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F.Y. B.Sc. (Sem. II) (CBCS)

BIOCHEMISTRY

[201]: CELL BIOLOGY

Unit 2
Structure, Chemical Composition, Enzymes and Functions of Different Cell Organelles

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CELL WALL, PLASMA MEMBRANE AND CYTOPLASM

CELL WALL

- A cell wall is an outer layer surrounding certain cells that is outside of the cell membrane. All cells have cell membranes, but generally only plants, fungi, algae, most bacteria, and archaea have cells with cell walls.
- The cell wall provides strength and structural support to the cell, and can control to some extent what types and concentrations of molecules enter and leave the cell.
- The materials that make up the cell wall differ depending on the type of organism. The cell wall has evolved many different times among different groups of organisms.

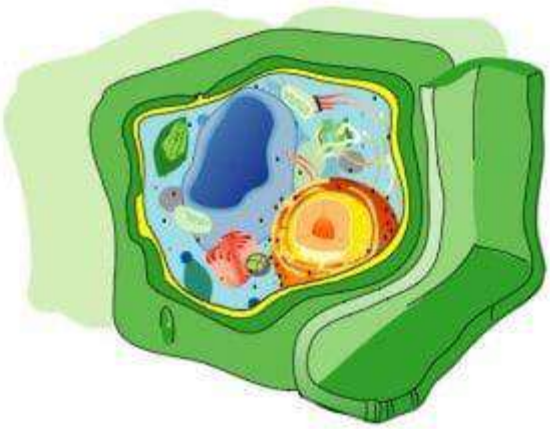
Cell Wall Functions

- The cell wall has a few different functions. It is flexible, but provides strength to the cell, which helps protect the cell against physical damage. It also gives the cell its shape and allows the organism to maintain a certain shape overall. The cell wall can also provide protection from pathogens such as bacteria that are trying to invade the cell. The structure of the cell wall allows many small molecules to pass through it, but not larger molecules that could harm the cell.

Cell Wall Structure

Plant Cell Walls:

- The main component of the plant cell wall is cellulose, a carbohydrate that forms long fibres and gives the cell wall its rigidity. Cellulose fibres group together to form bundles called microfibrils. Other important carbohydrates include hemicellulose, pectin, and lignin.
- These carbohydrates form a network along with structural proteins to form the cell wall. Plant cells that are in the process of growing have primary cell walls, which are thin. Once the cells are fully grown, they develop secondary cell walls.
- The secondary cell wall is a thick layer that is formed on the inside of the primary cell wall. This layer is what is usually meant when referring to a plant's cell wall. There is also another layer in between plant cells called the middle lamella; it is pectin-rich and helps plant cells stick together.
- The cell walls of plant cells help them maintain turgor pressure, which is the pressure of the cell membrane pressing against the cell wall. Ideally, plants cells should have lots of water within them, leading to high turgidity. Whereas a cell without a cell wall, such as an animal cell, can swell and burst of too much water diffuses into it, plants need to be in hypotonic solutions (more water inside than outside, leading to lots of water entering the cell) to maintain turgor pressure and their structural shape.
- The cell wall efficiently holds water in so that the cell does not burst. When turgor pressure is lost, a plant will begin to wilt. Turgor pressure is what gives plant cells their characteristic square shape; the cells are full of water, so they fill up the space available and press against each other.
- This diagram of a plant cell depicts the cell wall in green, surrounding the contents of the cell.



Algae Cell Walls

- Algae are a diverse group, and the diversity in their cell walls reflects this. Some algae, such as green algae, have cell walls that are similar in structure to those of plants.
- Other algae, such as brown algae and red algae, have cellulose along with other polysaccharides or fibrils. Diatoms have cell walls that are made from silicic acid. Other important molecules in algal cell walls include mannans, xylans, and alginic acid.

Fungi Cell Walls

- The cell walls of fungi contain chitin, which is a glucose derivative that is similar in structure to cellulose.
- Layers of chitin are very tough; chitin is the same molecule found in the rigid exoskeletons of animals such as insects and crustaceans. Glucans, which are other glucose polymers, are also found in the fungal cell wall along with lipids and proteins.
- Fungi have proteins called hydrophobins in their cell walls. Found only in fungi, hydrophobins give the cells strength, help them adhere to surfaces, and help control the movement of water into the cells.
- In fungi, the cell wall is the most external layer, and surrounds the cell membrane.

Bacteria and Archaea Cell Walls

- The cell walls of bacteria usually contain the polysaccharide peptidoglycan, which is porous and lets small molecules through. Together, the cell membrane and cell wall are referred to as the cell envelope.
- The cell wall is an essential part of survival for many bacteria.
- It provides mechanical structure to bacteria, which are single-celled, and it also protects them from internal turgor pressure.
- Bacteria have higher concentration of molecules such as proteins within themselves as compared to their environment, so the cell wall stops water from rushing into the cell. Differences in cell wall thickness also make Gram staining possible.
- Gram staining is used for the general identification of bacteria; bacteria with thick cell walls are gram-positive, while bacteria with thinner cell walls are gram-negative.
- While archaea are similar in many ways to bacteria, hardly any archaeal walls contain peptidoglycan.
- There are several different types of cell walls in archaea. Some are composed of pseudopeptidoglycan, some have polysaccharides, some have glycoproteins, and others have surface-layer proteins (called an S-layer, which can also be found in bacteria).

PLASMA MEMBRANE

- Plasma Membrane Definition: The plasma membrane of a cell is a network of lipids and proteins that forms the boundary between a cell's contents and the outside of the cell.
- It is also simply called the cell membrane. The main function of the plasma membrane is to protect the cell from its surrounding environment. It is semi-permeable and regulates the materials that enter and exit the cell. The cells of all living things have plasma membranes.

Functions of the Plasma Membrane

A Physical Barrier:

- The plasma membrane surrounds all cells and physically separates the cytoplasm, which is the material that makes up the cell, from the extracellular fluid outside the cell.
- This protects all the components of the cell from the outside environment and allows separate activities to occur inside and outside the cell.
- The plasma membrane provides structural support to the cell. It tethers the cytoskeleton, which is a network of protein filaments inside the cell that hold all the parts of the cell in place.
- This gives the cell its shape. Certain organisms such as plants and fungi have a cell wall in addition to the membrane. The cell wall is composed of molecules such as cellulose. It provides additional support to the cell, and it is why plant cells do not burst like animal cells do if too much water diffuses into them.

Selective Permeability:

- Plasma membranes are selectively permeable (or semi-permeable), meaning that only certain molecules can pass through them.
- Water, oxygen, and carbon dioxide can easily travel through the membrane. Generally, ions (e.g., sodium, potassium) and polar molecules cannot pass through the membrane; they must go through specific channels or pores in the membrane instead of freely diffusing through. This way, the membrane can control the rate at which certain molecules can enter and exit the cell.

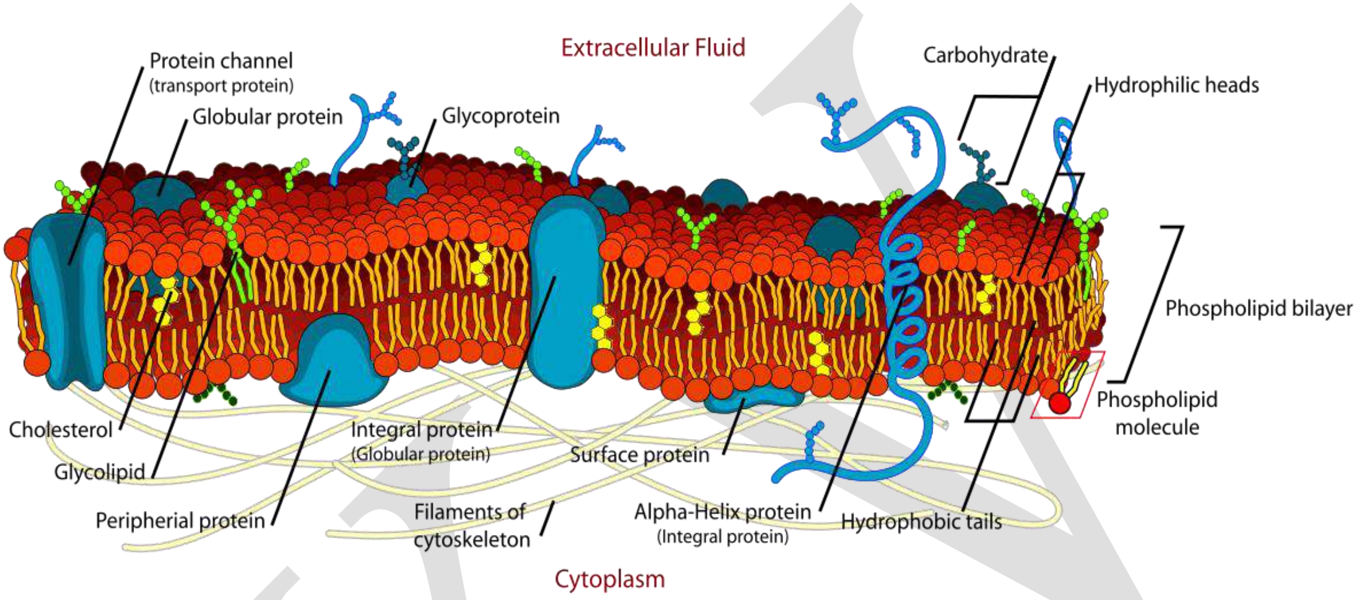
Endocytosis and Exocytosis

- Endocytosis is when a cell ingests relatively larger contents than the single ions or molecules that pass-through channels. Through endocytosis, a cell can take in large quantities of molecules or even whole bacteria from the extracellular fluid.
- Exocytosis is when the cell releases these materials. The cell membrane plays an important role in both of these processes. The shape of the membrane itself changes to allow molecules to enter or exit the cell. It also forms vacuoles, small bubbles of membrane that can transport many molecules at once, in order to transport materials to different places in the cell.

Cell Signalling

- Another important function of the membrane is to facilitate communication and signaling between cells. It does so through the use of various proteins and carbohydrates in the membrane.
- Proteins on the cell "mark" that cell so that other cells can identify it. The membrane also has receptors that allow it to carry out certain tasks when molecules such as hormones bind to those receptors.

Plasma Membrane Structure



Phospholipids

- The membrane is partially made up of molecules called phospholipids, which spontaneously arrange themselves into a double layer with hydrophilic ("water loving") heads on the outside and hydrophobic ("water hating") tails on the inside. These interactions with water are what allow plasma membranes to form.

Proteins

- Proteins are wedged between the lipids that make up the membrane, and these transmembrane proteins allow molecules that couldn't enter the cell otherwise to pass through by forming channels, pores or gates. In this way, the cell controls the flow of these molecules as they enter and exit. Proteins in the cell membrane play a role in many other functions, such as cell signaling, cell recognition, and enzyme activity.

Carbohydrates

- Carbohydrates are also found in the plasma membrane; specifically, most carbohydrates in the membrane are part of glycoproteins, which are formed when a carbohydrate attaches to a protein. Glycoproteins play a role in the interactions between cells, including cell adhesion, the process by which cells attach to each other.

Fluid Mosaic Model

- Technically, the cell membrane is a liquid. At room temperature, it has about the same consistency as vegetable oil. Lipids, proteins, and carbohydrates in the plasma membrane can diffuse freely throughout the cell membrane; they are essentially floating across its surface. This is known as the fluid mosaic model, which was coined by S.J. Singer and G.L. Nicolson in 1972.

CYTOPLASM

- Cytoplasm Definition**:** Cytoplasm refers to the fluid that fills the cell, which includes the cytosol along with filaments, proteins, ions and macromolecular structures as well as the organelles suspended in the cytosol.

- In eukaryotic cells, cytoplasm refers to the contents of the cell with the exception of the nucleus. Eukaryotes have elaborate mechanisms for maintaining a distinct nuclear compartment separate from the cytoplasm. Active transport is involved in the creation of these subcellular structures and for maintaining homeostasis with the cytoplasm.

- For prokaryotic cells, since they do not have a defined nuclear membrane, the cytoplasm also contains the cell's primary genetic material. These cells are usually smaller in comparison to eukaryotes, and have a simpler internal organization of the cytoplasm.

Structure of Cytoplasm

- The cytoplasm is unusual because it is unlike any other fluid found in the physical world. Liquids that are studied to understand diffusion usually contain a few solutes in an aqueous environment.

- However, the cytoplasm is a complex and crowded system containing a wide range of particles – from ions and small molecules, to proteins as well as giant multi protein complexes and organelles.

- These constituents are moved across the cell depending on the requirements of the cell along an elaborate cytoskeleton with the help of specialized motor proteins. The movement of such large particles also changes the physical properties of the cytosol.

- The physical nature of the cytoplasm is variable. Sometimes, there is quick diffusion across the cell, making the cytoplasm resemble a colloidal solution. At other times, it appears to take on the properties of a gel-like or glass-like substance.

- It is said to have the properties of viscous as well as elastic materials – capable of deforming slowly under external force in addition to regaining its original shape with minimal loss of energy.

- Parts of the cytoplasm close to the plasma membrane are also 'stiffer' while the regions near the interior resemble free flowing liquids.

- These changes in the cytoplasm appear to be dependent on the metabolic processes within the cell and play an important role in carrying out specific functions and protecting the cell from stressors.

- The cytoplasm can be divided into three components:
  - The cytoskeleton with its associated motor proteins
  - Organelles and other large multi-protein complexes
  - Cytoplasmic inclusions and dissolved solutes

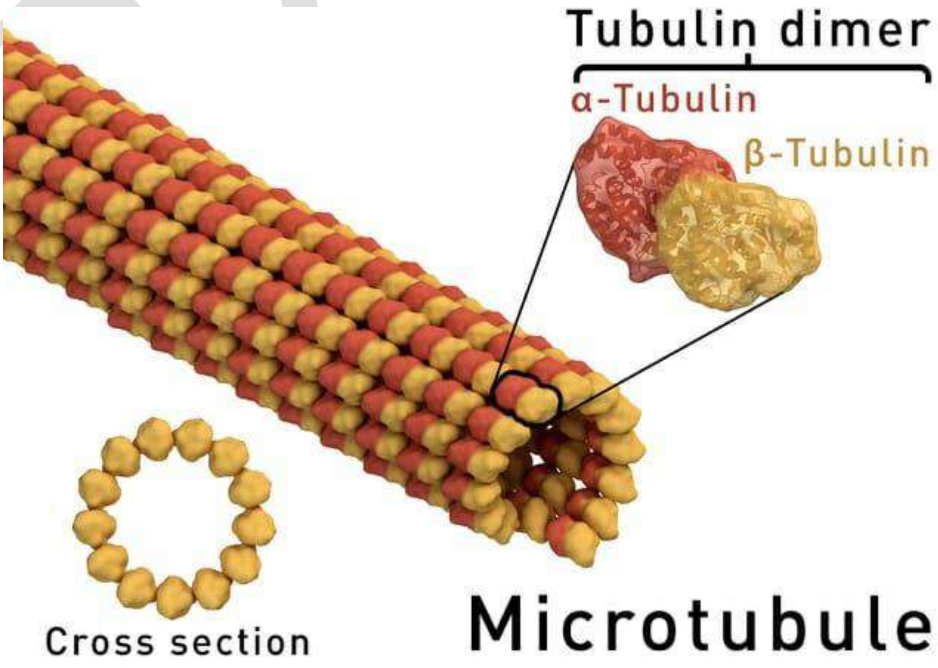
Cytoskeleton and Motor Proteins

- The basic shape of the cell is provided by its cytoskeleton formed primarily by three types of polymers – actin filaments, microtubules and intermediate filaments.

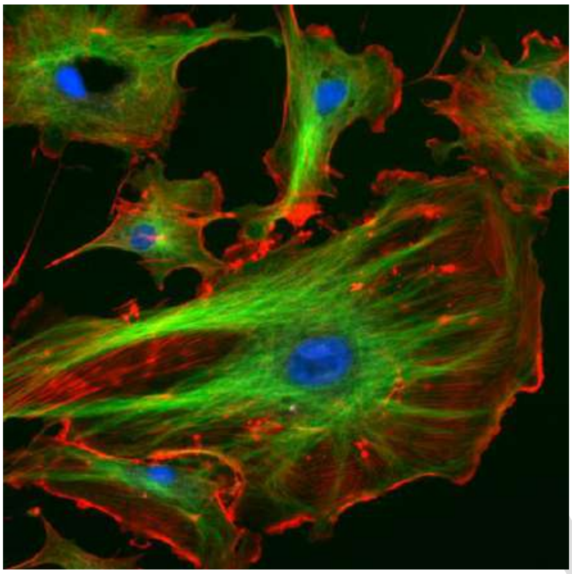
- Actin filaments or microfilaments are 7 nm in width and are made of double stranded polymers of F-actin. These filaments are associated with a number of other proteins that help in filament assembly and are also involved in anchoring them close to the plasma membrane.

- This cytoplasmic location helps the microfilaments become involved in rapid responses to signal molecules from the extracellular environment and produce cellular responses through signal transduction or chemotaxis. In addition, myosin, an ATP-based motor protein transmits cargo and vesicles along the microfilament and is also involved in muscle contraction.

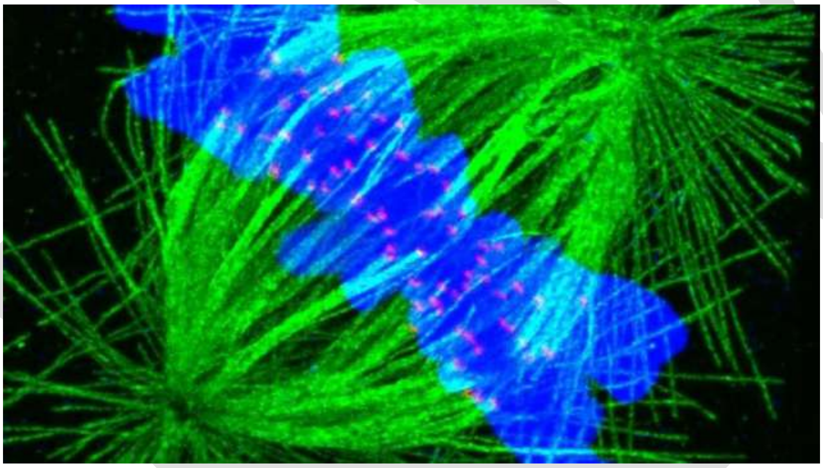
- Microtubules are polymers of α and β tubulin, which form a hollow tube by the lateral association of 13 protofilaments. Each protofilament is a polymer of alternating α and β tubulin molecules. The inner diameter of a microtubule is 12 nm and its outer diameter is 24 nm.



- Microtubules radiate towards the periphery of the cell from microtubule organizing centers (MTOCs) located close to the nucleus, and provide structure and shape to the cell.



- This image shows the nucleus in blue, the actin filaments on the cell periphery are labeled red and the extensive microtubule network is marked green. The cytoplasm undergoes rapid reorganization during cell division with microtubules forming the spindle, which binds to chromosomes and segregates them into two daughter cells.



- Similar to the previous image, chromosomes are stained blue and microtubules are green. Tiny red dots are kinetochores.

- Microtubules are involved in cytoplasmic transport, chromosome segregation and in forming structures such as cilia and flagella for cellular movement.

- Intermediate filaments are larger than microfilaments but smaller than microtubules and are formed by a group of proteins that share structural features. Though they are not involved in cell motility, they are important for cells to come together as tissues and to remain anchored to the extracellular matrix.

Organelles and Multi-protein Complexes

- Most eukaryotic cells have a number of organelles that provide compartments within the cytoplasm for specialized microenvironments. For instance, lysosomes contain a number of hydrolases in an acidic environment that is ideal for their enzymatic activity.

- These hydrolases are actively transported into the lysosome after being synthesized in the cytoplasm. Mitochondria, while containing their own genome, also need many enzymes synthesized in the cytosol, which are then selectively moved into the organelle.

- These organelles are placed in specific locations due to the physical gel-like nature of the cytoplasm and by anchoring to the cytoskeleton.

- In addition, the cytoplasm also plays host to multi-protein complexes like the proteasome and ribosomes. Ribosomes are large complexes of RNA and protein that are important for the translation of mRNA code into amino acid sequences of proteins.

- Proteasomes are giant molecular structures about 20,000 kilodaltons in mass and 15 nm in diameter. Proteasomes are important for targeted destruction of proteins that are no longer needed by the cell.

Cytoplasmic Inclusions

- Cytoplasmic inclusions can include a wide range of biochemicals – from small crystals of proteins, to pigments, carbohydrates and fats. All cells, especially in tissue like the adipose, contain droplets of lipids in their triglyceride form.

- These are used to create cellular membranes and are an excellent energy store. Lipids can generate twice as many ATP molecules per gram when compared to carbohydrates. However, the process of releasing this energy from triglycerides in intensive in oxygen consumption and therefore the cell also contains stores of glycogen as cytoplasmic inclusions.

- Glycogen inclusions are particularly important in cells like the skeletal and cardiac muscle cells where there can be a sudden increase in demand for glucose. Glycogen can be quickly broken down into individual molecules of glucose and used in cellular respiration before the cell can obtain more glucose reserves from the body.

- Crystals are another type of cytoplasmic inclusion found in many cells, and have special function in cells of the inner ear (maintaining balance). Presence of crystals in cells of the testis appears to be linked with morbidity and infertility.

- Finally, the cytoplasm also contains pigments such as melanin, which lead to the pigmented cells of the skin. These pigments protect the cell and internal body structures from the deleterious effects of ultraviolet radiation. Pigments are also prominent in the cells of the iris that surround the pupil of the eye.

- Each of these components affects the functioning of the cytoplasm in different ways, making it a dynamic region that plays a role in, and is influenced by the cell's overall metabolic activity.

Functions of Cytoplasm

- The cytoplasm is the site for most of the enzymatic reactions and metabolic activity of the cell. Cellular respiration begins in the cytoplasm with anaerobic respiration or glycolysis.

- This reaction provides the intermediates that are used by the mitochondria to generate ATP. In addition, the translation of mRNA into proteins on ribosomes also occurs mostly in the cytoplasm. Some of it happens on free ribosomes suspended in the cytosol while the rest happens on ribosomes anchored on the endoplasmic reticulum.

- The cytoplasm also contains the monomers that go on to generate the cytoskeleton. The cytoskeleton, in addition to being important for the normal activities of the cell is crucial for cells that have a specialized shape. For instance, neurons with their long axons need the presence of intermediate filaments, microtubules, and actin filaments in order to provide a rigid framework for the action potential to be transmitted to the next cell.

- Additionally, some epithelial cells contain small cilia or flagella to move the cell or remove foreign particles through coordinated activity of cytoplasmic extrusions formed through the cytoskeleton.

- The cytoplasm also plays a role in creating order within the cell with specific locations for different organelles. For instance, the nucleus is usually seen towards the centre of the cell, with a centrosome nearby. The extensive endoplasmic reticulum and Golgi network are also placed in relation to the nucleus, with the vesicles radiating out towards the plasma membrane.

Cytoplasmic Streaming

- Movement within the cytoplasm also occurs in bulk, through the directed movement of cytosol around the nucleus or vacuole. This is particularly important in large single celled organisms such as some species of green algae, which can be nearly 10 cm in length. Cytoplasmic streaming is also important for positioning chloroplasts close to the plasma membrane to optimize photosynthesis and for distributing nutrients through the entire cell.

- In some cells, such as mouse oocytes, cytoplasmic streaming is expected to have a role in the formation of cellular sub-compartments and in organelle positioning as well.

Cytoplasmic Inheritance

- The cytoplasm plays hosts to two organelles that contain their own genomes – the chloroplast and mitochondria. These organelles are inherited directly from the mother through the oocyte and therefore constitute genes that are inherited outside the nucleus.

- These organelles replicate independent of the nucleus and respond to the needs of the cell. Cytoplasmic or extranuclear inheritance, therefore, forms an unbroken genetic line that has not undergone mixing or recombination with the male parent.

MITOCHONDRIA, ENDOSYMBIOSIS HYPOTHESIS REGARDING ORIGIN OF MITOCHONDRIA AND CHLOROPLAST, MATERANAL ORIGIN OF MITOCHONDRIA

MITOCHONDRIA

- Mitochondria (singular: mitochondrion) are organelles within eukaryotic cells that produce adenosine triphosphate (ATP), the main energy molecule used by the cell. For this reason, the mitochondrion is sometimes referred to as “the powerhouse of the cell”.
- Mitochondria are found in all eukaryotes, which are all living things that are not bacteria or archaea. It is thought that mitochondria arose from once free-living bacteria that were incorporated into cells.

Function of Mitochondria

- Mitochondria produce ATP through process of cellular respiration—specifically, aerobic respiration, which requires oxygen. The citric acid cycle, or Krebs cycle, takes place in the mitochondria. This cycle involves the oxidation of pyruvate, which comes from glucose, to form the molecule acetyl-CoA. Acetyl-CoA is in turn oxidized and ATP is produced.

- The citric acid cycle reduces nicotinamide adenine dinucleotide (NAD^+) to NADH. NADH is then used in the process of oxidative phosphorylation, which also takes place in the mitochondria. Electrons from NADH travel through protein complexes that are embedded in the inner membrane of the mitochondria.

- This set of proteins is called an electron transport chain. Energy from the electron transport chain is then used to transport proteins back across the membrane, which power ATP synthase to form ATP.

- The number of mitochondria in a cell depends on how much energy that cell needs to produce. Muscle cells, for example, have many mitochondria because they need to produce energy to move the body. Red blood cells, which carry oxygen to other cells, have none; they do not need to produce energy.

- Mitochondria are analogous to a furnace or a powerhouse in the cell because, like furnaces and powerhouses, mitochondria produce energy from basic components (in this case, molecules that have been broken down so that they can be used).

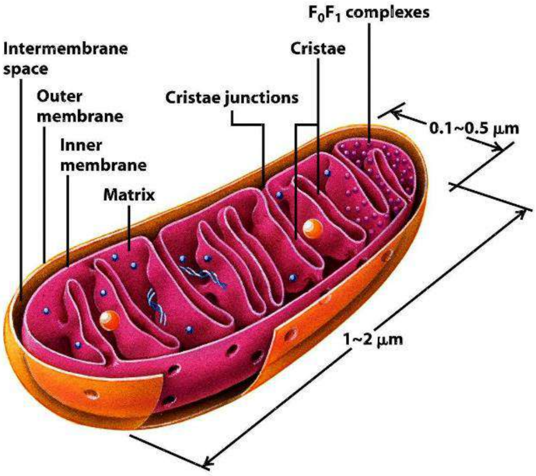
- Mitochondria have many other functions as well. They can store calcium, which maintains homeostasis of calcium levels in the cell. They also regulate the cell’s metabolism and have roles in apoptosis (controlled cell death), cell signalling, and thermogenesis (heat production).

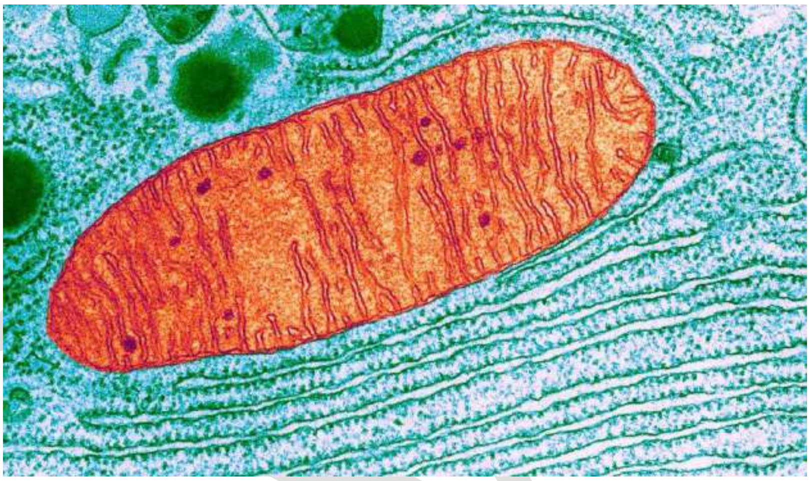
Structure of Mitochondria

- Mitochondria have two membranes, an outer membrane and an inner membrane. These membranes are made of phospholipid layers, just like the cell’s outer membrane. The outer membrane covers the surface of the mitochondrion, while the inner membrane is located within and has many folds called cristae.

- The folds increase surface area of the membrane, which is important because the inner membrane holds the proteins involved in the electron transport chain. It is also where many other chemical reactions take place to carry out the mitochondria's many functions. An increased surface area creates more space for more reactions to occur, and increases the mitochondria's output.

- The space between the outer and inner membranes is called the intermembrane space, and the space inside the inner membrane is called the matrix.





ENDOSYMBIOSIS HYPOTHESIS REGARDING ORIGIN OF MITOCHONDRIA AND CHLOROPLAST

Evolution of Mitochondria

- Mitochondria are thought to have evolved from free-living bacteria that developed into a symbiotic relationship with a prokaryotic cell, providing it energy in return for a safe place to live. It eventually became an organelle, a specialized structure within the cell, the presence of which are used to distinguish eukaryotic cells from prokaryotic cells.

- This occurred over a long process of millions of years, and now the mitochondria inside the cell cannot live separately from it. The idea that mitochondria evolved this way is called endosymbiotic theory.

- Endosymbiotic theory has multiple forms of evidence. For example, mitochondria have their own DNA that is separate from the DNA in the cell's nucleus. It is called mitochondrial DNA or mtDNA, and it is only passed down through females because sperm do not have mitochondria.

- You received your mtDNA from your mother, and you can only pass it on if you are a female who has a child. It is also circular, like bacterial DNA. Another form of evidence is the way new mitochondria are created in the cell. New mitochondria only arise from binary fission, or splitting, which is the same way that bacteria asexually reproduce.

- If all of the mitochondria are removed from a cell, it can't make new ones because there are no existing mitochondria there to split. Also, the genome of mitochondria and *Rickettsia* bacteria (bacteria that can cause spotted fever and typhus) have been compared, and the sequence is so similar that it suggests that mitochondria are closely related to *Rickettsia*.

- Chloroplasts, the organelles in plants where photosynthesis occurs, are also thought to have evolved from endosymbiotic bacteria for similar reasons: they have separate, circular DNA, a double membrane structure, and split through binary fission.

Evolution of Chloroplasts

- Chloroplasts are thought to have become a part of certain eukaryotic cells in much the same way as mitochondria were incorporated into all eukaryotic cells: by existing as free-living cyanobacteria that had a symbiotic relationship with a cell, making energy for the cell in return for a safe place to live, and eventually evolving into a form that could no longer exist separately from the cell. This is called the **endosymbiotic theory.**

- The evidence that chloroplasts evolved from bacteria is very similar to the evidence that mitochondria evolved from bacteria. Chloroplasts have their own, separate DNA that is circular, like that of a bacterial cell, and inherited maternally (only from the mother plant alga).

- New chloroplasts are formed through binary fission, or splitting, which is how bacteria reproduce. These forms of evidence are also found in mitochondria. The one difference is that chloroplasts are believed to have evolved from cyanobacteria, while mitochondria evolved from aerobic bacteria. (Mitochondria cannot photosynthesize; the process of cellular respiration occurs there instead.)

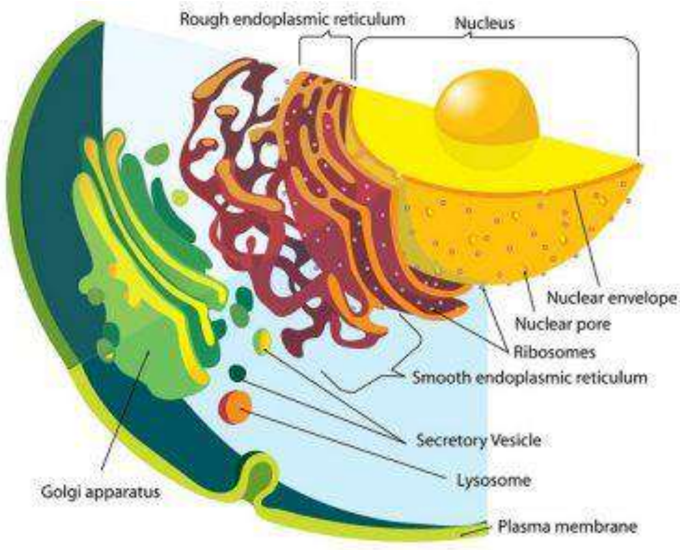
- The structure of chloroplasts is similar to that of cyanobacteria; both have double membranes, circular DNA, ribosomes, and thylakoids. Most chloroplasts are believed to have come from one common ancestor that engulfed a cyanobacteria between 600-1600 million years ago.

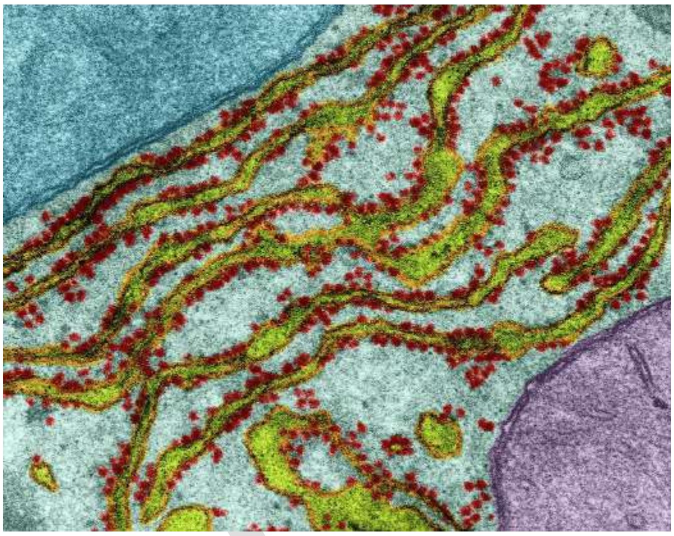
ENDOPLASMIC RETICULUM

- Endoplasmic Reticulum Definition**:** The endoplasmic reticulum (ER) is a large organelle made of membranous sheets and tubules that begin near the nucleus and extend across the cell. The endoplasmic reticulum creates, packages, and secretes many of the products created by a cell. Ribosomes, which create proteins, line a portion of the endoplasmic reticulum.

Endoplasmic Reticulum Overview

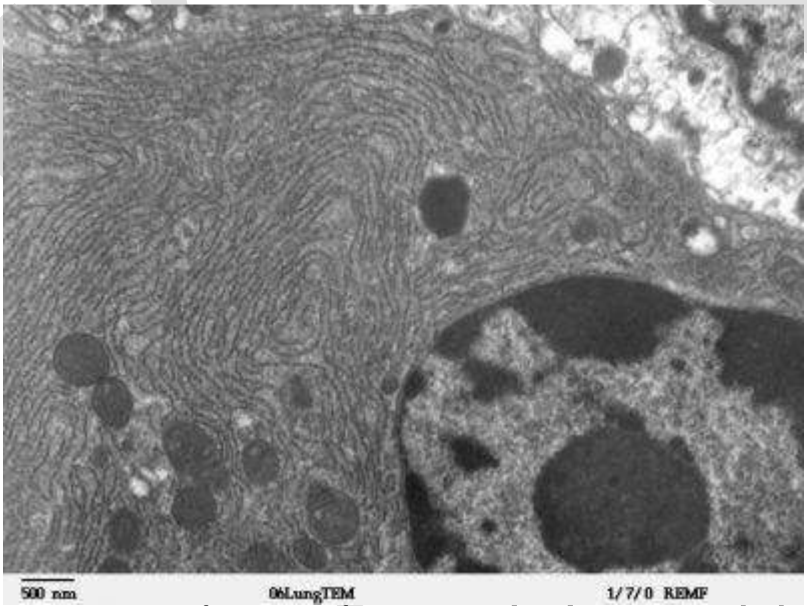
- The entire structure can account for a large proportion of the endomembrane system of the cell. **For instance, in cells such as liver hepatocytes that are specialized for protein secretion and detoxification, the ER can account for more than 50% of the total lipid bilayer of the cell.** Similarly, the ER membrane system is particularly prominent in pancreatic beta cells that secrete insulin, or within activated B-lymphocytes that produce antibodies.





- As seen in the image, the membranes of the endoplasmic reticulum are contiguous with the outer nuclear membrane, even though their compositions can be different.

- The ER contains special membrane-embedded proteins that stabilize its structure and curvature. **This organelle acts as an important regulator of cell function because it interacts closely with a number of other organelles.** Products of the endoplasmic reticulum often travel to the Golgi body for packaging and additional processing before being secreted.



- This is a microscopic image of a section from mammalian lung tissue. The bottom right corner of the image shows the nucleus and the rest of the picture illustrates the extensive nature of the ER. Small dark circles are mitochondria that exist in physical proximity with the membranes of the ER.

Endoplasmic Reticulum Function

- The ER plays a number of roles within the cell, from protein synthesis and lipid metabolism to detoxification of the cell. **Cisternae, each of the small folds of the endoplasmic reticulum, are**

commonly associated with lipid metabolism. This creates the plasma membrane of the cell, as well as additional endoplasmic reticulum and organelles.

- They also appear to be important in maintaining the Ca^{2+} balance within the cell and in the interaction of the ER with mitochondria. This interaction also influences the aerobic status of the cell.

- ER sheets appear to be crucial in the response of the organelle to stress, especially since cells alter their tubules-to-sheets ratio when the number of unfolded proteins increases.

- Occasionally, apoptosis is induced by the ER in response to an excess of unfolded protein within the cell. When ribosomes detach from ER sheets, these structures can disperse and form tubular cisternae.

- Although ER sheets and tubules appear to have distinct functions, there isn't a perfect delineation of roles. **For instance, in mammal tubules and sheets can interconvert, making the cells adaptable to various conditions.** The relationship between structure and function in the ER has not been completely elucidated.

Protein Synthesis and Folding

- Protein synthesis occurs in the rough endoplasmic reticulum. **Although translation for all proteins begins in the cytoplasm, some are moved into the ER in order to be folded and sorted for different destinations.** Proteins that are translocated into the ER during translation are often destined for secretion.

- Initially, these proteins are folded within the ER and then moved into the Golgi apparatus where they can be dispatched towards other organelles.

- For instance, the hydrolytic enzymes in the lysosome are generated in this manner. Alternately, these proteins could be secreted from the cell. This is the origin of the enzymes of the digestive tract.

- The third potential role for proteins translated in the ER is to remain within the endomembrane system itself. This is particularly true for chaperone proteins that assist in the folding of other proteins. The genes encoding these proteins are upregulated when the cell is under stress from unfolded proteins.

Lipid Synthesis

- The smooth endoplasmic reticulum plays an important role in cholesterol and phospholipid biosynthesis. **Therefore, this section of the ER is important not only for the generation and maintenance of the plasma membrane but of the extensive endomembrane system of the ER itself.**

- The SER is enriched in enzymes involved in sterol and steroid biosynthetic pathways and is also necessary for the synthesis of steroid hormones. Therefore, the SER is extremely prominent in the cells of the adrenal gland that secrete five different groups of steroid hormones that influence the metabolism of the entire body.

- The synthesis of these hormones also involves enzymes within the mitochondria, further underscoring the relationship between these two organelles.

Calcium Store

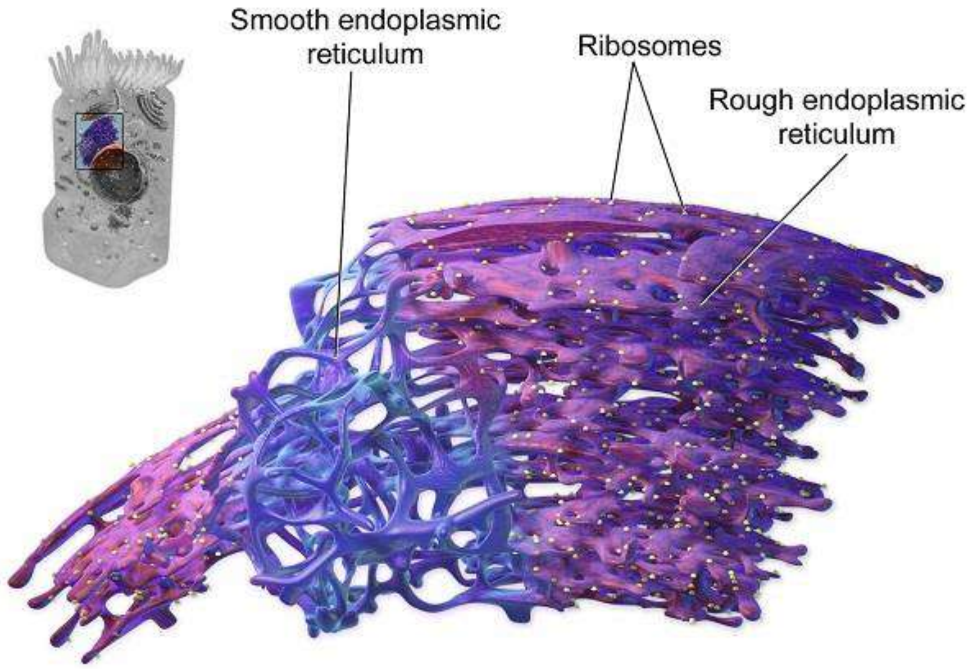
- **The SER is an important site for the storage and release of calcium in the cell.** A modified form of the SER called sarcoplasmic reticulum forms an extensive network in contractile cells such as muscle fibres. Calcium ions are also involved in the regulation of metabolism in the cell and can change cytoskeletal dynamics.

- The extensive nature of the ER network allows it to interact with the plasma membrane and use Ca^{2+} for signal transduction and modulation of nuclear activity. In association with mitochondria, the ER can also use its calcium stores to induce apoptosis in response to stress.

Structure of the Endoplasmic Reticulum

- The endoplasmic reticulum membrane system can be morphologically divided into two structures–cisternae and sheets. **Cisternae are tubular in structure and form a three-dimensional polygonal network.** They are about 50 nm in diameter in mammals and 30 nm in diameter in yeast. ER sheets, on the other hand, are membrane-enclosed, two-dimensional flattened sacs that extend across the cytoplasm.

- They are frequently associated with ribosomes and special proteins called translocons that are necessary for protein translation within the RER.



Endoplasmic Reticulum Structure

- The high-curvature of ER tubules is stabilized by the presence of proteins called reticulons and DP1/Yop1p. Reticulons are membrane-associated proteins encoded by four genes in mammals (RTN1-4).

- These proteins localize to ER tubules and the curved edges of ER sheets. DP1/Yop1p are a class of integral membrane proteins involved in stabilizing the structure of ER cisternae.

- Both reticulons and DP1/Yop1 proteins form oligomers and interact with the cytoskeleton. **Oligomerization seems to be one of the mechanisms used by these proteins to shape the lipid bilayer into a tubule.** Additionally, they also appear to use a wedge-like structural motif that causes the membrane to curve.

- These two classes of proteins are redundant, since the overexpression of one protein can compensate for the lack of the other protein.

- The construction of the ER is intimately involved with the presence of cytoskeletal elements, especially microtubules. ER membranes, especially cisternae, move and branch along microtubules. When microtubule structure is temporarily disrupted, the ER network collapses and reforms only after the microtubule cytoskeleton is re-established.

- In addition, changes to the pattern of microtubule polymerization are reflected in changes to ER morphology.

Endoplasmic Reticulum Location

- The endoplasmic reticulum processes most of the instructions from the nucleus. As such, the endoplasmic reticulum surrounds the nucleus and radiates outward. In cells that secrete many products for the rest of the body, the endoplasmic reticulum can account for more than 50% of the cell.

- **In general, the nucleus expresses mRNA (messenger RNA), which tells the cell how to build proteins.** The rough endoplasmic reticulum has many ribosomes, which are the primary location of protein production. This portion of the organelle creates proteins and begins to fold them into the proper formation.

- The smooth endoplasmic reticulum is the primary location for lipid synthesis. As such, it does not contain any ribosomes. Rather, it conducts a series of reactions which create the phospholipid molecules necessary to create various membranes and organelles.

- The rough version of the endoplasmic reticulum is often closer to the nucleus, whereas the smooth endoplasmic reticulum is further from the nucleus. However, both versions are connected to each other and the nucleus through a series of small tubules.

Types of Endoplasmic Reticulum

- There are two major types of ER within each cell – smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER). **Each has distinct functions, and often, differing morphology.** The SER is involved in lipid metabolism and acts as the calcium store for the cell.
- This is particularly important in muscle cells that need Ca^{2+} ions for contraction. The SER is also involved in the synthesis of phospholipids and cholesterol. It is often located near the periphery of the cell.
- On the other hand, the RER is commonly seen close to the nucleus. It contains membrane-bound ribosomes that give it the characteristic ‘rough’ appearance. These ribosomes are creating proteins that are destined for the lumen of the ER and are moved into the organelle as they are being translated.
- These proteins contain a short signal created by a few amino acids in their N-terminus and are initially translated in the cytoplasm.
- However, as soon as the signal is translated, special proteins bind to the growing polypeptide chain and move the entire ribosome and associated translation machinery to the ER.
- These polypeptides could be resident proteins of the RER, or be moved towards the Golgi network to be sorted and secreted.

GOLGI COMPLEX

- Golgi Apparatus Definition**:** The Golgi apparatus is an organelle in eukaryotic organisms that moves molecules from the endoplasmic reticulum to their destination. The organelle also modifies products of the endoplasmic reticulum to their final form. The Golgi apparatus is comprised of a series of flattened sacs that extend from the endoplasmic reticulum.

Golgi Apparatus Overview

- The main function of the Golgi apparatus is the ability to deliver vesicles, or packets of various cell products, to different locations throughout the cell. **The Golgi also has important functions in tagging vesicles with proteins and sugar molecules, which serve as identifiers for the vesicles so they can be delivered to the proper target.** The organelle is also called the Golgi complex or Golgi body.
- Typically, proteins and cellular products are manufactured in the endoplasmic reticulum. The rough endoplasmic reticulum has a number of ribosomes, which assemble proteins from instructions contained in messenger RNA. Throughout the rest of the endoplasmic reticulum, these protein products are folded and modified.
- As they reach the Golgi apparatus, more modifications are made. Finally, the products are packaged within vesicles which are “labeled” by other proteins and molecules. **The vesicles are**

released and based on their tags or labels they are carried to the appropriate location within the cell by the cytoskeleton.

Golgi Apparatus Functions

- The Golgi apparatus has many discrete functions. But, all functions are associated with moving molecules from the endoplasmic reticulum to their final destination and modifying certain products along the way. **The multiple sacs of the Golgi serve as different chambers for chemical reactions.** As the products of the endoplasmic reticulum move through the Golgi apparatus, they are continuously transferred into new environments, and the reactions that can take place are different.

- In this way, a product can be given modifications, or multiple products can be combined to form large macromolecules. The many sacs and folds of the Golgi apparatus allow for many reactions to take place at the same time, increasing the speed at which an organism can produce products.

Tagging Cellular Products

- Regardless of the product, the vesicles containing the product move from the endoplasmic reticulum and into the *cis* face of the Golgi apparatus. In layman's terms, this is the side facing the endoplasmic reticulum.

- The side furthest from the endoplasmic reticulum is known as the *trans* face of the Golgi apparatus, and this is where products are headed.

- After having any modifications or additions to their structure, the products are packaged in vesicles and tagged with markers that indicate where the vesicle needs to end up. **These tags can be molecules, such as phosphate groups, or special proteins on the surface of the vesicle.** Once tagged, the vesicle is excreted from the Golgi apparatus, on its way to its final destination.

Finalizing Cellular Products

- There are many products that are produced by eukaryotes, from proteins that can carry out chemical reactions to lipid molecules that can build new cell membranes. Some products are meant for the endoplasmic reticulum or the Golgi apparatus itself and travel in the opposite direction of most vesicles. **While the endoplasmic reticulum produces most of the products and bases used, it is the Golgi apparatus that is responsible for the final presentation and assembly of products.** Often, the environment must be slightly different from that present in the endoplasmic reticulum to obtain certain end products. The many sacs of the Golgi apparatus function to provide many different areas in which reactions can take place in the most favorable of conditions.

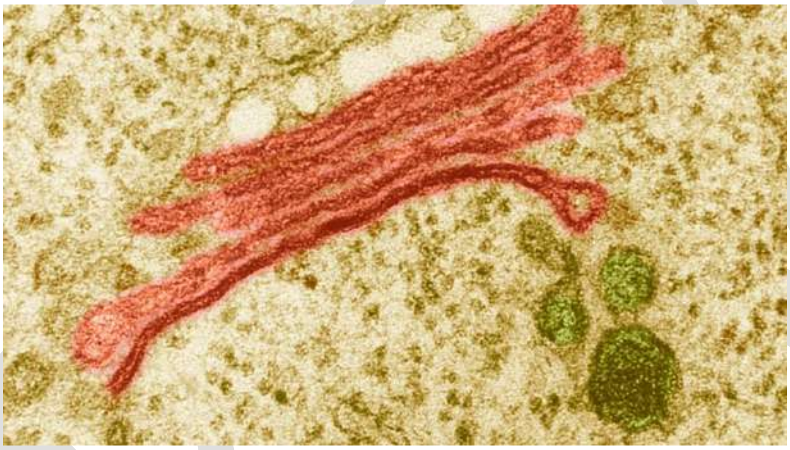
- In secretory cells, or cells which produce large amounts of a substance that your body needs, the Golgi apparatus will be very large. Consider the cells in your stomach that secrete acid.

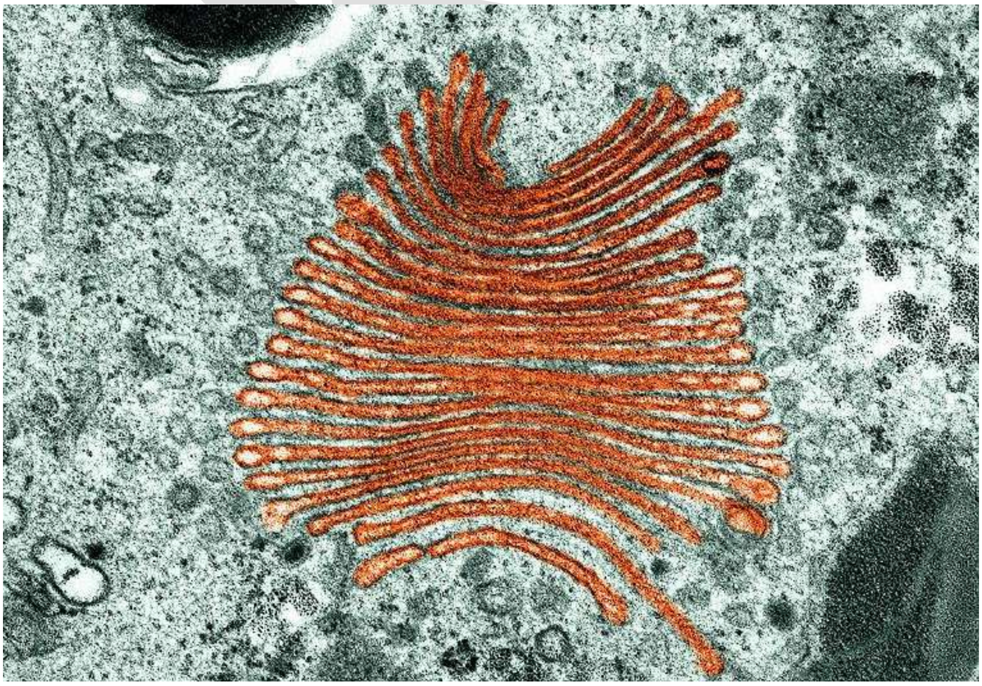
- The acid is produced by reactions in the endoplasmic reticulum and is modified as is goes through the Golgi apparatus. Once to the *trans* side of the Golgi apparatus, the acid is packaged in a vesicle and sent towards the cell's surface.

- As the vesicle joins with the plasma membrane, the acid is released into the stomach, so it can digest your food.

Golgi Apparatus Structure

- The image below shows the structure of the Golgi apparatus. The ***cis face*** of the organelle is closest to the endoplasmic reticulum. The ***trans face*** is the side furthest from the nucleus, which secretes vesicles to various parts of the cell. Further, **there are a number of lumens and cisternae** through which products flow. These appear as a series of flattened sacs stack on each other, much like the endoplasmic reticulum.





Golgi Apparatus Location

- The Golgi apparatus is situated in between the endoplasmic reticulum and the cell membrane. Most often, the Golgi appears to be an extension of the endoplasmic reticulum which is slightly smaller and smoother in appearance.

- However, the Golgi apparatus can be easily mistaken for smooth endoplasmic reticulum. Although they look similar, the Golgi is an independent organelle which has different functions.

Theory of Golgi Apparatus Function

- The most prevalent theory of how the Golgi apparatus forms is the *cisternal maturation model*. **This model suggests that the sacs themselves tend to move from the *cis* face to the *trans* face of the Golgi apparatus over time.** New sacs are formed closest to the endoplasmic reticulum. These sacs "age" as they move towards the *trans* face of the Golgi apparatus and their product becomes fully mature.

Specific Products

- It may seem like there could never be enough lipids to produce the continual flow of cell membrane needed to continually make transport vesicles between the endoplasmic reticulum and the Golgi apparatus.

- However, there are constantly segments of cell membrane being produced and recycled by the endoplasmic reticulum, Golgi apparatus, lysosomes, and other organelles in the cell, as well as the outer cell membrane itself. **The Golgi apparatus and endoplasmic reticulum work together to produce new cell membrane, as well as recycle the cell membranes of vesicles by merging two membranes when vesicles are absorbed.**

- The Golgi also creates lysosomes. These sacs contain digestive materials. The sacs are pinched off from the Golgi apparatus, and they are used to process materials which have been phagocytized or to digest organelles which no longer function. The lysosome delivers raw ingredients to the endoplasmic reticulum.

Golgi Apparatus in Plant Cells

- While this article primarily discusses the operation of the Golgi apparatus within animal cells, plant cells also have a Golgi apparatus. In fact, plant cells may contain hundreds of these organelles.

- **Within plant cells, the Golgi apparatus serves the additional function of synthesizing the major polysaccharide molecules which help form the *cell wall*.** To do this, plants often have many more Golgi bodies than an animal cell.

- Further, plant cells do not contain lysosomes. These digestive organelles are replaced in the plant with the central vacuole, which serves as a large lysosome as well as an organelle to store water.

Thus, many vesicles from the Golgi bodies of plants move to the vacuole and fuse their contents with this large organelle.

LYSOZOMES

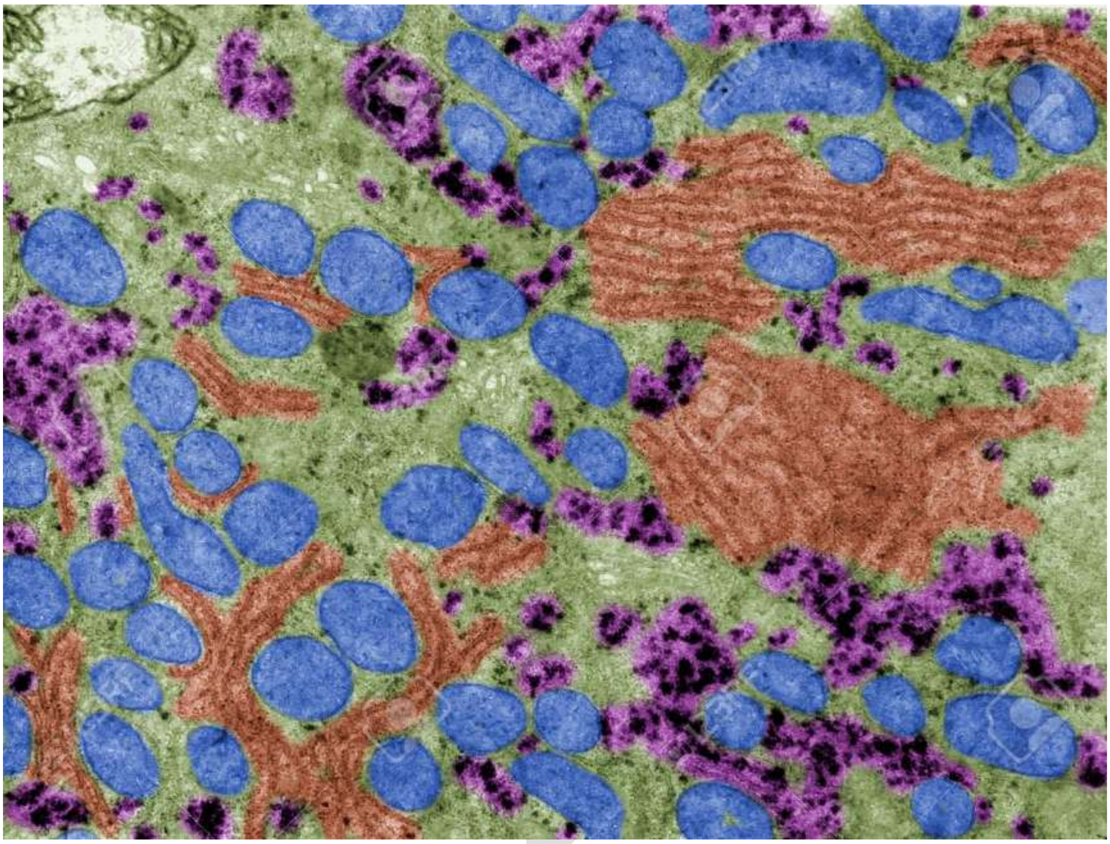
- A **lysosome** is a membrane-bound organelle found in many animal cells.
- They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules. A lysosome has a specific composition, of both its membrane proteins, and its lumenal proteins.
- The lumen's pH (~4.5–5.0) is optimal for the enzymes involved in hydrolysis, analogous to the activity of the stomach.
- Besides degradation of polymers, the lysosome is involved in various cell processes, including secretion, plasma membrane repair, apoptosis, cell signaling, and energy metabolism.[3]
- Lysosomes digest materials taken into the cell and recycle intracellular materials. Step one shows material entering a food vacuole through the plasma membrane, a process known as endocytosis.
- In step two a lysosome with an active hydrolytic enzyme comes into the picture as the food vacuole moves away from the plasma membrane. Step three consists of the lysosome fusing with the food vacuole and hydrolytic enzymes entering the food vacuole. In the final step, step four, hydrolytic enzymes digest the food particles.
- Lysosomes act as the waste disposal system of the cell by digesting in used materials in the cytoplasm, from both inside and outside the cell. Material from outside the cell is taken up through endocytosis, while material from the inside of the cell is digested through autophagy.[5] The sizes of the organelles vary greatly—the larger ones can be more than 10 times the size of the smaller ones.
- They were discovered and named by Belgian biologist Christian de Duve, who eventually received the Nobel Prize in Physiology or Medicine in 1974.
- Lysosomes are known to contain more than 60 different enzymes, and have more than 50 membrane proteins.
- Enzymes of the lysosomes are synthesised in the rough endoplasmic reticulum and exported to the Golgi apparatus upon recruitment by a complex composed of CLN6 and CLN8 proteins.
- The enzymes are trafficked from the Golgi apparatus to lysosomes in small vesicles, which fuse with larger acidic vesicles. Enzymes destined for a lysosome are specifically tagged with the molecule mannose 6-phosphate, so that they are properly sorted into acidified vesicles.

FUNCTION AND STRUCTURE

- Lysosomes contain a variety of enzymes, enabling the cell to break down various biomolecules it engulfs, including peptides, nucleic acids, carbohydrates, and lipids (lysosomal lipase). The enzymes responsible for this hydrolysis require an acidic environment for optimal activity.
- In addition to being able to break down polymers, lysosomes are capable of fusing with other organelles & digesting large structures or cellular debris; through cooperation with phagosomes, they are able to conduct autophagy, clearing out damaged structures.
- Similarly, they are able to break down virus particles or bacteria in phagocytosis of macrophages.
- The size of lysosomes varies from 0.1 µm to 1.2 µm.[24] With a pH ranging from ~4.5–5.0, the interior of the lysosomes is acidic compared to the slightly basic cytosol (pH 7.2). The lysosomal

membrane protects the cytosol, and therefore the rest of the cell, from the degradative enzymes within the lysosome.

- The cell is additionally protected from any lysosomal acid hydrolases that drain into the cytosol, as these enzymes are pH-sensitive and do not function well or at all in the alkaline environment of the cytosol. This ensures that cytosolic molecules and organelles are not destroyed in case there is leakage of the hydrolytic enzymes from the lysosome.
- The lysosome maintains its pH differential by pumping in protons (H^+ ions) from the cytosol across the membrane via proton pumps and chloride ion channels. Vacuolar-ATPases are responsible for transport of protons, while the counter transport of chloride ions is performed by ClC-7 Cl^-/H^+ antiporter. In this way a steady acidic environment is maintained.
- It sources its versatile capacity for degradation by import of enzymes with specificity for different substrates; cathepsins are the major class of hydrolytic enzymes, while lysosomal alpha-glucosidase is responsible for carbohydrates, and lysosomal acid phosphatase is necessary to release phosphate groups of phospholipids.



MICROBODIES

- Microbodies are different type of bodies present in the cytosol, also known as cytostomes. A microbody is usually a vesicle with a spherical shape, ranging from 0.2-1.5 micrometre in diameter.

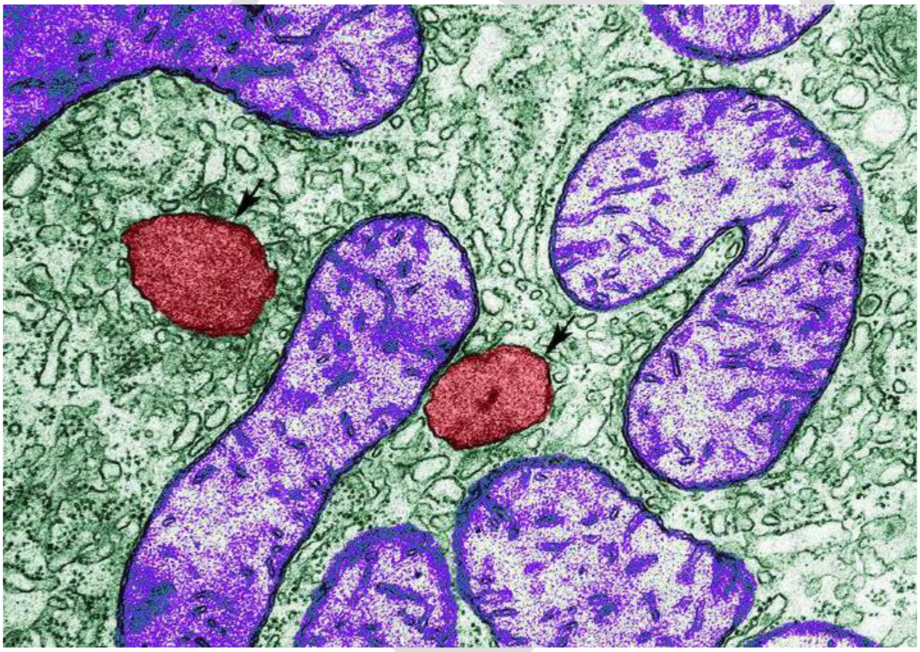
- Microbodies are found in the cytoplasm of a cell, but they are only visible with the use of an electron microscope. They are surrounded by a single phospholipid bilayer membrane and they contain a matrix of intracellular material including enzymes and other proteins, but they do not seem to contain any genetic material to allow them to self-replicate.

Function

- Microbodies contain enzymes that participate in the preparatory or intermediate stages of biochemical reactions within the cell. This facilitates the breakdown of fats, alcohols and amino acids.
- Generally, microbodies are involved in detoxification of peroxides and in photo respiration in plants. Different types of microbodies have different functions:

PEROXISOMES

- A **peroxisome** is a type of microbody that functions to help the body break down large molecules and detoxify hazardous substances. It contains enzymes like oxidase, react hydrogen peroxide as a by-product of its enzymatic reactions.
- Within the peroxisome, hydrogen peroxide can then be converted to water by enzymes like catalase and peroxidase. Discovered and named by C.de Duve.



GLYOXYSOMES

- **Glyoxysomes** are specialized peroxisomes found in plants and mold, which help to convert stored lipids into carbohydrates so they can be used for plant growth. In glyoxysomes the fatty acids are hydrolysed to acetyl-CoA by peroxisomal β-oxidation enzymes.
- Besides peroxisomal functions, glyoxysomes also possess the key enzymes of the Glyoxylate cycle.

HOMOGENIZATION METHOD AND ISOLATION OF CELL ORGANELLES BY DIFFERENTIAL CENTRIFUGATION AND MARKER ENZYMES

Cells/Tissue Homogenization:

- A ubiquitous first step in the isolation of subcellular organelles is to homogenize the cells. This process involves breaking open the cell membrane (and the cell wall if present). Commonly used methods of homogenization include
  - Dounce homogenization, where the cells are crushed between two revolving solid surfaces;
  - Filtration, where cells are forced through smaller pores in a filter;
  - Grinding, where cells are ground by swirling with glass beads;
  - Sonication, where cells are bombarded with ultrasonic vibrations; and
  - Solubilization, in which cell membranes are dissolved in detergents such as Triton X-100. Enzyme digestion is also used to remove cell-wall Organelle Isolation 131 constituents.
- The method of choice depends on the type of tissue to be homogenized and the specific purpose of the experiment. Usually during the homogenization process isotonic sucrose is added to the homogenization buffer to prevent osmotic rupture of organelle membranes since it is usually important to purify intact organelles.
- Following homogenization, the homogenate is normally spun at low speed to remove any intact cells along with other large cellular debris. When this is done, the next step is subcellular fractionation.

Differential Centrifugation

- Differential centrifugation is one of the widely-used techniques to separate cellular organelles. A slight modification of this technic known as rate zonal centrifugation is also used frequently in which organelles, after a single spin, band in a tube according to their sedimentation rate.
- Size, density, and shape influence the movement of a subcellular particle in a centrifugal field. This movement (sedimentation) results from the interaction between a particle's weight and the resistance it encounters in moving through a suspension medium and the relative centrifugal force exerted on the particle.
- Under a given centrifugal force, particles that are relatively large or dense will sediment more rapidly than particles that are smaller and lighter. With respect to the major components found in cells, the order of sedimentation is typically (from most to least dense): nuclei, mitochondria,

lysosomes, plasma membrane, endoplasmic reticulum, and contractile vacuoles. Depending on the specific cell type, however, this order can vary.

- Additionally, differences in the rate of sedimentation are sometimes not large enough to provide separation of one organelle from another.

Marker Enzymes

- Isolation of any organelle requires a reliable test for the presence of the organelle. Typically, this is done by following the activity of an enzyme that is known to be localized exclusively in the target organelle. Such enzymes are known as marker enzymes.
- For example, the enzyme acid phosphatase (that cleaves terminal phosphate group from substrates and has a pH optimum in the acidic range) is localized in lysosomes, while the enzyme succinate dehydrogenase is localized in mitochondria.
- By monitoring where each enzyme activity is found during a cell fractionation protocol, one can monitor the fractionation of lysosomes and mitochondria, respectively. Marker enzymes also provide information on the biochemical purity of the fractionated organelles.
- The presence of unwanted marker enzyme activity in the preparation indicates the level of contamination by other organelles, while the degree of enrichment for the desired organelle is determined by the specific activity of the target marker enzyme. Although marker enzymes reveal much concerning the purity of the organelle preparation, electron microscopy is generally used as a final step to assess the preparation's purity and the morphology of the isolated organelle.