Genetics & molecular biology

◉ Sheet ○ Slide

Number:	25
Done by:	تسنيم الرواضية
Corrected by:	-
Doctor:	- ديالا

Cell biology 3…

After the protein is translocated to the ER, it is going to be modified.

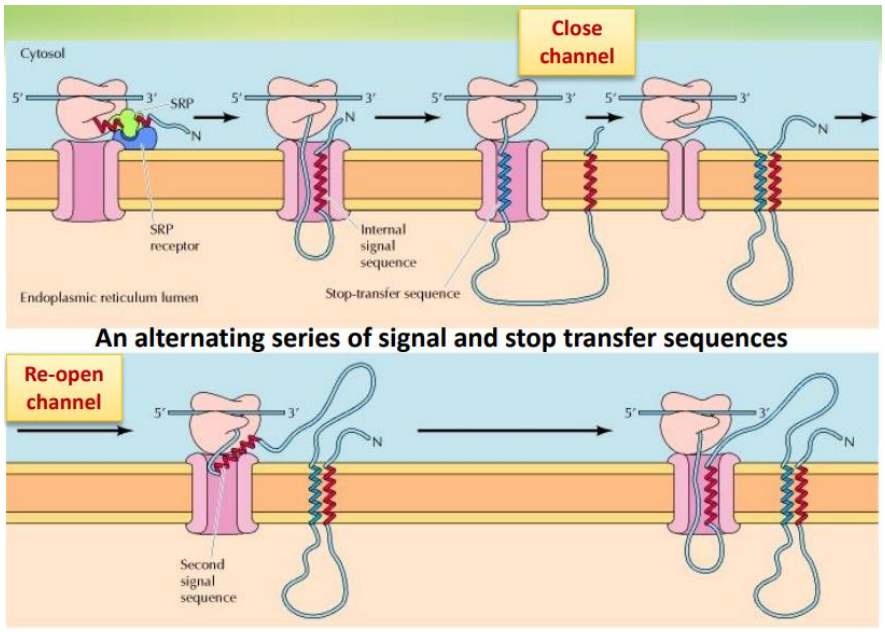
The modifications that are discussed in this sheet are:

1- Protein insertion (here is the last case).
2- Protein folding and processing in the ER.
3- Quality control in the ER.
4- Unfolded protein response (UPR).
5- Protein sorting and retention.
6- Synthesis of phospholipids in SER.
7- Synthesis of cholesterol and its derivatives.
8- Synthesis of ceramide.

Protein insertion

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

*The **internal signal sequence** forms a helical structure within the translocon (N-terminus on the *cytosolic side* of the membrane).
Translation proceeds then the helix will move laterally to the translocon.
*A **stop transfer sequence** is encountered, and this sequence will fold into another helix (N-terminus on the *luminal side* of the membrane) that will also move laterally to the translocon next to the first one.



An alternating series of signal and stop transfer sequences

* Another **internal signal sequence** appears again forming the third helix. Now we have three helices.

Note: It's an alternating process between having an internal signal sequence and stop signal sequence each one runs in an **opposite**

direction to the other; the internal signal sequence directs the N-terminus to the *cytosolic side* of the membrane, and the stop transfer sequence directs it to the *luminal side* of the membrane.

Protein folding and processing in the ER

-The folding of polypeptide chains into their correct three-dimensional conformations, the assembly of multisubunit proteins, and the covalent modifications occur either during translocation to the ER or in the ER lumen.

-Protein folding is happened by the assistance of a group of proteins called ***Chaperons***. After performing their function, Chaperons are either become part of the protein itself or dissociate without being part of it.

-Protein folding process includes non-covalent interactions and disulfide bridges formation (covalent).

THEREFORE, Chaperons' functions are:

1-Assist in protein folding.

2-Bind to the unfolded proteins preventing them from being improperly folded.

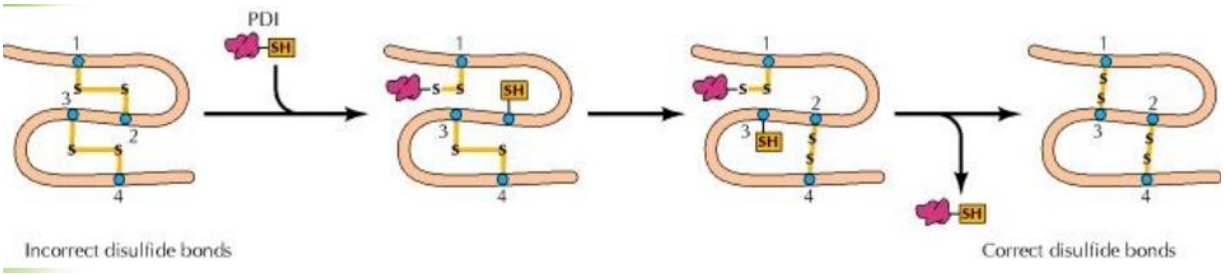
3-Bip (which pulls the polypeptide towards the ER lumen) is also a chaperon.

Always remember that: All the necessary information needed for the final 3D shape of the protein is inherited (present) in its Primary sequence. In addition, amino acids tend to interact with each other, by their nature, but these interactions may take place improperly that's why chaperons are important.

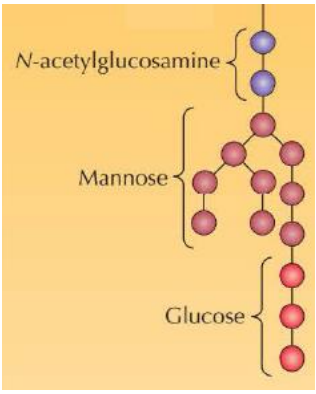
A- Protein folding and processing in the ER: *Disulfide bond:*

[Remember that: cysteine is a **thiol-containing** (-SH) amino acid**].**

The formation of disulfide bonds between the side chains of ***cysteine*** residues is an important aspect of protein folding within the ER. These bonds, generally, do not form in the cytosol, which is characterized by a reducing environment that maintains most cysteine residues in their reduced (-SH) state. In the ***ER***, however, ***an oxidizing environment*** promotes disulfide (S-S) bond by removing the hydrogen atoms. Disulfide bond formation is facilitated by the enzyme called ***protein disulfide isomerase (PDI)***, which is located in the ER lumen.



B- Protein processing in the ER: *N-linked glycosylation:*



In general, glycosylation is either N-linked or O-linked. N-linked glycosylation starts in the ER and further continues in Golgi apparatus. In N-linked glycosylation, the sugar moiety is linked to a nitrogen atom (amino group). The sugar moiety is an oligosaccharide that is composed of 2 N-acetylglucosamine, 9 mannose residues and 3 glucose residues.

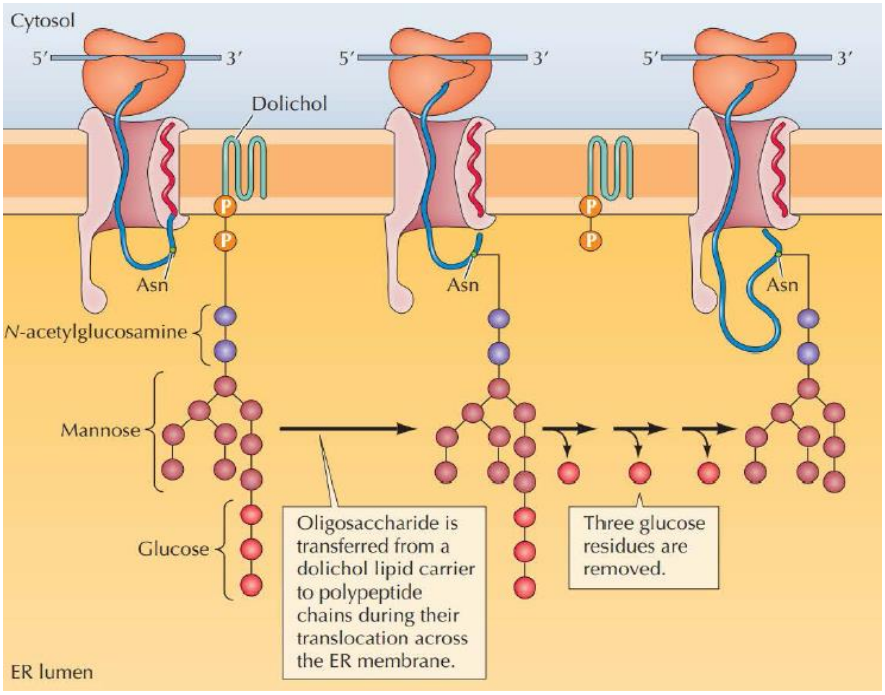
-A carrier, anchored in the ER membrane, called (***dolichol***) carries the sugar moiety.

- An enzyme called ***oligosaccharyl transferase*** is responsible for the transferring of the sugar moiety to the protein. The attachment site for the sugar moiety must be a nitrogen atom of an asparagine located in a certain amino acid sequence (Asn-X-Ser/Thr), where X referring to any amino acid, followed by either Serine or Threonine.

Once the oligosaccharide is attached to the protein, it undergoes some modifications:

1- Three glucose residues are removed.

2- One mannose residue is removed.



Note: The different types of N-linked glycosylated proteins have different oligosaccharide moieties; these further modifications will take place in Golgi apparatus.

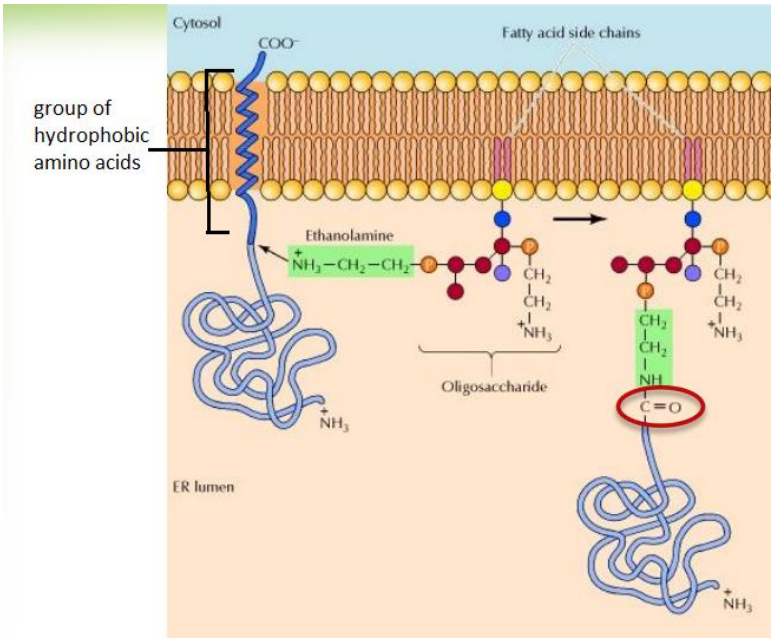
Functions of glycosylation:

1- Prevents protein aggregation in the ER.
2- Helps in further protein sorting.

c- Protein processing in the ER: *GPI anchors*:

Anchored proteins are attached to the membrane by several types of lipid anchors such as **glycosylphosphatidylinositol (GPI)**, so how does the addition to the GPI take place in the ER?

1- At the beginning, the protein will be attached to the membrane by a **(**group of hydrophobic amino acids**)** imbedded in the ER membrane. These hydrophobic amino acids have a helical structure.
2- The protein will be cleaved exposing the C-terminus.
3- The C-terminal sequence of the protein is attached to ethanolamine of the GPI anchor forming an amide linkage.
NOTES :-



- Proteins attach to anchored lipids instead of that group of AAs to be able to move and function as signaling molecules.

• Why is this protein attached to the group of hydrophobic amino acids in the ER membrane? Because if it was not attached to these amino acids, the polar nature of the cytoplasm would denature the protein.

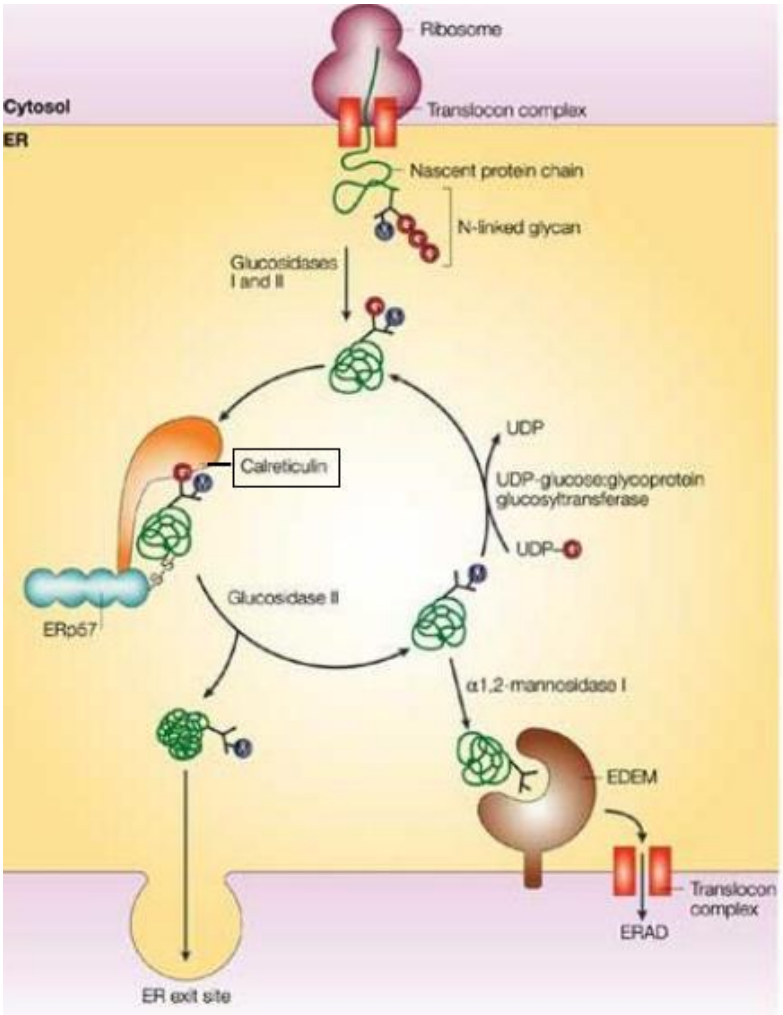
Quality control in ER " قسم ضبط الجودة "

One of the ER functions is to ensure that all its products, proteins specifically, are well synthesized and folded before being transferred to their destinations. ➔ Functioning as Quality Control Station.

Quality Control in ER is also called **ER-associated degradation (ERAD)**.

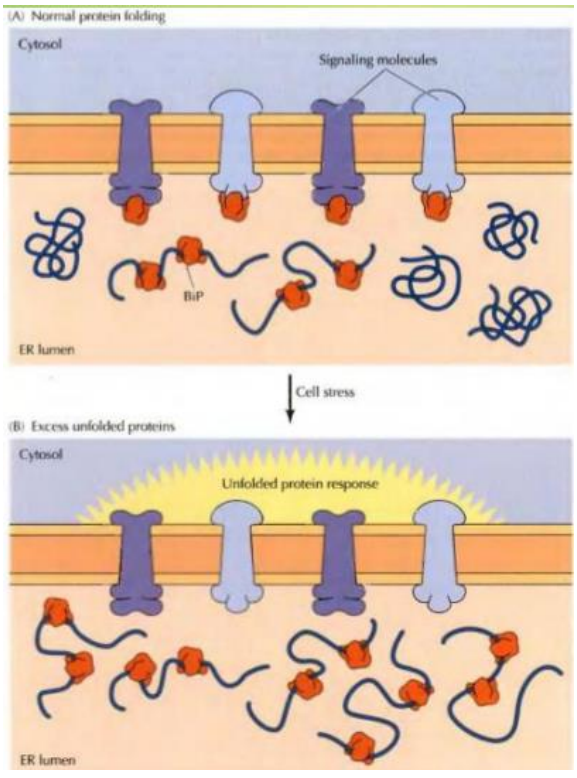
-Chaperones and protein-processing enzymes are misfolded-protein sensors that recognize or "sense" the folding position of the proteins. An example of such chaperons in the ER is ***calreticulin***.

- If the folding is correct, proteins move to transitional ER and then to Golgi AND if it is not, the following takes place:



1- Calreticulin <u>adds 3 glucose residues</u> to the misfolded protein indicating that it's not ready for the packaging and transferring to Golgi yet.

2-calreticulin, then, <u>unfolds</u> the protein and allows it to <u>refold again</u>. If it folds <u>*correctly*</u>, it will be packaged and transferred to the transitional ER and then to the Golgi. If it folds <u>*incorrectly*</u>, the protein will be <u>degraded</u> by ubiquitin proteosomal system (It will NOT be unfolded again, rather it will undergo DEGRADATION).



<u>From slides</u>:

- Calreticulin, a chaperone, helps in folding of glycoprotein, **and releases it when glucose is removed.**
- Misfolded proteins are identified, **returned to the cytosol** and degraded by ubiquitin proteosomal system.

Unfolded protein response (UPR)

UPR Coordinates protein-folding capacity of the ER with the physiological needs of the cell. It is activated when excess

unfolded proteins accumulate in the ER indicating that the cell is active producing large amounts of mRNA and proteins. ER accommodate that and maintain the folding of the protein through the unfolded protein response.

Activation of the unfolded protein response pathway leads to:

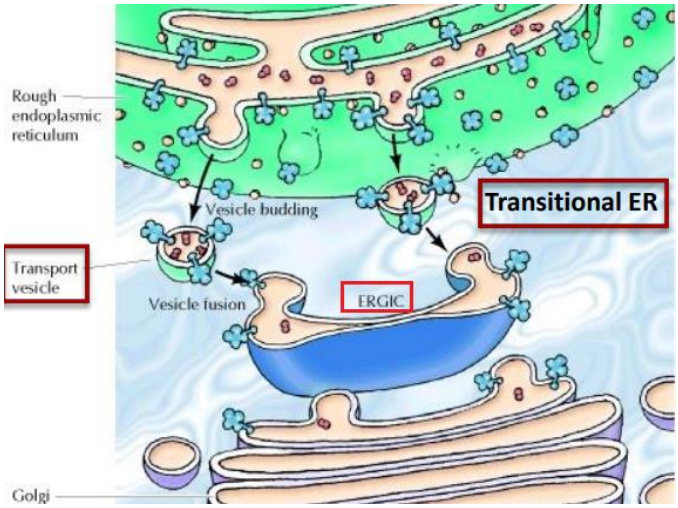
1. Expansion of the ER by increasing surface area.

2. Targeting of genes such as chaperones. (Production of additional chaperones to meet the need for increased protein folding).

3. Transient reduction in the amount of newly synthesized proteins entering the ER (by inhibiting the translation and translocation).

ER-Golgi intermediate compartment (ERGIC)

-After proteins are modified in ER, they will undergo further modifications in Golgi.
Transportation of proteins from the ER to the Golgi occurs through a compartment known as ***ERGIC***.
- When a vesicle buds from the ER, it will fuse with ERGIC and then moves towards Golgi.
-From slides: Note that topological orientation is maintained.



Protein sorting and retention

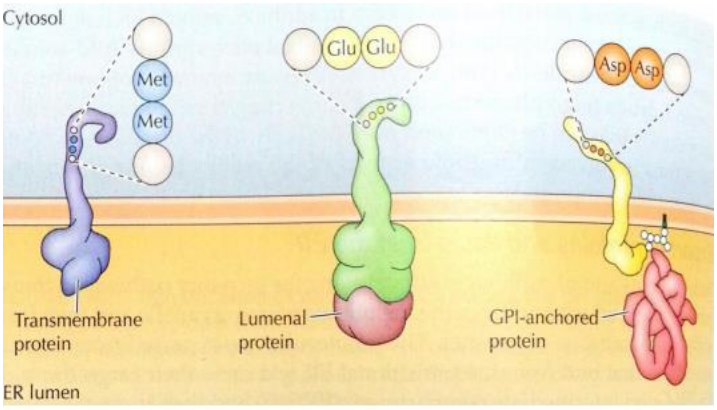
Each protein produced by the ER has to be transferred to Golgi apparatus before reaching its final destination.
One of these destinations is the ER itself. Therefore, proteins need to be recognized to head them back to the ER again, How?
-Through specific signal sequence such as:

1. ***KDEL*** sequence (Lys-Asp-Glu-Leu)
2. ***KKXX*** sequence (Lys-Lys-X-X)

Proteins bearing the KDEL and KKXX sequences are all to recycled back to the ER but are not prevented from being carried to Golgi. KDEL sequence is specific to the soluble proteins AND KKXX sequence is specific to the membrane proteins.

It was observed that all ER membrane proteins (from their **cytosolic side) share one point, which is, the replication of some amino acids in the form of binaries, these binaries could be:

1- Di-acidic amino acids (such as: Asp-Asp / Glu-Glu)
2- Di-hydrophobic amino acid (such as: met-met)



* They also function as carriers of GPI-anchored and lumenal proteins. They do NOT have any related function to the retention.

Synthesis of phospholipids in smooth ER (SER)

The SER is free of ribosomes, so it does not deal with protein modification, rather, it plays a major role in:

1. Membrane lipid synthesis.
2. Detoxification of chemicals.

The SER is responsible for synthesizing:

1. Glycerophospholipids (phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine).

2. Cholesterol and other steroid hormones, which are mainly synthesized in the testis and ovary (sex hormones).

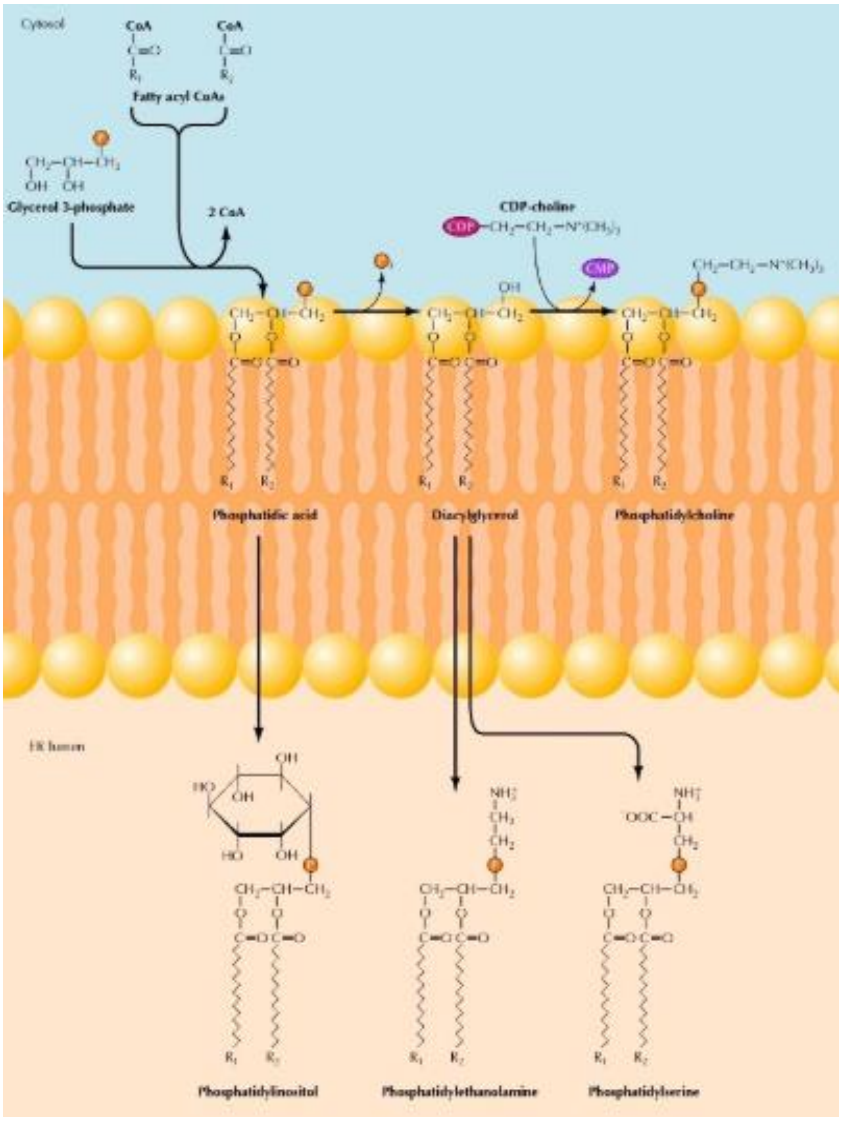
3. Ceramide.

The synthesis of glycerophospholipid (in brief):

To synthesize a glycerophospholipid, we need:

1. **Glycerol** in its active form (glycerol 3-phosphate).
2. **2 fatty acids** in their active form (attached to CoA)
3. **Different polar heads** (phosphate, amine, serine, etc...).

The synthesis process:



1- Addition of **2 fatty acids** to carbon 1,2 of the **glycerol 3-phosphate** producing **phosphatidic acid** (the "parent" molecule of the glycerophospholipids) that will be inserted directly to the membrane (THE OUTR LEAFLET, specifically).
2- A phosphate group is removed by a **phosphatase** enzyme producing **diacylglycerol**.

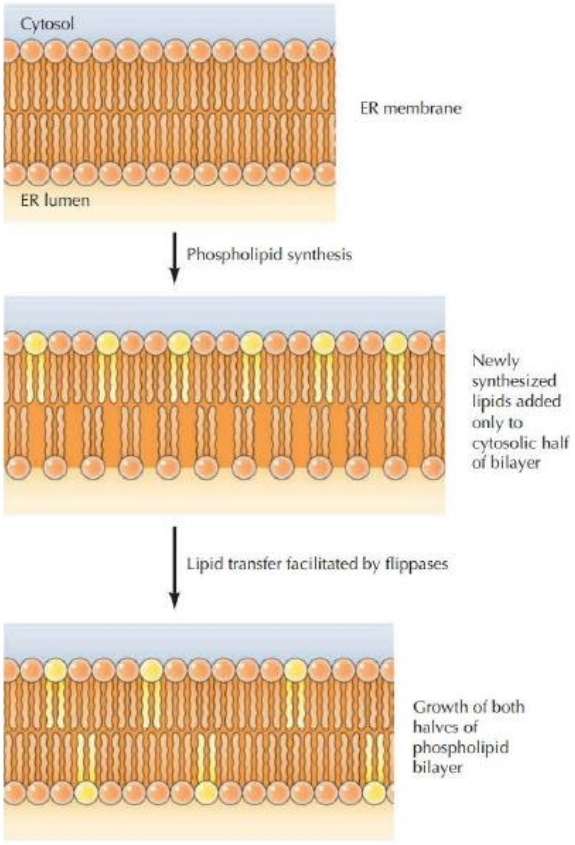
Now, diacylglycerol is used to produce the different types of glycerophospholipids:

A- Addition of phosphate group and choline (taken from CDP-choline molecule) to diacylglycerol produces **phosphatidylcholine**.
B- Addition of phosphate and inositol to diacylglycerol produces **phosphatidylinositol**.
C- Addition of phosphate and ethanolamine to diacylglycerol produces **phosphatidylethanolamine**.
D- Addition of carboxyl group to phosphatidylethanolamine produces **phosphatidylserine**.

TO SUMMARIZE:

1- (2 FAs) + (glycerol 3-Phosphate) = phosphatidic acid
2- (phosphatidic acid) – (Pi) = diacylglycerol
A. (diacylglycerol) + (Pi) + (choline) = phosphatidylcholine
B. (diacylglycerol) + (Pi) + (inositol) = phosphatidylinositol
C. (diacylglycerol) + (Pi) +(ethanolamine)= phosphatidylethanolamine
D. (phosphatidylethanolamine) + (carboxyl group) = phosphatidylserine

From slides: Enzymes are buried inside the membrane because the hydrophobic structure of lipids has to be maintained in close proximity to membranes.



These glycerophospholipids will insert themselves directly in the OUTER LEAFLET of the SER membrane, why?

- Because they are amphipathic molecules (hydrophobic fatty acid tail, polar heads), therefore they will be more stable in an amphipathic environment.

Increasing the number of the glycerophospholipid only in the outer leaflet will create some sort of inconsistency between the two leaflets.so, to achieve a balance between them; ***flippase*** enzyme is needed to flip some newly synthesized glycerophospholipids inserting them into the inner leaflet.

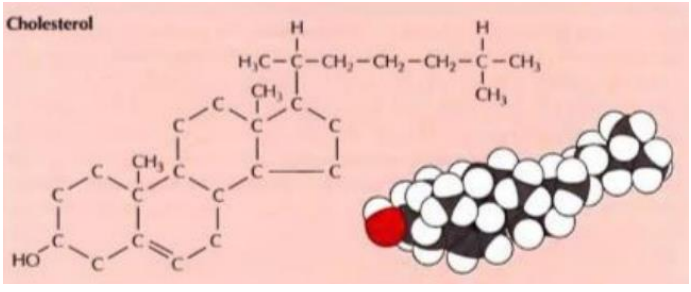
One of the results of this process is:

- Creating differences in the distribution and proportion of the different glycerophospholipid between the 2 leaflets (for example, the phosphatidylcholine present in a larger amount in the outer leaflet in comparison to the inner leaflet of the plasma membrane).

Synthesis of cholesterol and its derivatives

Steroid hormones are synthesized from cholesterol in ER, so we would expect large number of SER in:

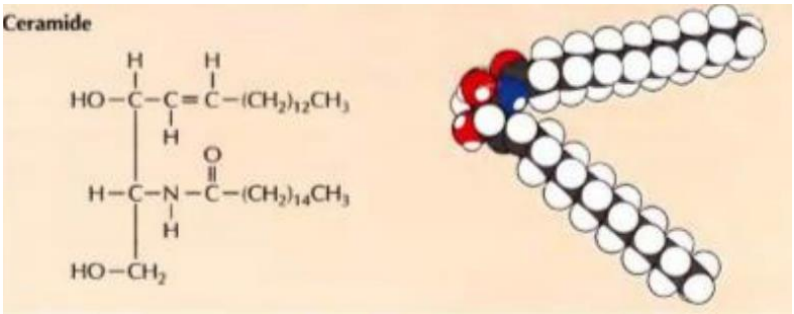
1- steroid producing cells such as in ovaries and testis (to produce the sex hormones).
2- Hepatocytes (produce cholesterol and bile acids, as well as their function in detoxification).



Note: A large set of isoprene molecules is needed to synthesize a cholesterol molecule.

Synthesis of Ceramide

Ceramide is the basic unit for sphingolipids. It is made of a sphingosine backbone attached to a fatty acid. Synthesis of ceramide happens inside the SER and then it can be further modified in the Golgi into sphingomyelin and glycolipids.



Synthesis of other lipids

-Smooth ER is abundant in the liver

- SER contains enzymes that metabolize various lipid-soluble compounds. The detoxifying enzymes inactivate a number of potentially harmful drugs (e.g., phenobarbital) **by converting them to water-soluble compounds** that can be eliminated from the body in the **urine**.
.Detoxification takes place mainly in Hepatocytes.

The End