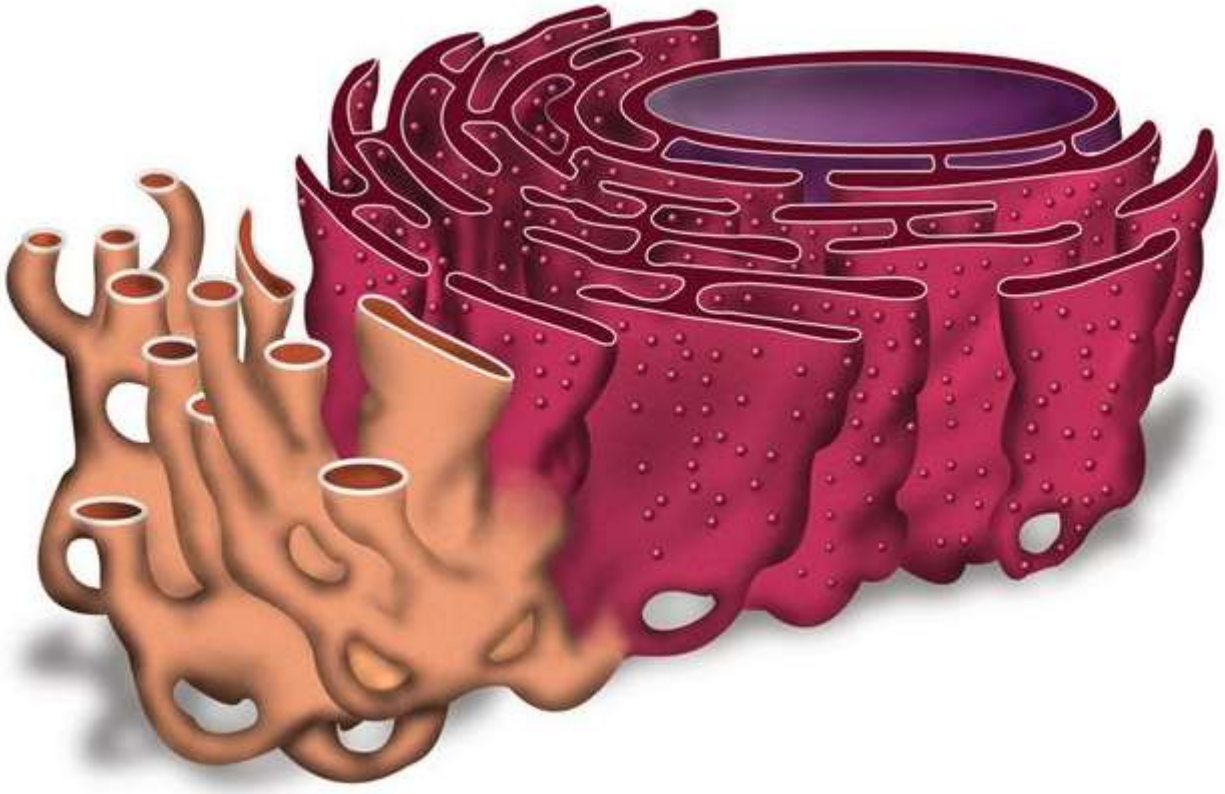
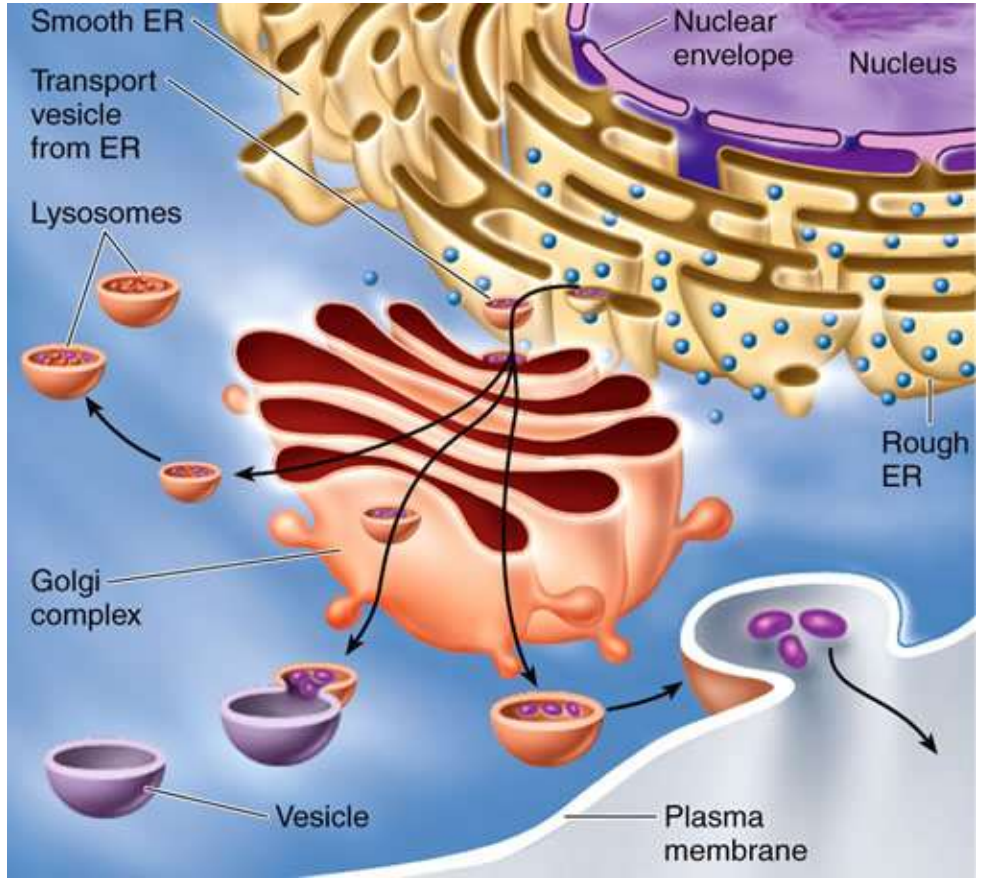


Endoplasmic Reticulum

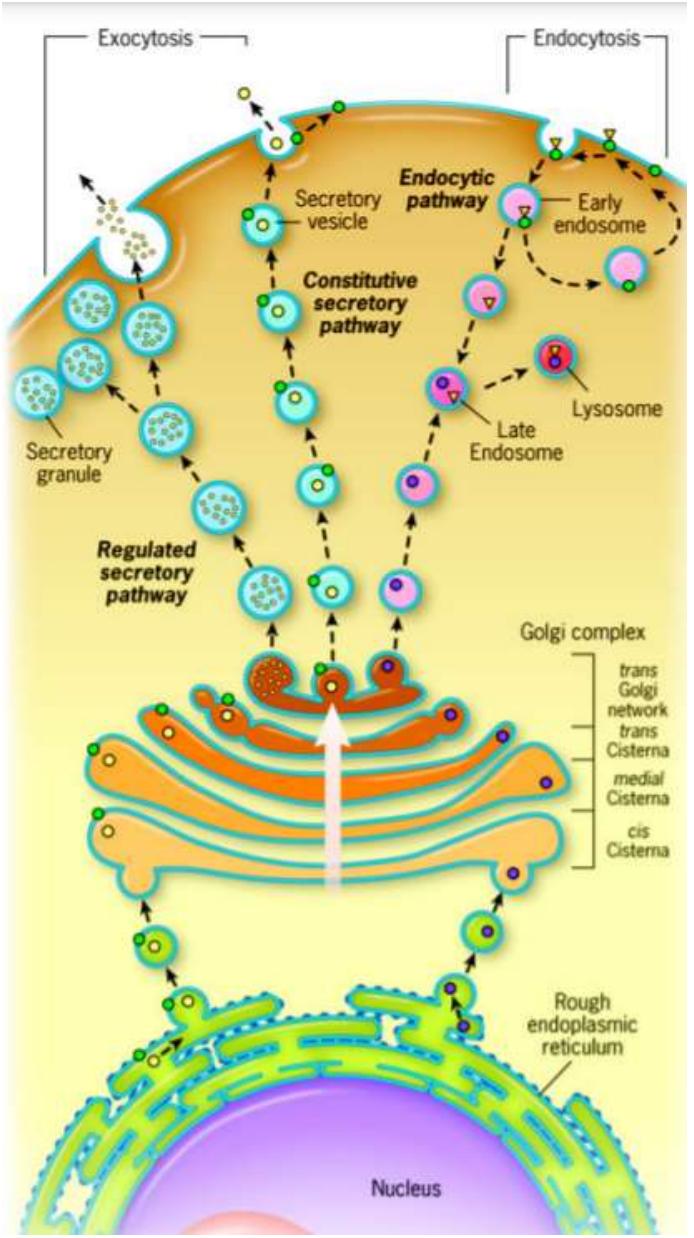
Endomembrane System

- Organelles form a system in which the individual components function as part of a coordinated unit→ endomembrane system
- Organelles: **Endoplasmic reticulum, Golgi complex, endosomes, lysosomes,** and **vacuoles**
- **Mitochondria** and **chloroplasts** are not part of this interconnected system
- In endomembrane system, materials are shuttled back and forth from one part of the cell to another.



Endomembrane System

- **Biosynthetic pathway:** proteins are synthesized in the endoplasmic reticulum→ modified during passage through the Golgi complex→ Golgi complex to various destinations, such as the plasma membrane, a lysosome, or the large vacuole of a plant cell (**secretory pathway**)
- **Constitutive secretion**: materials are transported in secretory vesicles from their sites of synthesis and discharged into the extracellular space in a continual manner
- Example: ECM, Plasma membrane
- **Regulated secretion:** materials are stored as membrane-bound packages and discharged only in response to an appropriate stimulus
- Example: endocrine cells, pancreatic cells, nerve cells
- Materials to be secreted are stored in large, densely packed, membrane-bound **secretory granules**



Endomembrane System

- The experimental approaches that have proven particularly useful in providing the foundation of knowledge on which current research on cytoplasmic organelles is based are as follows:

➢ Autoradiography

➢ Green Fluorescent Protein (GFP)

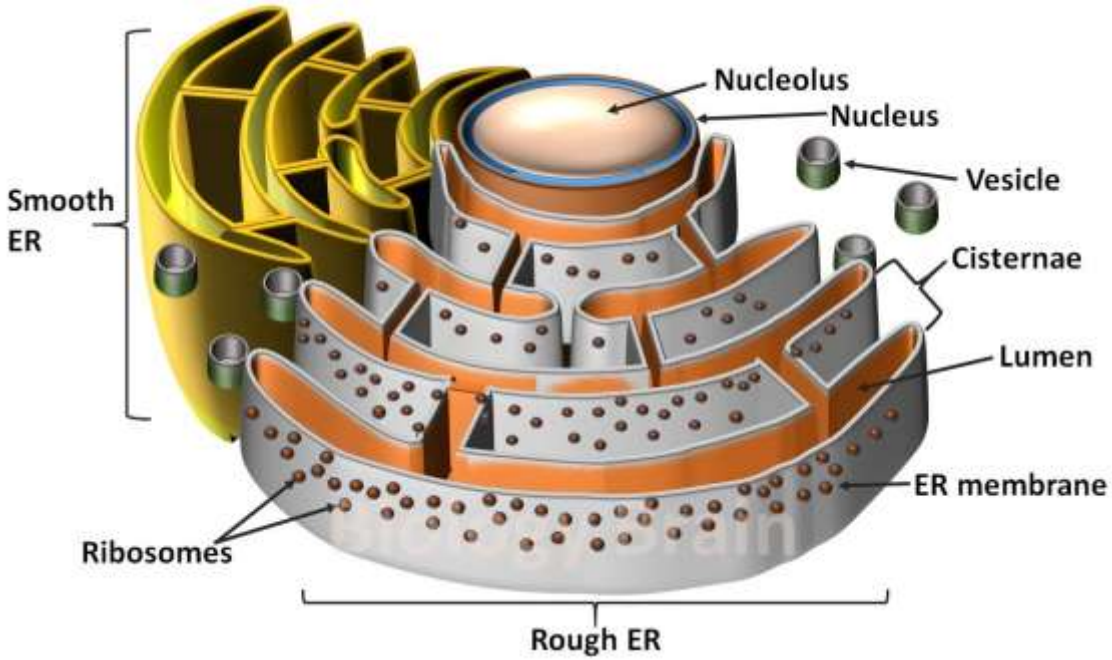
➢ Subcellular fractionation

➢ Cell-free systems

➢ Mutant phenotypes

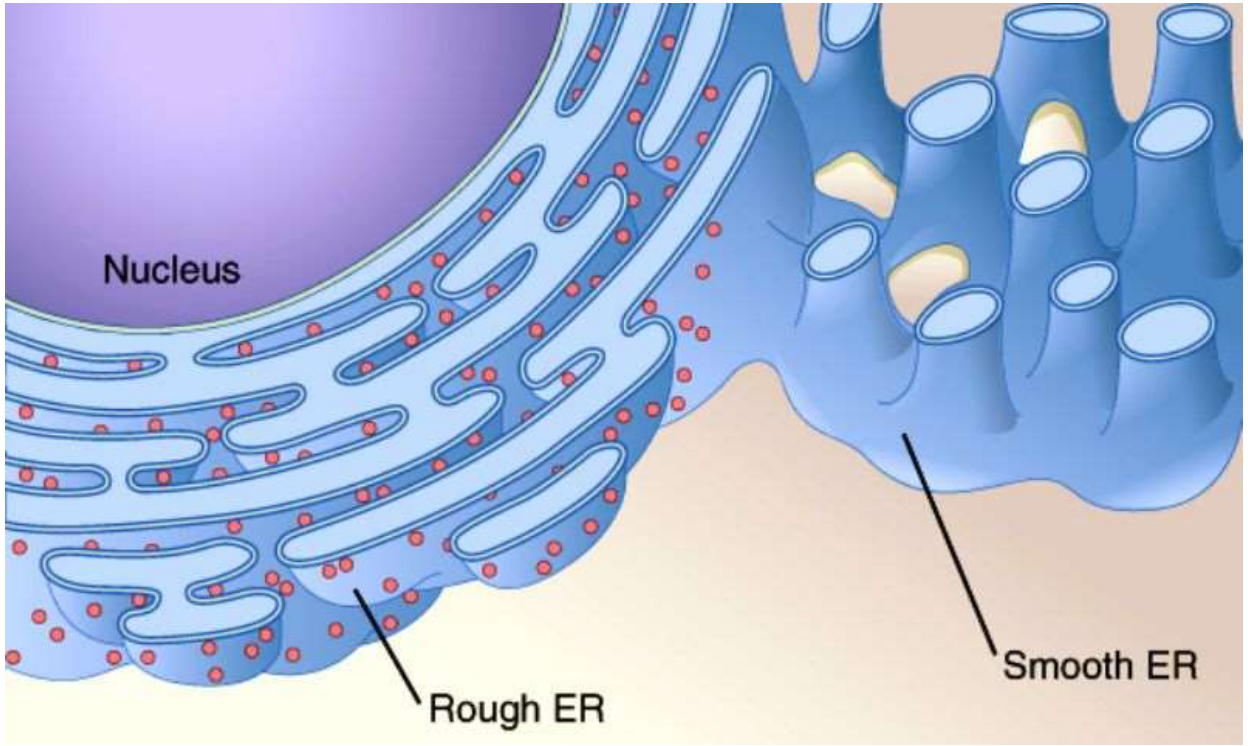
Endoplasmic Reticulum (ER)

- ER comprises a network of membranes that penetrates much of the cytoplasm
- Enclosed within the ER is an extensive space→ lumen
- Composition of the luminal (or cisternal) space is quite different from that of the surrounding cytosolic space
- ER is divided into two sub-compartments, the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER)



Difference in RER and SER

- RER→ ribosomes bound to its cytosolic surface
- SER→ lacks ribosomes
- RER is typically composed of a network of flattened sacs (cisternae)
- SER are highly curved and tubular
- The two types of ER share many of the same proteins and engage in certain common activities, such as the synthesis of certain lipids and cholesterol.



Smooth Endoplasmic Reticulum (SER)

- The SER is extensively developed in a number of cell types, including those of skeletal muscle, kidney tubules, and steroid-producing endocrine glands.
- SER functions include:

■ Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex

■ Detoxification in the liver of a wide variety of organic compounds, Detoxification: oxygenases, eg: cytochrome P450 family

■ The regulated release of Ca^{2+} from the SER of skeletal and cardiac muscle cells (known as the sarcoplasmic reticulum in muscle cells) triggers contraction.

Rough Endoplasmic Reticulum (RER)

Synthesis of Proteins on Membrane-Bound versus Free Ribosomes:

- Membrane bound ribosomes (RER):

(a) secreted proteins

(b) integral membrane proteins

(c) soluble proteins that reside within compartments of the endomembrane system

- Free ribosomes:

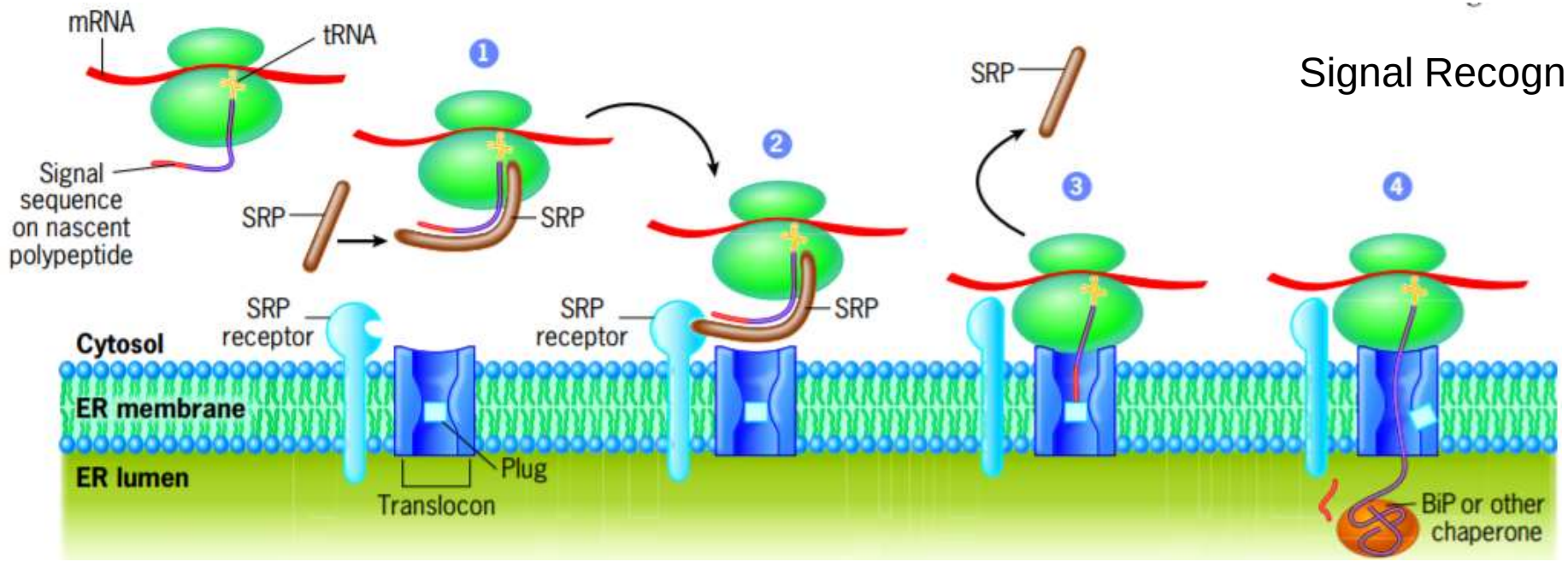
(a) cytosol proteins (eg: enzymes of glycolysis)

(b) peripheral proteins (eg: spectrin, ankyrin)

(c) proteins that are transported to the nucleus

(d) proteins to be incorporated into peroxisomes, chloroplasts, and mitochondria.

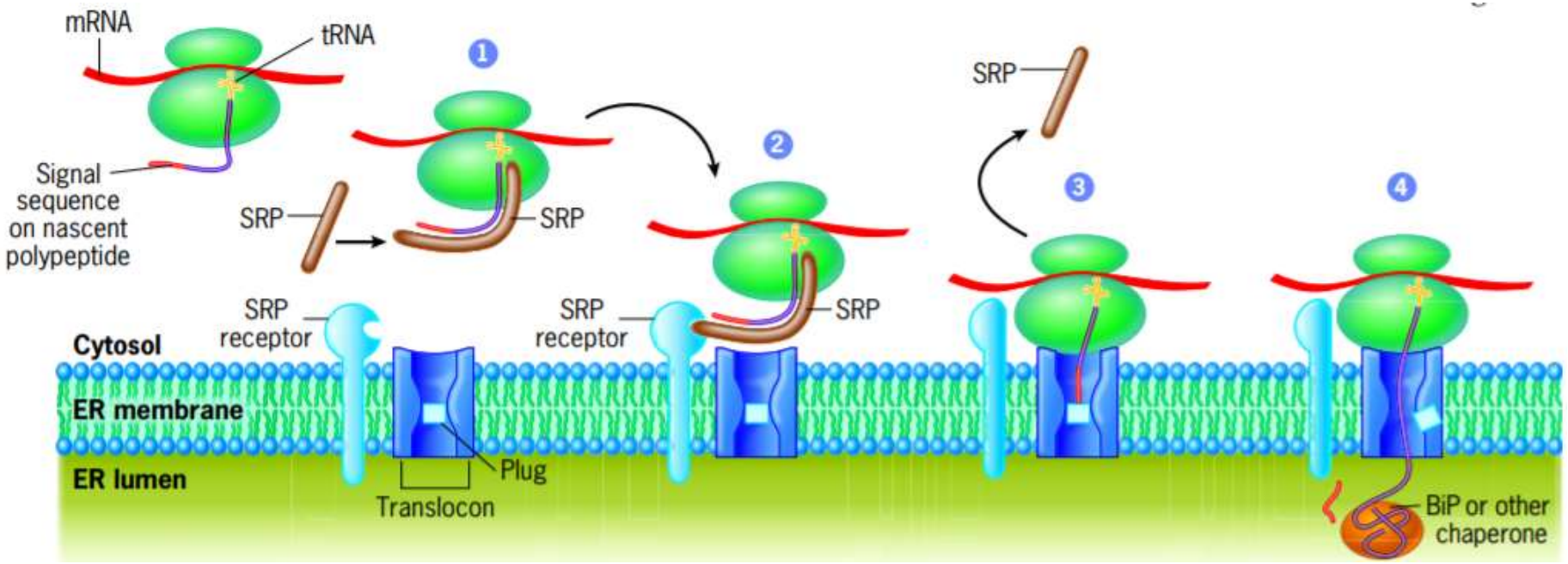
Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins on Membrane-Bound Ribosomes



Signal Recognition Particle (SRP)

(a) Synthesis of the polypeptide begins on a free ribosome. As the signal sequence (shown in red) emerges from the ribosome, it binds to the SRP (step 1), which stops further translation until the SRP-ribosome-nascent chain complex can make contact with the ER membrane.

(b) The SRP-ribosome complex then collides with and binds to an SRP receptor situated within the ER membrane (step 2).

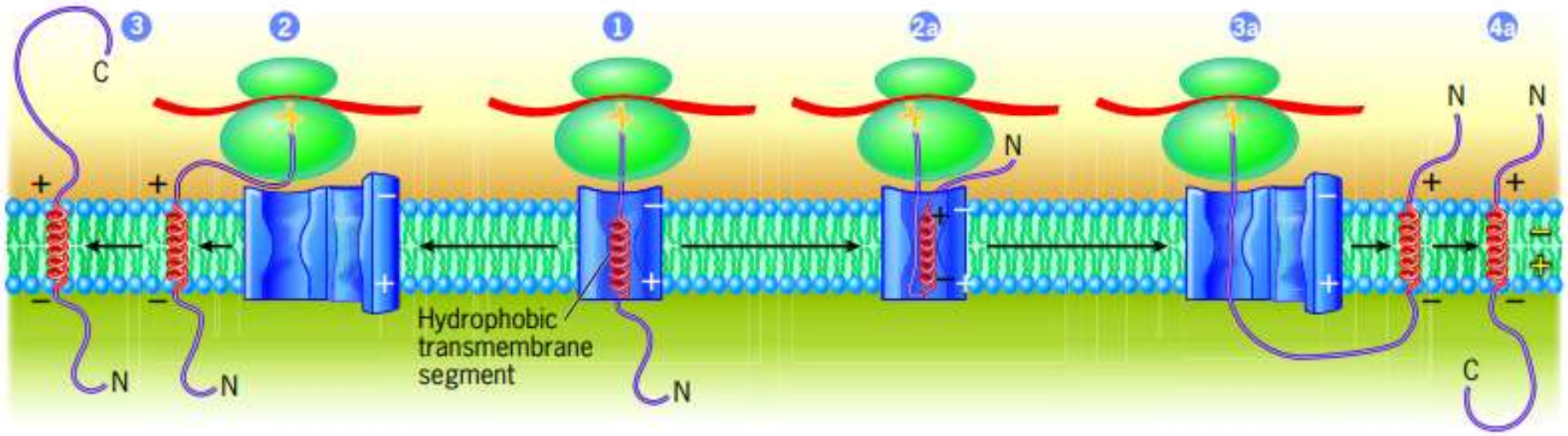


(c) Attachment of this complex to the SRP receptor is followed by release of the SRP and the association of the ribosome with a translocon of the ER membrane (step 3).

(d) The signal peptide then binds to the interior of the translocon, displacing the plug from the channel and allowing the remainder of the polypeptide to translocate through the membrane co-translationally (step 4).

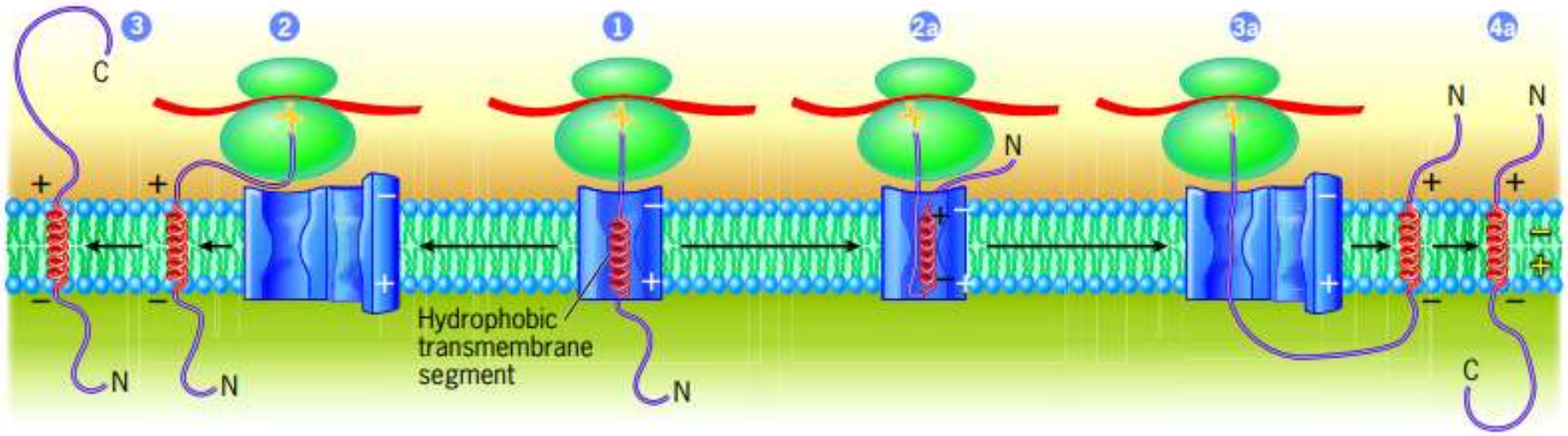
(e) After the nascent polypeptide passes into the lumen of the ER, the signal peptide is cleaved by a membrane protein (the signal peptidase), and the protein undergoes folding with the aid of ER chaperones, such as BiP.

Synthesis of Integral Membrane Proteins on Membrane-Bound Ribosomes



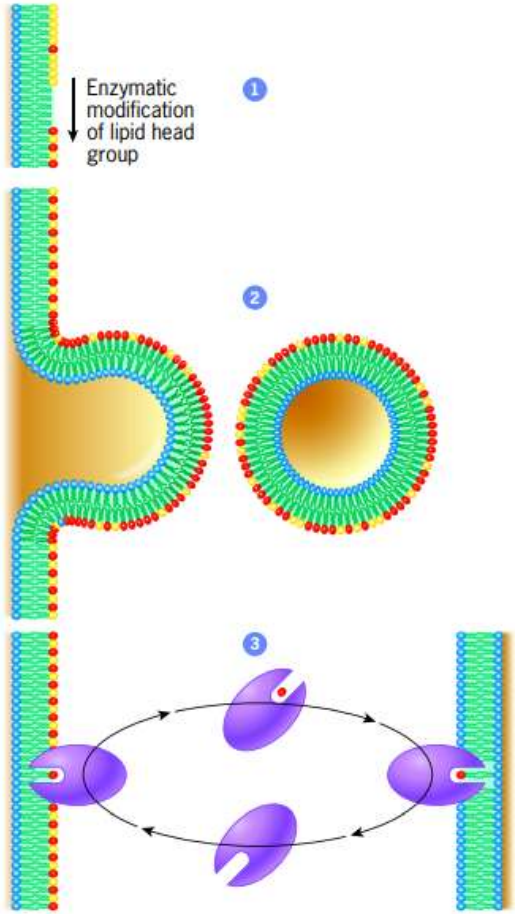
- The nascent polypeptide enters the translocon just as if it were a secretory protein (step 1).
- The entry of the hydrophobic transmembrane sequence into the pore blocks further translocation of the nascent polypeptide through the channel.
- In step 2, the lateral gate of the translocon has opened and expelled the transmembrane segment into the bilayer.
- Step 3 shows the final disposition of the protein.

Synthesis of Integral Membrane Proteins on Membrane-Bound Ribosomes

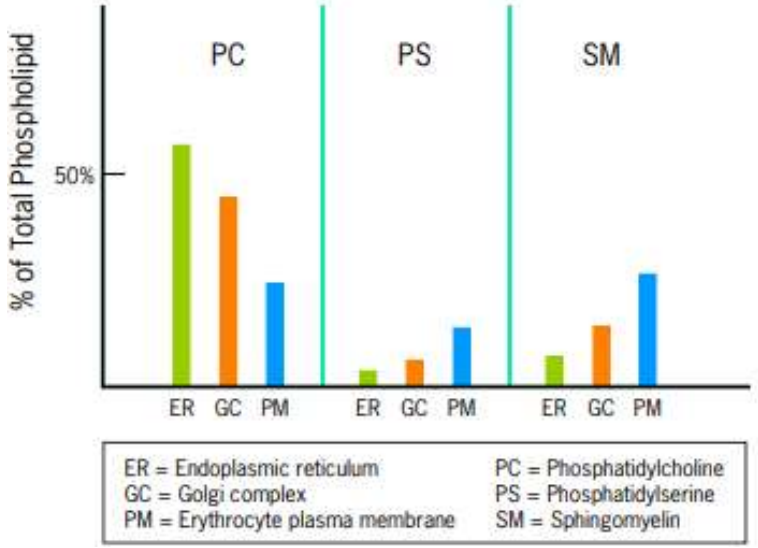


- In step 2a, the translocon has reoriented the transmembrane segment, in keeping with its reversed positively and negatively charged flanks.
- In step 3a, the translocon has opened laterally and expelled the transmembrane segment into the bilayer.
- Step 4a shows the final disposition of the protein

Synthesis of Membrane Lipids



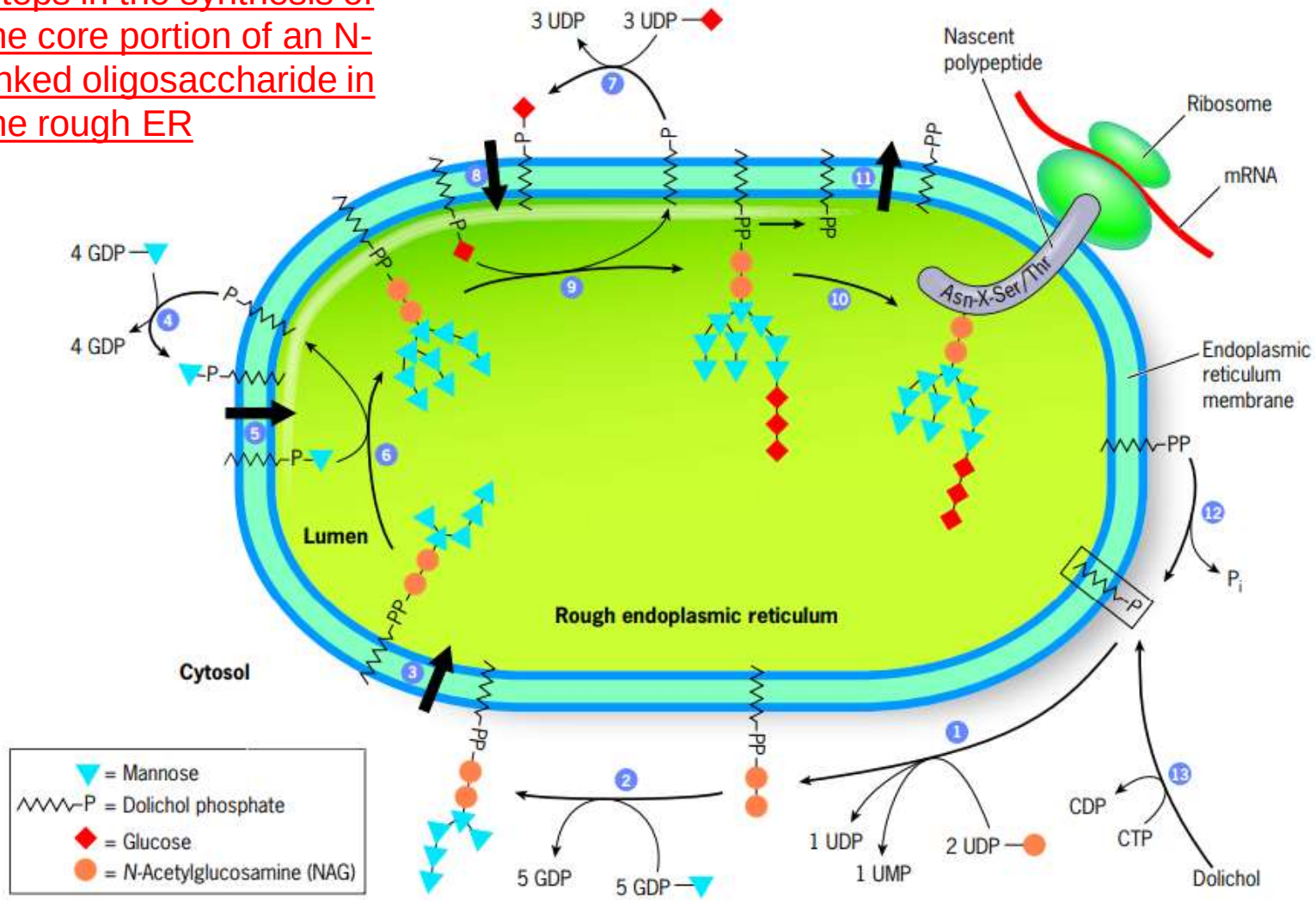
- Most membrane lipids are synthesized entirely within the endoplasmic reticulum
- The major exceptions are:
  (1) sphingomyelin and glycolipids, whose synthesis begins in the ER and is completed in the Golgi complex
  (2) some of the unique lipids of the mitochondrial and chloroplast membranes, which are synthesized by enzymes that reside in those membranes
- The enzymes involved in the synthesis of phospholipids are themselves integral proteins of the ER membrane.



Several factors that may contribute to changes in lipid composition across the membranes: 1) Enzyme modification 2) Preferential inclusion 3) Lipid-transfer proteins.

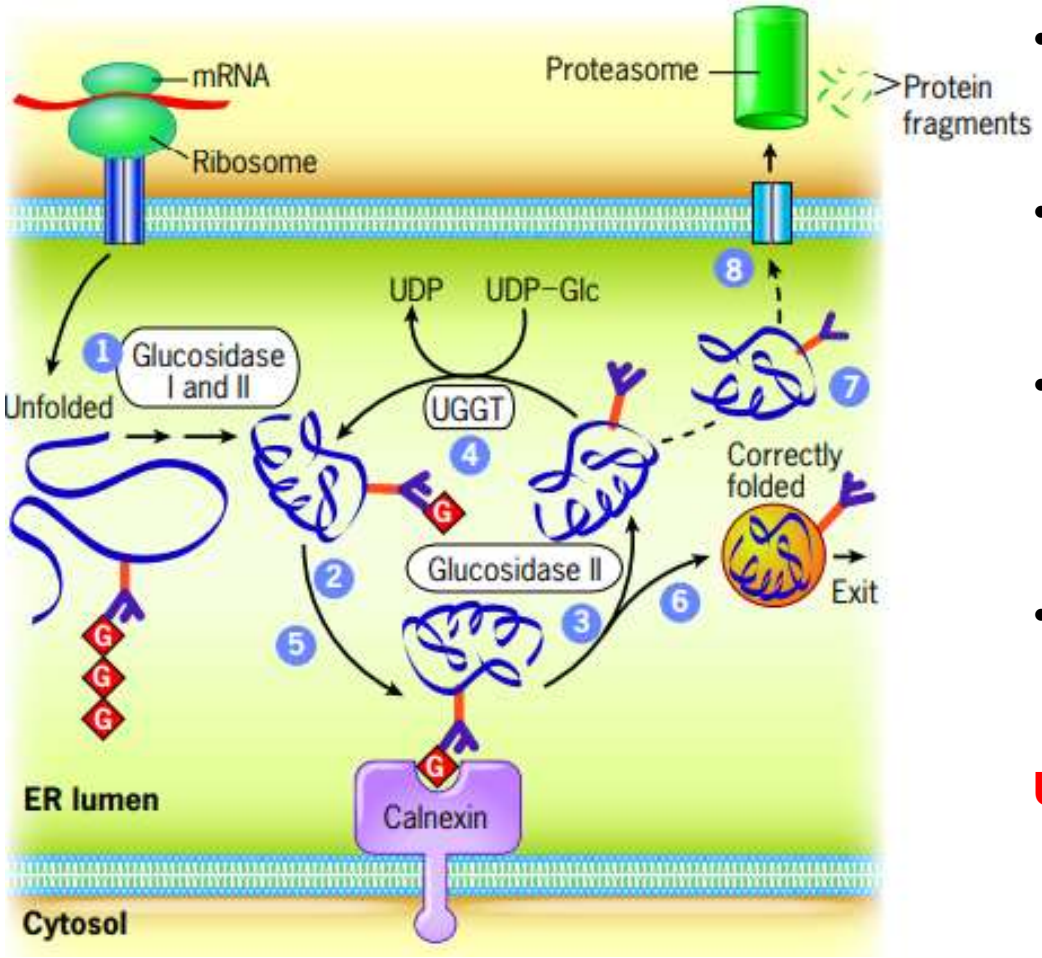
Glycosylation in the RER

Steps in the synthesis of the core portion of an N-linked oligosaccharide in the rough ER



- Addition of sugars to an oligosaccharide chain→ **glycosyltransferases**
- Transfer of oligosaccharide chain from dolichol phosphate to asparagine in the nascent polypeptide→ **oligosaccharyltransferase**

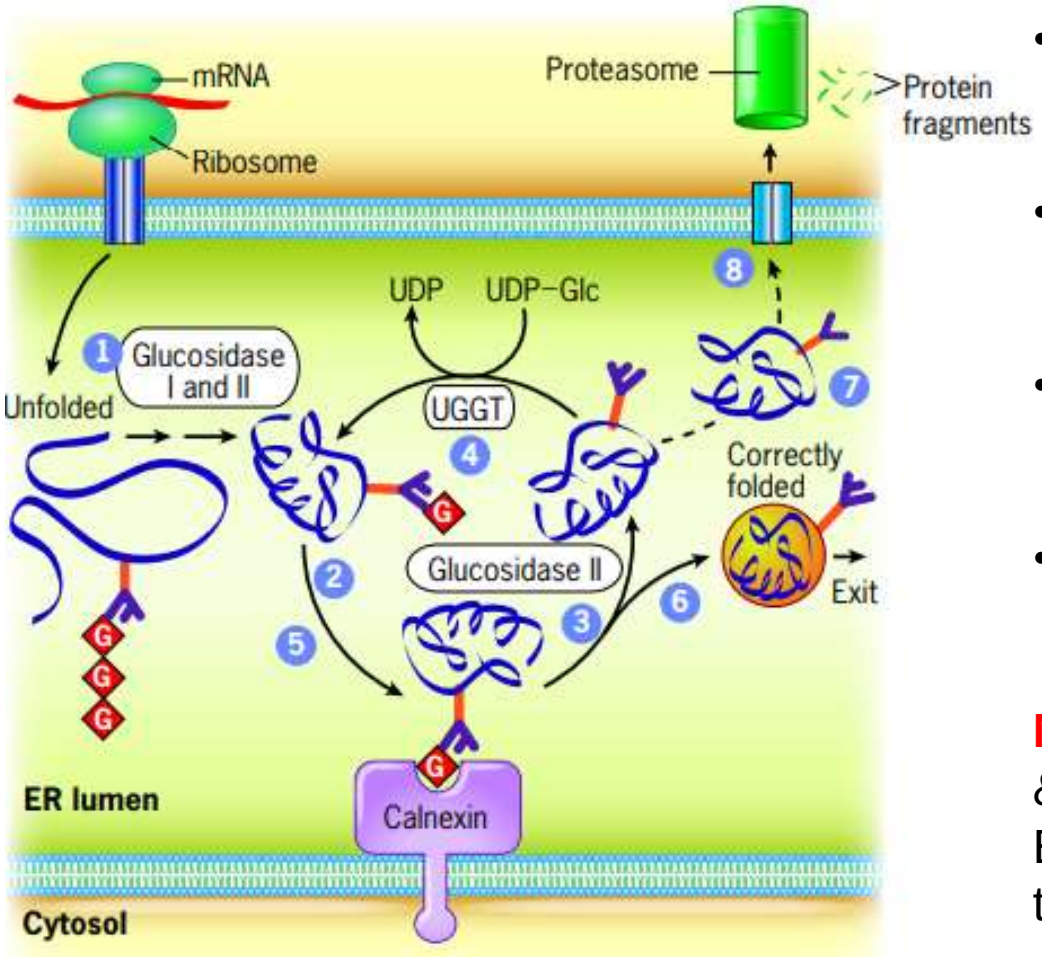
Quality control: ensuring that misfolded proteins do not proceed forward



- **<u>Step 1</u>:** removal of two of the three terminal glucose residues.
- **<u>Step 2</u>:** glycoprotein with a single remaining glucose, binds to an ER chaperone (calnexin or calreticulin)
- **<u>Step 3</u>:** removal of the remaining glucose by glucosidase II leads to release of the glycoprotein from the chaperone.
- **<u>Step 4</u>:** UGGT that adds a single glucose residue (only if the folding is incomplete or misfolded)

UGGT (UDP-glucose: glycoprotein glucosyltransferase)

Quality control: ensuring that misfolded proteins do not proceed forward



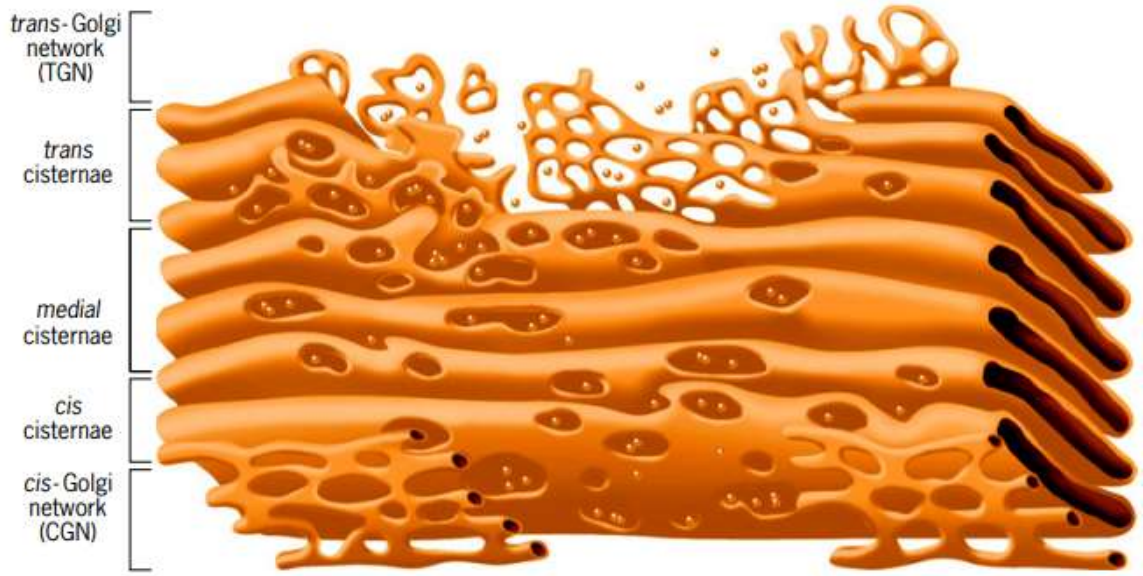
- **<u>Step 5</u>:** again the glycoprotein with a single glucose, binds to an ER chaperone
- **<u>Step 6</u>:** If the glycoprotein is folded correctly then it continues on its way.
- **<u>Step 7</u>:** Once one or more of these mannose residues have been removed.
- **<u>Step 8</u>:** the protein can no longer be recycled and, instead, is sentenced to degradation

Note: The “decision” to destroy the defective protein (step 7 & 8) begins with the activity of a slow-acting enzyme in the ER that trims a mannose residue from an exposed end of the oligosaccharide of a protein.

Golgi Complex

Golgi Complex

- Discovered by Camillo Golgi
- cis (entry face) closest to the ER
- trans (exit face) towards the plasma membrane
- CGN (cis Golgi network): function as a sorting station that distinguishes between proteins to be shipped back to the ER and those that are allowed to proceed to the next Golgi station



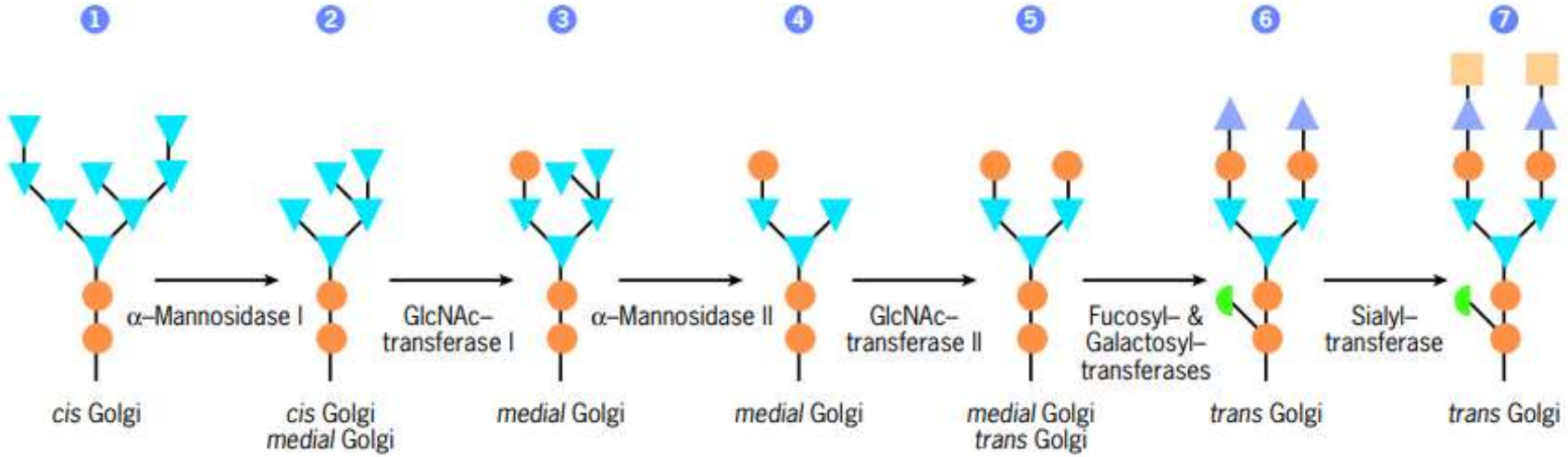
- The bulk of the Golgi complex consists of a series of large, flattened cisternae, which are divided into cis, medial, and trans cisternae

- TGN (trans Golgi network): sorting station where proteins are segregated into different types of vesicles heading either to the plasma membrane or to various intracellular destinations

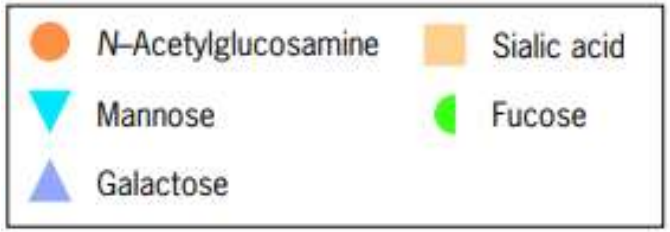
Glycosylation in the Golgi Complex

- The Golgi complex plays a key role in the assembly of the carbohydrate component of glycoproteins and glycolipids
- The sequence in which sugars are incorporated into oligosaccharides is determined by the spatial arrangement of the specific glycosyltransferases present in different Golgi stack
- Glycosylation steps in the Golgi complex are varied, producing carbohydrate domains of remarkable sequence diversity compared to glycosylation in ER
- Glycosaminoglycan chains of the proteoglycan and the pectins and hemicellulose found in the cell walls of plants→ synthesized in golgi complex

Glycosylation in the Golgi Complex



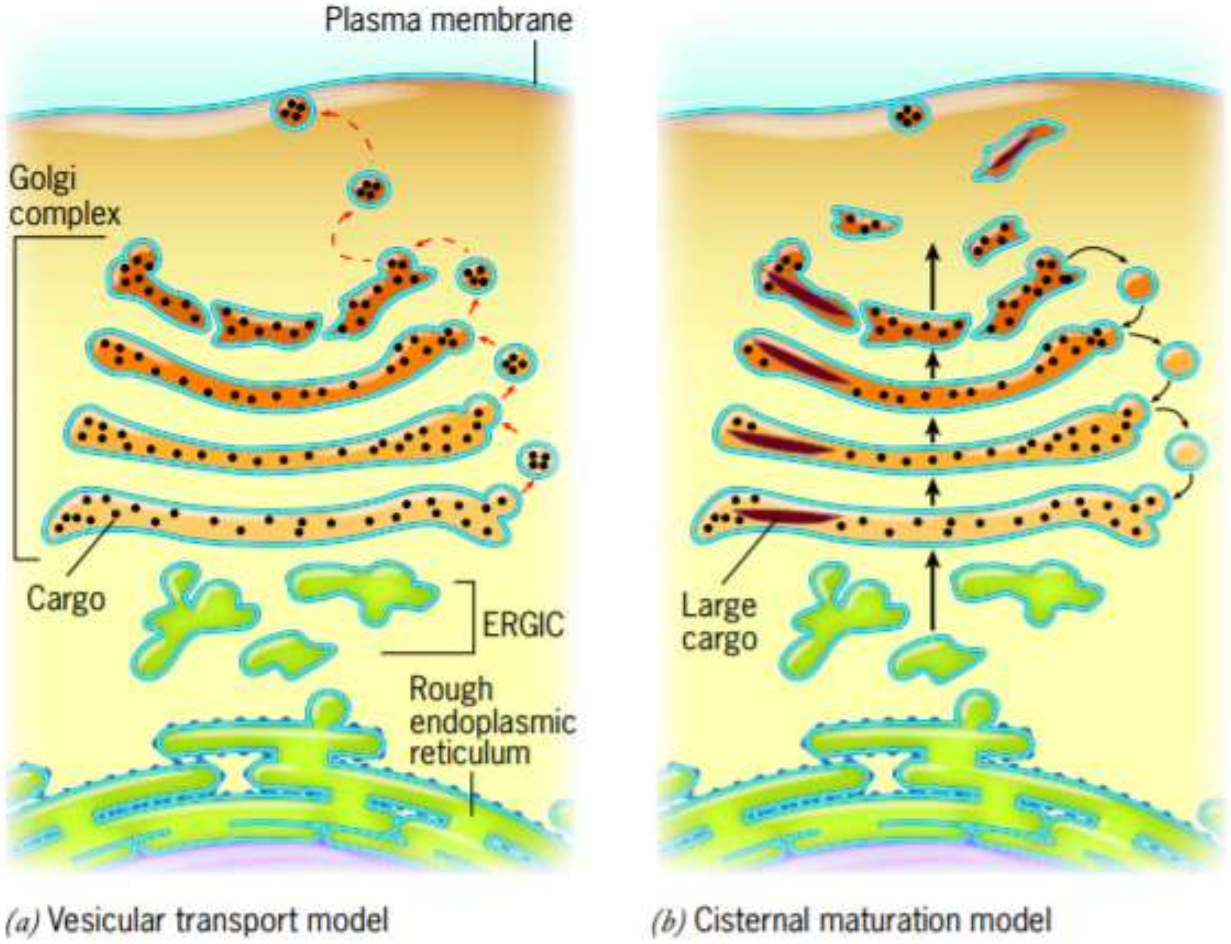
Steps in the glycosylation of a typical mammalian N-linked oligosaccharide in the Golgi complex



Glycosylation of newly synthesized proteins

- N-linked: oligosaccharide chain is linked to the amide nitrogen of asparagine (Asn) (in ER)
- O-linked: oligosaccharide chain is linked to the hydroxyl group of serine or threonine (in Golgi)

Movement of Materials through the Golgi Complex



(a) Vesicular transport model

(b) Cisternal maturation model

The dynamics of transport through the Golgi complex.

(*a*) In the vesicular transport model, cargo (black dots) is carried in an anterograde direction by transport vesicles, while the cisternae themselves remain as stable elements. (*b*) In the cisternal maturation model, the cisternae progress gradually from a *cis* to a *trans* position and then disperse at the TGN. Transport vesicles carry resident Golgi enzymes (indicated by the colored vesicles) in a retrograde direction. The red lens-shaped objects represent large cargo materials, such as procollagen complexes of fibroblasts.

Vesicle Transport and Their Functions

- Materials are carried between compartments by vesicles (or other types of membrane-bound carriers) that bud from donor membranes and fuse with acceptor membranes

- Membrane coating the vesicles are made of different proteins

- Protein coats have at least two distinct functions:

(1) act as a mechanical device that causes the membrane to curve and form a budding vesicle

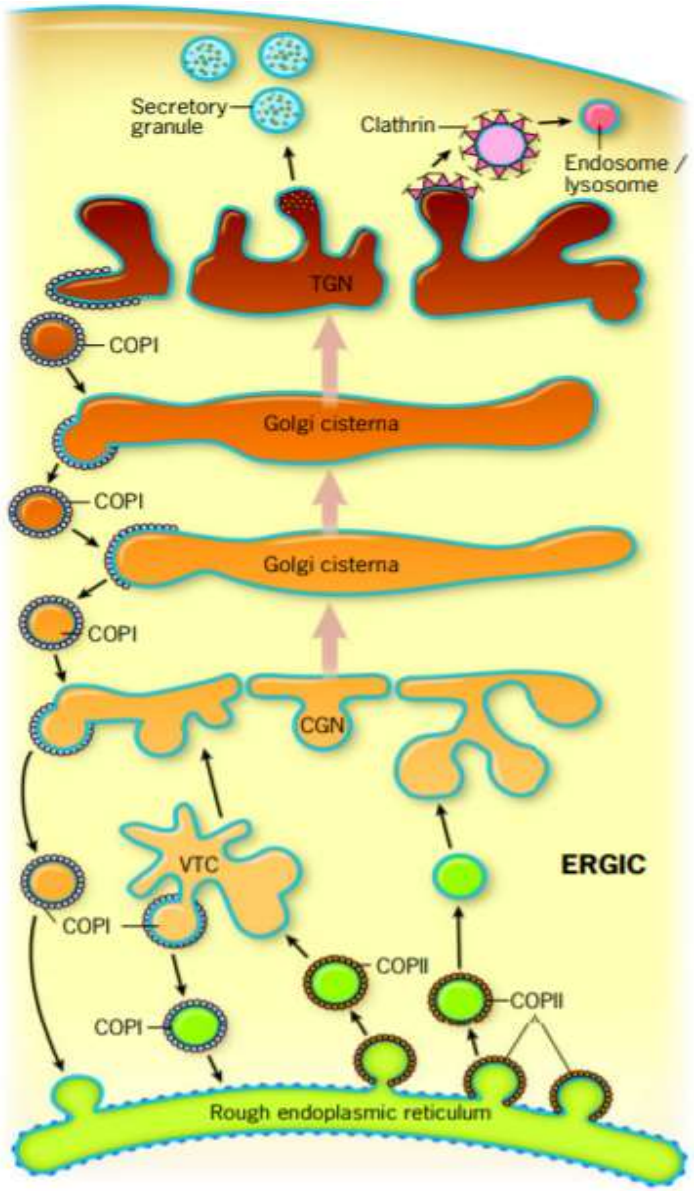
(2) provide a mechanism for selecting the components to be carried by the vesicle

- Selected components include:

(a) cargo consisting of secretory, lysosomal, and membrane proteins to be transported

(b) the machinery required to target and dock the vesicle to the correct acceptor membrane.

Types of Vesicle Transport and Their Functions



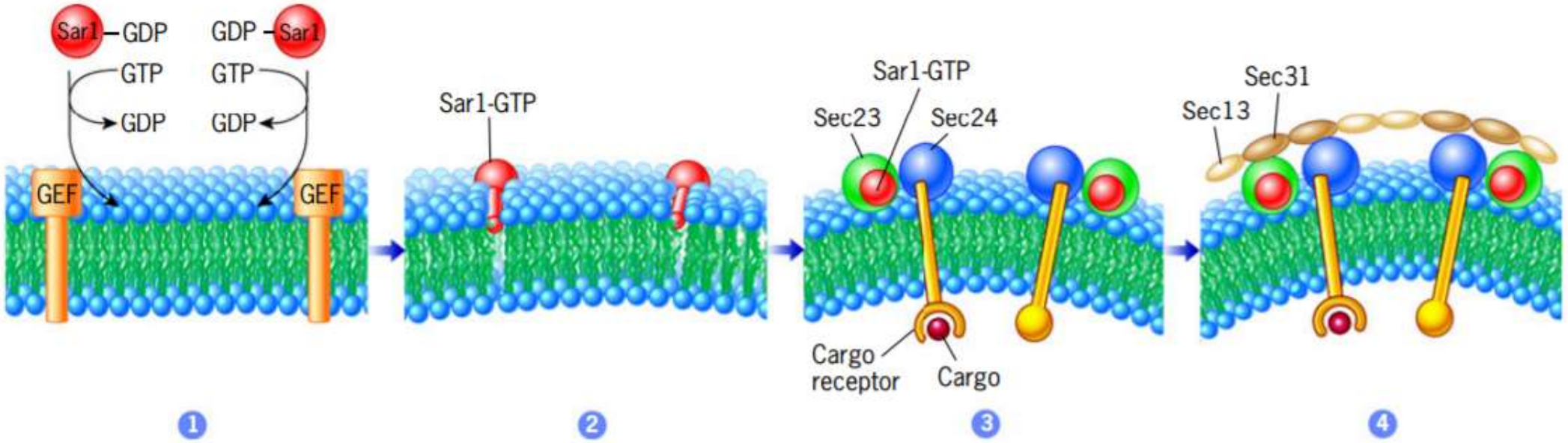
Several distinct classes of coated vesicles have been identified; they are distinguished by the proteins that make up their coat:

1. **COPII-coated vesicles:** move materials from the ER "forward" to the ERGIC and Golgi complex

2. **COPI-coated vesicles:** move materials in a retrograde direction:
   a. from the ERGIC and Golgi stack "backward" toward the ER
   b. from trans Golgi cisternae "backward" to cis Golgi cisternae

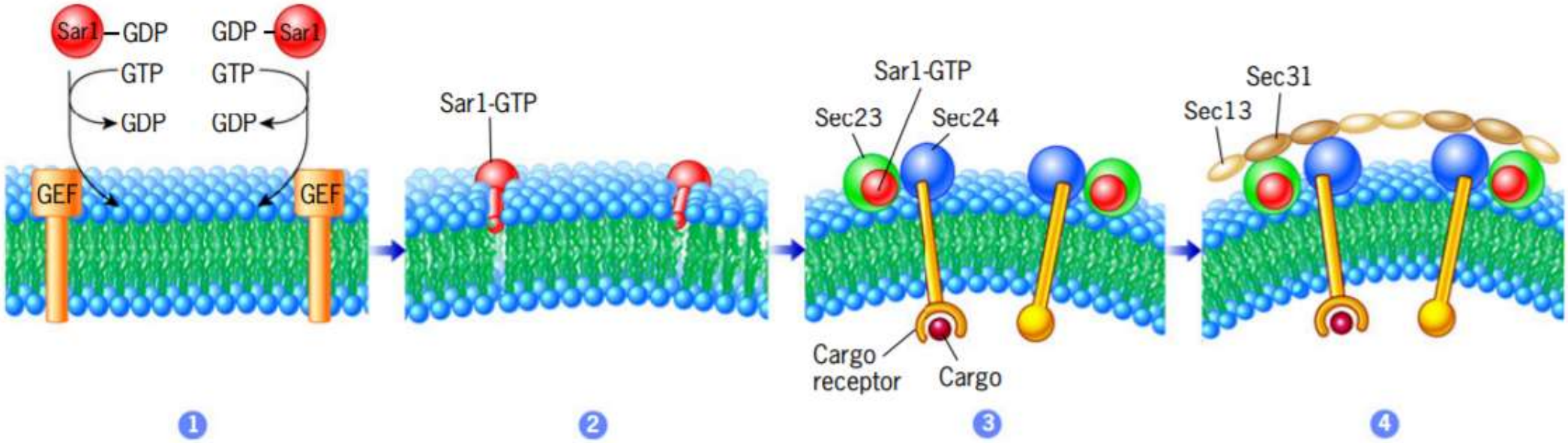
3. **Clathrin-coated vesicles:** move materials from the TGN to endosomes, lysosomes, and plant vacuoles.
They also move materials from the plasma membrane to cytoplasmic compartments along the endocytic pathway.
They have also been implicated in trafficking from endosomes and lysosomes.

COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex



Step 1: Sar1-GDP molecules have been recruited to the ER membrane by a protein called a GEF (guanine-exchange factor) that catalyzes the exchange of the bound GDP with a bound GTP.

Step 2: each Sar1-GTP molecule has extended a finger-like helix along the membrane within the cytosolic leaflet. This event expands the leaflet and induces the curvature of the lipid bilayer at that site.



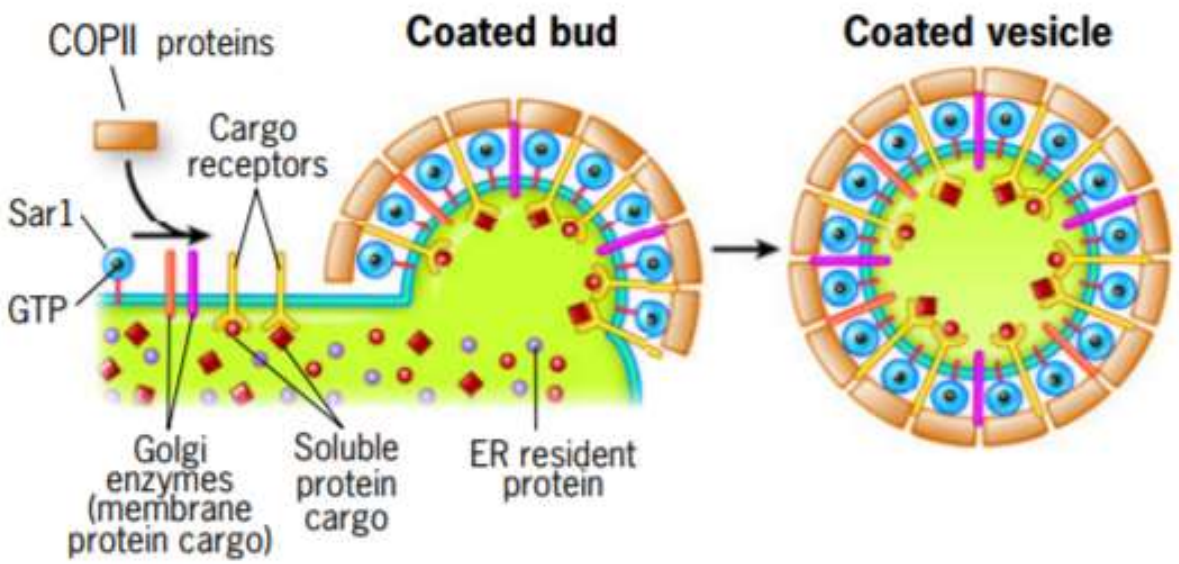
Step 3: dimer composed of two COPII polypeptides (Sec23 and Sec24) has been recruited by the bound Sar1-GTP.

The Sec23-Sec24 heterodimer further induce the curvature of the membrane in the formation of a vesicle.

Transmembrane cargo accumulates within the forming COPII vesicle as their cytosolic tails bind to the Sec24 polypeptide of the COPII coat.

Step 4: the remaining COPII polypeptides (Sec13 and Sec31) have joined the complex to form an outer structural scaffold of the coat.

COPII-Coated Vesicles



(3) membrane proteins that are able to bind soluble cargo (such as the secretory proteins, indicated by the red spheres and diamond)

❖ Example: mutations in one cargo receptor (ERGIC-53) have been linked to an inherited bleeding disorder.

- Proteins selected by COPII-coated vesicles include:

(1) enzymes that act at later stages in the biosynthetic pathway, such as the glycosyltransferases of the Golgi complex (indicated as orange membrane proteins in Figure)

(2) membrane proteins involved in the docking and fusion of the vesicle with the target compartment

- Membrane proteins of the ER are selectively captured because they contain “ER export” signals as part of their cytosolic tail.
- These signals interact specifically with COPII proteins (sec24) of the vesicle coat.

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

- COPI coated vesicles: retrograde transport of proteins, including the movement of:

(1) Golgi resident enzymes in a trans-to-cis direction

(2) ER resident enzymes from the ERGIC and the Golgi complex back to the ER

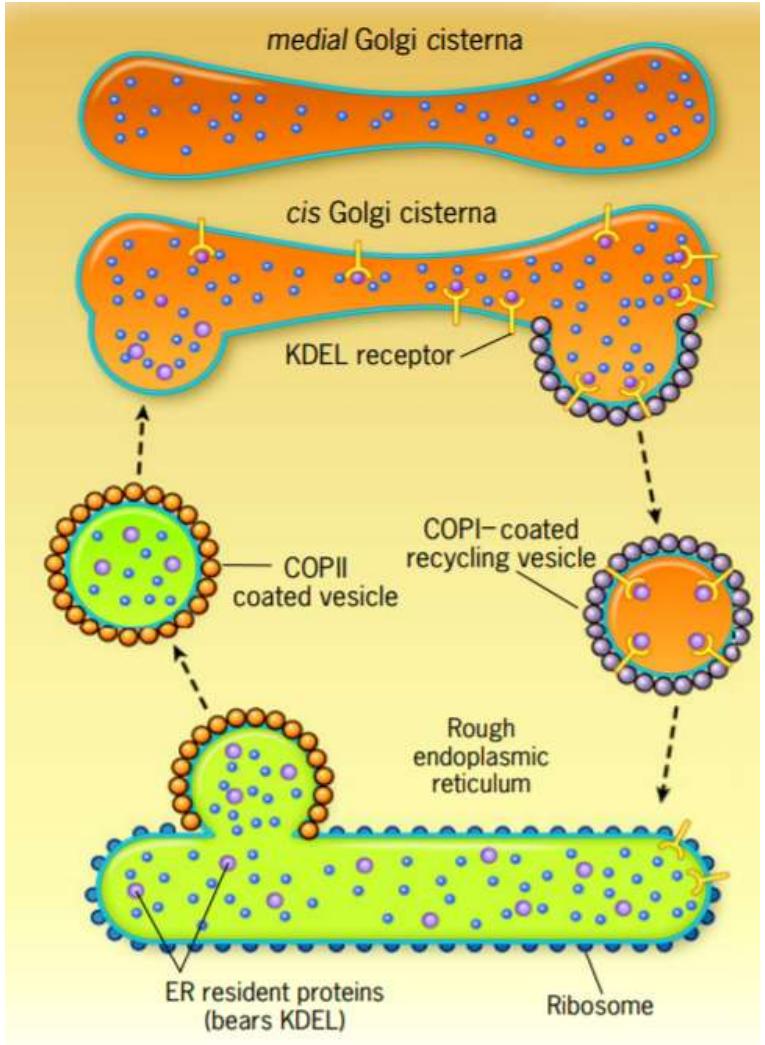
- COPI coat contains a small membrane bending GTP-binding protein, called Arf1, whose bound GTP must be hydrolyzed before the coat can disassemble
- Proteins are maintained in an organelle by a combination of two mechanisms:

1. **Retention of resident molecules that are excluded from transport vesicles:** Retention may be based primarily on the physical properties of the protein.

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

2. Retrieval of "escaped" molecules back to the compartment in which they normally reside:

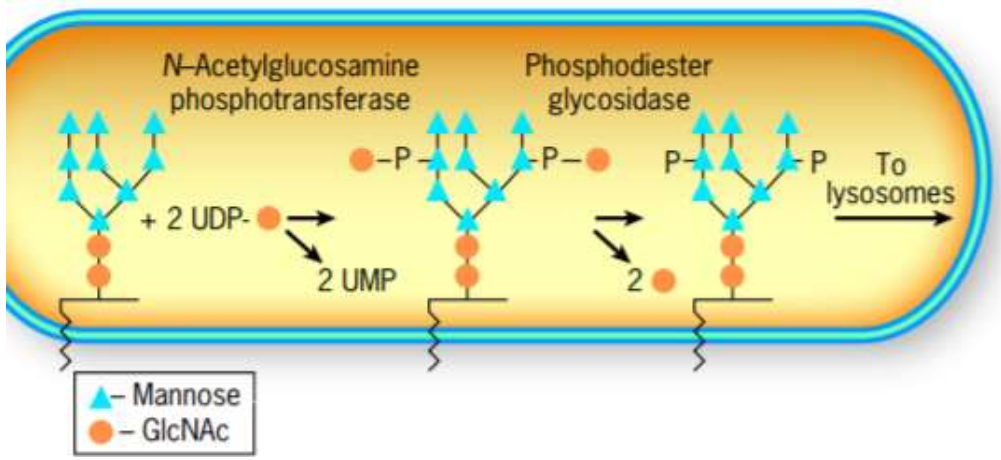
- Proteins that normally reside in the ER, those both in the lumen and in the membrane, contain short amino acid sequences at their C-terminus that serve as retrieval signals
- Soluble resident proteins of the ER lumen typically possess the retrieval signal "lys-asp-glu-leu" (or KDEL)
- These proteins are recognized and returned to the ER by the KDEL receptor, an integral membrane protein that shuttles between the cis Golgi and the ER compartments
- Membrane proteins that reside in the ER also have a retrieval sequences, most commonly KKXX (where K is lysine and X is any residue)



Sorting of Lysosomal Enzymes

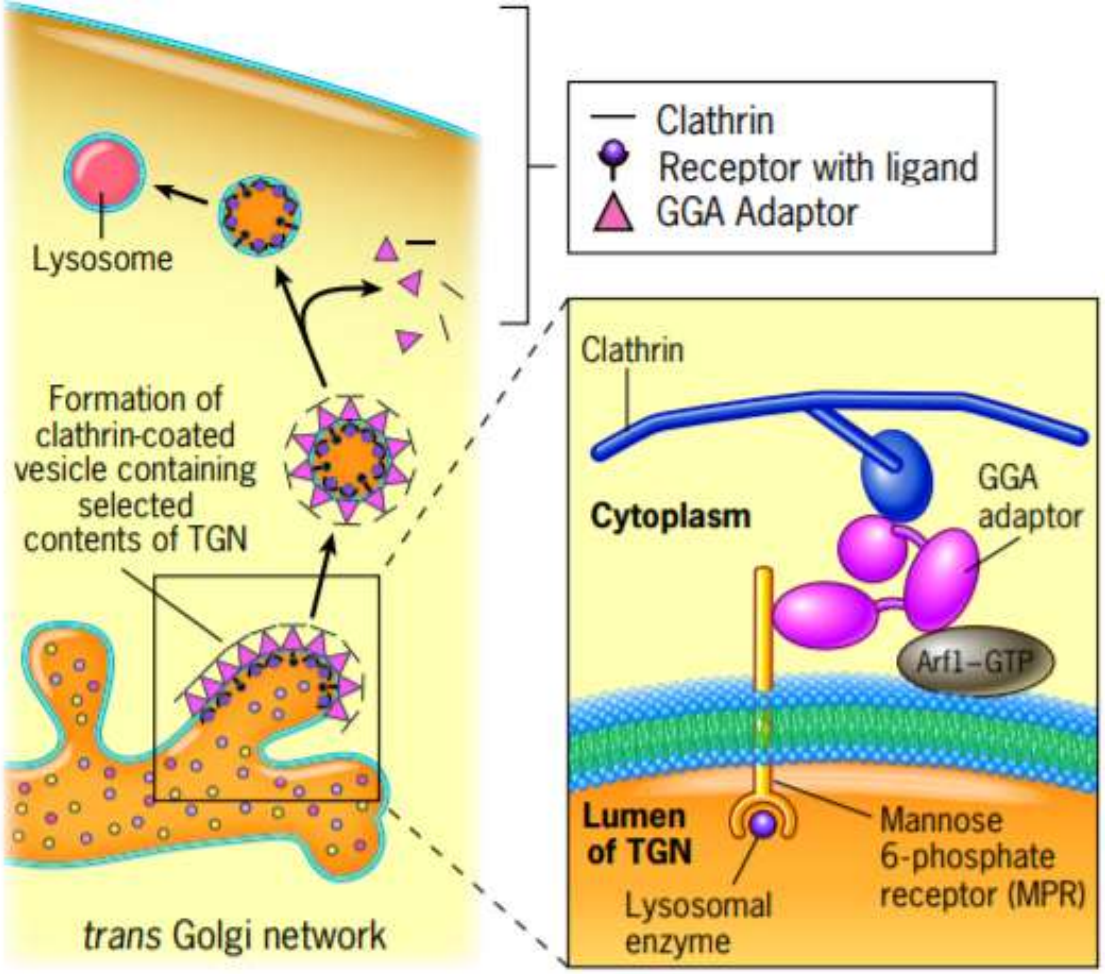
- Trans Golgi network (TGN), functions as a major sorting station, directing proteins to various destinations

- In the Golgi cisternae, soluble lysosomal enzymes are specifically recognized and phosphorylated



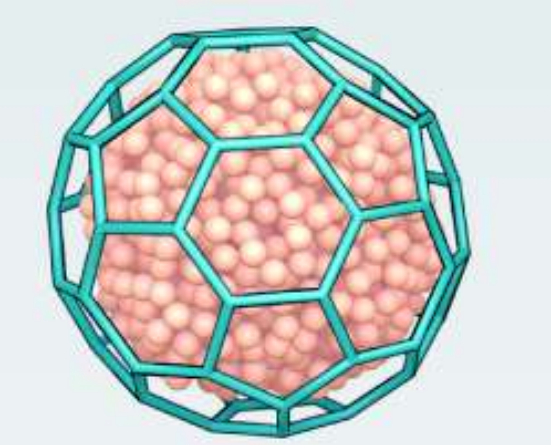
- Lysosomal enzymes possess phosphorylated mannose residues, which act as sorting signals
- Lysosomal enzymes carrying a mannose 6-phosphate signal are recognized and captured by mannose 6-phosphate receptors (MPRs), which are integral membrane proteins that span the TGN membranes

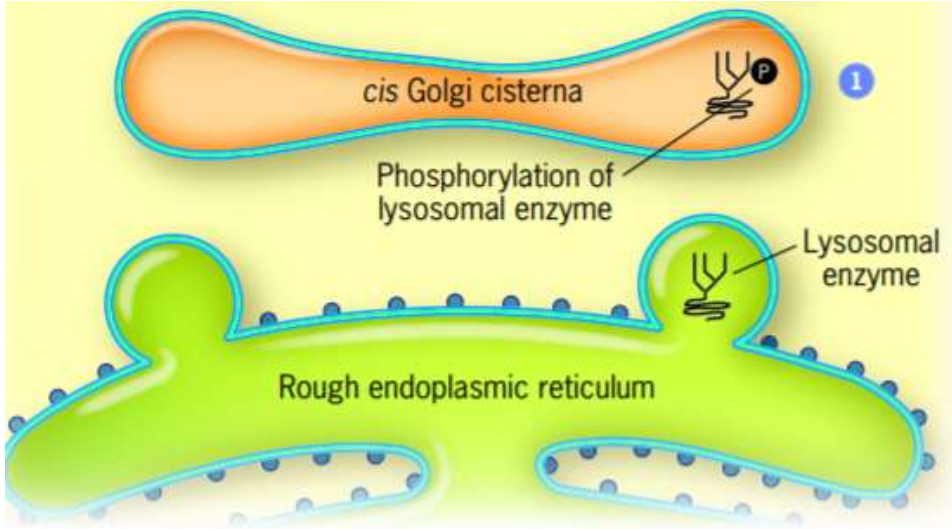
Transport (Clathrin coated vesicle) of Lysosomal Enzymes

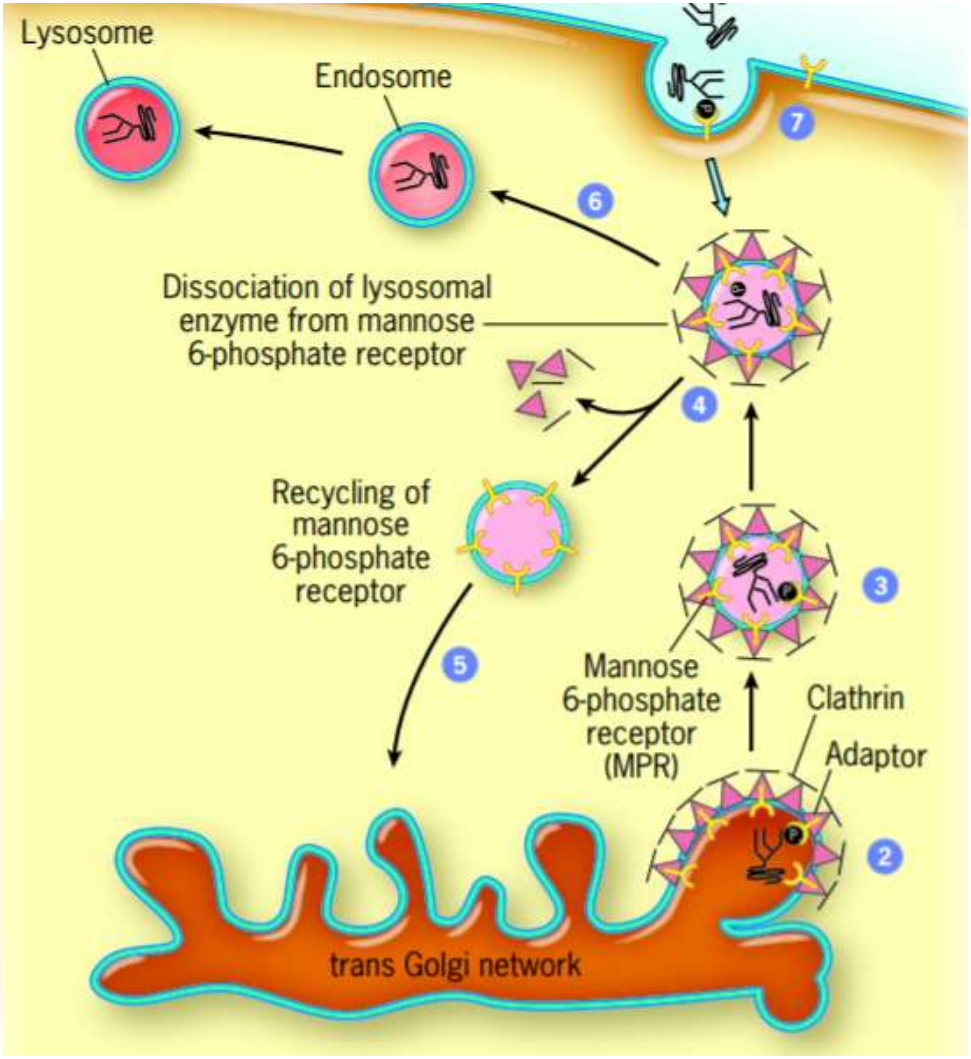


- **Clathrin protein**: forms outer scaffold
- **Adaptor protein**: interaction between outer clathrin scaffold and inner receptor bound with specific protein
- Example: GGA adaptor in lysosomal enzyme transport
- **Arf1** (small GTP-binding protein): recruits other clathrin-coated proteins

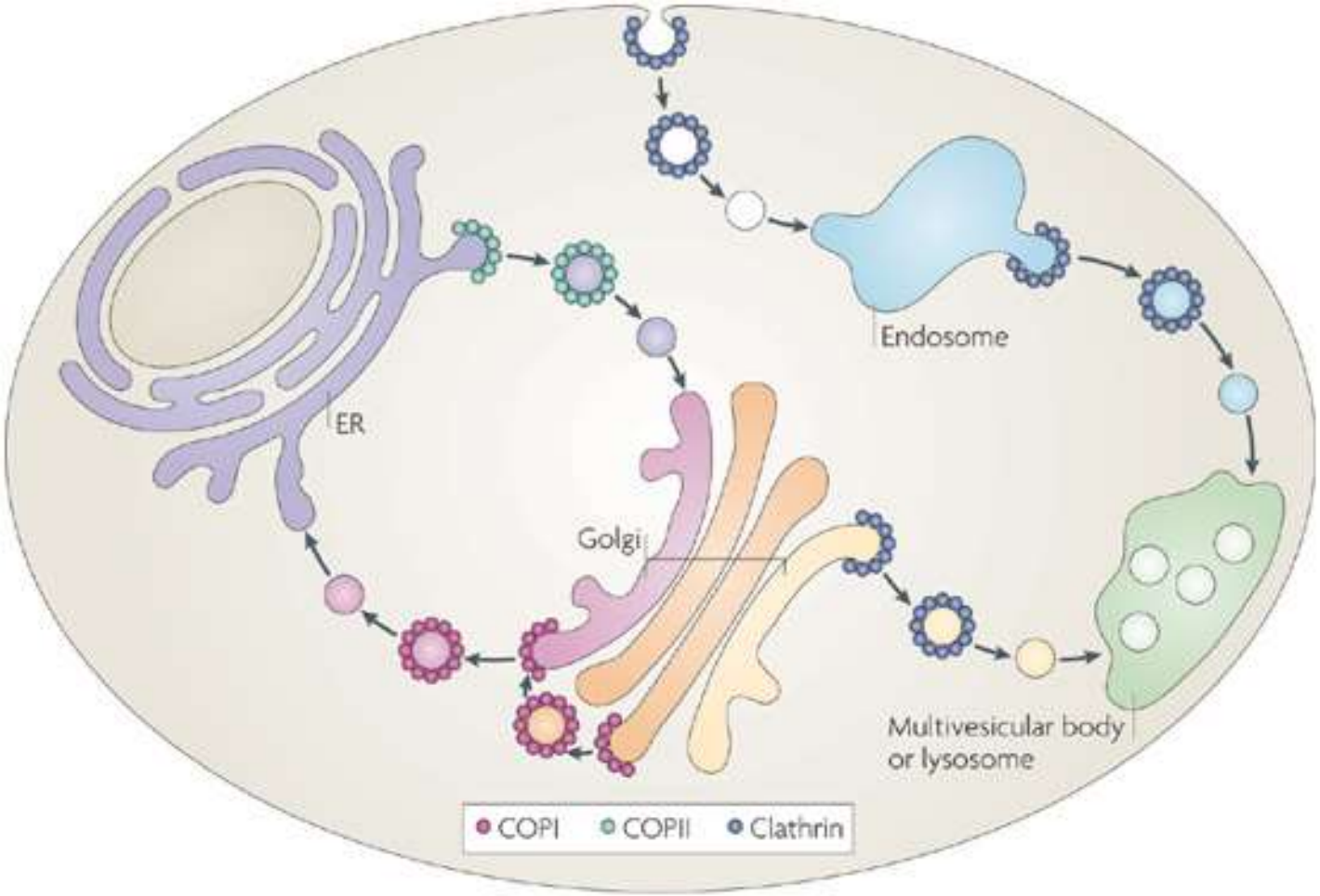
Sorting and Transport of Lysosomal Enzymes







Overview of types of vesicular transport



Targeting Vesicles to a Particular Compartment

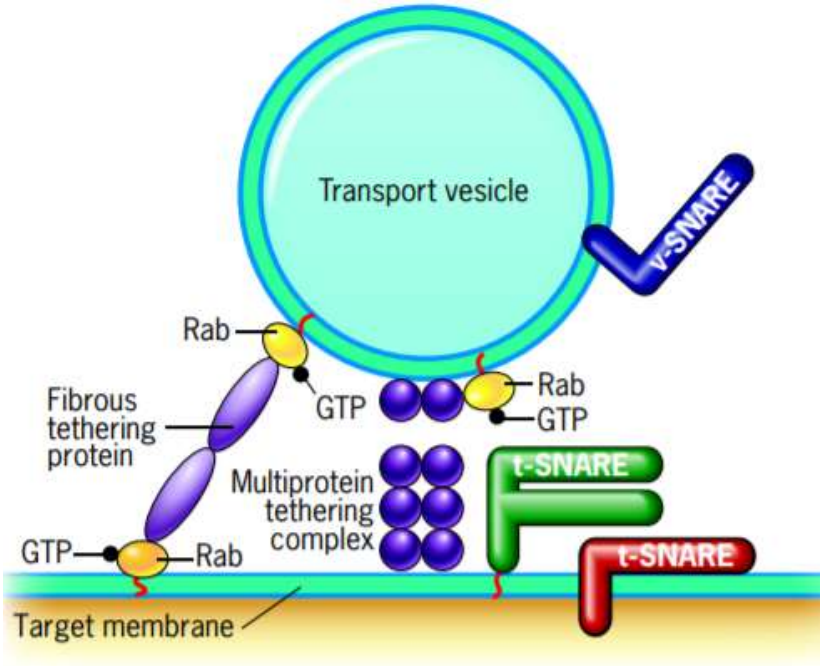
Step 1: Movement of the vesicle toward the specific target compartment

Step 2: Tethering vesicles to the target compartment: Tethering proteins

- Two groups of tethering proteins have been described:

➢ rod-shaped, fibrous proteins that are capable of forming a molecular bridge between the two membranes over a considerable distance (50–200 nm) (e.g., golgins and EEA1)

➢ large multiprotein complexes that appear to hold the two membranes in closer proximity (e.g., the exocyst and TRAPPI)



- Different tethering proteins initiate fusion between different types of membranes.

Targeting Vesicles to a Particular Compartment

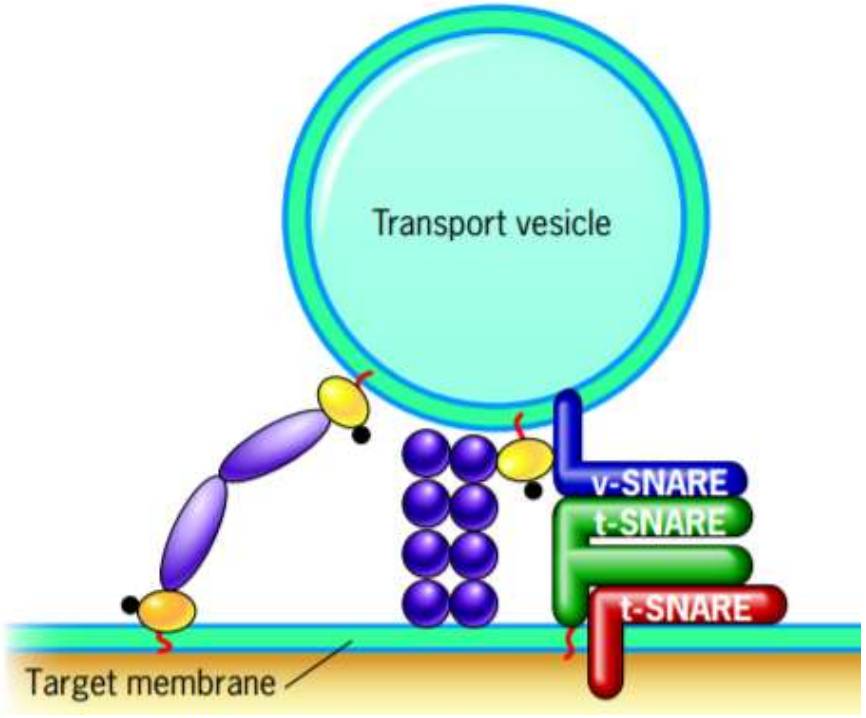
Step 2:

- In GTP-bound states, Rabs play a key role in vesicle targeting by recruiting specific cytosolic tethering proteins to specific membrane surfaces

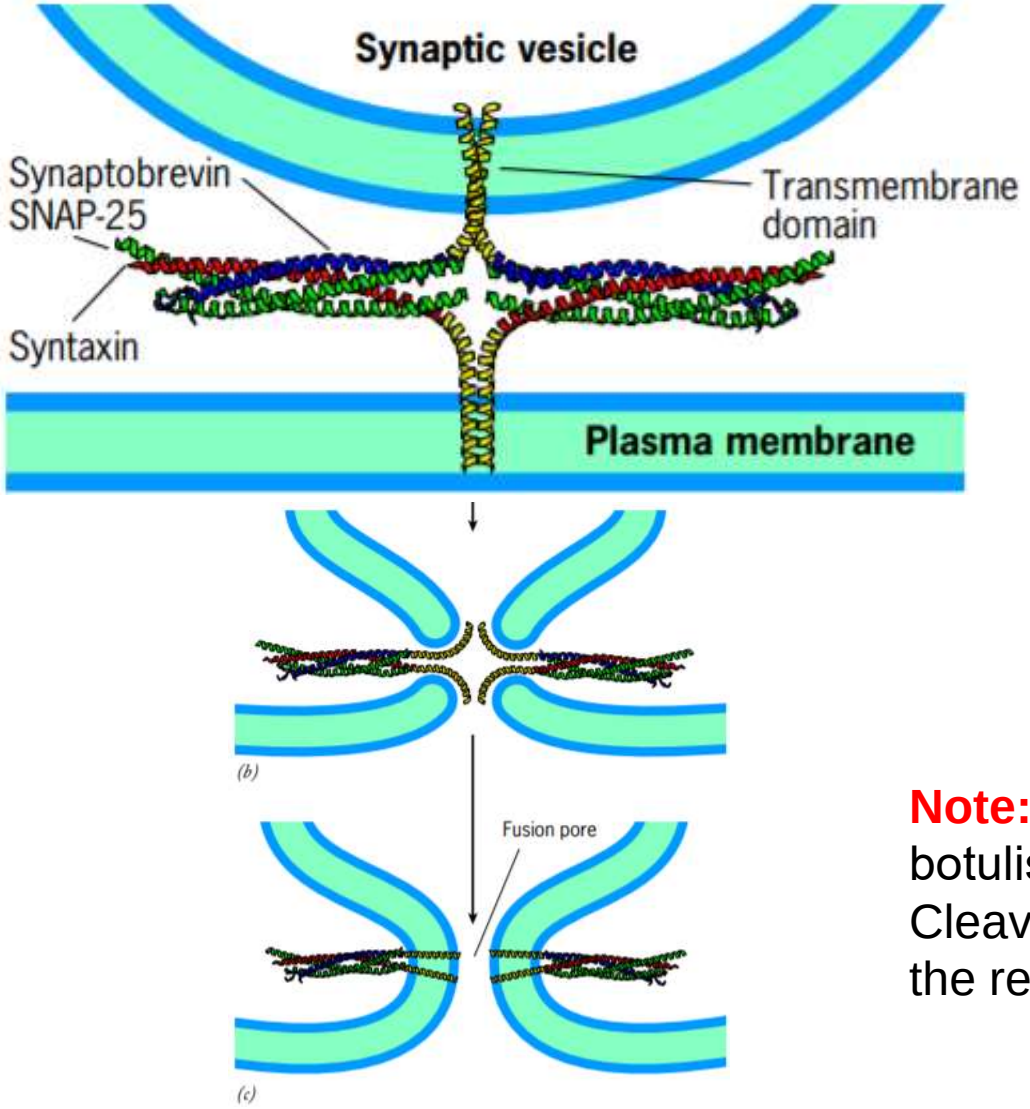
Step 3: Docking vesicles to the target compartment:

- Interaction between the cytosolic regions of integral proteins of the two membranes
- The key proteins that engage in these interactions are called SNAREs
- v-SNAREs: transport vesicles
- t-SNAREs: target compartments
- Cytosolic domain of SNARE: SNARE motif that consists of 60–70 amino acids capable of forming a complex with another SNARE motif

Step 4: Fusion between vesicle and target membranes



Fusion between synaptic vesicle and presynaptic membrane



- t-SNARE's of Plasma membrane of the nerve cell: **syntaxin** (red) and **SNAP-25** (green)
- v-SNARE of synaptic vesicle membrane: **synaptobrevin** (blue)
- Four helices: two donated by SNAP-25 and one each donated by syntaxin and synaptobrevin
- Interactions between t- and v-SNAREs are capable of pulling two lipid bilayers together with sufficient force to cause them to fuse

Note: The targets of bacterial toxins (acts as protease); botulism and tetanus are SNARE's.
Cleavage of the neuronal SNAREs by the toxins blocks the release of neurotransmitters, which causes paralysis.

Question 1

Which of the following sequences represents a possible pathway in the production of a secretory protein?

(a) Rough ER → Secretory vesicle → Ribosomes → Golgi apparatus

(b) Ribosomes → Rough ER → Golgi apparatus → Secretory vesicle

(c) Secretory vesicle → Golgi apparatus → Ribosomes → Rough ER

(d) Rough ER → Ribosomes → Secretory vesicles → Golgi apparatus

Question 2

Which one of the following is not a function of the Golgi apparatus?

(a) Protein synthesized by the ribosomes on the RER are transferred to Golgi, where it is accumulated in the sacs. These sacs may migrate to the surface of cell and discharge their content outside

(b) Protein filled sac may be retained within the cell as lysosome

(c) It is the site, where glycosylation of lipid takes place

(d) It is the site, where synthesis of phospholipid takes place

Question 3

BiP protein function in the ER. This has KDEL sequence, involved in the retrival pathway. What will happen to BiP protein if KDEL sequence will be lost

a. Ubiquitinated

b. Transported outside the cell

c. Will retain in golgi body

d. Will function normally in ER

Question 4

Q4. Which of the following organelle is the major site of new membrane synthesis in the cell

a) Golgi Body
b) Mitochondria
c) Endoplasmic Reticulum
d) Nucleolus

Question 5

Transport mediated from trans Golgi and from plasma membrane via:

1. COPII vesicles
2. Secretory vesicles
3. Clathrin coated vesicles
4. Endosomes