◉ Sheet ○ Slides

Number

1

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This sheet includes the first lecture and the first 17 minutes of the second lecture.

Cell biology is a branch of biology that studies the different structures and functions of the cell and focuses mainly on the idea of the cell as the basic unit of life. Cell biology explains the structure, organization of
the organelles they contain,
their physiological properties, metabolic processes, signaling pathways, life cycle, and interactions with their environment.

Cell membrane:

The cell membrane is best described by the fluid mosaic model, which represents the structure of a cellular membrane as a lipid bilayer irregularly interspersed with protein. The major lipid components are phospholipids and sphingolipids. Phospholipids can rotate and move laterally. Cholesterol, which controls the fluidity of membrane, is also found in animal cells.
Note: In plant cells and bacteria, we have *sterols* instead of cholesterol.

A- Membrane proteins:

Proteins are also found in the cell membrane. They can either be integral, which span the whole membrane (the two leaflets) or just one leaflet; or peripheral, which attach to the membrane either by lipid anchors or directly to some proteins.

- **Integral proteins**

The interior of the plasma membrane of the cell is **hydrophobic**, so accordingly, membrane-spanning portions of transmembrane proteins are usually α-helices of 20-25 **hydrophobic** amino acids, while ECM-facing and cytosol-facing portions are hydrophilic. In other words, imagine we have three layers on top of each other: the two outer layers are hydrophilic, and the inner one is hydrophobic. Most of the integral proteins' secondary structures have alpha-helices, but that doesn't mean there are no beta sheets. Integral proteins are usually glycosylated with the oligosaccharides exposed on the outer surface of the cell. 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.

- **Peripheral proteins:**

  Are proteins that dissociate from the membrane following treatments with polar solutions of extreme pH or high salt concentrations. Once dissociated, they are soluble in aqueous buffers. Peripheral proteins are anchored either by other proteins or lipid anchors. There are four types of lipid anchors:

1-**Myristoylation** (myristic fatty acid): the myristoyl group of the lipid is attached to the N-terminus of the protein. Ex: src protein

2-**Palmitoylation** (palmitate fatty acid): Palmitate is added to the –SH group of the side chains of *internal* cysteine residues. Ex: RAS protein

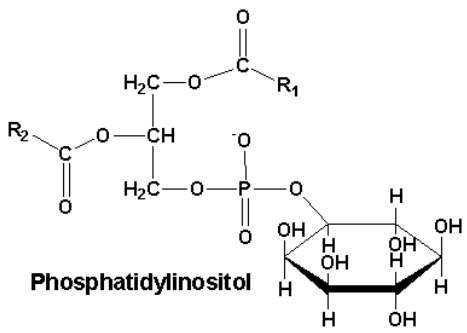
3-**Prenylation** (farnesylation): It refers to the linking of "isoprene"-based groups. The prenyl group is attached to the –SH group of cysteine *near C-terminus* of proteins.

4-**Glycosyl phosphatidylinositol** (GPI): The GPI system anchors proteins on the **outer** surface of the plasma membrane. It consists of phosphatidylinositol, carbohydrates and ethanolamine. We normally have phosphatidylinositol embedded in the plasma membrane. Its inositol group is linked to carbohydrates which are also linked to ethanolamine. The ethanolamine of the GPI attaches to the C-terminus of the peripheral protein. The two fatty acids of phosphatidylinositol are responsible for anchorage to the cell membrane.

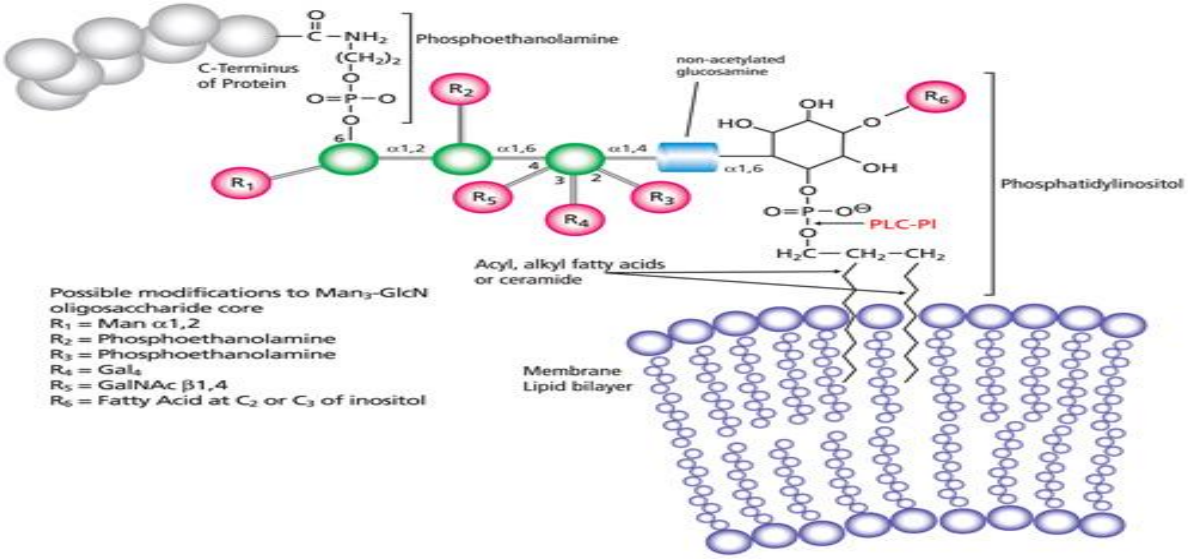
So to sum up:

The GPI system is composed of:

1-**Phosphatidylinositol** (*its two fatty acids anchor the system to the membrane*)
2-**Carbohydrates** attached to the inositol group of phosphatidylinositol.
3-**Ethanolamine** attached to the carbohydrates from one side and to the peripheral protein from the other side.



Have a look at this structure for better understanding:



-*Application*: Farnesylation inhibitors and disease treatment

RAS is an oncogene that needs farnesylation for its function and oncogenic activity, so by inhibiting the enzyme that does the farnesylation, which is *farnesyl transferase*, we can inhibit the action of RAS. Unfortunately, these inhibitors failed in human clinical trials because:

1- The anti-tumor inhibitors (farnesyl transferase inhibitors) don't act on RAS isoforms (N-RAS, K-RAS) and their tumorigenic activity.
2- Proteins other than mutated RAS need to be farnesylated in order to function; so inhibiting farnesyl transferase will result in losing their normal functions.

B- Lipids:

- **Phospholipids:**

Different types of phospholipids and sphingolipids have different types of head groups and a different composition of fatty acids as well; these differences provide a variety of functions. These different types of phospholipids aren't equally distributed between the two leaflets; some are found mainly in the inner leaflet, while others are found mainly in the outer leaflet (but that doesn't mean they are restricted to one leaflet).

The major components for each are:

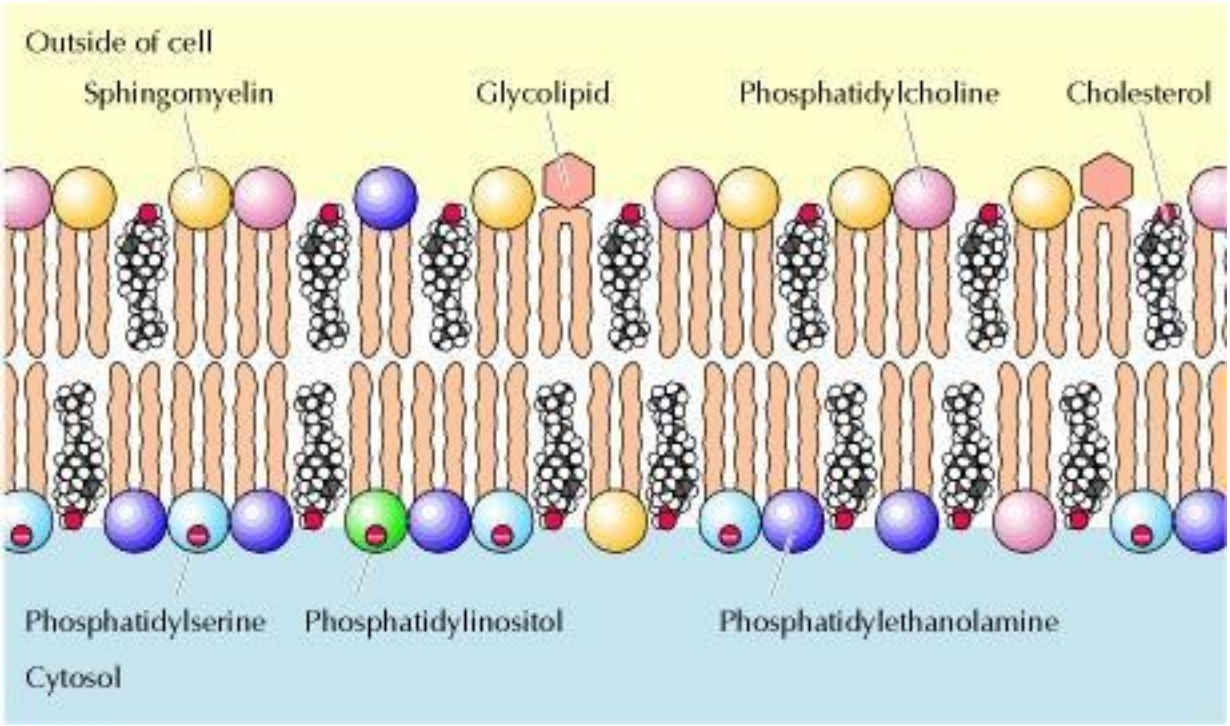
The outer leaflet: Phosphatidylcholine, sphingomyelin, glycolipids and phosphatidylinositol*.

The inner leaflet: Phosphatidylethanolamine, Phosphatidylserine and Phosphatidylinositol (minor)*.
**Note: P inositol is more abundant in the outer leaflet.*

P inositol has a role in cell signaling, cell junctions and endocytosis. The head groups of P inositol and P serine are negatively charged, thus the cytosolic face of the membrane has a negative charge compared to the extracellular face.

The concentrations of phospholipids aren't the same between

i. inner and outer surfaces
ii. one cell type and another, and
iii. surprisingly, between different regions of the same plasma membrane. These surprising differences in phospholipid concentrations are transient, and arise in response to some biological activities such as forming vesicles and in the leading region of the membrane (when the cell is about to move, it forms a leading region). The overall percentage of the cell membrane components is 50% lipids and 50% proteins.



- **Sphingolipids:**

Sphingolipids are also a component of the cell the membrane; they gather in structures called lipid rafts, which are semisolid clusters (10-200 nm) made of sphingolipids (sphingomyelin and glycolipids) with high concentrations of cholesterol. They are also enriched with a specific type of phospholipid called **phosphatidylinositol** that acts as one of the lipid anchors (remember: one of the two mechanisms used in anchoring peripheral proteins), also known as **Glycosylphosphatidylinositol anchors (GPI anchors)**. That's why we have high concentrations of phosphotidylinositol in these rafts.

These rafts move as a single unit. They have different functions; one of them is being able to mediate viral and prion infections. Lipid rafts are also enriched with proteins involved in signal transduction and intracellular trafficking.

Note: Prions are proteinaceous infectious particles.

To sum up:

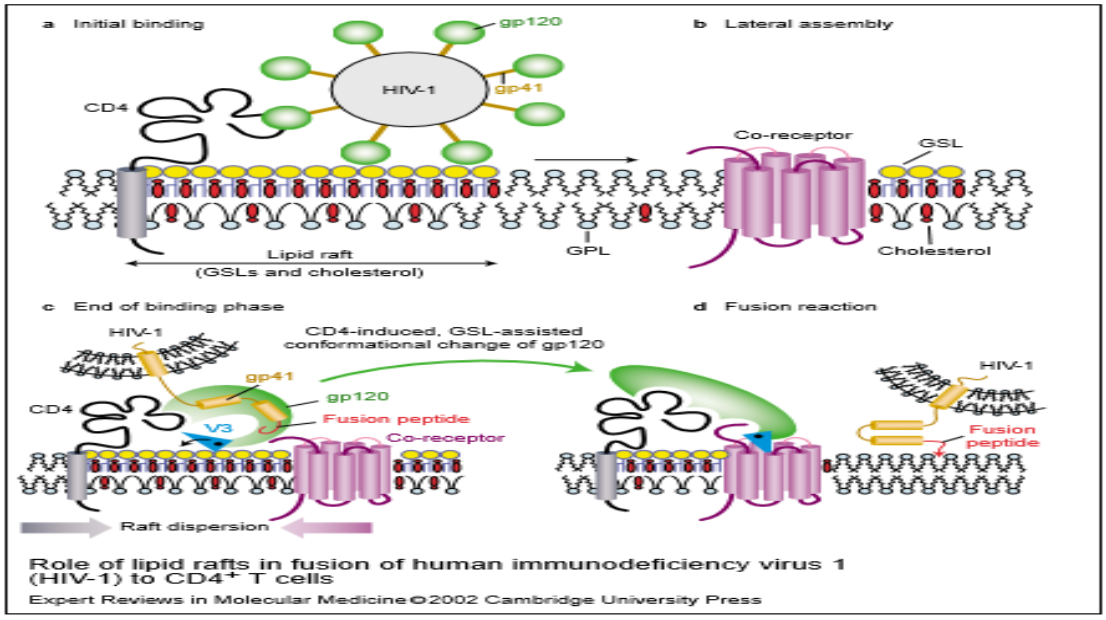
Lipid rafts are made of three components:

1- Clusters of Sphingolipids.
2- Phosphatidylinositol (part of the GPI anchoring system).
3- Proteins involved in signal transduction and intracellular trafficking.

Lipid rafts exist in both leaflets; the outer portion is more important in terms of function. Their normal function is not clear yet, but they are related to some diseases (diseases mediated by lipid rafts)**:**

1- HIV (infects CD4+ T cells):

GP120 (glycoprotein) of the virus will interact with the *polar heads of the sphingolipids that form the lipid rafts*. GP41 is another component of the viral glycoprotein. The terminal end of the GP120 has a fusion peptide on its top that facilitates the fusion of the virus with the membrane. When the virus is in close proximity, the fusion peptide binds to a molecule called a co-receptor then the fusion happens.



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

2- **influenza virus:** fusion happens in the same mechanism but with different glycoproteins.

3- **prion disease:** Prions are misfolded proteins that cause infection by aggregation. Prions are obtained by ingestion of infected meat; after ingestion, they get inserted into a lipid raft of a certain membrane, and *under the influence of the rapid movement of the raft*, they become close to a normal protein transforming it into an infectious prion, then these two prions reach another normal protein and so on….

Note:

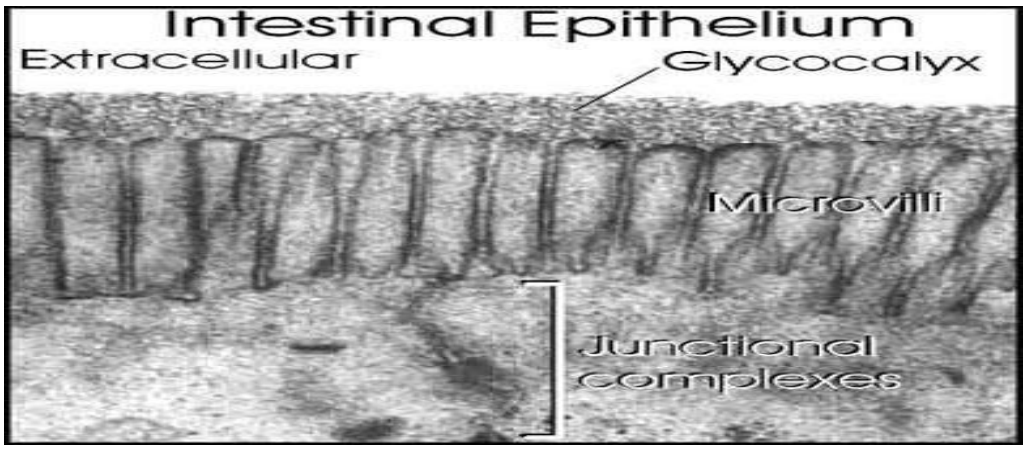
-*PrPc*: normal protein

-*PrPsc*: infectious (abnormal) protein.

So this is how lipid rafts facilitate some diseases, but surely they have good functions. (Not discovered yet)

Most membrane proteins are glycoproteins, which means they have a sugar moiety facing the outside of the cell, and these moieties together form a region (layer) around the cell called a glycocalyx, and their function is to protect the cell from infections. In the pathogenesis of a certain virus for example, the virus has to achieve a close proximity to the membrane in order to fuse, but with the presence of these molecules (glycocalyx) the virus must digest them before reaching the membrane. Another function of

the glycocalyx layer is facilitating cell-cell interaction and antigenic recognition (ABO blood system, antigens of viruses….etc). It also protects the cell from ionic and mechanical stresses.



-Protein mobility:

Membrane proteins move through the membrane, but their movement is restricted, especially when we are talking about polarized cells (e.g. epithelial cells of the intestines), which have an apical surface which gets the substances in the cell and a basolateral surface which moves these substances towards the gut stream. As such, we have certain protein transporters that are found at the apical surface that shouldn't be at the basolateral surface and vice versa. So we need to separate the proteins of the apical surface from the proteins of the basolateral surface, and this is achieved by tight junctions between epithelial cells (one of the restrictors of protein mobility).

The mobility of membrane proteins is also 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) restricts protein mobility.

"You didn't come this far to only come this far."

Good luck! ^_^