

# Lecture 7 Macromolecules transport II

## Vesicular Traffic

### Outline

- I. Overview of vesicular traffic
- II. Techniques to study vesicular traffic
- III. Transport from ER to Golgi apparatus
- IV. Transport from trans-Golgi to lysosome
- V. Endocytosis
- VI. Exocytosis

# I. Overview of vesicular traffic

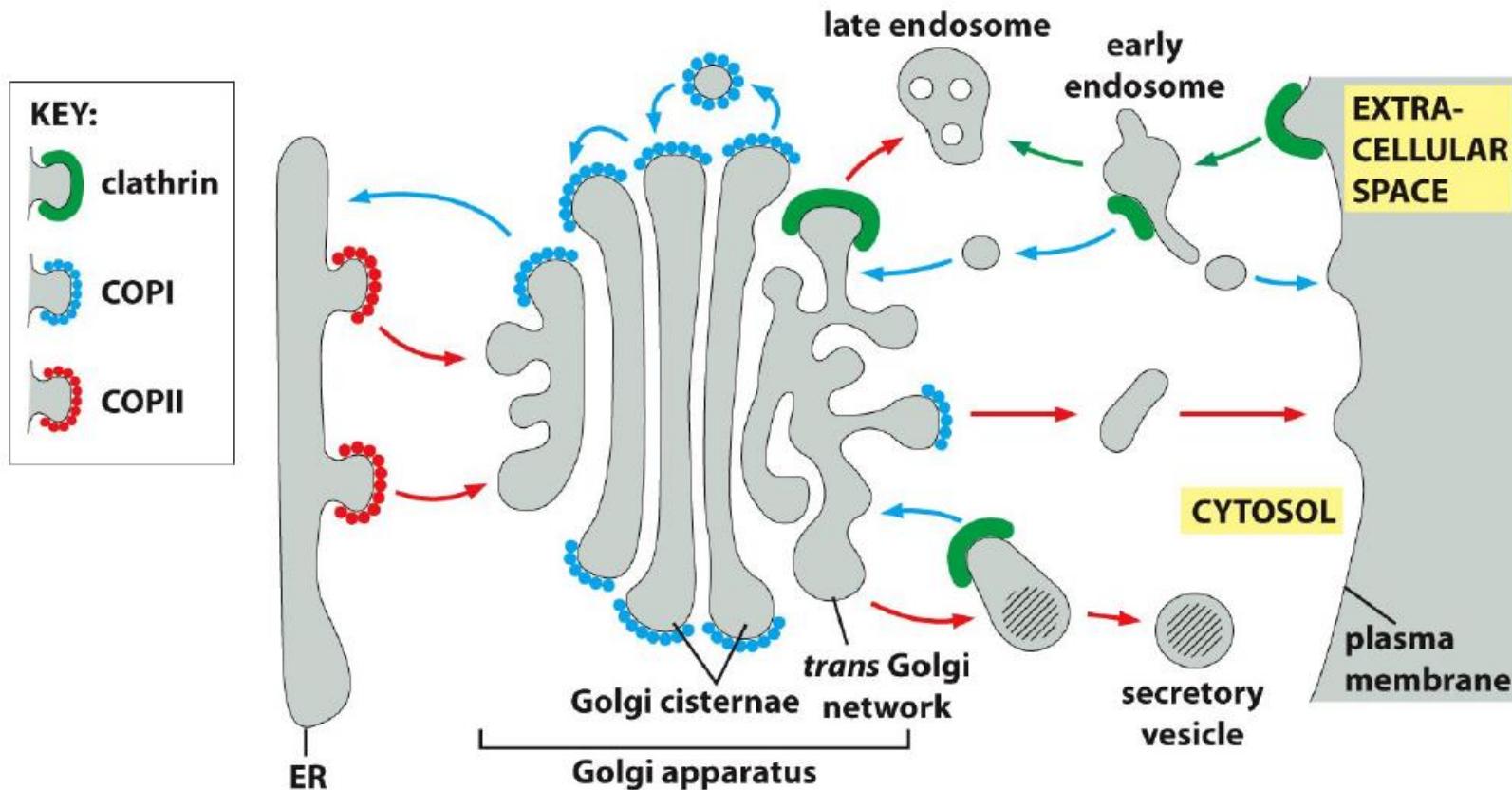
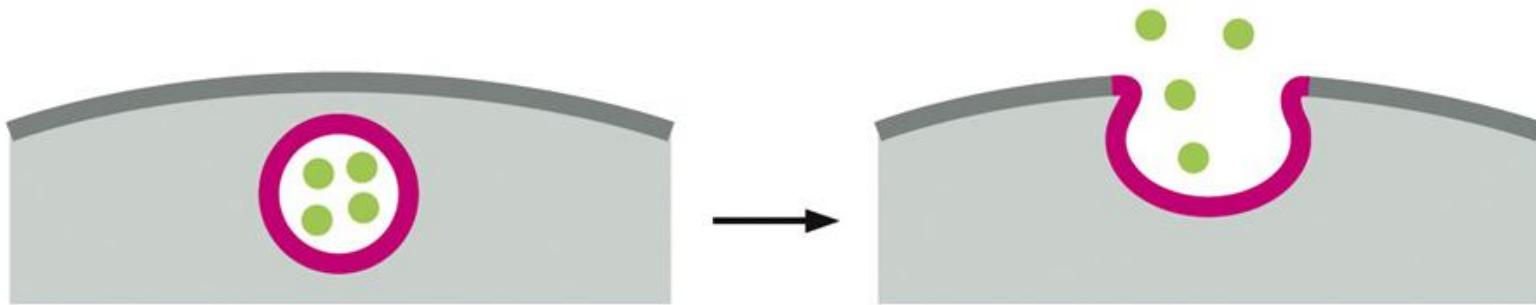
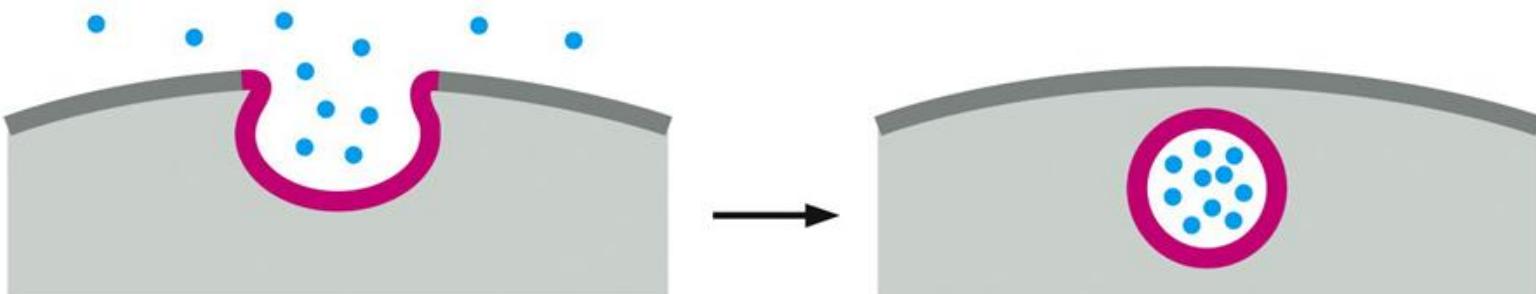


Figure 13-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Exocytosis and endocytosis



**(A) exocytosis**



**(B) endocytosis**

# Membrane orientation does not change during vesicular transport

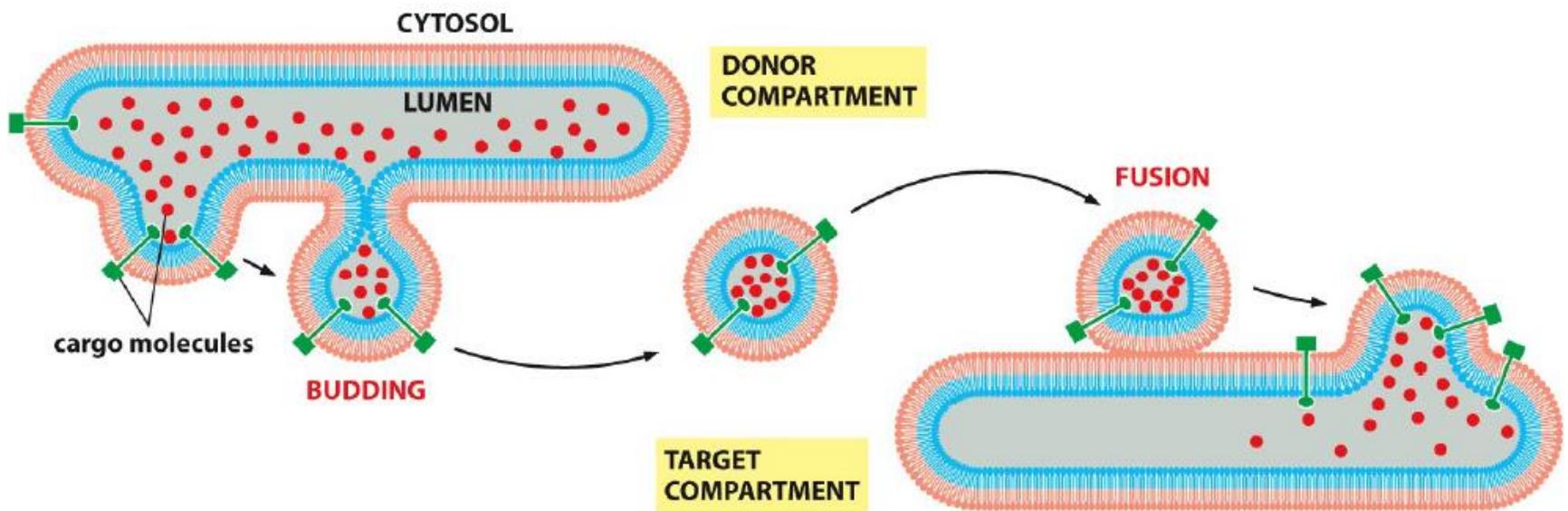


Figure 13-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

# What molecules undergo exocytosis in vesicular traffic ?

- ♥ Plasma membrane proteins:

- Cell surface receptors
  - transporters
  - ion channels

- ♥ Soluble proteins:

- digestive enzymes
  - peptide hormones
  - serum proteins
  - collagen
  - ECM proteins , etc

- ♥ From endosome to lysosome:

- unneeded proteins,
  - some amino acid for storage,
  - proteases, glycosidases, phosphatases, lipases,
  - lysosome membrane V-type proton pump, etc

# What molecules undergo endocytosis in vesicular traffic?

- ♥ Large nutrient macromolecules that are too large to be transported.

Such as:

- cholesterol-LDL

- iron-transferrin

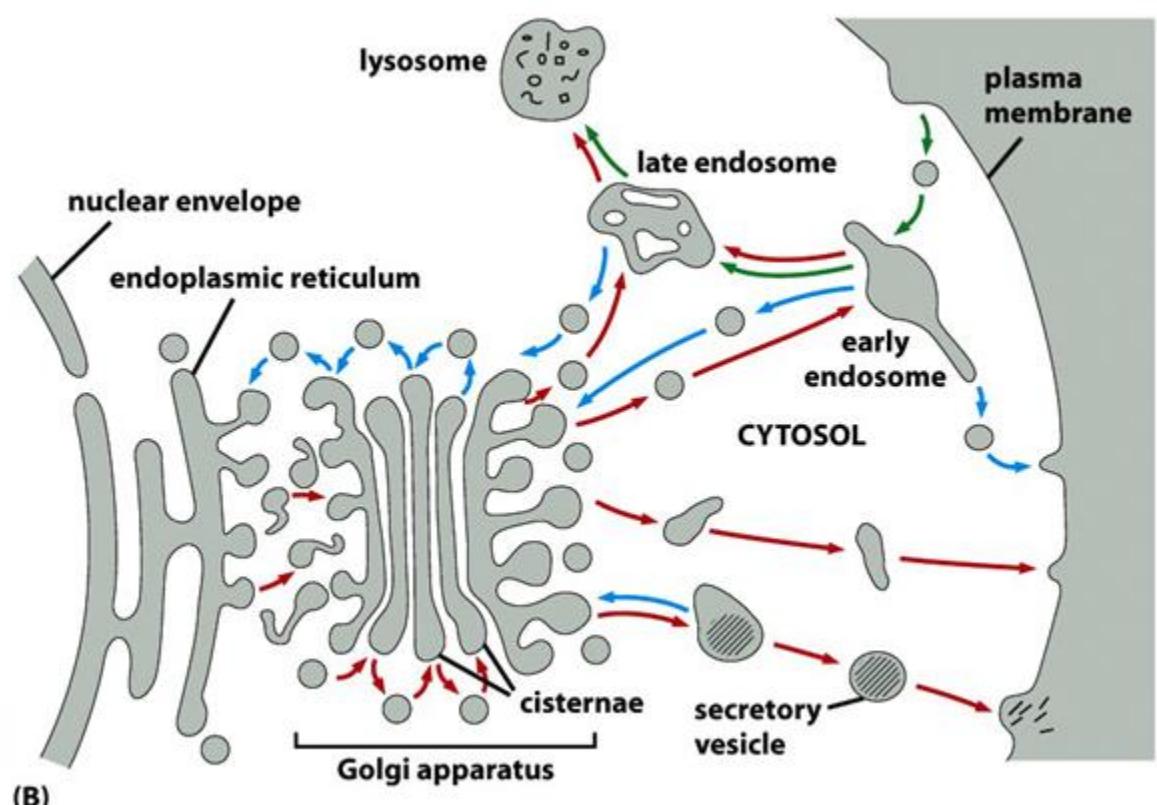
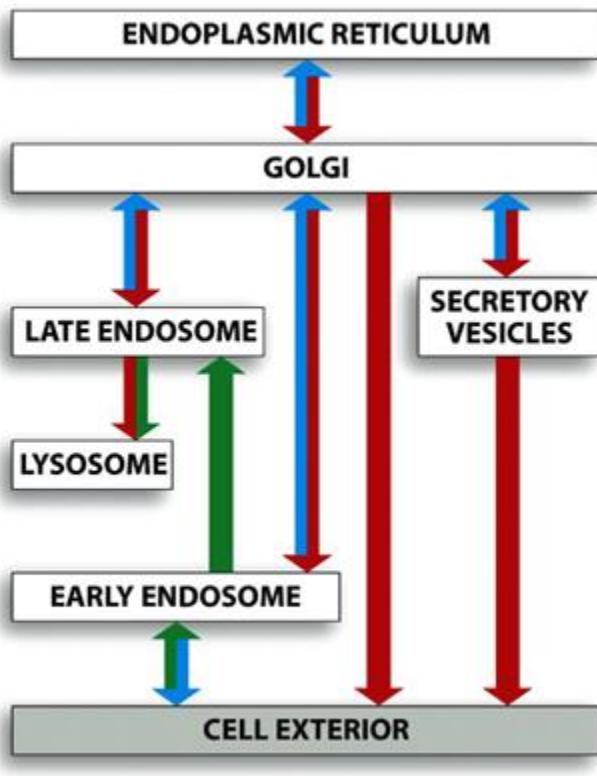
- vitamins, etc.

- ♥ Remove membrane receptors to down-regulate their activities.

- ♥ Some bacteria that are internalized into cells

- ♥ Some virus

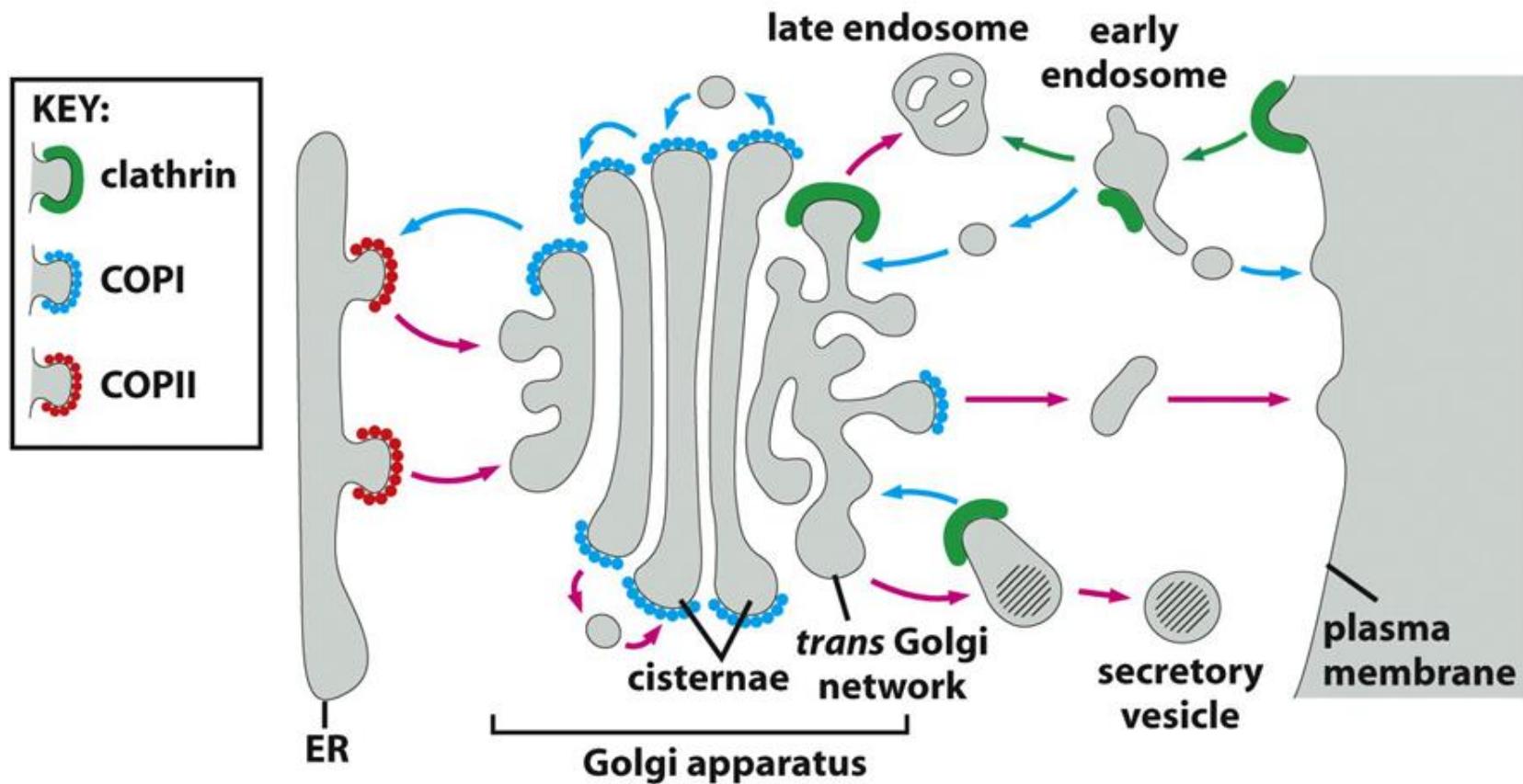
# Vesicular traffic all mediated by Golgi apparatus



Blue--- retrieval pathway  
Red--- secretory pathway  
Green--- endocytic pathway

Transport vesicle can be spherical and tubular

# Budding vesicle as dictated by special coating protein



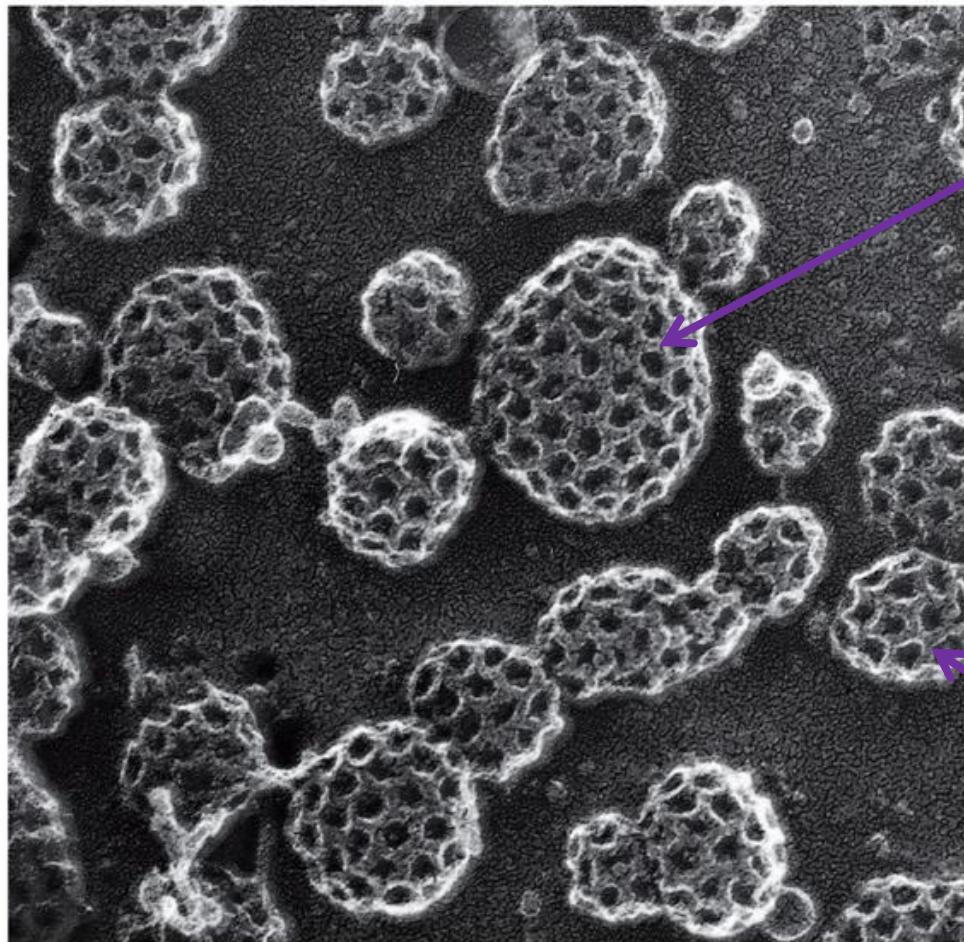
## Three major types of coating proteins

- **Clathrin**: transport proteins from the plasma membrane and the trans-Golgi network to late endosomes
- **COP I**: Transport proteins in the retrograde direction between Golgi cisternae and from the cis-Golgi back to the ER.
- **COP II**: Transport protein from the ER to Golgi

Main functions for the coating protein:

- ♥ Concentrate specific proteins for transport
- ♥ Molds the vesicle, assemble the membrane to curved basketlike lattice for budding

# Clathrin-coated pits and vesicles

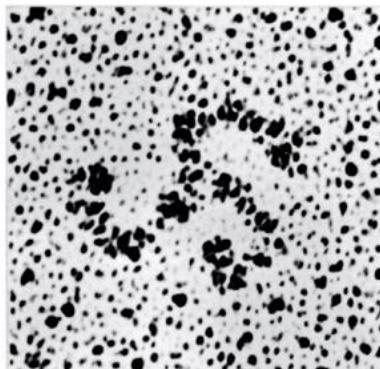
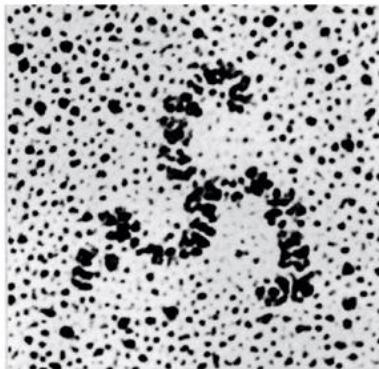


clathrin coat

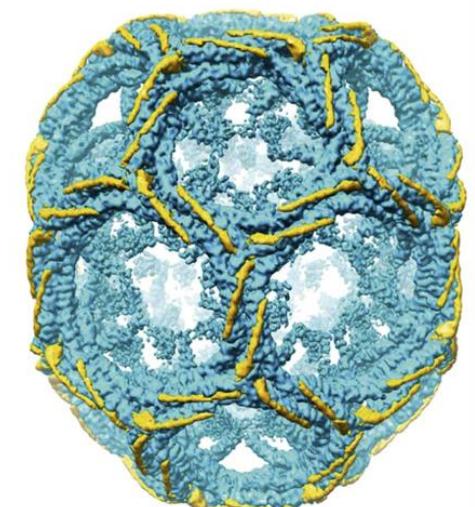
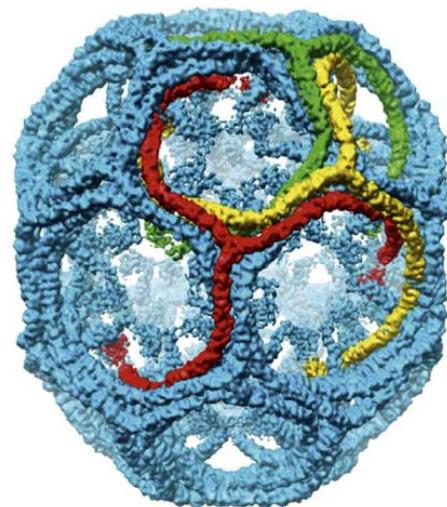
Electron Microscope (using Rapid-freeze and deep etch) images depicting clathrin-coated pits and vesicles in the inner membrane of fibroblasts.

0.2  $\mu\text{m}$

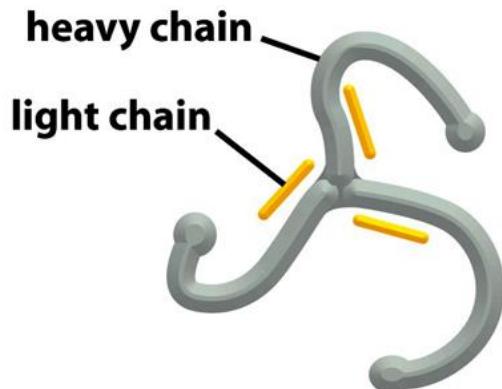
# Clathrin consists of 3 large and 3 small polypeptide chains---triskelion



(A)

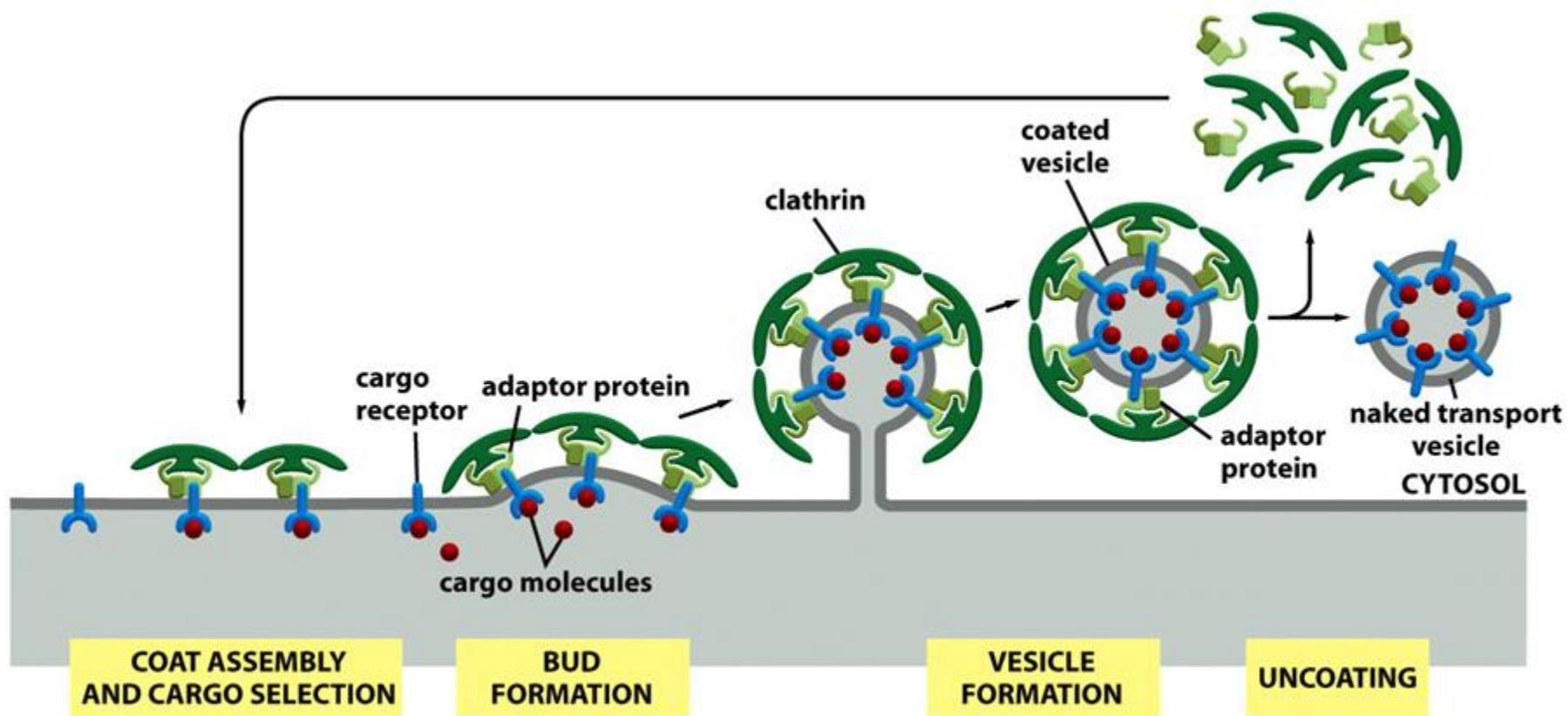


(B)



Triskelions further assemble into a framework of hexagons and pentagons

# How does clathrin coat assemble and disassemble?



## How does the coat protein control when and where to assemble?

- 1. Mediated by phosphoinositides (PIPs) on cell membrane.
- 2. Mediated by coat-recruited GTPase:
  - ♥ Arf proteins: responsible for COP I coat assembly and clathrin coat assembly
  - ♥ Sar I protein: COP II coat assembly

# Adaptor proteins select cargo into clathrin-coated vesicle

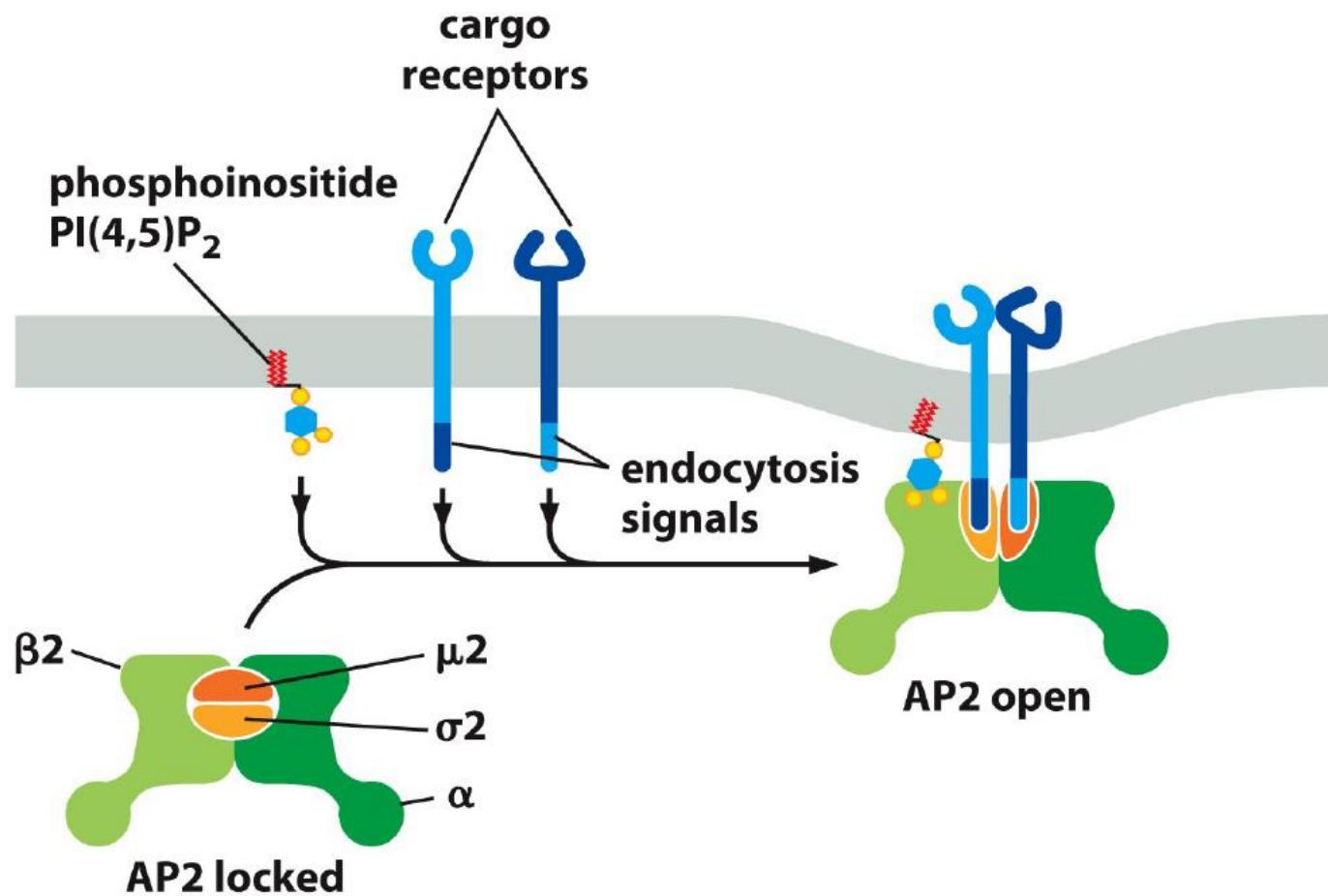
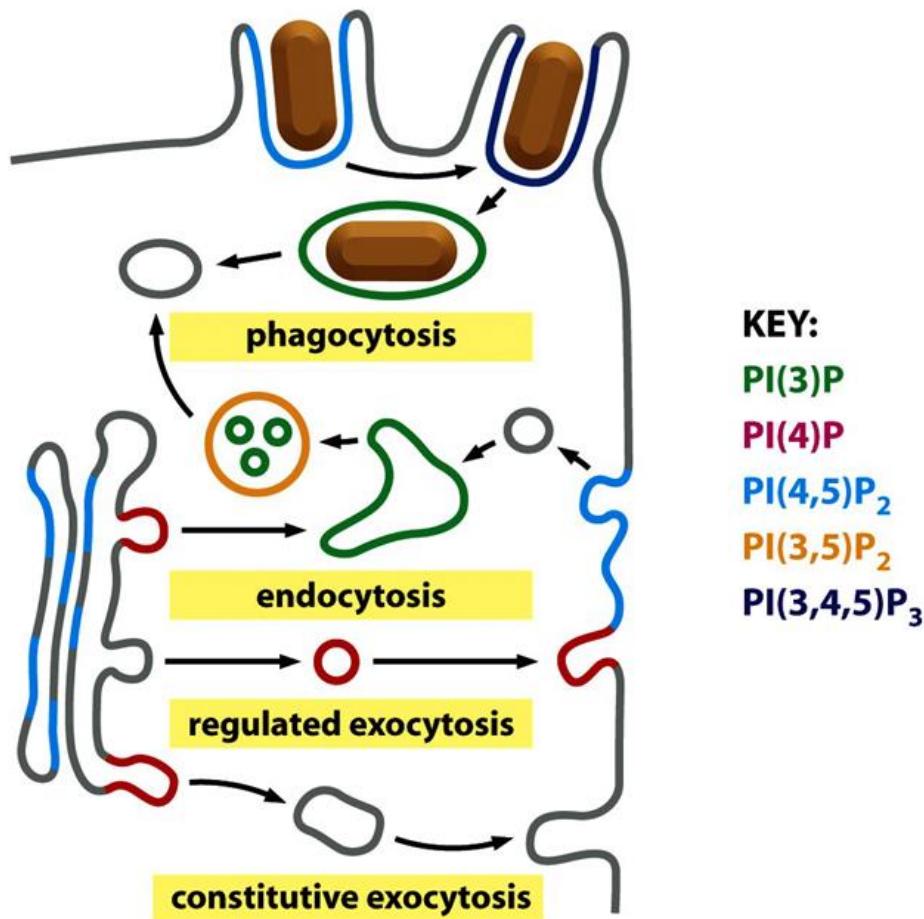
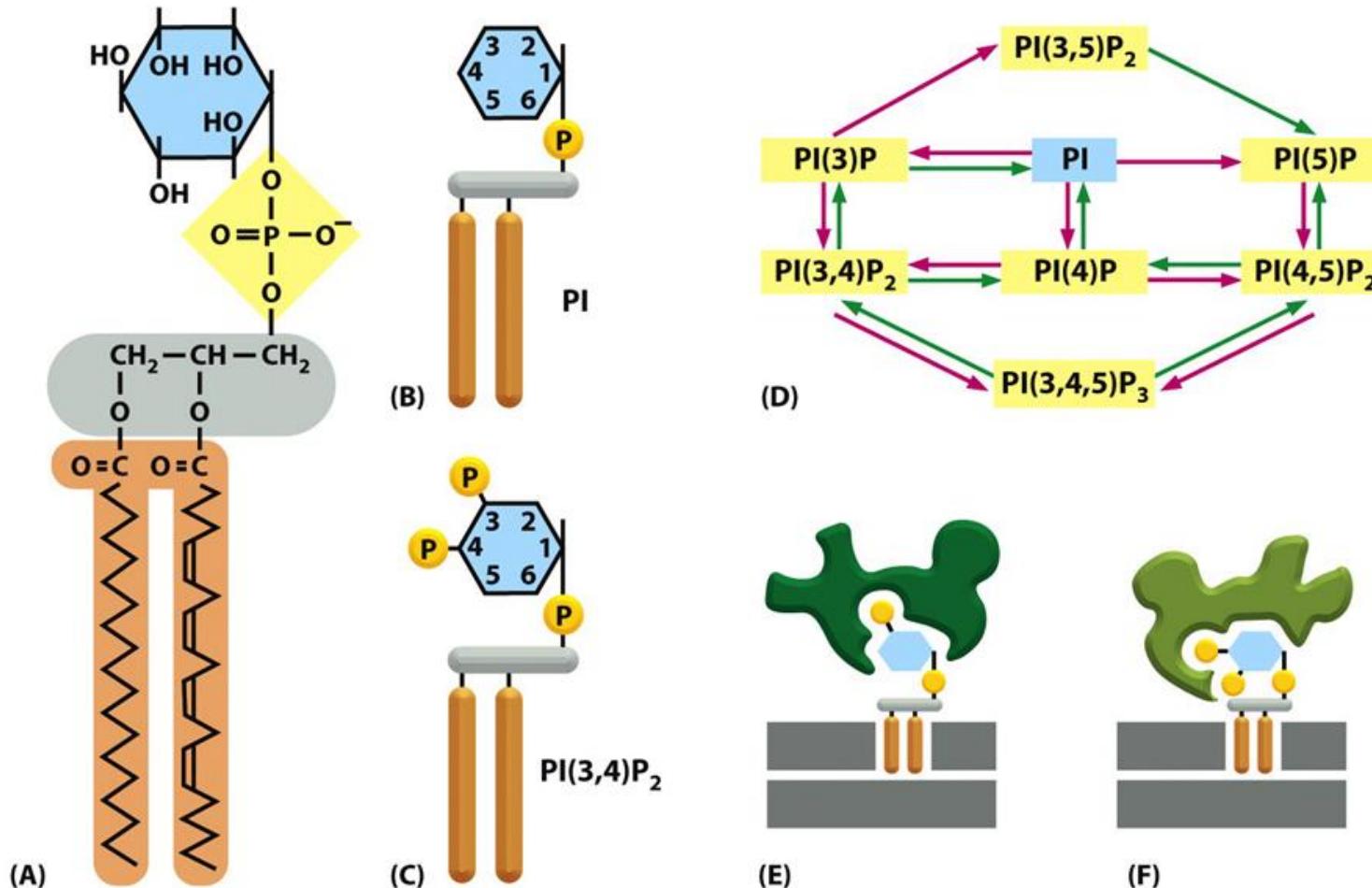


Figure 13-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Different locations for PIPs to regulate targeted vesicular transport



# Different receptors recognize different PIPs



- ♥ Membrane-bending proteins help deform the membrane during vesicle formation
- ♥ Cytoplasmic proteins regulate the pinching-off and uncoating of coated vesicles.

# Membrane-bending proteins help deform the membrane during vesicle formation

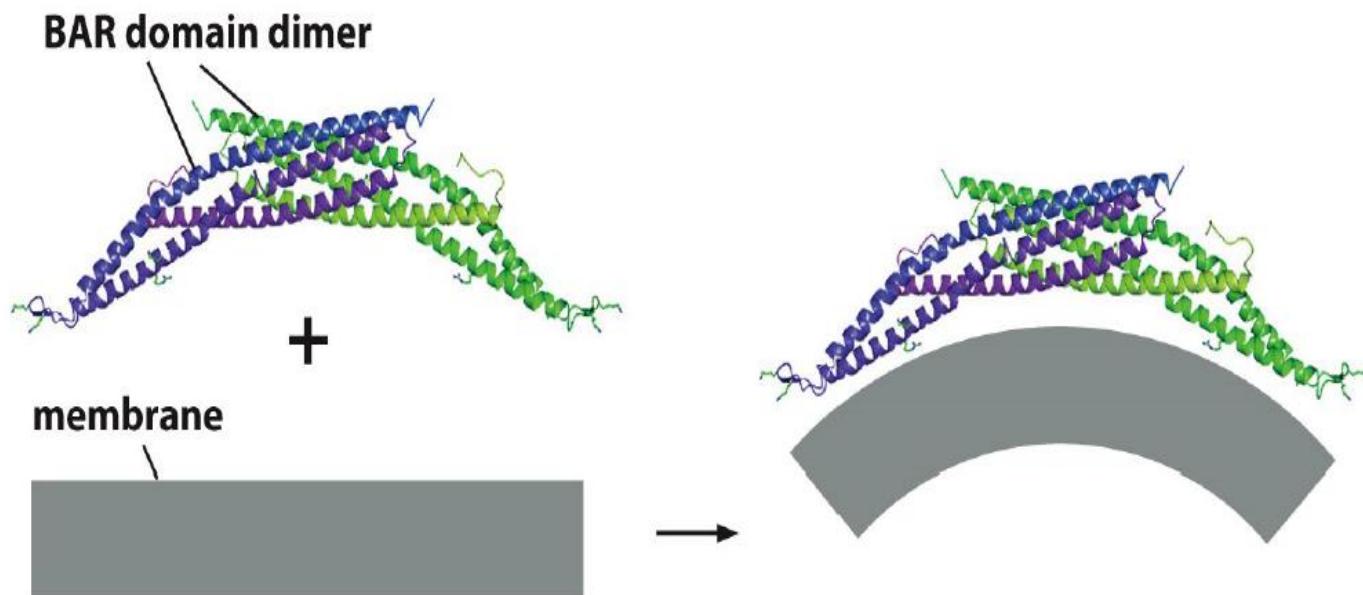
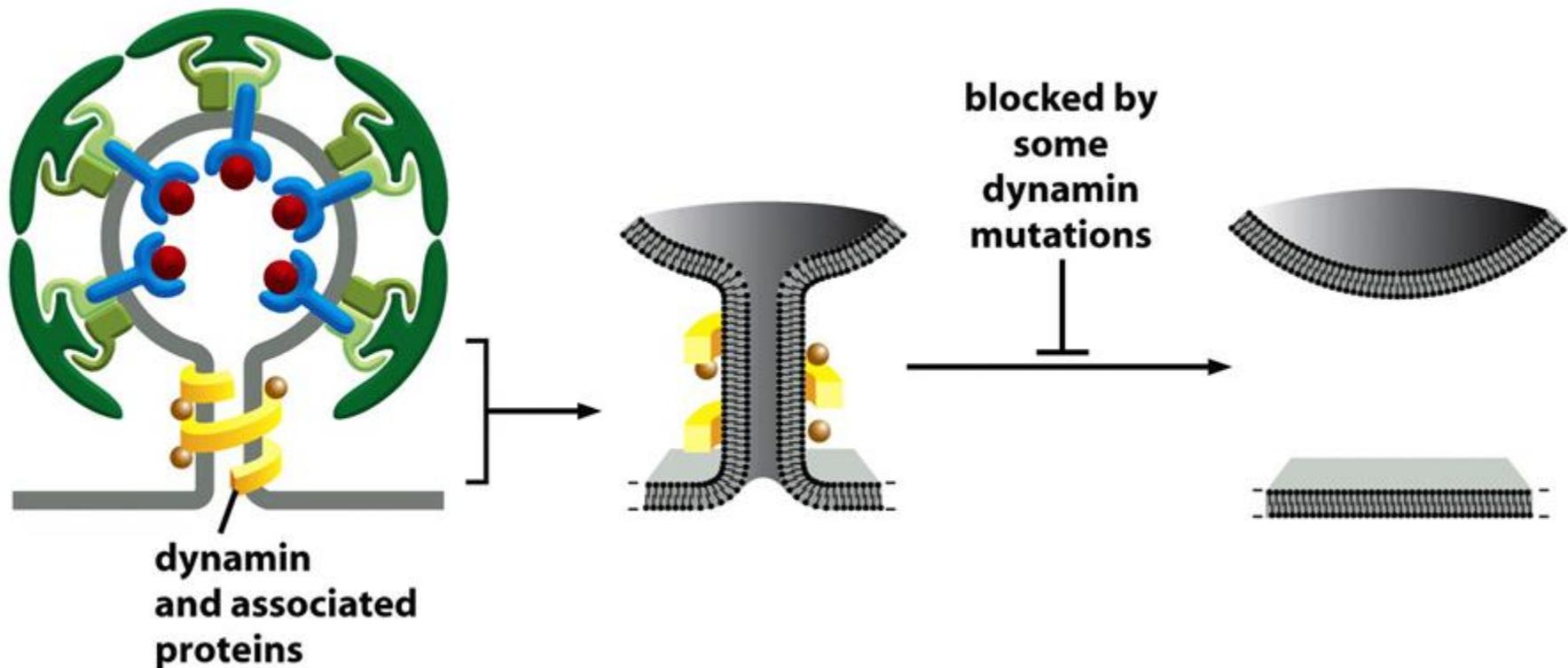


Figure 13-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

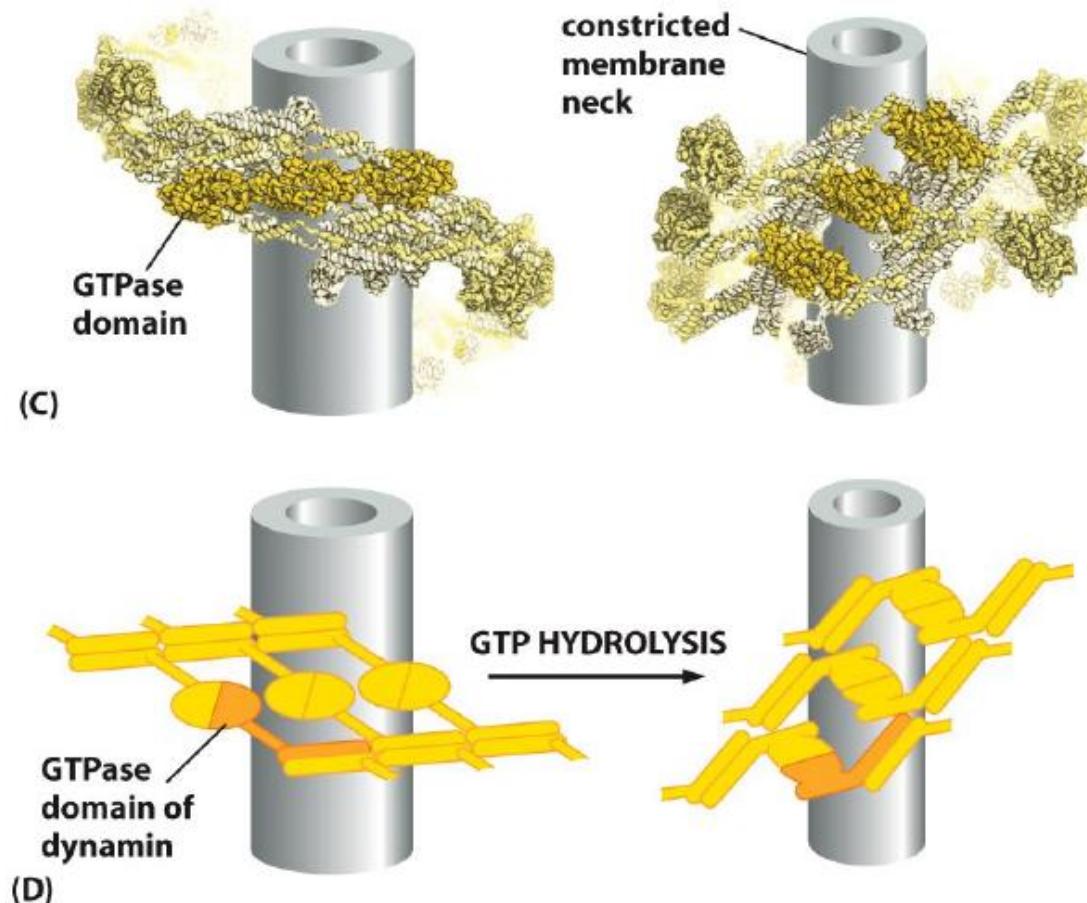
# Dynamin mediated vesicle budding off



Dynamin:

1. PI(4,5)-P<sub>2</sub>-binding domain tethers the protein to the membrane.
2. GTPase domain regulates the rate of budding

# Dynamin mediated vesicle budding off



## Pinching off of the vesicles under EM

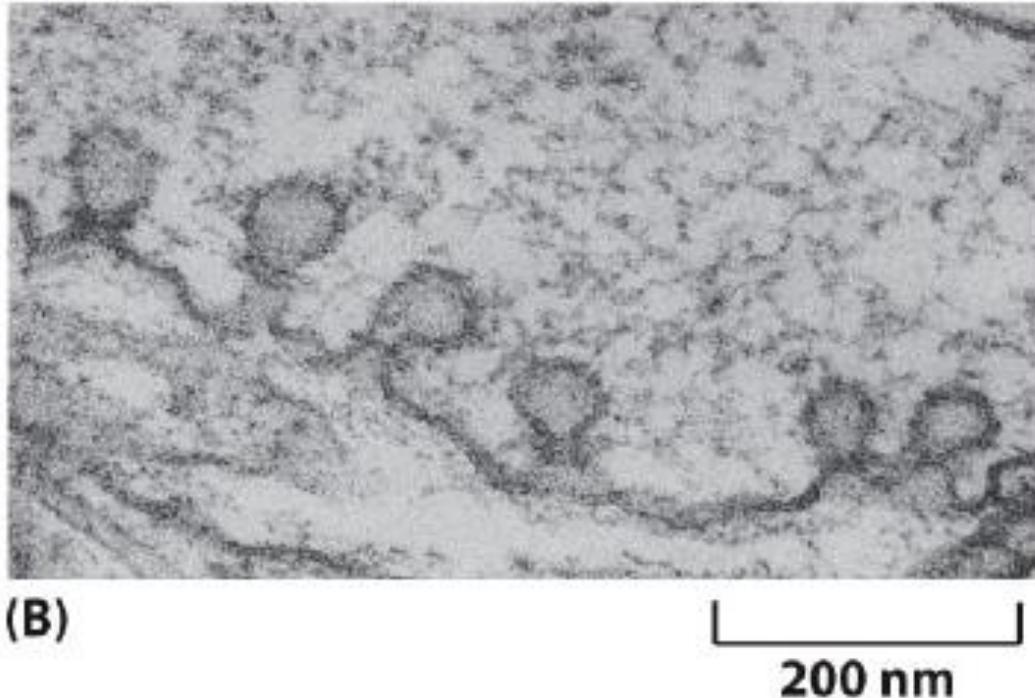
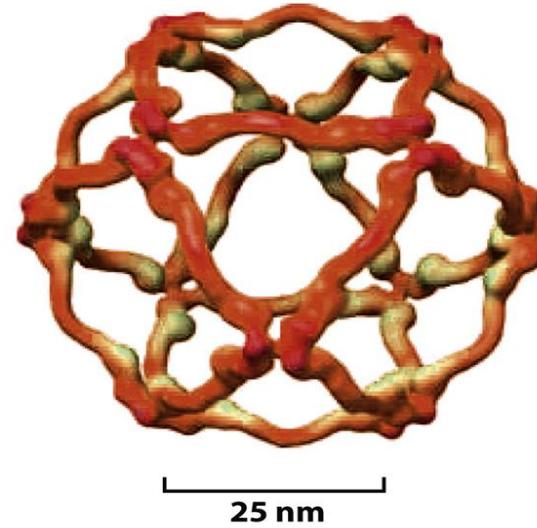
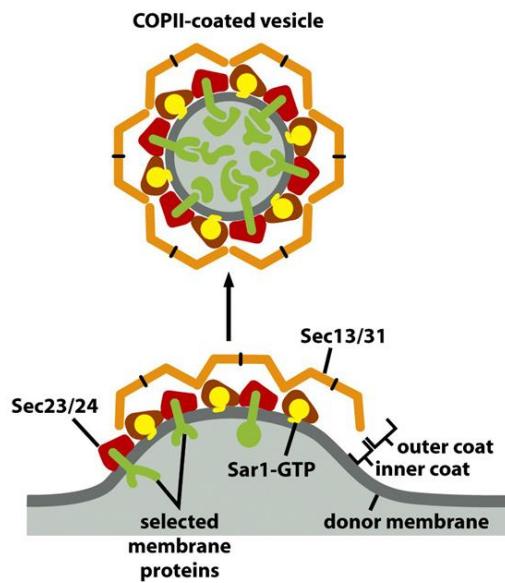
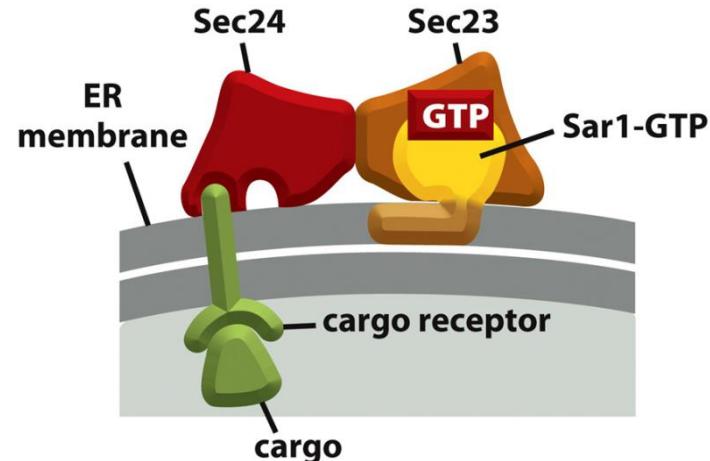
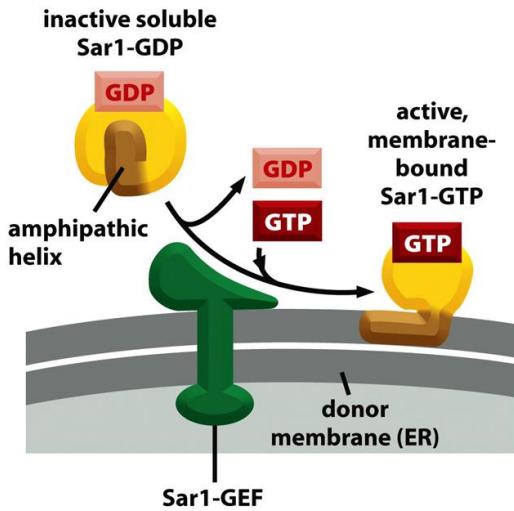


Figure 13-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

# how does GTPase control COP II assembly?



# How to ensure vesicles are targeted to specific sites for fusion?

- Rab proteins direct the vesicle to the correct target sites.
- *SNARE* proteins mediate the fusion of the lipid bilayer.

# Rab protein family--- monomeric GTPase and their subcellular localization

**TABLE 13-1 Subcellular Locations of Some Rab Proteins**

Protein	Organelle
Rab1	ER and Golgi complex
Rab2	<i>cis</i> Golgi network
Rab3A	Synaptic vesicles, secretory vesicles
Rab4/Rab11	Recycling endosomes
Rab5	Early endosomes, plasma membrane, clathrin-coated vesicles
Rab6	Medial and <i>trans</i> Golgi
Rab7	Late endosomes
Rab8	Cilia
Rab9	Late endosomes, <i>trans</i> Golgi

Table 13-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

> 60 family members, each associates with one or more biosynthetic secretory or endocytic pathways

# States of Rab GTPase

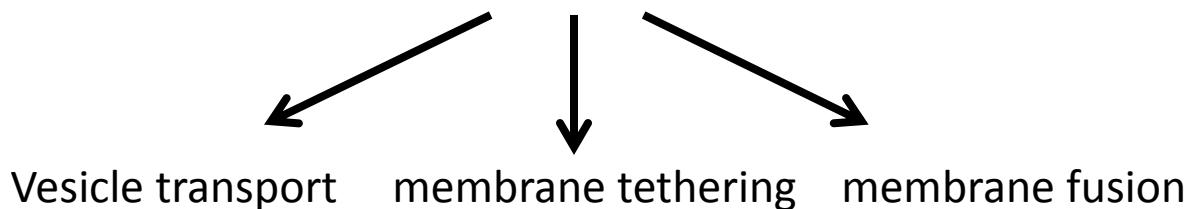
Rab- GDP is inactive and cytosolic, usually bound to  
Rab-GDP-dissociation inhibitor (GDI )



Activated by membrane bound Rab-GEF (Guanine exchange factor)  
and become Rab-GTP( active form)



Once in active form, Rab-GTP exposes hydrophobic anchor  
To be membrane-bound and recruit Rab effectors



# Rab proteins guide transport vesicle to their target membrane

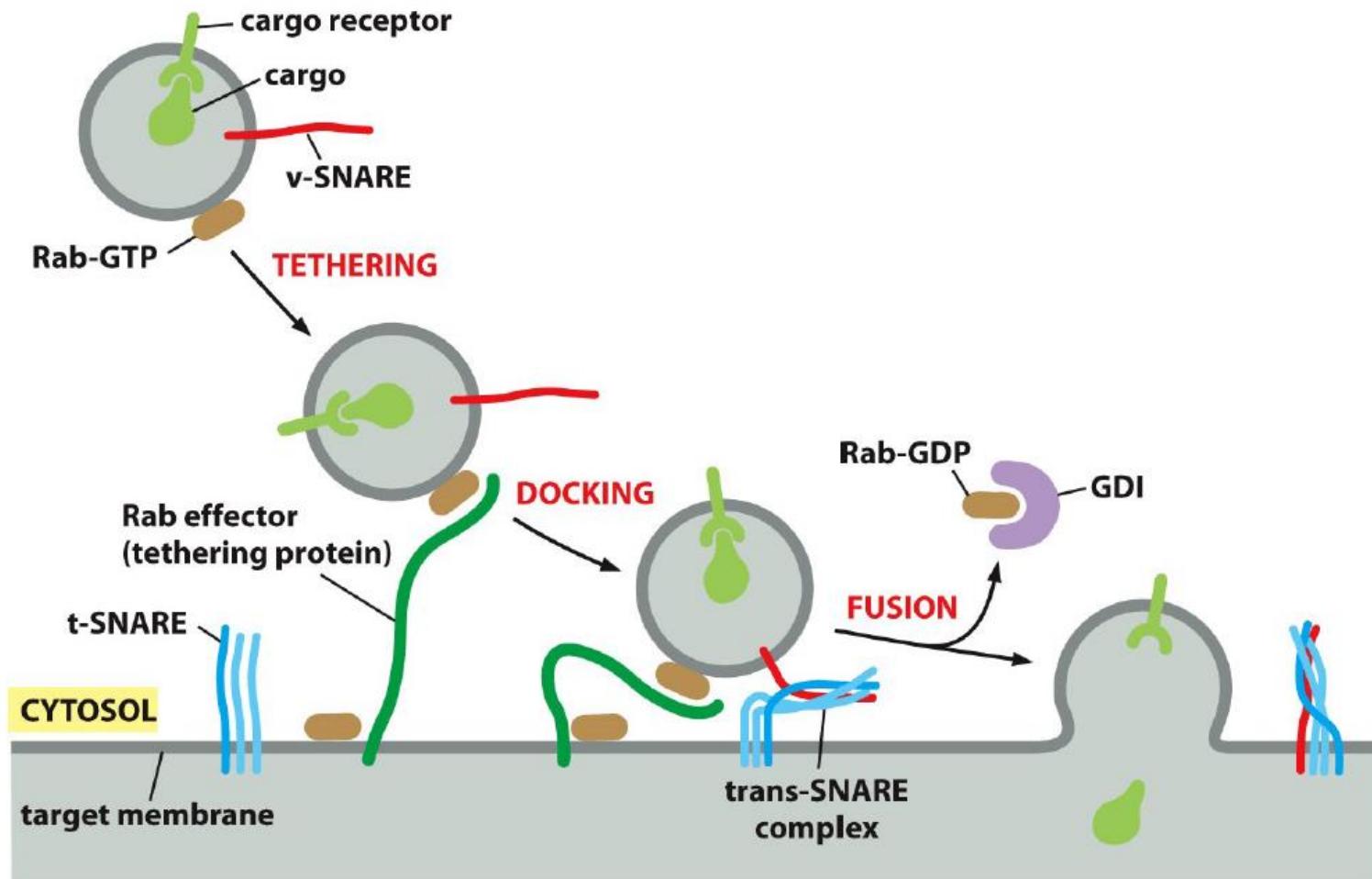


Figure 13-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

# The formation of a Rab5 domain on the endosome membrane

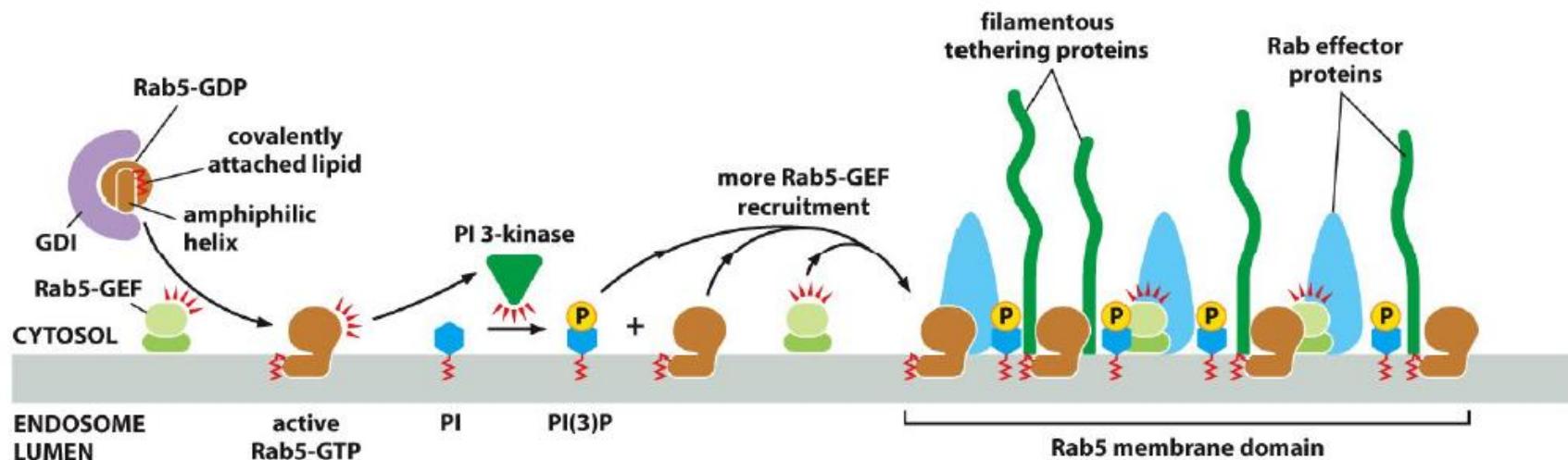


Figure 13-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Rab cascade can change the identity of an organelle

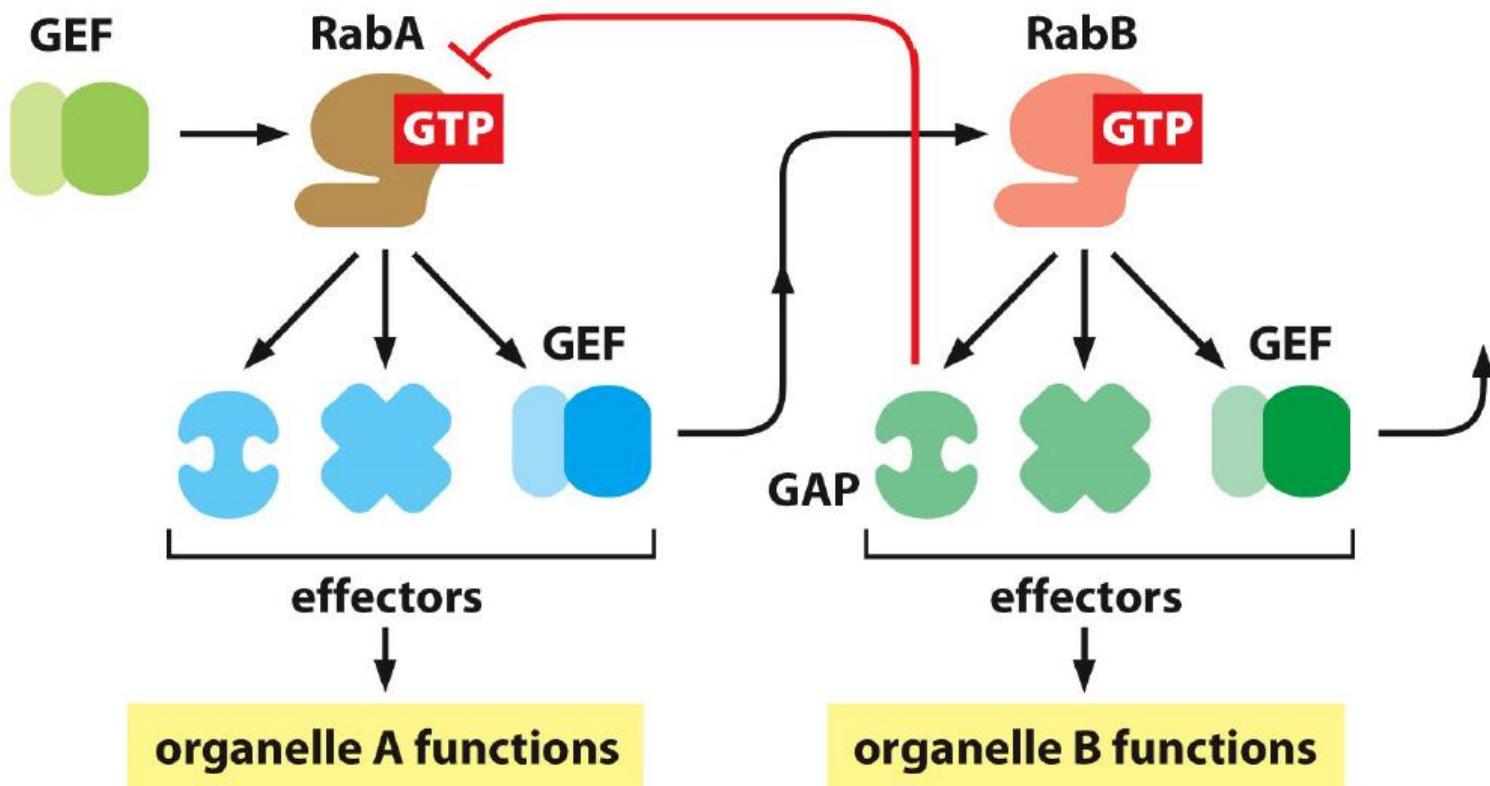
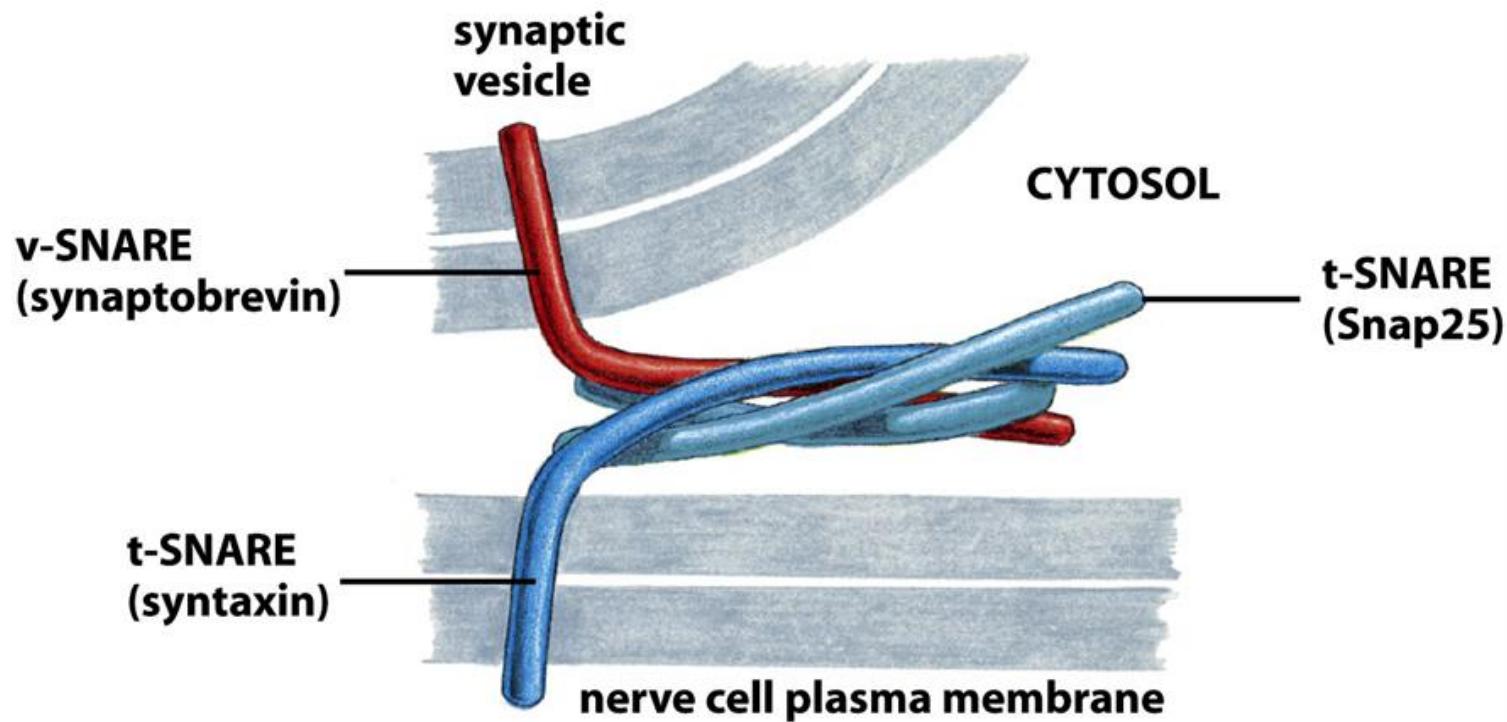


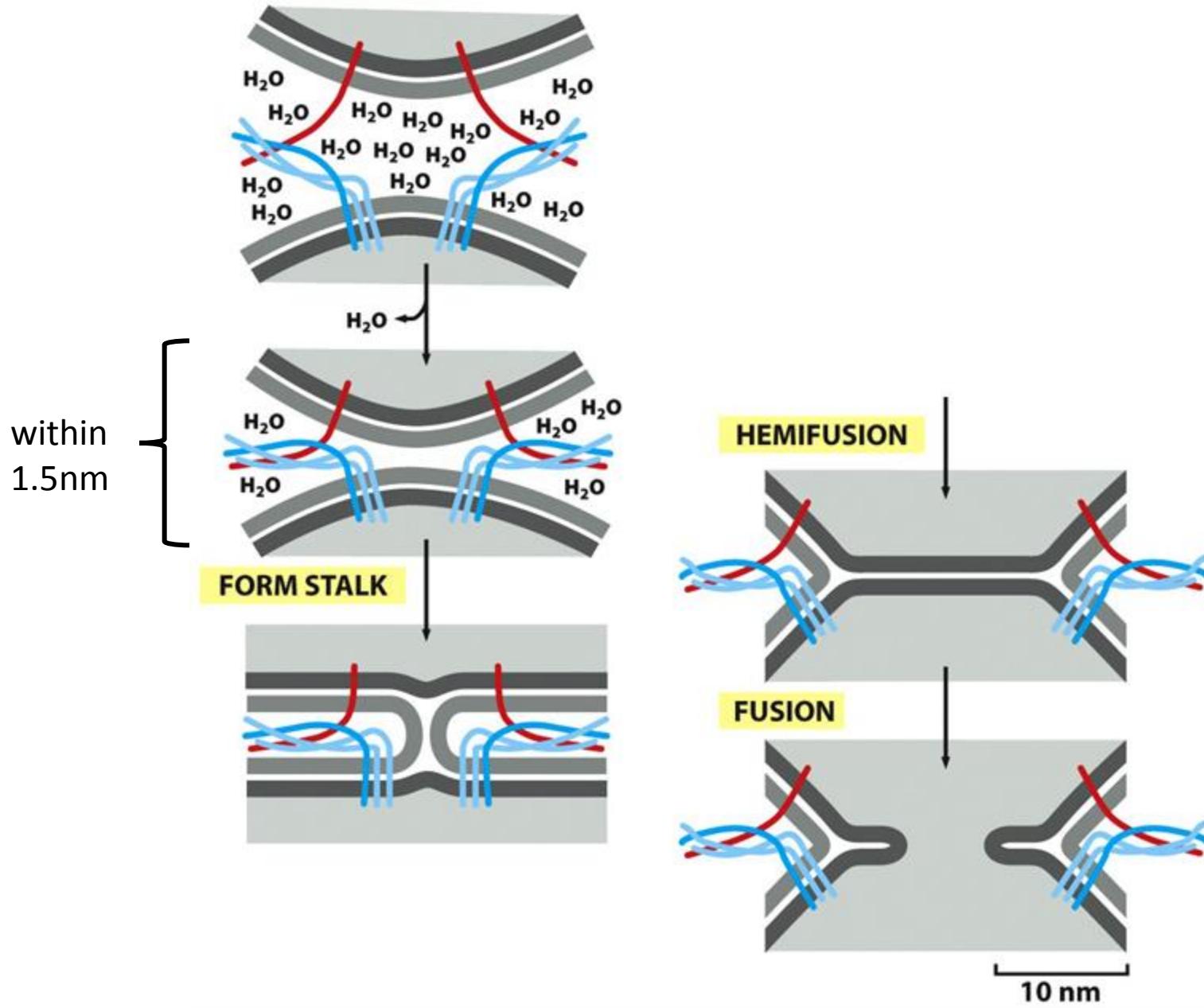
Figure 13-18 Molecular Biology of the Cell 6e (© Garland Science 2015)

# SNARES mediate membrane fusion

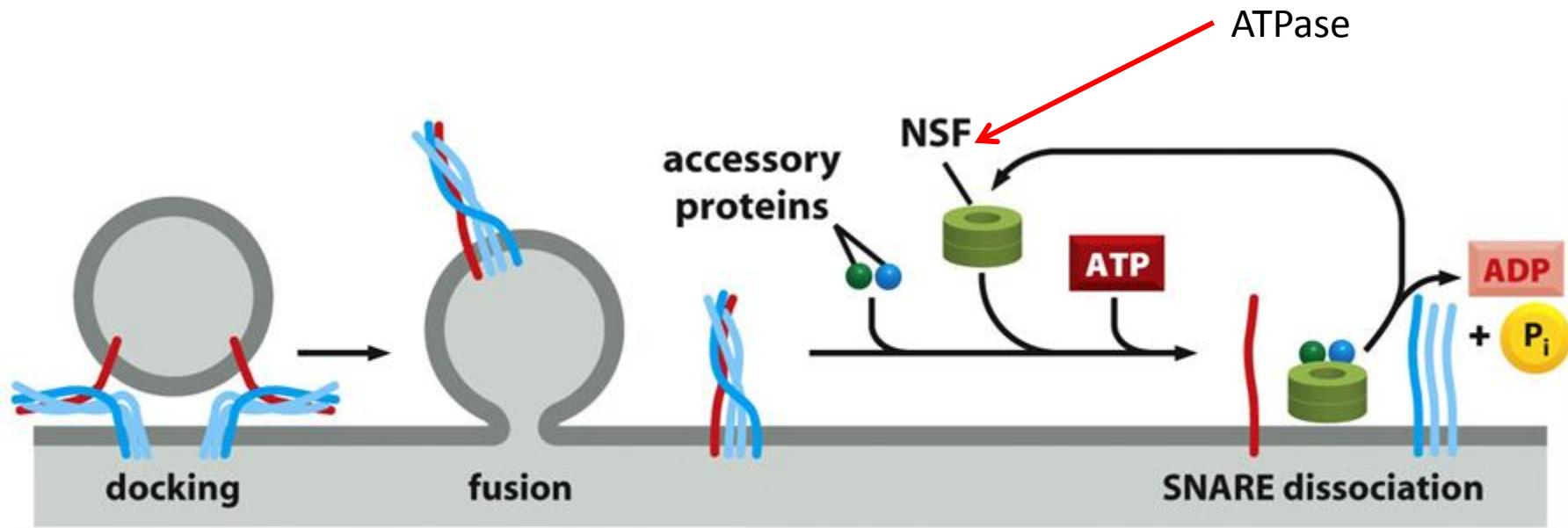


v-SNARE: single chain on vesicles

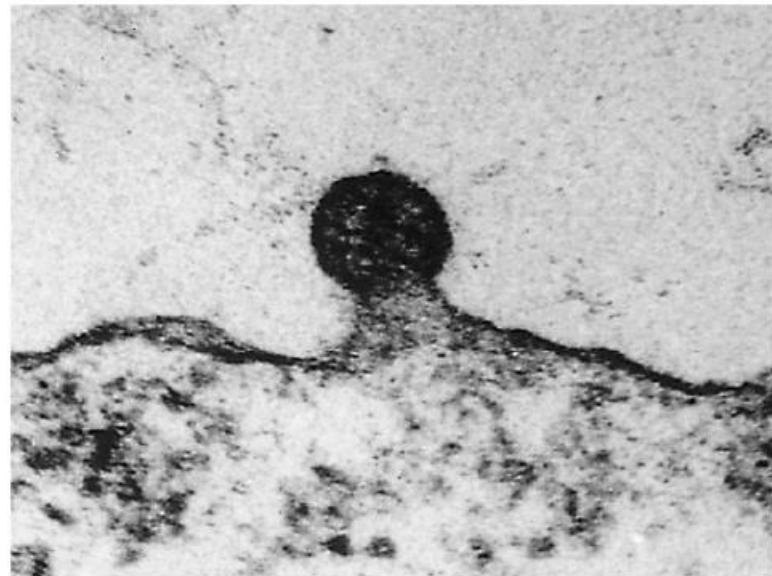
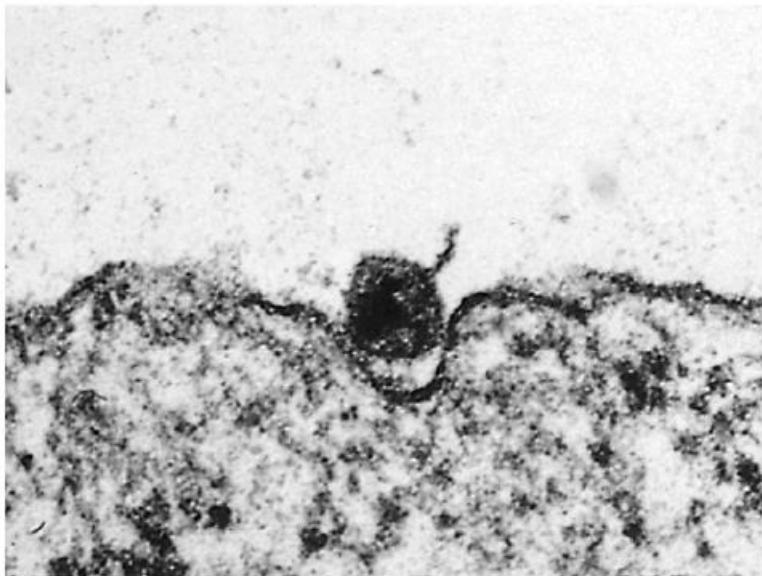
t-SNARE: 2-3 chains on target



# Dissociation of SNARE pairs by NSF after membrane fusion



# Entry of the enveloped virus into cells



200 nm

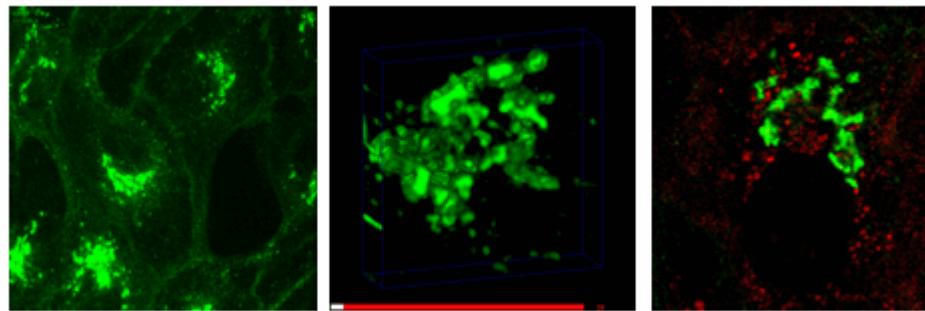
Figure 13-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

## II. Techniques to study vesicular transport

- ♥ Conventional radiolabelled amino acid---autoradiography
- ♥ GFP-fusion protein
- ♥ Cell-free system
- ♥ Genetic study with yeast temperature-sensitive mutants

# GFP fusion protein

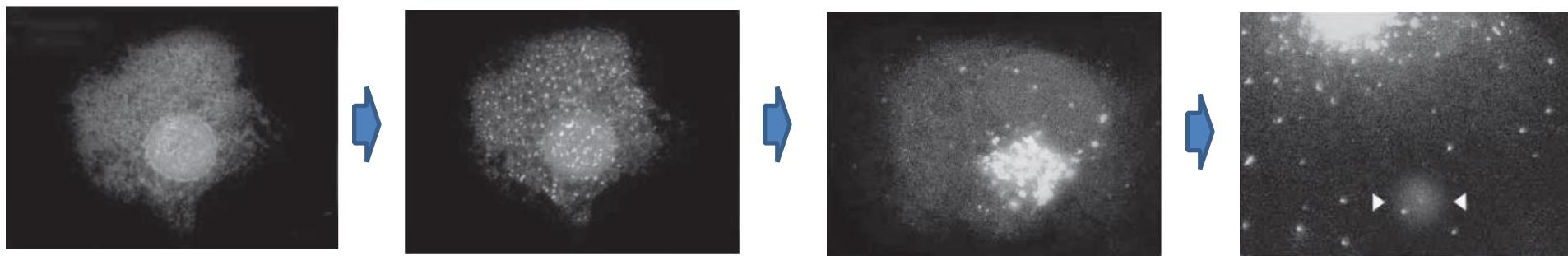
Secretory proteins were fused with GFP and then visualized by Fluorescent microscope  
Coupled With FRAP, FLIP .



Golgi in cells

Vesicle budding from Golgi

# A temperature-sensitive mutant secretory protein can be tracked by IF



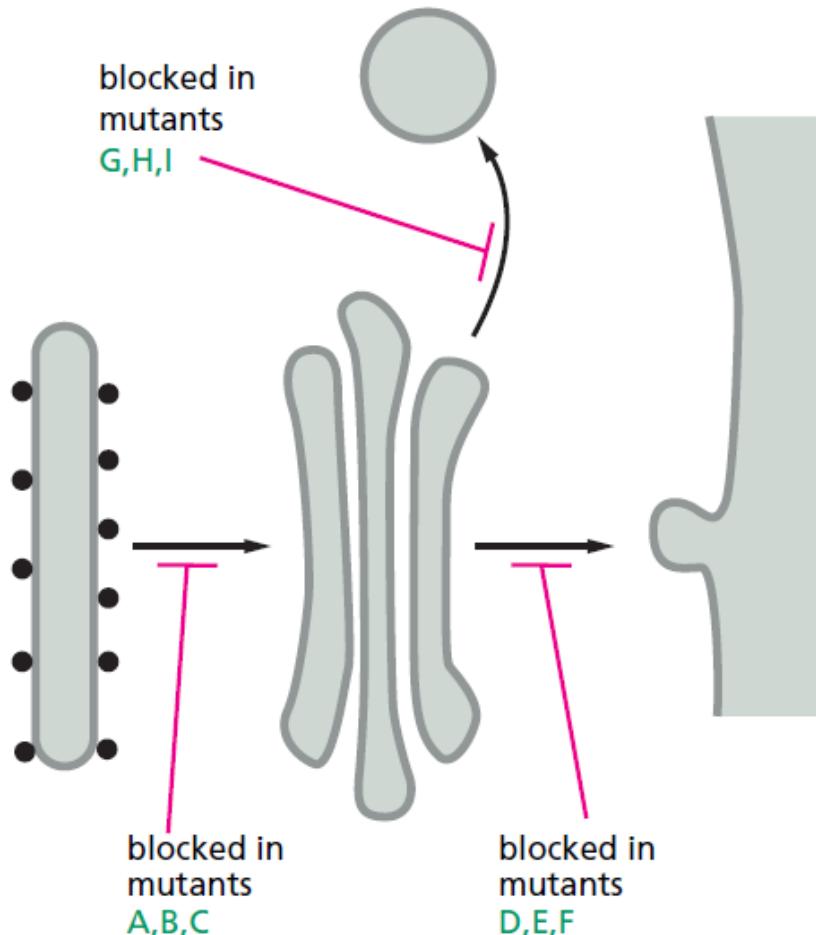
High temperature  
Retent in ER

low temperature  
exit in ER

low temperature  
Move to Golgi

Low temperature  
fuse to plasma  
membrane

# Yeast temperature mutant---genetic study

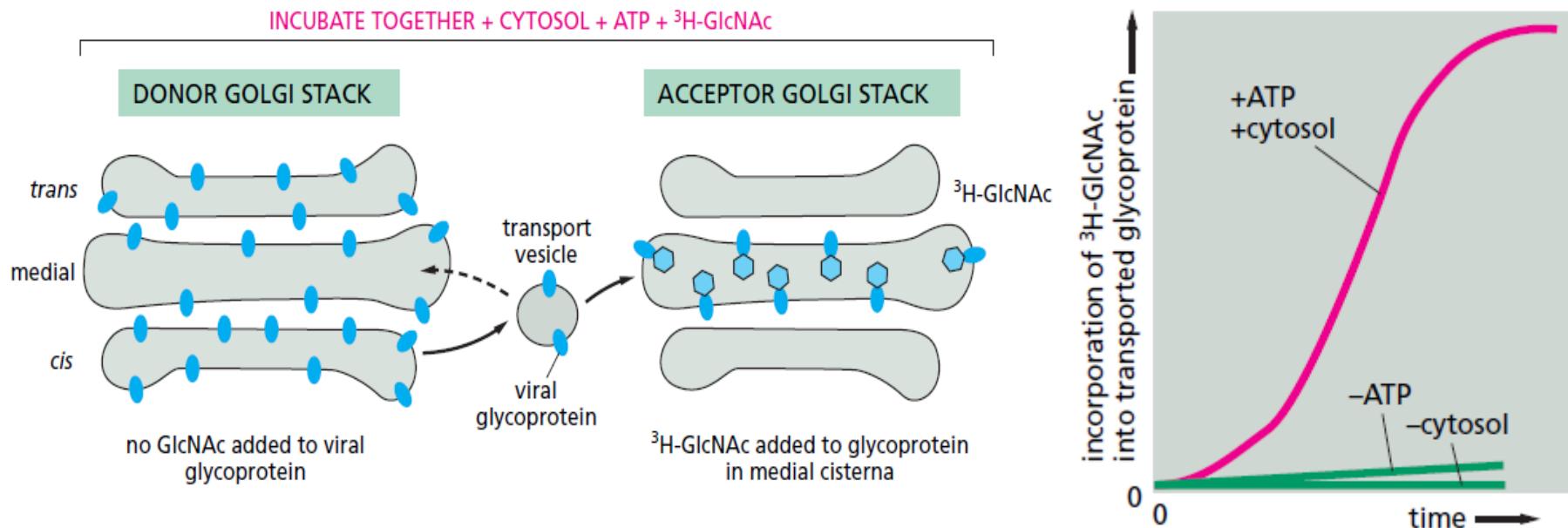


***multicopy suppression*** --- a key gene for Vesicular transport is mutated, to bypass this defect, an interacting protein that is produced by higher amounts will bind to the mutant protein vividly to cure this defect.

This is through library screening after transfecting of randomized plasmids into mutant yeast cells.

Through this screening, important binding partners for known vesicular transport protein will be identified.

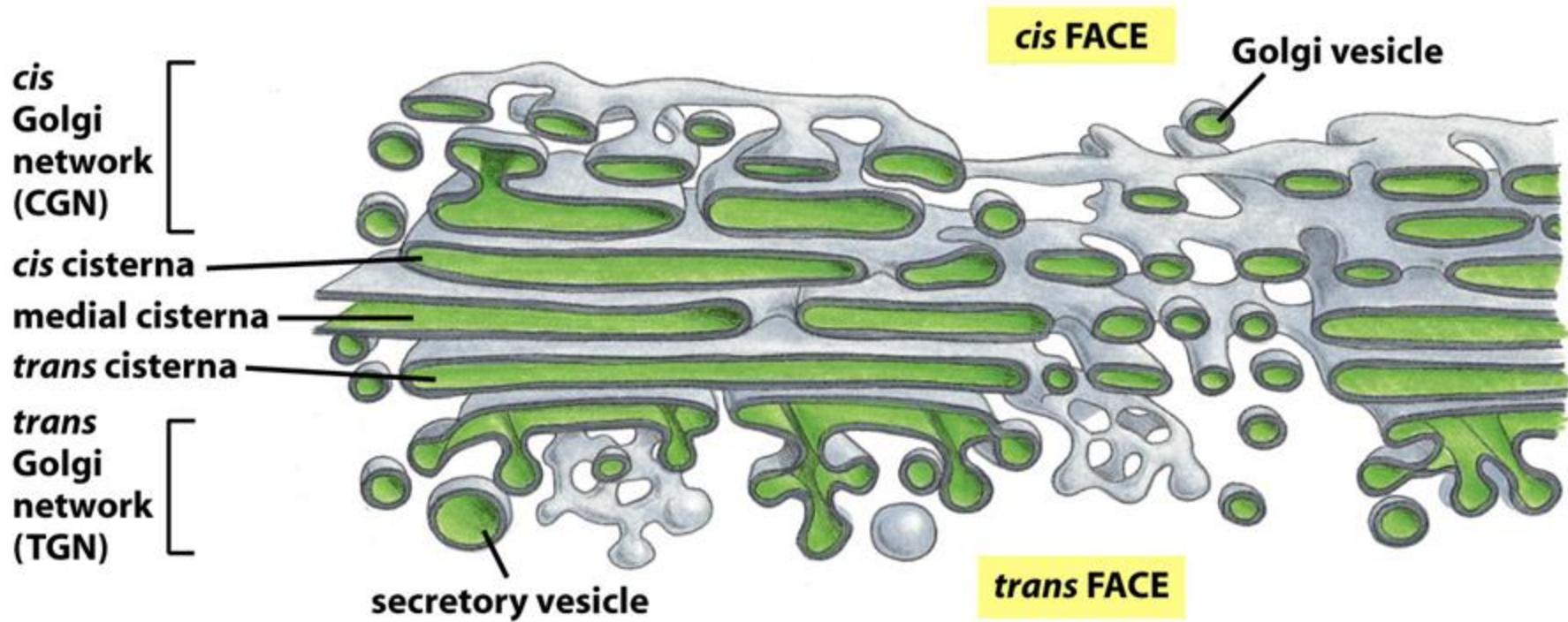
# Cell-free system to study vesicular transport



### III. Transport from ER to Golgi apparatus

- 1. Structure of the Golgi apparatus
- 2. Functions of the Golgi apparatus
- 3. Transport from ER to Golgi apparatus
- 4. Glycosylation in Golgi apparatus

# 1. Structure of the Golgi apparatus



Interconnected tubular and cisternal structures

cis-face is close to ER and Nucleus

# Golgi apparatus consists of an ordered series of compartments

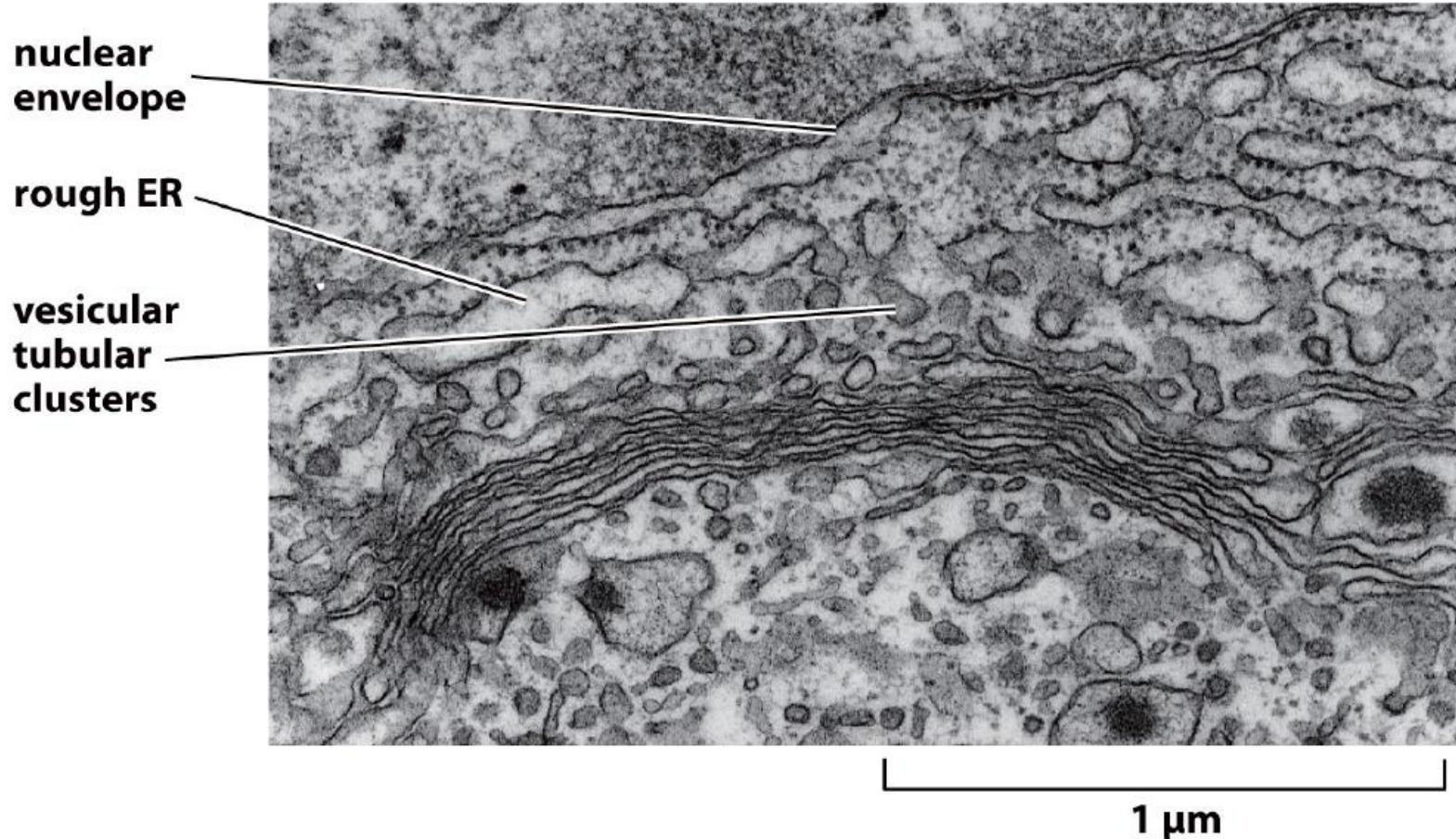
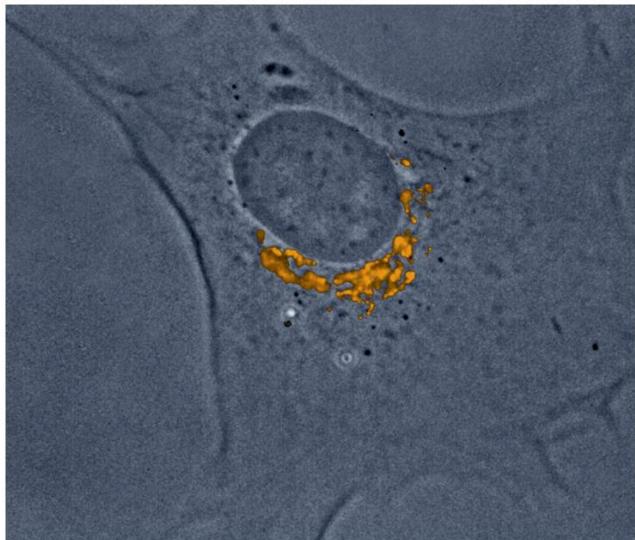


Figure 13-26b Molecular Biology of the Cell 6e (© Garland Science 2015)

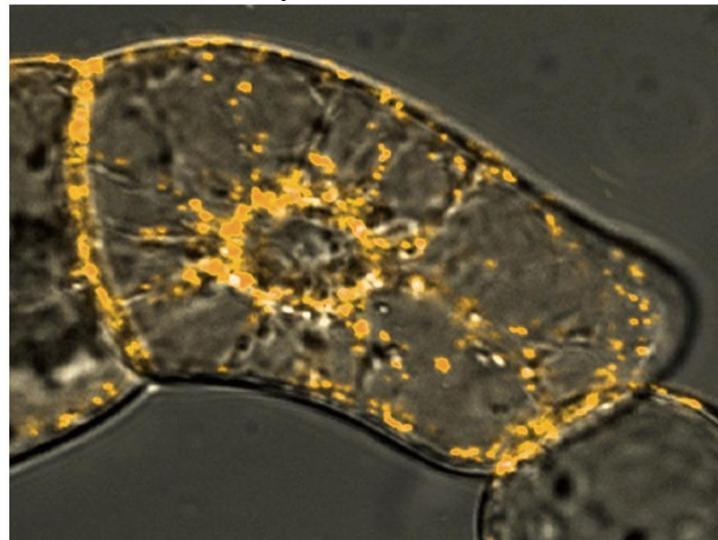
# Localization of the Golgi apparatus in animal and plant cells

Fibroblast



Close to the nucleus

plant cell



dispersed in the cytoplasm

In animal cells, many stacks are linked together into a single complex close to the nucleus and to the centrosome, which is dependent on [microtubule connection](#).

In most plant cells, individual Golgi stacks disperse in cytoplasm.

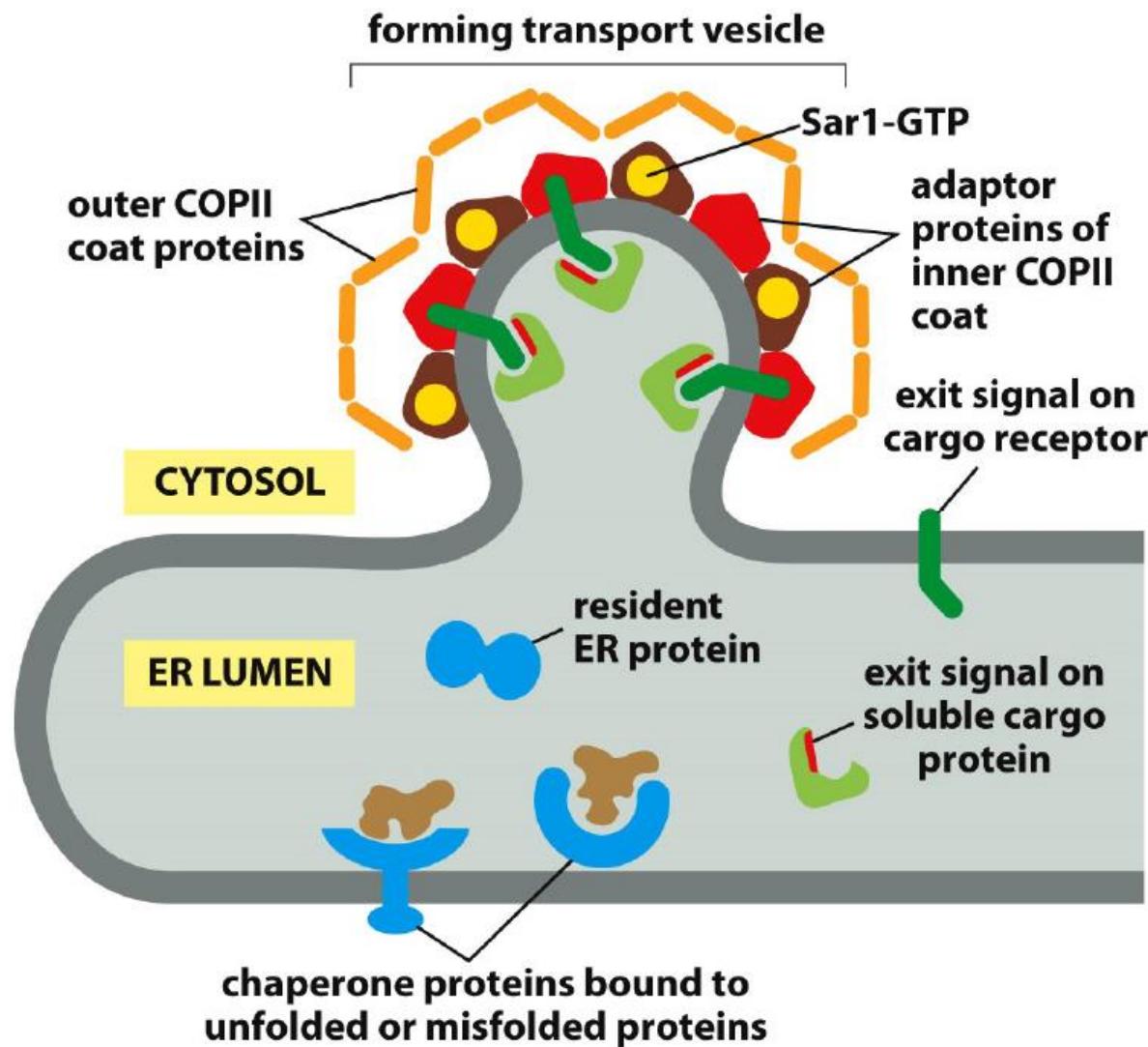
## 2. Function for Golgi apparatus

- ♥ A. Sorting and dispatching station for ER products
- ♥ B. Major site of carbohydrate synthesis and secretion of glycoproteins

### 3. Transport from ER to Golgi apparatus

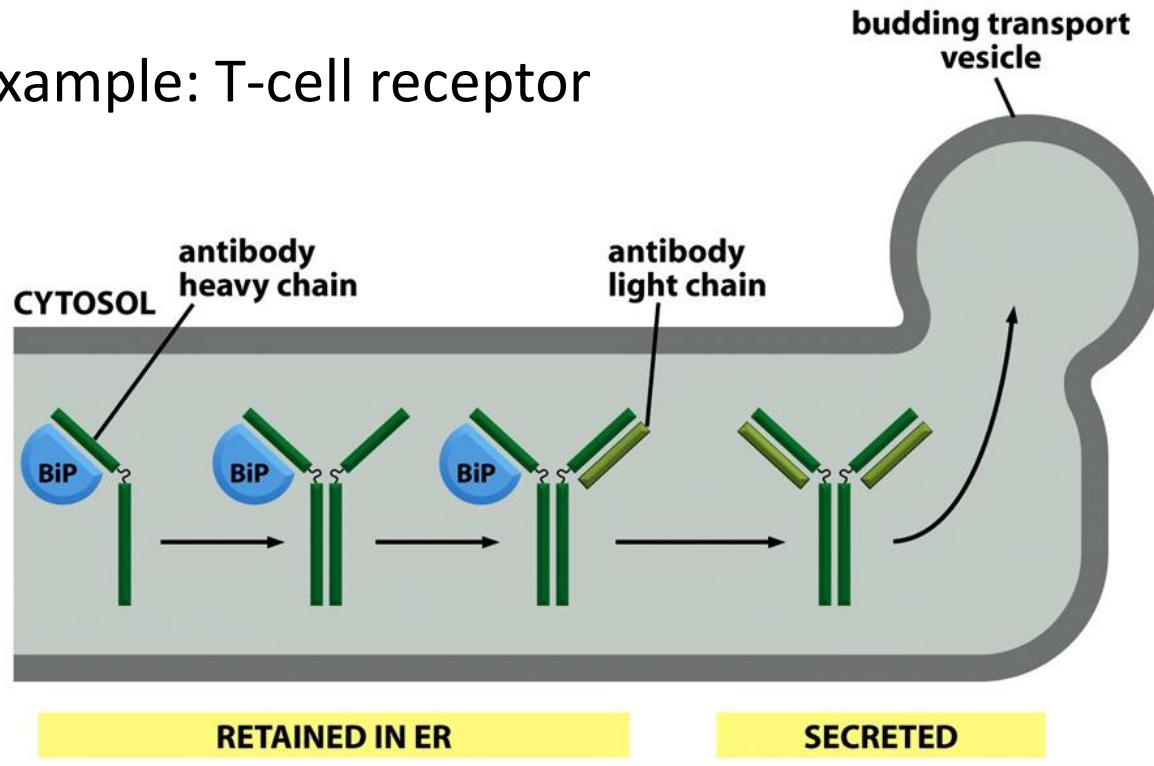
- ♥ COP II –coated transport vesicle
- ♥ bud from *ER exit* sites (without ribosomes)
- ♥ Cargoes display exit signals on their cytosolic sides
- ♥ COP II coat can recognize the exit signals directly or indirectly

# The assembly of COPII coat



# ER-exiting proteins are folded well

Example: T-cell receptor

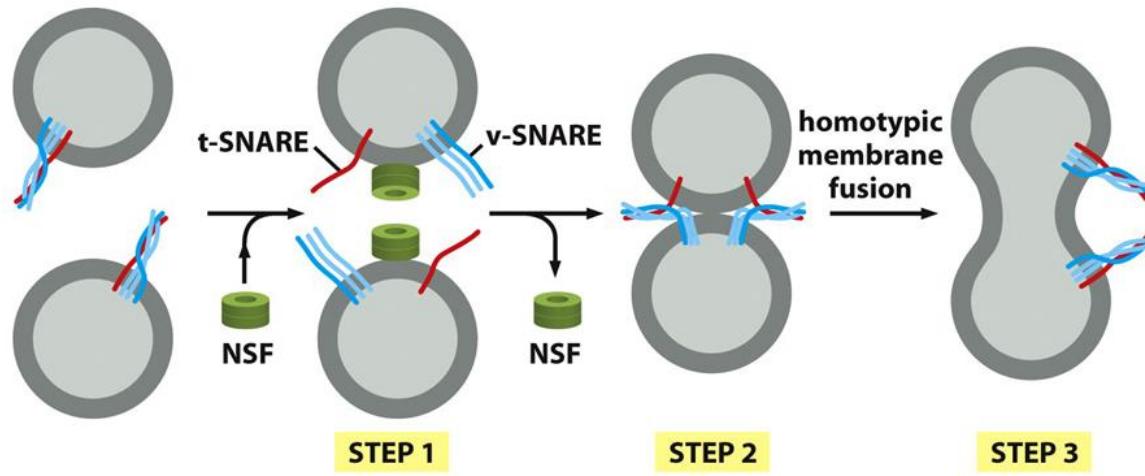


Molecular chaperons such as Bip and Calnexin help in folding.

Misfolded protein can't be transported further, rather they will be degraded in cytosol

# Transport vesicles can fuse with each other to form vesicular tubular clusters

- ♥ Homotypic fusion: fusion occurs between vesicles from the same compartment
- ♥ Hetertypic fusion: fusion occurs between vesicles from different compartments.
- ♥ Both are mediated by v-SNARE and t-SNARE



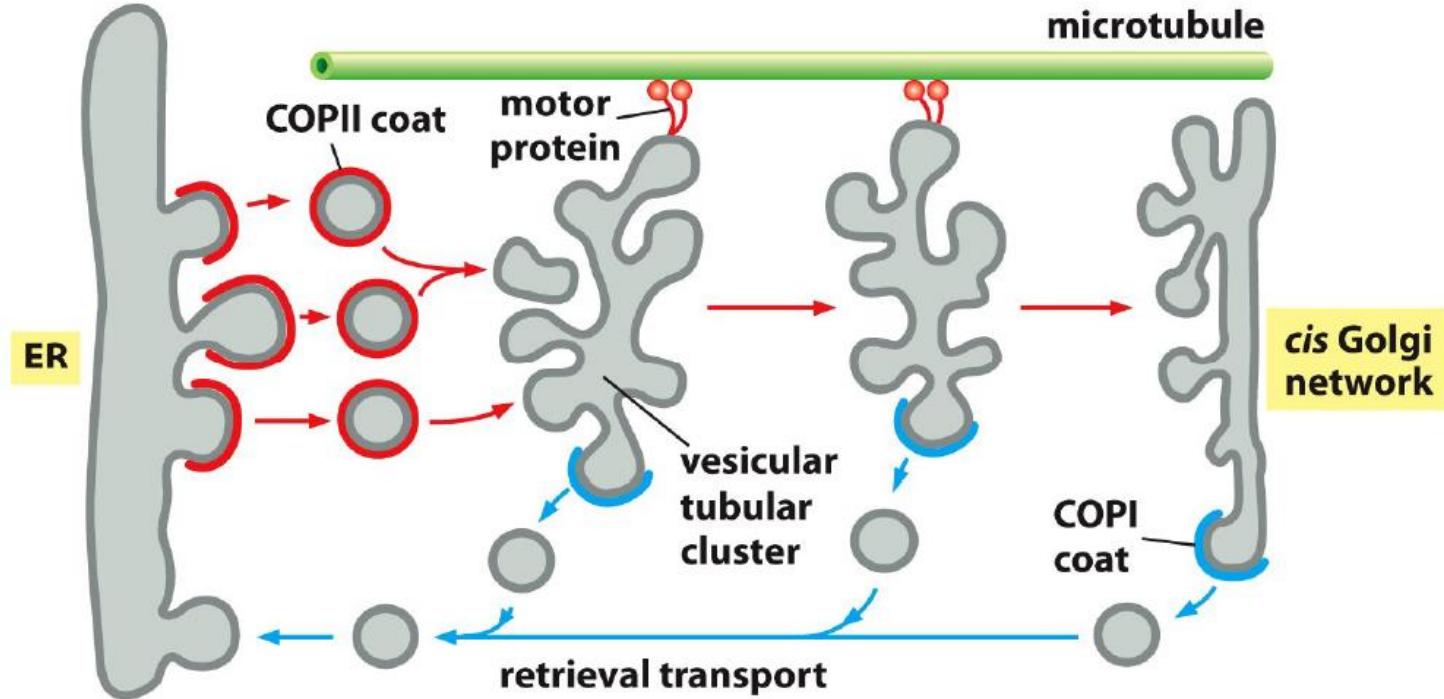


Figure 13-24b Molecular Biology of the Cell 6e (© Garland Science 2015)

- ♥ Vesicular tubular clusters are short lived and move quickly along the microtubule
- ♥ Vesicular tubular clusters can bud off on their own to send back ER resident protein--- a process called retrieval ( or retrograde) transport
- ♥ Retrieval occurs due to ER retrieval signals, which can be recognized and bound by COP I coats directly or indirectly

# Vesicular tubular cluster under EM

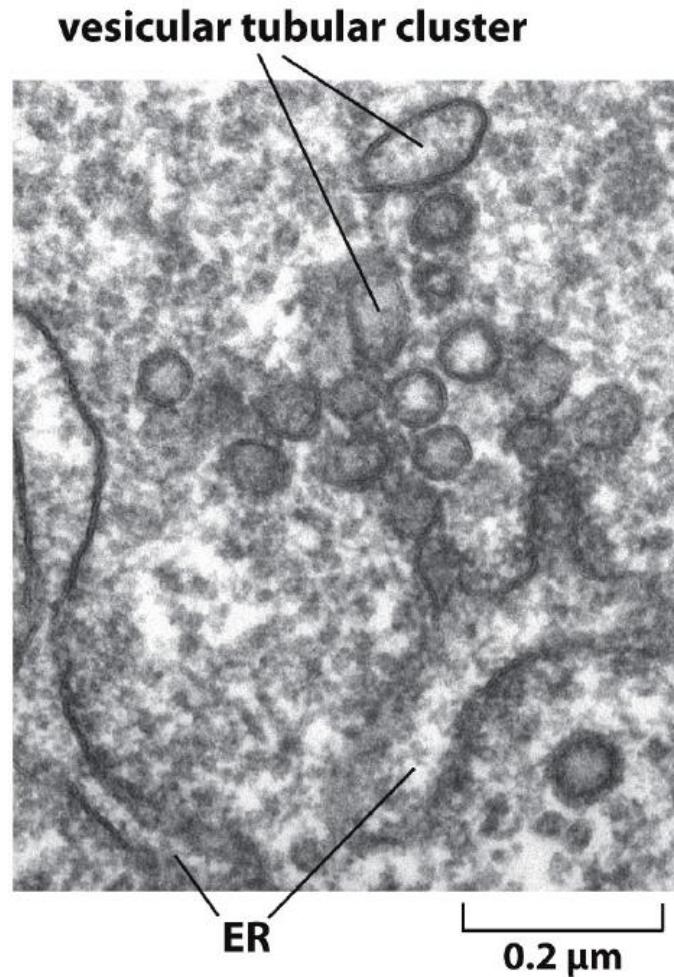


Figure 13-24a Molecular Biology of the Cell 6e (© Garland Science 2015)

# Retrieval for ER membrane and ER soluble proteins

- ♥ ER membrane protein: C-terminal KKXX sequence, which directly bind to COP I
- ♥ ER soluble protein: C-terminal KDEL sequence, binds to membrane-bound KDEL receptor

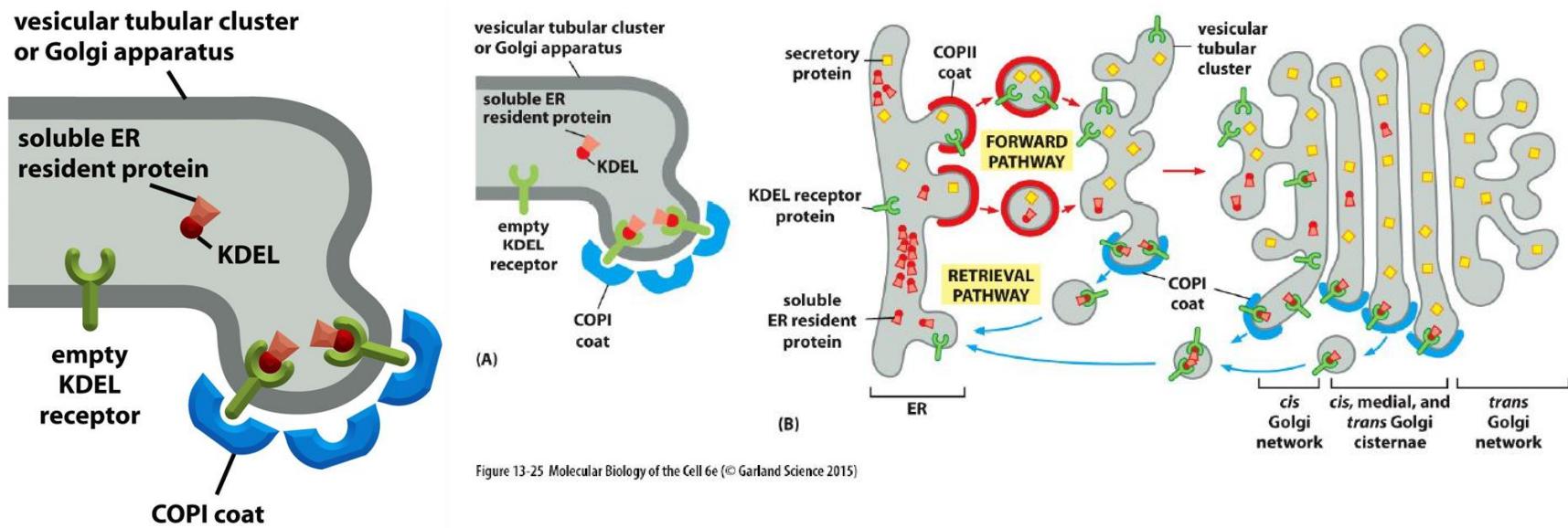


Figure 13-25 Molecular Biology of the Cell 6e (© Garland Science 2015)

## 4. Glycosylation in Golgi apparatus

- ♥ Functional compartmentalization
- ♥ Types of glycosylation
- ♥ Function of glycosylation
- ♥ Oligosaccharide processing in ER and Golgi apparatus

# Compartmentalization in Golgi apparatus

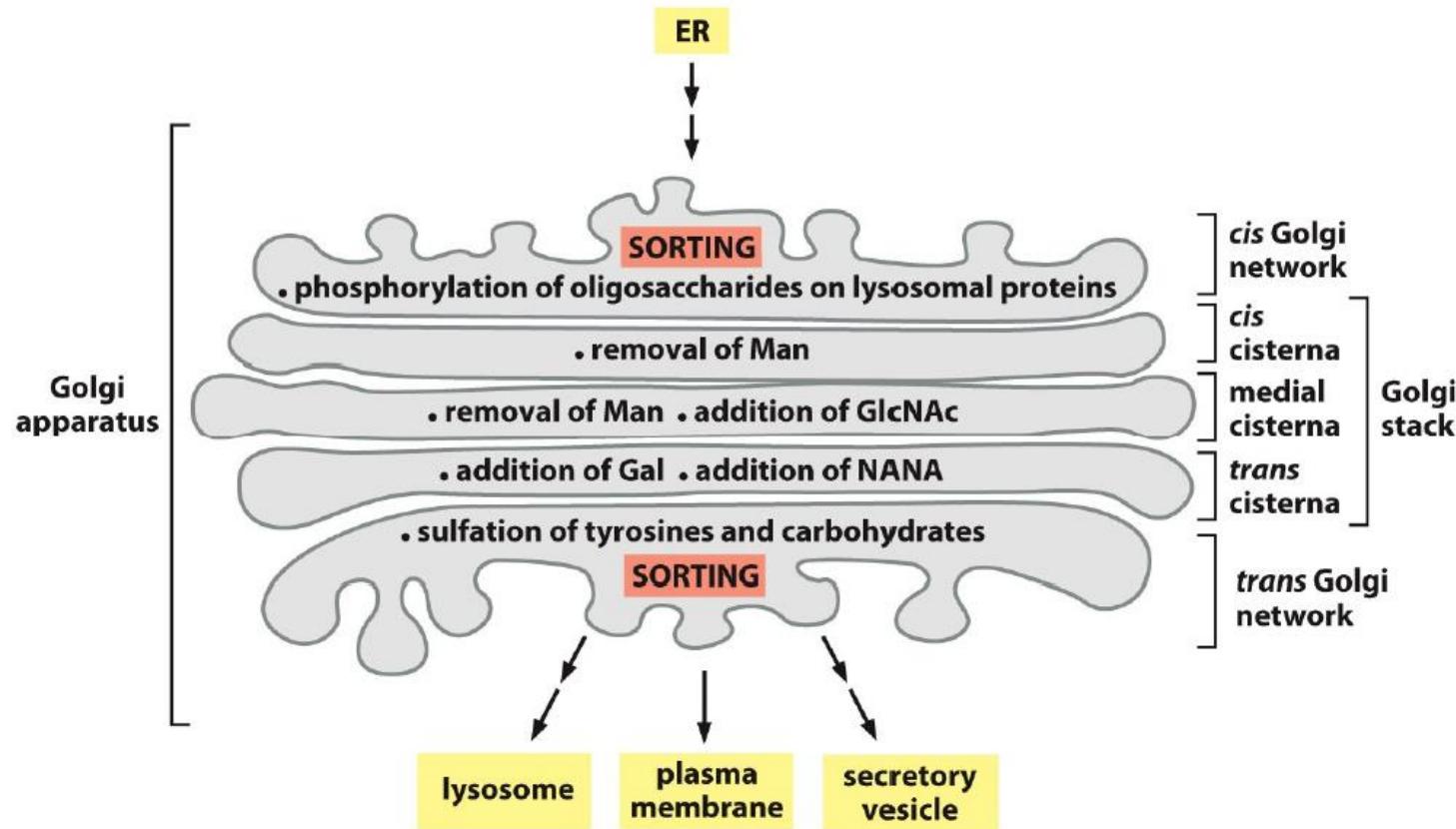
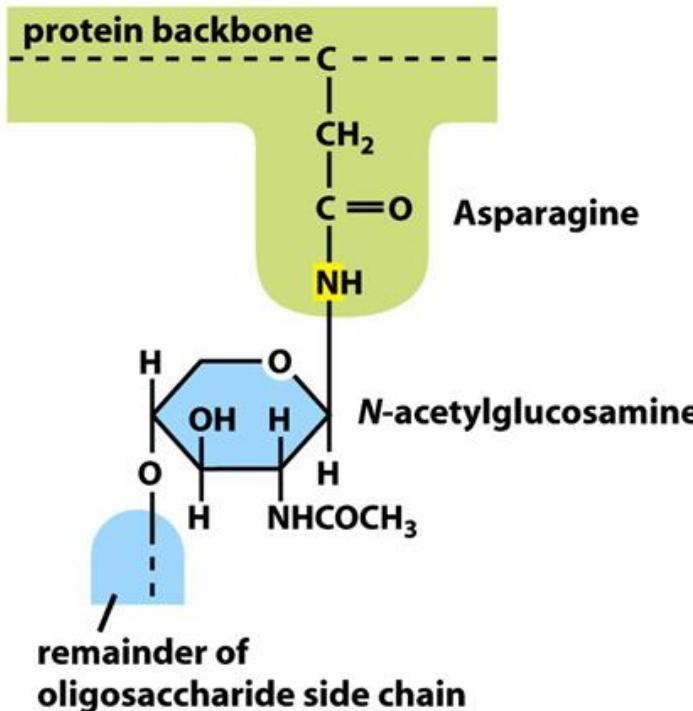


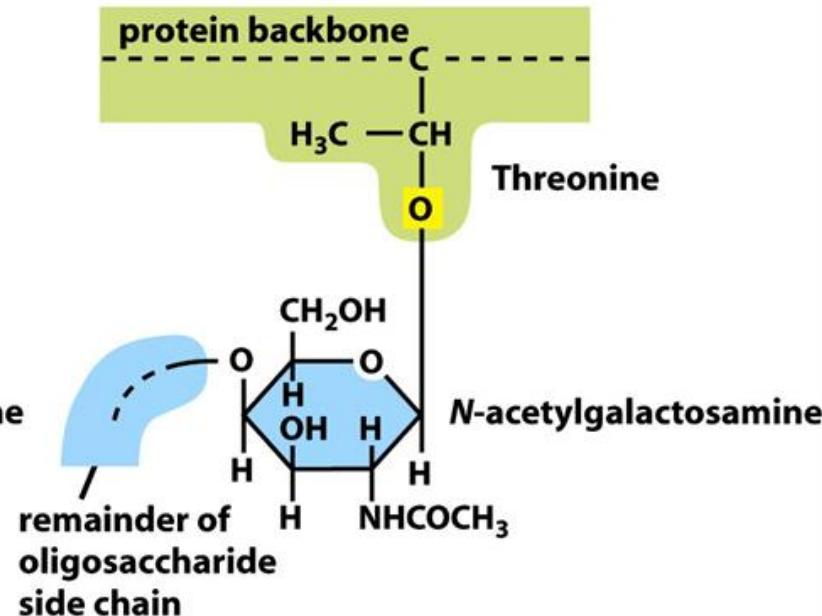
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# Types of glycosylation

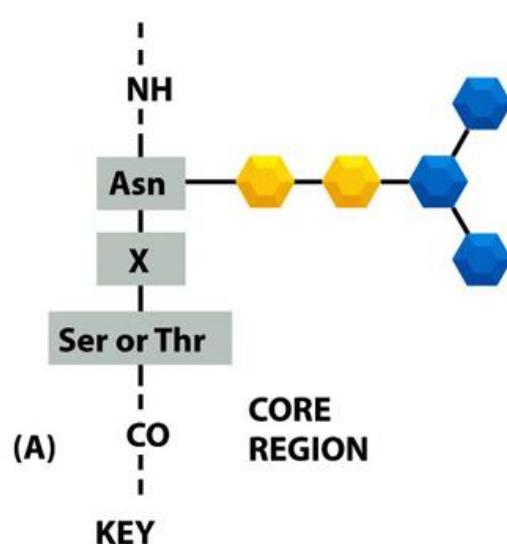
## N-LINKED GLYCOSYLATION



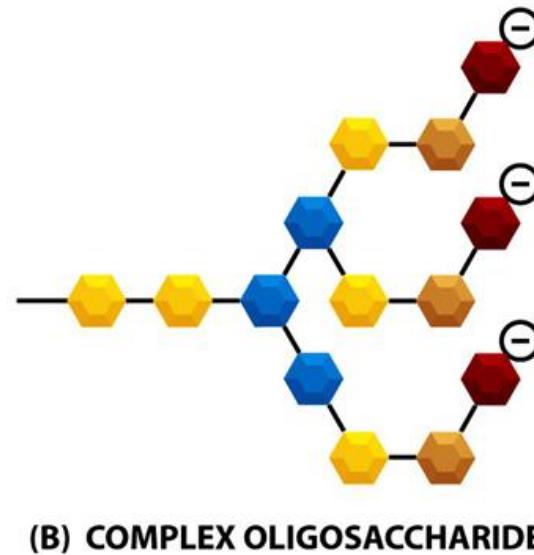
## O-LINKED GLYCOSYLATION



# Types of main N-linked glycosylation



- = *N*-acetylglucosamine (GlcNAc)
- = mannose (Man)
- = galactose (Gal)
- = *N*-acetylneuraminic acid (sialic acid, or NANA)



# Functions for glycosylation

- ♥ N-linked glycosylation promotes protein folding by:
  1. making it soluble and preventing aggregation
  2. marking protein folding state.
- ♥ Make protein more resistant to proteolytic enzymes
- ♥ Important for cell-cell recognition, e.g. selectin recognizes specific sugar group.
- ♥ Change specificity of protein in cell surface signaling molecules , e.g., Notch through O-linked glycosylation

# Golgi matrix proteins help organize the stack

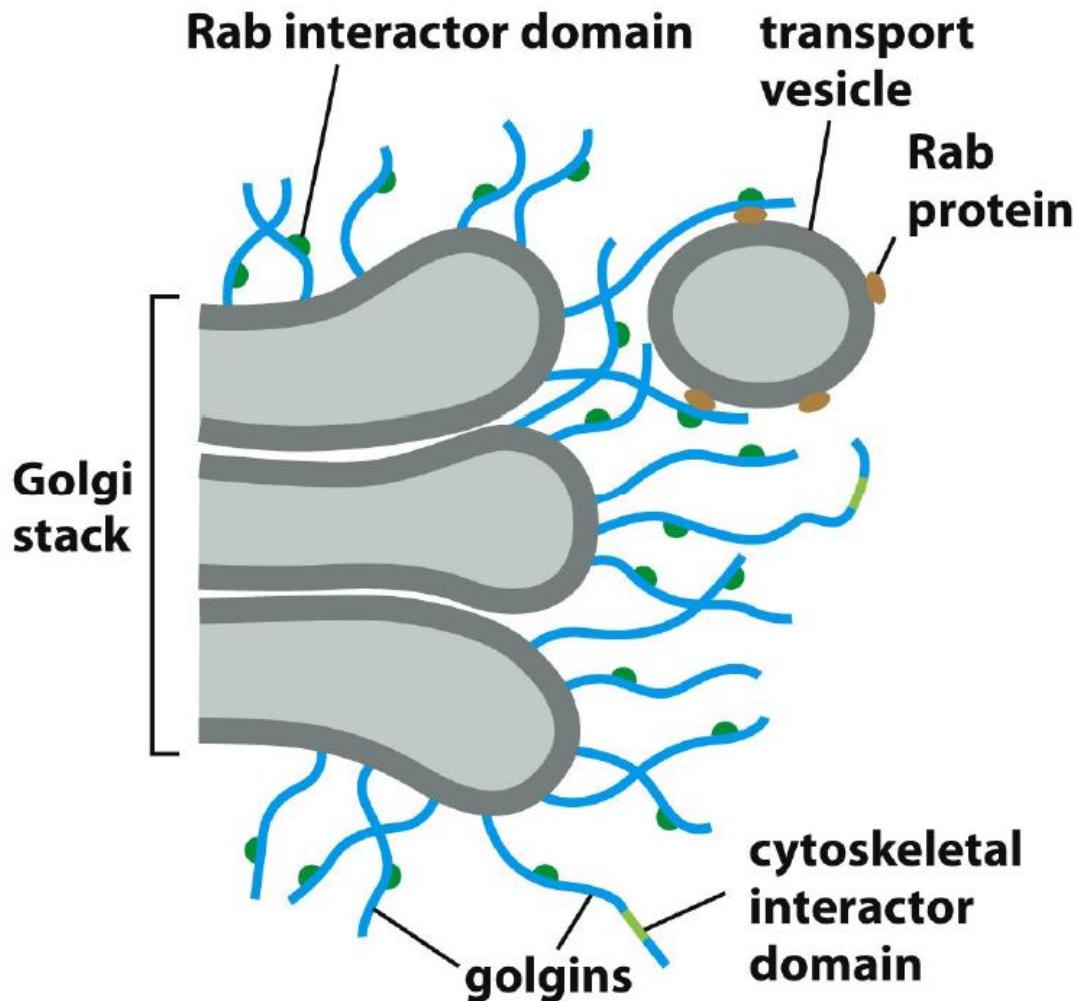
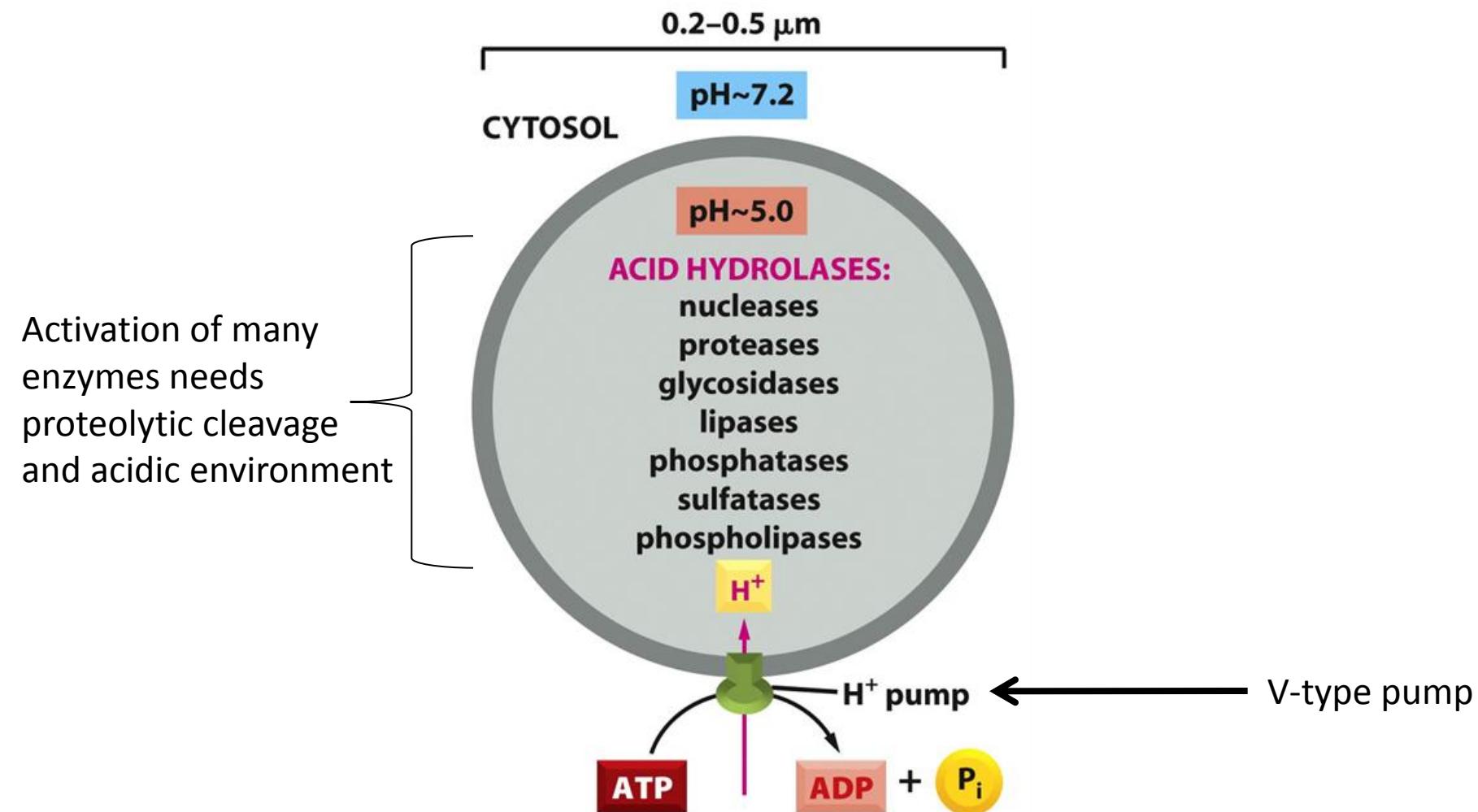


Figure 13-36 Molecular Biology of the Cell 6e (© Garland Science 2015)

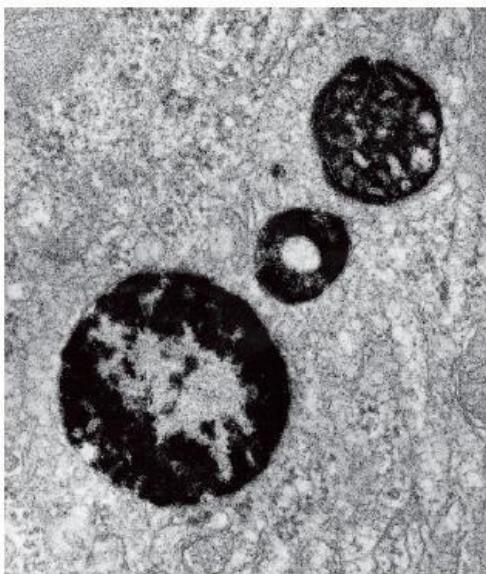
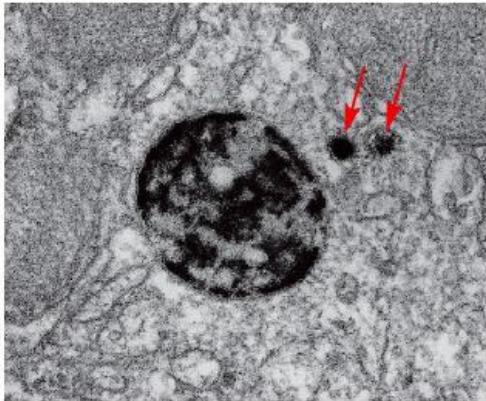
## IV. Transport from TGN to lysosome

- ♥ Introduction to lysosomes
- ♥ Transport from TGN to lysosomes

# Lysosome



# Lysosomes are heterogenous



200 nm

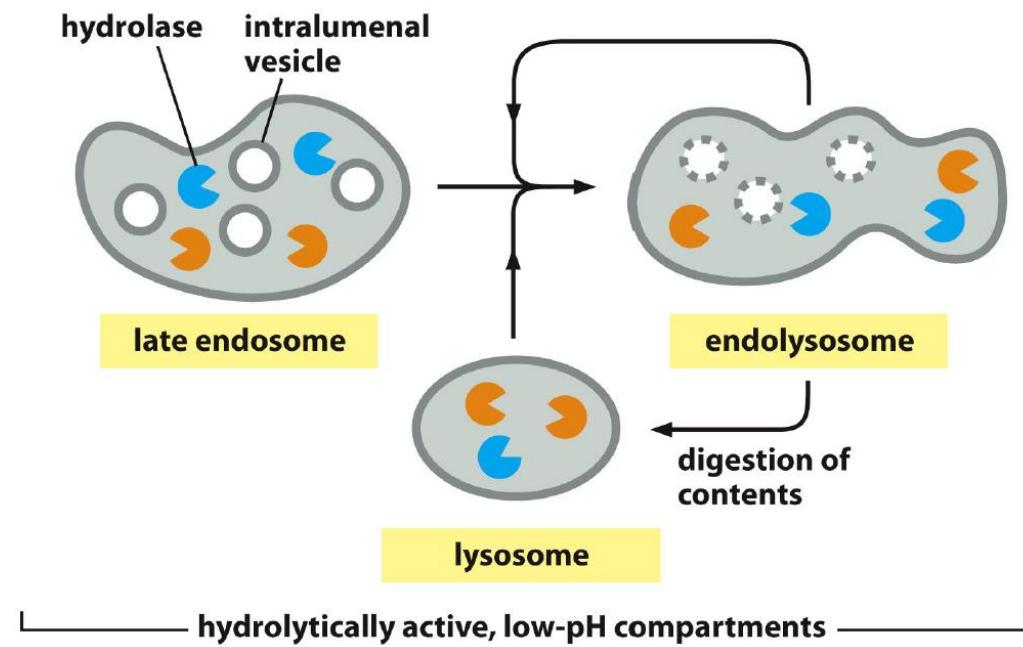
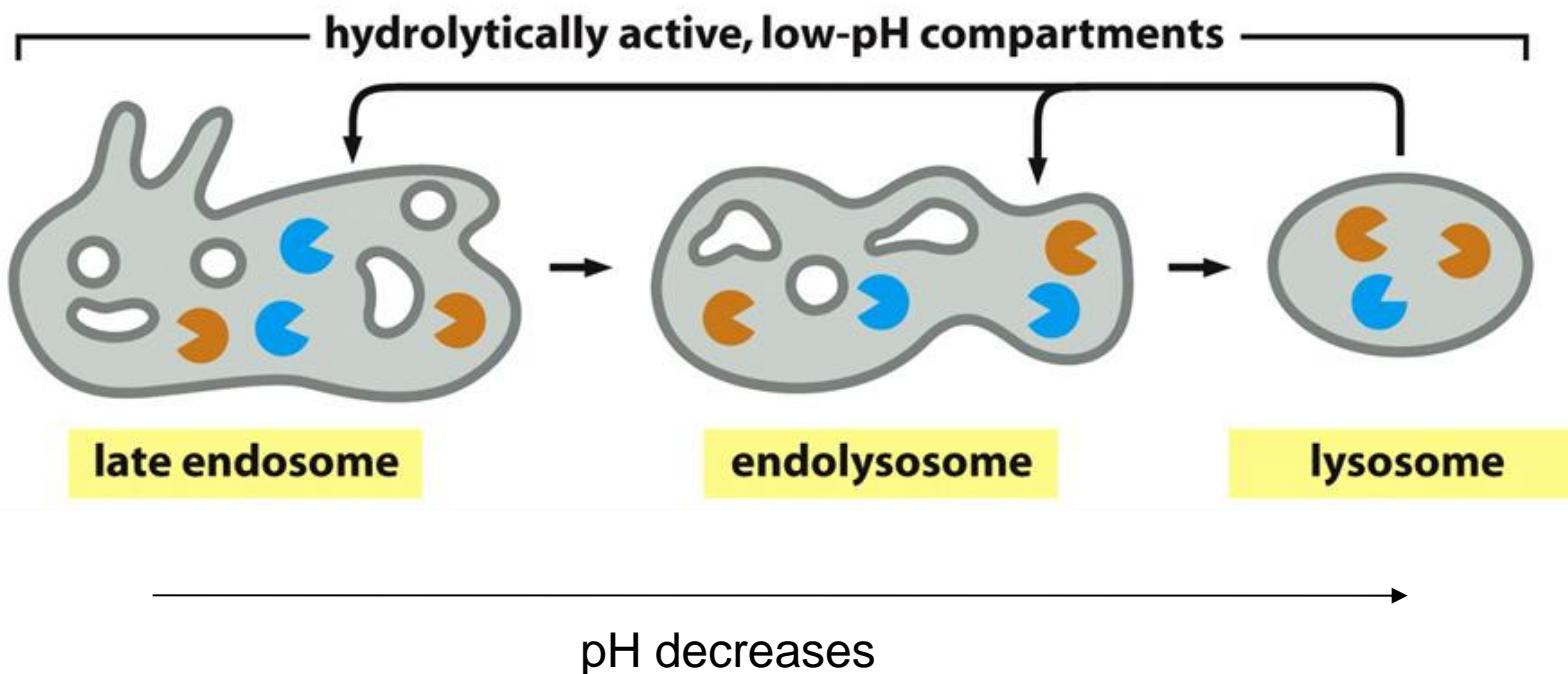


Figure 13-39 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Lysosome maturation



## Facts about lysosomes

♥ Found in all eukaryotic cells

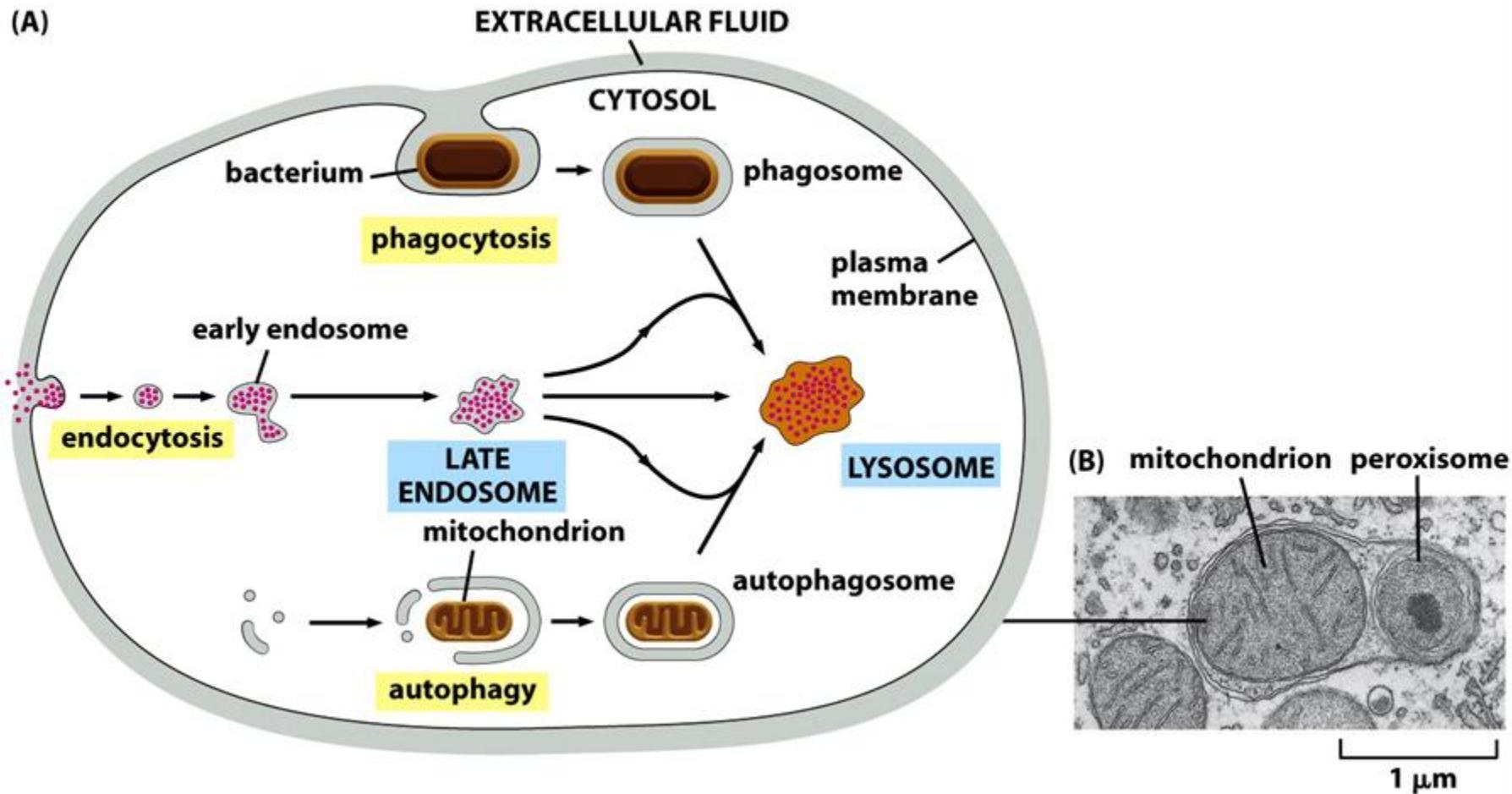
♥ Heterogeneity due to :

- ◆ multiple functions
- ◆ different maturation stages

♥ Functions of lysosomes:

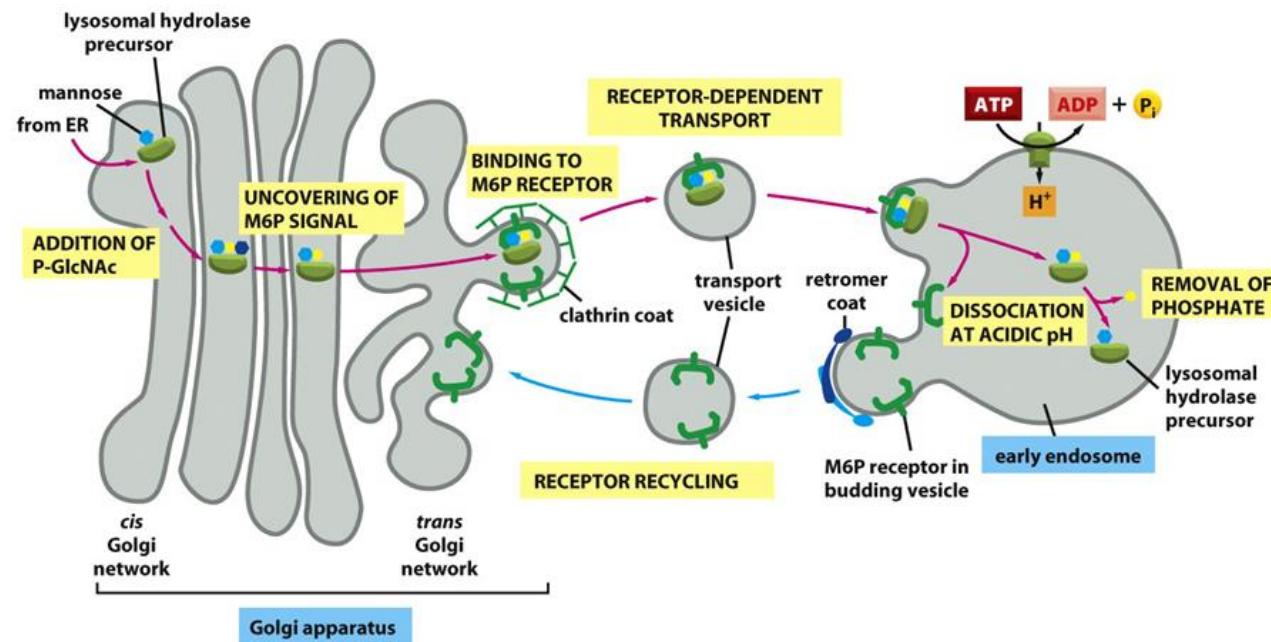
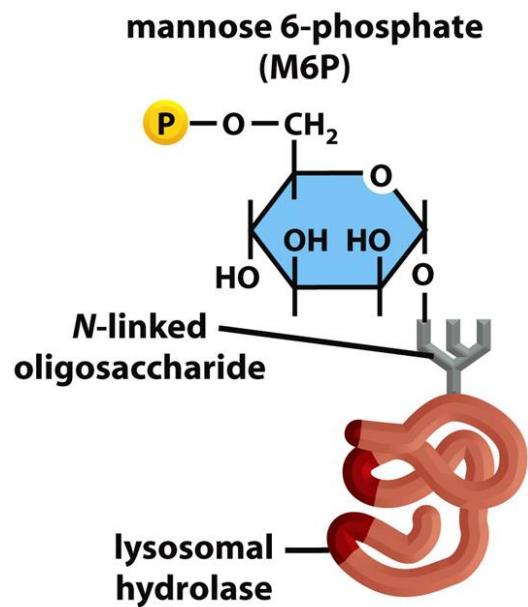
- ◆ breakdown intra- and extracellular debris
- ◆ destruction of phagocytosed microorganism
- ◆ production of nutrients for the cell

# Three pathways for degradation in lysosome



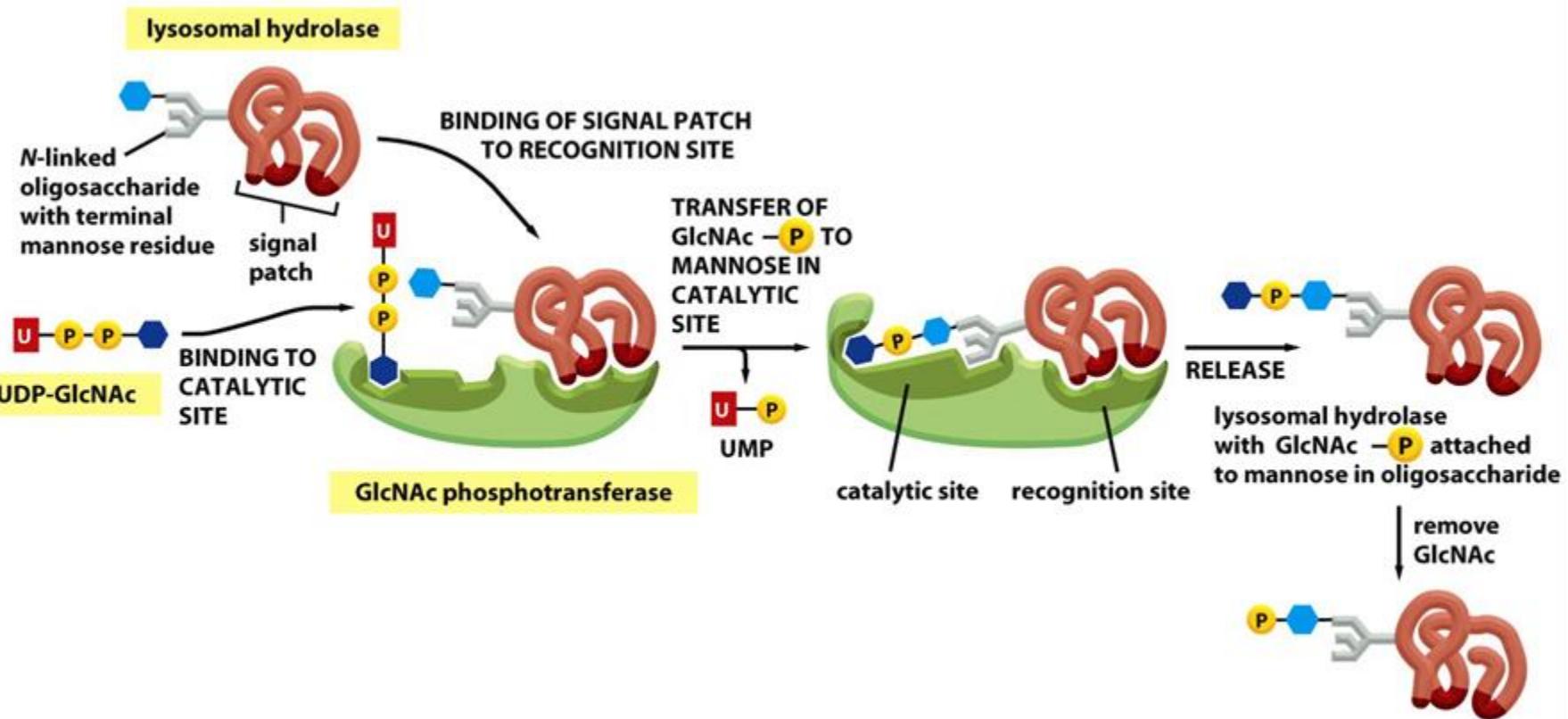
Indigested materials will be excreted out by exocytosis

# How are hydrolases selected to be transported to lysosome?



M6P receptor protein works best at pH 6.5-6.7,  
And release at pH 6.0( late endosome)  
M6P is then hydrolyzed inside endosome.

# The recognition of a lysosomal hydrolase



## Diseases associated with failure in TGN-lysosome transport

- ♥ **I-cell disease**: defect in GlcNAc-phosphotransferase, resulting in failure of lysosome hydrolases to be transported to lysosome, instead, they are secreted out.
- ♥ **Hurler's disease**: defect in a certain enzyme important for breakdown of glycosaminoglycan chains
- ♥ **Albinism**: defect in exocytosis of pigment lysosomes such as melanocytes

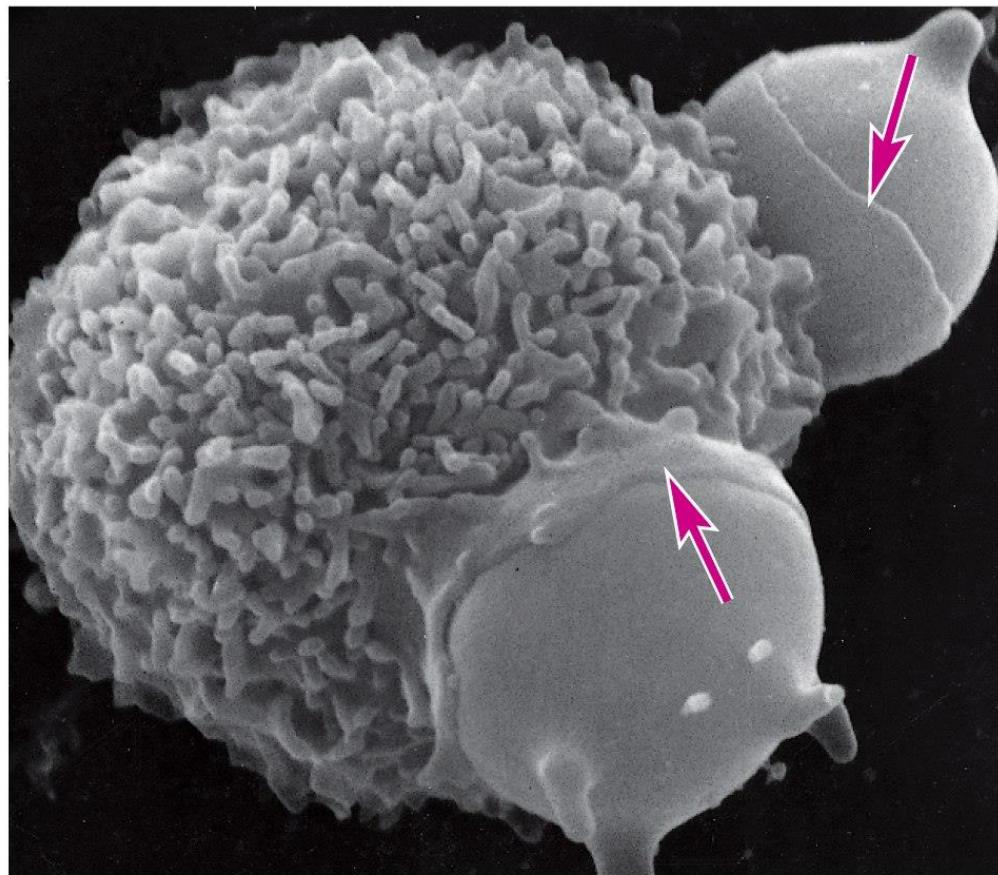
## V. Endocytosis

1. Overview of endocytosis
2. receptor mediated endocytosis
3. Fate of endocytic materials

## Two major types of endocytosis

- ♥ **phagocytosis**--- for large particles, a selective process, phagosomes are > 250nm in diameter, **is important to scavenge old and dead cells, and is important to eat outside pathogens.**
- ♥ **pinocytosis**--- fluid and solute, a continuous process, pinocytic vesicles ~ 100nm in diameter.

# Phagocytosis



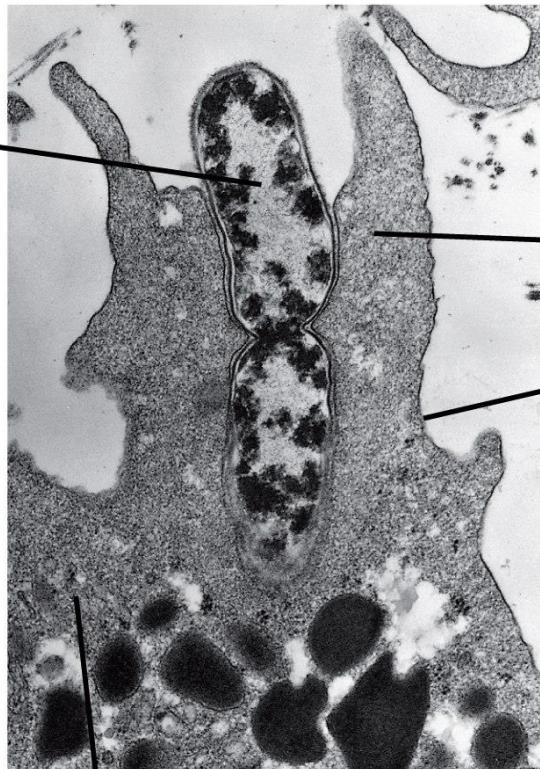
[

5  $\mu\text{m}$

A macrophage is “eating” two red blood cells, a macrophage can eat  $10^{11}$  old RBCs per day

# Phagocytosis

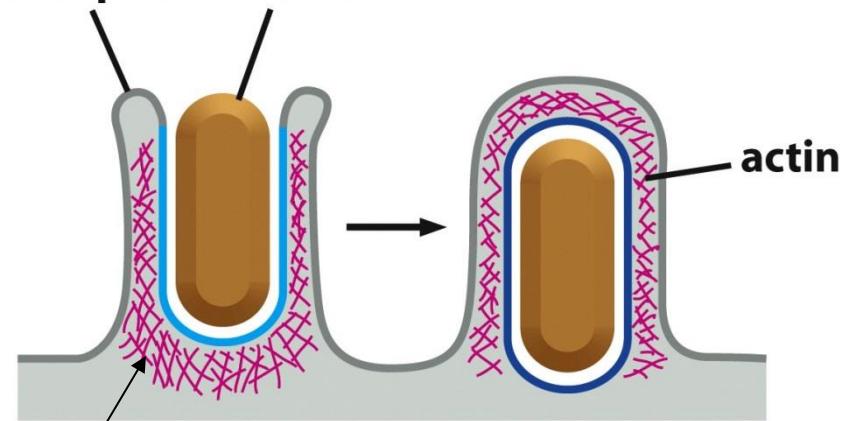
bacterium



phagocytic  
white blood cell

$1 \mu\text{m}$

pseudopod      bacterium



PI 3-kinase

Rho GTPase and Rho GEF trigger signaling  
Events to initiate actin polymerization,  
They also activate PIP kinases as shown.

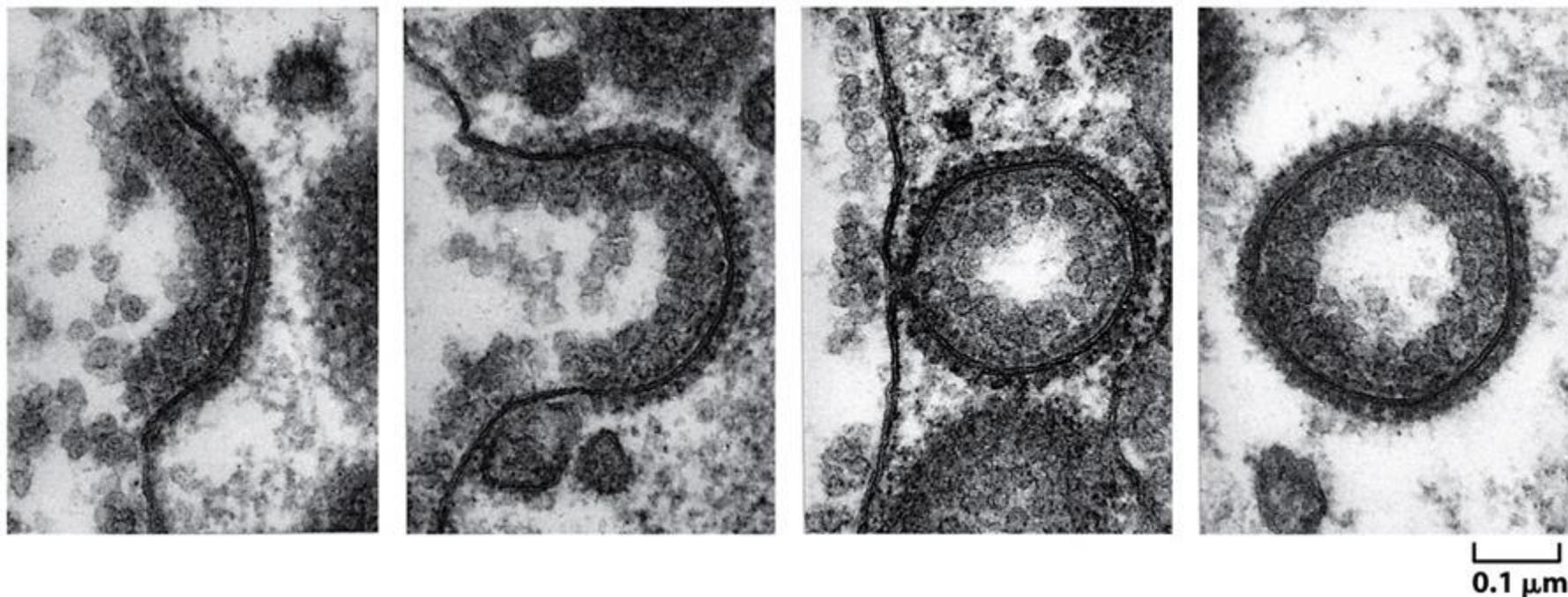
## Factors triggering phagocytosis the “eat-me” signals

- ♥ Antibody coated pathogens.
- ♥ Complements in pathogens.
- ♥ Oligosaccharides in microorganisms
- ♥ Phosphotidylserine in apoptotic cells

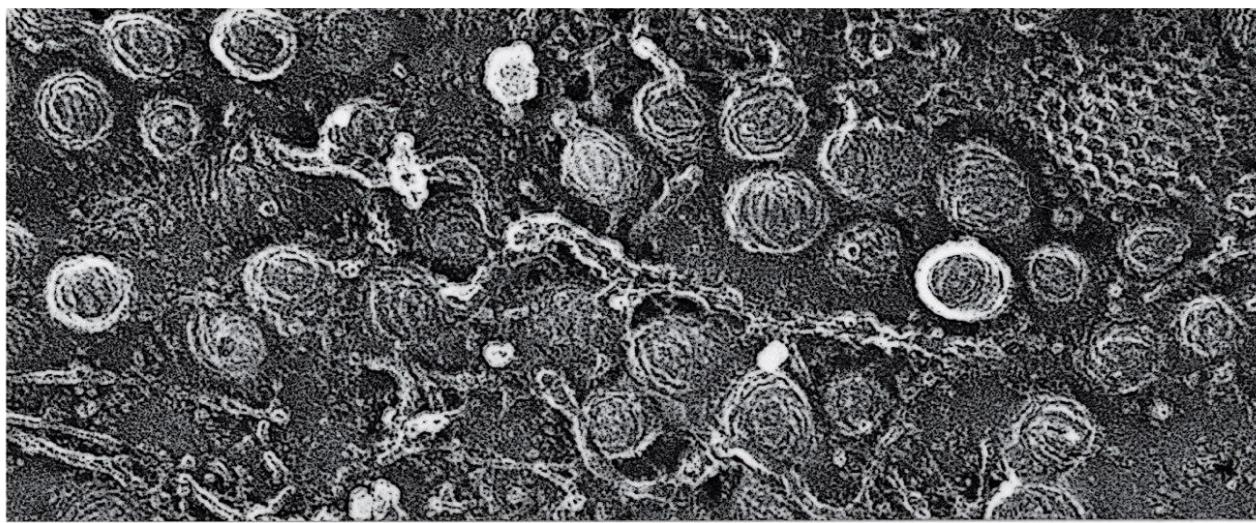
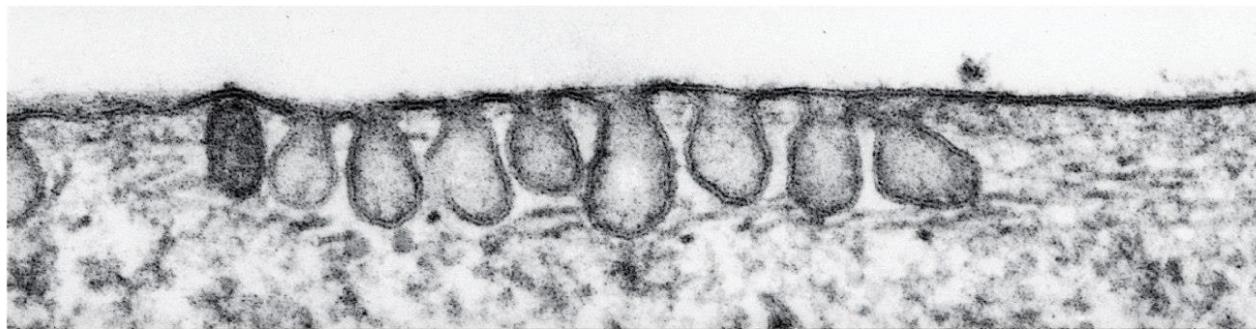
# Pinocytosis

- ♥ Occurs continuously and fast( several percent /min of plasma membrane)
- ♥ Well balance between endocytosis and exocytosis
- ♥ Occurs in several ways:
  1. clathrin-coated pits
  2. caveolae mediated, believed to start from lipid rafts. e.g. SV40 and papilloma virus

# Pinocytosis---clathrin-mediated



# Caveolae mediated pinocytosis



0.2  $\mu\text{m}$

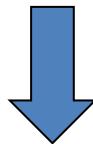
## Endocytosis mediated by Clathrin-coated pits --- receptor-mediated endocytosis

- 25 different receptors mediated this type of endocytosis
- Many different receptors cluster in the same pit
- The same receptors can cluster in different pits

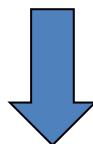
# Cells actively regulate receptor mediated endocytosis

For example:

Mono- and multi-ubiquitination of receptors



Ubiquitin binding protein recognizes these receptors

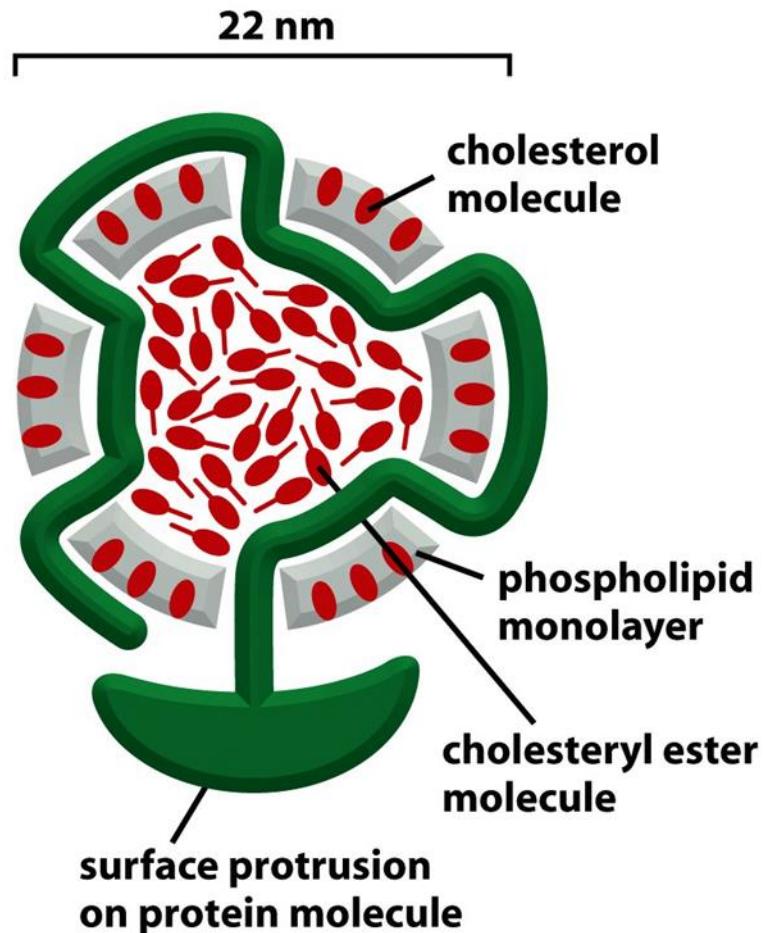


Direct clathrin coat assembly

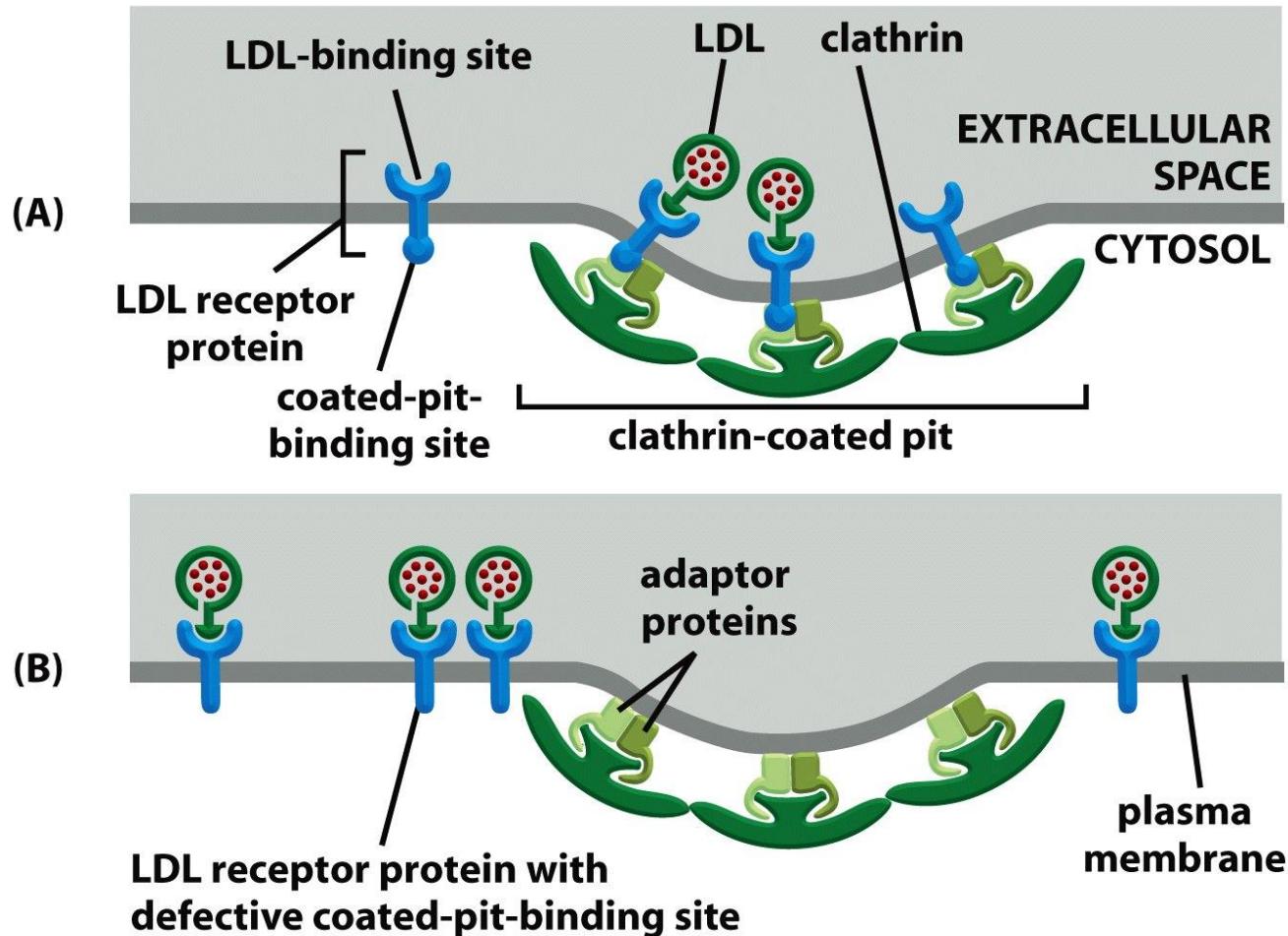
# Example: Taking up of cholesterol by LDL receptor on cells

## Structure of the LDL

Atherosclerosis : leads to stroke and heart attack. It occurs by Failure to take up LDL into cells, which causes accumulation of LDL in the blood, which then forms Atherosclerotic plaques.



# Endocytosis of LDL by normal and mutant LDL receptors

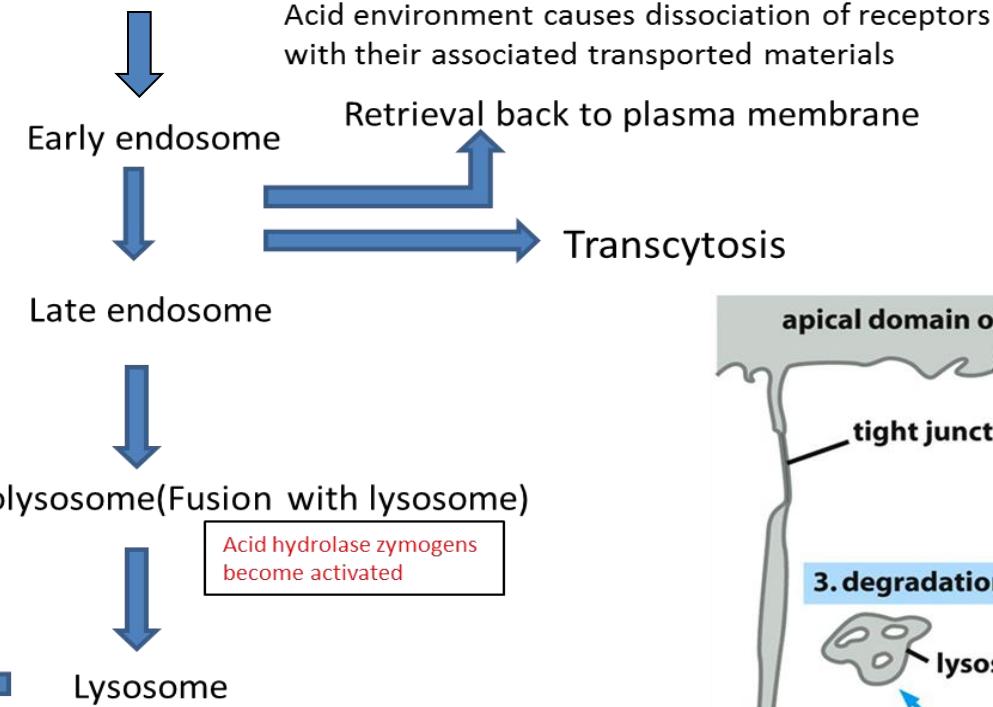




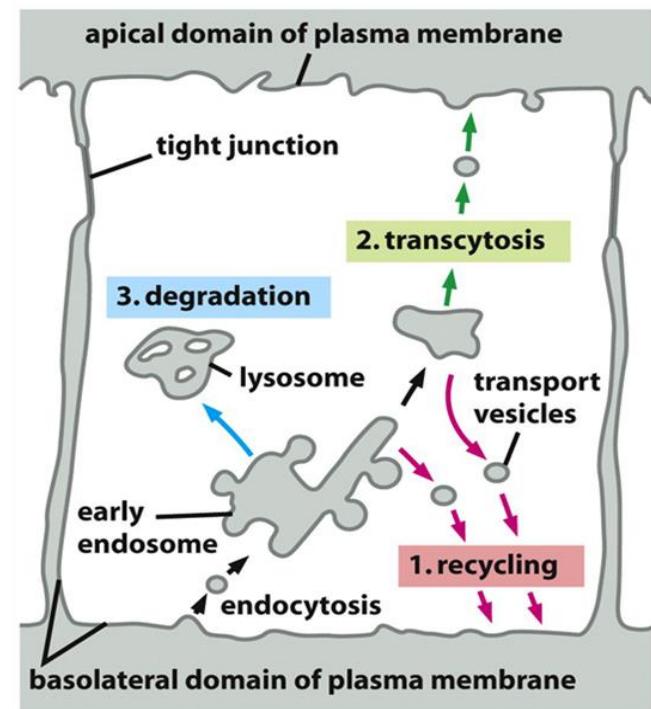
**13.3-receptor\_endocytosis.mov**

# Fate of endocytosed materials

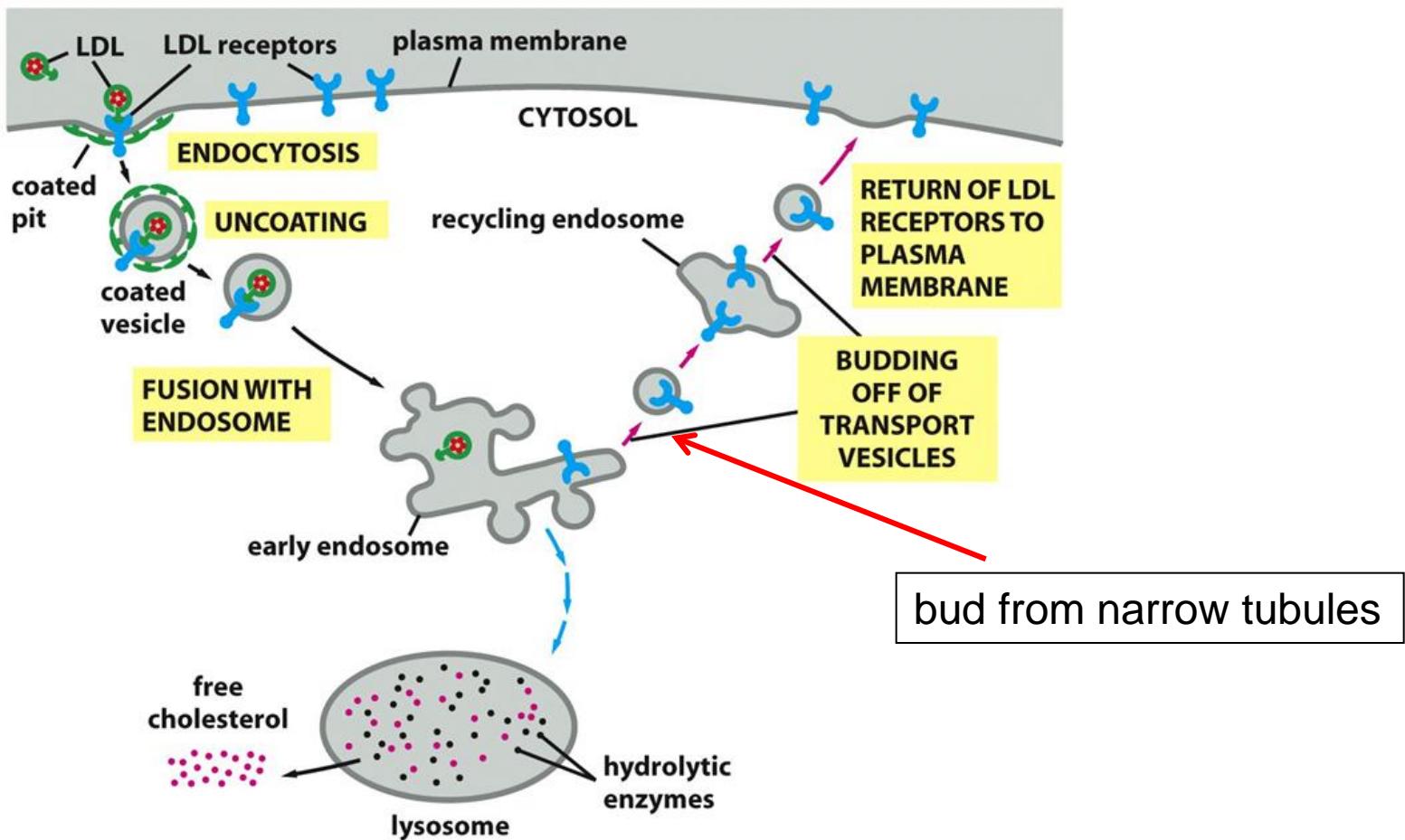
Endocytic particle fusion with endosome



Protein composition in each stage is different

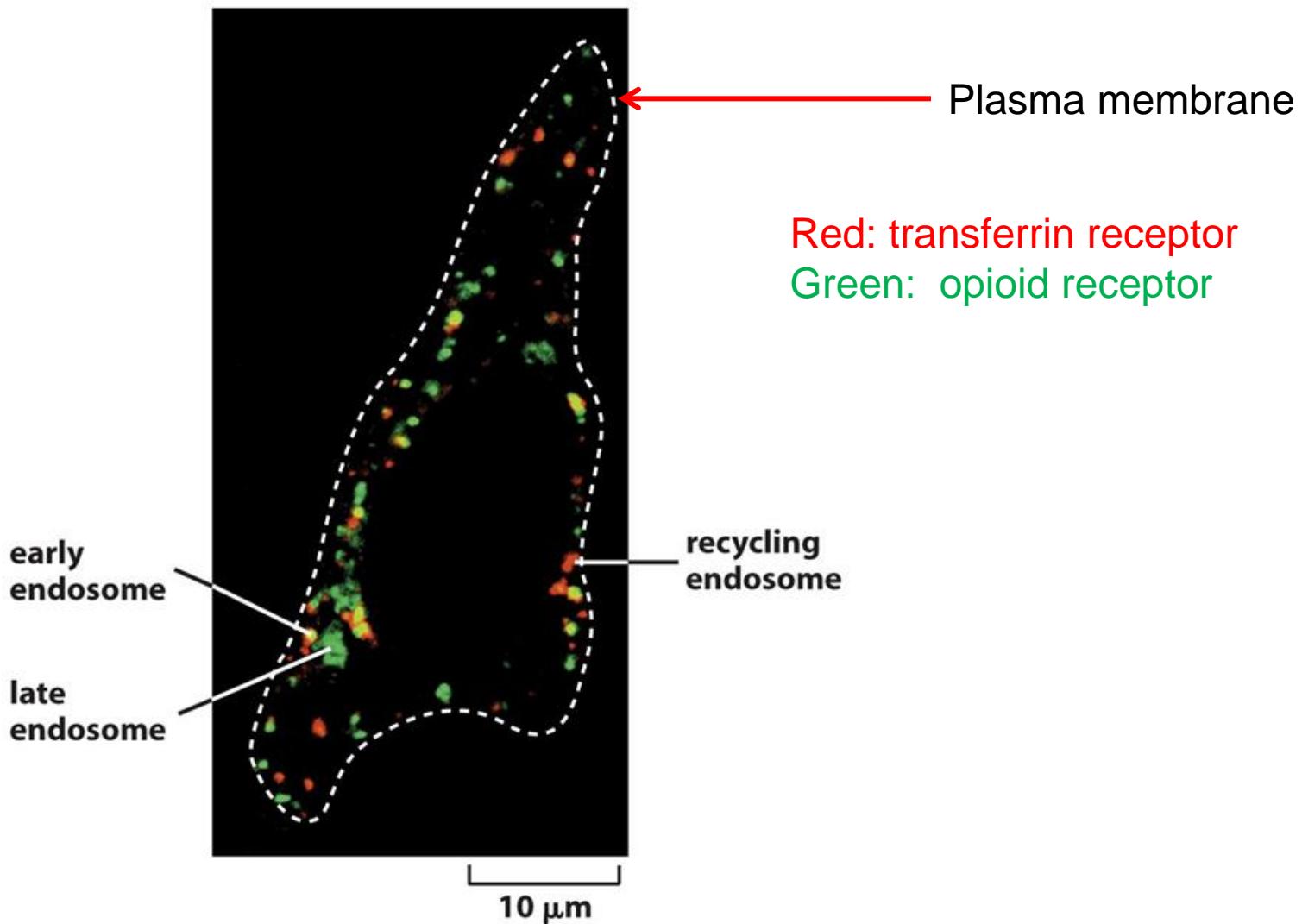


# (1) Retrieval: LDL-receptors are retrieved

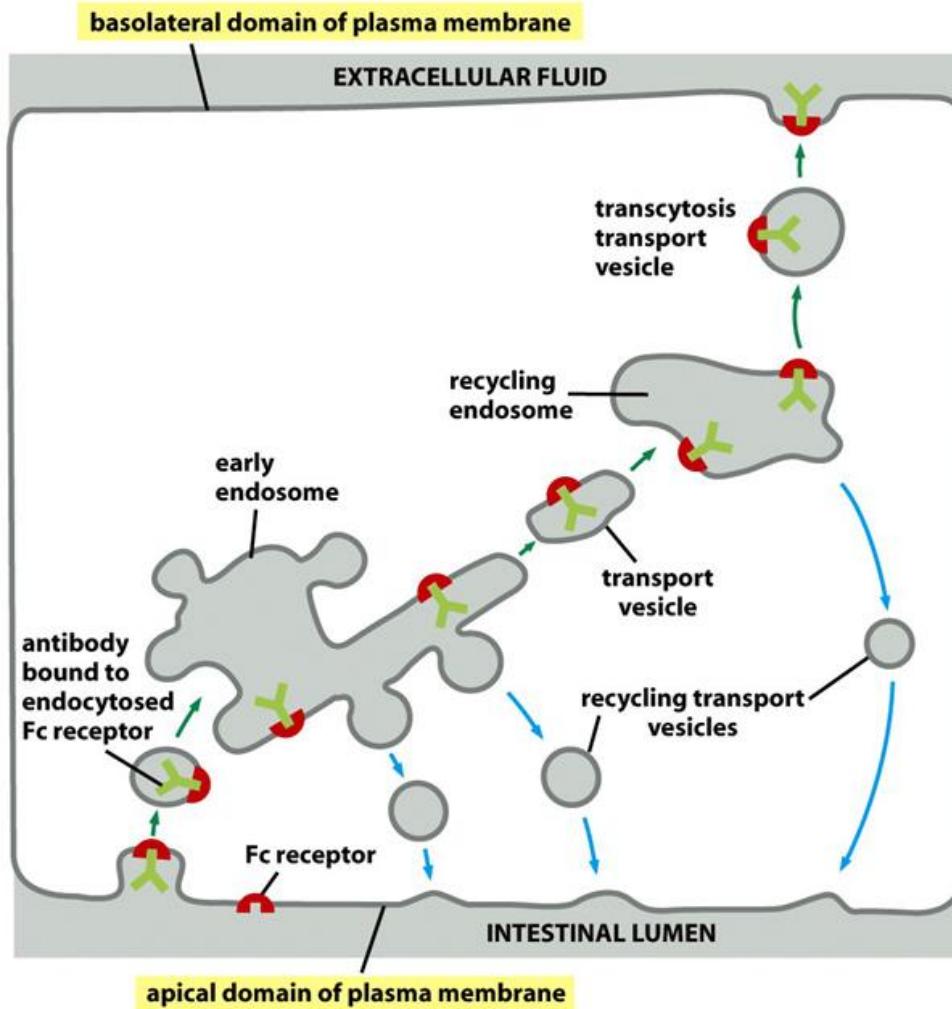


Some other receptors are degraded in lysosomes, such as EGFR, opioid receptors  
--- a process called *receptor down-regulation*.

# Example: transferrin receptor and opioid receptor are sorted differently



## (2). Transcytosis --- in polarized epithelial cells

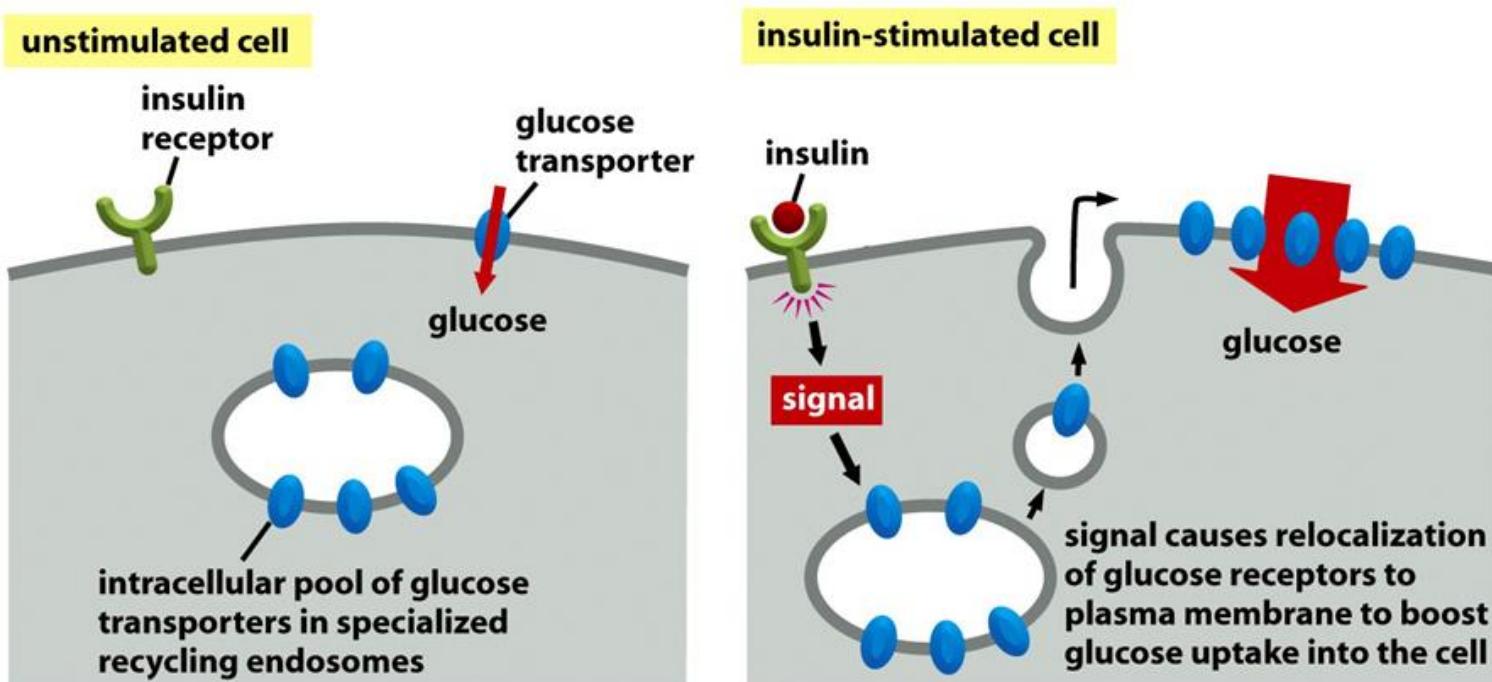


Different pH in two sides of cells decides association and dissociation between receptor and its ligand

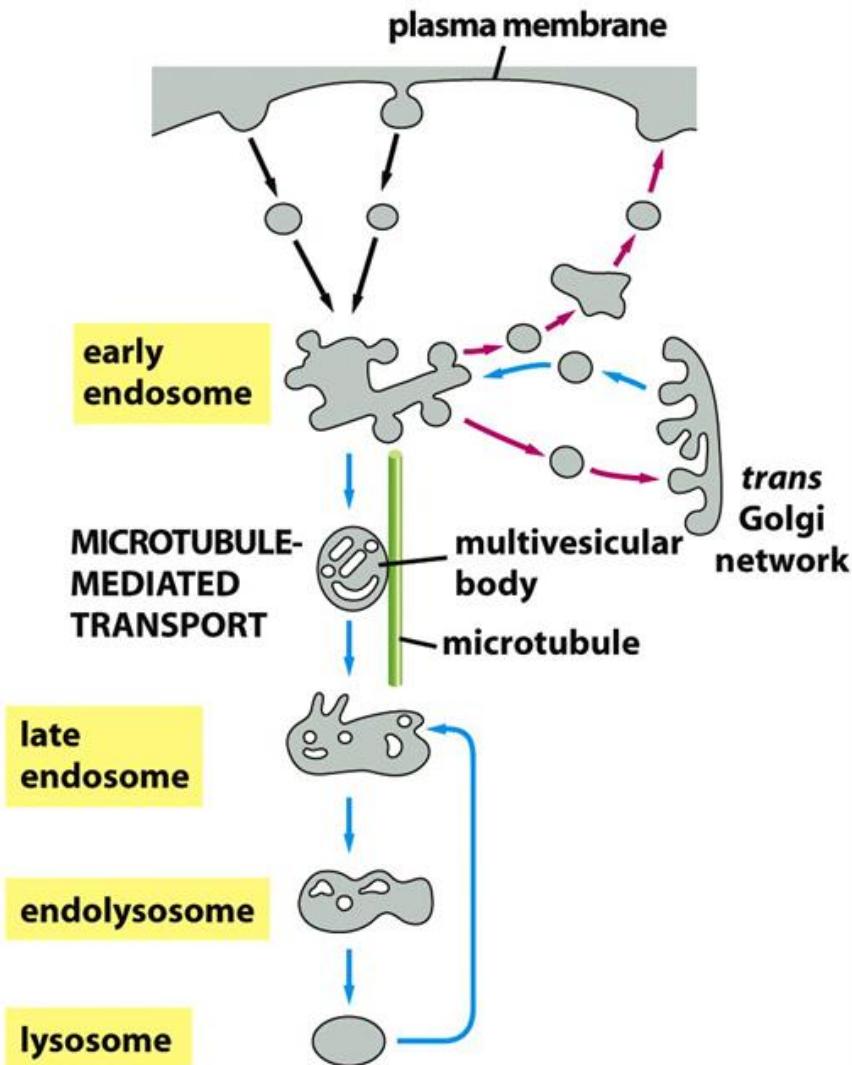
Different sorting signals in receptors may play a role in transcytosis

Mediated by recycling endosome (believed to be an important regulation site to adjust cell membrane protein in response to need)

Example:

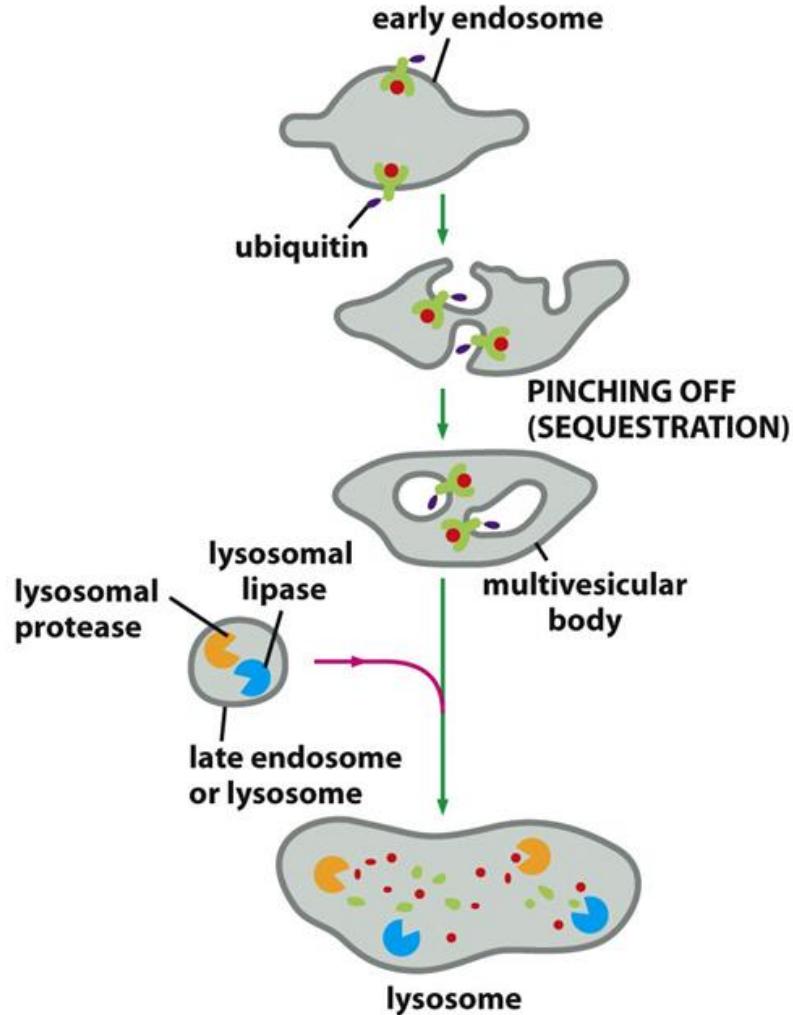


### (3). Late endosome-lysosome pathway--- mediated by multivesicular bodies



Multivesicular bodies derive from Early endosome, move along Microtubules, then turn to be late endosomes, which are targeted to degradation in lysosomes

# Ubiquitination tags serves as recognition marks for formation of multivesicular body



# ESCRT proteins recognize both ubiquitination and PIPs signals during multivesicular body formation

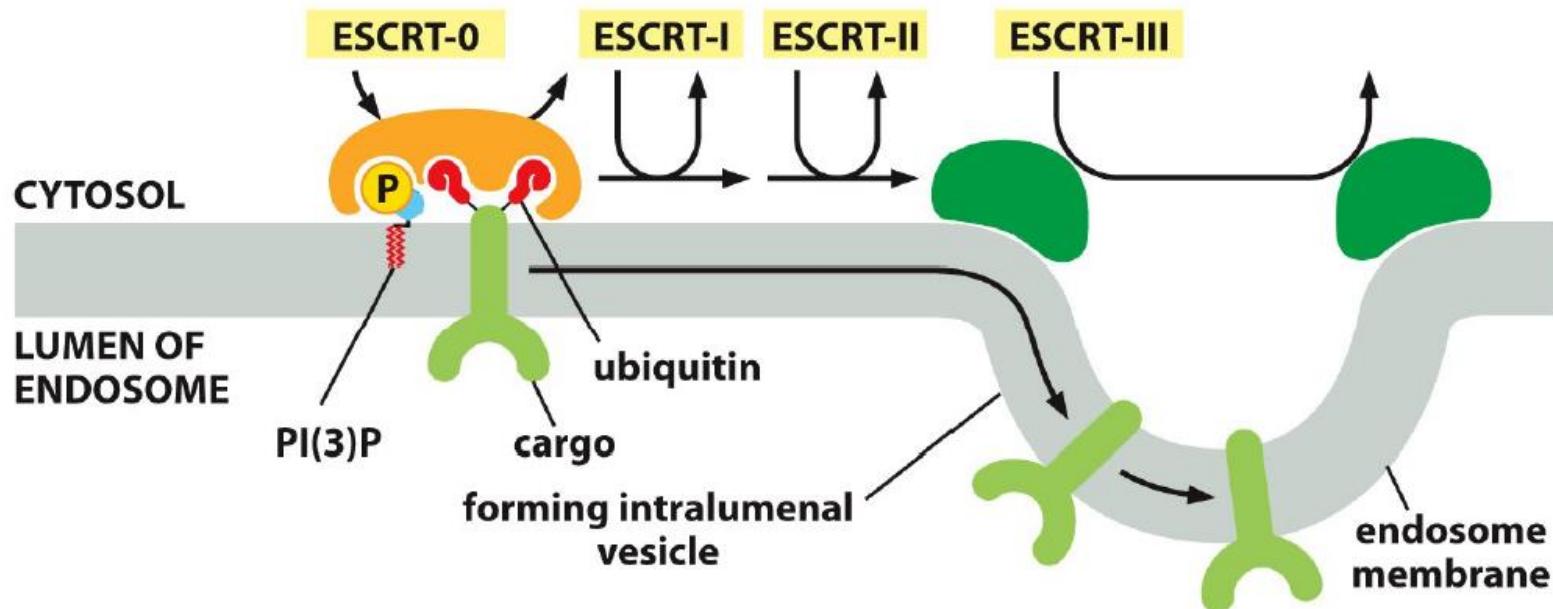


Figure 13-56 Molecular Biology of the Cell 6e (© Garland Science 2015)

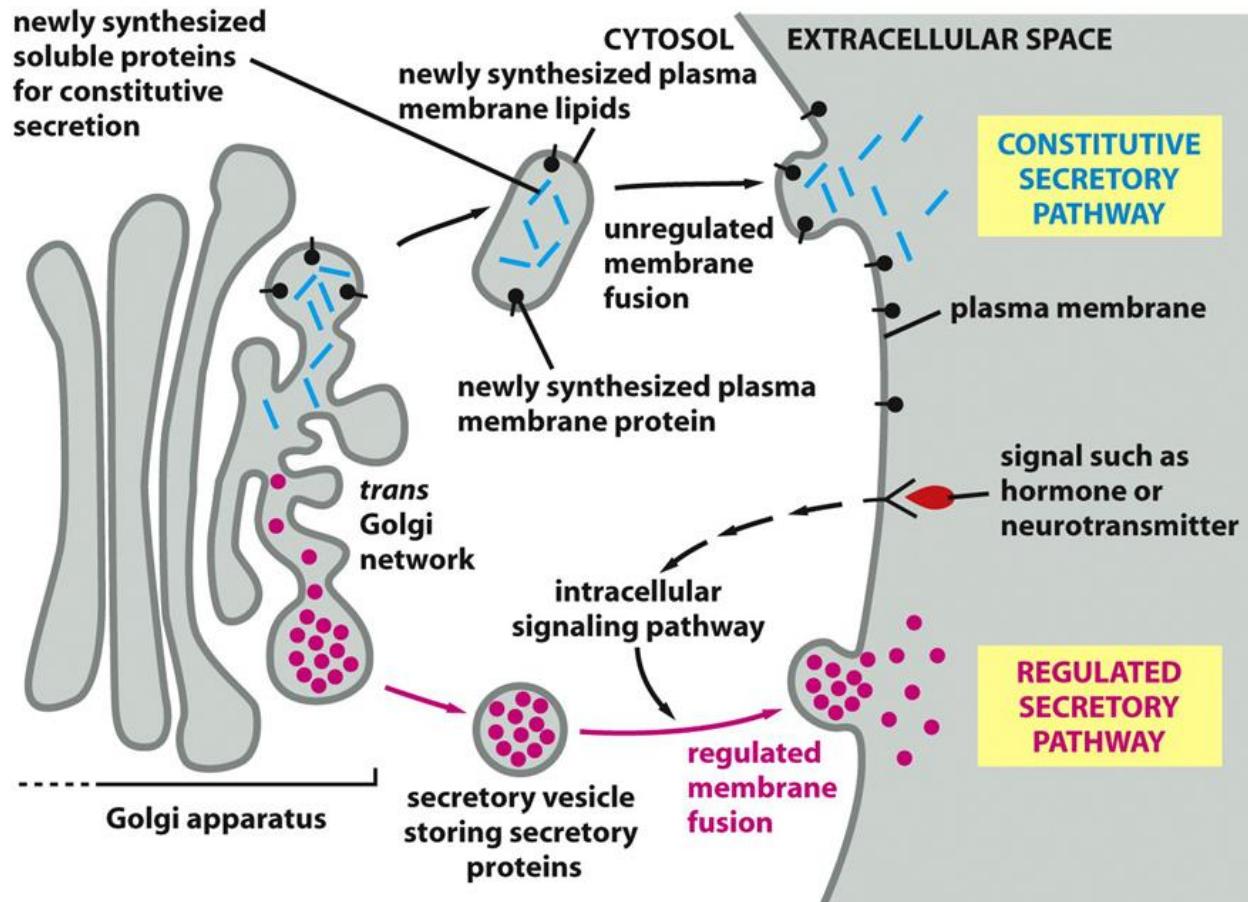
Mutations in ESCRT proteins are associated with prolonged signaling and cancer

# VI. Exocytosis

( From TGN to plasma membrane)

1. Overview of exocytosis
2. Formation of secretory particle
3. Signaling to release secretory content
4. Membrane lipid and protein after exocytosis

# Two different secretory pathways



# Constitutive and regulated secretory pathways

Constitutive pathway: all cells have it.

transport: membrane proteins  
lipid molecules  
ECM proteins

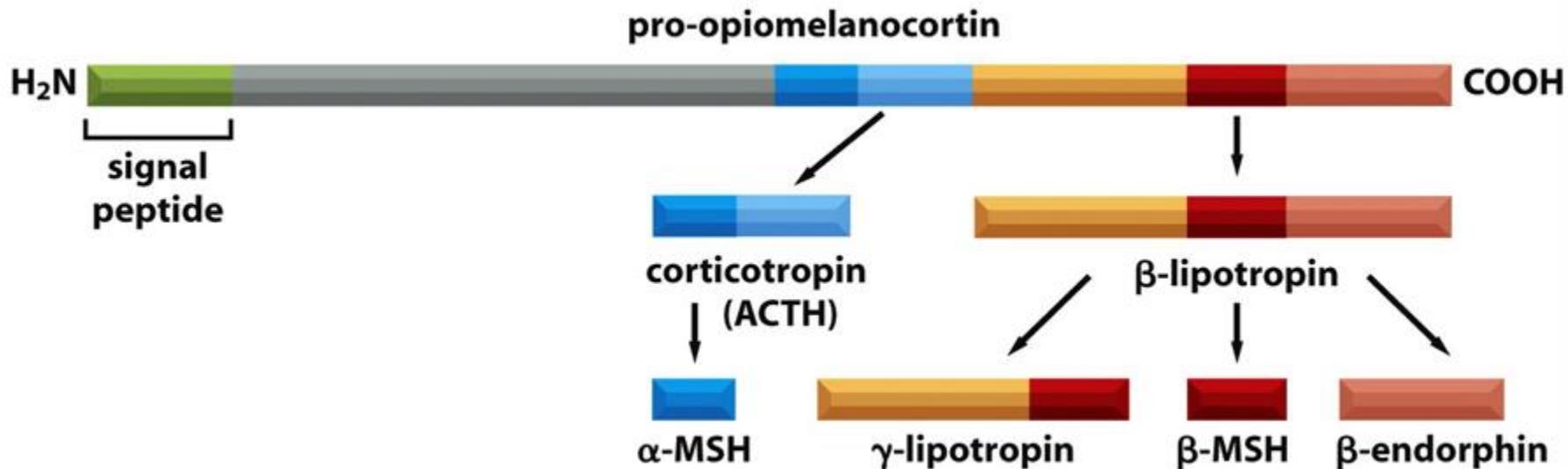
Regulated pathway: mainly in special secretory cells

transport:  
hormones, histamine, etc  
neurotransmitters  
digestive enzymes

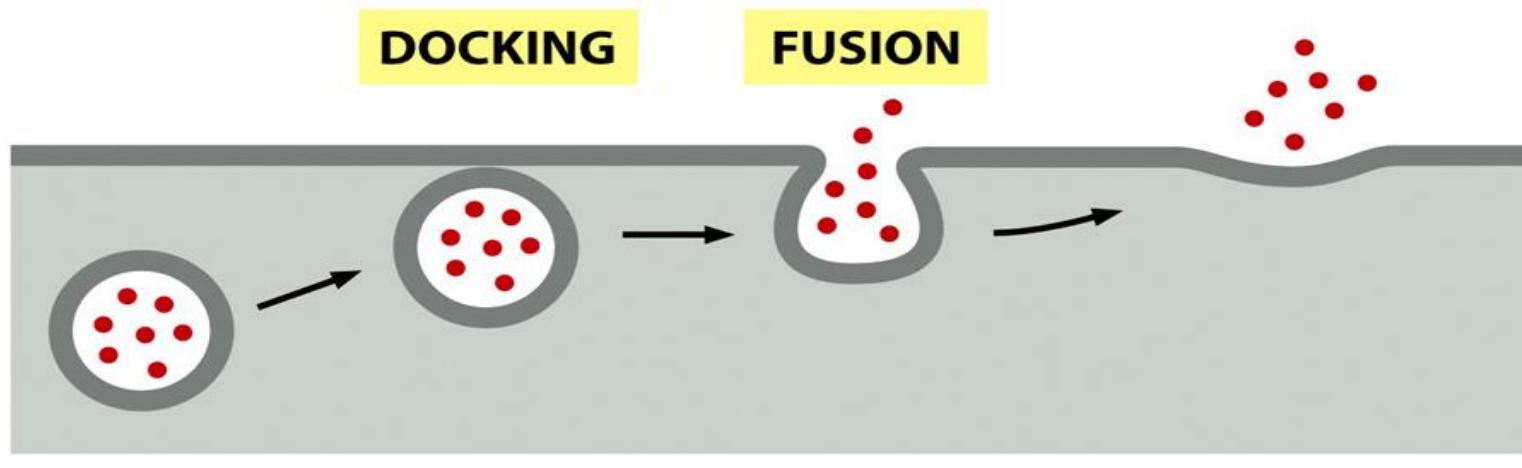
# Specialized secretory vesicles

- ★ Secretory particles aggregate in TGN lumen, occurs selectively (due to signal patch?)
- ★ Are electron-dense,
- ★ Occurs by two mechanism, aggregation triggered by:
  - (1). Different ionic environment
  - (2). clathrin coated retrieval of membrane and luminal content
- ★ Many of the proteins were previously synthesized as pro-peptide form
- ★ Proteocleavage starts in TGN, continue in secretory vesicles and ECM

Example:

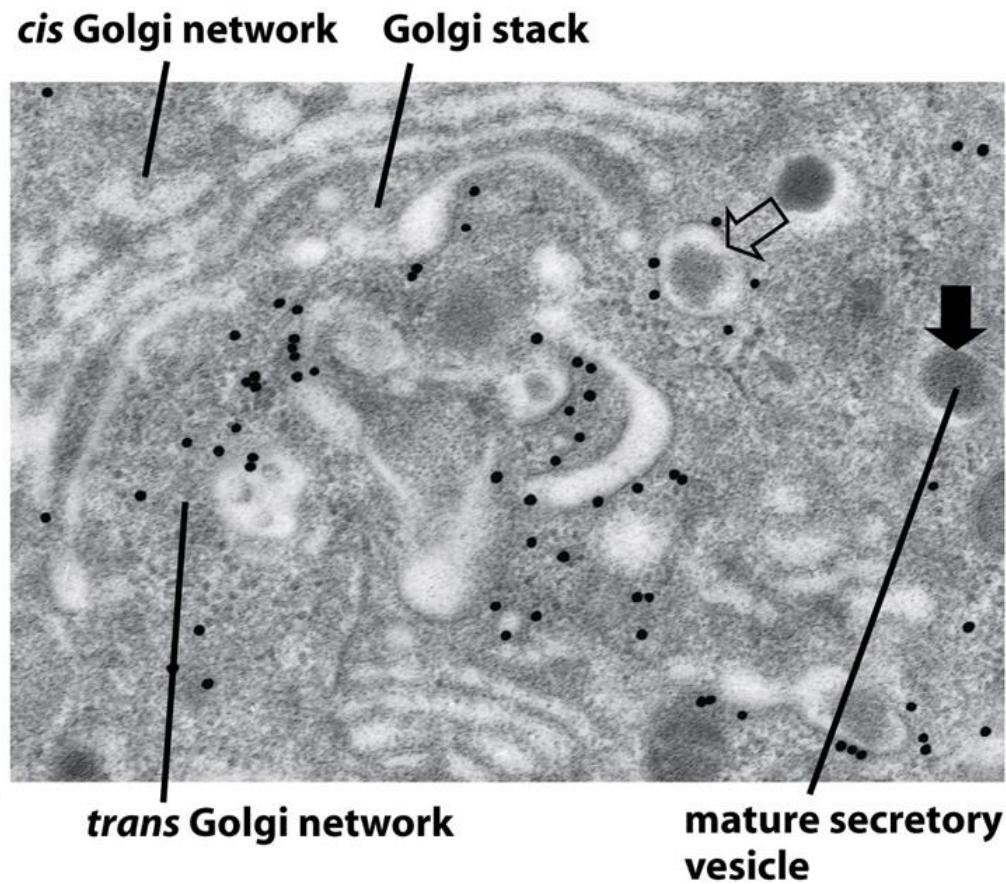
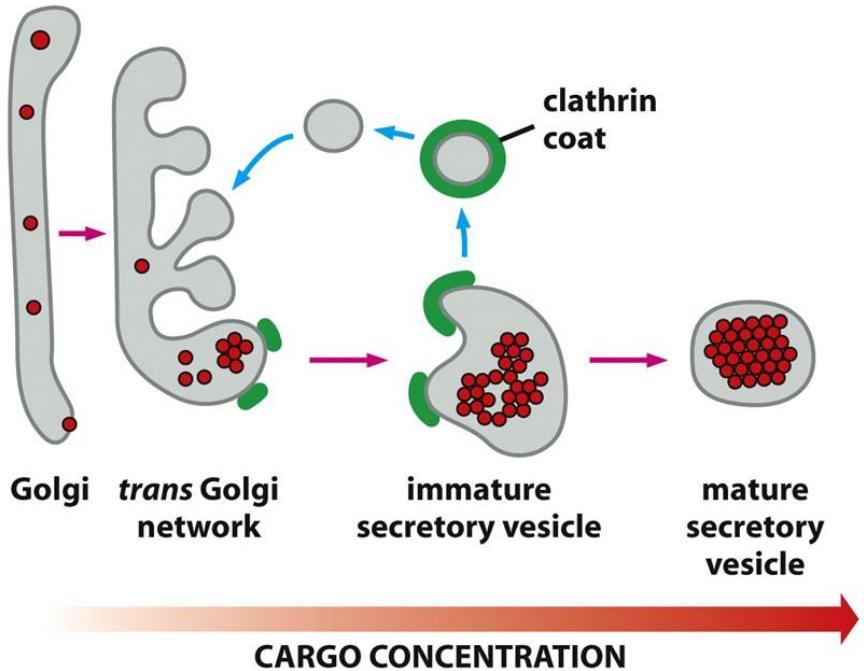


# Exocytosis of secretory vesicles



$0.2 \mu\text{m}$

# The formation of the secretory vesicles



Electron microscopy of pancreas  $\beta$ -cells  
Secreting vesicle formation.  
Antibody to clathrin is conjugated to gold

# How to trigger secretory vesicle to release its content

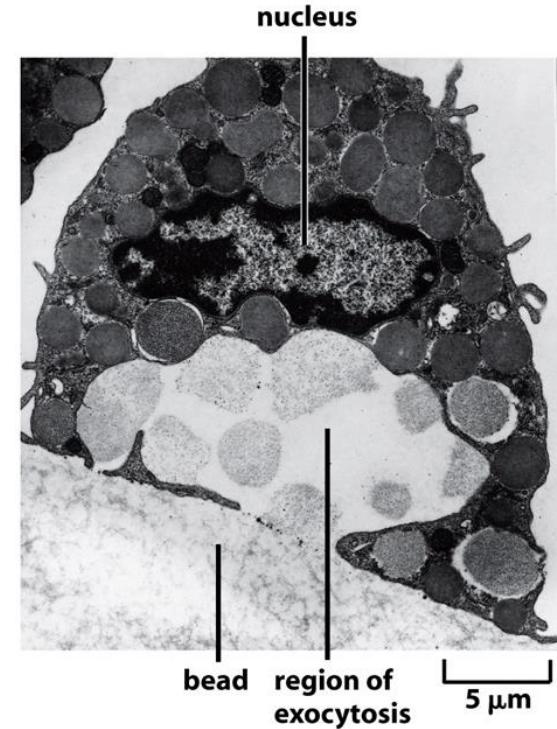
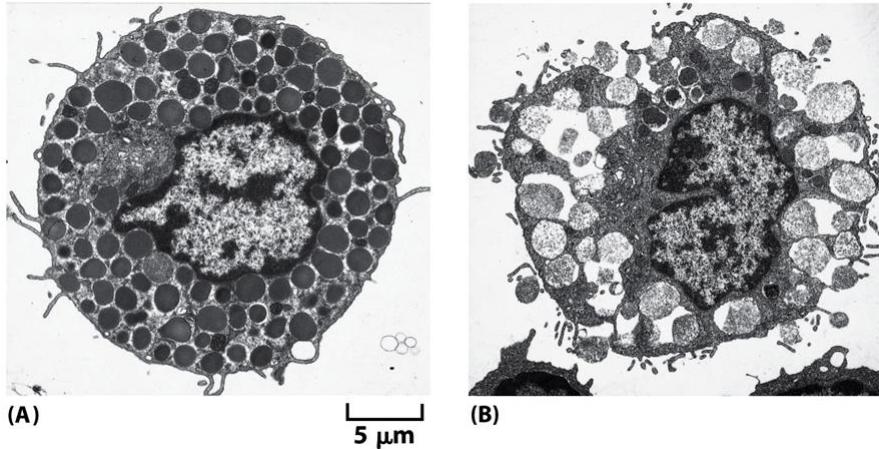
Example 1: in nerve cells, voltage-gated  $\text{Ca}^{2+}$  channel influx  $\text{Ca}^{2+}$ , which triggers nerve cells to release neurotransmitters.

Example 2: ligand binding stimulate mast cells to release histamine

# Can be local or all over in the cell

Ligand is fixed in a solid surface

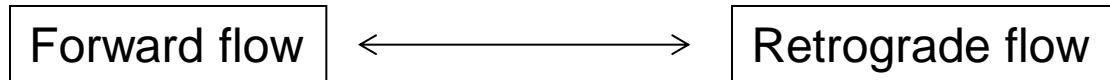
Soak mast cell in ligand-containing solution



# What happens to the membrane lipid and proteins after exocytosis?

Transient fused with plasma membrane--- **forward flow**

then recycled or to lysosomes for degradation--- **retrograde flow**

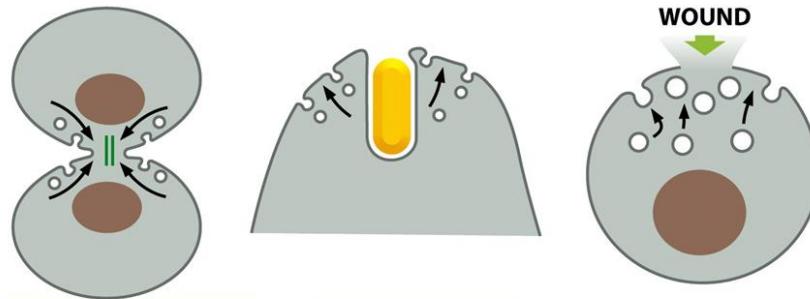


Balance --- no net cell grow

Forward flow dominates --- cell rapidly grows

Retrograde flow dominates --- cell shrinks

Examples to expand membrane:



(A) CLEAVAGE FURROW

(B) PHAGOCYTOSIS

(C) WOUND REPAIR