Chapter 14:

Vesicular Traffic, Secretion, and Endocytosis

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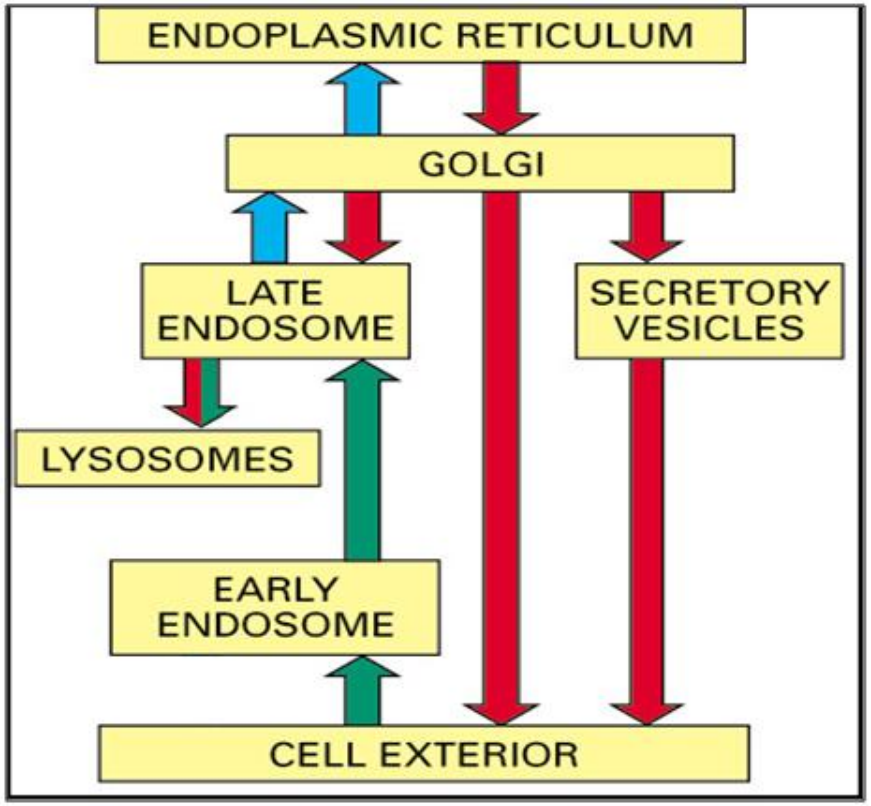
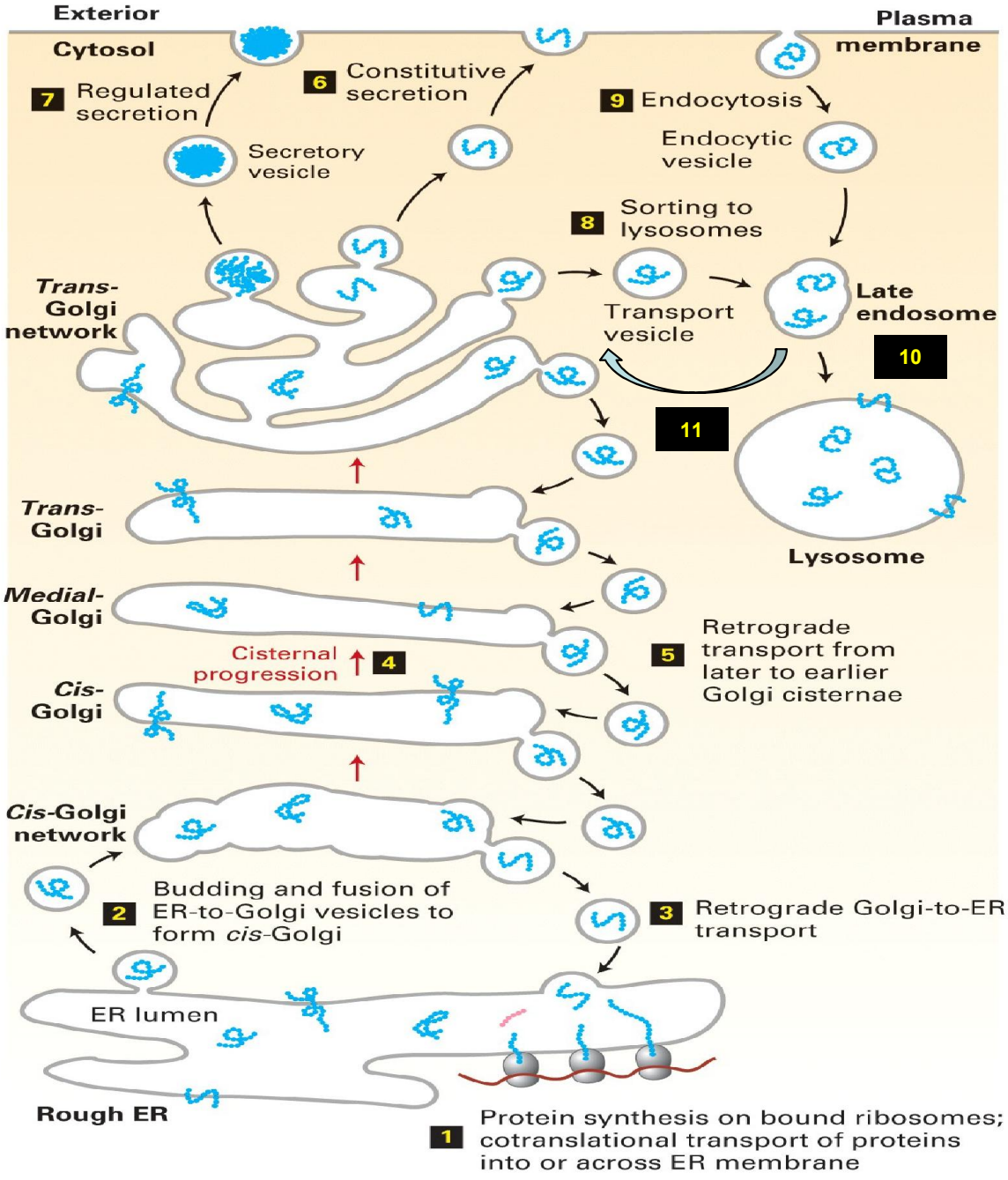
學習目標

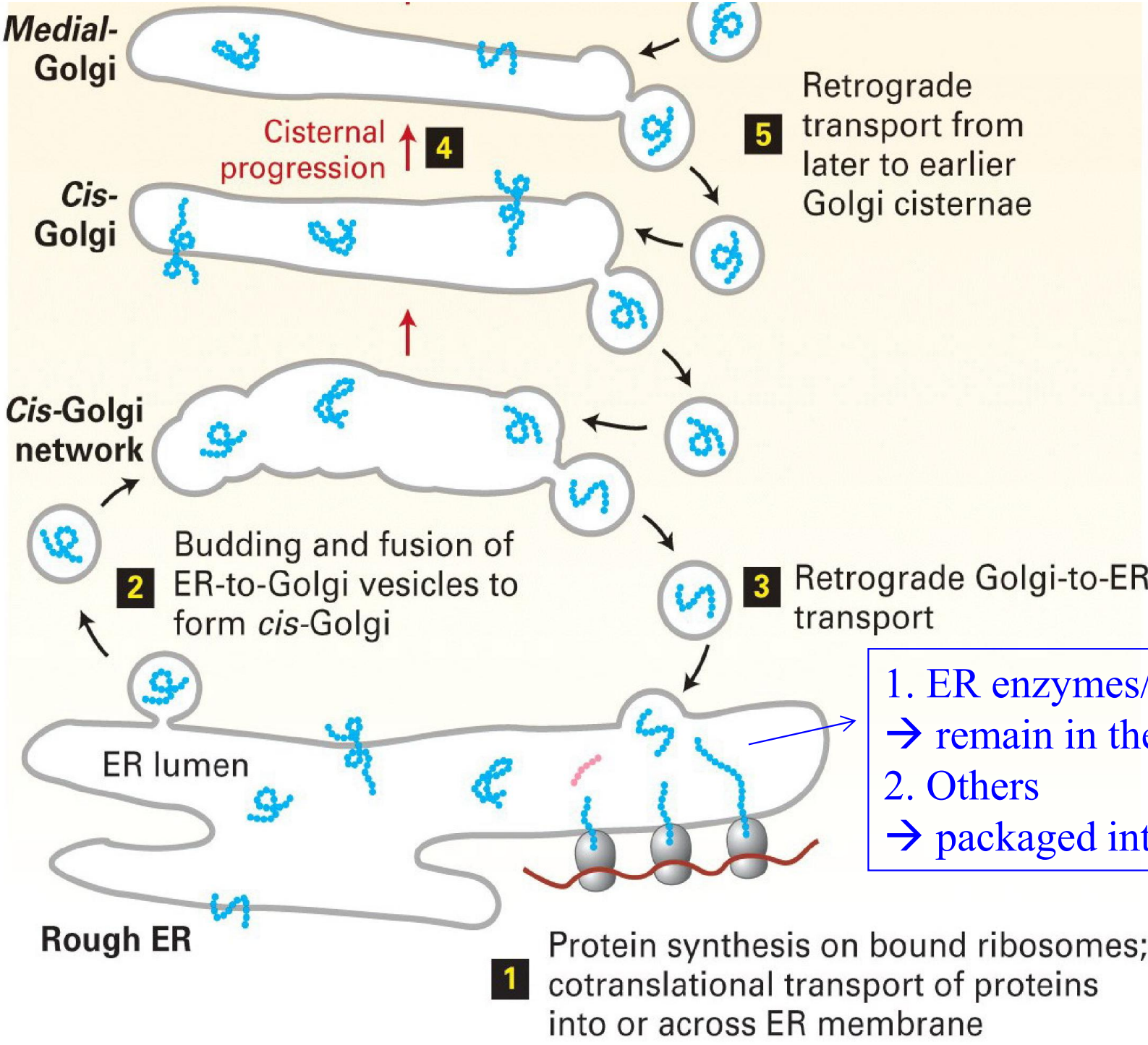
- Techniques for studying the secretory pathway
- Molecular mechanisms of vesicular traffic (泡囊傳輸)
- Vesicle trafficking in various stages of the secretory pathway
- Receptor-mediated endocytosis and the sorting of internalized proteins

Overview of vesicular trafficking and protein sorting



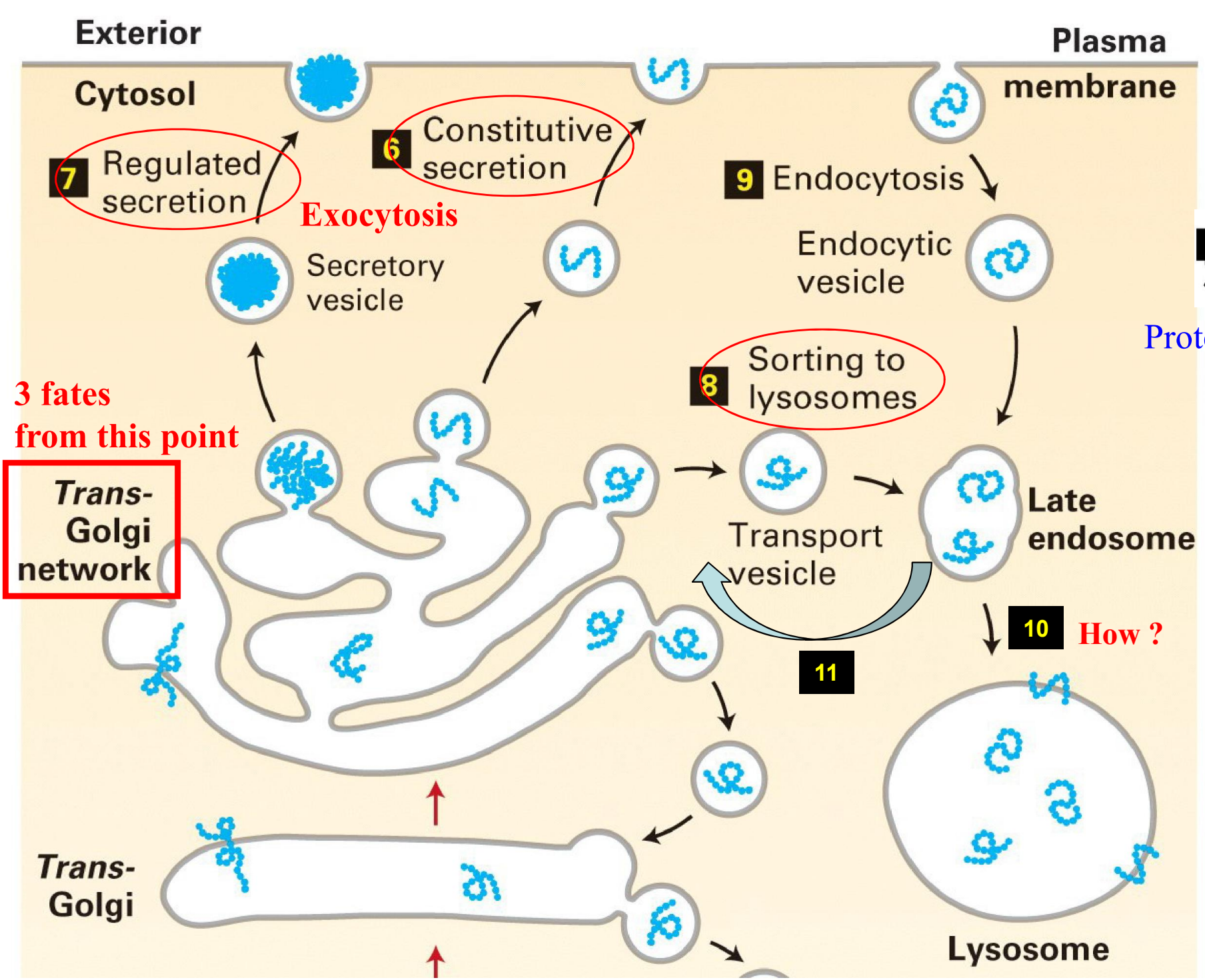
- **Biosynthetic – secretory pathways (1), (2), (4), (6), (7), (8)**
- **Endocytic pathways - (9), (10)**
- **Retrieval (Recycling) pathways – (3), (5), (11)**





1. ER enzymes/structural proteins
→ remain in the ER lumen
2. Others
→ packaged into transport vesicles

Fig 14-1

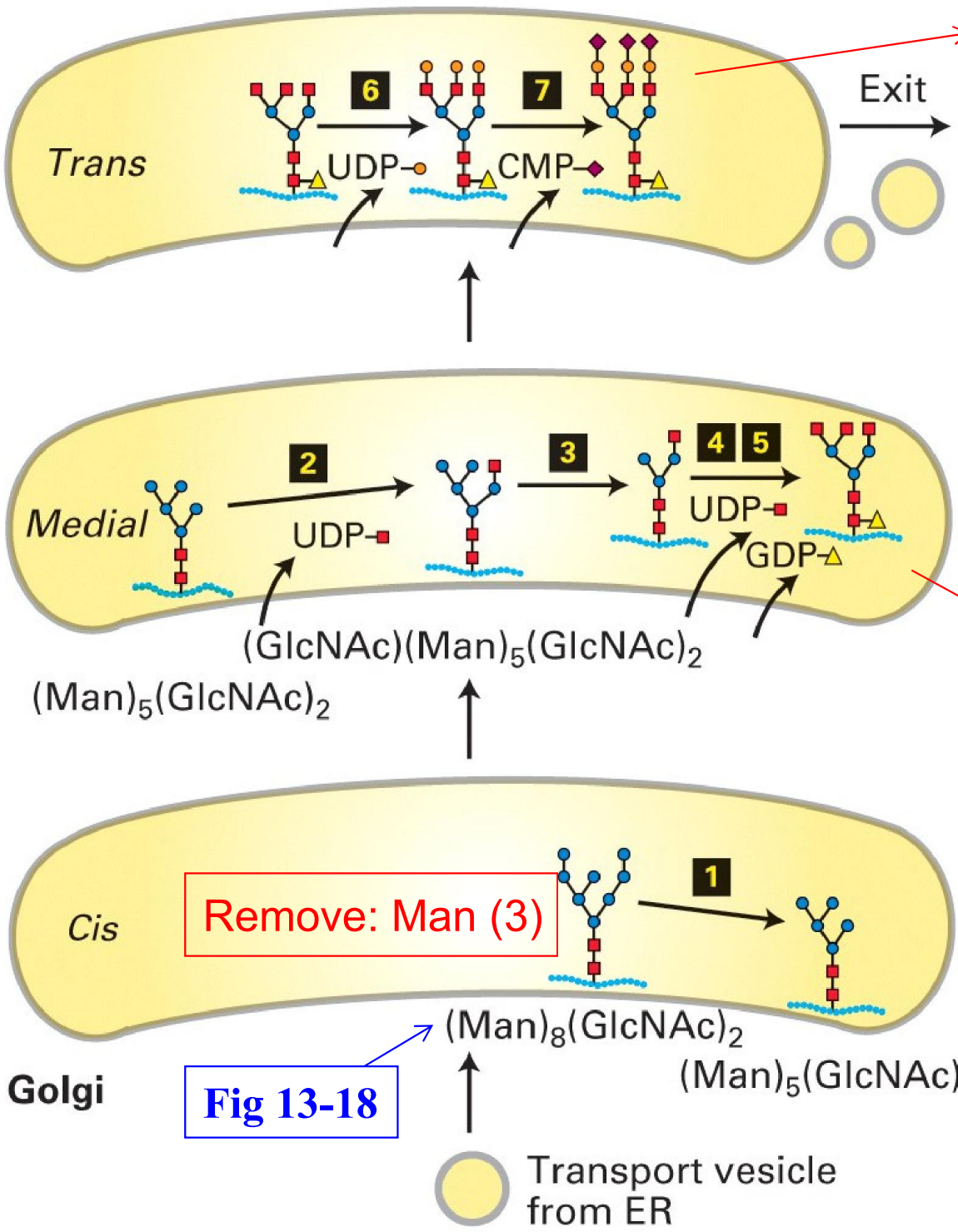


Protein secretion

Secretory proteins should NEVER be released into cytosol!!!

Fig 14-1

Processing of N-linked oligosaccharides in Golgi



Add: Gal. (3), NeuNAc (3)

Fig 14-14

■ = *N*-Acetylglucosamine
● = Mannose
▲ = Fucose
● = Galactose
◆ = *N*-Acetylneuraminic acid

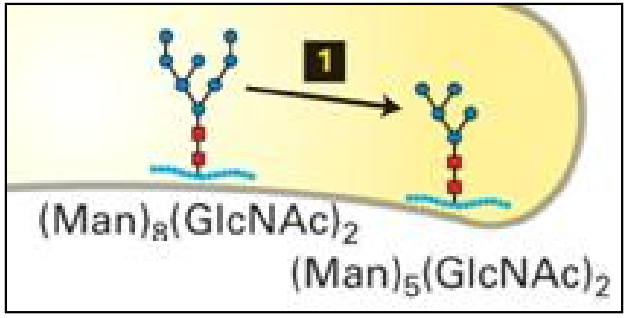
Add: GlcNAc (3), Fucose (1)
Remove: Man (2)

We can use **Golgi-specific glycosidase** to distinguish locations of glycosylated proteins along the pathway
→ e.g. *cis*-Golgi specific endoglycosidase D

14.1
Techniques for Studying the Secretory Pathway

cis Golgi-specific endoglycosidase D

- Does not cleave newly made N-linked oligosaccharides ($Man_8GlcNAc_2$) present in ER



- Can cleave *processed* N-linked oligosaccharides ($Man_5GlcNAc_2$) present in cis-Golgi
  - Cleaved $Man_5GlcNAc_2$ will have lower M.W. → so, run faster on SDS-PAGE (Fig. 14-3)

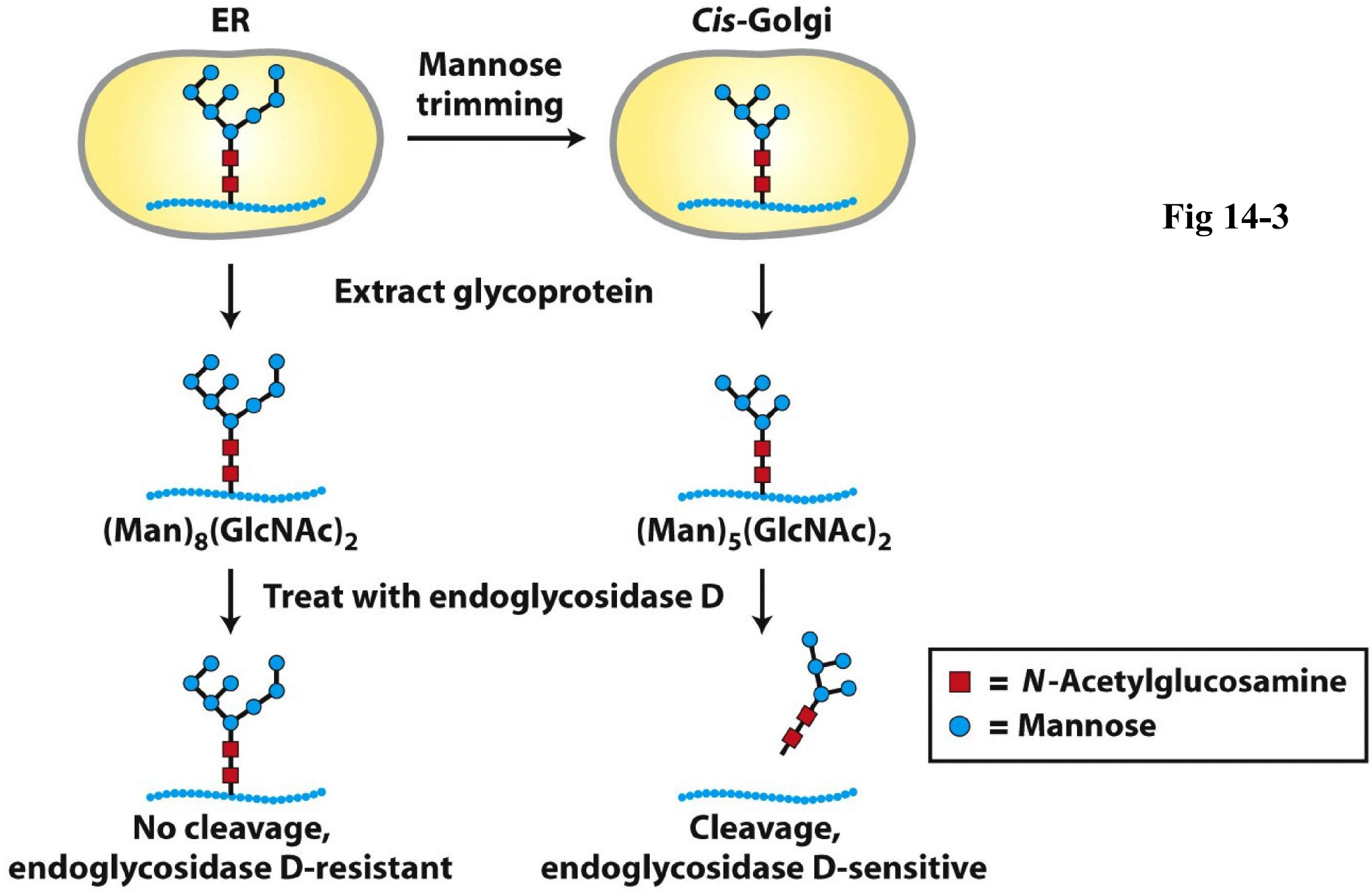
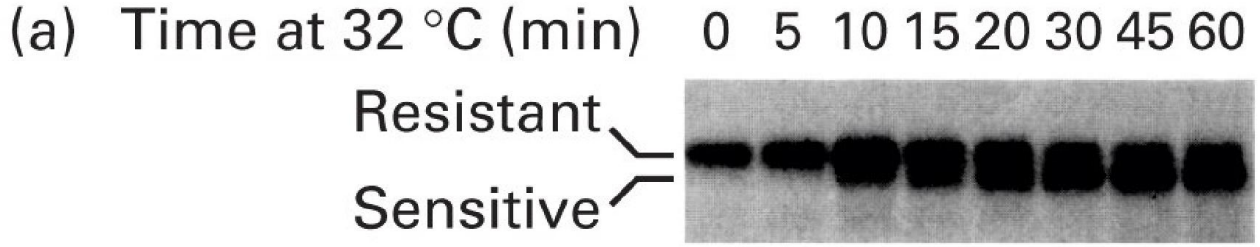
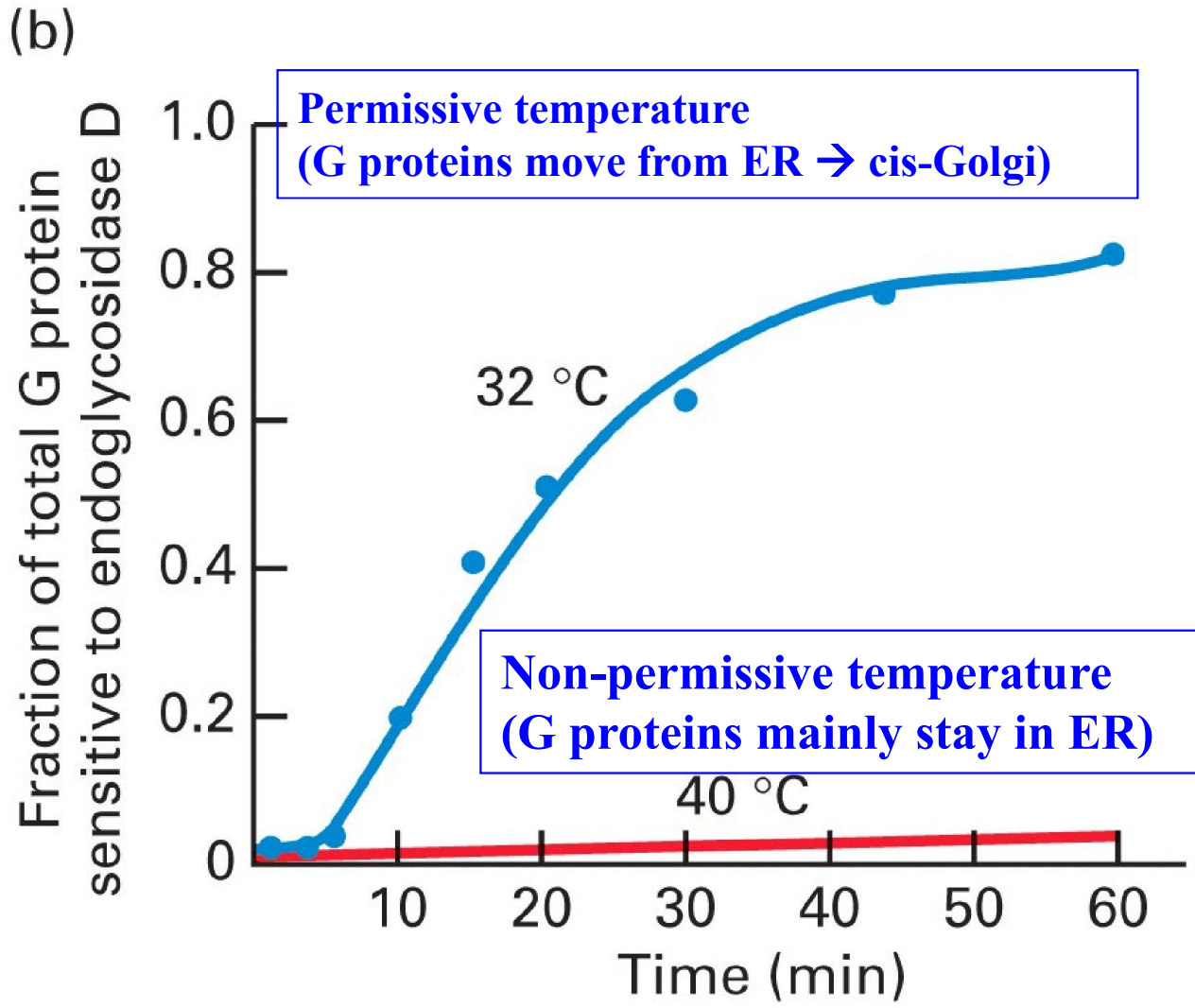


Fig 14-3

Figure 14-3a
Molecular Cell Biology, Sixth Edition

Analysis of protein transport (from ER → cis-Golgi) by endoglycosidase D digestion





1. Radioisotope label VSV G glycoproteins (0 min)
2. Return to permissive temp. at 32°C
3. VSG-G started to fold correctly and be transported from ER to cis-Golgi
4. Endoglycosidase D-sensitive VSV-G will be increased

Vesicular stomatitis virus (VSV): Member of Rhabdoviridae

Fig 14-3

Transport of GFP-tagged VSV-G protein in transfected cells

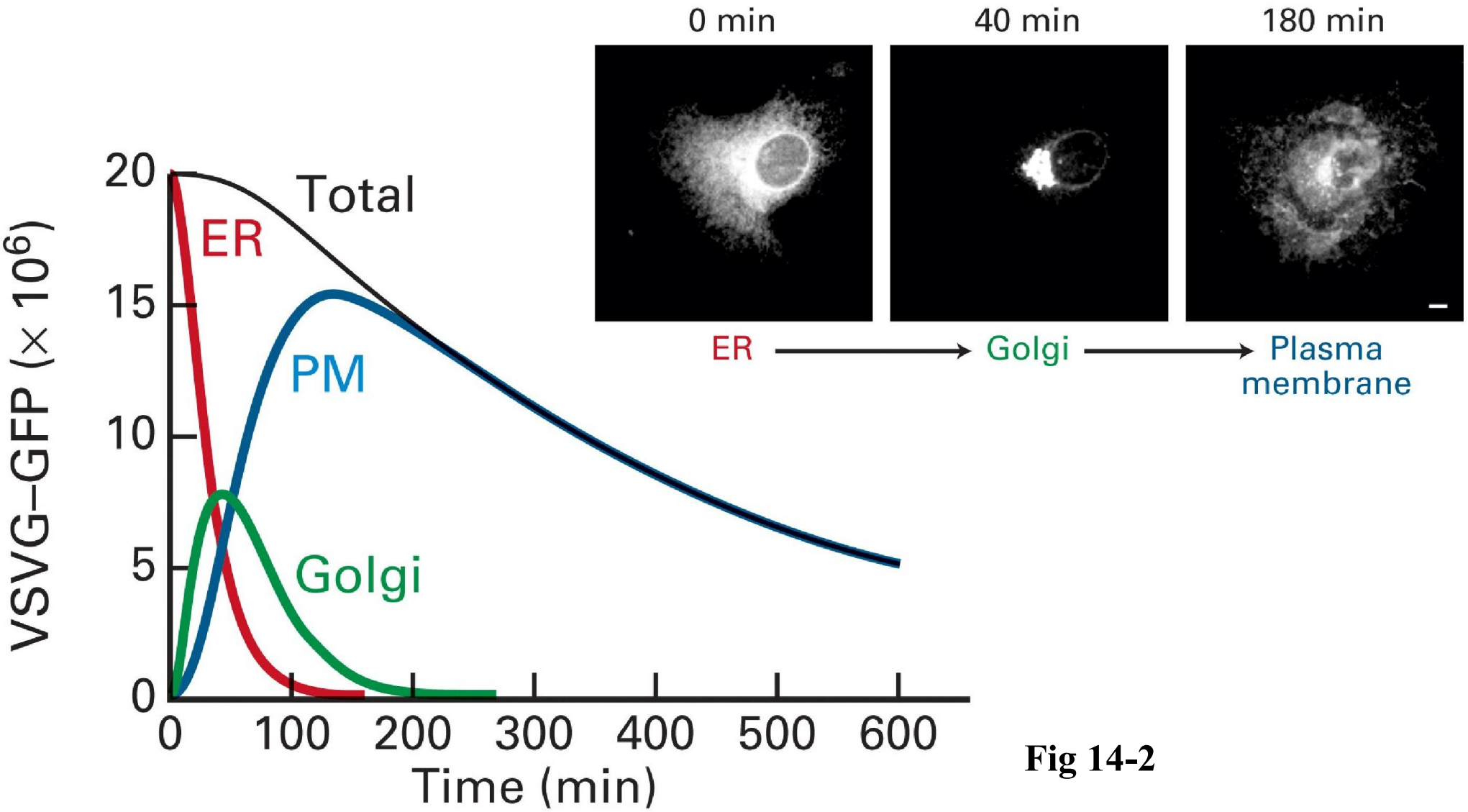
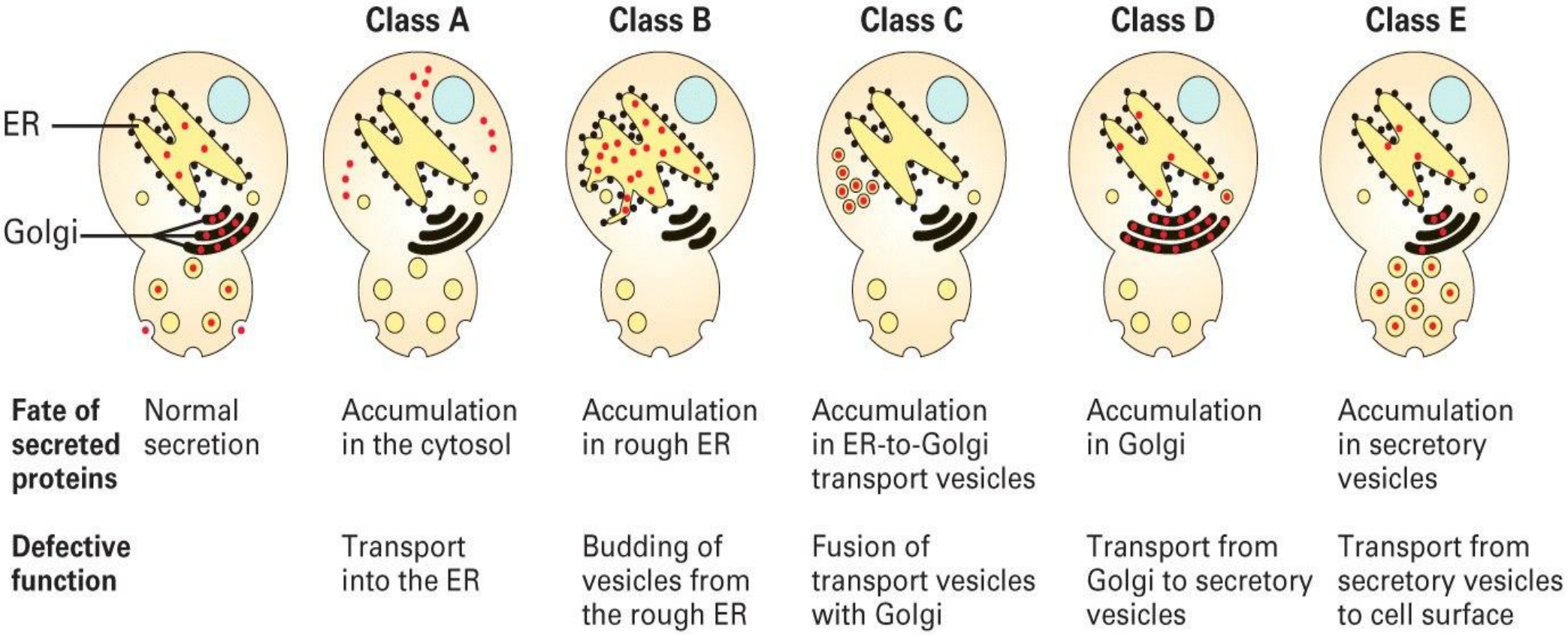


Fig 14-2

Yeast *sec* mutants for the identification of stages in secretory pathway

Fig 14-4



***sec* mutant = secretory mutant**

High temperature (non-permissive) causes transport of proteins stop at different stages in various secretion-defective mutants

Cell-free transport assays allow dissection of individual steps in vesicular transport

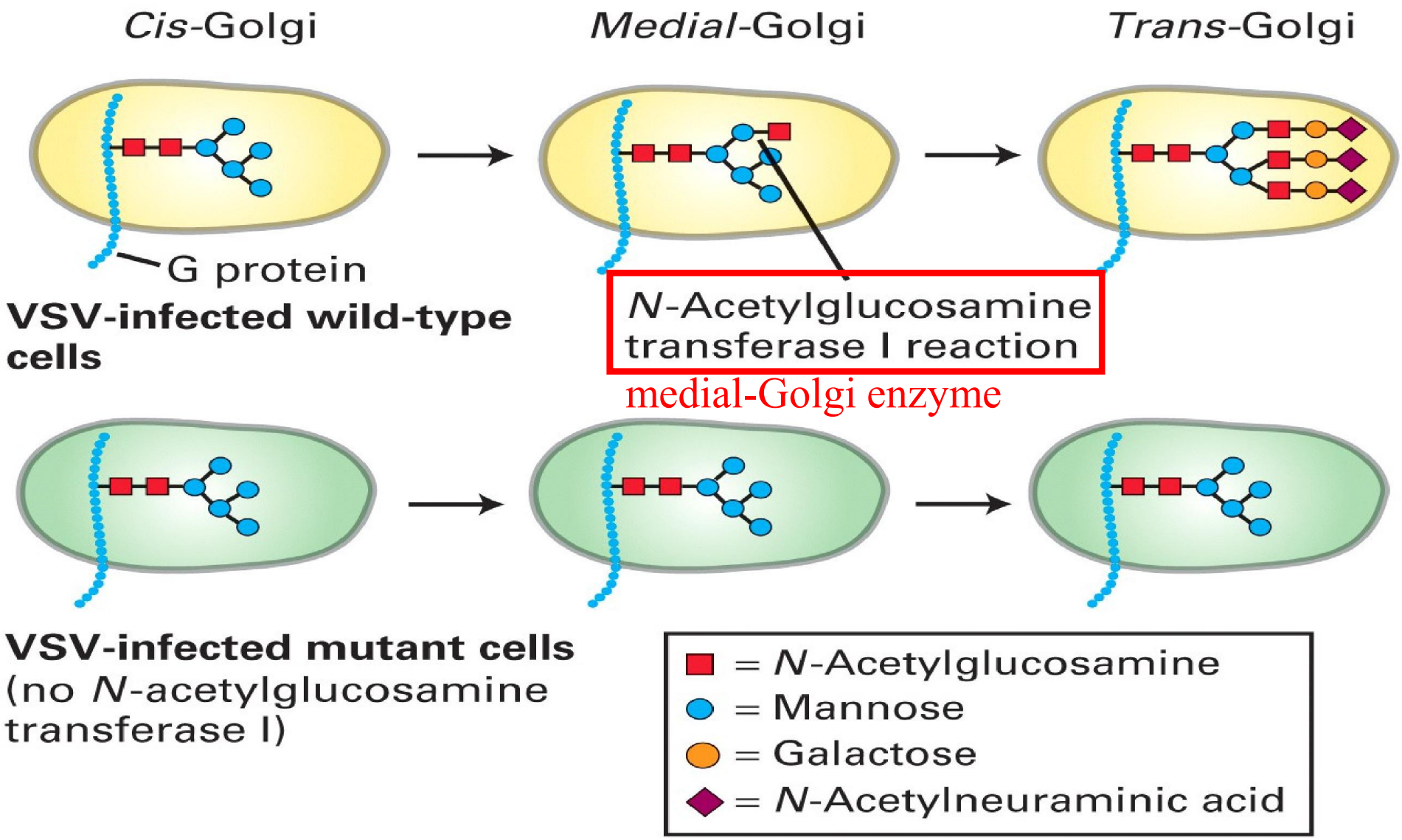
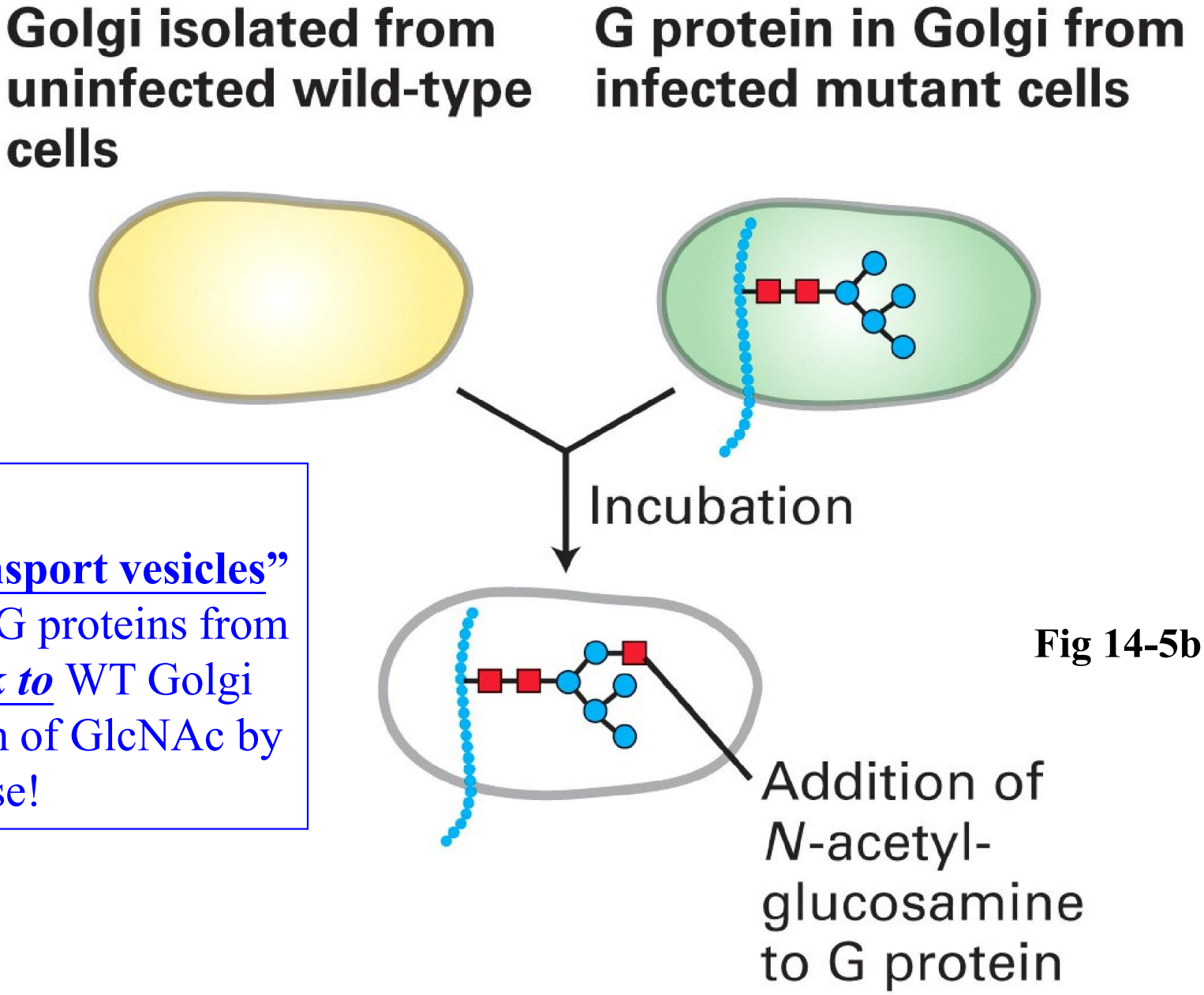


Fig 14-5a

Retrograde vesicular transport from wild type to mutant Golgi



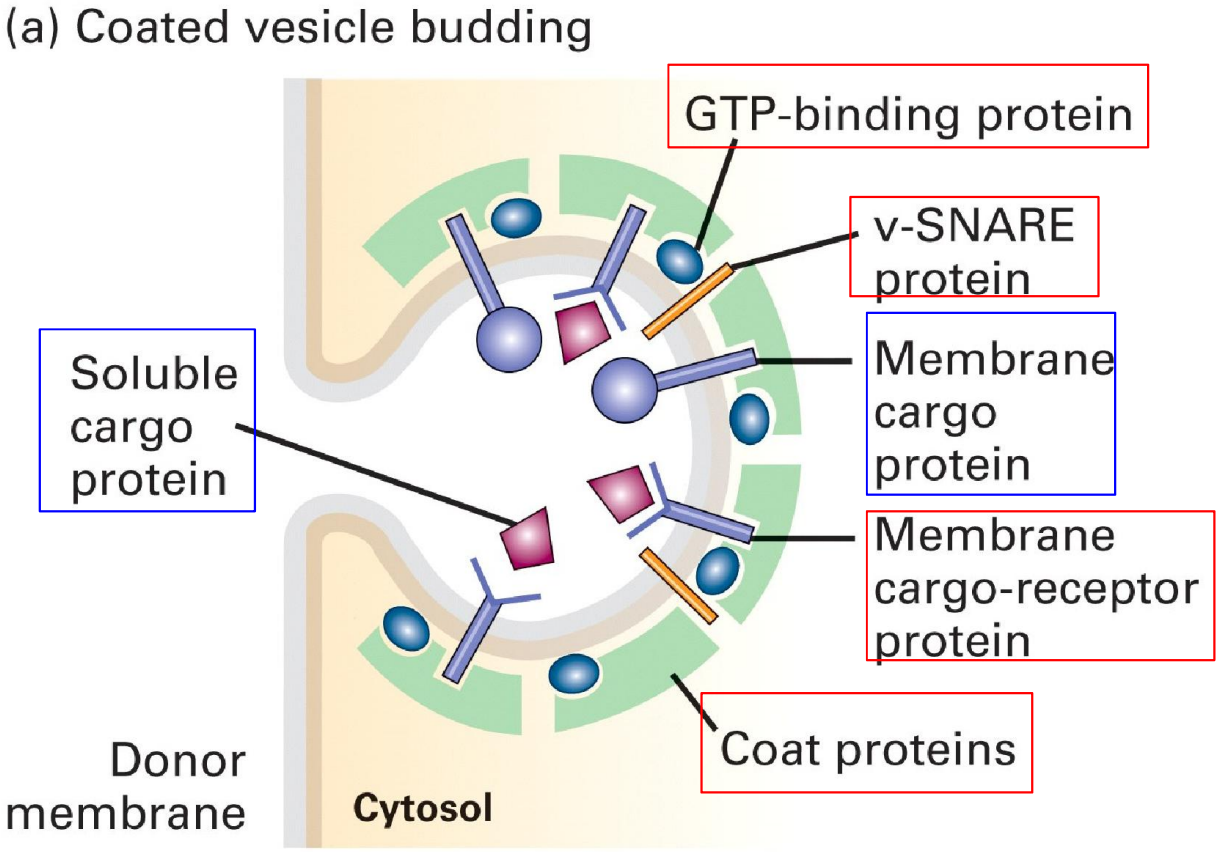
Why?
"**Retrograde transport vesicles**" bring unmodified G proteins from mutant Golgi ***back to*** WT Golgi for proper addition of GlcNAc by GlcNAc transferase!

Fig 14-5b

14.2
Molecular Mechanisms of Vesicular Traffic

Fig 14-6

Overview of vesicle budding (from 'parent' or donor organelle)



1. Formation of soluble protein complexes (a polymerization Rx)
   - Soluble cargo protein
   - Membrane cargo proteins
   - GTP-binding proteins (initiator of the formation)
   - v-SNARE protein
   - Membrane cargo receptor protein
   - Coat proteins
2. Release (budding)

"Cargo proteins" could be either soluble or membrane-bound

Overview of vesicle fusion (to 'daughter' or recipient organelle)

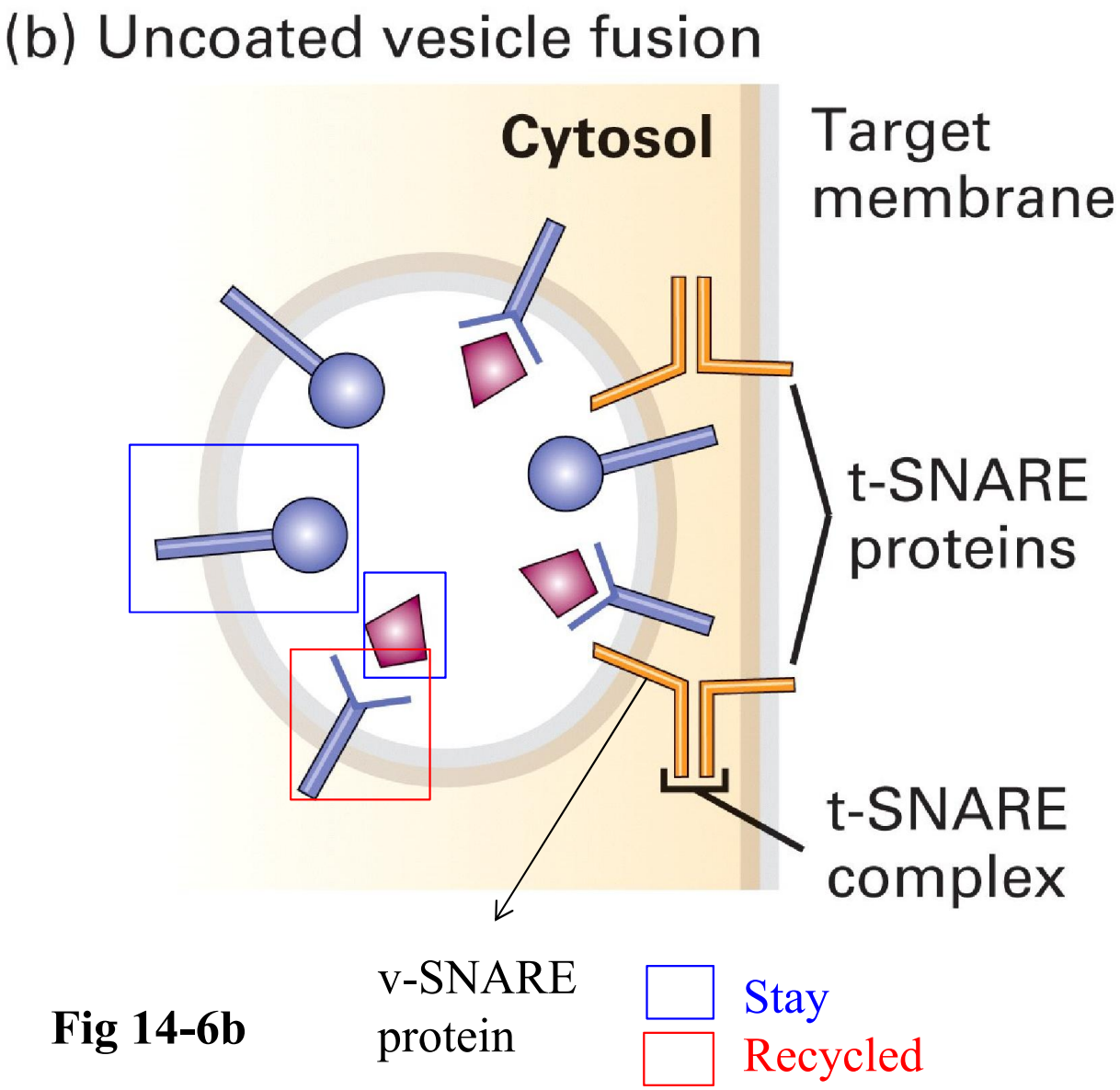
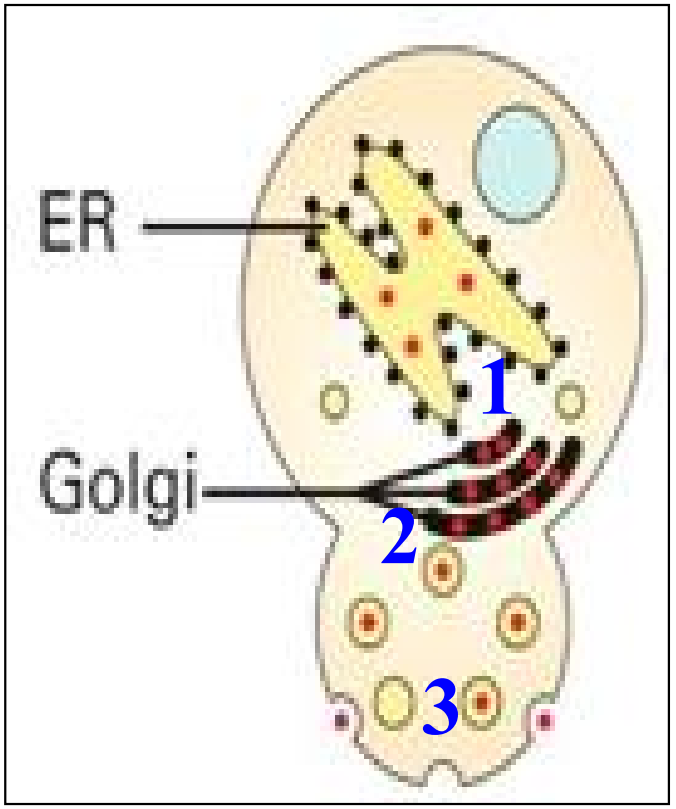


Fig 14-6b

2. Release (budding)
3. Shedding of
   - coat proteins
   - GTP-binding proteins
4. Exposure of v-SNARE
5. Fusion with target membrane
   - through interaction between v- and t-SNARE proteins
6. Release of cargo proteins into lumen

Coated vesicles (3 types)

1. COPII
   - Anterograde transport
     - Rough ER → Golgi transport
2. COPI
   - Retrograde transport
     - between Golgi cisternae, or
     - from *cis*-Golgi → rough ER
3. Clathrin
   - from plasma membrane → early/late endosomes (e.g. LDL receptor)
   - from *trans*-Golgi → late endosomes (e.g. mannose-6-phosphate receptor; M6PR)
   - from *trans*-Golgi → plasma membrane

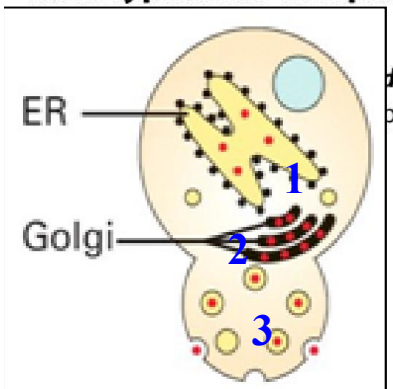


Coated vesicles involved in protein trafficking

TABLE 14-1 Coated Vesicles Involved in Protein Trafficking

VESICLE TYPE	TRANSPORT STEP MEDIATED	COAT PROTEINS	ASSOCIATED GTPase
COPII	ER to *cis*-Golgi	Sec23/Sec24 and Sec13/Sec31 complexes, Sec16	Sar1
COPI	*cis*-Golgi to ER Later to earlier Golgi cisternae	Coatomers containing seven different COP subunits	ARF
Clathrin and adapter proteins*	*trans*-Golgi to endosome	Clathrin + AP1 complexes	ARF
	trans-Golgi to endosome	Clathrin + GGA	ARF
	Plasma membrane to endosome	Clathrin + AP2 complexes	ARF
	Golgi to lysosome, melanosome, or platelet vesicles	AP3 complexes	ARF

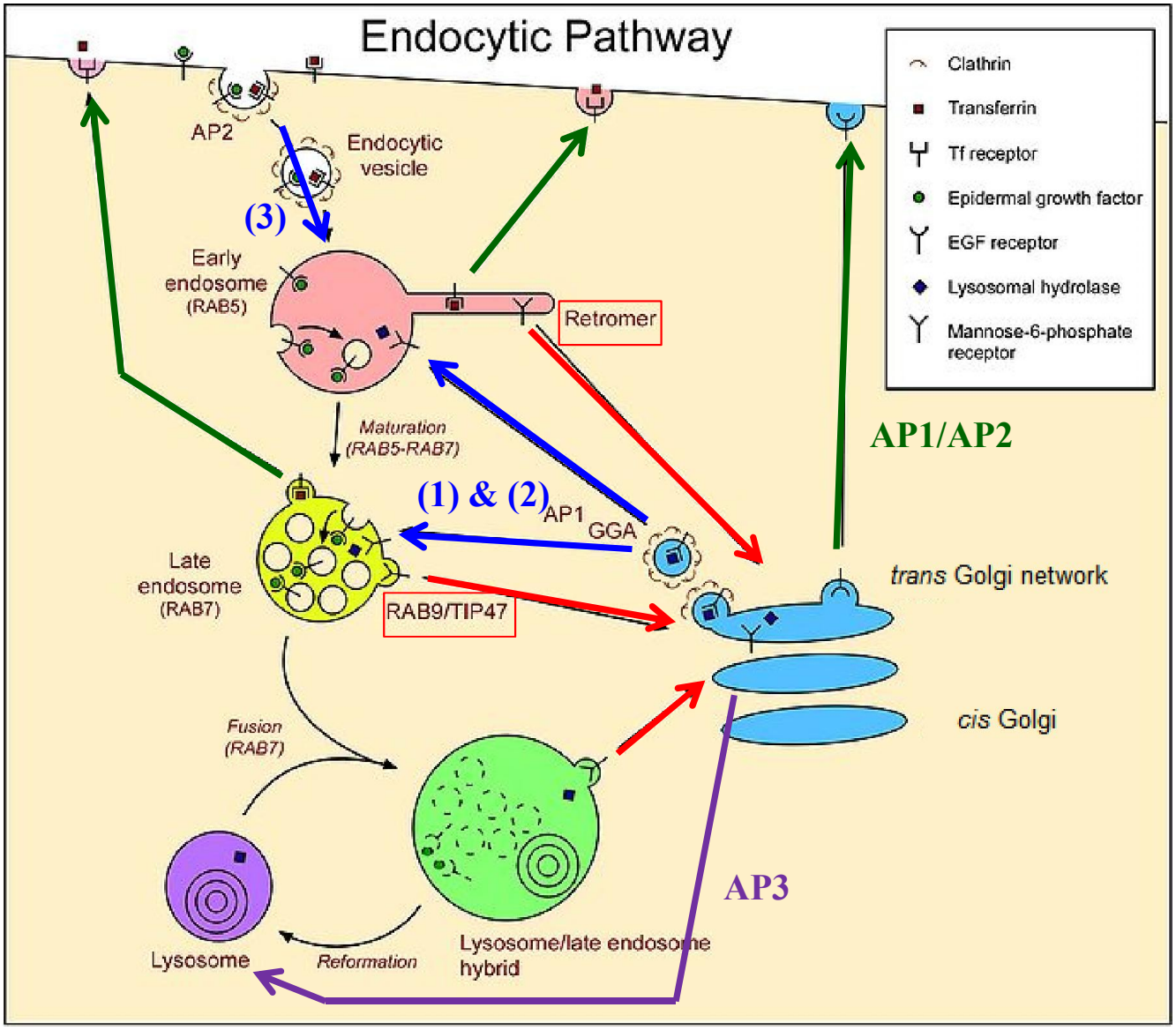
*Each type of AP complex consists of four different subunits. It is not known whether the coat of AP3 vesicles contains clathrin.



The endocytic pathway

The endocytic pathway

(Blue) To endosomes
(Red) To Golgi
(Green) To plasma membrane
(Purple) To lysosome



Clathrin and adapter proteins*	**(1)**	***trans*-Golgi to endosome**	**Clathrin + AP1 complexes**	**ARF**
		(e.g M6PR for lysosomal ***luminal*** enzymes)		
	(2)	***trans*-Golgi to endosome**	**Clathrin + GGA**	**ARF**
	(3)	**Plasma membrane to endosome**	**Clathrin + AP2 complexes**	**ARF**
		(e.g M6PR for ***secreted*** lysosomal enzymes, LDL receptor)		
	(4)	**Golgi to lysosome, melanosome, or platelet vesicles**	**AP3 complexes**	**ARF**

TABLE 14-2 Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

SIGNAL SEQUENCE*	PROTEINS WITH SIGNAL	SIGNAL RECEPTOR	VESICLES THAT INCORPORATE SIGNAL-BEARING PROTEIN
LUMENAL SORTING SIGNALS			
Lys-Asp-Glu-Leu (KDEL)	ER-resident soluble proteins	KDEL receptor in *cis*-Golgi membrane	COPI (Retrograde, *cis*-Golgi to ER)
Mannose 6-phosphate (M6P)	Soluble lysosomal enzymes after processing in *cis*-Golgi	M6P receptor in *trans*-Golgi membrane	Clathrin/AP1 (*trans*-Golgi to late endosomes, then to lysosome)
	Secreted lysosomal enzymes	M6P receptor in plasma membrane	Clathrin/AP2 (plasma membrane to endosomes)
CYTOPLASMIC SORTING SIGNALS → For sorting to ***membrane***			
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins	COPI α and β subunits	COPI (Retrograde, *cis*-Golgi to ER)
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER	COPII Sec24 subunit	COPII (Anterograde, ER to *cis*-Golgi)
Asn-Pro-X-Tyr (NPXY)	LDL receptor in plasma membrane	AP2 complex	Clathrin/AP2 (plasma membrane to endosomes)
Tyr-X-X-Φ (YXXΦ)	Membrane proteins in *trans*-Golgi	AP1 (μ1 subunit)	Clathrin/AP1 (*trans*-Golgi to lysosomal membrane)
	Plasma membrane proteins	AP2 (μ2 subunit)	Clathrin/AP2 (plasma membrane to endosomes)
Leu-Leu (LL)	Plasma membrane proteins	AP2 complexes	Clathrin/AP2 (plasma membrane to endosomes)

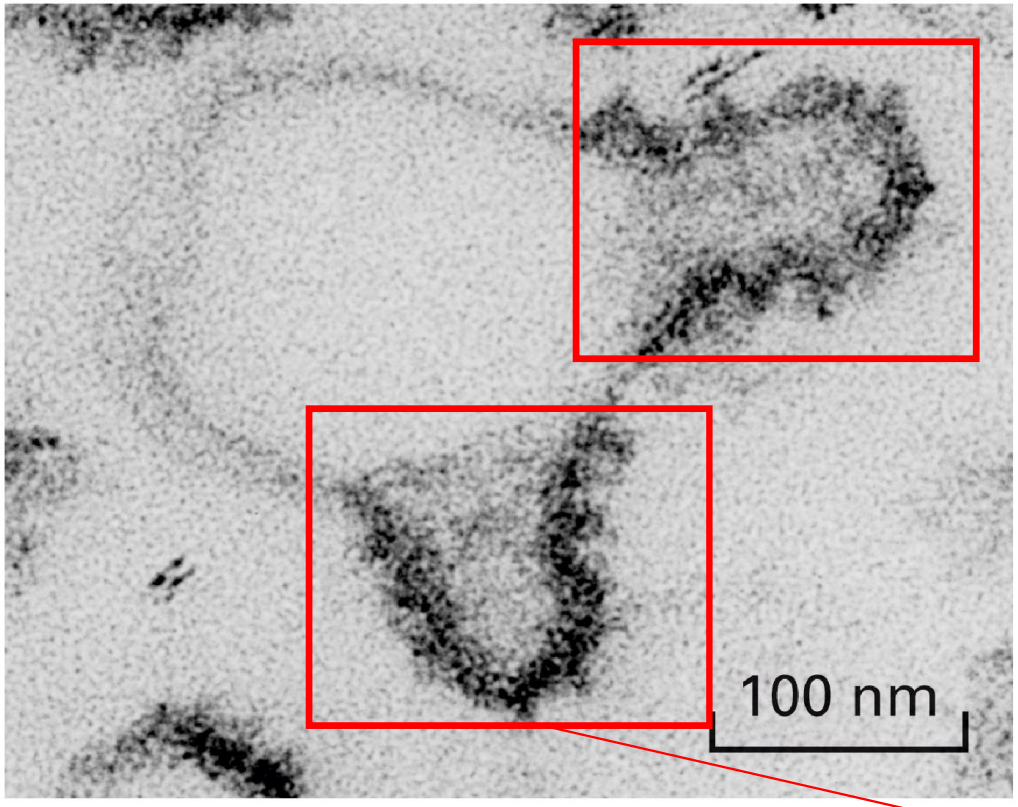
*X = any amino acid; Φ = hydrophobic amino acid. Single-letter amino acid abbreviations are in parentheses.

Table 14-2
Molecular Cell Biology, Sixth Edition

Coated vesicles - properties

- All three contain GTP-binding proteins (GTPases)
  - ARF (in COPI and Clathrin); Sar1 (in COPII)
  - Members of GTPase superfamily
    - 'switch' proteins (active vs. inactive)
      - Active: GTP-bound (via guanine-exchange factor to switch GTP onto inactive state → conformational change
      - Inactive: GDP-bound
  - Regulatory function
  - Control coat assembly process
    - Via GTP-binding (active) and hydrolysis (inactive)

In vitro budding reaction



1. Purified COPII coat proteins + isolated ER (or artificial phospholipd vesicles)
2. Vesicle buds can be visualized

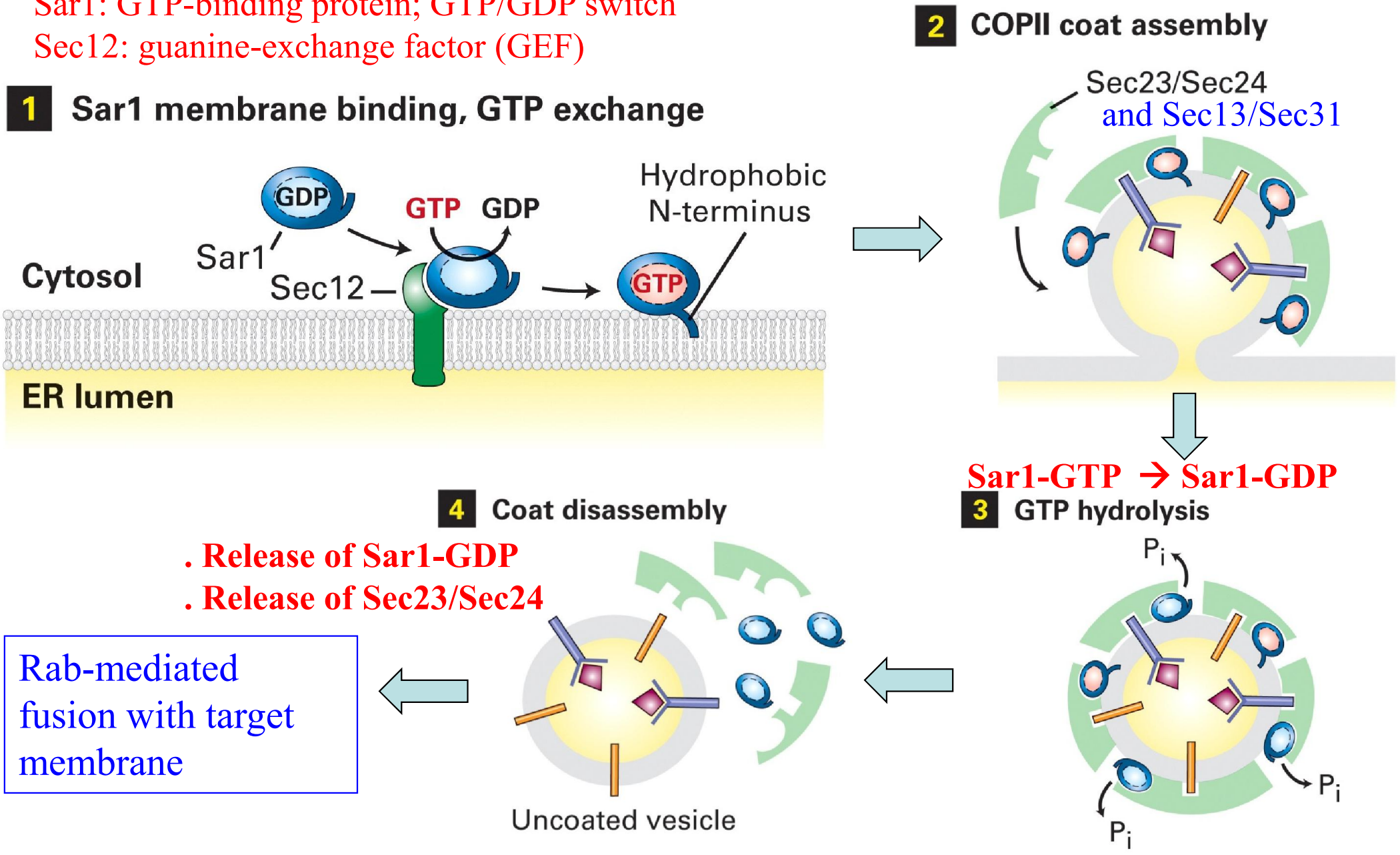
Fig 14-7

Vesicle bud (formed by the coat proteins)

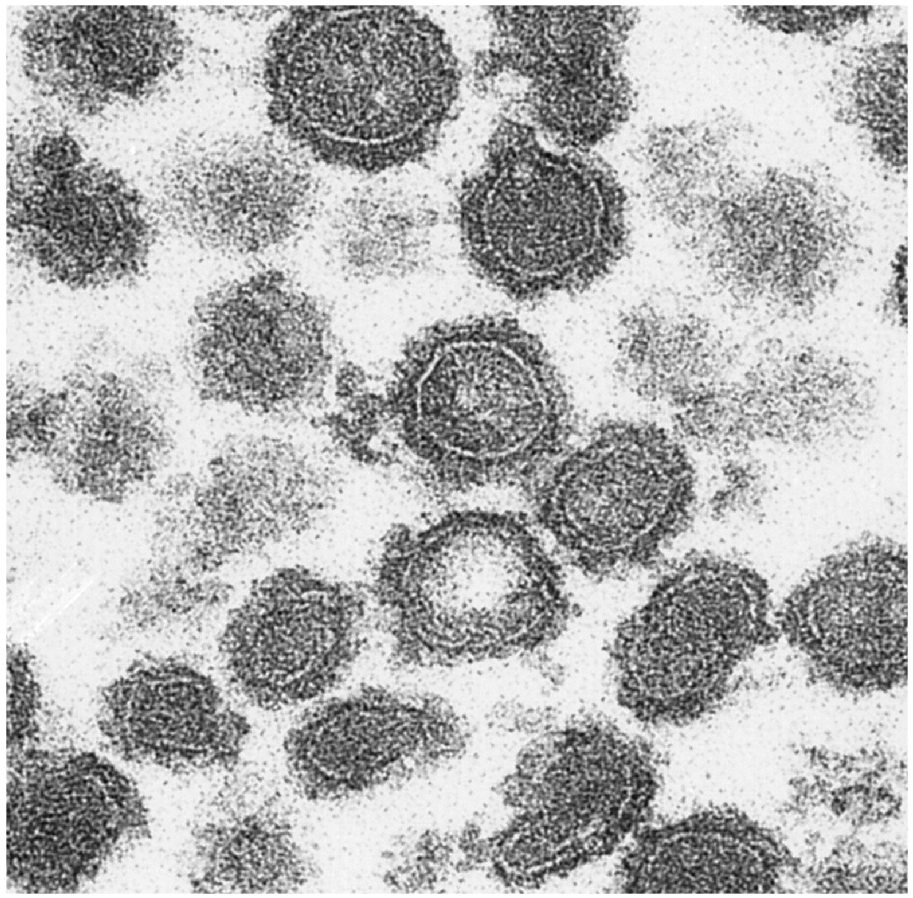
Action of Sar1 in the coating/uncoating of COPII vesicle

Fig 14-8

Sar1: GTP-binding protein; GTP/GDP switch
Sec12: guanine-exchange factor (GEF)



GTP hydrolysis is crucial during the disassembly process



Addition of non-hydrolyzable analog of GTP will prevent the coat disassembly

→Accumulation of coated vesicles

→Fails to uncoat/disassemble

Incubation of

1. Isolated Golgi
2. COPI protein extracts
3. Energy
4. **Non-hydrolyzable analog of GTP**

Fig 14-9

How do uncoated vesicles interact with target membrane?

- **Rab protein** helps in the docking of uncoated vesicles on target membrane
  - Member of the GTPase family
  - Rab-GDP (inactive) → Rab-GTP (active)
    - GTP exchange is catalyzed by GEF (**g**uanine-**e**xchange **f**actor)
  - Rab-GTP will undergo a conformational change (Fig 14-10)
  - Docks Rab-GTP onto target membrane
  - Process requires the interaction with Rab effector on target membrane
    - Rab effector = receptor for Rab-GTP

Docking/Fusion of vesicle with target membrane

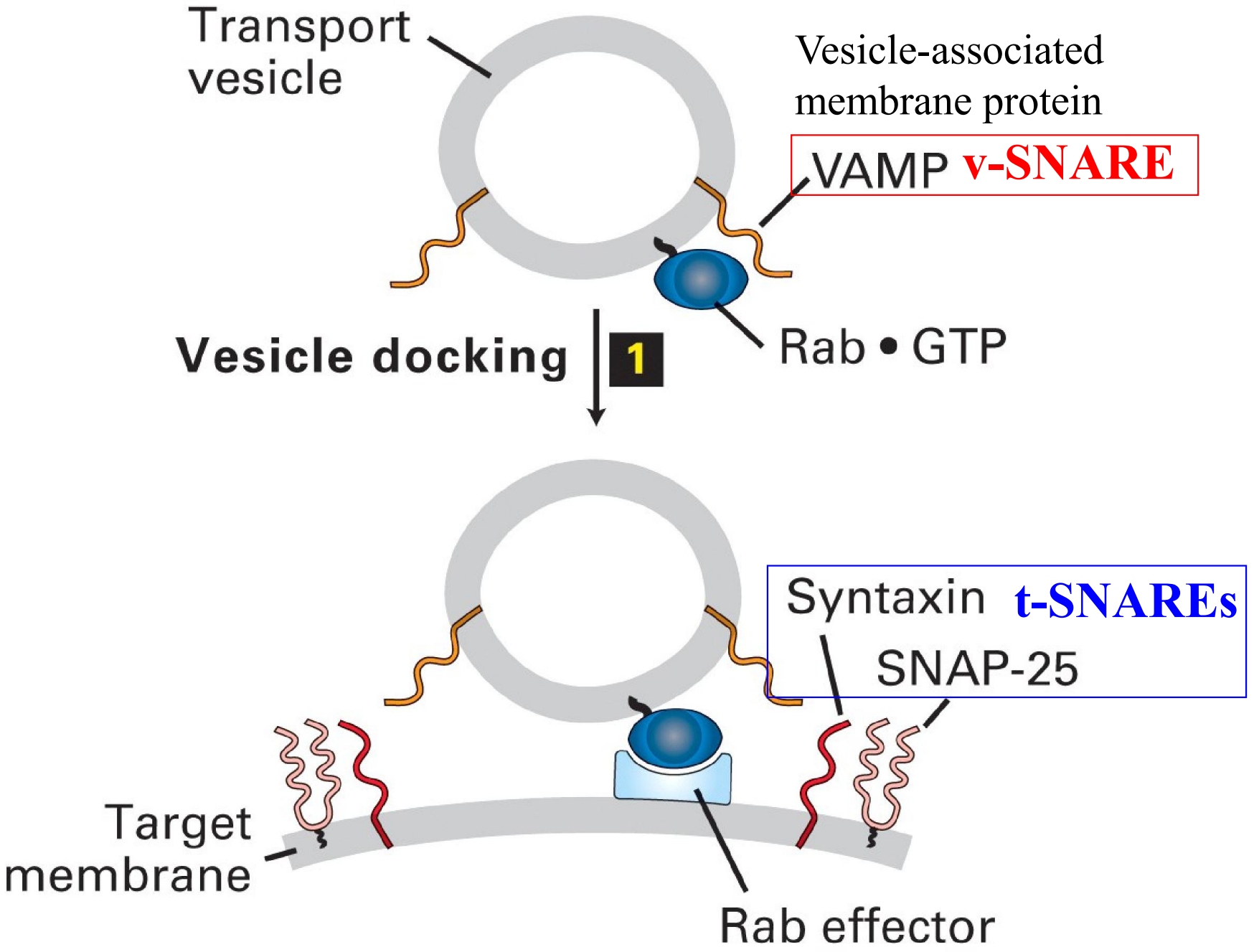
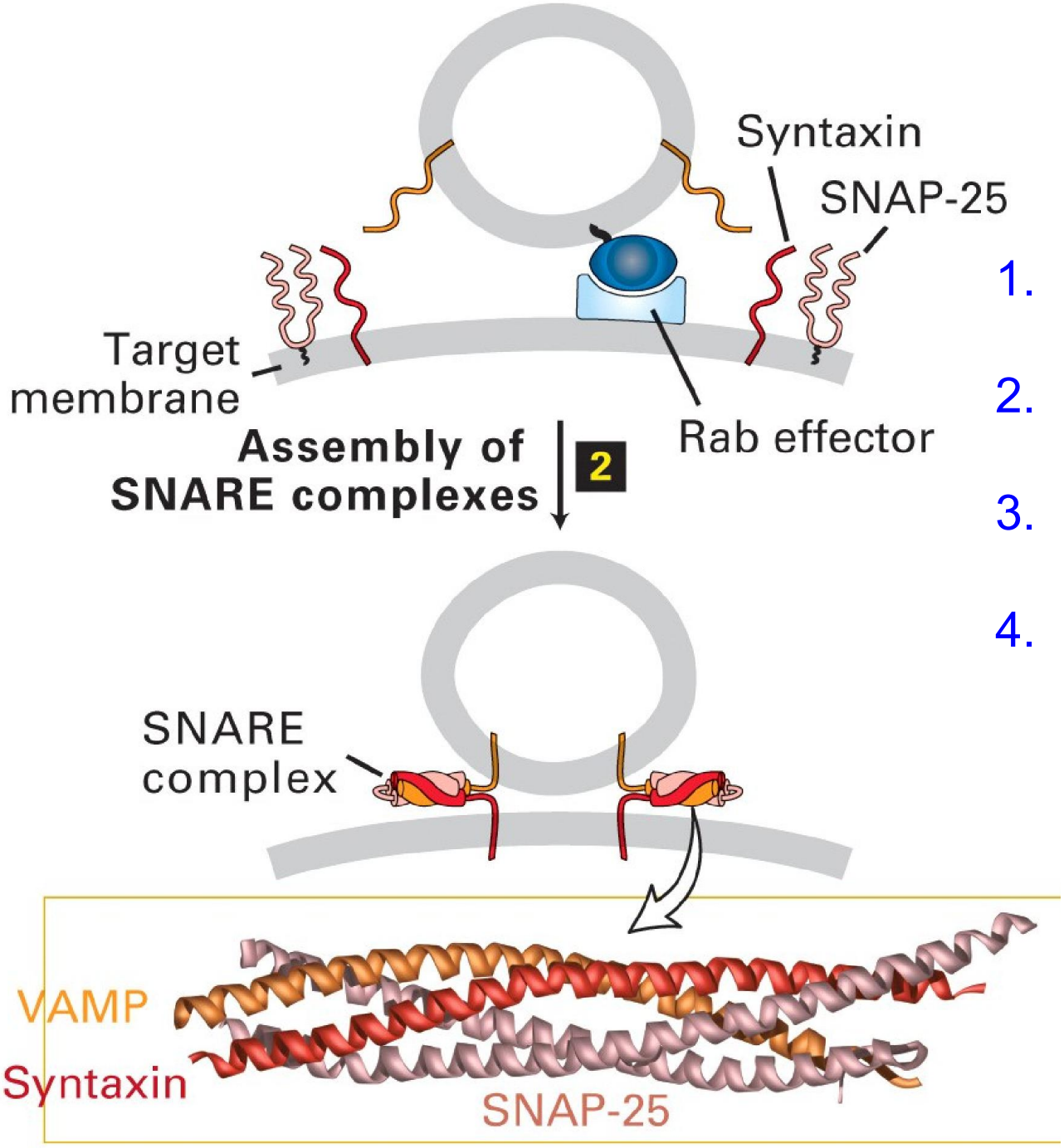


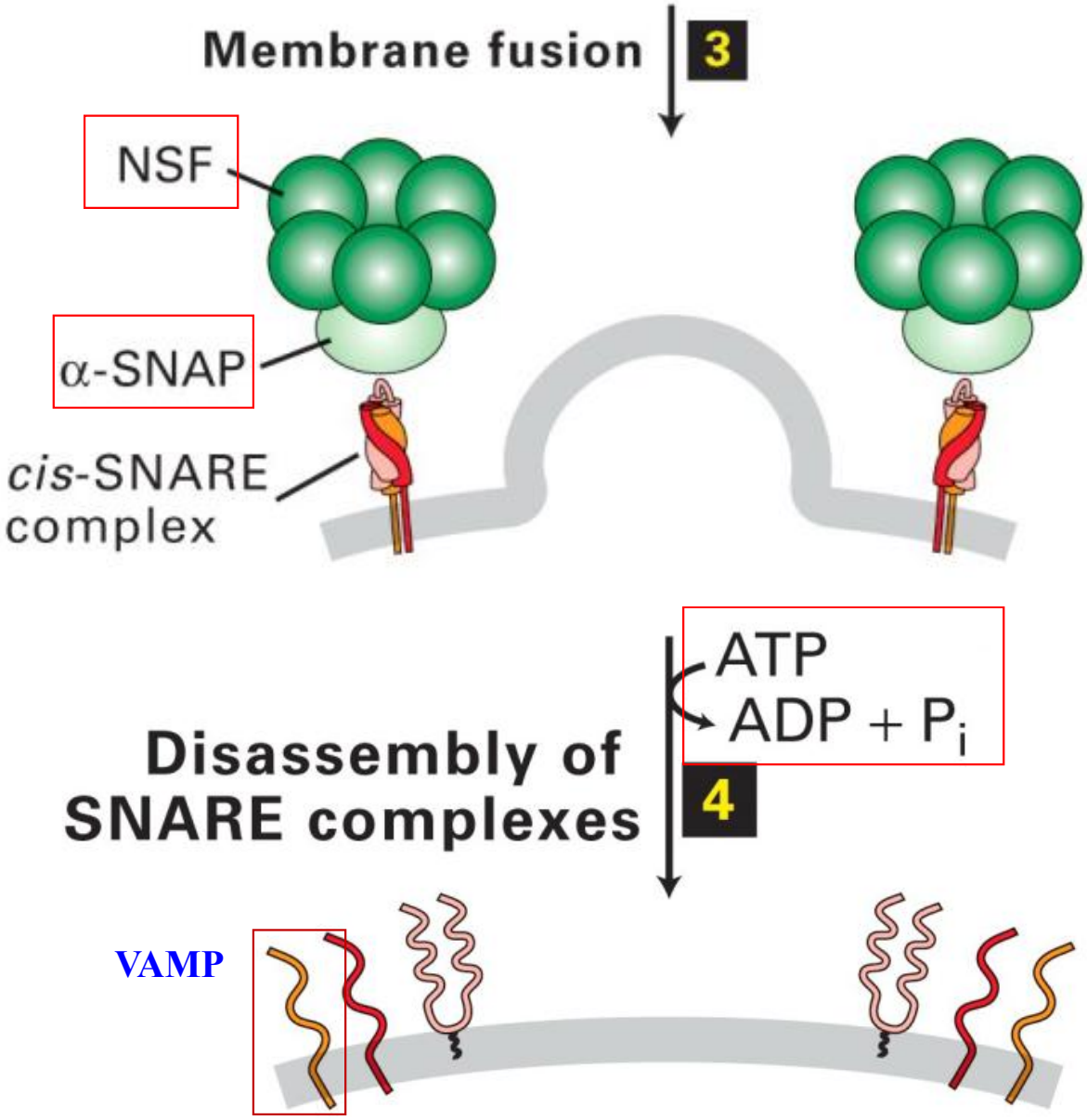
Fig 14-10

Fig 14-10



1. Interaction between v- and t-SNAREs
2. Formation of non-covalent coiled-coil
3. Vesicle and target are pulled close together
4. Fusion of vesicle with target membrane occurs (next slide)

Fig 14-10



4. Fusion of vesicle with target membrane occurs
5. Formation of cytosolic dimeric proteins
   - NSF & α-SNAP
6. Binding of NSF/α-SNAP w/ SNARE complex
7. ATP hydrolysis (by NSF)
8. Disassembly of SNARE complexes
9. Release of v-SNARE and t-SNARE

VAMP retrogradely transported back to ER via COPI pathway

The Nobel Prize in Physiology or Medicine 2013

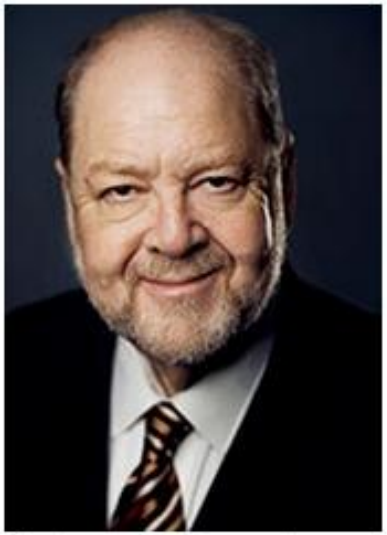


Photo: A. Mahmoud
James E. Rothman

Photo: A. Mahmoud
Randy W. Schekman

Photo: A. Mahmoud
Thomas C. Südhof

The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof *"for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"*.

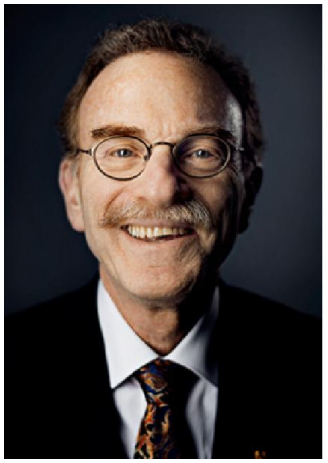
The Nobel Prize in Physiology or Medicine 2013

Summary

The 2013 Nobel Prize honours three scientists who have solved the mystery of how the cell organizes its transport system. Each cell is a factory that produces and exports molecules. For instance, insulin is manufactured and released into the blood and signaling molecules called neurotransmitters are sent from one nerve cell to another. These molecules are transported around the cell in small packages called vesicles. The three Nobel Laureates have discovered the molecular principles that govern how this cargo is delivered to the right place at the right time in the cell.

Randy Schekman discovered a set of genes that were required for vesicle traffic. James Rothman unravelled protein machinery that allows vesicles to fuse with their targets to permit transfer of cargo. Thomas Südhof revealed how signals instruct vesicles to release their cargo with precision.

Through their discoveries, Rothman, Schekman and Südhof have revealed the exquisitely precise control system for the transport and delivery of cellular cargo. Disturbances in this system have deleterious effects and contribute to conditions such as neurological diseases, diabetes, and immunological



Randy W. Schekman

Born: 30 December 1948, St. Paul, MN, USA

Affiliation at the time of the award: University of California, Berkeley, CA, USA, Howard Hughes Medical Institute

Prize motivation: "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"

Field: cell physiology, genetics

James E. Rothman

Born: 3 November 1950, Haverhill, MA, USA

Affiliation at the time of the award: Yale University, New Haven, CT, USA

Prize motivation: "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"

Field: biochemistry, cell physiology

Schekman's major achievements:

1. **Isolation of yeast *sec* mutants (Ch 14)**
2. **Isolation of the Sec61 translocation complex (Ch 13)**
3. **Isolation of the COPII coat complex (Ch 14)**

14.3
Early Stages of the Secretory Pathway

Early stages of the secretory pathways

- Anterograde transport ("move forward")
  - from ER to *cis*-Golgi
  - COPII vesicles
- Retrograde transport ("go backward")
  - from *cis*-Golgi to ER
  - in between Golgi (*cis*-, *medial*-, *trans*-)
  - COPI vesicles

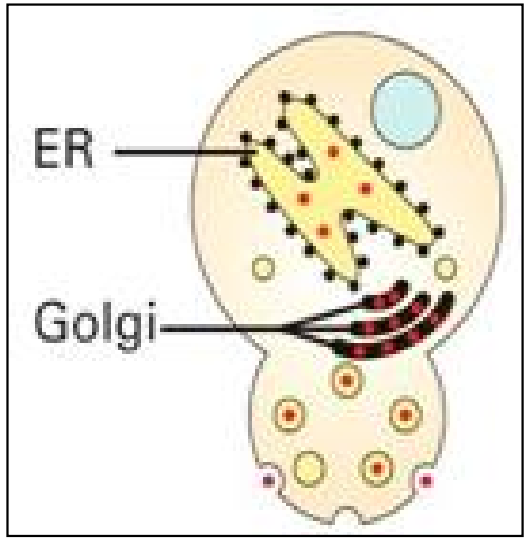
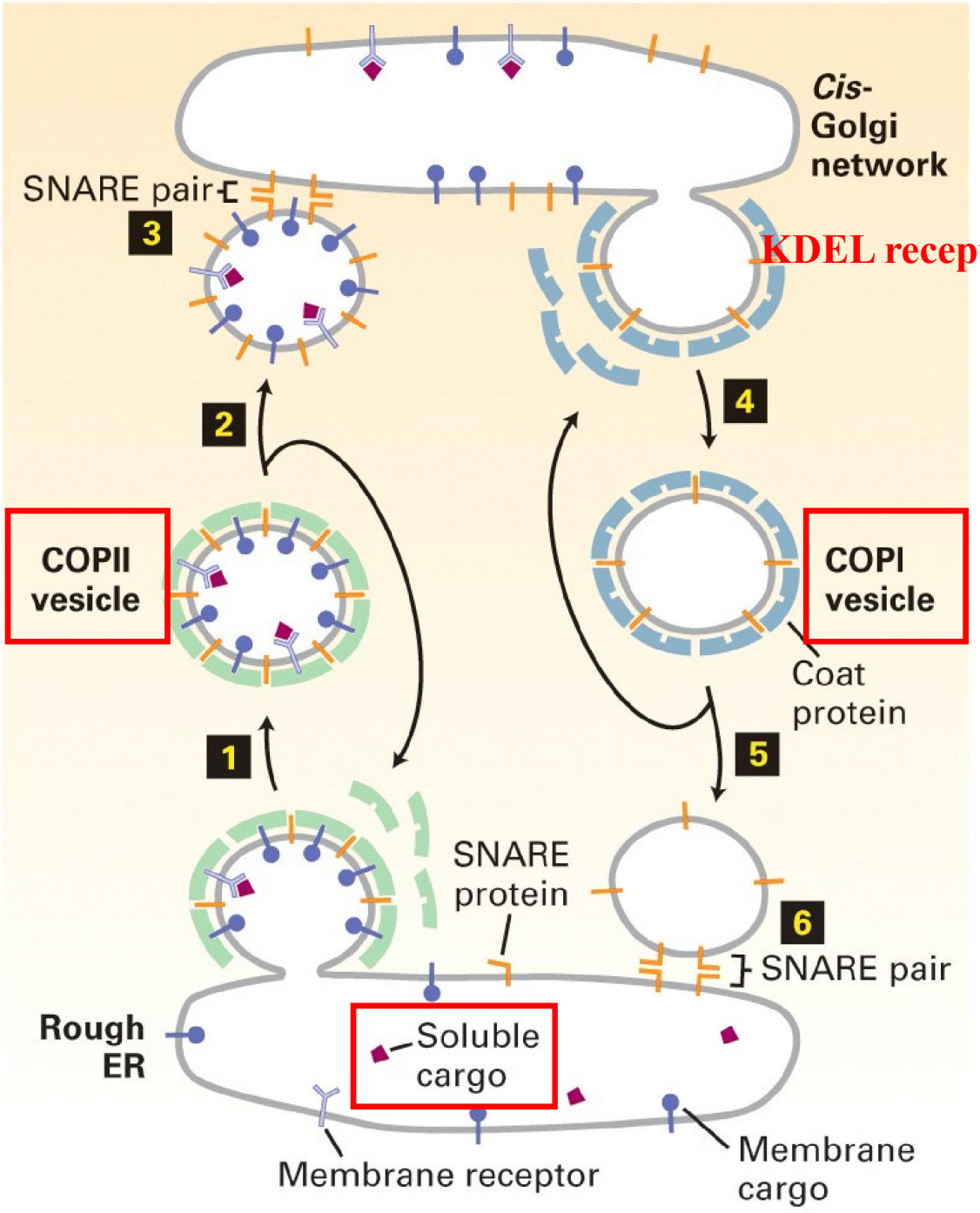


Fig 14-11

Vesicle-mediated protein trafficking between the ER and *cis*-Golgi



Lys-Asp-Glu-Leu (KDEL) — Signal sequence on cargo

ER-resident soluble proteins (e.g. PDI, PPI, BiP…etc.) — Cargo protein

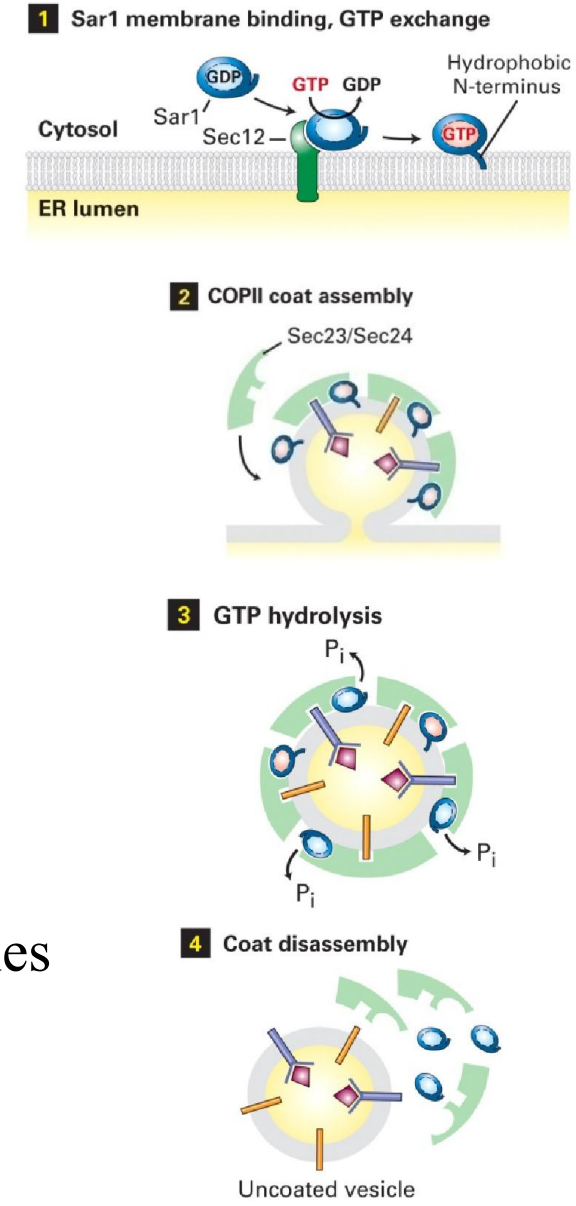
KDEL receptor in *cis*-Golgi membrane — Signal receptor

COPI — Vesicle involved

目的:
將COPII anterograde transport過程中誤被一起送到*cis*-Golgi的ER luminal protein再送回到ER

Formation of COPII vesicle
(ER to *cis*-Golgi)

- On rough ER membrane (Fig 14-8)
- Initiated by Sec12 (an ER-bound GEF)
  - Bound by Sar1-GDP (soluble)
  - Catalyzes GDP → GTP exchange on
  - Sar1-GTP → conformational change
  - Anchors onto membrane
- Additions of
  - Sec23/Sec24, Sec13/Sec31
  - Sec16 (Fibrous ER membrane protein)
    - Interacts with both Sec23/24 and Sec13/31 complexes
  - Other ER integral proteins (cargo proteins)
    - With di-acidic sorting signal **Asp-X-Glu (DXE)**
    - Interacts with the Sec24 protein (Fig 14-12)



Structure of COPII coat proteins (Sec23/Sec24 + Sar1-GTP)

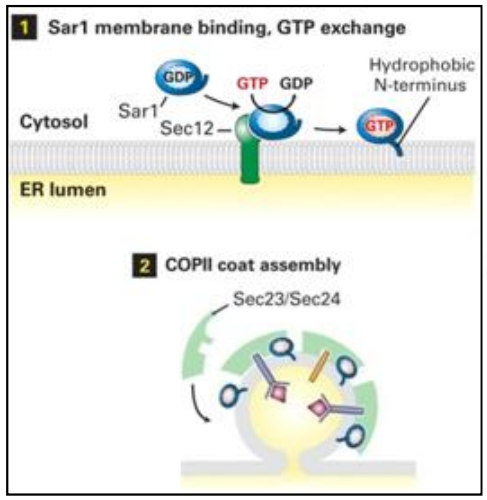


Fig 14-12

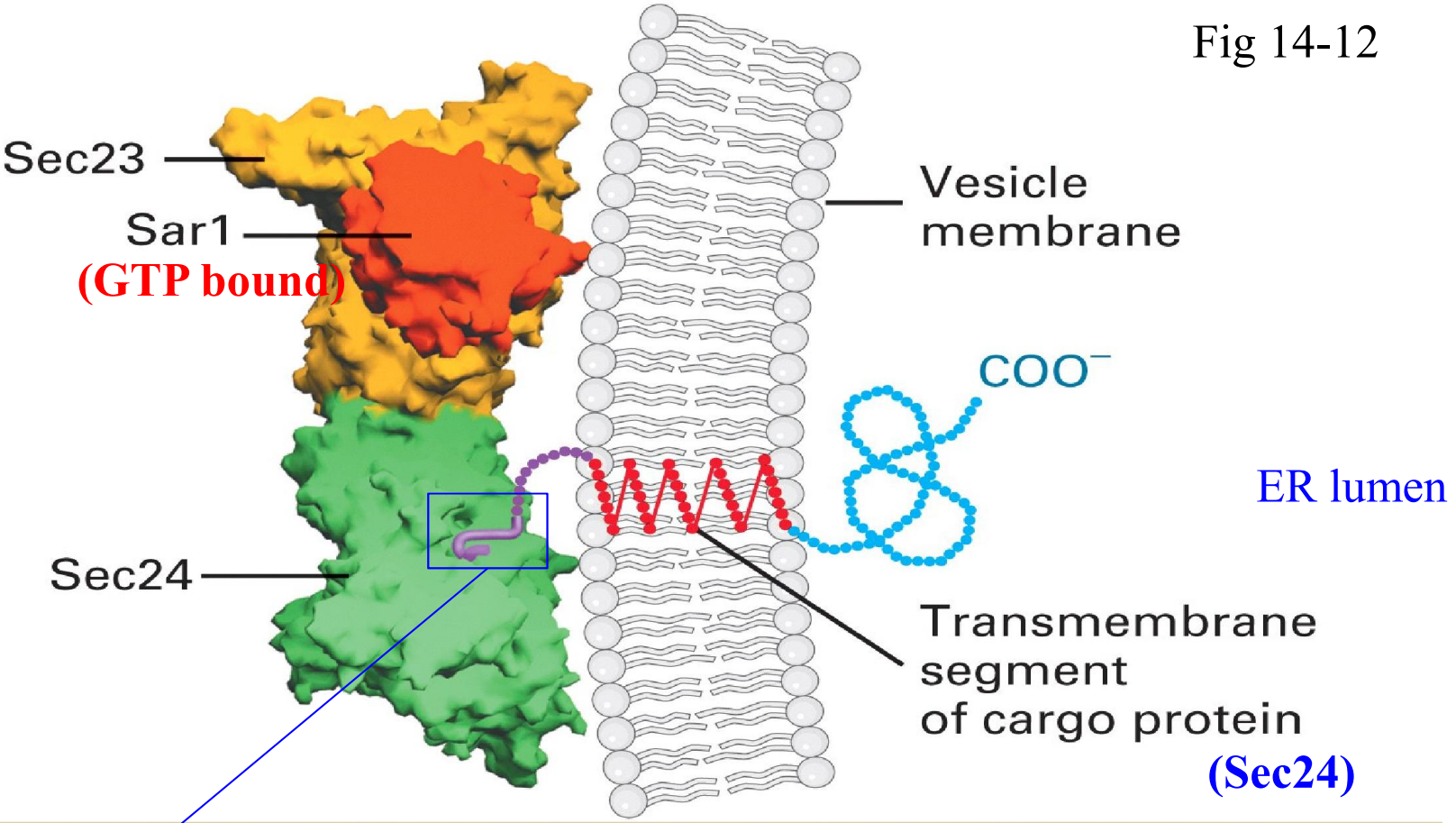


TABLE 17-2 Known Sorting Signals That Direct Proteins to Specific Transport Vesicles

Signal Sequence*	Proteins with Signal	Signal Receptor	Vesicles That Incorporate Signal-bearing Protein
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER (cytosolic domain)	COPII / subunit (Sec24)	COPII

Retrograde transport by COPI vesicles
(back to ER lumen or membrane)

- Transport of ER-resident proteins back to ER
  - e.g. Chaperone BiP, protein disulfide isomerase (PDI)
- Sorting signals
  - for ER ***luminal*** proteins
    - Lys-Asp-Glu-Leu (**KDEL**); located at C-terminus of the protein
    - e.g. BiP, PDI
  - For ER ***membrane*** proteins
    - Lys-Lys-X-X (**KKXX**); located on C-terminus of the protein
    - e.g. translocon Sec61, signal peptidase

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

Loading/Unloading of COPI cargo is pH-dependent

- KDEL receptor
  - Transmembrane protein
  - Present in
    - vesicles shuttling in between ER and cis-Golgi
      - COPI & COPII
    - cis-Golgi reticulum
  - Recognizes and binds to KDEL sorting signal
  - To transport misfolded ER luminal proteins back to ER
  - Binding with its cargo protein is pH-dependent

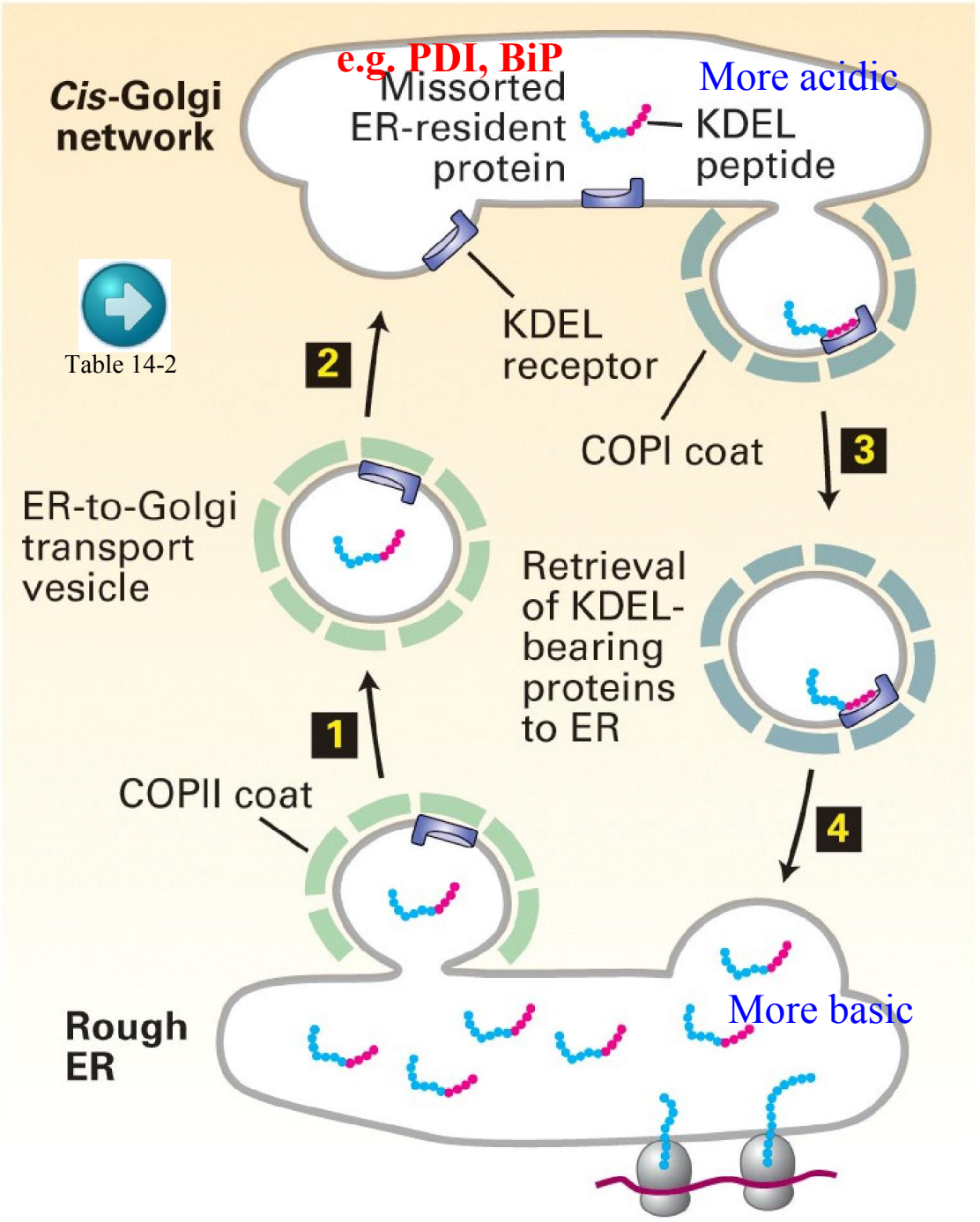
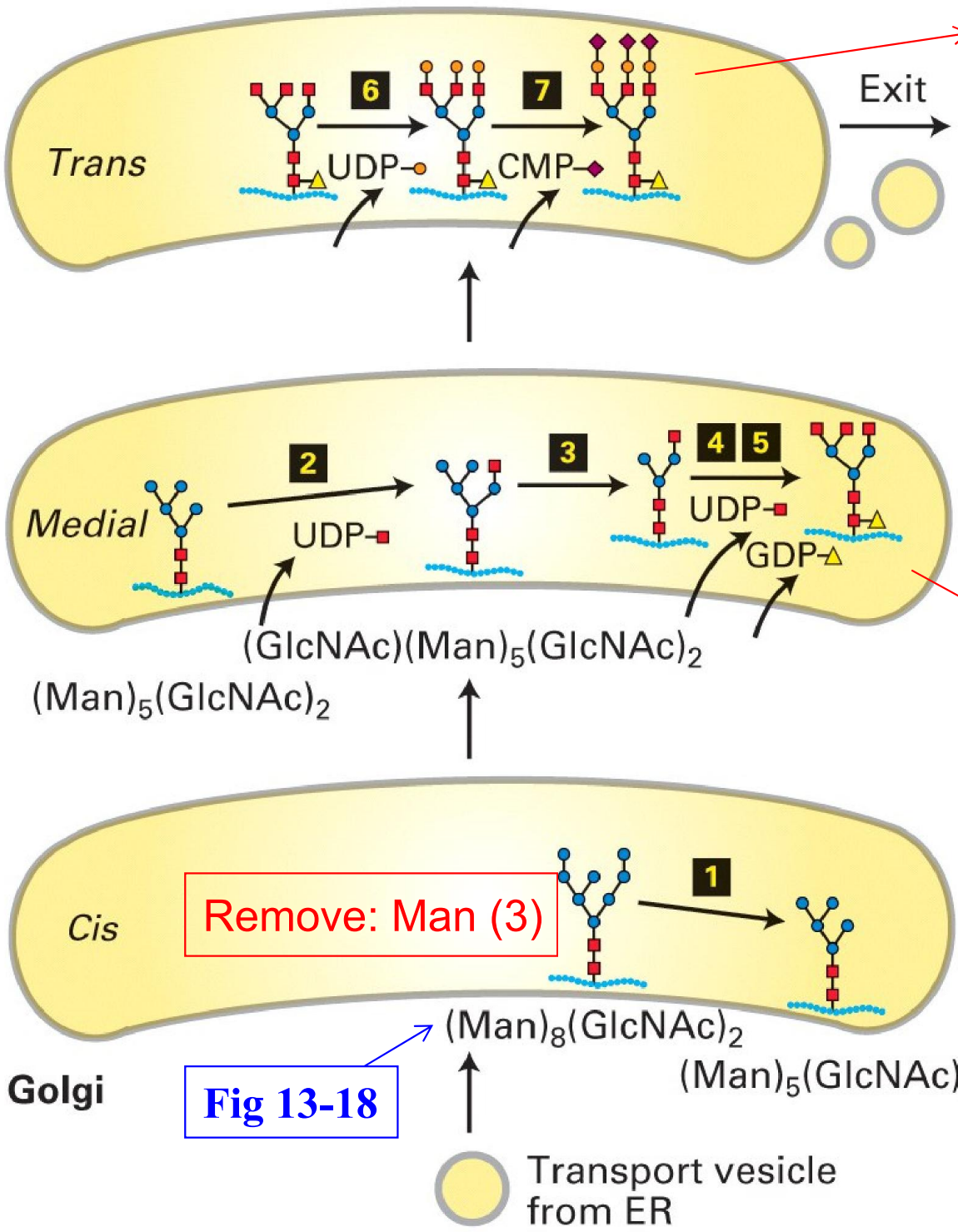


Table 14-2

Fig 14-13

Processing of N-linked oligosaccharides in Golgi



Add: Gal. (3), NeuNAc (3)

Fig 14-14

■ = *N*-Acetylglucosamine
● = Mannose
▲ = Fucose
○ = Galactose
◆ = *N*-Acetylneuraminic acid

Add: GlcNAc (3), Fucose (1)
Remove: Man (2)

Can use **Golgi-specific glycosidase** to distinguish locations of glycosylated proteins along the pathway
→ e.g. *cis*-Golgi specific endoglycosidase D

Vesicles transport through the Golgi (via *cisternal maturation/progression*)

Protein secretion

- Detailed mechanism remains unknown
- Current model:
  - (forward) non-vesicular mechanism
  - (backward) anterograde transport by COPI
- Various oligosaccacharides are added at different Golgi compartments

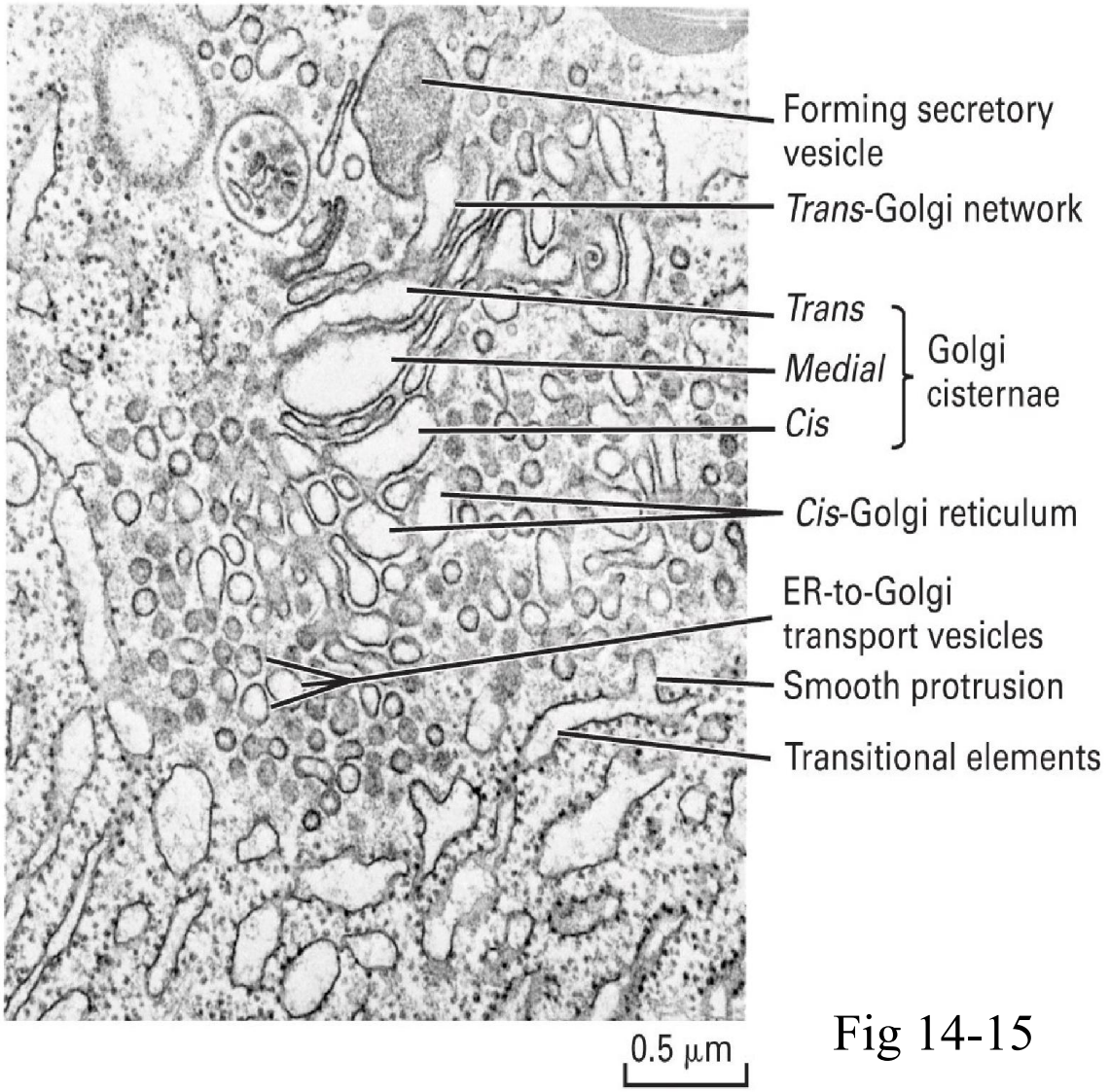


Fig 14-15

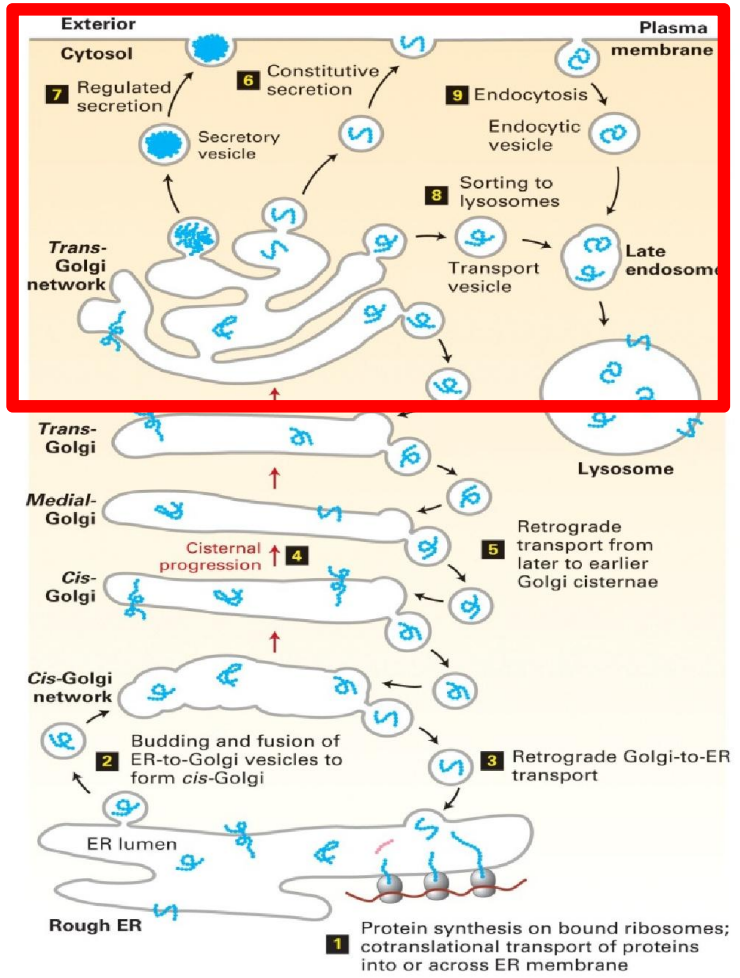
Cisternal maturation

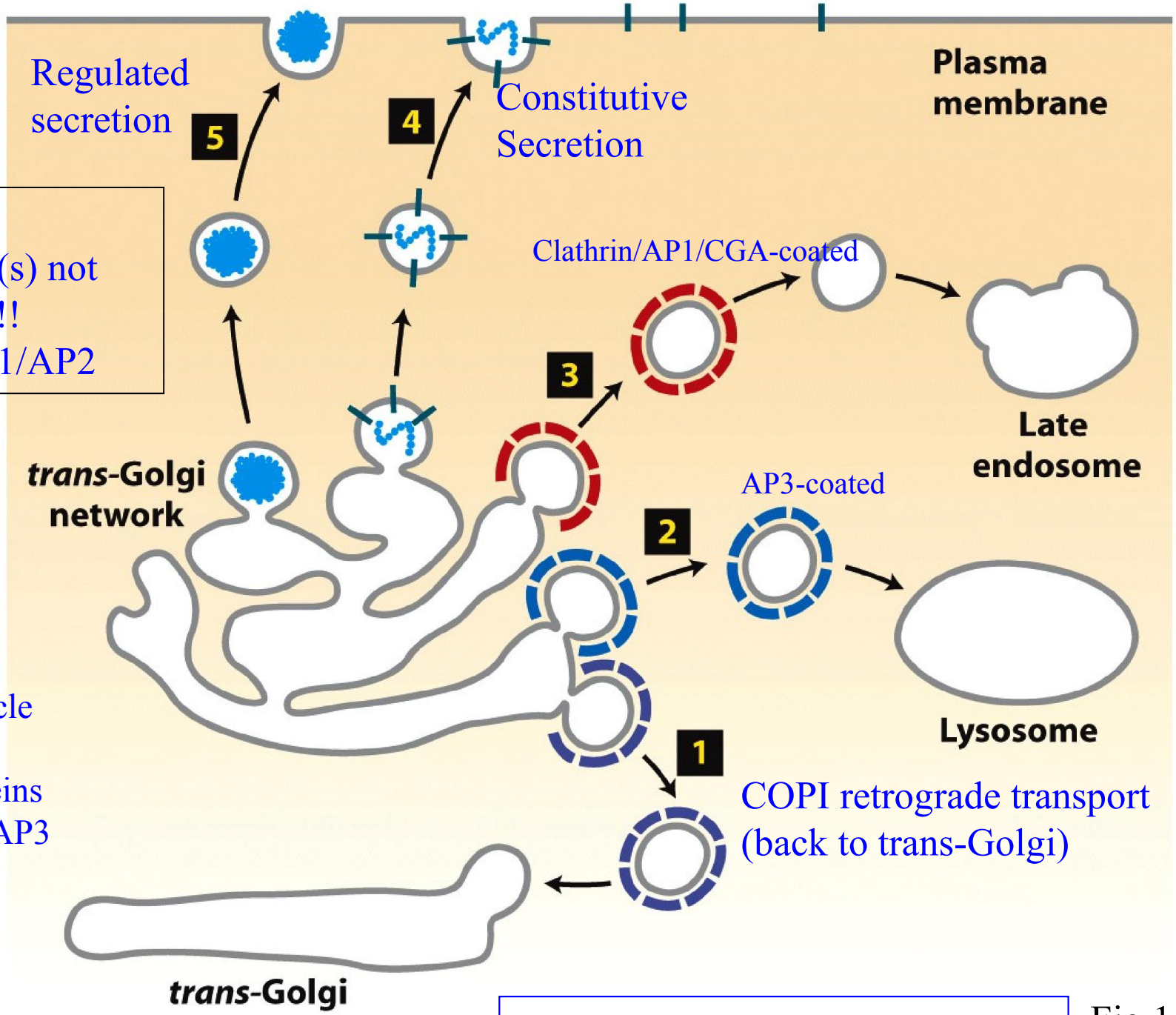
14.4
Later Stages of the Secretory Pathway

Later stages of the secretory pathways

Table 14-2

- Involves various vesicles budding from the *trans*-Golgi network (TGN)





(4) & (5),
- coat protein(s) not yet identified!!
- possible AP1/AP2

a complete vesicle
- Clathrin
- Adaptors proteins
  1. AP1, AP2, AP3
  2. CGA

Table 14-2

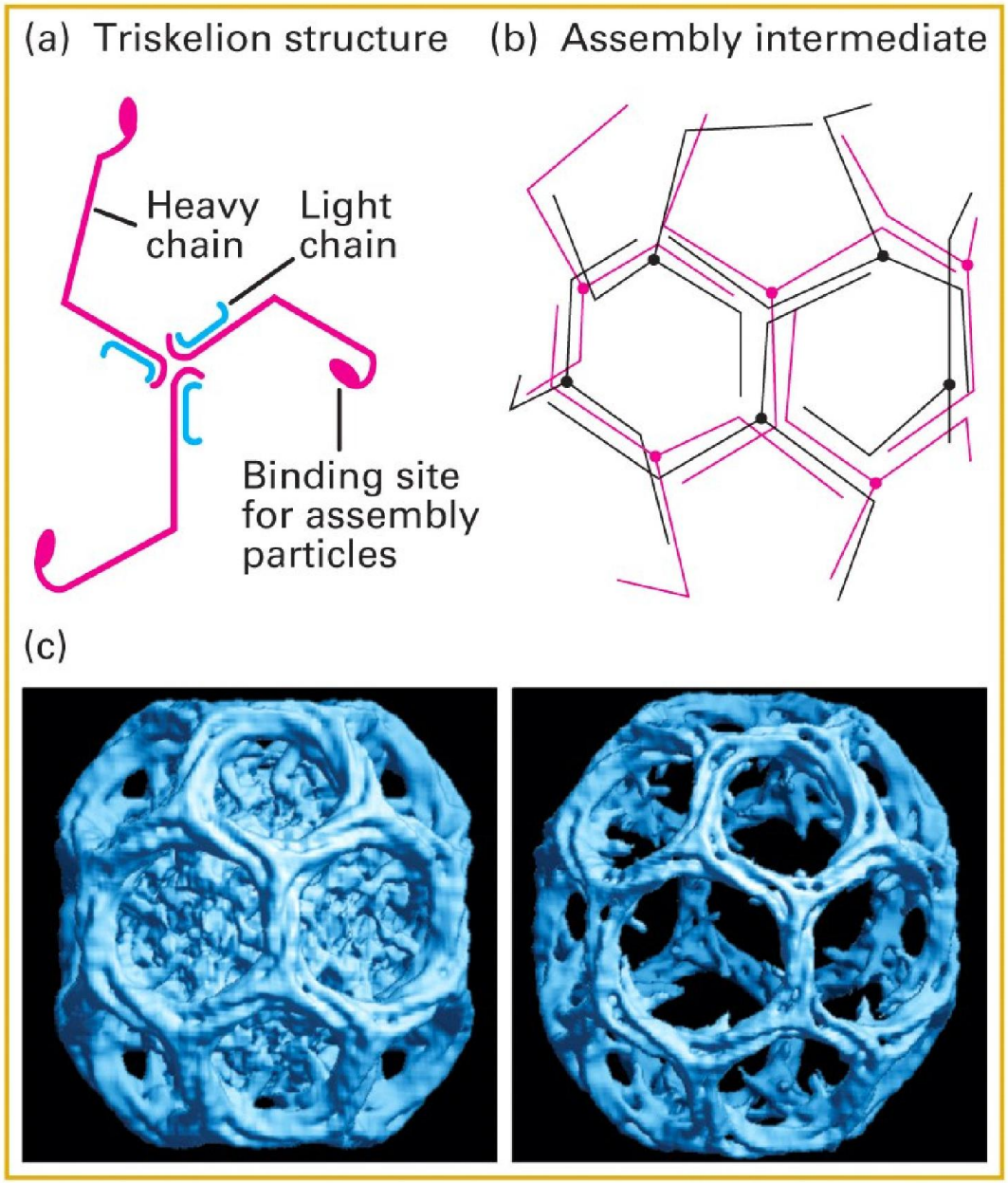
Figure 14-17
Molecular Cell Biology, Sixth Edition

Vesicle-mediated trafficking from *trans*-Golgi network

Fig 14-17

Clathrin-mediated vesicle budding from the *trans*-Golgi network

Lodish 5th Fig 17-19



Addition of adaptor proteins (APs) can assist stabilize the vesicular coat

Different AP complexes (e.g. AP1, AP2, AP3) mediate transport of different proteins

Formation of clathrin coat

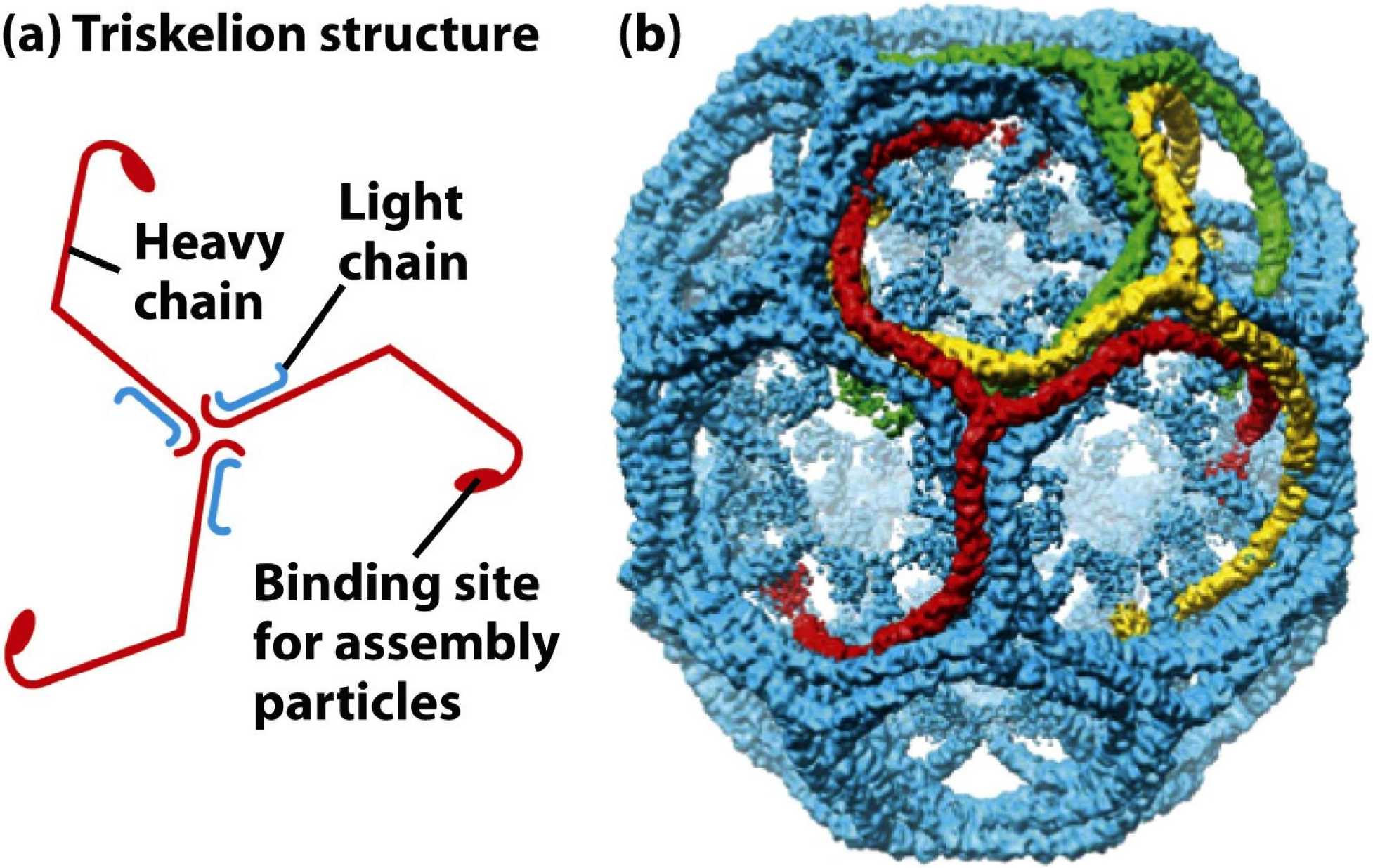
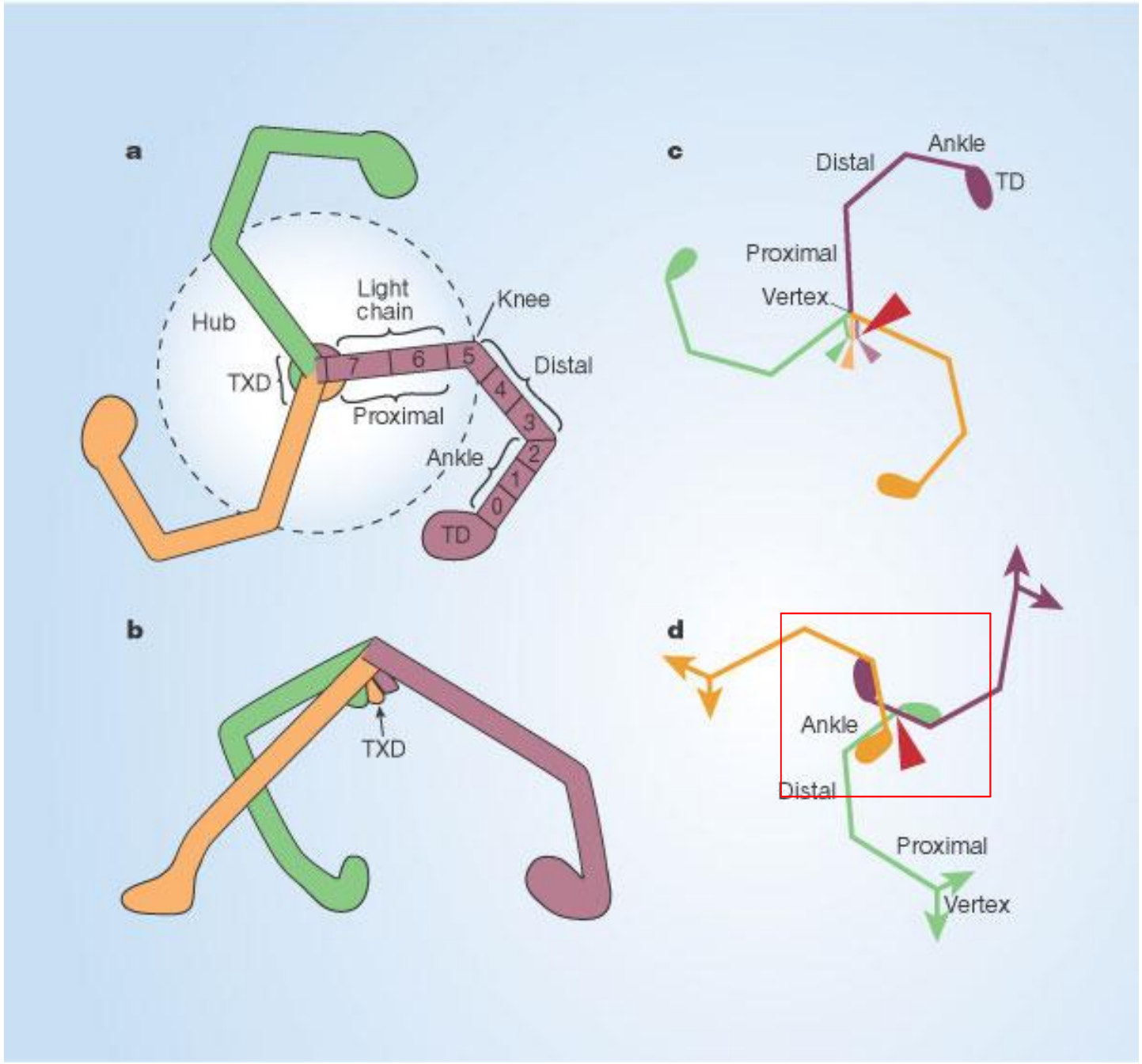


Figure 14-18
Molecular Cell Biology, Sixth Edition

Each clathrin coat is composed of 35 triskelions

Fig 14-18



The birth of clathrin

Cell biology: Clathrin's Achilles' ankle
Nature 432, 568-569 (2004)

How does clathrin vesicle pinch off host membrane?

1. Requires polymerization of dynamin monomers
2. Polymerized dynamin
   → Drives GTP hydrolysis
3. Contraction of dynamin
4. Pinching off of vesicles

(Note) Pinching off process in COPI and COPII vesicles DO NOT require dynamin!!!

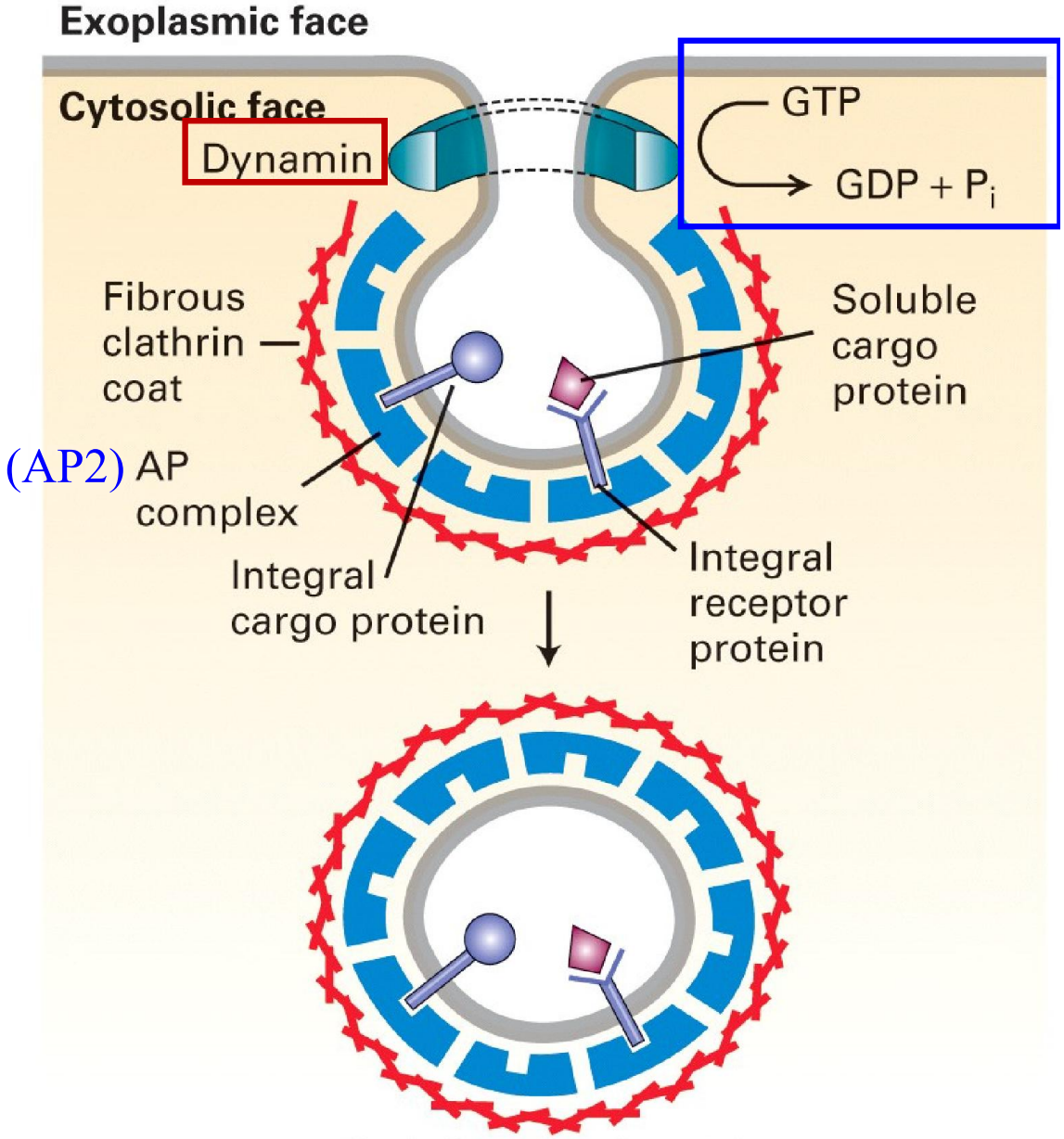


Table 14-2

Fig 14-19

GTP hydrolysis is required for the dynamin-mediated pinching-off

1. Actively endocytose nerve cells
2. Lysed and incubated with non-hydrolyzable GTP
3. Treated with gold-tagged anti-dynamin Ab
4. [Result] formation of clathrin/AP complexes, but **no** pinching-off from donor membrane

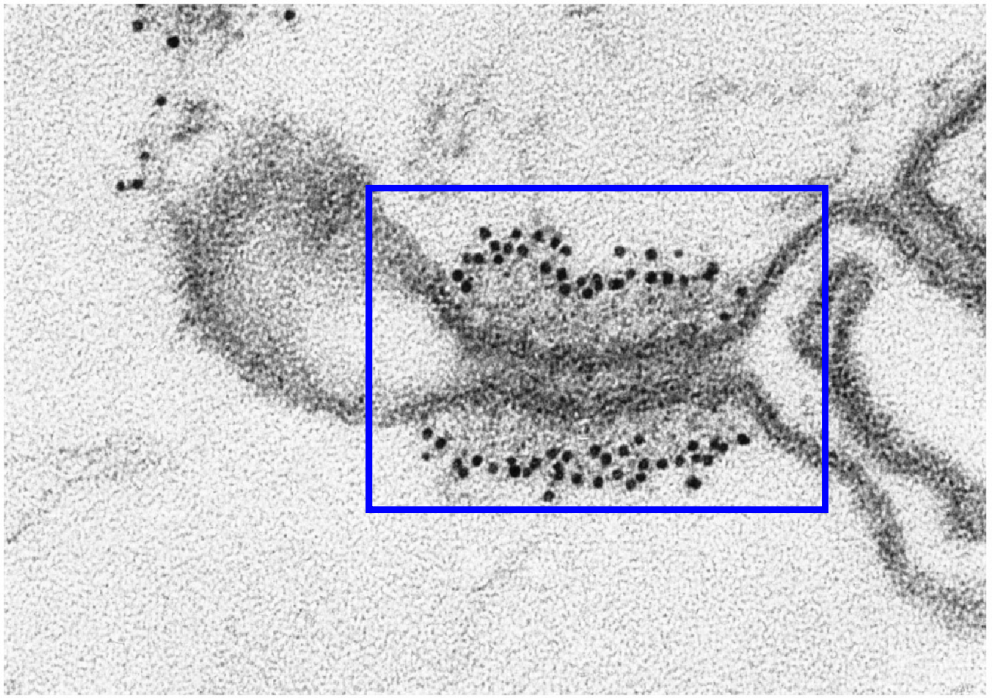
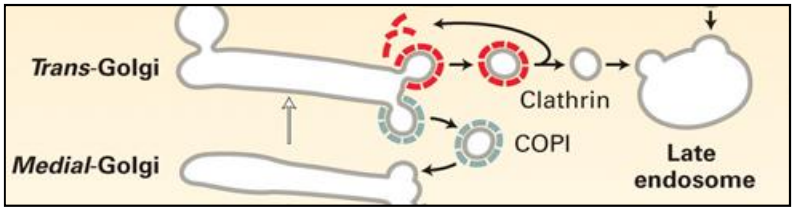


Fig 14-20

Vesicles can not pinch off due to the lack of GTPs

A special sorting signal for targeting proteins to lysosomes

Protein-M6P



(1) For soluble (cytosolic) lysosomal proteins

- From *trans*-Golgi to late endosome, then to lysosome
- Requires formation of mannose 6-phosphate (M6P) on cargo protein (occurs in the *cis*-Golgi)
  - The same first core Man8(GlcNAc)2 is formed as others in the rough ER (Fig 14-14)
  - The 2nd step involves 2 enzymes (Fig 14-21)
    - GlcNAc phosphotransferase (addition of GlcNAc-P onto the core)
    - Phosphodiesterase (removal of GlcNAc)

(2) For membrane lysosomal proteins

- Requires the Tyr-X-X-ϕ signal sequence

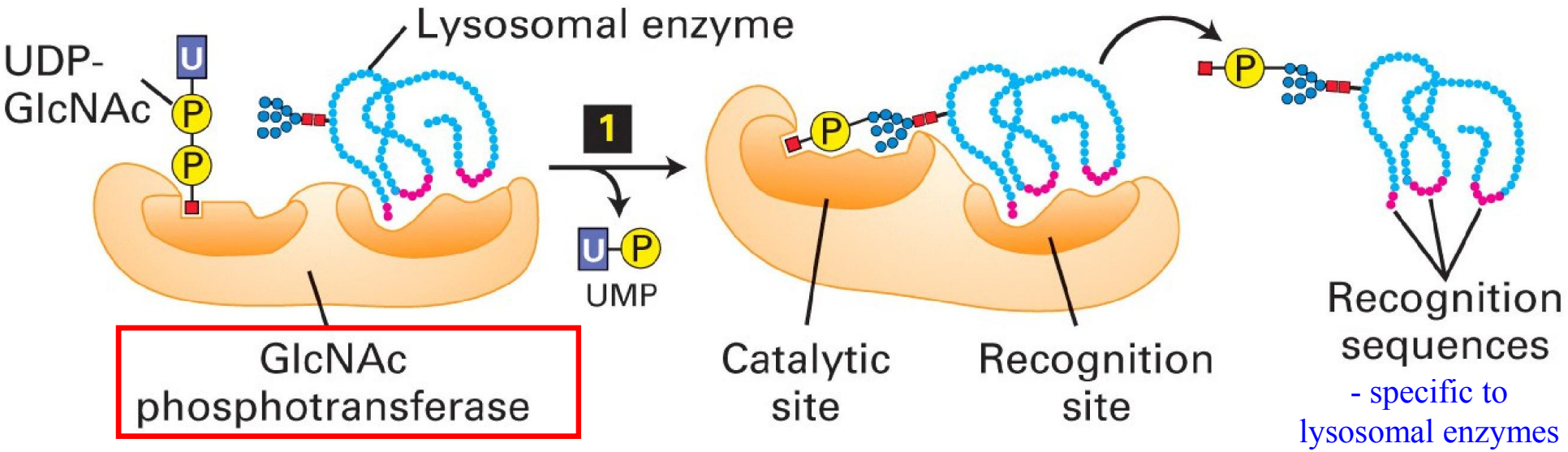
- Both via the clathrin/AP1 vesicle route

Table 14-2

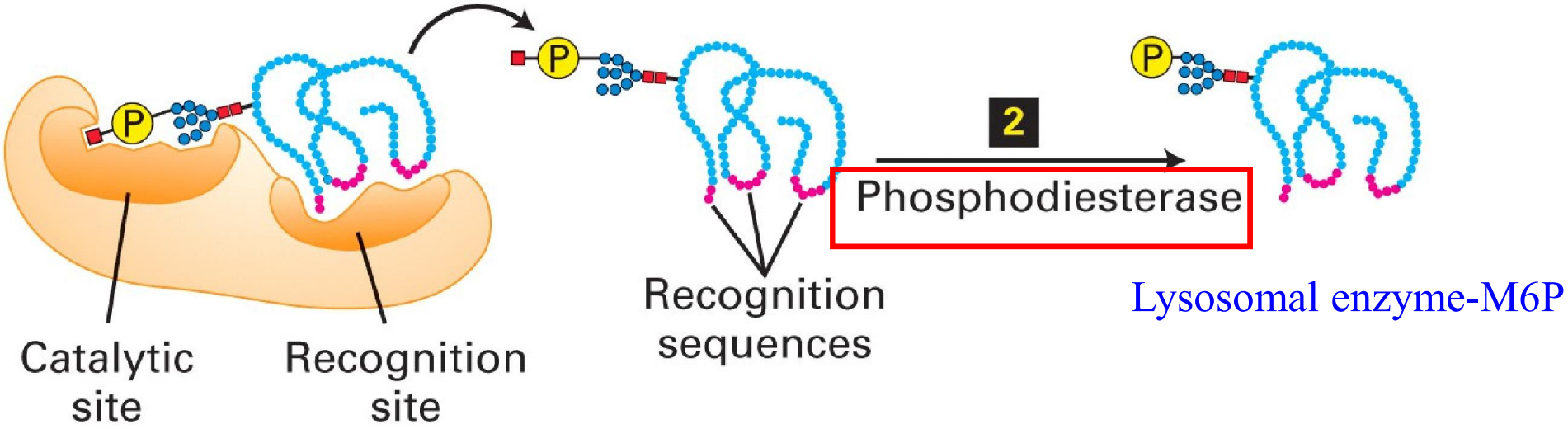
Formation of mannose 6-phosphate

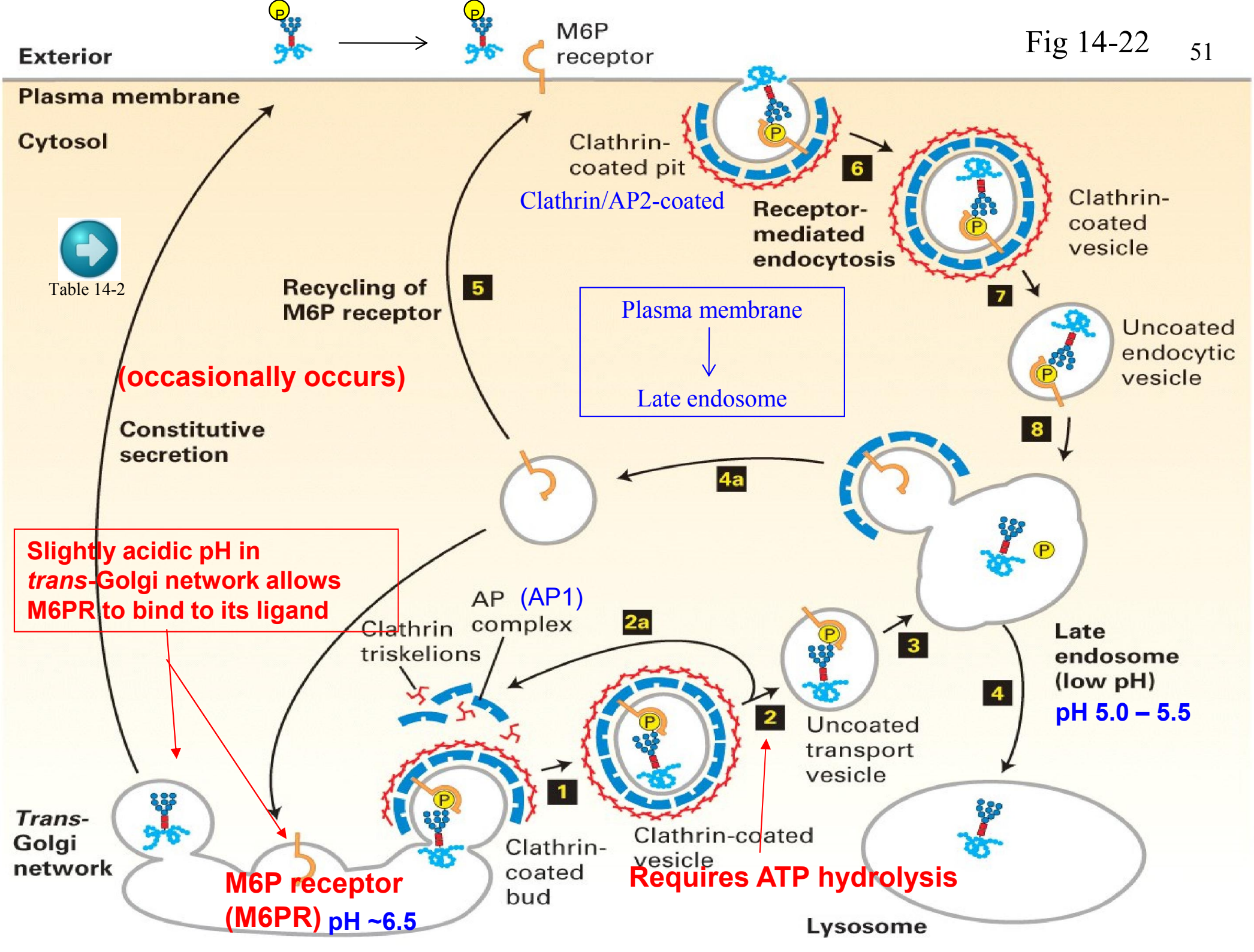
(1) Addition of GlcNAc-P

Fig 14-21



(2) Removal of GlcNAc




Exterior
M6P receptor
Plasma membrane
Cytosol
Clathrin-coated pit
Clathrin/AP2-coated
6
Receptor-mediated endocytosis
Clathrin-coated vesicle
7
Uncoated endocytic vesicle
8
Table 14-2
Recycling of M6P receptor
5
Plasma membrane
Late endosome
(occasionally occurs)
Constitutive secretion
4a
Slightly acidic pH in trans-Golgi network allows M6PR to bind to its ligand
AP (AP1) complex
Clathrin triskelions
2a
3
Late endosome (low pH)
pH 5.0 – 5.5
4
2
Uncoated transport vesicle
1
Clathrin-coated bud
Clathrin-coated vesicle
Requires ATP hydrolysis
Trans-Golgi network
M6P receptor (M6PR) pH ~6.5
Lysosome

Regulated and unregulated secretory pathways

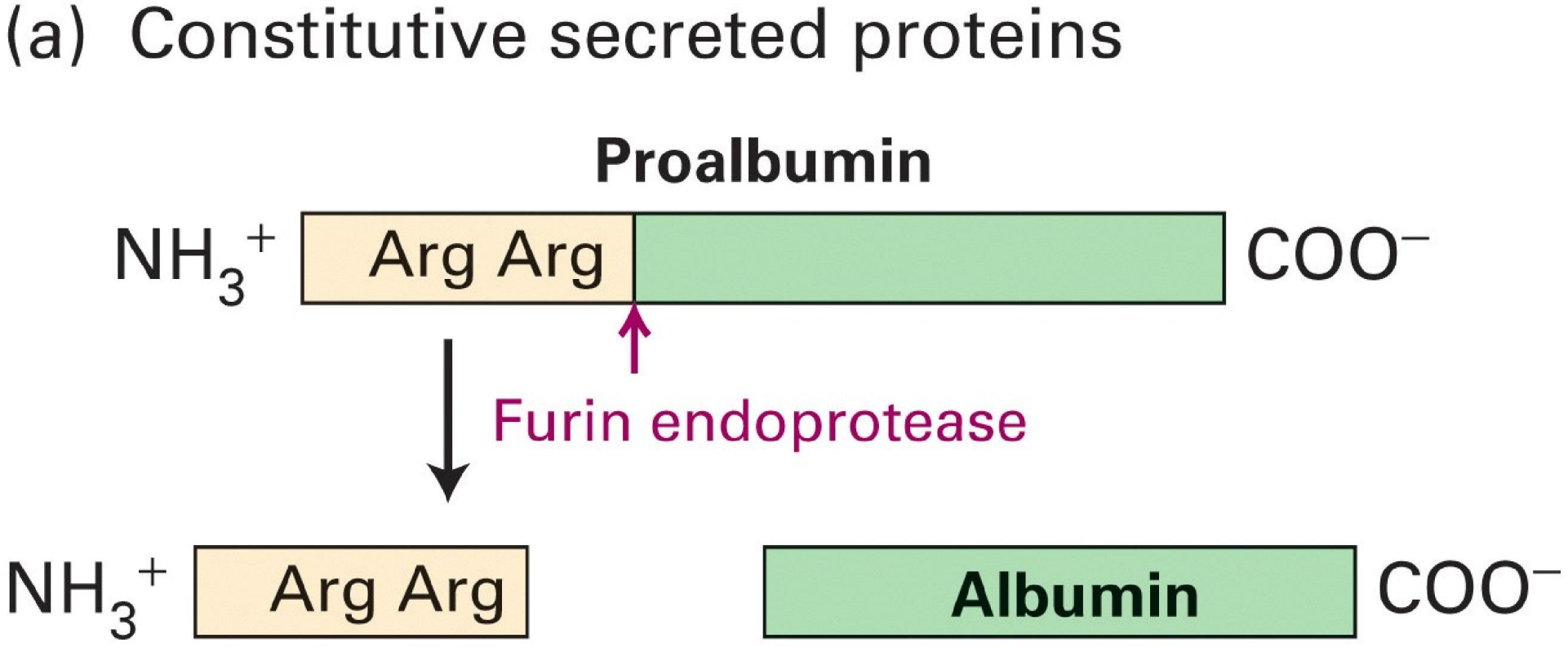
- Regulatory secretory pathway
  - Exocytosis
  - e.g. insulin secreted by pancreatic β cells
- Unregulated secretory pathway
  - Constitutive secretory pathway

- In general, both pathways often requires '<u>protein aggregation</u>' in the *trans*-Golgi network before forming the transport vesicle

Some proteins undergo proteolytic cleavage after leaving the *trans*-Golgi

- Required by some proteins for the conversion into mature protein
- Occurs after they leave *trans*-Golgi
- Lysosomal proproteins
  - Also called 'proenzymes' (inactive)
  - Sorted to lysosome via the same M6P receptor-mediated pathway (Fig 14-22)
  - Undergo proteolytic cleavage in late endosome/lysosome to generate active enzymes
- Other examples
  - Membrane protein: hemagglutinin (HA)
  - Secreted proteins: insulin, glucagon, albumin,…etc.

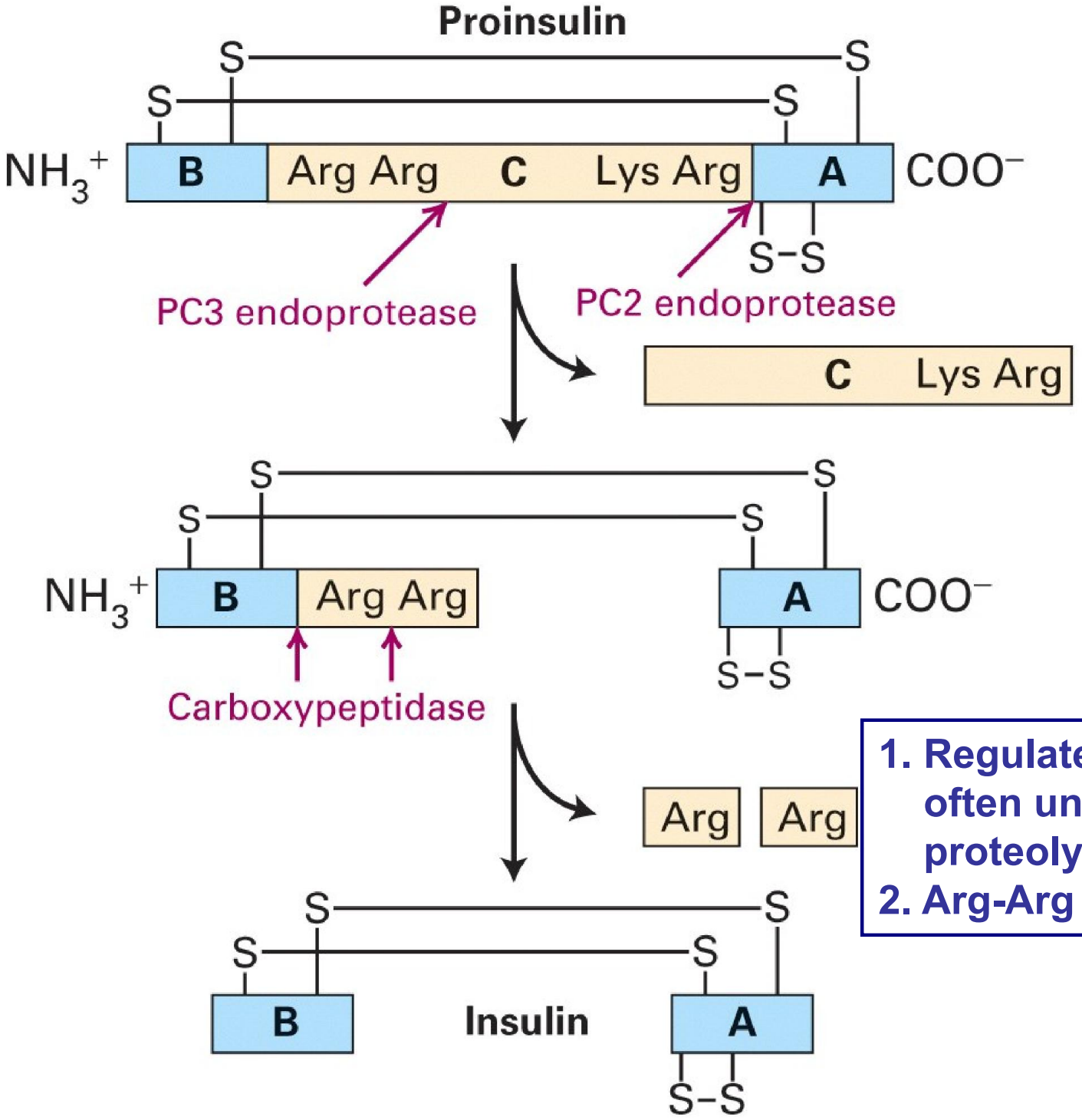
Proteolytic processing of proprotein



Arg-Arg or Lys-Arg are two common proenzyme recognition sites on proproteins

Fig 14-24a

(b) Regulated secreted proteins

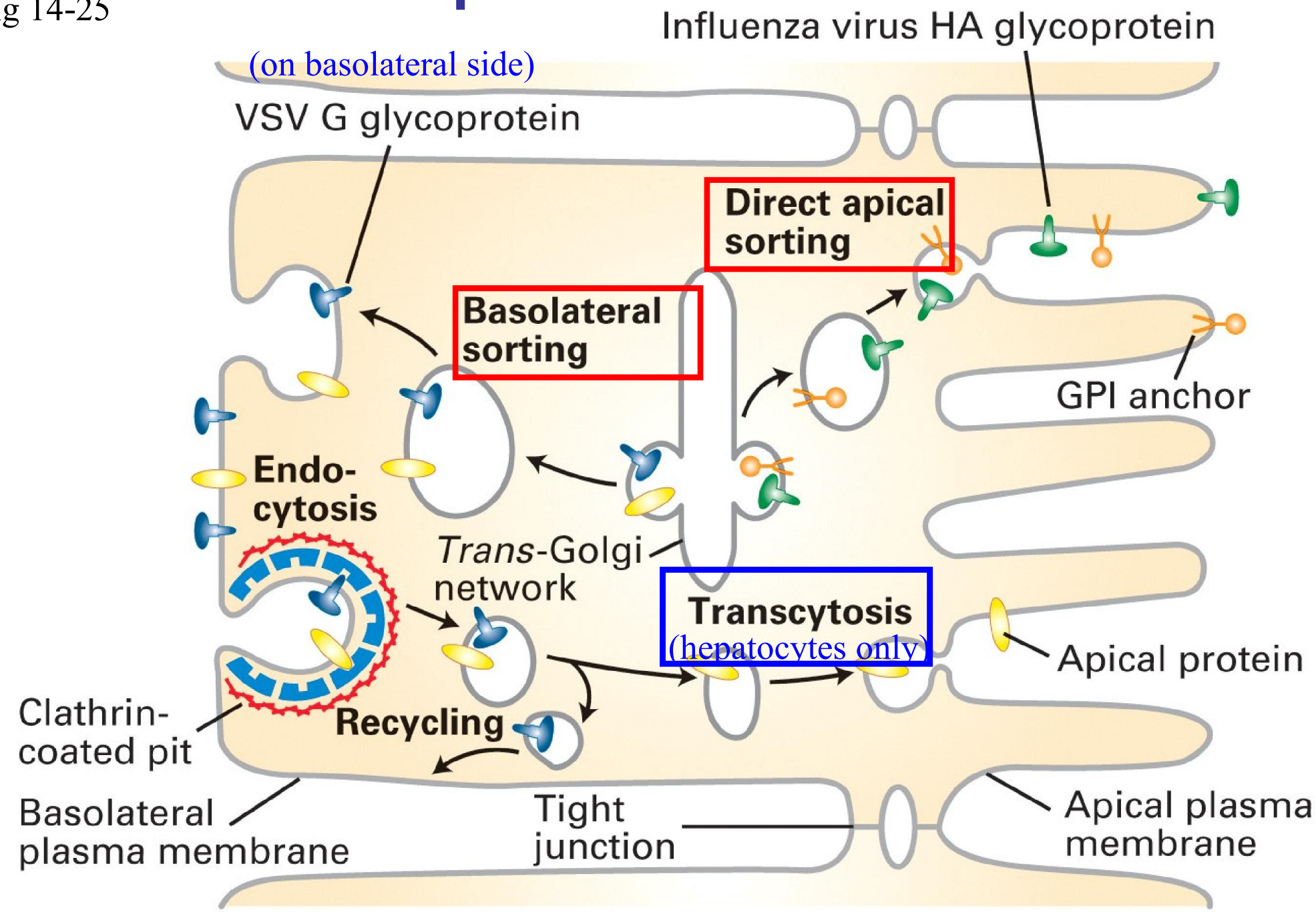


1. Regulated secreted proteins often undergo more extensive proteolytic cleavages
2. Arg-Arg or Lys-Arg sequences

Fig 14-24b

Sorting of membrane proteins in polarized cells

Fig 14-25



14.5
Receptor-mediated endocytosis

Endocytosis vs Phagocytosis

- Phagocytosis
  - "Cellular eating" of large particles (microorganisms, senescent and apoptotic cells)
  - Non-selective
  - Actin-mediated formation of plasma membrane extensions ('pseudopod')
  - Typical for only few cell types (e.g. macrophages)

Endocytosis vs Phagocytosis

- Endoctyosis
  - Uptake fluids (pinocytosis) and macromolecules
  - Requires the formation of endocytic vesicles
  - High rate and highly specific
    - e.g. receptor-mediated endocytosis
  - Essentially occurs in almost ALL eukaryotic cell types

Clathrin-mediated internalization of LDL

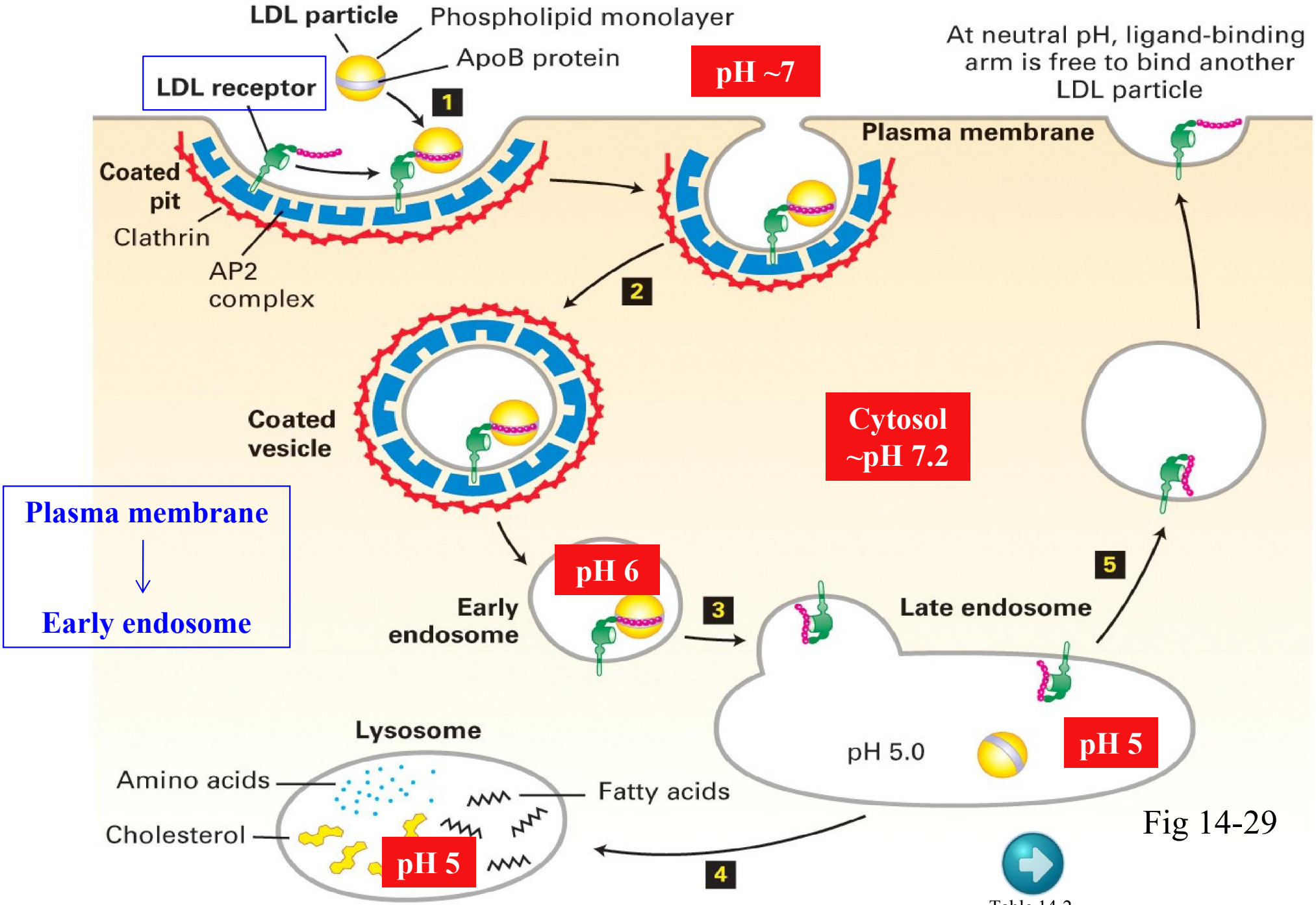


Fig 14-29

Table 14-2

pH-dependent binding of LDL by LDL receptor (pH=7)

Life span: ~20 hr
Recycled every 10-20 min

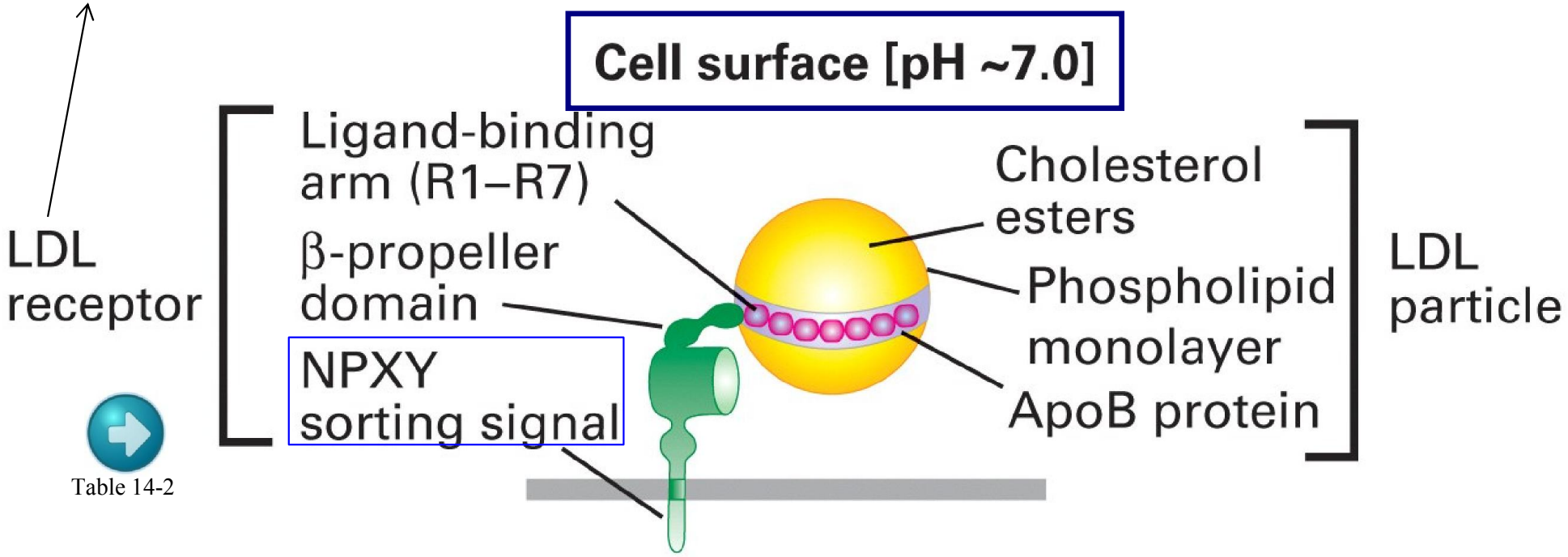
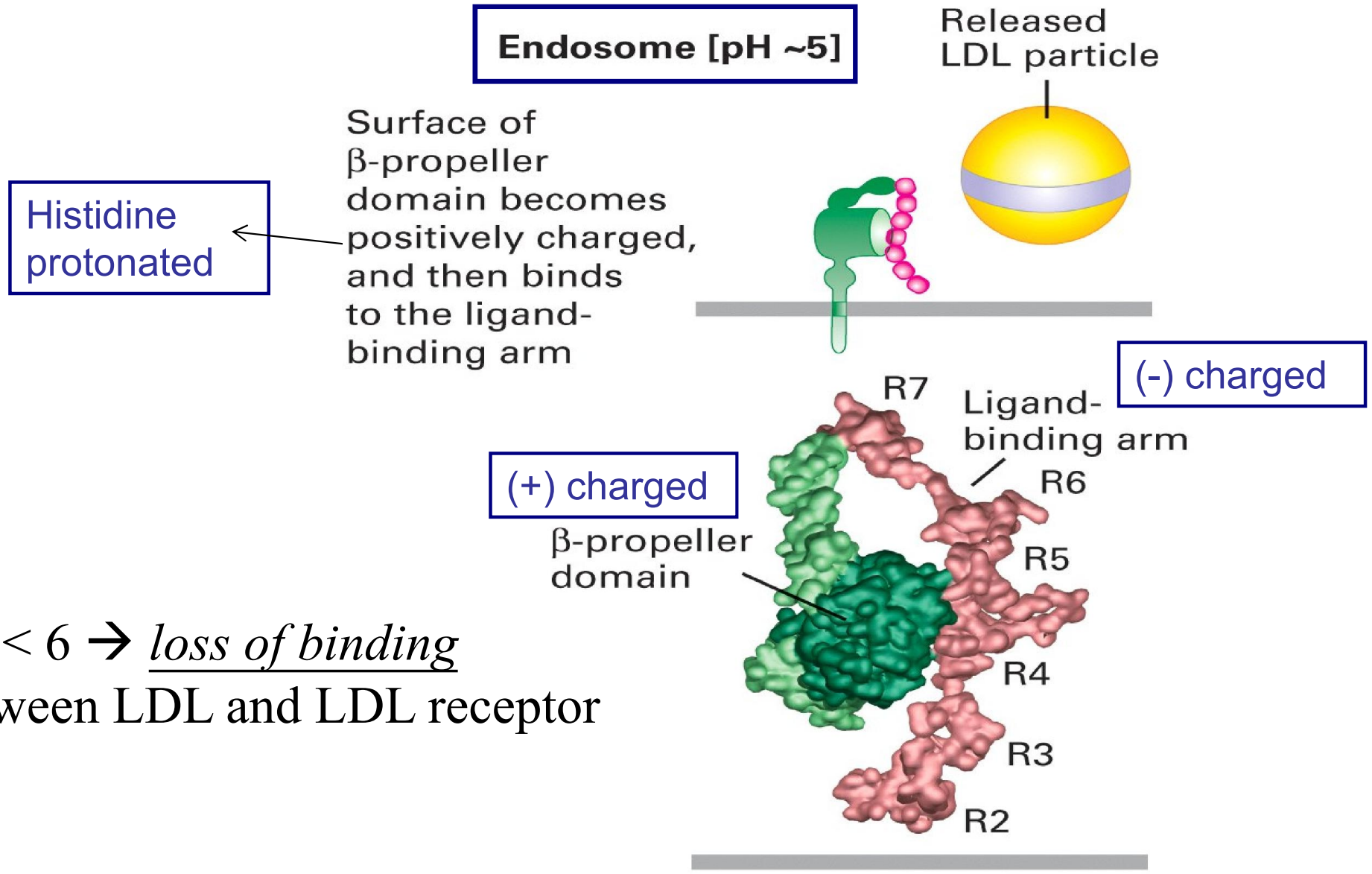


Table 14-2

- pH = 7 → *tight binding* between LDL and LDL receptor

Fig 14-30

pH-dependent binding of LDL by LDL receptor (pH<6)



- pH < 6 → *loss of binding* between LDL and LDL receptor

The transferrin cycle

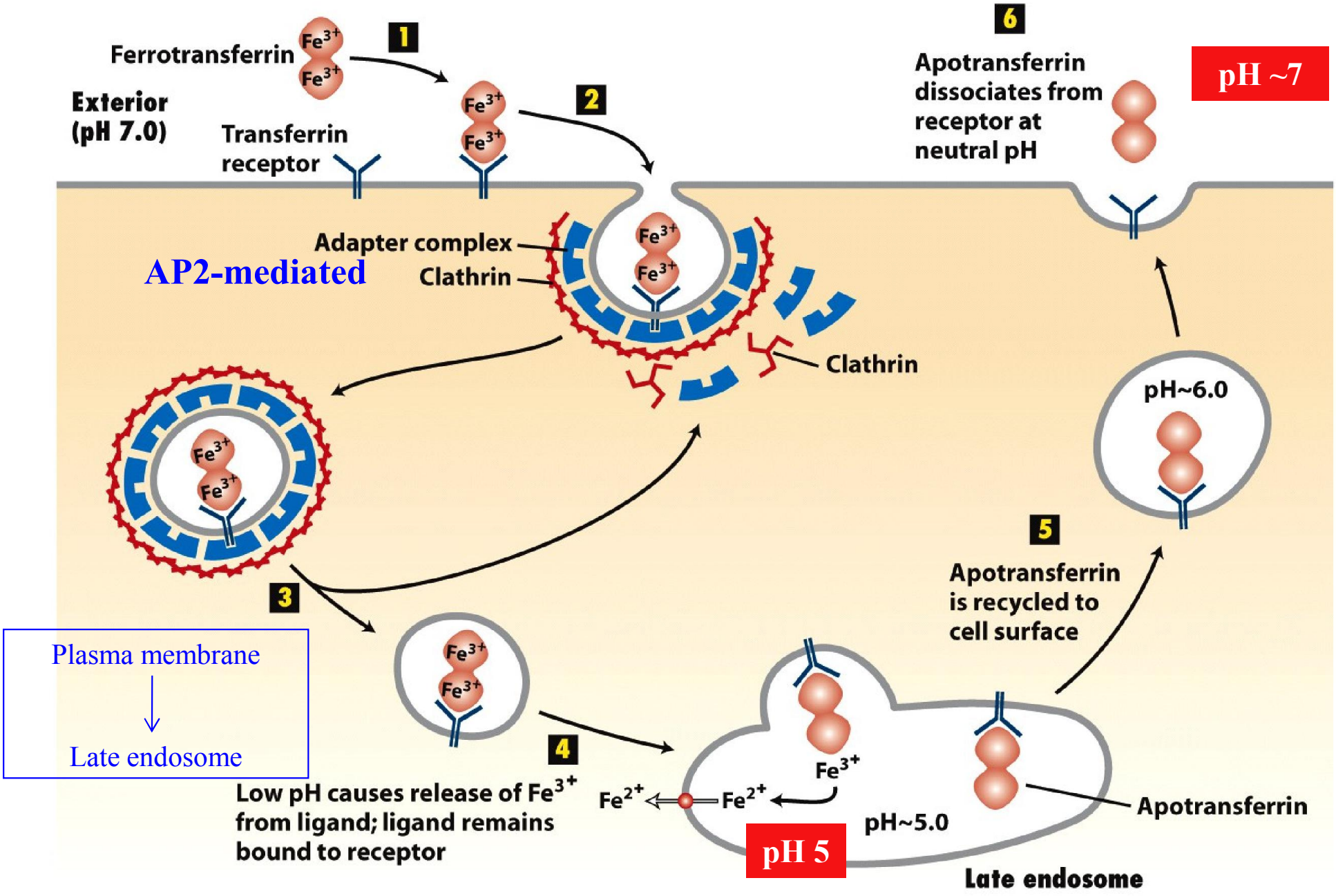


Figure 14-31
Molecular Cell Biology, Sixth Edition

14.6
Directing Membrane Proteins and Cytosolic Materials to the Lysosome

Delivery of membrane proteins to lysosome for degradation

Fig 14-32

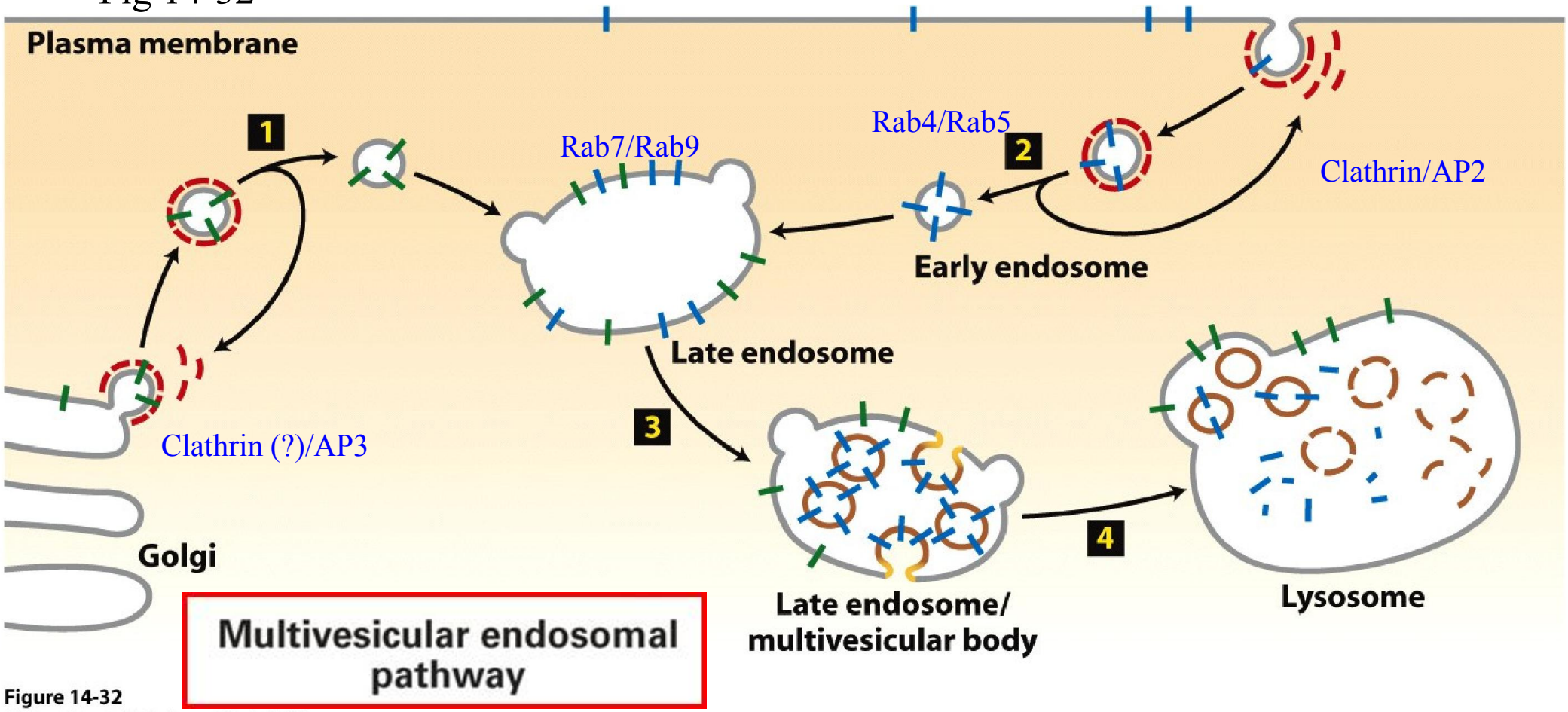


Figure 14-32
Molecular Cell Biology, Sixth Edition

Membrane proteins to be degraded are formed as **internal vesicles** in late endosome

Delivery of cytoplasmic components for degradation (Autophagy)

Inducible by
1. Nutrient starvation
2. Stress

Targets:
a. Entire organell (left)
b. Bulk cytosolic proteins (right)

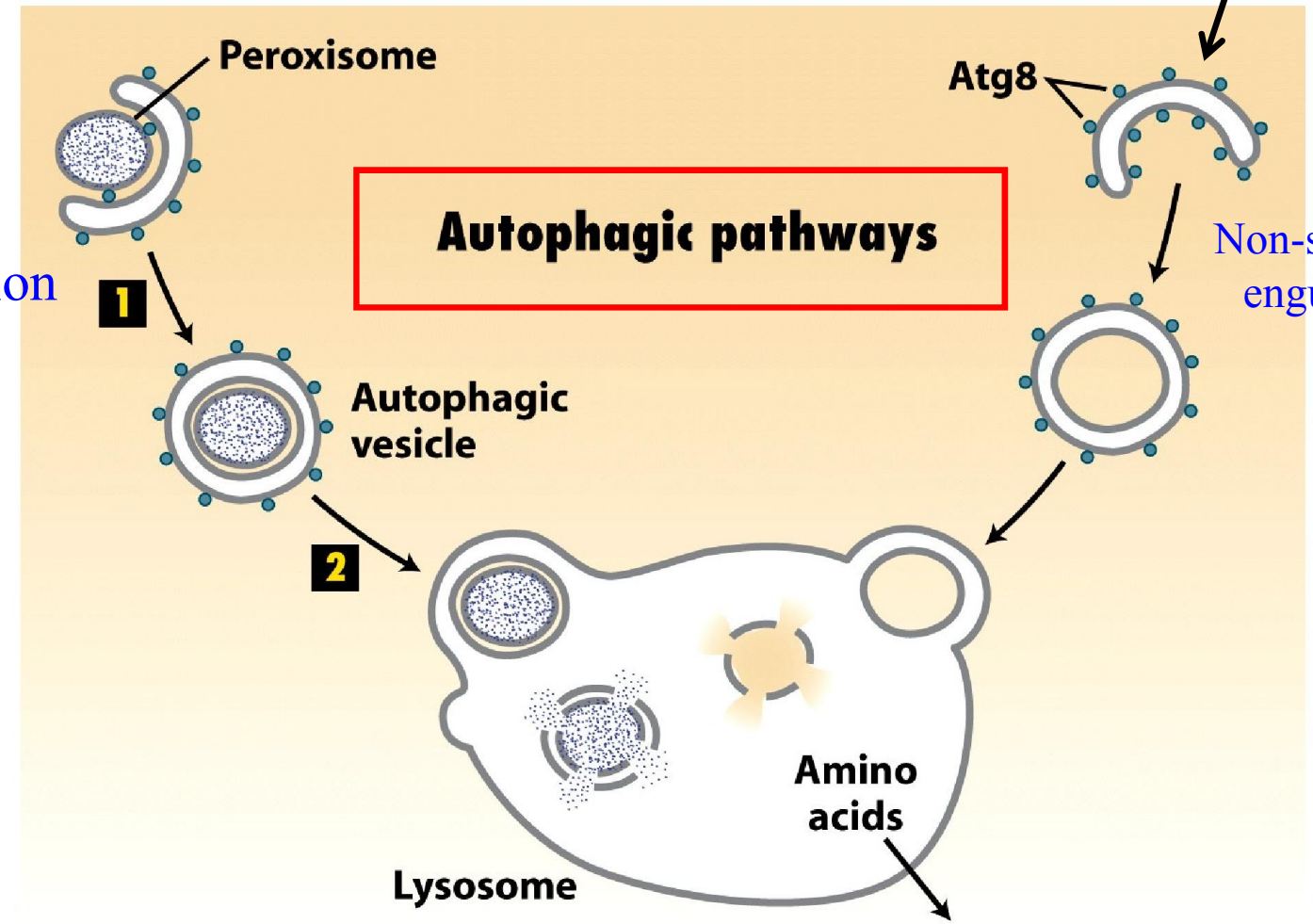
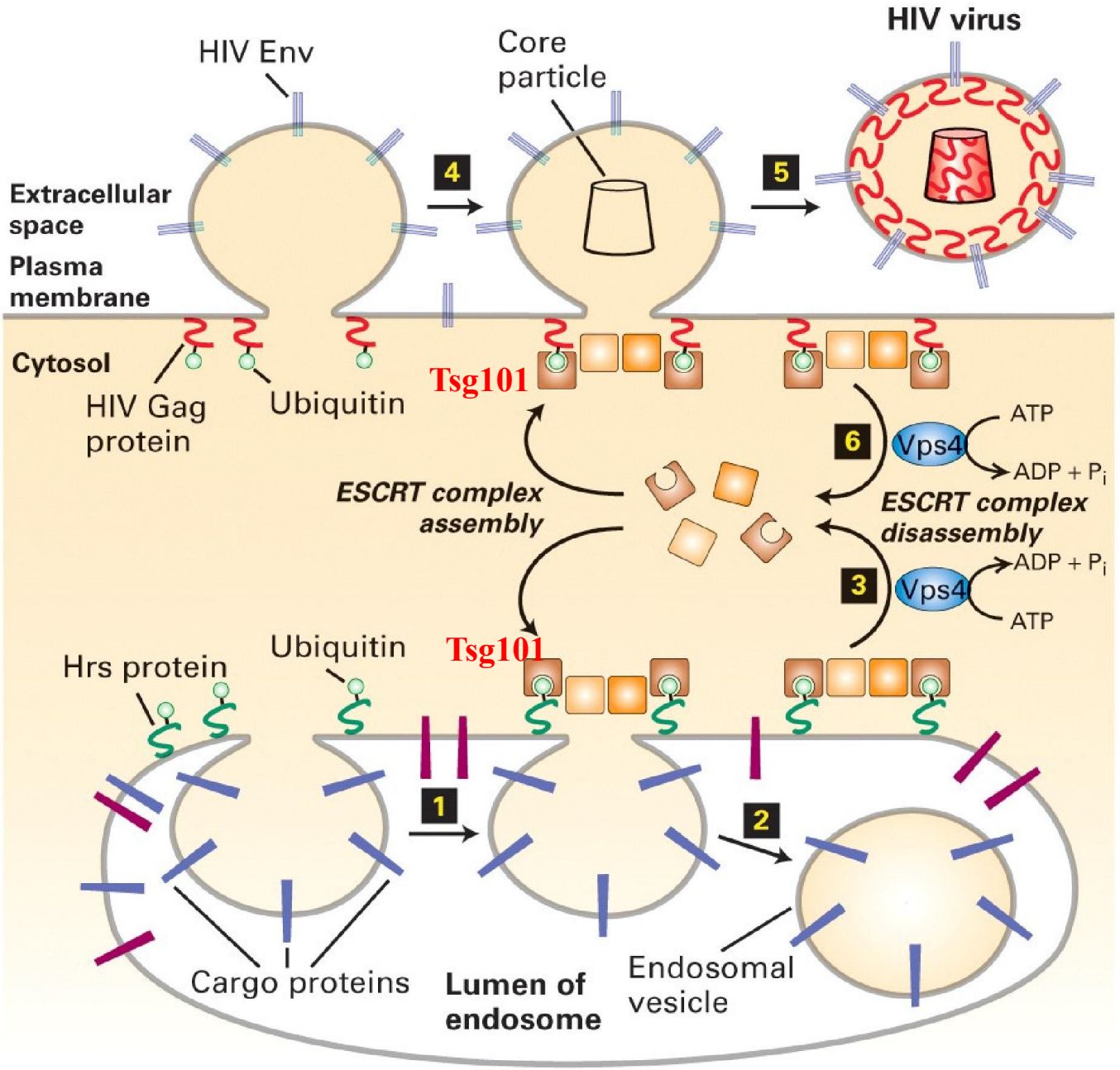


Figure 14-35
Molecular Cell Biology, Sixth Edition

Budding of retroviruses is similar to formation of multivesicular endosomes

Fig 14-33, 34

1. Requires ubiquitinated proteins to direct loading of cargo proteins.
   - Gag (HIV) = Hrs (endo.)
2. Cargo proteins are also ubiquitinated
3. Complete formation of vesicle requires assistance of the ESCRT complex
4. An ATP-driven process



結論

- Overview of protein secretion process.
- Golgi structure – 3D
- Golgi structure - SEM animation

Protein secretion

Golgi – 3D

Goigi - SEM

End of Chapter 14