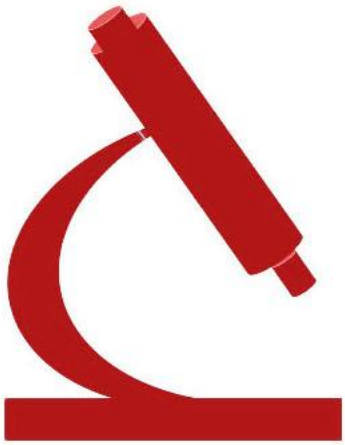


CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

7

Done by:

Nabil Sweis

Corrected by:

Ameen Alsaras & Ahmad Alazaidah

Doctor

Diala Abu-Hassan

❖ Insertion of proteins into the ER membrane:

➢ Brief Revision of previous lecture:

- Different proteins have different **final destinations**, one of which is the **membrane** (such as the *plasma membrane* or the *membrane of organelles* like the inner-mitochondrial membrane or lysosomal membrane). These proteins are called **membrane proteins.**

- Membrane proteins are initially inserted into the ER membrane during their synthesis.

- **Factors that affect protein insertion into the ER membrane:**

1) Single vs. Multiple membrane spanning regions

- Some membrane proteins **span the membrane by either:**

A. One helix.
B. Multiple helices.
C. Very few proteins span the membrane by beta sheets forming beta barrels.

2) Orientation of N- and C-termini

- Sometimes, the N-terminus of a plasma membrane protein for instance is extracellular and the C-terminus is intracellular. Sometimes it is the opposite.

Remember:

✓ The **cytosolic** part of the protein in the ER membrane → **REMAINS** → **cytosolic** when it reaches the plasma membrane.

✓ The **lumenal** part of the protein in the ER membrane → **BECOMES** → **extracellular** when it reaches the plasma membrane.

❖ Cases of membrane protein insertion:

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

✓ Before discussing case (1) specifically, the doctor briefly goes over the **general** steps during membrane protein synthesis:

✓ The ribosome starts the synthetic process ⇒ There is a **signal sequence** at the N-terminus ⇒ the protein (or more specifically the polypeptide) is transferred to the ER and the signal sequence attaches to the translocon as discussed earlier in translocation ⇒ it extends the rest of the polypeptide chain during translation ⇒ there is a protein inside called signal peptidase which cuts the signal sequence **(why ?)** because this sequence is not part of the protein's final structure (in this example)

In case 1, we are inserting part of the protein into the membrane (N-terminus in, C-terminus out):

A. The signal sequence at the N-terminus initiates translocation across the membrane. This signal sequence is cleaved (by **signal peptidase**) as the polypeptide chain crosses the membrane, so the N-terminus of the polypeptide is exposed in the ER lumen.

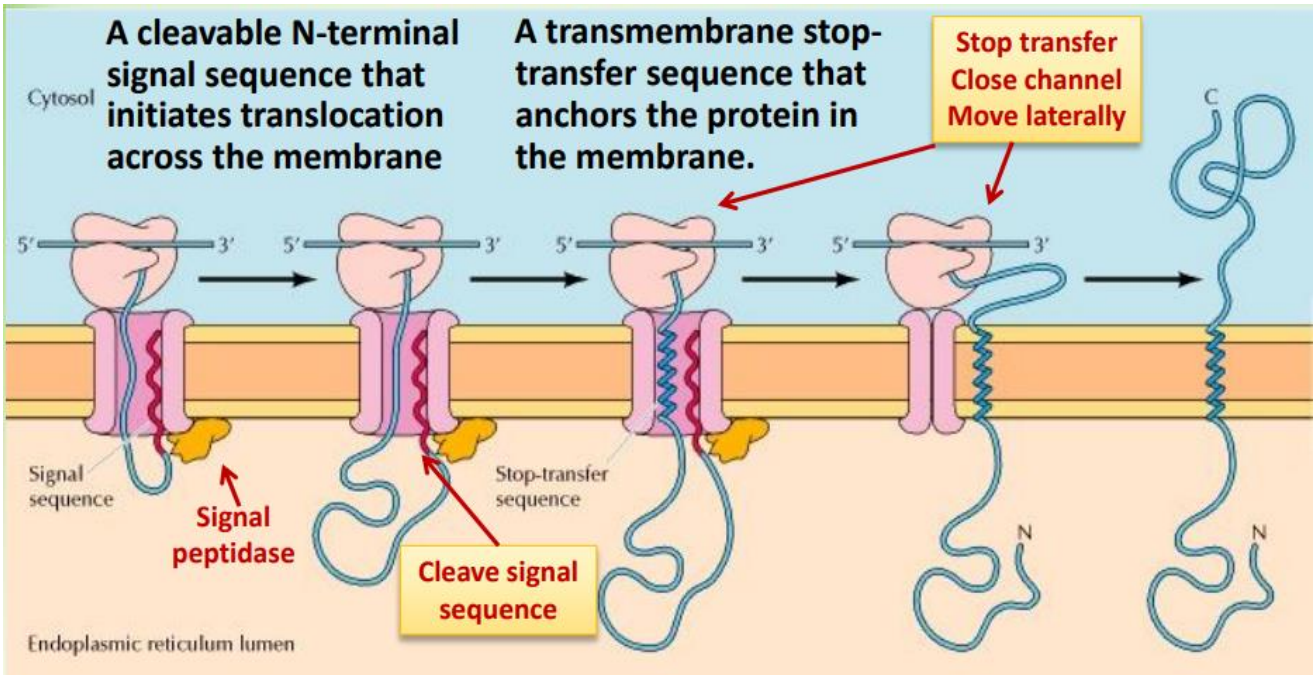
B. During translocation, we reach a **stretch of hydrophobic amino acids**, this **transmembrane sequence** is called a **stop transfer sequence**, and when it is recognized by the translocon (it attaches to its place on it), translocation is halted (stopped).

NOTE: What caused translocation to stop (what was the signal)?

- **The stop transfer sequence** (i.e. we faced a stretch of hydrophobic amino acids which will form a helix that is going to be inserted into the membrane-this hydrophobic sequence "fixes" the protein into the ER membrane and prevents further translocation)

C. A conformational change occurs (channel closes) and the helix then exits the translocon (moves laterally) into the ER membrane. Now the protein is inserted into the ER membrane.

✓ **Notice:** Because we allowed the entry of the polypeptide (the entry happens from the N-terminus to the C-terminus), and we left the N-terminus inside, the final protein has its N-terminus inside and its C-terminus outside.



-The previous image illustrates case 1 of membrane protein insertion.

---

2. Case 2 :

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

- The signal sequence in this case is **internal** (not at the N-terminus).

A. The transmembrane sequence (internal signal sequence) directs insertion of the polypeptide such that its N-terminus is directed outside (on the cytosolic side)

B. The transmembrane sequence exits the translocon to anchor the protein in the lipid bilayer and the remaining part of the polypeptide chain is translocated into the ER as translation proceeds.

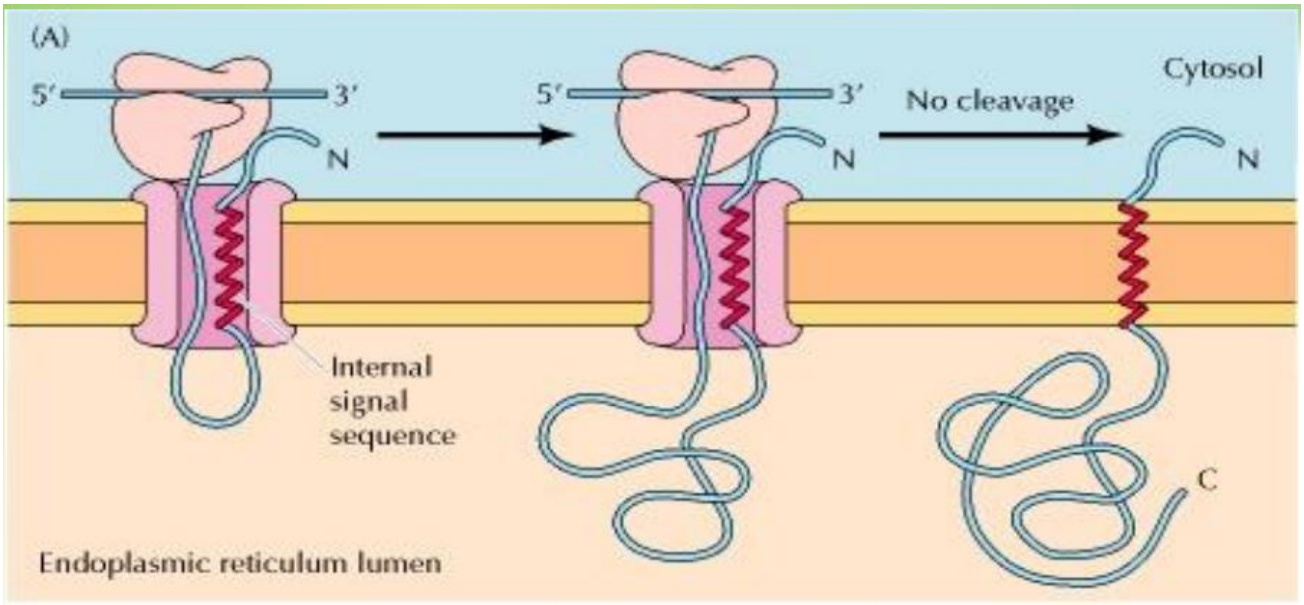
➢ Important Notes for Case 2a

✓ When the internal signal sequence enters, it has to be oriented in the opposite direction. (The start of the internal signal sequence (*its* ***N-terminus***) is directed ***outwards*****,** while its ***C-terminus*** is directed ***inwards***).

✓ The internal signal sequence itself is going to form a helical structure.

✓ The information necessary for the formation of the helical structure is inherent in the **primary sequence** (the primary sequence favors the formation of the helix in this direction).

- **Notice:** the signal sequence in this case is **NOT** cleaved by signal peptidase and it acts as a transmembrane alpha helix, because it is part of the protein structure unlike case 1.
- Also, in order for this case to occur, the signal sequence has to be internal, because this allows for the N-terminus to be placed outside.



- The previous image illustrates case 2a of membrane protein insertion.

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

(**Note**: In case 1, the N-terminus was also in and the C-terminus was out, but the difference between case 1 and this case is that in case 1, the signal sequence was cut by

signal peptidase, whereas in case 2b, signal peptidase is not needed, and the signal sequence is not cleaved).

In this case, an internal signal sequence is used to insert the protein with the N-terminus in and C-terminus out. How?

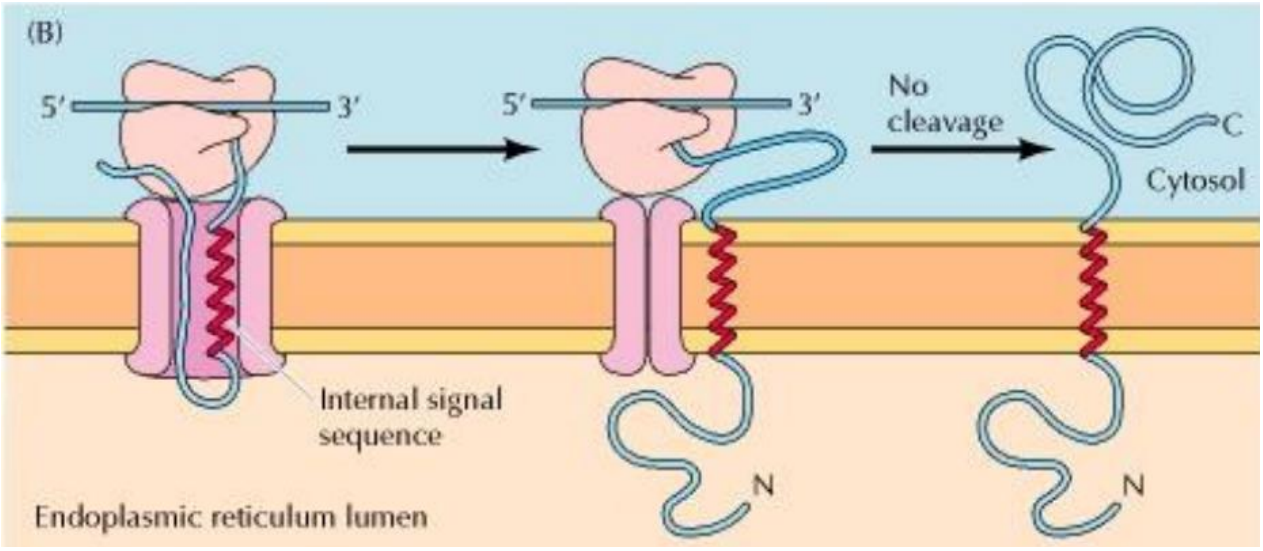
- Translation continues until it faces a stretch of hydrophobic amino acids that can form a helix, this stretch is actually the **internal signal sequence** (it will induce the formation of an alpha helix).

- The helix is oriented such that the N-terminus of the helix is inside while the C-terminus faces outside the ER
- The helix then exits into the ER membrane, with the rest of the structure entering inside the ER lumen, while the C-terminus is outside.

What governs this process (or what determines whether the helix is oriented in one way or another)?

-The **primary sequence** (which is the sequence of amino acids and the R groups) determines how the helix is oriented (which orientation it favors) and which side of the helix is inside, and which one is outside.

- The signal sequence is **NOT cleaved** by signal peptidase and acts as a transmembrane alpha helix.



The previous image illustrates case2b of membrane protein insertion

Notice that the internal signal sequence can be used to:

- Insert a protein with N-terminus out and C-terminus in as in case 2a
- Insert a protein with N-terminus in and C-terminus out as in case 2b (It all the depends on how the helix is oriented, that is, which side of the helix (the signal) is in and which side is out)

➢ The difference between case 2a and case2b is the orientation of the helix (internal signal sequence).

……

3. Case 3: Insertion of membrane proteins Multiple membrane spanning regions

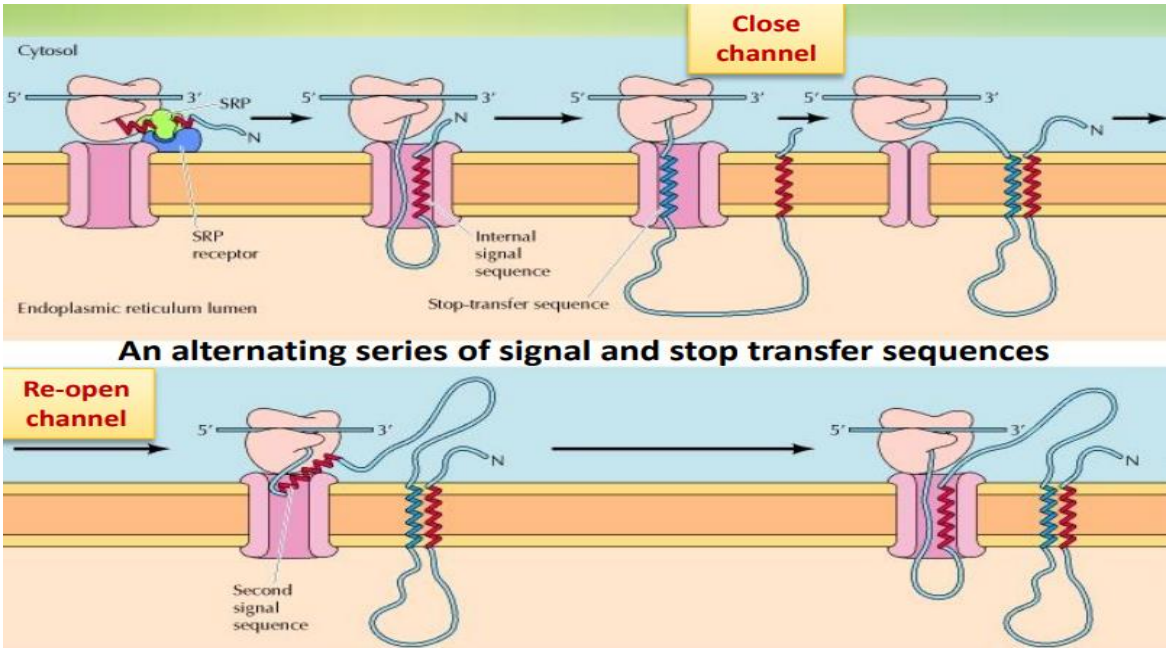
- Ribosome starts translation ⇒ SRP recognizes **internal** signal sequence and binds to it ⇒ SRP binds to its receptor close to translocon opening the structure of translocon ⇒ SRP is removed ⇒ entry of polypeptide into translocon ⇒ internal signal sequence forms a helix (with N-terminus out and C-terminus in as in this example) and then:

✓ The helix is going to exit the translocon into the ER membrane. Before it exits, we need to prepare for the next helix. Each helix is followed by a **loop** structure to start the next helix. So translation proceeds until a second transmembrane sequence is encountered.

✓ After the loop is finished, we are going to face a **stretch of hydrophobic amino acids** that form the second alpha helix. This long stretch of hydrophobic amino acids has the ability to stop the transferring process ⇒ Therefore, this new stretch is a **stop transfer sequence**. This second transmembrane helix then exits into the ER membrane. (**Note** that the translocon closes after it pushes out the two helices).

✓ To **reopen** the structure of translocon, we have another internal signal sequence (third transmembrane helix) which opens the structure and enters it , then it folds into an alpha helix, and then it exits the translocon into the ER membrane, followed by a loop, and another alpha helix (which is a stop transfer sequence) and the process is repeated , **alternating between an internal signal sequence with a stop transfer sequence , then another internal signal sequence with another stop transfer sequence…..etc.**

Note: Between every **internal signal sequence** and **stop transfer sequence**, there is a **loop.** Also, notice that one loop forms in the ER lumen and the next loop forms in the cytosol and vice versa. This pattern is repeated.



An alternating series of signal and stop transfer sequences

Note: a large percentage of proteins that act as channels consist of multiple helices that form the structure of the channel.

IMPORTANT: Notice that consecutive helices (in case 3) run in opposite directions.

- In the ER lumen, proteins undergo many processes which include:

❖ Protein folding and processing in the ER:

- When the protein is inside the ER lumen (whether all of it or part of it if it is a membrane protein), it can now undergo folding. (Also, if we have a protein with **quaternary structure** in which more than one polypeptide is present, we need to assemble them in the ER lumen to form the final functional structure of the protein)
- Protein folding is assisted by a group of proteins called **chaperones.**

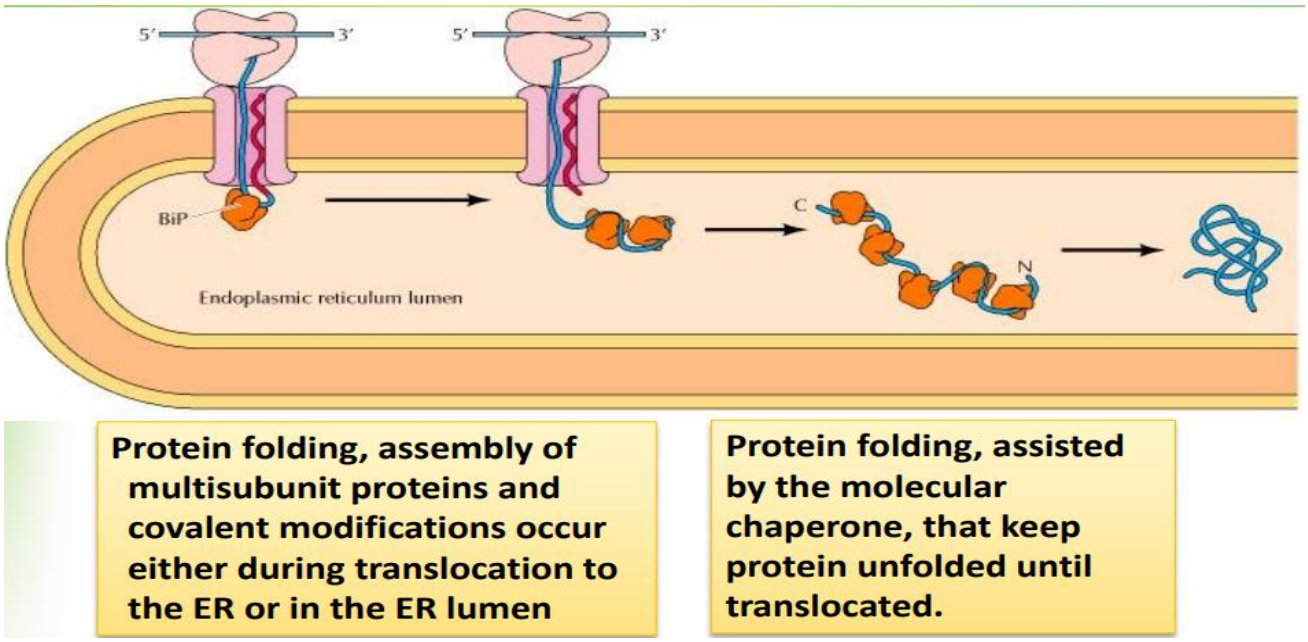
Chaperones = Folding assistants

- *Chaperones also play a role in keeping polypeptides (proteins) unfolded (refer to post-translational translocation)

- **Chaperones also check protein folding:**

If proteins are folded properly, they will be passed on into the next stage

If proteins are not folded properly, chaperones will open up the structure and allow it to refold.



The previous image illustrates the process of protein folding in in the ER

- Misfolded proteins are related to many diseases, such as **Alzheimer's disease**. (In some patients, they have what is called: *amyloid aggregates*, which are congestions of a misfolded version of a specific protein in cells).

- Another example of a disease caused by misfolded proteins is **sickle-cell anemia** in which:

- A **mutation** results in a change in the **hydrophilic amino acid Glutamate** which becomes **Valine (nonpolar and hydrophobic)** in hemoglobin (we know that hemoglobin is present in red blood cells in the blood, which is an aqueous environment).

- **(Notice that the doctor used the word "glutamate", which is the ionized form of glutamic acid. This amino acid (and its ionized form) are not to be confused with the other amino acid "glutamine" which is not part of our discussion)**

- Originally, **glutamate** was on the outer surface of the protein (in the final structure) because it is hydrophilic (exposed to the aqueous solution). **Valine** is generally **supposed** to be internal (when placed in an aqueous solution) because it is nonpolar. However, in sickle-cell disease, **valine** takes the place of **glutamate** on the exterior, and this is **unstable** in terms of energy because valine is hydrophobic and, as mentioned, valine is now on the exterior although it is supposed to be on the interior).

- The exterior "Valines" (hydrophobic) are uncomfortable with the aqueous environment, so they gather and accumulate forming **hydrophobic interactions** with each other, this results in a **sickle-shaped** accumulation of hemoglobin molecules, and this reflects on the shape of the whole cell.

❖ Protein folding and processing in the ER-Disulfide bonds

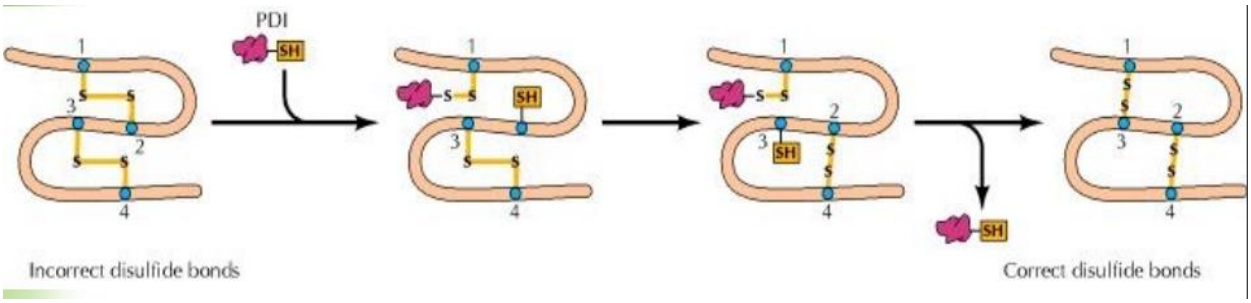
- **Dilsufide bridges** are the **only covalent bonds** that stabilize protein tertiary structure. Each disulfide bridge occurs between two **cysteine** amino acids
- For the formation of a disulfide bridge, we need to have an **oxidizing environment**.

The **cytosol** is ⇒ a **reducing** environment in comparison to the ER lumen.

The **ER lumen** is ⇒ an **oxidizing** environment in comparison to the cytosol

- So, the cytosol does not favor the formation of disulfide bridges, that is why this bond is formed in the ER lumen.
- Disulfide bridge formation is assisted by an enzyme called:
  Protein Disulfide Isomerase (PDI)

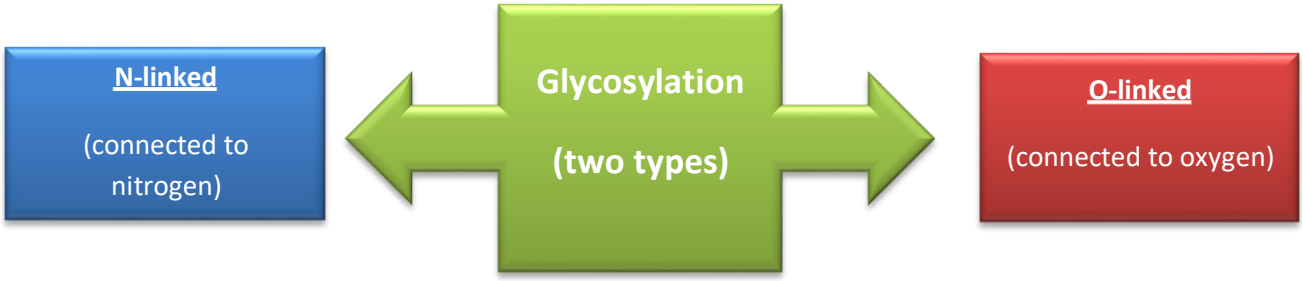
- **Disulfide Bridges could be either: *Intra*chain (within the same chain)**
  Interchain (between different chains)



❖ Protein processing in the ER … N-linked glycosylation:

- After protein folding and insertion into the membrane (insertion happens if it is a membrane protein), the next modification on proteins that occurs is:

Glycosylation: adding the sugar moiety (sugar moiety=sugar molecule/group or sugar portion of a complex molecule)



- The type of glycosylation that happens or starts in the ER lumen is **N-linked glycosylation.**
- Glycosylation can happen on **membrane proteins** or **soluble proteins (proteins that are going to be in the cytoplasm).** However, most of the time, glycosidic proteins are membrane proteins.

- (let's assume we have a membrane protein in this example):

➢ The protein (polypeptide) is being translocated into the ER lumen
- There is a certain amino acid in the polypeptide chain, which is **Asparagine** –an uncharged polar amino acid (has an amide group).

➢ A big sugar group (carbohydrate moiety) will be linked via a **covalent bond** to the **nitrogen** of the **Asparagine** in the polypeptide chain.

***When the sugar group is first added*, it is composed of:**

- ✓ **Two N-acetylglucosamine***
- ✓ **Nine mannose sugar molecules**
- ✓ **Three glucose molecules**

Total: 14

***N-acetylglucosamine**: is a modified glucose molecule in which an amino group is attached to the glucose, and an acetyl is added to the nitrogen of the amino group.

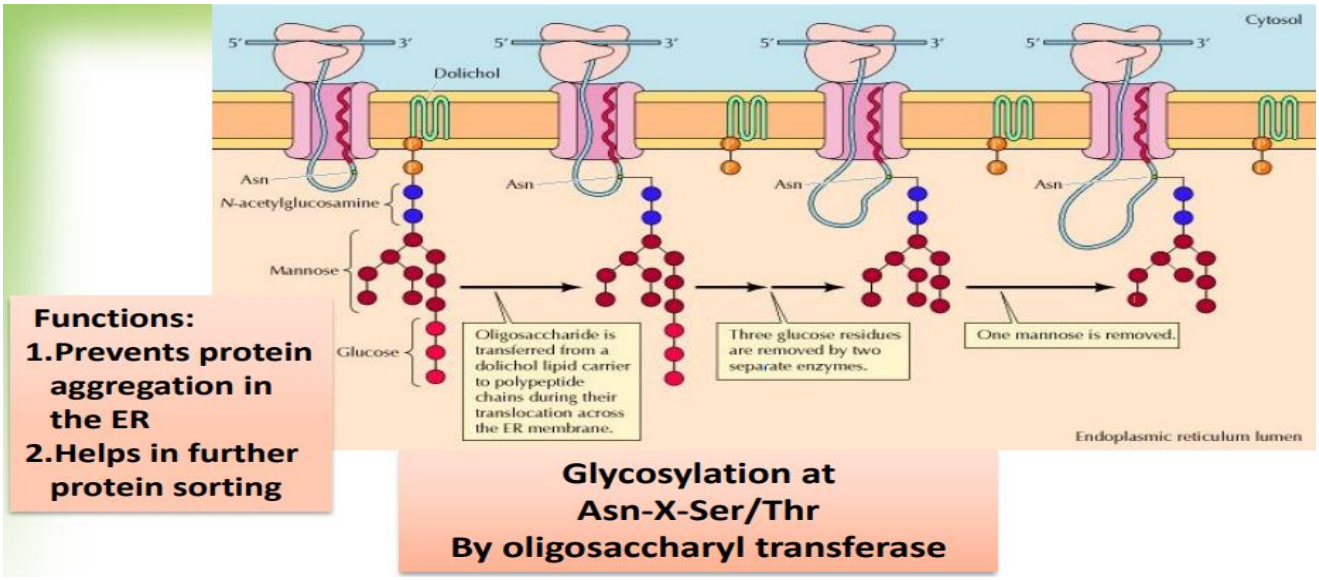
- **How is the sugar moiety (group) added?**

➢ In the first step, the sugar is transferred from a molecule called **Dolichol** (a lipid carrier of the sugar moiety) to the **nitrogen of the Asparagine** in the polypeptide chain. The sugar is now connected to the protein (polypeptide).

➢ **In the following steps, we start to modify the sugar portion:**

- **Three glucose molecules** are first **removed** by two separate enzymes
- Then **one mannose** sugar is **removed**

Note: This is not the final form of the N-linked sugar, as further modification will take place in the Golgi apparatus.



- **Note:**
  - ✓ **N-linked** glycosylation occurs at amino acids such as **Asparagine** in the polypeptide chain because it has nitrogen in its side chain.
  - ✓ **O-linked** Glycosylation occurs at amino acids such as **Serine** or **Threonine** because they have oxygen in their side chains

- **Importance of Glycosylation:**
  - Prevents protein aggregation in the ER.
  - Helps in further protein sorting

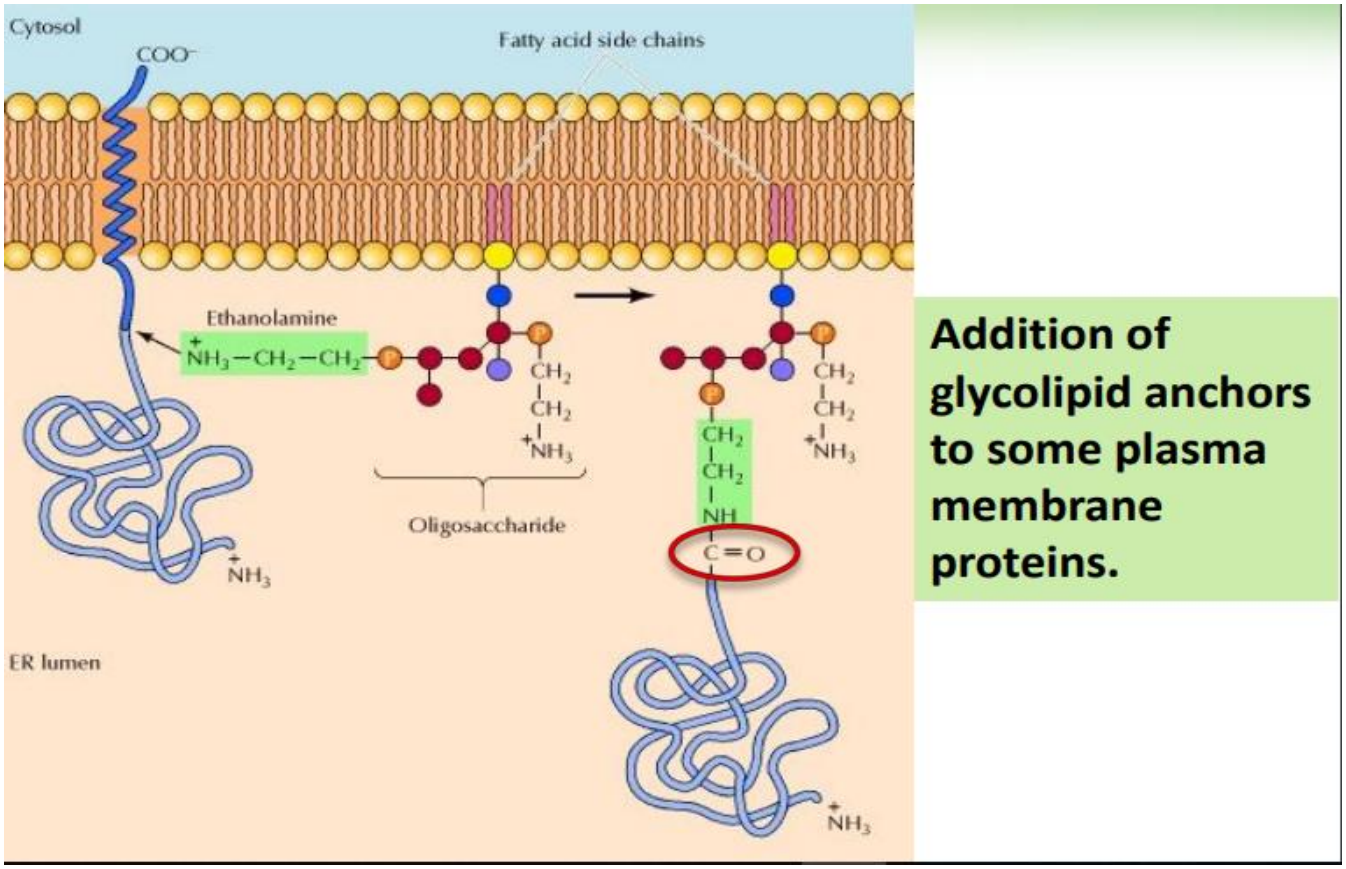
- The enzyme responsible for connecting the sugar group to the protein is called:
  Oligosaccharyl transferase.

❖ Protein processing in the ER-GPI anchors:

- Proteins (that will be anchored to the membrane) are first synthesized and translocated, but close to the end **(C-terminus**) of the polypeptide, **a helical structure forms** as part of the polypeptide chain and gets inserted into the membrane. (**BUT** this protein is not anchored yet, this is just a transitional and temporary stage)

- There is also a **phosphatidylinositol** molecule, its polar head consists of inositol, and it is attached to a group of sugars, and through these sugars it will get attached to the protein.
- Between the **protein** and **the sugars and phosphate group,** there is **Ethanolamine** which is going to get connected to the protein.
- We cut the protein separating it from the alpha helix and attach it to the ethanolamine part and thus connecting it to the GPI anchor.

- **Notes**: GPI anchors are added immediately after completion of protein synthesis to the **C-terminus**. The proteins remain attached to the membrane only by their associated glycolipid and will be transported to the cell surface as membrane components via the secretory pathway.



GOOD LUCK

Short Quiz:

Q1) A certain protein spans the membrane multiple times, if the first internal signal sequence (helix) is oriented such that its N-terminus faces outside the ER lumen and C-terminus faces inside, then the following helix will be:

A) An internal signal sequence with its N-terminus facing inside the ER lumen and C-terminus out.
B) An internal signal sequence with its N-terminus facing outside the ER-lumen and C-terminus in.
C) A stop translate sequence with its N-terminus facing inside the ER lumen and C-terminus out .
D) A stop transfer sequence with its N-terminus facing outside the ER lumen and C-terminus in.
E) A stop transfer sequence with its N-terminus facing inside the ER lumen and C-terminus out.

Q2) A mutation resulted in a defect in the enzyme "Protein Disulfide Isomerase" (PDI), this will most likely DIRECTLY impact:

A) Translocation of other proteins.
B) Translation of other proteins.
C) Tertiary structure of other proteins.
D) the oxidizing environment of the ER lumen and make it reducing.
E) None of the above.

Q3) Which of the following amino acid sequences will most likely form part of a stop transfer sequence:

A) Leucine-Methionine-Valine-Phenylalanine
B) Asparagine-Cysteine-Serine-Asparagine
C) Glutamic Acid-Arginine-Proline-Arginine
D) Cannot be determined**.**

Q4) A transmembrane protein has its N-terminus on the cytosolic side, <u>which of the following MUST be true:</u>

A) N-linked glycosylation cannot occur on this protein.
B) the signal sequence in the polypeptide must be internal.
C) the signal sequence is cleaved by signal peptidase.
D) A+B only.
E) All of the above.

Q5) Modification(s) on the sugar portion in N-linked glycosylation in the ER lumen include(s):

A) removal of 3 mannose sugars.
B) removal of 3 glucose molecules.
C) removal of one N-acetylglucosamine.
D) removal of one mannose sugar.
E) B+D.

FOR ANSWERS: LOOK AT PAGE FOOTER

Q1) Answer: **E**. Explanation: We know that in the case of proteins that span the membrane multiple times, we keep alternating between an internal signal sequence and a stop transfer sequence, and these helices run in opposite directions.

Q2) Ans: **C**. Explanation: PDI is responsible for disulfide bridge formation which is a key bond in the stabilization of protein tertiary structure.

Q3) Ans: **A**. Explanation: the stop transfer sequence is a stretch of **HYDROPHOBIC** amino acids. And so, we search for the sequence with nonpolar amino acids. (we are required to identify which amino acids are nonpolar or polar) .*Note that methionine can be found in the middle of an amino acid sequence, in addition to being found as a start codon at the beginning of sequences in general.

Q4) Ans: **B**. Explanation: We encountered this in case2a of membrane protein insertion, where we mentioned that the signal sequence must be internal. *The reason I put option A is because some students may think that because the N-terminus is outside the ER lumen, N-linked glycosylation cannot occur, but this is wrong because N-linked glycosylation has to do with the nitrogen atom in the side chain of some amino acids like Asparagine, not the nitrogen of the N-terminus. So (A) is wrong

Q5) Ans: **E**. Explanation: the answer is very direct. Modifications include removal of 3 glucose sugars then one mannose sugar.