Partie I : Aspects ultrastructuraux

Part I : Ultrastructural aspects

Specific course objectives

1) Understand the structure of the plasma membrane (concept of a fluid and asymmetric tri-stratified membrane),
2) The basic molecular components (lipids, proteins, and carbohydrates) of the erythrocyte membrane,
3) The properties of lipids (self-assembly, self-closure, and fluidity), proteins (fluidity), and carbohydrates (negative charge),
4) The molecular architecture of the membrane and clarify the concept of a fluid and asymmetric mosaic.

Introduction

The plasma membrane, plasmalemma, cytomembrane, is a continuous biological envelope that separates, but does not isolate, the intracellular and extracellular environments of all eukaryotic and prokaryotic cells. It possesses a specific molecular structure necessary for exchanges with the extracellular environment and for communication with other cells.

1. Méthodes de mise en évidence de la membrane plasmique (techniques de coupe simple et technique de réplique) voir le chapitre 5 (méthode et technique d'étude cellulaire)

2. Ultrastructure of the membrane (concept of a tristratified, fluid and asymmetric

1. Notion de membrane tristratifiée ou trilamellaire

This concept refers to the structural organization of biological membranes, notably observed in electron microscopy (TEM) after fixation with heavy metals; it appears in the form of three distinct layers:

- **Two dark (electron-dense) layers** (outer and inner) measuring 2 to 205 nm correspond to the hydrophilic (**osmiophile**) heads of phospholipids, which are strongly bound to metallic dyes (**OsO4**).
- **A central, light (osmiophobic) layer** measuring 3 to 4 nm (less dense) represents the hydrophobic tails of phospholipids, which are weakly bound to heavy metals. This organization is universal in eukaryotic and prokaryotic cells, as well as in the membranes of organelles (mitochondria, endoplasmic reticulum, Golgi apparatus, etc.).

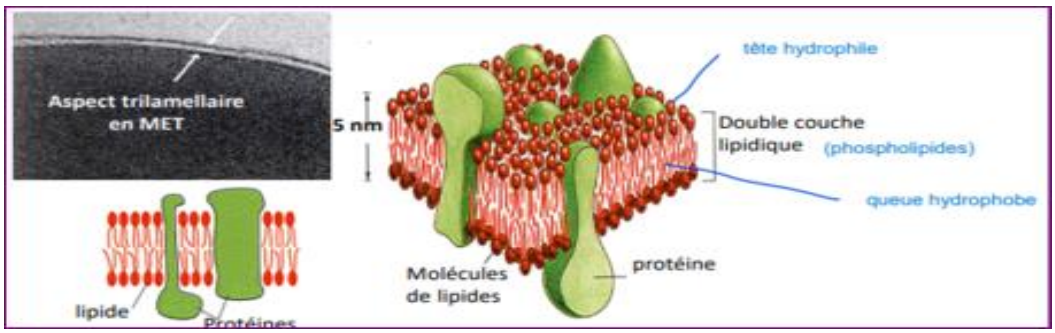
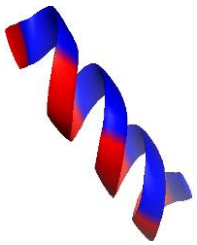


Figure 1 : TEM photograph, trilamellar aspect of a MP, with schematic 2D and 3D views.

The characteristic **tri-layered** appearance of biological membranes under electron microscopy results from the preferential binding of osmium to the polar regions of the phospholipids and proteins of the two membrane leaflets, while the hydrophobic central zone appears lighter. This organization reflects the amphiphilic nature of membrane lipids. The following two concepts should not be confused.

a) Amphiphily (chemical property): Amphiphily is a property of a molecule that possesses a hydrophilic (polar, charged) part and a hydrophobic (nonpolar) part. It is a chemical and structural concept.

Examples: Phospholipids, which have hydrophilic polar heads and two hydrophobic fatty acid tails. Amphiphily explains the spontaneous formation of lipid bilayers and the self-assembly of biological membranes.



b) Amphipathy describes a spatial organization: This is a structural and functional concept, not solely a chemical one. Hydrophilic and hydrophobic regions are separated in space, often within a three-dimensional structure. Example 1: An amphipathic α-helix has a hydrophobic face and a hydrophilic (or charged) face. **Example 2**: Mitochondrial signal peptides (MTS) are rich in positive charges on one side and hydrophobic charges on the other. Therefore, we speak of an amphipathic structure.

2. Membrane asymmetry

Asymmetry is a fundamental property of the plasma membrane. It results from the unequal distribution of lipids, proteins, and carbohydrates between the two layers of the bilayer.

A. Examples of asymmetry:

a) Lipides :

- ✓ **Phosphatidylcholine** and **sphingomyelin**: primarily located in the outermost leaflet.
- ✓ **Phosphatidylserine** and **phosphatidylethanolamine**: located in the innermost leaflet.

b) Cholestérol : distributed in a relatively homogeneous manner.

c) Protéines :

- ✓ **Peripheral proteins on the inner surface** (e.g., signaling proteins).
- ✓ **Glycosylated proteins on the outer surface** (e.g., receptors).

e) Carbohydrates: Exclusively on the outer surface, participating in cell recognition and interactions with the environment.

B. The glycocalyx: a consequence of asymmetry

The glycocalyx is a fibrillar coating called the cell coat; it is a layer of carbohydrates found only on the outer leaflet of the plasma membrane. It is composed of:

- ✓ **Glycoproteins**: membrane proteins linked to short, branched carbohydrate chains (oligosaccharides), generally attached by N-glycosidic bonds (on asparagine) or O-glycosidic bonds (on serine or threonine).

- ✓ **Proteoglycans**: composed of a protein core to which long chains of glycosaminoglycans (GAGs) are attached. These are linear, negatively charged polysaccharides, often sulfated (e.g., heparan sulfate).
- ✓ **Glycolipids** (lipids associated with carbohydrate residues).

Since carbohydrate chains are found only on the outer leaflet, they reinforce membrane asymmetry and play a key role in several functions.

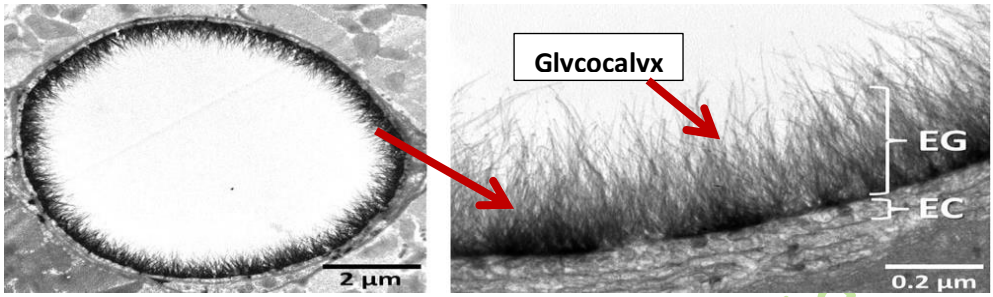


Figure 2 : The endothelial **glycocalyx**

As an indication: Deterioration of the **endothelial glycocalyx** is considered an early stage in the onset of all chronic vascular complications.

C. Roles of asymmetry:

- ✓ Maintenance of cell polarity.
- ✓ Cell signaling (e.g., exposure of phosphatidylserine on the outer leaflet can signal apoptosis).
- ✓ Interaction with the extracellular environment.

D. Proteins: Some proteins, such as lipid rafts, can locally stiffen the membrane.

E. Asymmetry and fluidity in interaction: These two properties are closely linked

- ❖ **The asymmetry** depends on specific enzymes (flippases, floppases, scramblases) that move lipids between the leaflets.
- ❖ Fluidity allows for dynamic reorganization of the membrane, while maintaining its functional asymmetry.

3. Results of the analysis of membrane replicas (concept of hemimembrane and intramembrane globular particles).

1. Membrane hémimembranaire

The term hemimembrane refers to the two layers that make up a biological membrane, notably observed in cryofracture. The lipid bilayer generally separates at the hydrophobic plane, giving rise to **two hemimembranes**:

- ➢ **P face (protoplasmic):** This corresponds to the innermost layer of the plasma membrane, facing the cytoplasm.
- ➢ **E face (exoplasmic):** This corresponds to the outermost layer, facing the outside of the cell.

These layers reveal the asymmetrical organization of lipids and transmembrane proteins.

2. Intramembranous globular particles

Intramembranous **g**lobular **p**articles (or IMPs) are structures observed under the electron microscope after **cryofracture**. They correspond to the transmembrane domains of integral proteins, which remain anchored in one of the leaflets after bilayer separation. They appear as spherical structures dispersed primarily on the P-face (although some may be visible on the E-face). Their density and distribution vary according to cell type and membrane function.

3. Role of IMPs :

- ✓ **Transport proteins**: Ion channels, transporters.
- ✓ **Receptors**: Involved in cell signaling.
- ✓ **Adhesion**: Attachment proteins for intercellular junctions.
- ✓ **Enzymes**: Associated with local metabolic activities.

4. Pathologies :

Abnormalities in the organization of **IMPs** or **hemimembranes** can reveal membrane dysfunctions (e.g., defects in ion channels in certain genetic diseases). Indeed, genetic mutations affecting **IMPs**, ion channels, or membrane-associated proteins can cause a wide range of genetic diseases such as: **Hereditary hemolytic anemia**, **Duchenne muscular dystrophy**, **Liddle syndrome** (mutation of the SCNN1A, SCNN1B, or SCNN1G genes, encoding subunits of the epithelial sodium channel (ENaC)).

4. Isolation method (hemolysis + centrifugation) for qualitative and quantitative chemical analysis of the red blood cell membrane.

Isolating the red blood cell (erythrocyte) membrane for qualitative and quantitative chemical analysis relies on specific steps involving hemolysis and centrifugation. The process is based on:

A. Hemolysis: Rupture of red blood cells in a hypotonic solution to release hemoglobin. Draw blood in an anticoagulant (e.g., EDTA or citrate). Separate the plasma and blood cells by slow centrifugation (approximately 1000 g, 10 minutes). Then, wash the red blood cells 2-3 times with isotonic saline (0.9% NaCl).

- ✓ **Rupture of red blood cells**: Resuspend the washed red blood cells in the hypotonic solution (distilled water). Leave for a few minutes at room temperature to induce hemolysis (release of hemoglobin).
- ✓ **Verification of hemolysis**: Obtain a clear or slightly red-colored solution, indicating hemoglobin release.

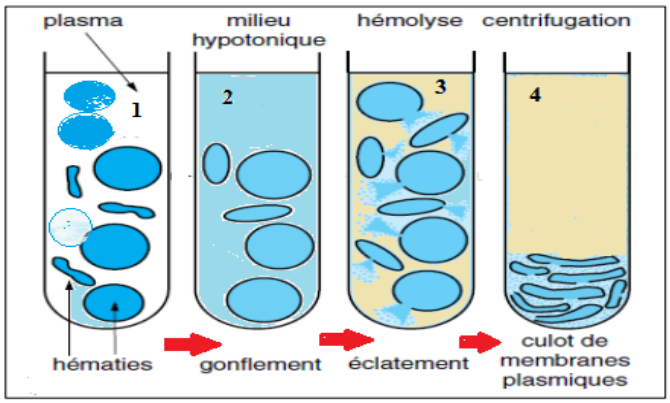


Figure 3 : Stages of hemolysis and formation of the plasma membrane

B. Centrifugation to isolate the membranes: Separation of membranes (or membrane ghosts) from other cellular components (hemoglobin, ions, etc.).

- ✓ **Differential centrifugation**: Centrifuge the hemolyzed solution at 10,000–20,000 g for 20–30 minutes at 4°C. The membranes (erythrocyte ghosts) form a whitish or pinkish pellet.
- ✓ **Membrane washing**: Resuspend the pellet in an isotonic buffer solution (e.g., 0.1 M NaCl phosphate buffer, pH 7.4). Repeat centrifugation 2–3 times to remove hemoglobin residues.
- ✓ Work at a low temperature (4°C) to limit enzymatic activity and the degradation of membrane components.

5. Basic molecular components (lipids; proteins, carbohydrates) of the erythrocyte membrane and the proportions and distribution of their varieties.

The molecular architecture of the plasma membrane is based on a dynamic and complex organization of lipids, proteins, and carbohydrates. This model is described by the fluid mosaic proposed by **Singer and Nicolson (1972),** which highlights the fluidity and **asymmetry** of the membrane.

The membrane of **erythrocytes** (**red blood cells**) is a highly organized structure composed of **lipids**, **proteins**, and **carbohydrates**. These components are distributed asymmetrically to meet the specific functional needs of erythrocytes, including their flexibility, stability, and ability to interact with the environment.

1. Overall composition of the erythrocyte membrane: in terms of weight proportion (expressed as a function of their weight) :

- ✓ Protein: ~ 50%; Fat: ~ 40%; Carbohydrates: ~ 10%

A. Membrane lipids: représentent ~40 % du poids sec de la membrane.

- ✓ Phospholipids : ~60-65 % des lipides.
- ✓ Cholesterol : ~30-35 % des lipides.
- ✓ Glycolipids : ~5-10 % des lipides.

1) Main phospholipids asymmetrical distribution between the two layers of the bilayer:

- **Internal leaflet** (cytoplasmic) :
  - ✓ Phosphatidylethanolamine (PE) : ~20 %.
  - ✓ Phosphatidylserine (PS) : ~10-15 %. The exposure of Phosphatidylserine on the outer leaflet is a signal of apoptosis (eat me !).
  - ✓ Phosphatidylinositol (PI) : ~2-5 % (rôle dans la signalisation).
- **Outer leaf** (exoplasmic) :
  - ✓ Phosphatidylcholine (PC) : ~30 %.
  - ✓ Sphingomyéline (SM) : ~25 %.

2) Cholesterol : Distributed evenly between the two leaflets. Plays a role in membrane fluidity and stability.

3) Bipolar and amphiphilic properties of membrane lipids:

Membrane lipids share a key property: they are **bipolar and amphiphilic**. This means they possess a hydrophobic region, which constitutes the majority by volume, and a hydrophilic region capable of interacting with water. The hydrophilic portion is generally composed of polar groups such as phosphoric acid, carbohydrate motifs, amine groups, or acid groups.

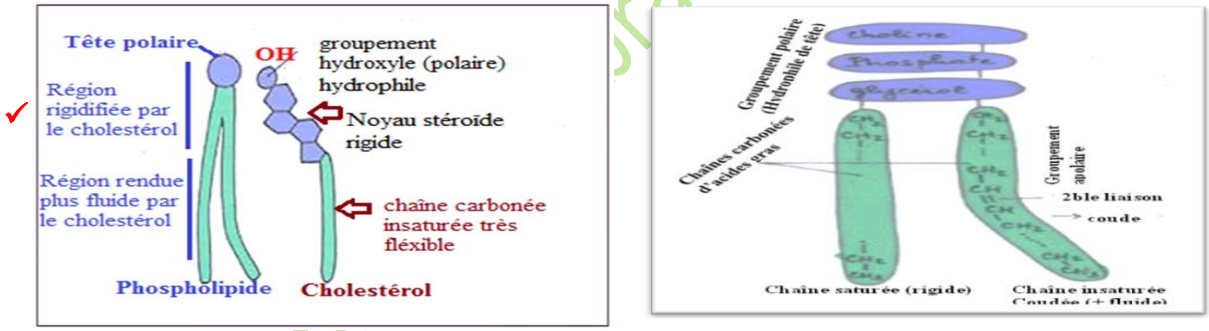
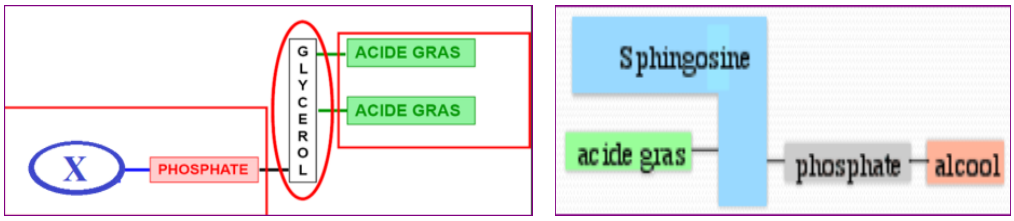


Figure 4 : Amphiphilic phospholipid molecule and the role of cholesterol in membrane fluidity.

There are three categories of membrane lipids: phospholipids, glycolipids, and sterols.

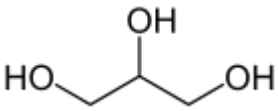
a) Phospholipids: These are complex molecules containing, in addition to C, H and O, phosphorus and possibly nitrogen.

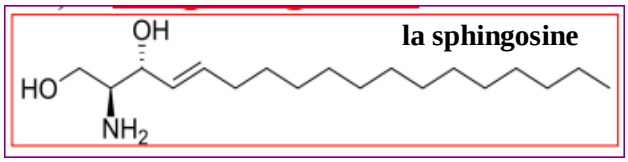


The alcohol, which is linked to one or two fatty acids, is either glycerol (HOH2C–CHOH–H2OH), or a fatty chain amino alcohol (hydrocarbon chain): sphingosine (C18H37O2N).

Depending on the case, we speak of: **Glycerophospholipid** (the most abundant).

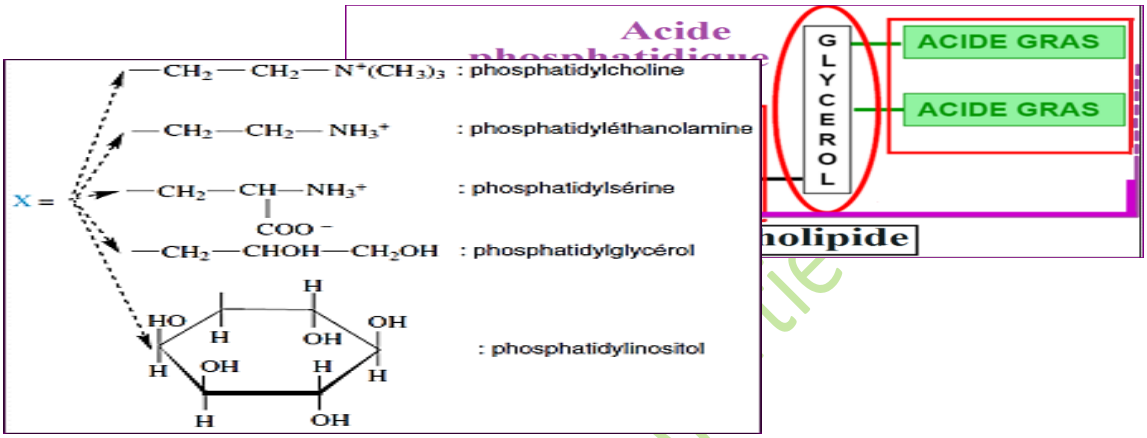
Sphingophospholipid.





a.1. Glycerophospholipid : he three alcohol groups of Glycerol are esterified by 2 fatty acids and phosphate, thus forming phosphatidic acid, as shown in the following diagram.

The last molecule **X** gives the identity to the lipid in question,

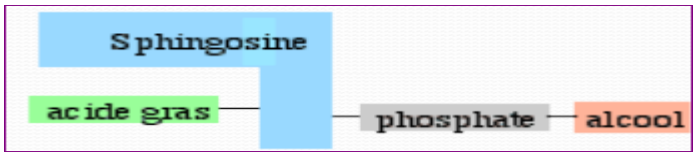


The final molecule is called, depending on the case,:

- **Phosphatidyl-glycerol** = **Diphosphatidylglycerol** (ou **cardiolipine** characteristic of the inner mitochondrial membrane),
- **Phosphatidyl- inositol**,
- **Phosphatidylethanolamine**,
- **Phosphatidylcholine**,
- **Phosphatidylserine.**

a. 2. Les Sphingolipides

Sphingosine associates on one side with a phosphate group, thus forming the hydrophilic (polar) "head" of the sphingolipid, and on the other side with a hydrocarbon chain of a fatty acid molecule, which represents the hydrophobic "tail" (see the following diagram).



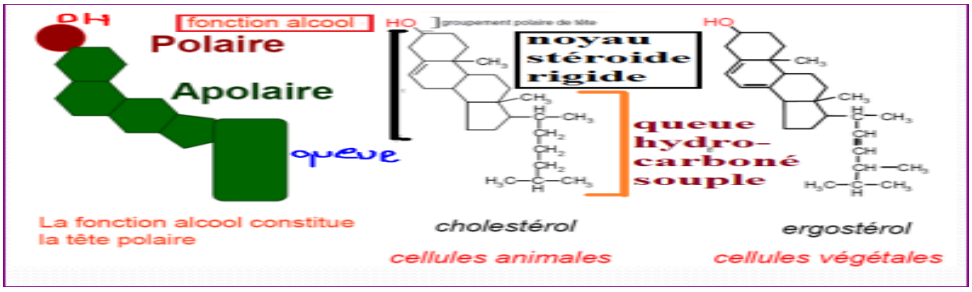
In humans, **sphingomyelins** constitute approximately 85% of all **sphingolipids**.

b. The Cholesterol

Cholesterol (Sterols); does not exactly fit the classic definition of lipids but are molecules related on a physicochemical level (**low affinity for water**).

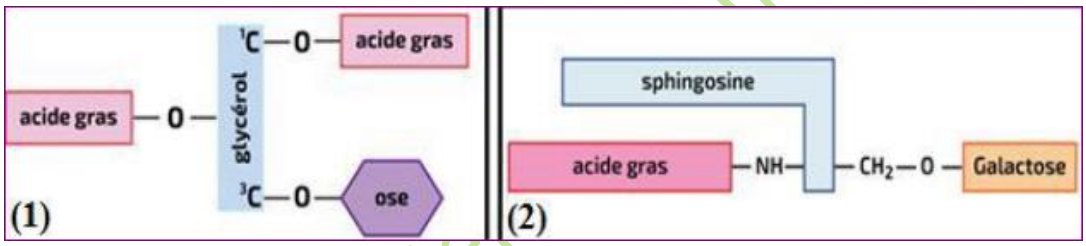
- It is abundant in eukaryotic cell membranes but absent in prokaryotes.

- It plays a role in the fluidity and mechanical stability of biological membranes.
- It is one of the components of lipid membrane rafts.
- It is also a precursor of steroid hormones.



c. Glycolipids

They do not contain **phosphoric acid**. They are constructed from **glycerol** (**glyceroglycolipids**) (1) or **sphingosine** (**sphingoglycolipids**) (2), but the third alcohol group of the former or the terminal alcohol group of the latter is directly esterified by **a sugar** or sugar derivative that constitutes the **polar "head" group**. **Neutral sphingosine glycolipids** are very abundant in animals, particularly in nerve cells.



6. Properties of lipids (self-assembly, self-closure, and fluidity), proteins (fluidity) and carbohydrates (negative charge).

I- Characteristics of membrane lipids

1) Spontaneous self-assembly in aqueous media

Membrane lipids possess an amphiphilic nature, characterized by a hydrophilic and **osmiophilic** (**lipophobic**) polar head and a hydrophobic and **osmiophobic** (**lipophilic**) nonpolar tail. This physicochemical property underlies their ability to spontaneously self-assemble into organized structures, such as lipid bilayers or micelles, when in an aqueous environment. Thanks to this amphiphilicity, lipids adopt specific arrangements in response to their environment, thus enabling the formation of various self-assembly structures, of which four main types can be distinguished.

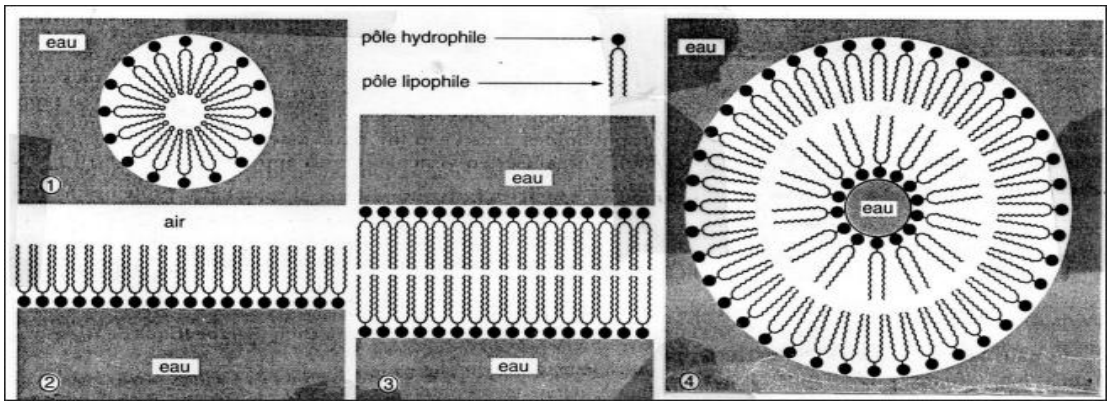


Figure 5 : Properties of amphiphilic molecules in aqueous media (specific self-assembly) of membrane lipids. (1) Micelles; (2) Planar monolayer; (3) Simple planar bilayer; (4) Unilamellar liposome.

2) membrane fluidity:

The flexibility, resistance, and malleability of the plasma membrane are due to the fluidity (viscosity) of lipid molecules.

- **Lateral movement**: Each lipid molecule can move laterally within the plane of each layer; these movements are rapid (2 µm/second).
- **Transverse movement**: The lipid molecule can move from one layer to another; this is a more difficult movement. This mechanism is called transverse diffusion or **flip-flop**; it requires the complete reversal of the molecule and demands an energy input (ATP) with the presence of specific enzymes called **flipases**.

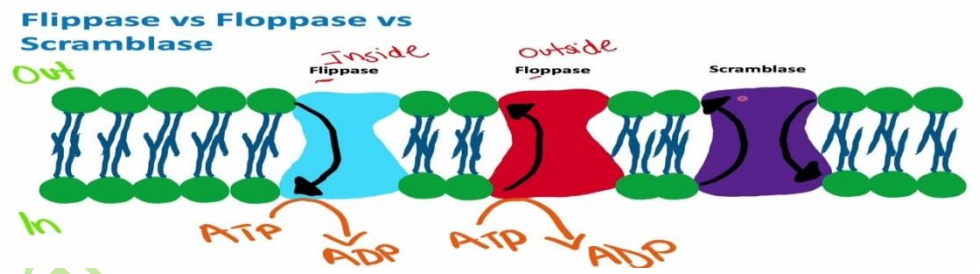


Figure 6: Flipase , Flopase and Scramblase

Flip: inward, **Flop**: outward, **Scramble**: mixes everything (Non-specific mixing of phospholipids between the two leaflets and loss of asymmetry. **It is ATP-independent**, activated by **increased intracellular Ca^{2+}**, **cellular stress**, exposure of **phosphatidylserine** to the cell surface, and finally **apoptosis**.

Factors influencing membrane fluidity

- **The fatty acid composition of membrane lipids:** The degree of fluidity of membranes is conditioned by the nature and length of the fatty acids of the membrane lipids; the more a bilayer is rich in short and unsaturated fatty acids, the more flexible and fluid it is.
  - Fatty acids possess one or more double bonds in their carbon chain, creating a rigid 30° angle or bend that gives the molecule a non-linear structure.

- These **double bonds** contribute to weakening the **Van der Waals** interactions between neighboring chains, making the membrane more fluid.

❖ **Temperature**: The degree of membrane fluidity is also conditioned by external factors. An increase in temperature leads to increased mobility and deformation of the fatty acid chains in lipids, giving the membrane a fluid appearance. Conversely, a decrease in temperature causes the membrane to become more viscous, especially if it is composed of saturated, long-chain phospholipids, and less able to maintain fluidity at low temperatures.

❖ **Cholesterol content**: The proportion of cholesterol in the bilayers can be high, sometimes reaching 25% of total lipids. Cholesterol acts through two mechanisms:

a) By its conformation: it is **rigid at the tetracyclic core**, and **flexible** at the hydrophobic chain.

b) By its position relative to other lipids: Cholesterol also has a buffering effect since it tends to prevent fatty acids from coming into close contact and establishing strong bonds when the low transition temperature is reached, and on the contrary to keep them associated at high temperatures.

Note: All cells, including the simplest ones like bacteria, are capable of regulating and adapting the lipid composition of their membranes according to environmental conditions in order to maintain optimal fluidity. For **example**, the growth temperature of *Escherichia coli* is normally 37°C; when cultured at 27°C, the relative amount of unsaturated hydrocarbon chains in its membrane lipids increases significantly, thus preserving fluidity.

B. Membrane proteins: Proteins represent approximately 50% of the membrane's weight.

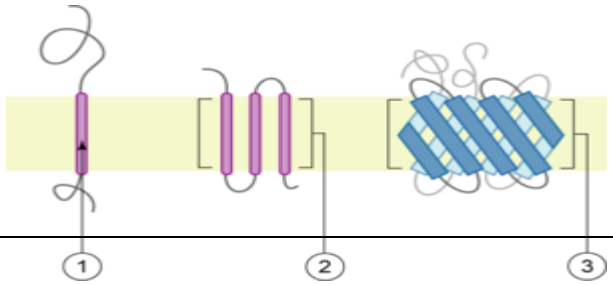
- **Extrinsic (peripheral) proteins**: either extracellular or cytosolic.
- **Intrinsic proteins**: either transmembrane, anchored in the inner leaflet, or anchored in the outer leaflet.

1. Integral (transmembrane) proteins: They span the membrane completely and interact with the hydrophobic part of the lipid bilayer. They are therefore intrinsic; their removal leads to membrane destabilization. These proteins possess:

- ✓ An **extracellular amino-terminal domain** (-NH2) often carrying carbohydrate residues.
- ✓ An intracellular **carboxyl end (COOH)** (see figure).
- ✓ A **hydrophobic body**, which interacts with the hydrophobic part of the lipid bilayer.

These proteins can be in the form of:

- ✓ **Single-transverse protein**: possessing a single helix or a single transmembrane segment.
- ✓ **Multi-crossing protein**: possessing several "**barrel**" segments (sometimes more than ten).



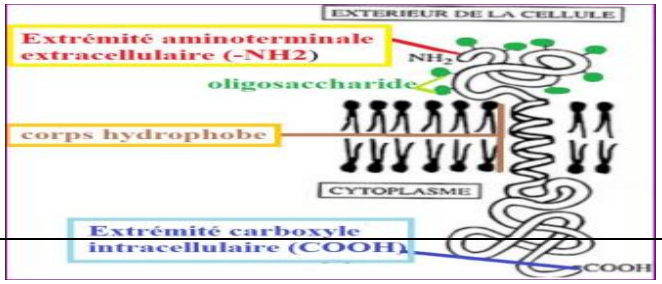


Figure 7: The different types of polytopic membrane proteins. (1) interaction via a transmembrane hydrophobic α-helix; (2) interaction via multiple transmembrane hydrophobic **α-helices**; (3) interaction via a transmembrane **β-barrel**.

2. Peripheral proteins: They are located on the extracellular, or cytosolic, membrane surface. They can be :

- ✓ **Extrinsic (surface) molecules**: detached from the membrane by a simple change in the ionic strength of the surrounding environment. They bind through primarily ionic interactions, either to the hydrophilic head groups of phospholipids or to the hydrophilic portions of proteins.
- ✓ **Intrinsic molecules anchored to the membrane by lipid anchoring**: their extraction requires destabilizing the hydrophobic interactions of the lipid bilayer.

3. Mobility or fluidity of membrane proteins: Some membrane proteins (especially integral ones) can move laterally within the lipid bilayer. This is achieved through the mechanism:

- ✓ Movement facilitated by lipid fluidity. Protein diffusion is limited by interactions with:
  - ❖ The underlying cytoskeleton.
  - ❖ Other membrane proteins.
  - ❖ Specific domains (lipid rafts).

Note: Proteins also have lateral movement, but more slowly. Some proteins have a more organized movement; they glide along cytoskeletal filaments thanks to cytoplasmic motor proteins attached to the inner leaflet of the plasma membrane. There is **no flip-flop** for proteins.

4. Functional importance of membrane proteins: They allow the aggregation or dispersion of receptors. They are essential for:

- ❖ Cell signaling, receptors, and intercellular interactions
- ❖ Membrane transport
- ❖ Adhesion/Cytoskeleton
- ❖ Respiratory chain (enzyme)

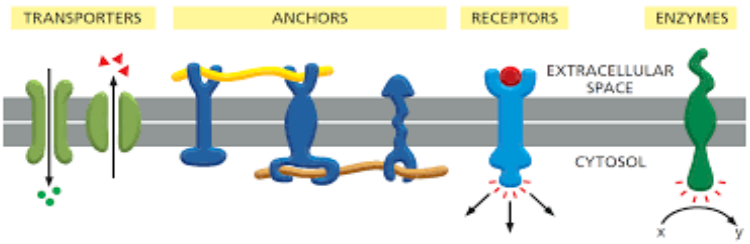


Figure 8 : Functional importance of membrane proteins

5. Anchoring of proteins to the membrane:

There are three main types of lipid-anchored proteins: **prenylated proteins**, **acylated proteins**, and **glycosylphosphatidylinositol** (**GPI**)-anchored proteins. Protein binding to GPI is mediated by a **GPI-transamidase** complex. The fatty acid chains of phosphatidylinositol insert into the outer leaflet of

the lipid bilayer of the plasma membrane. This type of protein plays a role in **embryogenesis**, **neurogenesis**, **development**, the **immune system**, and **fertilization**.

- **Pathologies**: Dysfunction of **GPI-anchored** proteins leads to sometimes serious diseases, the most important and classic of which is **Paroxysmal Nocturnal Hemoglobinuria** (**PNH**), characterized by the destruction of red blood cells (hemolysis), anemia, hemoglobinuria (dark urine), and a high risk of thrombosis. It is due to an acquired mutation of the **PIGA** gene (in hematopoietic stem cells). The **lack of CD55/CD59** in the **red blood cells** leads to an attack on the **complement system**, which induces hemolysis.

6. Membrane des érythrocytes : on trouve deux catégories principales :

a) Integral proteins (70%): Cross the membrane. Examples:

- ✓ **Band 3** (anionic exchange protein, AE1): ~25%. Transports HCO_3^-/Cl^-.
- ✓ **Glycophorin**: ~2-3%. Carries blood antigens (MN blood groups).

b) Peripheral proteins (30%): Attached to the submembranous cytoskeleton. Examples:

- ✓ **Spectrin**: ~60% of peripheral proteins. Maintains membrane elasticity and shape.
- ✓ **Ankyrin**: Links spectrin to band 3.
- ✓ **Actin**: Stabilizes the **spectrin-ankyrin** junction.

C. Membrane carbohydrates: Carbohydrates represent approximately 10% of the membrane's dry weight. They are found primarily in the form of glycoproteins and glycolipids.

- ✓ **Location and role**: Exclusively on the outer leaflet, forming the glycocalyx, which acts as a barrier against mechanical and enzymatic damage.
- ✓ **Key components**: Glycophorin (rich in sialic acid, imparting a negative charge to the surface of erythrocytes).
- ✓ **Glycolipids** (carrying ABO blood group antigens).
- ✓ **Cell recognition**: Carbohydrates serve as specific ligands for receptors (e.g., interactions with lectins).
- ✓ **Consequence**: Prevents cells from adhering to each other or to vessel walls through electrostatic repulsion.

7. Notion de microdomaine membranaire (radeau lipidique).

Membrane microdomains, or **lipid rafts**, are specialized regions of the plasma membrane enriched in specific lipids and associated proteins. They play a key role in cell signaling, membrane trafficking, and protein organization at the cell surface.

1. Characteristics of lipid rafts

a) Biochemical composition: Rich in sphingolipids and cholesterol, which give them a more ordered and less fluid structure than the rest of the membrane. They contain specific proteins such as caveolins, flottillins, and receptors involved in cell signaling.

b) Organization and structure:

- ✓ **Thickness**: Slightly thicker (~4–6 nm) than the rest of the membrane (~3–4 nm) due to the presence of long-chain sphingolipids.
- ✓ **Diameter:** From 10 to 200 nm, which makes them difficult to observe directly in conventional microscopy.

Membrane microdomains, or **lipid rafts**, are specialized regions of the plasma membrane, **enriched in specific lipids and associated proteins**. They play a key role in cell signaling, membrane trafficking, and the organization of proteins on the cell surface.

1. Caractéristiques des radeaux lipidiques

a) Biochemical composition: Rich in **sphingolipids** and **cholesterol**, which give them a more ordered and **less fluid structure** than the rest of the membrane. They contain specific proteins such as **caveolins**, **flottillins**, and **receptors** involved in cell signaling.

b) Organization and structure:

- ❖ **Thickness**: Slightly thicker (~4–6 nm) than the rest of the membrane (~3–4 nm) due to the presence of long-chain sphingolipids.
- ❖ **Diameter**: From 10 to 200 nm, making them difficult to observe directly using conventional microscopy.

2. Rôle fonctionnel :

- ❖ **Signaling platform**: Contains the receptors and proteins involved in cell communication.
- ❖ **Endocytosis and exocytosis**: Participates in the transport of certain molecules and pathogens.
- ❖ **Interaction with the cytoskeleton**: Binds scaffold proteins and stabilizes membrane domains that play a role in regulating cell signaling pathways.

3. Types of membrane microdomains:

- ❖ **Caveolae:** Invaginations rich in caveolin, playing a role in endocytosis and signal transduction.
- ❖ **Lipid plaques**: More rigid structures involved in anchoring specific proteins.

4. Biological importance: Lipid rafts are involved in several physiological and pathological processes, such as immune signaling, the response to viral infections (e.g., HIV virus which uses these rafts to enter cells).

8. Identification et localisation des spécialisations morphologiques de la membrane des Ⅽs polarisées (entérocytes, Ⅽs rénale, Ⅽs de l'épididyme).

Polarized cells have an asymmetric organization of their plasma membrane, with specialized domains depending on their role in absorption, secretion, or transport.

These specializations are found primarily in enterocytes (intestine), renal cells (renal tubules), and epididymal cells (male reproductive system).

1. Enterocytes (small intestine): Enterocytes are absorptive cells lining the epithelium of the small intestine.

a) Specializations

- ❖ **Apical pole** (facing the intestinal lumen):

- **Microvilli**: Increase the surface area for nutrient absorption.
- **Glycocalyx**: Covers the microvilli and contains digestive enzymes (e.g., lactase, sucrase)..
- **Tight junctions**: Maintain the seal between cells and regulate the passage of molecules.

b) Basolateral pole (facing the blood and connective tissue):

- **Adherent junctions and desmosomes**: Ensure cohesion between cells.
- **Membrane pumps and transporters**: Allow the passage of nutrients into the bloodstream.

2. Renal cells (renal tubules): The cells of the renal tubules play a key role in the reabsorption of nutrients and the filtration of waste.

a) Specializations

- **Apical pole** (**facing the tubule lumen**):
- **Microvilli (brush border**): Found mainly in the proximal tubule, they increase the surface area for the reabsorption of water, glucose, and ions.
- **Cadherins and tight junctions**: Limit the passive diffusion of molecules between cells.

b) Basolateral pole (facing the capillaries):

- **Na+/K+ pumps and ion channels**: Maintain ionic balance and ensure the passage of nutrients into the blood.
- **Membrane invaginations**: Increase the surface area for exchange with the blood capillary to optimize ion exchange.

3. Epididymal cells (male reproductive system): These cells ensure the maturation and storage of sperm in the epididymis.

a) Specializations

- **Apical pole** (facing the lumen of the epididymal duct):
- **Stereocilia**: Long, non-motile cytoplasmic extensions that increase the surface area in contact with the surrounding fluid to absorb secretions and nourish the sperm.
- **Tight junctions**: Maintain the blood-epididymal barrier and protect the sperm from immune attacks.

b) Basolateral pole (facing the blood vessels):

- **Membrane transporters**: Involved in ion exchange and the modification of the composition of the epididymal fluid.
- **Gap junctions**: Ensure communication between adjacent cells.

9. Ultrastructure of apical (microvilli) and laterobasal (invagination, zonulaoccludens, zonulaadherens, maculaadherens, Gap) specializations

Polarized cells exhibit apical and laterobasal membrane specializations that enable specific functions such as absorption, filtration, adhesion, and intercellular communication.

1. Apical specializations: Apical specializations concern the free surface of the cell, which is often in contact with an extracellular environment (e.g., intestinal lumen, renal tubules, epididymis).

- **Ultrastructure of microvilli**:
- Axes composed of **actin filaments** linked to the membrane by binding proteins (fimbrin, villin, fascin).
- Terminal **actin network anchored to the basement membrane** via intermediate filaments.
- Presence of a **glycocalyx** covering the microvilli, containing digestive enzymes and receptors.

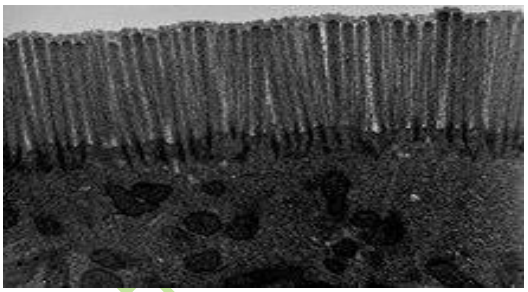


Figure 9 : Microvilli on a section of human jejunum under transmission electron microscopy.

2. Laterobasal Specializations: Laterobasal specializations allow cell adhesion and communication within epithelial tissue.

a) Zonula occludens (**Tight Junctions**): These junctions provide a barrier between adjacent cells. Tight, impermeable, or occlusive junctions (called zonula occludens) are also known as **zonula occludens**.

- **Ultrastructure**: Composed of transmembrane proteins (claudins, occludins, JAMs) forming a network of grooves and ridges visible under electron microscopy.
- Anchored to the **cytoskeleton** via adaptor proteins (ZO-1, ZO-2, ZO-3) connected to actin filaments.
- **Maintaining cell polarity** by separating the apical and basolateral domains.

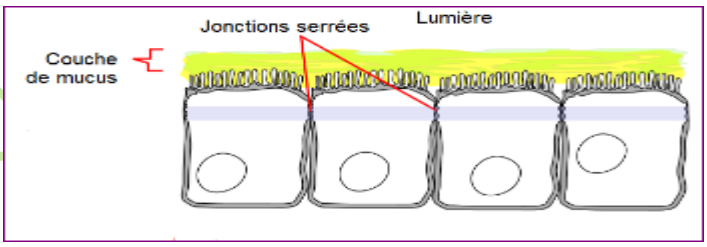


Figure 10 : Polarity of epithelial cells

b) Zonula adherens (Adhesive junctions) :These junctions ensure intercellular adhesion and anchoring of the cytoskeleton.

- **Ultrastructure**:
  - **Transmembrane proteins**: cadherins (E-cadherin) interacting with those of adjacent cells.
  - Submembrane plaque containing anchoring proteins (catenins, vinculin, α-actinin).
  - Association with actin filaments, forming a contractile belt.

- Participation in cell signaling and maintenance of tissue architecture.

c) Macula adherens (Desmosomes) : Desmosomes are very strong anchoring junctions, particularly abundant in tissues subjected to mechanical stress (epidermis, heart).

- **Ultrastructure**:
  - **Dense cytoplasmic plaque:** containing binding proteins (plakoglobin, desmoplakin).
  - **Transmembrane proteins**: desmogleins and desmocollins interacting between cells.
  - **Anchoring to intermediate filaments** (keratin or desmin depending on the cell type).
  - **Resisting physical stress**.

d) Gap junctions (Communicating junctions) : These are intercellular channels that allow the direct passage of ions and small molecules between adjacent cells.

- **Ultractructure**:
  - Composed of connexons (assemblies of 6 connexins).
  - Visible under electron microscopy as pitted plaques.
  - **Role**: Rapid intercellular communication. Transmission of electrical and metabolic signals (e.g., cardiac cells, neurons).

e) Basal invaginations: are folds of the basement membrane increasing the exchange surface with the underlying connective tissue.

- **Ultrastructure** :
  - Presence of numerous mitochondria aligned within the invaginations.
  - Association with Na+/K+ ATPase ion pumps for active transport.

Role : Facilitation of ionic and metabolic exchanges. And optimization of active transport (e.g., cells of renal tubules, exocrine glands).

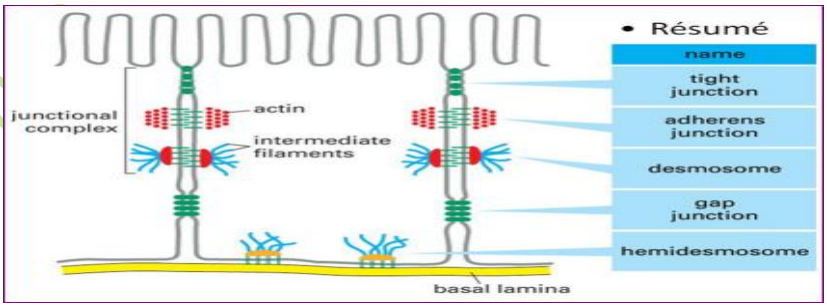


Figure11 : Representation of an epithelial cell connected to adjacent cells by the three main types of junctions: tight junctions, desmosomes, and open junctions.

10. Comparison of the molecular compositions and functions of junctional devices in different cell types (presented in tabular form).

Junction type	Main molecular composition	Fonction	Cell types involved

Junction type	Main molecular composition	Fonction	Cell types involved
Tight junctions (Zonula occludens)	Claudines, Occludines, JAMs, Protéines ZO-1, ZO-2, ZO-3	Assurent l'étanchéité entre Cs, empêchent la diffusion paracellulaire, maintiennent la polarité cellulaire	Épithéliums intestinaux, endothélium vasculaire, barrière hémato-encéphalique
Adhesive junctions (Zonula adherens)	Cadhérines (E-cadhérine), Caténines, Vinculine, α-actinine	They stabilize intercellular contacts and participate in cell signaling	Épitheliums, Cs endotheliales, cardiomyocytes
Desmosomes (Macula adherens)	Desmogléines, Desmocollines, Plakoglobine, Desmoplakine, Filaments intermédiaires (Kératine ou Desmine)	Cohesion and mechanical resistance to stress	Épiderme, Cs musculaires cardiac
Hémidesmosomes	Intégrines (α6β4), Plectine, Laminine 5, Filaments intermédiaires	Anchoring of the Cs to the basal plate	Keratinocytes, Cs epitheliales
Jonctions communicantes (Gap junctions)	Connexines (Cx26, Cx43, etc.), Connexons	They allow the passage of small molecules and ions, coordinating signals	cardiac Cs, neuron, Cs smooth muscul, hepatocytes

This table highlights the diversity of cell junctions according to their molecular composition and their functional role in tissues.

Congratulations and thank you for taking the time to read my course! Your dedication to learning is truly appreciated, and I hope it brings you valuable knowledge and success. Keep up the great work!. *Dr. Bouldjedri M.*

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