CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

8

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- **WHAT ARE THE TYPES OF GLYCOSYLATION?**

  N-linked glycosylation (because it links to the nitrogen atom).

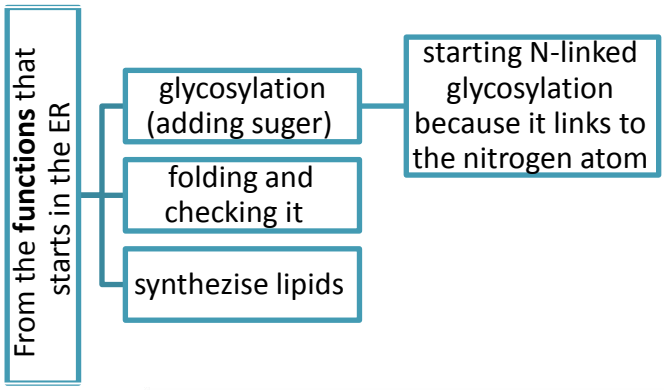
  O-linked glycosylation (because it connects to the oxygen atom).

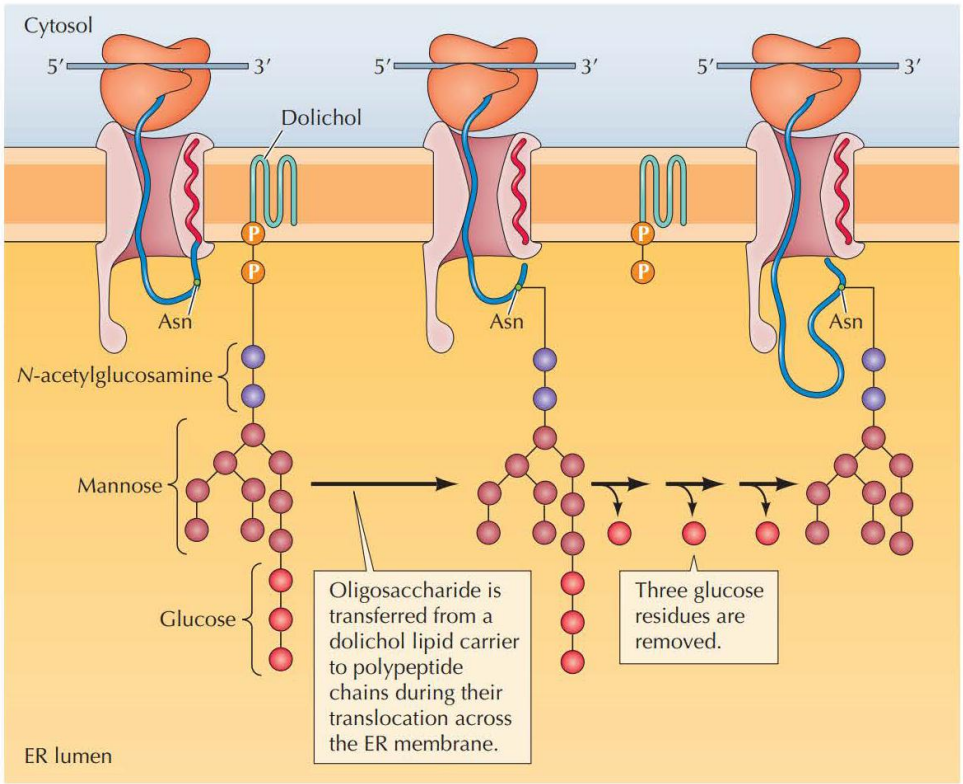
oligosaccharide units consisting of 14 sugar residues (which composed of 9 mannose & 3 glucose & 2 N-acetylglucosamine) are added to acceptor asparagine residues of growing polypeptide chains as they are translocated into the ER.

The oligosaccharide is synthesized on a lipid (dolichol) carrier anchored in the ER membrane. It is then transferred as a unit to acceptor asparagine residues in the consensus sequence (Asn-X-Ser/Thr) by a membrane-bound enzyme called oligosaccharyl transferase

Three glucose and **one mannose** are removed while the protein is still within the ER, and the protein is modified further after being transported to the Golgi apparatus

Glycosylation helps to prevent protein aggregation in the ER and provides signals that promote protein folding and subsequent sorting in the secretory pathway (starts in ER and finish in Golgi)





Protein processing in the ER-GPI anchors

- GPI anchors are assembled in the ER membrane
- 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
- These proteins transported to the cell surface as membrane components via the secretory pathway

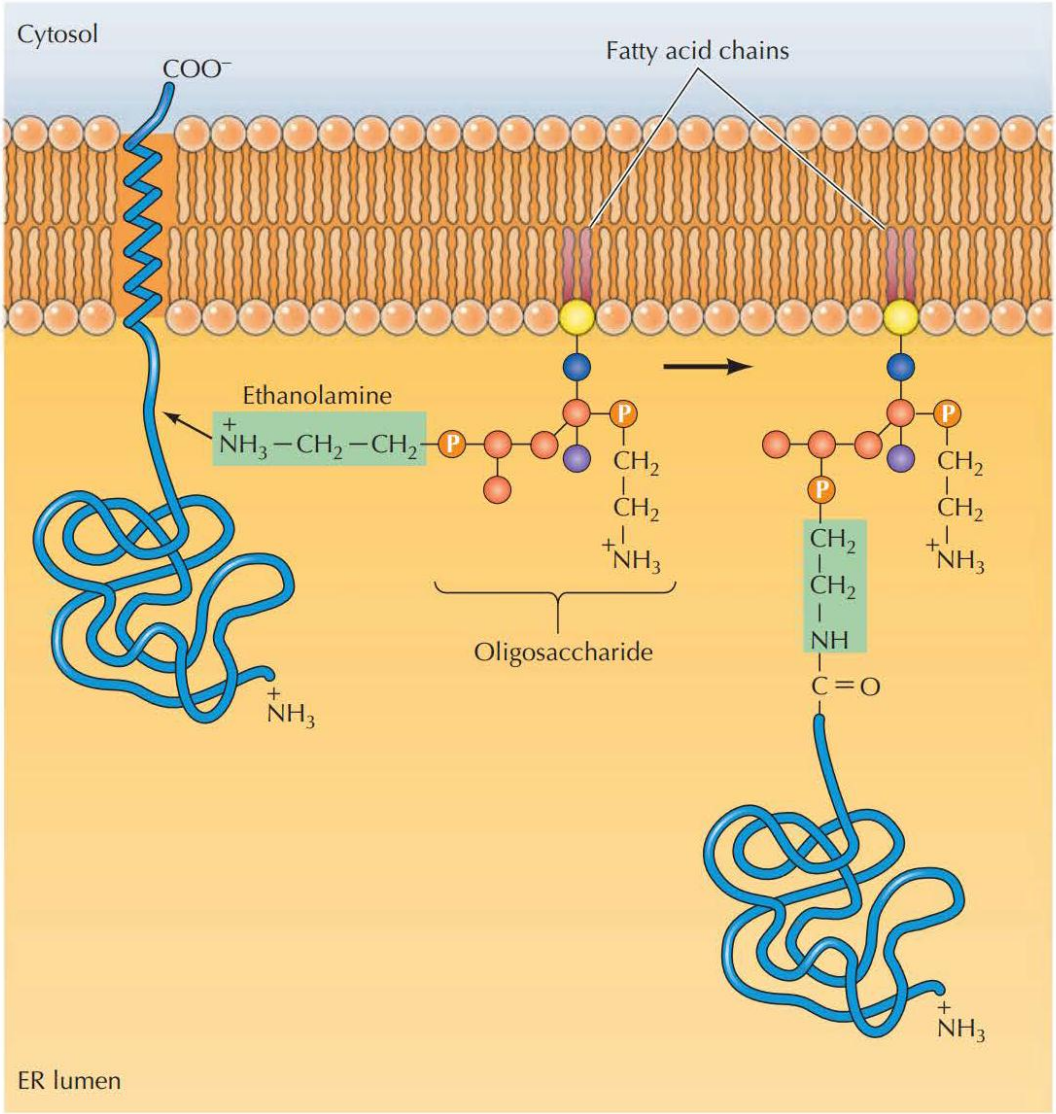


Figure 11.18 Addition of GPI anchors Glycosylphosphatidylinositol (GPI) anchors contain two fatty acid chains, an oligosaccharide portion consisting of inositol and other sugars, and ethanolamine (see Figure 9.38 for a more detailed structure). The GPI anchors are assembled in the ER and added to polypeptides anchored in the membrane by a carboxy-terminal membrane-spanning region. The membrane-spanning region is then cleaved, and the new carboxy terminus is joined to the NH_2 group of ethanolamine immediately after translation is completed, leaving the protein attached to the membrane by the GPI anchor.

ANOTHER FUNCTION OF THE proteins in the ER IS TO CHECK THE FOLDING OF PROTEINS

- Many proteins synthesized in the ER are rapidly degraded, because they fail to fold correctly. whether the proteins are folding they pass to Golgi for further modification if not they will stop in the ER, the process unfold it try to refold them again then if they are ok, they pass to Golgi if not they target to degradation. This function is called THE **Quality control in the ER or ER associated degradation (ERAD)**

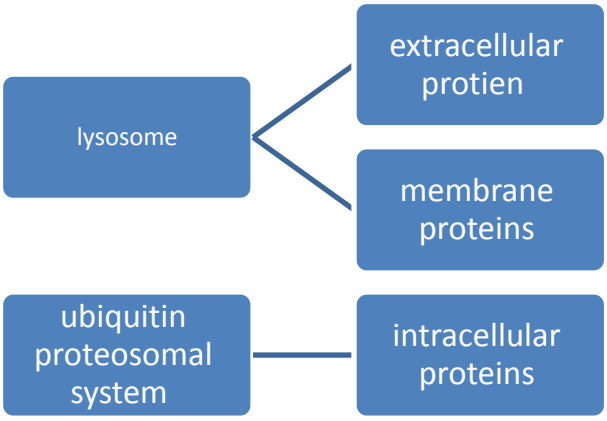
- *Different types of chaperons (*folding helpers of assistants) are involved in checking **the Quality** let's take an example:

  Calreticulin and its helper ''it is a protein complex'' (no need to know its name) are going to recognize glycosylation of a protein (Calreticulin is specific for glycosylation) IF THE STRUCTURE IS OK targeting it for packaging for vesicular formation and send it to Golgi

  IF NOT re-add glucose residues (that removed already) - *why?* because it ACTS AS A SIGNAL that means the protein is misfolded after addition is going to be checked again if it is OK it passes if it is abnormal protein there are different protein degradation mechanism one of them is LYSOSOME the other is **the ubiquitin proteasomal system**

UBIQUITIN PROTEASOMAL SYSTEM

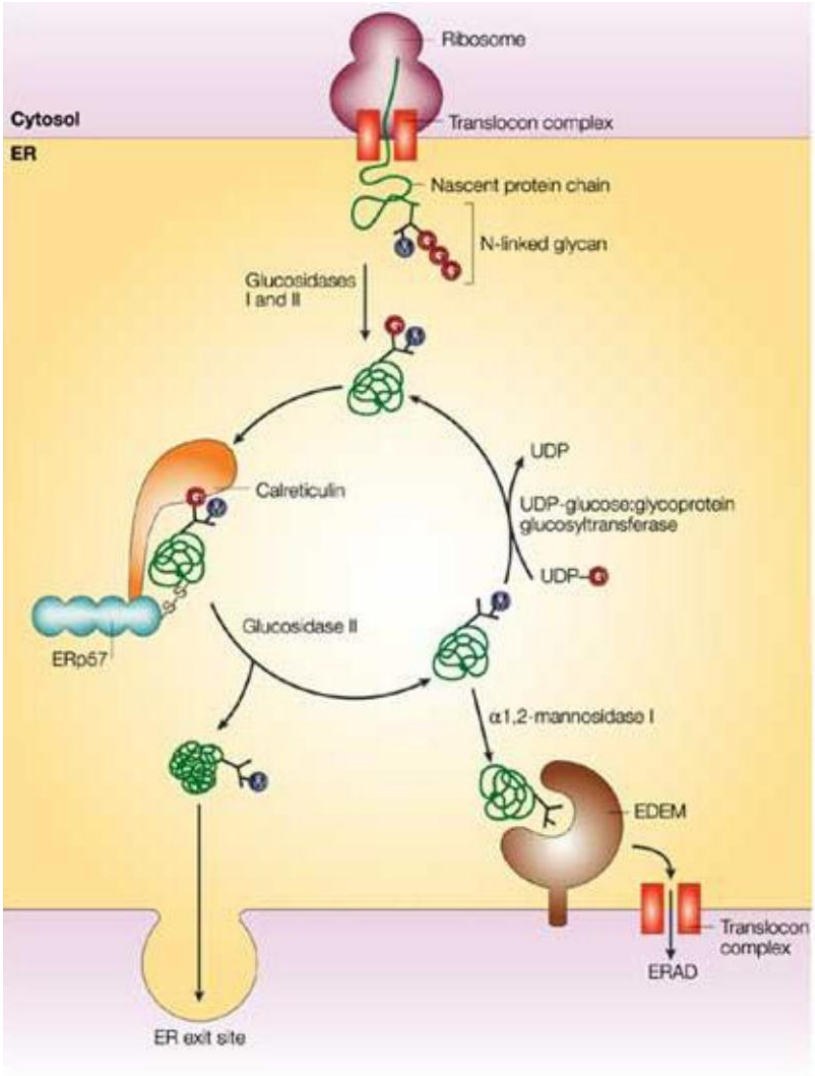
- UBIQUITIN *:(very small proteins) that tag the misfolded protein to get it degraded*
- PROTEASOMAL SYSTEM: *Proteasomal like a barrel shape structure is going to start the enzymatic reaction and cut the peptide bond between amino acids into fragments amino acids so get rid of abnormal protein (not organelle)*



Quality control in the ER (ER-associated degradation (ERAD))

- misfolded proteins are removed from the ER by a process referred to as **ER associated degradation (ERAD),** in which misfolded proteins are identified, returned from the ER to the cytosol, and degraded by the ubiquitin proteasomal system

- Chaperone and protein-processing enzymes are misfolded protein sensors
- Calreticulin (a chaperone) helps in folding of glycoprotein and releases it when glucose is removed
- A folding sensor binds to the protein.
  - If correctly folded, the protein moves to transitional ER.
  - If misfolded, glucose is added, Calreticulin re-folds the proteins.
  - If severely misfolded, the protein is degraded by ubiquitin proteasomal system (in this example or by lysosome in another example)



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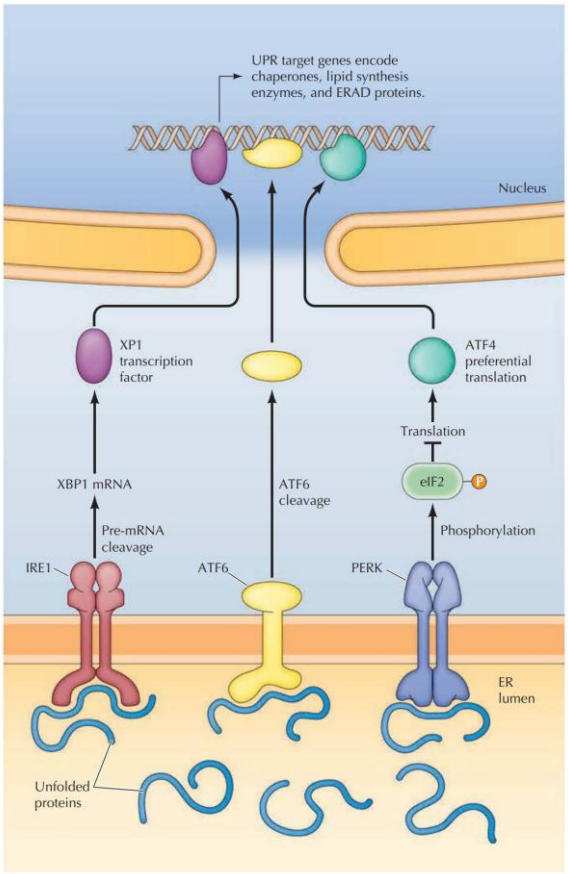
Unfolding protein response (UPR)

Activation of the unfolded protein response pathway leads to expansion of the ER and production of additional chaperones to meet the need for increased protein folding

- Coordinates protein folding capacity of the ER with the physiological needs of the cell
- Is activated when excess unfolded proteins accumulate in the ER
- ER expansion, activation of UPR target genes such as chaperones and transient reduction in new protein entry to ER

- Suppose under a certain condition a cell needs to synthesize so many proteins so the ribosome is active as well as the translocation to the ER so the ER feels like Overworked and it will be an accumulation of unfolding proteins in the ER so ***what will the cell do in this situation?***
- Unfolding protein response **WILL BE ACTIVATED**
- ER will expand the amount of it will increase. And **Reduce** protein synthesis **EXCEPT** chaperons
  - why we need to increase chaperons? Because they help these proteins to fold properly
- also reduce translocation of newly synthesized protein to ER as well as increase surface area increase capacity of ER
- Assume you have **A RESTAURANT (ER)** and you Increase **CHAPERONS (WORKERS)** + **AREA (ER CAPACITY)** so you host more **CUSTOMERS (PROTEINS)** than before then you will close the door and stop entry of new customers in this situation (no new proteins) until fixing the current misfolded proteins, once the process done ER makes them in



So far, we are done with changes that are going to happen to the proteins in the ER then we are going to packaging these from transitional ER side (the end of ER structure) through Golgi but before they go to Golgi there is an intermediate region called

ER Golgi intermediate compartment (ERGIC): separate membranous sacs in which vesicles are going to fuse from transitional ER and are going to bud and transported to Golgi apparatus Protein and lipids are carried from the ER to the Golgi in transport vesicles that bud from ER exit sites, fuse to form the vesicles and tubules of the ER-Golgi intermediate compartment (ERGIC), and are then carried to the Golgi

Orientation create a different surface features from a region to another in the membrane or from face to face with different compartment of Golgi Topologically cytosolic side remain cytosolic side but lumen side will be exterior.

Now proteins are ready to go to Golgi (*all of them)* for further modification once it will be ready it packaged to its final destination might be (plasma membrane; lysosome; mitochondria and might be to the ER itself)

Vesicular transport from the ER to the Golgi

- Proteins and lipids are carried from the ER to the Golgi in transport vesicles that bud from ER exit sites, fuse to form the vesicles and tubules of the ER-Golgi intermediate compartment (ERGIC), and are then carried to the Golgi
- Lumenal ER proteins targeted for the Golgi are bound by transmembrane proteins that are selectively packaged into vesicles
- Resident ER proteins destined to remain in the lumen of the ER are marked by KDEL retrieval sequences at their carboxy terminus

How vesicles can return the proteins to the ER?

- By specific signal sequence it might be amino acid sequence or sugar sequence, in this example the sequence is amino acids
- there are 2 types of amino acid sequence to return protein again to ER
  KDEL (Lys-Asp-Glu-Leu) and KKXX (two lysine and X: any amino acid)

✓ **So, if the protein has these sequences it will return again to the ER like translocon**

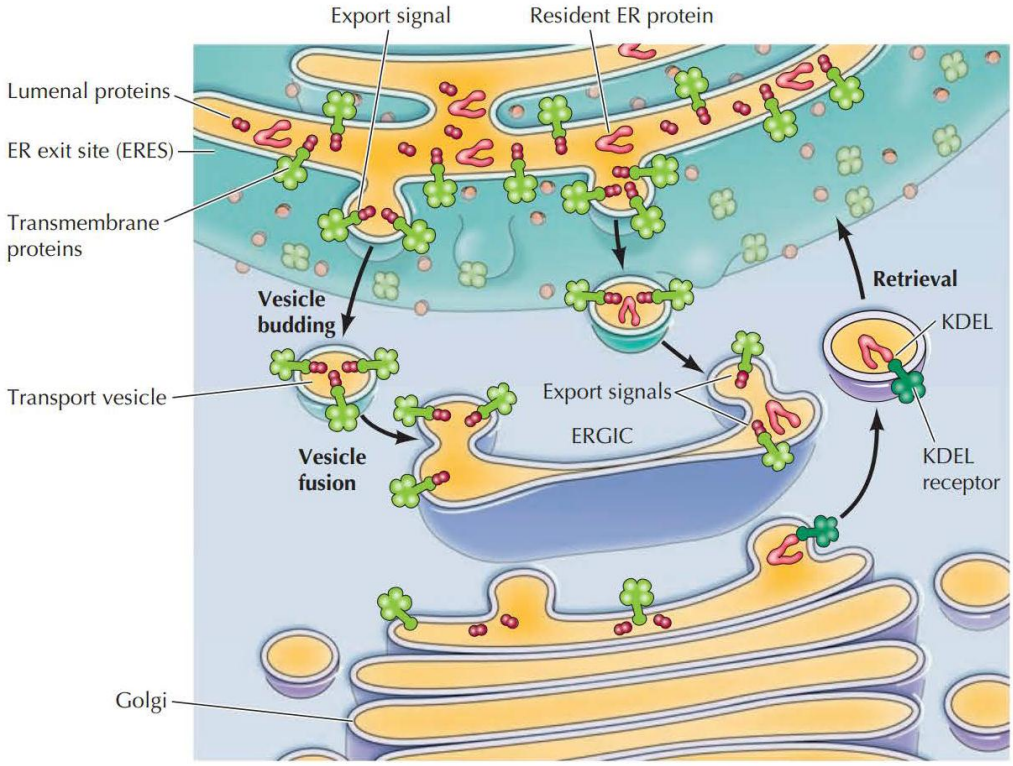
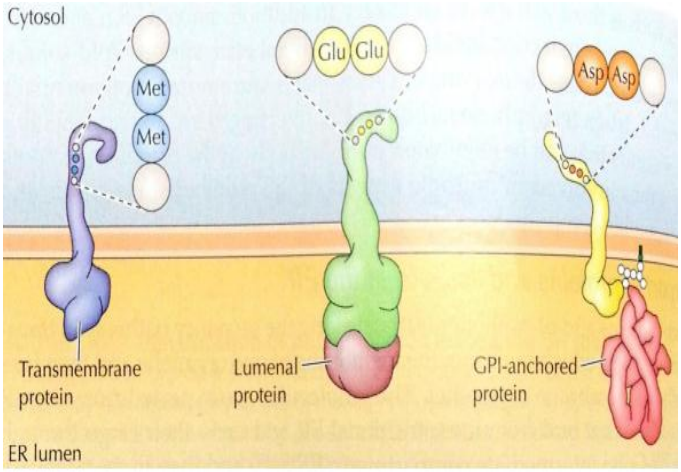


Figure 11.23 Vesicular transport from the ER to the Golgi Proteins and lipids are carried from the ER to the Golgi in transport vesicles that bud from ER exit sites (ERES), fuse to form the vesicles and tubules of the ER–Golgi intermediate compartment (ERGIC), and are then carried to the Golgi. Lumenal ER proteins targeted for the Golgi are bound by transmembrane proteins that are selectively packaged into vesicles. Resident ER proteins destined to remain in the lumen of the ER are marked by KDEL retrieval sequences at their carboxy terminus. If these proteins are exported from the ER to the Golgi, they are recognized by a recycling receptor in the ERGIC or the Golgi and selectively returned to the ER.

Protein sorting and retention

- Many proteins with **KDEL** at C-terminus are retained in the **ER lumen**
  - ✓ **If sequence is deleted**, the protein is transported to the Golgi and secreted from the cell
  - ✓ **Addition on of the sequence** causes a protein to be retained in the ER
- The retention of some **transmembrane proteins** in the ER is dictated by short C-terminal **KKXX** sequences
- Proteins bearing the KDEL and KKXX sequences are to recycled back to the ER but are not prevented from being carried to Golgi

Membrane proteins contain **di-acidic or dihydrophobic** amino acid signal sequences. They also function as **carriers** of GPI-anchored and luminal proteins



The smooth ER and lipid synthesis

Glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors; to be specific

- ✓ Phosphatidylcholine, Phosphatidylinositol, Phosphatidylethanolamine and Phosphatidylserine
  - Synthesis process starts by using an active form of glycerol molecule (glycerol 3-phosphate) phosphate group on the carbon number three an enzyme called fatty acyl transferase brings two fatty acid consequently (one by one not together) then these fatty acids are going to link with glycerol 3-phosphate on carbon number 1 and 2 from
  - Now the reaction will continue in the membrane this reaction made the parent of phospholipid which called phosphatidic acid before adding head group
  - We have reaction in which phosphate group is removed so it converts to diglycerol then add for example choline group.
  - choline is carried by CDP **REMEMBER** that nucleotides act as a carrier during reactions this is one example now **TAKE A PHOSPHATE GROUP** from CDP so it becomes CMP **REPLACE OH BY PHOSPHATE GROUP** from CDP then **ADD CHOLINE TO IT** now, I have phosphatidylcholine

Different types of phospholipids present differently between inner and outer - leaflet side of synthesis **HOW IS THAT?** it refers to the flipping moving by an action of enzyme called **flippases** that change the concentration between outer and inner leaflet of glycerophospholipids

Synthesis of phospholipids in SER

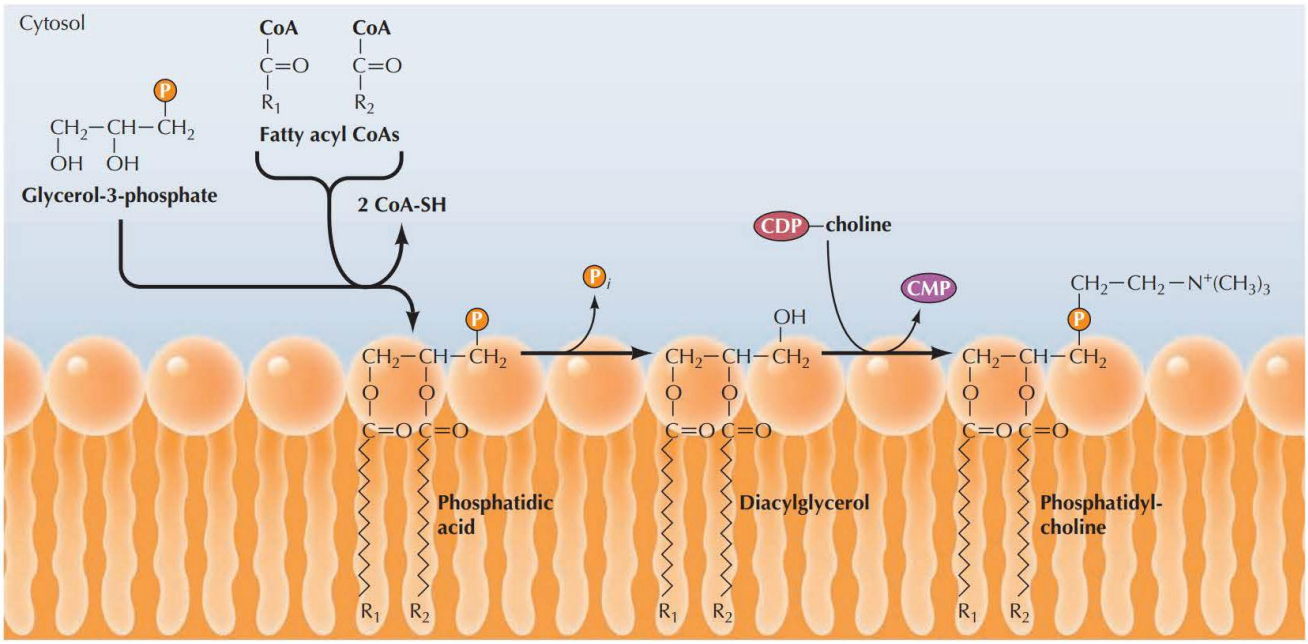


Figure 11.21 Synthesis of a phospholipid Glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors. Two fatty acids linked to coenzyme A (CoA) carriers are first joined to glycerol-3-phosphate, yielding phosphatidic acid, which is simultaneously inserted into the membrane. A phosphatase then converts phosphatidic acid to diacylglycerol, which is converted to phosphatidylcholine by addition of a polar phosphocholine head group.

Note on the figure: Enzymes are buried inside the membrane because the hydrophobic structure of lipids has to be maintained in close proximity to membranes

Two fatty acids linked to CoA carriers are first joined to glycerol 3-phosphate → **phosphatidic acid**

Phosphatidic acid —PHOSPHATASE→ **diacylglycerol**

Diacylglycerol —PHOSPHOCHOLINE→ **phosphatidylcholine**

Translocation of phospholipids across the ER membrane

- Because phospholipids are synthesized on the cytosolic side of the ER membrane, they are added only to the cytosolic half of the bilayer
- They are then translocated across the membrane by phospholipid flippases, resulting in even growth of both halves of the phospholipid bilayer

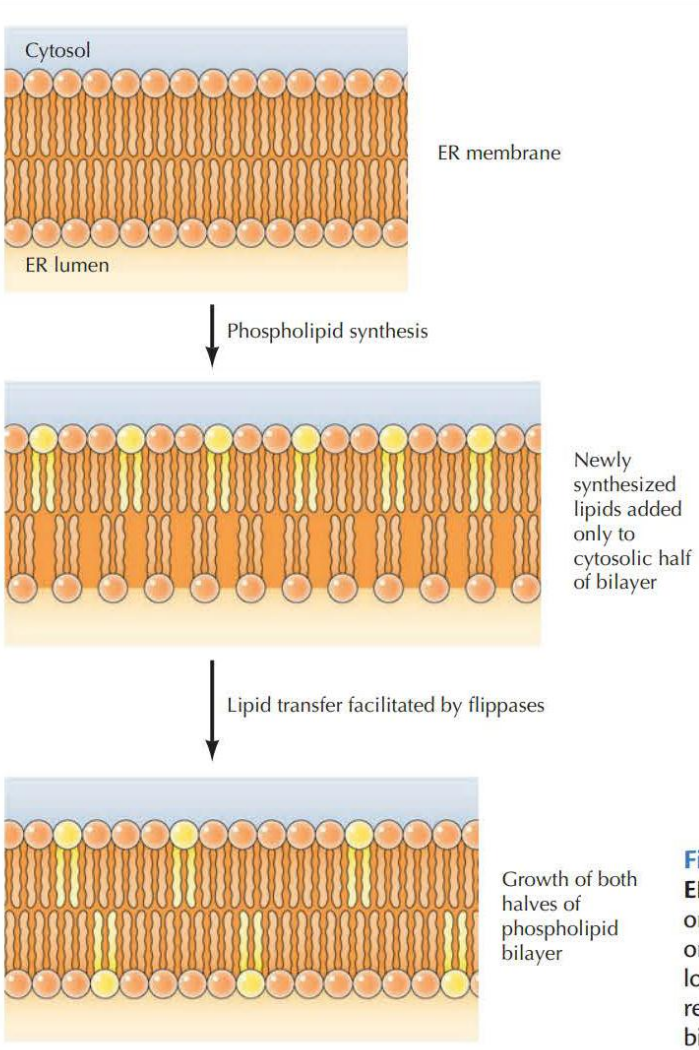


Figure 11.22 Translocation of phospholipids across the ER membrane Because phospholipids are synthesized on the cytosolic side of the ER membrane, they are added only to the cytosolic half of the bilayer. They are then translocated across the membrane by phospholipid flippases, resulting in even growth of both halves of the phospholipid bilayer.

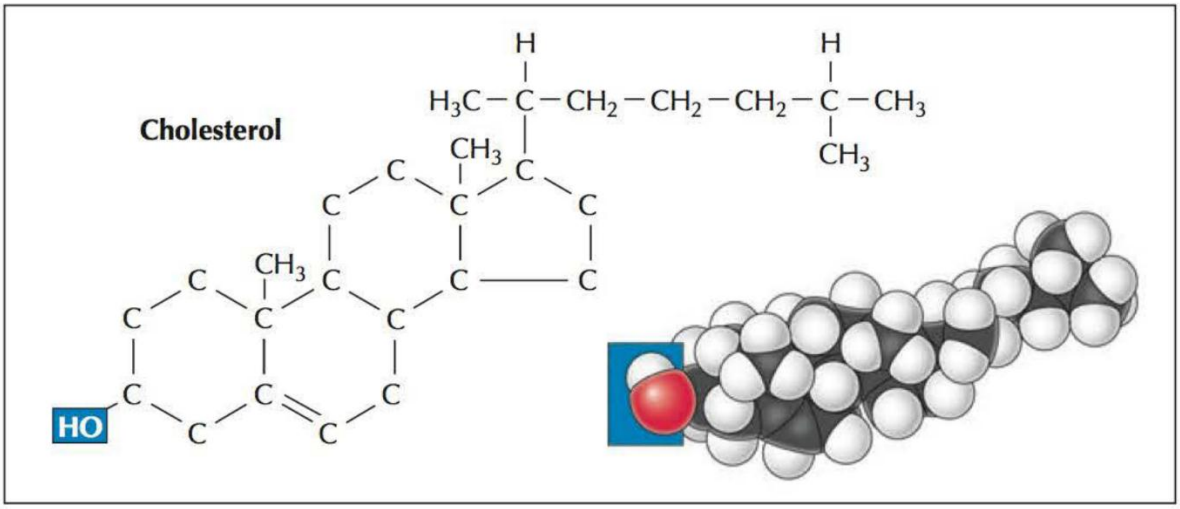
Synthesis of cholesterol and its derivatives

cholesterol

- ✓ Briefly its synthesis in our cells specifically liver cells to be used for making vitamin D and Bile
- ✓ to synthesize sex hormones in testes and ovaries briefly: we use (isoprene units) base structure of cholesterol it contains 30 carbon atom using multiple isoprene contains 5 carbon we gather it by very complex reaction

PAY ATTENTION if we talk about tests and ovaries, we need large ER because we need to synthesize these hormones same thing in liver cells because we want to synthesis vitamin D

Conclusion: ER is a dynamic structure that differs from one cell to another depends on the function of the cell



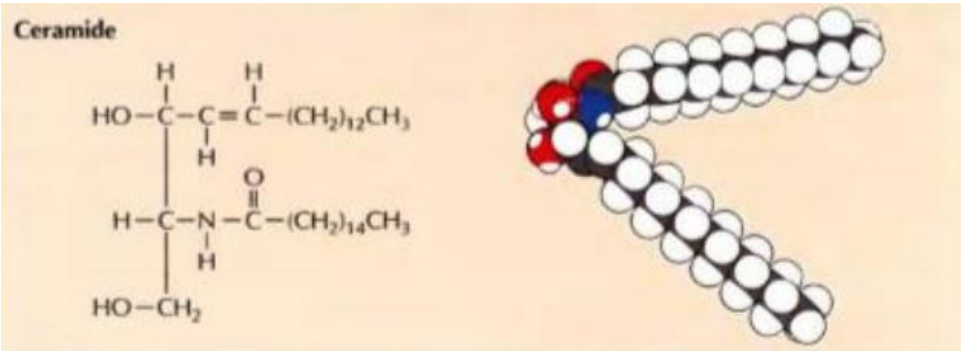
Note: Steroid hormones are synthesized from cholesterol in the ER Large amounts of smooth ER are found in steroid-producing cells, such as those in the testis and ovary

Synthesis of ceramide

Ceramide is the parent structure of sphingomyelin and glycolipid as phosphatidic is the parent structure of glycerophospholipids

Ceramide is synthesized in the ER, is converted either to sphingomyelin or to glycolipids in Golgi apparatus, How?

- ✓ A phosphorylcholine group is transferred from phosphatidylcholine to ceramide
- ✓ Alternatively, a variety of different glycolipids can be synthesized by the addition of one or more sugar residues (glucose)



Ceramide= sphingosine + fatty acid + free head group

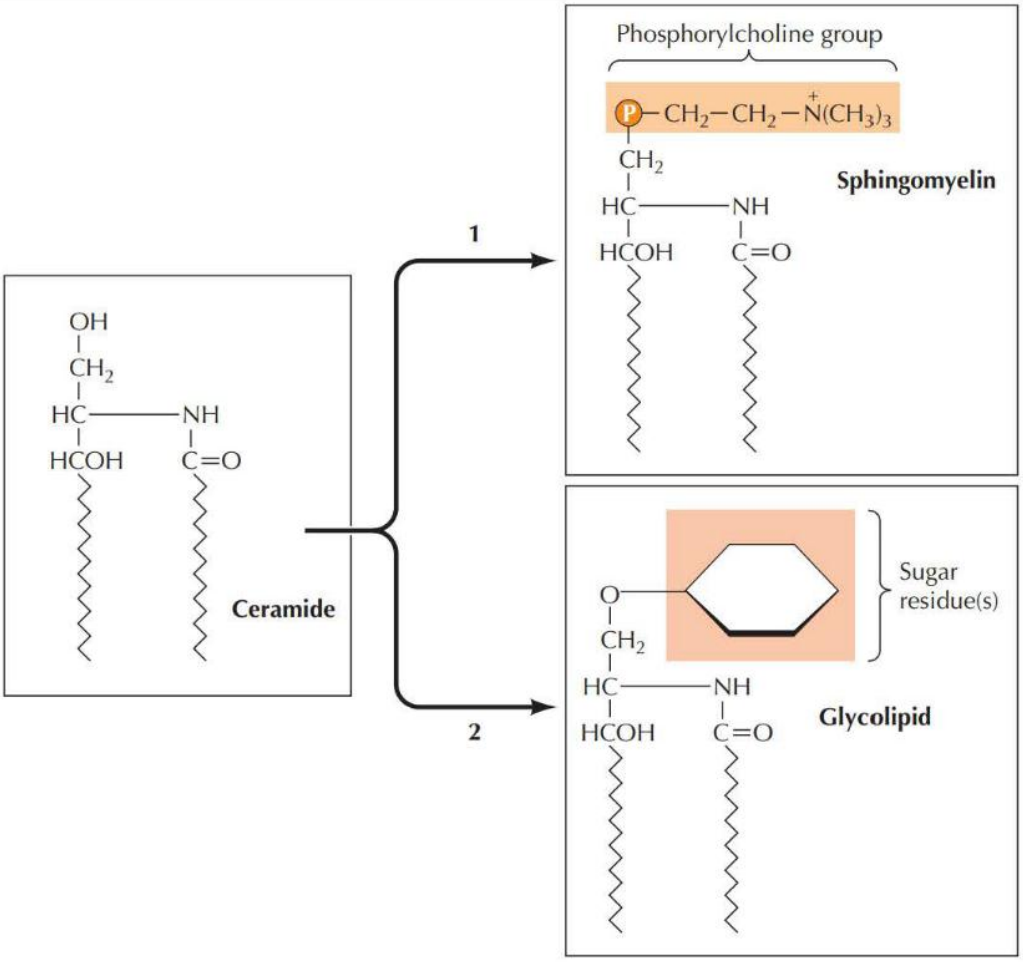


Figure 11.29 Synthesis of sphingomyelin and glycolipids Ceramide, which is synthesized in the ER, is converted either to sphingomyelin (a phospholipid) or to glycolipids in the Golgi apparatus. In the first reaction, a phosphorylcholine group is transferred from phosphatidylcholine to ceramide. Alternatively, a variety of different glycolipids can be synthesized by the addition of one or more sugar residues (e.g., glucose).

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