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Protein folding and processing in the ER

After the protein is translocated to the ER, whether it's a soluble or a membrane protein (it could span the membrane once or multiple times), it's going to be modified. Protein folding will happen by the assistance of a group of proteins called **chaperones**, which have many functions.

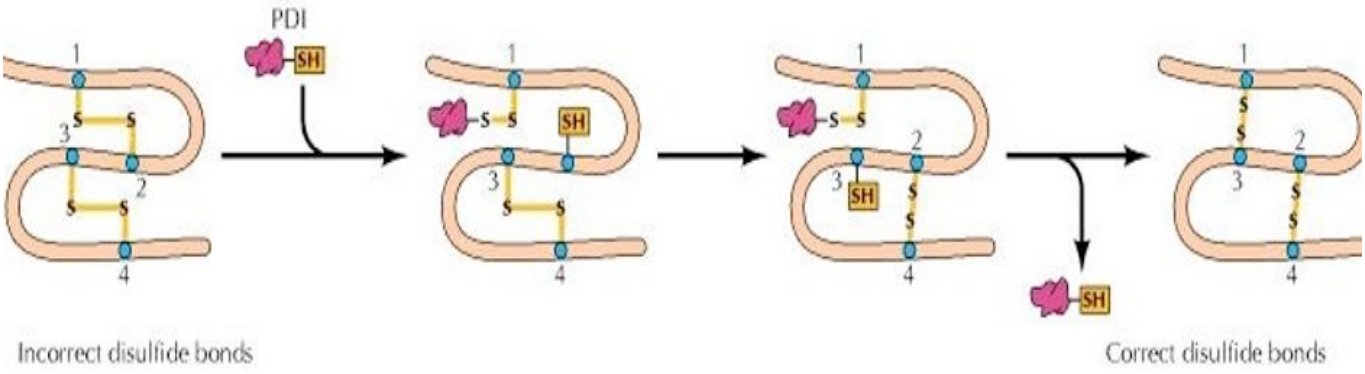
1. They assist in the formation of disulfide bridges.
2. They bind to unfolded proteins so that they won't fold improperly.
3. BiP (discussed earlier) is also a chaperone.

Folding is driven by the inherited information in the primary structure of the protein (the amino acid sequence) to produce the final shape of the protein, which is the most stable and functional shape. Folding of the protein means that new bonds are formed, these bonds are:

1. **Non-covalent** interactions between side chains, backbones, or both.
2. **Covalent** interactions like disulfide bridges.

Disulfide bridge formation

An oxidizing environment is needed to form disulfide bridges by removing hydrogen. The cytosol has a reducing environment assisted by **protein disulfide isomerase** (PDI). In the figure below, there are 4 cysteine residues; therefore 2 disulfide bridges will be formed. One between Cys1 and Cys2 because they are closer to each other and the other between Cys3 and Cys4 for the same reason, making the protein more stable than the unfolded form but not giving the functional form that we want. PDI modifies the disulfide bridges into the correct ones. PDI breaks the first disulfide bond between Cys1 and Cys2 by reducing Cys2 and then forming a disulfide bridge with Cys1. It then goes on and breaks the disulfide bridge between Cys3 and Cys4 by reducing Cys3. Now Cys4 is free to react with Cys2 forming a disulfide bridge. The PDI bonded to Cys1 is released leading to the formation of a new disulfide bridge between Cys1 and Cys3.



Another type of modification is **glycosylation**, which is the addition of sugar moieties to a protein forming a glycoprotein. Glycosylation is either **N-linked** or **O-linked** depending on whether the sugar is linked to a nitrogen atom (amino group) or an oxygen atom (OH group). N-linked glycosylation starts in the ER and is further modified in the Golgi apparatus, while O-linked glycosylation happens in the Golgi apparatus only.

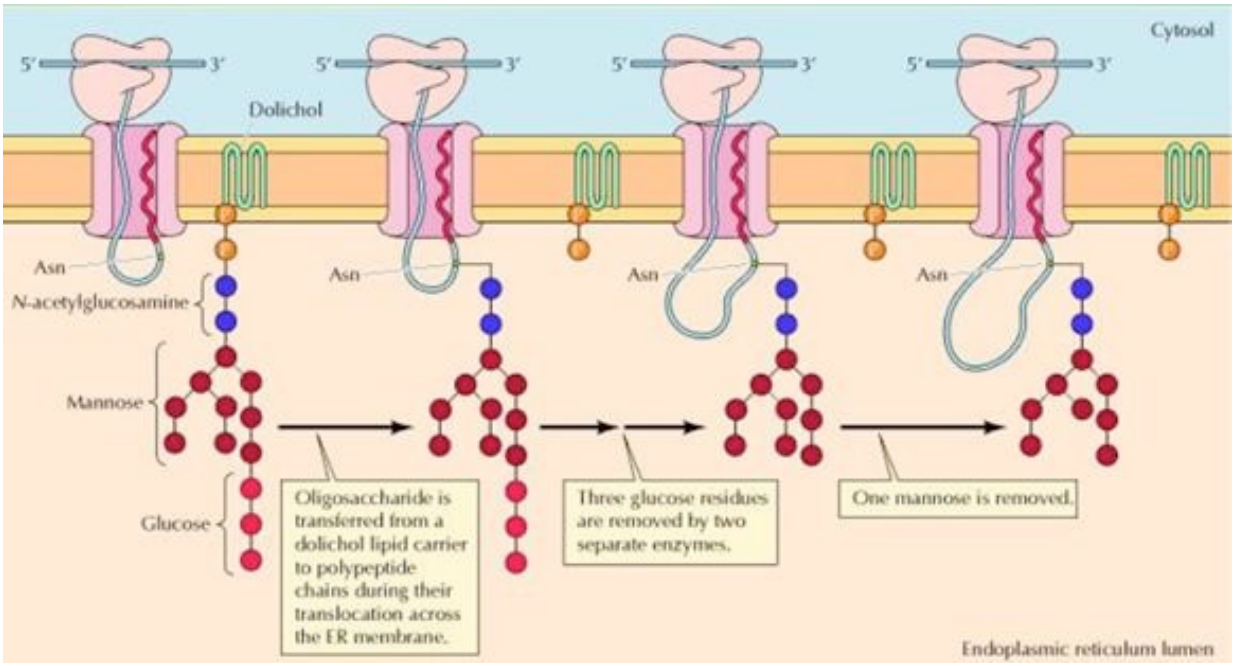
N-linked glycosylation

There is a basic sugar moiety that is going to be added to the protein and then modified. This sugar moiety is composed of 2 N-acetylglucosamine, 9 mannose residues and 3 glucose residues.

In the ER, this sugar moiety is first attached to **dolichol,** then transferred to the translocated protein and modified. An enzyme called oligosaccharyl transferase catalyzes the transfer process.

Dolichol is a lipid molecule that is inserted into the membrane of the ER and functions to attach to and carry a sugar moiety.

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), X referring to any amino acid.



The first modification is the removal of three glucose residues, which are removed by two separate enzymes. However, the second modification is the removal of one mannose residue.

Oligosaccharides are transferred from dolichol to the protein **during** the translocation.

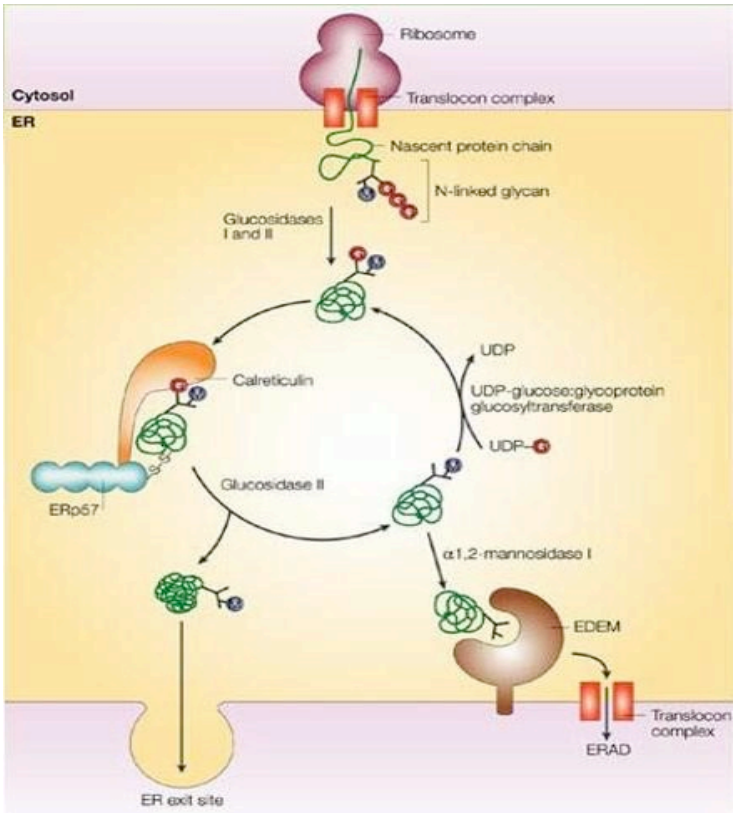
Functions of glycosylation:

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

Addition of GPI anchors

Peripheral proteins need to be attached to the membrane by different types of anchors, specifically **glycosylphosphatidylinositol** (GPI), which is a phospholipid that contains some sugars with an ethanolamine group at the end. First, the protein will be attached to the membrane by a group of hydrophobic amino acids, then it will be cleaved exposing the C-terminus. The NH_3^+ (positive charge) of ethanolamine in the GPI and the new C-terminus (negative charge) will form a covalent bond (amide linkage), thus establishing an attachment of the peripheral membrane protein.

Quality control in ER



The ER is considered the quality control station in the cell. This means that the ER is responsible for the correct folding of proteins through its chaperones. But despite this, mistakes will occur (even after the help of chaperones). These mistakes result in improper folding, so we need to check if the folding is correct or not before transporting the protein to the Golgi. If the folding is correct, it is sent to the Golgi and if it is not, we unfold it and try to refold again. If it stays misfolded, then it gets degraded. This process is known as the **ER associated degradation** (ERAD).

The ER checks the folding of proteins and senses the correct folding by chaperones. An example of the role of chaperones is **calreticulin**. In the figure below, there is a ribosome bound to a polypeptide chain that will be transferred by a translocon into the ER lumen. During this process, the protein (polypeptide) is glycosylated to become a glycoprotein.

Now, the polypeptide is inside the ER lumen and is ready for modification and folding. As the glycoprotein exits the translocon, 2 glucose residues are removed allowing calreticulin to bind and assist in folding.

Removal of another glucose residue terminates the interaction with calreticulin thereby releasing the glycoprotein. A protein (folding sensor) then assesses the extent of folding of the glycoprotein. There are 3 pathways depending on the extent of correct folding:

1. If it is folded correctly, the glycoprotein is packaged and transferred to the transitional ER and then to the Golgi.

2. If it is folded incorrectly, another glucose molecule is added from UDP-glucose, and then the protein will be unfolded and refolded again (it will undergo another cycle of folding by calreticulin).

3. If the protein is severely misfolded and cannot be properly folded, it will be degraded by the ubiquitin proteasome system.

Unfolded Protein Response (UPR)

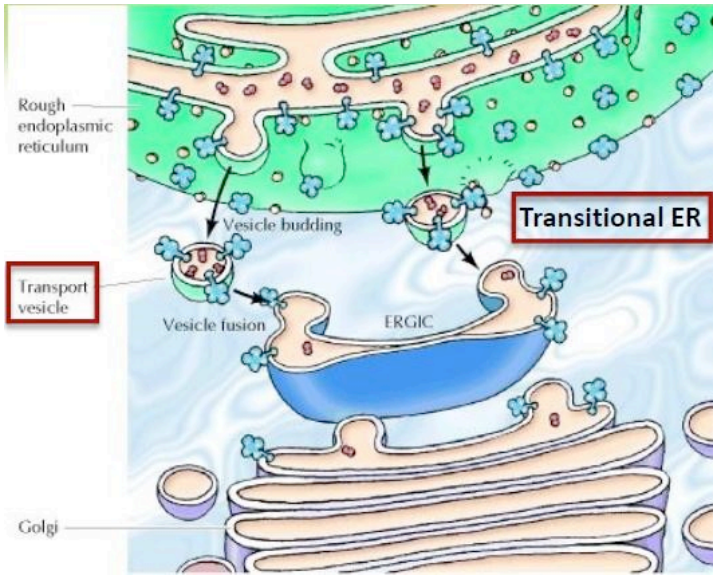
UPR coordinates protein-folding capacity of the ER with the physiological needs of the cell. It is activated when excess unfolded proteins accumulate in the ER. Examples of UPRs:

1. Expansion of the ER by increasing surface area.
2. Activation of UPR target genes such as chaperones.
3. Transient reduction in new protein entry to ER (inhibition of translation and translocation).

While quality control in the ER is always active, on the contrary, UPR is only activated in times of excess accumulation of unfolded proteins in the ER.

ER-Golgi intermediate compartment (ERGIC)

After the protein is modified in the ER, some proteins need more modifications that will occur in the Golgi apparatus. Transporting proteins from the ER to the Golgi occurs through a compartment known as ERGIC. A vesicle buds from the ER and fuses with the ERGIC and then goes to the Golgi. Note that the orientation of the membrane proteins is maintained through this pathway, which means that membrane proteins that were in the outer leaflet in the ER will stay in the outer leaflet in the ERGIC and Golgi. While the proteins that were in the lumen of the ER, will stay in the lumen of ERGIC and Golgi.



Protein Sorting and Retention

Some proteins need further modifications before transporting them to their final destination. These modifications happen in the Golgi apparatus such as O-glycosylation, which is limited to the Golgi. Some proteins head back to the ER through signal sequences like KDEL, KKXX, or specific regions within the protein.

1. **KDEL** sequence (Lys-Asp-Glu-Leu), this sequence is found on the C-terminus of the protein, the retention of many proteins is mediated by this signal.
2. **KKXX** sequence (Lys-Lys-X-X).also found on the c-terminus, The retention of transmembrane proteins is mediated by this signal.

Proteins that may have this sequence are not prevented from being transported to the Golgi (rather they are transferred to the Golgi and then carried back).

If this sequence is deleted, the protein is transported to the Golgi and then secreted from the cell.

Note that there are other amino acid sequences that function as the KDEL.

The Smooth Endoplasmic Reticulum (sER)

The sER is free of ribosomes, so it doesn't deal with protein modification or synthesis, but rather plays a major role in:

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

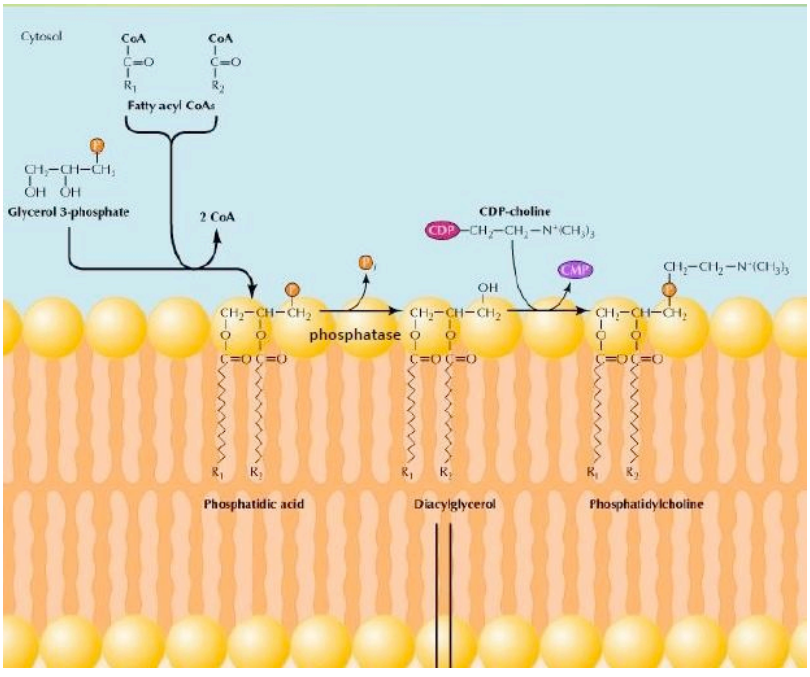
The sER is responsible for synthesizing:

1. Phospholipids, which are mainly synthesized in the liver.
2. Cholesterol and other steroid hormones, which are mainly synthesized in the testis and ovary.
3. Ceramide, which will be further modified into glycolipids or sphingomyelin in the Golgi apparatus.

To synthesize a glycerophospholipid, we need:

1. Glycerol in its active form (phosphorylated at carbon number 3).
2. 2 fatty acids in their active form (attached to CoA)
3. Different polar heads (phosphate, amine, serine, etc.).

Phospholipids consist of a polar head and hydrophobic tails. There are two types of phospholipids: sphingomyelin (which is also a sphingolipid) that is derived from a sphingosine backbone, and glycerophospholipids such as phosphatidyl choline, which are derived from a glycerol backbone.

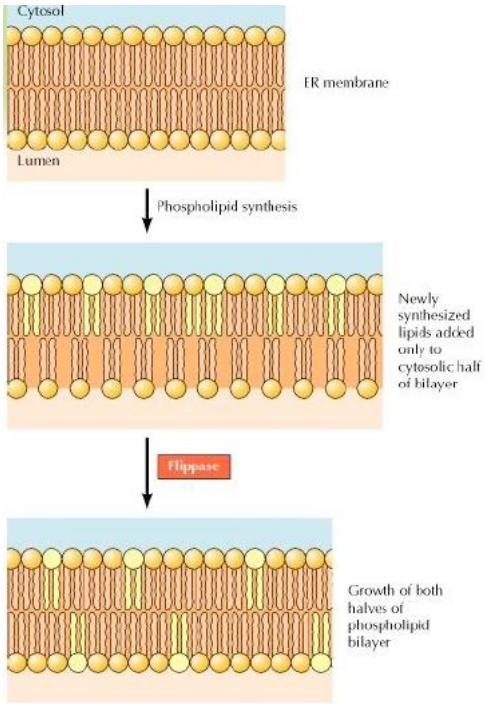


When we synthesize a phospholipid, we add 2 fatty

acids to carbon 1,2 of the phosphorylated glycerol producing two tails that will be inserted directly to the membrane because they are hydrophobic. This molecule is known as phosphatidic acid, which is the precursor to produce different types of phospholipids. After that, the phosphate group is removed with a phosphatase enzyme producing diacylglycerol, so we can add different types of polar heads.

The addition of a polar alcohol head to diacylglycerol yields a glycerophospholipid.

These processes occur in the outer leaflet of the sER membrane because we get the precursors (glycerol, polar heads) from the cytosol. These phospholipids will insert themselves directly to the sER membrane because they are amphipathic molecules, therefore they will be more stable in an amphipathic environment. After synthesizing these molecules, we add them to the inner leaflet of the sER membrane, because there are more phospholipids in the outer leaflet of the membrane in comparison with the inner leaflet. So to make the number of phospholipids in the inner and outer leaflet equal, an enzyme called **flippase** flips some phospholipids to the inner leaflet.



Synthesis of cholesterol and its derivatives

Steroid hormones are synthesized from cholesterol in the cytosolic part of the sER, so we would expect the sER in steroid producing cells such as ovaries, testes, adrenal glands (cortisol) and hepatocytes to be large.

Synthesis of Ceramide

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

Detoxification of chemicals

There are enzymes for metabolizing or detoxifying lipid soluble compounds (chemicals) inside some cells like liver cells. Detoxification of drugs mainly happens in the liver, and that's why the sER in liver cells is larger. The sER contains enzymes that metabolize various lipid-soluble compounds. These enzymes inactivate several potentially harmful drugs such as phenobarbital (which is used to treat epilepsy) or other sedatives by converting them to water-soluble compounds that can be eliminated from the body in the urine.

Golgi Apparatus

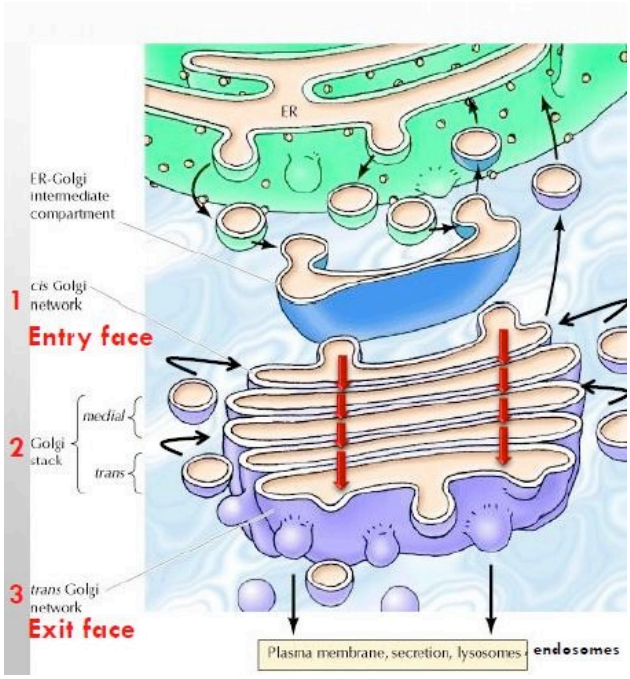
It is a group of unconnected sacs (membrane closed cisternae), which synthesizes some lipids in addition to making some modifications to proteins. It has three rows of different sacs:

1. The compartment that is close to ERGIC is called cis-Golgi network (CGN) or entry face.
2. The compartment that faces the plasma membrane and exports different types of proteins is called trans-Golgi network (TGN) or exit face.
3. In the middle region between cis and trans networks, there is a Golgi stack, and this region is split into 2 regions: medial and trans Golgi stacks.

There is a difference between the trans-Golgi network (located near the plasma membrane) and the trans-Golgi stack (part of the middle compartment).

Vesicles move through Golgi by this pathway:

Vesicles from ER ERGIC then they enter Golgi sac through the cis (entry) face medial stack trans stack trans (exit) face budding of the vesicle and its contents final destination. Now suppose that a vesicle enters the first sac, which is now in the entry face, this sac that contains the vesicle, and its content will move to the other compartment, which is the medial stack, then it will go to the trans stack, and finally to the trans face, and during this journey all modifications will happen. Then it will be packaged and will exit as a budding vesicle from the trans face to its destination. So the transportation of the proteins in Golgi doesn't occur by budding through vesicles, rather, the whole sac matures and progressively moves (for example, the cis face will become the medial compartment and then it will become the trans face).

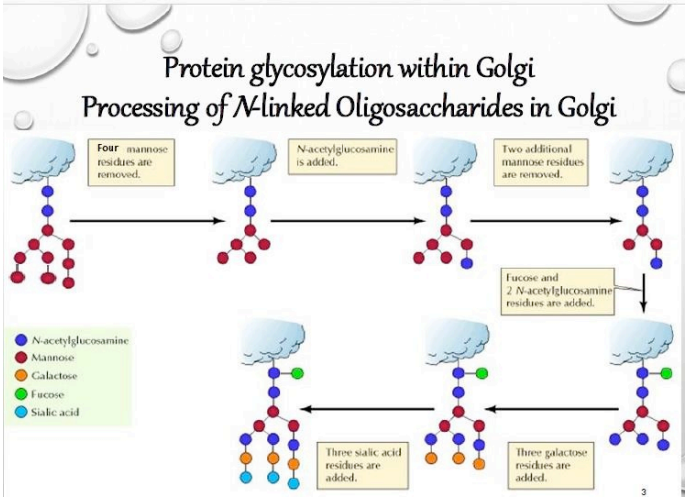


Golgi functions:

1. Protein glycosylation
2. Lipid metabolism.
3. Sorting and exporting modified proteins to their destination (main function).

Modification of N-linked glycoproteins

The Golgi already received N-glycoproteins, but they need further modification. First, 4 mannose residues are removed and 1 N-acetylglucosamine is then added. Next, 2 additional mannose residues are removed. After that, 1 fucose molecule, 2 N-acetylglucosamine, 3 galactose molecules and 3 sialic acid molecules are added.



*Numbers and exact details aren't required.

O-linked glycosylation

The carbohydrates are added to serine or threonine (not tyrosine although it has an OH group) because they have an OH group in their side chains. N-acetyl galactosamine, sialic acid, galactose and sialic acid again are added respectively.

Lipid metabolism

After ceramide was synthesized in the sER, different types of polar heads are added to produce sphingomyelin (phosphocholine is added) and glycolipids. Glucose is added to ceramide on the cytosolic side and then flips to the luminal side. On the other hand, sphingomyelin is synthesized on the luminal side of the Golgi, but can flip to the cytosolic side because glycolipids are mostly found on the outer surface

"If opportunity doesn't knock, build a door"

Good Luck