بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture #2

Endoplasmic Reticulum & Golgi Apparatus

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Fluid Mosaic Model

- The plasma membrane follows the fluid mosaic model, Therefore the distribution of structures is NOT random, they are present as clusters in certain regions of the cell. e.g different types of phospholipids are distributed depending on the function.
- One of these regions is a specialised region called **Lipid Raft,** this region is unique for having a special composition of lipids such as :

- Sphingolipids
- Glycolipids
- Cholesterols

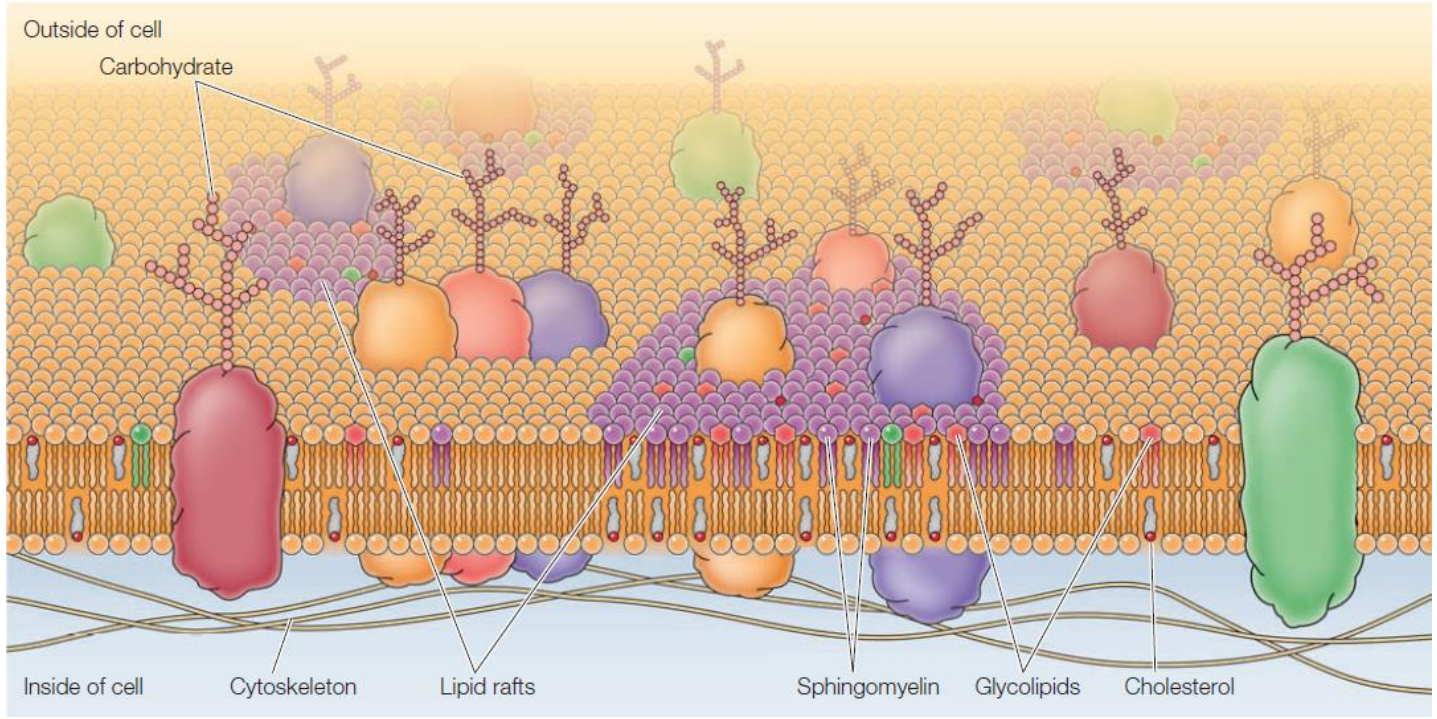
Which makes this region sort of rigid preventing lipids from moving out of this region.

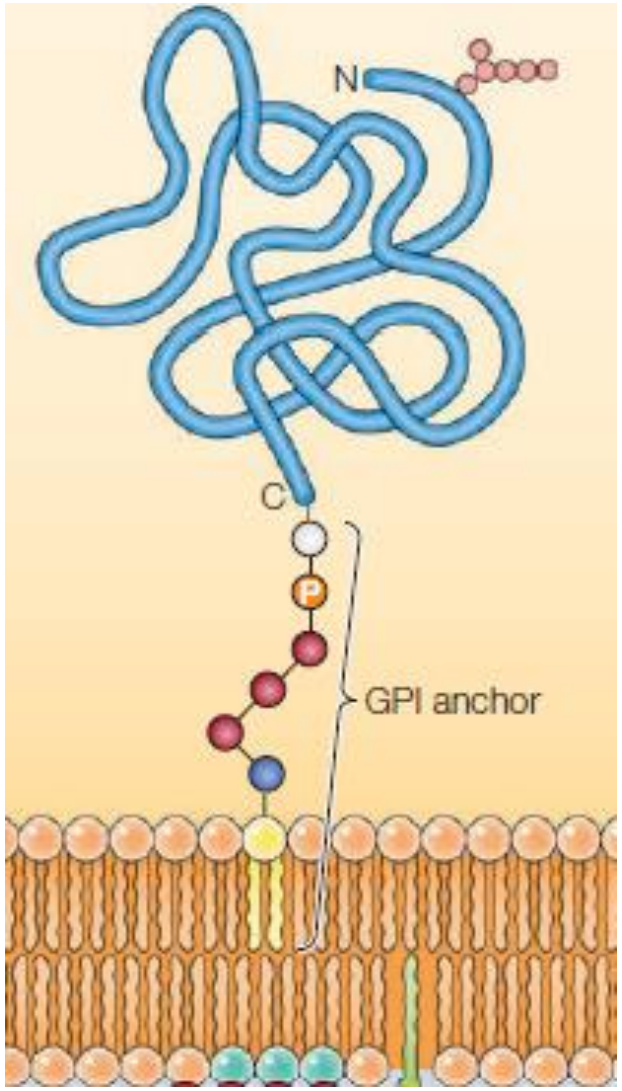
- Lipid rafts contains receptors and GPI anchored proteins and other molecules.

- The purpose of lipid rafts is to concentrate, organise, increase the efficiency of the signalling pathways, the presence of receptors and signalling molecule clustered in one region facilitate the ligands binding to receptors rather than when receptor being distributed randomly at the plasma membrane.

Lipid rafts

- Specialized membrane regions with clusters of **cholesterol** and the **sphingolipids** (sphingomyelin and **glycolipids**).
- Rafts are enriched in **glycosylphosphatidylinositol (GPI)-anchored proteins**, and proteins involved in signal transduction and intracellular vesicular trafficking (transport).





Caveolae
(Latin for “little caves”)

- They are a subset of lipid rafts that require cholesterol for their formation.
- They are formed the membrane protein caveolin, which interacts with cholesterol and the cytoplasmic protein **cavin**.
- They are important for several cellular activities, including endocytosis, cell signaling, regulation of lipid transport, and protection of the plasma membrane against mechanical stress.

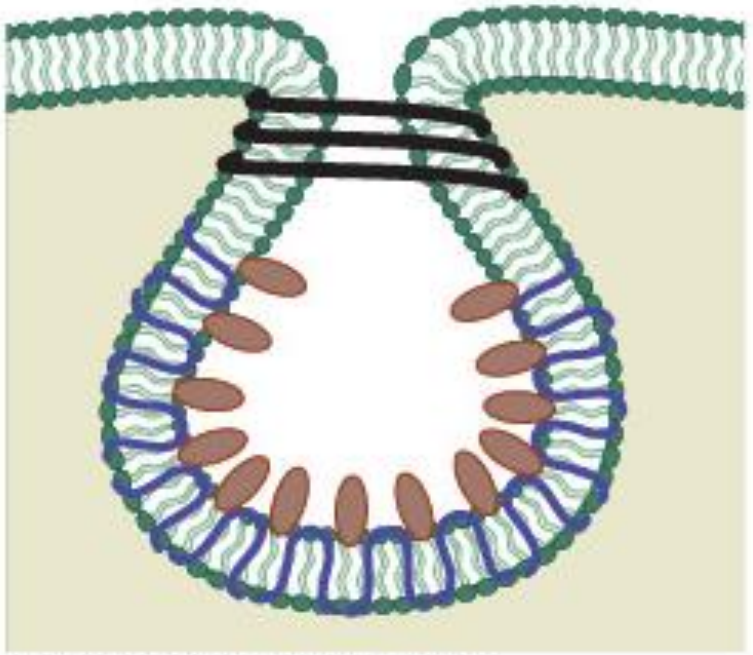
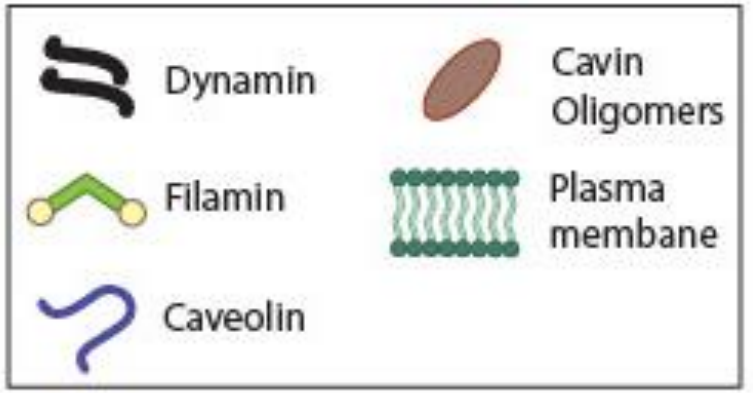
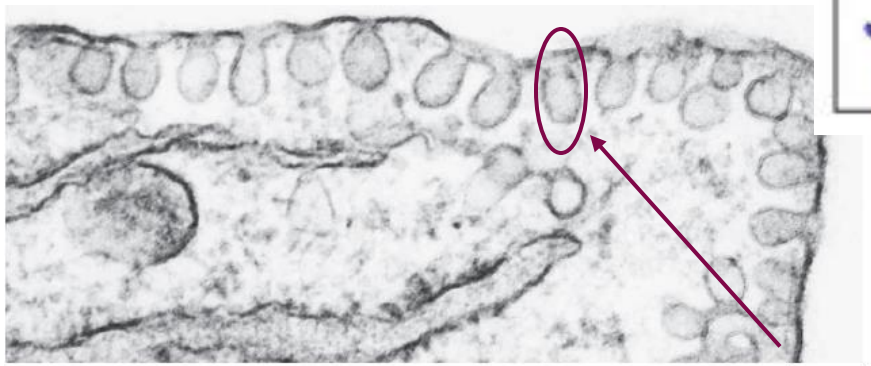


Fig. Simplified model of the Caveolae





Electron microscopic image of caveolae

Caveolae

- They are a specific type of a raft known as caveola.

- Caveola have specific types of proteins.

- Caveola are formed by a protein known as caveolin. This caveolin has an interaction with cholesterol and cytolytic protein cavin. the caveolin contains a cluster of signaling molecules (receptors).

- Caveolae are also very important for **transport processes like (endocytosis)** often happens in the caveolae regions. This shows that the **plasma membrane is not homogeneous**, but rather **contains specialized regions, such as lipid rafts and caveolae**, each playing specific roles in cell signaling and transport.

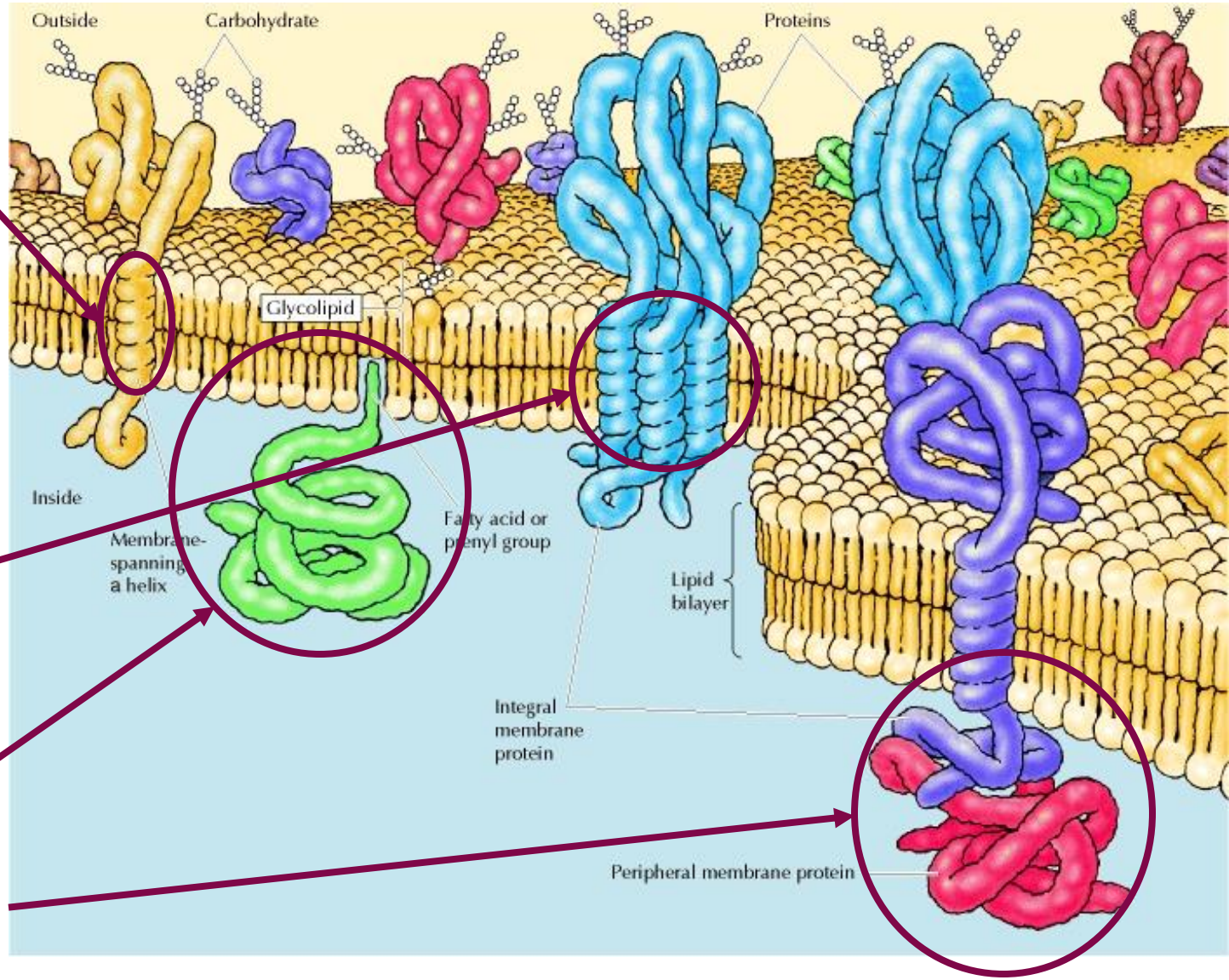
Membrane proteins

- **Integral membrane proteins:** the term integral means that **these proteins are embedded within or inserted into (integrated into) the lipid bilayer** – The region of the protein that spans the membrane **is helical in shape, made up of hydrophobic amino acids**, allowing it to **interact with the hydrophobic core of the lipid bilayer**.

- There are also integral proteins that are integrated in the membrane more than one time (**multi-transmembrane domains**).

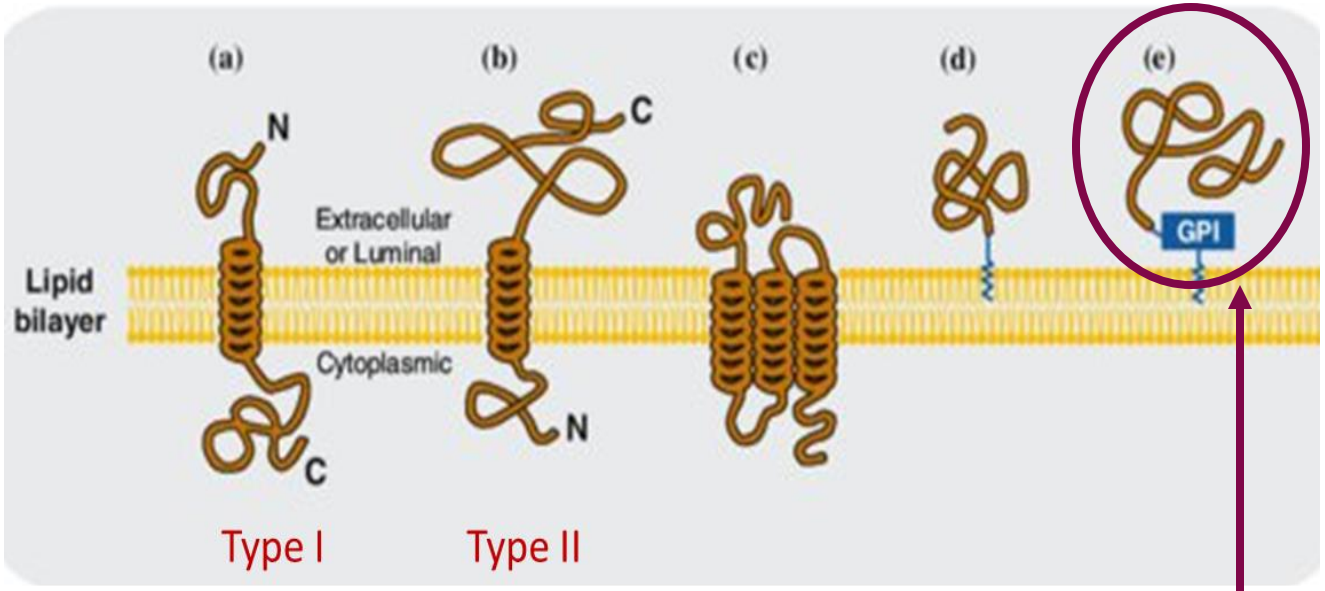
- There are a proteins that are adding a hydrocarbon chain (bind covalently to the protein) that is inserted inside the membrane.

- **Peripheral Proteins :** There are proteins that are **not integrated inside the membrane** but associated with integral proteins.



Membrane proteins

- An integral membrane protein **can cross the membrane once (single-pass), twice (double-pass) these two cases are the most popular , or multiple times** – for example, 7 times (like G-protein-coupled receptors) or even more. This means the protein may enter and exit the membrane several times, forming transmembrane segments.

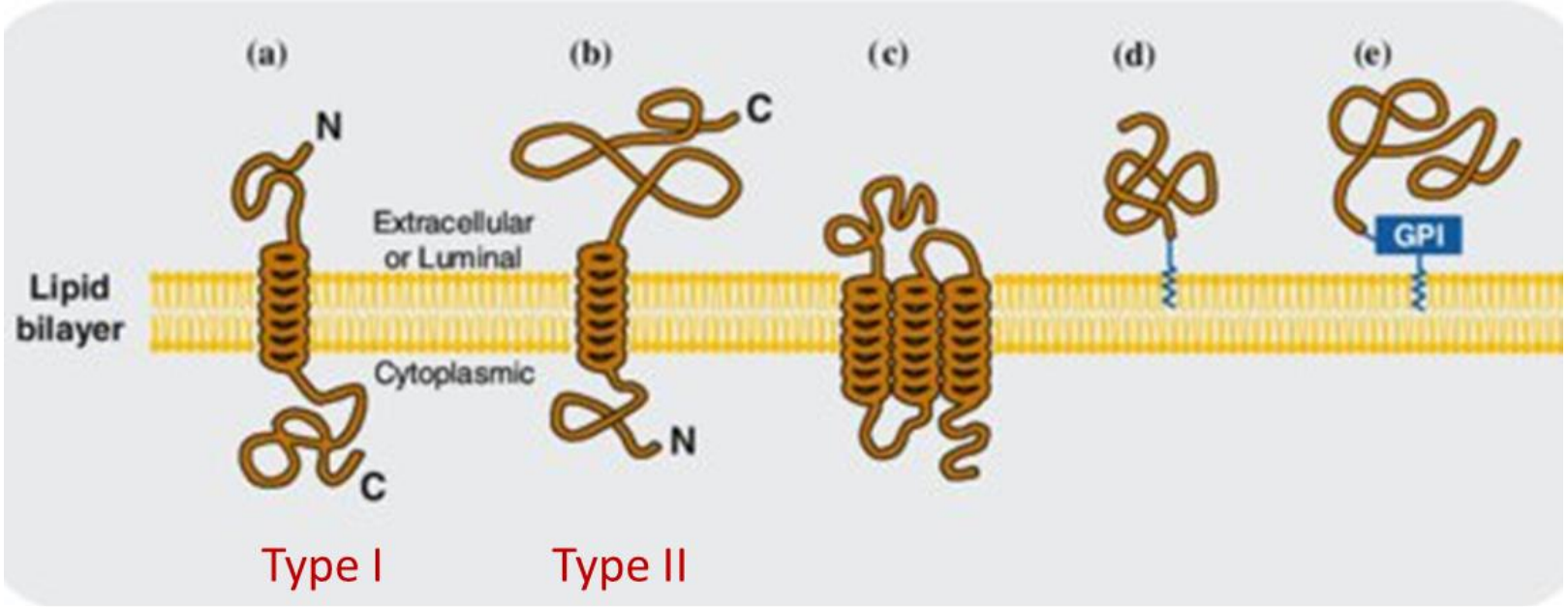


- Even a protein with a single transmembrane (or multi-transmembrane) domain **differs in its organization** (e.g the **N terminus can be outside (type I) or inside (type II)**) therefore, we have **different mechanisms of inserting proteins inside the cell.**

- GPI anchored proteins : a glycolipid molecule contains a lipid part (which is a part of the membrane) and a sugar part binds covalently with the protein.

Types of membrane proteins

- Peripheral membrane proteins are indirectly and loosely associated with membranes through protein-protein interactions, mainly ionic bonds.
- Integral membrane proteins have some of their helical parts inserted into the lipid bilayer.
  - Single-pass (type I or II) or multi-pass proteins.
- Lipid-anchored membrane proteins (myristoylation, palmitoylation , glycosyl-phosphatidylinositol)

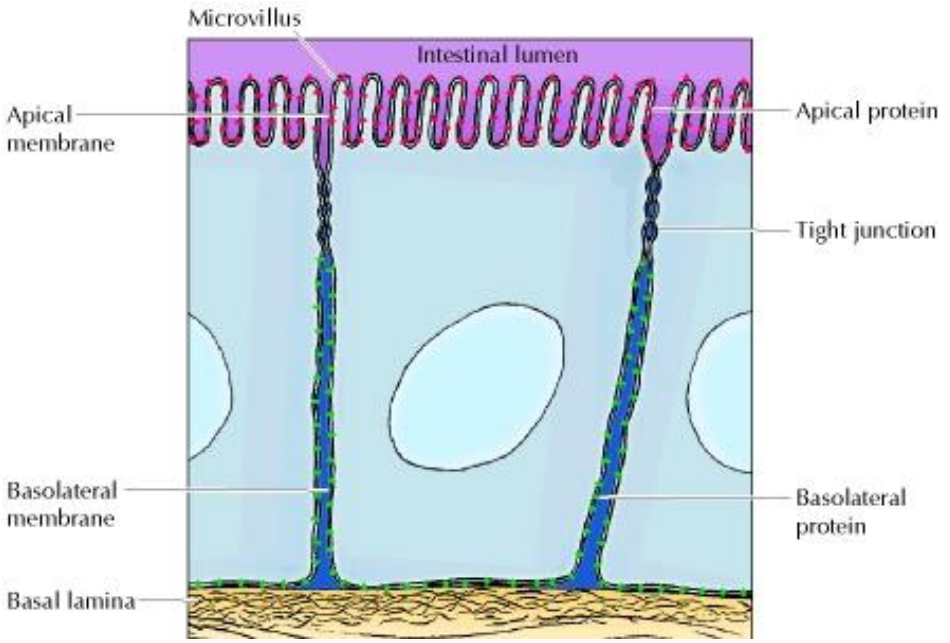


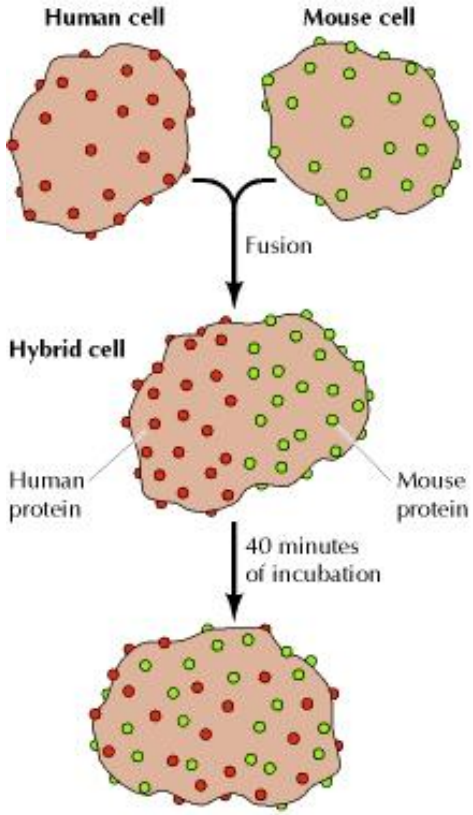
Protein mobility Membrane lipids and proteins aren't static, they are dynamic.

- Proteins and lipids are **able to diffuse laterally** through the membrane.

➢ **The mobility of membrane proteins is restricted by :**

- Their association with the cytoskeleton (filaments).
- Lipid rafts (high colestrol and other lipid restric the movement of poriteins).
- Specific membrane domains, which maintain the specific distribution of apical and basolateral proteins.
- Specific lipid composition (e.g. lipid rafts).

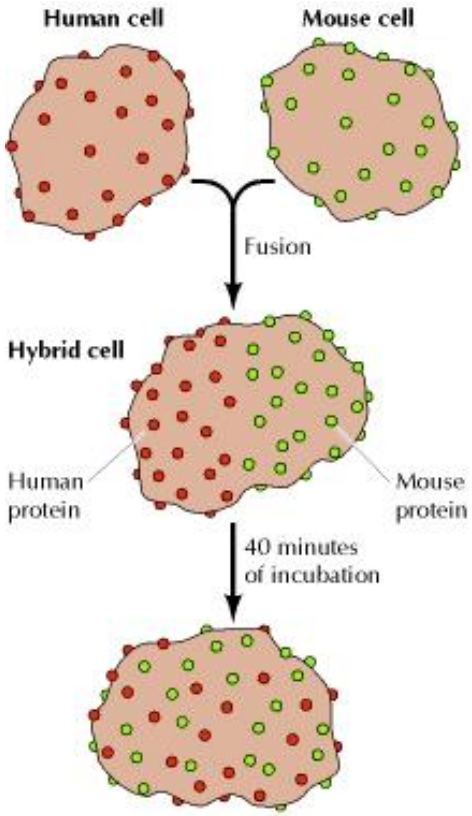


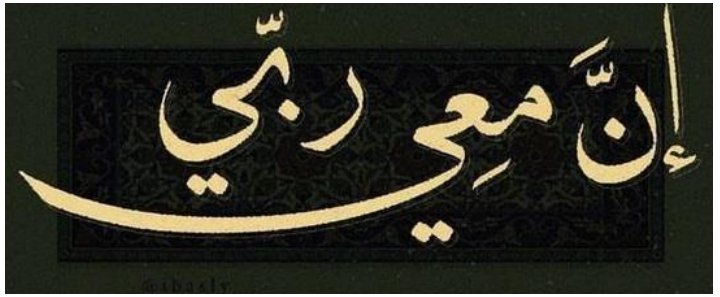


Protein mobility

- How do you know that membrane lipids and proteins are dynamic?

- ✓ We know that from experiments done a long time ago, where scientists took human cells, and they colored the membrane proteins with a red color, and they took a **mouse cell, and they colored the membrane proteins, with a green color**, and they fused the two cells into one cell. After a few hours, they noticed that **the green and red proteins are intermixed,** meaning that proteins move, even though they are integral proteins.





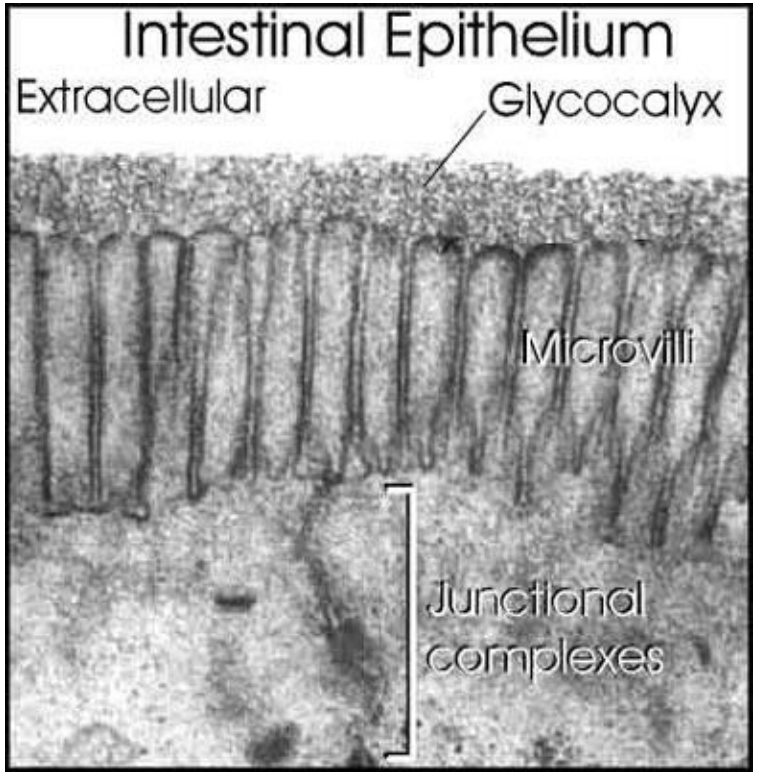
Glycocalyx Sugar Coating

- The surface of the cell is covered by a carbohydrate coat, known as the glycocalyx, **formed by the oligosaccharides of glycolipids and glycoproteins.**

When we are breathing, there are a very tiny particles that enter to our lung and could make friction with lung cells which cause damage to cells, the presence of this sugar coating prevent friction and cells damage.

Functions:

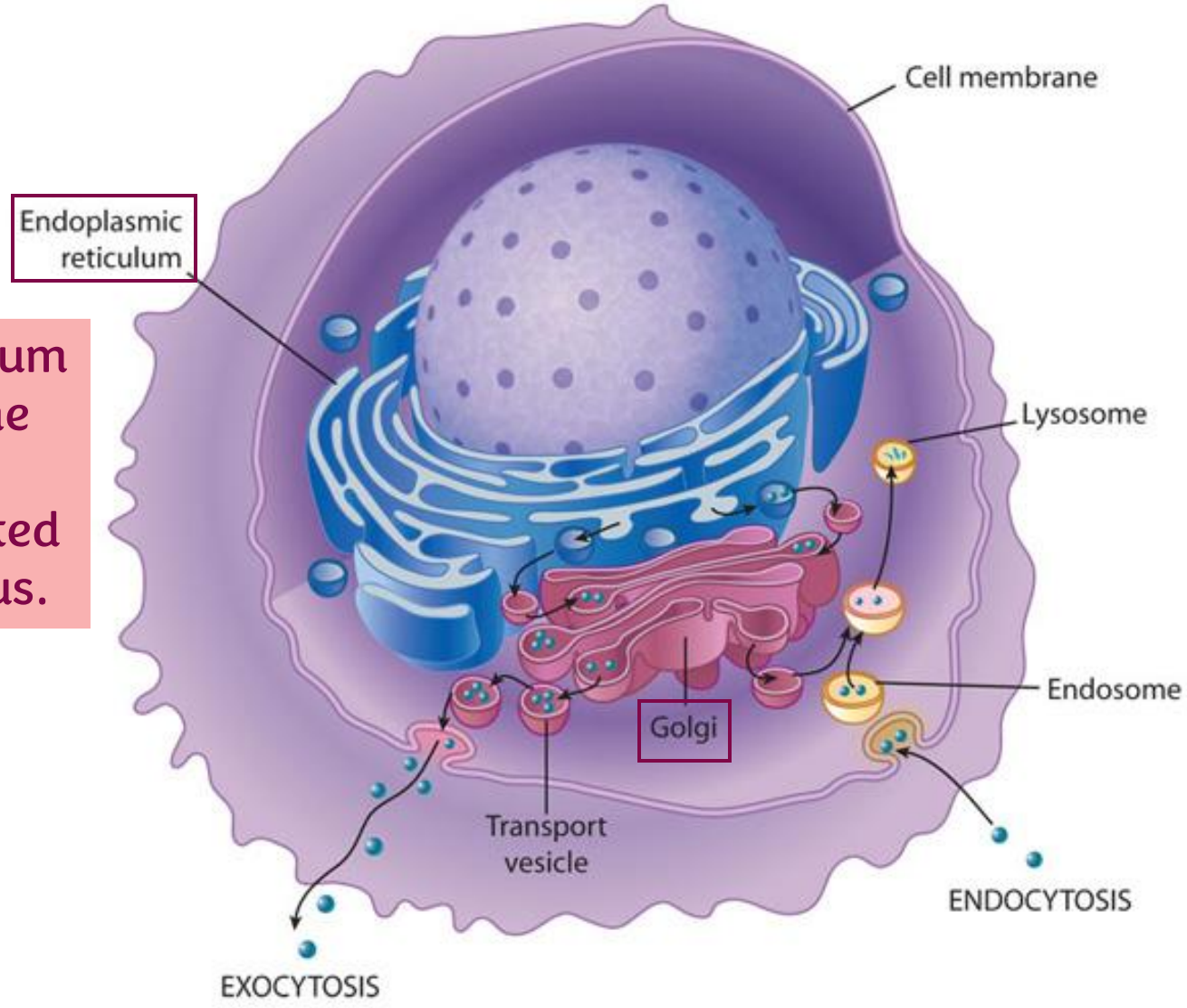
- Cell-cell interactions such as immune cells (label that allows immune cells to distinguish our cells from other cells).
- Protection of cell surface from ionic and mechanical stress.
- Formation of a barrier for microorganisms. (bacteria and viruses can't penetrate the cells because of glycocalyx).

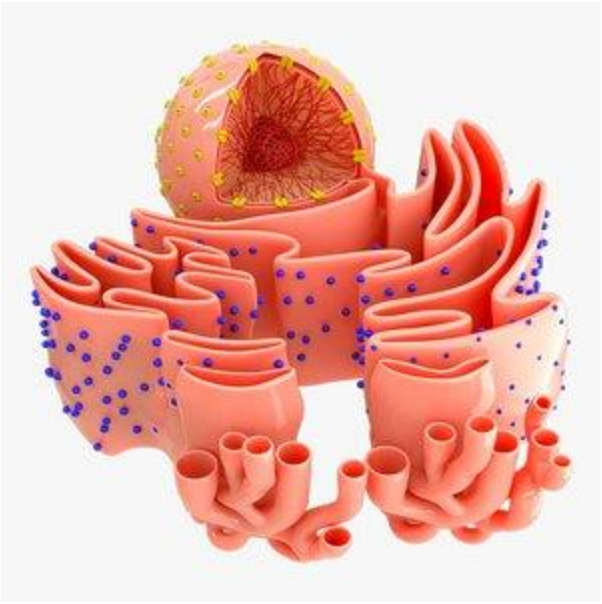


Protein sorting (endoplasmic reticulum)

An overview

Endoplasmic Reticulum is interacting with the nucleus, and it is functionally connected to the Golgi apparatus.





Endoplasmic reticulum (ER)

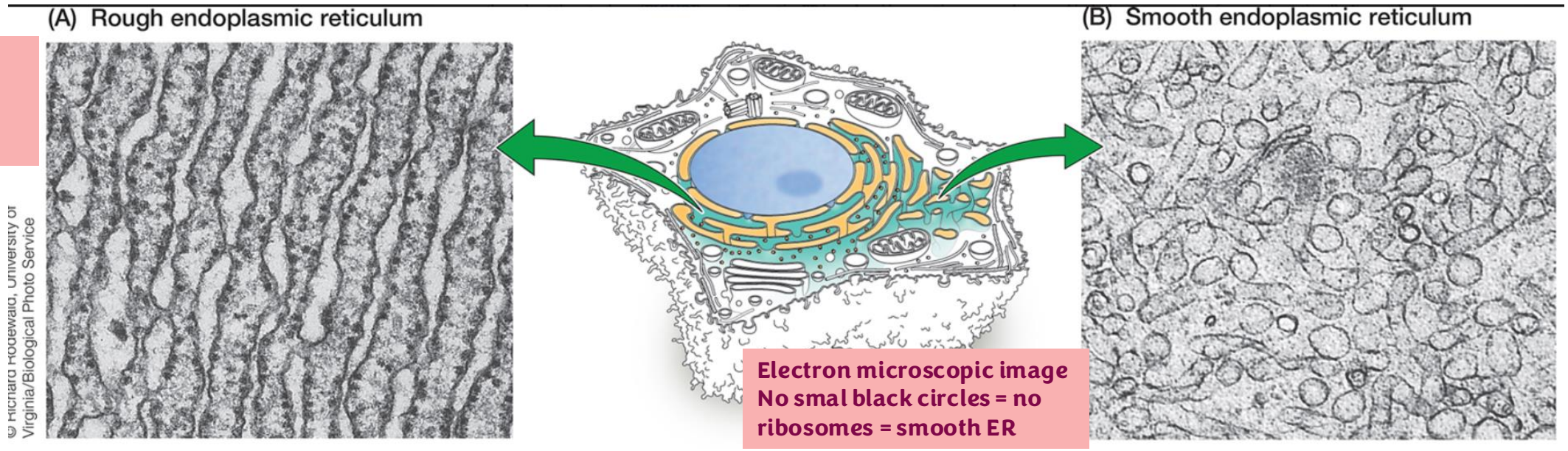
- It is a network of membrane-enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm.
- It is the largest organelle of most eukaryotic cells.

➢ **There are two types of endoplasmic reticulum :**

Translation

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

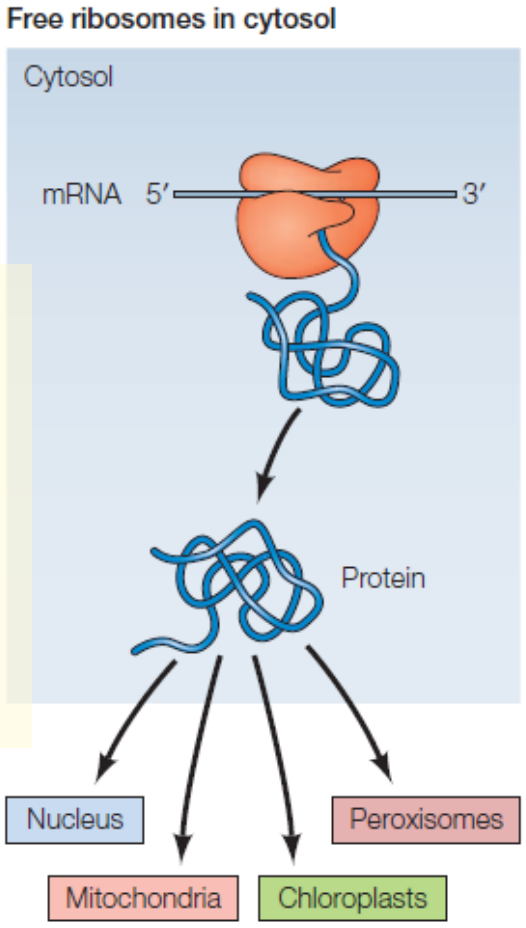
Electron microscopic image
Smal black circles = ribosomes = rough ER

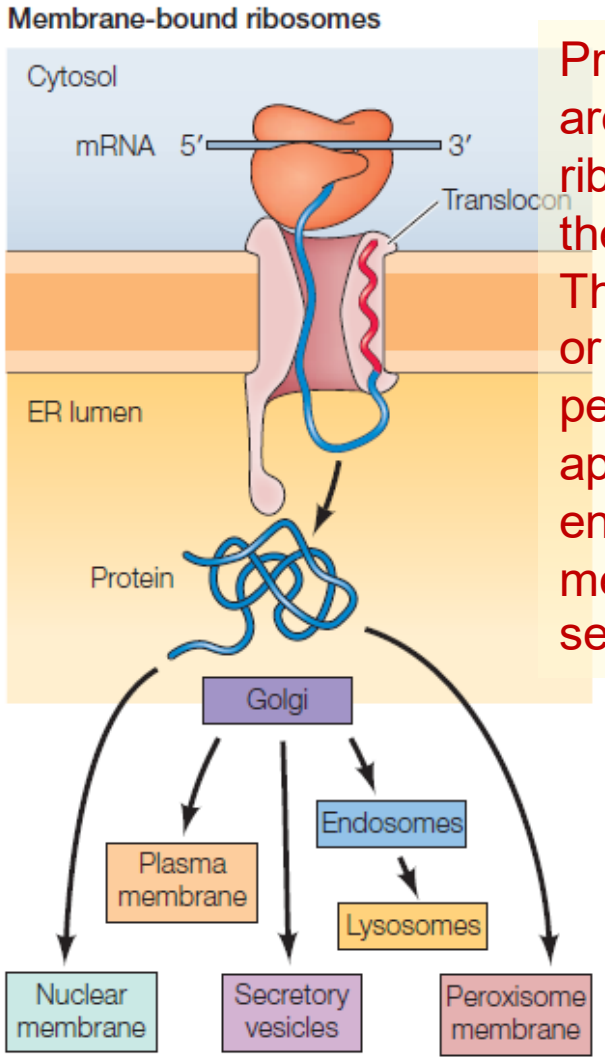


Electron microscopic image
No smal black circles = no ribosomes = smooth ER

Protein sorting

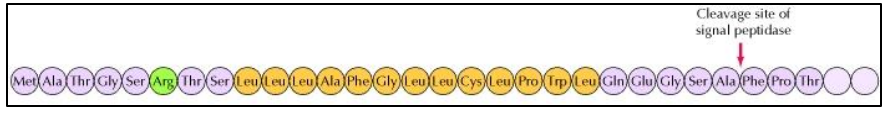
Proteins synthesized on free ribosomes either remain in the cytosol or are transported to the nucleus, mitochondria, or peroxisomes.





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

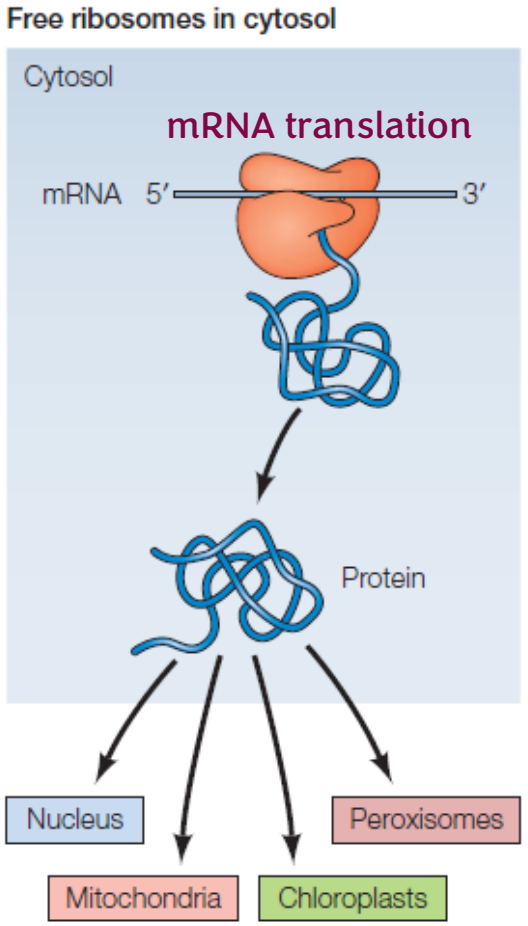


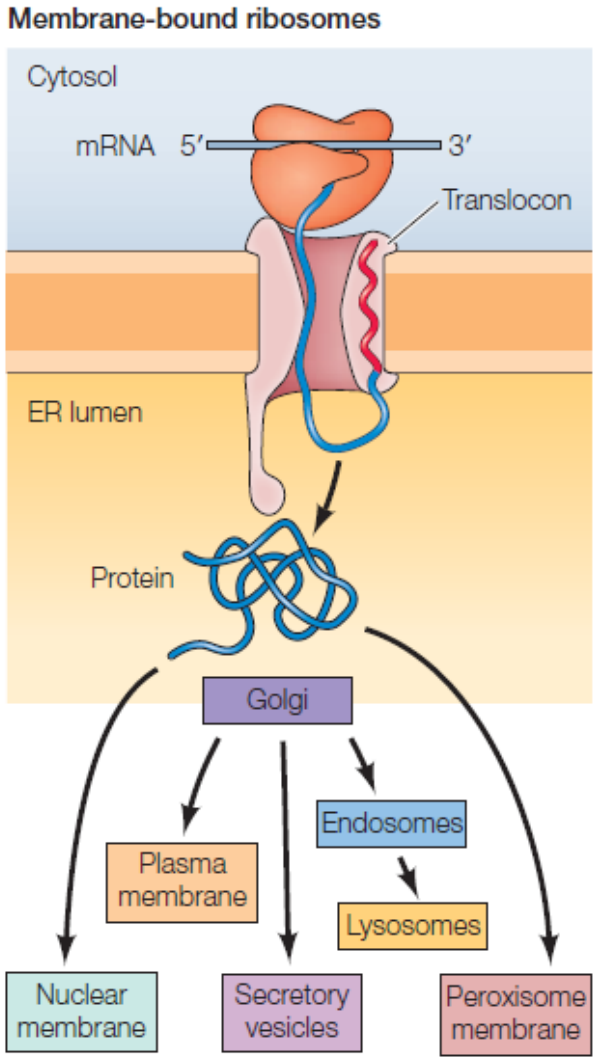
Signal sequence: a short sequence of amino acids of the polypeptide at the amino terminus. It is then cleaved from the polypeptide chain during its transfer into the ER lumen.

Protein sorting

- Many proteins are synthesised in the cytosol and once they are synthesised the cell decides where they will go whether to : Nucleus, Peroxisomes, Mitochondria, or stay in the cytosol.

- The cell sorting of proteins don't happen randomly but it happens depending on the presence of a code (شيفرة), this code is a sequence of amino acids that tells the cell where this protein should go.





- There are other proteins that are synthesised on the surface of the RER (rough endoplasmic reticulum).

- These proteins enter the lumun of the RER then the cell decides whether their destination : Nuclear membrane, Percisome membrane , or to the Golgi then to the Plasma membrane or Endosomes then lysosomes, or outside the cell.

Protein sorting

But this leads to a question, **what makes the cell know while the protein is being synthesised in the cytosol (or at RER membrane) the final distension of this protein?**

- We already mentioned that **the presence of a code called signal sequences on the N-terminus** allows a specific proteins to translocate the protein along with the ribosome (while protein is being synthesised) to RER surface and continue their synthesis in the lumen of RER.

- If this **signal sequence is not present**, the protein continues its synthesis at the cytosol.

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.

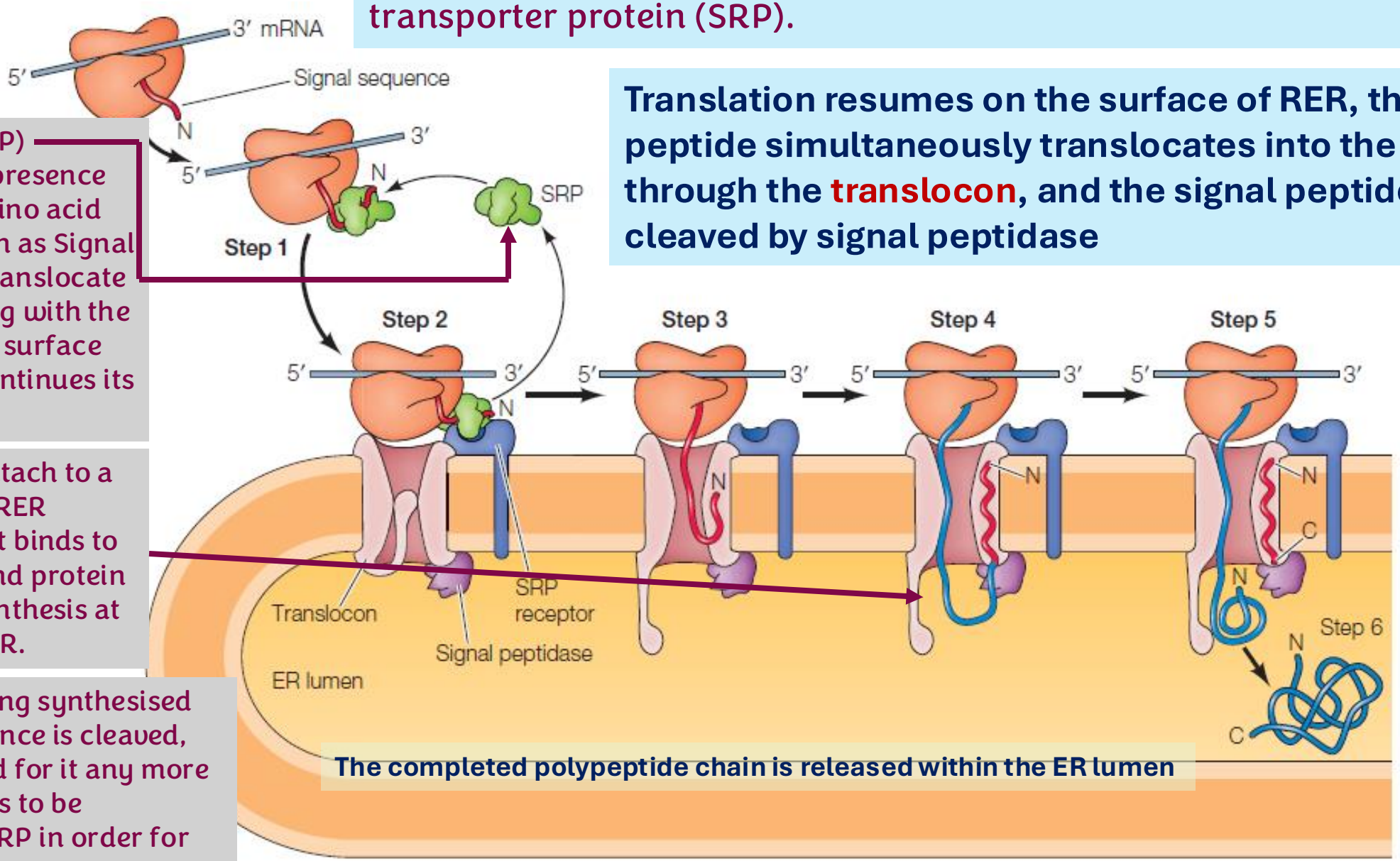
The signal sequence is recognized as the protein is synthesized and the ribosome is transported to the surface of the RER by a transporter protein (SRP).

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

This protein (SRP) recognises the presence of a specific amino acid sequence known as Signal sequence and translocate the protein along with the ribosome to the surface of RER and it continues its synthesis there.

The ribosome attach to a channel on the RER surface and as it binds to it, it opens up and protein continues its synthesis at the lumen of RER.

As protein is being synthesised the signal sequence is cleaved, because no need for it any more (it's purpose was to be recognised by SRP in order for translocation).

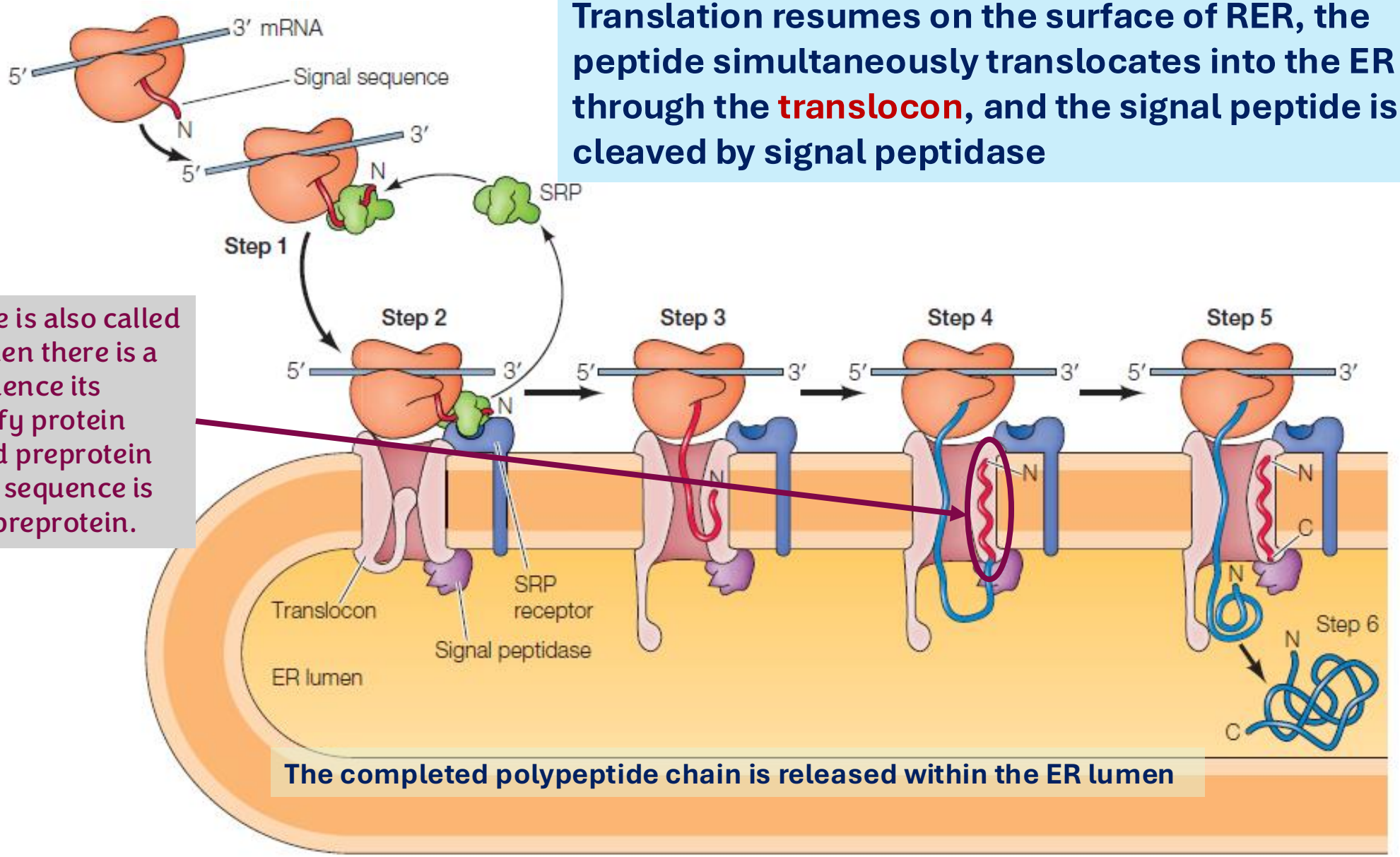


The completed polypeptide chain is released within the ER lumen

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

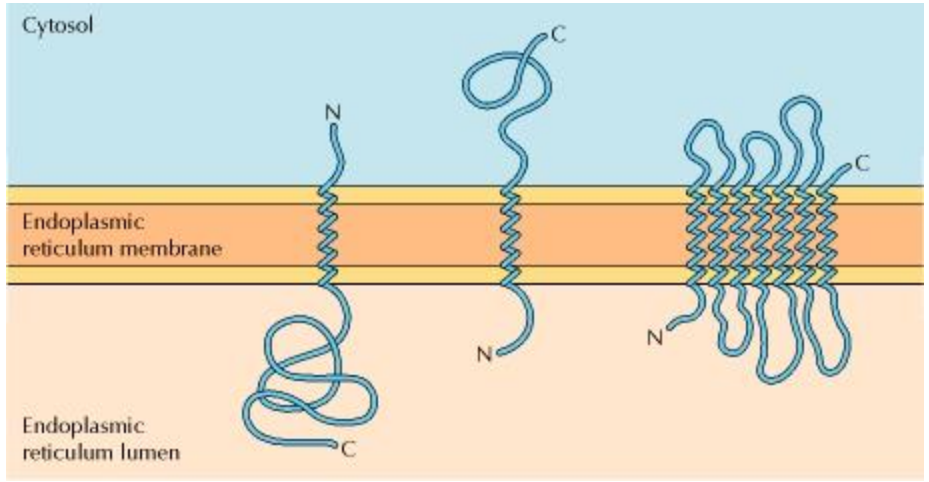
The signal sequence is also called the pre peptide (when there is a protein have a sequence its purpose is to identify protein destination is called preprotein and here the signal sequence is the pre part of the preprotein.



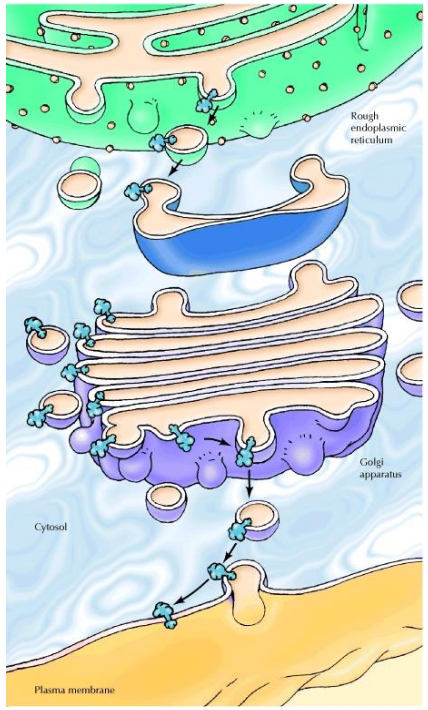
The completed polypeptide chain is released within the ER lumen

Pathways of protein sorting (sorting of the protein begins in the ER)

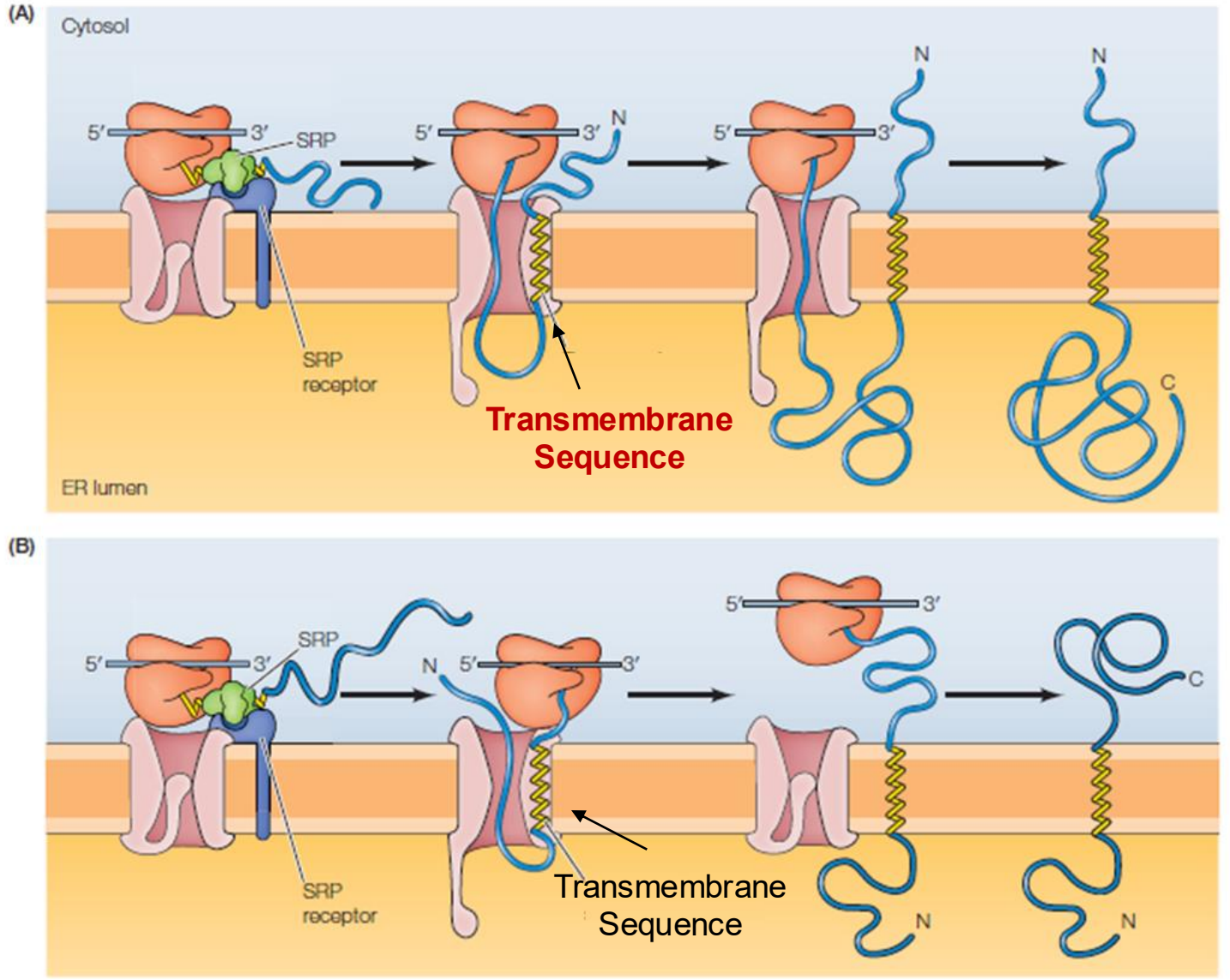
- Secretory, ER, Golgi apparatus, and lysosomal proteins are released into the lumen of the ER.
- Membranous proteins are initially inserted into the ER membrane.
- Considerations
  - Single vs. multiple membrane-spanning region
  - Orientation of N- and C-termini



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

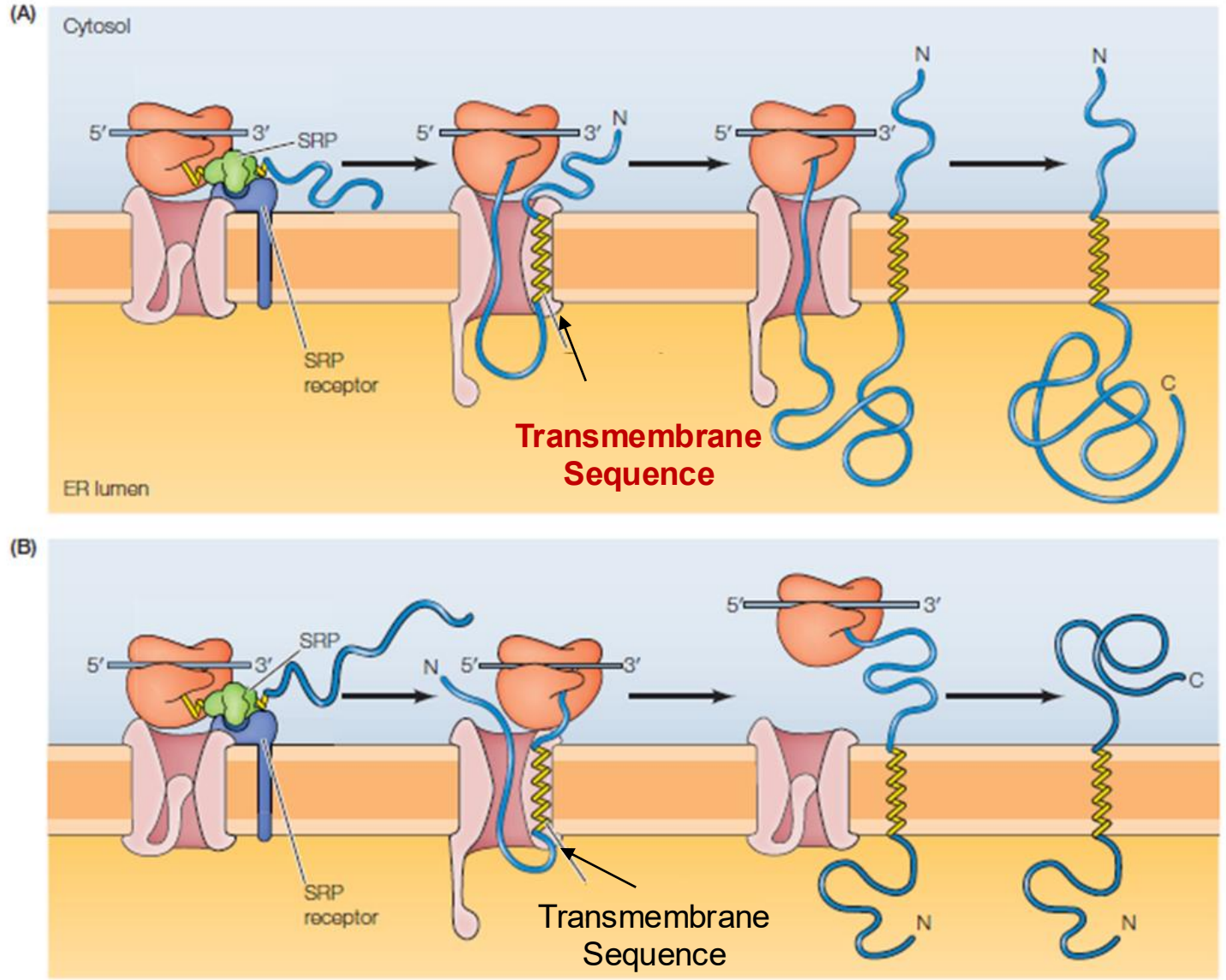


- When the protein being translated is going to be part of a membrane such as (peroxisomal, plasma, or lysosomal membrane), it doesn't get released entirely into the ER lumen.
- Instead, once the transmembrane domain/sequence (which is helical and hydrophobic) appears during protein formation, it gets pushed outside the translocon and into the ER membrane by other proteins. Synthesis of this protein is continued while it is part of the ER membrane.
- It remains a part of the ER membrane until it becomes part of the vesicle's membrane that is released from the ER.

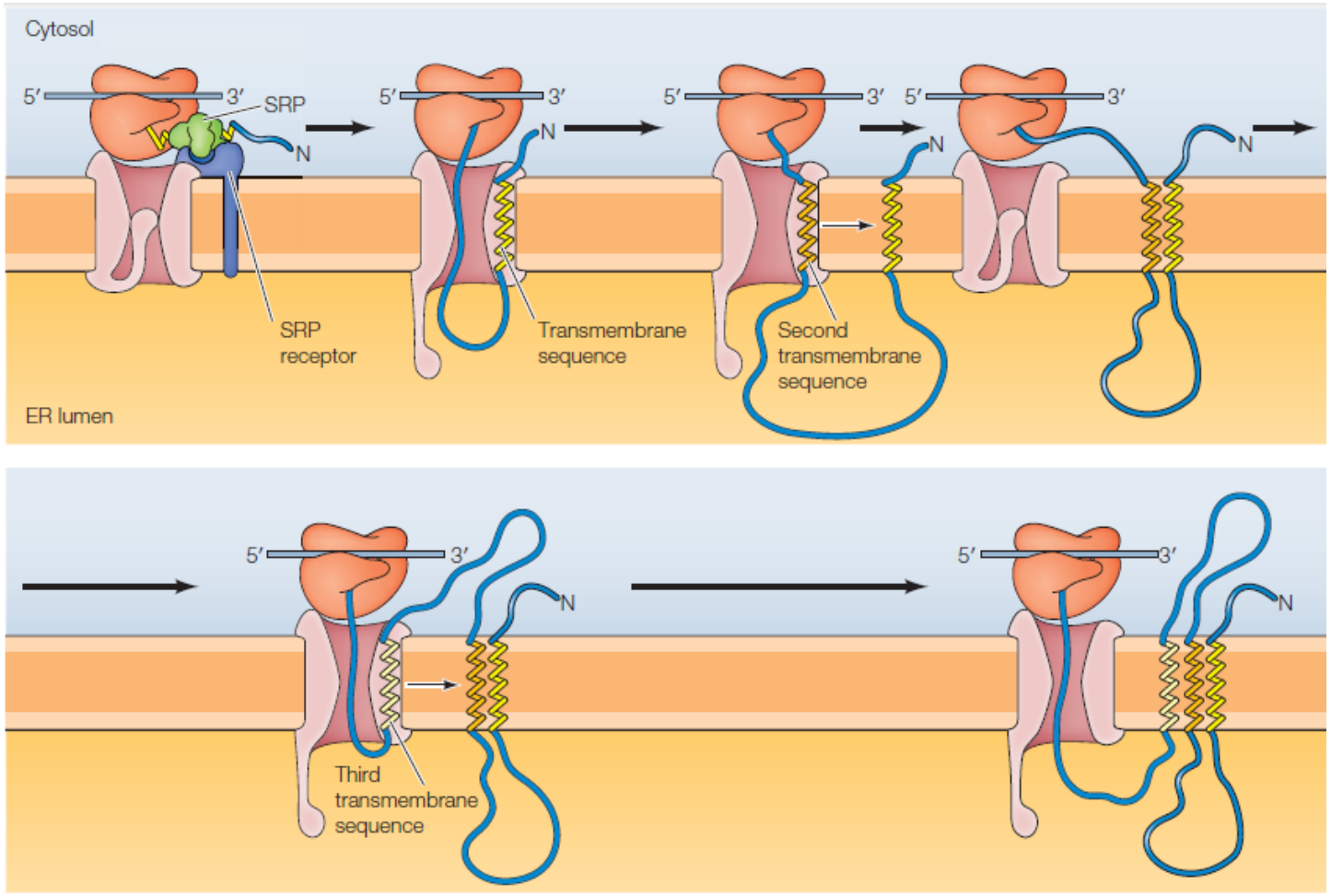


Insertion of membrane proteins via internal transmembrane sequences

- Translocation of the polypeptide chain stops when the translocon recognizes a transmembrane sequence allowing the protein to become anchored in the ER membrane.
- The direction of the internal transmembrane sequence determines the direction of insertion and orientation of the protein ends.



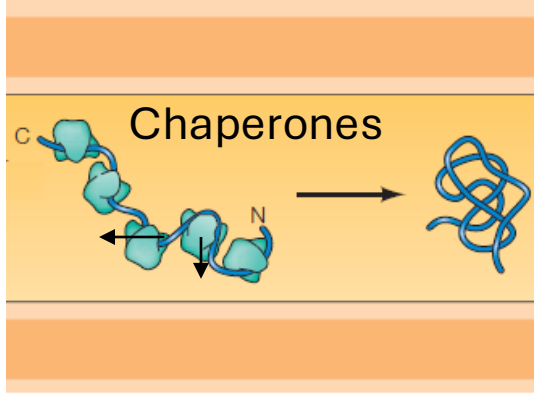
Multi-transmembrane domain proteins have multiple transmembrane sequences

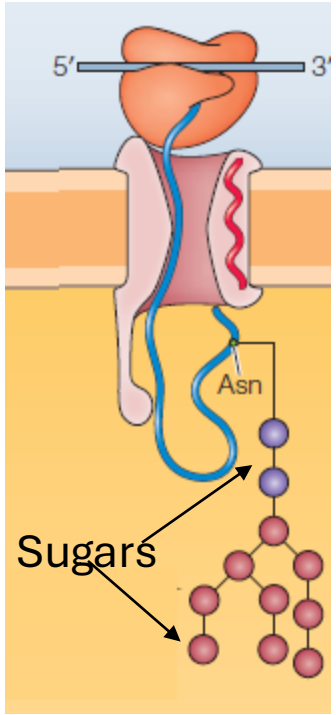


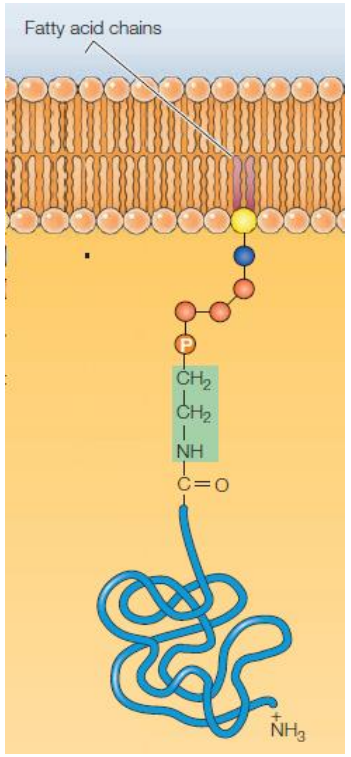
- When the protein formed has more than one transmembrane domain, each transmembrane domain that appears gets pushed into the ER membrane, and synthesis continues until all transmembrane domains become part of the ER membrane.

Once inside the ER, proteins are

- Folded (with the help of chaperones) **(proteins get their 3D shape).**
- Complexed (quaternary structure) **(For example, the 2 alpha and 2 beta chains of hemoglobin merge in the ER).**
- Modified by disulfide bonds **(between cysteine residues)** formed by by protein disulfide isomerase
- Glycosylated: **enzymatic addition of sugars to proteins.**
- Anchored by lipids

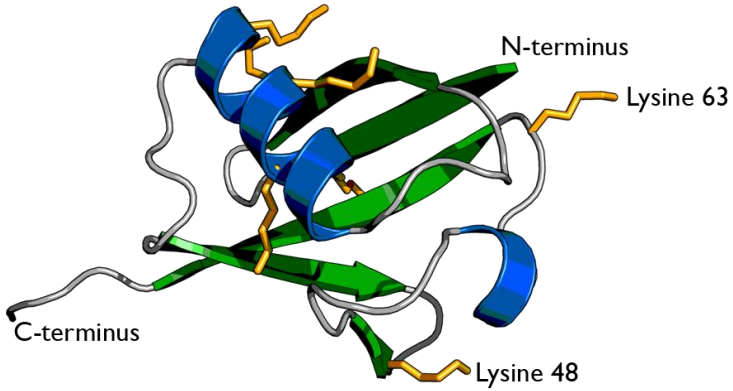




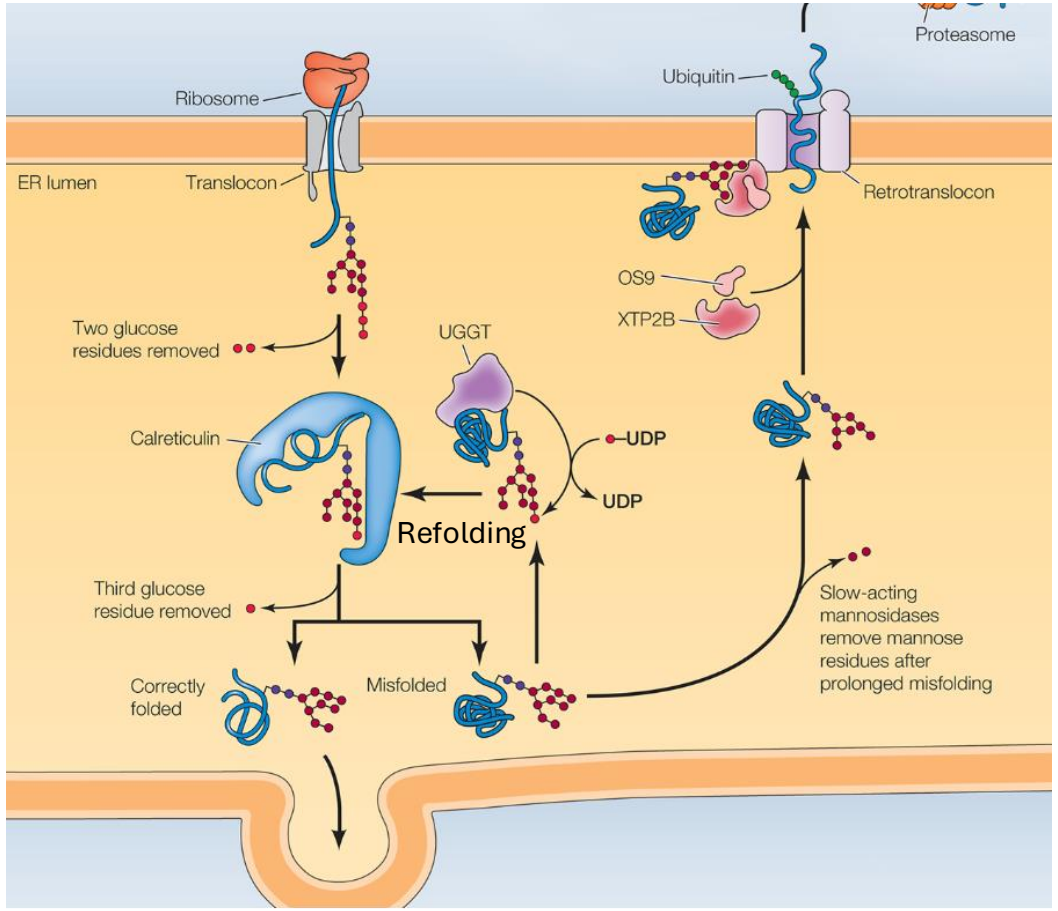


Protein folding and ER-associated degradation (ERAD)

- If correctly folded, proteins move on.
- If misfolded, proteins are refolded, and, if it does not work more than once, it is sent to the cytosol, ubiquitylated (addition of small proteins called ubiquitins), and degraded in the proteasome. When proteins are tagged with ubiquitins, these proteins must be degraded via proteasome and the amino acids are used for something cells.



- Note: pictures in the slides have a lot of names, they are not required to be memorized unless it is written in the slides or they were mentioned by the doc

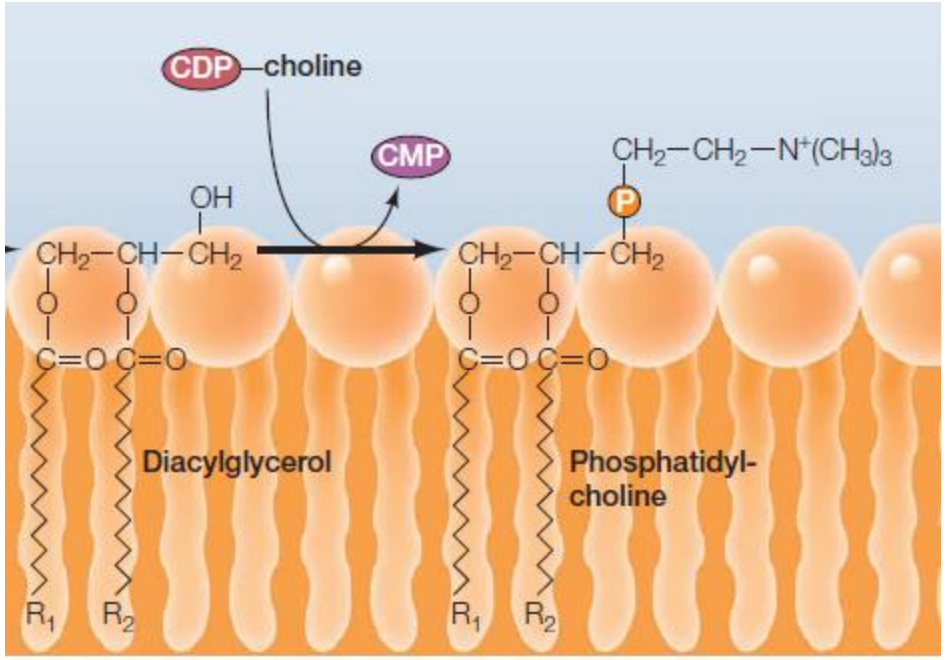


Protein folding and ER-associated degradation (ERAD)

- Smooth Endoplasmic Reticulum is the site of lipid metabolism, synthesis of glycerophospholipids, and ceramides (which is a sphingolipid), as well as synthesis of steroids and steroid hormones (such as androgens, estrogens, cortisol, etc.) from cholesterol.
- This means that organs that produce steroid hormones (such as ovaries and adrenal glands) are rich in SER

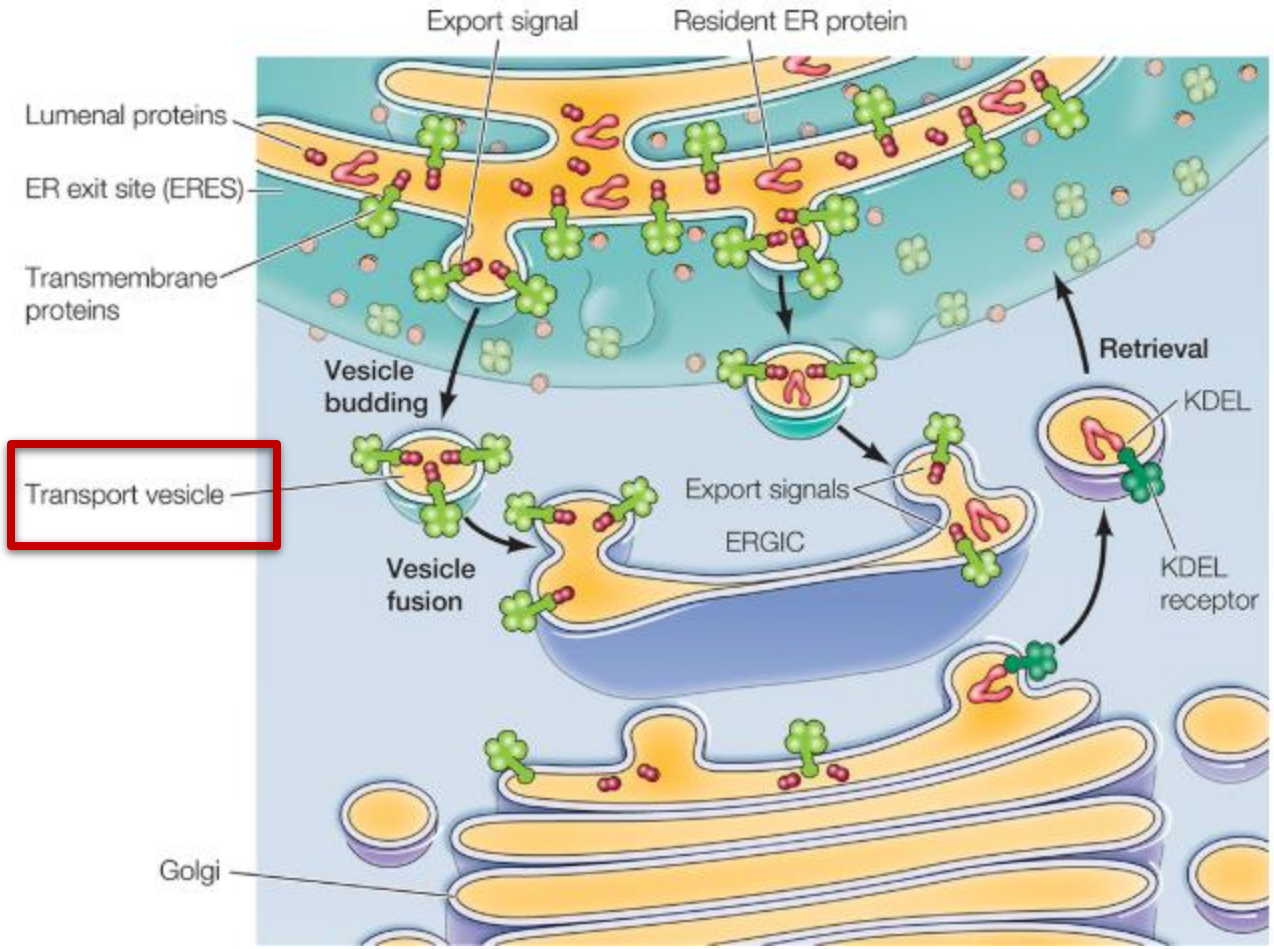
Synthesis of phospholipids in ER

- The smooth ER is the major site of synthesis of:
  - Membrane glycerophospholipids, which are then transported from the SER to other membranes.
  - Ceramides The precursor of sphingolipids)
    - Ceramide is converted to either glycolipids or sphingomyelin in the Golgi apparatus.
  - Steroids.
    - Large amounts of smooth ER are found in steroid-producing cells, such as those in the testis and ovary.
- SER is abundant in the liver, which contains enzymes that metabolize various lipid-soluble compounds.



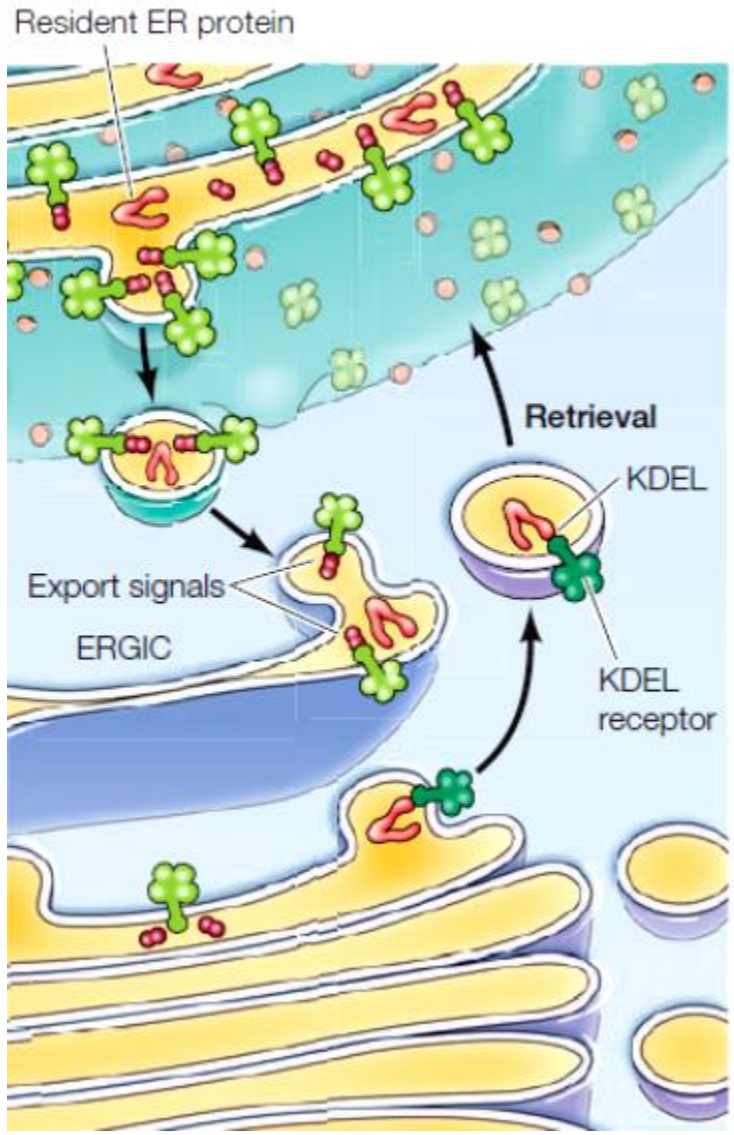
ER-Golgi intermediate compartment (ERGIC)

- 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.



Retention of ER protein

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



Amino Acids have unique one letter abbreviations. (K is Lysine, D is Aspartate, E is Glutamate, and L is leucine

Lecture 2: Golgi apparatus and vesicular transport

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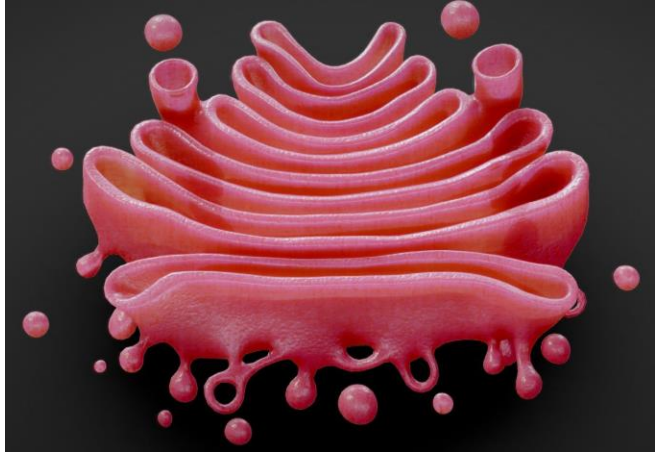
School of Medicine

Second year, First semester, 2025-2026

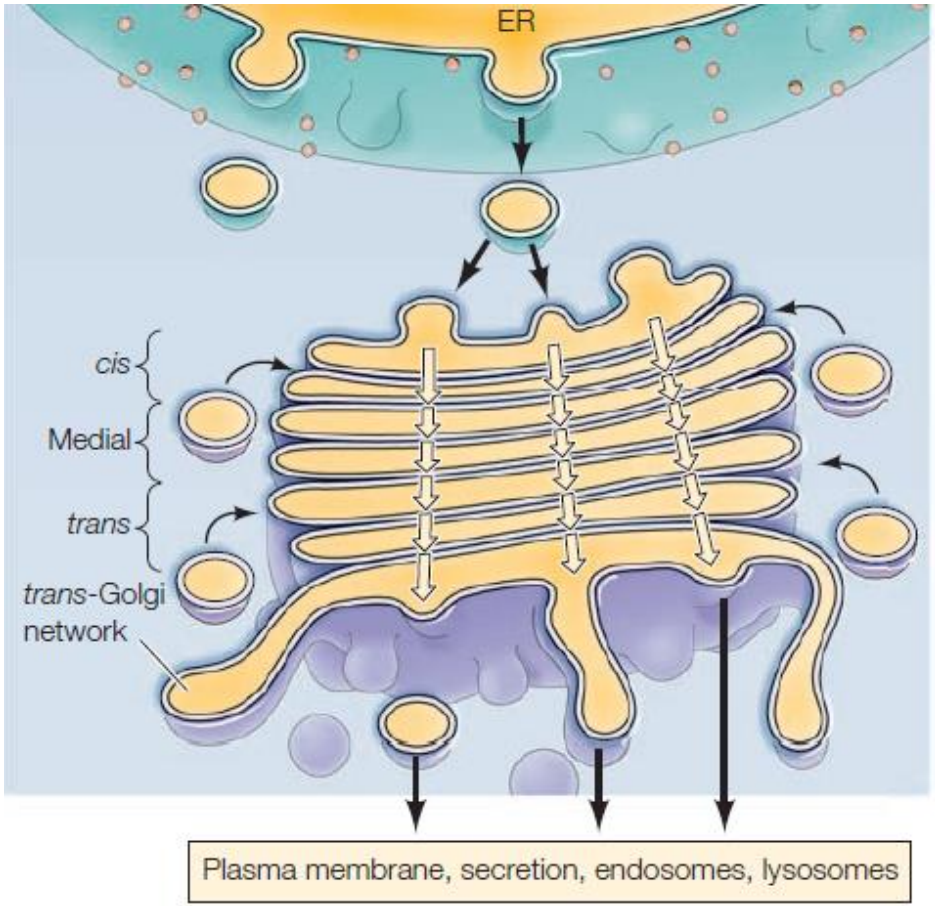
Functions of the Golgi apparatus

- Further protein processing and modification (which starts in the ER)
- Synthesis of glycolipids and sphingomyelin
- Protein sorting

Structure of the Golgi

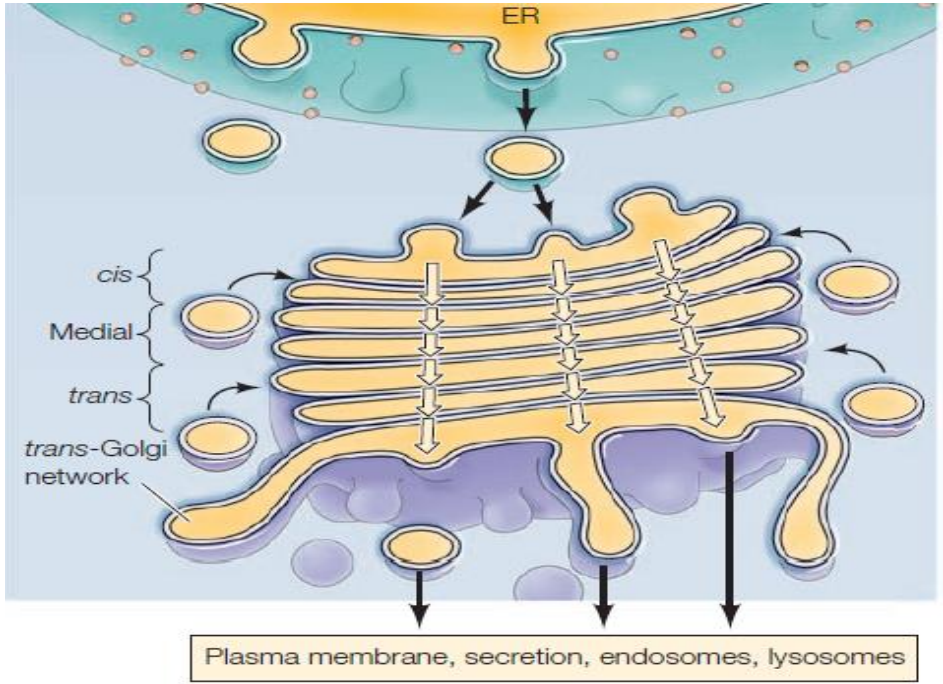


- The Golgi apparatus consists of a stack of flattened sacs (cisternae) on top of each other of four regions: *cis*, medial, and *trans* compartments and the *trans*-Golgi network.
- Proteins are carried through the Golgi apparatus in the *cis-to-trans* direction.
- Transport vesicles carry the Golgi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



Structure of the Golgi

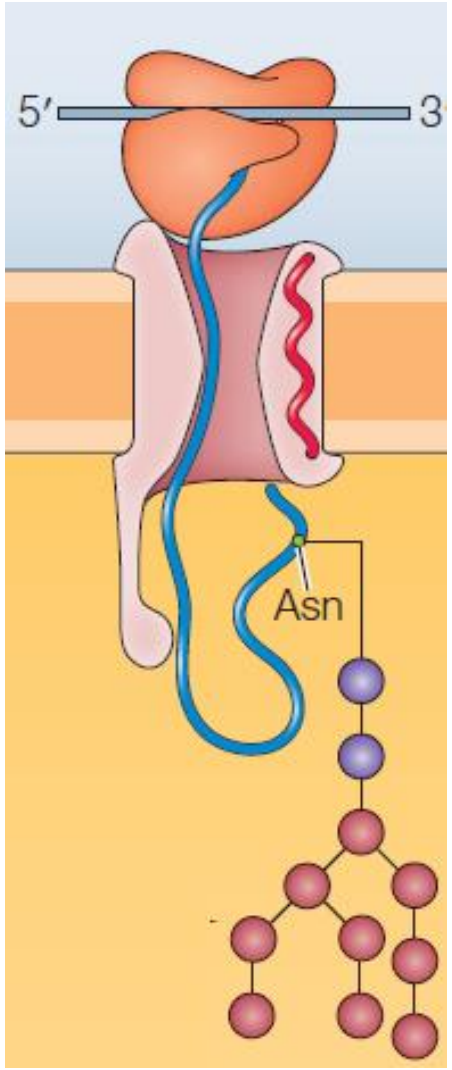
- The cis region of the golgi apparatus is the closest to the ER, so the vesicles that are released from the ER fuse with the cis region of the golgi then transfer to the medial region then trans region then trans-golgi network.
- Golgi specific proteins go from trans-golgi network to the cis region via vesicles. Other proteins are sent to the endosomes, lysosomes, plasma membranes, or are secreted outside the cells via secretary vesicles.



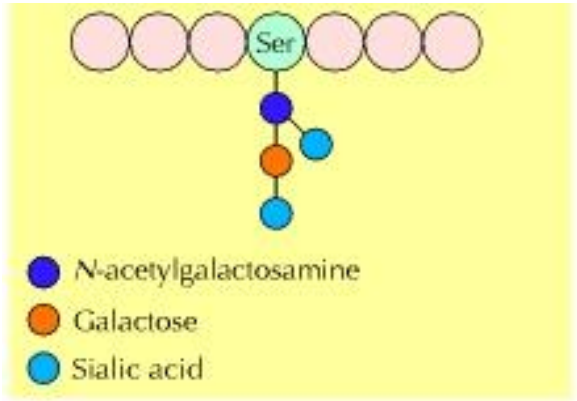
Processing of N-linked oligosaccharides in Golgi

- Glycosylation is the enzymatic addition of sugars to proteins. There are 2 ways of glycosalation:
- -N-Glycosylation: anomeric carbon of sugars is attached to a nitrogen in the amino acid (specifically asparagine)
- -O-Glycosylation: anomeric carbon of sugars is attached to an oxygen in the amino acid (specifically serine and threonine)

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



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



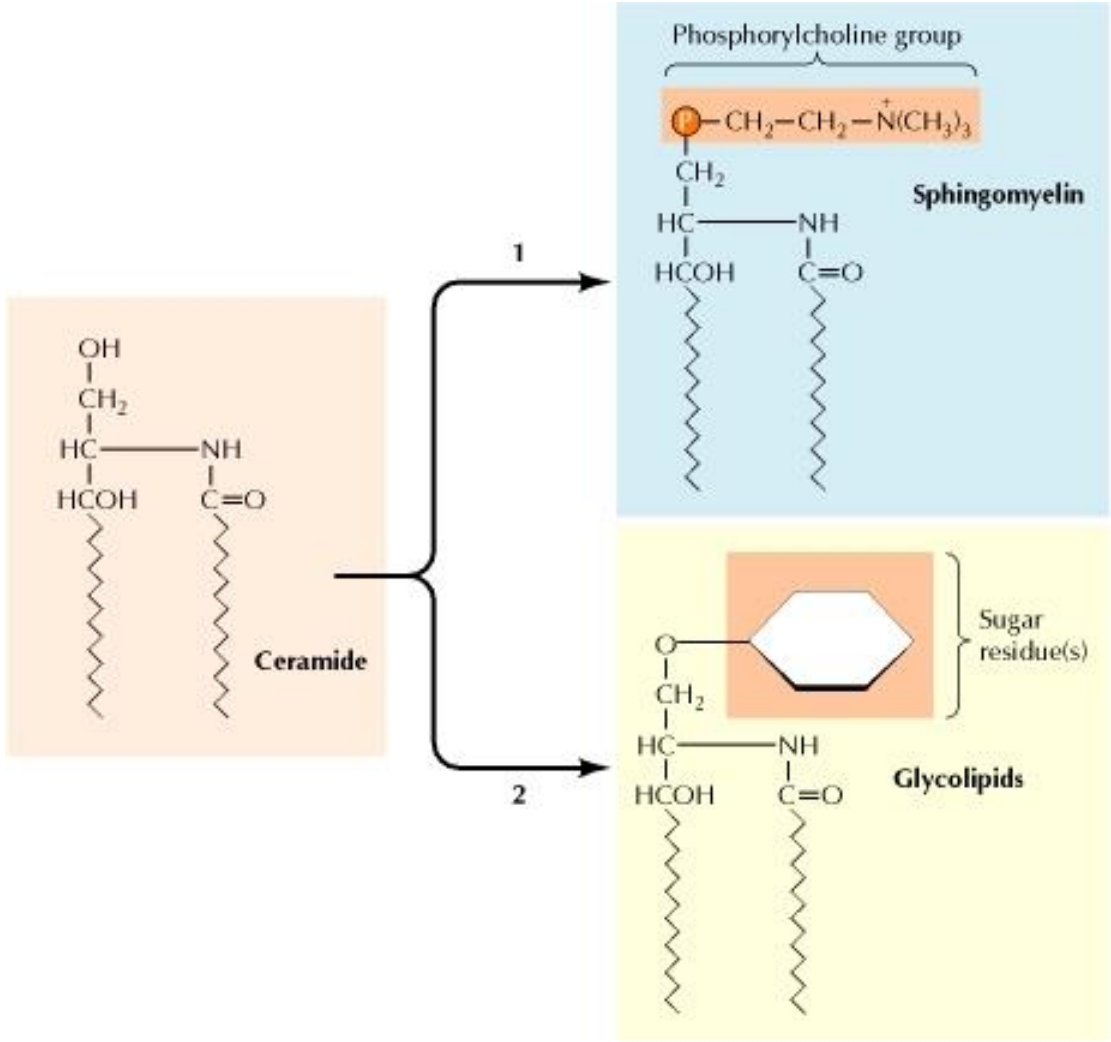
Lipid and Polysaccharide Metabolism in the Golgi

- The glycerol phospholipids, cholesterol, and ceramide are synthesized in the ER.
- Ceramide is then converted either to sphingomyelin (a phospholipid) **by addition of choline** or to glycolipids **by addition of sugars** in the Golgi apparatus.
- **Glycolipids have 3 types all of which are synthesized in the golgi apparatus:**

1. Cerebrosides: have one sugar (glucose or galactose)

2. Globosides: have 2 sugars or more

3. Gangliosides: have 3 sugars or more (including sialic acid)



Additional Resources:

رسالة من الفريق العلمي:

قَد تَنتَابَكَ مَخَاوِفُ حَولَ مُستَقبَلِكَ

لَن يُخرِجَكَ شَيءٌ مِن بِئرِ الهَوَاجِيسِ

سِوَى تَأَمُّلِكَ فِي قَولِهِ تَعَالَى:

﴿ أَلَيسَ اللَّهُ بِكَافٍ عَبدَهُ ﴾

فَيَتَنَامَى يَقِينُكَ بِأَنَّ الدُّنيَا لِلَّهِ

وَأَنَّ الرِّزقَ مِنَ اللهِ

وَأَنَّ مُستَقبَلَكَ المَجهُولَ بِيَدِ اللهِ وَحدَهُ

لَا عَلَيكَ سِوَى أَن تَحمِلَ هَمًّا وَاحِدًا

وَهُوَ: كَيفَ تُرضِي اللهَ؟ فَإِذَا أَرضَيتَ اللهَ

رَضِيَ عَنكَ، وَكَفَاكَ، وَأَغنَاكَ

For any feedback, scan the code or click on it

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	7	GPI anchored proteins : a glycolipid molecule contains a lipid part (which is a part of the membrane) and a sugar part binds non-covalently with the protein.	GPI anchored proteins : a glycolipid molecule contains a lipid part (which is a part of the membrane) and a sugar part binds covalently with the protein.
	34	O-Glycosylation: anomeric carbon of sugars is attached to an oxygen in the amino acid(specifically lysine and threonine)	O-Glycosylation: anomeric carbon of sugars is attached to an oxygen in the amino acid(specifically serine and threonine)
V1 → V2			