Cell Biology
Lecture 5
Part I

Membrane structure

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8.11.2023

Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 13
Intracellular Membrane Traffic

Pages: 697-752

Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview,* DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

Learning outcomes

- Can describe and analyze the molecular mechanisms of membrane transport, including:
  - Transport from the endoplasmic reticulum through the Golgi apparatus
  - Transport from the trans Golgi network to the cell exterior and endosomes
  - Transport into the cell from the plasma membrane
  - The degradation and recycling of macromolecules in lysosomes

MOVING BETWEEN COMPARTMENTS

GATED TRANSPORT

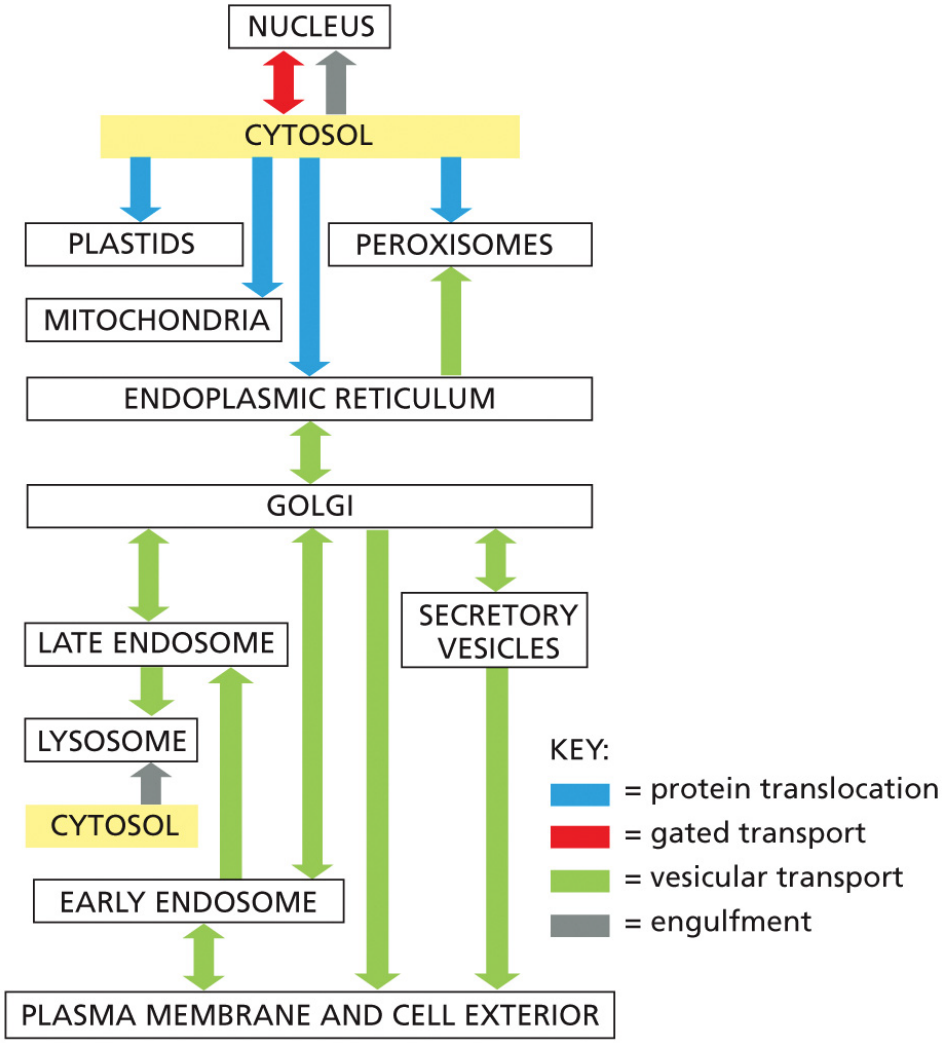
- Between cytosol and nucleus
- Nuclear pore complexes = selective gates

PROTEIN TRANSLOCATION

- From cytosol to topologically distinct compartments
- By transmembrane protein translocators
- Transported proteins typically unfolded

VESICULAR TRANSPORT

- Between topologically similar compartments
- By vesicles

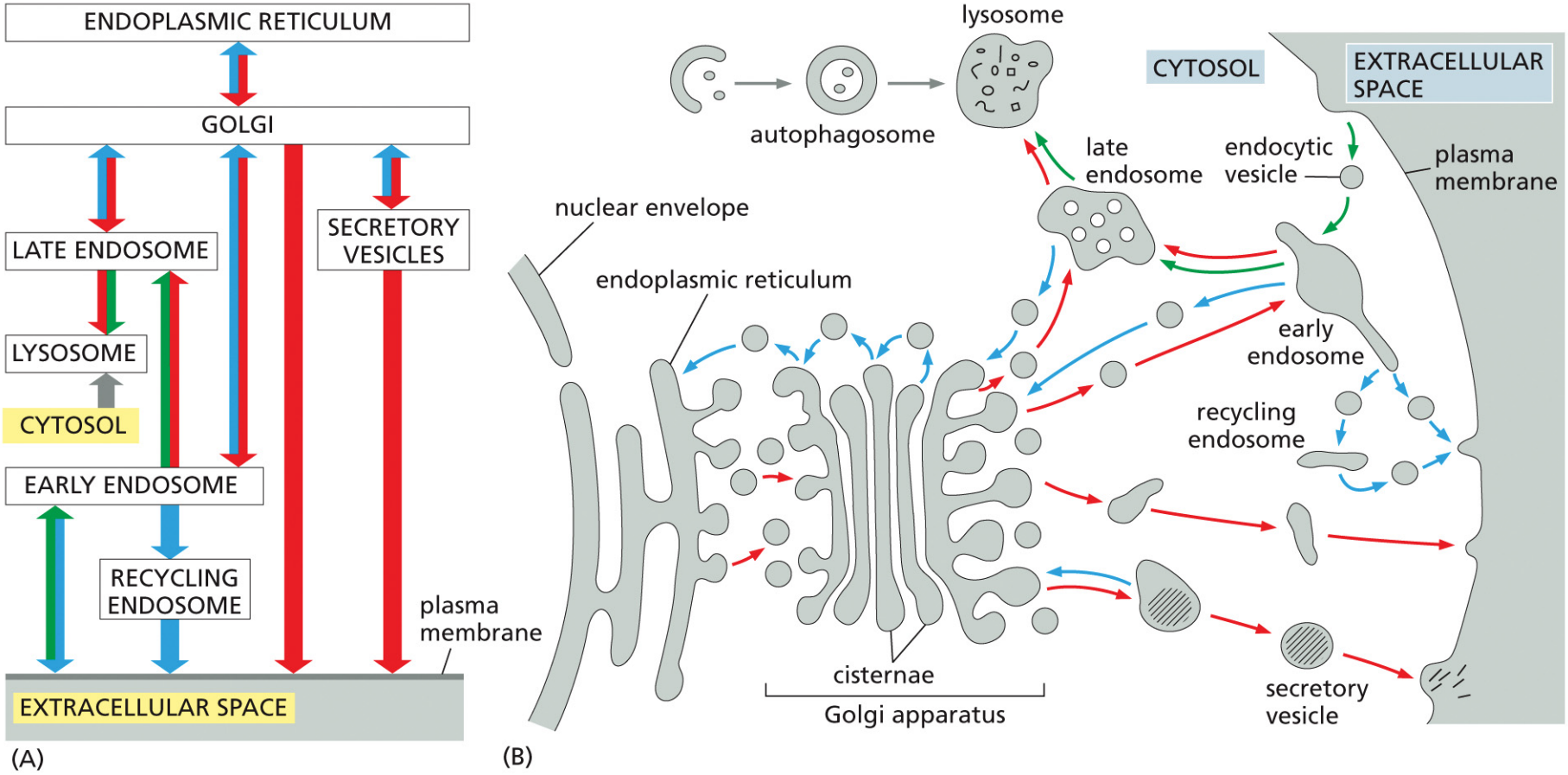


Note, these are all *compartments with membranes*!

endocytic and secretory pathways
+ retrieval pathways

***Selective* budding and fusion:**

- Selective molecules taken up
- At controlled time
- Brought into selected destinations



endocytic and secretory pathways
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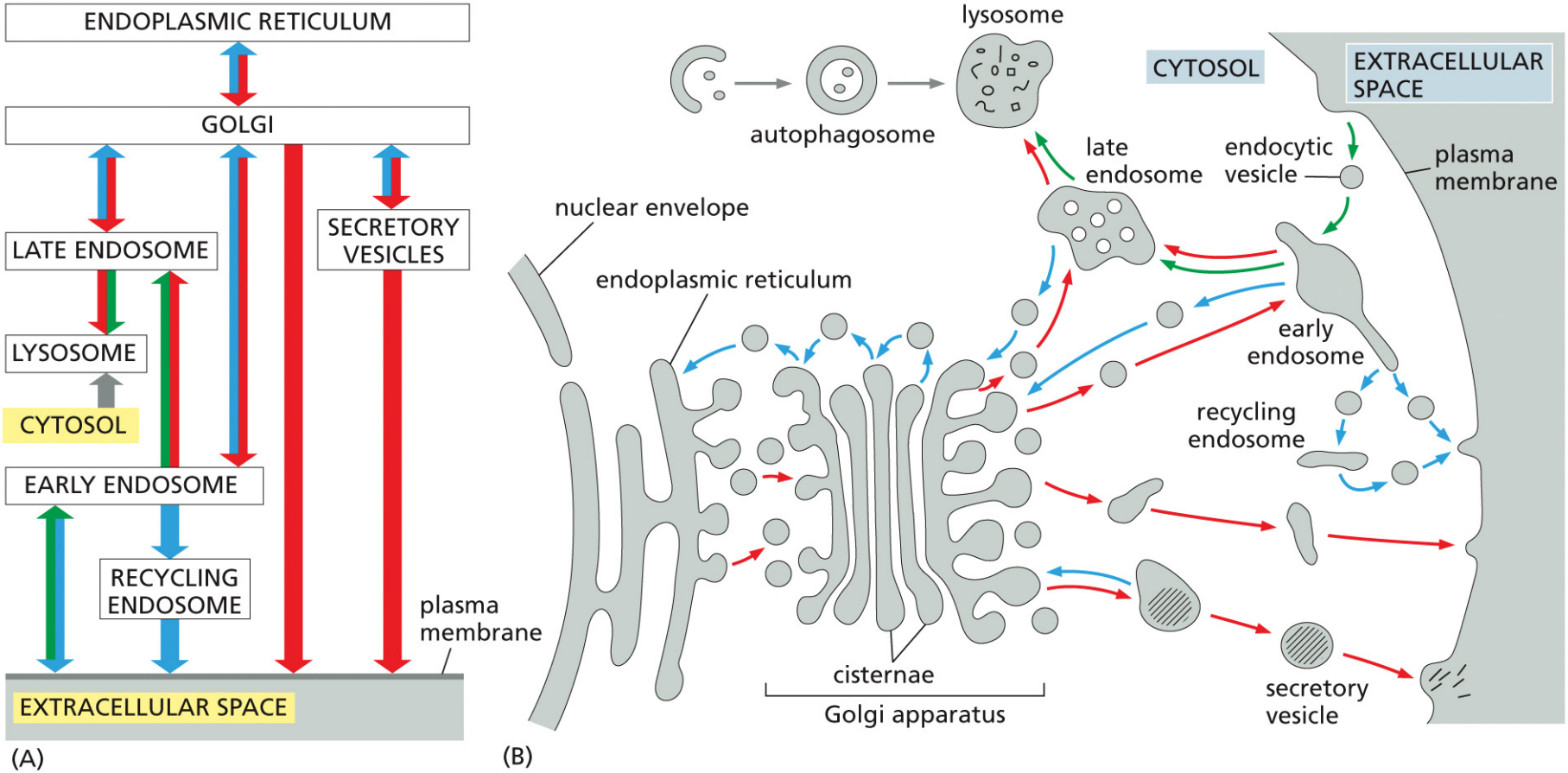
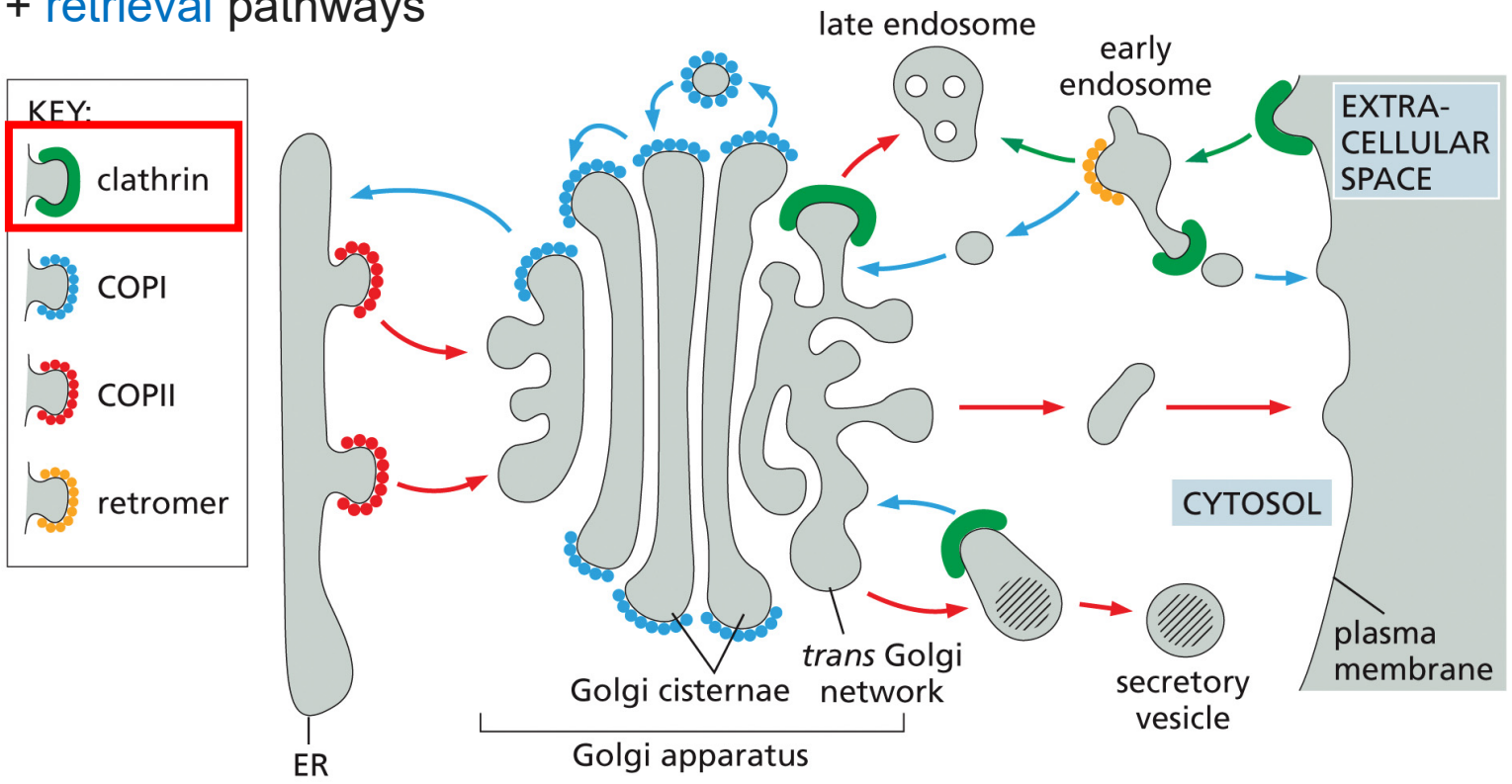


Identity of compartments defined by the composition of the membrane.

DIFFERENT COATS FOR DIFFERENT STEPS IN VESICLE TRAFFIC

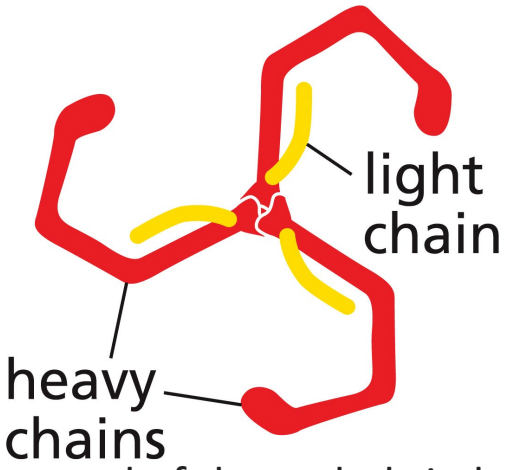
endocytic and secretory pathways
+ retrieval pathways



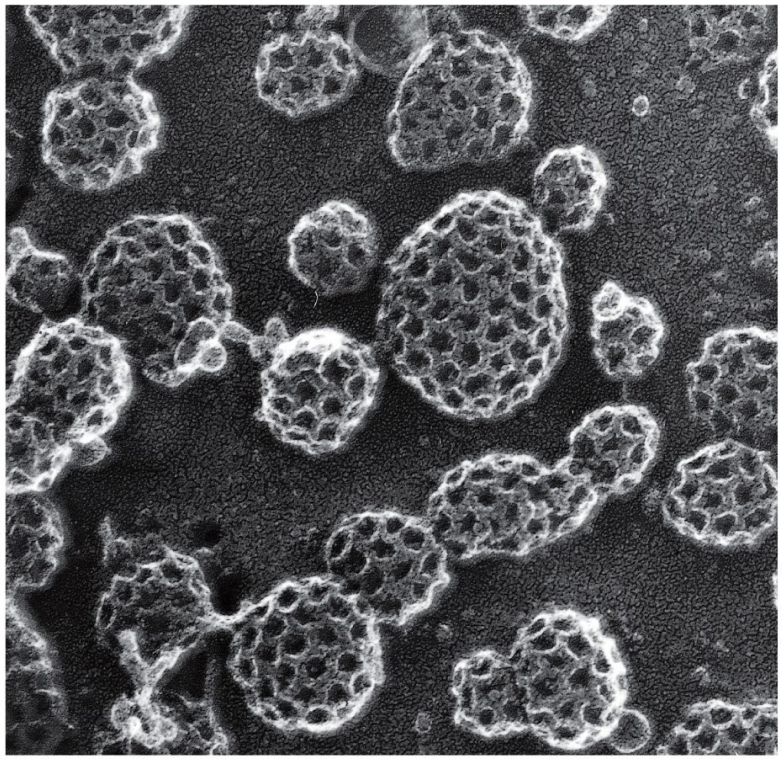
- Coat proteins recruit (and select) cargo + shape the transport vesicles
- Same coats in different places in the cell can incorporate different coat protein subunits that modify their properties
- Also other pathways exist

CLATHRIN COAT

- The assembly of a clathrin coat drives vesicle formation
- Form outer layer of the coat
- Associated with adaptor proteins



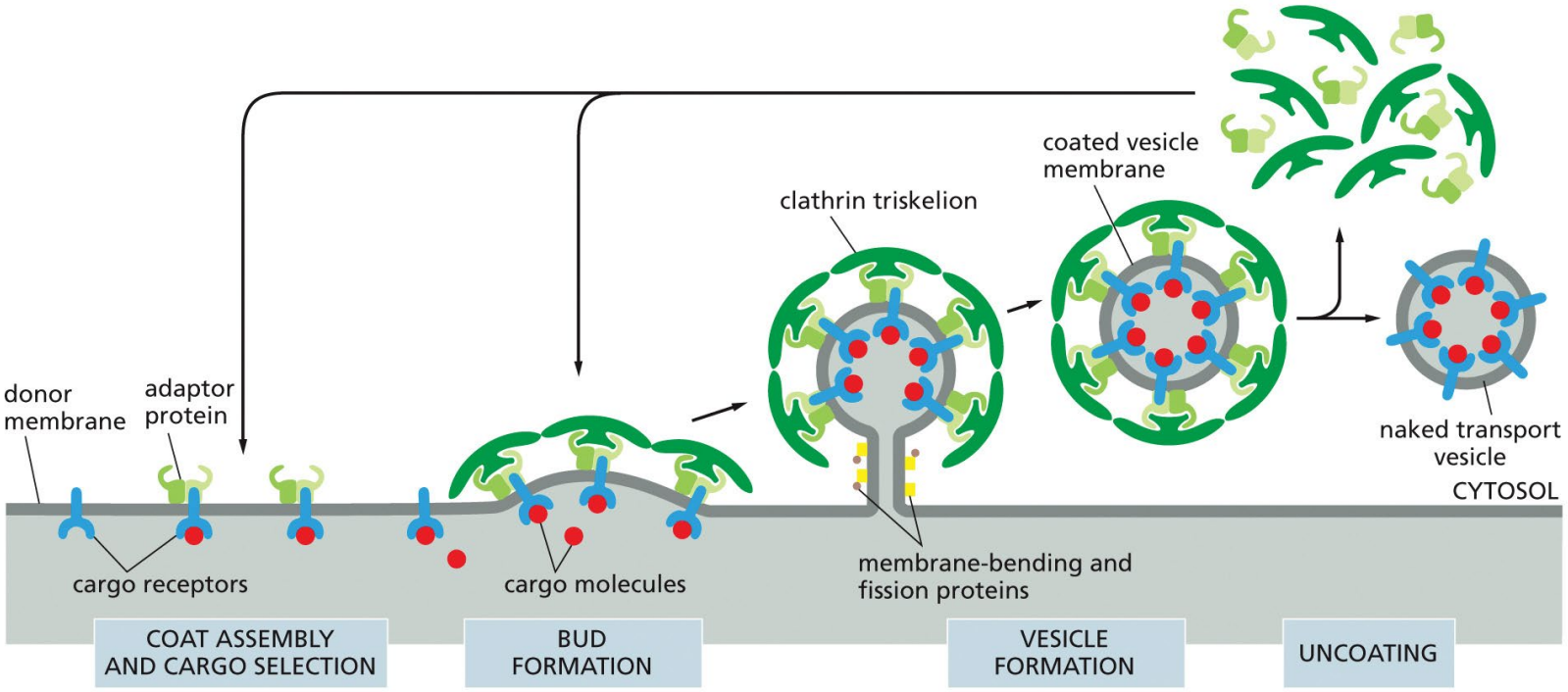
composed of three clathrin heavy chains and three clathrin light chains



Courtesy of John Heuser

ADAPTOR PROTEINS IN VEHICLES

- The **adaptor proteins** bind both **clathrin triskelions** and membrane-bound **cargo receptors**
- Mediates the *selective recruitment* of both *membrane* and *soluble cargo* molecules into the vesicle

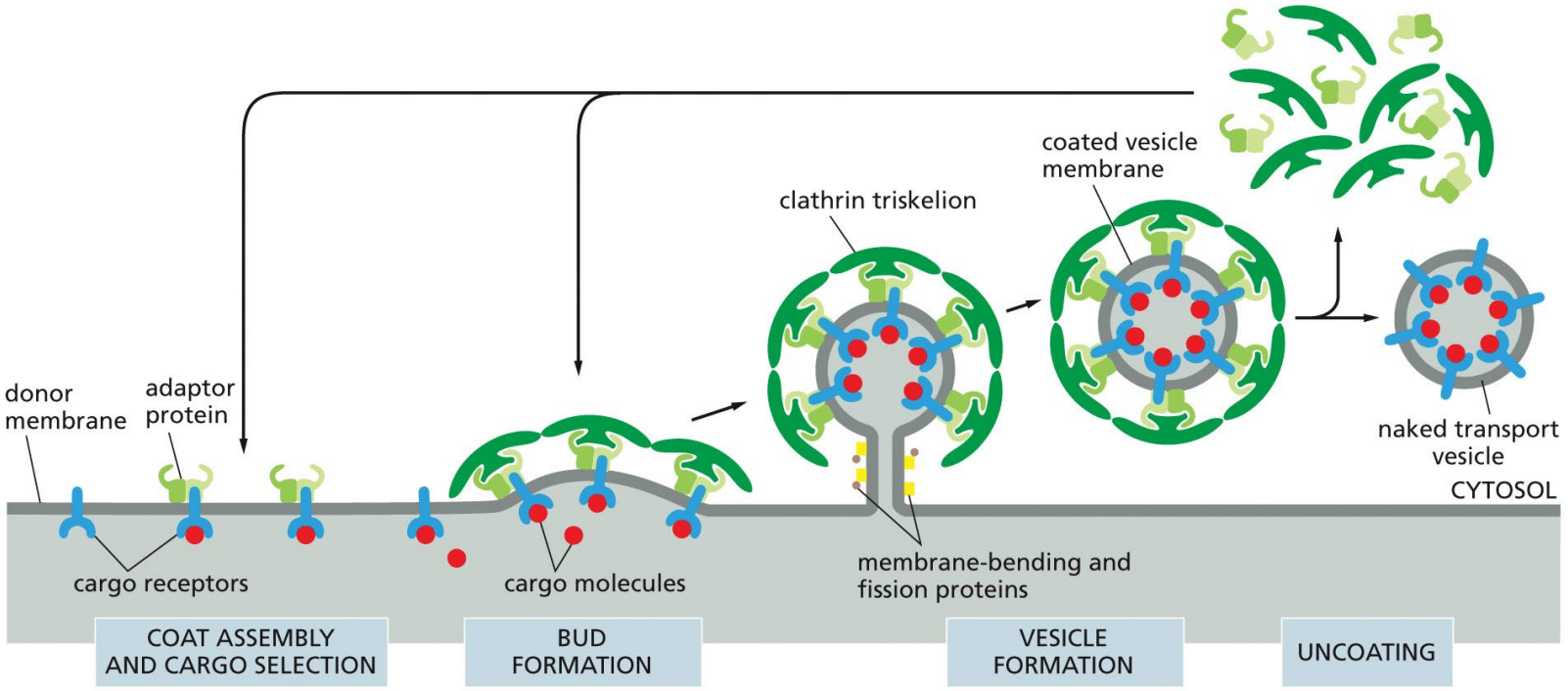


ADAPTOR PROTEINS IN VEHICLES

- The assembly of the coat introduces curvature into the membrane

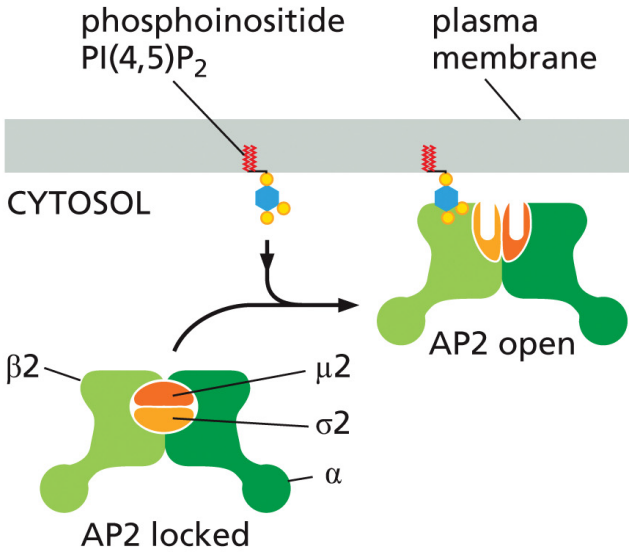
-> formation of a coated bud (called a coated pit if it is in the plasma membrane)

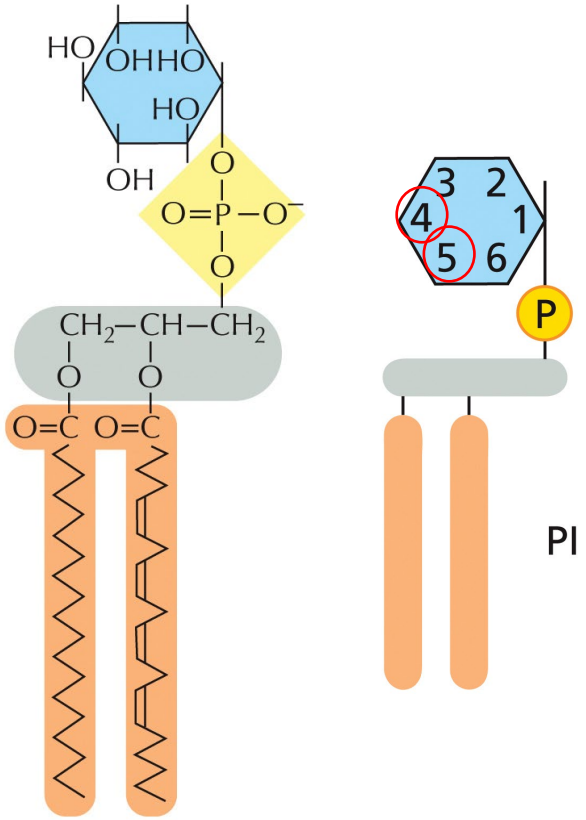
- Other *membrane-bending and fission proteins* are recruited to the *neck of the budding vesicle*, where sharp membrane curvature is introduced
- The coat is rapidly lost shortly after the vesicle buds off



AP2 AS AN EXAMPLE OF AN ADAPTOR PROTEIN

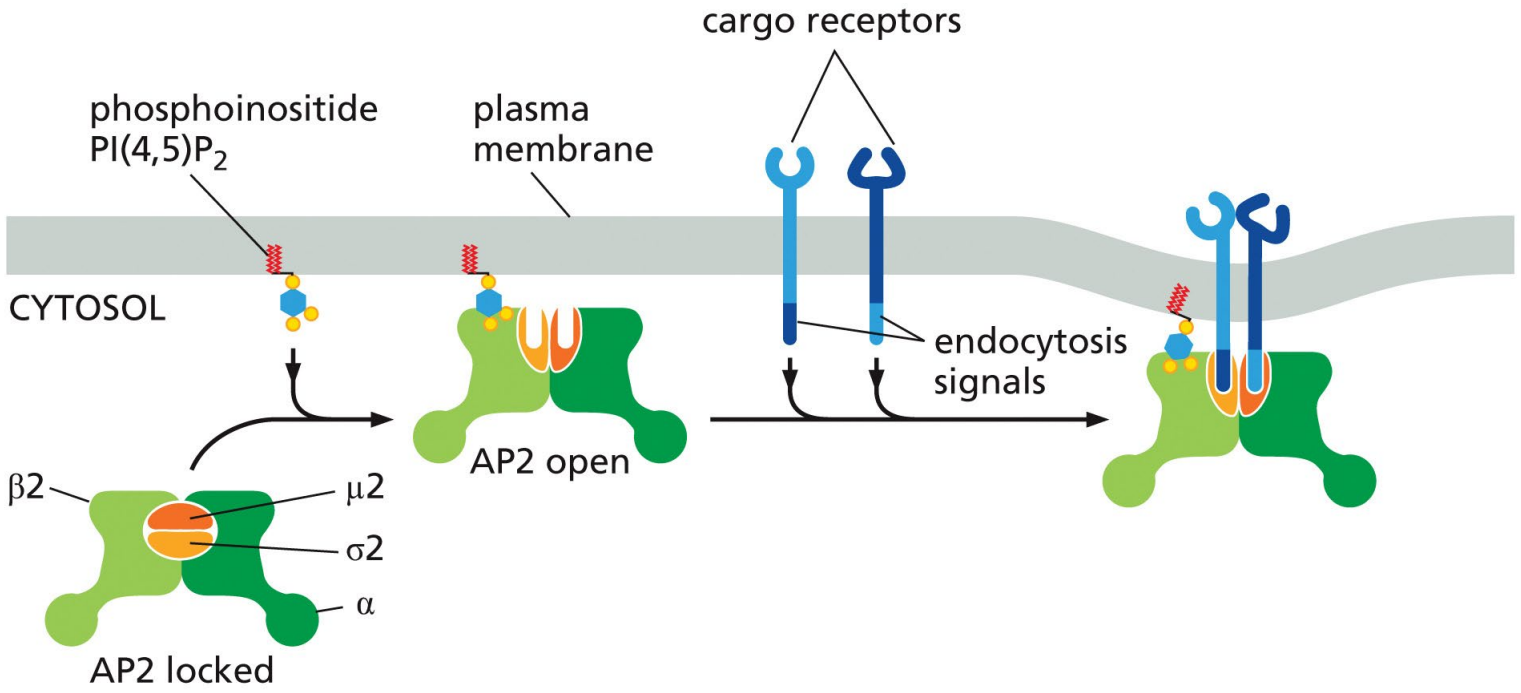
- Four subunits (α, β2, μ2, and σ2)
- Interaction with the **phosphoinositide PI(4,5)P_2** in the cytosolic leaflet of the plasma membrane → rearranges to expose binding sites for cargo = **Lipid-induced conformation switch**





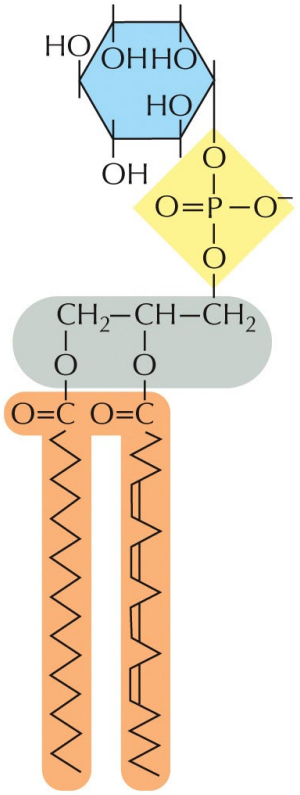
AP2 AS AN EXAMPLE OF AN ADAPTOR PROTEIN

- Each AP2 complex binds four $PI(4,5)P_2$ molecules
- μ2 and σ2 subunits bind the cytosolic tails of **cargo receptors** that display the appropriate endocytosis signals (short amino acid sequence motifs)
- Binds tightly to the membrane → induces *curvature* → favors the *binding of additional AP2 complexes* in the vicinity.



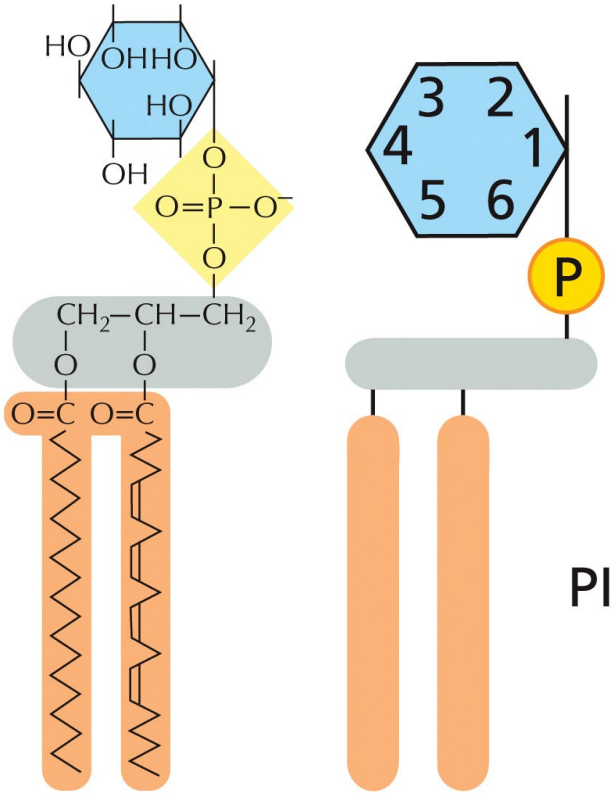
PHOSPHATIDYLINOSITOL (PI) AND PHOSPHOINOSITIDES (PHOSPHATIDYLINOSITOL PHOSPHATES, OR PIPS)

- **Phosphoinositides** mark organelles and membrane domains
- Also role in signalling

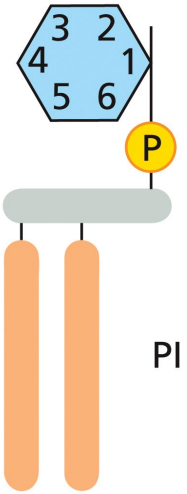


PHOSPHATIDYLINOSITOL (PI) AND **PHOSPHOINOSITIDES** (PHOSPHATIDYLINOSITOL PHOSPHATES, OR PIPS)

- **Phosphoinositides** mark organelles and membrane domains
- Also role in signalling
- Can be reversibly modified by **phosphorylation**

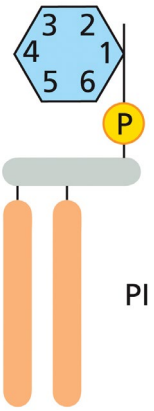


- **Free hydroxyl groups in the inositol sugar can be modified**
- **Phosphorylation** of *one, two, or three* of the hydroxyl groups on PI by PI and PIP kinases produces a variety of PIP species
- Named according to the ring position (in parentheses) and the number of phosphate groups (subscript) added to PI

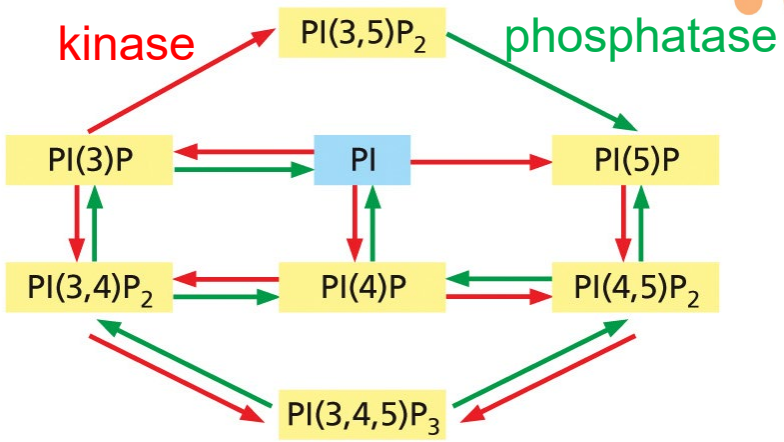


Phosphatidylinositol 3,4-bisphosphate [PI(3,4)P_2]

PHOSPHATIDYLINOSITOL (PI) AND **PHOSPHOINOSITIDES** (PHOSPHATIDYLINOSITOL PHOSPHATES, OR PIPS)

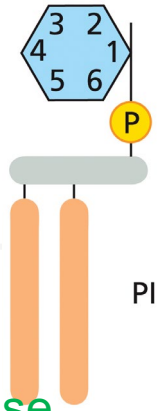


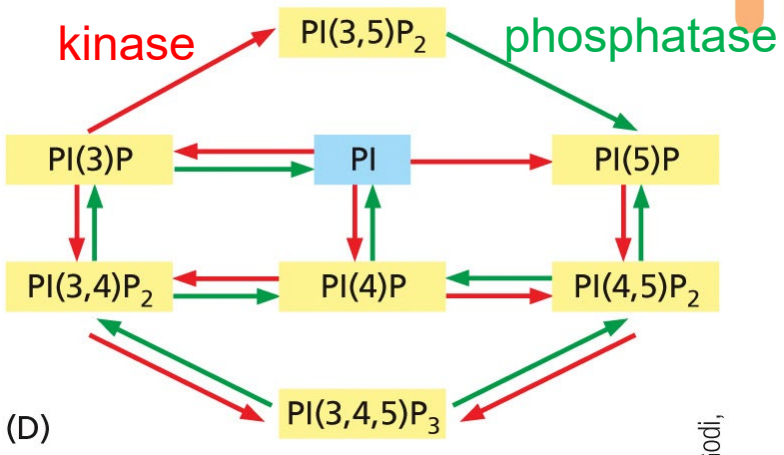
- **PI** and **PIP kinases** & **PIP phosphatases** *localized to different organelles*



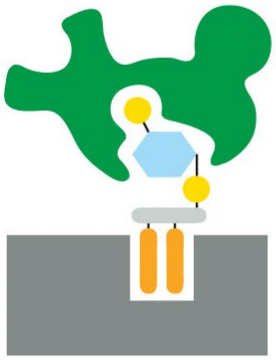
PHOSPHATIDYLINOSITOL (PI) AND **PHOSPHOINOSITIDES** (PHOSPHATIDYLINOSITOL PHOSPHATES, OR PIPS)

- PI and PIP kinases & PIP phosphatases localized to different organelles
- **Phosphoinositide head groups recognized by protein domains**
- Groups of proteins containing such domains are recruited to regions of membrane in which these phosphoinositides are present

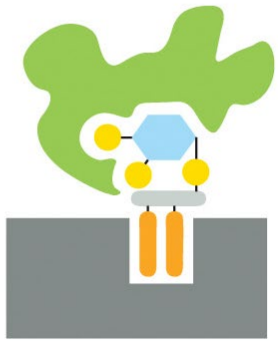




D, modified from M.A. De Matteis and A. Godi, *Nat. Cell Biol.* 6:487–492, 2004



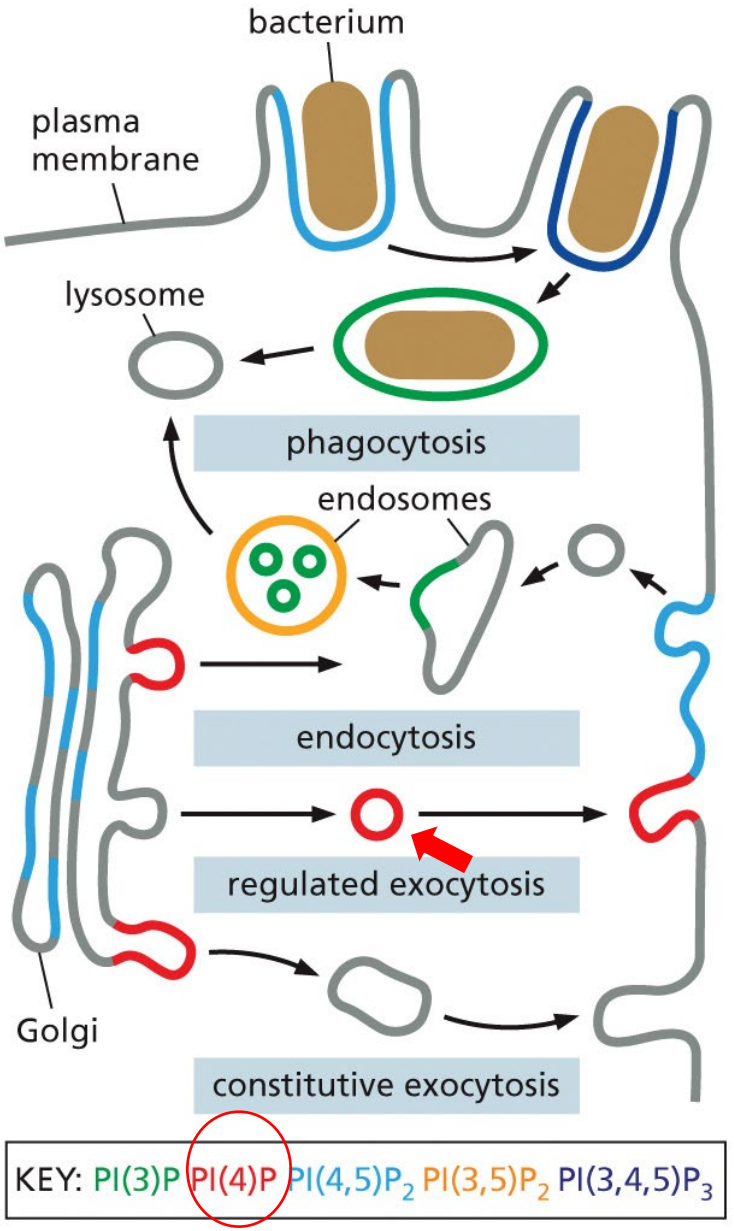
Phosphatidylinositol 3-phosphate [PI(3)P] in the endosome membrane



phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] in the plasma membrane

THE INTRACELLULAR LOCATION OF PHOSPHOINOSITIDES

- **Different types of PIPs are located in different membranes and membrane domains**
- Often associated with specific vesicle transport events.
- For example, the membrane of secretory vesicles contains PI(4)P



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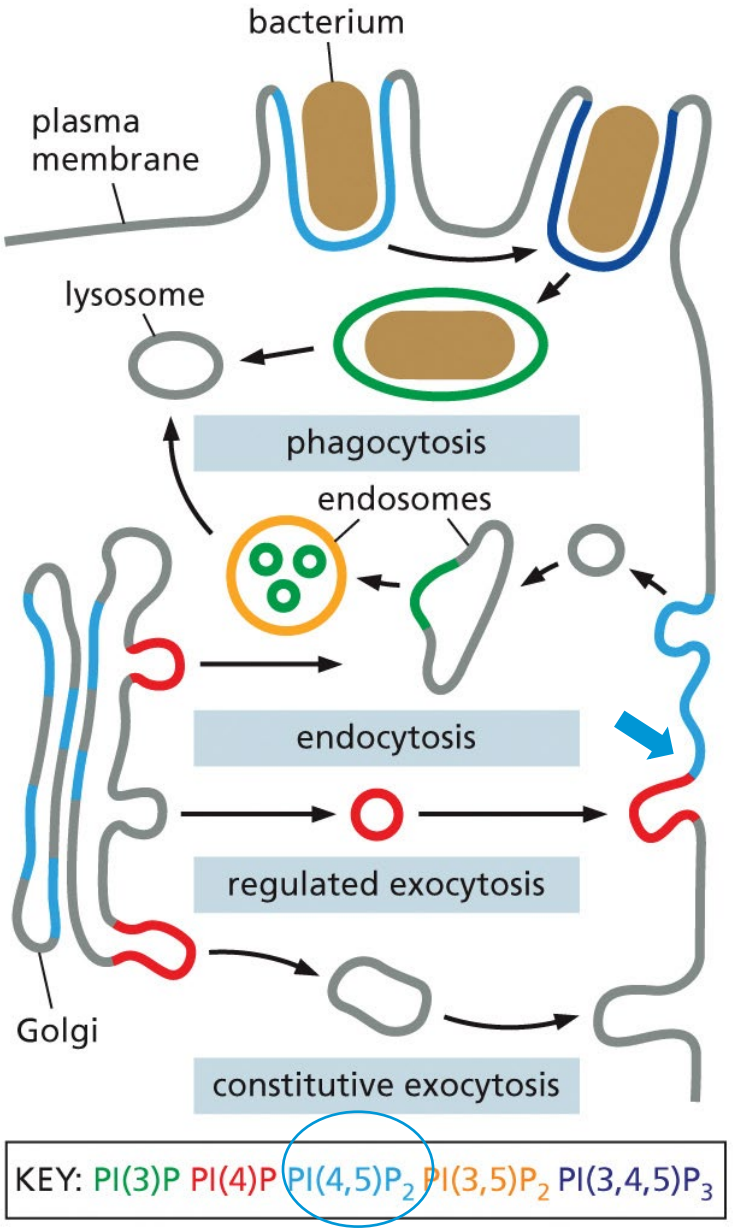
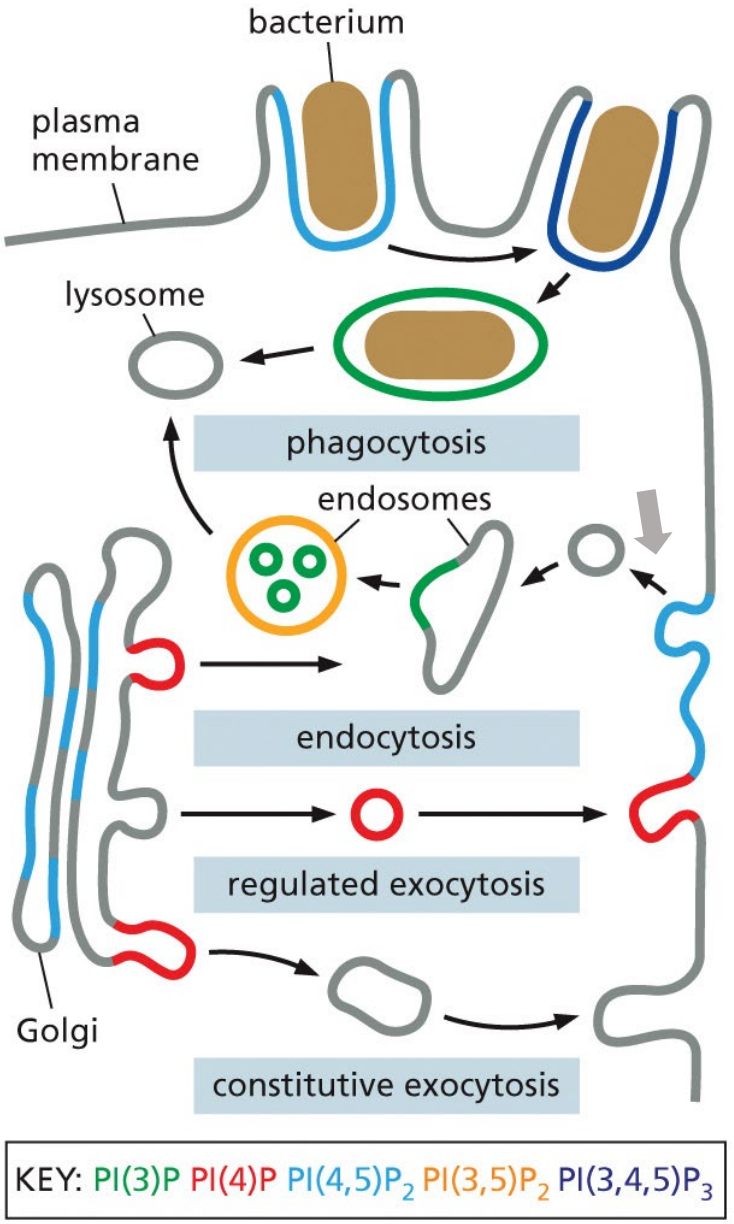
THE INTRACELLULAR LOCATION OF PHOSPHOINOSITIDES

- When the vesicles fuse with the plasma membrane, a phosphoinositide 5-kinase (PI 5-kinase) that is localized there converts the PI(4)P into PI(4,5)P_2



THE INTRACELLULAR LOCATION OF PHOSPHOINOSITIDES

- When the vesicles fuse with the plasma membrane, a phosphoinositide 5-kinase (PI 5-kinase) that is localized there converts the PI(4)P into $PI(4,5)P_2$
- $PI(4,5)P_2$ helps recruit adaptor proteins, → initiate the formation of a clathrin-coated pit, 1st step in clathrin-mediated endocytosis
- Clathrin-coated vesicle buds off from the plasma membrane, a PI(5)P phosphatase hydrolyzes $PI(4,5)P_2$, which weakens the binding of the adaptor proteins, promoting vesicle uncoating.



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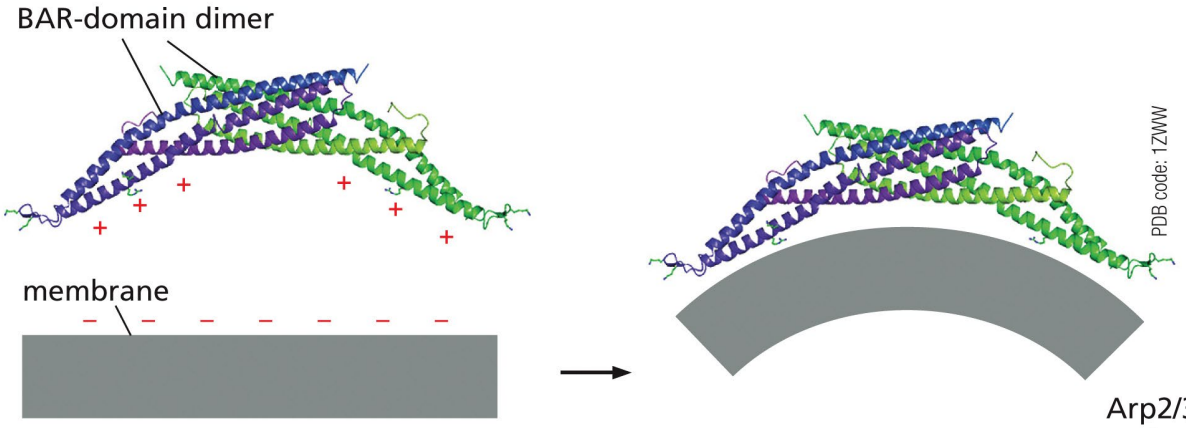
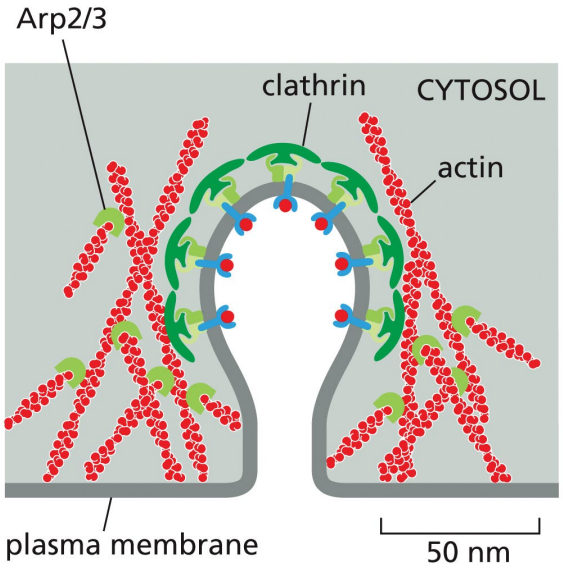
MEMBRANE-BENDING PROTEINS HELP DEFORM THE MEMBRANE DURING VESICLE FORMATION

- Coiled coils that dimerize into modules that have a positively charged inner surface that interacts with negatively charged lipid head groups



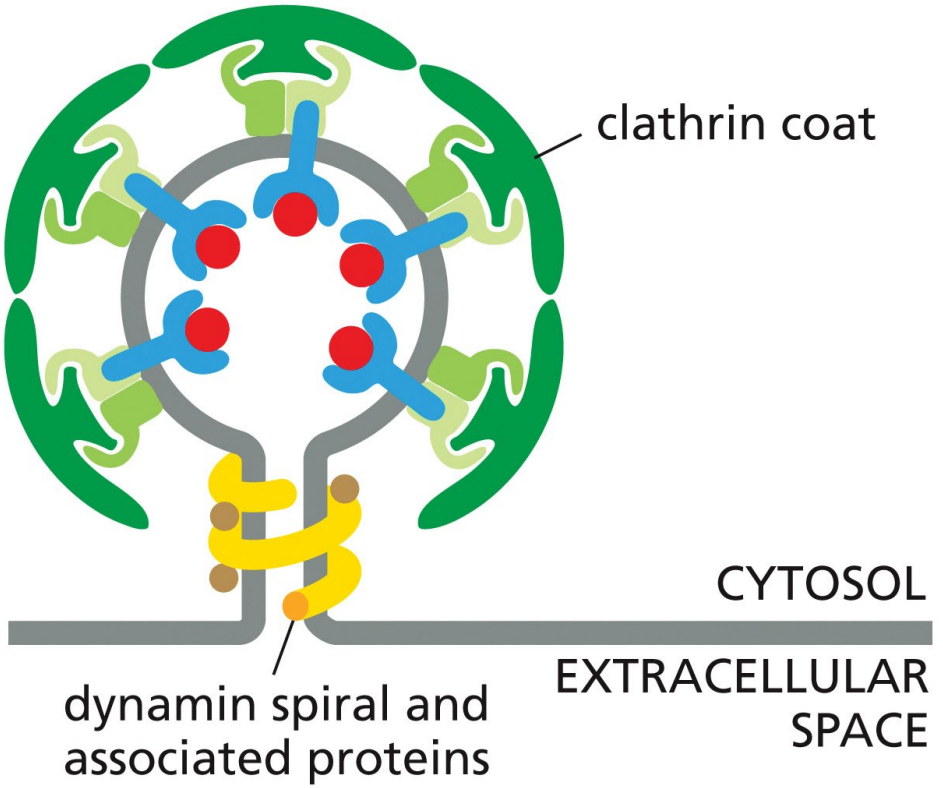
- **Local actin polymerization helps drive budding of membrane vesicles**
- Polymerization of actin filaments occurs near the vesicle neck, helping propel the budding vesicle away from the plasma membrane



CYTOPLASMIC PROTEINS REGULATE THE PINCHING OFF AND UNCOATING OF COATED VESICLES

- **Dynamin** molecules assemble into a spiral around the neck of the forming bud.
- Recruits other proteins to the bud neck, together destabilize the interacting lipid bilayers so that the **noncytoplasmic leaflets flow together**

- Conformational changes in the GTPase domains of membrane-assembled dynamin power a conformational change that constricts the neck of the bud

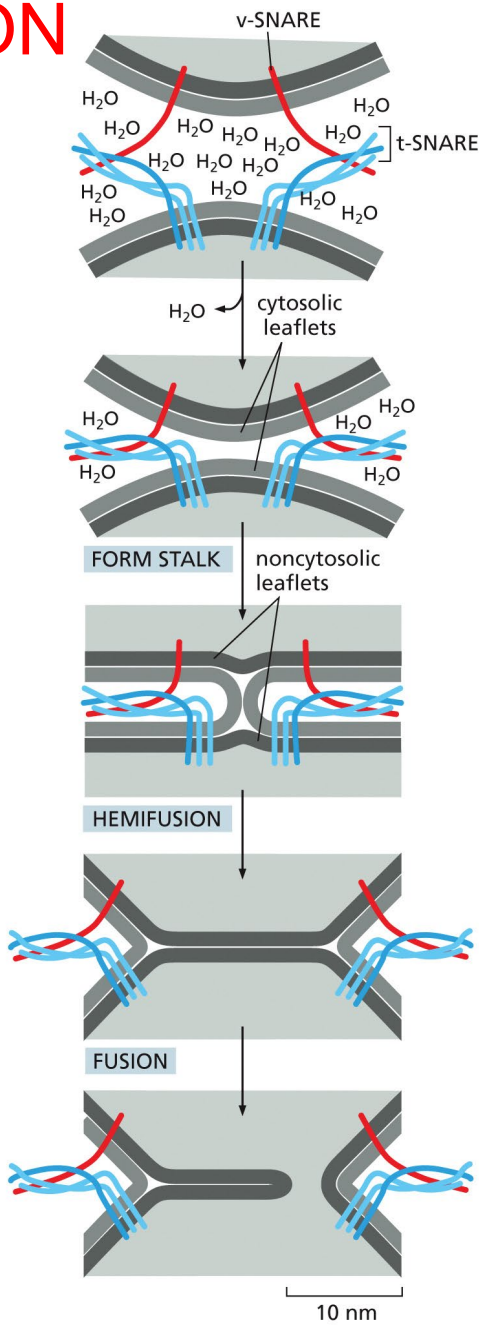


TARGETTING VESICLES TO CORRECT MEMBRANES

1. **Clathrin, COPI, and COPII** are different coat proteins
2. **PIPs and GTPases mediate & control budding**
3. **Adaptor proteins** recruit cargo
4. **SNARE** proteins and SNARE regulators mediate fusion
5. **Rab** proteins and Rab effectors direct to correct site

SNARES MEDIATE MEMBRANE FUSION

- Energy to replace water from membrane surface to enable fusion from SNARE assembly
- **vSNAREs** in vesicles and **t-SNAREs** (3 helix bundles) in target membrane
- Form a 4 helix bundle, highly **specific**



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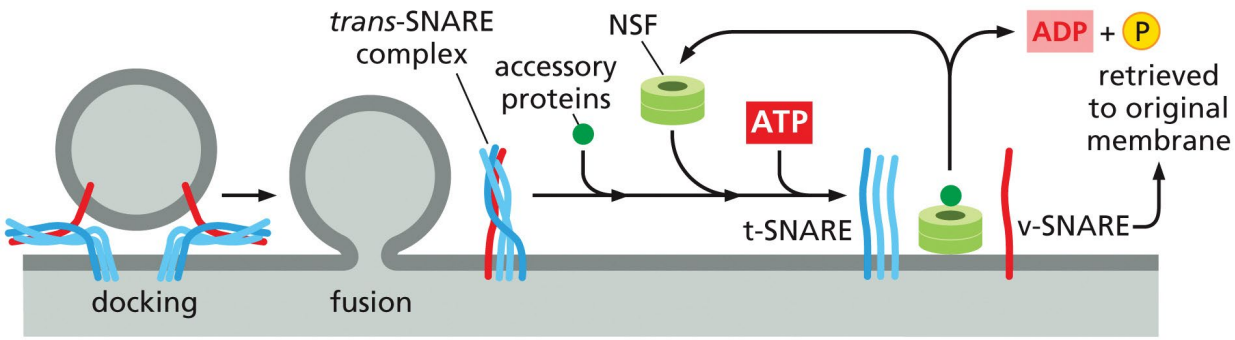
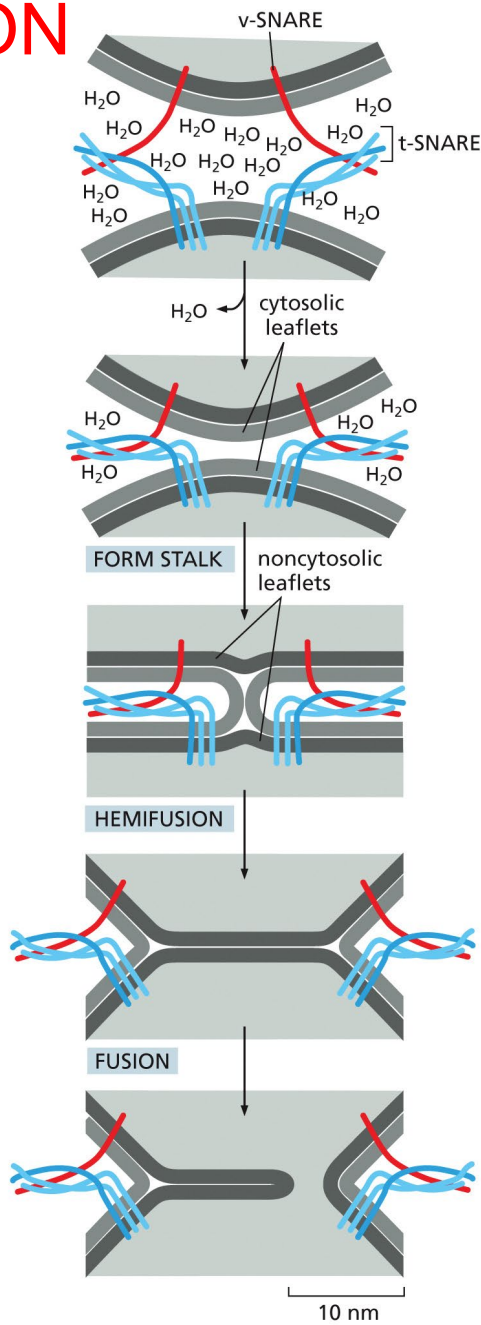
- vSNAREs in vesicles and t-SNAREs (3 helix bundles) in target membrane
- Form a 4 helix bundle, highly **specific**
- NSF ATPase dissembles SNARE complex





RAB PROTEINS GUIDE TRANSPORT VESICLES TO THEIR TARGET MEMBRANE

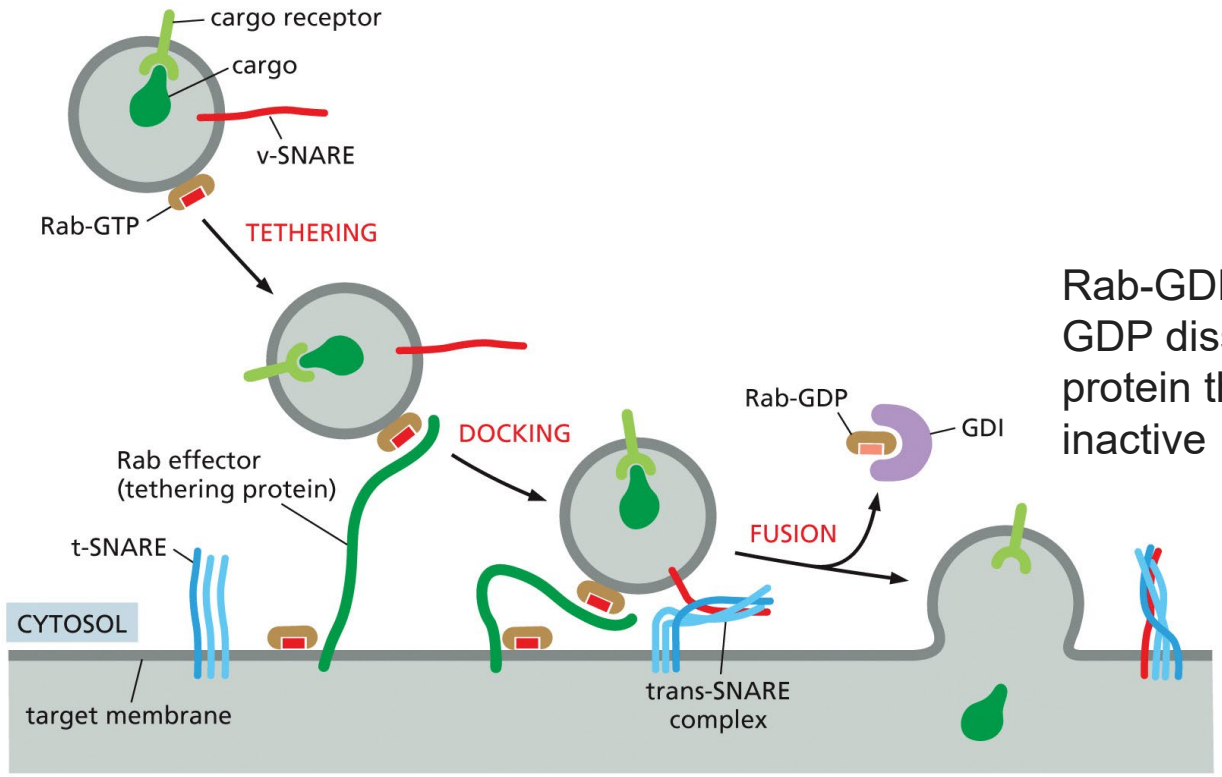
- Rab proteins are GTPases, >60 different known
- Rabs act as molecular markers to identify the organelles and guide vesicles

TABLE 13–1 Subcellular Locations of Some Rab Proteins

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

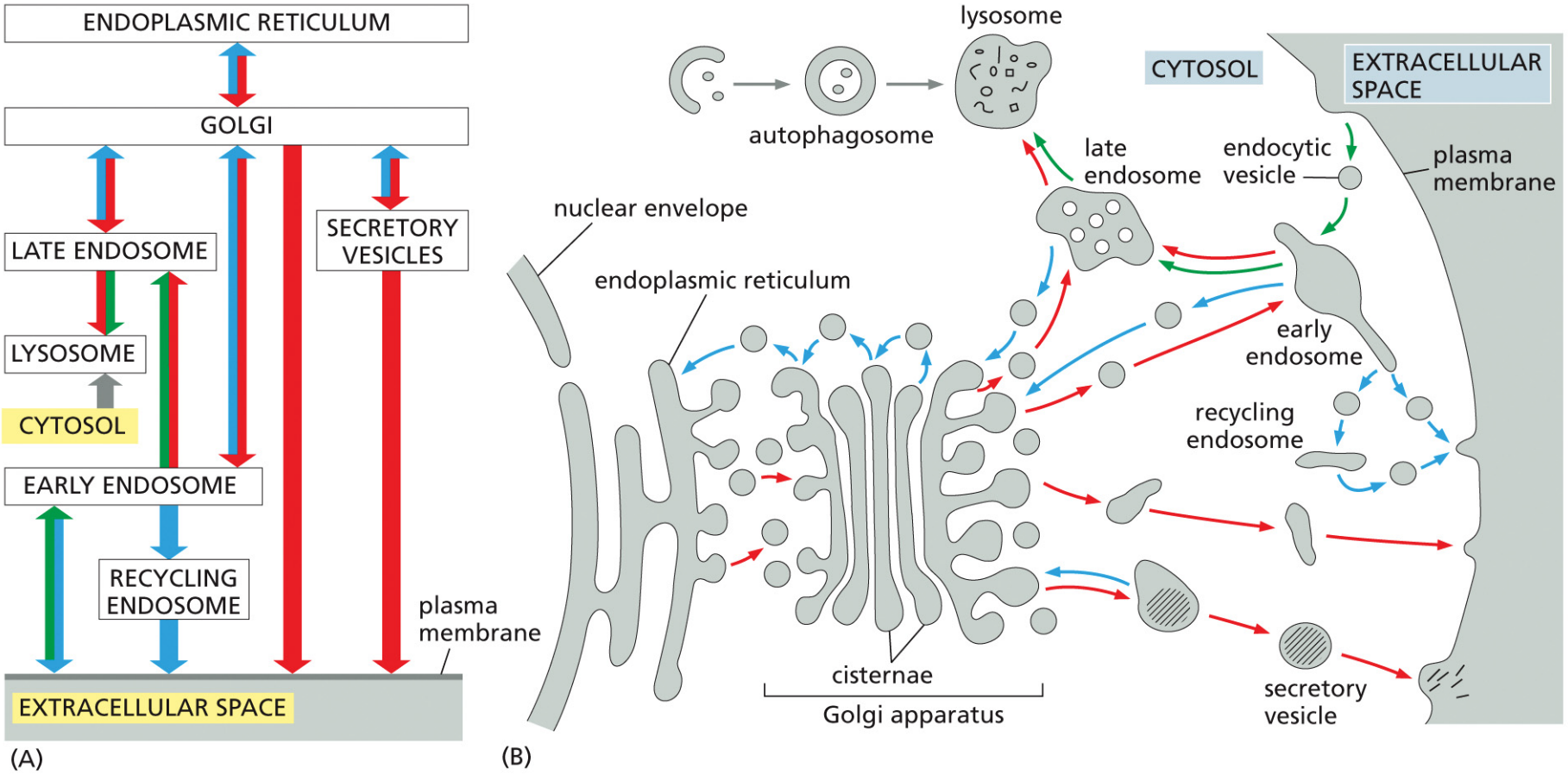
RAB PROTEINS GUIDE TRANSPORT VESICLES TO THEIR TARGET MEMBRANE

- Membrane bound state, bind GTP (active)
  - Interact with Rab effector proteins on the target and/or vesicle membrane
- Soluble state, bind GDP (+GDI) (inactive)
  - Hydrolysis of GTP to GDP during docking causes Rab to dissociate



Rab-GDP in cytosol is bound by a GDP dissociation inhibitor (GDI) protein that keeps it soluble and inactive

TRANSPORT FROM THE ENDOPLASMIC RETICULUM THROUGH THE GOLGI APPARATUS



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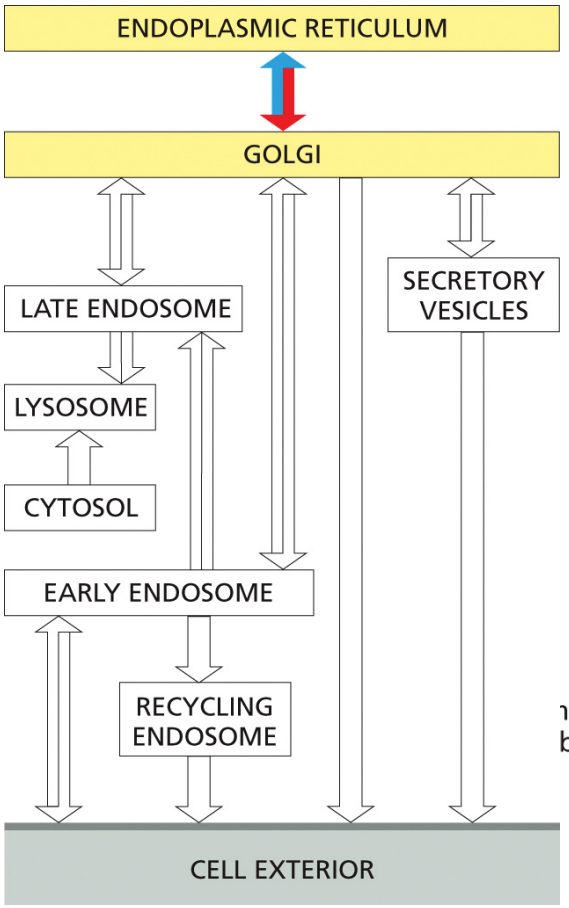
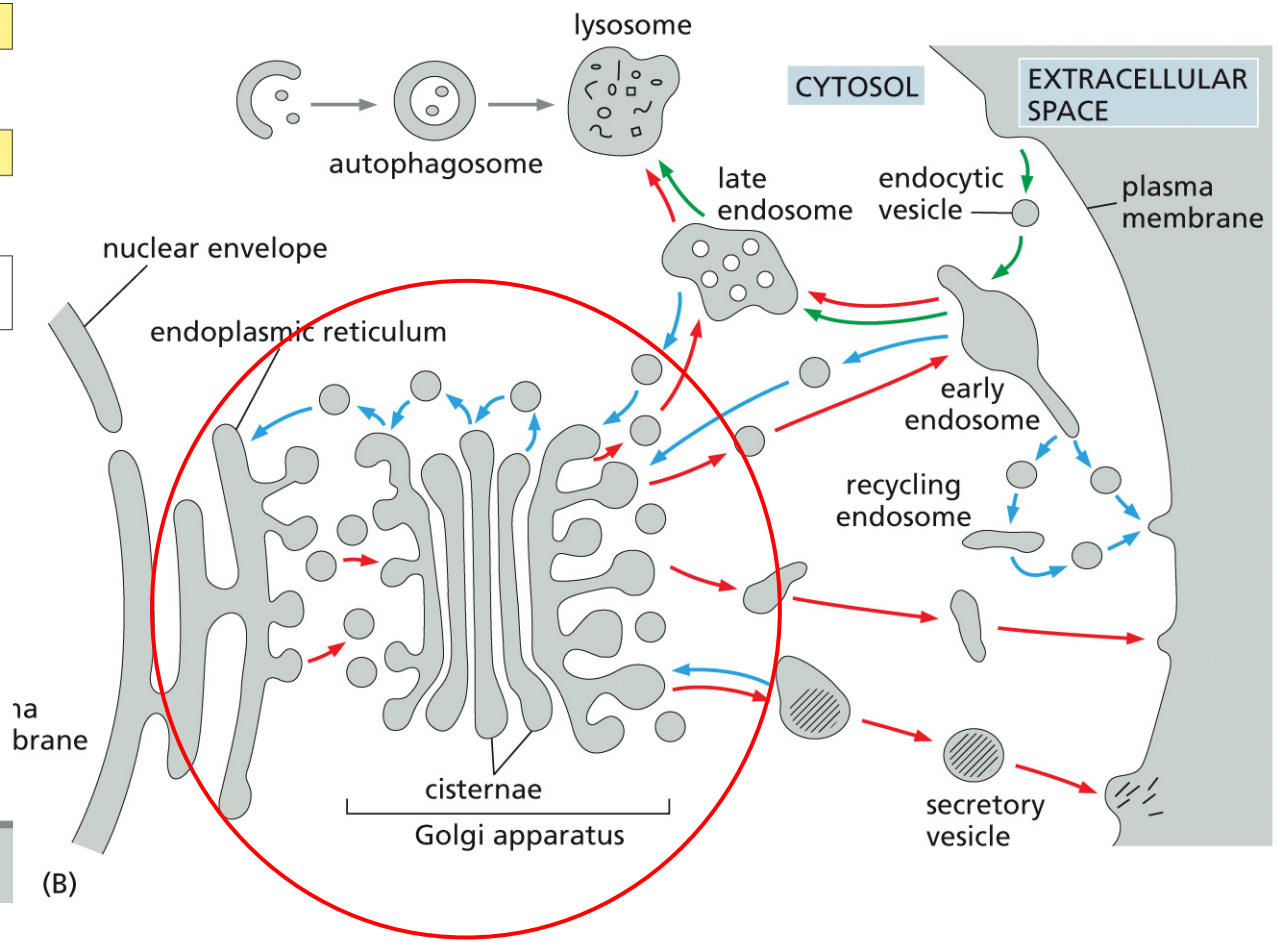
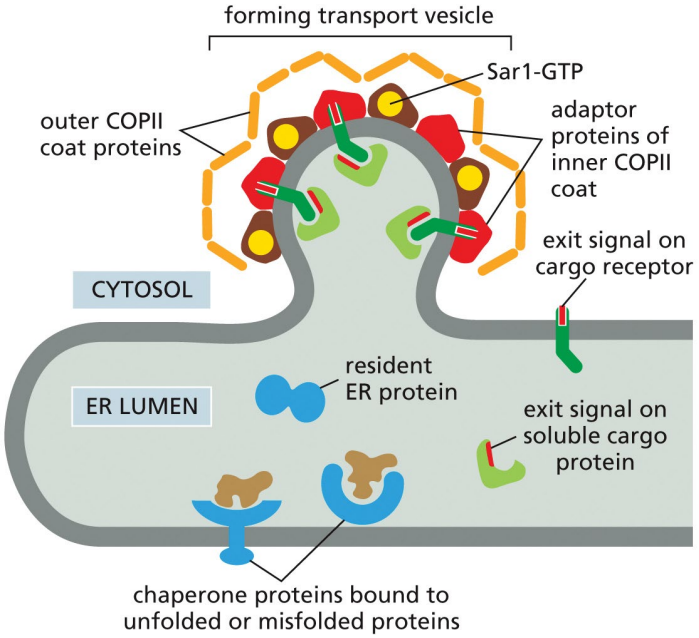


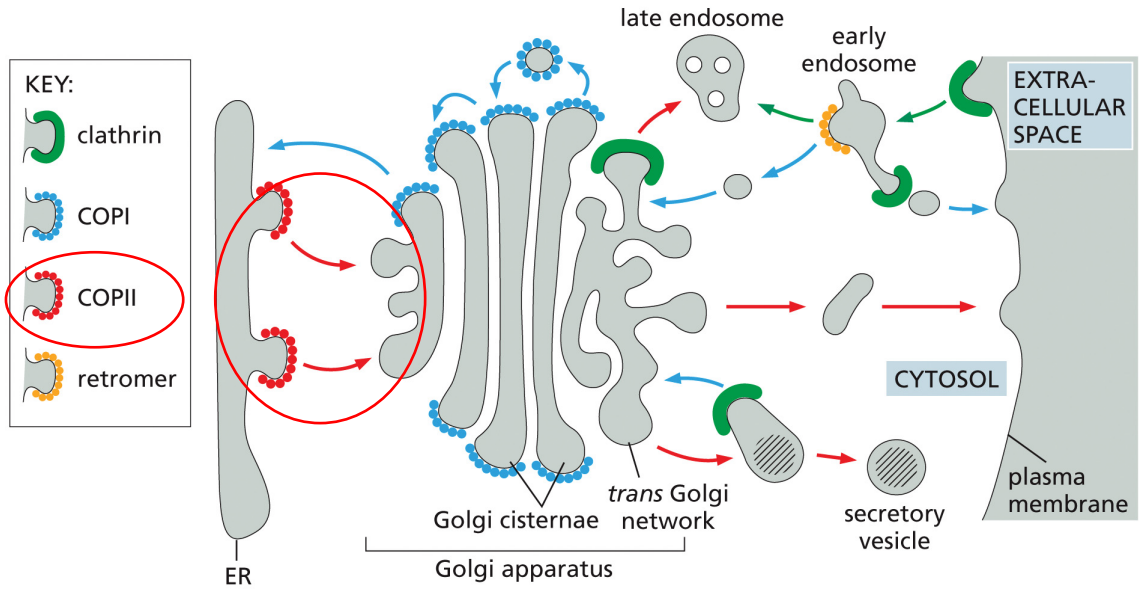


endocytic and secretory pathways
+ retrieval pathways

PROTEINS LEAVE THE ER IN COPII-COATED TRANSPORT VESICLES

- ER exit site contain no ribosomes
- Unfolded or incompletely assembled proteins are bound to chaperones and transiently retained in the ER compartment

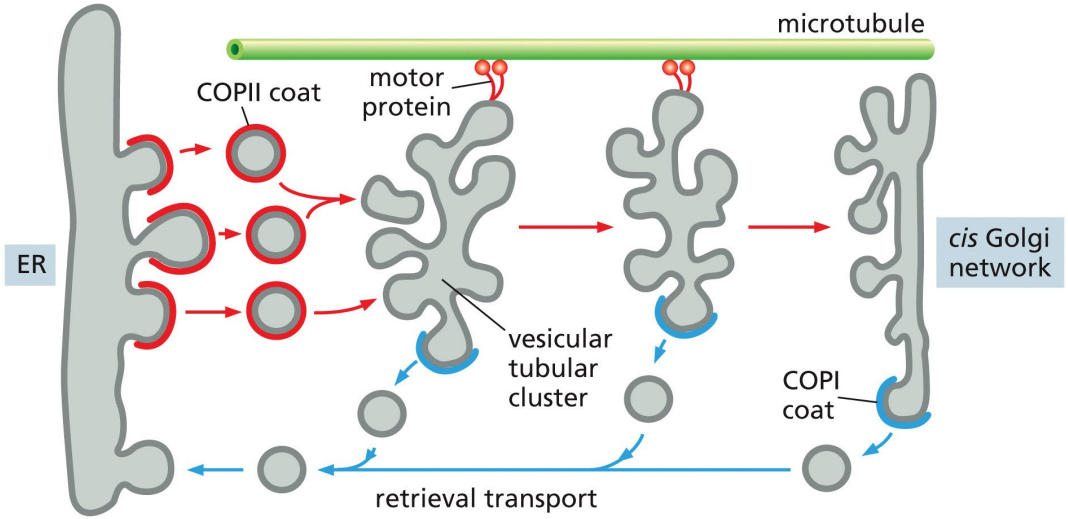


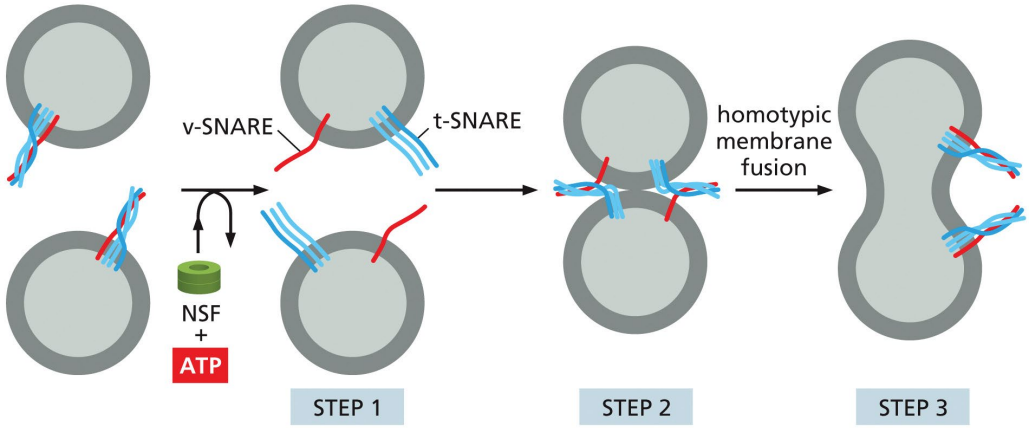


- Proteins leave the ER in COPII-coated transport vesicles
- COPI coats mediate the budding of vesicles that return to the ER from these clusters and from Golgi apparatus

VESICULAR TUBULAR CLUSTERS

- **Vesicular tubular clusters** move along microtubules to carry proteins from the ER to the Golgi apparatus
- Tubular clusters mature towards Golgi apparatus → Retrieval transport!
- ER resident proteins that escape from the ER are returned by vesicle transport.

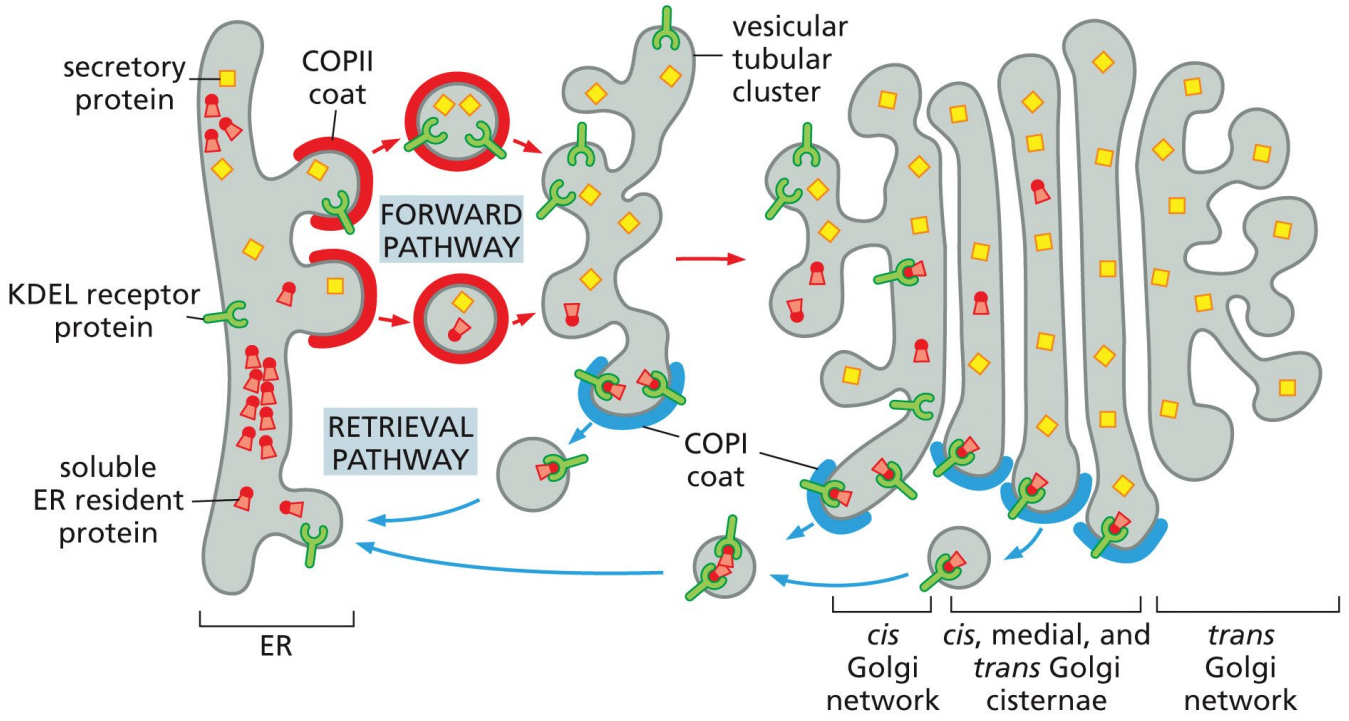




- Fusion of vesicles is mediated by SNAREs and controlled by Rab proteins

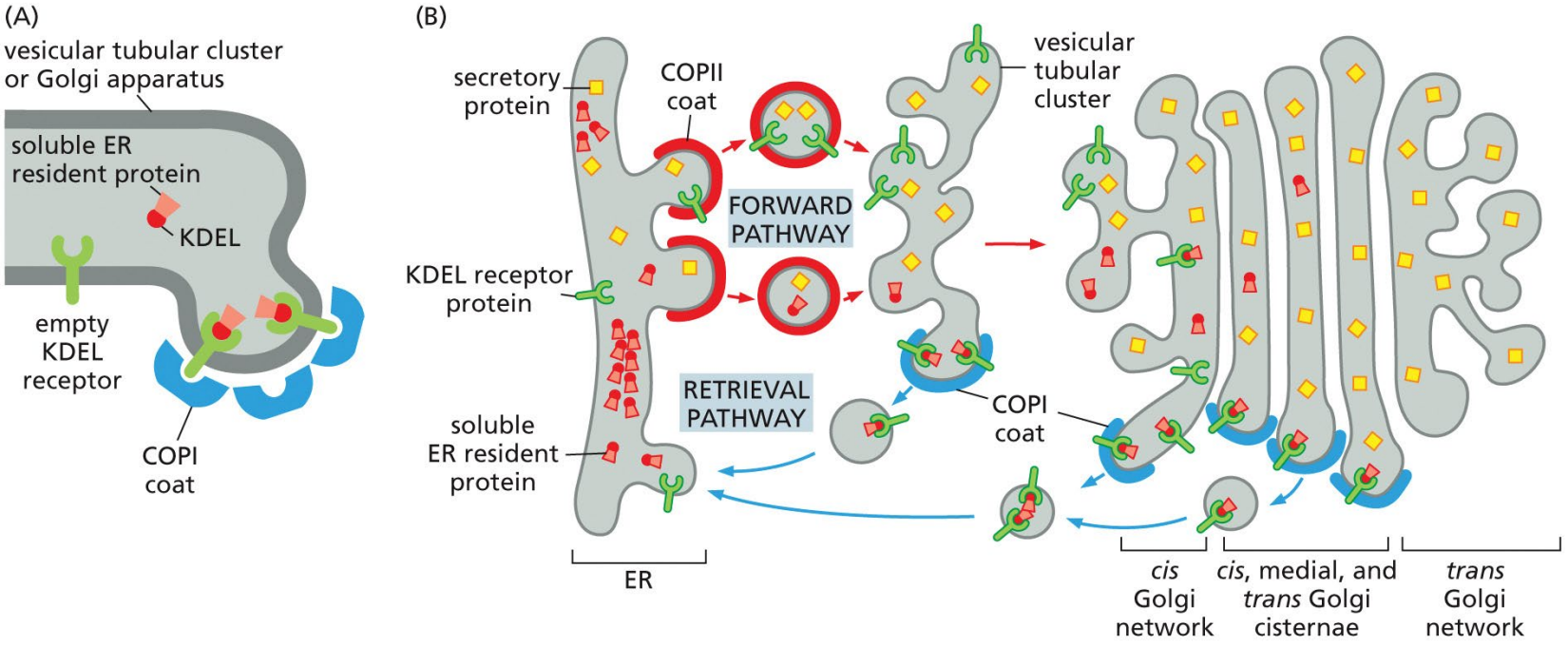
RETRIEVAL PATHWAY

- The retrieval of ER proteins begins in vesicular tubular clusters and continues in later parts of the Golgi apparatus
- In ER, the ER resident proteins dissociate from the KDEL receptor, which is then returned to the Golgi apparatus for reuse



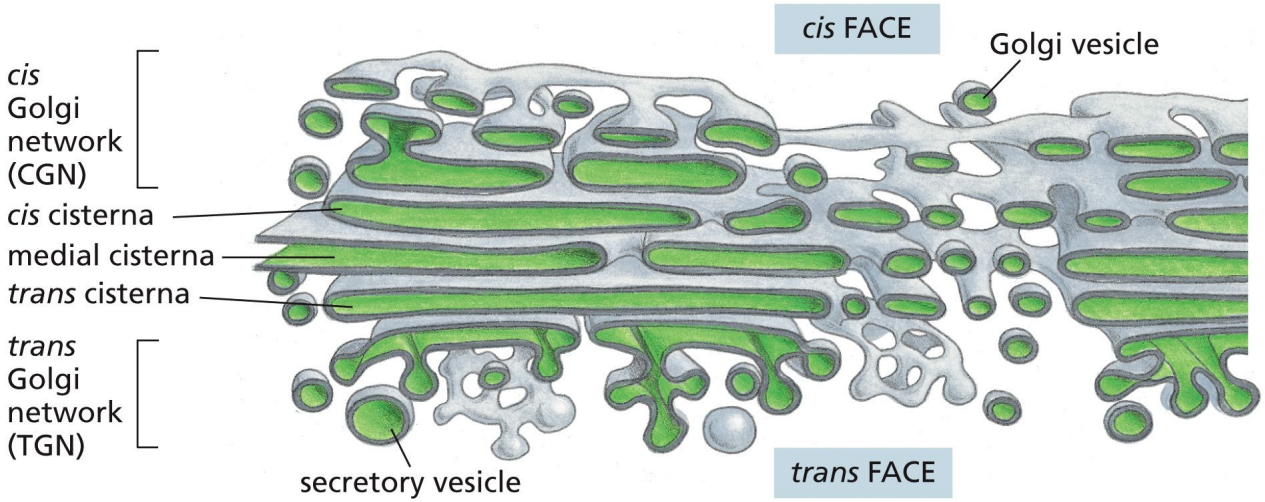
RETRIEVAL PATHWAY

- The retrieval pathway to the ER uses **sorting signals**
- **KDEL receptor** present in both vesicular tubular clusters and the Golgi apparatus captures the soluble ER resident proteins and carries them in COPI-coated transport vesicles back to the ER
- Upon binding its ligands in the tubular cluster or Golgi apparatus, the KDEL receptor may change conformation, so as to facilitate its recruitment into budding COPI-coated vesicles.

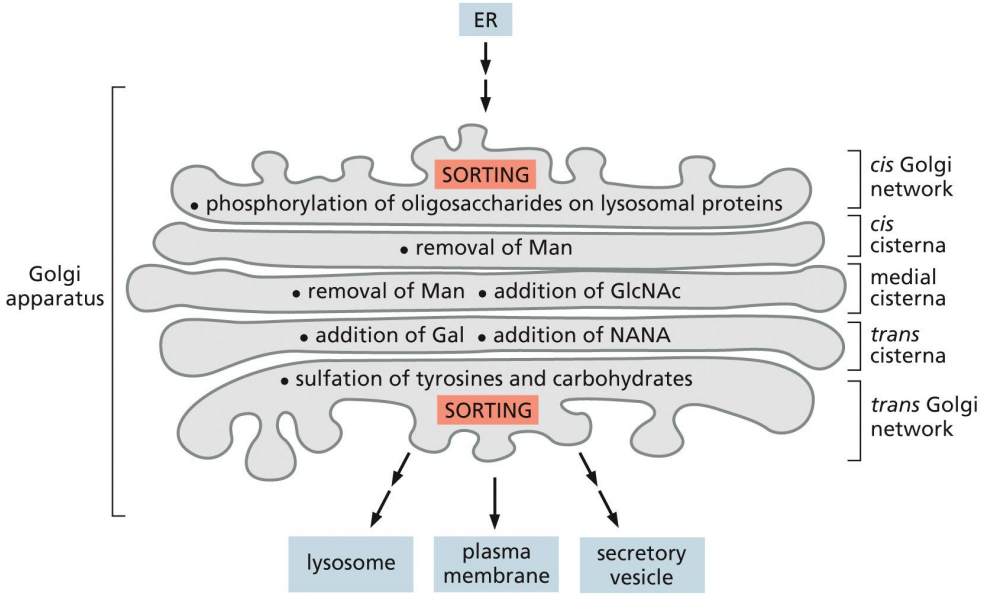


GOLGI APPARATUS

- Golgi apparatus consists of an ordered series of compartments
- Golgi proteins are membrane bound



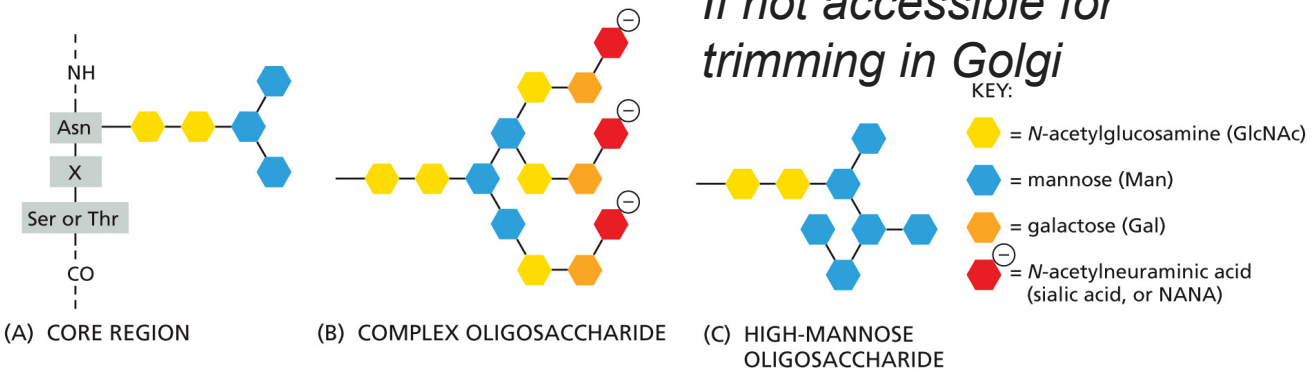
Redrawn from A. Rambourg and Y. Clermont, Eur. *J. Cell Biol.* 51:189–200, 1990.



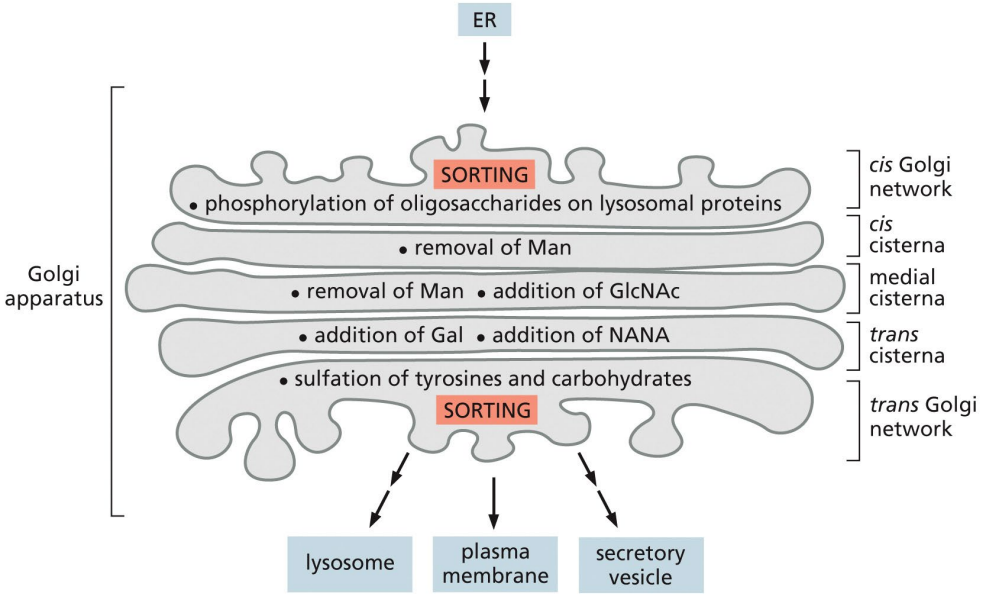
- Oligosaccharides are processed in Golgi compartments

OLIGOSACCHARIDE CHAINS ARE PROCESSED IN THE GOLGI APPARATUS

- The two main classes of *N*-linked oligosaccharides



If accessible for trimming in Golgi



Added in ER:

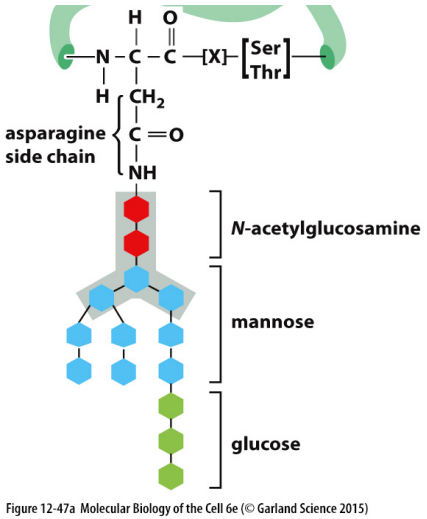
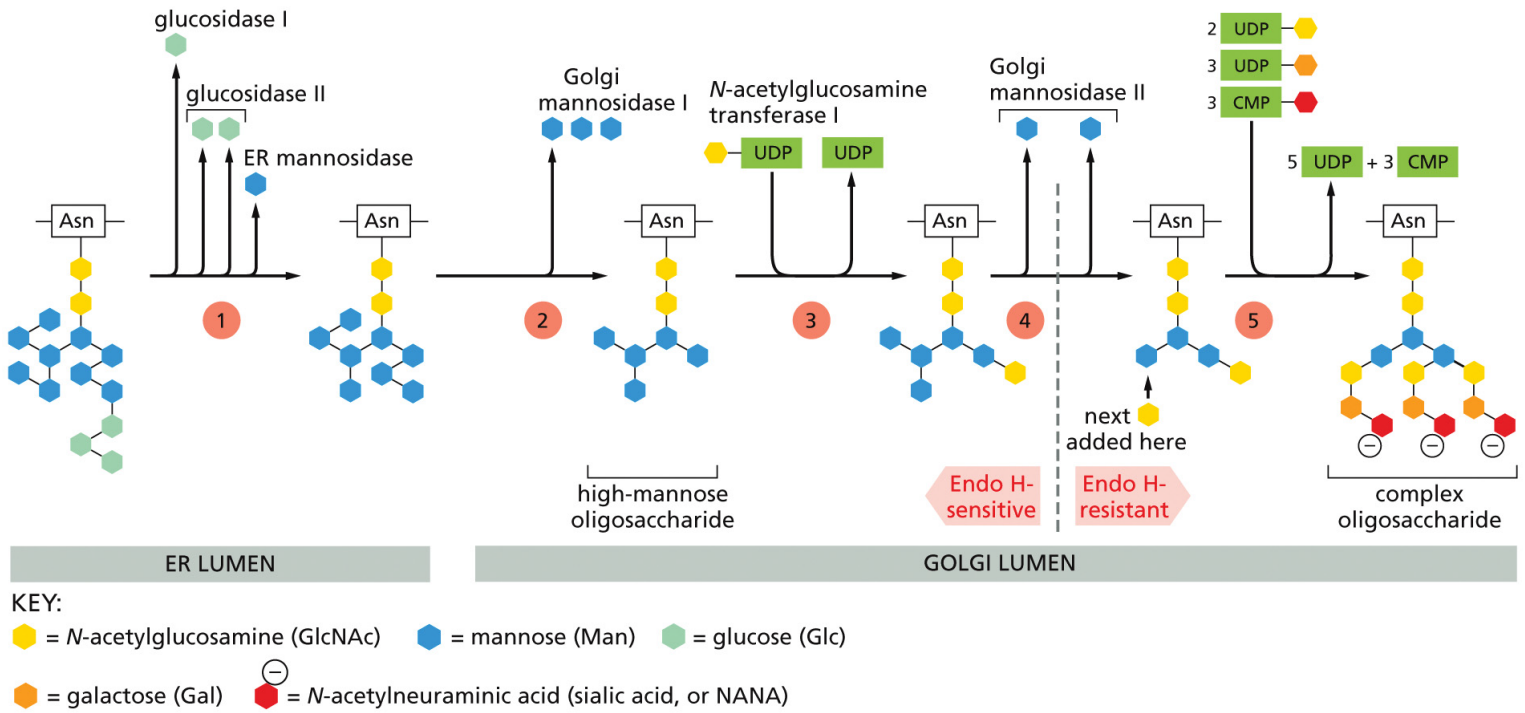


Figure 12-47a Molecular Biology of the Cell 6e (© Garland Science 2015)

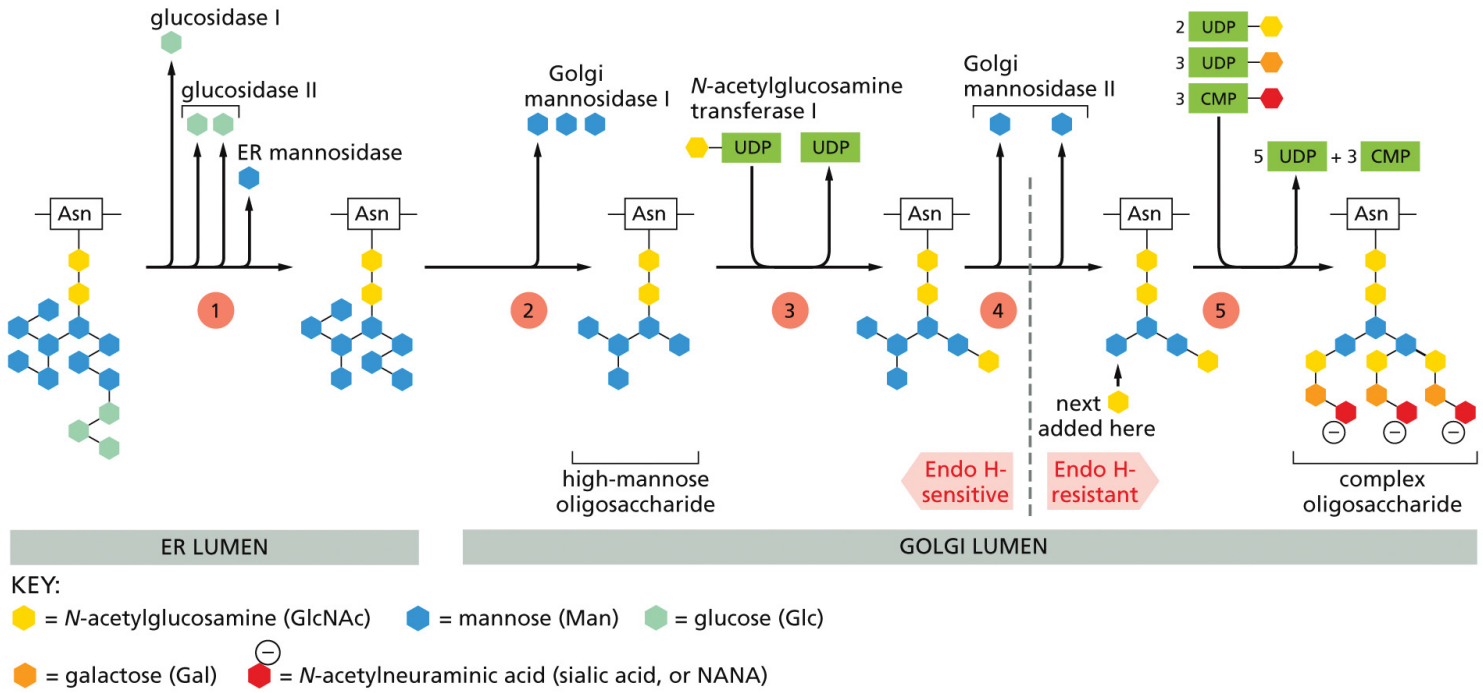
OLIGOSACCHARIDE CHAINS ARE PROCESSED IN THE GOLGI APPARATUS

- All glycosidases and glycosyl transferases are single-pass transmembrane proteins, often complexes
- Both complex oligosaccharides and high-mannose oligosaccharides share a common *core region*
- **High-mannose oligosaccharides** are not trimmed back all the way to the core region and contain additional mannoses



OLIGOSACCHARIDE CHAINS ARE PROCESSED IN THE GOLGI APPARATUS

- **Complex oligosaccharides** consists of a *core region*, together with a *terminal region* that contains a variable number of copies of a special trisaccharide unit (*N-acetylglucosamine–galactose–sialic acid*) linked to the three core mannoses
- Terminal region is often truncated and contains only GlcNAc and galactose (Gal) or just GlcNAc. In addition, a fucose may be added, usually to the core GlcNAc attached to the asparagine (Asn).
- *Complex oligosaccharides are heterogeneous!*



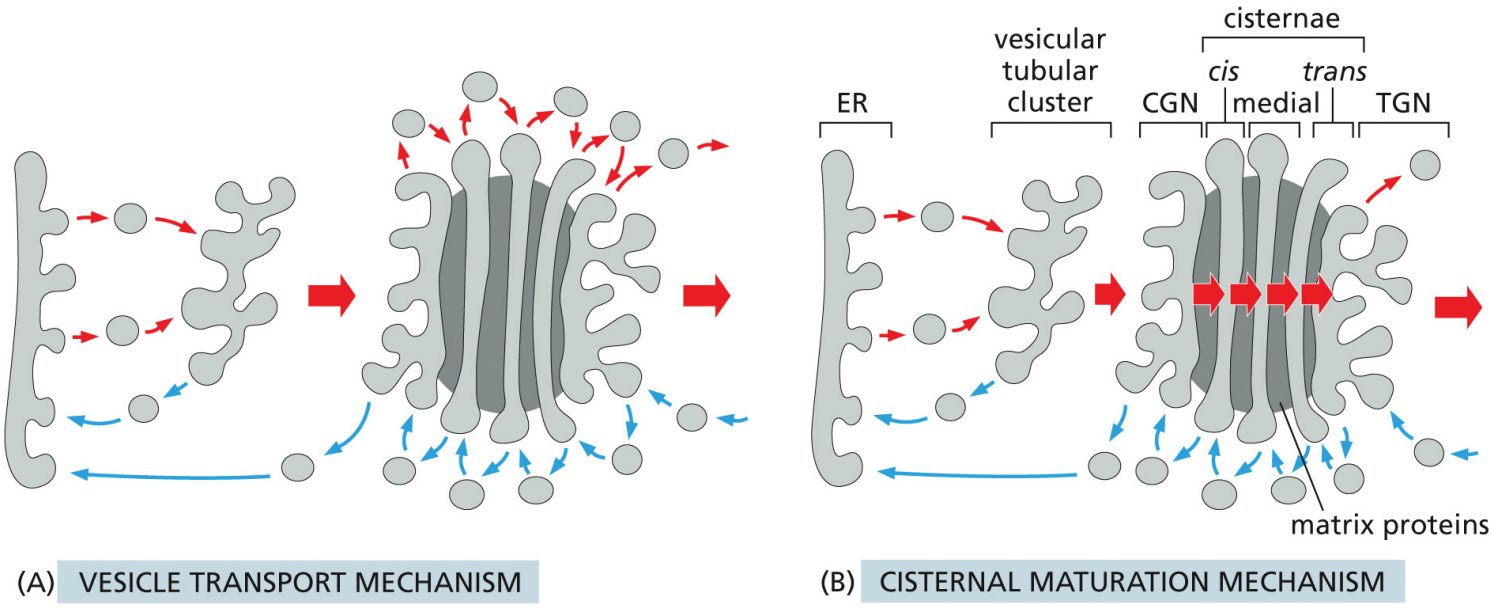
TRANSPORT THROUGH THE GOLGI APPARATUS OCCURS BY MULTIPLE MECHANISMS

- Cisternae long-lived structures between which vesicles transport material

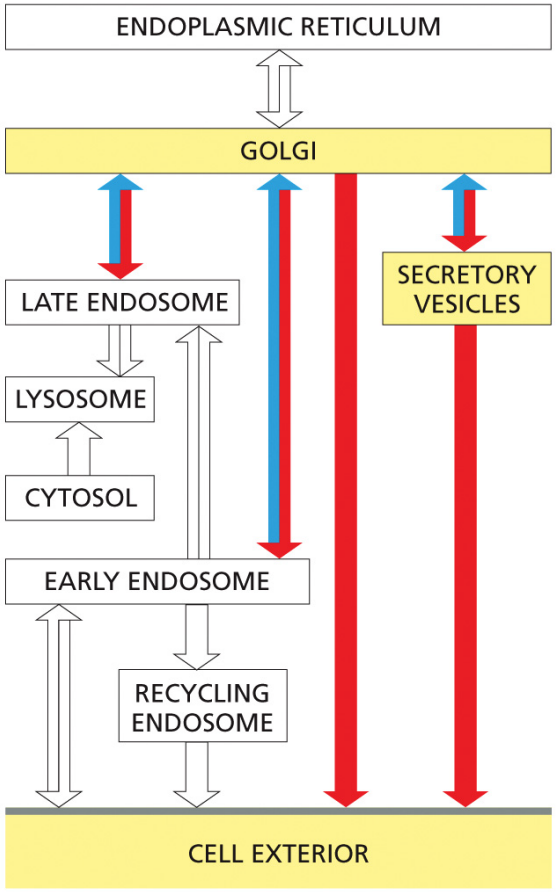
Or

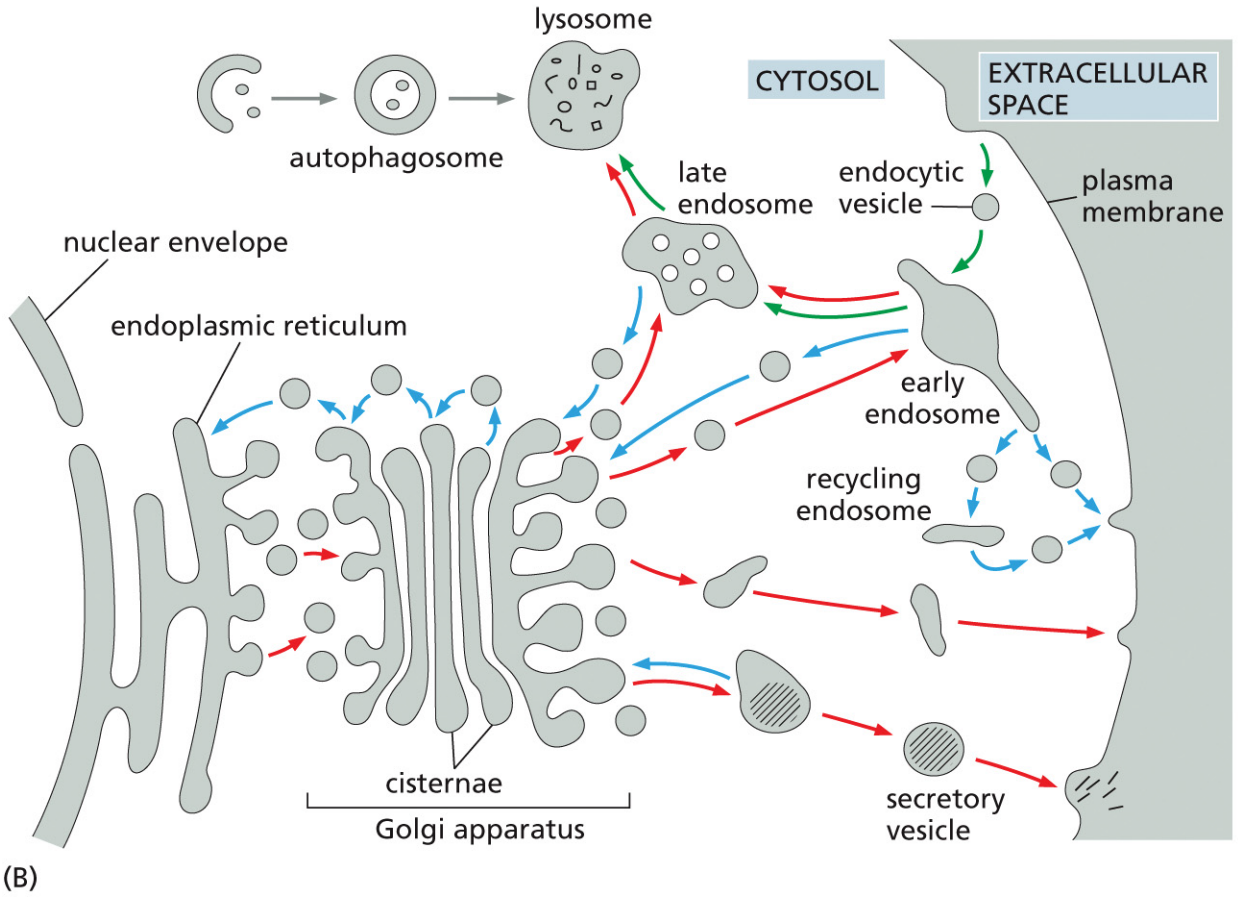
- Cisternae mature and vesicles move components backwards

Both might be relevant



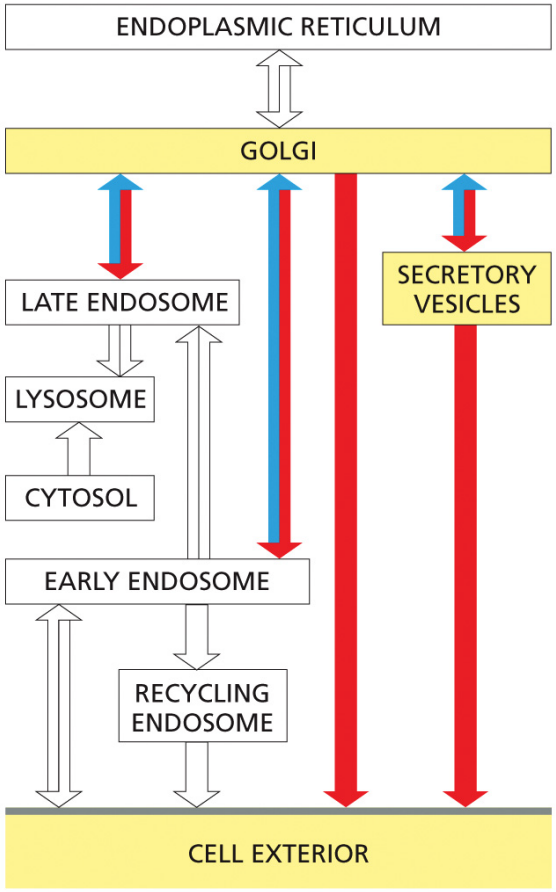
TRANSPORT FROM THE TRANS GOLGI NETWORK TO THE CELL EXTERIOR AND ENDOSOMES





endocytic and secretory pathways
+ retrieval pathways

TRANSPORT FROM THE TRANS GOLGI NETWORK TO THE CELL EXTERIOR AND ENDOSOMES



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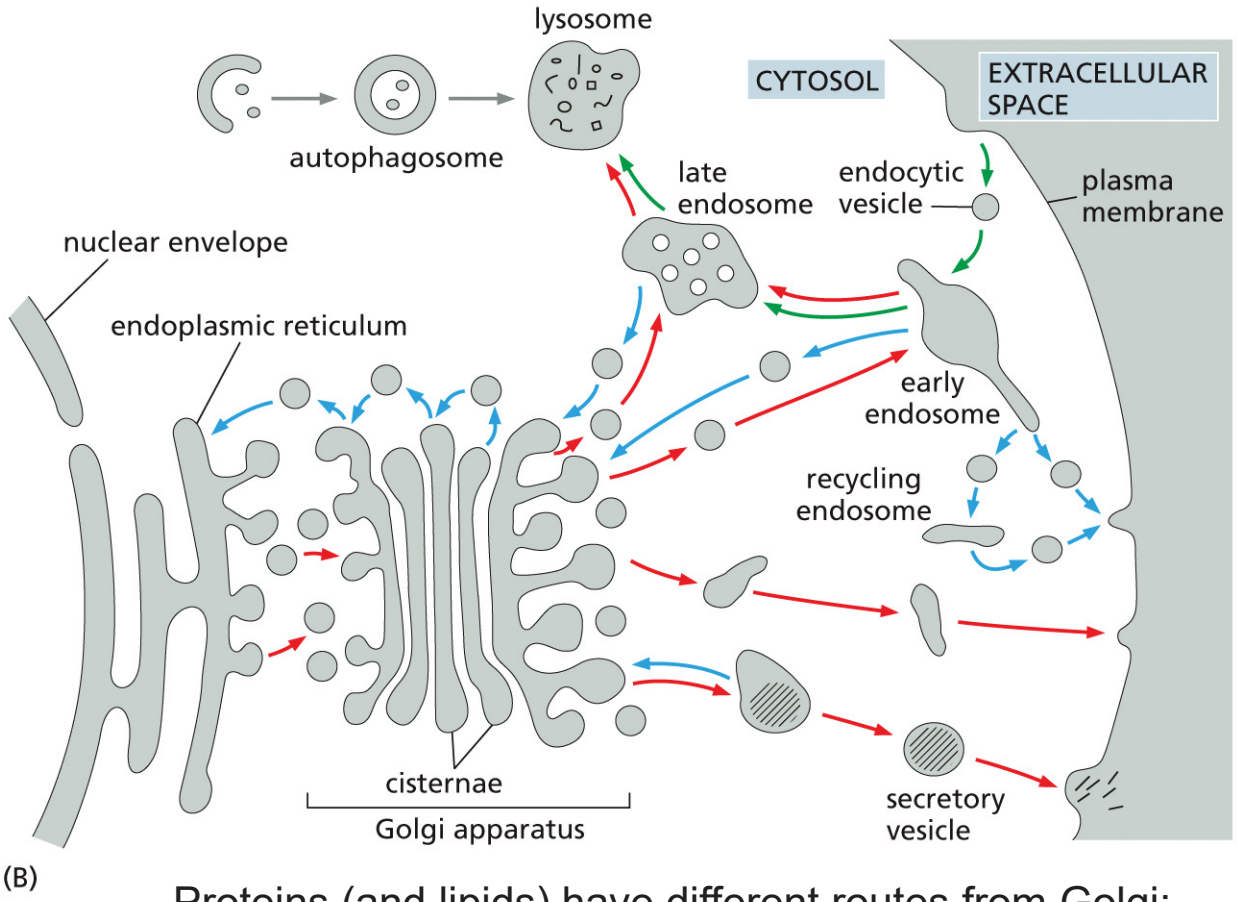
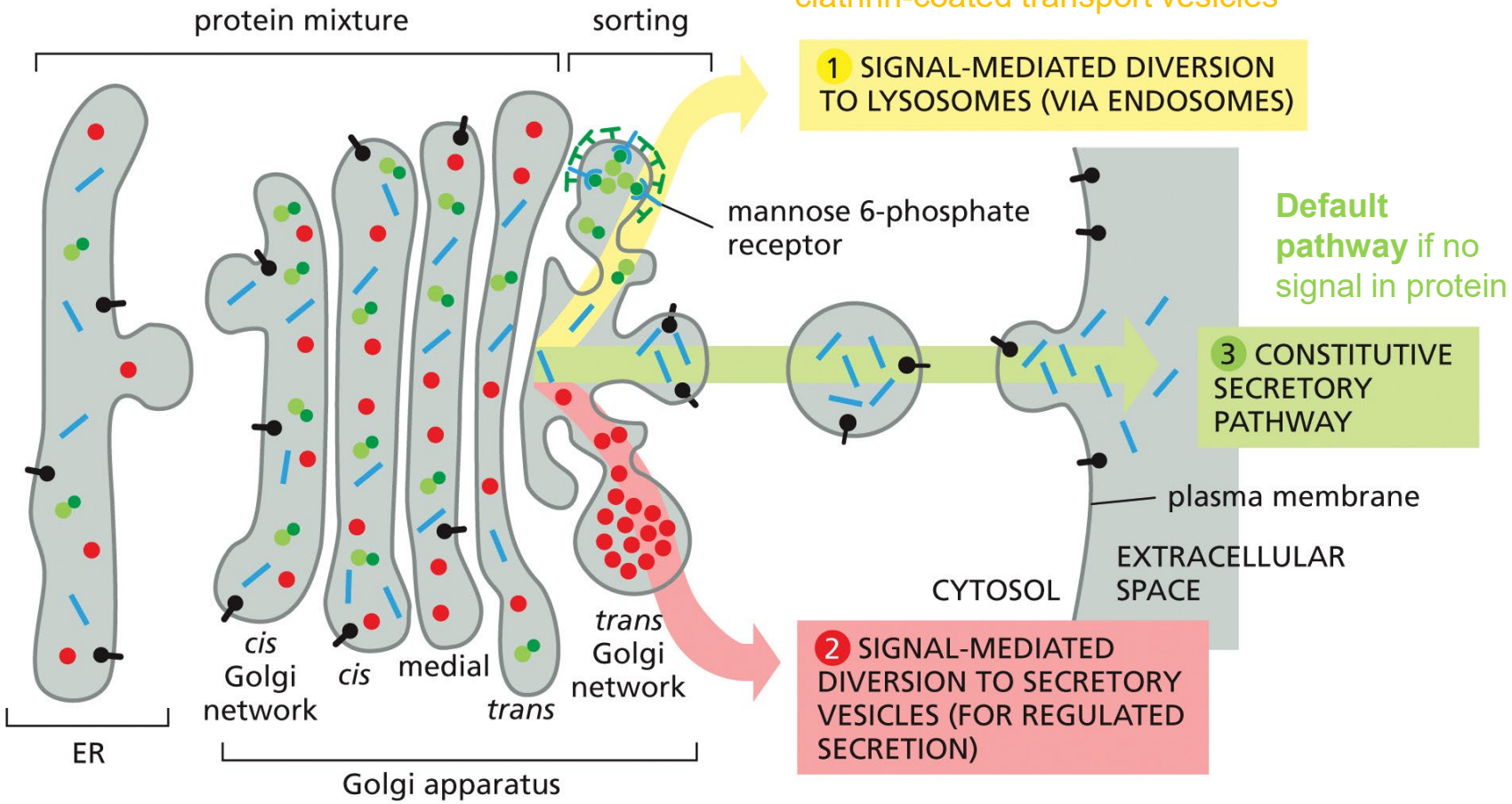


Proteins (and lipids) have different routes from Golgi:

- Lysosomes (via endosomes)
- Secretion
  - Constitutive secretion
  - Regulated secretion

THREE PATHWAYS OF PROTEIN SORTING IN THE *TRANS* GOLGI NETWORK

Proteins with mannose 6-phosphate **(M6P) marker** → **lysosomes** (via endosomes) in clathrin-coated transport vesicles



Regulated secretory pathway present only in specialized secretory cells

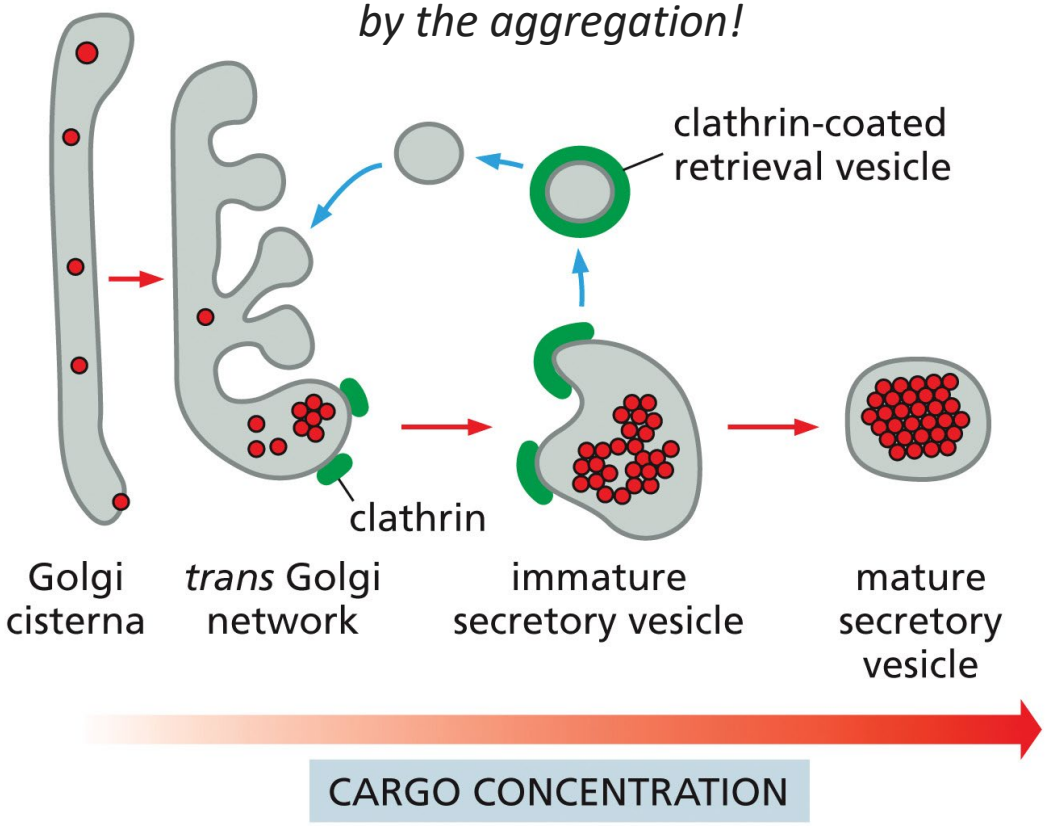
- proteins *with signals directing for secretion*

SECRETORY VESICLES BUD FROM THE TRANS GOLGI NETWORK

- Secretory proteins become segregated and highly concentrated in secretory vesicles

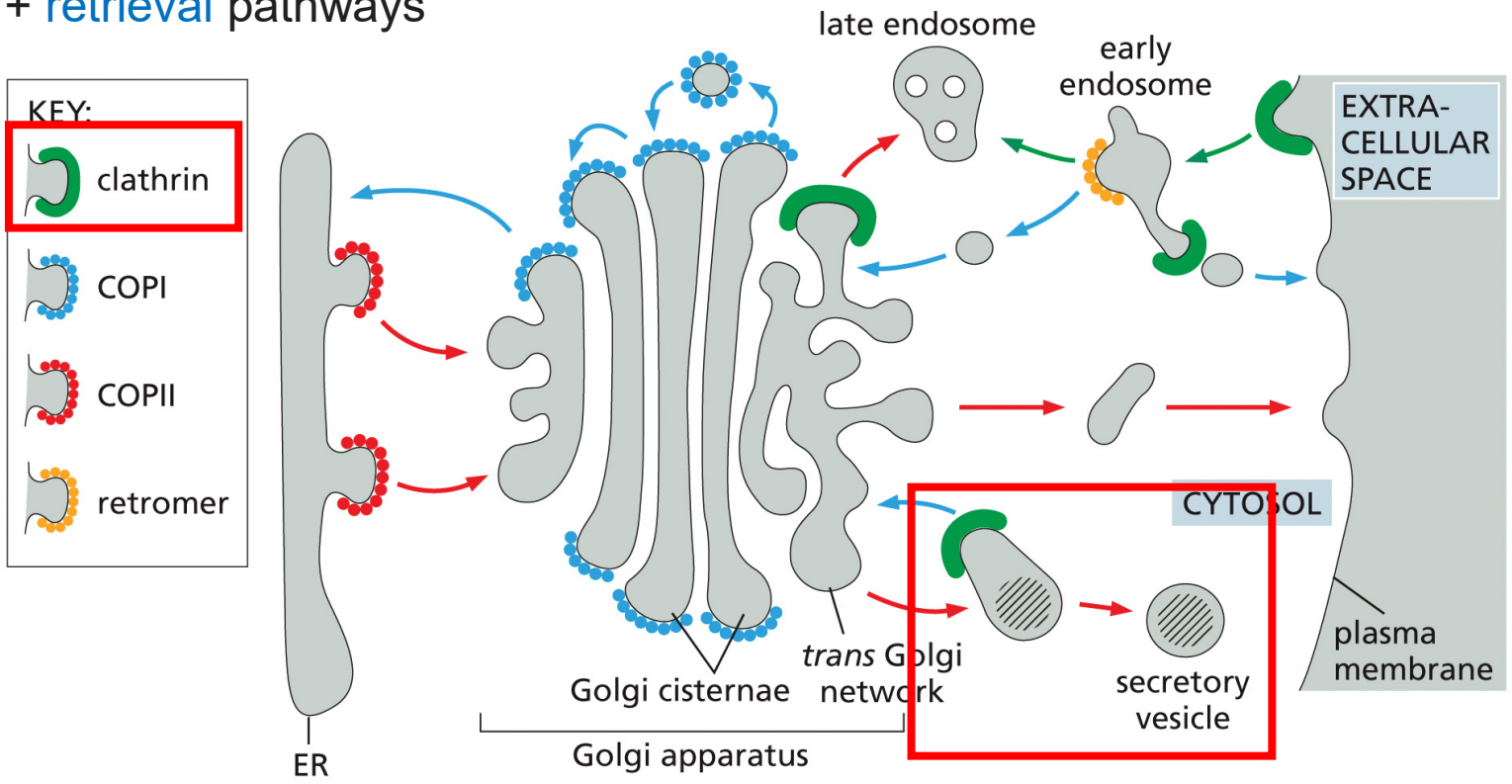
1. *Aggregation* in the ionic environment of the TGN
2. *Clathrin-coated* vesicles *retrieve* excess membrane and lumenal content present in immature secretory vesicles as the secretory vesicles mature (-> 200-400x increase in concentration)
3. Acidification by V-type ATPases

The exact signal for secretion is not known, might be induced by the aggregation!



DIFFERENT COATS FOR DIFFERENT STEPS IN VESICLE TRAFFIC

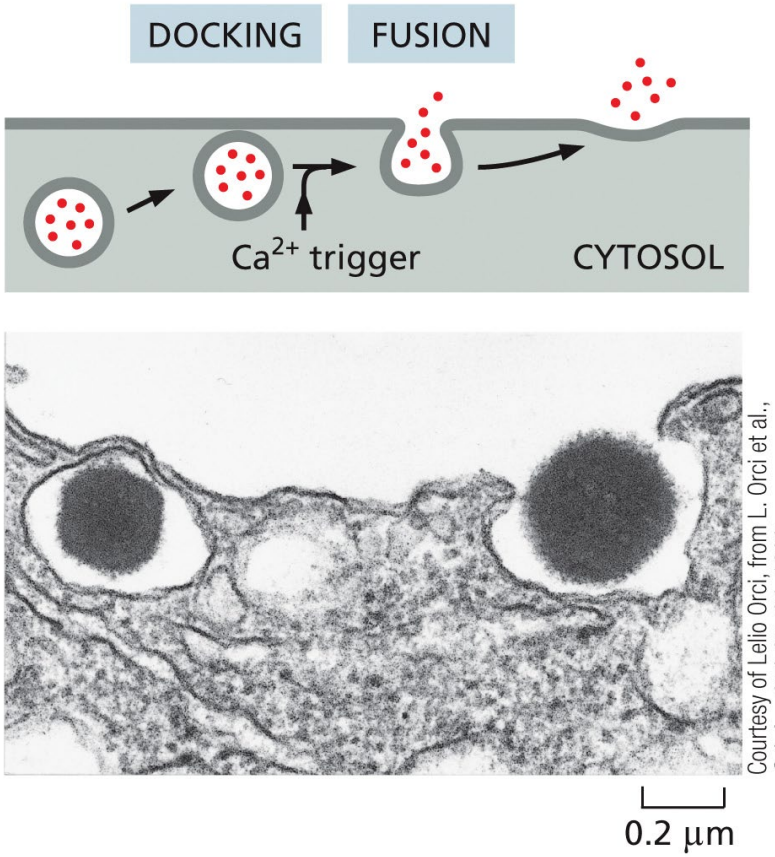
endocytic and secretory pathways
+ retrieval pathways



- Clathrin coating directs components of vesicles back to trans Golgi

EXOCYTOSIS OF SECRETORY VESICLES

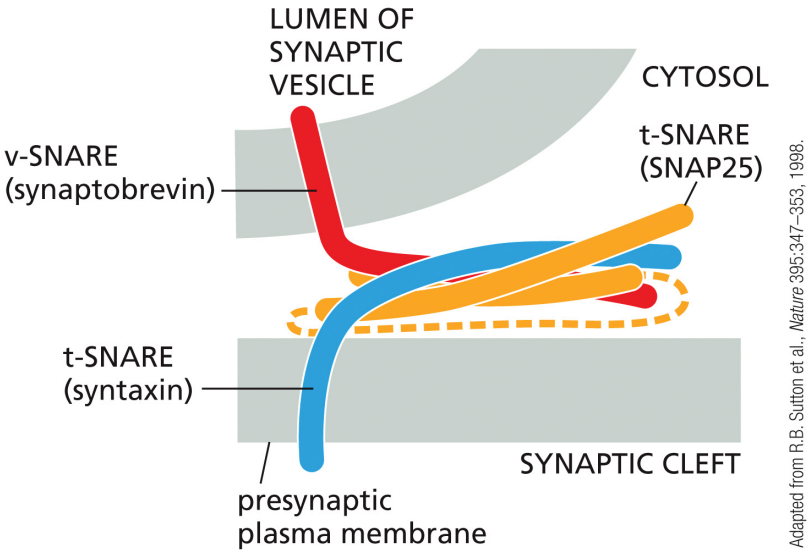
- Secretory vesicles wait near the plasma membrane until signaled to release their contents
- The release of insulin from a secretory vesicle of a pancreatic β cell



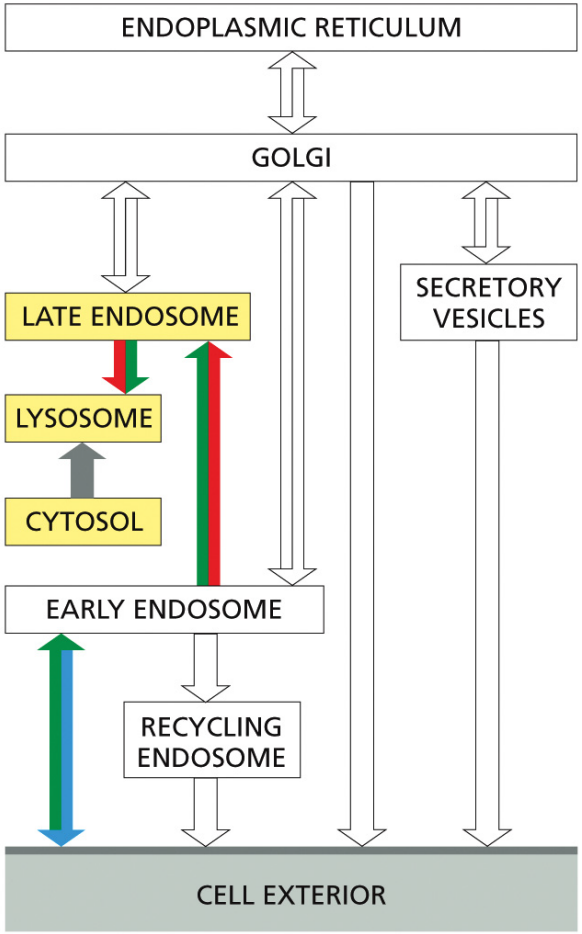
EXOCYTOSIS OF SYNAPTIC VESICLES

- The **trans-SNARE complex** responsible for **docking** synaptic vesicles at the plasma membrane of nerve terminals consists of three proteins
- The v-SNARE *synaptobrevin* and the t-SNARE *syntaxin* are both transmembrane proteins, and each contributes one α helix to the complex
- t-SNARE *SNAP25* is a peripheral membrane protein that contributes two α helices to the four-helix bundle; the two helices are connected by a loop *(dashed line)* that lies parallel to the membrane and has fatty acyl chains (not shown) attached to anchor it there

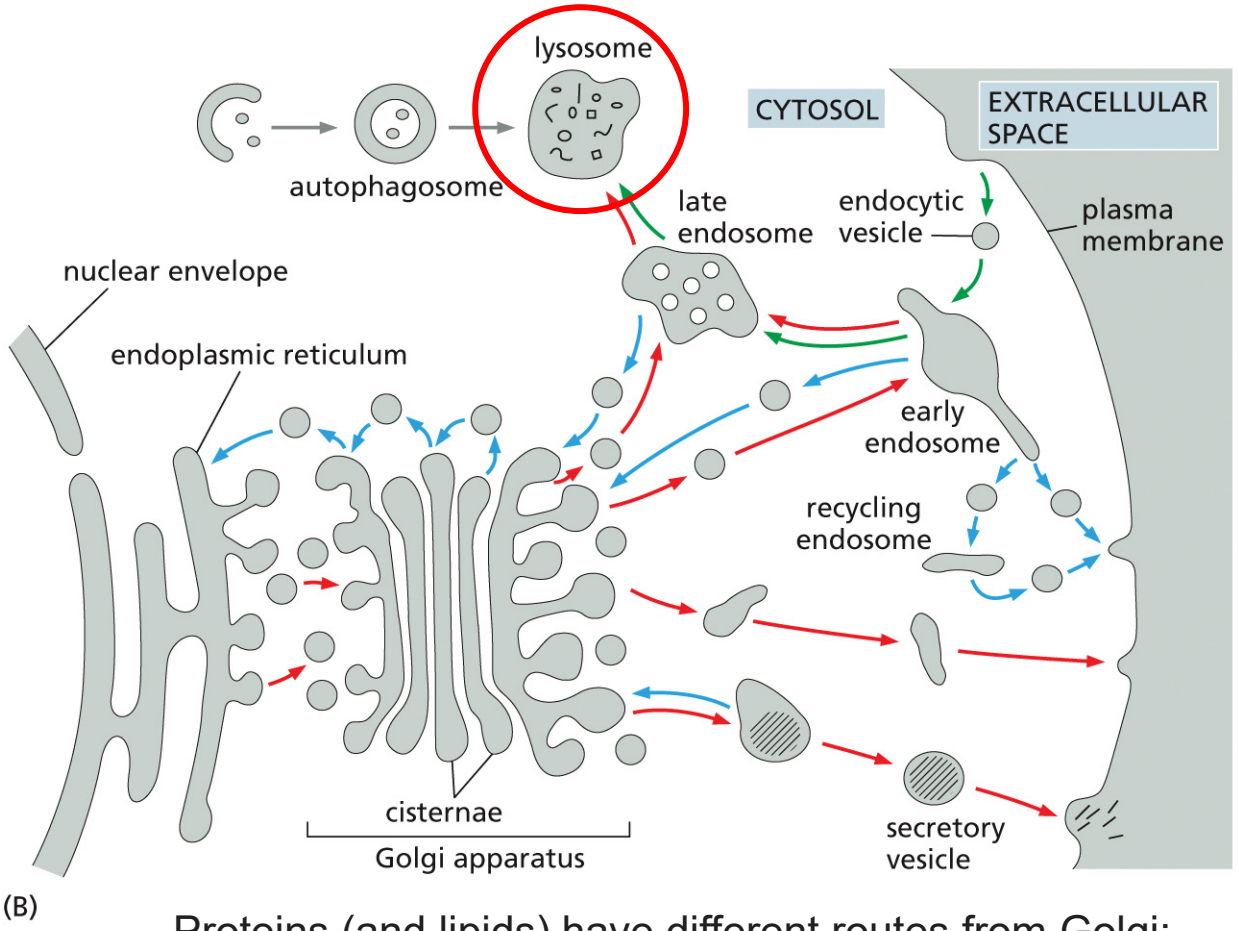
For rapid exocytosis, synaptic vesicles are primed at the presynaptic plasma membrane



TRANSPORT FROM THE TRANS GOLGI NETWORK TO THE CELL EXTERIOR AND ENDOSOMES



endocytic and secretory pathways
+ retrieval pathways



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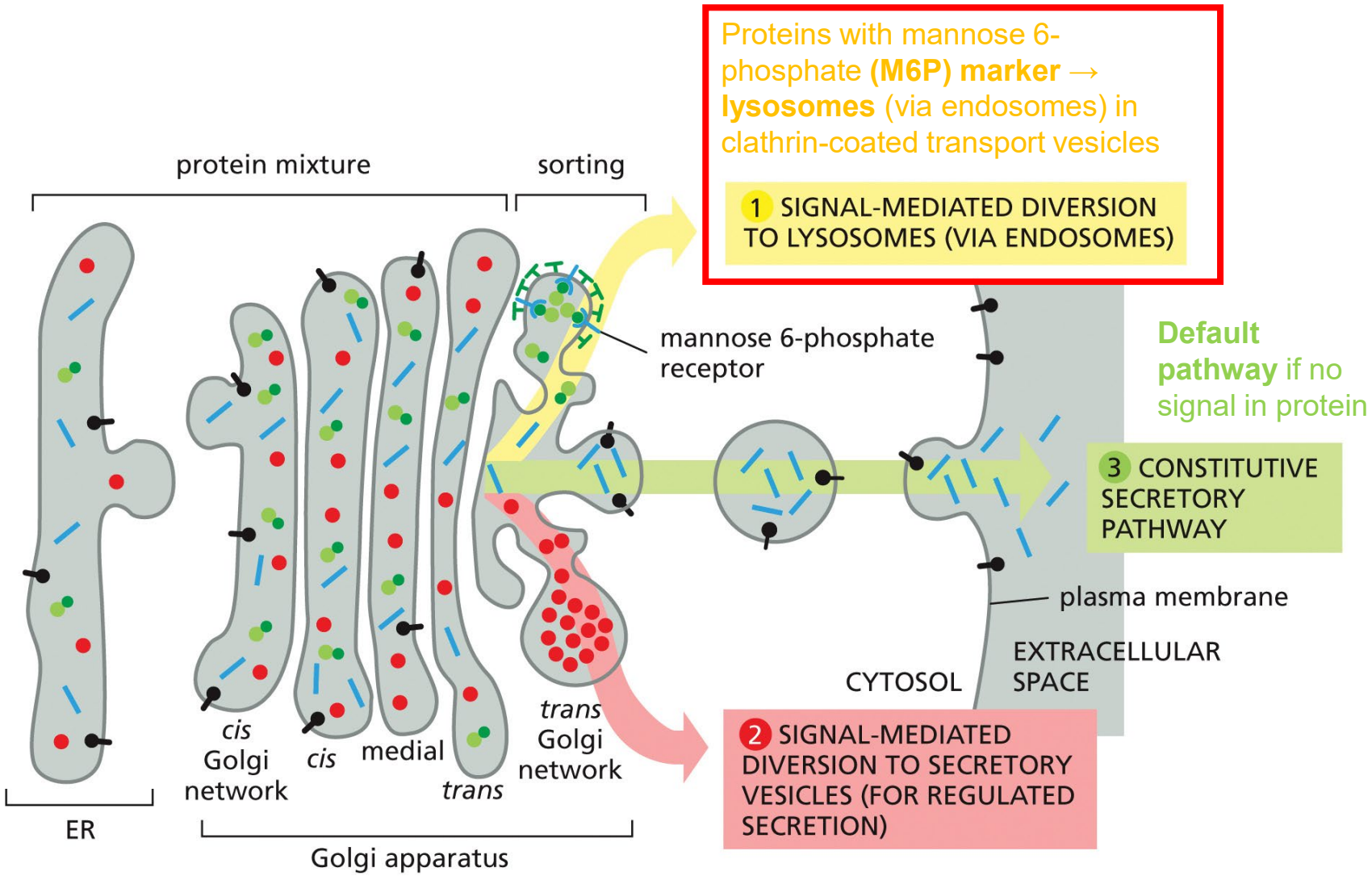
THREE PATHWAYS OF PROTEIN SORTING IN THE *TRANS* GOLGI NETWORK



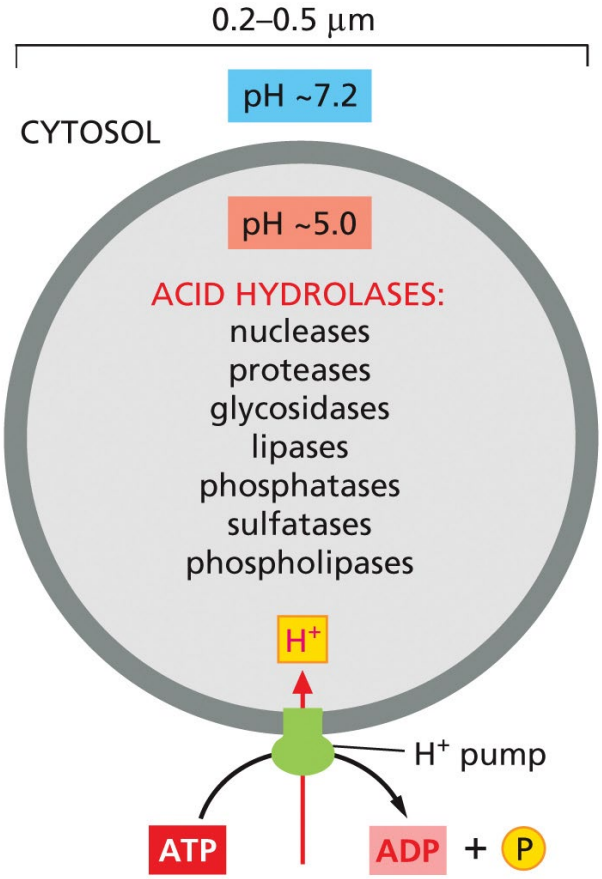
Regulated secretory pathway present only in specialized secretory cells

- proteins *with signals directing for secretion*

THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

- Lysosomes are the principal sites of **intracellular digestion**

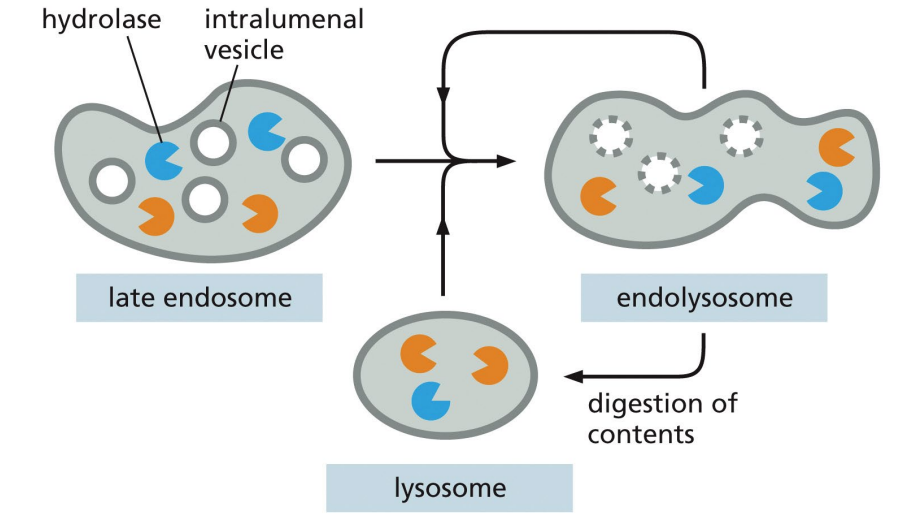
- The **acid hydrolases** are *hydrolytic enzymes* that are *activated* and *active* under *acidic conditions*
  → protection of cell
- An **H^+ ATPase** in the membrane pumps H^+ into the lysosome, maintaining its *lumen at an acidic pH*

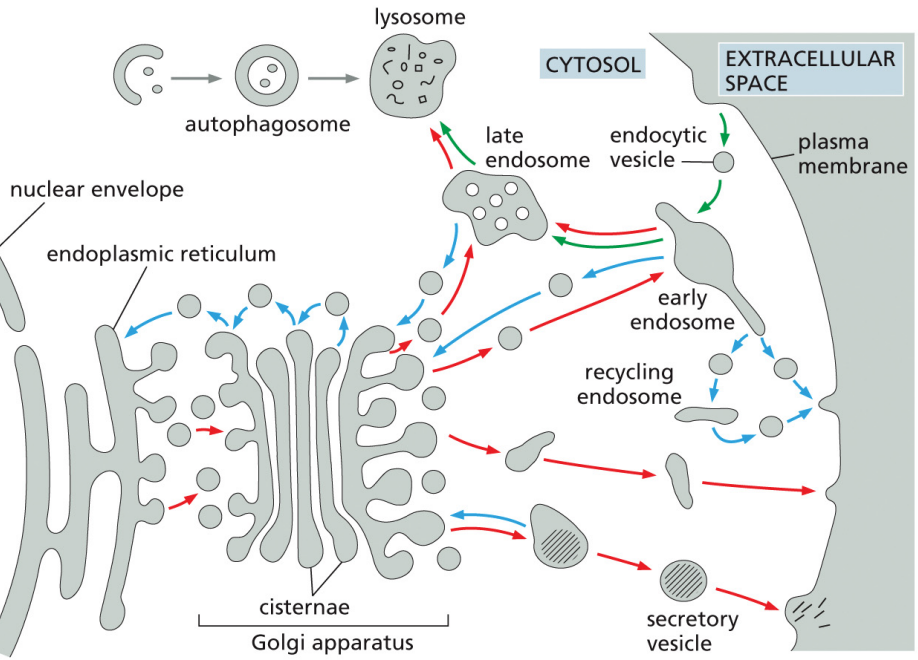


LYSOSOMES ARE HETEROGENEOUS AND DYNAMIC

Lysosome maturation

- Endolysosomes mature into lysosomes as hydrolases complete the digestion of their contents



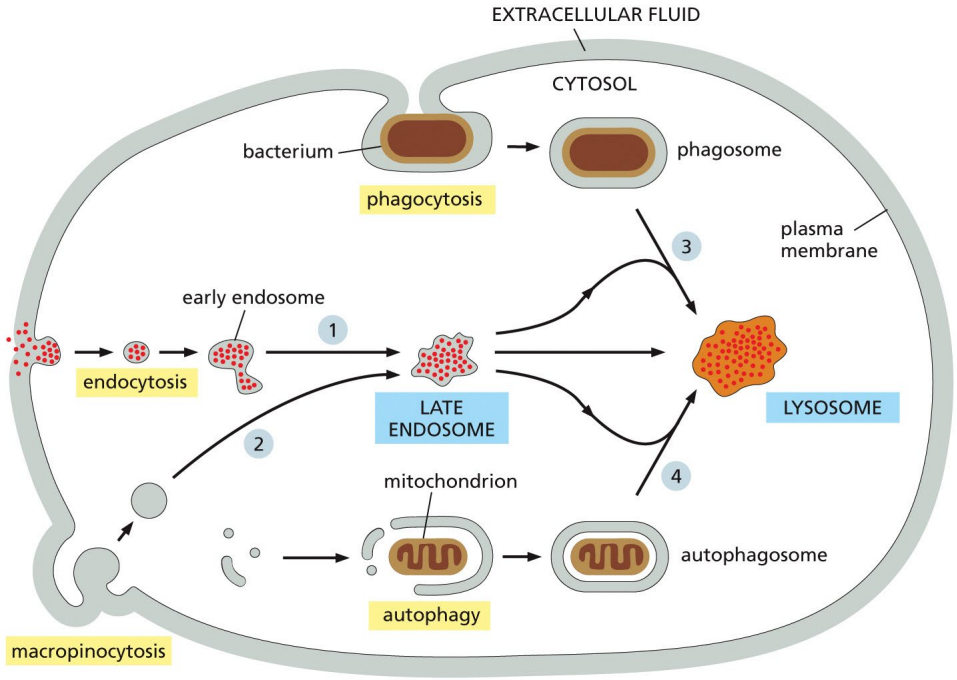


- Late endosomes fuse with preexisting lysosomes or endolysosomes
- Lysosomes also fuse with phagosomes

THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

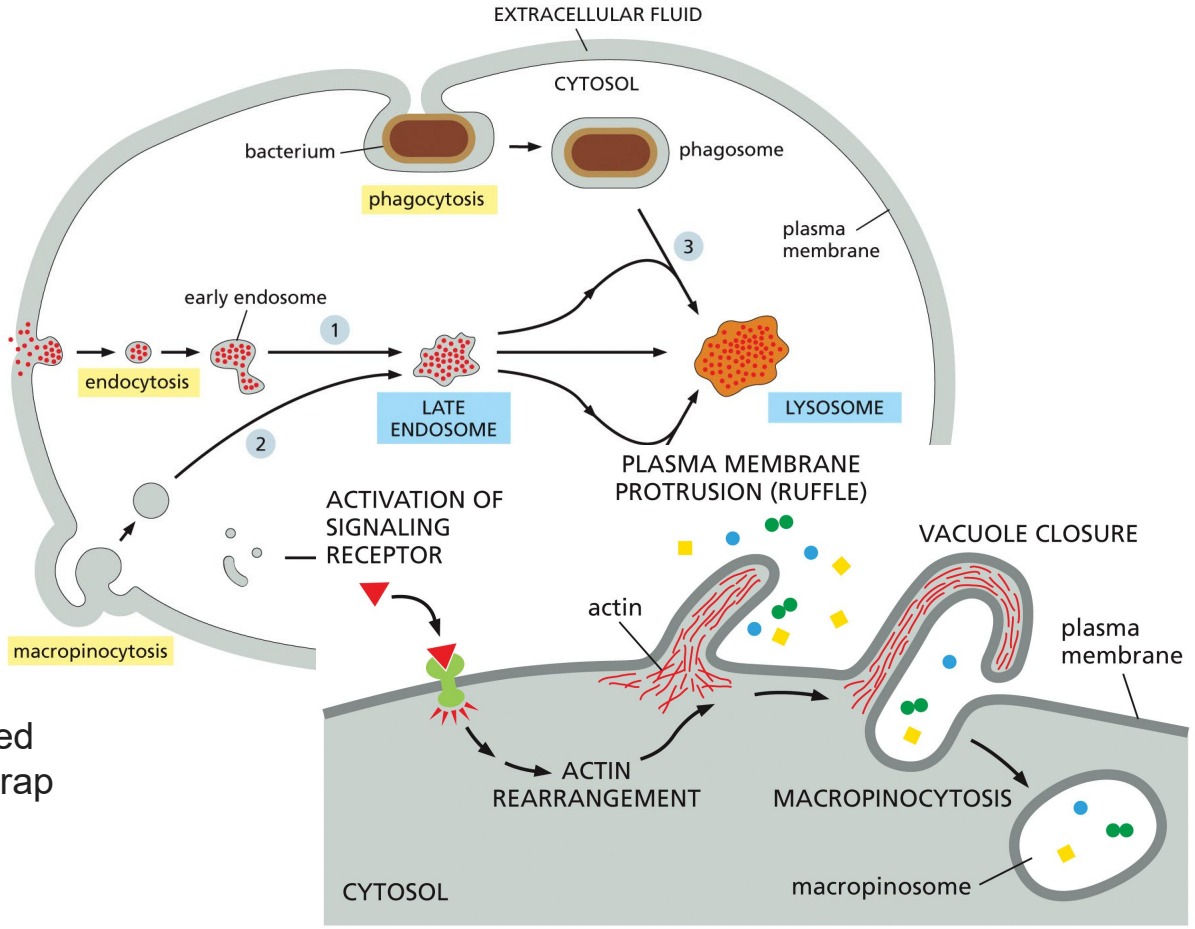
- Multiple pathways deliver materials to lysosomes

Endocytosis



THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

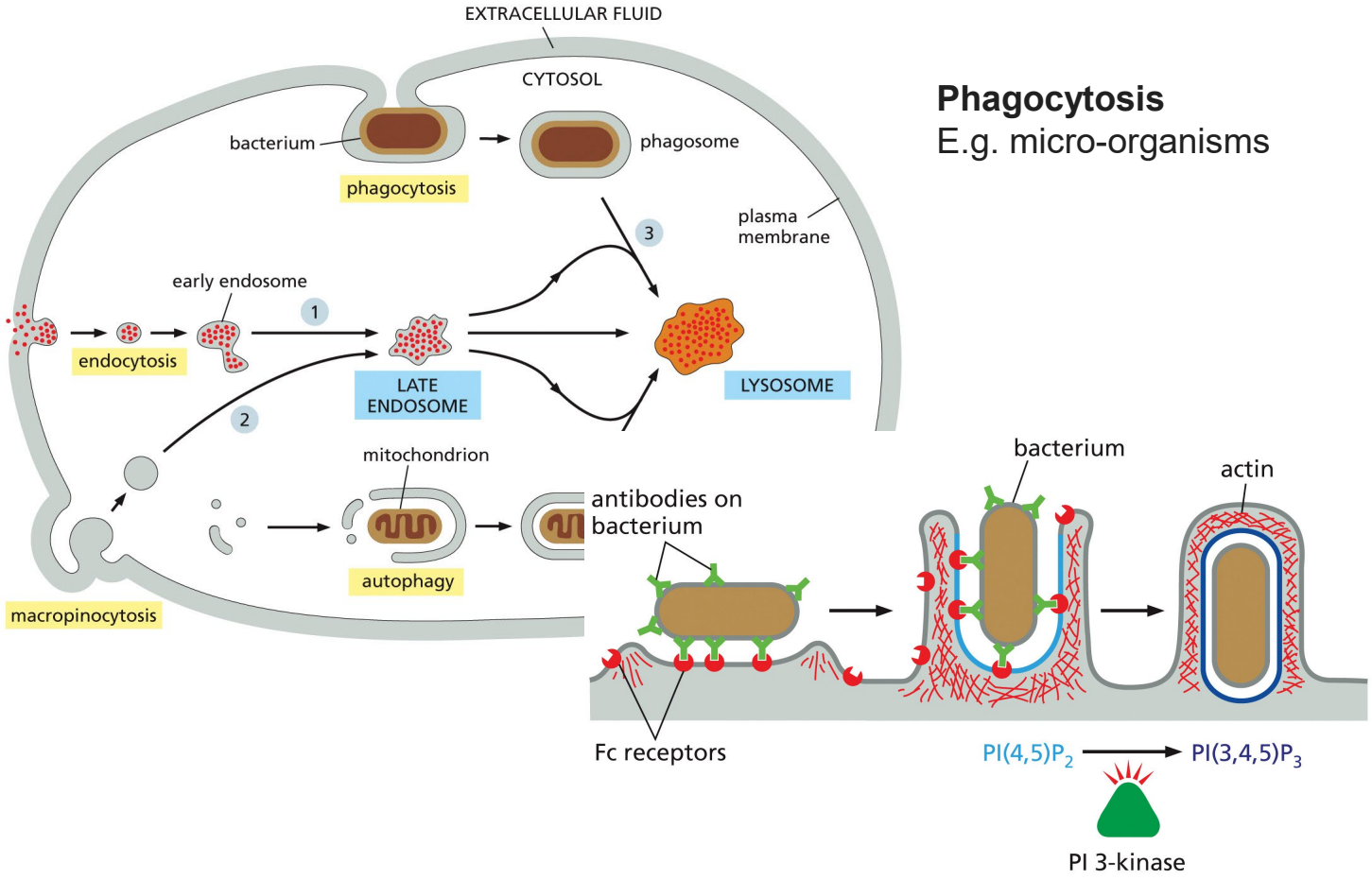
- Multiple pathways deliver materials to lysosomes



Macropinocytosis
Cell-surface ruffles formed by actin nonspecifically trap extracellular fluid and macromolecules and particles

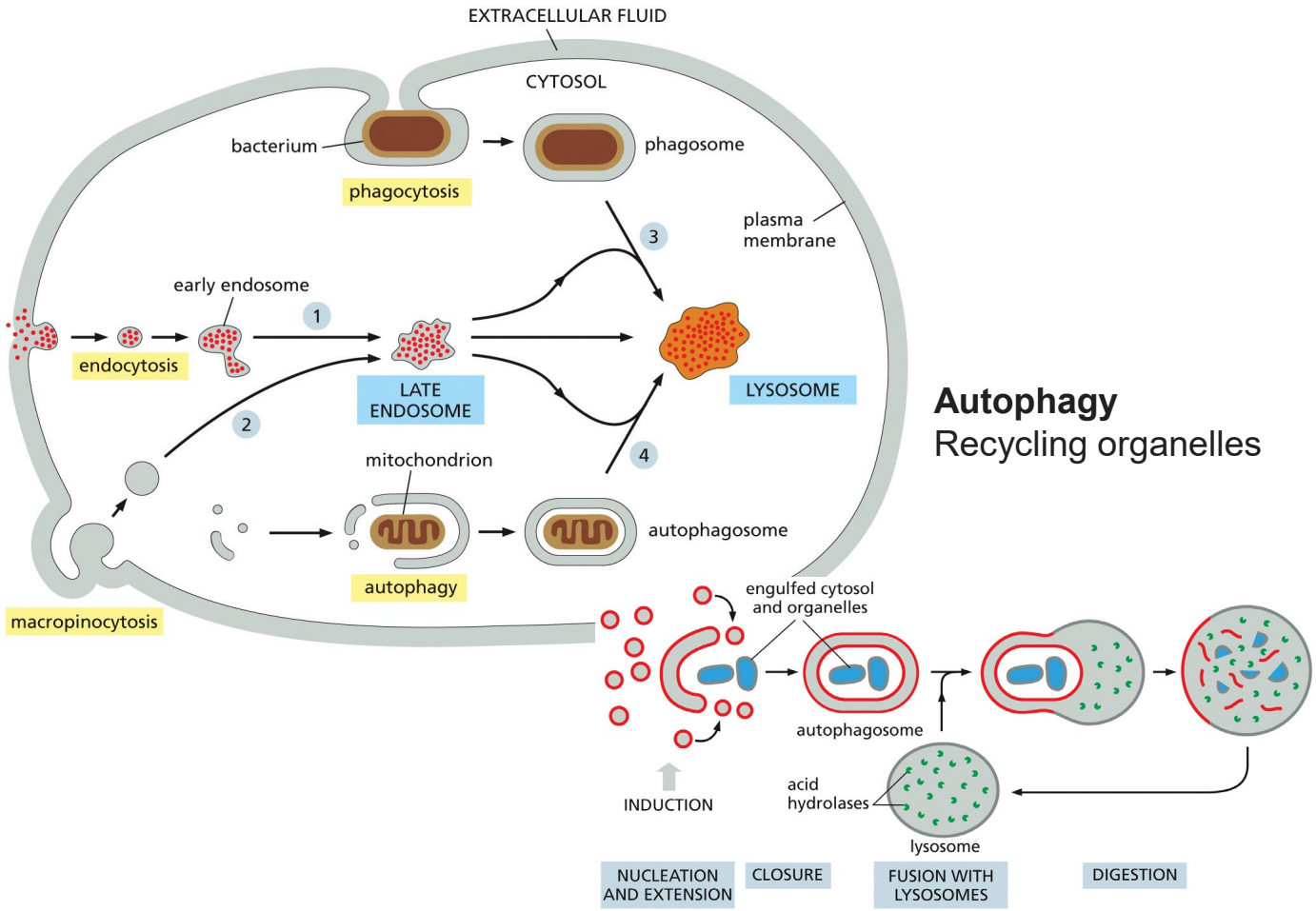
THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

- Multiple pathways deliver materials to lysosomes



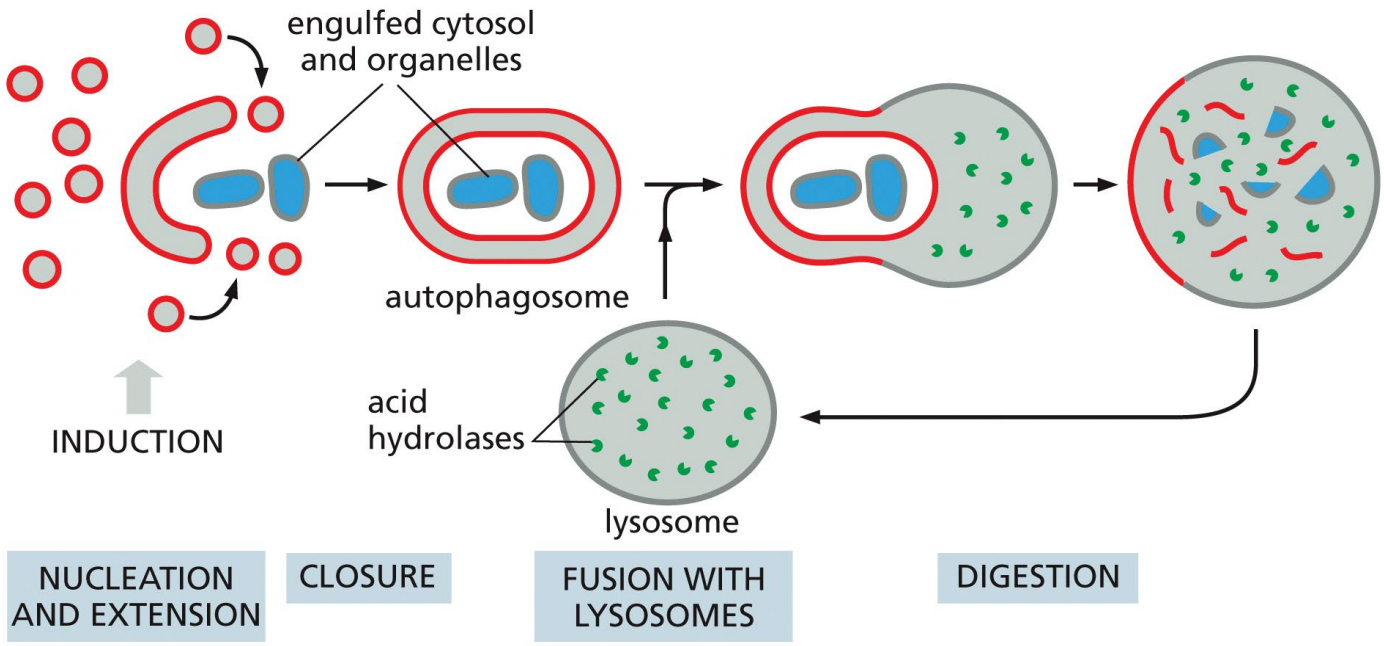
THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

- Multiple pathways deliver materials to lysosomes



THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

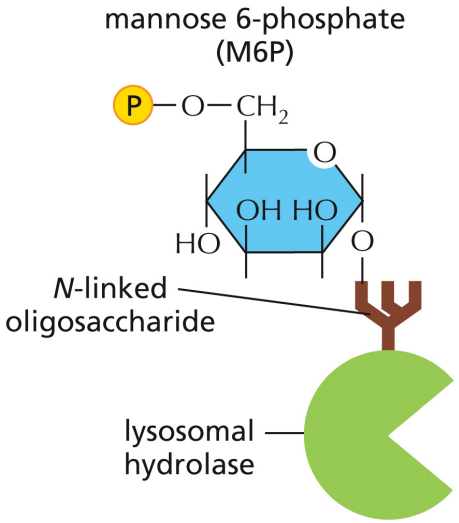
- **Autophagy degrades unwanted proteins and organelles**



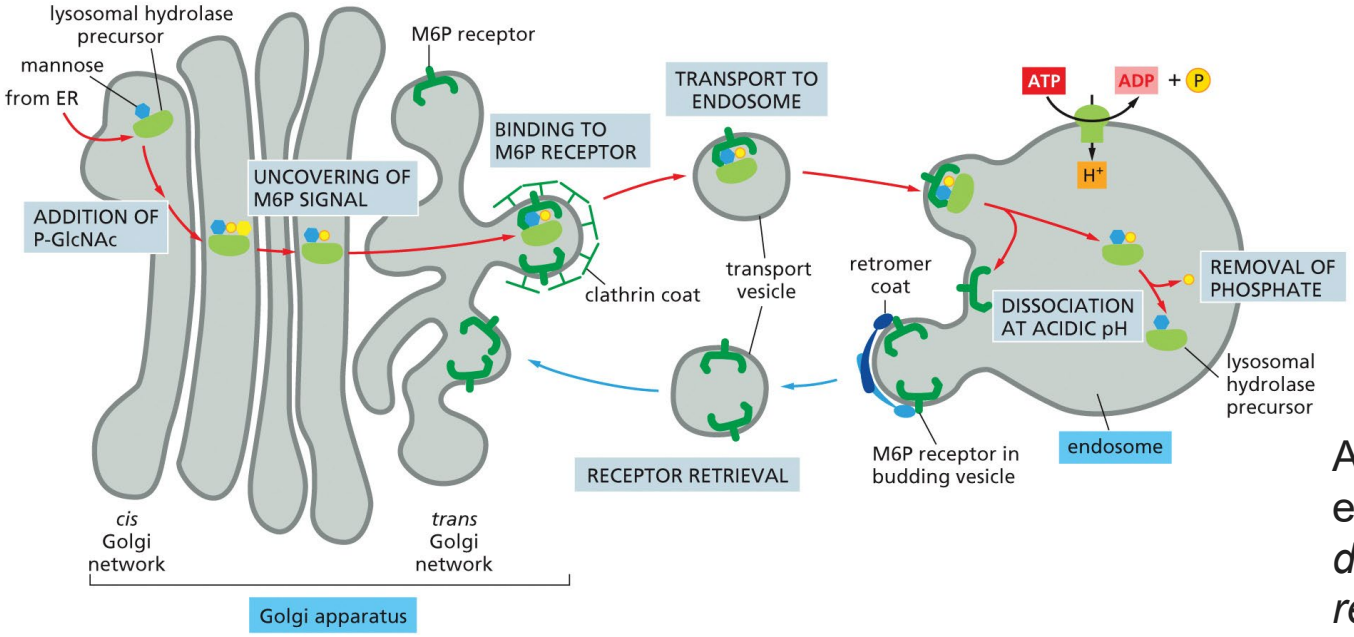
1. Activation of a signaling pathway initiates a nucleation event in the cytoplasm
2. Autophagosomal membrane grows by fusion of vesicles of unknown origin → fuses to form a double-membrane-enclosed autophagosome → sequesters a portion of the cytoplasm
3. The autophagosome then fuses with lysosomes containing acid hydrolases that digest its content

TRANSPORT OF LYSOSOMAL HYDROLASES TO ENDOSOMES

- A mannose 6-phosphate (M6P) receptor sorts lysosomal hydrolases in the trans Golgi network



Adaptor proteins in the *clathrin coat* bind *M6P receptors*, which bind the *M6P-modified lysosomal hydrolases*

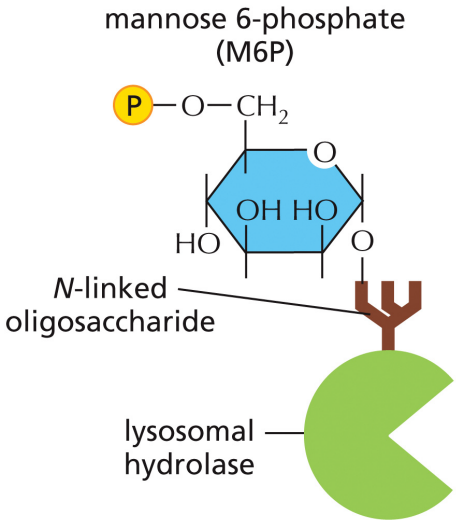


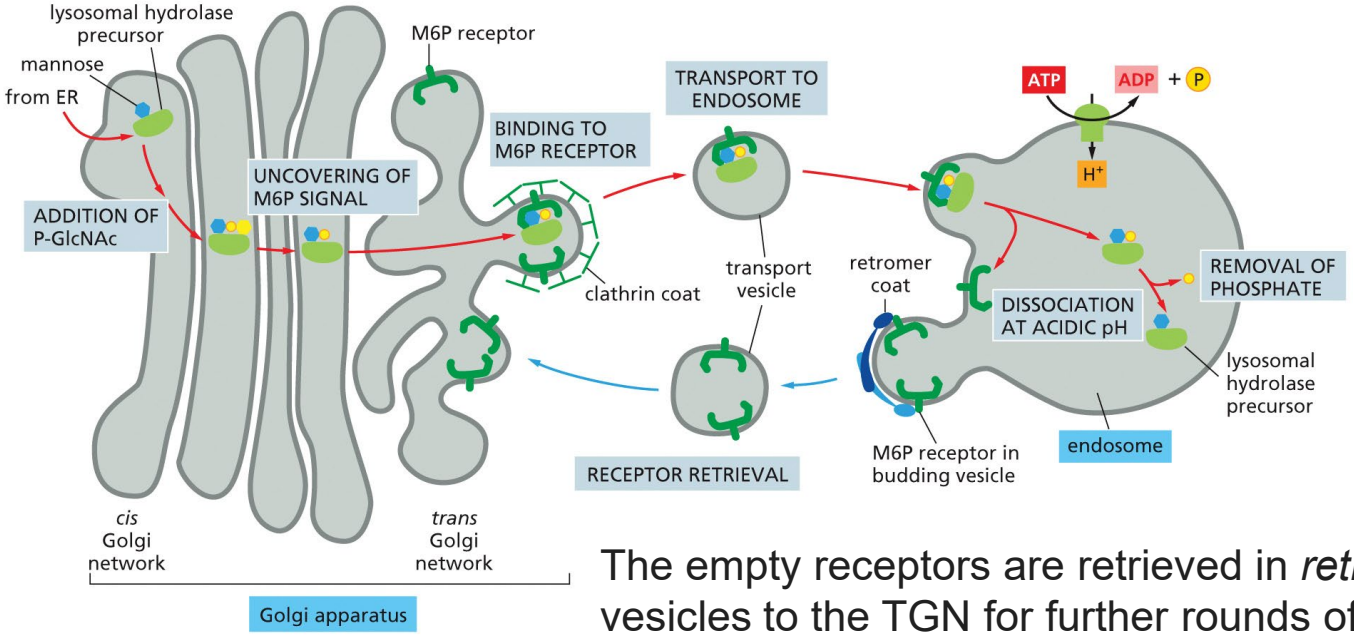
Clathrin-coated vesicles bud off from the TGN, shed their coat, and fuse with early endosomes.

At the *lower pH* of the endosome, the *hydrolases dissociate from the M6P receptors*

TRANSPORT OF LYSOSOMAL HYDROLASES TO ENDOSOMES

- A mannose 6-phosphate (M6P) receptor sorts lysosomal hydrolases in the trans Golgi network

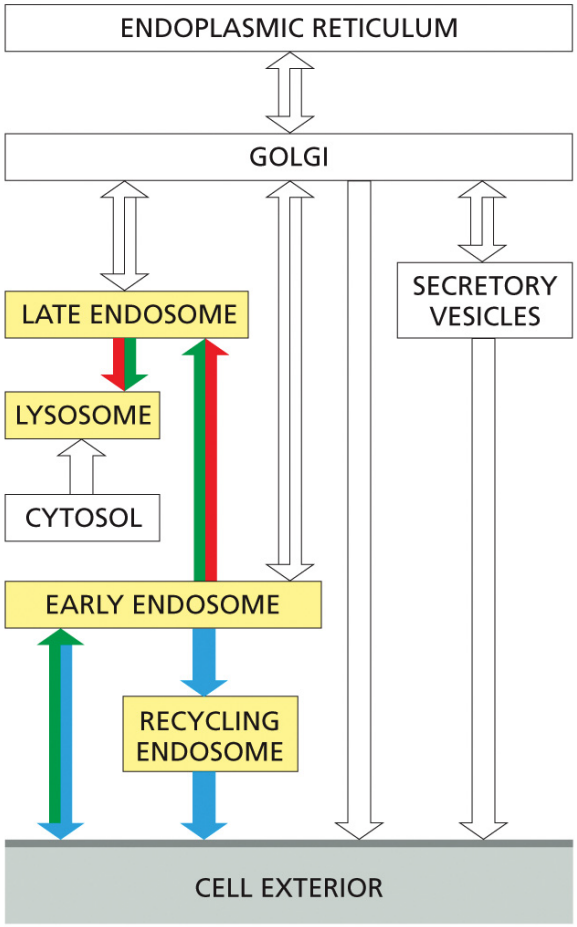


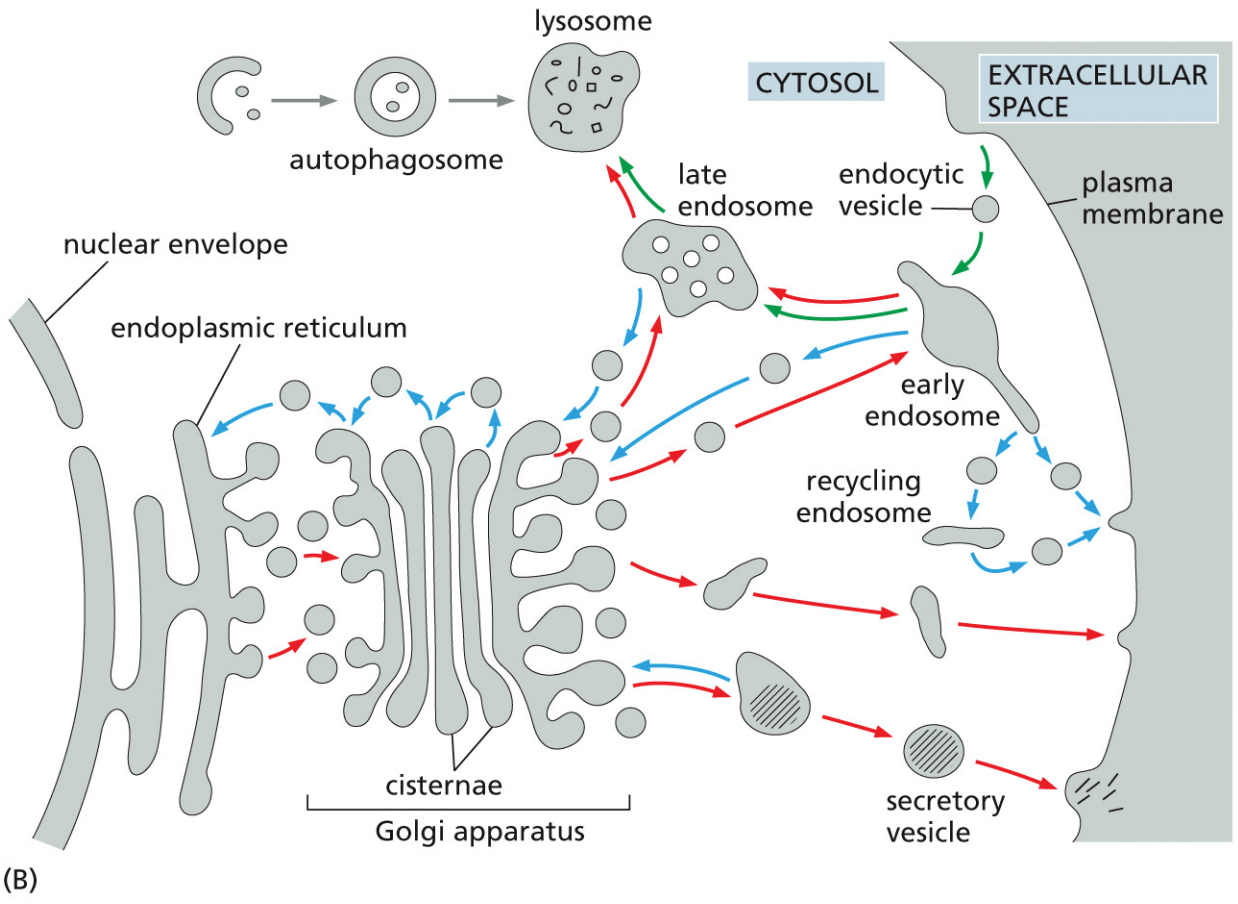


The phosphate is removed from the M6P attached to the hydrolases (further ensures that the hydrolases do not return to the TGN with the receptor)

The empty receptors are retrieved in *retromer-coated* vesicles to the TGN for further rounds of transport

THE **ENDOCYTIC PATHWAY** FROM THE PLASMA MEMBRANE TO LYSOSOMES



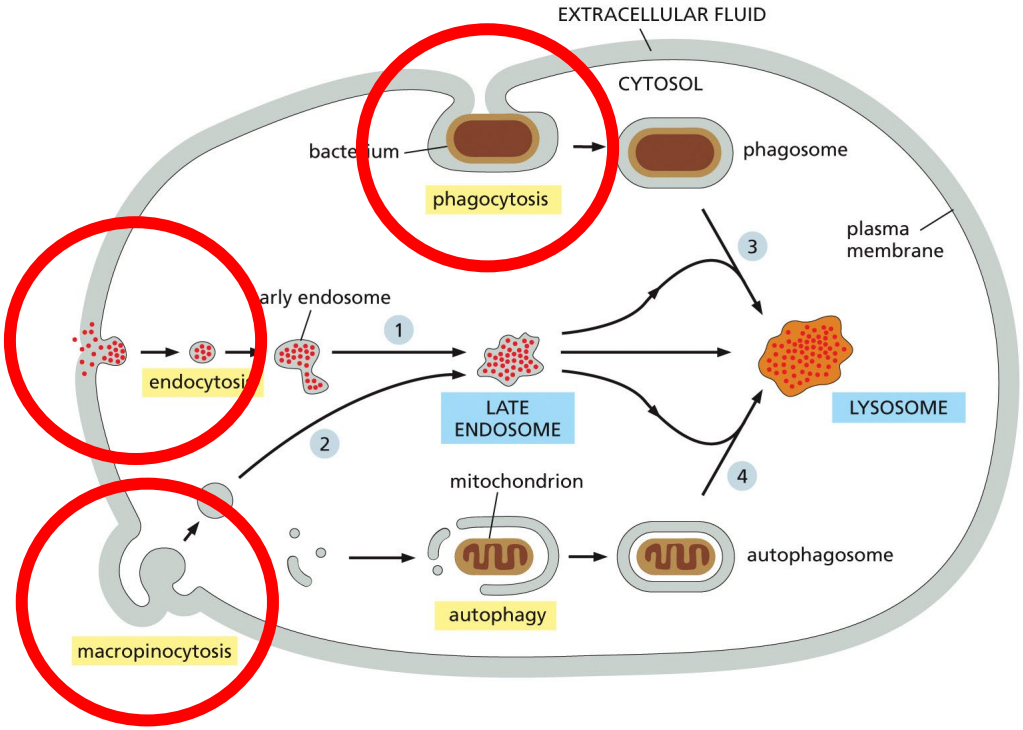


endocytic and secretory pathways
+ retrieval pathways

The route inward

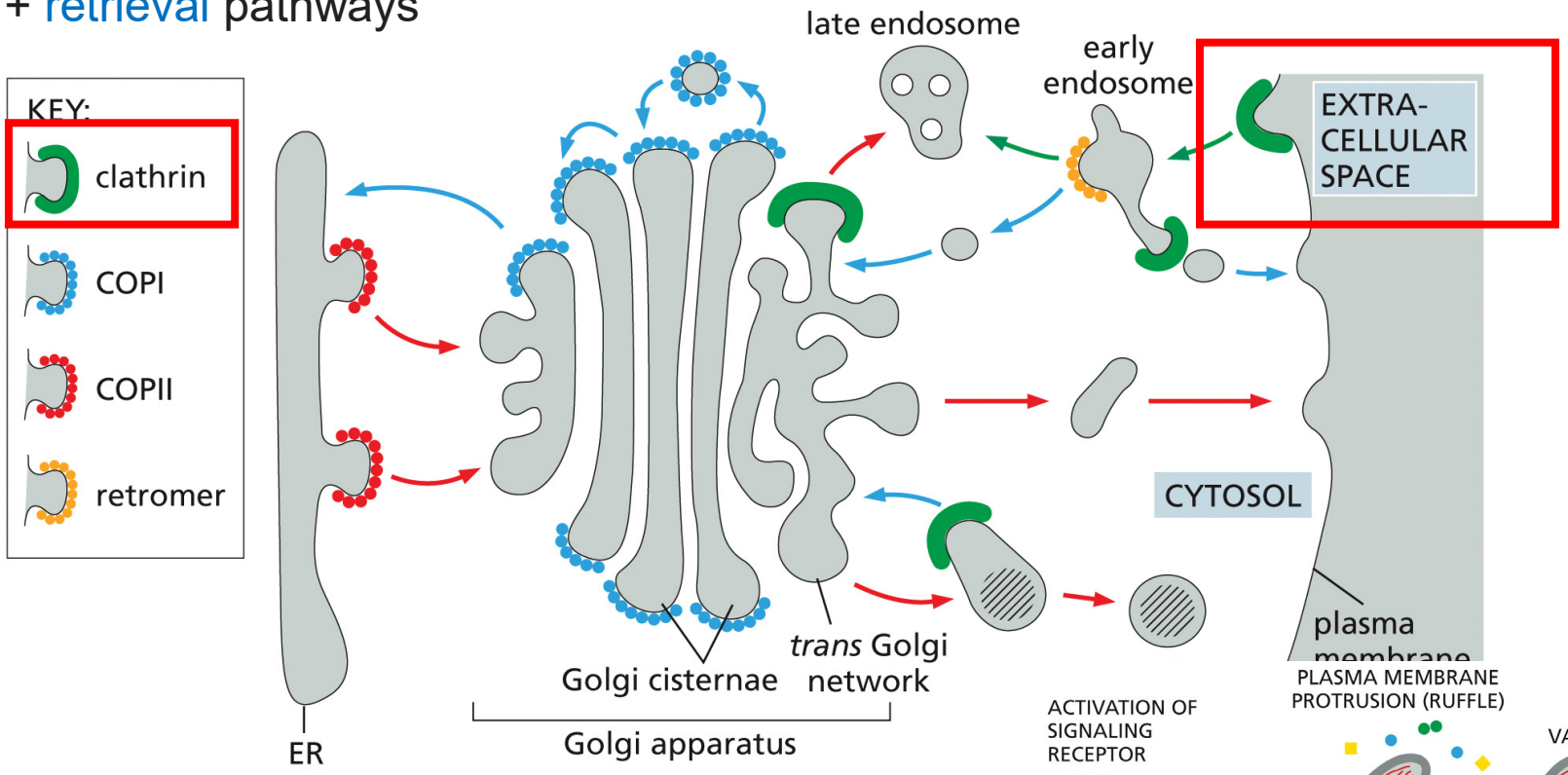
THE DEGRADATION AND RECYCLING OF MACROMOLECULES IN LYSOSOMES

- Multiple pathways deliver materials to lysosomes

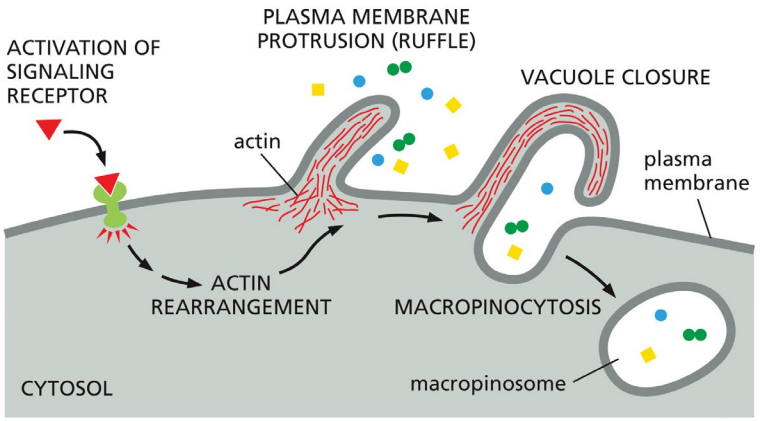


DIFFERENT COATS FOR DIFFERENT STEPS IN VESICLE TRAFFIC

endocytic and secretory pathways
+ retrieval pathways

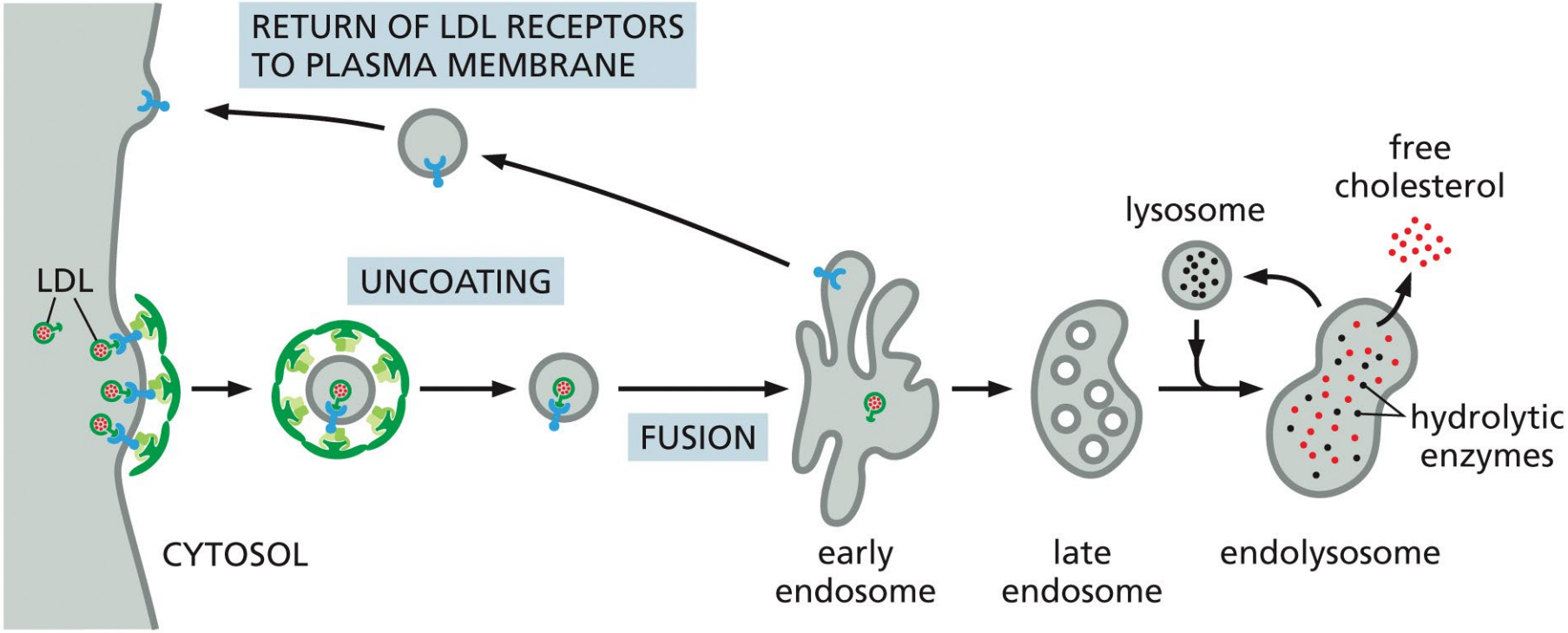


- Clathrin-coated pits initiate pinocytosis
- Pinocytosis can also take place without clathrins, e.g. in macropinocytosis



TRANSPORT INTO THE CELL FROM THE PLASMA MEMBRANE: ENDOCYTOSIS

- Specific proteins are retrieved from early endosomes and returned to the plasma membrane



SUMMARY

endocytic and secretory pathways
+ retrieval pathways

- Mechanisms of membrane transport and compartment identity
- Transport from the endoplasmic reticulum through the Golgi apparatus
- Transport from the trans Golgi network to the cell exterior and endosomes
- Transport into the cell from the plasma membrane: endocytosis
- The degradation and recycling of macromolecules in lysosomes