Lecture 1: Introduction & endoplasmic reticulum

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Second year, First semester, 2024-2025

Me!

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- Come in groups

Course outline (1)

Focus on diseases

- Introduction and biomembranes
- Endoplasmic reticulum and protein sorting
- Golgi apparatus
- Vesicular network
- Mitochondria and mitochondrial diseases
- Peroxisomes
- The nucleus
- Cytoskeletal networks
- The extracellular network
- Cell signaling, proliferation, differentiation, and death
- Cancer cells

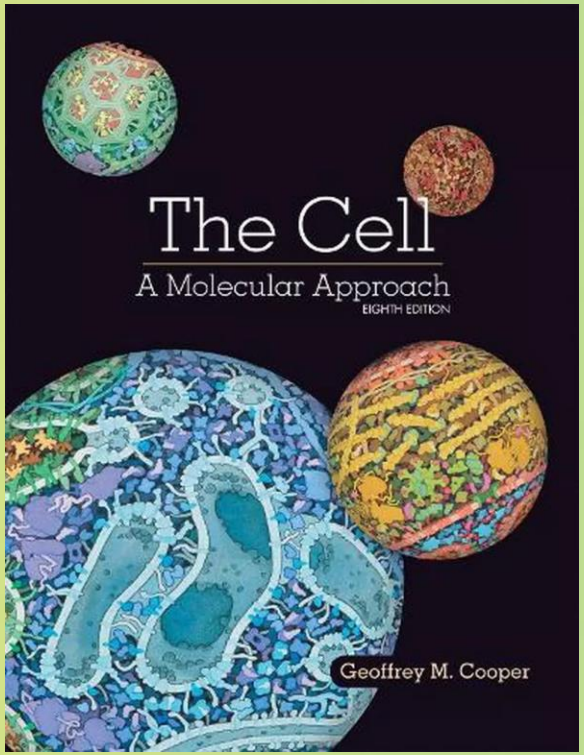
Course outline (2)

Focus on processes and techniques

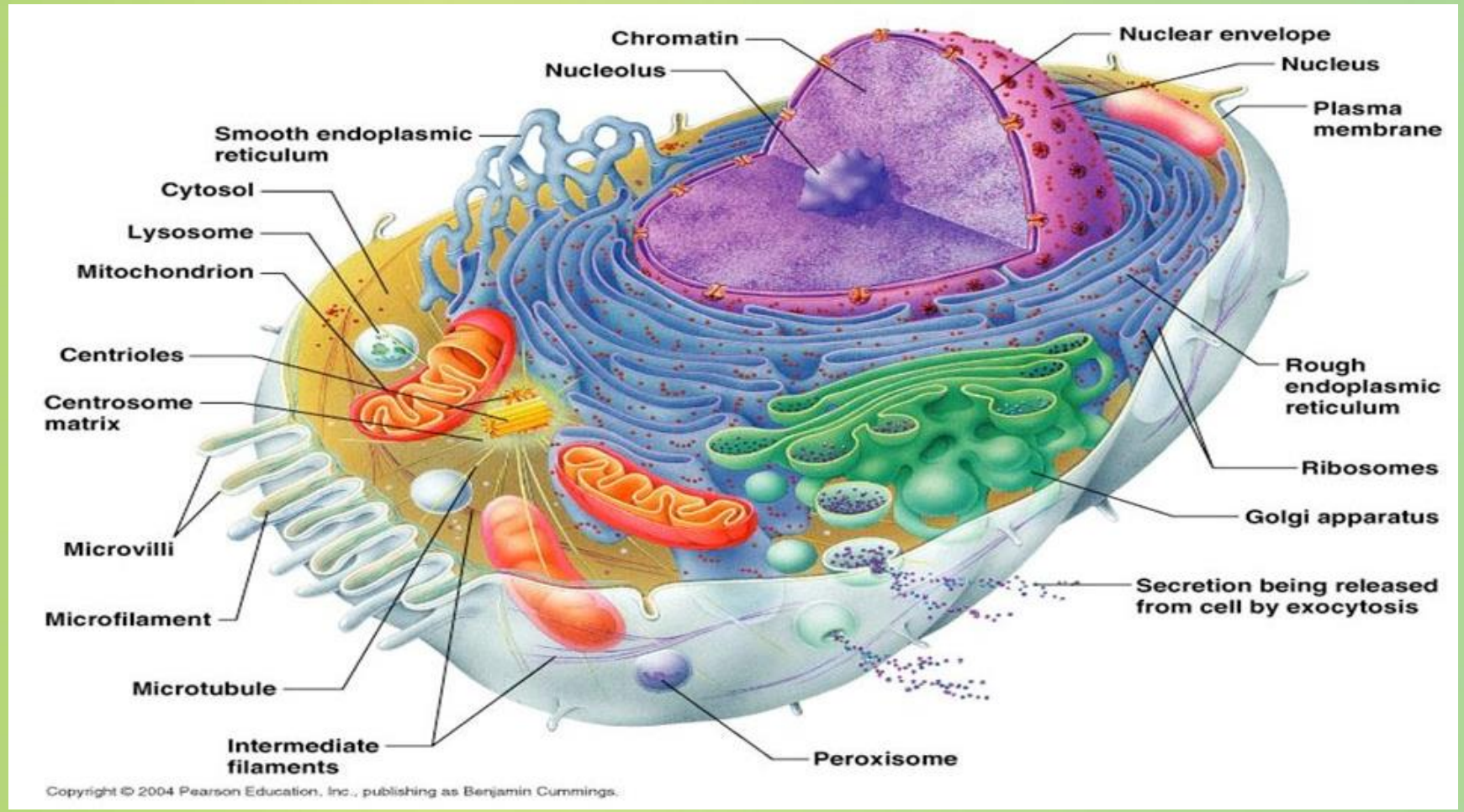
- Introduction and the central dogma of molecular biology
- Gel electrophoresis, restriction endonucleases, recombinant DNA technology, DNA cloning, and RFLP
- The utilization of denaturation/renaturation concepts
  - Dot blotting and Southern blotting
- DNA replication
- PCR and DNA sequencing
- The human genome
- Transcription, mechanisms of regulation, and epigenetics
  - Coding and non-coding RNAs
- RNA detection, quantification, and detection
- Translation
- Yeast two-hybrid system
- DNA mutations
- DNA repair and CRISPR-Cas9

The textbook

- **The Cell: A Molecular Approach 8th Edition** by Geoffrey Cooper, Sinauer Associates is an imprint of Oxford University Press.



The cell


Chromatin
Nuclear envelope
Nucleolus
Nucleus
Plasma membrane
Smooth endoplasmic reticulum
Cytosol
Lysosome
Mitochondrion
Centrioles
Centrosome matrix
Rough endoplasmic reticulum
Ribosomes
Golgi apparatus
Microvilli
Secretion being released from cell by exocytosis
Microfilament
Microtubule
Intermediate filaments
Peroxisome
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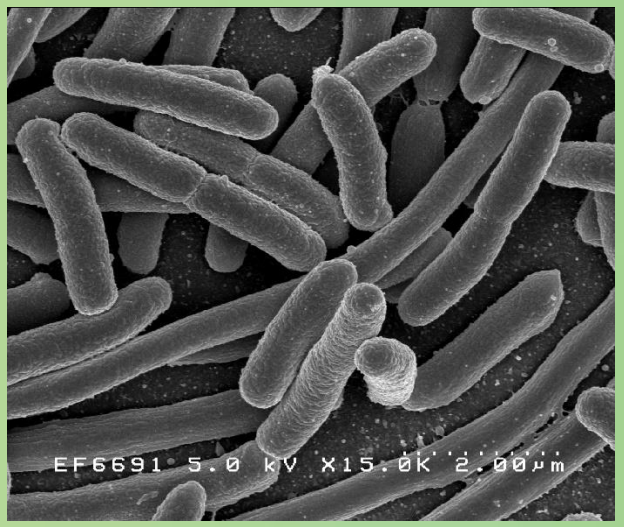
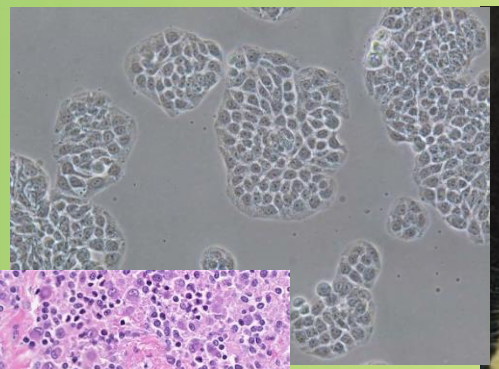
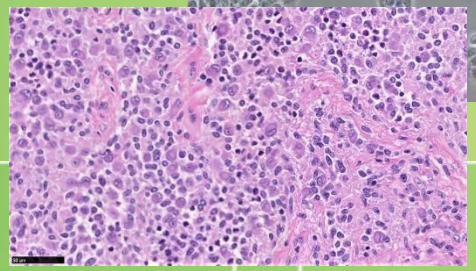
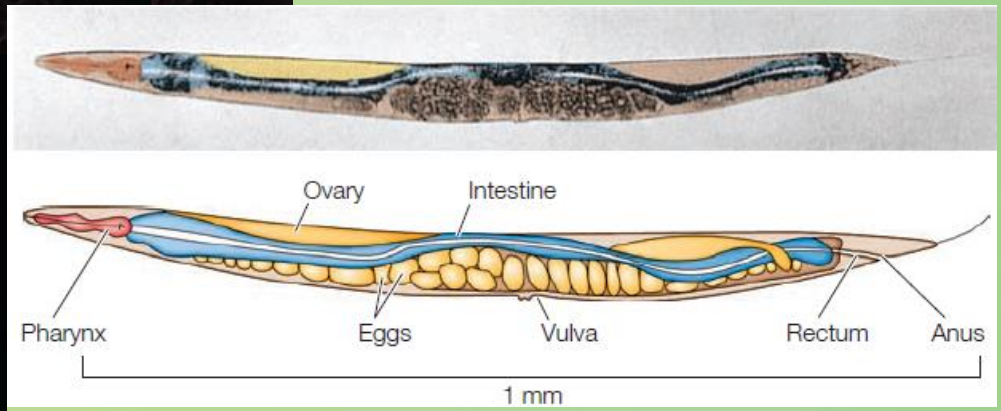
What organisms do we use to study cells?

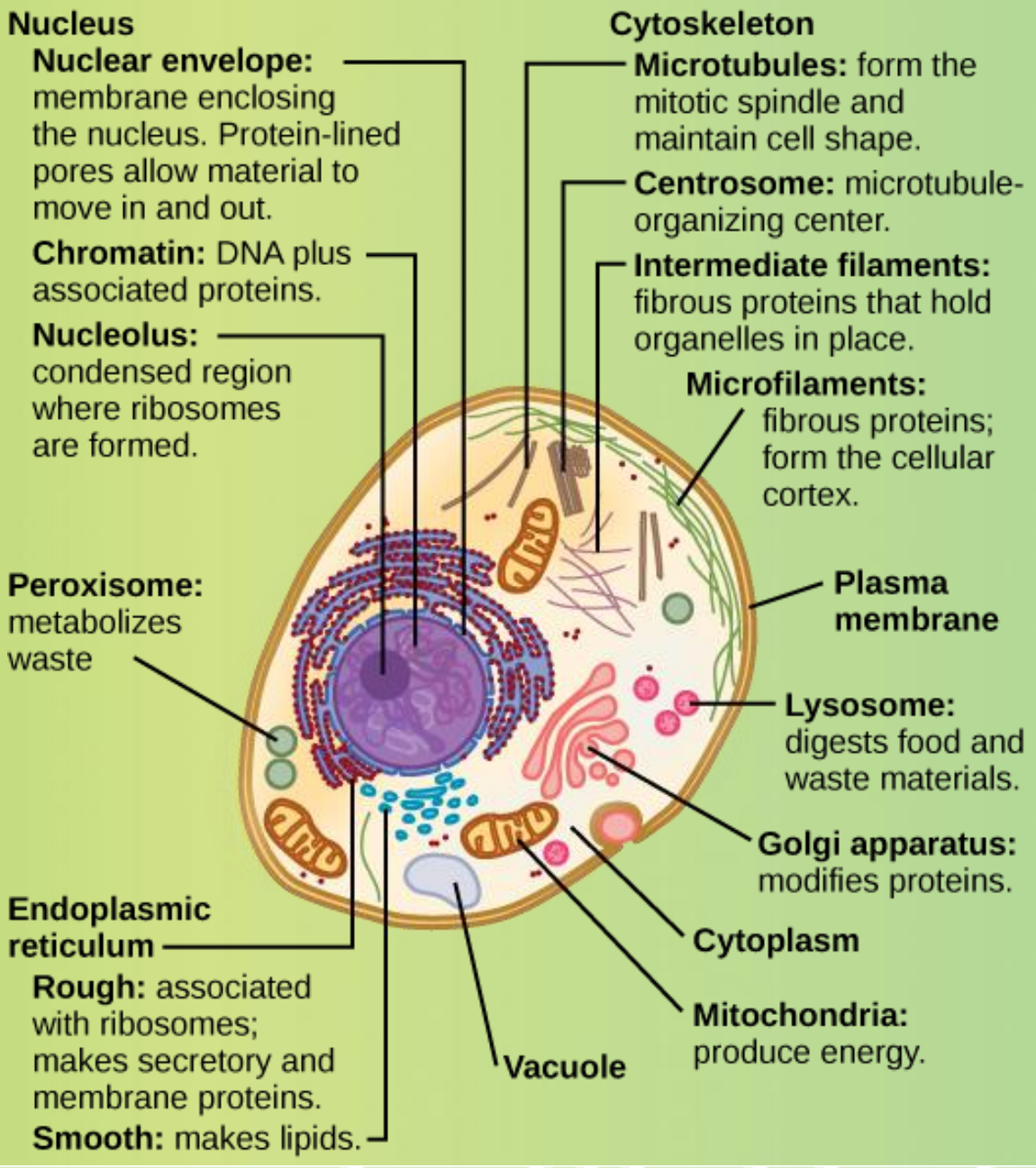
- Escherichia coli (E. coli)
- Yeast (Saccharomyces cerevisiae)
- Caenorhabditis elegans
- Drosophila melanogaster
- Mice
- Cultured cells and tissues










Nucleus
Nuclear envelope: membrane enclosing the nucleus. Protein-lined pores allow material to move in and out.
Chromatin: DNA plus associated proteins.
Nucleolus: condensed region where ribosomes are formed.
Peroxisome: metabolizes waste
Endoplasmic reticulum
Rough: associated with ribosomes; makes secretory and membrane proteins.
Smooth: makes lipids.
Cytoskeleton
Microtubules: form the mitotic spindle and maintain cell shape.
Centrosome: microtubule-organizing center.
Intermediate filaments: fibrous proteins that hold organelles in place.
Microfilaments: fibrous proteins; form the cellular cortex.
Plasma membrane
Lysosome: digests food and waste materials.
Golgi apparatus: modifies proteins.
Cytoplasm
Mitochondria: produce energy.
Vacuole

Organelles

TABLE 4-2 *Organelles*

Organelle	Function
Mitochondrion	transfers energy from organic compounds to ATP
Ribosome	organizes the synthesis of proteins
Endoplasmic reticulum (ER)	prepares proteins for export (rough ER); synthesizes steroids, regulates calcium levels, breaks down toxic substances (smooth ER)
Golgi apparatus	processes and packages substances produced by the cell
Lysosome	digests molecules, old organelles, and foreign substances
Microfilaments and microtubules	contribute to the support, movement, and division of cells
Cilia and flagella	propel cells through the environment; move materials over the cell surface
Nucleus	stores hereditary information in DNA; synthesizes RNA and ribosomes
Cell wall*	supports and protects the cell
Vacuole*	stores enzymes and waste products
Plastid*	stores food or pigments; one type (chloroplast) transfers energy from light to organic compounds

*Cell walls, large vacuoles, and plastids are found in the cells of plants and some other eukaryotes, but not in the cells of animals.

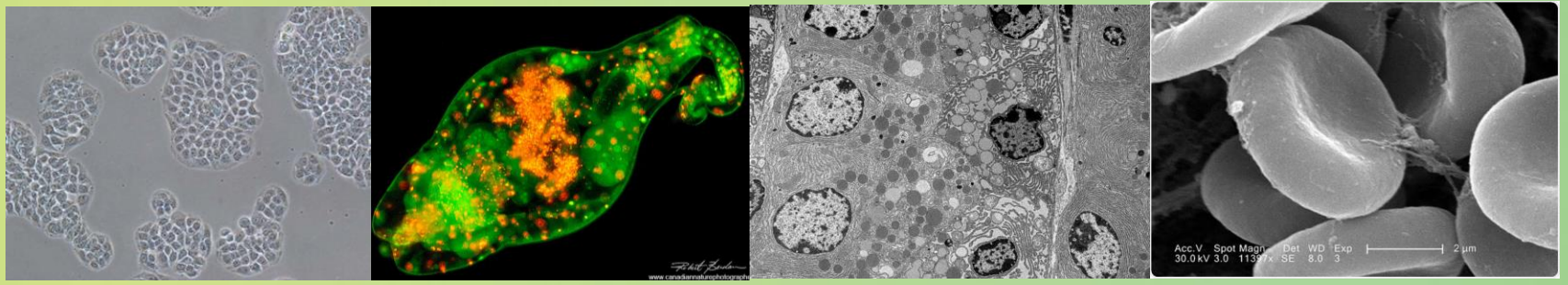
Major molecular components of cells

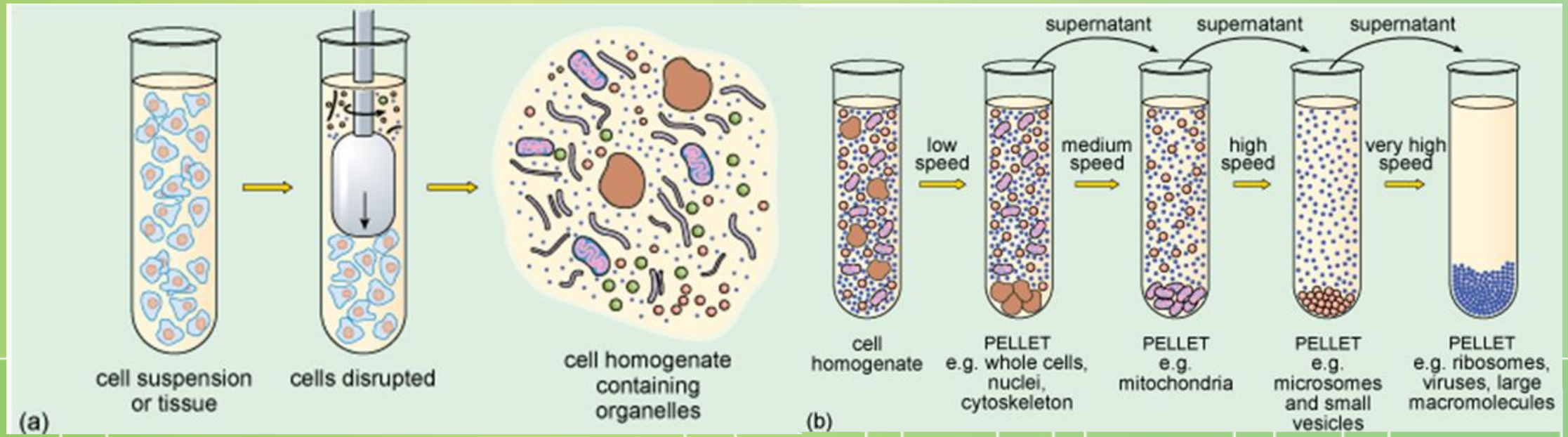
- Nucleic acids
- Carbohydrates
- Proteins
- Lipids (50% of mass of plasma membranes, 30% of mitochondrial membranes)

- Molecules function by interacting with each non-covalently.

How do we study cell components?
Cell and protein detection

- Microscopy
  - Light, fluorescence (immunofluorescence), electron, scanning electron
- Cell fractionation





Biochemical composition of plasma membranes

Membrane	Protein (%)	Lipid (%)	Carbohydrate (%)	Weight fraction of protein	Ratio of protein to lipid
Plasma membranes					
Myelin	18	79	3	0.18	0.23
Blood platelets	33—42	51—58	7.5	0.4	0.7
Mouse liver cells	46	54	2—4	0.46	0.85
Human erythrocytes	49	43	8	0.49	1.1
Amoeba	54	42	4	0.54	1.3
Rat liver cells	58	42	(5—10)*	0.58	1.4
HeLa cells	60	40	2.4	0.6	1.5
Nuclear envelope of rat liver cells	59	35	2.9	0.59	1.6
Retinal rods, bovine	51	49	4	0.51	1.0
Mitochondrial outer membrane	52	48	(2—4)*	0.52	1.1
Sarcoplasmic reticulum	67	33	—	0.67	2.0
Chloroplast lamellae, spinach	70	30	(6)*	0.7	2.3
Mitochondrial inner membrane	76	24	(1—2)*	0.76	32
Gram-positive bacteria	75	25	(10)*	0.75	3.0
Halobacterium purple membrane	75	25		0.75	3.0

Lipid composition of organelles

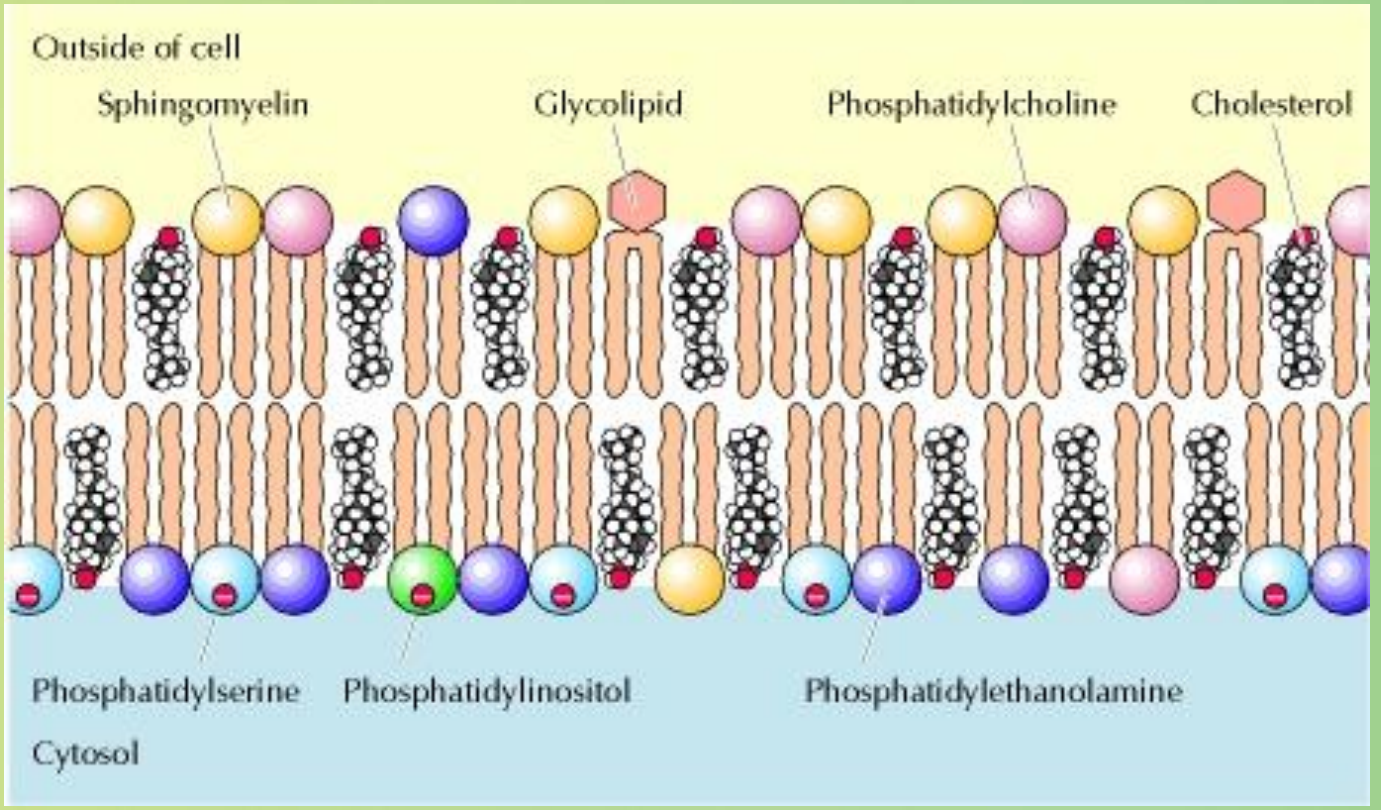
Table 1: *Head group composition of the membranes of some mammalian liver cells, erythrocytes , and nerve cells in weight percent. Adapted from Jamieson and Robinson (1977). Abbreviations: PC = phosphatidylcholines, PE = phosphatidylethanolamines, PS = phosphatidylserines, PI = phosphatidylinositols, SM = sphingomyelin, CL = cardiolipin.*

Membrane	PC	PE	PS	PI	SM	CL	Glycolipid	Cholesterol	Others
Erythrocyte (human)	20	18	7	3	18	–	3	20	11
Plasma (rat liver)	18	12	7	3	12	–	8	19	21
ER	48	19	4	8	5	–	tr	6	10
Golgi	25	9	3	5	7	–	0	8	43
Lysosome	23	13	–	6	23	≈ 5	–	14	16
Nuclear membrane	44	17	4	6	3	1	tr	10	15
Mitochondria	38	29	0	3	0	14	tr	3	13
Neurons	48	21	5	7	4	–	3	11	1
Myelin	11	17	9	1	8	–	20	28	6

Cholesterol is an essential component of animal plasma membranes.
It is not present in bacteria and plant cells, but the latter cells contain sterols.

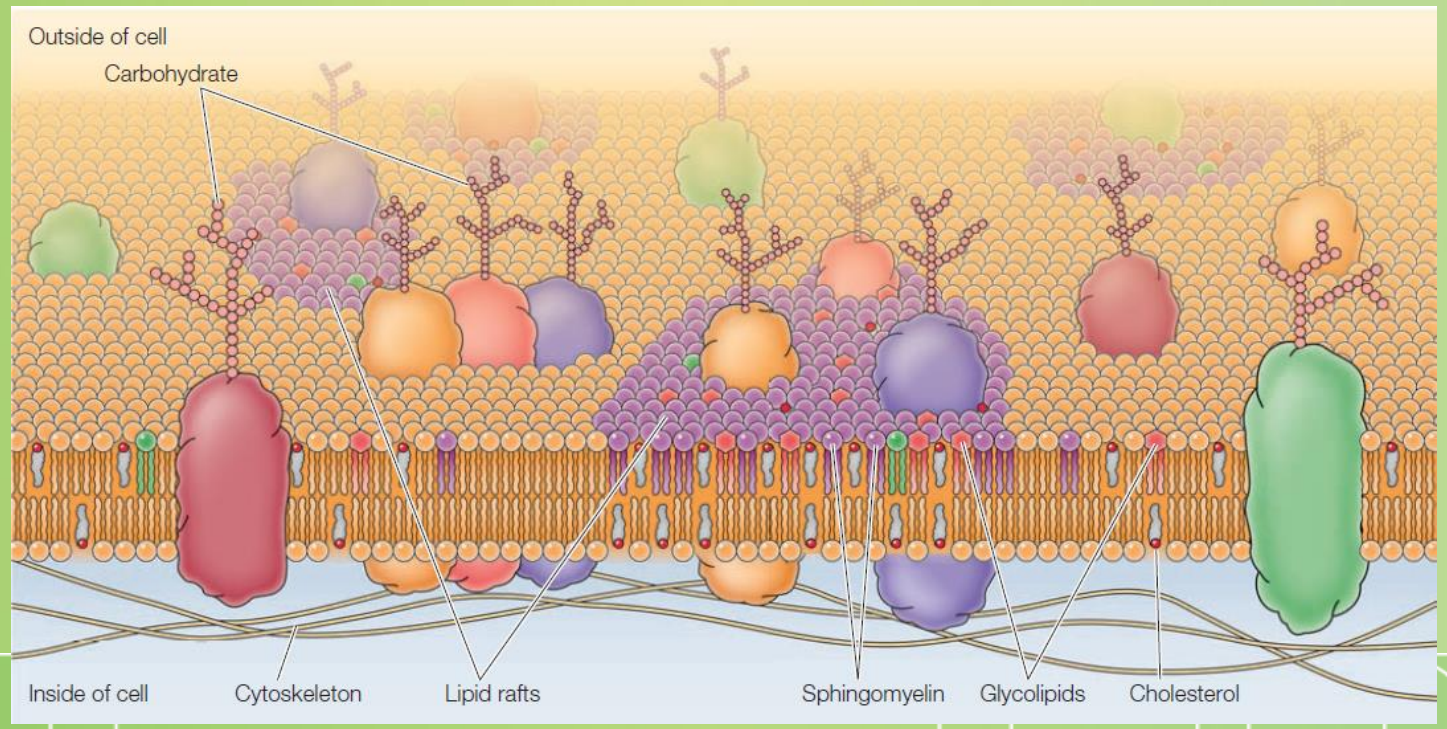
Composition and properties of plasma membranes

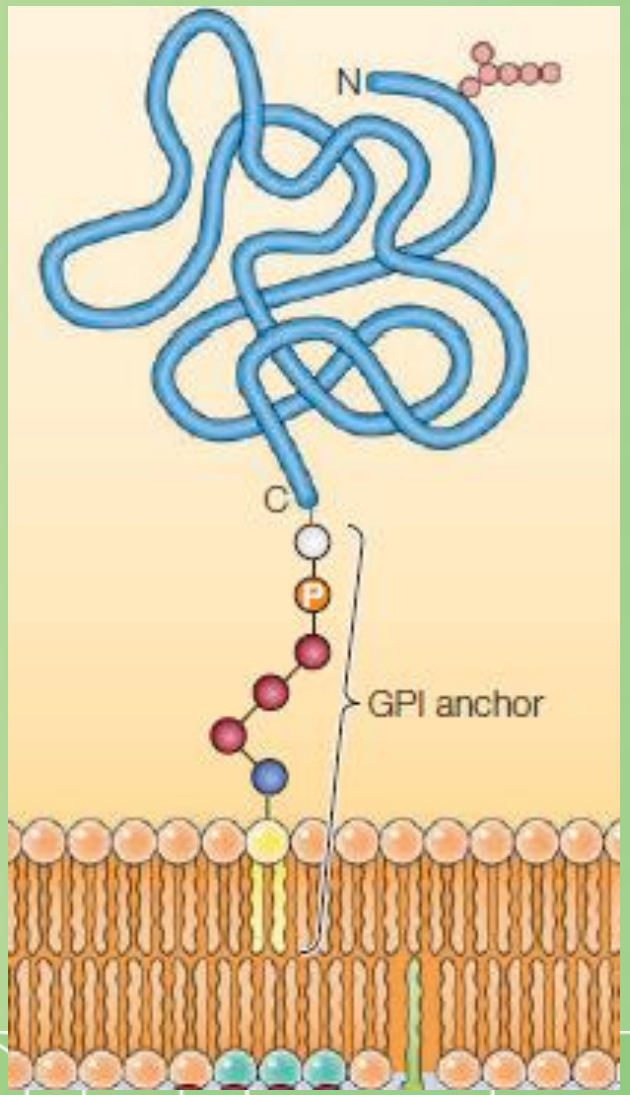
- The phospholipids are asymmetrically distributed between the two halves of the membrane bilayer.
  - The outer leaflet: Ⓟcholine, sphingomyelin
  - The inner leaflet:Ⓟethanolamine, Ⓟserine, Ⓟinositol (minor)
    - Ⓟinositol has a role in cell signaling.
  - Glycolipids are found exclusively on the outer 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.

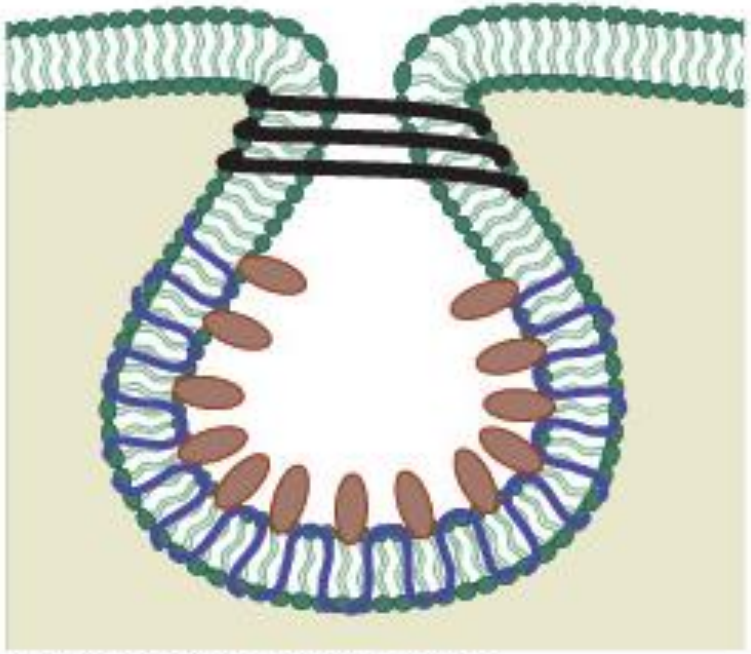
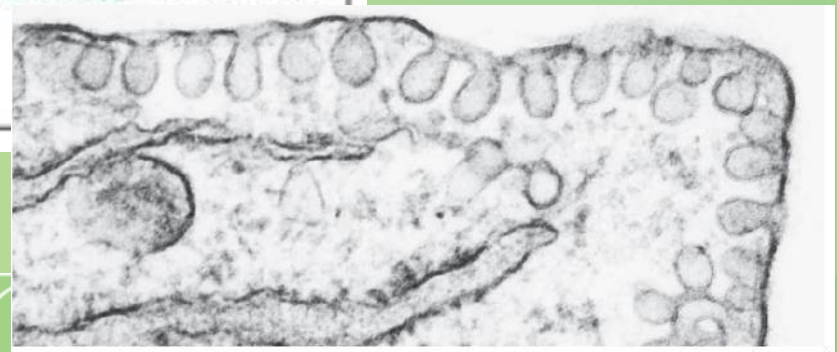
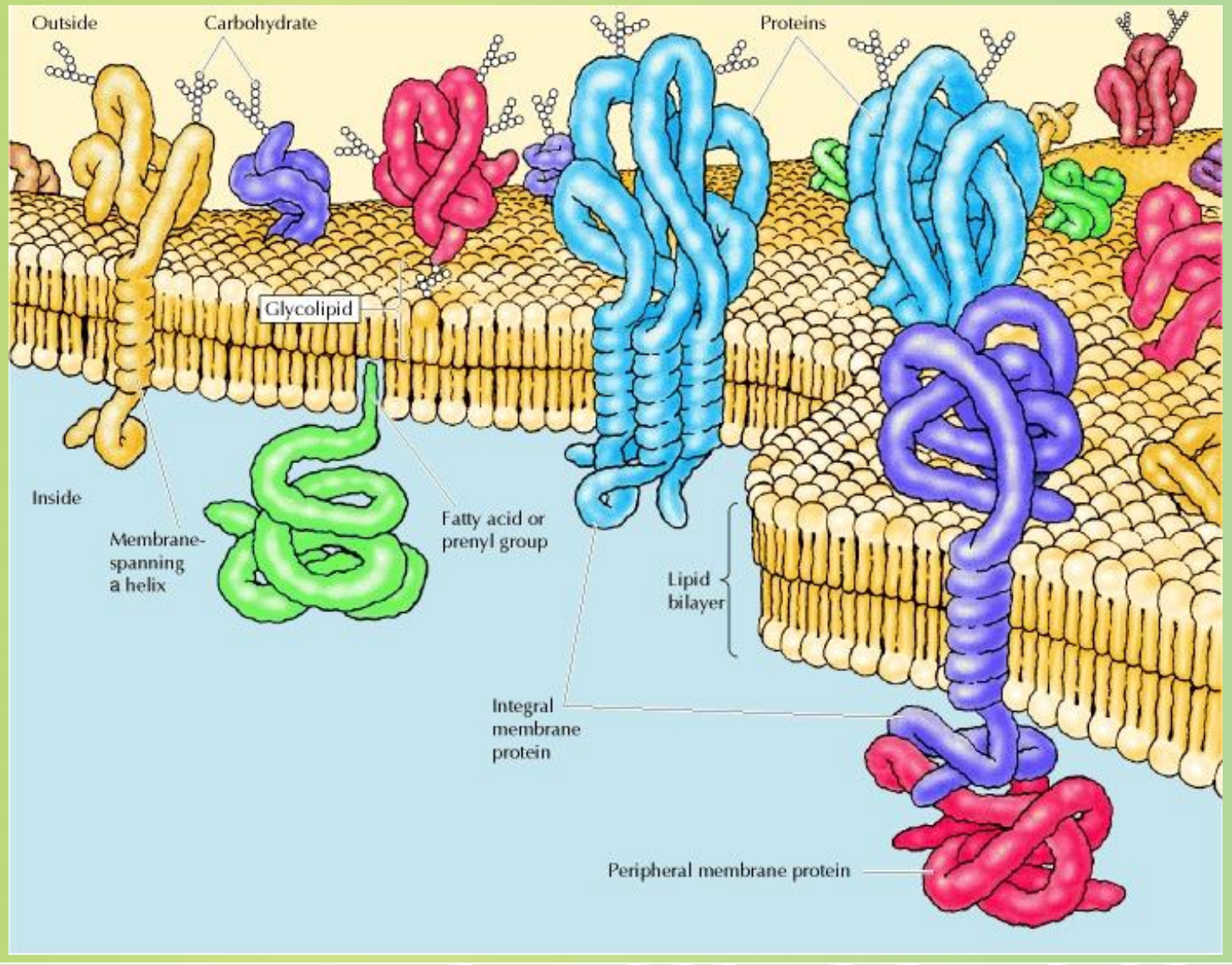


Fig. Simplified model of the Caveolae

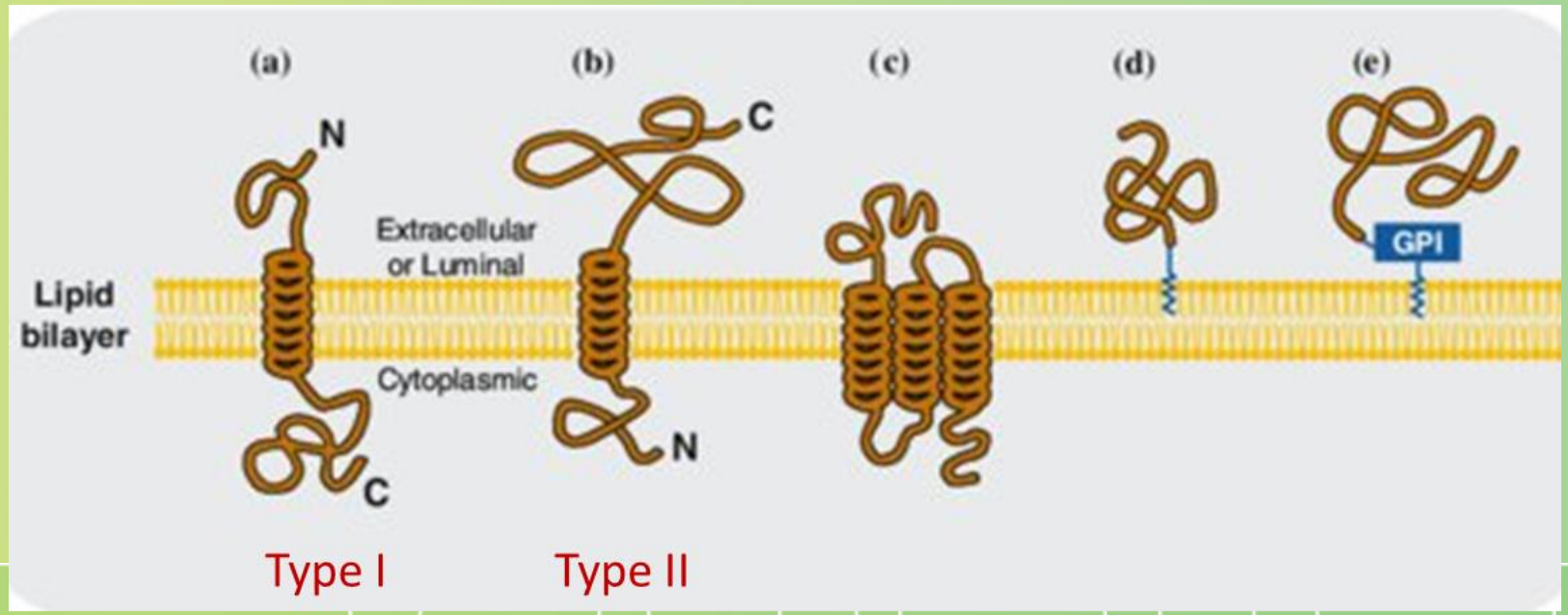


Membrane proteins



Types of membrane proteins

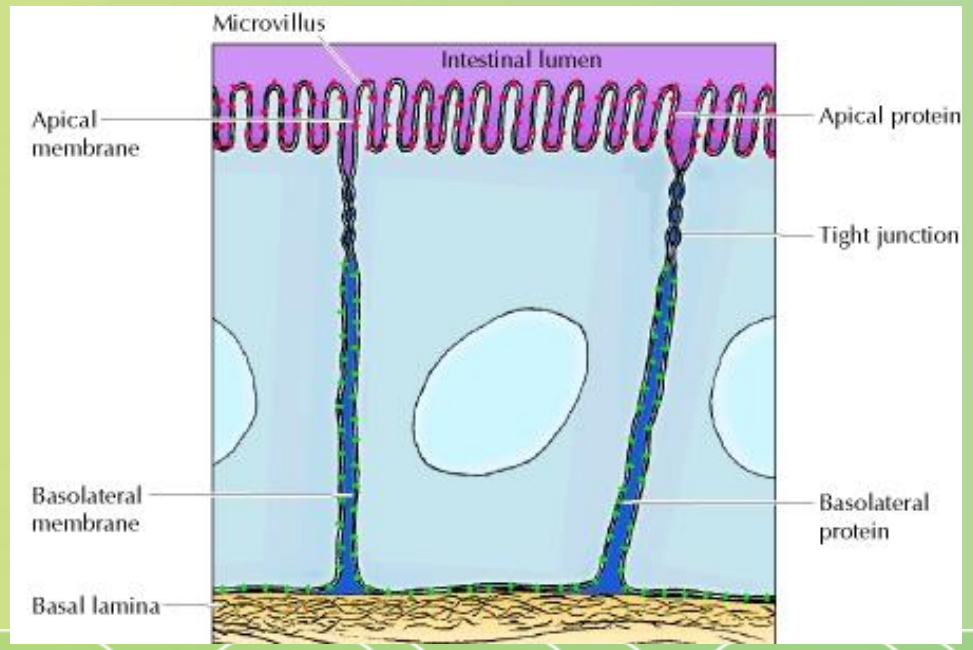
- Peripheral membrane proteins are indirectly and loosely associated with membranes through protein-protein interactions, mainly ionic bonds.
- Integral membrane proteins have some of their helical parts inserted into the lipid bilayer.
  - Single-pass (type I or II) or multi-pass proteins.
- Lipid-anchored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

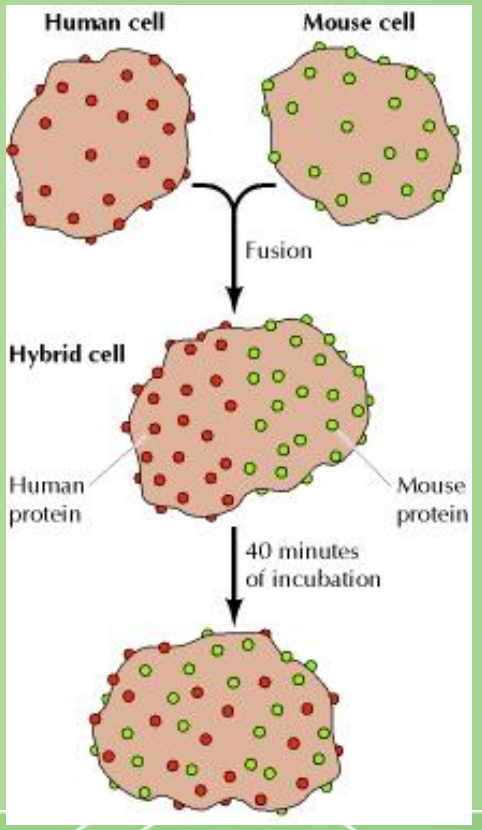


Protein mobility

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

- The mobility of membrane proteins is restricted by
  - Their association with the cytoskeleton
  - Specific membrane domains, which maintain the specific distribution of apical and basolateral proteins
  - Specific lipid composition (e.g. lipid rafts).



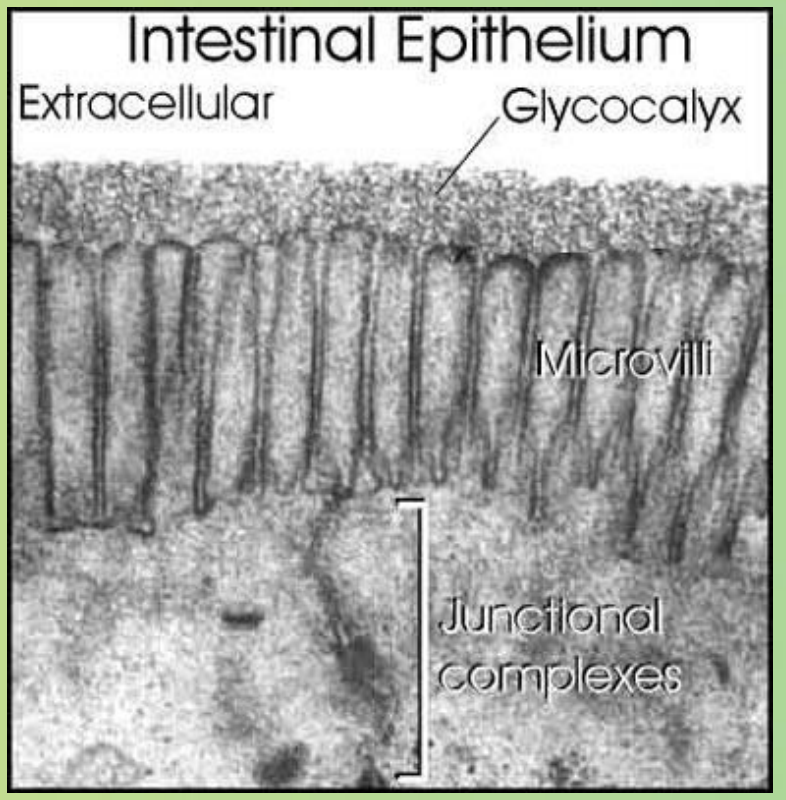


Glycocalyx

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

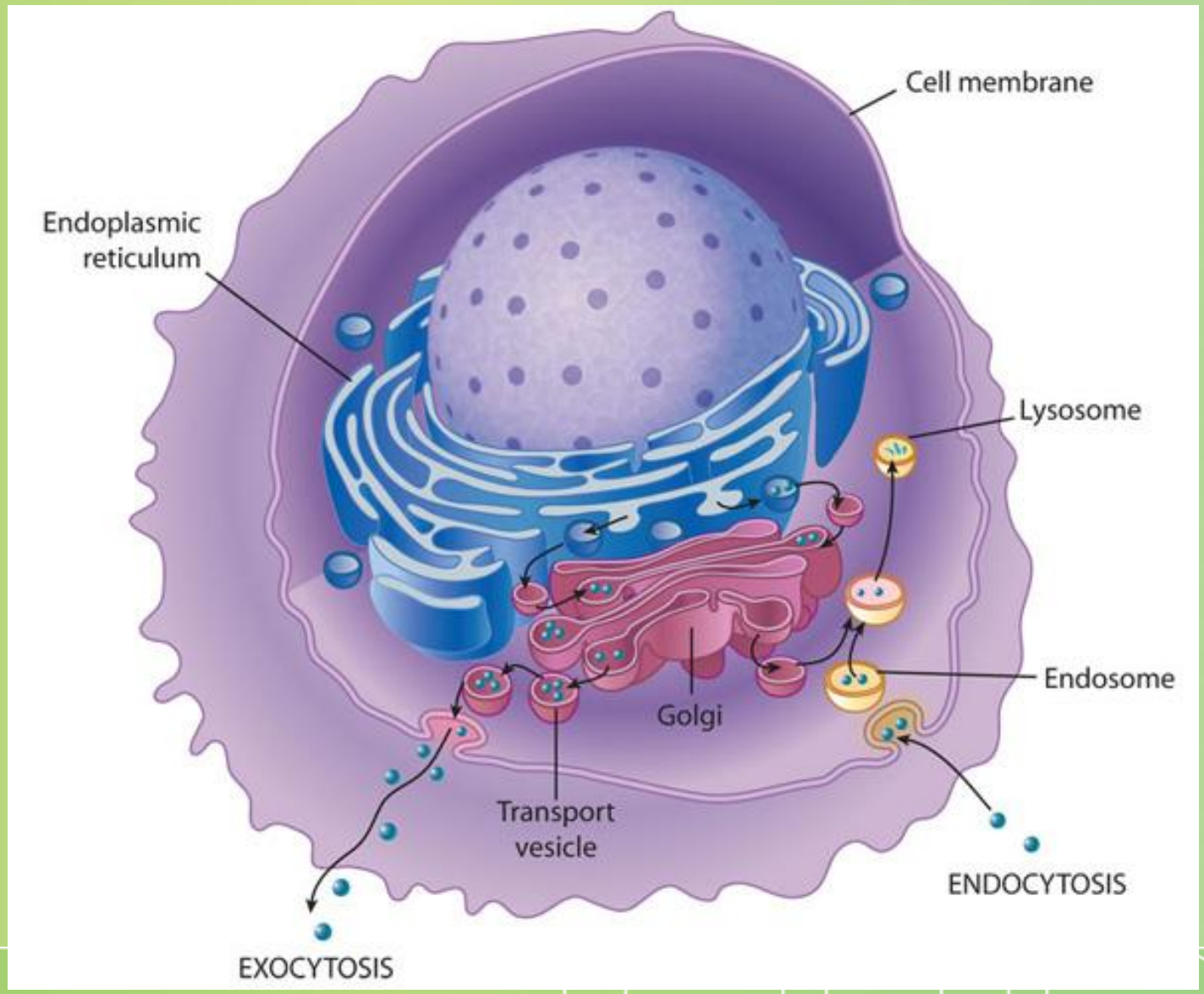
Functions:

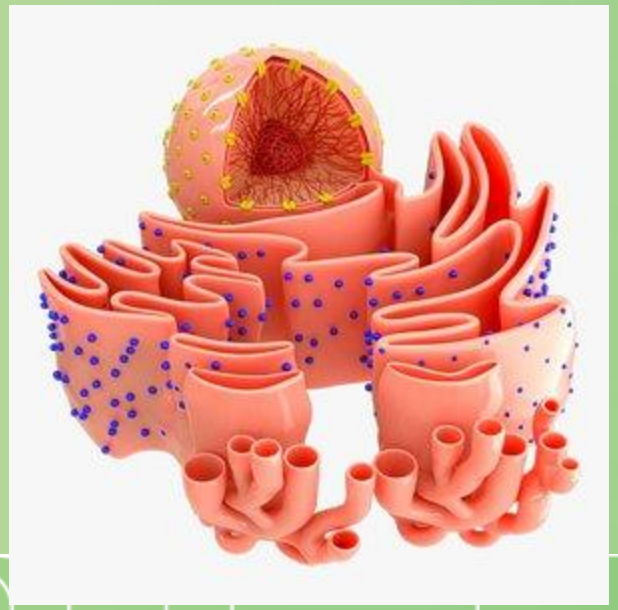
- Cell-cell interactions such as immune cells
- Protection of cell surface from ionic and mechanical stress
- Formation of a barrier for microorganisms



Protein sorting (endoplasmic reticulum)

An overview

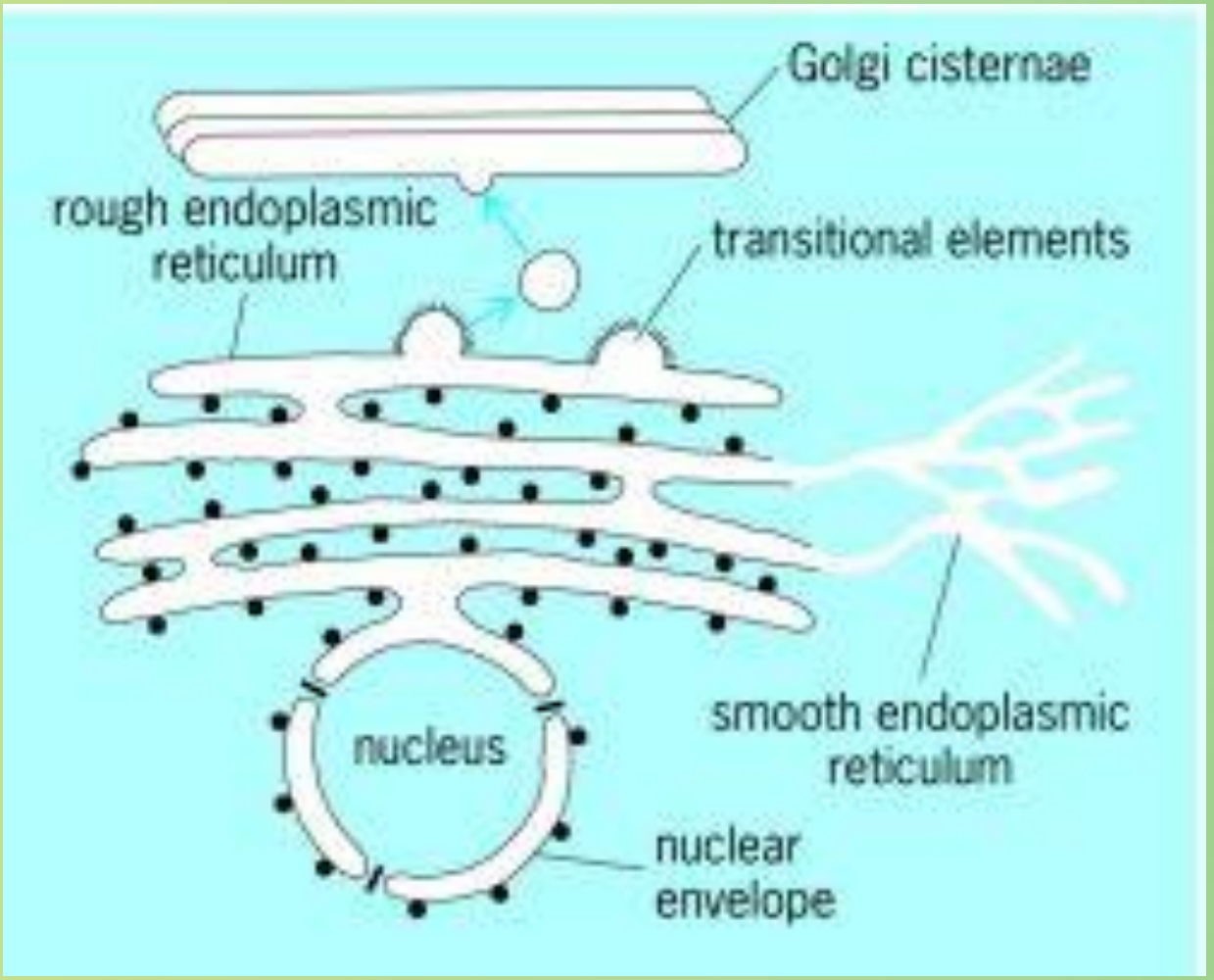

Cell membrane
Endoplasmic reticulum
Lysosome
Endosome
Golgi
Transport vesicle
ENDOCYTOSIS
EXOCYTOSIS



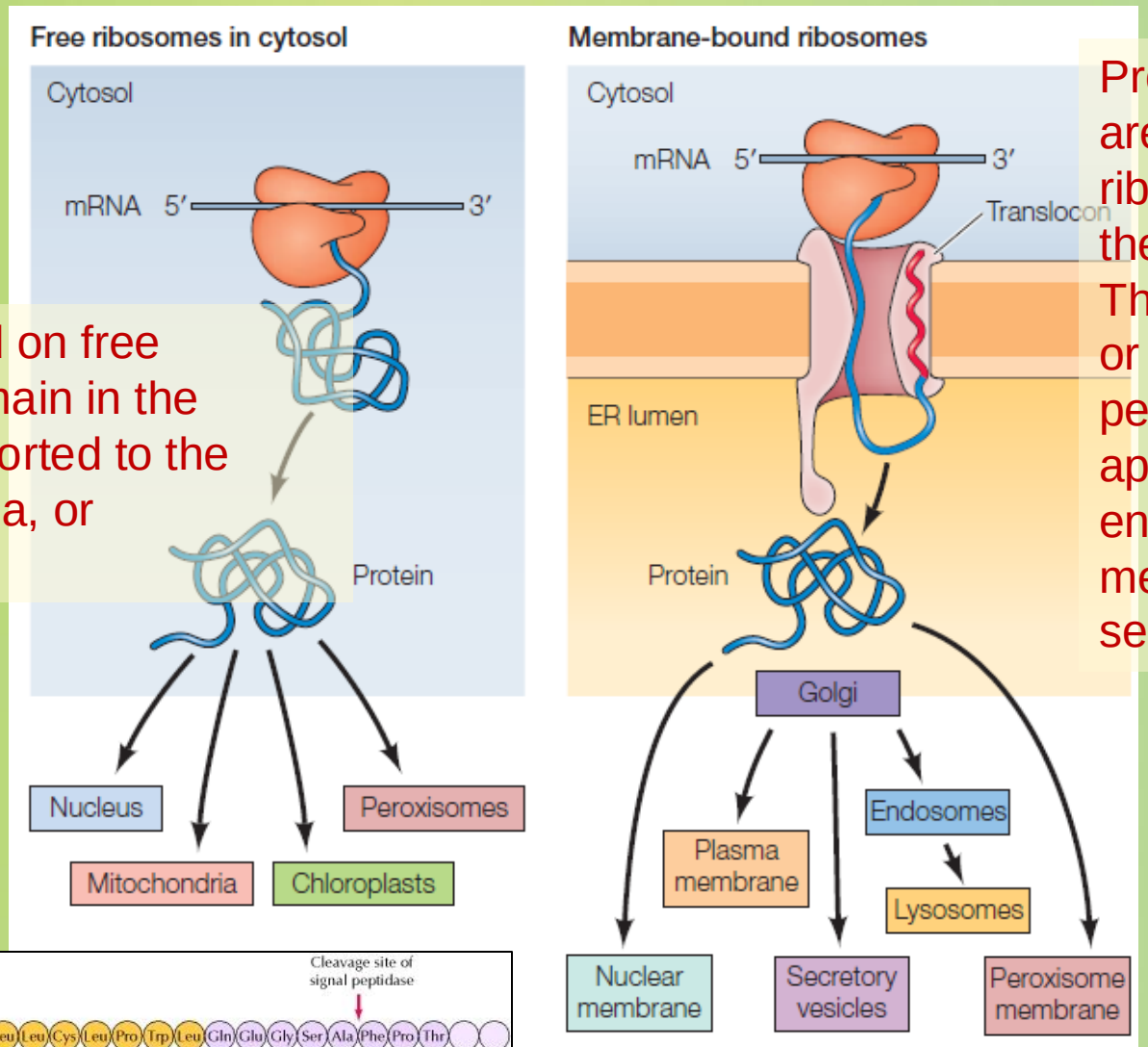
Endoplasmic reticulum (ER)

- 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.

- 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



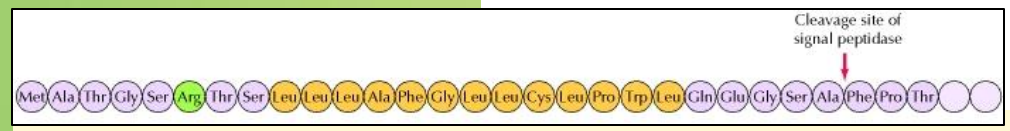
Protein sorting



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.

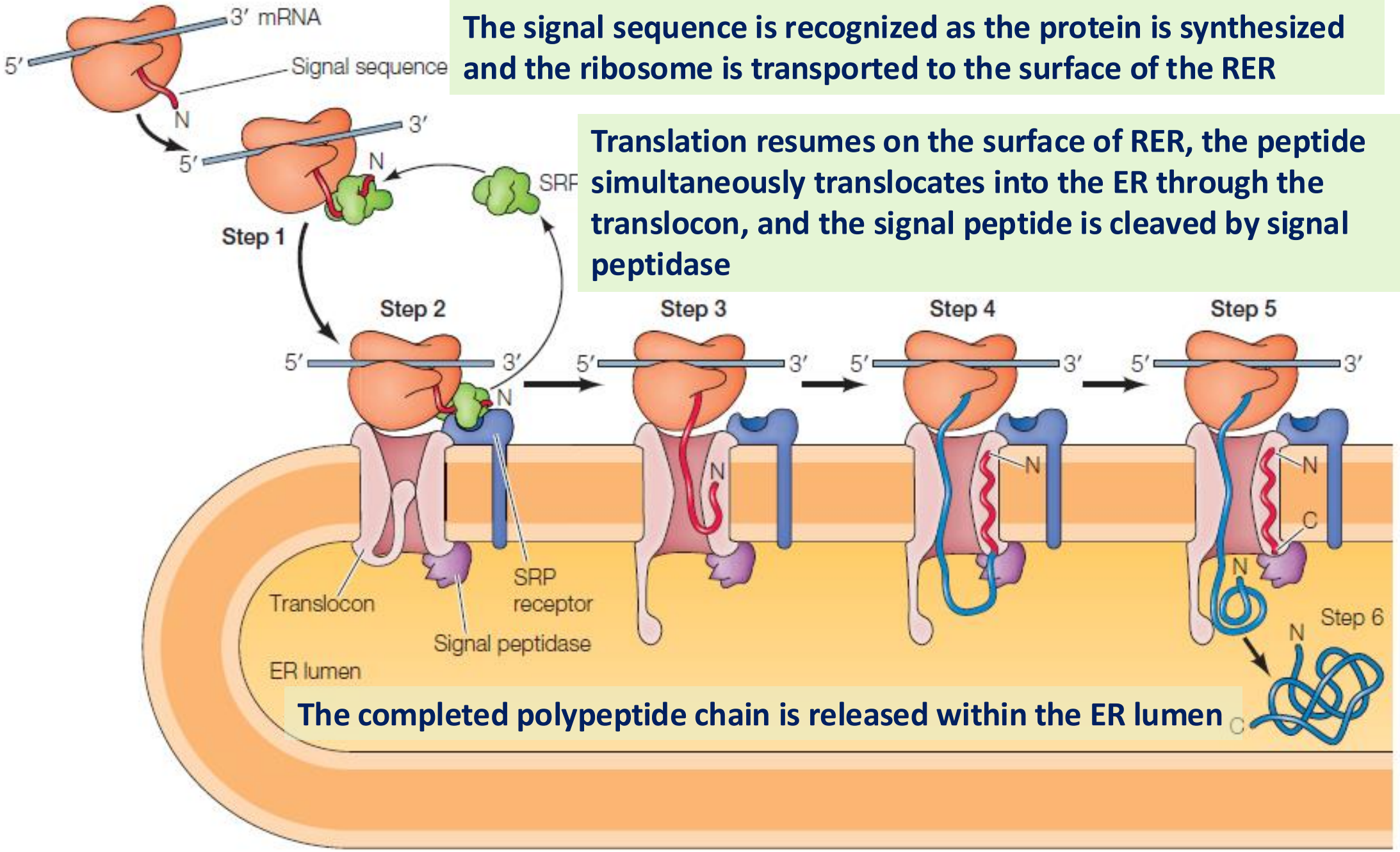
In cell biology, a lumen is a membrane-defined space that is found inside several organelles, cellular components, or structures



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.

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

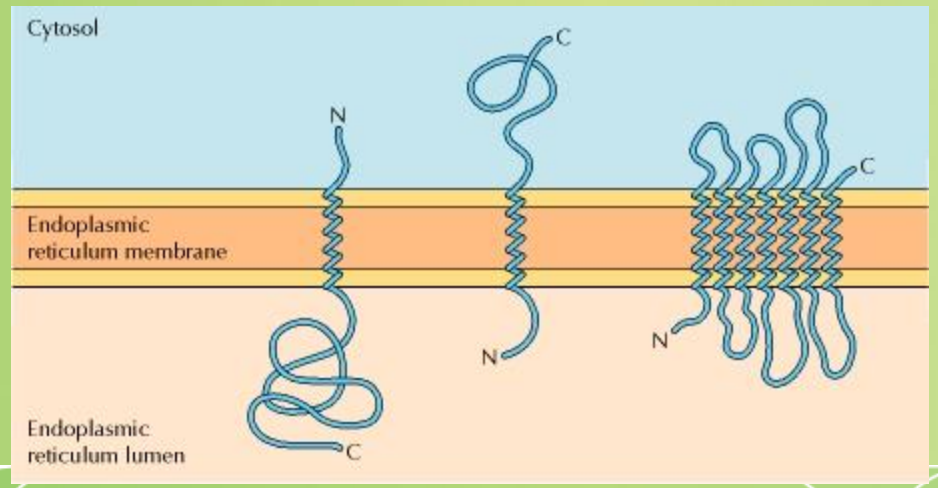
Translation resumes on the surface of RER, the peptide simultaneously translocates into the ER through the translocon, and the signal peptide is cleaved by signal peptidase



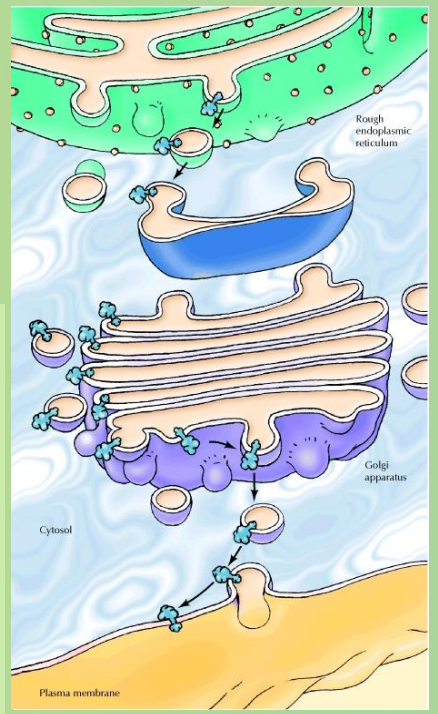
The completed polypeptide chain is released within the ER lumen

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

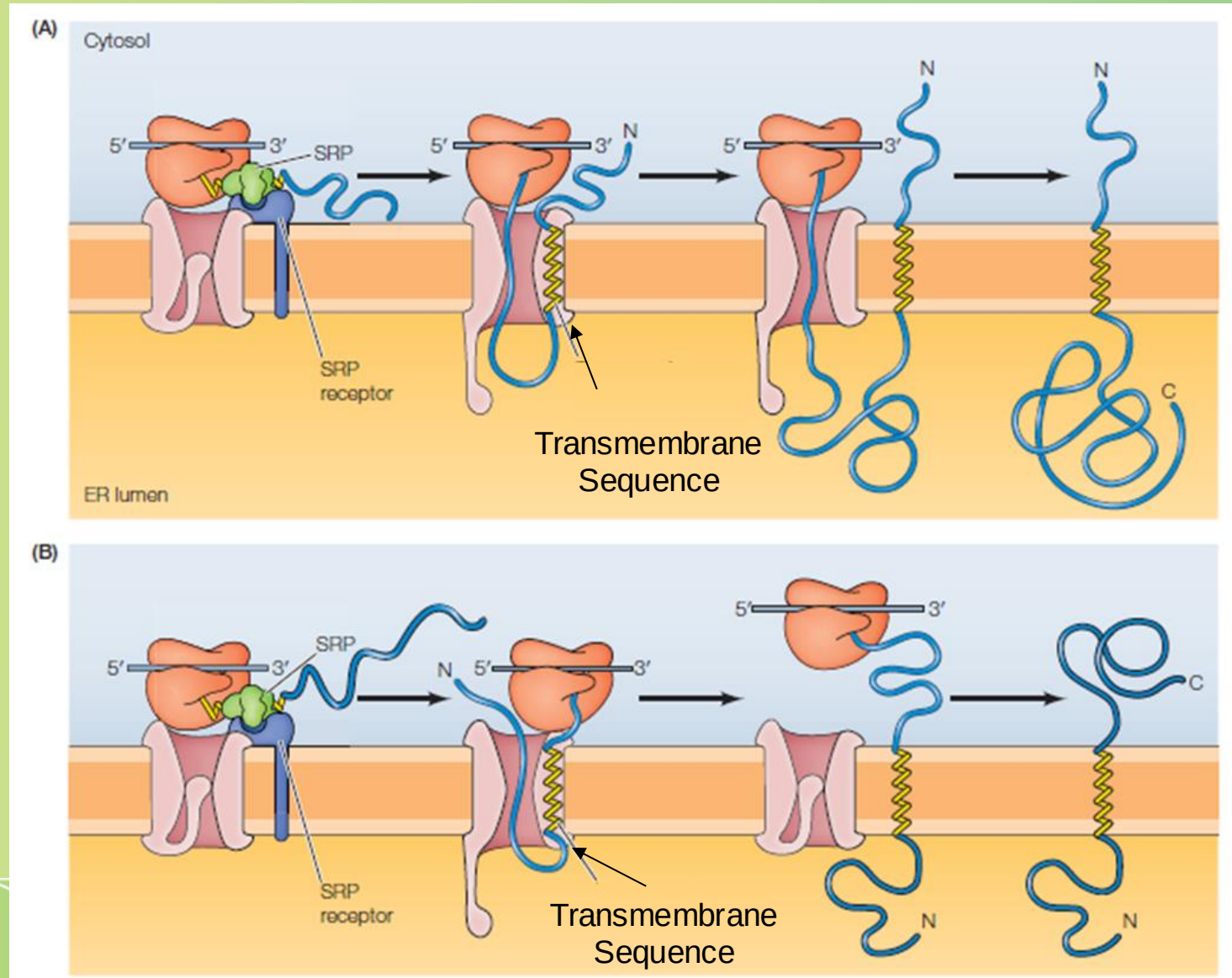


The lumens of the ER and Golgi apparatus are topologically equivalent to the exterior of the cell.

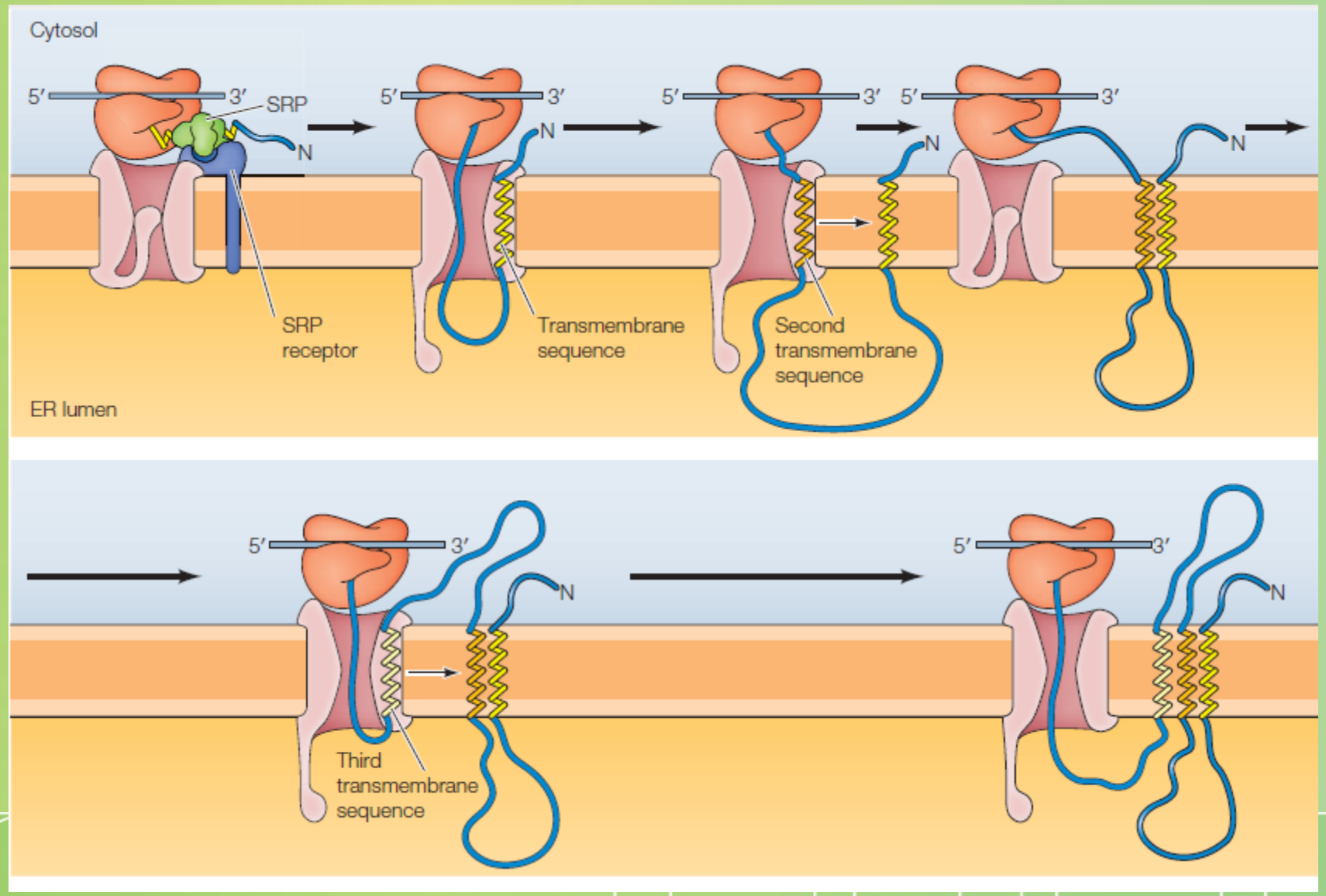


Insertion of membrane proteins via internal transmembrane sequences

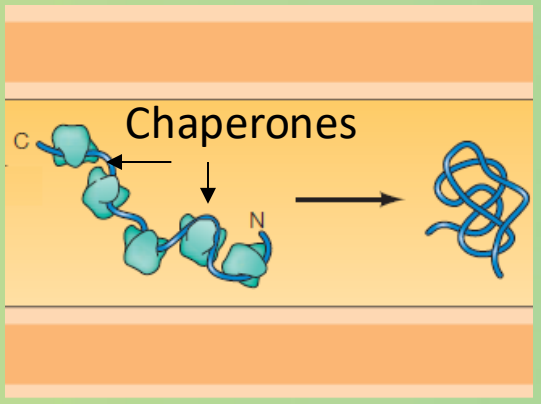
- Translocation of the polypeptide chain stops when the translocon recognizes a transmembrane sequence allowing the protein to become anchored in the ER membrane.
- The direction of the internal transmembrane sequence determines the direction of insertion and orientation of the protein ends.



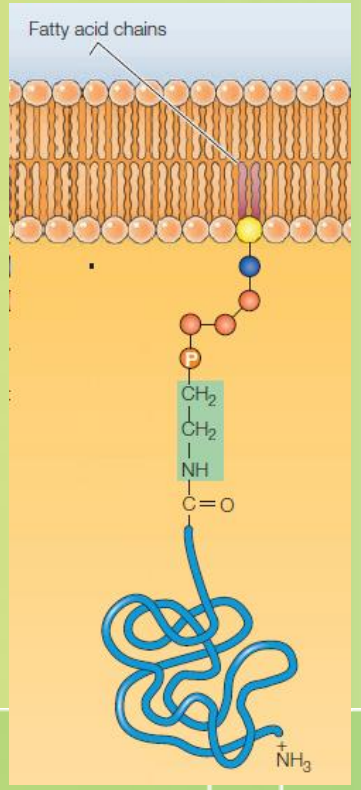
Multi-transmembrane domain proteins have multiple transmembrane sequences

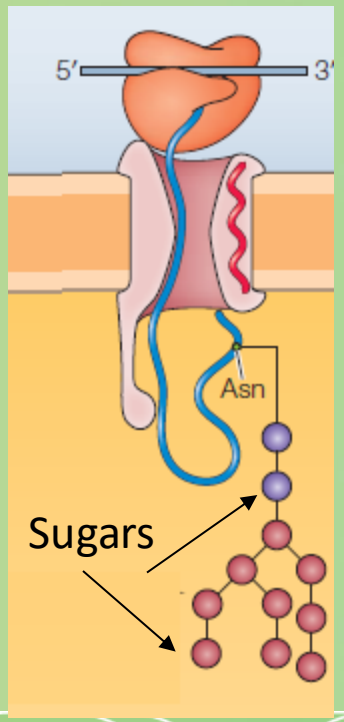


Once inside the ER, proteins are



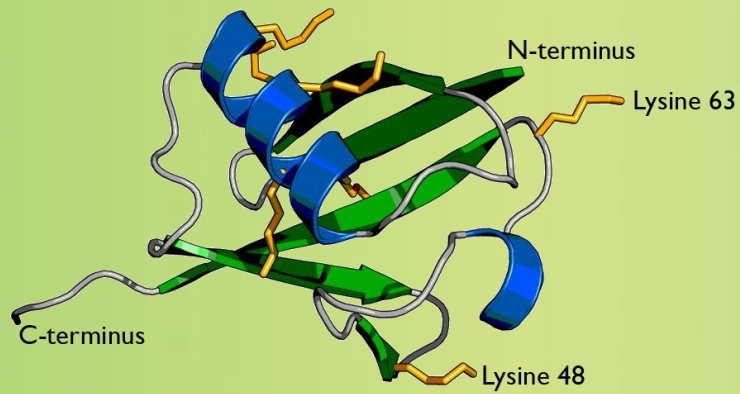
- Folded (with the help of chaperones)
- Complexed (quaternary structure)
  - Disulfide bond formation by protein disulfide isomerase
- Glycosylated
- Anchored by lipids

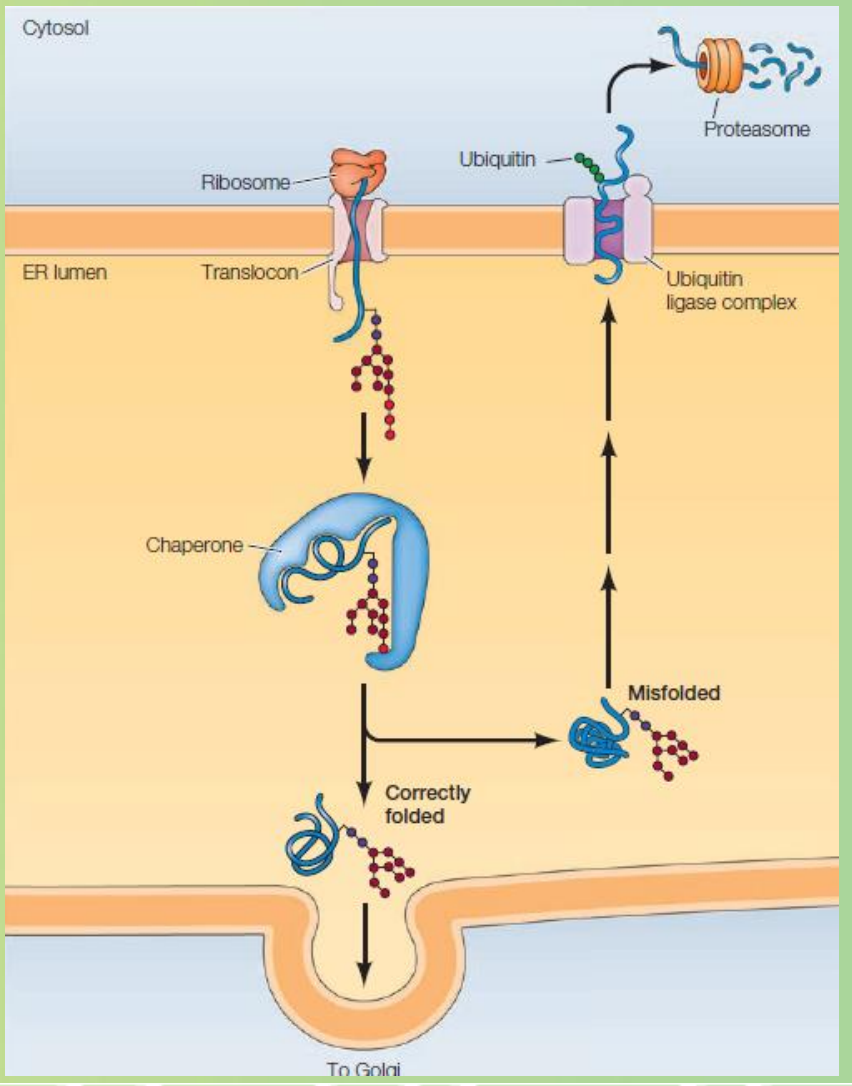




Protein folding and ER-associated degradation (ERAD)

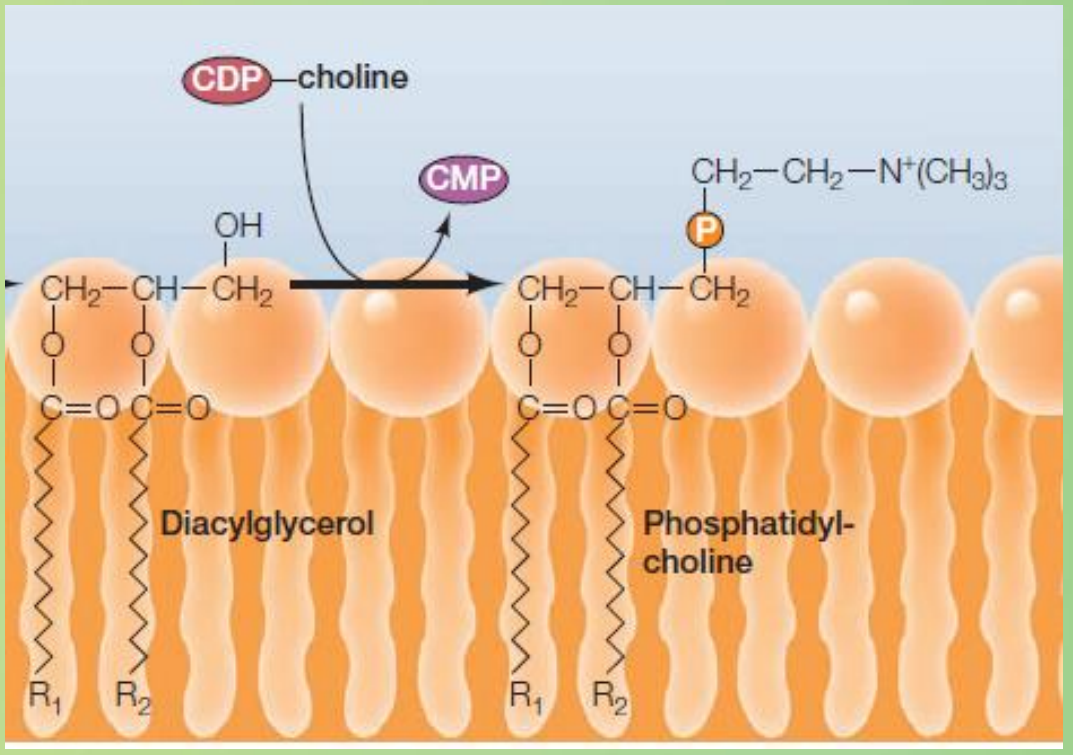
- If correctly folded, proteins move on.
- If misfolded, proteins are sent to the cytosol, ubiquitylated (addition of small proteins called ubiquitins), and degraded in the proteasome.





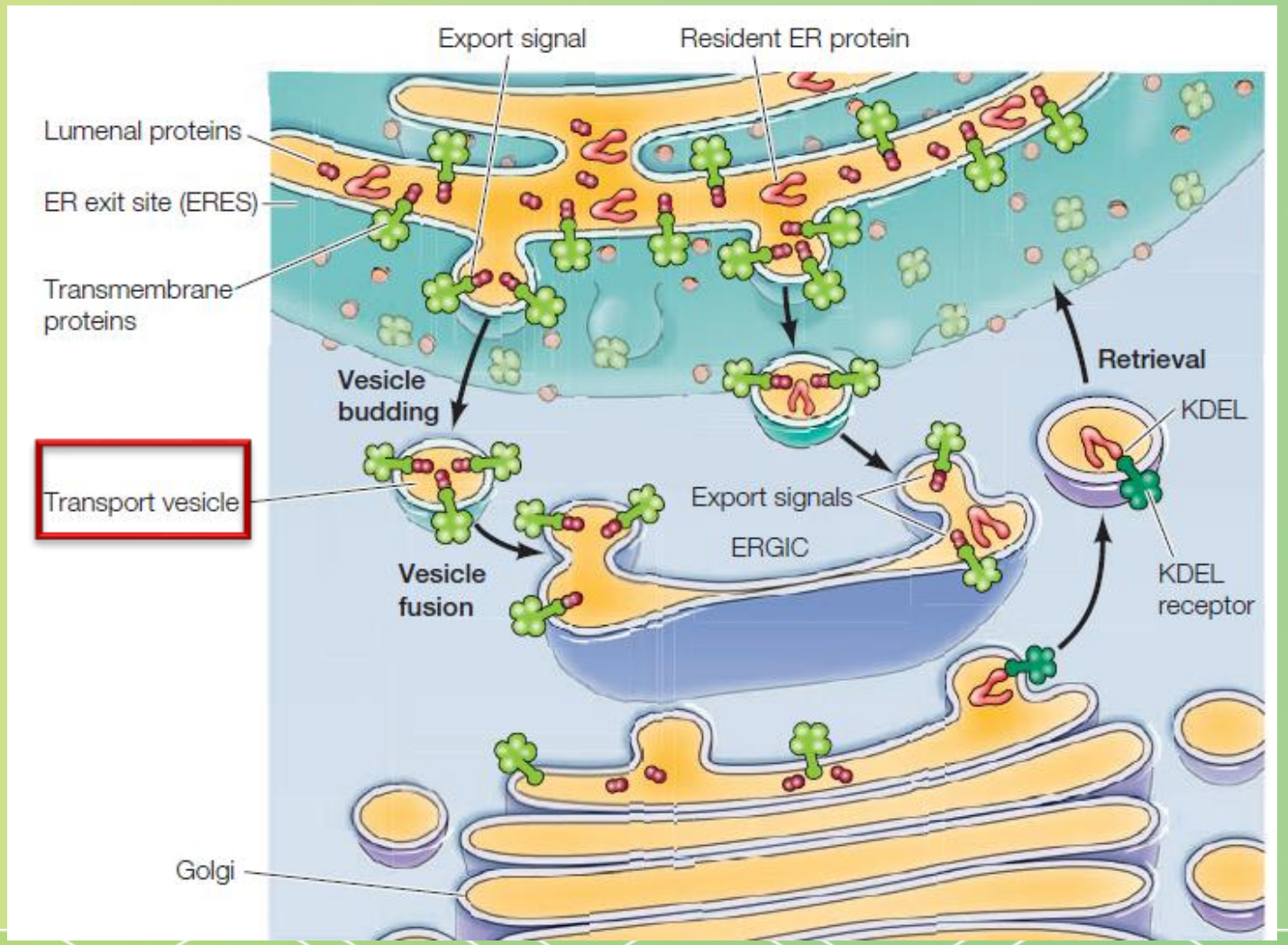
Synthesis of phospholipids in ER

- The smooth ER is the major site of synthesis of:
  - Membrane glycerophospholipids, which are then transported from the SER to other membranes.
  - 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.
- 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.
  - 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.