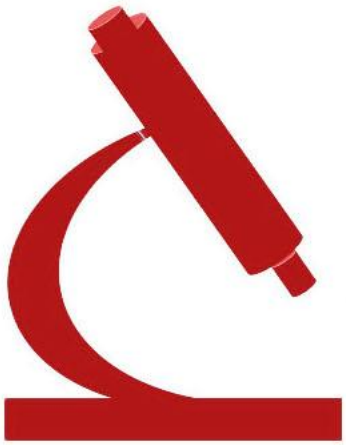

CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

6

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Application: farnesylation inhibitors and disease treatment

Until now we have studied that farnesylation is a way of anchoring proteins. This piece of information was invested in research.

➡ **Ras**, which is a protein, is a part of signaling pathways. It acts downstream the receptors. Once the ligand binds to the receptor, the receptor is active, then **Ras** gets activated.

Ras is going to activate another protein, and this protein is going to activate another one, until a transcription factor (which is a protein) enters the nucleus and activates certain genes to synthesis certain proteins.

These proteins, that get synthesized downstream of **Ras**, are going to activate cell cycle and cell division **normally** and then they turn off.

➡ Suppose, under certain circumstances, **Ras** becomes active all the time, the cell continues cell division (uncontrolled cell division), and it will transform from a normal cell to a cancer cell.

In other words, the over activation of Ras will lead to some types of Cancer.

***Note**: when Ras changes from the normal function to the abnormal one, it is an oncogene. (onco means cancer or tumor.)

➡ **Ras** is not an integral membrane protein. It is an anchored protein, and it is anchored through farnesylation.

So, if we inhibit farnesylation, **Ras** will not be attached to the membrane, it is going to stay in cytosol as a soluble protein and will not get the signal from the receptor. So, the whole pathway is going to be downward. (is going to be inhibited.)

The inhibition of farnesylation can be done by inhibiting the enzyme that does this process. This enzyme facilitates the attachment of the protein to the farnesyl group, and it is called **Farnesyl Transferase**.

So, if we designed chemical inhibitors that inhibit **Farnesyl Transferase**, we would stop Farnesylation.

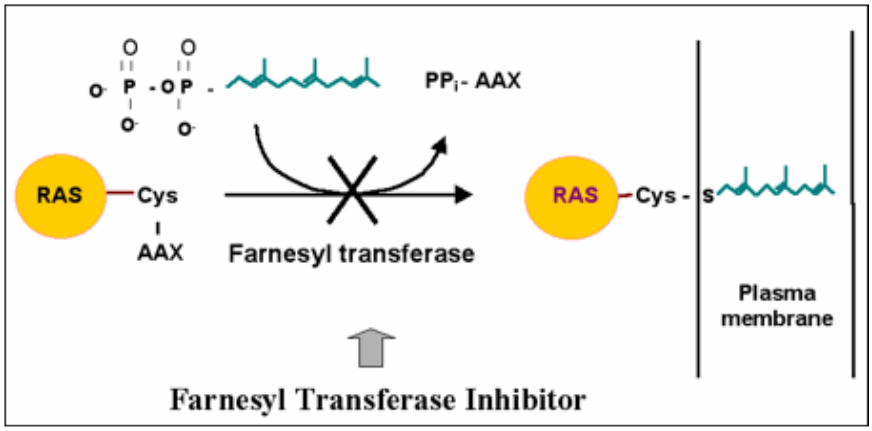
Scientists tested **Farnesyl Transferase Inhibitors** (**FTIs**) to check if **FTIs** are suitable to treat cancer in human beings.

The testing was firstly done on cells growing in a dish (cell/tissue culture), specifically on cancer cells, to see if the inhibitors will contribute in decreasing the uncontrolled cell division. These inhibitors passed the first test.

The second test for inhibitors was done using animal models (small animals) like rats, rabbets, etc. The inhibitors passed this test too.

Then, they moved on the experiment to human beings. (third/clinical trial)

When FTIs were moved to clinical trials / experiments on human beings, they failed.



Why did the FTIs Fail?

1) There are different forms of RAS (N-RAS, K-RAS, etc..). one Ras is presented in this cell type, the other RAS is presented in another cell type and the FTI doesn't act on different Rases.

2) Because FIT is not specific just for the farnesylation of Ras, it is specific for farnesyl transferase that does the job for **all** proteins that are anchored to the membrane through farnesylation.

Any proteins in the cell that must get attached or anchored to the membrane through farnesylation are going to get away from the membrane (they aren't going to get anchored, instead they are going to stay in cytosol and this stops their functions).

These proteins may have important roles in cell including growth regulation.

FTIs fail in treating cancer in human beings. However, they show positive results for the treatment of malaria (which isn't a cancer or tumor).

FTIs are considered for the treatment of other diseases such as Hutchinson-Gilford Progeria Syndrome (AKA progeria), caused by mutated farnesylated lamin A protein.

**** what is important to know here that Ras is a protein that turns on cell cycle and cell division (proliferation) normally then it turns off at normal situations. When there is a cancer, Ras stays active all the time, it leads to uncontrolled division.**

Protein sorting (endoplasmic reticulum)

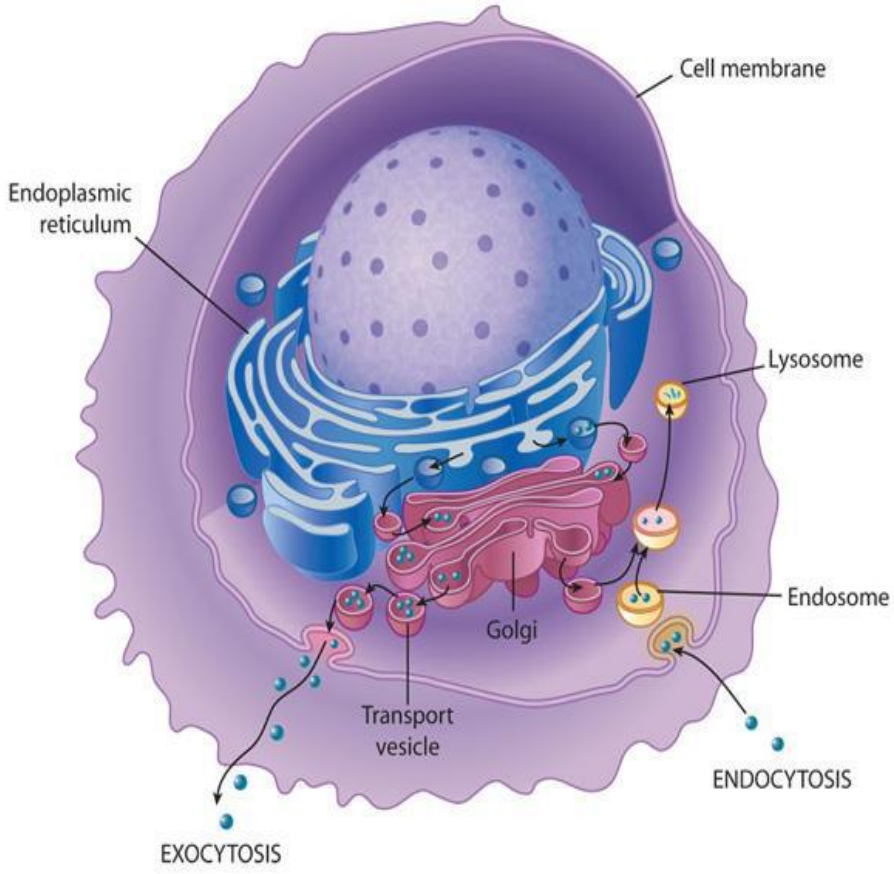
Endoplasmic Reticulum, which is the first part of the secretory pathway, consists of flatten membranous sacs (cisternae) that is attached to (continuation of) the nuclear envelope. Part of it is covered (loaded) by ribosomes which is called the **Rough Endoplasmic Reticulum**, another part is free of ribosomes creating the **Smooth Endoplasmic Reticulum**.

*ER is the largest organelle of most eukaryotic cells.

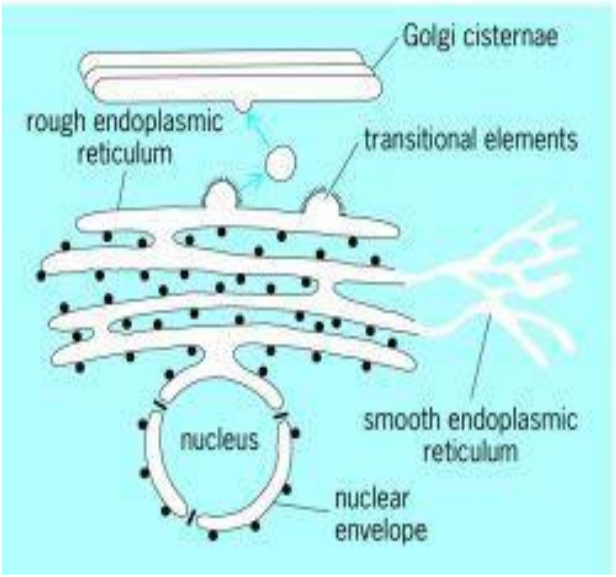
An overview of cellular components

In the picture below, we can see how the nucleus of the cell is surrounded by the membranes of the **ER** from all sides.

When the structure of the **ER** finishes, the next structure which is **Golgi Apparatus** starts. Different molecules including proteins, lipids, etc. get packaged and released from Golgi Apparatus to their final destinations (mitochondria, plasma membrane, lysosomes, peroxisomes, etc.…).



The **Rough Endoplasmic Reticulum**, as we can see, is loaded with ribosomes. We can also see the point that we have mentioned, it is a continuation of the **Nuclear Envelope**.



The **Smooth Endoplasmic Reticulum** is somewhat away from the **Nucleus**, because it doesn't contain ribosomes, that are concerned with the translation of messages carried by **mRNA**.

At the end of the **ER**, there is a structure (that is part of the **ER**) called **Transitional ER**, which packages molecules from **ER** to be sent to **Golgi Apparatus** for further modification and synthesis of other molecules as well.

***Note:**

1) The rough ER: covered by ribosomes on its outer surface and functions in protein processing.
2) The smooth ER: lipid metabolism.
3) Transitional ER: exit of vesicles to Golgi apparatus.

ER, as an organelle, expands and shrinks. The amount of membranes in the **ER** isn't fixed.

The cell may need more synthesis of proteins or it may need more synthesis of lipids molecules in the smooth ER, so the membranes of the **ER** will expand (larger structure of ER).

(In other words, ER will be larger or smaller to accommodate cell needs.)

ER is the first part of the secretory pathway.

Proteins are synthesized on ribosomes, whether they are free or attached to the ER. then proteins get modified in the ER, further modification in Golgi Apparatus. Finally, they get packaged and released to their final destinations.

These steps are called **secretory pathway**.

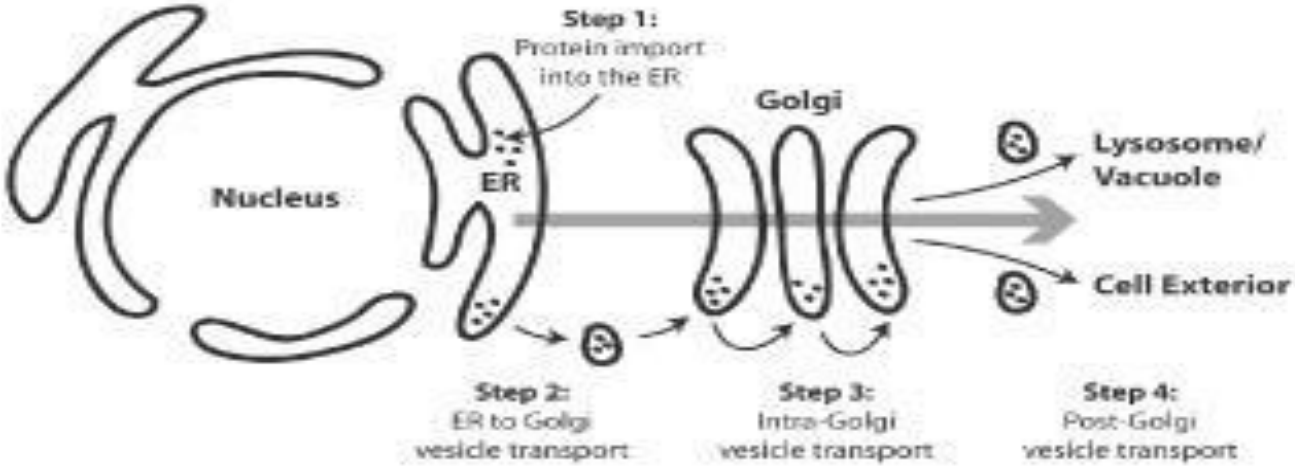
*Scientists discovered this pathway by labeling molecules, like proteins, using fluorenes light or radioactive molecules. The continuous labeling can't show the order of these steps, there must be stops in some points. This technique is called **Pulse Chase (ضُخ والحق)**, in which we must follow the signals of these labels...

You add the labeling to the molecules. you get them move along the secretory pathway. Then you follow the colors of the labels or tags, New molecules are synthesized now without label signs. It is like turning light on and off.

[The fluorenes turn on first in ER, indicating that proteins are presented in the ER. When proteins move from the ER to Golgi Apparatus, the fluorenes turn off in the ER, and turn on in Golgi Apparatus. The new proteins are synthesized without labeling, so we are making **temporary** labeling for some proteins.

When proteins move to the vesicles, the fluorenes turn off in Golgi Apparatus and turn on in the vesicles. This continues until the proteins reach their final destinations.]

You can read more about this technique/ pages 374-375 in the book. (Fourth edition)



ER-Golgi- secretory vesicles- cell exterior

ER, Golgi apparatus, and lysosomal proteins are initially targeted to the ER.

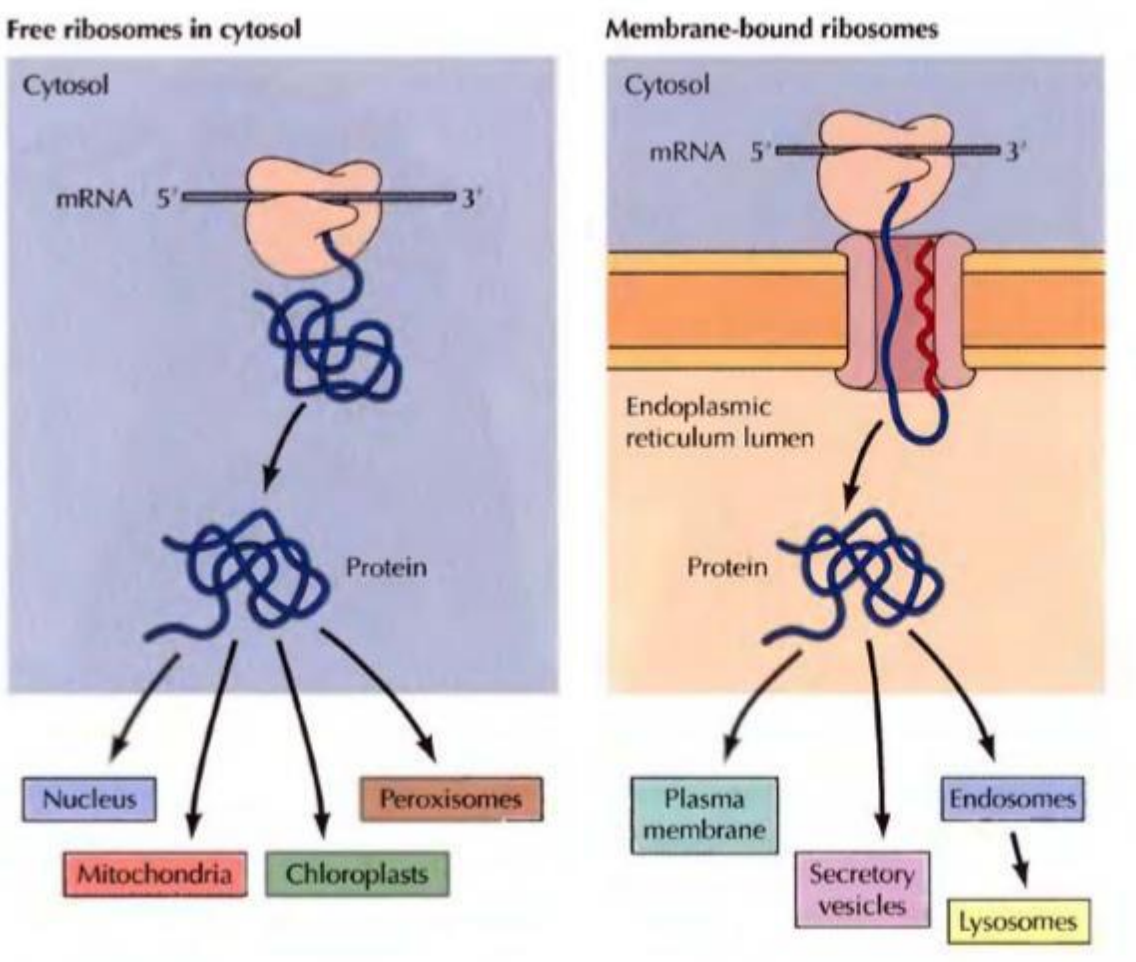
In all circumstances/ in all examples of proteins, protein synthesis starts on ribosomes free in the cytosol.

Depending on the final destination that the proteins are going to, different types of ribosomes are used for protein synthesis. (in other words, final destination governs the type of ribosomes on which the protein is synthesized.)

for example,

1) If the protein is going into the plasma membrane, secretory vesicles (for secretion outside of the cell) or to the endosomal or lysosomal systems, it is going to be synthesized on **ribosomes** of the **Rough Endoplasmic Reticulum** / it starts on free ribosomes that get then attached to the Rough ER.

2) Whereas if the protein is ending up in the nucleus, mitochondria, chloroplasts (in plant cells) or peroxisomes, it is going to be synthesized on **free ribosomes** / it starts on free ribosomes that stay free until the translation of protein finishes.



Ribosomal and protein targeting

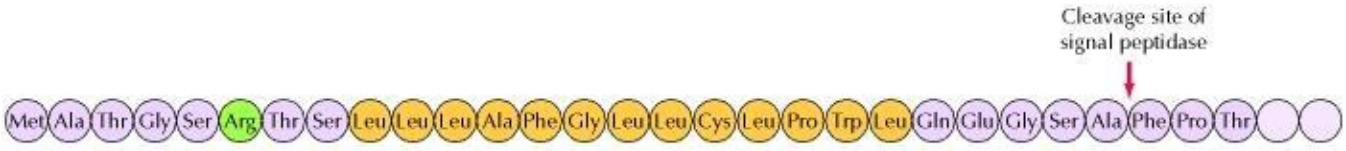
The synthesis of proteins, whether on **free ribosomes** or on **attached ribosomes**, needs the ribosomes to transport the polypeptide chain to **the lumen of the ER** (for folding, adding sugar groups, post translational modification, etc..). so, a process called **translocation** (transporting) must happen.

Most of the time translocation happens co-translating (that is translocation occurs while translation is happening.). but in some examples, it is not (that is translation must finish first, then translocation starts.)

*What takes these proteins to the ER?

-There is **a signal sequence** within the amino acid sequence of the protein and it is short stretch of hydrophobic amino acids at the N- terminus. *like the example below in yellow*. Signal sequence transports the growing polypeptide chain to the ER (inside the ER)

These short stretches of hydrophobic amino acids are then cleaved from the polypeptide chain during its transfer into the ER lumen.



You can find links of Translocation animation in the slides. ☺

Co-translational Translocation of polypeptides to ER

For proteins that are synthesized on attached ribosomes,

* Synthesis starts on free ribosome.

* **SRP** (signal recognitional particle) is the important molecule for translocation.

1) Translation starts by synthesizing a signal sequence which grows until it has enough length, so it can be recognized by SRP.

2) There is a receptor on the membrane of the ER which SRP is going to bind to. This will pull the ribosome with the growing polypeptide chain to the membrane of the ER.

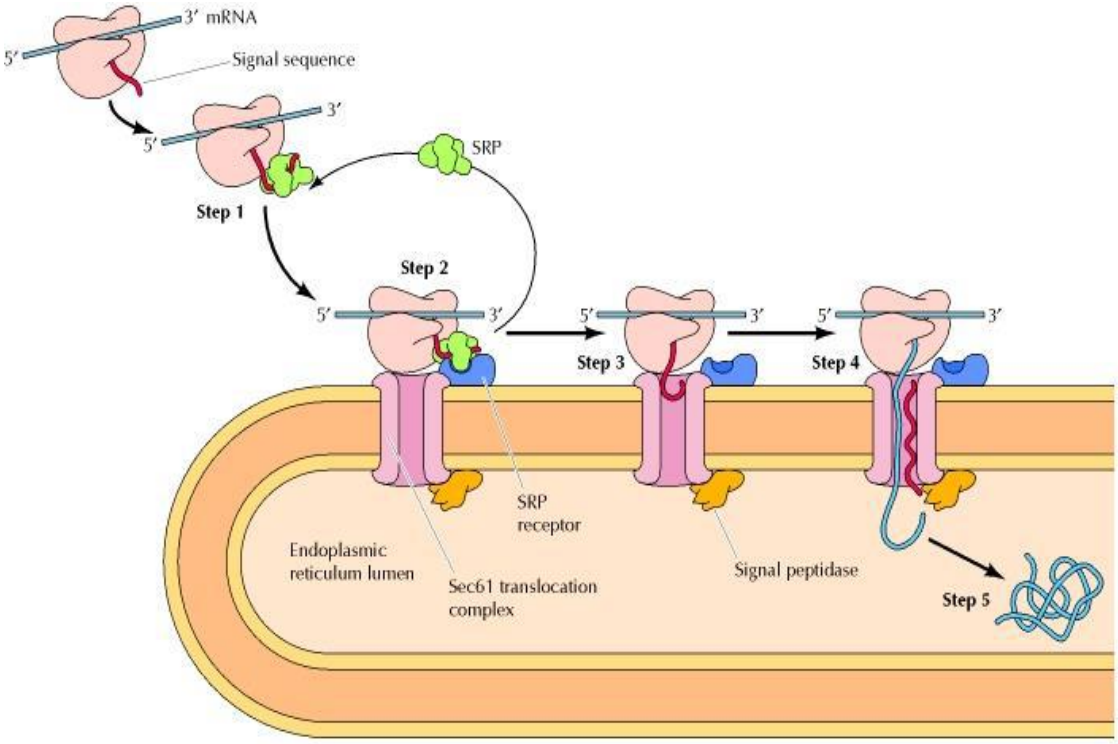
Now the ribosome is attached to the ER. ✓

3) The SRP must be removed to allow the growing polypeptide chain to enter the protein that is going to transport it. But, this protein is still in closed conformation, it doesn't allow the passage of the polypeptide chain.

Once the ribosome and SRP bind, they are going to make **conformational changes** within the protein.

***Conformational changes**: minor changes in the 3D shape. these changes do not change the shape of protein from globular to fibrous. They may do very slight movement (in range of angstroms (Angstrom= 10^-10 m) that opens the structure.

So, the channel of the protein opens. (This protein is called **translocon**.) SRP detaches.



4) The growing polypeptide chain enters the translocon until it is inside the lumen. Once the polypeptide chain reaches the lumen of the ER, it faces a protein called **signal peptidase**.

Signal peptidase: enzyme that cuts the signal peptide and leaves the rest of the polypeptide chain.

NOTE:

What is the force that pushes the polypeptide chain through the translocon?

Because the ribosome is attached to the ER membrane and translation doesn't finish, the ribosome isn't allowed to move along the mRNA, it must stay in that position. So, the growing polypeptide chain must be pushed due to the translation process. (in other words, translation is the force that pushes the polypeptide chain into the channel of the translocon. / the movement of mRNA on the ribosome with the growing polypeptide chain that is coming out of the ribosomal structure is pushing through the translocon by the action of translation process.)

5) The whole polypeptide chain is now in the lumen for modification.

Posttranslational translocation

*happens when a protein is synthesized on free ribosomes.

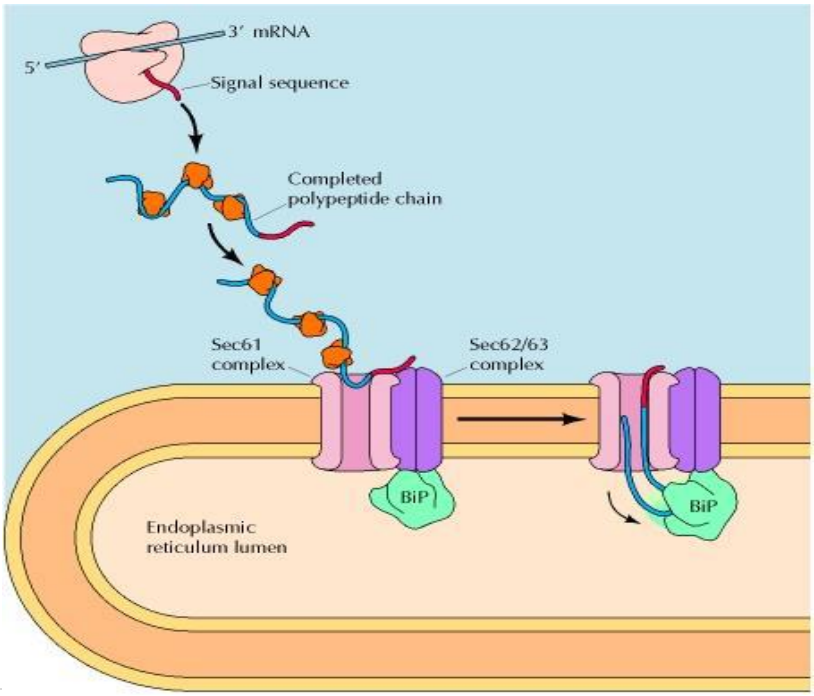
1) Synthesis starts on free ribosomes and will finish the whole polypeptide chain in the cytosol. Once the whole chain is ready, the signal sequence is going to bind to the translocon and the co-protein that forms a complex with it. (Sec62/63)

Sec62/63: a protein complex made of translocon and its associated protein (co-protein).

2) The polypeptide chain binds with the complex and enters to the translocon.

Remember that: conformational changes have opened the translocon channel to allow the polypeptide chain to enter.

3) There is a protein on the luminal side of the ER called **BiP** which is going to pull the polypeptide chain. (because the translation is already done, the force of translation isn't pushing the chain! So, there must be another way to pull the chain, which is the action of **BiP** protein.)



Note that **BiP** is part of a family called chaperons (like HSP40, HSP70, etc.). This family helps in different steps of protein folding that includes the entry of the polypeptide chain.

Insertion of proteins into the ER membrane

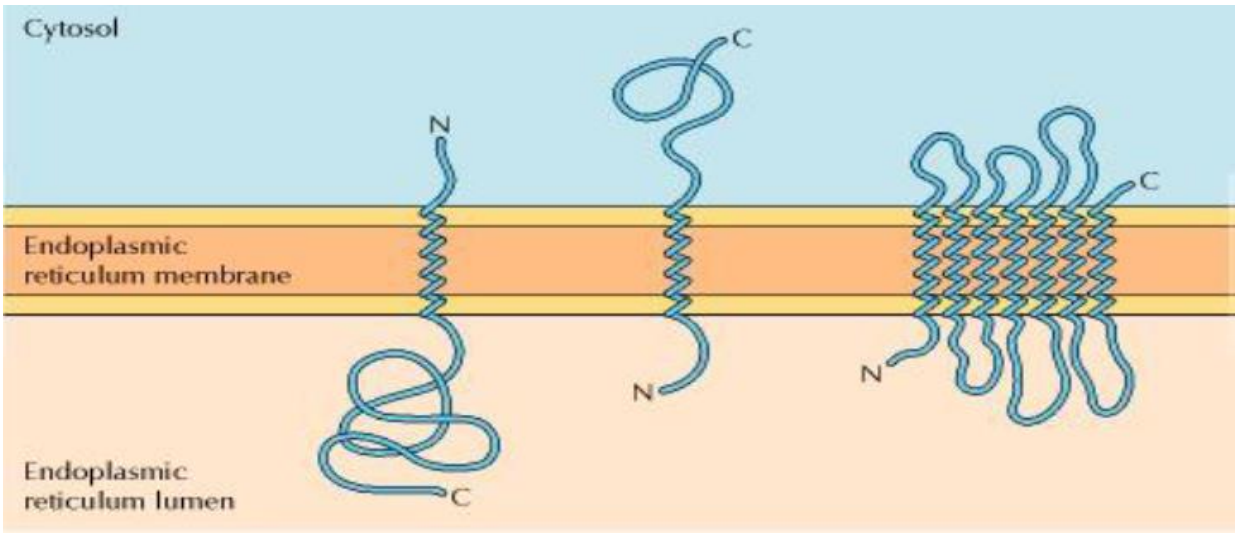
The transmembrane part of the protein must be inserted from the beginning of the synthesis process. In other words, it must be inserted firstly in the ER membrane regardless of the final destination of the membrane protein (lysosome membrane, plasma membrane, etc.).

- Why?

Because that membrane part is mostly hydrophobic. So, if I inserted it right away, I'm going to give it its final structure which is part of its folding that occurs in the ER lumen.

Whereas if I leave it until reaching the plasma membrane or the mitochondrial membrane, I may lose the structure and the proper folding.

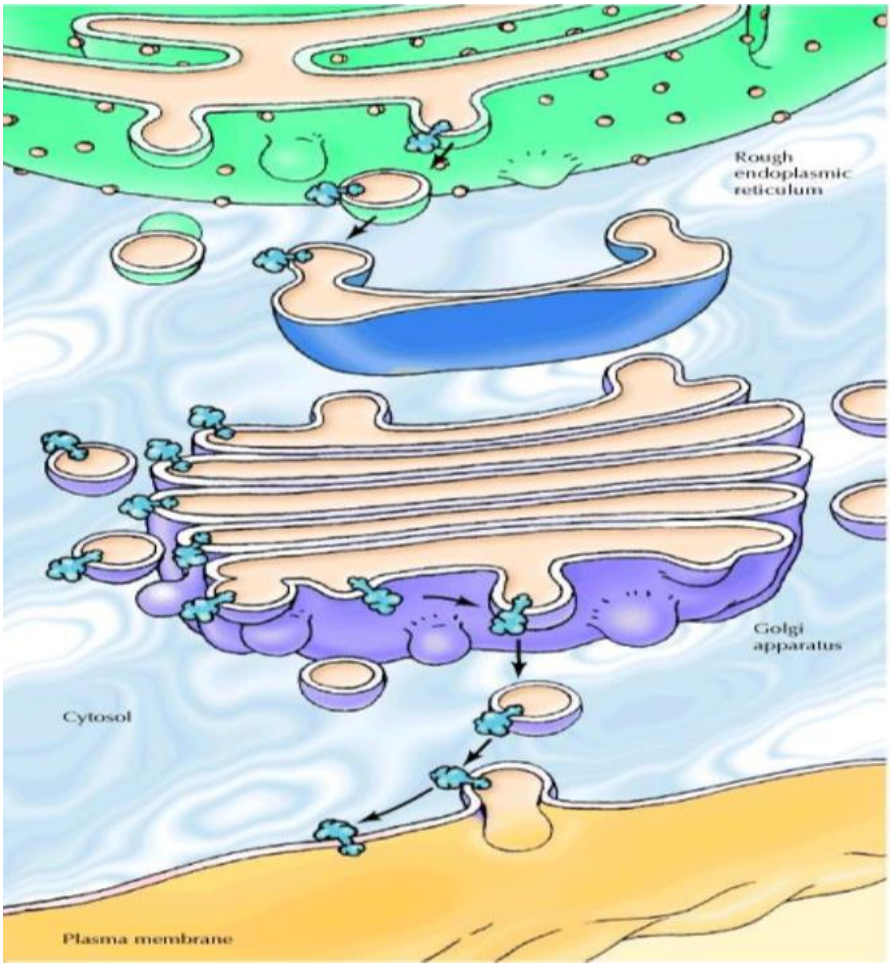
Some proteins span the membrane just by one helix, other span the membrane by multiple helixes. Very few of them span the membrane by beta-sheet.



Membrane protein orientation

Sometimes the N-terminus is on the outside (Extracellular) and the C-terminus is Intracellular. And they may be in opposite orientation, that is the N-terminus is inside and the C-terminus is outside. So, it is different from one protein to another and the cell has all these different possibilities and it can deal with them.

Suppose a protein was inserted in the ER membrane. **The big part of it facing the cytosol, and the small part facing the lumen of the ER.** when it is transported to the vesicle, the orientation stays the same, the big part of it facing the cytosol, and the small part facing the lumen. When this vesicle fuses with Golgi Apparatus, the orientation is also the same and then it exit through different compartments of Golgi apparatus. Once the vesicle reaches the plasma membrane and fuses with it, the big part faces the cytosol, and the small one faces the EC (extracellular) region.



Note:

Don't use "inside" or "outside". Use "lumenal", "cytosolic" and "EC". So what was first cytosolic, stays cytosolic. And what was firstly lumenal, becomes EC.

In the situation of inserting the protein in the Plasma membrane.

Question 😳:

A mutation happened in a protein that lead to insertion in the membrane in opposite way and this protein was a receptor on the cell membrane. What would do you expect to happen?

Any receptor has a ligand binding domain that has to be on the outside, now it is on the inside, so it is not going to bind the ligand that is going to get active.

Some notes from the slides (about Co-translational Translocation of polypeptides to ER):

* The SRP inhibits translation and escorts the complex to the ER membrane, where it binds to the SRP receptor.

* Translation resumes when the growing polypeptide chain is translocated across the membrane.

☆ قتل الزهور لن يؤخر الربيع ☆