CYTOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 2 + 3
endoplasmic reticulum & Golgi

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

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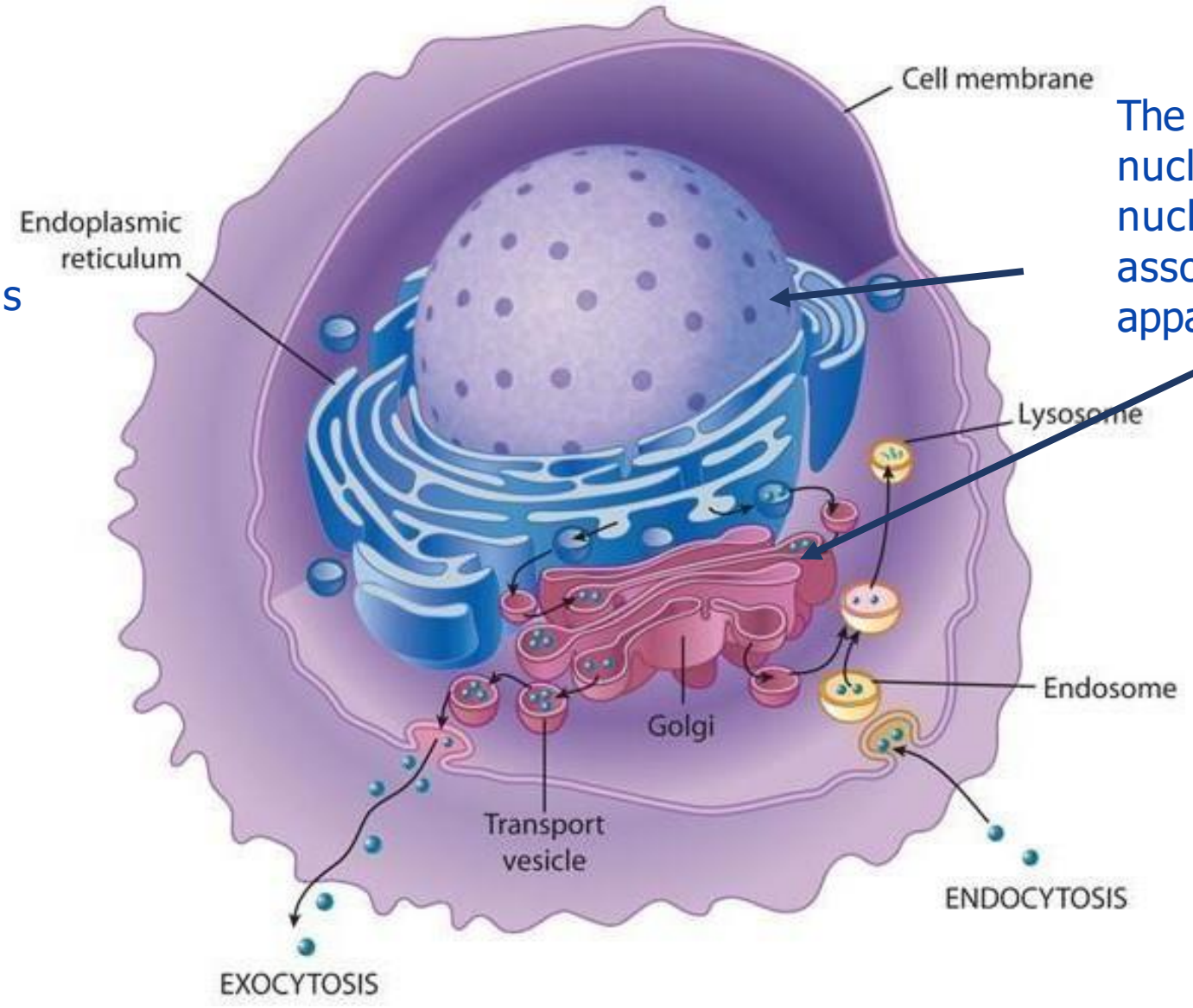
Protein sorting (endoplasmic reticulum)

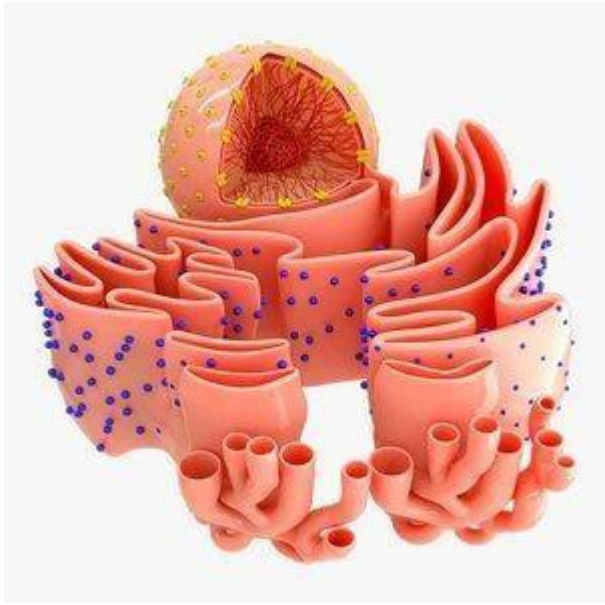
An overview

The proteins found in the organelles, cytosol, and those secreted outside the cell, what is the mechanism that determines their destination?
The endoplasmic reticulum is the main contributor

The ER is the largest organelle in the cell following the nucleus



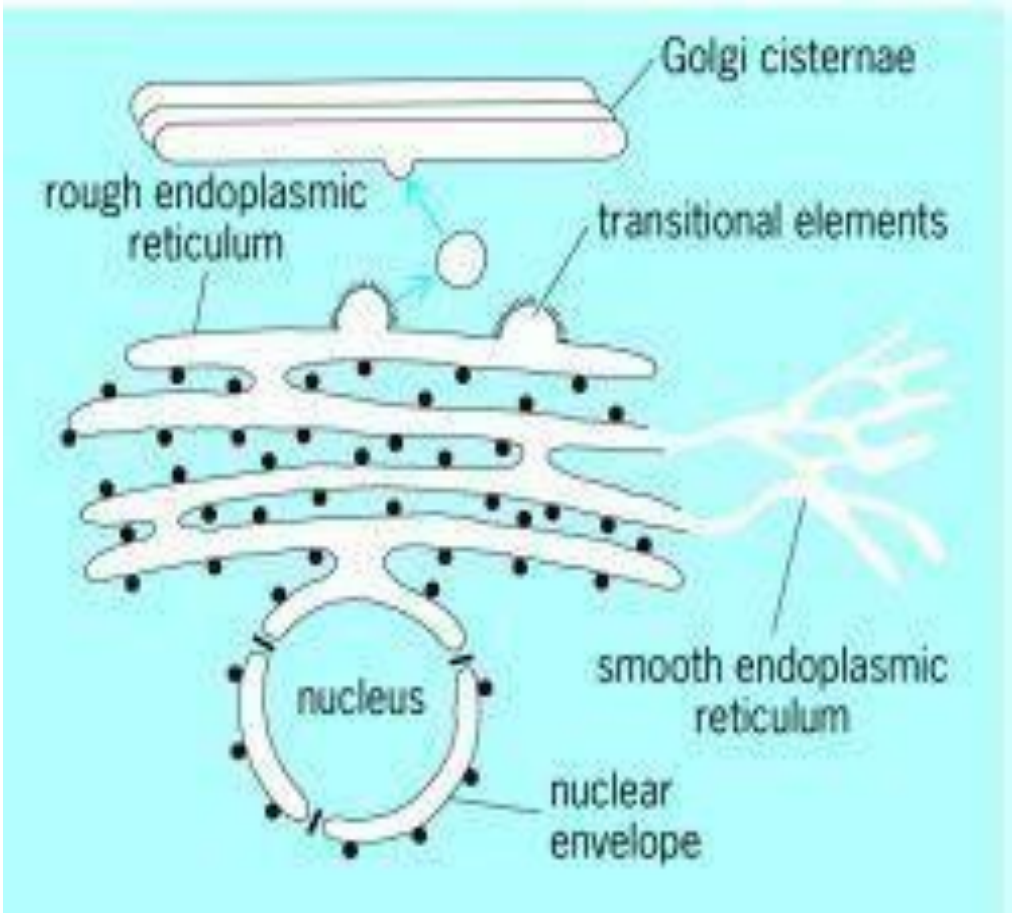
The ER is associated with nucleus (specifically the nuclear membrane) and associated with Golgi apparatus



Endoplasmic reticulum (ER)

It's organized as small tubes and composed of sacs that contain a lot of fluids (high flow) so it's very dynamic organelle

- It is a network of membrane-enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm.
- It is the largest organelle of most eukaryotic cells.



- The rough ER: covered by ribosomes on its outer surface and functions in protein processing.
- The smooth ER: lipid metabolism
- Transitional ER: exit of vesicles to Golgi apparatus

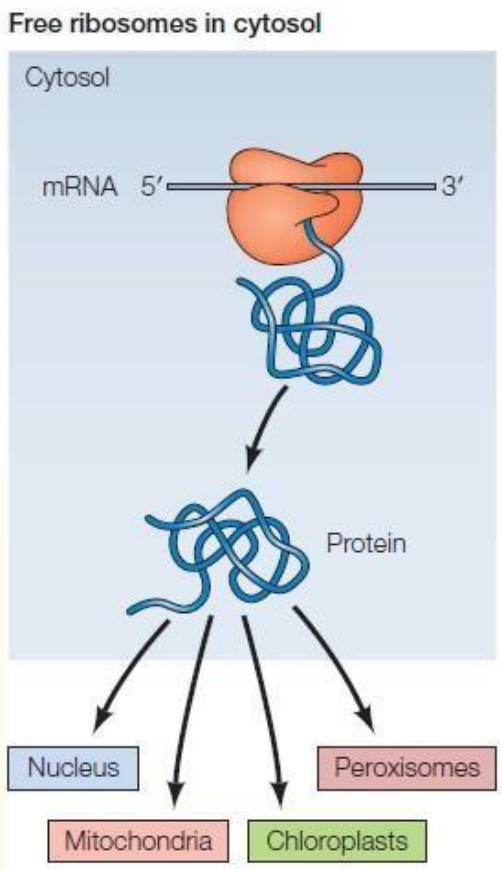
Synthesis of proteins occurs on the surface of rough ER through ribosomes

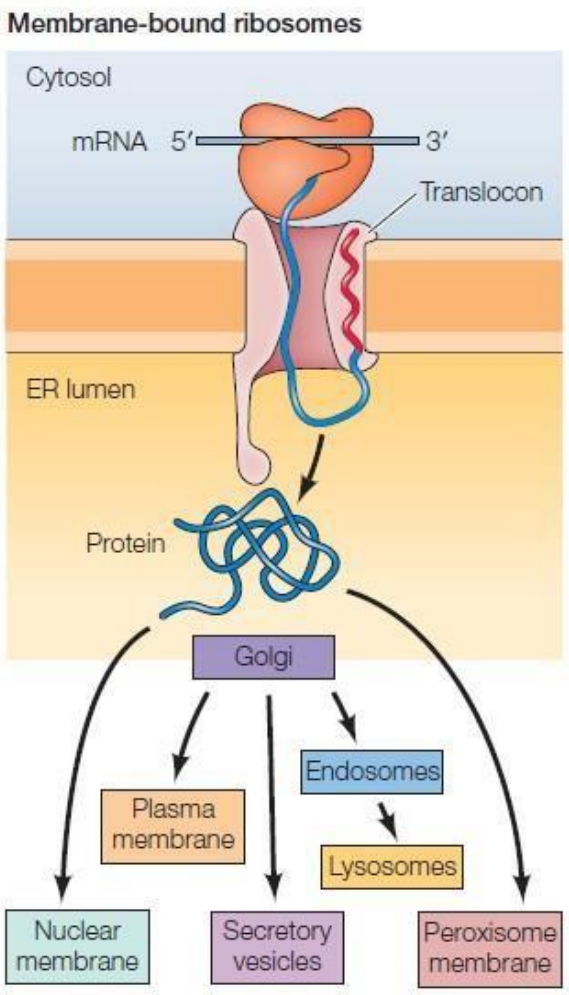
Cisternae : جيرهصلا لثم

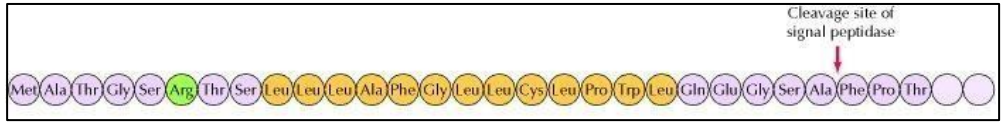
Protein sorting

- ✓ If the signal sequence is present on the N terminus of the protein while it is being synthesized in the ribosome, the protein will be directed to the surface of rough ER and protein synthesis continues into the ER lumen. If there are no signal sequences, protein synthesis will be completed in the cytosol.

Proteins synthesized on free ribosomes either remain in the cytosol or are transported to the nucleus, mitochondria, or peroxisomes.







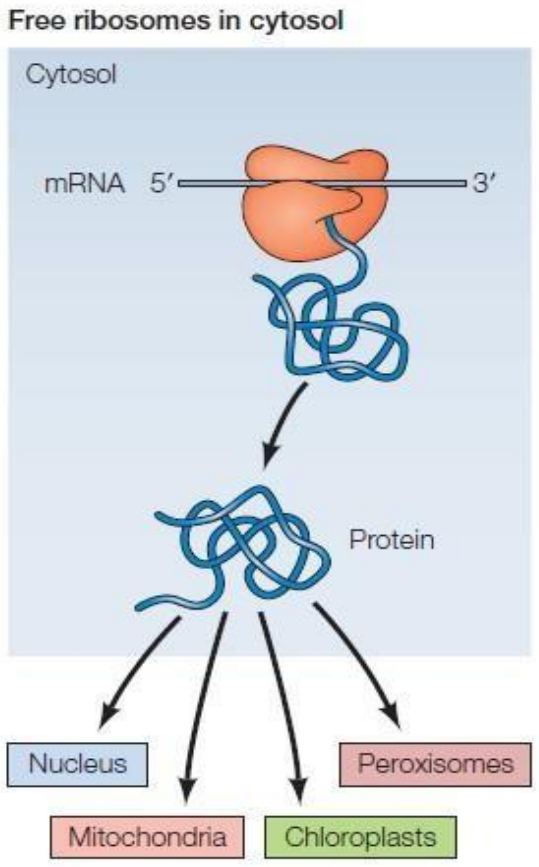
- ✓ Proteins containing **signal sequences** are synthesized on membrane-bound ribosomes and translocated directly into the ER.
- ✓ These proteins may stay within the ER or transported to nuclear membranes, peroxisomal membranes, or the Golgi apparatus and, from there, to endosomes, lysosomes, the plasma membrane, or outside the cell via secretory vesicles.

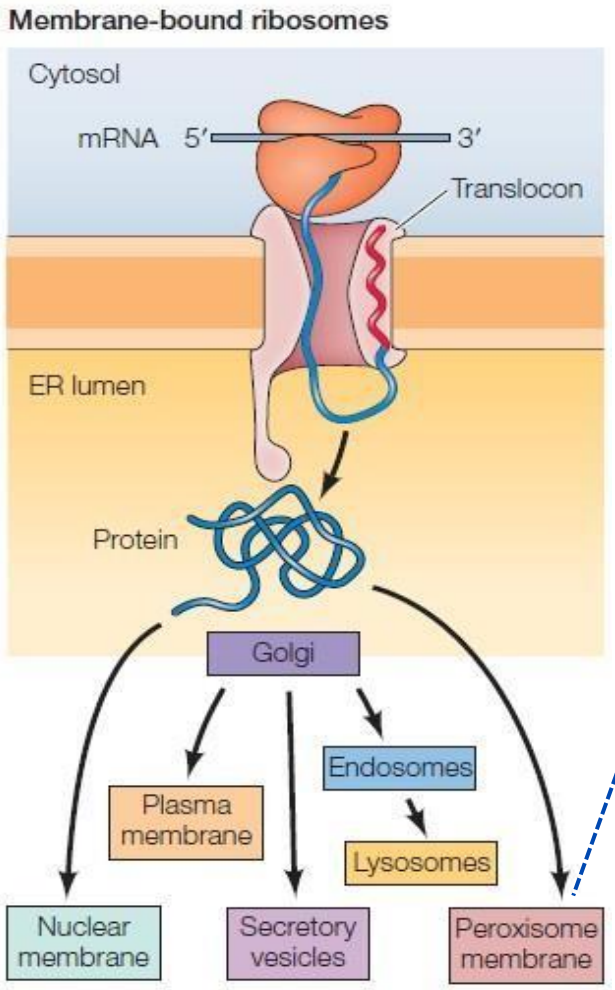
In cell biology, a lumen is a membrane-defined space that is found inside several organelles, cellular components, or structures

Signal sequence: a short sequence of amino acids of the polypeptide at the amino terminus. It is then cleaved from the polypeptide chain during its transfer into the ER lumen.

Protein sorting

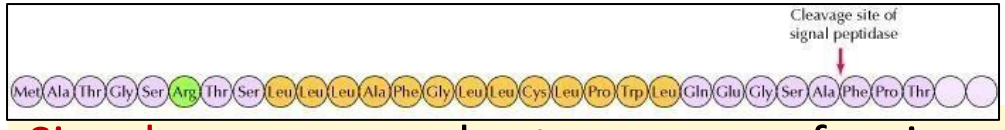
but if it is directed to the peroxisome, it will come from free ribosomes in the cytosol.





If the protein is bound to the peroxisomal membrane it was synthesized in the endoplasmic reticulum .

You should memorize where 2 types of proteins bound



Signal sequence: a short sequence of amino acids of the polypeptide at the amino terminus. It is then cleaved from the polypeptide chain during its transfer into the ER lumen.

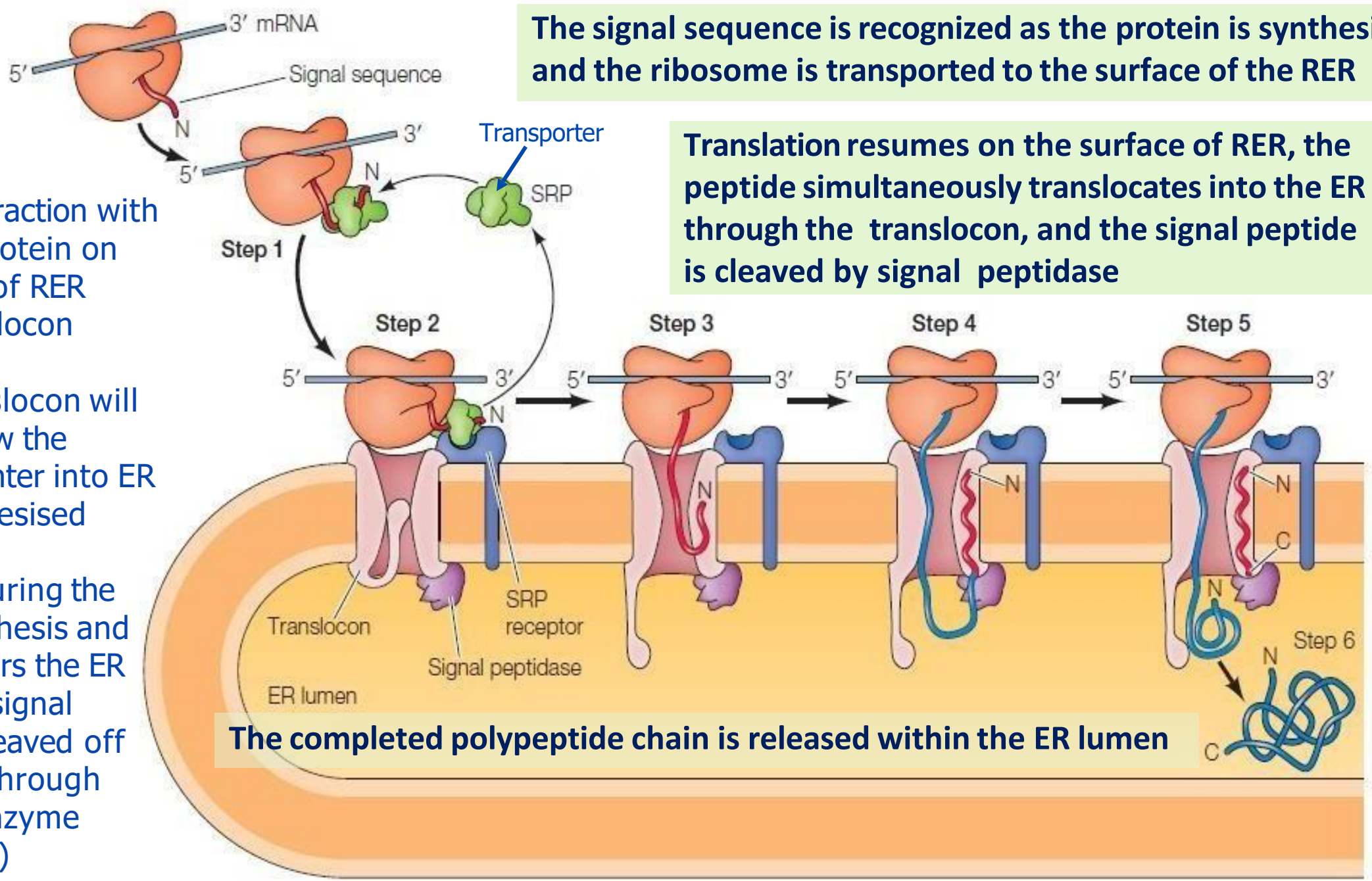
The signal sequence is recognized as the protein is synthesized and the ribosome is transported to the surface of the RER

Translation resumes on the surface of RER, the peptide simultaneously translocates into the ER through the translocon, and the signal peptide is cleaved by signal peptidase

step 2) Interaction with a channel protein on the surface of RER called Translocon

Step 3) translocon will open to allow the protein to enter into ER as it is synthesised

Step 4+5) During the protein synthesis and when it enters the ER lumen , the signal peptide is cleaved off (removed) through peptidase enzyme (hydrolysis)



The completed polypeptide chain is released within the ER lumen

Pathways of protein sorting

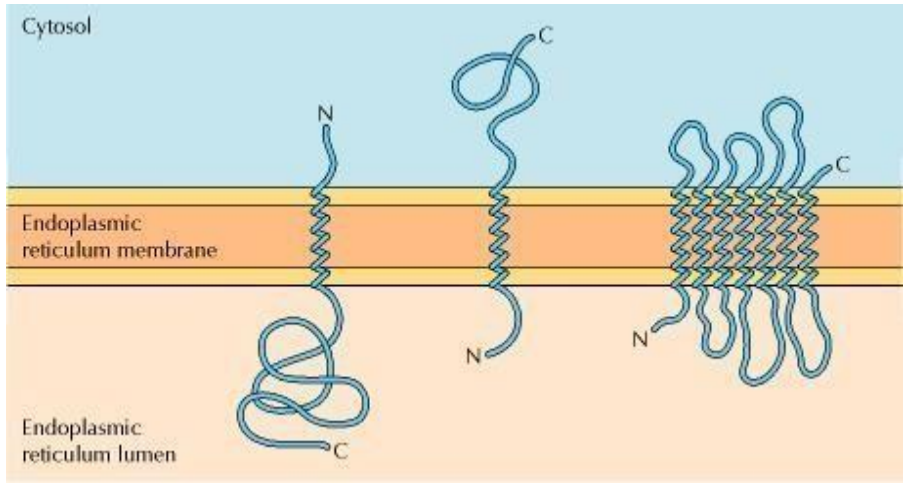
secretory vesicles to the plasma membrane

- Secretory, ER, Golgi apparatus, and lysosomal proteins are released into the lumen of the ER.
- Membranous proteins are initially inserted into the ER membrane. (Integrated)
- Considerations
  - Single vs. multiple membrane-spanning region
  - Orientation of N- and C-termini

Number of domains doesn't change during the transport process

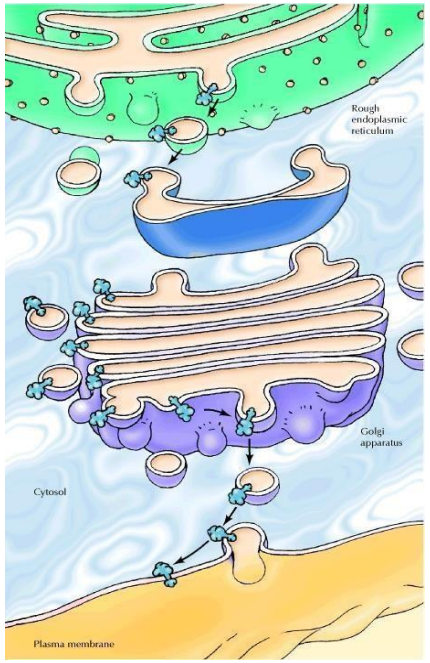
Then they transport to their specific membrane (plasma membrane , peroxisome membrane and etc .....) with the same shape as they had in the ER

So if the N terminus exists in the ER lumen , it will be found outside the cell in the plasma membrane (ولا في صب) And the C terminus will be inside the cell (into the cytosol)



ةيلخادلا في ةلثمم
the same environment

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



Insertion of membrane proteins via internal transmembrane sequences

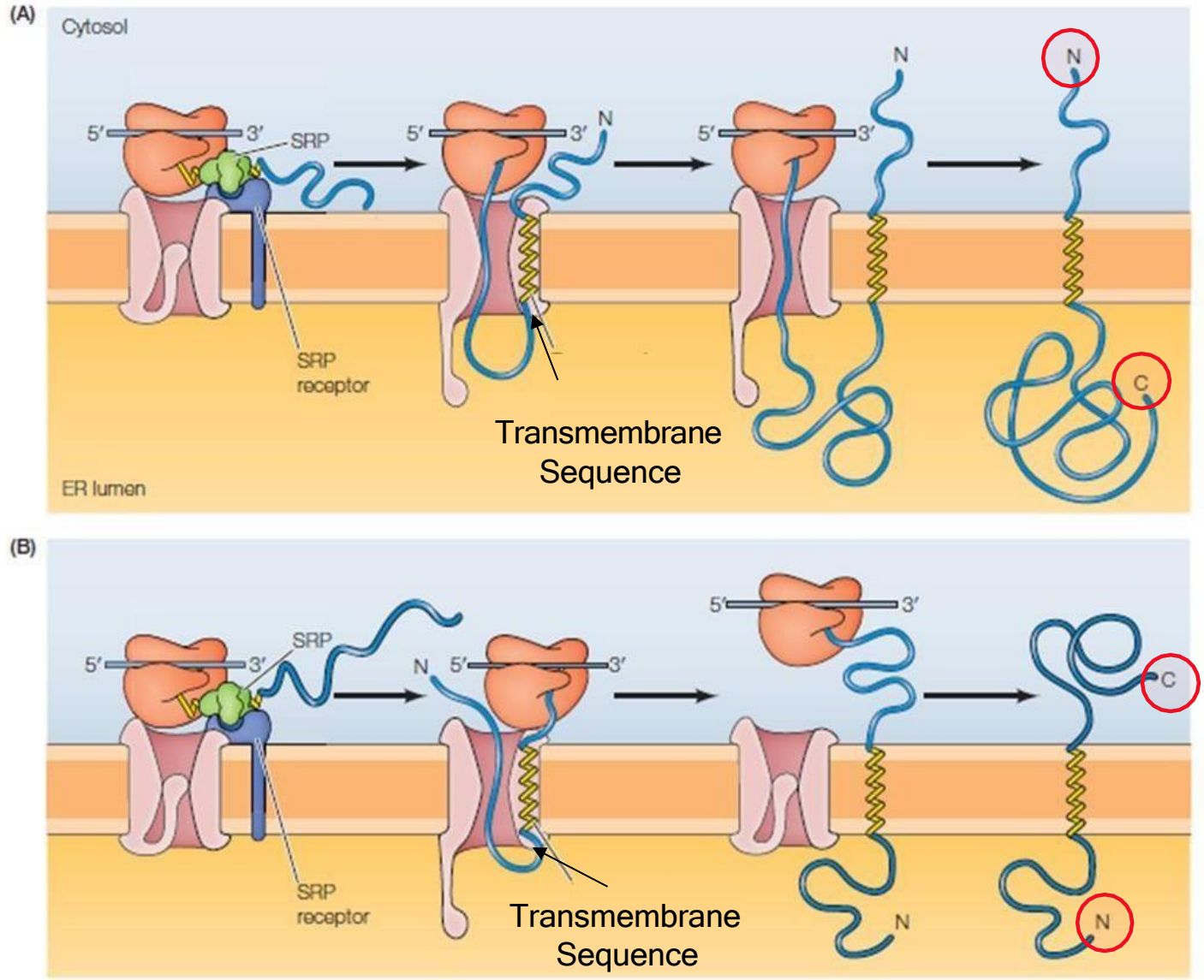
- Translocation of the polypeptide chain stops when the translocon recognizes a transmembrane sequence allowing the protein to become anchored in the ER membrane.

How do membrane proteins integrate into the ER membrane?

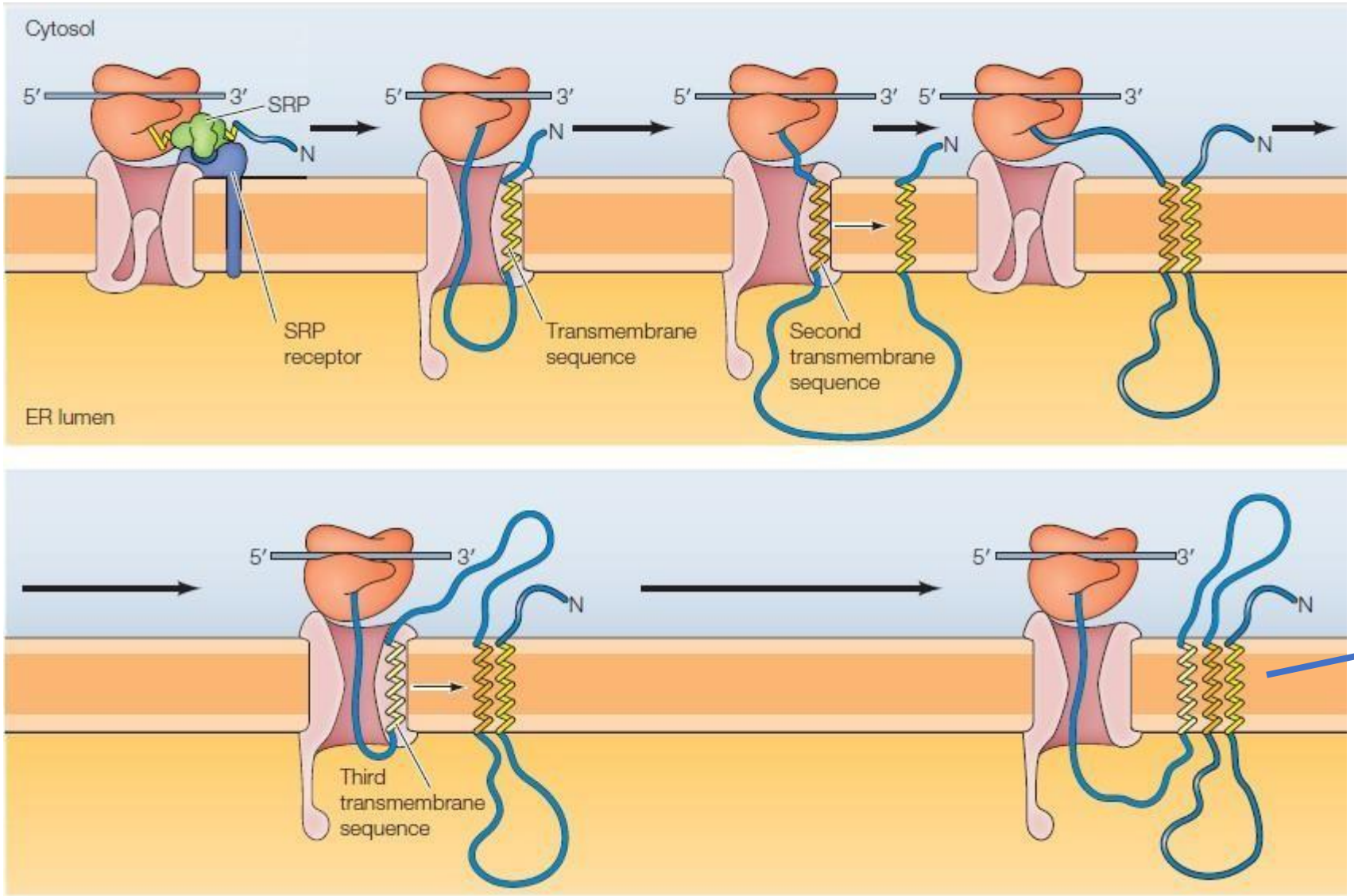
There is a specific sequence of amino acids called (transmembrane sequence) During the protein synthesis process this part will become anchored in the ER

- The direction of the internal transmembrane sequence determines the direction of insertion and orientation of the protein ends.

the N terminus is oriented toward the cytosol and the C terminus is in the lumen, if the transmembrane domain sequence is flipped, the direction of both of them will change .



Multi-transmembrane domain proteins have multiple transmembrane sequences



A protein with multiple transmembrane domains on the ER membrane

Once inside the ER, proteins are

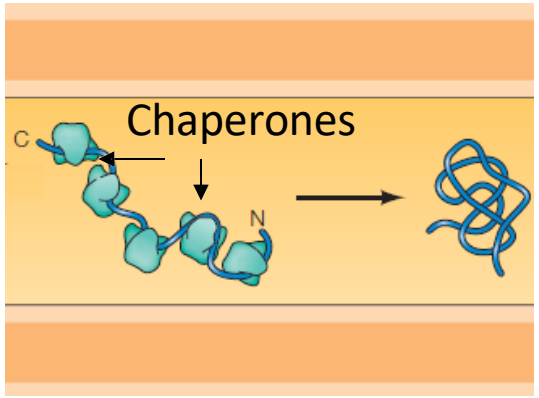
- Folded (with the help of chaperones)
- Complexed (quaternary structure)
- Disulfide bond formation by protein disulfide isomerase
- Glycosylated
- Anchored by lipids

If the protein have quaternary structure mean that it is made of more than one polypeptide and also it will form quaternary structure in endoplasmic reticulum and maybe chaperones can help as well

If the protein get inside the lumen , what will happen ?

-protein folding
(protein will take the 3D structure (tertiary structure))

-some proteins need help from the chaperones because they cannot takes their proper 3D structure so they need help (chaperones: proteins that help in protein folding)



Chaperones: بروتينات تساعد البروتينات

Once inside the ER, proteins are

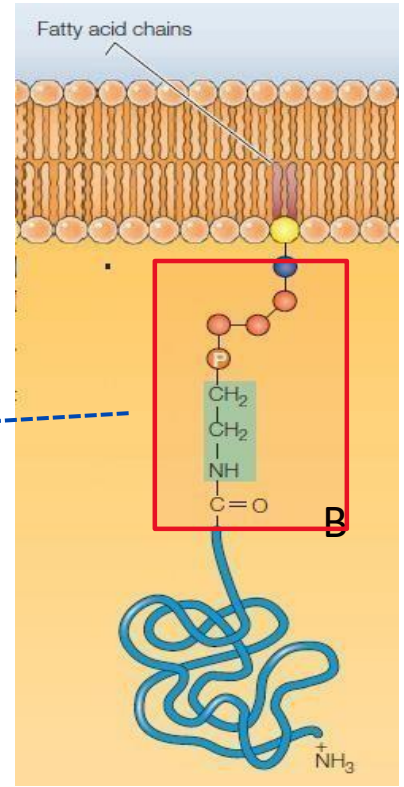
- Folded (with the help of chaperones)
- Complexed (quaternary structure)
- Disulfide bond formation by protein disulfide isomerase -------
- Glycosylated----------
- Anchored by lipids

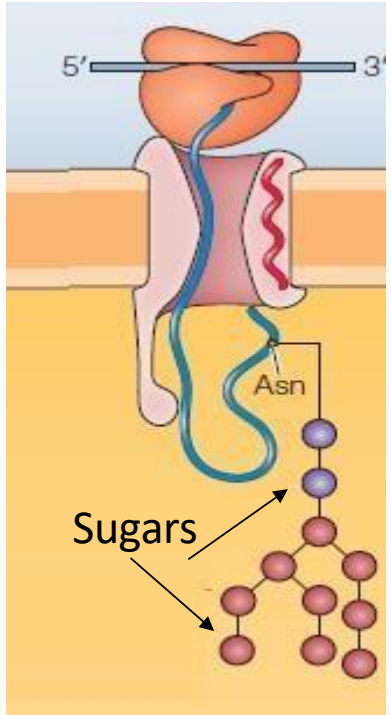
If the protein have disulfide bond there is an enzyme called protein disulfide Isomerase will help to form the proper disulfide bonds or the right bonds between cysteine residues

Like asparagine via glycosidic bond called N-linked glycosidic bond

Either directly connect fatty acid chain with amino acid
or sugar residue mediating fatty acid molecule with protein molecule

We call it GPI (glycosyl phosphatidyl inositol)



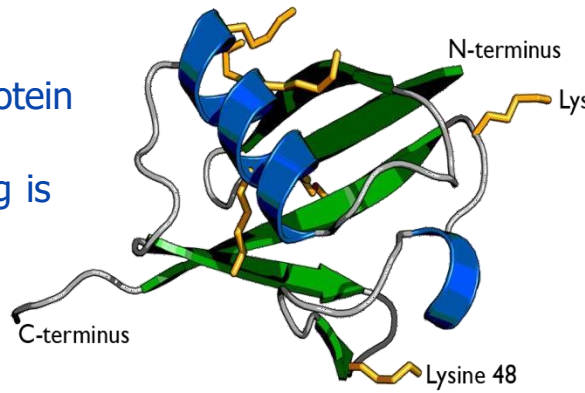


Protein folding and ER-associated degradation (ERAD)

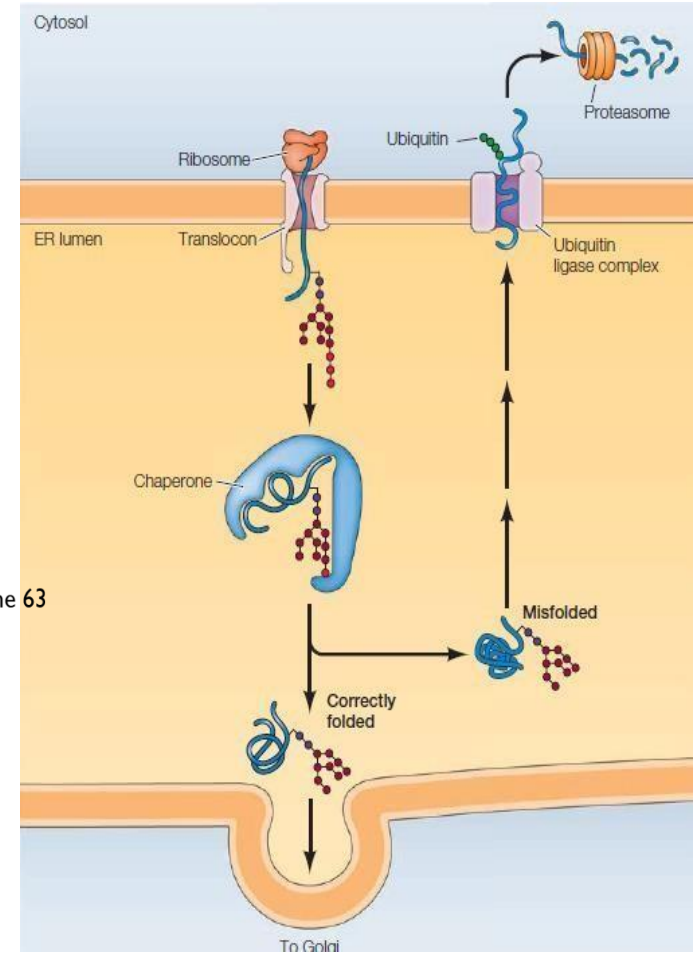
- If correctly folded, proteins move on.
- If misfolded, proteins are sent to the cytosol, ubiquitylated (addition of small proteins called ubiquitins), and degraded in the proteasome.

If the protein folding incorrectly, there is a system in cell called (protein folding and ERAD) this system is sensor in quality control
The system wathches how the protein folding is going ,if the folding is right the protein move on ,but if there is misfolding in this case the protein will be unfolded and then folded again.

If the protein is misfolded again so the protein is sent outside the endoplasmic reticulum(it happen to the protein what's called Ubiquitination) What does it mean? there is small proteins called ubiquitins , these proteins are added to the protein so Ubiquitination will happen to the protein
and from here we will go to large protein complex called proteasome doing protein degradation to misfolded proteins or proteins that the cell no longer needs so the cell will replace (turnover) these proteins



some: structure/ object(like chromosome)



Ubiquitination: The process of adding small proteins called ubiquitins for the purpose of protein degradation or polypeptide degradation.

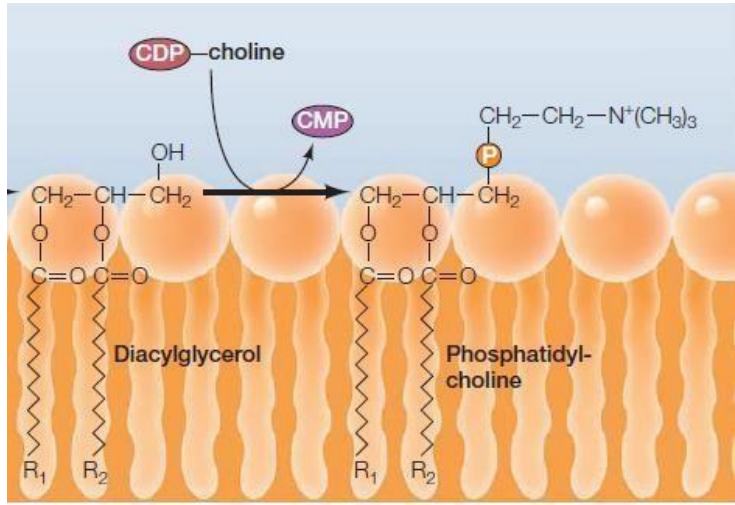
Synthesis of phospholipids in ER

Responsible for lipid metabolism specifically formation of glycerophospholipids that composed of glycerol , two fatty acid , phosphate group (phosphocholine as an example that form phosphatidylcholine) . Then Formation of phosphatidylethanolamine , phosphatidylinositol, Phosphatidylserine, etc…

- The smooth ER is the major site of synthesis of:
  - Membrane glycerophospholipids, which are then transported from the SER to other membranes.
  - Sphingophospholipids (like ceramides and glycolipids) and steroids.
    - Large amounts of smooth ER are found in steroid-producing cells, such as those in the testis and ovary.

Because they are responsible for the production of cholesterol hormones such as estrogen, progesterone and androgens.

Glycolipid : ceramides with sugar molecule or disaccharide Forming globosides or gangliosides that composed of sialic acid



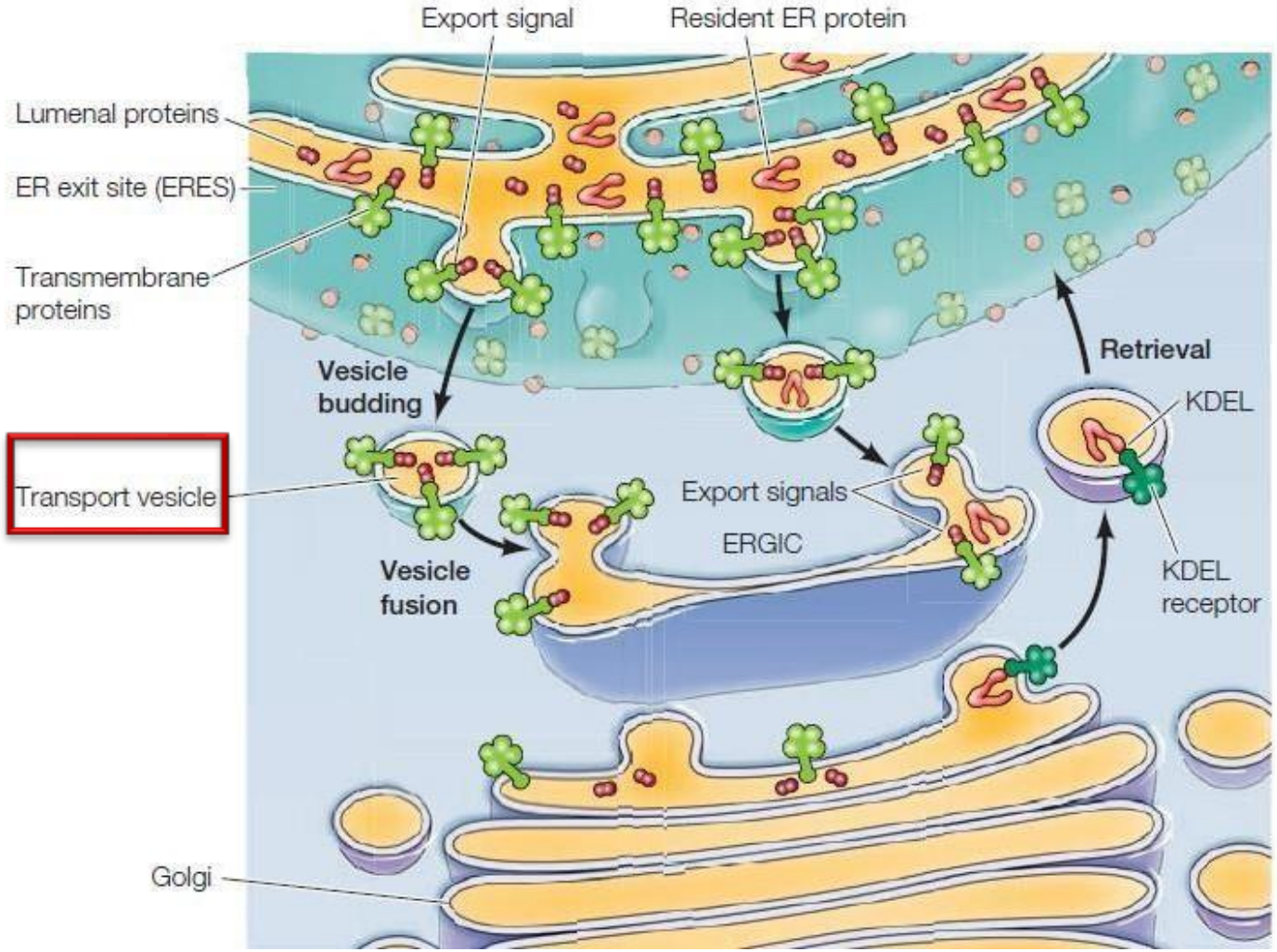
- SER is abundant in the liver, which contains enzymes that metabolize various lipid-soluble compounds.

In addition, for drugs that are lipophilic, it is difficult for the body to eliminate them. Therefore, they are sent to the liver, specifically to the smooth endoplasmic reticulum (SER) in liver cells, where the lipophilic molecules are modified to become hydrophilic, allowing the body to easily excrete them

ER-Golgi intermediate compartment (ERGIC)

- Proteins and lipids are carried from the ER to the Golgi in transport vesicles, which fuse with the ER-Golgi intermediate compartment (ERGIC), and are then carried to the Golgi.

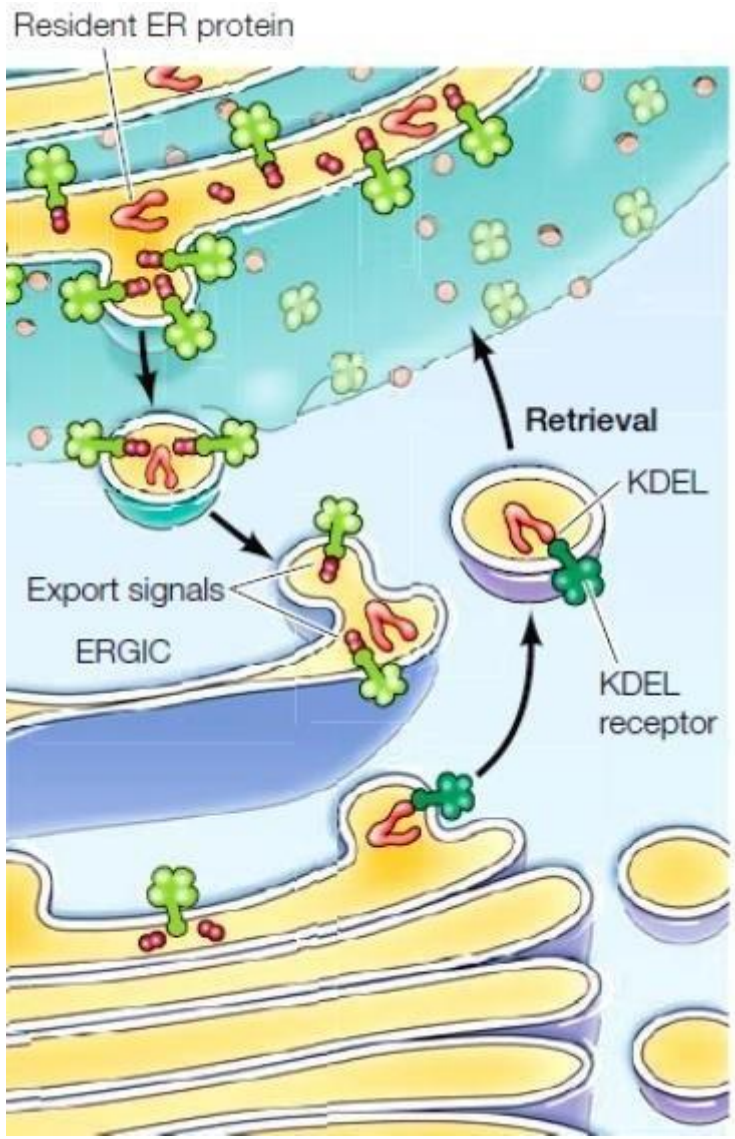
ERGIC: Check points



Retention of ER protein

- Many proteins with KDEL sequence (Lys-Asp-Glu-Leu) at C-terminus are retained in the ER lumen.
  - If the sequence is deleted, the protein is transported to the Golgi and secreted from the cell.
  - Addition of the sequence causes a protein to be retained in the ER.

If the protein must remain in the endoplasmic reticulum , it will be sent to ER via vesicles because they have amino acid sequence called KDEL so it is signal that the protein must go back to ER



KDEL
K: Lysine
D: Aspartic Acid
E: Glutamate
L: Leucine

Functions of the Golgi apparatus

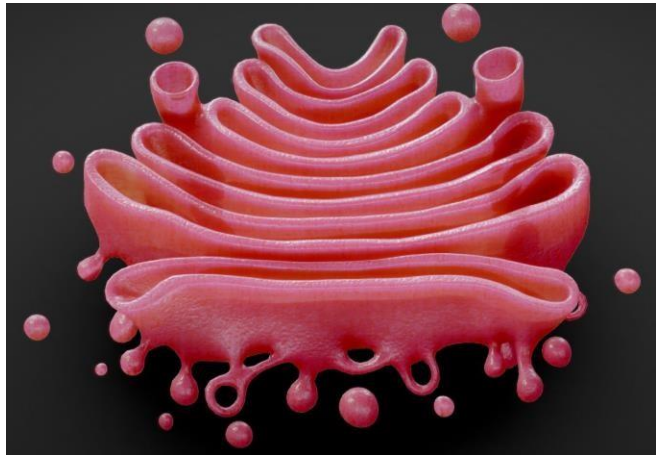
- Further protein processing and modification
- Further protein sorting
- Synthesis of glycolipids and sphingomyelin

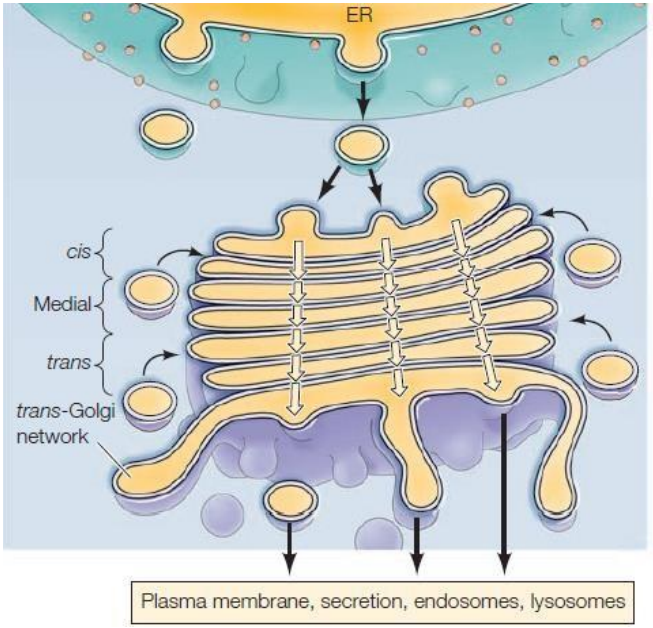
A quick comparison

ER	Golgi
Synthesis of:	Synthesis of:
-Glycerophospholipids especially cyramides	-Sphingolipids especially glycolipids and sphingomyelin
-Steroids	

Structure of the Golgi

- The Golgi apparatus consists of a stack of flattened sacs (cisternae) of four regions: *cis*, medial, and *trans* compartments and the *trans*-Golgi network.
- Proteins are carried through the Golgi apparatus in the *cis-to-trans* direction.
- Transport vesicles carry the Golgi proteins back to earlier compartments for reuse.





- Golgi apparatus is a dynamic structure

Transport vesicles carry the proteins from cis to trans-golgi region inside golgi , and then they may go back to cis region feeding it

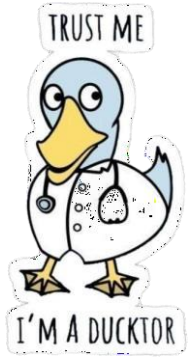
In other words , golgi can carry out a forward and backward movement of vesicles , that's why we considered it as a dynamic structure

Watch this animation for further explanation

https://learninglink.oup.com/access/content/cooper8e-student-resources/cooper8e-chapter-2-noitamina-12

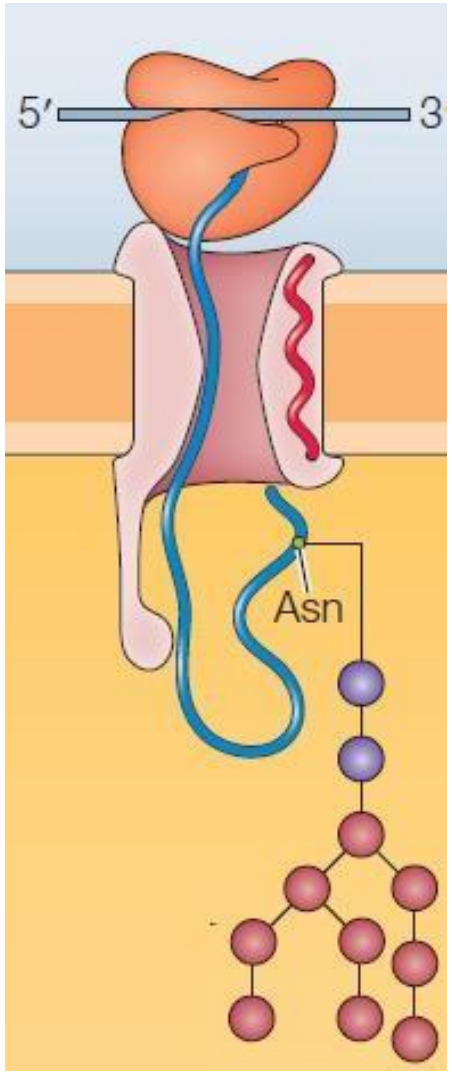
- After cyramide synthesis in the ER , cyramide goes to golgi , where a sugar or phosphocholine is added to it making a glycolipid or sphingomyelin

Processing of *N*-linked oligosaccharides in Golgi

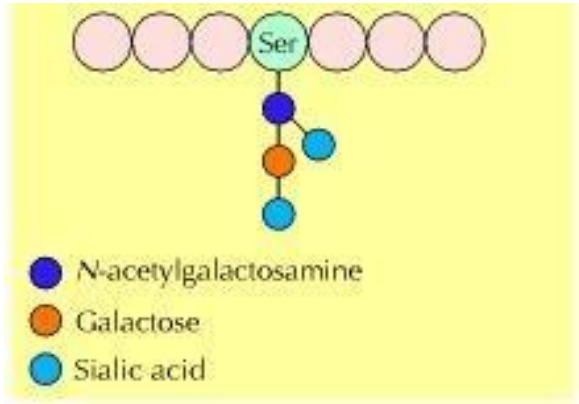


The *N*-linked oligosaccharides, which are added to asparagine residues of glycoproteins and transported from the ER, are further modified enzymatically in different compartments of the Golgi.

N–linked glycosylation starts in the ER and continues in golgi apparatus
While O–linked glycosylation starts in golgi apparatus

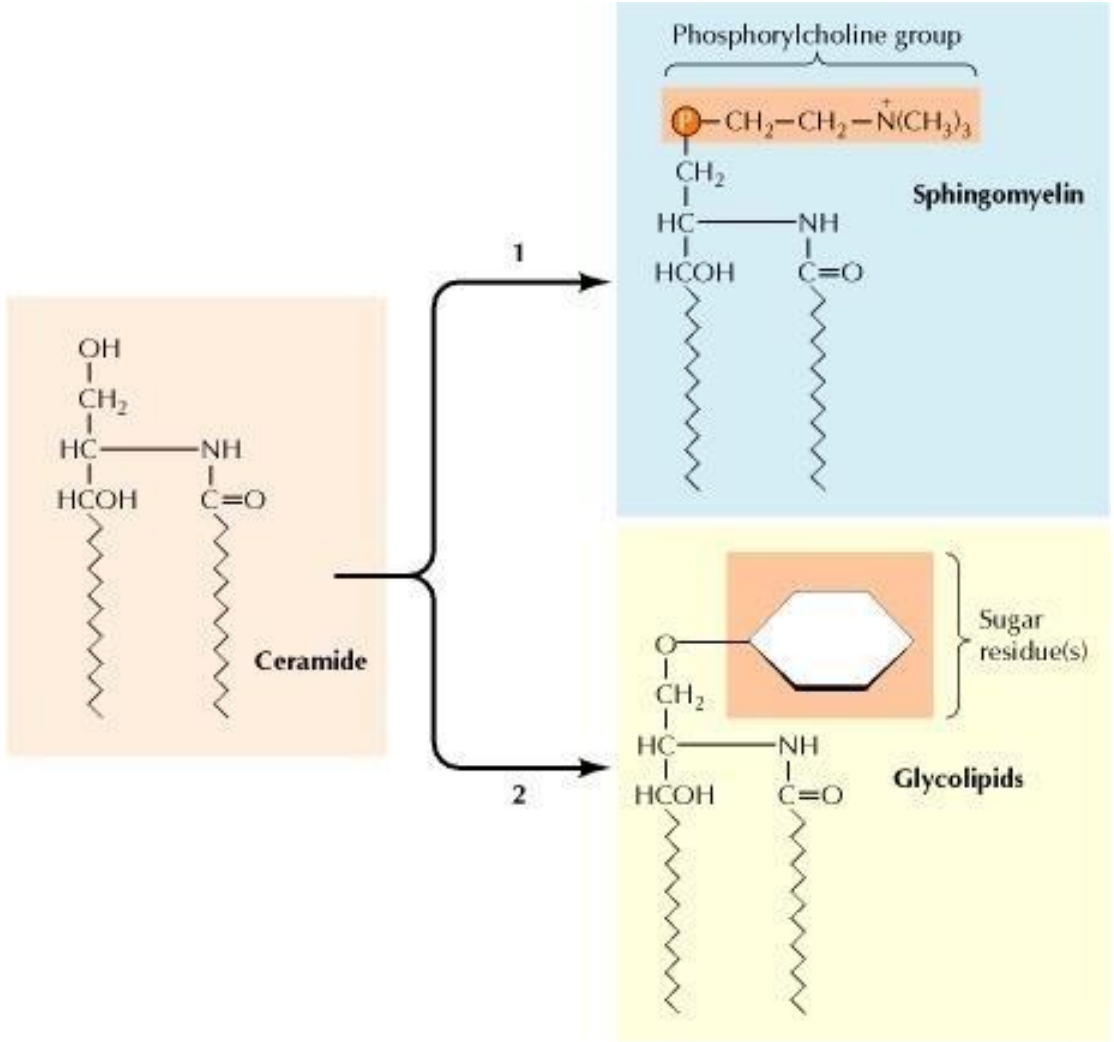


Proteins can also be modified by the addition of carbohydrates to the hydroxyl side chains of serine and threonine residues, hence called O-linked sugars.



Lipid and Polysaccharide Metabolism in the Golgi

- Ceramide is converted either to sphingomyelin (a phospholipid) or to glycolipids in the Golgi apparatus.

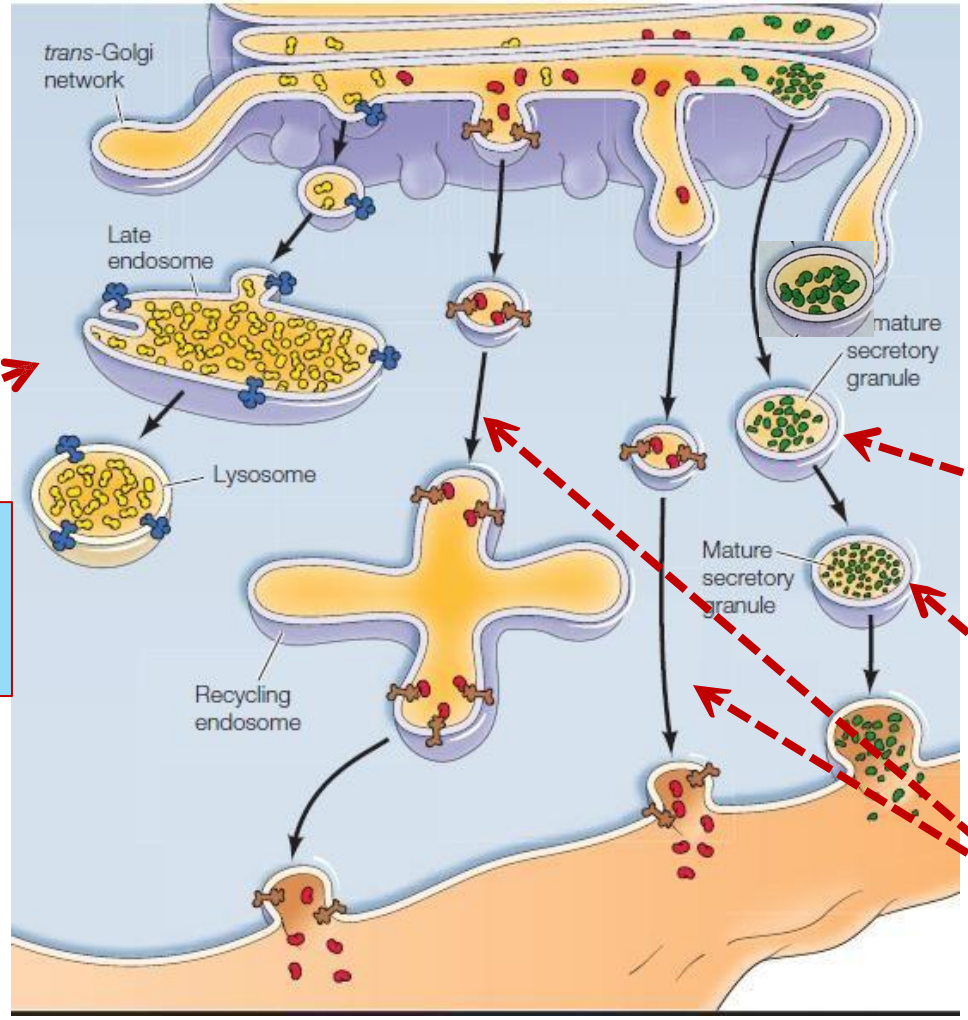


Ceramide is synthesized in the ER

-EXTRA-

Remember
-Sphingomyelin is the only sphingophospholipid
-Glycolipids are 3 types :
Globosides , cerebrosides and gangliosides

Protein Sorting and export



In contrast to the ER, all of the proteins retained within the Golgi complex are associated with the Golgi membrane rather than being soluble proteins within the lumen

Proteins can be targeted to late endosomes, which develop into lysosomes

Protein processing in Immature secretory vesicles

Regulated secretion after signaling from specialized vesicles

Continuous, unregulated secretion

- Exporting proteins from golgi to cell surface can occur via many routes :

- Direct transport from trans-golgi network to the plasma membrane via vesicles which leads to continuous secretion *unregulated *from the cell
- Vesicle remains in the cytosol waiting for a specific signal to come to fuse with the plasma membrane and release its contents *regulated secretion*

For example, the digestive enzymes produced by pancreatic acinar cells are stored in mature secretory granules until the presence of food in the stomach and small intestine triggers their secretion .
Another example , vesicles containing neurotransmitters remain in the cytosol waiting for calcium ions to inter the cell and bind to proteins on the vesicular membrane allowing these vesicles to interact with the plasma membrane and release their content outside the cell

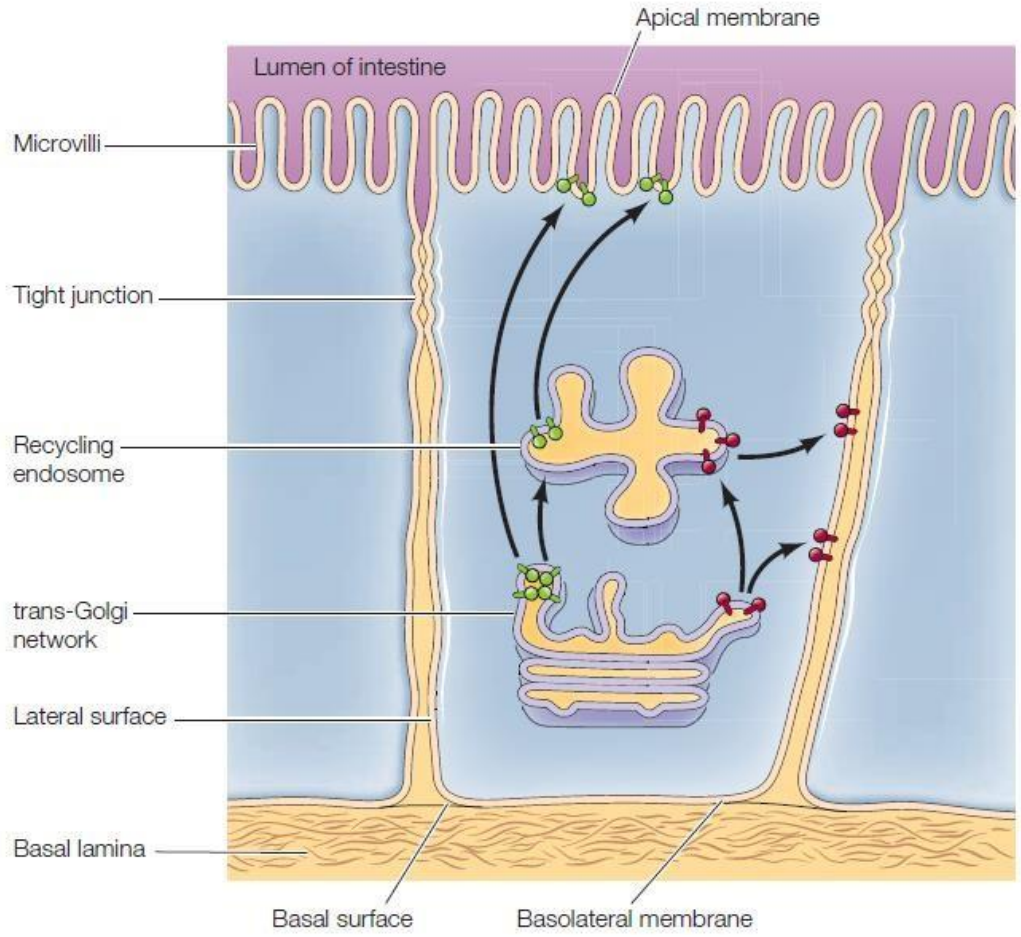
-What about lysosomal proteins ?
The transport vesicle fuses with what we call a late endosome, which then matures into a lysosome

Transport to the plasma membrane of polarized cells

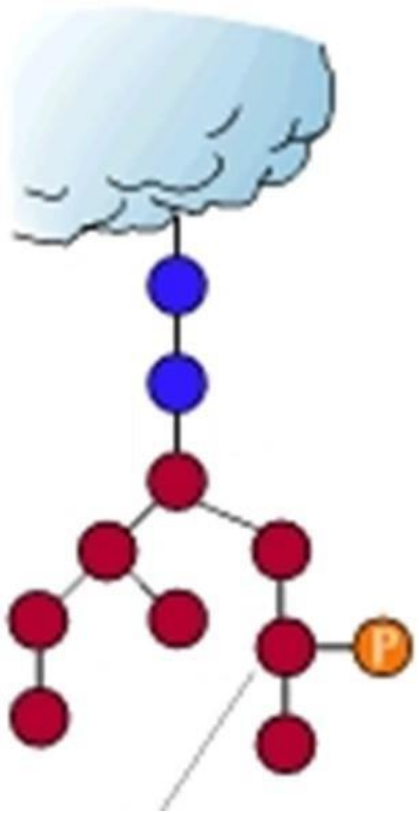
- Proteins are selectively packaged into transport vesicles from the trans-Golgi or recycling endosomes.
- Targeting is determined by special sequences (basolateral) or GPI sugar modification (apical).

Upon vesicle fusion with the plasma membrane, the specific site of fusion doesn't matter; because all sides of the membranes are similar ,EXCEPT for polarized cells

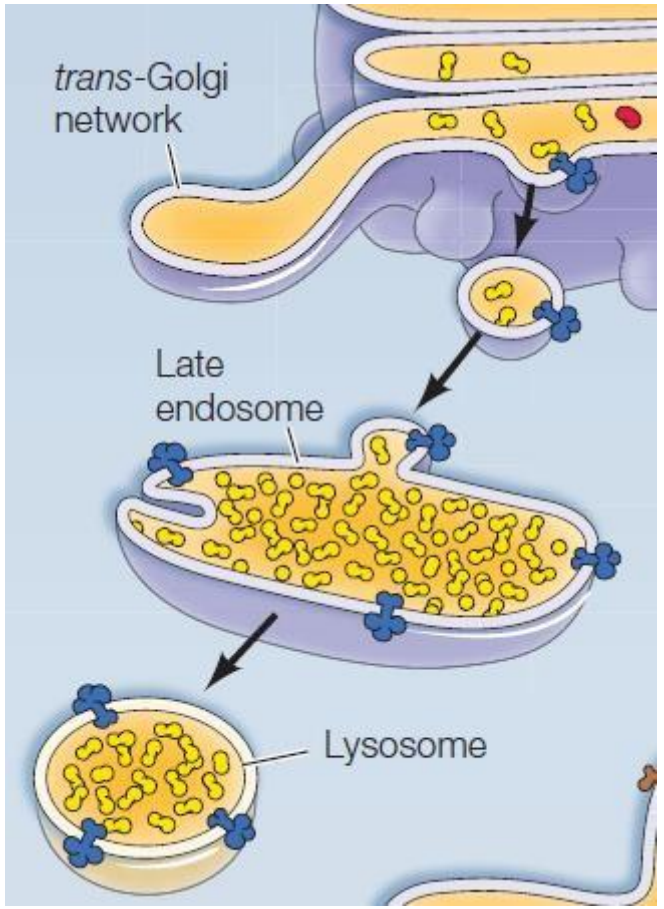
Polarized cells are cells with different apical and basolateral surfaces like intestinal cells



Processing of lumenal lysosomal proteins



Protein destined to lysosomes have a signal patch (a three-dimensional structural determinant), which is recognized by modifying enzymes that add mannose-6-phosphate to the proteins.



Lumenal lysosomal proteins bind to a mannose-6-phospahte receptor and are transported to late endosome, which mature into lysosomes.

- Lysosomal proteins are glycosylated proteins
- One of these sugars is mannose, which is then phosphorylated and bound to a specific receptor on golgi surface
- So a vesicle pinches out with the phosphorylated lysosomal protein inside , and fuses with the late endosome which is then matured into a lysosome