Genetics
& Cell biology

◉ Sheet ○ Slides

Number

2

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Doctor

Diala

Endoplasmic reticulum (ER):

Is the largest organelle of most eukaryotic cells. It is a network of membrane enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm and they are directed to outside where they interact with golgi apparatus. ER has three regions:

-Rough ER: its outer surface is covered by ribosomes, and it functions in protein synthesis.

-Smooth ER: Lipid synthesis and metabolism.

-Transitional ER: where vesicles exit to Golgi apparatus.

The Secretory Pathway:

It is responsible for the secretion of proteins and ECM components (collagen, elastin, GAGs) to the outside of the cell.

The secretory pathway: ER → Golgi apparatus → secretory vesicles → cell membrane→ cell exterior /or/ lysosomal vacuoles (inside)

The order of the secretory pathway was discovered by the **pulse chase** experiment. Read it from the book, the doctor said it's included in the exam.

Unlike the name implies, the secretory pathway also includes the proteins that will be used **inside** the cell.

Ribosomes

There are 2 types of Ribosomes:

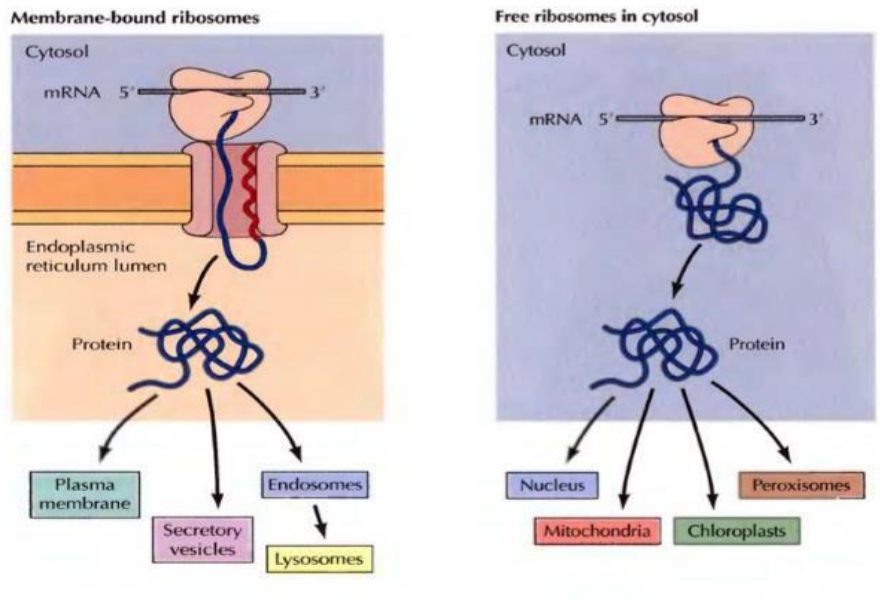
1) Free Ribosomes
2) ER-associated Ribosomes (bound ribosomes)

The final destination of a protein is what determines the site of synthesis of that protein (whether it will be synthesized in the free or bound ribosomes).

-Plasma membrane proteins, endosomal proteins, lysosomal proteins and proteins that will be secreted outside the cell are synthesized in the bound ribosomes.

- Cytosolic, nuclear, peroxisomal and mitochondrial proteins are synthesized in the free ribosomes.

-Generally proteins that will be used by the cell organelles are synthesized by free ribosomes, while proteins that will be secreted outside are synthesized by bound ribosomes.



All protein synthesis is initiated on free ribosomes.

During protein synthesis, ribosomes are targeted for binding to the ER membrane by the **signal sequence**.

Signal sequence is a short stretch of N-terminal hydrophobic amino acids present at the N terminus of the polypeptide and that sequence eventually will be cleaved.

Conclusion: **since the signal sequence isn't a part from the final functional protein since it will be cleaved.**

Translocation of the polypeptide to ER membrane happens either during translation (Co-translational) or after translation (Post-translational).

1) Co-translational translocation

Most of the time, proteins are translocated to the ER during translation.

Mechanism of co-translational translocation:

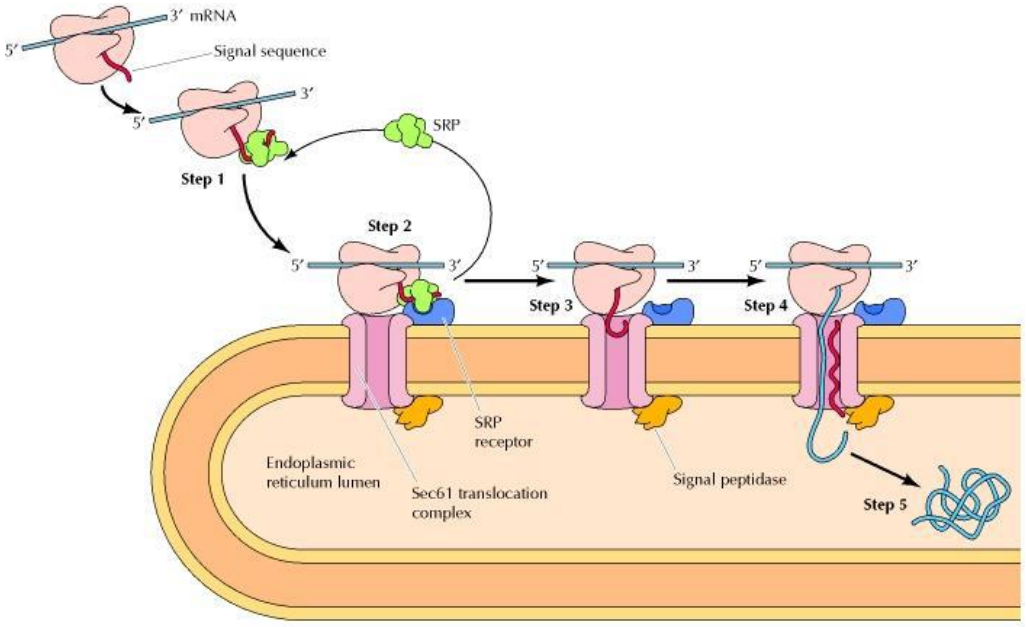
Step 1: As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP).

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 (Sec61 proteins), and the signal sequence is inserted into a membrane channel.

Step 4: Translation resumes and the growing polypeptide chain is translocated (**pushed**) across the membrane into the ER lumen.

Step 5: Cleavage of the signal sequence by signal peptidase releases the polypeptide into the lumen of the ER.



Notes:

- **Pushing** of the growing polypeptide chain across the ER membrane is the result of the translation process.

- The translocon is enclosed initially and the binding of the SRP-ribosome complex to the SRP receptor induces a conformational change that opens it.

- Signal peptidase is a peripheral protein that is bound to the inner part of the ER membrane.

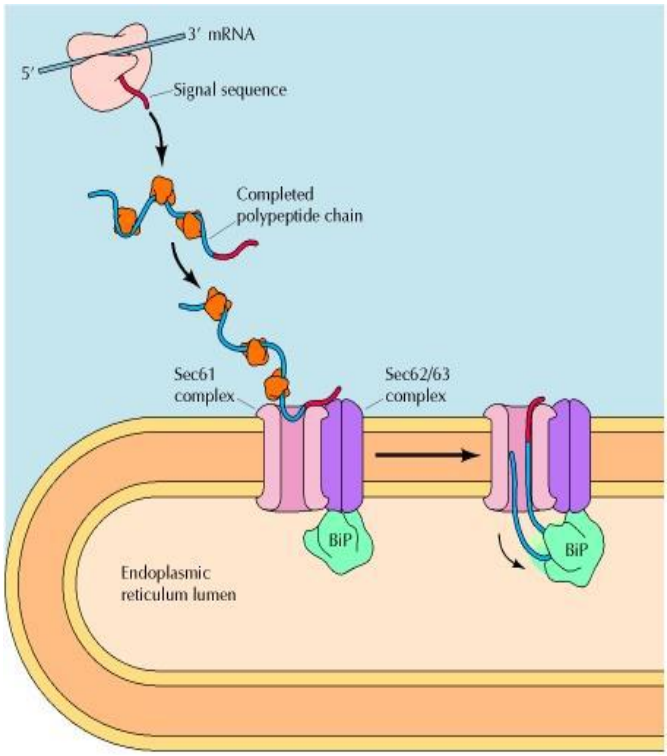
2) Post-translational translocation

Sometimes proteins are translocated to the ER **after** synthesis.

1. Proteins are synthesized on free ribosomes and **remain unfolded** by cytosolic chaperones (HSP70 and HSP40).

2. Their signal sequences are recognized by a protein complex (Sec62/63) (also a chaperone), which is associated with the translocon (SEC61) in the ER membrane. Here also, the binding of the chaperone-protein complex induce a conformational change that result in opening the channel.

3. The protein complex is also associated with a HSP70 chaperone protein (BiP), which **pulls** the polypeptide through the channel.



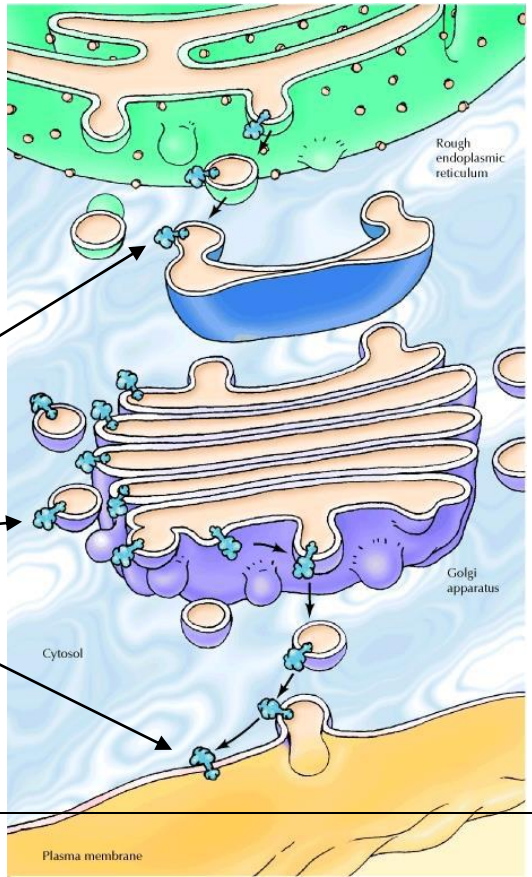
- ❖ Secretory, ER, Golgi apparatus, and lysosomal proteins are released into **the lumen of the ER**. (Soluble proteins)
- ❖ Membrane proteins are initially inserted into the **ER membrane.** (Hydrophobic proteins)

Membrane protein orientation

Membrane proteins are oriented in a specific way that allows them to function properly, for example plasma membrane proteins have 2 parts, one part facing the cytosol, and the other part facing the outside of the cell. plasma membrane proteins are synthesized and inserted into the ER membrane, and then leave via vesicles along the secretory pathway keeping their orientation (cytosolic part facing the cytosol and the extracellular part facing the inside of the vesicle), so when the vesicle fuses with the plasma membrane, the orientation and thus the function of the protein are maintained.

Note: **The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.**

- Generally, the part that faces the cytosol remains facing the cytosol and the part that faces the lumen will face the exterior of the cell.



Factors that affect protein insertion into the ER membrane:

1. Single vs. multiple membrane spanning region.

2. Orientation of N-and C-termini (often, each one of them exist in one side of the membrane).

Case 1: Insertion of membrane proteins N-terminus in and C-terminus out and composed of one helix

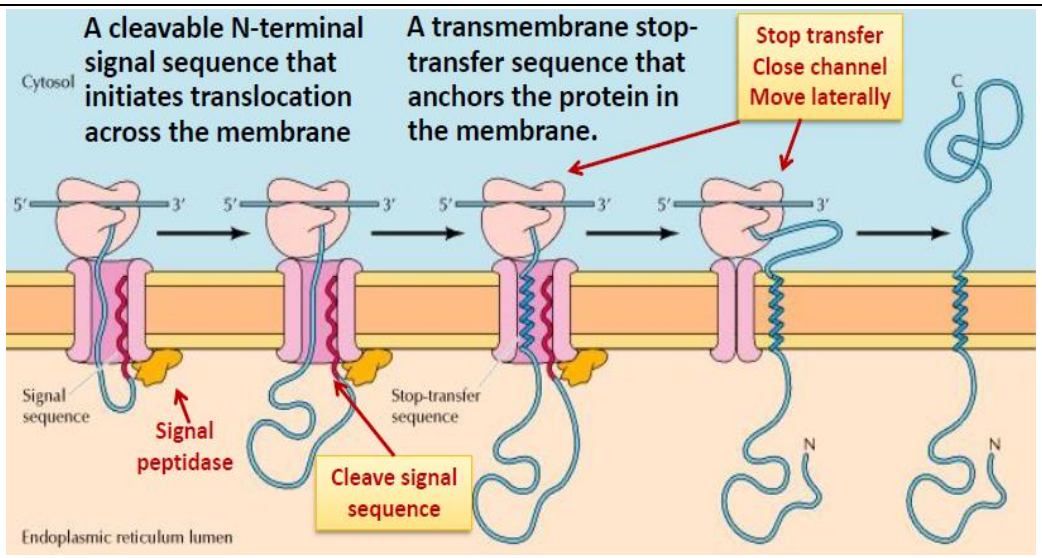
Note: Translation starts at the N- terminus and ends at the C- terminus.

- The ribosome is directed towards the translocon.
- The signal sequence is an N-terminal hydrophobic stretch of amino acids. At the beginning of translation, as the signal sequence enters the translocon, it forms a specific structure that **remains** inside the translocon.
- Then translation continues and we have another sequence known as the ''Stop Transfer sequence'', which is, again, a hydrophobic sequence that can form a helix due to the forces between the amino acids composing the sequence and therefore stop the transfer process.

Note: The Helix starts at the N-terminus and ends at the C-Terminus

- Now, a conformational change occurs in the translocon (canal) causing it to close, which pushes this structure laterally outside the translocon (it remains embedded in the ER membrane).
- There is a protein associated with translocon known as signal peptidase. It cleaves the signal sequence, therefore releasing the N-terminus into the lumen and the C terminus remain outside (the exact time of the cleavage process is not will determined but we most know that it's not at the beginning or at the end).
- Now we have a protein integrated inside the membrane with one helix, N-terminus inside and C-terminus outside.

Note: **The orientation of Stop Transfer Sequence determines the orientation of the protein (N-terminus + C-terminus).**

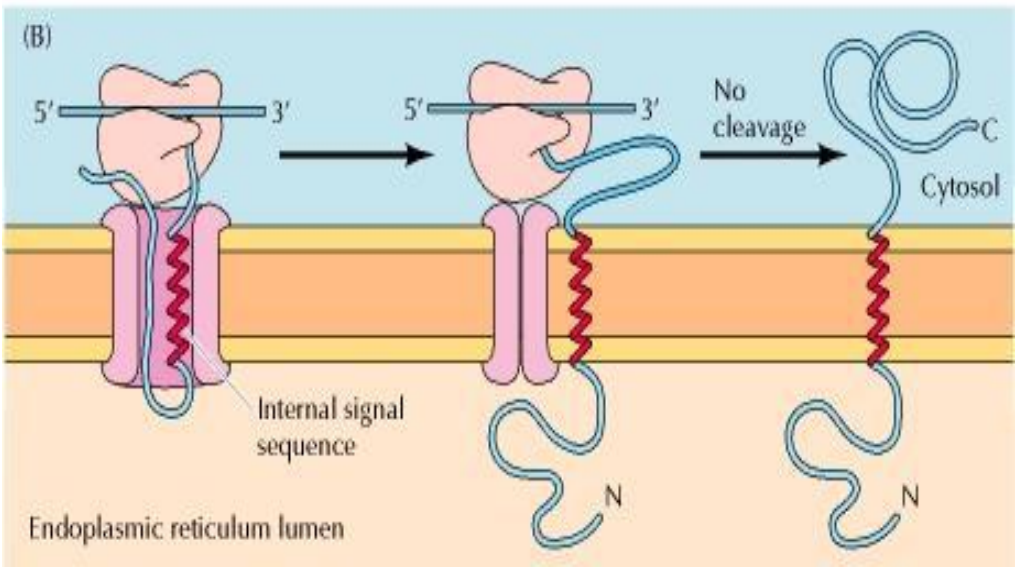


Case 1

Case (2A): Insertion of membrane proteins N-terminus in and C-terminus out.

- Here, the signal sequence is an Internal signal, unlike case 1, where the signal sequence was at the N-terminus (At the beginning of the growing chain), and **it won't be cleaved**, it remains as a part of the protein. So, NO need for signal peptidase.
- Translation is going on, and the N-terminus is facing the lumen and the C-terminus facing the outside. The internal signal sequence will fold quickly forming a helix inside the channel of translocon, and then move laterally through the lipid bilayer, keeping N-terminus inside and the C-terminus outside.

***Note*: The signal sequence is always hydrophobic whether in soluble proteins or in membrane proteins.**

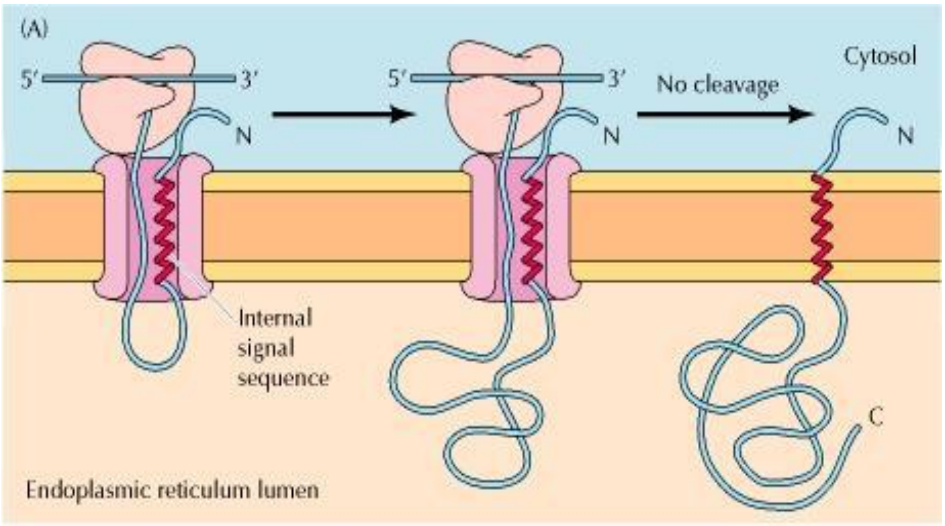


Case 2A

Case (2B): Insertion of membrane proteins C-terminus in and N-terminus out

- Again, the signal sequence is an internal sequence; notice the different in orientation, N-terminus facing the outside "cytosol", and the C-terminus facing inside "lumen". The internal signal sequence will fold quickly forming a helix inside the channel of translocon, and then move laterally through the lipid bilayer.

- **Note: the orientation of the helix folding and formation (the internal signal sequence) is what determines whether the N/C terminus is IN/OUT in the last two cases (2A and 2B)**

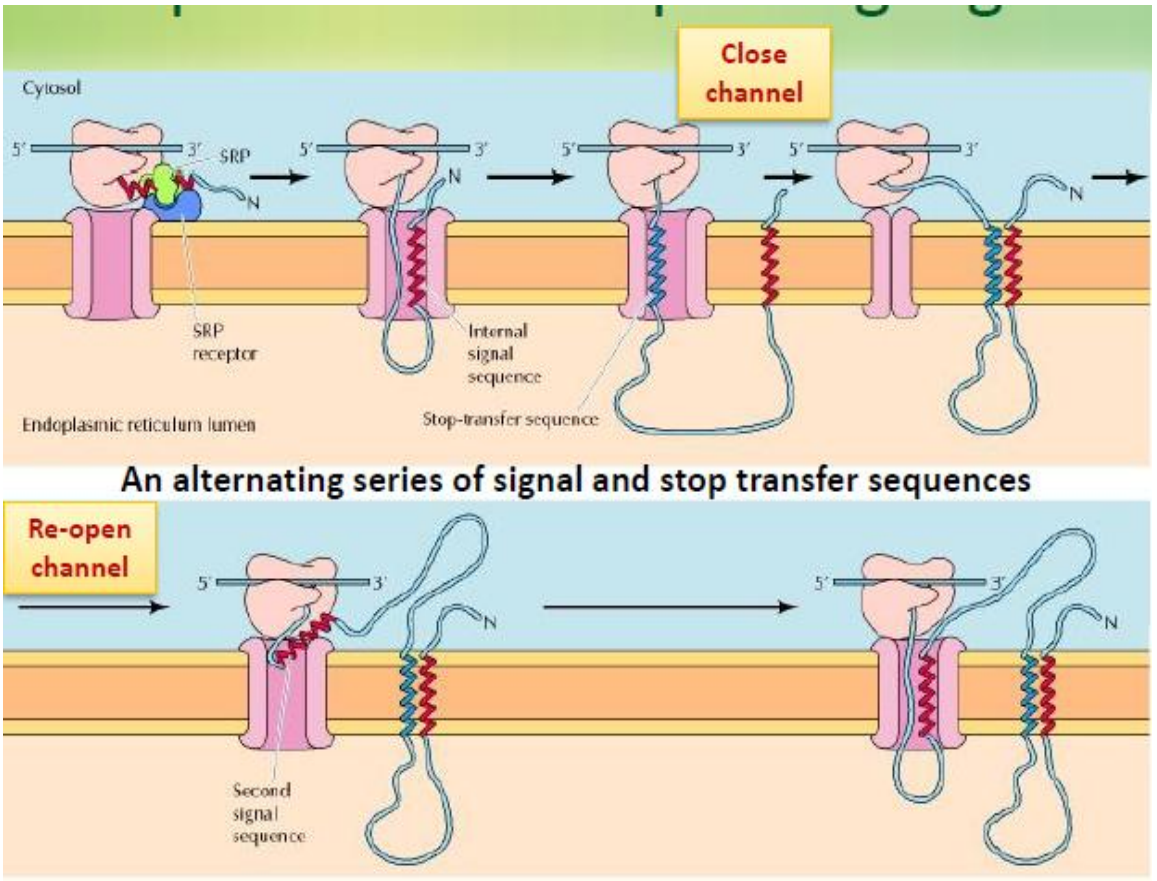


Case 2B

Case (3): Insertion of membrane proteins, multiple membrane spanning regions.

- The internal signal sequence forms a helical structure within the translocon (N-terminus outside, C-terminus inside), that will move laterally to the ER membrane.
- The stop transfer sequence is shown up again, (a sequence that is going to stop the transfer), and this sequence is going to fold into another helix that will also move laterally to ER membrane forming a second helix.

- We have again another signal sequence in the middle that is going to form a helical structure inside the translocon, move laterally through ER membrane. Now we have three helices within the membrane.



Case 3

An alternating series of signal and stop transfer sequences

The End