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GENETICS & Molecular Biology

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Subject: Cell membrane & the endoplasmic reticulum.

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Last time we started talking about the composition of the membrane including different components of lipids and proteins.

We have different types of lipids present within the membrane. Cholesterol is present with its fused structure, it has a very rigid structure which will give the membrane more rigidity and less fluidity.

On the other hand there is another advantage of having cholesterol which is preventing membrane freezing, the membrane as we know it's semi-solid ; gel-like structure, when it gets exposed to very low temperatures the cholesterol is going to make the tails of phospholipids (hydrophobic) far away from each other reducing their interactions to prevent it from freezing.

(as we know converting liquid to solid requires increasing of non-covalent interactions, so having cholesterol between them will interrupt these reactions and reduce them)

Movement of the components of the membrane

We have

flip flop : vey occasionally happening, it has to be mediated and assisted by “flippase” enzyme; because it's not a stable movement (moving polar molecules through hydrophobic regions of the membrane).

lateral movement: more common, it happens within the same leaflet.

Rotation : each phospholipid molecule around itself.

The distribution of different types of phospholipids as well as sphingolipids is different between the inner and outer leaflet, e.g : in the outer leaflet I would find more **phosphatidylcholine** , more sphingomyelin and exclusively **glycolipids** (only found in outer leaflet).

On the other leaflet (the inner) I will find **phosphatidylethanol amine, phosphatidylserine** and a small amount of **phosphatidylinositol** which is important for cell signalling (the picture in the slide summaries it all), the presence of serine and ethanolamine give the negative charge to the inner leaflet of the membrane , making it relatively negative in

comparison with the outer leaflet (due to asymmetric distribution of types of lipids in both leaflets).

Lipid rafts

Sphingolipids (glucosylceramide) especially sphingomyelin, as well as cholesterol, are gathered in small patches within the membrane called **lipid rafts.**

Lipid raft : just like a patch, moves as a single unit from one place to another, they contain lipids and proteins, the have a wide range in sizes, the might be just 10nm up to 200nm.

What's the importance of lipid rafts?

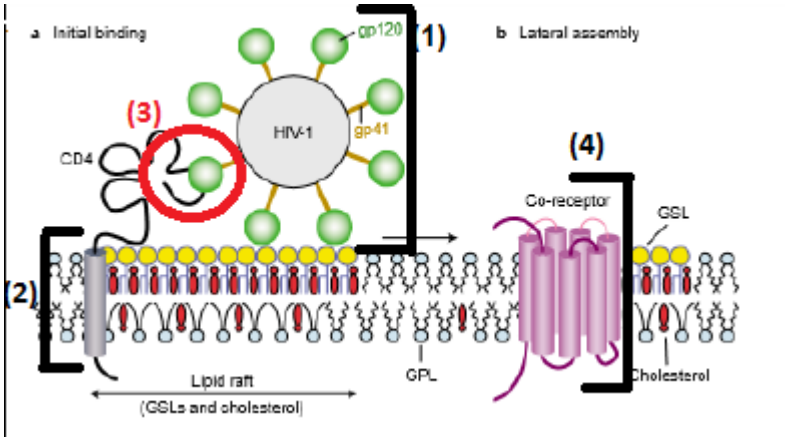
Mediating specific signalling pathway in a specific signalling disease, infection.. etc

Mediating infections

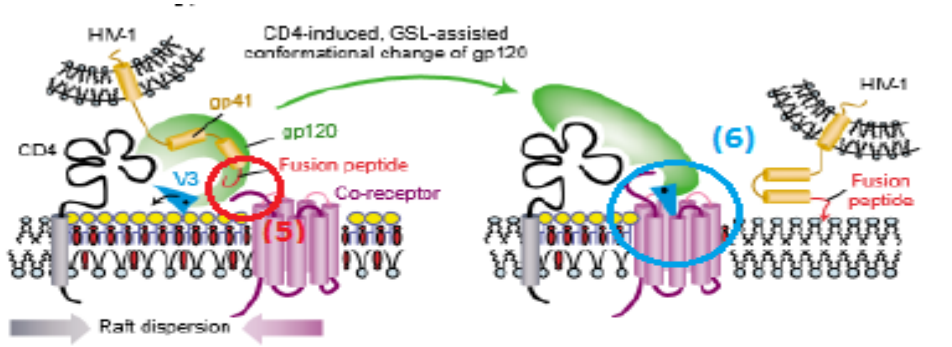
Like HIV, influenza

These infections are mediated by proteins present in the lipid rafts.

1. HIV has spikes coming out with a glycoprotein structure called GP120 found on its surface
2. The virus is going to attack the immune system specifically CD4+cells (named by the glycoprotein on its membrane called CD4 which is a one simple helix crossing the membrane)
3. The CD4 is going to interact with certain regions on the GP120 (alpha helix)
4. The interaction is going to move and bring a co-receptor in close proximity to this raft (the co-receptor is in a different raft than the CD4) because they are both present in different rafts this interaction through the cytosolic part of the protein and the interaction with the lipid membrane is going to bring the co-receptor from the adjacent raft towards the raft with the CD4, causing a raft dispersion



5. There is a small sequence within the GP120 that will interact with the co-receptor by a fusion peptide
6. The virus inserts its protein through the channel of the co-receptor diffusing the virus and infecting the cell.



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

So the GP120 interacts with 2 proteins , CD4 and the co-receptor which are present in rafts.

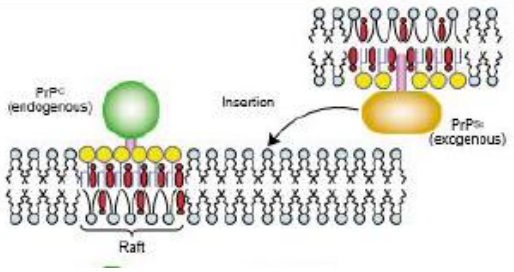
If we design a peptide to compete with the fusion peptide on binding to the co-receptor, this will prevent HIV binding, but HIV is always changing itself so if we design these inhibitors now, later on they might not be helpful.

Another application on rafts

Prions

Small proteinous infectious particles, in normal situation they are normal proteins that are present in our cells and have a normal function.

From the picture (refer to the slides), the one in green is a normal protein and the yellow is a prion. These proteins tend to be in lipid rafts.



Once we get the abnormal form of this protein from where ever the source is (e.g: mad cow) it will be present as a segment in the membrane of the infecting particle with the abnormal protein. This protein is going to fuse (with its lipid raft) to the membrane of the normal cell as a single unit.

Then the raft with the infectious protein will move quickly with closer proximity to the raft of the normal protein. Once they are in contact the infectious particle is going to infect the normal one and transform it to an abnormal protein (prion). Later on the newly formed prion will move and interact with more normal ones (amplification).

Another component of the membrane

Membrane proteins

There are 2 groups of membrane proteins

-integral (membrane spanning proteins)

-peripheral membrane proteins

Let's talk about the integral for a moment, it can be one segment or more than one inserted into the membrane, the segment has to have hydrophobic amino acids so they can interact with the hydrophobic tails of the sphingolipids.

So if I want to isolate this protein from the membrane , the best solvent would be an amphipathic one, because the membrane has hydrophobic and hydrophilic regions , so adding the amphipathic solvent the hydrophobic part will interact to prevent disruption of the molecule or changing its folding , and the polar heads will interact with the hydrophilic part .

But why can't I use a polar solvent? Simply I will disrupt the alpha helix, it won't be stable out of the membrane surrounded by a hydrophilic environment causing it to change its interactions ,therefore changing its shape.

Integrins are mostly made from alpha helix either one or more with different orientations and numbers. But in some situations I might find beta sheets making a beta barrel (channel like) e.g porin proteins.

Most of the integral proteins are glycoproteins, they have a sugar moiety attached to them specifically on the outside.

Now let's talk about peripheral proteins, they might be attached to the outer or the inner leaflet, they are attached by lipid anchors.

Types of lipid anchors

*Myristoyl group, it is basically a carboxylic acid, with a long hydrocarbon chain, to be inserted between the hydrophobic tails of the phospholipids. Through interacting with glycine residue of a protein they can form an anchor to that protein in the inner leaflet.

* Palmitate, it is derived from palmitic acid (carboxylic acid with a large hydrocarbon chain) it is inserted to the membrane with the 16 carbons, it has 2 interactions with the sulfhydryl group of cysteine residue of a protein forming an anchor to it in the inner side of the membrane.

*Another way to anchor proteins is by farnesyl (prenyl), made out of several isoprene units making the hydrocarbon part to be easily inserted in to the membrane , and through the sulfhydryl group of the cysteine residue of a protein it anchors the protein to the inner leaflet. (remember: since it has different isoforms, isoprene unit was found in ubiquinone and cholesterol formation, also in vitamin K).

*The last way to anchor is by GPI (glycosyl phosphatidylinositol)

Phosphatidylinositol together is a phospholipid, so expect to see 2 hydrophobic tail ((CH2)12CH3) Each tail) attached to the 2 carbons of glycerol and the third carbon is attached to a phosphate group, the phosphate group is attached to inositol sugar. Now I have this (phospholipid attached to inositol) attaching to another set of sugars ; mannose, N- acetyl glucosamine ..etc these sugars is where the protein

will be connected to by covalent bonds. GPI is the only one that attaches proteins on the outside leaflet.

Pharmacological applications on these anchors :

RAS

It's a transducer, part of a signalling pathway (oncogene, if it is activated it will cause more proliferation and if uncontrolled eventually cancer)

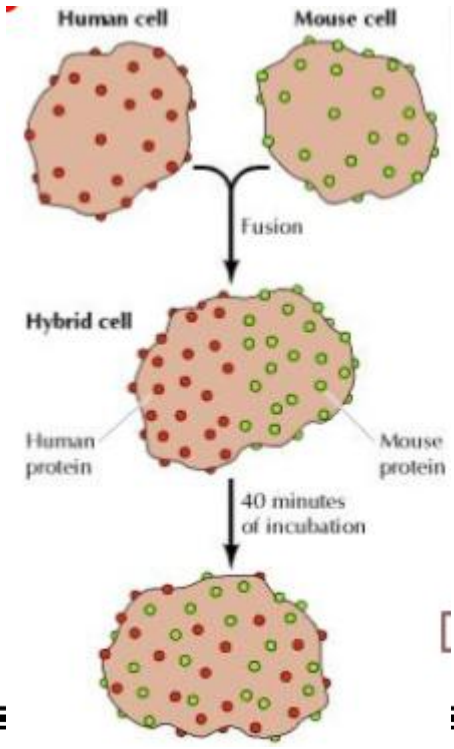
It is attached to the membrane via farnesylation, so if I stop its attachment to its lipid anchor I will stop its activity(it has to be in close proximity to its receptor on the membrane to transmit signals) from that point came the idea of farnesyltransferase enzyme inhibitors, it worked out well in animals but not in humans; because in humans RAS has many isoforms (mRAS, kRAS) so these inhibitors were only for a specific type of RAS and at the same time they worked on other types of proteins that some of them are beneficial for normal wellbeing of the cell.

These inhibitors can be used to treat other diseases like malaria and Hutchinson-Gilford progeria that is associated with a mutation in the Lamin A protein that is attached to the inner leaflet via farnesylation.

Another point we need to consider about proteins is that proteins can move, either as a single unit (a raft) or within the phospholipid structure.

? How did they discover that proteins move within different regions of the membrane?

They did a very simple experience –in concept-, yet it's very sophisticated. They marked the membrane proteins of a mouse cell with a green color, and the membrane proteins of a human cell with a red color. They fused the two cells together. If proteins do NOT move, then one side will stay red and the other side will stay green. But what happened actually is that the two colors were present on both sides, which means that proteins move within the membranes of the



mouse cell and the human cell. (see the figure)

Why do we consider this as an important issue?

Because of the polarity of cells, for example: small intestinal cells. These cells have to do two functions: first they absorb and transfer nutrients from the intestinal lumen to their cytosol. Then they transfer them from the cytosol to the portal circulation. So once we have the nutrients inside the cytosol we don't want them to go out, which means that we need to have the transporter that gets the nutrients in at one side (the apical side only) and the transporter that takes them out to the blood at the other side (the basolateral side -> under the level of the brush border).

So I need to restrict the movement of these proteins so they can be separated at two sides, and this is achieved by tight junctions.

** **Remember**: tight junctions hold cells together, they're the only type of cell-cell junctions considered impermeable to any molecule, they don't allow anything to pass across membranes.

These junctions are present in between the apical and the basolateral surfaces, their presence is going to restrict the movement of proteins, and even substances in between cells, because we want absorption to happen through cells (intracellular) not in between cells (intercellular).

Another way to restrict the movement is that proteins interact with other proteins in the ECM and with cytoskeletal proteins from the inside. So they're somehow tied by these proteins, and they can't move freely.

Another way is by GPI anchors. These can't restrict movement completely, but they restrict it within the raft in which they're present. So being part of a raft does some sort of restriction because as we said rafts move as a single whole unit.

The forth way for restriction is by interaction with other proteins on adjacent cells. So it's kind of tying a protein on one cell with a protein on the adjacent cell.

Mostly, the integral membrane proteins are glycoproteins (having a sugar or a carbohydrate molecule projecting out of the cell) and this sugary component is very important for cell-cell interactions, protection against mechanical stress, immune responses and prevention of invasion by microorganisms (because what a microorganism basically does is that it needs to remove the obstacles in its way like sugars to be able to cause infection, and it does so by releasing some substances to remove these sugars). Remember when we talked about HIV virus and how there has to be an interaction between its spikes and membrane proteins, so if this protein has a sugar moiety it needs to be cut and removed so the virus can cause the infection.

The oligosaccharides from glycoproteins and glycolipids form a structure called **Glycocalyx ,** a layer of carbohydrates around the cell outside the membrane.

Now we move to another topic which is cellular components. The picture in slide 2 is just a quick reminder of the organization of organelles inside the cell.

The endoplasmic reticulum:

Once mRNA is synthesized and processed inside the nucleus it has to be transported into the cytosol so it can assemble with ribosomes and translation starts. What happens to the polypeptide chain after translation? Folding, packaging and transport; depending on its function.

Insulin ,for example, is a protein hormone, it has to be excreted. But before it gets transported it must be modified. It has many disulfide bridges that contribute to its 3D shape and folding, that's why modification happens before it can be packed and transferred outside the cell.

Other proteins are plasma proteins and mitochondrial membrane proteins and they need to be transported to their final destination. And when we talked about the nucleus, we mentioned that its membranes are continuous with the membrane of the RER to which ribosomes are attached.

The ER is the largest organelle in the cell, it's made up of membranes forming trabeculae and they're arranged into sacs (or cisternae), and is attached to ribosomes for protein synthesis. The part at the end of the ER is called **transitional ER**, very close to the next station which is Golgi Apparatus, and then to the rest of the components of what we call "the secretory pathway".

The secretory pathway:

The ER → transitional ER → Golgi → different destinations (Lysosomes, cell surface, excretion,…).

How did they know that this is the route for proteins?

They used a series of experiments known as "pulse chase", using radioactive isotopes to label these proteins and follow their movement. (Make sure to read it from the book it's only one page 374-375, it's a very interesting subject).

Now as we know what the secretory pathway is, do you think that protein synthesis has to be completed first then proteins are transported to the ER for modification (Post-translational modification)? Or does modification take place while the protein is still being translated (Co-translational)?

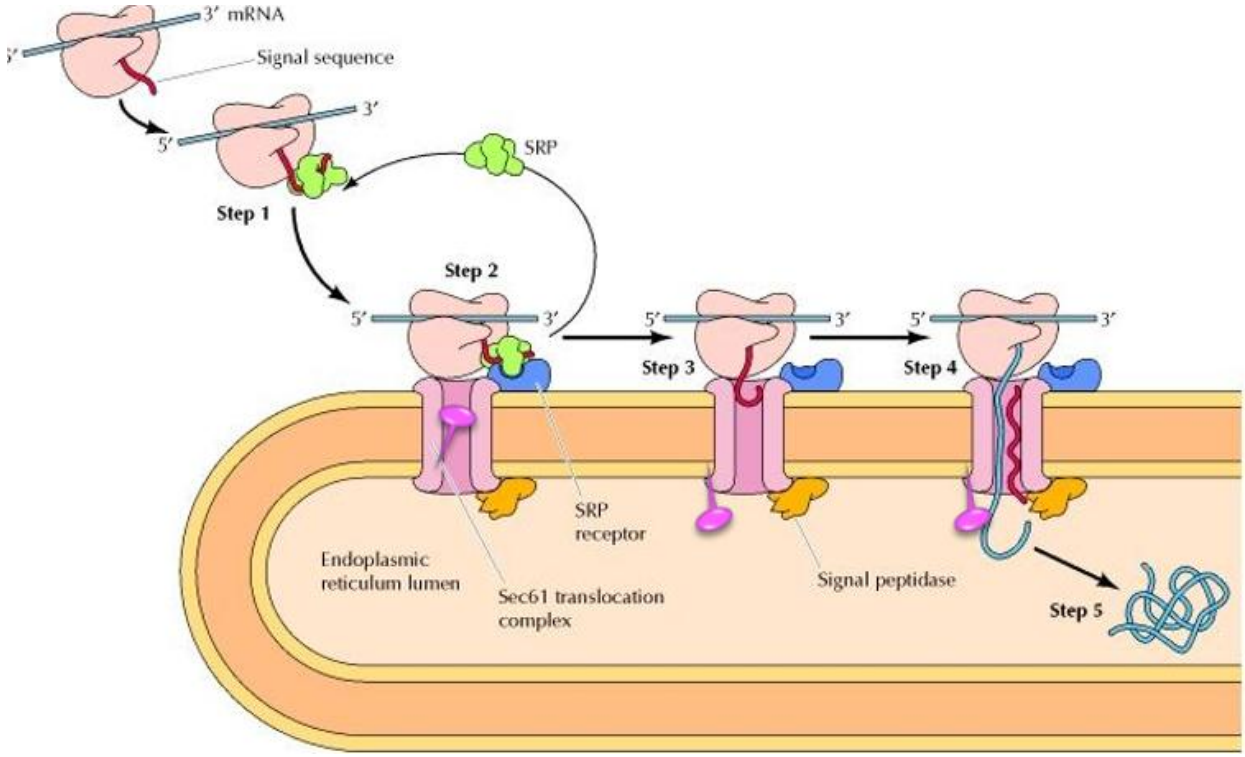
Well, in most cases it's post-translational, specifically the ones synthesized by free ribosomes. But if proteins are synthesized by ER-bound ribosomes the modification is co-translational.

It also depends on the fate protein (its destination). In case it's going to plasma membranes, secretory vesicles, endosomes then lysosomes; then these proteins are synthesized by ribosomes attached to RER (and the modification is co-translational). Nuclear proteins, mitochondrial proteins, and proteins going to peroxisomes are synthesized by free ribosomes (post-translational modification).

Basically, translation starts on the ribosome when it's free, then it either attaches to the ER (and the modification is co-translational), or it stays free (and the modification is post-translational).

In case of **co-translational modification**, how can ribosomes target the growing polypeptide chain into the lumen of the ER for modification?

By a signal sequence, a certain code mostly made of hydrophobic amino acids.



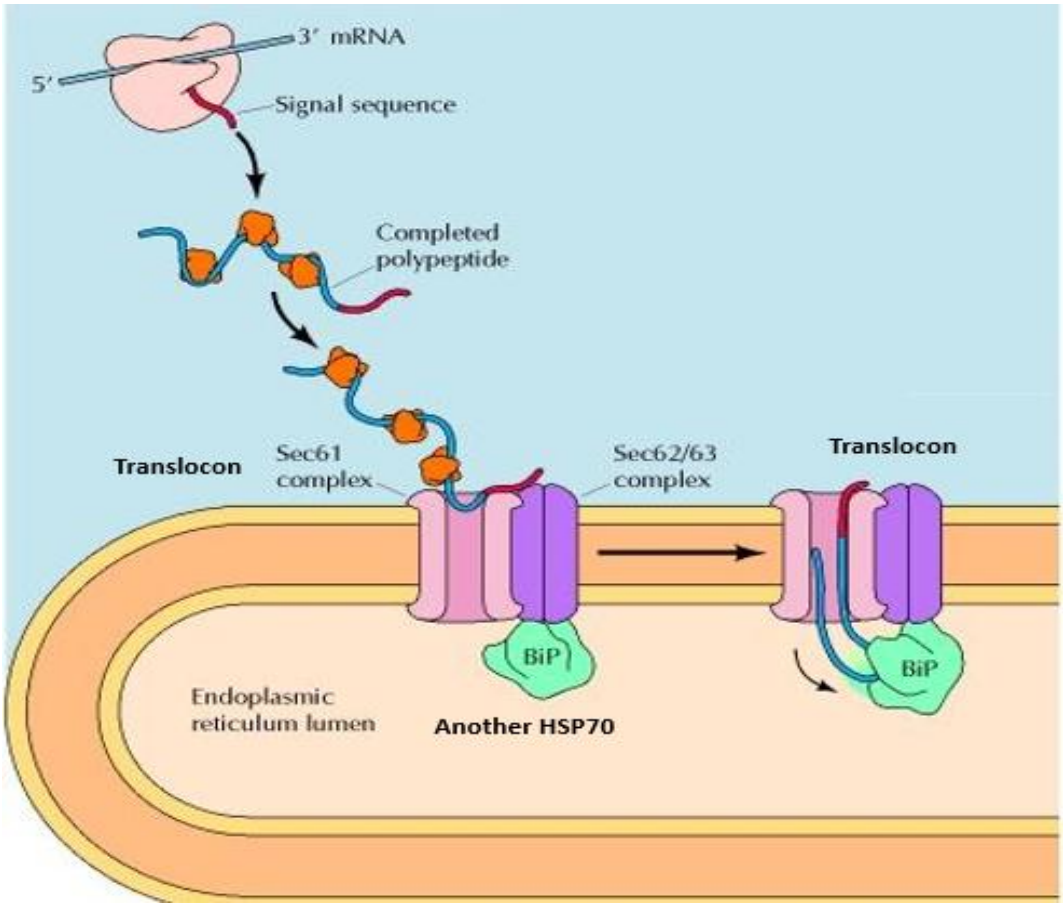
So the free ribosome starts translation, it translates the signal sequence and now this sequence is part of the growing polypeptide chain (the red part is the signal sequence) and translation hasn't stopped yet. The sequence is going to be recognized by a protein called SRP (signal recognition particle) and is bound to it (step 1). Then SRP guides the ribosome and the growing polypeptide chain as it moves to bind to the SRP-receptor on the ER and now the ribosome is considered a part of the ER (step 2).

The protein that's going to act as a channel, through which the growing polypeptide chain is going to be transported to the lumen of the ER, is called **Translocon** (Pink).

Then there is the arm (Purple) closing the channel, so the channel isn't open yet for the transfer of the polypeptide chain. Once SRP binds to its receptor it induces conformational changes and SRP is released (because its function is done, it transferred the whole complex to the Translocon

). These conformational changes orient the signal sequence inside the Translocon and the arm is open, allowing the entrance of the polypeptide chain (Step 3). The signal sequence now leads the polypeptide chain through the Translocon, and as translation continues moving along codons it pushes the polypeptide chain into the lumen (Step 4). When translation is done and the polypeptide chain is inside the lumen the signal sequence is cleaved by a **signal peptidase** enzyme on the inner surface of the ER membrane (so the signal sequence isn't part of the functional protein, it only guides it through the Translocon to the ER lumen) (Step 5).

Now if the modification is **post-translational** it goes as follows:



Synthesis of the polypeptide chain is completed by a free ribosome, and again they have a signal sequence to direct it though the Translocon protein, but this sequence isn't recognized by SRP.

Here we need to keep the protein in the unfolded form because folding needs to happen in the ER lumen and checked after that. The ER is the

“Quality control station”, so folding can’t occur in the cytosol because it won’t be checked if it’s proper or not.

This sequence binds to another type of receptor associated with the Translocon then allows the entrance of the polypeptide chain. Once part of the chain gets inside the lumen, it binds to a protein called **Bip** (a chaperon, heat shock protein 70). Bip binds to part of the polypeptide chain and pulls the entire polypeptide chain to the inside. Here since translation is completed and is inactive there’s nothing to push the polypeptide chain through the Translocon as it happens in the co-translational process, so we need to have something to pull in to the inside and this is the function of Bip.

Everything mentioned above only applies to soluble proteins. If I need to synthesize membrane proteins then it has to be anchored to the membrane of the ER, then to the membrane of Golgi, then the membrane of the endosome, Lysosome and then to the plasma membrane. In membrane proteins we could have a protein with just one time spanning the membrane, or multiple spanning sequences within the membrane. A protein with its N-terminus to the inside and C-terminus to the outside, or the other way around.

Different orientations, different times of spanning the membrane, different mechanisms of inserting the proteins into the membrane, and they will be discussed in the next lecture.

Sorry for any mistake.

“When life gives you lemons, complain.”