Unit VI

Cell Biology, Molecular Biology and Bioinformatics

Module 1. Cell biology

Cell and Cell Theory

The microscopes we use today are far more complex than those used in the 1600s by Antony van Leeuwenhoek, a Dutch shopkeeper who had great skill in crafting lenses. Despite the limitations of his now-ancient lenses, van Leeuwenhoek observed the movements of protista (a type of single-celled organism) and sperm, which he collectively termed "animalcules. "

In a 1665 publication called Micrographia, experimental scientist Robert Hooke coined the term "cell" for the box-like structures he observed when viewing cork tissue through a lens. In the 1670s, van Leeuwenhoek discovered bacteria and protozoa. Later advances in lenses, microscope construction, and staining techniques enabled other scientists to see some components inside cells.

By the late 1830s, botanist Matthias Schleiden and zoologist Theodor Schwann were studying tissues and proposed the unified cell theory. The unified cell theory states that: all living things are composed of one or more cells; the cell is the basic unit of life; and new cells arise from existing cells. Rudolf Virchow later made important contributions to this theory.

Schleiden and Schwann proposed spontaneous generation as the method for cell origination, but spontaneous generation (also called abiogenesis) was later disproven. Rudolf Virchow famously stated "Omnis cellula e cellula"... "All cells only arise from pre-existing cells. "The parts of the theory that did not have to do with the origin of cells, however, held up to

scientific scrutiny and are widely agreed upon by the scientific community today.

The generally accepted portions of the modern Cell Theory are as follows:

- The cell is the fundamental unit of structure and function in living things.
- All organisms are made up of one or more cells.
- Cells arise from other cells through cellular division.

The expanded version of the cell theory can also include:

- Cells carry genetic material passed to daughter cells during cellular division
- All cells are essentially the same in chemical composition
- Energy flow (metabolism and biochemistry) occurs within cells

Key Points

- The cell theory describes the basic properties of all cells.
- The three scientists that contributed to the development of cell theory are Matthias Schleiden, Theodor Schwann, and Rudolf Virchow.
- A component of the cell theory is that all living things are composed of one or more cells.
- A component of the cell theory is that the cell is the basic unit of life.
- A component of the cell theory is that all new cells arise from existing cells.

Cell membrane- Structure and function

The cell membrane (plasma membrane) is a thin semi-permeable membrane that surrounds the cytoplasm of a cell. Its function is to protect the integrity of the interior of the cell by allowing certain substances into the cell while keeping other substances out. It also serves as a base of attachment for the cytoskeleton in some organisms and the cell wall in others. Thus the cell membrane also serves to help support the cell and help maintain its shape.

Another function of the membrane is to regulate cell growth through the balance of endocytosis and exocytosis. In endocytosis, lipids and proteins are removed from the cell membrane as substances are internalized. In exocytosis, vesicles containing lipids and proteins fuse with the cell membrane increasing cell size. Animal cells, plant cells, prokaryotic cells, and fungal cells have plasma membranes. Internal organelles are also encased by membranes.

1. Cell Membrane Structure

The cell membrane is primarily composed of a mix of proteins and lipids. Depending on the membrane's location and role in the body, lipids can make up anywhere from 20 to 80 percent of the membrane, with the remainder being proteins. While lipids help to give membranes their flexibility, proteins monitor and maintain the cell's chemical climate and assist in the transfer of molecules across the membrane.

Dry Weight
40% lipid – E.g. phospholipid molecules and cholesterol.
60% protein – E.g. channel proteins and carrier proteins.
1-10% carbohydrate – Often found attached to proteins/lipids on the outside of the cell membrane – a coat of carbohydrate surrounding a cell is often called the glycocalyx.

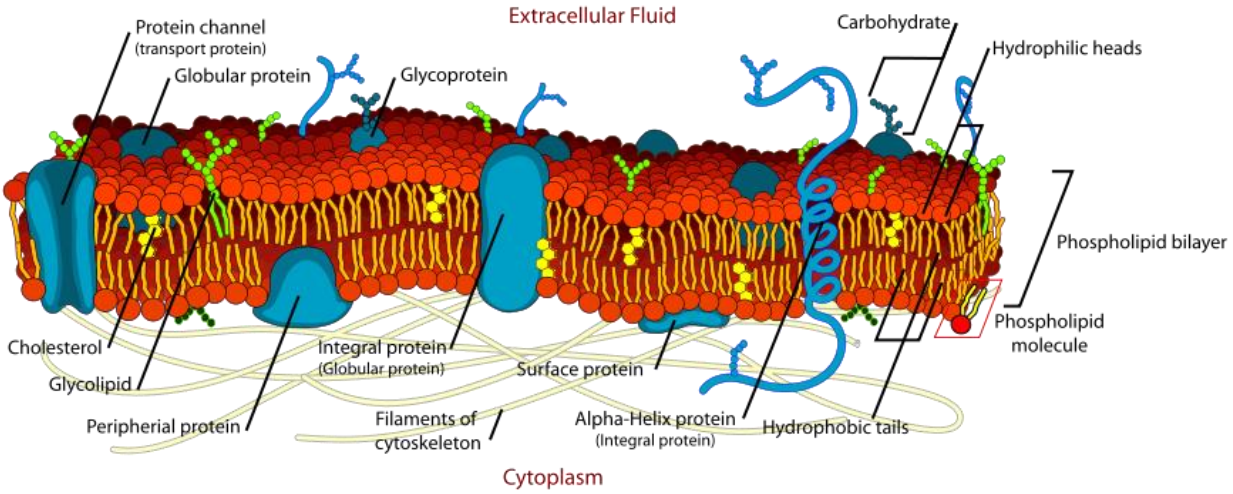


Fig: Structure of Cell membrane

Phospholipids

The membrane bilayer contains many kinds of phospholipid molecules, with different sized head and tail molecules.

These consist of a head molecule, a phosphate molecule, a glycerol and two fatty acid chains.

- **Head group-** This is a polar group e.g. a sugar or choline – meaning that the head end of the phospholipid is hydrophilic.
- **Tail of 2 fatty acid chains** – normally consisting of between 14-24 carbons (but the most common carbon lengths are 16 and 18). If the chain contains a cis double bond then the chain is kinked – therefore reducing the tight packing of the membrane and so increasing its movement. As the tail is made of fatty acids, it does not form hydrogen bonds with water and therefore is hydrophobic and non-polar.

Phospholipid molecules are therefore amphipathic – being both hydrophilic and hydrophobic. They spontaneously form bilayers in the water with the head groups facing out and the tail groups facing in.

In the bilayer, there are van der Waal forces between the fatty acid tails of the phospholipid, with electrostatic and hydrogen bonds between the hydrophilic groups and water.

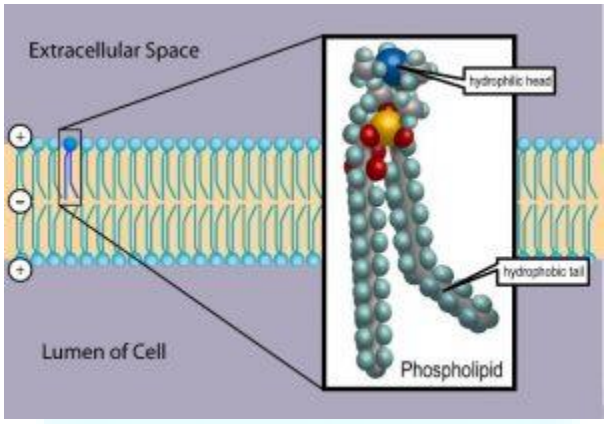


Fig: Structure of Phospholipid

Cholesterol

- Cholesterol is vital for many functions in a cell, including very importantly, a major constituent of the cell membrane.
- Cholesterol itself consists of a polar head, a **planar steroid ring** and a non-polar hydrocarbon tail.
- Cholesterol is important in the membrane as it helps to maintain cell membrane stability and fluidity at varying temperatures.
- Cholesterol is bound to neighbouring phospholipid molecules via hydrogen bonds and therefore at low temperatures, reduces their packing.
- Overall this means at low temperatures, when rate of movement is lowest, a fluid phase is maintained.
- At high temperatures, cholesterol helps to stop the formation of crystalline structures and the rigid planar steroid ring prevents intrachain vibration and therefore making the membrane less fluid.

Cell Membrane Proteins

- The cell membrane contains two types of associated proteins.
- **Peripheral membrane proteins** are exterior to and connected to the membrane by interactions with other proteins.
- **Integral membrane proteins** are inserted into the membrane and most pass through the membrane.
- Portions of these transmembrane proteins are exposed on both sides of the membrane. Cell membrane proteins have a number of different functions.
- **Structural proteins** help to give the cell support and shape.
- Cell membrane **receptor proteins** help cells communicate with their external environment through the use of hormones, neurotransmitters, and other signaling molecules.
- **Transport proteins**, such as globular proteins, transport molecules across cell membranes through facilitated diffusion.
- **Glycoproteins** have a carbohydrate chain attached to them.
- They are embedded in the cell membrane and help in cell to cell communications and molecule transport across the membrane.

Functions of the Cell Membrane

Cell membranes are vital for the normal functioning of all the cells in our bodies. Their main functions consist of:

- Forming a continuous, highly selectively permeable barrier – both around cells and intracellular compartments.
- Allowing the control of an enclosed chemical environment – important to maintain ion gradients.
- Communication – both with the extracellular and extra-organelle space.
- Recognition – including recognition of signaling molecules, adhesion proteins and other host cells (very important in the immune system).

- Signal generation – in response to a stimulus creating a change in membrane potential.

In a cell, different parts of the membrane have different functions and therefore their structure is specialised for this. An example of this specialisation can be seen in the different parts of a nerve; the cell membrane in the axon is specialised for electrical conduction whereas the end of the nerve is specialised for synapsing, meaning the composition of the membrane is different.

Organization based on fluid mosaic model

The fluid mosaic model was proposed by S.J. Singer and Garth L. Nicolson. This model explains the structure of the plasma membrane of animal cells as a mosaic of components such as phospholipids, proteins, cholesterol, and carbohydrates. These components give a fluid character to the membranes.

Each phospholipid has a hydrophilic head pointing outside and a hydrophobic tail forming the inside of the bilayer.

Cholesterol and proteins are embedded in the bilayer that gives the membrane a mosaic look. Each component has a specific function to perform.

Components of Plasma membrane

- **Phospholipids**
    - Phospholipids are amphipathic molecules with a hydrophilic head and a hydrophobic tail. These are attached to a glycerol molecule by a covalent bond.

- **Cholesterol**

- It helps the plasma membrane to retain the fluidity. It is present between the phospholipids and prevents the compaction of hydrophilic tails at low temperatures and their expansion at high temperatures.

- **Proteins**

  The plasma membrane has three types of proteins:

  - **Integral Proteins:** These proteins form channels to allow the movement of large molecules and ions across the hydrophobic layer of the membrane.
  - **Peripheral Proteins:** These are found embedded in a single leaflet of the membrane. They carry signals from one segment of the membrane and relay it to the another.
  - **Glycoproteins:** They stabilize the membrane and are responsible for intercellular communication.

Factors Affecting Fluidity of Plasma Membrane

The fluidity of the cell membrane is influenced by three factors:

- **Temperature**
  - Phospholipids are found close together when it is cold. When it's hot, they move apart.
- **Cholesterol**
  - The cholesterol molecules are randomly distributed along the phospholipid bilayer and hold it preventing it from separating too far, or compact too tightly.

- **Saturated and Unsaturated Fatty Acids**
  - Fatty acids make up the phospholipid tails. Saturated fatty acid chains have a single bond between the carbon atoms whereas, unsaturated fatty acid chains have double bonds between the carbon atoms.

- Double bonds make it harder for the chain to pack tightly by creating kinks. These kinks increase the fluidity of the membrane.

Restriction to Fluidity of Plasma Membrane

- **Lipid Rafts**
  - These are the lipid domains found on the external leaflet of the plasma membrane. Cholesterol, glycosphingolipids, glycosylphosphatidylinositol are the building blocks of lipid rafts.

- **Protein Complexes**
  - Proteins and glycoproteins are diffused within the plasma membrane. These help in the transport of ions and metabolites, cell signalling, adhesion, and migration.

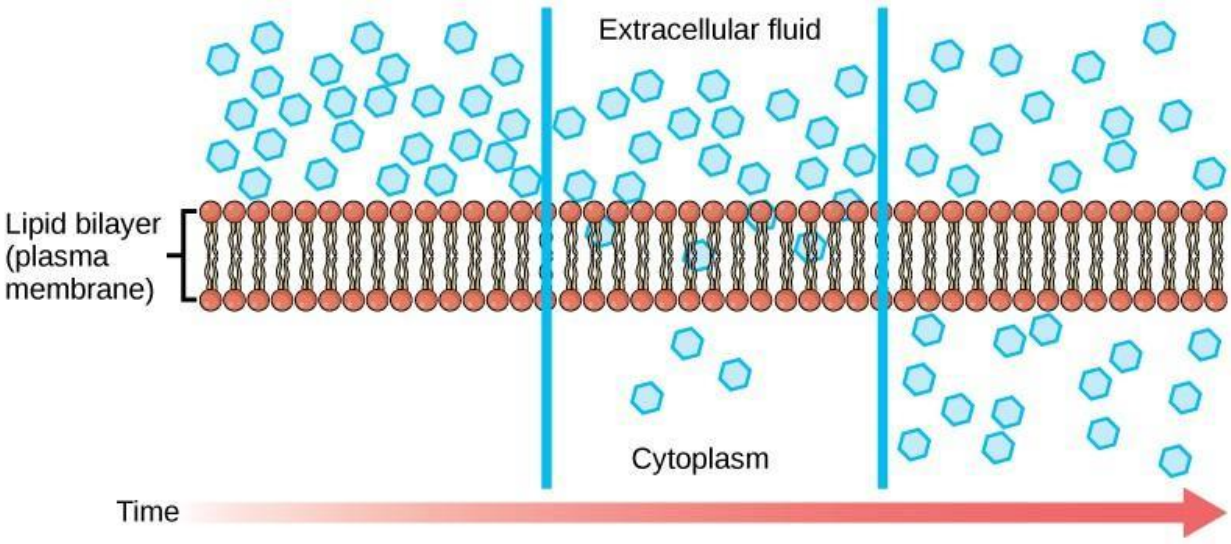
Key Points on Fluid Mosaic Model

- The plasma membrane comprises amphiphilic, phospholipid molecules.
- The second important component of the plasma membrane is integral proteins that are integrated completely into the membrane.
- Carbohydrates are found on the external surface of the membrane where they are bound to proteins or lipids.

Membrane transport - diffusion, active transport, ion pumps, bulk transport

Diffusion

- Diffusion is a **passive process of transport**.



- A single substance tends to move from an area of high concentration to an area of low concentration until the concentration is equal across a space.
- For example, think about someone opening a bottle of ammonia in a room filled with people.
- The ammonia gas is at its highest concentration in the bottle; its lowest concentration is at the edges of the room.
- The ammonia vapor will diffuse, or spread away, from the bottle, and gradually, more and more people will smell the ammonia as it spreads.
- Materials move within the cell's cytosol by diffusion, and certain materials move through the plasma membrane by diffusion.
- Diffusion expends no energy.
- On the contrary, concentration gradients are a form of potential energy, dissipated as the gradient is eliminated.
- Each separate substance in a medium, such as the extracellular fluid, has its own concentration gradient, independent of the concentration gradients of other materials.
- In addition, each substance will diffuse according to that gradient.
- Within a system, there will be different rates of diffusion of the different substances in the medium.

Factors That Affect Diffusion

Several **factors** affect the rate of diffusion:

- **"Steepness" of the concentration gradient:** The greater the difference in concentration, the more rapid the diffusion. The closer the distribution of the material gets to equilibrium, the slower the rate of diffusion becomes.
- **Mass of the molecules diffusing:** Heavier molecules move more slowly; therefore, they diffuse more slowly.
- **Temperature:** Higher temperatures increase the energy and therefore the movement of the molecules, increasing the rate of diffusion.
- **Solvent density:** As the density of a solvent increases, the rate of diffusion decreases. The molecules slow down because they have a more difficult time getting through the denser medium. If the medium is less dense, diffusion increases.

Active transport

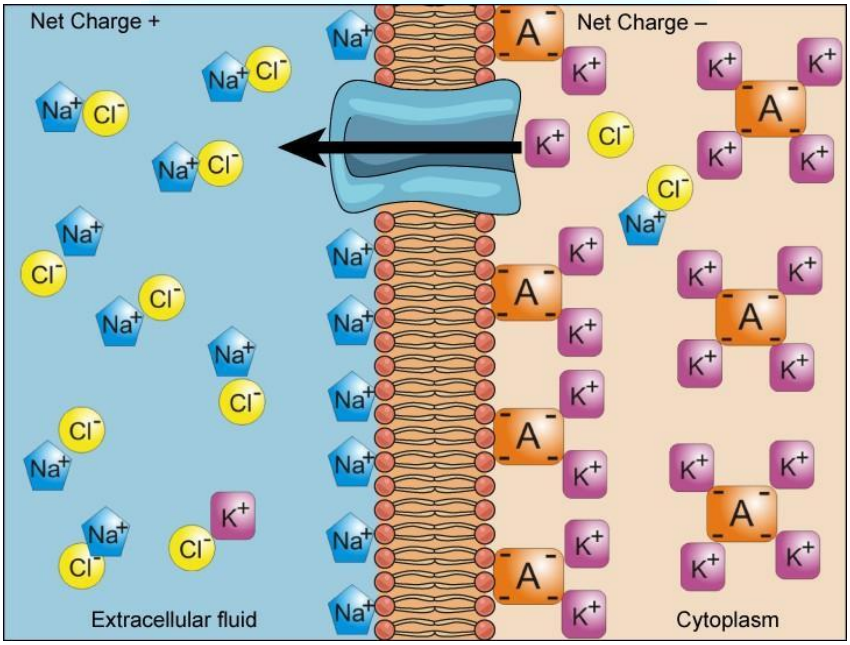
- Active transport mechanisms require the use of the cell's energy, usually in the form of adenosine triphosphate (ATP).
- If a substance must move into the cell against its concentration gradient—that is, if the concentration of the substance inside the cell is greater than its concentration in the extracellular fluid (and vice versa)—the cell must use energy to move the substance. Some active transport mechanisms move small-molecular weight materials, such as ions, through the membrane. Other mechanisms transport much larger molecules.

Electrochemical Gradient

We have discussed simple concentration gradients—different concentrations of a substance across a space or a membrane—but in living systems, gradients are more complex. Because ions move into and out of cells and because cells contain proteins that do not move across the membrane

and are mostly negatively charged, there is also an electrical gradient, a difference of charge, across the plasma membrane.

The interior of living cells is electrically negative with respect to the extracellular fluid surrounding them. At the same time, cells have a lower concentration of (Na+) than does the extracellular fluid. Therefore, both the concentration gradient and the electrical gradient tend to drive Na+ into the cell. Conversely, cells have a higher concentration of K+ than the extracellular fluid does. Therefore, the concentration gradient tends to drive K+ out of the cell, while the electrical gradient tends to drive it inside the cell. The combined gradient of concentration and electrical charge that affects an ion is called its electrochemical gradient.



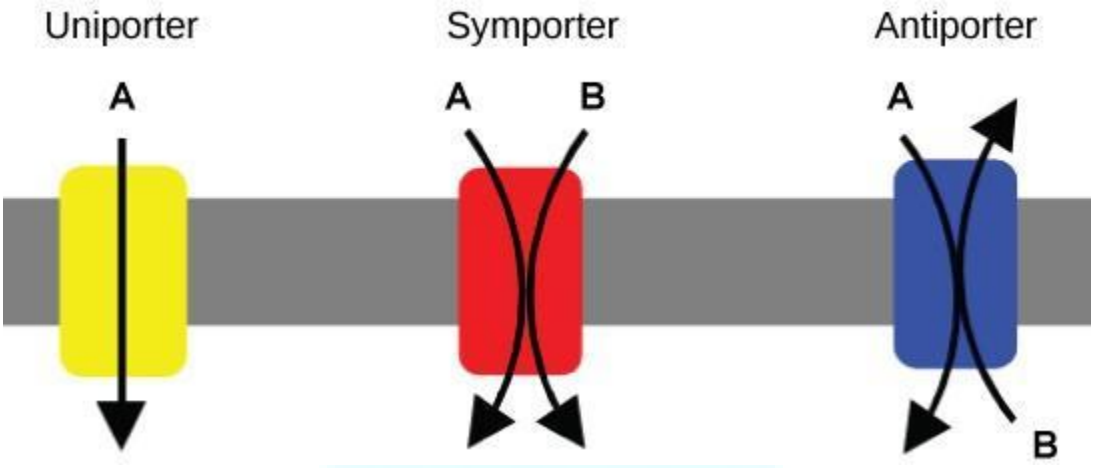
Moving Against a Gradient

- To move substances against a concentration or electrochemical gradient, the cell must use energy, usually in the form of ATP.
- Active transport proteins, called pumps, work against electrochemical gradients.
- Small substances constantly pass through plasma membranes.

- Active transport maintains concentrations of ions and other substances needed by living cells in the face of these passive movements.
- Much of a cell's supply of metabolic energy may be spent maintaining these processes.

Proteins for Active Transport

The specific proteins that facilitate active transport are called transporters. There are three types of transporters (Figure 8.17). A uniporter carries one specific ion or molecule. A symporter carries two different ions or molecules, both in the same direction. An antiporter carries two different ions or molecules in different directions. All of these transporters can transport small, uncharged organic molecules such as glucose.



- Two mechanisms exist for the transport of small-molecular weight material and small molecules.
- **Primary active transport** is directly dependent on ATP.
- **Secondary active transport** does not directly require ATP, because it uses electrochemical gradients established by primary active transport for fuel.
- Primary active transport must occur first to in order to allow secondary active transport to occur. Although it does not use ATP, secondary active transport is still considered active because it requires energy.

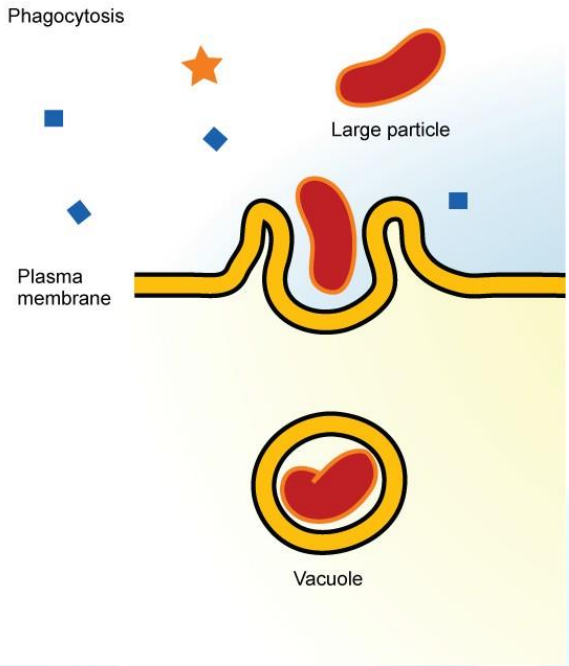
Bulk Transport

In addition to moving small ions and molecules through the membrane, cells also need to remove and take in larger molecules and particles. Some cells are even capable of engulfing entire unicellular microorganisms. You might have correctly hypothesized that the uptake and release of large particles by the cell requires energy. A large particle, however, cannot pass through the membrane, even with energy supplied by the cell.

- **Endocytosis**
  - Endocytosis is a type of active transport that moves particles, such as large molecules, parts of cells, and even whole cells, into a cell.
  - There are different variations of endocytosis, but all share a common characteristic: The plasma membrane of the cell invaginates, forming a pocket around the target particle.
  - The pocket pinches off, resulting in the particle being contained in a newly created intracellular vesicle formed from the plasma membrane.
  - The **three types of endocytosis** are:
    - Phagocytosis
    - Pinocytosis
    - Receptor-mediated endocytosis

  - **Phagocytosis**
    - Phagocytosis ("cell eating") is the process by which large particles, such as other cells or relatively large particles, are taken in by a cell.
    - For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil will "eat" the invaders through phagocytosis, surrounding and engulfing

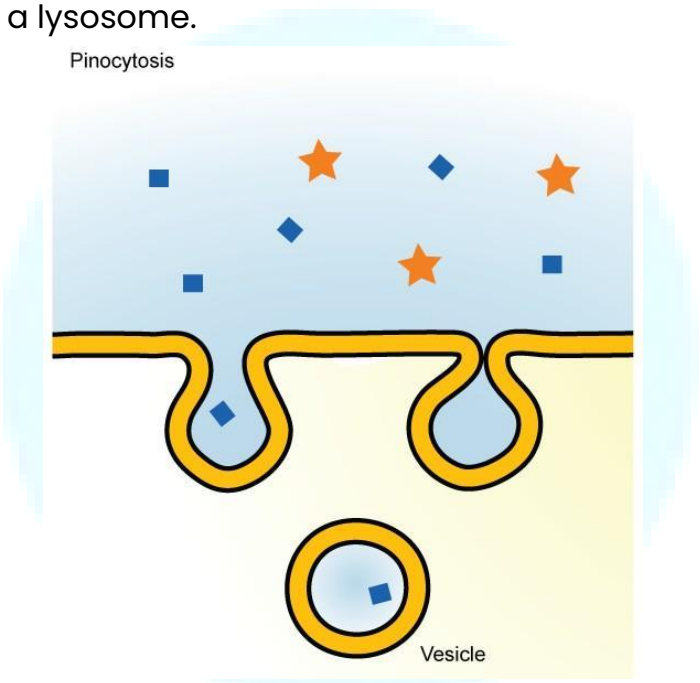
the microorganism, which is then destroyed by lysosomes inside the neutrophil.



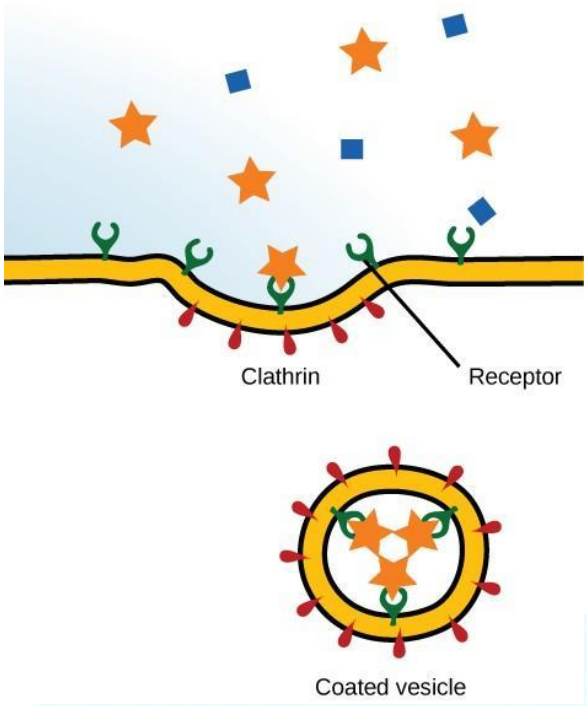
- In preparation for phagocytosis, a portion of the inward-facing surface of the plasma membrane becomes coated with a protein called **clathrin,** which stabilizes this section of the membrane.
- The coated portion of the membrane then extends from the body of the cell and surrounds the particle, eventually enclosing it.
- Once the vesicle containing the particle is enclosed within the cell, the clathrin disengages from the membrane and the vesicle merges with a lysosome for the breakdown of the material in the newly formed compartment.
- When accessible nutrients from the degradation of the vesicular contents have been extracted, the newly formed endosome merges with the plasma membrane and releases its contents into the extracellular fluid.

- The endosomal membrane again becomes part of the plasma membrane.

- **Pinocytosis**
  - Through pinocytosis ("cell drinking"), cells take in molecules, including water, which the cell needs from the extracellular fluid. Pinocytosis results in a much smaller vesicle than does phagocytosis, and the vesicle does not need to merge with a lysosome.



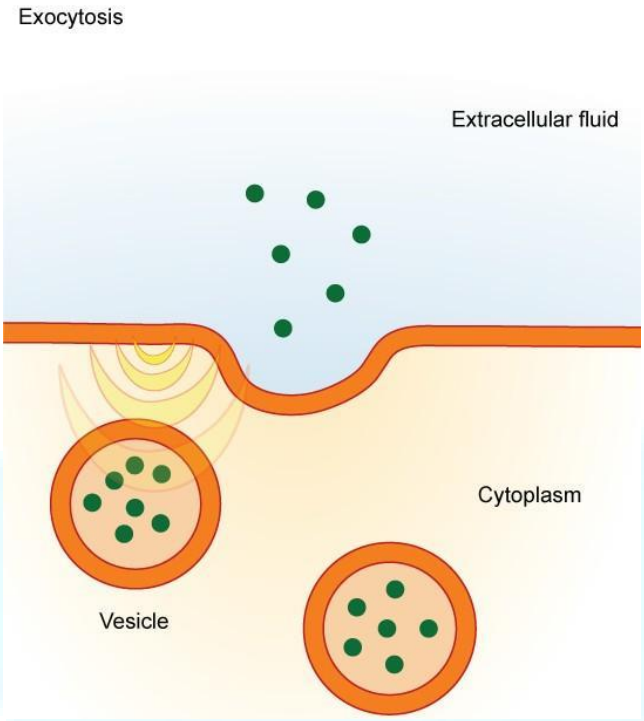
- **Receptor-mediated Endocytosis**
  - Receptor-mediated endocytosis is a targeted variation of endocytosis that employs receptor proteins in the plasma membrane that have a specific binding affinity for certain substances .



- Receptor-mediated endocytosis, as in phagocytosis, uses clathrin protein attached to the cytoplasmic side of the plasma membrane.
- Some human diseases are caused by the failure of receptor-mediated endocytosis.
- For example, the form of cholesterol termed low-density lipoprotein or LDL (also referred to as “bad” cholesterol) is removed from the blood by receptor-mediated endocytosis.
- In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely.
- People with this condition have life-threatening levels of cholesterol in their blood, because their cells cannot clear LDL particles from their blood.
- Although receptor-mediated endocytosis is designed to bring specific substances that are normally found in the extracellular fluid into the cell, other substances may gain entry into the cell at the same site.

- Flu viruses, diphtheria, and cholera toxin all have sites that cross-react with normal receptor-binding sites and gain entry into cells.

- **Exocytosis**



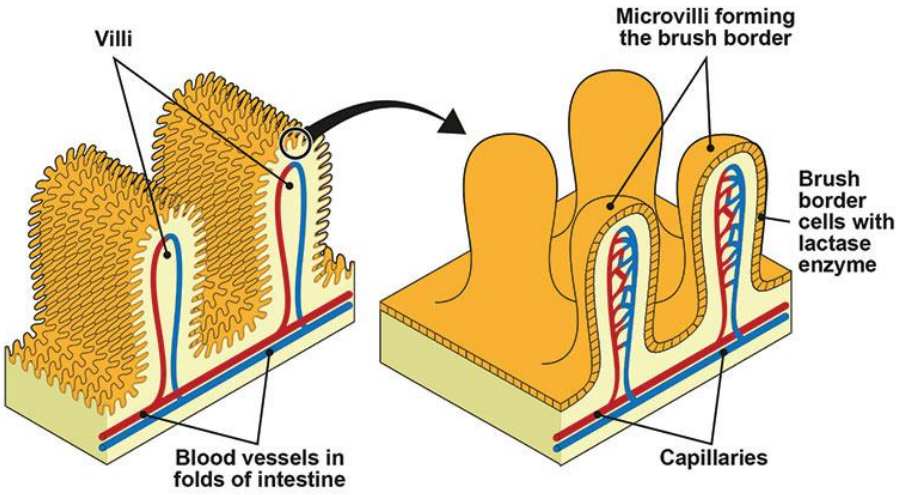
  - The reverse process of moving material into a cell is the process of exocytosis.
  - The purpose of exocytosis is to expel material from the cell into the extracellular fluid.
  - Waste material is enveloped in vesicle, which fuses with the interior of the plasma membrane, expelling the waste material into the extracellular space.
  - Cells also use exocytosis to secrete proteins such as hormones, neurotransmitters, or parts of the extracellular matrix.

Differentiation of cell membrane

Microvilli

- Microvilli, in the most simplistic terms, are tiny little microscopic projections that exist in, on, and around cells.
- They can exist on their own or in conjunction with villi (projections of some mucous membranes, most specifically of the small intestine, which are tiny folds that project out like numerous fingers).
- On each of the villi, there are even smaller folds that stick out like fingers called microvilli.
- Microvilli are most often found in the small intestine, on the surface of egg cells, as well as on white blood cells.
- Thousands of microvilli form a structure called the brush border that is found on the apical surface of some epithelial cells, such as the small intestines.

Structure of Microvilli



- Microvilli form a rather polymorphic class of surface protuberances that are regularly packed in some tissues and loosely positioned in oth-ers.
- Generally, they are shorter and smaller in diameter than cilia. They are commonly about 0.1 µm diameter and range in length from a fraction of a micrometer to about 2 µm.
- Microvilli are essentially bundles of cross-linked actin fibers.
- Although they are cellular extensions, there are little or no cellular organelles present in the microvilli.

- However, they are covered in their own plasma membrane, which encloses cytoplasm and microfilaments.
- Each microvillus has a dense bundle of cross-linked actin filaments, which serves as its structural core.
- 20 to 30 tightly bundled actin filaments are cross-linked by bundling proteins fimbrin (or plastin-1), villin and espin to form the core of the microvilli.
- Actin filaments, present in the cytosol, are most abundant near the cell surface. These filaments are thought to determine the shape and movement of the plasma membrane.
- The nucleation of actin fibers occurs as a response to external stimuli, allowing a cell to alter its shape to suit a particular situation.
- In the enterocyte microvillus, the structural core is attached to the plasma membrane along its length by lateral arms made of myosin 1a and Ca2+ binding protein calmodulin.
- The space between microvilli at a cell's surface is called the intermicrovillous space. Intermicrovillous space increases with the contractile activity of myosin II and tropomyosin and decreases when contraction ceases.

Tight junctions

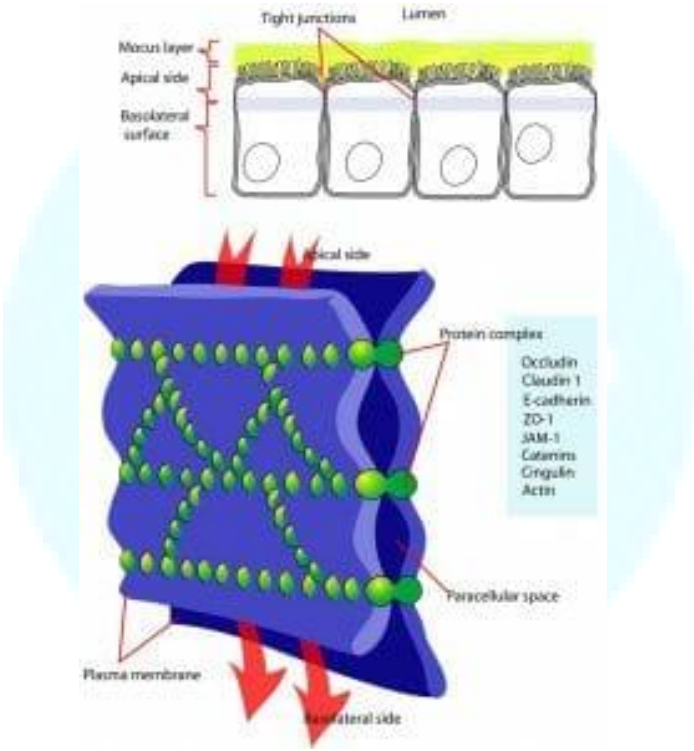
- Tight junctions are areas where the membranes of two adjacent cells join together to form a barrier.
- The cell membranes are connected by strands of transmembrane proteins such as claudins and occludins.
- Tight junctions bind cells together, prevent molecules from passing in between the cells, and also help to maintain the polarity of cells.
- They are only found in vertebrates, animals with a backbone and skeleton; invertebrates have septate junctions instead.

Function of Tight Junctions

- Tight junctions have several different functions.
- Their most important functions are to help cells form a barrier that prevents molecules from getting through, and to stop proteins in the cell membrane from moving around.
- Tight junctions are often found at epithelial cells, which are cells that line the surface of the body and line body cavities. Not only do epithelial cells separate the body from the surrounding environment, they also separate surfaces within the body.
- Therefore, it is very important that the permeability of molecules through layers of epithelial cells is tightly controlled.
- If molecules are blocked by tight junctions and physically unable to pass through the space in between cells, they must enter through other methods that involve entering the cells themselves.
- They could pass through special proteins in the cell membrane, or be engulfed by the cell through endocytosis.
- Using these methods, the cell has greater control over what materials it takes in and allows to pass through.
- However, in endothelial cells, certain proteins must be kept on certain sides of the cell.
- The apical, or outside layer, of the sheet of cells contains proteins that only let certain substances pass through.
- The basal, or inside layer, is where cells let molecules pass through them by expelling them from their membrane in a process called **exocytosis**.
- **Exocytosis** also relies on specific proteins in order to work correctly.
- Tight junctions keep the correct proteins on the correct sides of the cell in order for these functions to occur. This also helps maintain the polarity of cells.
- Another function of tight junctions is **simply to hold cells together**.

- The branching protein strands of tight junctions link adjacent cells together tightly so that they form a sheet.
- These strands are anchored to microfilaments, part of the cell's cytoskeleton that is made up of long strands of actin proteins.
- Microfilaments are located inside the cell, so the combination of microfilaments and sealing strands anchors the cells together from the inside and the outside.

Structure of Tight junctions

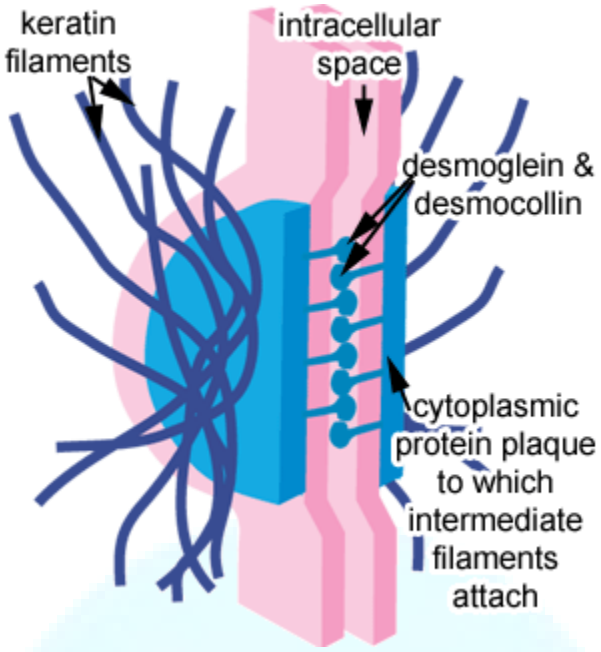


- Tight junctions are a branching network of protein strands on the surface of a cell that link with each other throughout the surface of the membrane.
- The strands are formed by transmembrane proteins on the surfaces of the cell membranes that are adjacent to each other.
- There are around 40 different proteins at tight junctions.

- These proteins can be grouped into four main types. Transmembrane proteins are wedged in the middle of the cell membrane and are responsible for adhesion and permeability.
- Scaffolding proteins organize transmembrane proteins.
- Signaling proteins are responsible for forming the tight junction and regulating the barrier.
- Regulation proteins regulate what proteins are brought to the cell membrane in vesicles.
- **Claudins and occludins** are the two main types of proteins present at tight junctions, and they are both transmembrane proteins.
- Claudins are important in forming tight junctions, while occludins play more of a role in keeping the tight junction stable and maintaining the barrier between cells that keeps unwanted molecules out.

Belt and spot desmosomes

- **Desmosomes** are anchoring junctions between neighboring cells.
- A similar anchoring junction is the hemidesmosome.
- Both desmosomes and **hemidesmosomes** use intermediate filaments as their cytoskeletal anchor.
- However, the transmembrane linker of desmosomes is cadherin whereas that of hemidesmosomes is integrins.
- Furthermore, desmosomes link cell to another cell whereas hemidesmosomes link cell to the extracellular matrix.



- Desmosomes are typically found in simple and stratified squamous epithelium.
- They aid in resisting shearing forces. They are also found between myocytes where they bind these cells to one another.
- There are basically two types of desmosomes identified:
    - **Spot desmosomes** - Spot desmosomes appear spot-like or circular in outline.
    - **Belt desmosomes -** The belt desmosomes are belt-like surrounding the cell completely, hence, the name.

Gap junctions

- Gap junctions are a type of cell junction in which adjacent cells are connected through protein channels.
- These channels connect the cytoplasm of each cell and allow molecules, ions, and electrical signals to pass between them.
- Gap junctions are found in between the vast majority of cells within the body because they are found between all cells that are directly touching other cells.

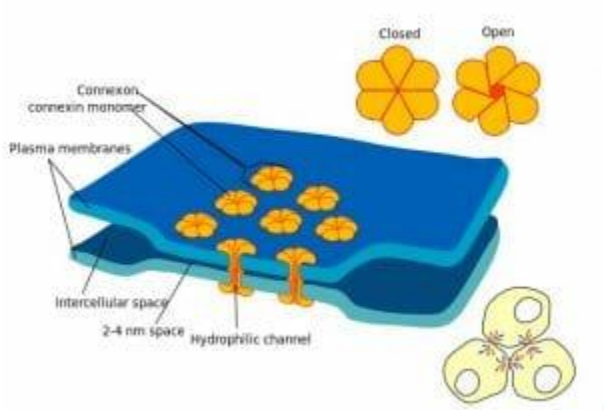
- Exceptions include cells that move around and do not usually come into close contact with other cells, such as sperm cells and red blood cells.
- Gap junctions are only found in animal cells; plant cells are connected by channels called plasmodesmata instead.

Function of Gap Junctions

- The main function of gap junctions is to connect cells together so that molecules may pass from one cell to the other.
- This allows for cell-to-cell communication, and makes it so that molecules can directly enter neighboring cells without having to go through the extracellular fluid surrounding the cells.
- Gap junctions are especially important during embryonic development, a time when neighboring cells must communicate with each other in order for them to develop in the right place at the right time.
- If gap junctions are blocked, embryos cannot develop normally.
- Gap junctions make cells chemically or electrically coupled.
- This means that the cells are linked together and can transfer molecules to each other for use in reactions.
- Electrical coupling occurs in the heart, where cells receive the signal to contract the heart muscle at the same time through gap junctions.
- It also occurs in neurons, which can be connected to each other by electrical synapses in addition to the well-known chemical synapses that neurotransmitters are released from.
- When a cell starts to die from disease or injury, it sends out signals through its gap junctions.
- These signals can cause nearby cells to die even if they are not diseased or injured.
- This is called the “bystander effect”, since the nearby cells are innocent bystanders that become victims.
- However, sometimes groups of adjacent cells need to die during development, so gap junctions facilitate this process.

- In addition, cells can also send therapeutic compounds to each other through gap junctions, and gap junctions are being researched as a method of therapeutic drug delivery.

Gap Junction Structure



- In vertebrate cells, gap junctions are made up of connexin proteins. (The cells of invertebrates have gap junctions that are composed of innexin proteins, which are not related to connexin proteins but perform a similar function.)
- Groups of six connexins form a connexon, and two connexons are put together to form a channel that molecules can pass through.
- Other channels in gap junctions are made up of pannexin proteins.
- Relatively less is known about pannexins; they were originally thought only to form channels within a cell, not between cells.
- Hundreds of channels are found together at the site of a gap junction in what is known as a gap junction plaque. A plaque is a mass of proteins.

Cell organelles: Structure and function, Nucleus - nuclear envelope, nuclear pore complex, Mitochondria , Golgi apparatus, Ribosomes, Lysosomes, Endoplasmic reticulum, Peroxisomes and Centriole.

Important Cell organelles

Within the cytoplasm, the major organelles and cellular structures include:

(1) Nucleolus
(2) Nucleus
(3) Ribosome
(5) Endoplasmic reticulum
(6) Golgi apparatus
(7) Cytoplasm
(8) Mitochondria
(9) Vacuole
(10) Lysosome
(12) Centriole

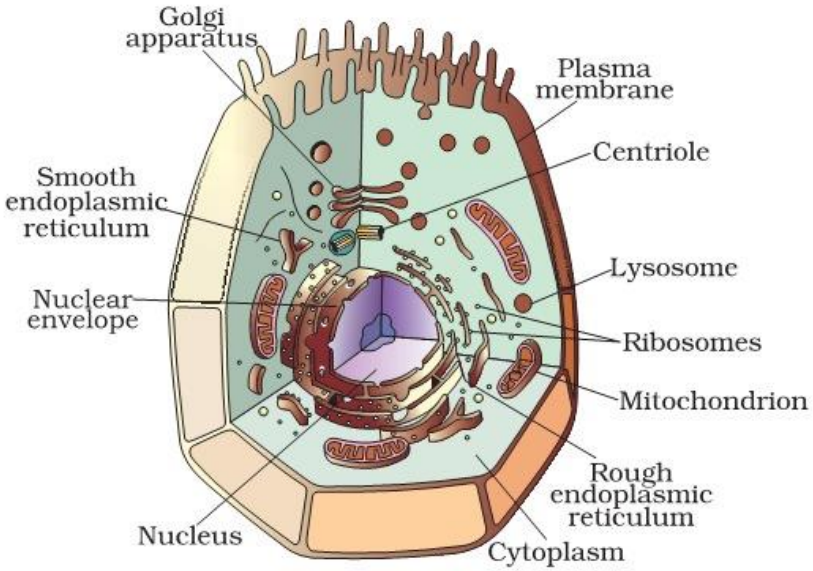


Fig. 5.5: *Animal cell*

Plasma Membrane or Cell Membrane

- Cell membrane is also called the plasma membrane.
- It can be observed only through an electron microscope.
- Plasma membrane is the outermost covering of the cell that separates the contents of the cell from its external environment.

Cell Wall

- Cell wall is **absent in animals**.
- Plant cells, in addition to the plasma membrane, have another rigid outer covering called the cell wall. The cell wall lies outside the plasma membrane.
- The plant cell wall is mainly composed of cellulose. Cellulose is a complex substance and provides structural strength to plants.

Cytoplasm

- It is the jelly-like substance present between the cell membrane and the nucleus.
- The cytoplasm is the **fluid content** inside the plasma membrane.
- It also contains many specialized cell organelles [mitochondria, golgi bodies, ribosomes, etc].
- Each of these organelles performs a specific function for the cell.
- Cell organelles are enclosed by membranes.
- The significance of membranes can be illustrated with the example of viruses.
- Viruses lack any membranes and hence do not show characteristics of life until they enter a living body and use its cell machinery to multiply.

Nucleus

- It is an important component of the living cell.
- It is generally spherical and located in the center of the cell.

- It can be stained and seen easily with the help of a microscope.
- Nucleus is separated from the cytoplasm by a double layered membrane called the nuclear membrane.
- This membrane is also porous and allows the movement of materials between the cytoplasm and the inside of the nucleus [diffusion].
- With a microscope of higher magnification, we can see a smaller spherical body in the nucleus. It is called the nucleolus.
- In addition, nucleus contains thread-like structures called chromosomes. These carry genes and help in inheritance or transfer of characters from the parents to the offspring. The chromosomes can be seen only when the cell divides.
- Gene is a unit of inheritance in living organisms. It controls the transfer of a hereditary characteristic from parents to offspring. This means that your parents pass some of their characteristics on to you.
- Nucleus, in addition to its role in inheritance, acts as control center of the activities of the cell.
- The entire content of a living cell is known as protoplasm [cytoplasm + nucleus]. It includes the cytoplasm and the nucleus. Protoplasm is called the living substance of the cell.
- The nucleus of the bacterial cell is not well organized like the cells of multicellular organisms. There is no nuclear membrane.
- Every cell has a membrane around it to keep its own contents separate from the external environment.
- Large and complex cells, including cells from multicellular organisms, need a lot of chemical activities to support their complicated structure and function.
- To keep these activities of different kinds separate from each other, these cells use membrane-bound little structures (or 'organelles') within themselves.

Nuclear Envelope

- The nuclear envelope (NE) consists of **two membranes: the outer and inner nuclear membranes (ONM and INM)**, which are bound by nuclear pore complexes (NPCs) and perforated by nuclear pores.
- Although the NE is a continuous membrane system, the protein composition differs significantly between the ONM and the INM.
- The ONM is contiguous to the endoplasmic reticulum and sprinkled on the cytoplasmic side with ribosomes.
- At the same time, INM has indirect interaction with a number of nuclear components, such as chromatin and nuclear lamina, which is an intermediate filament meshwork essential for the maintenance of the nuclear architecture.
- The main function of NE is to protect the genome and ensure the safe transport of proteins between the cytoplasm and the nucleus.
- Adequate functioning of the NE, in particular the INM, allows for the maintenance of the nuclear structure and position.

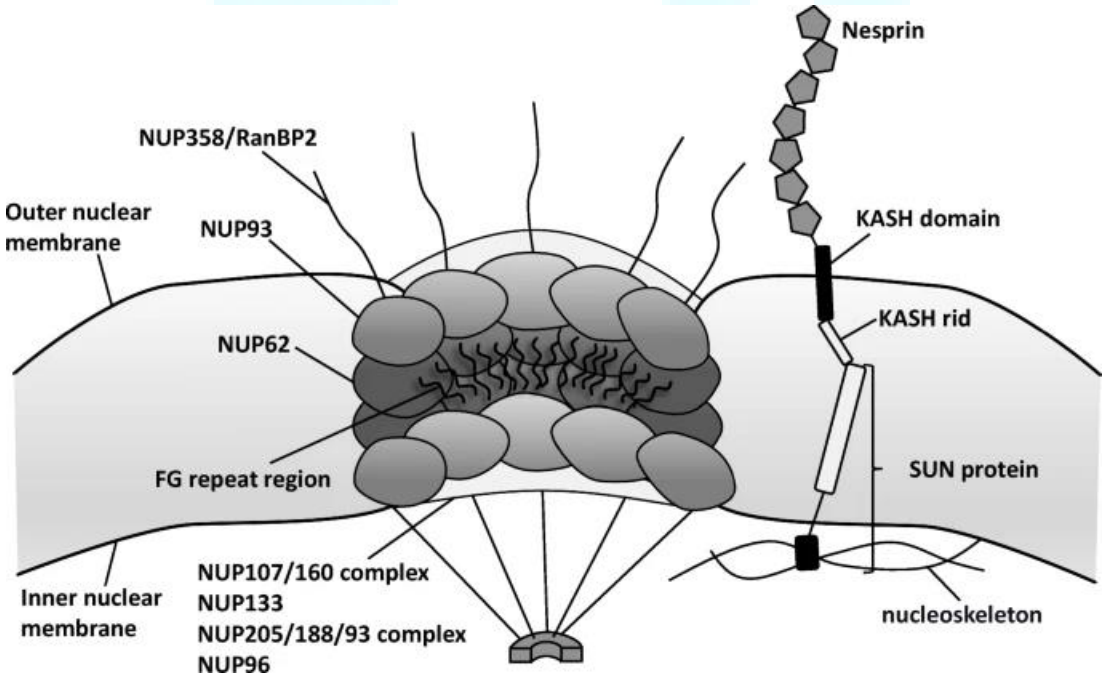


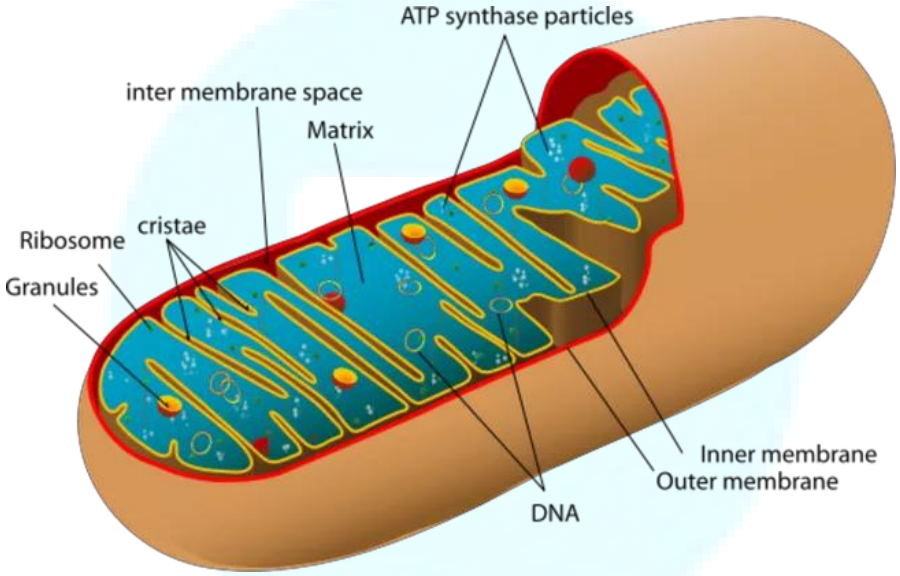
Fig: Schematic diagram of nuclear envelope (NE) and nuclear pore complex (NPC) proteins

Nuclear Pore Complexes(NPC)

- The majority of enzymes and their substrates that are engaged in cell proliferation, differentiation, and other vital functions are shuttled between intranuclear and cytosolic compartments through NPCs.
- The NPCs are aqueous channels formed by multicomponent protein complexes of the nuclear envelope, regulating the movement of cell components from the cytosol to the nucleus and vice versa.
- Consequently, defective NPC function could lead to inappropriate localization of a large number of nuclear and cellular components.
- The NPC composition and structure are age-dependent, as cells lose essential nucleopore proteins with age.
- Cell cultures exposed to oxidative stress also show marked changes in **phosphorylation and O-glycosylation** of nucleoporins and alterations in the localization of these proteins and their interaction with other transport components.
- These types of changes in the structure and function of the NPC could lead to aberrant intracellular trafficking of crucial proteins involved in signaling and cell cycle regulation.
- The NPCs show a broad degree of compositional and structural conservation in all eukaryotes. It has a doughnut-shaped structure consisting of eight spokes, which are arranged radially around a central channel that serves as the conduit for macromolecular transport. Additionally, NPCs can interact with chromatin and contribute to the formation of specific genomic loops.
- A growing body of evidence, both from human brain tissue and model animal studies, indicates that the disturbances of NPC structure and function resulting from neuronal oxidative stress are typical features of degenerating neurons. Thus, it can be hypothesized that abnormal structure and disturbed function of NPC underlie the pathogenesis of neurodegeneration.

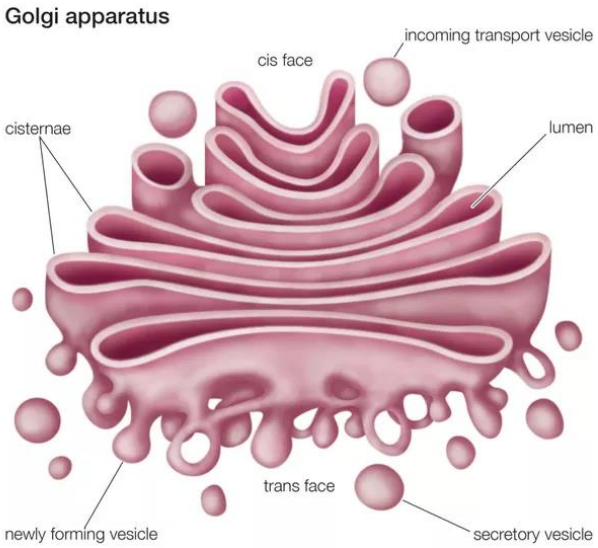
Mitochondria

- Mitochondria are known as the powerhouse of the cell.
- The energy required for various chemical activities needed for life is released by mitochondria in the form of ATP (Adenosine Triphosphate) molecules.
- If Mitochondria is the Power Plant. ATP is the Electricity.
- ATP is known as the energy currency of the cell.
- The body uses energy stored in ATP for making new chemical compounds and for mechanical work.



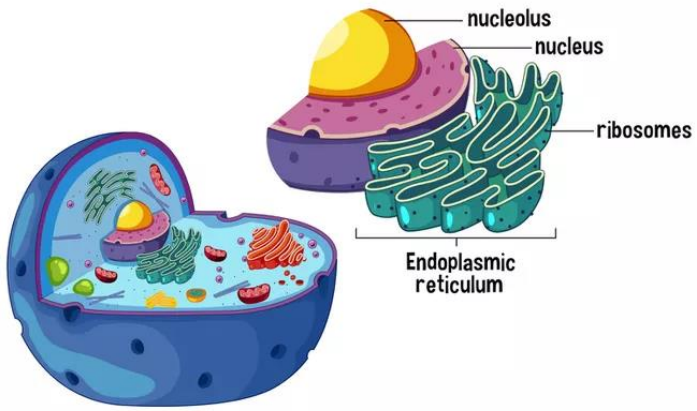
- Mitochondria have two membrane coverings instead of just one.
- The outer membrane is very porous while the inner membrane is deeply folded. These folds create a large surface area for ATP-generating chemical reactions.
- Mitochondria are strange organelles in the sense that they have their own DNA and ribosomes. Therefore, mitochondria are able to make some of their own proteins [ribosomes prepare proteins].

Golgi Apparatus or Golgi Complex



- The golgi apparatus consists of a system of membrane-bound vesicles arranged approximately parallel to each other in stacks called cisterns.
- These membranes often have connections with the membranes of ER and therefore constitute another portion of a complex cellular membrane system.
- The material synthesized near the ER is packaged and dispatched to various targets inside and outside the cell through the golgi apparatus.
- Its functions include the storage, modification and packaging of products in vesicles.
- In some cases, complex sugars may be made from simple sugars in the golgi apparatus.
- The golgi apparatus is also involved in the formation of lysosomes.

Ribosome



- A ribosome is a complex of RNA and protein and is, therefore, known as a ribonucleoprotein. It is composed of two subunits – smaller and larger.
- The smaller subunit is where the mRNA binds and is decoded, and in the larger subunit, the amino acids get added. Both of the subunits contain both protein and ribonucleic acid components.
- The two subunits are joined to each other by interactions between the rRNAs in one subunit and proteins in the other subunit.
- Ribosomes are located inside the cytosol found in the plant cell and animal cells.

The **ribosome structure** includes the following:

- It is located in two areas of cytoplasm.
- Scattered in the cytoplasm.
- Prokaryotes have 70S ribosomes while eukaryotes have 80S ribosomes.
- Around 62% of ribosomes are comprised of RNA, while the rest is proteins.
- The structure of free and bound ribosomes is similar and is associated with protein synthesis.

The important ribosome **function** includes:

- It assembles amino acids to form proteins that are essential to carry out cellular functions.
- The DNA produces mRNA by the process of DNA transcription.

- The mRNA is synthesized in the nucleus and transported to the cytoplasm for the process of protein synthesis.
- The ribosomal subunits in the cytoplasm are bound around mRNA polymers. The tRNA then synthesizes proteins.
- The proteins synthesized in the cytoplasm are utilized in the cytoplasm itself, the proteins synthesized by bound ribosomes are transported outside the cell.

Lysosomes

- Lysosomes are a kind of waste disposal system of the cell.
- Lysosomes help to keep the cell clean by digesting any foreign material as well as worn-out cell organelles.
- Foreign materials entering the cell, such as bacteria or food, as well as old organelles end up in the lysosomes, which break them up into small pieces. Lysosomes are able to do this because they contain powerful digestive enzymes capable of breaking down all organic material.
- During the disturbance in cellular metabolism, for example, when the cell gets damaged, lysosomes may burst and the enzymes digest their own cell. Therefore, lysosomes are also known as the 'suicide bags' of a cell.
- Structurally, lysosomes are membrane-bound sacs filled with digestive enzymes. These enzymes are made by RER.

Endoplasmic Reticulum (ER)

- The endoplasmic reticulum (ER) is a large network of membrane-bound tubes and sheets. It looks like long tubules or round or long bags (vesicles).
- The ER membrane is similar in structure to the plasma membrane.
- There are two types of ER –– **rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER)**.

- Rough Endoplasmic Reticulum RER – Ribosomes

  - RER looks rough under a microscope because it has particles called ribosomes attached to its surface.
  - The ribosomes, which are present in all active cells, are the sites of protein manufacture.
  - The manufactured proteins are then sent to various places in the cell depending on need, using the ER.

- Smooth Endoplasmic Reticulum SER
  - The SER helps in the manufacture of fat molecules, or lipids, important for cell function.

Functions of Endoplasmic Reticulum (ER):

- Some of these proteins and lipids help in building the cell membrane. This process is known as membrane biogenesis.
- Some other proteins and lipids function as enzymes and hormones.
- Although the ER varies greatly in appearance in different cells, it always forms a network system.
- Thus, one function of the ER is to serve as channels for the transport of materials (especially proteins) between various regions of the cytoplasm or between the cytoplasm and the nucleus.
- The ER also functions as a cytoplasmic framework providing a surface for some of the biochemical activities of the cell.
- In the liver cells of the group of animals called vertebrates, SER plays a crucial role in detoxifying many poisons and drugs.

Peroxisomes

- Peroxisomes are small vesicles, single membrane-bound organelles found in the eukaryotic cells.

- They contain digestive enzymes for breaking down toxic materials in the cell and oxidative enzymes for metabolic activity.
- They are a heterogeneous group of organelles and the presence of the marker enzymes distinguished them from other cell organelles.
- Peroxisomes play an important role in lipid production and are also involved in the conversion of reactive oxygen species such as hydrogen peroxide into safer molecules like water and oxygen by the enzyme catalase.
- Mostly peroxisomes occur as an individual organelle, e.g. in fibroblasts. They also exist in the form of interconnected tubules in liver cells known as peroxisome reticulum.

Peroxisome Structure

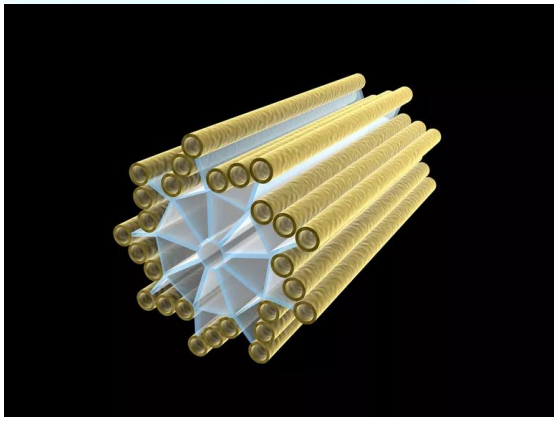
- Peroxisomes vary in shape, size and number depending upon the energy requirements of the cell. These are made of a phospholipid bilayer with many membrane-bound proteins.
- The enzymes involved in lipid metabolism are synthesised on free ribosomes and selectively imported to peroxisomes. These enzymes include one of the two signalling sequences- Peroxisome Target Sequence 1 being the most common one.
- The phospholipids of peroxisomes are usually synthesised in smooth Endoplasmic reticulum. Due to the ingress of proteins and lipids, the peroxisome grows in size and divides into two organelles.
- Peroxisomes do not have their own DNA. Proteins are transported from the cytosol after translation.

Peroxisome Function

- The main function of peroxisome is the lipid metabolism and the processing of reactive oxygen species. Other peroxisome functions include:
- They take part in various oxidative processes.
- They take part in lipid metabolism and catabolism of D-amino acids, polyamines and bile acids.
- The reactive oxygen species such as peroxides produced in the process is converted to water by various enzymes like peroxidase and catalase.
- In plants, peroxisomes facilitate photosynthesis and seed germination. They prevent loss of energy during photosynthesis carbon fixation.

Centrioles

- Centrioles are cylindrical cell structures that are composed of groupings of microtubules, which are tube-shaped molecules or strands of protein.
- Without centrioles, chromosomes would not be able to move during the formation of new cells.
- Centrioles help to organize the assembly of microtubules during cell division. To put it simply, chromosomes use the centriole's microtubules as a highway during the cell division process.



<u>Where Centrioles Are Found?</u>

- Centrioles are found in all animal cells and only a few species of lower plant cells.
- Two centrioles—**a mother centriole** and **a daughter centriole**—are found within the cell in a structure called a **centrosome**.

Composition

- Most centrioles are made up of nine sets of microtubule triplets, with the exception of some species, such as crabs which have nine sets of microtubule doublets.
- There are a few other species that deviate from the standard centriole structure.
- **Microtubules** are composed of a single type of globular protein called **tubulin**.

Two Main Functions

- During mitosis or cell division, the centrosome and centrioles replicate and migrate to opposite ends of the cell. Centrioles help to arrange the microtubules that move chromosomes during cell division to ensure each daughter cell receives the appropriate number of chromosomes.
- Centrioles are also important for the formation of cell structures known as cilia and flagella. Cilia and flagella, found on the outside surface of cells, aid in cellular movement. A centriole combined with several additional protein structures is modified to become a basal body. Basal bodies are the anchoring sites for moving cilia and flagella.

Cytoskeleton: Microtubules, microfilaments and intermediate filaments; molecular motors

The Cytoskeleton

- The cytoskeleton is a network of different protein fibers that provide many functions. It maintains or changes the shape of the cell, secures some organelles in specific positions, enables the movement of cytoplasm and vesicles within the cell, and enables the cell to move in response to stimuli.
- There are **three types of fibers** within the cytoskeleton:
    - **microfilaments (actin filaments)**
    - **intermediate filaments**
    - **microtubules**
- Some of the cytoskeletal filaments work in conjunction with molecular motors that move along the filaments within the cell to carry out a diverse set of functions.

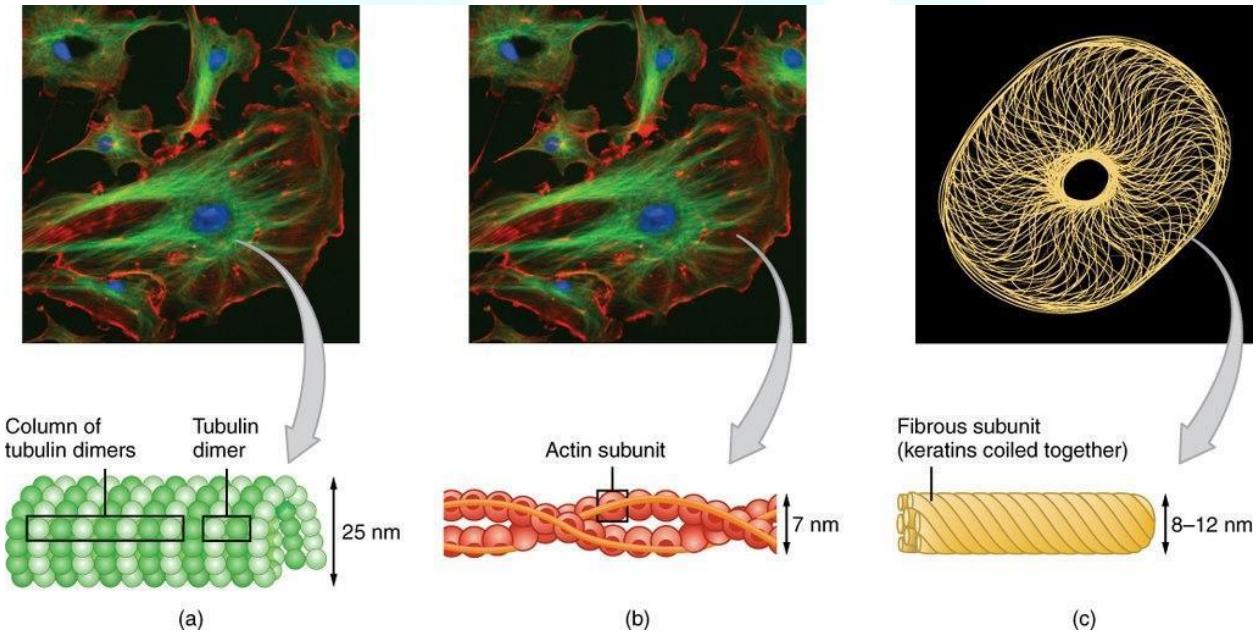
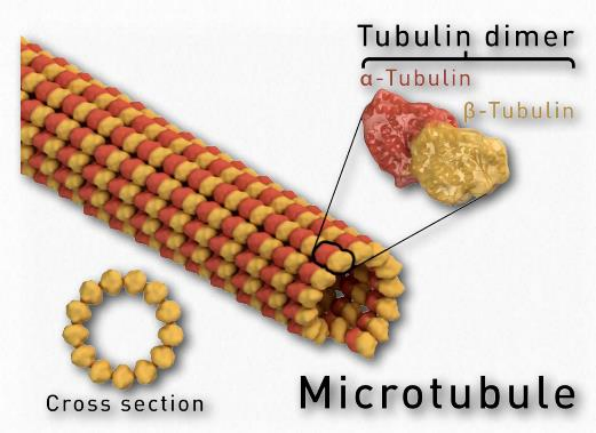


Fig: The three types of cytoskeletal filaments. (a) microtubules, (b) microfilaments and, (c) intermediate filaments.

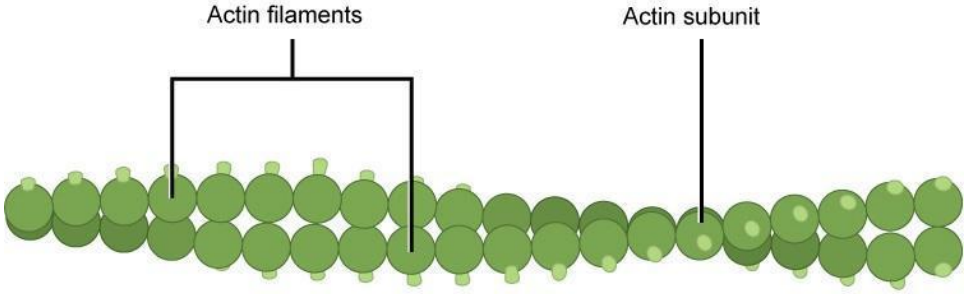
Microtubules

- Microtubules are the thickest type of cytoskeleton with a diameter of 25nm and are found throughout the cytoplasm.
- These polymers are made up of globular protein subunits called α-tubulin and β-tubulin that attach to the polymer as tubulin dimers.
- The tubulin dimers stack to form individual protofilaments, thirteen protofilaments associate to form the hollow tube of the final polymerized microtubule.



- Structure of microtubules: microtubule made up of dimers of α-tubulin and β-tubulin stacked to form protofilaments that assemble to form a hollow tube structure.

Microfilaments (Actin Filaments)



- Microfilaments (also called actin filaments) are cytoskeleton fibers composed of actin subunits.
- Microfilaments/actin filaments are the thinnest type of cytoskeleton with a diameter of 7nm.

- Actin is one of the most abundant proteins in eukaryotic cells.
- Actin can be present as either a free monomer that is globular in shape or bound together in a helical polymer.
- Actin must be bound to ATP to assemble into a polymer.
- The actin filament itself has structural polarity similar to microtubules. Again, this term "polarity" refers to the fact that there are two distinct ends to the filament. These ends are called the "(-)" end and the "(+)" end. At the "(+)" end, actin subunits are adding onto the elongating filament, and at the "(-)" end, actin subunits are disassembling or falling off of the filament.
- As seen in microtubules, the ability of the actin filament to assemble and disassemble, thereby changing its length, is termed dynamic instability. This dynamic instability is integral for some cellular functions, including cell crawling, which depends on rapid actin polymerization and de-polymerization.

Intermediate Filaments

Fig: Intermediate filaments consist of several intertwined strands of fibrous proteins.

- Intermediate filaments are made of several strands of fibrous proteins that are wound together.
- These elements of the cytoskeleton get their name from the fact that their diameter, 8 to 10 nm, is between those of microfilaments and microtubules.
- Intermediate filaments have no role in cell movement.
- Their function is purely structural.

- They bear tension, thus maintaining the shape of the cell, and anchor the nucleus and other organelles in place.
- The intermediate filaments are the most diverse group of cytoskeletal elements.
- Several types of fibrous proteins are found in the intermediate filaments (eg: Keratin)

Molecular motor/ Motor protein

- Motor proteins, such as **myosins and kinesins**, move along cytoskeletal filaments via a force-dependent mechanism that is driven by the hydrolysis of ATP molecules.
- Nucleotide hydrolysis and controlled inorganic phosphate release by motor proteins causes restructuring of core domains that control the association of the motor protein with the filaments, other proteins, and the fresh supply of nucleotides.
- Motor proteins propel themselves along the cytoskeleton using a mechanochemical cycle of filament binding, conformational change, filament release, conformation reversal, and filament rebinding.
- In most cases, the conformational change(s) on the motor protein prevents subsequent nucleotide binding and/or hydrolysis until the prior round of hydrolysis and release is complete.

Controlled hydrolysis of nucleotides and inorganic phosphate release by motor proteins can generate mechanical forces that can be used for:

- Translocating the motor proteins themselves along the filaments
- Stabilizing and/or moving the filaments (i.e., contractile stress fibers) & escorting cargo that is attached to the motor protein (e.g., vesicles, organelles, other proteins) to specific regions in the cell.

- Transport of substances in a particular direction, or polarity, along the filaments. This directionality is achieved by specific conformational changes that allow movement in only one direction.

Cell Division- Mitosis, meiosis, Cell cycle and regulation of cell cycle

Cell Division

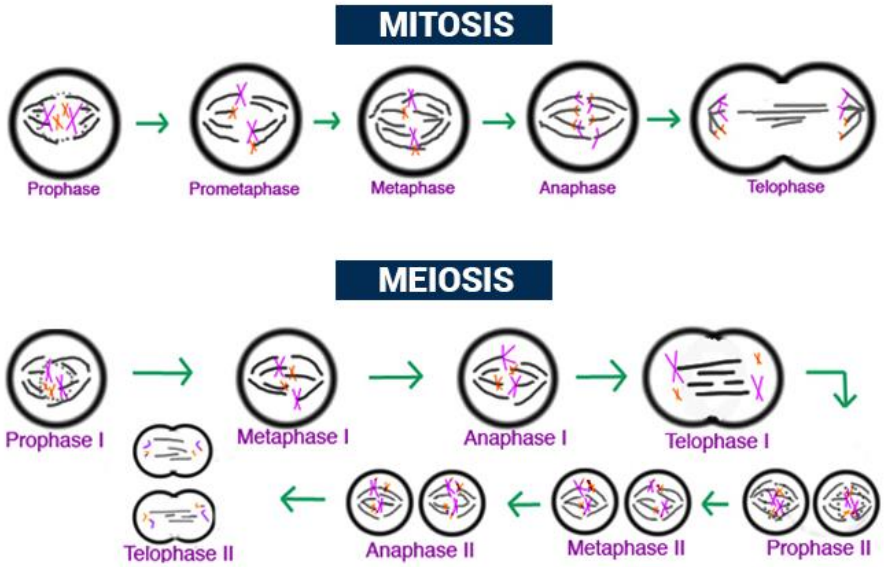
Cell division happens when a parent cell divides into two or more cells called daughter cells. Cell division usually occurs as part of a larger cell cycle. All cells reproduce by splitting into two, where each parental cell gives rise to two daughter cells. These newly formed daughter cells could themselves divide and grow, giving rise to a new cell population that is formed by the division and growth of a single parental cell and its descendant.

Types of Cell Division

There are two distinct types of cell division out of which the first one is vegetative division, wherein each daughter cell duplicates the parent cell called **mitosis**. The second one is **meiosis**, which divides into four haploid daughter cells.

To put it straight, **mitosis creates new body cells**, whereas **meiosis generates sperm and egg cells.**

Types of Cell Division



Mitosis

- Cell duplication and distribution is the aim of Mitosis.
- This is an asexual mode of reproduction.
- Nuclei form in 4 stages namely prophase, metaphase, anaphase, and telophase.
- 2 diploid cells are formed.
- Spindle fibers disconnect after sister chromatids get separated.

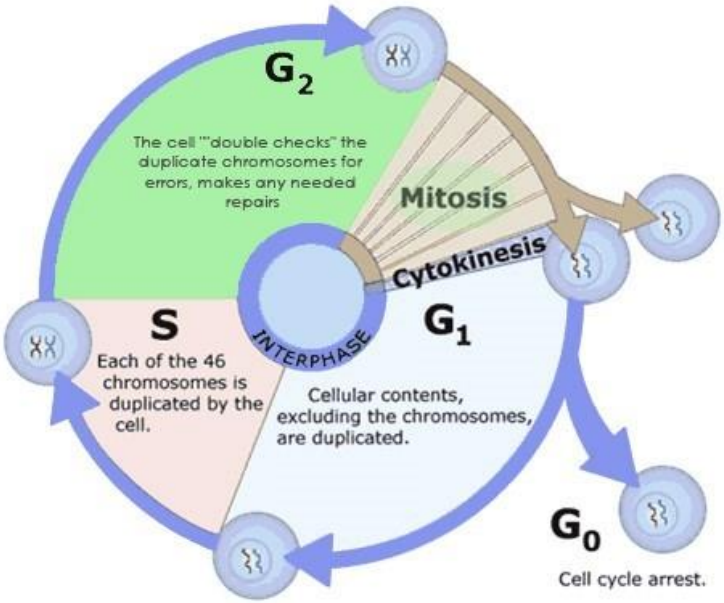
Meiosis

- Meiosis is a form of sexual reproduction.
- The resulting daughter cells are reduced in their chromosome number by half.
- Eukaryotic chromosomes are formed.
- Similar to mitosis, S-Phase is dominant in the meiosis form of cell replication.
- A cell undergoing meiosis will either become human sperm or an egg.

Difference Between Mitosis and Meiosis:

	Mitosis	Meiosis
Meaning	It refers to the cell division that forms two daughter cells each with the same number and type of chromosomes as the parent cell.	It refers to the cell division which forms four daughter cells each with half the number of chromosomes as the parent cell.
Type of Reproduction	It is asexual	It is sexual
Number of Divisions	one	two
Mother cells	They can either be haploid or diploid	They are always diploid
Function	General growth and repair and reproduction of cells	Genetic diversity through sexual reproduction
Creation	Creates everything except sex cells	Creates sex cells only
Occurrence	Takes place in somatic cells	Takes place in germ cells

Cell Cycle



The cell cycle was discovered by Prevost and Dumas (1824) while studying the cleavage of zygote of Frog. It is a series of stages a cell passes through, to divide and produce new cells. This entire process where with the help of one single parent cell a new cell population grows and develops is known as the cell cycle.

Phases of Cell Cycle

- Cell cycle or cell division refers to the series of events that take place in a cell leading to its maturity and subsequent division.
- These events include duplication of its genome and synthesis of the cell organelles followed by division of the cytoplasm.
- Human cells exhibit typical eukaryotic cell cycle and take around 24 hours to complete one cycle of growth and division. The duration of the cycle, however, varies from organism to organism and cell to cell.

A typical eukaryotic cell cycle is divided into **two main phases:-**

- **Interphase**

Also known as the resting phase of the cell cycle; interphase is the time during which the cell prepares for division by undergoing both cell growth and

DNA replication. It occupies around 95% time of the overall cycle. The interphase is divided into **three phases:-**

- **G1 phase (Gap 1)** – G1 phase is the phase of the cell between mitosis and initiation of replication of the genetic material of the cell. During this phase, the cell is metabolically active and continues to grow without replicating its DNA.
- **S phase (Synthesis)** – DNA replication takes place during this phase. If the initial quantity of DNA in the cell is denoted as 2N, then after replication it becomes 4N. However the number of chromosomes does not vary, viz., if the number of chromosomes during G1 phase was 2n, it will remain 2n at the end of S phase. The centriole also divides into two centriole pairs in the cells which contain centriole.
- **G2- phase (Gap 2)** –During this phase, the RNA, proteins, other macromolecules required for multiplication of cell organelles, spindle formation, and cell growth are produced as the cell prepares to go into the mitotic phase.

Some cells like cardiac cells in the adult animals do not exhibit division and some others only divide to replace those cells which have been either damaged or lost due to cell death. Such cells which do not divide further attain an inactive G0 phase also known as quiescent phase after they exit the G1 phase. These cells remain metabolically active but do not divide unless called upon to do so.

- **Mitosis Phase or M Phase**
  - This is the most dramatic period of the cell cycle, involving a major reorganization of virtually all components of the cell.
  - Since the number of chromosomes in the parent and progeny cells is the same, it is also called as equational division.

- Though for convenience mitosis has been divided into four stages of nuclear division, it is very essential to understand that cell division is a progressive process and very clear-cut lines cannot be drawn between various stages.
- Mitosis is the process in which a eukaryotic cell nucleus splits in two, followed by division of the parent cell into two daughter cells.
- The word “mitosis” means “threads,” and it refers to the threadlike appearance of chromosomes as the cell prepares to divide.
- Early microscopists were the first to observe these structures, and they also noted the appearance of a specialized network of microtubules during mitosis.
- These tubules, collectively known as the spindle fibres, extend from structures called centrosomes — with one centrosome located at each of the opposite ends, or poles, of a cell.
- As mitosis progresses, the microtubules [spindle fibres] attach to the chromosomes, which have already duplicated their DNA and aligned across the center of the cell.
- The spindle tubules then shorten and move toward the poles of the cell. As they move, they pull the one copy of each chromosome with them to opposite poles of the cell.
- This process ensures that each daughter cell will contain one exact copy of the parent cell DNA.

Mitosis consists of **five morphologically distinct phases**:

- **prophase**
- **prometaphase**
- **metaphase**
- **anaphase**
- **telophase**

- Each phase involves characteristic steps in the process of chromosome alignment and separation.

- Once mitosis is complete, the entire cell divides in two by way of the process called
  - **In animals, mitotic cell division is only seen in the diploid somatic cells.**
  - **But plants can show mitotic divisions in both haploid and diploid cells.**

Prophase

- During prophase, the "first phase," several events must occur to provide access to the chromosomes in the nucleus.
- The nuclear envelope starts to break down.
- This is caused by phosphorylation of nuclear pore proteins and lamins, the intermediate filament cytoskeletal protein that provides structure to the nuclear envelope, by the cell cycle regulatory protein M-cyclin/Cdk
- Later, in telophase, these proteins will be dephosphorylated to reform the nuclear envelope.

Metaphase

- During metaphase, all of the chromosomes are aligned on the metaphase plate, or the equatorial plane, midway between the two poles of the cell.
- The sister chromatids are still tightly attached to each other, and the chromosomes are maximally condensed.

Anaphase

- During anaphase, the sister chromatids at the equatorial plane are held together by cohesins.
- Before the chromatids can be separated, the cohesins must be removed.
- This is regulated by a protein called Anaphase Promoting Complex (APC).
- Prior to anaphase, APC is in its inactive (dephosphorylated) state.

- At the beginning of anaphase, APC is phosphorylated by M-cyclin/Cdk and becomes active.
- APC is a ubiquitin ligase, meaning it can add a small peptide called ubiquitin to proteins.
- When ubiquitin ligases add certain polymers of ubiquitin to proteins (called polyubiquitination), it serves to "tag" the proteins for degradation by the proteasome.
- At the beginning of anaphase, APC polyubiquitinates the protein securin, causing secruin to be degraded by the proteasome.
- The degradation of securing, releases the protein separase.
- Separase is the enzyme that physically breaks down the cohesin proteins that held the sister chromatids together.

Telophase

- During telophase, all of the events that set up the duplicated chromosomes for mitosis during the first three phases are reversed.
- The chromosomes reach the opposite poles and begin to decondense (unravel).
- The mitotic spindles are broken down into monomers that will be used to assemble cytoskeleton components for each daughter cell.
- As previously mentioned, lamins and nuclear pore proteins are dephosphorylated, leading to the reformation of the nuclear envelope chromosomes.

Cytokinesis

- Cytokinesis is the second part of the mitotic phase during which cell division is completed by the physical separation of the cytoplasmic components into two daughter cells.
- Although the stages of mitosis are similar for most eukaryotes, the process of cytokinesis is quite different for eukaryotes that have cell walls, such as plant cells.

- In animal cells, cytokinesis begins following the onset of anaphase.
- A contractile ring composed of actin filaments forms just inside the plasma membrane at the former metaphase plate.
- The actin filaments pull the equator of the cell inward, forming a fissure.
- This fissure, or "crack," is called the cleavage furrow. The furrow deepens as the actin ring contracts, and eventually, the membrane and cell are cleaved in two.

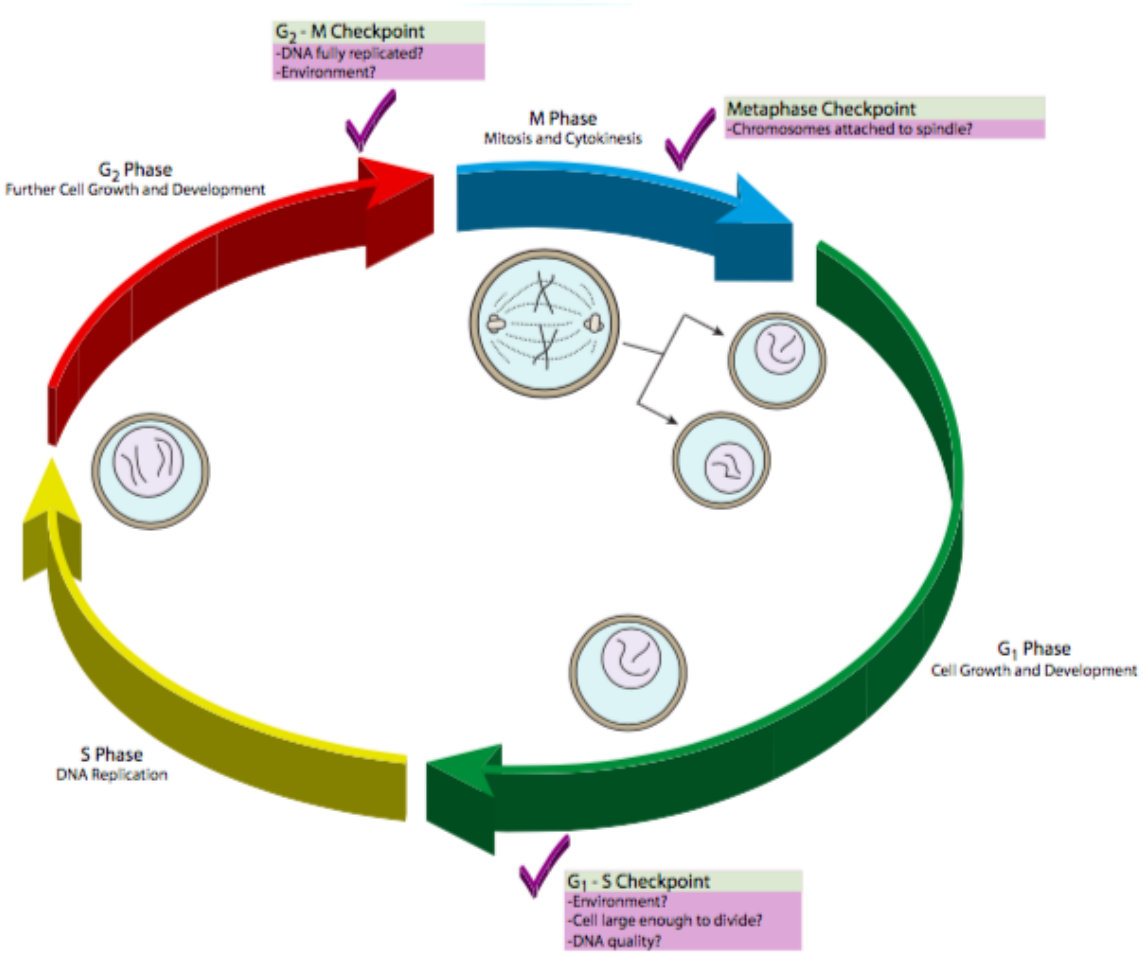
Regulation of cell cycle

- The length of the cell cycle is highly variable even within the cells of an individual organism.
- In humans, the frequency of cell turnover ranges from a few hours in early embryonic development to an average of two to five days for epithelial cells or an entire human lifetime spent in G0 by specialized cells such as cortical neurons or cardiac muscle cells.
- There is also variation in the time that a cell spends in each phase of the cell cycle.
- When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is approximately 24 hours.
- The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

Regulation at Internal Checkpoints

- Daughter cells must be exact duplicates of the parent cell.
- Mistakes in the duplication or distribution of the chromosomes can lead to mutations that may be passed forward to every new cell produced from the abnormal cell.

- To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints.
- The cell cycle can be stopped until conditions are favorable or errors have been corrected.
- These checkpoints occur at
    - at the end of **G1 (G1-S checkpoint)**
    - at the end of **G2 (G2–M checkpoint)**
    - during **metaphase (M checkpoint)**.



The G1-S Checkpoint

- The G1-S checkpoint determines whether all conditions are favorable for cell division to proceed and for DNA replication to occur during S phase.

- The G1-S checkpoint, also called the restriction point, is the point at which the cell irreversibly commits to the cell division process.
- In addition to adequate reserves and cell size, there is a check for damage to the genomic DNA and ensure there is space for a near cell at the G1-S checkpoint.
- A cell that does not meet all the requirements will "arrest" (pause or become quiescent) in G1 and will attempt to correct these deficiencies (e.g., repair DNA damage).
- If, during cell cycle arrest, the cell can meet the G1-S checkpoint criteria, the cell cycle will proceed to the S phase.
- If not, the cell will undergo apoptosis (programmed cell death)

The G2-M Checkpoint

- The G2-M checkpoint bars the entry to the mitotic phase if certain conditions are not met.
- As in the G1-S checkpoint, cell size and protein reserves are assessed.
- However, the most important role of the G2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged.
- Also similar to the G1-S checkpoint, the cell cycle will arrest at the G2-M transition if checkpoint criteria are not met and will attempt to repair the damage or undergo apoptosis if damaged severely.

The M Checkpoint

- The M checkpoint occurs near the end of the metaphase stage of mitosis.
- The M checkpoint is also known as the spindle checkpoint because it determines if all the sister chromatids are correctly attached to the spindle microtubules.
- Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each

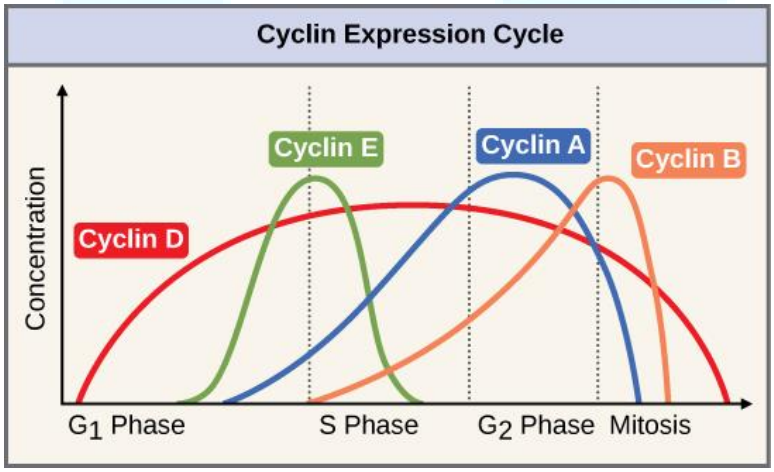
pair of sister chromatids are firmly anchored to spindle fibers arising from opposite poles of the cell.

- As with the previous checkpoints, cells that cannot proceed past this checkpoint will be eliminated by apoptosis.

Regulator Molecules of the Cell Cycle

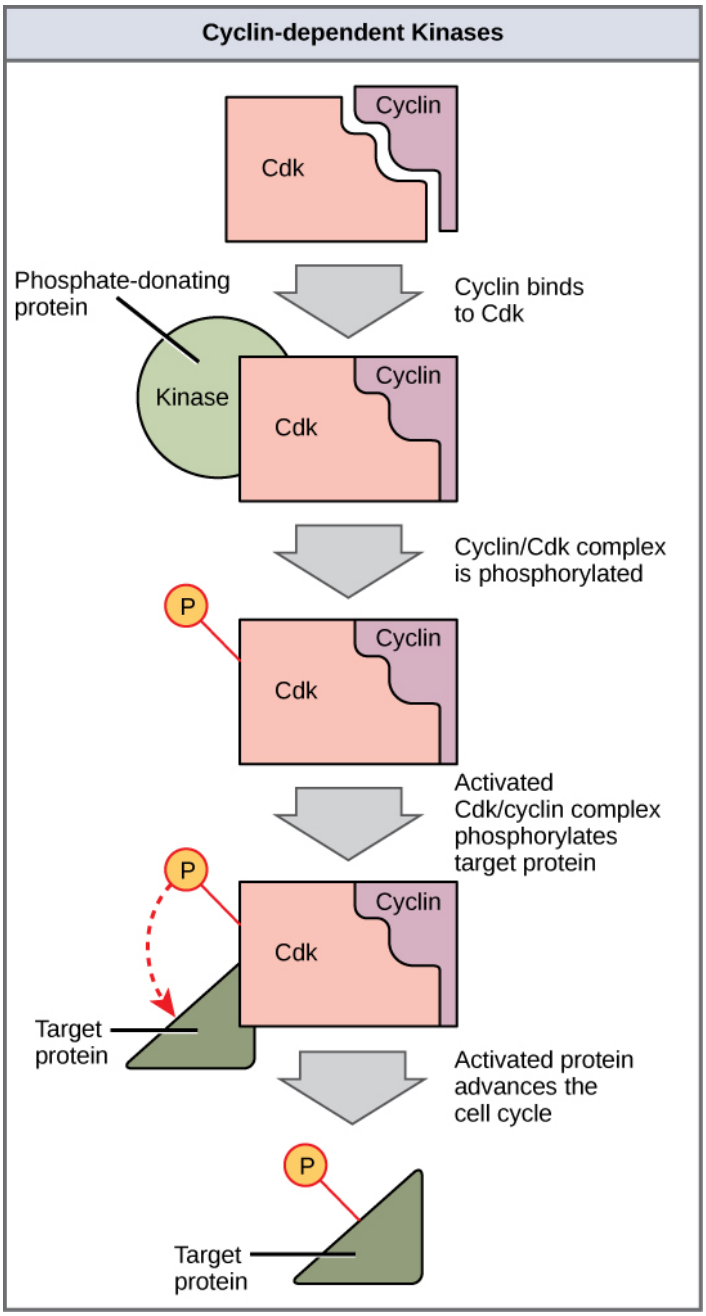
Positive Regulation of the Cell Cycle

- Two groups of proteins, called **cyclins and cyclin-dependent kinases (Cdks)**, are responsible for the progress of the cell through the various checkpoints.
- The levels of the four cyclin proteins fluctuate throughout the cell cycle in a predictable pattern.
- Increases in the concentration of cyclin proteins are triggered by both external and internal signals.
- After the cell moves to the next stage of the cell cycle, the cyclins that were active in the previous stage are degraded.



- Cyclins regulate the cell cycle only when they are tightly bound to Cdks.
- To be fully active, the Cdk/cyclin complex must also be phosphorylated in specific locations. Like all kinases, Cdks are enzymes (kinases) that phosphorylate other proteins.

- Phosphorylation activates the protein by changing its shape.
- The proteins phosphorylated by Cdks are involved in advancing the cell to the next phase.
- The levels of Cdk proteins are relatively stable throughout the cell cycle; however, the concentrations of cyclin fluctuate and determine when Cdk/cyclin complexes form.
- The different cyclins and Cdks bind at specific points in the cell cycle and thus regulate different checkpoints.
- Although the cyclins are the main regulatory molecules that determine the forward momentum of the cell cycle, there are several other mechanisms that fine tune the progress of the cycle with negative, rather than positive, effects.
- These mechanisms essentially block the progression of the cell cycle until problematic conditions are resolved.
- Molecules that prevent the full activation of Cdks are called Cdk inhibitors.
- Many of these inhibitor molecules directly or indirectly monitor a particular cell cycle event.
- The block placed on Cdks by inhibitor molecules will not be removed until the specific event being monitored is completed.



Negative Regulation of the Cell Cycle

- The second group of cell cycle regulatory molecules are negative regulators.
- Negative regulators halt the cell cycle.
- Remember that in positive regulation, active molecules cause the cycle to progress.

- The best understood negative regulatory molecules are **retinoblastoma protein (Rb), p53, and p21.**
- Retinoblastoma proteins are a group of tumor-suppressor proteins common in many cells.
- Much of what is known about cell cycle regulation comes from research conducted with cells that have lost regulatory control.
- All three of these regulatory proteins were discovered to be damaged or non-functional in cells that had begun to replicate uncontrollably (became cancerous).
- In each case, the main cause of the unchecked progress through the cell cycle was a faulty copy of the regulatory protein.
- Rb, p53, and p21 act primarily at the G1 checkpoint.
- p53 is a multi-functional protein that has a major impact on the cell's commitment to division; it acts when there is damaged DNA in cells that are undergoing the preparatory processes during G1.
- If damaged DNA is detected, p53 halts the cell cycle and recruits enzymes to repair the DNA. If the DNA cannot be repaired, p53 can trigger apoptosis (cell suicide) to prevent the duplication of damaged chromosomes.
- As p53 levels rise, the production of p21 is triggered.
- p21 enforces the halt in the cycle dictated by p53 by binding to and inhibiting the activity of the Cdk/cyclin complexes.
- As a cell is exposed to more stress, higher levels of p53 and p21 accumulate, making it less likely that the cell will move into the S phase.
- Rb exerts its regulatory influence on other positive regulator proteins.
- Rb monitors cell size. In the active, dephosphorylated state, Rb binds to proteins called transcription factors, most commonly to E2F.
- Transcription factors "turn on" specific genes, allowing the production of proteins encoded by that gene.
- When Rb is bound to E2F, production of proteins necessary for the G1/S transition is blocked.

- As the cell increases in size, Rb is slowly phosphorylated until it becomes inactivated.
- Rb releases E2F, which can now turn on the gene that produces the transition protein and this particular block is removed.
- For the cell to move past each of the checkpoints, all positive regulators must be "turned on" and all negative regulators must be "turned off."

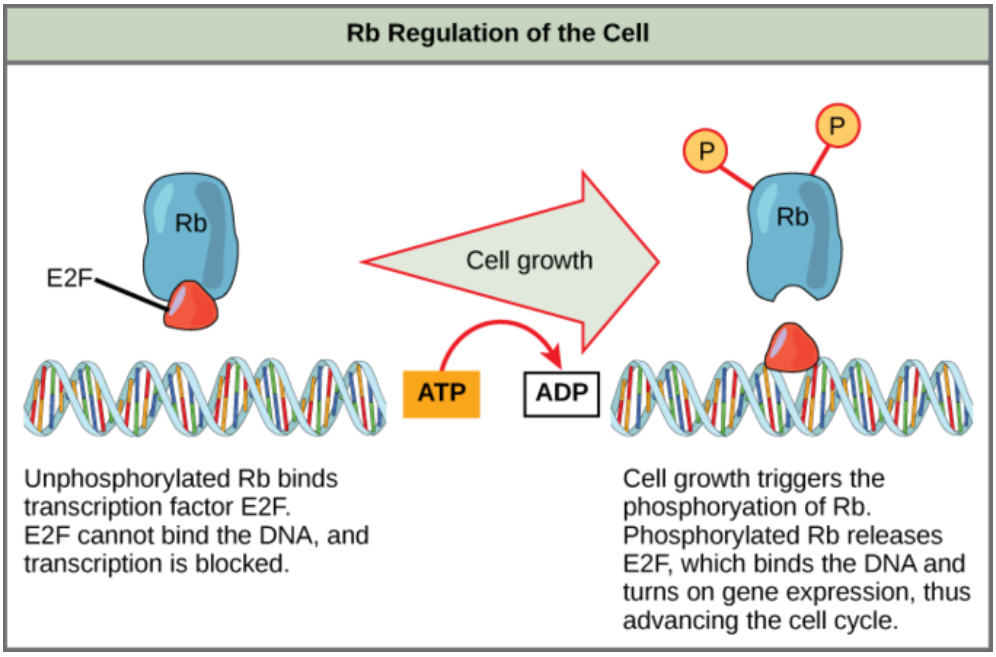


Figure: Function of the Rb Regulator Molecule

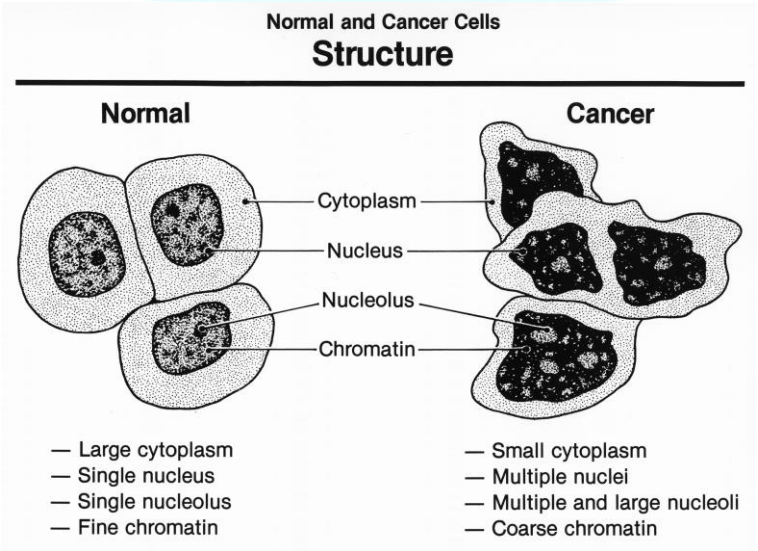
Key Points

- Two groups of proteins, cyclins and cyclin-dependent kinases (Cdks), are responsible for promoting the cell cycle.
- Cyclins regulate the cell cycle only when they are bound to Cdks; to be fully active, the Cdk/cyclin complex must be phosphorylated, which allows it to phosphorylate other proteins that advance the cell cycle.
- Negative regulator molecules (Rb, p53, and p21) act primarily at the G1 checkpoint and prevent the cell from moving forward to division until damaged DNA is repaired.
- p53 halts the cell cycle and recruits enzymes to repair damaged DNA; if DNA cannot be repaired, p53 triggers apoptosis to prevent duplication.

- Production of p21 is triggered by p53; p21 halts the cycle by binding to and inhibiting the activity of the Cdk/cyclin complex.
- Dephosphorylated Rb binds to E2F, which halts the cell cycle; when the cell grows, Rb is phosphorylated and releases E2F, which advances the cell cycle.

Cancer – Types and causes

- Cancer is one of the most feared diseases in the world and it affects over 11 lakh people every year in India alone.
- Worldwide, more than 10 million people succumb to this disease every year.
- In humans, cell differentiation and proliferation are highly manipulated and regularized by the cell division mechanism.
- **Uncontrolled cell division occurs when a process called contact inhibition fails.**
- In healthy organisms, during this process, when cells come in contact with other cells, the process of cell replication ceases.
- As a result, contact inhibition becomes a powerful anti-cancer mechanism, but it is lost in cancer cells. Hence, most types of cancer have tumours (except for cancers of the blood).



Types of Tumour

A tumour is classified into one of these three types based on its ability to undergo metastasis (spreading):

- Benign Tumour
    - These tumours are localized at a particular location in the body. Moreover, it does not spread to the other parts of the body and is generally harmless. However, when a benign tumour occurs in areas such as the brain, it can turn fatal. Treatment often involves surgery and it does not grow back.
- Malignant Tumour
    - These tumours are cancerous – meaning that they will grow quickly and spread to other normal tissues of the body. This ability to spread is called metastasis. Usually, cancer cells metastasize when it gets into the bloodstream or the lymph nodes and form secondary tumours across various sites in the body.
- Premalignant Tumour
    - This type of tumour may be benign but is observed to have the characteristics of a malignant tumour. It may not have metastasized yet, but it has the potential to turn cancerous. In other words, a premalignant tumour is a type of tumour that has an increased risk of becoming cancer. Benign tumours become premalignant and eventually, malignant.

Types of Cancer

From a medical perspective, cancer types can be classified based on the type of cell they originated from. Therefore cancer can be classified into:

- **Carcinoma**
    - The most common form of cancer, it originates from the epithelial cells.
- **Sarcoma**

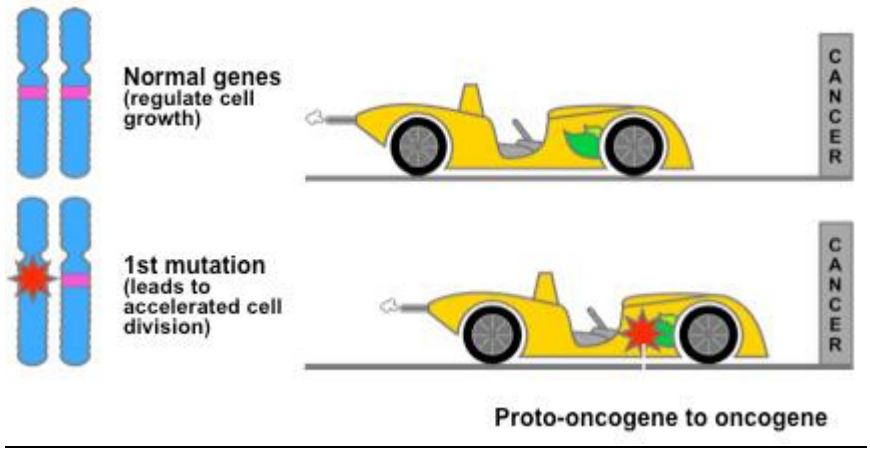
    - Originates from the connective tissues such as cartilage, fat and bone tissues.
- **Melanoma**
    - Originates from melanocytes, which are a type of cell that contains pigments.
- **Lymphoma & Leukaemia**
    - Originates from the cells that comprise blood (such as b lymphocytes or white blood cells)

Causes of cancer

- tobacco use
- high alcohol consumption
- an unhealthy diet, characterized by red and processed meat, sugary drinks and salty snacks, starchy foods, and refined carbohydrates including sugars and processed grains
- a lack of physical activity
- exposure to air pollution
- exposure to radiation
- unprotected exposure to UV light, such as sunlight
- infection by certain viruses including H. pylori, human papillomavirus (HPV), hepatitis B, hepatitis C, HIV, and the Epstein-Barr virus, which causes infectious mononucleosis.

Oncogenes and Tumour suppresser genes

Oncogenes



Proto-oncogene to oncogene

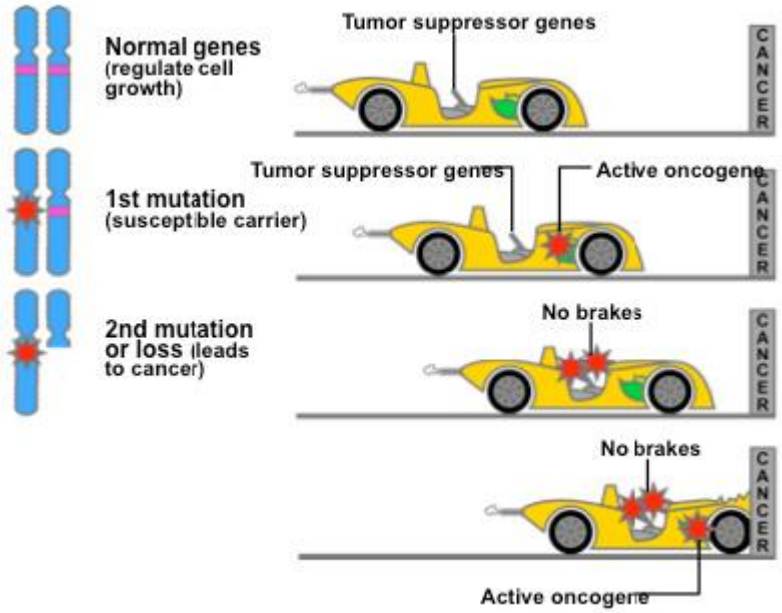
- An oncogene is a proto-oncogene that has been mutated in a way that leads to signals that cause uncontrolled growth- i.e., cancer.
- This is like pushing down on the gas pedal - you now have a gene that is telling the cell, "go, go, go; don't pay attention to the checkpoint and stop".
- Not all genes can mutate and form oncogenes.
- Only **"proto-oncogenes"** can mutate to form an oncogene.
- There are about 70 proto-oncogenes in our DNA.
- Oncogenes are mutated genes whose presence can stimulate the development of cancer.
- When oncogenes arise in normal cells, they can contribute to the development of cancer by telling cells to make proteins that stimulate excessive cell growth and division.
- Oncogenes are **"gain of function"** genes.
- They gain the ability to drive non-stop growth.
- In spite of their dominant activities, a single mutated oncogene usually isn't enough to cause cancer all by itself because tumor-suppressor genes are acting to put the brakes on to keep cell growth from getting out of control.
- Oncogenes aren't usually involved in inherited forms of cancer because most occur as somatic mutations and can't be passed from parent to child.

A few important oncogenes:

- **HER-2/neu**
  - HER-2/neu encodes for a cell surface receptor that can stimulate cell division. The HER-2/neu gene is amplified in up to 30% of human breast cancers.
- **RAS**
  - The Ras gene products are involved in kinase signaling pathways that ultimately control transcription of genes, regulating cell growth and differentiation.
- **MYC**
  - The Myc protein is a transcription factor and controls expression of several genes.
- **SRC**
  - Src was the first oncogene ever discovered. The Src protein is a tyrosine kinase, which regulates cell activity.
- **hTERT**
  - hTERT codes for an enzyme (telomerase) that maintains chromosome ends.

Tumour suppresser genes

- Tumor suppressor genes in normal cells act as braking signals during phase G1 of the cell cycle, to stop or slow the cell cycle before S phase.



- If tumor-suppressor genes are mutated, the normal brake mechanism will be disabled, resulting in uncontrolled growth, i.e. cancer.
- Mutations in tumor-suppressor genes cause loss-of-function.
- Loss-of-function mutations generally only show up when both copies of the gene are mutated. In other words, if a pair of tumor suppressor genes are lost or mutated, their functional absence might allow cancer to develop.
- Individuals who inherit an increased risk of developing cancer often are born with one defective copy of a tumor suppressor gene.
- Because genes come in pairs (one inherited from each parent), an inherited defect in one copy will not lead to cancer because the other normal copy is still functional.
- But if the second copy undergoes mutation, the person then may develop cancer because there no longer is any functional copy of the gene.

A few important tumor-suppressor genes:

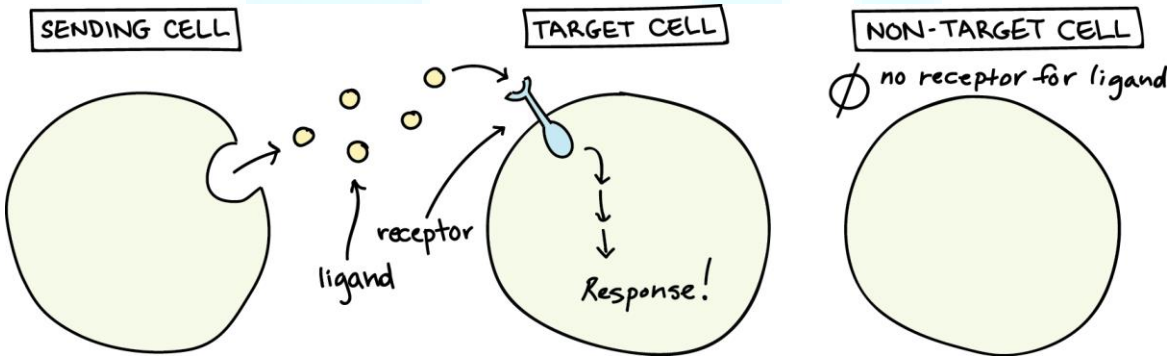
- **p53:** a transcription factor that regulates cell division and cell death.

- **Rb:** alters the activity of transcription factors and therefore controls cell division.
- **APC:** controls the availability of a transcription factor.

Cell signalling, signalling molecules, second messengers, ligands and receptors.

Cell signalling

- Cells typically communicate using chemical signals.
- These chemical signals, which are proteins or other molecules produced by a sending cell, are often secreted from the cell and released into the extracellular space.
- There, they can float – like messages in a bottle – over to neighboring cells.



- Not all cells can "hear" a particular chemical message.
- In order to detect a signal (that is, to be a target cell), a neighbor cell must have the right receptor for that signal.
- When a **signaling molecule** binds to its **receptor**, it alters the shape or activity of the receptor, triggering a change inside of the cell.
- Signaling molecules are often called **ligands**, a general term for molecules that bind specifically to other molecules (such as receptors).

- The message carried by a ligand is often relayed through a chain of chemical messengers inside the cell.
- Ultimately, it leads to a change in the cell, such as alteration in the activity of a gene or even the induction of a whole process, such as cell division.
- Thus, the original intercellular (between-cells) signal is converted into an intracellular (within-cell) signal that triggers a response.

Forms of signaling

There are four basic categories of chemical signaling found in multicellular organisms:

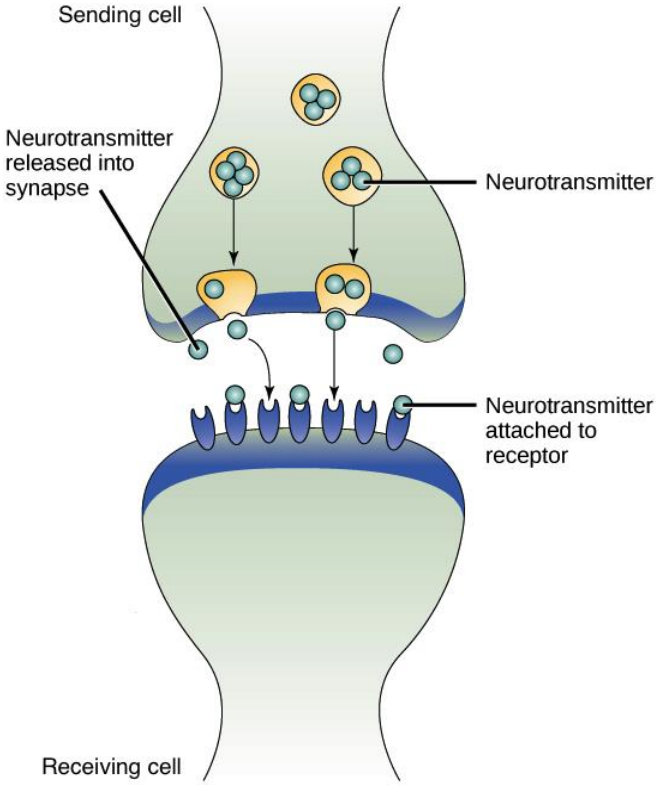
- **paracrine signaling**
- **autocrine signaling**
- **endocrine signaling**
- **signaling by direct contact**

1. **Paracrine signaling**

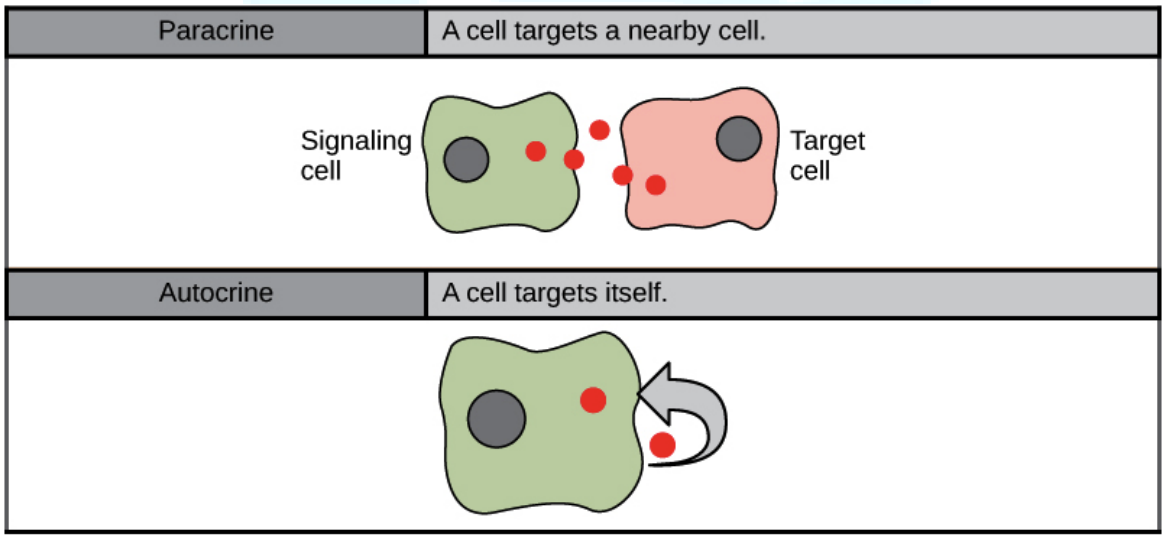
- Often, cells that are near one another communicate through the release of chemical messengers (ligands that can diffuse through the space between the cells).
- This type of signaling, in which cells communicate over relatively short distances, is known as paracrine signaling.
- Paracrine signaling allows cells to locally coordinate activities with their neighbors.
- Although they're used in many different tissues and contexts, paracrine signals are especially important during development, when they allow one group of cells to tell a neighboring group of cells what cellular identity to take on.

  - **Synaptic signaling**

- **One unique example of paracrine signaling** is synaptic signaling, in which nerve cells transmit signals.
- This process is named for the synapse, the junction between two nerve cells where signal transmission occurs.
- When the sending neuron fires, an electrical impulse moves rapidly through the cell, traveling down a long, fiber-like extension called an axon.
- When the impulse reaches the synapse, it triggers the release of ligands called neurotransmitters, which quickly cross the small gap between the nerve cells.
- When the neurotransmitters arrive at the receiving cell, they bind to receptors and cause a chemical change inside of the cell (often, opening ion channels and changing the electrical potential across the membrane).
- The neurotransmitters that are released into the chemical synapse are quickly degraded or taken back up by the sending cell.
- This "resets" the system so they synapse is prepared to respond quickly to the next signal.



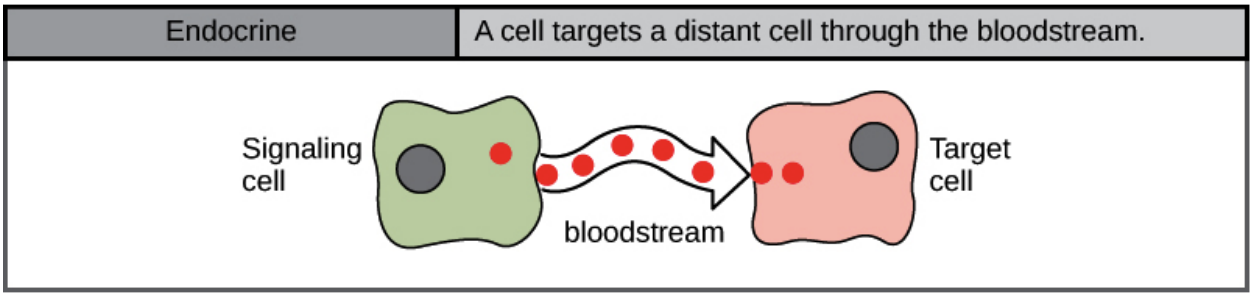
2. Autocrine signaling



- In autocrine signaling, a cell signals to itself, releasing a ligand that binds to receptors on its own surface (or, depending on the type of signal, to receptors inside of the cell).

- This may seem like an odd thing for a cell to do, but autocrine signaling plays an important role in many processes.
- For instance, autocrine signaling is important during development, helping cells take on and reinforce their correct identities.
- From a medical standpoint, autocrine signaling is important in cancer and is thought to play a key role in metastasis (the spread of cancer from its original site to other parts of the body).
- In many cases, a signal may have both autocrine and paracrine effects, binding to the sending cell as well as other similar cells in the area.

3. Endocrine signaling



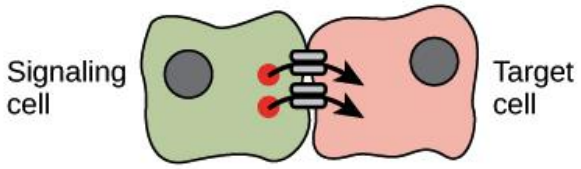
- When cells need to transmit signals over long distances, they often use the circulatory system as a distribution network for the messages they send.
- In long-distance endocrine signaling, signals are produced by specialized cells and released into the bloodstream, which carries them to target cells in distant parts of the body.
- Signals that are produced in one part of the body and travel through the circulation to reach far-away targets are known as hormones.
- In humans, endocrine glands that release hormones include the thyroid, the hypothalamus, and the pituitary, as well as the gonads (testes and ovaries) and the pancreas.
- Each endocrine gland releases one or more types of hormones, many of which are master regulators of development and physiology.

- For example, the pituitary releases growth hormone (GH), which promotes growth, particularly of the skeleton and cartilage.
- Like most hormones, GH affects many different types of cells throughout the body. However, cartilage cells provide one example of how GH functions: it binds to receptors on the surface of these cells and encourages them to divide.

4. Signaling through cell-cell contact

- Gap junctions in animals and plasmodesmata in plants are tiny channels that directly connect neighboring cells.
- These water-filled channels allow small signaling molecules, called intracellular mediators, to diffuse between the two cells.
- Small molecules and ions are able to move between cells, but large molecules like proteins and DNA cannot fit through the channels without special assistance.
- The transfer of signaling molecules transmits the current state of one cell to its neighbor. This allows a group of cells to coordinate their response to a signal that only one of them may have received.
- In plants, there are plasmodesmata between almost all cells, making the entire plant into one giant network.

Signaling across gap junctions	A cell targets a cell connected by gap junctions.



- In another form of direct signaling, two cells may bind to one another because they carry complementary proteins on their surfaces.
- When the proteins bind to one another, this interaction changes the shape of one or both proteins, transmitting a signal.

- This kind of signaling is especially important in the immune system, where immune cells use cell-surface markers to recognize “self” cells (the body's own cells) and cells infected by pathogens.

Second messengers

- Second messengers are intracellular signaling molecules released by the cell in response to exposure to extracellular signaling molecules—the first messengers. (Intercellular signals, a non-local form or cell signaling, encompassing both first messengers and second messengers, are classified as autocrine, juxtacrine, paracrine, and endocrine depending on the range of the signal.)
- Second messengers trigger physiological changes at cellular level such as proliferation, differentiation, migration, survival, apoptosis and depolarization.
- They are one of the triggers of intracellular signal transduction cascades.
- **Examples** of second messenger molecules include **cyclic AMP, cyclic GMP, inositol triphosphate, diacylglycerol, and calcium.**

Types of second messenger molecules

There are three basic types of secondary messenger molecules:

- **Hydrophobic molecules:** water-insoluble molecules such as diacylglycerol, and phosphatidylinositols, which are membrane-associated and diffuse from the plasma membrane into the intermembrane space where they can reach and regulate membrane-associated effector proteins.
- **Hydrophilic molecules:** water-soluble molecules, such as cAMP, cGMP, IP3, and Ca2+, that are located within the cytosol.

- **Gases:** nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H2S) which can diffuse both through cytosol and across cellular membranes.

These intracellular messengers have some **properties** in common:

- They can be synthesized/released and broken down again in specific reactions by enzymes or ion channels.
- Some (such as Ca2+) can be stored in special organelles and quickly released when needed.
- Their production/release and destruction can be localized, enabling the cell to limit space and time of signal activity.

Common mechanisms of second messenger systems

- There are several different secondary messenger systems (cAMP system, phosphoinositol system, and arachidonic acid system), but they all are quite similar in overall mechanism, although the substances involved and overall effects can vary.
- In most cases, a **ligand** binds to a membrane-spanning receptor protein molecule.
- The binding of a ligand to the receptor causes a conformation change in the receptor.
- This conformation change can affect the activity of the receptor and result in the production of active second messengers.
- In the case of **G protein-coupled receptors**, the conformation change exposes a binding site for a G-protein.
- The G-protein (named for the GDP and GTP molecules that bind to it) is bound to the inner membrane of the cell and consists of **three subunits: alpha, beta and gamma.**
- The G-protein is known as the **"transducer".**

- When the G-protein binds with the receptor, it becomes able to exchange a GDP (guanosine diphosphate) molecule on its alpha subunit for a GTP (guanosine triphosphate) molecule.
- Once this exchange takes place, the alpha subunit of the G-protein transducer breaks free from the beta and gamma subunits, all parts remaining membrane-bound.
- The alpha subunit, now free to move along the inner membrane, eventually contacts another membrane-bound protein - the "primary effector."

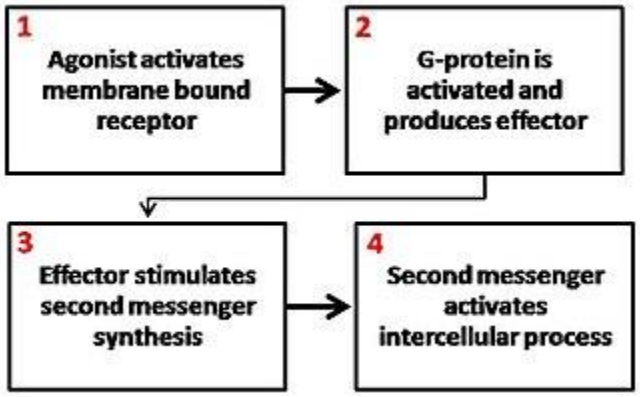


Fig: General Schematic of Second Messenger Mechanism

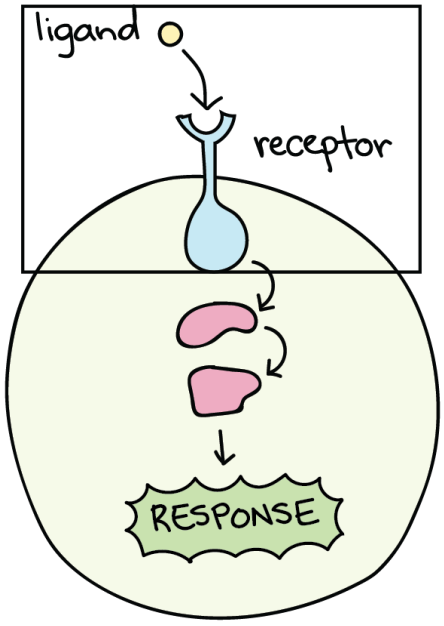
- The primary effector then has an action, which creates a signal that can diffuse within the cell.
- This signal is called the **"second (or secondary) messenger."**
- The secondary messenger may then activate a **"secondary effector"** whose effects depend on the particular secondary messenger system.
- **Calcium ions** are one type of second messengers and are responsible for many important physiological functions including muscle contraction, fertilization, and neurotransmitter release.
- The ions are normally bound or stored in intracellular components (such as the endoplasmic reticulum(ER)) and can be released during signal transduction.

- The enzyme phospholipase C produces diacylglycerol and inositol trisphosphate, which increases calcium ion permeability into the membrane.
- Active G-protein open up calcium channels to let calcium ions enter the plasma membrane.
- The other product of phospholipase C, diacylglycerol, activates protein kinase C, which assists in the activation of cAMP (another second messenger).

Ligands and receptors

A complex signaling pathway inside of a cell begins with a single key event – the binding of a **signaling molecule**, or **ligand,** to **its receiving molecule, or receptor.**

Receptors and ligands come in many forms, but they all have one thing in common: they come in closely matched pairs, with a receptor recognizing just one (or a few) specific ligands, and a ligand binding to just one (or a few) target receptors. Binding of a ligand to a receptor changes its shape or activity, allowing it to transmit a signal or directly produce a change inside of the cell.

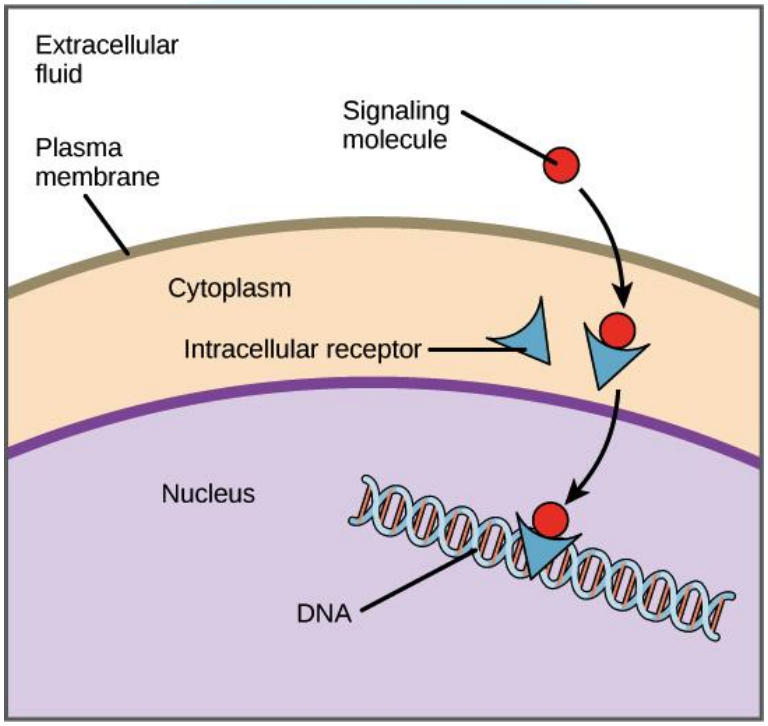


Types of receptors

Receptors come in many types, but they can be divided into two categories: intracellular receptors, which are found inside of the cell (in the cytoplasm or nucleus), and cell surface receptors, which are found in the plasma membrane.

1. Intracellular receptors

- Intracellular receptors are receptor proteins found on the inside of the cell, typically in the cytoplasm or nucleus.



- In most cases, the ligands of intracellular receptors are small, hydrophobic (water-hating) molecules, since they must be able to cross the plasma membrane in order to reach their receptors.
- For example, the primary receptors for hydrophobic steroid hormones, such as the sex hormones estradiol (an estrogen) and testosterone, are intracellular.

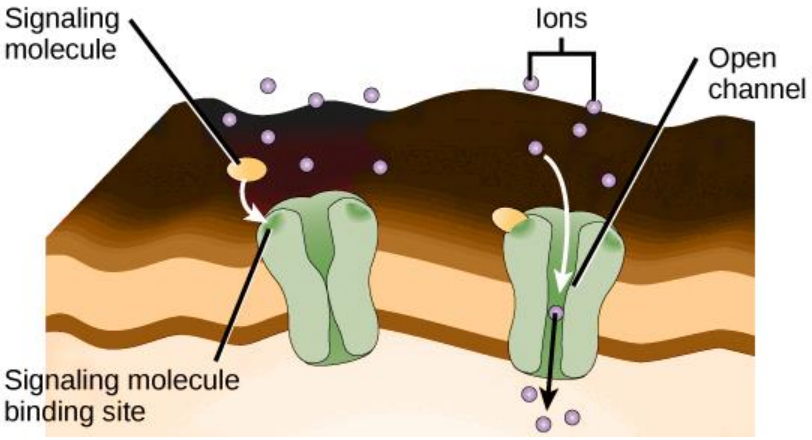
- When a hormone enters a cell and binds to its receptor, it causes the receptor to change shape, allowing the receptor-hormone complex to enter the nucleus (if it wasn't there already) and regulate gene activity.
- Hormone binding exposes regions of the receptor that have DNA-binding activity, meaning they can attach to specific sequences of DNA.
- These sequences are found next to certain genes in the DNA of the cell, and when the receptor binds next to these genes, it alters their level of transcription.
- Many signaling pathways, involving both intracellular and cell surface receptors, cause changes in the transcription of genes.
- However, intracellular receptors are unique because they cause these changes very directly, binding to the DNA and altering transcription themselves.

2. Cell-surface receptors

- Cell-surface receptors are membrane-anchored proteins that bind to ligands on the outside surface of the cell.
- In this type of signaling, the ligand does not need to cross the plasma membrane. So, many different kinds of molecules (including large, hydrophilic or "water-loving" ones) may act as ligands.
- A typical cell-surface receptor has three different domains, or protein regions: a extracellular ("outside of cell") ligand-binding domain, a hydrophobic domain extending through the membrane, and an intracellular ("inside of cell") domain, which often transmits a signal.
- The size and structure of these regions can vary a lot depending on the type of receptor, and the hydrophobic region may consist of multiple stretches of amino acids that criss-cross the membrane.
- There are many kinds of cell-surface receptors, but here we'll look at three common types:
    - **ligand-gated ion channels**
    - **G protein-coupled receptors**

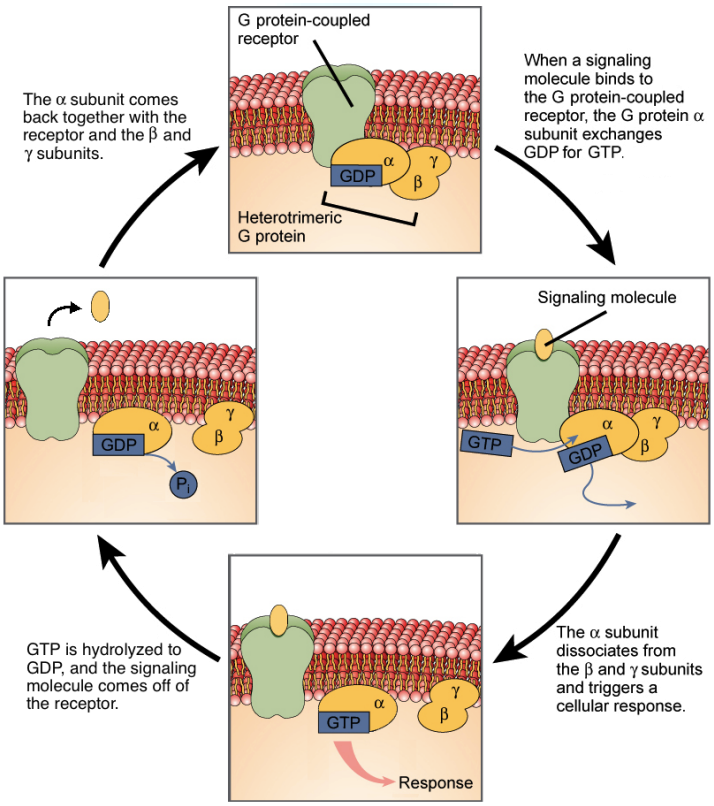
- **receptor tyrosine kinases**

- **Ligand-gated ion channels**
  - Ligand-gated ion channels are ion channels that can open in response to the binding of a ligand.
  - To form a channel, this type of cell-surface receptor has a membrane-spanning region with a hydrophilic (water-loving) channel through the middle of it.
  - The channel lets ions to cross the membrane without having to touch the hydrophobic core of the phospholipid bilayer.
  - When a ligand binds to the extracellular region of the channel, the protein's structure changes in such a way that ions of a particular type, such as Ca^{2+} or Cl^-, can pass through.
  - In some cases, the reverse is actually true: the channel is usually open, and ligand binding causes it to close.
  - Changes in ion levels inside the cell can change the activity of other molecules, such as ion-binding enzymes and voltage-sensitive channels, to produce a response.
  - Neurons, or nerve cells, have ligand-gated channels that are bound by neurotransmitters.



- **G protein-coupled receptors**
  - G protein-coupled receptors (GPCRs) are a large family of cell surface receptors that share a common structure and method of signaling.
  - The members of the GPCR family all have seven different protein segments that cross the membrane, and they transmit signals inside the cell through a type of protein called a G protein.
  - GPCRs are diverse and bind many different types of ligands.
  - One particularly interesting class of GPCRs is the odorant (scent) receptors. There are about 800 of them in humans, and each binds its own "scent molecule" – such as a particular chemical in perfume, or a certain compound released by rotting fish – and causes a signal to be sent to the brain, making us smell a smell.
  - When its ligand is not present, a G protein-coupled receptor waits at the plasma membrane in an inactive state. For at least some types of GPCRs, the inactive receptor is already docked to its signaling target, a G protein.

- G proteins come in different types, but they all bind the nucleotide guanosine triphosphate (GTP), which they can break down (hydrolyze) to form GDP.
- A G protein attached to GTP is active, or "on," while a G protein that's bound to GDP is inactive, or "off."
- The G proteins that associate with GPCRs are a type made up of three subunits, known as heterotrimeric G proteins.
- When they're attached to an inactive receptor, they're in the "off" form (bound to GDP).

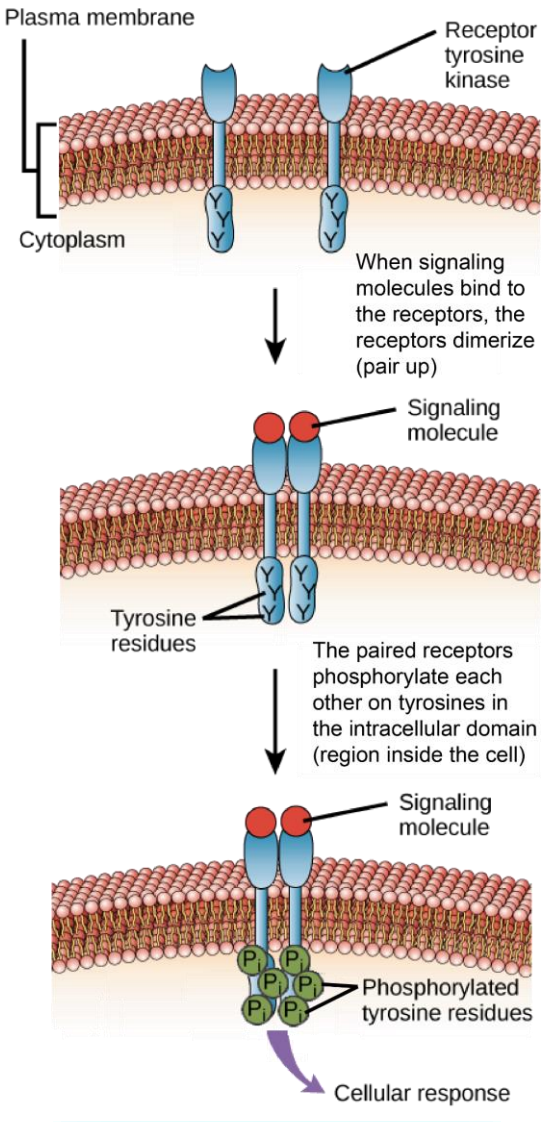


- Ligand binding, however, changes the picture: the GPCR is activated and causes the G protein to exchange GDP for GTP.
- The now-active G protein separates into two pieces (one called the α subunit, the other consisting of the β and γ subunits), which are freed from the GPCR.

- The subunits can interact with other proteins, triggering a signaling pathway that leads to a response.
- Eventually, the α subunit will hydrolyze GTP back to GDP, at which point the G protein becomes inactive.
- The inactive G protein reassembles as a three-piece unit associated with a GPCR.
- Cell signaling using G protein-coupled receptors is a cycle, one that can repeat over and over in response to ligand binding.
- G protein-coupled receptors play many different roles in the human body, and disruption of GPCR signaling can cause disease.

- **Receptor tyrosine kinases**
  - Enzyme-linked receptors are cell-surface receptors with intracellular domains that are associated with an enzyme.
  - In some cases, the intracellular domain of the receptor actually is an enzyme that can catalyze a reaction. Other enzyme-linked receptors have an intracellular domain that interacts with an enzyme.
  - Receptor tyrosine kinases (RTKs) are a class of enzyme-linked receptors found in humans and many other species.
  - A kinase is just a name for an enzyme that transfers phosphate groups to a protein or other target, and a receptor tyrosine kinase transfers phosphate groups specifically to the amino acid tyrosine.
  - Signaling molecules first bind to the extracellular domains of two nearby receptor tyrosine kinases.

- The two neighboring receptors then come together, or dimerize.
- The receptors then attach phosphates to tyrosines in each others' intracellular domains.
- The phosphorylated tyrosine can transmit the signal to other molecules in the cell.
- In many cases, the phosphorylated receptors serve as a docking platform for other proteins that contain special types of binding domains.
- A variety of proteins contain these domains, and when one of these proteins binds, it can initiate a downstream signaling cascade that leads to a cellular response.
- Receptor tyrosine kinases are crucial to many signaling processes in humans.
- For instance, they bind to growth factors, signaling molecules that promote cell division and survival.
- Growth factors include **platelet-derived growth factor (PDGF)**, which participates in wound healing, and **nerve growth factor (NGF)**, which must be continually supplied to certain types of neurons to keep them alive.
- Because of their role in growth factor signaling, receptor tyrosine kinases are essential in the body, but their activity must be kept in balance: overactive growth factor receptors are associated with some types of cancers.

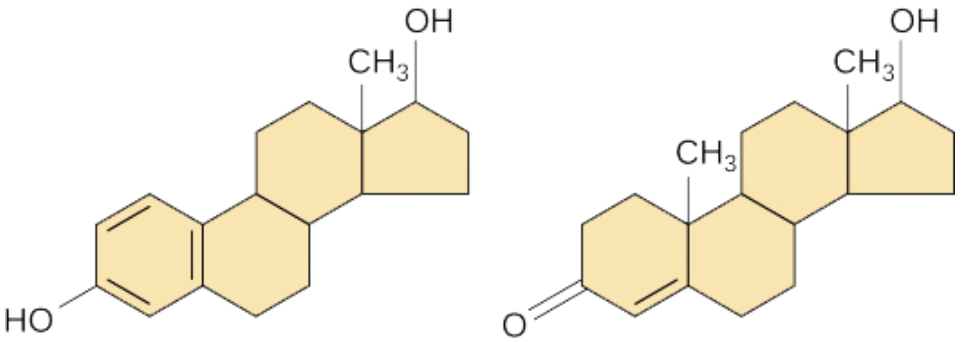


Types of ligands

Ligands, which are produced by signaling cells and interact with receptors in or on target cells, come in many different varieties. Some are proteins, others are hydrophobic molecules like steroids, and others yet are gases like nitric oxide. Here, we'll look at some examples of different types of ligands.

Ligands that can enter the cell

- Small, hydrophobic ligands can pass through the plasma membrane and bind to intracellular receptors in the nucleus or cytoplasm.

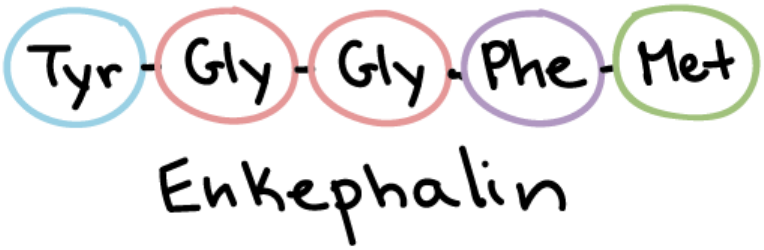


Estradiol **Testosterone**

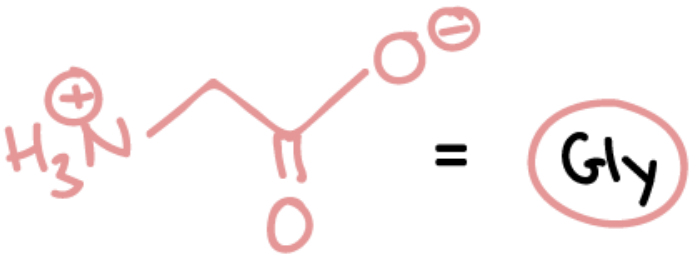
- In the human body, some of the most important ligands of this type are the steroid hormones.
- Familiar steroid hormones include the female sex hormone estradiol, which is a type of estrogen, and the male sex hormone testosterone.
- Vitamin D, a molecule synthesized in the skin using energy from light, is another example of a steroid hormone.
- Because they are hydrophobic, these hormones don't have trouble crossing the plasma membrane, but they must bind to carrier proteins in order to travel through the (watery) bloodstream.
- Nitric oxide (NO) is a gas that acts as a ligand.
- Like steroid hormones, it can diffuse directly across the plasma membrane thanks to its small size.
- One of its key roles is to activate a signaling pathway in the smooth muscle surrounding blood vessels, one that makes the muscle relax and allows the blood vessels to expand (dilate).
- In fact, the drug nitroglycerin treats heart disease by triggering the release of NO, dilating vessels to restore blood flow to the heart.
- NO has become better-known recently because the pathway that it affects is targeted by prescription medications for erectile dysfunction, such as Viagra.

Ligands that bind on the outside of the cell

- Water-soluble ligands are polar or charged and cannot readily cross the plasma membrane.
- So, most water-soluble ligands bind to the extracellular domains of cell-surface receptors, staying on the outer surface of the cell.
- Peptide (protein) ligands make up the largest and most diverse class of water-soluble ligands.
- For instance, growth factors, hormones such as insulin, and certain neurotransmitters fall into this category. Peptide ligands can range from just a few amino acids long, as in the pain-suppressing enkephalins, to a hundred or more amino acids in length.



Enkephalin



Glycine

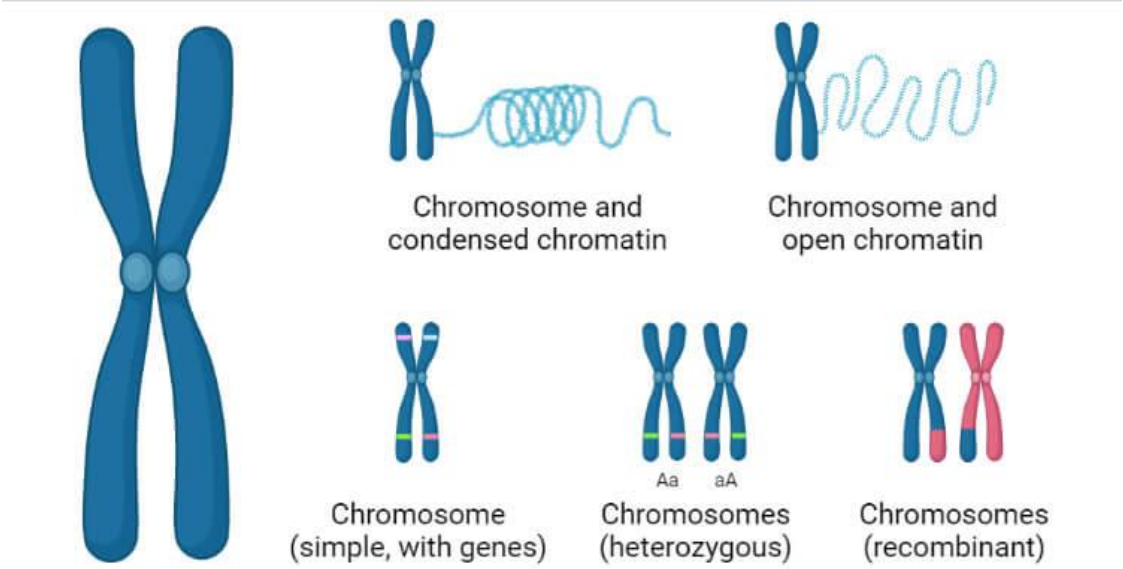
- Some neurotransmitters are proteins.
- Many other neurotransmitters, however, are small, hydrophilic (water-loving) organic molecules. Some neurotransmitters are standard amino acids, such as glutamate and glycine, and others are modified or non-standard amino acids.

ENTRI

Chromosome-Structure, types

What are Chromosomes?

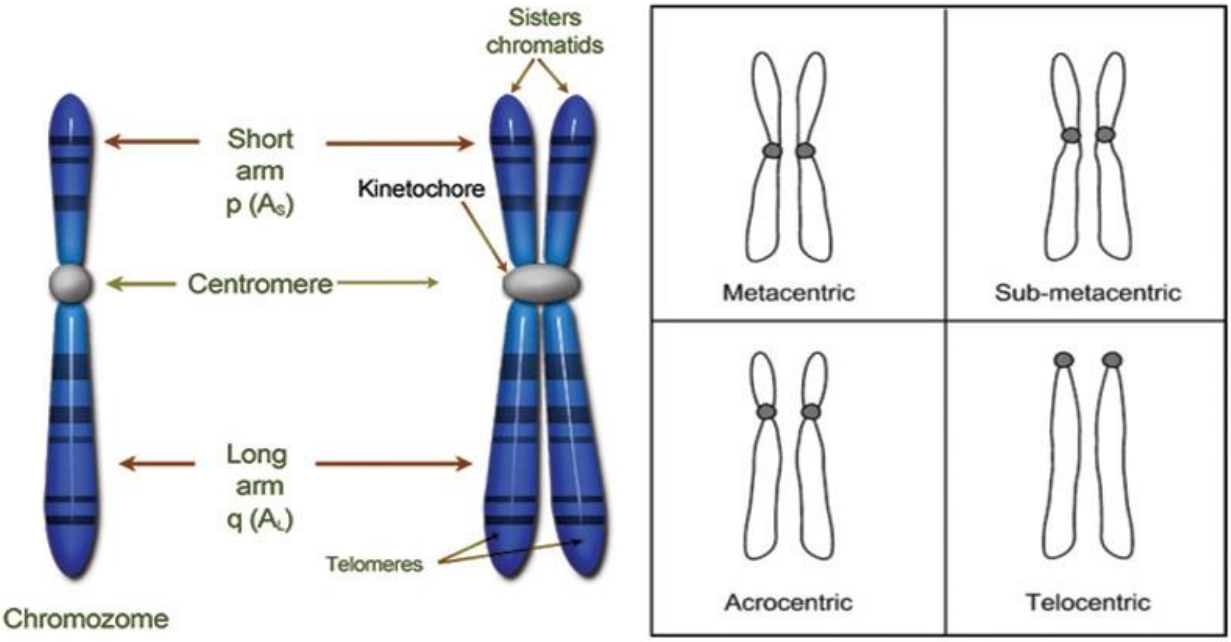
- In the nucleus of each cell, the DNA molecule is packaged into thread-like structures called chromosomes.
- Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure.
- Chromosomes were first described by Strasburger (1815), and the term 'chromosome' was first used by Waldeyer in 1888.
- They appear as rod-shaped dark stained bodies during the metaphase stage of mitosis when cells are stained with a suitable basic dye and viewed under a light microscope.



- Chromosomes are the nuclear components of the special organization, individuality, and function that are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species.
- Each chromosome is made up of DNA tightly coiled many times around proteins that support its structure.

- The proteins that bind to the DNA to form eukaryotic chromosomes are traditionally divided into two classes: the histones and the non-histone chromosomal proteins.
- The complex of both classes of protein with the nuclear DNA of eukaryotic cells is known as chromatin.
- Chromatin are a highly compacted structure consisting of packaged DNA and necessary so as to fit DNA into the nucleus.
- The assembly of DNA into chromatin involves a range of events, beginning with the formation of the basic unit, the nucleosome, and ulti-mately giving rise to a complex organization of specific domains within the nucleus.
- In the first step of this process, DNA is condensed into an 11 nm fiber that represents an approximate 6-fold level of compaction. This is achieved through nucleosome assembly.
- The nucleosome is the smallest structural component of chromatin and is produced through interactions between DNA and histone proteins.
- Each nucleosome consists of histone octamer core, assembled from the histones H2A, H2B, H3 and H4 (or other histone variants in some cases) and a segment of DNA that wraps around the histone core. Adjacent nucleosomes are connected via “linker DNA”.

Structure of a Chromosome



- In eukaryotes the chromosomes are multiple large, linear and are present in the nucleus of the cell.
- Each chromosome typically has one centromere and one or two arms that project from the centromere.
- Structurally, each chromosome is differen-tiated into three parts—
    - **Pellicle**
    - **Matrix**
    - **Chromonemata**

    - **Pellicle**
        - It is the outer envelope around the substance of chromosome.
        - It is very thin and is formed of achromatic substances.

    - **Matrix**
        - It is the ground substance of chromosome which contains the chromonemata.
        - It is also formed of non-genic materials.

- **Chromonemata**
  - Embedded in the matrix of each chromosome are two identical, spirally coiled threads, the chromonemata.
  - The two chromonemata are also tightly coiled together that they appear as single thread of about 800A thickness.
  - Each chromonemata consists of about 8 mi-crofibrils, each of which is formed of a double helix of DNA.

In mitotic metaphase chromosomes, the following structural feature (except chromomere) can be seen under the light mi-croscope:

(1) Chromatid
(2) Chromonema
(3) Chromomeres,
(4) Centromere
(5) Secondary constriction or Nucleolar organizer
(6) Telomere
(7) Satellite

Centromere

- A small structure in the chromonema, marked by a constriction which is recognised as permanent structure in the chromosome is termed as the centromere.
- At this point the two chromonemata are joined together.
- It is known as centromere or kinetochore or primary constriction.
- It divides the chromosome into two sections, or “arms.” The short arm of the chromosome is labeled the “p arm.” The long arm of the chromosome is labeled the “q arm.”
- Its position is constant for a given type of chromosome and forms a feature of identification.

- In thin electron microscopic sections, the kinetochore shows a trilaminar structure, i.e., a 10 nm thick dense outer protein aceous layer, a middle layer of low density and a dense inner layer tightly bound to the centromere.
- The chromosomes are attached to spindle fibres at this region during cell division.

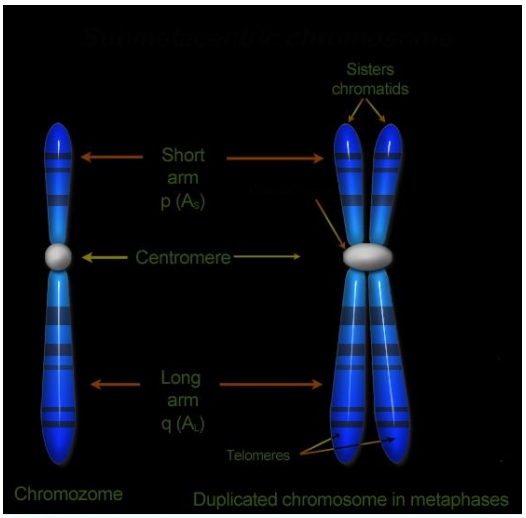
Secondary Constriction or Nucleolar Organiser

- The chromosome besides having the primary constriction or the centromere possesses second-ary constriction at any point of the chromosome.
- Constant in their position and extent, these constrictions are useful in identifying particular chromosomes in a set.
- The chromosome region distal to the secondary constriction i.e., the region between the secondary constriction and the nearest telomere is known as satellite.
- Therefore, chromosomes having secondary constrictions are called satellite chromo-somes or sat-chromosomes.
- Nucleolus is always associated with the secondary constriction of sat-chromosomes. There-fore, secondary constrictions are also called nucleolus organiser region (NOR) and sat-chromo-somes are often referred to as nucleolus organiser chromosomes.

Telomeres

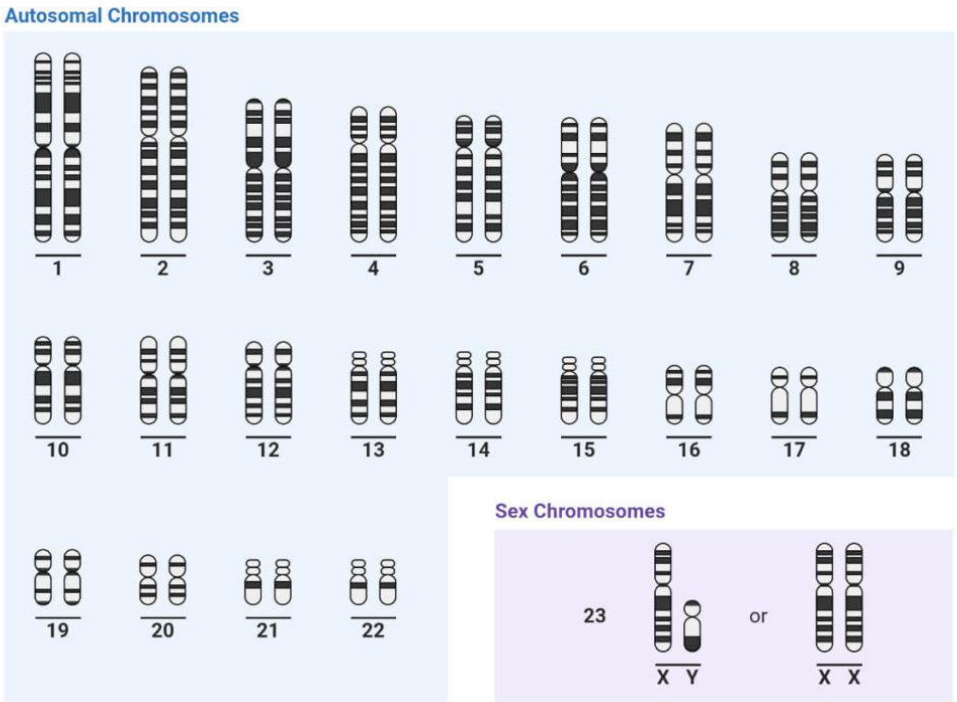
- These are specialized ends of a chromosome which exhibits physiological differentiation and polarity.
- Each extremity of the chromosome due to its polarity prevents other chromosomal seg-ments to be fused with it. The chromosomal ends are known as the telomeres.

- If a chromosome breaks, the broken ends can fuse with each other due to lack of telomere.



Types of Chromosomes

A. Autosomes and Sex Chromosomes

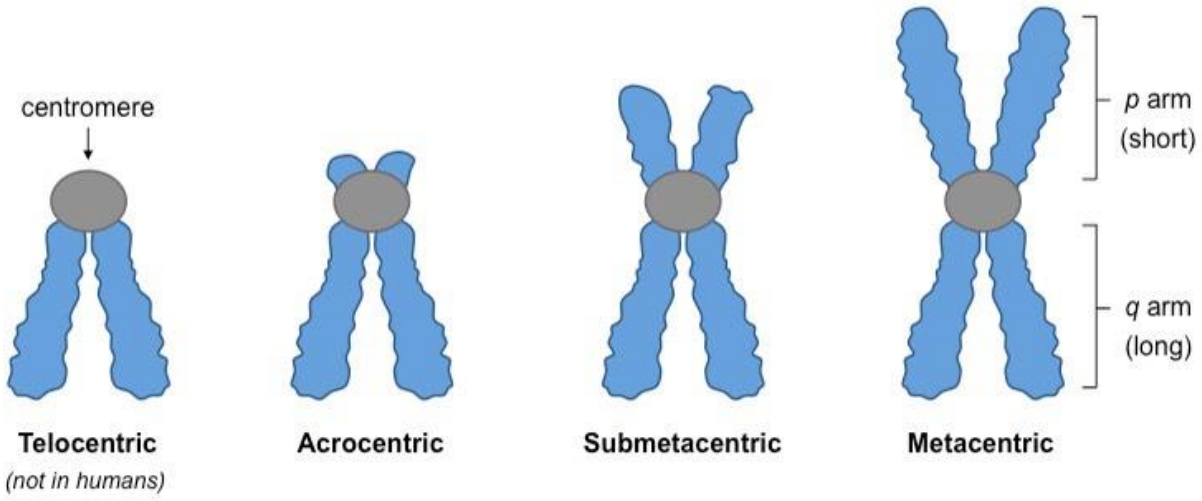


- Human chromosomes are of two types- autosomes and sex chromosomes.
- Genetic traits that are linked to the sex of the person are passed on through the sex chromosomes. The rest of the genetic information is present in the autosomes.
- Humans have 23 pairs of chromosomes in their cells, of which 22 pairs are autosomes and one pair of sex chromosomes, making a total of 46 chromosomes in each cell.

B. On the Basis of Number of Centromeres

- **Monocentric** with one centromere.
- **Dicentric** with two centromeres.
- **Polycentric** with more than two centromeres
- **Acentric** without centromere. Such chromosomes represent freshly broken segments of chromosomes which do not survive for long.
- **Diffused or non-located** with indistinct centromere diffused throughout the length of chromosome.

C. On the Basis of Location of Centromere



- **Telocentric** are rod-shaped chromosomes with centromere occupying the terminal position, so that the chromosome has just one arm.
- **Acrocentric** are also rod-shaped chromosomes with centromere occupying a sub-terminal position. One arm is very long and the other is very short.
- **Sub-metacentric** chromosomes are with centromere slightly away from the mid-point so that the two arms are unequal.
- **Metacentric** are V-shaped chromosomes in which centromere lies in the middle of chro-mosome so that the two arms are almost equal.

Prokaryotic Chromosomes

- The DNA of a bacterial cell, such as Escherichia coli, is a circular double-stranded molecule often referred to as the bacterial chromosome.
- The circular DNA is packaged into a region of the cell called the nucleoid where it is organized into 50 or so loops or domains that are bound to a central protein scaffold, attached to the cell membrane.
- The DNA is negatively supercoiled, that is, it is twisted upon itself.
- It is complexed with several DNA-binding proteins, the most common of which are proteins HU, HLP-1 and H-NS. These are histone-like proteins.

Eukaryotic Chromosomes

- The large amount of genomic DNA in a eukaryotic cell is tightly packaged in chromosomes contained within a specialized organelle, the nucleus.
- With the exception of the sex chromosomes, diploid eukaryotic organisms such as humans have two copies of each chromosome, one inherited from the father and one from the mother.
- Chromosomes contain both DNA and protein.
- Most of the protein on a weight basis is histones, but there are also many thousands of other proteins found in far less abundance and these are collectively called non- histone proteins (NHP).
- This nuclear DNA–protein complex is called chromatin.

- In the nucleus, each chromosome contains a single linear double-stranded DNA molecule.
- The length of the packaged DNA molecule varies. In humans, the shortest DNA molecule in a chromosome is about 1.6 cm and the longest is about 8.4 cm.
- The extensive packaging of DNA in chromosomes results from three levels of folding involving nucleosomes, 30 nm filaments and radial loops.

Euchromatin and Heterochromatin

- The DNA sequence is maintained through a series of processes and is condensed into 46 Chromosomes in Humans.
- The number of Chromosomes varies for every species.
- These Chromosomes undergo further condensation through two ways called mitosis or meiosis.
- On the other hand, interphase Chromosomes also undergo a series of events like DNA folding, wrapping, and bending which are facilitated by Histones.
- The combination of DNA and Histone Proteins in the nuclear matter is termed as **Chromatin**.
- Chromatin consists of 1147 base pairs of DNA wrapped around the Protein core histone. The histone is made of 2 units of H2A, H2B, H3, and H4 forming an octamer.
- The chromatin in the interphase is generally classified into **two parts:**
    - **Euchromatin**
    - **Heterochromatin**

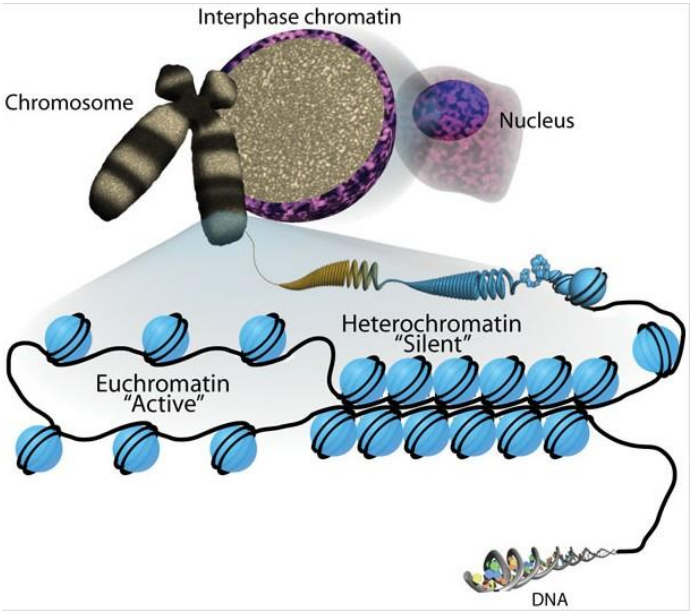
Euchromatin

- A region in which DNA is accessible and is present in an open confrontation because of the relaxed state of Nucleosome arrangements is referred to as Euchromatin.

- Euchromatin is associated with the presence of high levels of proteins in the chromatin.
- In other words, Euchromatin is made up of histones and protamines.
- Histones are a group of DNA binding proteins.
- They play a vital role in regulating the process of gene transcription and thus play an important role in the maintenance of chromosomal organization and cellular function.
- Histone also maintains the condensation of the chromatin fiber in the nucleus.
- Protamines are a sub-family of the histone proteins and play an important role in regulating the transition of the chromatin into the Euchromatin.
- Euchromatin and Heterochromatin are two structural units that help in maintaining the condensation of the chromatin.
- There are also other structural units called facultative Heterochromatin and constitutive Heterochromatin.

Structure of Euchromatin

- Euchromatin majorly has unmethylated first gene exons.
- They exist in decondensed form and are present in the distal arms of the Chromosome.
- Euchromatin is spread all around the nucleus and is replicated during the whole S Phase.
- It is generally known as the transcriptionally active form of chromatin. Euchromatin has less compact structure and is usually referred to as 11 nm fiber with the presence of beads on a string.
- The beads represent nucleosomes and string refers to DNA.



Functions of Euchromatin

- The chromatin which is involved in the active transcription of DNA into mRNA is Euchromatin.
- As Euchromatin is more open in order to allow the recruitment of RNA polymerase complexes and gene regulatory Proteins, transcription can be initiated.

Heterochromatin

A functionally different genomic compartment which has relatively low gene density along with a highly compact chromatin structure is referred to as Heterochromatin.

- There are **two kinds of Heterochromatin:**
    - **'Constitutive Heterochromatin'** is virtually present in all stages of an organism's life cycle.
    - **'Facultative Heterochromatin'** occurs in one of a pair of homologs.
- Heterochromatin can epigenetically administer the expression of nearby genes resulting in varied phenotypes in genetically identical cells.

- Biochemical and genetic approaches show that the RNAi machinery plays an important role in the formation of Heterochromatin.
- Heterochromatin is the opposite of Euchromatin.
- Euchromatin and Heterochromatin are structural units.
- It is associated with the presence of DNA or **histone protein.**
- Heterochromatin is a part of the chromatin.
- It is associated with the presence of **histone H3** and Heterochromatin proteins.
- These Heterochromatin proteins consist of proteins of the family of HP1, PH1, and HIRA.
- The Heterochromatin proteins are associated with the repressive histone marks. These proteins are enriched in the Heterochromatin regions.
- The organization of the genetic material into distinct compartments, or domains, within the nucleus is called **chromatin structure.**
- The DNA in a Euchromatin region is loosely packaged and is relatively accessible.
- In this form, the DNA is in a transcriptionally active state.
- A key process that contributes to this accessibility is DNA replication.
- In this process, the genetic material in Euchromatin is replicated and distributed in multiple replication sites, or replication forks, along the length of a Chromosome.
- The DNA in Heterochromatin is tightly packaged.
- These tightly packaged regions have no or very few replication sites.
- Therefore, the DNA in Heterochromatin is in a transcriptionally repressed state.
- Transcriptional activity is also repressed in Heterochromatin.
- This is due in large part to the presence of methyl groups on the DNA in Heterochromatin.
- Methylation of the DNA prevents transcriptional activity.
- This process is called **transcriptional silencing.**

Structure of Heterochromatin

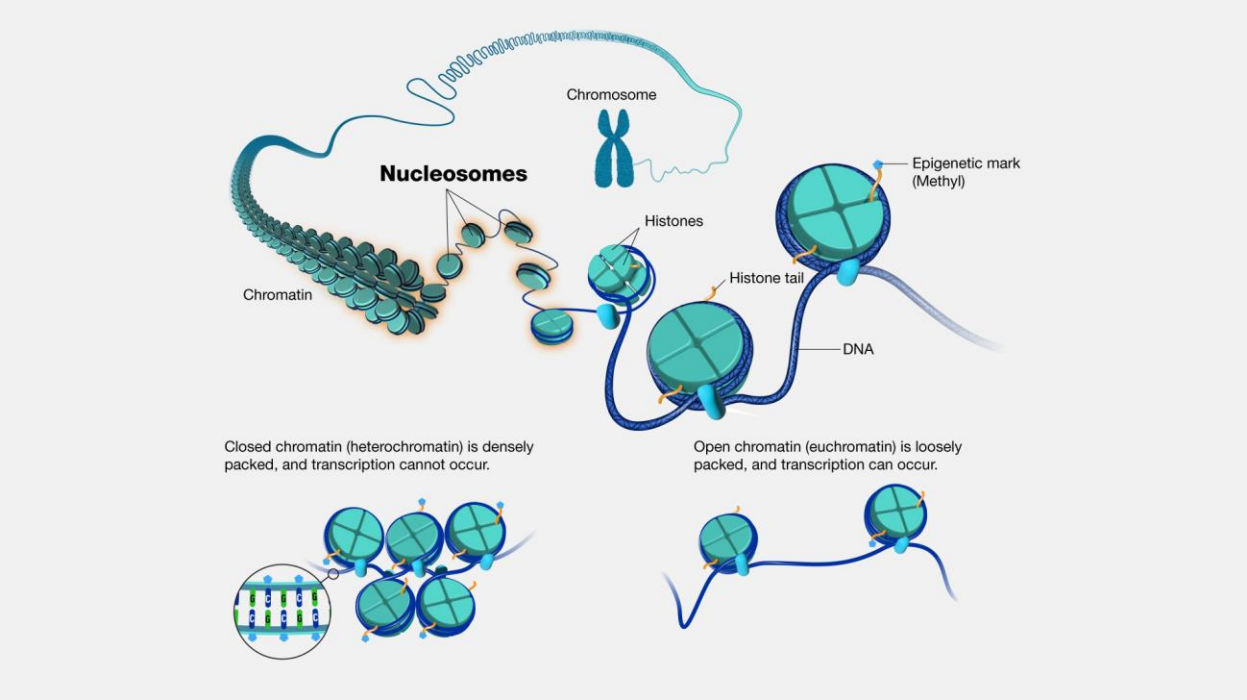
- The structure of Heterochromatin is tightly packed and condensed.
- The changes in Heterochromatin occurs due to the modifications to histones and spreading of silencing complexes causing the changes in structure of chromatin.
- Due to its repressive structure, Heterochromatin does not completely express the genes within it.
- Heterochromatin usually folds into higher order structures and this induces an increase in negative supercoiling of DNA.
- The structure of Heterochromatin is stable and is also dynamic and changes with the cell cycle.
- The formation of chromatin is promoted due to the DNA elements called barriers which promote the formation of active chromatin and remove the nucleosomes.
- This allows the Heterochromatin to spread.
- The structure of Heterochromatin is easily explained by analyzing the **'Constitutive Heterochromatin' and 'Facultative Heterochromatin'.**
- **Constitutive Heterochromatin** is the stable form which consists of repeated sequences of DNA called Satellite DNA.
- The structural functions are regulated by this form of Heterochromatin and are found in centromeres and telomeres.
- Facultative Heterochromatin is known to change its structure according to the cell cycle.
- This consists of repeated DNA sequences termed as **'LINE Sequences'.**
- This can be seen to change its structure in the inactivated X-Chromosome of females.
- The structure of Heterochromatin can also be determined by the density gradient data in which the Heterochromatin appears as a regular structure and Euchromatin has an irregular structure.

Functions of Heterochromatin

- The functional aspects of Heterochromatin are determined by the modifications of chromatin.
- The Heterochromatin core histones present in yeast are hypoacetylated which makes the lysine residues to become more positively charged, allowing an increase in the interaction between the histone and DNA, making the nucleosome more closed in structure.
- The closed chromatin structure of Heterochromatin is due to the low acetylation of **Histone H4-K16** in Heterochromatin, further promoting the folding of Chromatin to high structure orders.
- The active transcriptional activity is due to the Hypomethylation of Heterochromatin at **H3-K4 and K79.**

Nucleosome

- A nucleosome is the basic structural unit of DNA packaging in eukaryotes.
- The structure of a nucleosome consists of a segment of DNA wound around eight histone proteins and resembles thread wrapped around a spool.
- The nucleosome is the fundamental subunit of chromatin.
- Each nucleosome is composed of a little less than two turns of DNA wrapped around a set of eight proteins called histones, which are known as a histone octamer.
- Each histone octamer is composed of two copies each of the histone proteins H2A, H2B, H3, and H4.
- DNA must be compacted into nucleosomes to fit within the cell nucleus.
- In addition to nucleosome wrapping, eukaryotic chromatin is further compacted by being folded into a series of more complex structures, eventually forming a chromosome.
- Each human cell contains about 30 million nucleosomes.



- The nucleosome core particle consists of approximately 146 base pairs (bp) of DNA wrapped in 1.67 left-handed superhelical turns around a histone octamer, consisting of 2 copies each of the core histones H2A, H2B, H3, and H4.
- Core particles are connected by stretches of linker DNA, which can be up to about 80 bp long.
- Technically, a nucleosome is defined as the core particle plus one of these linker regions; however the word is often synonymous with the core particle.
- Genome-wide nucleosome positioning maps are now available for many model organisms including mouse liver and brain.
- Linker histones such as H1 and its isoforms are involved in chromatin compaction and sit at the base of the nucleosome near the DNA entry and exit binding to the linker region of the DNA.
- Non-condensed nucleosomes without the linker histone resemble "beads on a string of DNA" under an electron microscope.

- In contrast to most eukaryotic cells, mature sperm cells largely use protamines to package their genomic DNA, most likely to achieve an even higher packaging ratio.
- Histone equivalents and a simplified chromatin structure have also been found in Archaea, suggesting that eukaryotes are not the only organisms that use nucleosomes.