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Essential Cell Biology

By

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This course handout is intended for students that begin in biology, especially those in first-year Bachelor's degree students in Biology. It may also be used by second-year students in Biological Sciences (Biochemistry, Genetic) or students in Master of Applied Biochemistry, Fundamental Biochemistry or Fundamental & Applied Genetic.

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I-Introduction & History of Cell Biology

Currently, he is admitted by all seeing it as completely natural to talk about human, animal, plant, or bacterial cells. However, throughout history this has not always been the case. It is to Mister Robert Hooke that we owe the first time observation of cells.

In fact, Mr. Hooke was a prolific inventor who, among other things, built a microscope with a magnification of 30- which was revolutionary for the time-. This is how in 1667, Robert Hooke use for the first time the term of cell. This term of cell was used by Mr Hooke because he observed regular structures during microscopic observation of cork.

But it was necessary to wait almost two centuries – Exactly 172 years- for the cell to be considered as the basic unit of life and not the tissue. It was in 1837 that the German botanist, Professor Matthias Jakob Schleiden discovered that the structure observed by Hooke almost two centuries earlier was present in all the plants he examined under a microscope. Professor Schleiden discussed this discovery with his colleague and friend Professor Théodore Schwann, who informed him that he had also observed regular structures of this type in the animal tissues he had studied.

In 1839, Professor Schwann expose his cellular theory in his famous book ‘’*Microscopic investigations into the correspondence in the structure and growth of animals and plants*’’ – Original title in german “ ***Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen’’*** and affirm that the cell is the basic unit of life. The cell theory developed by schwann is based on three fundamental principles:

1- All of plants and animals are made of cells.
2- Cells possess the ability to assimilation, growth and reproduction.
3- Cells are arising from division of pre-existing cells.

II- Organizational Aspect of the Cell

II.1-Eukaryotic Cell Structure

Eukaryotic animal cells have different forms and sizes. In fact, these cells can be with rounded, cylindrical forms. The typical width size of animal eukaryotic is a range between 10 to 30µm, but in terms of length, the animal eukaryotic cells can reach length size greater than one meter (ex: neuron).

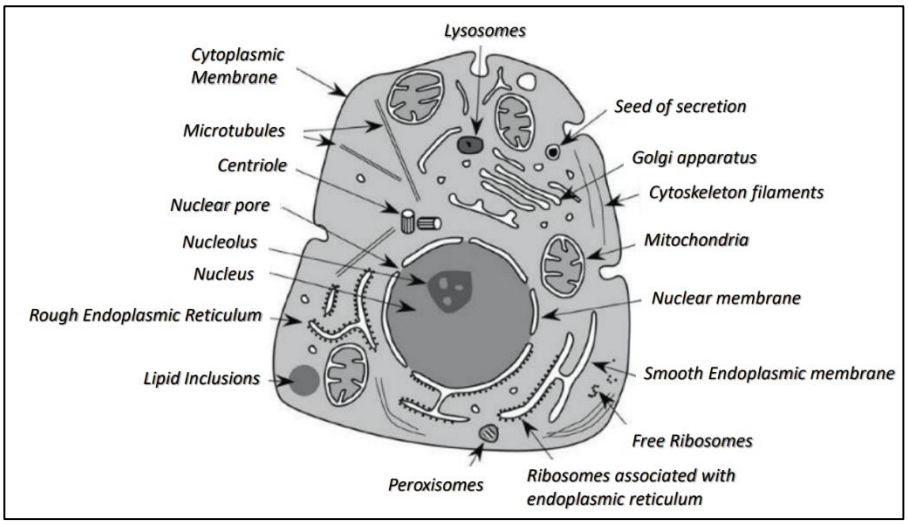

Figure 1: Schematic organization of eukaryotic animal cell, based on ultrafine section by transmission electron microscopy

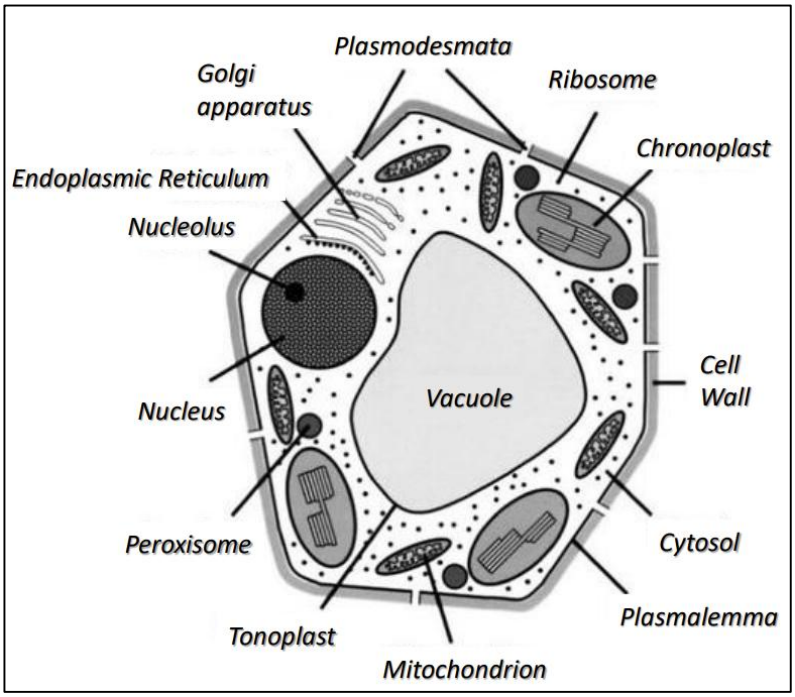

Figure 2: Schematic organization of eukaryotic plant cell, based on ultrafine section by transmission electron microscopy

Despite differences in forms and size, the eukaryotic animal cells exhibit the same structural characteristics. Indeed, these cell have:

- **Cytoplasmic membrane:**

True frontiers of the cell, cytoplasmic membrane composed of a phospholipid bilayer with embedded proteins, separate internal components from the cell from external environment. This membrane is not completely hermetic, because it allows-under specific condition- the bidirectional transport of organic molecules, ions, water, and oxygen.

- **Nucleus:**

We do not have exact information about discoverer of the nucleus, but we know that this intracellular structure has been known by scientists since the beginning of the 18th century, but it had to wait until the 19th century that it was named *"nucleus"* by the Scottish botanist Robert Brown.

Largest cellular organelle, nucleus allows us to differentiate a eukaryotic and prokaryotic cells. It is considered as a cellular command center, because at this level take place, several essential biological processes, notably those linked to genetic material (transcription, processing and transport of RNA precursors, as well as DNA replication, repair and recombination).

- **Cytoskeleton:**

Like a road network that extends throughout the cell, the cytoskeleton is dynamic structure formed from the association of microtubules, actin microfilaments, and intermediate microfilaments. In addition to its main role that assists cells maintain their shape and internal organization, cytoskeleton participates to essential cell function like cell division, motility and vesicular traffic.

- **Endomembrane system:**

The famous scientist ***Camillio Golgi*** was the first in the late to 1800_s to describe the endomembrane system. This intracellular structure containing a group of membrane and organelles including Endoplasmic reticulum *(Rough & Smooth)*, Golgi Appartus, Lysosomes, Nuclear membrane, Vesicles *(seed of*

secretion). The different components of endomembrane system work together in many some biochemical process, especially to ensure transport, packaging and modification of lipids and proteins.

- **Mitochondria:**

Considered as cell power plant, mitochondria *(single : mitochondrion)* . Scientific Community admitted that mitochondria have for the origin aerobic procaryote that integrated inside eukaryotic cells by endosymbiotic process since 1.5-2 billion years. Interestingly, contrary to the other cellular organelles, mitochondria are surrounded by two membranes and contain their own DNA.

- **Peroxisome:**

Should be not to be confused with the lysosome of which it is structurally close, peroxisome is not a compound of endomembrane system. Peroxisome is a round organelle with approximately 1µm diameter that participate in variety biochemical in process –especially oxidation of fatty acids and amino acids- through more than fifty enzymes it contains.

- **Centriole:**

Discovered in 1880_s by the independent works of two great researchers: *Theodor Heinrich Boveri* & *Édouard Van Beneden*. The centriole is a structure found exclusively in animal cells with length may vary around 0.5µm and 0.2 µm diameter. It is cylinders composed of nine triplet microtubules with symmetrical organization. Besides the primordial role in cell division and the formation of the mitotic spindle, the centriole participate in the formation of cilia and flagella of eukaryotic cells

The first big difference between prokaryotic and eukaryotic organisms, is that eukaryotes are multicellular organisms, when prokaryotes are unicellular. In addition to a size up to 10,000 times smaller, prokaryotic cells do not have an endomembrane system especially the genetic material is not protected by a membrane structure – DNA is directly stored in the cytoplasm-. Unlike eukaryotic cells where DNA is associated with histones in several chromosomes, in prokaryotic cells, DNA is unique and circular with the presence of one or more plasmids.

Many prokaryotic cells possess the ability to move, through various cytoplasmic extensions namely flagella.

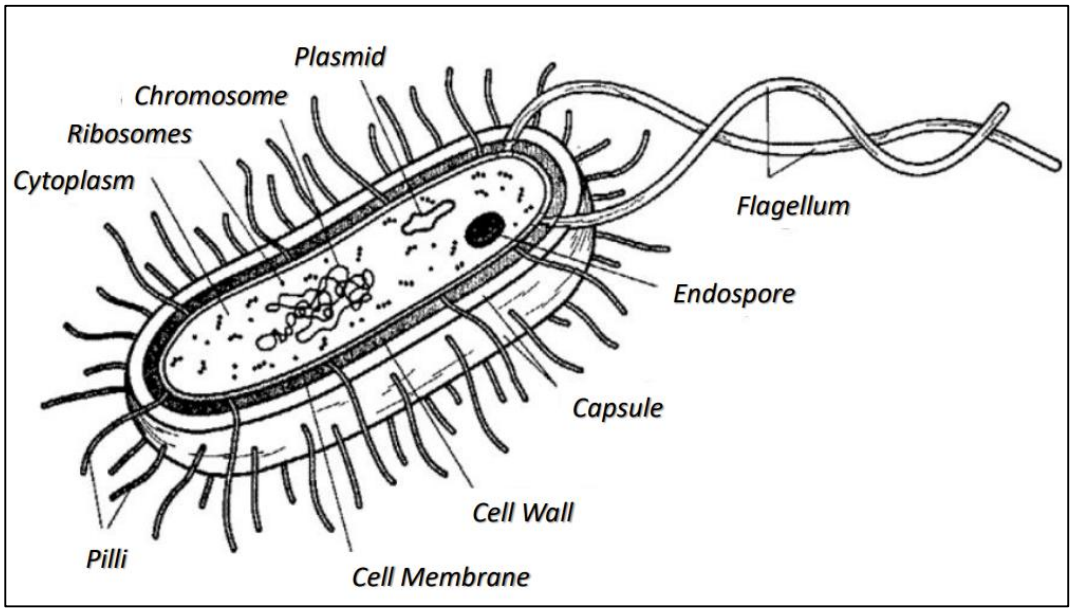

Figure 3: Schematic organization of bacterial (prokaryotic) cell.

II.2-Protein Synthesis and Intracellular Flow

The cell - in this case we will continue to talk about the eukaryotic cell- is a dynamic structure with thousands of reactions per millisecond. Protein synthesis is among the most important metabolic processes at the cellular level. Either constitutively or following external signals, the DNA contained in the nucleus produced by the transcription process of different types of RNA (transfer RNA "*tRNA*", messenger RNA "*mRNA*", ribosomal RNA "*rRNA*") that leaves the nucleus towards the cytoplasm via the nuclear pores. By the translation process, ribosomes transform the message contained in the mRNA into a polypeptide chain that will become a protein. The proteins produced will either be released into the extracellular medium, integrated into the plasma membrane or remain in the cytoplasm to play many roles including that of transcription factor translocated in the nucleus to activate genes.

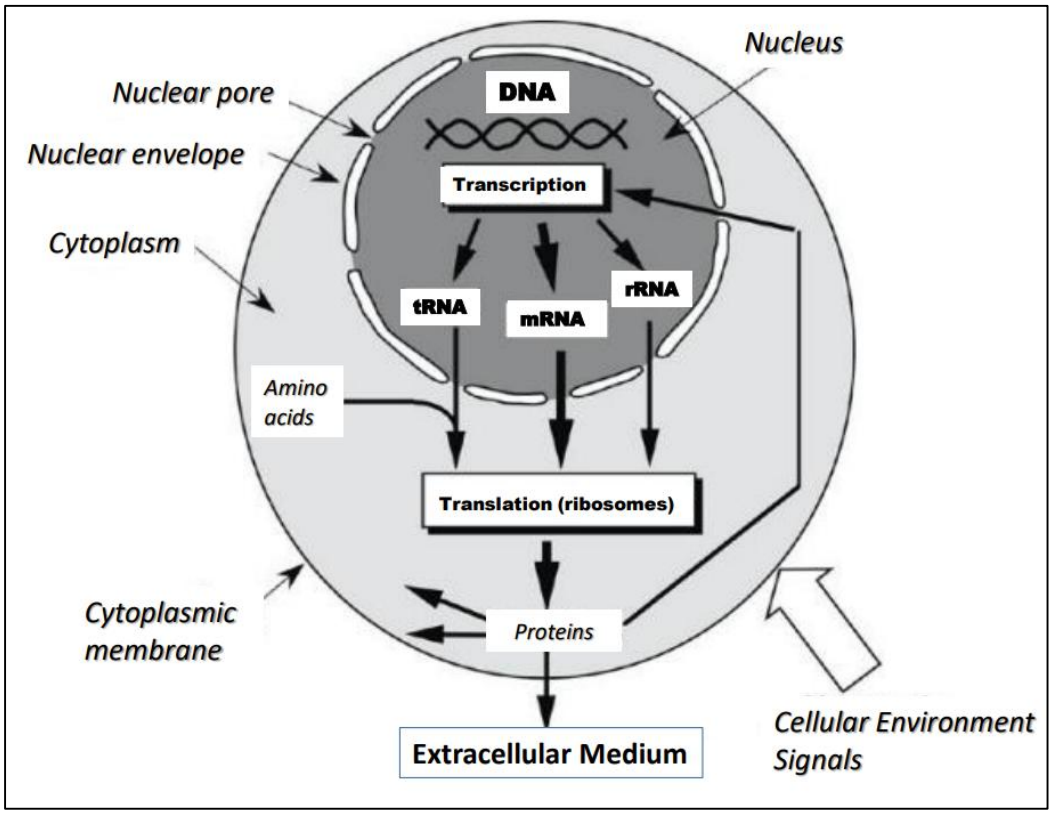

Figure 4: Schematic intracellular flow of eukaryotic cell.

II.3-Metabolic Activities in Cells

The intrinsic ability of eukaryotic and prokaryotic cells to produce proteins from DNA allows them to possess metabolic autonomy, produce energy and also reproduction. Isolated viruses have no biological activity because they need to infect cells in order to hijack their cellular machinery (decreased expression of the host proteins and increased expression of viral proteins)

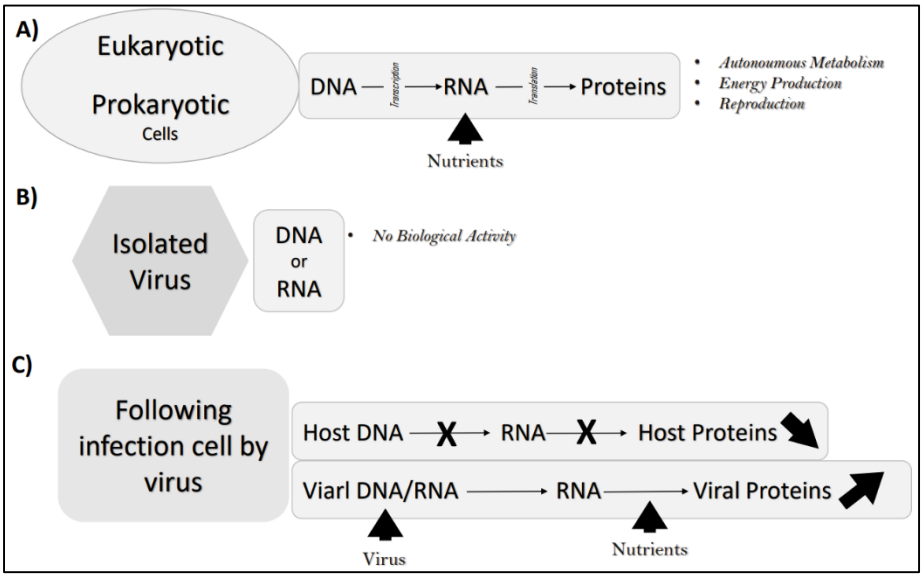

Figure 5: Schematic comparison of the metabolic and functional activities of cells and virus. **A**: eukaryotic Prokaryotic cell, **B**: isolated virus, **C**: Virus infecting eukaryotic/prokaryotic cell

II.4-The Cell Cycle and Checkpoints

As mentioned above, one of the main functions of cells is to reproduce by division. Cell division is a set of steps that follow each other in a specific order - Cell cycle, where the cell undergoes structural and metabolic modifications resulting in the formation of daughter cells from a single parent cell. The cell cycle can be divided into two principal steps: ***Interphase*** & ***Division***.

The interphase is the time period during which the cell prepares to enter into the next division phase. Depending on metabolic processes, cell structure and DNA quantity, the interphase is divided into three main stages: $\mathbf{G_1}$ (*Gap1*) corresponding to the stage where cells are metabolically active and copy essential organelles and biochemical molecules, but the DNA quantity stays constant and is always in configuration 2n. In **S** (*Synthesis*), the cell starts the duplication of their DNA and centriole. During this **S** stage, the DNA quantity of cell is comprised between 2n to 4n. In $\mathbf{G_2}$ (*Gap2*) stage, the DNA quantity is 4n and the cell ensures the maintain of the integrity and the closeness of the recently duplicated chromosomes (sister chromatids).

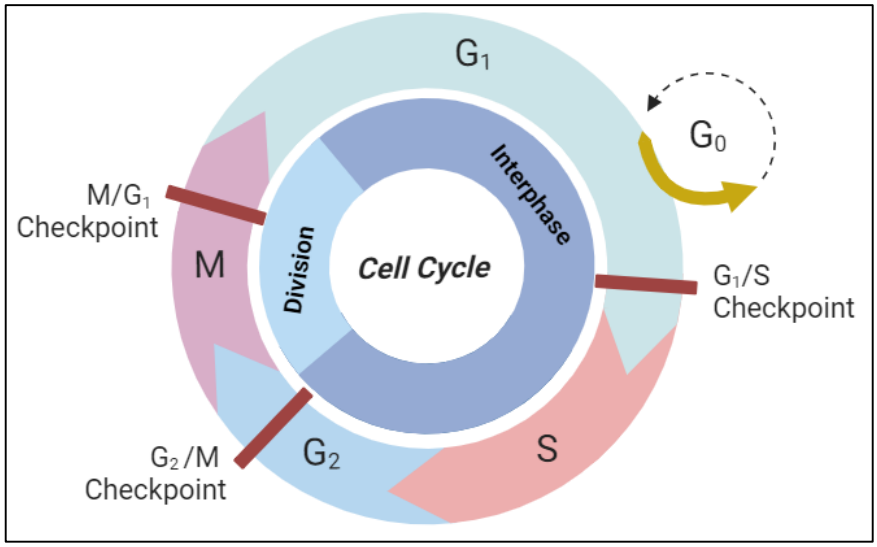

Figure 6: Schematic representation of cell cycle phases.

It is important to note that there is the particular case of phase **G_0** or resting phase. This particular phase, which can be short or very long, is characterized by the fact that the cell is neither dividing nor preparing to divide, but all other cellular functions are maintained.

During division step or mitosis, the chromosomes are observable under microscope, because the chromatin is hundreds or even thousands of times more condensed than it in interphase.

Mitosis is a complex process that can be subdivided into five different stages : ***Prophase***, ***Prometaphase***, ***Metaphase***, ***Anaphase***, and ***Telophase*** – with addition of *Cytokinesis*-.

During Prophase, following the move of the centrioles towards the poles, the mitotic spindle is set up. Prometaphase begins with the breakdown of nuclear envelope-this step is important to spindle assembly-. Microtubules are very dynamic in prometaphase and they interact with chromosomes kinetochores. In Metaphase, chromosomes are at their maximum condensation. Due to microtubules dynamism and the interaction with kinetochores, the centromeres of all cell's chromosomes are line-up at the equator of the spindle. In Anaphase, we observe the abrupt separation of sister chromatids. This separation into two distinct types of movements, the first part is the kinetochore microtubules retraction and the chromosome move toward the spindle pole. In the second

part, the non-kinetochore microtubules move and the spindle poles are separated. Telophase is the end of mitosis process, where chromosomes achieve the move to the pole. After that the nuclear membrane is reformed, chromosome begins to decondense-return to interphase conformation-. The division of cytoplasm into two daughter cells is called **cytokinesis** process.

Cell division is a process involving many molecules and where the cell undergoes significant changes. To guarantee that the division occurs without any dysfunction and produces daughter cells that are physiologically functional, there are within the cells protein complexes -mainly formed by cyclins- that exert control the quality of cell division, these are the ***cell cycle checkpoints***. The three principal cell cycle checkpoints are: G_1/S Checkpoint, G_2/S Checkpoint & M/G_1 Checkpoint. The quality of DNA and division processes is monitored during these checkpoints. In the case of anomaly detection, the division is stopped and a repair system is involved. In the event that the dysfunction has not been addressed by the various operating systems, the cell is directed towards the apoptotic pathway.

II.5-Cell Death Mechanisms

In addition to cell division -proliferation-, cell death is one of the main mechanisms of a cell's life. Cell death can be divided into three type: Type I (apoptosis), Type II (Autophagy), Type III (Necrosis).

Without going into molecular details, Apoptosis Considered as programmed apoptotic cell death, the apoptosis is managed by complex and tightly regulated cellular signaling pathways, with activation of caspase proteases. It is characterized by cell shrinkage, membrane blebbing, and condensation of the chromatin. Apoptosis can be triggered when cells cannot repair the damage caused by any stress, such as DNA damage, accumulation of unfolded proteins (Endoplasmic reticulum stress). The lack of signal via growth factors can be induce apoptosis.

The cell death type II or programmed non-apoptotic cell death is Autophagy. Unlike the apoptotic cell, the autophagic cells appear with a large intracellular

vesicles, plasma membrane blebbing, enlarged organelles and the depletion of cytoplasmic organelles in the absence of chromatin condensation

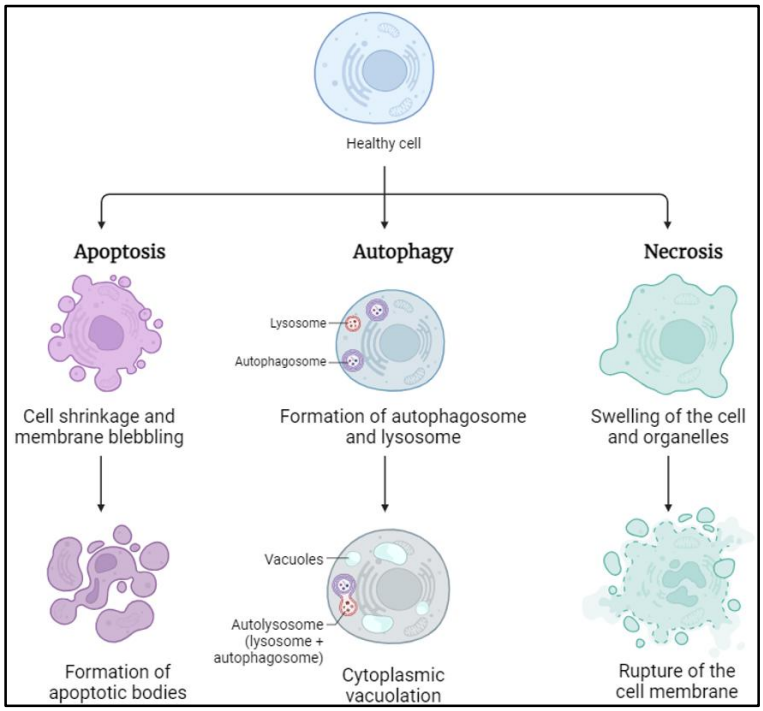

Figure 7: Cell death mecanisms

Three types of autophagy (*Macro-autophagy*, *micro-autophagy* and *chaperone-mediated autophagy*) are triggered when the cell is exposed to metabolic stress, with a significant decrease in the production of ATP, amino acids, and nutrients. It is important to note that in some cases, the cell uses autophagy as a mechanism of survival, but it transforms into a cellular death mechanism when metabolic stress is irreversible.

The Necrosis can be programmed or non-programmed. Non-programmed necrosis is induced by various external factors such as biological agents (virus, bacteria), toxins, ischemia and trauma (physical injury). Necrosis can be also the response of the activation of specific signaling pathways (programmed necrosis or Necroptosis), especially thus mediated by RIP-kinase-dependent necrosis. Morphological changes (cytoplasmic swelling) and plasma membrane rupture are characteristic of necrosis, with a loss of intracellular organelle structure and random DNA degradation without chromatin condensation.

III- Cell Membrane

The plasma membrane is the physical boundary between the inside of a cell and the external environment. It also delimits the different intracellular organelles.

III.1-Composition

The primary constituents of plasma membranes are the lipids, particulary phospholipids. As they separate the water compartments on both sides, phospholipids have a spontaneous tendency to arrange themselves in such a way that their polar head is present groups face the water and the hydrocarbon fatty acid tails face of the hydrophobic core, this arrangement is named the hydrophobic effect.

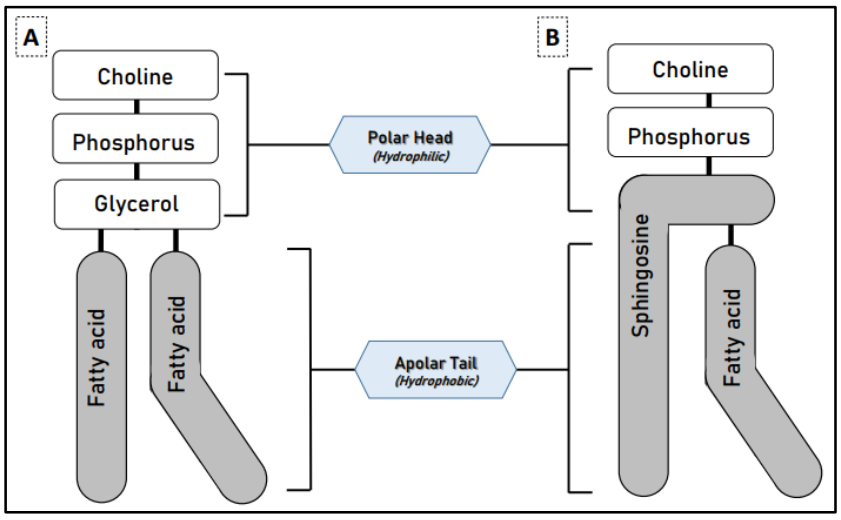

Figure 8: Organization of phospholipids.
A: *Glycerophospholipid/ Phosphatidylcholine.* **B**:*Sphingomyelin*

The amphipatic characteristic's of phospholipids allows them to adopt different organization in function of the aqueous solution (ex: water).

III.2-Membrane Dynamics and Phospholipid Movements

When placed in water, amphipathic phospholipids try to bury their hydrophobic fatty acyl chains away from water by forming spherical micelles in which the fatty acyl chains face the center of the sphere, and the polar head groups are at its surface.

Alternatively, bilayers are formed in which sheets of two phospholipid monolayers or leaflets separate water compartments. The bilayers eventually must form a spherical vesicle so that there is no hydrophobic edge facing the water. When phospholipids are put on top of water they can form monolayers, where the polar head groups face the water and fatty acids stick up in the air. In order to demonstrate that the plasma membrane behaves in the same way as phospholipids, many researchers have initiated experiments that remain relevant. In 1925, two German pharmacists *(Evert Gorter* & *Francois Grendel)* utilized the red blood cell membrane to extract lipids and examine the plasma membrane's behavior. They observed that when phospholipids are put in water and compressed with a physical barrier, their surface is twice as large as when they are not subjected to any pressure. They thus advanced the theory - still true- that the plasma membrane is organized in bilayers.

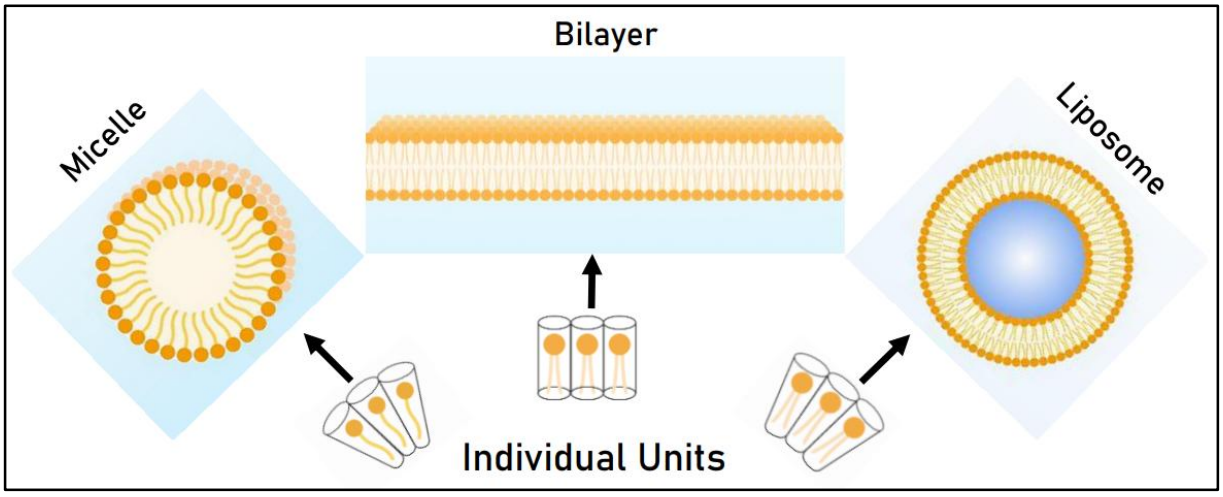

Figure 9: Types of amphipatic lipid aggregates.

Forty years later –1965-, at Babraham Institute in Cambridge-United Kingdom-, British hematologist and biophysicist ***Alec Douglas Bangham*** and his team observe under electron microscope, that phospholipids in a polar solvent, forms the spherical lipid bilayer structure's separating the solvent into intra & extra compartment. He referred to these vesicles as liposomes. Bangham thought – rightly so – that these liposomes are an excellent model for studying the properties of plasma membranes and especially that it would be an excellent way (intermediate) to administer drugs inside the cell.

This Bangham hypothesis proved to be true more than 50 years later when mRNA vaccines against Sars-Cov2 use lipid vesicles -like those of Bangham-, as vehicles during vaccination.

It is important not to confuse liposomes with micelles. Micelles are colloid structures composed of a monolayer of fatty acids, where the hydrophobic tail is directed towards the interior of the sphere.

As previously written, liposomes and modified red blood cells (Ghost red blood cells) have paved the way for the study of cell membranes. Cell membranes can be defined as fluid, mobile and packing systems. The dynamics of lipid bilayer depends on three important parameters: temperature, cholesterol levels and the nature of phospholipids-particularly fatty acids-.

The dynamics of phospholipids allow them to perform several movements' types in the plasma membrane:

A. Rapid rotation around central long axis

B. Contortion of fatty acids chains of phospholipids in different directions and flexion towards the center of the lipid bilayer (the hydrophobic center).

C. Lateral movement (diffusion) of phospholipids along the plasma membrane.

D. Flip-Flop movement: the phospholipids can move across the bilayer and change the position between extracellular and intracellular layer and vice-versa.

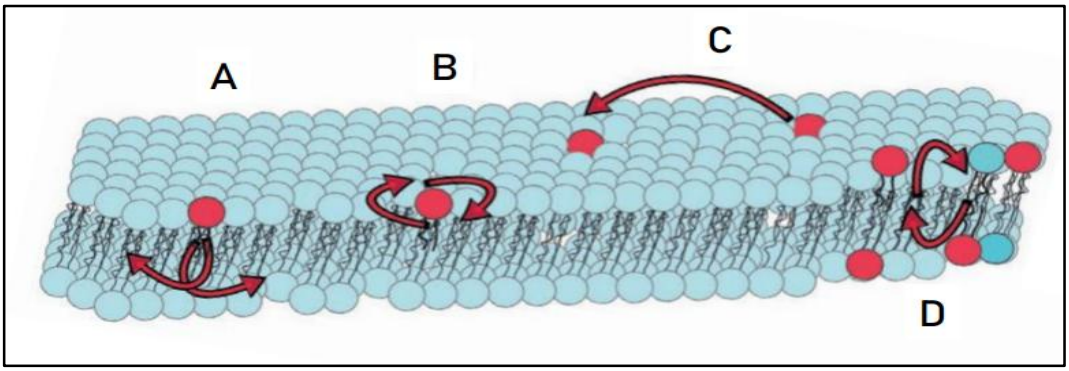

Figure 10: Phospholipids motion in lipid bilayer.

Indeed, lateral diffusion of phospholipids occurs at a frequency of 10^7 per second with an estimated speed of 1µm per second. The speed of the flip-flop

is conditioned by the presence or not of Flippase/Floppase. In the absence of these proteins, the flip-flop is a very long process that is measured in days,

while the presence of Flippase/Floppase accelerates this process that occurs then in a few seconds.

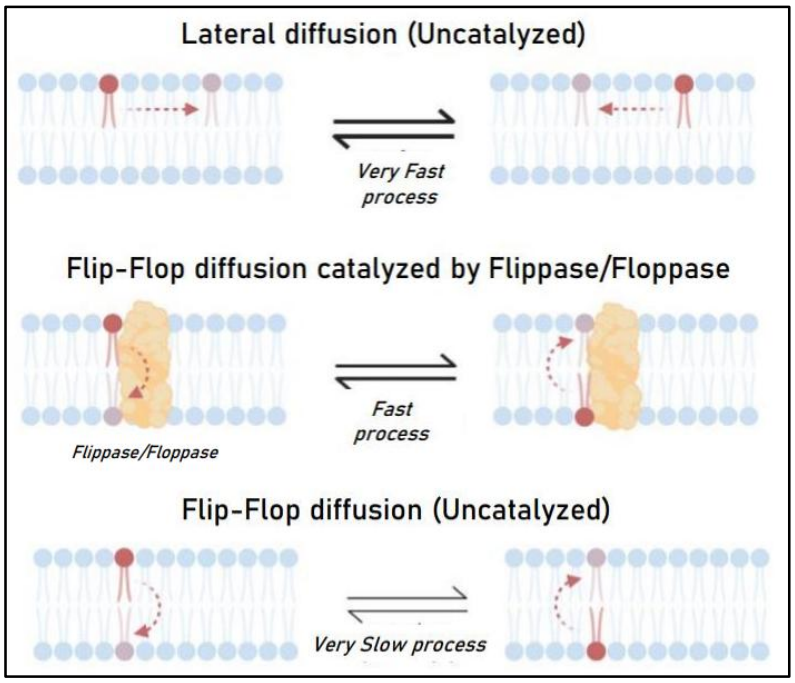

Figure 11: Speed movement of phopholipids in lipid bilayer.

III.3-Membrane Proteins Pages

The different types of lipids represent only 50% of the plasma membrane mass. The proteins constitute the other 50% of membrane weight-this percentage can be change in function of the specificity of the membrane-.

Due to their larger structure, it is considered that at the membrane, the protein/lipid ratio is between 1/50 to 1/100. The protein diversity allows the specific functions of plasmic membrane.

Independently of their amino-acid constitution, membrane proteins can be divided into two groups: peripheral & integral. This basic membrane proteins, can be distinguished by their operational definition, based on the ability to extract them from the lipid bilayer.

- **Integral membrane proteins :**

Due to their embedded in the lipid bilayer, these proteins can be removed only by disrupting the bilayer, by using detergent. Detergeant allows the formation of mixed phospholipid-detergeant micelles where the integral proteins are coated by the hydrophobic domain.

The major type of integral membrane proteins are transmembrane. Some transmembrane proteins make a single pass across the membrane, while others make multiple passes. The part (one or more) of the protein within hydrophobic core of lipid bilayer is a hydrophobic stretch of 20–25 nonpolar amino acids forming an α-helix. Hydrophilic part of transmembrane protein contains charged and polar amino acids in aqueous environment outside and inside of the cell.

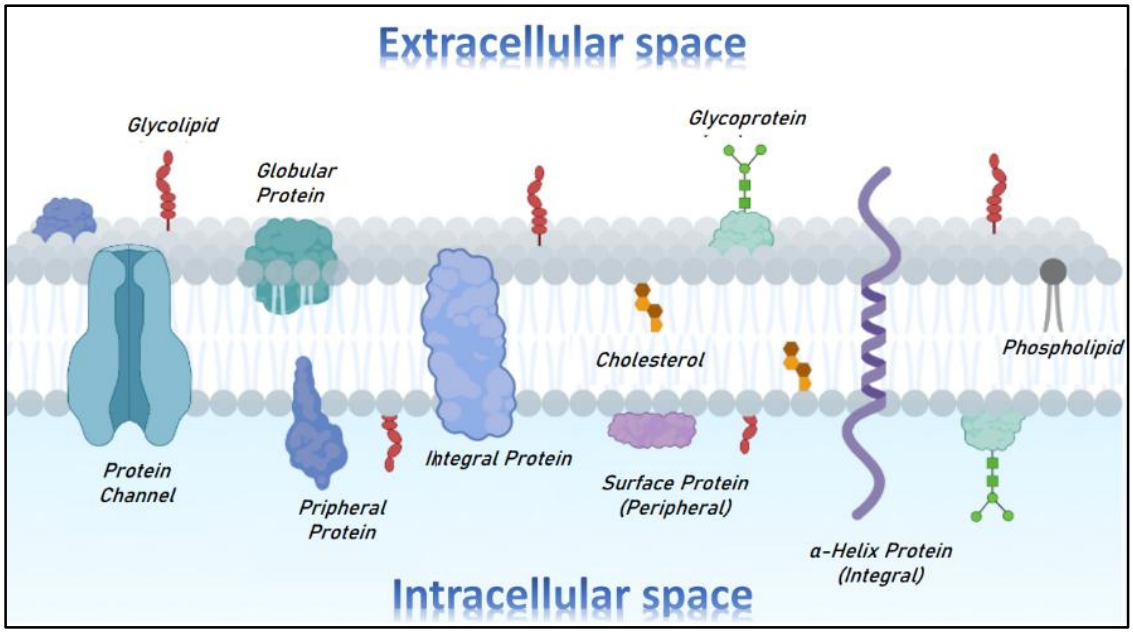

Figure 12: Protein composition in the lipid bilayer.

It is important to note that all transmembrane proteins are integral membrane proteins, but the reverse is not true. Indeed, some fatty acids or phospholipids present in the bilayer (example: Glycosylphophatidylinositol –GPI-), link covalently various proteins. Thus, these proteins are strongly bound to the plasma membrane, but they have a relatively high mobility compared with other proteins with amino acid chains embedded in the bilayer.

- **Peripheral membrane proteins** :

Unlike integral membrane proteins, the peripheral membrane proteins are bound to polar head of membrane lipids or integral (or another peripheral) membrane proteins by ionic interactions. So, these peripheral membrane proteins can be removed from the membrane without dissolving the bilayer.

Post-translational modifications of proteins including covalent interaction of certain proteins with fatty acids such as myrstic acid (Myristoylation) or palmitic acid (palmitylation), increases the hydrophobicity of proteins and thus their binding to the plasma membrane.

Like lipids, proteins are not static in the plasma membrane but perform many-lateral- movements.

Lipids and proteins (peripheral and integral) undergo changes consisting on the addition of sugar residues, this phenomenon is called glycosylation. These proteins and lipids are called glycoproteins and glycolipids.

III.4-The Fluid Mosaic Model and Membrane Asymmetry

The scientific knowledges acquired over many years -especially between the 1950s and 1960s-on the physicochemical characteristics of the lipid bilayer and membrane proteins, allowed the two American researchers *Seymour Jonathan* ***Singer*** *and Garth L.* ***Nicolson****,* to propose in 1972 the model of the fluid mosaic. The fluid mosaic model describes the dynamic nature of the cell membrane. *Singer & Nicolson* states that the various proteins floating within the lipid bilayer like boats or iceberg in the sea. The fluid mosaic model suggests that the membrane is not static but rather is constantly in motion, with lipids and proteins able to move laterally within the membrane. This perpetual dynamic of the membrane allows it to perform many functions

In addition to being dynamic, one of the major characteristics of the plasma membrane is its asymmetry. Scientific research demonstrates a remarkable difference in the composition (lipids and proteins) and organization of the internal and external layer of the cell membrane.

This cell membrane asymmetry is not by chance but accurately controlled, conferring distinct functionalities to each membrane layer and governing a myriad of physiological cell processes.

The main points regarding plasma membrane asymmetry are:

- The lipid composition of each of the two sheets is different. For example, phosphatidylserine (PS) and phosphatidylthanolamine (PE) are more abundant on the internal side, while phosphatidylcholine (PC) and sphingomyelin are more abundant in the external sheet.

✓ Protein Asymmetry: Different proteins are selectively distributed between the inner and outer layers. For instance, certain signaling receptors and transport proteins are predominantly found on one side of the membrane, allowing them to interact with specific molecules or participate in specific cellular processes.

✓ Oligosaccharide chains of glycoproteins and glycolipids are located on the extracellular side.

✓ S-S disulfide bonds between cysteine residues are of interest to external peripheral proteins or the extracellular domain of transmembrane proteins,

✓ The asymmetry of lipids and proteins contributes to the functional specialization of the cell membrane. For example, receptors on the extracellular side can interact with signaling molecules to initiate cellular responses, while transport proteins on the cytoplasmic side regulate the movement of ions and molecules into and out of the cell. The membrane components are combined with the cytoskeleton on the cytosolic side with the intervention of specialized peripheral proteins.

This asymmetry is an important hallmarks of physiology of cell membrane, but it makes the cell consume a lot of energy. Maintaining the asymmetry of the membrane is not a passive phenomenon, it involves various mechanisms, such as lipid transporters, flippases, floppase and scramblases. These mechanisms ensure that specific lipids and proteins are localized to their appropriate sides of the membrane.

As explained above, the plasma membrane is a barrier whose main role is to protect the homeostasis of the intracellular medium. The barrier created by the membrane is not impassable, because there are permanent exchanges between intra and extracellular media. The selectivity of membrane permeability can be divided into two types: *without plasma membrane movement* & *with plasma membrane movements*.

III.5-The Fluid Mosaic Model and Membrane Asymmetry

Membrane transport ***without*** plasma membrane movement take place at the molecular level and not at the cellular level. These transports have three characteristics: direct passage of transported materials through the membrane, no intervention of the endo-membrane system, no intervention of the cytoskeleton. Depending on the intervention of specific proteins and the need for Energy (ATP), this membrane transport can be occurring in three different ways:

III.5.1- Passive transport without permease:

Processes that do not involve specific protein transporters (permeases) or energy consumption. Thus, the membrane allows for the passage of molecules through simple diffusion. This transport concerns the *Small, Non-Polar Molecules.* This is the case for many small gaseous molecules, such as ***Oxygen*** *(O_2),* ***Carbon Dioxide*** *(CO_2)* ***and Nitrogen*** *(N_2).* **Ethanol** *(C_2H_6O)*, **steroid hormones** (e.g., testosterone, estrogen), and some **vitamins** (e.g., vitamin A, vitamin D) – *also called lipid soluble molecules*- can diffuse through the lipid bilayer without the need for transport proteins. It is important to note that the urea – a polar wast product of proteins metabolism- can diffuse across the phosphlipid bilayer due to its small size and the interaction with lipid tail.

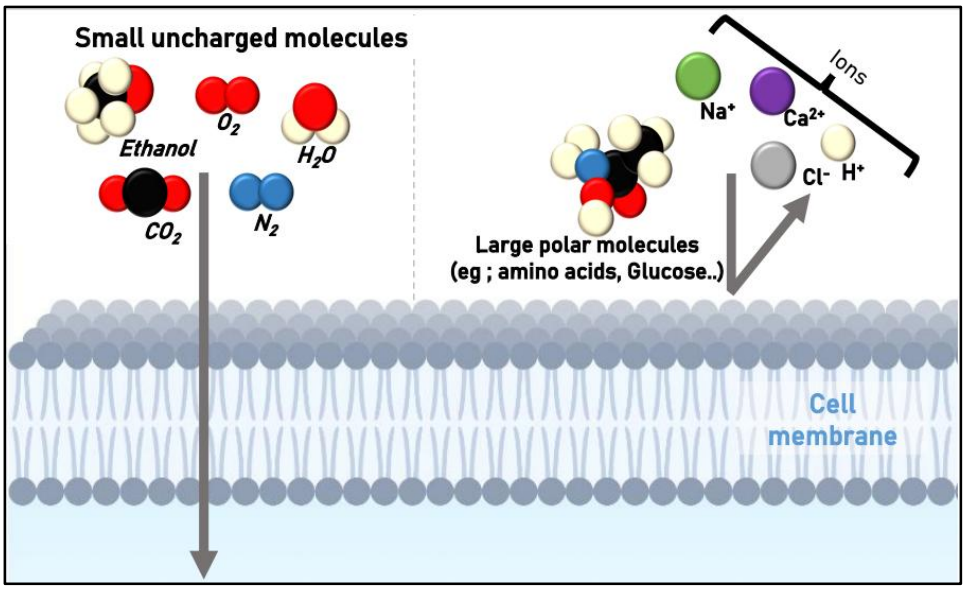

Figure 13: schematic representation of cell membrane passive permeability.

But the passive diffusion speed of urea is slower than non-polar molecules such as oxygen or carbon dioxide.
It is important to note that the passive transport process without permease occurs on both sides of the plasma membrane bidirectionally, but since the concentration of molecules is always higher on one side compared to another, the net flow of simple diffusion is always from the most concentrated medium to the least concentrated medium – until it reaches equilibrium-.

The diffusion of molecules according to the concentration gradient has been theorized by the German physiologist **Adolf Eugene Fick**, through these two laws (*First & Second Fick's law*).

III.5.2-Passive transport witht permease

As we have mentioned above, ions and charged molecules do not diffuse through the membrane. However, an exchange of this type of molecules between the intra and extracellular medium is almost permanent. Thus, the movement of these types of molecules – through the membrane – is mediated by specific proteins, but without energy consumption because the diffusion is always in accordance of gradient's concentration.

This passive transport also called facilitated transport is extremely regulated process. In fact, the proteins (permeases) that ensure this transport are

specific for each molecule or group of molecules. A water crosses the membrane via specific transporter called Aquaporines. The $GLUT_s$ are specific transporters of glucose that is found in almost mammalian cells. For ions, the different transporters can be activated by ligand association (eg., neurotransmitters receptors Acetylcholine, GABA, 5-HT) or by physical stimulus such as change in membrane voltage (voltage-gated Calcium channels, voltage-gated Potassium channels, voltage-gated Calcium channels)

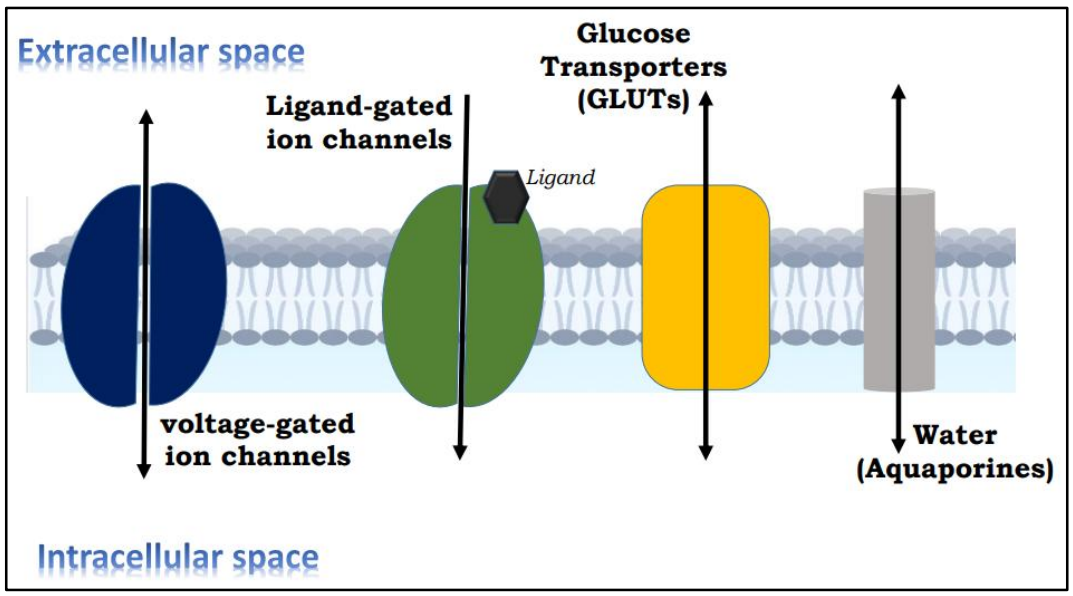

Figure 14: schematic representation of different types of permease in passive transport.

The net movement of water through a selectively permeable membrane is ensured by integral membrane proteins called Aquaporines. This fundamental biological process allowing the water molecules to move from a medium of high concentration to a medium of low concentration is called: osmosis. The types solutions in osmosis can be divided into three types:

- ***Isotonic Solution***: The solute concentration is the same inside and outside the cell. There is no net movement of water, so the cell retains its shape.
- ***Hypotonic Solution***: The solute concentration is lower outside the cell than inside. Water moves into the cell, causing it to swell and potentially burst (lyse).

- ***Hypertonic Solution***: The solute concentration is higher outside the cell than inside. Water moves out of the cell, causing it to shrink (crenate).

III.5.3-Active transport

Unlike passive transport (with or without permease), active transport occurs against gradient's concentration. This process need the intervention of specific transporters and requires the input of energy. The transport of ions or small molecules usually used the ATP as source of energy is also called **Primary active transport**.

The most case of primary active transport is the Na^+/K^+ pump. This transmembrane carrier transfers - against concentration of the gradient- the Na & K ions to equilibrate the concentration in both sides of plasma membrane. The ATP-Binding Cassette (ABC) transporter are another family of membrane proteins expressed by prokaryotic and eukaryotic cells. The ABC transporters play critical roles in various physiological processes, including nutrient uptake, lipid metabolism, detoxification, immune response, and drug resistance.

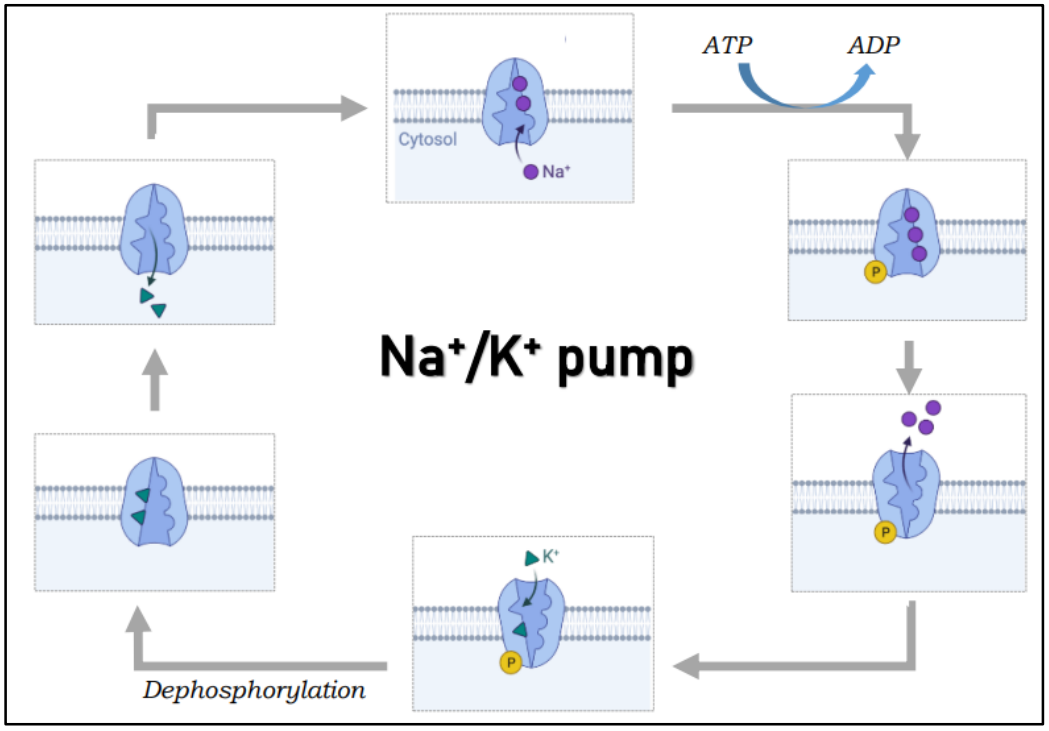

Figure 15: Representation of Sodium-Potassium pump mecanism.

The **secondary active transport** mainly corresponds to **symport** and **antiport**. The symport referred to transported molecule and co-transported molecule can move in the same direction. This process allows the movement of substances against their concentration gradient by utilizing the energy provided by the movement of another molecule down its concentration gradient.

One common example of symport is the sodium-glucose co-transporter (SGLT), which is found in the membranes of cells lining the small intestine and renal tubules. In the small intestine, SGLT transports both sodium ions (Na^+) and glucose molecules (or other sugars) into the epithelial cells from the intestinal lumen, against their respective concentration gradients. The energy for glucose uptake is provided by the electrochemical gradient of sodium ions, which is maintained by the sodium-potassium pump (Na^+/K^+-ATPase) on the basolateral membrane of the epithelial cells. Similarly, in the renal tubules, SGLT mediates the reabsorption of glucose from the urine into the blood. The antiport is process in which two different molecules or ions are transported across a biological membrane simultaneously, but in opposite directions, coupled by a single transporter protein. This process allows the movement of substances against their concentration gradients by utilizing the energy provided by the movement of another molecule or ion down its concentration gradient. One common example of antiport is the chloride-bicarbonate exchanger found in the plasma membrane of red blood cells, which exchanges bicarbonate ions (HCO_3^-) for chloride ions (Cl^-) across the membrane, helping to regulate pH and carbon dioxide transport.

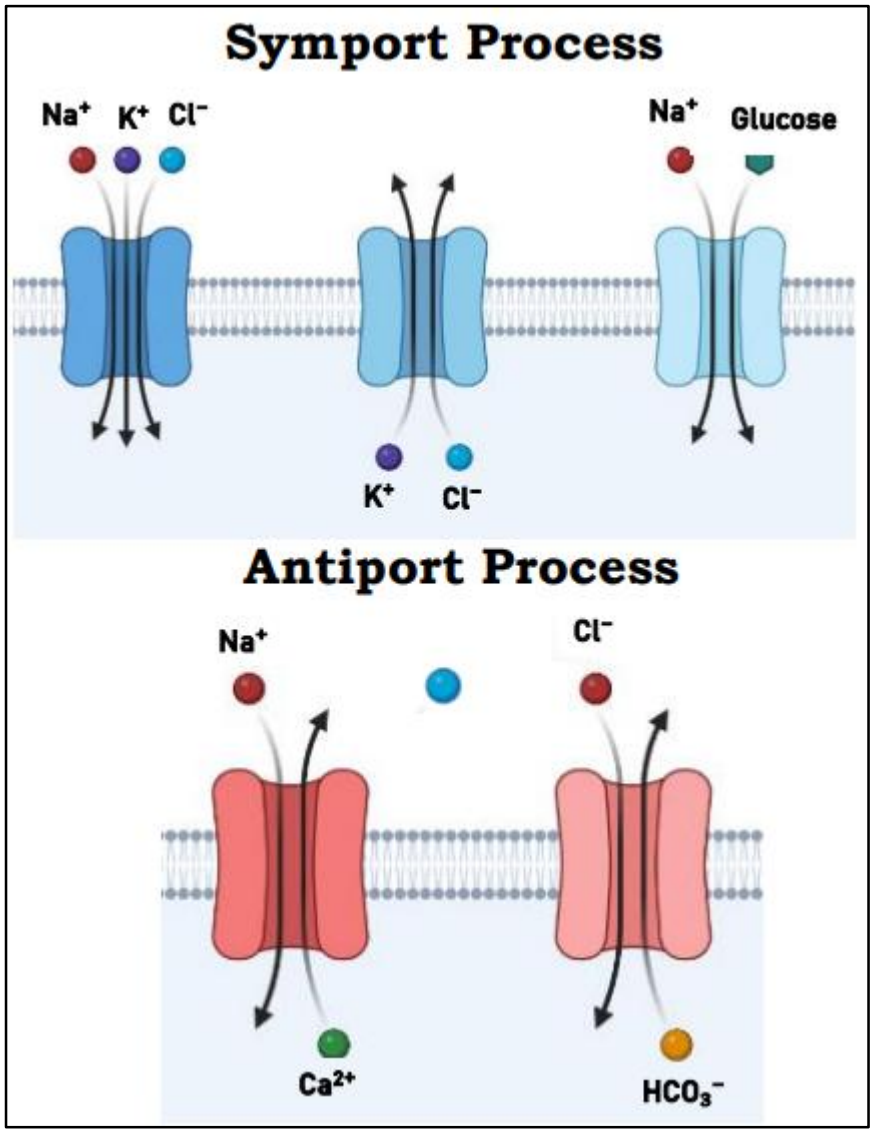

Figure 16: Examples of secondary active transporters.

The selective permeability of the cell membrane is vital for several reasons:

- **Regulation of Molecular Traffic:** The membrane controls the entry and exit of ions, nutrients, waste products, and other molecules, ensuring that the cell maintains the appropriate concentrations of substances for proper function.

- **Protection and Support:** It provides structural support to the cell and protects it from harmful substances in the extracellular environment.

- **Cell Communication:** Specialized proteins in the membrane facilitate cell signaling and communication with other cells or the external environment.

- **Maintaining Electrochemical Gradient:** The membrane maintains an electrochemical gradient across its surface, which is crucial for processes such as nerve impulse transmission and muscle contraction.

III.6-Vesicular Transport

Vesicular transport is also defined as membrane transport with plasma membrane movement. Unlike transport of small molecules and ions, the transport of larger particles and –in some cases- unicellular organisms passes through cell membrane, is observable under microscope. This transport into and out the cell, with plasma membrane movement has four major characteristics:

a) Involvement of the plasma membrane and the endomembrane system,
b) The molecules are contained during part of their intracellular transport in a vesicle or vacuole surrounded by an envelope membrane,
c) The cytosol is the origin of part of the transported material or its final destination,
d) Intervention of the cytoskeleton and they energy consumption.

The primary types of membrane transport involving plasma membrane movement are *endocytosis*, *exocytosis*, and *phagocytosis*.

III.6.1-Endocytosis

Endocytosis is the process by which cells internalize substances (process nutrients, hormones, and other essential substances), as well as to remove debris and pathogens from the extracellular environment by engulfing them with their plasma membrane. There are several types of endocytosis:

- **Phagocytosis**:

Often referred to as "cell eating," phagocytosis involves the engulfment of large particles such as bacteria, dead cells, or foreign particles (debris). This mechanism generally takes place during immunological process and involves specialized cells, like macrophages and neutrophils, perform phagocytosis.

The plasma membrane extends around the target (particle), forming a large vesicle called phagosome, which then fuses with a lysosome, where the engulfed material is degraded by lysosomal enzymes.

- **Pinocytosis**:

Known as "cell drinking," pinocytosis involves the non-specific uptake of extracellular fluid and small molecules. The plasma membrane invaginates, forming a vesicle that internalizes the fluid and its contents.

In this part we only describe the movements of the membrane. It is important to note that the molecular process of phagocytosis and pinocytosis is more complex than what we have just described.

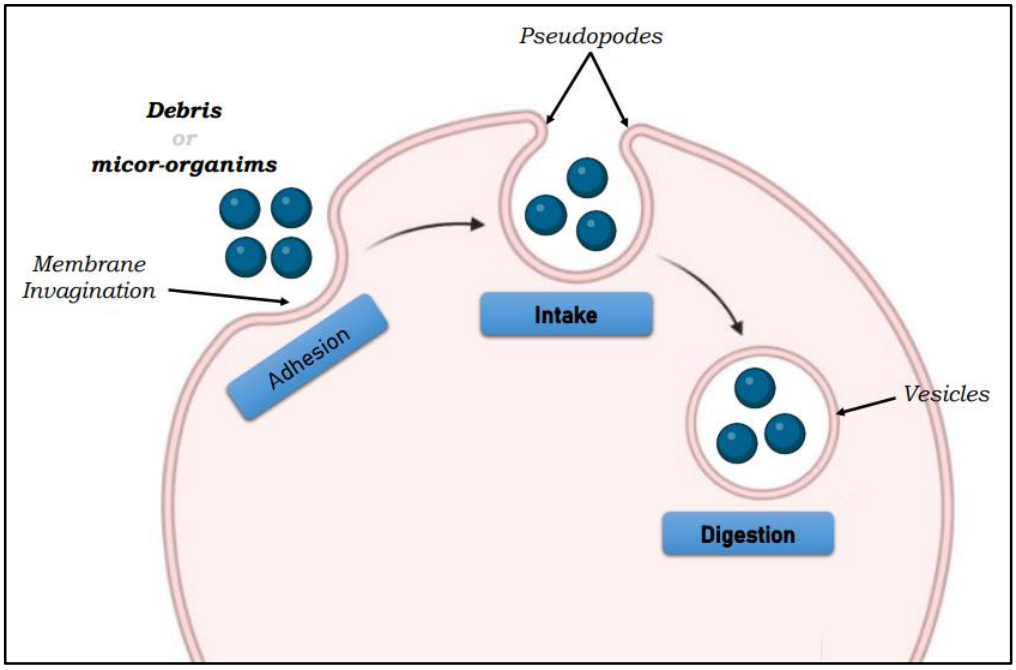

Figure 17: Phagocytosis and Pinocytosis process.

- **<u>Receptor-Mediated Endocytosis</u>**:

This is a highly selective process where cells take in specific molecules based on receptor-ligand interactions. Ligands (molecules) bind to specific receptors on the cell surface, triggering the invagination of the plasma membrane to form a vesicle containing the ligand-receptor complexes. This important process is used by various viruses (eg. HIV, Influenza A) and Bioparticules (eg. cholesterol, iron), to break into the cell.

There are different mechanisms of receptor-mediated Endocytosis, but the most widely studied is *Clathrin-mediated endocytosis (CME).*

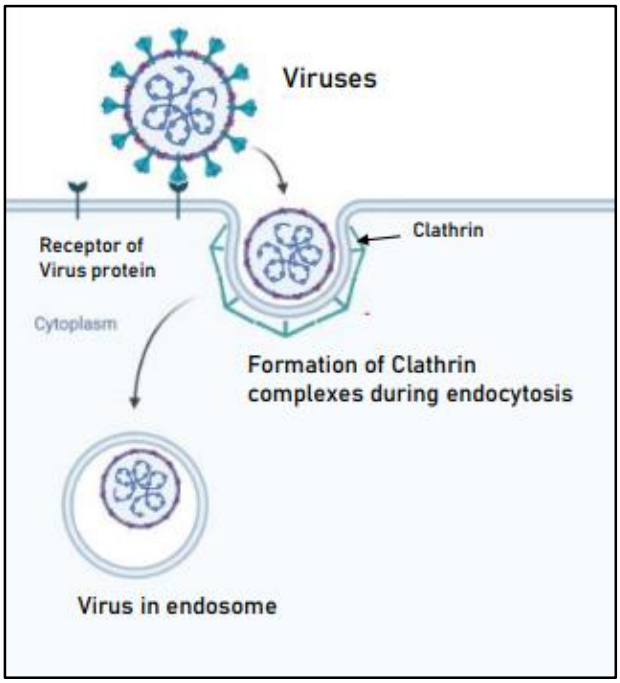

Figure 18: Clathrin mediated endocytosis of viruses.

III.6.2-Exocytosis

Discovered in the 1950s, exocytosis is the process by which cells expel materials to the extracellular space. This is crucial for the secretion of various substances, including hormones, neurotransmitters, and digestive enzymes. Exocytosis serves the following purposes:

- *Removing toxins or waste products from the cell's interior***:** To maintain homeostasis, waste or toxins created by cells must be removed from the cell. For instance, in aerobic respiration, cells produce the waste products carbon dioxide and water during ATP formation. Carbon dioxide and water are removed from these cells via exocytosis.
- *Facilitating cellular communication:* Cells create signaling molecules like hormones and neurotransmitters. They are delivered to other cells following their release from the cell through exocytosis.

- *Facilitating cellular membrane growth, repair, signaling and migration:* When cells absorb materials from outside the cell during endocytosis, they use lipids and proteins from the plasma membrane to create vesicles. When certain exocytotic vesicles fuse with the cellular membrane, they replenish the cell membrane with these materials.

There are two main pathways of exocytosis:

- **Constitutive Exocytosis**:

This is a continuous process where cells constantly transport and secrete molecules such as proteins and lipids.

This is a fundamental cellular process by which cells continuously transport materials from the inside of the cell to the extracellular space. Ubiquitous exocytosis occurs continuously and plays a key role in:

- *Plasma Membrane Maintenance*: Constitutive exocytosis replenishes lipids and proteins to the plasma membrane, ensuring its growth and repair.
- *Extracellular Matrix Production*: Cells secrete extracellular matrix components like collagen and proteoglycans, which are essential for tissue structure and function.
- *Protein and Lipid Delivery*: Vital for delivering proteins and lipids to the cell surface, including receptors, transporters, and adhesion molecules.
- *Waste Removal*: Helps in the removal of cellular waste and unwanted materials from the cell.

Constitutive exocytosis involves many proteins and takes place in five steps:

1. *Cargo Packaging:* Proteins and lipids destined for the plasma membrane or extracellular space are synthesized in the endoplasmic reticulum (ER) and sorted and packaged into transport vesicles at the trans-Golgi network (TGN).

2. *Vesicle Transport:* Vesicles are transported along microtubules and actin filaments towards the plasma membrane using motor proteins like kinesin and dynein.

3. *Vesicle Docking and Tethering:* Upon reaching the plasma membrane, vesicles are tethered to specific sites by tethering proteins (homodimeric coiled-coil proteins and multi-subunits tethering complexes).

4. *Vesicle Fusion*: The **s**oluble **N**SF **a**ttachment protein **re**ceptor complex (vesicle SNAREs & target SNAREs) mediates the fusion of the vesicle membrane with the plasma membrane. This fusion process is often aided by accessory proteins like SNAP (soluble NSF attachment protein) and NSF (N-ethylmaleimide-sensitive factor), which help disassemble SNARE complexes post-fusion.

5. *Cargo Release:* Following membrane fusion, the vesicle's contents are released into the extracellular space or become part of the plasma membrane.

- **Regulated Exocytosis**:

Unlike Constitutive Exocytosis, regulated exocytosis occurs in specialized cells in response to specific stimuli (from the cell itself or from extracellular origin). This process is considered as the final event in the pathway of regulated secretion. Cells store secretory vesicles filled with specific substances and release them upon receiving a stimulus.

Regulated exocytosis is essential for functions such as:

- *Neurotransmitter Release:* Critical for communication between neurons in the nervous system (eg. GABA, 5-HT, Epinephrine…).
- *Hormone Secretion:* Important for the release of hormones from endocrine glands into the bloodstream (eg. Insulin)
- *Enzyme and Protein Release*: Involved in the secretion of digestive enzymes, immune responses, and other cellular functions (eg. Granzyme, Perforin).

Like ubiquitous exocytosis, regulated exocytosis involves many proteins and proceeds in defined steps:

1. *Vesicle Formation and Storage*: The secretory vesicles -formed in the Golgi apparatus and are filled with specific cargo- are transported to specific sites within the cell, often near the plasma membrane, where they are stored until a signal triggers their release.

2. *Signal Detection:* Often linked to the activation of cell surface receptors that trigger intracellular signaling cascades.

3. *Vesicle Docking and Priming*: Upon receiving the signal, vesicles are transported to the plasma membrane and dock at specific sites. Docking involves the interaction of vesicle-associated membrane proteins (VAMPs) with target membrane proteins (t-SNAREs) at the plasma membrane. Priming prepares the vesicles for fusion and may involve partial assembly of the SNARE complex and other regulatory proteins.

4. *Vesicle Fusion and Release:* The fusion of the vesicle with the plasma membrane is triggered by a rapid increase in intracellular calcium concentration, often through voltage-gated calcium channels. The membrane fusion is mediated by the SNARE complex, which includes v-SNAREs (e.g., synaptobrevin) and t-SNAREs (e.g., syntaxin, SNAP-25). Accessory proteins like synaptotagmin act as calcium sensors, promoting the final fusion step upon calcium binding.

In the end of the process, the contents of the vesicle are released into the extracellular space or the lumen of an organelle.

In addition to the proteins involved in this process, the regulated exocytosis requires:

- *Calcium Ions (Ca^{2+}):* The key trigger for regulated exocytosis, where a rise in intracellular calcium concentration initiates vesicle fusion.
- *Phosphorylation:* Modifications of SNARE proteins, synaptotagmin, and other regulatory proteins can enhance or inhibit exocytosis.

- *Second Messengers:* Cyclic AMP (cAMP) and other signaling molecules can modulate the sensitivity and efficiency of the exocytic machinery.

Ca^{2+}.

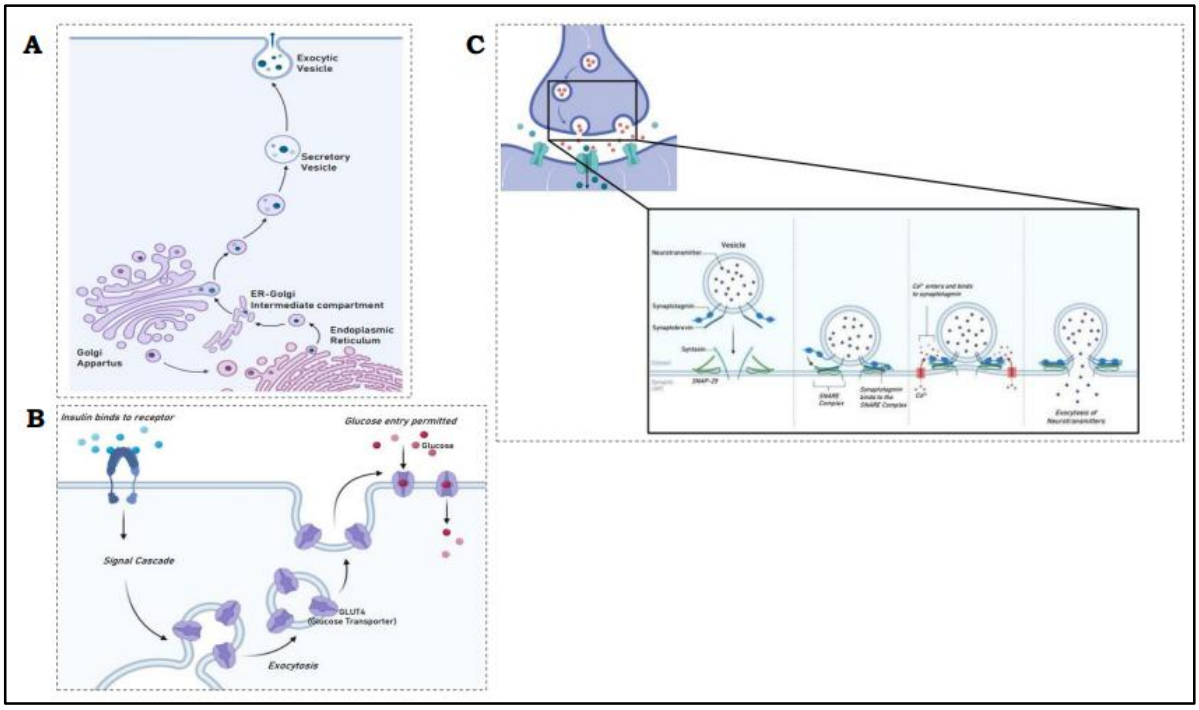

Figure 19: Constitutive and regulated exocytosis

A: *Constitutive Exocytosis.* **B**:*Exocytosis induced by ligand bind to receptor*
C:*Neurotransmitter exosytosis .*

IV-Extracellular matrix

The cell is not isolated from its immediate environment. This immediate microenvironment of the cell is referred as the extracellular matrix (ECM) interacts with the cell via the plasmatic membrane.

In this chapter we will review the different components of the extracellular matrix, its interaction with the plasma membrane and in the last part, the cell polarity

IV.1- Introduction of extracellular matrix

The extracellular matrix (ECM) is an intricate and dynamic network of proteins, glycoproteins, proteoglycans, and other macromolecules organized in a cell/tissue-specific manner and provide structural and biochemical support to surrounding cells. Components of the ECM link together to form a structurally stable composite, contributing to play a crucial role in tissue organization, cell signaling, and the regulation of cellular behavior (eg, proliferation, adhesion, migration, differentiation, apoptosis & polarity).

The ECM is a complex network of macromolecules, including polysaccharides and more than 300 proteins. The combination of distinct physical and biochemical properties ECM components, confers to it these unique characteristics.

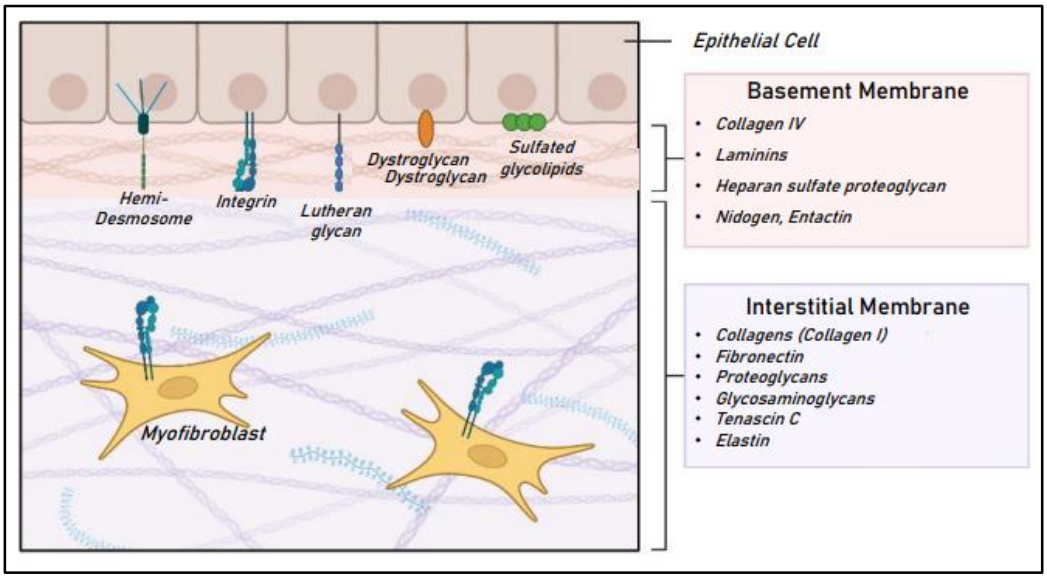

Figure 20: Mammalian Extracellular Matrix architecture

Main constituents of the Extracellular Matrix are a network of hydrophilic, extended gels of glycosaminoglycans (GAGs) and fibrous proteins.

IV.2-Collagens

Correspond to the most abundant proteins in the Extracellular Matrix. Collagens confer tensile strength of ECM.

Representing approximately one-third of protein in human body, collagens are characterized by their unique triple polypeptide α chain structure (*primary structure consists of repeating tripeptide units of Gly-X-Y, where X is often proline, and Y is often hydroxyproline*). The amino-acid composition of collagen is unique and contribute to their structural properties. The different amino-acid found in collagen are:

- **Glycine (Gly)**: Approximately one-third of the amino acids in collagen are glycine. This small amino acid is essential for the tight helical structure of collagen.

- **Proline (Pro)** & **Hydroxyproline (Hyp)**: Proline and its hydroxylated form, hydroxyproline, make up around 25-30% of the amino acids in collagen. proline residues in collagens are hydroxylated by *prolyl 4-hydroxylase*. Hydroxyproline is unique to collagen and is critical for stabilizing the triple helix structure through hydrogen bonding.

- **Alanine (Ala)**: Alanine constitutes about 11% of collagen. It is a non-polar amino acid that contributes to the stability of the collagen molecule.

- Other amino-acids occur in smaller proportions but are still significant for the overall function and structure of collagen. They are : *Glutamic acid (Glu), Arginine (Arg), Lysine (Lys), Aspartic acid (Asp), Serine (Ser), Leucine (Leu)*

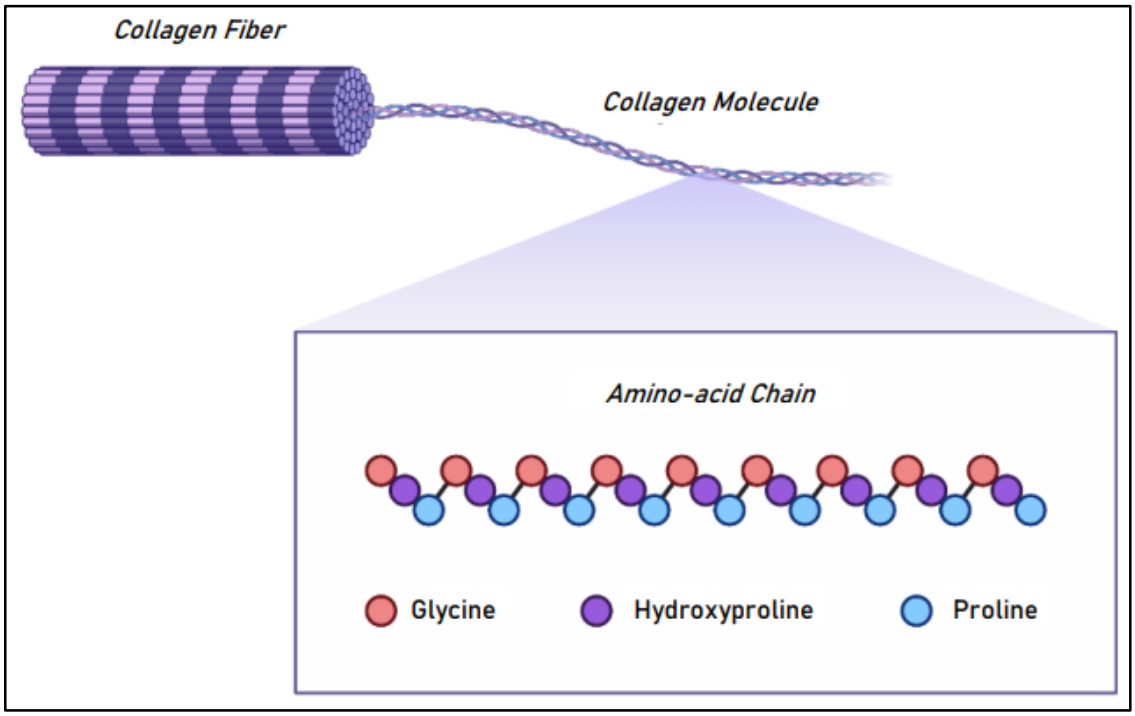

Figure 21: Collagen primary structure

In vertebrates, we found 46 different types of α helix that are organized into 28 types of collagens **(Table I)**. This different types can be regrouped into five families:

- **Fibril-forming collagens**: Composed of three polypeptide chains wound around each other. They form fibrils through self-assembly, which then bundle together to create larger fibers that contribute to tissue strength and elasticity. Fibril formin Collagen biosynthesis involves several steps, starting with the translation of collagen polypeptides in the endoplasmic reticulum (ER), followed by post-translational modifications (eg, hydroxylation and glycosylation). The triple helix forms in the ER and is then transported to the Golgi apparatus and subsequently secreted into the extracellular space. In the extracellular space, procollagen molecules are cleaved to form mature collagen, which self-assembles into fibrils.

- **Network-forming collagens**: Unlike fibrillar collagens, network-forming collagens create branched, web-like structures. These collagens have interrupted triple-helical regions interspersed with non-helical (globular) domains, allowing for the formation of flexible, interconnected networks.

The biosynthesis of network-forming collagens involves the production of precursor molecules (procollagens) that undergo post-translational modifications in the endoplasmic reticulum and Golgi apparatus.After secretion into the extracellular space, these procollagens are processed into mature collagens and self-assemble into networks.

- **Fibril-Associated Collagens with Interrupted Triple helices (FACIT):**

A unique subclass of collagens that are closely associated with the surfaces of collagen fibrils but do not form fibrils themselves.

FACIT collagens have a triple-helical domain interrupted by non-helical regions. This interruption allows flexibility and enables these collagens to bind to fibrillar collagens and other extracellular matrix molecules. They typically consist of a central triple-helical region flanked by non-helical terminal domains.

FACIT collagens biosynthesis takes place in the endoplasmic reticulum as procollagens, where they undergo post-translational modifications. After secretion into the extracellular space, they associate with existing collagen fibrils and other matrix components.

- **Membrane-associated collagens with interrupted triple helices (MACIT)**

Distinct subgroup of collagens that are integral membrane proteins.

MACIT collagens have a triple-helical domain interrupted by non-helical regions, providing flexibility and functional diversity. The structure of these collagens typically includes a cytoplasmic domain, a transmembrane domain, and an extracellular collagenous domain.

MACIT collagens are synthesized in the endoplasmic reticulum and transported to the Golgi apparatus, where they undergo post-translational modifications. After, they are transported to the cell membrane, where their transmembrane domain anchors them in place, and their extracellular collagenous domain interacts with the ECM.

- **Multiple tripe-helix domains and interruptions (MULTIPLEXINs) :**

This collagen subgroup are characterized by multiple triple-helical domains separated by non-collagenous interruptions, which contribute to their ability to interact with various ECM components and cell surface receptors. This structure allows for greater flexibility and diverse functional interactions.

Synthesized in the endoplasmic reticulum, where they undergo post-translational modifications. MULTIPLEXINs are transported to the Golgi apparatus and secreted into the extracellular space. In the ECM, they integrate into existing structures and interact with other ECM components and cells.

Table I: Collagens types

Collagen Type	α Chain	Molecular species	Class
Collagen **I**	α1(I), α2(I)	$[\alpha1(I)]_2$, α2(I) $[\alpha1(I)]_3$	Fibril-Formin(Fibrillar)
Collagen **II**	α1(II)	$[\alpha1(II)]_3$	
Collagen **III**	α1(III)	$[\alpha1(III)]_3$	
Collagen **IV**	α1(IV), α2(IV), α3(IV), α4(IV), α5(IV), α6(IV)	[α1(IV)]2, α2(IV) α3(IV), α4(IV), α5(IV) $[\alpha5(IV)]_2$, α6(IV)	Network-Forming
Collagen **V**	α1(V), α2(V), α3(V), α4(V)[a]	$[\alpha1(V)]_2$, α2(V) $[\alpha1(V)]_3$ $[\alpha1(V)]_2\alpha4(V)$ α1(XI)α1(V)α3(XI)	Fibril-Formin(Fibrillar)
Collagen **VI**	α1(VI), α2(VI), α3(VI), α4(VI)[b] α5(VI)[c], α6(V)		Network-Forming
Collagen **VII**	α1(VII)	$[\alpha1(VII)]_3$	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)
Collagen **VIII**	α1(VIII)	$[\alpha1(VIII)]_2$, α2(VIII) α1(VIII), $[\alpha2(VIII)]_2$ $[\alpha1(VIII)]_3$ [α2(VIII)]3	Network-Forming
Collagen **IX**	α1(IX), α2(IX), α3(IX)	[α1(IX), α2(IX), α3(IX)]	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)
Collagen **X**	α1(X)	$[\alpha1(X)]_3$	Network-Forming
Collagen **XI**	α1(XI), α2(XI), α3(XI)[d]	α1(XI)α2(XI)α3(XI) α1(XI)α1(V)α3(XI)	Fibril-Formin(Fibrillar)
Collagen **XII**	α1(XII)	$[\alpha1(XII)]_3$	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)

Collagen **XIII**	α1(XIII)	$[\alpha1(XIII)]_3$	Membrane-associated collagens with interrupted triple helices (MACIT)
Collagen **XIV**	α1(XIV)	$[\alpha1(XIV)]_3$	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)
Collagen **XV**	α1(XV)	$[\alpha1(XV)]_3$	Multiple tripe-helix domains and interruptions (MULTIPLEXINs)
Collagen **XVI**	α1(XVI)	$[\alpha1(XVI)]_3$	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)
Collagen **XVII**	α1(XVII)	$[\alpha1(XVII)]_3$	Membrane-associated collagens with interrupted triple helices (MACIT)
Collagen **XVIII**	α1(XVIII)	$[\alpha1(XVIII)]_3$	Multiple tripe-helix domains and interruptions (MULTIPLEXINs)
Collagen **XIX**	α1(XIX)	$[\alpha1(XIX)]_3$	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)
Collagen **XX**	α1(XX)	$[\alpha1(XX)]_3$	
Collagen **XXI**	α1(XXI)	$[\alpha1(XXI)]_3$	
Collagen **XXII**	α1(XXII)	$[\alpha1(XXII)]_3$	
Collagen **XXIII**	α1(XXIII)	$[\alpha1(XXIII)]_3$	Membrane-associated collagens with interrupted triple helices (MACIT)
Collagen **XXIV**	α1(XXIV)	$[\alpha1(XXIV)]_3$	Fibril-Formin(Fibrillar)
Collagen **XXV**	α1(XXV)	$[\alpha1(XXV)]_3$	Multiple tripe-helix domains and interruptions (MULTIPLEXINs)
Collagen **XXVI**	α1(XXVI)	$[\alpha1(XXVI)]_3$	Fibril-Associated Collagens with Interrupted Triple helices (FACIT)
Collagen **XXVII**	α1(XXVII)	$[\alpha1(XXVII)]_3$	Fibril-Formin(Fibrillar)
Collagen **XXVIII**	α1(XXVIII)	$[\alpha1(XXVIII)]_3$	Network-Forming

[a] The α 4(V) chain is solely synthesized by Schwann cells. [b] The α 4(VI) chain does not exist in humans. [c] The α 5(VI) has been designated as α 1(XXIX). [d] The α 3(XI) chain has the same sequence as the α 1(II) chain but differs in its posttranslational processing and cross-linking.

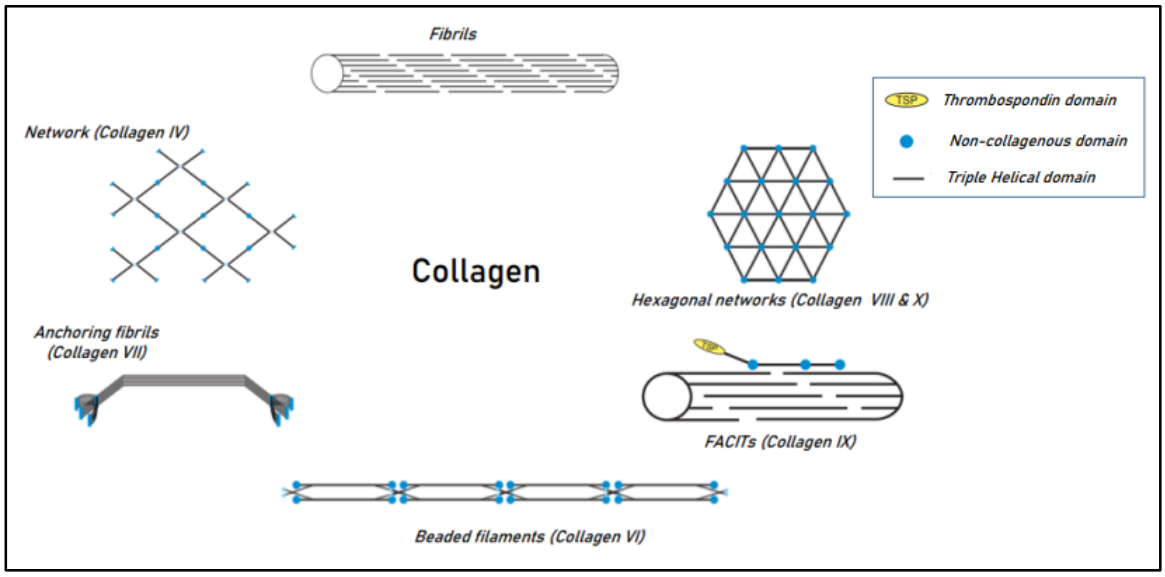

Figure 22: Supramolecular structures formed by Collagen

IV.3-Proteoglycans and Glycosaminoglycans

In addition to collagen, proteoglycans are the most components of extracellular matrix (ECM).

Proteoglycans consist of a core protein with one or more covalently attached glycosaminoglycan (GAG) chains. The core protein of a proteoglycan provides the scaffold for the attachment of GAG chains. The size and amino acid sequence of the core protein can vary widely among different proteoglycans, influencing their specific functions and interactions within the ECM.

Glycosaminoglycan (GAG) chains are long, linear polysaccharides composed of repeating disaccharide units. Each disaccharide unit typically consists of an amino sugar (such as N-acetylglucosamine or N-acetylgalactosamine) and an uronic acid (such as glucuronic acid or iduronic acid). GAGs are highly negatively charged due to the presence of sulfate and carboxyl groups, which allows them to attract water and interact with a variety of molecules.

Depending on the different constituents, Glycosaminoglycan can be classified into four groups:

- **Chondroitin sulfate:** Found in all connective tissues, especially in articular cartilage. Chondroitin Sulfate is composed of glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) covalently attached to a sulfate. Negatively charged, Chondroitin Sulfate interacts with proteins in the Extracellular Matrix, and helps regulate various cellular processes.
- **Dermatan sulfate:** Comprising *N*-acetyl-D-galactosamine (GalNAc) and L-iduronic acid residues with 50–200 repeats, Dermatan Sulfate side chains are linear polysaccharides covalently attached to core proteins that form Proteoglycans. Dermatan sulfate is ubiquitously expressed in various tissues, with relative abudance in skin, cartilage, and the aorta.
- **Heparan sulfate:** Made of linear chains of repeating disaccharide units consisting of an amino sugar (either N-acetylglucosamine or N-sulfoglucosamine) and an uronic acid (either glucuronic acid or iduronic acid). The O-sulfonation of disaccharides residues can be occurring in various sites, generating a diversity and complexity of heparan sulfate structures.
- **Keratan sulfate:** found predominaly at the cornea- first tissue where discovered- and nervous system. Like Chondroitin, Dermatan and Heparan sulfates, Keratan sulfate is composed of repeating disaccharide units, which consist of galactose and N-acetylglucosamine, to which is associated -via the hydroxyl groups- of the sulfate groups. Interestingly, Kertan sulfate are characterized by the absence of iduronic acid –major component of uronic acid- and the presence of fucose and sialic acid.
- **Hyaluronic acid:** Unlike other glysoaminoglycan groups, Hyaluronic acid does not contain a sulfate group. Playing principal role in skin aging, Hyaloronic acid is composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronidic β bond.The physico-chemical properties of Hyaluronic acid allow us to interact and retain water molecules.

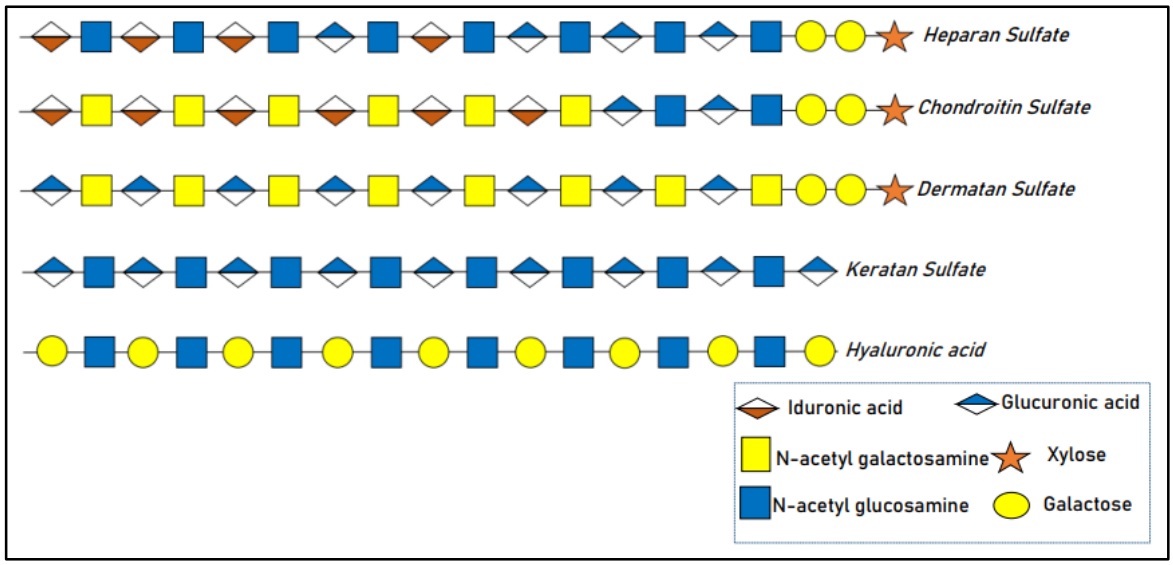

Figure 23: Schematic representation of glycosaminoglycans composition

As mentioned already, the proteoglycan represent the core protein with attached glycosaminoglycans. In addition of the variety of glycosaminoglycans, the core protein of proteoglycan are diverse. The association between glycosaminoglycan and core proteins allow to classify proteoglycans into five group:

- **Glypicans:** Considered as cell surface proteoglycan. Glypicans are a family of Heparan Sulfte proteoglycans, anchored by a glycosylphosphatidylinositol moiety to the outer membrane leaflet, and can be released from the cell surface by the GPI-phospholipase. The core protein of glypicans has 14 cysteine residues necessary for forming a compact three-dimensional structure and that is relatively conserved among the different family members, but the genes coding for the core protein are located in different chromosomes. Glypicans are implicated in various biological process, especially in Cell Adhesion and Migration, Regulation of Growth Factor Signaling and in Developmental Processes and pathologies such as cancers.
- **Syndecans:** Commonly co-expressed in cell surface with Glypicans, Syndecans family is composed of four members in human. In addition to Heparan sulfate, glycosaminoaglycans chain of Syndecans can be constituted by Chondroitin sulfate or Dermatan sulfate. Mammalian genome encodes four syndecans, considered as transmembrane receptor type I. They are syndecans implicated in various biological processes, especially in cell adhesion and

migration, growth factor signaling and cancer, inflammation, but also in and wound healing.

- **Small Leucine-Rich Proteoglycans (SLRPs):** as the name suggests, this group are characterized by protein core with leucine rich-repeat (LRR) motifs covalently linked to glycosaminoglycan (GAG) side chains. In this group, GAGs can be composed of chondroitin sulfate, dermatan sulfate or keratan sulfate. Based on their amino acid sequences and structural features, SLRPs are classified into five groups. The different SLRPs play different biological roles such as cell signaling modulator, collagen fibrillogenesis, wound healing and tissue repair, regulation of inflammatory response,

- **Lecticans:** it constitutes a large family of proteoglycans. Lecticans are structurally characterized by the presence of globular domains at the N-terminal- which contains the attachment sites for chondroitin sulfate or Keratan sulfate chains- and C-terminal ends of their core proteins that has lectin-like propertie. Different members of this family can be divided into four major groups: *Aggrecan, Versican, Neurocan, Brevican.* Lecticans are known for their prominent role in neural development and plasticity.

- **Other proteoglycans:** Many proteoglycans do not belong to the families we have previously mentioned. The core protein of this proteoglycans has different sizes ranging from 10 amino acids (Serglycin) up to 450 amino acids (Perlecan), the predominant glycosaminoglycans are Heparan Sulfate & Chondroitin sulfate. These proteoglycans play various roles in the different biological process. The the best-known member of that group is CD44. Exiting in various isoforms (more than 20 isoforms), the Cluster of Differentiation 44 “CD44” is a transmembrane glycoprotein type I expressed in all human cell types except on red blood cells. It is implicated in various biological process, such as cell migration, adhesion, lymphocyte activation and in pathological process such as cancer, inflammation and autoimmune diseases.

Table II: Human Proteoglycans

Type	Core Protein (kDa)	GAG Chains	Chromosome Localization	Tissue Location
Glypicans				
Glypican 1	56	Heparan Sulfate	2q35-q37	GPI-anchored cell surface
Glypican 2	59		7q22.1	
Glypican 3	59		Xq26.1	
Glypican 4	58			
Glypican 5	59		13q32	
Glypican 6	58			
Syndecans				
Syndecan-1	33	Heparan Sulfate , Chondroitin sulfate & Dermatan sulfate	2p24.1	Transmembrane, extracellular
Syndecan-2	23	Heparan Sulfate	8q22-23	
Syndecan-3	43	Heparan Sulfate , Chondroitin sulfate & Dermatan sulfate	1pter-p22.3	
Syndecan-4	22	Heparan Sulfate	20q12	
Lecticans				
Aggrecan	208–220	Chondroitin sulfate & Keratan sulfate	15q26.1	Extracellular
Versican (isoform 0)	373	Chondroitin sulfate	5q14.3	
Versican (isoform 1)	265		5q14.3	
Versican (isoform 2)	180		5q14.3	
Versican (isoform 3)	72		5q14.3	
Neurocan	145		19p12	
Brevican	96		1q31	
SLRPs				
Decorin	36	Chondroitin sulfate & Dermatan sulfate	12q21.33	Extracellular
Biglycan	38	Dermatan sulfate & Chondroitin sulfate	Xq28	
Fibromodulin	42	Keratan sulfate	1q32	Extracellular, intracellular
Lumican	38		12q21.3-q22	Extracellular
Keratocan	37		12q22	
Mimecan	25		9q22	
Others				
Thrombomodulin	58	Chondroitin sulfate	20p11.2	Transmembrane

CD44	37-81	Chondroitin sulfate & Dermatan sulfate	11p13	Transmembrane, extracellular, intracellular
NG2/CSPG4	251	Chondroitin sulfate	15q24.2	Transmembrane
Invariant chain	31	Chondroitin sulfate	5q32	Cell surface, intracellular
Neuroglycan-C	120-150	Chondroitin sulfate	3p21.3	Transmembrane
Type XVIII collagen	180-200	Heparan Sulfate	21q22.3	Extracellular
Perlecan	400-450	Heparan Sulfate	1p36.1	Extracellular
Agrin	212	Heparan Sulfate	1p36.33	Transmembrane, extracellular
Betaglycan	110	Heparan Sulfate & Chondroitin sulfate	1p33-p32	Transmembrane
SV2	20	Keratan sulfate	1q21.2	Transmembrane
Serglycin	10-19	Heparan Sulfate & Chondroitin sulfate	10q22.1	Intracellular
Endocan	50	Dermatan sulfate	5q11.2	Circulating extracellular
Neuropilin-I	130	Heparan Sulfate & Chondroitin sulfate	10p12	Transmembrane
Type IX collagen	270	Chondroitin sulfate	6q12-14	Extracellular
Testican 1	48	Heparan Sulfate & Chondroitin sulfate	5q31.2	Extracellular
Testican 2	45	Heparan Sulfate & Chondroitin sulfate	10pter-q25.3	Extracellular

IV.4-The Basement membrane

Known since the mid-19th century, the basement membrane is considered as highly specialized form of the extracellular matrix. Under electron microscopy, the average thickness of the basement membrane is 50nm and can reach 200nm. According to electron microscope density, the basement membrane consists of two zones, a transparent zone to electrons: *lamina lucida*, and a dense zone to electrons: *lamina dense*.

The basement membrane surrounds and separates the polarized cells (epithelial & endothelial cells) from the tissues, thus allowing the underlying connective tissues to be separated. It is found in different at the interface of:

- **Epithelial Tissues**: The basement membrane separating epithelial cells from the underlying connective tissue. It provides structural support and regulates cell behavior from: skin epidermis, gastrointestinal tract, respiratory epithelium and glandular epithelium

- **Endothelial Cells**: The basement membrane plays a crucial role in blood vessel function and stability. It surrounds blood vessels, providing a selective barrier and structural integrity.
- **Muscle Cells**: Encasing muscle fibers, providing support and maintaining the structural integrity of muscle tissue. The basement membrane also plays a role in muscle repair and regeneration.
- **Nerve Cells**: Schwann cells, which myelinate peripheral nerves, are surrounded by a basement membrane. This structure supports nerve regeneration and function.
- **Kidneys**: The glomerular basement membrane is a critical component of the filtration barrier in the kidneys. It separates the blood in the glomerular capillaries from the urinary space in Bowman's capsule.
- **Adipocyte**: Basement membrane supports the structure and function of adipocyte.

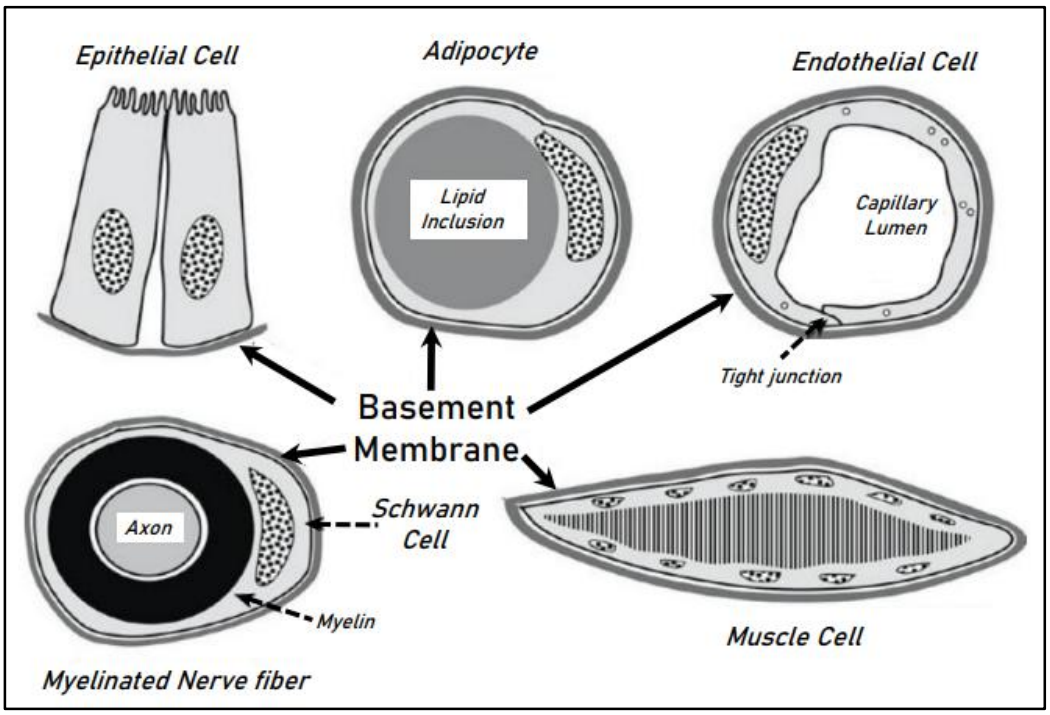

Figure 24: Distribution of basement membrane in different cell types.

Due to the development of biochemical and molecular techniques, the composition of the basal membrane has been analyzed through the work of many researchers (eg; *Nicholas Kefalides*, *Peter Bruckner*, *Rupert Timpl*, *Klaus kunn*).

It has been demonstrated that, the basement membrane is a composite of: ***laminins***, ***collagen IV***, ***nidogens/entactin***, ***perlecan*** and ***agrin***. These different components become organized into insoluble cell scaffoldings and constitute a complex meshwork.

- **Laminins** : Most abundant noncollagenous protein in basement membranes. Laminins are a fairly large family (16 members in mammalian) of heterotrimers each consisting of an α (five), a β (four) and a γ (three) subunit joined together through a long coiled-coil domain.

Laminins are either a cross-shaped (three short arms and one long coiled-coil arm), Y-shaped (due to absence of the α-short arm), or rod-shaped (with truncated short arms). The major receptor-binding domains are the C terminal laminin-type globular domains located at the C-terminal moiety of α subunit. Laminin interact with cell surface receptors and other basement membranes components, influencing cell adhesion, differentiation, migration, and signaling. The two important Laminin in basement membrane are:

Laminin-332: Important for the adhesion of epithelial cells.
Laminin-511: Plays a role in cell differentiation and basement membrane assembly.

- **Collagen IV:** Constitutes the primary structural component, forming a network that provides tensile strength and scaffold of basement membrane. Collagen IV structure consist of three alpha chains (α1, α2, α3, α4, α5, α6), each of which has a non-collagenous domain at the C-terminal (NC1 domain) and at the N-terminal (7S domain). The alpha chains coil around each other to form a triple-helical structure. This structure is unique in collagen IV because it contains interruptions in the Gly-X-Y repeats, allowing flexibility. The most ubiquitous trimer consists of two α1 and one α2 chains. The α3α4α5

and α5α5α6 heterotrimers, on the other hand, have restricted distributions; notably in the renal glomeruli, neuromuscular junctions, eyes, inner ears and other locations.

The collagen IV provide structural support to the basement membrane, contributing to its mechanical strength and stability, but also in cell-matrix interactions, barrier function and tissue repair/regeneration.

- **Nidogen/Entactin:** are a family of highly conserved, sulfated glycoproteins. Members of the nidogen family are composed of a series of sulfated monomeric glycoproteins.

Structure of *nidogen/entactin* consists of several domains, including three globular domains (G1, G2, and G3) connected by rod-like segments. The structure allows it to interact with various other basement membrane components, especially with laminin-111 & collagen IV. To date, there are two known isoforms of nidogen: Nidogen-1 and Nidogen-2. They have similar structures and functions but may be expressed in different tissues or at different stages of development. Nidogen allows to linking laminins and collagen IV and stabilizing the structure of basement membrane. It also contributes to cell adhesion, migration, and differentiation by interacting with cell surface receptors and other extracellular matrix components. Nidogens control cell growth, survival, and differentiation by influencing cell signaling pathway.

- ***Perlecan:*** With average molecular weight of 467kDa, perlecan widely distributed in the basement membrane of skin, breast, heart, pituitary gland, thymus, prostate, colon, lung, kidney, ear and placenta. Throughout their Heparan Sulfate chain, perlecan interact with laminin, type IV collagen, and fibronectin. Nidogen/Entactin interact with perlecan throughout core protein G2 and G3 domains.
- ***Agrin:*** *Heparan sulfate proteoglycan with average weigh mass of* 400 kDa, argin is a large, multidomain protein with several distinct regions that allow it to interact with a variety of molecules. The different domains are Key

domains *N-terminal Domain, LG (laminin G-like) Domains, SEA Domain, EGF (epidermal growth factor)-like Domains*. Argin play an important role in neuromuscular junction in influencing the Synapse formation and maintenance.

IV.5-Cell Polarity

Different microscopic technics, but also the development of biochemical methods made it possible to observe that the architecture of the cells is not symmetrical. This asymmetric architecture of the cell is called *Cell Polarity*.

IV.5.1-Definition and Types

Firstly described by the American biologist and zoologist *Edwin Grant Conklin*. The National Library of Medicine define Cell polarity as *Orientation of intracellular structures especially with respect to the apical and basolateral domains of the plasma membrane. Polarized cells must direct proteins from the Golgi apparatus to the appropriate domain since tight junctions prevent proteins from diffusing between the two domains*.

The asymmetric spatial arrangement and protein composition distribution of different cellular components, allows the establishment and maintain of functionally specialized domains in the plasma membrane and cytoplasm. The cell polarity is a fundamental characteristic of all cells in invertebrate and vertebrate. In response to different extracellular or intracellular molecular signals, cell polarity is organized in an apico-basal axis.

The cell polarity is a critical state in some biological cell process such as, signal transduction, transmission of nervous system information, cell migration, phagocytosis, epithelial secretion and absorption, immune response and epithelial cell growth. In each cell type, extracellular and/or intracellular signals triggering cell polarity are different, but it is orchestrated by a same mechanistic pattern: activation of compartmentalized signaling pathways, major rearrangements of the cytoskeleton, regulation of lipid landscape and polarized membrane trafficking.

Cell polarization can be categorized into several types based on the spatial organization and functions of different cell types. Here are the main types of cell polarization:

- **Apical-Basal Polarity**: Often refers to epithelial cells, this type of polarity is characterized by distinct apical (*top*) and basal (*bottom*) surfaces. The apical surface faces the lumen or external environment and often has specializations such as microvilli or cilia, while the basal surface interfaces with the basement membrane and underlying tissues.

Apical-Basal polarity involves complexes like *Par*, *Scribble*, and *Crumbs*, which help establish and maintain this polarity by controlling the distribution of proteins and lipids.

- **Planar Cell Polarity (PCP)**: In epithelial tissue, cells within a plane are polarized to coordinate structures and functions, such as hair follicle orientation or cilia beating direction. The PCP pathway, help orient cells within the tissue plane. This pathway involve proteins such as *Frizzled*, *Van Gogh*, and *Dishevelled*.
- **Front-Back Polarity (Migratory Polarity)**: Migrating cells like fibroblasts, neutrophils, and cancer cells exhibit front-back polarity during movement. The leading edge is characterized by actin-rich protrusions (lamellipodia and filopodia) that explore and move forward, while the trailing edge retracts. The *Rho GTPases* (*Rac*, *Cdc42*, and *Rho*), *PI3K/Akt* signaling, and the cytoskeleton (actin and microtubules) play crucial roles in establishing and maintaining this polarity.
- **Neuronal Polarity**: Neurons exhibit distinct axonal and dendritic domains essential for signal transmission and neural circuit formation. The axon is specialized for sending signals, while dendrites receive signals. Neuronal polarity involves signaling pathways such as *PI3K/Akt*, Par proteins, and cytoskeletal elements (microtubules and actin filaments) to establish and maintain polarity.

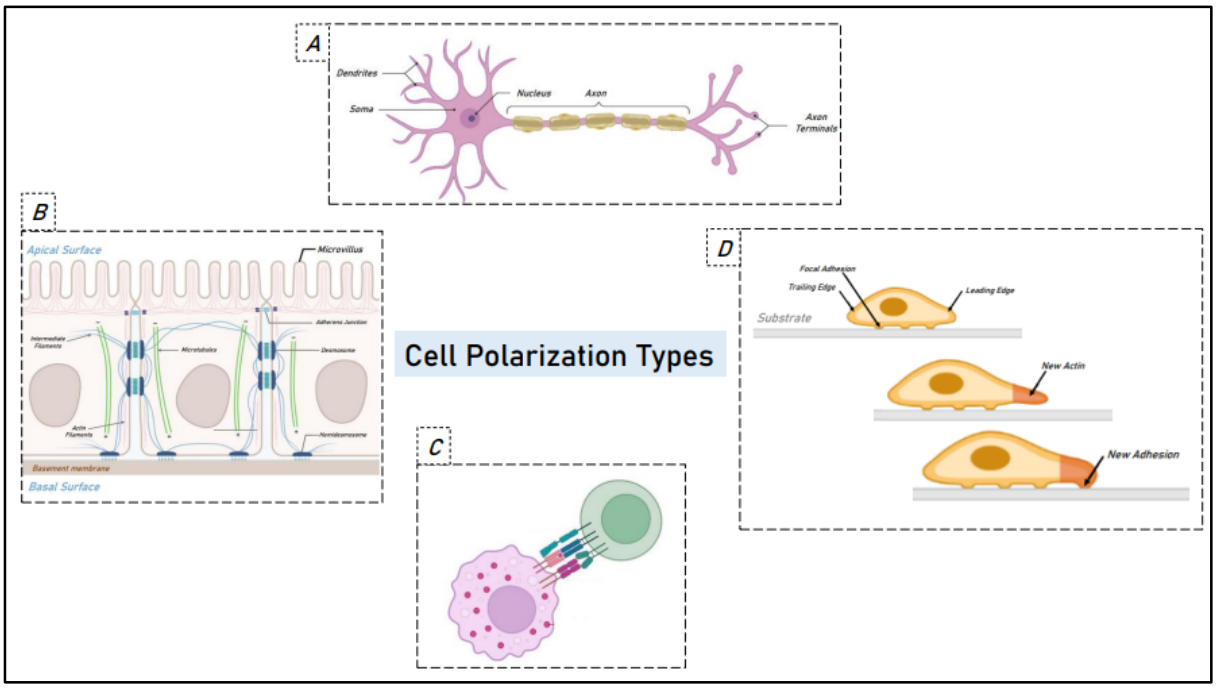

Figure 25: Examples of cell polarization types.

A: *Neuron polarization.* **B**:*Apical-Basal polarization of epithelial cells .* **C**: *T- cell polarization and formation of immunological synapse.* **D**: *Polarization of cell during migration.*

- **Hematopoietic Cell Polarity**: Immune cells such as T cells and neutrophils, exhibit polarity when responding to chemotactic signals. This polarization is essential for directed movement towards infection sites. Mechanisms of T cells and neutrophils polarity Involves chemokine receptors, Rho GTPases, and cytoskeletal rearrangements to establish front-back polarity for effective migration.
- **Asymmetric Cell Division**: Stem cells and Progenitor cells exhibit polarity during division to produce two daughter cells with different fates—one remains a stem cell, and the other differentiates. They involves the Par complex, spindle orientation, and differential distribution of cell fate determinants.

IV.5.2-Cell-Cell Junction

Cells in tissues form specialized junctions like tight junction, adherens junction, and desmosomes that exhibit polarized distribution and are essential for tissue integrity and function. This type of polarization involves adhesion molecules (cadherins, integrins), cytoskeletal elements, and signaling pathways that regulate junction assembly and maintenance.

- **Tight Junctions***:*

Specialized connections between adjacent cells that form a barrier to regulate the movement of substances between them. TJs serve as barrier/gate function (barrier to the free diffusion of ions and small solutes along the paracellular pathway) and to form an intramembrane diffusion barrier for lipids and integral membrane proteins (also called fence function).

Tight junctions are composed of transmembrane proteins that interact with each other on the surfaces of neighboring cells. The majors proteins involved in tight junctions are:

- **Claudins**:

Family proteins composed at least 24 members; these proteins form the backbone of tight junctions, creating a selective barrier that regulates the passage of ions and small molecules.

- **Occludin**:

Member of claudins family. Occludin is a transmembrane protein with a molecular weight of 65kDa. It play an important role in the junctions sealing and regulation of tissues permeability.

- **JAMs (Junctional Adhesion Molecules)***:*

members of immunoglobulin superfamily. JAMs are type I transmembrane proteins characterized by two type V/C2 extracellular immunoglobulin domains, a single transmembrane domain and a carboxy-terminal intracellular part. These proteins also help in the formation and maintenance of tight junctions. JAMs are important regulators of the barrier function in various organs. They act predominantly as signalling molecules rather than classical adhesion receptors.

These different roles of Tight Junctions are realized through interaction with PDZ domain-containing scaffolding proteins inside the cell , such as Zonula Occludens proteins, which anchor the transmembrane proteins to the cytoskeleton to recruit and assemble signaling complexes.

- **Adherens Junction:**

Adherens junctions (AJs) are cell–cell adhesion complexes observed in a variety of cell types, as well as in different animal species. AJ is characterized by a pair of plasma membranes apposed with a distance of 10–20 nm between them, whose intercellular space is occupied by rod-shaped molecules bridging the membranes.

One prominent AJ is the zonula adherens . Found in most epithelial cells, the zonula adherens forms belts that link the cells into a continuous sheet and separate the apical and basolateral membranes of each highly polarized cell. AJs can also function as clusters during zonula adherens assembly and dynamic cell–cell interactions, and in mature tissues.

Cadherin adhesion molecules (a calcium-dependent cell-cell adhesion glycoproteins) are core AJ component. Most classic cadherins operate as homophilic adhesion receptors to facilitate cell–cell recognition and adhesion. All cadherins contain two or more extracellular cadherin domains. Classic cadherins additionally have a highly conserved cytoplasmic tail that interacts with a defined set of cytoplasmic proteins particularly members of the catenin family, which locally regulate the organization of the actin cytoskeleton, cadherin stability and intracellular signaling pathways that control gene transcription.

- **Gap junctions:**

Specialized connexion between cells. Gap junctions form a space with a size of 2 to 4nm between two neighbouring cells. They are formed by a family of integral proteins (21 members) called connexins.

The channel that spans the gap junction is the connexon, that corresponding to hexameric assembly of connexins proteins. This a continuous aqueous channel, allowing small molecules (such as ions, second messengers, and metabolites) to pass between the cells.

Desmosomes:

Unlike adherens junctions (AJs), which connect to the actin cytoskeleton network, desmosomal junctions – also called *maculae adherentes*- are tethered to the intermediate filament network. The junctions formed by desmosomes provide a strong adhesion between cells, because they also link intracellularly to the intermediate filament cytoskeleton they form the adhesive bonds in a network that gives mechanical strength to tissues (it appear as electron dense discs approximately 0.2–0.5 µm in diameter). This essential structural and mechanical function is highlighted by the prominent distribution of desmosomes in tissues that are routinely subjected to physical forces, such as the heart and skin.

A variety of proteins they participate in the composition of desmosomes in différents tissues. It is the case of cadherins members: *Desmogleins (Dsg) & Desmocollins (Dsc)* ; Plakins members : *Plakoglobin (γ-catenin) & Plakophilins (Pkp)*; Intermediate filaments proteins members : *Keratin & Desmin*.

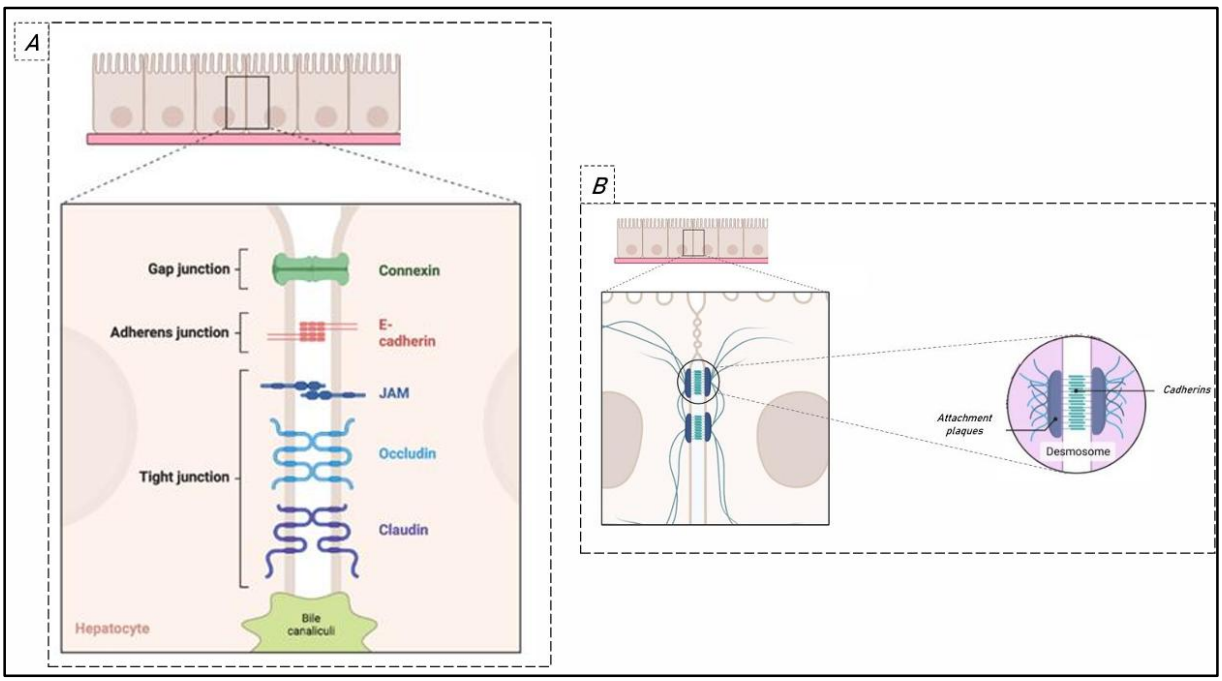

Figure 26: Types of cell-cell junctions.

A: *Cell junction organization.* **B**: *Desmosomes.* *Attachment plaques is composed of: Desmoplakin, Plakophilins & Plakoglobin.*

V-Cytosolic Compartment

The inside of a cell is not a bag that contains only organelles and proteins. Indeed, the organelles are suspended in the aqueous, gel-like fluid called Cytosol.

V.1-Chemical Composition and Cytoplasmic Organelles

The cytosol is mainly composed of water, which represent ≈70% of its components. This water serves as a solvent for various solutes and facilitates the transport of molecules within the cell. Thus, promoting many chemical reactions and molecular interactions, that are essentials for cellular metabolism and homeostasis.

Despite the fact that is mainly water-based, the cytosol is a dense medium with relatively high viscosity. For this reason, macromolecules diffuse over a short distance of a few nanometers or by active transport on the cytoskeleton tracks over longer distances of micrometers.

The cytosol viscosity is due to the fact that, it contains many dissolved ions, especially: *Potassium, Sodium, Calcium, Magnesium, Chloride, Bicarbonate, Phosphate, Sulfate and Hydrogen* (Table III). These ions play vital roles in maintaining the structural and functional integrity of the cell and are involved in numerous physiological processes like signal transduction, metabolism, and homeostasis.

In addition to ions and because it is the site of many processes *(we will detail this below)*, cytosol also contains small molecules, and macromolecules (e.g., proteins, RNA, metabolites).

Table III: The main ions present in the cytosol.

Ions	Characteristics in cytosol
Potassium (K^+)	• The concentration is typically much higher inside the cell compared to outside. • Plays a key role in maintaining the resting membrane potential. • Involved in cell signaling and volume regulation.
Sodium (Na^+)	• Higher concentration outside the cell compared to the cytosol. • The sodium-potassium pump (Na^+/K^+-ATPase) helps maintain this gradient. • Important in processes like action potentials in neurons and muscle cells.
Calcium (Ca^{2+})	• The concentration is usually very low in the cytosol, with a much higher concentration in the endoplasmic reticulum (ER) and extracellular space. • Acts as a signal in numerous cellular processes, including muscle contraction, neurotransmitter release, and enzyme activity.
Magnesium (Mg^{2+})	• Relatively low concentration compared to calcium. • Essential cofactor for many enzymes. • Involved in the regulation of ATP, protein synthesis, and nucleic acid function.
Chloride (Cl^-)	• Higher concentration outside the cell compared to inside. • Plays a role in maintaining osmotic balance and electrical neutrality.
Bicarbonate (HCO_3^-)	• Acting as a buffer and help to regulate pH in the cytosol. • The concentration can fluctuate, particularly during metabolic processes that produce or consume acids.

Phosphate (PO_4^{3-})	• Important in energy storage and transfer, as part of ATP and other nucleotides. • Plays a role in regulating cellular signaling through phosphorylation and dephosphorylation.
Sulfate (SO_4^{2-})	• Generally present in lower concentrations. • Important in the synthesis of certain molecules, like proteoglycans.
Hydrogen (H^+)	• Not typically abundant in free form in the cytosol. • Concentration rate is closely linked to the pH of the cytoplasm. • Cellular processes such as respiration influence the concentration of H^+ ions.

It is important to note that the viscous nature of cytosol, allows this gel to encoat many cellular organelles. Among the organelles suspended in the cytosolic compartment, we find *Ribosomes*, *Mitochondria*, *Endoplasmic Reticulum*, *Golgi Apparatus*, *Cytoskeleton*, *Lysosomes* and Peroxisomes **(Table IV)**

Table IV: Organelles suspended in the cytosol.

Organelle	Characteristics
Ribosomes	• Biomolecules composed of rRNA and dozens of proteins. • Considered as a site of protein translation. • Free in the cytosol or attached to the endoplasmic reticulum (rough ER). • Implicated in cellular proliferation, differentiation and homeostasis.
Mitochondria	• Membrane bound organelle and cell energy unit. • Performs oxidative phosphorylation to generate ATP.

	• Contributes to many processes central to cellular function and dysfunction including calcium signalling, cell growth and differentiation, cell cycle control and cell death.
Endoplasmic Reticulum (ER)	• Part of the endomembrane system. • Network of membranes, which proteins and other molecules move. • Rough ER is involved in protein synthesis and folding. • Smooth ER is involved in lipid synthesis and detoxification.
Golgi Apparatus	• Membrane-bound organelles. • Recognizable by electron microscope due to its flat-perforated cisternae structure's. • Involved in modifying, sorting, and packaging glycoproteins and glycolipids for secretion or delivery to other parts of the cell.
Cytoskeleton	• Scaffolding-structure for the cell. • A network of protein filaments formed by actin filaments, microtubules, intermediate filaments. • Roles in processes such as endocytosis, cell division, intra-cellular transport, motility, force transmission, reaction to external forces, adhesion and preservation, and adaptation of cell shape.
Lysosomes	• Highly heterogeneous cellular organites, with size ranges between 0.05 and 0.5 μm in animal cells. • Whose number is between 50 and 1000 per cell • Contains fifty-one enzymes (phosphatase, glycosidase, lipase, nuclease, sulfatate and proteases) for digesting macromolecules and cellular debris.

	• Considered as the *garbage-disposal system* of animal cells.
Peroxisomes	• Contains about 50 enzymes, including catalase, urate oxidase, and d-amino acid oxidase. These enzymes degrade uric acid and amino acids. • An important function of this microbody is provided by other enzymes that degrade long chain fatty acids by β-oxidation. • Synthesizes bile acids, cholesterol, and plasmalogen, and metabolizes amino acids and purine.

Hence, the cytosol or cytoplasmic matrix in addition to organelles-cited before-represents the cytoplasm. In other words, the **cytoplasm** is the entire internal environment of the cell, while the **cytosol** is just the liquid portion that surrounds the cell's organelles.

Cytosol plays a crucial role in supporting various cellular functions. Here are the main functions of the cytosol:

V.2-Metabolism (Glycolysis):

The cytosolic compartment is crucial for energy production, particularly through glycolysis and the initial stages of cellular respiration.

Glycolysis also known as Embden–Meyerhof pathway, corresponds to the first step in the breakdown of glucose to extract energy and does not require oxygen (anaerobic).

This process entails the use of glucose, which by a series of reactions including oxidation and consumption of ATP molecules (2 ATP molecules per glucose molecule), allows the produce -from one glucose molecule- : 04 ATP, 02 NADH & 02 Puryvate molecules. The pyruvate can be used in the citric acid cycle (Krebs cycle) or serve as a precursor for other reactions.

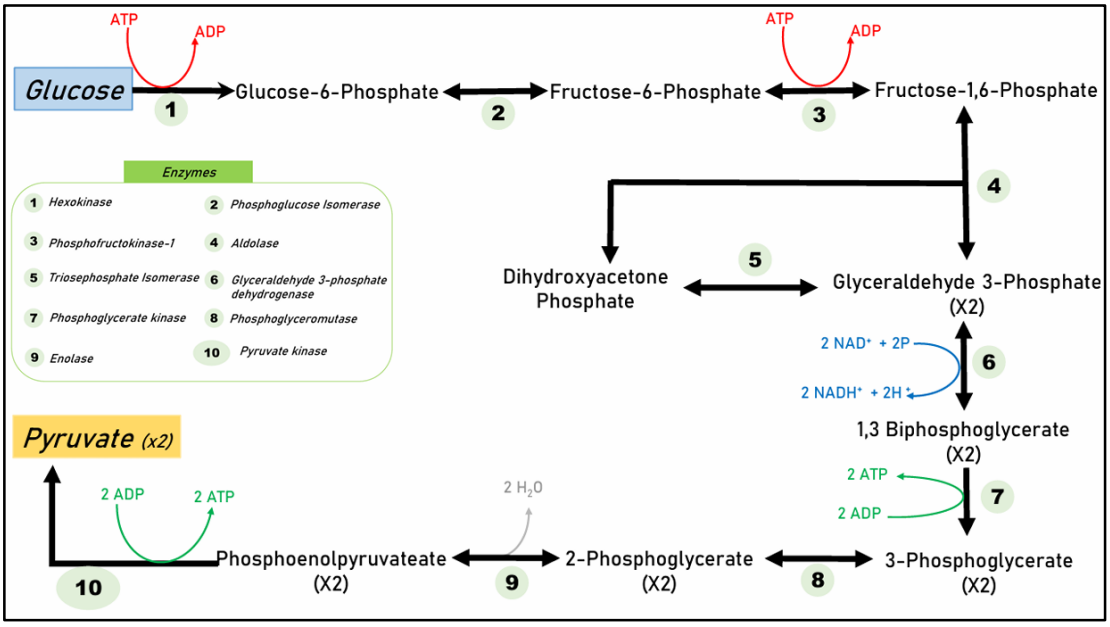

Figure 27: Schematic representation of glycolysis pathway.

In addition to energy metabolism, the cytosol is the site of another type of metabolism essential for cell survival and function: protein synthesis.

V.3-Protein Metabolism and Synthesis (Translation):

Protein synthesis requires the presence of a sufficient quantity and variety of amino acids within the cytosol. In Human, in addition to the essential amino acids that the organism cannot produce (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine)—which are obtained through dietary intake or protein recycling—non-essential amino acids are produced within the cytosol.

In addition to its central role in energy production, the tricarboxylic acid (TCA) cycle or Krebs cycle is also involved in amino acid metabolism. In fact, the TCA cycle provides carbon skeletons for amino acids. The action of Glutamate Dehydrogenase (GDH) produces the L-Glutamate from α-Ketoglutarate.This mechanism is called ***the reductive amination***. The α-Ketoglutarate may also transaminased (amino group transfer) via transaminase enzymes (Alanine Aminotransferase "ALT") from another amino acid donor—specifically alanine—to produce Glutamate and Pyruvate. This process is called ***transamination***. This pathway can be also synthetize the Aspartate from

oxaloacetate and glutamate -as amino acid donor- following the action of Aspartate Aminotransferase ‘’AST” enzyme. The pentose phosphate pathway can provide an amino acids. In fact, the Erythrose 4-Phosphate an intermediate of pentose phosphate pathway, play a role of precursor of aromatic amino acids. The table below **(Table V)** gives an overview of different TCA intermediate involved in amino acids synthesis, in animal and plant cells.

Table V: TCA cycle intermediates and amino acids synthesis in cytosol.

Entry Point	Amino acids
Pyruvate	Alanine/Cysteine/Glycine/Serine/Threonine/Tryptophan
Acetyl-CoA	Isoleucine/Leucine/Lysine/Threonine/Tryptophan
Acetoactyl-CoA	Leucine/Lysine/Phenylalanine/Tryptophan/Tyrosine
α-Ketoglutarate	Arginine/Glutamine/Glutamate/Histidine/Proline
Succinyl-CoA	Isoleucine/Methionine/ Threonine/Valine
Fumarate	Phenilalanine/Tyrosine
Oxaloacetate	Asparagine/Aspartate

In addition to the ribosome present at the endoplasmic reticulum or the nucleus membranes, free ribosomes-unattached to any membrane- are present are present at the cytosol level either alone or in the form of polysomes. These ribosomes are responsible for the synthesis of polypetides chains of proteins addressed for the cytosol, nucleus, mitochondria or peroxisomes. In other words, cytosolic ribosomes ensure the step of translation of mRNA of proteins **(Figure 28)**, especially thus that function in the cytosol (eg., actin, tubulin, kinase) or destined for the nucleus (eg.,histones) and mitochondria, chloroplasts, or peroxisomes.

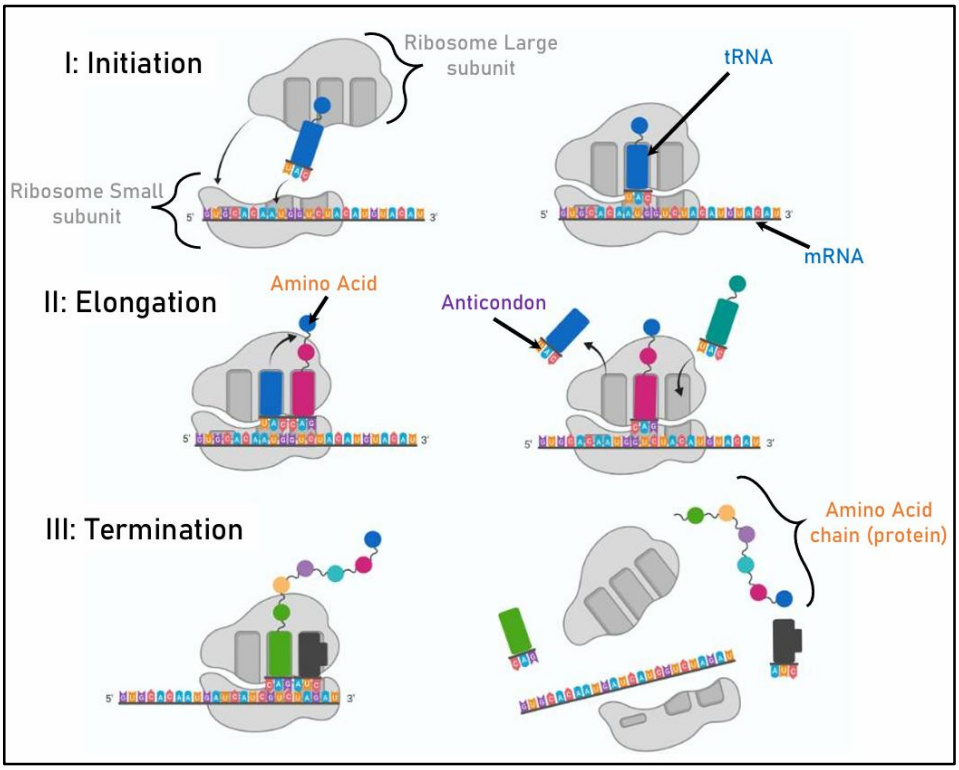

Figure 28: Molecular process of protein translation.

V.4-Protein Folding and Chaperones

Following the translation step, the newly synthesized polypeptide chain undergoes a series of modifications to attain its definitive three-dimensional structure. This protein folding process is essential for their functions and facilitated by cytosolic proteins known as chaperones. Categorized into several families **(Table VI)**, the role of chaperone molecules was only elucidated during the 1990s. Indeed, in addition to their role in protein folding and unfolding, chaperones prevent protein aggregation, particularly under stress conditions. They also participate in the transport of proteins within the cytosol, as well as their translocation from one cellular compartment to another (e.g., from the cytosol to the nucleus or to the endoplasmic reticulum, and vice versa). The formation of heteromeric protein complexes (proteins composed of multiple different subunits) is also facilitated by chaperone molecules. Thus, these various molecular chaperones play a predominant role in maintaining protein homeostasis (proteostasis).

Table VI: Major Families of Cytosolic Chaperone.

Chaperone Family	Key Representatives	Mechanism of Action	Primary Cellular Functions
Hsp70 System	Hsp70 (HSPA8), Hsp40 (J-proteins), NEFs (e.g., BAG-1, Hsp110)	ATP-dependent cycle of binding/releasing hydrophobic client peptides. Regulated by co-chaperones.	*De novo* folding, prevention of aggregation, protein translocation, co-factor in disaggregation.
Chaperonins (Group II)	TRiC/CCT (TCP-1 Ring Complex)	ATP-dependent folding inside an isolated barrel-shaped compartment.	Folding of complex proteins (e.g., actins, tubulins).
Hsp90 System	Hsp90α/Hsp90β, Co-chaperones (p23, CDC37)	ATP-dependent conformational regulation and stabilization of metastable clients.	Late-stage maturation and activation of signaling proteins (kinases, transcription factors).
Small Heat Shock Proteins (sHsps)	Hsp27 (HSPB1), αB-crystallin	ATP-independent "holdases." Form dynamic oligomers to bind unfolding proteins.	Prevent aggregation during stress, holding clients for refolding.
Disaggregases	Hsp104 (yeast/plants), Hsp70-Hsp40-Hsp110 complex (mammals)	ATP-dependent threading of aggregated proteins through a central pore.	Solubilization and reactivation of protein aggregates.
Folding Enzymes	Peptidyl-prolyl Isomerases (e.g., FKBP, Cyp40)	Catalyze *cis-trans* isomerization of proline peptide bonds.	Accelerate folding kinetics.

V.5- The Ubiquitin-Proteasome System :

In addition to protein synthesis, an essential process for maintaining cellular homeostasis, particularly that of proteins, occurs in the cytosol. This process is the degradation of proteins by the proteasome. In eukaryotic cells the Ubiquitin-Proteasome system is an essential degradation pathway of protein. Briefly, ubiquitinases (E1, E2 and E3 ubiquitine enzymes) add ubiquitin – a

small protein of 8.5 kDa- to target proteins, which subsequently directs them to the proteasome for rapid degradation by proteasome **(Figure 29)**.

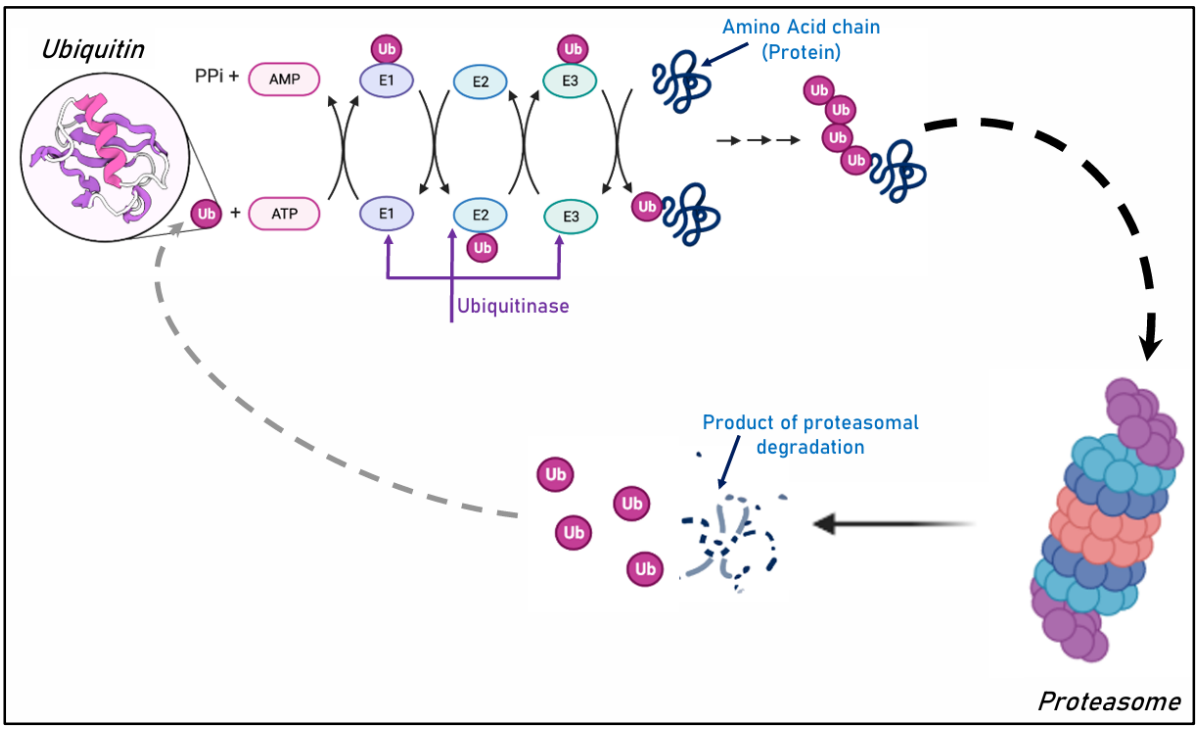

Figure 29: Ubiquitin-Proteasome pathway

In Eukaryotic cells, the proteasome, so called Proteasome 26S is a heteromeric macromolecular complex composed of multiple subunits organized into two primary structures: the 20S core particle, which constitutes the central catalytic core responsible for protein degradation, and the 19S regulatory particle, which caps one or both ends of the 20S core **(Figure 30)**.

The 19S particle facilitates the unfolding of ubiquitinated proteins and their translocation into the 20S core to enable degradation. It is important to note that in antigen-presenting cells, the proteasome is distinct from the standard 26S proteasome. Notably, in antigen-presenting cells, cytokine signaling induces the replacement of the standard 26S proteasome with a specialized form known as the immunoproteasome. This substitution involves quantitative and qualitative alterations to the complex, markedly increasing its proteolytic efficiency.

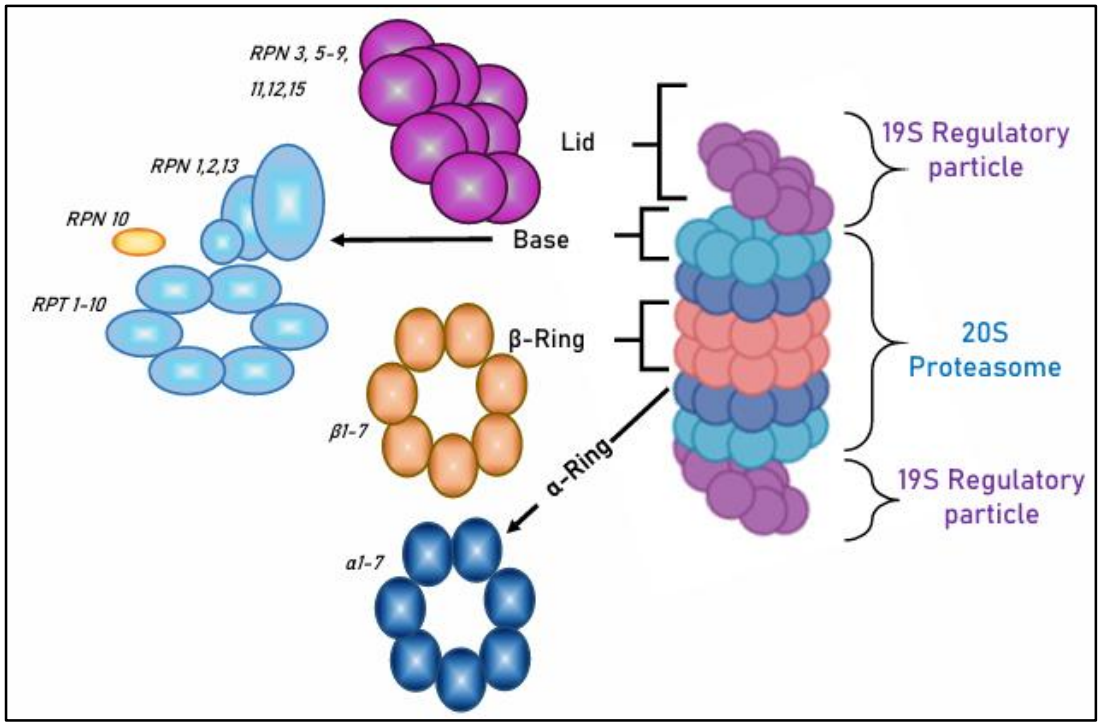

Figure 30: Schematic representation of Proteasome 26S.

RPT: *Regulatory Particle Triple-A*, **RPN**: *Regulatory Particle Non-ATPase cyclic.*

V.6- Signal Transduction :

The cell receives numerous external signals through cell-cell interactions or via soluble molecules. From the receptors that bind the external signal to the intracellular target—typically genes within the nucleus—a series of biochemical events occurs in the cytosol. This sequence of events is referred to as signal transduction, commonly known as cell signaling. The cytosol act as wiring system where numerous key molecules are involved in signal transduction, notably ***kinases***. Kinases are proteins responsible for transferring one or more phosphate groups onto specific target proteins. The addition of phosphate groups induces a conformational change in the downstream protein. This change activates the protein, enabling it, in turn, to transfer phosphate groups onto other proteins and activate them, propagating the signal to its final endpoint. This process is termed a kinase cascade **(Figure 31) (Table VII)**. Kinases responsible for adding phosphate groups to serine or threonine residues are called Serine/Threonine Kinases. Tyrosine

Kinases are proteins that add phosphate groups specifically to tyrosine residues.

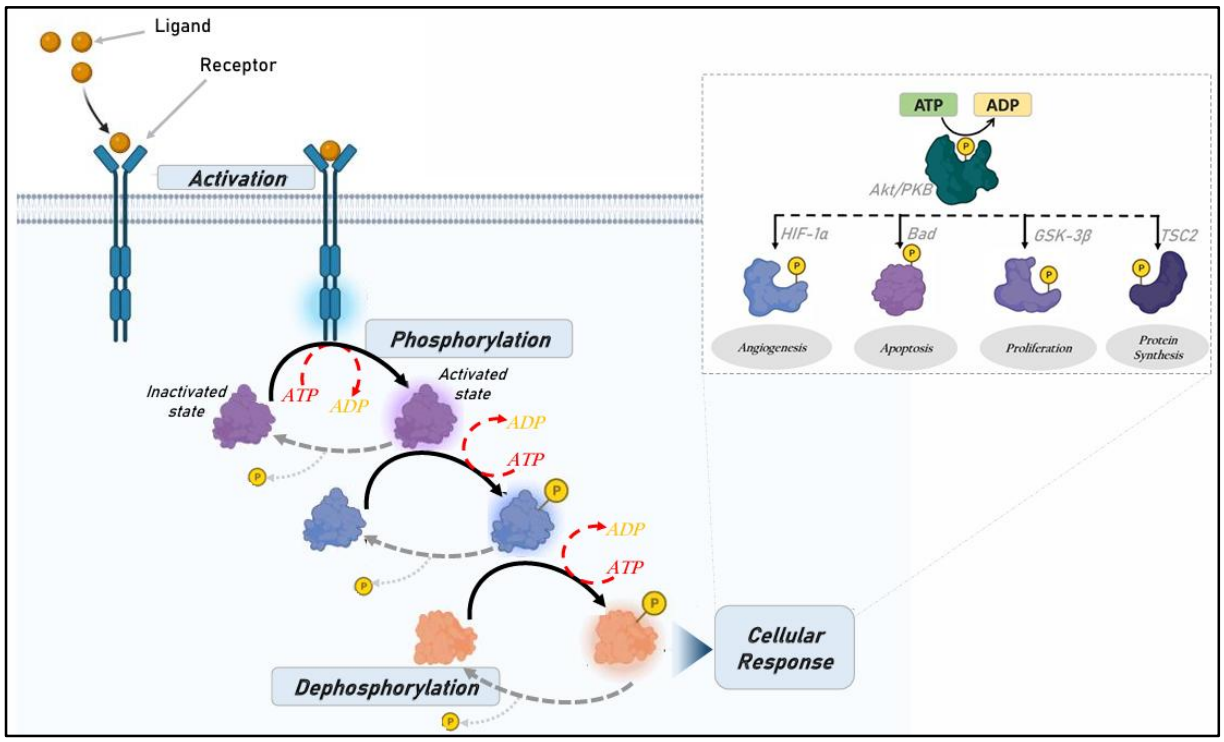

Figure 31: Phosphorylation signaling pathway.

Akt/PKB: *Protein Kinase B.* **HIF-1α**: *Hypoxia-Inducible Factor 1a.* **GSK-3β**: *Glycogen Synthase Kinase-3β .* **TSC2**: *Tuberous sclerosis complex 2 (Tuberin).* **ATP**: *Adenosine TriPhosphate.* **ADP**: *Adenosine DiPhosphate*

The arrest of cellular signaling induced by the kinase-mediated phosphorylation cascade is facilitated by another class of enzymes, whose role is to remove phosphate groups; these are the ***phosphatases***.

Table VII: Key cellular kinases signaling pathways**.**

Pathway	Receptor Type	Key Components	Cellular Effects
GPCR (G-protein coupled receptors)	7-transmembrane receptors	Gα, Gβγ, cAMP, PKA, IP_3, DAG, Ca^{2+}	Fast responses (heart rate, metabolism, sensory signals)
RTK (Receptor tyrosine kinases)	Single-pass transmembrane receptors	Ras, MAPK, PI3K, Akt	Immune response, cell growth
JAK-STAT	Cytokine receptors (no intrinsic kinase activity)	JAK kinases, STAT transcription factors	Late-stage maturation and activation of signaling proteins (kinases, transcription factors).
TGF-β/Smad	Ser/Thr kinase receptors	Smad proteins	Development, differentiation
Wnt/β-catenin	Frizzled receptors	β-catenin stabilization	Development, cell fate
Notch signaling	Notch receptor	Intracellular domain → nucleus	Cell-cell communication, development
NF-κB pathway	Cytokine/TLR receptors	IκB degradation → NF-κB activation	Inflammation, immune response
Steroid hormone signaling	Intracellular nuclear receptors	Hormone–receptor complex → DNA	Gene expression regulation

In addition to kinase cascades, numerous cellular signaling events that occur in the cytosol involve non-protein molecules. These are referred to as second messengers. Second messengers most commonly exist as cyclic nucleotides (eg., cAMP, cGMP), lipid derivatives (eg., IP_3, DAG) or ions (eg., Ca^{2+}) . Other molecules, such as nitric oxide(NO), carbon monoxide (CO), and reactive oxygen species (ROS), can also function as second messengers in specific contexts.

Second messengers perform numerous functions, their primary role is to amplify, diversify, and propagate the initial signal to elicit a coordinated biochemical cell response **(Table VIII)**. In fact, a single ligand-bound receptor can trigger the production of many second messenger molecules. Each second

messenger can then activate multiple downstream effector proteins (e.g., kinases), dramatically amplifying the original signal. This allows a weak extracellular stimulus to provoke a robust intracellular response. Second messengers provide nodes for crosstalk between different signaling pathways. The cell can integrate signals from multiple receptors by monitoring the concentrations of various second messengers (e.g., simultaneous Ca^{2+} and cAMP flux), leading to a tailored and specific response.

Table VIII: Functions of most important second messenger.

Second Messenger	Main Target (s)	Produced by	Key Functions
cAMP	PKA, EPAC, ion channels	Adenylate Cyclase	Glycogen & lipid metabolism, gene transcription (via CREB), cardiac contractility
cGMP	PKG, ion channels, PDEs	Guanylyl cyclase	Smooth muscle relaxation, vasodilation (NO pathway), phototransduction (vision)
IP_3	IP_3 receptors on ER	PIP_2	Mobilizes intracellular Ca^{2+} → regulates secretion, contraction
DAG	PKC	PIP_2	Cell proliferation, differentiation, secretion, immune activation
Ca^{2+}	Calmodulin, PKC, CaMKs, calcineurin	Channels, Enoplasmic Reticulum release	Muscle contraction, neurotransmitter release, exocytosis, enzyme regulation
NO	Soluble guanylyl cyclase → ↑ cGMP	NO synthase	Vasodilation, neurotransmission, immune defense
CO	Guanylyl cyclase	Heme oxygenase	Neuromodulation, vascular regulation
cADPR	Ryanodine receptors (ER)	ADP-ribosyl cyclase	Sustained Ca^{2+} release, long-term signaling (immune cells, neurons)

ROS	MAPK, NF-κB, redox proteins	Mitochondria, NADPH oxidase	Stress response, apoptosis, innate immunity
S1P	GPCRs (S1P receptors)	Sphingomyelin	Cell survival, migration, angiogenesis, inflammation

cAMP: cyclic Adenosine MonoPhosphate**, cGMP**:*cyclic Guanosine MonoPhosphate*, **IP_3:** *Insositol 1,4,5-trisPhosphate*, **DAG**: *DiAcylGlycerol*, **cADPR**: *Cyclic ADP-Ribose*, **S1P**: *Sphingosine 1-Phosphate*.

Of note, the cytosol plays a crucial role in other processes, notably cell division –especially during cytokinesis- and the transport of molecules in vesicles throughout the cell (vesicular trafficking), mediated by the microtubule network—which will be detailed later-.

V.7- Intracellular Defense and Immune Surveillance

The cytosol play also an important role in the control of infection progression and the elimination of bacteria and viruses. It is a hub of intense immune surveillance and execution, equipped with multiple, overlapping systems to detect, contain, and eliminate bacterial and viral invaders, often at the ultimate cost of the cell's own life to protect the organism as a whole.

V.7.1- Eliminating Bacteria

Generally, bacteria are destroyed outside the cell or within a membrane-bound compartments phagosomes. However, some facultative or obligate intracellular bacteria (e.g., *Listeria monocytogenes*, *Shigella flexneri, Salmonella enterica,Mycobacterium tuberculosis,Mycobacterium tuberculosis*) have evolved to escape or modify the phagosome and enter the cytosol to survive and replicate. The cytosol is not a safe haven; it is a battleground. The various mechanisms employed by the cytosol to eliminate bacteria include:

- **The xenophagy :** it is a specific autophagy mechanism where the cell recognizes invaders bacteria in the cytosol via the PAMPs-PRR(Pattern Recognition Receptor) interaction tags and engulfed encloses cytosolic invaders in a double-membrane structure called an ‘’autophagosome’’, which fuses with a lysosome that ensures the degradation and destruction of

bacteria through the action of powerful digestive enzymes (acid hydrolases) and a low pH it contains.

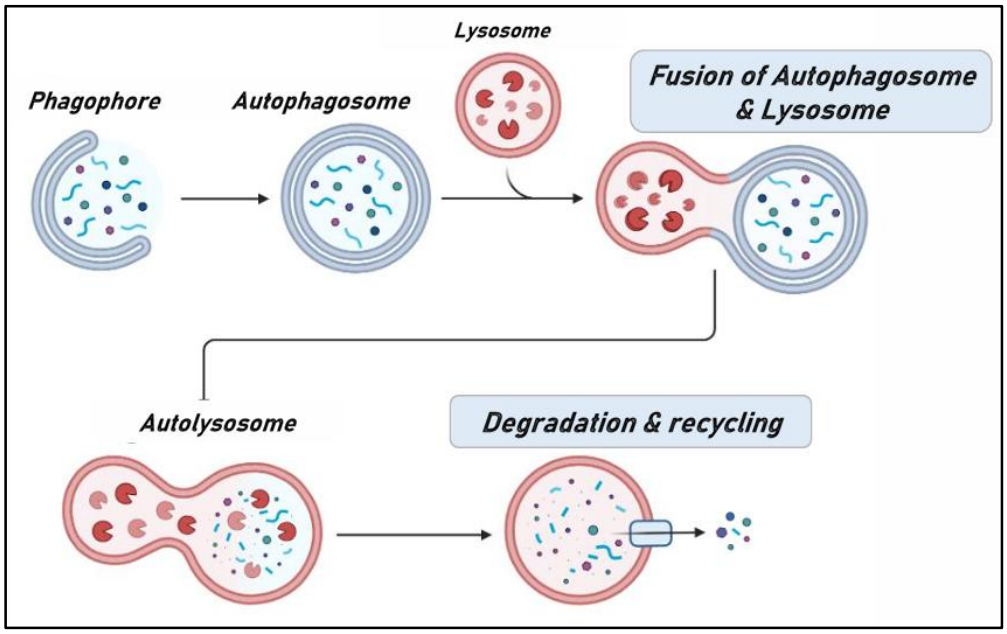

Figure 32: Steps of Autophagy Steps.

- **<u>Inflammasome activation</u> :** The term "inflammasome" refers to innate immune receptors that, upon detecting infectious agents or host-derived danger molecules, activate caspase-1 to induce an inflammatory response. The inflammasome is activated when bacterial components (cell wall fragments, toxine, LPS..) activate molecule PRR that act as sensor. Leading to the activation of senor proteins such as NLRP3, NLRP1, NLRC4, AIM2. The ASC (Apoptosis-associated speck-like protein containing a CARD) protein acts as a molecular bridge, linking the sensor protein (eg., NLRP3) to the effector protein via homotypic interactions. The pro-Caspase-1 is the key enzyme that is activated upon inflammasome assembly. This process entails the cleavage of pro-caspase-1, generating active caspase-1, which triggers pyroptosis (a programmed cell death induced by inflammation) and the release of pro-inflammatory cytokines (eg., IL-1β and IL-18).

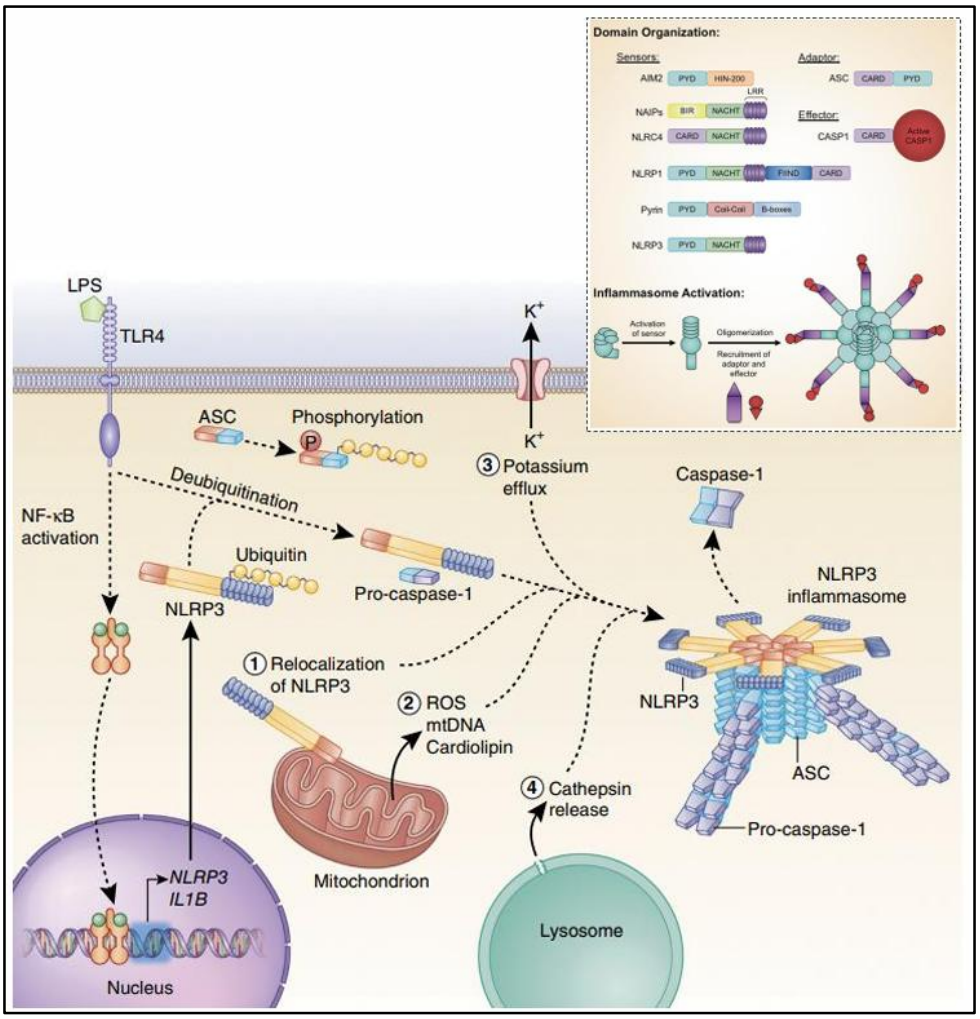

Figure 33: Molecular mechanism of NRLP3 inflammasome activation.

- **<u>Ubiquitin-Mediated Targeting and the Proteasome</u> :** As noted earlier, the cell employs the ubiquitin-proteasome system to degrade "unneeded" proteins. The cell use ubiquitin to coat the surface of intracellular bacteria, thereby triggering their degradation via the proteasome, as well as the activation of inflammatory mediators.

V.7.2- Eliminating Viruses

Beyond its antibacterial functions, the cytosol plays a paramount role in antiviral defense. Since viruses are obligate intracellular parasites that co-opt the cytosol for replication, this compartment becomes the central arena for host processes designed to block viral replication. The cytosol contributes to viral clearance through mechanisms that share similarities with its

antibacterial action, a key example being the induction of apoptosis or pyroptosis. Other mechanisms are specific to antiviral defense, such as:

- **RNA Interference** : the cytosolic ribonuclease III enzyme Dicer recognizes and cleaves long double-stranded RNA (dsRNA), a pathogen-associated molecular pattern (PAMP) produced during viral replication. This processing generates small interfering RNAs (siRNAs) of defined length. These siRNAs are subsequently incorporated into the RNA-induced silencing complex (RISC). Upon loading, RISC is guided by the siRNA to identify and catalytically cleave complementary viral RNA transcripts, thereby mediating sequence-specific degradation and post-transcriptional gene silencing of the viral genome.

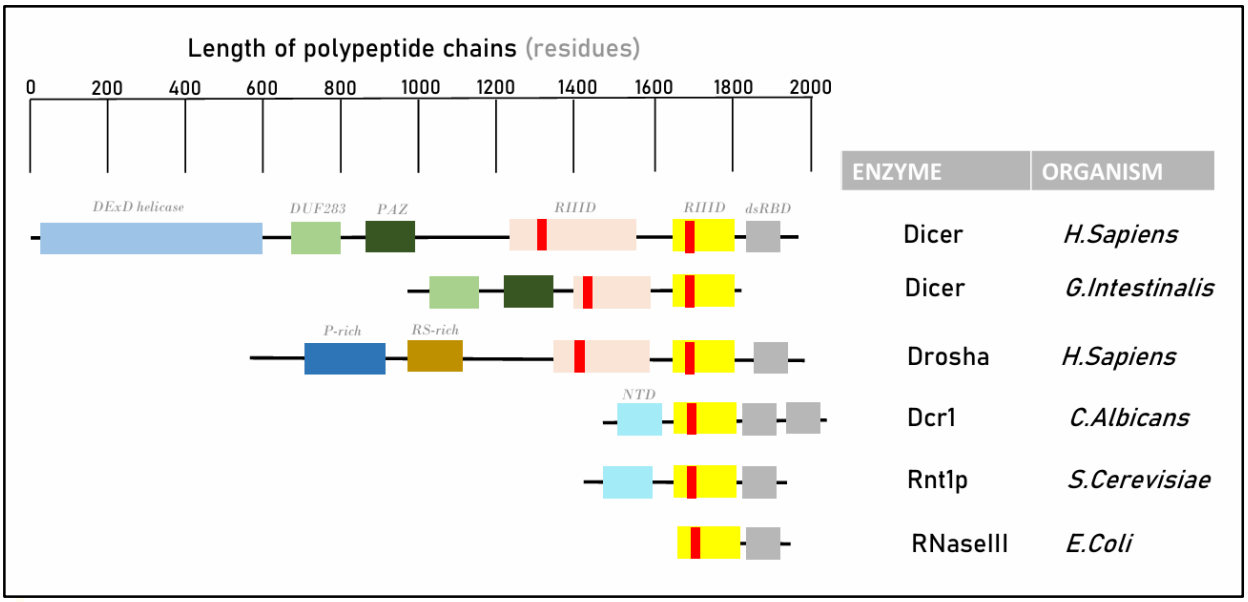

Figure 34: Representative RNase III proteins in different organisms.

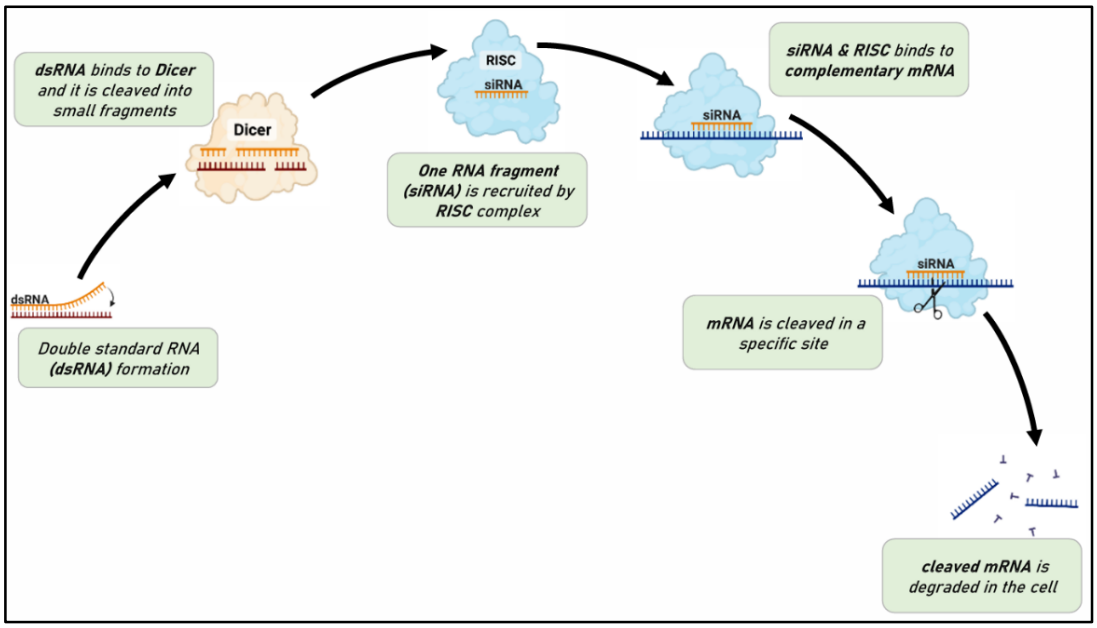

Figure 35: Schematic Representation of RNAi mechanism.

- **Activation of adaptative immune system:** Cytosolic ubiquitination of viral proteins directs their proteasomal degradation. The resulting peptide fragments are loaded onto Major Histocompatibility Complex (MHC) class I molecules for presentation to CD8+ T cells, thereby activating a cytotoxic T cell response. Following endocytosis, viruses are degraded within endolysosomal vesicles in the cytosol. This lysosomal processing generates antigenic peptides that are loaded onto MHC class II molecules for presentation to CD4+ T cells, activating them.

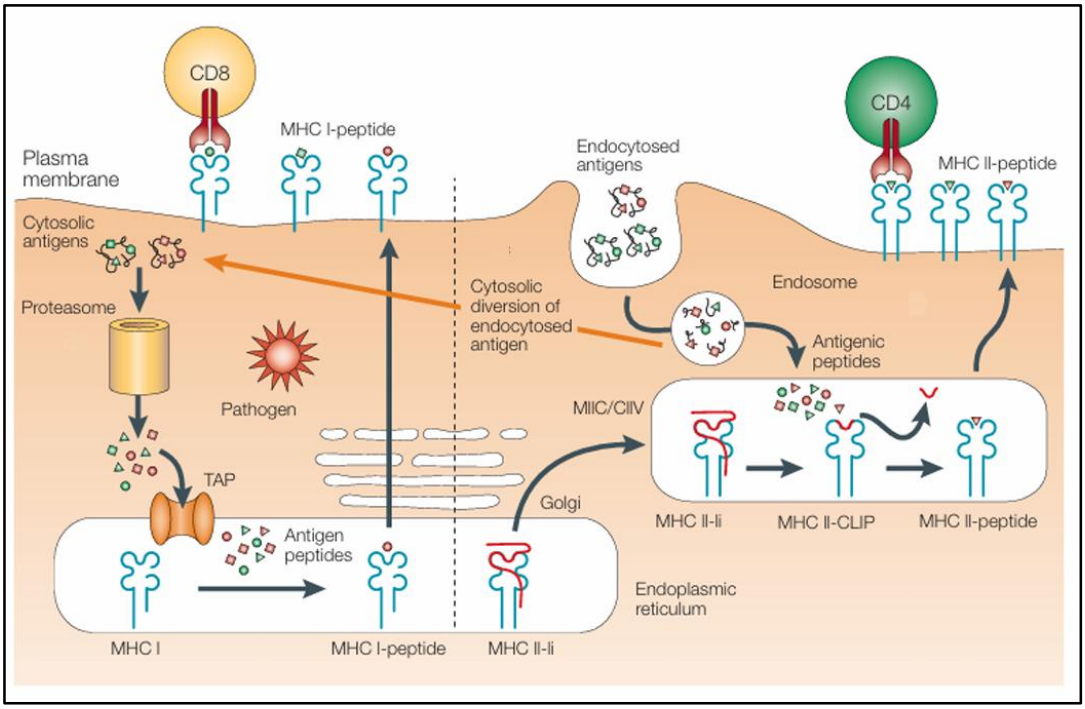

Figure 36: Mechanism of MHC antigens presentation.

VI-Mitochondria

VI.1Endosymbiotic Origin

As noted at the beginning of this manuscript, mitochondria have a prokaryotic origin. The theory describing the integration of a prokaryotic cell into a eukaryotic cell is termed endosymbiosis. The concept that endosymbiotic bacteria gave rise to eukaryotic organelles dates back to 1905, when the Russian biologist/botanist ***Konstantin Mereschkowsky*** suggested that plastids originated from cyanobacteria (blue-green algae). Between 1925 and 1927, the American anatomist ***Ivan E. Wallin*** was the first to propose the idea – without providing reproducible experimental evidence – that mitochondria originate from bacteria. It was in 1967 that the endosymbiotic theory was formally advanced by the renowned American biologist ***Lynn Margulis*** (then Lynn Sagan) in her seminal paper entitled "*On the Origin of Mitosing Cells*." She proposed that the ancestors of mitochondria are archaeobacteria - specifically alphaproteobacteria - and that plastids and flagella also originated from prokaryotic cells, thereby suggesting a major role for symbiosis in the evolution of eukaryotic cells.

The development of molecular biology and phylogenetic studies, particularly focusing on genes such as **C**ytochrome c **Ox**idase Subunit **I** (**Cox I**) and the large subunit of **R**ibulose-1,5-**b**isphosphate **c**arboxy**l**ase/oxygenase (**rbcL**), has provided undeniable evidence demonstrating that mitochondria belong to the Alphaproteobacteria group and chloroplasts to the Cyanobacteria group.

VI.2-Structure and Characteristics

The simplest and most common definition of a mitochondrion is *a semi-autonomous intracellular organelle, possessing a double membrane and its own genome, present in the majority, but not all, eukaryotic organisms.*

The number of mitochondria in eukaryotic organisms varies significantly between species, and more notably, within the same organism depending on the cell type **(Table IX)**. For instance, in humans, lymphocytes contain approximately 100-300 mitochondria, whereas hepatocytes possess a mitochondrial population ranging from 1,000 to 1,700. This number increases substantially, reaching 100,000 to 500,000 in mature oocytes, and up to 2 x 10^6 (2 million) in neurons. It is also important to note that a single cell can drastically alter its mitochondrial number according to physiological

Table IX: Estimated number of mitochondria in various mammalian cells.

Cell Type	Number of mitochondria per cell
Cardiomyocytes	3000-8000
Hepatocytes	1000-1700
Skeletal muscle	120-160
Fibroblasts	200-350
Neurons	~ $2x10^6$
Macrophage	100-700
Erythrocytes	00
Patelets	4-10
Spermatozoa	50-75
Mature Oocyte	10^5
Femal germ cells *(excluding mature oocyte)*	10-5000

Typically, mitochondria exhibit an ovoid or elongated cylindrical morphology, with an average size of approximately 0.5 to 3 µm. However, it is noteworthy that this size can also vary; reaching lengths of 5 to 10 µm, as observed in osteoclasts or muscle cells **(Figure 37)**.

The variations in mitochondrial number, shape, and size demonstrate that this organelle possesses a remarkable adaptive capacity – or plasticity – as it undergoes constant change.

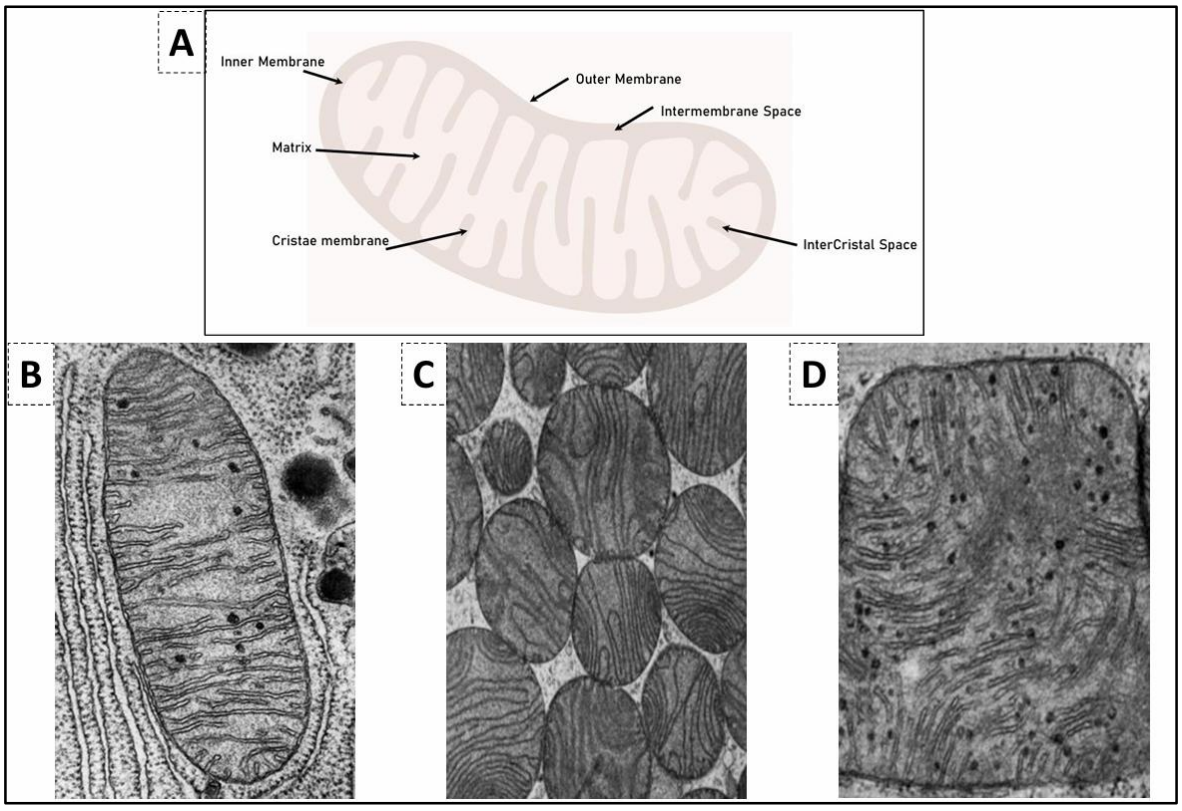

Figure 37: Structure and morphological diversity of mitochondria.

A *Schematic representation of mitochondria structure*. **B**: *a longitudinal section of a mitochondrion from bat pancreas.* **C**: *mitochondria from cat ventricular cardiac muscle.* **D**: *mitochondria from an interscapular brown adipose cell from the bat.*

Mitochondria are complex organelles characterized by distinct structural components:

- **<u>Membranes</u>**:

The initial observation of the mitochondrial double plasma membrane is credited to the work of the Swedish histologist ***Fritiof Stig Sjöstrand***. Subsequent research has revealed that the mitochondrial membrane system

is complex and partitions the organelle into four distinct compartments: the Outer Mitochondrial Membrane (OMM), the Inner Mitochondrial Membrane(IMM), the Intermembrane Space, and the Mitochondrial Matrix.

- Outer Mitochondrial Membrane

The Outer Mitochondrial membrane is a permeable, protein-rich, and dynamic structure. It occupy a unique location as the cell's central signaling hub. Its physical placement at the crossroads of the cytosol, ER, and other organelles is not passive; it is an active, structured, and functionally critical arrangement.

The location of Outer Mitochondrial Membrane can be described in two contexts: topological position within the organelle and strategic placement within the cell. Indeed, in the organelle. It is considered as primary interface between the mitochondrial contents and the cytosol. It also considered as ‘’outermost boundary” of the mitochondrion and enclosing the entire organelle. It surrounds the Inter-membrane Space (IMS), which separates it from the Inner Mitochondrial Membrane (IMM). For the location within the cell, the Outer Mitochondrial Membrane play critical role as the interface with the Cytosol.

A structured, protein-tethered microdomains of the OMM is also found in close apposition (~10-30 nm) with the Endoplasmic Reticulum (ER) membrane. This structure is called Organelle Contact Sites ‘’MAMs”. Due to its interaction with the ER and the cytoskeleton are often clustered near the nucleus.

This perinuclear location allows the OMM to influence nuclear signaling, such as the release of mtDNA fragments (DAMPs) that can impact innate immune responses.

The structural composition of OMM is unique. It differs significantly from that of the Inner Mitochondrial Membrane. The functions of the OMM are greatly influenced by its composition.

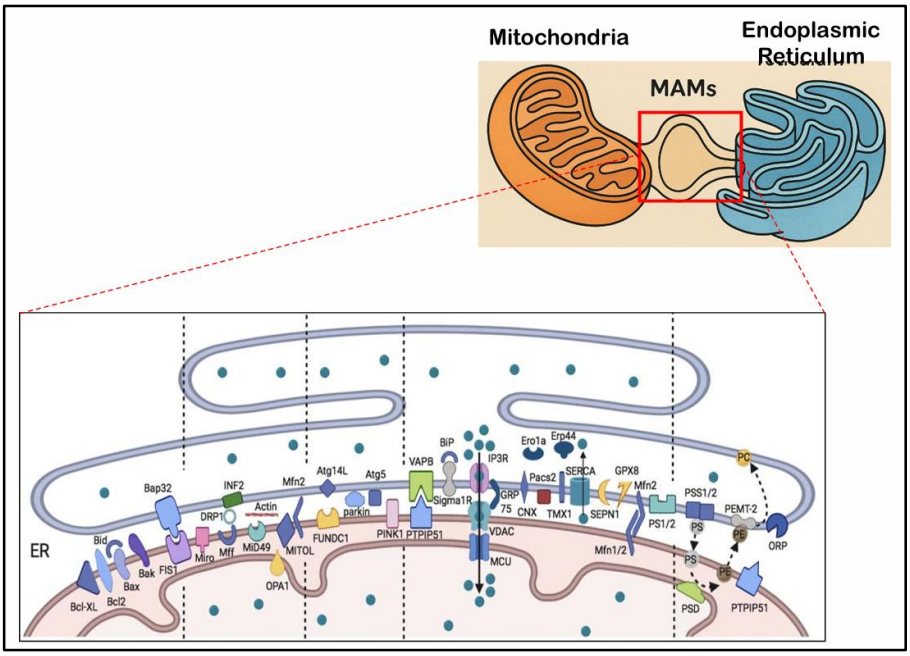

Figure 38: Structure and molecular composition of MAMs

Indeed, the OMM lipid bilayer has a high lipid-to-protein ratio, but it is uniquely enriched in **porins**, with **Voltage-Dependent Anion Channel (VDAC) -**1000 to 10000 copies per mitochondrion- being the most abundant protein. The VDAC$_s$ are *ß-barrel* proteins forming aqueous pores with an interior bearing a positive charge, facilitating the binding and transport- *passive diffusion*- of ATP and ADP –corresponding to their primary substrates- , but also they allow the passive diffusion of ions, nutrients, metabolites, and molecules with molecular weights up to ~5 kDa. The characteristics of VDACs creates a *sieve-like structure*.

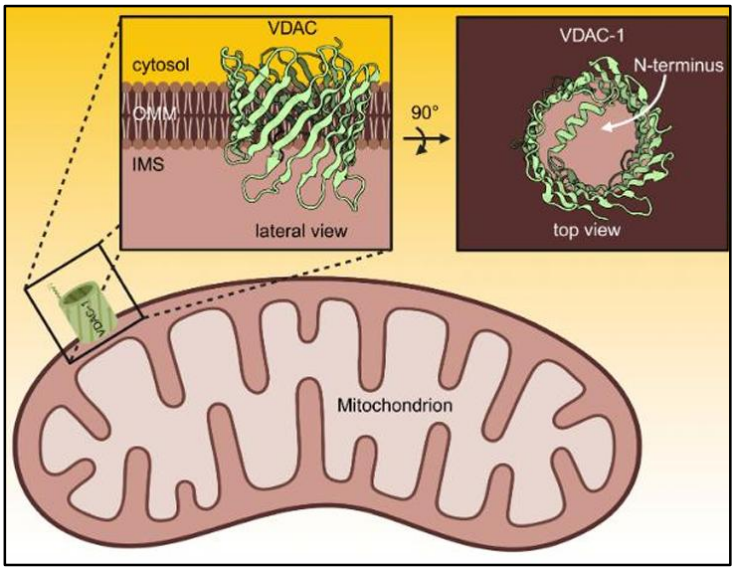

Figure 39: Structure of Voltage-Dependent Anion

The lipid composition- rich in phosphatidylcholine and cholesterol- of bilayer OMM is more similar to the endoplasmic reticulum (ER) and the plasma membrane than to IMM. This lipid bilayer composition support the theory of endosymbiotic origin of mitochondria.

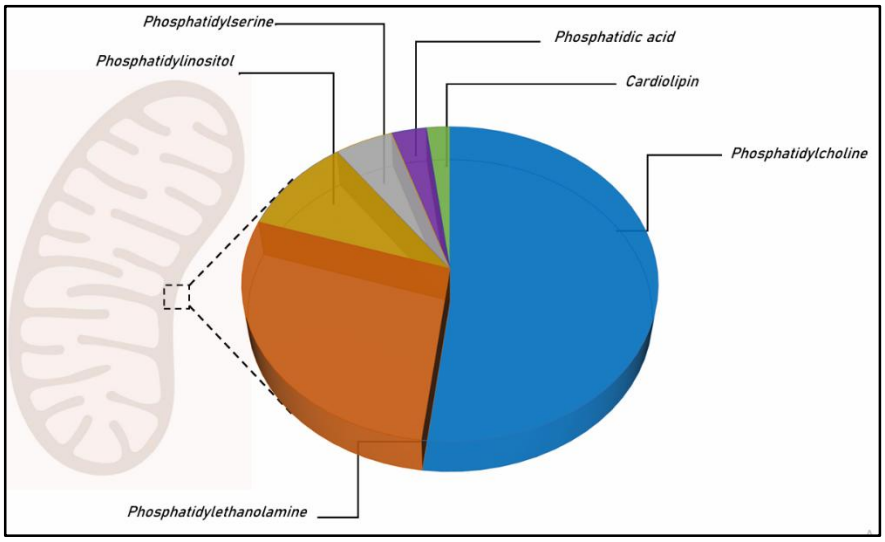

Figure 40: Phospholipids composition of the Outer Mitochondrial Membrane.

In addition to its lipids, the protein composition of the OMM is also unique. This distinct proteome influences its structure and function. Thus, making the OMM a non-static shell

Key protein types found in the OMM include Permeability Pores, Protein Import Machinery, SAM Complex, Fusion Machinery, Fission Machinery, Organelle Tethering Complexes, Apoptotic Pore Complex and Immune Signaling Platform **(Tableau X)**. The distinct lipidomic and proteomic profile of the OMM defines it as a specialized structure. It constantly undergoes *Fusion* & *Fission* but also Remodeling his protein composition at contact sites (eg., MAMs) to facilitate lipid transfer and calcium signaling. In fact, Mitochondria-Associated Membranes (MAMs) correspond to specialized regions where the OMM and the Endoplasmic Reticulum (ER) membrane are closely (~10-30 nm) apposed.

Table X: Key proteins complexes in Outer Mitochondrial Membrane.

Structural/Functional Element	Key Proteins	Primary Function
Permeability Pores	VDAC	Forms aqueous channels for metabolite/ion exchange
Protein Import Machinery	Translocase of the Outer Membrane	The main entry gate for ~99% of mitochondrial proteins.
Sorting and Assembly Machinery Complex	Sam50 (core subunit)	Assembles beta-barrel proteins (like VDAC and TOM40) into the OMM after they pass through the TOM complex
Fusion Machinery	Mitofusins (Mfn1, Mfn2)	GTPases that protrude from the OMM to tether and fuse adjacent mitochondria
Fission Machinery	Receptors: Mff, MiD49/51	Anchor the cytosolic dynamin-related protein Drp1 to the OMM, enabling membrane constriction
Organelle Tethering Complexes	Mfn2, VAPB-PTPIP51, FIS1-BAP31	Form physical bridges between the OMM and the ER membrane, creating specialized contact sites
Apoptotic Pore Complex	Bax, Bak	Upon activation, these proteins oligomerize to form large, permeant pores in the OMM during apoptosis
Immune Signaling Platform	Mitochondrial Antiviral Signaling	Forms large, prion-like aggregates on the OMM to amplify antiviral interferon signals

As mentioned above, the diversity of OMM proteins enables them to play roles in:

Metabolism:

Considered as metabolic gatekeeper, the OMM's role in metabolism is primarily mediated by the VDAC proteins. The VDAC play role of central metabolic conduit of ions, nucleotides and respiratory substrates. Is it important to note, that VDAC doesn't just allow passive diffusion. It facilitates the formation of supramolecular complexes (metabolons) that include

hexokinase (HK), *creatine kinase* (CK), and proteins from the inner membrane. This direct channeling optimizes efficiency by passing intermediates directly from the cytosol to the metabolic machinery, minimizing diffusion times.

The ER-Mitochondria Nexus

The Mitochondria-Associated Membranes is formed by the presence of anchor proteins that enable the interaction between the OMM and the ER. Key among them is **Mitofusin-2** (Mfn2), which can form homotypic or heterotypic bridges with Mfn1/Mfn2 on the ER. Other critical tethers include the **VAPB-PTPIP51 complex**. It is also observed that the narrow (~10-30 nm) gap at MAMs creates a privileged space for Ca^{2+} signaling or *Calcium Microdomains*. Ca^{2+} released from the ER through IP3 receptors is efficiently taken up by the mitochondria through the OMM (permeably) and the Inner Membrane MCU complex. This Ca^{2+} signal is a key activator of dehydrogenases in the TCA cycle, directly linking cellular stimulation to ATP production.

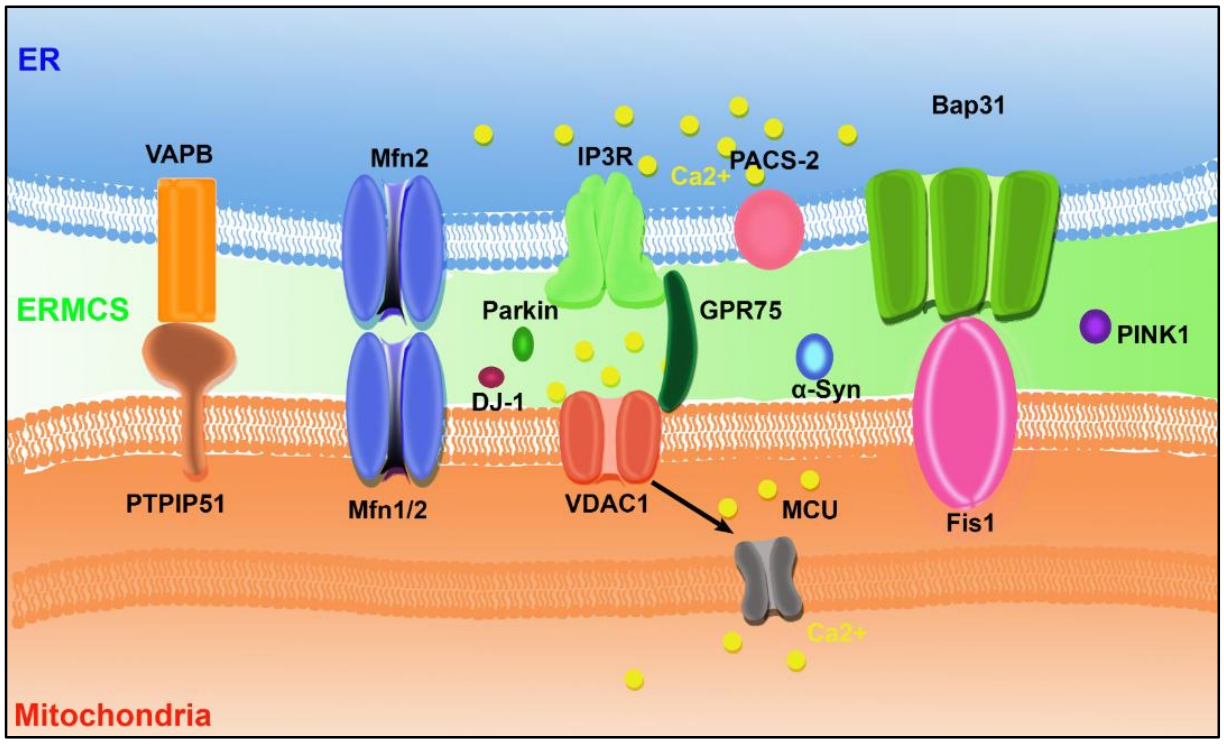

Figure 41: The protein organization at ER–mitochondria contact sites.

The OMM is also considered as a major site for phospholipid synthesis, particularly *phosphatidylethanolamine* (PE) and *cardiolipin*. *Phosphatidylserine* (PS) is transferred from the ER to the OMM, where it is decarboxylated to PE. This intimate coupling is essential for maintaining the unique lipid composition of mitochondrial membranes.

Survival and Apoptosis

The OMM is the pivotal platform for the intrinsic apoptosis pathway. The status of the OMM is determined by the balance between pro-apoptotic (e.g., Bax, Bak, Bid) and anti-apoptotic (e.g., Bcl-2, Bcl-xL) proteins. In health, anti-apoptotic proteins guard the OMM. Upon severe stress, pro-apoptotic proteins are activated, oligomerize, and permeabilize the OMM (MOMP). **M**itochondrial **O**uter **M**embrane **P**ermeabilization leads to the irreversible release of cytochrome c and other factors, triggering caspase activation and cellular dismantling.

Control of Mitochondrial Form and Function

Mfn1/2 are OMM GTPases that mediate the tethering and fusion of two mitochondria. This mixing of contents allows for complementation of DNA and metabolites, a key quality control mechanism. In addition to fusion machinery, the OMM hosts receptors like **Mff**, **MiD49**, and **MiD51,** which recruit the cytosolic GTPase **Drp1** that influence the *mitochondrial fission*. This fission process is essential for distributing mitochondria during cell division and for isolating damaged portions for degradation via mitophagy by PINK1-Parkin pathway. When damaged, PINK1 stabilizes on the OMM and recruits Parkin, which ubiquitinates OMM proteins, marking the entire organelle for autophagic engulfment.

Immune sentury

Due to the presence of The **M**itochondrial **A**nti**V**iral-**S**ignaling protein (MAVS) in OMM, the Outer Mitochondrial Membrane serves as a signaling platform for innate immunity. Upon viral infection, cytosolic sensors (RIG-I-like receptors) activate MAVS, which forms large prion-like aggregates on the OMM, launching a potent interferon-based antiviral defense.

Inflammasome Activation

Mitochondrial **O**uter **M**embrane **P**ermeabilization allows the mitochondrial DNA (mtDNA) escape into the cytosol through the OMM, where it can activate immune sensors like the NLRP3 inflammasome, triggering inflammation.

- Inner Mitochondrial Membrane

The mitochondrial inner membrane (IMM) represents one of nature's most sophisticated architectural achievements. Far more than a passive envelope, it is a dynamic, protein-dense, and lipidically unique structure that serves as the primary bioenergetic control center of the eukaryotic cell. Its complex, invaginated design is not accidental but the result of a billion-year evolutionary optimization, integrating ancient bacterial machinery with novel eukaryotic innovations to create a supremely efficient energy-transducing surface.

It is important to mention that, the evolutionary theory states that the modern IMM is the product of a transformative evolutionary journey. Its precursor was the intracytoplasmic membrane (ICM) of an α-proteobacterial endosymbiont. This ancestor possessed a rudimentary shaping system—a Mic60-like protein capable of generating negative curvature. The transition to the eukaryotic cristae was driven by synergistic, co-evolutionary steps:

Protein Innovation: The acquisition of ATP synthase dimerization provided a powerful new source of positive curvature.

Lipid Innovation: The specialization of cardiolipin metabolism created a membrane environment uniquely suited to stabilize high curvature.

Functional Synergy: These innovations converged. The new dimeric ATP synthase and the refined MICOS complex, operating within the curvature-favoring lipid matrix of remodeled CL, co-operatively forged the complex, stable, and highly efficient cristae.

The Internal Mitochondrial Membrane possesses numerous characteristics (**Table XI**), we can mention :

Structure:

The IMM's defining characteristic is its transformation into a labyrinth of folds known as ***cristae***. This is a calculated structural adaptation, not a random crumpling. These folds serve a singular, vital purpose: to exponentially increase the available surface area for the machinery of **ox**idative **phos**phorylation (**OXPHOS) (Figure 41)**.

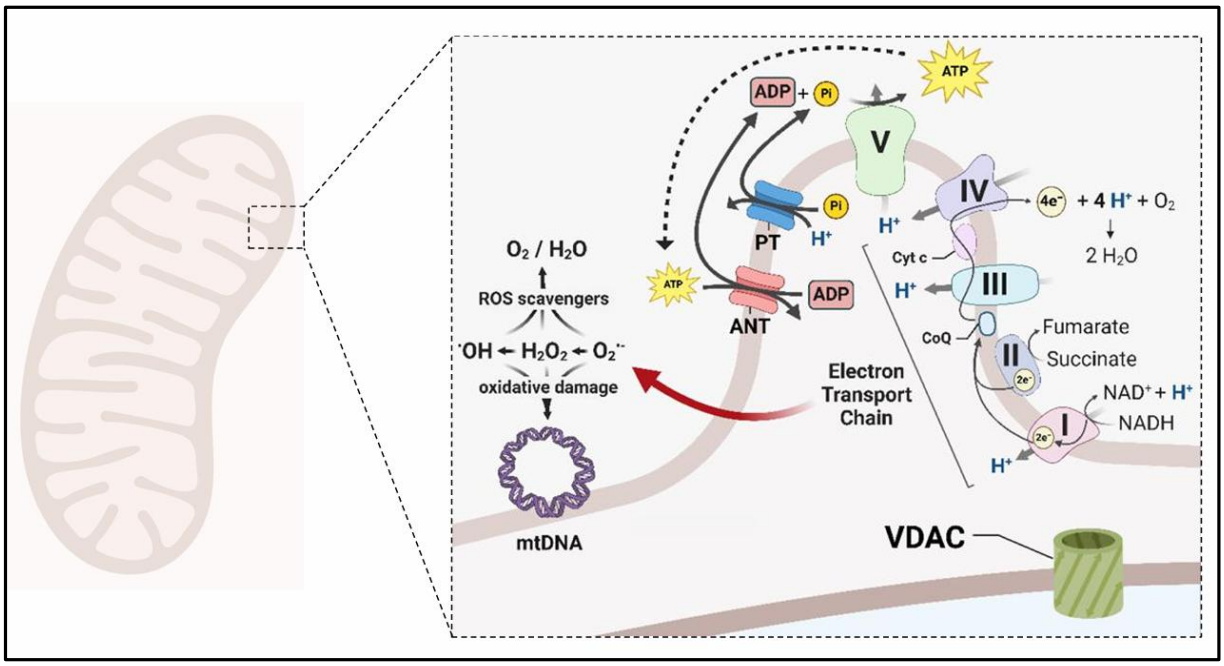

Figure 42: The OXPHOS machinery.

The morphology of these cristae is exquisitely variable and adaptive, shaped by cellular energy demands, and can be broadly categorized:

Lamellar Cristae: Predominant in high-energy-demand mammalian tissues like cardiac and skeletal muscle, these are dense, parallel, sheet-like folds maximizing protein complex packing.

Tubular Cristae: Found in organisms like yeast (S. cerevisiae), these interconnected, tube-like structures represent an alternative solution for surface area expansion.

Discoidal Cristae: Observed in certain protists (e.g., Trypanosoma), these stacks of disk-like membranes indicate the structural plasticity of the cristae-forming system.

Connecting each crista to the peripheral inner boundary membrane is a specialized portal: the *cristae junction* (CJ). These narrow, regulated necks are not mere openings; they are active structural elements that maintain the crista as a distinct biochemical subcompartment, regulating metabolite and protein exchange-will be explained further below-.

Protein Machinery :

The IMM's intricate shape is not self-assembling; it is actively built and maintained by a suite of dedicated protein complexes. Among these protein complexes, we can mention in particular :

ATP Synthase Dimers (Complex V): Functioning as positive curvature generators, rows of these dimers are the principal architects of the sharply curved cristae tips. Their oligomerization bends the lipid bilayer with mechanical precision. This dimerization, enabled by specific eukaryotic subunits like *"e"* and *"g"*, is a key evolutionary innovation absent in bacterial ancestors.

The MICOS Complex: In a complementary role, the Mitochondrial Contact Site and Cristae Organizing System generates negative curvature at the cristae junctions. It acts as a structural scaffold, defining the neck's architecture and bridging the IMM to the outer membrane. Its core subunit, Mic60, traces its origins to α-proteobacteria, where its homologs shape intracytoplasmic membranes (ICMs), revealing the ancient, pre-mitochondrial roots of this essential machinery.

OPA1: This dynamin-related GTPase adds a layer of dynamic control. It works in concert with MICOS to regulate CJ tightness, drive inner membrane fusion, and orchestrate global cristae remodeling in response to metabolic shifts and apoptotic signals—a sophisticated regulatory layer superimposed upon the foundational structure.

Lipid Matrix:

The protein machinery is embedded within a uniquely tailored lipid environment, the mitochondrial "lipidome," which is fundamental to both structure and function.

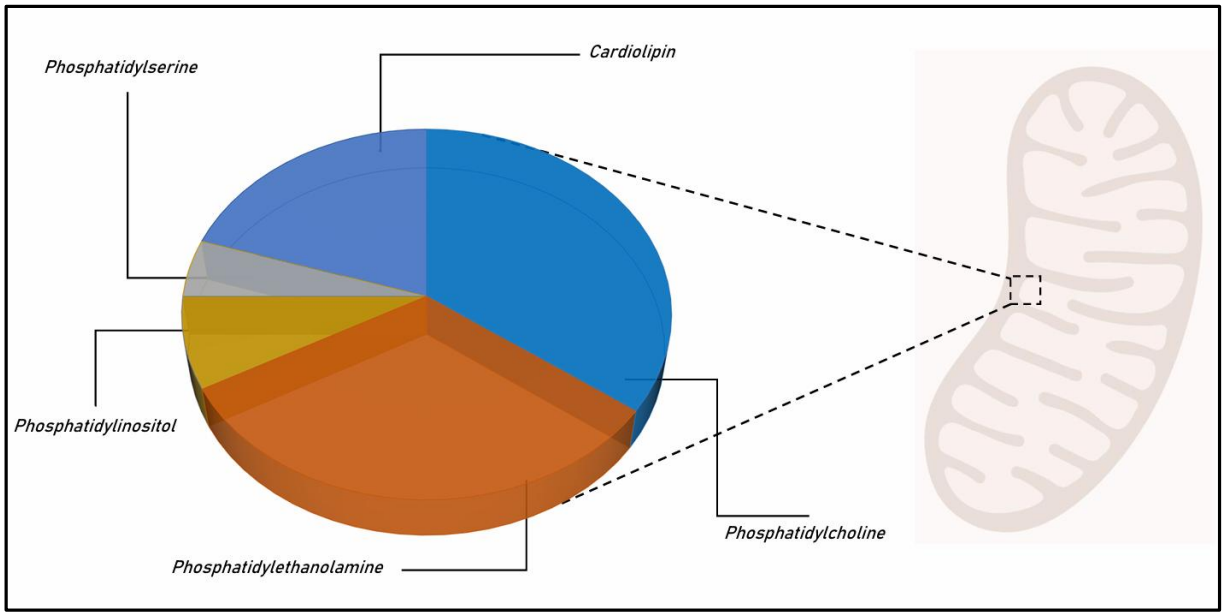

Figure 43: Phospholipids composition of the Inner Mitochondrial Membrane.

Cardiolipin (CL): This unique "dimeric" phospholipid, with its four acyl chains and cone-shaped geometry, is the signature lipid of the IMM (**Figure 44**). Its biophysical properties make it a natural stabilizer of highly curved, non-bilayer structures. CL is enriched at sites of extreme curvature (cristae tips and junctions), where it reduces membrane stress and acts as a molecular glue, binding to and optimizing the function of ETC complexes and ATP synthase.

Evolutionary Lipid Specialization: The eukaryotic IMM underwent a profound lipidomic shift. Crucially, the abundance of CL was amplified, and a dedicated two-step remodeling pathway (involving enzymes like tafazzin) evolved to produce CL with a highly uniform, unsaturated fatty acid composition. This increases membrane fluidity and intrinsic curvature, a key adaptation for cristae stability. Concurrently, phosphatidylcholine (PC) became the dominant

bilayer-forming lipid, while phosphatidylglycerol (PG) was largely relegated to being a CL precursor.

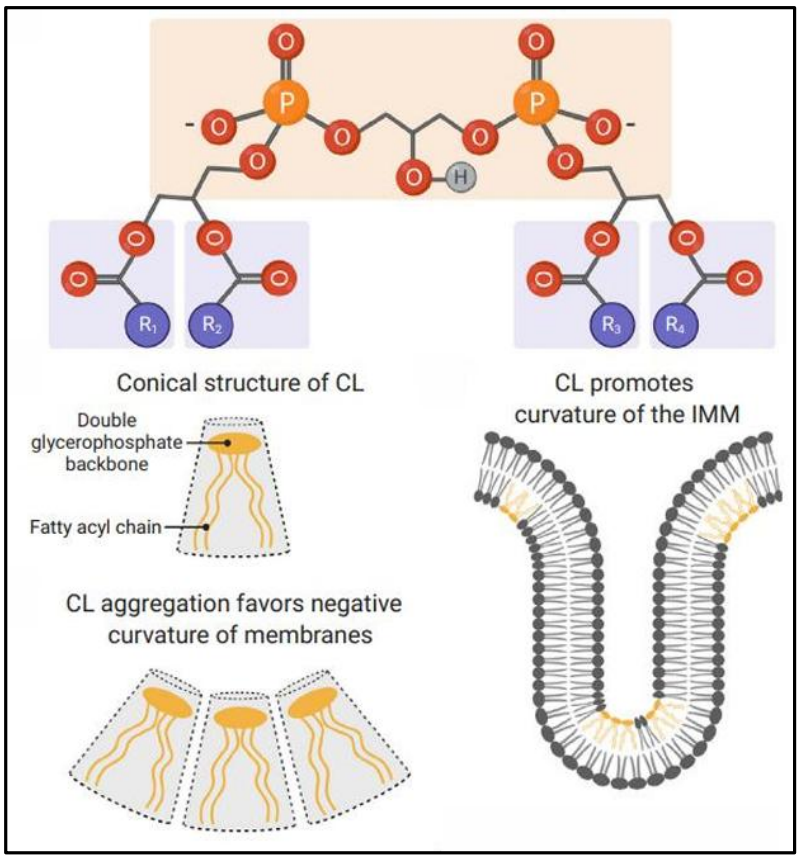

Figure 44: Major characteristics and structure of Cardiolipids.

Integrated Functions:

The co-evolved structure and composition of the IMM enable a staggering array of essential cellular functions:

Energy Transduction: The IMM is the exclusive site of OXPHOS. Cristae house the electron transport chain (I-IV), which builds a proton gradient across the IMM's impermeable barrier. This proton-motive force (Δp) is then harnessed by ATP synthase dimers to phosphorylate ADP into ATP.

Selective Gateway: The IMM is a tightly controlled interface. It contains specific transporters (e.g., ANT for ADP/ATP exchange, MCU for regulated Ca^{2+} uptake) and sophisticated protein import complexes (TIM23/TIM22) to govern the flux of molecules and the biogenesis of the organelle itself.

Signaling Hub: The IMM is central to critical signaling pathways. It buffers cellular calcium, contributes to redox balance via enzymes like NNT, and plays

a key role in apoptosis. Under severe stress, IMM dysregulation can trigger mitochondrial permeability transition (mPTP), leading to the release of pro-apoptotic factors.

Table XI: The Essential Characteristics of the IMM.

Aspect	Core Description	Evolutionary & Functional Significance
Architecture	Highly invaginated into specialized folds (**cristae**) connected by regulated **cristae junctions (CJs)**.	Maximizes surface area for bioenergetics Creates controlled subcompartments.
Structural Engineers	**ATP synthase dimers** (positive curvature at tips); **MICOS complex** (negative curvature at CJs); **OPA1** (dynamic regulation).	Eukaryotic dimerization innovated sharp cristae MICOS has ancient bacterial roots. OPA1 adds eukaryotic regulatory finesse.
Chemical Foundation	Lipidome dominated by **Cardiolipin (CL)** and **Phosphatidylcholine (PC)**; CL is highly unsaturated and remodeled.	CL stabilizes extreme curvature; specialized eukaryotic remodeling promotes fluidity and function.
Primary Role	Site of **Oxidative Phosphorylation (OXPHOS)**; generates the **proton-motive force** and synthesizes ATP.	The core bioenergetic function that powered eukaryotic complexity.
Integrative Functions	Selective metabolite/ion transport (**ANT, MCU**); protein import (**TIM**); Ca^{2+} buffering; redox balance; apoptosis initiation.	Positions the IMM as a central hub integrating energy production, cellular signaling, and life/death decisions.

- InterMembrane Space

Far from an inert "gap," the mitochondrial **i**nter**m**embrane **s**pace **(IMS)** is a topologically complex, biochemically unique, and dynamically regulated cellular compartment. It is a compartment of very small volume since the outer and inner membrane are ~8 nm apart which is about the diameter of a membrane. Its strategic position, sandwiched between two membranes of opposing permeability, creates a specialized environment essential for energy transduction, protein homeostasis, redox signaling, and the orchestration of cell death.

Structure

The IMS is a topologically complex compartment defined by its position between the two mitochondrial membranes. Its structure is not uniform. As mentioned above, the IMS is the lumen between the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), and can subdivided into two compartments:

Peripheral IMS: The space between the OM and the **inner boundary membrane (IBM)**, which is the region of the IM lying parallel and close to the OM.

Intracristae Space (ICS): The interior space of the cristae, which are tubular or lamellar invaginations of the IM. This is not a closed space but is connected to the peripheral IMS.

Cristae Junctions (CJs): These are narrow, tubular connections (12–40 nm in diameter) that link the intracristae space to the peripheral IMS. They are formed by proteins like mitofilin/Fcj1 and are regulated by proteins such as OPA1/Mgm1 and prohibitins. CJs act as selective diffusion barriers**,** physically separating the two IMS subcompartments and creating distinct microenvironments.

Contact Sites: Zones where the OM and IM are in close proximity, facilitated by protein complexes like the TOM-TIM supercomplexes during protein import. These are visible in electron micrographs.

Composition

Physicochemical Milieu

The IMS environment is distinct from both the cytosol and the matrix, shaped by the permeability properties of the two bordering membranes. It is resumed in table XII.

Table XII: Physicochemical properties of IMS.

Characteristic	Detail	Implication
pH	**More acidic** than the cytosol by 0.2–0.7 pH units.	Creates a proton gradient; influences protein function and redox state.
Redox State	**More oxidizing** than the cytosol or matrix. The glutathione (GSH/GSSG) buffer is more oxidized.	Favors disulfide bond formation; required for the Mia40/Erv1 import pathway; influences signaling.
Small Molecule Permeability	The OM is porous via **Porins/VDAC**, allowing free diffusion of molecules <5 kDa. The IM is selectively permeable via dedicated carriers.	The peripheral IMS equilibrates rapidly with the cytosol for ions (K^+, Na^+, Ca^{2+}, Cl^-) and metabolites. The ICS environment can differ due to CJ restriction.
Metabolite & Ion Concentrations	Similar to cytosol for small ions. Metabolite levels (ATP, ADP, etc.) are dynamically set by IM transporters.	Serves as the transit and buffering pool for all cytosol-matrix exchange.

Protein Composition (The IMS Proteome)

The IMS contains a unique and diverse set of proteins, all nuclear-encoded and imported via specific pathways. It is resumed in **Table XIII**.

Table XIII: IMS Proteome.

Category	Component/Protein	Primary Function/Notes
Respiratory Chain	Cytochrome c	Mobile electron carrier between Complex III and IV; apoptotic signal.
	Cox12	Subunit of Cytochrome c Oxidase (Complex IV).
Metabolic Enzymes	Adenylate kinase	Maintains cellular ATP/ADP/AMP balance (interconverts adenine nucleotides).
	Creatine kinase	Buffers ATP levels via the phosphocreatine system.
Protein Import Machinery	Small Tim proteins (Tim9, Tim10, Tim8, Tim13)	Chaperones for importing hydrophobic carrier proteins across the intermembrane space.
	Mia40	Key oxidoreductase; imports and folds proteins via disulfide bond formation.
	Erv1	Sulfhydryl oxidase; works with Mia40 in the disulfide relay system, re-oxidizes Mia40.
Redox Control & Assembly	Superoxide dismutase 1 (Sod1)	Converts superoxide radicals to hydrogen peroxide and oxygen (localized to IMS and cytosol).
	Copper chaperones (Cox17, Sco1, Sco2)	Deliver copper to Cytochrome c Oxidase (Complex IV).
	Cytochrome c heme lyase	Catalyzes covalent attachment of heme to apocytochrome c.
Proteases	i-AAA protease (Yme1)	ATP-dependent protease in the inner membrane (active towards the intermembrane space).
	Omi/HtrA2	Serine protease; released during apoptosis to degrade anti-apoptotic proteins.
Apoptotic Factors	Cytochrome c	Upon release, triggers apoptosome formation and caspase activation.
	Smac/DIABLO	Neutralizes Inhibitor of Apoptosis Proteins (IAPs).
	AIF (Apoptosis-Inducing Factor)	Induces caspase-independent chromatin condensation and DNA fragmentation.
	Endonuclease G	Contributes to caspase-independent DNA degradation.
Lipid Transport/Homeostasis	PRELI/Ups family proteins (Ups1, Ups2)	Mediate lipid transfer between mitochondrial membranes (e.g., phosphatidic acid, cardiolipin).

Key Characteristics of IMS Proteins:

Targeting & Folding: Many lack cleavable presequences. Import often depends on acquiring a folded conformation via cofactor binding (heme) or oxidative folding (disulfide bond formation via the Mia40/Erv1 pathway).

Cysteine Motifs: A hallmark is the presence of **twin Cx_9C or twin Cx_3C motifs**, which are substrates for the Mia40/Erv1 disulfide relay system.

Dual Localization: Some, like Sod1, are present in both the cytosol and IMS.

Due to its diversity, the intermembrane space, play a various functions. This is the case of:

Bioenergetic Optimization & Compartmentalization

Proton Compartmentalization: The cristae junctions restrict proton diffusion**.** Protons pumped into the ICS by the respiratory chain are temporarily "trapped," creating a strong local proton-motive force right at the ATP synthase complexes densely packed on cristae ridges. This dramatically increases the efficiency of oxidative phosphorylation.

ROS Confinement: The architecture helps confine ROS production by the respiratory chain to the ICS, minimizing oxidative damage to other cellular components.

Protein Import, Folding, and Quality Control (Biogenesis Hub)

Transit Corridor: All nuclear-encoded proteins for the IM and matrix must cross the IMS via TOM and TIM complexes.

Chaperone Function: Small Tim complexes (e.g., Tim9-Tim10) act as chaperones to guide hydrophobic carrier proteins and β-barrel precursors through the aqueous IMS to their membrane insertase complexes (TIM22, SAM/TOB).

Oxidative Protein Folding: The *Mia40-Erv1 disulfide relay* system imports and folds a large family of cysteine-rich IMS proteins by catalyzing disulfide bond formation.

Proteolysis: AAA proteases (e.g., i-AAA) degrade misfolded proteins, contributing to quality control and even participating in the import of some proteins.

Redox Signaling and Defense

ROS Signaling Hub: As a primary site of ROS generation, the IMS is a source of redox signals (e.g., H_2O_2) that regulate processes like transcription factor activation and apoptosis.

Antioxidant Defense: Contains key enzymes like Sod1 (dismutates $O_2^{\bullet -}$ to H_2O_2) and Cytochrome c Peroxidase (Ccp1) to detoxify ROS.

Redox Buffer Regulation: Maintains a distinct, oxidized glutathione pool, influencing the activity of redox-sensitive proteins.

Initiation and Regulation of Apoptosis

Storage Depot: Sequesters pro-apoptotic factors like cytochrome C, AIF**,** and Smac/DIABLO in the ICS.

Controlled Release: Apoptotic signals trigger cristae junction remodeling (involving OPA1) to mobilize cytochrome C, followed by OM permeabilization (via BCL-2 family proteins), releasing IMS proteins into the cytosol to execute cell death.

Logistics and Homeostasis

Metal Ion Trafficking: The IMS contains specialized chaperones for essential cofactors:

Copper: Cox17 delivers copper to Sco1/Sco2 and Cox11 for assembly into cytochrome C oxidase.

Zinc: Proteins like Hot13 and metallothioneins regulate zinc homeostasis.

Lipid Transport: The PRELI/Ups family proteins (e.g., Ups1, Ups2) are implicated in the transport and regulation of phospholipids (especially cardiolipin) between the OM and IM.

Metabolite Exchange: Serves as the transit hub for all metabolites (ATP/ADP, phosphates, Krebs cycle intermediates) shuttled between cytosol and matrix by IM carriers.

Fe/S Cluster Export: The sulfhydryl oxidase Erv1 is essential for exporting a precursor needed for cytosolic iron-sulfur cluster assembly.

- Mitochondrial matrix

The **M**itochondrial **M**atrix **(MM)** is the innermost compartment of the mitochondrion, enclosed by the inner mitochondrial membrane. It is a dense, gel-like space containing numerous enzymes, mitochondrial DNA (mtDNA), ribosomes, metabolites, and ions essential for mitochondrial function.

Structural and Dynamic Aspects

Surrounded by Cristae: The IMM invaginates into the matrix as **cristae**, increasing surface area for OXPHOS complexes.

Dynamic Environment: Matrix composition changes with metabolic state, calcium levels, and redox balance.

Connected to Mitochondrial Dynamics: Matrix contents mix during *mitochondrial fusion*, supporting quality control and metabolic efficiency.

Key Features and Functions

mtDNA and Nucleoids

- The matrix houses multiple copies of mitochondrial DNA **(mtDNA)**, compacted into nucleoprotein complexes called nucleoids.
- mtDNA encodes 13 polypeptides (subunits of oxidative phosphorylation complexes), 22 tRNAs, and 12S/16S rRNAs.

Metabolic Hub

Site of the **tricarboxylic acid (TCA) cycle**, which oxidizes fuel molecules and provides intermediates for biosynthesis (e.g., amino acids, lipids, nucleotides).

Produces **reducing equivalents (NADH, $FADH_2$)** for the electron transport chain (ETC).

Protein Synthesis

Contains mitochondrial ribosomes (mitoribosomes) for translation of mtDNA-encoded proteins.

Recently discovered **small open reading frames (sORFs)** in rRNA regions encode mitochondrial-derived peptides (e.g., humanin, MOTS-c) with metabolic and protective roles.

Biosynthesis

Synthesizes key biomolecules such as:

- *Heme* (starting from TCA intermediates)
- *Iron-sulfur* (Fe-S) clusters
- *Ketone bodies* (in liver mitochondria)
- Certain *amino acids* (e.g., glutamate, aspartate)

Calcium Buffering : Takes up **Ca^{2+}** via the mitochondrial calcium uniporter (MCU), helping regulate cytosolic Ca^{2+} levels and modulating mitochondrial metabolism.

Redox Balance: Contains antioxidants (e.g., glutathione peroxidase, peroxiredoxin) to mitigate reactive oxygen species (ROS) produced by the ETC.

Protein Import and Folding: Nuclear-encoded mitochondrial proteins are imported via **TIM23 complex** and refolded in the matrix with chaperones (e.g., Hsp60, Hsp70).

- Mitochondrial DNA

After a brief overview of mitochondrial DNA in the previous paragraphs, in this section we will discuss in more detail the characteristics of mtDNA.

The genetics of the mitochondrial genome follow rules that are fundamentally different from Mendelian inheritance, as summarized in **Table XIV.**

Table XIV: Genetic of mtDNA.

Concept	Definition	Clinical Relevance
Heteroplasmy	Coexistence of wild-type and mutant mtDNA within a single cell	Mutation load determines whether phenotypic threshold is crossed; explains variable expressivity
Threshold effect	Clinical manifestation appears only when mutant mtDNA exceeds ~60–90% depending on tissue	High energy-demand tissues (brain, muscle, heart) are most vulnerable and reach threshold first
Mitotic segregation	Random distribution of mtDNA at cell division shifts heteroplasmy levels over time	Explains why disease can worsen with age as mutant copies drift upward in post-mitotic cells
Homoplasmy	All mtDNA molecules are identical (wild-type or mutant)	Severe mutations are rarely homoplasmic — lethal in pure form; maintained through selection

Human mtDNA is a closed, circular, double-stranded molecule. Its two strands are distinguished by their base composition and termed the *heavy* (H) strand rich in guanines and the *light* (L) strand. What fits into this tiny circle is astonishing: 37 genes encoding everything needed to sustain oxidative phosphorylation, plus a critically important non-coding regulatory region **(Table XV)**.

Interestingly, the D-Loop (~1.1 kb) non-coding region play role as a command center of the mitochondrial genome. It contains the promoters for both H- and L-strand transcription (HSP and LSP), the origin of H-strand replication (OH), and — crucially — it is the most rapidly evolving segment of the human genome, making it indispensable for population genetics and forensic identification.

Table XV: Category of genes encoded by mtDNA.

Gene Category	Number	Encoded Products	Functional Role
Protein-coding genes	13	OXPHOS subunits	7 subunits of Complex I; 1 of III (Cyt b); 3 of IV (COX I–III); 2 of ATP synthase (ATP6, ATP8)
Ribosomal RNA genes	2	12S rRNA, 16S rRNA	Form mitoribosomal RNA scaffold for mt translation
Transfer RNA genes	22	mt-tRNAs	Decode all mitochondrial codons; use non-standard wobble rules
Non-coding D-loop region	1 (~1.1 kb)	HSP, LSP promoters; OH replication origin	Master switch for mtDNA replication and transcription

Unlike nuclear DNA, mtDNA replicates continuously and independently of the cell cycle, driven by the need to maintain copy number. When mitochondria divide or a cell's energy demands increase, more copies are needed and the replication machinery fires accordingly.

The mitochondrial transcription machinery is stripped down to its essentials : a single RNA polymerase (POLRMT), two transcription factors (TFAM and TFB2M), and one termination factor (MTERF1) . Yet it produces a complex suite of RNA molecules that must be carefully processed before they can be used. Transcription initiates at two promoters within the D-loop: the Heavy Strand Promoter (HSP) and the Light Strand Promoter (LSP). Rather than

producing individual mRNAs, mitochondrial transcription generates two near-genome-length polycistronic transcripts- one from each strand. These massive precursor molecules are then resolved into individual mRNAs, rRNAs, and tRNAs by endonucleolytic cleavage, a process guided by the 'tRNA punctuation model': tRNAs are folded and excised by RNase P and ELAC2, releasing the flanking coding RNAs.

This translation in the mitochondrial matrix is carried out by the ***mitoribosome*** — one of the most divergent ribosomes in nature. The human mitoribosome (55S) is composed of a large subunit (39S, mt-LSU) and a small subunit (28S, mt-SSU). Strikingly, it has a much higher protein-to-RNA ratio than cytoplasmic or bacterial ribosomes: the 12S and 16S rRNAs are remarkably small, while the protein complement has expanded to **82 subunits**.

VI.3-Biochemical processes '' A Cell Powerhouse'' :

We have already addressed, in an undetailed way, certain functions of the mitochondria. In this section, we will discuss in a little more detail the biochemical role played by mitochondria.

As mentioned above, the inner mitochondrial membrane (IMM) is extensively folded into cristae — invaginations that dramatically increase surface area and house the respiratory chain supercomplexes. The transmembrane electrochemical potential ($\Delta\Psi m \approx -180$ mV) generated across the IMM serves as the driving force for ATP synthesis.

VI.3.1- Pyruvate Dehydrogenase Complex (PDH) :

Before entering the TCA cycle, pyruvate that corresponds to the end-product of glycolysis, must be oxidatively decarboxylated to acetyl-CoA. This irreversible reaction is catalyzed by the pyruvate dehydrogenase complex (PDH), a multi-enzyme megacomplex (**~9.5 MDa** in mammals) located in the mitochondrial matrix.

Structurally, PDH comprises three catalytic components and five cofactors:

- E1 (pyruvate decarboxylase, $\alpha_2\beta_2$ tetramer) — requires thiamine pyrophosphate (TPP); decarboxylates pyruvate and transfers the acetyl group to lipoamide.
- E2 (dihydrolipoamide acetyltransferase) — bears the swinging lipoamide arm; transfers acetyl group to CoA, generating acetyl-CoA.
- E3 (dihydrolipoamide dehydrogenase) — FAD-dependent; reoxidizes dihydrolipoamide, ultimately reducing NAD^+ to NADH.

Thus, the net reaction of PDH complex can be summarized as follows:

Pyruvate + CoA + NAD^+ → Acetyl-CoA + CO_2 + NADH + H^+

Standard Gibbs free energy Change: **$\Delta G^{\circ\prime} \approx -33.5$ kJ/mol** (thermodynamically irreversible).

It is important to mention that PDH activity is tightly regulated by product inhibition (Feedback loop) and reversible phosphorylation:

- Inhibition by acetyl-CoA, NADH, and ATP : signals energy sufficiency
- PDH kinase (PDK1–4) phosphorylates Ser293/Ser300/Ser232 on E1α, inactivating the complex

PDH phosphatase (PDP1/2) dephosphorylates and reactivates PDH in response to Ca^{2+}, insulin, and low energy charge

VI.3.2- Tricarboxylic Acid (TCA) Cycle:

As already noted, The TCA cycle is an eight-step amphibolic pathway in the mitochondrial matrix that completes the oxidation of acetyl-CoA carbons, generates reducing equivalents (NADH, $FADH_2$), and provides biosynthetic precursors. It is the metabolic hub integrating carbohydrate, lipid, and amino acid catabolism. Per Acetyl-CoA Cycle Yield is: 3 NADH, 1 $FADH_2$, 1 GTP (or ATP) and 2 CO_2. Total ATP equivalent via OXPHOS is ~10 ATP per acetyl-CoA **(Figure 45)**.

The TCA cycle intermediates are continuously withdrawn for biosynthesis (**cataplerosis**). In fact, α-ketoglutarate feeds *glutamate/glutamine* synthesis; oxaloacetate (OAA) is a gluconeogenic precursor; succinyl-CoA is incorporated into heme biosynthesis. **Anaplerotic** reactions replenish the cycle most

notably via pyruvate carboxylase (OAA generation) and glutaminolysis. This pathway is critically upregulated in cancer cells (Warburg-associated reprogramming).

It is important to note that, fain-of-function mutations in cytosolic **i**socitrate **de****h**ydrogenase IDH1 and mitochondrial IDH2 produce the oncometabolite 2-hydroxyglutarate (2-HG), competitively inhibiting α-KG–dependent dioxygenases (TET methylcytosine hydroxylases, histone demethylases), driving epigenetic reprogramming in gliomas and Acute Myeloid Leukemia.

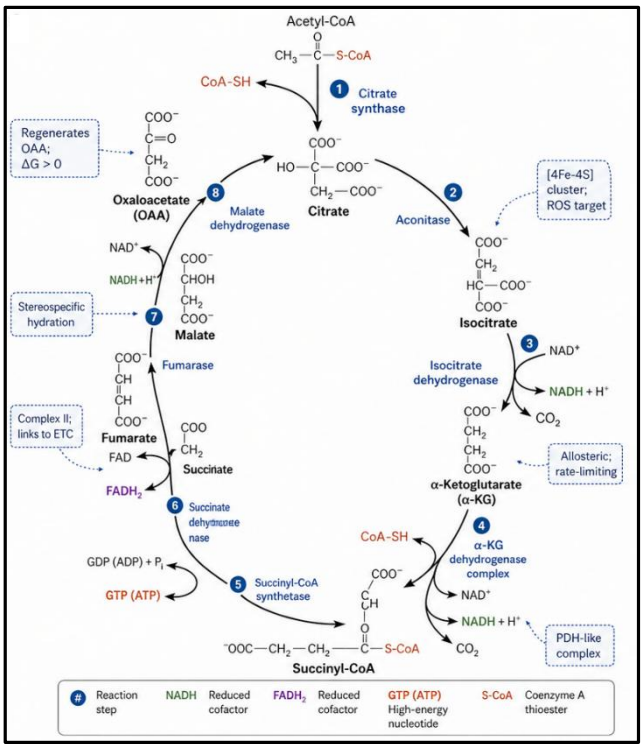

Figure 45: Tricarboxylic Acid Cycle.

VI.3.3- Electron Transport Chain & Oxidative Phosphorylation:

OXPHOS is the principal ATP-generating process in aerobic organisms. It is carried out by five large multi-subunit protein complexes (Complexes I–V) embedded in the IMM, plus the mobile electron carriers ubiquinone (CoQ) and cytochrome c. The four complex playing a crucial role in proton pumping are NADH-CoQ reductase, Succinate-CoQ reductase, $CoQH_2$-Cyt c reductase and Cytochrome c oxidase **(Table XVI)**.

Table XVI: Proton Pumping Complexes.

Complex	Name	Reaction	H^+ pumped / $2e^-$	Inhibitors
I	NADH-CoQ reductase	NADH → NAD^+; CoQ → $CoQH_2$	4	Rotenone, Piericidin A
II	Succinate-CoQ reductase	$FADH_2$ → FAD; CoQ → $CoQH_2$	0 (no pumping)	TTFA, Carboxin
III	$CoQH_2$-Cyt c reductase	$CoQH_2$ → CoQ; Cyt c (Fe^{3+}) → Fe^{2+}	4 (Q cycle)	Antimycin A
IV	Cytochrome c oxidase	Cyt c → Cu centers → O_2 → H_2O	2–4	CN^-, CO, Azide

Electron transfer through Complexes I, III, and IV is coupled to vectorial proton pumping from the matrix to the IMS. This generates the proton motive force (pmf), composed of:

- ΔΨm: electrical potential (−180 mV) — dominant component in mitochondria.
- ΔpH: chemical gradient (matrix is ~0.5–1 pH unit more alkaline than IMS).

ATP synthase (F_1F_o-ATPase) -*Complex V*- harnesses the proton motive force to synthesize ATP via rotary catalysis. The enzyme consists of two functional domains:

- F_o domain (membrane-embedded): c-ring (8–15 subunits), subunit a, b_2 forms the proton channel; rotation driven by H^+ translocation
- F_1 domain (matrix-facing): $\alpha_3\beta_3\gamma\delta\varepsilon$ — catalytic core; the asymmetric γ subunit rotates within the $(\alpha\beta)_3$ hexamer, sequentially driving three catalytic sites through conformational states: Open (O) → Loose (L) → Tight (T).

''P/O ratio'' : Approximately 2.5 ATP per NADH and 1.5 ATP per $FADH_2$ are produced experimentally (lower than the theoretical 3 and 2 due to proton leak and transport costs).

Complexes I, III, and IV associate into higher-order structures — respiratory supercomplexes or 'respirasomes' (stoichiometry $I_1III_2IV_1$). These mega-assemblies enhance electron channeling efficiency (substrate channeling of CoQ and cytochrome c), stabilize individual complexes against degradation, and reduce reactive oxygen species (ROS) generation. The assembly factor COX7A2L (SCAF1) governs supercomplex stoichiometry.

VI.3.4- β-Oxidation of Fatty Acids:

Mitochondrial β-oxidation is the major pathway for fatty acid catabolism, generating acetyl-CoA for the TCA cycle and large quantities of NADH and $FADH_2$ for OXPHOS. It accounts for up to 40–60% of ATP production in the heart and liver under fasting conditions.

Fatty acids cannot traverse the IMM in their free form. They must first be activated in the cytoplasm and then transported via the carnitine shuttle:

- *Step 1* "Activation": Fatty acid + CoA + ATP → Fatty acyl-CoA + AMP + PPi (catalyzed by acyl-CoA synthetases on OMM).
- *Step 2* "Outer transfer": CPT-I (carnitine palmitoyl transferase I) exchanges CoA for carnitine on the OMM cytosolic face; produces acylcarnitine.
- *Step 3* "Translocation": Acylcarnitine crosses the IMM via the acylcarnitine/carnitine translocase (CACT/SLC25A20).
- *Step 4* "Inner transfer": CPT-II regenerates acyl-CoA in the matrix, releasing free carnitine.

VI.3.4- Mitochondria-Linked Signaling and Pathophysiology:

Beyond bioenergetics, mitochondria orchestrate a broad spectrum of cellular signaling pathways including apoptosis, calcium homeostasis, innate immunity, and redox sensing. Dysfunction in these roles underlies major diseases spanning neurodegeneration, cancer, and metabolic syndrome.

For the ROS and redox signaling, complexes I and III are major sites of superoxide ($O_2^{\bullet -}$) production, arising from single-electron transfer to O_2. Superoxide is rapidly dismutated to H_2O_2 by Mn-SOD (SOD2) in the matrix and Cu/Zn-SOD (SOD1) in the IMS. At physiological levels, H_2O_2 acts as a second messenger, but at pathological levels it causes oxidative damage to mtDNA, lipids, and proteins.

Cellular stress signals can lead to initiation of the intrinsic mitochondrial apoptotic pathway :

- Pro-apoptotic BAX/BAK oligomerize and permeabilize the OMM (MOMP).
- MOMP releases cytochrome c, Smac/DIABLO, and AIF into the cytoplasm.
- Cytochrome c binds APAF-1 and ATP → forms the apoptosome (heptameric ring) → activates caspase-9 → caspase-3/7 cascade → cell death.
- The BCL-2 protein family, especially BCL-2, BCL-XL, MCL-1 sequester BH3-only proteins and prevent BAX/BAK oligomerization.

Mitochondria is also implicated in the Calcium signaling Mitochondria buffer cytosolic Ca^{2+} via the mitochondrial Ca^{2+} uniporter (MCU) complex. Matrix Ca^{2+} stimulates PDH phosphatase, IDH3, and α-KG dehydrogenase, directly coupling excitation-metabolism coupling in excitable cells. Pathological Ca^{2+} overload triggers opening of the mitochondrial permeability transition pore (mPTP) — composed of ANT, CypD, and potentially the F_1F_o-ATPase — causing ΔΨm collapse, matrix swelling, and OMM rupture.

As mentioned in a previous paragraph, the quality control of mitochondria is ensured by mitophagy mechanism. Indeed, damaged or depolarized mitochondria are selectively eliminated by mitophagy. The PINK1-Parkin pathway is the best-characterized route: loss of ΔΨm stabilizes the kinase PINK1 on the OMM, which phosphorylates ubiquitin (Ser65) and recruits the E3 ligase Parkin. Parkin ubiquitinates OMM proteins (VDAC1, MFNL1/2), enabling recognition by autophagy receptors (p62/SQSTM1, NDP52, OPTN) and LC3-decorated phagophores. Loss-of-function mutations in PINK1 and Parkin cause early-onset familial Parkinson's disease.

VII-Questions & Answers (Q&A)

VII.1- Questions

1. *Who first used the term "cell" and when?*
2. *What are the three principles of the cell theory according to Schwann?*
3. *Which two scientists are credited with establishing that cells are the basic unit of life?*
4. *What is the typical size range of an animal eukaryotic cell?*
5. *Name three main components of the cytoskeleton.*
6. *What is the endomembrane system, and what organelles does it include?*
7. *What is the main function of mitochondria?*
8. *How do prokaryotic cells differ from eukaryotic cells in terms of genetic material?*
9. *What are the two main steps of the cell cycle?*
10. *What happens during the S phase of interphase?*
11. *What are the five stages of mitosis?*
12. *What are the three main types of cell death?*
13. *What are the primary constituents of the plasma membrane?*
14. *Who proposed the fluid mosaic model, and when?*
15. *What is the difference between integral and peripheral membrane proteins?*
16. *What is membrane asymmetry?*
17. *Give an example of a molecule that can passively diffuse through the membrane without a permease.*
18. *What is the role of aquaporins?*
19. *How does the sodium-potassium pump work?*
20. *What is the difference between symport and antiport?*
21. *Define phagocytosis and pinocytosis.*
22. *What is clathrin-mediated endocytosis?*
23. *What are the two main pathways of exocytosis?*
24. *What are the main fibrous proteins in the extracellular matrix?*
25. *What is the primary structure of collagen characterized by?*
26. *How many types of collagen are there in vertebrates?*
27. *What are proteoglycans composed of?*

28. *Name three types of glycosaminoglycans.*

29. *What is the basement membrane, and where is it found?*

30. *What are the main components of the basement membrane?*

31. *Define cell polarity.*

32. *What are tight junctions composed of?*

33. *What is the function of gap junctions?*

34. *How do adherens junctions differ from desmosomes?*

35. *What is the main difference between cytosol and cytoplasm?*

36. *What are the main ions present in the cytosol?*

37. *Where does glycolysis occur, and what are its end products?*

38. *What is the role of cytosolic chaperones?*

39. *How does the ubiquitin-proteasome system work?*

40. *What are second messengers? Give two examples.*

41. *What is xenophagy?*

42. *How does RNA interference (RNAi) defend against viruses?*

43. *What is the role of MHC molecules in antiviral defense?*

44. *What is the endosymbiotic origin of mitochondria?*

45. *How many mitochondria are typically found in a human hepatocyte?*

46. *What are the four compartments of mitochondria?*

47. *What is the function of VDAC in the OMM?*

48. *What are MAMs?*

49. *What is the primary role of the inner mitochondrial membrane?*

50. *What is stored in the mitochondrial matrix?*

51. A 22-year-old male with cystic fibrosis presents with thick mucus in his lungs and recurrent infections. Genetic testing reveals a mutation in the CFTR gene. *What is the most likely cellular transport mechanism affected by this mutation?*

A) Simple diffusion

B) Primary active transport

C) Secondary active transport (symport)

D) Chloride channel (facilitated diffusion)

52. A researcher treats cultured neurons with a toxin that inhibits microtubule polymerization. *Which cellular process will be most directly impaired?*

A) DNA replication

B) Vesicular transport

C) Glycolysis

D) Protein translation

53. A patient with suspected McLeod syndrome shows abnormalities in red blood cell shape and fragility. *Which membrane component is likely deficient?*

A) Cholesterol

B) Glycophorin

C) Spectrin (cytoskeletal protein)

D) Phosphatidylserine

54. A cell is placed in a hypertonic solution. *What will happen?*

A) It will swell and lyse.

B) It will shrink and crenate.

C) No change.

D) It will divide.

55. A genetic defect prevents the addition of mannose-6-phosphate to lysosomal enzymes. *Where will these enzymes likely accumulate?*

A) Cytosol

B) Golgi apparatus

C) Mitochondria

D) Extracellular matrix

56. A patient presents with muscle weakness and fatigue. Biopsy reveals enlarged, abnormal mitochondria. *Which process is most likely impaired?*

A) Glycolysis

B) Oxidative phosphorylation

C) Protein synthesis

D) Autophagy

57. A toxin inhibits the Na^+/K^+ ATPase. *What is the most immediate effect on the cell?*

A) Increased intracellular Na^+

B) Increased intracellular K^+

C) Depletion of ATP

D) Inactivation of voltage-gated channels

58. A mutation disrupts the gene for vinculin, a protein linking integrins to actin. *Which cell function is most affected?*

A) Cell–ECM adhesion

B) DNA repair

C) Protein folding

D) mRNA splicing

59. A chemotherapy drug causes rapid cell shrinkage, membrane blebbing, and DNA fragmentation. *What type of cell death is this?*

A) Necrosis

B) Autophagy

C) Apoptosis

D) Pyroptosis

60. A patient with a collagen IV mutation presents with hematuria and renal failure. *Which structure is primarily affected?*

A) Epidermis

B) Glomerular basement membrane

C) Bone matrix

D) Cartilage

61. A lab cell line lacks functional flippases. *What membrane property is disrupted?*

A) Fluidity

B) Asymmetry

C) Thickness

D) Glycosylation

62. A patient with metastatic cancer shows elevated hyaluronidase activity. *Which ECM component is being degraded?*

A) Collagen

B) Hyaluronic acid

C) Laminin

D) Fibronectin

63. A neuron is treated with a drug that blocks voltage-gated calcium channels. *Which process is immediately inhibited?*

A) Action potential propagation

B) Neurotransmitter release

C) Myelin formation

D) ATP synthesis

64. A genetic disease causes accumulation of gangliosides in neurons. *Which organelle is likely deficient?*

A) Peroxisome

B) Lysosome

C) Golgi

D) Mitochondria

65. A cell is engineered to lack all cholesterol in its plasma membrane. *What will happen to membrane fluidity at low temperatures?*

A) Increase

B) Decrease

C) No change

D) Become asymmetric

66. A bacterium escapes the phagosome and enters the cytosol. *Which host defense mechanism may still target it?*

A) Complement system

B) Xenophagy

C) Antibody production

D) MHC class II presentation

67. A mutation in a gene encoding a component of the proteasome is identified. *What cellular process is most compromised?*

A) Protein synthesis

B) Protein degradation

C) Lipid synthesis

D) Carbohydrate metabolism

68. A patient presents with poor wound healing and fragile skin. Genetic testing reveals a defect in collagen fibril assembly. *Which collagen type is most likely affected?*

A) Type I

B) Type IV

C) Type VII

D) Type XVIII

69. A drug inhibits co-translational translocation into the ER. *Where will secretory proteins accumulate?*

A) Nucleus

B) Cytosol

C) Mitochondria

D) Peroxisomes

70. A researcher knocks out the gene for Drp1 in mouse cells. *What will happen to mitochondria?*

A) They will fragment.

B) They will elongate/fuse.

C) They will lose their DNA.

D) They will increase in number.

71. A patient has a mutation affecting the cardiolipin synthase gene. *Which organelle's function is most compromised?*

A) Lysosome

B) Golgi

C) Mitochondrion

D) Peroxisome

72. A toxin blocks the binding of ATP to Hsp70 chaperones. *What process is directly inhibited?*

A) Protein degradation

B) Protein folding

C) DNA replication

D) mRNA splicing

73. A patient has an autoimmune disease targeting desmoglein 3. *Which tissue is most affected?*

A) Heart muscle

B) Skin epidermis

C) Liver

D) Kidney tubules

74. A cell is treated with a drug that inhibits PI3K. *Which signaling pathway is most directly affected?*

A) MAPK pathway

B) Akt/PKB pathway

C) JAK-STAT pathway

D) Wnt pathway

75. A mutation prevents the hydroxylation of proline in collagen. *What is the consequence for collagen structure?*

A) Enhanced stability

B) Triple helix cannot form

C) Increased glycosylation

D) Faster secretion

76. A virus enters cells via clathrin-mediated endocytosis. *What would blocking clathrin function do?*

A) Enhance viral replication

B) Block viral entry

C) Trigger apoptosis

D) Increase viral budding

77. A patient has a defect in the synthesis of dolichol phosphate. *Which post-translational modification is impaired?*

A) Phosphorylation

B) N-linked glycosylation

C) Ubiquitination

D) Acetylation

78. A researcher depletes cellular calcium stores in the ER. *Which process in the cytosol will be directly affected?*

A) Glycolysis

B) Calcium-dependent signaling

C) Protein synthesis

D) Fatty acid oxidation

79. A patient with a genetic disorder has neurons with aggregated proteins and impaired proteasome function. *What might be a secondary consequence?*

A) Increased autophagy

B) Enhanced protein synthesis

C) Decreased glycolysis

D) Mitochondrial fission

80. A cell line lacks functional peroxisomes. *Which metabolic pathway is impaired?*

A) Glycolysis

B) Beta-oxidation of very-long-chain fatty acids

C) TCA cycle

D) Pentose phosphate pathway

81. A mutation in a centrosomal protein causes abnormal spindle formation. *What is the likely outcome during cell division?*

A) Increased ATP production

B) Chromosome mis-segregation

C) Enhanced DNA repair

D) Faster cytokinesis

82. A drug inhibits the enzyme cyclooxygenase (COX). *Which class of lipid signaling molecules is reduced?*

A) Phosphatidylinositols

B) Prostaglandins

C) Sphingomyelins

D) Cardiolipins

83. A patient presents with severe sunburns and dry skin. Genetic analysis reveals a defect in lipid secretion by sebaceous glands. *Which transport process is likely impaired?*

A) Phagocytosis

B) Exocytosis

C) Pinocytosis

D) Receptor-mediated endocytosis

84. A toxin inhibits the mitochondrial ATP synthase. *What immediate effect occurs in the inner mitochondrial membrane?*

A) Increased proton gradient

B) Decreased proton gradient

C) Collapse of membrane potential

D) Increased oxygen consumption

85. A mutation prevents the addition of GPI anchors to proteins. *Where will these proteins likely accumulate?*

A) Cytosol

B) ER lumen

C) Mitochondrial matrix

D) Nucleus

86. A patient has a mutation in the gene encoding the GLUT4 transporter. *Which tissue is most affected?*

A) Liver

B) Muscle and adipose tissue

C) Brain

D) Red blood cells

87. A cell is treated with a drug that caps actin filaments. *Which process is most directly inhibited?*

A) Cytokinesis

B) Nuclear import

C) Transcription

D) Translation

88. A patient has a defect in the synthesis of heparan sulfate. *Which cell signaling process might be affected?*

A) Growth factor binding

B) Steroid hormone reception

C) Thyroid hormone signaling

D) Retinoic acid signaling

89. A researcher knocks out the gene for Bcl-2 in a cell line. *What is the likely cellular response to stress?*

A) Increased autophagy

B) Enhanced proliferation

C) Increased apoptosis

D) Senescence

90. A toxin blocks the fusion of autophagosomes with lysosomes. *What accumulates in the cell?*

A) Double-membrane vesicles containing organelles

B) Free fatty acids

C) Misfolded proteins in the ER

D) Lipid droplets

91. A patient has a mutation in the laminin-332 gene. *Which tissue is primarily affected?*

A) Bone

B) Skin epidermis

C) Cardiac muscle

D) Nervous tissue

92. A drug inhibits the enzyme phospholipase C. *Which second messengers are not produced?*

A) cAMP and cGMP

B) IP_3 and DAG

C) Ca^{2+} and NO

D) PIP_2 and PIP_3

93. A cell lacks functional t-SNARE proteins on the plasma membrane. *Which process is blocked?*

A) DNA replication

B) Vesicle docking/fusion

C) Transcription

D) Translation

94. A patient has a genetic defect affecting the synthesis of all proteoglycans. *Which property of the ECM is most compromised?*

A) Tensile strength

B) Hydration and resilience

C) Elasticity

D) Adhesion

95. A researcher treats cells with a drug that collapses the mitochondrial membrane potential. *What is the immediate consequence for protein import into mitochondria?*

A) No effect

B) Import is enhanced

C) Import is blocked

D) Only matrix proteins are affected

96. A patient presents with an inflammatory disease caused by overactive NLRP3 inflammasome. *Which cytokine is excessively released?*

A) IL-1β

B) TNF-α

C) IFN-γ

D) IL-10

97. A mutation disrupts the KDEL receptor in the Golgi apparatus. *Where will ER-resident proteins end up*?

A) Secreted from the cell

B) Stuck in the Golgi

C) Degraded in lysosomes

D) Sent to the nucleus

98. A cell is engineered to lack all integrins. *What is the primary cellular defect?*

A) Cannot adhere to the ECM

B) Cannot divide

C) Cannot transcribe genes

D) Cannot produce ATP

99. A toxin inhibits the enzyme acetylcholinesterase at a neuromuscular junction. *What is the effect on the postsynaptic muscle cell?*

A) Sustained contraction

B) Relaxation

C) Apoptosis

D) Atrophy

100. A patient has a mutation in the gene for the mitochondrial phosphate carrier. *Which process is directly impaired?*

A) Glycolysis

B) ATP synthesis

C) Protein import

D) Citric acid cycle

VII.1- Answers

1.Robert Hooke in 1667, while observing cork.

2.

A. All plants and animals are made of cells.

B. Cells possess the ability to assimilate, grow, and reproduce.

C. Cells arise from the division of pre-existing cells.

3. Matthias Jakob Schleiden (plants) and Theodor Schwann (animals).

4. 10 to 30 µm in width, but can exceed one meter in length (e.g., neurons).

5. Microtubules, actin microfilaments, intermediate filaments.

6. A group of membranous organelles including the endoplasmic reticulum (rough and smooth), Golgi apparatus, lysosomes, nuclear membrane, and vesicles.

7. To produce ATP through oxidative phosphorylation (cell powerhouse).

8. Prokaryotic DNA is circular and free in the cytoplasm, not enclosed in a nucleus, while eukaryotic DNA is linear and housed in a nucleus.

9. Interphase and division (mitosis).

10. DNA replication occurs, changing DNA content from 2n to 4n.

11. Prophase, Prometaphase, Metaphase, Anaphase, Telophase (plus cytokinesis).

12. Type I (Apoptosis), Type II (Autophagy), Type III (Necrosis).

13. Phospholipids, which form a bilayer.

14. Seymour Jonathan Singer and Garth L. Nicolson in 1972.

15. Integral proteins are embedded in the lipid bilayer and require detergents for removal, while peripheral proteins are attached via ionic interactions and can be removed without disrupting the bilayer.

16. The difference in lipid and protein composition between the inner and outer leaflets of the plasma membrane.

17. Oxygen (O_2), carbon dioxide (CO_2), or ethanol.

18. They are integral membrane proteins that facilitate the transport of water across the membrane (osmosis).

19. It uses ATP to transport 3 Na^+ out and 2 K^+ into the cell against their concentration gradients.

20. Symport transports two molecules in the same direction, while antiport transports them in opposite directions.

21. Phagocytosis is "cell eating" (engulfment of large particles), while pinocytosis is "cell drinking" (uptake of fluid and small molecules).

22. A receptor-mediated process where clathrin-coated pits form vesicles to internalize specific ligands (e.g., viruses, cholesterol).

23. Constitutive exocytosis (continuous) and regulated exocytosis (triggered by signals).

24. Collagens and elastin.

25. Repeating tripeptide units of Gly-X-Y, where X is often proline and Y is hydroxyproline.

26. 28 types, formed from 46 different α chains.

27. A core protein with one or more covalently attached glycosaminoglycan (GAG) chains.

28. Chondroitin sulfate, heparan sulfate, hyaluronic acid, keratan sulfate, dermatan sulfate.

29. A specialized ECM layer found at the interface of epithelial/endothelial cells and underlying connective tissue (e.g., skin, kidneys, blood vessels).

30. Laminins, collagen IV, nidogens/entactin, perlecan, and agrin.

31. The asymmetric spatial arrangement and distribution of cellular components, leading to specialized domains (e.g., apical-basal polarity).

32. Transmembrane proteins such as claudins, occludin, and JAMs (Junctional Adhesion Molecules).

33. To allow direct communication between adjacent cells via connexin channels, permitting the passage of small molecules and ions.

34. Adherens junctions connect to the actin cytoskeleton and use cadherins, while desmosomes connect to intermediate filaments and use desmogleins/desmocollins.

35. Cytosol is the liquid portion surrounding organelles, while cytoplasm includes both cytosol and organelles.

36. K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}, H^+.

37. In the cytosol; end products are 2 pyruvate, 2 ATP (net), and 2 NADH per glucose molecule.

38. To assist in protein folding, prevent aggregation, and facilitate protein transport.

39. Ubiquitin is attached to target proteins by E1-E2-E3 enzymes, marking them for degradation by the 26S proteasome.

40. Small intracellular signaling molecules that amplify signals; examples include cAMP, Ca^{2+}, IP_3, DAG.

41. A selective autophagy process that targets intracellular bacteria for degradation.

42. Dicer cleaves viral dsRNA into siRNAs, which guide RISC to degrade complementary viral RNA.

43. They present viral peptide antigens to T cells, activating adaptive immune responses.

44. Mitochondria evolved from aerobic alphaproteobacteria that were engulfed by ancestral eukaryotic cells.

45. Between 1,000 and 1,700.

46. Outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), intermembrane space (IMS), and matrix.

47. It forms aqueous pores for the passive diffusion of metabolites, ions, and small molecules (≤5 kDa).

48. Mitochondria-Associated Membranes—specialized contact sites between the OMM and endoplasmic reticulum.

49. To host the electron transport chain and ATP synthase for oxidative phosphorylation.

50. Mitochondrial DNA (mtDNA), ribosomes, enzymes for the TCA cycle, and metabolites.

51. *D)* Chloride channel (facilitated diffusion)

Explanation: CFTR is a chloride channel (a permease). Its dysfunction disrupts ion and water balance, leading to thick mucus.

52. *B)* Vesicular transport

Explanation: Microtubules serve as tracks for vesicular transport via motor proteins.

53. *C)* Spectrin (cytoskeletal protein)

Explanation: Spectrin links the membrane to the cytoskeleton; its deficiency reduces RBC elasticity.

54. *B)* It will shrink and crenate.

Explanation: Water moves out of the cell in a hypertonic solution.

55. *B)* Golgi apparatus

Explanation: Mannose-6-phosphate tags target enzymes to lysosomes. Without it, they are not packaged correctly.

56. *B)* Oxidative phosphorylation

Explanation: Mitochondrial diseases often affect ATP production via OXPHOS.

57. *A)* Increased intracellular Na^+

Explanation: The pump normally exports Na^+; inhibition leads to Na^+ accumulation inside.

58. *A)* Cell–ECM adhesion

Explanation: Vinculin is part of focal adhesions, connecting integrins to the cytoskeleton.

59. *C)* Apoptosis

Explanation: Apoptosis features cell shrinkage, blebbing, and organized DNA cleavage.

60. *B)* Glomerular basement membrane

Explanation: Collagen IV is a key component of the glomerular basement membrane.

61. *B)* Asymmetry

Explanation: Flippases help maintain phospholipid asymmetry between membrane leaflets.

62. *B)* Hyaluronic acid

Explanation: Hyaluronidase degrades hyaluronic acid, facilitating tumor invasion.

63. *B)* Neurotransmitter release

Explanation: Calcium influx via these channels triggers exocytosis of synaptic vesicles.

64. *B)* Lysosome

Explanation: Lysosomal storage diseases (e.g., Tay-Sachs) involve undegraded gangliosides.

65. *B)* Decrease

Explanation: Cholesterol maintains fluidity at low temps; without it, membranes become too rigid.

66. *B)* Xenophagy

Explanation: Cytosolic bacteria can be targeted by autophagy (xenophagy).

67. *B)* Protein degradation

Explanation: The proteasome is responsible for degrading ubiquitin-tagged proteins.

68. *A)* Type I

Explanation: Type I collagen is the major fibrillar collagen in skin and connective tissue.

69. *B)* Cytosol

Explanation: Proteins destined for secretion start translation on free ribosomes; without ER signal recognition, they remain cytosolic.

70. *B)* They will elongate/fuse.

Explanation: Drp1 is required for mitochondrial fission; its absence promotes fusion.

71. *C)* Mitochondrion

Explanation: Cardiolipin is a signature lipid of the inner mitochondrial membrane, essential for cristae structure and ETC function.

72. *B)* Protein folding

Explanation: Hsp70 uses ATP to bind/release client proteins during folding.

73. *B)* Skin epidermis

Explanation: Desmoglein 3 is a key desmosomal cadherin in the skin; its disruption causes blistering (e.g., pemphigus).

74. *B)* Akt/PKB pathway

Explanation: PI3K produces PIP3, which recruits Akt to the membrane for activation.

75. *B)* Triple helix cannot form

Explanation: Hydroxyproline stabilizes the collagen triple helix via hydrogen bonds.

76. *B)* Block viral entry

Explanation: Clathrin-coated pits are essential for this entry mechanism.

77. *B)* N-linked glycosylation

Explanation: Dolichol phosphate is the lipid carrier for oligosaccharides in N-glycosylation in the ER.

78. *B)* Calcium-dependent signaling

Explanation: The ER is a major calcium store; its release into the cytosol triggers many signaling events.

79. *A)* Increased autophagy

Explanation: When the proteasome is overwhelmed, autophagy is upregulated to clear aggregates.

80. *B)* Beta-oxidation of very-long-chain fatty acids

Explanation: Peroxisomes handle beta-oxidation of very-long-chain fatty acids; mitochondria handle shorter chains.

81. *B)* Chromosome mis-segregation

Explanation: Centrosomes organize the mitotic spindle; defects lead to aneuploidy.

82. *B)* Prostaglandins

Explanation: COX produces prostaglandins from arachidonic acid.

83. *B)* Exocytosis

Explanation: Sebaceous glands secrete lipids via exocytosis.

84. *A)* Increased proton gradient

Explanation: ATP synthase uses the proton gradient to make ATP; inhibition prevents proton flow back into the matrix, maintaining a high gradient.

85. *B)* ER lumen

Explanation: GPI anchors are added in the ER; without them, proteins are not attached to the membrane and may be retained.

86. *B)* Muscle and adipose tissue

Explanation: GLUT4 is the insulin-responsive glucose transporter in muscle and fat cells.

87. *A)* Cytokinesis

Explanation: Actin forms the contractile ring during cytokinesis.

88. *A)* Growth factor binding

Explanation: Heparan sulfate proteoglycans bind and present many growth factors (e.g., FGF) to their receptors.

89. *C)* Increased apoptosis

Explanation: Bcl-2 is an anti-apoptotic protein; its loss makes cells more prone to apoptosis.

90. *A)* Double-membrane vesicles containing organelles

Explanation: Autophagosomes that cannot fuse with lysosomes accumulate.

91. *B)* Skin epidermis

Explanation: Laminin-332 is crucial for epithelial basement membrane adhesion; mutations cause blistering diseases.

92. *B)* IP_3 and DAG

Explanation: PLC cleaves PIP_2 into IP_3 and DAG.

93. *B)* Vesicle docking/fusion

Explanation: t-SNAREs on target membranes pair with v-SNAREs on vesicles to drive fusion.

94. *B)* Hydration and resilience

Explanation: Proteoglycans attract water, providing hydration and resistance to compression.

95. *C)* Import is blocked

Explanation: The membrane potential across the inner membrane is required for driving the import of many mitochondrial proteins.

96. *A)* IL-1β

Explanation: The NLRP3 inflammasome activates caspase-1, which processes pro-IL-1β into active IL-1β.

97. *A)* Secreted from the cell

Explanation: The KDEL receptor retrieves ER-resident proteins from the Golgi; without it, they are secreted.

98. *A)* Cannot adhere to the ECM

Explanation: Integrins are the primary transmembrane receptors for ECM adhesion.

99. *A)* Sustained contraction

Explanation: Acetylcholinesterase degrades ACh; inhibition leads to persistent signaling and muscle contraction.

100. *B)* ATP synthesis

Explanation: The phosphate carrier imports phosphate into the matrix, which is essential for ATP synthesis (ATP synthase requires ADP and Pi).

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NB : We used Biorender.com website and generative AI to generate figures and to correct the English language.