Cell and Cell Organelles:

Introduction- Higher eukaryotes have multiple organs to perform specific functions such as liver, kidney and heart. Each Organ has specific tissue and each tissue is composed of cells. **"Cell is the structural and functional unit of life"** and it contains all necessary infrastructure to peform all functions. Based on cellular structure, cells are classified as prokaryotic and eukaryotic cells. In most of the cases, prokaryotes are single cells where as eukaryotes are either single cells or part of multicellular tissues system. Besides this, both types of cells have several structural and metabolic differences as given in Table 3.1 and are discussed later in the lecture.

Feature	**Prokaryote**	**Eukaryote**
Size	Small, in μm range	Variable size, upto 40μm in diameter.
Genetic material	Circular DNA present in cytosol as free material	DNA in the form of linear chromosome present in well defined double membrane nucleus, no direct connection with cytosol
Replication	Single origin of replication	Multiple origin of replication.
Genes	No Intron	Presence of Intron
Organelles	No membrane bound organelles	Membrane bound orgelles with well defined function.
Cell walls	Very complex cell wall	Except Fungi and plant, eukaryotic cells are devoid of a thick cell wall.
Ribosome	70S	80S
Trancription and translation	Occurs together	Transcription in nucleus and translation in cytosol

Prokaryotic and Eukaryotic cells

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:

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 complexity. 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).

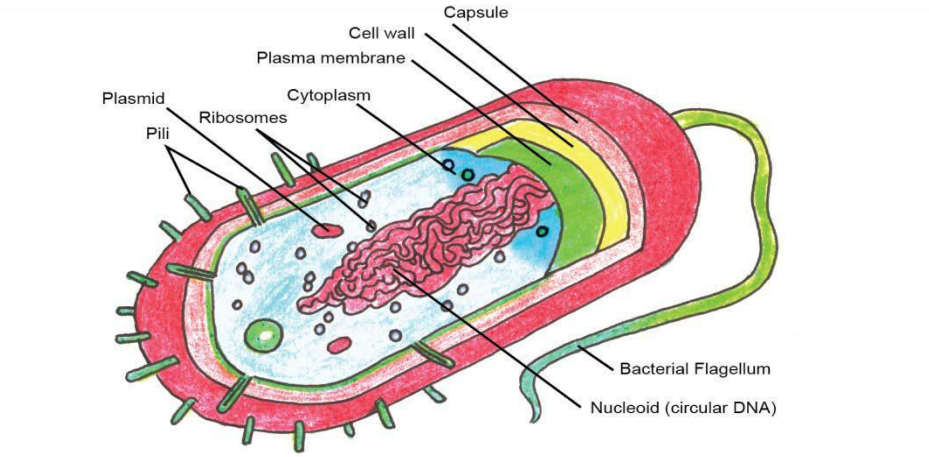


Figure 1: Schematic diagram of a prokaryotic cell

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.

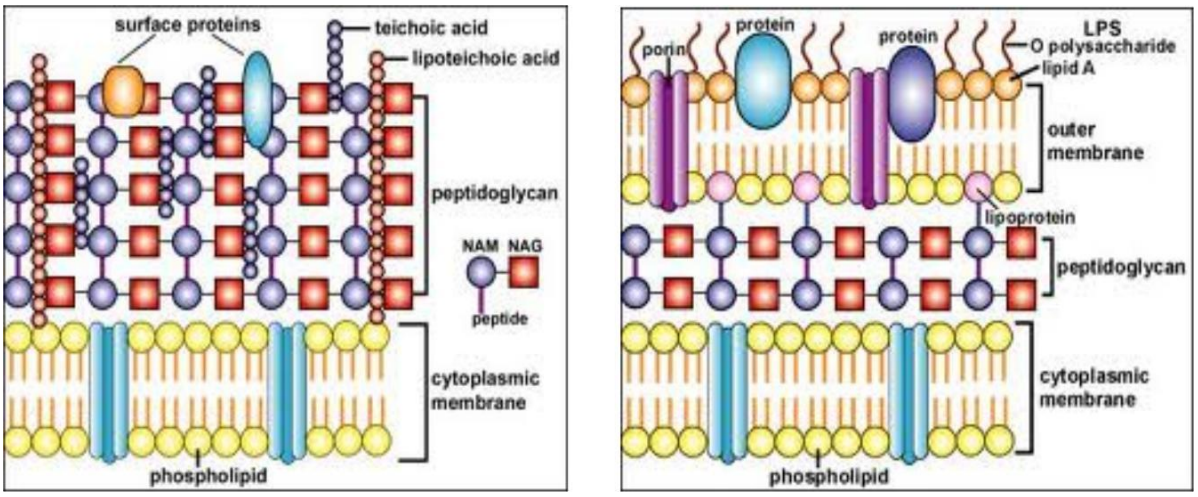
Capsule: The capsule is found in some bacterial cells, this additional outer covering protects the cell when it is engulfed by phagocytes and by viruses, assists in retaining moisture, and helps the cell adhere to surfaces and nutrients. The capsule is found most commonly among Gram-negative bacteria. *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.

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

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.

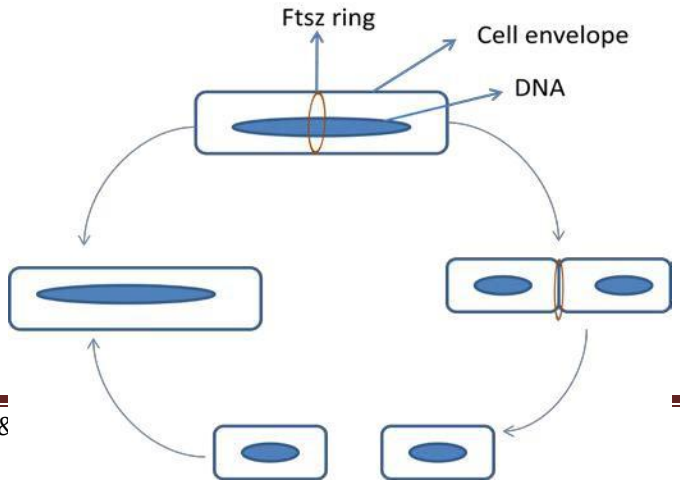


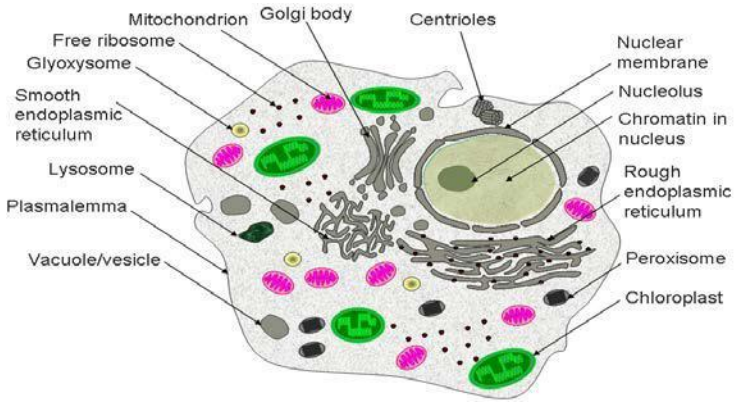
Figure 4: Binary fission in prokaryotes

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.



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.

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

Plant and animal cells

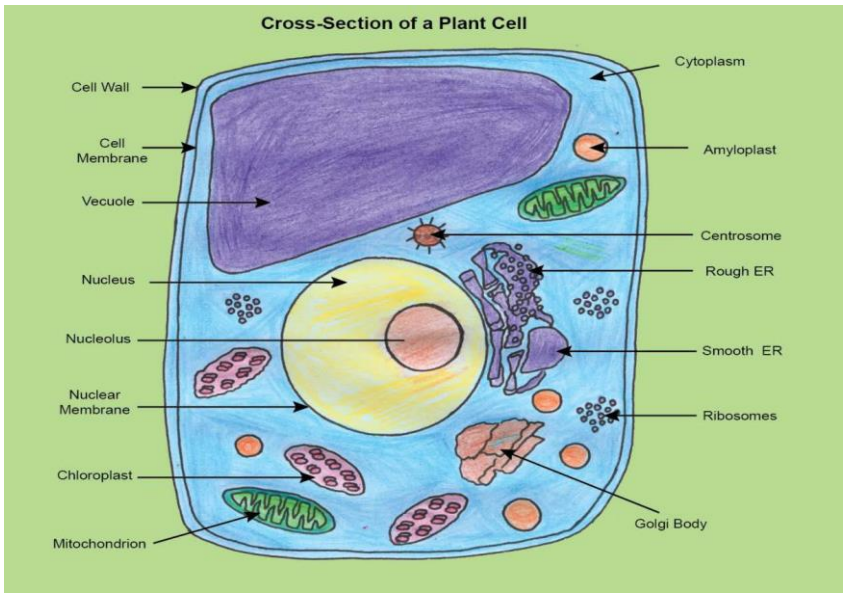
Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. Their distinctive features include the following organelles:

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.

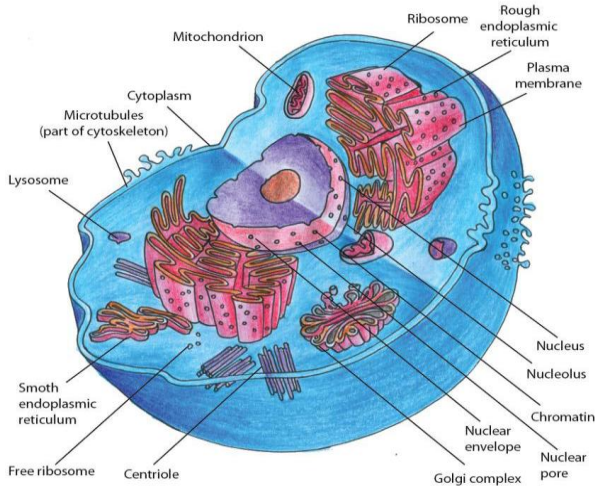


Schematic representation of a plant cell.

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.



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.

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.

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.

Plasma Memebrane:

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.

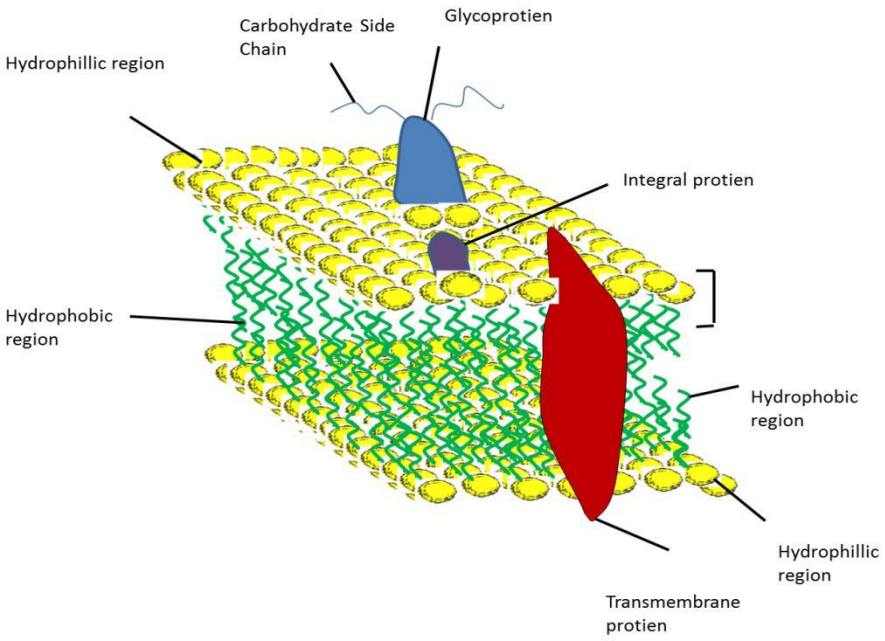
3. Mediated Transport: Nutrients are moved across the membrane by special proteins called transport proteins or permeases which are quite specific, recognizing and transporting only a limited group of chemical substances, often even only a single substance.

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.



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

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.

Membrane Lipid

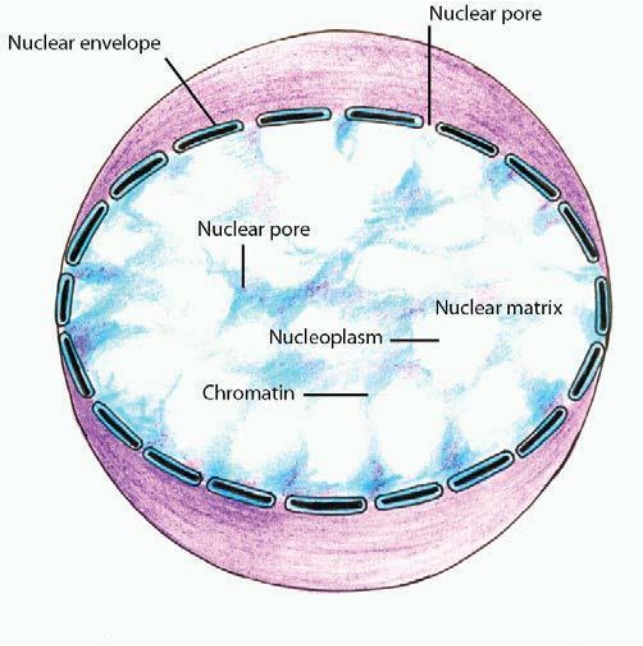
The plasma membrane is made of a combination of glycosphingolipids and protein receptors organized in glycolipoprotein microdomains termed lipid rafts which are 10– 200 nm in size. In addition to an external cell membrane (called the plasma membrane) eukaryotic cells also contain internal membranes that 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.

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.

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



The schematic representation of nucleus.

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.

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.

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

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.

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.

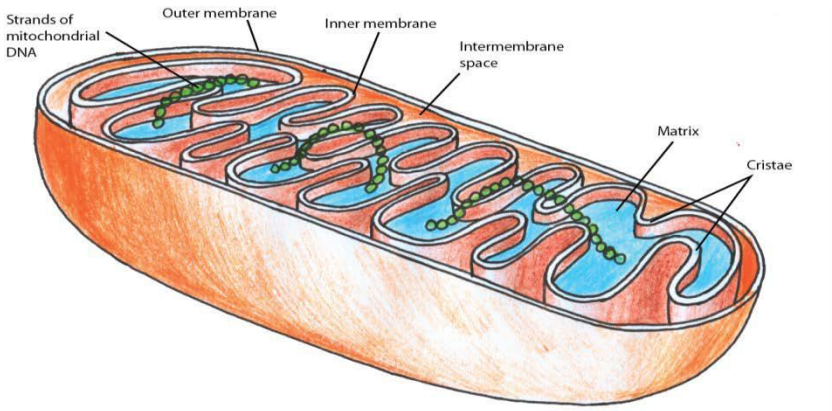
As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell. Overall, the cell nucleus stores all the chromosomal DNA of an organism.

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.



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.

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.

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.

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.

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.

Ribosome:

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 endoplasmic 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, 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. 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.

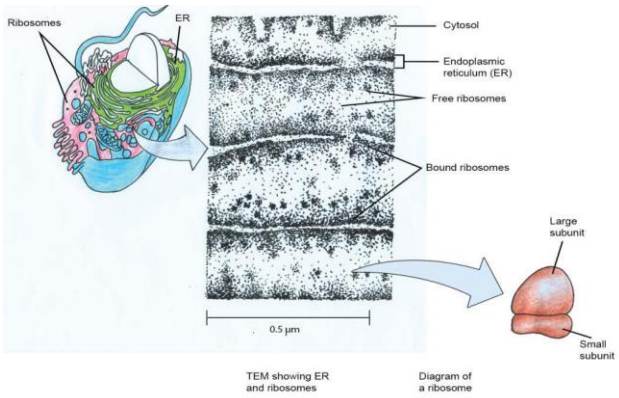
Structure

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

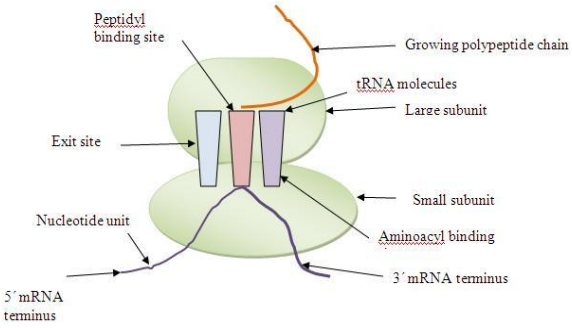
each subunit, and consequently the sizes of the subunits differ in bacterial and eukaryotic cells. The assembled ribosome is 70S in bacteria and 80S in vertebrates. There are great structural and functional similarities between ribosomes from all species which is another reflection of the common evolutionary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



Schematic representation of the ribosome.



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

Endoplasmic reticulum:

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

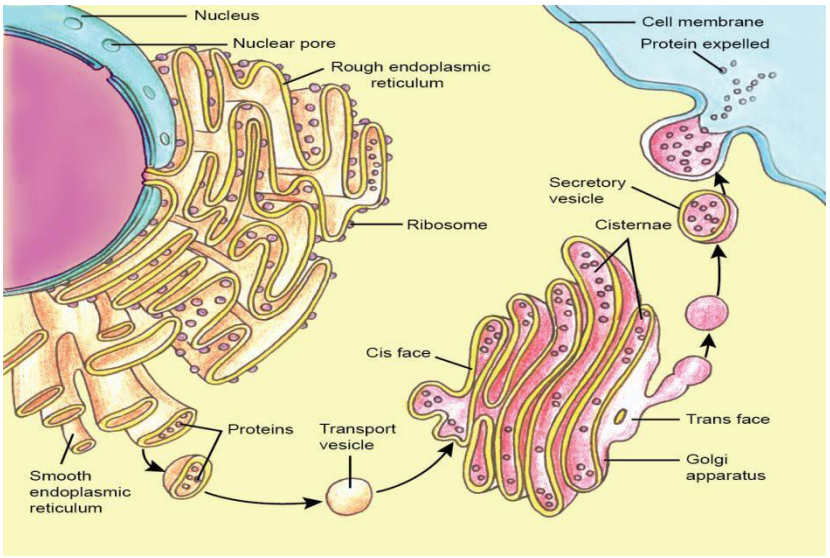


Figure 3. The Endoplasmic reticulum.

Occurrence:

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

Morphology:

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

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

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

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

Ultrastructure:

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

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

Types of endoplasmic reticulum:

Agranular or smooth endoplasmic reticulum:

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

Granular or rough endoplasmic reticulum:

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

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.

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:

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

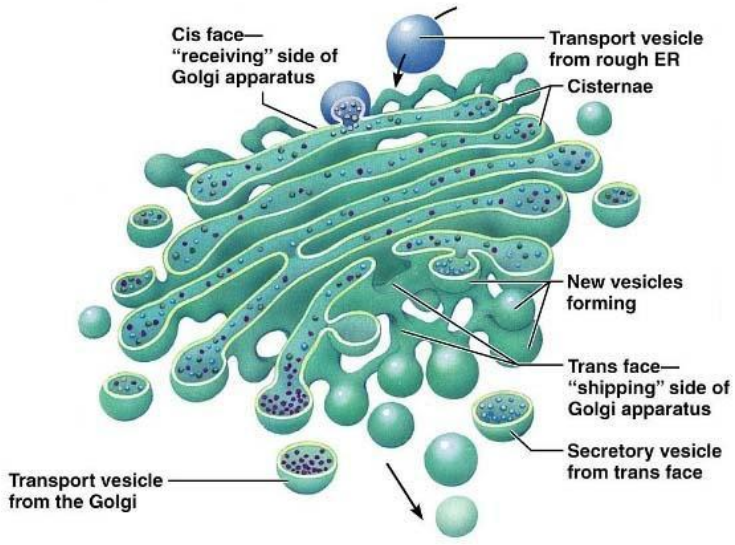
A complex array of associated vesicles and tubules (30 to 50 nm diameter) surround the dictyosome and radiate from it. The peripheral area of dictyosome is fenestrated or lace-like in structure.

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.



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.

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

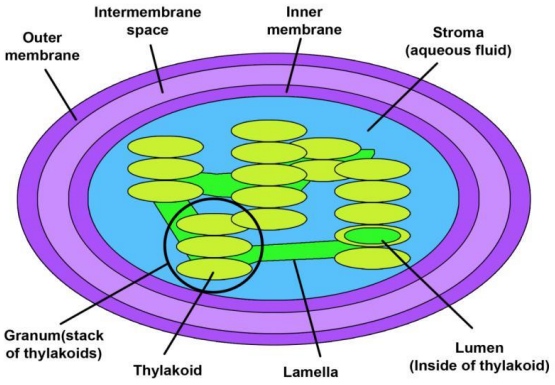
The matrix or stroma fills most of the volume of the chloroplasts and is a kind of gel-fluid phase that surrounds the thylakoids (grana). It contains about 50 per cent of the proteins of the chloroplast, most of which are soluble type. The stroma also contains ribosomes and DNA molecules both of which 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

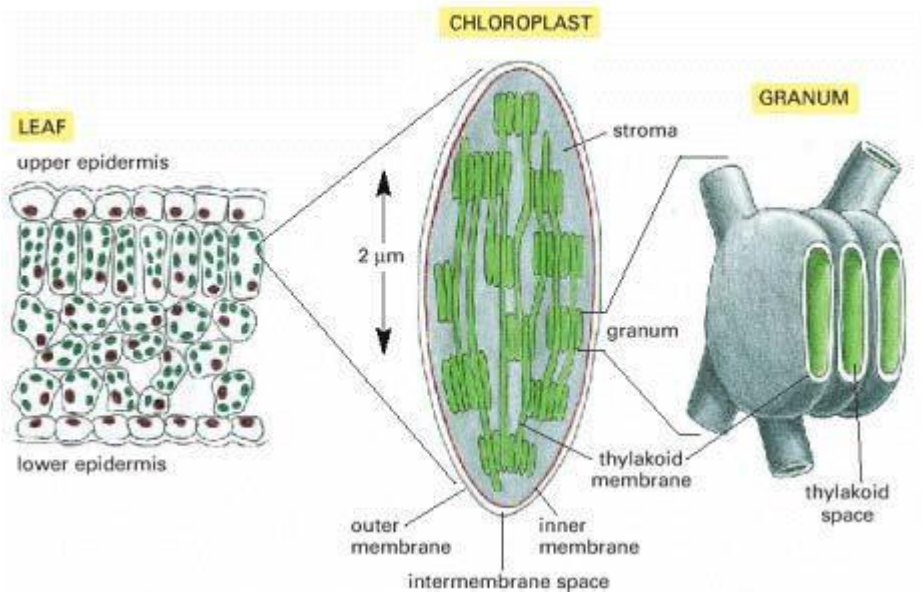
nuclear DNA and are synthesized in the cytosol, the proteins encoded by mitochondrial or chloroplast DNA is synthesized on ribosomes within the organelles.



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 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.



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.

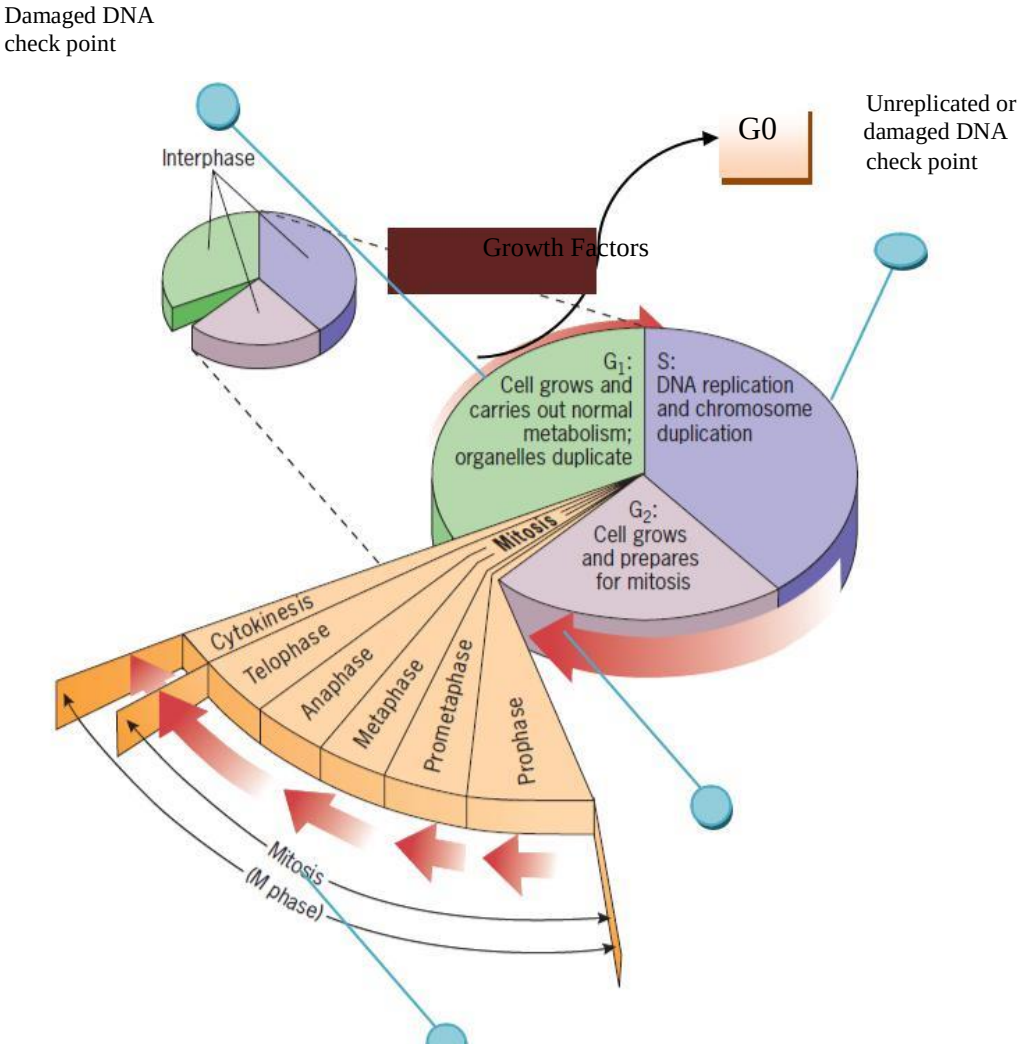
Cell division and its significance:

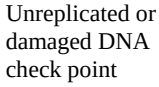
Continuity of life depends on cell division. All cells are produced by divisions of pre-existing cell (Please recall our discussion about the cell theory in our first lecture). A cell born after a division, proceeds to grow by macromolecular synthesis, and divides after reaching a species-determined

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.





An overview of the cell cycle.

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.

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:

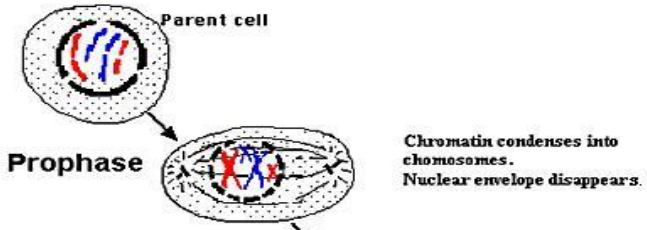


Fig.3: Mitosis cell cycle

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.

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.

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.

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.

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

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.

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.

Stages of meiosis

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

Meiosis I

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

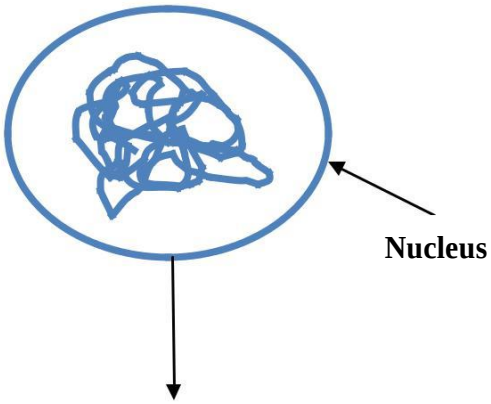
A. Prophase I

During this phase DNA is exchanged between homologous chromosomes or sister chromatids in a process called

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

1. Leptotene

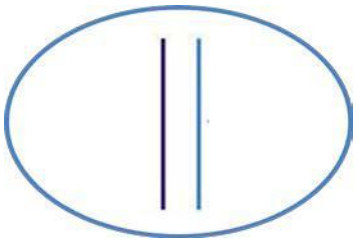
It is a very short duration stage and progressive condensation of chromosomes takes place. In this stage the chromosomes are first observed as thin threads and are said to be in a diffused state. The sister chromatids are tightly packed and indistinguishable from one another.



2. Zygotene

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

Purple and blue represent homologous duplicated chromosomes.



3. Pachytene

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

4. Diplotene

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

5. Diakinesis

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

Chiasmata 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.

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

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

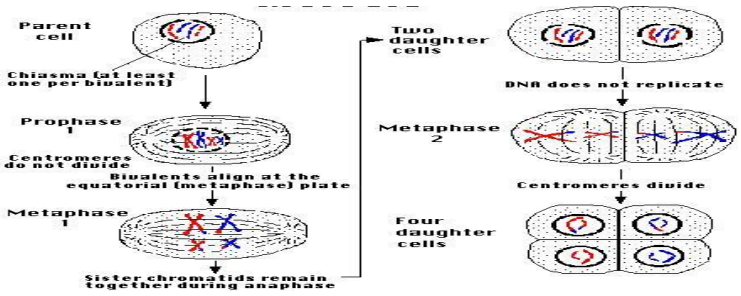
In metaphase II, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes (centrioles) at each pole. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate (see Fig 3).

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).



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.

Giant chromosomes:

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

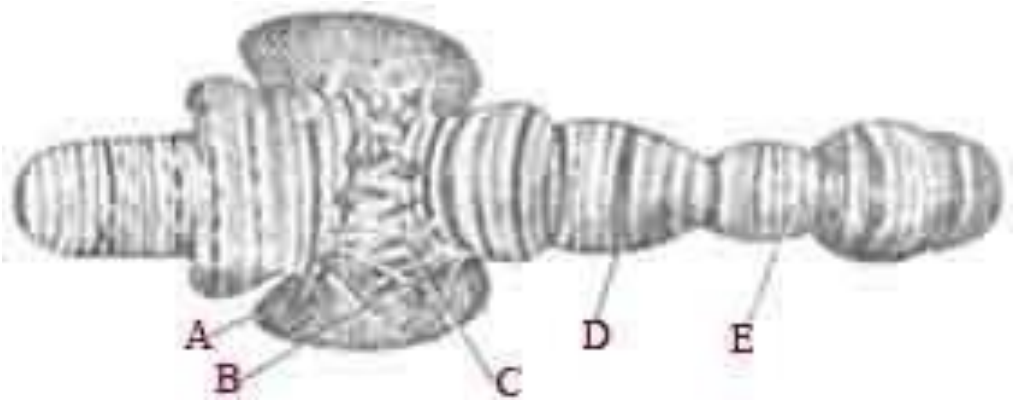
Polytene Chromosome

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

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

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

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



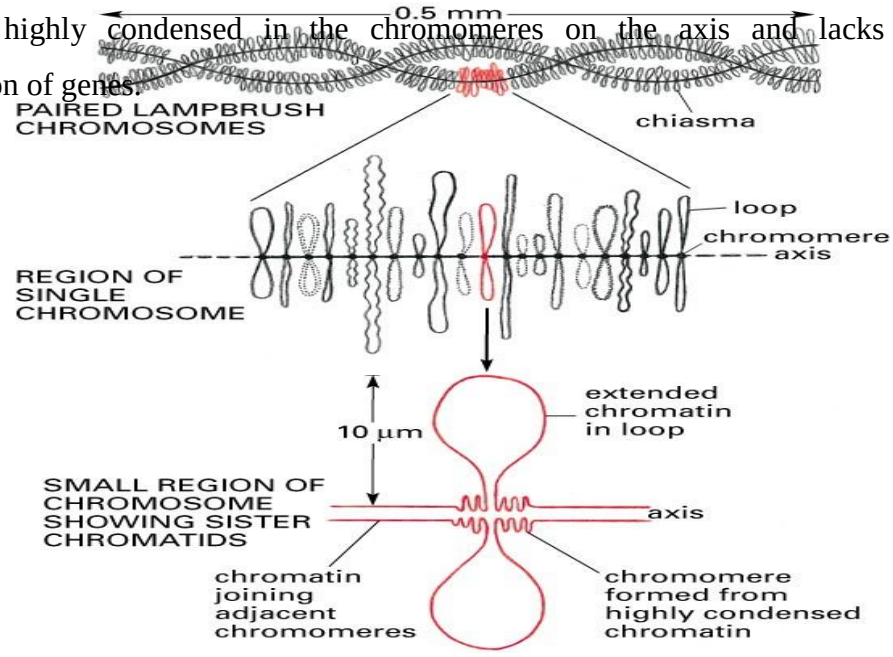
The structure of Drosophilla polytene chromosome. A: mRNA; B-Chromosome puff; C: Chromonemata; D: Dark band; E: Interband.

Lampbrush chromosome

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

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

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



Lampbrush chromosome.

The loops perform intense transcription of heterogenous RNA (precursors of mRNA molecules for ribosomal and histone proteins). Thus each lateral loop is covered by an assymetrical matrix of RNA transcripts; thicker at one end of the loop than other. The number of pairs of loops gradually increases during meiosis till it reaches maximum at diplotene. This stage may persist for

months or years as oocytes build up supply of mRNA required for further development. As meiosis proceeds further number of loops gradually decrease and loops ultimately disappear due to reabsorption into the chromosome or disintegration.

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

Interesting Facts

- If unfolded the DNA in each cell's nucleus would be 2 meters long. Humans have an estimated 100 trillion cells. In other words, if the all the DNA from every cell in a person's body were patched up together they would form a strand of 200 billion kilometers, or more than 1,000 times the distance between Earth and the Sun.
- Genes for the same feature appear in the same locus (place) on each matching pair of chromosomes in every human body cell.
- The 23rd chromosome pair in humans decides what sex you are, and the sex chromosomes are called X and Y.
- In some rare cases people are born with one extra chromosome. Those born with three chromosome 21 have Down's syndrome.
- It takes about 8 hours for one of your cells to completely copy its DNA.
- Human beings share 7% of genes with *E. coli* bacterium, 21% with worms, 90% with mice and 98% with chimpanzees.