

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
الَّذِي عَلَّمَ بِالْقَلَمِ ۖ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ۝

CYTOLOGY

MID | Lecture 2

Introduction to Cell Biology and ER pt.2

Muhannad Barhoom

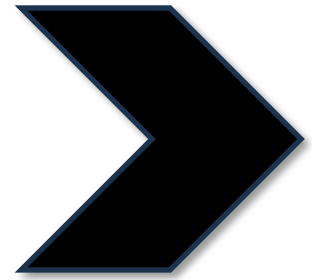
Reviewed by:

Yaman Feras

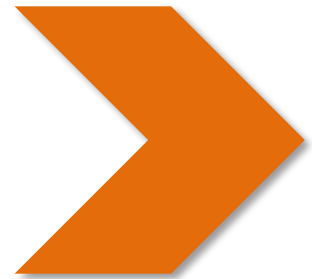
Ali Alsharaeah



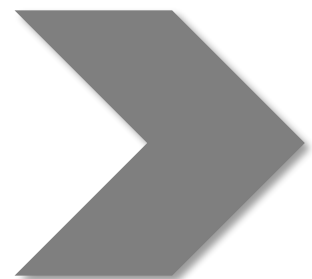
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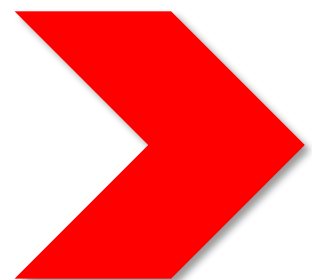
Black: the original slides



Orange: the doctor's explanation/words



Gray: additional information and explanation

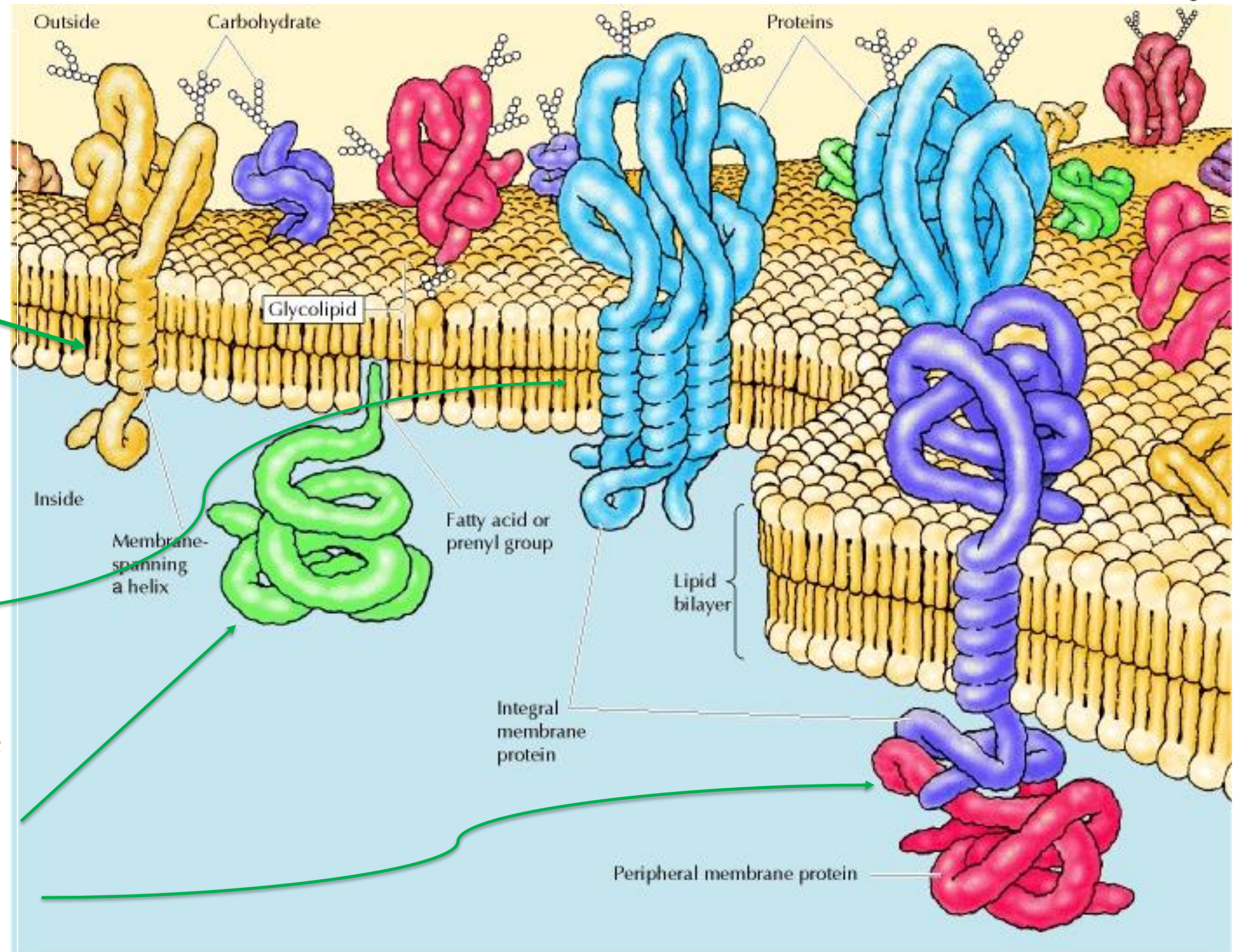


Red: important information

Membrane proteins

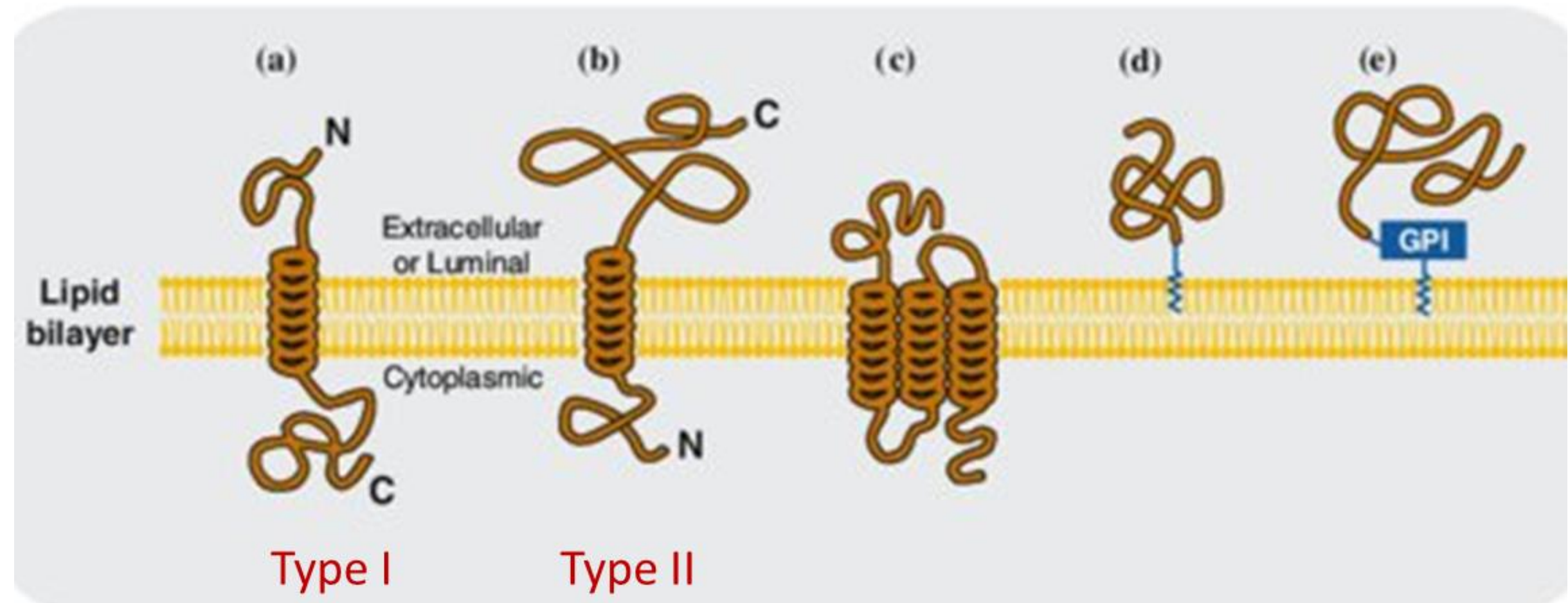


- **Proteins that are exposed to the environment in the cytosolic or extracellular part are hydrophilic (the exposed part) and usually coupled with sugars and the part that integrates in the membrane is hydrophobic except channel proteins which have amphipathic helices.**
- **Integral membrane proteins:** 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 proteins that are anchored to a lipid molecule (bind covalently to the protein) that is inserted inside the membrane like (GPI).
- **Peripheral Proteins :** There are proteins that are not integrated inside the membrane but associated with integral proteins.



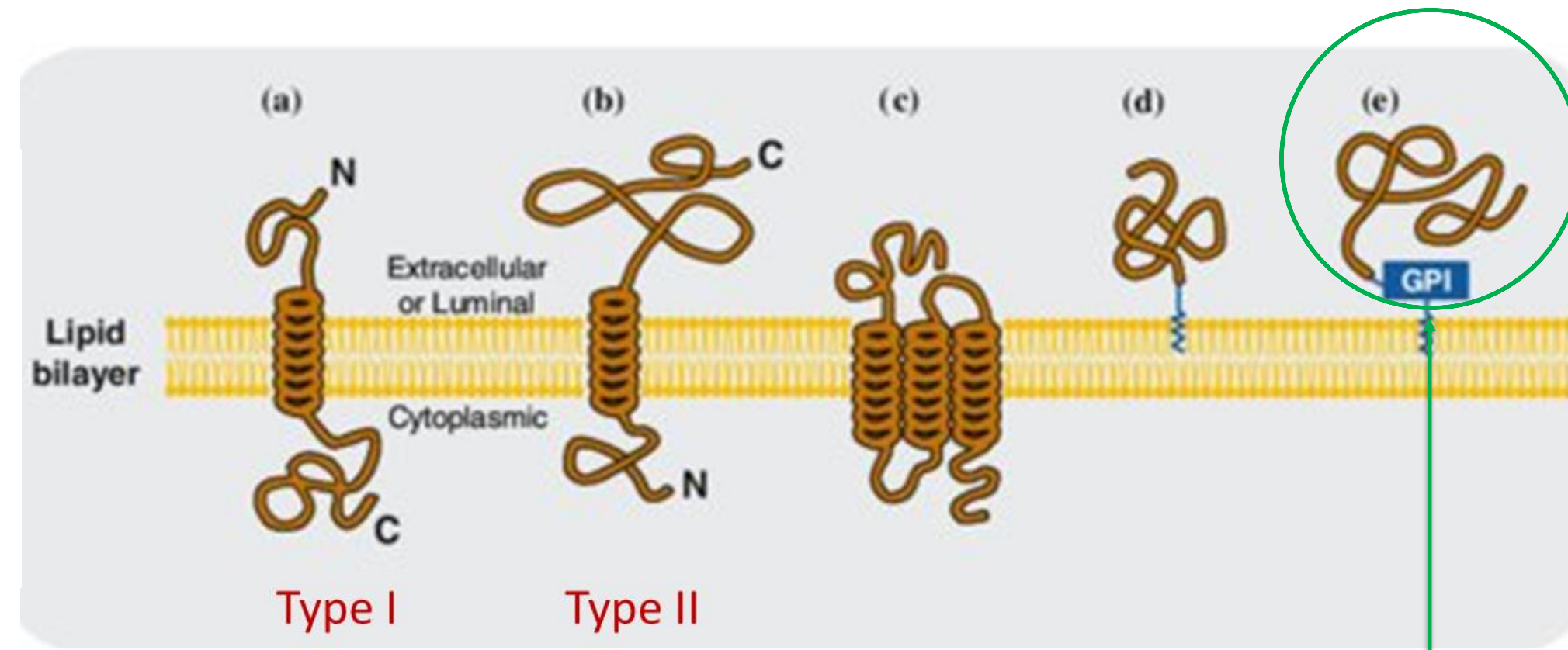
Types of membrane proteins

- 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)



Membrane proteins

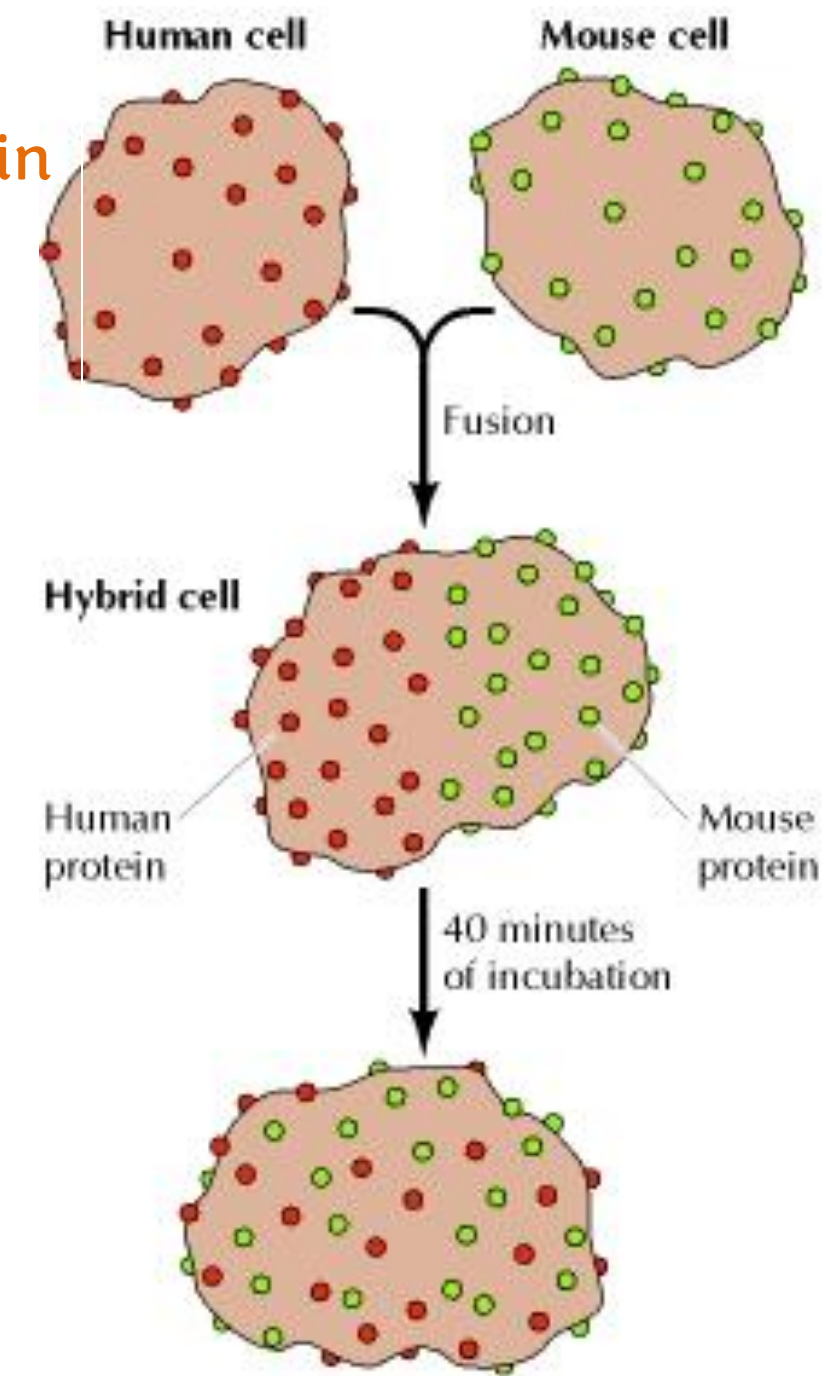
- An integral membrane protein can cross the membrane once (single-pass), twice (double-pass) these two cases are the most popular, or multiple times – for example, 7 times (like G-protein-coupled receptors) or even more. This means the protein may enter and exit the membrane several times, forming transmembrane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

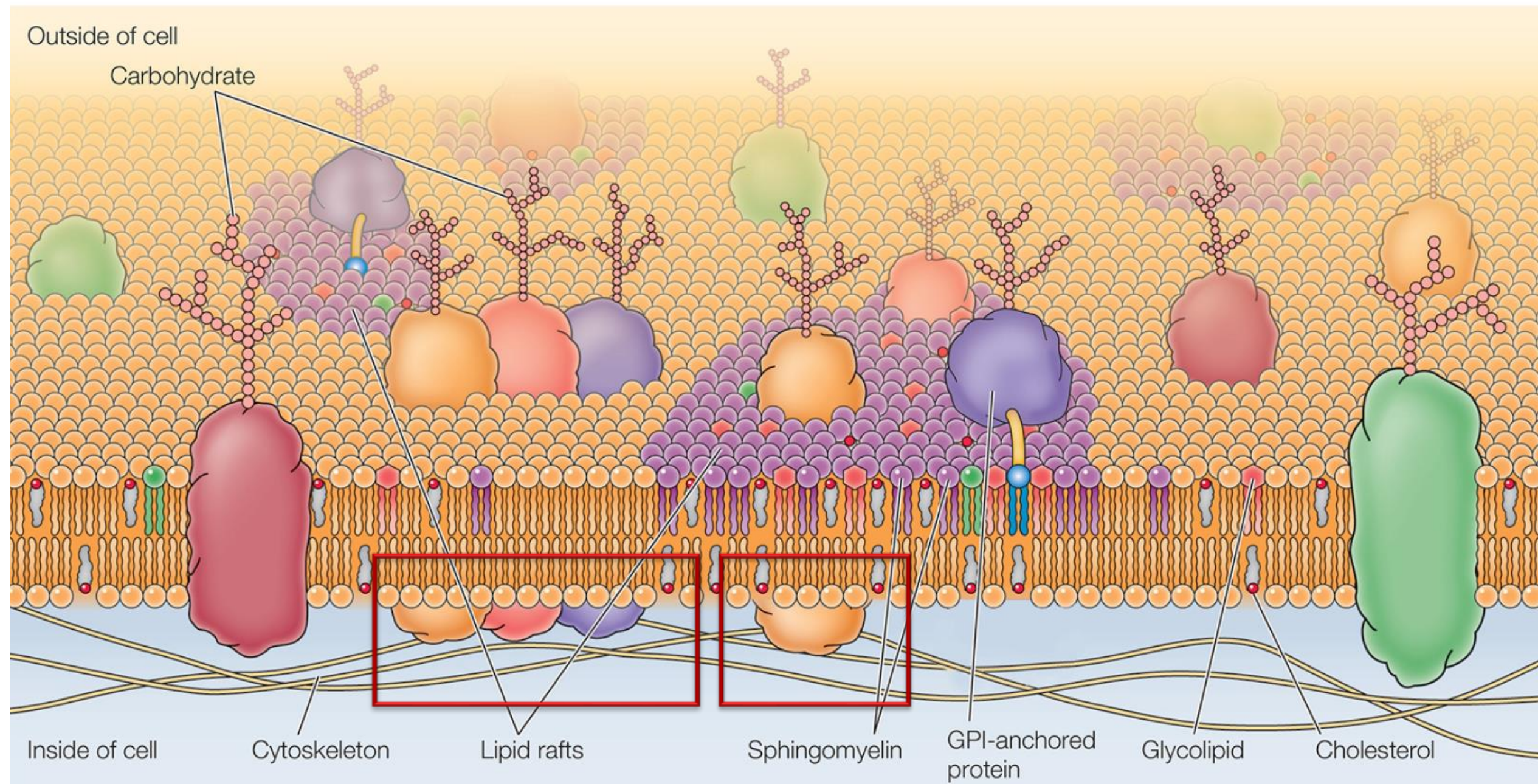
Protein mobility

- We knew that proteins move in the membrane by conducting an experiment in which we stain proteins from animal cell and mouse cell the we fuse the two cell after some time u will find that the proteins are mixed across the membrane
- Proteins and lipids are able to diffuse laterally through the membrane.
- BUT, in specialized epithelial cells, the mobility of membrane proteins by three mechanisms



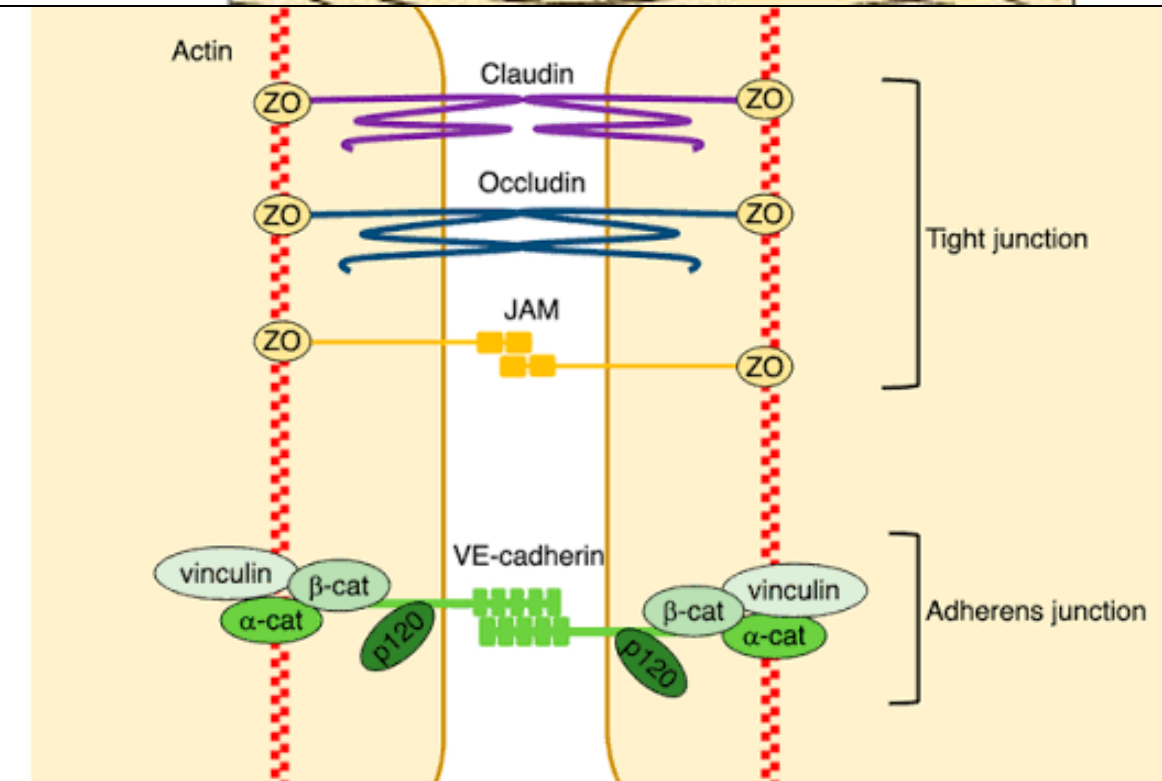
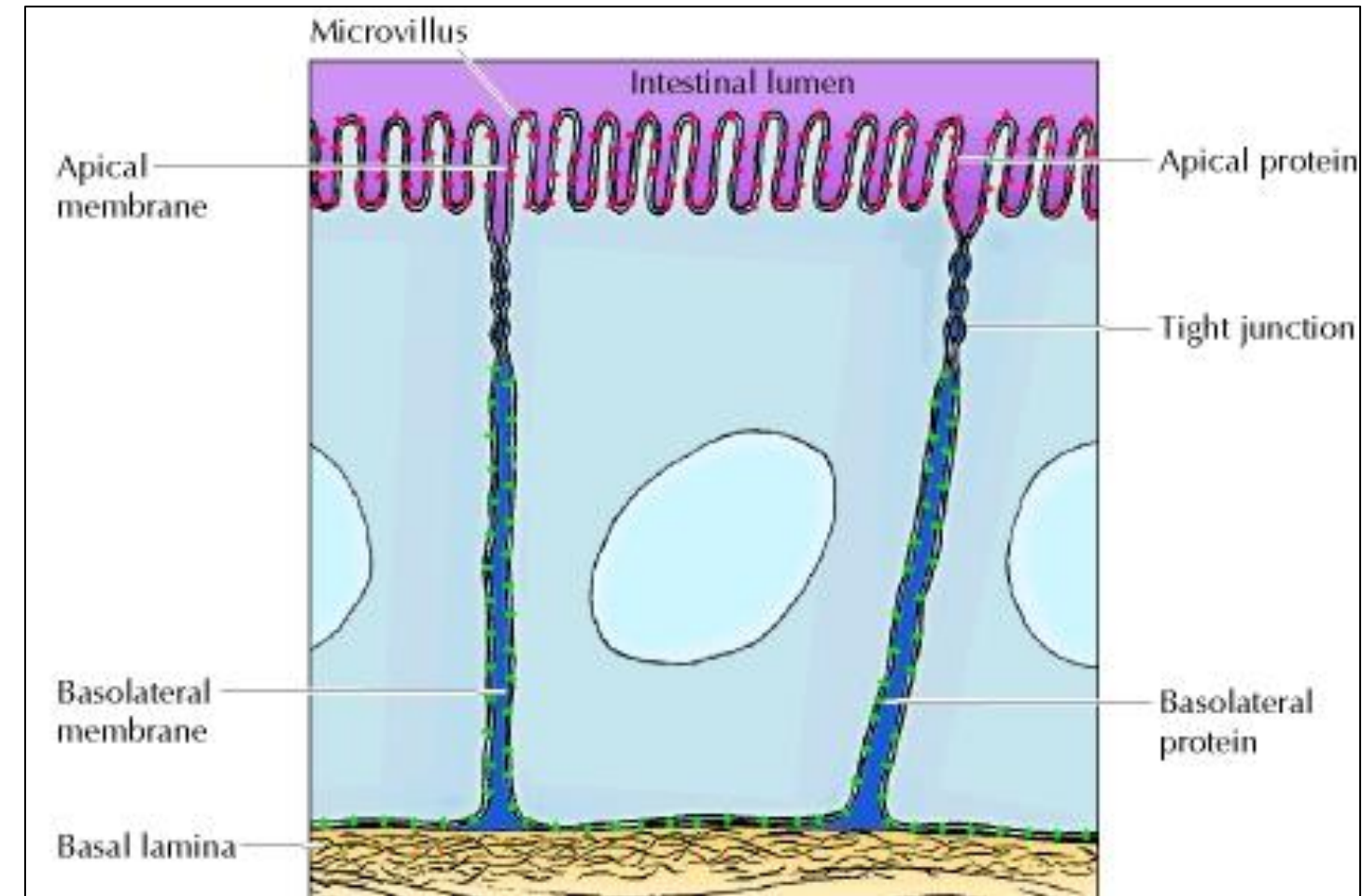
Mechanism 1

- Membrane protein association with the cytoskeleton (**the network that determines the shape of the cell**)



Mechanism 2

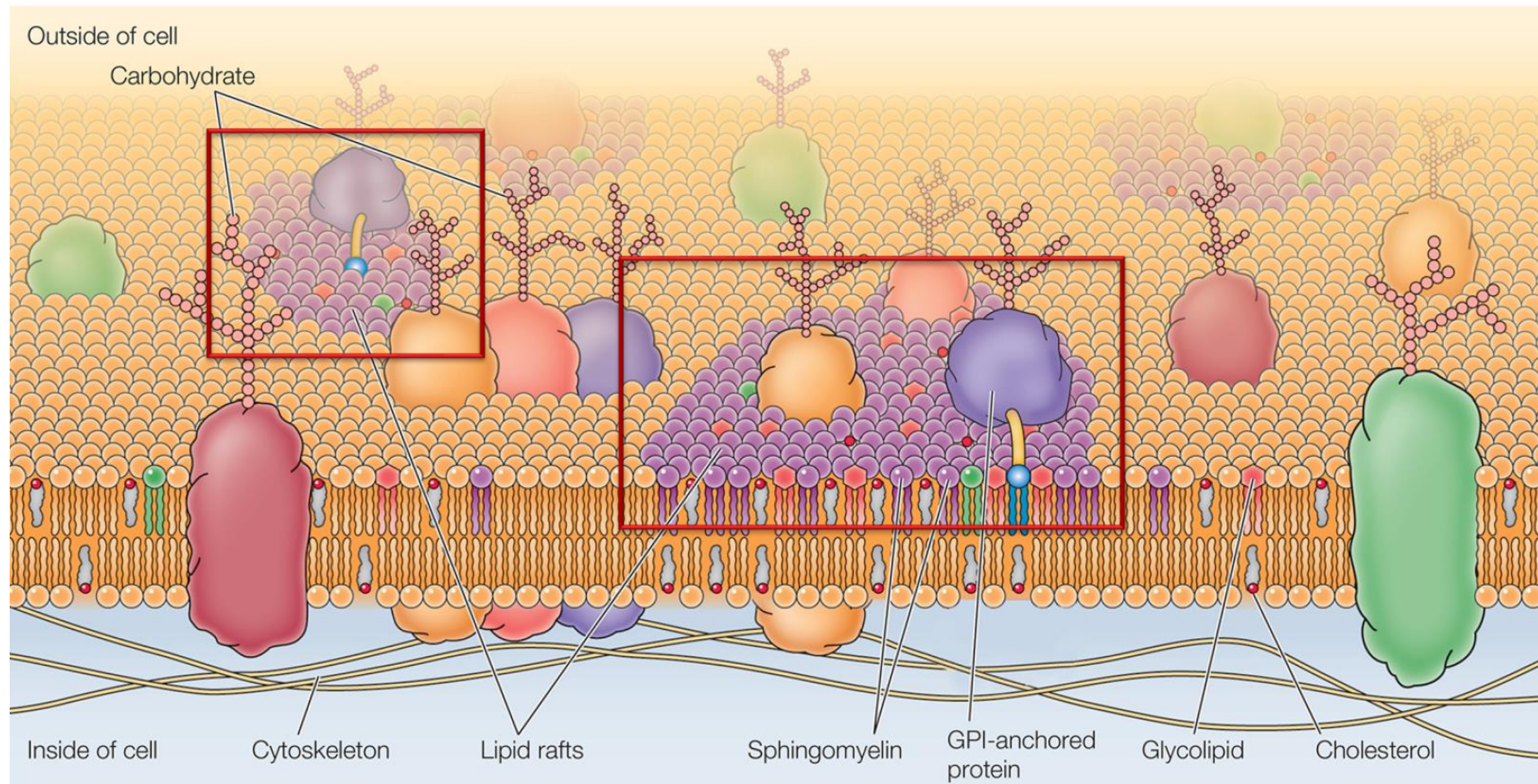
- Specific membrane domains can be created by **tight junctions**.
 - Specialized seals between adjacent cells that prevent fluid leakage and control the movement of molecules between tissue compartments.
- Tight junctions divide the plasma membrane into apical and basolateral surface.
- The proteins are also names as apical and basolateral.
- **Tight junctions prevent the movement of proteins from one domain to another.**



Mechanism 3

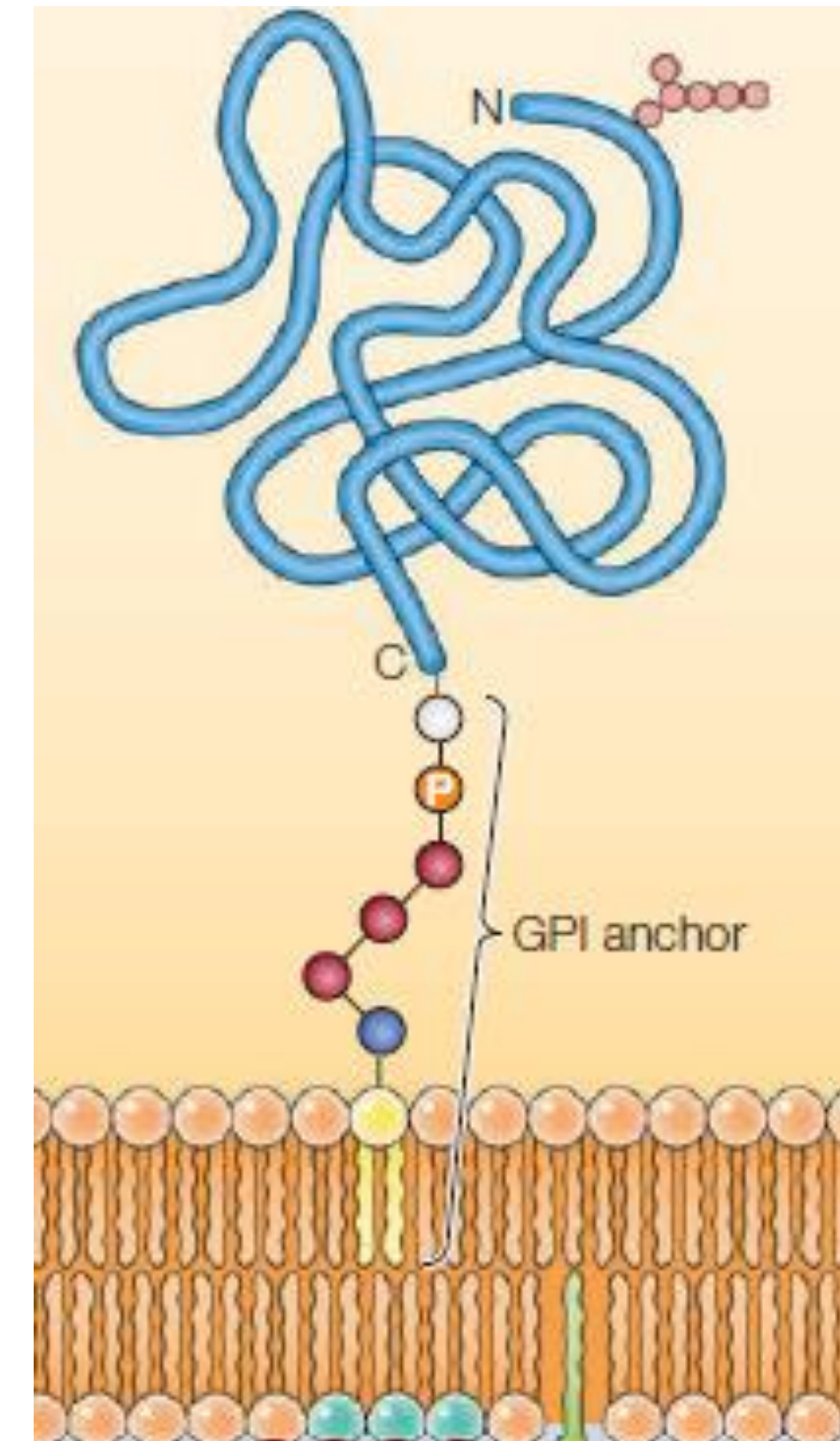
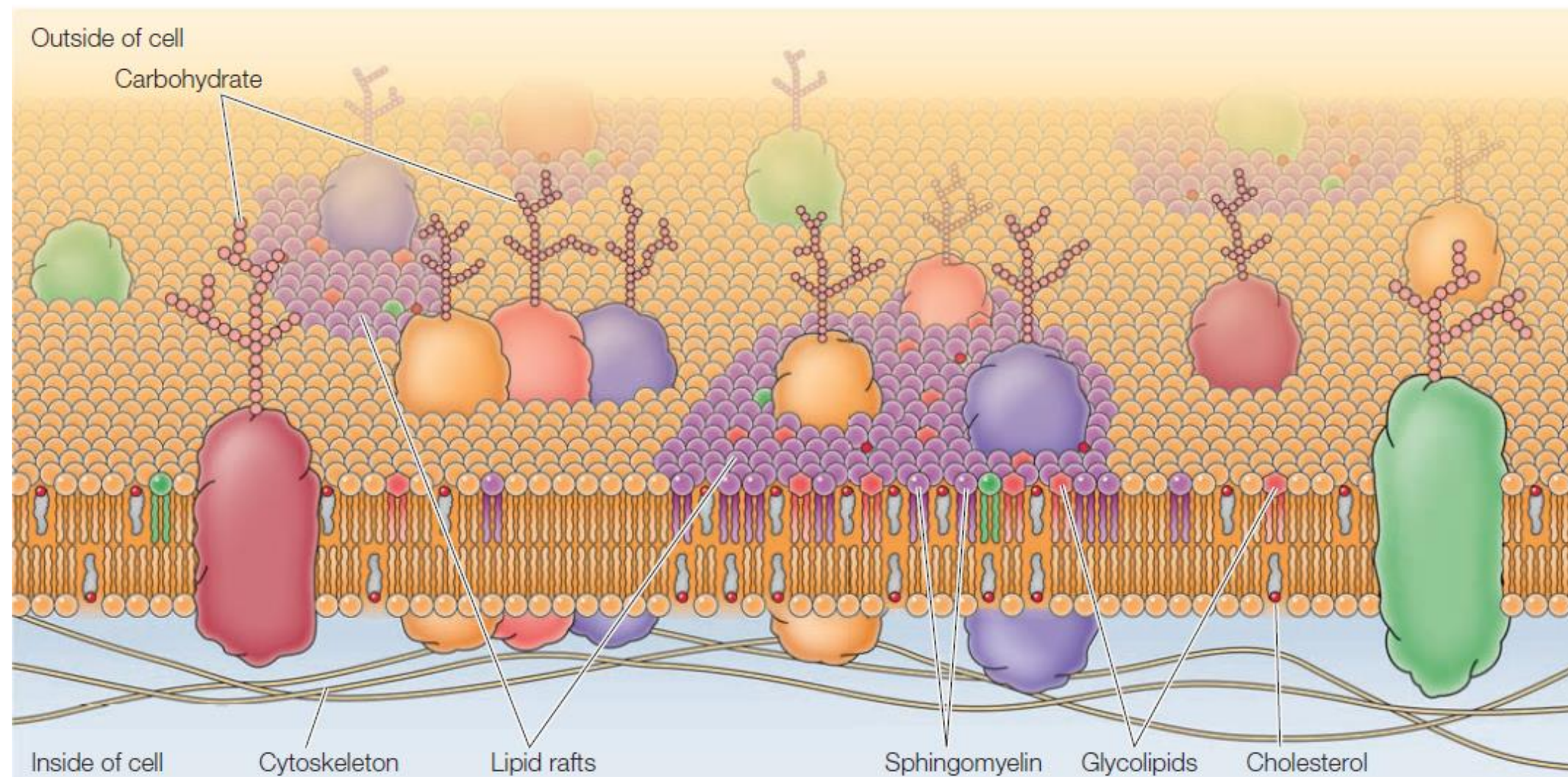
- Lipid distribution across the membrane isn't homogeneous

- Specific lipid composition of the membrane (e.g. lipid rafts)



Lipid rafts

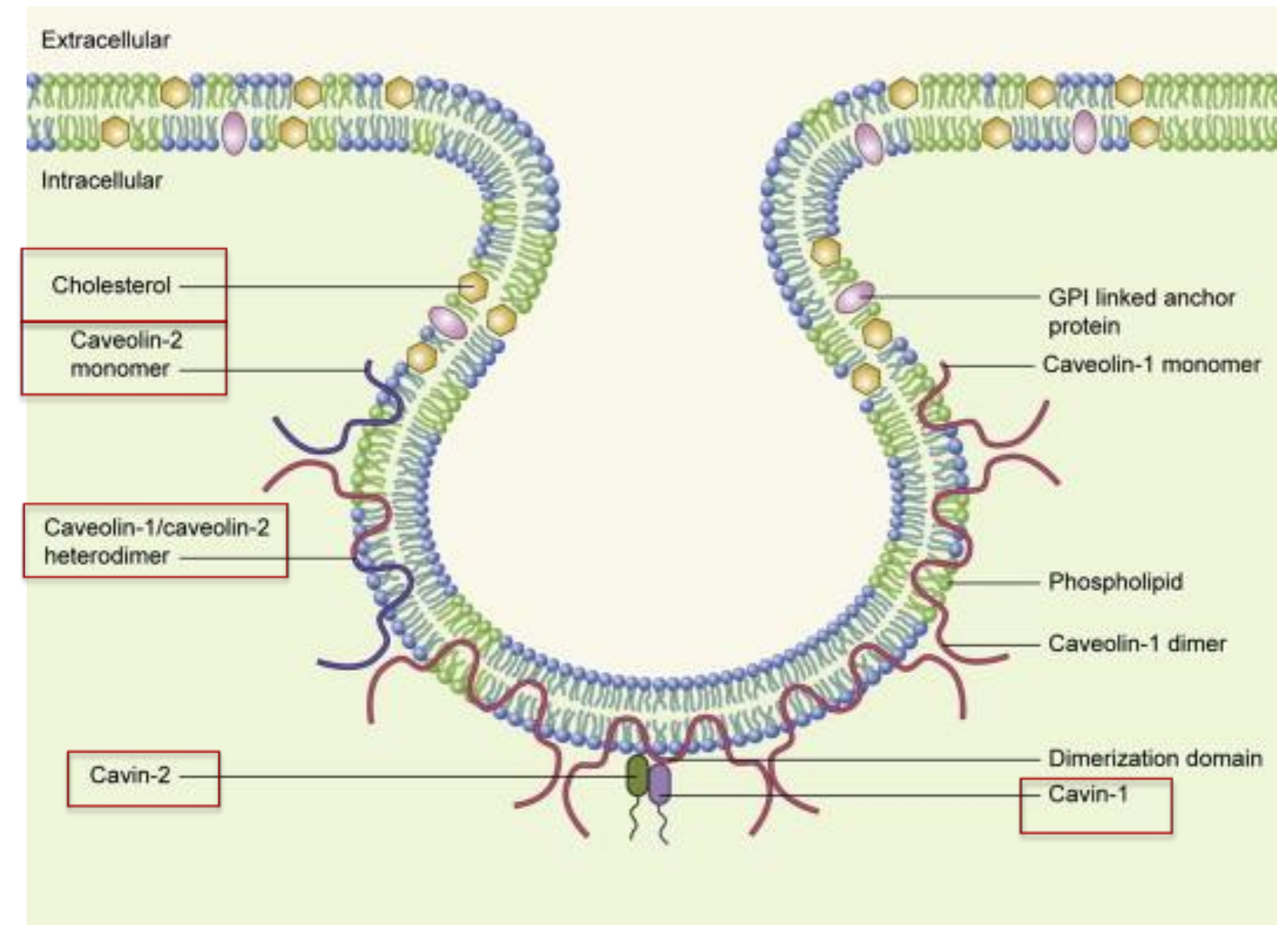
- Specialized membrane regions with clusters of **cholesterol** and the **sphingolipids** (sphingomyelin and glycolipids).
- Rafts are enriched in **glycosylphosphatidylinositol (GPI)-anchored proteins**, and proteins involved in signal transduction and intracellular vesicular trafficking (transport).



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.
- Caveolae contains signaling molecules , allows the endocytosis process (endo =inside & cytosin=eating)
- Caveolae have specific types of proteins , **caveolin & cavin** which form it . In addition to **cholesterol and lipids**



Electron microscopic image of caveolae
(the invagination is directed toward [-Z] axis)

Glycocalyx **Sugar Coating**

- The surface of the cell can be 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 sugarcoating prevent friction and cells damage.

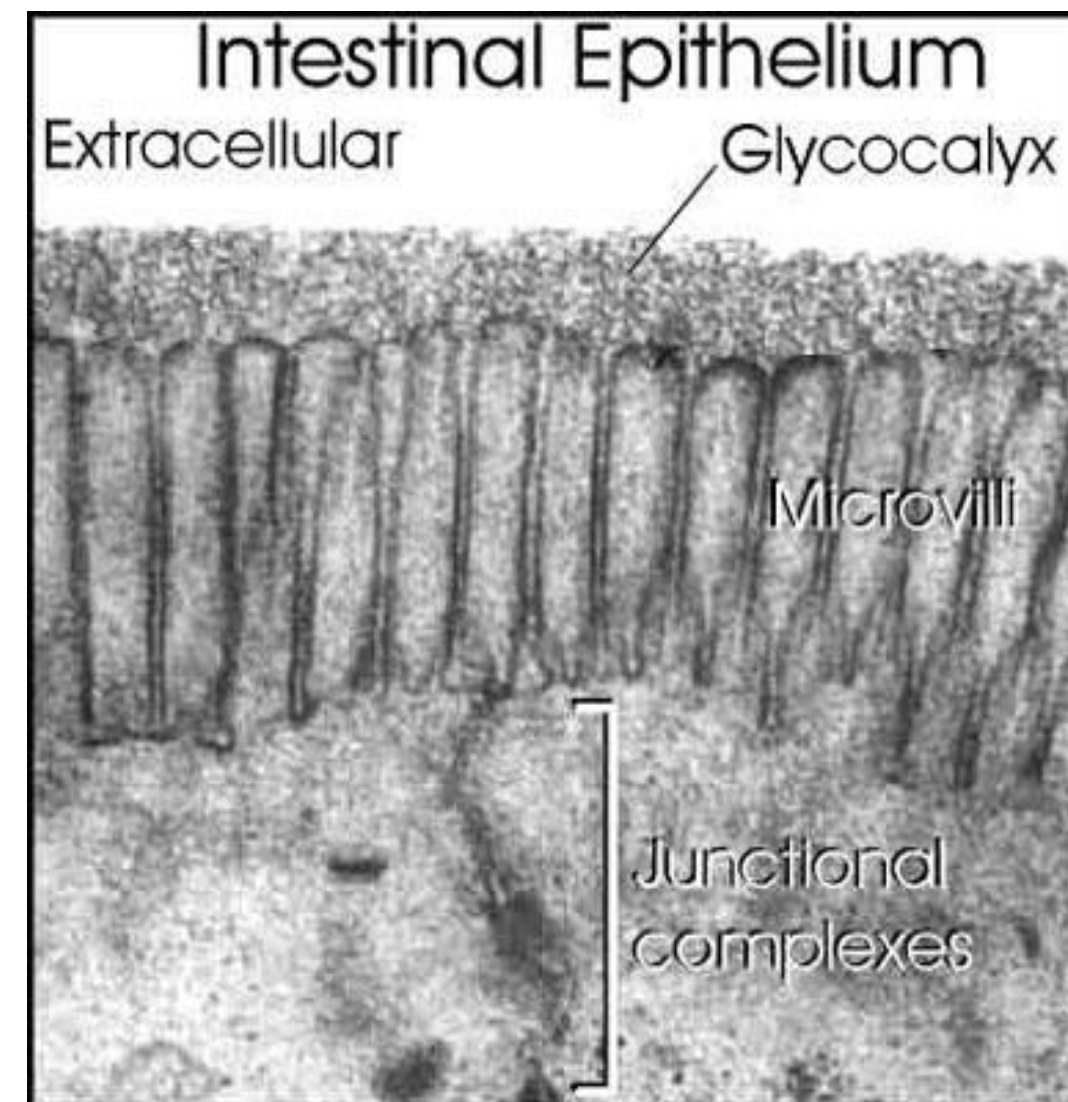
Functions:

- 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

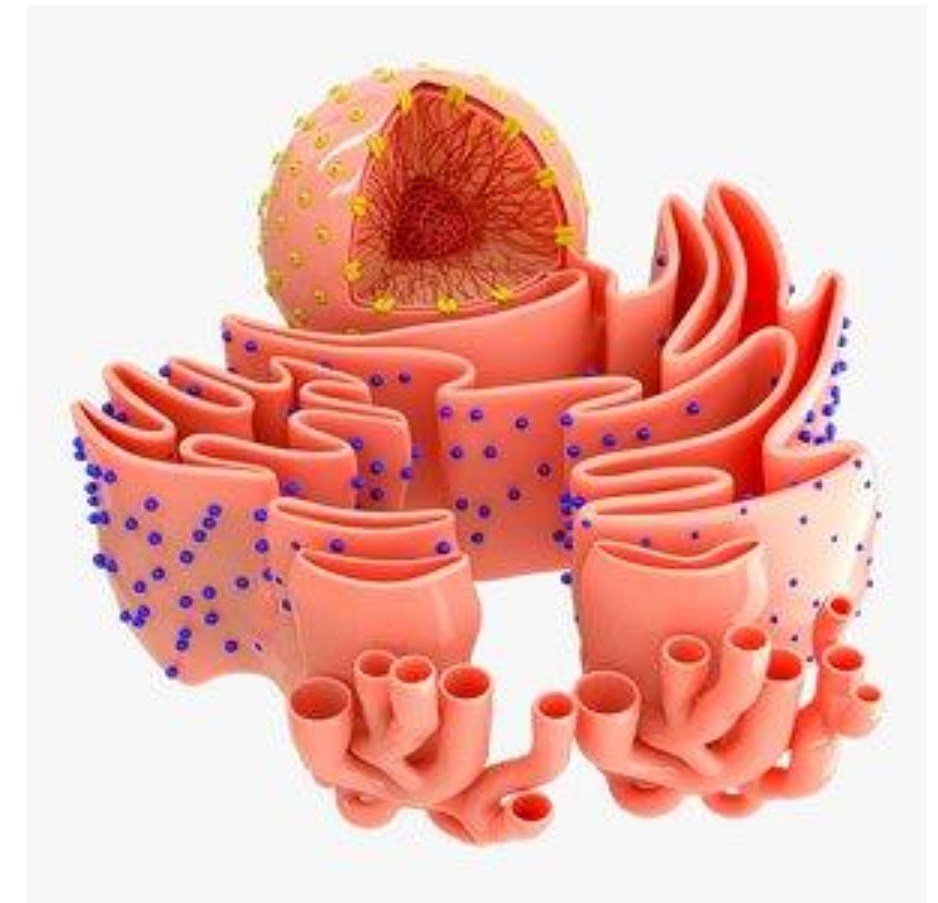
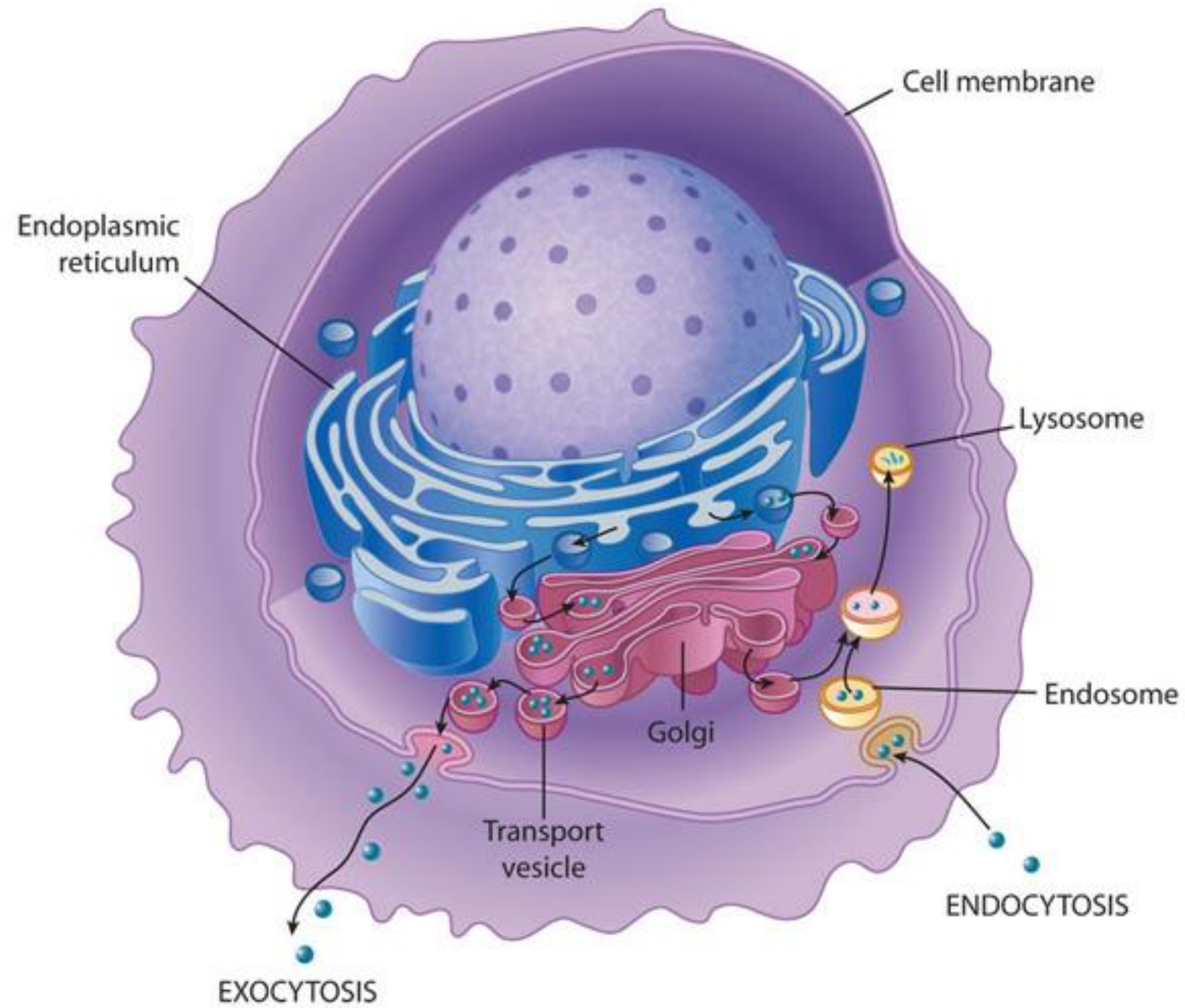
- Formation of a barrier for microorganisms
(bacteria and viruses can't penetrate the cells because of glycocalyx)





Protein sorting (endoplasmic reticulum)

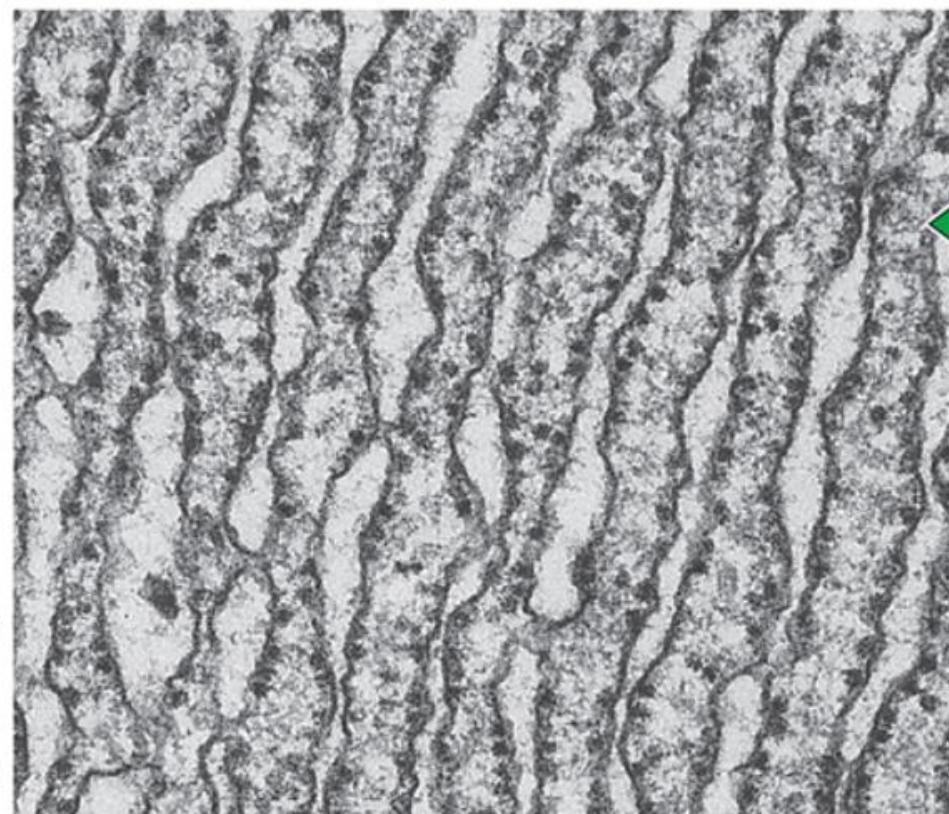
An overview



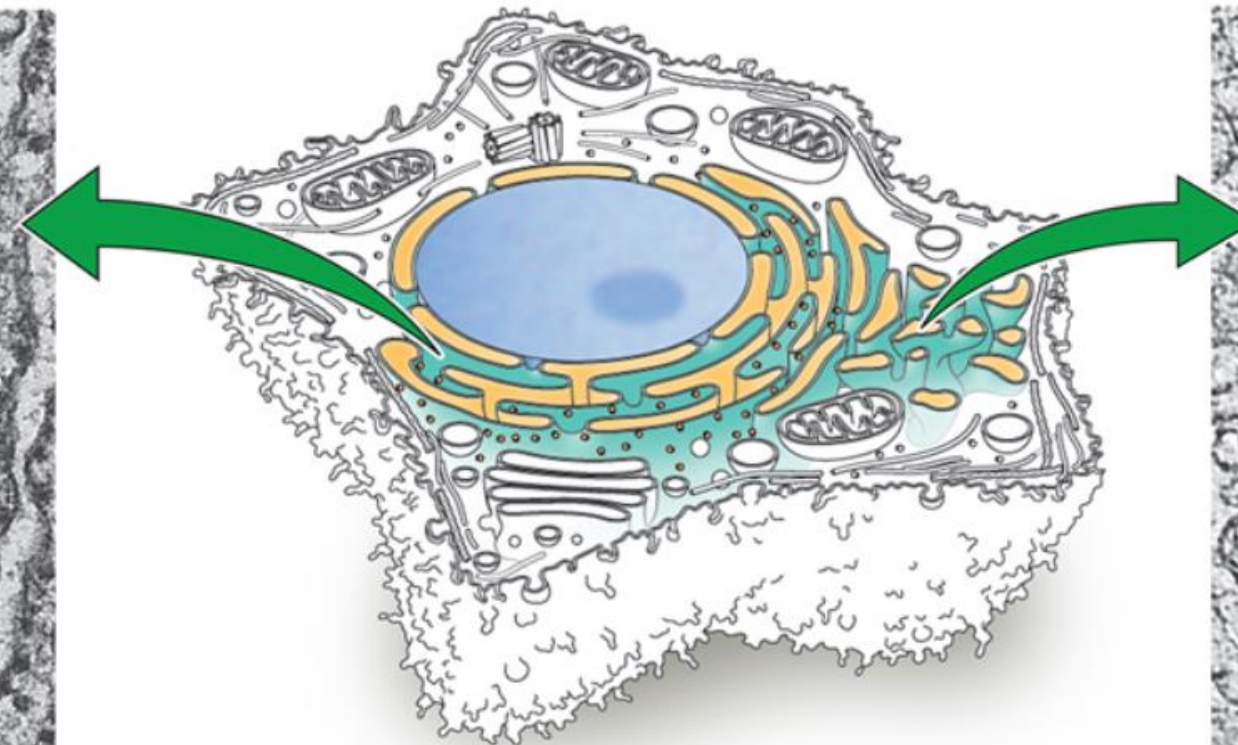
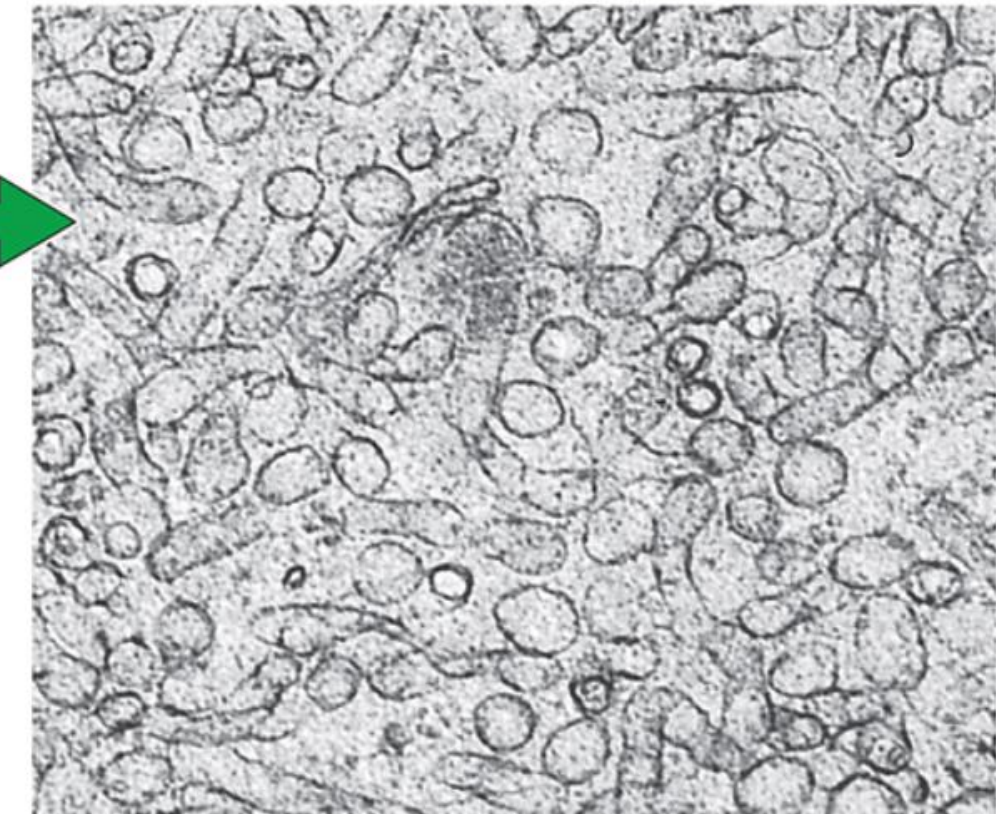
Endoplasmic reticulum (ER)

- It is a network of membrane-enclosed **tubules** and **sacs (cisternae)** that extends from the nuclear membrane throughout the cytoplasm. **It is a part of the nuclear membrane**
- It is the **largest organelle** of most eukaryotic cells.
- **There are two types of Endoplasmic Reticulum :**
 - 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

(A) Rough endoplasmic reticulum

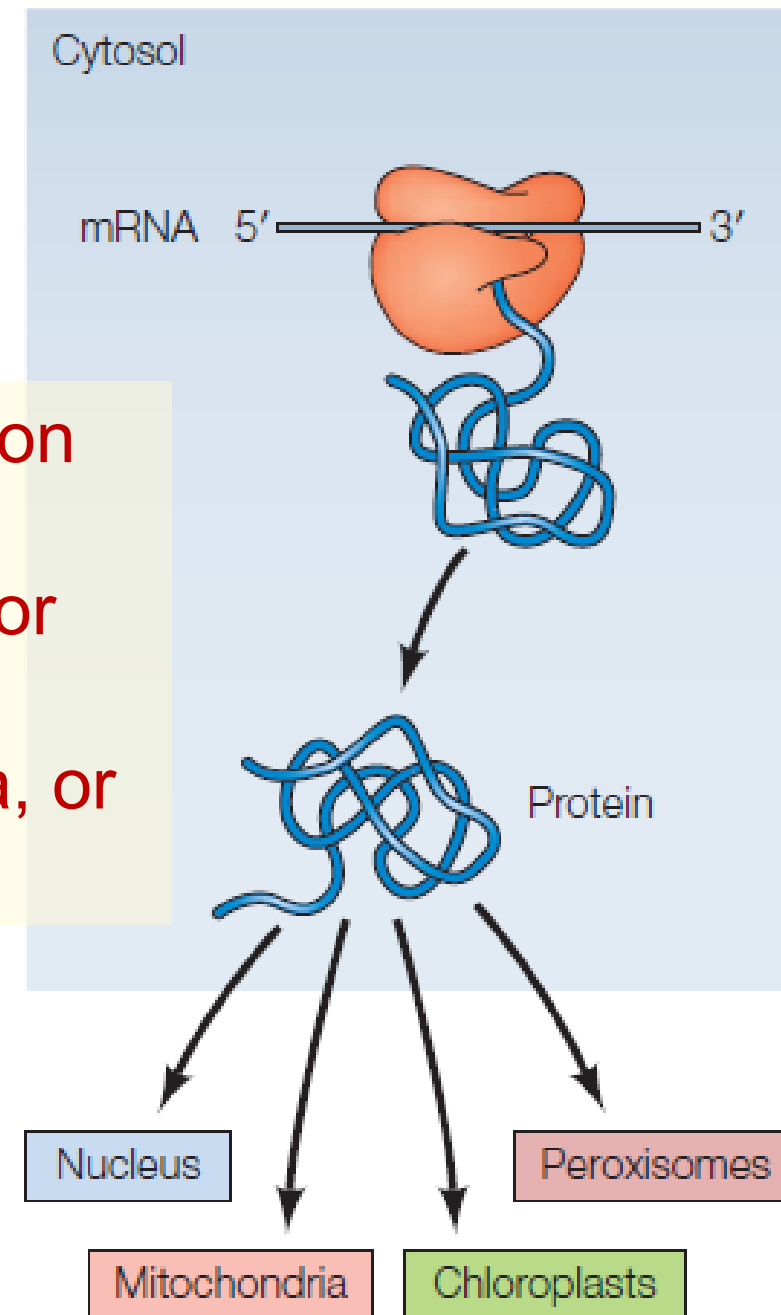


(B) Smooth endoplasmic reticulum



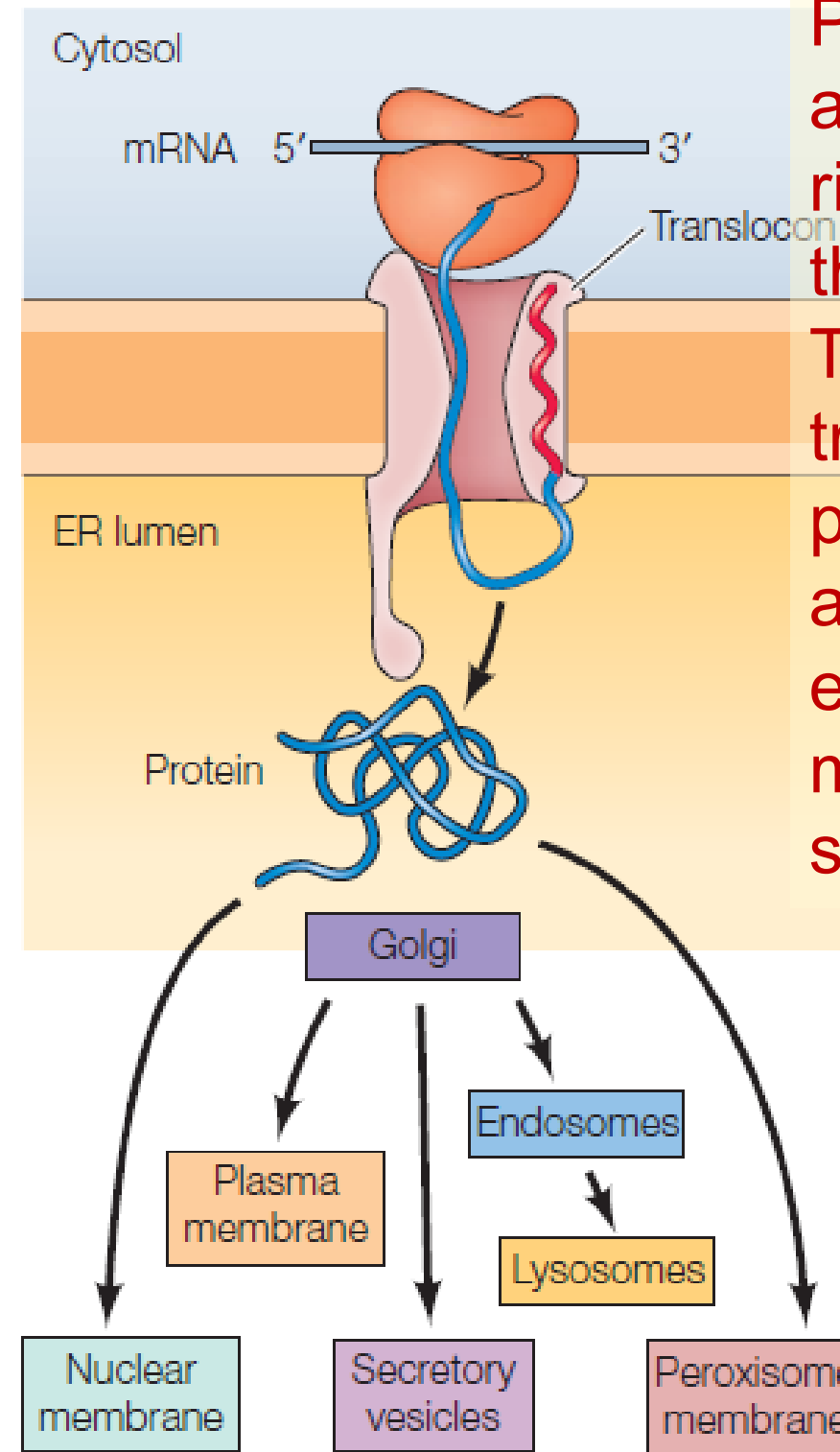
Protein sorting

Free ribosomes in cytosol



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

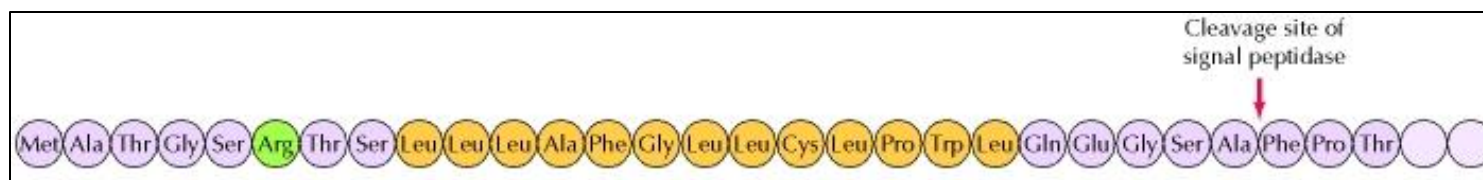
Membrane-bound ribosomes



Proteins containing **signal sequences** are synthesized on membrane-bound ribosomes and translocated directly into the ER.

These proteins may stay within the ER or be 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.

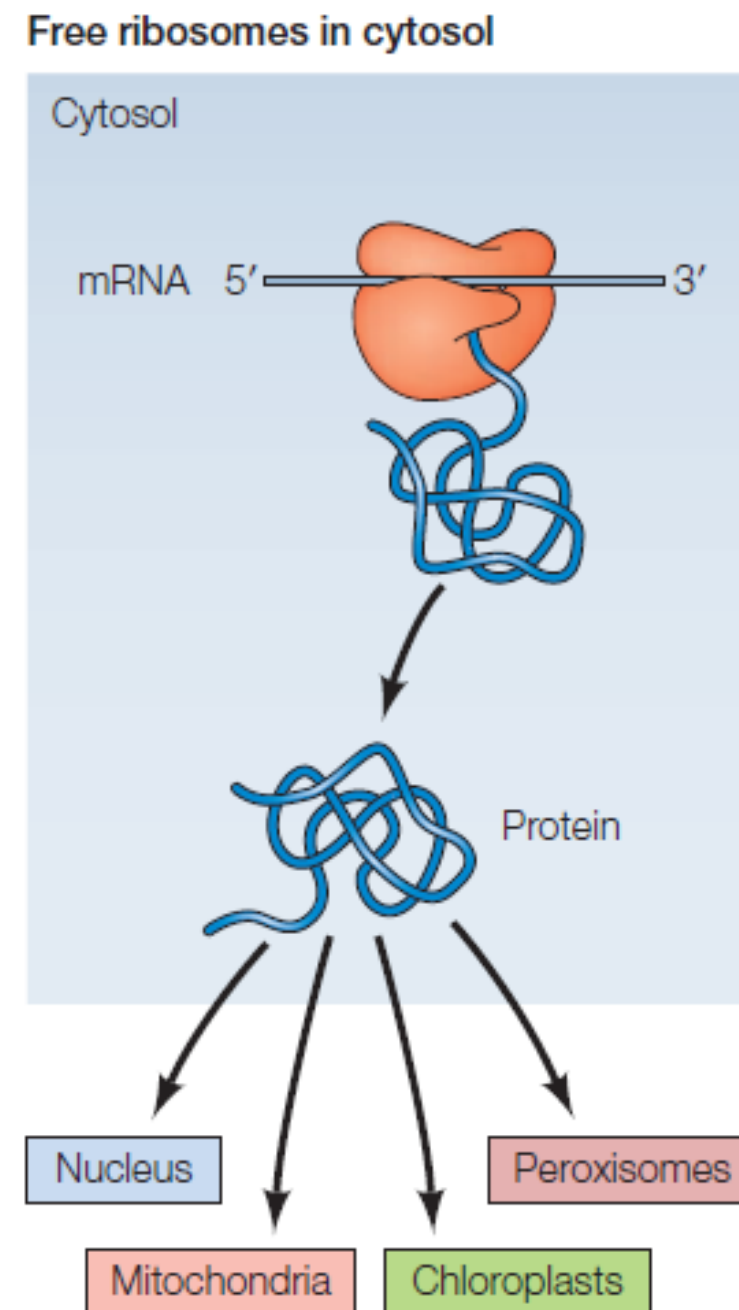
In cell biology, a lumen is a membrane-defined space that is found inside several organelles, cellular components, or structures



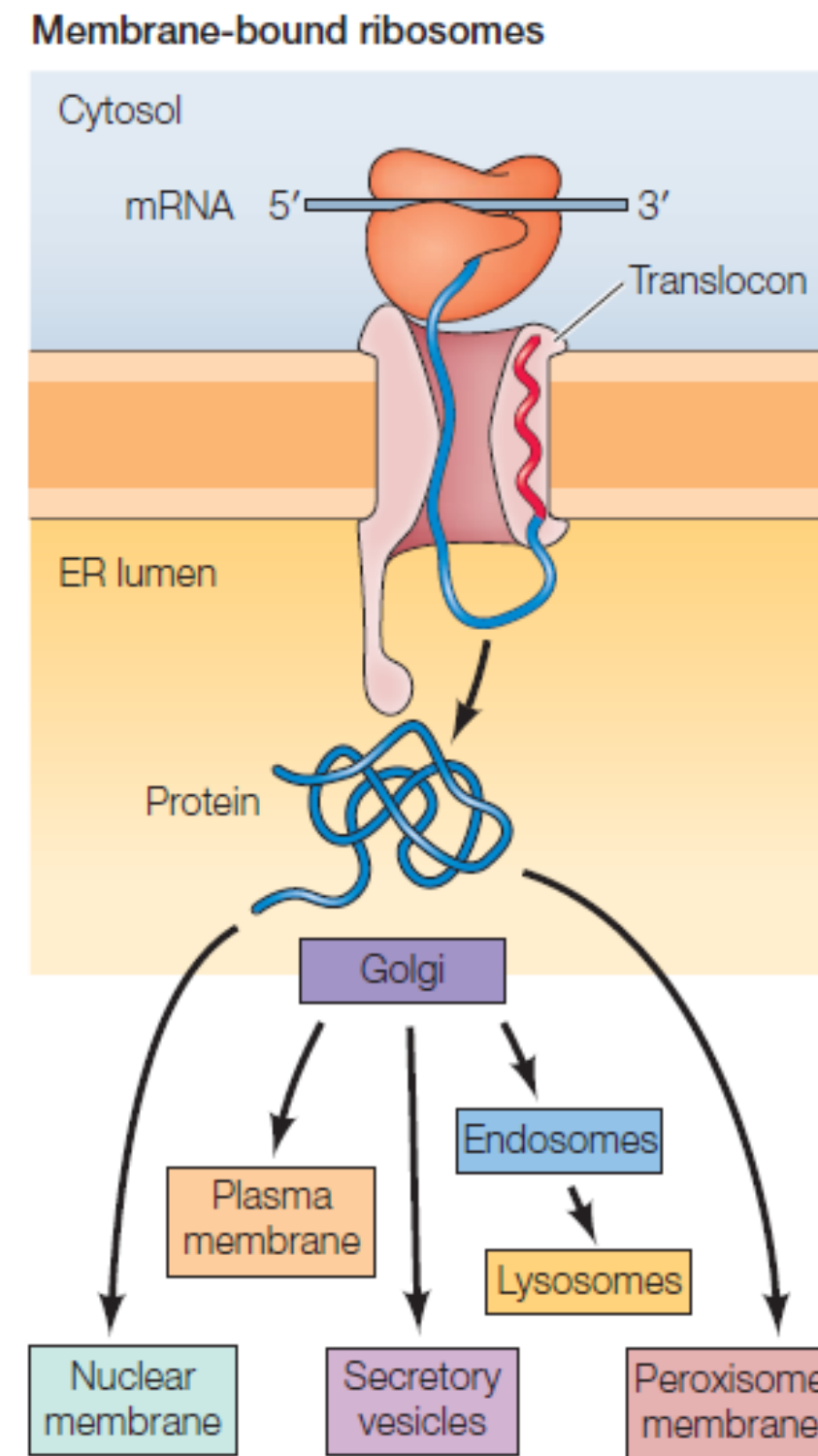
Signal sequence: a short sequence of amino acids of the polypeptide at the amino terminus. It is then cleaved from the polypeptide chain during its transfer into the ER lumen.

Protein sorting

- Many proteins are synthesized in the cytosol and once they are synthesized 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.

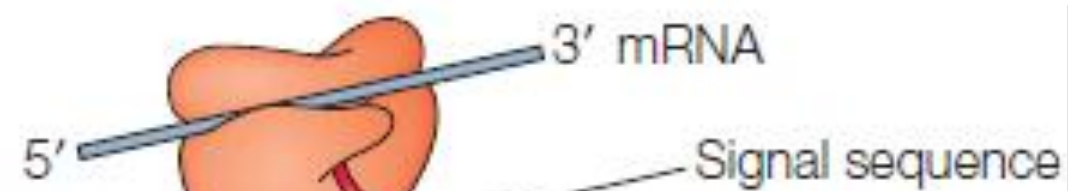


- If the signal exists proteins that are synthesized on the surface of the RER(rough endoplasmic reticulum).
- These proteins enter the lumen of the RER then the cell decides whether their destination :Nuclear membrane,Peroxisome membrane , or to the Golgi then to thePlasma membrane or Endosomes the lysosomes , or out the cell

Protein sorting

- what makes the cell know while the protein is being synthesized in the cytosol (or at RER membrane) the final destination of this protein ?
- 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 synthesized) 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. (Also called **The signal peptide + the prepeptide**)



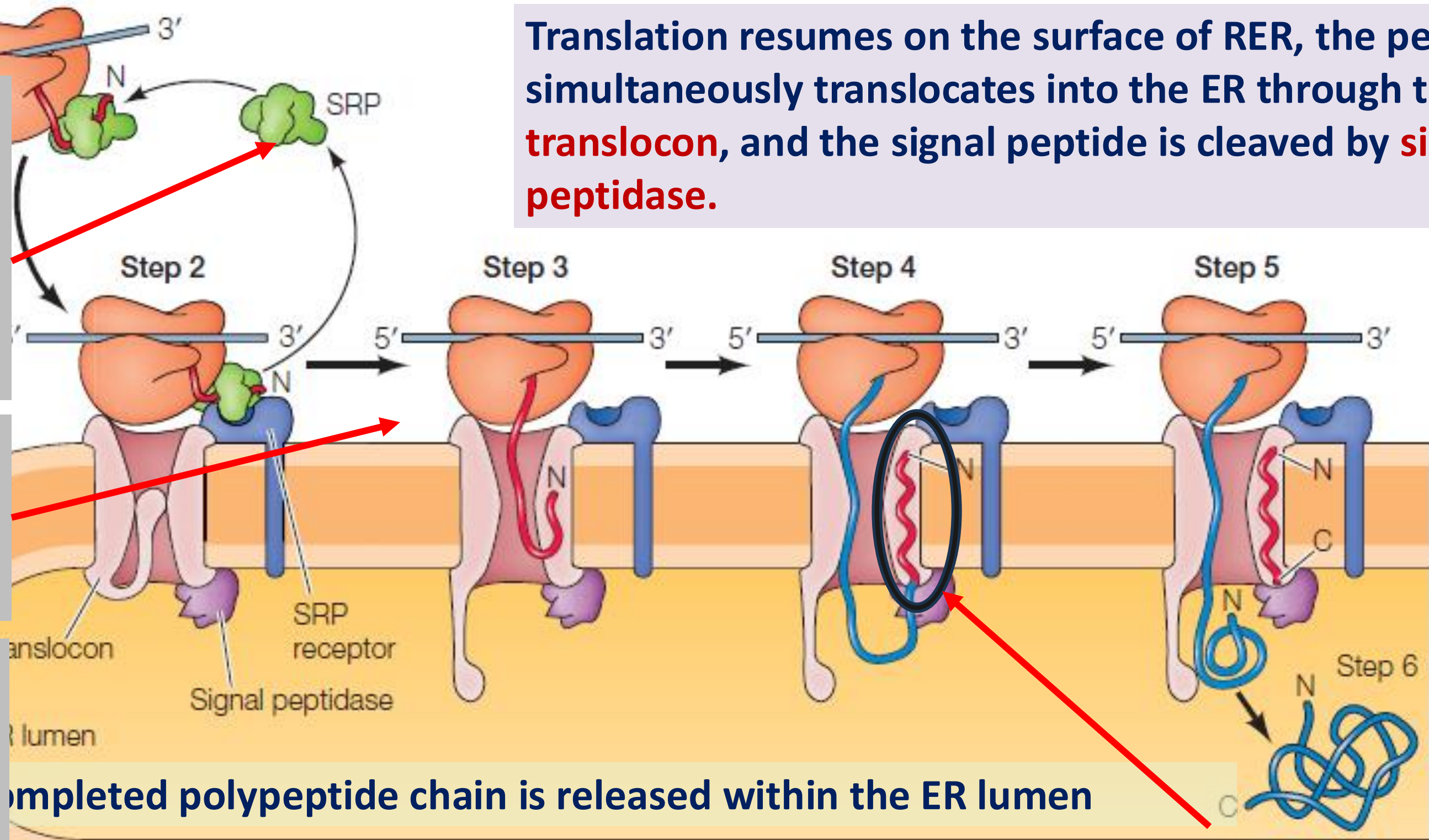
The signal sequence is recognized as the protein is synthesized and the ribosome is transported to the surface of the RER

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**.

This protein recognize 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 it's 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 synthesized the signal sequence is cleaved by **protease**, because no need for it any more (it's purpose was to be recognized in order for translocation)

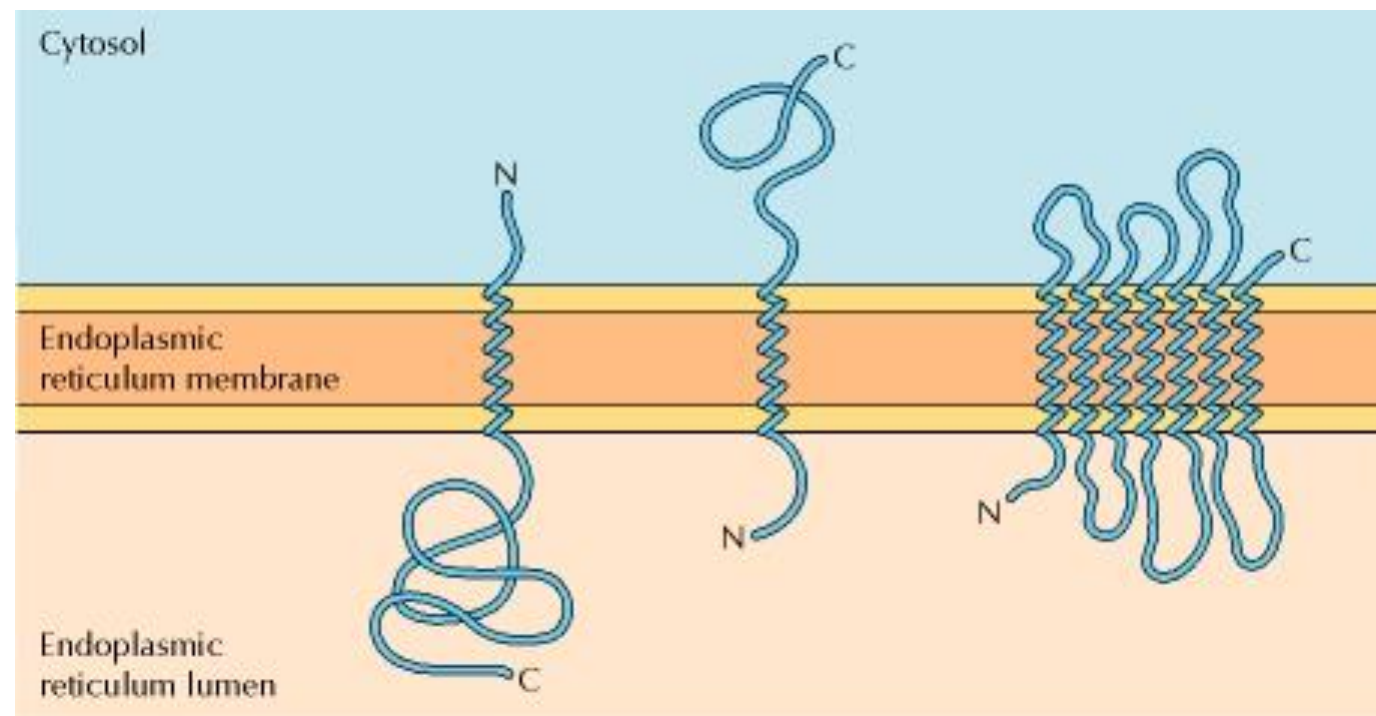


Completed polypeptide chain is released within the ER lumen

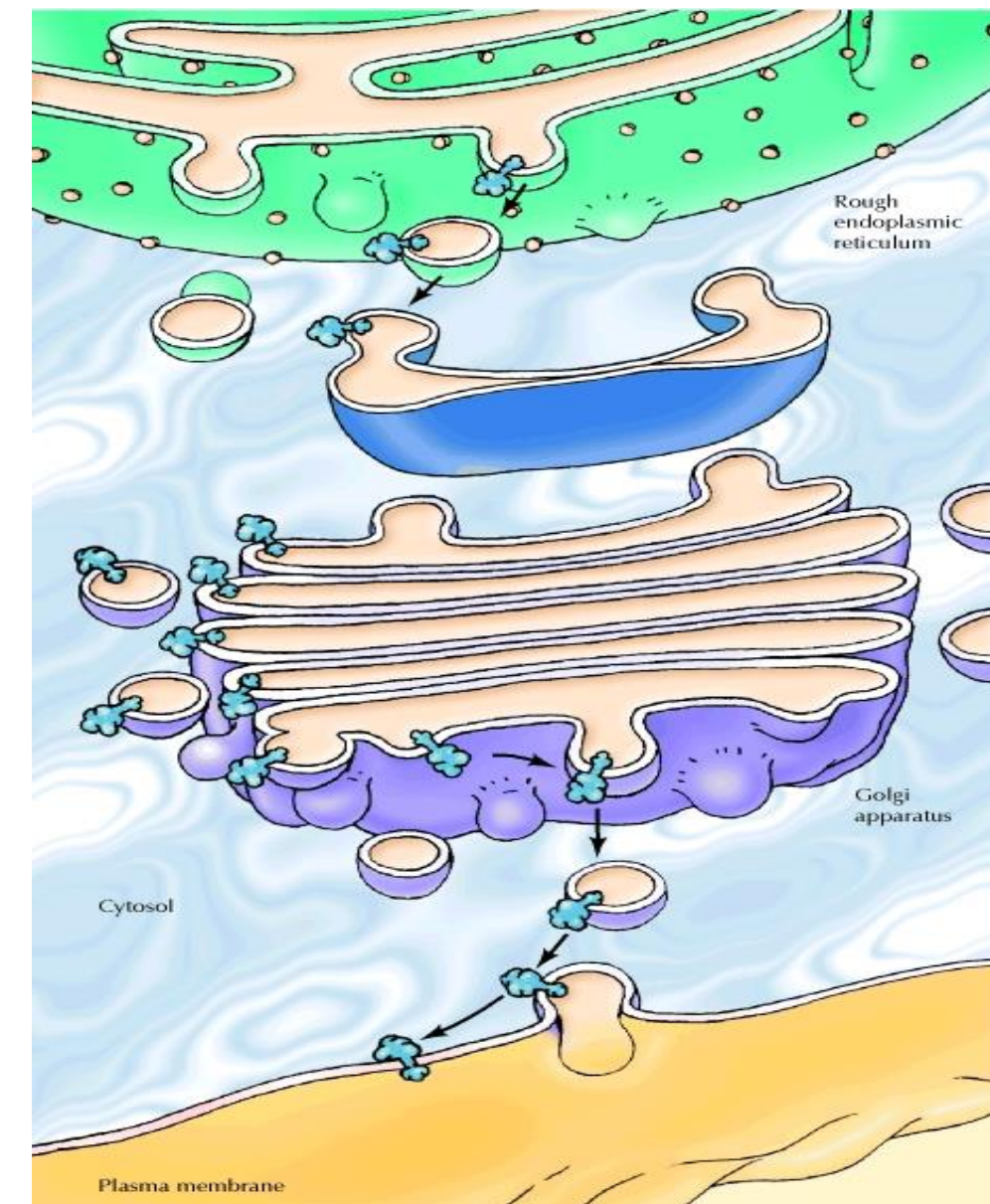
The signal sequence is also called the pre peptide (when there is a protein have a sequence its purpose is to identify protein destination is called preprotein and here the signal sequence is the pre part of the preprotein)

Pathways of protein sorting

- 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

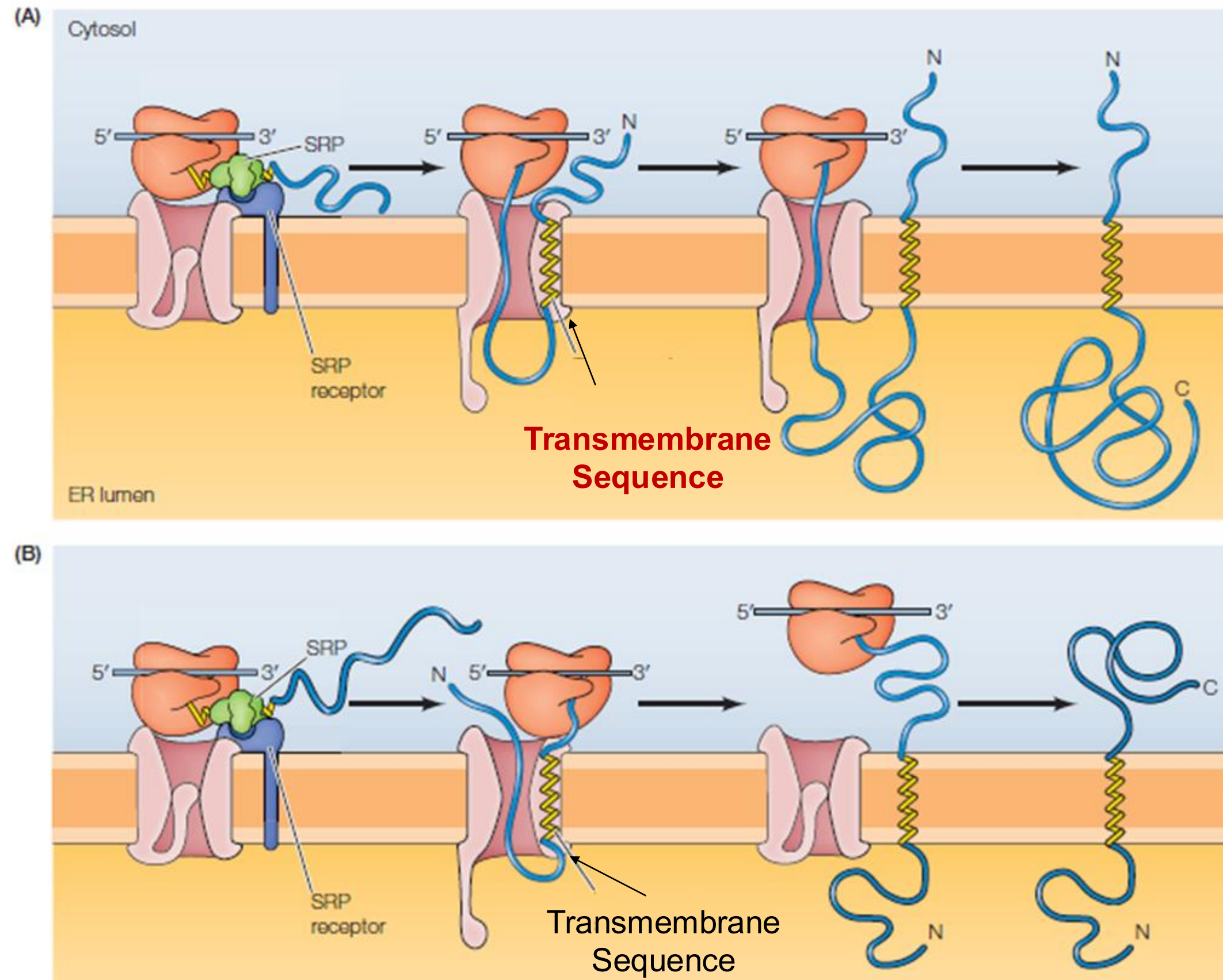


The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.



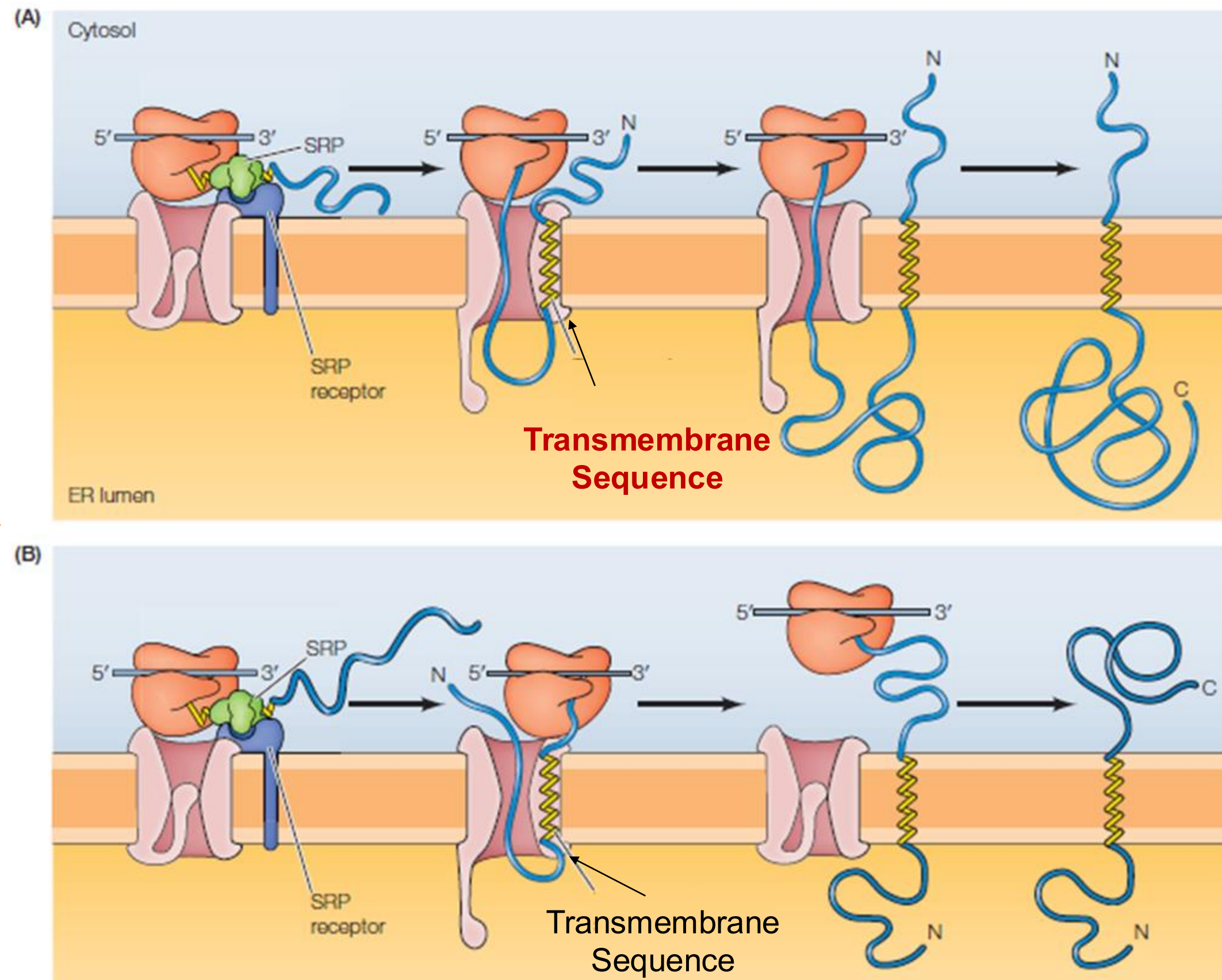
Insertion of membrane proteins via internal transmembrane sequences

- 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.

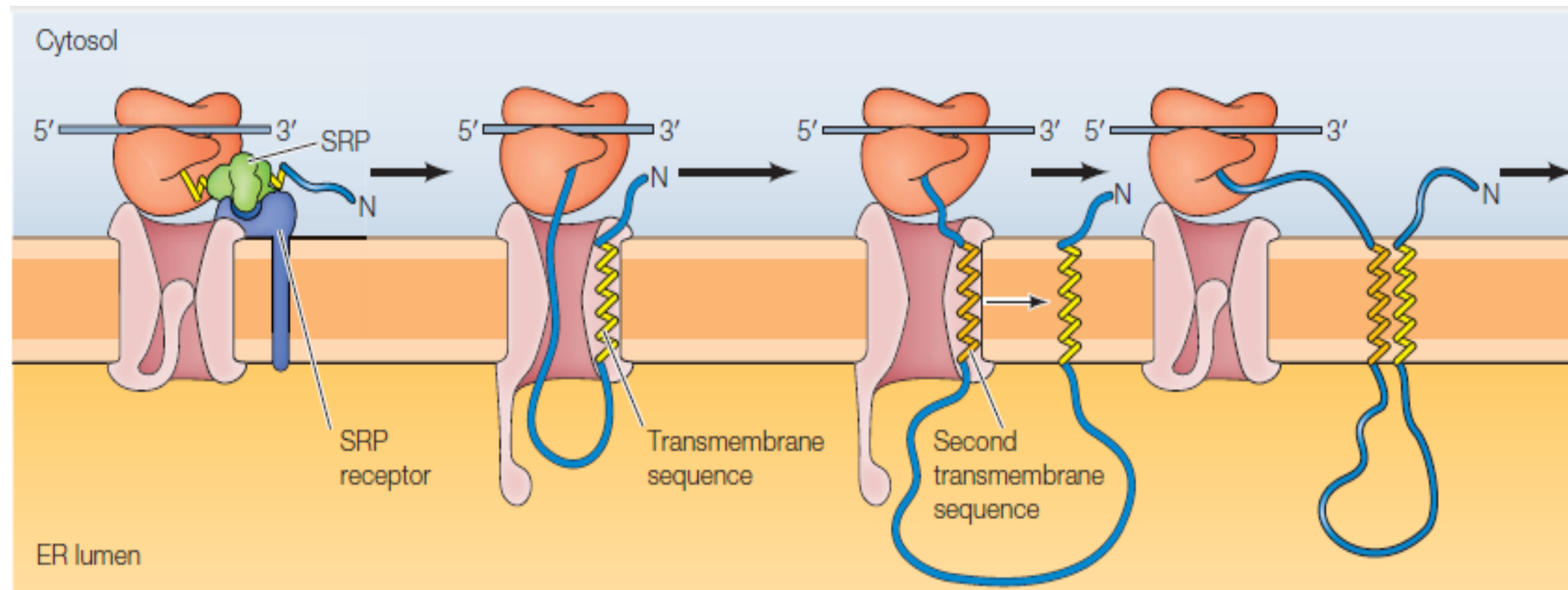


Insertion of membrane proteins via internal transmembrane sequences

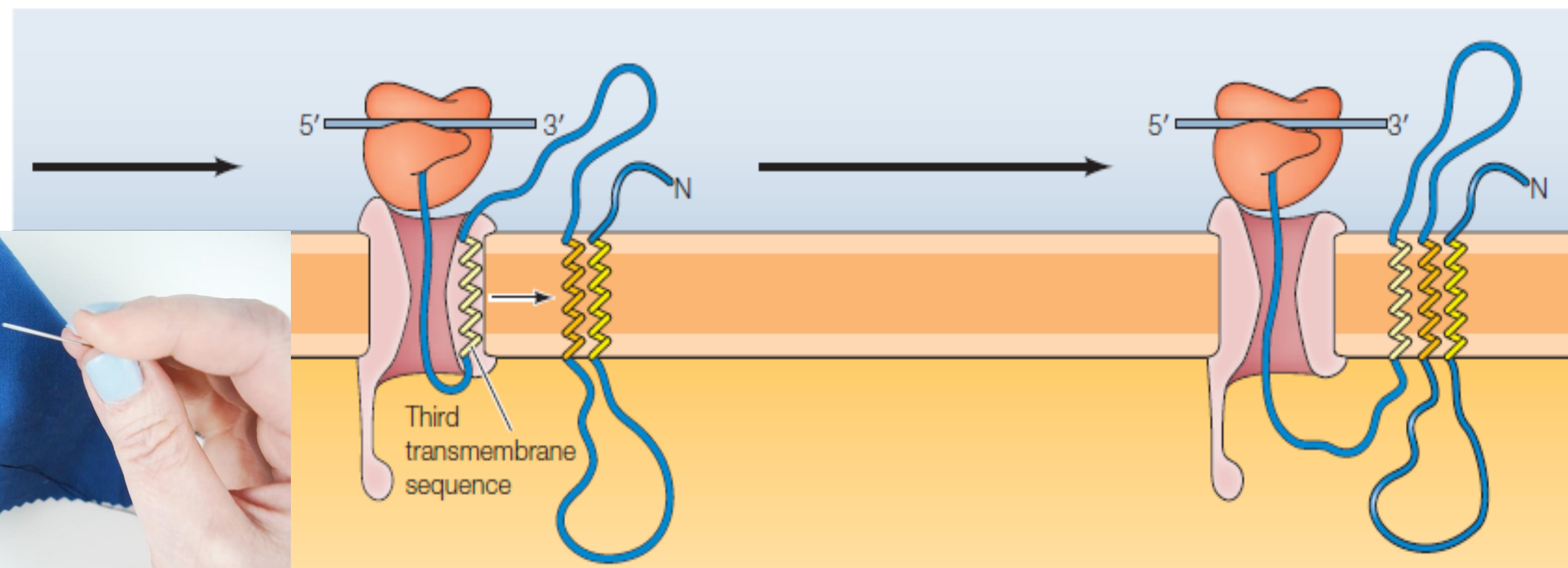
- When the protein being translated is going to be **part of a membrane** such as (peroxisomal, plasma, or lysosomal membrane), it doesn't get released entirely into the ER lumen
- Instead, once the transmembrane domain/sequence (which is helical and hydrophobic) appears during protein formation, it 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.



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.

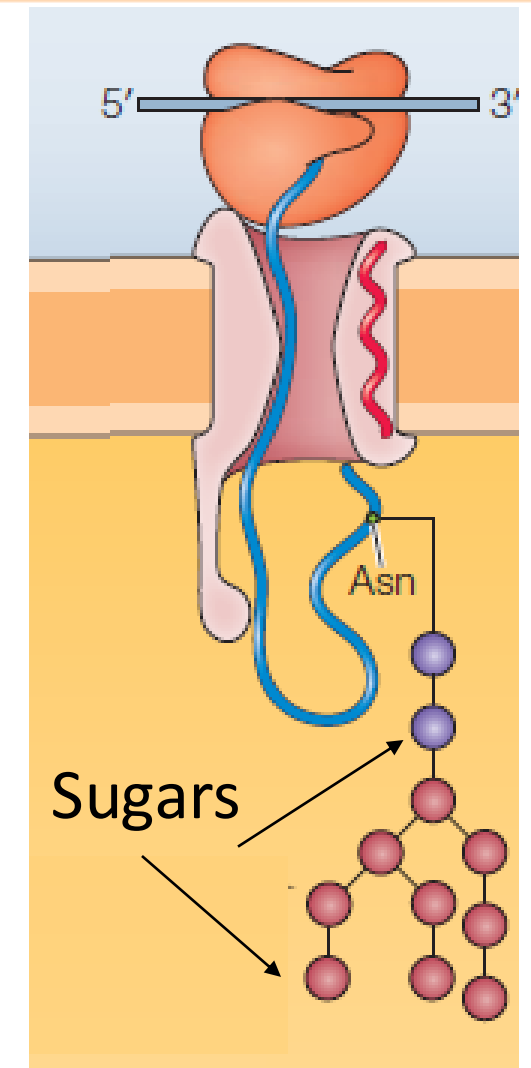
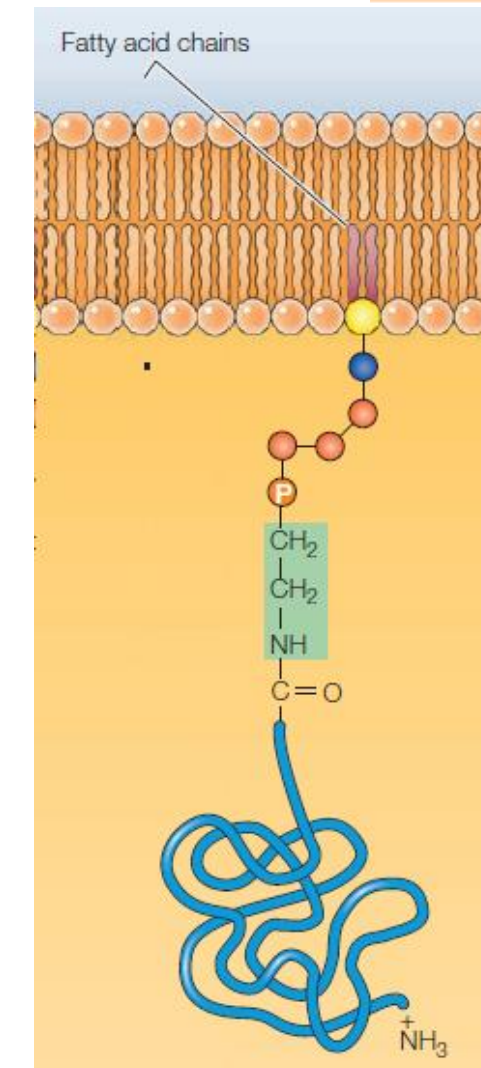
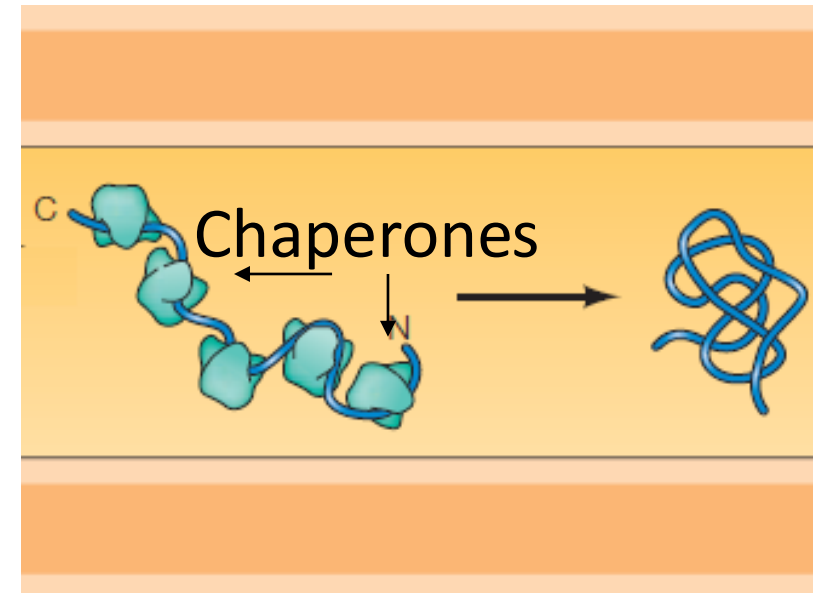


- The sequence of transmembrane domain determines the orientation of N- and C- termini (inside the ER lumen or outside)



Once inside the ER, proteins are

- Folded (with the help of chaperones)
- Complexed (quaternary structure) ,such as hemoglobin, where the alpha and beta polypeptide chains are linked together in the ER
- Modified by disulfide (cys residue) bonds formed by protein disulfide isomerase
- Glycosylated (addition of sugar molecules)
- Anchored by lipids (inside the ER)

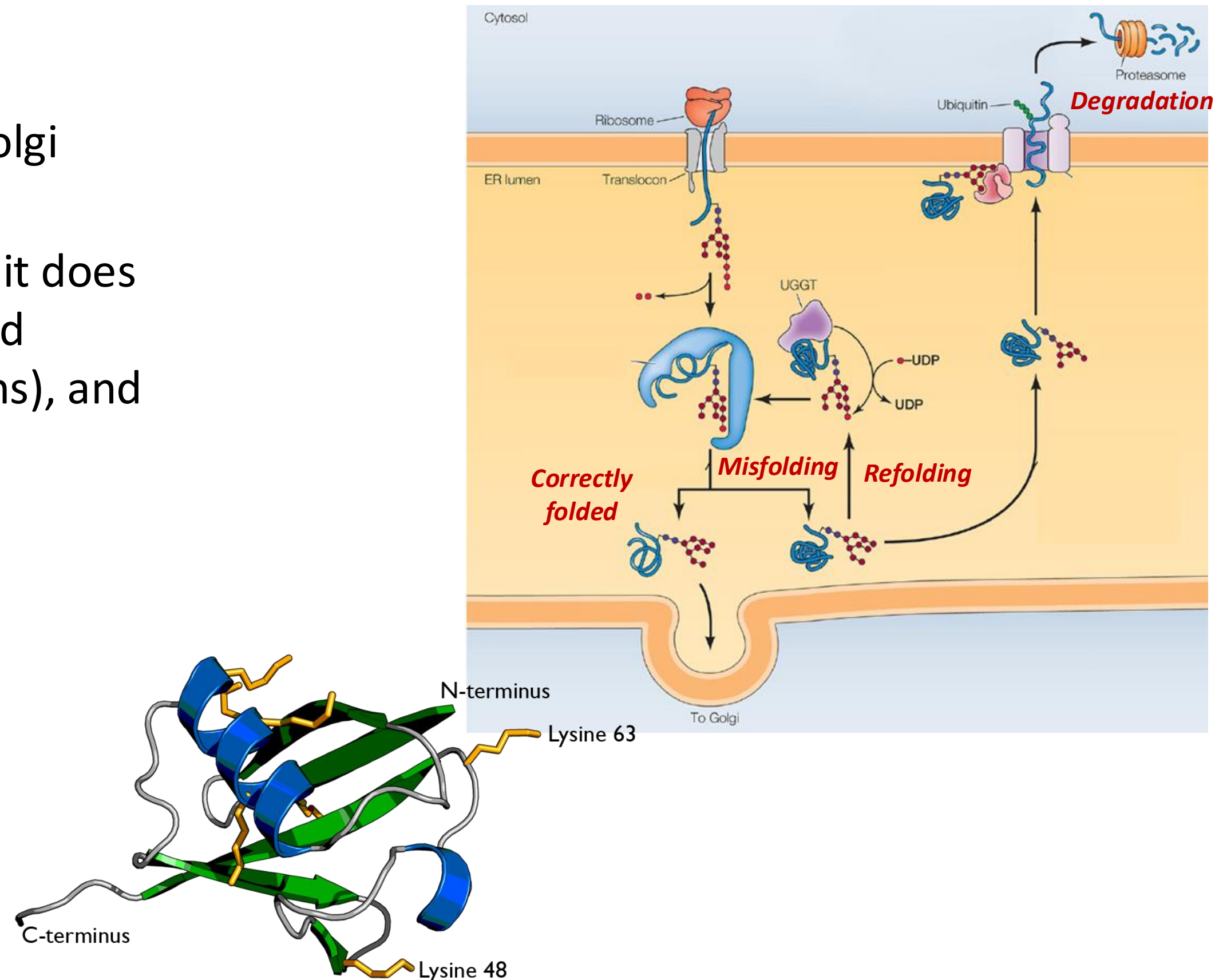


➤ (recap from BioChem) In the primary structure, amino acids are linked in a linear sequence, but protein folding brings amino acids that are far apart in the chain close together through interactions between their side chains. Proteins, such as chaperones, assist this process by helping proteins fold correctly and preventing misfolding and aggregation.

Protein folding and ER-associated degradation (ERAD)

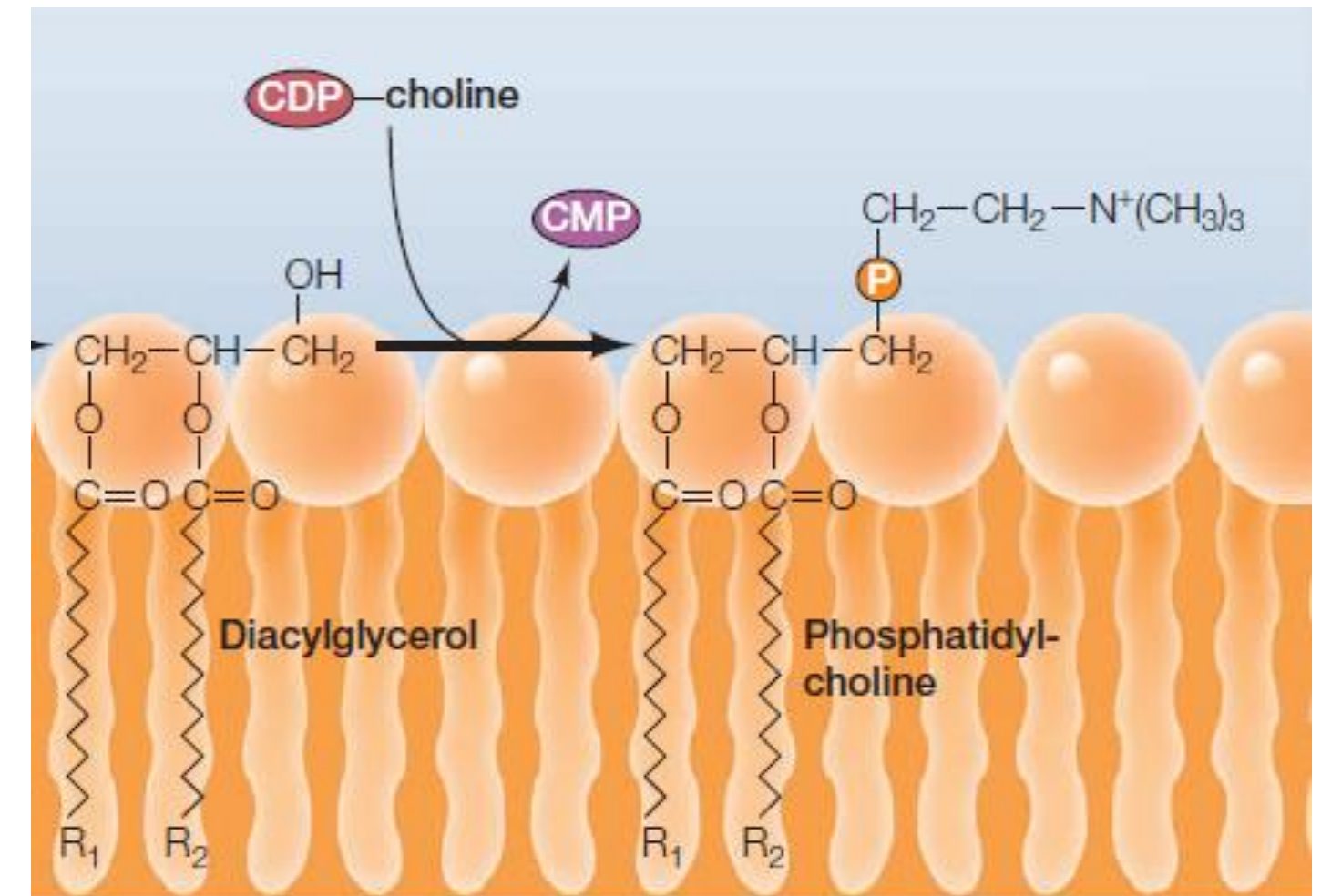
- If correctly folded, proteins move on to Golgi apparatus
- If misfolded, proteins are refolded, and, if it does not work, sent to the cytosol, ubiquitylated (addition of small proteins called ubiquitins), and degraded in the proteasome. (-some → structure)

➤ Protein misfold → refold it to fix it → still misfold occurs → the polypeptide chain is sent to the cytosol and ubiquitylated in it (adding small proteins called ubiquitins to the chain and is degraded in the proteasome back to amino acids.)



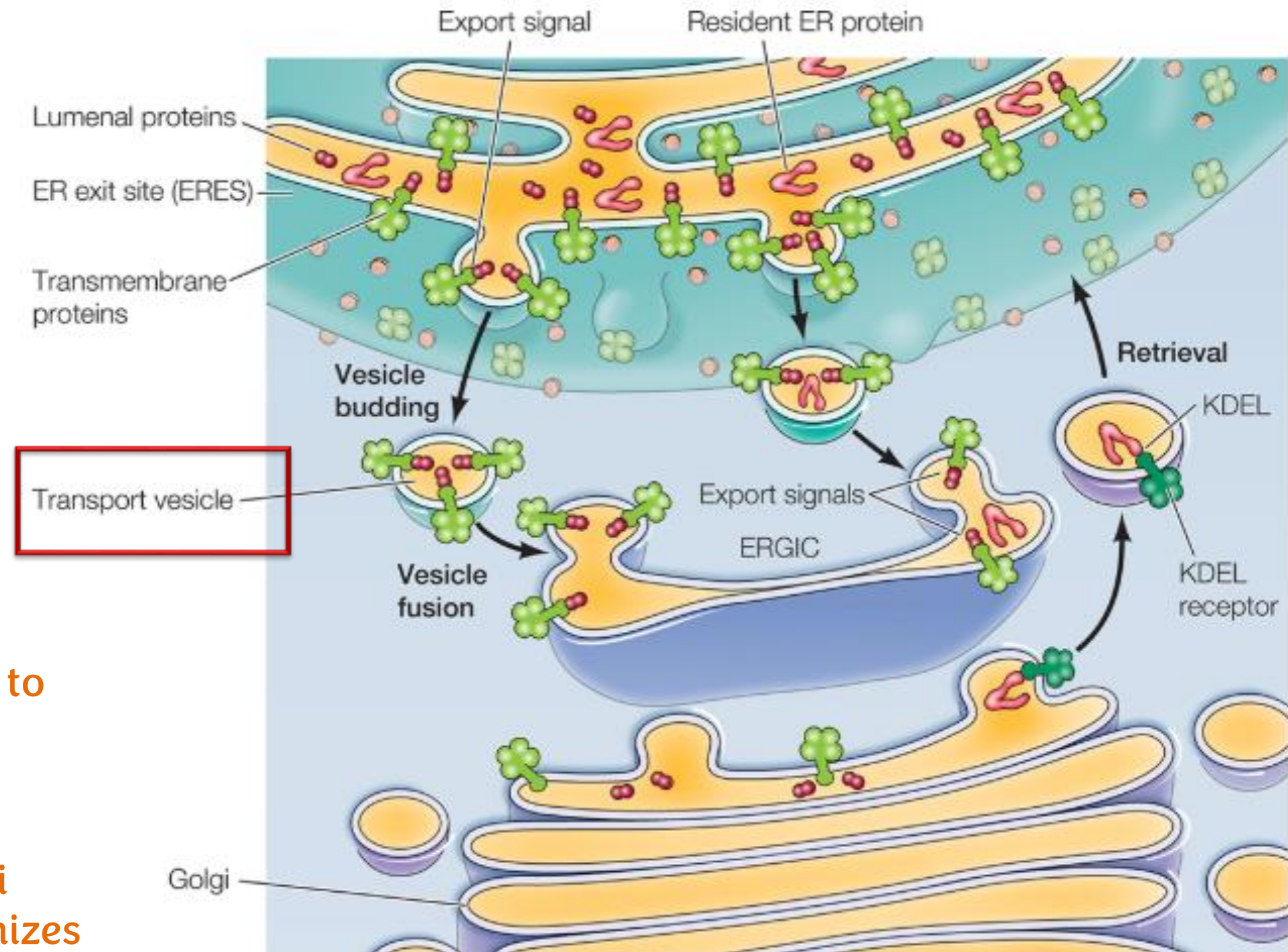
Synthesis of phospholipids in ER

- 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.



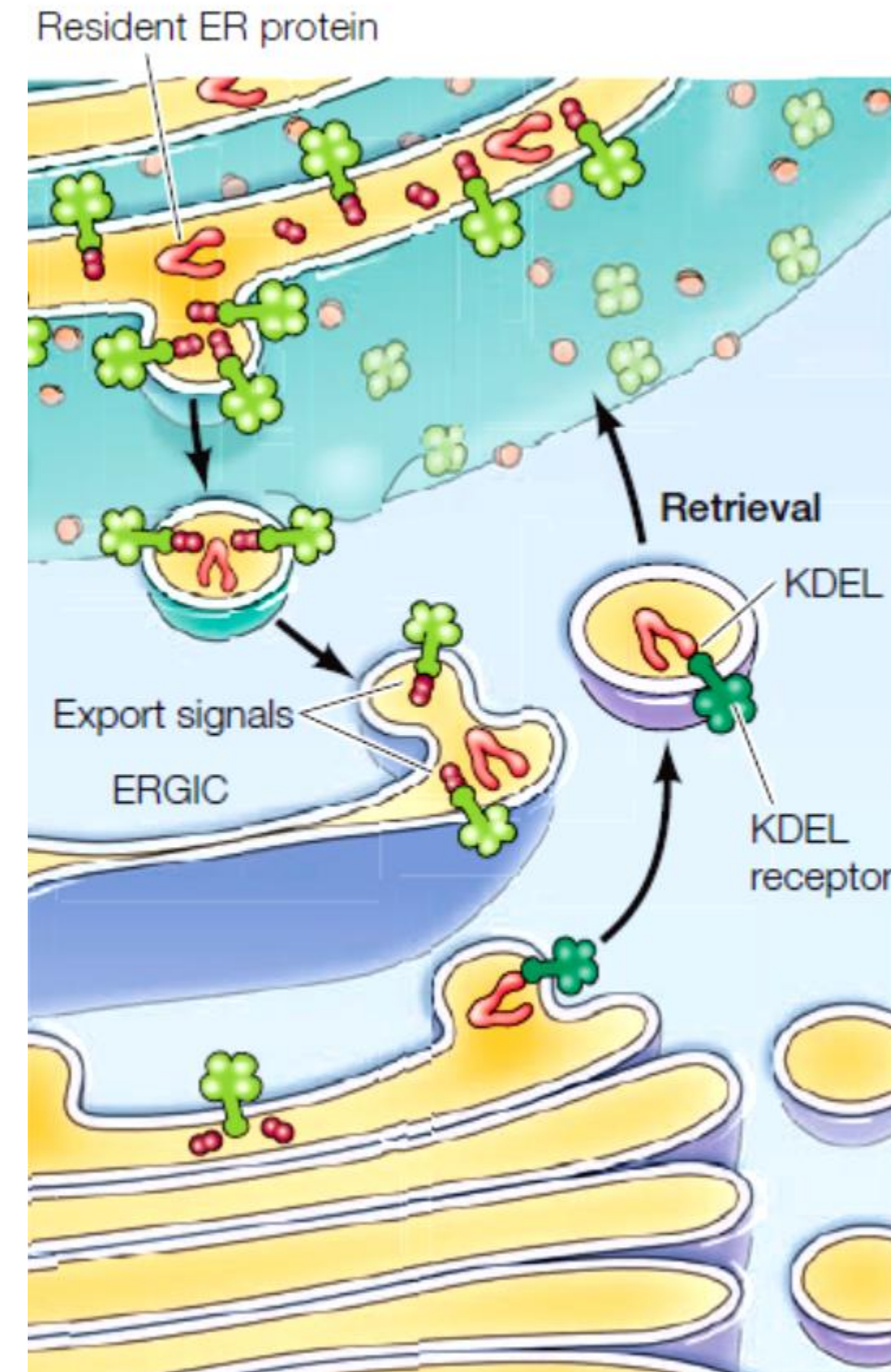
ER-Golgi intermediate compartment (ERGIC)

- 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.
- Some Proteins go from the ER membrane to the nuclear membrane, others go to the Plasmalemma. Some Proteins go from the ER itself to outside the cell (secretion) (explained later on in the course)
- Most proteins are taken from the ER to the Golgi apparatus via transport vesicles. The cell recognizes which protein to retain in the ER by using Retention.



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 to a protein causes this protein to be retained in the ER.



Quiz time



Good luck!!

For any feedback, scan the code or click on it.



Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional resources:

References Used:

Prof. Mamoun Ahram recorded lecture

Extra References for The Reader to Use:

اللَّهُمَّ إِنِّي تَوَكَّلْتُ عَلَيْكَ وَاسَلَمْتُ أَمْرِي إِلَيْكَ ، لَا مَلْجَأَ
وَلَا مَنجَى مِنْكَ إِلَّا إِلَيْكَ اللَّهُمَّ لَا سَهْلَ إِلَّا مَا قَدَّ جَعَلْتَهُ
سَهْلًا ، اللَّهُمَّ اجْعَلِ الصَّعْبَ لِي سَهْلًا ، اللَّهُمَّ ذَكِّرْنِي مِنْهُ
مَا نَسِيتُ وَلَا حَوْلَ وَلَا قُوَّةَ إِلَّا بِاللَّهِ ، لَا إِلَهَ إِلَّا أَنْتَ
سُبْحَانَكَ إِنِّي كُنْتُ مِنَ الظَّالِمِينَ ، يَا حَيُّ يَا قَيُّوْمُ بِرَحْمَتِكَ
أَسْتَغِيثُ أَنْ يَمْسُنِي الضَّرَرُ

رسالة من الفريق العلمي: