Genetics & molecular biology

◉ Sheet ○ Slide

Number: -24

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Protein sorting (endoplasmic reticulum)

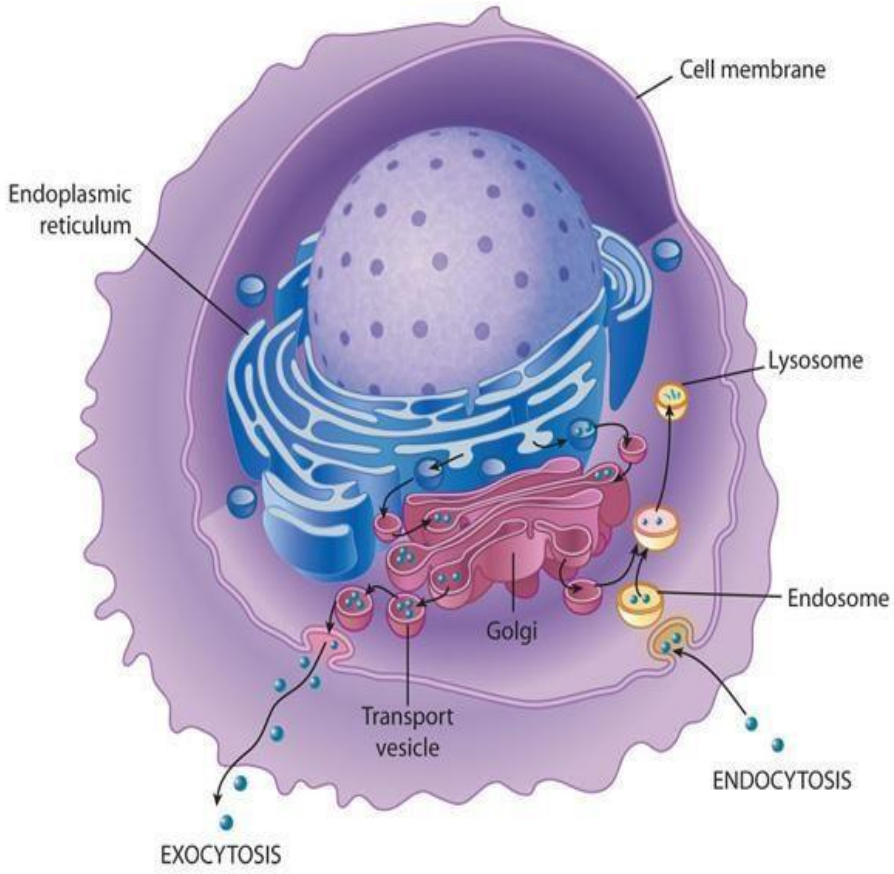
Endoplasmic Reticulum, which is the first part of the secretory pathway, consists of flattened membranous sacs (cisternae) that is attached to (continuation of) the nuclear envelope. Part of it is covered (loaded) by ribosomes which is called the **Rough Endoplasmic Reticulum**, another part is free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

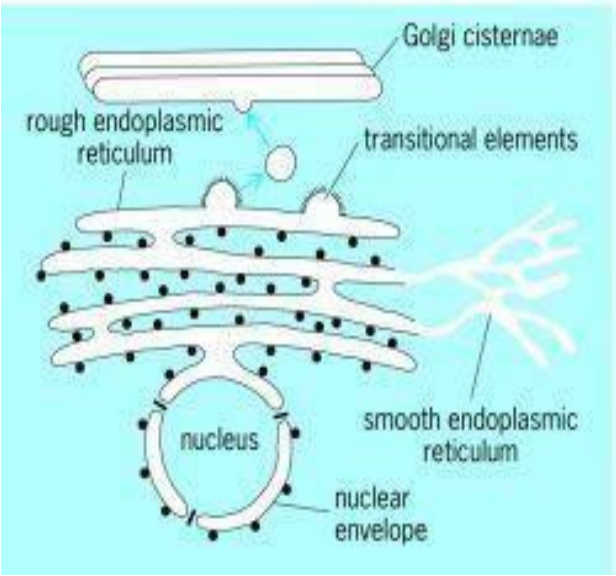
ER is the largest organelle of most eukaryotic cells .

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 **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,to sum up functions ,

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.

Note:ER, as an organelle, expands and shrinks,moves and divides . 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.

What is the secretory pathway?

The pathway by which proteins are synthesized by 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.

Scientists discovered this pathway by labeling molecules , like proteins, using florescent light or radioactive molecules in a technique called Pulse chase analysis .

pulse-chase analysis is a method for examining a cellular process occurring over time by successively exposing the cells to a labeled compound (pulse) and then to the same compound in an unlabeled form (chase)

Mechanism: A selected cell or a group of cells is first exposed to a labeled compound (the pulse) that is to be incorporated into a molecule or system that is studied. The compound then goes through the metabolic pathways and is used in the synthesis of the product studied.

Shortly after introduction of the labeled compound , excess of the same, but unlabeled, substance (the chase) is introduced into the environment.

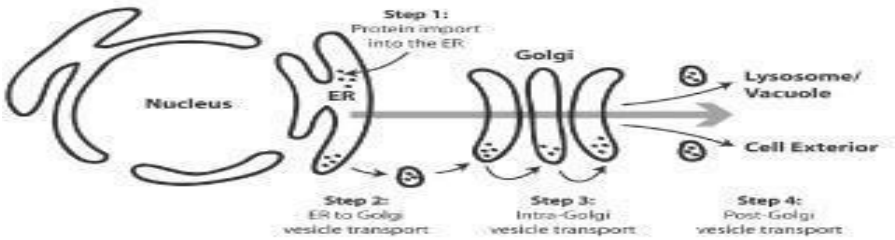
Note that only limited amount of amino acid is added in the beginning so that directionality of synthesis is clear

Under the microscope : [The florescence turns on first in ER(right next to nucleus), indicating that proteins are presented in the ER. When proteins move from the ER to Golgi Apparatus, the florescence turns off in the ER, and turns on in Golgi Apparatus..

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

Why this method? Because continuous labelling can't show the order of these steps moving from one station to the next in the secretory pathway ,so it is labelled at one point that moves sequentially ,all of this to clearly show directionality of synthesis ,and that's why we introduce a limited amount of labelled material only .

ER-Golgi- secretory vesicles- cell exterior



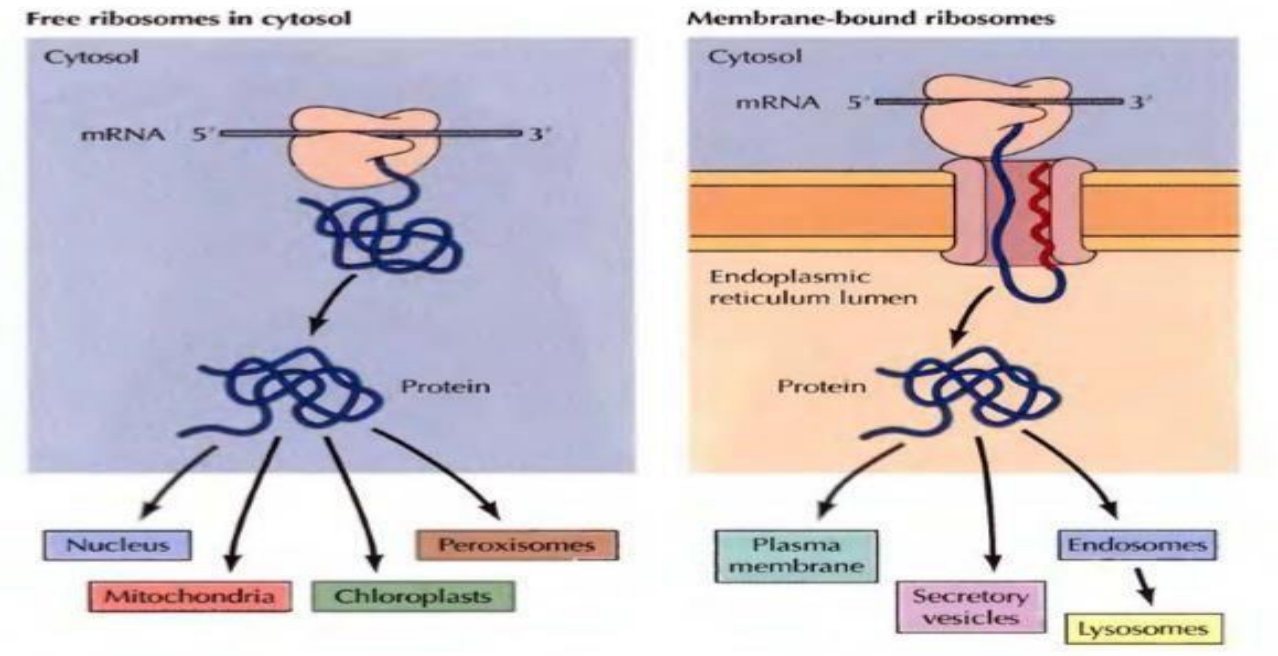
Next,

In the secretory pathway ,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 and membranes of organelles ,it is going to be synthesized on **ribosomes** of the **Rough Endoplasmic Reticulum** , it starts on free ribosomes that go and attach 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.

Note figure bellow



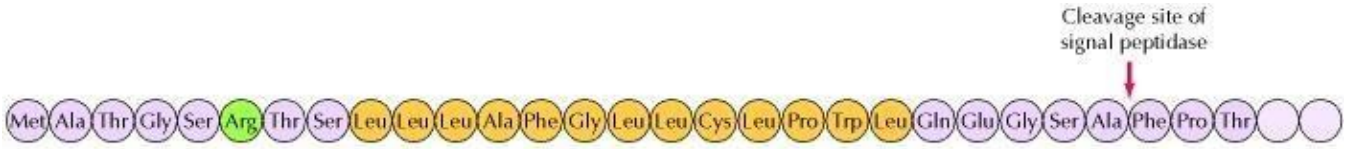
Ribosomal and protein targeting

Whether the synthesis of proteins is on **free ribosomes** or on **attached ribosomes**, **the** polypeptide chain needs to be transported to **the lumen of the ER** for modification . this is called **translocation** .

What signals these proteins to be translocated to the ER ?

A short stretch of **hydrophobic** amino acids at the N- terminus called **signal sequence** (1-15 amino acids) directs the growing polypeptide chain translocation to the ER lumen .

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

Translocation can be of two types:

Most proteins are translocated co translation (during translation),however some only get translocated post translation ,for a certain protein its either this or that .

1-Co-translational Translocation of polypeptides to ER(refer to figure)

Mechanism of translocation

translation always starts on free ribosomes whether it will continue to be free or become bound ribosome.

Step 1: As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP).

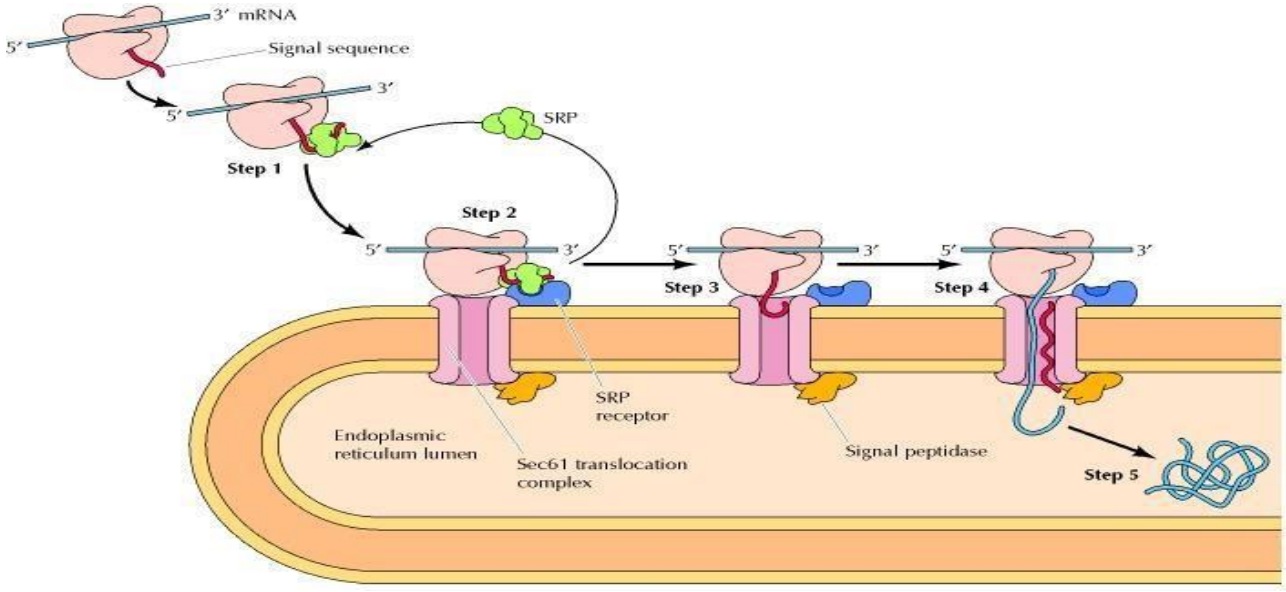
SRP(signal recognition particle):recognizes signal sequence, has a receptor next to the protein that translocate the polypeptide chain called (TRANSLOCONE)

Step 2: The SRP inhibits translation and escorts the complex to the ER membrane, where it binds to the SRP receptor.

Step 3: The SRP is released, the ribosome binds to a translocon protein ,and the signal sequence is inserted into a membrane channel.

Step 4: Translation resumes, and the growing polypeptide chain is translocated across the membrane through translocone.

Step 5: Cleavage of the signal sequence by signal peptidase(enzyme on the luminal side,a member of chaperon family of proteins) releases the polypeptide into the lumen of the ER.



NOTE:

1-*conformational changes have opened the translocon channel to allow the polypeptide chain to enter.*

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

Because the ribosome is attached to the ER membrane and translation is ongoing , 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. In other words 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 itself.)

finally, The whole polypeptide chain is now in the lumen for modification.

2-Posttranslational translocation

1) Synthesis like always starts on free ribosomes and will finish the whole polypeptide chain in the cytosol, remaining unfolded by cytosolic chaperons.

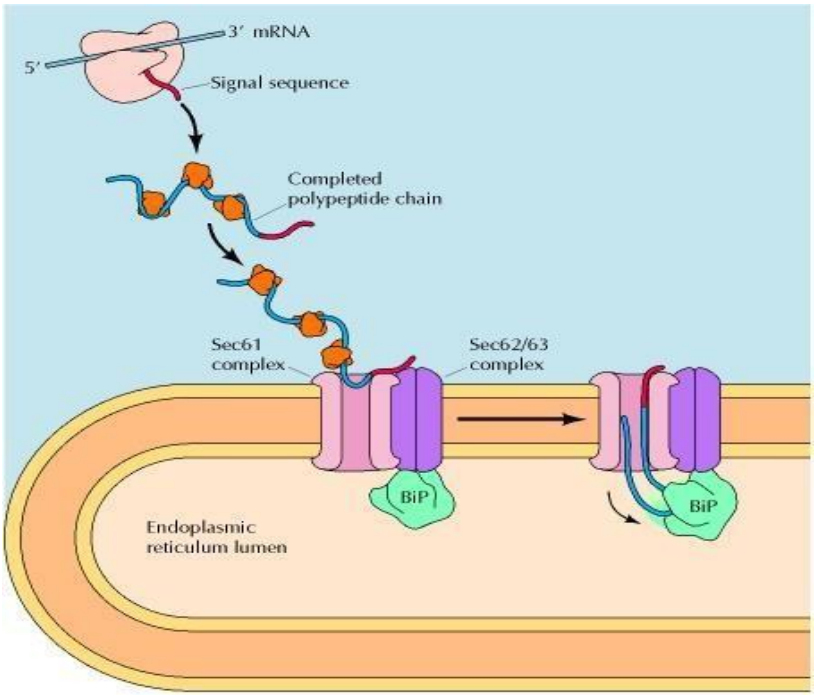
2) Once the whole chain is ready, the signal sequence is going to bind to a specific sequence on the translocon and permitting its entry,

   The translocon here is different it's complexed with other proteins

Chaperons; (orange particles in diagram) family of proteins responsible for the whole process of protein folding (stop self folding , folding to prevent misfolding,unfold to refold correctly and so on)

3) In order to facilitate entry of protein ,on the luminal side of the ER a chaperon protein called **BiP** pulls the polypeptide chain.

   unlike co translational translocation here 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 pull instead of push.



Insertion of proteins into the ER membrane

Secretory, ER, Golgi apparatus, and lysosomal proteins are released into the lumen of the ER.

Membrane proteins are initially inserted into the ER membrane.

Factors that affect protein insertion into the ER membrane:

1. Single vs. multiple membrane spanning region

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

2. Orientation of N- and C-terminus

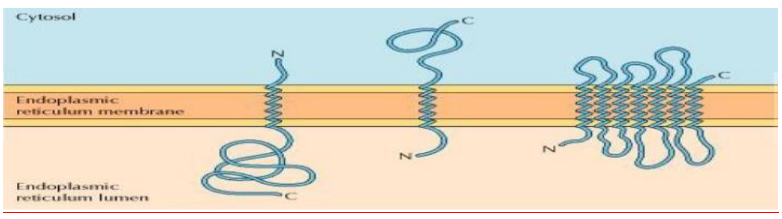
Insertion:

The transmembrane part of the protein must be inserted from the beginning of the synthesis process into the ER membrane . 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 the transmembrane part of the protien is mostly hydrophobic. So, if it was inserted from the get go , this is going to give it its final structure which is part of its folding, which is one of the functions of the ER (quality control early on)

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

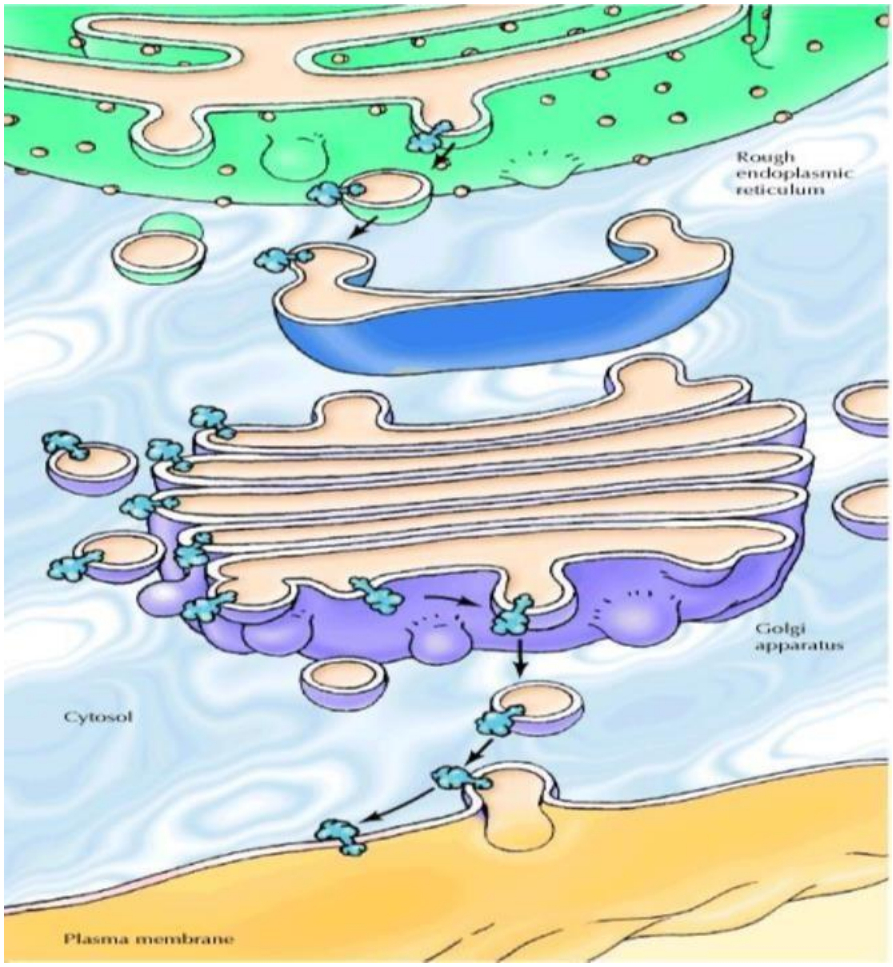


From the figure note that Sometimes the N-terminus is Extracellular and the C-terminus is Intracellular and vice versa

Membrane protein orientation

Suppose a protein was inserted in the ER membrane. **One part of it facing the cytosol(part A) and another part facing the lumen of the ER(part B).** when it is transported to the vesicle, the orientation stays the same, part A of it facing the cytosol, and part B 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, Part A faces the cytosol, and part B faces the EC (extracellular) region.



Since part B that was facinf the lumen of ER and Golgi before fusion with plasma membrane is now facing the exterior of the cell,

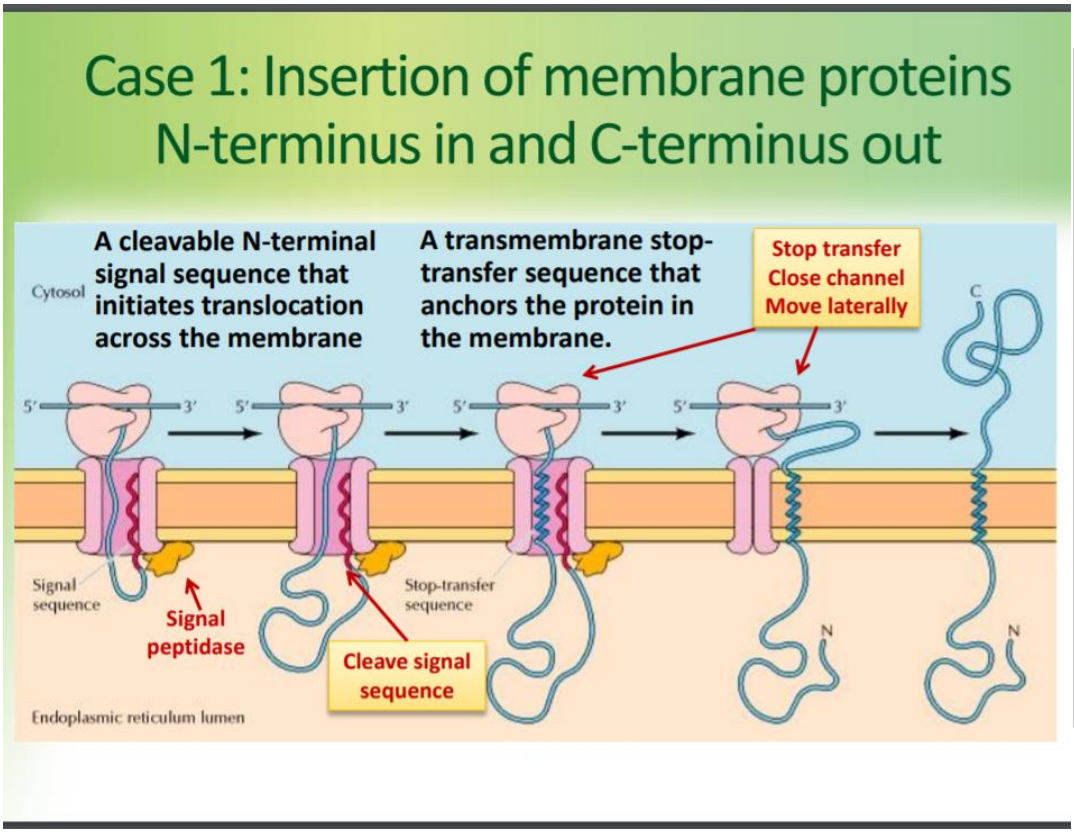
we can say :The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.

Question :

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

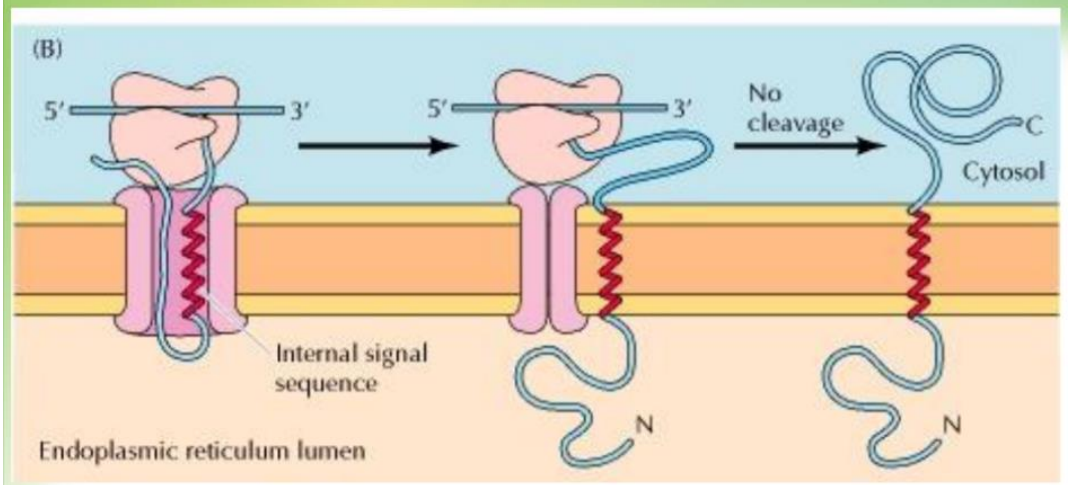
Any receptor has a ligand binding domain that has to be on the outside, now it is on the inside, so it is not going to bind the ligand ,thus receptor rendered dysfunctional .

Next, Different cases of insertion : (study diagrams thoroughly)



Stop transfer sequence formed by alpha helix of a 25-30 stretch of hydrophobic amino acids blocking the translocon , and then shutting it close. Protein is moved laterally ,translocation continuous until reaching end of c terminus

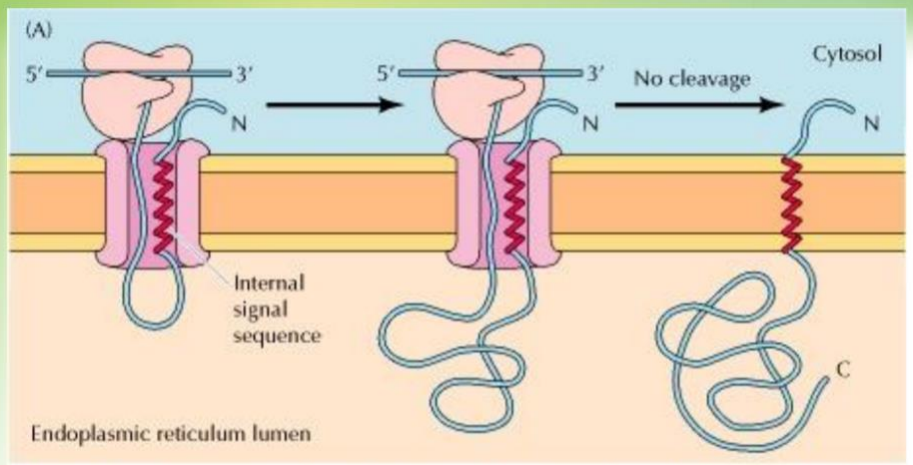
Case 2b: Insertion of membrane proteins N-terminus in and C-terminus out



The signal sequence is not cleaved by signal peptidase and acts as a transmembrane alpha helix.

,here signal sequence isn't in the beginning of n terminus but in the middle , so Internal signal sequence remains part of final protein

Case 2a: Insertion of membrane proteins C-terminus in and N-terminus out



The signal sequence is not cleaved by signal peptidase and acts as a transmembrane alpha helix.

Case 2a is the same as case2b ,but with reverse n and c terminus orientation,with n terminus on the outside , and reason for this is different primary amino acid sequence

Note that in the diagrams the translocon is removed in the last steps for simplicity purposes only .