Molecular biology of the cell
BIO 207

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1) Sign-in to IS-Academia as usual, and select “retour indicatif” on the right.

2) Chose the course you want to evaluate.

Retour indicatif des enseignements (dès 2022-2023)

Répondre au questionnaire pour l'ensemble des matières :
Ingénierie des sciences du vivant, 2022-2023, Bachelor semestre 4

Répondre au questionnaire par matière :
Analyse numérique
Analysis IV (for SV, MT)
Biological chemistry II
Cellular and molecular biology II
Fluid mechanics (for SV)
Labo intégré en sciences de la vie II
Physique générale : quantique
Probability and statistics II
Technologie, économie et politique: en face des crises

3) Evaluate the course.

N'oubliez pas de sauver votre questionnaire

Vos réponses à ce questionnaire donneront à l'enseignant, aux directeurs de section concernés et à la Direction de l'Ecole une indication sur l'appréciation de cet enseignement par les étudiants. Donnez librement votre opinion personnelle. Ce questionnaire est anonyme.

Enseignement

Le déroulement du cours permet ma formation et un climat de classe approprié

Sans avis | Pas du tout d'accord | Pas d'accord | D'accord | Tout à fait d'accord

Merci de faire part de vos éventuelles remarques sur ce cours.
(Vos remarques seront lues par votre enseignant. Soyez constructifs et éviter les remarques malpolies ou injurieuses.
L'anonymat ne peut pas être garanti en cas de remarques potentiellement répréhensibles d'un point de vue légal).

Remarques

Intracellular Compartments and Protein Sorting

CHAPTER 12

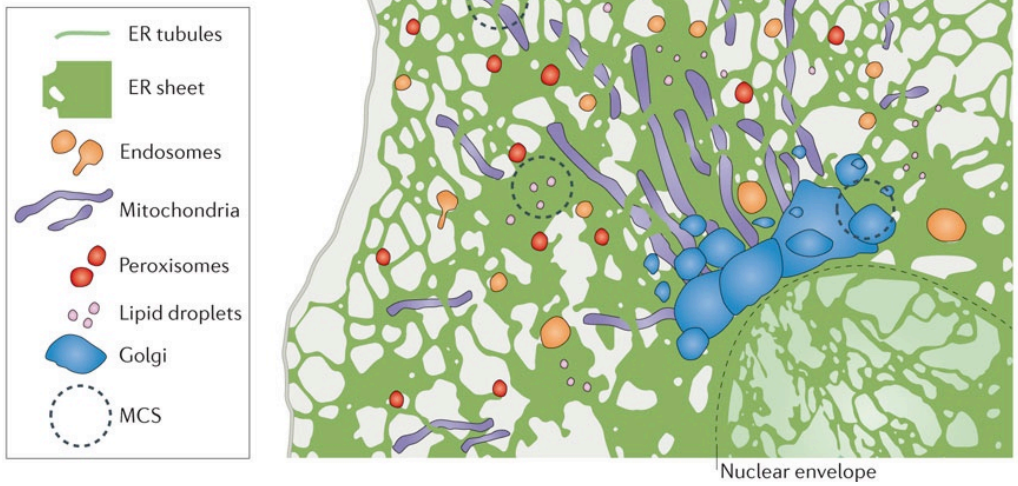
IN THIS CHAPTER

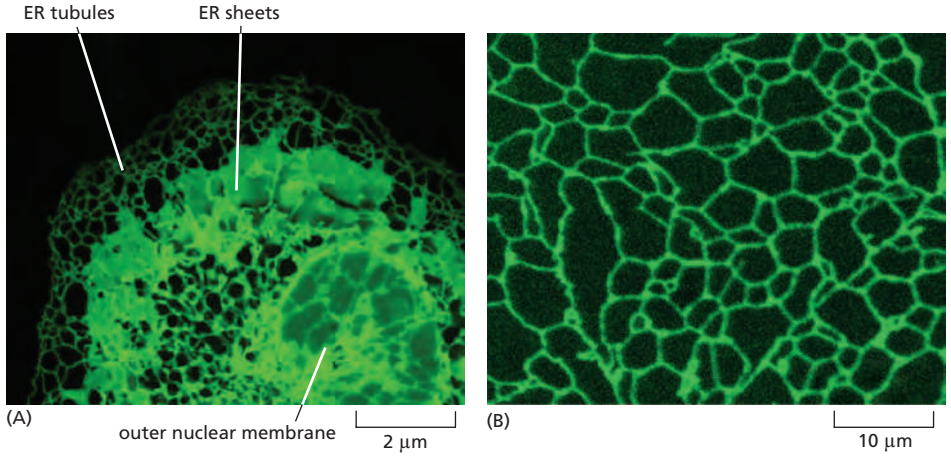
CHAPTER 13

Intracellular Membrane Traffic

IN THIS CHAPTER

Endoplasmic reticulum





ER lumen = ER cisternal space

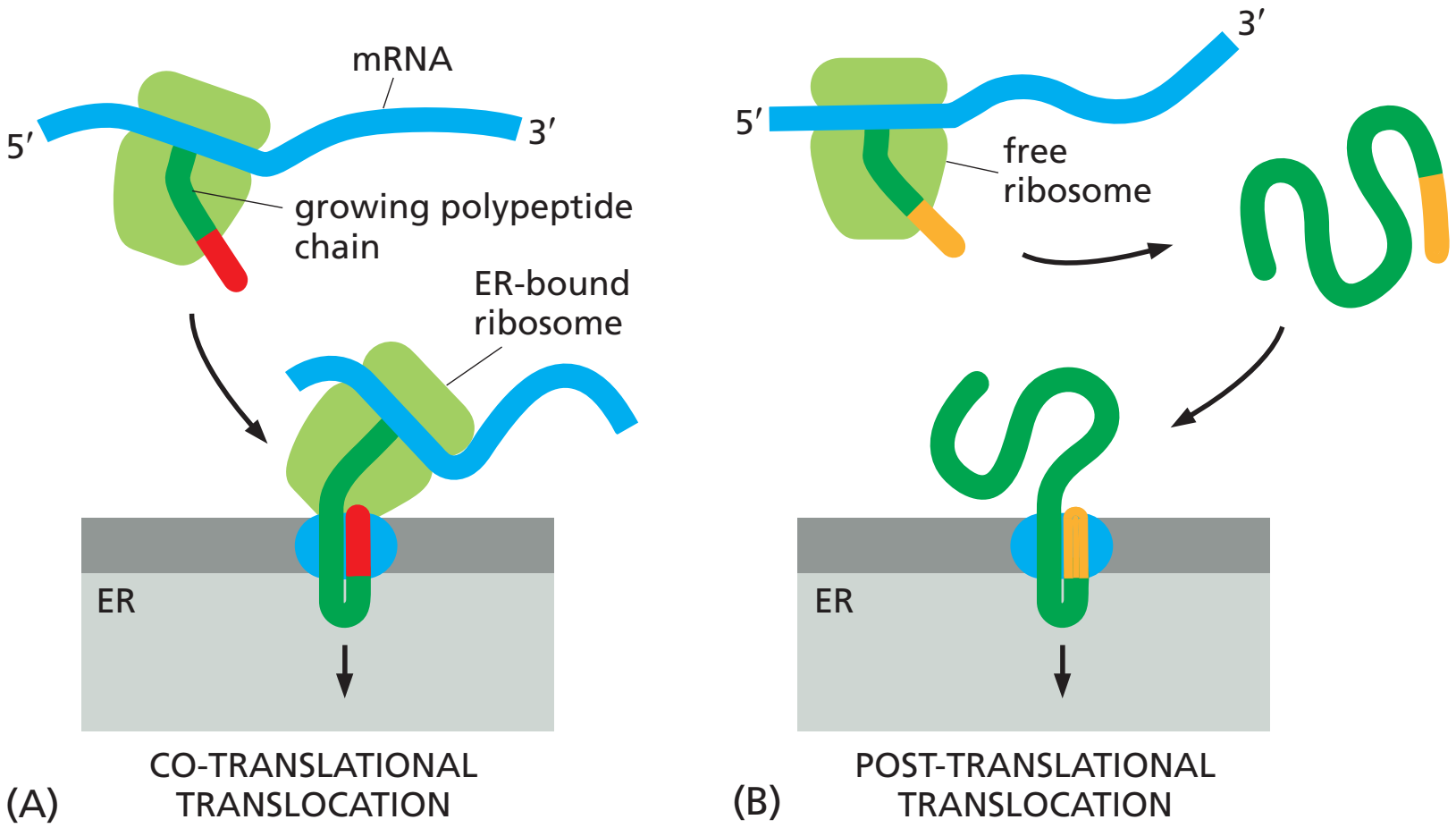
Lipid & Protein Biosynthesis

Site of production of all transmembrane proteins and lipids for all the organelles

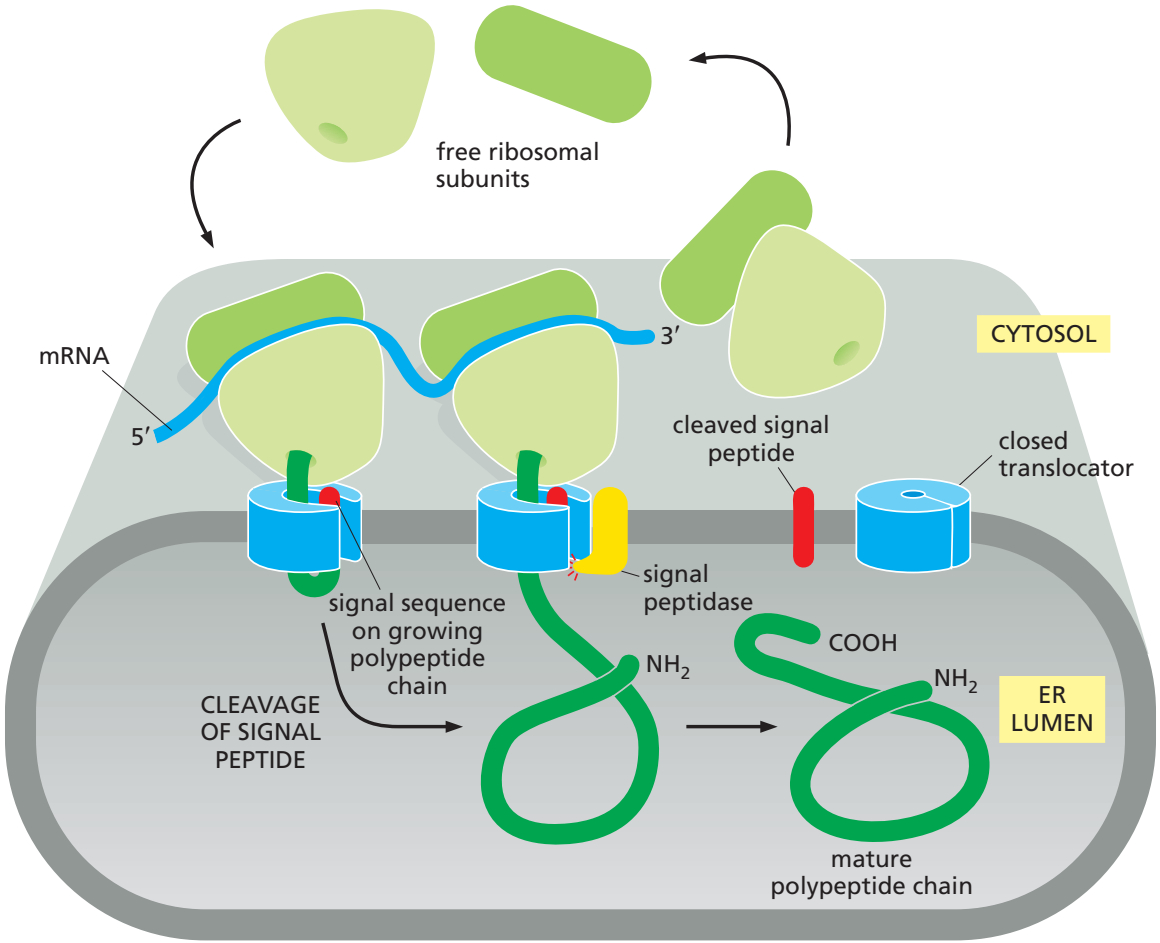
Ca2+ storage in the cell (SERCA pump)

Protein translocation

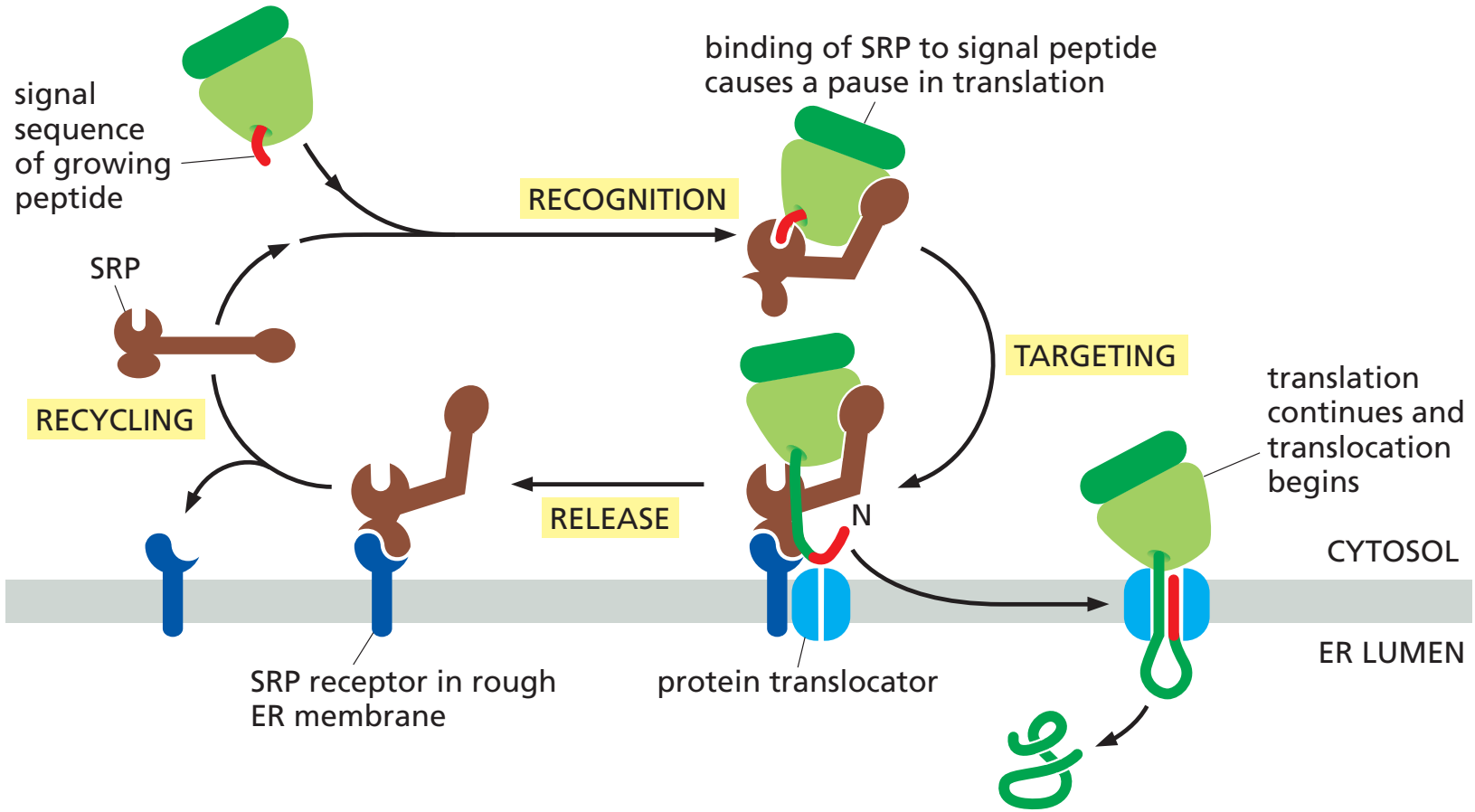
Co-translational and post- translational protein translocation to the ER



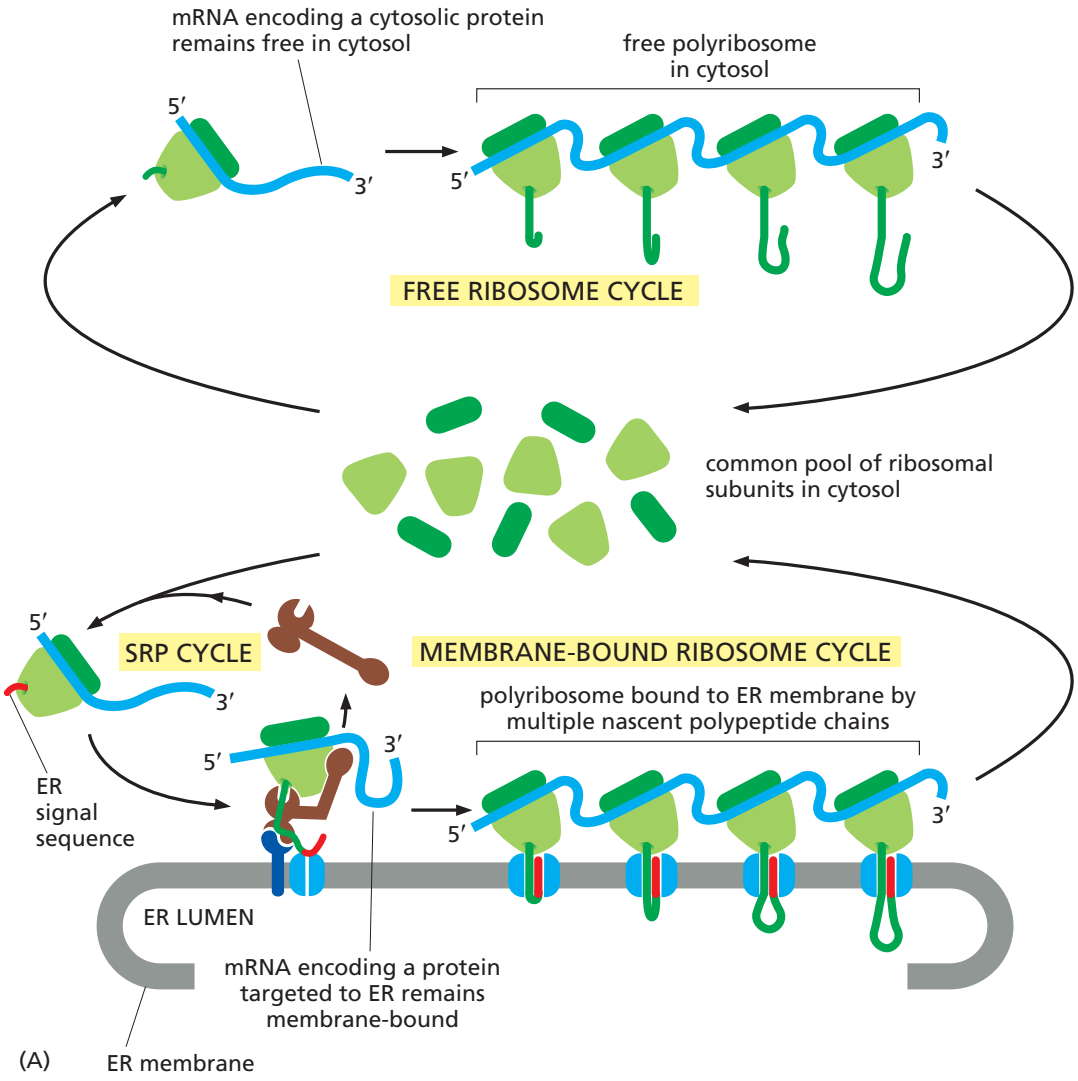
Co-translational translocation

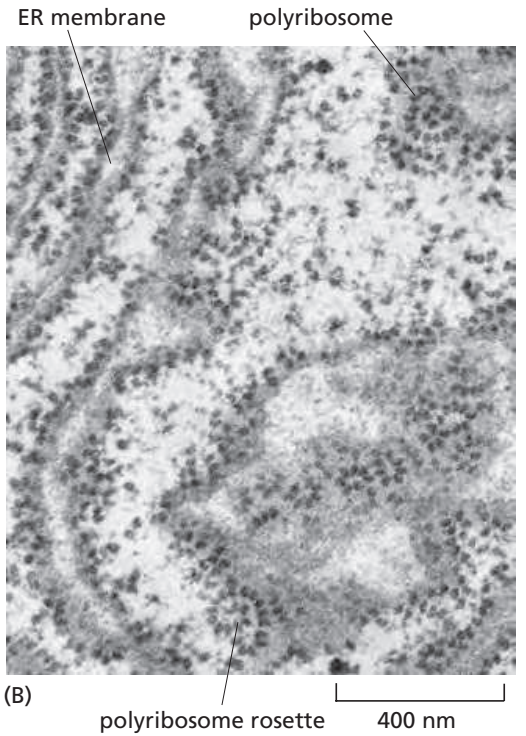


SRP directs ribosomes to the ER membrane



Free and membrane-bound polyribosomes





Structure of the Sec61 translocator complex

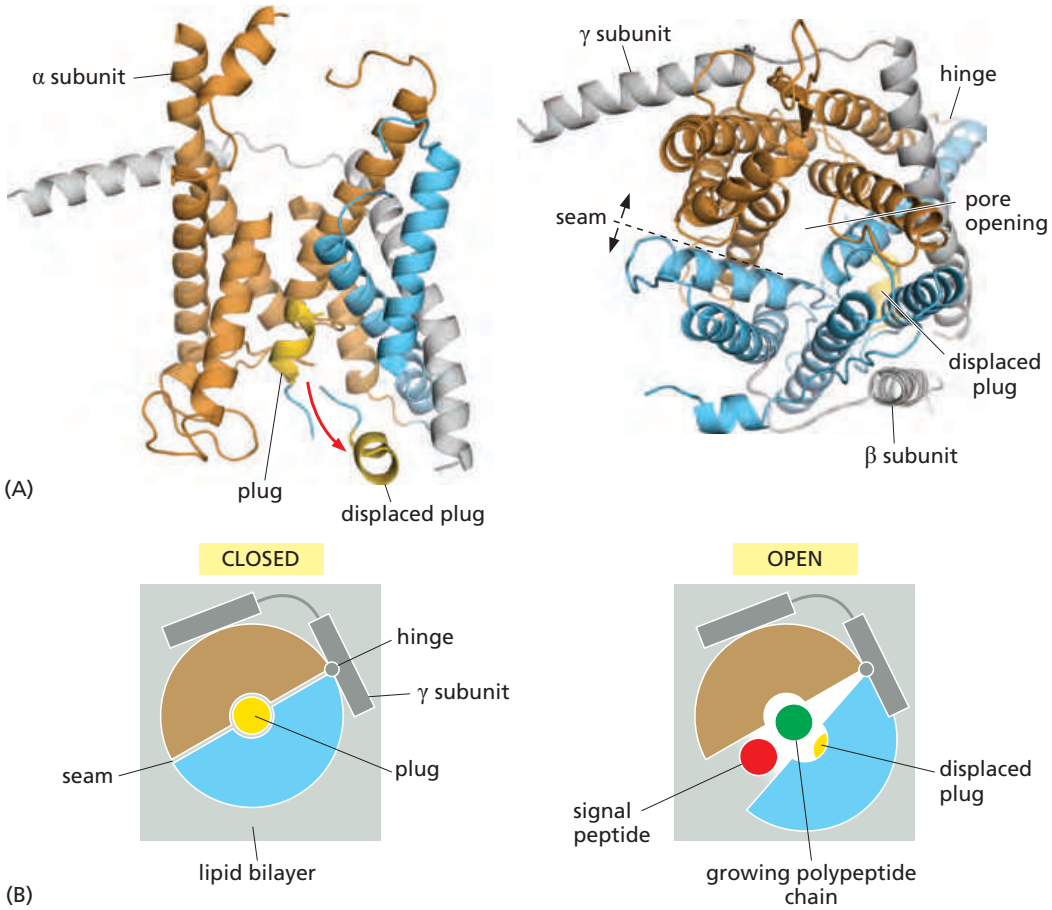
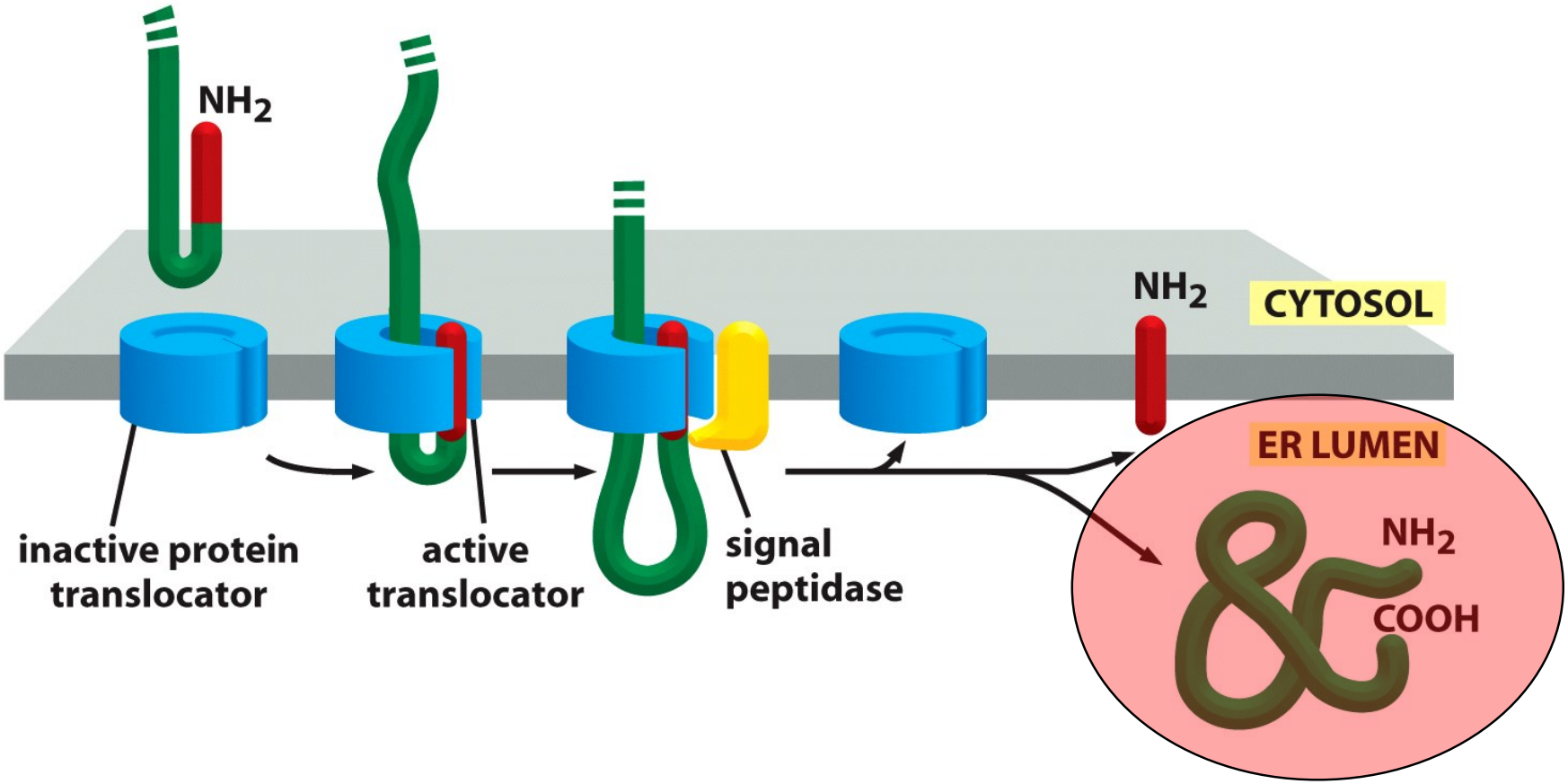
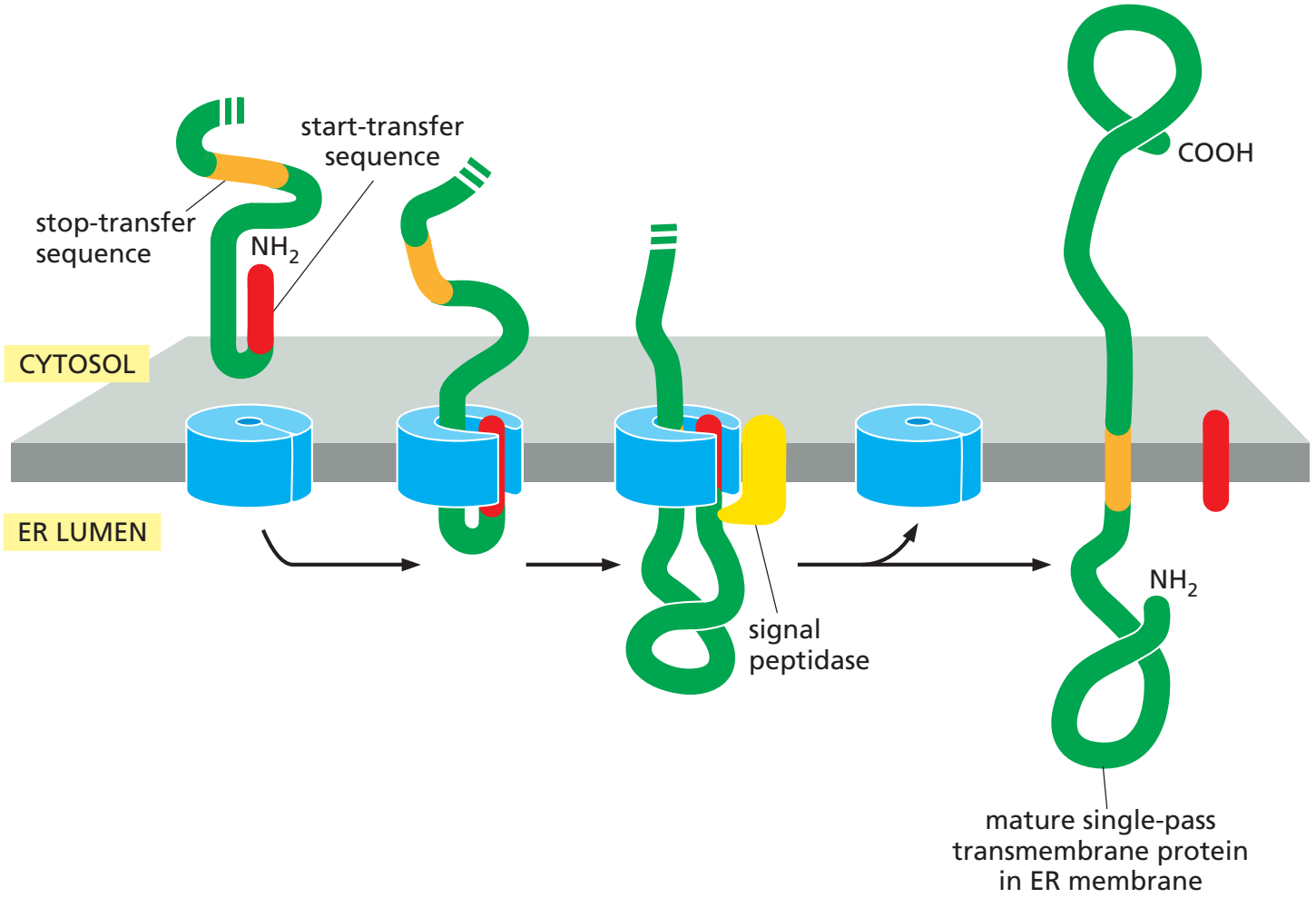


Figure 12–39 Structure of the Sec61 complex. (A) A side view *(left)* and a top view (*right*, seen from the cytosol) of the structure of the Sec61 complex of the archaeon *Methanococcus jannaschii*. The Sec61α subunit has an inverted repeat structure (see Figure 11–10) and is shown in *blue* and *beige* to indicate this pseudo-symmetry; the two smaller β and γ subunits are shown in *gray*. In the side view, some helices in front have been omitted to make the inside of the pore visible. The *yellow* short helix is thought to form a plug that seals the pore when the translocator is closed. To open, the complex rearranges itself to move the plug helix out of the way, as indicated by the *red* arrow. A ring of hydrophobic amino acid side chains is thought to form a tight-fitting diaphragm around translocating polypeptide chain to prevent leaks of other molecules across the membrane. The pore of the Sec61 complex can also open sideways at a lateral seam. (B) Models of the closed and open states of the translocator are shown in top view, illustrating how a signal sequence (or a transmembrane segment) could be released into the lipid bilayer after opening of the seam. (PDB codes: 1RH5 and 1RHZ.)

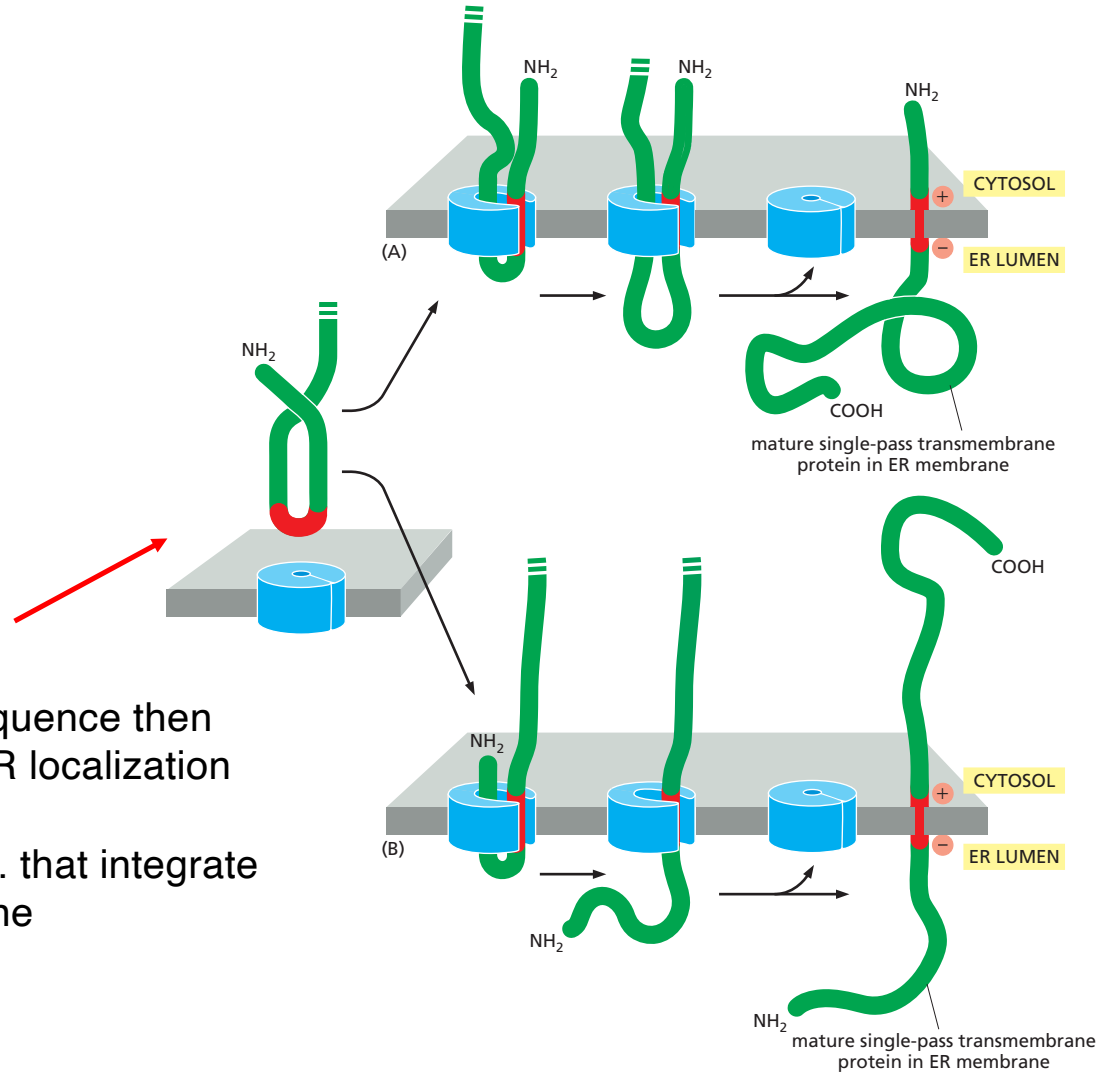
A protein translocating to the ER lumen



A single-pass transmembrane protein with a cleaved ER signal sequence

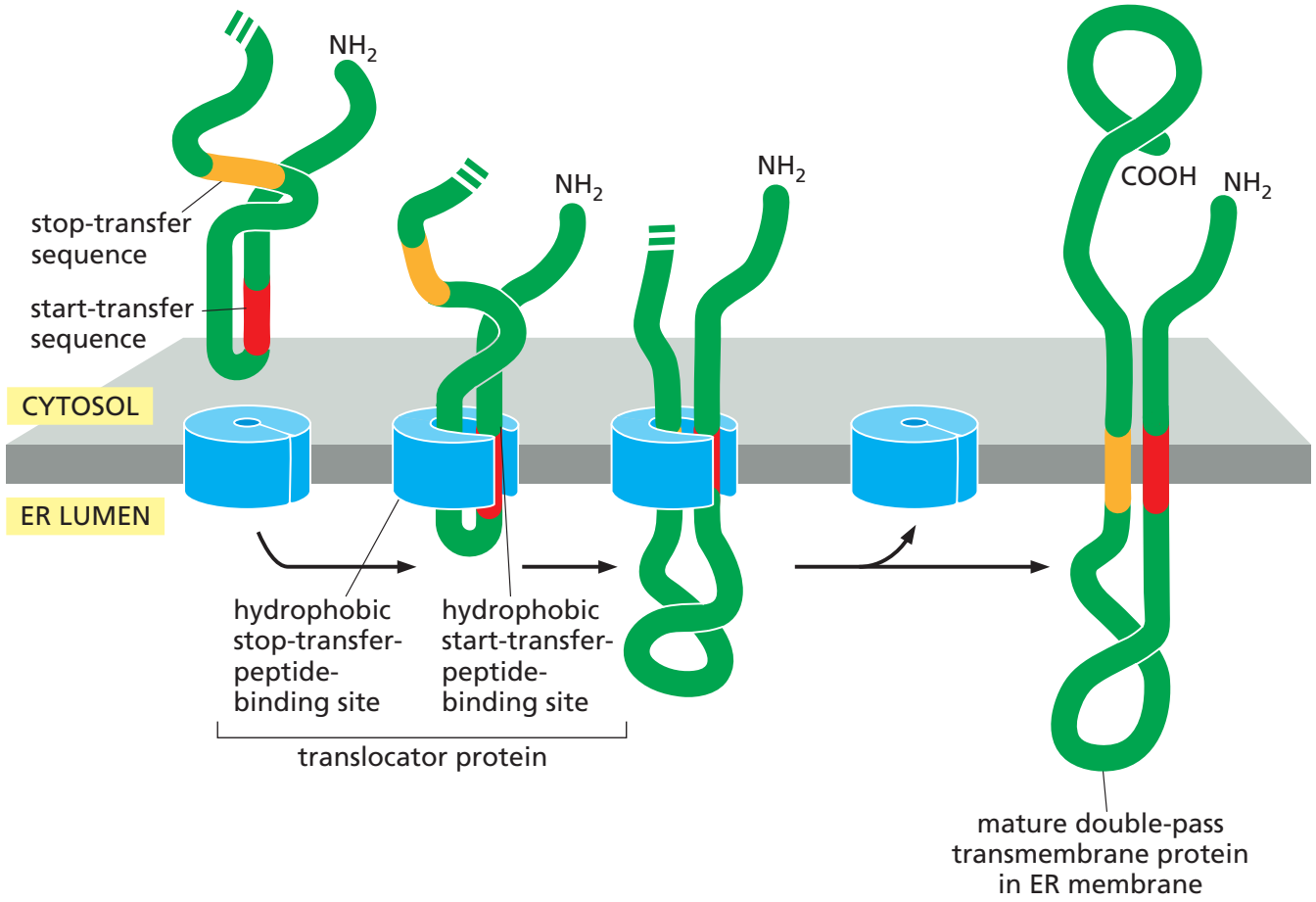


Integration of a single- pass transmembrane protein with an internal signal sequence into the ER membrane

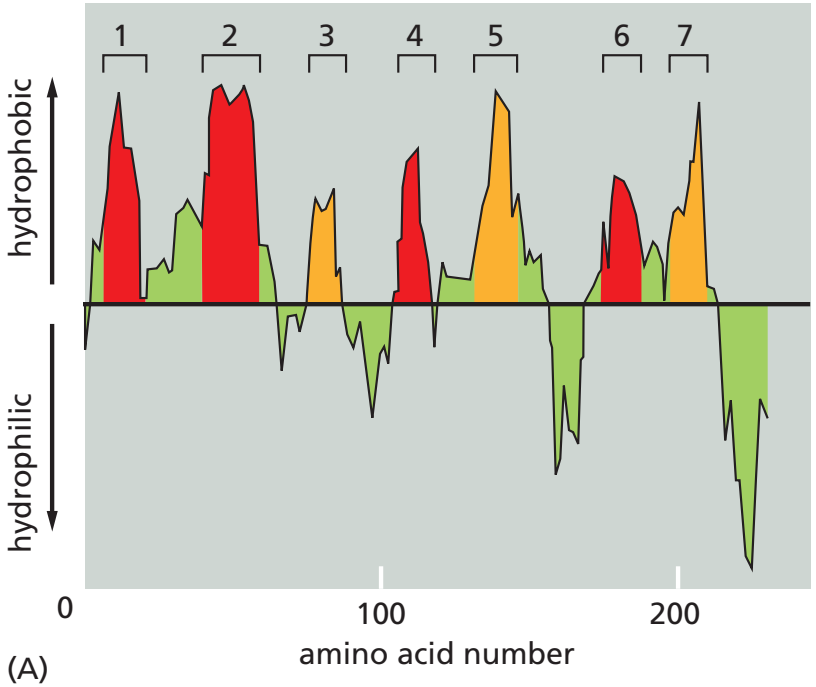


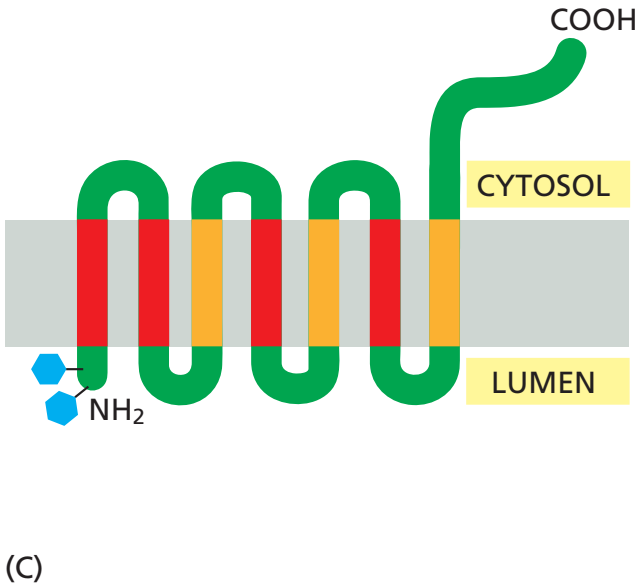
Different A.A. sequence then the N-terminal ER localization sequence!
Hydrophobic A.A. that integrate into the membrane

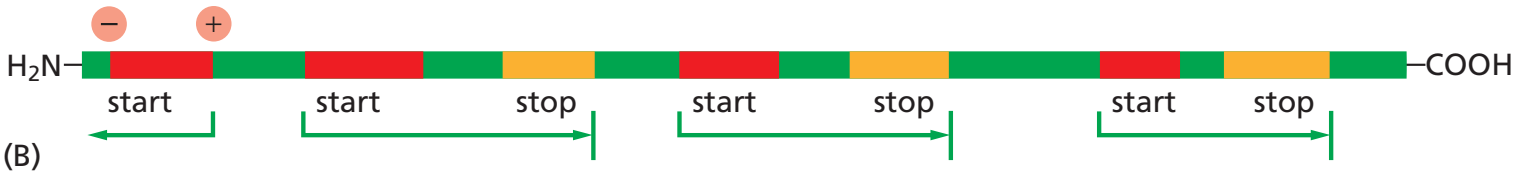
Integration of a double- pass transmembrane protein with an internal signal sequence into the ER membrane



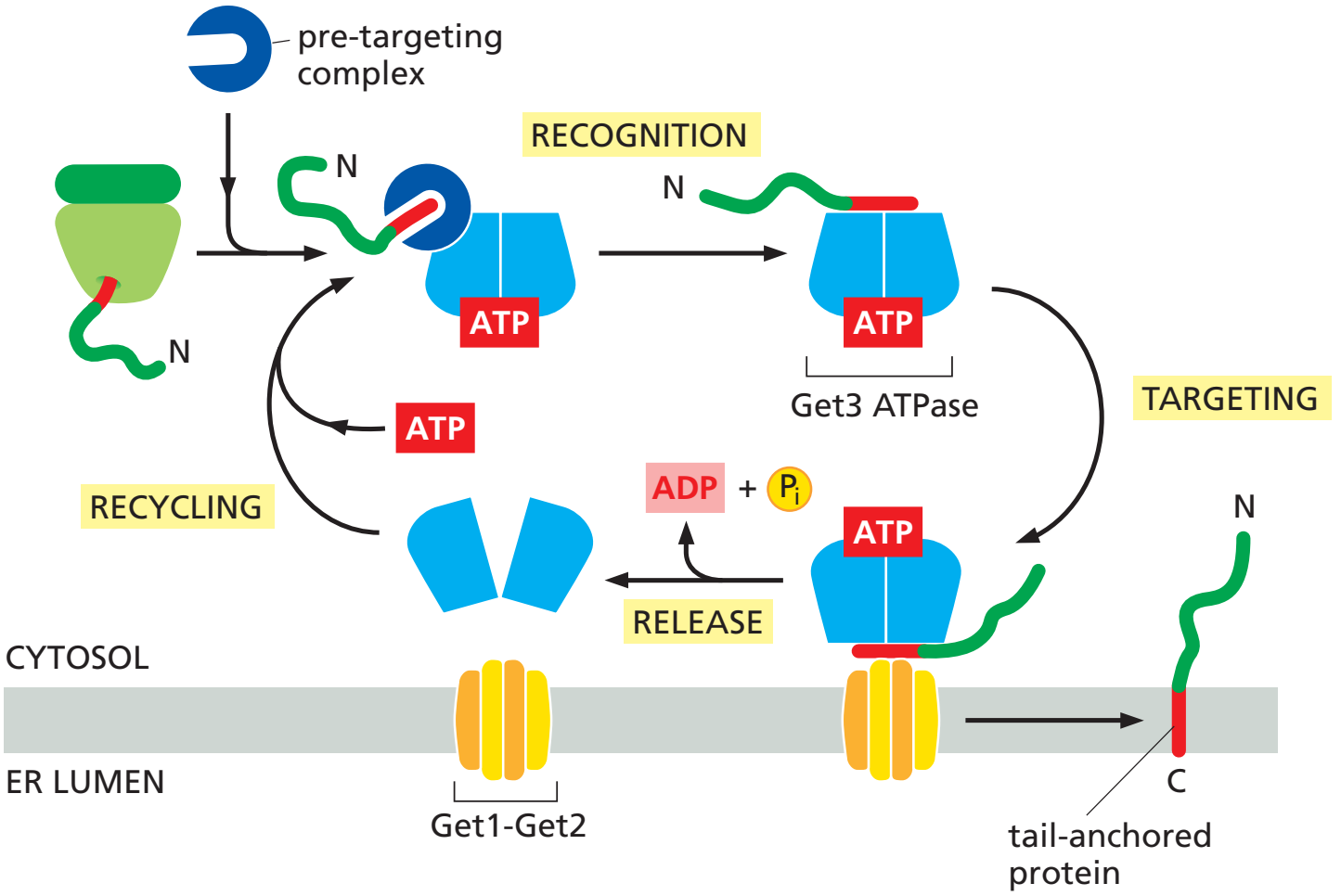
The insertion of the multipass membrane protein rhodopsin into the ER membrane







Special case insertion of tail-anchored proteins



The attachment of a GPI anchor to a protein in the ER

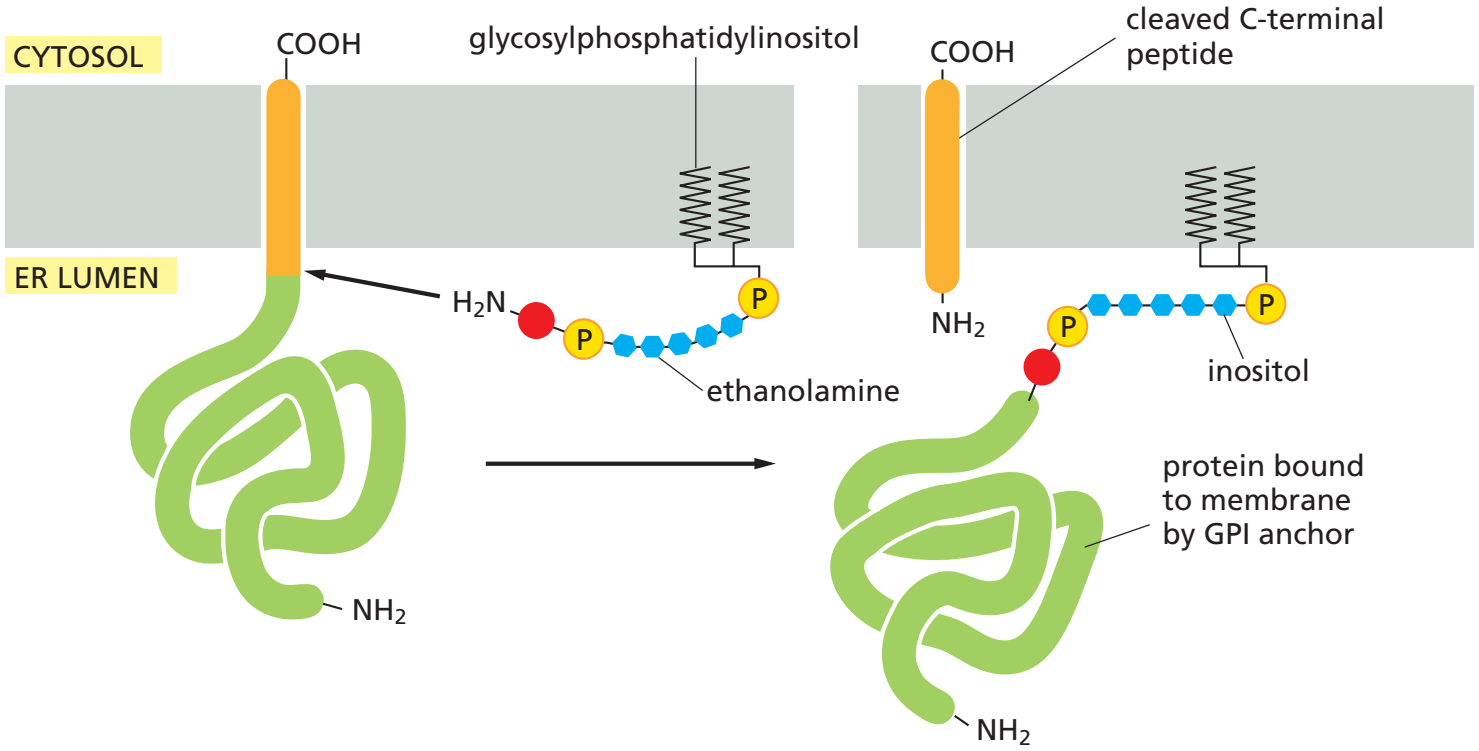


Figure 12–52 The attachment of a GPI anchor to a protein in the ER. GPI-anchored proteins are targeted to the ER membrane by an N-terminal signal sequence (not shown), which is removed (see Figure 12–42). Immediately after the completion of protein synthesis, the precursor protein remains anchored in the ER membrane by a hydrophobic C-terminal sequence of 15–20 amino acids; the rest of the protein is in the ER lumen. Within less than a minute, an enzyme in the ER cuts the protein free from its membrane-bound C-terminus and simultaneously attaches the new C-terminus to an amino group on a preassembled GPI intermediate. The sugar chain contains an inositol attached to the lipid from which the GPI anchor derives its name. It is followed by a glucosamine and three mannoses. The terminal mannose links to a phosphoethanolamine that provides the amino group to attach the protein. The signal that specifies this modification is contained within the hydrophobic C-terminal sequence and a few amino acids adjacent to it on the lumenal side of the ER membrane; if this signal is added to other proteins, they too become modified in this way. Because of the covalently linked lipid anchor, the protein remains membrane-bound, with all of its amino acids exposed initially on the lumenal side of the ER and eventually on the exterior of the plasma membrane.

Summary of ER protein translocation

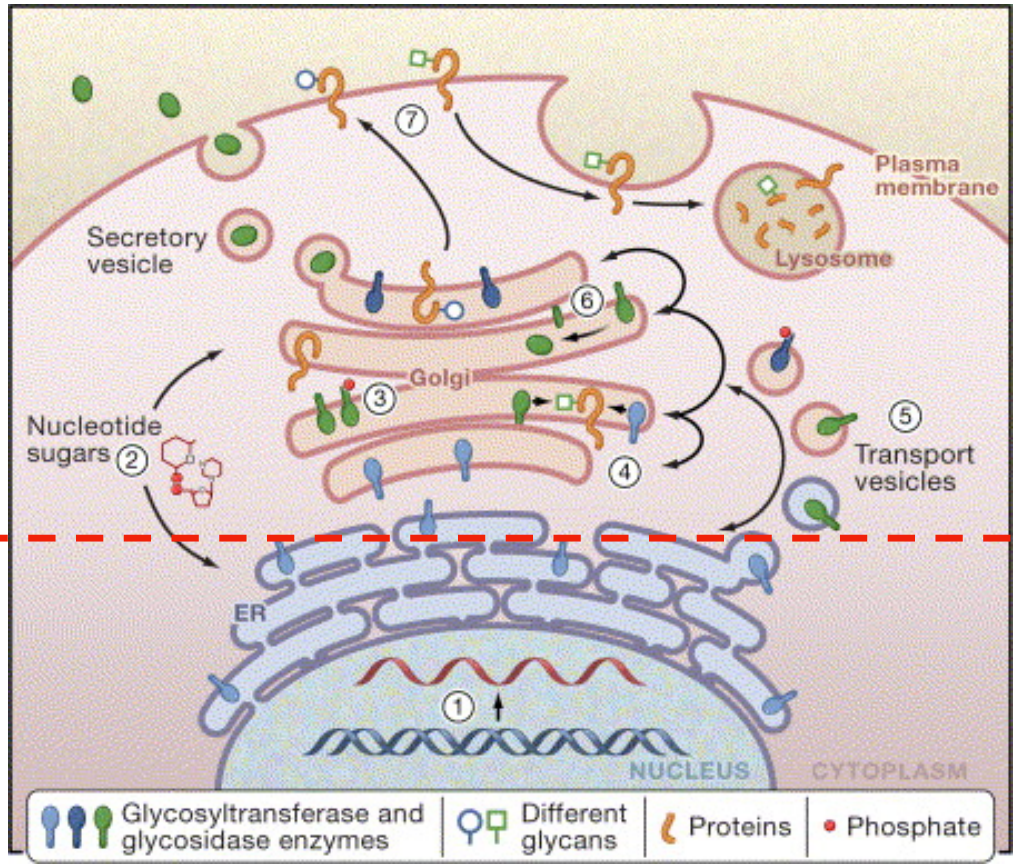
Translocation of proteins to the ER

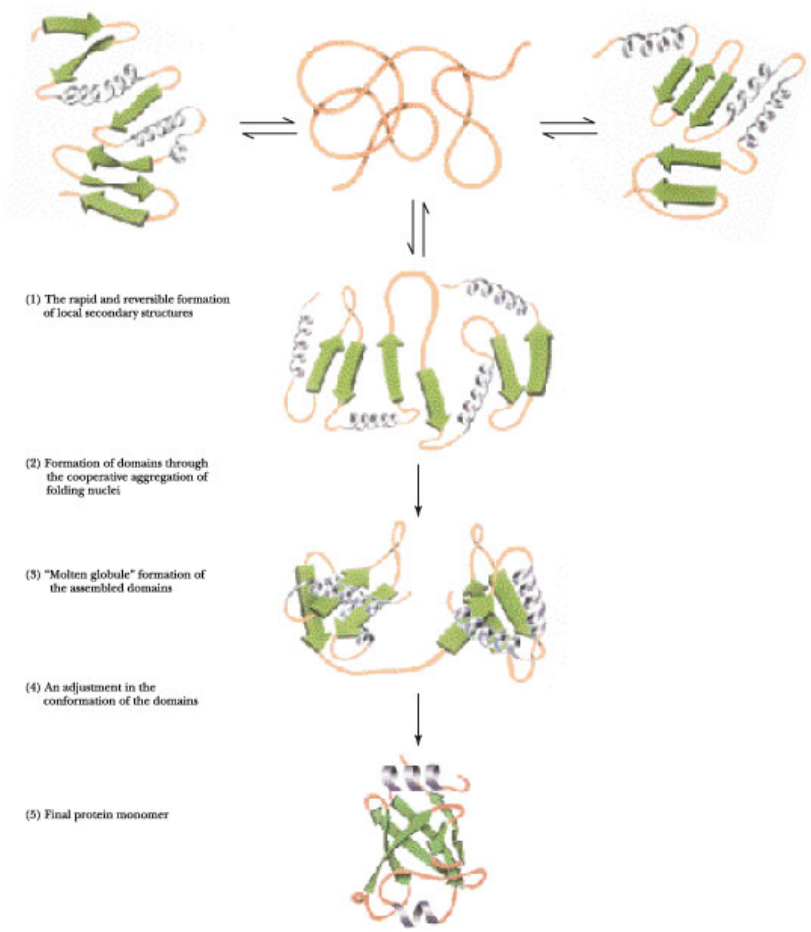
1: Soluble proteins. All soluble (non membrane bound) proteins must have an ER localization signal -> N-Terminal Amino Acid sequence, which is cleaved off.
2: Membrane proteins

- Membrane proteins can have an N-Terminal ER localization signal: this is generally the case for proteins whose N-terminus is in the lumen/extracellular
- Multipass integration of membrane proteins is driven by START/STOP sequences.
- This N-Terminal ER localization signal is not obligatory! Proteins where the N-terminus is cytoplasmic have an "internal" Signal sequence (=at a transmembrane domain), or a tail anchor
- GPI anchors are a special case of membrane attachment

What happens (can happen) to a protein inside the lumen of the ER and Golgi?

Quality control of proteins takes places in the ER





Protein folding often requires assistance in particular to avoid aggregation during phases when hydrophobic amino acids are exposed

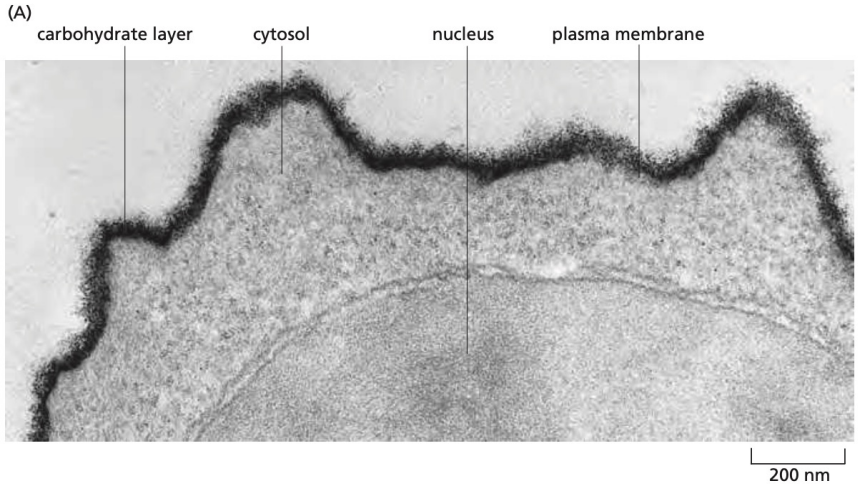
This assistance is provided by proteins called "chaperones".

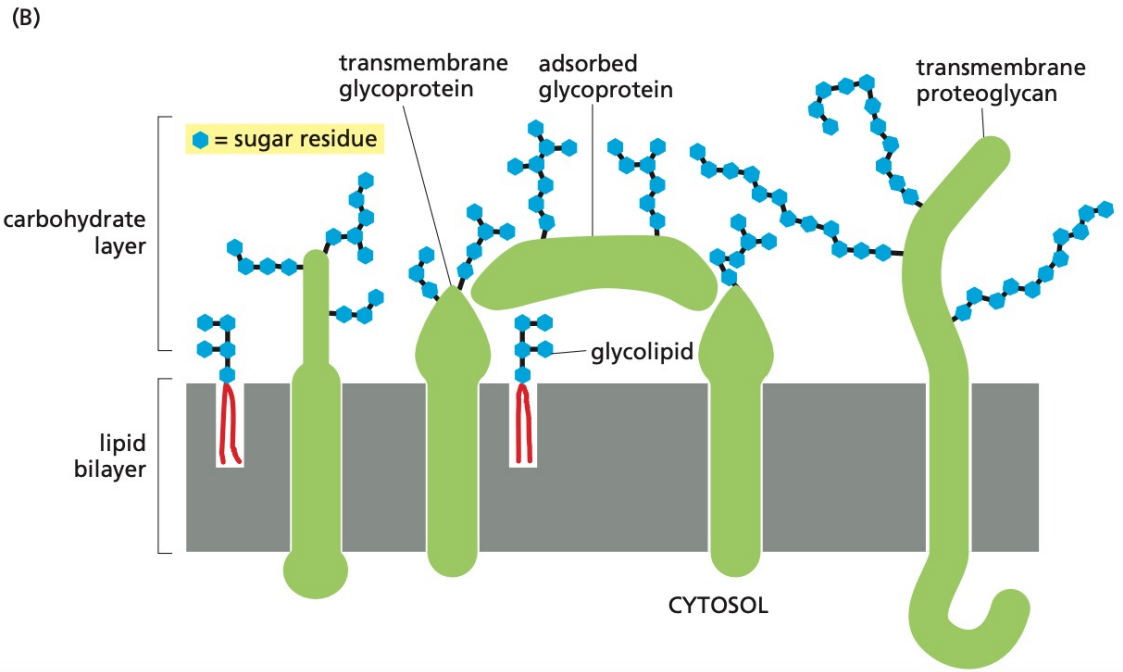
Protein folding can be influenced by post translational modifcations

Reversible
- Formation of disulfide bridges
- Isomerization of prolines
- Phosphorylation(Not in the ER)
- Ubiquitination (Not in the ER)

Irreversible (meaning a cell does not edit these once done, during degradation this can be different!)
- Proteolytic cleavage
- Glycosylation
- Anchoring on a GPI

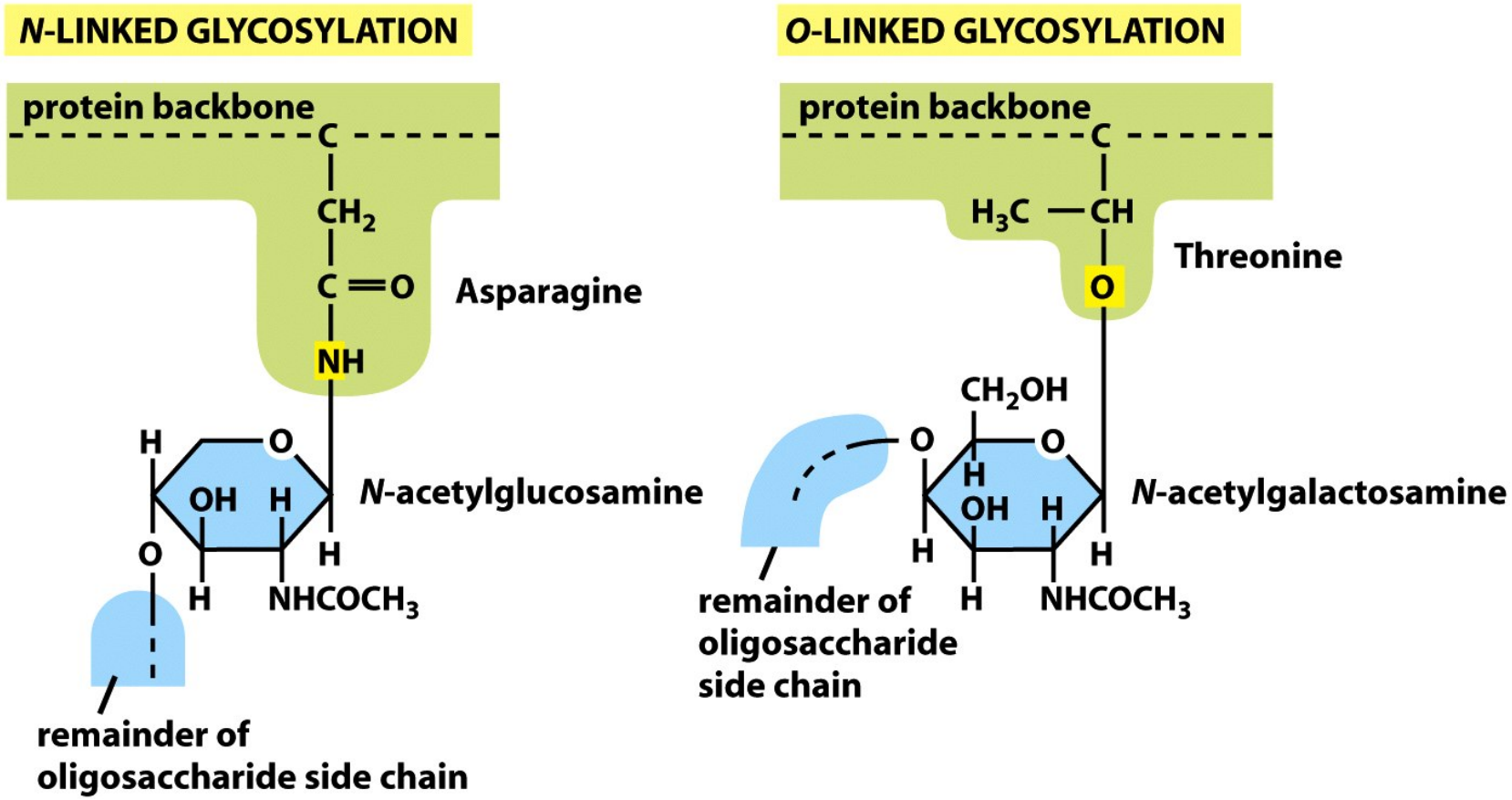
The carbohydrate layer on the cell surface





Directionality as a consequence of location of discussed last week!

N- and O- linked glycosylation



ER

Golgi

Synthesis of the lipid- linked precursor oligosaccharide in the rough ER membrane

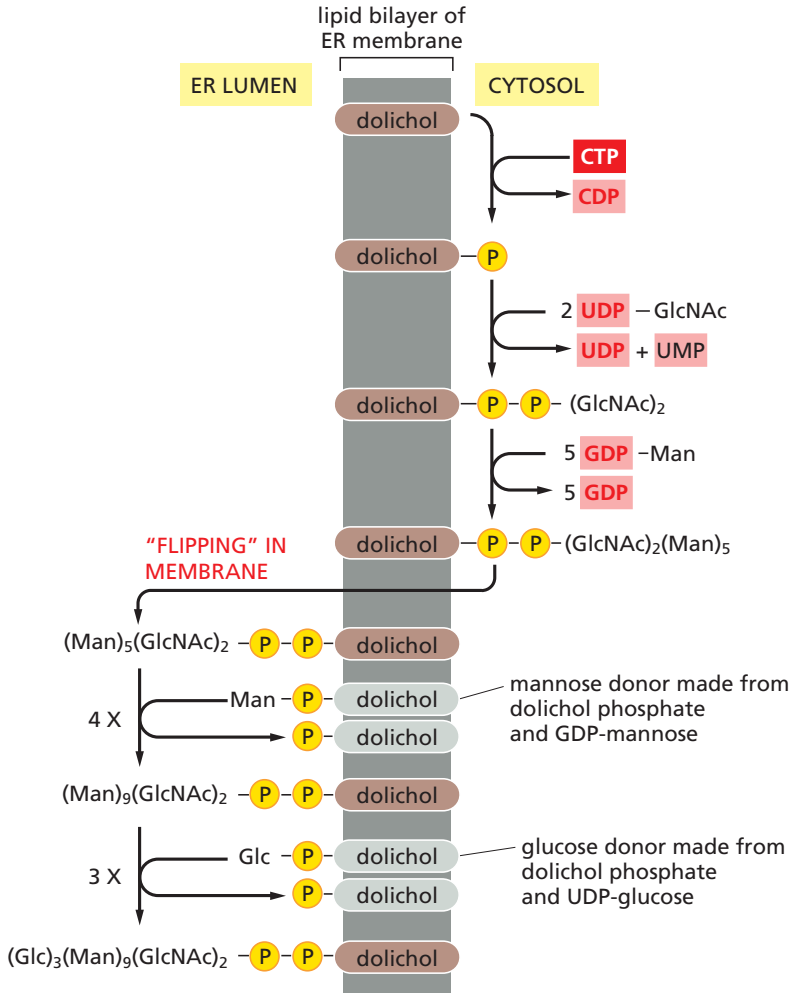
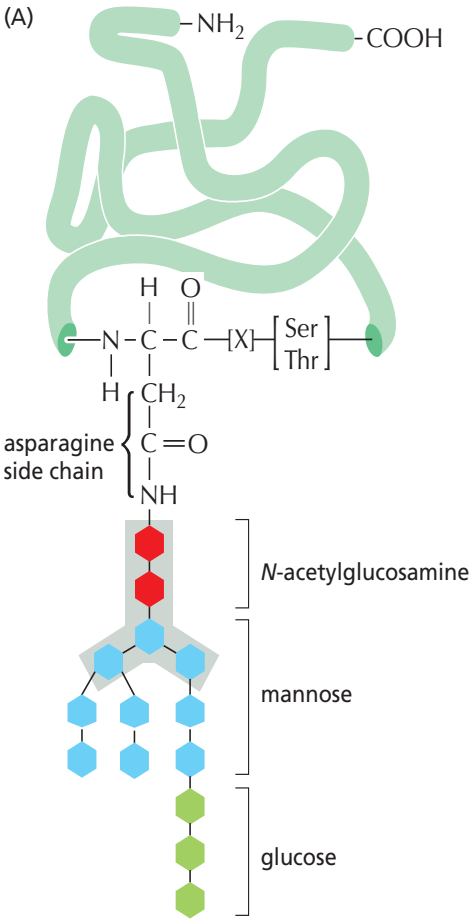
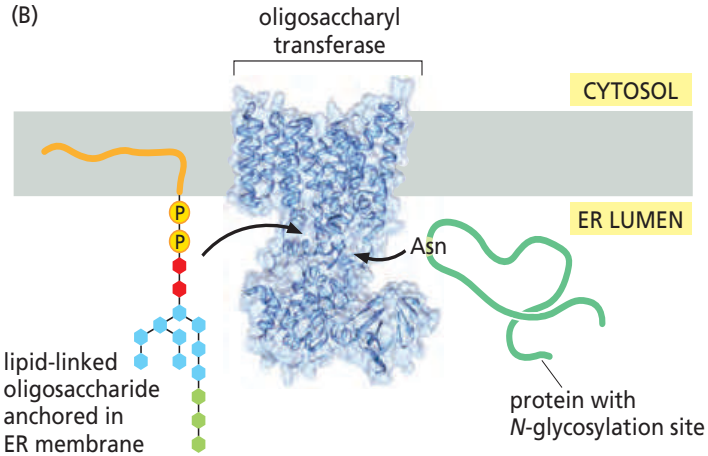


Figure 12–48 Synthesis of the lipid-linked precursor oligosaccharide in the rough ER membrane. The oligosaccharide is assembled sugar by sugar onto the carrier lipid dolichol (a polyisoprenoid; see Panel 2–5, pp. 98–99). Dolichol is long and very hydrophobic: its 22 five-carbon units can span the thickness of a lipid bilayer more than three times, so that the attached oligosaccharide is firmly anchored in the membrane. The first sugar is linked to dolichol by a pyrophosphate bridge. This high-energy bond activates the oligosaccharide for its eventual transfer from the lipid to an asparagine side chain of a growing polypeptide on the lumenal side of the rough ER. As indicated, the synthesis of the oligosaccharide starts on the cytosolic side of the ER membrane and continues on the lumenal face after the $(Man)_5(GlcNAc)_2$ lipid intermediate is flipped across the bilayer by a transporter (which is not shown). All the subsequent glycosyl transfer reactions on the lumenal side of the ER involve transfers from dolichol-P-glucose and dolichol-P-mannose; these activated, lipid-linked monosaccharides are synthesized from dolichol phosphate and UDP-glucose or GDP-mannose (as appropriate) on the cytosolic side of the ER and are then flipped across the ER membrane. GlcNAc = *N*-acetylglucosamine; Man = mannose; Glc = glucose.

N-linked protein glycosylation in the rough ER

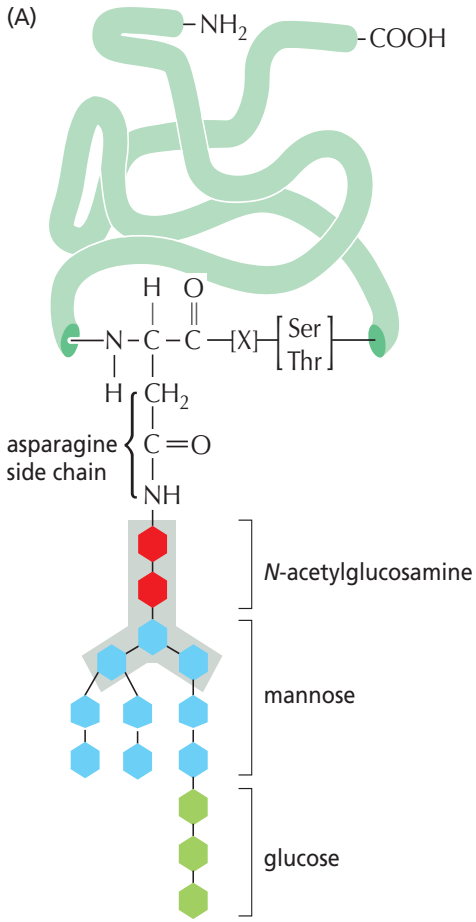




Most plasma membrane proteins and secreted proteins are N-glycosylated (>80%)
This helps their folding, protects them against proteases, and has other functions as well

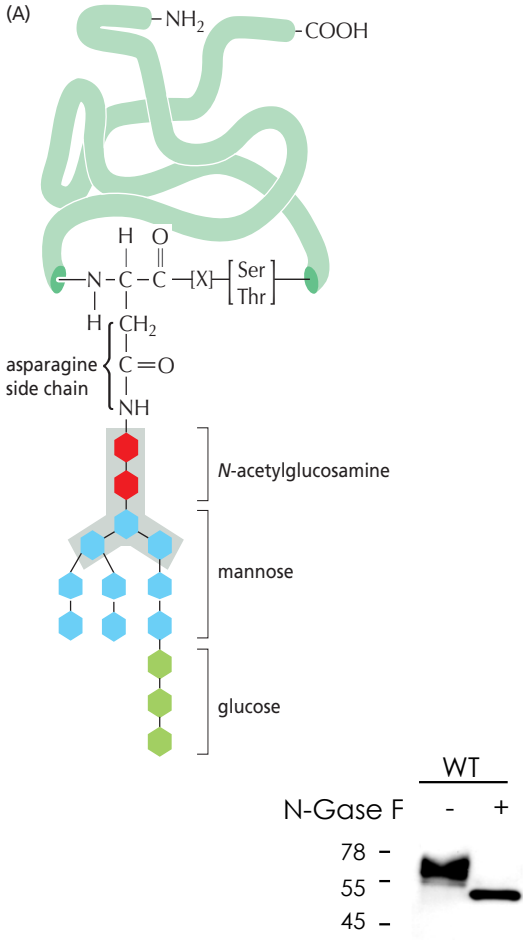
Glycosylation

What is the purpose of these sugar "trees"?
- The presence of this hydrophilic group favors the folding of the protein
- Their presence on the surface of the protein, on the surface of the cell, protects from extracellular protease
- The sugars are codes that inform the cell of what is happening and what must follow:
- Is the protein already folded? - How long has the protein been trying to fold? protein been trying to fold?
- Etc... (some of which are still to be discovered)



Necessary but not necessarily sufficient condition, consensus sequence:
Asn-X-Ser/Thr
The sugar "tree" is added en bloc to the ER lumen

Glycosylation influences analysis



Addition of sugars changes the mobility of a protein on an SDS gel

Migration varies with the number of glycosylation sites, complexity and heterogeneity of sugars

N-glycosidase F: enzymatically removes N-linked sugars.

A change in gel mobility following N-Gly-F treatment is evidence of N-glycosylation

N-linked protein glycosylation in the rough ER

N-glycosylation
The tree of sugars is pre-assembled and added to the protein
by an oligosaccharyl-transferase

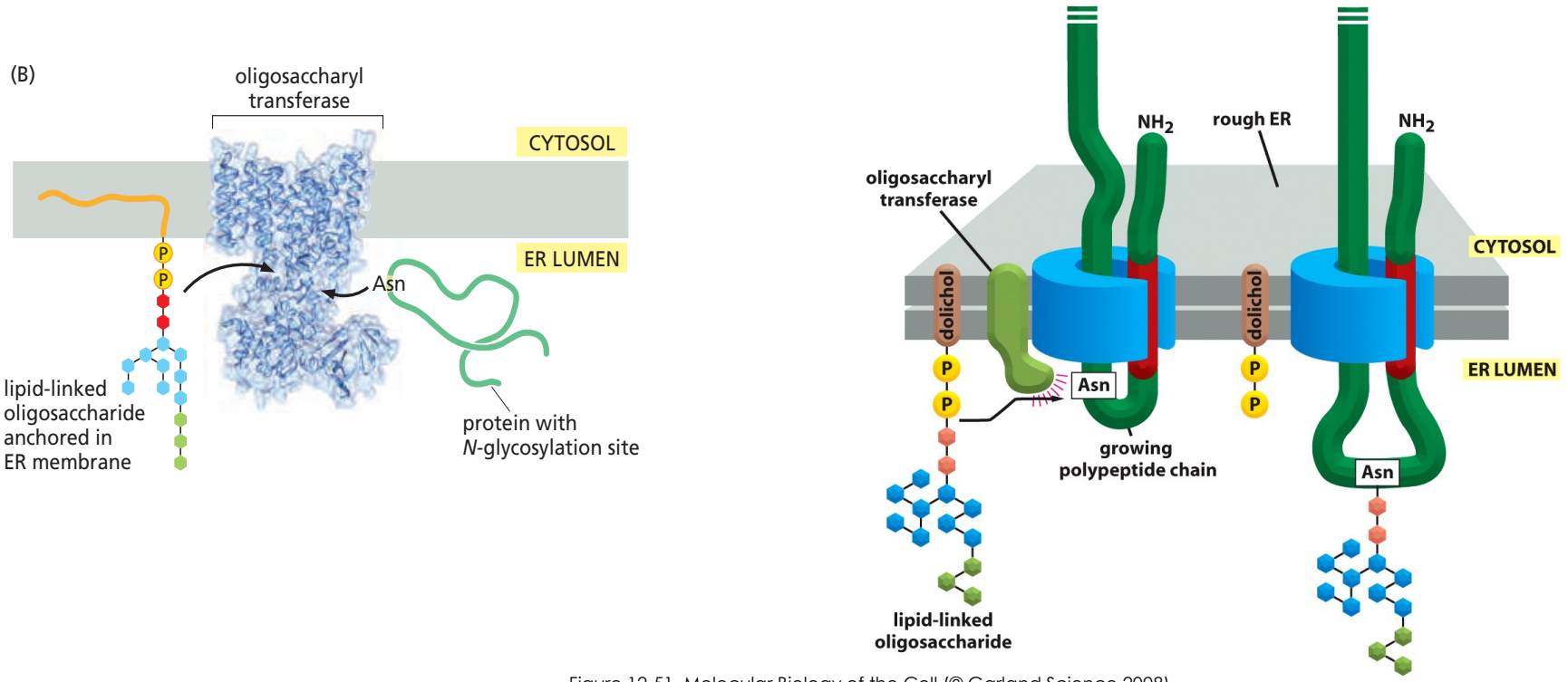
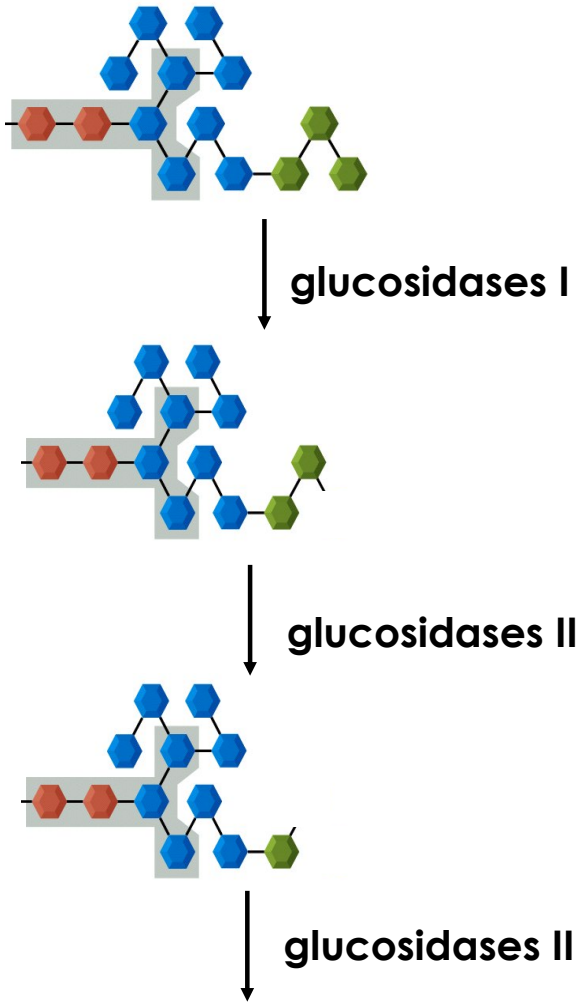


Figure 12-51 Molecular Biology of the Cell (© Garland Science 2008)

N-linked protein glycosylation in the rough ER



As soon as the sugars are added, the mannoses start to be removed in the ER by glucosidases

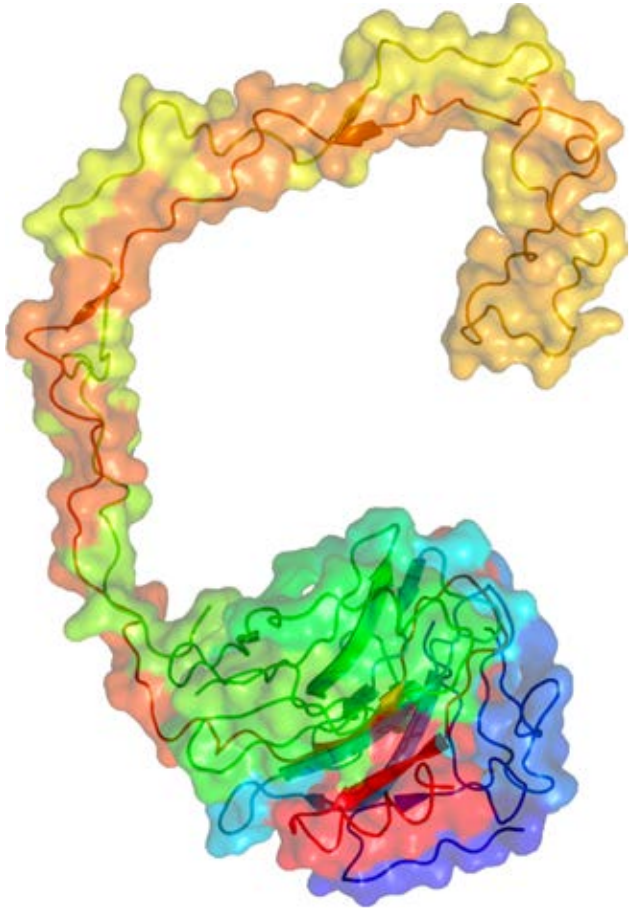
This allows the recognition of the newly synthesized protein to interact with chaperones, proteins promoting folding

The presence of all mannose and glucose indicates that the protein is young (newly synthesized)

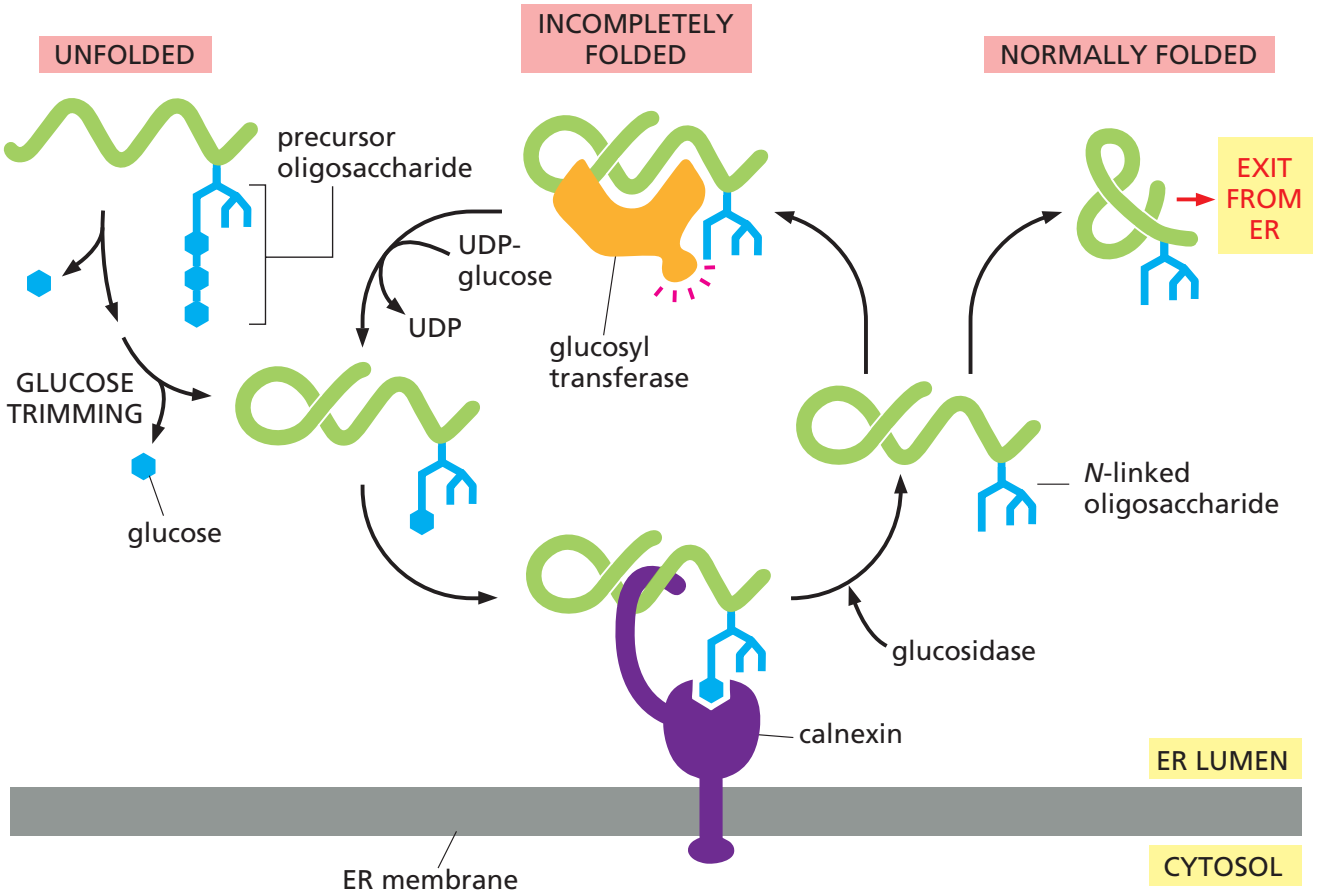
Mannoses can also be removed, but at a slower rate: "timer" of folding
Trimming of mannoses is recognized by quality control as a "problem".

The role of *N*-linked glycosylation in ER protein folding

Calnexin and calreticulin
Two chaperones recognizing mono-glucose on an N-linked sugar
Calnexin: type I transmembrane Calreticulin: soluble analogue
Role: bind to a protein during folding to prevent it from aggregating during the process No enzymatic activity



The role of *N*-linked glycosylation in ER protein folding



The calnexin/calreticulin cycle
- There is a glucosylation-deglucosylation cycle,
- where the enzyme, the glucosyl-transferase, is able to recognize if
whether a protein has completed its folding or not.
- If the folding is complete, this enzyme will give a glucose
But this cycle must be able to be interrupted, otherwise the ER would be full of protein stuck for a long time in this cycle
The ER also contains mannosidase, but this one has a slower activity
The cleavage of mannose is therefore an indication of the time spent in the ER
This acts as an extraction signal from the cycle, where the protein will be "abandoned" by the cell and put in the "trash".

Calnexin/Calreticulin cycle & ER protein folding

The calnexin/calreticulin cycle

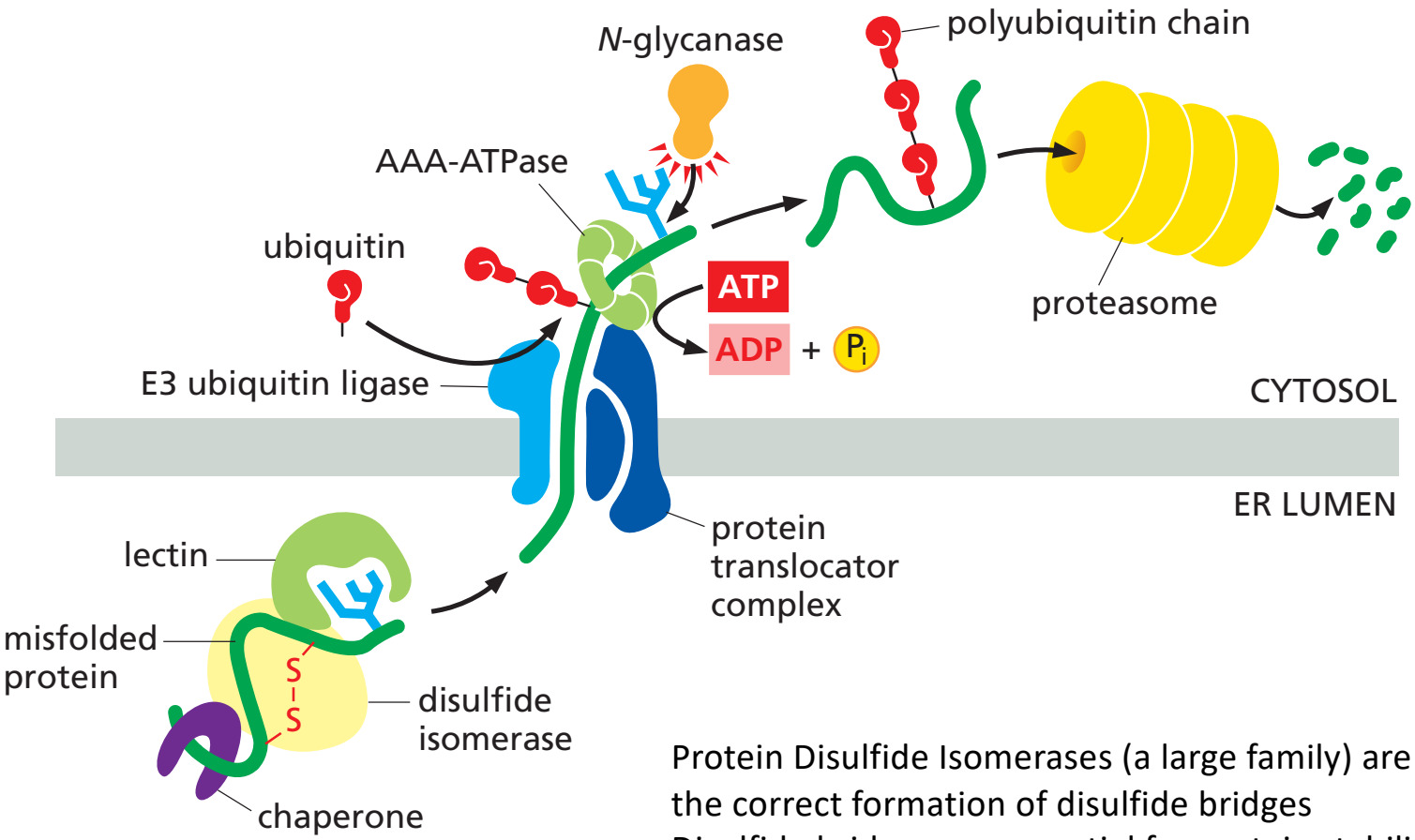
1: There is a glucosylation-deglucosylation cycle, where the enzyme, the glucosyl-transferase, is able to recognize if a protein has completed its folding or not. What the recognition signal is for all proteins to be correctly folded is not clear.

- If the folding is complete, this enzyme will give a glucose.

2: This cycle must be able to be interrupted, otherwise the ER would be full of proteins stuck for a long time in this cycle
The ER also contains mannosidase, but this one has a slower activity than the glucosyl-transferase
The cleavage of mannose is therefore an indication of the time spent in the ER
This acts as an extraction signal from the cycle, where the protein will be "abandoned" by the cell and put in the "trash".

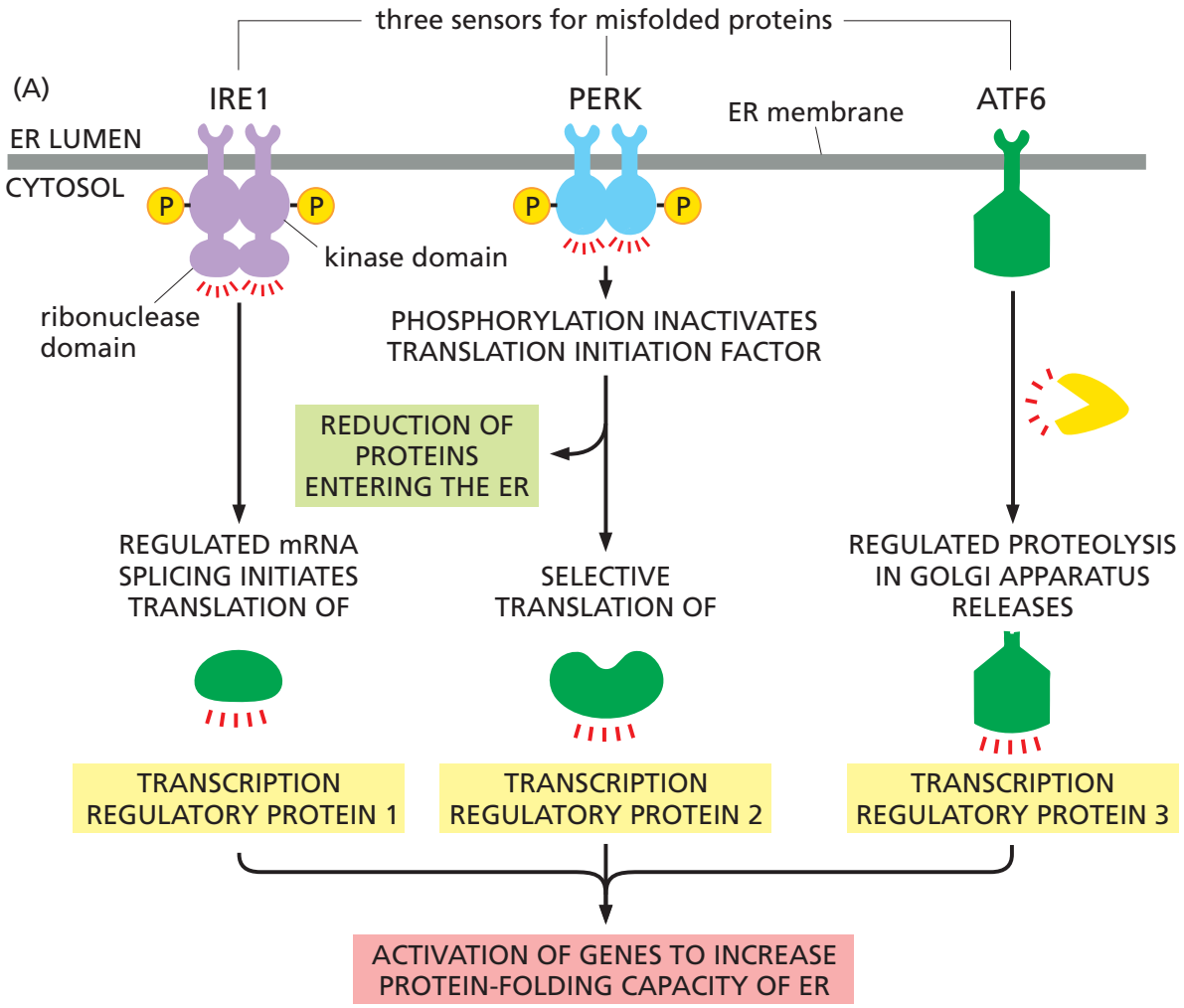
What happens if a protein is not properly folded in the ER?

The export and degradation of misfolded ER proteins



Protein Disulfide Isomerases (a large family) are necessary for the correct formation of disulfide bridges
Disulfide bridges are essential for protein stability, function and probably also guide folding

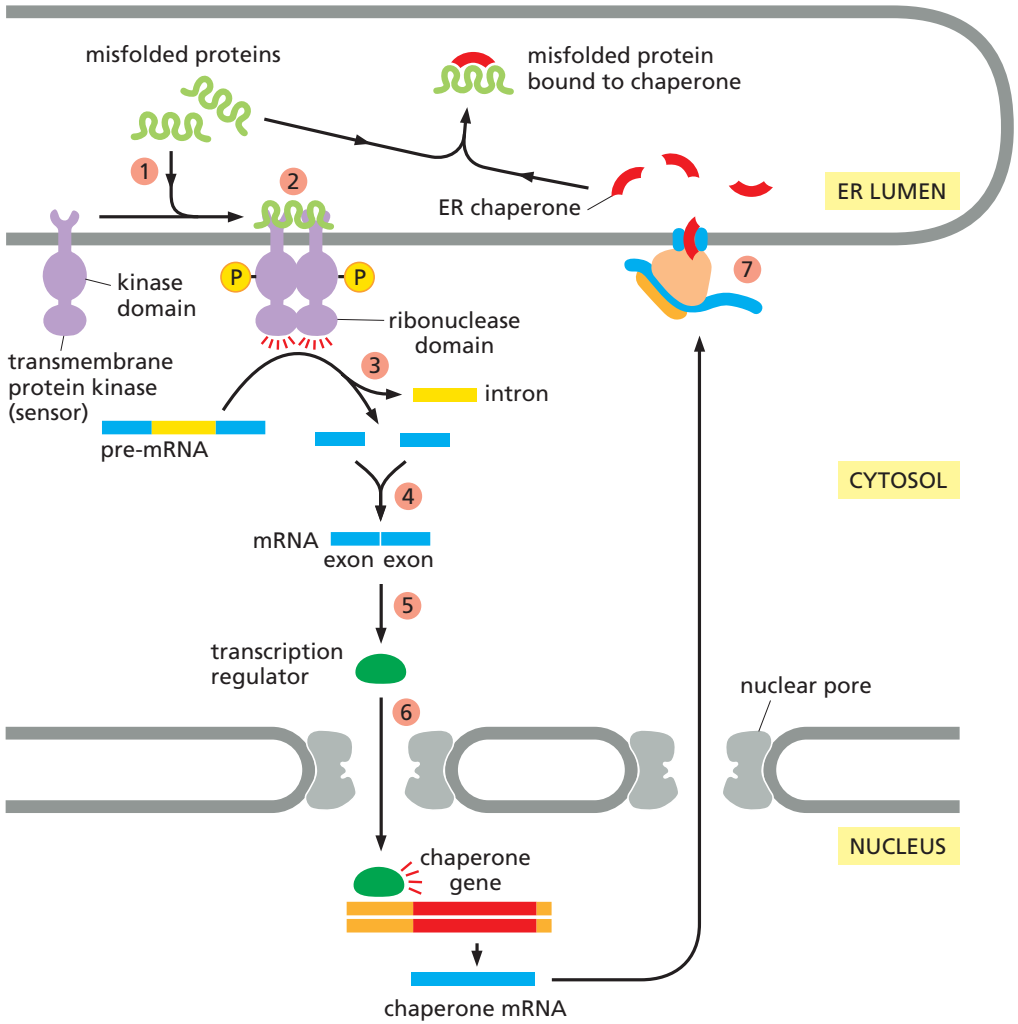
Too many improperly fold proteins leads to the unfolded protein response



The unfolded protein response

(B)

1. MISFOLDED PROTEINS IN ER SIGNAL THE NEED FOR MORE ER CHAPERONES. THEY BIND TO AND ACTIVATE A TRANSMEMBRANE KINASE
2. ACTIVATED KINASE UNMASKS AN ENDORIBONUCLEASE ACTIVITY
3. ENDORIBONUCLEASE CUTS SPECIFIC RNA MOLECULES AT TWO POSITIONS, REMOVING AN INTRON
4. TWO EXONS ARE LIGATED TO FORM AN ACTIVE mRNA
5. mRNA IS TRANSLATED TO MAKE A TRANSCRIPTION REGULATOR
6. TRANSCRIPTION REGULATOR ENTERS NUCLEUS AND ACTIVATES GENES ENCODING ER CHAPERONES
7. CHAPERONES ARE MADE IN ER, WHERE THEY HELP FOLD PROTEINS



Synthesis of lipids in the ER

The synthesis of phosphatidylcholine

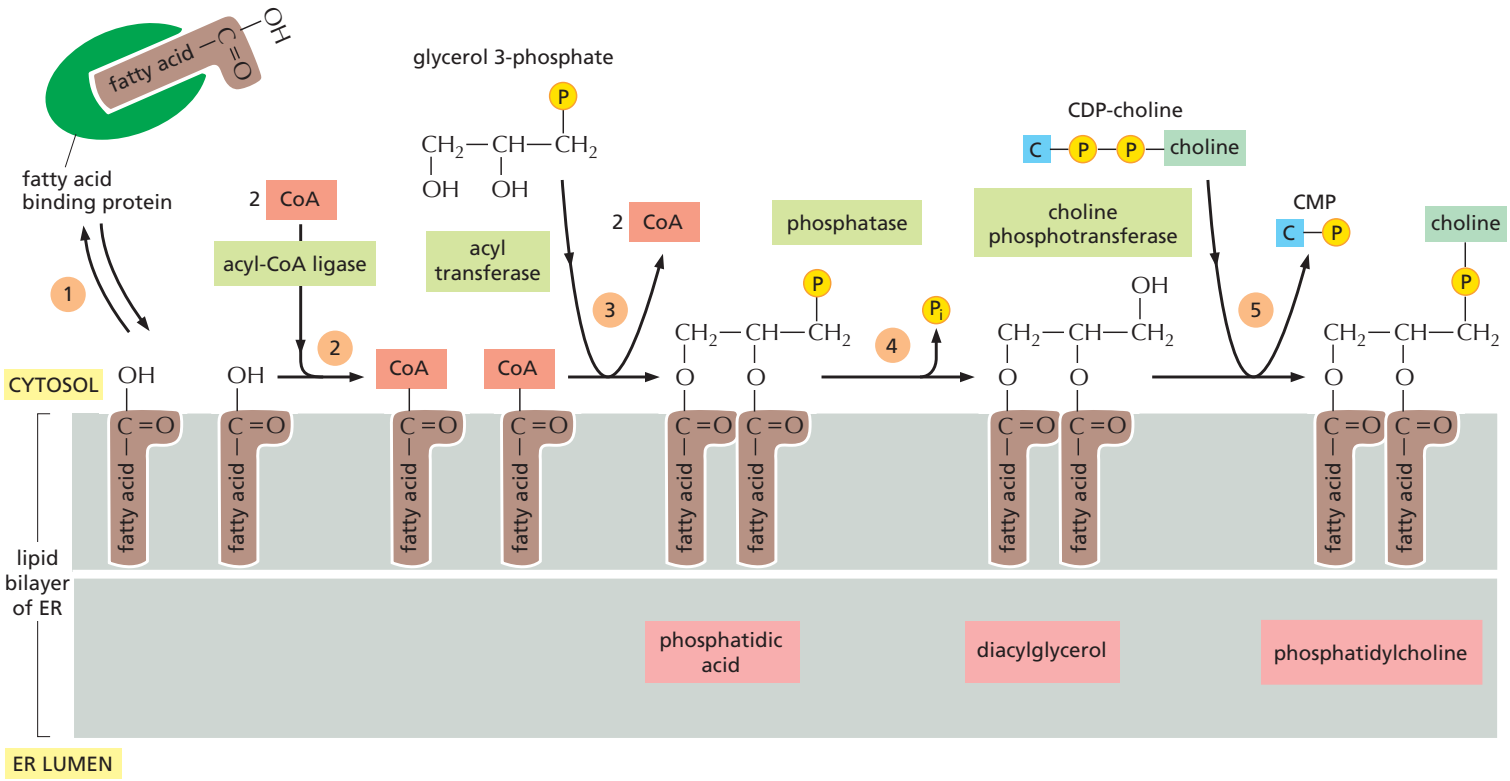


Figure 12–53 The synthesis of phosphatidylcholine. As illustrated, this phospholipid is synthesized from glycerol 3-phosphate, cytidine-diphosphocholine (CDP-choline), and fatty acids delivered to the ER by a cytosolic fatty acid binding protein.

The role of phospholipid translocators in lipid bilayer synthesis

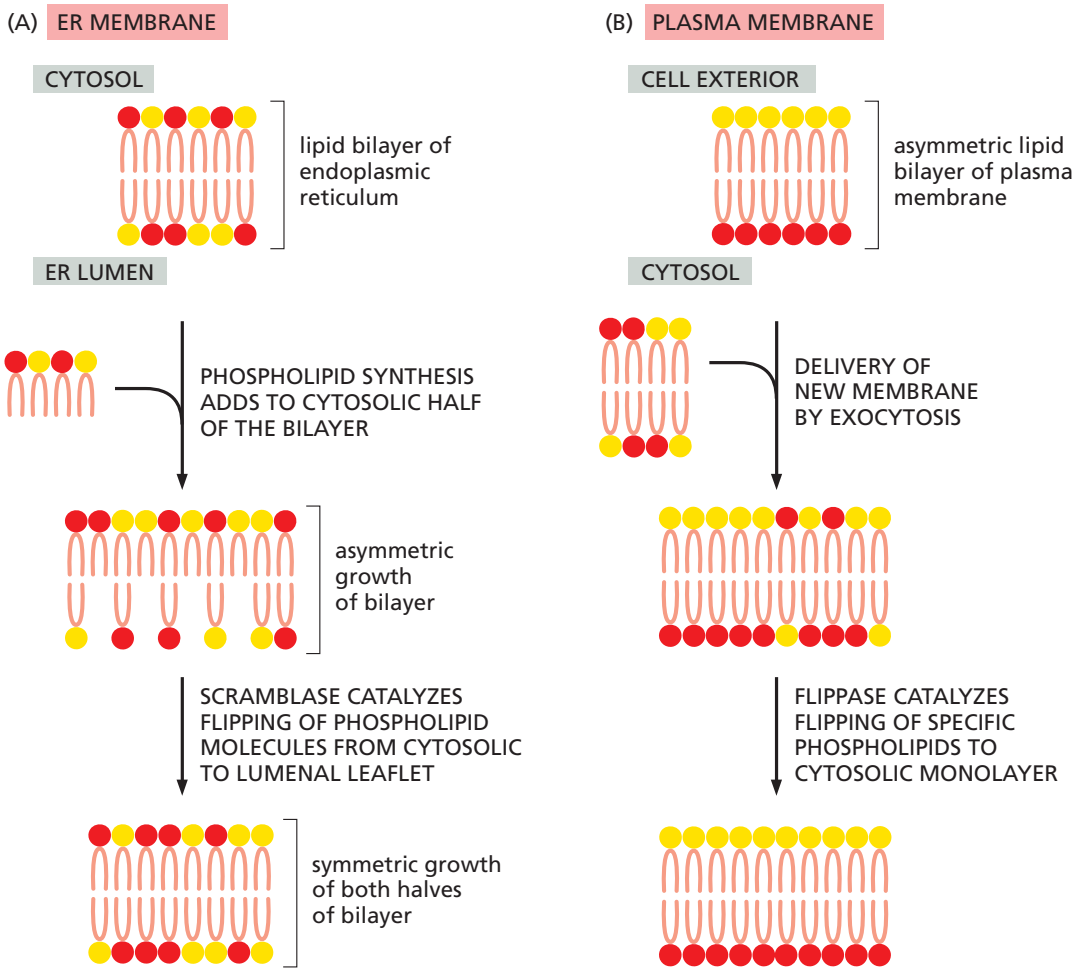


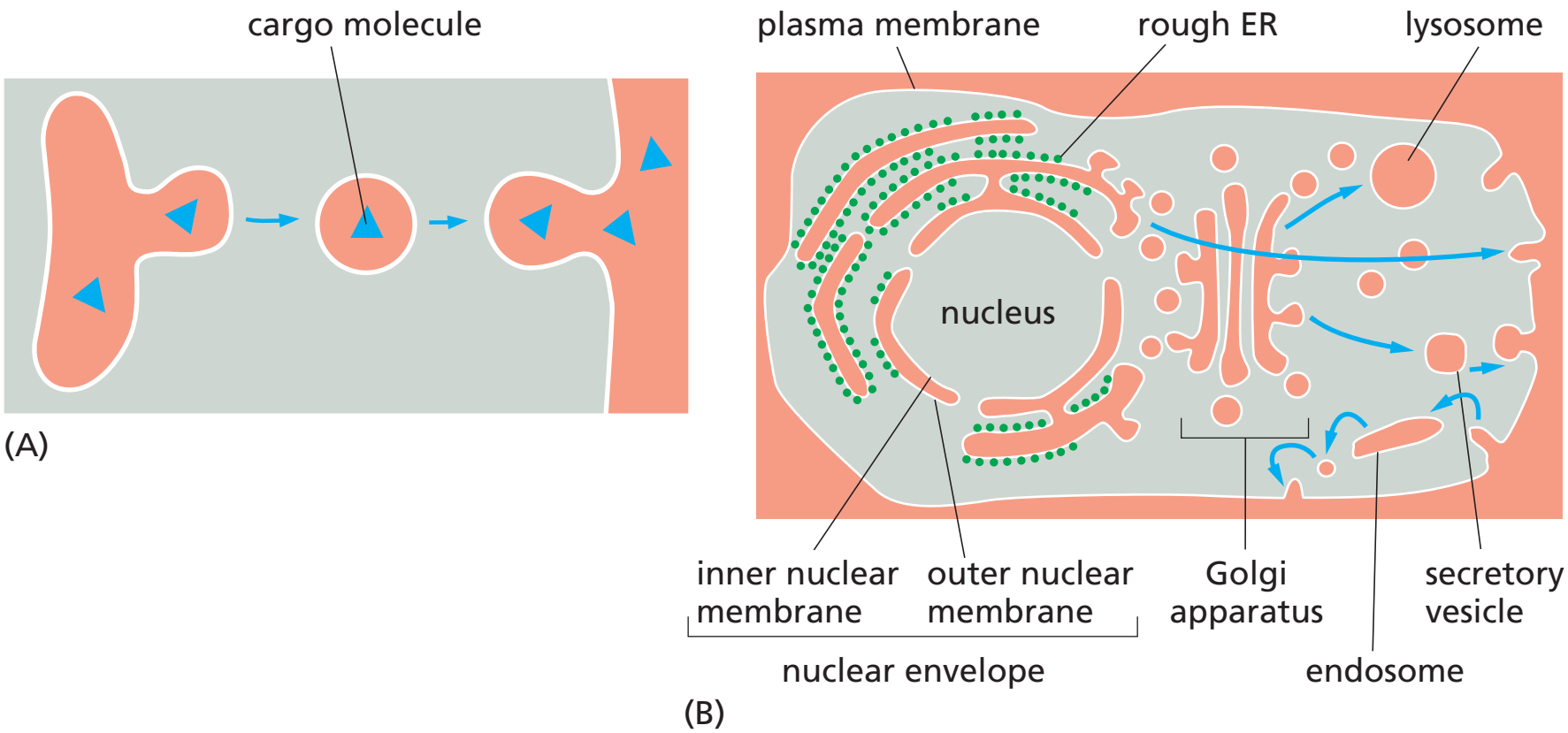
Figure 12–54 The role of phospholipid translocators in lipid bilayer synthesis. (A) Because new lipid molecules are added only to the cytosolic half of the ER membrane bilayer and lipid molecules do not flip spontaneously from one monolayer to the other, a transmembrane phospholipid translocator (called a scramblase) is required to transfer lipid molecules from the cytosolic half to the lumenal half so that the membrane grows as a bilayer. The scramblase is not specific for particular phospholipid head groups and therefore equilibrates the different phospholipids between the two monolayers. (B) Fueled by ATP hydrolysis, a head-group-specific flippase in the plasma membrane actively flips phosphatidylserine and phosphatidylethanolamine directionally from the extracellular to the cytosolic leaflet, creating the characteristically asymmetric lipid bilayer of the plasma membrane of animal cells (see Figure 10–15).

CHAPTER 13

Intracellular Membrane Traffic

IN THIS CHAPTER

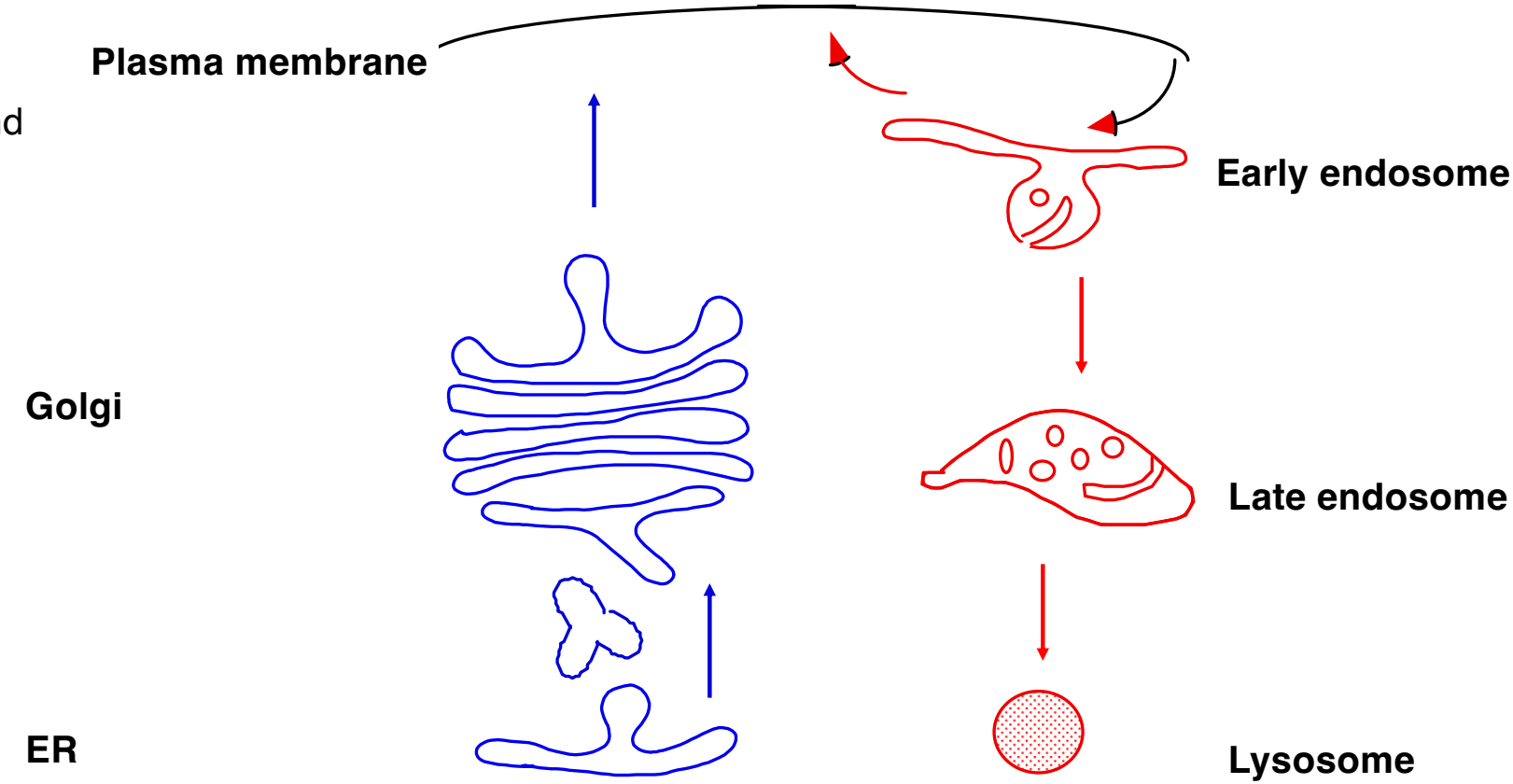
Topologically equivalent compartments, are able to “communicate”



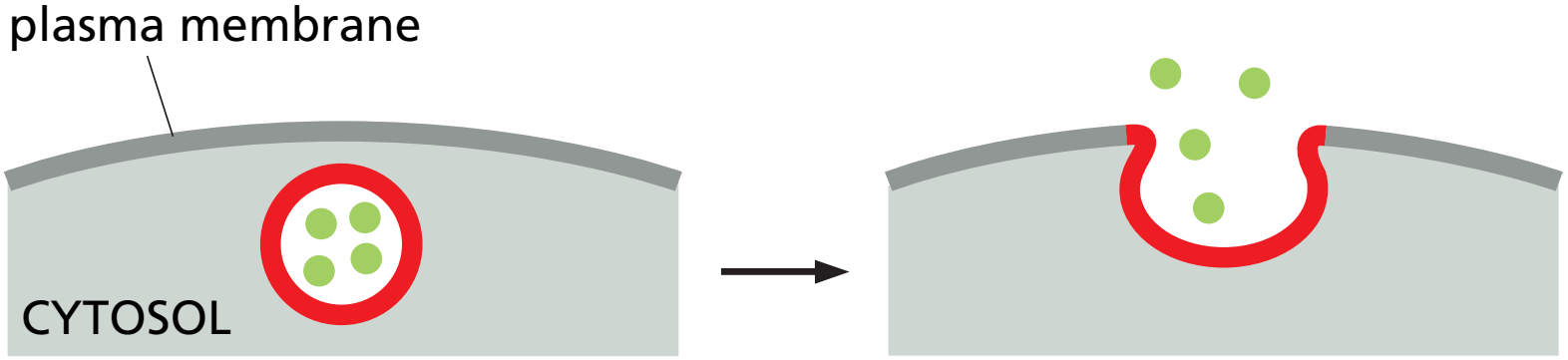
Topology of cellular spaces: the interior of the compartments of the endomembrane system is topologically equivalent to the extracellular space This is a consequence of vesicular transport

Transport pathways

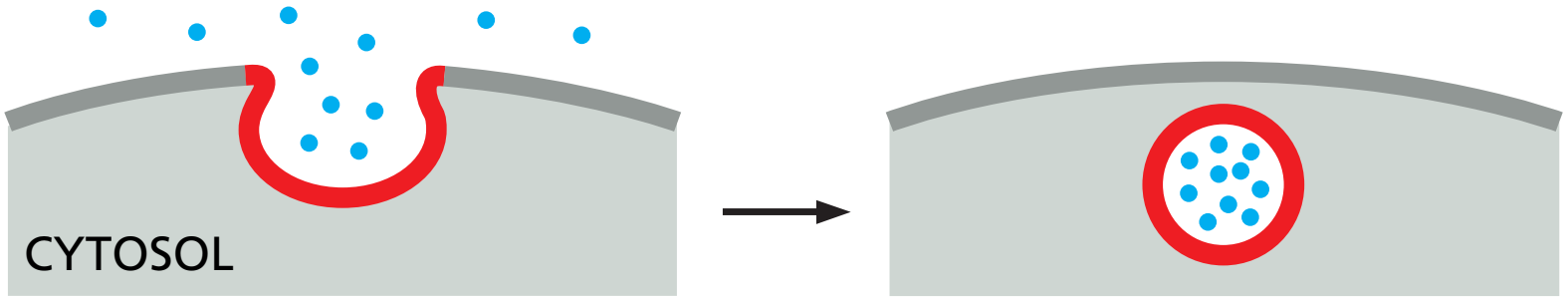
two major intracellular transport pathways: biosynthesis and endocytosis



Uptake and Expulsion at the plasma membrane

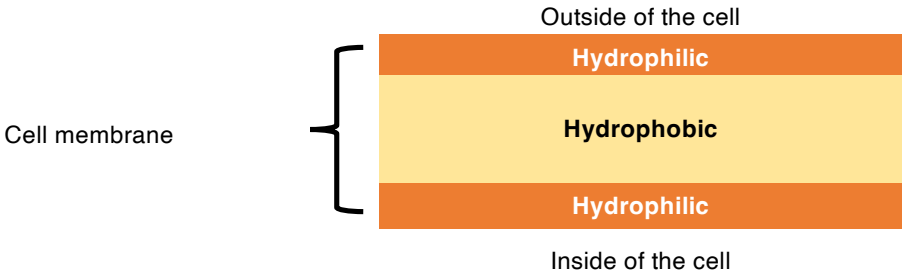


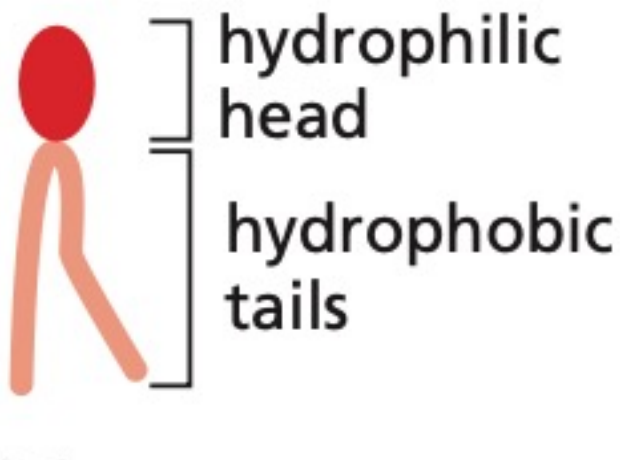
(A) exocytosis

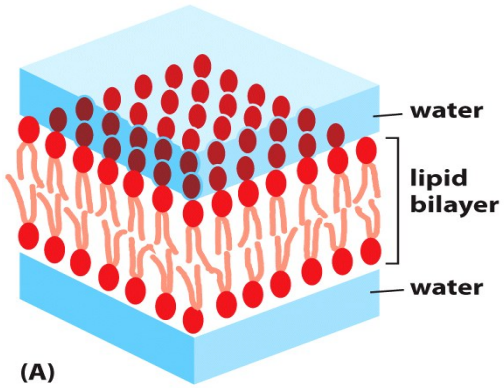


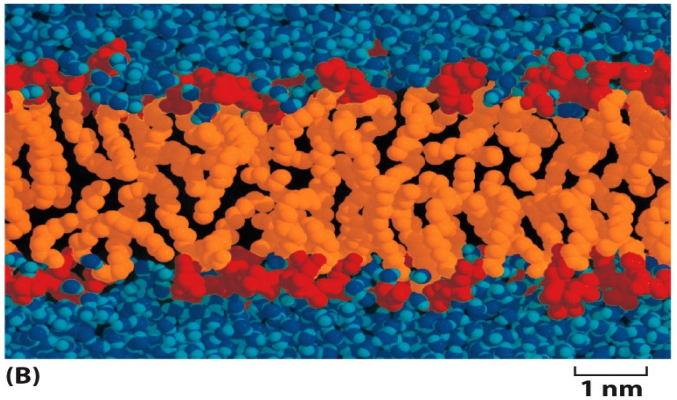
(B) endocytosis

Cell membranes are composed of lipids 50:50 mass ratio

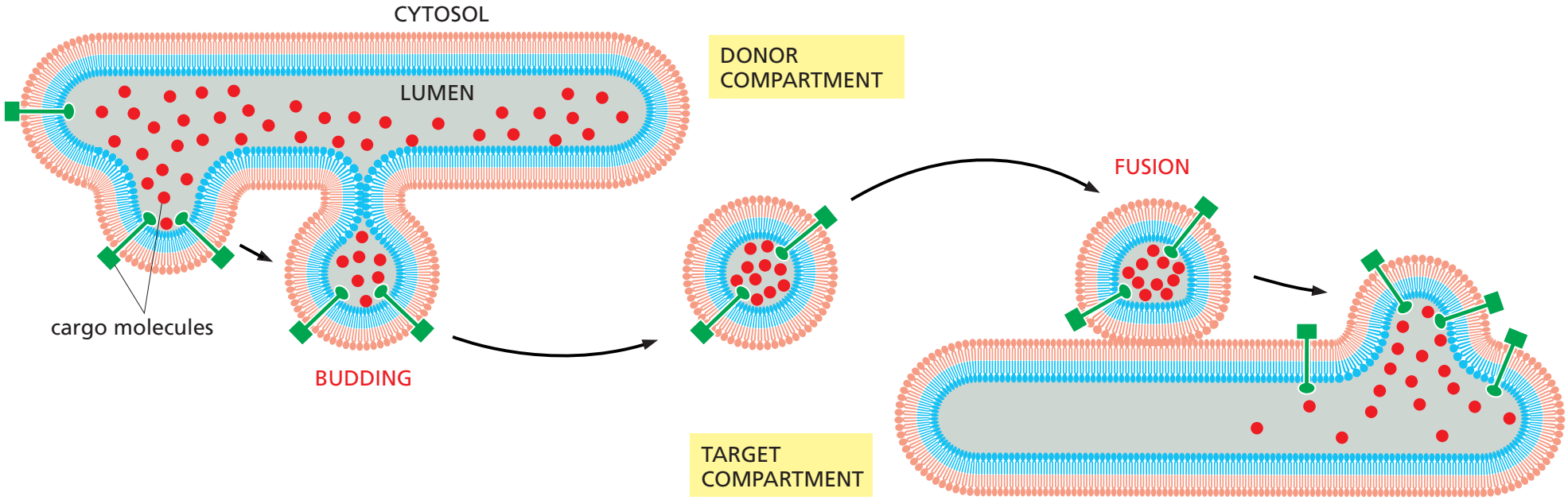




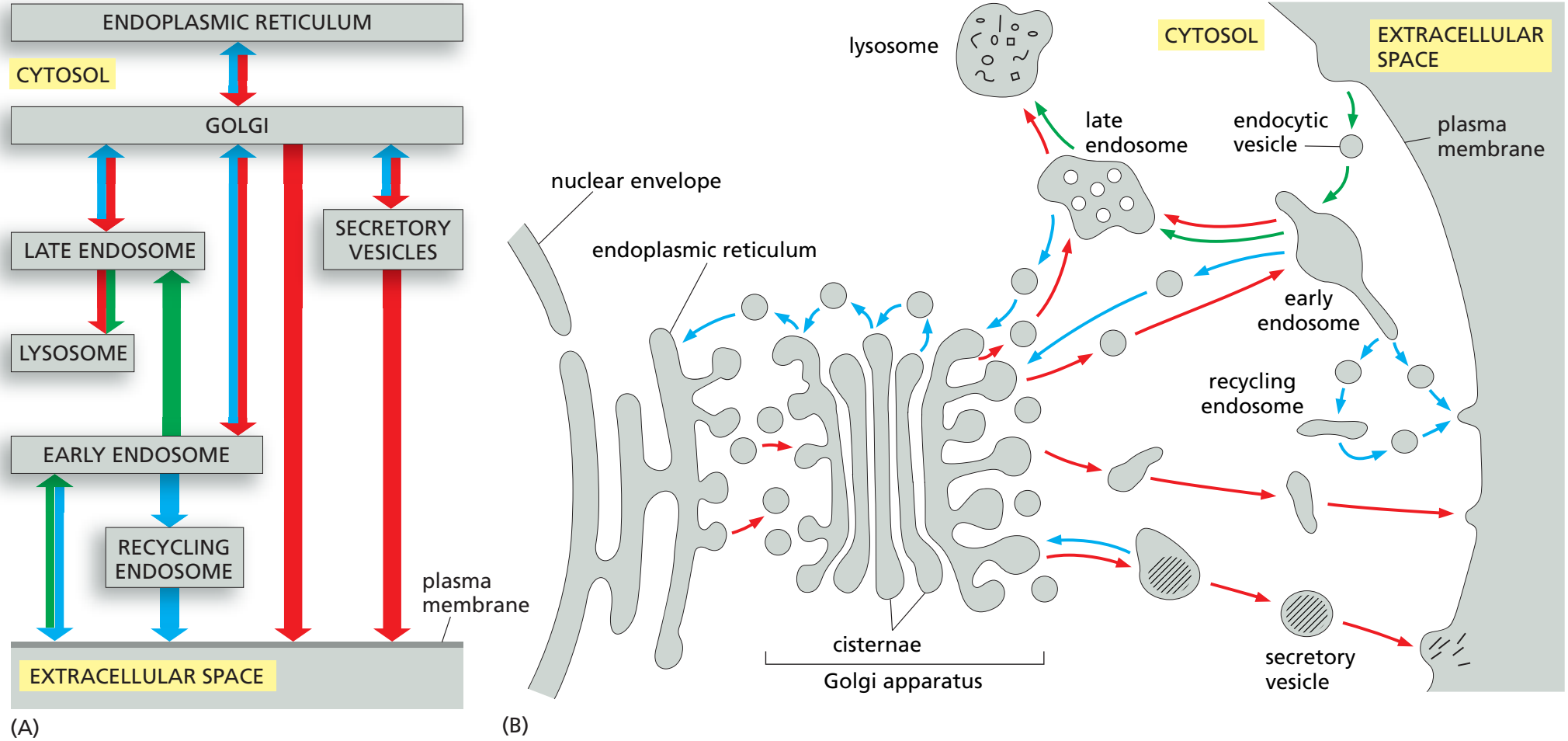




Basics of vesicle transport



Vesicles in the secretory and endocytic pathways



Two “problems” to solve in vesicle transport

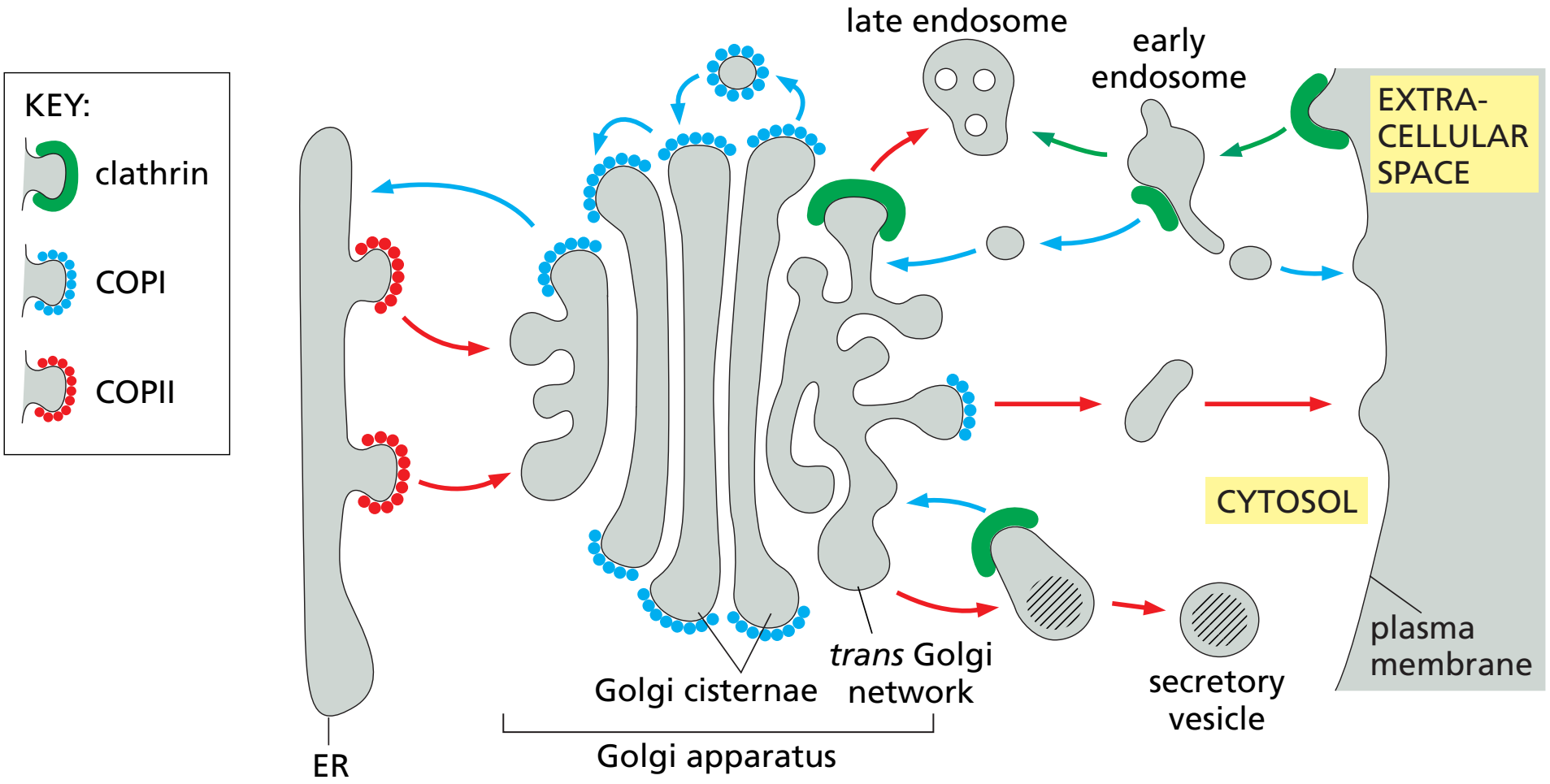
Mechanism

- Form a bud
- Detach a vesicle
- Move the vesicle optimally through the cell
- Merge the vesicle with an acceptor compartment
- Ensure that the compartments remain constant in size

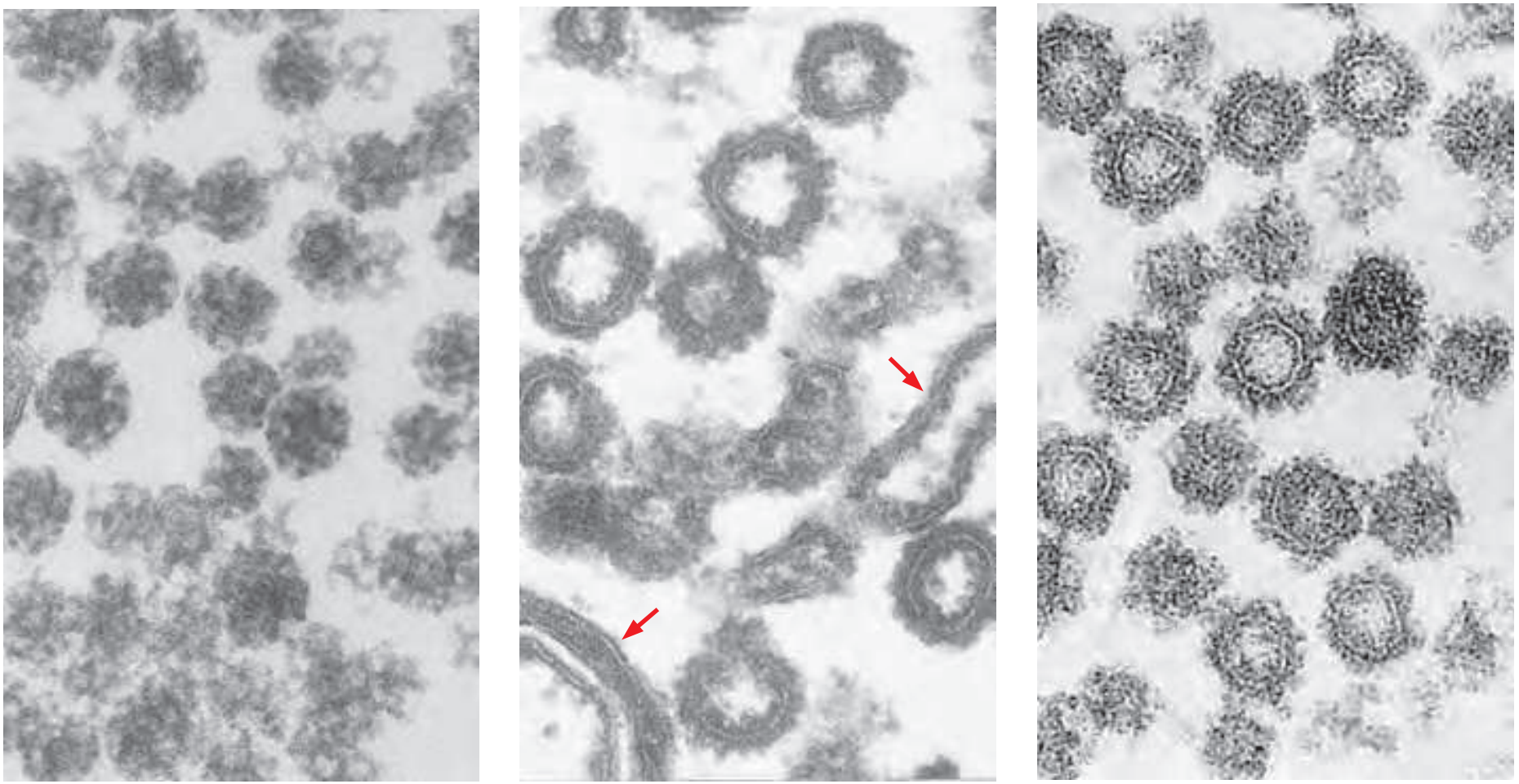
Specificity

- Put the right proteins in the nascent vesicle
- Address the vesicle to the right compartment

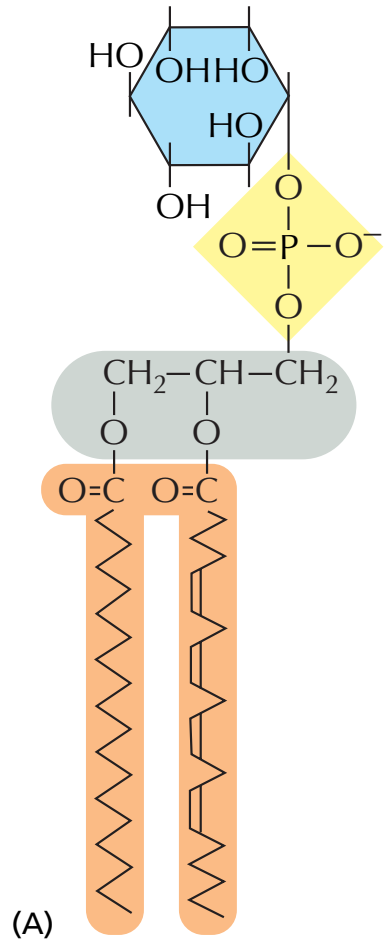
Use of different coats for different steps in vesicle traffic



Electron micrographs of clathrin-coated, COPI-coated, and COPII-coated vesicles



Phosphatidylinositol (PI) and phosphoinositides (PIPs)



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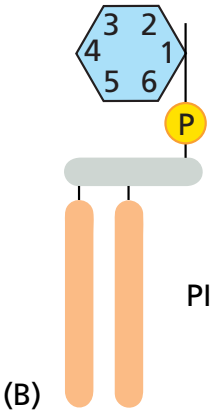

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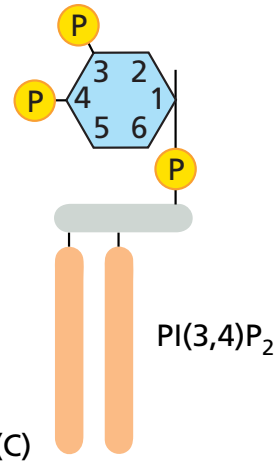

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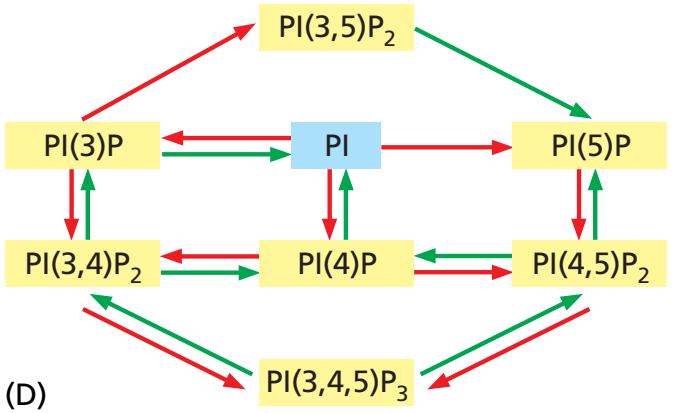

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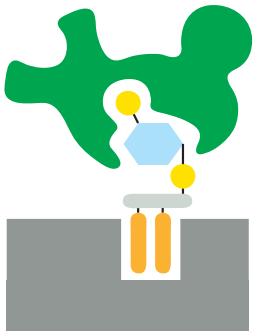

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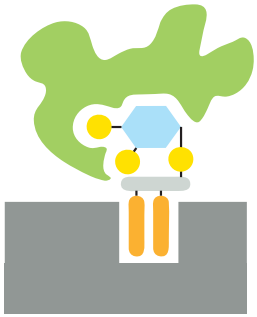
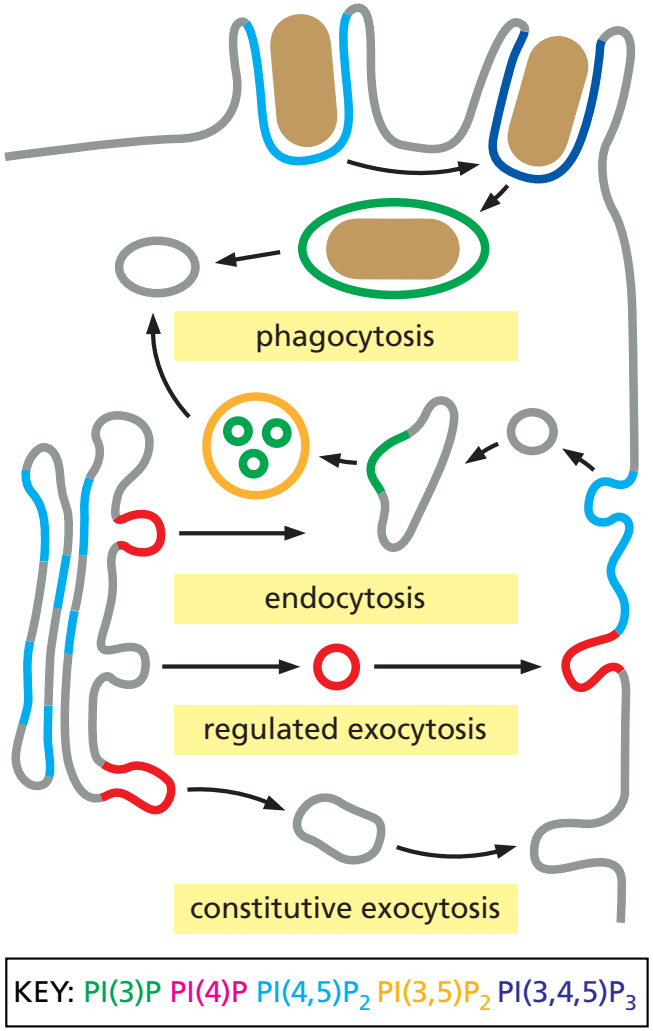


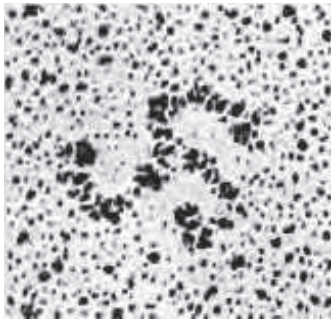
(F)

The intracellular location of phosphoinositides



Clathrin Coated vesicles

The structure of a clathrin coat



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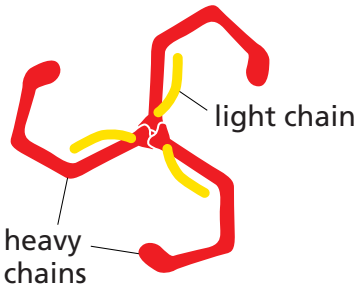

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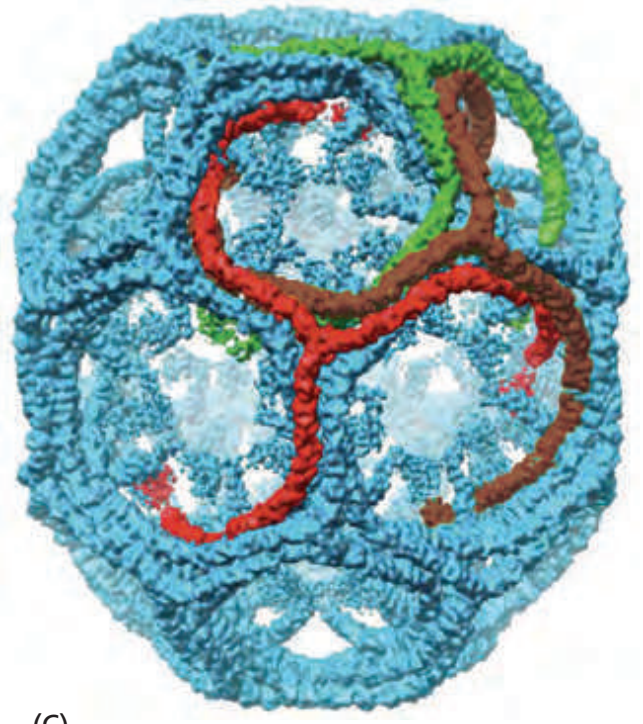

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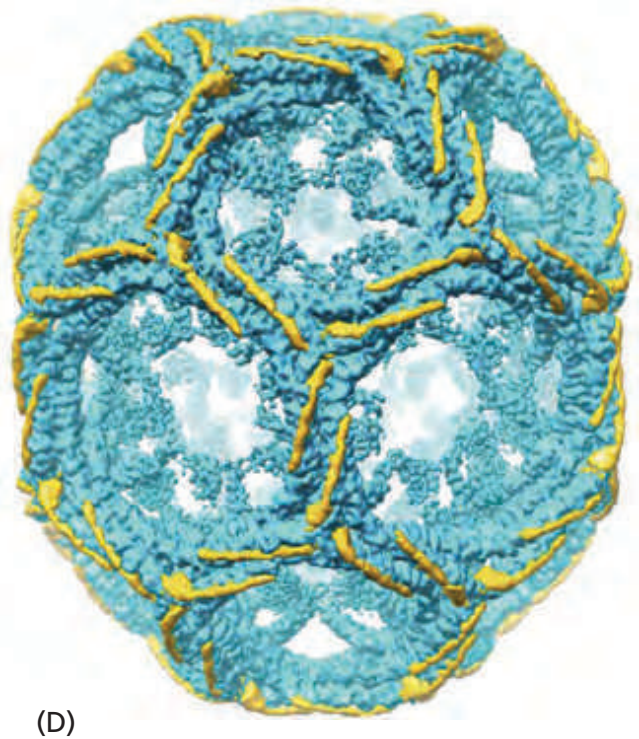
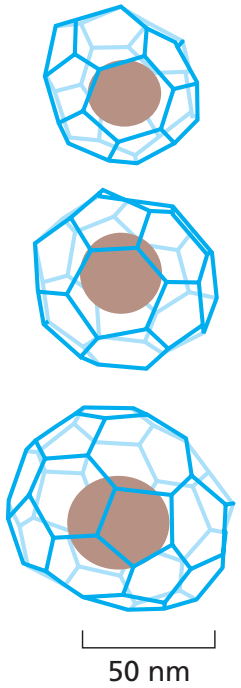


(D)

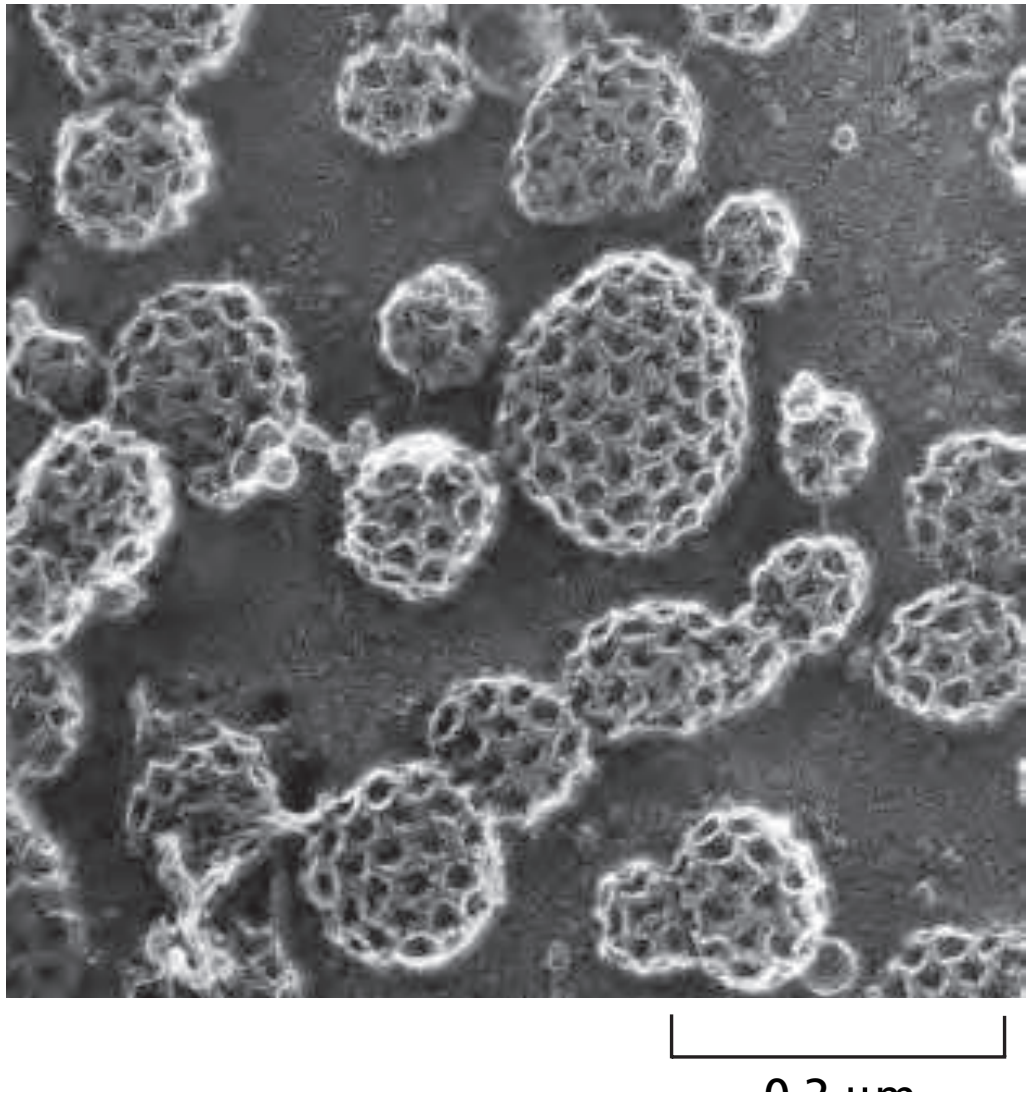
25 nm



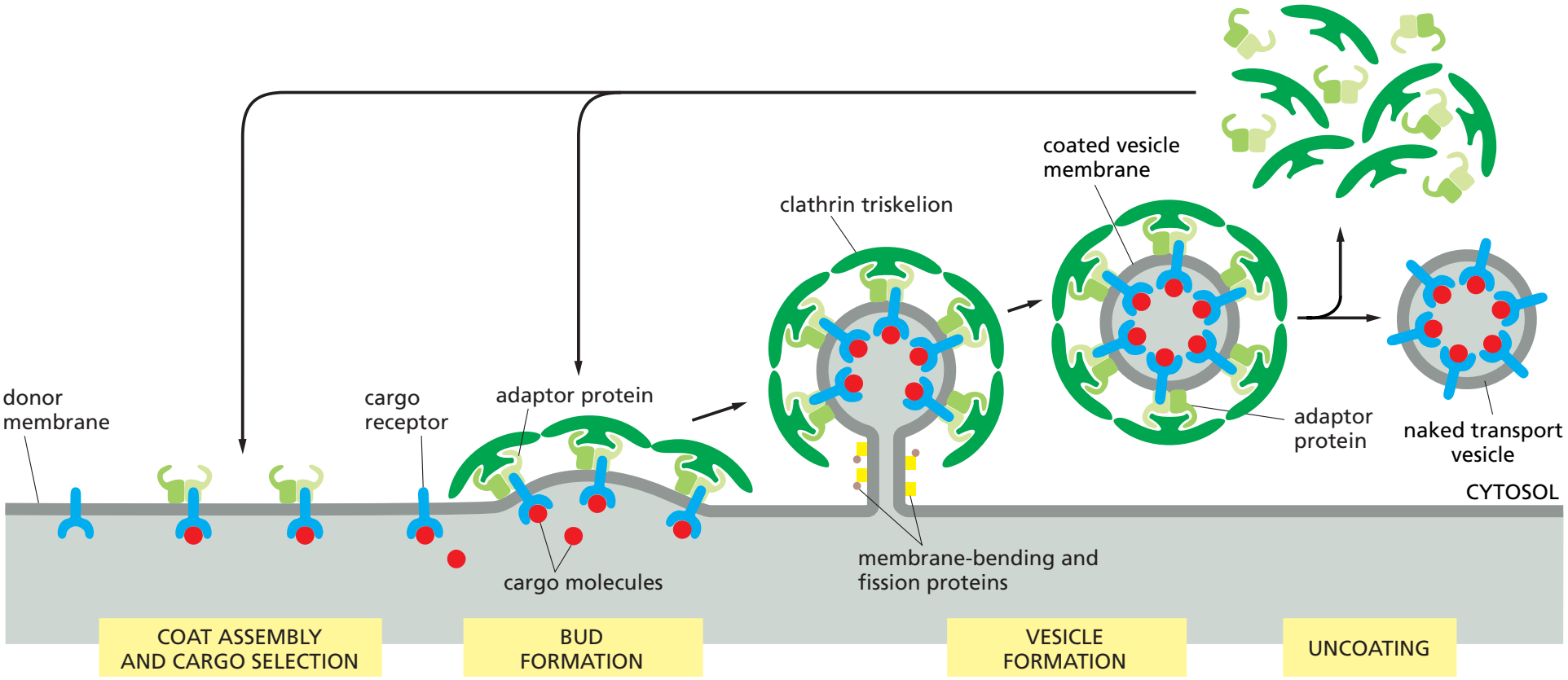
(E)

50 nm

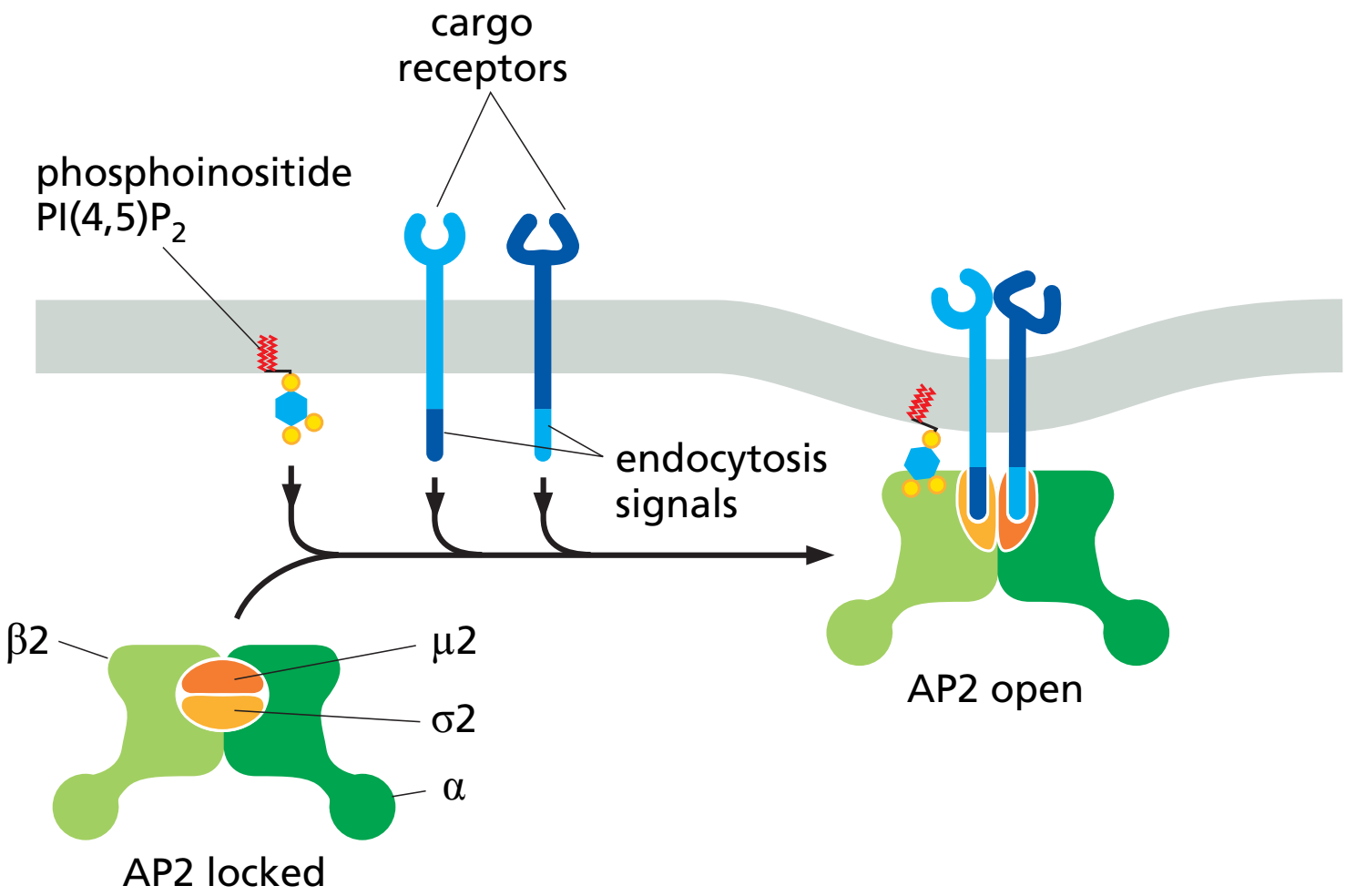
Clathrin-coated pits and vesicles



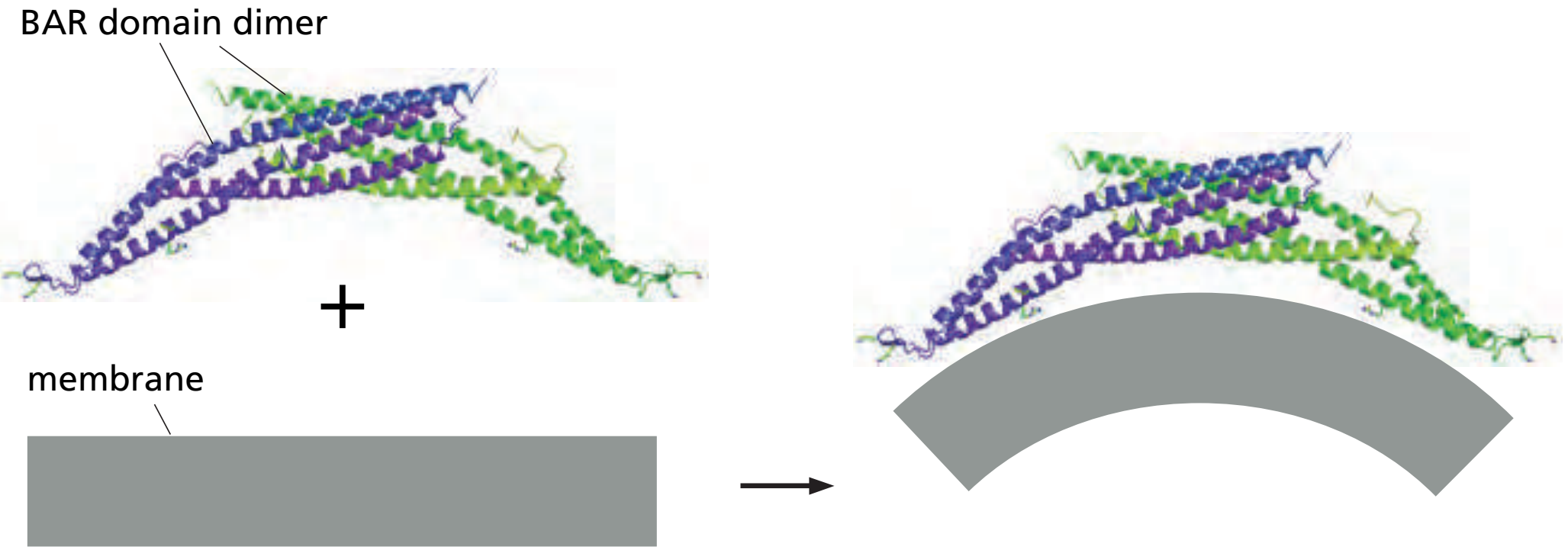
The assembly and disassembly of a clathrin coat



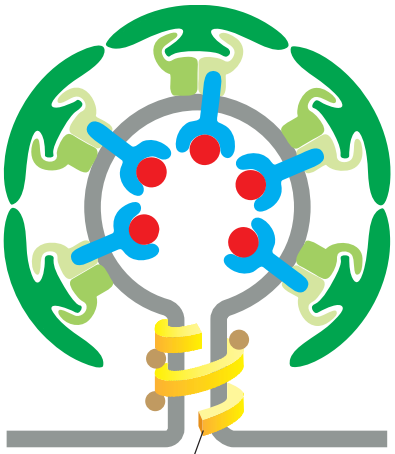
Lipid-induced conformation switching of AP2



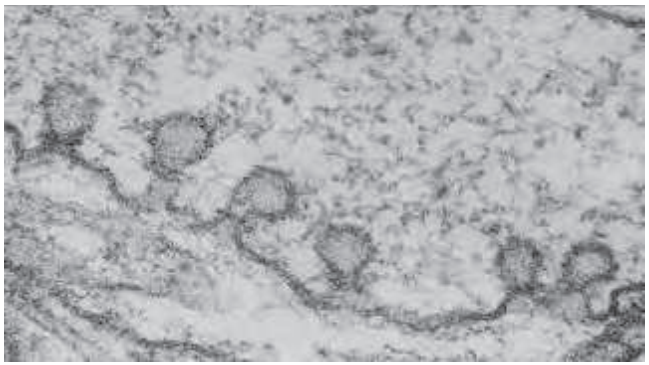
The structure of BAR domains and membrane bending



pinching off clathrin-coated vesicles: Dynamin

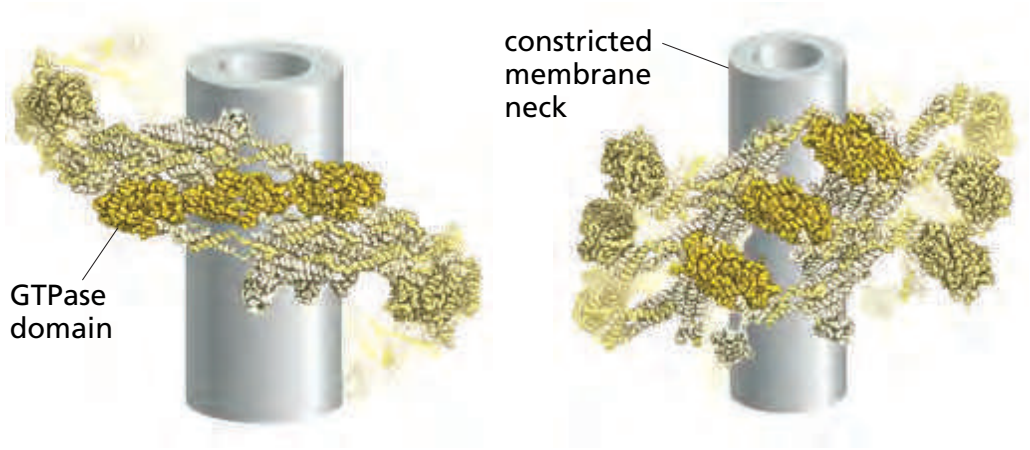


(A)



(B)

200 nm



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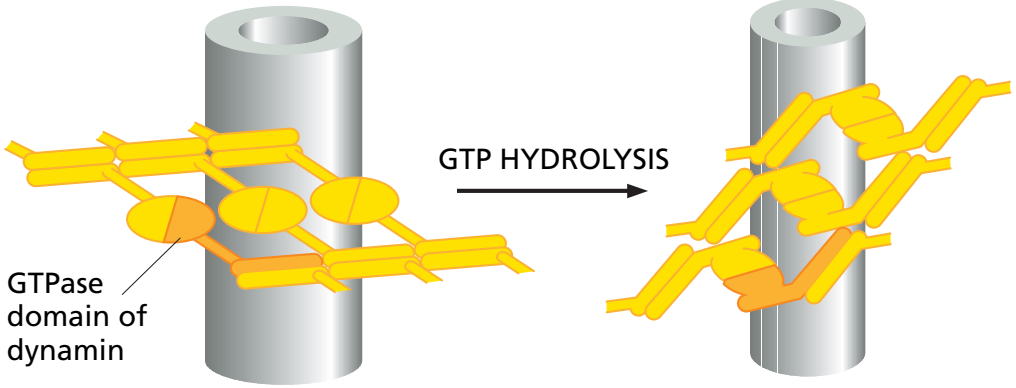
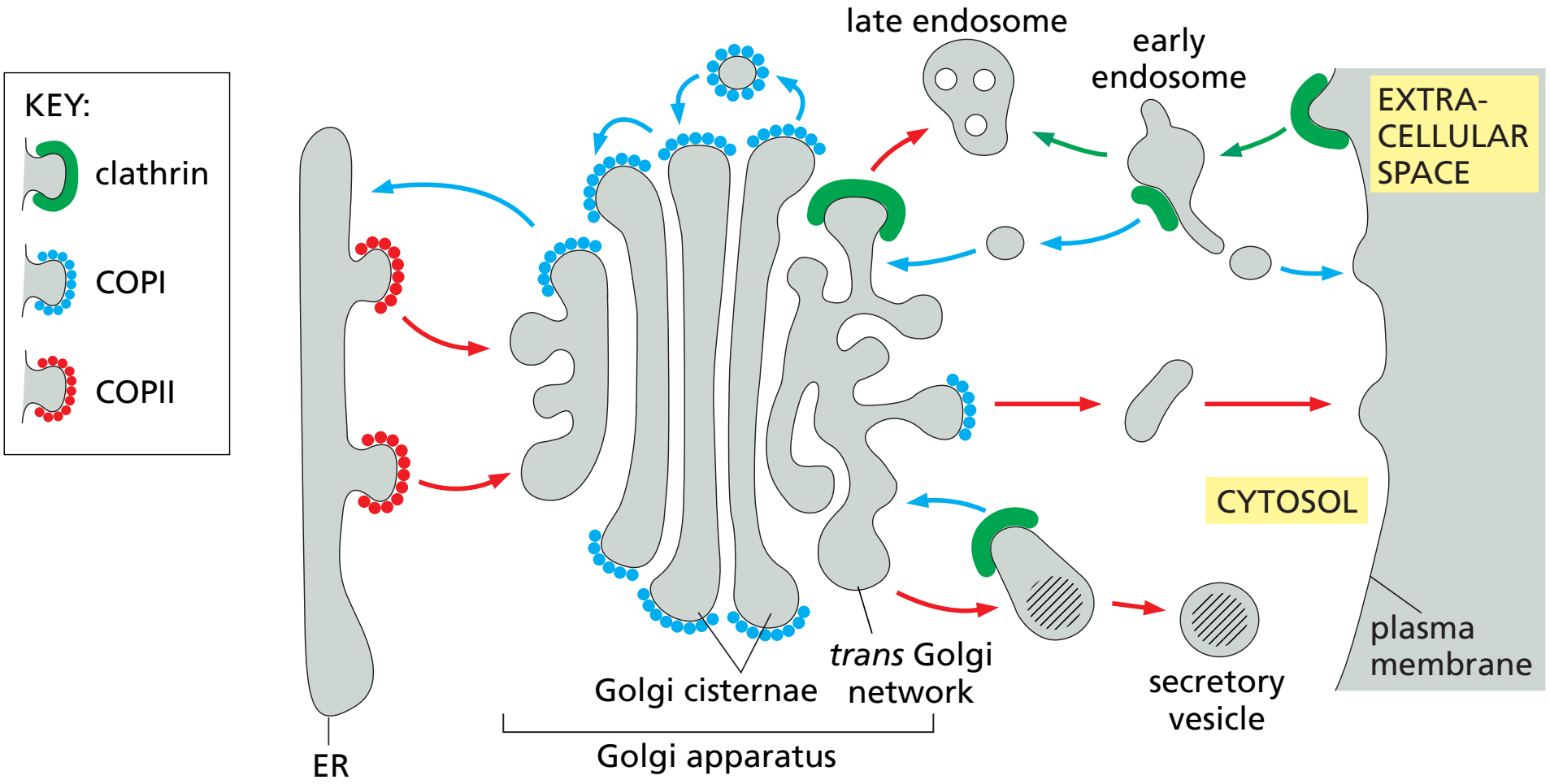


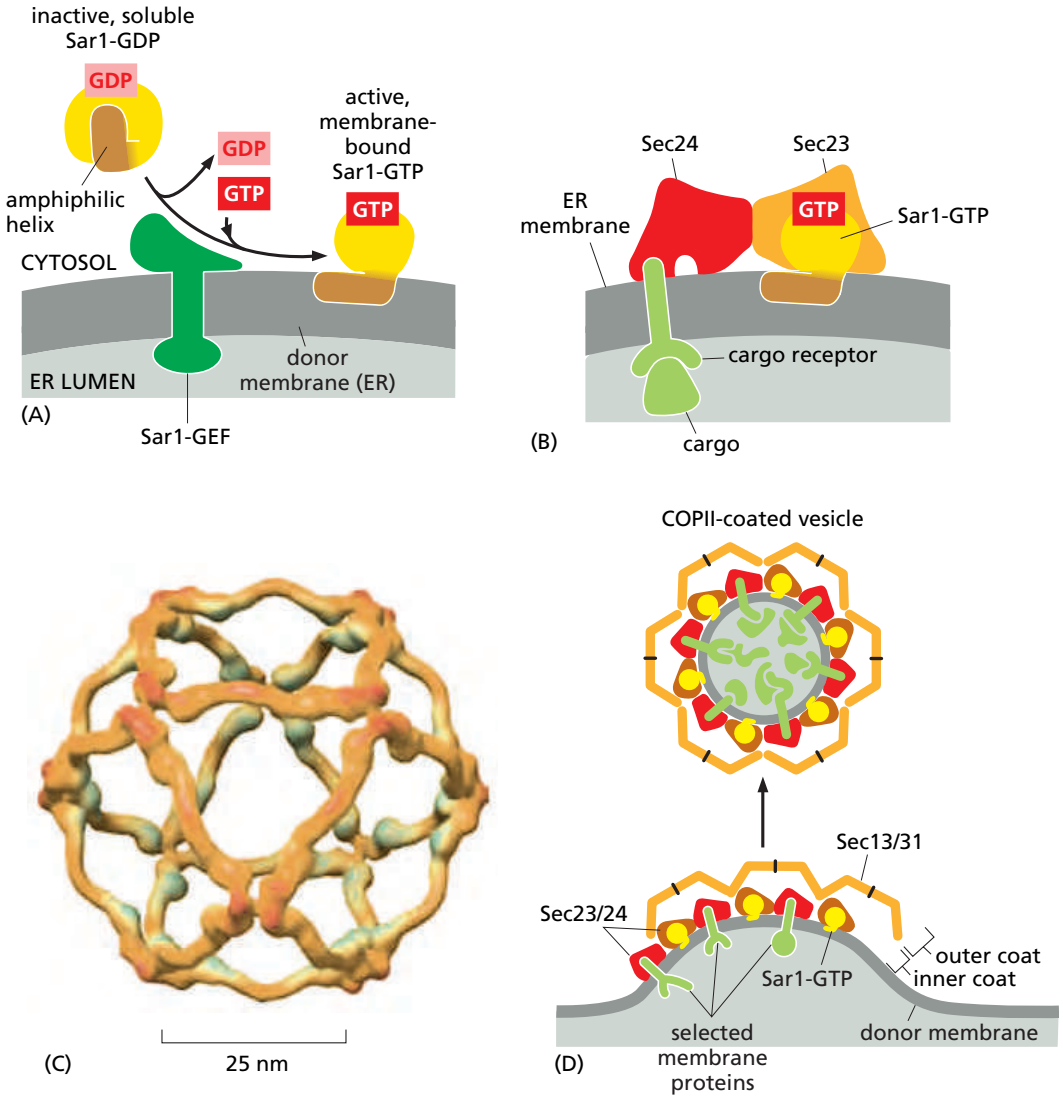
(D)

CopII Coated vesicles

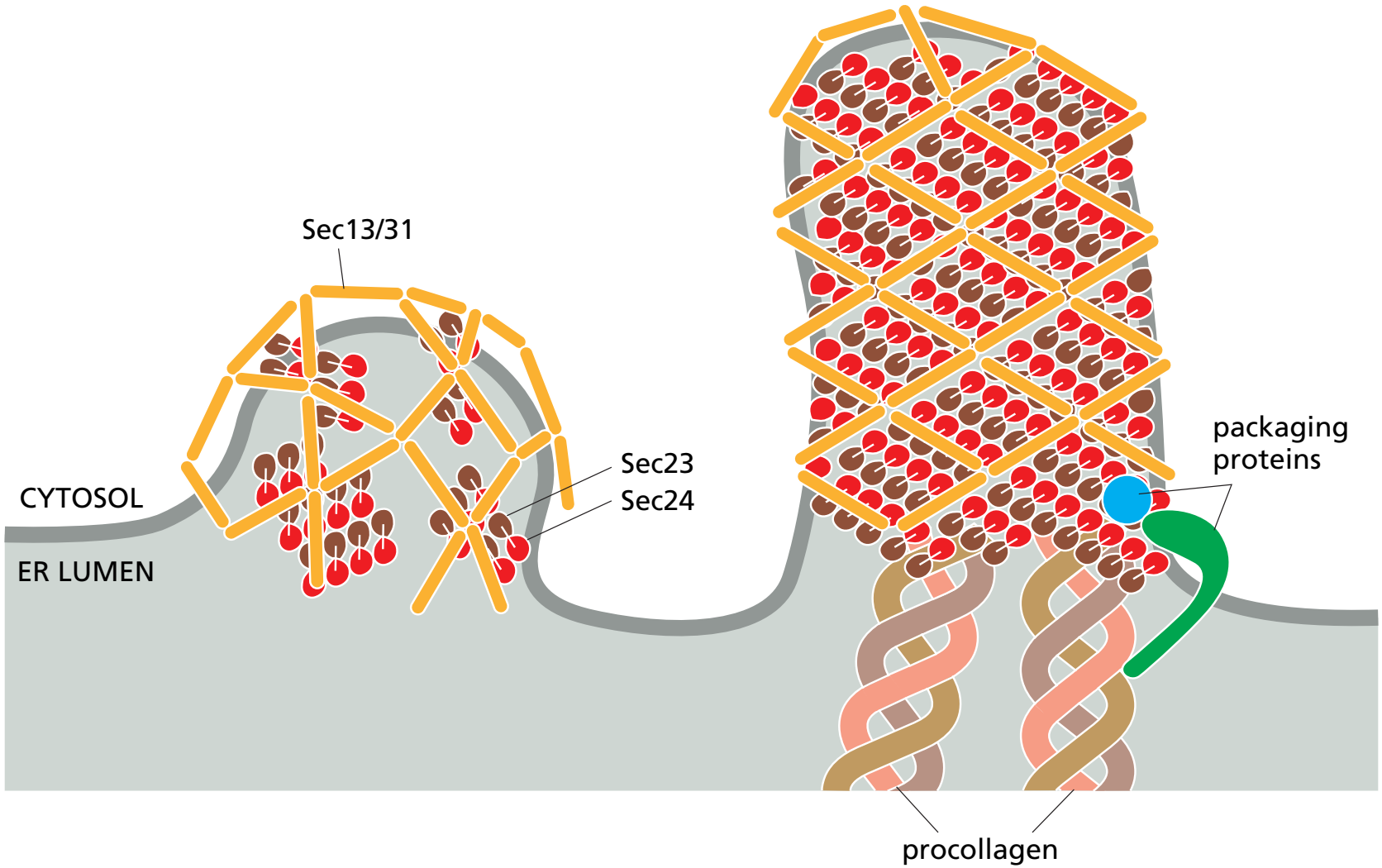
Use of different coats for different steps in vesicle traffic



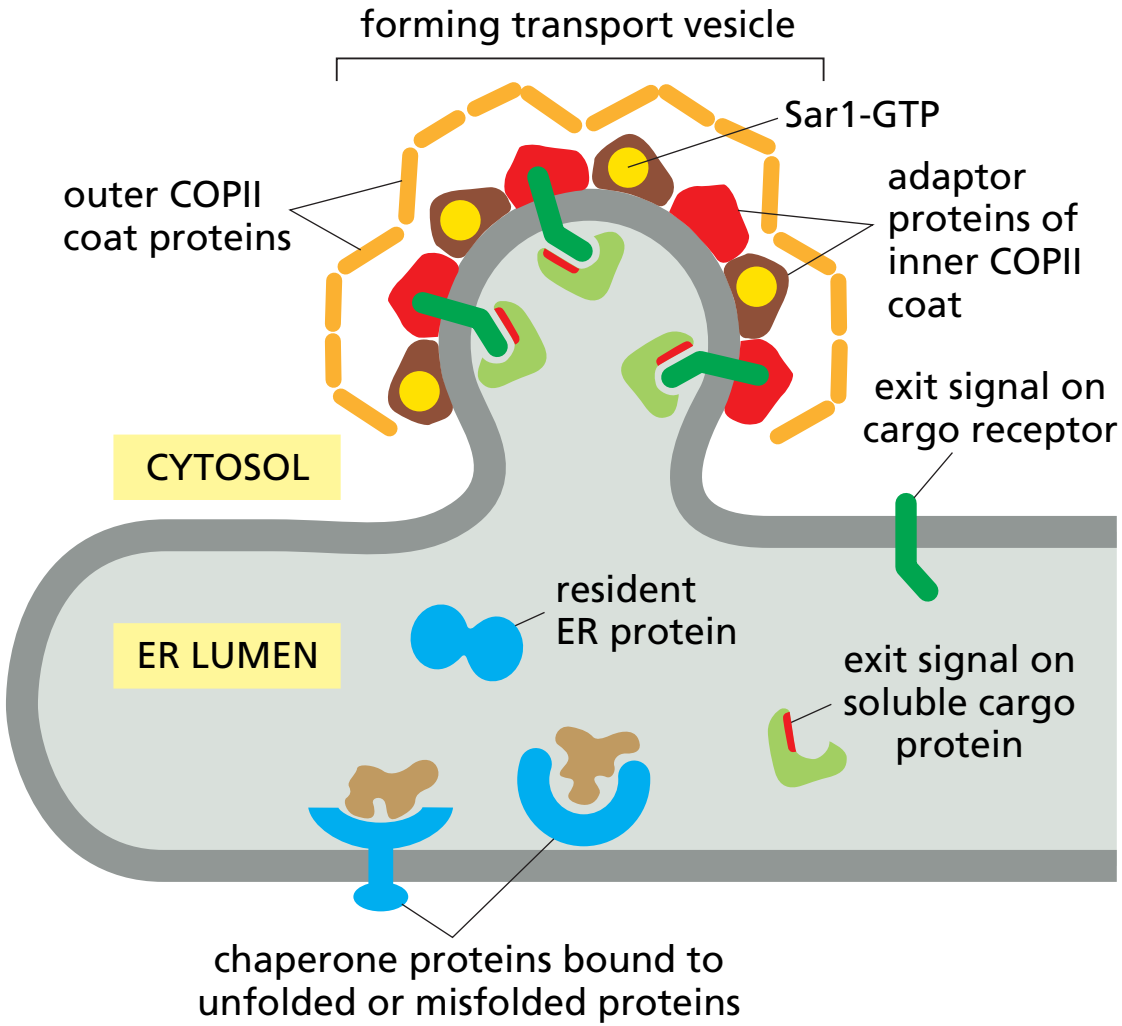
Formation of a COPII-coated vesicle



Non spherical vesicle: packaging of procollagen into large tubular COPII-coated vesicles

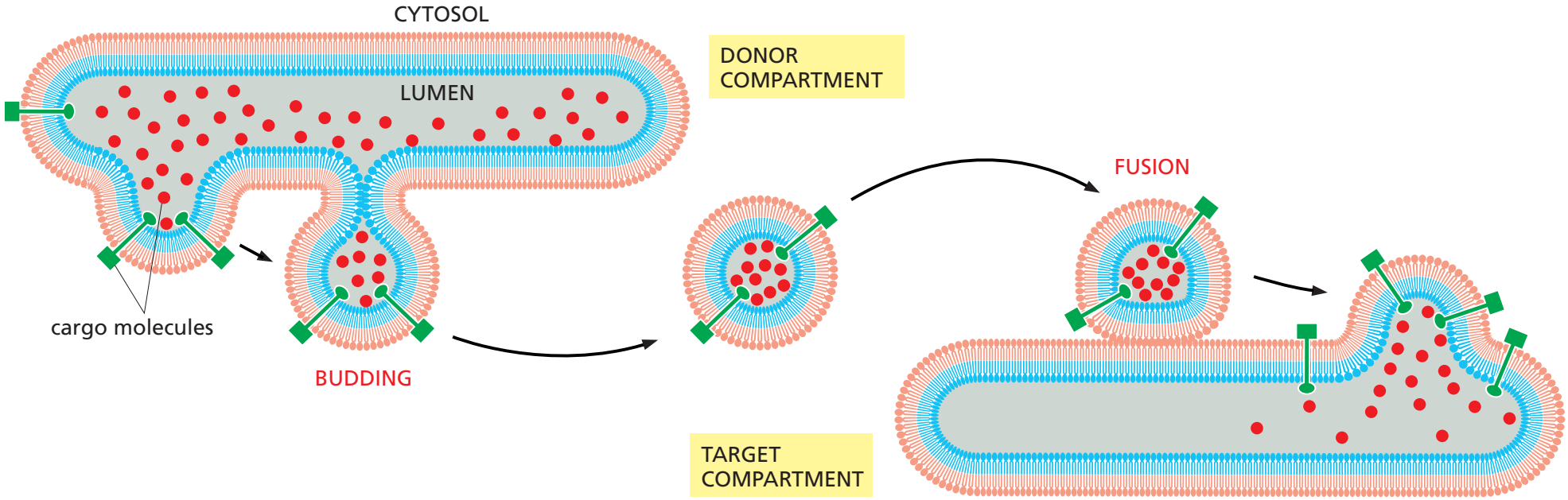


The recruitment of membrane and soluble cargo molecules into ER transport vesicles

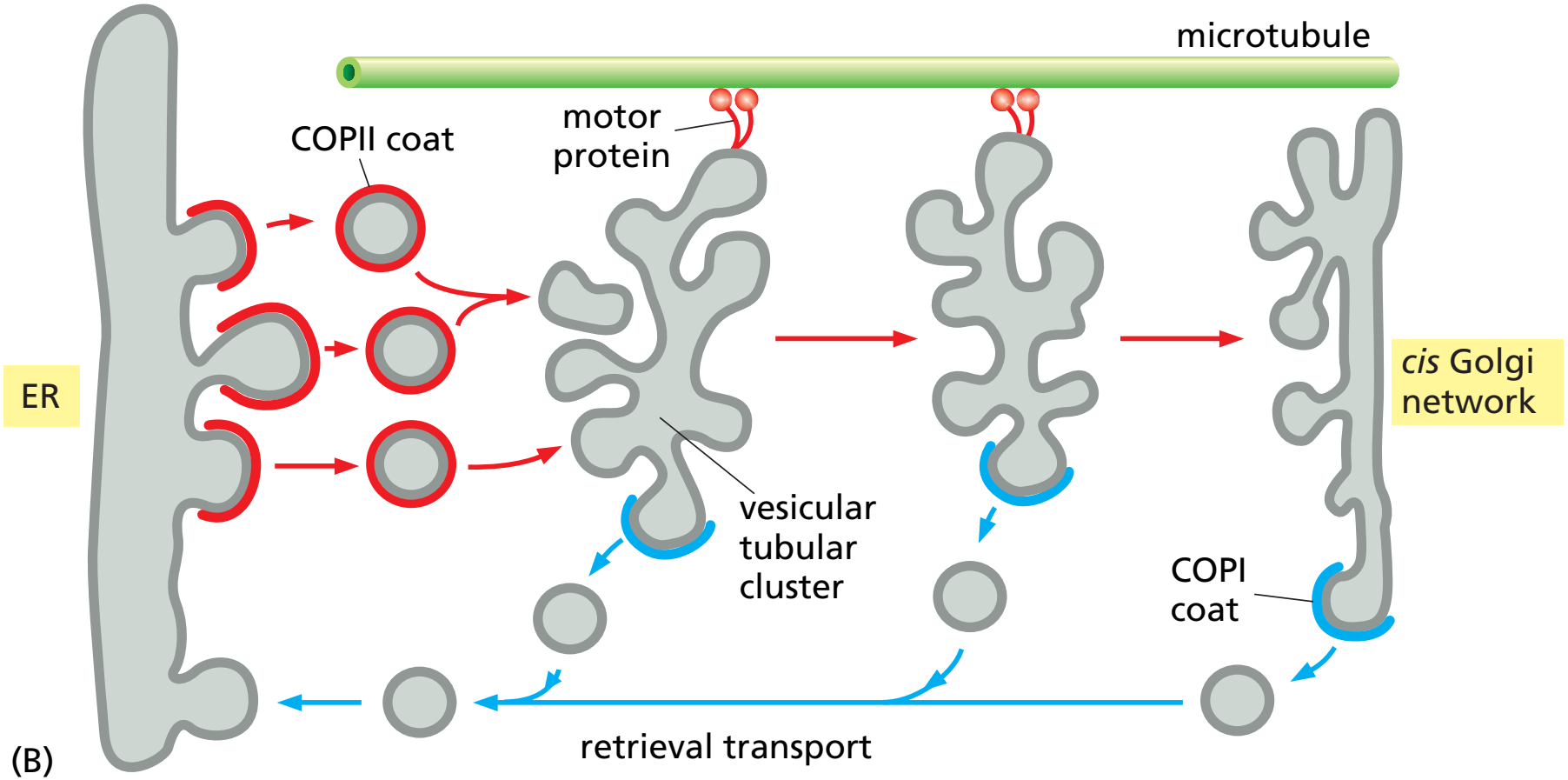


Movement of vesicles is aided by the cytoskeleton

Basics of vesicle transport



Movement of vesicles goes across microtubules

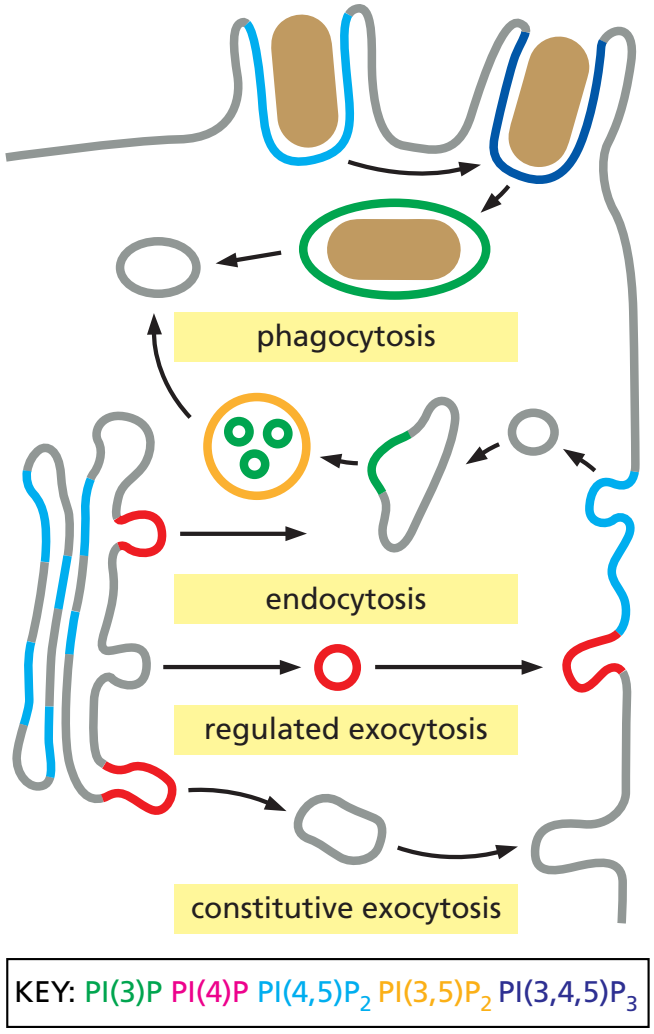


http://www.youtube.com/watch?v=7sRZy9PgPvg

How are vesicles recognized by the correct destination compartment?

Addressing implies recognition, which implies a notion of identity
What contributes to the identity of a compartment or a domain of a compartment?

Part1 PI's

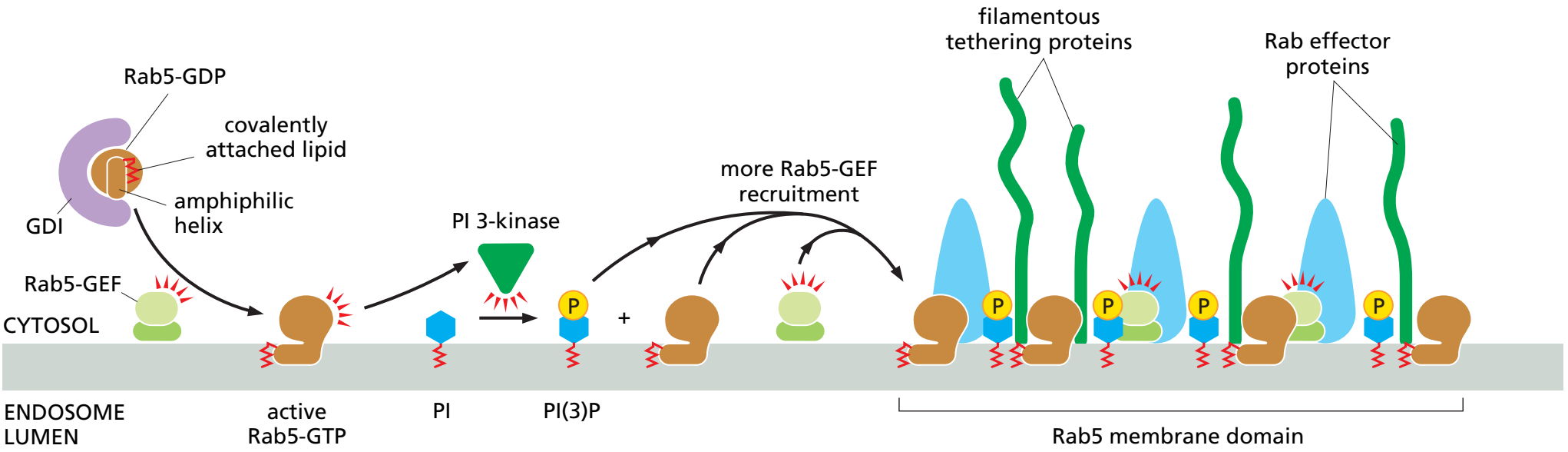


Part 2 RAB proteins: Subcellular locations of Some Rab Proteins

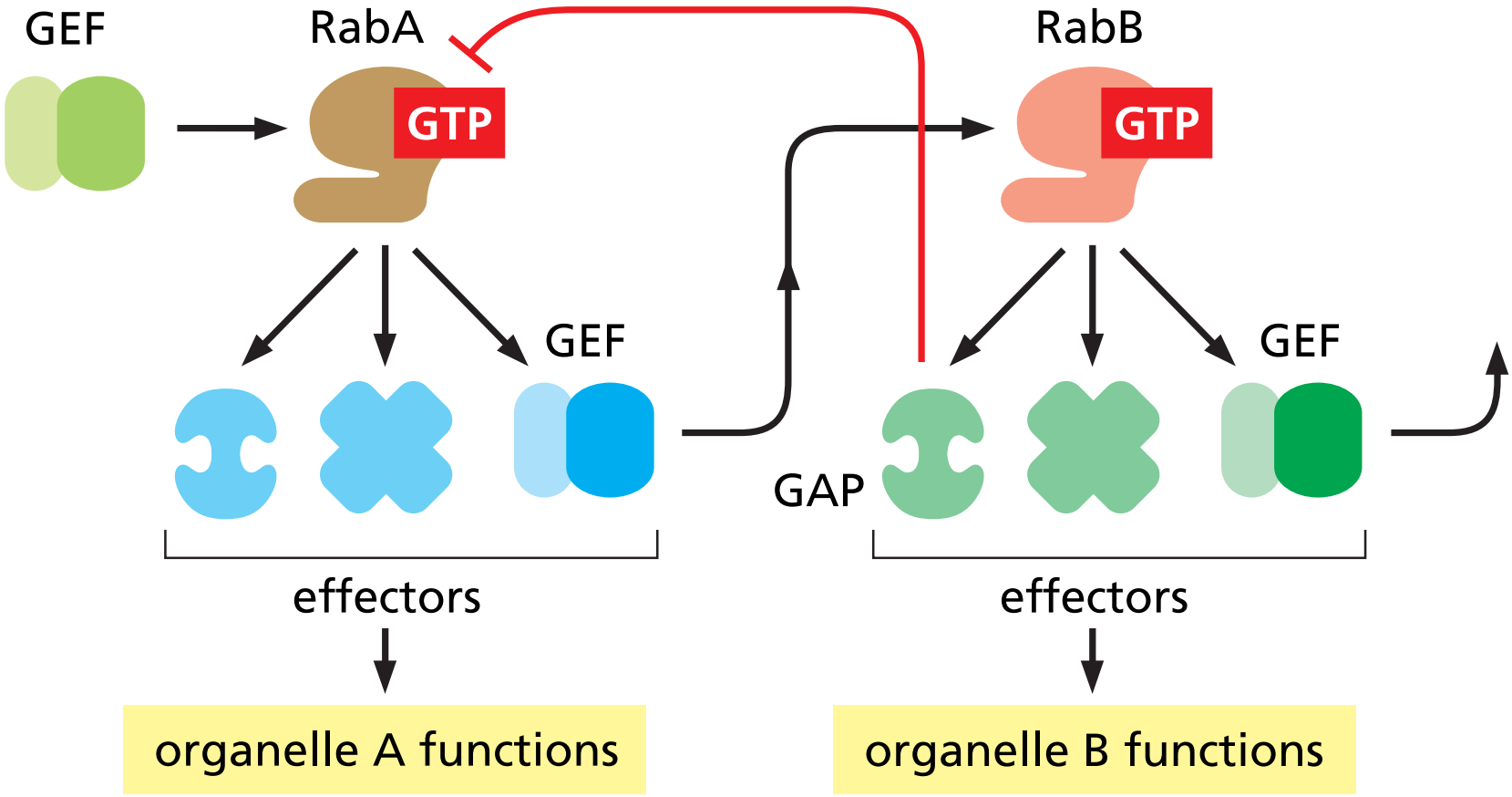
the Rab family of small GTPases: identity markers involved in vesicle recognition

TABLE 13–1 Subcellular Locations of Some Rab Proteins	
Protein	Organelle
Rab1	ER and Golgi complex
Rab2	*cis* Golgi network
Rab3A	Synaptic vesicles, secretory vesicles
Rab4/Rab11	Recycling endosomes
Rab5	Early endosomes, plasma membrane, clathrin-coated vesicles
Rab6	Medial and *trans* Golgi
Rab7	Late endosomes
Rab8	Cilia
Rab9	Late endosomes, *trans* Golgi

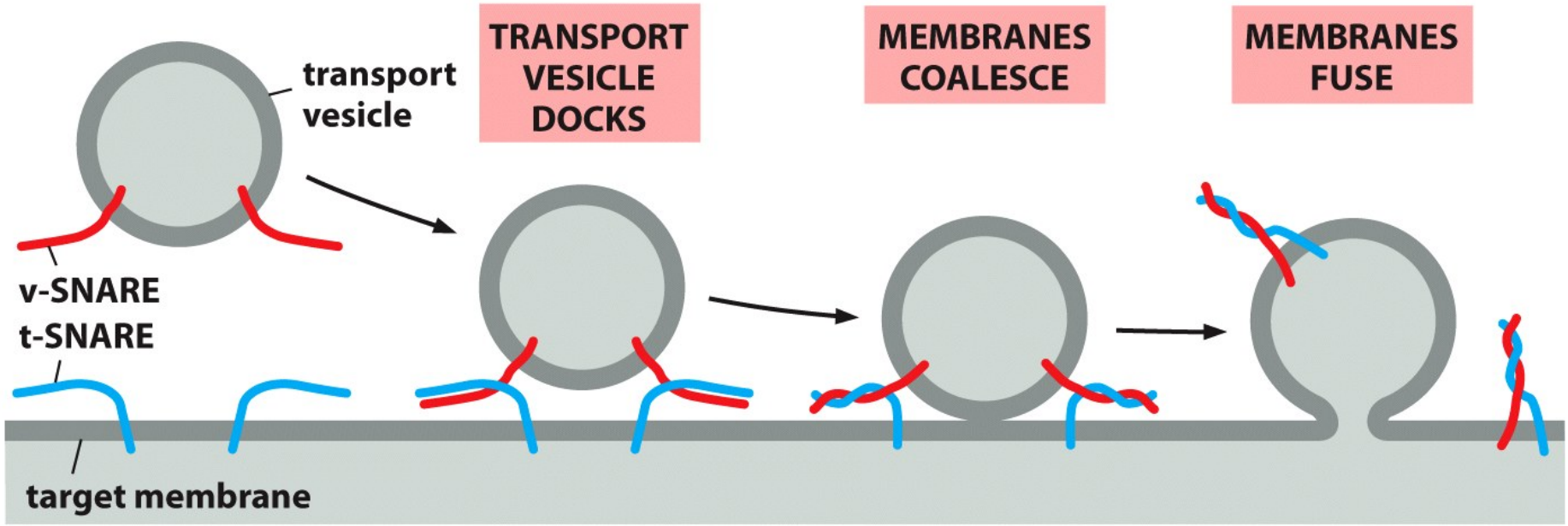
The formation of a Rab5 domain on the endosome membrane



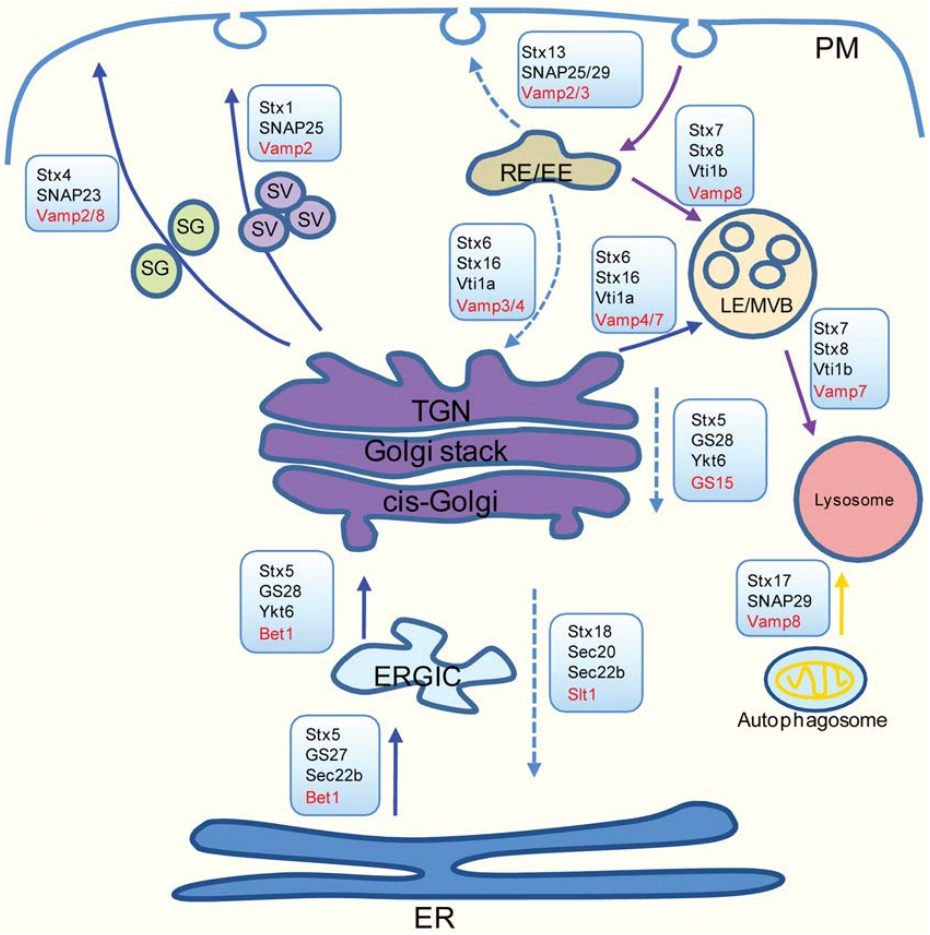
A model for a generic Rab cascade



Part 3 the fusion proteins: SNARE proteins



SNARE complexes can only be made with certain combinations and thus define specificity



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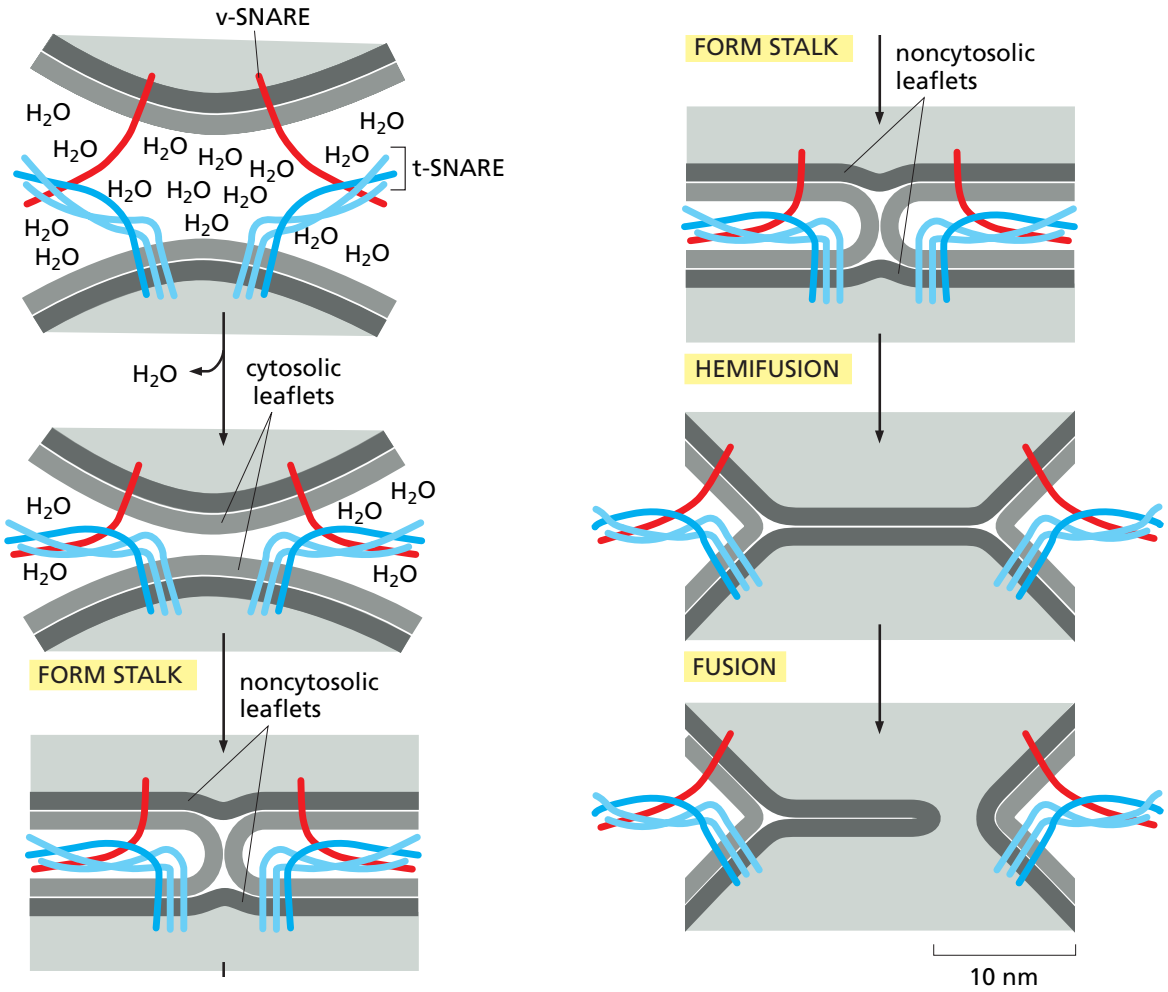
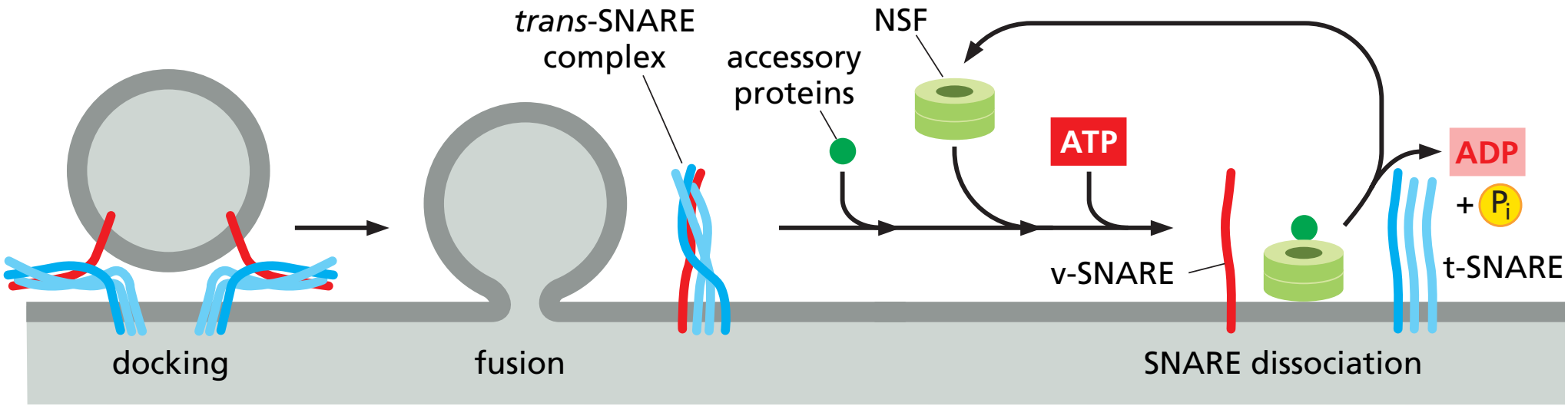
A model for how SNARE proteins may catalyze membrane fusion

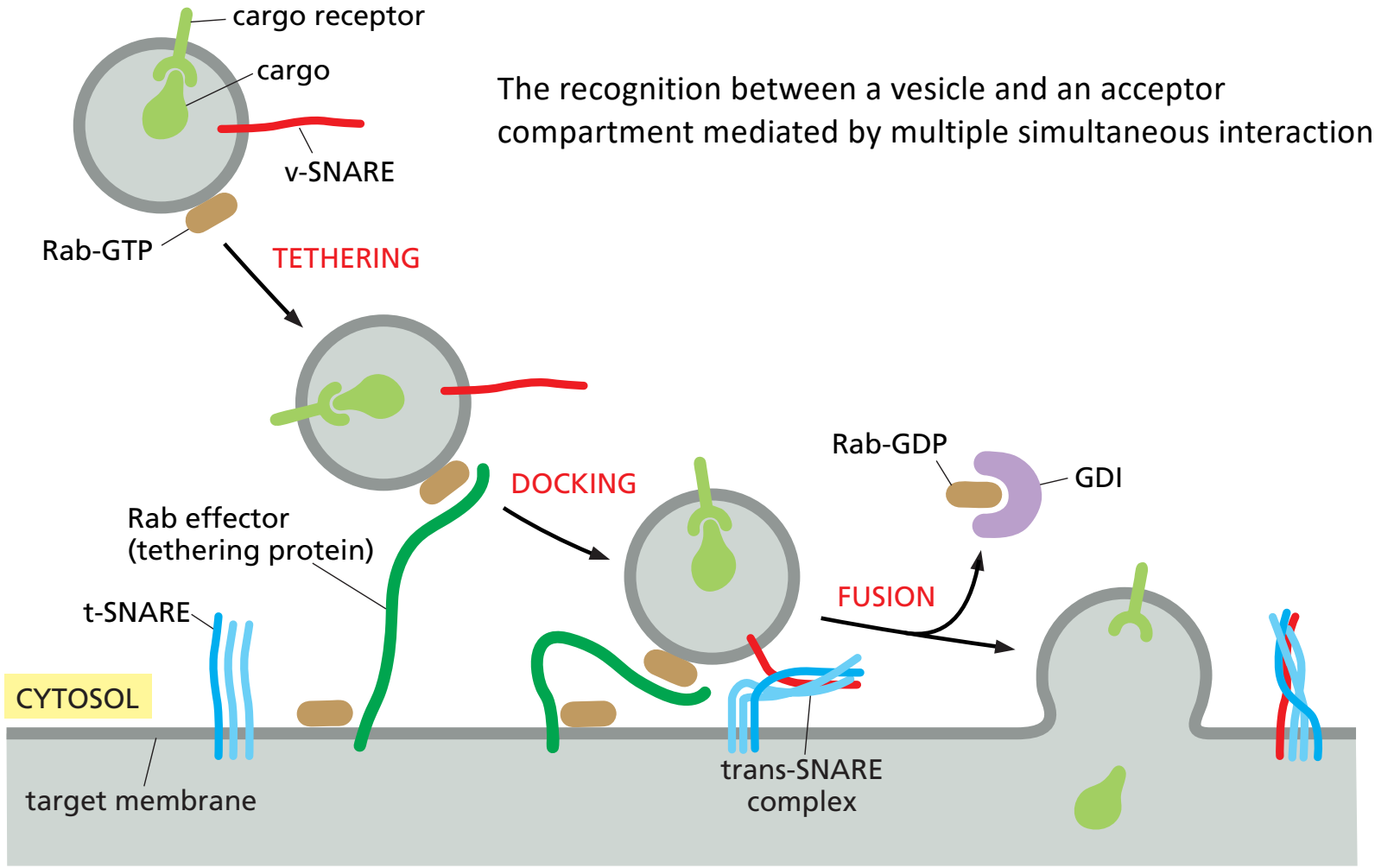


Dissociation of SNARE pairs by NSF after a membrane fusion cycle



SNARE complexes are very stable; therefore, it costs energy to dissociate them

Tethering of a transport vesicle to a target membrane



The recognition between a vesicle and an acceptor compartment mediated by multiple simultaneous interactions