CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

5

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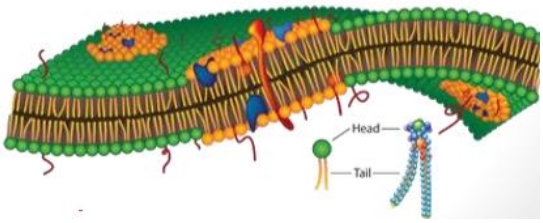
➢ Lipid Rafts

- Lipid rafts are aggregates (accumulations) of sphingolipids.
- They're semisolid clusters (10-200 nm) of cholesterol and sphingolipids (sphingomyelin and glycolipids). They also contain proteins as well as some types of phospholipids, specifically (phosphatidylinositol) that makes up the glycolipid glycosylphosphatidylinositol (GPI) which anchor membrane proteins.
- Proteins present in the lipid raft, which are anchored to the membrane by the glycolipid glycosylphosphatidylinositol (GPI), are involved in signal transduction and intracellular trafficking.



➢ Functions of lipid rafts :

Lipid rafts are ,relatively, newly discovered and their functions are still not completely understood. Most of their discovered functions are related to disease.

- **In normal situations :**

  Proteins that are present as a part of the lipid raft mostly contribute to membrane trafficking (transport of certain molecules in and out) and signal transduction (receptors related to certain different signaling pathways).

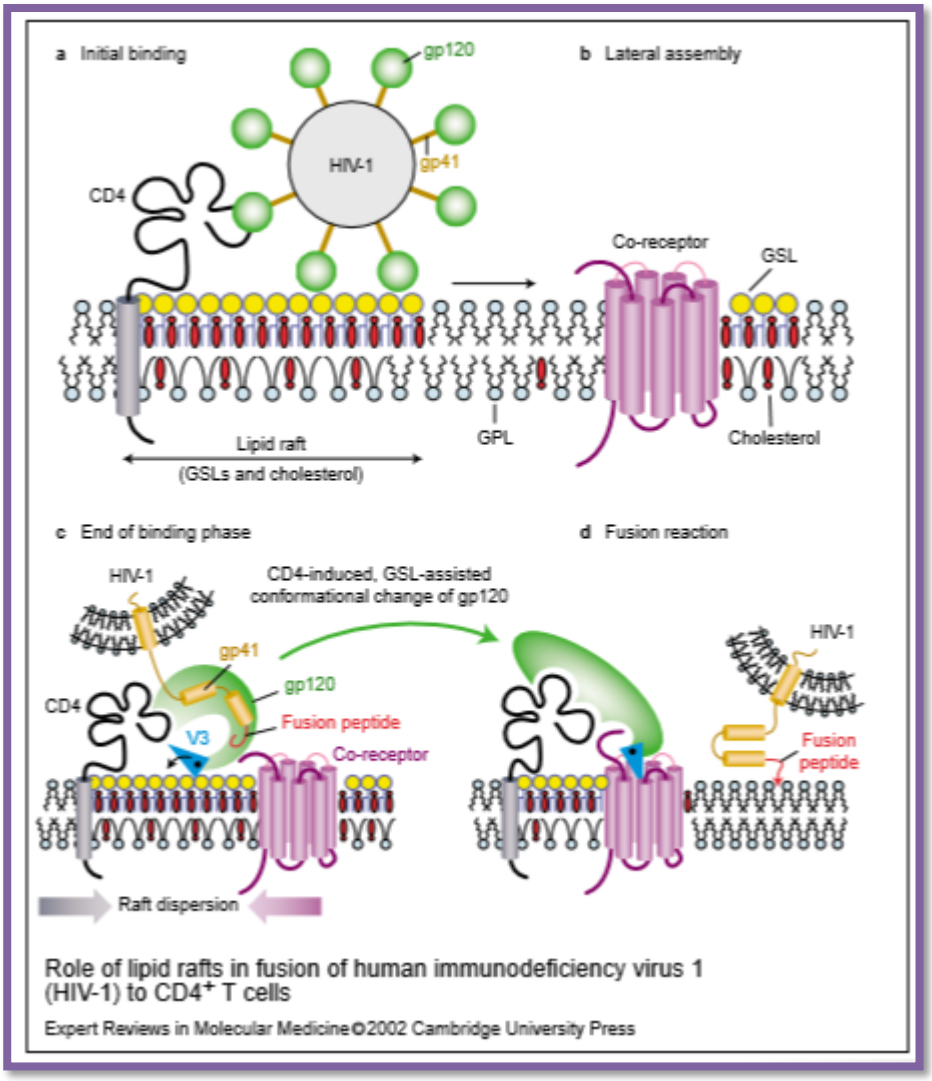
- **In abnormal situations:** they contribute to viral infections as they facilitate the invasion of a virus to the cell. For example :

1) : HIV Virus

- ✓ Budding may occur from lipid rafts.
- ✓ Viral fusion to $CD4^+$ T cells
- ✓ T cells (a type of lymphocyte that plays a central role in the immune system) are the target of the HIV Virus.
- ✓ In the membrane of a T cell there's a protein called **CD4** which functions as a receptor of the HIV Virus. **CD4** has **an extracellular part.**
- ✓ The spikes of HIV Virus are glycoproteins coming out of the capsule of the virus.
- ✓ HIV Virus contains two types of glycoproteins : **1.glycoprotein 120. 2.glycoprotein 41.**

- ✓ The fusion of the HIV Virus with the membrane happens through the glycoproteins (Virus' spikes). Viruses don't have their own machines, so they infect (invade) a cell to take over its metabolic machinery and reprogram it to produce more copies of the virus.
- ✓ The **spikes** come in close proximity to the **CD4** receptor. The binding of the virus with the **CD4** receptor isn't enough to infect a T cell. Another component is needed to facilitate the fusion which is a co-receptor called **CCR5**. The co-receptor **CCR5** isn't part of the lipid raft.
- ✓ **CCR5** is going to move until it reaches the lipid raft and becomes part of it. Once it's inside the raft, it becomes in close proximity to the virus, then the glycoproteins **(120,41)** can fuse with **the co-receptor**.
- ✓ Once the HIV Virus is bound (fused) with both the receptor **CD4** and the co-receptor **CCR5**, it infects (invades) the T cell.

Note :



Role of lipid rafts in fusion of human immunodeficiency virus 1 (HIV-1) to $CD4^+$ T cells

Sphingolipids of the lipid raft are represented in **red.**

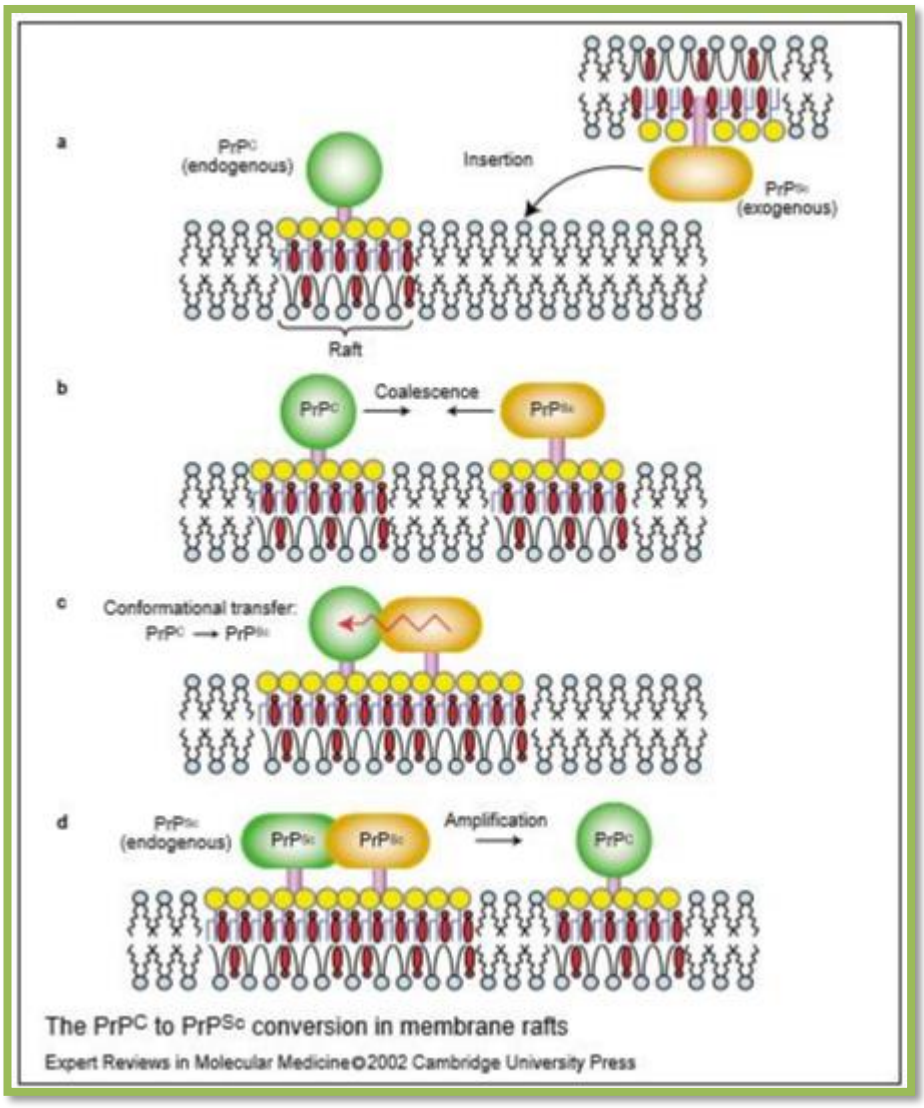
2) Influenza Virus :

- ✓ Raft-associated glycoproteins in envelope.
- ✓ Very similar to the invading mechanism of the HIV Virus.
- ✓ The glycoproteins of the lipid raft interact with the glycoproteins of the Influenza Virus, which makes the virus in very close proximity to the cell membrane.
- ✓ This proximity creates some sort of tension which facilitates the fusion of the virus with the cell membrane rather than their salvation (parting away).

3) Prion disorder :

- ✓ Prion stands for : proteinaceous infectious particle.
- ✓ Normal prion protein (PrPc) is converted to abnormal proteins (PrPsc) in lipid rafts.
- ✓ The protein as a molecule, like viruses and bacteria, is going to infect and cause changes.
- ✓ The lipid rafts in our membranes contain **normal prions(PrPc).**
- ✓ Prion disorder is related to mad cow disease. Infected cows have abnormal membrane proteins **(PrPsc)**, which are misfolded proteins.
- ✓ The abnormal prion has a three-dimensional shape that differs from the shape of the normal one (for example : instead of having a Beta-Pleated sheet, it could have an alpha-helix). Notice that in the figure shown below, **the normal prion** is rounded while **the abnormal prion** is oval.
- ✓ When we eat an infected cow that's not cooked well, **the abnormal prion** in the mad cow cell will fuse with a lipid raft in the membrane of a normal cell in our body.
- ✓ **The normal prion** is inserted into a raft structure and the abnormal prion is inserted into **another** raft structure. The two lipid rafts are going to move closer and fuse with each other.
- ✓ **The normal prion** and **the abnormal one** become in close proximity which enables **the abnormal prion** to infect the normal prion.
- ✓ **The abnormal prion** converts **the normal prion** into an abnormal one. The prion loses its structure as it becomes misfolded which means that it loses its function as well.
- ✓ After that, there will be amplification. There are 2 abnormal prions. The newly infected prion will infect a normal prion and the (cow prion) will infect another normal prion resulting in 4 abnormal prions.

- ✓ The total number of abnormal prions (resulting by the infection of 2 abnormal prions) will be equal to 2^n , n: how many times infection (by 2 abnormal prions) has occurred.

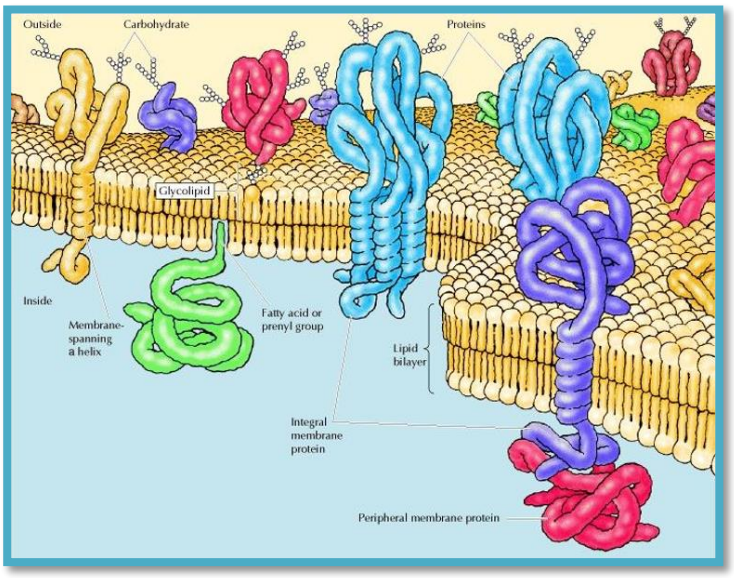


- ✓ How does this appear on an infected person?

  Their neural cells (brain cells) become sponge-like structures. When the brain loses its structure, it loses its function as well. It'll cause Bovine Spongiform Encephalopathy (BSE) or more commonly known as "mad cow" disease.

- ➢ **In conclusion: Lipid rafts bring molecules closer to each other which facilitates their fusion.**

➢ Membrane Proteins

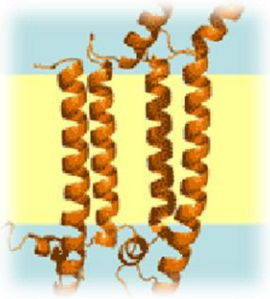


- Proteins are present in various structures, functions and shapes. Some proteins are anchored to the membrane.
- Membrane proteins are divided into :
  1) Integral proteins (transmembrane proteins).
  2) Peripheral proteins.
  3) Lipid-anchored membrane proteins.

Integral proteins :

- ✓ Portions of integral membrane proteins are inserted into the lipid bilayer. They are dissociated by reagents of small amphipathic molecules.
  - The hydrophobic portions of detergents disrupt hydrophobic interactions.
  - The hydrophilic part makes the detergent-protein complexes soluble in aqueous solutions.
- ✓ Depending on their function, some membrane proteins are anchored to the outside leaflet and others are anchored to the inner leaflet of the membrane.
- ✓ Some membrane proteins have sugars (Carbohydrates) attached to them. They're called glycoproteins.
  - Integral proteins have both hydrophobic and hydrophilic regions.
- ✓ The portion that passes through the membrane passes through the hydrophobic region. In order to have a stable structure, the amino acids making up that part are expected to be non-polar. (If a hydrophilic amino acid comes in contact with a hydrophobic environment the resulting structure will be unstable).
- ✓ The hydrophobic tails of the phospholipid bilayer are in very close proximity to the hydrophobic region(s) of the membrane proteins.
- ✓ The portions that are exposed to the cytosol or to the extracellular fluid tend to be hydrophilic. These portions are made up of polar amino acids since both the cytosol and the ECF have aqueous solutions.

- Alpha-helices vs. Beta-pleated sheets.

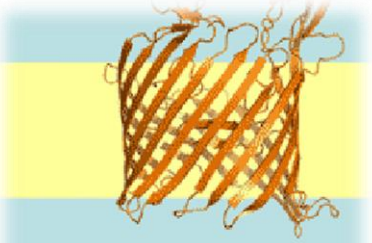


✓ The parts that pass through the membrane are mostly helices. A protein could have one or more helices embedded in the membrane.

✓ In order for an alpha-helix to pass (span) through the whole membrane, it needs to be composed of (20-25) hydrophobic amino acids. They are usually glycosylated with the oligosaccharides exposed on the outer surface of the cell.

✓ Even though it's rare, but there's a possibility to find a Beta-pleated sheet spanning the membrane.

✓ When embedded in the membrane, Beta pleated sheets form barrels called Beta Barrels. They're hollow from the inside for the passage of molecules. They serve as transporters. For example : porins.



Beta-barrel (example: porins)

- Hydrophilic amino acids might be present within the part that spans (passes) the membrane.

✓ As mentioned before, the parts that span the membrane must be hydrophobic to have a stable structure. However, if it's a channel protein that provides a hydrophilic passageway for water or small polar ions, there should be hydrophilic parts spanning the membrane.

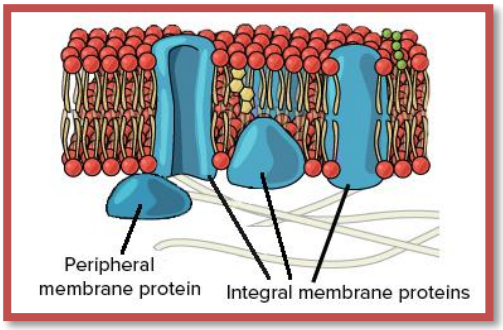
✓ Alpha-helices making up the channel protein have two sides, a hydrophilic side and a hydrophobic one. The side that faces the hydrophobic tails is hydrophobic and the side that faces the inner side of the channel is hydrophilic.

✓ The R-groups (side chains) of amino acids facing the inner side of the channel are polar while the R-groups facing the hydrophobic tails are non-polar.

✓ Integral proteins are very hard to study because if you take them out of the membrane and expose them to different environmental factors they might denature and lose their structure. If their structure is lost then so is

their function. Therefore, in order to study their structure and function well they must remain attached to the membrane.

Peripheral proteins :

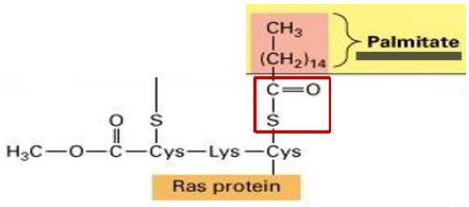


- ✓ Peripheral proteins are associated with either the inner leaflet or the outside leaflet of the membrane. They can be dissociated from the membrane following treatments with polar solutions of extreme pH or high salt concentration.
- ✓ Peripheral proteins are not embedded in the phospholipid bilayer, instead they're exposed to aqueous solutions either from the cytosol or the extracellular fluid. Therefore, the amino acids making up their outer surfaces must be polar.
- ✓ Once dissociated, they are soluble in aqueous buffers.
- ✓ They're indirectly associated with membranes in different ways through **non-covalent** protein-protein interactions, mainly ionic bonds.

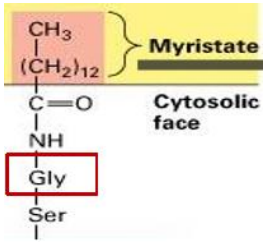
Lipid-anchored membrane proteins :

- ✓ Proteins anchored to the inner or to the outer leaflet of the membrane.
- ✓ They're linked to the phospholipid bilayer by being **covalently** bonded to a lipid.

- Types of anchors :



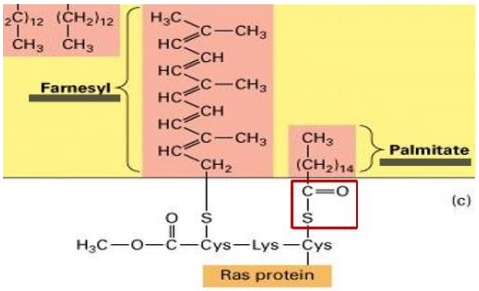
1) Palmitoylation :
   - ✓ Palmitate (Palmitic Acid) is a fatty acid which contains a hydrophobic tail.
   - ✓ Its hydrophobic tail interacts with the (-SH) of the R-group of the final cysteine near the **C-terminus** of the protein.
   - ✓ It attaches proteins to the **inner** surface of the membrane.
   - ✓ Ras protein is anchored to the membrane in this way.



2) Myristoylation :
   - ✓ Myristoyl group is a long hydrocarbon chain that's attached to an amino acid (for example : Glycine) on the **N-terminus** end of the protein.

- ✓ They anchor proteins on the **inner** surface of the membrane (facing the cytosol).

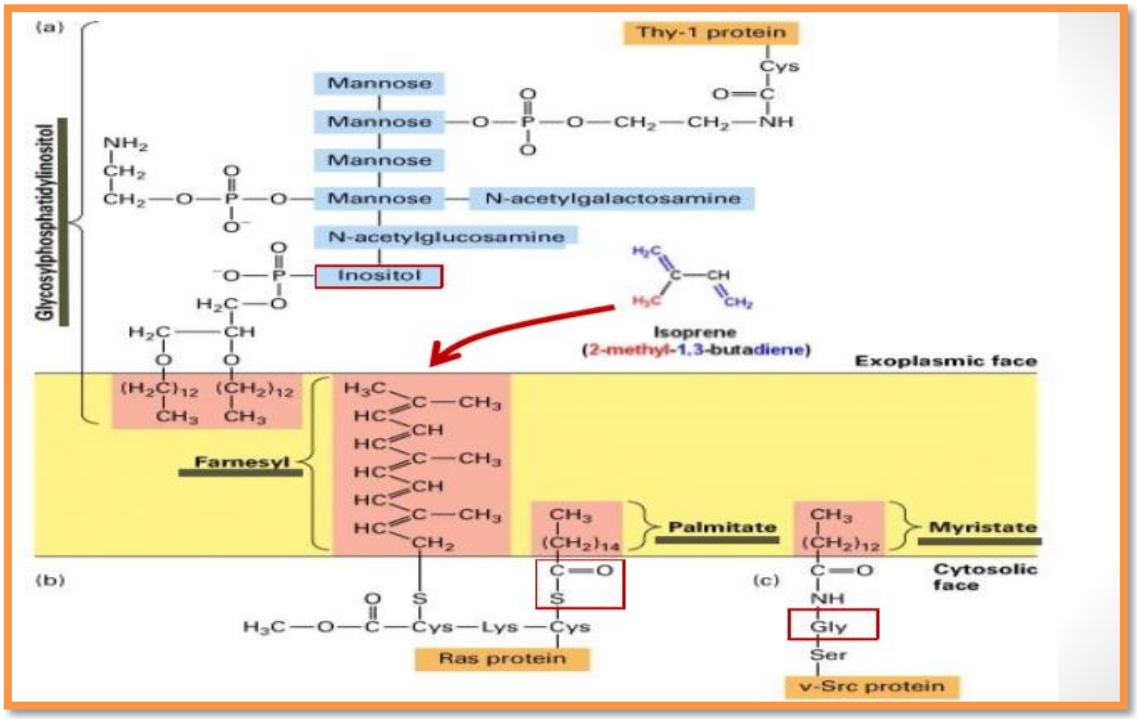
3) Prenylation (Farnesylation):



- ✓ Long hydrocarbon chain consisting of repeated isoprene groups (5 carbons) forming a farnesyl structure.
- ✓ Prenyl group is attached to –SH group of cysteine near **C-terminus** end of proteins (Covalent bonding).
- ✓ They anchor proteins on the **inner** surface of the membrane (facing the cytosol).
- ✓ A covalent bond is formed between the protein and the anchor so the protein can't be easily removed.

4) GPI Anchors :

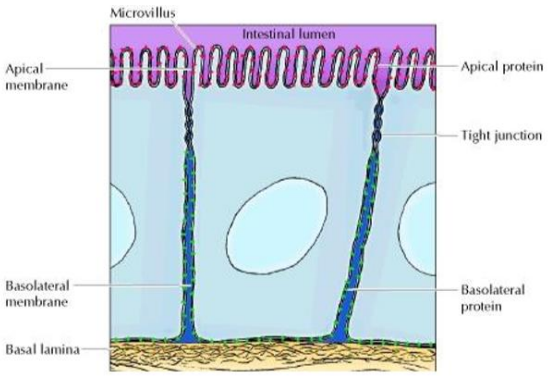
- ✓ The carbohydrate bridges the protein with the fatty acid chains of the phospholipid (usually ethanolamine).
- ✓ Glycosylphosphatidylinositol is a glycolipid that contains inositol sugar. Inositol sugar interacts with sugars that connect the protein to the **outside** leaflet of the membrane. (facing the extracellular matrix).
- ✓ GPI anchors are added to the **C-terminus** of a protein in the ER.



➢ Protein Mobility :

- ✓ Proteins, like lipid molecules, move within the membrane.
- ✓ Protein mobility is important regarding to polar cells.

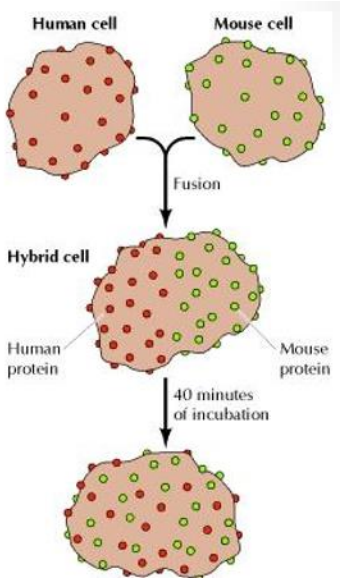
- ✓ Polar cells : cells that have two sides (2 poles). For example: intestinal cells have an apical surface (brush border) and a basolateral surface. They have a basolateral side and an apical side.



- ✓ Glucose molecules move from the lumen of the small intestine to the blood stream (blood vessels). The movement of glucose should always go in that direction. Glucose moves from the brush border (of intestinal cells) to the basolateral surface and then to blood vessels.
- ✓ The channel protein on the brush border allows the entry of glucose molecules into the cell and another channel protein on the basolateral surface allows the exit of glucose to the blood stream.
- ✓ The membrane must be separated into two parts; the apical side (where the apical proteins can move) and the basolateral side (where the basolateral protein can move) in order to direct the movement of glucose in that direction.
- ✓ The apical side and the basolateral side are separated by certain mechanisms such as tight junctions.
- ✓ Tight junctions connect cells. They're very firm and rigid and they don't allow the passage of any molecule. That's why they are efficient in separating the apical surface from the basolateral surface.
- ✓ In non-polar cells any type of protein can be found at any time and on any side or area of the plasma membrane.

- The experiment that confirms the movement of proteins within the membrane :

1. The proteins of one cell were labeled in green while the proteins of another cell were labeled in red.
2. The membranes of the two cells were then fused together.



➢ Result of the fusion : the red proteins were found everywhere as well as green proteins.

- The mobility of membrane proteins is restricted by :

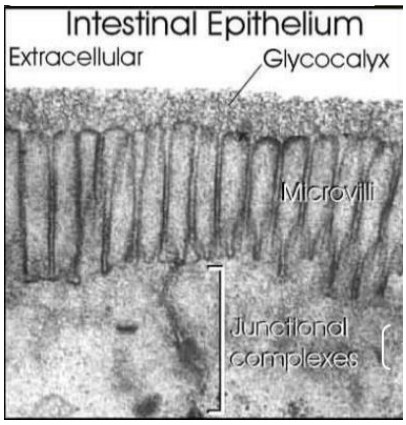
✓ Association with the cytoskeleton, ECM proteins, proteins on the surface of adjacent cells.

✓ Specific membrane domains such as tight junctions, that maintain the spatial distribution of apical and basolateral proteins.

✓ Lipid composition (lipid rafts rich in GPI anchored-proteins) restrict protein mobility.

➢ Glycocalyx :

- Carbohydrates are present in the membrane either as glycoproteins (attached to proteins) or as glycolipids (attached to lipids).
- Carbohydrates are found on the outer surface of the cell membrane facing the extracellular matrix. They are hydrophilic molecules.
- Sugars form a layer (coat) that covers the surface of the cell. This carbohydrate coat is called Glycocalyx. It's formed by the oligosaccharides of glycolipids and transmembrane glycoproteins.
- Glycocalyx has a protective function. It protects the cell by separating its membrane from viruses and making it harder for them to invade cells. Viruses first need to release enzymes that can digest these sugars in order to get to the membrane.



- Other functions including :
  1) Cell-cell interactions (leukocytes).
  2) Protection of cell surface from ionic and mechanical stress.
  3) Acts as a barrier for microorganisms.