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).
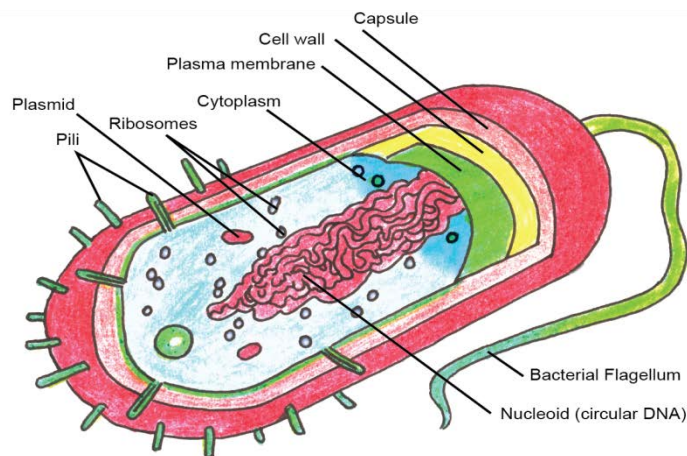
Figure 1: Schematic diagram of a prokaryotic cell

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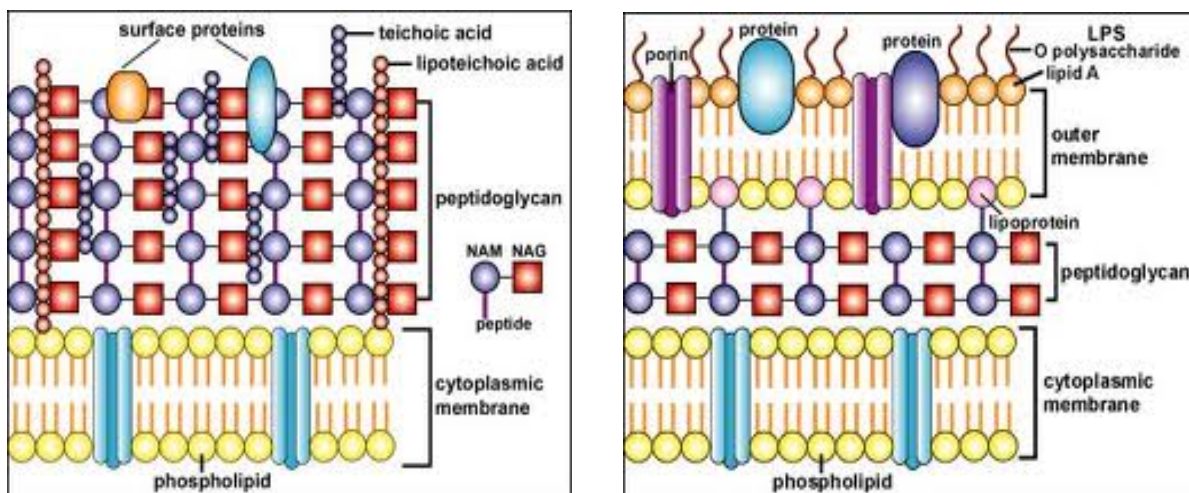
A: Gram positive cell wall   **B: Gram negative cell wall**

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
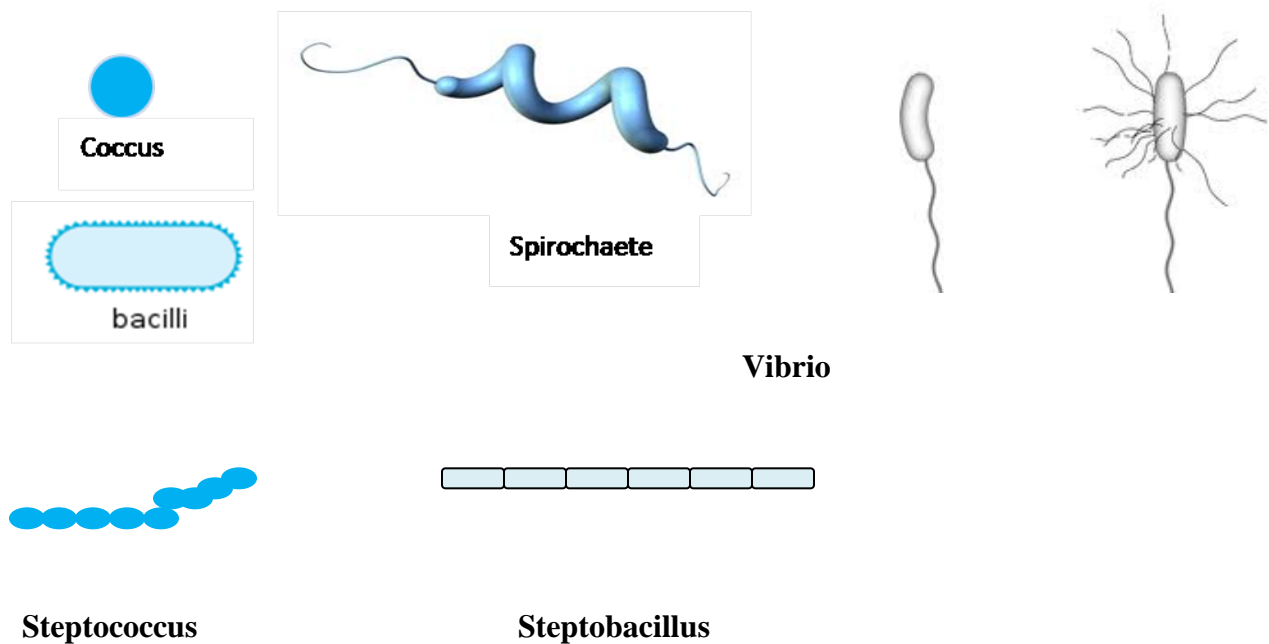- Spirochaete - spiral-shaped
- Vibrio - comma-shaped

Coccus

bacilli

Spirochaete

Vibrio

Steptococcus

Steptobacillus

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.
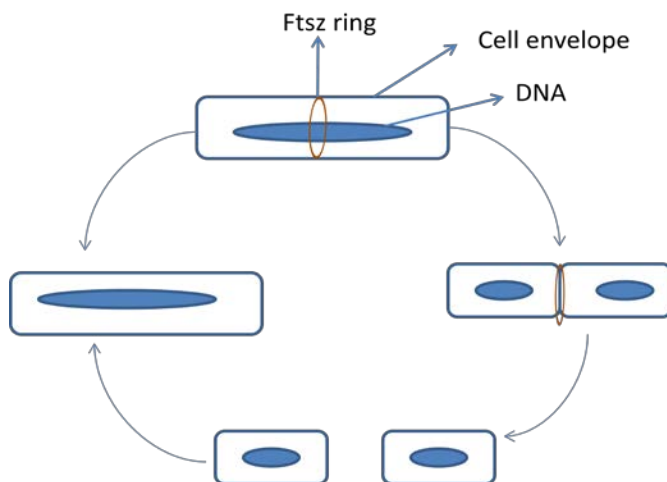

Figure 4: Binary fission in prokaryotes

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.
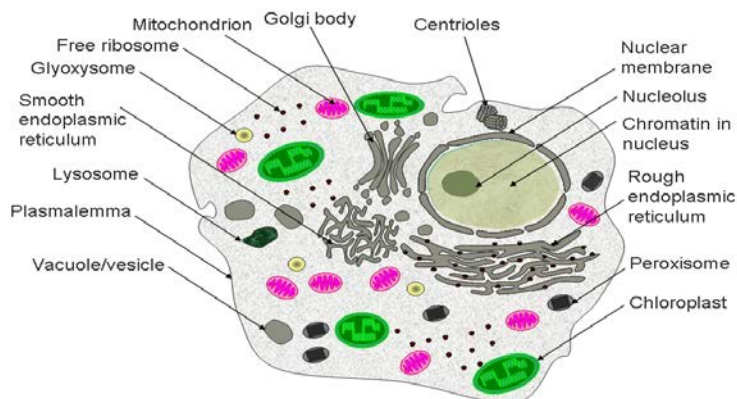
Figure 5 Eukaryotic cell:

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.
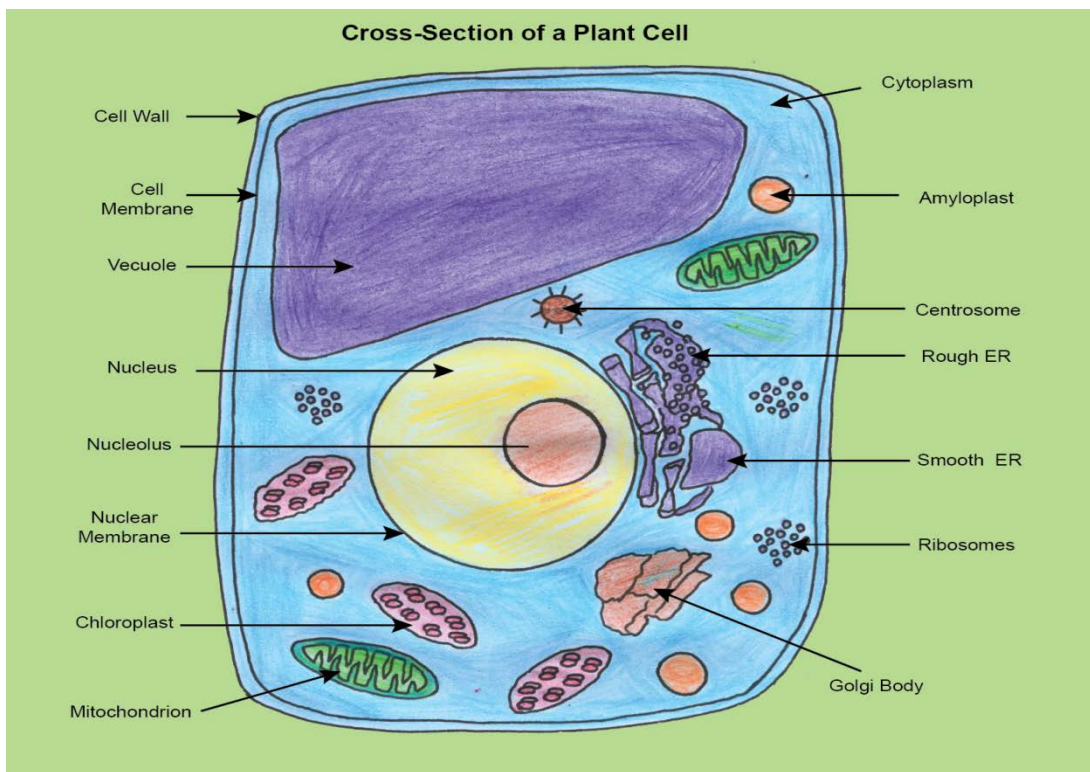
Figure 1: Schematic representation of a plant cell.

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).
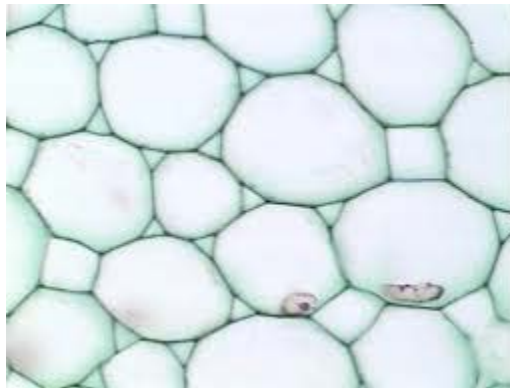

Figure 2a: Parenchyma cells which have thin primary cell wall.

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).
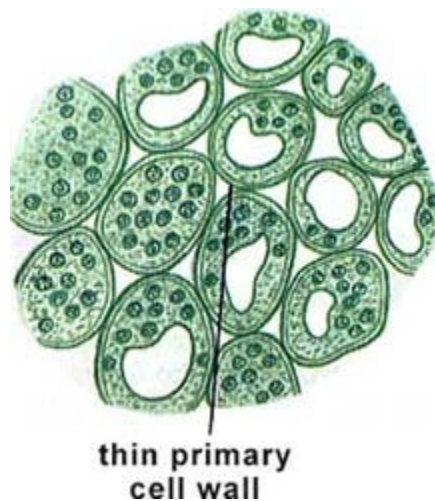

Figure 2b: Typical collenchyma cell.

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.
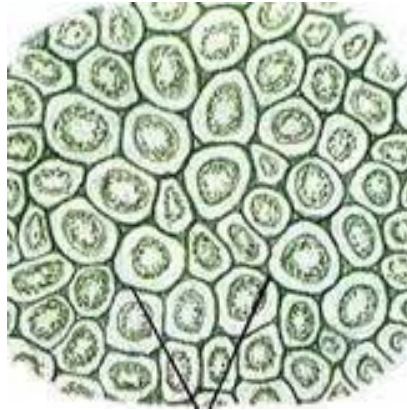

Figure 2c: Sclerenchyma cells with irregularly thickened cell wall.

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.
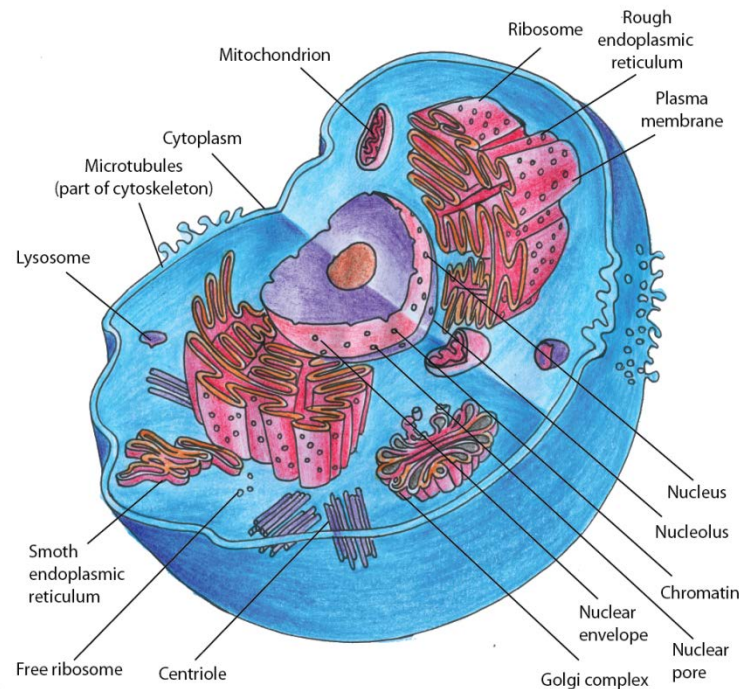

Figure 3: Schematic representation of a typical animal cell.

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.
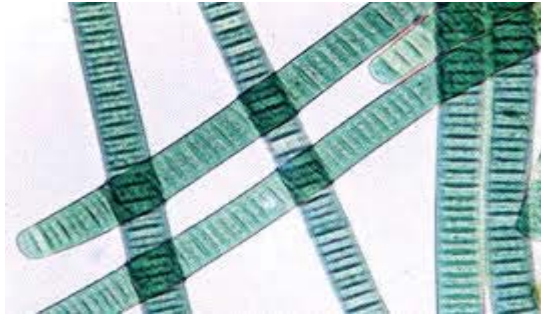

Figure 4: Cyanobacteria

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.
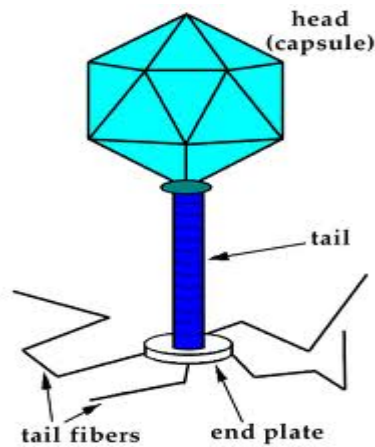

Figure 5: Virus

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.
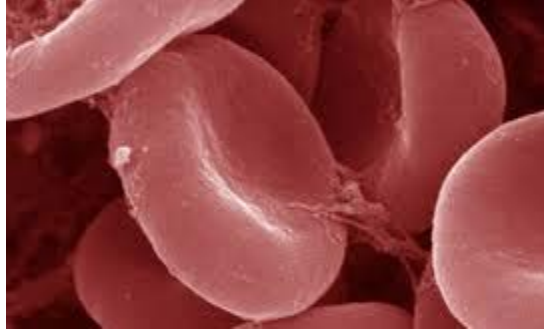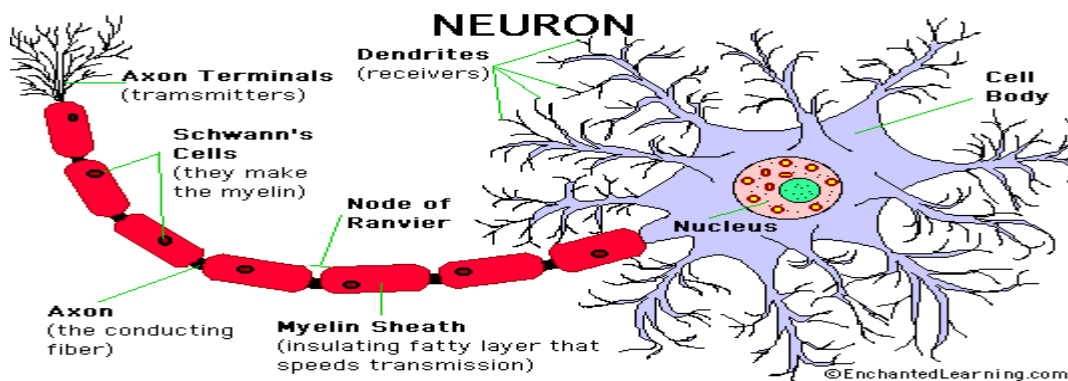

Figure 6: Red blood cell.
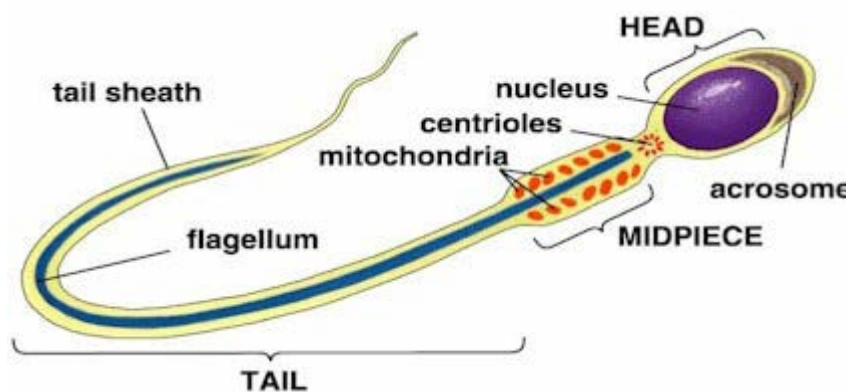
Figure 7: Nerve cell

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.
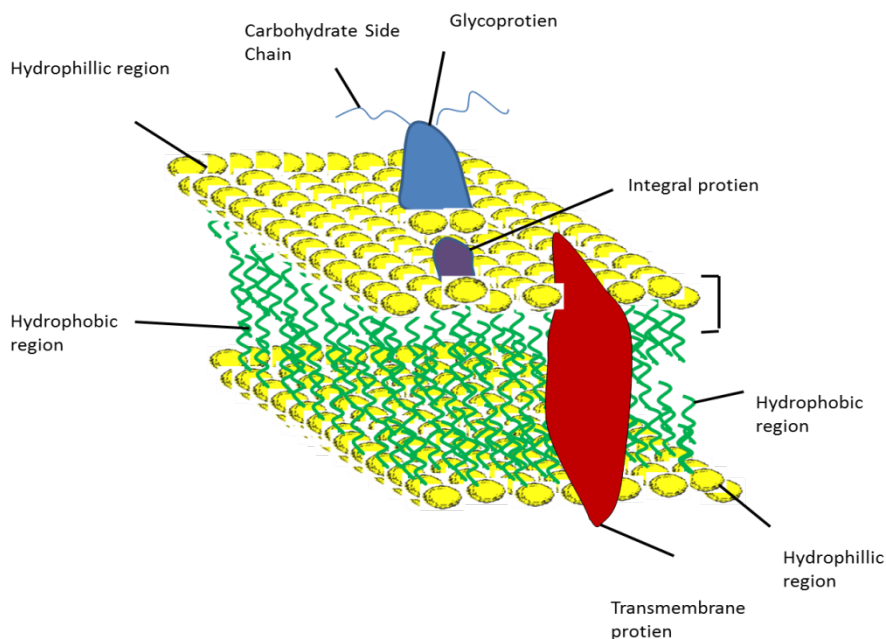

Figure 1: The architecture of the cell membrane

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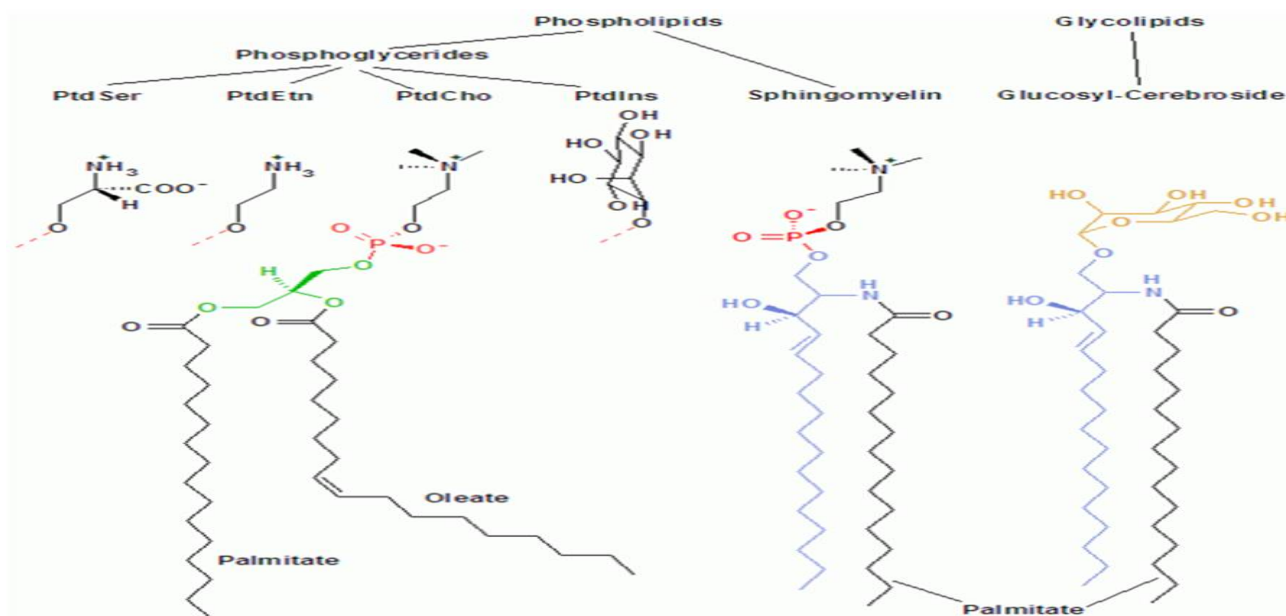
Figure 2 The major membrane phospholipids and glycolipids. The figure has been adapted from the "Membrane Organization and Lipid Rafts" by Kai Simons and Julio L. Sampaio, 2011, Cold Spring Harbor Laboratory Press.

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.
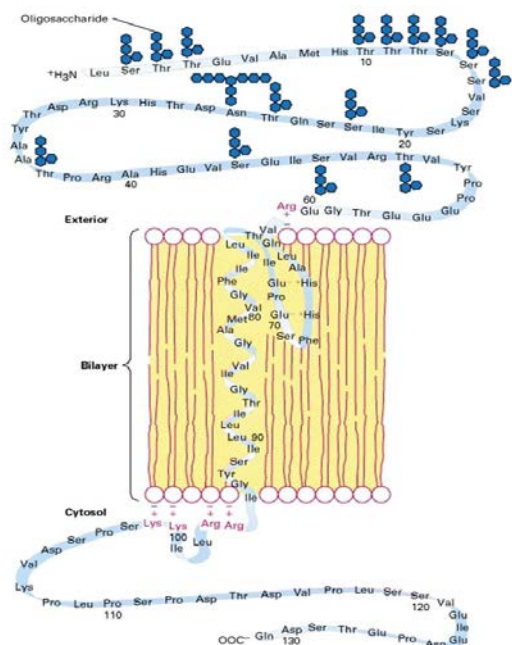
Figure 3a, 3b. Figure 3a This protein is a homodimer, but only one of its polypeptide chain is shown. Residues 62–95 are buried in the membrane, with the sequence from position 73 through 95 forming an α helix. The ionic interactions shown between positively charged arginine and lysine residues and negatively 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.**
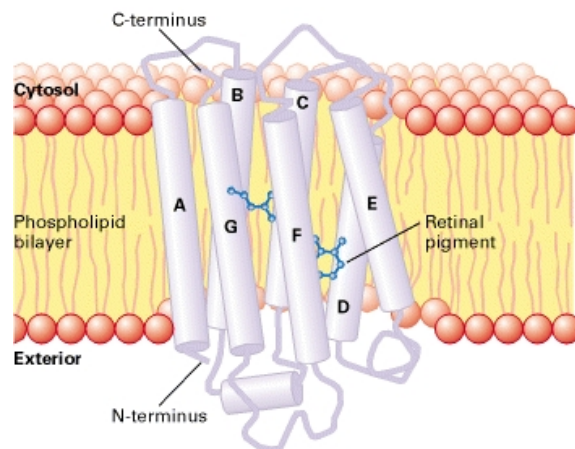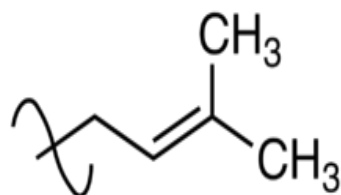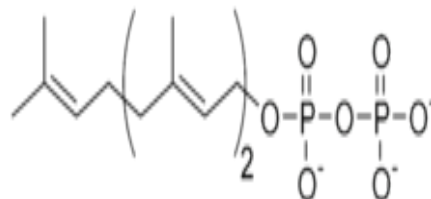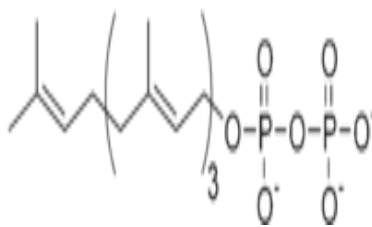
Fig 3b The seven membrane-spanning α helices are labeled A–G. The retinal pigment is covalently attached to lysine 216 in helix G. The approximate position of the protein in the phospholipid bilayer is indicated. Adapted from R. Henderson et al., 1990, *J. Mol. Biol.* **213:899.**

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.
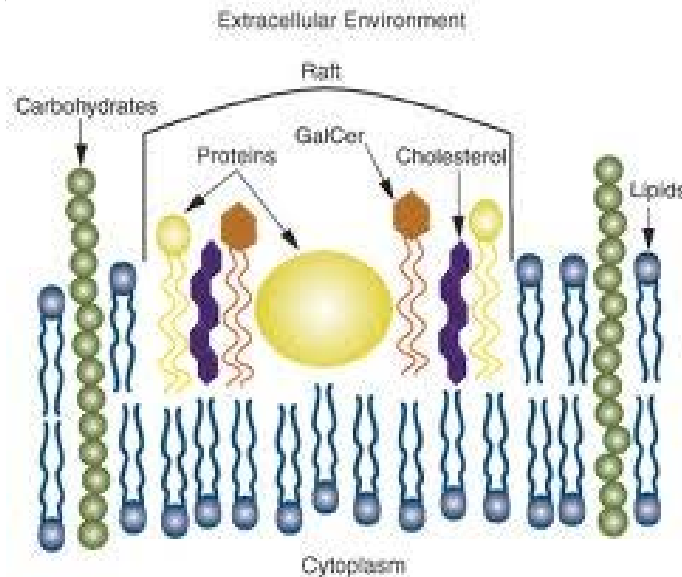
Figure 5: Lipid raft

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			Simons 2000
	Cholesterol, glycoshingolipid, low in PI and other anionic phospholipids	Signalling?	
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.
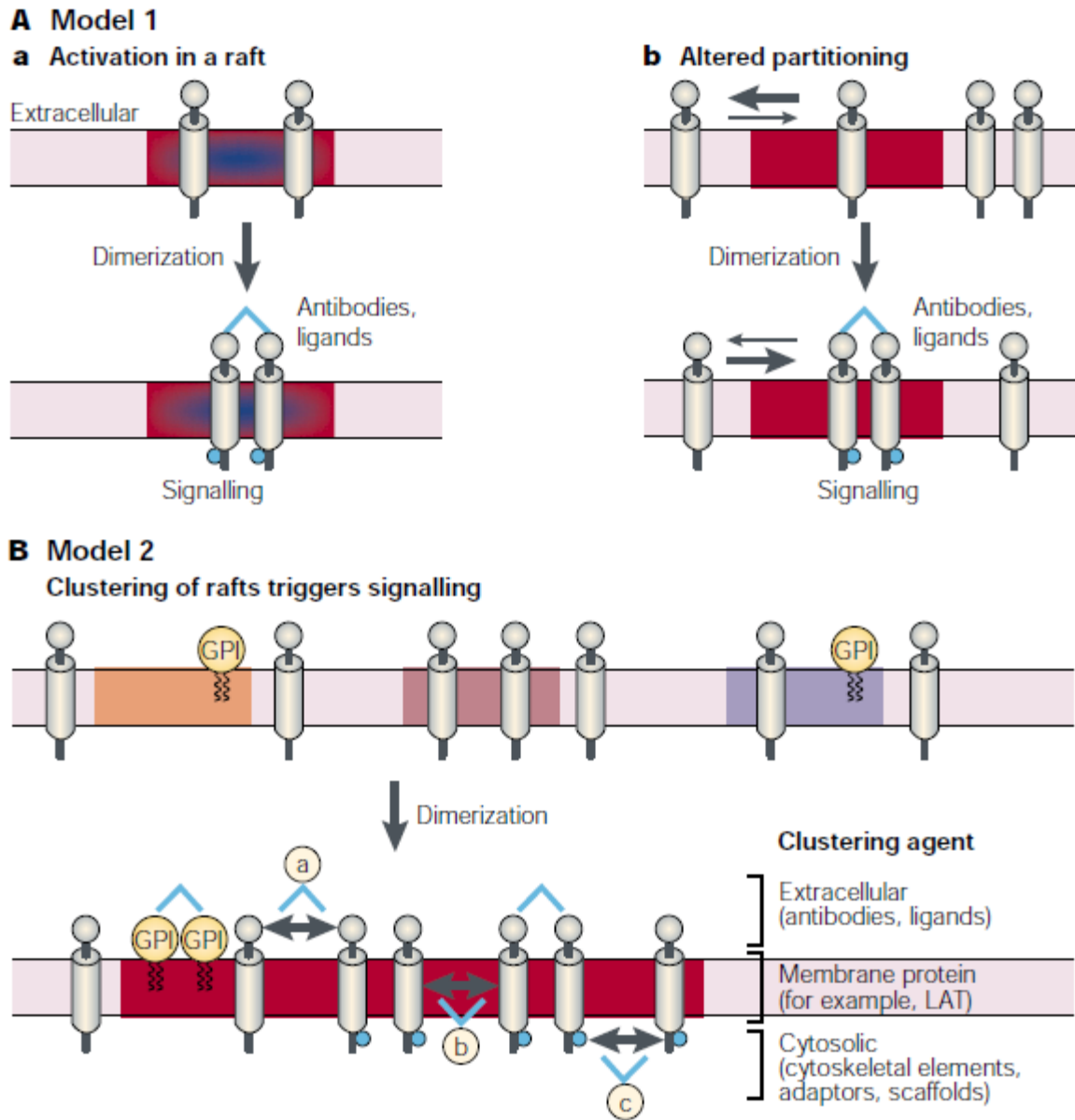
Figure 6: Models of how signalling could be initiated through rafts. A. In these models, signalling occurs in either single rafts (Model 1) or clustered rafts (Model 2). Following dimerization the protein becomes phosphorylated in rafts. B. In the second model we assume that there are several rafts in 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.

References:

1. Alberts B et al. (2002) in "The Molecular biology of the cell", 4th edition. Garland Science, New York.

2. Fricke H.The electrical capacity of suspensions with special reference to blood. Journal of General Physiology 1925. 9:137-152.

3. Futerman AH, Hannun YA. The complex life of simple sphingolipids. EMBO Rep. 2004. 5(8):777-82.

4. Henderson R. et al. 1990. Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. J. Mol. Biol. 213:899.

5. Hertwig O, Campbell M, Campbell H J. The Cell: Outlines of General Anatomy and Physiology. 1895. New York: Macmillan and Co.

6. Janes P W, Ley S C, Magee A I. 1999. Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor.J. Cell Biol. 147, 447–461.

7. Laux, T. et al. 2000.GAP43, MARCKS, and CAP23 modulate PI(4,5)P2 at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. J. Cell Biol. 149, 1455–1472.

8. Lingwood D, et al. 2009. Lipid Rafts As a Membrane-Organizing Principle Science 327:46.

9. Lodish H, Berk A, Zipursky LS, *et al.* (2004) in Molecular Cell Biology (4th ed.). New York: Scientific American Books.

10. Marchesi V T, Furthmayr H, Tomita M. 1976. The red cell membrane. Ann. Rev. Biochem. 45:667.

11. Moulton GE. Ed.D. (2004) in "Excerpted from The Complete Idiot's Guide to Biology". Alpha Books, (USA) Inc.

12. Pike, L   J, Miller J M. 1998. Cholesterol depletion delocalizes phosphatidylinositol   bisphosphate   and   inhibits   hormonestimulated phosphatidylinositol turnover. J. Biol. Chem. 273, 22298–22304.

13. Simons K   Sampaio J L. Membrane Organization and Lipid Rafts. Cold Spring Harbor Perspective Biology. 2011.

14. Thomas S, Pais A P, Casares S, Brumeanu T D. 2004. Analysis of lipid rafts in T cells. Molecular Immunology 41: 399-409.

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.
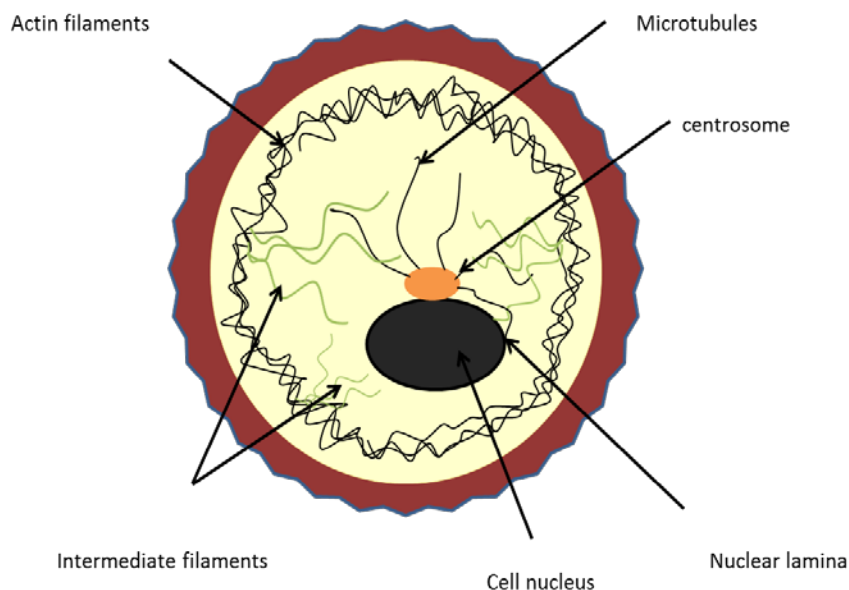

Figure 1: The cytoskeletal system

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
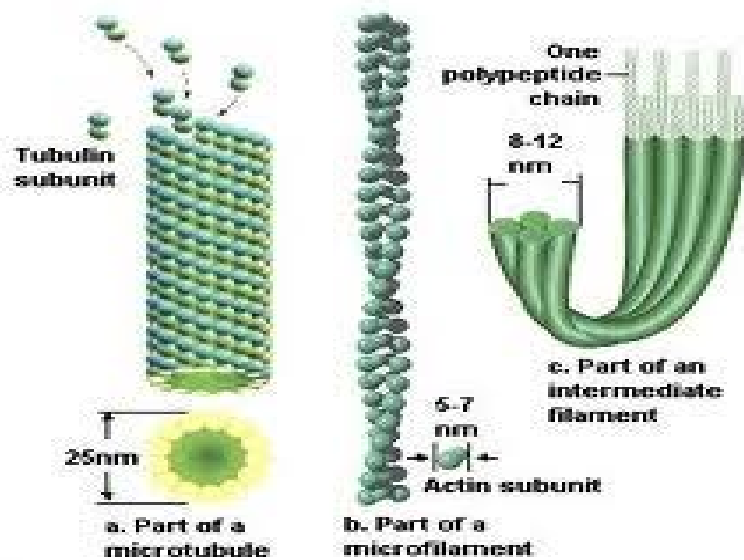
Figure 2: The difference among the various cytoskeletal systems

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.
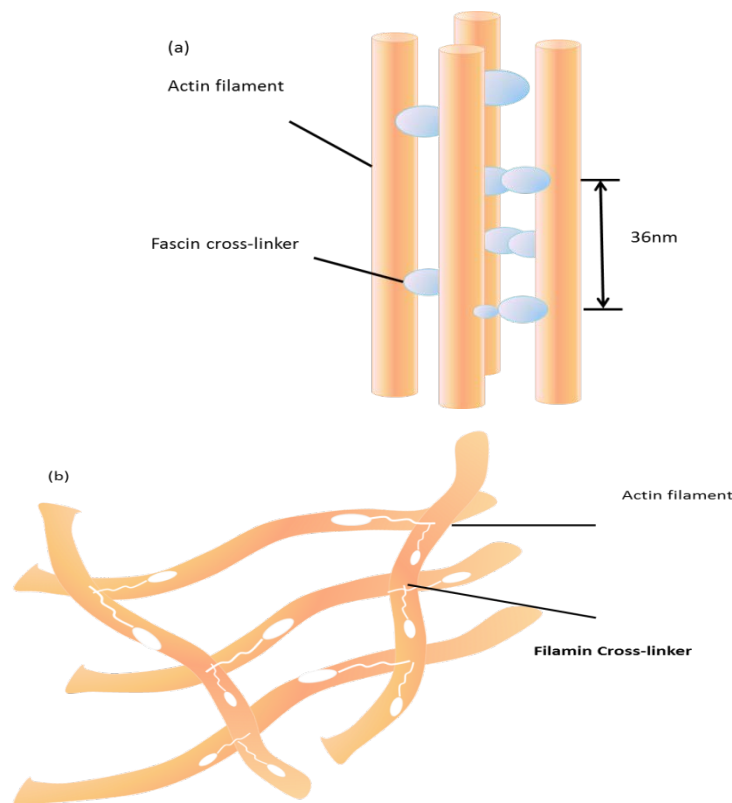

Figure 3: Actin cross-linking proteins bridging pairs of actin filaments

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.
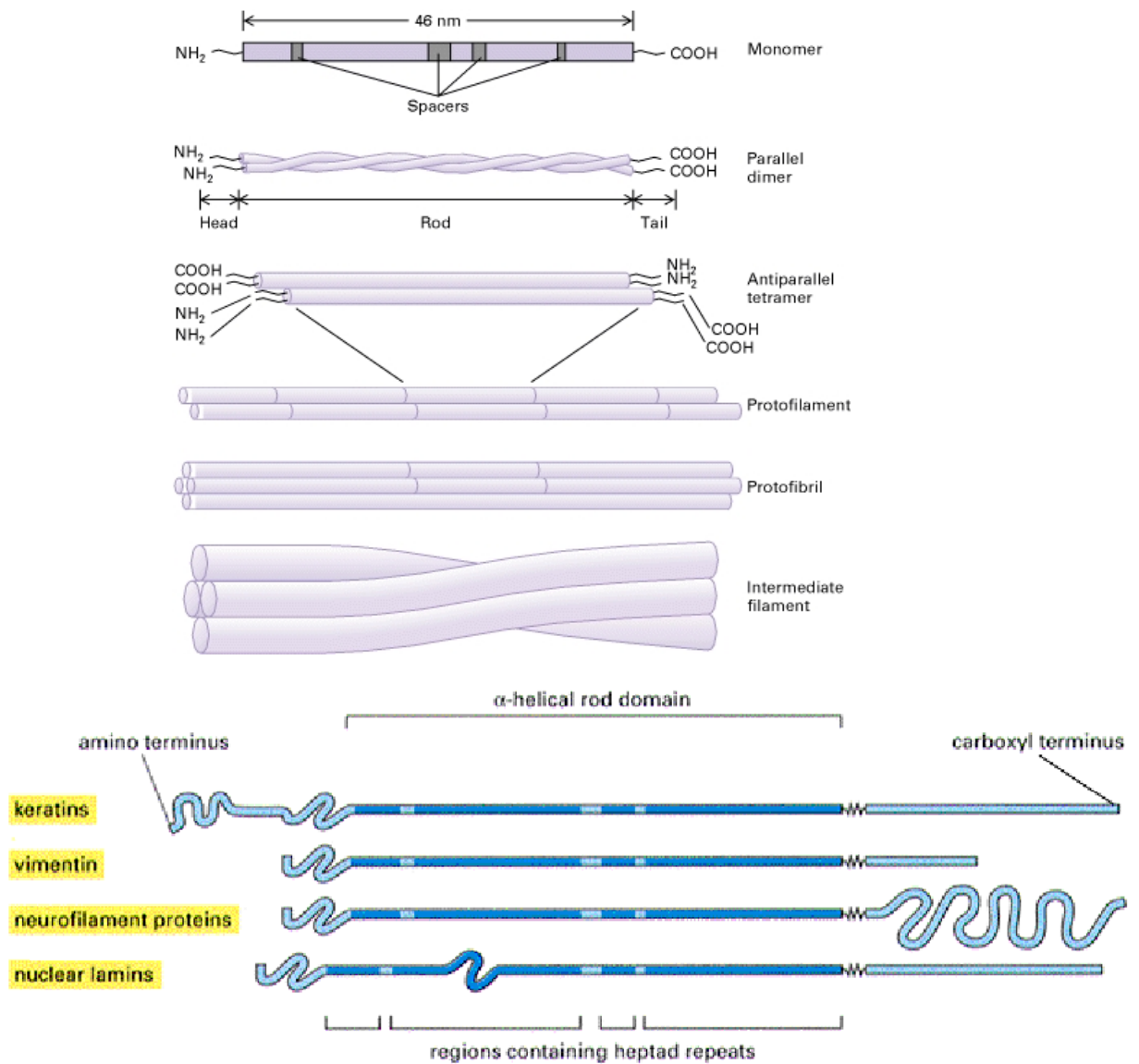

Figure 4 Monomer of Intermediate filaments. The above figure is from Alberts et al, Molecular Biology of the Cell, Garland Publishing, NY, 1996.
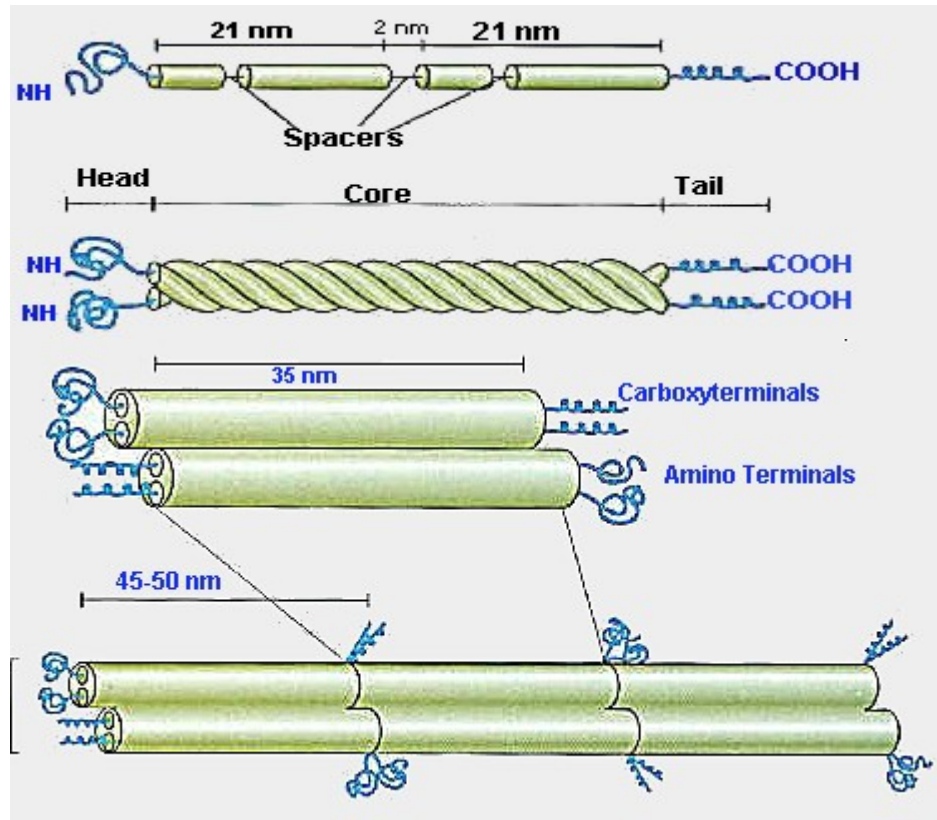
Figure 5: Formation of Intermediate filaments. The rods coil around another filament like a rope to form a dimer. The N and C terminals of each filament are aligned. Some Intermediate filaments form homodimers; other form heterodimers. These dimers then form staggered tetramers that line up head-tail. 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.
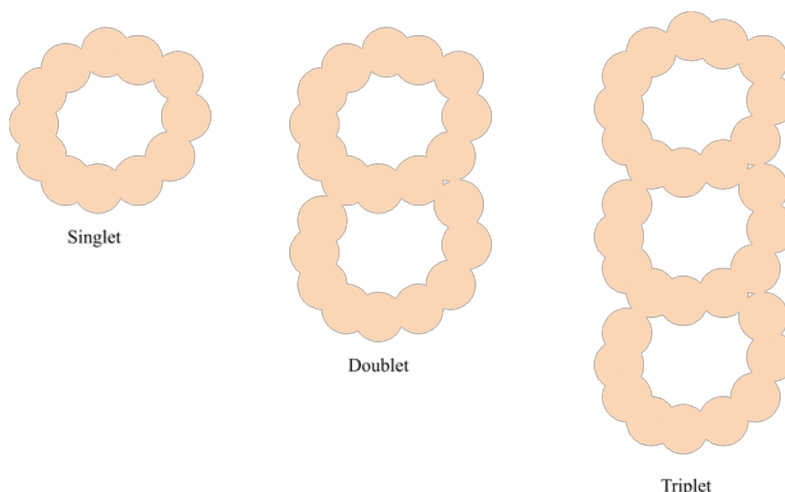
Figure 6: In cross section, a typical microtubule, a singlet, is a simple tube built from 13 protofilaments. In a doublet microtubule, an additional set of 10 protofilaments forms a second tubule (B) by fusing to the wall of a singlet (A) microtubule. Attachment of another 10 protofilaments to the B 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).
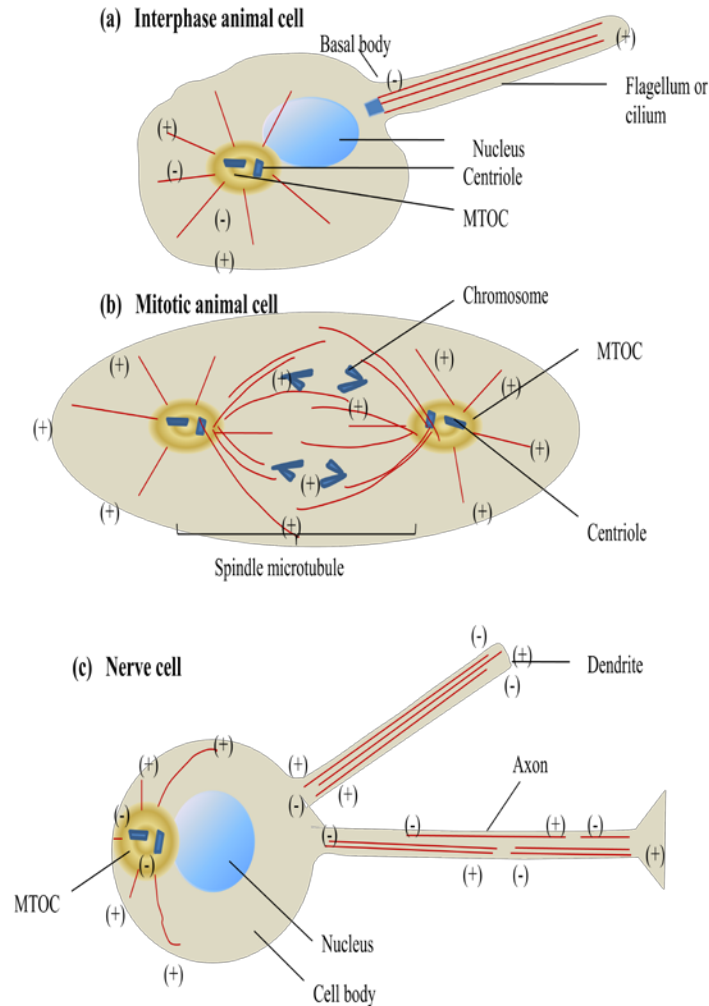
Figure 7: (a) In interphase animal cells, the (−) ends of most microtubules are proximal to the MTOC. Similarly, the microtubules in flagella and cilia have their (−) ends continuous with the basal body, which acts as the MTOC in these structures. (b) As cells enter mitosis, the microtubule network rearranges, 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.

References:

1. Alberts B, Johnson A, Lewis J, et al. (2002). Molecular Biology of the Cell (4th ed.). New York: Garland Science. USA.

2. Brinkley B R. (1985). Microtubule Organizing Centers. Annual Review of Cell Biology, Vol. 1: 145-172.

3. Frixione E. (2000). Recurring views on the structure and function of the cytoskeleton: a 300-year epic. Cell motility and the cytoskeleton, 46 (2): 73–94.

4. Graumann P L. (2004). Cytoskeletal elements in bacteria. Current Opinion in Microbiology, 7:565–571.

5. Lodish H, Berk A, Zipursky LS, et al. (2000). Molecular Cell Biology. New York: Scientific American Books.

6. Remedios C G D, Chhabra D, Kekic M, Dedova I V, Tsubakihara M, Berry D A, Nosworthy N J. (2003). Actin Binding Proteins: Regulation of Cytoskeletal Microfilaments. Physiological Reviews, 83(2): 433-473.

7. Wolosewick JJ, Porter KR. (1979). Microtrabecular lattice of the cytoplasmic ground substance. Artifact or reality. Journal of Cell Biology, 82 (1): 114–39.

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.
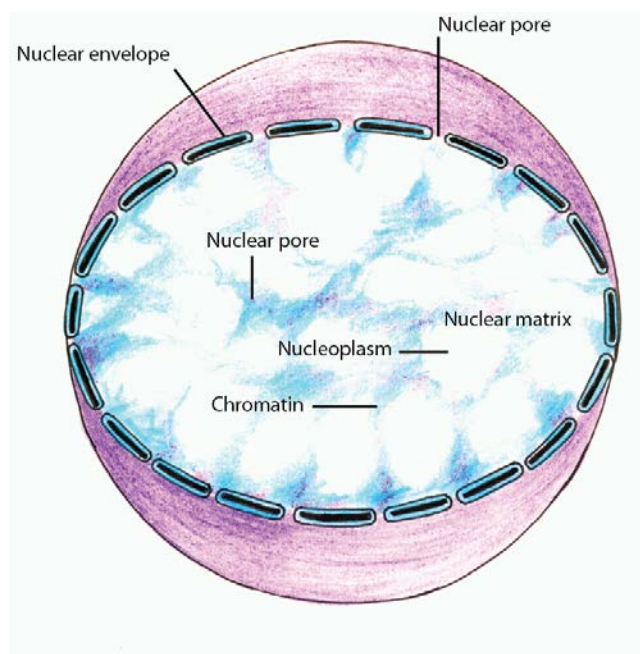

Figure 1: The schematic representation of nucleus.

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.
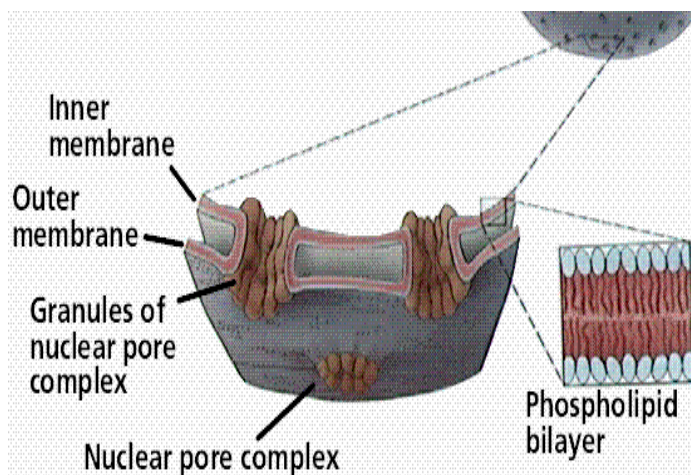

Figure 2: An illustration of the nuclear membrane

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.
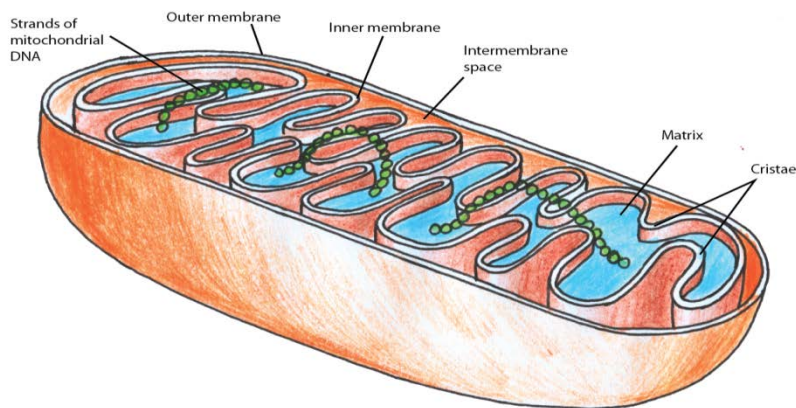

Figure 3: Schematic representation of mitochondria

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.
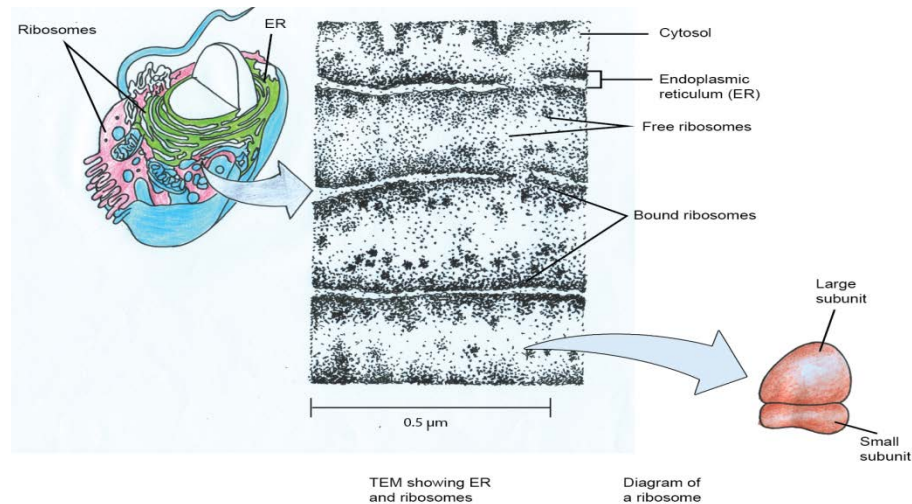

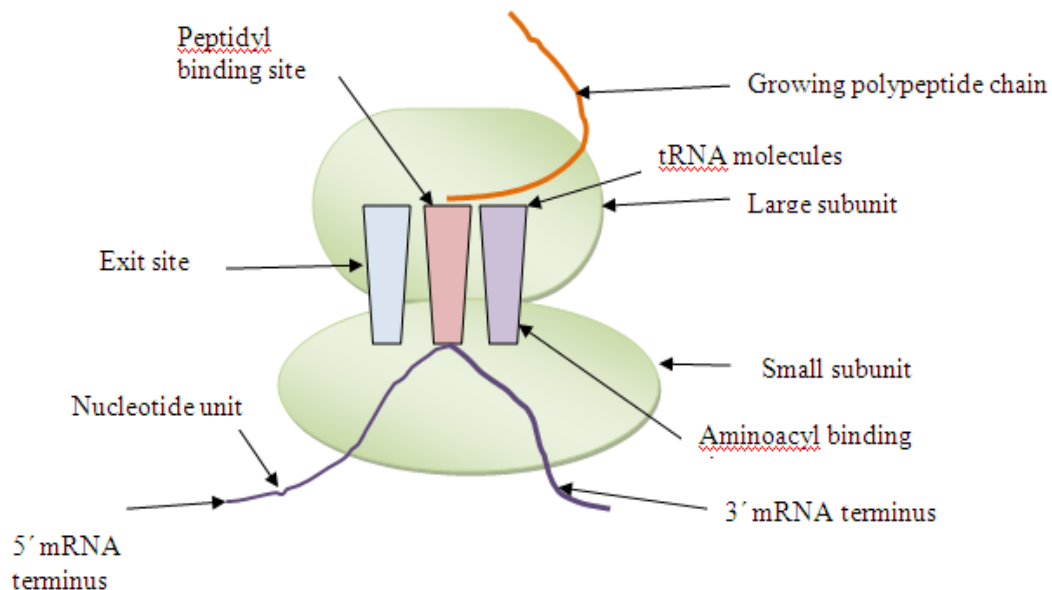**Figure 1: Schematic representation of the ribosome.**

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.
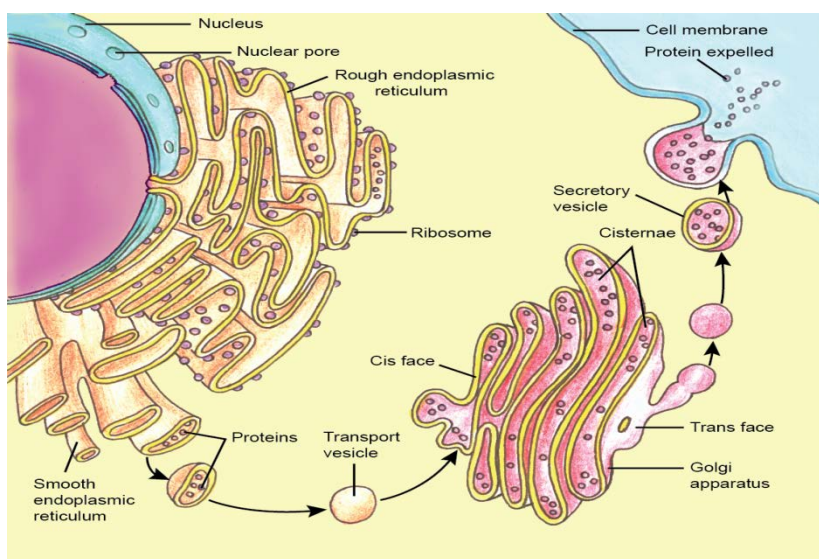

Figure 3. The Endoplasmic reticulum.

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 A° 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.
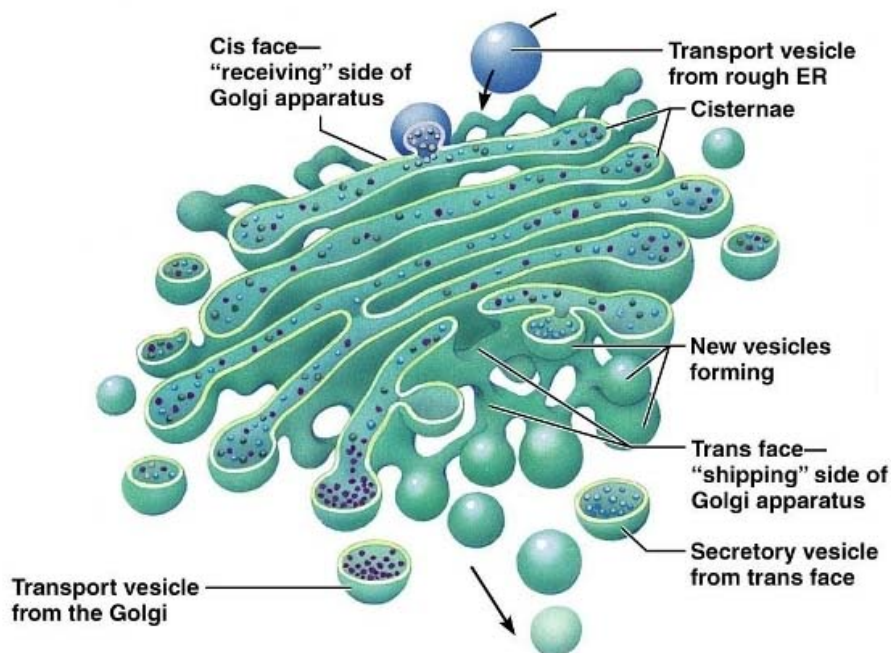

Figure 5: The Golgi complex.

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.

Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000.

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)**.
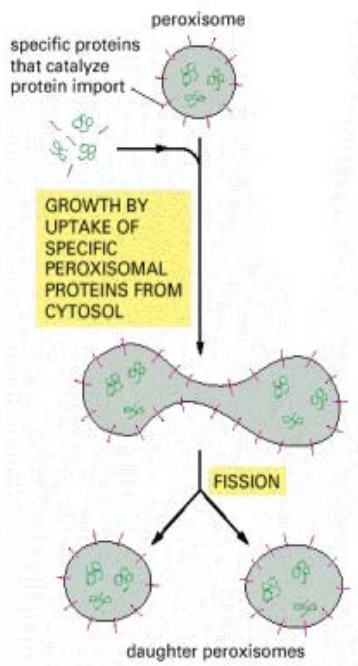

Figure 1 Production of new peroxisomes. The figure has been printed with permission from Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.
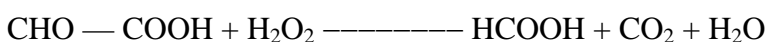
Functions:

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:

$$CH_2OH. COOH + O_2 ————————— CHO – COOH + H_2O_2$$

$$CHO — COOH + H_2O_2 ———————— HCOOH + CO_2 + H_2O$$

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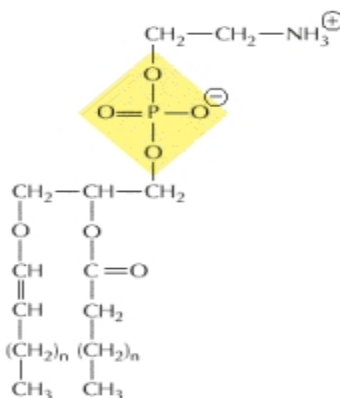
Figure 2: The structure of plasmalogen. The figure has been printed with permission from Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.

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.
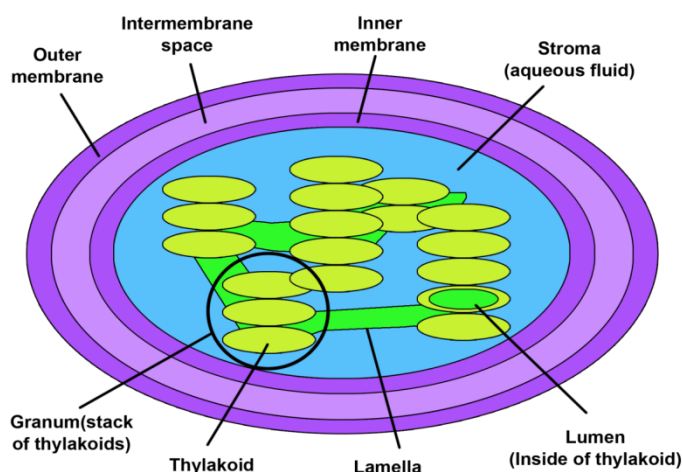
Figure 3: Structure of chloroplast.

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.
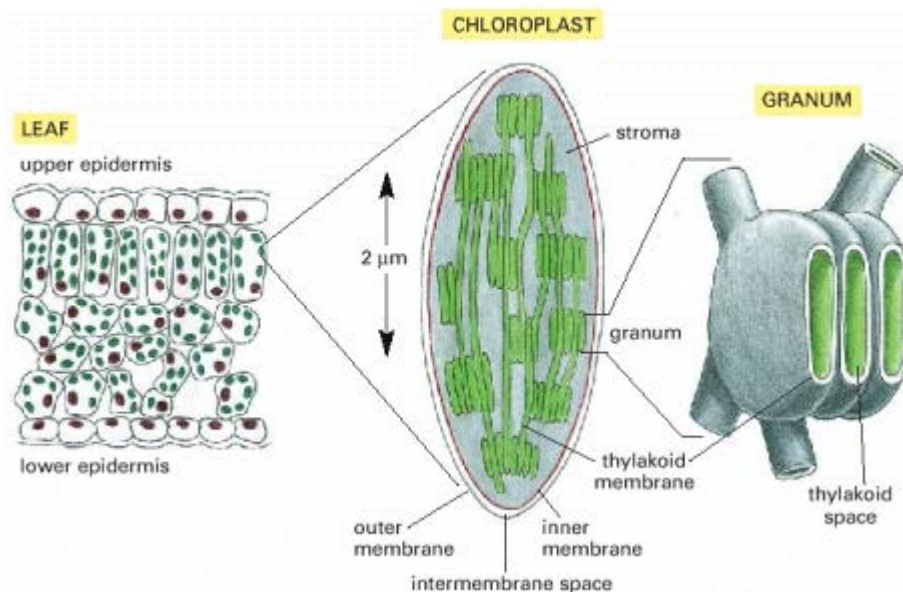

Figure 4. The structure of chloroplast and thylakoid. The figure has been printed with permission from Molecular Biology of the Cell. 4th edition. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.

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.
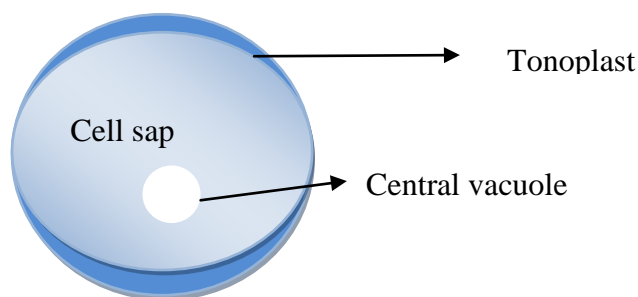

Figure 5: Plant cell central vacuole.

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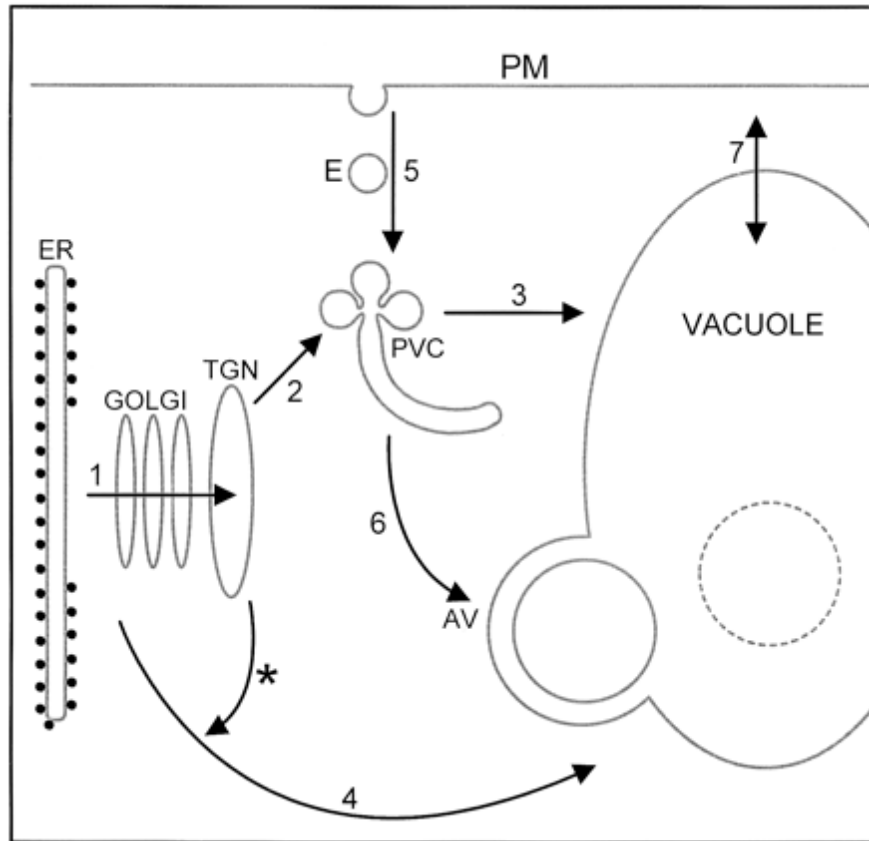
Figure 6: Vascular transport pathway. This Figure has been reprinted with permission from Plant Vacuoles by Francis Martya (1999), Plant Cell, Vol. 11, 587-600.

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.

2. Cooper GM. Sunderland (MA): Sinauer Associates; 2000. The Cell: A Molecular Approach. 2nd edition.

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.
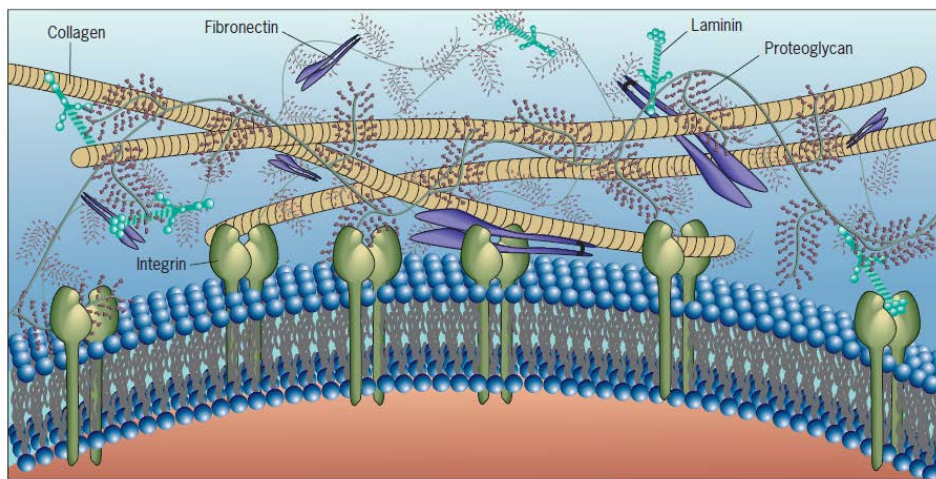
Figure 1: An overview of the extracellular matrix molecular organization. The proteins; fibronectin, collagen, and laminin contain binding sites for one another, as well as binding sites for receptors like integrins that are located at the cell surface. The proteoglycans are huge protein polysaccharide 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.
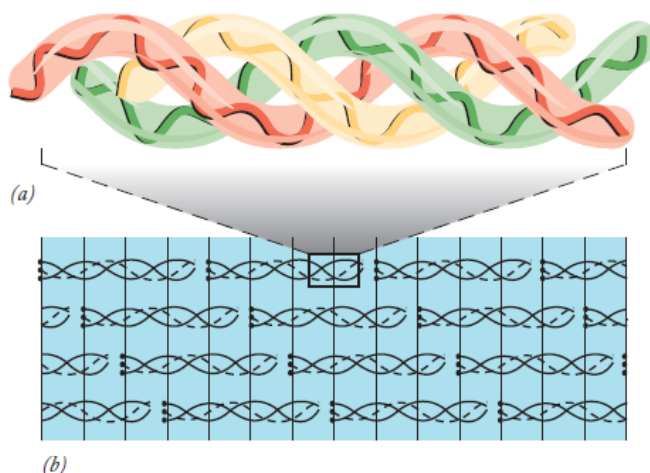
Figure 2: The structure of collagen I. (*a*) The monomer of collagen. (*b*) Collagen I molecules become aligned in and a bundle of collagen I molecules, such as that shown here, form a collagen fibril.

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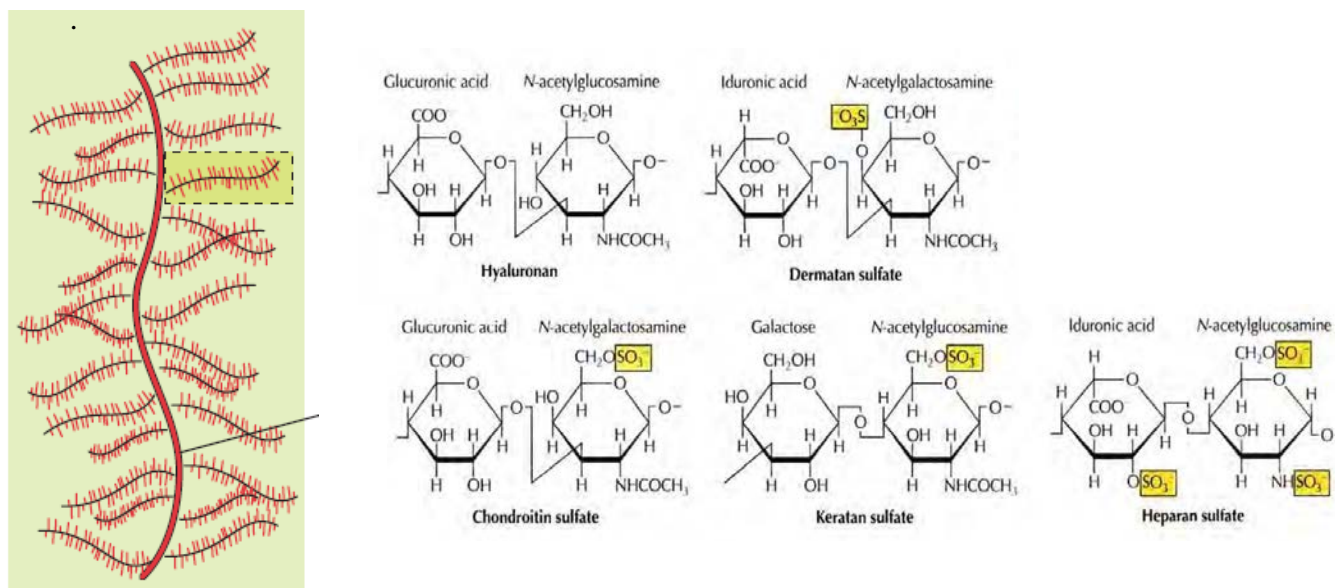
Figure 3: Proteoglycan complex with the major types of matrix glucosaminoglycans. Glycosaminoglycans consist of repeating disaccharide units. With the exception of hyaluronan, the sugars frequently contain sulfate.

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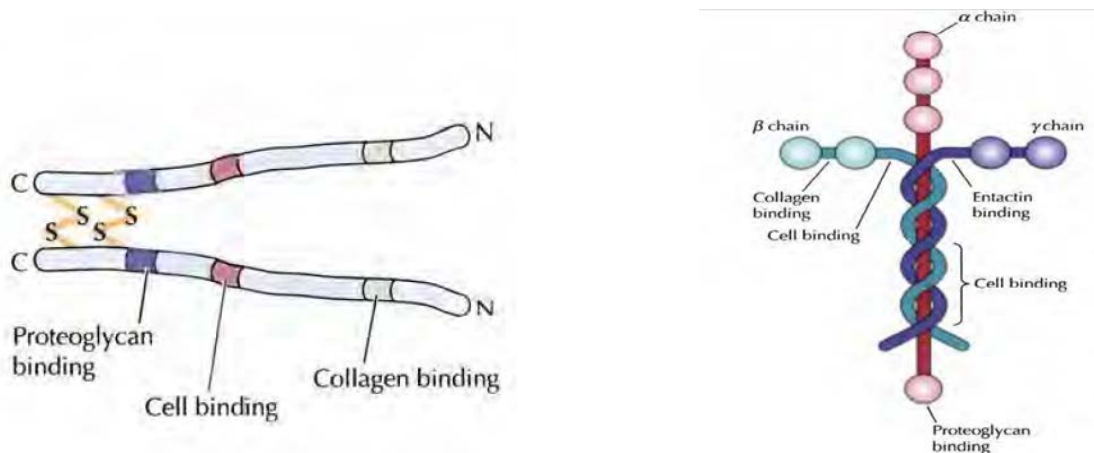
Figure 4: An illustration of matrix associated proteins. A. Fibronectin. B. Laminin

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).
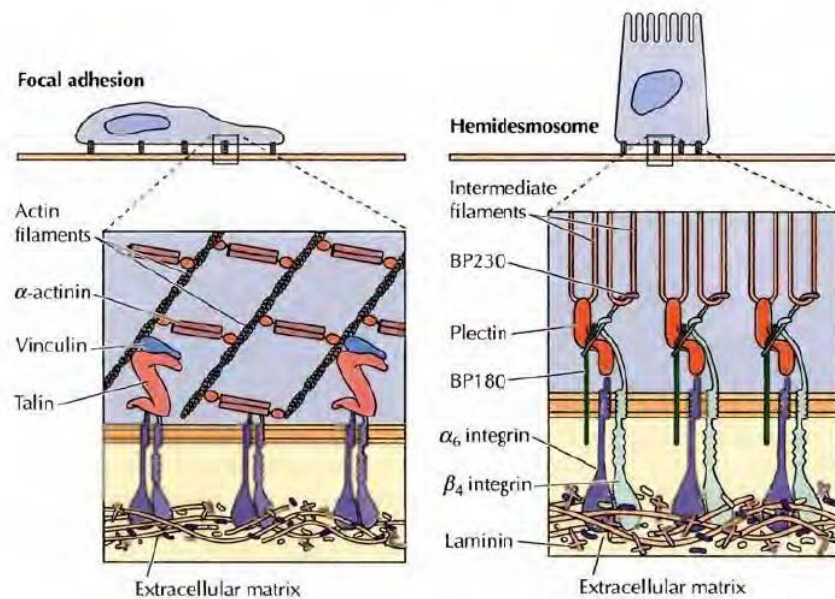
Figure 5: Cell-matrix junctions mediated by integrins. Integrins mediate two types of stable junctions the focal adhesions where bundles of actin filaments are anchored to integrins through associations with a number of other proteins, including α-actinin, talin, and vinculin. In hemidesmosomes, integrin 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.
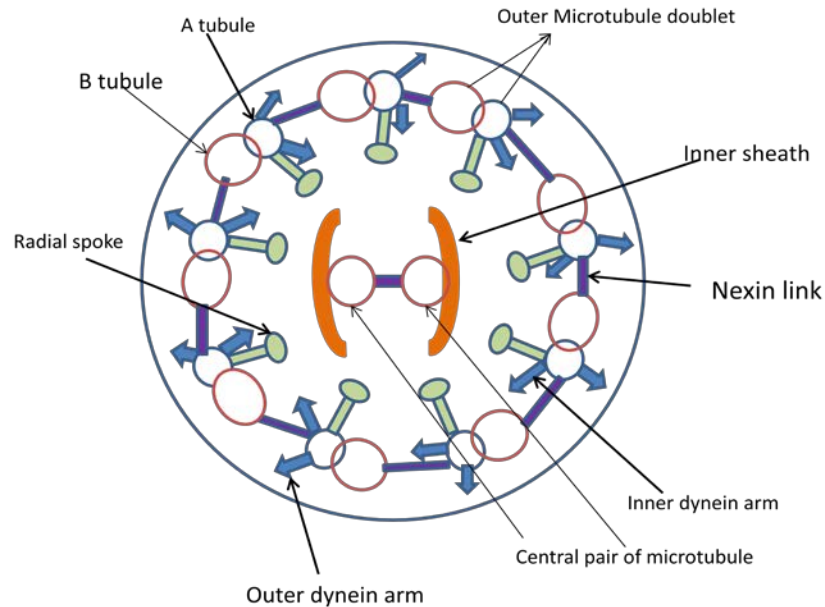

Figure 1: Structure of axoneme of cilia and flagella

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.
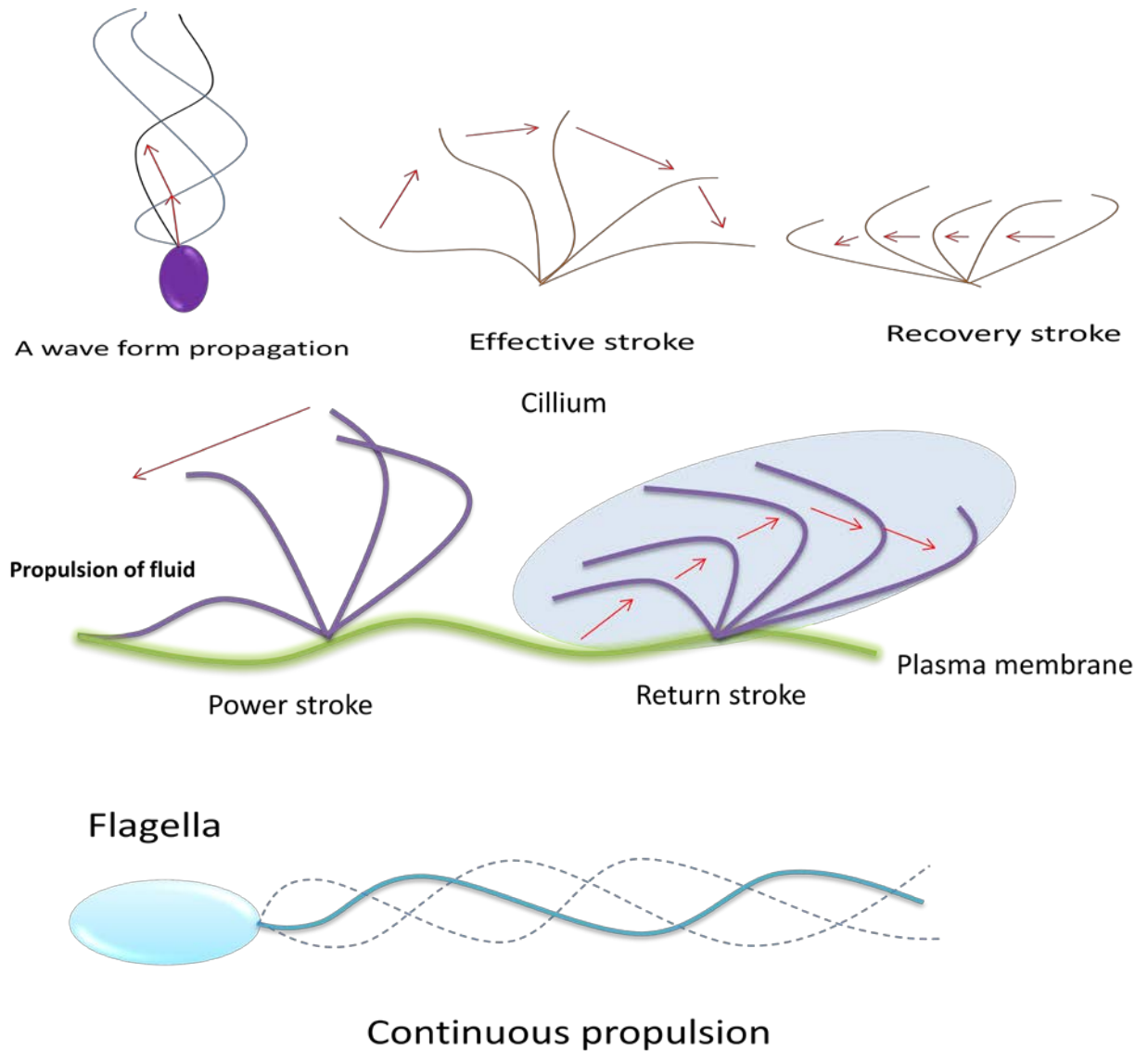
A wave form propagation

Effective stroke

Recovery stroke

Cillium

Propulsion of fluid

Power stroke

Return stroke

Plasma membrane

Flagella

Continuous propulsion

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).
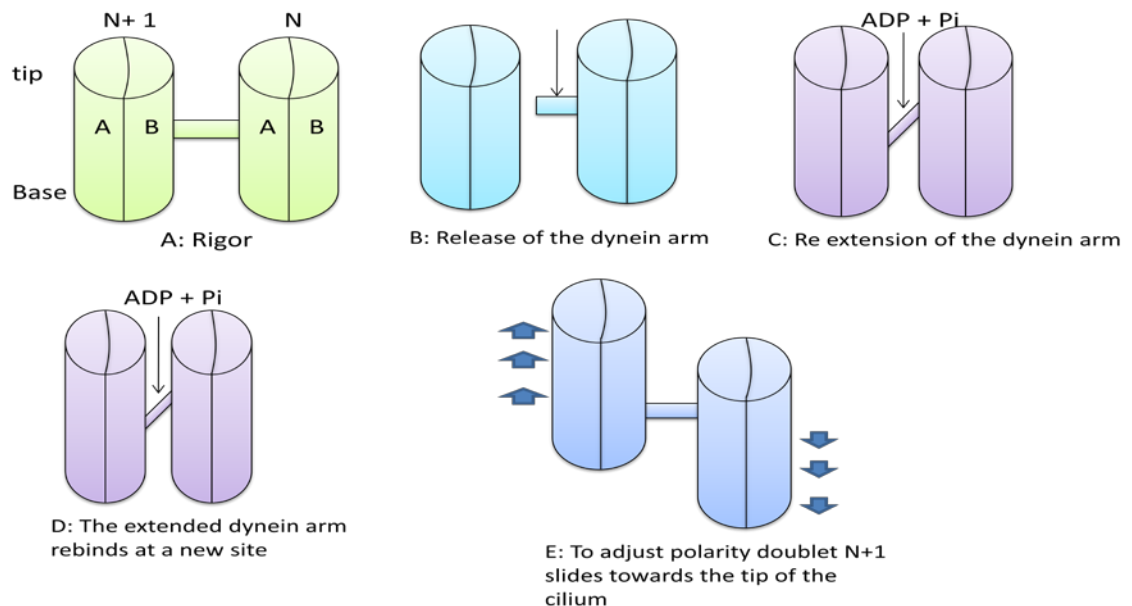

Figure 3: Schematic representation of the mechanochemical cycle involved in sliding of filament in ciliary movement.

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.
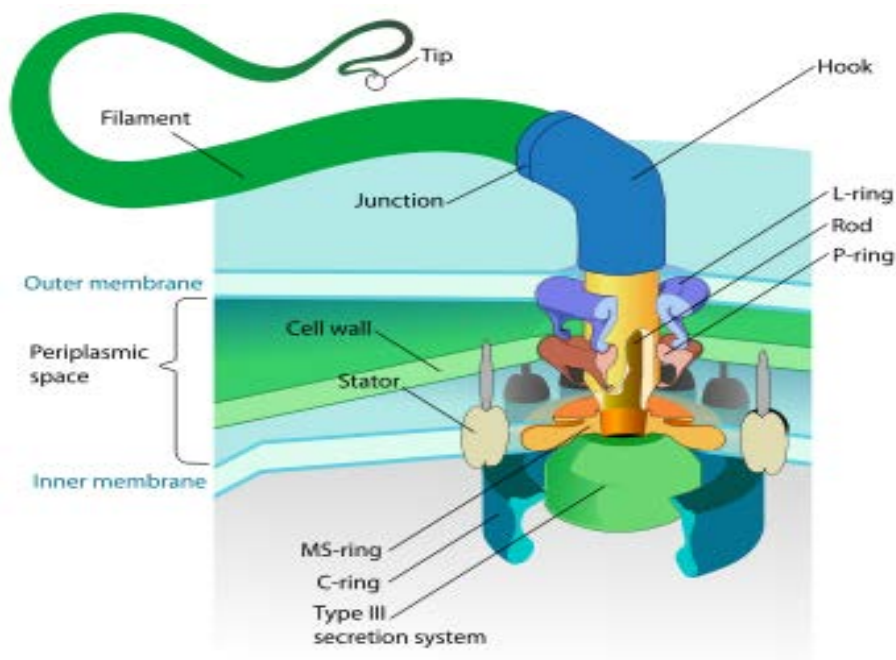

Figure 4: Flagellum of gram negative bacteria

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.
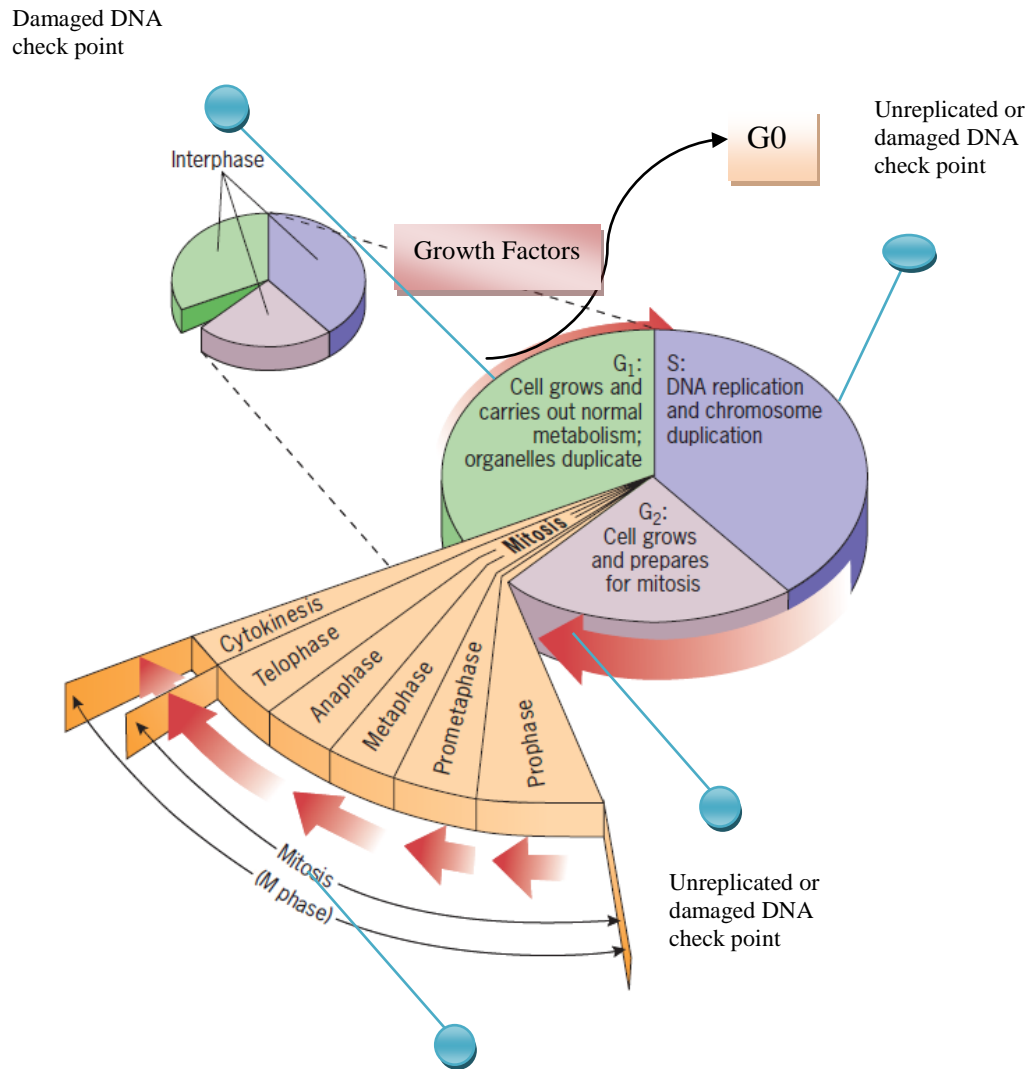
Figure 1: An overview of the cell cycle.

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.
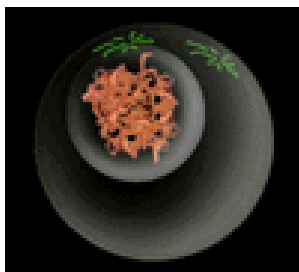

Fig.2: Interphase

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:
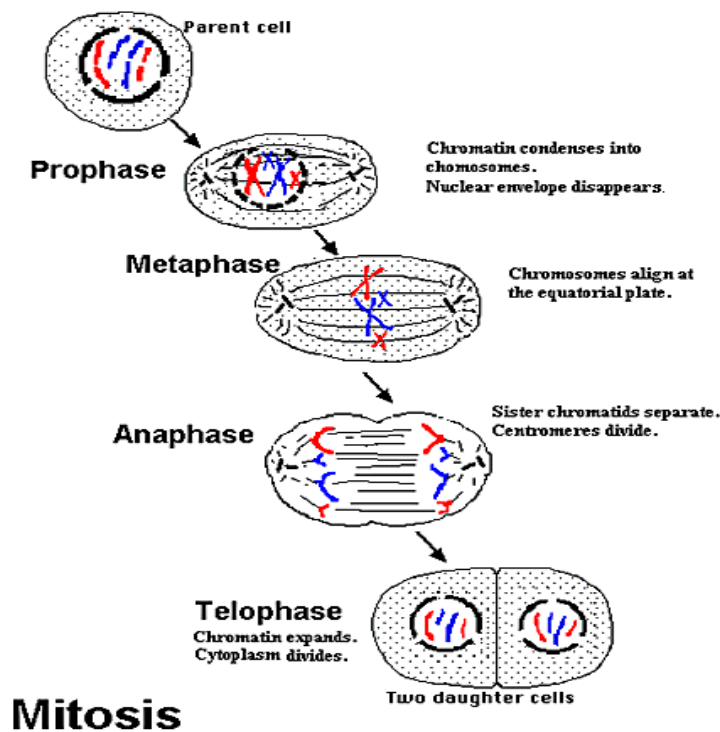
Fig.3: Mitosis cell cycle

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.
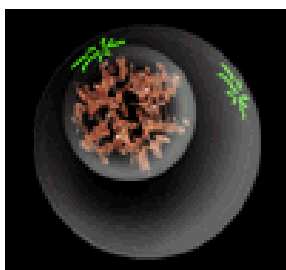
Fig.4: Prophase

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.
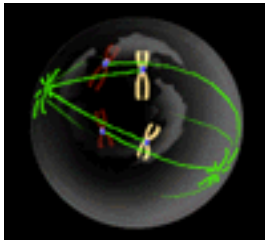
Fig.5: Prometaphase
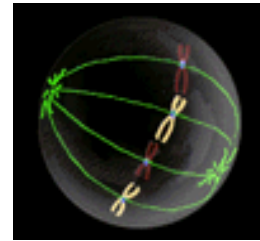
Fig. 6: Metaphase

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.
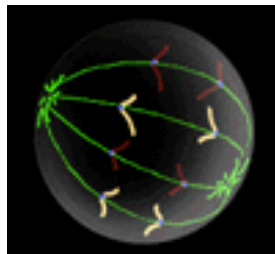

Fig.7: Anaphase

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.
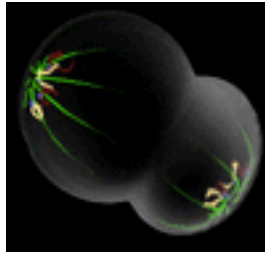

Fig.8: Telophase

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.
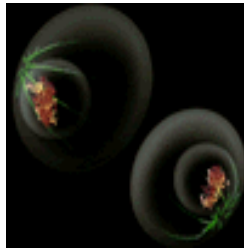

Fig.9: Cytokinesis

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.
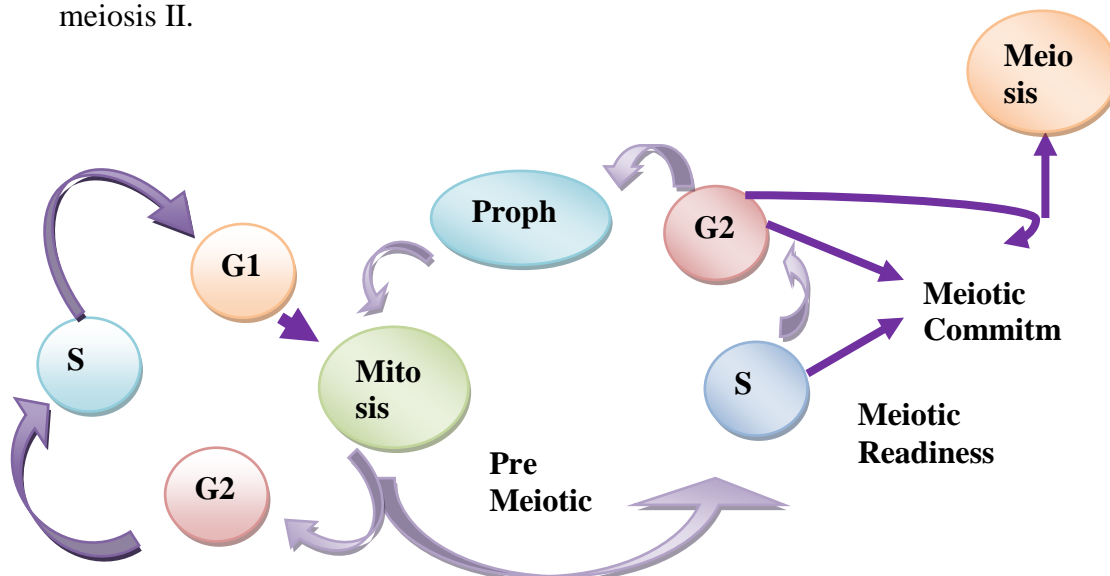

Fig 1: Position of meiosis in the Cell cycle.

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.

Nucleus
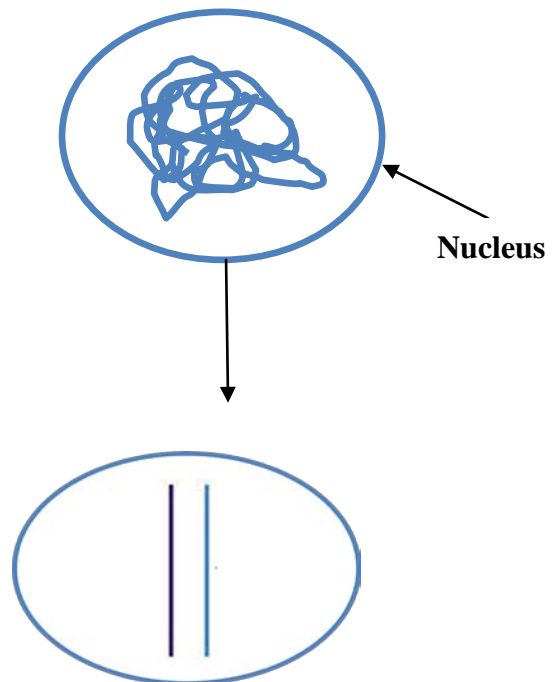
2. Zygotene

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.

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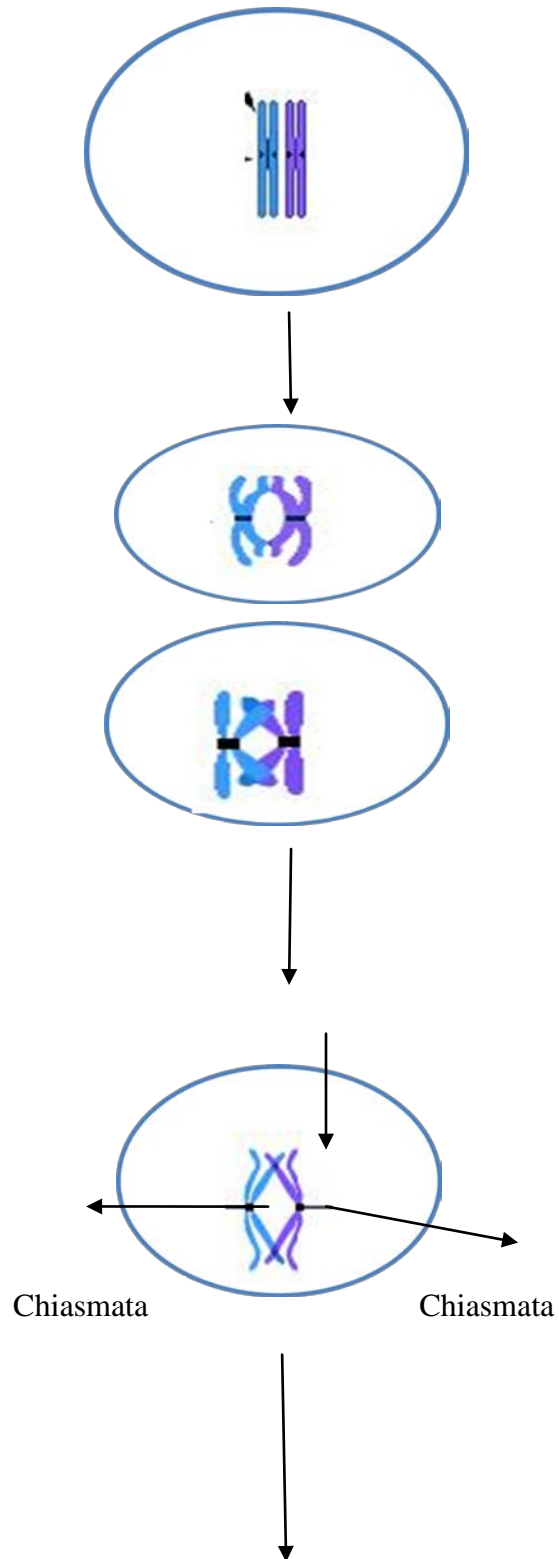
entangle together, effectively overlapping, making chiasmata clearly visible. The rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.
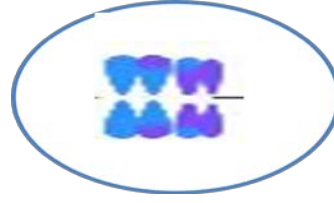

Figure 2: Stages of Meiosis I

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).
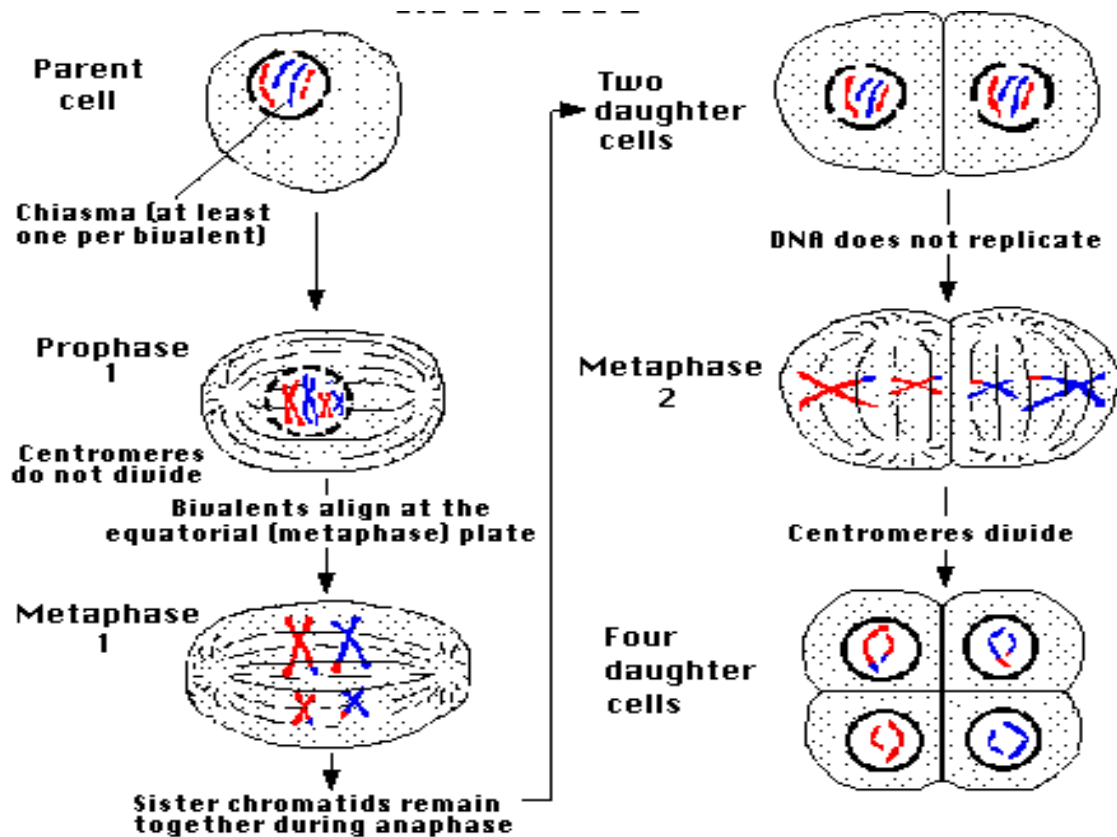
Figure 3: Events in meiosis I and II

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.
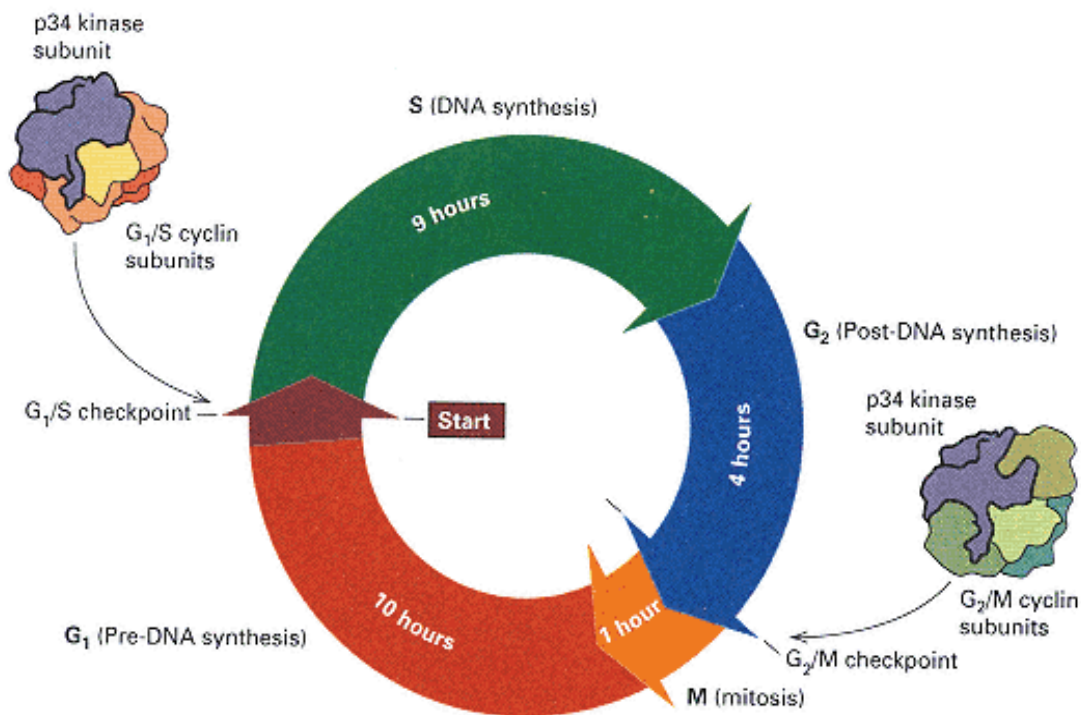

Figure 1: The cell cycle of a typical mammalian cell growing in tissue culture with a generation time of 24 hours. The critical control points for the G$_1$S and G$_2$M transitions are governed by a p34 kinase that is activated by stage-specific cyclins and that regulates the activity of its target proteins 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.
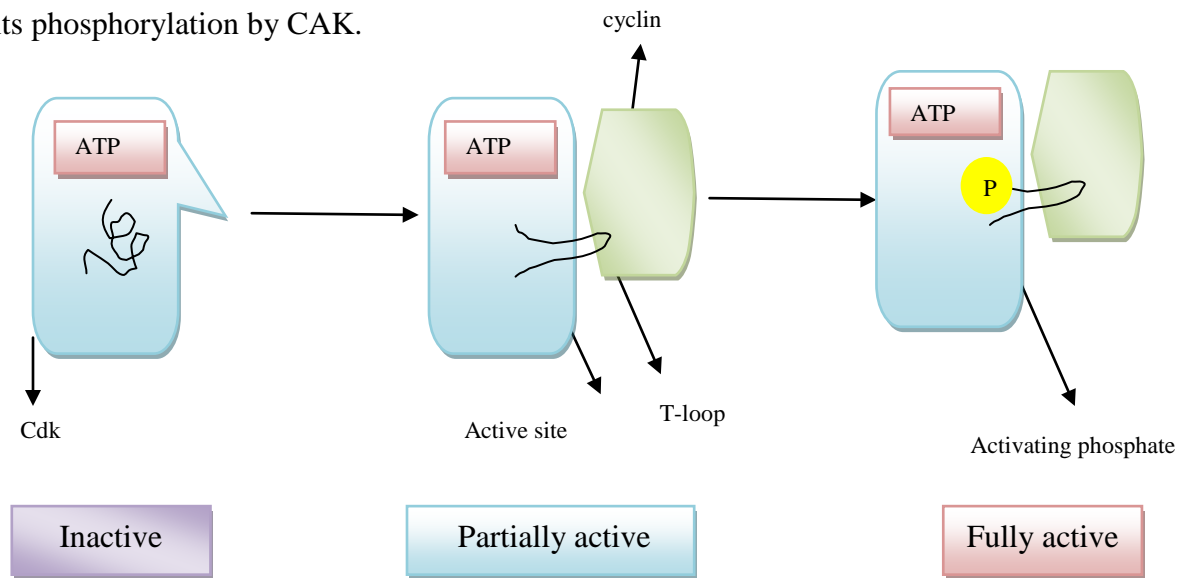

Figure 2: The structural basis of Cdk activation.

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.**
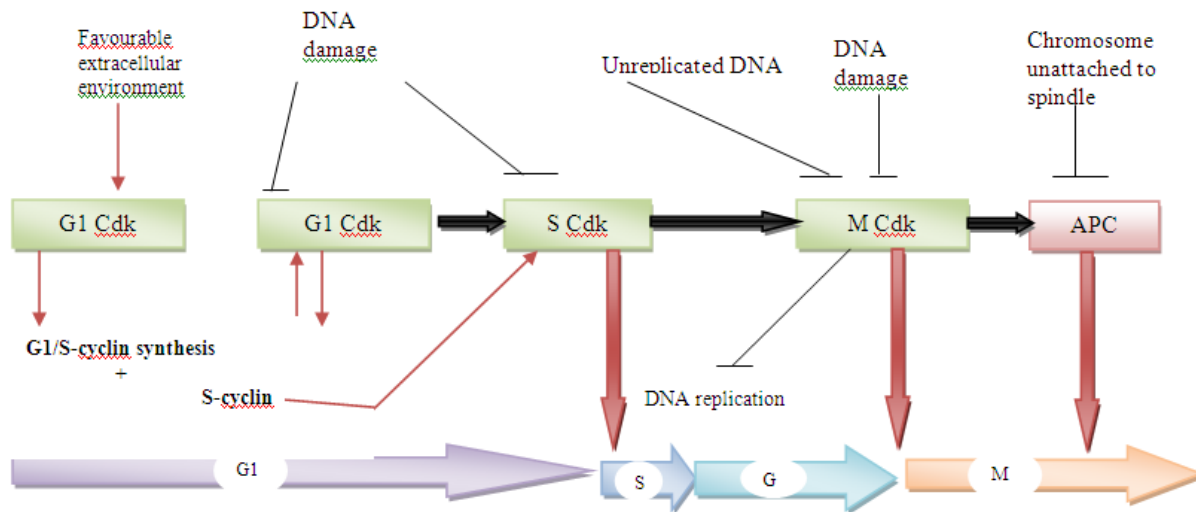

Figure 3: An overview of the cell cycle control system. Activation of G1-Cdk is stimulated through various external and internal signals. This in turn activates genes encoding G1/S and S cyclins. G1/S Cdk results in wave of S-Cdk activity which initiates chromosome replication in S-phase and contributes 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.
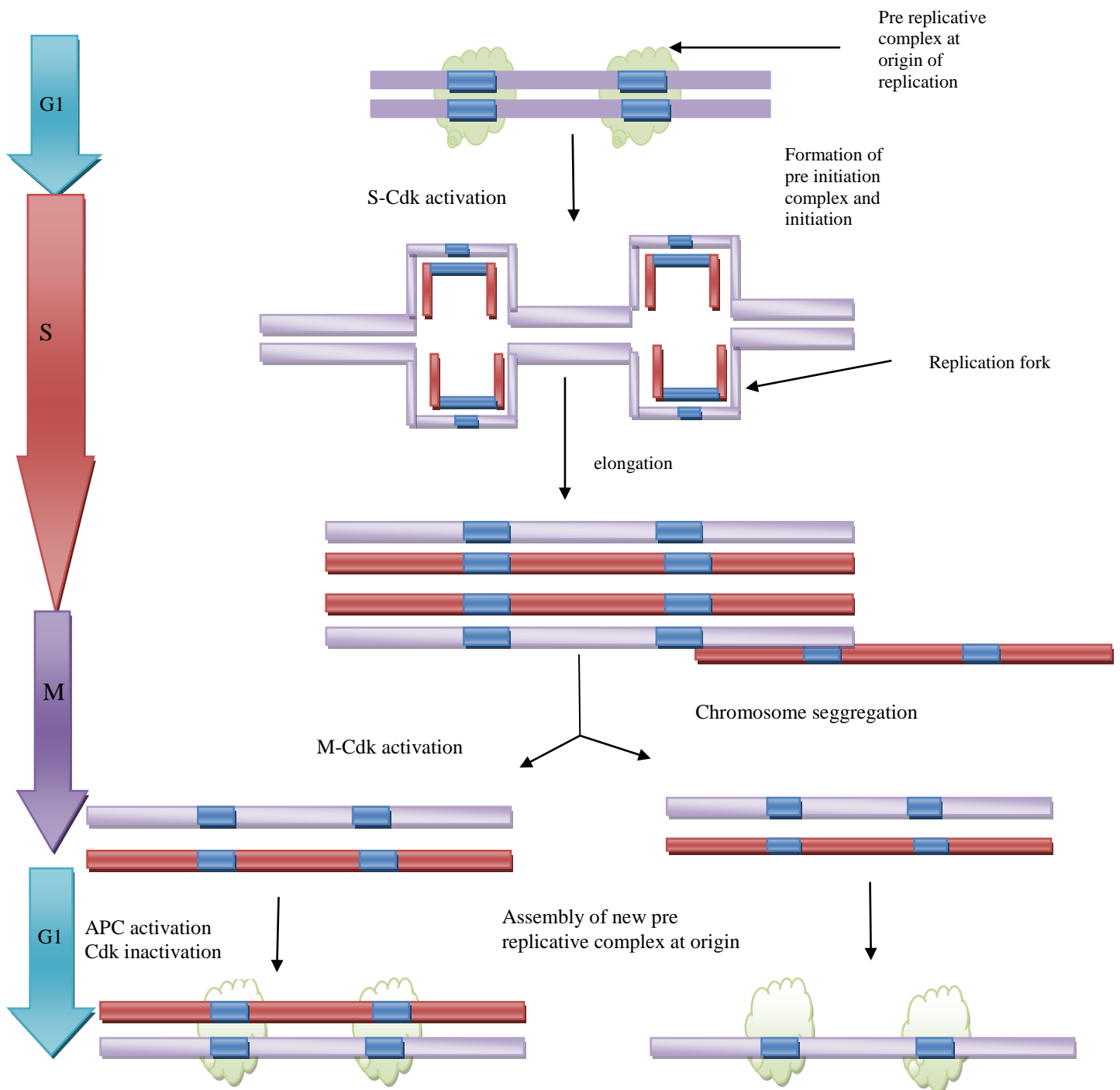
G1

S

M

G1

Pre replicative complex at origin of replication

S-Cdk activation

Formation of pre initiation complex and initiation

Replication fork

elongation

Chromosome seggregation

M-Cdk activation

APC activation
Cdk inactivation

Assembly of new pre replicative complex at origin

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.
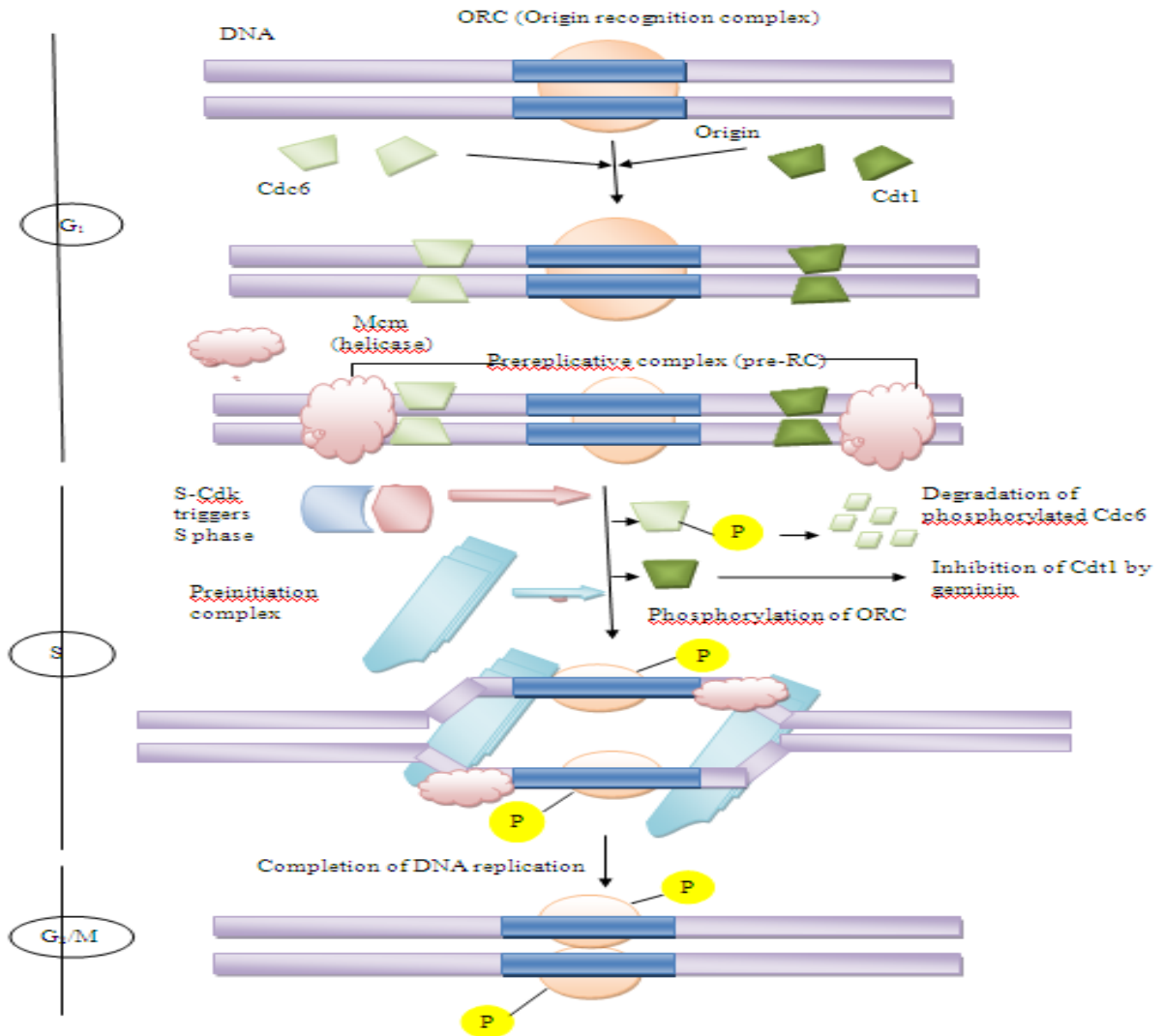
Figure 5: Control of the initiation of DNA replication.

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?