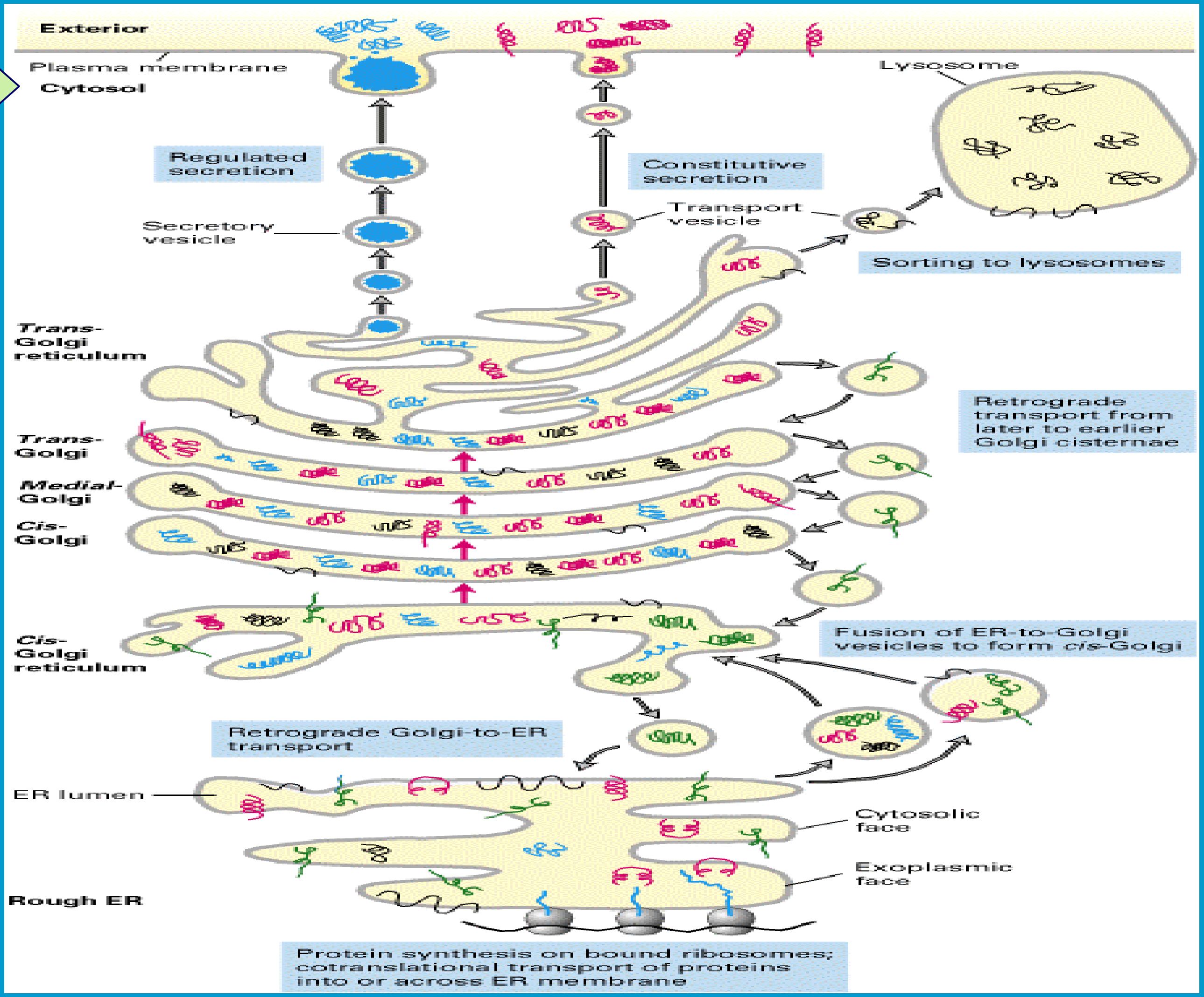
The secretory pathway

Protein synthesis and sorting

Destination

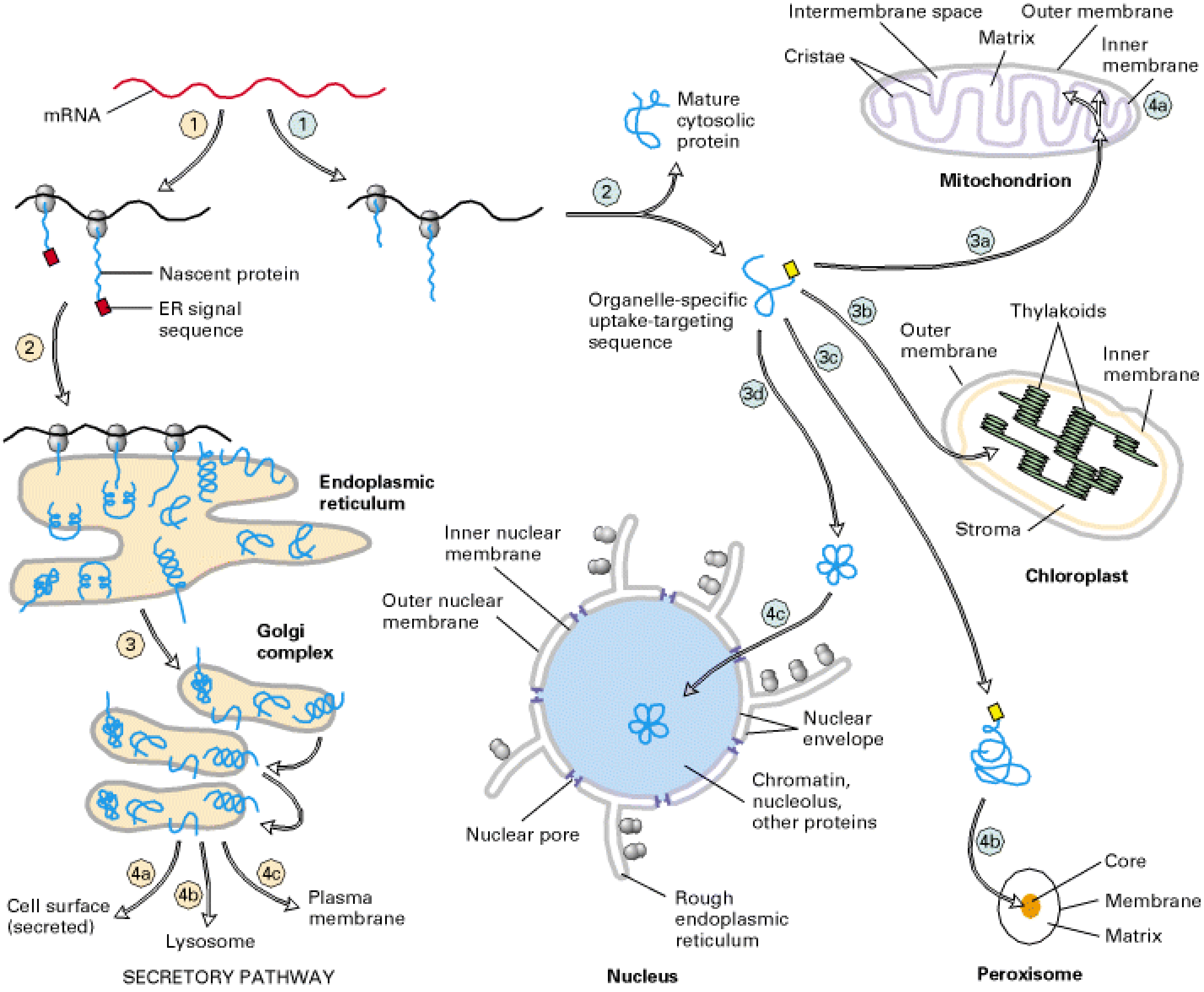
Sorting packaging

Synthesis

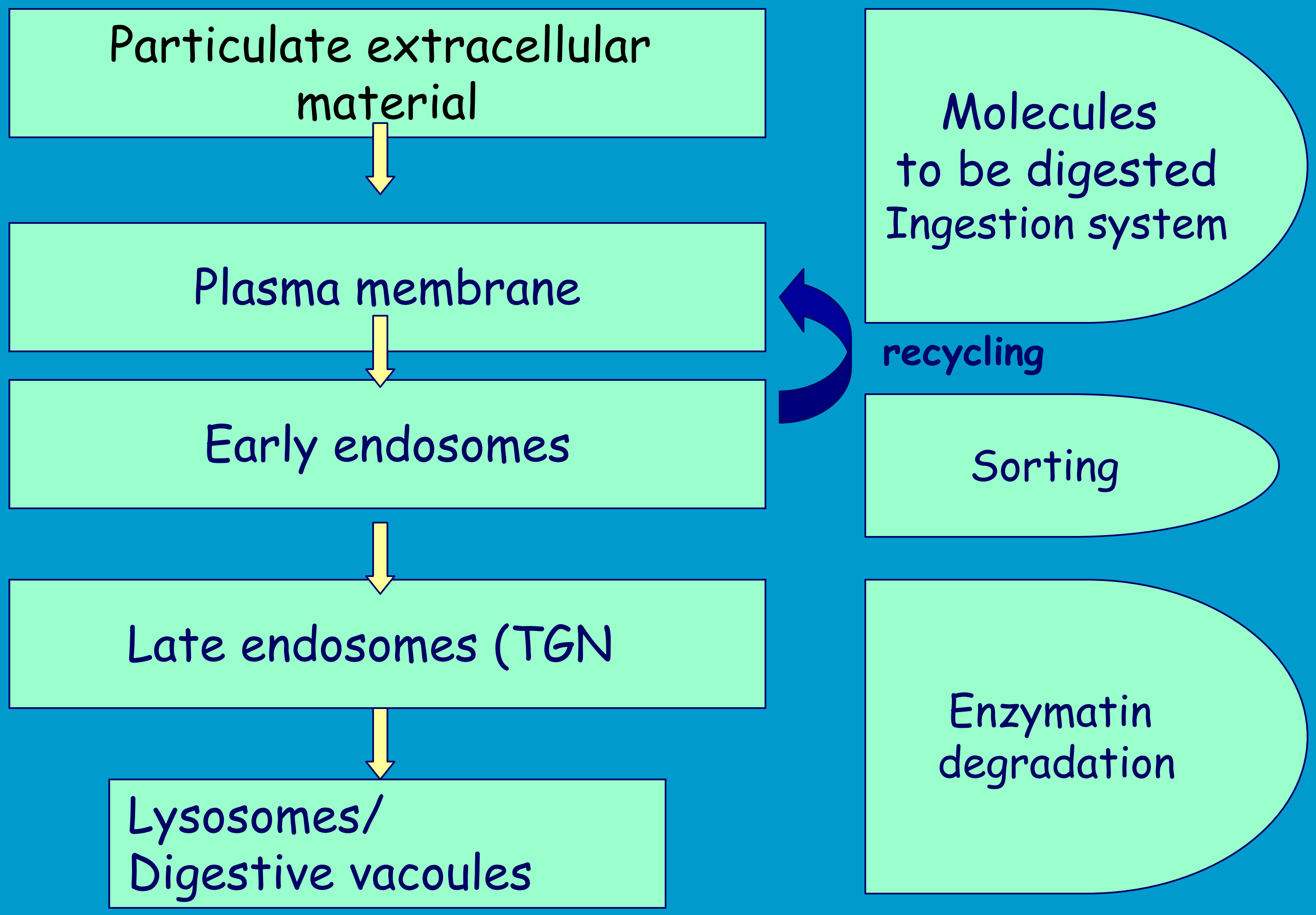


Molecular Cell Biology. Lodish *et als*. Figure 17-13

Secretory pathway



Endocytotic pathway



Particulate extracellular material

Plasma membrane

Early endosomes

Late endosomes (TGN

Lysosomes/
Digestive vacoules

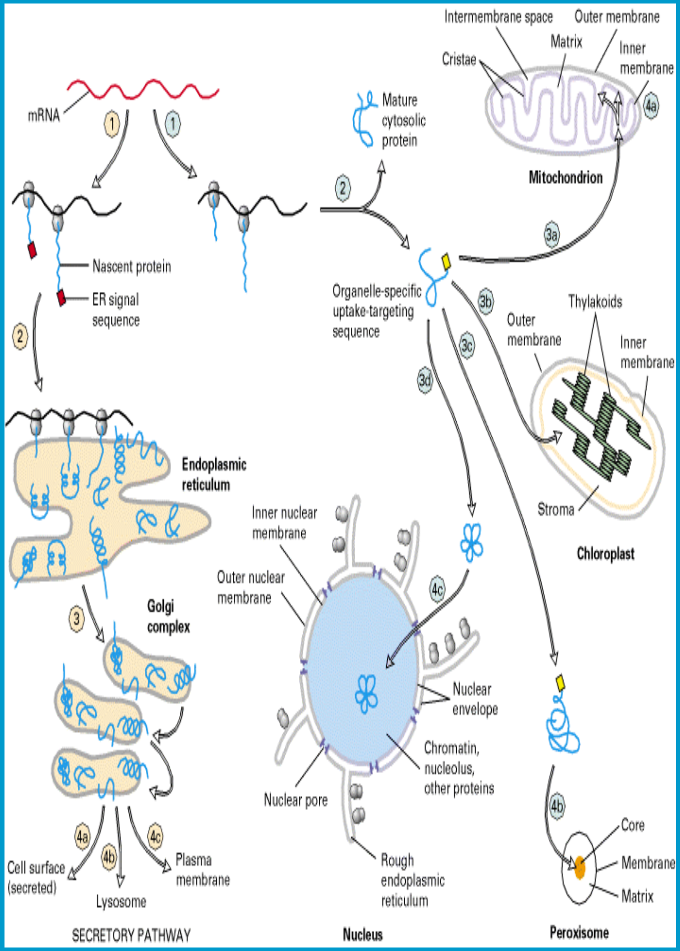
Molecules
to be digested
Ingestion system

recycling

Sorting

Enzymatin
degradation

Overview of the secretory pathway



1. Co-translational translocation. Newly made secretory proteins are located in the lumen of the rough ER.

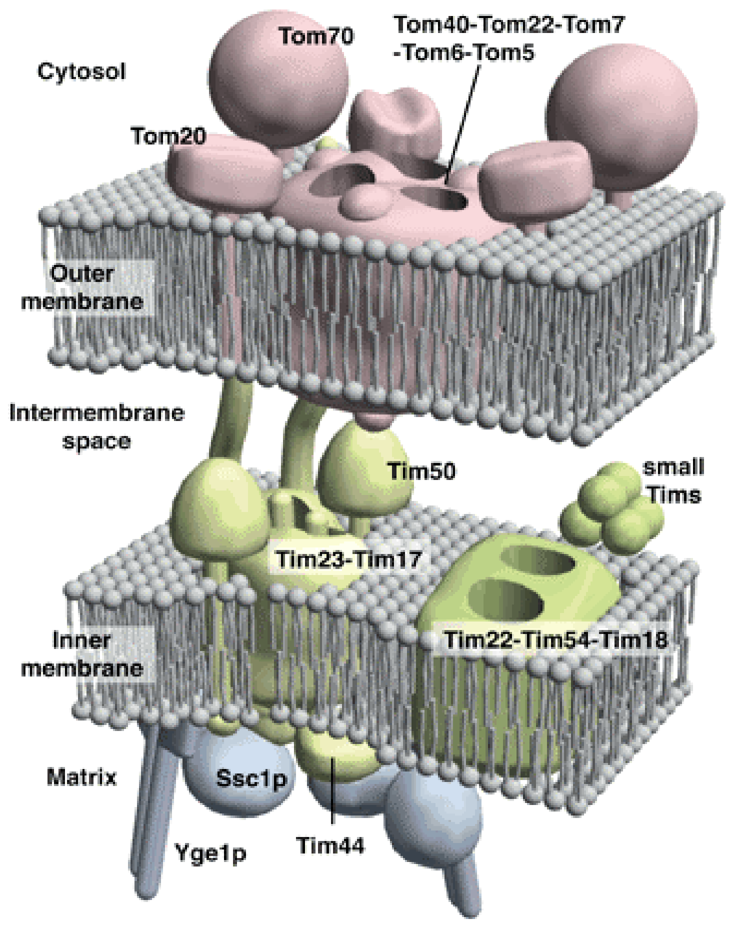
2. Moving in the secretory pathway

- Rough ER
- ER-to-Golgi transport vesicles
- Golgi cisternae
- Transport vesicles
- Cell surface (exocytosis)

Secreted and integral membrane proteins undergo modifications in the secretory pathway.

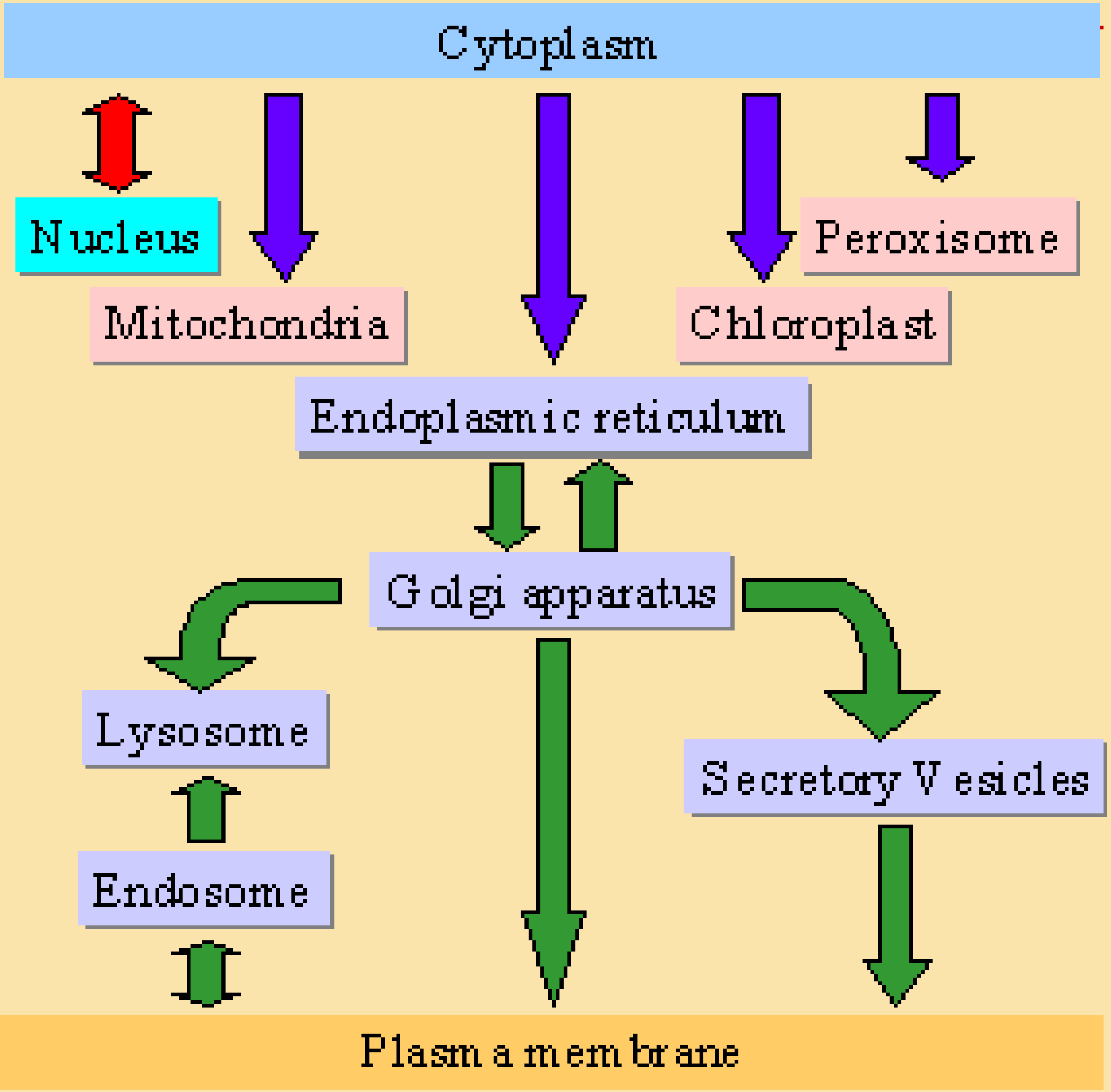
Translocation across membranes

Common principles



1. Multiple pathways of protein translocation across membrane exit.
2. Molecular chaperones are required in the cytosol, inside the organelle and often within the organelle membrane.
3. ATP and/or GTP hydrolysis is often required.
4. A proton-motive force across the membrane is often required.
5. Protein translocation occurs through gated, aqueous channels

The basic targeting pathways


Cytoplasm

Nucleus

Mitochondria

Peroxisome

Chloroplast

Endoplasmic reticulum

Golgi apparatus

Lysosome

Secretory Vesicles

Endosome

Plasma membrane

Eukaryotic targeting signals

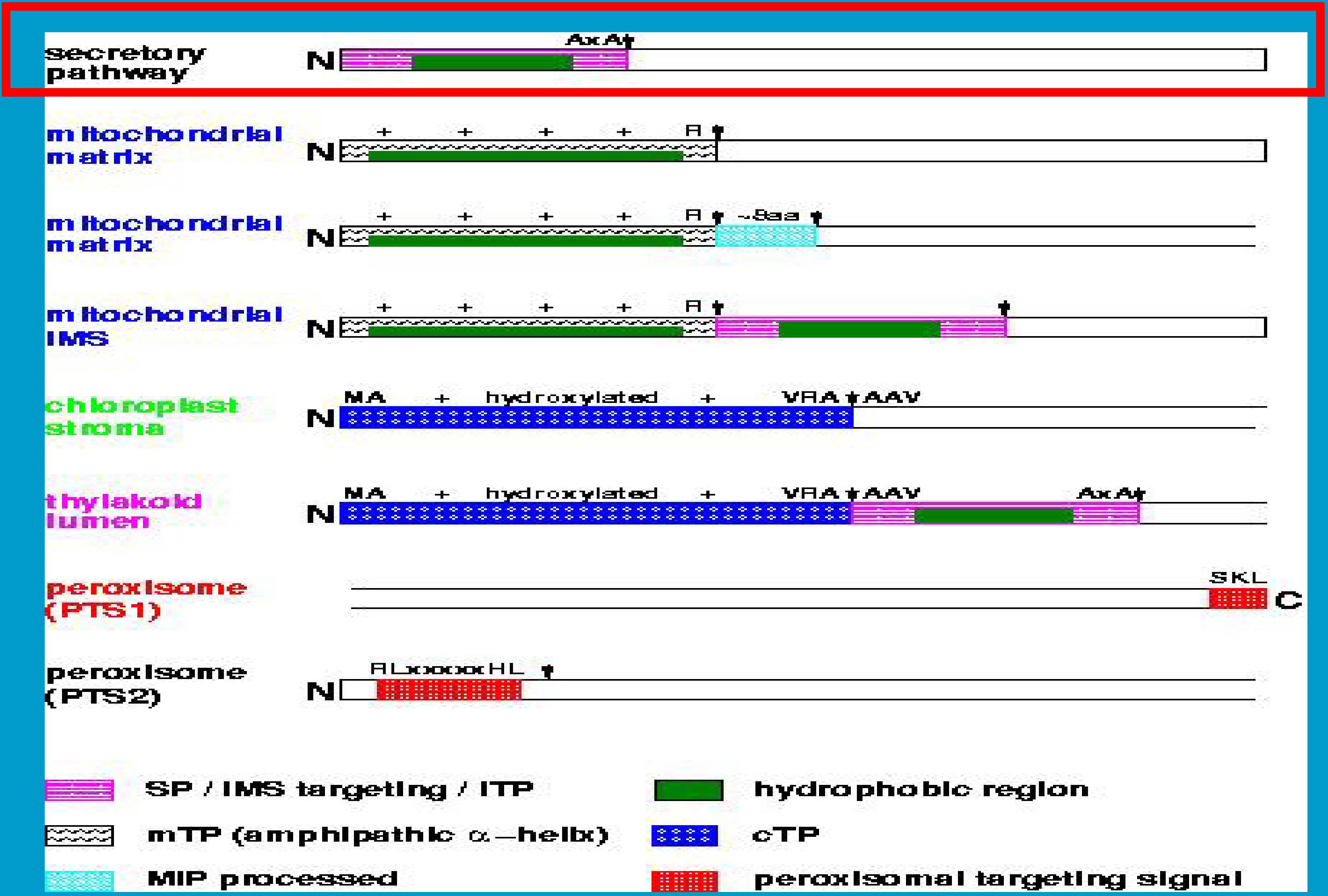


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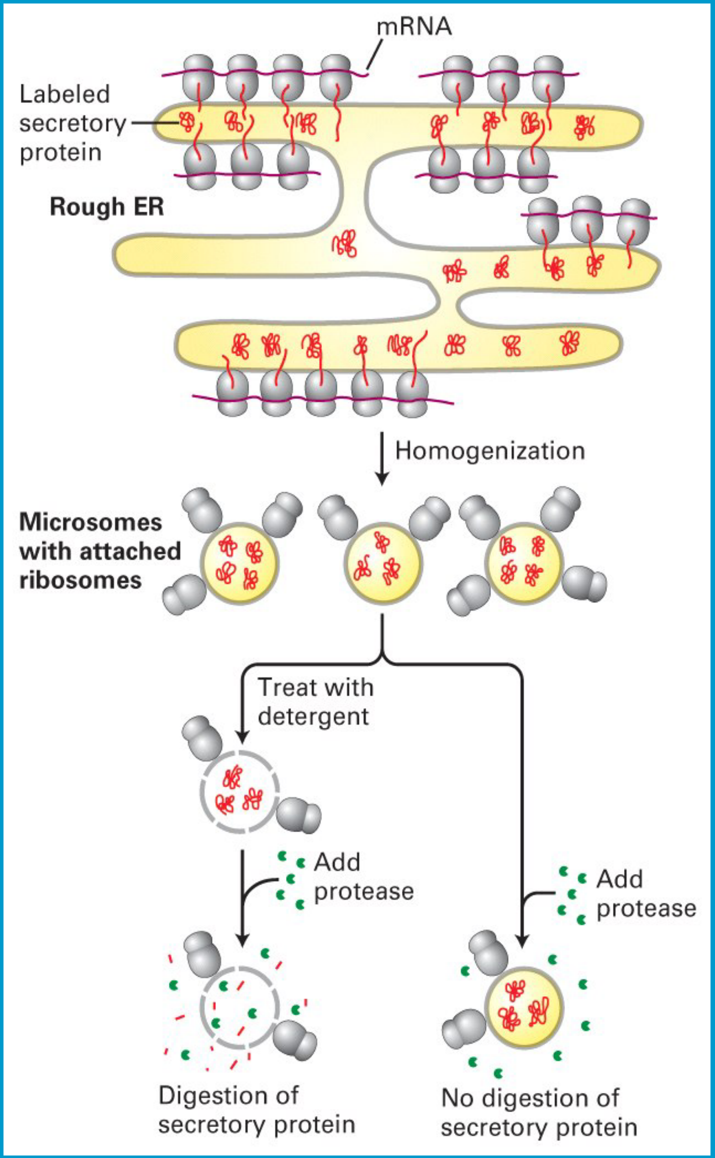
Table 17-1. Properties of Uptake-Targeting Signal Sequences That Direct Proteins from the Cytosol to Organelles

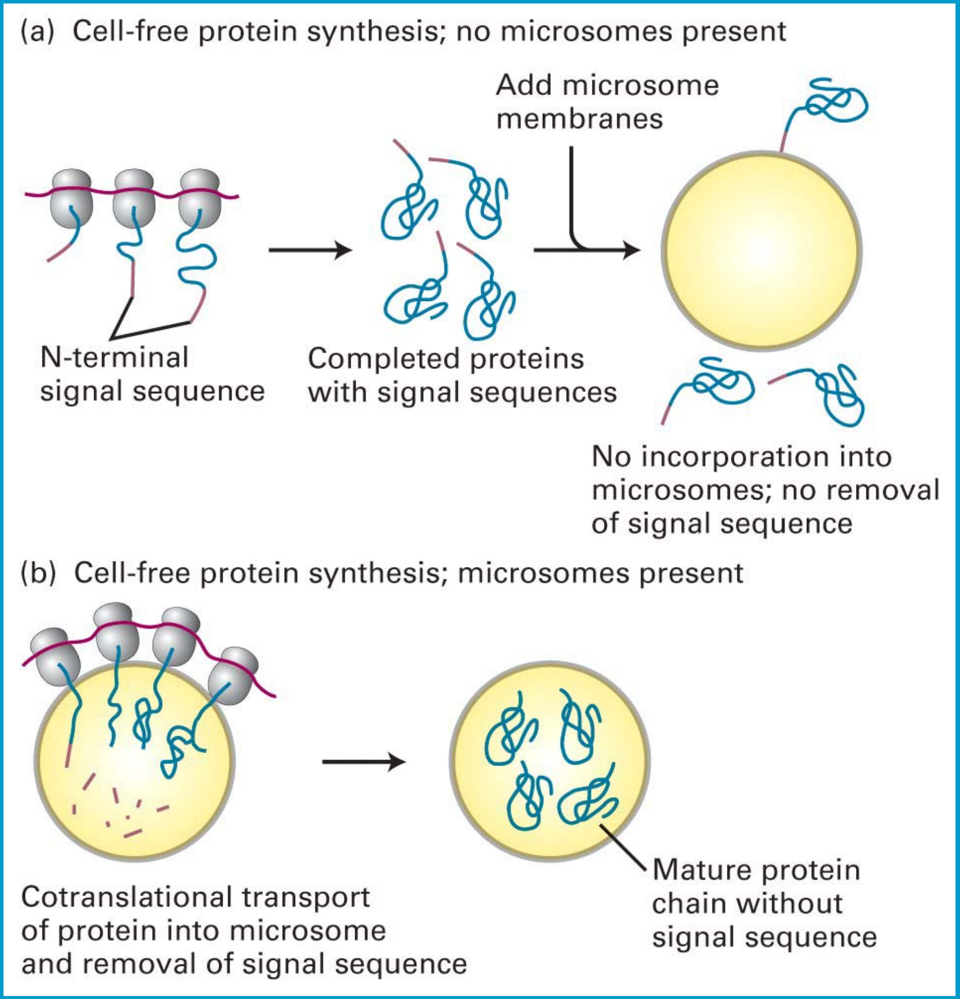
Target Organelle	Usual Signal Location within Protein	Signal Removal*	Nature of Signal
Endoplasmic reticulum	N-terminal	(+)	"Core" of 6 12 mostly hydrophobic amino acids, often preceded by one or more basic amino acids
Mitochondrion	N-terminal	(+)	3 5 nonconsecutive Arg or Lys residues, often with Ser and Thr; no Glu or Asp residues
Chloroplast	N-terminal	(+)	No common sequence motifs; generally rich in Ser, Thr, and small hydrophobic amino acid residues and poor in Glu and Asp residues
Peroxisome	C-terminal	(−)	Usually Ser-Lys-Leu at extreme C-terminus
Nucleus	Internal	(−)	One cluster of 5 basic amino acids, or two smaller clusters of basic residues separated by ≈10 amino acids

** Indicates whether signal sequence usually is (+) or is not ( ) removed after a protein enters its target organelle.*

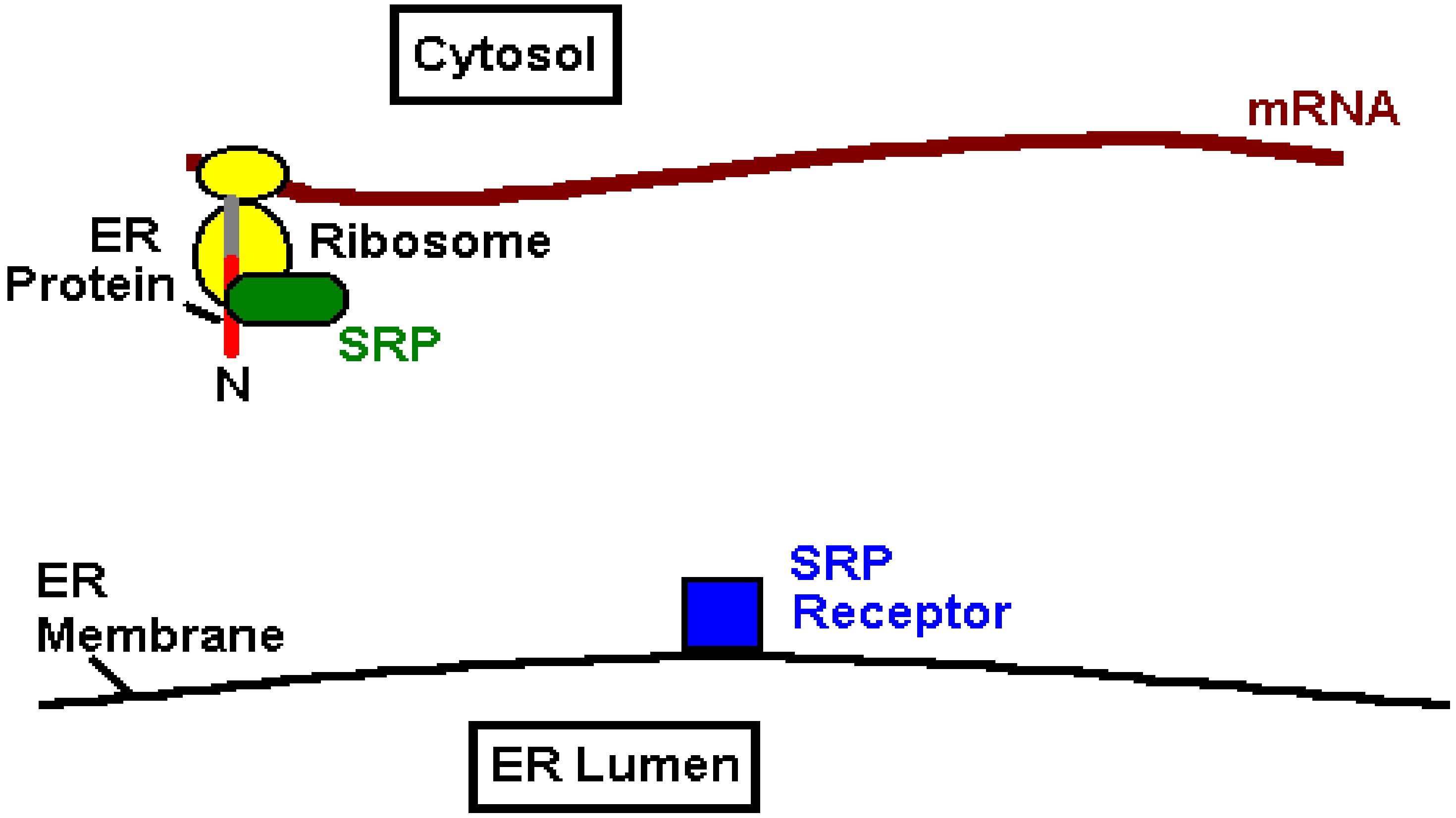
These signals direct the protein from the cytosol into the matrix space of the mitochondrion or the corresponding stroma of the chloroplast; other signals discussed in the text redirect proteins into other subcompartments of these organelles.

Translocation of secretory proteins across the ER membrane

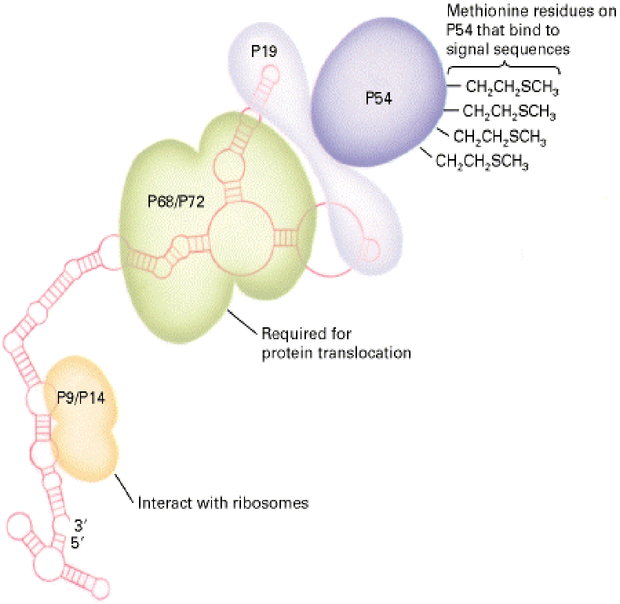




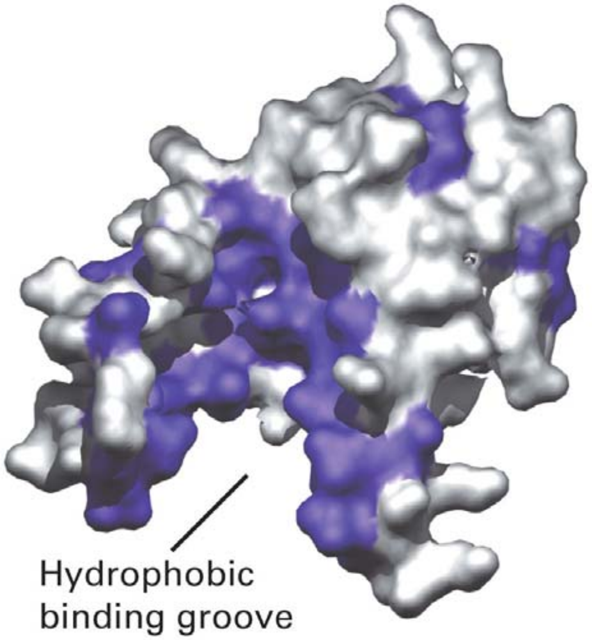
Targeting proteins to the ER



Structure of the signal-recognition particle (SRP)

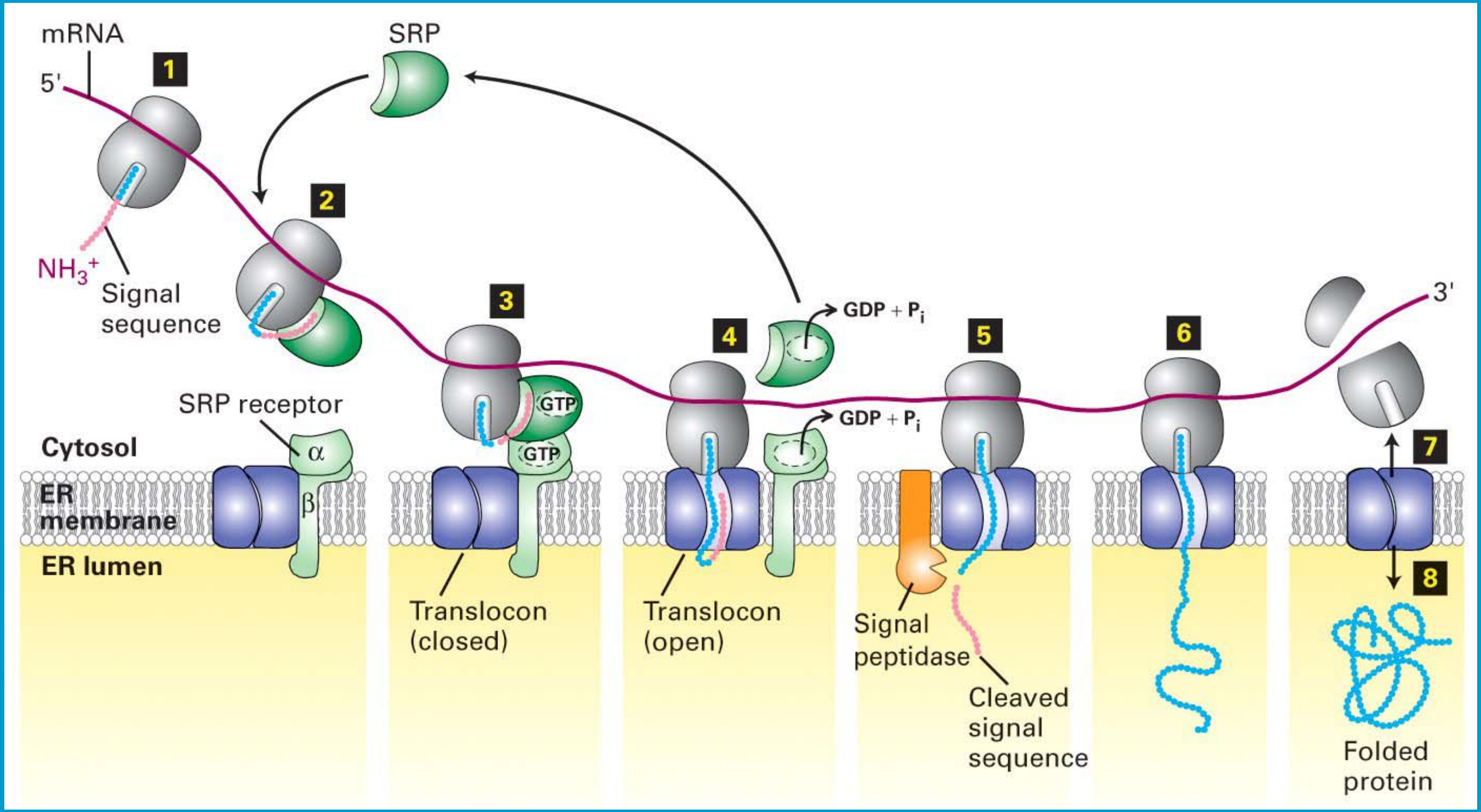


(b) Ffh signal sequence–binding domain (related to P54 subunit of SRP)



SRP comprises:
1. One 300-nucleotide RNA.
2. 6 proteins: P9, P14, P19, P54, P68, P72
3. P54 binds to hydrophobic core of the protein signal sequence .
4. SRP receptor and P54 are GTPases.

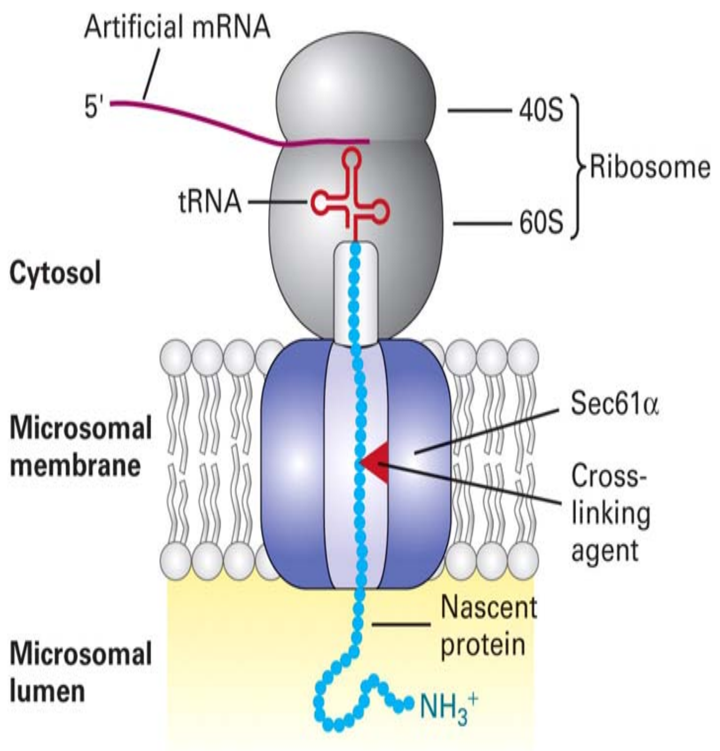
Synthesis of secretory proteins on the rough ER

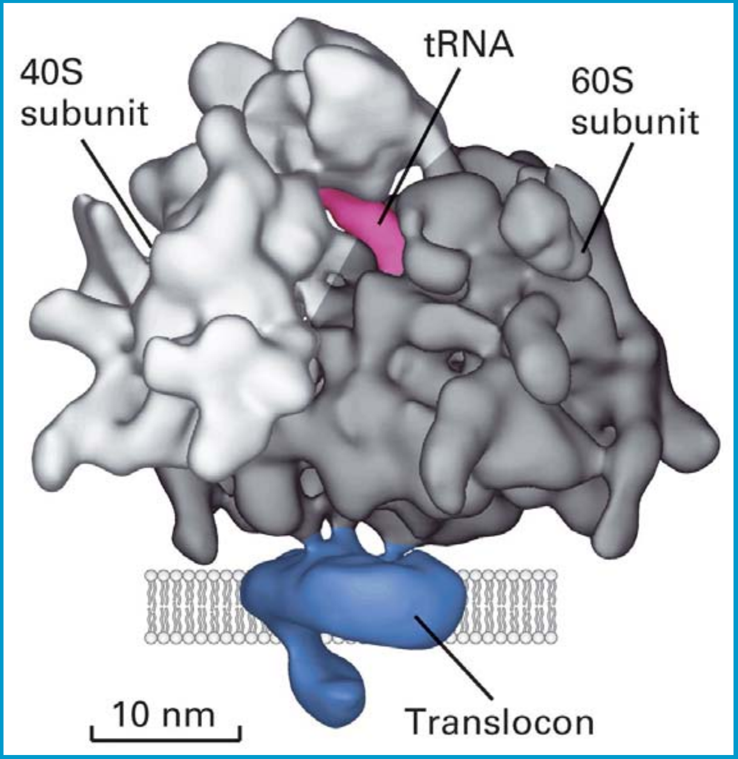


Molecular Cell Biology. Lodish *et als*. Figure 16-6

Basic components of the ER translocon

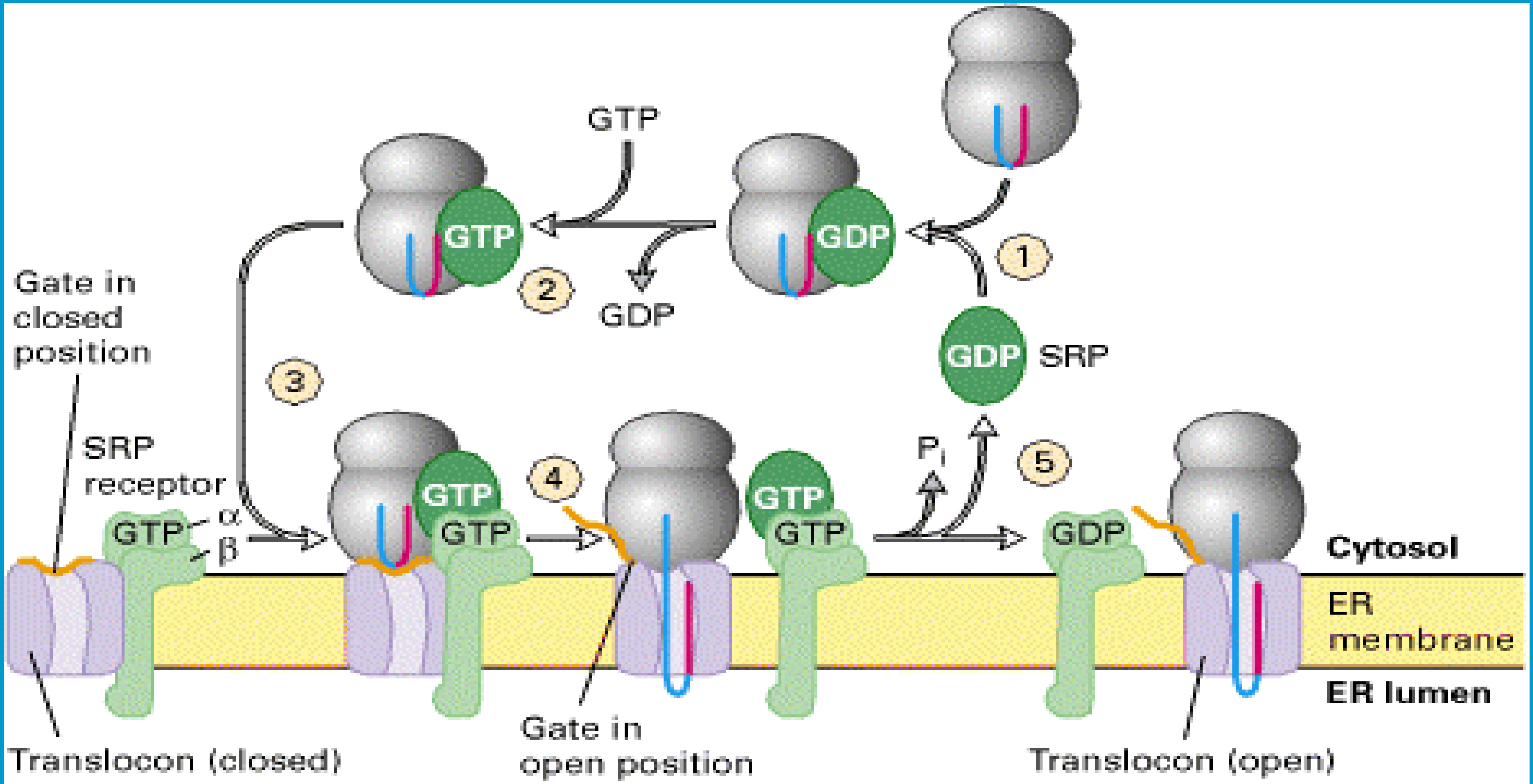
Molecular Cell Biology. Lodish *et als*. Figure 16-7,8





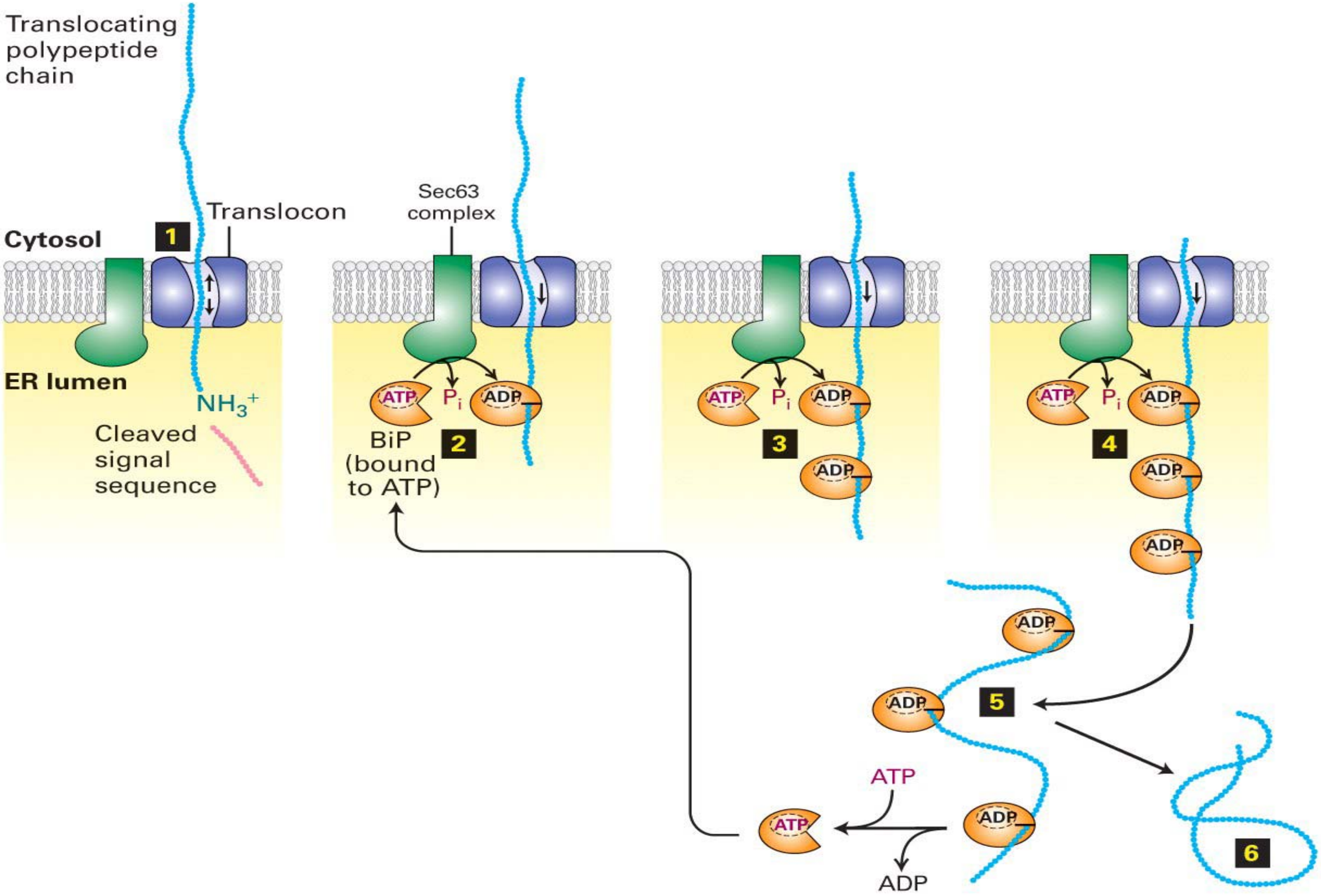
- ER translocon channel: 3-4 Sec61 complexes.
- Channel is a roughly pentagonal cilinder
- (5x8 nm / 2nm pore)
  - Absence of ribosome: closed channel.
- \- Ribosome attached:opened gate.

GTP hydrolysis: driving force for protein transport through the ER



Molecular Cell Biology. Lodish *et als*. Figure 17-20

Post-translational translocation across the ER



SUMMARY

I. Synthesis of this class of proteins is initiated on membrane-unattached ribosomes

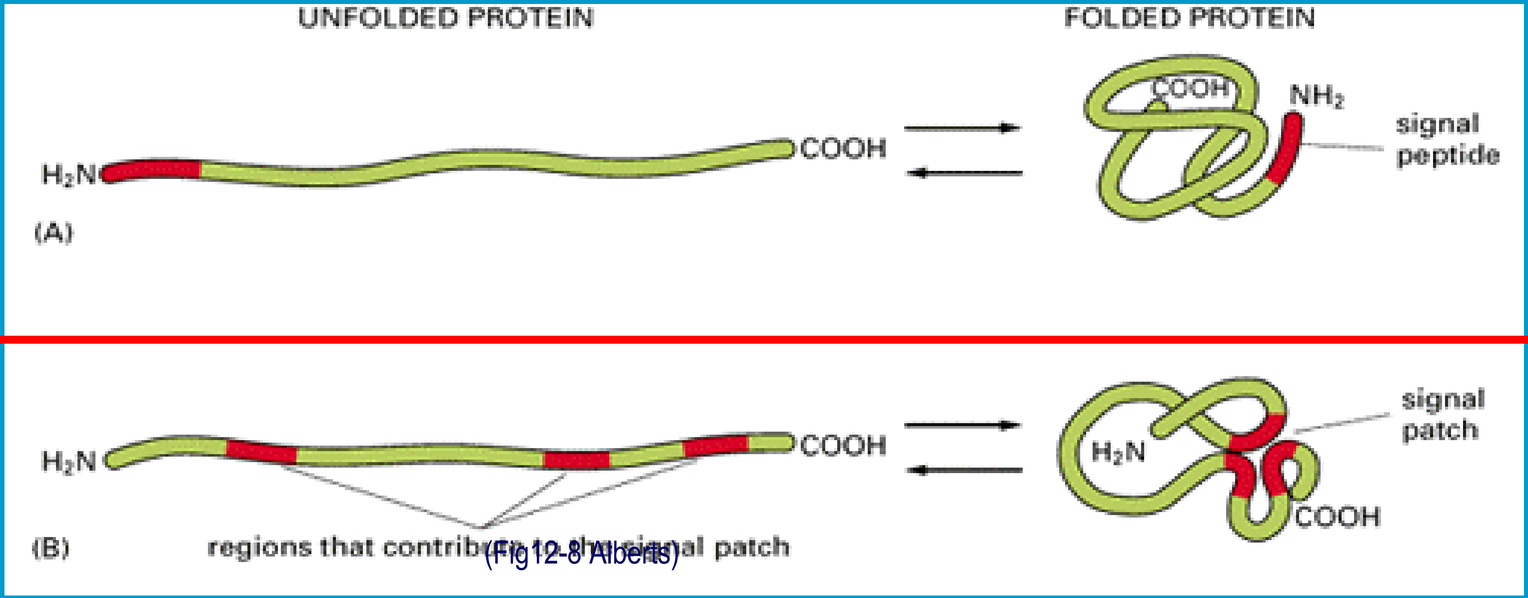
II. ER signal sequence, is recognized and bound by a signal-recognition particle (SRP), which in turn is bound by an SRP receptor on the rough ER membrane

III. The SRP directs both the binding of the ribosome to the ER membrane and the insertion of the nascent protein into the transmembrane channel.

IV. Driving force: hydrolysis of GTP by the P54 subunit of SRP and the α subunit of the SRP receptor is essential for this process.

V. Proteins cross the ER membrane in an unfolded state through a protein-lined channel, the translocon, a multiprotein complex composed of TRAM protein and the Sec61 complex.

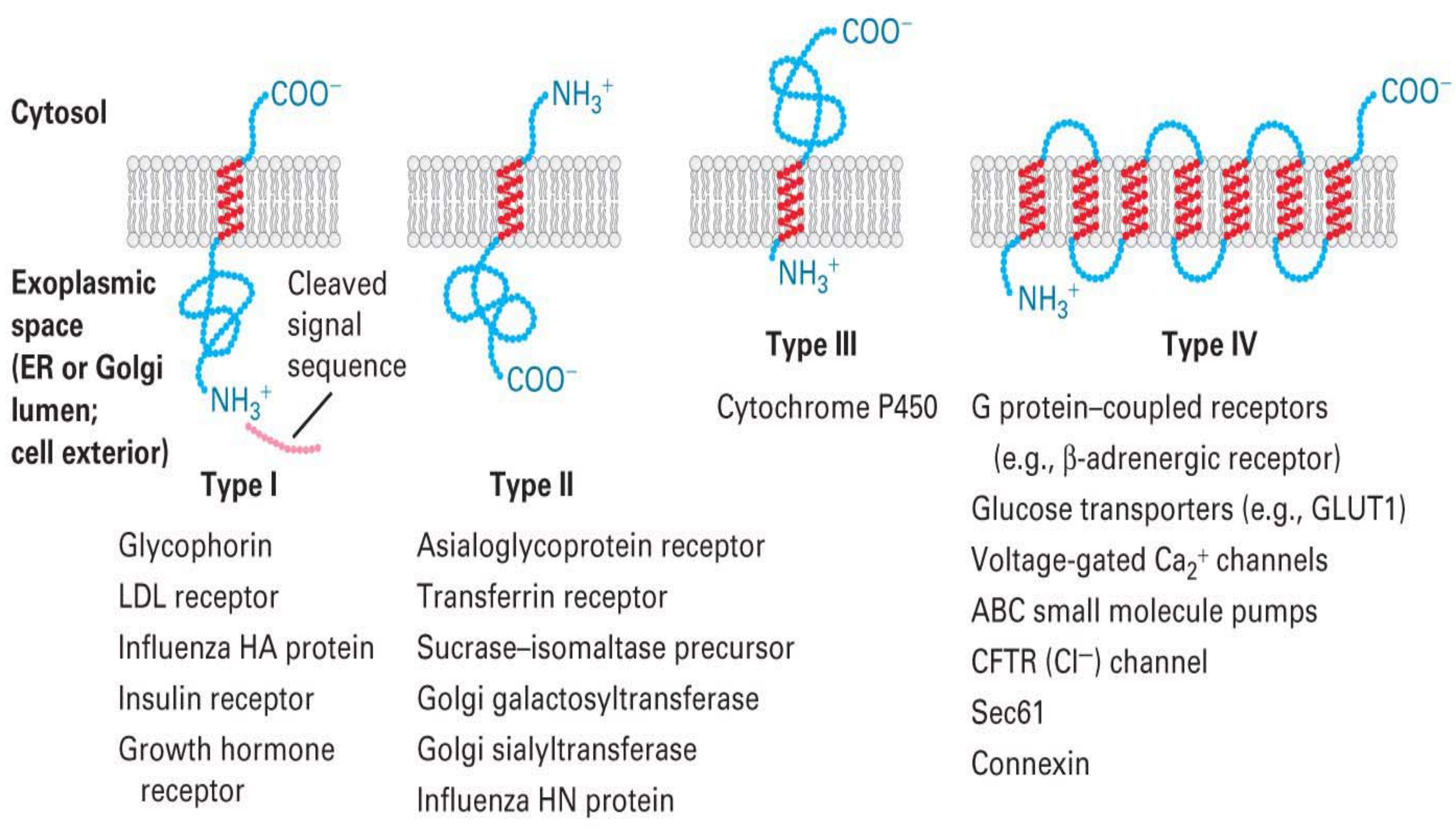
Targeting signals



(Fig12-8 Alberts)

- For membrane proteins, the targeting signal are internal hydrohobic segments.

Integral membrane protein topology



Type I

- Glycophorin
- LDL receptor
- Influenza HA protein
- Insulin receptor
- Growth hormone receptor

Type II

- Asialoglycoprotein receptor
- Transferrin receptor
- Sucrase–isomaltase precursor
- Golgi galactosyltransferase
- Golgi sialyltransferase
- Influenza HN protein

Type III

- Cytochrome P450

Type IV

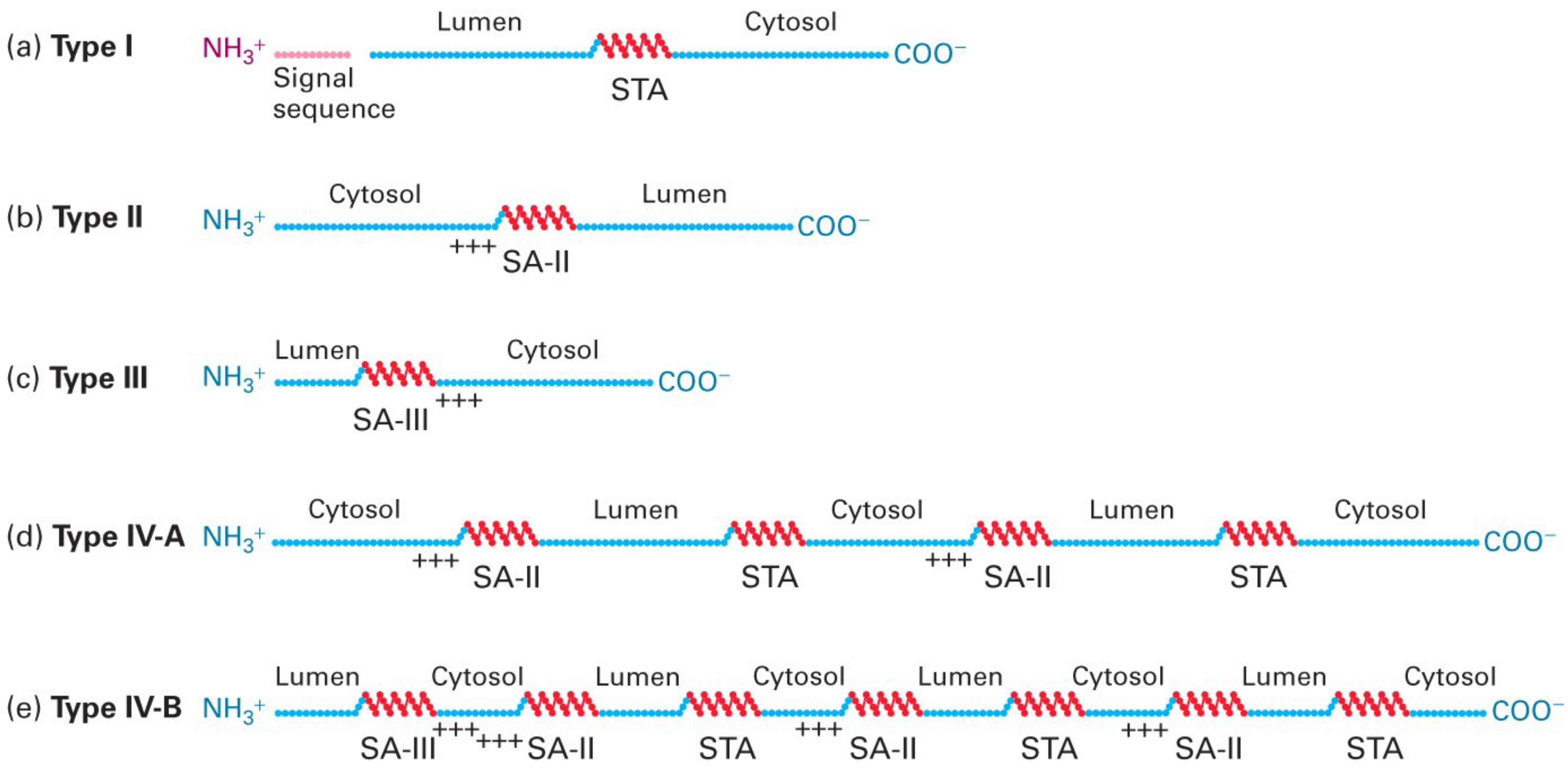
- G protein–coupled receptors (e.g., β-adrenergic receptor)
- Glucose transporters (e.g., GLUT1)
- Voltage-gated Ca$_2^+$ channels
- ABC small molecule pumps
- CFTR (Cl$^-$) channel
- Sec61
- Connexin

Molecular Cell Biology. Lodish *et als*. Figure 16-10

Integral membrane protein topology

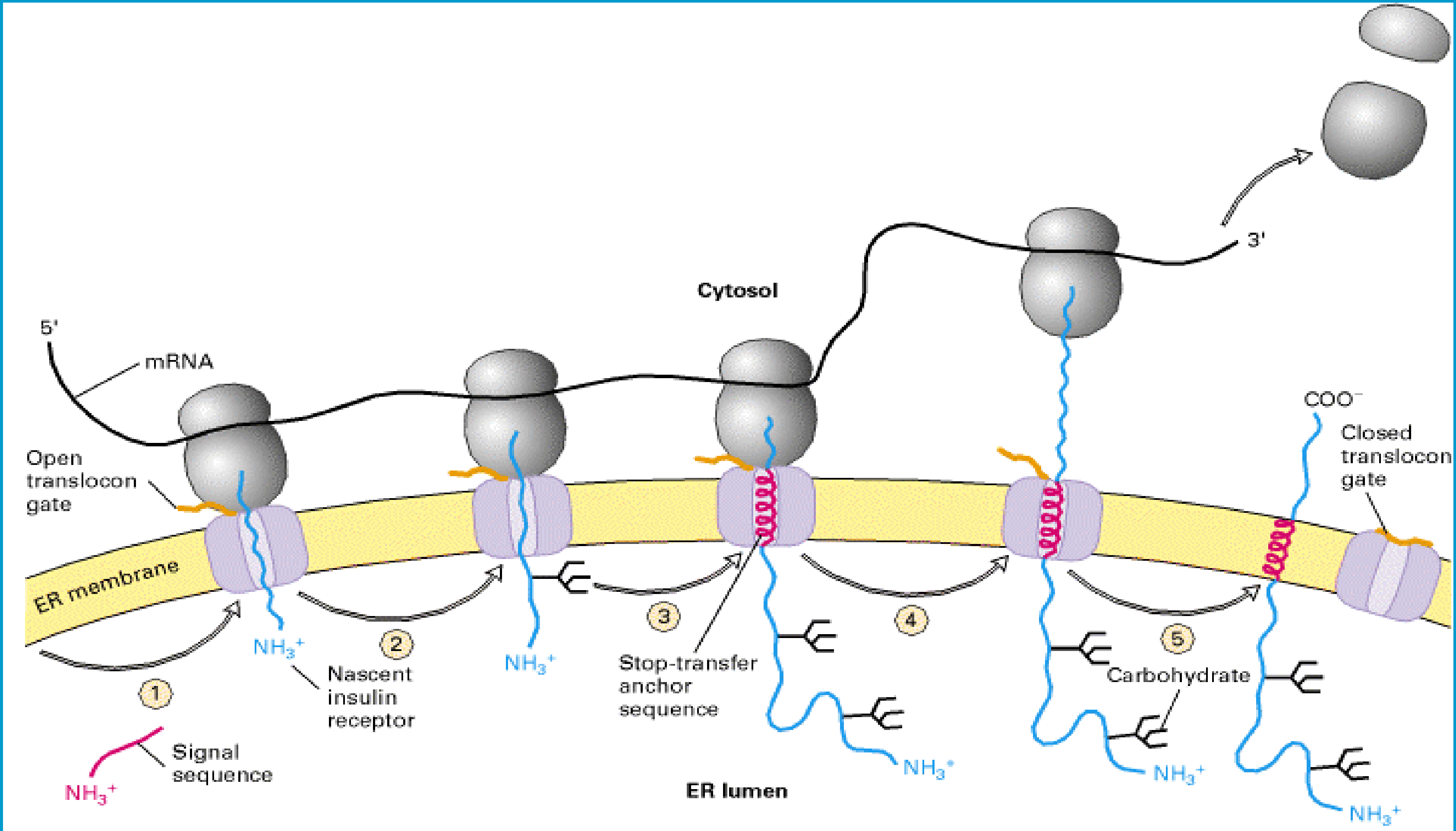
Arrengements

STA = Internal stop-transfer anchor sequence
SA-II = Internal signal-anchor sequence
SA-III = Internal signal-anchor sequence



Monotopic membrane protein insertion

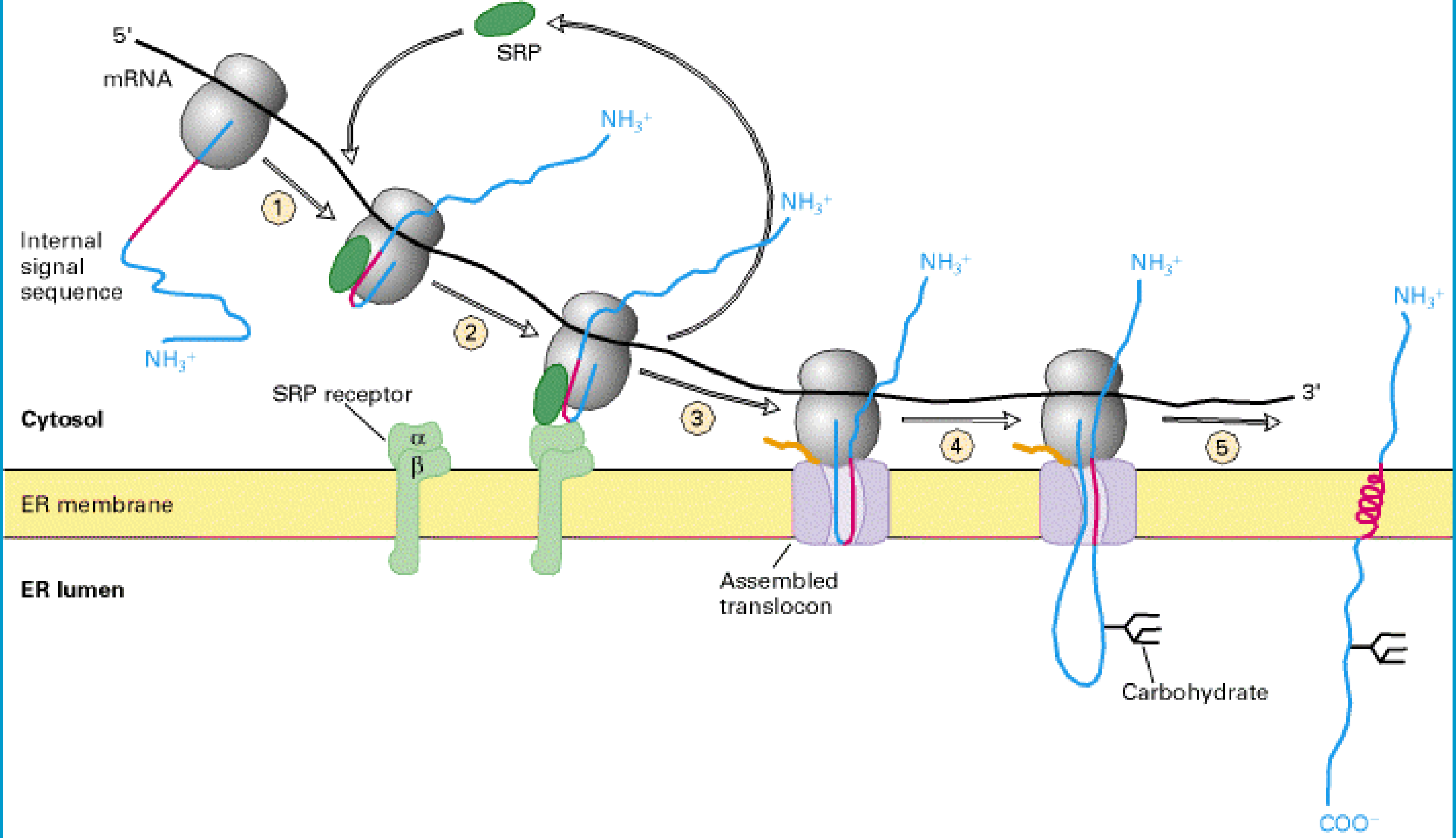
N_{lumen}-$C_{cytosol}$



Molecular Cell Biology. Lodish *et als*. Figure 17-22

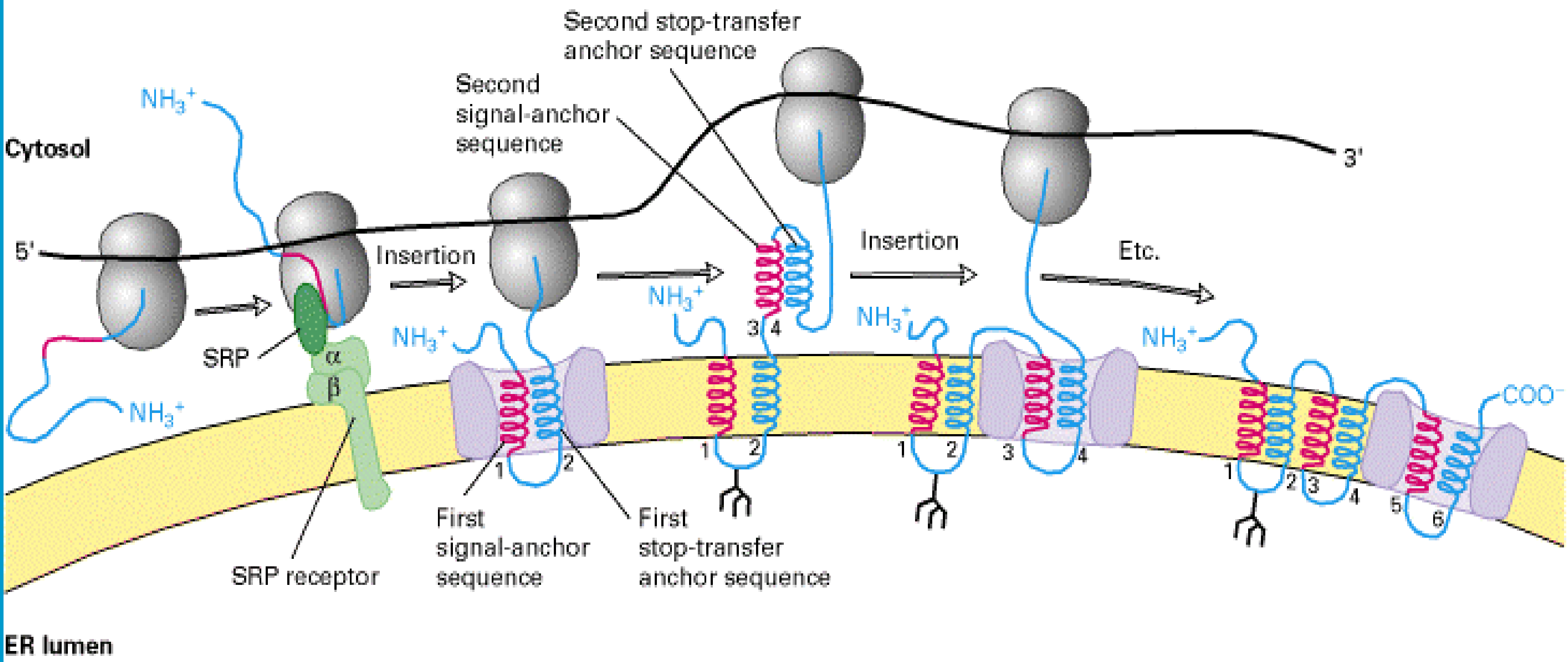
Monotopic membrane protein insertion

$N_{cytosol}$-C_{lumen}



Molecular Cell Biology. Lodish *et als*. Figure 17-23

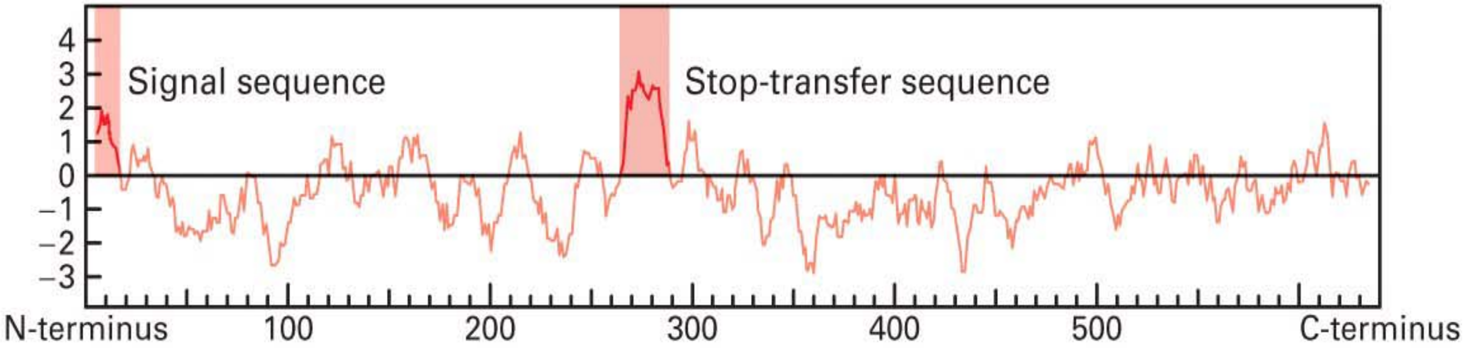
Polytopic membrane protein insertion

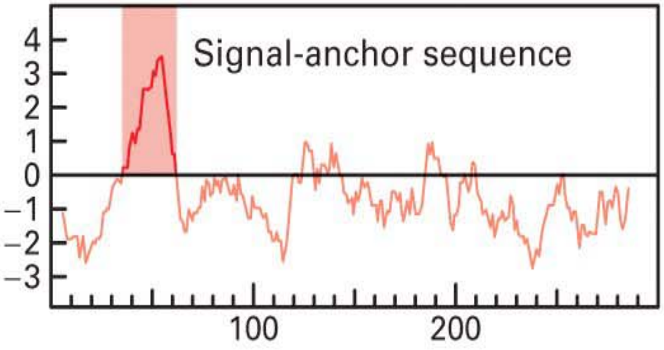


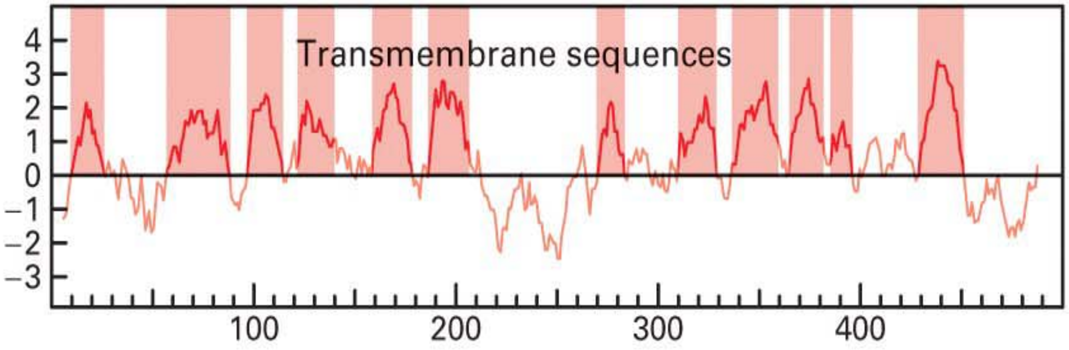
Molecular Cell Biology. Lodish *et als*. Figure 17-24

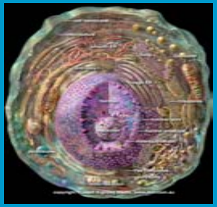
Integral membrane protein topology

Hydropathy profiles


(a) Human growth hormone receptor (type I)


(b) Asialoglycoprotein receptor (type II)


(c) GLUT1 (type IV)



Properties of the different protein translocation systems

	Co/post-translational	Driving force ATP/GTP	Driving force ΔpH/Δϕ	Molecular chaperones	Substrate unfolding	Signal peptidase
ENDOPLASMIC RETICULUM	Co-translational	ATP/GTP	No	yes	Yes	yes
MITOCHONDRIA						
Outer membrane						
Inner membrane						
CHLOROPLAST						
Outer envolope						
Inner envelope						
PEROXISOME						

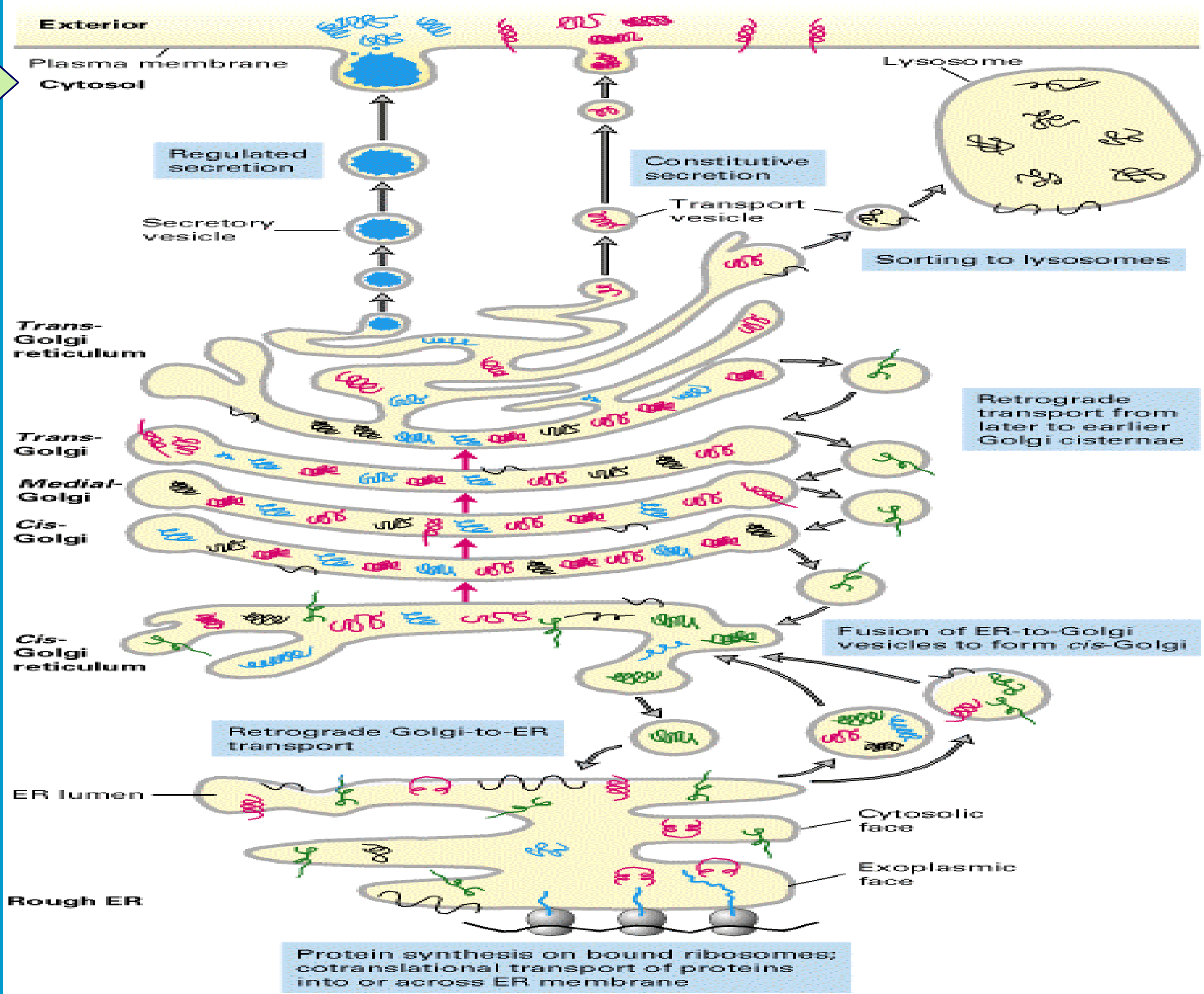
The secretory pathway

Protein synthesis and sorting

Destination

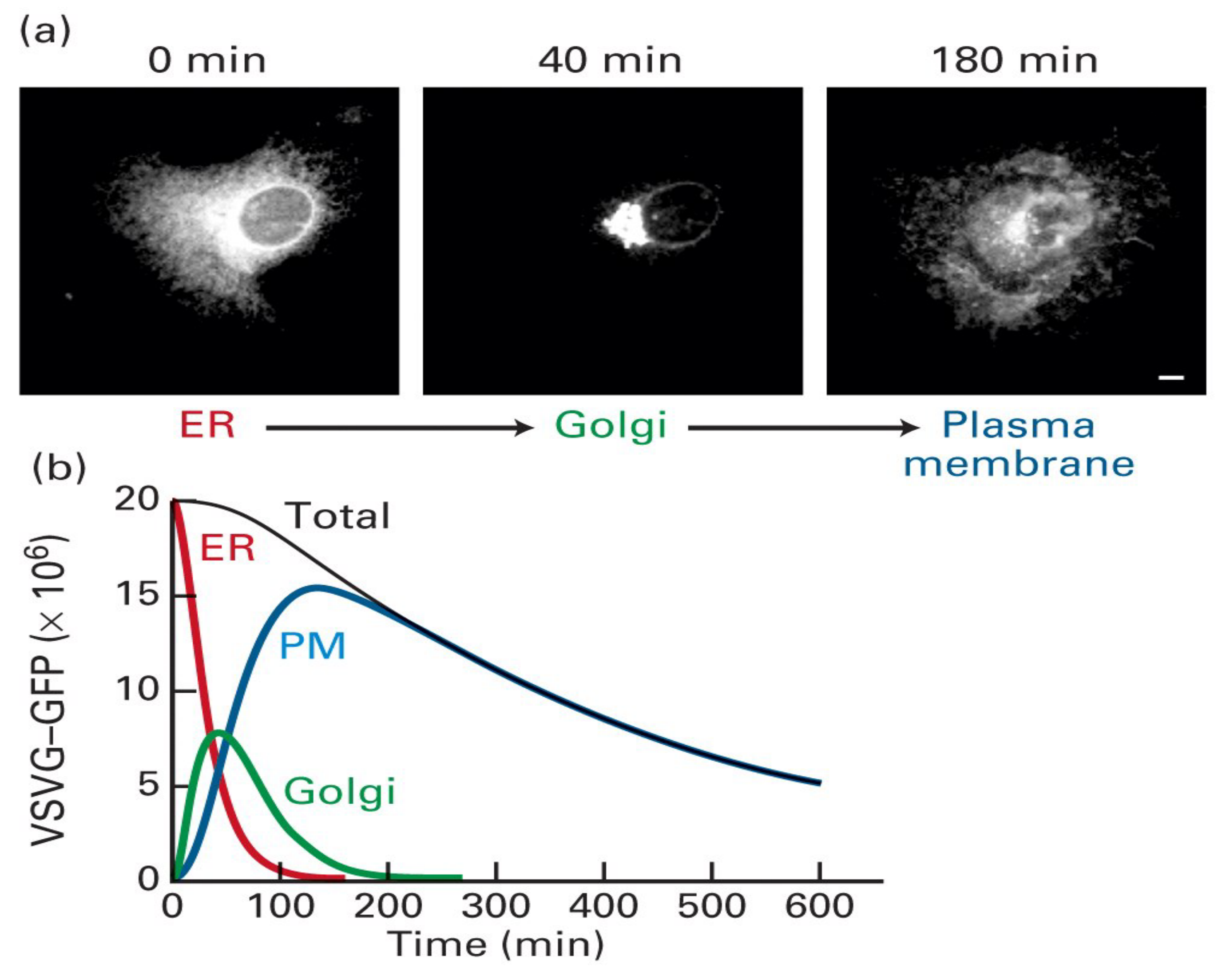
Sorting packaging

Synthesis

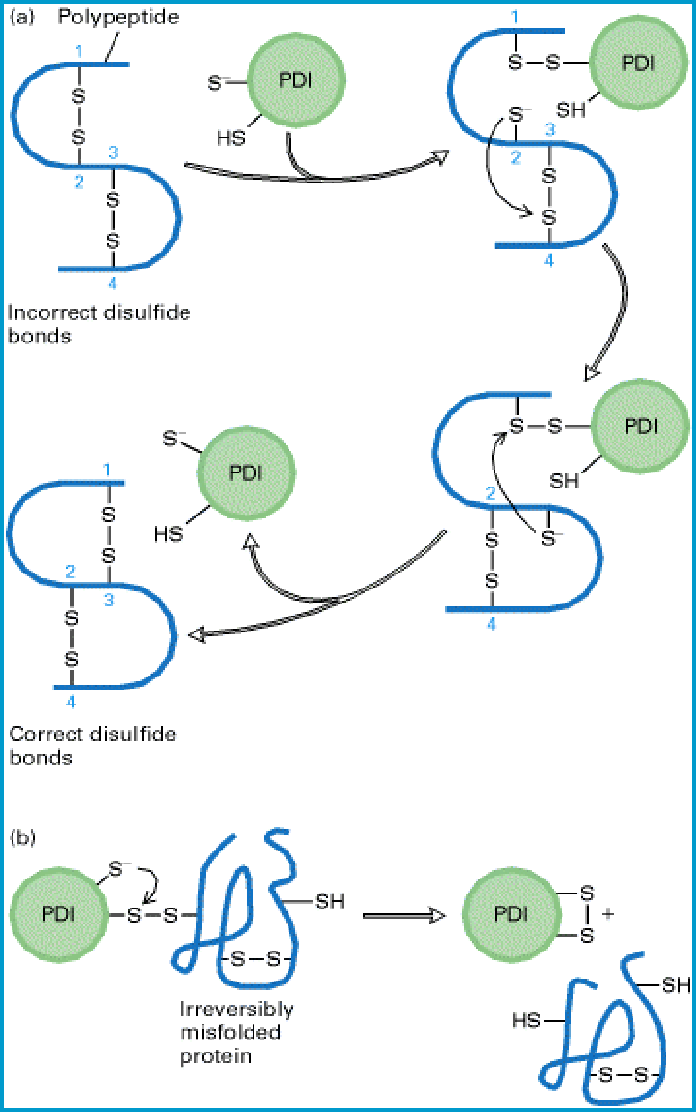


Molecular Cell Biology. Lodish *et als*. Figure 17-13

Techniques for studying the secretory pathway



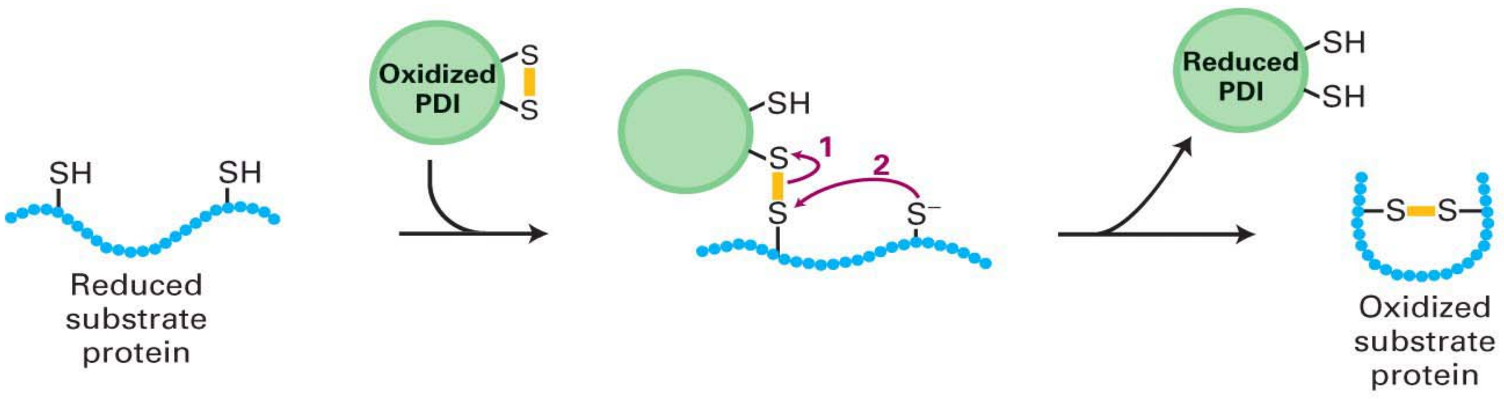
Post-translational modifications



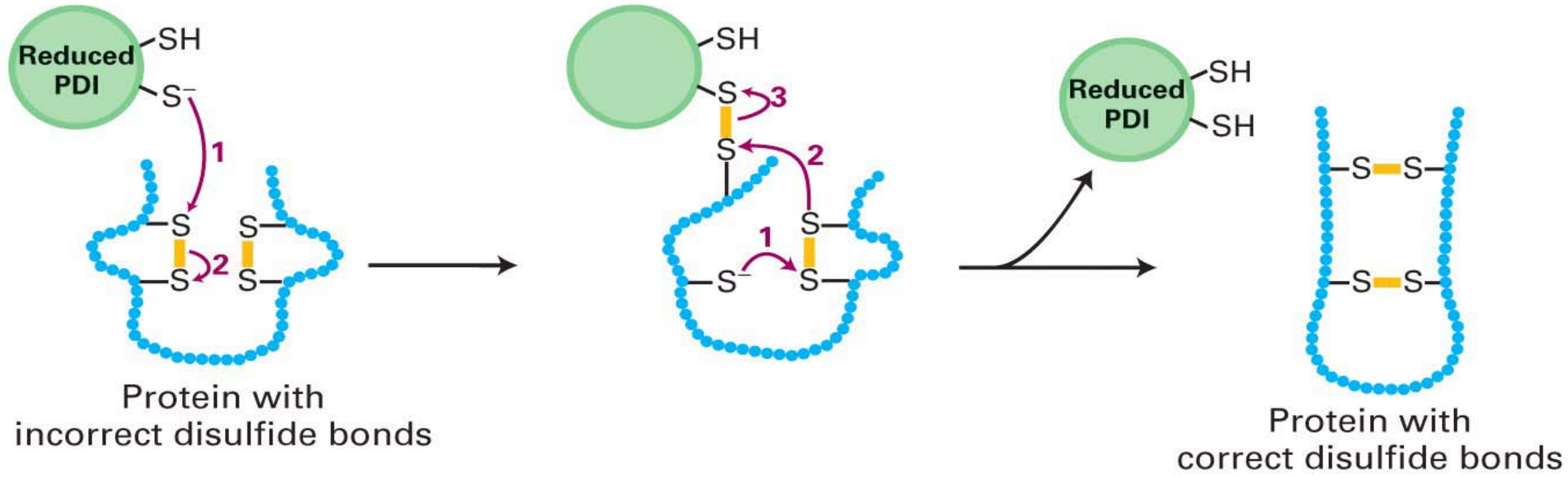
1. Formation of disulfide bonds
2. Proper folding
3. Addition and processing of carbohydrates
4. Specific proteolytic cleavages
5. Assembly into multimeric proteins

Disulfide bonds formation

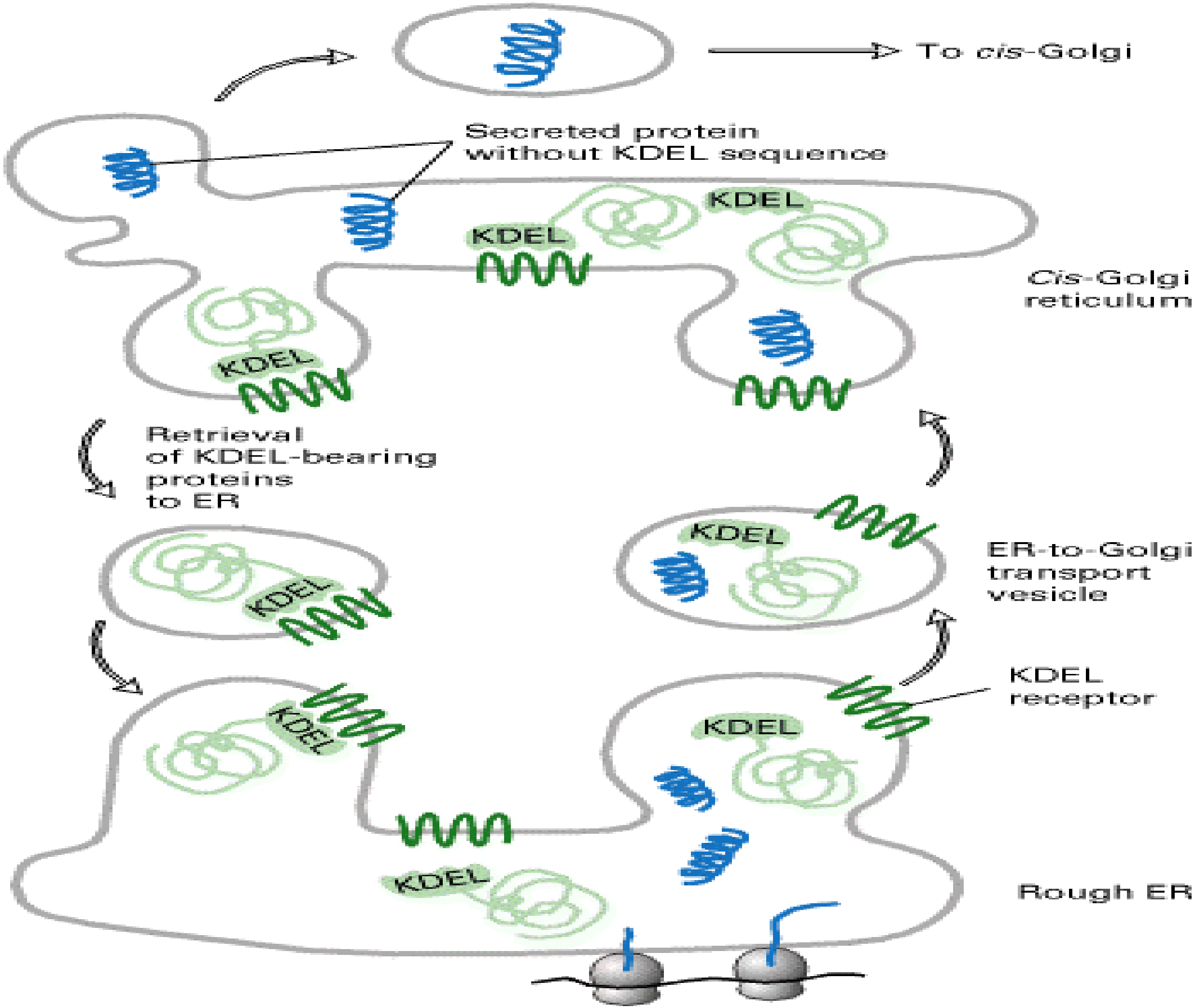
(a) Formation of a disulfide bond



(b) Rearrangement of disulfide bonds

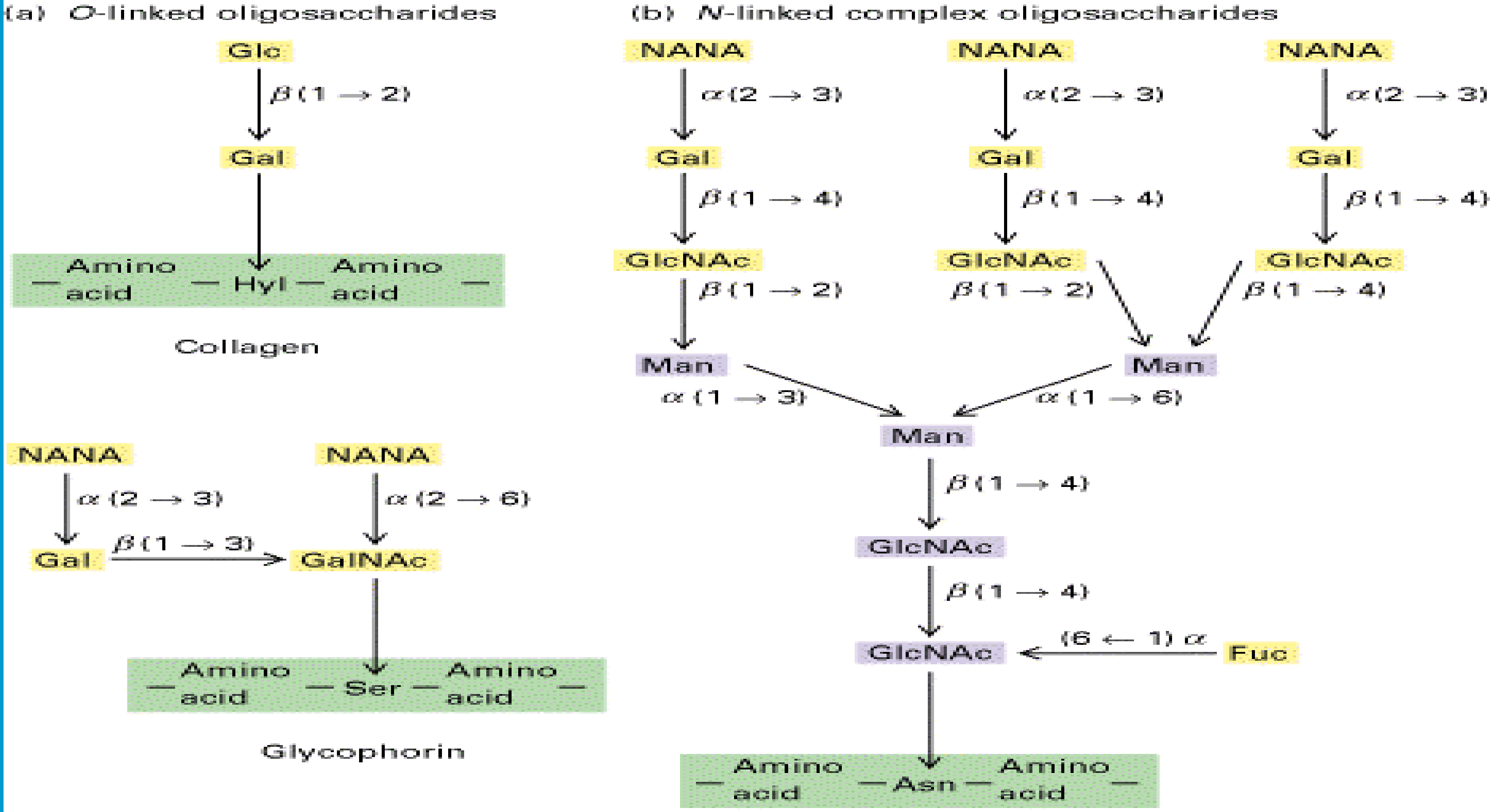


Retrieval of ER-resident proteins



Molecular Cell Biology. Lodish *et als*. Figure 17-29

Structure of *O*-linked and *N*-linked oligosaccharides



(a) *O*-linked oligosaccharides

Glc

β(1 → 2)

Gal

— Amino acid — Hyl — Amino acid —

Collagen

NANA

α(2 → 3)

Gal

β(1 → 3)

NANA

α(2 → 6)

GalNAc

— Amino acid — Ser — Amino acid —

Glycophorin

(b) *N*-linked complex oligosaccharides

NANA

α(2 → 3)

Gal

β(1 → 4)

GlcNAc

β(1 → 2)

Man

α(1 → 3)

NANA

α(2 → 3)

Gal

β(1 → 4)

GlcNAc

β(1 → 2)

NANA

α(2 → 3)

Gal

β(1 → 4)

GlcNAc

β(1 → 4)

Man

α(1 → 6)

Man

β(1 → 4)

GlcNAc

β(1 → 4)

GlcNAc

(6 ← 1) α

Fuc

— Amino acid — Asn — Amino acid —

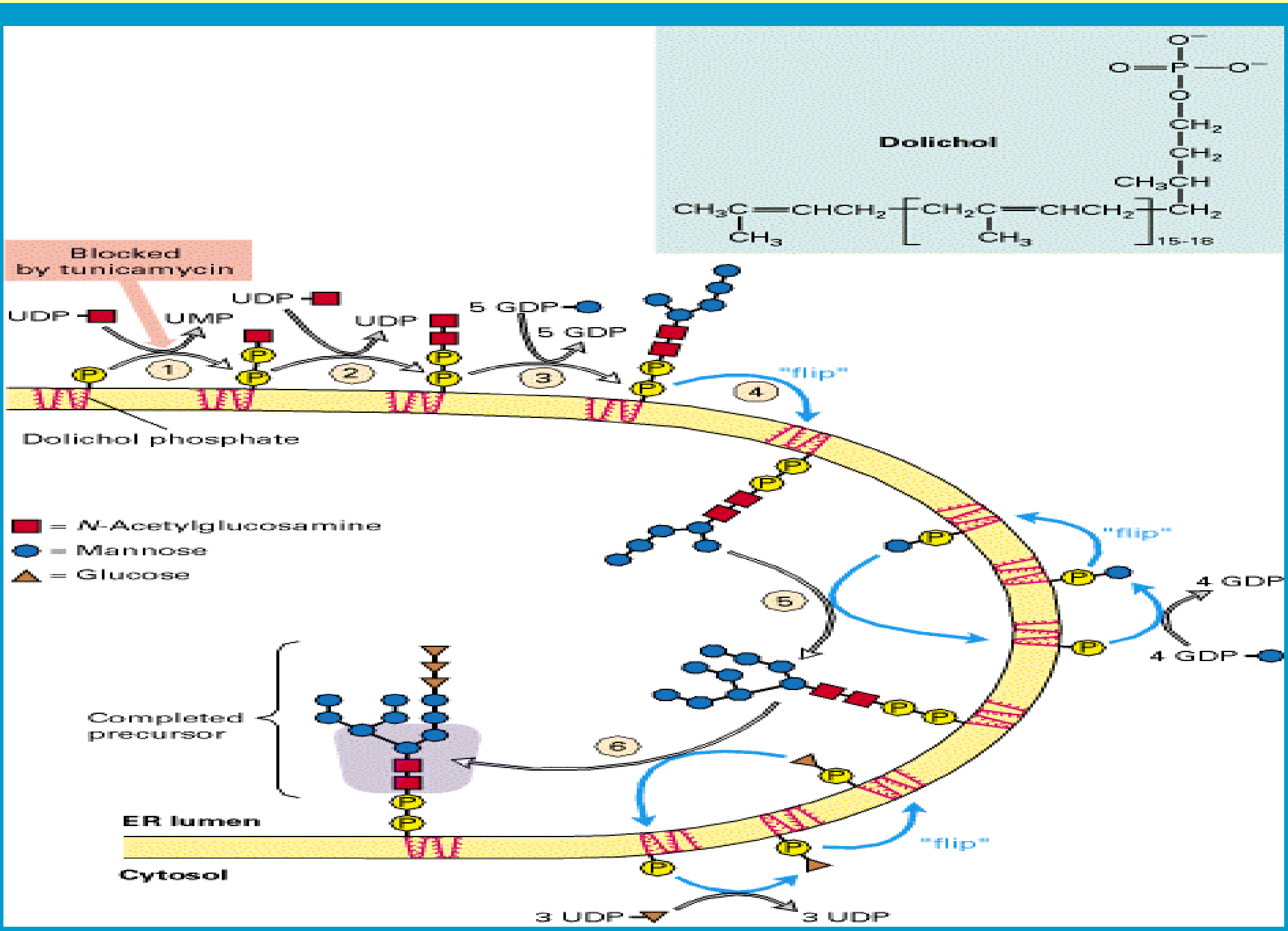
NANA = *N*-Acetylneuraminic acid (sialic acid)
GalNAc = *N*-Acetylgalactosamine
GlcNAc = *N*-Acetylglucosamine (conserved)
GlcNAc = *N*-Acetylglucosamine

Gal = Galactose
Man = Mannose
Fuc = Fucose
Glc = Glucose

Molecular Cell Biology. Lodish *et als*. Figure 17-30

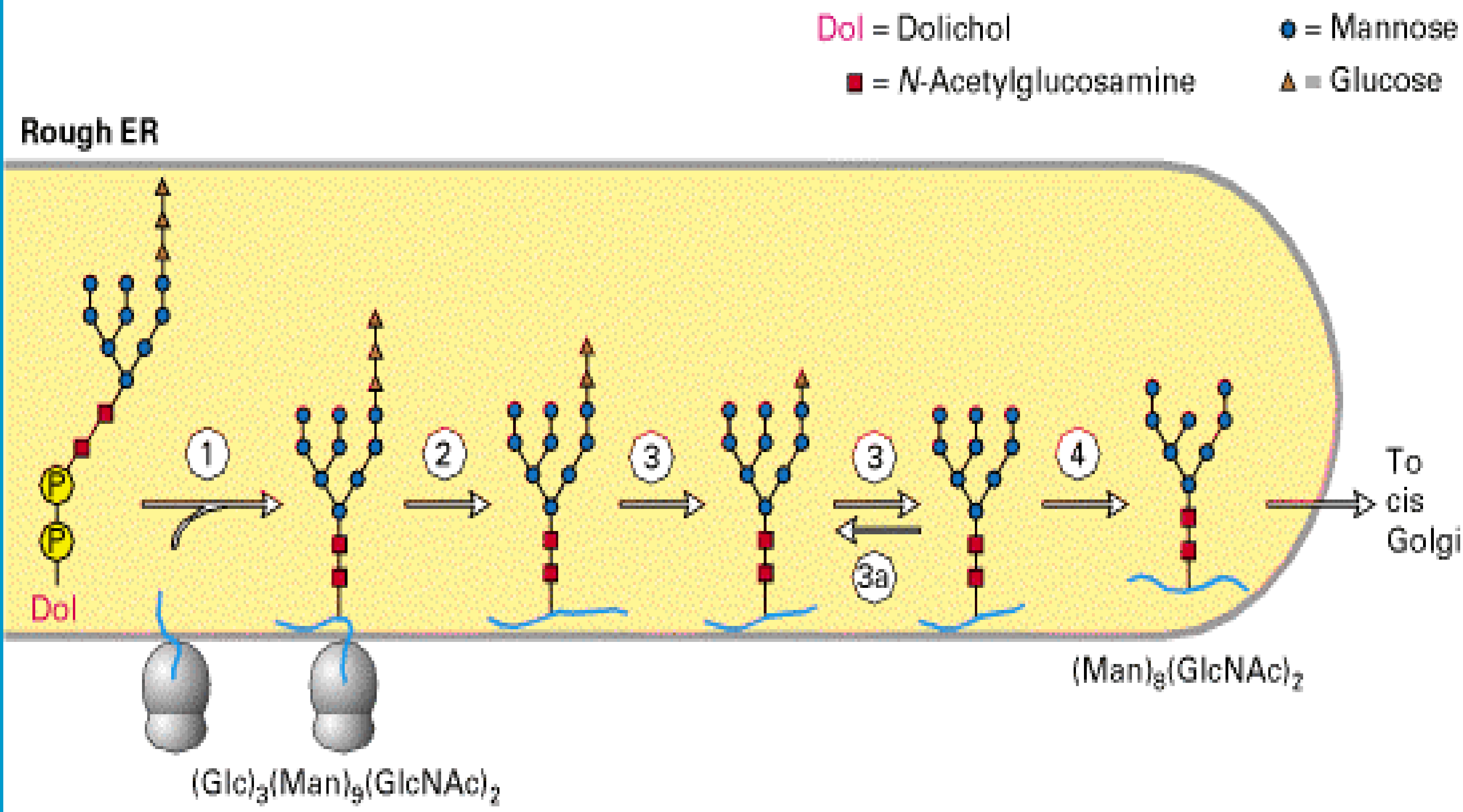
N-linked glycosylation

Initial steps

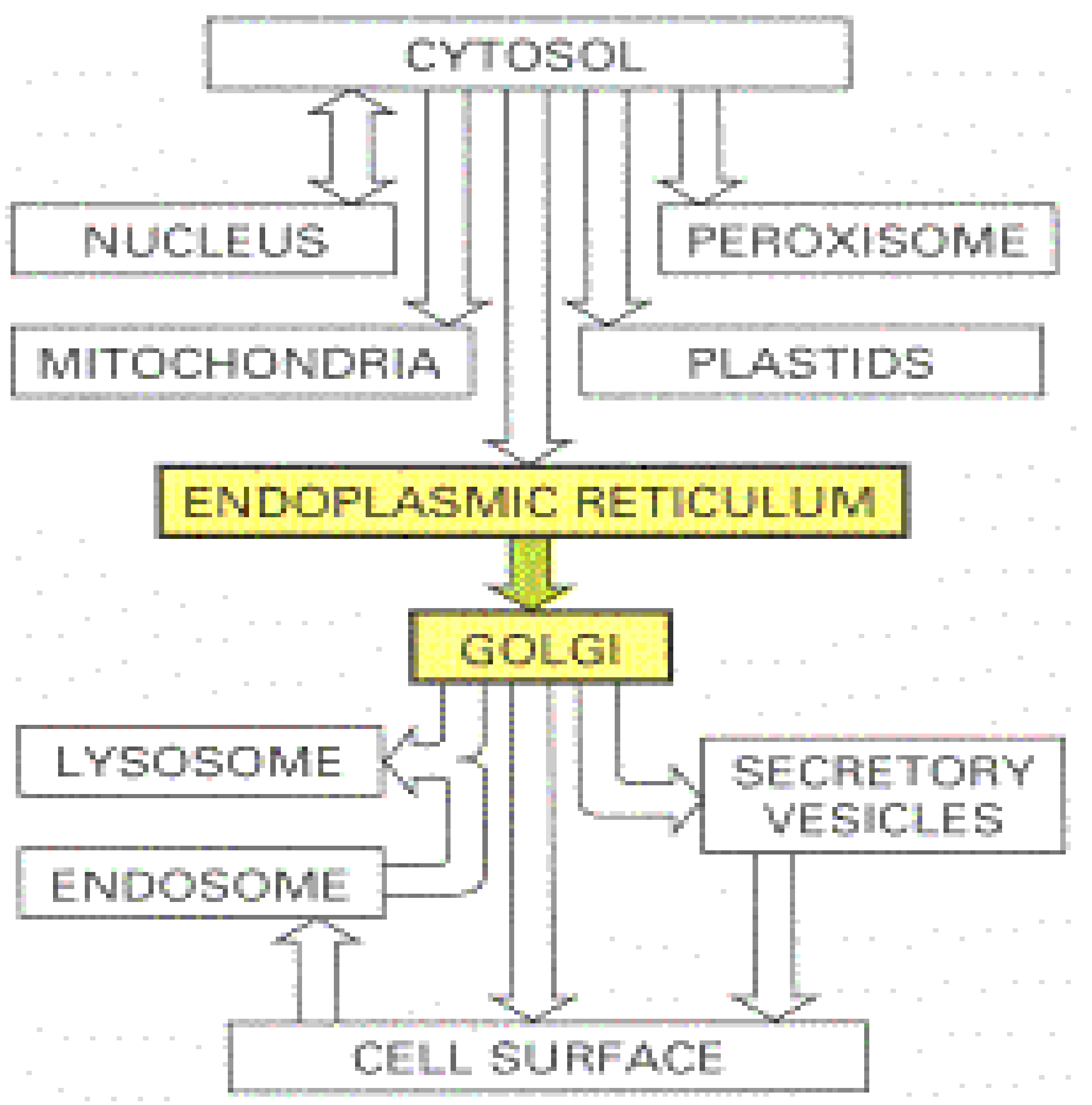


Molecular Cell Biology. Lodish *et als*. Figure 17-35

Processing of *N*-linked oligosaccharides



Molecular Cell Biology. Lodish *et als*. Figure 17-36



CYTOSOL

NUCLEUS

PEROXISOME

MITOCHONDRIA

PLASTIDS

ENDOPLASMIC RETICULUM

GOLGI

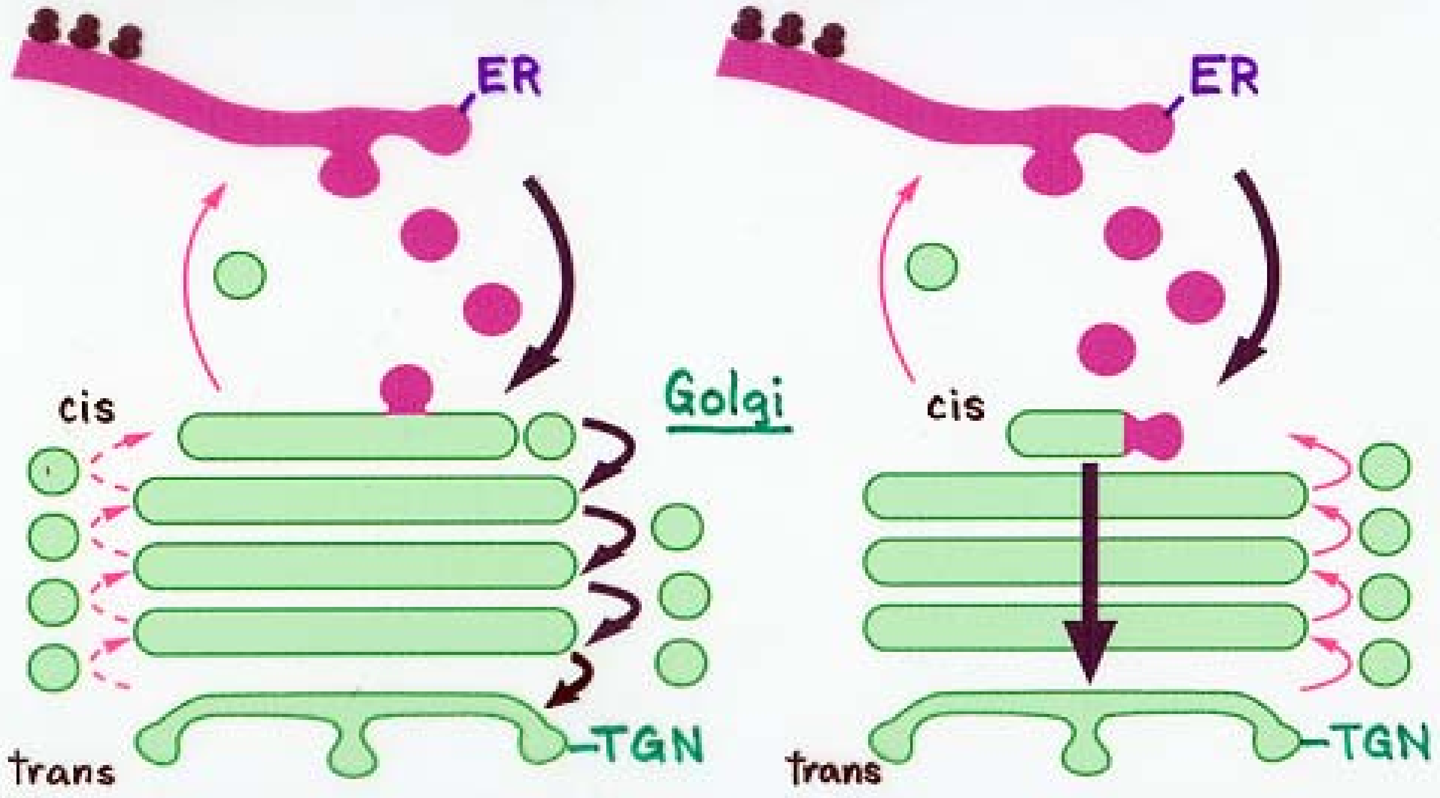
LYSOSOME

SECRETORY VESICLES

ENDOSOME

CELL SURFACE

MODELS OF GOLGI TRAFFICKING



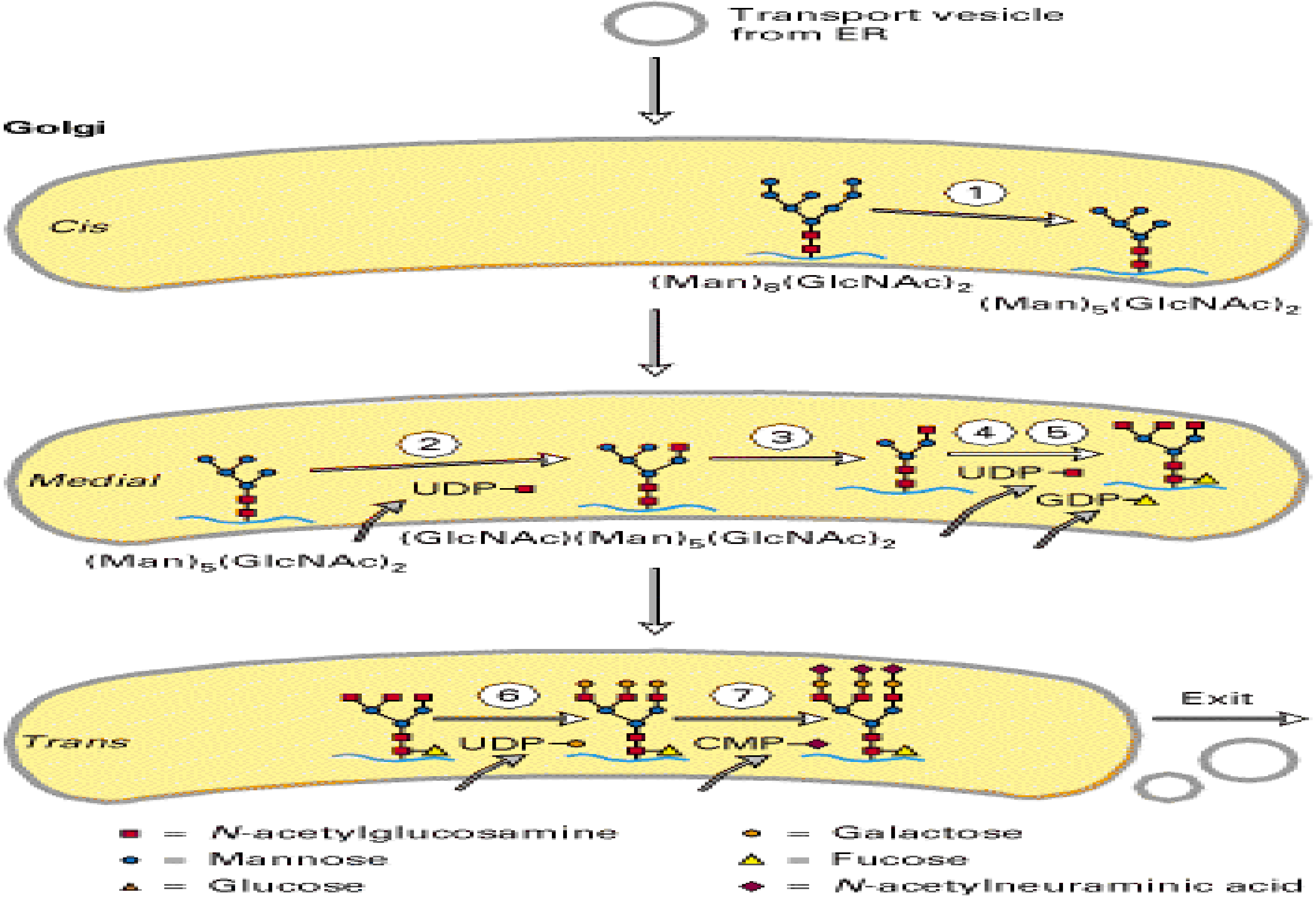
Vesicular shuttle model

- stable Golgi cisternae
- products in vesicles

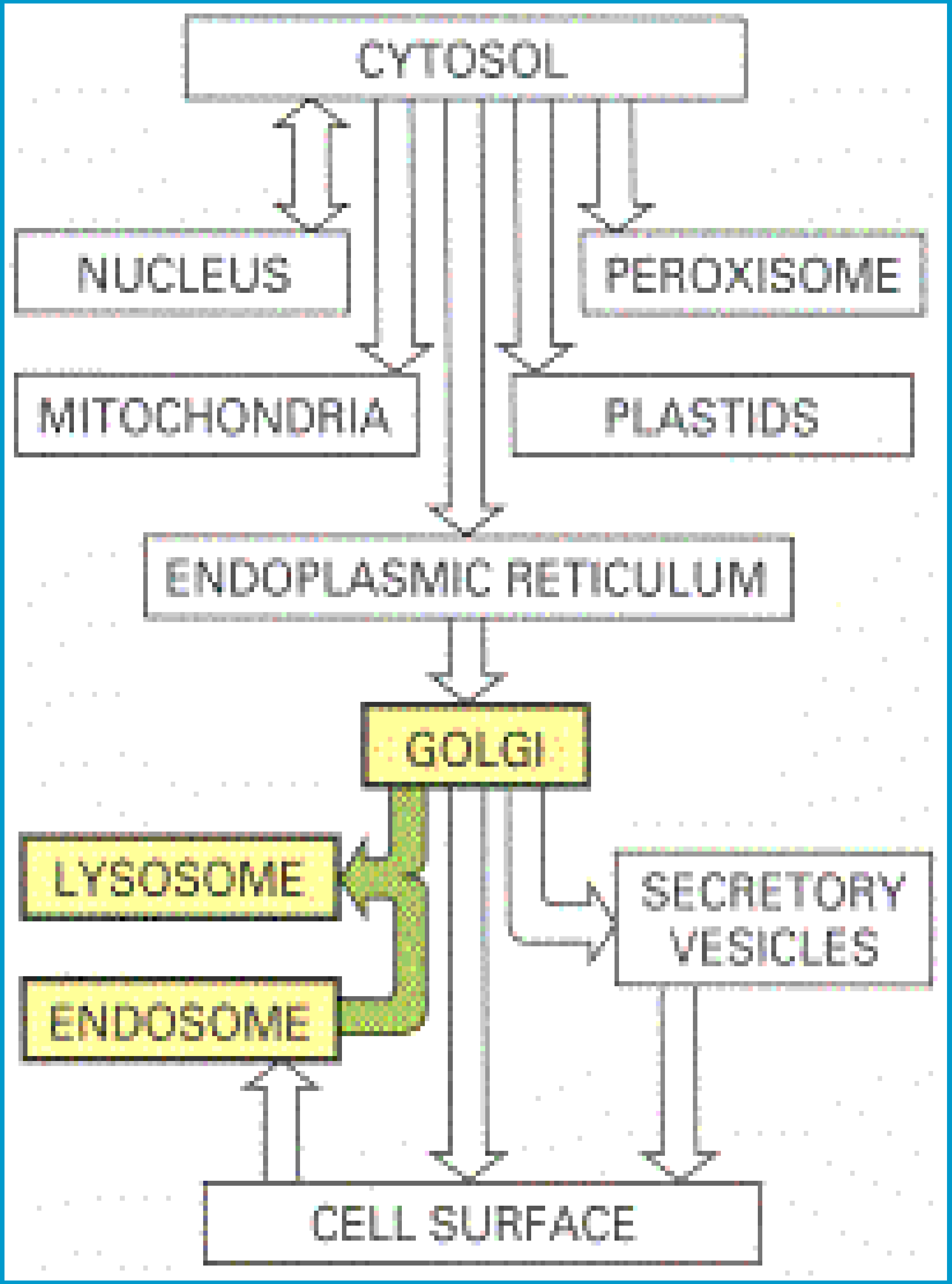
Progression/maturation model

- transient, migrating cisternae
- vesicles recycle Golgi enzymes

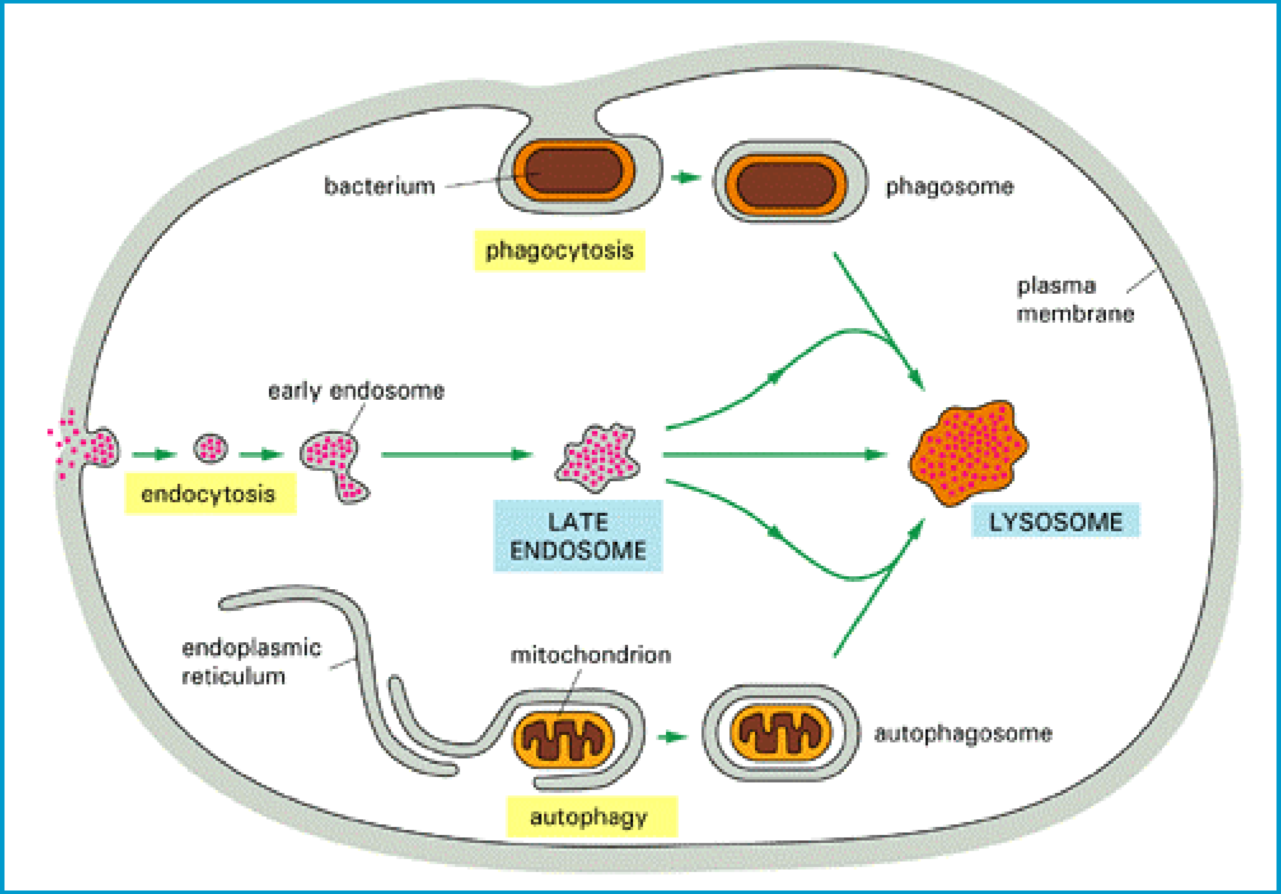
Processing of glycoproteins in the Golgi apparatus



Molecular Cell Biology. Lodish *et als*. Figure 17-38


CYTOSOL
NUCLEUS
PEROXISOME
MITOCHONDRIA
PLASTIDS
ENDOPLASMIC RETICULUM
GOLGI
LYSOSOME
SECRETORY VESICLES
ENDOSOME
CELL SURFACE

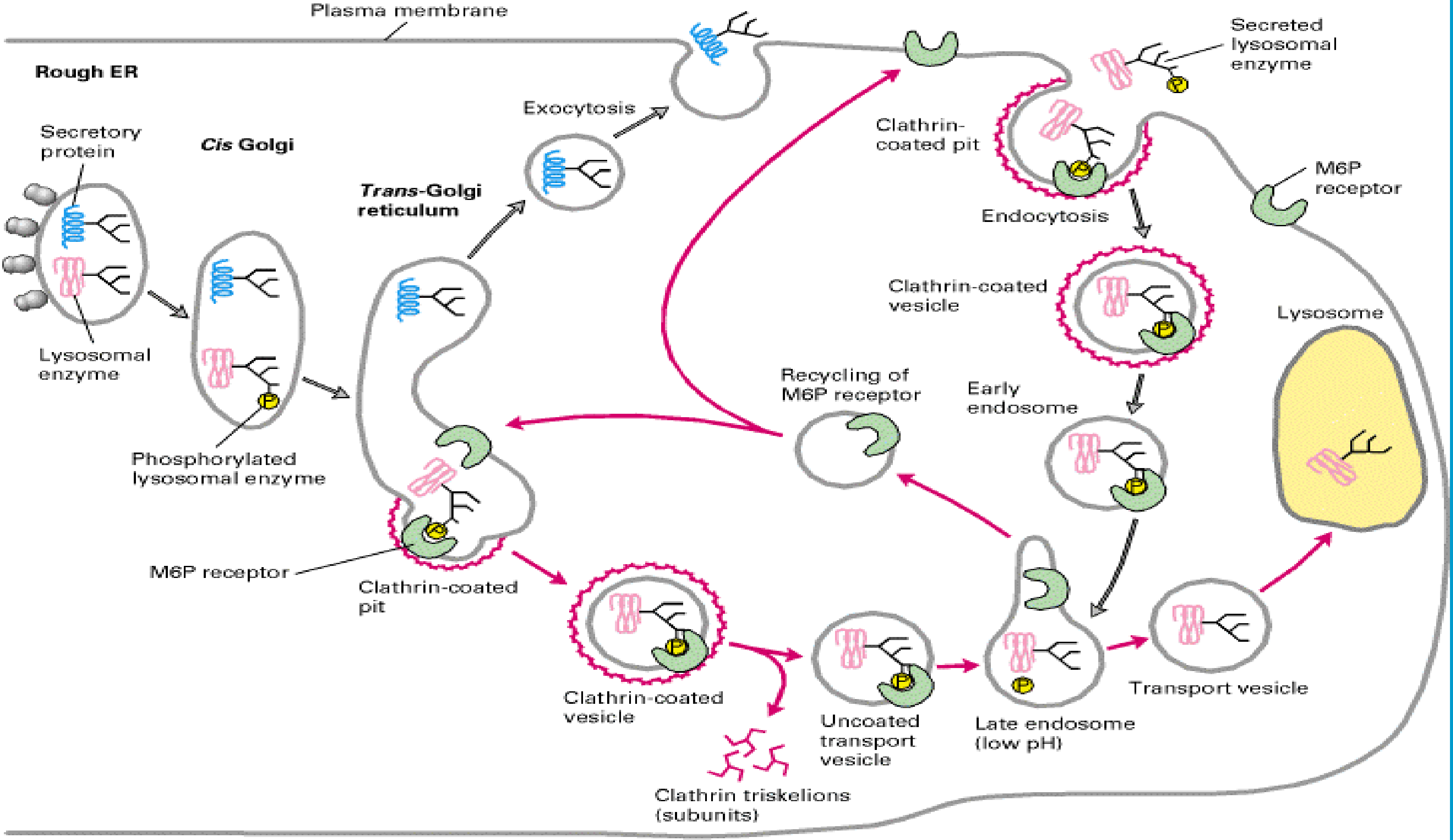
Three pathways to degradation in lysosomes



(Fig 13-22.Alberts)

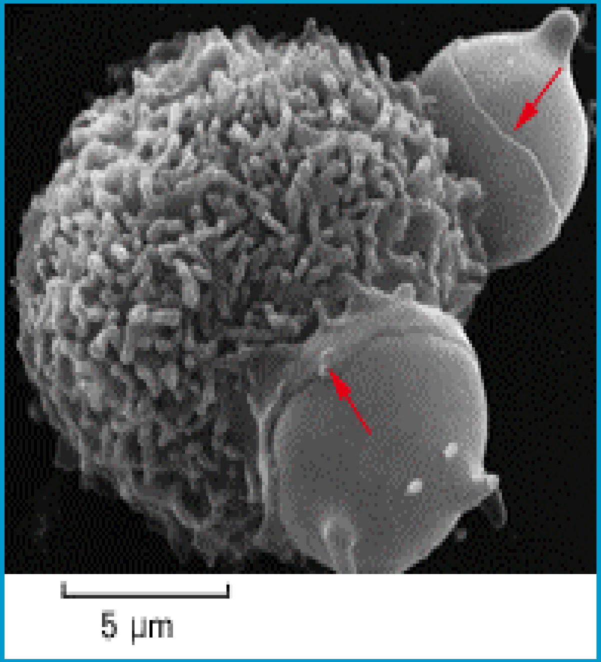
Mannose6-P pathway

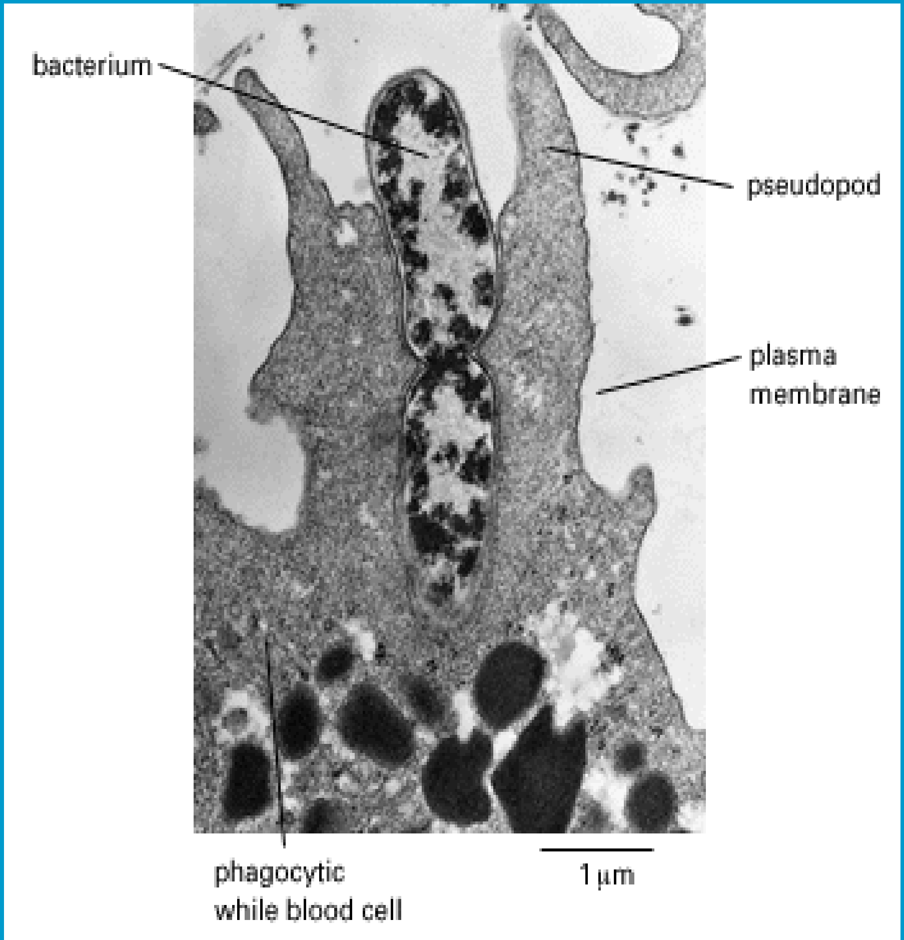
Targeting lysosomal enzyme to lysosomes



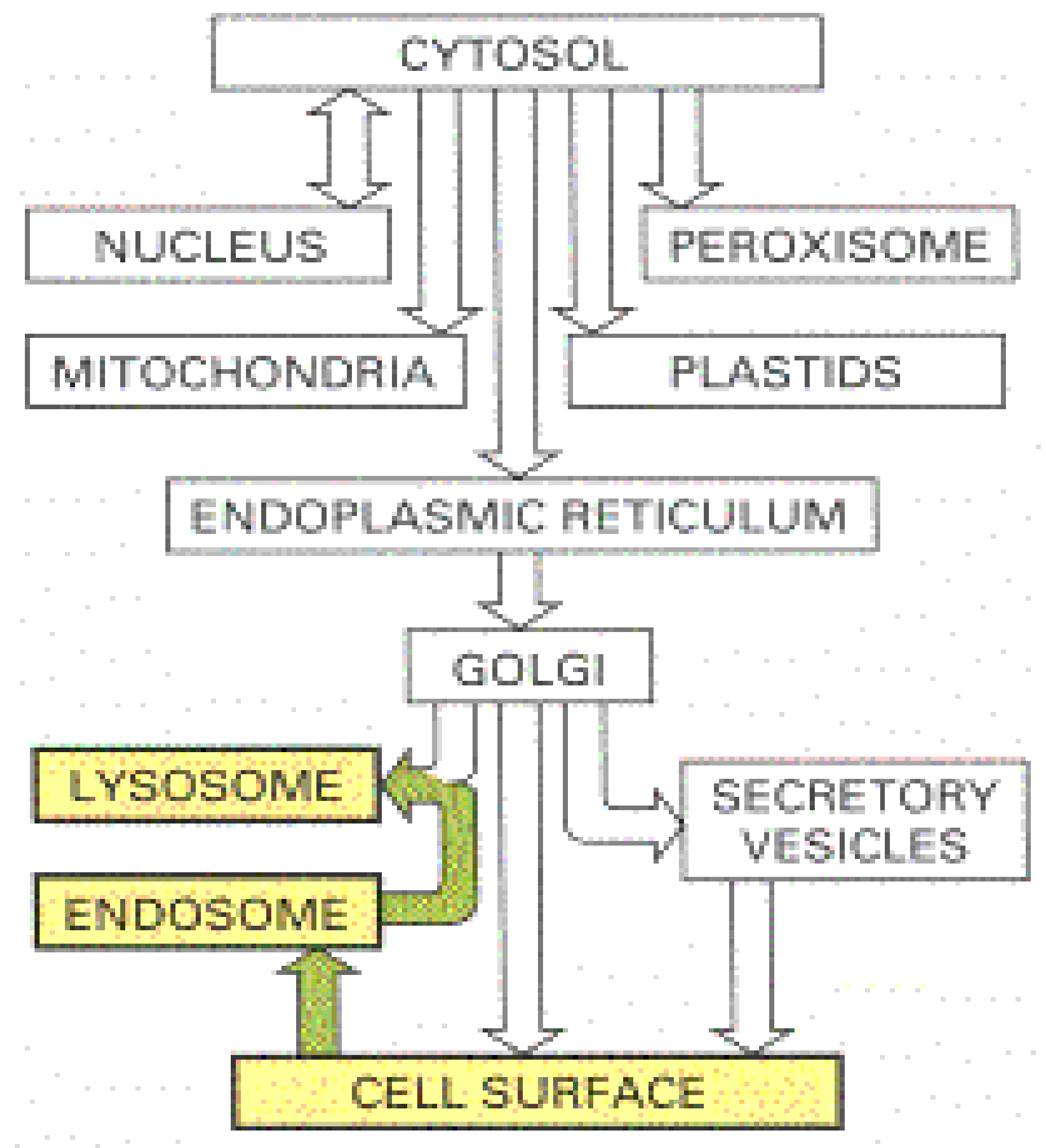
Molecular Cell Biology. Lodish *et als*. Figure 17-40

Phagocytosis

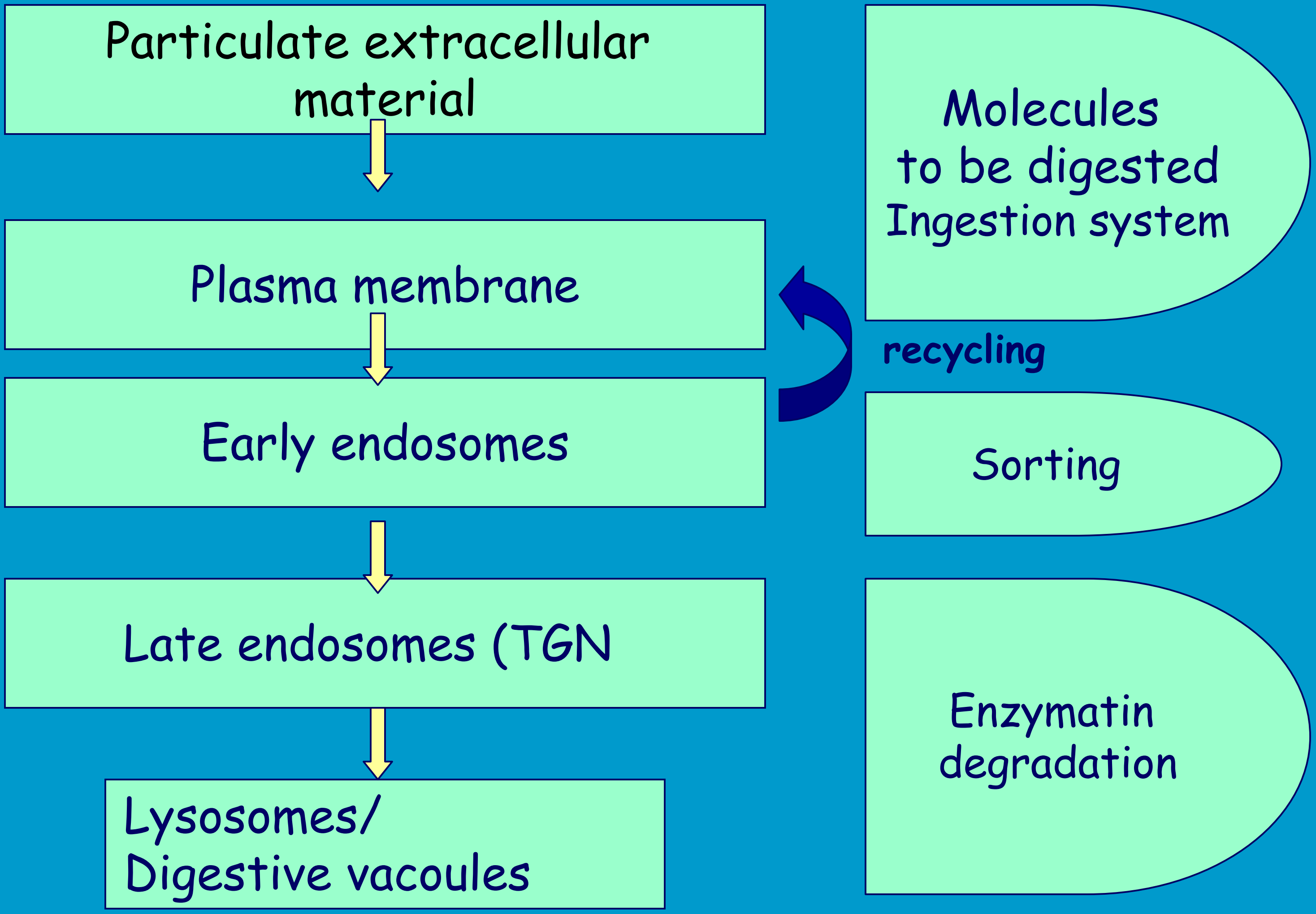




(Fig 13-26,27.Alberts)


CYTOSOL
NUCLEUS
PEROXISOME
MITOCHONDRIA
PLASTIDS
ENDOPLASMIC RETICULUM
GOLGI
LYSOSOME
SECRETORY VESICLES
ENDOSOME
CELL SURFACE

Endocytotic pathway



Particulate extracellular material

Plasma membrane

Early endosomes

Late endosomes (TGN

Lysosomes/
Digestive vacoules

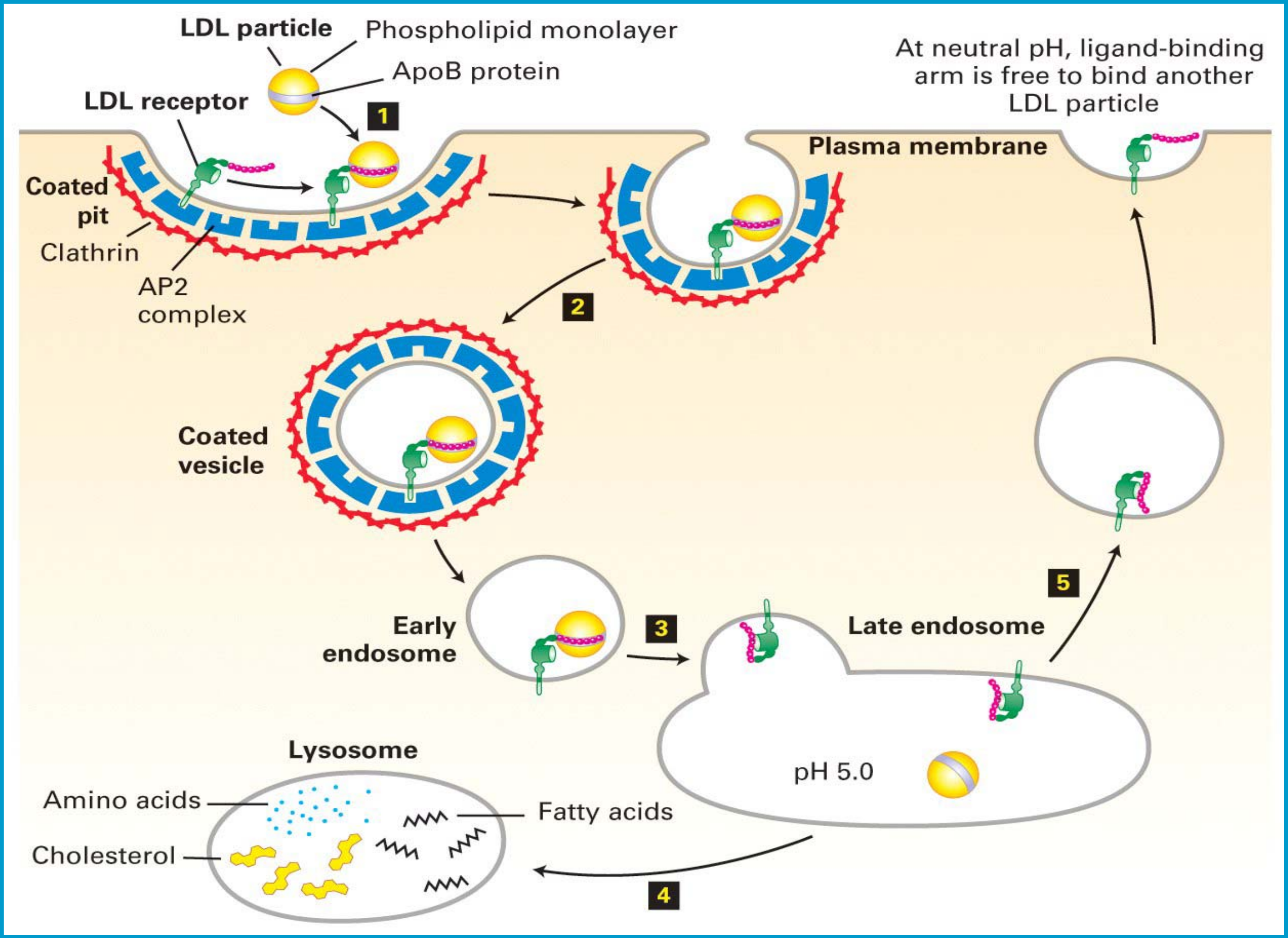
Molecules
to be digested
Ingestion system

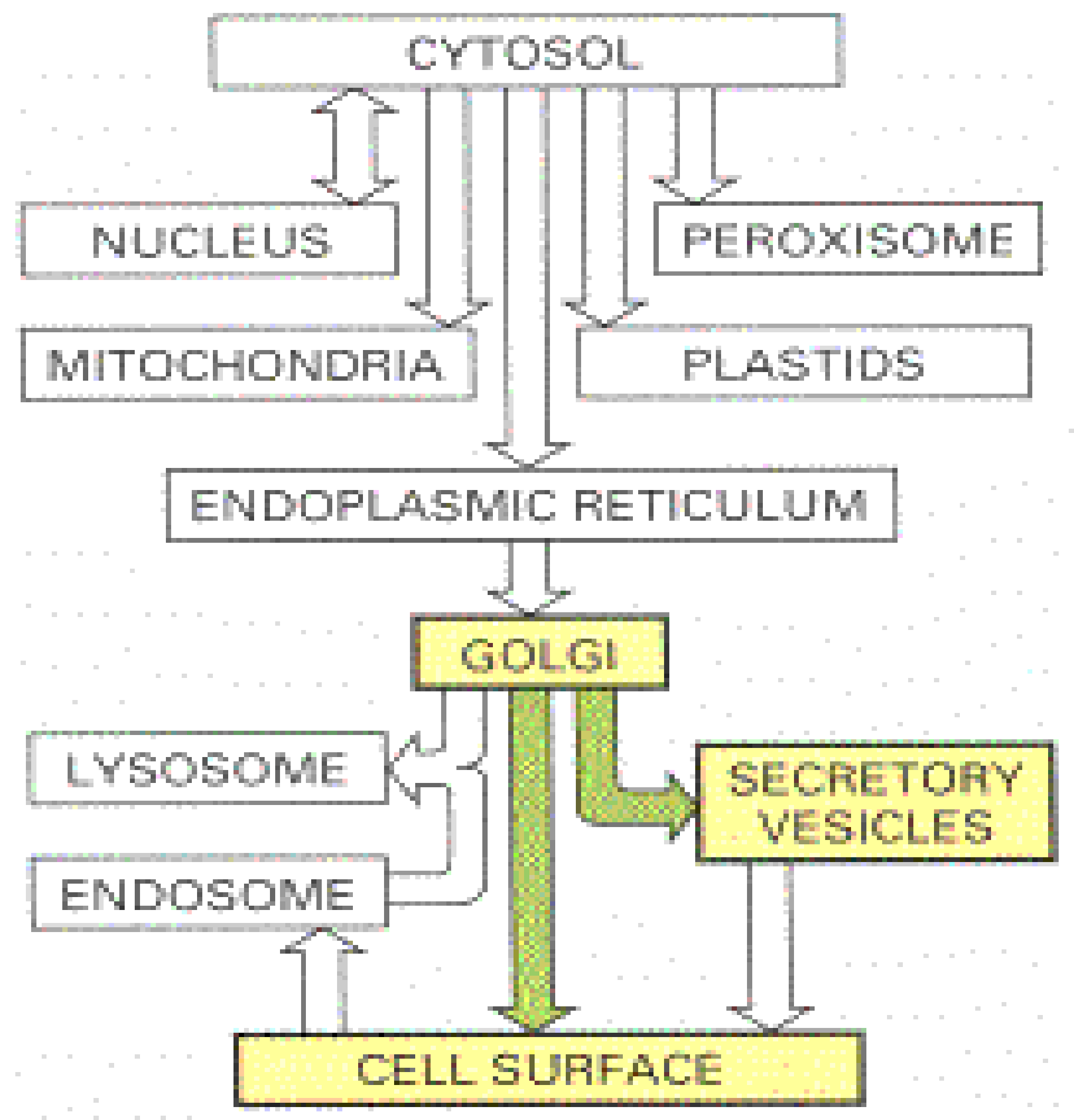
recycling

Sorting

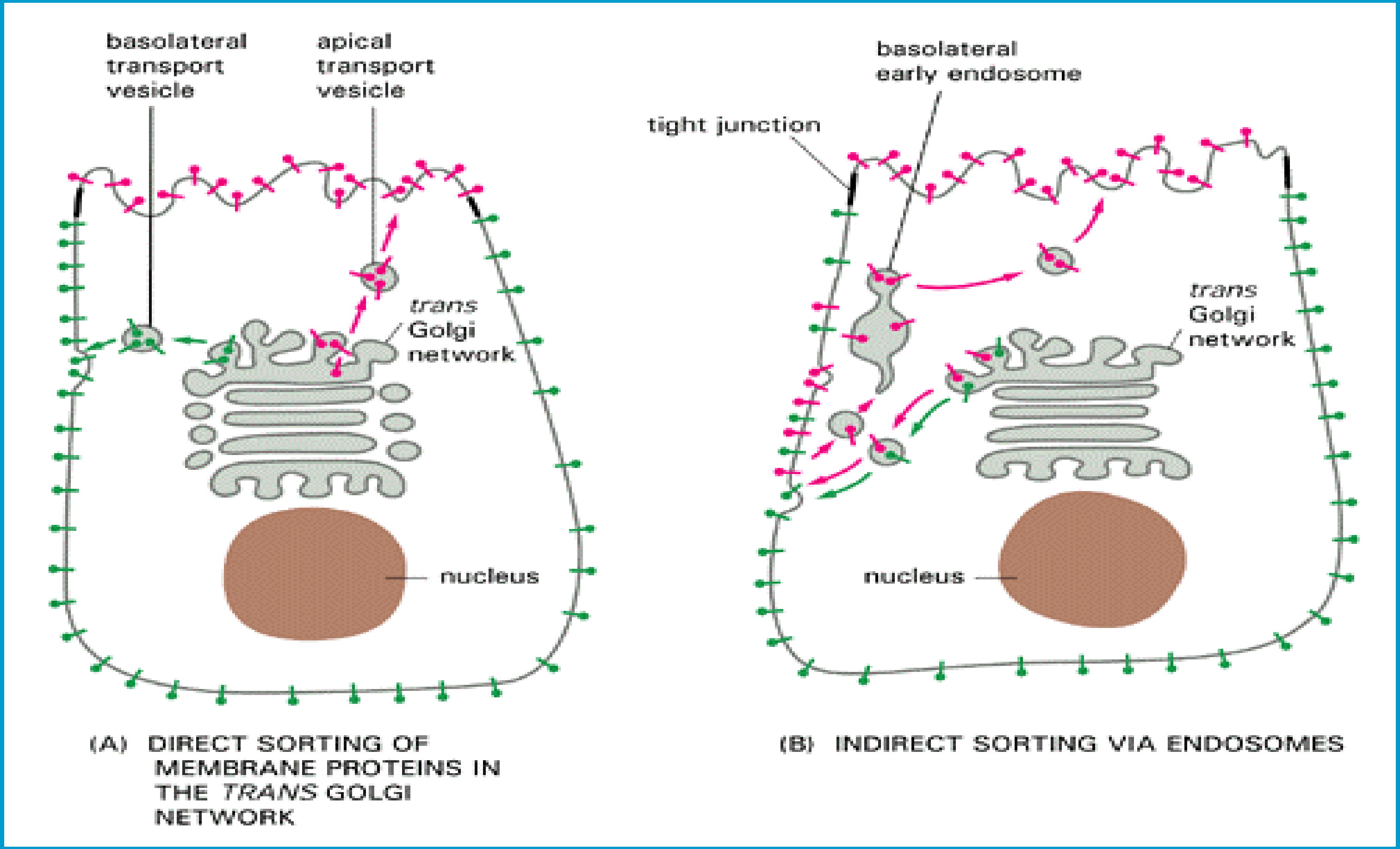
Enzymatin
degradation

Receptor-mediated endocytosis of LDL



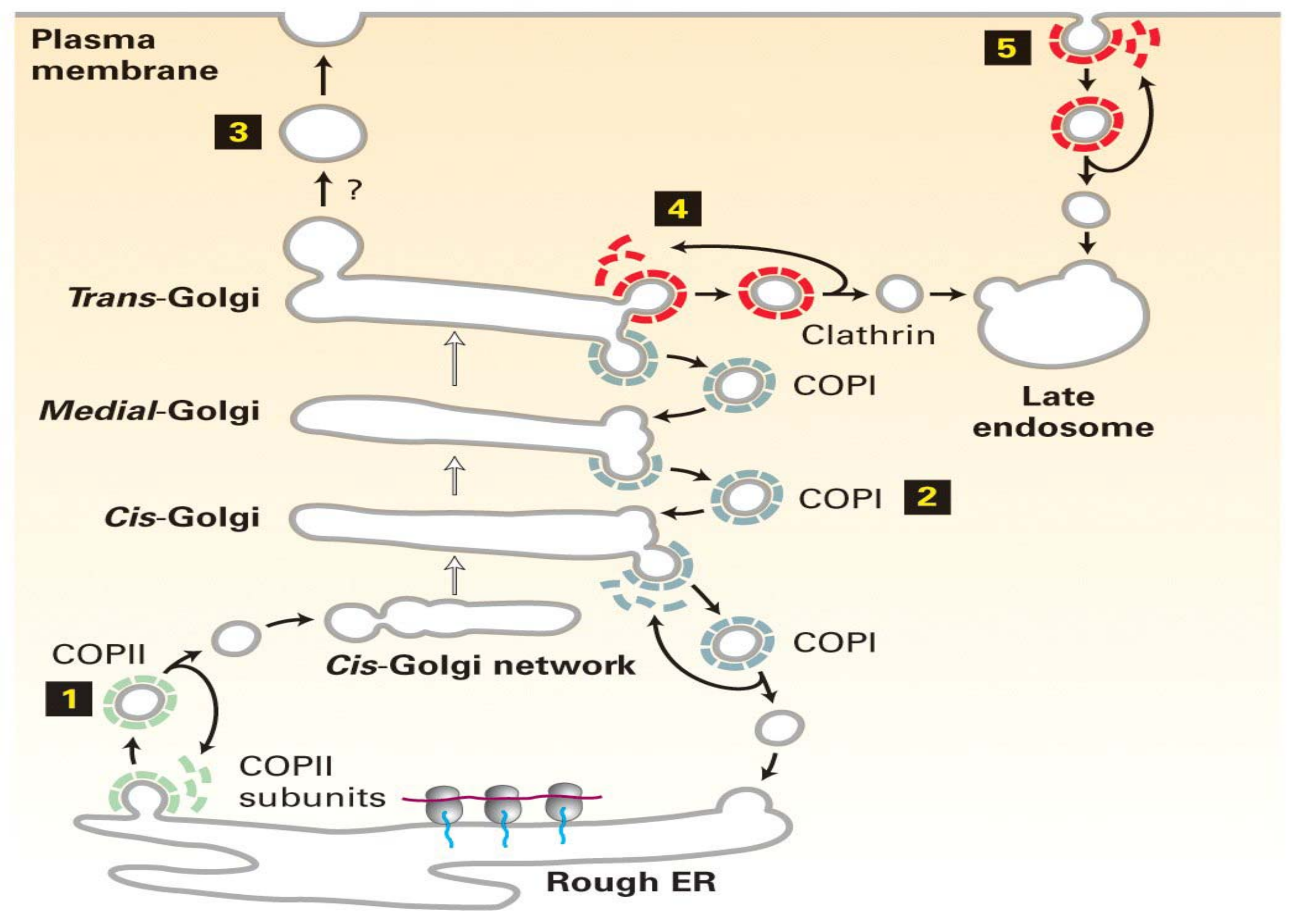

CYTOSOL
NUCLEUS
PEROXISOME
MITOCHONDRIA
PLASTIDS
ENDOPLASMIC RETICULUM
GOLGI
LYSOSOME
SECRETORY VESICLES
ENDOSOME
CELL SURFACE

Sorting of plasma membrane proteins in a polarized epithelial cell



(Fig 13-44 Alberts)

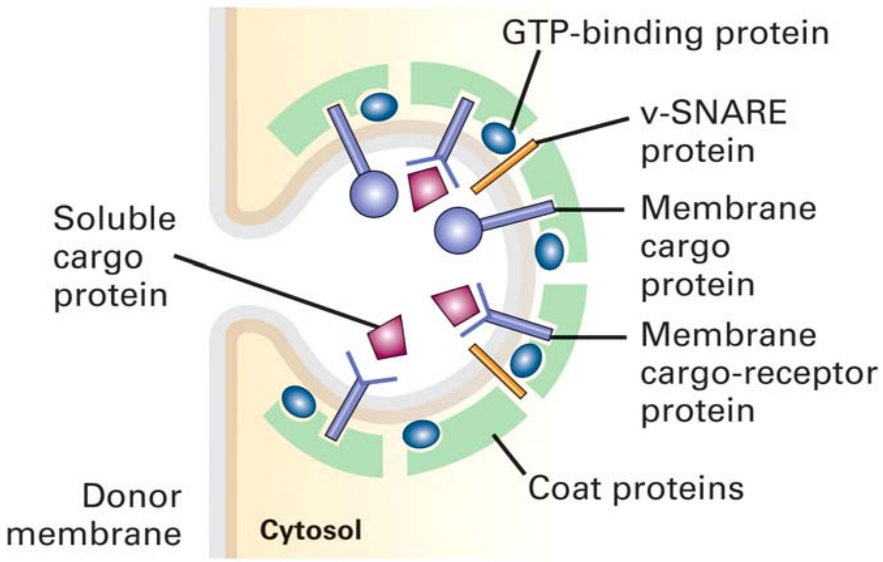
Vesicular traffic in the secretory and endocytic pathways



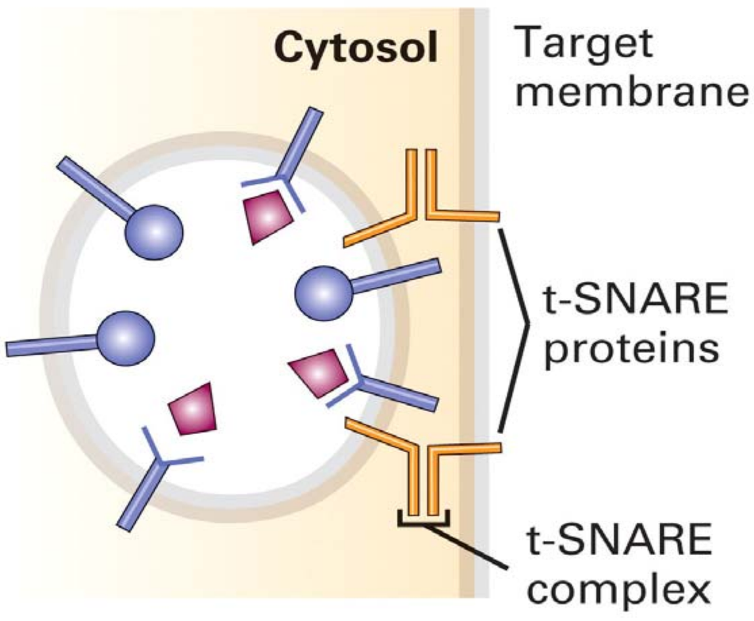
Molecular Cell Biology. Lodish et al. Figure 17-30

Molecular mechanism of vesicular transport

(a) Coated vesicle budding



(b) Uncoated vesicle fusion



1. Budding of vesicles is driven by the polimerization ofsoluble protein complexes onto the membrane to form a proteinaceous coat
2. Coat add cuvature to the membrane and filter proteins admitted into the vesicles
3. Fusion: specific joining of v-SNARE in teh vesicle membrane with t-SNARE in the target membrane, bring the membraen into colse position to fuse.
4. Typical vesicle: 50nm diameter.

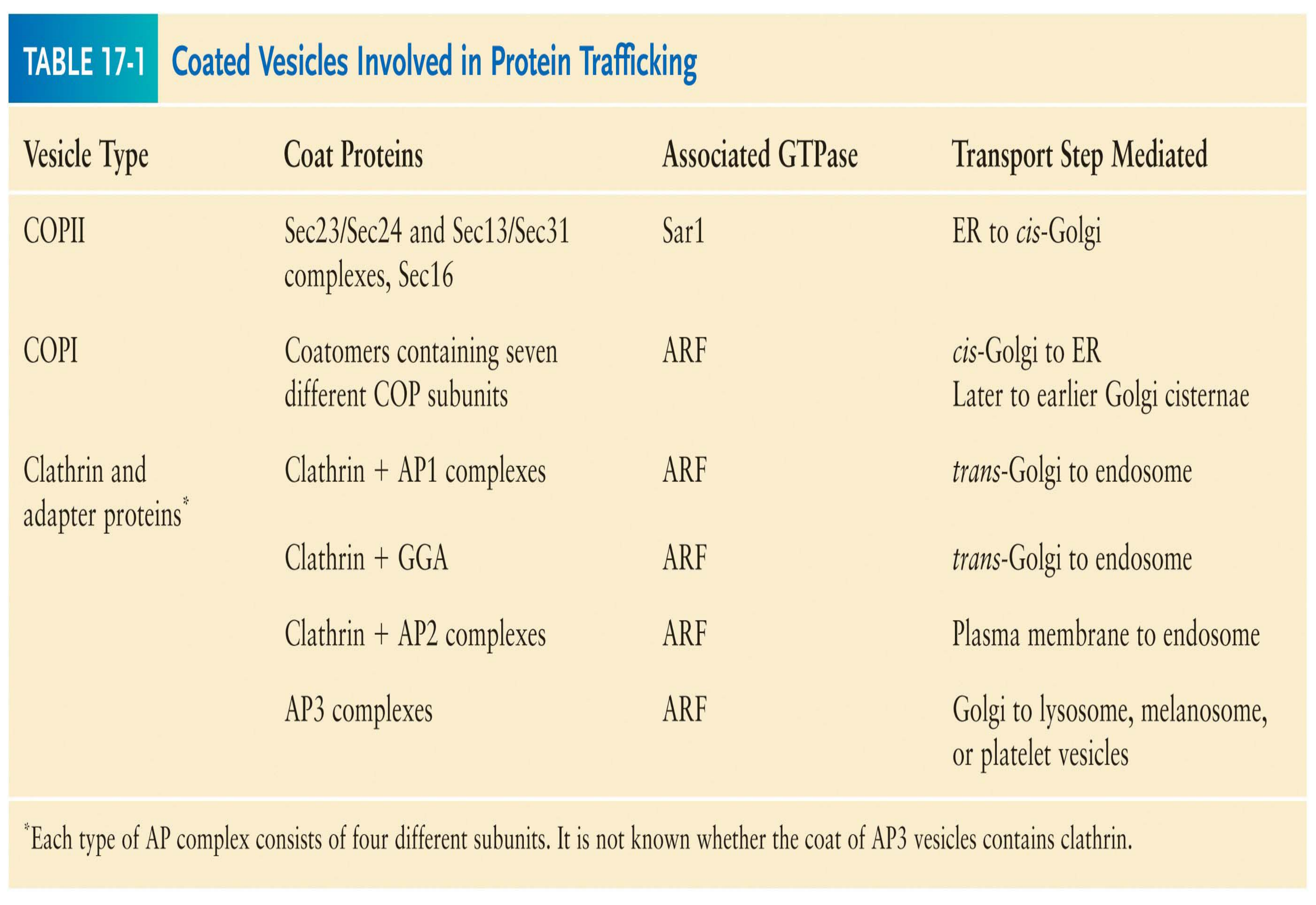


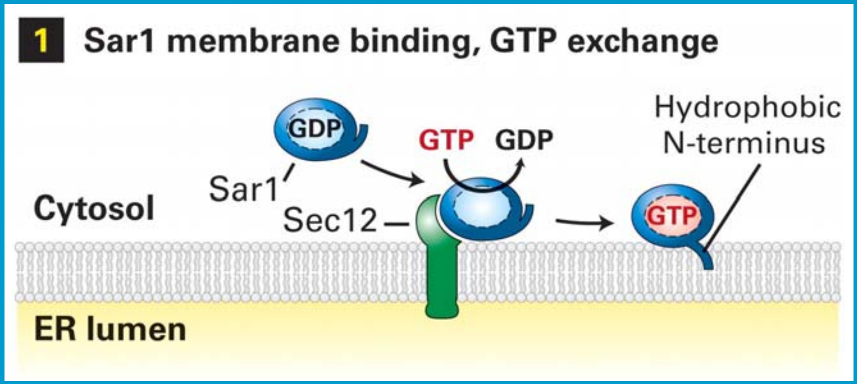
TABLE 17-1 Coated Vesicles Involved in Protein Trafficking

Vesicle Type	Coat Proteins	Associated GTPase	Transport Step Mediated
COPII	Sec23/Sec24 and Sec13/Sec31 complexes, Sec16	Sar1	ER to *cis*-Golgi
COPI	Coatomers containing seven different COP subunits	ARF	*cis*-Golgi to ER Later to earlier Golgi cisternae
Clathrin and adapter proteins*	Clathrin + AP1 complexes	ARF	*trans*-Golgi to endosome
	Clathrin + GGA	ARF	*trans*-Golgi to endosome
	Clathrin + AP2 complexes	ARF	Plasma membrane to endosome
	AP3 complexes	ARF	Golgi to lysosome, melanosome, or platelet vesicles

*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.

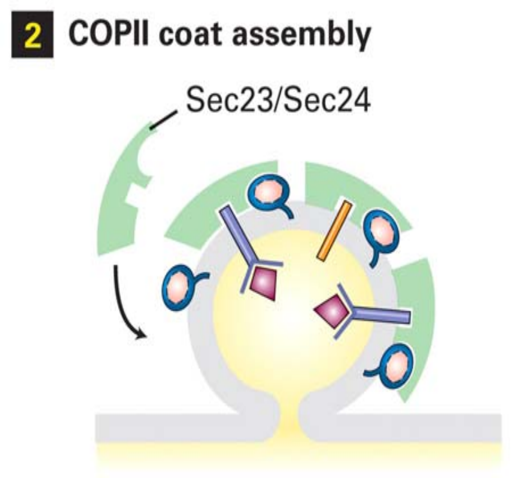
Molecular Cell Biology. Lodish *et als*. table 17-1

Minimal component of a coated vesicle

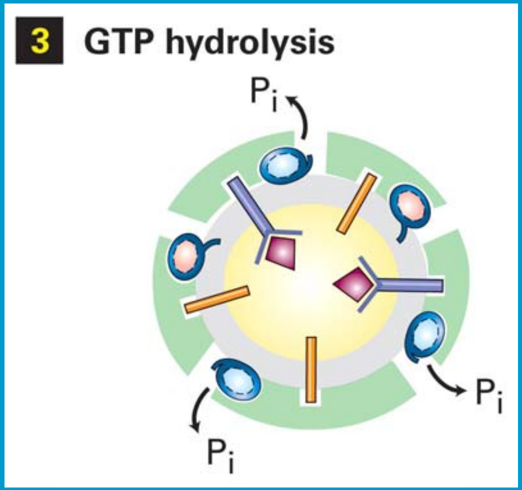


1 Sar1 membrane binding, GTP exchange

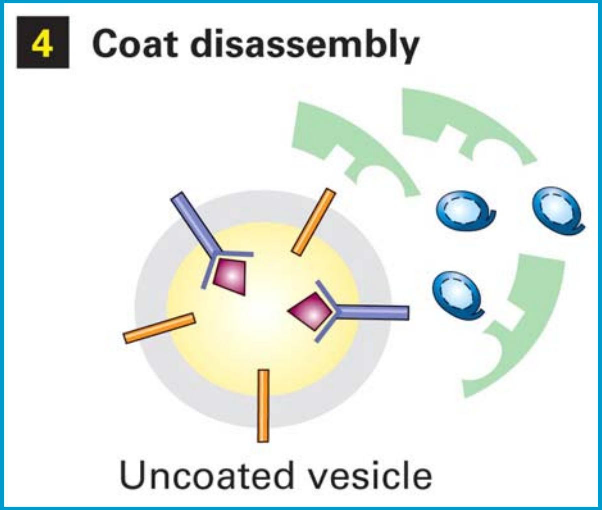
1. GTP- binding proteins.
2. V-SNARE
3. Coat proteins
4. Membrane cargo receptor proteins



2 COPII coat assembly

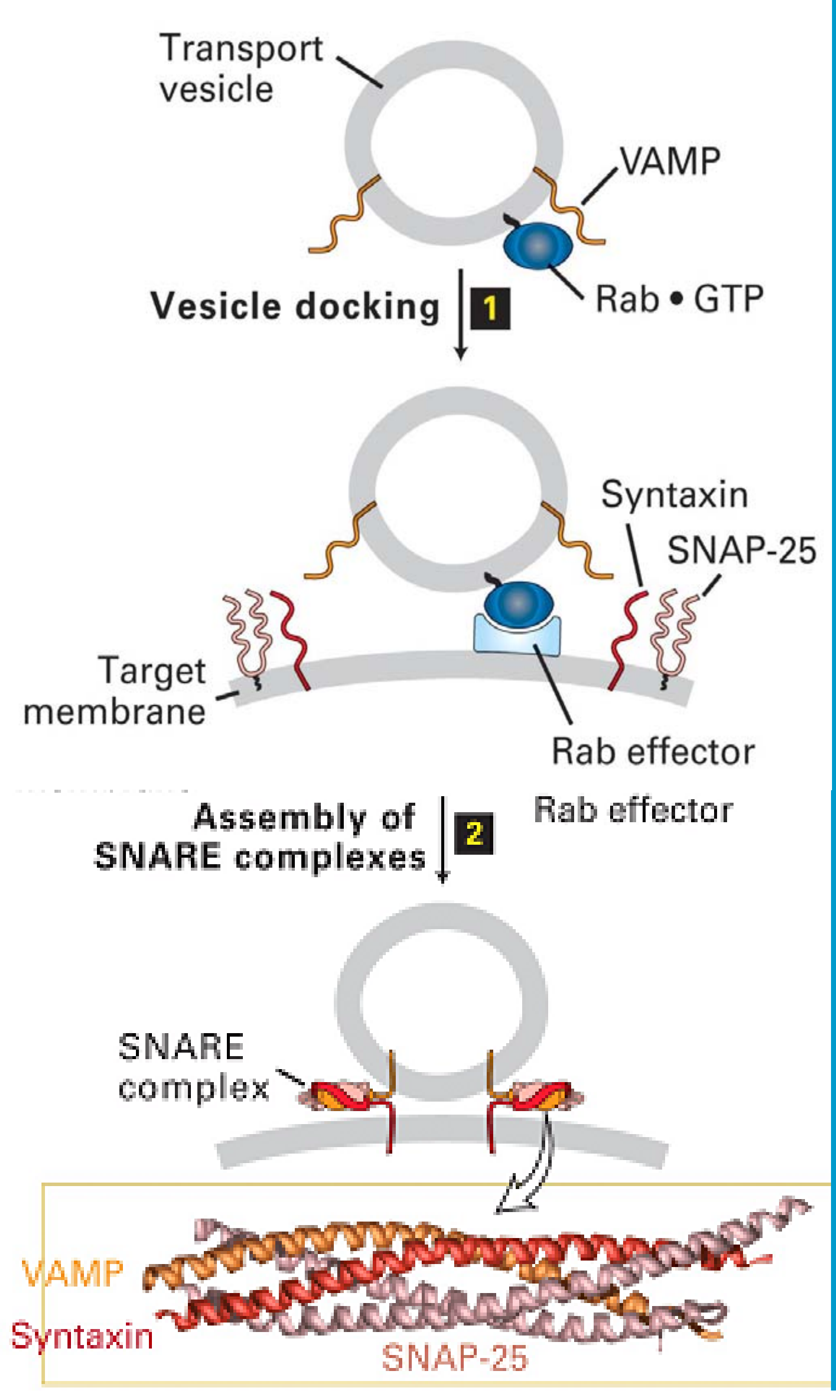


3 GTP hydrolysis

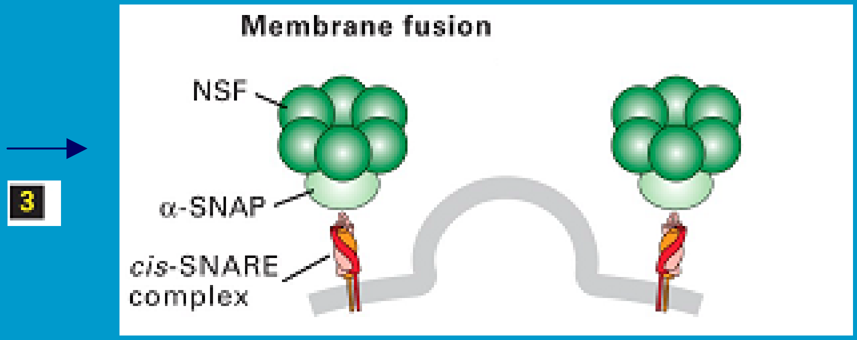


4 Coat disassembly

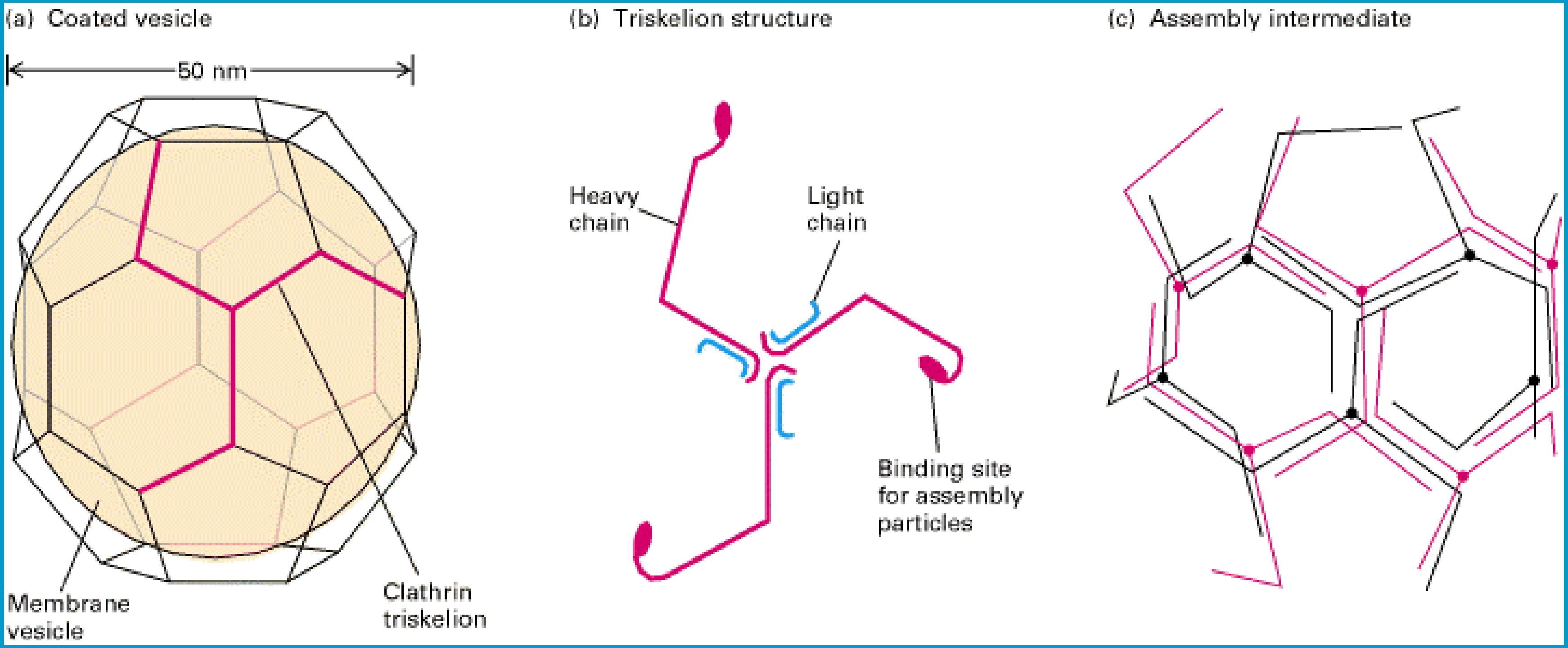
Model for docking and fusion of transport vesicles



- Step1: Rab-GTP protein bind to an effector protein in the plasma membrane
- Step 2: Formation of a very stable coil-coiled SNARE complex
- Step 3: Immediately fusion of membranes
- Step 4: NSF binds to SNARE complex. ATP hydrolysis catalized dissociation of SNARe complex

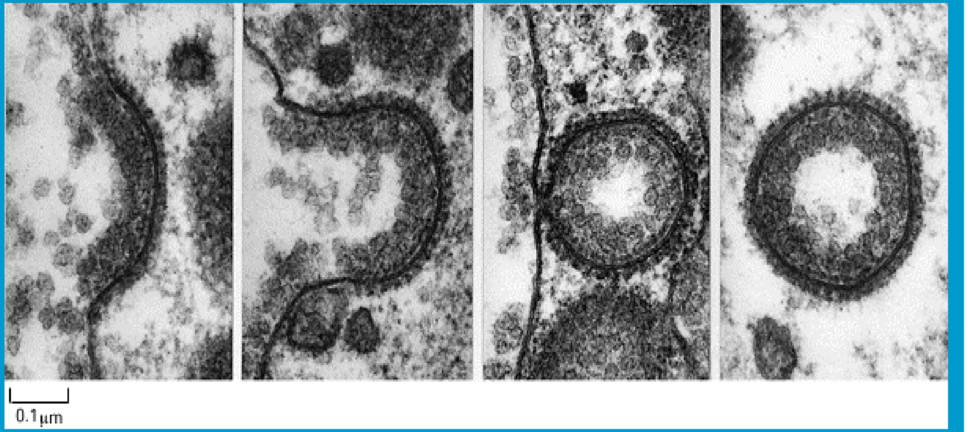


Structure of clathrin-coated vesicles



Molecular Cell Biology. Lodish *et als*. Figure 17-53

Model for the formation of a clathrin-coated pit



Components:

- Integral receptor proteins
- Adapter proteins (ex. AP1)
- Clathrin coat
- Dynamin: is required for pinching off

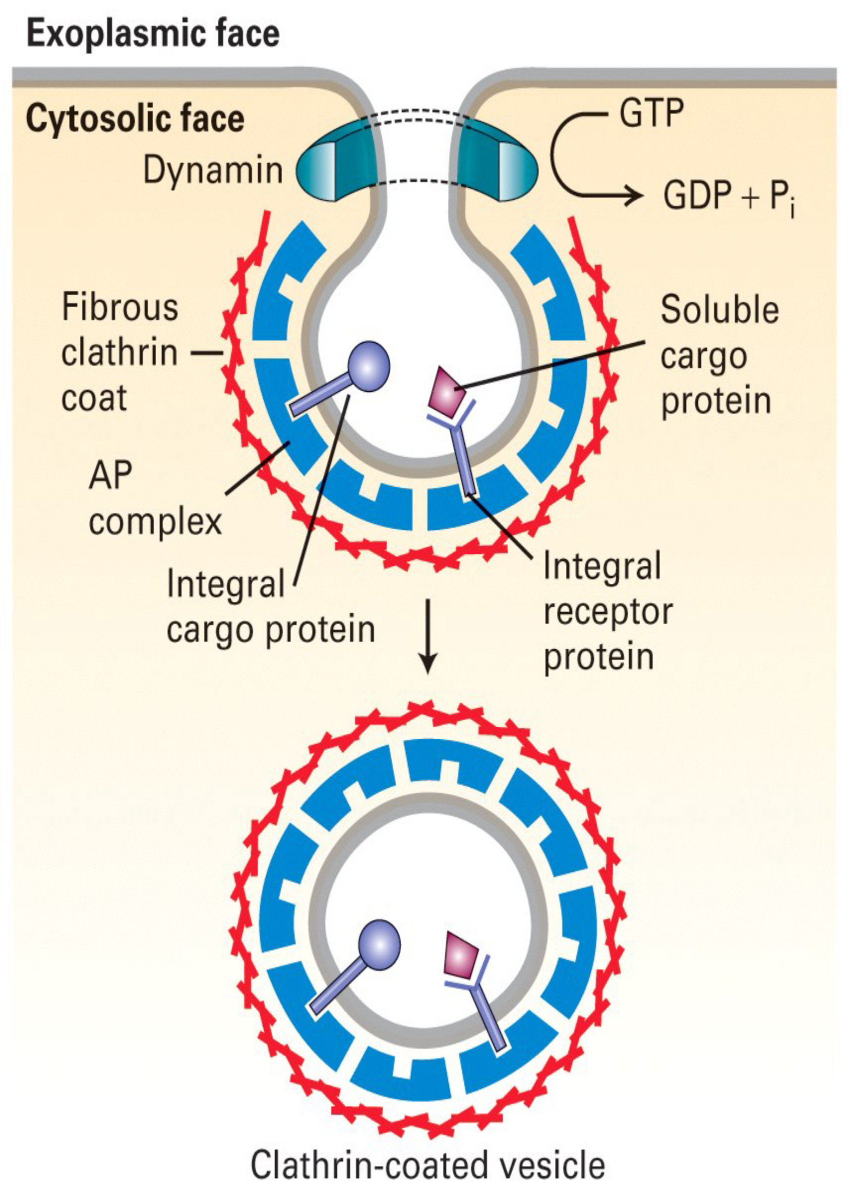


Fig 13-28.Alberts

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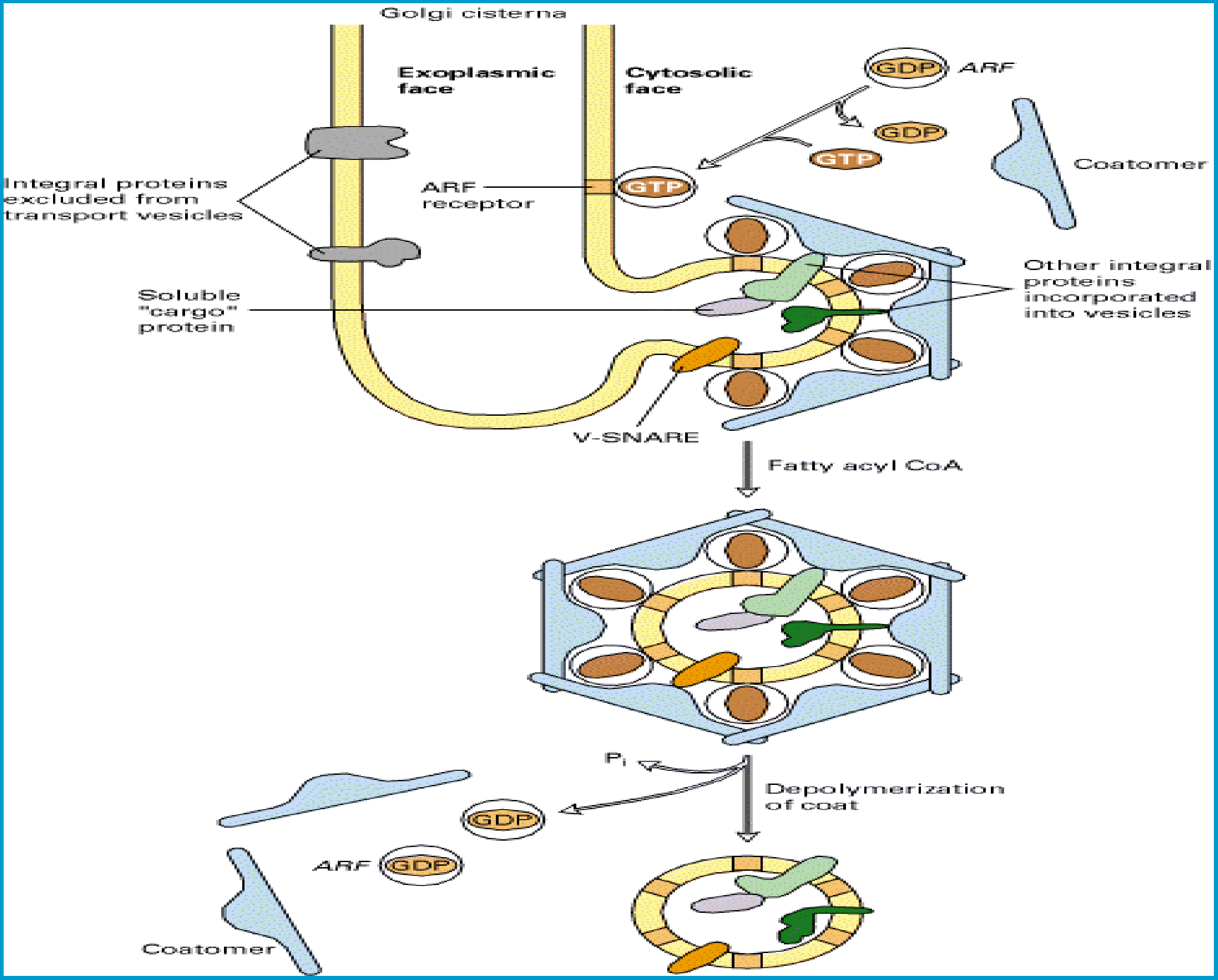
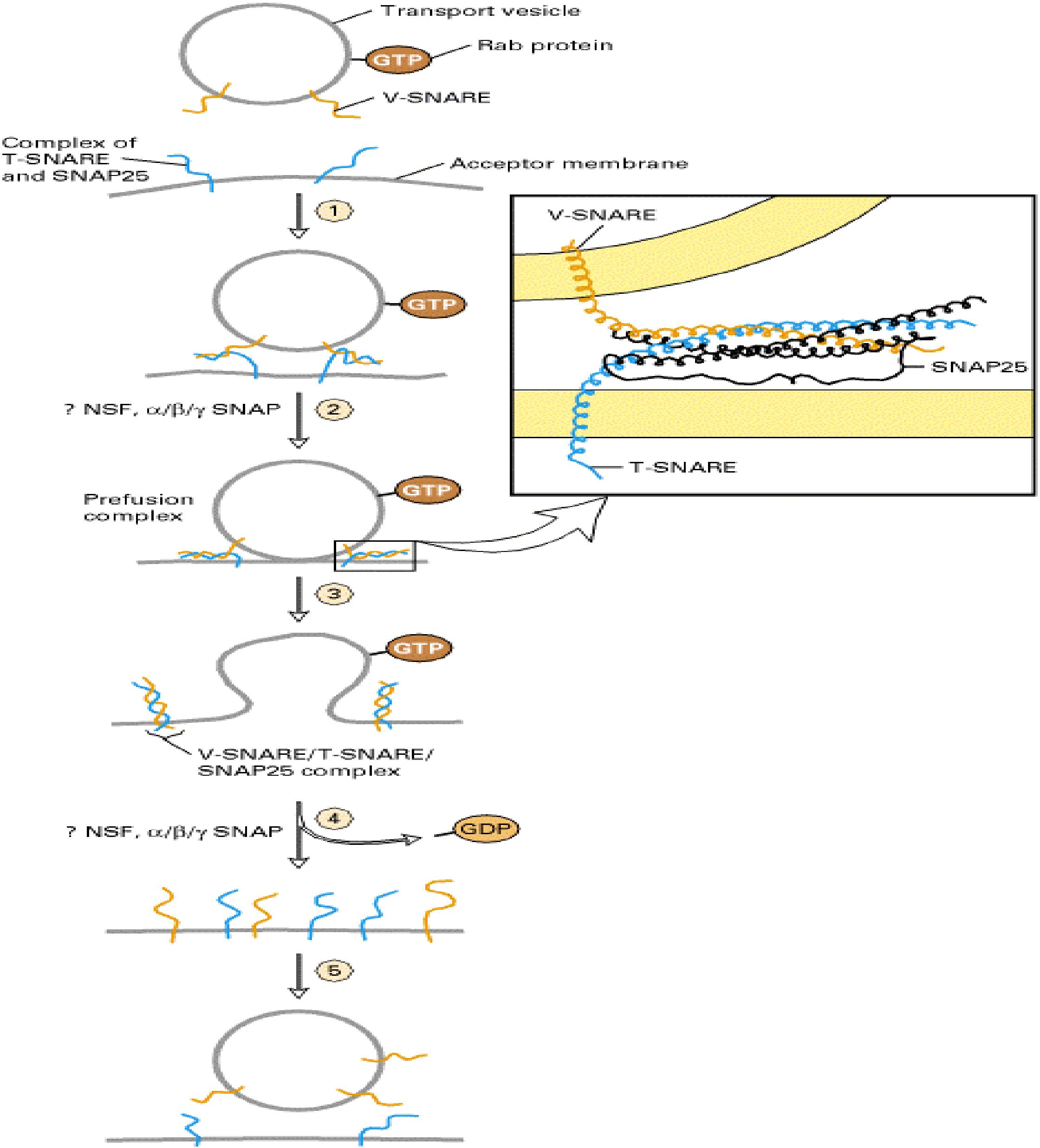
Model for formation of COPI-coated vesicles



Molecular Cell Biology. Lodish *et als*. Figure 17-58



Model for targeting and fusion of transport vesicles with their acceptor membranes

Molecular Cell Biology. Lodish *et als*. Figure 17-59

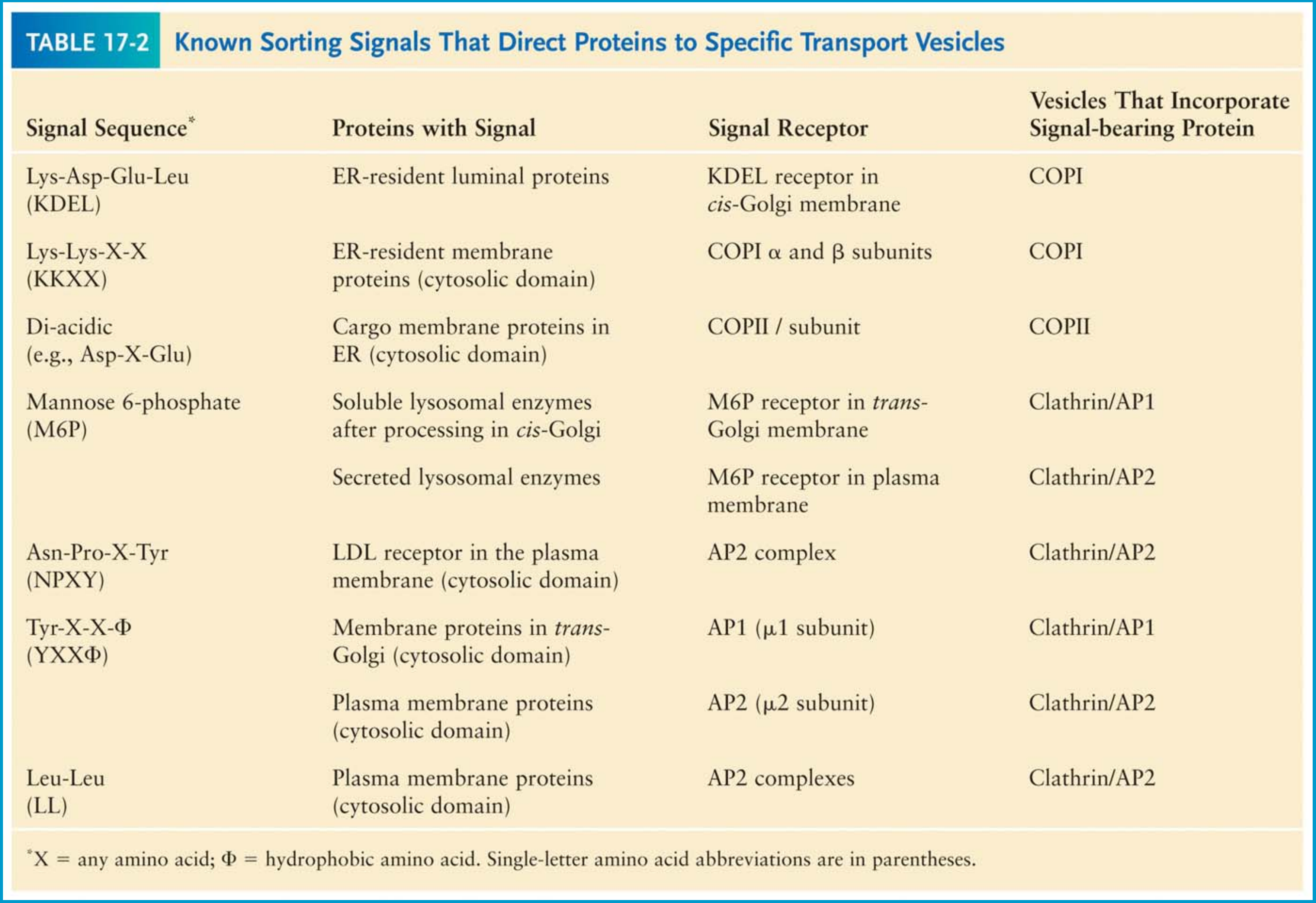


TABLE 17-2 Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

Signal Sequence*	Proteins with Signal	Signal Receptor	Vesicles That Incorporate Signal-bearing Protein
Lys-Asp-Glu-Leu (KDEL)	ER-resident luminal proteins	KDEL receptor in *cis*-Golgi membrane	COPI
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins (cytosolic domain)	COPI α and β subunits	COPI
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER (cytosolic domain)	COPII / subunit	COPII
Mannose 6-phosphate (M6P)	Soluble lysosomal enzymes after processing in *cis*-Golgi	M6P receptor in *trans*-Golgi membrane	Clathrin/AP1
	Secreted lysosomal enzymes	M6P receptor in plasma membrane	Clathrin/AP2
Asn-Pro-X-Tyr (NPXY)	LDL receptor in the plasma membrane (cytosolic domain)	AP2 complex	Clathrin/AP2
Tyr-X-X-Φ (YXXΦ)	Membrane proteins in *trans*-Golgi (cytosolic domain)	AP1 (μ1 subunit)	Clathrin/AP1
	Plasma membrane proteins (cytosolic domain)	AP2 (μ2 subunit)	Clathrin/AP2
Leu-Leu (LL)	Plasma membrane proteins (cytosolic domain)	AP2 complexes	Clathrin/AP2

*X = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.