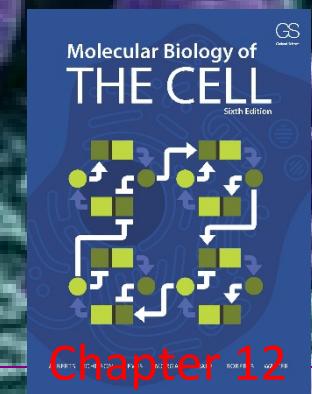


Lecture 6

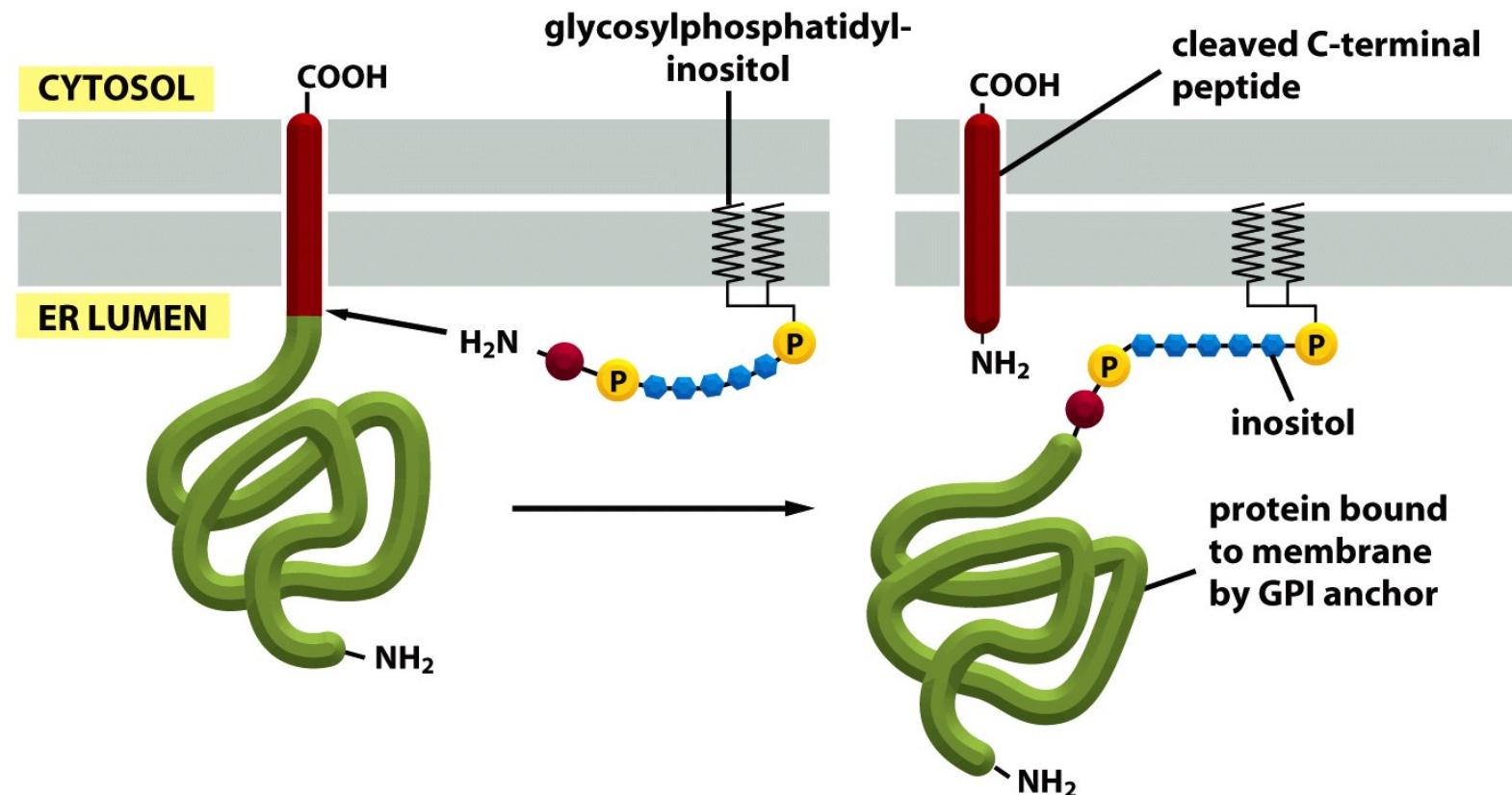
Transport of macromolecules Part I

Outline

- I. Cell compartments
- II. Transport between nucleus and cytosol
- III. Transport to mitochondria and chloroplast
- IV. Transport to the peroxisome
- V. Transport to the endoplasmic reticulum



Glycosylphosphatidylinositol (GPI)-anchored proteins: how to covalently attach the GPI anchor to a protein



Phosphoethanolamine: provides the amino group for the attachment of the protein by an enzymatic “cleavage-transfer” reaction

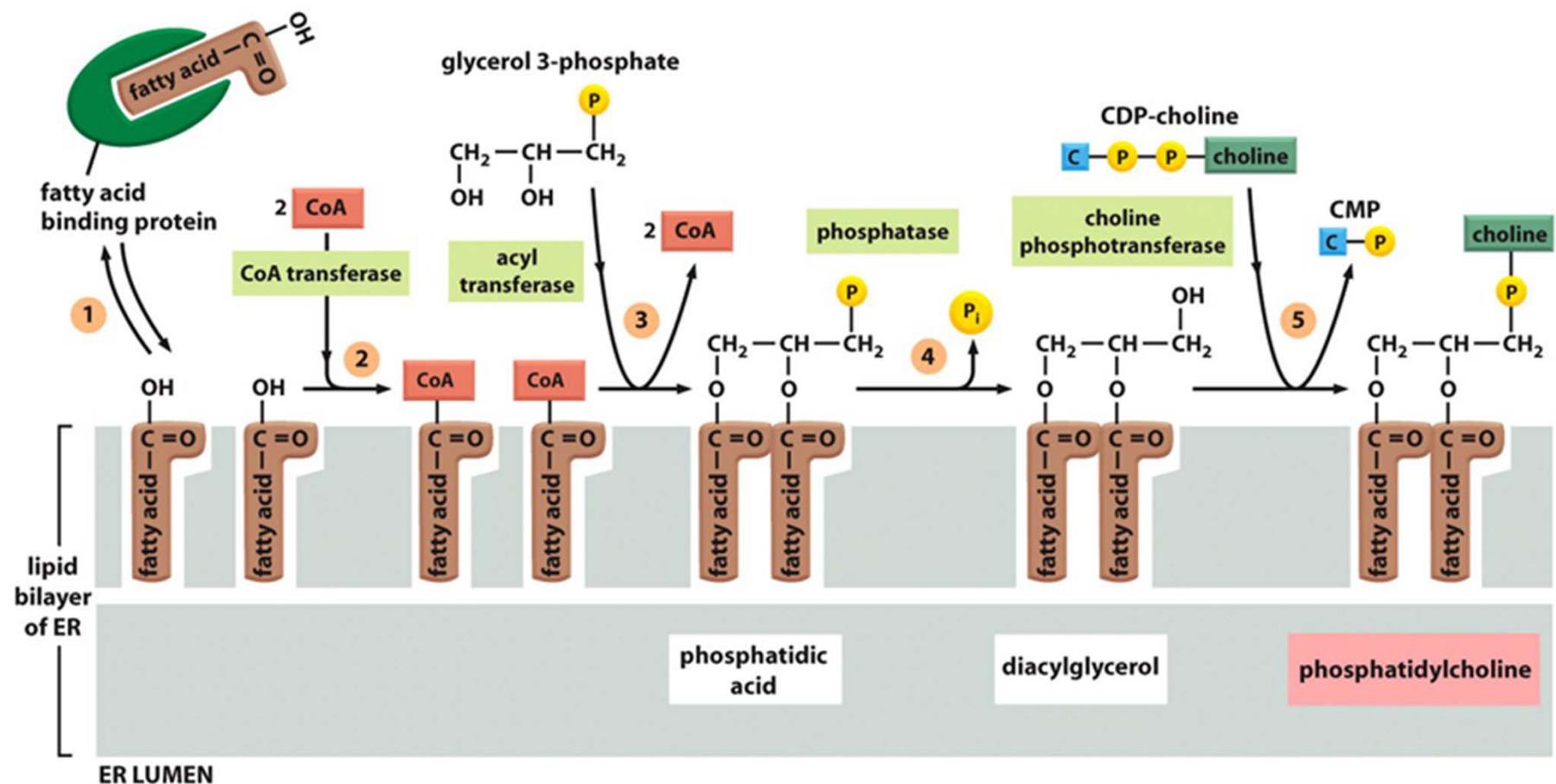
Phospholipid synthesis in ER: synthesis & distribution

Facts:

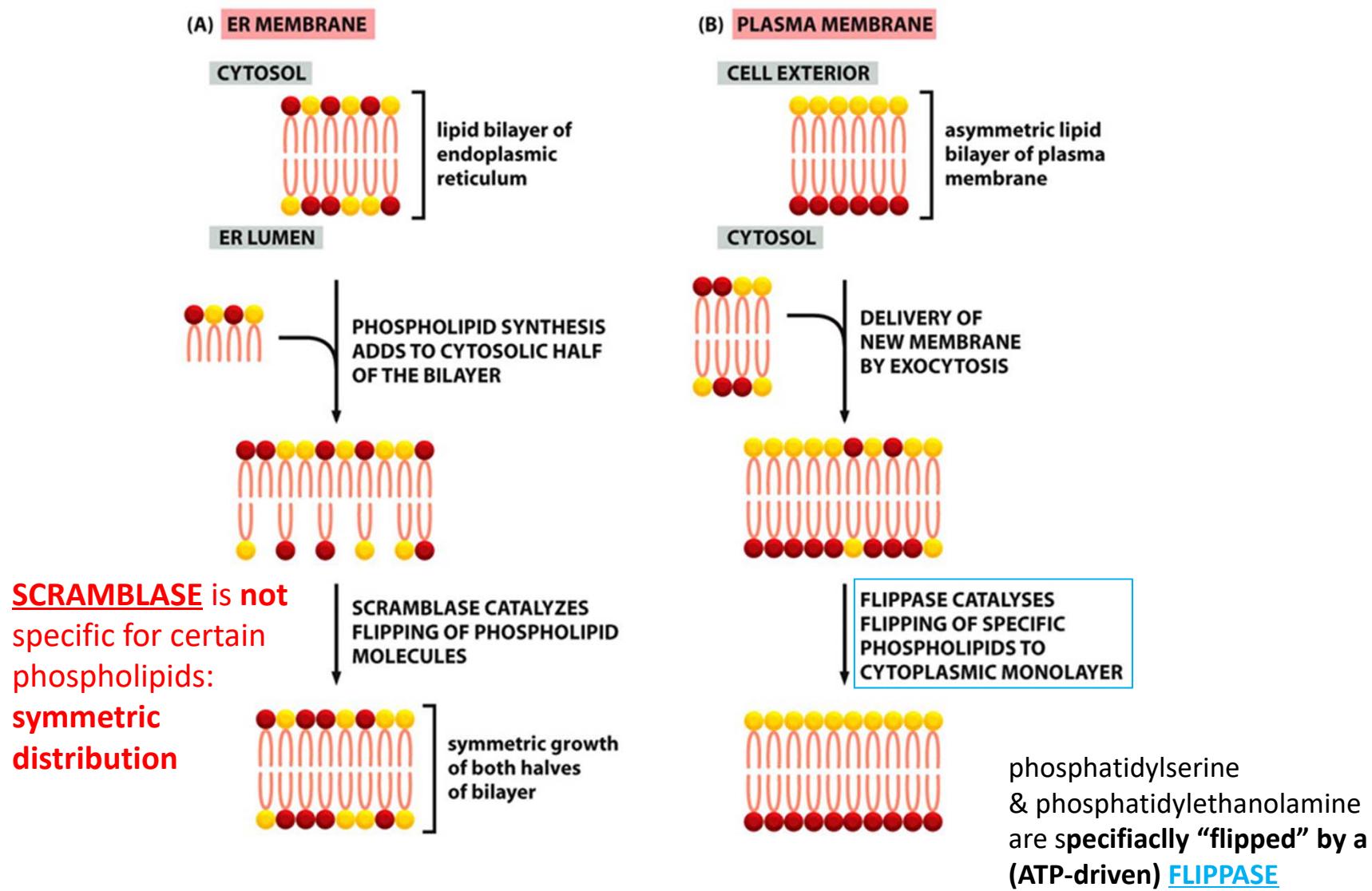
- Nearly all major lipids are **synthesized in the ER**, these include:
 - **Phospholipids**
 - **Cholesterol**
 - **Ceramide** (precursor for Sphingomyelin)
- **Synthesis is on the cytosolic side of ER**, where key enzymes are located.
- **Equal distribution** for these lipids between **both leaflets of ER** after synthesis due to **scramblase**, which **catalyzes rapid flip-flop**.

(1). Synthesis of **phosphatidylcholine** as the major lipid from two fatty acids, glycerol phosphate and choline

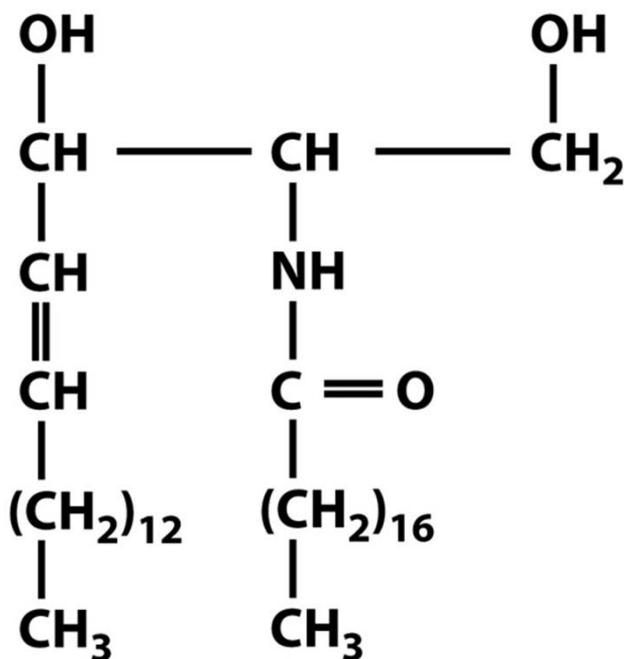
Similar mechanism for other major phospholipids



(2). Symmetry and asymmetry in ER and plasma membrane



(3). Synthesis of sphingolipids in the ER: ceramide



1st step: condensing between **serine** and a fatty acid to form **sphingosine**.

2nd step: a second fatty acid is added to form ceramide.

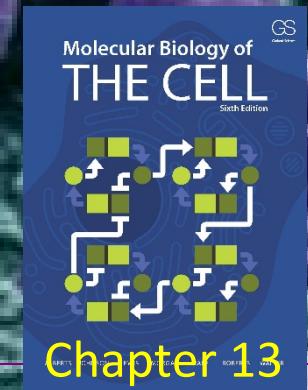
Ceramide is transported to the Golgi for **Sphingomyelin synthesis**
(transfer of a phosphocholine head group)
or for the addition of oligosaccharide chains to form **glycosphingolipids**

Lecture 7

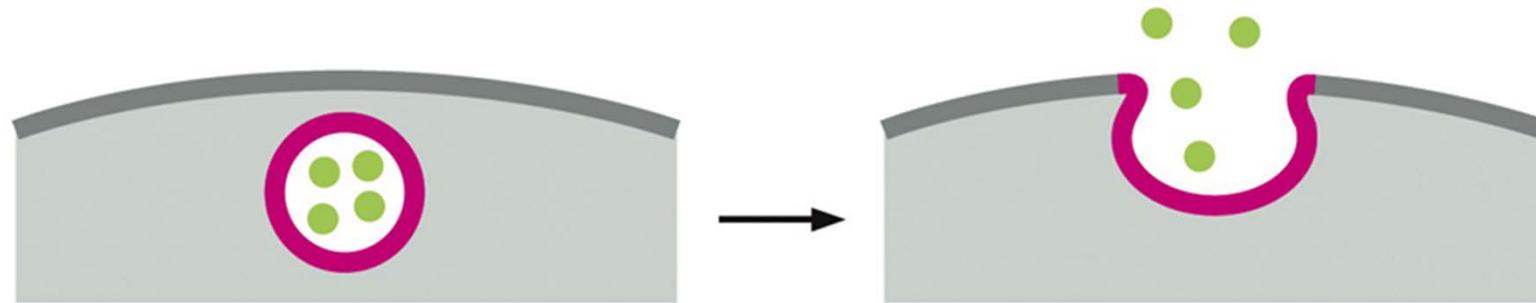
Transport of macromolecules Part II - Vesicular transport -

Outline

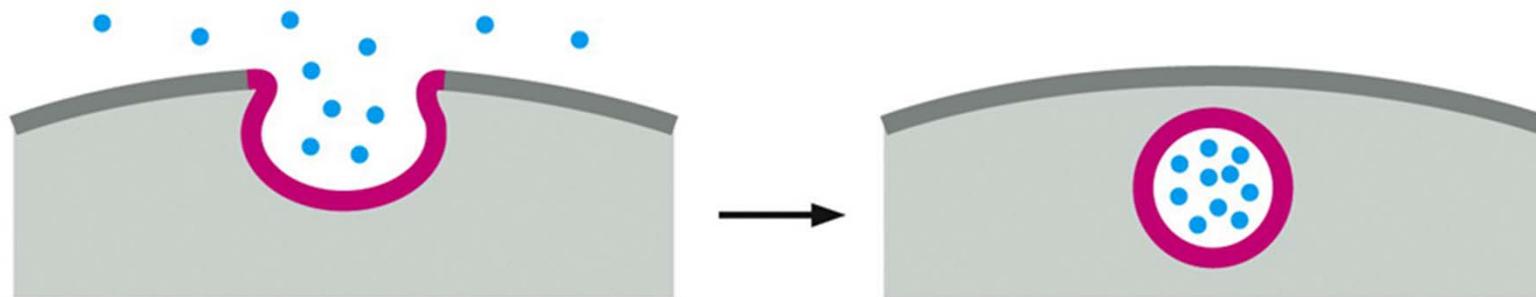
- I. Overview of vesicular traffic
- II. Techniques to study vesicular traffic
- III. Transport from ER to Golgi apparatus
- IV. Transport from trans-Golgi to lysosome
- V. Endocytosis
- VI. Exocytosis



Transport directions: exocytosis (secretion) vs. endocytosis



(A) **exocytosis** soluble proteins are **exported out** of the cell,
membrane proteins **remain** in the **PM**



(B) **endocytosis** (soluble proteins **and** membrane proteins
are **imported into** the cell)

The orientation of the membrane does not change during vesicular transport processes

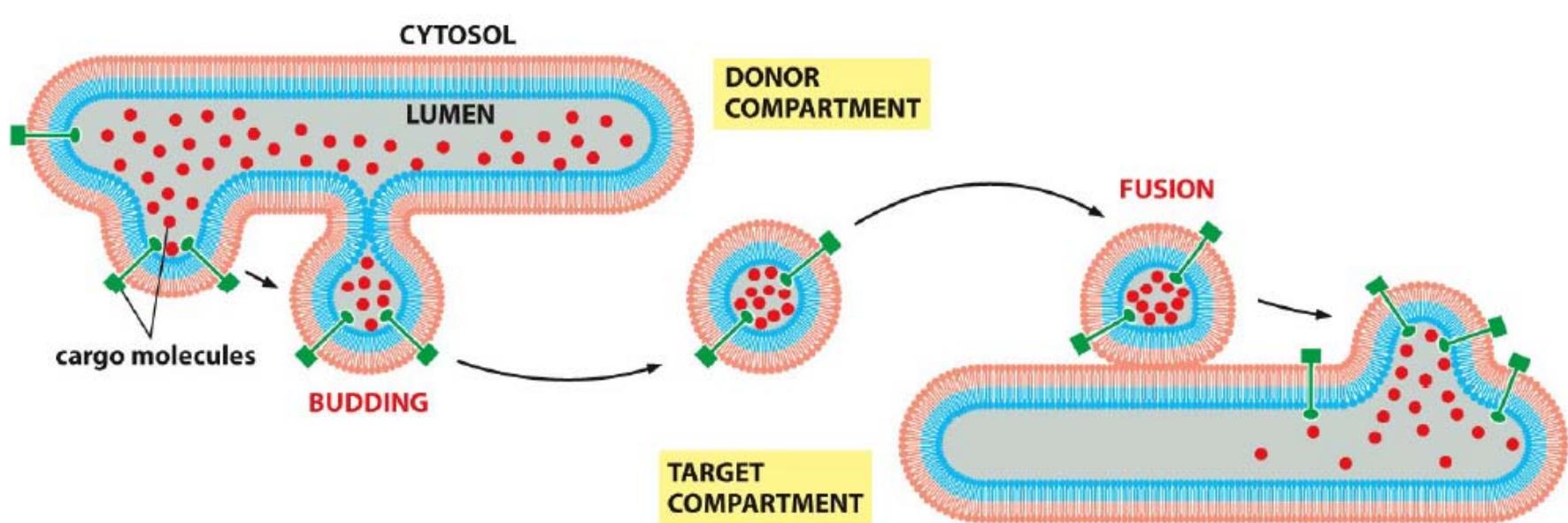


Figure 13-2 Molecular Biology of the Cell 6e (© Garland Science 2015)

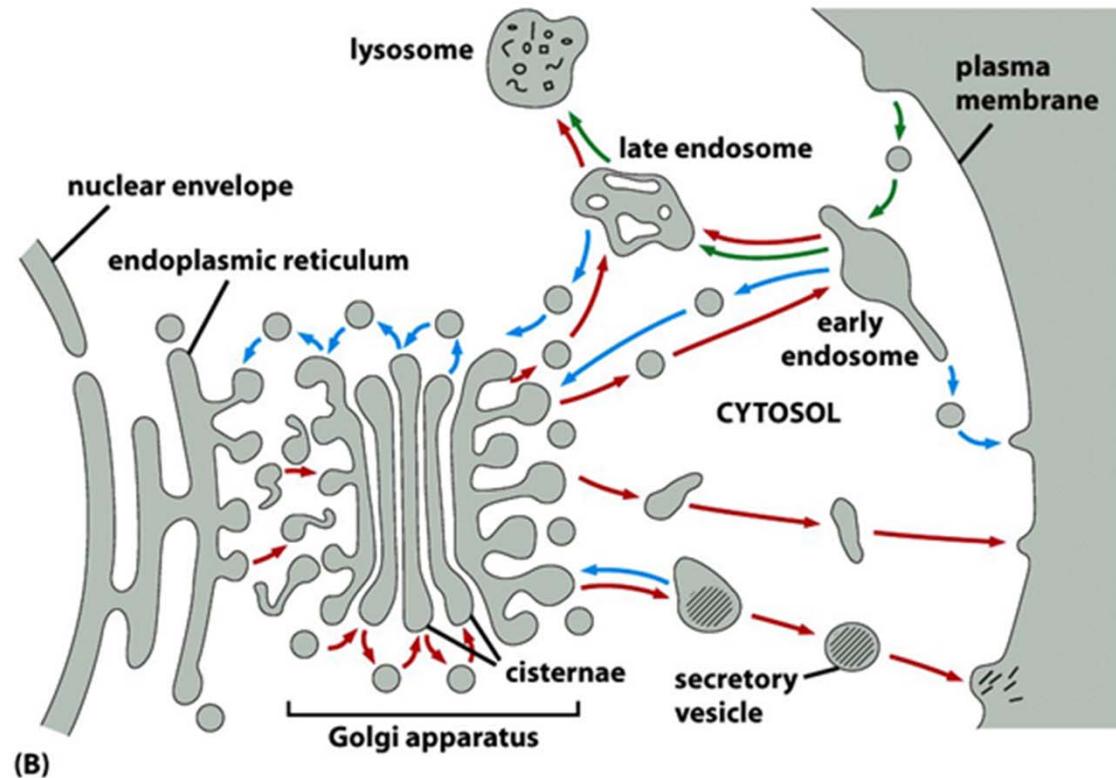
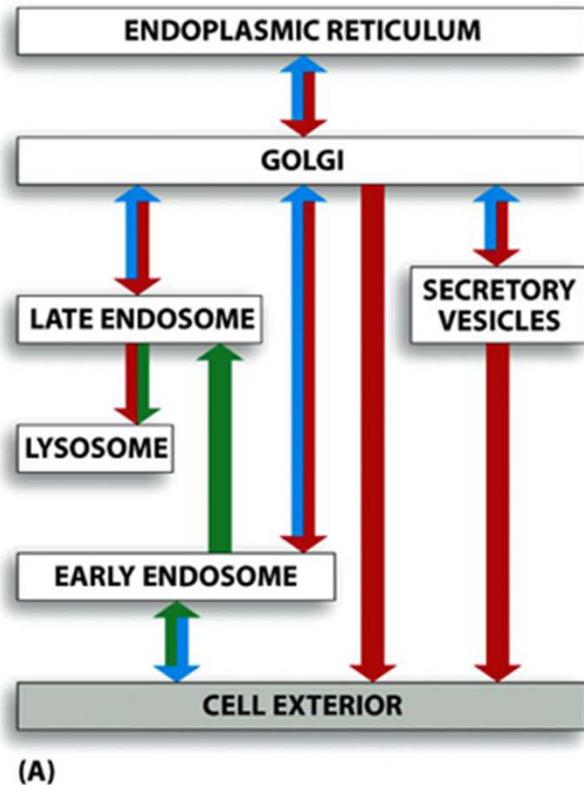
What molecules undergo **exocytosis** in vesicular traffic ?

- **Plasma membrane proteins:**
 - cell surface receptor
 - transporters
 - ion channels
- **Soluble proteins:**
 - digestive enzymes
 - peptide hormones
 - serum proteins
 - collagen
 - ECM (extra cellular matrix) proteins , etc.
- **From endosome to lysosome (forward/biosynthetic):**
 - hydrolases: proteases, glycosidases, phosphatases, lipases
 - some amino acid for storage
 - unneeded proteins (turn-over/degradation)

What molecules undergo **endocytosis** in vesicular traffic?

- Large nutrient macromolecules that are too large to be transported (by transporters/channels):
Such as:
 - cholesterol-LDL
 - iron-transferrin
 - vitamins, etc.
- Receptors, translocators, ATPases of the PM are endocytosed and degraded to down-regulate their activities.
- Some bacteria that are internalized into cells
- Some viruses

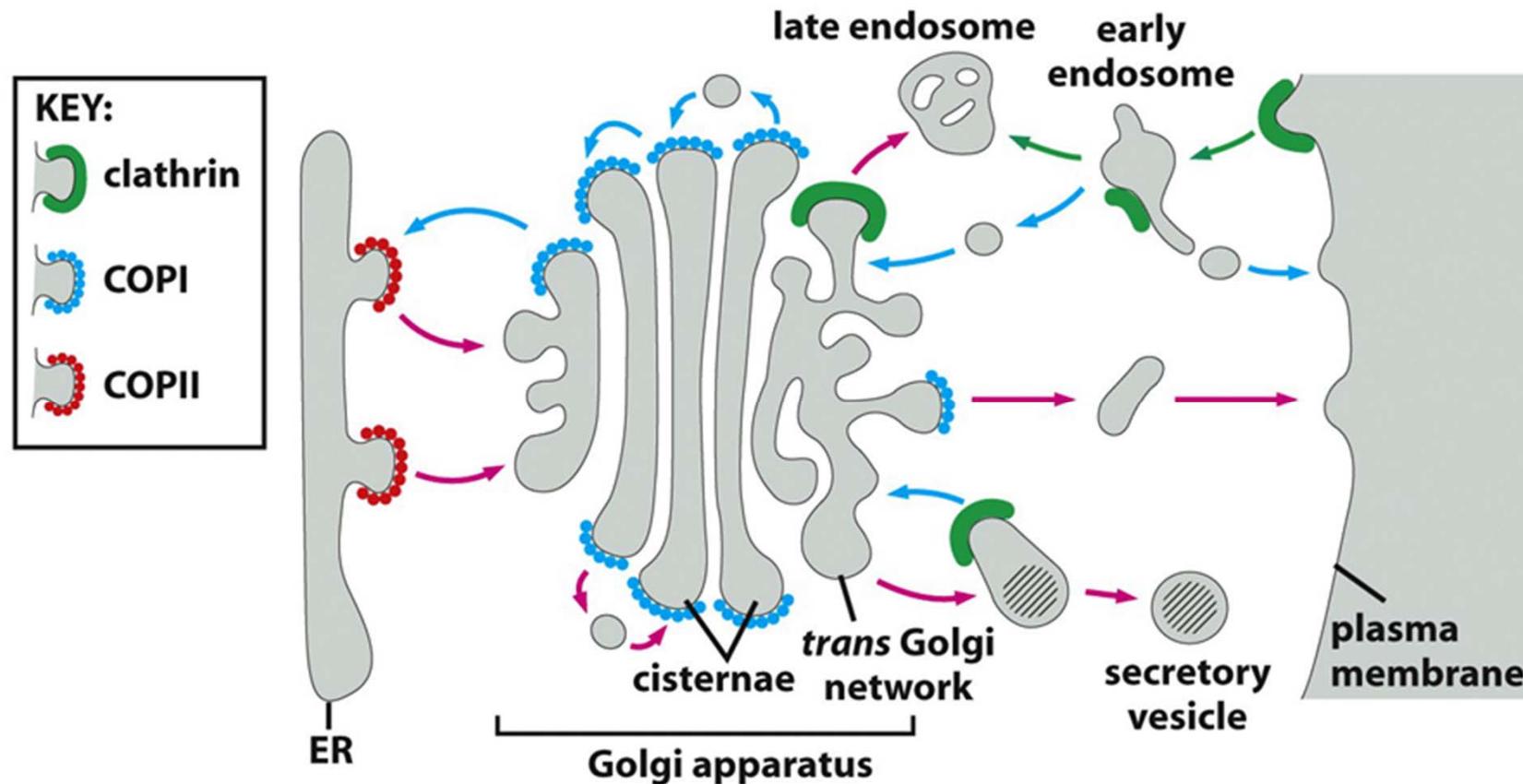
Vesicular traffic routes towards, within and from the Golgi apparatus



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

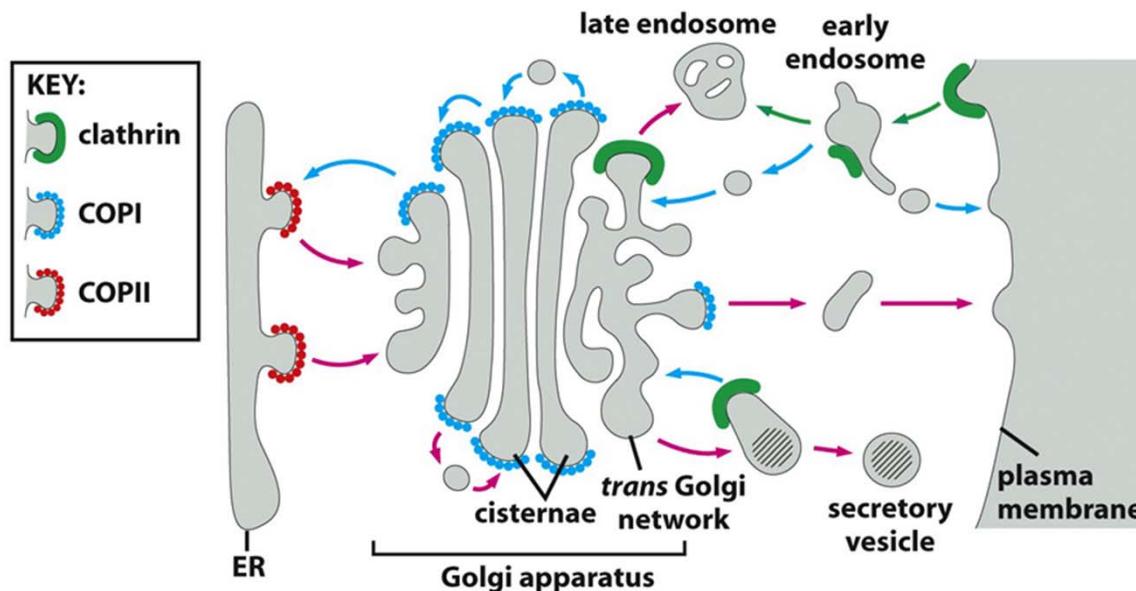
Transport vesicle can be spherical and tubular

Overview of vesicular traffic in the secretory pathway



- Transport vesicles are characterized by a specific set of “coat” proteins, which drive formation of the vesicles

Transport vesicles are characterized by a specific set of “coat” proteins, which drive formation of the vesicles



- **Clathrin** : transport proteins from the **plasma membrane (endocytosis)** and **anterograde** from ***trans*-Golgi network (TGN)** to **early** and **late endosomes**
- **COP (coat-protein) I**: Transport proteins in the **retrograde** direction **between Golgi cisternae** and **retrograde from the *cis*-Golgi back to the ER (retrieval)**.
- **COP (coat-protein) II**: Transport proteins from the **ER to the *cis*-Golgi**

Why do transport vesicles need coat proteins?

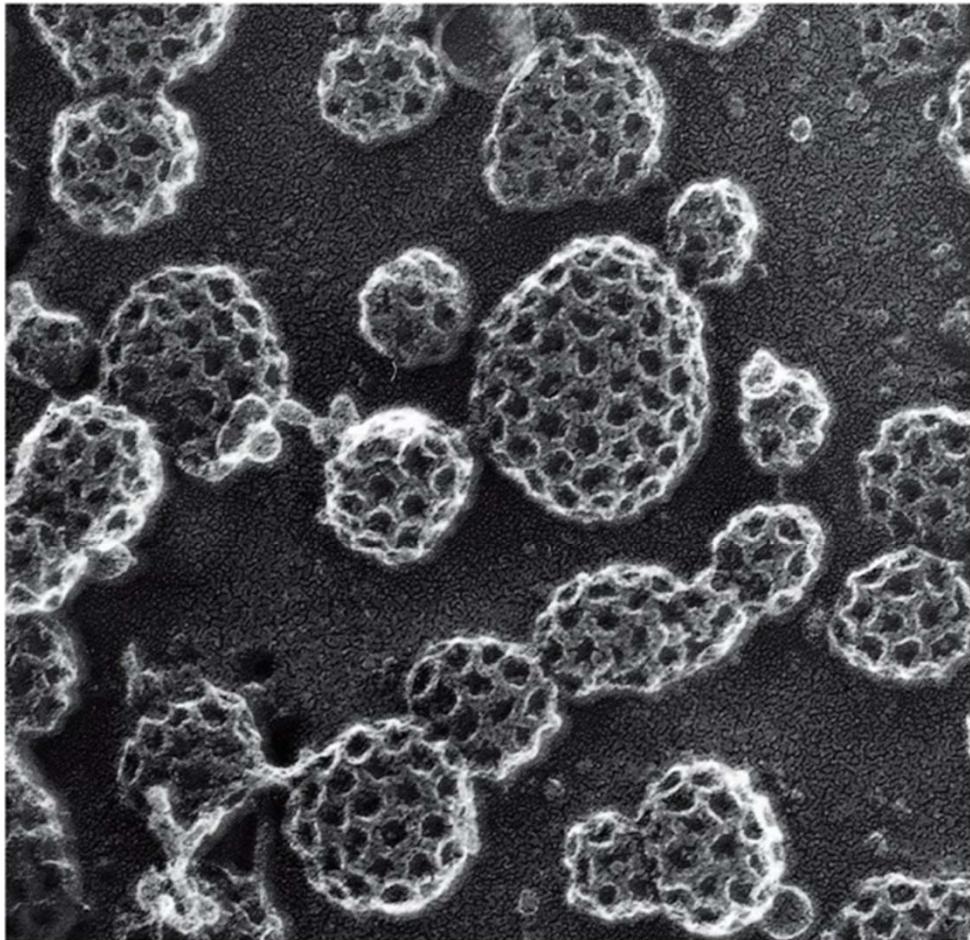
Main functions for the coating proteins:

- **Selection & concentration** of specific proteins for transport (selection of cargo/freight proteins)
- **Mold the vesicle**
 - assembly of the coat proteins **also** induces **membrane curvature** (formation of curved basket-like lattice that facilitates/triggers vesicle budding)

In conclusion:

**coat proteins link cargo selection to vesicle formation
= cargo sorting into the budding vesicle !!!**

Clathrin-coated pits and budding of clathrin-coated vesicles at the PM



rapid-freeze, deep etch
electron micrograph

0.2 μm

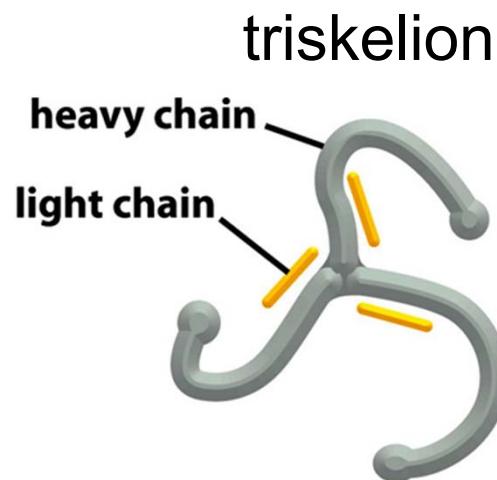
Clathrin was named after
clatratus (*latin*) means lattice-like
by Barbara Pearse 1975

“The protein that forms a lattice”

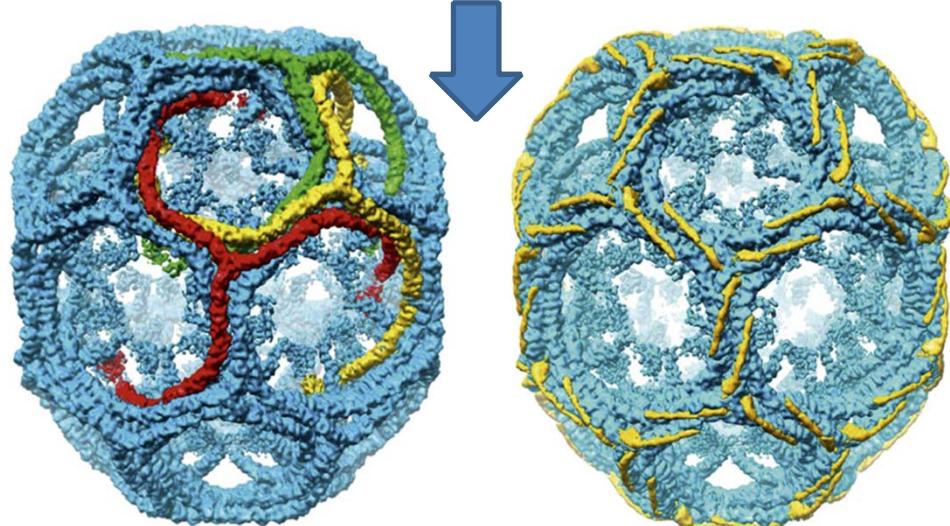
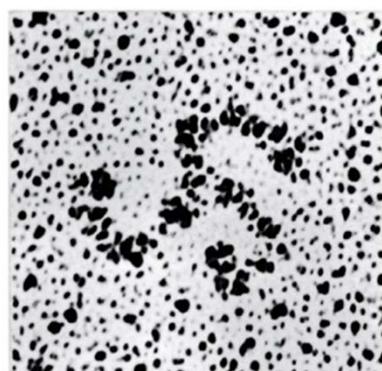
Electron micrograph showing
Clathrin coated pits/buds and
budding of CCVs from the inner
leaflet of the PM of fibroblasts

“Clathrin” consists of 3 large and 3 small polypeptide chains, which assemble into a “three-legged” triskelion

Clathrin, a part of the coat, is the scaffold that **curves** the membrane



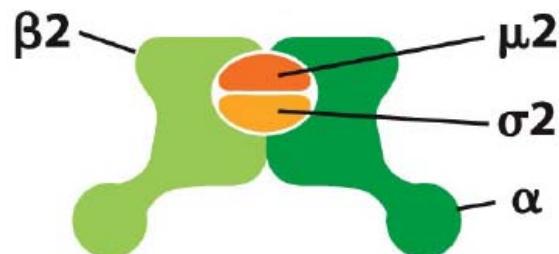
Triskelia assemble further into a framework of hexagons and pentagons (clathrin “baskets”)



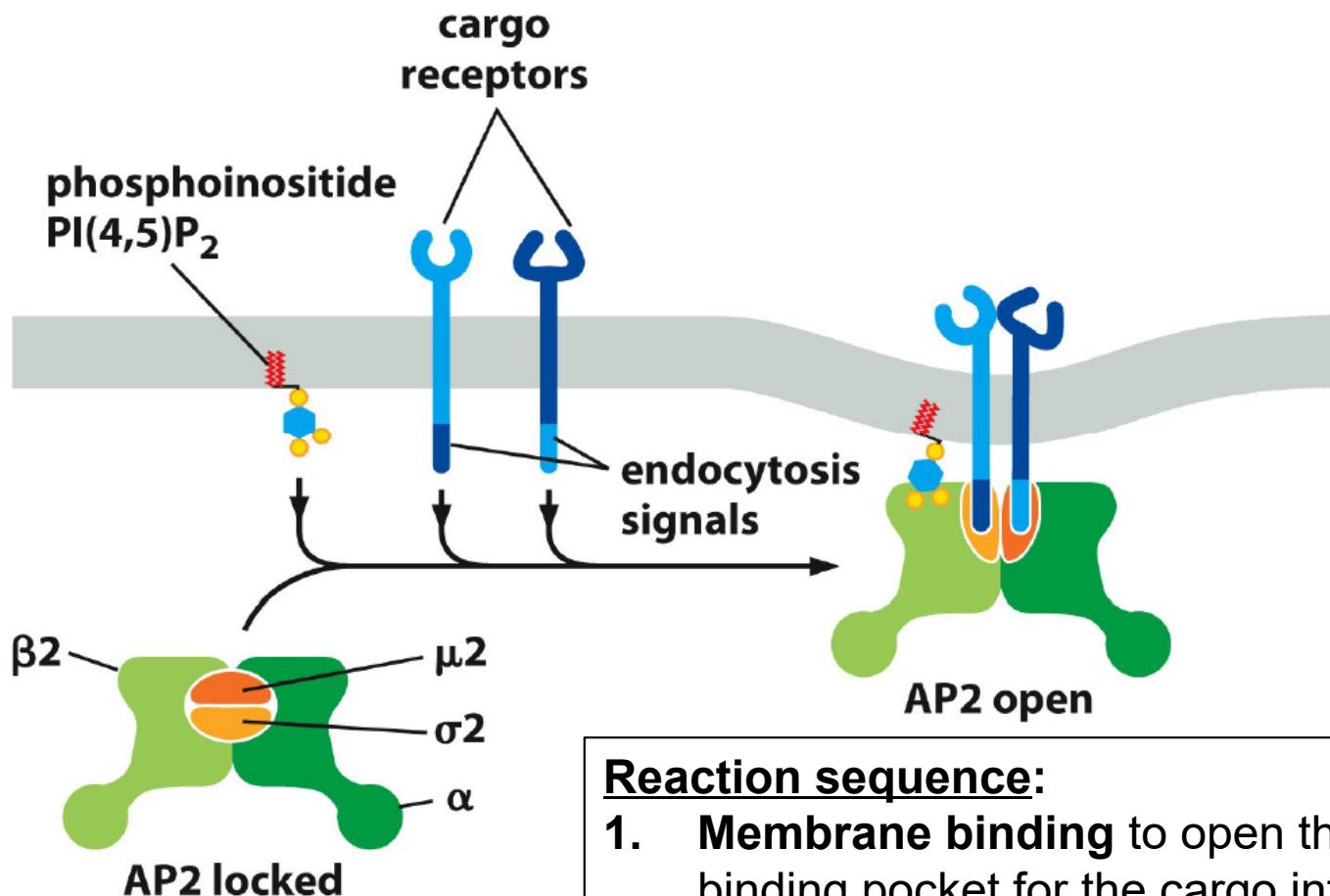
CCV cargo is recognized by tetrameric adaptor protein (AP) complexes

AP complexes:

- hetero-tetramer (4 subunits, termed **adaptins**)
 - 2 large subunits (α - / β -adaptin)
 - 1 medium subunit (μ -adaptin)
 - 1 small subunit (σ -adaptin)
- 5 different APs exist
- Bind to **membranes**, to **cargo** and to **clathrin**



AP complexes bind to phospholipids and to cargo proteins...
... before they can recruit clathrin to form the vesicle

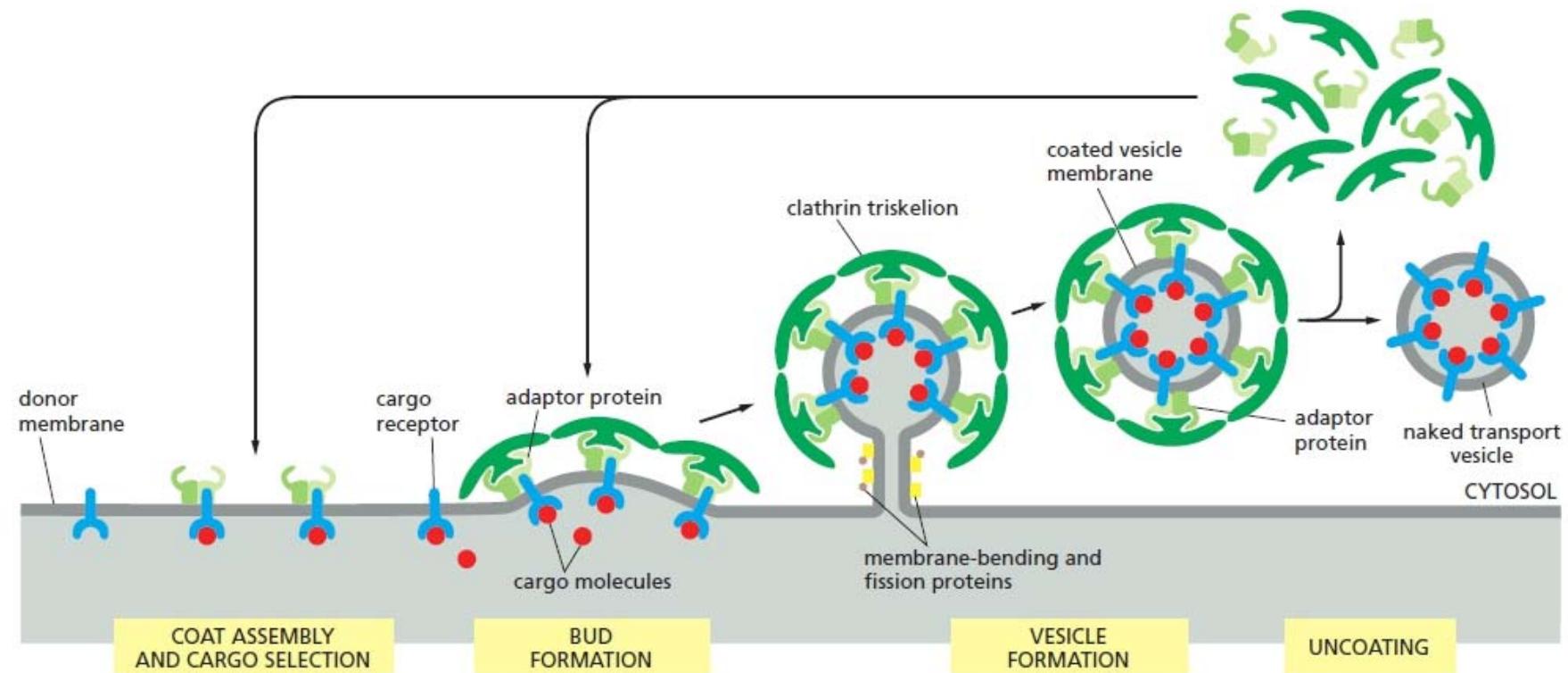


Reaction sequence:

1. **Membrane binding** to open the binding pocket for the cargo interaction
2. **Cargo binding**
3. **Clathrin recruitment**

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

How does a clathrin coat assemble and disassemble?



**Coat proteins sort cargo into the budding vesicle:
They link cargo selection to vesicle formation**

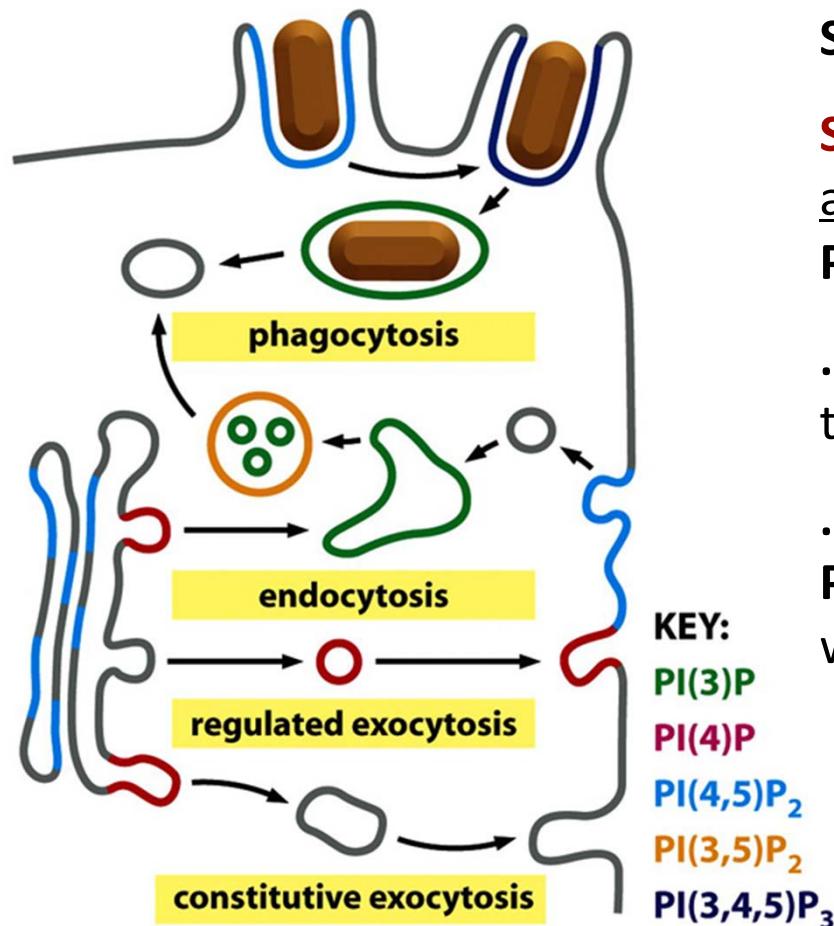
How does the coat protein control **when** and **where** to assemble?

Multiple factors confer location-specificity:

- **Presence of specific phosphoinositides (PIPs)** within a respective membrane of a cellular compartment can trigger coat recruitment
- Activation and recruitment of **coat-recruiting GTPases**:
 - **ARF** (ADP-ribosylation factor) **GTPases**:
 - responsible for **COP I coat assembly** and **clathrin coat assembly**
 - **Sar** (secretion-associated ras-superfamily related protein) **1 GTPase**:
 - **COP II coat assembly**

Differential locations of phosphoinositides (PIPs) regulate targeted vesicular transport

Specific phosphoinositides mark compartments and membrane domains



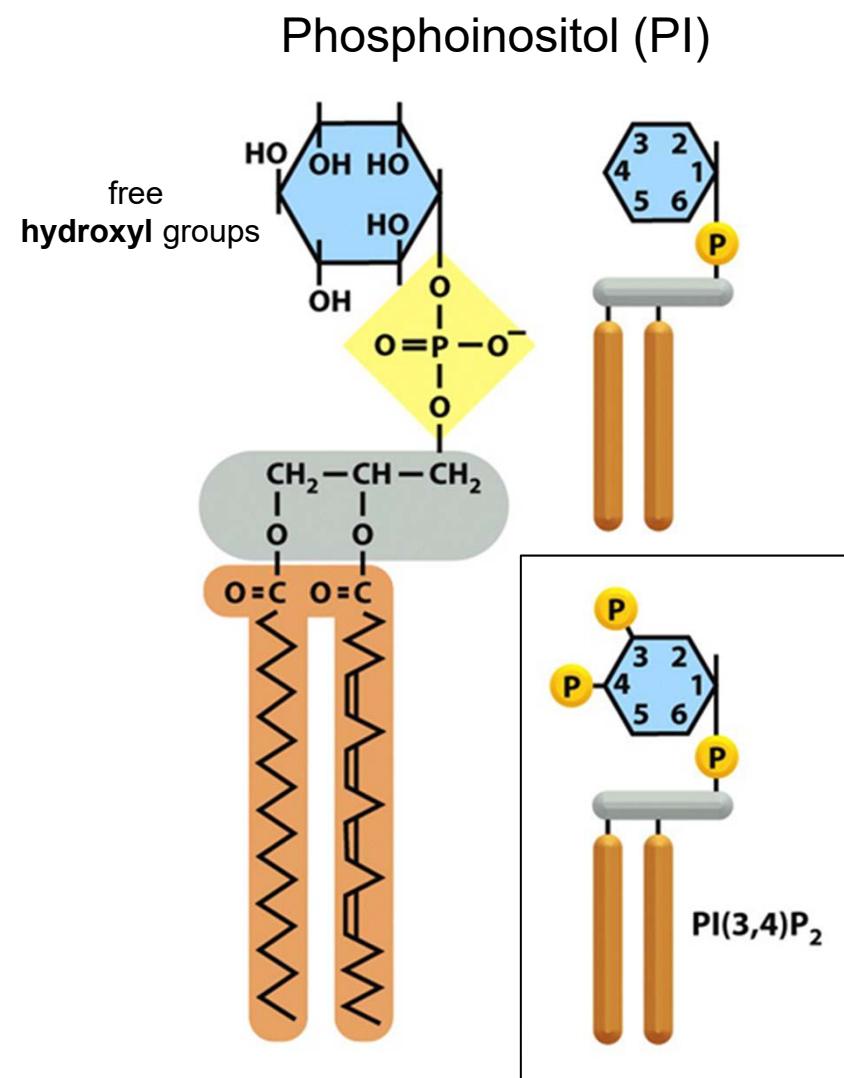
Specificity of vesicle formation by PIPs:

Secretory vesicles (SVs) contain **PI(4)P**, after fusion with PM, a PM-localizing **PI 5-kinase** converts **PI(4)P** to **PI(4,5)P₂** ...

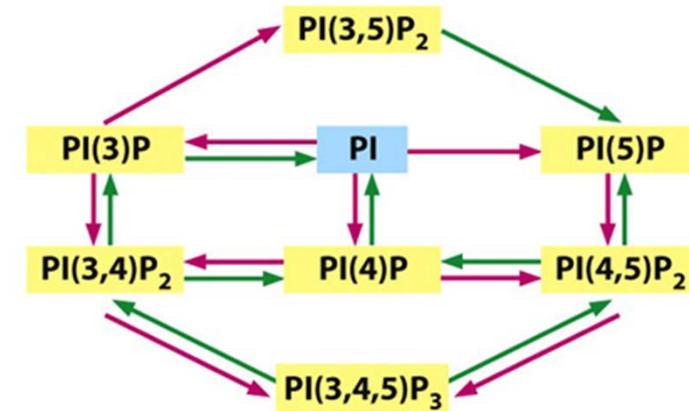
... **PI(4,5)P₂** recruits the AP complex for the formation of endocytic CCVs....

... after CCV budding, a cytosolic **PI(5)P phosphatase** hydrolyses **PI(4,5)P₂**, which promotes uncoating of the vesicle

Different head groups allow for specific interaction with proteins



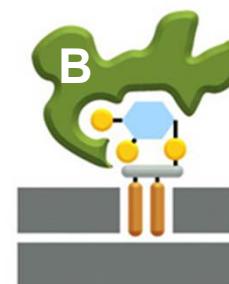
Conversion of PIPs by PIP kinases & phosphatases



Specific interaction between respective headgroups and proteins



Protein A
binds PI(3)P



Protein B
binds PI(4,5)P₂

Curving membranes: brute force & ignorance

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

Membrane-bending proteins help deform the membrane during vesicle formation: **BAR (Bin/Amphiphysin/Rvs) domains**

BAR domains:

- are **coiled-coils** that dimerize
- BAR domain-containing proteins are **curved**
- dimers have **positively charged** inner surface
- dimers **interact/associate with negatively charged lipid headgroups**
- **induce curvature of membranes**

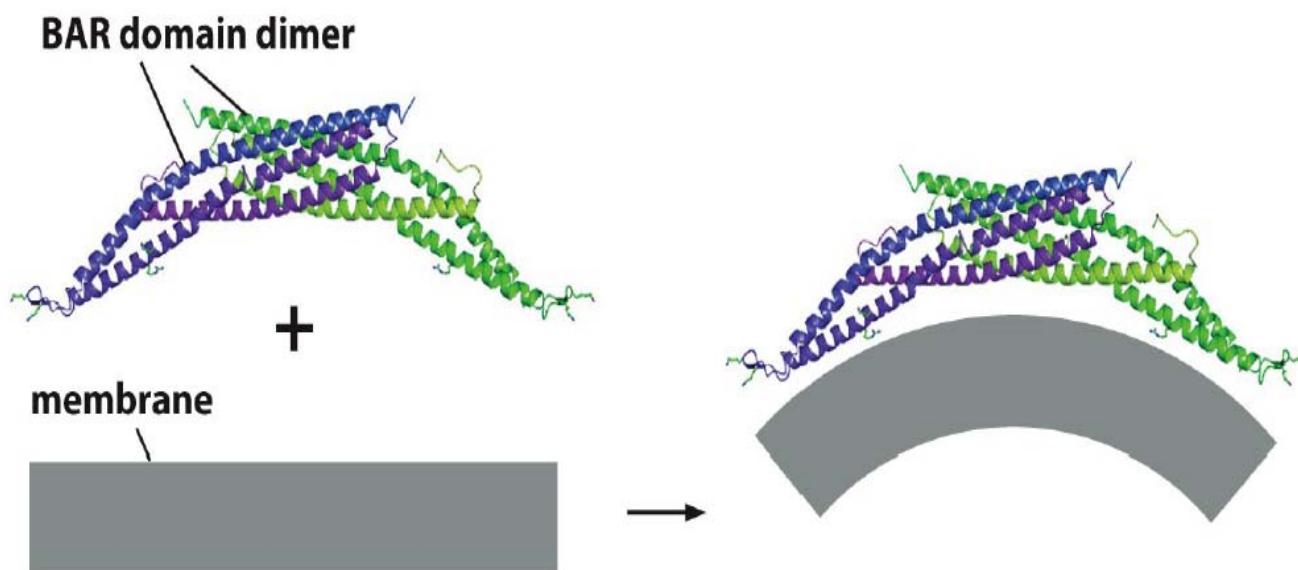
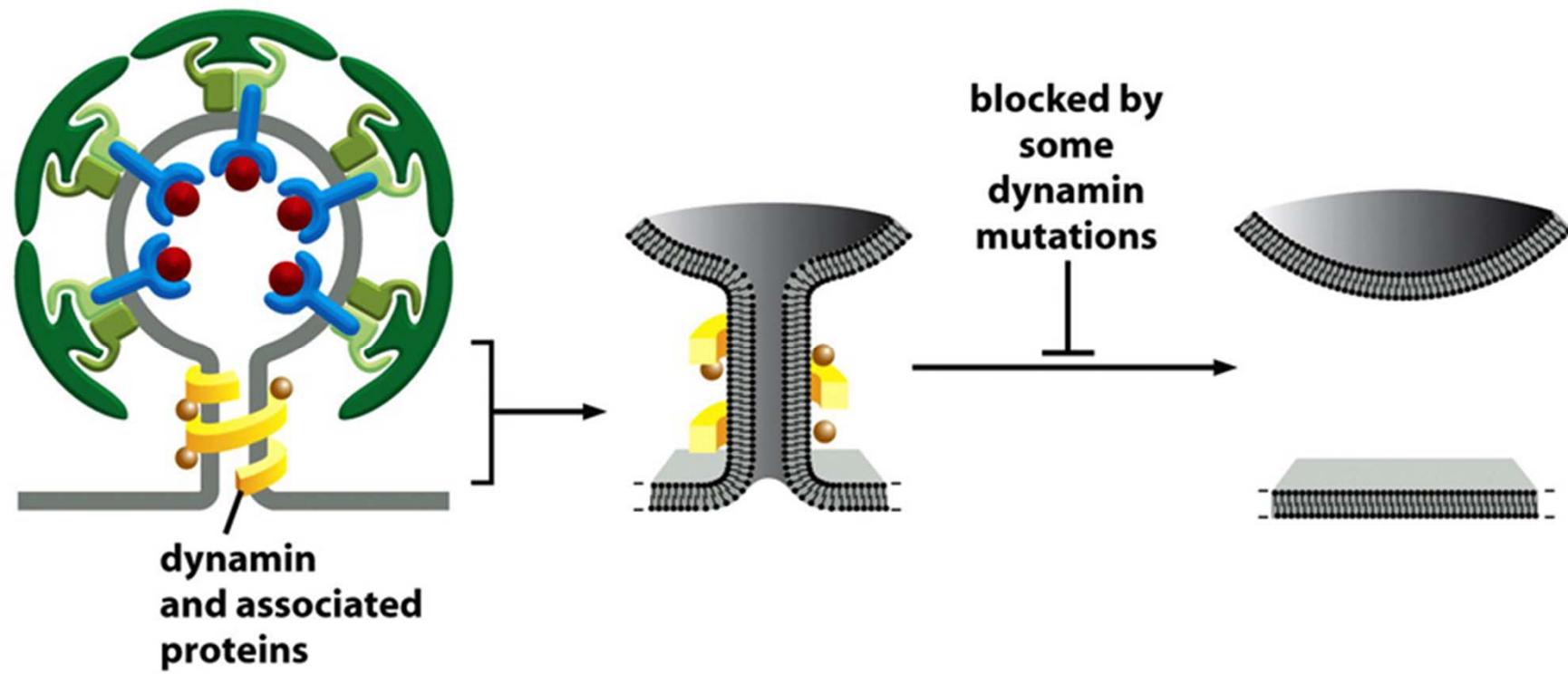


Figure 13-12 Molecular Biology of the Cell 6e (© Garland Science 2015)

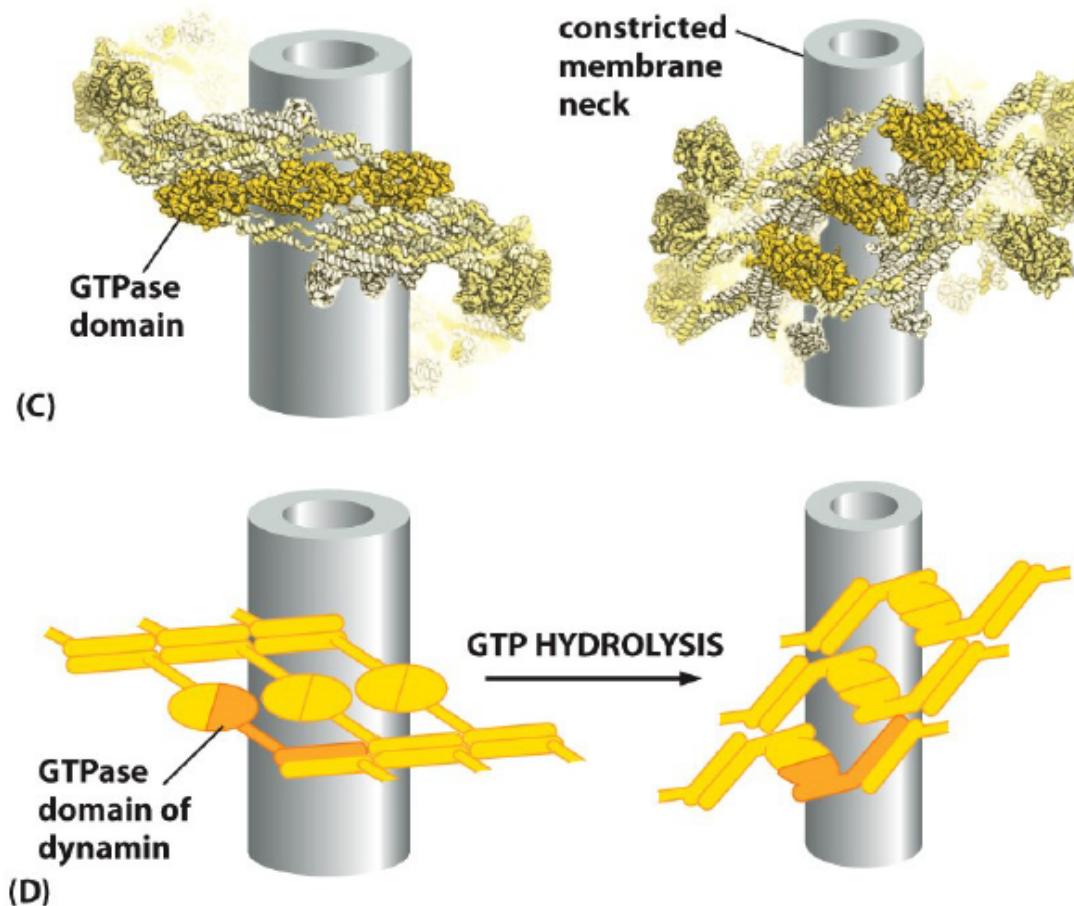
Fission of the vesicle: Dynamin assists!



Dynamin:

1. PI(4,5)-P₂-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

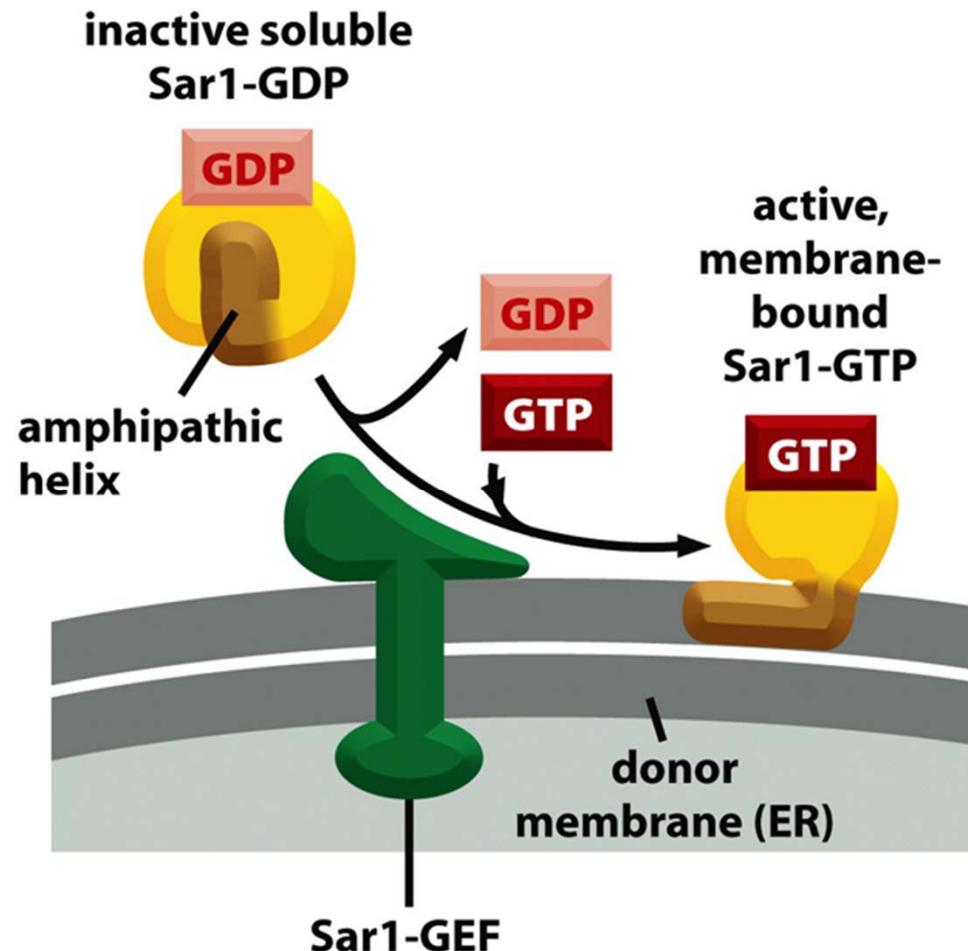


(B)



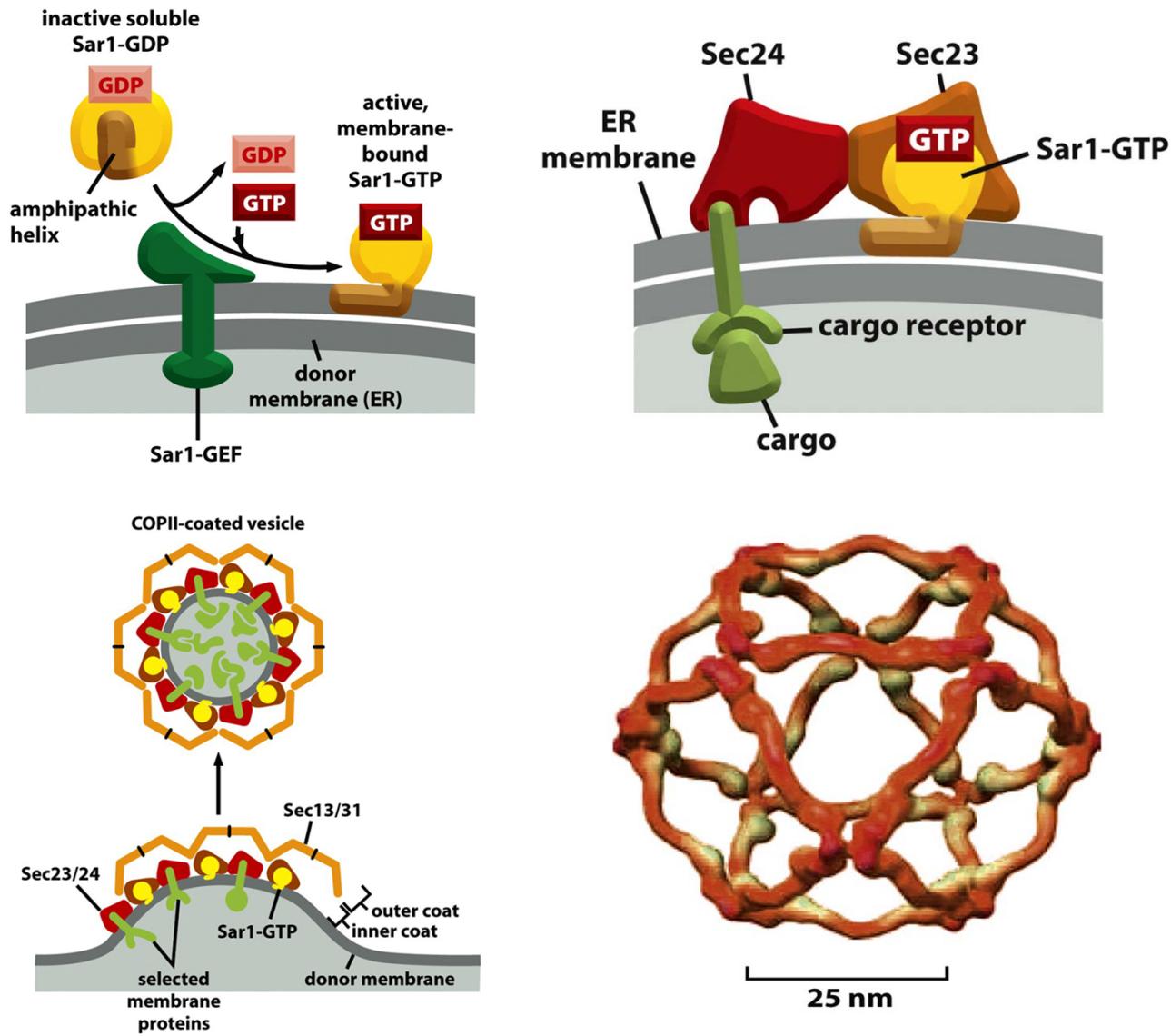
200 nm

How does a GTPase control COP II assembly?



Remember the principle of the GTPase switch?

How does a 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** (soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptors) **proteins** mediate the fusion of the lipid bilayer.

Rab protein family: monomeric GTPases and their subcellular localizations

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 a Rab GTPase

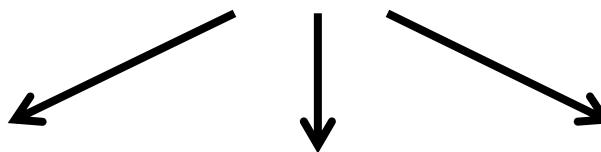
Rab-GDP is **inactive** and **cytosolic**, usually bound to
Rab-GDP-dissociation inhibitor (**GDI**)



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



Once in active form, **Rab-GTP exposes hydrophobic anchor**
to be membrane-bound **and** recruits Rab effectors



Vesicle transport membrane tethering membrane fusion

Rab proteins guide transport vesicle to their target membrane

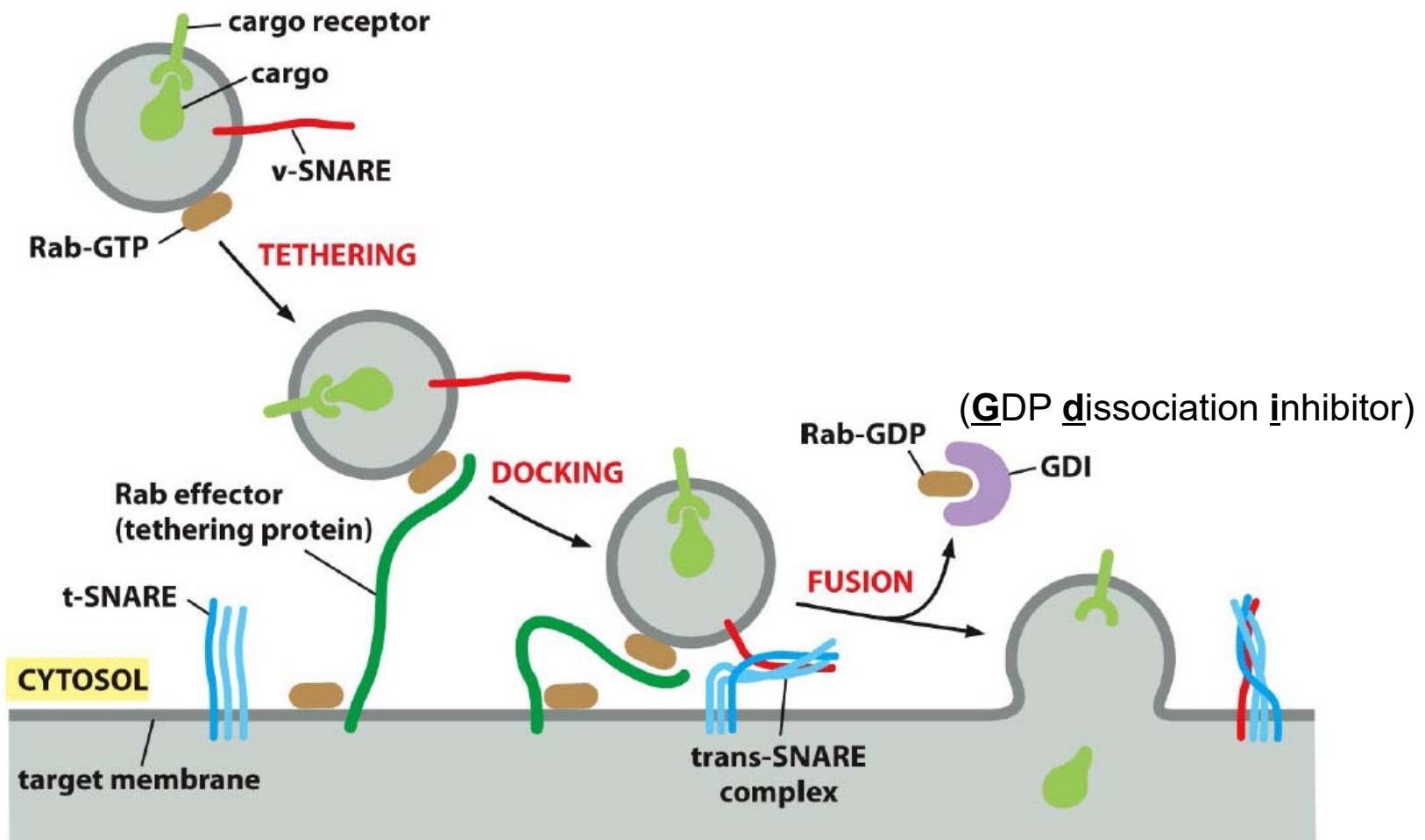


Figure 13-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

The formation of a Rab5 domain on the endosome membrane

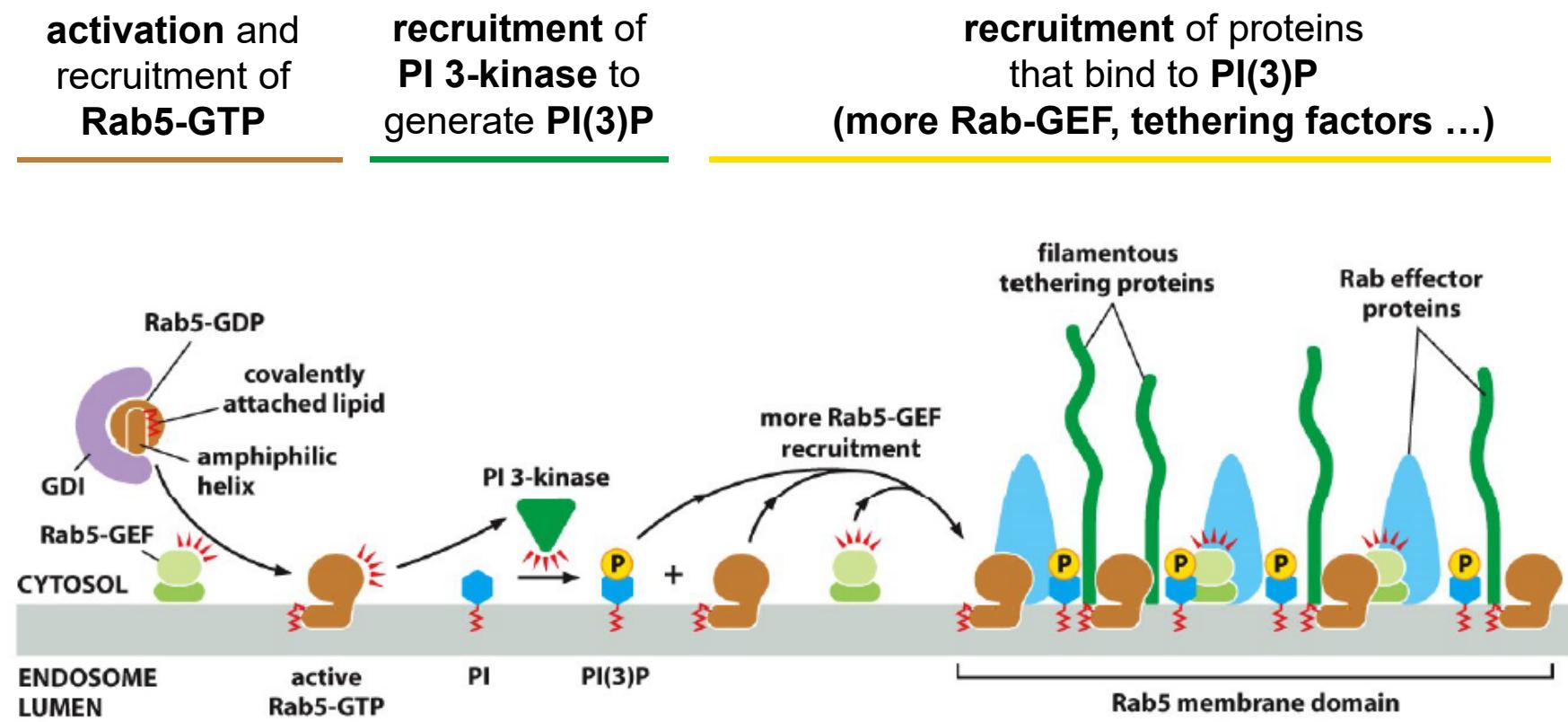


Figure 13-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

Size of the “patch” is controlled by the GTP/GDP cycle

Rab cascade can change the identity of an organelle
(Replacement of a RabA domain by a RabB domain)

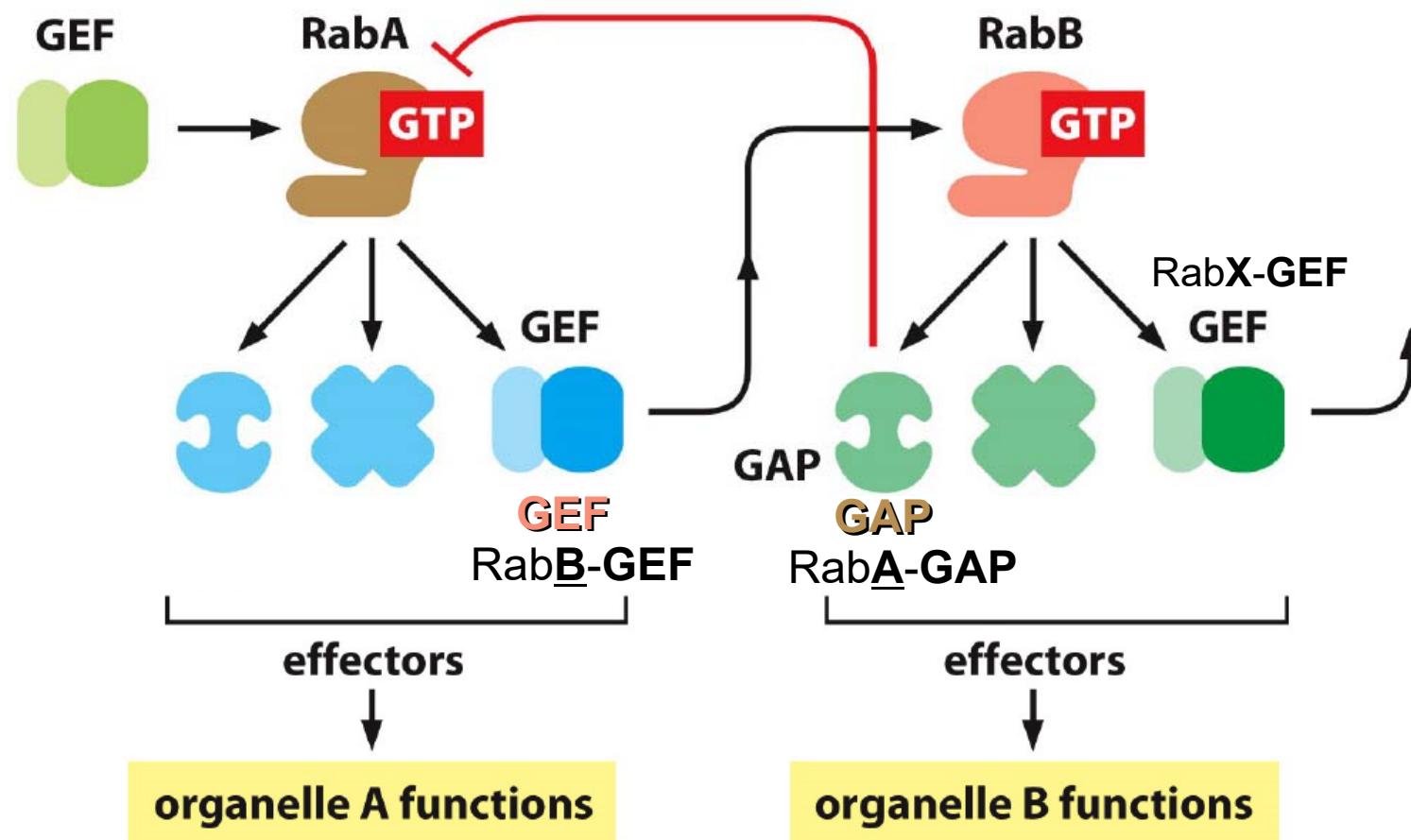
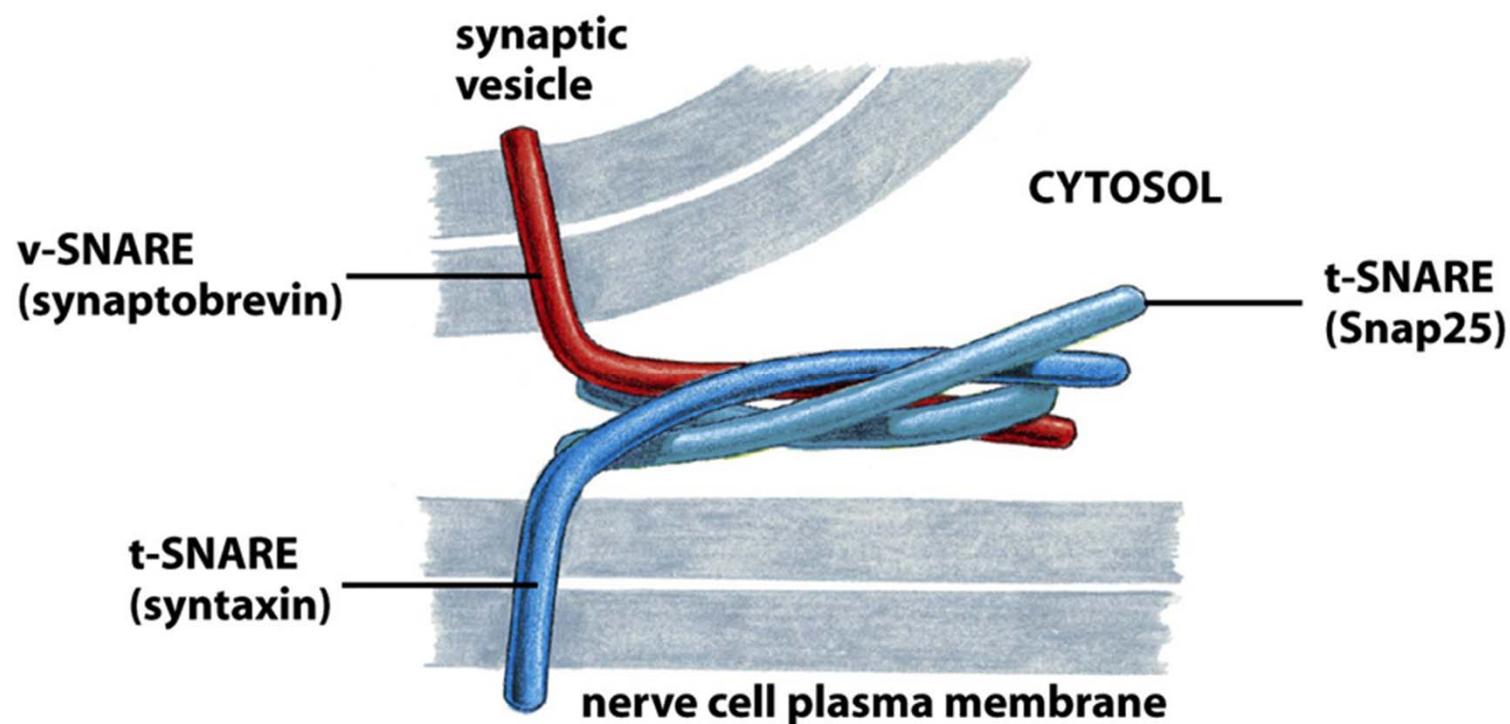


Figure 13-18 Molecular Biology of the Cell 6e (© Garland Science 2015)

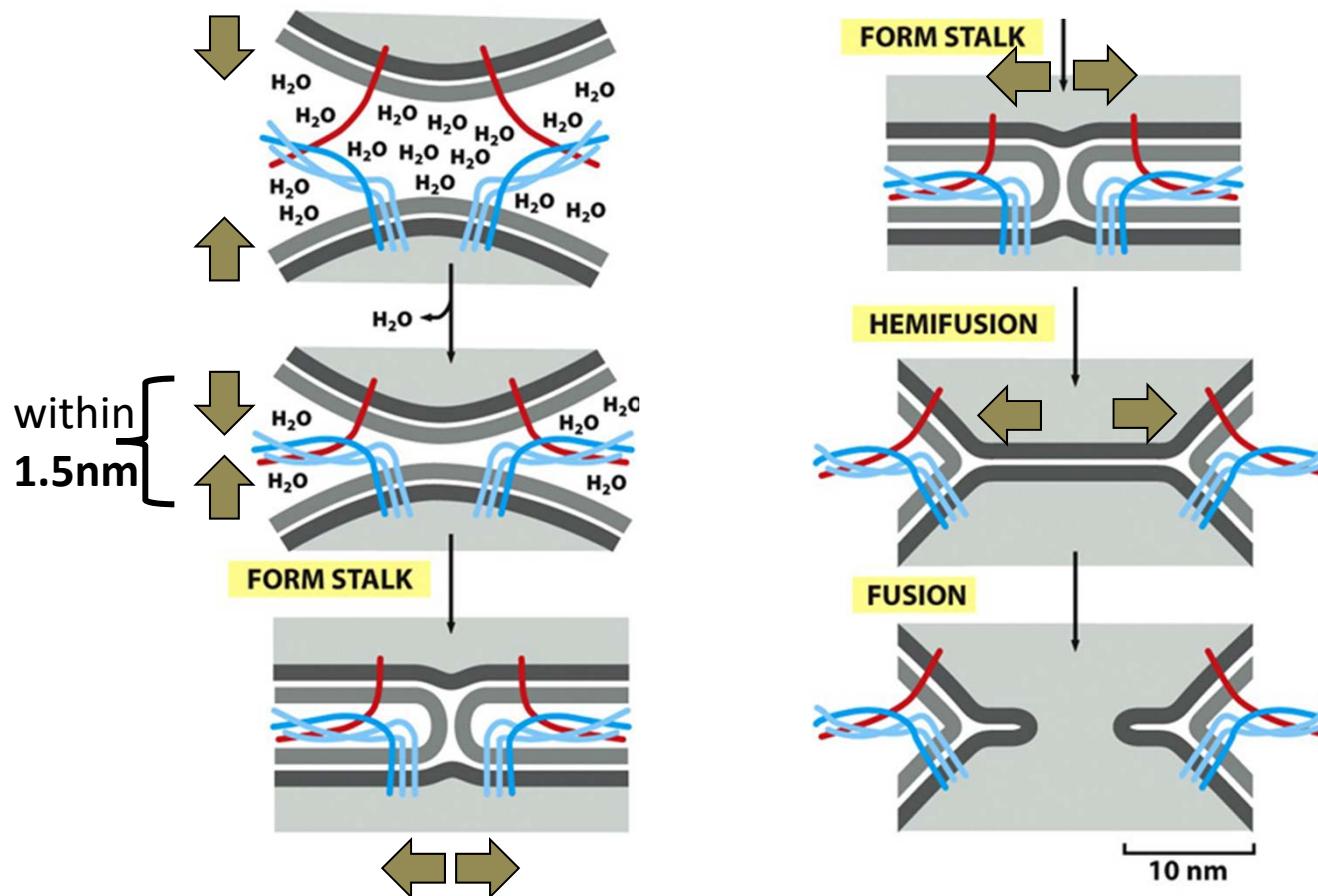
Example: Maturation of early endosomes to late endosomes is Rab5 to Rab7 conversion

SNARES mediate membrane fusion



v-SNARE: single chain on vesicles
t-SNARE: 2-3 chains on target

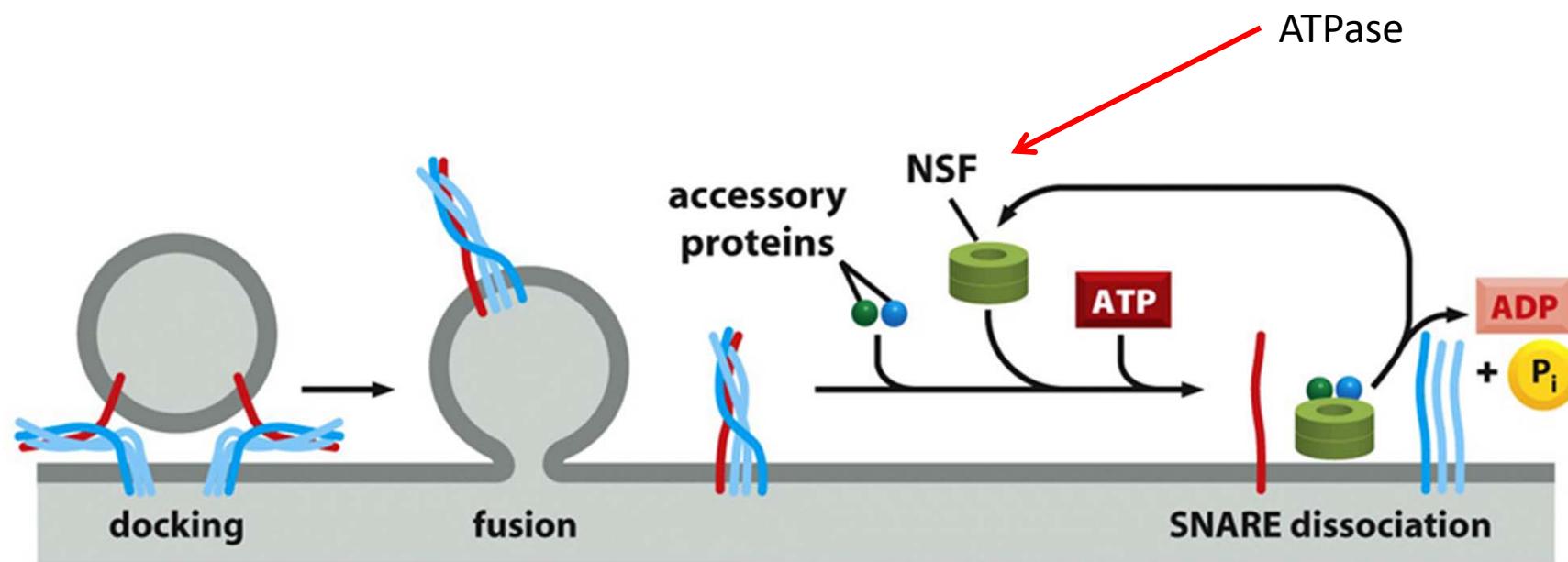
How SNARE proteins may catalyze membrane fusion



SNARE-mediated bilayer fusion occurs in multiple steps

1. Pairing between v- and t-SNAREs forces lipid bilayers into close apposition and expels water molecules
2. Lipid molecules in the two interacting (cytosolic) leaflets flow between the membranes to form a connecting stalk.
3. Lipids of the two noncytosolic leaflets then contact each other, forming a new bilayer, which widens the fusion zone (*hemifusion*, or half-fusion).
4. Rupture of the new bilayer completes the fusion reaction.

Dissociation of SNARE pairs after membrane fusion requires ATP hydrolysis by the AAA-ATPase NSF



NSF (N-ethylmaleimide-sensitive factor)

SNARE (soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptors) protein

Entry of the enveloped HIV virus into cells: the virus membrane fuses with the PM of the cell

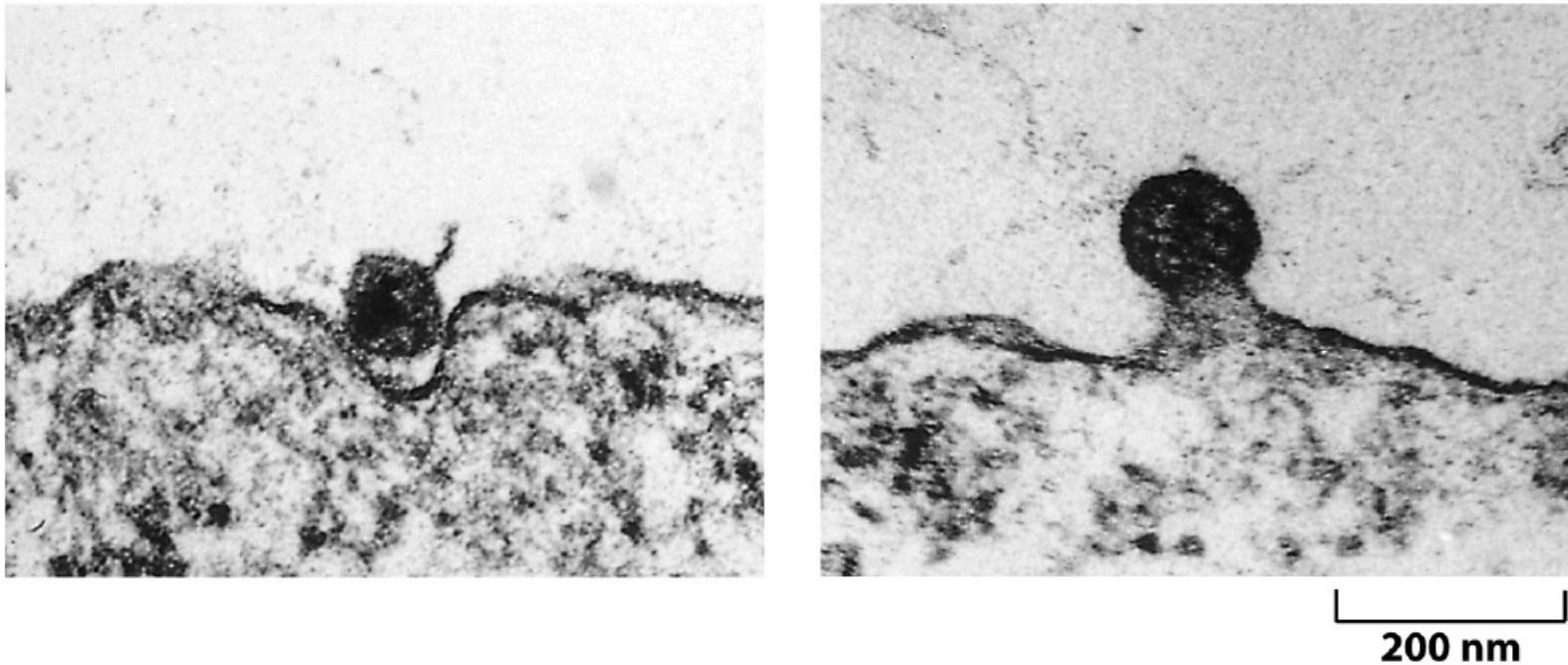


Figure 13-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

The entry of enveloped viruses into cells.
Electron micrographs showing how **HIV enters a cell by fusing its membrane with the plasma membrane of the cell.**

Lecture 7 Macromolecules transport II

Vesicular Traffic

Outline

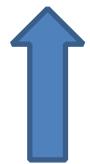
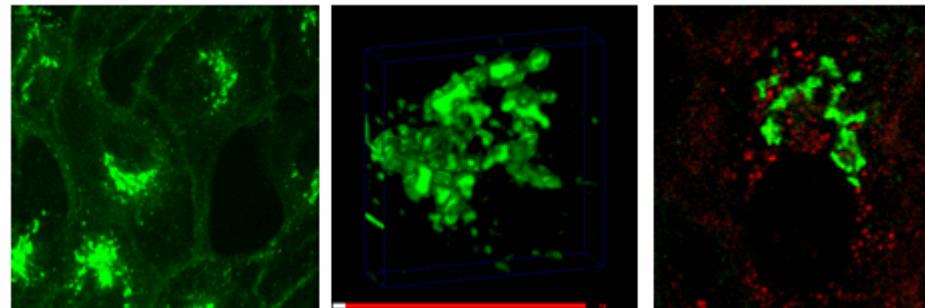
- I. Overview of vesicular traffic
- II. Techniques to study vesicular traffic
- III. Transport from ER to Golgi apparatus
- IV. Transport from trans-Golgi to lysosome
- V. Endocytosis
- VI. Exocytosis

II. Techniques to study vesicular transport

- **Radiolabelled amino acids (pulse-chase)**
 - protein-protein interaction (function & properties)
 - visualization by autoradiography
- **GFP-fusion proteins**
 - visualization of fluorescent reporter proteins by life cell imaging techniques (**FRAP/FLIP/FLIM/FRET**)
- **Cell-free systems**
 - identify minimal requirements
 - isolate individual sequences of a complex mechanism
- **Genetic study with yeast temperature-sensitive mutants**
 - identification of interacting genes

Analysis by tracing of green fluorescent (GF) protein fusions

Secretory proteins were fused with GFP and then visualized by fluorescence microscopy , coupled With FRAP, FLIP .

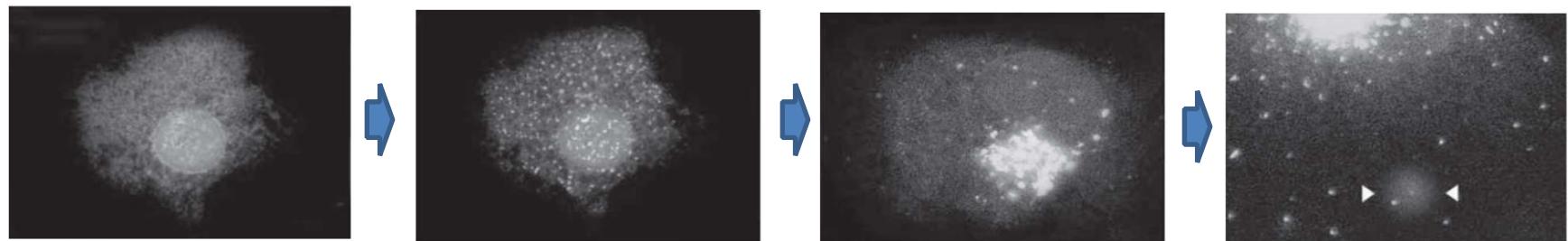


Golgi in cells



Vesicle budding from Golgi

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



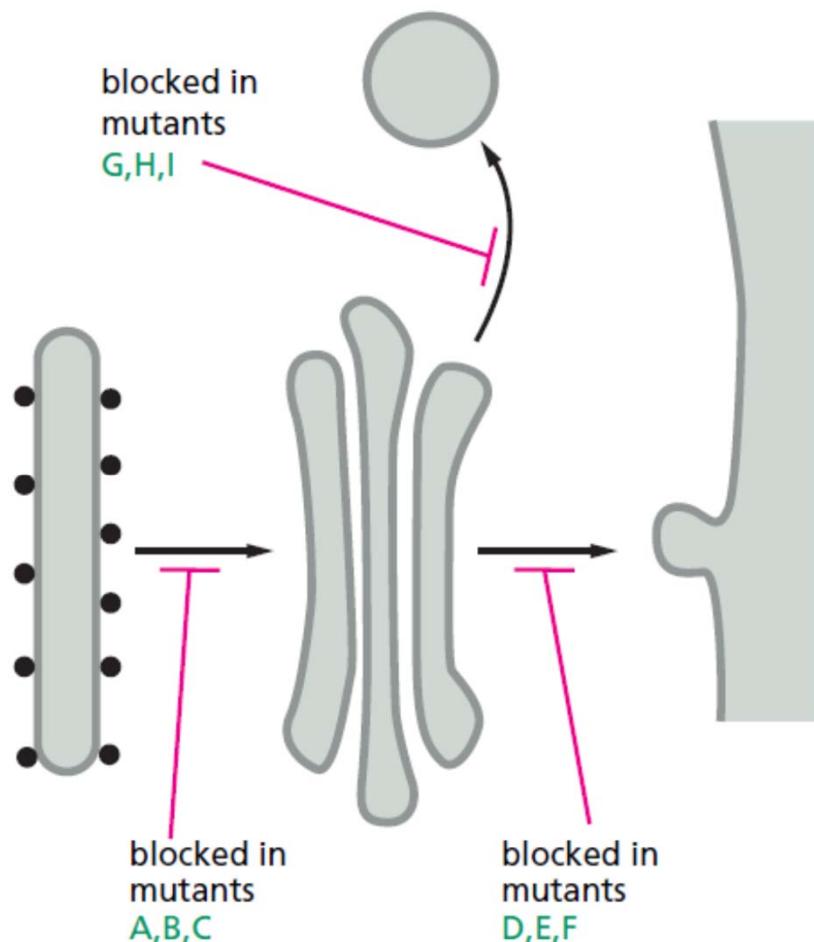
High temperature:
Retention in the ER

Low temperature:
Exit from the ER

Low temperature:
Move to the Golgi

Low temperature:
Fusion with the PM

Yeast temperature mutant: genetic study



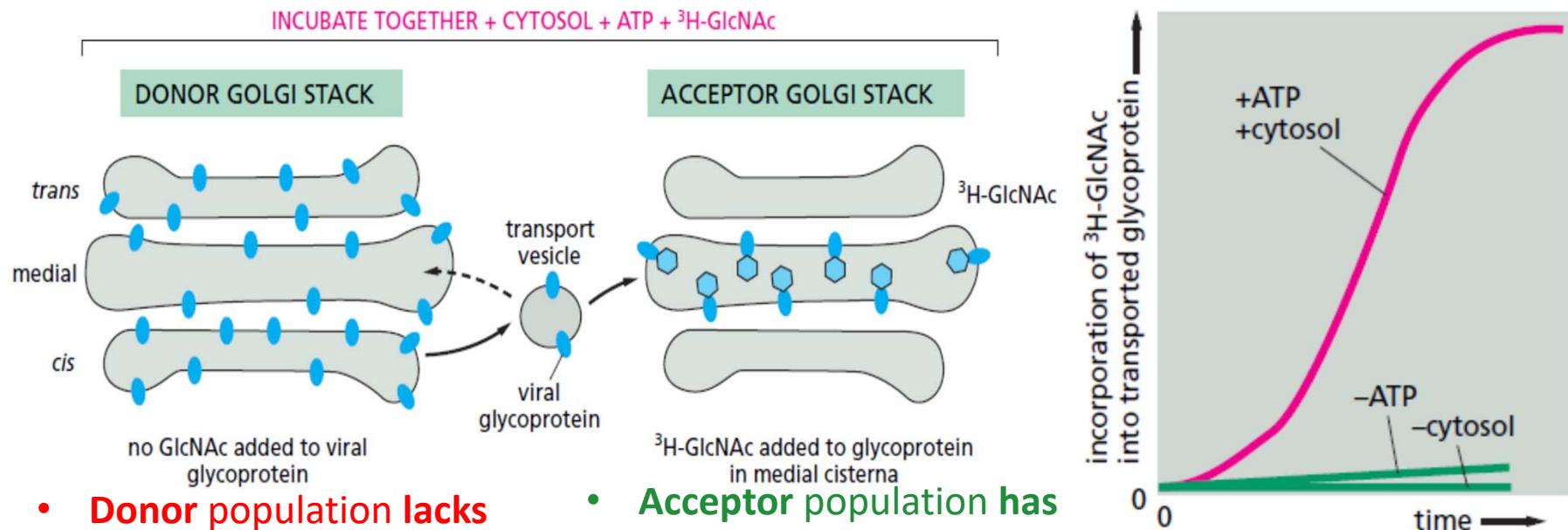
multicity suppression

Firstly, a **key gene** for vesicular transport is **mutated**; to bypass this defect, an **interacting protein** that is **produced to 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 a known vesicular transport protein** can be identified.

Cell-free (*in vitro*) transport system to study vesicular transport intra Golgi transport, using Golgi stacks from two different cell populations



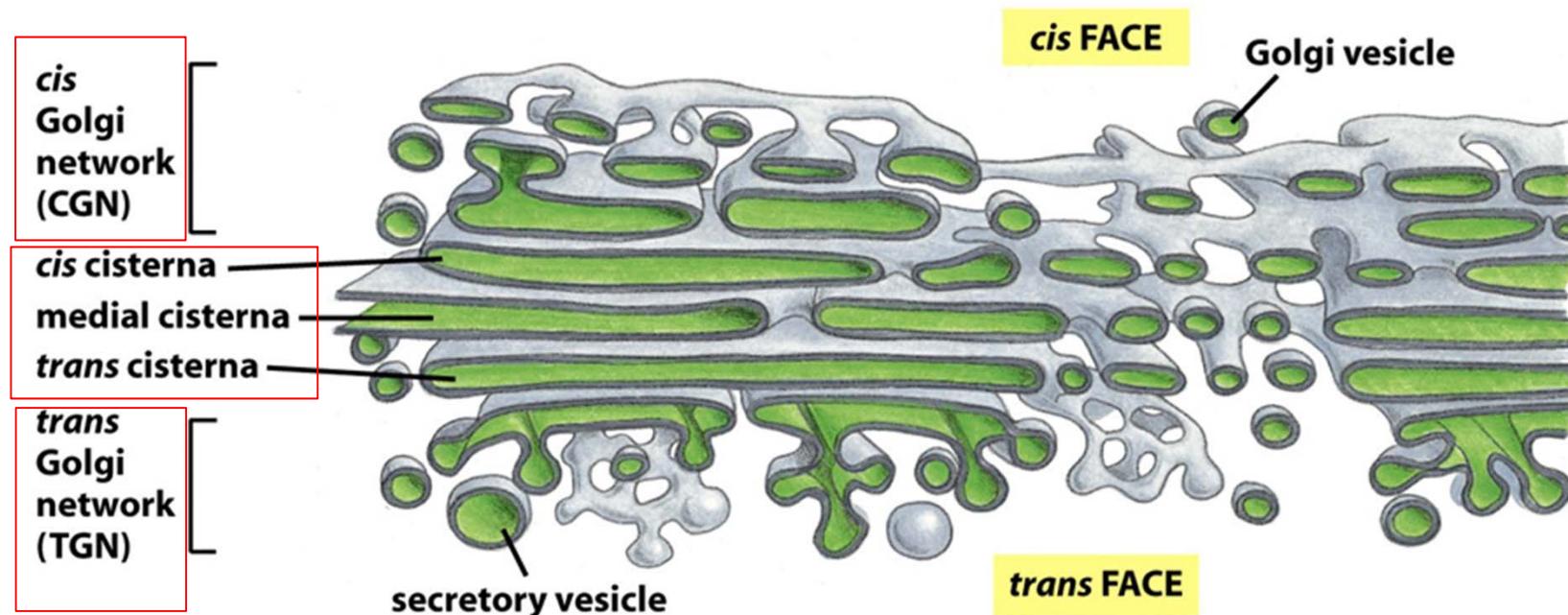
Experimental strategy:

Both Golgi populations are incubated “at cellular conditions” (with cytosol and ATP and “detectable” radiolabeled sugars ($^3\text{H}\text{GlcNAc}$)) to reconstitute transport: **Detection of $^3\text{H}\text{GlcNAc}$ -reporters demonstrates 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 a mammalian Golgi apparatus



Interconnected tubular and cisternal structures

cis-face of the Golgi is close to ER and nucleus

A “**Golgi apparatus**” consists of an ordered series of functionally distinct compartments:

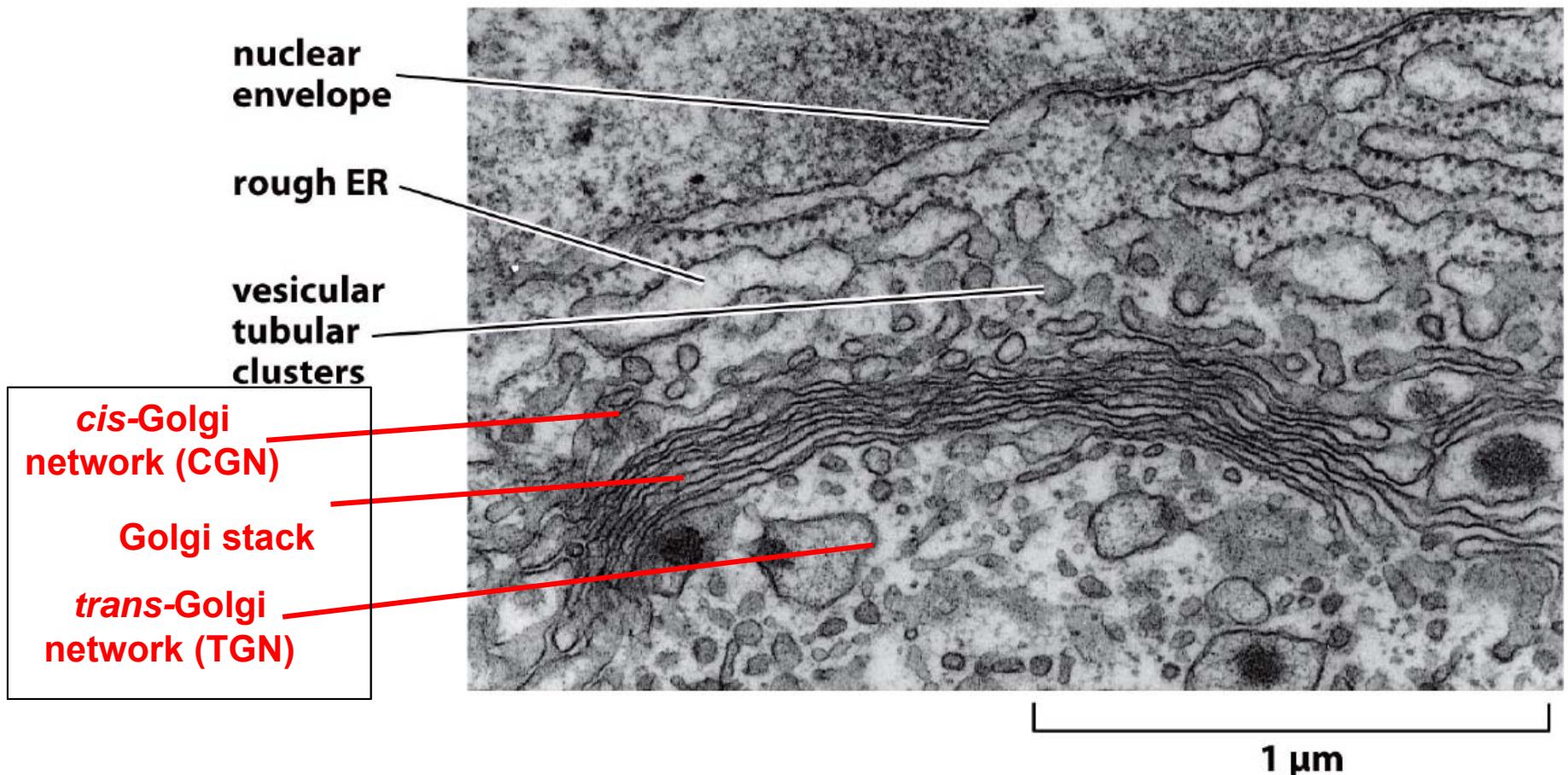
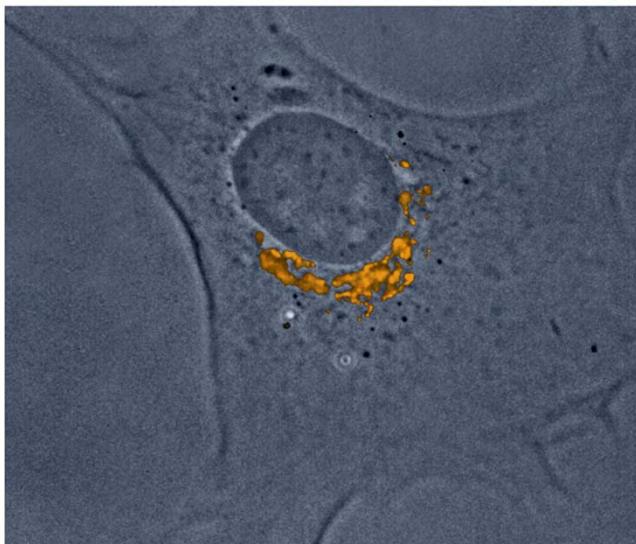


Figure 13-26b Molecular Biology of the Cell 6e (© Garland Science 2015)

Localization of the Golgi apparatus in animal and plant cells

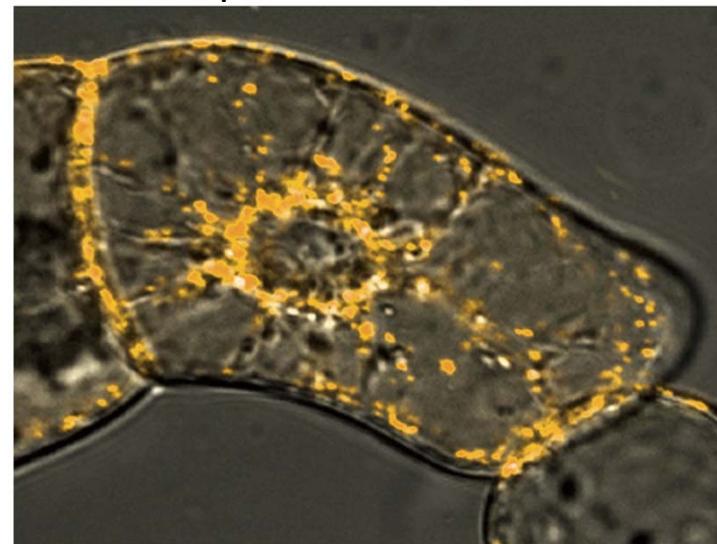
fibroblast



Close to the nucleus

In **animal cells**,
many stacks are linked together to a
single complex close to the nucleus and
to the centrosome, which is dependent
on **microtubule connection**.

plant cell



dispersed in the cytoplasm

In **plant cells**,
Golgi stacks, termed dictyosomes,
are individually, disperse in the cytoplasm.
Cells contain **hundreds** of individual stacks

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 (coat protein) II-coated transport vesicle (COP II vesicles)** mediate protein transport from the ER to the Golgi
- **COP II vesicles bud from ER exit sites (ERES) at the ribosome-free **transitional** ER (**tER**)**
- **Membrane proteins** display sorting signals (exit signals) on their **cytosolic domain** for ER exit
- **COP II coat can recognize** the exit signals **directly** (in case of membrane proteins) or **indirectly** (on soluble proteins that are sorted via a membrane protein that acts as sorting receptor)

The assembly of COPII coat

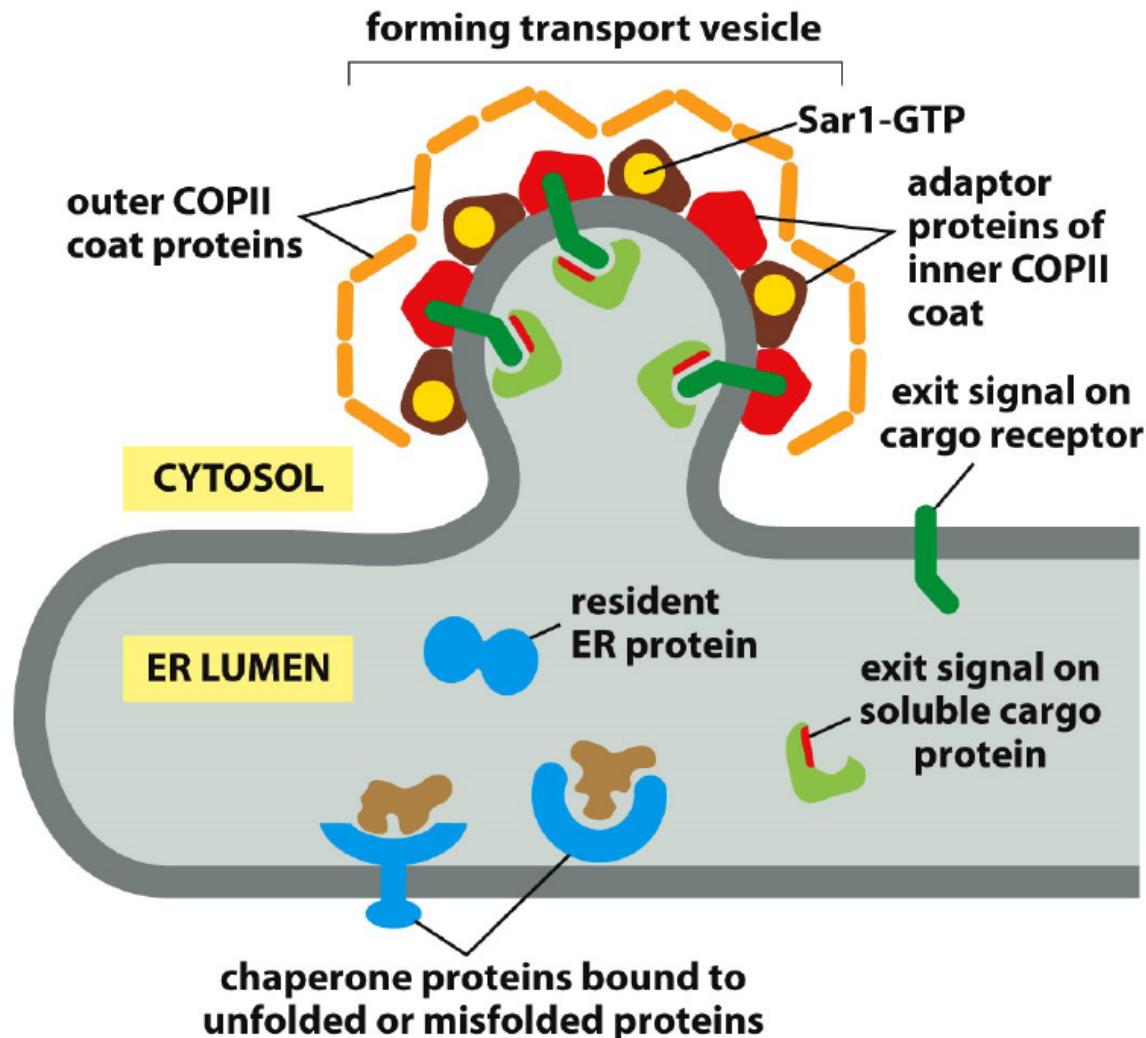
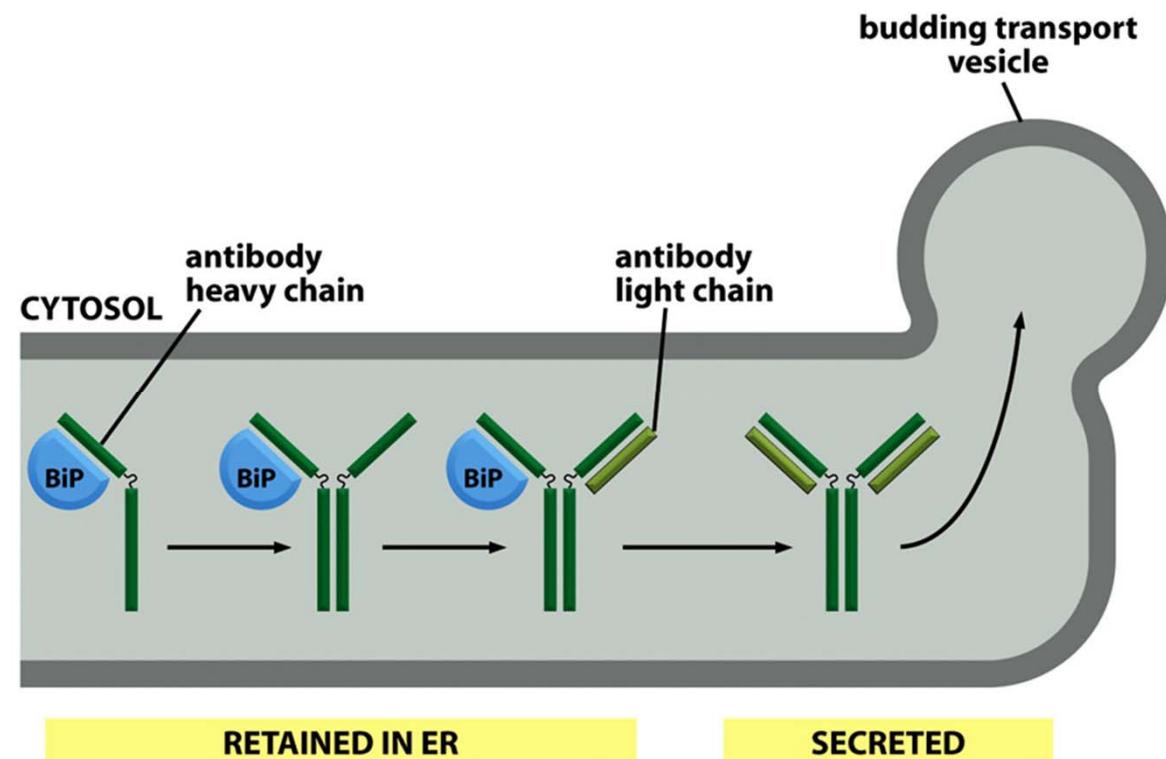


Figure 13-22 Molecular Biology of the Cell 6e (© Garland Science 2015)

ER-exiting proteins are folded well

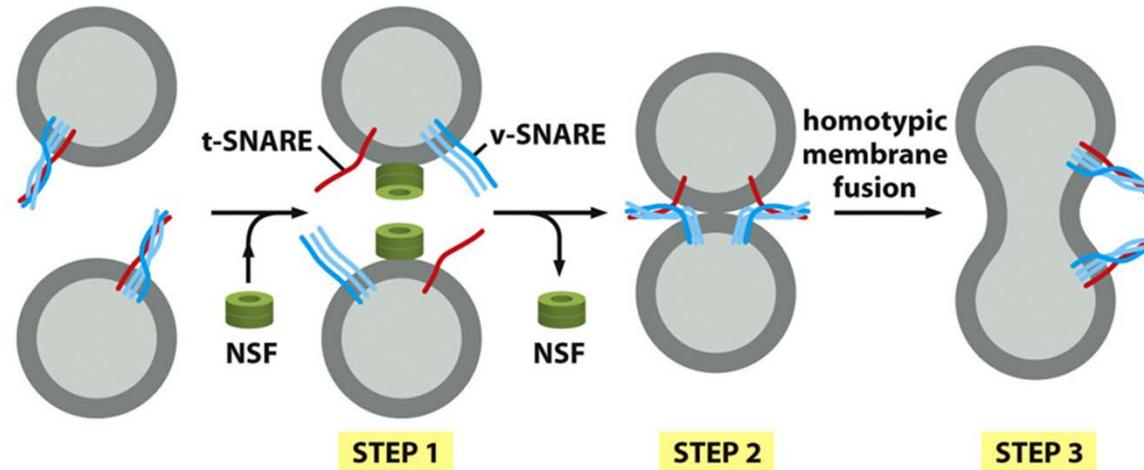
Example: Assembly of T-cell receptors



Molecular chaperons such as **BiP (binding protein)** 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
- **Heterotypic fusion:** fusion occurs between vesicles from different compartments.
- Both are mediated by v-SNARE and t-SNARE



In mammals, vesicular-tubular cluster (VTC) mediate transport between the ER and the Golgi apparatus

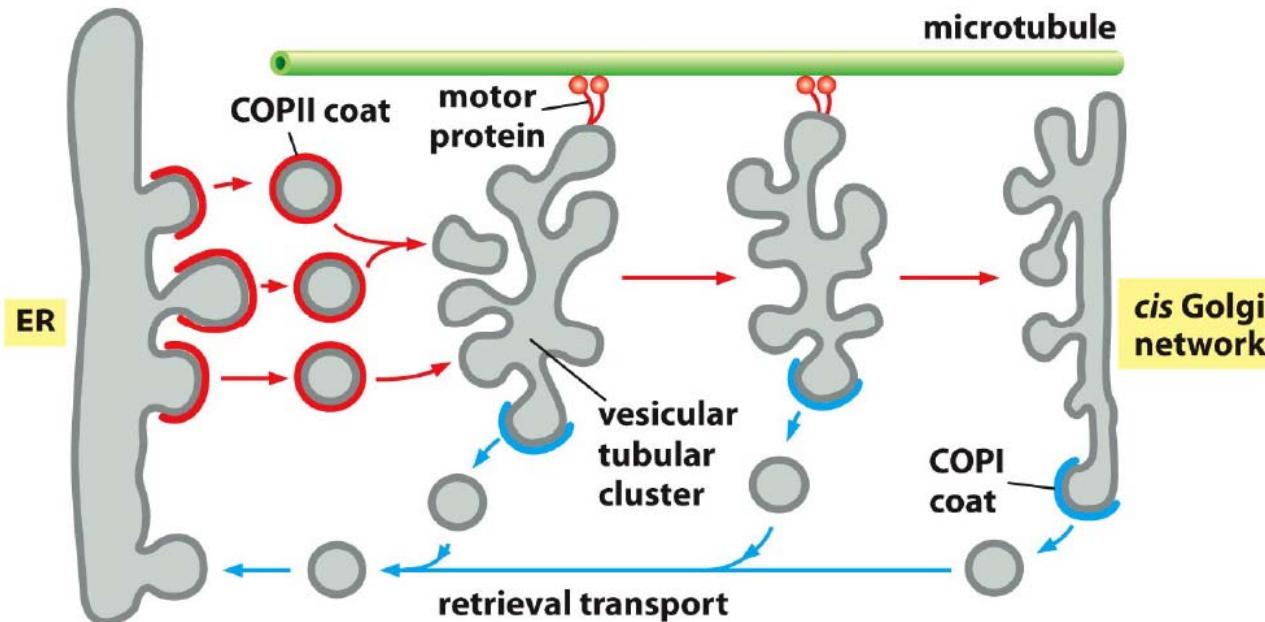
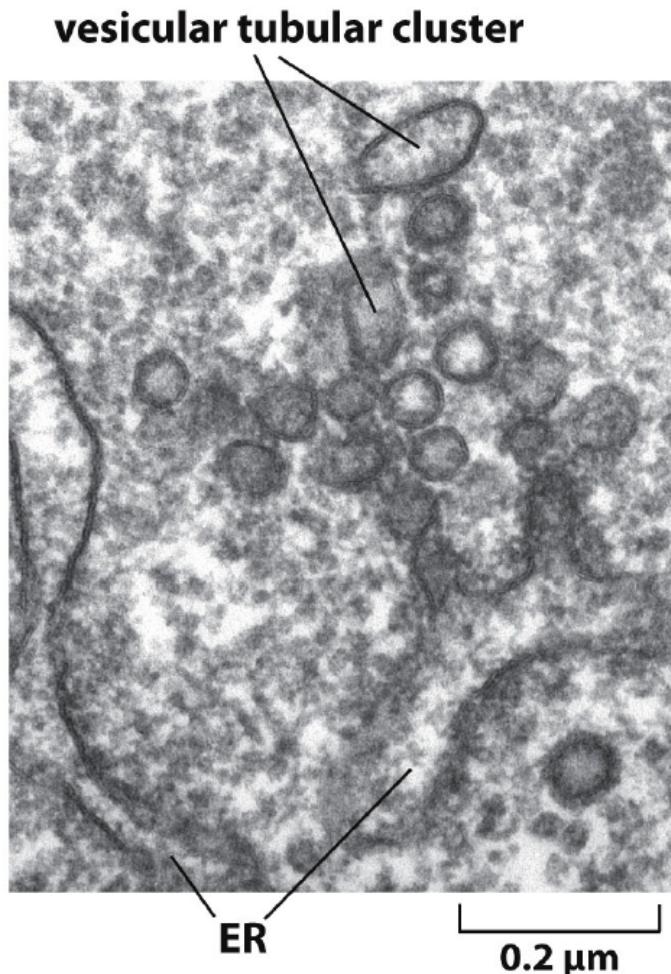


Figure 13-24b Molecular Biology of the Cell 6e (© Garland Science 2015)

- **Vesicular tubular clusters (VTCs)** are short lived and move quickly along the microtubule
- **Vesicular tubular clusters** can bud off COP I vesicles on their own to send back ER resident protein--- a process called retrieval (or retrograde) transport
- **Retrieval** requires **ER retrieval signals**, which are recognized by COP I coats directly (on membrane proteins) or indirectly (on soluble proteins via receptor)

Vesicular tubular cluster (VTC) arise from homotypic fusion of COP II vesicles



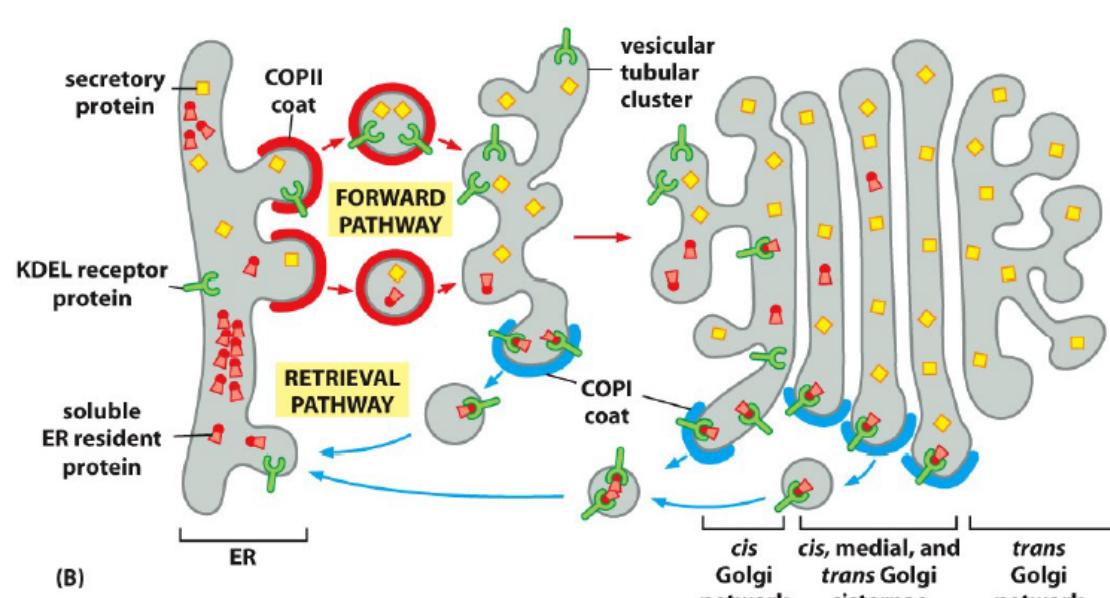
**Vesicular tubular cluster (VTC)
forming around an ER exit site.**

Many of the vesicle-like structures seen in the micrograph are **cross sections of tubules** that extend above and below the plane of this thin section and are interconnected.

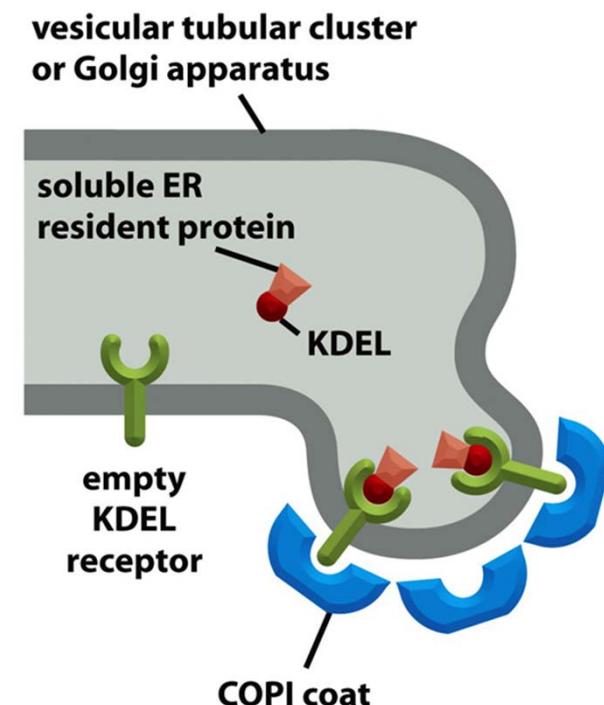
Figure 13-24a Molecular Biology of the Cell 6e (© Garland Science 2015)

Retrieval of ER membranes and of soluble ER-resident proteins from VTCs or from the Golgi requires signals

- ER **membrane** proteins: C-terminal KKXX sequence, which directly binds to COP I coat
- ER **soluble** protein: C-terminal H/KDEL sequence, binds to membrane-bound KDEL receptor (ERD2)



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4. Glycosylation in the Golgi apparatus

1. Functional compartmentalization of the Golgi apparatus
2. Types of Golgi-specific glycosylations
3. Function of glycosylation
4. Oligosaccharide processing in ER and Golgi apparatus

Compartmentalization in Golgi apparatus

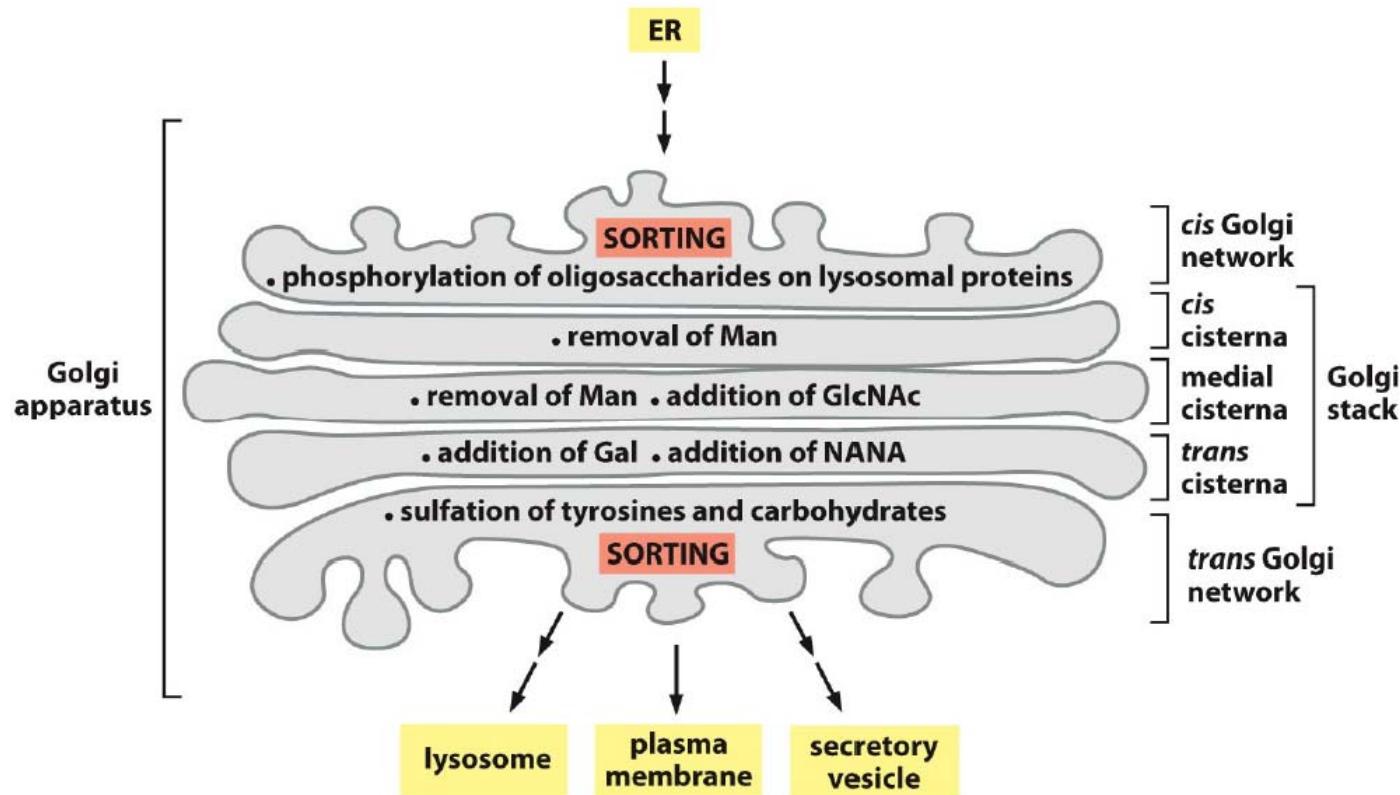


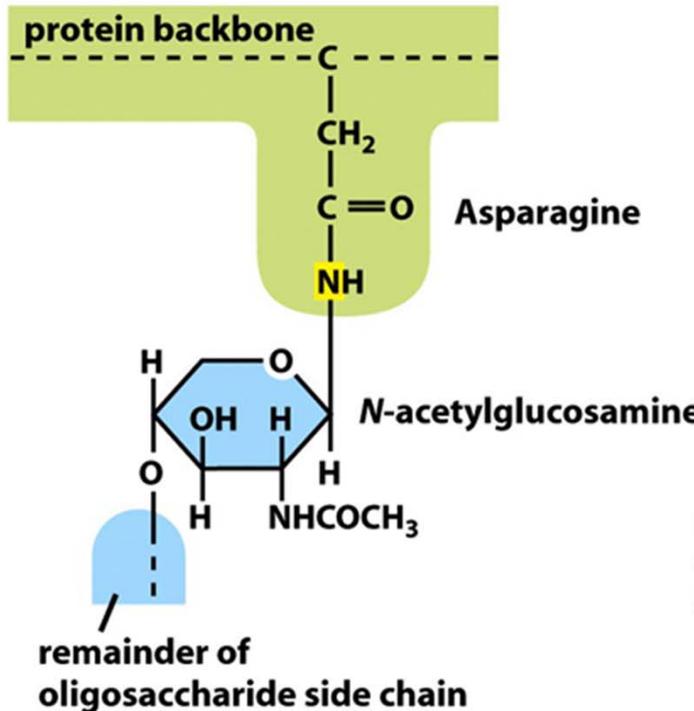
Figure 13-29 Molecular Biology of the Cell 6e (© Garland Science 2015)

Processing enzymes are not restricted to a particular cisterna; instead, their distribution is graded across the stack:

- **early-acting** enzymes are present mostly in the ***cis*-Golgi** cisternae
- **later-acting** enzymes are mostly in the ***trans*-Golgi** cisternae

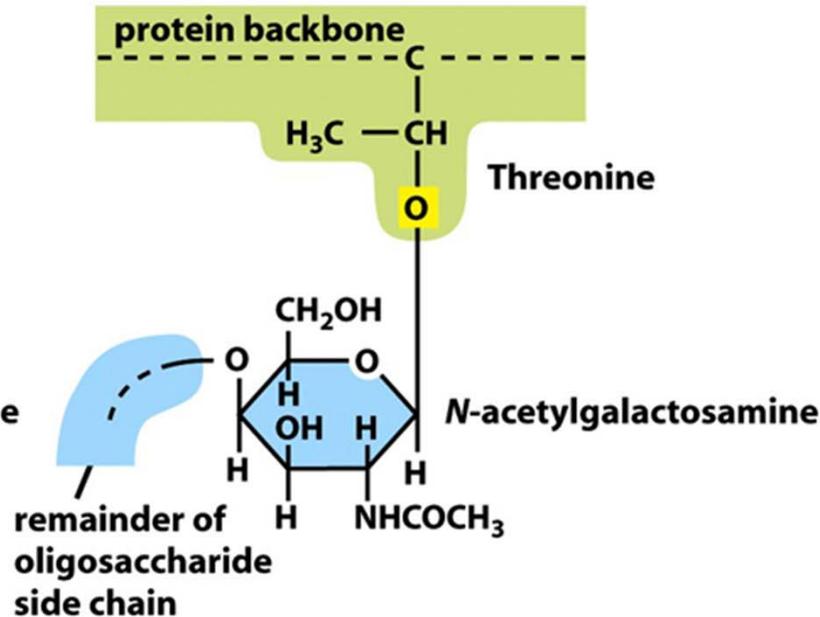
Different types of glycosylation:

N-LINKED GLYCOSYLATION



Attachment of a sugar to an **nitrogen of an amino group** of asparagine in the ER

O-LINKED GLYCOSYLATION

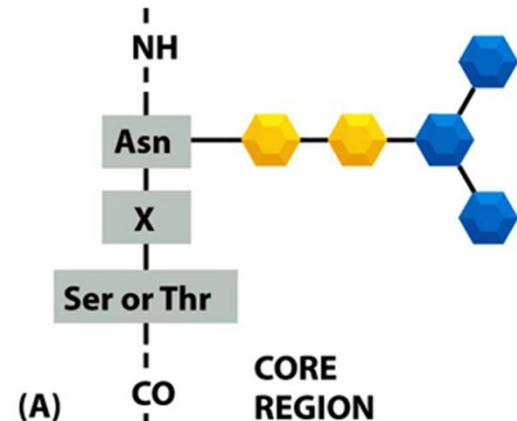


Attachment of a sugar to an **oxygen of a hydroxyl group** of serine/threonine in the **Golgi apparatus**

Mammals have two main classes of N-linked oligosaccharides:
“complex” & “high-mannose” forms

A common core:

2 N-acetylglucosamines
(GlcNAc)
3 mannoses (Man).



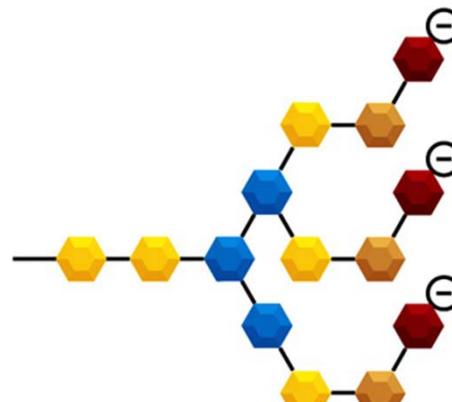
Yellow hexagon = N-acetylglucosamine (GlcNAc)

Blue hexagon = mannose (Man)

Brown hexagon = galactose (Gal)

Red hexagon with minus sign = N-acetylneurameric acid (sialic acid, or NANA)

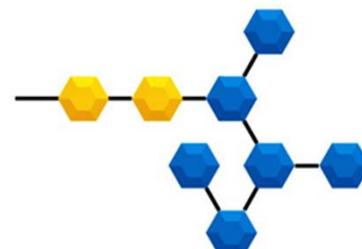
Complex oligosaccharides:



core + variable number
of copies of a special
trisaccharide unit

GlcNAc-galactose-sialic acid

High-mannose oligosacch.:



core + variable number
mannose residues

Functions of 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

Golgins, Golgi matrix proteins, help organize the stack

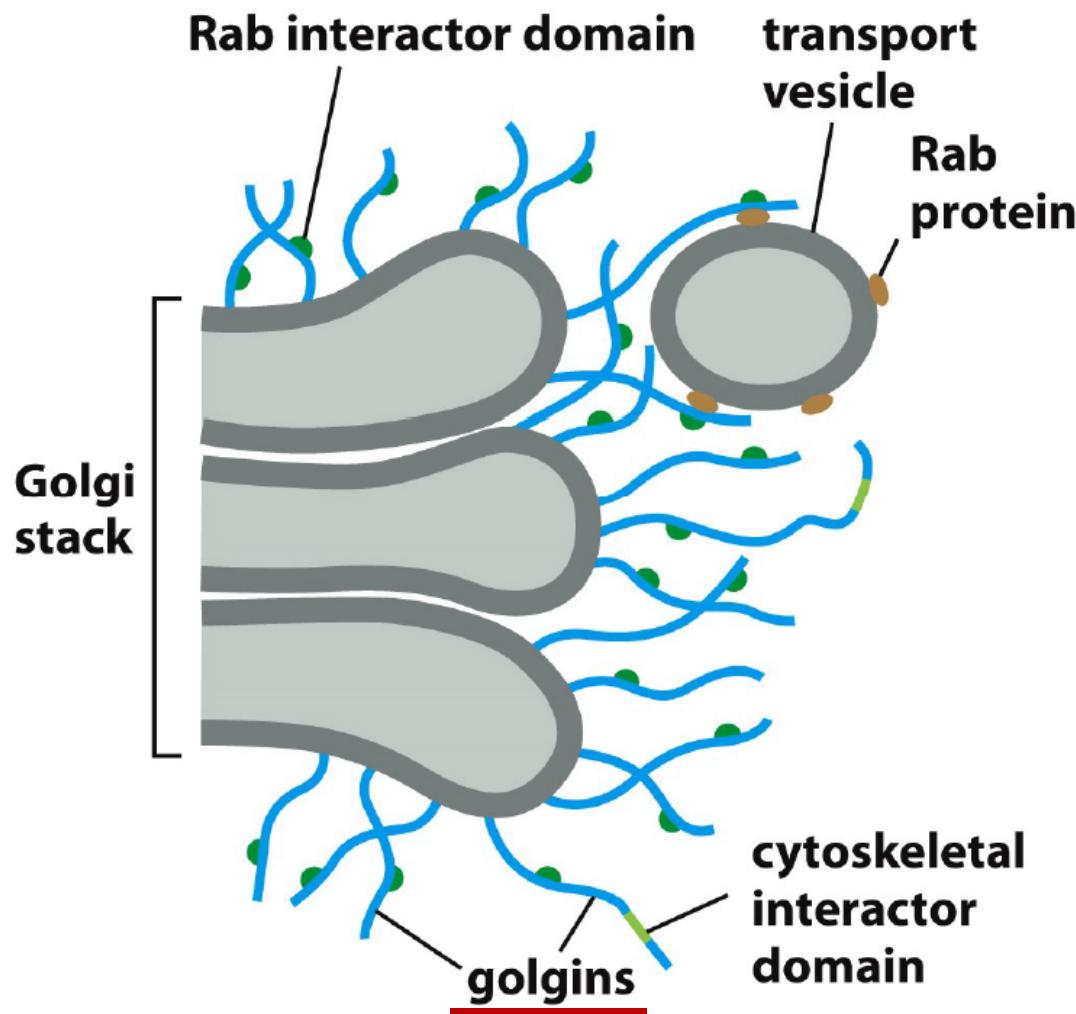
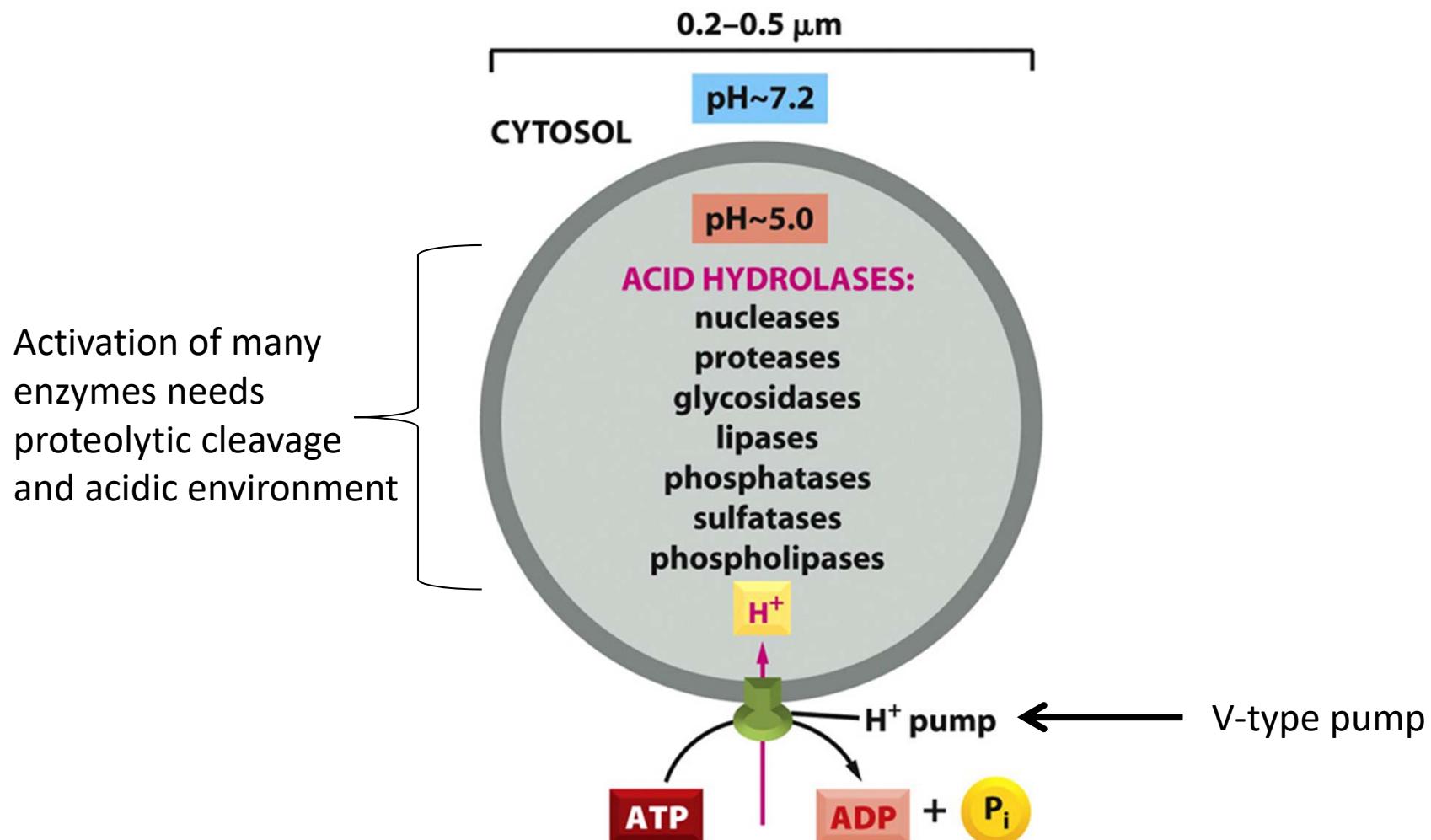


Figure 13-36 Molecular Biology of the Cell 6e (© Garland Science 2015)

IV. Transport from TGN to lysosome

- Introduction to lysosomes
- Transport from TGN to lysosomes

What is a lysosome?



Lysosomes are heterogenous

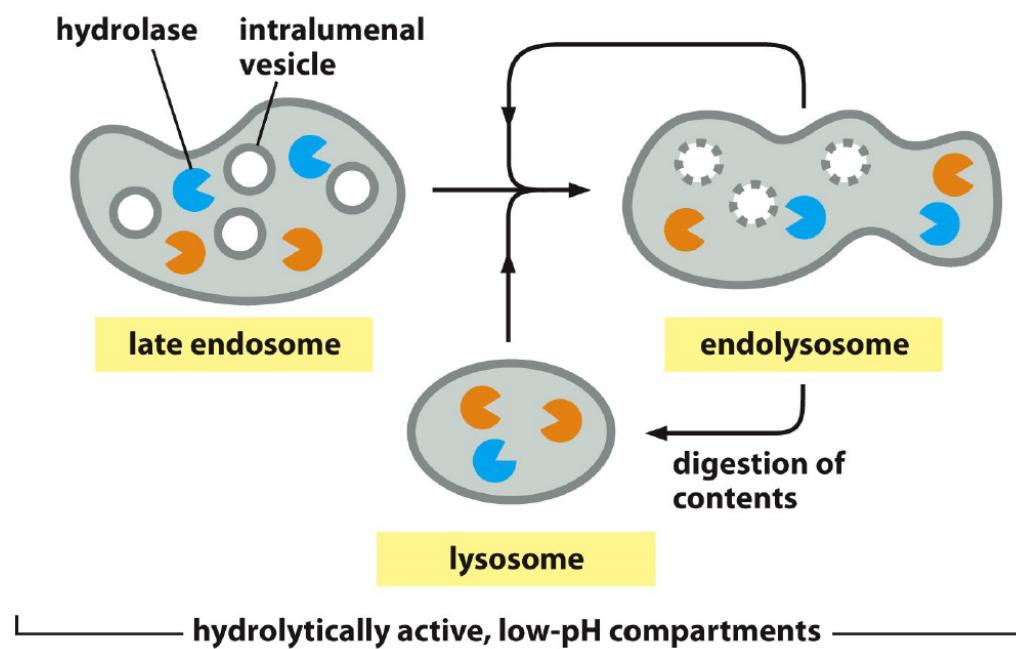
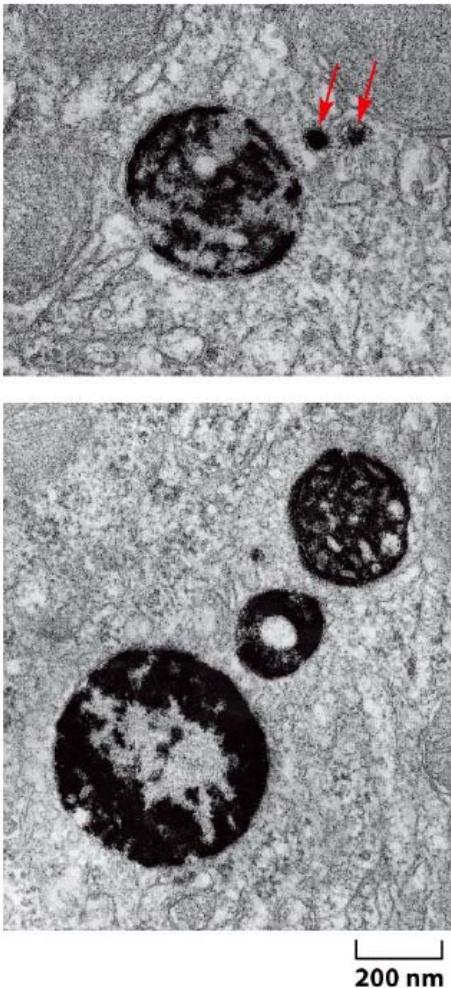
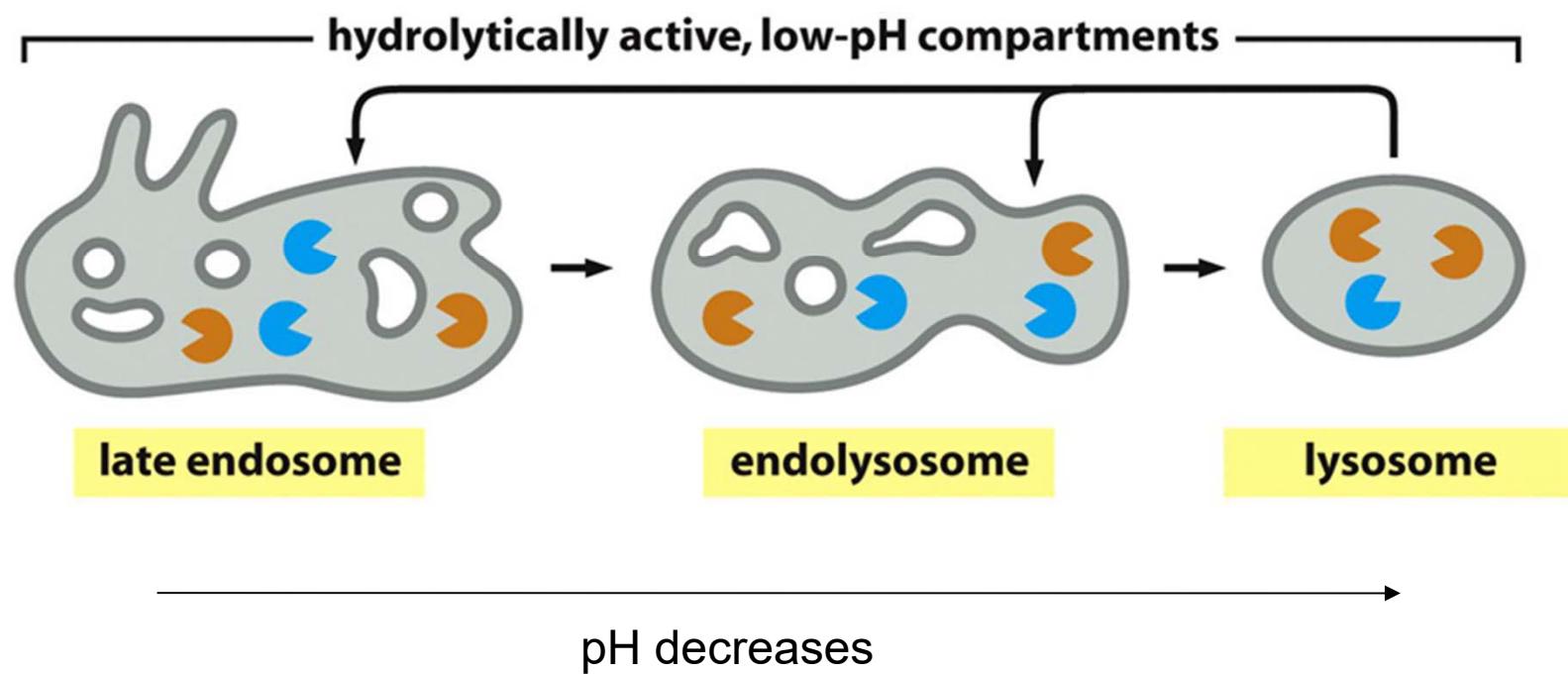


Figure 13-38 Molecular Biology of the Cell 6e (© Garland Science 2015)

Lysosome maturation



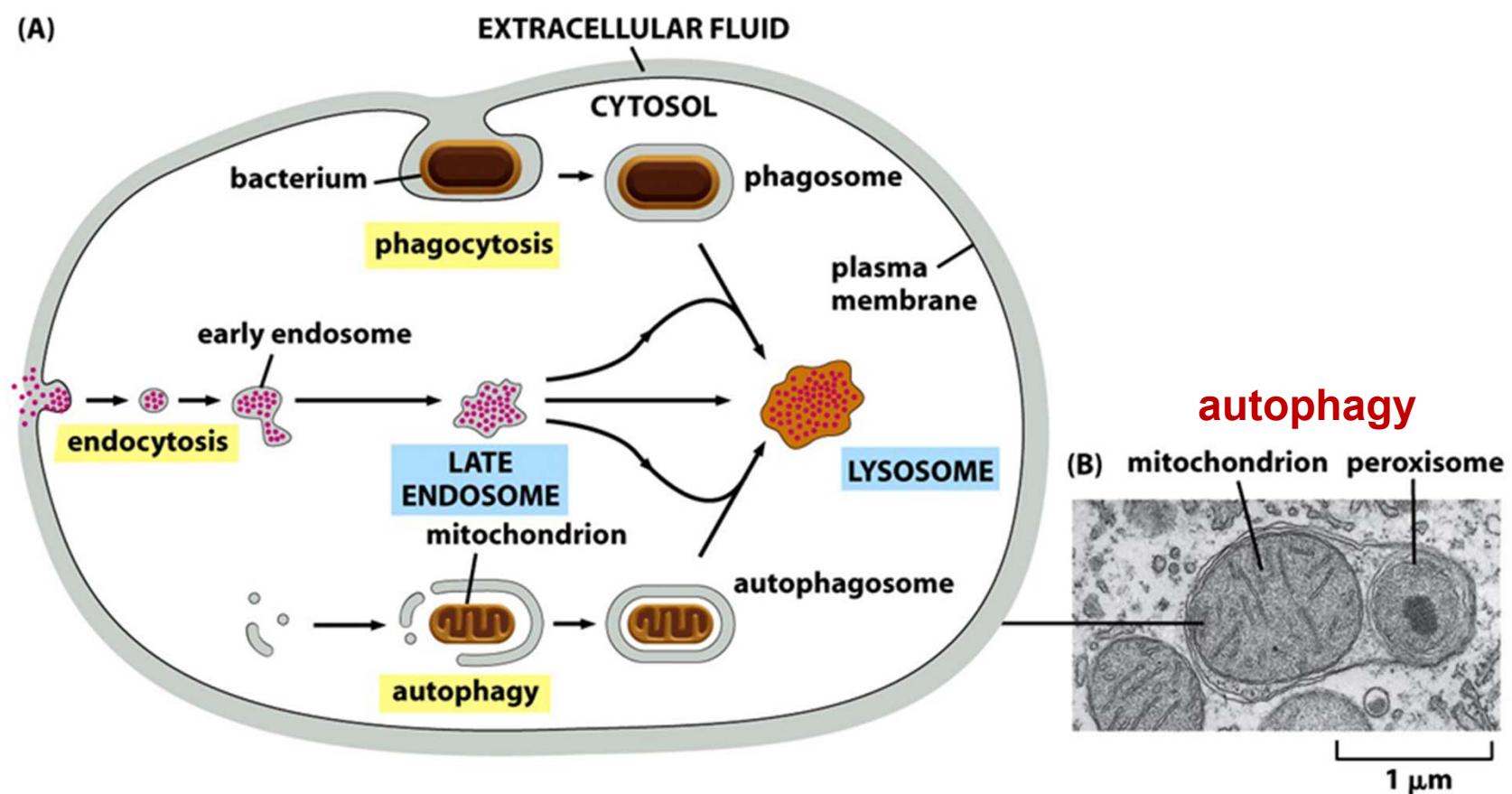
Facts about lysosomes

- Found in all eukaryotic cells
- Heterogeneity due to :
 - multiple function
 - different maturation stages

Functions of lysosomes:

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

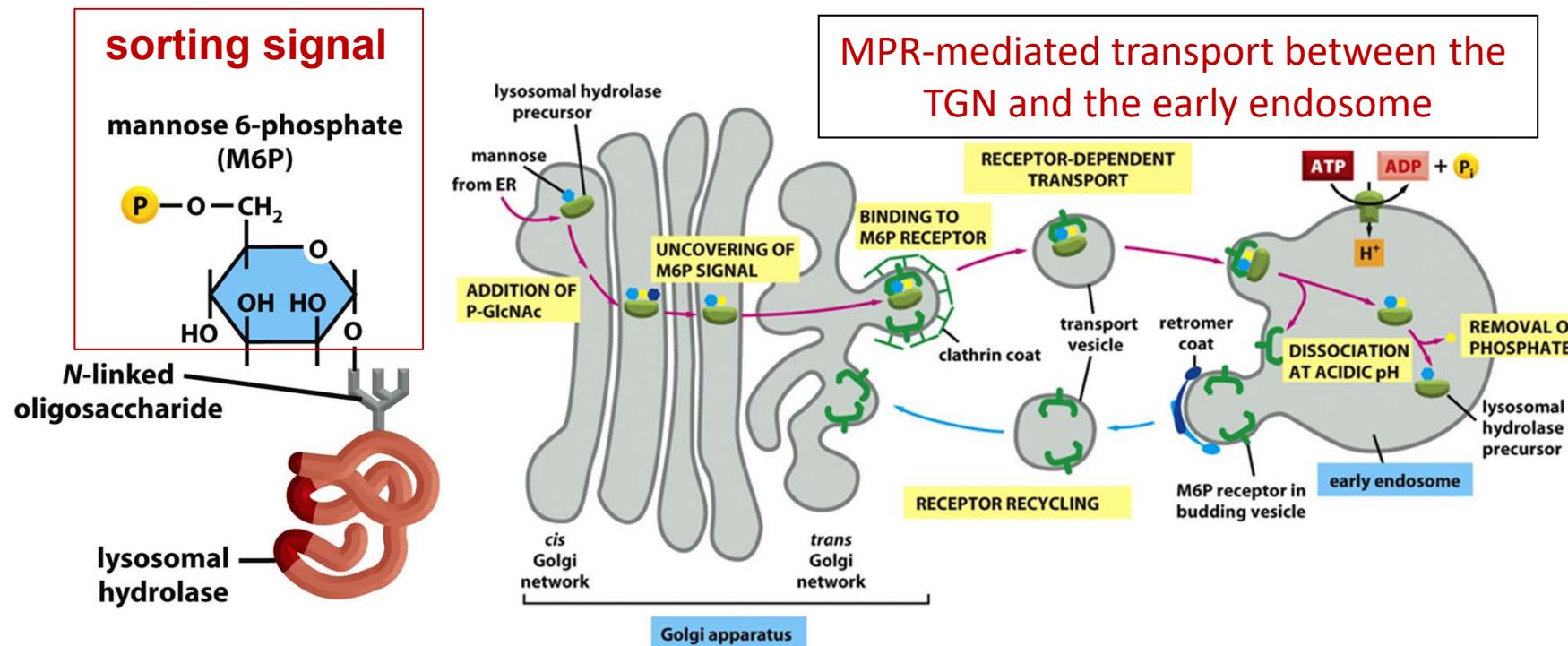
Three pathways for degradation in lysosome



Indigested materials will be excreted out by exocytosis

How are hydrolases selected for transported to lysosomes?

Lysosomal sorting receptor: mannose 6-phosphate receptor (MPR)

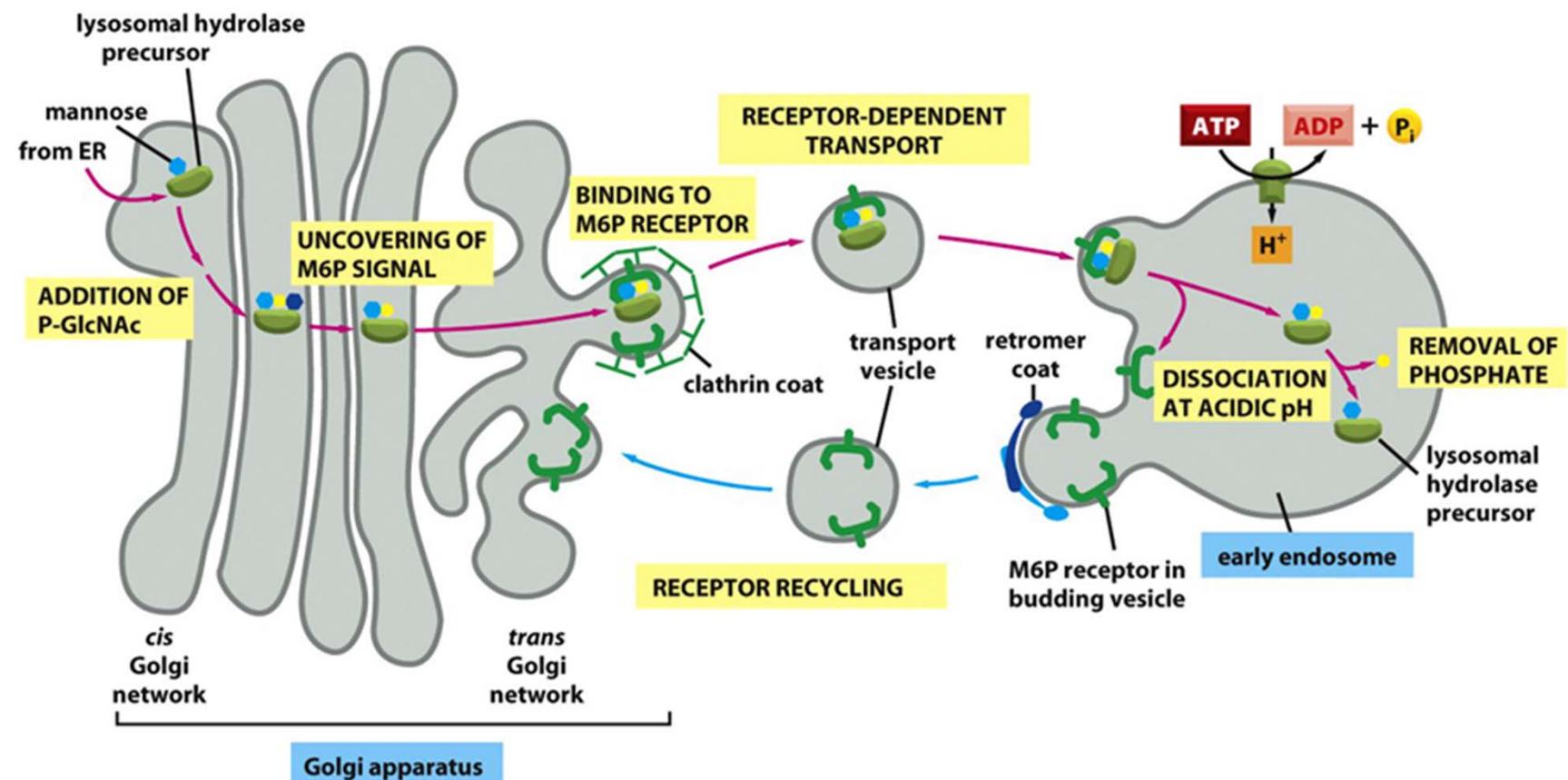


M6P receptor protein works best at pH 6.5-6.7,
and release at pH 6.0(late endosome)
M6P is then hydrolyzed inside endosome.

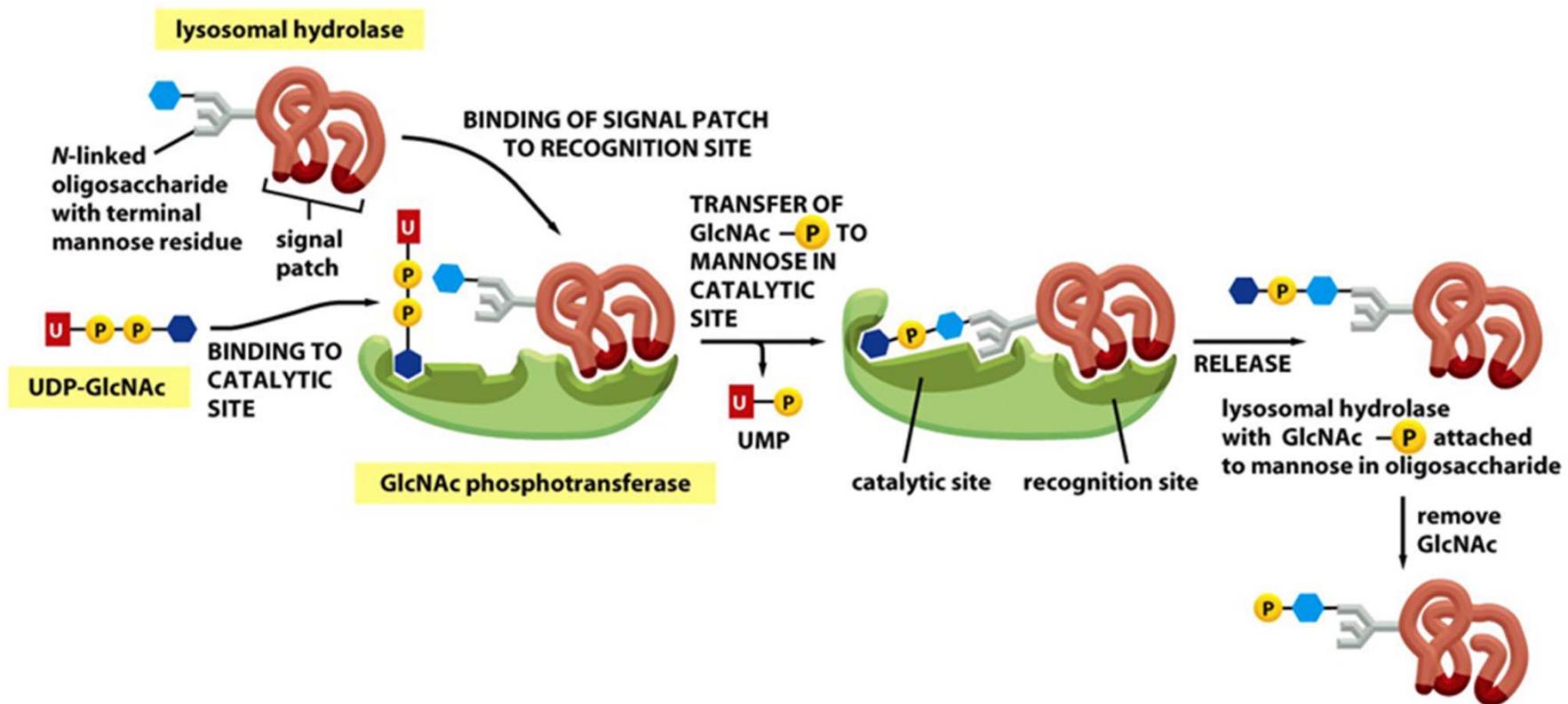
How are hydrolases selected for transported to lysosomes?

Bi-directional MPR transport between TGN and the early endosome:

- Anterograde from TGN to EE: MPRs transport lysosomal hydrolases
- Retrograde from EE to TGN: recycling of MPRs



The recognition of a lysosomal hydrolase: synthesis of the lysosomal sorting signal



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

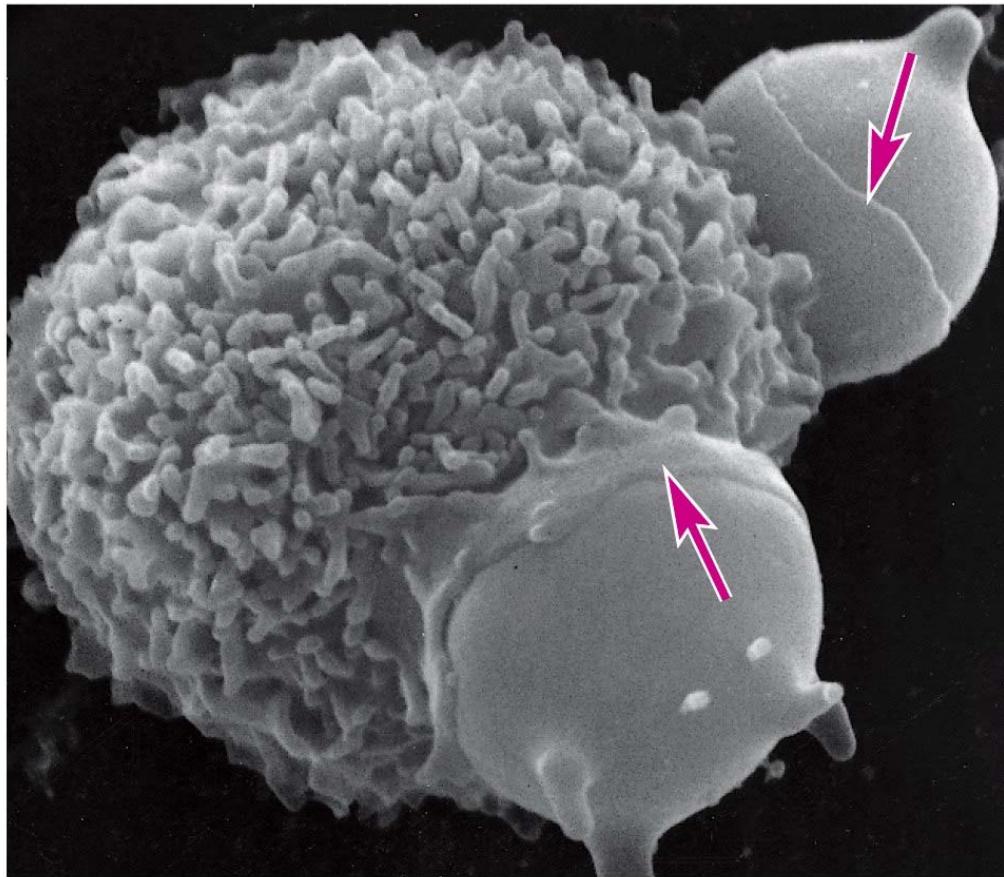
V. Endocytosis

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

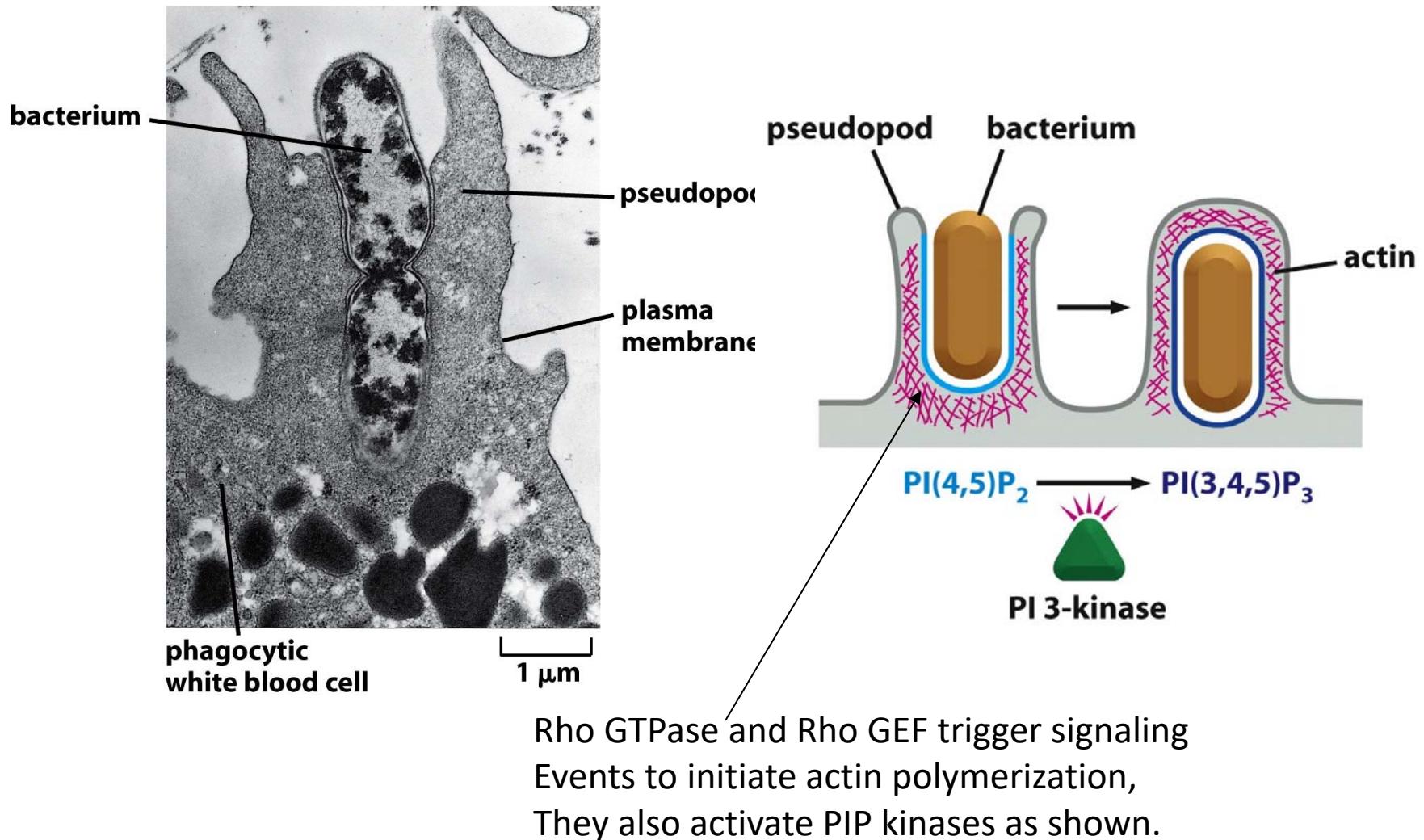


[

5 μm

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

Phagocytosis



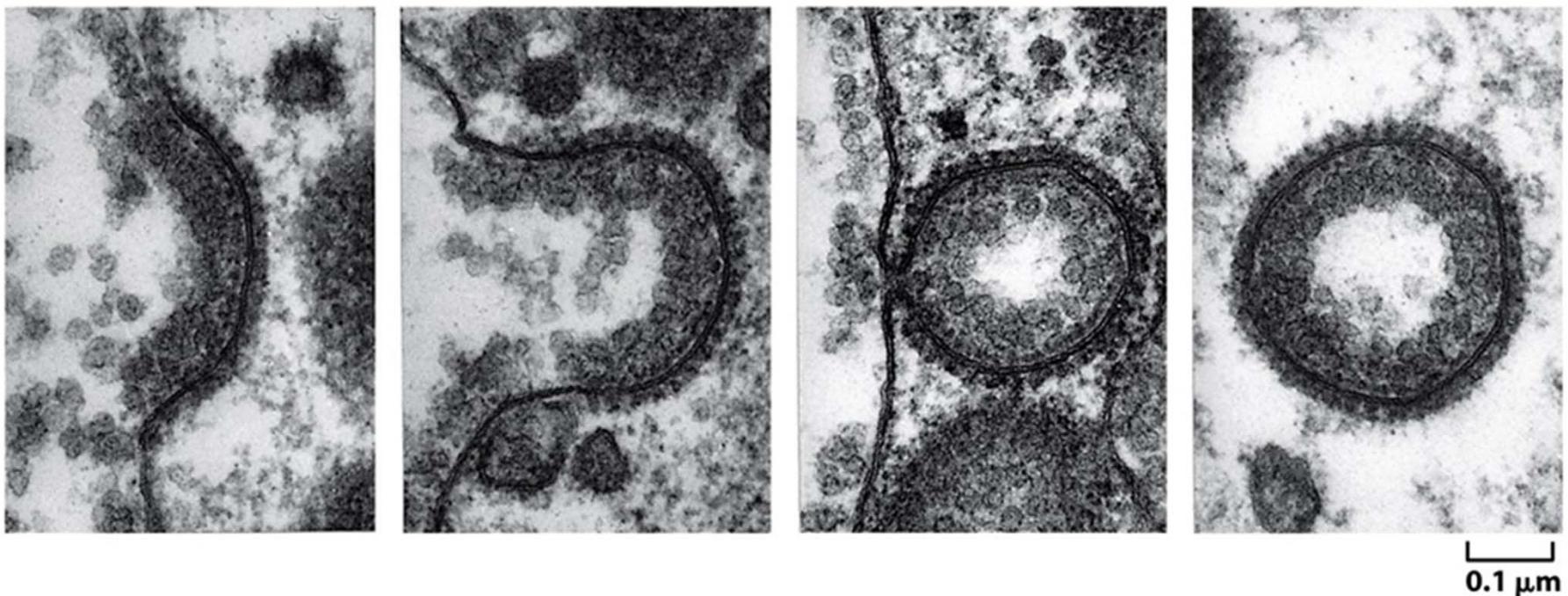
Factors triggering phagocytosis the “eat-me” signals

- 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

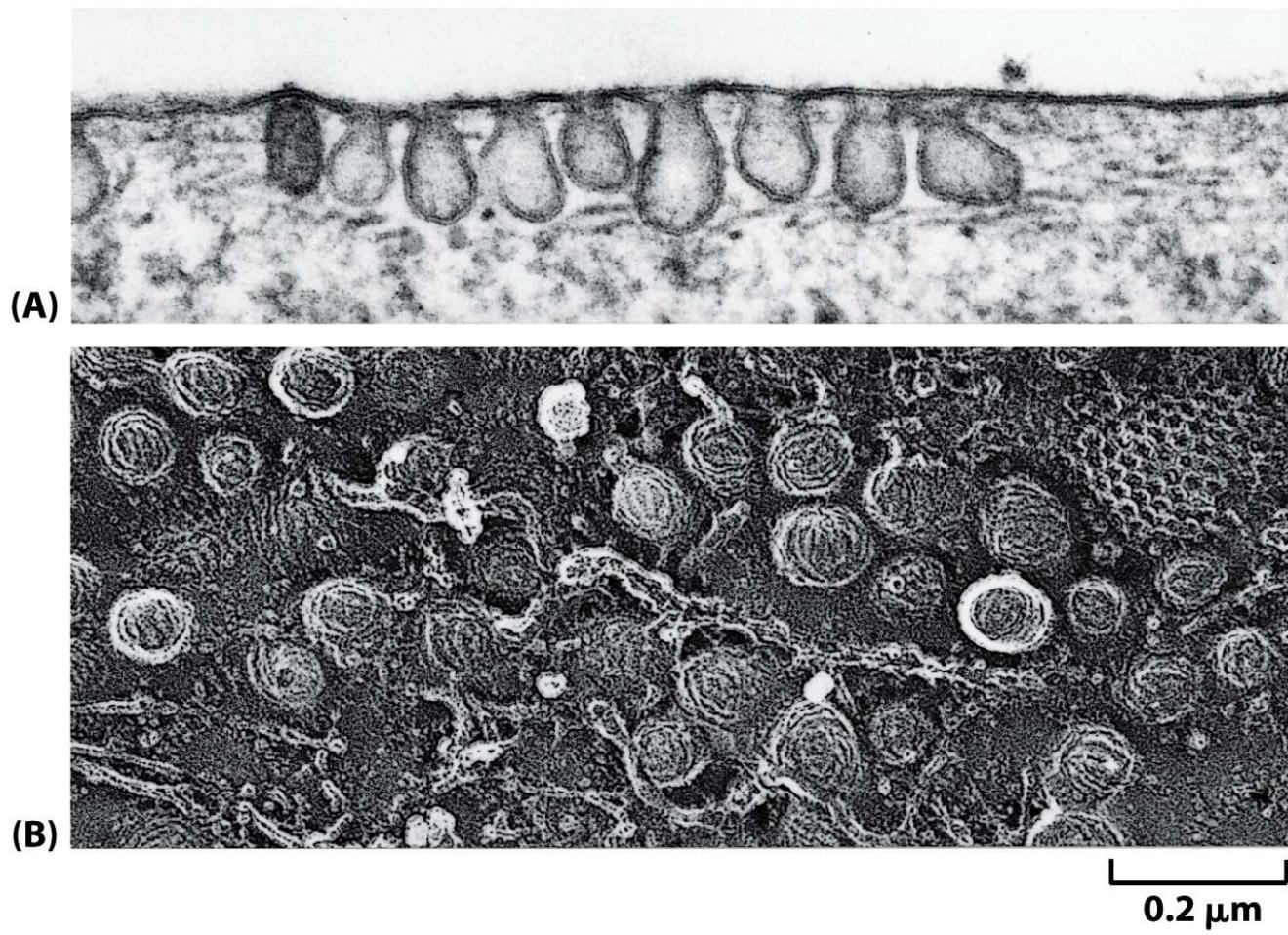
Pinocytosis: clathrin-mediated



0.1 μm

CCVs from large hen oocyte, they **take up lipoprotein** particles to form yolk. The lipoprotein particles bound to their membrane-bound receptors appear as a dense, fuzzy layer on the extracellular surface of the plasma membrane, which is the inside surface of the coated pit and vesicle

Caveolae mediated pinocytosis



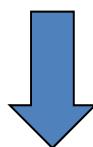
Endocytosis mediated by Clathrin-coated pits --- receptor-mediated endocytosis

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

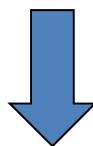
Cells actively regulate receptor mediated endocytosis

For example:

Mono- and multi-ubiquitination of receptors



Ubiquitin binding protein recognizes these receptors

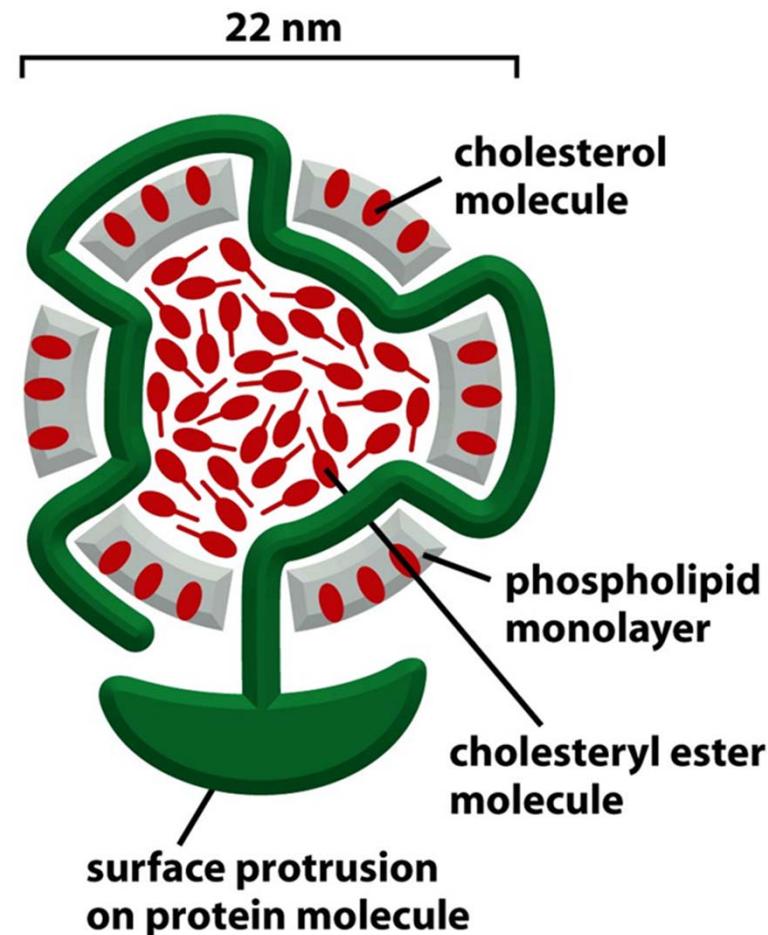


Direct clathrin coat assembly

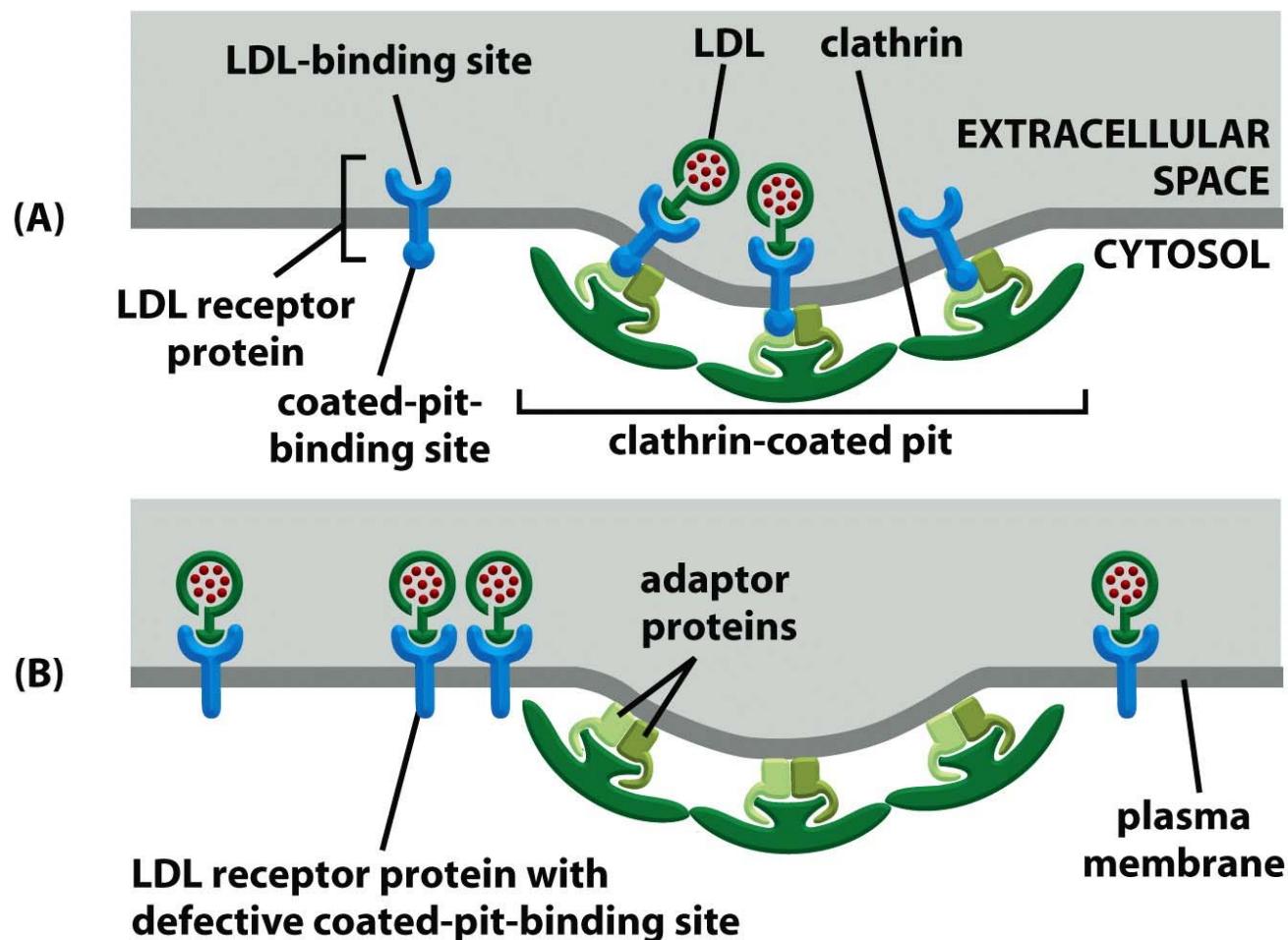
Example: Taking up of cholesterol by LDL receptor on cells

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.

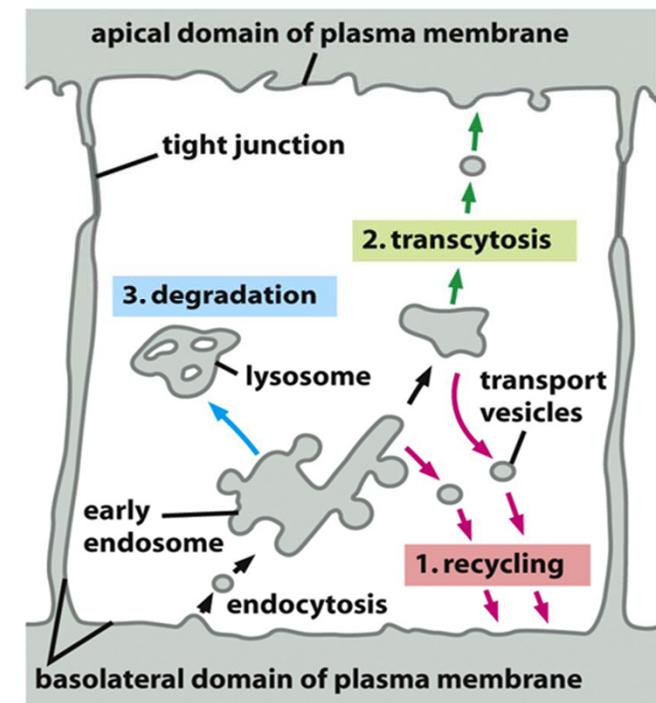
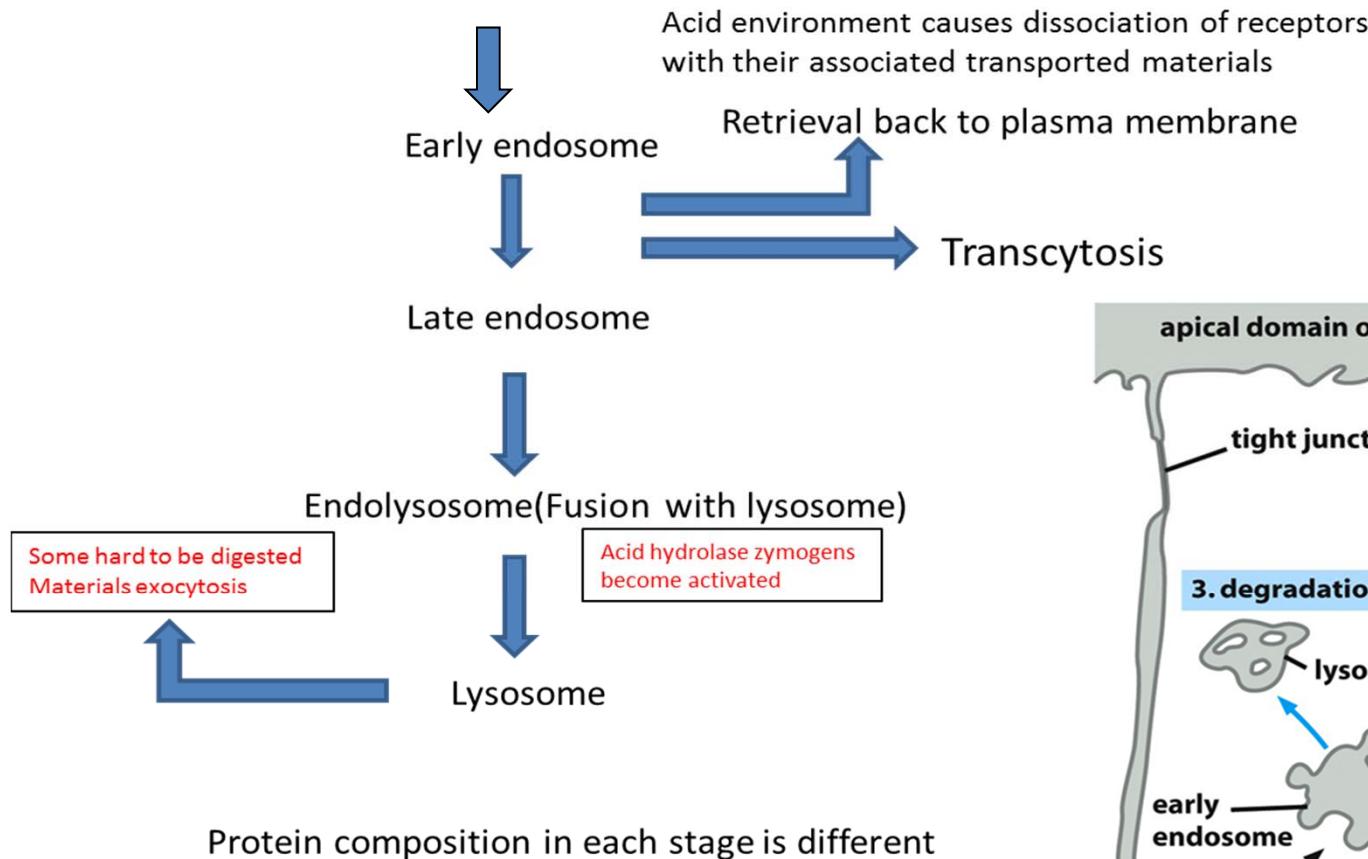


Endocytosis of LDL by normal and mutant LDL receptors

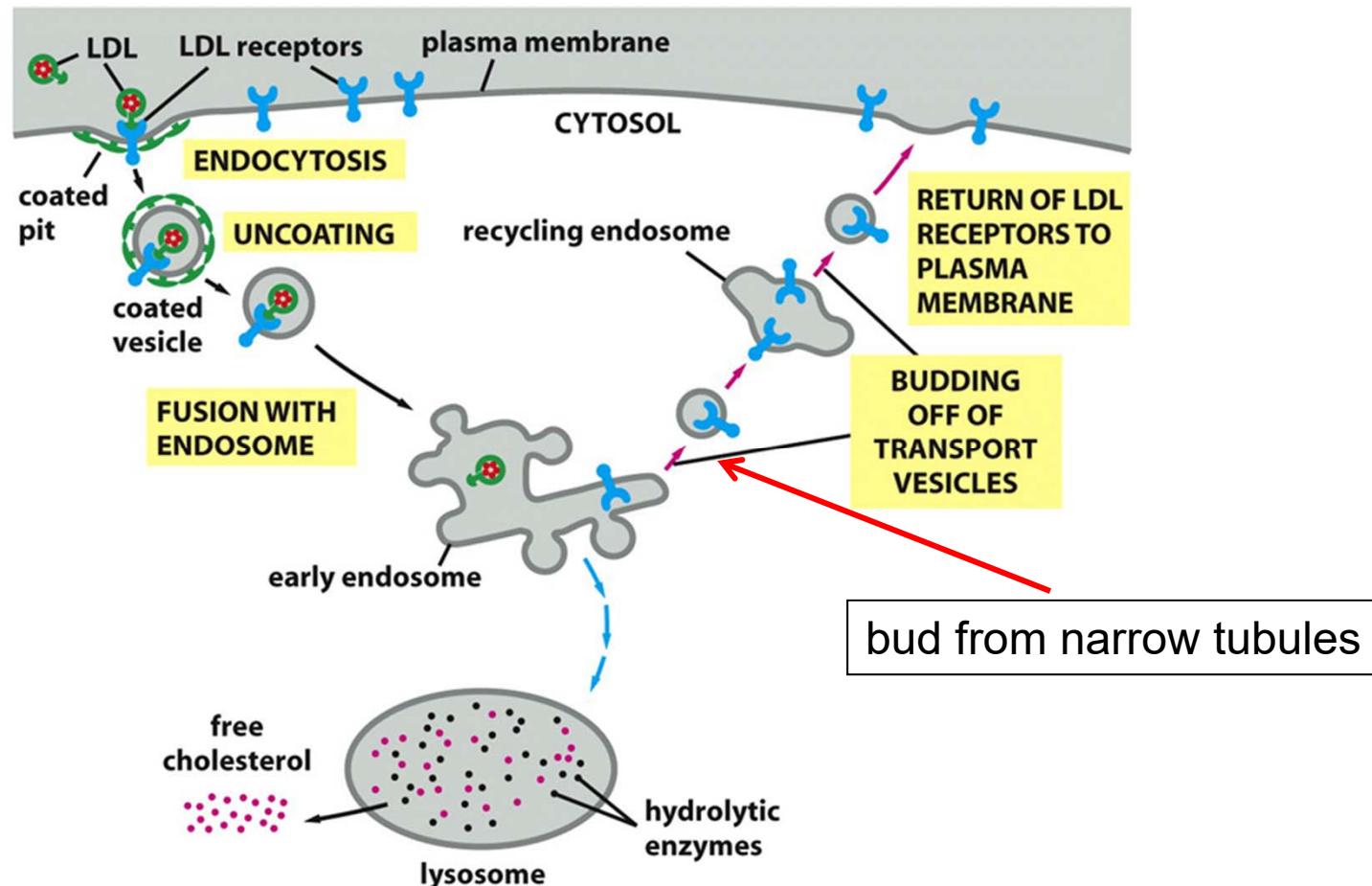


Fate of endocytosed materials

Endocytic particle fusion with endosome

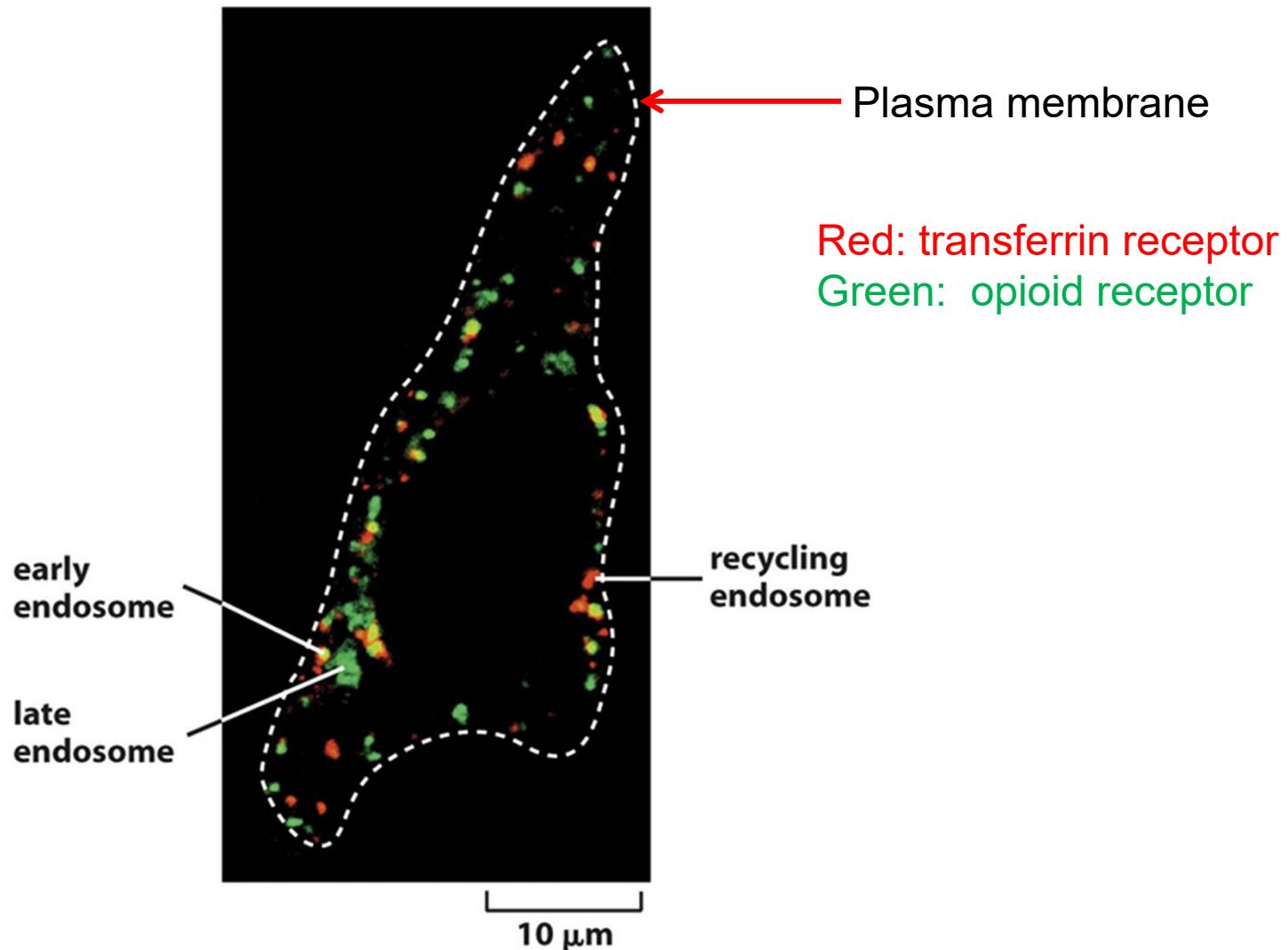


(1) Retrieval: LDL-receptors are retrieved

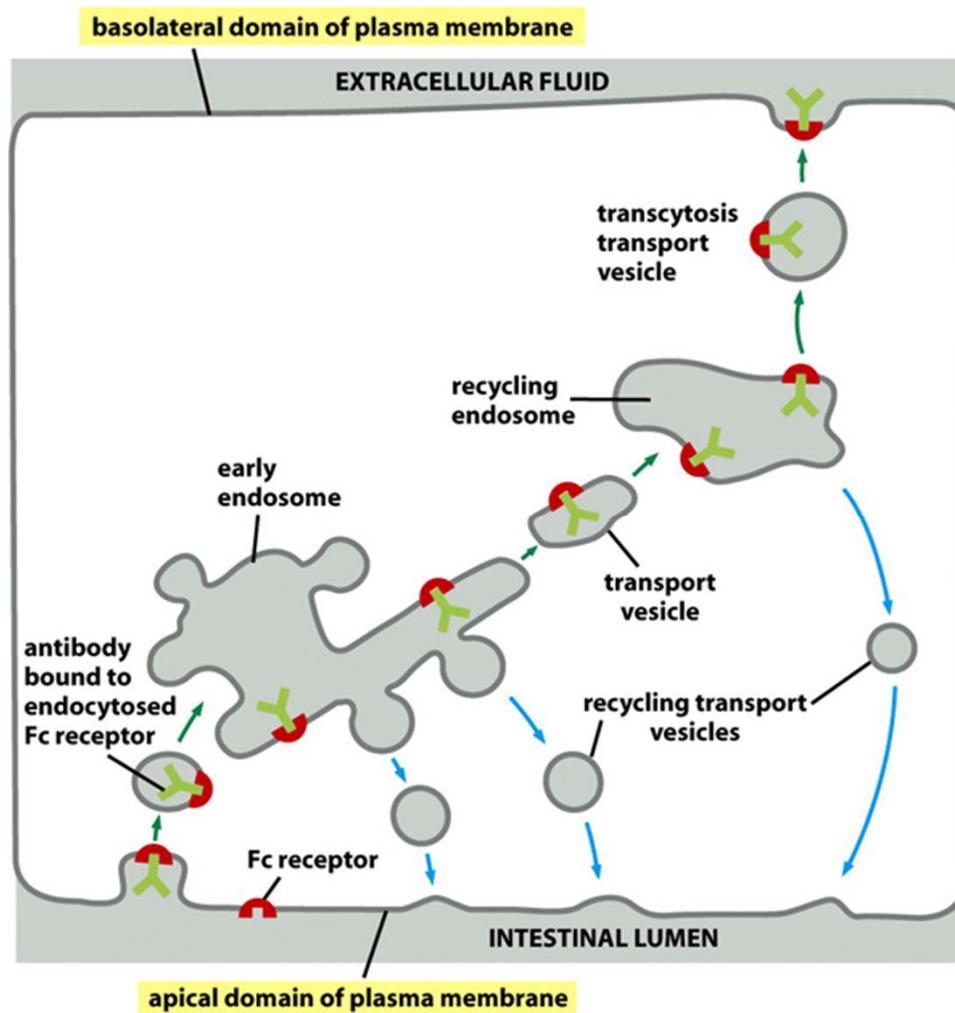


Some other receptors are degraded in lysosomes, such as EGFR, opioid receptors
--- a process called *receptor down-regulation*.

Example: transferrin receptor and opioid receptor are sorted differently



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



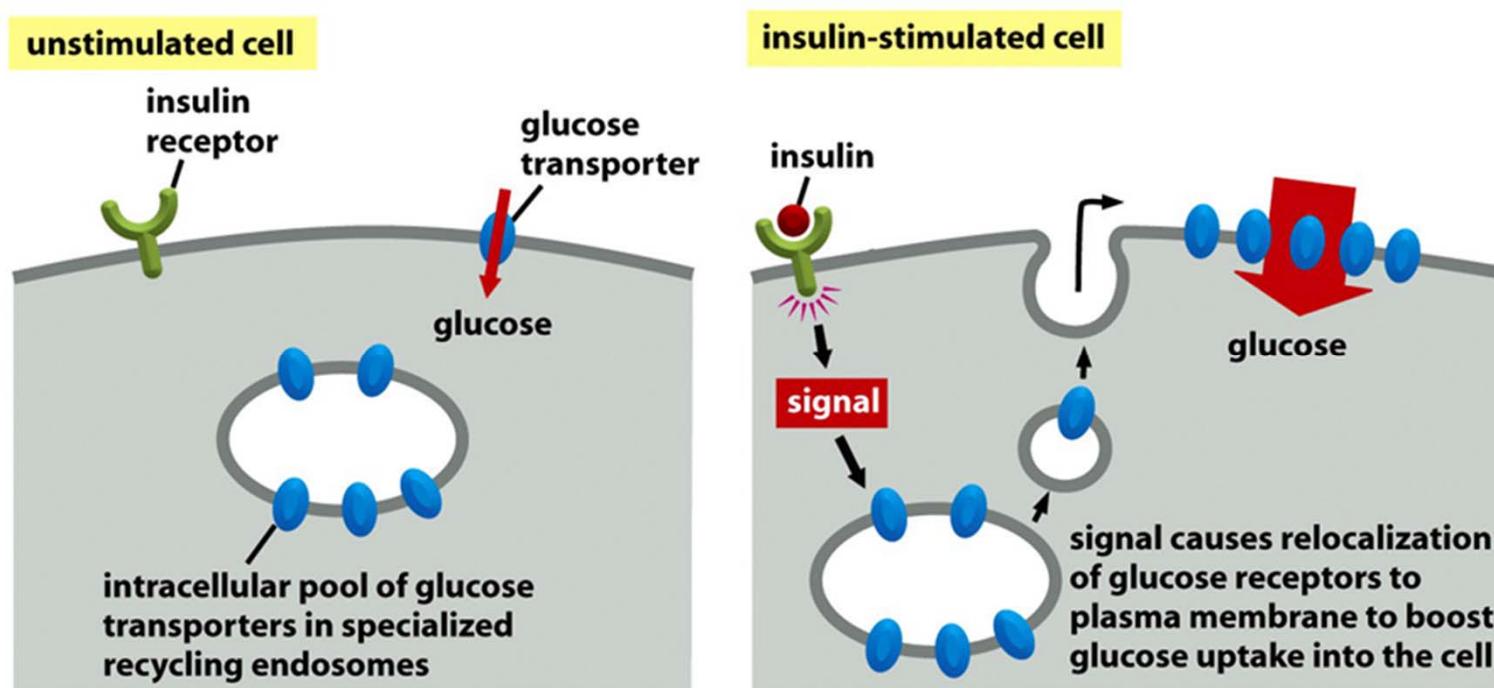
Different pH in two sides of cells decides association and dissociation between receptor and its ligand

Different sorting signals in receptors may play a role in transcytosis

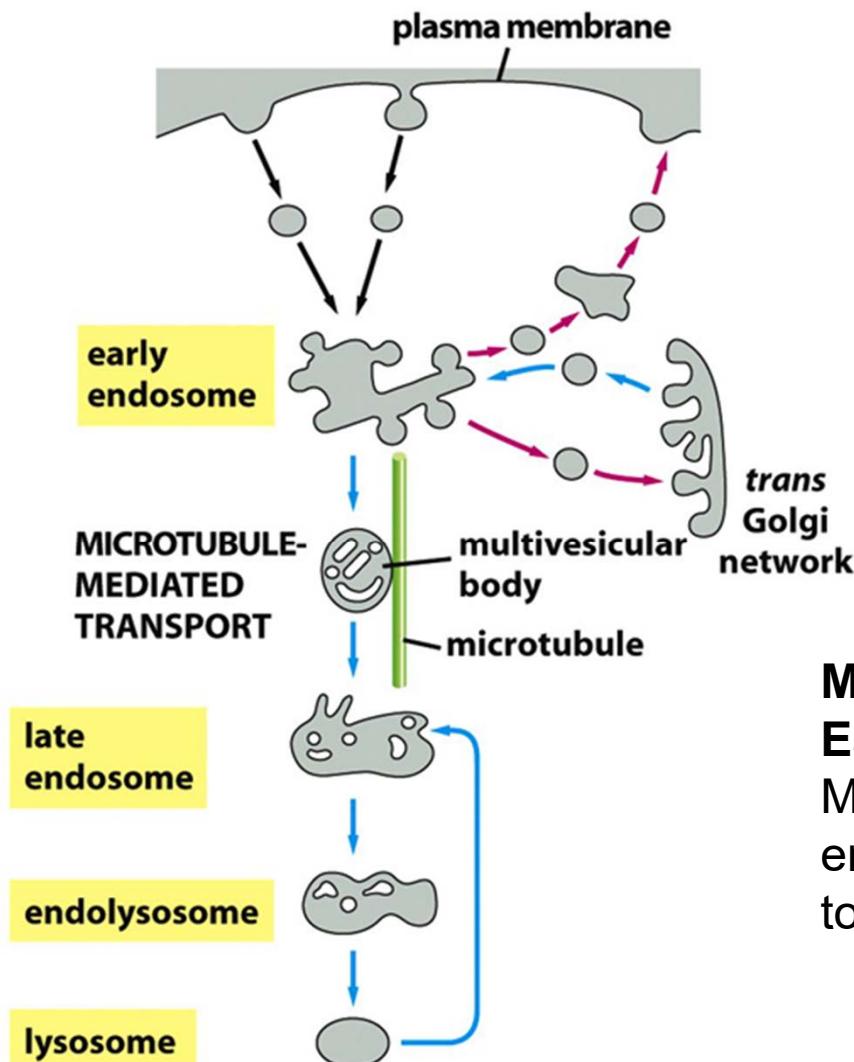
Storage of plasma membrane proteins in recycling endosomes

Recycling endosomes can serve as an intracellular storage site for specialized plasma membrane proteins that can be mobilized when needed

Example:

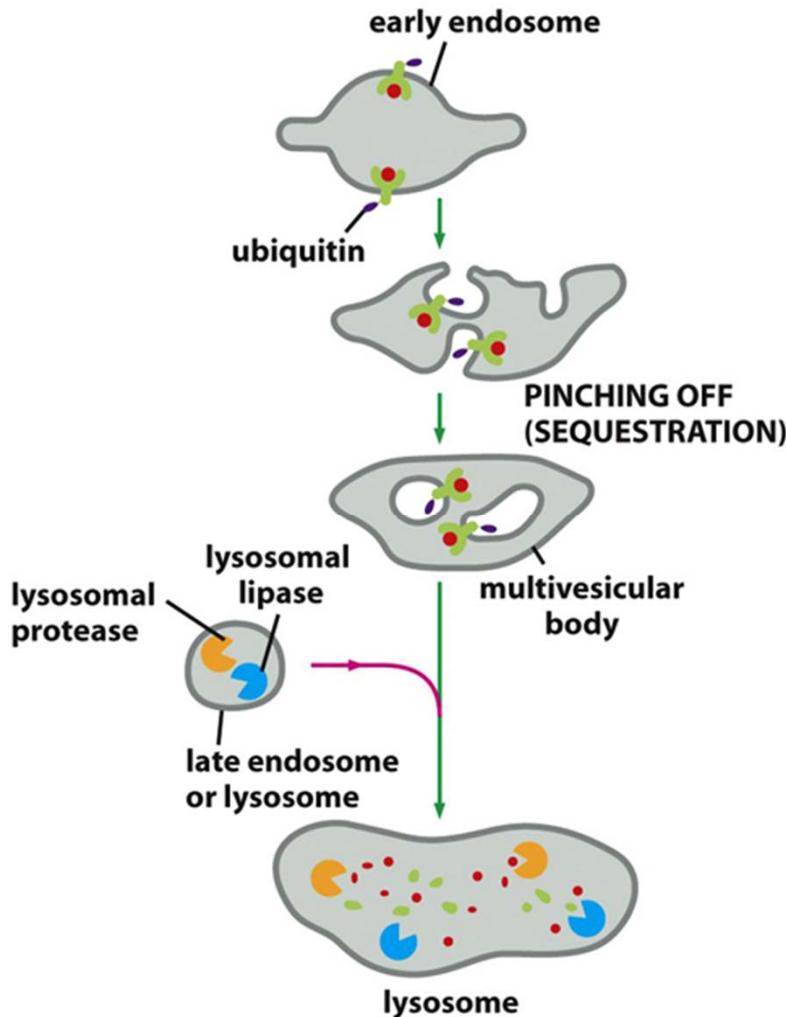


(3). Late endosome-lysosome pathway--- mediated by multivesicular bodies



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

Ubiquitination tags serve as recognition marks for formation of multivesicular body



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

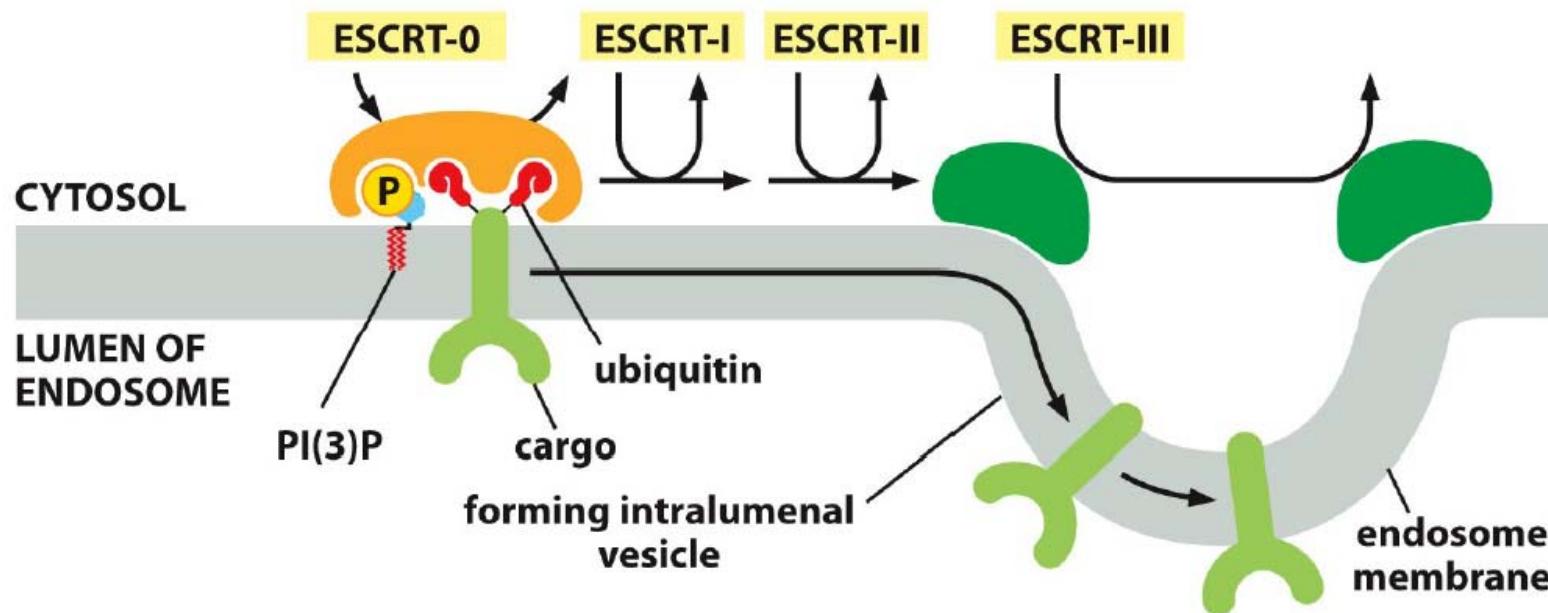


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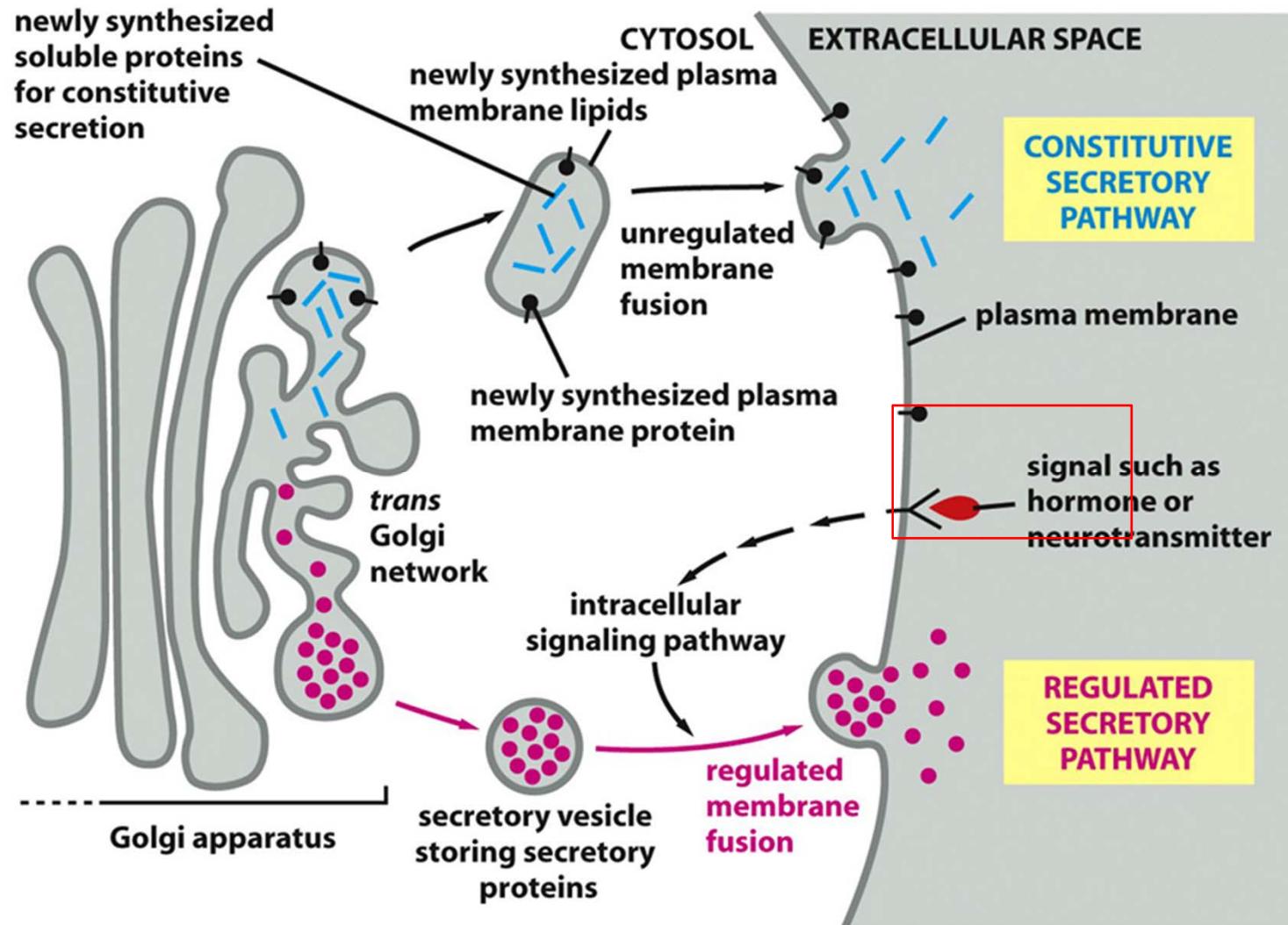
Mutations in ESCRT proteins are associated with prolonged signaling and cancer

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 secretion vs. regulated secretion



Constitutive and regulated secretory pathways

Constitutive pathway: all cells have it.

transport: membrane proteins
lipid molecules
ECM proteins

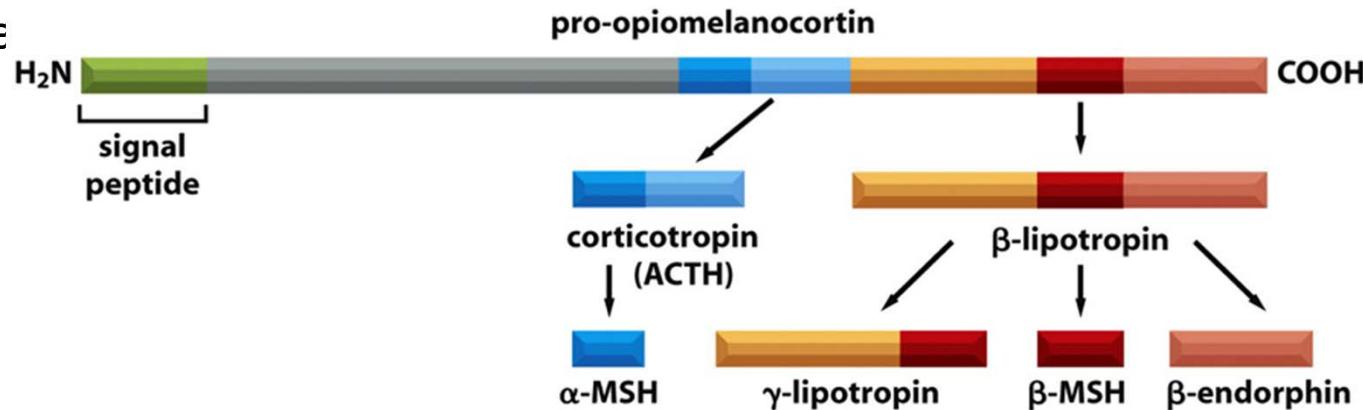
Regulated pathway: mainly in special secretory cells

transport:
hormones, histamine, etc
neurotransmitters
digestive enzymes

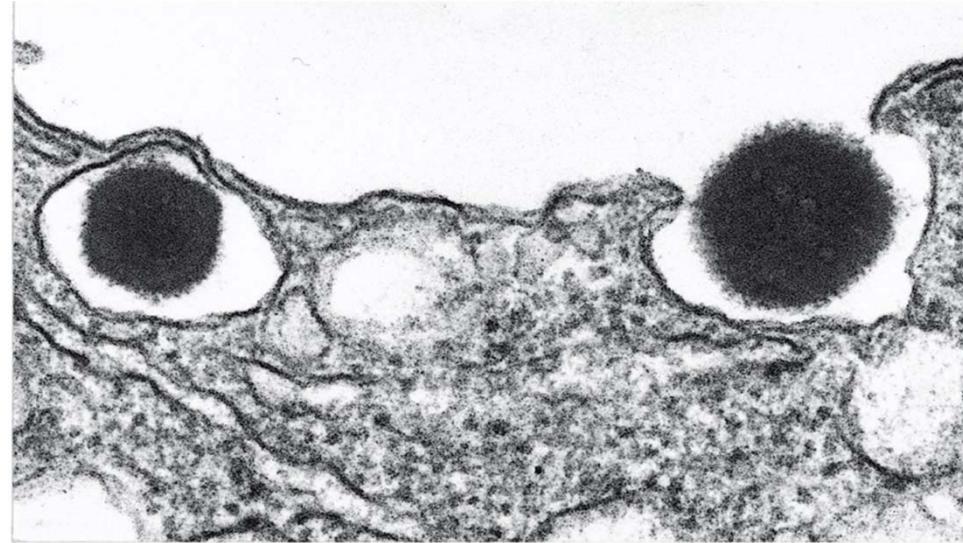
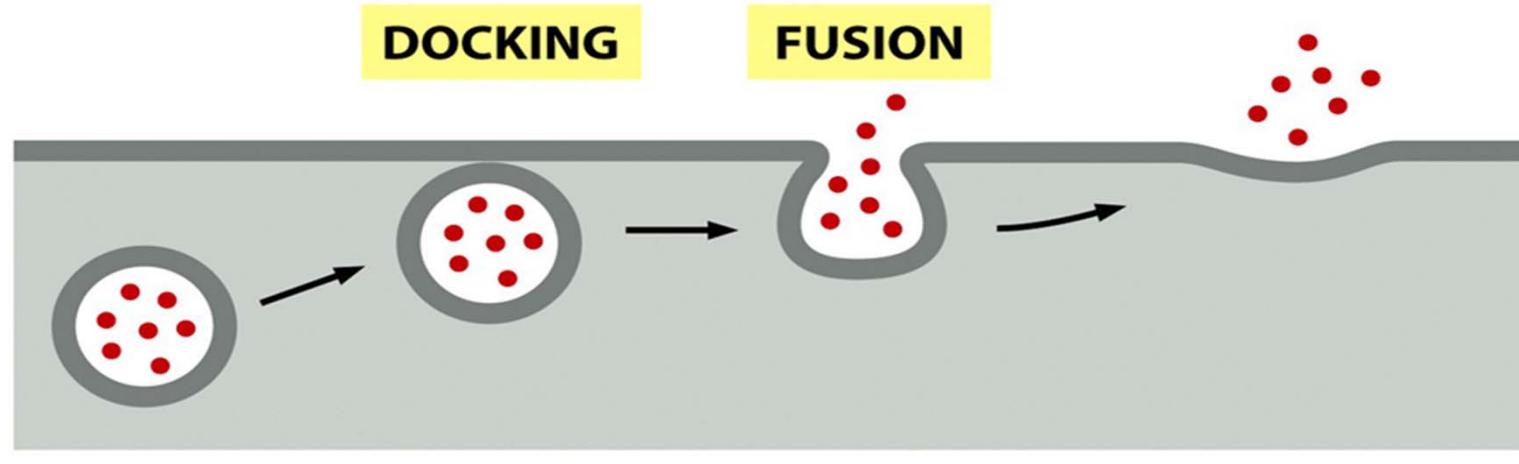
Constitutive and regulated secretory pathways

- Secretory particles aggregate in TGN lumen, occur selectively (due to signal patch?)
- are electron-dense
- Occur by two mechanisms, 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, continues in secretory vesicles and ECM

Example

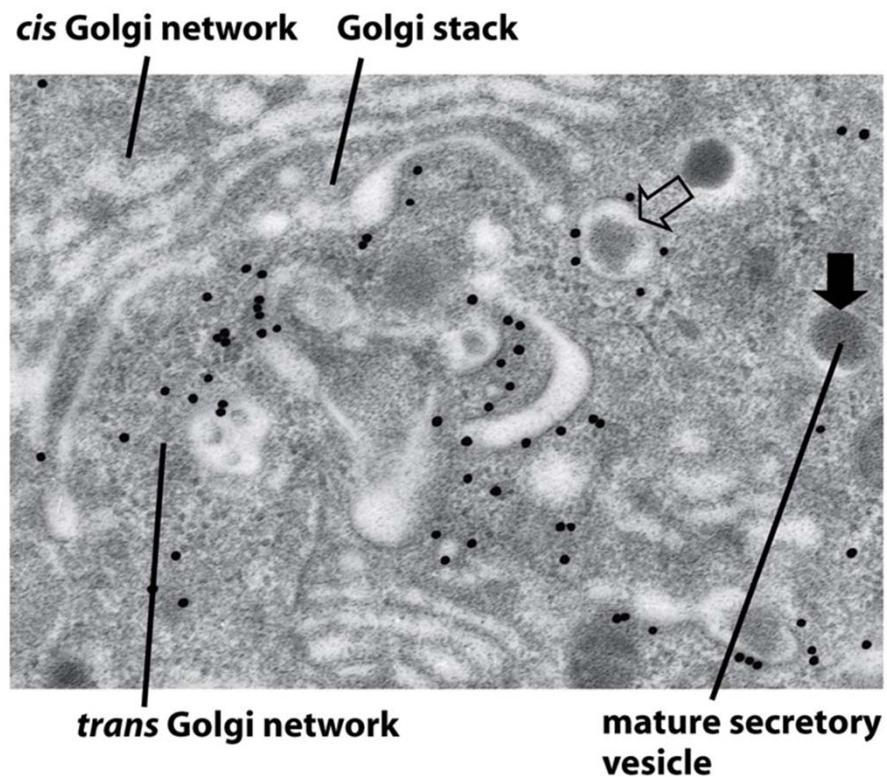
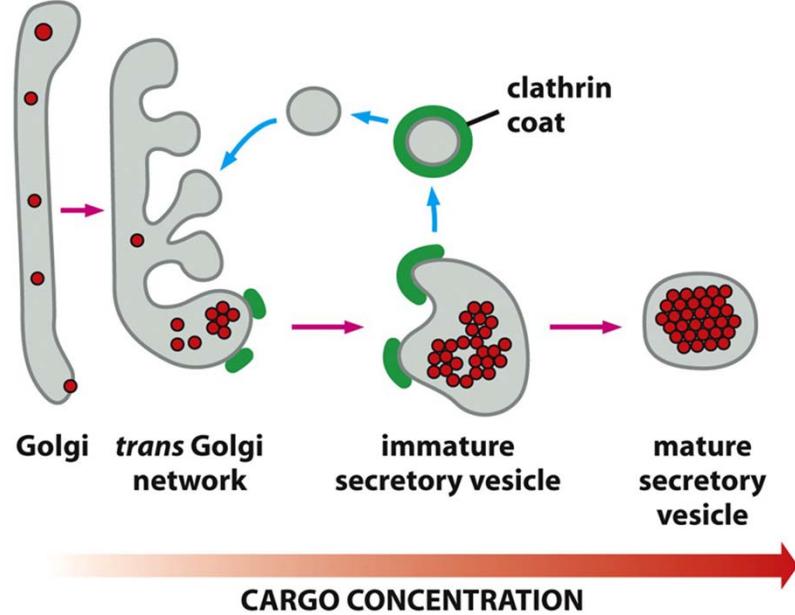


Exocytosis of secretory vesicles



0.2 μm

The formation of the secretory vesicles



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

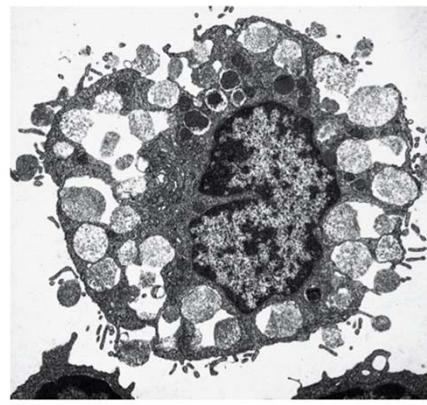
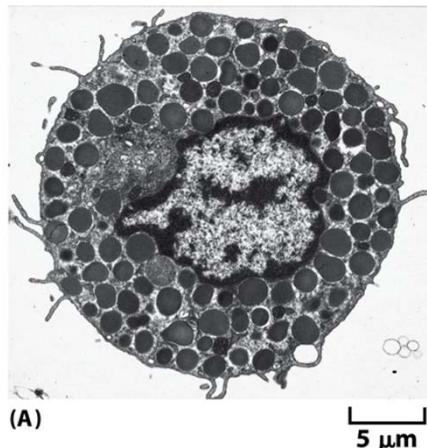
How to trigger secretory vesicle to release its content

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

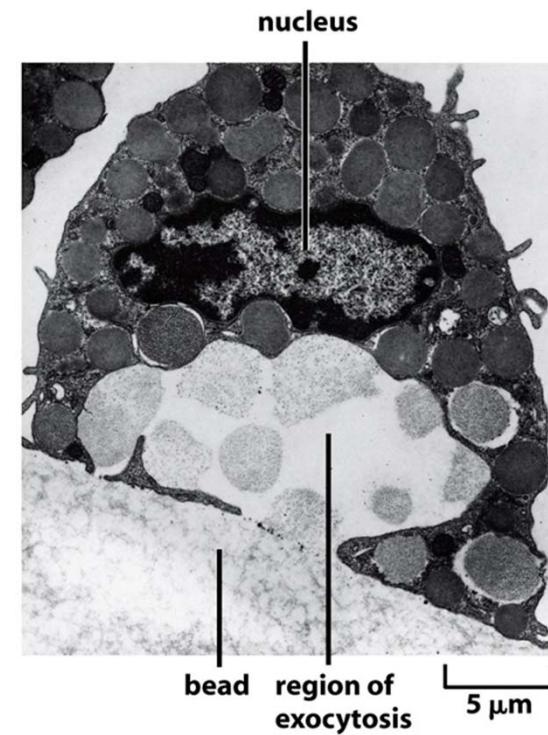
Example 2: ligand binding stimulate mast cells to release histamine

Can be local or all over in the cell

Soak mast cell in ligand-containing solution



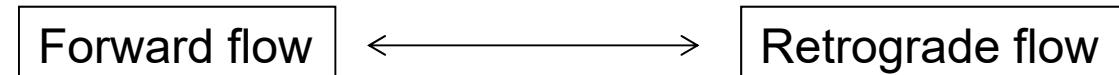
Ligand is fixed in a solid surface



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**

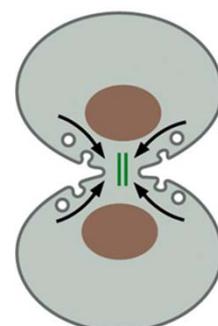


Balance --- no net cell grow

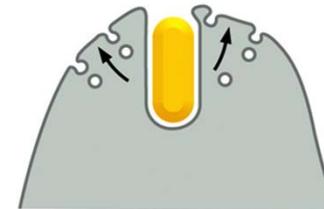
Forward flow dominates --- cell rapidly grows

Retrograde flow dominates --- cell shrinks

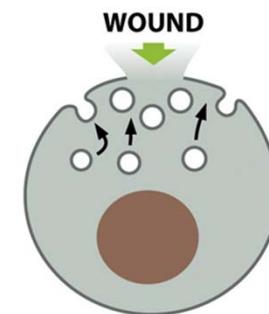
Examples to expand membrane:



(A) CLEAVAGE FURROW



(B) PHAGOCYTOSIS



(C) WOUND REPAIR