University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: **18**

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Subject: ER

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والنّيّة اجعل لوجه الله خالصة إنّ البناء دون الأصل لم يقم

This sheet was written according to section 1. **

Last time we start talking about **ER**, the **largest organelle** inside the cell, and the major function of ER was **protein modification and folding**. Modifications include different types; covalent modifications (add some lipids, or lipid anchors, disulfide bridges), these covalent modifications have to happen in ER .Also disulfide bridges are parts of protein folding.

For example, insulin protein has two chains; there are interchain and intrachain disulfide bridges that hold the 3D structure of this protein. '' again: structure is related to function, it is needed for proper function".

****

There are **two types of Ribosomes**; either attached to ER or free in the cytosol. Generally, **translation process starts in the cytosol on the free ribosomes**, then some of these ribosomes attach to the ER and some of them stay free.

** In the case of **attached** ribosomes; after the beginning of translation in the cytosol, they will attach to the ER and translocation is going to be mostly **co-translational**.

** In the case of **free** ribosome, mostly what we see is completion of translation and then translocation. "**Post-translational translocation**".

Targeting the growing polypeptide chains to the ER is dedicated by the presence of **signal sequence**, there are several signal sequences, of these, one that has a short stretch (short range) of hydrophobic amino acids .

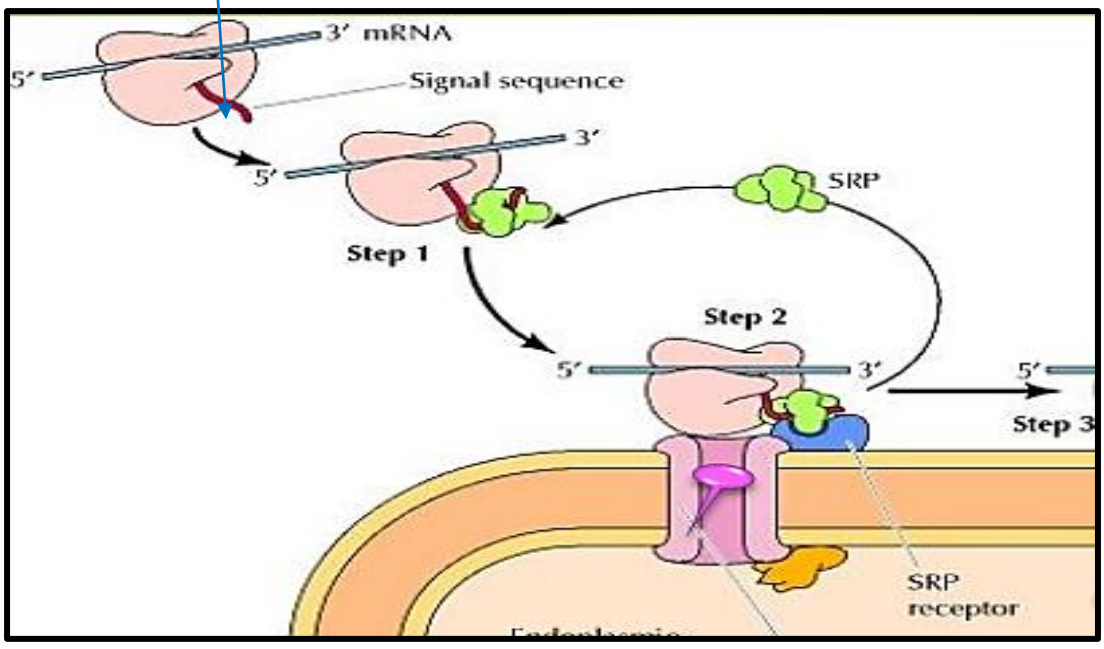
Note Slide 8 contains animations about how these proteins get inside the ER lumen.

Co-translational translocation of polypeptides to ER

starting with free ribosome, it attaches to the mRNA, and starts translation, once we have *a short stretch of growing polypeptide chain that contains the signal sequence* "showed in red color", this signal sequence is going to be identified by a protein called : **SRP** (signal recognition particle). Then SRP binds to signal sequence, and stops the translation process then it is going to bind to its receptor (**SRP receptor**) pulling the ribosoms with growing polypeptide chain with him.

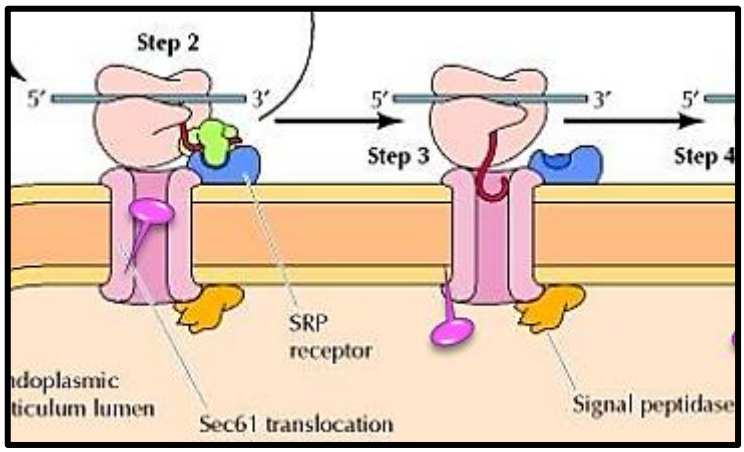
SRP receptor is a peripheral membrane protein attaches to a protein called ; Translocon .

Translocon: is a protein that has a channel through it the growing polypeptide chain is going to move to the ER lumen .



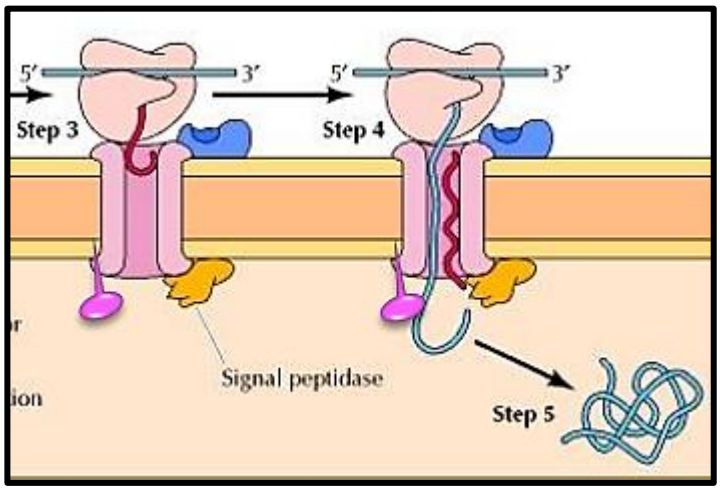
Notice that the the channel of the translocon is still closed by mean of something like arm, Once the SRP binds to its receptor, this binding will induce conformational changes that will open this arm and allow the entry of the growing polypeptide chain .

The growing polypeptide chain must be disassociated from SRP to enter (SRP will remain outside, while the growing chain will enter inside ER lumen through the translocon)



There is also another peripheral membrane protein attached to the inner surface of ER membrane called : (Signal Peptidase) , that is going to cut the signal sequence from growing polypeptide chain. This also will reactivate the translation process, **because when SRP had bound to the signal sequence, it inhibited the translation .**

- So we have the separation of growing chain from SRP, the translocation into the lumen through the translocon and translation resuming.



Now we have the whole polypeptide chain of this protein inside the ER lumen. so now it can start folding. *** see the next page

NOTE

This protein that is in the lumen is a **soluble protein**, it is not a membrane protein," if it is a membrane protein it must be anchored to the ER membrane as the first step, and then it going to be transferred by a vesicle into Golgi, then to lysosome, plasma membrane or whatever the destination.

Yes, chaperons are proteins that help and assist in protein folding and checks for proper folding... "We saied (help) because **the information needed for protein folding are inherited in the primary sequence of amino acids**.

Is that mean there is a chaperon in ER lumen?! ***

→ Here, the doctor showed us an animation about translocation :p

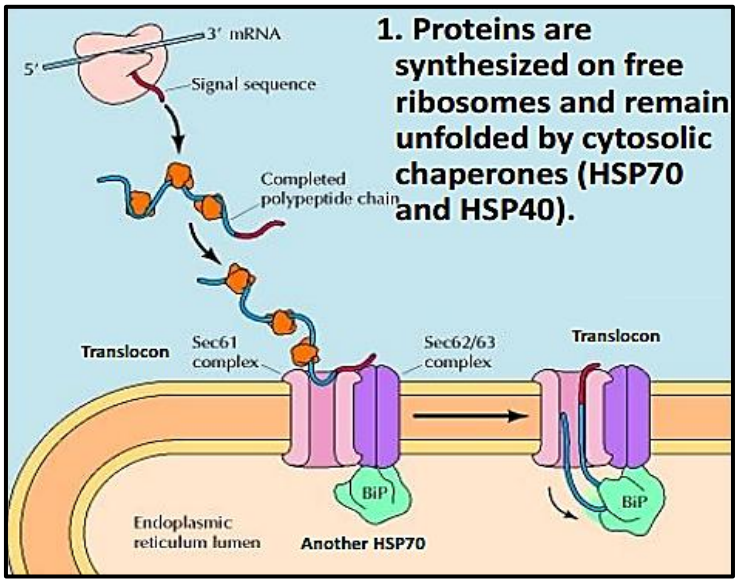
** In **co-translational translocation**, the N-terminus enters ER lumen before C-terminus.

Post-translational translocation

(The picture)... We have the ribosome bound to mRNA and growing polypeptide chain, we have a **signal sequence** (*different one*), during translation, **there are some chaperones that keep polypeptide chain unfolded until the translation is completed.**

**After that, the polypeptide chain is directed to translocon.

The **signal sequence is going to bind a complex (co-receptor) next to the translocon called: " Sec 62\63 " complex.



**This binding will open the translocon, and the growing chain will move through the channel into the lumen.

**Once the polypeptide chain is inside "next to ER membrane" is going to interact with a chaperon called: (Bip) and Bip will help in pulling of this protein.

Notes concerning post-translational translocation:

-No SRP.

-This type of signal sequence is going to be recognized by a sec 62\63.

-No signal peptidase, instead Bip will bind the growing chain and help in protein folding.

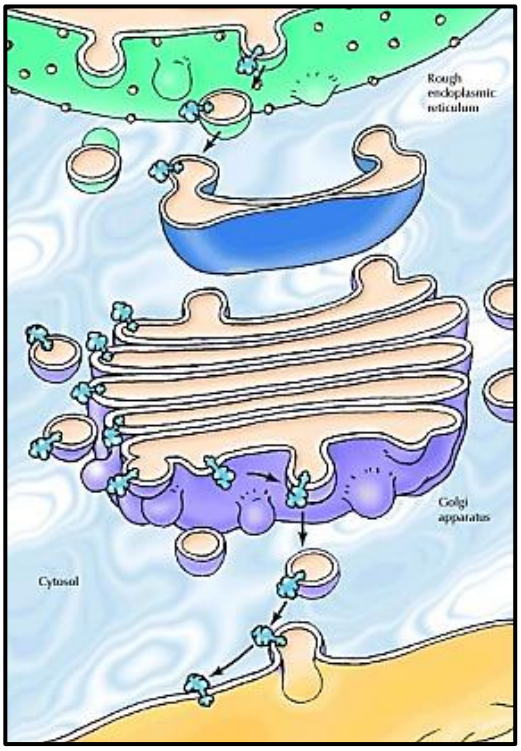
All what we discussed previously either co-translational or post-translational translocation all about **soluble proteins** not membrane proteins.

**** What about **integral membrane proteins**? They might span the membrane once or more, have the N-terminus inside and C-terminus outside or vice versa (the C-terminus inside and the N-terminus outside).

The orientation and how many times it will span the membrane determine the way it's going to be translocated as a membrane protein..

All the membrane proteins have to be inserted in the ER membrane (even if they aren't ER membrane protein) and then translocated as a part of viscular membrane to Golgi, plasma membrane, lysosome or whatever the destination.

Some of these proteins will stay in the ER membrane as a membrane protein like Translocon.



How about the direction (orientation) of the protein?

Any membrane protein has a direction, for example, plasma membrane proteins have a cytosolic part (face) and extracellular part (face). what determine this is the orientation and the direction of the insertion of the protein into the ER membrane.
** The part that will face the cytosol is going to be directed toward the cytosol.
** The part that will face the extracellular matrix (if we are talking about plasma membrane protein) it has to be inside the ER lumen.

About the above figure: *

First, the membrane protein has been inserted into the ER membrane, '' the big part of the protein is directed outside (cytosol), and the small part is directed into the ER lumen". A vesicle will come from ER, fuse with Golgi and finally fuse with the plasma membrane.

** **As the vesicle fuses with the plasma membrane the small part, which was luminal, now it is extracellular (outside), and the large part -which was cytosolic- it will stay facing the cytosol (inside)**

The lumens of ER and Golgi are topologically equivalent to the exterior of the cell

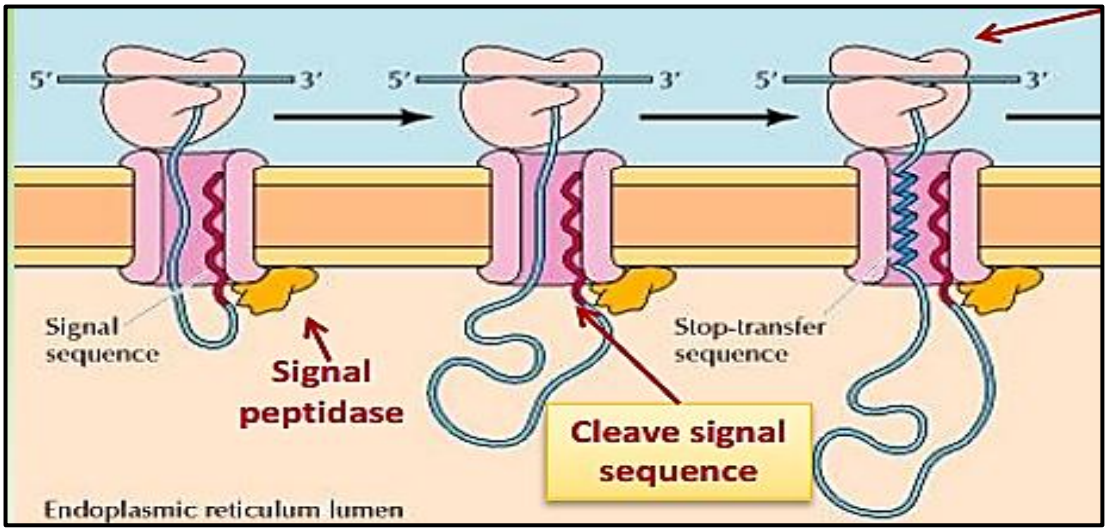
How about the insertion?

As we said, the orientation may be different and it's affected by how many times the polypeptide spans the membrane.

Case 1: Insertion of membrane proteins N-terminus in and C terminus out.

- Suppose I have the ribosome with the growing polypeptide chain, the signal sequence is directed into the translocon.
- If you remember that the signal sequence was a hydrophobic stretch of amino acids, this sequence will form a helical structure from the N-terminus to the C-terminus.

> Notice the helical structure (the signal sequence) is located at the beginning of the polypeptide chain (N-terminus)



*Signal peptidase is going to cut the first signal sequence, because the function of the signal sequence is just to direct the growing chain into the translocon on ER.

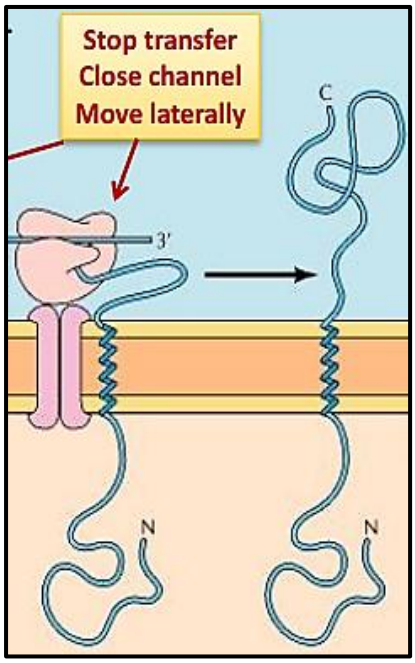
*Then translation continues and we have another sequence **"Stop Transfer sequence"**, which is, again, a hydrophobic sequence that can form a helix. " stop the *transfer* process".

*Now, the **stop transfer sequence** is folded into a helix, the direction of the helix determines the orientation or the placement of the N-terminus versus the C-terminus.

In this case, the N-terminus is directed inside, while the C-terminus is directed outside "cytosolic side".

Now, the helix since it is a hydrophobic sequence, it is inserted in the membrane, and move laterally.

After translation is done, the C-terminus will be the end of the polypeptide chain, so, the N-terminus is inside and the C-terminus is outside.



The signal sequence is always hydrophobic either in soluble proteins or in membrane proteins.

Very important note:

In this case, the **"Stop Transfer Sequence", determines the orientation** of the protein

(N-terminus + C-terminus).

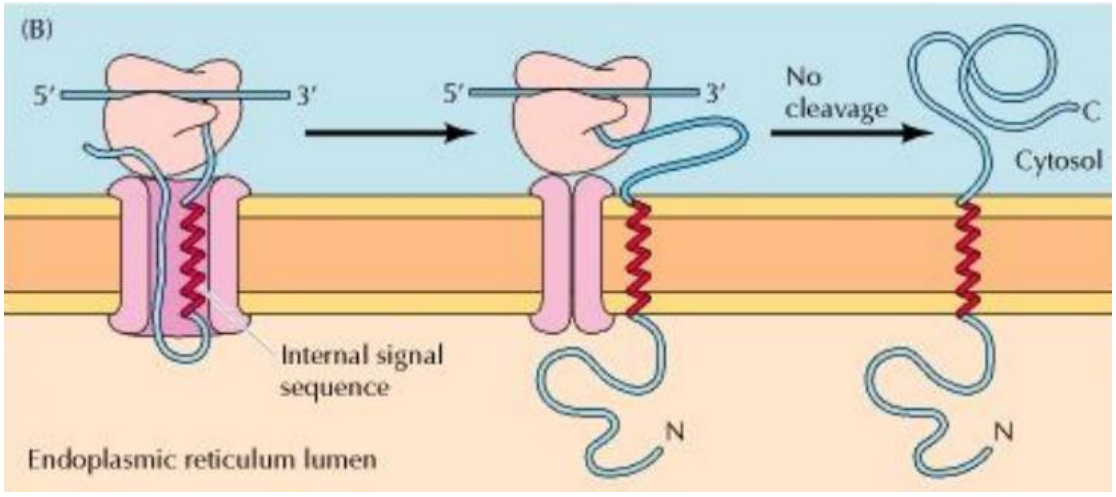
Case 2 (2b): **Insertion of membrane proteins N-terminus in and C-terminus out.** (slide 16)

*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 direction of internal signal sequence is the N-terminus facing the lumen and the C-terminus facing the outside.

*The internal signal sequence will form a helix inside the channel of translocon, and then move laterally through the lipid bilayer, keeping N-terminus inside and the C-

terminus outside.



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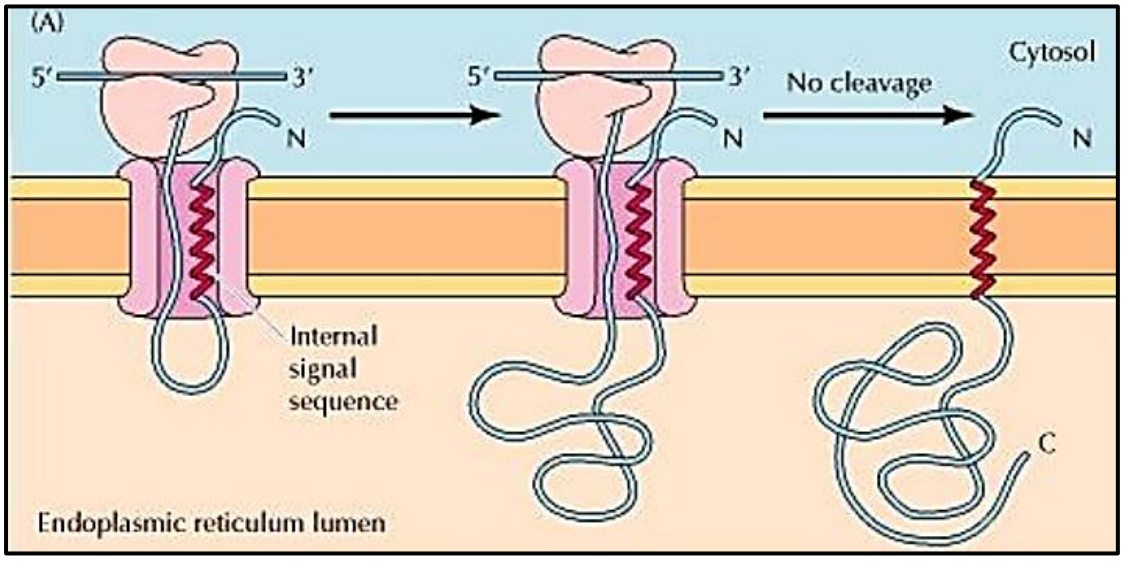
Case 2a: **Insertion of membrane proteins C-terminus in and N-terminus out.**



* 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" . (*Unlike case 2b*).

* After the translation is completed, the ribosome will dissociate, the rest of the protein will be inside, so, the C-terminal also inside.

Conclusion **Two ways to make the C-terminus outside, and only one-way to make the N-terminus outside.**

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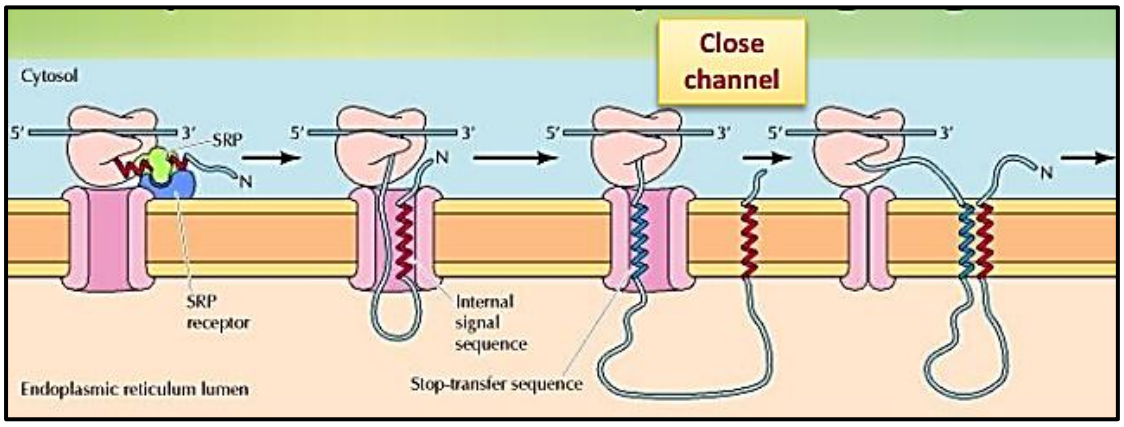
Case 3: Insertion of membrane proteins, multiple membrane spanning regions.



*Again, the growing polypeptide chain has an **Internal signal sequence** that will bind the SRP, then the whole complex "ribosome +growing polypeptide chain +SRP" will bind to SRP receptor on the surface of ER. (The sequence grow to the inside the lumen).

*The internal signal sequence forms a helical structure within the translocon (N-terminus outside, C-terminus inside), and the polypeptide sequence will grow to the inside.

I need to have more than one helix,,,, what will happen?

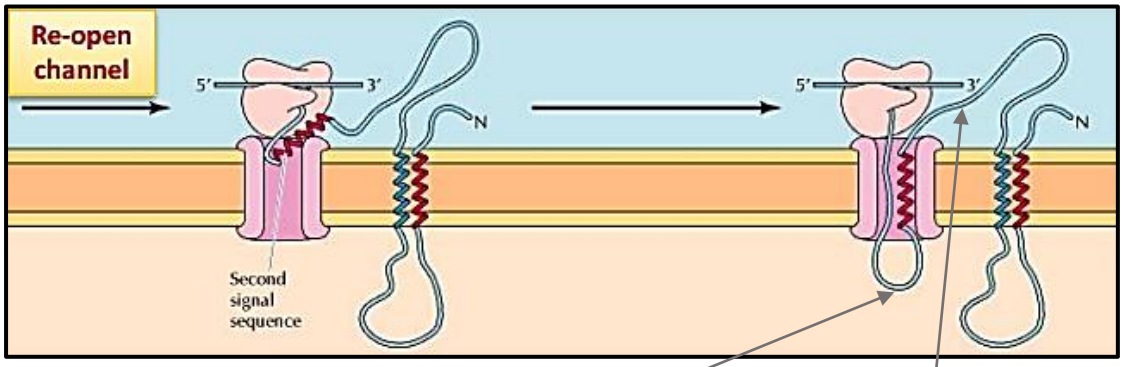
The **stop transfer sequence** is shown up again, (a sequence that going to stop the transfer, and this stretch of 20-25 hydrophobic amino acids is going to fold to a helix and form another helix).

*The first helix of **the internal signal sequence** is going to move laterally and the second one of **the stop transfer sequence**, again, is going to move laterally to form the second helix of the protein.

→ notice the loop structure that has been formed inside the lumen of ER. These loops are the most movable and variable region in any protein; (we have them between two helices because of the strike hindrance, ex: Trp cannot be followed by another Trp). Here in section 3 the doctor said that what determine the folding of the helix its primary sequence and

the strike hindrance *between Trp*, when it's at the beginning of the helix and *translocon's amino acids* , which affect the orientation (N\C terminus at outside\inside the ER)

To form more helices:



I have again **another signal sequence** in the middle that going to direct itself according to its primary sequence of amino acids, so we have the N-terminus outside "to the cytosol" and the C-terminus inside "lumen". This signal sequence will form a helical structure inside the translocon, move laterally through ER membrane.

→ Now we have three helices within the membrane, and the second loop protrude/ locate outside the ER.

The orientation of sequences and loops:

** **Internal sequence → stop transfer sequence →** 2^{nd} **Internal sequence.**

** The first loop is inside → the second one is outside …

Protein folding and processing in the ER

After we have the proteins in the ER, as a soluble protein or a membrane protein (whether it spans the membrane just once or more than that)-

→ Protein folding will happen by the assistance of chaperons.

In ER chaperon binds the protein and helps to fold properly, in addition to other modifications (add some lipids, disulfide bridges formation,).

How the ER assist in Disulfide bridge formation?

I need an **oxidizing reaction** to remove hydrogen to form disulfide bridges, so I need an oxidizing environment, (the cytocol has a reducing environment) in addition to enzyme called: "**Protein Disulfide Isomerase (PDI)**".

Note -→ the cytosol is more reducing environment

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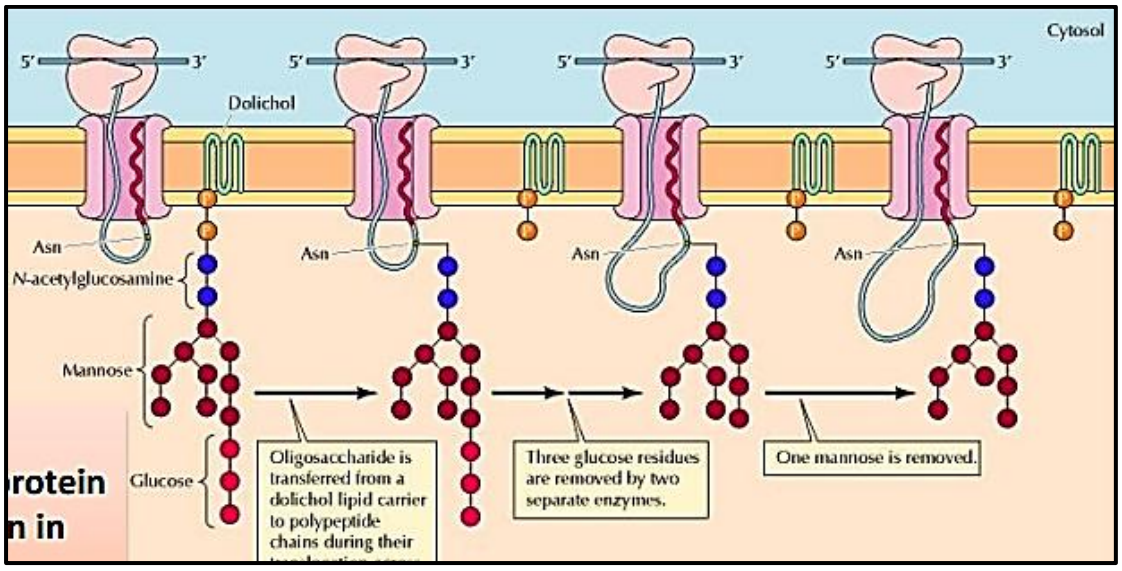
#Protein processing in the ER, N-linked glycosylation



*Glycosylation is the addition of sugar moiety to a protein forming glycoprotein.

in ER there is a sugar moiety that is attached to a lipid carrier: "**Dolichol** ", and this sugar will be added to the protein, that is being translocated into the ER, as the first step, then it is going to be modified\ changed (the sugar will not stay the same) .

** A Lipid part, inserted into the membrane and attached to a complex of carbohydrate (sugar).

**The sugar moiety is composed of two N-acetylglcoseamine, 9 mannose, 3 glucose residues.

The next step → cutting the sugar part from the **Dolichol** and attachment of it to the protein by a covalent bond.

"**It binds covalently to the N atom of the Asn residue (N-linked)**"

→ The modifications start by the removal of three glucose residues, then one mannose.

*** Glycosylation process needs an enzyme called:

"**Oligosaccharyl transferase**" → this enzyme will recognize certain amino acid sequence. (Asn -X- Ser/Thr), X any amino acid.

Some notes: (based on student questions)

ER function → modifications and folding, BUT translocation is not main function, it needs the transfer of the protein synthesized in the cytosol (or just after beginning of translation) into it -ER- for modifications and folding.

Translocon has another name "Sec 61", but for simplicity, we're going to use the general name (translocon).

What determine the direction of the N-terminus and the C-terminus within the membrane is the primary sequence of amino acids.

About case 2a: No SRP, there is nothing to pull the growing polypeptide chain toward the translocon; the hydrophobic sequence put itself in the translocon, to get away from the hydrophilic environment.

Sheet 17- page 10, when the SRP binds to the signal sequence, the translation process pauses for a while, until the whole complex is bound to the ER membrane and when SRP is released from its receptor, then the translation continues.

End of text ☺

Sorry for any mistake.