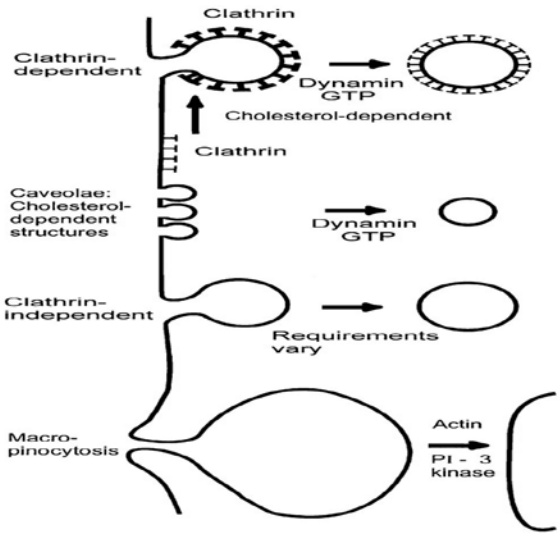

Figure 3: Structures proposed to be involved in endocytosis.

On the other hand, Shiga toxin is endocytosed preferentially by the clathrin-coated pathway, although it is bound to a glycolipid receptor (globotriasylceramide; Gb3).

Bacterial toxin transport using macromolecular syringes:

The pathogens inject their toxins into the cytosol of host cells through bacterial transport machines that function as macromolecular syringes are either bacterial flagella or conjugative pili and facilitate the direct passage of toxin effectors from bacterial donor cells into eukaryotic cells by processes of Type III or Type IV secretion mechanisms or by constructing large pores within the plasma membrane of target cells that function as for direct effectors delivery.

As the first step in cellular entry, AB toxins bind to one or more plasma membrane surface receptors. They incorporate two discrete and essential functional components that vary in physical arrangement but are generally conserved in terms of function. Thus, toxins “A fragments” are the active moiety that can modify intracellular target molecules by one of the enzymatic activities, including ADP-ribosylation, UDP-glucosylation, or proteolysis. Meanwhile, the “B fragments” serve as delivery vehicles for their A components by binding to plasma membrane surface receptors and facilitating translocation of the A components into the cytosol through available portals.

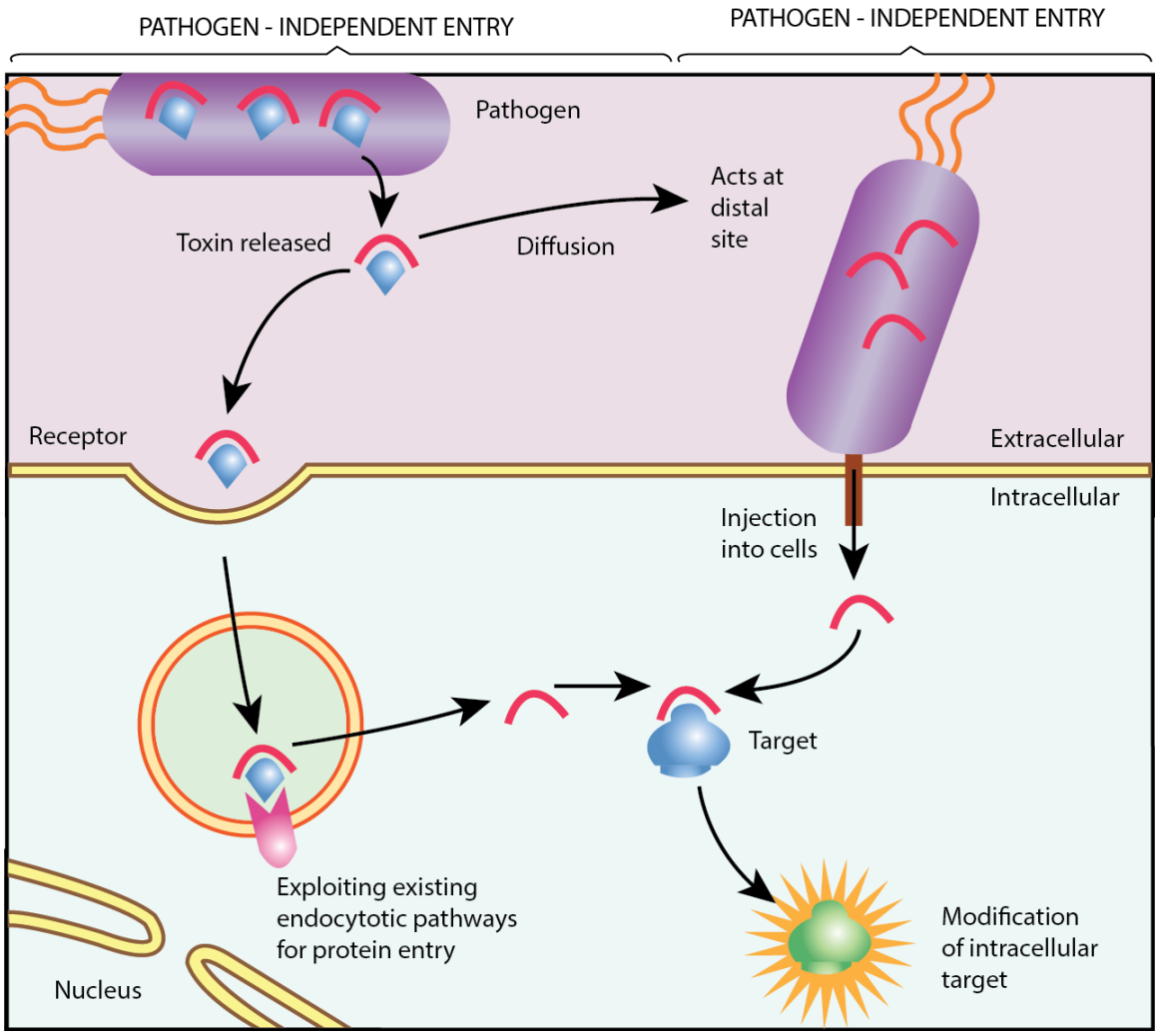

Figure 4: Intracellularly acting bacterial toxins access their substrates within host cells by one of several mechanisms. Some gram-negative pathogens directly inject toxin effectors through flagellar- or pilus-adapted transport machines into eukaryotic cells by Type III or IV secretion systems, respectively. Alternatively, bacteria release intracellular-acting toxins, also called AB toxins, into the host environment where they act locally or diffuse to act distally to the site of colonization. AB toxins commonly exploit endocytic pathways that eukaryotic cells use for importing proteins. Finally, *Bordetella* adenylate cyclise toxins directly enter the cytosol from the plasma membrane. Redraw this

Toxins Exploit the Acidic Environment of Endosomal Compartments

Some AB toxins like diphtheria, anthrax and the botulinum neurotoxins, exploit the drop in pH to between 5.0 and 6.0 as endocytic vesicles are trafficked from the plasma membrane into the cell. This acidification results in the insertion of B fragments into the membrane and the formation of ion-conducting channels. Partially unfolded A fragments use these B fragment-derived channels as conduits into the cytosol.

Toxins Exploit the Sec61 Retro-Translocon in the Endoplasmic Reticulum

The second group of AB toxins includes cholera toxin, shiga toxin, and *Pseudomonas aeruginosa* exotoxin A and exploit the degradation pathway for misfolded proteins. The secretion pathway is at least partially reversible to the fate of nascent proteins for secretion, enabling several bacterial toxins to travel this “retrograde” pathway from the plasma membrane to the ER lumen through pores to the cytosol. Within the lumen of the ER, the A fragments are transported through an existing membrane complex whose primary protein is Sec61. The B fragments of these toxins bind to receptors to facilitate the trafficking of catalytic A fragments to the ER.

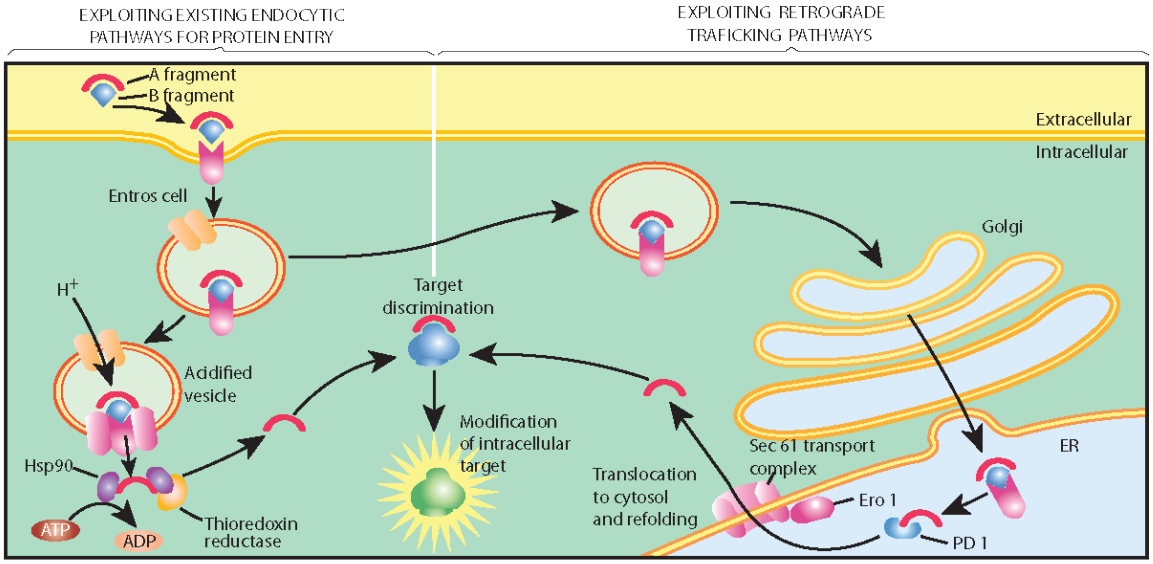

Figure 5: Entry of AB toxins into target cells. Some toxins use host endocytic pathways that cells use for degrading exogenous proteins within lysosomes. Redraw this

Entry of toxin with influx of Ca^{2+}:

In the absence of $Ca^{2+,}$ cells were not sensitive to the toxic proteins like abrin and modeccin and also the sensitivity to ricin and diphtheria toxin was reduced. Calcium deprivation leads to negative effect on the binding and endocytosis of these toxins. Some studies indicate that Ca^{2+} is involved in the entry mechanism for abrin, modeccin, and ricin, possibly as a Ca^{2+} flux together with the toxin.

The plant toxins abrin, modeccin, and ricin consist of two polypeptide chains connected by a disulfide bond. B-chain binds the toxins to cell surface receptors while A-chain enters the cell and inhibits protein synthesis.

Diphtheria toxin is synthesized as one polypeptide chain which is cleaved by proteolytic enzymes into an A- and a B-fragment. “A fragment” possesses enzymatic activity and inactivates components of the protein-synthesizing machinery. Recently it was shown that low pH is required for entry of diphtheria toxin into the cytosol before transport of the A-fragment across the membrane. The low pH induces exposure of a hydrophobic domain in the B fragment that forms ion permeable channels across the membrane and interacts with the membrane lipids and hence facilitates entry of the A-fragment (**Sandvig et al., 1982**).

Interesting facts:

- “A” denotes to the active protein and “B” dentotes to the binding protein in AB toxin.
- Some toxins like diphtheria, anthrax and the botulinum neurotoxins, exploit the drop in pH for its entry as endocytic vesicles are trafficked from the plasma membrane into the cell.
- Some toxins like cholera toxin, shiga toxin, and *Pseudomonas aeruginosa* exotoxin A exploit the sec61 retro-translocon in the endoplasmic reticulum for its entry.
- Calcium deprivation leads to negative effect on the binding and endocytosis of these abrin, modeccin, ricin and diphtheria toxin. The entry mechanism for these toxins depends on Ca^{2+} flux together with the toxin.

Questions:

1. Which of the following is not an A-B exotoxin?
    a. Streptolysin O
    b. Staphylococcus aureus enterotoxin
    c. Cholera toxin
    d. Diphtheria toxin

e. Tetanus toxin

2. All of the following are true of A-B exotoxins except:
   a. The B portion of the toxin binds to surface receptors on host cells.
   b. They consist of two polypeptide components.
   c. They are only produced by gram-negative bacteria.
   d. The A portion of the toxin is the active component.
   e. Many exotoxins are A-B toxins.
3. Which of the following bacterial toxins binds to nerve cells, preventing chemical communication between nerve and muscle cells?
   a. Diphtheria toxin
   b. Erythrogenic toxin
   c. Staphylococcal enterotoxin
   d. E. coli endotoxin
   e. Botulinum toxin
4. Which is true of endotoxins?
   a. They increase blood pressure.
   b. They are produced by gram-positive bacteria.
   c. They are disease-specific.
   d. They are released upon cell lysis.
   e. They are proteins.

5. In a bacterial exotoxin:
   a. The A subunit allows the toxin to bind to the surface of specific host cells.
   b. The A subunit is part of the outer bacterial membrane, released when the bacterialcell dies.
   c. The A subunit is able to interfere with a specific host cell activity, once it has beentaken into the host cell.

d. We expect the A subunit for all exotoxins to operate in the same manner.

6. What effect does botulin toxin have on the body?
   a. It causes excitation of neurons.
   b. It stimulates the removal of fluid from the tissues.
   c. It stimulates increased intestinal motility.
   d. It paralyzes the muscles of the respiratory tract.
   e. It prevents gastrointestinal motility.
7. Where are the target cells of diphtherotoxin located?
   a. The throat
   b. The skin
   c. The skeletal muscles
   d. The lungs
   e. The heart and nervous system
8. What does the A in AB toxin stand for?
   a. Active
   b. Agglutination
   c. Adhesion
   d. Accumulation
   e. None of these is correct.
9. Which bacterium produces the hemolysin streptolysin?
   a. *S. pyogenes*
   b. *S. aureus*
   c. *C. diphteriae*
   d. *C. botulinum*
   e. *C. tetani*
10. Which toxin contains LPS and triggers fever?
    a. Endotoxin
    b. Exotoxin
    c. Both
    d. None

11. CDCs regonize:
    a. Cholesterol as a receptor
    b. Carbohydrates moieties as a receptor
    c. Glycolipids receptor
    d. Glycoproteins
12. How the toxins enter into the cell?
13. What are macromolecular syringes?
14. What are the functions of A- and B-fragments in A/B toxin for the entry of toxin into the cell?
15. What are the two mechanisms that are used by pore forming toxins for the formation of pores in the cell membrane? Explain with an example.

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M4 L1

Cell signalling models and hormones

In this lecture we will study about the various cell signalling models and in the later part about hormones which mediate cell signalling.

Cell Signaling: Secreted molecules mediate via three forms of signaling namely Paracrine, Autocrine, and Endocrine. Signaling molecules that a cell secretes may be carried far afield to act on distant targets, or they may act as local mediators, affecting only cells in the immediate environment of the signaling cell.

1. Autocrine signaling
2. Paracrine signaling
3. Endocrine signaling

1. Autocrine signaling:

In the autocrine signaling, cells respond to substances which they themselves release (as shown in Figure 1(a) and thus changes takes place in the cell itself. A cell secretes a hormone or chemical messenger that stimulates its own growth and proliferation. An example of an autocrine agent is the cytokine interleukin-1 in monocytes. It is produced in response to external stimuli and binds to cell-surface receptors on the same cell that produced it. Autocrine signaling is a characteristic feature of tumor cells, many of which overproduce and release growth factors that stimulate inappropriate, unregulated self-proliferation as well as influencing adjacent non-tumor cells; this process may lead to formation of a tumor mass. In autocrine signaling, a group of identical cells produces a higher concentration of a secreted signal than does a single cell as shown in Figure 1 (b).

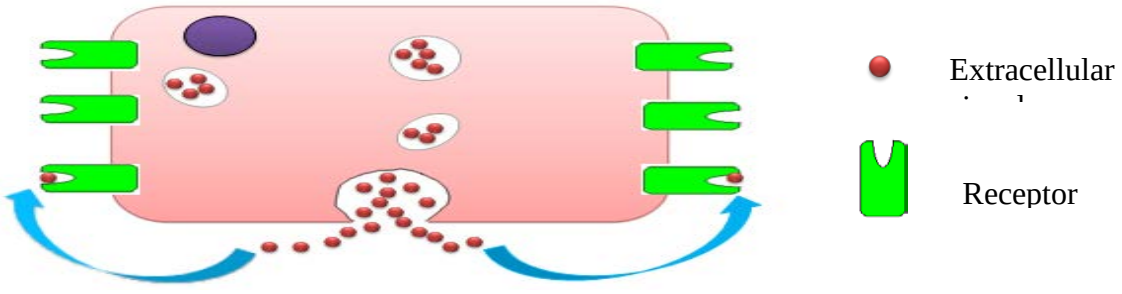
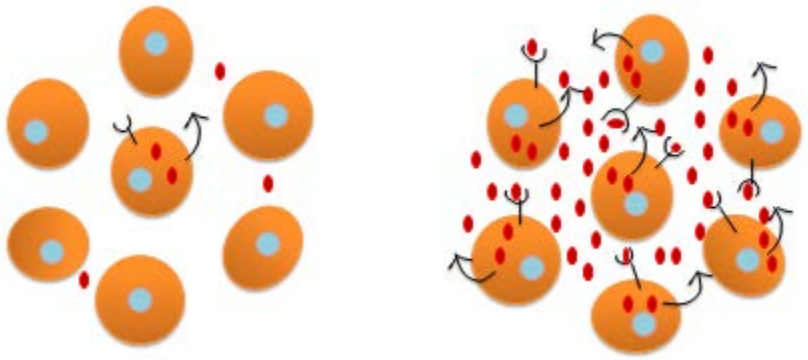


Figure 1 (a): Autocrine signaling: Cells responding to substances which they themselves release.



A single signalling cell receives weak autocrine signal

In a group of identical signalling cells, each cell receives a strong autocrine signal

Figure 1 (b): Autocrine signaling A group of identical cells produces a higher concentration of a secreted signal than does a single cell.

2. Paracrine signaling:

In paracrine signaling (para = near), the signaling molecules released by a signal-releasing cell (secretory cell), affect only those target cells in close proximity as shown in Figure 2 (a) & (b). For paracrine signals to be delivered only to their proper targets, the secreted signaling molecules must not be allowed to diffuse too far; for this reason they are often rapidly taken up by neighboring target cells, destroyed by extracellular enzymes, or immobilized by the extracellular matrix. Many growth factors regulating development in multicellular organisms also act at a short range. Some of these molecules bind tightly to the extracellular matrix, unable to signal, but subsequently can be released in an active form. Many developmentally important signals diffuse away from the signaling cell, forming a concentration gradient and inducing different cellular responses depending on the distance of a particular target cell from the site of signal release. The conduction by a neurotransmitter of a signal from one nerve cell to another or from a nerve cell to a muscle cell occurs via paracrine signaling.

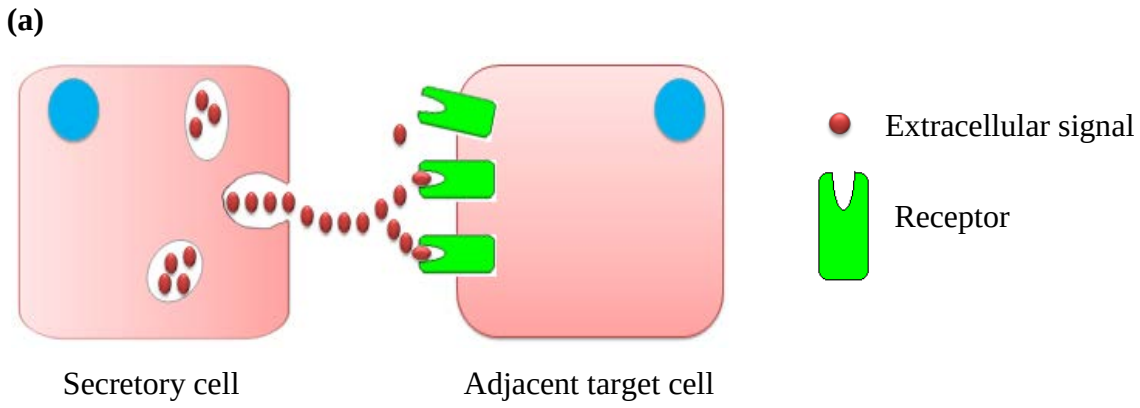


Figure 2 (a): Signaling molecules released by a secretory cell affect only the adjacent target cells.

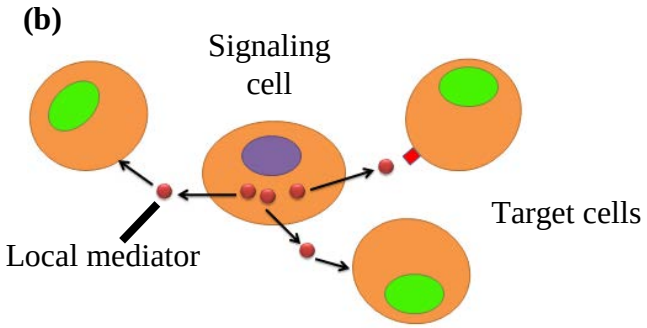


Figure 2 (b): Paracrine signaling: Signaling molecules released by a secretory cell affect only the adjacent target cells in close proximity

3. Endocrine model:

In endocrine signaling, the signaling molecules are synthesized and secreted by endocrine cells, transported through the circulatory system or the tissue fluid of the organism and finally act on target cells distant from their site of synthesis as shown in Figure 3. Endocrine signals are called hormones. The target cells have receptors for binding specific hormones and thereby "pull" the appropriate hormones from the extracellular fluid. Endocrine hormones, for example, insulin and epinephrine, are synthesized and released in the bloodstream by specialized ductless endocrine glands.

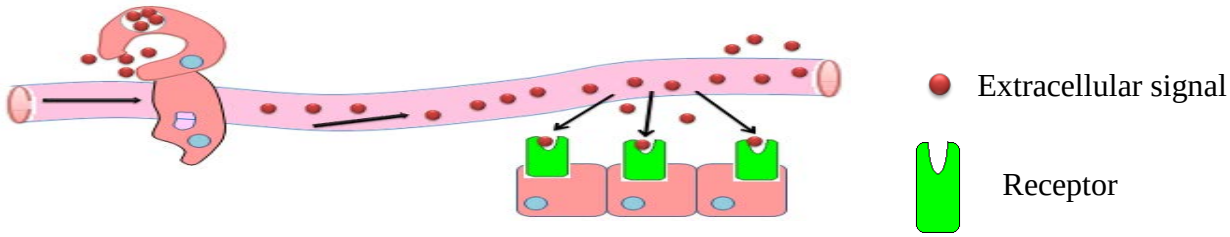


Figure 3: Endocrine signaling: Signaling molecules are synthesized and secreted by endocrine cells, transported through the circulatory system or the tissue fluid of the organism and finally act on target cells distant from their site of synthesis

Hormone: The term hormone is derived from the Greek word ‘horman’ which means ‘to excite’ or ‘to activate’. Hormones are chemical signalling molecules in animals. Endocrine system consists of specialized glands known as endocrine glands which on stimulation secrete powerful chemicals called hormones into the blood stream. The hormones have specific target cells located somewhere else in the body. A **hormone** is a chemical messenger which is released by one or more cells that affects cells in other parts of the organism. It is essentially a chemical that transports a signal from one cell to another. Most hormones initiate a cellular response by initially combining with either a specific intracellular or cell membrane associated receptor protein. A cell may have several different receptors that recognize the same hormone and activate different signal transduction pathways, or alternatively different hormones and their receptors may invoke the same biochemical pathway. For example, insulin is a hormone that is made by the beta cells in the pancreas. When it's released into the blood, insulin helps regulate how the cells of the body use glucose for energy.

Biochemical nature of hormones: Hormones may belong to different biochemical nature. Accordingly, they are classified as:

1. Peptide hormone
2. Steroid hormone
3. Monoamines

1. Peptide hormone: Peptide hormone is a class of peptides which have endocrine functions and is secreted into the blood stream. Peptide hormones are synthesized in cells from amino acids. Numerous significant peptide hormones are secreted from the pituitary gland. Examples of peptide hormones are adrenocorticotrophic hormone (ACTH), growth hormone etc. ACTH is secreted by the anterior pituitary and acts on the adrenal cortex to regulate the secretion of glucocorticoids while growth hormone acts on bone, muscle and the liver.

Peptide hormones cannot pass through the plasma membrane of the cell. So the receptors for these hormones have to be on the plasma membranes of the peptide hormone sensitive cells. When contacted by hormone, these receptors activate a **second messenger** system i.e. a cascade of internal chemical signals that culminate in the secretion of the hormone into the bloodstream. These messengers enter the nucleus and influence gene expression as shown in Figure 4.

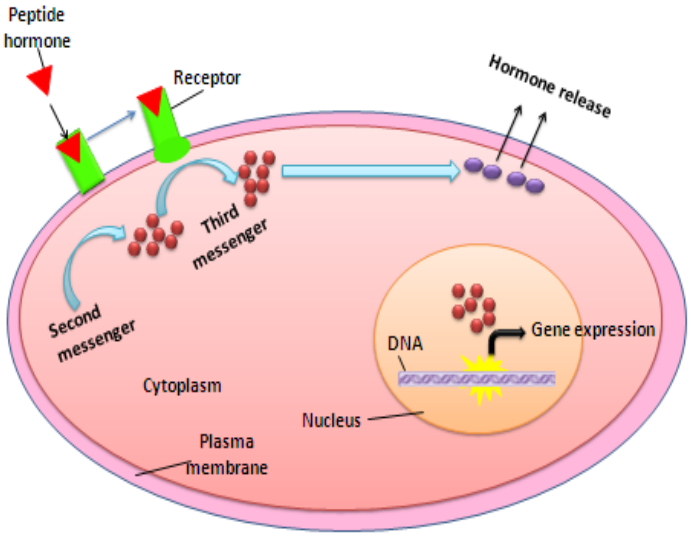


Figure 4: Peptide hormone regulation in vertebrates

2. Steroid hormone: Steroid hormones are derived from cholesterol and eicosanoids. They are lipid soluble. Examples of steroid hormones are testosterone and cortisol. The gonads and the adrenal cortex are the primary sources of steroid hormones. Examples of eicosanoids are prostaglandins.

Steroid hormones being lipid soluble can diffuse through the plasma membrane of the cell very easily. The steroid hormone binds to its receptor in the cytoplasm and the activated hormone-receptor complex enters the nucleus influencing gene expression as shown in Figure 5.

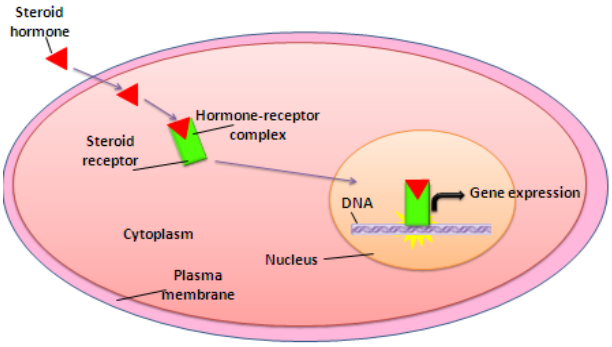


Figure 5: Steroid hormone regulation in vertebrates

3. Monoamines: Monoamines are derived from the aromatic amino acids like tyrosine, tryptophan and phenylalanine by the action of aromatic amino acid decarboxylase enzymes. Examples of monoamines are epinephrine, norepinephrine etc.

Endrocrine system and function: The endocrine system consists of the system of glands. Each gland secretes different types of hormone directly into the bloodstream to regulate the body.

Hormones of the pituitary gland: The pituitary is a small, pea-sized gland situated at the base of the brain. It sends signals to the thyroid and adrenal glands and the ovaries and testes, directing them to produce thyroid hormone, cortisol, estrogen, testosterone, and other hormones. These hormones have an effect on the metabolism, blood pressure, sexuality, reproduction and other essential functions of the body. In humans, the pituitary gland consists of three lobes:

- Anterior Lobe (Adenohypophysis)
- Posterior Lobe (Neurohypophysis)
- Intermediate lobe

1. Anterior Lobe: The anterior lobe contains six types of secretory cells. All of them secrete their hormone in response to hormones reaching them from the hypothalamus of the brain.The anterior pituitary gland secretes the following vital endocrine hormones:

(a) Thyroid stimulating hormone (TSH): Thyroid-stimulating hormone (thyrotropin) is a glycoprotein consisting of an alpha chain of 92 amino acids and a beta chain of 118 amino acids. TSH is synthesized and secreted by thyrotrope cells in the anterior pituitary gland. Thyroid stimulating hormone stimulates the thyroid gland to secrete the hormone thyroxine (T_4). T_4 is converted to triiodothyronine (T_3), which is the active hormone that stimulates metabolism. The TSH receptor is found mainly on thyroid follicular cells. A deficiency of TSH causes hypothyroidism. TSH deficiency has also been implicated as a cause of osteoporosis.

(b) Follicle-Stimulating Hormone (FSH): FSH is synthesized and secreted by gonadotrophs present in the anterior pituitary gland. FSH is a heterodimeric glycoprotein consisting of an alpha chain of 92 amino acids and a beta chain of 118 amino acids. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. Increase in FSH secretion causes ovulation. In sexually-mature males, FSH (assisted by testosterone) acts on spermatogonia stimulating the production of sperm. In sexually-mature females, FSH (assisted by LH) acts on the follicle to stimulate it to release estrogens. In female who has recently undergone menopause has a high level of FSH concentration in the serum.

(c) Luteinizing hormone (LH): Luteinizing hormone is a hormone produced by gonadotroph cells in the anterior pituitary gland. It is also a heterodimeric glycoprotein consisting of 92-amino acid alpha chain and a beta chain of 121 amino acids. In females, an acute rise of luteinizing hormone triggers ovulation and development of the corpus luteum. LH levels are normally low during childhood and high after menopause. In males, LH stimulates the Leydig cell to produce testosterone. Low secretion of LH can result in hypogonadism. These conditions lead to hypothalamic suppression, Kallmann syndrome, Hyperprolactinemia etc.

(d) Prolactin: Prolactin is also known as luteotropic hormone. It is a protein which is made up of 198 amino acids. During pregnancy it helps in the preparation of the breasts for future milk production. Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands of the breasts and prepare for the production of milk. After birth, prolactin promotes the synthesis of milk. Prolactin promotes neurogenesis in maternal and foetal brains. Prolactin has important cell cycle related functions as a growth factor, differentiating factor and anti-apoptotic factor.

(e) Growth Hormone (GH): Growth Hormone is also known as somatotropin. GH is a protein made up of 191 amino acids. The growth hormone-secreting cells are stimulated to produce and release ggrowth hormone by the intermittent arrival of growth hormone releasing hormone from the hypothalamus. Growth hormone stimulates growth, cell reproduction and regeneration in vertebrates. Hyposecretion of growth hormone produces a short body. It can also cause delayed sexual maturity. In adults, deficiency causes pituitary adenoma or other structural lesions or trauma and rarely idiopathic GHD. Hypersecretion of growth hormone leads to gigantism in childhood. In adults, it leads to acromegaly.

(f) Adrenocorticotropic hormone (ACTH): ACTH is also known as corticotrophin. ACTH is a peptide of 39 amino acids. It is an important component of the hypothalamic-pituitary-adrenal axis. ACTH acts on the cells of the adrenal cortex and stimulates them to produce glucocorticoids, mineralocorticoids, androgens and in foetus, dehydroepiandrosterone sulphate. Rapid actions of ACTH include stimulation of delivery of cholesterol to mitochondria where P450scc enzyme (Cholesterol side-chain cleavage

enzyme) is located. Hyposecretion of ACTH in the pituitary leads to hypocorticism, Addison's disease. Hypersecretion of ACTH causes Cushing's disease.

2. Posterior Lobe: The posterior pituitary comprises the posterior lobe of the pituitary gland. It consists mainly of axons extending from the supraoptic and paraventricular nuclei of the hypothalamus. Posterior lobe of the pituitary releases two hormones into the circulation, both synthesized in the hypothalamus.

(a) Vasopressin: Vasopressin is also known as arginine vasopressin, argipressin or antidiuretic hormone. Vasopressin is a peptide made up of 9 amino acids. Vasopressin acts on the collecting ducts of the kidney to facilitate the reabsorption of water into the blood and reduce the volume of urine formed. Deficiency of vasopressin leads to diabetes insipidus, a condition where there is excessive loss of urine and hypernatremia. High levels of vasopressin secretion may lead to hyponatremia.

(b) Oxytocin: Oxytocin is a mammalian hormone that acts mainly as a neuromodulator in the brain. Oxytocin is a peptide of 9 amino acids. Oxytocin is best known for its roles in sexual reproduction, in particular during and after childbirth. It acts on certain smooth muscles stimulating contractions of the uterus at the time of birth and release of milk when the baby begins to suckle.

3. Intermediate lobe: The intermediate lobe is the boundary between the anterior and posterior lobes of the pituitary. It consists of three types of cells - basophils, chromophobes, and colloid-filled cysts.

The intermediate lobe of the pituitary secretes the melanocyte-stimulating hormone. The melanocyte-stimulating hormone stimulates the production and release of melanin by melanocytes present in the skin and hair.

Pheromones:

Pheromones are chemicals that are secreted in our sweat and other bodily fluids which release neurotransmitters that directly modify the behaviour of the opposite sex, such as triggering sexual excitement. The word ‘pheromone’ comes from the Greek word ‘phero’ which means "to bear" and ‘hormone’ which means "impetus". There are alarm pheromones, food trail pheromones, sex pheromones, and many other pheromones affect behaviour or physiology. The first pheromone that was identified in 1956 was a powerful sex attractant for silkworm moths. The least amount of it made male moths beat their wings madly in a flutter dance. The term "pheromone" was introduced by Peter Karlson and Martin Luscher in 1959. Strong pheromones can be a warning signal to predators to stay away or it can be also a signal that the prey animal is indigestible. Certain plants emit alarm pheromones when grazed upon by herbivorous animals resulting in tannin production in neighbouring plants. These tannins make the plants less appetizing.

Growth factors: Growth factors are signaling molecules which bind specifically to the receptor molecule embedded in either the cytoplasm or plasma membrane or nucleus of a cell. The vast majority of receptors are activated by binding to secreted growth factors. Many growth factors, regulating development in multicellular organisms act at short range as in paracrine signaling or act where the cells can respond to substances that they themselves release as in case of autocrine signaling. For example, the insulin receptor binds insulin and related hormones called insulin-like growth factors 1 and 2.

Individual growth factor proteins tend to occur as members of larger families of structurally and evolutionarily related proteins. Few families of growth factors are listed below:

(a) Epidermal growth factor (EGF): Epidermal growth factor is a growth factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. Human EGF is a 6045-Da protein with 53 amino acid residues and three intramolecular disulfide bonds.

(b) Fibroblast growth factor (FGF): Fibroblast growth factors are a family of growth factors involved in angiogenesis, wound healing and embryonic development. The FGFs are heparin-binding proteins and interactions with cell-surface-

associated heparan sulphate proteoglycans are essential for FGF signal transduction. FGFs play an important role in the processes of proliferation and differentiation of wide variety of cells and tissues.

(c) Insulin-like growth factor (IGF): The insulin-like growth factors (IGFs) are proteins with high sequence similarity to insulin. IGFs are part of a complex system that cells use to communicate with their physiological environment. This complex system consists of two cell-surface receptors (IGF1R and IGF2R), two ligands (IGF-1 and IGF-2), a family of six high-affinity IGF-binding proteins (IGFBP-1 to IGFBP-6), as well as associated IGFBP degrading enzymes, referred to collectively as proteases.

Role of growth factors in medicine:

Growth factors have been increasingly used in the treatment of hematologic and oncologic diseases and cardiovascular diseases like leukemias, angiogenesis for cardiovascular diseases, aplastic anaemia neutropenia, myelodysplastic syndrome (MDS) and bone marrow transplantation.

Keywords: Hormone, autocrine, paracrine, endocrine, pheromones, growth factors

Interesting facts:

- The first hormone to be discovered is “Secretin”.
- William Bayliss (1860-1924) and Ernest Starling (1866-1927) discovered secretin in 1902.
- Glutamate is a positive autocrine signal for glucagon release.

Questions:

1. Based on the biochemical nature, how are hormones classified?
2. Describe the various types of endocrine glands.
3. What are pheromones?
4. What are growth factors?
5. Classify cell signaling in animals with example.

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Module 4 Lecture 2

Plant growth factors

Plants need certain optimum conditions for its growth and development which are summed up as plant growth factors. These are specific requirements of plant cells. The plant growth factors can be studied under:

- Nutritional plant growth factors
- Environmental plant growth factors

Nutritional plant growth factors: Plants require adequate amount of nutrition for their better growth. The basic nutrients required for plant growth are:

(1) Macronutrients

(2) Micronutrients

(3) Water

Macronutrients: The nutrients which are required by the plants in large quantities are termed as macronutrients. There are six elements in the soil which fall into this category. These are nitrogen, potassium, magnesium, calcium, phosphorus and sulfur.

a) Nitrogen: Nitrogen is essential for growth as it is an important part of all proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy. It is an essential part of chlorophyll involved in photosynthesis. Nitrogen improves the quality of the leaf and the foliage crops and also increases seed and fruit production; thus helping in rapid plant growth.

b) Potassium: Potassium activates enzymes necessary for starch synthesis, photosynthesis, energy metabolism, nitrate reduction and sugar degradation in plants. Potassium plays a vital role in reducing water loss from leaves and increases the ability of the roots to take up water from the soil. Potassium improves winter hardiness, drought tolerance, reduction to diseases.

c) Magnesium: Magnesium is essential for photosynthesis as it is an essential part of chlorophyll in all green plants. It also activates many plant enzymes essential for growth.

d) Calcium: Calcium is an essential part of the structure of cell wall in plants, providing normal transport and retention of other elements and strength in the plants. It also counteracts the effect of organic acids and alkali salts within plants.

e) Phosphorus: Phosphorus is essential for photosynthesis, plant maturation, withstanding stress, rapid growth of roots and encourages blooming. It is also involved in the formation of starches, sugars, oils etc.

f) Sulfur: Sulfur improves growth of roots, seed production and makes the plant resistant to cold. It is essential for protein production in plants. Sulfur also promotes activity and development of vitamins and enzymes and helps in chlorophyll formation.

Micronutrients:

The nutrients that are required by the plants in smaller quantities are termed as micronutrients. There are eight elements which are termed as micronutrients. These eight micronutrients include iron, zinc, molybdenum, manganese, boron, copper, cobalt and chlorine.

Iron: Iron is essential for the production of chlorophyll. Iron is a fundamental component of many enzymes associated with nitrogen reduction and fixation, energy transfer and lignin formation. Iron is associated with sulfur in plants to form compounds that catalyze other reactions. Lipoxygenases is a very common example of iron containg enzyme involved in deoxygenation of polyunsaturated fatty acids in plant.

Zinc: Zinc is an important component of various enzyme systems for protein synthesis, energy production and growth regulation. Zinc is essential for the transformation of carbohydrates and regulates the consumption of sugars. Zinc is essential for proper mobility of solutes and essential nutrients in plants. Few common examples of Zn containing enzymes are alcohol dehydrogenase, Cu-*Zn* superoxide dismutase, carbonic anhydrase, and RNA polymerase.

Molybdenum: Molybdenum is an essential component of the enzymes relating to nitrogen fixation by bacteria. Molybdenum is involved in protein synthesis, nitrogen metabolism and sulfur metabolism. Molybdenum plays a significant role in pollen formation.

Manganese: Manganese has a significant role to play in nitrogen metabolism, photosynthesis and other plant metabolisms. Manganese functions with the enzyme systems involved in carbohydrates breakdown.

Boron: Boron is essential for cell wall formation, production of sugar and carbohydrates. Boron helps in seed, fruit and grain development.

Copper: Copper is essential for reproductive growth. Copper is essential for the utilization of proteins and helps in root metabolism. It is essential for the metabolism of carbohydrate and nitrogen metabolism and is required for lignin synthesis needed for the strength of cell wall.

Cobalt: Cobalt is essential for nitrogen fixation and plays a vital role in protein synthesis.

Chlorine: Chlorine plays an important role in plant metabolism. It is essential for stomatal opening and electrical charge balance in physiological functions in plants. Further, it restricts wilting.

Water: Water is one of the most essential factors required for the growth of plants. A majority of growing plants contain as much as 90% water. Water plays a crucial role for efficient photosynthesis, respiration, transpiration and transportation of minerals and other nutrients through the plant. Water is responsible for functioning of opening of stomata in leaves and is also the source of pressure for the directed growth of roots through the soil. The role of water in plant growth is emphatically summarized in Table 1.

Table 1. Role of water in plant growth vs reduced water supply to plants

Role of water in plants	**Effect of reduced water supply to plants**
Primary component of photosynthesis and transpiration	Reduced plant growth and vigour
Turgor pressure	Wilting
Solvent to move minerals from the soil upto the plant NO_3^-, NH_4^+, $H_2PO_4^-$, HPO_4^{2-}, K^+, Ca^{2+}, Mg^{2+}, HSO_4^{2-}, $H_2BO_3^-$, Cl^-, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, MoO_4^{2-}	Reduced plant growth and vigour Nutrient deficiencies
Solvent to move products of photosynthesis throughout the plant, including down to the root system	Reduced health of roots which leads to reduced health of plant
Regulation of stomatal opening and closure, thus regulating transpiration and photosynthesis	Reduced plant growth and vigour Reduced cooling effect leading to warmer micro climate temperatures
Source of pressure to move roots through soil	Reduced root growth leading to reduced plant growth and vigour
Medium for biochemical reactions	Reduced plant growth and vigour

Environmental plant growth factors:

There are various environmental factors which has a significant role to play in enhancing the growth of plants. The environmental plant growth factors include:

(1) Light
(2) Temperature
(3) Relative humidity
(4) Carbon dioxide and Oxygen
(5) Soil

(1) Light: Light quality refers to the color or wavelength reaching the plant's surface. Adequate light is one of the most important factors that influence the growth of plants and it is the quality, quantity and duration of light exposure which matters. Various natural and artificial light sources can be used to provide light to the plants. Red and blue light have the greatest impact on plant growth whereas green light is least effective. Blue light is essential for the vegetative growth of the leaves of plants whereas combination of red and blue light promotes flowering in plants. There are certain plants which require less light for their growth. In such cases, light can be filtered using protective shelters so that the plants are exposed to minimum required amount of sunlight. The more sunlight a plant receives, the higher will be its photosynthetic rate. However, leaves of plants growing in low light readily excoriate when moved to a bright location having intense sunlight. Over time, as the wax content on a leaf increases and the plant grows it becomes more sun tolerant. When starting indoor transplants, generally plants are given 12-14 hours of light per day. Plants are generally intolerant to continuous light for 24 hours.

Light quality is a major consideration for indoor growing of the plants.

- Fluorescent cool white lamps are high in the blue range and are used for starting seeds germination.
- For flowering plants that need more red light, broad spectrum fluorescent bulbs are used.
- Incandescent lights are high in red and red-orange, but generally it produces too much heat for use in supplementing plant growth. Figure 1 shows the graph of relative efficiency of various colors of light in photosynthesis.

Figure 1. Relative efficiency of various colours of light in photosynthesis.
(X axis: Various colours of light; Y axis: Relative efficiency of flowering plant)

Photoperiod: The flowering response of many plants is controlled by the photoperiod which is the length of uninterrupted darkness. The plant's reproductive cycle is timed to the amount of light available. Photoperiod response can be divided into three types.

(a) Short day plants (SDP): SDPs flower in response to long periods of night darkness as shown in Figure 2. *Short-day plants flower when day lengths are less than the critical photoperiod.* Some short-day obligate plants are: Chrysanthemum, Poinsettia, Strawberry, Coffee, Tobacco, Common duckweed (*Lemna minor*), Cocklebur (*Xanthium*), Maize (tropical cultivars only). Some examples of short-day facultative plants are: Sugar cane, Rice, Cotton (*Gossypium*), Hemp (*Cannabis*).

(b) Long day plants (LDP): LDPs flower in response to short periods of night darkness as shown in Figure 2. *Long-day plants flower when day lengths exceed the critical photoperiod.* Examples include onions and spinach. Some examples of long-day obligate plants are: Carnation (*Dianthus*), Oat (*Avena*), Clover (*Trifolium*), Henbane (*Hyoscyamus*), Ryegrass (*Lolium*), Bellflower (*Campanula carpatica*). Some long-day facultative plants are: Pea (*Pisum sativum*), Lettuce (*Lactuca sativa*), Wheat (*Triticum aestivum*, spring wheat cultivars), Barley (*Hordeum vulgare*), Turnip (*Brassica rapa*), *Arabidopsis thaliana*.

(c) Day neutral plants (DNP): DNP flower without regard to the length of the night but typically flower earlier and more profusely under long daylight regimes. Day neutral strawberries provide summer long harvesting (except during heat extremes).

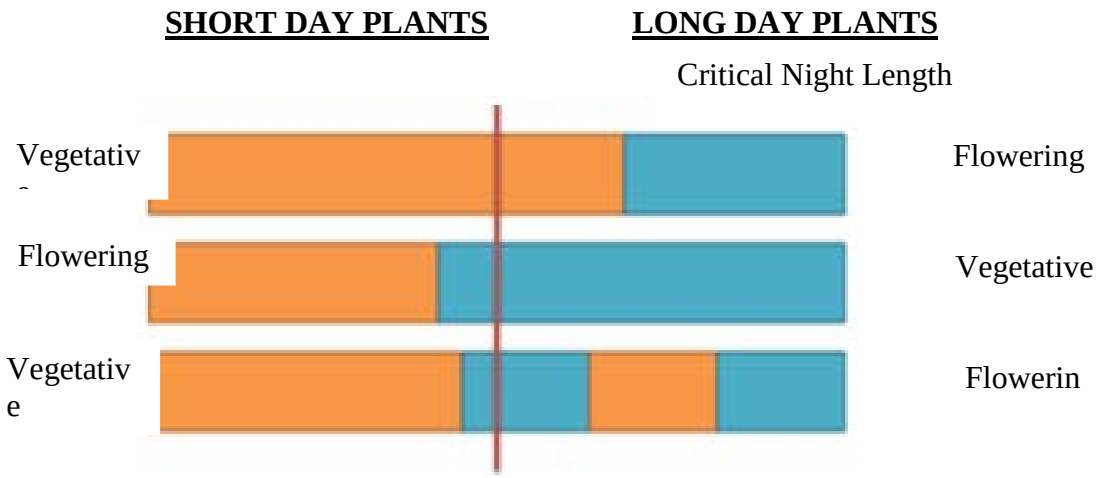

Figure 2: Photoperiod and Flowering
Short day plants flower with uninterrupted long nights.
Long day plants flower with short nights or interrupted long nights
(Left side: Short day plants flower with uninterrupted long nights.
Right side: Long-day plants flower with short nights or interrupted long nights.)

(2) Temperature: Temperature is a crucial factor that has an influence on the growth of plants. Temperature of the surrounding atmosphere and the temperature of the soil play an important role. For many of the plant processes like photosynthesis, respiration, germination and flowering, optimum temperature is one of the pre-requisites. Usually cold-season plants have 12.7 – 18.3 °C as the optimum temperature for germination whereas warm-season plants germinate at 18.3 – 23.8 °C. The **Temperature Comparison between Cool Season and Warm Season Vegetables is as shown in Table 2.** The temperature range for optimum photosynthesis and respiration vary with individual requirements of the plants and also among the species of plants.

Table 2 Temperature Comparison between Cool Season and Warm Season Vegetables

	Cool Season: broccoli, cabbage, and cauliflower	**Warm Season:** tomato, pepper, squash and melons
Germination	4.4 °C – 32.2 °C 26.6 °C optimum	10°C -37.7 °C 26.6 °C optimum
Growth	**Daytime** 18.3 °C – 26.6 °C preferred 4.4 °C minimum **Night time** greater than 0 °C for tender transplants down to mid -6.7 °C for established plants	**Daytime** 30 °C optimum 15.5 °C minimum Below 12.8 °C will stunt plant, reducing yields. **Night time** above 0 °C
Flowering	Temperature extremes lead to bolting and buttoning.	**Daytime**: Greater than 35 °C by 10 a.m., blossoms abort. **Night time**: Below 12.8 °C, non- viable pollen (use blossom set hormones).
Soil	**Winter** Use organic mulch to cool soil. Since seeds germinate best in warm soils, use transplants for spring planting, and direct seeding for mid-summer plantings (fall harvest).	**Summer** Use black plastic mulch to warm soil, increasing yields and earliness of crop

(3) Relative humidity: Moisture is defined as the ratio of water vapour in the air to the amount of water in the air. It is a very important factor in growth of plants. The relative humidity in the air is used by the plants and is very crucial for the transpiration of the plants. Transpiration is highest during hot, dry and windy days while transpiration slows down during cool and humid days. Water moves from areas of high relative humidity to areas of lower relative humidity. Inside a leaf, the relative humidity between cells approaches 100%. When the stomata opens, water vapors inside the leaf rush out forming a bubble of higher humidity around the stomata on the outside of the leaf and carbon dioxide move into the leaf through the stomata as shown in Figure 3. The difference in relative humidity around the stomata and adjacent air regulates transpiration rates and pulls water up through the xylem tissues. When the supply of water from the roots is inadequate, the stomata closes, photosynthesis shuts down, and plants can wilt.

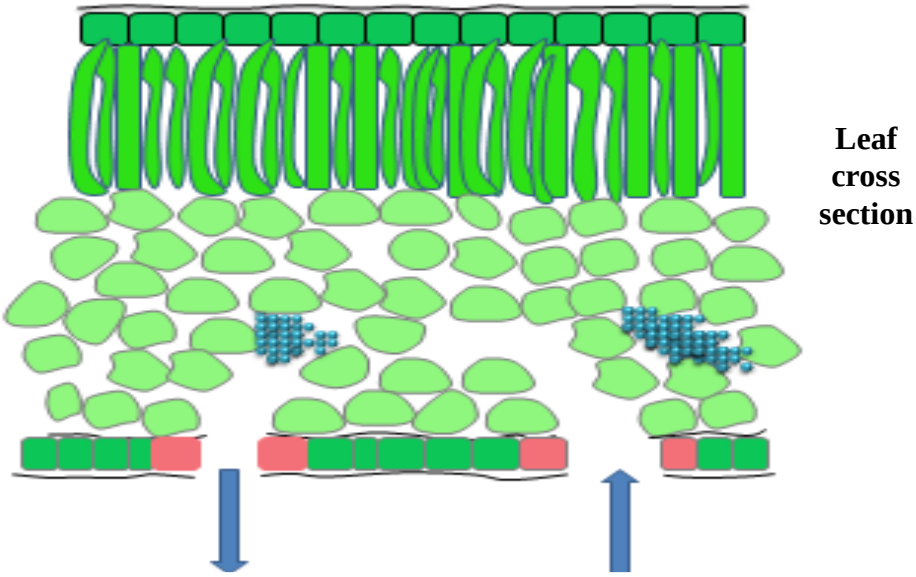

Figure 3: Water vapor moves out and carbon dioxide move into the leaf through the stomata

(4) Carbon dioxide and oxygen: Carbon dioxide is one of the vital elements for plant growth. The manufacturing of sugar by plants requires the presence of carbon dioxide. Plant growth requires a tremendous amount of carbon dioxide. Plants can use as much as 1500 ppm of carbon dioxide. When all other growth influencing factors are kept in their ideal ranges, CO_2 becomes the limiting factor. This means as carbon dioxide is increased, growth rates and yields also increases. Oxygen is essential for plant respiration and utilization of photosynthesis byproducts. No nutrient absorption occurs at the root zone unless oxygen is present. At a molecular level, oxygen is required to transmit nutrients across the cell wall and into the roots. As oxygen levels are increased at the root zone, nutrient absorption continues to increase as well. Plants produce oxygen gas during photosynthesis to produce glucose but then require oxygen to undergo aerobic cellular respiration and break down this glucose to produce ATP. When oxygen is absent, hypoxia occurs which affect nutrient uptake of a plant and inhibits respiration within the root cells.

(5) Soil: Soil is the outermost layer of the surface of the earth in which plants grow. Soil with proper humidity and the right balance of all the minerals and nutrients is one of the essential factors instrumental in plant growth. The type of soil and the quality and the nutrients required in it vary according to the plant species. The right pH balance, which measures the alkalinity or acidity of the soil and presence of certain chemicals, is also instrumental in the growth of plants. pH influences availability of certain nutrients. For most plant life, the most favorable pH value is between 6 - 6.8. The soil serves the needs of the plant by providing: water, air, nutrients and stability. The ability of a soil to provide these services may be evaluated by key soil attributes as shown in Table 3)

Table 3: Soil attributes relevant to plants

Key soil attribute	Relevance to plants
Wetness	Water supply, exclusion of air and, consequently, exclusion of oxygen
Root barrier	Controls the depth of soil available for roots to extract water and nutrients, and to anchor the plant
Stoniness	Stones and rocks dilute the volume of soil within the root depth that is available for water storage and nutrients
Porosity	Promotes stability by allowing deep rooting. Drains excess water, and circulates air to roots
Natural nutrient status	Controls nutrient supply and reserves
Drought proneness	An interaction between climate and soil attribute

Interesting Facts:

- Photoperiodism is responsible for the geographical distribution of many plants worldwide.
- Bulbing in onions is primarily controlled by photoperiod - day length - as the days become longer, plants begin to bulb.

Questions:

1. What are plant growth factors? How can it be classified?
2. What are macronutrients? Name them.
3. What are micronutrients? Name them.
4. What are environmental plant growth factors? Give example.
5. What are short day plants? Give two examples of short day plants.
6. What are long day plants? Give two examples of long day plants.
7. What are day neutral plants? Give two examples of day neutral plants.
8. _____ flower in response to short periods of night darkness.
   - **(a)** Short day plants
   - **(b)** Long day plants
   - **(c)** Day neutral plants
9. What are nutritional plant growth factors? Classify each of them in details.
10. What are macronutrients? Describe the function of each of the macronutrients.
11. What are micronutrients? Describe the function of each of the micronutrients.
12. Describe the role of water in plant growth.
13. What are environmental plant growth factors? Describe each factor in details.
14. Describe how the effect of light can contribute to the growth of plants.

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Module 4 Lecture 3
Plant hormones

We have studied animal hormones in previous lectures. Plants also have analogous signaling molecules, called phytohormones. These are a group of naturally occurring, organic substances which influence the physiological processes at low concentrations. Plant hormones are physiological intercellular messengers which are needed to control the complete plant lifecycle, including growth, germination, rooting, fruit ripening, flowering, foliage and death. They are secreted in response to environmental factors such as light, temperature, abundance of nutrients, drought conditions, chemical or physical stress. Levels of hormones change over the lifespan of a plant and they are also dependent upon environment and seasons.

Every aspect of plant growth and development is under hormonal control to some degree. A single hormone can regulate a diverse array of cellular and developmental processes, while at the same time multiple hormones often influence a single process. Right combination of hormones is vital to achieve the desired behavioral characteristics of cells and the productive development of plants as a whole. The application of growth factors allows synchronization of plant development to occur. For example, ripening bananas can be regulated by using desired atmospheric ethylene levels. Other applications include rooting of seedlings or the suppression of rooting with the simultaneous promotion of cell division as required by plant biotechnologists.

Five major classes of plant hormones are known in plants. With progressing research, more active molecules are being found and new families of regulators are emerging, one example being polyamines such as putrescine or spermidine.

(1) Auxin
(2) Gibberellin
(3) Cytokinin
(4) Abscisic acid
(5) Ethylene

(1) Auxin:

The term auxin is derived from the Greek word ‘auxein’ which means to grow. They are a class of plant hormones which has a cardinal role in coordination of many growth and behavioral processes in the plant's life cycle essential for development of plant. Auxin is the first plant hormone to be identified. They have the ability to induce cell elongation in stems and resemble indoleacetic acid (the first auxin to be isolated) in physiological activity.

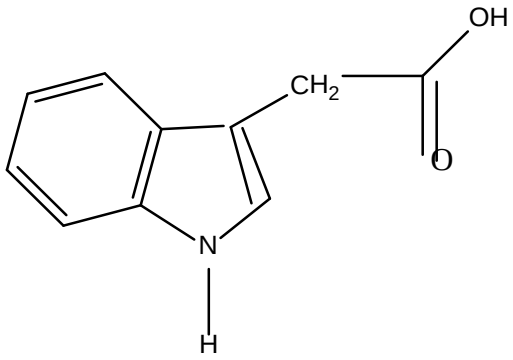


Figure 1: Auxin

Indole-3-acetic acid (IAA) is the main auxin in most plants. IAA is transported is cell to cell.

Discovery of auxin: Darwin (1881) was the first person who discovered the existence of auxin in plants, the first phytohormone known. He noted that the first leaf (coleoptile) of canary grass (*Phalaris canariensis*) was very sensitive and responsive to light and he demonstrated the bending of the grass coleoptiles towards unilateral source of light. This bending occurred only when the coleoptile was also illuminated. When the tip of the coleoptiles was covered with a black cap, it resulted in loss of sensitivity of the plant towards the light as shown in Figure 2. Darwin concluded that some influence that causes curvature is transmitted from the coleoptile tip to the rest of the shoot. Boysen – Jensen (1913) also made similar observations on oat (*Avena*) coleoptiles as shown in Figure 2. Paal (1918) demonstrated that when the decapitated coleoptiles tip was replaced on the cut end eccentrically, more growth resulted on the side which causes bending even when this is done in complete darkness.

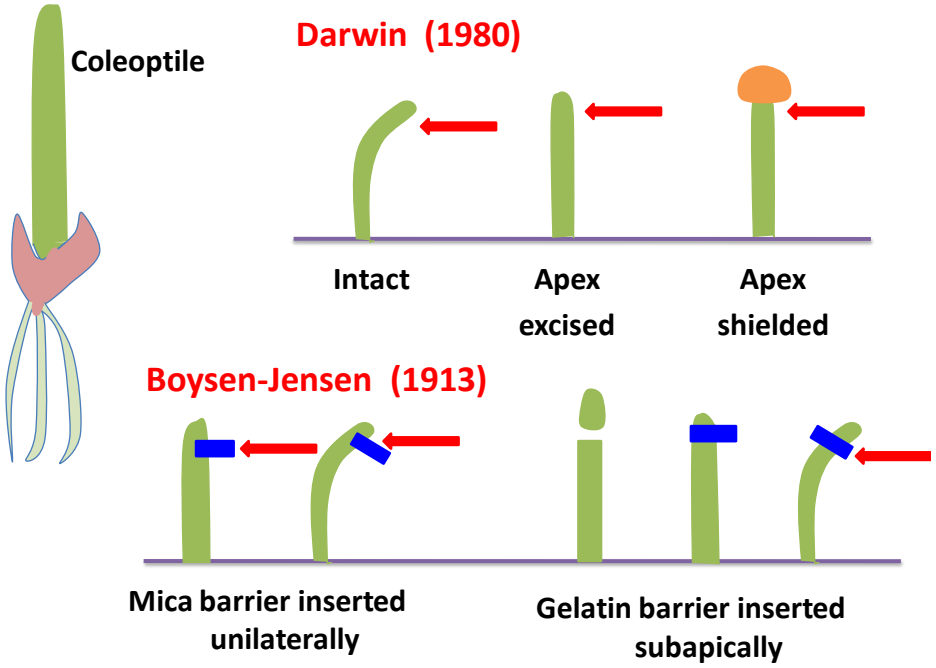


Figure 2: Discovery of auxin: phototropic response of grass seedlings

Sites of biosynthesis of auxin: IAA is synthesized primarily in actively growing tissue in leaf primordia and young leaves, fruits, shoot apex and in developing seeds. It is made in the cytosol of cells.

Tryptophan-dependent Pathways for auxin synthesis: Tryptophan, one of the protein amino acids, is the precursor of auxin biosynthesis. The conversion of tryptophan to Indole Acetic Acid can occur by either transamination followed by a decarboxylation or decarboxylation followed by a transamination. Formation of IAA via an oxime (C=NOH) and nitrile (CN) is shown in Figure 3.

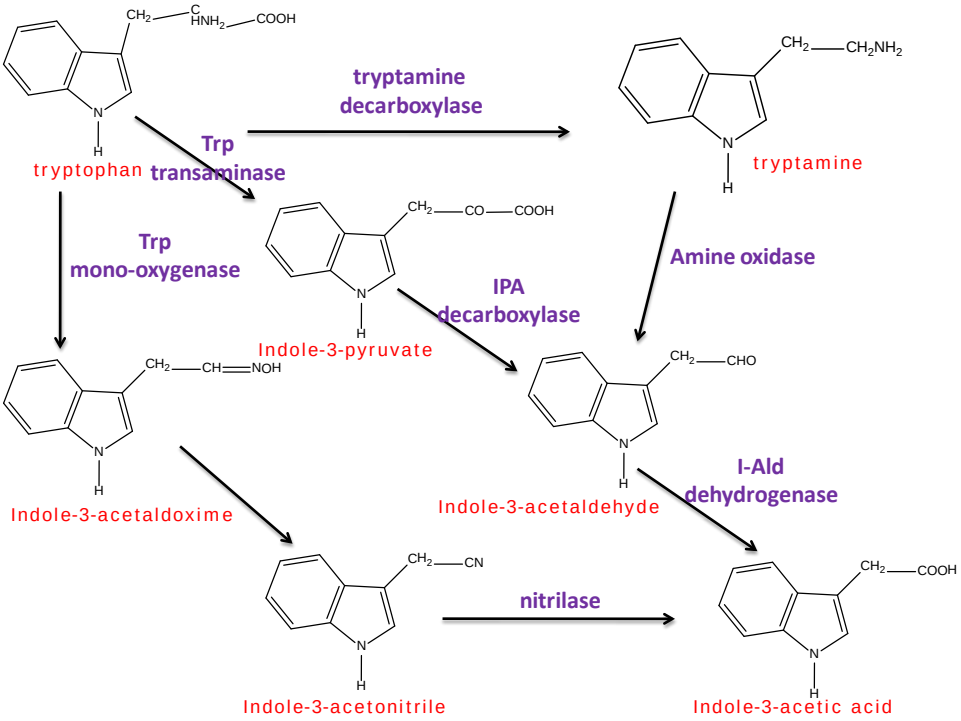


Figure 3: Biosynthesis of auxin from Tryptophan

Classification of auxins: Auxins are classified into two types based on its occurrence, if they occur naturally or are synthesized artificially.

1. Natural auxins

2. Synthetic auxins

Natural auxins: The four naturally occurring (endogenous) auxins are Indole-3-acetic acid, 4-chloroindole-3-acetic acid, phenylacetic acid and indole-3-butyric acid; only these four are synthesized by plants.

Synthetic auxins: Synthetic auxin analogs include 1-naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D) and many others. Some synthetic auxins, such as 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), are used also as herbicides. Broad-leaf plants (dicots), such as dandelions, are much more susceptible to auxins than narrow-leaf plants (monocots) such as grasses and cereal crops, so these synthetic auxins are useful as synthetic herbicides.

Auxin signaling: Auxin binds to a receptor with ubiquitin ligase activity. This stimulates ubiquitination and degradation of a specific transcriptional repressor further leading to transcription of auxin-induced genes as shown in Figure 4.

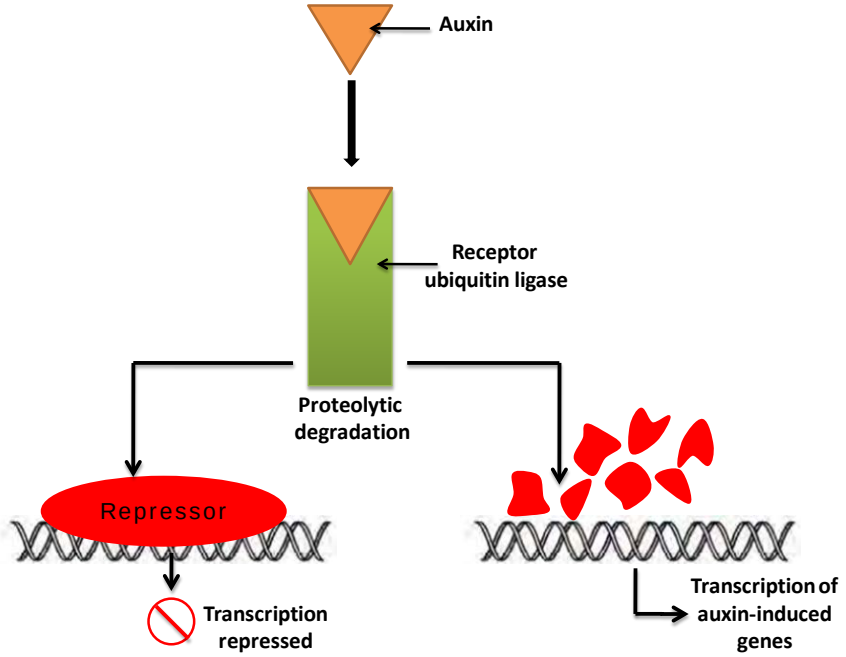


Figure 4: Auxin signaling

Functions of Auxin: Indole acetic acid regulates many responses: Cell elongation and wall relaxation and cell differentiation. It promotes differentiation of vascular tissue (i.e., xylem & phloem). IAA apparently stimulates the production of ethylene. IAA at more than 10^{-6} M concentration inhibits root elongation. However, very low concentration (>10^{-8} M) favor root elongation. It stimulate root initiation both lateral roots and adventitious roots. Most plants do not initiate the production of flowers after auxin treatment except pineapple and its relatives belonging to Bromeliaceae. Once flowers are initiated, in many species, IAA promotes the formation of female flowers, especially in cucurbits (gourd family). Parthenocarpic fruit development is regulated by auxins. The apical meristem (apex) controls or dominates the development of the lateral buds. Apical dominance also occurs in roots. Auxin mediates the tropistic (bending) response of shoots and roots to gravity and light. It delays leaf senescence. Auxin may inhibit or promote (via ethylene) leaf and fruit abscission.

(2) Gibberlin:

Gibberellins (GAs) are a group of diterpenoid acids that function as plant growth regulators inflencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence.

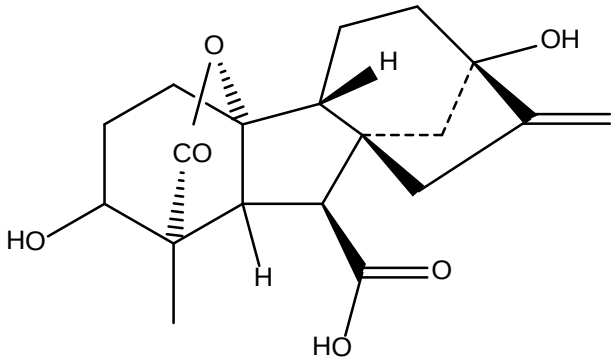


Figure 5: Gibberellin

Discovery of Gibberellin: Kurusawa, a Japanese plant pathologist, discovered gibberellin in 1926. When he was working in the rice fields, he observed that some of the rice seedlings grew much taller than the others which were found to be infected by a fungus, *Gibberella fujikuroi*. Yabuta and Yabuta and Sumiki (1938) demonstrated that some substances secreted by the fungus were probably responsible for more elongation (growth) of the seedlings. Till date, about 62 different gibberellins are known out of which 25 have been isolated from the fungus *Gibberella fujikuroi.*

Chemical structure of Gibberellins:

All gibberellins are derived from the ent-gibberellane skeleton. During the synthesis of gibberellins, the central 6-member ring is reduced to 5 carbons to make the basic gibberellin. The carbons are numbered 1 through 20. Gibberellins are diterpenes synthesized from acetyl CoA via the mevalonic acid pathway. They all have either 19 or 20 carbon units grouped into either four or five ring systems. The fifth ring is a lactone ring. They have been depicted in Figure 7.

Biosynthesis: In the formation of gibberellins 3 acetyl CoA molecules are oxidized by 2 NADPH molecules to produce 3 CoA molecules as a side product and mevalonic acid. Further Mevalonic acid is then phosphorylated by ATP and decarboxylated to form isopentyl pyrophosphate. 4 of these molecules form geranylgeranyl pyrophosphate which serves as the donor for all GA carbon atoms. This compound is then converted to copalylpyrophosphate which has 2 ring systems. Copalylpyrophosphate is then converted to kaurene which has 4 ring systems.

Subsequent oxidations reveal kaurenol (alcohol form), kaurenal (aldehyde form), and kaurenoic acid respectively. Kaurenoic acid is converted to the aldehyde form of GA_{12} by decarboxylation. GA_{12} is the 1st true gibberellane ring system with 20 carbons. From the aldehyde form of GA_{12} arise both 20 and 19 carbon gibberellins but there are many mechanisms by which these other compounds arise. Transport of Gibberellin in plants is non-polar. It moves from one part to another in the phloem. Due to the lateral movements between the two vascular bundles, gibberellins are translocated in the xylem.

Functions of Gibberellin: Gibberellins are involved in stem elongation. Many seedlings (eg. radish, lettuce, tomatoes etc.) when grown in petri dishes containing GA_3 solution, show elongation of hypocotyl. GA1 also causes hyperelongation of stems by stimulating both cell division and cell elongation. GAs cause stem elongation in response to long days GAs can cause seed germination in some seeds that normally require cold (stratification) or light to induce germination as shown in Figure 7. Barley is one such example. Gibberellins are known to stimulate the de-novo synthesis of numerous hydrolases, notably α-amylase in the aleurone cells that surround the starchy endosperm in barley. In seed germination in lettuce, the main signal stimulating gene expression of amylase and other germination-initiating enzymes is light. Thus the photoactivation is achieved by phytochrome in its Pfr form. GA stimulates the production of numerous enzymes, notably a-amylase, in germinating cereal grains.

3. Cytokinin:

Cytokinins are a class of phytohormones with a structure resembling adenine which promote cell division and have other similar functions to kinetin. This hormone is termed as "cytokinin" because they stimulate cell division (cytokinesis). Cytokinins promote cell division or cytokinesis, in plant roots and shoots. They are involved primarily in cell growth and differentiation, but also affect axillary bud growth, apical dominance and leaf senescence. Kinetin was the first cytokinin to be discovered and it is so named because of the compounds' ability to promote cytokinesis (cell division). Though it is a natural compound, it is not made in plants and therefore it is usually considered a "synthetic" cytokinin. The most common form of naturally occurring cytokinin in plants today is called zeatin which was isolated from corn (*Zea mays*). Cambium and other actively dividing tissues also synthesize cytokinins. Approximately 40 different structures of cytokinin are known. Other naturally occurring cytokinins include dihydrozeatin (DHZ) and isopentenyladenosine (IPA). Cytokinin concentrations are highest in meristematic regions and areas of continuous growth potential such as roots, young leaves, developing fruits, and seeds. Cytokinins have been found in almost all higher plants as well as mosses, fungi, bacteria and also in tRNA of many prokaryotic and eukaryotic organisms. Today there are more than 200 natural and synthetic cytokinins combined.

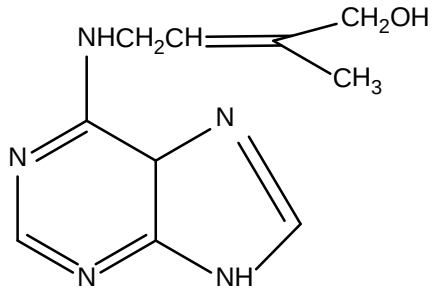


Figure 6: Cytokinin

Chemical nature of cytokinin:

Chemical nature of cytokinins is based on two types:

1. Adenine-type cytokinins
2. Phenylurea-type cytokinins

Adenine-type: These cytokinins are represented by kinetin, zeatin and 6-benzylaminopurine. Majority of the adenine-type cytokinins are synthesized in the roots. Cytokinin biosynthesis also takes place in the cambium and other actively dividing tissues.

Phenylurea-type: These cytokinins are represented by diphenylurea and thidiazuron (TDZ). Till now there is no evidence that the phenylurea cytokinins occur naturally in plant tissues.

Cytokinins are involved in both local and long distance signaling. Cytokinins are transported within the xylem.

Classes of cytokinin:

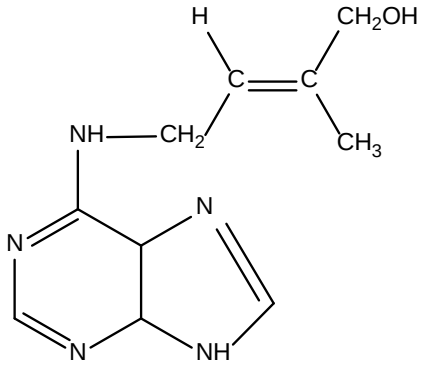
There are two classes of cytokinin hormone**:** They are as follows:

(i) Natural cytokinin

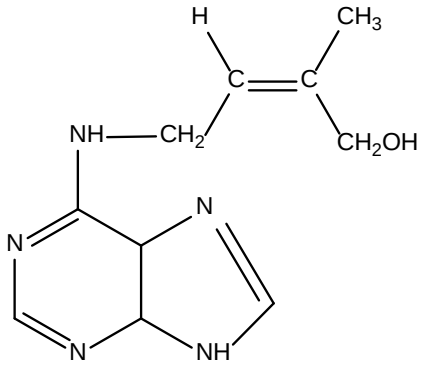
(ii) Synthetic cytokinins

(i) Natural Cytokinin:

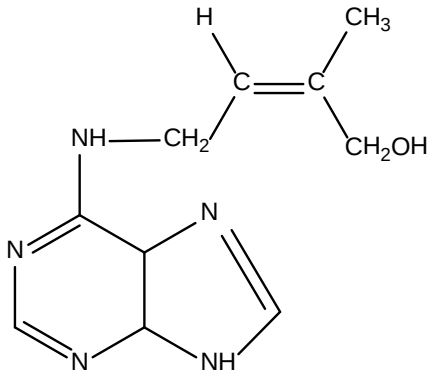
The most common form of naturally occurring cytokinin in plants today is called zeatin which was isolated from corn (*Zea mays*). Approximately 40 different structures of cytokinin are known. Other naturally occurring cytokinins include dihydrozeatin (DHZ) and isopentenyladenosine (IPA). Figure 7depicts some cytokinins.



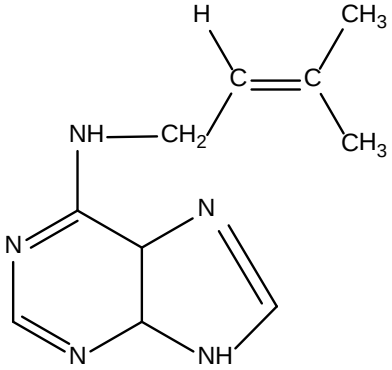
Trans-zeatin



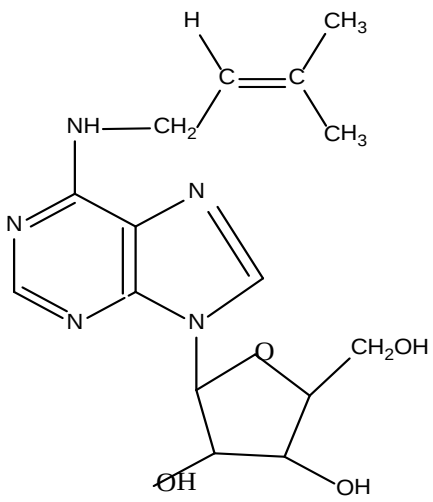
Cis-zeatin



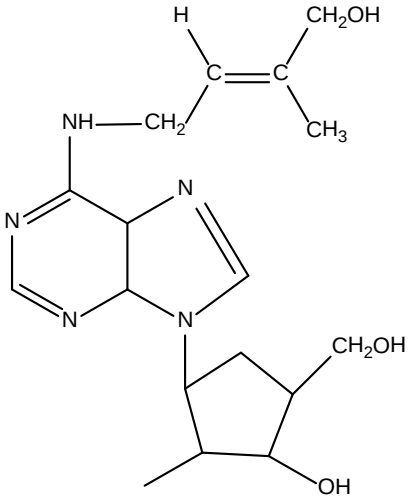
Dihydrozeatin



Isopentyladenine



Isopentyladenine riboside



Trans-zeatin riboside

Figure 7: Cytokinins

(ii) Synthetic Cytokinin:

Kinetin is also known as synthetic cytokinin.

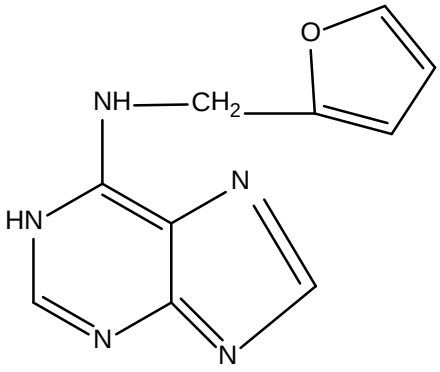


Figure 8: Kinetin

Function of Cytokinin:

Stimulates cell division: Especially by controlling the transition from G2 mitosis. This effect is moderated by cyclin-dependent protein kinases (CDK's) and cyclins. Stimulates morphogenesis (shoot initiation/bud formation) in tissue culture: In plant tissue cultures, cytokinin is required for the growth of a callus. Stimulates the growth of lateral buds- Cytokinin application to dormant buds causes them to develop. Witches' broom is caused by a pathogen *Corynebacterium fascians* (or *Agrobacterium tumefaciens*) that produces cytokinin which, in turn, stimulates lateral bud development. Thus apical dominance may be related to cytokinin too. Stimulates leaf expansion resulting from cell enlargement: Cytokinins stimulate the expansion of cotyledons. The mechanism is associated with increased plasticity of the cell wall. May enhance stomatal opening in some species. Promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis.

4. Abscicic acid:

Abscisic acid (ABA) is the major phytohormone that controls plant's ability to survive in harsh, changing environment. ABA promotes abscission of leaves and fruits; hence it is this action that gave rise to the name of this hormone 'abscisic acid'. ABA is a naturally occurring compound in plants. The ABA signaling pathway is conserved across all plants, including mosses, and it is considered as an early adaptation to the terrestrial environment. ABA is found in leaves (where it is partially synthesized), stems, and green fruits. It is generally associated with negative-feedback interactions or stress-related environmental signals such as drought, freezing temperatures and environmental pollutants

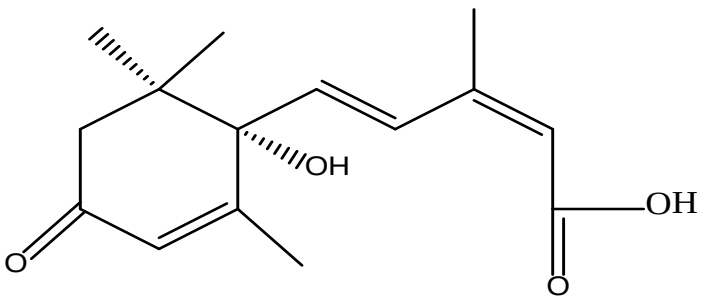


Figure 9: Abscisic acid

Chemical nature of abscisic acid:

Abscisic acid is a naturally occurring compound in plants. Abscisic acid (ABA) is an isoprenoid plant hormone, which is synthesized in the plastids by the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway; It is a sesquiterpenoid (15-carbon) which is produced partially via the mevalonic pathway in chloroplasts and other plastids. Since it is sythesized partially in the chloroplasts, biosynthesis of ABA primarily occurs in the leaves. The production of ABA is accentuated by stresses such as water loss and freezing temperatures.

Functions of Abscisic acid:

Some of the physiological responses of Abscisic acid are listed below:

(1) Antitranspirant: Stimulates the closure of stomata by decreasing transpiration to prevent water loss (water stress brings about an increase in ABA synthesis). In angiosperms and gymnosperms (but not in ferns and lycopsids), ABA triggers closing of stomata when soil water is insufficient to keep up with transpiration.

Mechanism: ABA binds to receptors at the surface of the plasma membrane of the guard cells.

The receptors activate several interconnecting pathways which converge to produce a rise in pH in the cytosol. Transfer of Ca^{2+} from the vacuole to the cytosol. These changes stimulate the loss of negatively-charged ions (anions), especially NO_3^- and Cl^-, from the cell and also the loss of K^+ from the cell. The loss of these solutes in the cytosol reduces the osmotic pressure of the cell and thus turgor. The stomata close. ABA also promotes abscission of leaves and fruits (in contrast to auxin, which inhibits abscission). It is, in fact, this action that gave rise to the name abscisic acid. The dropping of leaves in the autumn is a vital response to the onset of winter when ground water is frozen — and thus cannot support transpiration — and snow load would threaten to break any branches still in leaf. Inhibits shoot growth but will not have as much affect on roots or may even promote growth of roots, induces seeds to synthesize storage proteins. It inhibits the affect of gibberellins on stimulating de novo synthesis of alpha-amylase. It also has some effect on induction and maintanance of dormancy. ABA inhibits seed germination in

antagonism with gibberellins and induces gene transcription especially for proteinase inhibitors in response to wounding which may explain an apparent role in pathogen defense. Inhibits fruit ripening and is responsible for seed dormancy by inhibiting cell growth. It downregulates enzymes needed for photosynthesis.

5. Ethylene:

Ethylene (IUPAC name: ethene), unlike the rest of the plant hormone compounds is a gaseous hormone. Of all the known plant growth substance, ethylene has the simplest structure. It contains a carbon-carbon double bond, ethylene is classified as an unsaturated hydrocarbon. It is produced in all higher plants and is usually associated with fruit ripening. Ethylene which is also known as the 'death hormone' or 'ripening hormone' plays a regulatory role in many processes of plant growth, development and eventually death. Fruits and vegetables contain receptors which serve as bonding sites to absorb free atmospheric ethylene molecules. The overall effect is to hasten ripening, aging and eventually spoilage.

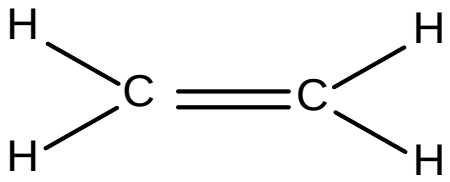


Figure 10: Ethylene

Functions of ethylene:

Ethylene has various physiological responses which are listed below:

Ethylene plays vital role in fruit ripening: The changes which typically takes place due to the stimulating effect of ethylene includes softening of the fruit due to the enzymatic breakdown of the cell walls, sugar accumulation, starch hydrolysis and disappearance of organic acids and phenolic compounds including tannins. Ethylene has the ability to initiate germination in certain seeds, such as cereals and break the dormancy. Ethylene increases the rate of seed germination of several species. Ethylene can also break bud dormancy and ethylene treatment is used to promote bud sprouting in tubers such as potatoes etc. It stimulates shoot and root growth along with differentiation. Ethylene induces abscission. Abscission takes place in specific layers of cells, called abscission layers, which become morphologically and biochemically differentiated during organ

development. Weakening of the cell walls at the abscission layer depends on cell wall–degrading enzymes such as polygalacturonase and cellulase. Ethylene induces flowering in Bromeliaceae family plants which includes pineapple and its relatives. Flowering of other species of plants, such as mango, is also initiated by ethylene. Ethylene may change the sex of developing flowers on monoecious plants (plants which have separate male and female flowers).

The femaleness of diocious flowers in plants is stimulated by the production of ethylene. The promotion of female flower formation in cucumber is one example of this effect.Flower and leaf senescence stimulation is caused by ethylene. Exogenous applications of ethylene accelerate leaf and flower senescence. Enhanced ethylene production in plants is associated with the loss of chlorophyll and the fading of colours.

Role of plant hormones in tissue culture:

The culture of plant tissues or plant cells in a synthetic culture medium under controlled aseptic conditions is known as plant tissue culture. Plant tissue culture is the aseptic culture of plant protoplasts, cells, tissues or organs under conditions which lead to cell multiplication or regeneration of organs or whole plants. Tissue culture produces clones, in which all product cells have the same genotype. The culture medium provides all minerals and growth hormones necessary for the growth of cells. The controlled conditions give the culture a suitable microenvironment for the cell growth, proliferation and morphogenesis. During plant tissue culture, cells of small segments of plant tissue undergo repeated divisions to form masses of cells called calli. Plant tissue culture techniques are central to pioneering areas of applied plant science, including plant biotechnology and agriculture. Selected plants can be cloned and cultured as suspended cells from which plant products can be harvested. The management of genetically engineered cells to form transgenic whole plants requires tissue culture procedures; tissue culture methods are also required in the formation of somatic haploid embryos from which homozygous plants can be generated.

Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without protoplasts, pieces of leaves, or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones. The composition of the medium, particularly the plant hormones and the nitrogen source has profound effects on the morphology of the tissues that grow from the initial explant. Thus, plant hormones are one of the most essential components of the medium used in plant tissue culture. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots. A balance of both auxin and cytokinin will often produce an unorganized growth of cells or callus. Effect of different auxin and cytokinin concentration on tissue development is shown in Figure 11. The ratio of these two hormones can determine plant development:

↑ Auxin ↓Cytokinin = Root Development

↑ Cytokinin ↓Auxin = Shoot Development

Auxin = Cytokinin = Callus Development

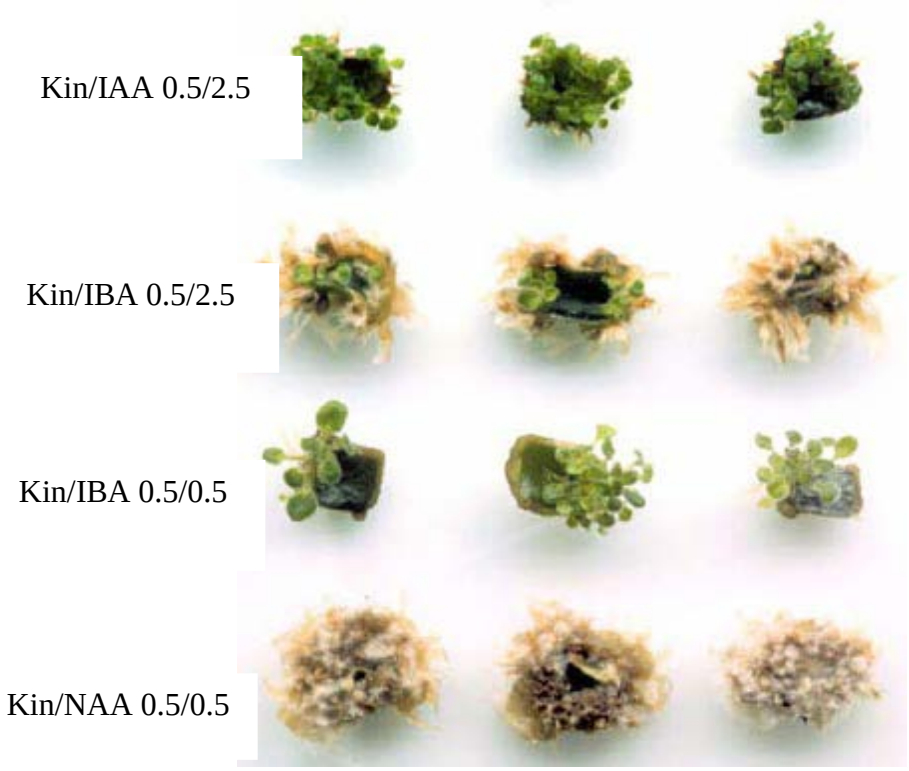
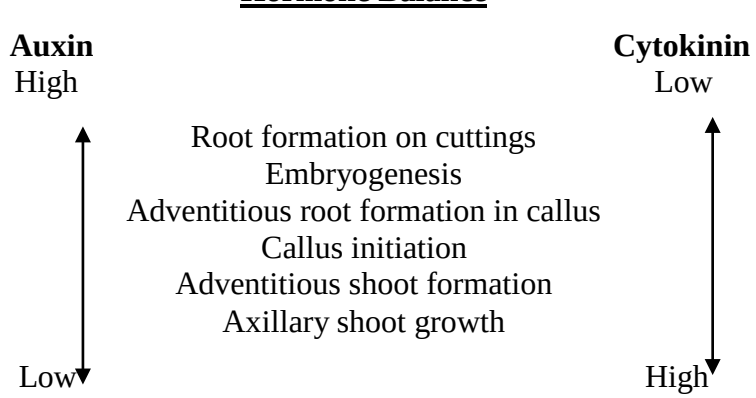


Figure 11: Effect of different auxin and cytokinin concentration on tissue development

(Kin– Kinetin; IAA– Indole acetic acid; IBA-Indole-3-butyric acid; NAA-Naphthaleneacetic acid)

Cytokinins are derived from adenine and produce two immediate effects on undifferentiated cells: the stimulation of DNA synthesis and increased cell division in tissue culture. Cytokinins also produce a delayed response in undifferentiated tissue which is the formation of shoot primordia. Although low tissue concentrations of cytokinins (e.g., 1×10^{-8} M zeatin) have clear effects during tissue culture, higher concentrations are found in actively dividing tissues such as those of plant embryos and developing fruits. Auxins are indole or indole-like compounds that stimulate cell expansion, particularly cell elongation in tissue culture. Auxins also promote adventitious root development. Only small amounts of auxin (1×10^{-6} M) are required to demonstrate an IAA response and even smaller amounts of synthetic auxin (e.g., NAA) are required for a tissue response during tissue culture. Synthetic auxins are more effective hormones that last for an extended length of time. Furthermore, light influences the physiological activity of IAA while synthetic auxins are not as light sensitive.



Cytokinin by itself does not induce vascular tissues, but in the presence of IAA, cytokinin promotes vascular differentiation and regeneration. Cytokinin, which promotes cell divisions in the vascular tissues, is a limiting and controlling factor that increases the number of xylem fibers in tissue culture and along the plant axis. In tissue cultures, low IAA concentrations induce sieve elements but not tracheary elements, whereas high IAA concentrations resulted in the differentiation of both phloem and xylem. However, even in cultures grown at a high IAA concentration, only phloem developed at the surface

further away from the high auxin-containing medium. Abscisic acid, ethylene, gibberellins, and other hormone-like compounds have regulatory roles which cannot be ignored in tissue culture. For instance, although abscisic acid, ethylene and gibberellins are not added to cultured cells to ensure organogenesis or cell proliferation, these hormones are synthesized in the tissues and are playing an active, but hidden role in growth and development of the plant tissues.

Plant hormones do not function in isolation within the plant body, but, instead, function in relation to each other. Hormone balance is apparently more important than the absolute concentration of any one hormone. Both cell division and cell expansion occur in actively dividing tissue, therefore cytokinin and auxin balance plays a role in the overall growth of plant tissue.

Interesting facts:

- The first plant hormone to be discovered is Auxin.
- The first cytokinin to be discovered was the synthetic analog kinetin.
- Ethylene is also known as ‘ripening hormone’ or ‘death hormone’.

Questions:

9. What are plant growth hormones? Give examples.
10. _____ is the first plant hormone to be identified.
    (a) Auxin
    (b) Gibberellin
    (c) Cytokinin
    (d) Abscisic acid
11. Draw the structure of auxin.
12. What are natural auxins? Give examples.
13. What are synthetic auxins? Give examples.
14. Give an example of natural and synthetic cytokinin.
15. _____is known as the ‘ripening hormone’.

(a) Abscisic acid
(b) Auxin
(c) Ethylene

8. What are plant growth hormones? Describe each of the phytohormones in detail.

9. What are auxins? How can auxins be classified based on its occurrence in plants? Add a note to it.

10. How was gibberellin discovered? Mention the functions of gibberellin.

12. Discuss the effect of cytokinin in plant growth and development.

13. Discuss the effect of abscisic acid in plant growth and development.

14. Which plant hormone is known as the 'ripening hormone'? How was it discovered? Discuss its effect in plant growth and development.

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M5 L1

Cell signalling

As living organisms we are constantly receiving and interpreting signals from our environment. These signals can be light, heat, odours, touch or sound. The cells of our bodies are also constantly receiving signals from other cells. These signals are important to keep cells alive and functioning as well as to stimulate important events such as cell division and differentiation. In animals, rapid responses to the changes in the environment are mediated primarily by the nervous system and by hormones including small peptides, small nonpeptide molecules such as the catecholamines (Dopamine, epinephrine, norepinephrine). We have already studied role of hormones like epinephrine, ACTH and norepinephrine etc in signaling events. We shall discuss downstream process when signaling molecule interacts with receptor. We shall also talk about various secondary messengers involved in signaling process. These signaling molecules are released from the cells and they travel through the blood to their specific target cells as shown in Figure 1. Some molecules are transported long distances by the blood while others have more of local effects. Certain membrane-bound proteins on one cell can directly signal an adjacent cell.

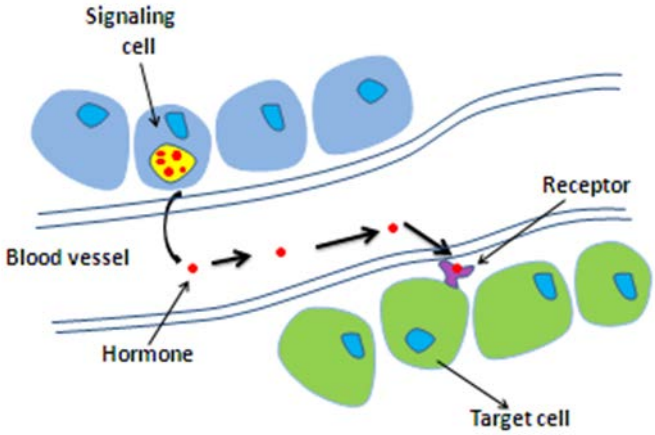

Figure 1: Signaling molecules released from cell and transported by the blood to the target cell

Cell signaling can be divided into 3 stages:

1. Reception: A cell detects a signaling molecule from the outside of the cell. A signal is detected when the ligand binds to a receptor protein on the surface of the cell or inside the cell.

2. Transduction: When the signaling molecule binds to the receptor, it changes the receptor protein. This change initiates the process of transduction. Each relay molecule in the signal transduction pathway changes the next molecule in the pathway.

3. Response: Finally, the signal triggers a specific cellular response as shown in Figure 2.

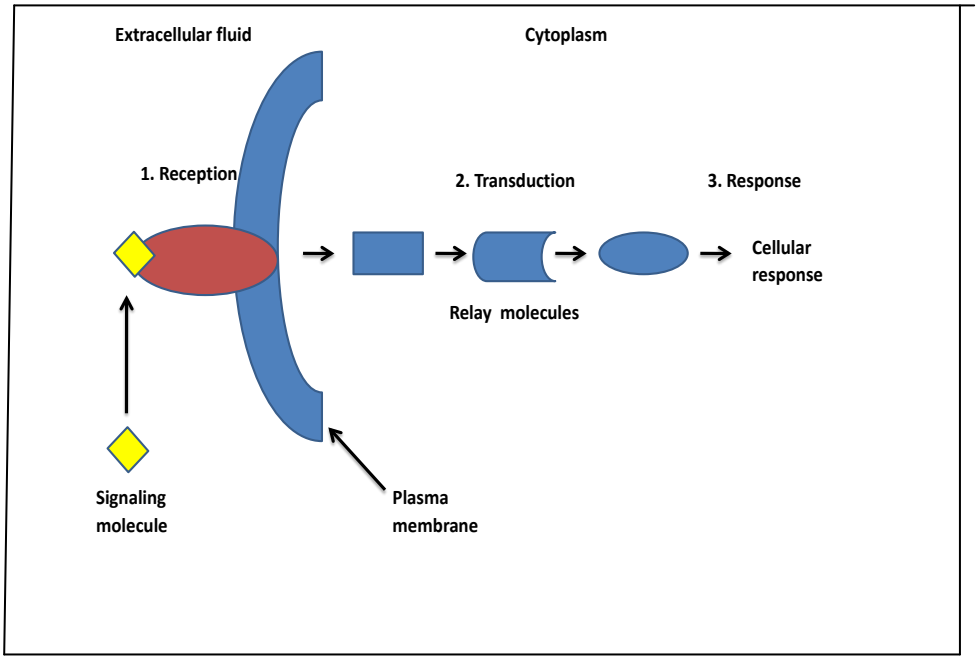

Figure 2: Cell signalling stages

Signal Transduction:

Signal transduction is phenomenon which involves in the transfer of signal from extracellular to intracellular environment through the cell surface receptor protein that stimulate intracellular target enzymes, which may be either directly linked or indirectly coupled to receptors by G proteins. These intracellular enzymes serve as downstream signalling elements that propagate and amplify the signal initiated by ligand binding. Thus, signal transduction pathway allows cells to respond to extracellular environmental signals. These signals can be physical and chemical such as light, oxygen, nutrient, hormones. Figure 3 represents the signal transduction pathway.

Signal transduction is the combination of following phenomenon:

1. Signal reception
2. Integration
3. Amplification
4. A target that is affected
5. Termination

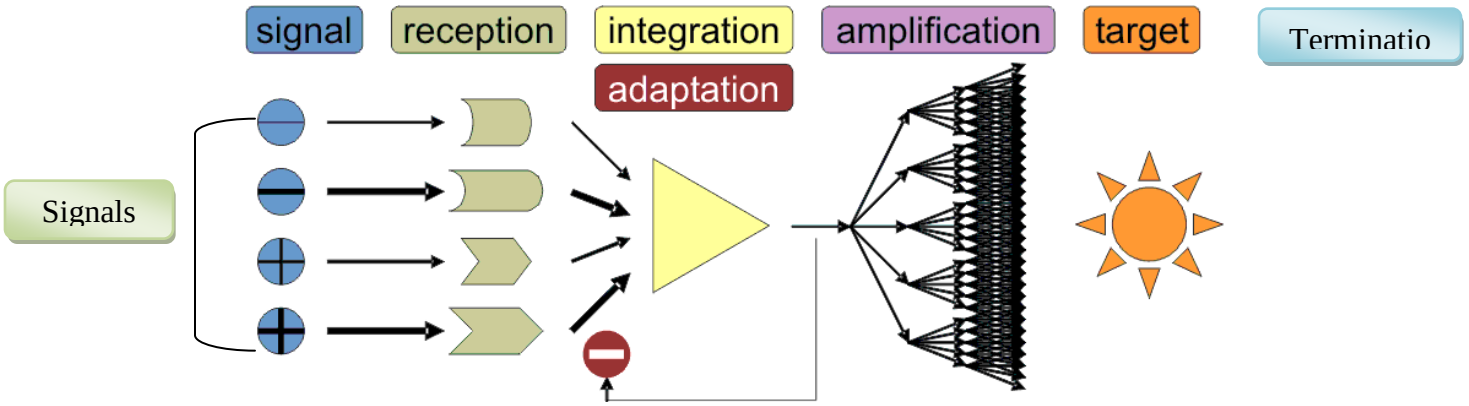

Figure 3: Representation of Signal Transduction pathway

Thus signal transduction begins with receiving signal to the cell receptor and end with a change in cellular function. The cell receptor can be of various types- G-protein coupled receptor, tyrosin kinase receptor etc. The transduction process is typically mediated via a cascade of some important second messengers including cAMP, cGMP, calcium ion, inositol 1, 4, 5-trisphosphate, (IP_3), and diacylglycerol (DAG). Second messengers are intracellular molecules that change in concentration in response to environmental signals and involve in conveying information inside the cell.

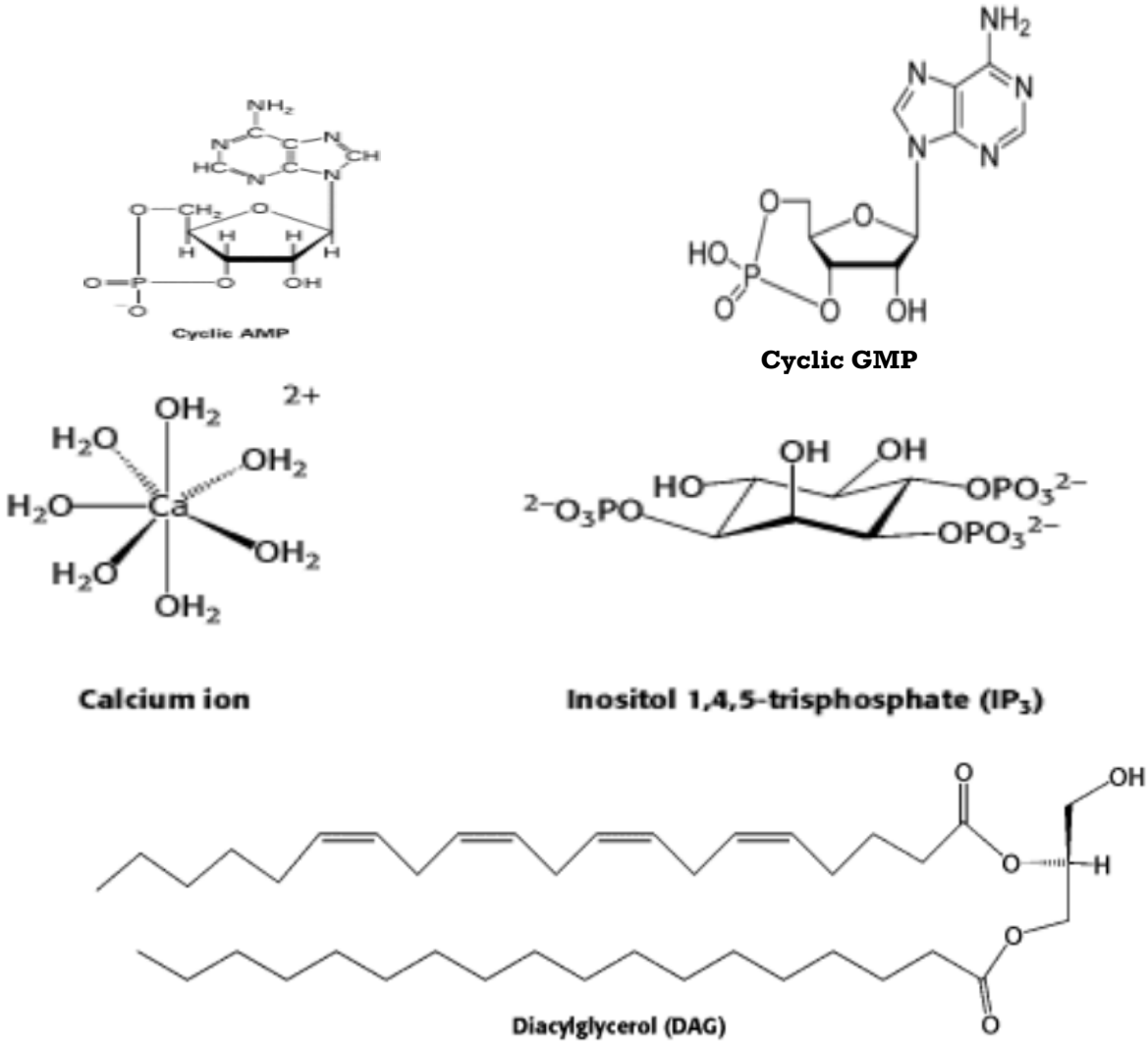

Figure 4: Common second messengers

Signal transduction pathways act similar to molecular circuit. This pathway depends on following factors during transformation of signal from extracellular environment to intracellular.

1. Signal reception by cell membrane receptor: Some non polar signaling molecules such as estrogens and other steroid hormones are able to cross the bilipid membrane and hence make entry inside the cell. Once inside the cell, these molecules can bind to proteins that interact directly with DNA and involve in regulation of gene transcription. Thus, a chemical signal enters the cell and directly alters gene-expression patterns. However, most of signalling molecules are too large and too polar so they are unable to cross the membrane, hence there is no appropriate transport system. In this case these signaling

molecules transmit signals through cell surface receptor protein without crossing the cell membrane. We will discuss about cell receptors in upcoming lecture notes.

These receptors are intrinsic membrane protein which consist both extracellular and intracellular domain. A binding site present in extracellular domain specifically recognizes the signaling molecule (i.e. well known as ligand). Such binding sites are analogous to enzyme active sites except that no catalysis takes place within them. When these signal molecules comes and bind to binding site on receptor protein in extracellular region then some conformational change occurs in tertiary and quaternary structure of the receptor which results in the drastic change in the intracellular domain of the receptor. These structural changes are not sufficient to yield an appropriate response, because they are restricted to a small number of receptor molecules in the cell membrane. The information embodied by the presence of the ligand, often called the primary messenger, must be transduced into other forms that can alter the biochemistry of the cell.

2. Second messengers: Second messengers act as the intermediate molecule that relay signals from receptors on cell surface to target molecule inside cells, in cytoplasm or nucleus.

   The use of second messengers has several consequences:

a) The second messengers are able to diffuse frequently into other compartment of the cell such as nucleus where they can influence gene expression and other process.
b) Generation of second messengers leads to amplification of signal. Each signaling molecule is involved in the generation of several second messengers in the cell. Thus, a low concentration of signal in the environment, even as little as a single molecule, can yield a large intracellular signal and response
c) Since common second messengers generate in different signaling pathway, thus the coordination of signal transduction is driven by interaction between these pathways. Multiple signaling pathways create both opportunities and potential problems. Interactions between signaling pathways enables the cell to process and interpret multiple inputs differently in different contexts leading to cross-talk. Cross talk between second messengers cause oscillation of various second messengers and also creates biostability between two steady states. Thus cross talk more precisely involves

in regulation of cell activity than individual independent pathways without cross talk. However, inappropriate cross-talk can cause second messengers to be misinterpreted.

3. Protein phosphorylation: Protein phosphorylation is most common route for transferring information coming through second messenger which involve elicit responses by activating protein kinases. Protein phosphorylation is a post-translational modification of proteins by phosphorylation at serine, threonine or tyrosine residues by a protein kinase by the addition of a covalently bound phosphate group from ATP.

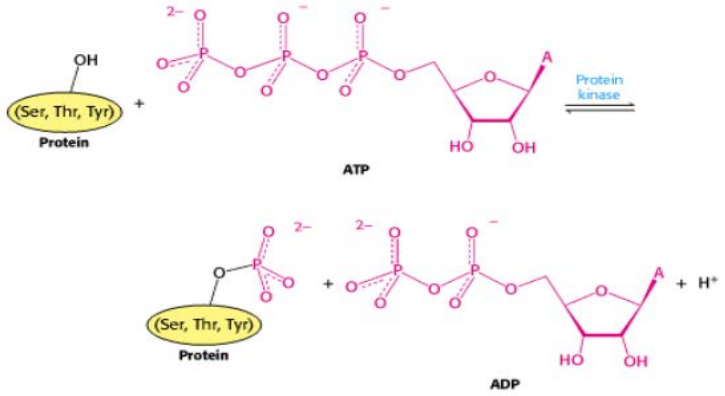

Figure 5: Action of cAMP-dependent protein kinase

4. Signal termination by protein phosphatase: Signal termination is final step of signal transduction. The well known route for signal termination is by protein phosphatase enzyme. The signalling process must be terminated after signaling process has been initiated and the information has been transduced to affect other cellular processes, because without such termination cells lose their responsiveness to new signals. Additionally, if termination fails in signaling processes, it may lead to uncontrolled cell growth and thus increases the risk of cancer.

Signal amplification

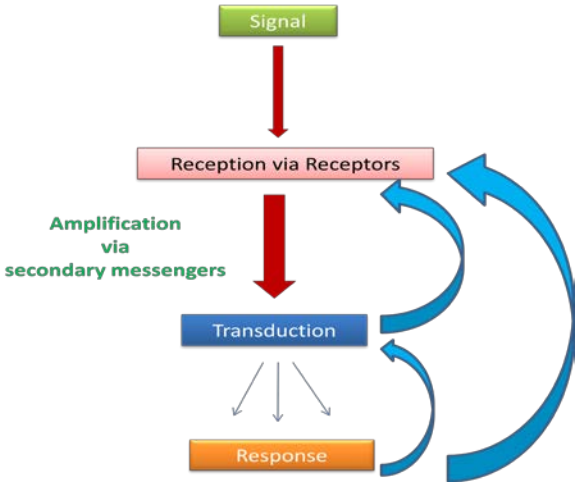

Figure 6: Signal amplification Pathways

Signal amplification is phenomenon in which when receptor proteins interact with the signal molecules at the surface of the cell, in most cases signals are relayed to the cytoplasm or the nucleus by second messengers which influences the activity of one or more enzymes or genes inside the cell. However, most signalling molecules are found in such a low concentration that their effect in cytoplasm would be minimal unless the signal was amplified. Therefore, most enzymes linked and G-protein linked receptor use a chain of other protein messenger to amplify the signal as it is being relayed. Thus in case of protein kinase one cell surface receptor activates many G protein molecules. Each G protein activates many adenylyl cyclases. Each cyclic AMP in turn will activate protein kinase which then activates several molecules of a specific enzyme.

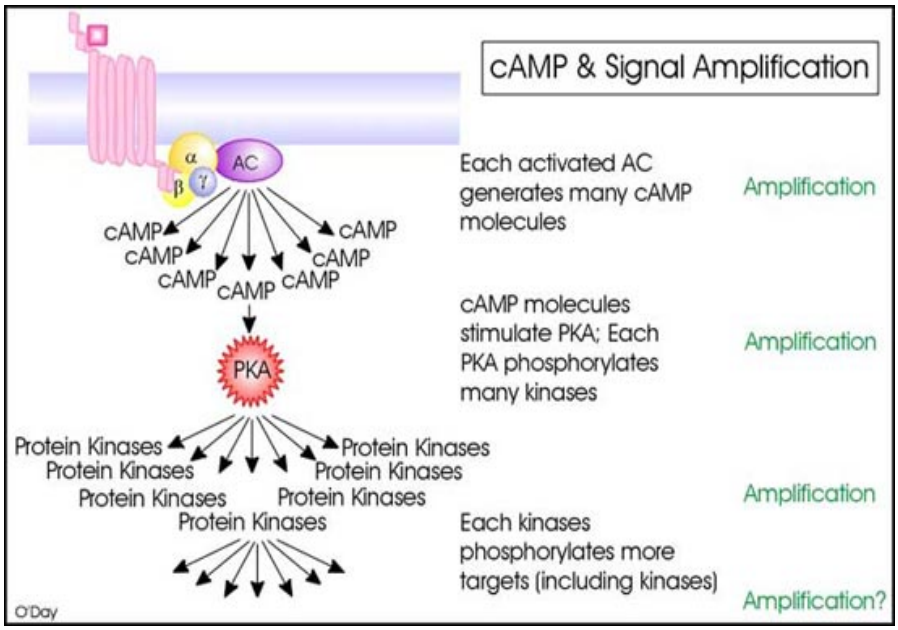

Figure 7: Signal amplification and cAMP

For example, the binding of a single molecule (such as glycogen or epinephrine) at the cell surface can activate many effector G proteins and an adenylyl cyclase each of which can produce a large number of cAMP messengers in a short period of time. Thus, the production of a second messenger provides a mechanism to greatly amplify the signal generated from the original message. There are many steps in the reaction cascade, amplification of the signal via cAMP molecules which activate protein kinase K which involve in phosphorylation of Ser, Thr and tyrosine of target protein. PKA is tertameric protein which is made up of two catalytic and two regulatory subunits. Binding of cAMP to the regulatory subunits induces a conformational change that leads to dissociation of the catalytic subunits, which elicit formation of enzymatically active form of protein kinase A, are now able to phosphorylate Ser and Thr residues on their target proteins. In signal amplification, each PKA catalytic subunit phosphorylates a large number of phosphorylase kinase molecules, which in turn phosphorylate an even larger number of glycogen phosphorylase molecules, which in turn can catalyze the formation of a much larger number of glucose phosphates. Thus, what begins as a hardly noticeable stimulus at the cell surface is rapidly transformed into a major mobilization of glucose within the cell.

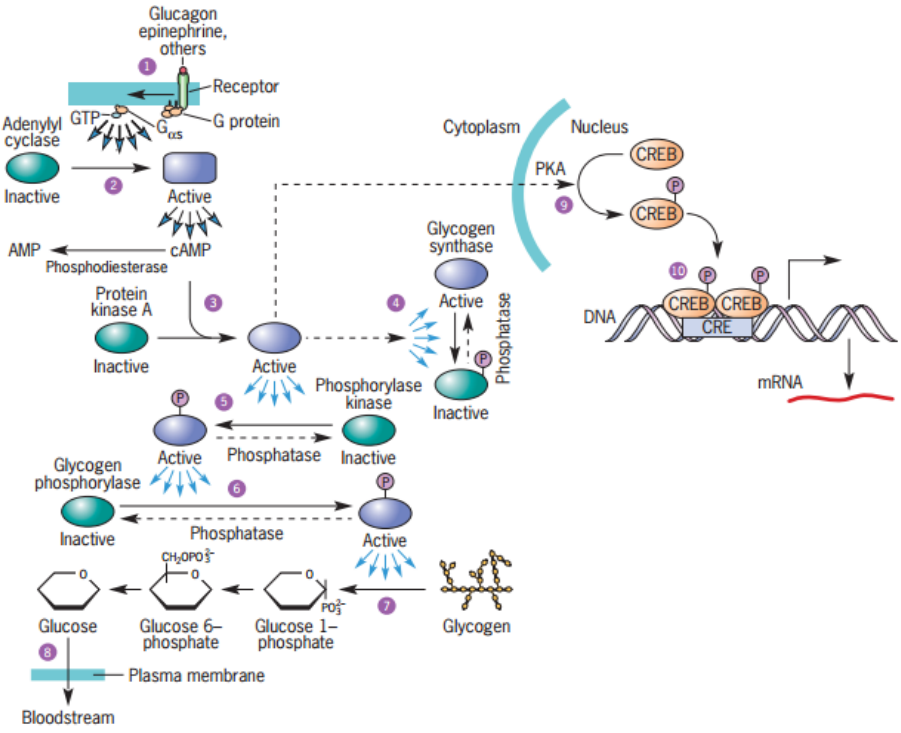

Figure 8: Signal amplification Pathways for Glycogen degradation

The different effect of some hormone in different tissues in shown in the following table:

Table 1: Example of hormone induced response mediated by cAMP

Tissue	Hormones	Response
Liver	Epinephrine and glucagon	Glycogen breakdown, glucose synthesis (glucogenesis), inhibition of glycogen synthesis
Skeletal muscle	Epinephrine	Glycogen breakdown, inhibition of glycogen synthesis
Cardiac muscle	Epinephrine	Increase contractility
Adipose	Epinephrine, ACTH and glucagon	Triacyglycerol catabolism
Kidney	Vasopressin(ADH)	Increase permeability of epithelial cells to water
Thyroid	TSH	Secretion of thyroid hormones
Bone	Parathyroid hormone	Increase calcium resorption
Ovary	LH	Increase secretion of steroid hormones
Adrenal cortex	ACTH	Increase secretion of glucocorticoids

In the upcoming chapter we will study about each component of signal transduction in detail.

Interesting Facts:

1. Most signaling molecules are found in such low concentration that their effect in cytoplasm would be minimal unless the signal is amplified.
2. Most enzymes linked and G-protein linked receptor use a chain of other protein messenger to amplify the signal as it being relayed.

Questions:

1. What is signal amplification?
2. What is Signal transduction? Explain diagrammatically.
3. Which are the possible factors which influence signal amplification?
4. How second messenger take part in the signal amplification pathway?

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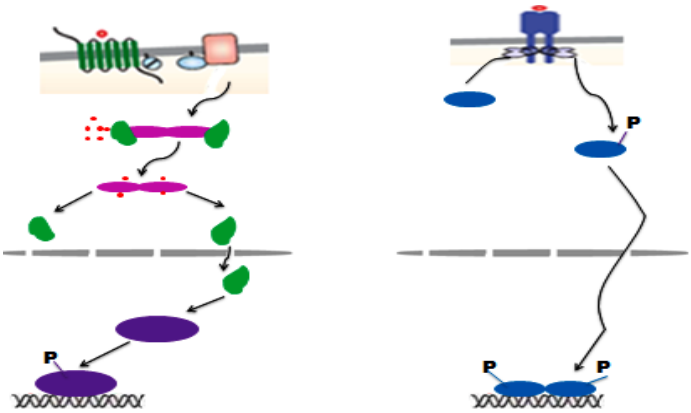
5. Stryer. Biochemistry, 5^{th} edition.

M5 L2

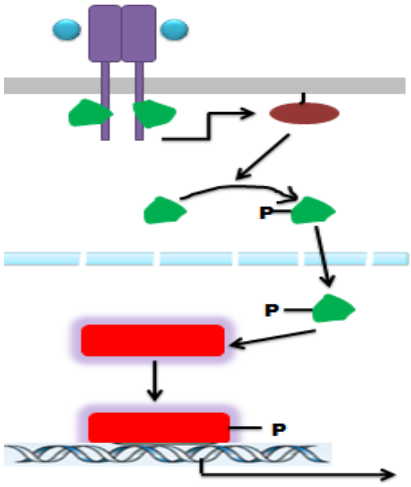
Cell receptors

Receptor: A receptor is a protein molecule found on the surface of a cell which receives chemical signals originating externally from the cell. Binding of specific signalling molecules to a receptor directs a cell to allow certain molecules to enter or exit or directs a cell to divide or die. Cells within multicellular organisms communicate via extracellular mediators: either through diffusible molecules or by direct cell–cell contact. Examples of receptors are G-Protein coupled receptors, Cytokine receptors as shown in Figure 1.

Receptors are located in either the cytoplasm or plasma membrane or nucleus of a cell. A molecule which binds specifically to a receptor is called a ligand. A ligand may be a peptide or other small molecules, such as a hormone, a neurotransmitter, a pharmaceutical drug or a toxin. Each type of receptor recognizes and binds only certain ligand shapes. Binding of a ligand to its receptor causes a conformational change in the cytosolic domain of the receptor which then triggers the subsequent signalling cascade; i.e. it activates or inhibits a specific biochemical pathway. Ligand-induced changes in receptors result in cellular changes which constitute the biological activity of the ligands. Most signalling molecules bind to receptors expressed on the target cell surface but some signalling molecules are able to cross the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus.



G protein-coupled receptor Cytokine receptor



Receptor tyrosine kinases

Figure 1: Examples of receptors

Receptor types based on cellular location:

There are mainly three types of receptors present in the cell based on their location in the cell. They are:

1. Cytosolic receptors
2. Nuclear receptors
3. Membrane bound receptors

1. Cytosolic receptors: Cytosolic receptors are specialized integral membrane proteins that take part in communication between the cell and the outside world. Extracellular signalling molecules (usually hormones, neurotransmitters, cytokines, growth factors or cell recognition molecules) attach to the receptor, triggering changes in the function of the cell. In this way the receptors play a unique and important role in cellular communications and signal transduction. Most steroid hormones have receptors within the cytoplasm which acts by stimulating the binding of their receptors to the promoter region of steroid-responsive genes. Examples of cytosolic receptors are Estrogen receptors, Glucocorticoid receptors etc.

Mechanism of action of cytosolic receptors: Estrogen diffuses across the plasma membrane and binds to its receptor in the nucleus. The estrogen receptor is bound to Hsp 90 chaperones in the absence of the estrogen hormone as shown in Figure 2. The binding of the estrogen induces a conformational change in the receptor, displacing Hsp 90 and then leading to the formation of receptor dimers which binds to DNA, associate with coactivators with histone acetyltransferase (HAT) activity, and stimulate transcription of their target genes. In other cases, the receptor binds the DNA either in the presence or absence of hormone. But hormone binding alters the activity of the receptor as a transcriptional regulatory molecule. For example, in the absence of hormone, thyroid hormone receptor is associated with a corepressor complex and represses transcription of its target genes. Hormone binding induces a conformational change that results in the interaction of the receptor with co activators rather than co repressors, leading to transcriptional activation of thyroid hormone-inducible genes.

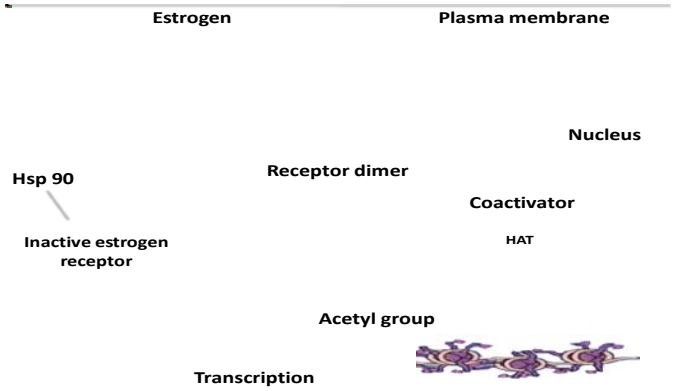


Figure 2: Estrogen receptor action

2. Nuclear receptors: Nuclear receptors are intracellular proteins expressed in the nucleus of a cell. These receptors are members of a family of proteins known as the nuclear receptor super family. Nuclear receptors constitute a superfamily of dimeric C4 zinc-finger transcription factors that bind lipid-soluble hormones and interact with specific response elements in DNA. Steroid receptors are homodimers of zinc-finger proteins that reside within the nucleus (except for the glucocorticoid receptor which resides in the cytosol until it binds its ligand). Until their ligand finds them, some steroid receptors within the nucleus associate with histone deacetylases (HDACs), keeping gene expression repressed in those regions of the chromosome.

Structure of nuclear receptors: Nuclear receptors constitute a superfamily of dimeric C4 zinc-finger transcription factors. They are modular in structure and contain the following structural domains:

N-terminal regulatory domain (A-B): The A-B domain is highly variable in sequence between various nuclear receptors. It contains the activation function 1 (AF-1) whose action is independent of the presence of ligand. The transcriptional activation of AF-1 is normally very weak but it synergizes with AF-2 in the E-domain to produce an upregulation of gene expression.

DNA-binding domain; DBD (C): It is a highly conserved domain containing two zinc fingers that binds to specific sequences of the DNA called hormone response elements (HRE) as shown in Figure 3.

Hinge region (D): It is the flexible domain that connects the DBD with the LBD. It influences subcellular distribution and intracellular trafficking.

Ligand binding domain LBD (E): Its sequence is moderately conserved but it is highly conserved in structure between the various nuclear receptors. The structure of the LBD is referred to as an alpha helical sandwich fold in which three anti parallel alpha helices (the sandwich filling) are flanked by two alpha helices on one side and three on the other (the bread). The ligand binding cavity is within the interior of the LBD and just below is present three anti parallel alpha helical sandwich filling. Along with the DBD, the LBD

contributes to the dimerization interface of the receptor. In addition, it binds coactivator and corepressor proteins. The LBD also contains the activation function 2 (AF-2) whose action is dependent on the presence of bound ligand.

C-terminal domain (F): It is highly variable in sequence between various nuclear receptors.

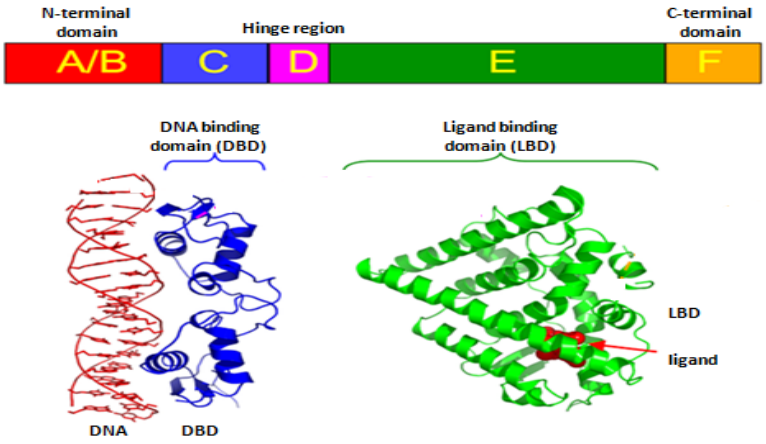


Figure 3: Structural Organization of Nuclear Receptors (estrogen receptor)
Top – Schematic amino acid sequence of a nuclear receptor.
Bottom – 3D structures of the DBD (bound to DNA) and LBD (bound to hormone) regions of the nuclear receptor

Mechanism of action: The intracellular nuclear receptors respond to small hydrophobic signaling molecules that diffuse readily across the plasma membrane. These molecules bind to the receptors and a conformational change takes place in the receptor. This is followed by a series of intracellular signal transduction cascade. The steroid hormones like thyroid hormone, vitamin D_3 and retinoic acid differ greatly from one another in both chemical structure and function. Once inside the cell, these signaling molecules bind to intracellular receptors that are expressed by the hormonally responsive target cells. These receptors are transcription factors that contain domains for ligand binding, DNA binding and transcriptional activation. Ligand binding regulates their function as activators or repressors of their target genes. So the steroid hormones and related molecules directly regulate gene expression. In response, these receptors work with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism.

The nuclear receptor is kept in the cytoplasm by interaction between its ligand-binding domain (LBD) and inhibitor proteins in the absence of a steroid hormone. When hormone is present, it diffuses readily through the plasma membrane and binds to the ligand-binding domain. This causes a conformational change thus releasing the receptor from the inhibitor proteins. The receptor with bound ligand is then translocated into the nucleus, where its DNA-binding domain (DBD) binds to response elements, allowing the ligand-binding domain and an additional activation domain (AD) at the N-terminus to stimulate transcription of target genes as shown in Figure 4.

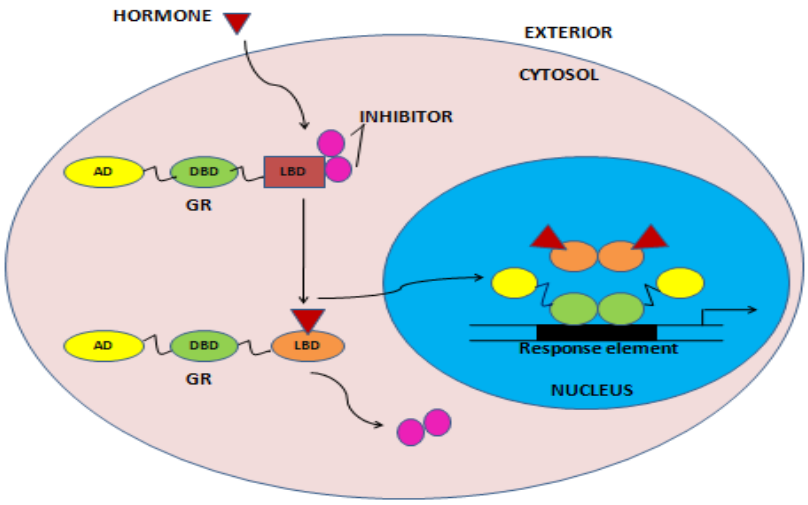


Figure 4: Model of hormone-dependent gene activation by a homodimeric nuclear receptor.

3. Membrane bound receptors: Membrane bound receptors are proteins that are associated with the cell membrane. They can span across the membrane and can transmit a signal from outside the cell to inside the cell. Outside the cell, a ligand (e.g. Hormone) will bind to the receptor. A few chemical stimuli, including steroid hormones and the gas nitric oxide cross the plasma membrane and bind receptors inside the cell. Thus the receptor undergoes a conformational change. This change in the shape of the receptor is detected inside the cell. It is the shape change that is the transmission of the signal from the outside to the inside. Inside the cell, other proteins can interact with the receptor in its new shape and be turned 'on' to continue the signal pathway.

Selective expression of certain receptors and their associated cytoplasmic transduction machinery allows differentiated cells to respond specifically to particular ligands. Members of each family of receptors share one or more structurally homologous domains. In some families, the members share both ligand binding and signal transducing strategies (seven helix receptors, G-protein coupled receptors, cytokine receptors). Members of other families share either a similar ligand-binding structure (Tumour Necrosis Factor receptor family) or a common signal transducing method (receptor tyrosin kinases).

Membrane receptors are found also in the cis Golgi network which captures the proteins during protein sorting and carries them in transport vesicles back to the ER. Also most cholesterol is transported in the blood bound to protein in the form of particles known as low density lipoproteins or LDL. When a cell needs cholesterol for membrane synthesis, it makes transmembrane receptor for LDL and inserts them into its plasma membrane. Once in the plasma membrane, the LDL receptors diffuse until they associate with clathrin-coated pits as shown in Figure 5.

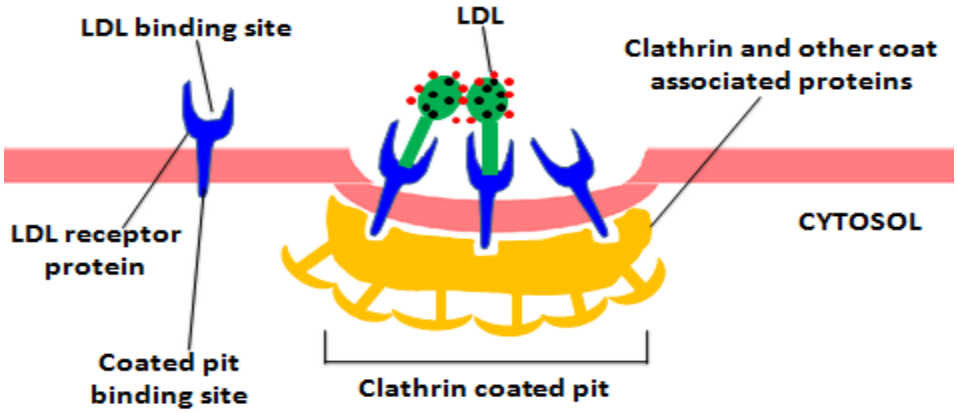


Figure 5: LDL receptor proteins binding to a coated pit in the plasma membrane of a cell

The seven major classes of cell surface receptors are:

1. G protein-coupled receptors
2. Cytokine receptors
3. Receptor tyrosine kinases
4. TGFβ receptors
5. Hedgehog receptors
6. Wnt receptors
7. Notch receptor

Structure of Seven helix receptors: Membrane bound receptors constitute the members of the largest family of plasma membrane receptors built from a serpentine arrangement of seven transmembrane α helices. G protein–coupled receptors (GPCRs) are examples of seven transmembrane α helices receptors. GPCRs are found in all eukaryotic cells from yeast to man. All G protein–coupled receptors contain seven membrane-spanning regions with their N-terminal segment on the exoplasmic face and their C-terminal segment on the cytosolic face of the plasma membrane.

As shown in Figure 6 (a), all receptors of this type have the same orientation in the membrane and contain seven transmembrane - helical regions (H1–H7) - four extracellular segments (E1–E4), and four cytosolic segments (C1–C4). The carboxyl-terminal segment (C4), the C3 loops and, in some receptors, also the C2 loops are involved in interactions with a coupled trimeric G protein. The long C3 loop between helices 5 and 6 is important for interactions between a receptor and its coupled G protein. This superfamily of seven-pass transmembrane receptor proteins includes rhodopsin, the light-activated protein in the vertebrate eye as shown in Figure 6 (b).

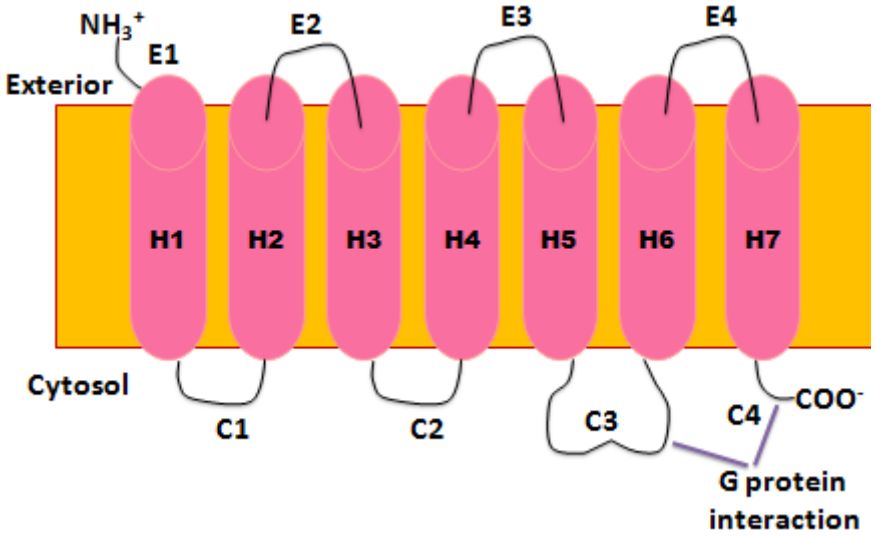


Figure 6 (a): Schematic diagram of G protein–coupled receptors

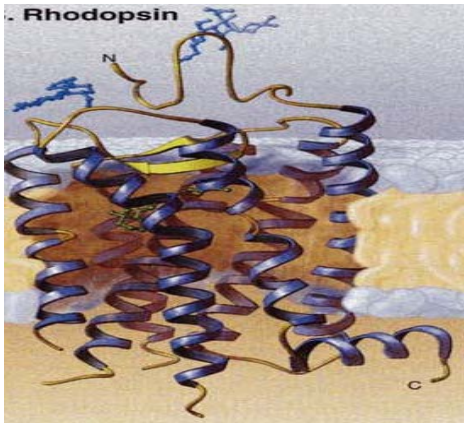


Figure 6 (b): Atomic structure of seven helix receptor of bovine rhodopsin, the light activated protein in vertebrate eye

Mechanism of action of seven helix receptors: The seven helix receptors use trimeric GTP-binding proteins to relay signals to effector proteins inside the cells. Human genome encodes several thousand GPCR. These include receptors in the visual, olfactory (smell) and gustatory (taste) systems, neurotransmitter receptors and most of the receptors for hormones that control the metabolism of carbohydrate, amino acid and fat. GPCRs are coupled to signal-transducing trimeric G proteins. In mammals, olfactory cells alone use 500 – 1000 different seven-helix receptors to discriminate odorant molecules.

Phosphorylation of the C-terminal tail inactivates many types of seven helix receptors. Two different strategies, sometimes acting on the same receptor, provide negative feedback. One strategy is for the second messengers, which are produced in response to receptor activation to stimulate general protein kinases (including cAMP), protein kinase A (PKA) and protein kinase C (PKC) which phosphorylate the activated receptor. Phosphorylation inhibits the receptor thus allowing for crosstalk between receptors. The second strategy involves a class of protein kinases specific for the receptor themselves. They are called G-protein coupled receptor kinases. These kinases phosphorylate multiple serines or threonines on the C-terminal cytoplasmic tail of active receptors. Phosphorylation promotes binding of a regulatory protein called arrestin which inactivates the receptor by blocking interaction of the receptor with trimeric G-proteins. Arrestin binding to some seven helix receptors promotes their removal from the plasma membrane by endocytosis. G protein–coupled receptors transduce signals from extracellular hormones to associated effector proteins. In the resting state, when no ligand is bound to the receptor, the G_{α} subunit is bound to GDP and complexed with $G_{\beta\gamma}$. As shown in the Figure 6, ligand binding shifts the equilibrium from the resting conformation towards the active conformation. Active receptor promotes dissociation of GDP from α subunit of multiple trimeric G-proteins, allowing GTP to bind. This dissociates G_{α} from $G_{\beta\gamma}$, each of which activate downstream effectors that produce the second messengers cAMP and diacylglycerol (DAG) as shown in Figure 7. cAMP and DAG activates PKA and PKC, which phosphorylate active receptors on their C-terminus. This attracts arrestin, putting the receptor into the inactive adapted state.

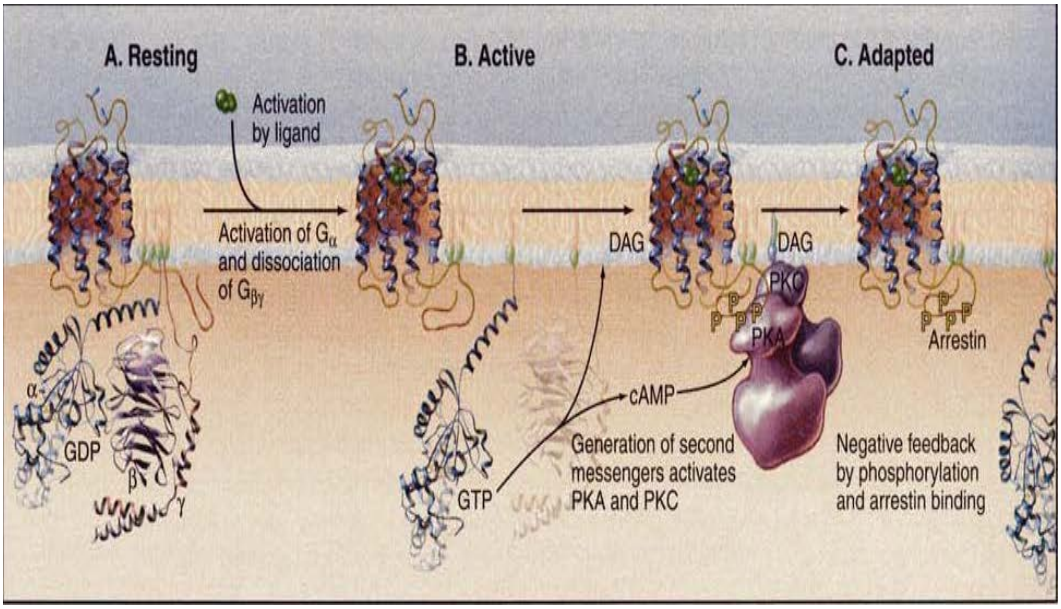


Figure 7: **Activation and adaptation of a seven helix receptor: A, resting; B, active; C, adapted.**

Epinephrine Binds to Several Different G Protein–Coupled Receptors. All epinephrine receptors are G protein– coupled receptors, the different types are coupled to different G proteins. These receptors are of interest because they trigger different intracellular signal-transduction pathways. Both subtypes of β-adrenergic receptors, termed β1 and β2, are coupled to a stimulatory G protein (Gs) that activates the membrane-bound enzyme adenylyl cyclase. Once activated, adenylyl cyclase catalyzes synthesis of the second messenger cAMP. That binding of epinephrine to β-adrenergic receptors induces a rise in cAMP.

Interesting facts:

- Receptor tyrosine kinase is the first receptor to be discovered.
- G proteins were discovered by Alfred G. Gilman and Martin Rodbell when investigating stimulation of cells by adrenaline.
- Skin contains approximately 640,000 sense receptors scattered unevenly over the body's surface.

Questions:

1. What are receptors? Give examples.
2. How many types of receptors are present on the cell?
3. What are cytosolic receptors? Give example.
4. What are nuclear receptors?
5. What is membrane bound receptors? Give example.

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M5 L3

Second messengers - cAMP

Second messengers: As mentioned din lecture 1 of this module, **s**econd messengers are molecules that relay signals received at receptors on the cell surface such as hormones, growth factors, etc. to appropriate target molecules in the cytosol and/or nucleus. In addition to their job as relay molecules, second messengers serve to amplify the strength of the signal. Binding of a ligand to a single receptor at the cell surface may end up causing massive changes in the biochemical activities within the cell.

There are 3 major classes of second messengers:

1. Cyclic nucleotides (**cAMP** and **cGMP**)
2. Inositol trisphosphate (**IP_3**) and diacylglycerol (**DAG**)
3. Calcium ions (Ca^{2+})

We will discuss about all of them in the upcoming lectures.

Cyclic adenosine monophosphate (cAMP)

cAMP is an important second messenger involved in a plethora of cellular effects and biological roles by regulating various metabolic process and mediating the effects of many hormones that binds to a specific receptor on the cell membrane of target cells including catecholamines, ACTH, and vasopressin. It also plays imperative role in the transcription of some genes. Earl Sutherland won a Nobel Prize in Physiology or Medicine in 1971 for his discoveries regarding the mechanisms of the action of hormones, especially epinephrine, via second messengers such as cyclic AMP. cAMP is represented by $C_{10}H_{12}N_5O_6P$ and the molecular mass is 329.206.

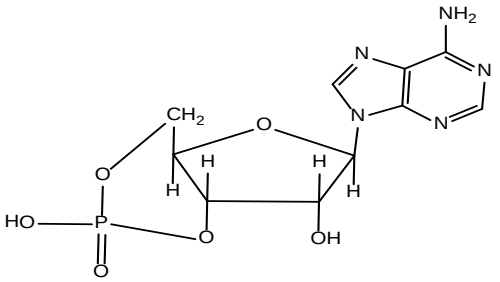

Figure 1: Cyclic Adenosine monophosphate or 3'-5'-cyclic adenosine monophosphate

[Compositions of cAMP - Adenine base + Ribose sugar + 3', 5'-cyclic phosphate]
Adenosine in cAMP is a nucleoside composed of the pentose sugar D-ribose and adenine, a base. Cyclic AMP contains an ester linkage between the phosphate and ribose units.
Some of the hormones that achieve their effects through cAMP as a second messenger:

- Adrenaline
- Glucagon
- Luteinizing hormone (LH)

Binding of the hormone to its receptor activates a G protein which, in turn, activates adenylyl cyclase. The resulting rise in cAMP turns on the appropriate response in the cell by either (or both): changing the molecular activities in the cytosol, often using Protein Kinase A (PKA) — a cAMP-dependent protein kinase that phosphorylates target proteins; turning on a new pattern of gene transcription.

Functions of cAMP

1. **cAMP as a second messenger:** cAMP is a second messenger, used for intracellular signal transduction. It is involved in transmitting signal from outside the cell to the interior via the process of binding of hormones like glucagon and epinephrine or other signal molecules to cell membrane receptor. It is involved in the activation of protein kinases and regulates the effects of adrenaline and glucagon. cAMP also binds to and regulates the function of ion channels such as the HCN channels and a few other cyclic nucleotide-binding proteins such as Epac1.

2. Regulation of protein kinase A by cAMP (Phosphorylation of protein kinase): The most important function of cAMP in animal cell is regulation of protein kinase A activity. Protein kinase A is found primarily in inactive form in the cell in which it consists of two regulatory (R) and two catalytic (C) subunits together. Binding of cAMP to the regulatory subunits induces a conformational change that leads to dissociation of the catalytic subunits, which elicits formation of enzymatically active form of protein kinase A, and are now able to phosphorylate Ser and Thr residues on their target proteins.

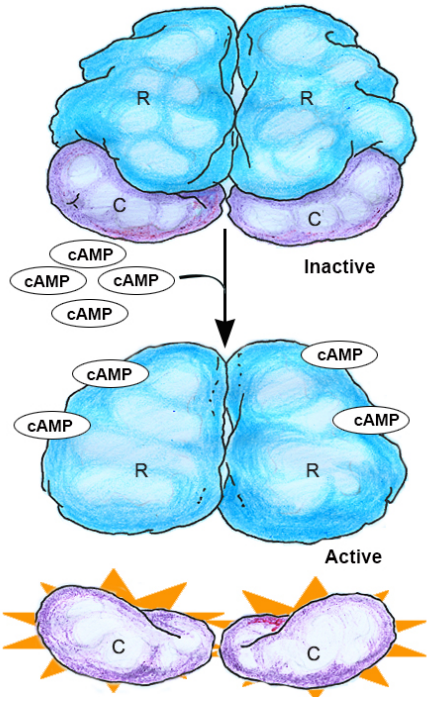

Figure 2: Regulation of protein kinase A

3. Regulation of Cyclic AMP-inducible gene expression via protein kinase A:

In many animal cells, increase in cAMP activates the transcription of specific target genes that contain a regulatory sequence called the cAMP response element, or CRE. In this case, the signal is passed from the cytoplasm to the nucleus by the catalytic subunit of protein kinase A, which is able to enter the nucleus after its release from the regulatory subunit. Within the nucleus, protein kinase A phosphorylates a transcription factor called CREB (CRE-*b*inding protein), leading to the activation of cAMP-inducible genes. Thus such type of regulation of gene expression by cAMP plays significant role in controlling proliferation, survival, and differentiation of a wide variety of animal cells.

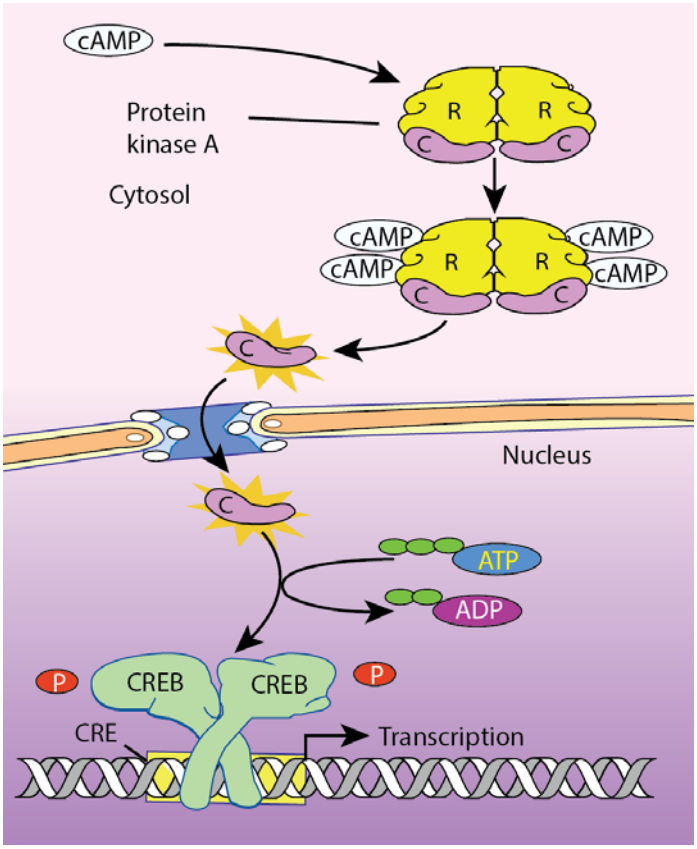

Figure 3: Cyclic AMP inducible gene expression

4. In eukaryotic cells: cAMP and its associated kinases play key role in numerous biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism. There are several minor PKA-independent functions of cAMP like activation of calcium channels, providing a minor pathway by which growth hormone-releasing hormone causes a release of growth hormone. The GEF (guanine nucleotide exchange factor)

domain is usually covered by the N-terminal region containing the cAMP binding domain. When cAMP binds, the domain dissociates and exposes the active GEF domain, allowing Epac to activate small Ras-like GTPase proteins, such as Rap1.

5. In bacteria: In bacteria, cAMP plays a crucial role and its level varies depending on the medium used for growth. In *E.coli*, cAMP involves in the positive regulation of the lac-operon. cAMP is synthesized from ATP by adenylyl cyclase, as a result increase in the level of cAMP causes decrease in glucose concentration which is the carbon source. cAMP then binds to the transcriptional regulatory protein, cAMP receptor protein (CRP) also called catabolic activator protein(CAP). After binding of cAMP to CAP enhance the binding capacity of CAP to its binding site (CAP binding site) on target DNA sequence which in lac operon located 60 nucleotides upstream of transcription start site, making it easier for RNA polymerase to bind to the adjacent promoter to start transcription of the lac-operon, increasing the rate of lac-operon transcription. With a high glucose concentration, the cAMP concentration decreases, and the CRP disengages from the lac-operon.

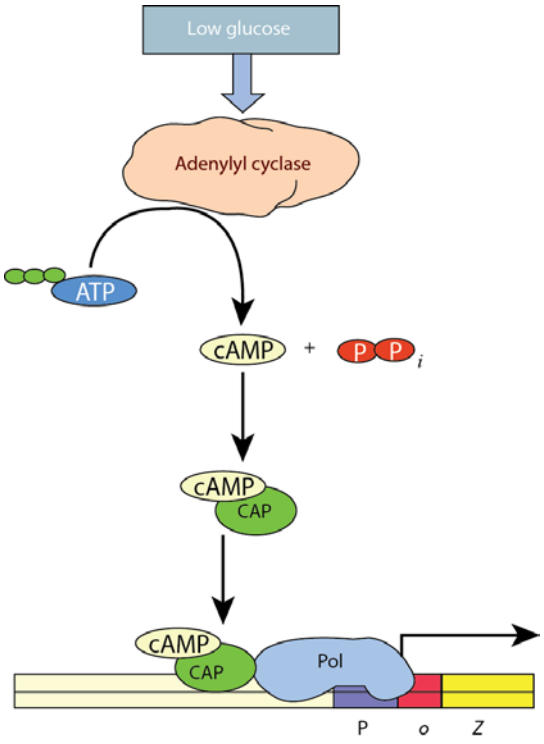

Figure 4: Positive regulation of lac operon by glucose repression coupled to enhance level of cAMP.

6. In some slime moulds: In some slime mold species such as *Dictyostelium discoideum*, the chemotactic movement of cells is organized by periodic waves of cAMP that propagates through the cell. The waves are the result of a regulated production and secretion of extracellular cAMP and a spontaneous biological oscillator that initiates the waves at centers of territories.

7. Mitochondrial Biogenesis: cAMP/PKA has key role in mitochondrial compartment biogenesis. Mitochondria takes part in several vital cellular functions. For example they are involved in ATP production, via the process of oxidative phosphorylation, the ATP production via mitrochondria is 15 times more than glycolysis alone. They also take central part in metabolic regulation and assist diverse cell signalling events. Mitochondria are therefore essential for the maintenance, adaptability and survival of eukaryotic cells. cAMP/PKA signaling balances respiratory activity with mitochondria dependent apoptosis via transcriptional regulation.

Areas of action of cyclic AMP:

There are so many application of cAMP for the prevention of several disorders in human beings. Some are shown below:

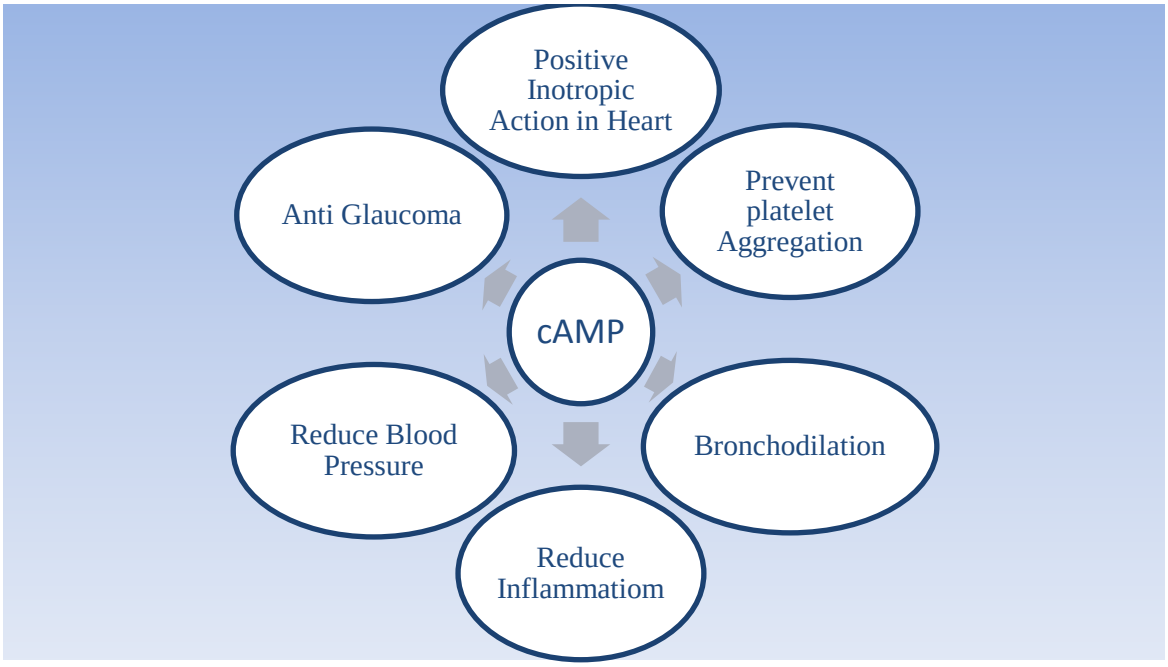

Figure 5: Action of cAMP in our body

Mechanism of Regulation of cAMP: When stimulatory hormone binds to G-protein coupled receptor some conformational changes occur in the receptor by which its unexposed catalytic part gets exposed due to which it can interact and activate G-protein with exchange of GDP by GTP at G_α subunit. Now G_α subunit goes and activates membrane bound adenylyl cyclase that facilitates the conversion of ATP to cAMP. This cAMP is now ready to activate protein kinase A which is involved in phosphorylation of several proteins that takes part in the further cellular responses. At the same time of cAMP production by adenylyl cyclase, the cAMP level is controlled by other enzyme that is called cAMP phosphodiesterase which converts cAMP into 5'-AMP. On the other hand, when inhibitory hormone binds to G-protein coupled receptor, it counters the effect as shown in Figure 6. In this process, the adenylyl cyclase is inhibited because GPCR activates the G protein that contains inhibitory alpha subunit which binds to enzyme and inhibits cAMP production.

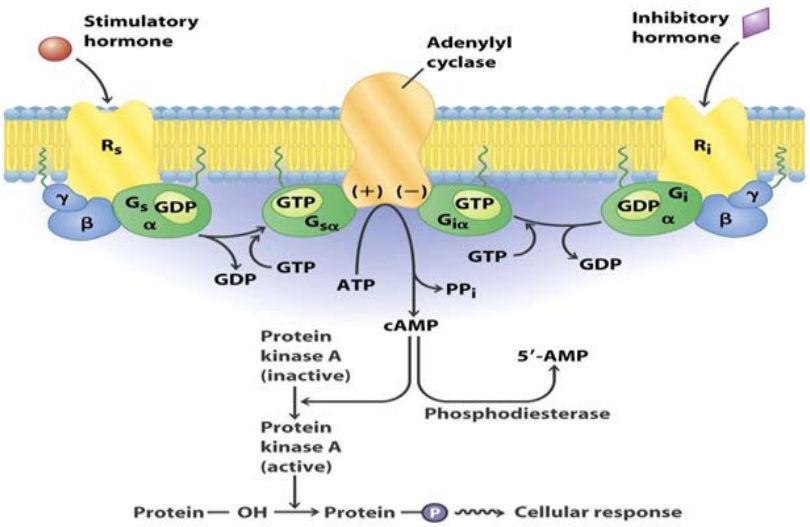

Figure 6: Synthesis and Regulation of cAMP

Synthesis and Degradation of cAMP: cAMP is a cyclic nucleotide which serves as an intracellular and in some cases extracellular secondary messenger. It is derived from adenosine triphosphate (ATP) by adenylyl cyclase located on inner side of plasma membrane and used for intracellular signal transduction in many different organisms, conveying the cAMP-dependent pathway. The elevated cAMP level is regulated by degradation pathway which takes place by cAMP phosphodiesterase enzyme.

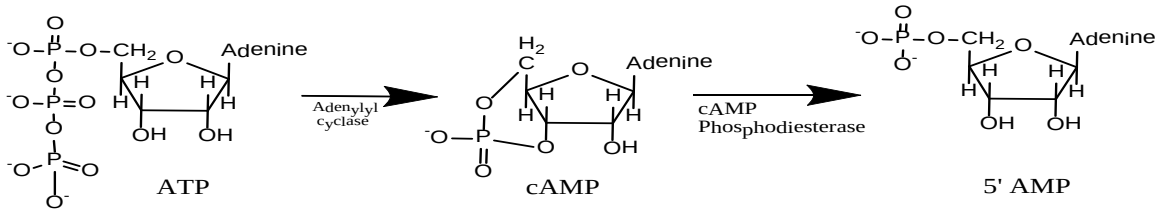

Figure 7: Synthesis and degradation of cAMP

Interesting facts:

1. Cyclic AMP is synthesized from ATP by the action of the enzyme adenylyl cyclase.
2. cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase.

3. cAMP and its associated kinases function in several biochemical processes, including the regulation of glycogen, sugar, and lipid metabolism.

Questions:

1. Explain the effect of glucose concentration on the cAMP and catabolic activator protein?
2. How cAMP involve in signal transduction pathway?
3. How cAMP synthesizes and regulated in our body?

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M5 L4
Second messengers - cGMP

Cyclic Guanosine monophosphate: Cyclic Guanosine monophosphate or cGMP, is a cyclic nucleotide derived from guanosine triphosphate(GTP). cGMP is a multi-functional second messenger molecule, similar in action to cAMP but generally producing opposite effects on cell function. It has molecular formula of $C_{10}H_{12}N_5O_7P$ and has molecular weight of 345.2 g/mol. It has composition of Guanine nucleotide base, ribose sugar and cyclic phosphate between 3' and 5' positions of ribose sugar as shown in figure 1. Cyclic GMP is synthesized from the nucleotide GTP using the enzyme guanylyl cyclise as shown in figure 2.

Structure:

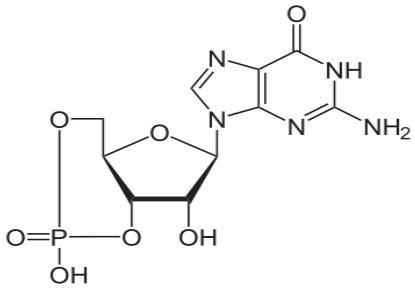

Figure 1: Guanosine 3',5'-cyclic phosphate

Synthesis of cGMP: cGMP is an important signal molecule that carries different messeges in different tissues. cGMP is generated via two pathways distinguished by the nature of Guanylyl Cyclase (GC) that mediate its conversion from guanosine triphosphate (GTP). The guanyl cyclase is generally found in cell in two forms - soluble form and membrane- bound form. They are generated via two pathways as described below:

1. The soluble pathway, where cGMP is generated via nitric oxide (NO)-activated guanylyl cyclase which is cytosolic protein with tightly associated heme group. NO is sufficiently non-polar which can easily cross the plasma membrane of target cell without any carrier and binds to the heme group of guanylyl cyclase and activates the cGMP production.

2. In the membrane-bound pathway, GCs is transmembrane protein with extracellular ligand binding domain, share some homology with those activated by NO. The ligands for a subset of membrane GCs are members of the Natriuretic Peptide (NP) hormone family including atrial NP hormone, B-type NP hormone and C-type NP hormone.

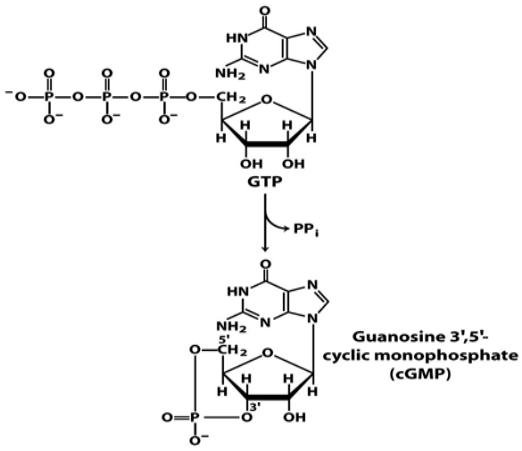

Figure 2: Conversion of GTP to cGMP

Functions of cGMP:

1. cGMP is an important molecule of the cell that takes part in various activities in cellular system. When guanylyl cyclase stimulation leads to elevated levels of cGMP, it then mediates biological responses, such as blood vessel dilation which increases blood flow.

2. The action of cGMP is regularly facilitated by stimulation of cGMP dependent protein kinases, although cGMP is a common regulator of ion channel conductance, glycogenolysis, cellular apoptosis and phosphodiesterases.

3. Another well-known role of cGMP is in the vertebrate eye, where it serves as the second messenger responsible for converting the visual signals received as light to nerve impulses. The photoreceptor in rod cells of the retina is a G protein-coupled receptor called rhodopsin. When light falls on the extracellular side of rhodopsin, then some conformational changes occurs in it by which its bounded chromophore 11-cis retinal is converted to all-trans retinal form, ultimately rhodopsine's unexposed catalytic cytoplasmic side gets exposed which interacts with the G protein transducin and activates them by replacement of GDP by GTP on its α- subunit. The activated G_α then activates cGMP phosphodiesterase 6 which converts all cGMP into 5' GMP. Due to this cGMP level gradually decreases, the cGMP dependent sodium ion-channel becomes closed. This

channel is also entry site of calicium ions so Ca^{++} level also decrease. This critical situation created in the cell is called hyperpolyrisation. But after decreasing Ca^{++} level, guanylyl cyclase is activated and again cGMP synthesis starts. Thus we can summarize the whole phenomenon as, change in cGMP level in retinal rod cells is translated to a nerve impulse by a direct effect of cGMP on ion channels in the plasma membrane.

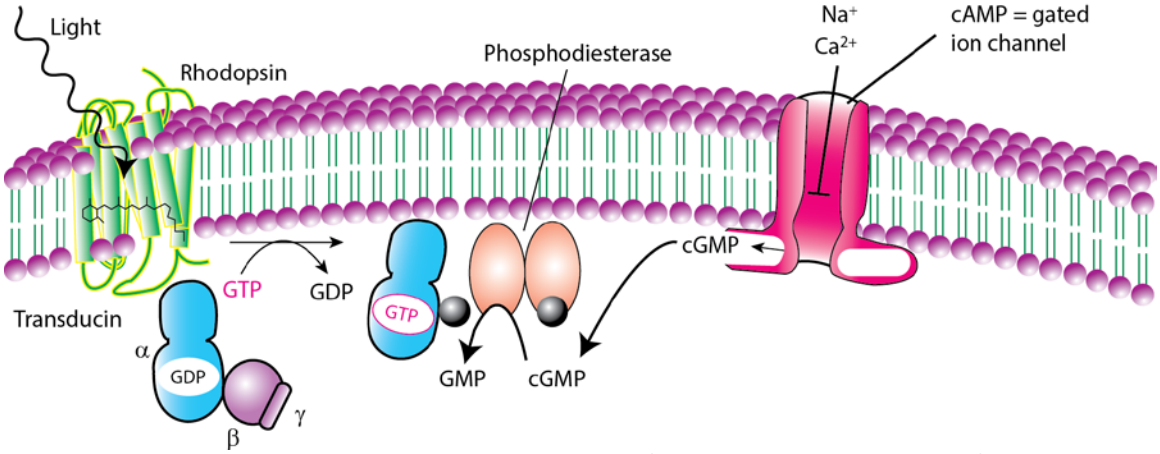

Figure 3: Visual Signal Transduction. The light-induced activation of rhodopsin leads to the hydrolysis of cGMP, which in turn leads to ion channel closing and the initiation of an action potential.

4. In kidney, membrane bound guanylyl cyclase is activated by the hormone atrial natriuretic factor (ANF), which is released by cells in atrium of the heart when the heart is stretched by increased blood volume. ANF comes to kidney with blood from heart and activates membrane bound guanylyl cyclase in the cells of collecting ducts. The resulting rise in cGMP level in cells triggers increase renal excretion of Na^{+} and water, driven by change in osmotic pressure. Water loss causes reduction of blood volume which counters the production of ANF. Vascular smooth muscle also contains ANF receptor-guanylyl cyclase which release. On binding to these receptor , ANF causes relaxation of blood vessels which increases the blood flow with decreasing blood pressure.

cGMP, like cAMP get synthesized by receiving odourous input by olfactory receptor. cGMP is produced slowly and has a more sustained life than cAMP. cGMP in the olfactory is synthesized by both membrane guanylylcylcase (mGC) as well as soluble guanylyl cyclase (sGC). cGMP synthesis in the olfactory is due to sGC activation by nitric oxide, a neurotransmitter. cGMP also requires increased intracellular levels of

cAMP and the cross-link between the two second messengers seems to be due to rising intracellular calcium levels.

Vasodilation: NO is an extracellular gaseous second messenger. NO is unusual because it acts both as an extracellular messenger, mediating intercellular communication, and as a second messenger, acting within the cell in which it is generated. NO is synthesized by L-arginine which is catalyzed by nitric oxide synthesae. The NO formed in the endothelial cell diffuses across the plasma membrane and into the adjacent smooth muscle cells, where it binds and stimulates guanylyl cyclase which synthesizes cyclic GMP (cGMP).

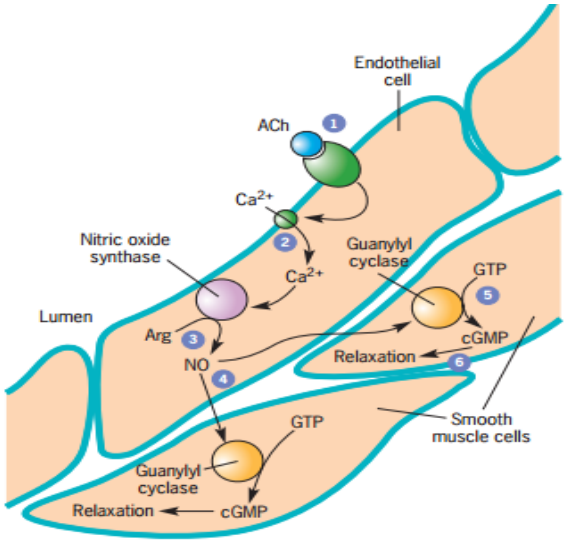

Figure 4: Signal transduction pathway leads to dilation of blood vessel through NO and cGMP.

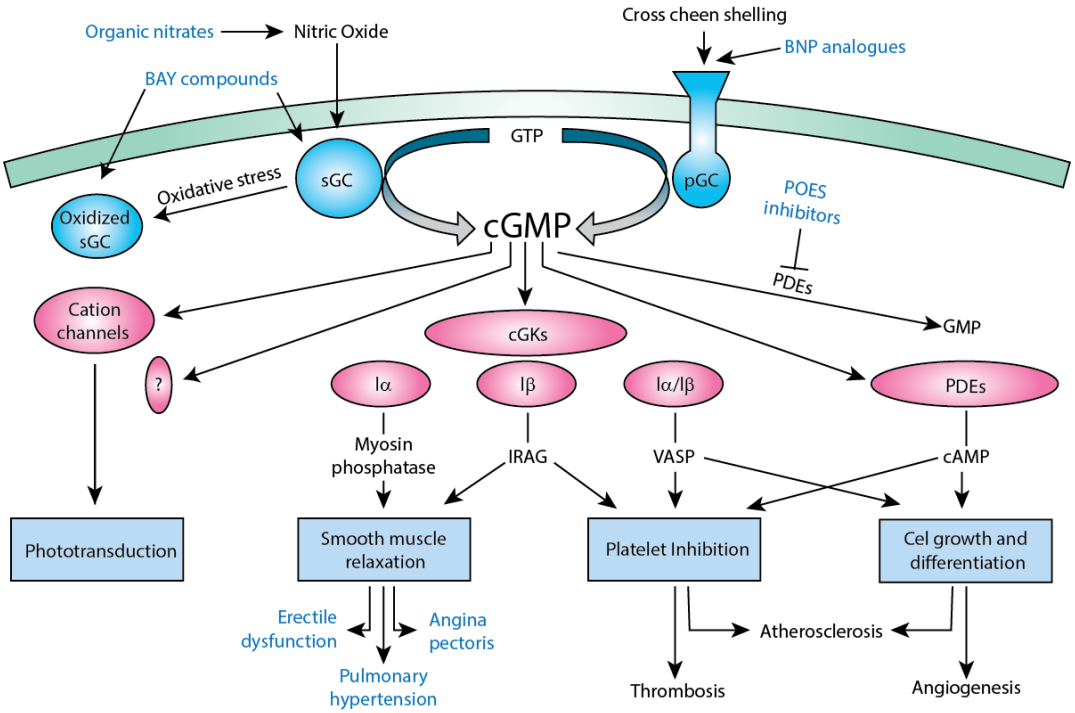

Figure 5: Pathways of generation of cGMP and several areas of effect

The conversion mechanism of GTP to cGMP by guanyl cyclase is shown below:

Regulation Pathway of cGMP: In animals, most of the actions of cGMP are supposed to be mediated by cGMP-dependent protein kinase, which is also called protein kinase G, abbreviated as PKG. After activation of PKG by cGMP, phosphorylates Ser and Thr residues in target proteins. PKG has catalytic and regulatory domains on a single polypeptide chain (*Mr*, 80,000). A part of regulatory domains fit comfortably in substrate-binding site. After binding of cGMP forces this part of regulatory domain out of the binding site, activating the catalytic domain.

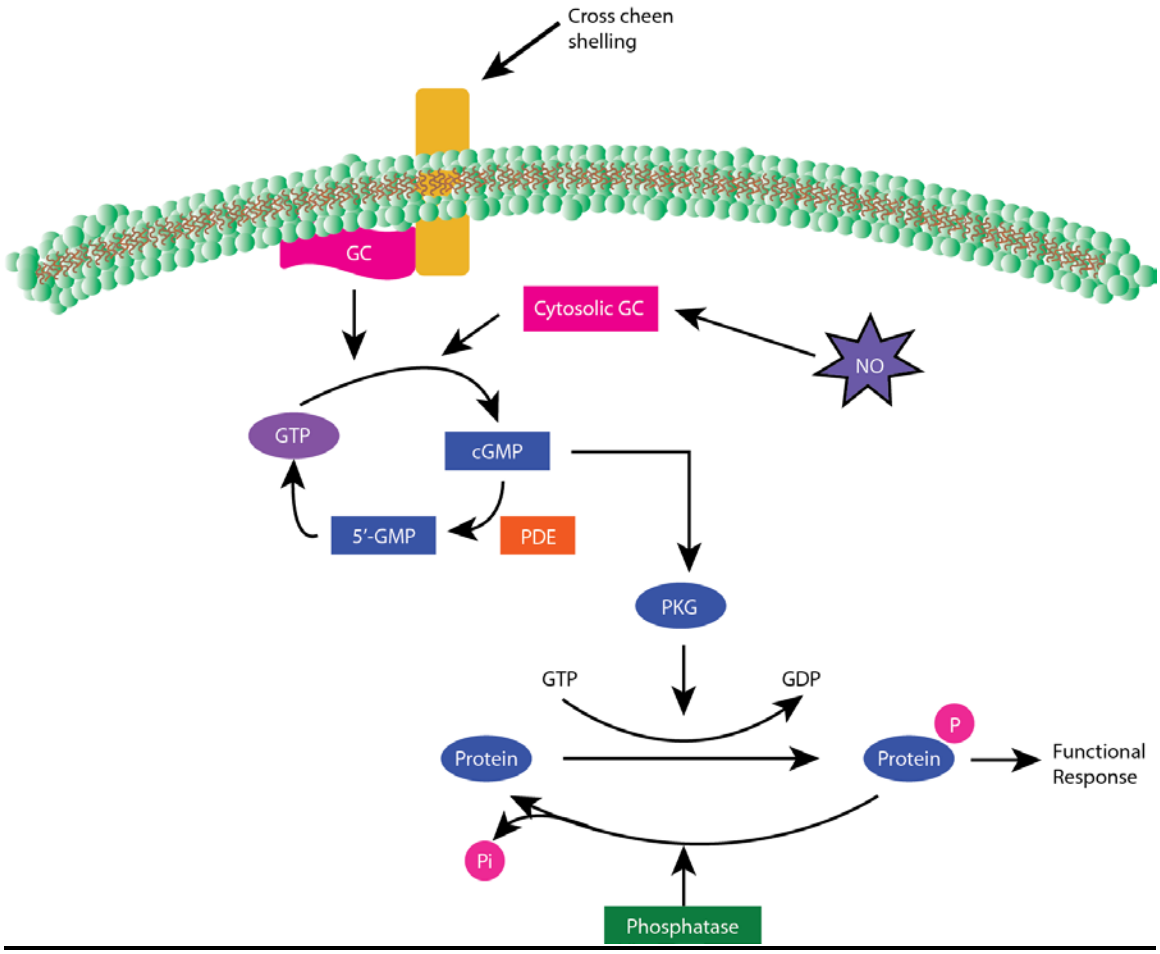

Figure 6: Regulatory Pathway of cGMP

Interesting facts:

1. Cyclic GMP is synthesized from the nucleotide GTP using the enzyme guanylyl cyclase.

2. Cyclic GMP serves as the second messenger for atrial natriuretic peptide (ANP), nitric oxide (NO), the response of the rods of the retina to light.

3. Some of the effects of cGMP are mediated through Protein Kinase G (PKG) a cGMP-dependent protein kinase that phosphorylates target proteins in the cell.

Questions:

1. How cGMP is involved in vasodilation or smooth muscle relaxation?
2. What is the visual pathway for light transduction in brain?
3. How cGMP regulation take place and what are the enzyme involve in its regulation?

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M5 L5
Calcium ion flux and its role in cell signalling

Calcium ion flux: Calcium ions are also important intracellular messengers. In fact, calcium ions are probably the most widely used intracellular messengers. Calcium (Ca^{2+}) plays an essential role in the physiology and biochemistry of organisms and the cell. It plays role in common signalling mechanism because once it enters the cytoplasm it exerts allosteric regulatory affects on many enzymes and proteins. Calcium is a second messenger produced by indirect signal transduction pathways such as G-protein coupled receptors. Calcium ions (Ca^{2+}) impact nearly every aspect of cellular life. The principles of Ca^{2+} signaling, from changes in protein conformations driven by Ca^{2+} to the mechanisms that control Ca^{2+} levels in the cytoplasm and organelles. The highly localized nature of Ca^{2+}-mediated signal transduction and its specific roles in excitability, exocytosis, motility, apoptosis, and transcription. Normally, cytosolic calcium [Ca^{2+}] ions is kept very low (10^{-7} M) by the action of Ca^{2+} pumps in the ER, mitochondria and plasma membrane. Hormonal, neural, or other stimuli cause either an influx of Ca^{2+} into the cell through specific Ca^{2+} channels in the plasma membrane or the release of sequestered Ca^{2+} from the ER or mitochondria, in either case raising the cytosolic [Ca^{2+}] and triggering a cellular response. This phenomenon is called Calcium ion flux.

Role of Calcium in cell signalling:

In response to many different signals, a rise in the concentration of Ca^{2+} in the cytosol triggers many types of events such as:

- Muscle contraction
- Exocytosis

  a) Release of neurotransmitters at synapses (and essential for the long-term synaptic changes that produce Long-Term Potentiation (LTP) and Long-Term Depression (LTD)

  b) Secretion of hormones like insulin
- Activation of T cells and B cells when they bind antigen with their antigen receptors (TCRs and BCRs respectively)

- Adhesion of cells to the extracellular matrix (ECM)
- Apoptosis
- A variety of biochemical changes mediated by Protein Kinase C (PKC)

The concentration of calcium ions in a particular cellular compartment is guarded by the regulated activity of Ca^{2+} pumps, Ca^{2+} exchangers, and/or Ca^{2+} ion channels located within the membranes that surrounds the compartment. There are two major types of signaling receptors- G-protein coupled receptor (GPCRs) and receptor protein-tyrosine kinases (RTKs). One of the most important pathways of intracellular signalling is based on the use of second messengers derived from the membrane phospholipids phosphatidylinositol 4, 5-bisphosphate (PIP_2). PIP_2 is a minor component of the plasma membrane, localized to the inner leaflet of the phospholipids bilayer. Hydrolysis of PIP_2 by phospholipase C-β is stimulated by variety of hormones and neurotransmitters. After hydrolysis PIP_2 is cleaved into two components- Diacyl glycerol (DAG) and inositol l,4,5- triphosphate (IP_3), both are also the secondary messengers of cell. Diacylglycerol and IP_3 stimulate distinct down-stream signalling pathways (protein kinase C and Ca^{2+} mobilization, respectively), so PIP_2 hydrolysis triggers a two-armed cascade of intracellular signalling.

The first secondary messenger diacylglycerol produced by hydrolysis of PIP_2 remains associated with the plasma membrane and activates protein-serine/threonine kinases belonging to the protein kinase C family, many of which play important roles in the control of cell growth and differentiation.

The other second messenger produced by PIP_2 cleavage, IP_3, is a small polar molecule that is released into the cytosol, where it acts to signal the release of Ca^{2+} from intracellular stores.

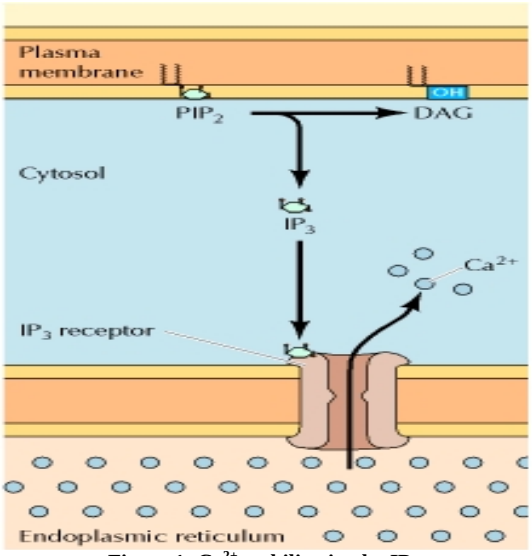

Figure 1: Ca^{2+} mobilization by IP_3

Whereas the other important pathway in which extracellular messengers that signal through RTKs can trigger a similar response as in GPCR signal pathway. The major difference is that RTKs activate members of the phospholipase C-γ subfamily, which possess an SH_2 domain that allows them to bind to the activated, phosphorylated RTK.

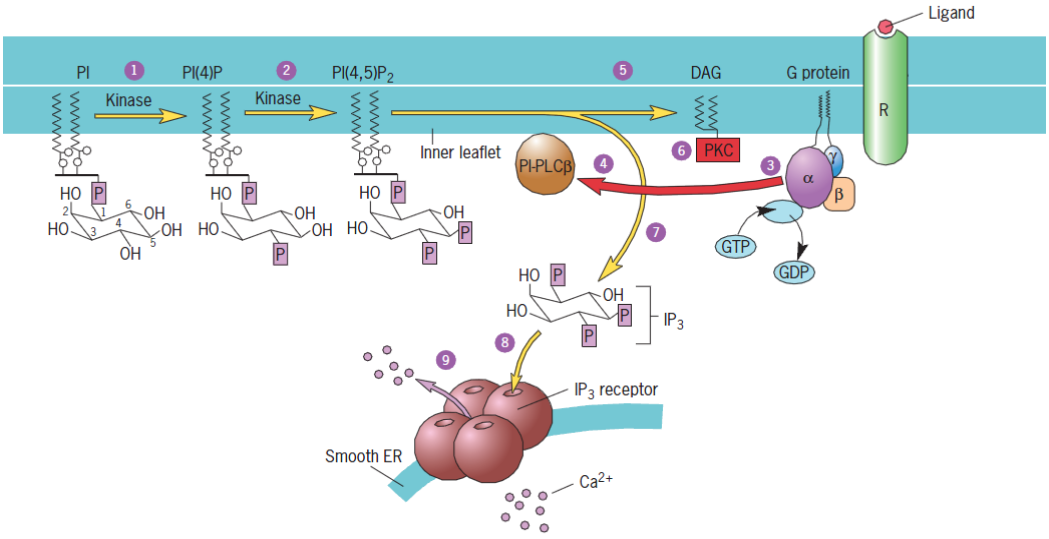

Figure 2: Comparative pathway of GPCR and RTKs for release of calcium from ER

There are numerous other PLC isoforms. For example, PLC δ is activated by Ca^{2+} ions, and PLCε is activated by Ras-GTP. All PLC isoforms carry out the same reaction,

producing IP_3 and linking a multitude of cell surface receptors to an increase in cytoplasmic Ca^{2+}. There is another major route leading to elevation of cytosolic [Ca^{2+}] which involve in synaptic transmission. In this case, a nerve impulse leads to a depolarization of the plasma membrane, which triggers the opening of voltage-gated calcium channels in the plasma membrane, allowing the influx of Ca^{2+} ions from the extracellular medium.

Properties of Calcium Ion (Ca^{2+})

There are two major properties that allow Calcium (Ca^{2+}) ion to work effectively as a signaling mechanism:

- Ca^{2+} levels inside the cell are readily detectable. This is because the levels of Ca^{2+} are highly regulated by transport systems that expel Ca^{2+} from the cell. The level of Ca^{2+} in the cytoplasm is approximately 100nM, which are several orders of magnitude lower than outside the cell as a result of Ca^{2+} pumps that actively export Ca^{2+} from the cell. Ca^{2+} is pumped not only across the plasma membrane but also into the endoplasmic reticulum, which therefore serves as an intracellular Ca^{2+} store. IP_3 acts to release Ca^{2+} from the endoplasmic reticulum by binding to receptors that are ligand-gated Ca^{2+} channels. As a result, cytosolic Ca^{2+} levels increase to about 1 μM, which affects the activities of a variety of target proteins, including protein kinases and phosphatases.

- Ca^{2+} can readily bind to proteins and cause conformational changes. Ca^{2+} is attracted to the negatively charged oxygen atoms in the side chains of glutamate and asparagines, and the uncharged oxygen in both the side chains and main chains of glutamine and asparagine. Ca^{2+} is readily able to cause large conformational changes due to the fact that it can form ligand with up to eight oxygen atoms. This can lead to cross linking of amino acids in a protein that did not exist before Ca^{2+} was introduced.

Function of Ca^{2+} in cell:

- Protein function is governed by shape and charge. Ca^{2+} binding triggers changes in protein shape and charge. Similarly, phosphorylation imparts a negative charge, altering protein conformations and their interactions. Protein kinases, comprising ~2% of eukaryotic genomes, remove phosphate from ATP and covalently attach it to the free hydroxyl groups of serine, threonine, or tyrosine residues. The abilities of Ca2+ and phosphate ions to alter local electrostatic fields and protein conformations are the two universal tools of signal transduction.
- Ca^{2+} had a new and totally unexpected function: it carried the information necessary for the contraction of heart.
- Changes in intracellular [Ca^{2+}] are detected by Ca^{2+}-binding proteins that regulate a variety of Ca^{2+}-dependent enzymes.

Example of Ca^{2+}-dependent enzymes**:**

- **Calmodulin (CaM)** (*M*r 17,000) is an acidic protein with four high-affinity Ca^{2+}-binding sites. When intracellular [Ca^{2+}] rises to about 10^{-6} M (1 μM), the binding of Ca^{2+} to calmodulin drives a conformational change in the protein. Calmodulin associates with a variety of proteins and, in its Ca^{2+}-bound state, modulates their activities. Calmodulin is a member of a family of Ca^{2+}-binding proteins that also includes troponin, which triggers skeletal muscle contraction in response to increased [Ca^{2+}]. This family shares a characteristic Ca^{2+}-binding structure.
- **C**a*l*modulin is also an integral subunit of a family of enzyme[s,] the Ca^{2+}/calmodulin-dependent protein [k]inases (CaM kinases [I]–IV). *W*hen intracellular [Ca^{2+}] increases in response to some stimulus, calmodulin binds Ca^{2+}, undergoes a change in conformation, and activates the CaM ki[na]se. The kinase then phosphorylates a number of target enzymes, regulating their ac[ti]vities. Calmodulin is also a regulatory subunit of phosphorylase *b* kinase of muscle, which is activated by Ca^{2+}. Thus $^{Ca2+}$ triggers ATP-requiring muscle contract[io]ns while also activating glycogen breakdown, providing fuel for ATP synthesis. Many other enzymes are also known to be modulated by Ca^{2+} through calmodulin.

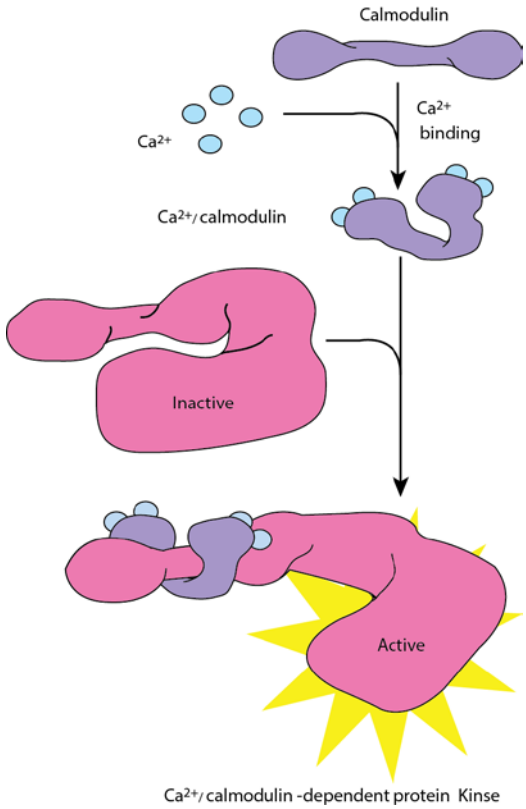

Figure 3: Calcium ion dependent enzyme- calmodulin and Ca2+/calmodulin-dependent protein kinases

- The rise of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) is an crucial triggering signal for T-cell activation by antigen and other stimuli that cross-link the T cell antigen receptor (TCR).
- The downstream consequences of Ca^{2+} signalling, also involve in gene expression on the influence of signal complexity.

Some Proteins Regulated by Ca^{2+} and Calmodulin:

- Adenylyl cyclase (brain)
- Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases I to IV)
- Ca^{2+}-dependent Na^{+} channel (*Paramecium*)
- Ca^{2+}_-release channel of sarcoplasmic reticulum
- Calcineurin (phosphoprotein phosphatase 2B)
- cAMP phosphodiesterase
- cAMP-gated olfactory channel
- cGMP-gated Na^{+}, Ca^{2+} channels (rod and cone cells)
- Glutamate decarboxylase
- Myosin light chain kinases
- NAD^{+} kinase
- Nitric oxide synthase
- Phosphoinositide 3-kinase
- Plasma membrane Ca^{2+} ATPase (Ca^{2+} pump)
- RNA helicase (p68)

Interesting facts:

1. Calcium ions are probably the most widely used intracellular messengers.

2. Normally, the level of calcium in the cell is very low (~100 nM).

3. Getting Ca^{2+} into (and out of) the cytosol is via Voltage-gated channels.

Questions:

1. Ca^{2+}, IP_3 and cAMP have all been described as second messengers. In what ways are their mechanisms of action similar? In what ways are they different?

2. Describe the function of Ca^{2+} as a second messenger.

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M5 L6
G proteins in signal transduction

G- Protein: G proteins (guanine nucleotide-binding proteins) are a family of proteins involved in transmitting chemical signals outside the cell, and causing changes inside the cell. They communicate signals from many hormones, neurotransmitters, and other signaling factors.

Type of G protein:

G protein can refer to two distinct families of proteins.

- **Heterotrimeric G proteins:** sometimes also known as the large G proteins that are activated by G protein-coupled receptors and made up of alpha (α), beta (β), and gamma (γ) subunits.

- **Small G proteins:** They are proteins of 20-25kDa that belong to the Ras superfamily of small GTPases. These proteins are homologous to the alpha (α) subunit found in heterotrimers, and are in fact monomeric. However, they also bind GTP and GDP and are involved in signal transduction.

Heterotrimeric G-protein

Heterotrimeric G proteins are more complex protein which were first characterized by Martin Rodbellare. It consists of three different subunits- α, β, and γ having molecular weight of these are 45, 37, and 9 kD respectively, among these α subunit binds to GDP in unactive state or GTP in active state, hence heterotrimeric G-protein is known as a member of the G protein superfamily. Heterotrimeric G proteins are held at the plasma membrane by lipid chains that are covalently attached to the α and γ subunits.

Structure of Heterotrimeric G-protein:

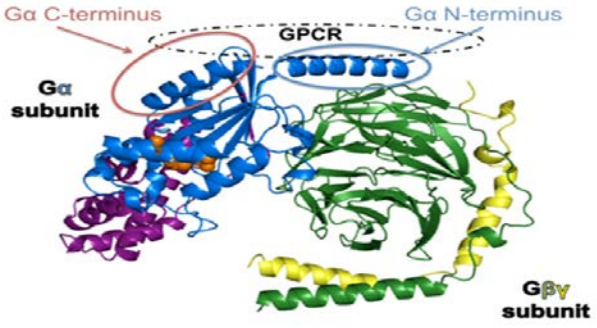

Figure 1: Structure of Heterotrimeric G-protein

The alpha subunit has two domains - the transducing insertion domain fold whereas the other is a P-loop containing nucleoside triphosphate hydrolase fold. P-loop or a phosphate-binding loop is an ATP or GTP- binding site motif found in many nucleotide-binding proteins. It is a glycine-rich loop led by a beta sheet and followed by an alpha helix. It interacts with the nucleotide phosphate groups and with the Mg^{2+} ion that coordinates the β- and γ-phosphates in GTP. Upon nucleotide hydrolysis the P-loop does not significantly change conformation, but stays bound to the remaining phosphate groups. β- and γ- subunits are usually anchored to the membrane by covalently attached fatty acids. $G_{\beta\gamma}$ can also directly participate in signal transduction. It activates a wide variety of signaling proteins including several isoforms of Adenylate Cyclase.

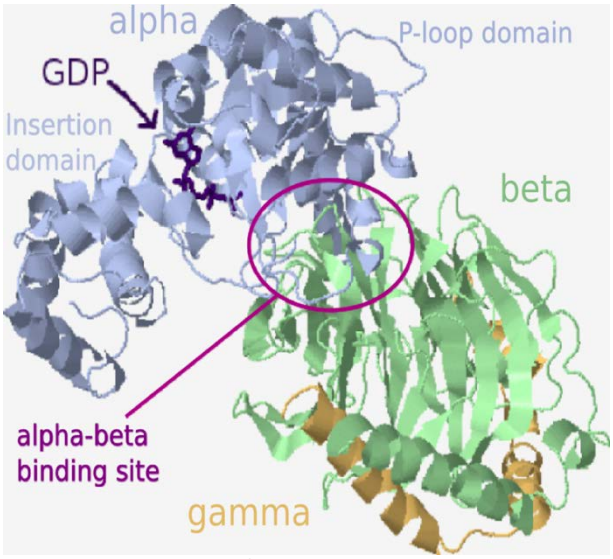

Figure 2: Close view of G-protein with loops and subunits

Small G protein

These proteins belong to a large superfamily referred to as small G proteins based on their low M_r of 20,000 to 35,000. The small G proteins, like the heterotrimeric G proteins, bind guanine nucleotides, possess intrinsic GTPase activity and cycle through GDP- and GTP-bound forms. One unifying feature of the various classes of G protein is that the binding of GTP versus GDP dramatically alters the affinity of the protein for some target molecule, apparently by inducing a large conformational change. Small G proteins appear to function as molecular switches that control several cellular processes.

Examples of small G-protein: These are the following cellular actions which are performed by small G-protein.

Table 1: Classification of small G-proteins on the basis of their functions

Sl. no.	Class	Proposed cellular function
1.	Ras	Signal transduction (control of growth factor and MAP- kinase pathway)
2.	Rac, CDC42	Signal transduction (control of cellular stress responses and MAP-kinase pathways)
3.	Rab	Localized to synaptic vesicle, where it regulates vesicle trafficking and exocytosis
4.	Rho	Assembly of cytoskeleton structures (e.g. actin microfilament)
5.	ARF	ADP-ribosylation
6.	EFTU	Associated with ribosomes where it regulates protein synthesis
7.	Ran	Nuclear-cytoplasmic trafficking of RNA and protein

Conformational changes occur in G protein during nucleotide exchange:

When G- protein couple receptor comes in contact of ligand then some conformational changes occurs in GPCR; then the activated GPCR interact with G-protein, causes conformational change in alpha subunit of G-protein. Due to conformational change, the G-protein that bound to GDP prior to activation get exchange by GTP through which the three switch regions on the α- subunit close to the nucleoside triphosphate, generating the active conformation.

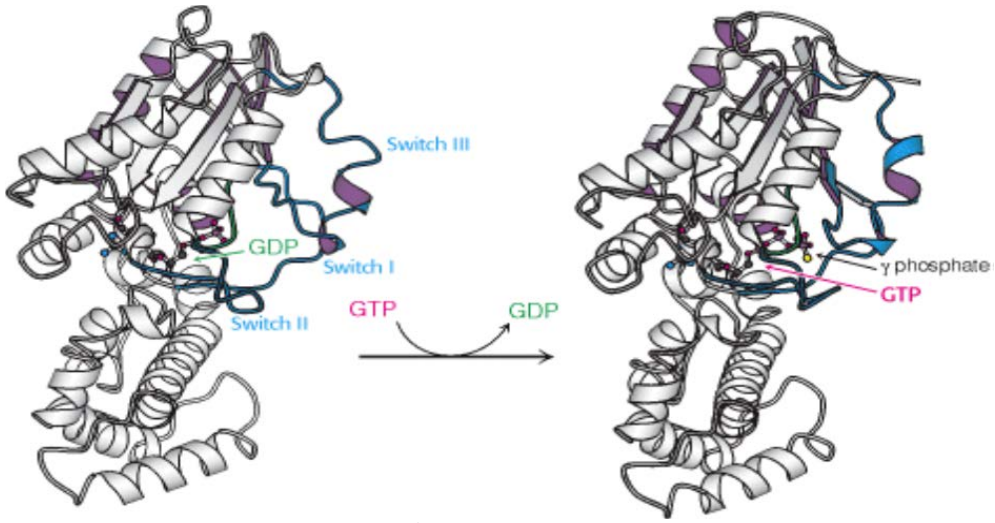

Figure 3: Conformational change in G-protein

Nature of G protein: G-Protein generally found in two states- *active form and unactive form.*

In unactivated state, the guanylyl nucleotide bound to the G-Protein is GDP. In this form, the G-protein exists as a heterotrimer consisting of α, β and γ subunits in which the α subunit (G_α) binds to nucleotide. The role of the hormone- bound receptor is to catalyze the exchange of GTP for GDP. Thus, inactive G-protein converted to active form and G_α subunit has no much affinity for $G_{\beta\gamma}$ subunits, hence α-subunit dissociated from βγ-subunits. Now this is called activated state of G-protein. In activated state, G_α subunit stimulate effector protein such as adenylyl cyclase that lead to production of second messenger cAMP which may activate one or more signalling molecules. Other effectors are cGMP phosphodiesterase, phospholipase C-β. After dissociation from the G_α subunit, the βγ complex also has a signaling function and it can couple to at least four different types of effectors: PLC-β, K^+ ion channels, adenylyl cyclase, and PI 3-kinase.

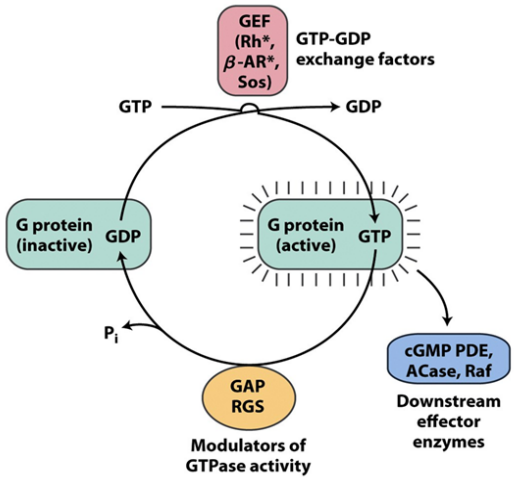

Figure 4: Regulation of G- protein activation and inactivation

After certain amount of time passed , the G_α turn themselves off by hydrolysis of bound GTP to GDP and inorganic phosphate (Pi). This results in conformational change caused decrease in affinity for effector and increase in affinity for βγ complex, thus G_α subunit dissociate from effector and associate with βγ subunit for the reformation of inactive form of G-protein.

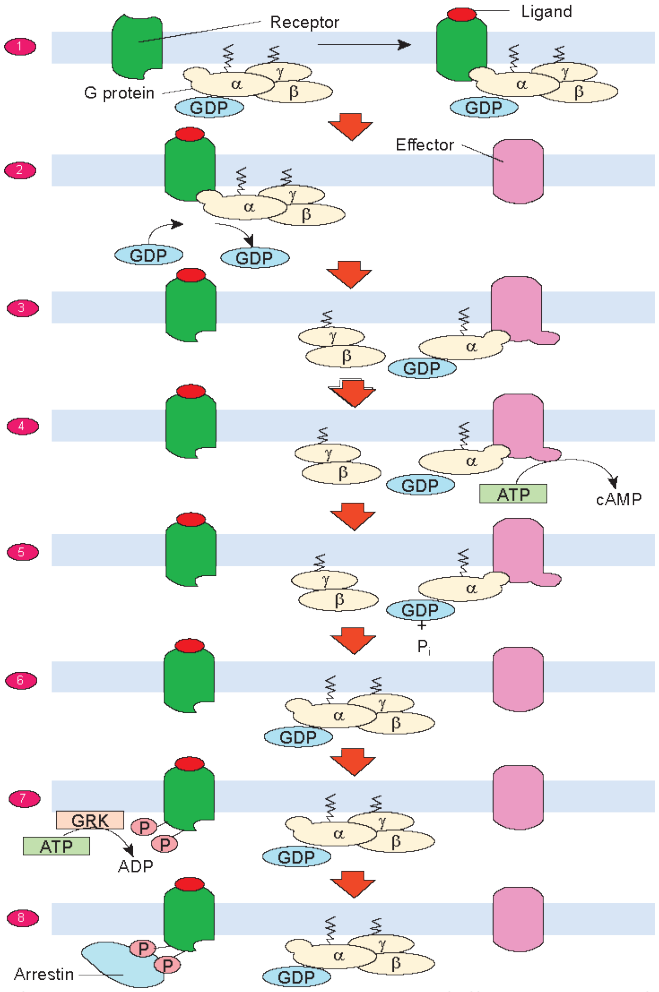

Figure 5: The mechanism of receptor-mediated activation (or inhibition) of effectors by means of heterotrimeric G proteins

Heterotrimeric G proteins come in five flavors, G_s, G_q, $G_{t\alpha}$, G_i, and $G_{12/13}$. This classification is based on the G_α subunits and the effectors to which they couple. The particular response produced by an activated GPCR depends on the type of G protein with which it interacts, although some GPCRs can interact with different G proteins and trigger more than one physiologic response.

1. **G_s** family members couple receptors to adenylyl cyclase. Adenylyl cyclase is activated by GTP-bound G_s subunits.

2. **$G_{q\alpha}$,** Gq family members contain G_α subunits that activate PLC-β. PLC-β hydrolyzes phosphatidylinositol bisphosphate, producing inositol trisphosphate and diacylglycerol.
3. **Transducin ($G_{t\alpha}$)**, a variant of $G_{i\alpha}$, which transduces visual stimuli by coupling the light-induced conformational change of rhodopsin to the activation of a specific phosphodiesterase, which then hydrolyzes cGMP to GMP. This cGMP-phosphodiesterase (cGMP-PDE) is an $\alpha\beta\gamma_2$ heterotetramer that is activated by the displacement of its inhibitory subunits (PDE) by their tighter binding to $G_{t\alpha}$. GTP. A cation-specific transmembrane channel that is held open by the binding of cGMP closes on the resulting reduction in [cGMP], thereby triggering a nerve impulse indicating that light has been detected.
4. **G_i** , Activated G_i subunits function by inhibiting adenylyl cyclase
5. **G_{olf},** a variant of $G_{s\alpha}$, which is expressed only in olfactory sensory neurons and participates in odorant signal transduction.
6. **$G_{12\alpha}$**and **$G_{13\alpha}$,** $G_{12/13}$ members are less well characterized than the other G protein families although their inappropriate activation has been associated with excessive cell proliferation and malignant transformation.

Thus heterogeneity in G proteins occurs in the β and γ subunits as well as in the α subunits. In fact, 21 different α subunits, 6 different β subunits, and 12 different γ subunits have been identified in humans, some of which appear to be ubiquitously expressed whereas others are expressed only in specific cells.Thus, a cell may contain several closely related G proteins of a given type that interact with varying specificities with receptors and effectors.This complex signalling system presumably permits cells to respond in a graded manner to a variety of stimuli.

Role of G protein in signal transduction

Since, organs or cells in order to do their function properly within an organism must have capacity to respond signals from distant cells as well as from its local environment. Thus the process in which information carried by extracellular messenger molecules is translated into changes that occur inside a cell is referred to as **signal transduction**. In this process:

- Cells in different organs communicate with one another through extracellular signalling molecules released by one set of cells and received by the other.
- Not all molecules can pass through the lipid bilayer of a Cell, and so signal transduction systems are used in order to transmit an external signal to the cell interior.

G-proteins play key role in signal transduction with the help of G- protein couple receptor which abbreviated as GPCR.

G- protein couple receptor: GPCR comprise a large protein family of transmembrane receptors that sense molecules outside the cell and activate inside signal transduction pathways and ultimately, cellular responses. G protein-coupled receptors are found only in eukaryotes, including yeast, choanoflagellates and animals. The ligands that bind and activate these receptors include light-sensitive compounds, odors, pheromones, hormones, and neurotransmitters, and vary in size from small molecules to peptides to large proteins. G protein-coupled receptors are involved in many diseases, and are also the target of approximately 40% of all modern medicinal drugs.

Classification of GPCR: The exact size of the GPCR superfamily is unknown but nearly 800 different human genes (or ≈4% of the entire protein-coding genome) have been predicted from genome sequence analysis. Although numerous classification schemes have been proposed, the superfamily is classically divided into three main classes (A, B, and C) with no detectable shared sequence homology between classes. The largest class so far is class A, which accounts for nearly 85% of the GPCR genes. Of class A GPCRs, over half of these are predicted to encode olfactory receptors while the remaining receptors are liganded by known endogenous compounds or are classified as orphan receptors. Despite the lack of sequence homology between classes, all GPCRs share a common structure and mechanism of signal transduction.

In general, GPCRs can be classified into 5 classes based on sequence homology and functional similarity:

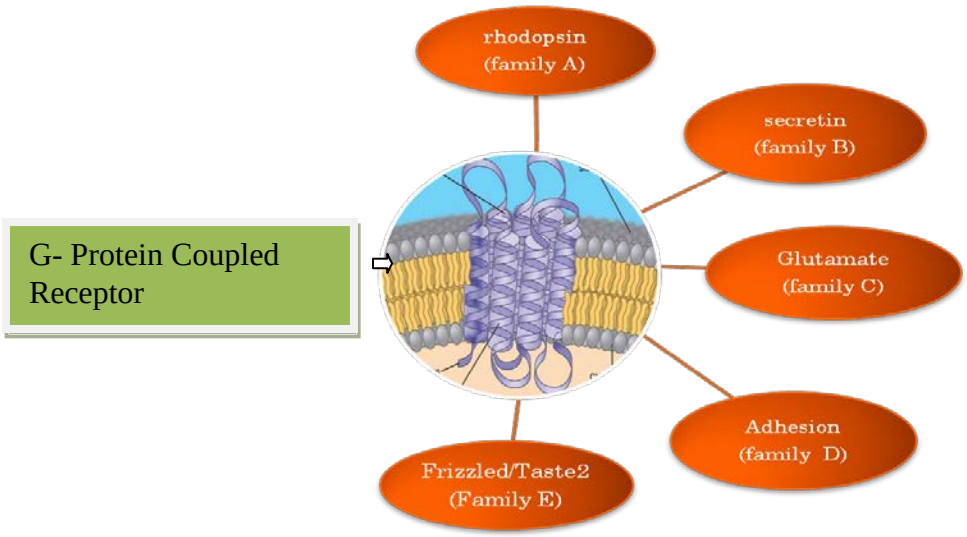

Figure 6: Classification of G-protein

Role of G- protein coupled receptor: GPCRs are involved in a wide variety of physiological processes. Some examples of their physiological roles are as follows:

1. The visual sense: Rhodopsine which is complex of opsins and chromophore 11-cis retinal, use a photoisomerization reaction to translate electromagnetic radiation into cellular signals due to the conversion of *11-cis*-retinal to *all-trans*-retinal.
2. The sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors)
3. Behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin, dopamine, GABA, and glutamate
4. Regulation of immune system activity and inflammation: Chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response
5. Autonomic nervous system transmission: Both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways, responsible for control of many automatic functions of the body such as blood pressure, heart rate, and digestive processes
6. Cell density sensing: A novel GPCR role in regulating cell density sensing.
7. Homeostasis modulation (water balance).

Mechanism of action: When a ligand binds to the GPCR it causes a conformational change in the GPCR, which allows it to act as a guanine nucleotide exchange factor (GEF). The GPCR then activate an associated G-protein by exchanging its bound GDP for a GTP. The G-protein's α subunit, together with the bound GTP then dissociate from the β and γ subunits to further affect intracellular signaling proteins or target functional proteins directly depending on the α subunit type ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, $G_{\alpha 12/13}$).

There are two principal signal transduction pathways followed by the G protein-coupled receptors:

- the cAMP signal pathway
- the Phosphatidylinositol signal pathway.

Adenylate Cyclase regulated by G_s and G_i type of G_α

The adenylate cyclase pathway regulated by both stimulatory and inhibitory subunits of G protein G_s and G_i respectively.

In case of stimulatory subunit, G_α have intrinsic GTPase activity, which is used to hydrolyze bound GTP to GDP and P_i. This hydrolysis reaction is slow, however, requiring from second to minutes. Thus the GTP form of G_α is able to activate downstream components of signal transduction pathway before GTP hydrolysis that deactivates the subunit. In essence, the bound GTP acts as a built in clock that spontaneously resets the G_α subunit after a short time period. After GTP hydrolysis and release of P_i, the GDP –bound form of G_α then reassociates with $G_{\beta\gamma}$ to re-form the inactive heterotrimeric protein. Since G_α hydrolyzes its bound GTP at a characteristic rate, it functions as a molecular clock that limits the length of time that both G_α. GTP and $G_{\beta\gamma}$ can interact with their effectors.

On the other hands, $G_{\beta\gamma}$ can also directly participate in signal transduction by activation of wide variety of signaling proteins including several isoforms of AC, certain Na^+, K^+ and Ca^{2+}-specific ion channels, various protein tyrosine kinases and phospholipase C- β (PLC-β; a component of the phospho-inositide signaling system; Section. $G_{\beta\gamma}$ thereby provides an important source of cross talk between signaling systems.

“Several types of ligand–GPCR complexes may activate the same G protein.This occurs, for example, in liver cells in response to the binding of the corresponding hormones to

glucagon receptors and to β-adrenergic receptors. In such cases, the amount of cAMP produced is the sum of that induced by the individual hormones."

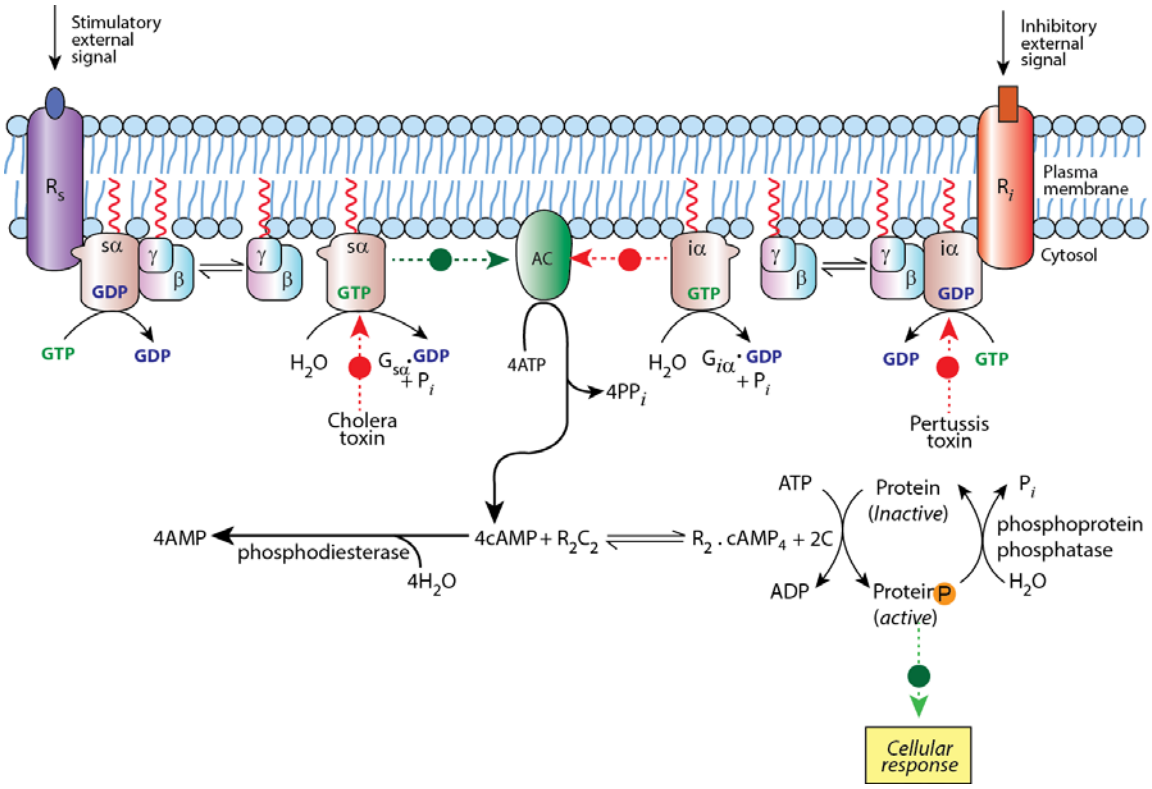

Figure 6: Mechanism of receptor-mediated activation/inhibition of Adenylate Cyclase.

In case of inhibitory type of α-subunit, some ligand–GPCR complexes inhibit rather than activate AC. These include the *α_2-adrenergic receptor* and receptors for *somatostatin and opioids*. The inhibitory effect is mediated by "inhibitory" G protein, **G_i**, which may have the same β and γ subunits as does "stimulatory" G protein, G_s, but has a different α subunit,$G_{iα}$ (41 kD). G_i acts analogously to G_s in that on binding to its corresponding ligand–GPCR complex, its $G_{iα}$ subunit exchanges bound GDP for GTP and dissociates from G. However, $G_{iα}$inhibits rather than activates AC, through direct interactions and possibly because the liberated $G_{βγ}$ binds to and sequesters **$G_{sα}$**. The latter mechanism is supported by the observation that liver cell membranes contain far more G_i than G_s. The activation of G_i in such cells would therefore release enough $G_{iα}$ to bind than available G_s.

Cholera Toxin Stimulates Adenylate Cyclase by Permanently Activating $G_{s\alpha}$:

The major symptom of cholera, an intestinal disorder caused by the bacterium *Vibrio cholerae*, is massive diarrhea that, if untreated, frequently results in death from dehydration. This dreaded disease is not an infection in the usual sense since the vibrio neither invades nor damages tissues but merely colonizes the intestine, much like E. coli. The catastrophic fluid loss that cholera induces (often over 6 liters per hour!) occurs in response to a bacterial toxin. Indeed, merely replacing cholera victims' lost water and salts enables them to survive the few days necessary to immunologically eliminate the bacterial infestation.

Cholera toxin (CT; also known as choleragen) is an 87-kD protein of subunit composition AB5 in which the B subunits (103 residues each) form a pentagonal ring to which the A subunit (240 residues) is bound its A subunit is cleaved at a single site by a bacterial protease to yield two fragments, A1 (the N-terminal ~195 residues) and A2 (the C-terminal ~45 residues), that remain joined by a disulfide bond. In the cytoplasm, A1 catalyzes the irreversible transfer of the ADP–ribose unit from NAD to a specific Arg side chain of Gs. This reaction is greatly accelerated by the interaction of A1 with the small Ras-like G protein ADP- ribosylation factor (ARF) in complex with GTP, which normally functions to prime the formation of clathrin-coated vesicles.

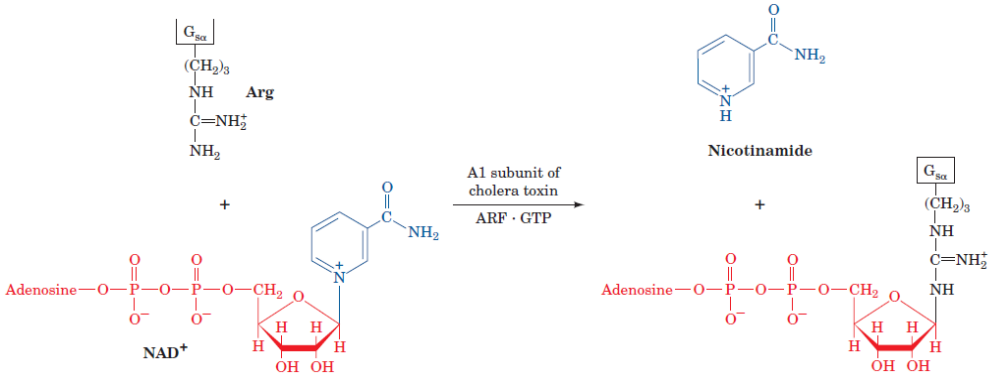

Figure 7: Mechanism of action of cholera toxin. The cholera toxin's A1 fragment in complex with ARF. GTP catalyzes the ADP- ribosylation of a specific Arg residue on $G_{s\alpha}$ by NAD^+, thereby rendering this subunit incapable of hydrolysing GTP.

ADP-ribosylated $G_{s\alpha}$ GTP can activate AC but is incapable of hydrolyzing its bound GTP. As a consequence, the AC remains "locked" in its active state. The epithelial cells of the small intestine normally secrete digestive fluid (an – HCO_3^- rich salt solution) in response to small increases in [cAMP] that activate intestinal Na^+ pumps through their phosphorylation by PKA. The ~100-fold rise in intracellular [cAMP] induced by CT causes these epithelial cells to pour out enormous quantities of digestive fluid, thereby producing the symptoms of cholera. $G_{s\alpha}$ and $G_{i\alpha}$ are members of a family of related proteins, many of which have downstream effectors other than AC.

Termination of GPCR and interrupt binding with G protein

The hormone-bound activated receptor must be reset as well to prevent the continuous activation of G proteins. This resetting is accomplished by two processes.

First, the hormone dissociates, returning the receptor to its initial, unactivated states. The likelihood that the receptor remains in its unbound states depends on the concentration of hormone.

Second, the hormone- receptor complex is deactivated by the phosphorylation of serine and threonine residues in the carboxyl-terminal tail of the receptor kinase (also called G protein receptor kinase 2, GRK 2) phosphorylates the carboxyl terminal tail of hormones-receptor complex but not the unoccupied receptor. Finally, the molecule β-arrestin binds to the phosphorylated receptor and further diminish its ability to activate G-protein.

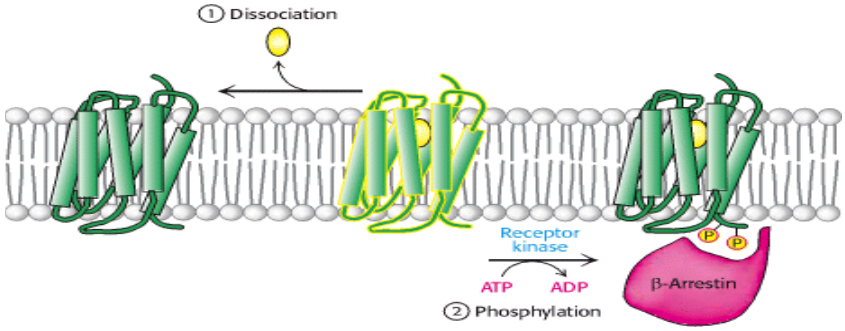

Figure-8: Signal termination

Human Diseases Linked to the G Protein Pathway

There are so many human diseases are related to disfunction of G-protein and G-protein coupled receptor which may be due to mutation in the genes that encode for these proteins. Some of them are given in the following table:

Table 2: Disease due defective G-protein

Sl. No.	Disease	Defective G-protein
1.	Albright's hereditary osteodystrophy and pseudohypathyroidism	$G_{S\alpha}$
2.	McCune-Albright syndrome	$G_{s\alpha}$
3.	Pituitary, thyroid tumors(gsp oncogene)	$G_{s\alpha}$
4.	Adernocortical, ovarian tumors (*gip* oncogene)	$G_{i\alpha}$
5.	Combined precocious puberty and pseudohypoparathyroidism	$G_{s\alpha}$

Table 3: Disease due to defective G-protein couple receptor

Sl. No.	Disease	Defective G- protein couple receptor
1.	Family hypocalciuric hypercalcemia	Human analogue of BoPCAR1 receptor
2.	Neonatal severe hyperparathyroidism (thyroid adenomas)	Human analogue of BoPCAR1 receptor
3.	hyperparathyroidism (thyroid adenomas)	Thyrotrophin receptor
4.	Familial male precocious puberty	Luteinizing Hormone receptor
5.	X-linked nephrogenic diabetes inspidus	V2 vasopressin recept
6.	Retinitis pigmentosa	Rhodopsine receptor
7.	Color blindness, spectral sensitivity variations	Cone opsin receptor
8.	Familial glucocorticoid deficiency and isolated glucocorticoid deficiency	Adernocorticotrophic hormone (ACTH) receptor

The mechanism for transmitting signals across the plasma membrane by G proteins is of ancient evolutionary origin and is highly conserved.

G Proteins often Require Accessory Proteins to Function

The proper physiological functioning of a G protein often requires the participation of several other types of proteins which are described below:

1. A GTPase-activating protein (GAP), which as its name implies, stimulates its corresponding G protein to hydrolyze its bound GTP. This rate enhancement can be > 2000-fold. The downstream effectors of $G_{t\alpha}$ and G_{qt}, cGMP-PDE and PLC-β respectively, exhibit GAP activities toward $G_{t\alpha}$ and $G_{q\alpha}$ (which otherwise would hydrolyze GTP at physiologically insignificant rates), but AC does not exhibit GAP activity toward either $G_{s\alpha}$ or $G_{i\alpha}$. However, in humans, a diverse family of 37 RGS proteins (for regulators of G protein signaling) function as GAPs for G_{α} subunits by binding most avidly to them when they are in the transition state conformation for hydrolyzing GTP.

2. A guanine nucleotide exchange factor [GEF; alternatively guanine nucleotide releasing factor (GRF)], which induces its corresponding G protein to release its bound GDP. The G protein subsequently binds another guanine nucleotide (GTP or GDP, which most G proteins bind with approximately equal affinities), but since cells maintain a GTP concentration that is 10-fold higher than that of GDP, this, in effect, exchanges the bound GDP for GTP. For heterotrimeric G proteins, the agonist–GPCR complexes function as GEFs.

3. A guanine nucleotide-dissociation inhibitor (GDI). A $G_{\beta\gamma}$ may be regarded as its associated G_{α}'s GDI because GDP dissociates slowly from isolated G_{α} subunits but is essentially irreversibly bound by heterotrimers.

Interesting facts:

1. G proteins are molecular switches that use GDP to control their signaling cycle. G protein is inactive when GDP bounds. To activate the protein, the GDP is replaced with GTP, and then G protein will deliver its signal.
2. The G protein system plays a central role in many signalling tasks, so it became sensitive target for many drugs and toxins.

3. The diversity of GPCRs is observed not only by the multiplicity of stimuli to which they respond, but also by the variety of intracellular signalling pathways they activate

Questions:

1. How G-protein activated when light fall on eye? What is the role of G-protein in signalling in eye?
2. What are the effect of cholera toxin in cell signaling?
3. Which are the enzyme dependent on G-protein activation? How G-protein involve in signal transduction?

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Module 6 Lecture 1

Cell Culture

Cell culture is the multifaceted process through which cells are isolated from animal or plant and their subsequent growth under controlled artificial conditions, generally outside their natural environment. In this procedure cells are directly isolated from body or disaggregated by enzymatic or mechanical procedure or they may be derived from cell lines or cell strains. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. The in vitro propagation of cells has become a common practice in many laboratories for a huge numbers of applications. The ranges of cell types grown are vast. Generally the cells are sensitive to a wide range of compounds and it is therefore necessary to ensure that they come into contact only with those under study and not with extraneous materials. Adherent mammalian cells require a suitable surface for attachment.

Primary cell culture: Primary cell culture is the primary step of cell culturing in which the cell is first isolated from tissue and then proliferated under the appropriate conditions until they consume all available contents for their growth. Now the cell is ready for subculturing by transferring them to new growth medium that furnish more opportunity for continued growth.

Cell lines: A cell line is a permanently established cell culture that will proliferate indefinitely in appropriate fresh medium and space. Cell lines differ from cell strains in that they have absconded the Hayflick limit and become immortalised. The Hayflick limit (or Hayflick Phenomenon) is the number of times a normal cell population will divide before it stops, presumably because the telomeres reach a critical length. A cell line arises from a primary culture at the time of the first successful subculture. The terms finite or continuous are used as prefixes if the status of the culture is known.

Cell Strain: By applying cloning, the positive population of cell lines are selected, therefore this cell lines now becomes a cell strain. A cell strain often acquires additional genetic changes resulting to the initiation of the parent line.

There are two types of cell culture:

Continuous cell culture: A continuous cell culture is one that is apparently capable of an unlimited number of population doublings, often referred to as an immortal cell culture. Such cells may or may not express the characteristics of in vitro neoplastic or malignant transformation. Continuous cell lines are usually aneuploid and often have a chromosome number between the diploid and tetraploid values. There is also considerable variation in chromosome number and constitution among cells in the population (heteroploidy).

Some important properties of Continuous cell lines:

- Reduced serum requirement
- Reduced density limitation of growth
- Growth in semisolid media
- Aneuploidy

Several normal cells do not give rise to continuous cell lines. The classical example are normal human fibroblasts that remain euploid throughout their life span and at crisis (usually around 50 generations) will stop dividing, although they may remain viable for up to 18 months thereafter. Human glia and chick fibroblasts behave similarly. Epidermal cells, on the other hand, have shown gradually increasing life spans with improvements in culture techniques.

Finite cell culture: A finite cell culture is capable of only a limited number of population doublings after which the culture ceases proliferation.

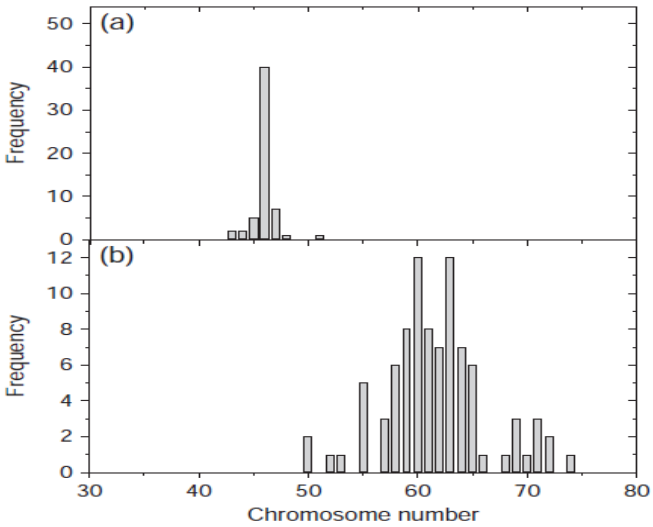


Figure 1: Chromosome Numbers of Finite and Continuous Cell Lines.

(a) A normal human glial cell line. (b) A continuous cell line from human metastatic melanoma.

Table 1: Properties of finite and continuous cell lines

Properties	Finite	Continuous(transformed)
Ploidy	Euploid, Diploid	Aneuploid, Hetroploid
Transformation	Normal	Immortal growth control altered and tumerigenic
Anchorage dependence	Yes	No
Contact inhibition	Yes	No
Density limitation of cell proliferation	Yes	Reduced or lost
Mode of growth	Monolayer	Monolayer or suspension
Maintainance	Cyclic	Steady state possible
Serum Requirement	High	Low
Cloning efficiency	Low	High
Markers	Tissue specific	Chromosomal, enzymatic, antigenic
Special function (e.g. virus susceptibility and differentiation)	May be retained	Often lost
Growth rate	Slow(T_D of 24-96 h)	Rapid(T_D of 12-24 h)
Yield	Low	High
Control parameter	Generation time, tissue specific marker	Stain characteristics

Features of cell line: To use any cell line for the production of biological product, one should have knowledge of following things related to cell lines:

- Age, sex and species of the donor tissue.
- For human cell lines, the donor's medical history and if available, the results of tests performed on the donor for the detection of adventitious agents
- Culture history of the cell line including methods used for the isolation of the tissues from which the line was derived, passage history, media used and history of passage in animals, etc.
- Previous identity testing and the results of all available adventitious agents testing

Characteristics of Cell lines: Each cell line is distinguished by characteristic features which render these cells unique and biomedically or biotechnologically useful.

- The growth pattern and morphological appearance of the cell line should be determined and should be stable from the master cell bank to the end-of-production cells.
- If there are specific markers that may be useful in characterizing the cell line (such as marker chromosomes, specific surface markers), these should be characterized for stability.
- Mostly cultured cell lines are allowed to generate their own ECM (extra cellular matrix), but primary culture and propagation of some specialized cells, exogenous provision of ECM.
- Many transformed cell lines have provided the best model for the induction of differentiation.
- Since normal cells has limited dividing capacity, therefore after a fixed number of population doublings cell lines derived from normal tissue will die out. This is a genetically determined event involving several different genes and this phenomenon is known as senescence. If the cells have an identified finite life expectancy, the total number of population doubling levels through senescence should be determined.

- Some cell lines may avoid senescence and give rise to continuous cell lines. The ability of a cell line to grow continuously probably reflects its capacity for genetic variation, allowing subsequent selection.
- A common feature of many human continuous cell lines is the development of a subtetraploid chromosome number. The alteration in a culture that gives rise to a continuous cell line is commonly called in vitro transformation and may occur spontaneously or be chemically or virally induced.

Table 2: Selection in cell line Development

Stage	Factor influencing Selection	
	Primary explant	Enzymatic disaggregation
Isolation	Mechanical damage	Enzymatic damage
Primary culture	Adhesion of explant, outgrowth(migration), cell proliferation	Cell adhesion and spreading, cell proliferation
First subculture	Trypsine sensitivity, nutrient, hormone, proliferative ability	
Propagation as cell lines	Relative growth rate of different cell, selective growth rate of one lineage, nutrient, hormone and subculture limitation Effect of cell density on predominance of normal or transformed phenotypes	
Senescence, transformation	Normal cell die out, transformed cell grow	

Requirement of cell lines: For the maintenance of Cell line some basic conditions are required. These are described as follows.

1. **pH:** Most cell lines grow well at pH 7.4. Although the optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast lines perform best at pH 7.4 to pH 7.7, and transformed cells may do better at pH 7.0 to pH 7.4.
2. **Buffering:** Culture media must be buffered under two sets of conditions:
   a) Open dishes, where the evolution of CO_2 causes the pH to rise
   b) Overproduction of CO_2 and lactic acid in transformed cell lines at high cell concentrations, when the pH will fall.
3. **Temperature:** The temperature recommended for most human and warm-blooded animal cell lines is 37°C, closely to body heat, but generally set a little lower for safety, because overheating may become major problem than under heating.

4. **Media:** Although many cell lines are still propagated in medium supplemented with serum, in many instances cultures may now be propagated in serum-free media. Media that have been produced commercially will have been tested for their capability of sustaining the growth of one or more cell lines. However under certain circumstance we can use our own media.
5. **Growth curve:** A growth curve gives three parameters of measurement: (1) the lag phase before cell proliferation is initiated after subculture, indicating whether the cells are having to adapt to different conditions; (2) the doubling time in the middle of the exponential growth phase, indicating the growth promoting capacity of the medium; and (3) the maximum cell concentration attainable indicating whether there are limiting concentrations of certain nutrients. In cell lines whose growth is not sensitive to density (e.g., continuous cell lines), the terminal cell density indicates the total yield possible and usually reflects the total amino acid or glucose concentration.

Generation of cell lines: Stably transfected cell lines are used extensively in drug discovery. Cell lines expresses a target of interest, such as a G-protein coupled receptor (GPCR) or a reporter gene, form the basis for most cell-based compound screening campaigns. In establishing new assays for high throughput screening, creation of the appropriate cell line is a bottleneck. Typically, a stable cell line is created by transfection with a plasmid encoding the target of interest or reporter gene construct, and an additional gene which allows for chemical selection of successfully transfected cells (usually an antibiotic resistance gene). Through a lengthy selection process and subsequent limiting dilution to obtain clones, the desired stable cell line is generated. This process takes approximately 2-3 months, usually yielding 5-10 usable clones and allowing little control over the end result throughout the process.

Technique for cell line generation:

LEAP (Laser-Enabled Analysis and Processing) has been developed for high-throughput laser-mediated cell elimination for cell purification. It is a precise laser-based cell ablation enables cell purification based on fluorescent and morphological criteria. It has whole well imaging system i.e all cells in the well can be analysed. It has F-theta scanning optics i.e Image up to 40X faster than typical HCS (High-content screening) systems. Image magnifications of 3X, 5X, 10X, or 20X can be obtained. Combinations of

8 excitation and 8 emission wavelengths is possible. LEAP images all cells within a well, selects a specific population of cells by gating, and eliminates selected cells at $>10^3$ per second. LEAP can select cells of interest based on fluorescent properties, morphological properties, or a combination of both. By replacing the antibiotic resistance gene used for chemical selection with a gene encoding a fluorescent protein, transfected cells can be selected based on fluorescence. These cells can then be purified using LEAP by specifically eliminating non-fluorescent cells using laser elimination. By selecting cells that remain fluorescent and proliferate over a period of time, stable cell lines are isolated. In addition, fluorescence level may be used to identify cell lines with a specific desired expression level of the transfected construct. The fluorescent reporter gene may also be replaced by a variety of fluorescent cell physiology read outs, enabling the selection of cells based on functional responses.

Applications:

The generation of stably-transfected cell lines is essential for a wide range of applications:

- Cell line can be used for gene function studies
- Drug discovery assays or the production of recombinant proteins can be carried out by cell lines.
- In contrast to transient expression, stable expression of cell line allows long term, as well as defined and reproducible expression of the gene of interest.

Table 3: Types of culture system for cell lines

Culture System	Advantage	Application
Batch culture - Polyclonal	Fast, useful for cells which do not grow in single cell culture	Over expressions, protein expression system (e.g. for Basic research)
Limiting Dilution-monoclonal	Defined cell clone	Study of gene function, protein production(e.g. for therapeutic applications)

In a batch culture system, a mixed population of drug resistant cells is selected on plates or in flasks and can be used directly for experimental analysis. During a limiting dilution procedure, cells are usually diluted and selected e.g., in a 96-well plate for outgrowth of cell clones or single colony growth. Subsequently, colonies can be picked and used to generate monoclonal cell lines.

Culture conditions for generation of stable cell lines:

As for transient transfection experiments, culture conditions (passage number, split rhythm, etc.) of selected cell type are very important for the generation of stably-transfected cell lines. The American Type Culture Collection (ATCC®; www.atcc.org) is a reliable source for various cell types. Generally, the cell line should be passaged two days before the experiment to promote good proliferation and cell physiology. Cell passage should not be higher than 30. Interference of higher passage numbers with integration efficiency is possible and may be cell-type dependent. Depending on the scope of experiment, cells can be cultivated as polyclonal batches or monoclonal single cell clones post transfection.

Transfection Method: Stable expression can be influenced by the transfection method used. The choice of transfection method determines which cell type can be targeted for stable integration. While biochemical transfection reagents can be used to transfer DNA into standard cell lines, efficient delivery of DNA into difficult-to-transfect suspension cell lines or even primary cells is only possible with viral methods or Nucleofection. Unfortunately, viral methods suffer from several limitations, such as time consuming production of vectors and safety concerns.

Table 4: Experimental outlines for the Generation of cell lines

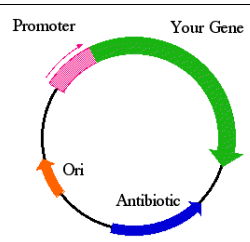
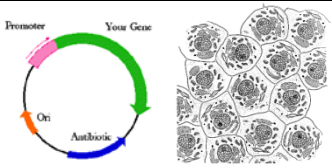
	Procedure Outline	Important Information
 Expression Plasmid	Design experiment and choose cell type, expression vector and transaction method	Make sure that transfection method and expression vector are suitable for the cell type
	Determine the appropriate cell number per plate (only for limiting dilution) and G418 concentration	Cells differ in their susceptibility to G418. The activated concentration of stock G418 can vary from batch to batch
 Expression plasmid + cells	Transfect expression vector into cells.	Amount of expression vector per expression is dependent on transfection method and cell type.
	Plant transfect cells and cultivate cells into medium without G418	Do not add G418 to culture medium immediately after transfection as this may drastically increase mortallity
	Dilute cell into culture plate and start selection 24-48 hour post transfection. Feed every 2-3 days (for batch culture) or 10 days (for limiting dilution)	Choose culture condition [batch culture limiting dilution] depending upon the experimental design. Refresh selection medium is important to avoid false positive cells.
	Analyze stably transfected cell	Make sure the chosen cell is suitable for your application.

Table 5: Commonly used cell lines

Type of cell line	Cell lines	Morphology	Origin	Species	Age	Ploidy	Characteristics
Finite from normal tissue	IMR-90	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection contact inhibited
	MRC-5	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection, contact inhibited
	MRC-9	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection, contact inhibited
	WI-38	Fibroblast	Lung	Human	Embryonic	Diploid	Susceptible to human viral infection, contact inhibited
Continuous from normal tissue	3T3-A31	Fibroblast		Mouse BALB/c	Embryonic	Aneuploid	contact inhibited, radily transformed
	BEAS-2B	Epithelial	Lung	Human	Adult		
	BHK21-C13	Fibroblast	Kidney	Syrian Hamster	Adult	Aneuploid	Transformable by polyoma
	BRL3A	Epithelial	Liver	Rat	New born		Produce IGF-2
	C2	Fibroblastoid	Skeletal muscle	Mouse	Embryonic		Myotubes
	C7	Epitheloid	Hypothalamous	Mouse			Neurophysin, Vasopressin
	COS-1, COS-7	Epitheloid	Kidney	Pig	Adult		Good hosts for DNA transfection
	CHO-K1	Fibroblast	Overy	Chinise Hamster	Adult	Diploid	FactorVIII, Angiotensin II converting enzyme

	HaCaT	Epithelial	Keratinocytes	Human	Adult	Diploid	Comification
	LLC-PKI	Epithelial	Kidney	Pig	Adult	Diploid	Na^+ dependent glucose uptake
	NRK49F	Fibroblast	Kidney	Rat	Adult	Aneuploid	Induction of suspension growth by TGF-α, β
Continuous from neuroplastic tissues	A2780	Epithelial	Overy	Human	Adult	Aneuploid	Chemosensitive with resistant variant
	A549	Epithelial	Lung	Human	Adult	Aneuploid	Synthesize surfactant
	B16	Fibroblast	Melanoma	Mouse	Adult	Aneuploid	Melanin
	HeLa	Epithelial	Cervix	Human	Adult	Aneuploid	G6PD Type A
	HeLa-S3	Epithelial	Cervix	Human	Adult	Aneuploid	High plating efficiency, will grow well in suspension
	HEP-G2	Epitheloid	Heptoma	Human	Adult	Aneuploid	Retain some microsomal metabolizing enzyme
	K-562	Suspention	Myeloid leukemia	Human	Adult	Aneuploid	Hemoglobin
	SK-HEP-1	Endothelial	Hepatoma, Endothelium	Human	Adult	Aneuploid	Factor VIII
	MCF-10	Epithelial	Fibrocytic mammary tissue	Human	Adult	Near Diploid	Dome formation
	HT-29	Epithelial	Colon	Human	Adult	Aneuploid	Differentiation inducible with NaBt

Interesting facts:

- There are several different types of cell lines that can be finite or continuous, prepared from normal tissue as well as neuroplastic tissues.
- Cell bank system is generally used for maintenance of cell line with constant supply of starting material as well as it perform cell lines characterization and detection of cell line cross contamination.
- In drug discovery, stably transfected cell lines are generally used.

Questions:

1. What are the basic types of cell lines and which type of tissue involve in formation of cell line?
2. What are the conditions required for cell line growth?
3. What are the criteria for choosing cell lines?
4. Which type of precaution one should take during cell line maintenance?
5. Give some example of human cell lines and what are the characteristic of those cell lines?

References:

1. Fresheny, I. “Culture of animal cell – A manual of basic technique and specialized application” by; chaptor-2: Biology of cultured cells; page-23
2. http://www.biology online.org/dictionary/Cell_line
3. http://atcc.custhelp.com/app/answers/detail/a_id/355/~/definition-of-%22cell-line%22
4. http://www.biology-online.org/dictionary/Stock_culture
5. http://bio.lonza.com/uploads/tx_mwaxmarketingmaterial/Lonza_TechREF_Generation_of_Stable_Cell_Lines_low_res.pdf

M6 L2 Characterization of Cells

Characterization of a cell line is vital for determining its functionality and in proving its authenticity as pure cell line. Special attention must be paid to the possibility that the cell line has become cross-contaminated with an existing continuous cell line or misidentified because of mislabeling or confusion in handling DNA profiling. This has now become the major standard procedure for cell line identification, and a standard procedure with universal application.

The various important factors for cell line characterization are:

(1) It leads to authentication or confirmation that the cell line is not cross-contaminated or misidentified

(2) It is confirmation of the species of origin

(3) It is used for correlation with the tissue of origin, which comprises the following characteristics:

a) Identification of the lineage to which the cell belongs
b) Position of the cells within that lineage (i.e., the stem, precursor, or differentiated status)

(4) For determination whether the cell line is transformed or not:

a) Whether the cell line is finite or continuous?
b) Whether the cell line expresses properties associated with malignancy?

(5) It indicates whether the cell line is prone to genetic instability and phenotypic variation

(6) Identification of specific cell lines within a group from the same origin, selected cell strains, or hybrid cell lines, all of which require demonstration of features unique to that cell line or cell strain

Table 1: Decisive factors for characterization of cell lines and corresponding methods

Decisive factor	**Method**
DNA profile	PCR of microsatellite repeats
Karyotype	Chromosome spread with banding
Isoenzyme analysis	Agar gel electrophoresis
Genome analysis	Microarray
Gene expression analysis	Microarray
Proteomics	Microarray
Cell surface antigen	Immunohistochemistry
Cytoskeleton	Immunocytochemistry with antibodies specific cytokeratins

Parameters of Characterization

The nature of the technique used for characterization depends on the type of work being carried out. Some of the parameters are:

1. In case molecular technology, DNA profiling or analysis of gene expression are most useful.
2. A cytology laboratory may prefer to use chromosome analysis coupled with FISH (fluorescence in situ hybridization) and chromosome painting. Chromosomal analysis also known as karyotyping, is one of the best traditional methods for distinguishing among species. Chromosome banding patterns can be used to distinguish individual chromosomes. Chromosome painting, explicitly using combinations of specific molecular probes that hybridize to individual chromosomes, adds further resolution and specificity to this technique. These probes identify individual chromosome pairs and are species specific. Chromosome painting is a good method for distinguishing between human and mouse chromosomes in potential cross-contaminations.

3. A laboratory with immunological capability may prefer to use MHC (Major Histo compatibility complex) analysis (e.g., HLA typing) coupled with lineage specific markers.

   Combined with a functional assay related to our own interests, these procedures should provide sufficient data to authenticate a cell line as well as confirm that it is suited to the concerned.

4. **Lineage or Tissue markers:** The progression of cells down a particular differentiation pathway towards a specific differentiated cell type and can be considered as a lineage, and as cells progress down this path they acquire lineage markers specific to the lineage and distinct from markers expressed by the stem cells. These markers often reflect the embryological origin of the cells from a particular germ layer. Lineage markers are helpful in establishing the relationship of a particular cell line to its tissue of origin. There are some lineage markers which are described as follows:

a) **Cell surface antigen:** These markers are particularly useful in sorting hematopoietic cells and have also been effective in discriminating epithelium from mesenchymally derived stroma with antibodies such as anti- and anti-HMFG 1 and, distinguishing among epithelial lineages, and identifying neuroectodermally derived cells (e.g., with anti-A2B5).

b) **Intermediate filament proteins**: These are among the most widely used lineage or tissue markers. Glial fibrillary acidic protein (GFAP) for astrocytes and desmin for muscle are the most specific, whereas cytokeratin marks epithelial cells and mesothelium.

c) **Differentiated products and functions**: Haemoglobin for erythroid cells, myosin or tropomyosin for muscle, melanin for melanocytes, and serum albumin for hepatocytes are examples of specific cell type markers, but like all differentiation markers, they depend on the complete expression of the differentiated phenotype.

Transport of inorganic ions, and the resultant transfer of water, is characteristic of absorptive and secretary epithelia. Polarized transport can also be demonstrated in epithelial and endothelial cells using Boyden chambers or filter well inserts. Other tissue-specific functions that can be expressed in vitro include muscle contraction and depolarization of nerve cell membrane.

d) **Enzymes:** Three parameters are available in enzymatic characterization:

- The constitutive level (in the absence of inducers or repressors)
- The induced or adaptive level (the response to inducers and repressors)
- Isoenzyme polymorphisms

Table 1: Enzymatic markers used for cell line

Enzyme	Cell types	Inducer	Repressor
Alkaline ephosphatase	Type II pneumocyte (in lung alveolus)	Dexamethasone, Oncostain, IL-6	TGF-β
Alkaline Phosphatase	Enterocytes	Dexamethanose, NaBt collagen, Matrigel	-
Angiotensin-converting enzyme	Endothelium	Collagen, Matrigel	-
Creatine Kinase BB	Neurons, neuroendocrine cells, SCLC	-	-
Creatine Kinase MM	Muscle cells	IGF-II	FGF-1,2,7
DOPA-decarboxylase	Neuron, SCLC	-	-
Glutamyl synthetase	Astroglia (brain)	Hydrocortisone	Glutamine
Neuron specific enolase	Neuron, neuroendocrine cell	-	-
Non-specific esterase	Macrophage	PMA, Vitamin D3	-
Proline hydrolase	Fibroblasts	Vitamin C	-
Sucrase	Enterocytes	NaBt	-

e) **Regulation:** The level of expression of many differentiated products is under the regulatory control of environmental influences, such as nutrients, hormones, the matrix, and adjacent cell. Hence the measurement of specific lineage markers may require preincubation of the cells in, for example, a hormone such as hydrocortisone, specific growth factors, or growth of the cells on extracellular matrix of the correct type.

f) Lineage fidelity: Lineage markers are more properly regarded as tissue or cell type markers, as they are often more characteristic of the function of the cell than its embryonic origin.

5.**Unique Markers:** Unique markers include specific chromosomal aberrations (e.g., deletions, translocations, polysomy), major histocompatibility (MHC) group antigens (e.g., HLA in humans), which are highly polymorphic, and DNA fingerprinting or SLTR DNA profiling. Enzymic deficiencies, such as thymidine kinase deficiency (TK−) and drug resistance such as vinblastine resistance (usually coupled to the expression of the P-glycoprotein by one of the *mdr* genes that code for the efflux protein) are not truly unique, but they may be used to distinguish among cell lines from the same tissues but different donors.

6. Transformation: The transformation status forms a major element in cell line characterization and is dealt with separately.

a) **Cell Morphology:** Observation of morphology is the simplest and most direct technique used to identify cells. Most of these are related to the plasticity of cellular morphology in response to different culture conditions. For example, epithelial cells growing in the center of a confluent sheet are usually regular, polygonal, and with a clearly defined birefringent edge, whereas the same cells growing at the edge of a patch may be more irregular and distended and, if transformed, may break away from the patch and become fibroblast-like in shape.

b) **Microscopy:** The inverted microscope is one of the most important tools in the tissue culture laboratory, but it is often used incorrectly. As the thickness of the closed culture vessel makes observation difficult from above, because of the long working distance, the culture vessel is placed on the stage, illuminated from above, and observed from below. As the thickness of the wall of the culture vessel still limits the

working distance, the maximum objective magnification is usually limited to 40X. The use of phase-contrast optics, where an annular light path is masked by a corresponding dark ring in the objective and only diffracted light is visible, enables unstained cells to be viewed with higher contrast than is available by normal illumination. Because this means that the intensity of the light is increased, an infrared filter should be incorporated for prolonged observation of cells.

It is useful to keep a set of photographs at different cell densities for each cell line, prepared shortly after acquisition and at intervals thereafter, as a record in case a morphological change is subsequently suspected. Photographs of cell lines in regular use should be displayed above the inverted microscope. Photographic records can be supplemented with photographs of stained preparations and digital output from DNA profiling and stored with the cell line record in a database or stored separately and linked to the cell line database.

c) **Staining:** A polychromatic blood stain, such as Giemsa, provides a convenient method of preparing a stained culture. Giemsa stain is usually combined with May–Grunwald stain when staining blood, but not when staining cultured cells. Alone, it stains the nucleus pink or magenta, the nucleoli dark blue, and the cytoplasm pale gray-blue. It stains cells fixed in alcohol or formaldehyde but will not work correctly unless the preparation is completely anhydrous.

Chromosome Content: Chromosome content or karyotype is one of the most characteristic and best-defined criteria for identifying cell lines and relating them to the species and sex from which they were derived. Chromosome analysis can also distinguish between normal and transformed cells because the chromosome number is more stable in normal cells (except in mice, where the chromosome complement of normal cells can change quite rapidly after explantation into culture).

Chromosome Banding: This group of techniques was devised to enable individual chromosome pairs to be identified when there is little morphological difference between them. For Giemsa banding, the chromosomal proteins are partially digested by crude trypsin, producing a banded appearance on subsequent staining. Trypsinization is not required for quinacrine banding. The banding pattern is characteristic for each chromosome pair.

Other methods for banding are:

a) Using trypsin and EDTA rather than trypsin alone
b) Q-banding, which stains the cells in 5% (w/v) quinacrine dihydrochloride in 45% acetic acid, followed by rinsing Giemsa banding the slide, and mounting it in deionized water at pH 4.5
c) C-banding, which emphasizes the centromeric regions

Techniques have been developed for discriminating between human and mouse chromosomes, principally to aid the karyotypic analysis of human-mouse hybrids. These methods include fluorescent staining with Hoechst 33258, which causes mouse centromeres to fluoresce more brightly than human centromeres.

Chromosome painting: Chromosome paints are available commercially from a number of sources. The hybridization and detection protocols vary with each commercial source, but a general scheme is available. Karyotypic analysis is carried out classically by chromosome banding, using dyes that differentially stain the chromosomes. Thus each chromosome is identified by its banding pattern. However, traditional banding techniques cannot characterize many complex chromosomal aberrations. New karyotyping methods based on chromosome painting techniques—namely spectral karyotyping (SKY) and multicolour fluorescence in situ hybridization (M-FISH)—have been developed. These techniques allow the simultaneous visualization of all 23 human chromosomes in different colours.

Chromosome Analysis

The following are methods by which the chromosome complement may be analyzed:

(1) Chromosome count: Count the chromosome number per spread for between 50 and 100 spreads. (The chromosomes need not be banded.)

(2) Karyotype: Digitally photograph about 10 or 20 good spreads of banded chromosomes. Image analysis can be used to sort chromosome images automatically to generate karyotypes.

Chromosome counting and karyotyping allow species identification of the cells and, when banding is used, distinguish individual cell line variations and marker chromosomes. However, karyotyping is time-consuming, and chromosome counting with a quick check on gross chromosome morphology may be sufficient to confirm or exclude a suspected cross-contamination.

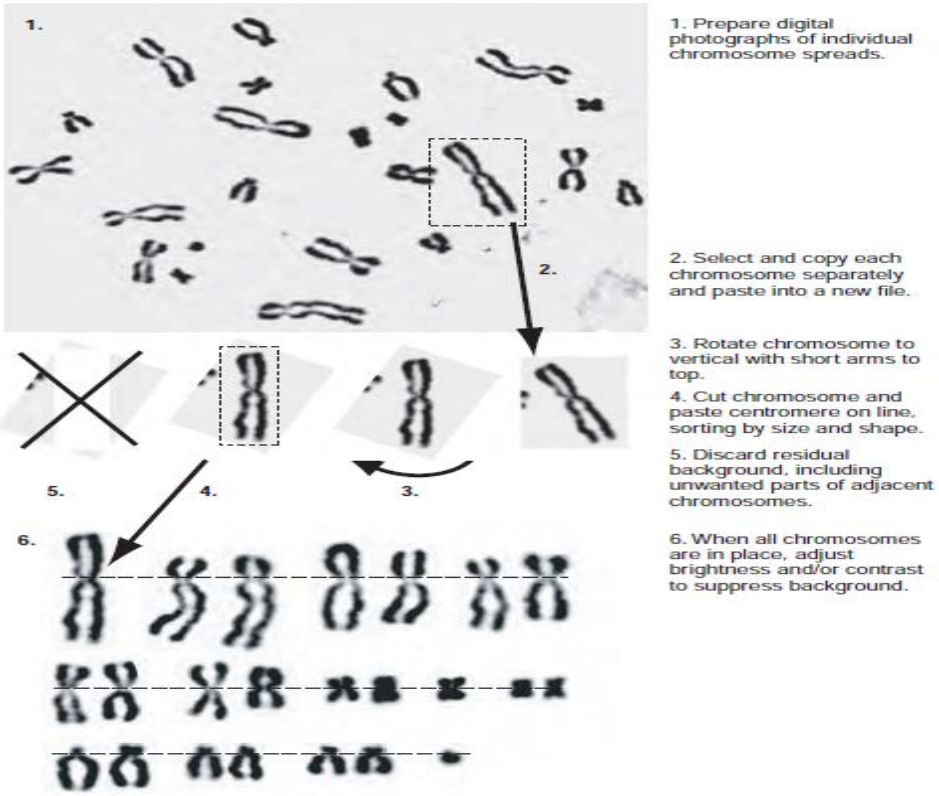


Figure 1: Karyotype Preparation Steps in the preparation of a karyotype from digital microphotographs of metaphase spread. Chinese hamster cells recloned from the Y-5 strain.

DNA Analysis: DNA content can be measured by propidium iodide fluorescence with a CCD camera or flow cytometry, although the generation of the necessary single-cell suspension will, of course, destroy the topography of the specimen. DNA can be estimated in homogenates with Hoechst 33258 and other DNA fluorochromes such as DAPI, propidium iodide, or Pico Green (Molecular Probes). Analysis of DNA content is particularly useful in the characterization of transformed cells that are often aneuploid and heteroploid.

DNA Hybridization: Hybridization of specific molecular probes to unique DNA sequences (Southern blotting) can provide information about species specific regions, amplified regions of the DNA, or altered base sequences that are characteristic to that cell line. Thus strain-specific gene amplifications, such as amplification of the dihydrofolate reductase (DHFR) gene, may be detected in cell lines selected for resistance to methotrexate; amplification of the MDR gene in vinblastine-resistant cells overexpression of a specific oncogene, or oncogenes in transformed cell lines or deletion, or loss, of heterozygosity in suppressor genes. Although DNA aberrations can be detected in restriction digests of extracts of whole DNA, this is limited by the amount of DNA required.

It is more common to use the polymerase chain reaction (PCR) with a primer specific to the sequence of interest, enabling detection in relatively small numbers of cells. Alternatively, specific probes can be used to detect specific DNA sequences by in situ hybridization having the advantage of displaying topographical differences and heterogeneity within a cell population.

DNA fingerprinting: DNA fingerprints appear to be quite stable in culture, and cell lines from the same origin, but maintained separately in different laboratories for many years, still retain the same or very similar DNA fingerprints. DNA fingerprinting is a very powerful tool in determining the origin of a cell line, if the original cell line, or DNA from it or from the donor individual, has been retained. This emphasizes the need to retain a blood, tissue, or DNA sample when tissue is isolated for primary culture. Furthermore, if a cross-contamination or misidentification is suspected, this can be investigated by fingerprinting the cells and all potential contaminant.

Antigenic Markers: Immunostaining and ELISA assays are among the most useful techniques available for cell line characterization facilitated by the abundance of antibodies and kits which is commercially available. Antibody is essential to be certain of its specificity by using appropriate control material. This is true for monoclonal antibodies and polyclonal antisera alike; a monoclonal antibody is highly specific for a particular epitope.

Immunostaining: Antibody localization is determined by fluorescence, wherein the antibody is conjugated to a fluorochrome, such as fluorescein or rhodamine, or by the deposition of a precipitated product from the activity of horseradish peroxidase or alkaline phosphatase conjugated to the antibody. Various methods have been used to enhance the sensitivity of detection of these methods, particularly the peroxidase linked methods. In the peroxidase–anti-peroxidase (PAP) technique, a further amplifying tier is added by reaction with peroxidase conjugated to anti-peroxidase antibody from the same species as the primary antibody. Even greater sensitivity has been obtained by using a biotin-conjugated second antibody with streptavidin conjugated to peroxidase or alkaline phosphatase or gold-conjugated second antibody with subsequent silver intensification.

Differentiation: Many of the characteristics described under antigenic markers or enzyme activities may also be regarded as markers of differentiation, and as such they can help to correlate cell lines with their tissue of origin as well as define their phenotypic status. Although sometimes constitutively expressed (e.g., melanin in B16 melanoma or Factor VIII in endothelial cells), expression of differentiated lineage markers may need to be induced before detection is possible.

Interesting facts:

- EDTA, a chelator of divalent cations, is added to trypsin solutions to enhance activity.
- The calcium and magnesium in the extracellular matrix, which aids in cell-cell adhesion, also obliterates the peptide bonds that trypsin acts on.
- The EDTA is added to remove the calcium and magnesium from the cell surface which allows trypsin to hydrolyze specific peptide bonds. This activity can be arrested by adding a serum media mixture or a trypsin inhibitor (from soybean, for example) in serum-free systems.

Questions:

1. Why characterization of cell line is necessary?
2. What are the parameters on which characterization depends?
3. What is the role of trypsin and EDTA in cell culture? How EDTA help trypsin in cell detachment?

References:

1. Fresheny, I.; "Culture of animal cell – A manual of basic technique and specialized application" by; chaptor-15: Characterization

M6 L3 Contamination in cell culture

Contamination: Contamination is the presence of a minor and unwanted constituent (contaminant) in material, physical body, natural environment, at a workplace, etc. In biological sciences accidental introduction of foreign material (contamination) can seriously distort the results of experiments where small samples are used. In cases where the contaminant is a living microorganism, it can often multiply and take over the experiment, especially cultures, and render them useless.

Source of Contamination: Maintaining asepsis is one of the most difficult challenges to work with living cells. There are several potential routes to contamination including failure in the sterilization procedures for solutions, glassware and pipettes, turbulence and particulates (dust and spores) in the air in the room, poorly maintained incubators and refrigerators, faulty laminar-flow hoods, the importation of contaminated cell lines or biopsies, and lapses in sterile technique.

Table 1: Route of Contamination

Technique	Route or cause	Prevention
(Manipulations, pipetting, dispensing, etc.)	Nonsterile surfaces and equipment	Work area of items not in immediate use should be clear.
	Spillage on necks and outside of bottles and on work surface	Swab regularly with 70% alcohol. Do not pour liquids. Dispense or transfer by pipette, auto dispenser or transfer device. If pouring is unavoidable: (1) do so in one smooth movement, (2) discard the bottle that you pour from, and (3) wipe up any spillage.
	Touching or holding pipettes too low down, touching necks of bottles, inside screw caps.	Hold pipettes above graduations.
	Splash-back from waste beaker	Discard waste into a beaker with a funnel or, preferably, by drawing off the waste into a reservoir by means of a vacuum pump.
	Sedimentary dust or particles of skin settling on the culture or bottle; hands or apparatus held over an open dish or bottle	Do not work over (vertical laminar flow and open bench) or behind and over (horizontal laminar flow) an open bottle or dish.
Work surface	Dust and spillage	Swab the surface with 70% alcohol before during, and after work. Mop up spillage immediately.

Operator hair, hands, breath, clothing	Dust from skin, hair, or clothing dropped or blown into the culture	Wash hands thoroughly or wear gloves. Wear a lint-free lab coat with tight cuffs and gloves overlapping them.
	Aerosols from talking, coughing, sneezing, etc.	Keep talking to be minimum, Avoid working with a cold or throat infection, or wear a mask. Long hair should be tie back or wear a cap. Generally wear a lab coat different from the one which wear in the general lab area or animal house.
Materials and reagents		
Solutions	Non-sterile reagents and media	Filter or autoclave solutions before using them
	Dirty storage conditions	Clean up storage areas and disinfect regularly.
	Inadequate sterilization procedures	Monitor the performance of the autoclave with a recording thermometer or sterility indicator. Check the integrity of filters with a bubble-point or microbial assay after using them. Test all solutions after sterilization.
	Poor commercial supplier	Test solutions; change suppliers.
Glassware and screw caps	Dust and spores from storage	Shroud caps with foil. Wipe bottles with 70% alcohol before taking them into the hood. Replace stocks from the back of the shelf. Do not store anything unsealed for more than 24 h.
	Ineffective sterilization (e.g., an overfilled oven or sealed bottles, preventing the ingress of steam)	Check the temperature of the load throughout the cycle. In the autoclave; keep caps slack on empty bottles. Stack oven and autoclave correctly.
Instruments, pipettes	Ineffective sterilization	Sterilize items by dry heat before using them. Monitor the performance of the oven.
	Contact with a nonsterile surface or some other material	Do not grasp any part of an instrument or pipette that will pass into a culture vessel.

Culture flasks and media bottles in use	Dust and spores from incubator or refrigerator	Use screw caps instead of stoppers. Swab bottles before placing in hood. Box plates and dishes.
	Dirty storage or incubation conditions.	Cover caps and necks of bottles with aluminum foil during storage or incubation. Wipe flasks and bottles with 70% alcohol before using them. Clean out stores and incubators regularly.
	Media under the cap and spreading to the outside of the bottle.	Discard all bottles that show spillage on the outside of the neck. Do not pour.
Equipment and facilities	Room air Drafts, eddies, turbulence, dust, aerosols	Clean filtered air. Reduce traffic and extraneous activity. Wipe the floor and work surfaces regularly.
Laminar-flow hoods	Perforated filter	Check filters regularly for holes and leaks.
	Change of filter needed	Check the pressure drop across the filter.
	Spillages, particularly in crevices or below a work surface	Keep around and below the work surface clear regularly. Let alcohol run into crevices.
Equipment and facilities		
Room air	Drafts, eddies, turbulence, dust, aerosols	Clean filtered air. Reduce traffic and extraneous activity. Wipe the floor and work surfaces regularly.
Laminar-flow hoods	Perforated filter	Check filters regularly for holes and leaks.
	Change of filter needed	Check the pressure drop across the filter.
	Spillages, particularly in crevices or below a work surface	Clear around and below the work surface regularly. Let alcohol run into crevices.
Dry incubators	Growth of molds and bacteria on spillages	Wipe up any spillage with 70% alcohol on a swab. Clean out incubators regularly.
CO_2, humidified incubators	Growth of molds and bacteria on walls and shelves in a humid atmosphere.	Clean out with detergent followed by 70% alcohol

	Spores, etc., carried on forced-air circulation	Enclose open dishes in plastic boxes with close-fitting lids (but do not seal the lids). Swab incubators with 70% alcohol before opening them. Put a fungicide or bacteriocide in humidifying water (but check first for toxicity).
Other equipment	Dust on cylinders, pumps, etc	Wipe with 70% alcohol before bring in
Mites, insects, and other infestations in wooden furniture, or benches, in incubators, and on mice, etc., taken from the animal house	Entry of mites, etc., into sterile packages	Seal all sterile packs. Avoid wooden furniture if possible; use plastic laminate, one-piece, or stainless steel bench tops. If wooden furniture is used, seal it with polyurethane varnish or wax polish and wash it regularly with disinfectant. Keep animals out of the tissue culture lab.
Importation of biological materials		
Tissue samples	Infected at source or during dissection	Do not bring animals into the tissue culture lab. Incorporate antibiotics into the dissection fluid. Dip all potentially infected large-tissue samples in 70% alcohol for 30 s.
Incoming cell lines	Contaminated at the source or during transit	Handle these cell lines alone, preferably in quarantine, after all other sterile work is finished. Swab down the bench or hood after use with 2% phenolic disinfectant in 70% alcohol, and do not use it until the next morning. Check for contamination by growing a culture for two weeks without antibiotics. (Keep a duplicate culture in antibiotics at the first subculture.) Check for contamination visually, by phase-contrast microscopy and Hoechst stain for mycoplasma. Using indicator cells allows screening before first subculture.

Monitoring For Contamination:

Potential sources of contamination are enumerated along with the precautions that should be taken to avoid them. Even in the best laboratories contaminations do arise, so the following procedure is generally recommended:

(1) Contamination by eye and with a microscope at each handling of a culture should be checked properly.

(2) If it is suspected that a culture is contaminated and the fact cannot be confirmed in situ, the hood or bench should be kept clear except suspected culture and Pasteur pipettes. Because of the potential risk to other cultures, this should be better to do after all your other culture work is finished. A sample should be removed from the culture and placed on a microscope slide. Slide should be checked with a microscope, preferably by phase contrast. If it is confirmed that the culture is contaminated, pipettes should be discarded, hood or bench should be swabbed with 70% alcohol containing a phenolic disinfectant. The hood or bench should not be used until the next day.

(3) Nature of the contamination should be recorded.

(4) If the contamination is new and is not widespread, the culture, the medium bottle used to feed it, and any other reagent (e.g., trypsin) that has been used in conjunction with the culture should be discarded properly into disinfectant, preferably in a fume hood and outside the tissue culture area.

(5) If the contamination is new and widespread all media, stock solutions, trypsin, and so forth in current use should be discarded immediately.

(6) If the same kind of contamination has occurred before check stock solutions for contamination (a) by incubation alone or in nutrient broth (b) by plating out the solution on nutrient agar. If (a) and (b) prove negative, but contamination is still suspected, 100 mL of solution should be incubated, filtered it through a 0.2-μm filter, and plated out filter on nutrient agar with an uninoculated control.

(7) If the contamination is widespread, multispecific, and repeated then one should check (a) the laboratory's sterilization procedures (e.g., the temperatures of ovens and autoclaves, particularly in the center of the load, the duration of the sterilization cycle), (b) the packaging and storage practices, (e.g., unsealed glassware should be resterilized every 24 h), and (c) the integrity of the aseptic room and laminar-flow hood filters.

(8) One should not be attempting to decontaminate cultures unless they are irreplaceable.

Visible Microbial Contamination: Characteristic features of microbial contamination are as follows:

(1) A sudden change in pH, usually a decrease with most bacterial infections, very little change with yeast until the contamination is heavy, and sometimes an increase in pH with fungal contamination.

(2) Cloudiness in the medium, sometimes with a slight film or scum on the surface or spots on the growth surface that dissipate when the flask is moved

(3) Under a 10X objective, spaces between cells will appear granular and may shimmer with bacterial contamination. Yeasts appear as separate round or ovoid particles that may bud off smaller particles. Fungi produce thin filamentous mycelia and, sometimes, denser clumps of spores which may be blue or green. With toxic infection, some deterioration of the cells will be apparent.

(4) Under a 100X objective, it may be possible to resolve individual bacteria and distinguish between rods and cocci. At this magnification, the shimmering that is visible in some infections will be seen to be caused by mobility of bacteria. Some bacteria form clumps or associate with the cultured cells.

(5) With a slide preparation, the morphology of the bacteria can be resolved with a 100× objective, but this is not usually necessary. Microbial infection may be confused with precipitates of media constituents (particularly protein) or with cell debris, but can be distinguished by their regular morphology. Precipitates may be crystalline or globular and irregular and are not usually as uniform in size. Clumps of bacteria may be confused with precipitated protein, but, particularly if shaken, many single or strings of bacteria will be seen. If you are in doubt, plate out a sample of medium on nutrient agar.

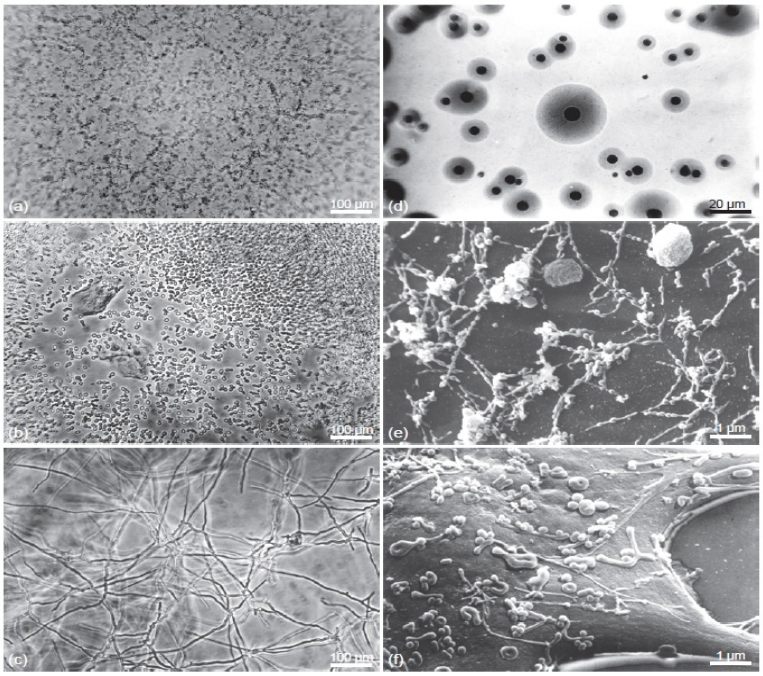


Figure-1: Types of Contamination. Examples of microorganisms found to contaminate cell cultures. (a) Bacteria. (b) Yeast. (c) Mold. (d) Mycoplasma colonies growing on special nutrient agar (e, f). Scanning electron micrograph of mycoplasma growing on the surface of cultured cells. From where has this figure been taken

Ways of Disposal of Contaminated Cultures: The following procedures are generally used for disposal of contaminated culture:

- It is important to ensure that all contaminated material is disposed of correctly. Culture vessels should be removed from the culture area, unopened if possible, and autoclaved.
- Open items, such as Petri dishes with the lids in place, and pipettes or other items that have come in contact with a contaminated culture should be immersed in hypochlorite disinfectant (Petri dishes can be opened while submerged).
- If only one of a series of similar cultures is contaminated, it is necessary to discard the bottle of medium that was used with it, but if the contamination is widespread, then all medium as well as all other stock solutions and reagents, used with these cells, should be discarded into hypochlorite.

Eradication of Contamination: Eradication of Contamination in cell culture is a challenging job during safe culturing. There are different way for different organism, some example are given as follows:

Case-I: Bacteria, Fungi, and Yeasts: The most reliable method of eliminating a microbial contamination is to discard the culture and the medium and reagents used with it as treating a culture may be unsuccessful or lead to the development of an antibiotic-resistant microorganism. This procedure is optimal; however, the majority of cell lines do not form spheroids in this manner. Alternatively, aggregates may be formed from cell suspensions in stationary flasks, previously base-coated with agar. Aggregates may be left in the original flasks or transferred individually (by pipette) to multi well plates, where continued growth over weeks will yield spheroids of maximum size, about 1000 μm.

Decontamination should be attempted only in extreme situations, under quarantine, and with expert supervision. If unsuccessful, the culture and associated reagents should be autoclaved as soon as failure becomes obvious. The general rule remains that contaminated cultures are discarded and that decontamination is not attempted unless it is absolutely vital to retain the cell strain. In any event, complete decontamination is difficult to achieve, particularly with yeast, and attempts to do so may produce hardier, antibiotic-resistant strains.

Case-II: Eradication of Viral Contamination: There are no reliable methods for eliminating viruses from a culture at present; disposal or tolerances are the only options.

Cross Contamination: During the development of tissue culture, a number of cell strains have evolved with very short doubling times and high plating efficiencies. Although these properties make such cell lines valuable experimental material, they also make them potentially hazardous for cross-infecting other cell lines.

Interesting facts:

1. Once cell lines are infected, they may undergo spontaneous differentiation or altered function due to adaptation, which may have a profound impact on experimental results.

Questions:

1. What are the possible routes of contamination in cell culture?
2. What are possible methods for monitoring contamination?
3. What are the general procedures for disposal of contaminated culture?

References:

1. Fresheny**,** I.; "Culture of animal cell – A manual of basic technique and specialized application"
2. http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Stem-Cell-Research/Stem-Cell-Analysis/Cell-Line-Contamination.html.

M6 L4 Cell line differentiation

Differentiation: Differentiation in cell line is the process which leads to the expression of phenotypic properties and characteristic of the functionally mature cell in vivo. It is the phenomenon in which less specialized cell develops or matures to become more distinct in form and function. This may be irreversible when there is cessation of DNA synthesis in the erythroblast nucleus, neuron, or mature keratinocyte. The process may be reversible, when the dedifferentiation of mature hepatocytes into precursors happens during liver regeneration. Some of the properties of the differentiated cells are adaptive, such as albumin synthesis in differentiated hepatocytes, which is often lost in culture but can be reinduced. Differentiation is the combination of constitutive (stably expressed without induction) and adaptive (subject to positive and negative regulation of expression) properties found in the mature cell.

Terminal differentiation: Terminal differentiation is another type of differentiation in which a cell has progressed down a particular lineage to a point at which the mature phenotype is fully expressed and beyond which the cell cannot progress. This stage may be reversible in some cells, such as fibrocytes, that can revert to a less differentiated phenotype, or even a stem cell, and resume proliferation. It may be irreversible in cells like erythrocytes, neurons, skeletal muscle, or keratinized squamous cells. The growth of cells on floating collagen has been used to improve the survival of epithelial cells and promote terminal differentiation

Pluripotent cell: A cell that is able to differentiate into all cell types of the adult organism is known as pluripotent. Such cells are called embryonic stem cells in animals and meristematic cells in higher plants.

Totipotent cell: A cell that is able to differentiate into all cell types, including the placental tissue, is known as totipotent. In mammals, only the zygote and subsequent blastomeres are totipotent.

Control of Differentiation: Differentiation is controlled by various parameters. There are five major parameters that control differentiation. Figure 1 shows the diagrammatic representation of the figures.

1. Cell–cell interaction
2. Cell–matrix interaction
3. Cell shape and polarity
4. Oxygen tension
5. Soluble systemic factors

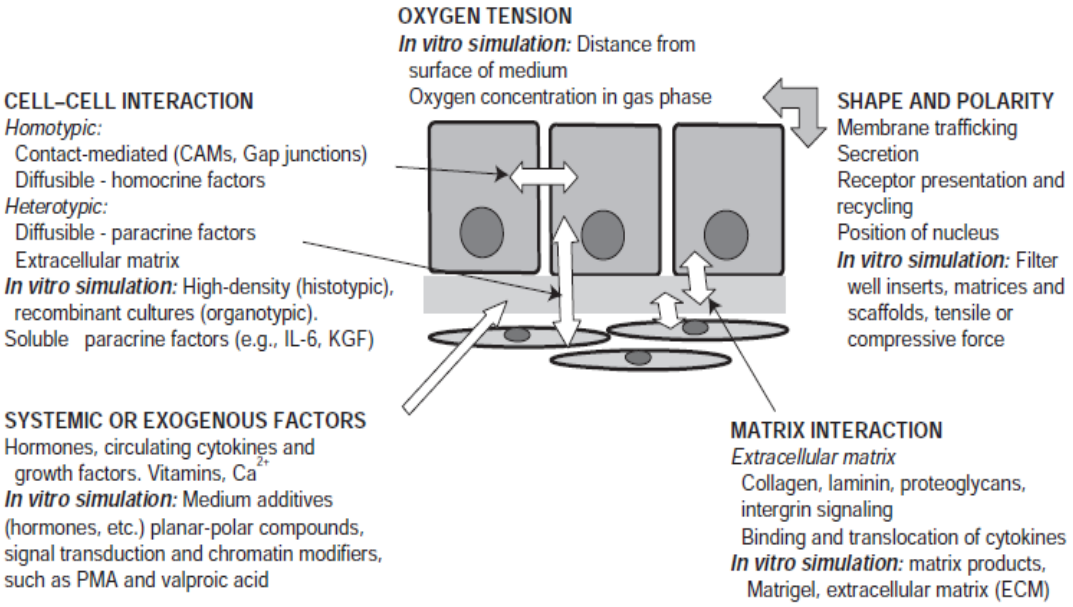


Figure 1: Parameter factors for cell differentiation.

1. Cell- cell Interaction: Cell-cell interactions are of two types, Homotypic and heterotypic. They can be detailed as follows:

Homotypic cell-cell interaction: Homologous cell interaction occurs at high cell density. It may involve gap junctional communication in which metabolites, second messengers such as cyclic AMP, diacylglycerol (DAG), Ca^{2+}, or electrical charge may be involved. This interaction harmonizes the expression of differentiation within a population of similar cells, rather than initiating its expression. Homotypic cell–cell adhesion molecules, (CAMs) or cadherins, which are calcium-dependent, provide another mechanism by which contacting cells may interact. These adhesion molecules promote interaction primarily between like cells via identical, reciprocally acting,

extracellular domains, and they appear to have signal transduction potential via phosphorylation of the intracellular domains.

Heterotypic cell-cell interaction: Heterologous cell interaction such as between mesodermally and endodermally or ectodermally derived cells is responsible for initiating and promoting differentiation. During and immediately after gastrulation in the embryo, and later during organogenesis, mutual interaction between cells originating in different germ layers promotes differentiation.

2. Cell–Matrix Interactions

Animal cells are not surrounded by cell walls. Animal cells are surrounded by a plasma membrane which is complex mixture of glycoproteins and proteoglycans surface that is highly specific for each tissue, and even for parts of a tissue. Recreation of this complex microenvironment, involving cell–cell and cell–matrix interactions has been shown to be vital in the expression of the mature keratinocyte phenotype in the reconstruction of skin equivalents and the maintenance of the stem cell compartment. Collagen has been found to be essential for the functional expression of many epithelial cells and for endothelium to mature into capillaries. Small polypeptides containing this sequence effectively block matrix-induced differentiation, implying that the intact matrix molecule is required. Defined matrices are required; although fibronectin, laminin, collagen, and numerous other matrix constituents are commercially available, the specificity probably lies largely in the proteoglycan moiety, within which there is the potential for wide variability, particularly in the number, type, and distribution of the sulfated glycosaminoglycan, such as heparan sulfate. The extracellular matrix may also play important role in the modulation of growth factor activity. One type of extracellular matrix is exemplified by the thin, sheet-like basal laminae, previously called basement membranes, upon which layers of epithelial cells rest. In addition to supporting sheets of epithelial cells, basal laminae surround muscle cells, adipose cells, and peripheral nerves. Extracellular matrix is most abundant in connective tissues.

3. Polarity and cell shape: Various studies shows that growth of the cells on collagen gel and the subsequent release of the gel from the bottom of the dish with a spatula or bent Pasteur pipette are required for full maturation of cell. This process allows shrinkage of the gel and modification in the shape of the cell from flattened to cuboidal or even columnar shape. Following the shape change and also possibly due to contact to medium through the gel, the cells develop polarity which is visible by electron microscopy. When the nucleus becomes asymmetrically distributed nearer to the bottom of the cell an active Golgi complex is formed and secretion is observed towards the apical surface.

4. Oxygen Tension: Gas exchange enhances when positioning the cells at the air–liquid interface, particularly facilitating oxygen uptake without raising the partial pressure and risking free radical toxicity. It is also possible that the thin film above mimics the physicochemical conditions in vivo (surface tension, lack of nutrients) as well as oxygenation.

Table 1: Common Physiological Inducers of Differentiation

Type of Inducer	Common Inducers	Cell type
Steroid and related	Hydrocorticosone	Glia, glioma Lung alveolar typeII cells Hepatocytes Mammary epithelium Myloid leukemia
	Retinoid	Tracheobranchial epithelium Endothelium Enterocytes (Caco-2) Embryonal carcinoma Malanoma Myeloid leukemia Neuroblastoma
Peptide Hormones	Melanotropin	Melanocytes
	Thyotropin	Thyroid
	Erythropoietin	Erythroblasts
	Prolactin	Mammary epithelium
	Insulin	Mammary epithelium
Cytokines	Nerve growth factor	Neuron
	Glia maturation factor, CNTF, PDGF, BMP2	Glial cell
	Epimorphin	Kidney epithelium
	Fibrocyte-pneumocyte factor	Type II pneumocyte
	Interferon-α, β	A549 cells

		HL60, myeloid leukemia
	Interferon-γ	Neuroblastoma
	CNTF	Type 2 astrocytes
	IL-6, OSM	AS49
	BMP	1 OT 1/2
	KGF	Keratinocytes Prostatic epithelium
	HGF	Kidney (MDCK) Hepatocyte
	TGF-β	Bronchial epithelium Melanocyte
	Endothelium	Melanocyte
Vitamins	Vitamin E	Neuroblastoma
	Vitamin D3	Monocytes (U937) Myeloma Enterocyte (IEC-6)
	Vitamin K	Hepatoma Kidney epithelium
Minerals	Ca++	Keranocytes

Table 2: Common Non-Physiological Inducers of Differentiation

Type of Inducer	Common Inducers	Cell type	Fate
Planar- polar compound	DMSO	Murin erytholeukemia	Immature erythrocytes
		Myeloma	Granulocyte
		Neuroblastoma	Neurons
		Mammary epithelium	Secretory epithelium
		Hepatocyte precursors, HepaRG hepatoma	Hepatocyte
	Sodium butyrate	Erythroleukemia	Immature erythrocytes
		Colon cancer	Absorptive epithelium
	N- methyl acetamide	Glioma	Astrocyte
	N- methyl formamide, Dimethyl formamide	Colon cancer	Absorptive epithelium
	HMBA	Erythroleukemia	Immature erythrocytes
	Butylated hydroxyanisole	Adipose derived stem cells	Neuron
	Benzodiazepines	Erythroleukemia	Immature erythrocytes
Cytotoxic drugs	Genistein	Erythroleukemia	Immature erythrocytes
	Cytosin arabinoside	Myeloid leukemia	Granulocyte
	Mitocin C, anthracyclines	Melanoma	Melanocyte
	Metotrexate	Colorectal carcinoma	Adsorptive & mucin-secreting epithelium
Chromatin modifiers	Valproic acid	Adipose derived stem cells	Neuron Neurite extension

		PC12 cells	
	Azacytidine	NG108-15 neuronal cells	Cholinergic neuron
Signal transduction modifiers	Isobutylmethyl xanthine	Adipose derived stem cells	Adipocytes
	Forkoline	Adipose derived stem cells	Neuron
	PMA	Bronchial epithelium	Squamous epithelium
		Mammary epithelium	
		Colon(HT29, Caco-2)	Ductal morphogenesis
		Monocyte leukemia(U937)	Monocytes
		Erythroleukemia(K562)	Immature erythrocytes
		Neuroblastoma	Neurite growth

Relationship between Differentiation and Malignancy:

With increasing progression of cancer, histology of a tumor indicates poorer differentiation, and from a prognostic standpoint, patients with poorly differentiated tumors generally have a lower survival rate than patients with differentiated tumors. Cancer is principally a malfunction of cells to differentiate normally. It is therefore surprising to find that many tumors grown in tissue culture can be induced to differentiate. Indeed much of the fundamental data on cellular differentiation has been derived from murine leukemia, mouse and human myeloma, hepatoma, and neuroblastoma. Nevertheless, there appears to be an inverse relationship between the expression of differentiated properties and the expression of malignancy associated properties, even to the extent that the induction of differentiation has often been proposed as a mode of therapy. In correct environmental conditions, and assuming that the appropriate cells are present, partial, or even complete, differentiation is achievable in cell culture. As a general approach to promoting differentiation, as opposed to cell proliferation and propagation, the following aspects need to be taken care.

i. Selection of the correct cell type by use of appropriate isolation conditions and a selective medium.
ii. Growing the cells to a high cell density (>1×10^5 cells/cm^2) on the appropriate matrix. The matrix may be collagen of a type that is appropriate to the site of origin of the cells, with or without fibronectin or laminin, or it may be more complex, tissue derived or cell derived, such as Matrigel or a synthetic matrix (e.g., poly-D-lysine for neurons).
iii. Changing the cells to a differentiating medium rather than a propagation medium for other cell types this step may require defining the growth factors appropriate to maintaining cell proliferation and those responsible for inducing differentiation.
iv. Addition of differentiation-inducing agents, such as glucocorticoids, retinoids, vitamin D_3, DMSO, HMBA, prostaglandins and cytokines, such as bFGF, EGF, KGF, HGF, IL-6, OSM, TGF-β, interferons, NGF, and melanocyte-stimulating hormone (MSH), as appropriate for the type of cell.
v. Addition of the interacting cell type during the growth phase, the induction phase or both phases. Selection of the correct cell type is not always clear, but lung fibroblasts for lung epithelial maturation, glial cells for neuronal maturation, and bone marrow stromal cells for hematopoietic cells are some of the better-characterized examples.

Equilibrium between cell proliferation and differentiation:

Cells in culture can be thought to be in a state of equilibrium between cell proliferation and differentiation. Normal culture conditions i.e. in low cell density, mitogens in the medium will favor cell proliferation, whereas high cell density and addition of differentiation factors will induce differentiation. The position of the equilibrium will depend on culture conditions. Dedifferentiation of the culture may be due to the effect of growth factors or cytokines inducing a more proliferative phenotype, reprogramming of gene expression, or overgrowth of a precursor cell type. The relationship between differentiation and cell proliferation may become relaxed but it is not lost. For example, B16 melanoma cells still produce more pigment at a high cell density and at a low rate of cell proliferation than at a low cell density and a high rate of cell proliferation.

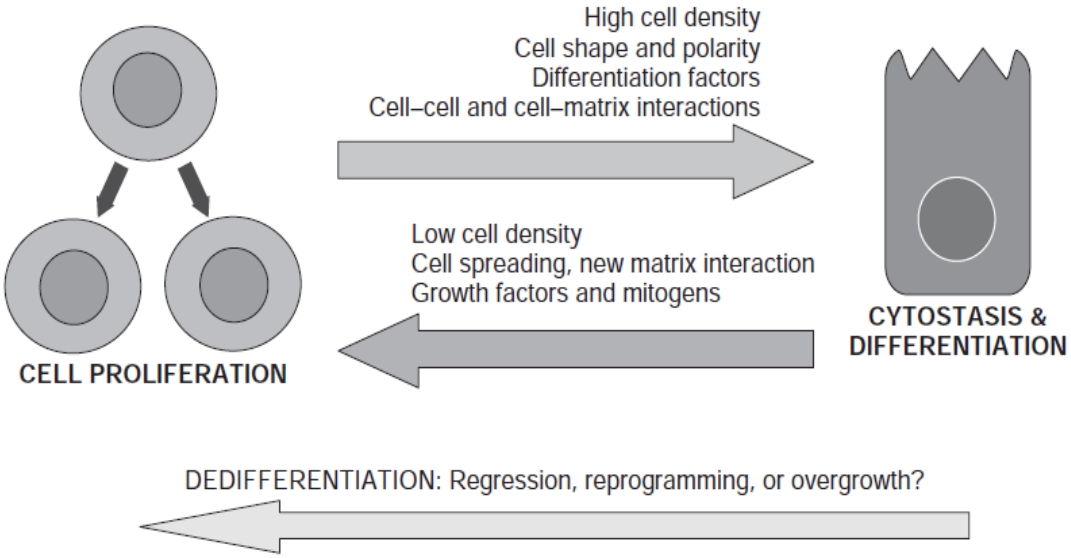


Figure 2: Differentiation and Proliferation

As differentiation progresses, cell division is reduced and ultimately ceases. In most cell systems, cell proliferation is incompatible with the expression of differentiated properties. Tumor cells can sometimes break this restriction, and in melanoma, for example, melanin continues to be synthesized while the cells are proliferating. Even in these situation, synthesis of the differentiated product increases when division stops.

Differentiation from stem cells:

It may be useful to think of a cell culture as being an equilibrium between stem cells, undifferentiated precursor cells, and mature differentiated cells and to suppose that the equilibrium may shift according to the environmental conditions.

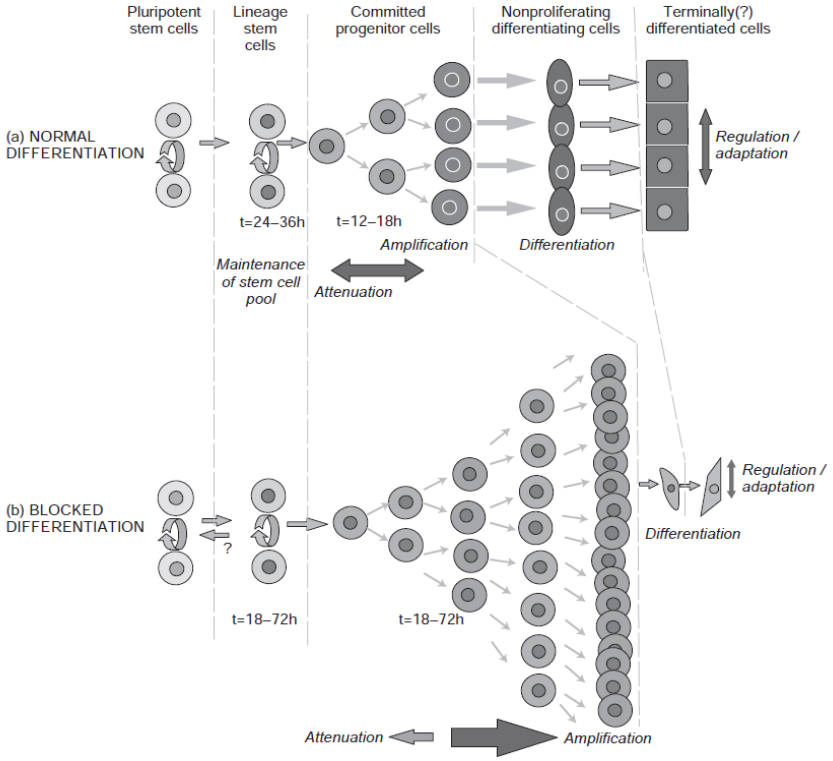


Figure 3: (a) In vivo, a small stem cell pool gives rise to a proliferating progenitor compartment that produces the differentiated cell pool. (b) In vitro, differentiation is limited proliferation, and the population becomes predominantly of progenitor cells, though stem cells may also be present.

Diamond nanoparticles have also been used to modify the substrate for the proliferation and differentiation of neural stem cells and the configuration of the growth surface can also be altered by photoetching. Treatment of the substrate with denatured collagen improves the attachment of many types of cells, such as epithelial cells, and the nondenatured gel may be necessary for the expression of differentiated functions.

Interesting facts:

1. Cells in culture can be thought to be in a state of equilibrium between cell proliferation and differentiation. Normal culture conditions i.e. in low cell density, mitogens in the medium will favor cell proliferation, whereas high cell density and addition of differentiation factors will induce differentiation.

2. Patients with poorly differentiated tumors generally have a lower survival rate than patients with differentiated tumors.

Questions:

1. What is differentiation? What are the major pathways of differentiation?
2. What are the parameters that are required for control of differentiations?
3. What are the relationship between differentiation and malignancy?

References:

1. Fresheny, I.; “Culture of animal cell – A manual of basic technique and specialized application.

M6 L5 Three dimensional cell culture

Three Dimentional Culture: Three-Dimensional (3-D) cell cultures have been widely used in biomedical research since the early decades of this century. The potential of 3-D cell cultures is currently being exploited in various areas of biomedical research. One reason for the recent progress in research on multi cell systems may be the increasing interaction between researchers working in different fields of biomedical science and using similar 3-D culture techniques. Such a research effort mirrors the common need for improved and more refined in vitro models as a link between cell-free systems or single cells and organs or whole organisms in vivo. One major advantage of 3-D cell cultures is their well-defined geometry, which makes it possible to directly relate structure to function and which enables theoretical analyses such as diffusion fields. Subsequently, the most promising data on these cultures may be obtained by using techniques allowing for spatial resolution. Combining such approaches with molecular analysis has clearly confirmed that, in comparison with conventional cultures, cells in 3-D cultures more closely resemble the in vivo situation with regard to cell shape and cellular environment. These parameters (shape and environment) can determine gene expression and the biological behaviour of the cells. In contrast to 2D monolayer, 3D cell culture models are modular, adaptable biomedical systems consisting range in complexity from a single cell type (monotypic), representing the minimum unit of the differentiated tissue *in vivo* to complex co-culture models that recapitulate both the 3D architecture and the multicellular complexity of the parental tissue. There will always be a number of questions that can only be answered by investigations using single cells or cell-free systems. At the same time, 3-D cultures cannot completely replace the testing of biological mechanisms for their relevance in vivo, e.g., in knockout animals

Effect of Cell Density:

Cell–cell interaction is manifested at the simplest level when a cell culture reaches confluence and the constituent cells begin to interact more strongly with each other because of contact mediated signaling, formation of junctional complexes and increased potential for exchange of homocrine factors. The first noticeable effect is cessation of cell motility (contact inhibition) and withdrawal from cell cycle (density limitation of cell proliferation) in normal cells and reduced cell proliferation and increased apoptosis in transformed cells.

Reciprocal Interaction:

When different cells interact in their population, they have tendency to show reciprocal effect on their respective phenotypes, and the resultant phenotypic changes lead to new interactions. Cell interaction is therefore not just a single event, but a continuing cascade of events. Similarly exogenous signals do not initiate a single event, as may be the case with homogeneous populations, but initiate a new cascade, as a result of the exogenously modified phenotype of one or both partners.

Choice of Model for Three Dimensional Cultures:

There are two major way to approach these goals.

- One is to accept the cellular distribution within the tissue, explant it and maintain it as an organ culture.
- The second is to purify and propagate individual cell lineages, study them alone under conditions of homologous cell interaction, recombine them, and study their mutual interactions.

Types of Three Dimensional cultures: There are three main types of three dimensional cultures:

- Organ culture
- Histotypic culture
- Organotypic culture

1) **Organ culture:** Organ culture in which whole organs or representative parts are maintained as small fragments in culture and retain their intrinsic distribution, numerical and spatial, of participating cells. Organ culture seeks to retain the original structural relationship of cells of the same or different types, and hence their interactive function in order to study the effect of exogenous stimuli on further development. Organ culture seeks to retain the original structural relationship of cells of the same or different types and hence their interactive function, in order to study the effect of exogenous stimuli on further development.
2) **Histotypic culture:** Histotypic culture in which propagated cells are grown alone to high density in a three-dimensional matrix or are allowed to form three-dimensional aggregates in suspension
3) **Organotypic culture:** Organotypic culture in which cells of different lineages are recombined in experimentally determined ratios and spatial relationships to recreate a component of the organ under study

Organ Culture:

Gas and Nutrient Exchange: A major deficiency in tissue architecture in organ culture is the absence of a vascular system, limiting the size (by diffusion) and potentially the polarity of the cells within the organ culture. When cells are cultured as a solid mass of tissue, gaseous diffusion and the exchange of nutrients and metabolites is from the periphery, and the rate of this diffusion limits the size of the tissue. The dimensions of individual cells cultured in suspension or as a monolayer are such that diffusion is not limiting, but survival of cells in aggregates beyond about 250 μm in diameter (~5000 cell diameters) starts to become limited by diffusion, and at or above 1.0 mm in diameter (~2.5×10^5 cell diameters) central necrosis is often apparent. To alleviate this problem, organ cultures are usually placed at the interface between the liquid and gaseous phases, to facilitate gas exchange while retaining access to nutrients. This is achieved by most

system by positioning the explant in a filter well insert on a raft or gel exposed to the air, but explants anchored to a solid substrate can also be aerated by rocking the culture, exposing it alternately to a liquid medium and a gas phase or by using a roller bottle or rotating tube rack. Anchorage to a solid substrate can lead to the development of an outgrowth of cells from the explant and resultant alterations in geometry even though this effect can be minimized by using a hydrophobic surface. One of the advantages of culture at the gas–liquid interface is that the explant retains a spherical geometry if the liquid is maintained at the correct level. If the liquid is too deep, gas exchange is impaired whereas if it is too shallow, surface tension will tend to flatten the explants and promote outgrowth. Permeation of oxygen increases by using increasing O_2 concentrations up to pure oxygen or by using hyperbaric oxygen. As increasing the O_2 tension will not facilitate CO_2 release or nutrient metabolite exchange, the benefits of increased oxygen may be overridden by other limiting factors.

Structural Integrity: The maintenance of structural integrity is the main reason for adopting organ culture as an in vitro technique in preference to cell culture. Whereas cell culture utilizes cells dissociated by mechanical or enzymatic techniques or spontaneous migration, organ culture deliberately maintains the cellular associations found in the tissue.

Growth and Differentiation: There is a relationship between growth and differentiation such that differentiated cells no longer proliferate. It is also possible that cessation of growth, regardless of cell density, may contribute to the induction of differentiation, if only by providing a permissive phenotypic state that is receptive to exogenous inducers of differentiation. Because of density limitation of cell proliferation and the physical restrictions imposed by organ culture geometry, most organ cultures do not grow or if they do proliferation is limited to the outer cell layers. Hence the status of the culture is permissive to differentiation and the appropriate cellular interactions and soluble inducers are provided as an ideal environment for differentiation to occur.

Limitations of Organ Culture:

- Organ cultures depend largely on histological techniques and they do not impart themselves readily into biochemical and molecular analyses.
- Biochemical monitoring requires reproducibility between samples, which is less easily achieved in organ culture than in propagated cell lines, because of sampling variation in preparing an organ culture, minor differences in handling and geometry, and variations in the ratios of cell types among cultures.
- Organ cultures are also more difficult to prepare than replicate cultures from a propagated cell line and do not have the advantage of a characterized reference stock to which they may be related.
- Organ culture is essentially a technique for studying the behaviour of integrated tissues rather than isolated cells.
- Organ culture has contributed a great deal to our understanding of developmental biology and tissue interactions and that it will continue to do so in the absence of adequate synthetic systems.

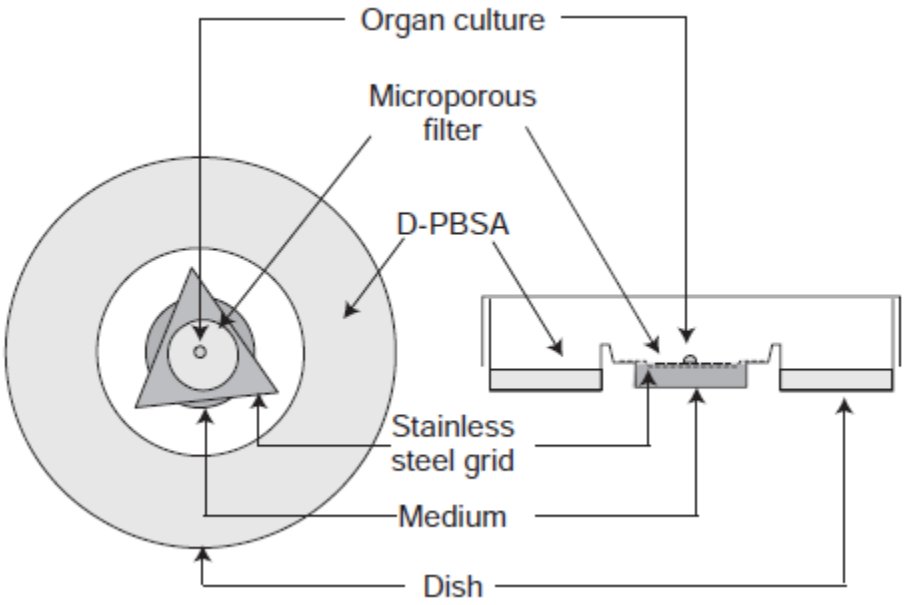


Figure 1: Organ Culture Small fragment of tissue on a filter laid on top of a stainless steel grid over the central well of an organ culture dish

Histotypic culture:

Histotypic culture is defined as high-density cell culture with the cell density approaching that of the tissue in vivo. Various attempts have been made to regenerate tissue-like architecture from dispersed monolayer cultures. As cells reach a high density, medium nutrients will become limiting. To avoid this, the ratio of medium volume to cell number should remain approximately as it was in low density culture. This can be achieved by seeding cells on a small coverslip in the center of a large non–tissue-culture grade dish or by use of filter well inserts, which give the opportunity for the formation of both high-density polarized cultures and heterotypic combinations of cell types to create organotypic cultures. A high medium-to-cell ratio can also be maintained by perfusion.

Gel and Sponge Techniques: Use of three-dimensional sponges and gels has increased extensively with the development of tissue engineering. Two commonly used gel in this technique.

- **Collagen gel:** Collagen gel (native collagen, as distinct from denatured collagen coating) provides a matrix for the morphogenesis of primitive epithelial structures. Many different types of cell can be shown to penetrate such matrices and establish a tissue-like histology.
- **Matrigel:** Matrigel is a commercial product derived from the extracellular matrix of the Engelbreth–Holm–Swarm (EHS) mouse sarcoma which has been used for coating plastic but can also be used in gel form. It is composed of laminin, collagen, fibronectin, and proteoglycans with a number of bound growth factors, although it can be obtained in a growth factor-depleted form. It has been used as a substrate for epithelial morphogenesis formation of capillaries from endothelial cells and in the study of malignant invasion.

Organotypic Culture:

High density three-dimensional culture involving the recombination of different cell lineages may be referred to as organotypic culture, a term that used to distinguish these reconstruction techniques from organ culture where the original cells are not dissociated. The key event that distinguishes these constructs from histotypic culture is the introduction of heterotypic cell interaction including diffusible paracrine effects and signaling implicating the extracellular matrix. The relationship of the cells allows the

generation of a structured microenvironment, cell polarity and enhanced differentiation. Creation of organotypic culture by mixing cells randomly and allowing them to interact and sort, as can happen spontaneously particularly with embryonic cells or the construct may be design to keep the interacting cells separate so that their interactions may be studied.

Tissue Equivalents: The advent of filter well technology boosted by its commercial availability, has produced a rapid expansion in the study of organotypic culture methods.

Tissue Engineering: Just as organotypic culture needs cell interaction, constructs for tissue engineering often require similar interactions, as in the interaction between endothelium and smooth muscle in blood vessel reconstruction. In addition to biological interactions, some constructs require physical forces; skeletal muscle needs tensile stress, bone and cartilage needs compressive stress, and vascular endothelium in a blood vessel construct needs pulsatile flow.

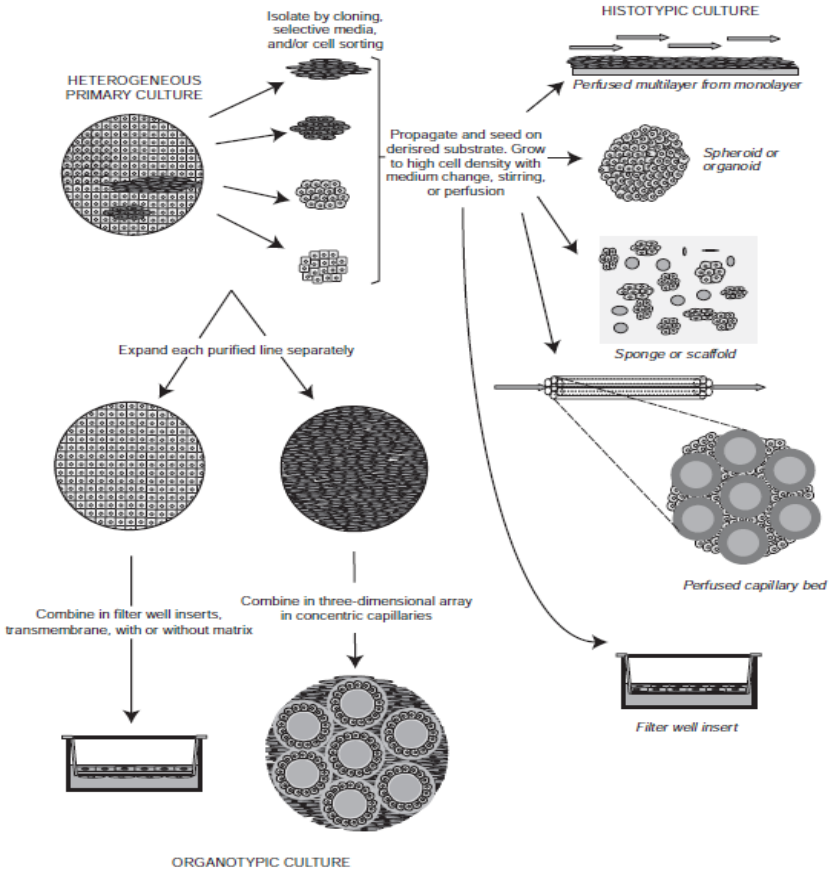


Figure 2: Histotypic and Organotypic Culture

Imaging Cells in 3-D Constructs: As we know that microscopic observation becomes difficult when cells are incorporated into a scaffold in a three-dimensional organotypic construct, alternative methods must be used to visualize the status of the cells within the construct. This can be done by NMR if the bioreactor housing the constructs is placed within an NMR detector and the output displayed as an MRI, and the emission spectrum being analysed.

Interesting facts:

1. Organ culture is a technique for studying the behaviour of integrated tissues.
2. Organ culture has contributed a great deal to our understanding of developmental biology and tissue interactions.

Questions:

1. What are the types of three dimensional cultures?
2. What are the advantages of three dimensional cultures?
3. What are Gel and Sponge Techniques and how its use increase with increasing genetic engineering?

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M6 L6 Role of matrix in cell growth

Role of matrix in cell growth: Matrix is an insoluble, dynamic gel in the cytoplasm, believed to be involved in cell shape determination and locomotive mechanism, across a solid substrate. It consists of polymeric microtubules, actin microfilaments and intermediate filaments interacting with a number of other proteins.

Extracellular matrix (ECM): The extracellular matrix (ECM) is a part of three connective tissue layers (endomysium, perimysium, and epimysium) surrounding muscle fibres. Extracellular matrix is composed of proteins including collagens and proteoglycans.

Component of extra cellular matrix: ECM is comprised variously of collagen, laminin, fibronectin, hyaluronan and proteoglycans such as beta glycan, decorin, perlecan, and syndecan-1, some of which bind to growth factors or cytokines.

- Proteoglycans in extracellular matrix form a cross-linked meshwork with fibrous proteins
- Some proteins bind multiple other proteins and glycosaminoglycans (fibronectin).
- Integrin is a family of proteins that mediate signalling between cell interior and extracellular matrix
- Mass of interactions between cells and matrix not only anchors cells to matrix but also provides paths that direct migration of cells in developing tissue and (through integrin) conveys information in both directions across plasma membrane. Figure 1 depicts structure of cell matrix.

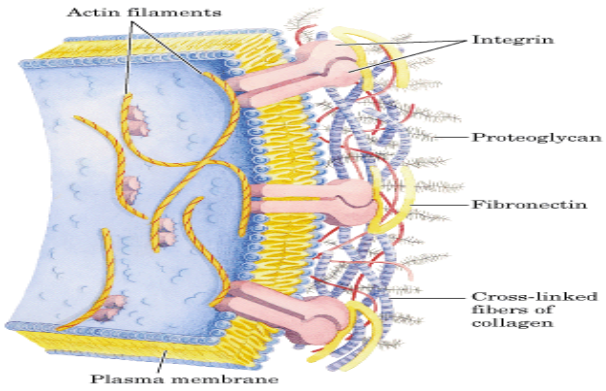

Figure 1: Cell matrix

ECMs are composed of proteins such as collagen and elastin that serve as scaffolds for cells as well as networks of various adhesion ligands and growth factors, which promote cell signalling. ECM is complex in both structure and composition.

Role of matrix: There are some important roles that matrix play in biological system which is described as follows.

- Matrixes are generally used for providing support
- It involve in segregating tissues from one another
- It takes part in regulation of intercellular communication
- Extracellular Matrix cells have been found to cause regrowth and healing of tissue.
- Some time it acts as fibrosis
- The use of ECM constituents can be highly beneficial in enhancing cell survival, proliferation, or differentiation, but unless recombinant molecules are used
- In human foetuses, the extracellular matrix works with stem cells to grow and regrow all parts of the human body and foetus can regrow anything that gets damaged in the womb
- In case of injury repair and tissue engineering, the extracellular matrix serves two main functions

a) It prevents the immune system by triggering from the injury and responding with inflammation and scar tissue

b) It facilitates the surrounding cells to repair the tissue instead of forming scar tissue

Molecular components: Components of the ECM are produced intracellularly by resident cells and secreted into the ECM through exocytosis. Once secreted, they then aggregate with the existing matrix. The ECM is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs).

Proteoglycans: Since we know that, GAGs are carbohydrate polymers and are usually attached to extracellular matrix proteins to form proteoglycans (exception-hyaluronic acid). Proteoglycans have a net negative charge that attracts positively charged sodium ions (Na^+) which attracts water molecules via osmosis, keeping the ECM and resident cells hydrated. Proteoglycans may also help to trap and store growth factors within the ECM. There are the different types of proteoglycan found within the extracellular matrix.

1. Heparin sulphate: Heparin sulphate (HS) is a linear polysaccharide found in all animal tissues. It occurs as a proteoglycan (PG) in which two or three HS chains are attached in close proximity to cell surface or extracellular matrix proteins. HS binds to a variety of protein ligands and involve in regulation of a wide variety of biological activities, including developmental processes, angiogenesis, blood coagulation and tumour metastasis. In the extracellular matrix, particularly basement membranes, the multi-domain proteins perlecan, agrin and collagen XVIII are the main proteins to which heparin sulphate is attached.

2. Chondroitin sulphate: Chondroitin sulfates help to provide the tensile strength of cartilage, tendons, ligaments and walls of the aorta. They have also been known to affect neuroplasticity.

3. Keratan sulphate: Keratan sulfates have variable sulfate content and unlike many other GAGs, do not contain uronic acid. They are present in the cornea, cartilage, bones and the horns of animals.

Non-proteoglycan polysaccharide: There are various non-proteoglycan polysaccharides.

1. **Hyaluronic acid:** Hyaluronic acid (or hyaluronan at physiological pH) is a polysaccharide containing alternating residues of D-glucuronic acid and *N*-acetyl glucosamine. Unlike other glycosaminoglycan (GAGs) it is not found as a proteoglycan. Like cellulose and chitin, it is synthesized at the plasma membrane by a transmembrane hyaluronan synthase. Hyaluronan is the only GAG that occurs as a single long polysaccharide chain. Hyaluronate is also an essential component of the extracellular matrix of cartilage and tendons, to which it contributes tensile strength and elasticity as a result of its strong interactions with other components of the matrix. A number of proteoglycans interact with hyaluronan to form large complexes in the extracellular

matrix. A well-characterized example is aggrecan, the major proteoglycan of cartilage. Hyaluronic acid acts as an environmental sign that regulates cell behaviour during embryonic development, healing processes, inflammation and tumour development. It interacts with a specific trans-membrane receptor, CD44.

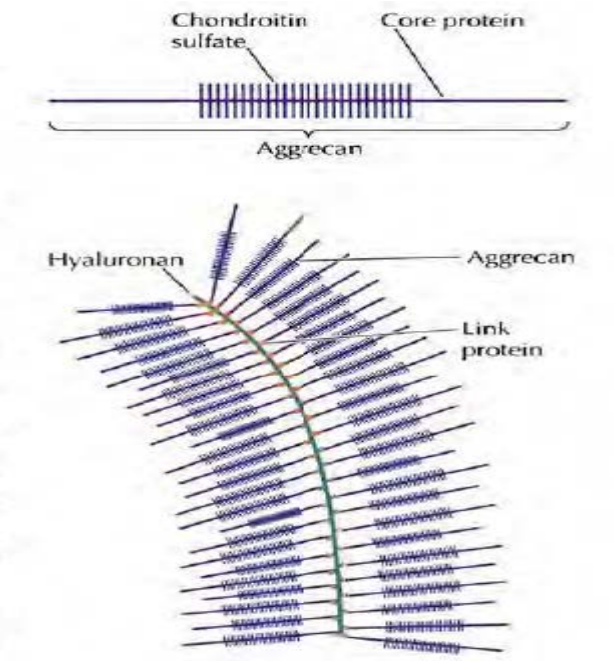

Figure 2: Structure of hyaluronan and aggrecan

2. Matrix Structural Proteins: Extracellular matrices are composed of tough fibrous proteins embedded in a gel-like polysaccharide ground substance-a design basically similar to that of plant cell walls.

Collagen: In ECM of most animals, collagens are the abundantly found structural protein. In fact, collagen is the most abundant protein in the human body and accounts for 90% of bone matrix protein content. Collagens are present in the ECM as fibrillar proteins and give structural support to resident cells. Collagens are a large family of proteins containing at least 27 different members. They are characterized by the formation of triple helices in which three polypeptide chains are wound tightly around one another in a rope-like structure. The different collagen polypeptides can assemble into 42 different trimers. The triple helix domains of the collagens consist of repeats of the amino acid sequence Gly-X-Y. A glycine (the smallest amino acid, with a side chain consisting only of hydrogen) is required in every third position, so that the polypeptide chains can pack together close enough to form the collagen triple helix. Proline is

frequently found in the X position and hydroxyproline in the γ position; because of their ring structure these amino acids stabilize the helical conformations of the polypeptide chains. The unusual amino acid hydroxyproline is formed within the endoplasmic reticulum by modification of proline residues that have already been incorporated into collagen polypeptide chains. Lysine residues in collagen are also frequently converted to hydroxylysines. The hydroxyl groups of these modified amino acids are thought to stabilize the collagen triple helix by forming hydrogen bonds between polypeptide chains. These amino acids are rarely found in other proteins although hydroxyproline is also common in some of the glycoproteins of plant cell walls.

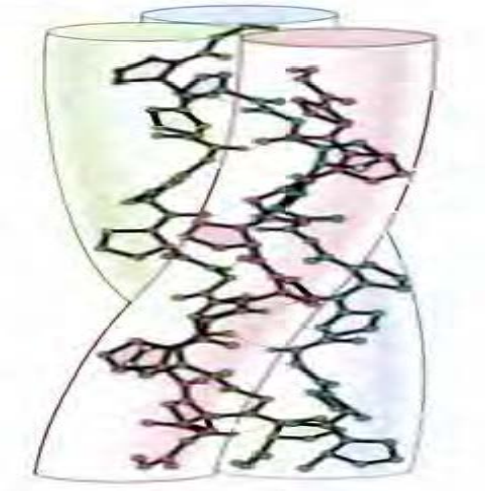

Figure 3: Structure of triple helix collagen

The collagen can be divided into several families according to the types of structure they form:

Table 1: Classification of collagens

Collagen class	Type	Tissue Distribution
Fibril- forming	I	Most connective tissue
	II	Cartilage and vitreous humors (e.g. skin and lung)
	III	Tissue containing collagen I
	V	Cartilage
	XI	Bone and cornea
	XXIV	Eye, ear and lung
	XXVII	Cartilage
Fibril-associated	IX	Cartilase
	XII	Tissue containing collagen I
	XIV	Tissue containing collagen I
	XVI	Many tissue
	XIX	Many tissue
	XX	Cornea
	XXI	Many tissue
	XXII	Cell junctions
	XXVI	Testis and overy
Network forming	IV	Basal laminae
	VIII	Many tissues
	X	Cartilase
Anchoring fibrils	VII	Attachement of basal laminae to underlying connective tissue
Transmembrane	XVII	Skin hemidesmosomes
	XXV	Nerve cells

The most abundant type of collagen (type I collagen) is one of the fibril forming collagens that are the basic structural components of connective tissues. The polypeptide chains of these collagens consist of approximately a thousand amino acids or 330 Gly-X-Y repeats. After being secreted from the cell these collagens assemble into collagen fibrils in which the triple helical molecules are associated in regular staggered arrays. These fibrils do not form within the cell because the fibril forming collagens are synthesized as soluble precursors (procollagens) that contain nonhelical segments at both ends of the polypeptide chain. Procollagen is cleaved to collagen after its secretion, so the assembly of collagen into fibrils take place only outside the cell. The association of collagen molecules in fibrils is further strengthened by the formation of covalent

crosslinks between the side chains of lysine and hydroxylysine residues. Frequently, the fibrils further associate with one another to form collagen fibers, which can be several micrometers in diameter.

Elastin: In contrast to collagens, Elastins give elasticity to tissues, allowing them to stretch when needed and then return to their original state. This is useful in blood vessels, the lungs, in skin, and the ligaments. Elastins are synthesized by fibroblasts and smooth muscle cells. Elastins are highly insoluble, and tropoelastins are secreted inside a chaperone molecule, which releases the precursor molecule upon contact with a fiber of mature elastin. Tropoelastins are then deaminated to become incorporated into the elastin strand. Diseases such as cutis laxa and Williams syndrome are associated with deficient or absence of elastin fibers in the ECM.

Matrix adhesion proteins: Matrix adhesion proteins, the final class of extracellular matrix constituents are responsible for linking the components of the matrix to one another and to the surfaces of cells. They interact with collagen and proteoglycans to specify matrix organization and are the major binding sites for integrins.

1. Fibronectin: Fibronectin is the principal adhesion protein of connective tissues. Fibronectin is a dimeric glycoprotein consisting of two polypeptide chains, each containing nearly 2500 amino acids. Fibronectin are proteins that connect cells with collagen fibers in the ECM, allowing cells to move through the ECM. Within the extracellular matrix, fibronectin is often cross-linked into fibrils. Fibronectin has binding sites for both collagen and GAGs so it cross-links these matrix components. Fibronectins bind collagen and cell surface integrins, causing a reorganization of the cell's cytoskeleton and facilitating cell movement. Fibronectin are secreted by cells in an unfolded, inactive form. Binding to integrins unfolds fibronectin molecules, allowing them to form dimers so that they can function properly. Fibronectins also help at the site of tissue injury by binding to platelets during blood clotting and facilitating cell movement to the affected areas during wound healing.

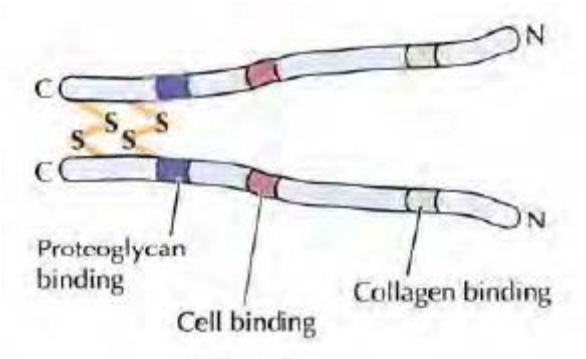

Figure 4: Structure of Fibronectin

2. Laminin: In almost all animals, Basal laminae contain distinct adhesion proteins of the laminin family. Laminins are heterotrimers of α, β and γ subunits which are the products of five α genes, four β genes, and three γ genes. Like type IV collagen, laminins can self-assemble into meshlike polymers. Such laminin networks are the major structural components of the basal laminae synthesized in very early embryos, which do not contain collagen. The laminins also have binding sites for cell surface receptors such as integrins, type IV collagen, and the heparan sulfate proteoglycan, perlecan. In addition, laminins are tightly associated with another adhesion protein, called entactin, which also binds to type IV collagen. As a result of these multiple interactions, laminin, entactin, type IV collagen, and perlecan form cross-linked networks in the basal lamina. They also support in cell adhesion.

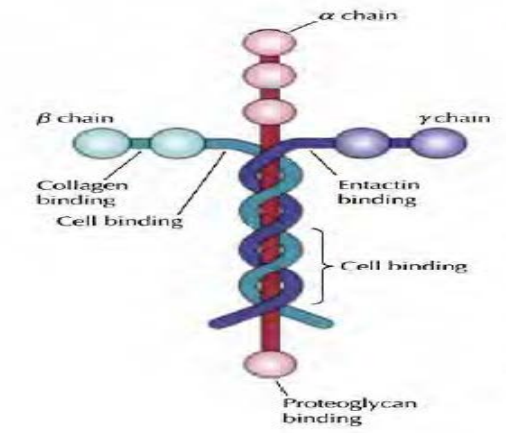

Figure 5: Structure of laminin

Cell adhesion to the ECM: Many cells bind to components of the extracellular matrix. Cell adhesion can occur in two ways by focal adhesions, connecting the ECM to actin filaments of the cell, and hemi-desmosomes, connecting the ECM to intermediate filaments such as keratin. This cell-to-ECM adhesion is regulated by specific cell surface cellular adhesion molecules (CAM) known as integrins. The integrins are a family of transmembrane proteins consisting of two subunits, designated α and β. Integrins are cell surface proteins that bind cells to ECM structures such as fibronectin and laminin, and also to integrin proteins on the surface of other cells.

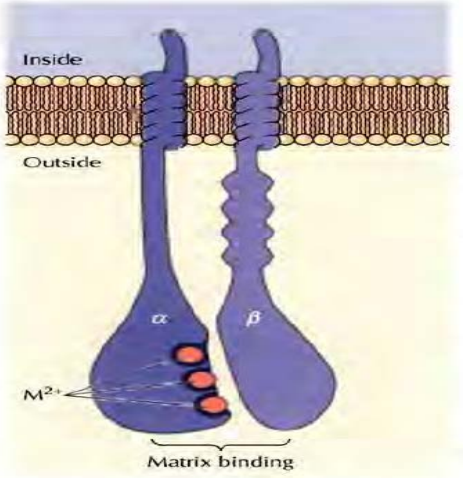

Figure 6: Structure of Integrin

Fibronectins bind to ECM macromolecules and facilitate their binding to transmembrane integrins. The attachment of fibronectin to the extracellular domain initiates intracellular signaling pathways as well as association with the cellular cytoskeleton via a set of adaptor molecules such as actin.

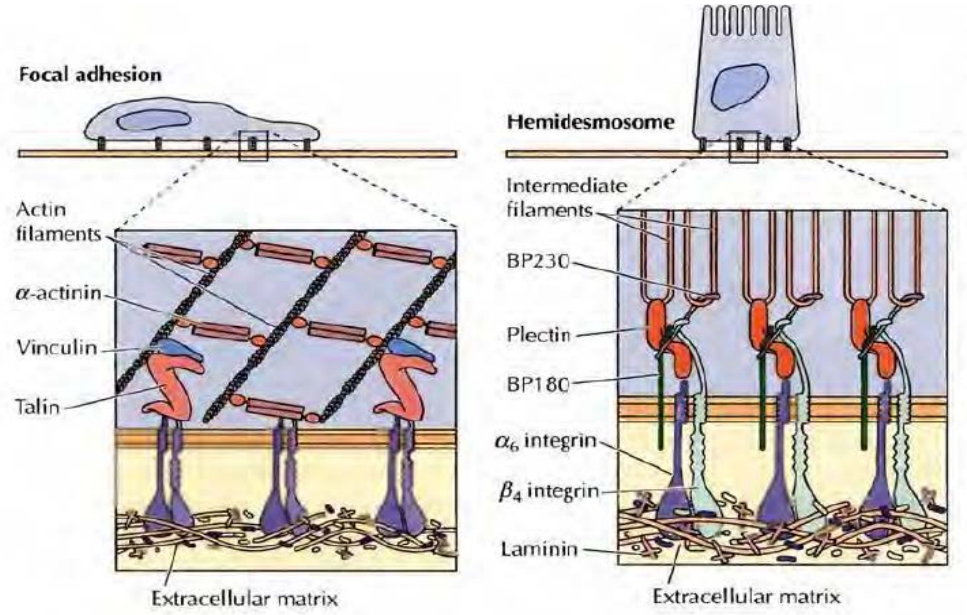

Figure 7: Focal adhesion and Hemidesmosome

Commercially available matrices:

Commercially available matrices such as Matrigel™ (Becton Dickinson) from the Engel breth–Holm–Swarm (EHS) sarcoma, contain laminin, fibronectin, and proteoglycans, with laminin predominating. Other matrix products include Pronectin F (Protein Polymer Technologies), laminin, fibronectin, vitronectin entactin (UBI), heparan sulfate, EHS Natrix (BD Biosciences), ECL (US Biological), and Cell-tak (BD Biosciences). Some of these products are purified, whereas others are a mixture of matrix products that have been poorly characterized and may also contain bound growth factors. If cell adhesion for survival is the main objective, and defined substrates are inadequate, the use of these matrices is acceptable, but if mechanistic studies are being carried out, they can only be an intermediate stage on the road to a completely defined substrate.

Table 2: Examples of some matrix

Material	Composition	Source
Matrigel	Laminin, fibronectin, collagen IV, proteoglycans, growth factors (growth factor depleted available)	EHS sarcoma
EHS Natrix	Laminin, fibronectin, collagen IV, proteoglycan, growth factors	Cell line from EHS sarcoma
Cell-Tak	Polyphenolic protein	Mytilus edulis
Collagens(various)	Collagen I, II, III, IV	Human, bovine, rat tail
ProNectin F	Protein polymer with multiple copies of RGD containing epitops	Recombinant
Laminin	Attachement protein from basement membrane	Natural
Laminin	Attachement protein from from basement membrane	Recombinant
Fibronectin	Attachement protein from from extracellular matrix	Natural
Fibronectin	Attachement protein from from extracellular matrix	Recombinant
Heparan sulfate	Matrix proteoglycan	Natural
ECL	Enactin-collagen IV-laminin	Natural
Vitronectin	Attachement protein from extracellular matrix	Natural
ECM	Extracellular matrix	natural

Interesting facts:

1. Collagens are abundantly found structural protein in the human body and accounts for 90% of bone matrix protein content.

2. Elastins give elasticity to tissues, allowing them to stretch when needed and then return to their original state.

3. Fibronectins bind to ECM macromolecules and facilitate their binding to transmembrane integrins.

4. Basal laminae contain distinct adhesion proteins of the laminin family in almost all animals.

Questions:

1. Explain the role of matrix in cell culture?
2. Give the detail about matrix material that are generally used?
3. What is the difference between proteoglycan and non-proteoglycan polysaccharide and how can they involve in formation of matrix?

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3. Fresheny, I. “Culture of Animal Cells- A Manual of Basic Technique and Specialized Applications”
4. What are the types of matrixes? What are the advantages of matrix adhetion protein?

Module 7 Introduction to Hematology

The present module details the components of blood plasma cell (RBC, WBC, and platelets). We will see how these cells are different from other cells which we have studied till now. Further it also describes Haemopoiesis, erythropoiesis and leucopoiesis.

Lecture 1 Components of blood plasma cell

The blood plasma is mainly composed of red blood corpuscles (RBC), white blood corpuscles (WBC) and platelets. These cell types are different from most of the cells you have come across.

RBC

The first person to describe red blood cells or erythrocytes was a Dutch biologist Jan Swammerdam, in 1658. Anton van Leeuwenhoek provided further descriptions of the RBCs in 1674. Red blood cells are the most common type of blood cell (about 4-6 millions/mm^3). Its role is to deliver oxygen to the body tissues. A typical human mature erythrocyte is 6–8 μm in diameter and 2 μm thick. The RBCs mature in the bone marrow. In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles as mitochondria, Golgi apparatus and endoplasmic reticulum. The shape of the red blood cells become sickle shaped in the disease called sickle-cell anaemia. The mean life of erythrocytes is about 120 days. When they come to the terminal end of their life, they are retained by the spleen where they are phagocyted by macrophages. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. This role is accomplished by haemoglobin which is an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color. Haemoglobin structure was discovered in 1959 through X-ray crystallography by Dr. Max Perutz who unraveled the structure of hemoglobin. Hemoglobin in the erythrocytes also carries carbon dioxide back from the tissues which is transported back to the pulmonary capillaries of the lungs as bicarbonate dissolved in the blood plasma. Other than hemoglobin, the cell membrane of red blood cells play many roles that regulate surface deformability, flexibility, adhesion to other cells and immune recognition. The red blood cell membrane is composed of 3 layers which are the glycocalyx on the exterior (rich in carbohydrates) and the lipid bilayer which comprising of transmembrane proteins.

WBC

White blood cells (WBCs) or Leukocytes confirm immunity to organisms. The density of the leukocytes in the blood has been reported to be 5000-7000 /mm^3. They are of two types namely granulocytes (cytoplasm having granules) and agranulocytes (cytoplasm lacking granules). Further, granulocytes can be distinguished into neutrophil, eosinophil and basophil. The granules in the granulocyte lineage have different affinity towards neutral, acid or basic stains giving the cytoplasm different colors. The agranulocytes are lymphocytes and monocytes. The proportion of each type of leucocyte along with their primary function in blood is listed in Table 1.

Table 1: Proportions of leukocyte present in the blood

Leukocyte type	Percentage proportion %	Function
	Granulocyte	
Neutrophil	50 - 70	Phagocyting bacteria
Eosinophil	2 - 4	Attack parasites and phagocyte antigen-antibody complexes.
Basophil	0.5 - 1	Secrete anti-coagulant and vasodilatory substances as histamines and serotonin
	Agranulocyte	
Lymphocyte	20 - 40	Main constituent of the immune system
Monocyte	3 - 8	Replenish resident macrophages and dendritic cells

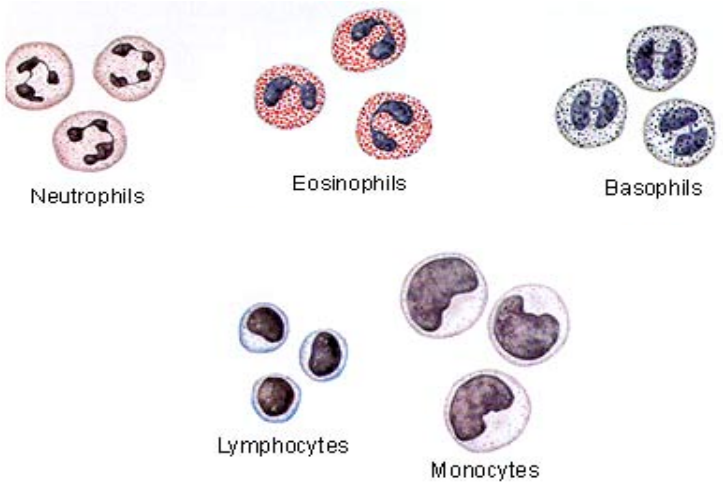

Figure 1: Different types of Leucocytes

Neutrophils: The neutrophils have a diameter of 12-15 µm. Their nucleus is divided into 2 - 5 lobes connected by a fine nuclear strand or filament. The cytoplasm is transparent due to the presence of small granules. Interestingly in the nucleus of the neutrophil from females, we can observe a Barr body which is the inactivated X chromosome (Figure 1).

Eosinophils: The eosinophils are quite rare in the blood. They have the same size as the neutrophils and generally have bilobed nucleus. The cytoplasm is granular which stains pink to orange with acidic dyes (Figure 1).

Basophils: Basophils are the rarest leukocytes having a diameter of 9-10 µm. Their cytoplasm is granular which stains dark purple with basic dyes. Their nucleus is bi or tri-lobed.

Lymphocytes: Lymphocytes are 8-10 µm in diameter and generally they are smaller than the other leukocytes (Figure 1). The cytoplasm is transparent. The nucleus is round and large occupying most of the cellular space. With respect to the amount of cytoplasm, lymphocytes are divided into small, medium and large. The lymphocytes are the main constituents of the immune system which is a defense against the attack of pathogenic micro-organisms such as viruses, bacteria, fungi and protista. Lymphocytes yield antibodies and arrange them on their membrane. An antibody is a molecule able to bind itself to molecules of a complementary shape called antigens, and recognize them. Lymphocytes can be further divided into B and T cells and the natural killer cells. The natural killer cells are characterized by their cytotoxic activity. They kill viruses, bacteria, infected and neoplastic cells and also regulate the production of other hematic cells such as erythrocytes and granulocytes.

Monocytes: Monocytes are the largest leukocytes having the diameter of 16-20 µm (Figure 1). They have a horseshoe-shaped nucleus with a transparent cytoplasm. Most monocytes are the precursors of macrophages which are larger blood cells. In the presence of an inflammation site, monocytes quickly migrate from the blood vessel and start an intense phagocytory activity. They produce substances which have defensive functions such as lysozyme, interferons and other substances which modulate the functionality of other cells. Macrophages cooperate in the immune defense. They expose molecules of digested bodies on the membrane and present them to more specialized cells, such as B and T lymphocytes.

Platelets

Platelets or thrombocytes are only about 20% of the diameter of red blood cells and are the most numerous cell of the blood. Their diameter is about 2-3 µm; hence they are much smaller than erythrocytes. Their density in the blood is 200000-300000 /mm^3 and a normal platelet count in a healthy individual is between 150,000 and 450,000 per µl (microlitre) of blood (150–450)×10^9/L). Platelets are not only the smallest blood cell, they are also the lightest. Therefore they are pushed out from the center of flowing blood to the wall of the blood vessel. There they roll along the surface of the vessel wall, which is lined by cells called endothelium. The endothelium is a very special surface, like Teflon, that prevents anything from sticking to it. However when there is an injury or cut, and the endothelial layer is broken, the tough fibers that surround a blood vessel are exposed to the liquid flowing blood. It is the platelets that react first to injury. The tough fibers surrounding the vessel wall, like an envelope, attract platelets like a magnet, stimulate the shape change that is shown in the pictures above, and platelets then clump onto these fibers, providing the initial seal to prevent bleeding, the leak of red blood cells and plasma through the vessel injury. Their function is to stop the loss of blood from wounds (hematostasis). They do so by releasing factors like serotonin which reduce the diameter of lesioned vessels and slow down the blood flux, the fibrin which trap cells and forms the clotting. Even if platelets appear roundish in shape, they are not real cells and with Giemsa stain they have an intense purple color. Platelets are produced in the bone marrow from cells known as megakaryocytes by the process of fragmentation which results in the release of over 1,000 platelets per megakaryocyte. The dominant hormone controlling megakaryocyte development is thrombopoietin.

Functions of Platelet:

When there is a wound, platelets aggregate using the protein fibrinogen and von Willebrand factor (vWF) as a connecting agent. The most abundant platelet aggregation receptor is glycoprotein IIb/IIIa which is a calcium-dependent receptor for fibrinogen, fibronectin, vitronectin, thrombospondin vWF. Activated platelets adhere with the help of glycoprotein Ia, to the collagen that is exposed by endothelial damage. Aggregation and adhesion act together to form the platelet plug. Platelet aggregation is stimulated by ADP, thromboxane, and α2 receptor-activation, but inhibited by other inflammatory products

like PGI2 and PGD2. Other role of platelets includes modulation of inflammatory processes by interacting with leukocytes and by secreting cytokines, chemokines, and other inflammatory mediators. They Platelets also secrete platelet-derived growth factor (PDGF). Diseases like thrombocytopenia and thrombocytosis may present with coagulation problems due to low platelet counts which increase bleeding risks.

Lecture 2 Haemopoiesis, erythropoiesis and leucopoiesis

Haemopoiesis

Haemopoiesis or haematopoiesis is the of process formation of new blood cellular components. It has been estimated that in an adult human, approximately 10^{11}–10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation. The mother cells from which the progeny daughter blood cells are generated are known as haematopoietic stem cells. In an embryo yolk sac is the main site of haemopoiesis whereas in human the basic sites where haemopoiesis occurs are the bone marrow (femur and tibia in infants; pelvis, cranium, vertebrae, and sternum of adults), liver, spleen and lymph nodes (Table 1). In other vertebrates haemapoiesis occurs in loose stroma of connective tissue of the gut, spleen, kidney or ovaries.

Table 1: Sites of Haemopoiesis in humans

Stage	Sites
Fetus	0–2 months (yolk sac) 2–7 months (liver, spleen) 5–9 months (bone marrow)
Infants	Bone marrow
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

The process of haemopoiesis

Pluripotent stem cells with the capability of self renewal, in the bone marrow known as the haemopoiesis mother cell give rise to the separate blood cell lineages. This haemopoietic stem cell is rare, perhaps 1 in every 20 million nucleated cells in bone marrow. Figure 1 illustrates the bone marrow pluripotent stem cell and the cell lines that arise from it. Cell differentiation occurs from a committed progenitor haemopoietic stem cell and one stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions. The process leads to division of stem cells and commitment of each cell to differentiate into one of the different blood progenitor cells. The cell lineage chosen by the progenitor cells is a matter both chance and on the external stimuli received by progenitor cells. Internal transcription factors like PU.1 commits cells to the myeloid lineage whereas GATA-1 leads to erythropoietic and megakaryocytic differentiation. The proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells is in turn regulated by glycoprotein hormones like Granulocyte colony

stimulating factor or G-CSF. The growth factors may cause cell proliferation but can also stimulate differentiation, maturation, prevent apoptosis and affect the function of mature cells. The other growth factors that act at various levels of haemopoiesis are interleukin (IL-1 and IL-3); macrophage colony-stimulating factor; stem cell factor; and tumour necrosis factor (Table 2).

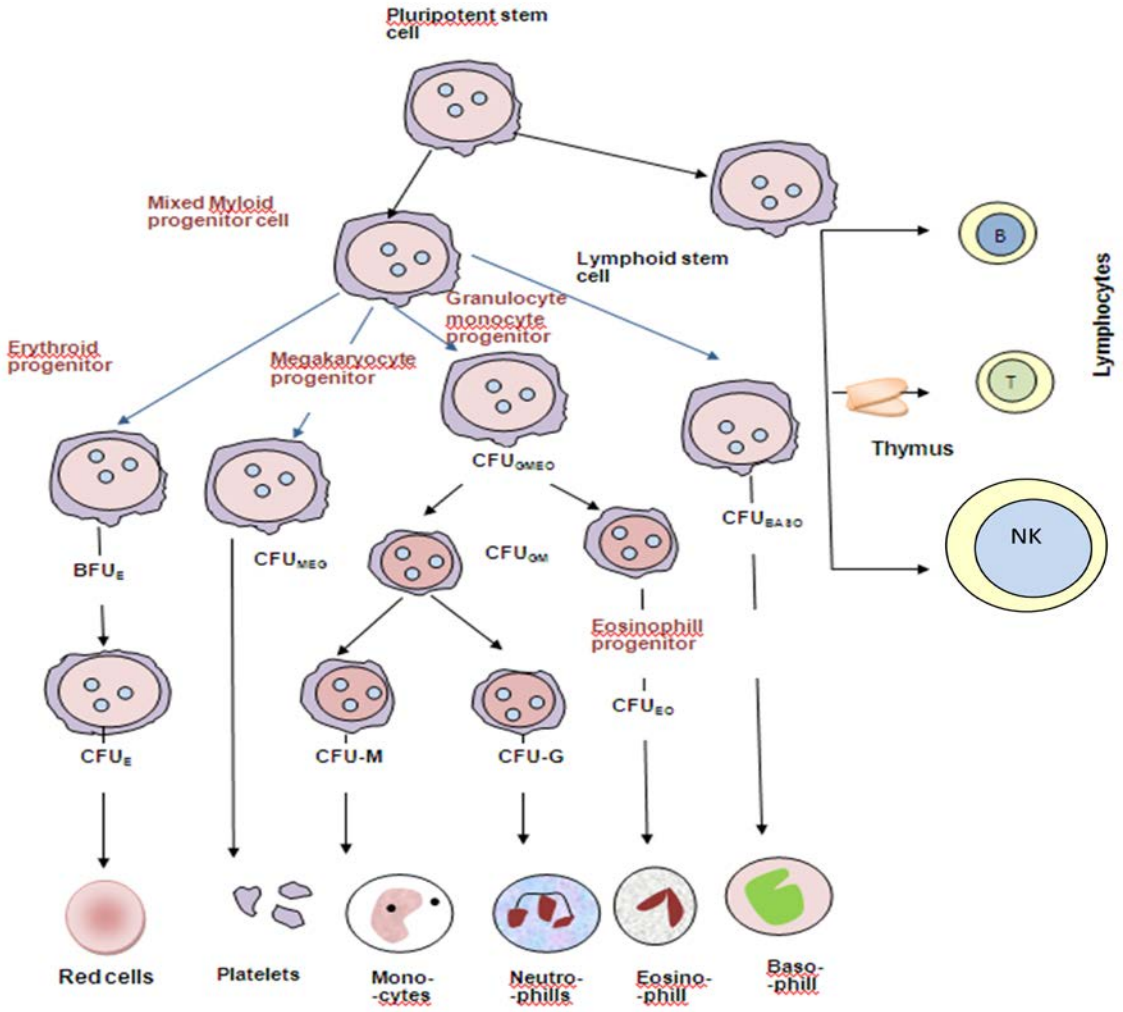

Figure 1: Diagrammatic representation of the bone marrow pluripotent stem cell and the cell lines that arise from it. Various progenitor cells can be identified by culture in semi-solid medium by the type of colony they form. Baso, basophil; BFU, burst-forming unit; CFU, colony-forming unit; E, erythroid; Eo, eosinophil; GM, granulocyte, monocyte; Meg, megakaryocyte; NK, natural killer cell (Hoffbrand et al. 2011).

Table 2 Growth factors in haemopoiesis

Acts On	Growth factor type
stromal cells	IL-1, TNF
pluripotential stem cells	SCF, Flt-L
multipotential progenitor cells	IL-3, GM-CSF, IL-6, G-CSF, Thrombopoietin
committed progenitor cells	G-CSF, M-CSF, IL-5, Thrombopoietin

Legend: Flt-L, Flt ligand; G- and GM-CSF, granulocyte and granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; SCF, stem cell factor; TNF, tumour necrosis factor.

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target progenitor cells. Receptors like granulocyte macrophage colony-stimulating factor GMCSF-R are from the haematopoietin receptor superfamily which possesses the capacity to dimerize after binding their ligand. This results in cascade of intracellular signal transduction pathways of which the three major ones are the Janus associated kinase or JAK/STAT, the mitogen activated protein (MAP) kinase and the phosphatidylinositol 3 (PI3) kinase pathways (see Figure 2). The JAK proteins are tyrosine-specific protein kinases that associate with the intracellular domains of the growth factor receptors. A growth factor molecule binds simultaneously to the extracellular domains of two or three receptor molecules, resulting in their aggregation. JAKs then phosphorylate members of the signal STAT family of transcription factors resulting in their dimerization and translocation from the cell cytoplasm across the nuclear membrane to the cell nucleus where specific genes are transcribed. JAK also activates the MAPK pathway which is in turn controlled by Ras. Different domains of the intracellular receptor protein may signal for the different processes (e.g. proliferation or suppression of apoptosis) mediated by growth factors. Other growth factors like SCF, Flt-3L and macrophage colony-stimulating factor (M-CSF) bind to receptors that have an extracellular immunoglobulin-like domain linked. Growth factor binding results in dimerization of these receptors and consequent activation of the tyrosine kinase domain. Phosphorylation of tyrosine residues in the receptor itself generates binding sites for signalling proteins which initiate complex cascades of biochemical events resulting in changes in gene expression, cell proliferation and prevention of apoptosis.

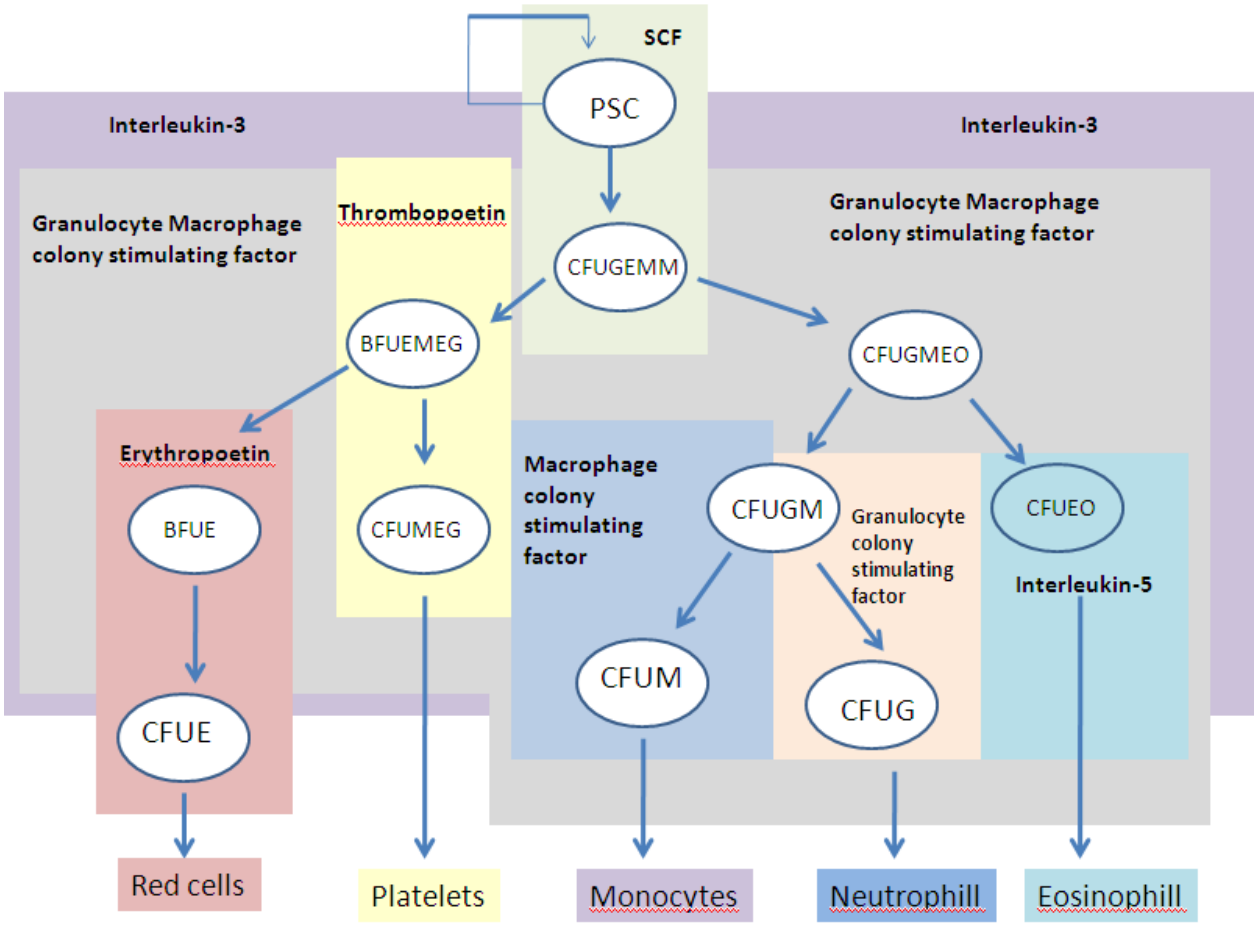

Figure 2: Schematic representation of the role of growth factors in normal haemopoiesis. PSC, pluripotential stem cell; SCF, stem cell factor. For other abbreviations see Figure 1.

Erythropoiesis

Erythropoiesis is the name for the process which leads to the formation of red blood cells (RBCs) or more properly termed as the erythrocytes. The normal life span of RBC's is about 120 days, thus new erythrocytes need to be formed. The overall process occurs in five days and the bone marrow is the site for the production of RBCs. A condition known as hypoxia which is shortage in RBC's oxygen carrying capacity leads to the release of the growth factor erythropoietin. Other growth factors which are released are IL-1, IL-4, IL-6, IL-11, IL-12, and SCF. Furthermore, Insulin, Growth hormone, and steroid hormones are very crucial in RBC production. EPO acts on precursor RBC cells which are Burst Forming Unit-Erythroid (BFUE) and Colony Forming Unit-Erythroid cells (CFUE) leading to their proliferation. The scheme given below summarizes the process of erythropoiesis. During sudden hypoxia due to massive blood loss the entire aforesaid process takes place in three days.

↓ O2 tension → ↑ EPO → ↑ RBC's precursors (BFU-E and CFU-E) → ↑ differentiation & proliferation → ↑ mature RBC's release in 5 days.

They are six morphologically identifiable stages in erythroid differentiation which can be visualized under the microscope using Romanowsky (or Geimsa) stained slides. The different stages are namely:

a. **Pronormoblasts**: These cells makes up about 1-2% of all nucleated cells in the bone marrow. The cytoplasm is very basophilic, i.e., has very dark blue color.

b. **Basophilic normoblasts**: These cells constitutes up to 4% of all nucleated cells in the bone marrow. Under the microscope the cytoplasm shows deep blue color.

c. **Polychromatophilic normoblasts**: These cells makes up to 10-20% of all nucleated cells in the bone marrow. The cytoplasm varies in color due to the synthesis of hemoglobin, which leads to a wide range of colors consisting of a mixture of gray, blue, mauve, and/or violet.

d. **Orthochromic normoblasts**: The cytoplasm of these cells has a resultant color of pale grayish-blue-violet due to the presence of hemoglobin

e. **Reticulocytes**: The retics appear slightly larger than normal erythrocytes, with a varying degree of color. The cytoplasm may be irregular and might have inclusions known as "basophilic stippling", which are the residual RNA remaining in the cells.

f. **The mature erythrocyte (RBC):** The erythrocyte has a diameter of about 7μ and width of about 2μ. The cell lacks nucleus, and mitochondria.

Leucopoiesis

Leucopoiesis is the process by which white blood cells or lymphocytes (B-cells and T-cells) are produced and developed from the lymphoid progenitor cells, it is also known as leukocytopoiesis or lymphopoesis. Lymphocytes are formed in the six constituents of the lymphomyeloid complex (LMC) which are namely the bone marrow, thymus, lymph nodes, subepithelial lymphoid tissue, spleen, connective tissue (including blood). The existence of specific markers on the lymphocyte membrane (CD-antigens) has enabled the differentiation of lymphocytic subpopulations. The largest number of the lymphocytes in the peripheral blood belongs to the subpopulations of the mature T-cells. A considerable smaller number of the lymphocytes belong to mature B-cells. The precursors of T- and B-cells are of the least number. Lymphoblast is the earliest

morphologically recognizable cell of the lymphocytic lineage. During the lymphocytopoiesis, three developing cell forms can be seen. This process mainly comprises the formation of functional antigen receptors of the T-cells in the thymus and the ability to form and secrete immunoglobulins by the B cells in the bone marrow. Leucopoesis also results in development of natural killer cells (NKC). The process starts with the primitive reticular cell, which on activation developsinto cytoplasmic basophilia and finally becomes a lymphoblast. A series of cell divisions (6-8 cell divisions) results reduction in the amount of cytoplasm leading to the development of small lymphocyte.

B-cell development

B cell development occurs through several stages, each stage representing a change in the genome content at the antibody. When the B cell fails in any step of the maturation process, it will die by a mechanism called apoptosis. B cell leucopoesis is dependent on the integration of extracellular stimuli by transcription factors that specify hematopoietic progenitors to differentiation into highly-specialized effector B-cells. The B cell factor-1 or Ebf1 is expressed in the early stages of the B cell lineage and in the stromal cells of the bone marrow. Ebf1 functions in a complex regulatory network with other transcription factors to establish the B cell program. B cell membrane receptors evolve and change throughout the B cell life span. Examples of such receptors are the TACI, BCMA and BAFF-R which are present on both immature B cells and mature B cells. CD20 is expressed on all stages of B cell development except the first and last; it is present from pre-B cells through memory cells, but not on either pre-pro-B cells or plasma cells. Figure 3 illustrates the stages of B-cell development.

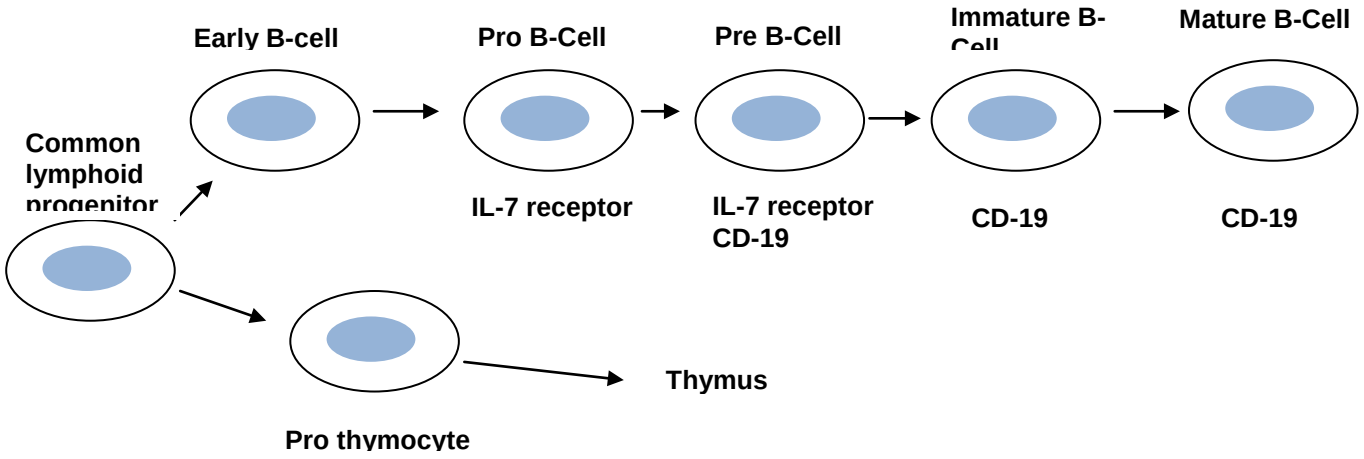

Figure 3: B cell developmental stages.

T-cell leucopoesis

T cells are formed in bone marrow and then they migrate to the cortex of the thymus to undergo maturation in an antigen-free environment for about one week. About 2-4% of the T cells succeed to mature and the other 96-98% of T cells undergoes apoptosis and is phagocytosed by macrophages in the thymus. This process is termed as thymus education wherein T-cells capable of recognizing self antigens undergo apoptosis.

The mature forms of T-cells are:

1. T-helper: Activatates of other cells such as B cells and macrophages.
2. T-cytotoxic: Kills virally infected cells.
3. T-memory: Remembers previously encountered antigens.
4. T-suppressor cells: Moderates the immune response of other leukocytes.

Interesting facts:

1. Blood makes up around 7% of the weight of a human body which is about 5 liters.
2. Red blood cells develop in bone marrow and circulate in the body for around 120 days.
3. Mature RBC lacks nucleus, mitochondria, Golgi apparatus and endoplasmic reticulum
4. In some vertebrates, haematopoiesis can occur wherever there is a loose stroma of connective tissue and slow blood supply, such as the gut, spleen, kidney or ovaries.
5. Approximately 1013 new myeloid cells (all blood cells excluding lymphocytes) are produced each day.
6. Vitamin B12 (as well as B6 and folic acid) are crucial for the development of RBC's, as they are required for protein synthesis
7. Hemophilia A is the X-linked genetic disease in which the individual does not produce factor VIII and so is more susceptible to severe hemorrhages.
8. In Medicine anticoagulants like heparin are used in surgeries in which tissue injuries made by the surgical act could trigger undesirable systemic blood clotting.
9. Macrophages breakdown Hb to biliverdin and iron.

Questions:

Q1. What are granulocytes and agranulocytes?
Q2. Enumerate the structure of lymphocytes?
Q3. How does platelet aggregation occur?
Q4. Explain the process of haemopoiesis?
Q5. What is Erythropoiesis?
Q6. What are the functions of B cell and T cell?

Further Readings

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