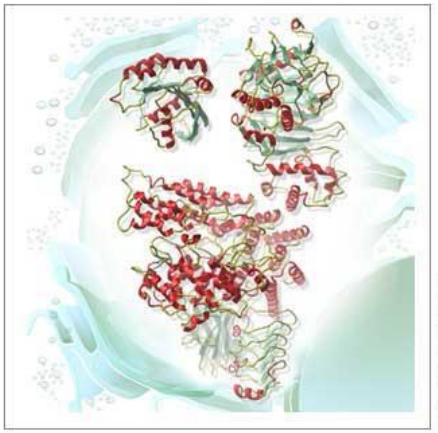


Vesicular transport

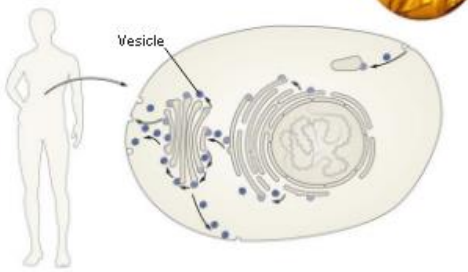
Prof. Dr. Kőhidai László

Department of Genetics, Cell- and Immunobiology
Semmelweis University

2023. October 03.

The Nobel Prize in Physiology or Medicine 2013

Proper functioning of the cells in the body depends on getting the right molecules to the right place at the right time. Some molecules, such as insulin, need to be exported out of the cell, whereas others are needed at specific sites inside the cell. Molecules produced in the cell were known to be packaged into vesicles (pictured in blue), but how these vesicles correctly deliver their cargo was a mystery.

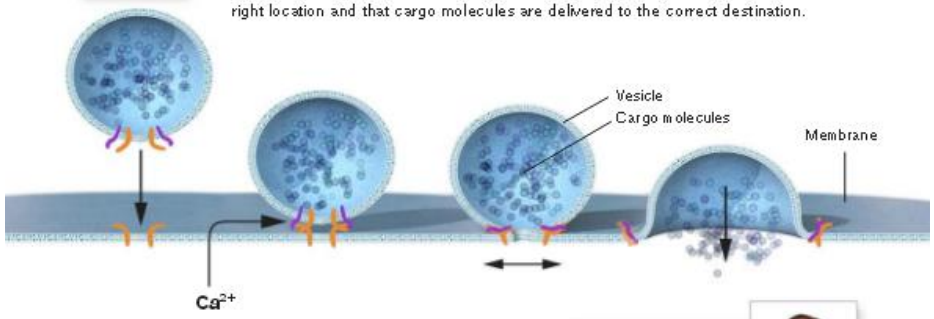


Randy W. Schekman

Randy W. Schekman discovered genes encoding proteins that are key regulators of vesicle traffic. Comparing normal (left) with genetically mutated yeast cells (right) in which vesicle traffic was disturbed, he identified genes that control transport to different compartments and to the cell surface.

James E. Rothman

James E. Rothman discovered that a protein complex (pictured in orange) enables vesicles to fuse with their target membranes. Proteins on the vesicle bind to specific complementary proteins on the target membrane, ensuring that the vesicle fuses at the right location and that cargo molecules are delivered to the correct destination.



Thomas C. Südhof

Thomas C. Südhof studied how signals are transmitted from one nerve cell to another in the brain, and how calcium controls this process. He identified molecular machinery (pictured in purple) that senses calcium ions (Ca^{2+}) and triggers vesicle fusion, thereby explaining how temporal precision is achieved and how signaling substances can be released from the vesicles on command.

Illustration and layout: Mattias Karlén

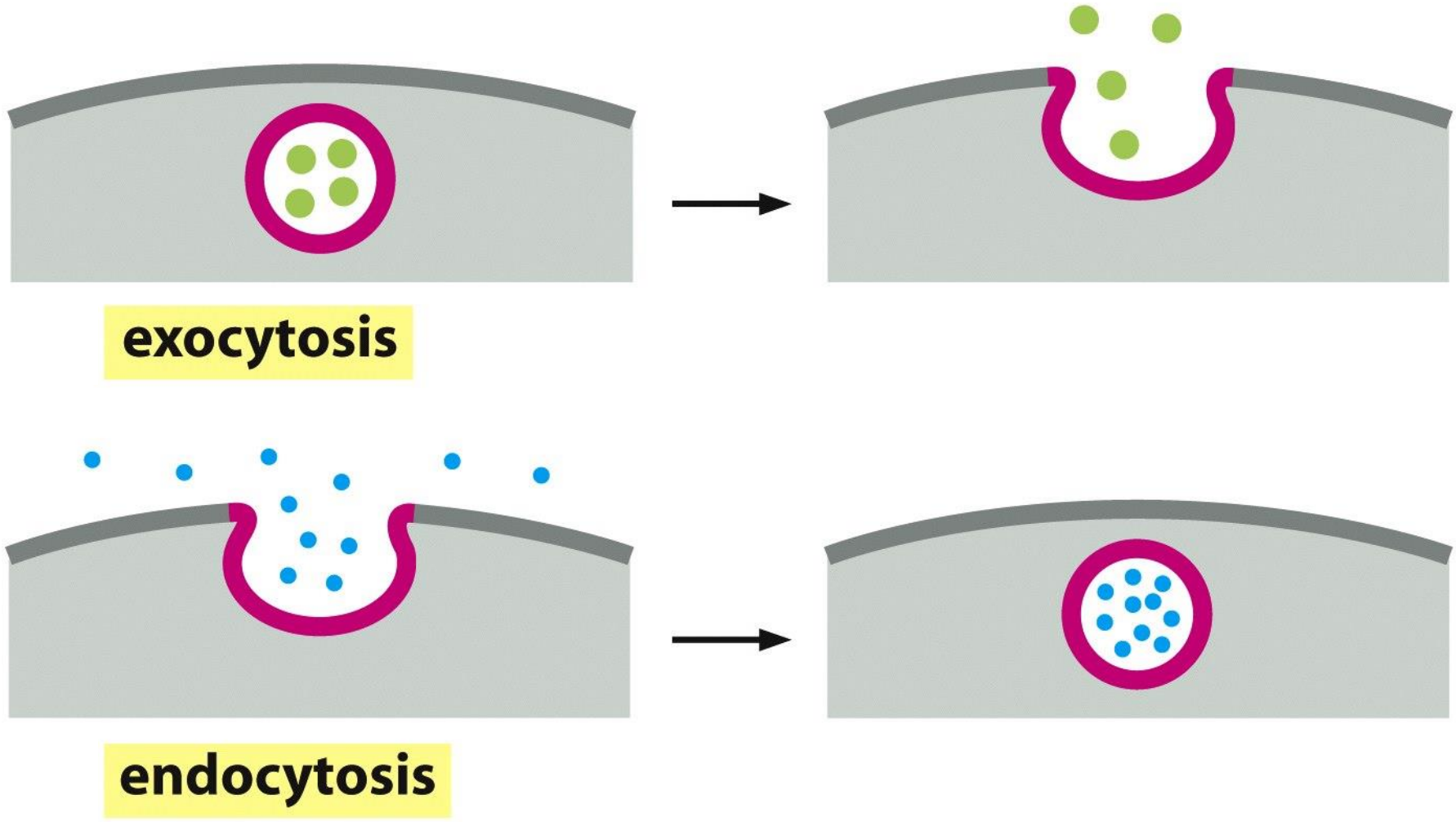


Figure 13-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Intracellular vesicular transport

!

Communication – intracellular;
– cell-environment

- Membrane system
- Uptake of substances and transport to the place of digestion (lysosomes) - endocytosis
- Transport of proteins between ER-Golgi and to the surface membrane - exocitózis

Main pathways of vesicular transport

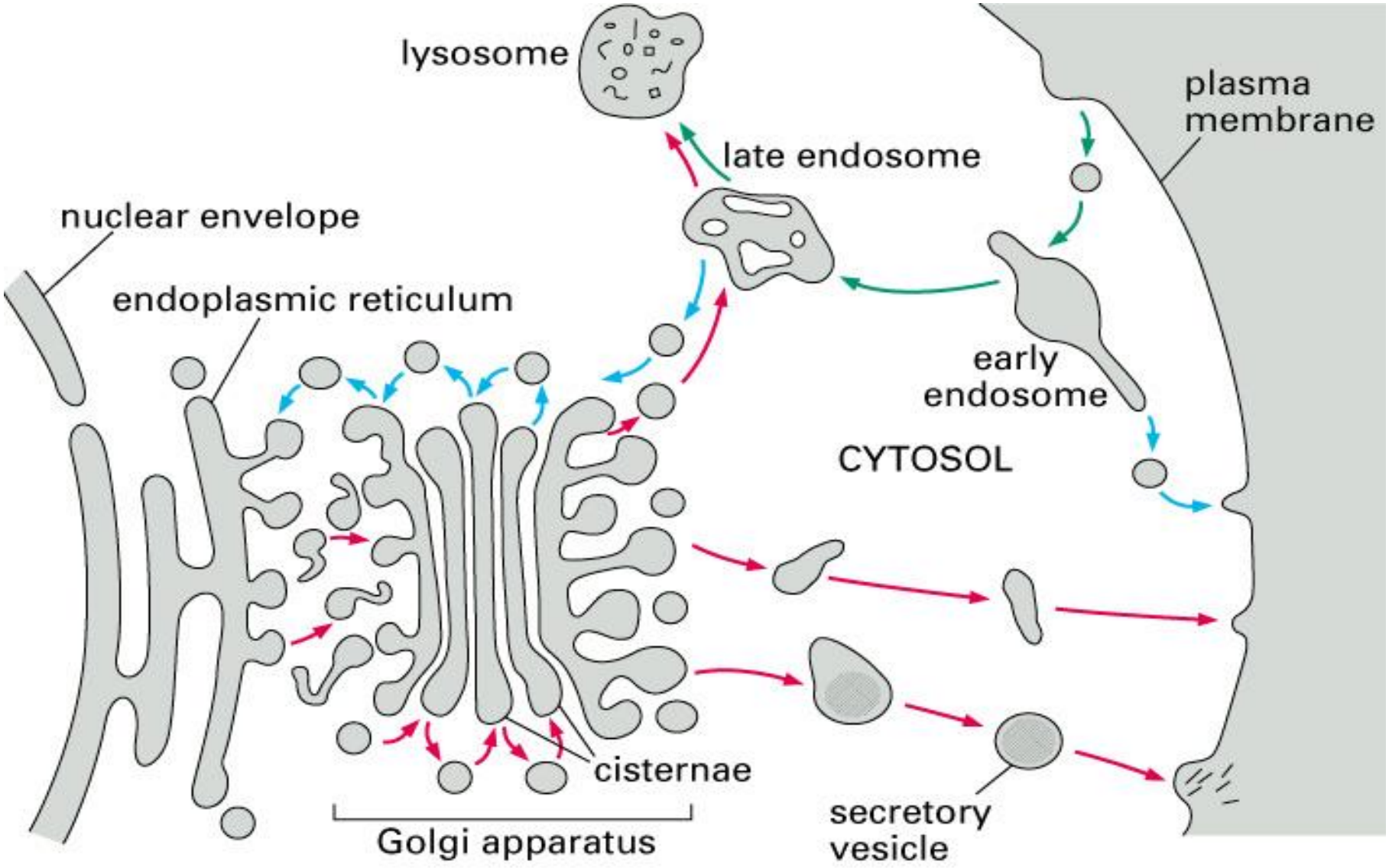


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Intracellular vesicular transport

!

- Bidirectional, transport between compartment is balanced
- Continous recyclicling of membrane proteins

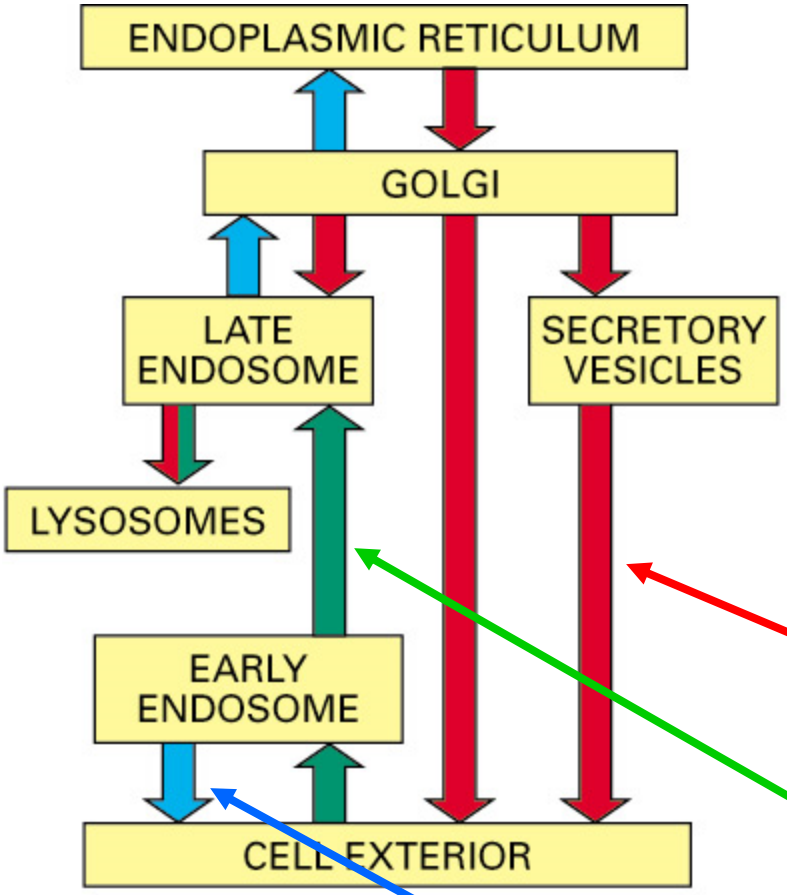


Figure 13–1. Molecular Biology of the Cell, 4th Edition.

Pathway of biosynthetic- and secretory processes

Endocytosis

Recycling

Transport vesicles !

- Membrane bounded vesicles filled with different cargos
  - Secretion
  - Lysosomal enzymes
  - Components of surface membrane and the ECM

- Direction of transport is determined by the components of the membrane see: donor and target compartments

Molecular bases of vesicular transport !

- Biosynthetic-secretory and endocytotic pathways join 10 or more compartments

- Direction of the transport and fusion are determined by molecular matching (receptor/ligand)

Coated vesicles

!

Role of the coat:

- Components of the membrane (e.g. receptors) are concentrated into patches

- Removal of coated surfaces and formation of vesicles

Types of coated vesicles

!

- Clathrin-coated vesicles
- COPI-coated vesicles
- COPII-coated vesicles

Each type of vesicle has its own transport mechanism

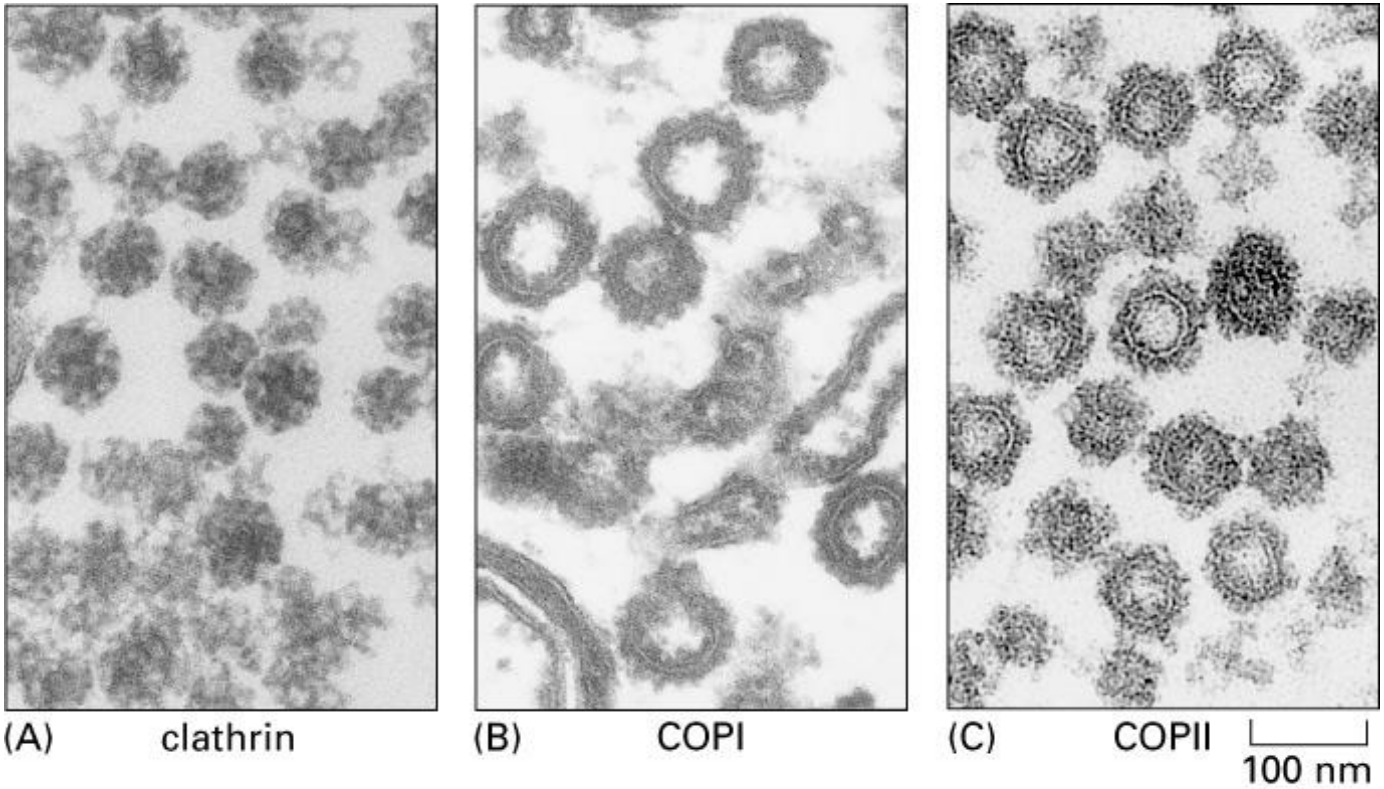
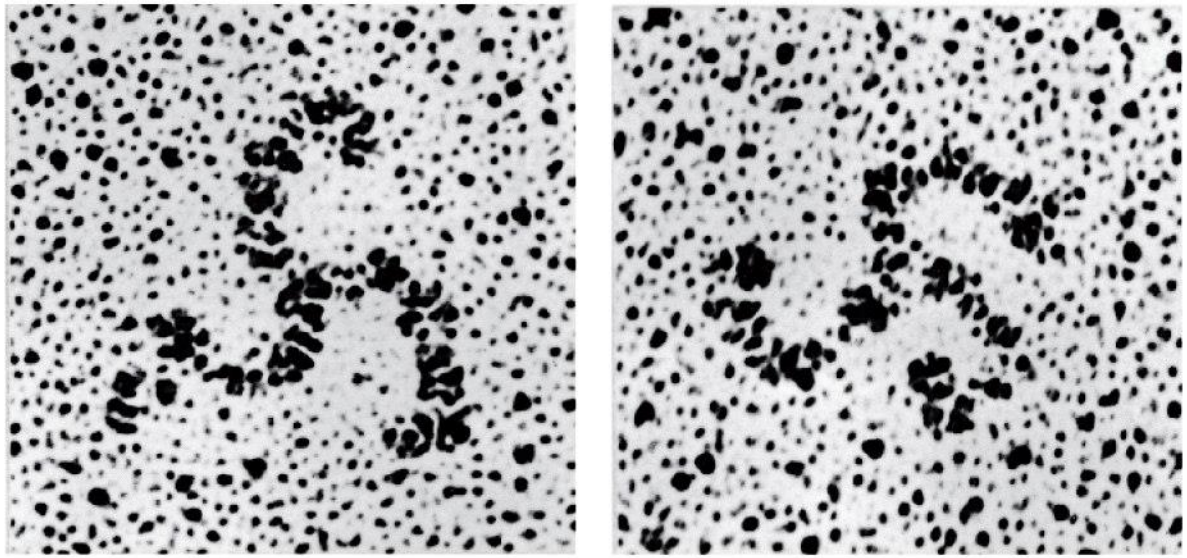


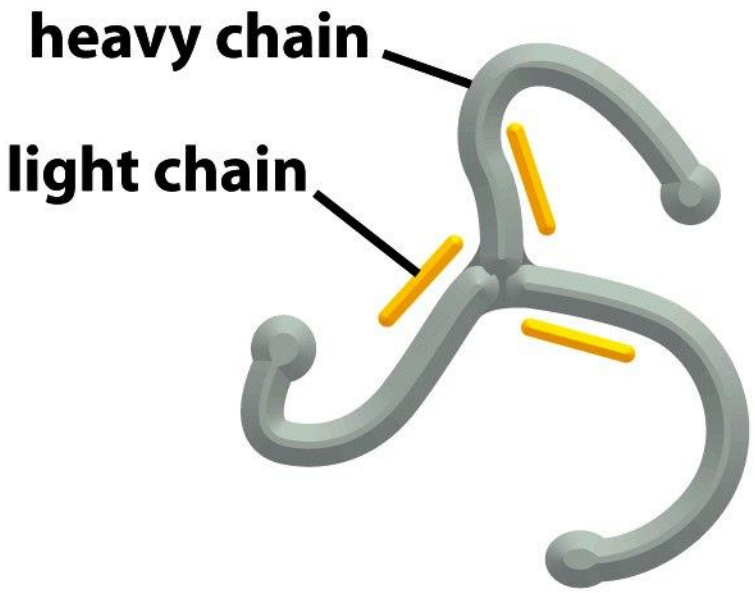
Figure 13–4. Molecular Biology of the Cell, 4th Edition.

The TEM morphology
of the three types of vesicles

Clathrin coated vesicles

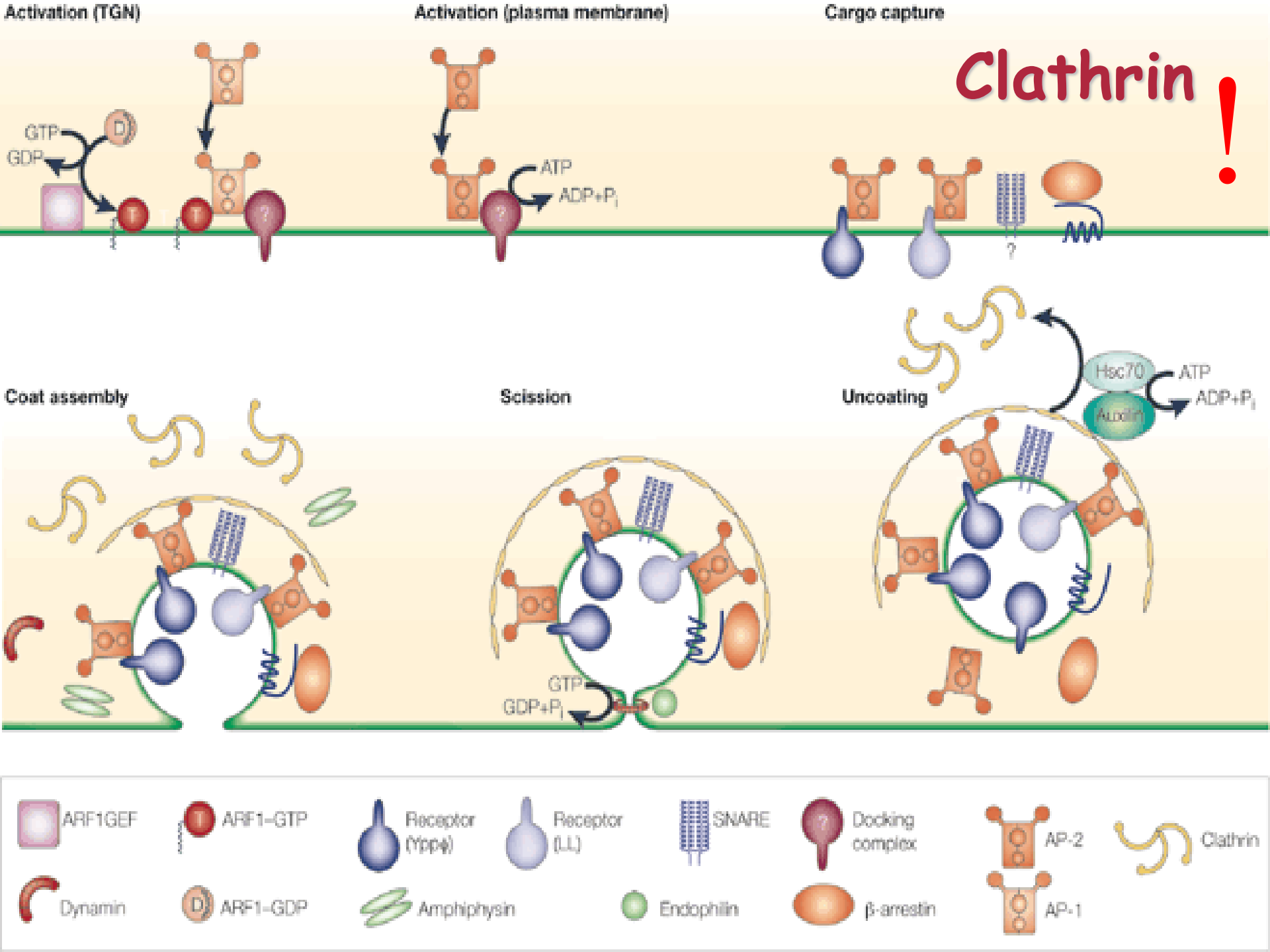


(A)

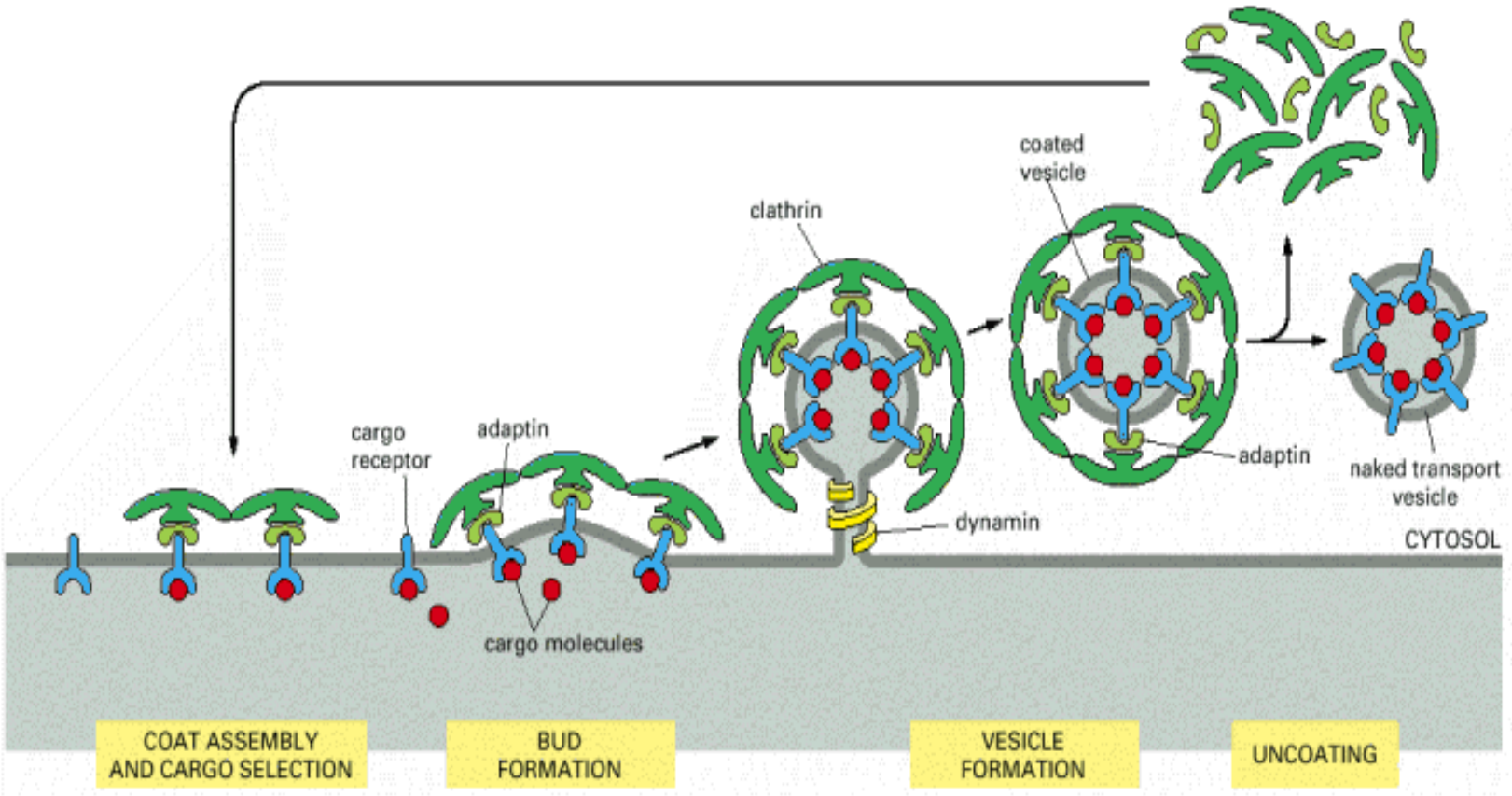


(B)

Figure 13-7a, b *Molecular Biology of the Cell* (© Garland Science 2008)


Activation (TGN)
Activation (plasma membrane)
Cargo capture
Clathrin
!
GTP
GDP
ATP
ADP+Pi
Coat assembly
Scission
Uncoating
Hsc70
ATP
ADP+Pi
Auxilin
GTP
GDP+Pi
ARF1GEF
ARF1-GTP
Receptor (YppΦ)
Receptor (LL)
SNARE
Docking complex
AP-2
Clathrin
Dynamin
ARF1-GDP
Amphiphysin
Endophilin
β-arrestin
AP-1

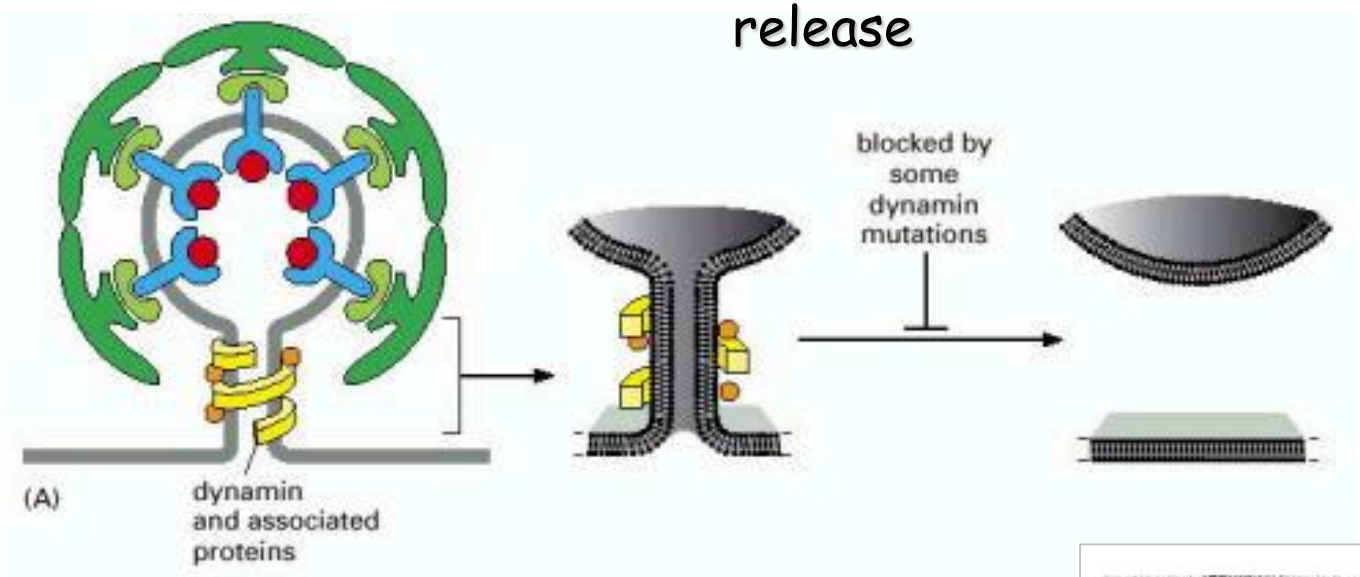
Releasing of clathrin-coated vesicles !



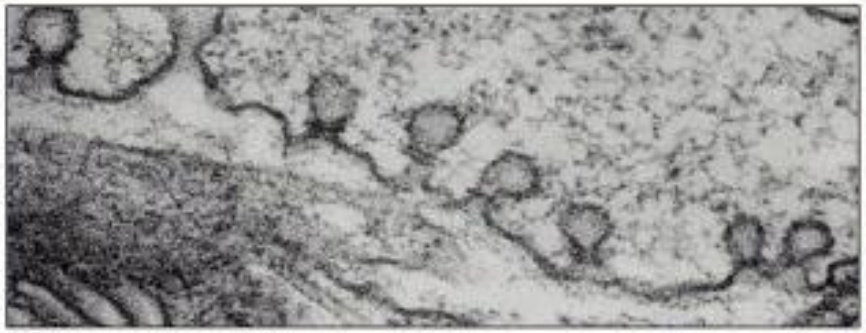
Alberts et all. Molecular Biology of the cell

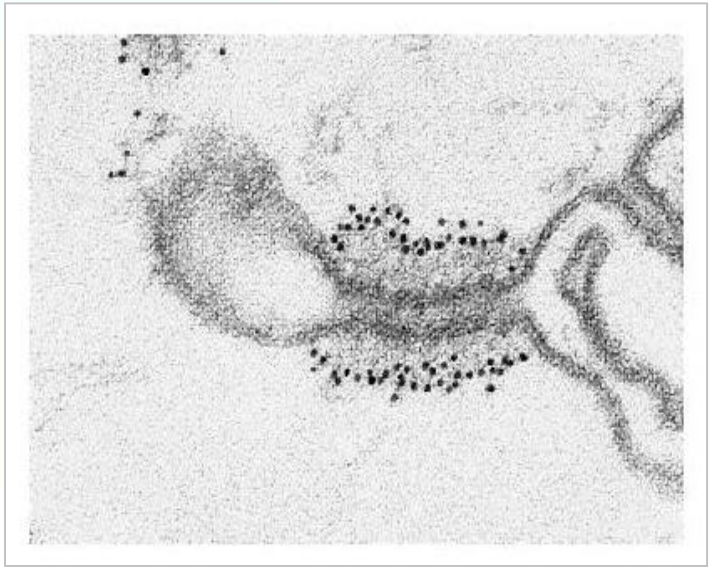
Receptor-mediated endocytosis

Role of dynamin in the process of vesicle release

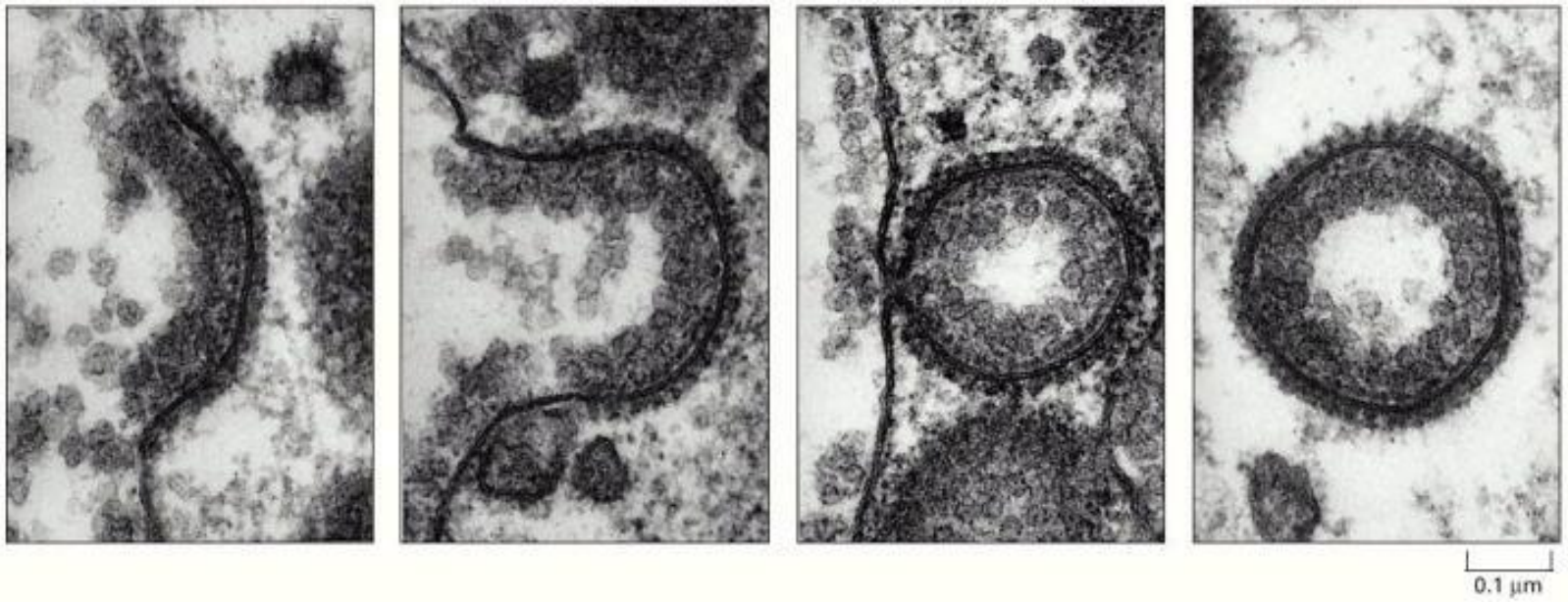


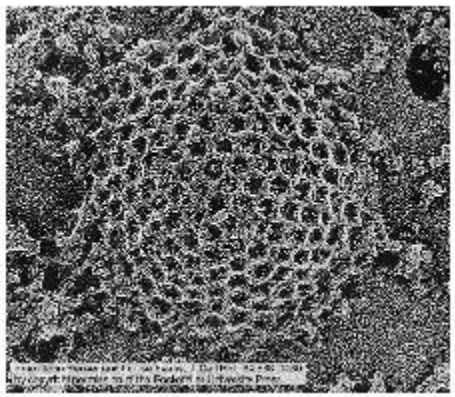
Dynamin is a G-protein, it binds and cleaves GTP



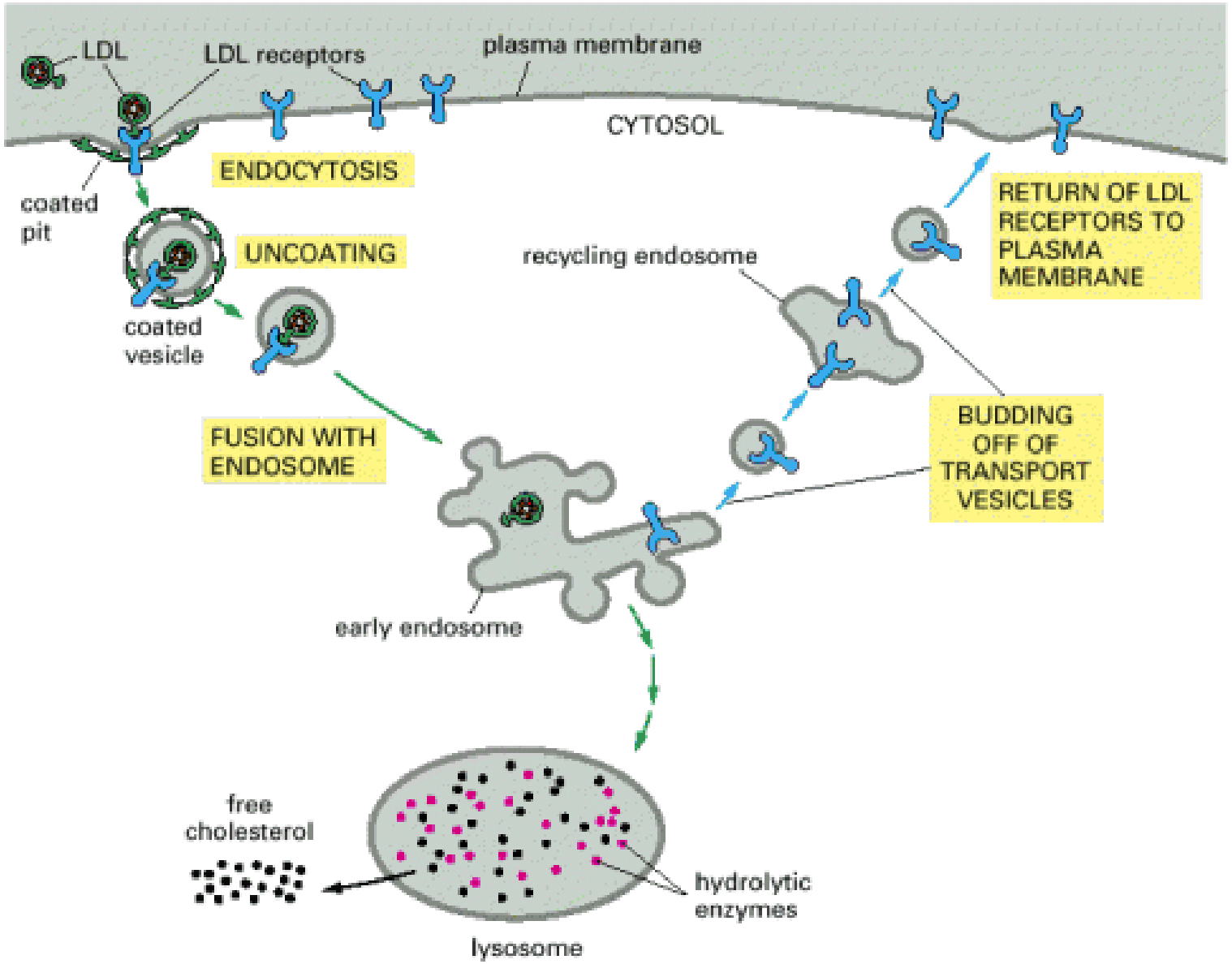


Alberts et all. Molecular Biology of the cell


0.1 µm



Receptor-mediated endocytosis !



Alberts et all. Molecular Biology of the cell

Vesicular transport between rER and Golgi

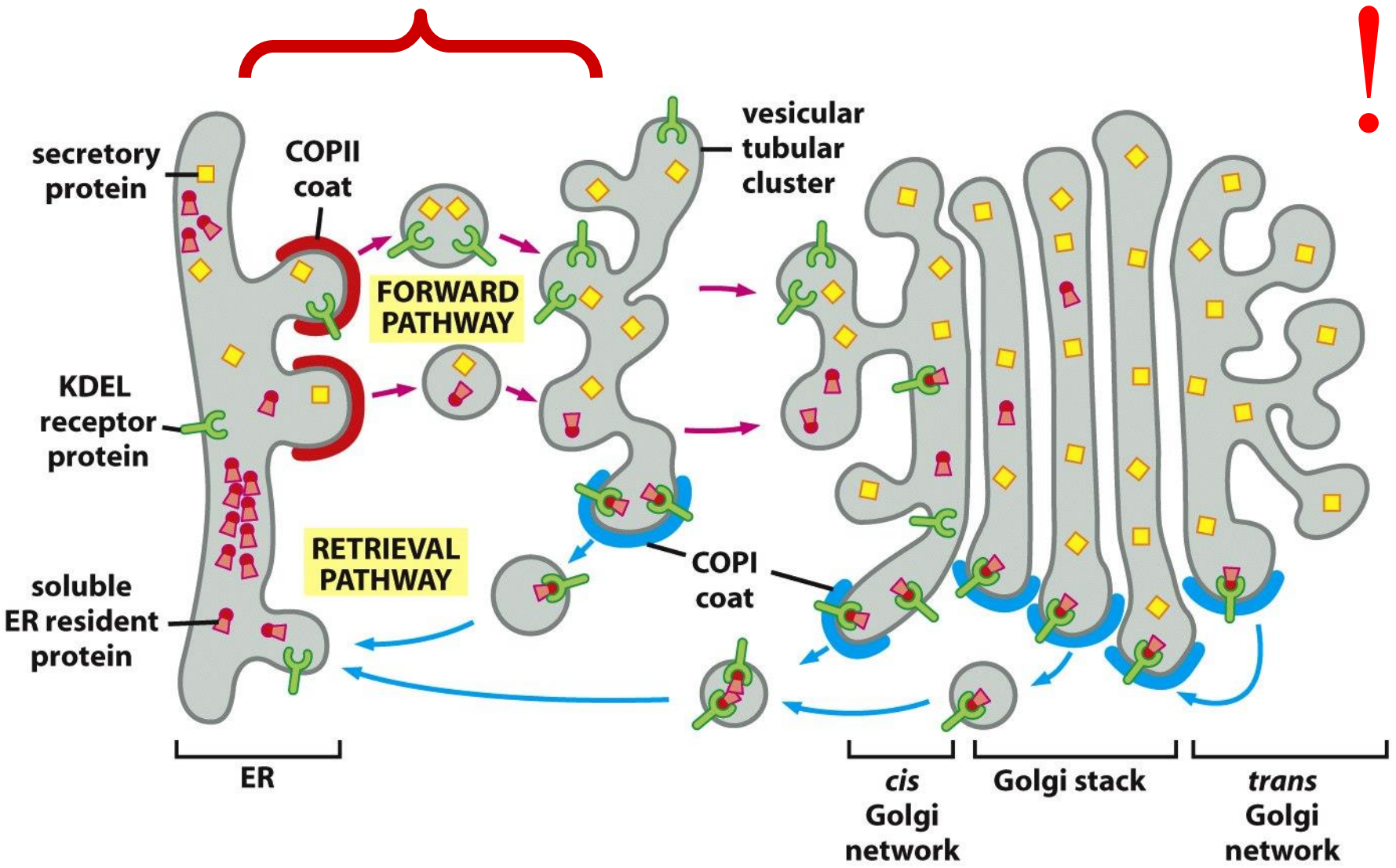
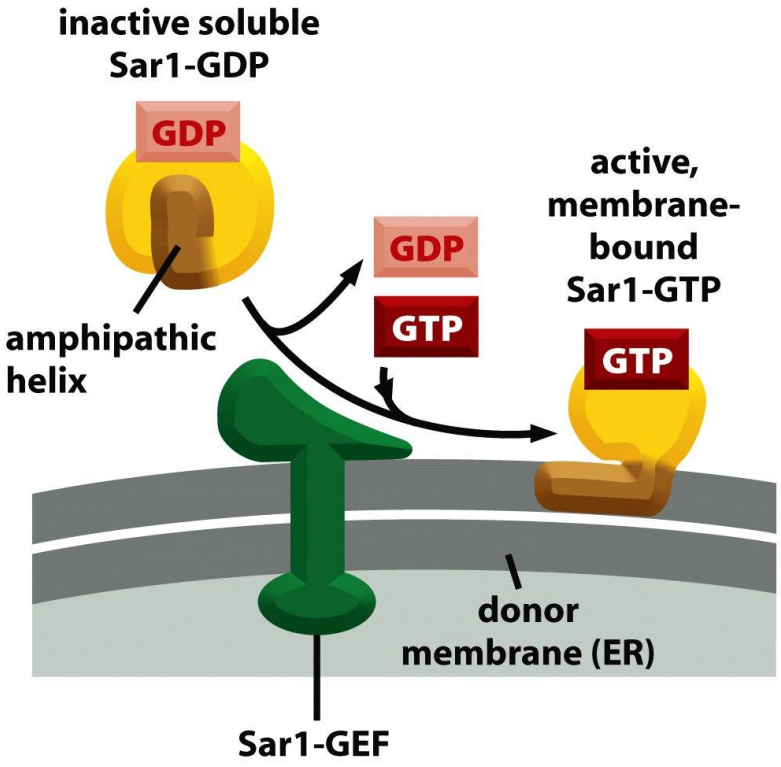
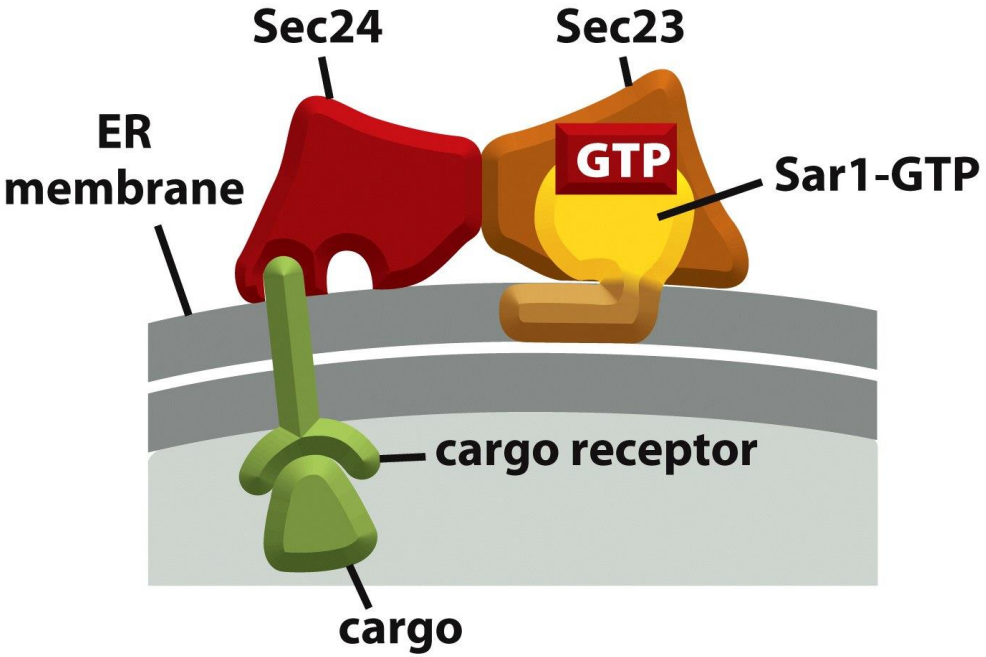


Figure 13-24b *Molecular Biology of the Cell* (© Garland Science 2008)

rER-Golgi: Forward transport – COPII (1)





GDI = GDP dissoc. inhibitor
GAP = GTP-ase act. Prot.
GDF = GDI-displacement factor
GEF = guanin nucl. exch. factor

rER-Golgi: Forward transport – COPII (2)

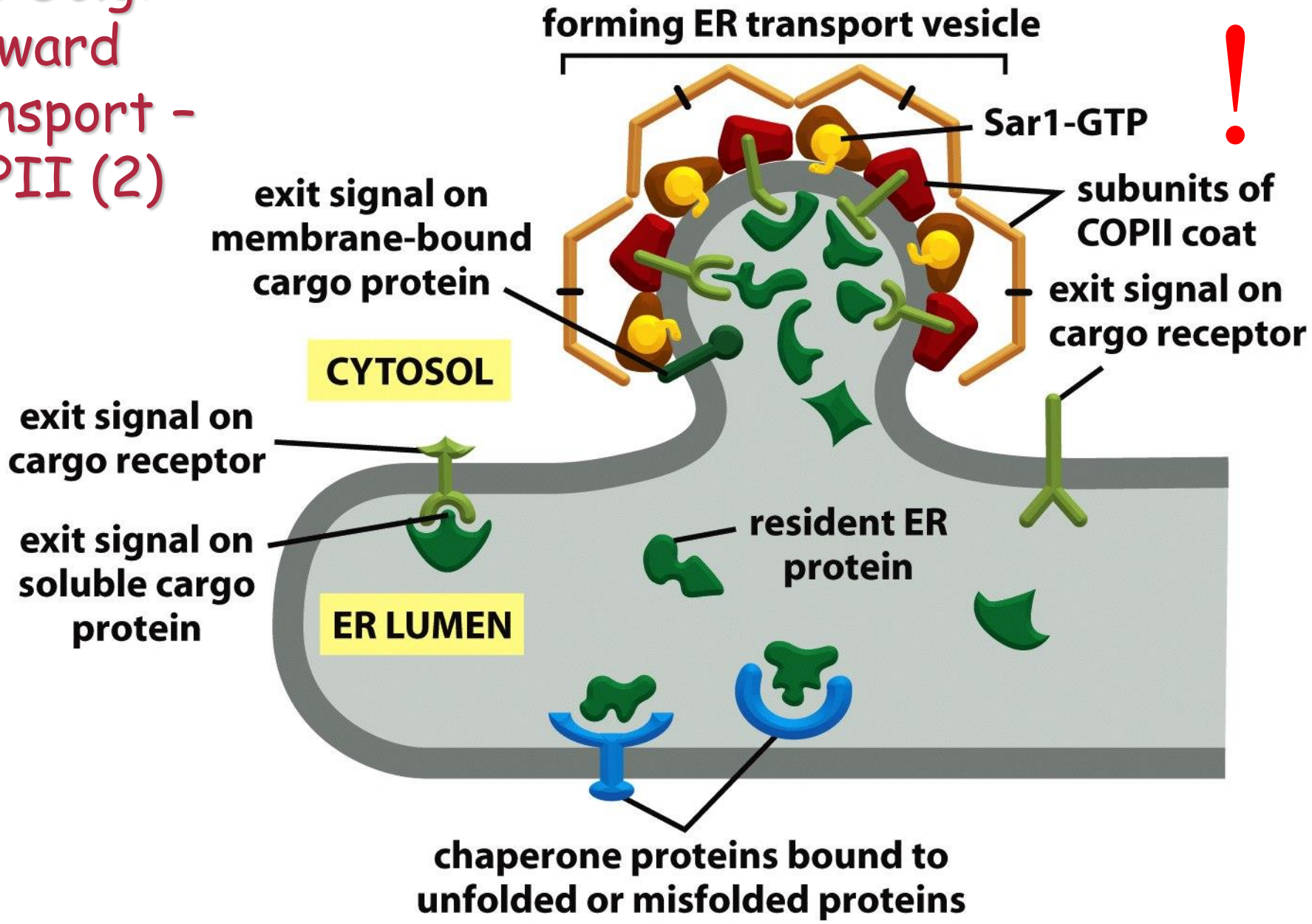


Figure 13-20 *Molecular Biology of the Cell* (© Garland Science 2008)

rER-Golgi: Forward transport – COPII (3)

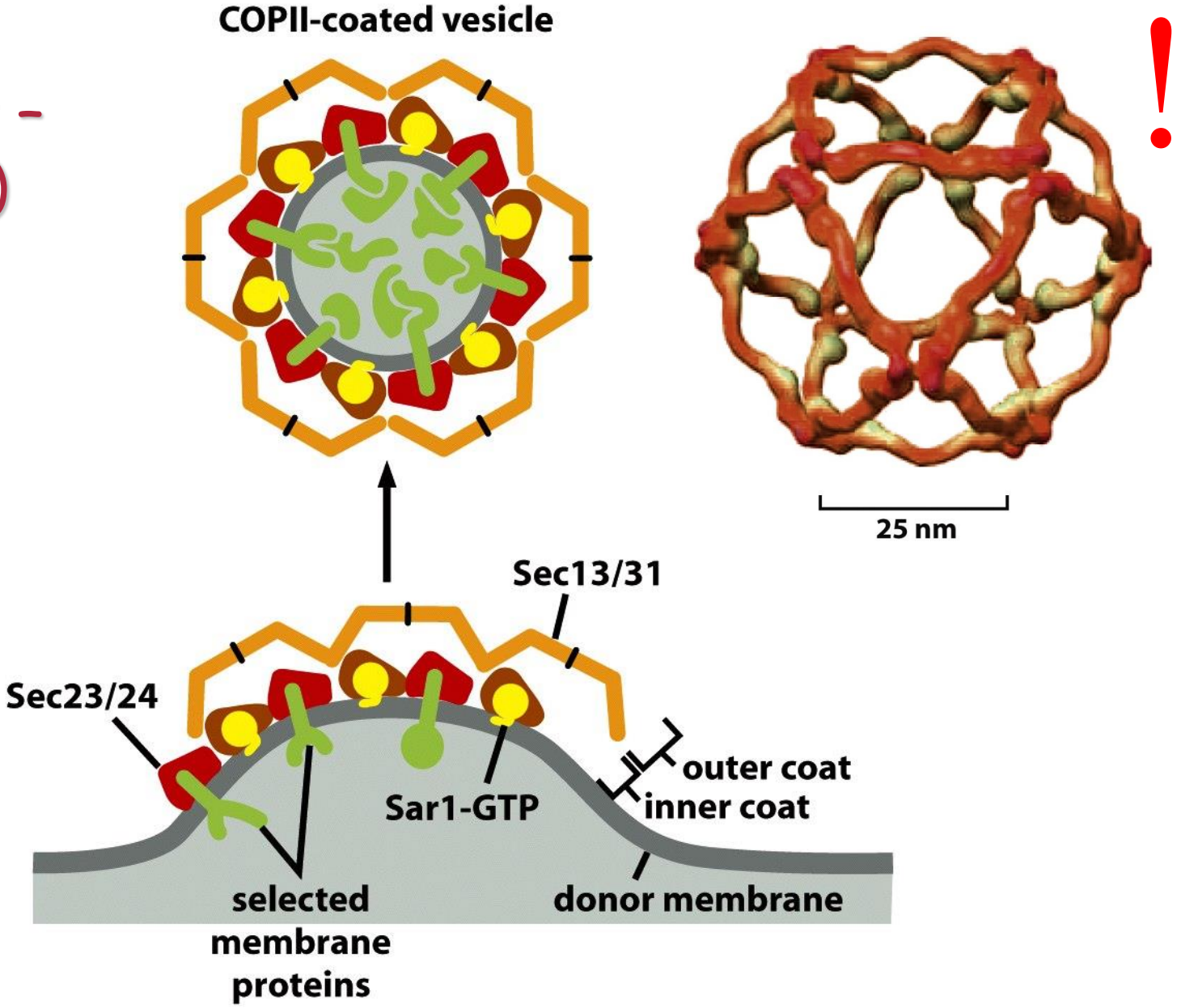


Figure 13-13d *Molecular Biology of the Cell* (© Garland Science 2008)

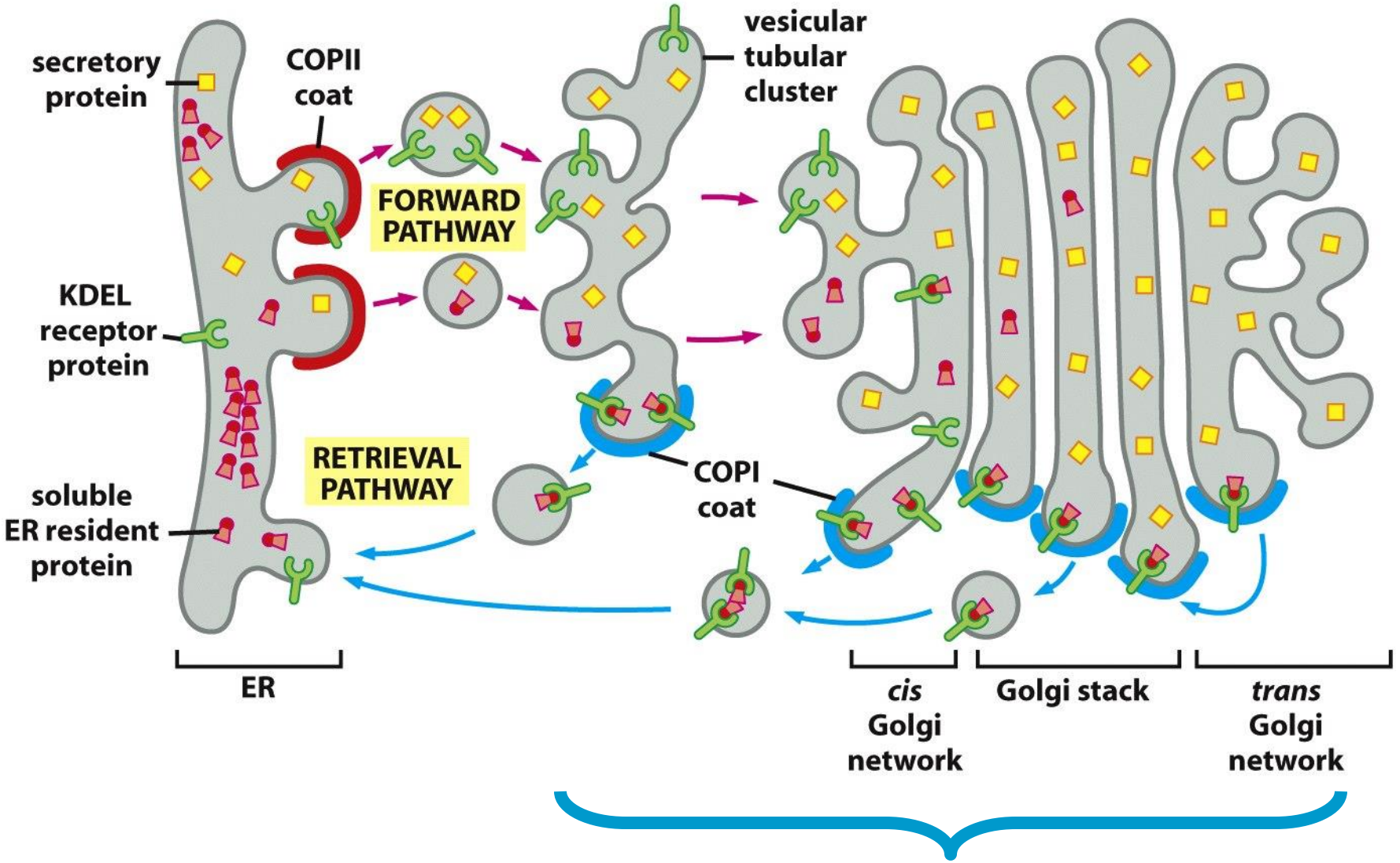


Figure 13-24b *Molecular Biology of the Cell* (© Garland Science 2008)

Golgi- rER: Retrograde transport – COPI

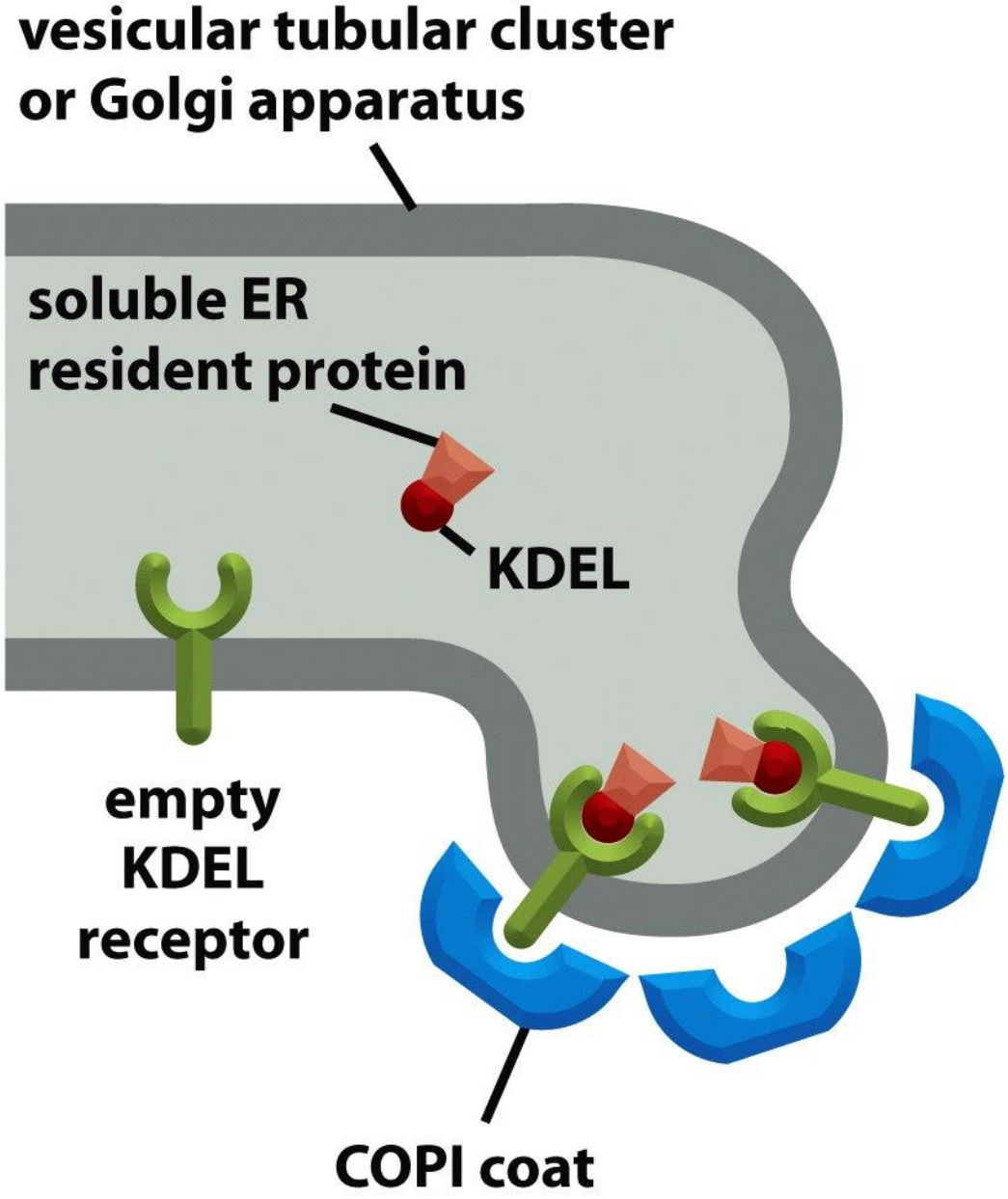
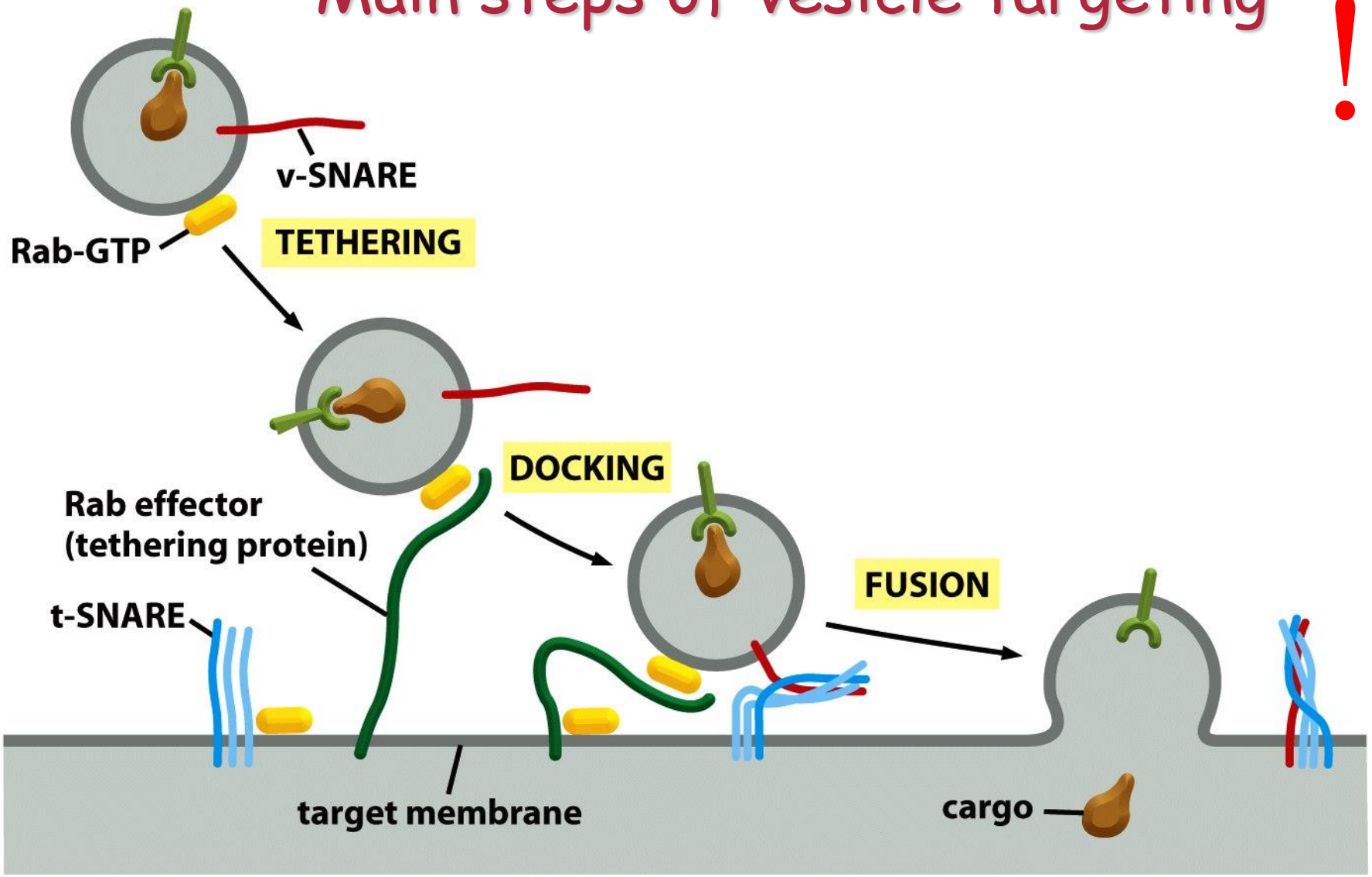


Figure 13-24a *Molecular Biology of the Cell* (© Garland Science 2008)

Vesicular transport in the cytoplasm

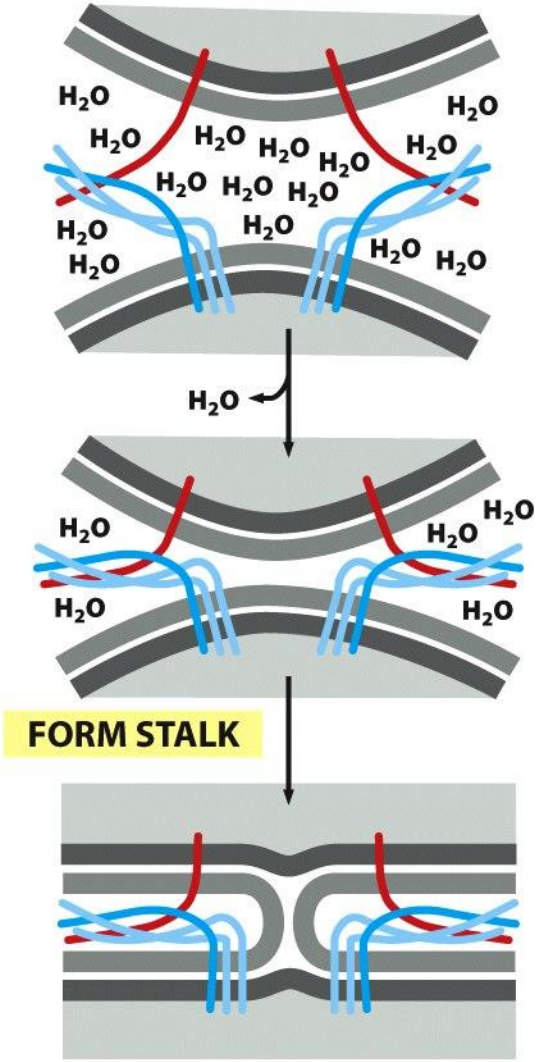
Main steps of vesicle targeting

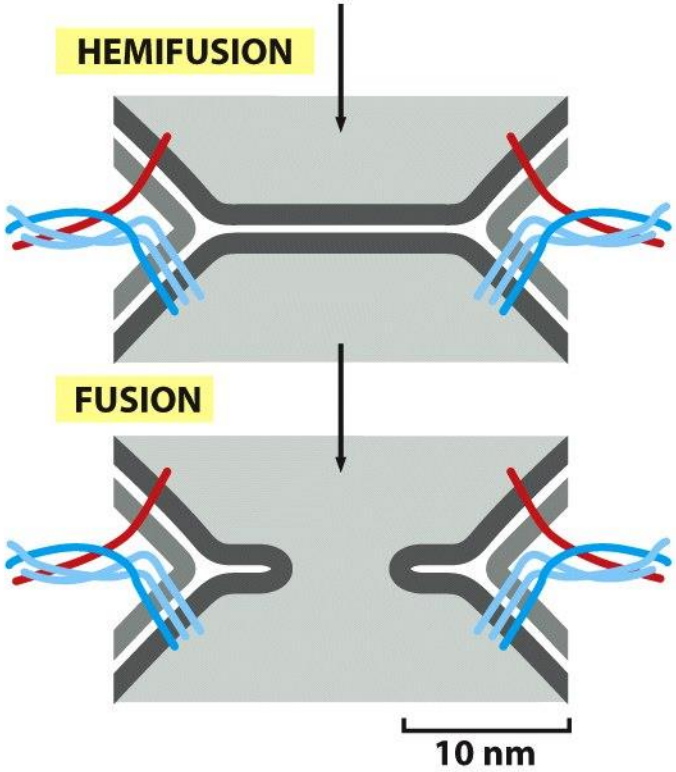


SNARE – soluble N-ethylmaleimide sensitive factor attachment protein receptor

Figure 13-14 *Molecular Biology of the Cell* (© Garland Science 2008)

Main steps of vesicle fusion





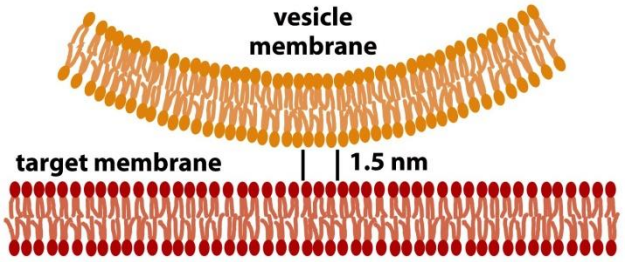


Figure 13-17 *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular interactions in fusion
- Trans-SNARE -

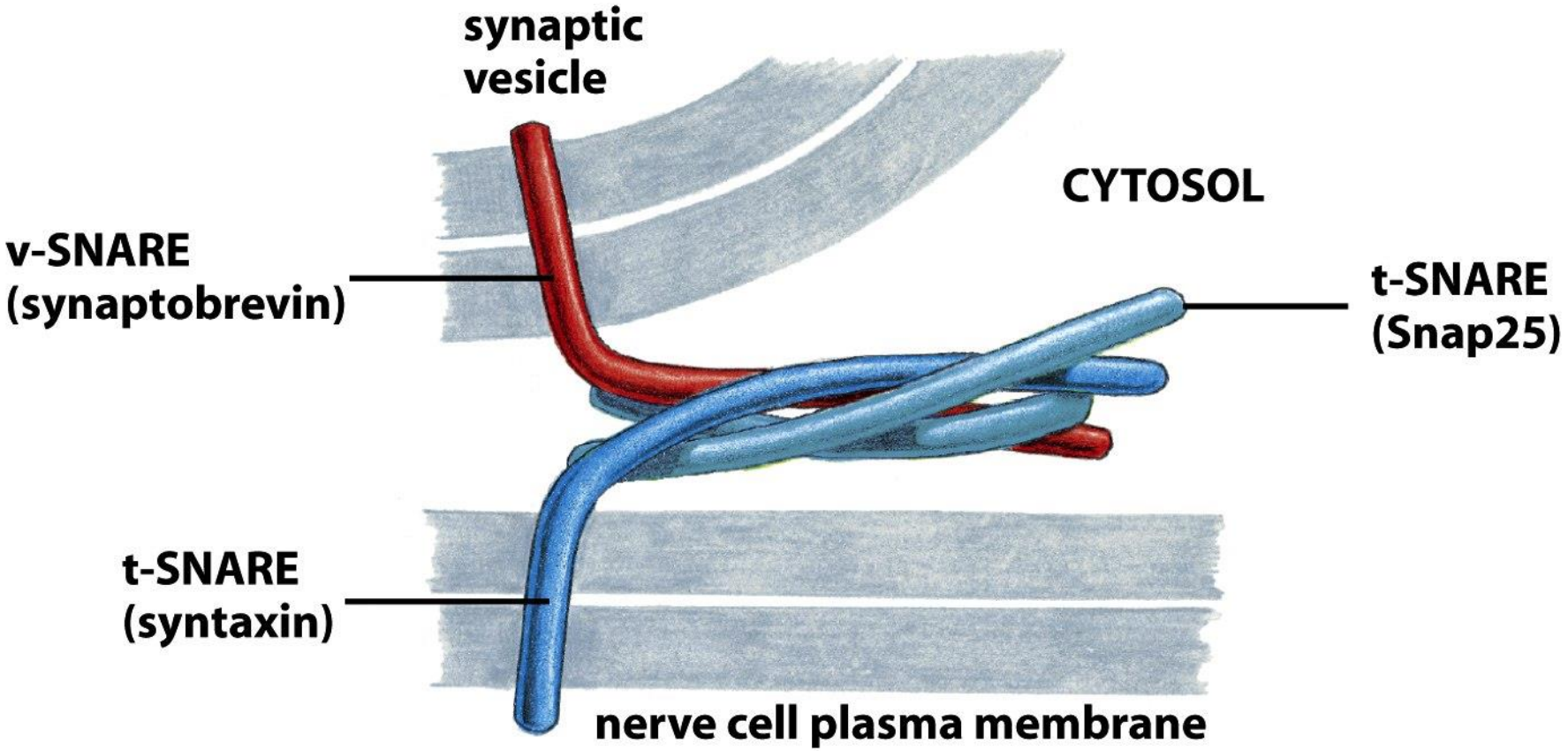
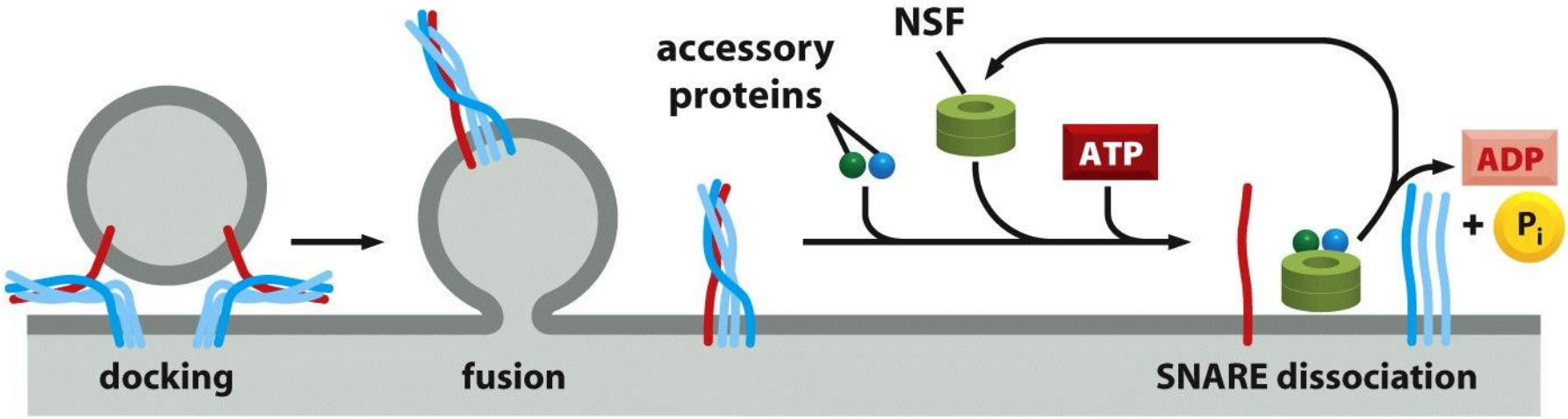


Figure 13-16 *Molecular Biology of the Cell* (© Garland Science 2008)

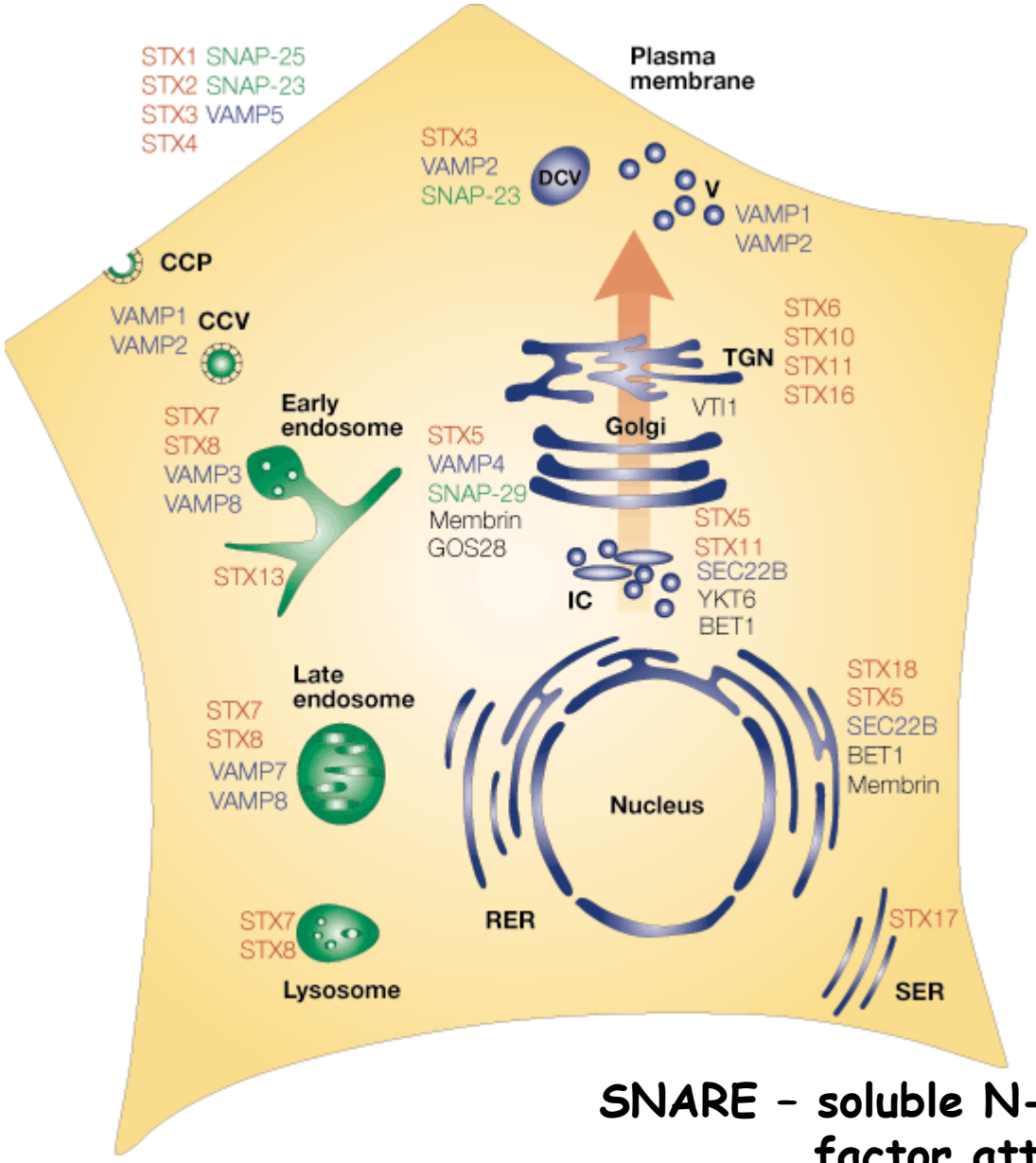
Regeneration phase of targeting system !



NSF – N-ethylmaleimide sensitive factor

Figure 13-18 *Molecular Biology of the Cell* (© Garland Science 2008)

Subcellular distribution of SNARE proteins



syntaxin

VAMP

SNAP-25

egyéb

CCP – cl.-coated pit
CCV – cl-coated vesic.
DCV – dense core vesic.
IC – intermed. comp.

SNARE – soluble N-ethylmaleimide sensitive factor attachment protein receptor

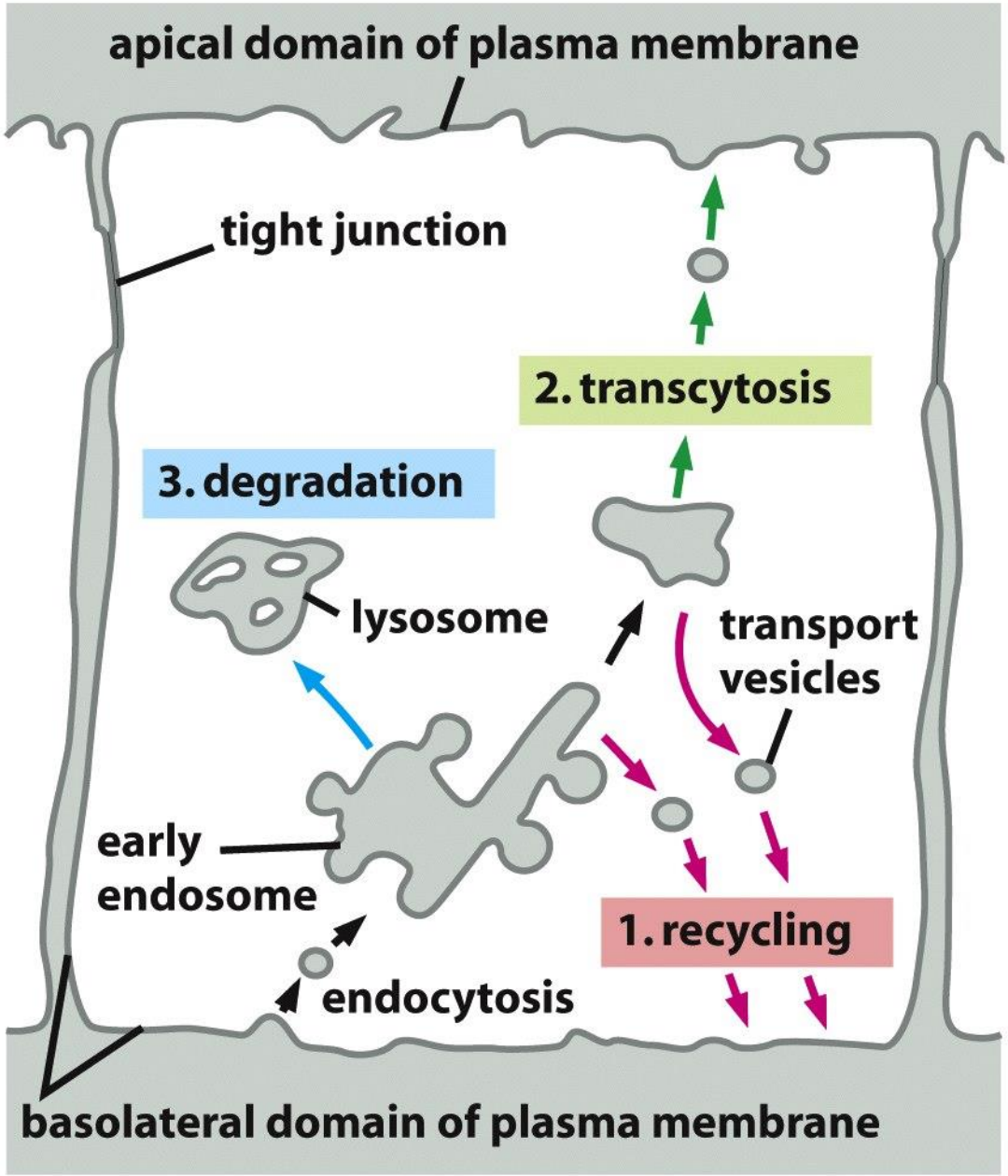
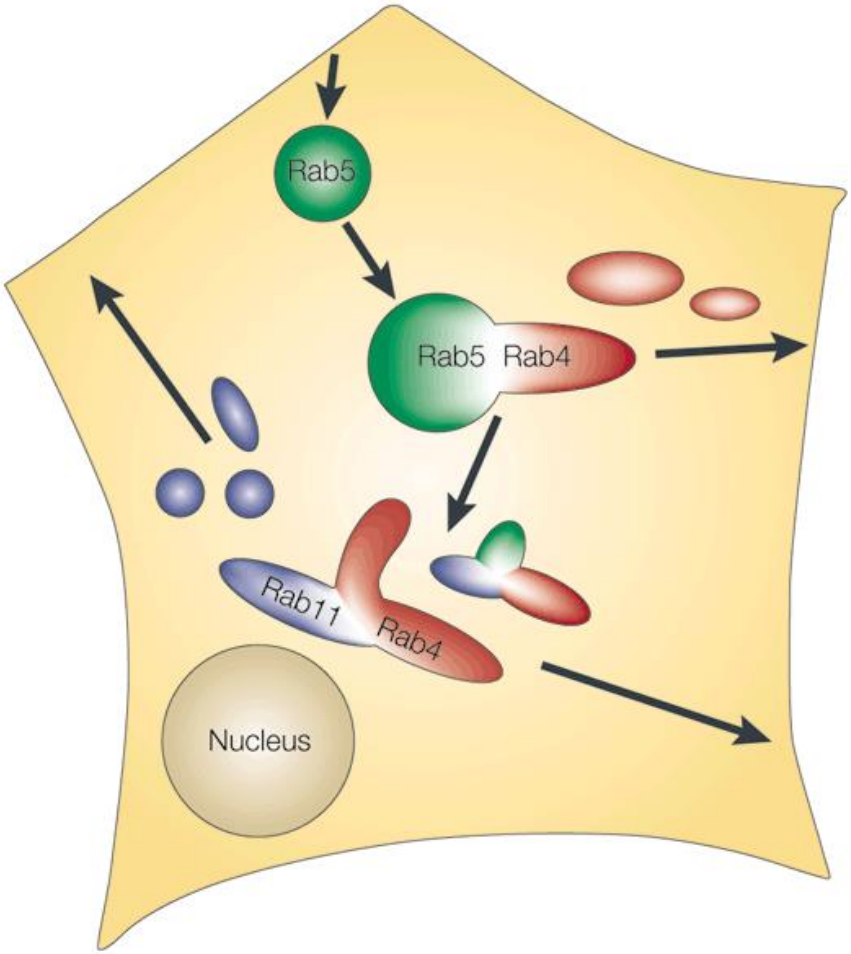


Figure 13-52 *Molecular Biology of the Cell* (© Garland Science 2008)

Chief cofactors of transport

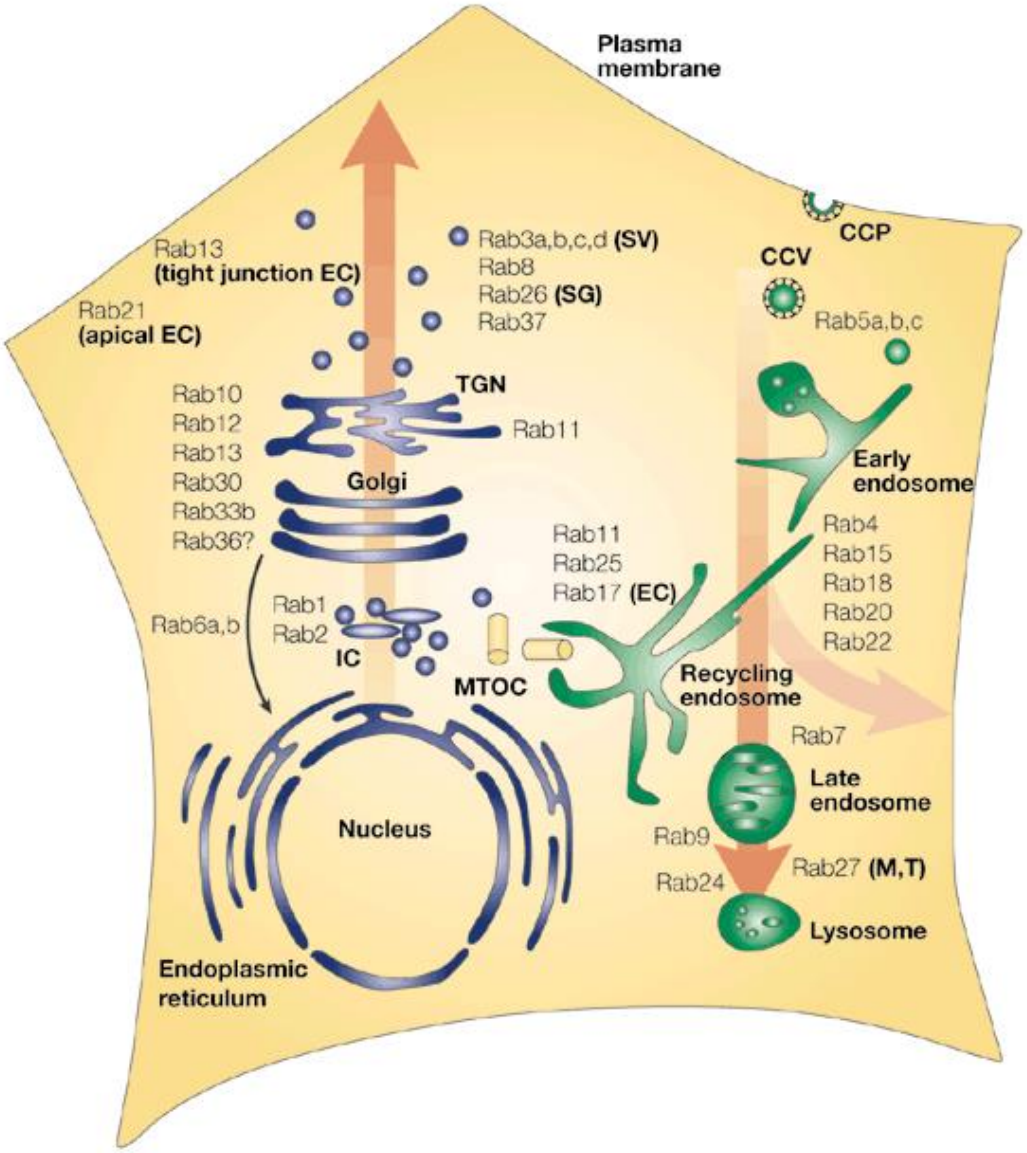
Role of G-proteins in vesicular trasport (1)



Subcellular Locations of Some Rab Proteins

PROTEIN	ORGANELLE
Rab1	ER and Golgi complex
Rab2	*cis* Golgi network
Rab3A	synaptic vesicles, secretory granules
Rab4/Rab11	recycling endosomes
Rab5A	plasma membrane, clathrin-coated vesicles, early endosomes
Rab5C	early endosomes
Rab6	medial and *trans* Golgi cisternae
Rab7	late endosomes
Rab8	early endosomes
Rab9	late endosomes, *trans* Golgi network

Role of G-proteins in vesicular trasport (2)



Exocytosis

Main steps of exocytosis

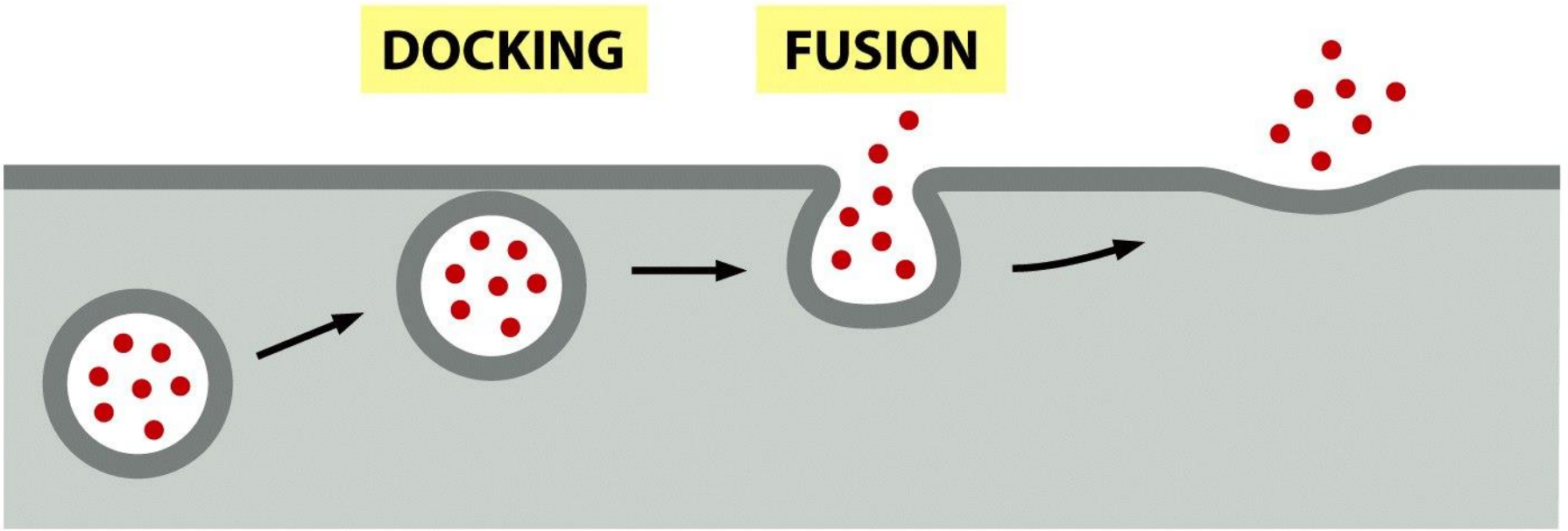


Figure 13-66a *Molecular Biology of the Cell* (© Garland Science 2008)

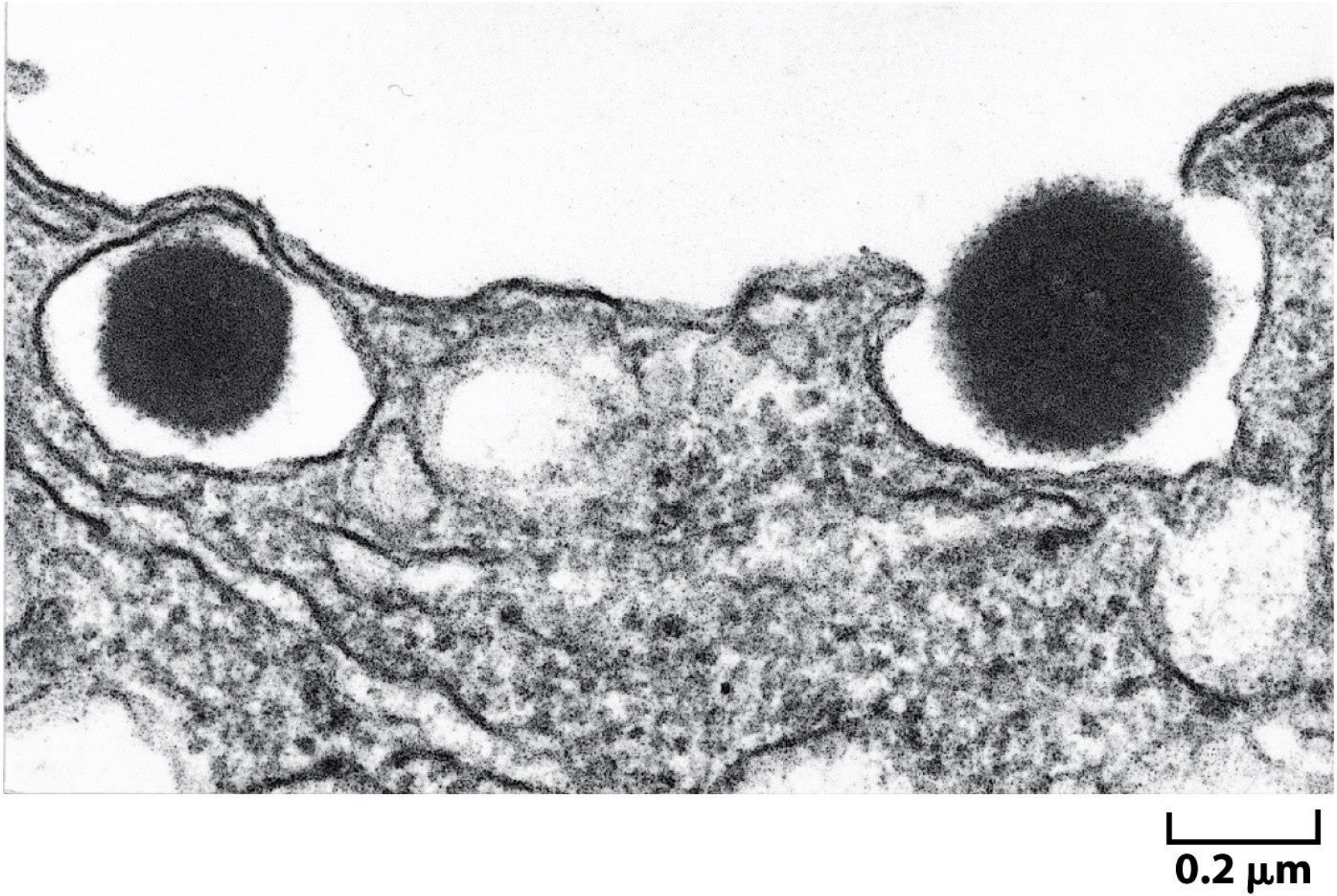


Figure 13-66b *Molecular Biology of the Cell* (© Garland Science 2008)

Membrane removal in cargo concentration

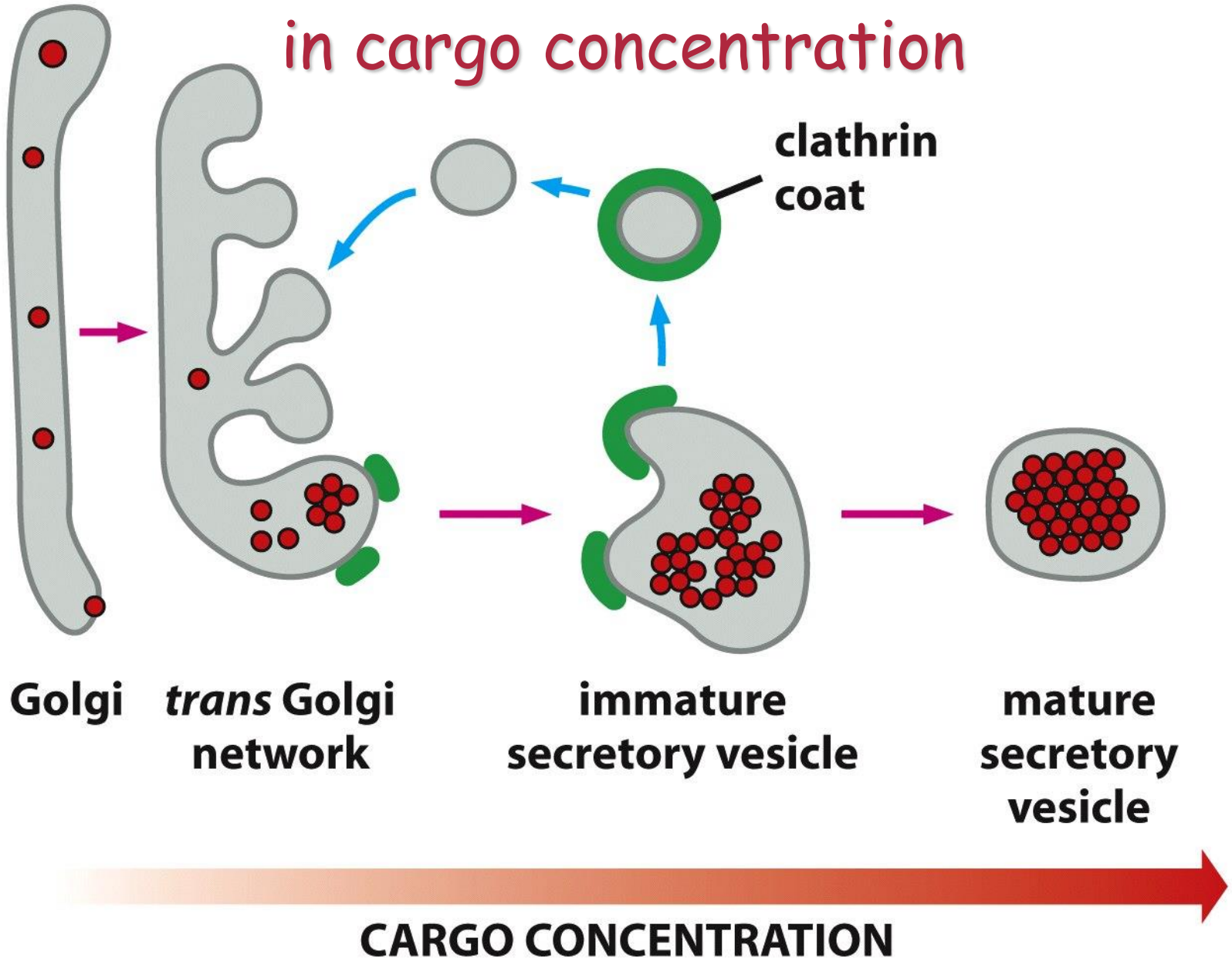


Figure 13-65a *Molecular Biology of the Cell* (© Garland Science 2008)

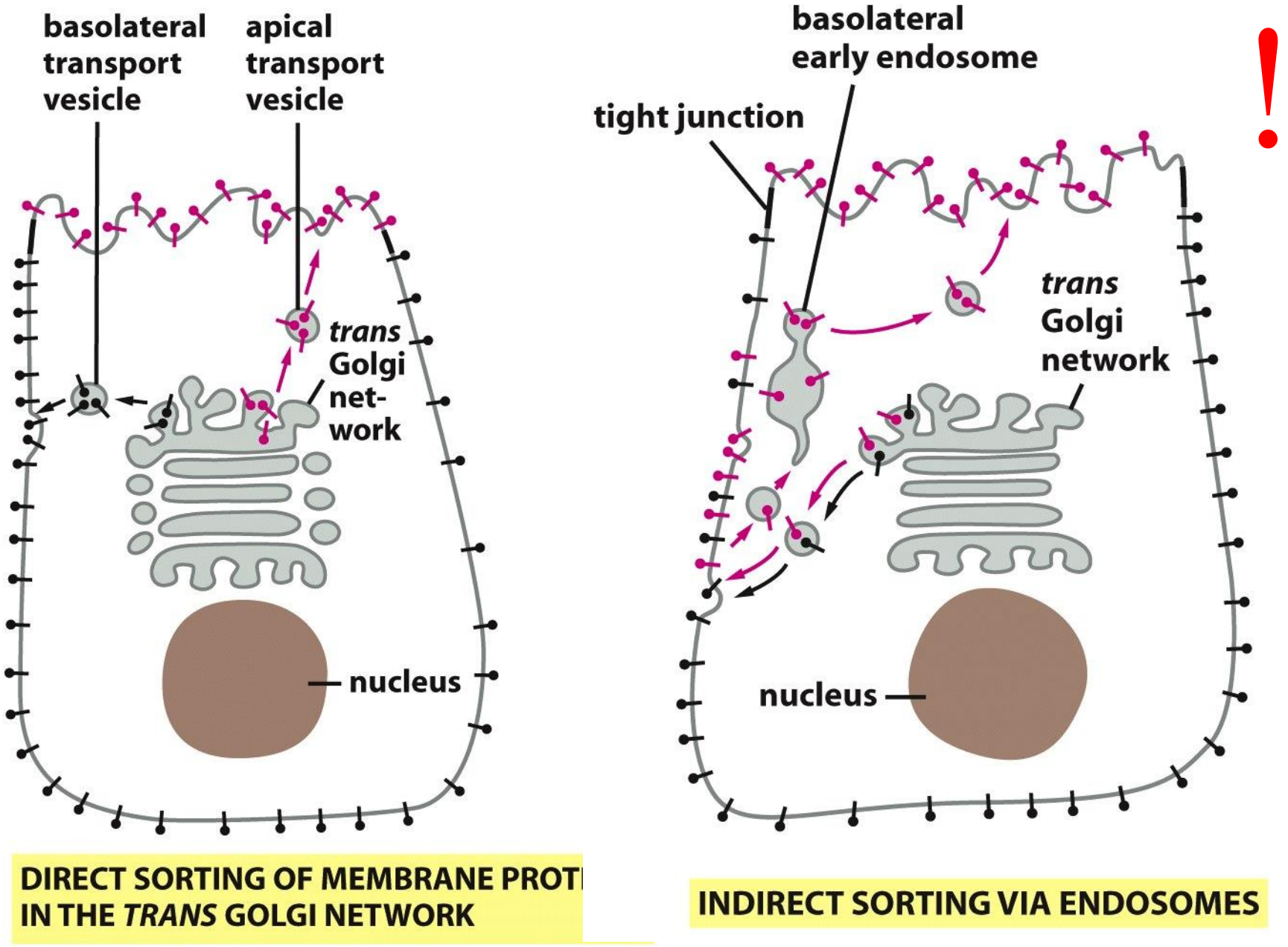


Figure 13-72a *Molecular Biology of the Cell* (© Garland Science 2008)

Appendix

Information on the next slides are only to inform students about the subjetc, they do not belong to the core subjects of Cell Science.

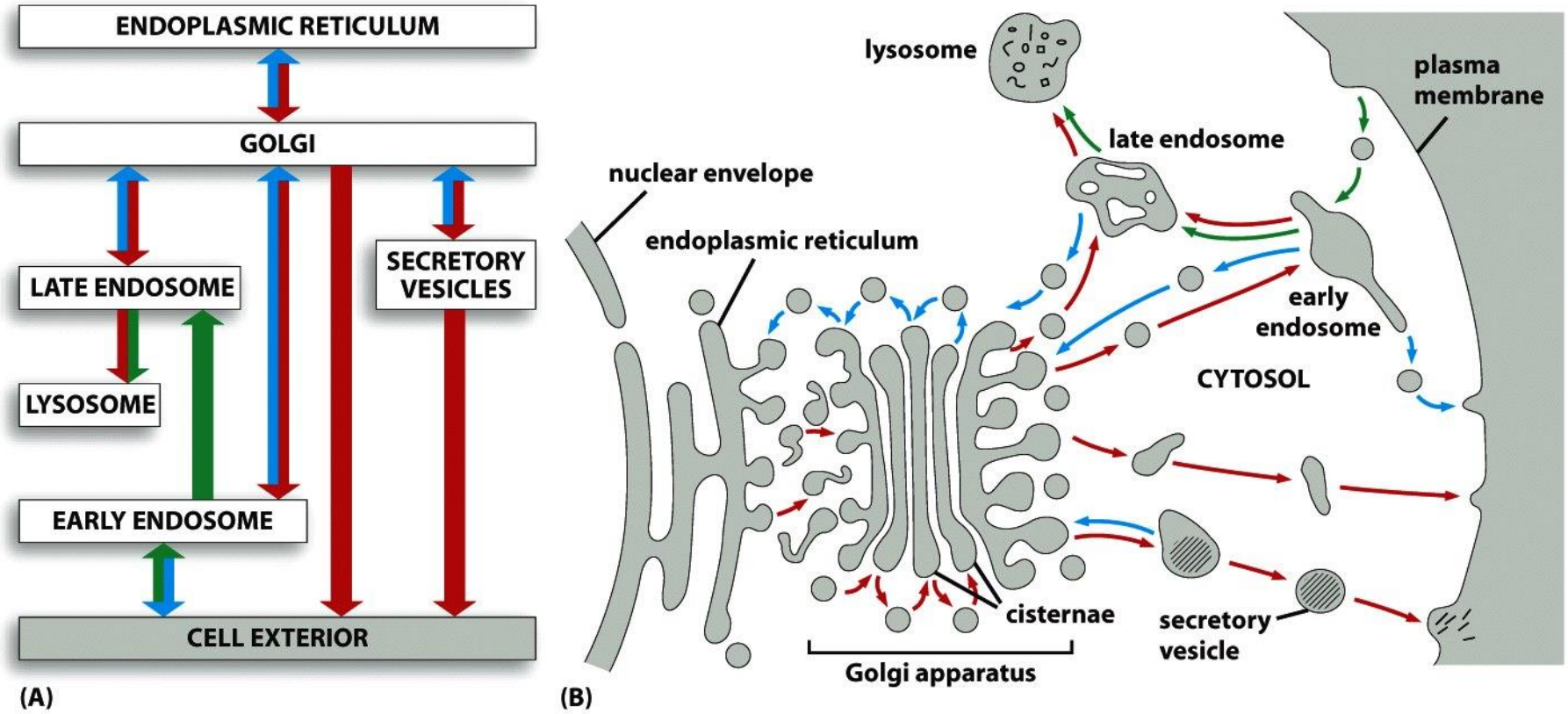
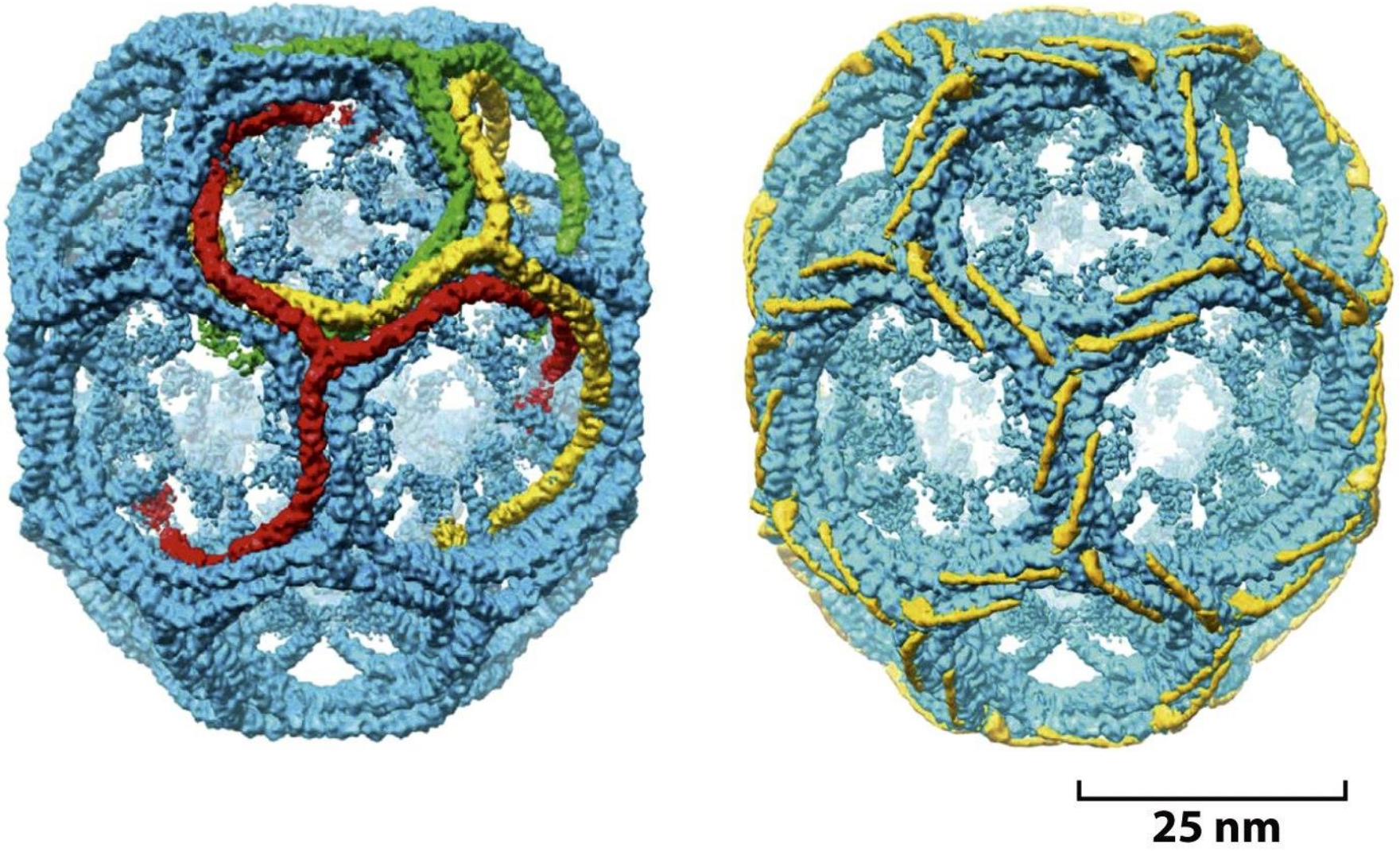


Figure 13-3 *Molecular Biology of the Cell* (© Garland Science 2008)

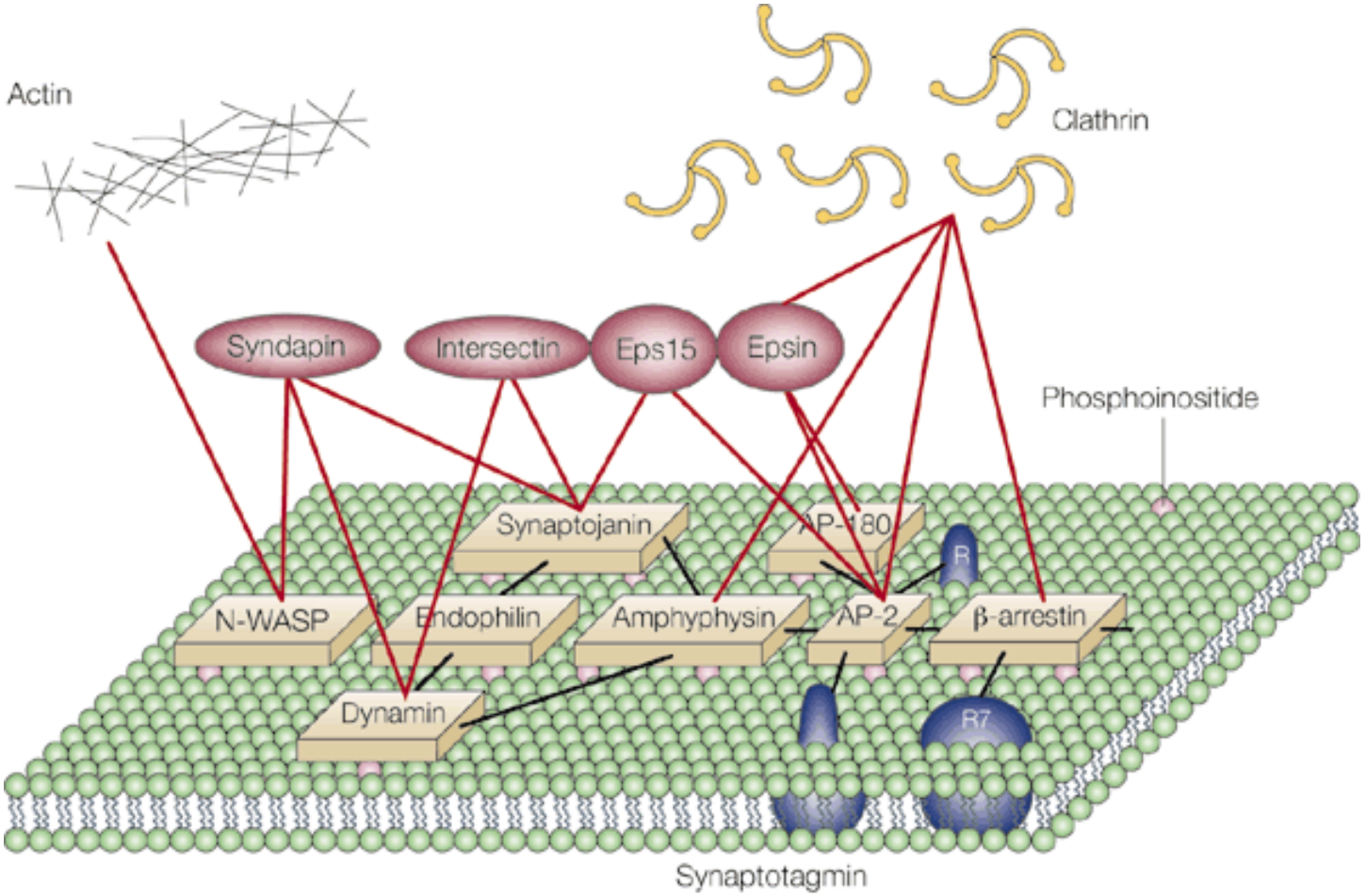
Clathrin-coat
Position of heavy and light chains in the wall



Comparison of clathrin composition

Light chain (LC)	
Homo sapiens	100% / 228 aa
R. norvegicus	93% / 237 aa
C. elegans	25% / 222 aa
Heavy chain (HC)	
H. sapiens	100% / 1674 aa
R. norvegicus	100% / 1674 aa
C. elegans	71% / 1666 aa
D. melanogaster	80% / 1662 aa
S. cerevisiae	50% / 1642 aa

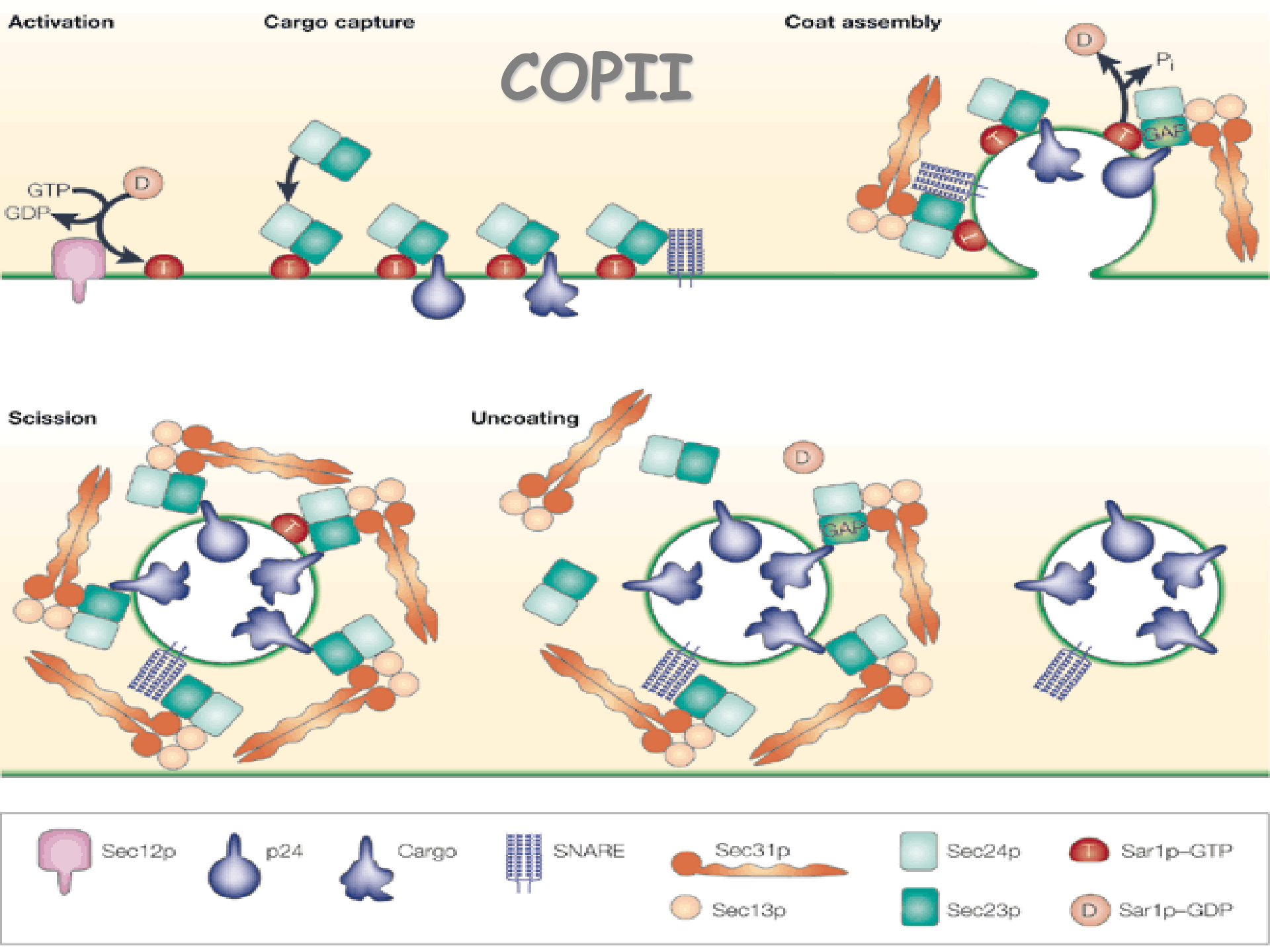
Protein-protein and protein-lipid associations under clathrin coat

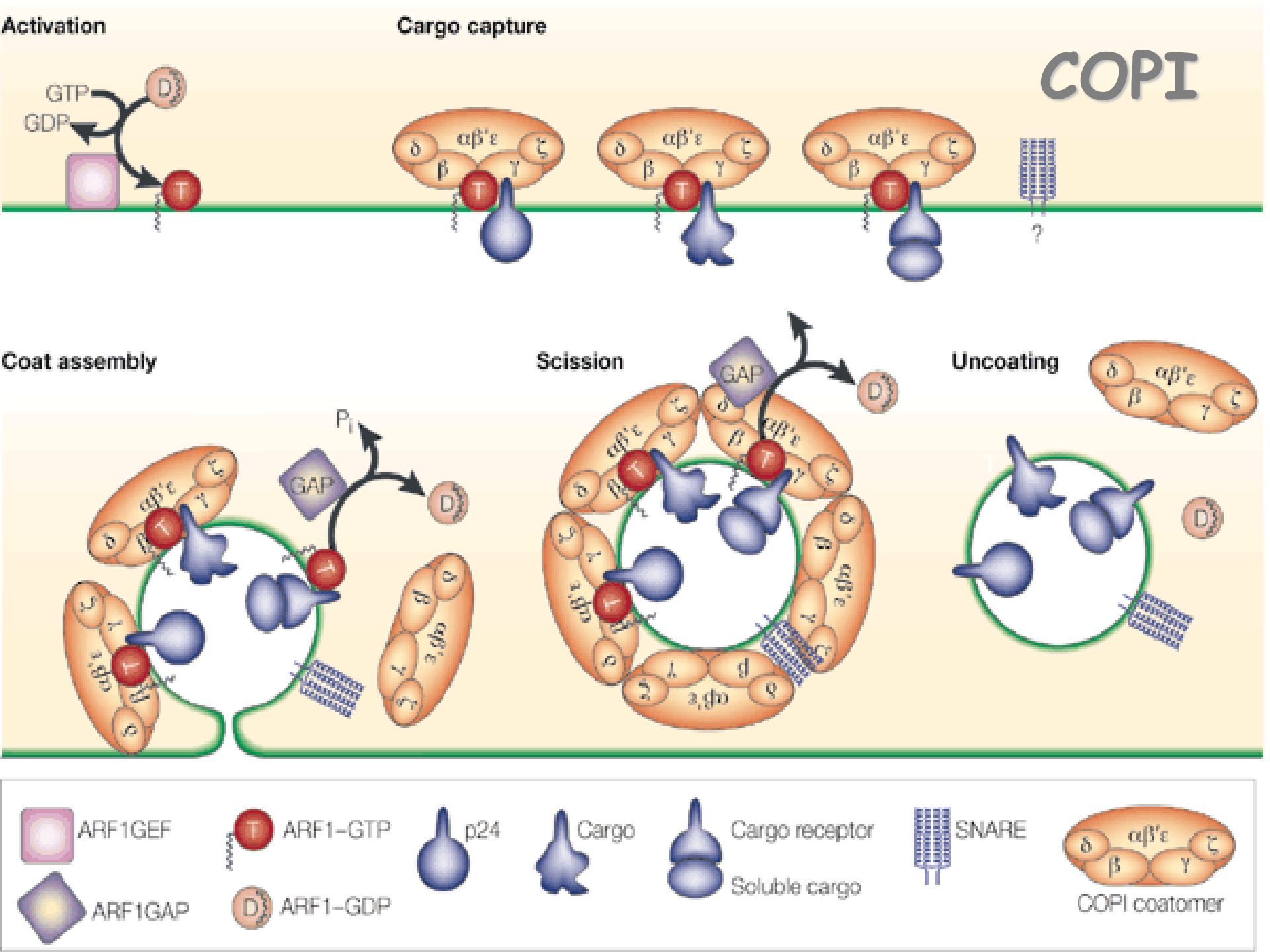


Clathrin binding proteins in Mammals

AP-1	Ankyrin
AP-2	β-Arrestin
AP-3	β-Arrestin 2
Amphiphysin 1	Epsin 1
Amphiphysin 2	Epsin 2
ACK1	GGA1
Auxilin	GGA2
Auxilin 2	GGA3
Synaptojanin 1	AP180
PI3K-C2α	CALM

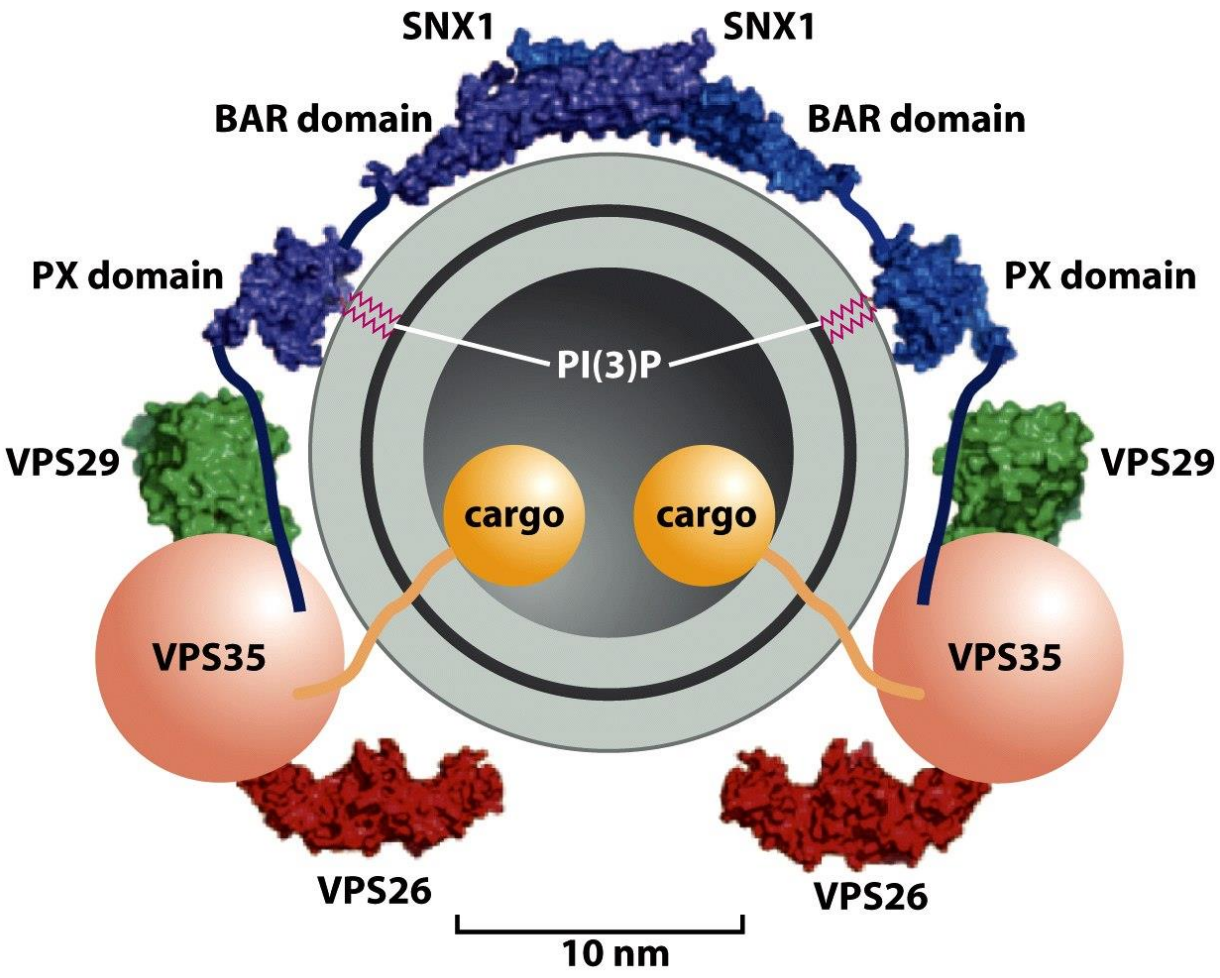
COPII


Activation
Cargo capture
Coat assembly
GTP
GDP
D
T
GAP
P_i
Scission
Uncoating
Sec12p
p24
Cargo
SNARE
Sec31p
Sec13p
Sec24p
Sec23p
Sar1p–GTP
Sar1p–GDP


Activation
Cargo capture
COPI
GTP
GDP
D
T
αβ'ε
δ
β
γ
ζ
?
Coat assembly
Scission
Uncoating
Pi
GAP
ARF1GEF
ARF1GAP
ARF1–GTP
ARF1–GDP
p24
Cargo
Cargo receptor
Soluble cargo
SNARE
COPI coatomer

Coating molecular complex of retromer

- Retrograde transport of proteins from endosomes to the TGN -



Homotypic fusion

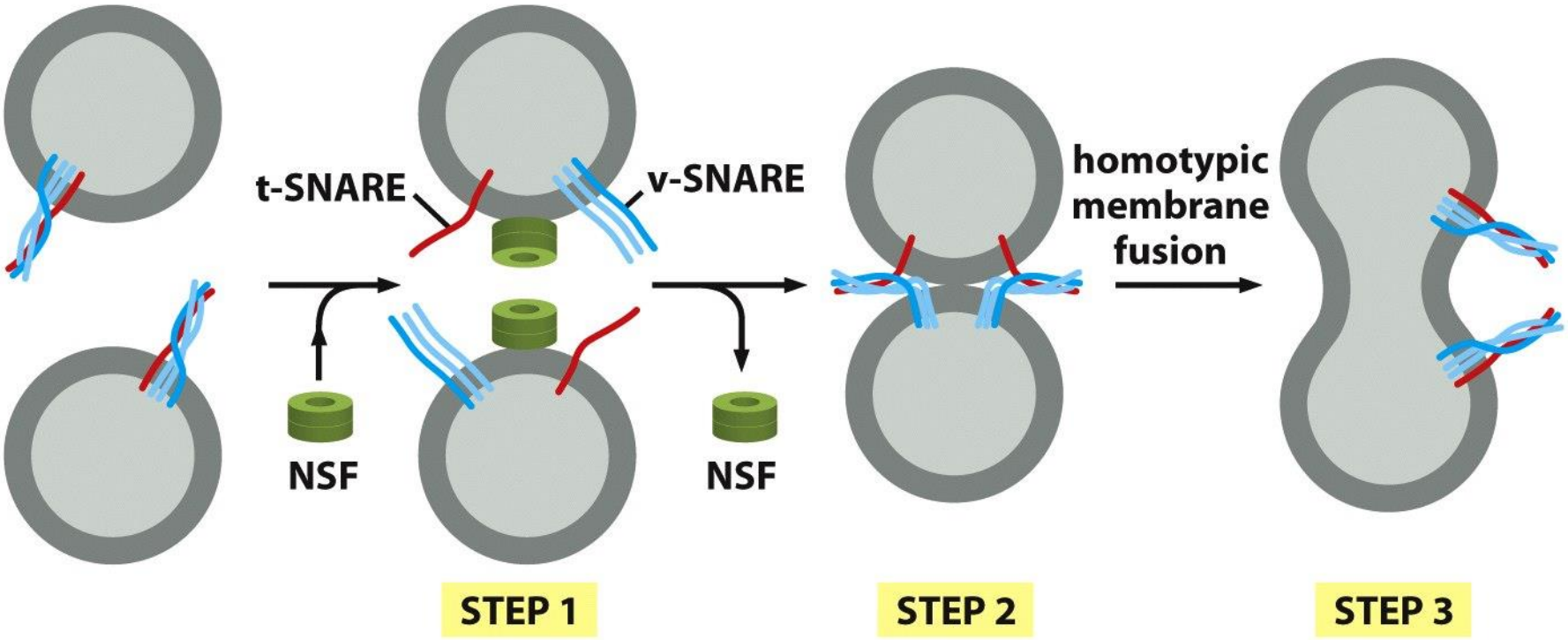


Figure 13-22 *Molecular Biology of the Cell* (© Garland Science 2008)

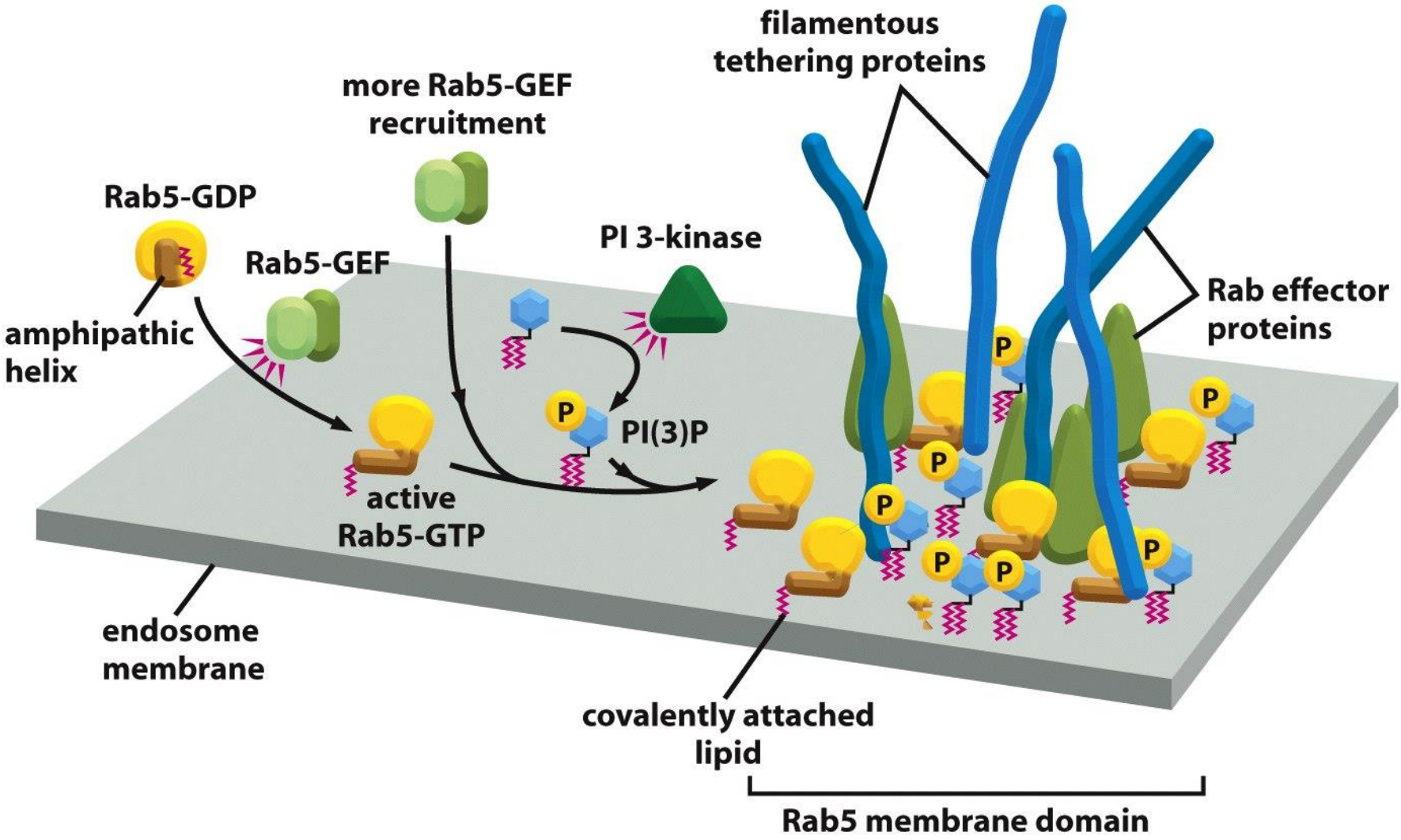


Figure 13-15 *Molecular Biology of the Cell* (© Garland Science 2008)

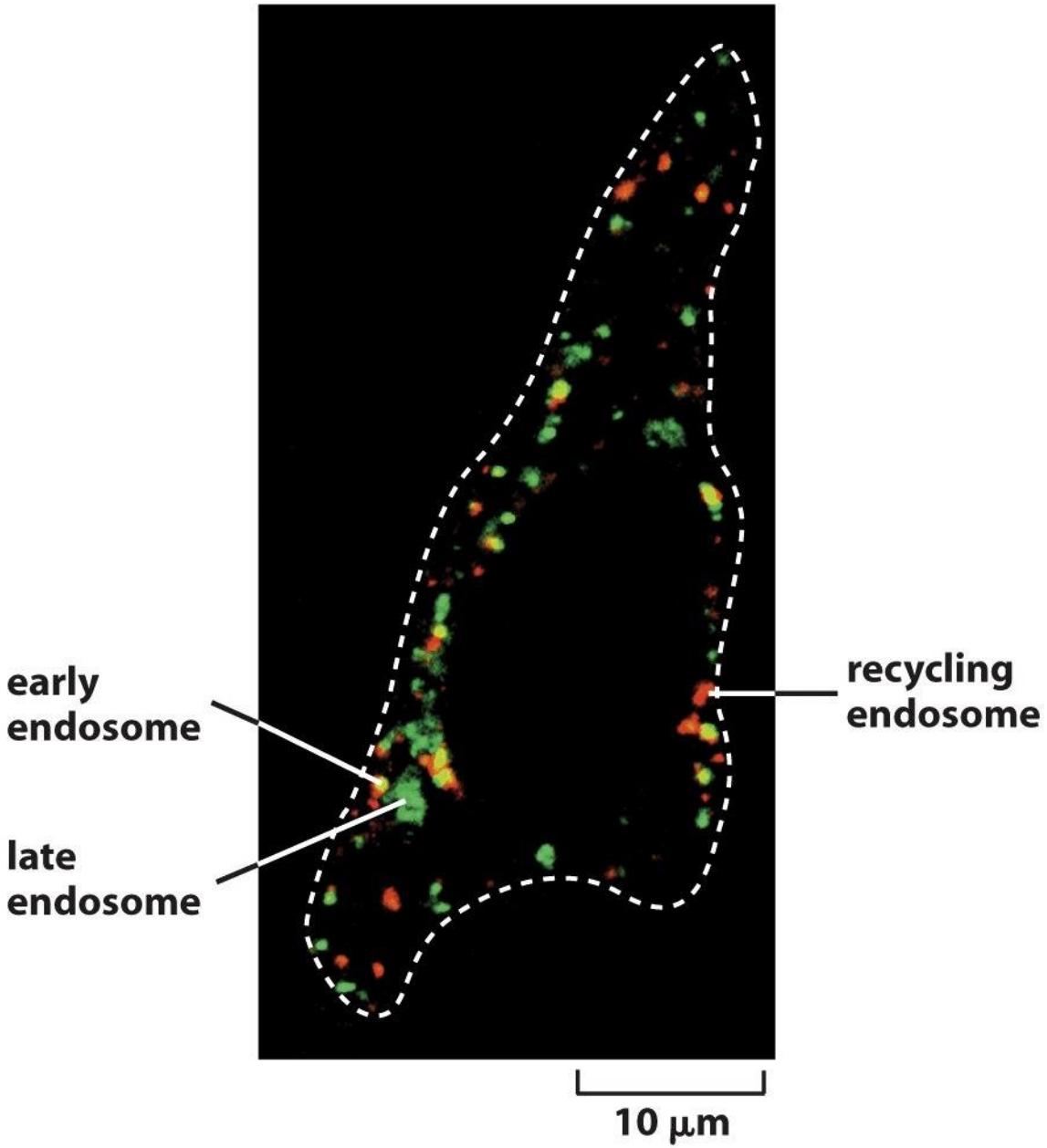
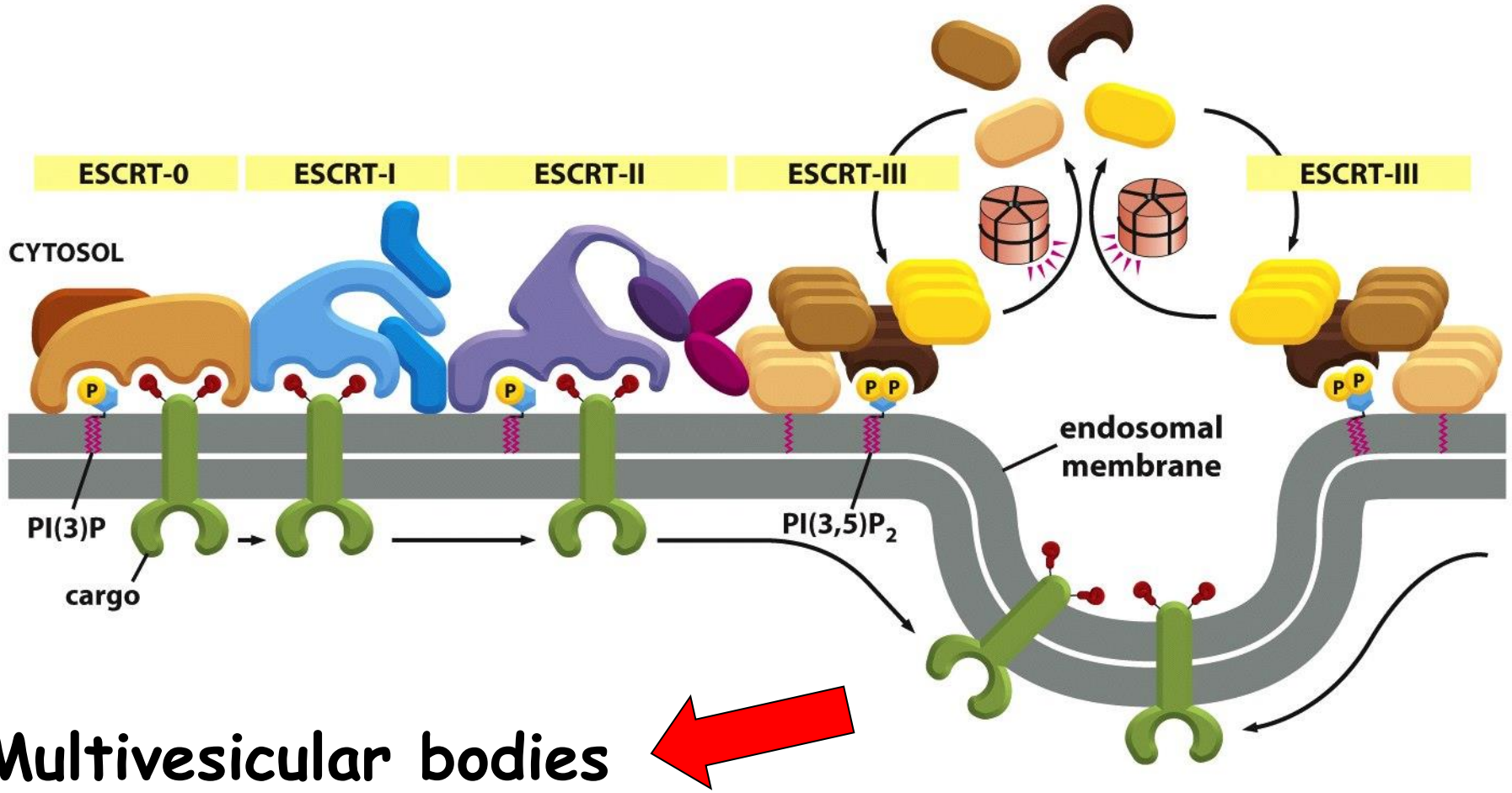


Figure 13-54 *Molecular Biology of the Cell* (© Garland Science 2008)

Endosomal sorting of ubiquitinated cargo proteins

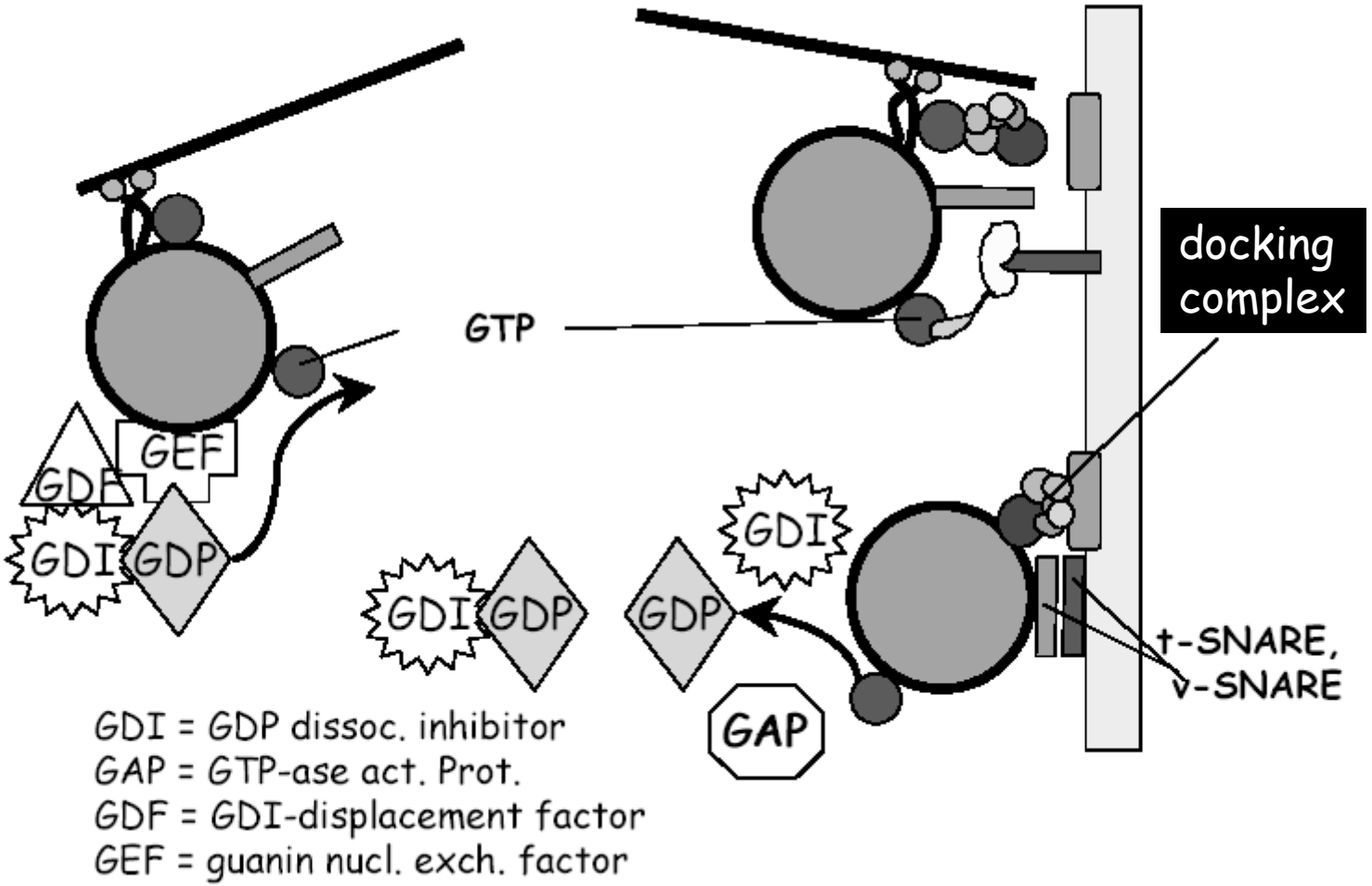


Multivesicular bodies

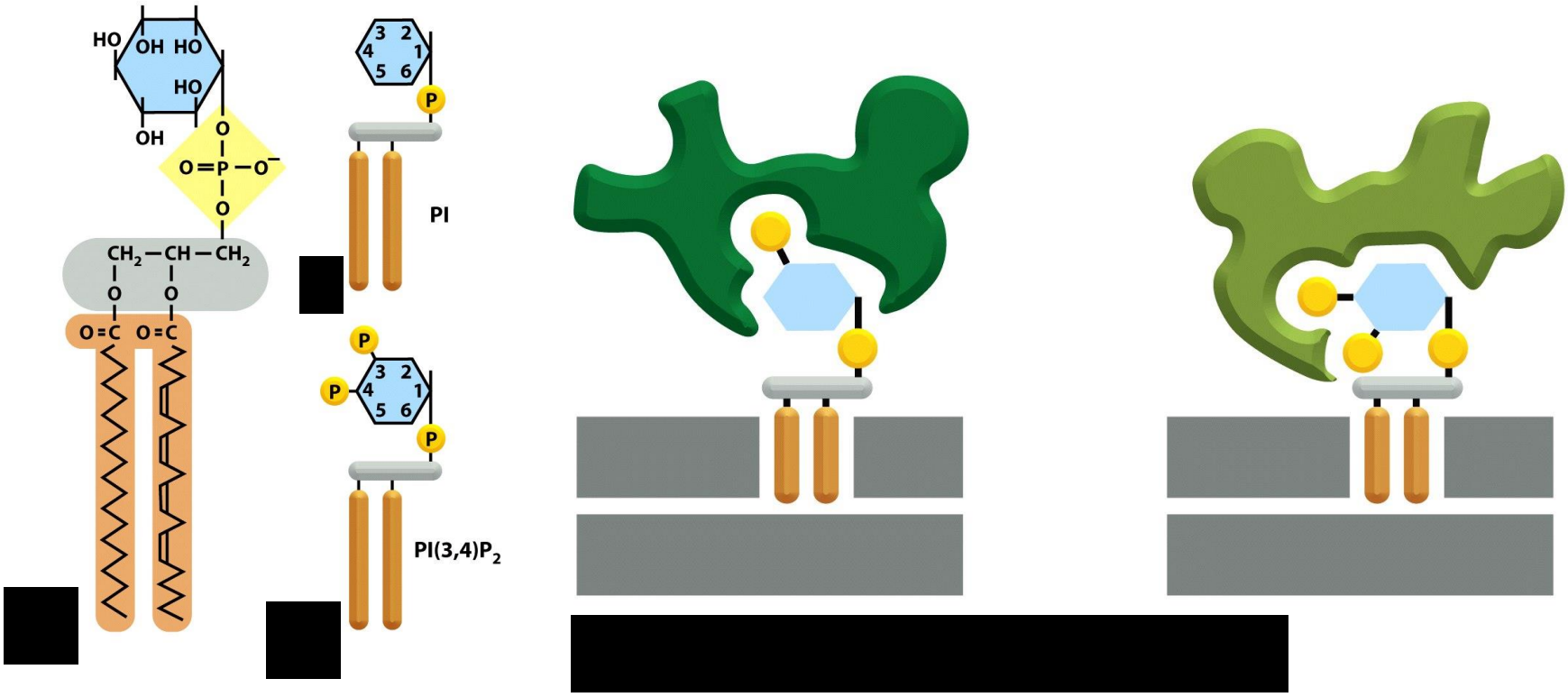
ESCRT - endosomal sorting complex required for transport

Figure 13-58 *Molecular Biology of the Cell* (© Garland Science 2008)

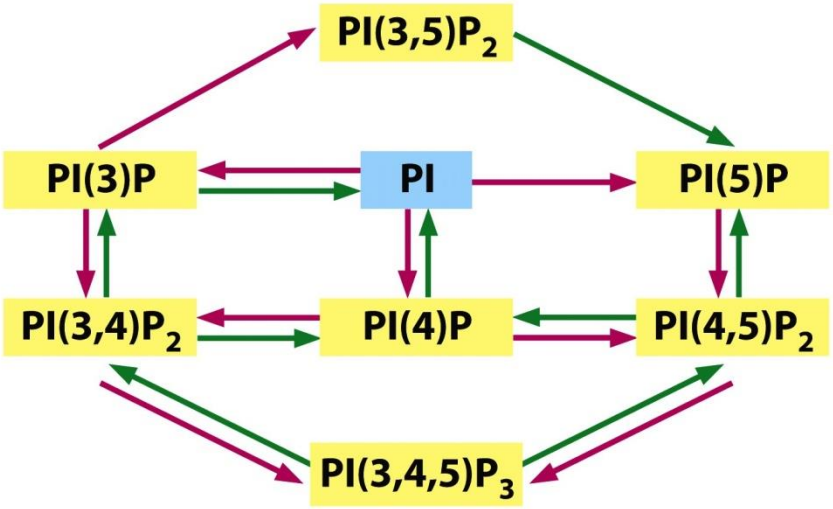
Rab-cycle

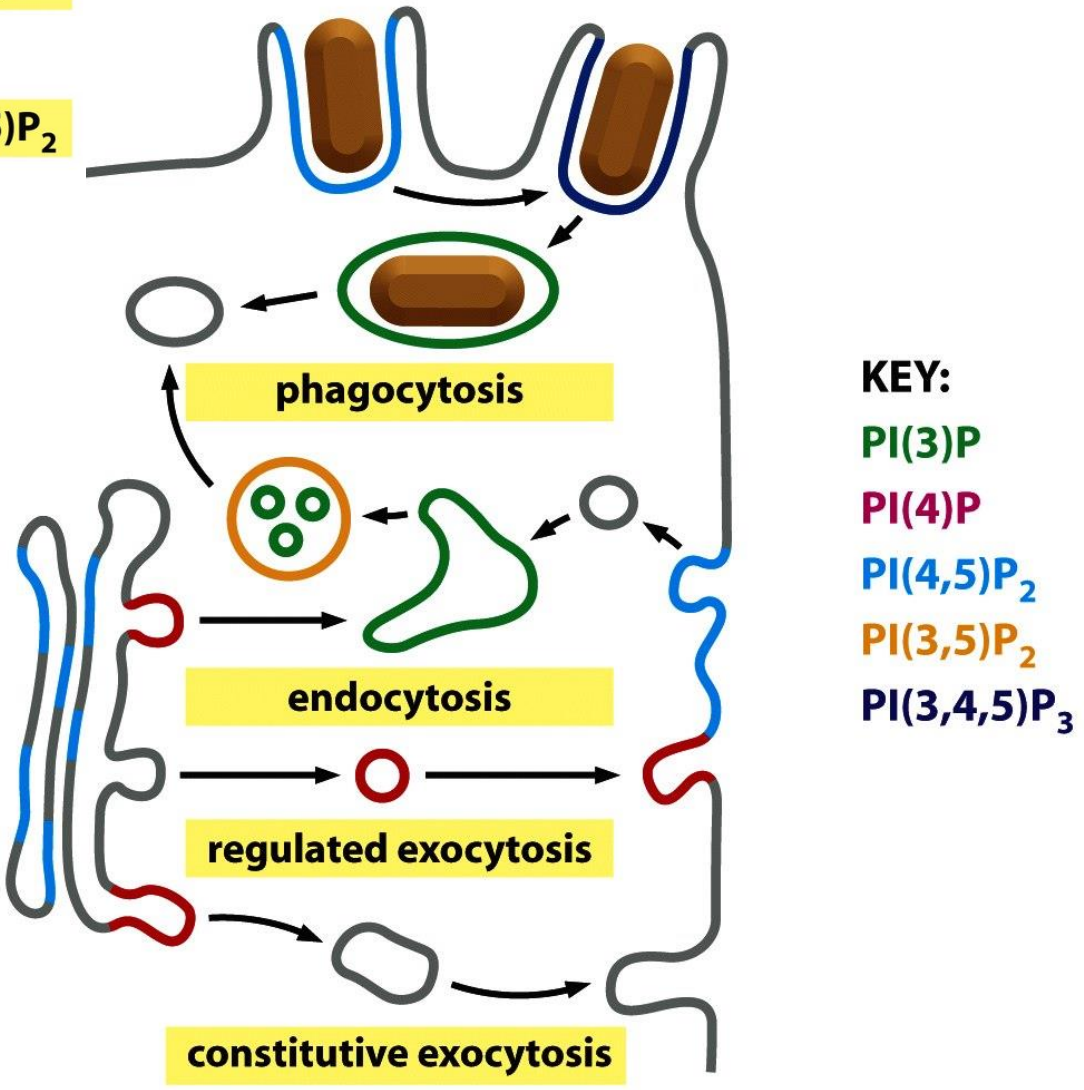

docking complex
GTP
GEF
GDF
GDI
GDP
GDI
GDP
GDI
GDP
GAP
t-SNARE,
v-SNARE
GDI = GDP dissoc. inhibitor
GAP = GTP-ase act. Prot.
GDF = GDI-displacement factor
GEF = guanin nucl. exch. factor

Significance of phosphatidylinositol phosphates (1)



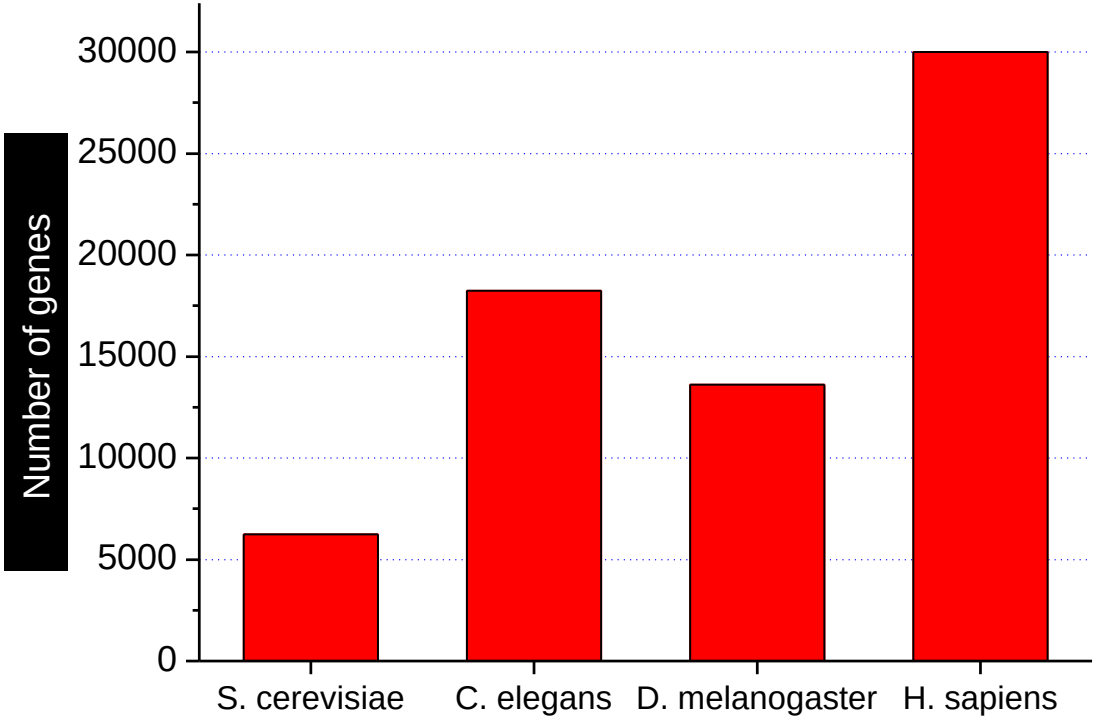
Significance of phosphatidylinositol phosphates (2)



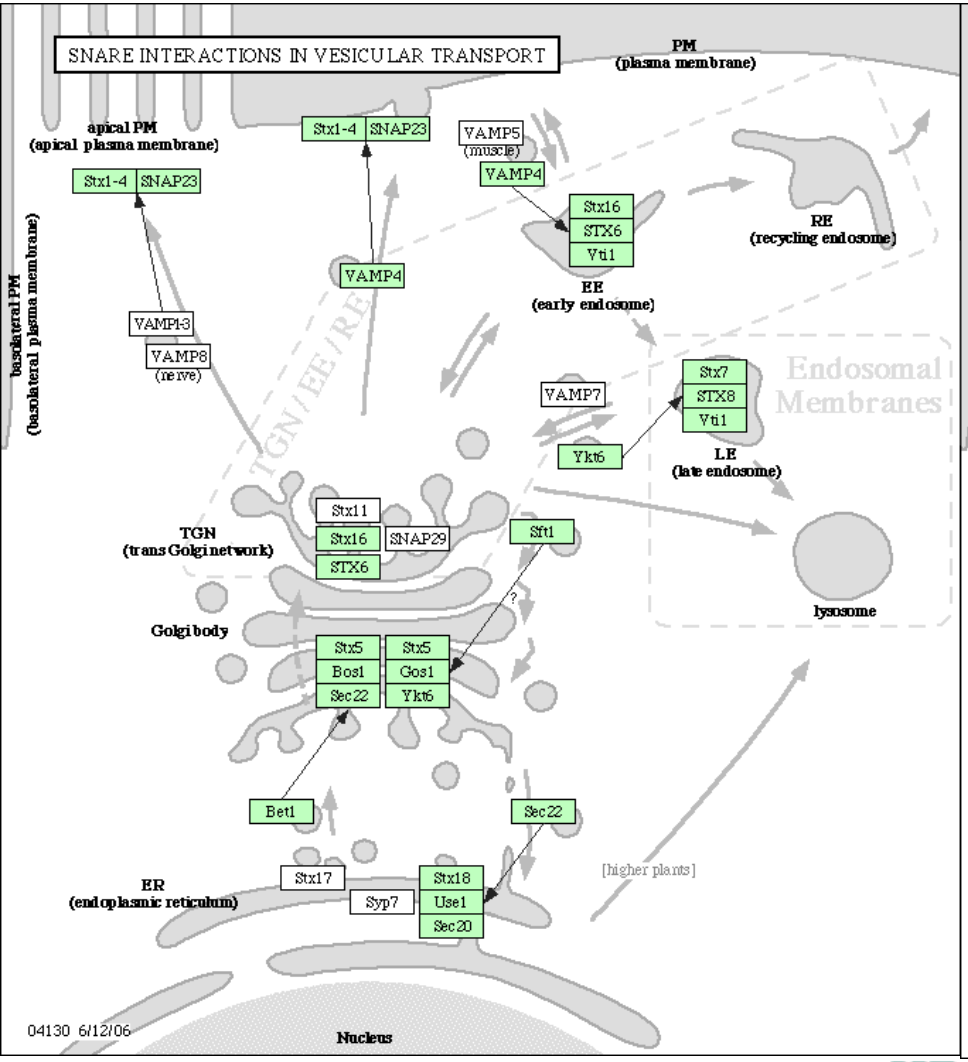


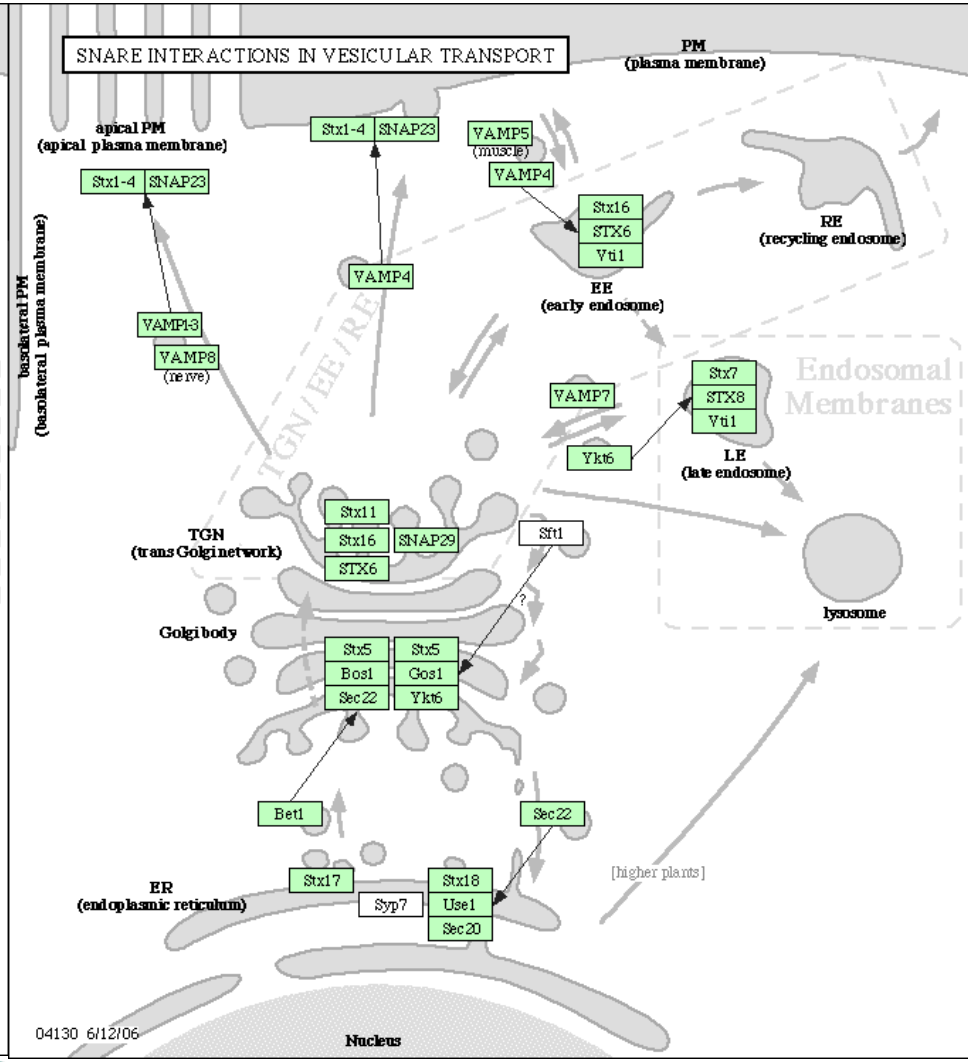
Phylogeny of vesicular transport

Number of genes responsible for vesicular transport Phylogenetical approaches



Evolution of SNARE-s





KEGG