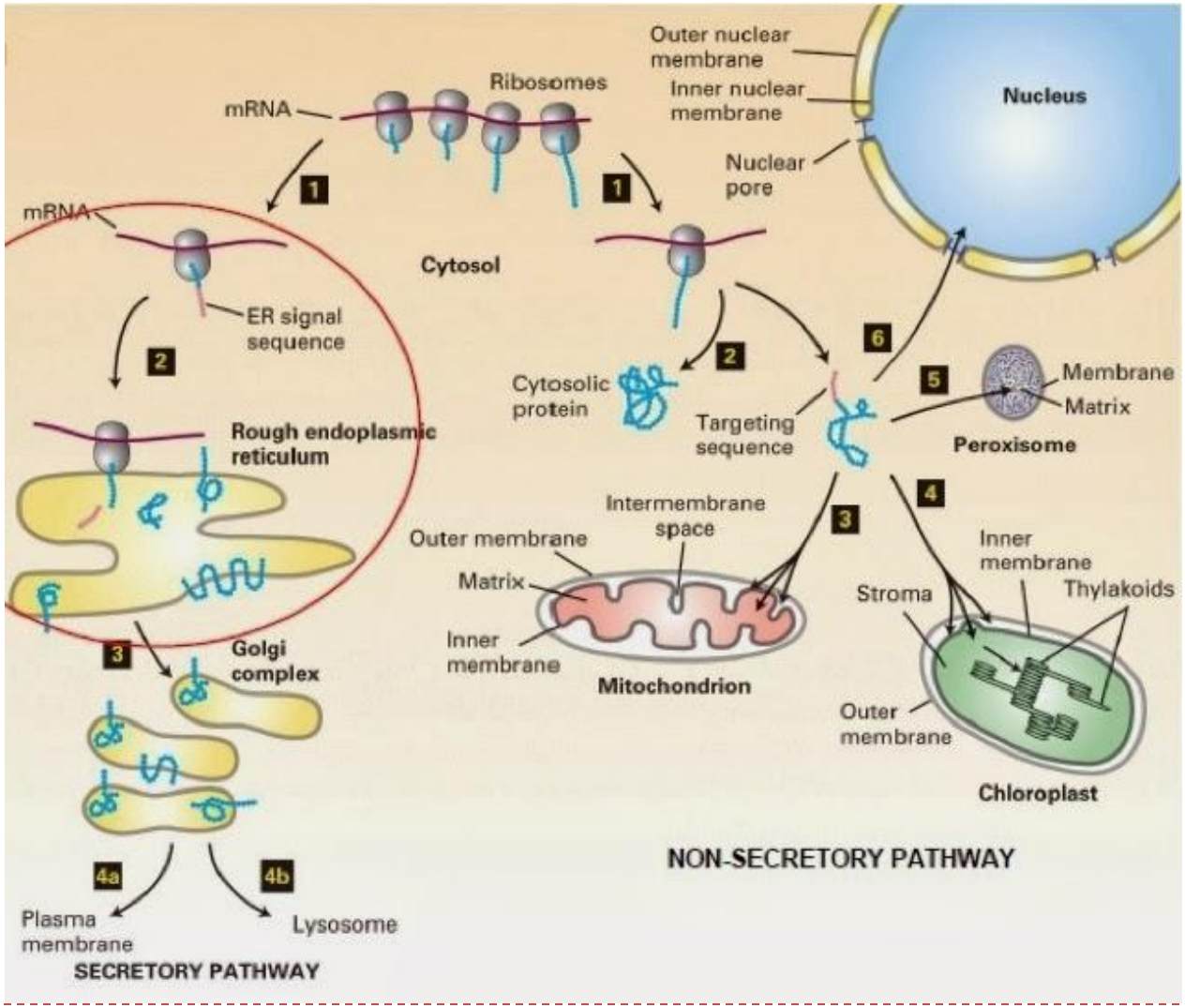
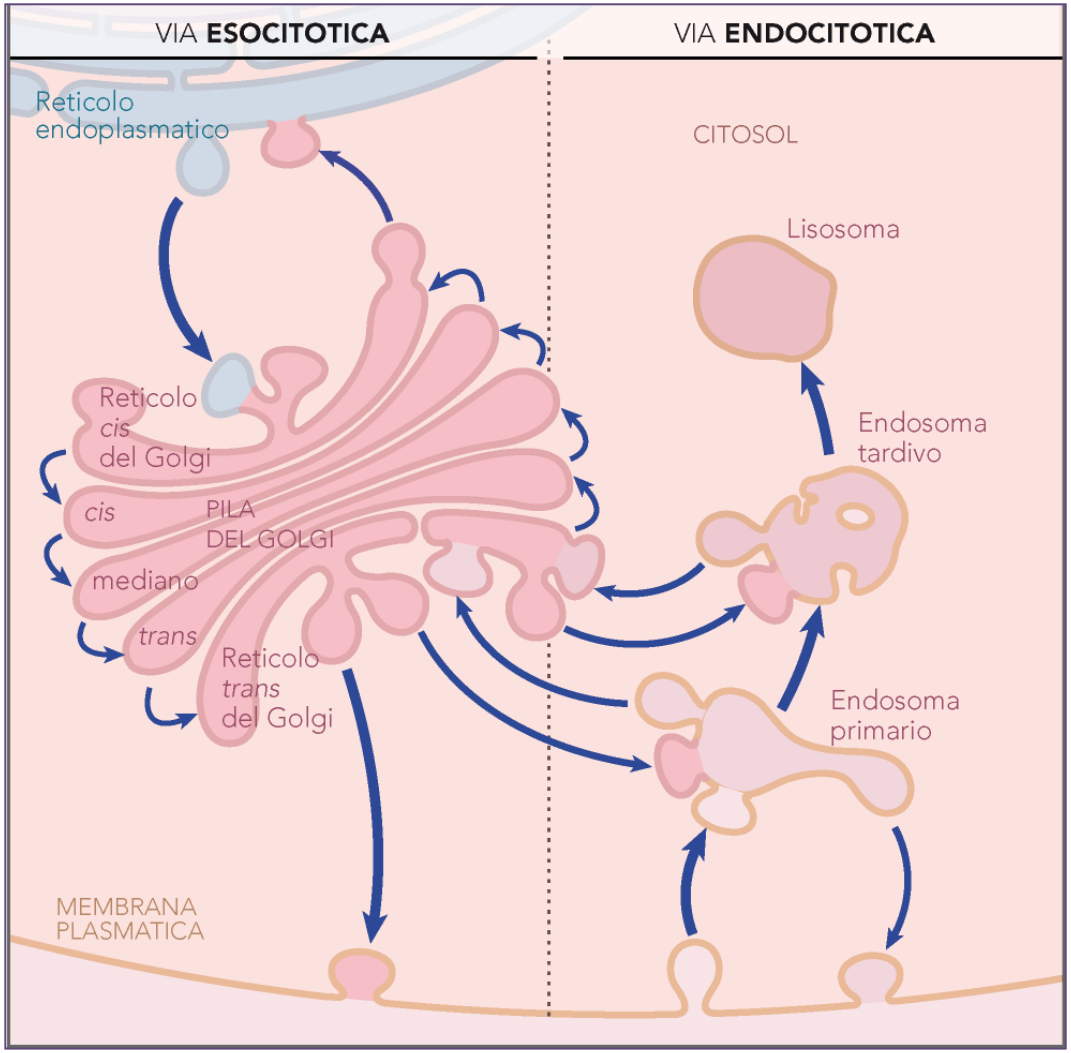
Chapter 1: Vesicular traffic

Biochimica cellulare parte B – 2018/19

Major Protein-sorting pathways in eukaryotic cells



Secretory and endocytic pathways

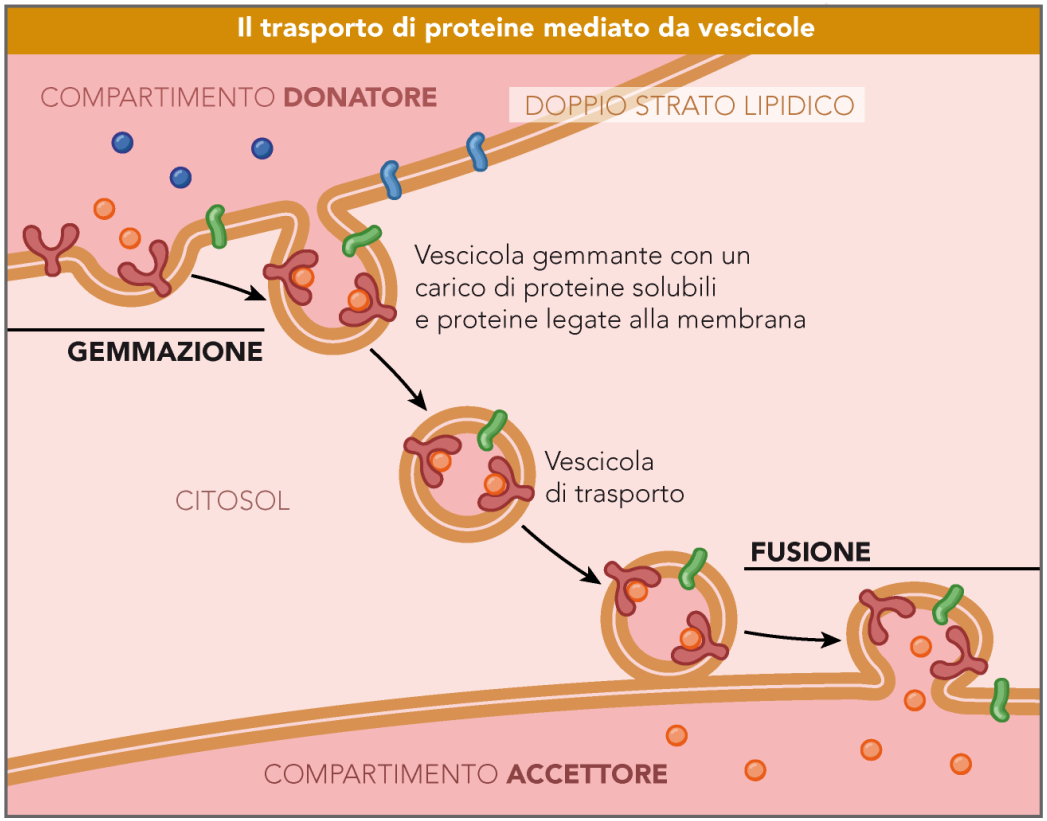


Vesicular transport

✓Unifying principle governs all protein trafficking in **secretory** and **endocytic pathways**: transport of membrane and soluble proteins from one membrane-bounded compartment to another is mediated by **transport vesicles.**

✓Vesicles collect **"cargo" proteins** in buds arising from the membrane of one compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment

✓The same face of the membrane remains oriented toward the cytosol



Each step in the secretory and endocytic pathways employs a different type of vesicle, but each of the different vesicular transport steps is simply a variation on a common theme.

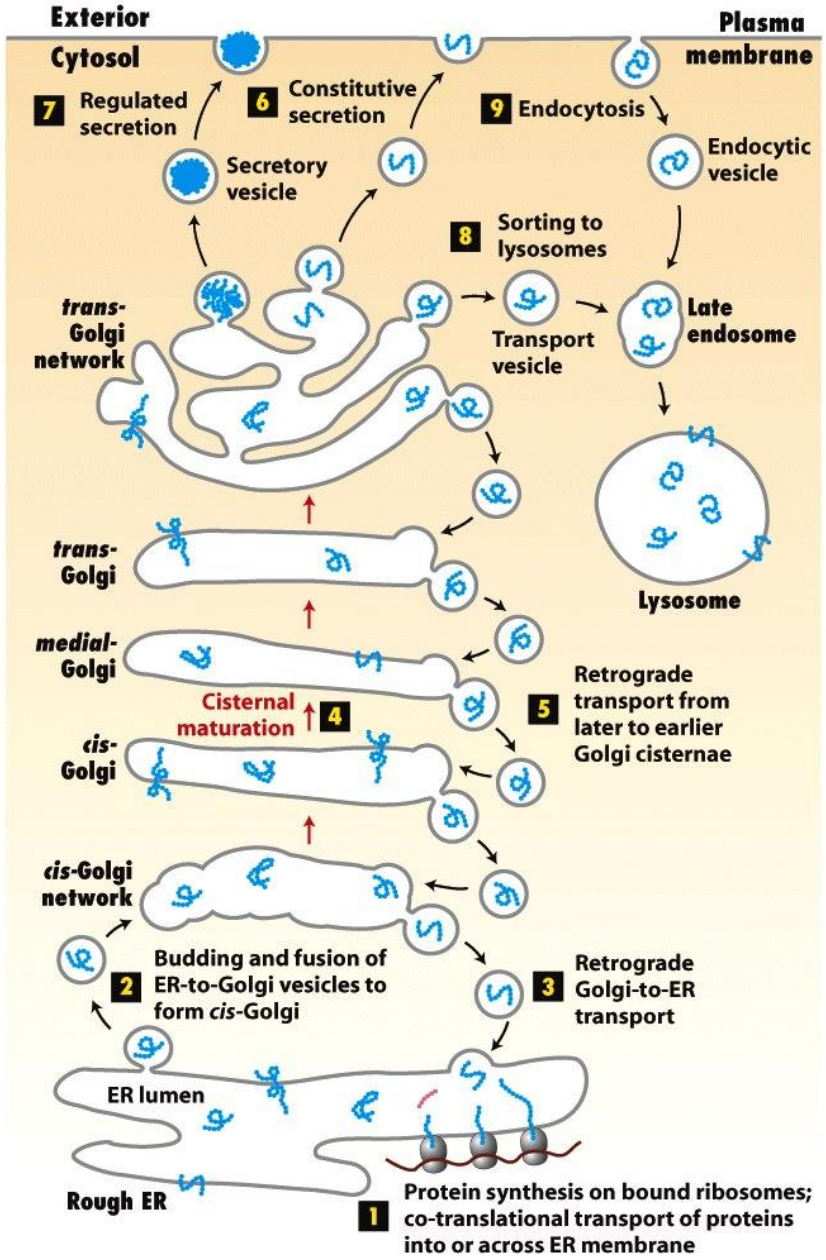


Figure 14-1
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Major routes for protein trafficking in the secretory pathway

Anterograde and retrograde transport vescicles

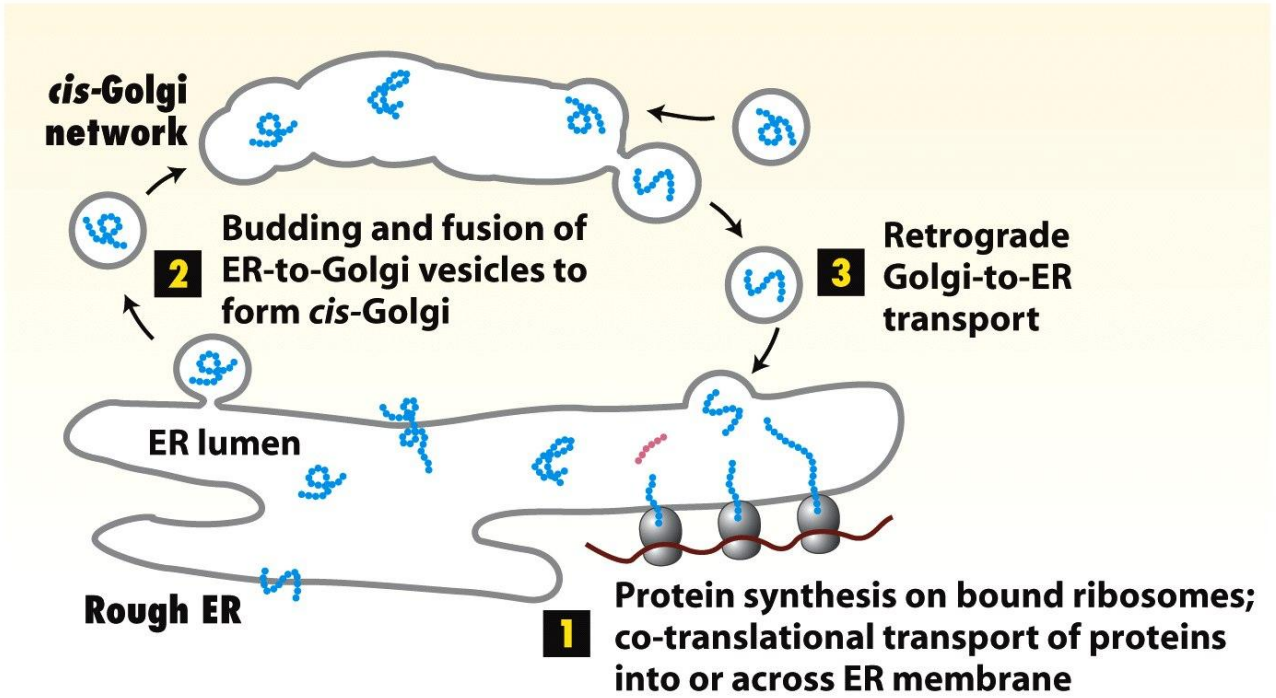

Figure 14-1 part 1
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Exocytosis and endocytosis

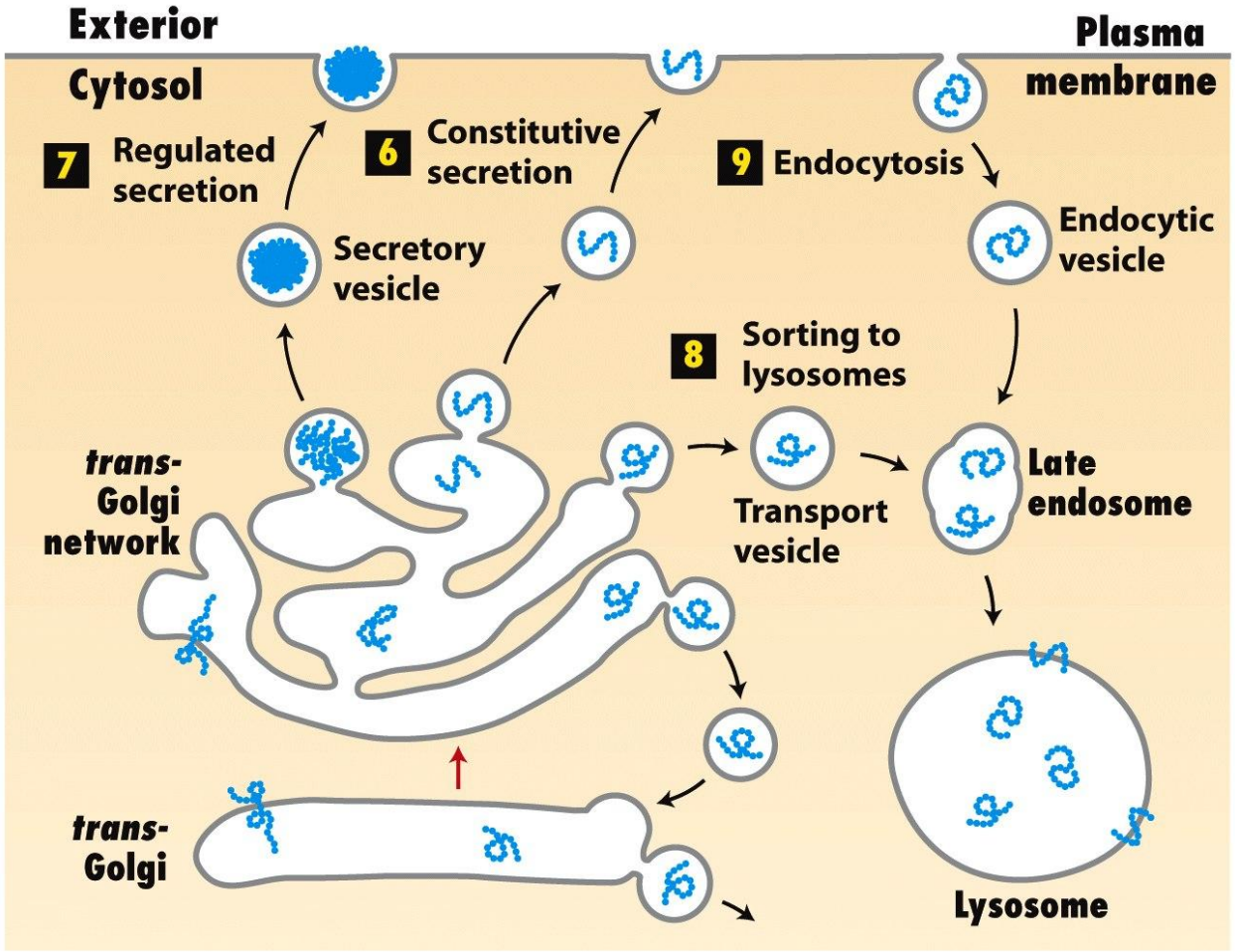

Figure 14-1 part 3
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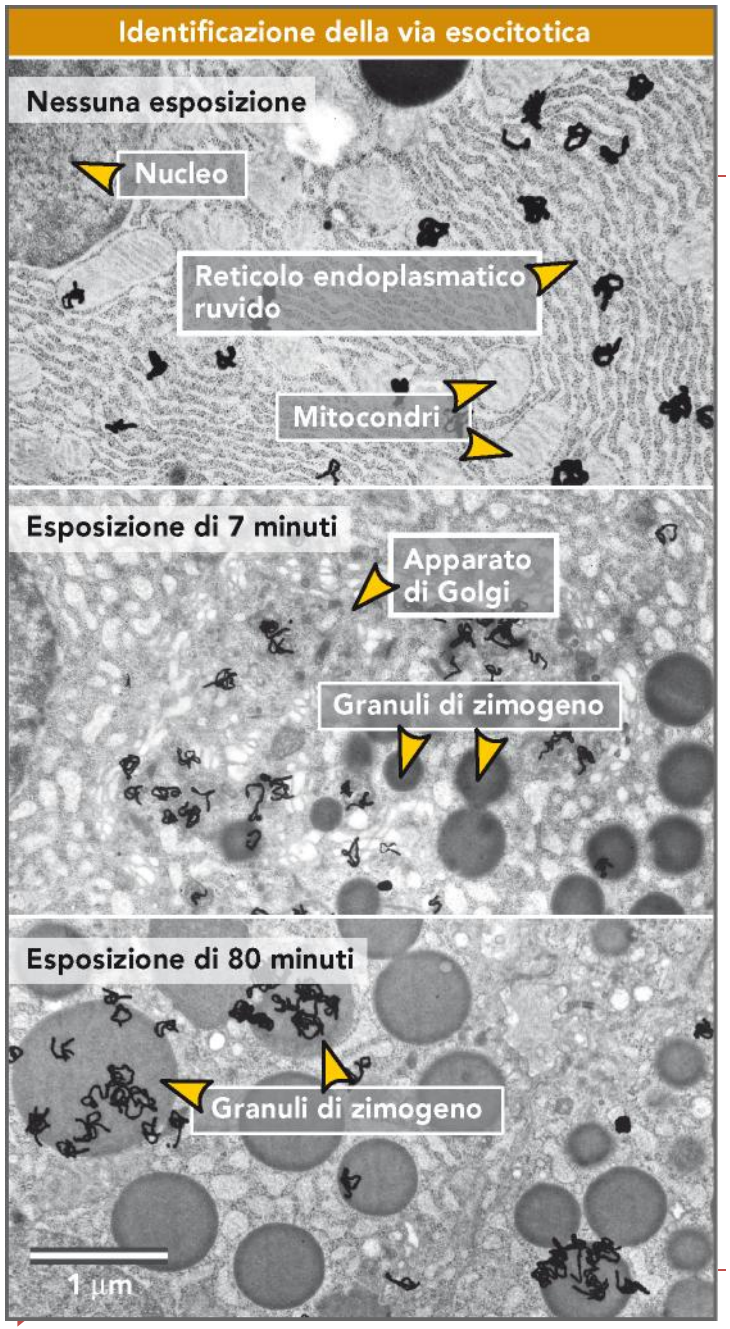

Studies to establish the order in which proteins move from organelle to organelle in the secretory pathway.

Many components required for the formation and fusion of transport vesicles have been identified in the past decade by a remarkable convergence of genetic and biochemical approaches.

The classic studies of G. Palade (1960s) first established 1) the order in which proteins move from organelle to organelle in the secretory pathway 2) These studies showed that secretory proteins were never released into the cytosol.

pulse-chase labeling on pancreatic acinar cells

Fluorescence microscopy of VSVG-GFP fusion protein

A procedure for observing the intracellular trafficking of a secretory protein in almost any type of cell

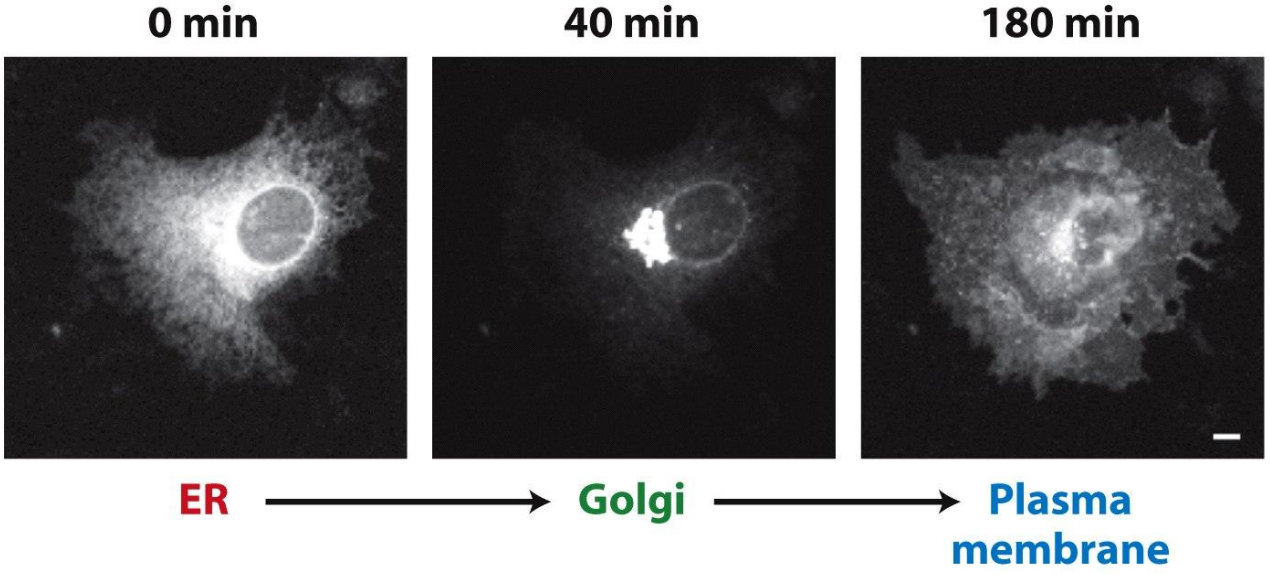

Figure 14-2a
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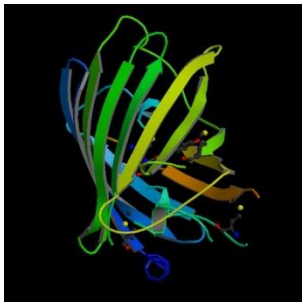

GFP protein

Gene encoding **a temperature-sensitive mutant** of the membrane **glycoprotein G** from vesicular stomatitis virus (VSV), fused to GFP protein has been introduced into cultured mammalian cells by transfection (VSVG-GFP).

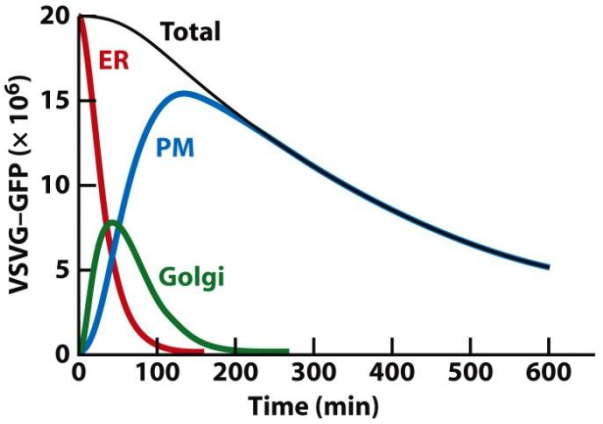

Figure 14-2b
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Transport from the ER to the Golgi can be assayed based on sensitivity to cleavage by endoglycosidase D

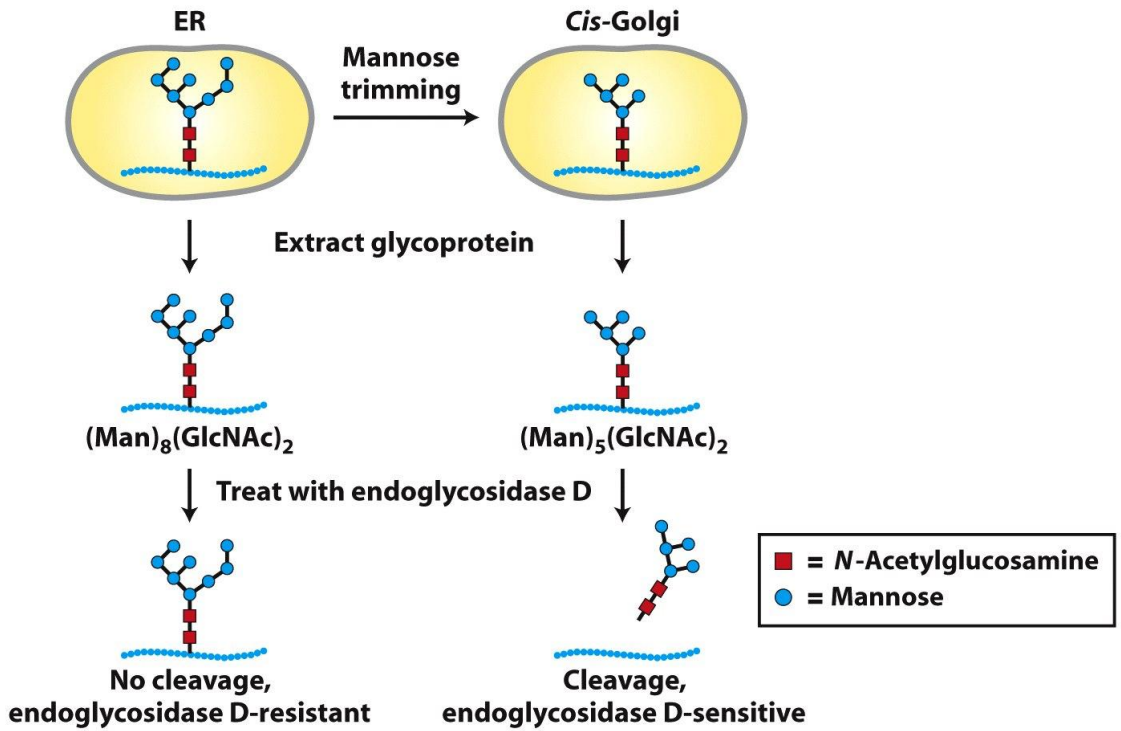

Figure 14-3a
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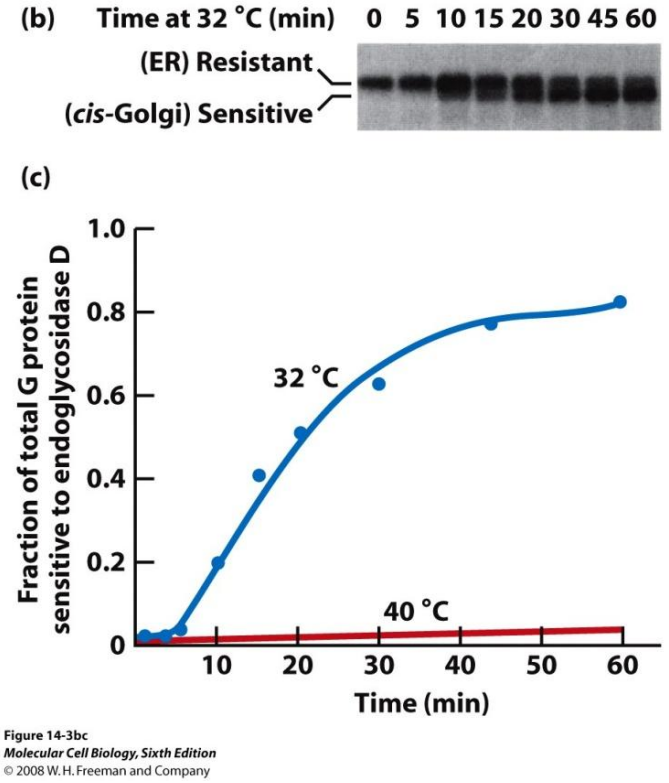

Figure 14-3bc
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Cis-Golgi maturation of VSV-G protein from vesicular stomatitis virus (VSV)

Tracking movement of VSV-G protein in virus-infected cells pulse-labeled with radioactive amino acids.

Phenotypes of yeast *sec* mutants identified stages in the secretory pathway

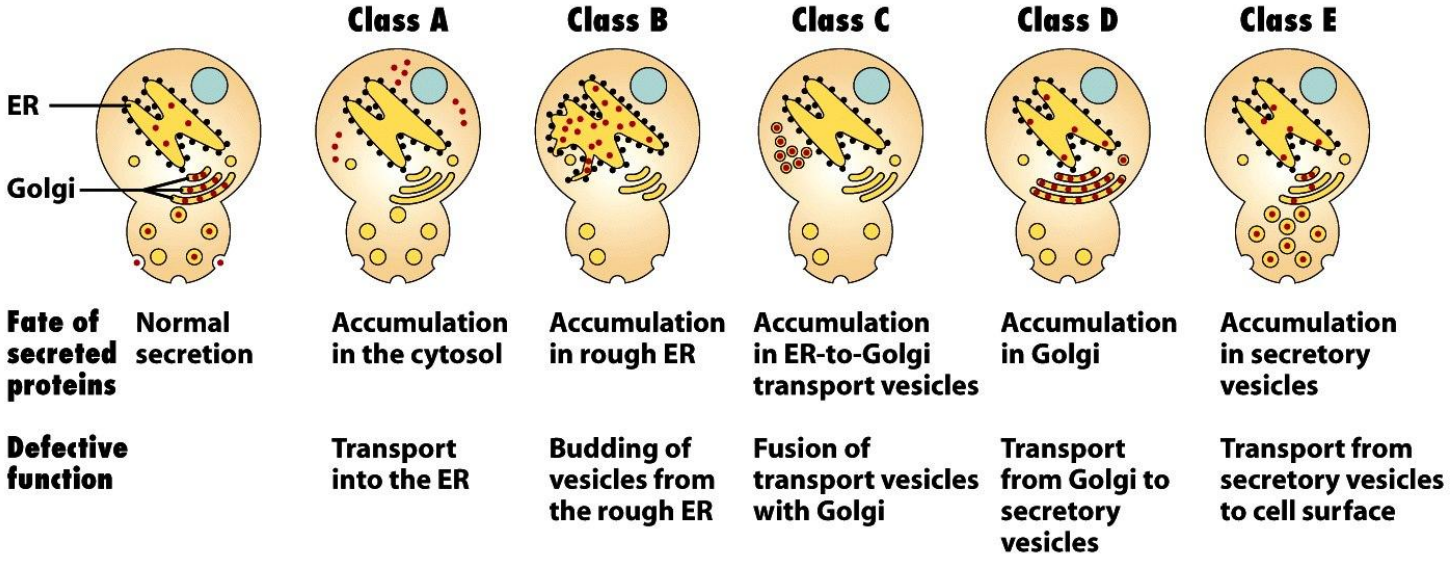

Figure 14-4
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Many of the components required for intracellular protein trafficking have been identified in yeast by analysis of **temperature-sensitive sec mutants** defective for the secretion of proteins at the **nonpermissive** temperature. Analysis of such mutants identified **5 classes** (A–E) characterized by protein accumulation in different compartments.

Characterization of *sec* mutants in the various classes has helped elucidate the fundamental components and molecular mechanisms of vesicle trafficking.

Analysis of haploid double *sec* mutants determined the order of the steps in the pathway.

Basic mechanisms underlying vesicle budding and fusion.

Each step in the secretory and endocytic pathways employs **a different type of vesicle**. Each of the different vesicular transport steps is simply a **variation on a common theme.**

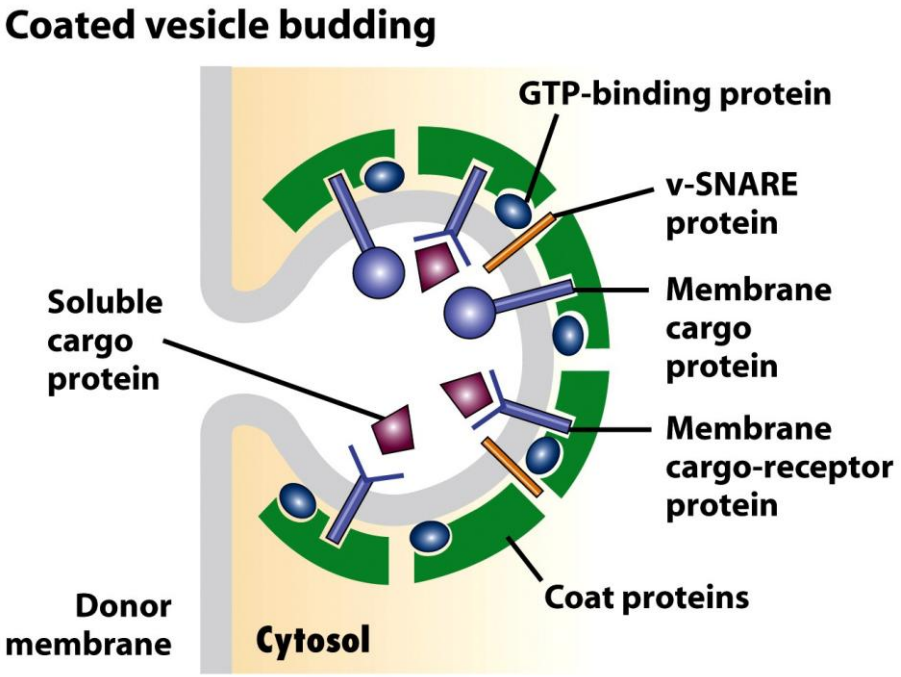

Figure 14-6a
Molecular Cell Biology, Sixth Edition
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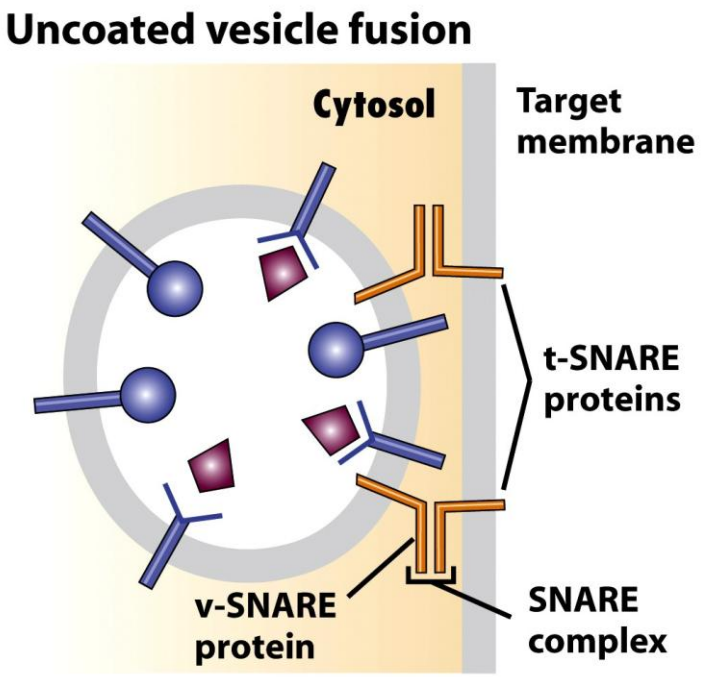

Figure 14-6b
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The budding of vesicles is driven by the polymerization of soluble protein complexes onto the membrane to form a proteinaceous vesicle coat. The integral membrane proteins include v-SNAREs, which are crucial to eventual fusion of the vesicle with the correct target membrane.

In vitro budding reactions of a coated vescicle

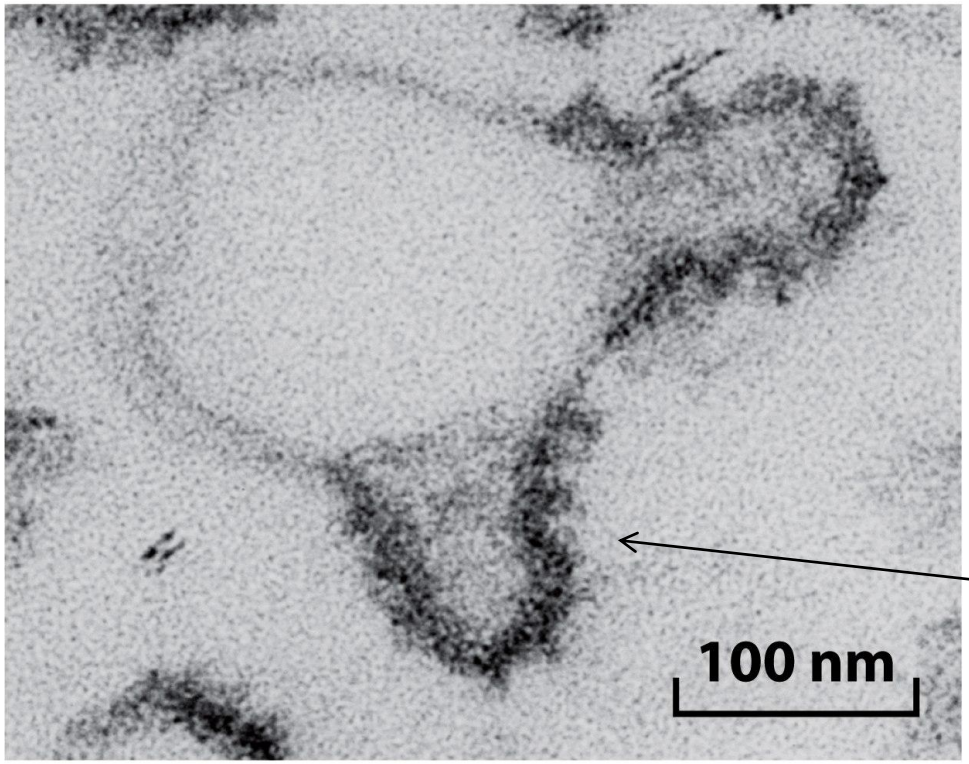


Figure 14-7
Molecular Cell Biology, Sixth Edition

Isolated or artificial membranes and **purified coat proteins**. Polymerization of the coat proteins onto the cytosolic face of the parent membrane is necessary to produce the high curvature of the membrane

Vesicle buds

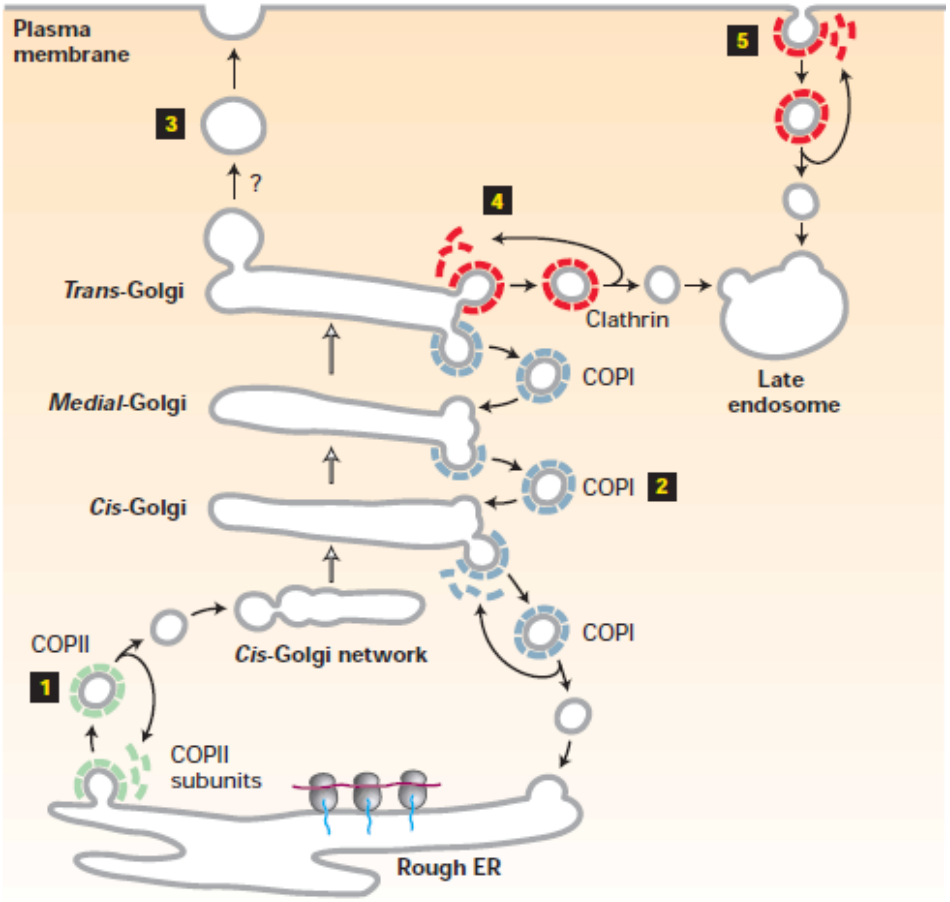
Types of coated vesicles

TABLE 17-1 Coated Vesicles Involved in Protein Trafficking

Vesicle Type	Coat Proteins	Associated GTPase	Transport Step Mediated
COPII	Sec23/Sec24 and Sec13/Sec31 complexes, Sec16	Sar1	ER to *cis*-Golgi
COPI	Coatomers containing seven different COP subunits	ARF	*cis*-Golgi to ER Later to earlier Golgi cisternae
Clathrin and adapter proteins*	Clathrin + AP1 complexes	ARF	*trans*-Golgi to endosome
	Clathrin + GGA	ARF	*trans*-Golgi to endosome
	Clathrin + AP2 complexes	ARF	Plasma membrane to endosome
	AP3 complexes	ARF	Golgi to lysosome, melanosome, or platelet vesicles

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

Major types of coat proteins in vesicular traffic in the secretory and endocytic pathways.

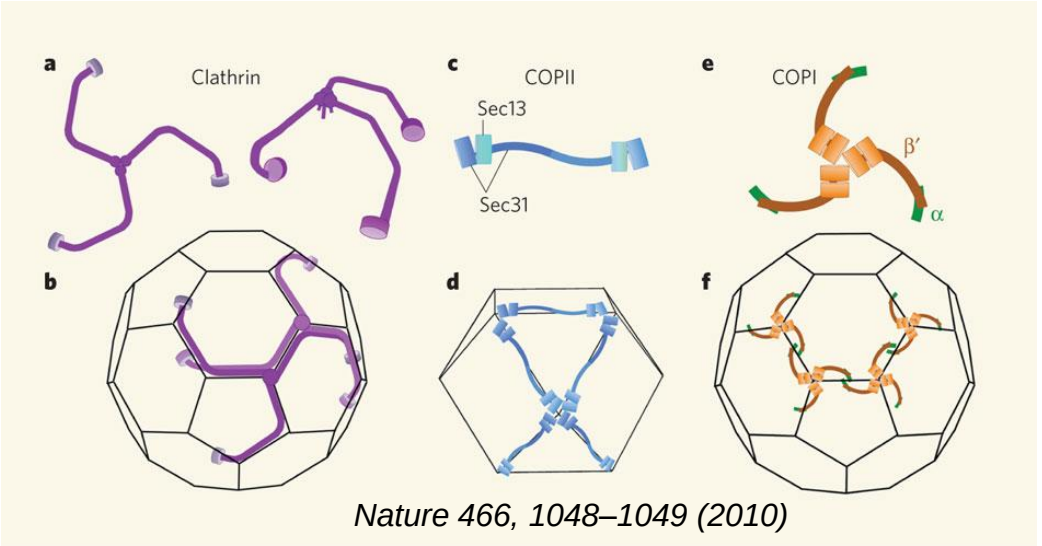


The budding of vesicles is driven by the polymerization of soluble protein complexes onto the membrane to form a **proteinaceous vesicle coat** The coat functions:

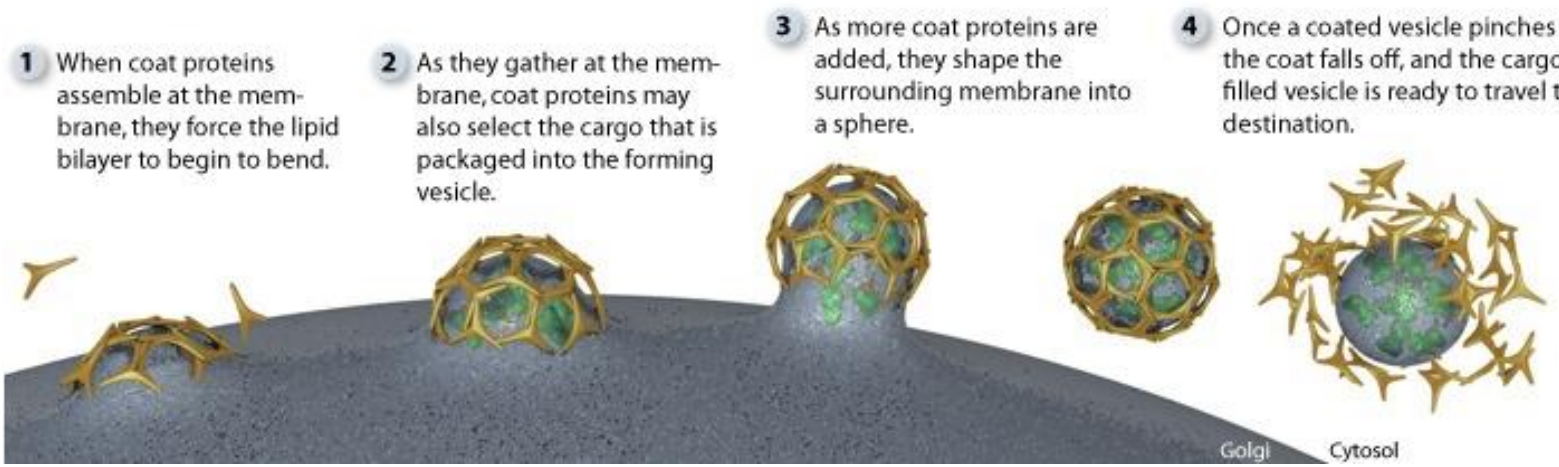
1) **acts as the filter** to determine which proteins are admitted into the vesicle.
2) **adds curvature** to the membrane to form a vesicle

Budding Vescicles wear coats

Some vesicles form with the help of coat proteins. Geometrically arranged coat proteins on the surface of the membrane help the vesicle to bud off.



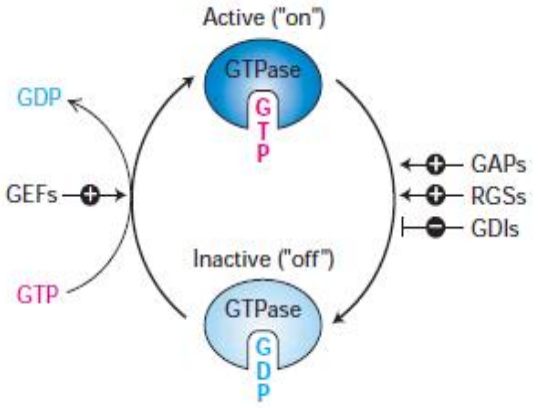
Nature 466, 1048–1049 (2010)



http://learn.genetics.utah.edu/content/cells/vesicles/

A Set of GTPase Switch Proteins Controls Assembly of Vesicle Coats

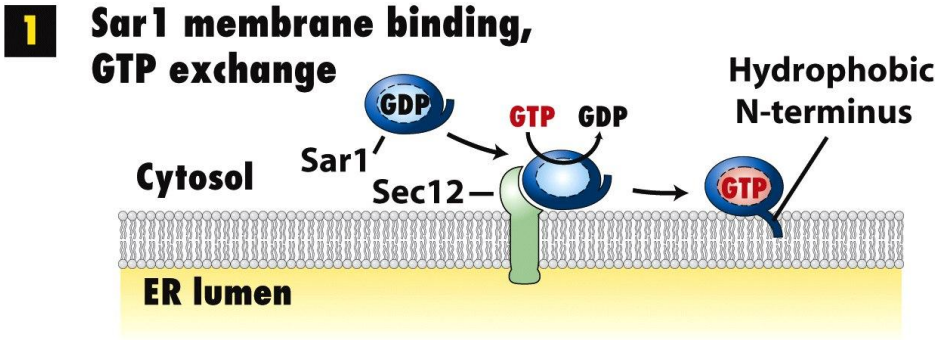
The coats of all three vesicles contain a **small GTP-binding protein:** acts as a **regulatory subunit** to control coat assembly.
Sar1 is present in the coat of COPII vesicles. **ARF** is the GTPase used by COPI and clathrin vesicles.



Both ARF and Sar1 are **monomeric GTPase** of **switch proteins** that exchange GDP/GTP.

Cycling of GTPase switch proteins between the active and inactive forms. Activation is promoted by GEFs (guanine nucleotide–exchange factors).

Binding of GTP to Sar1 (ARF) promoted by the GEF **Sec12** causes a conformational change in **Sar1** that exposes its hydrophobic N-terminus,



Sar1 couples a cycle of GTP binding and hydrolysis to the formation and then dissociation of the COPII coat

Figure 14-8 part 2
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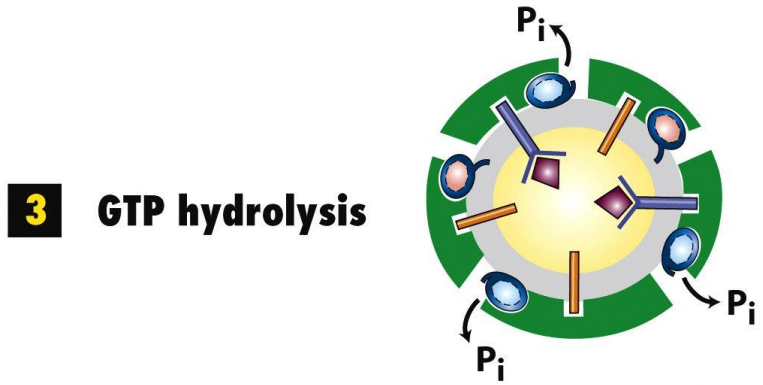
2. The membrane-attached Sar1 GTP drives polymerization of cytosolic complexes of COPII subunits on the membrane, eventually leading to formation of vesicle buds.

3. Once COPII vesicles are released from the donor membrane, the Sar1 GTPase activity hydrolyzes Sar1 GTP in the vesicle membrane to Sar1 GDP



Disassembly of COPII coat

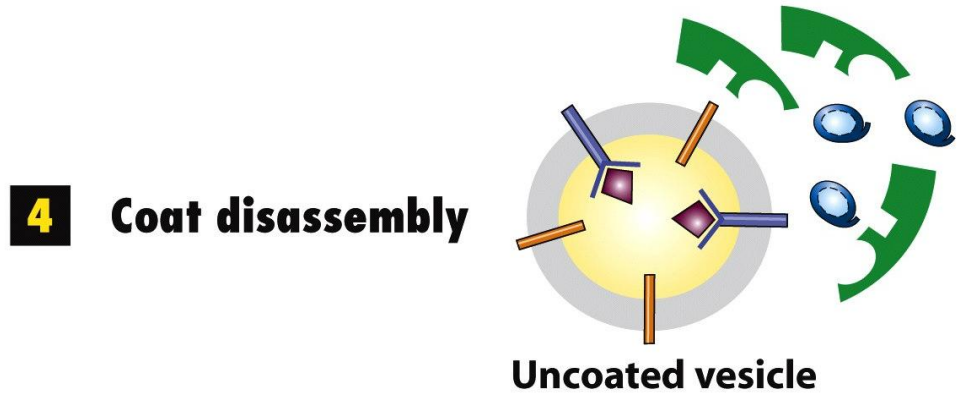


Figure 14-8 part 4
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4. This hydrolysis triggers disassembly of the COPII coat.

With mutant versions of Sar1 that **cannot hydrolyze GTP**, vesicle coats form and vesicle buds pinch off. However, all available coat subunits eventually become permanently assembled into coated vesicles that are unable to fuse with target membranes.

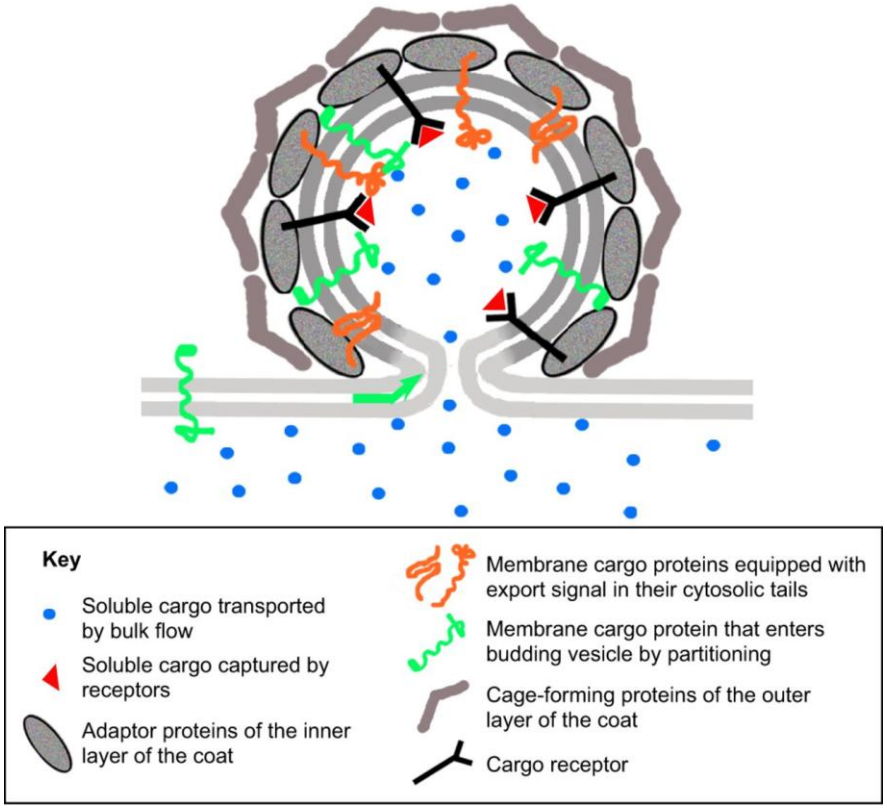
ARF protein undergoes a similar cycle of nucleotide exchange and hydrolysis coupled to the assembly of vesicle coats composed either of COPI or of clathrin and other coat proteins.

Different mechanisms of recruitment of cargo to transport vesicles

Vesicle buds must be able to discriminate among potential membrane and soluble cargo proteins.

Membrane cargo proteins: the mechanism by which the vesicle coat selects cargo molecules is by directly binding to specific sequences, or **sorting signals,** in the cytosolic portion of membrane cargo proteins.

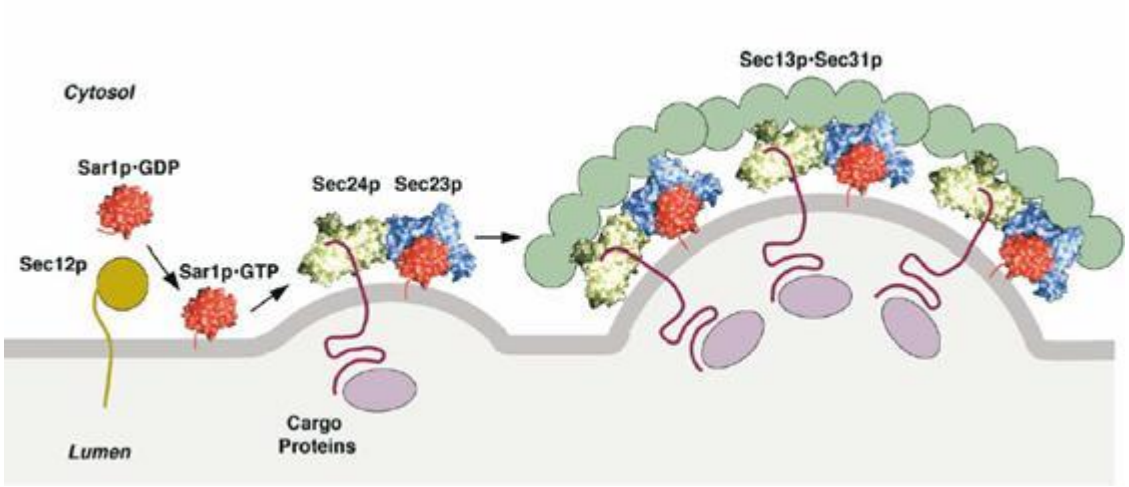
Soluble proteins within the lumen of parent organelles can in turn be selected by binding to the luminal domains of certain membrane cargo proteins, which act as receptor.

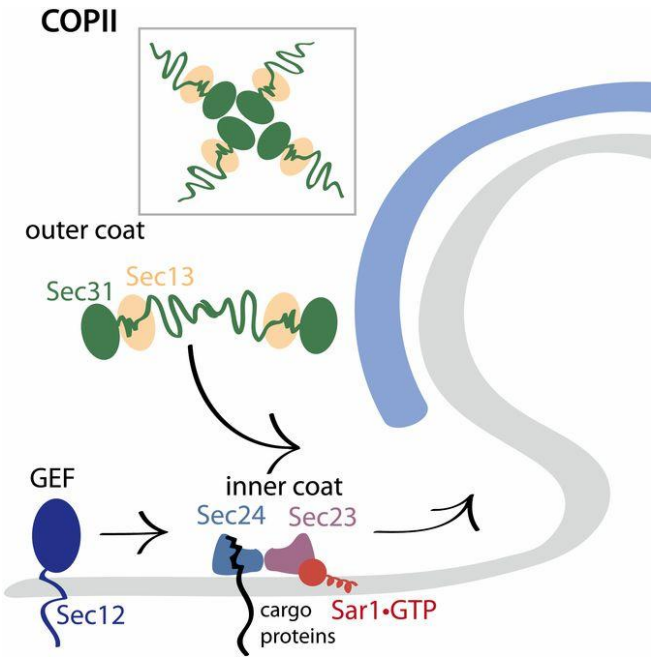


Nica Borgese J Cell Sci 2016;129:1537-1545

COPII Vesicles Mediate Transport from the ER to the Golgi

COPII vesicles were first recognized when cell-free extracts of yeast rough ER membranes were incubated with cytosol, ATP, and a nonhydrolyzable analog of GTP.



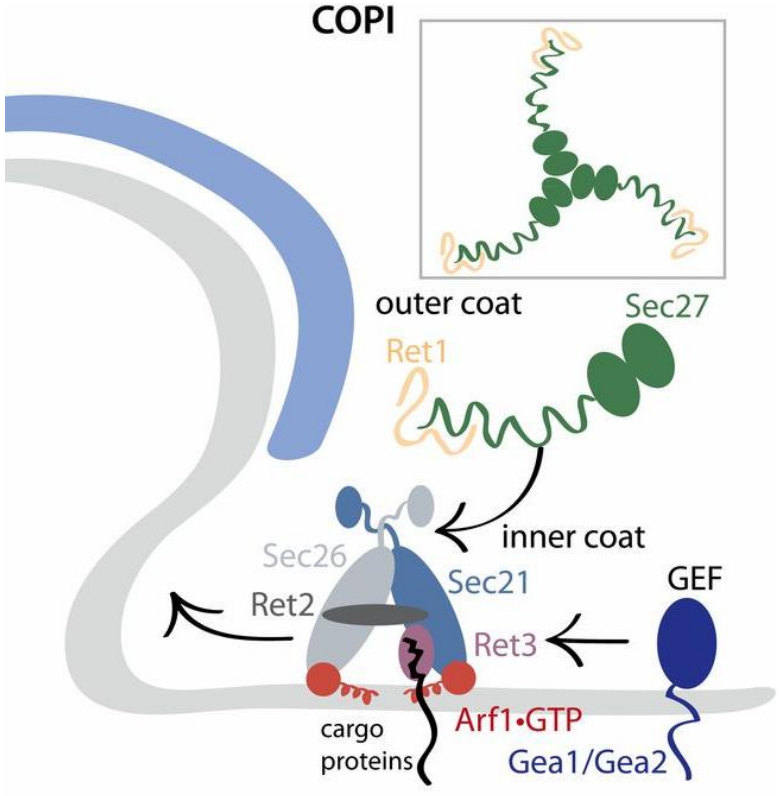


Genetics 1, 2013 vol. 193 no. 2 383-410

The cytosolic segments of Integral ER membrane proteins are specifically recruited into **COPII** contain a **di-acidic** sorting signal (**Asp-X-Glu**) which binds to the **Sec24** subunit of the COPII coat and is essential for the selective export of certain membrane proteins from the ER.

Few receptors for soluble cargo proteins are known.

The COPI coat assembles upon activation of Arf1



Genetics 2013 vol. 193 no. 2 383-410

Arf1 in turn recruits the **inner coat complex** (Sec21/Sec26/Ret2/Ret3) (similar to AP-2 adaptor complex).

The COPI outer coat is formed by 3 proteins which assembles in a triskelion structure via interactions of three domains of Sec27

Targeting Sequences on Cargo Proteins Make Specific Molecular Contacts with Coat Proteins

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

Signal Sequence*	Proteins with Signal	Signal Receptor	Vesicles That Incorporate Signal-bearing Protein
Lys-Asp-Glu-Leu (KDEL)	ER-resident luminal proteins	KDEL receptor in *cis*-Golgi membrane	COPI
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins (cytosolic domain)	COPI α and β subunits	COPI
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins in ER (cytosolic domain)	COPII Sec24 subunit	COPII
Mannose 6-phosphate (M6P)	Soluble lysosomal enzymes after processing in *cis*-Golgi	M6P receptor in *trans*-Golgi membrane	Clathrin/AP1
	Secreted lysosomal enzymes	M6P receptor in plasma membrane	Clathrin/AP2
Asn-Pro-X-Tyr (NPXY)	LDL receptor in the plasma membrane (cytosolic domain)	AP2 complex	Clathrin/AP2
Tyr-X-X-Φ (YXXΦ)	Membrane proteins in *trans*-Golgi (cytosolic domain)	AP1 (μ1 subunit)	Clathrin/AP1
	Plasma membrane proteins (cytosolic domain)	AP2 (μ2 subunit)	Clathrin/AP2
Leu-Leu (LL)	Plasma membrane proteins (cytosolic domain)	AP2 complexes	Clathrin/AP2

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

Rab proteins are required for the targeting of vesicles to the target membrane

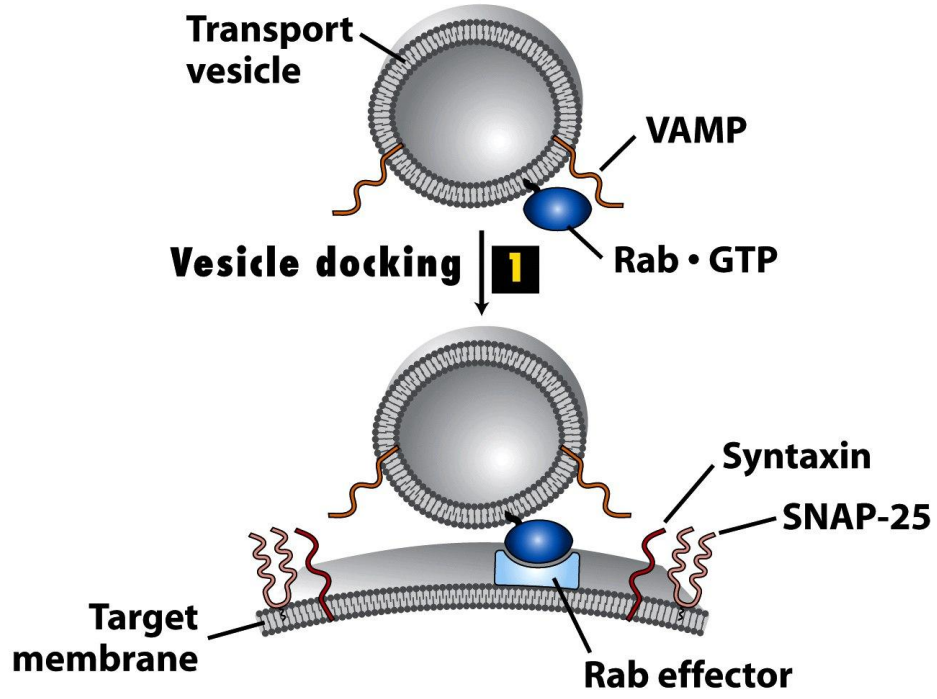


Figure 14-10a part 1
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Targeting of vesicles to the appropriate target membrane is mediated by **Rab proteins,** GTPase superfamily of **switch proteins.**

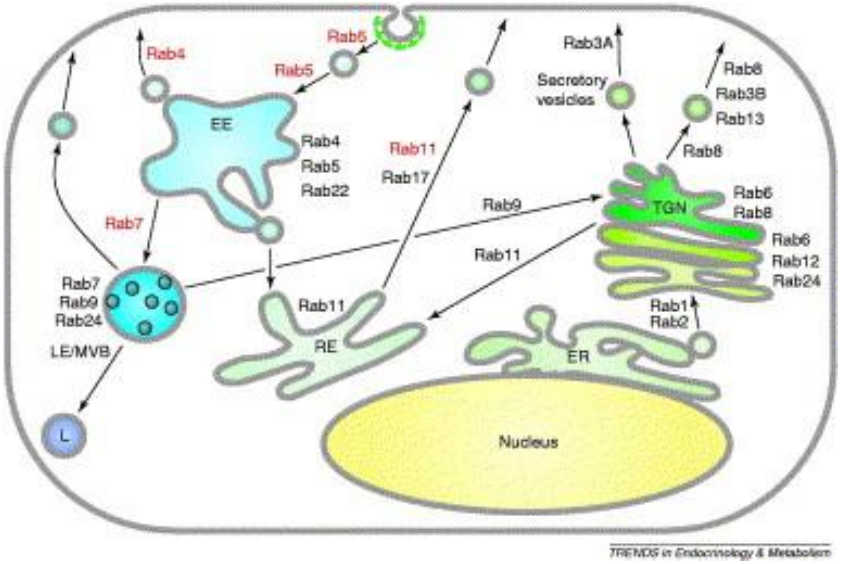
Conversion of cytosolic Rab GDP to Rab GTP, enables it to interact with a particular transport vesicle and insert its isoprenoid anchor into the vesicle membrane.

Once Rab GTP is tethered to the vesicle surface, it interacts with one of a number of different large proteins, known as **Rab effectors,** attached to the target membrane.

After vesicle fusion occurs, the GTP bound to the Rab protein is hydrolyzed to GDP, triggering the release of Rab -GDP, which then can undergo another cycle of GDP-GTP exchange, binding, and hydrolysis.

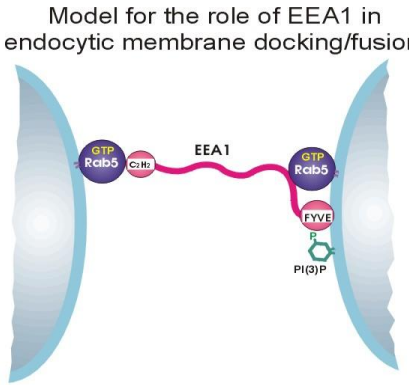
A different type of Rab and Rab effector appears to function for each vesicle type

Each type of vescicles and organelles has at least one **Rab** protein on its cytosolic surface. Sec4 mutant (yeast cells) accumulate secretory vesicles that are unable to fuse with the plasma membrane (class E mutants).



Z. Gáborik , L. Hunyady Trends in Endocrinology and Metabolism, V. 15, 2004, 286 -93

Rab1 is essential for ER-to-Golgi transport reactions, **Rab7** associate with late endosome.



Example: **Rab5 protein** is localized to endocytic vesicles (EE). A long coiled protein known as **EEA1** (early endosome antigen 1), which resides on the membrane of the early endosome, functions as the **Rab effector** for Rab5.

Paired Sets of SNARE Proteins Mediate Fusion of Vesicles with Target Membranes

After Rab-mediated docking of a vesicle on its target membrane, the interaction of cognate **SNAREs** brings the two membranes close enough together that they can fuse. They provide a layer of specificity.

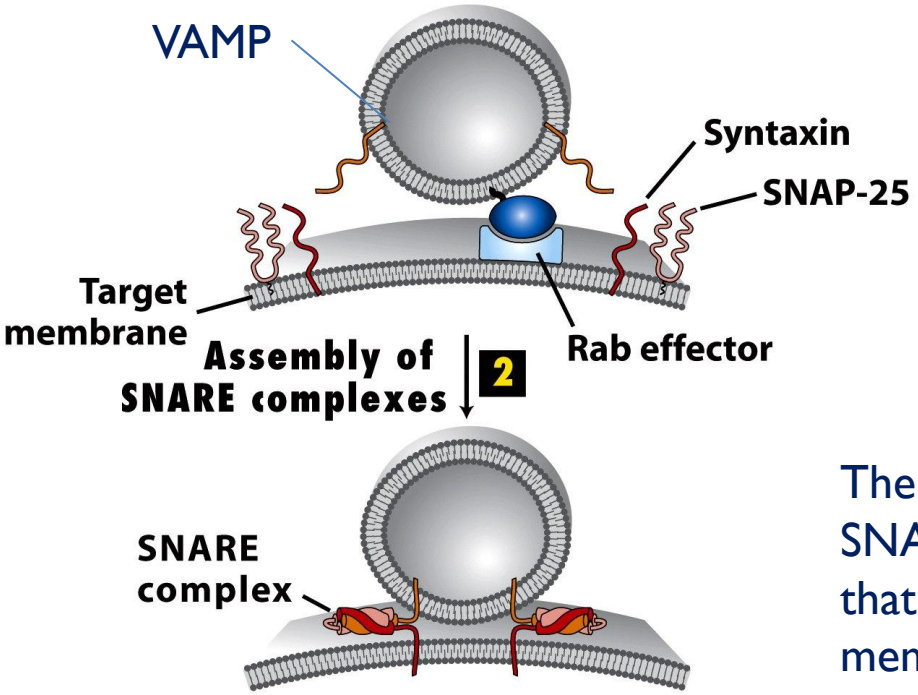


Figure 14-10a part 2
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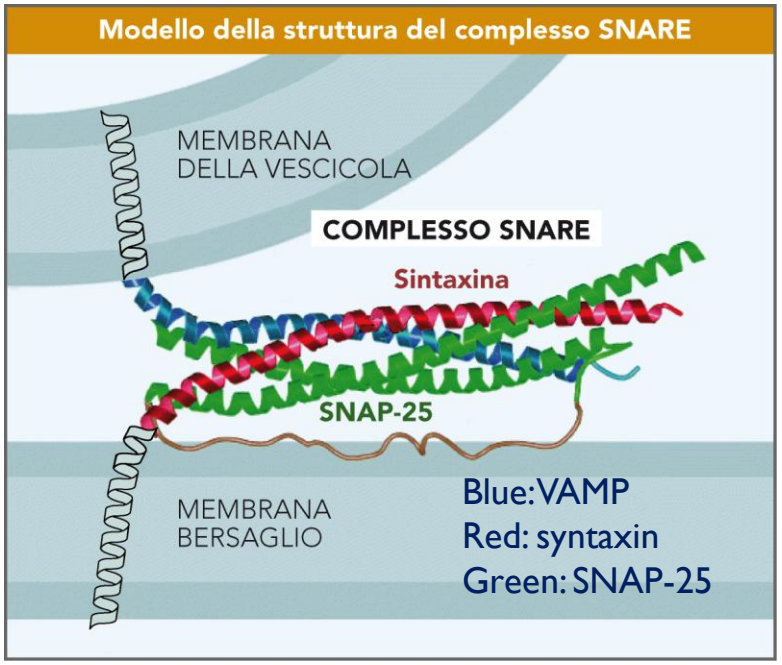
The best-understood examples of SNARE-mediated fusion occurs during **exocytosis of secreted proteins.**

The cognate **SNAREs:**
V-SNARE: = VAMP (*vesicle-associated membrane protein*)
T-SNARE: Syntaxin and SNAP-25.

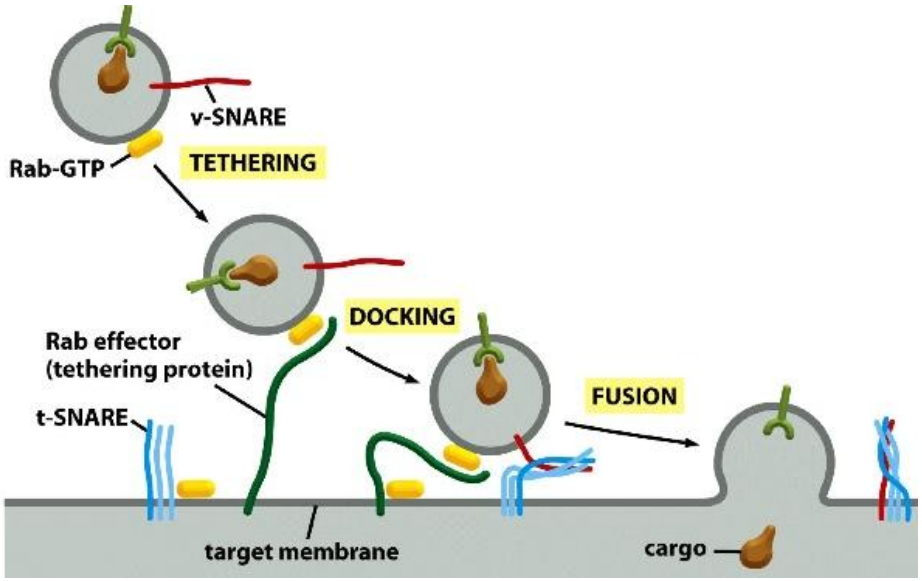
The cytosolic region in each of these three SNARE proteins form a **four-helix bundle** that anchor vesicles to the target membrane.

Model of the structure of the SNARE complex



The cytosolic region in each of these three SNARE proteins contains a repeating **heptad sequence** that allows four helices—one from VAMP, one from syntaxin, and two from SNAP-25 to coil around one another to form a **four-helix bundle** with unusual stability **.**

In **liposomes,** formation of SNARE complexes is sufficient to bring about **membrane fusion**



Dissociation of SNARE complexes is driven by ATP Hydrolysis

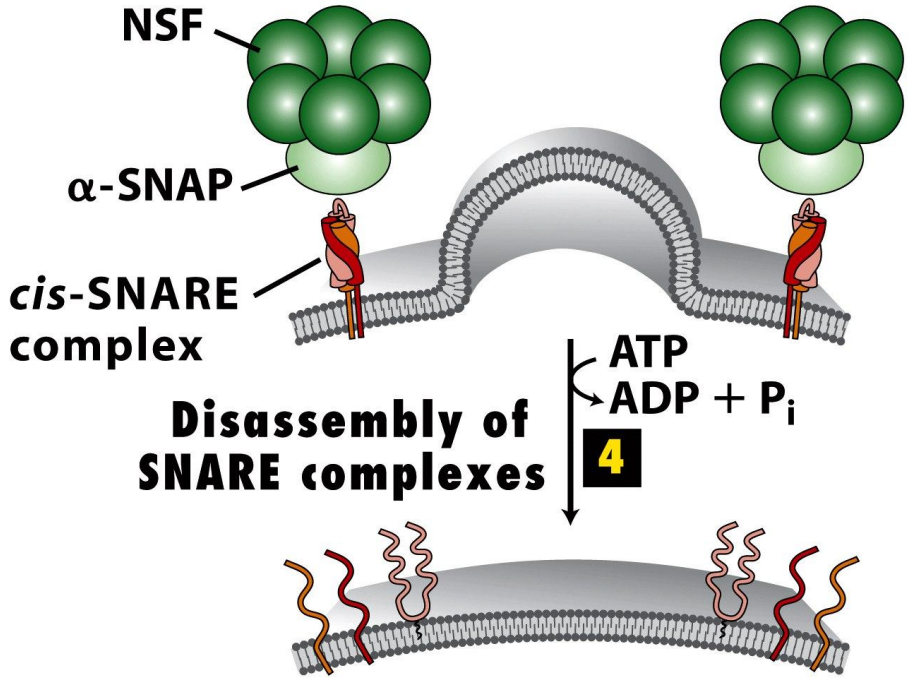


Figure 14-10a part 4
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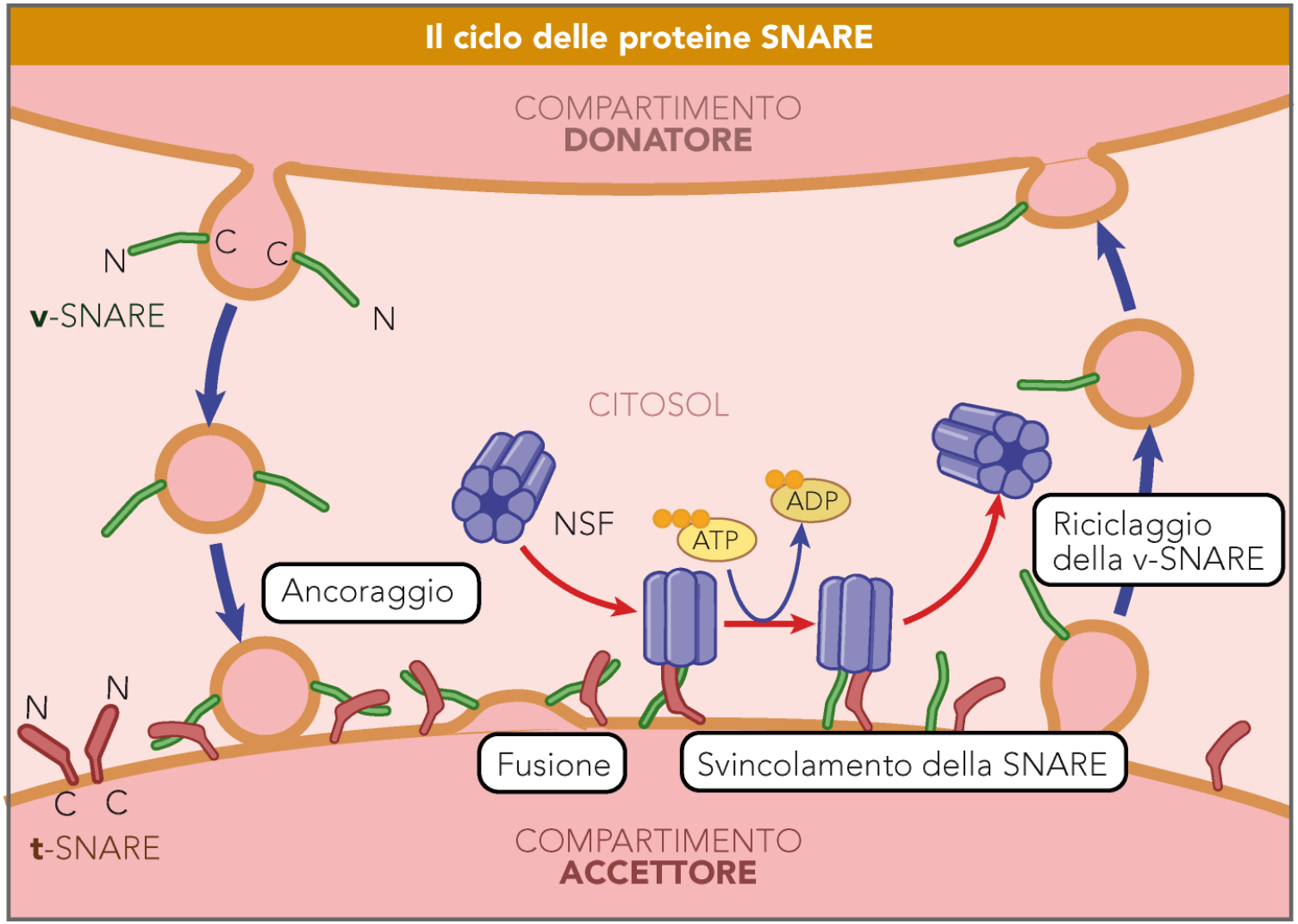
Because of the stability of SNARE complexes, their dissociation depends on additional proteins and the input of energy.

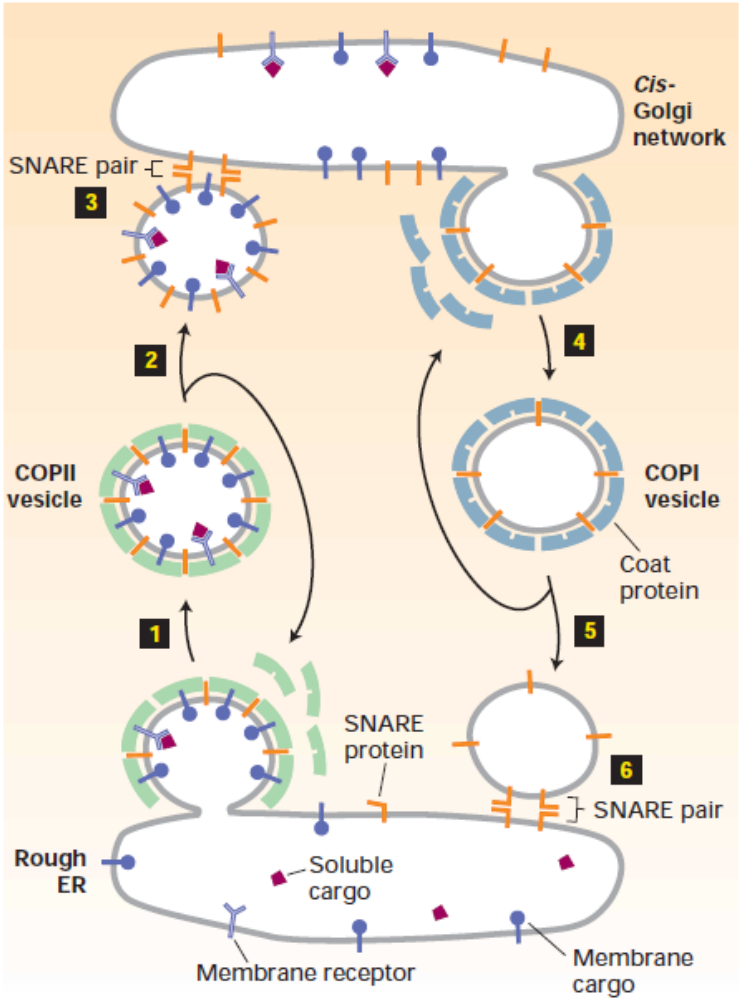
NSF examer and **α-SNAP**, are required for regeneration of free SNARE proteins and not for ongoing vesicle fusion

Sec yeast mutants homologues to NSF and alpha-SNAP belong to the mutants of class C (accumulate ER-to-Golgi transport vesicles). NSF and α-SNAP proteins are not necessary for actual membrane fusion, but rather are required for **regeneration** of free SNARE proteins.

NSF is required to recycling of SNARE proteins



Early Stages of the Secretory Pathway

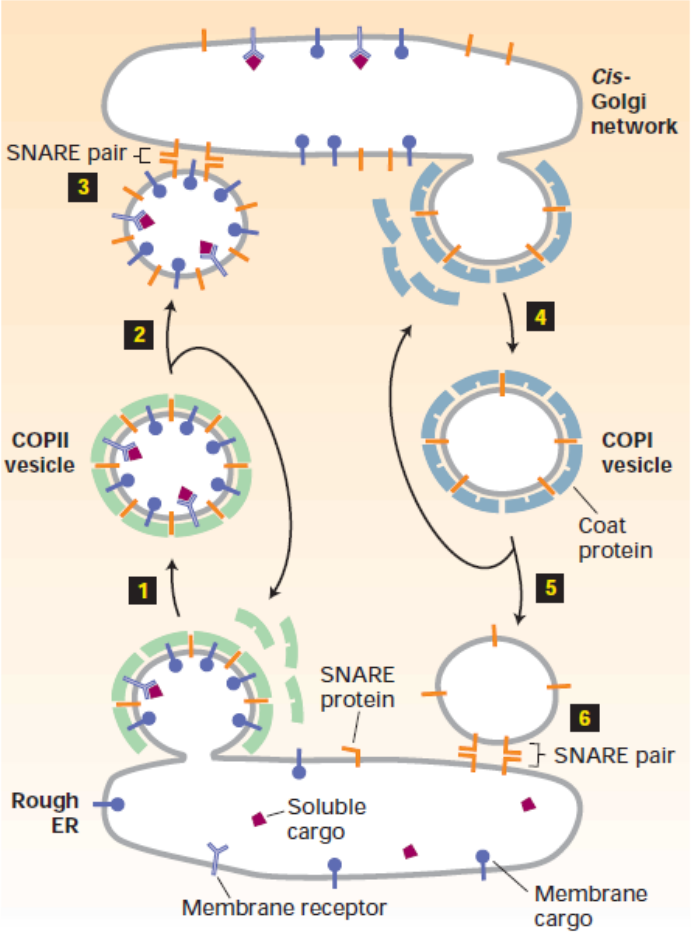


Take a closer look at vesicular traffic through the ER and Golgi stages of the secretory pathway

Vesicular traffic through the ER and Golgi stages of the secretory pathway is mediated by COPII (anterograde transport) and by COPI vesicles (retrograde transport)

COPI Vesicles Mediate Retrograde Transport within the Golgi and from the Golgi to the ER

Yeast cells containing temperature sensitive mutations in COPI proteins have been categorized as class B *sec mutants*



Functions of retrograde transport from the cis-Golgi to the ER:

✓Recycling of vesicle membranes

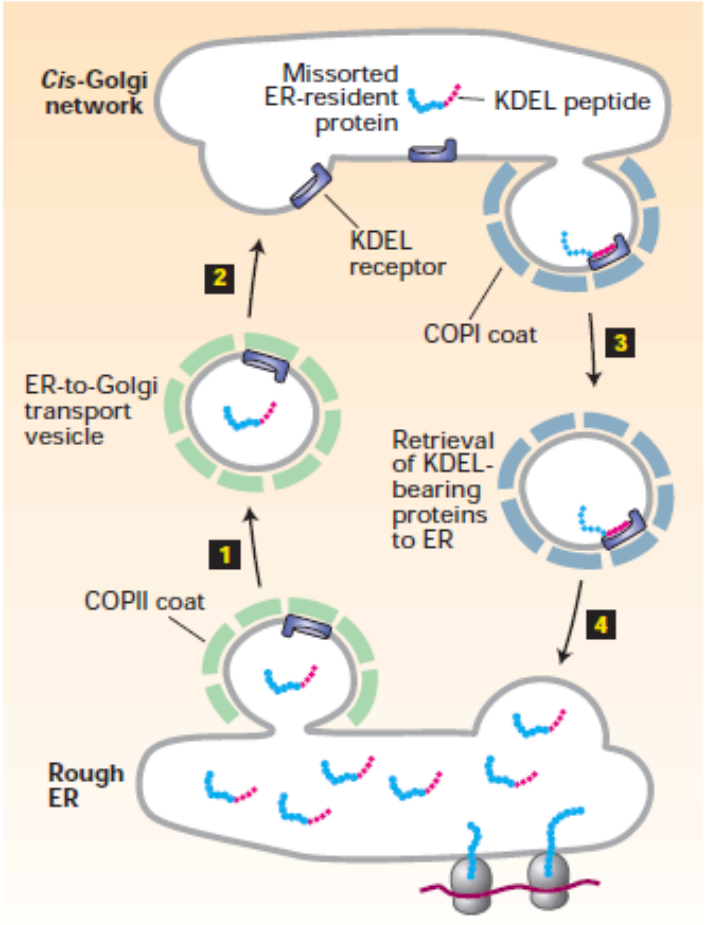
✓Recycling of v-SNARE

- COPI mutants cannot recycle key membrane proteins back to the rough ER, the ER gradually becomes depleted of ER proteins such as v-SNAREs and eventually vesicle formation from the rough ER is halted.

✓Retrieval of missorted ER-resident proteins (sorting mistakes).

- ER contains several soluble resident proteins (chaperone BIP and protein disulfide isomerase) loaded passively into vesicles destined for the cis-Golgi.

Retrograde transport from the cis-Golgi rescues missorted ER-resident proteins (sorting mistakes).

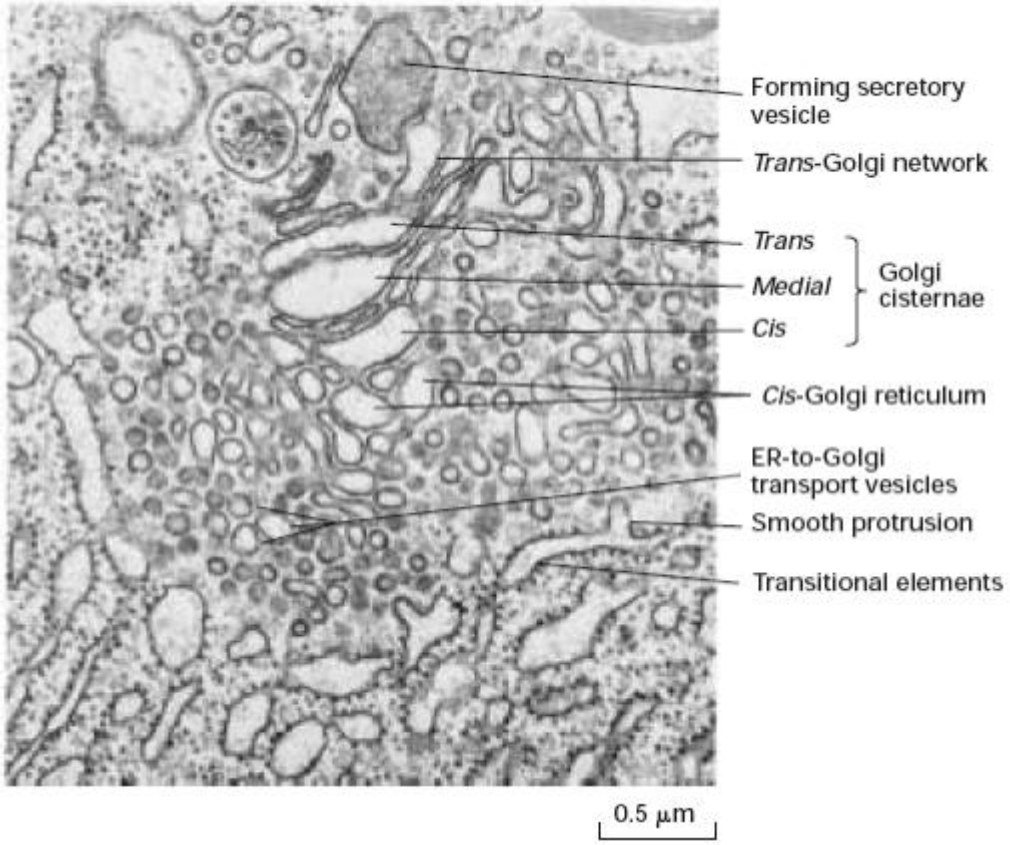


Most soluble ER-resident proteins carry a Lys-Asp-Glu- Leu (KDEL) sorting signal at their C-terminus. KDEL is recognized and bound by the **KDEL receptor**, found on transport vesicles shuttling between the ER and the cis-Golgi and on the cis-Golgi reticulum.

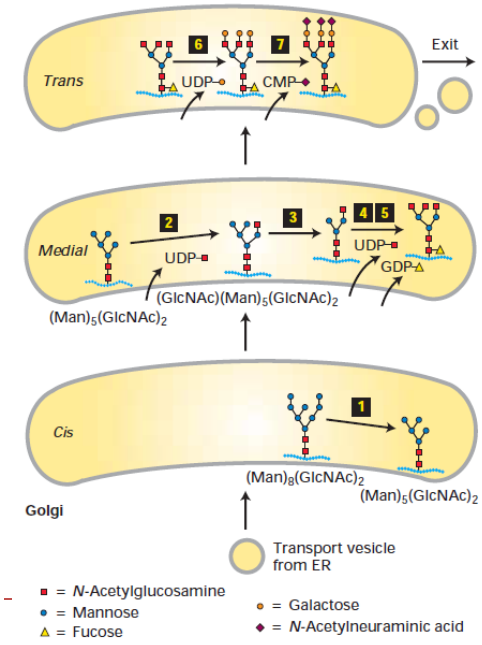
The KDEL receptor and other membrane proteins that are transported back to the ER from the Golgi contain a **Lys-Lys-X-X** sequence at the very end of their C-terminal segment, which faces the cytosol. This is necessary and sufficient to incorporate proteins into COPI vesicles for retrograde transport.

Mutant protein disulfide isomerase lacking these four residues is secreted

Anterograde Transport Through the Golgi could occur by Cisternal Progression

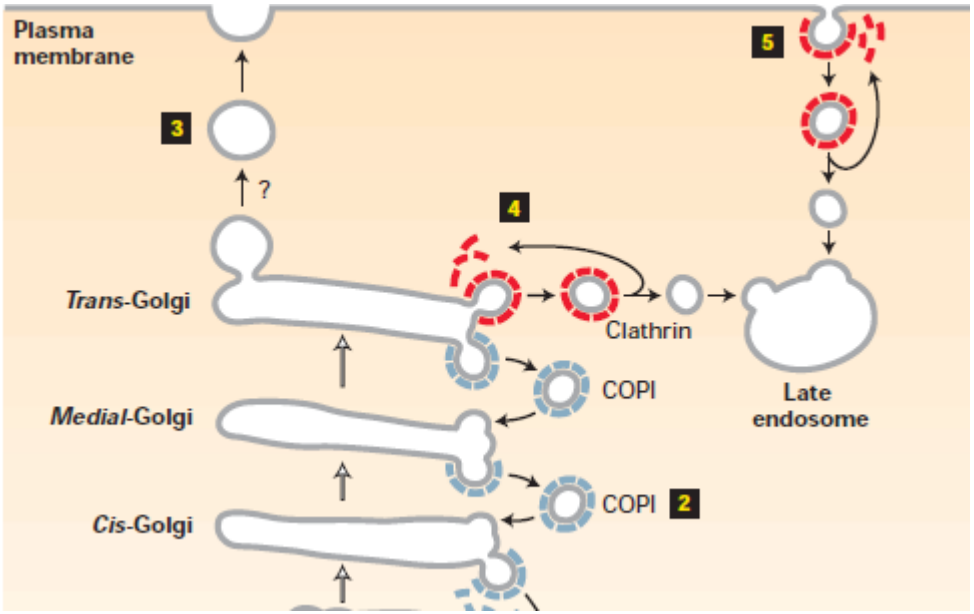


Some protein aggregates (e.g. collagen) are too large to be incorporated into small transport vesicles, and aggregates have never found in transport vesicles. It has been suggested that the forward movement of these and perhaps all secretory proteins from one Golgi compartment to another occurs via cisternal progression.



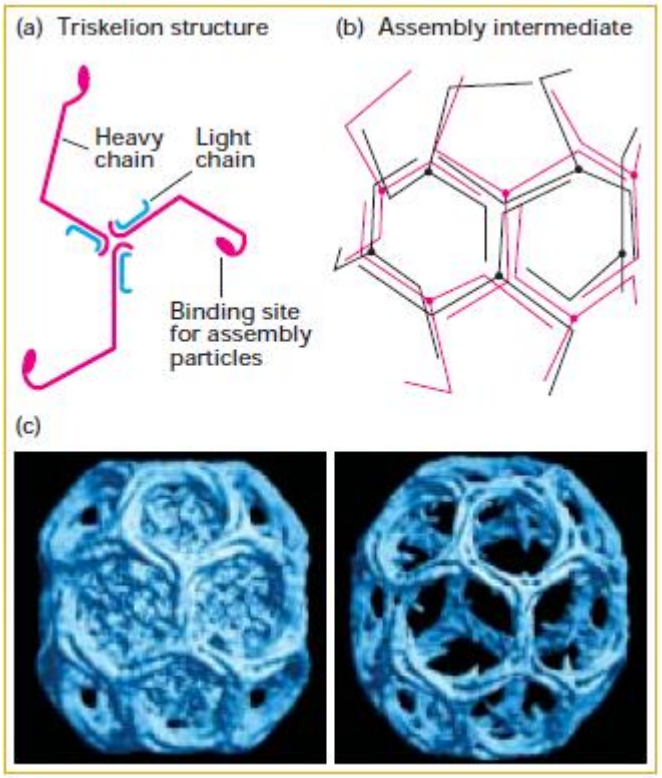
The retrograde trafficking of COPI vesicles from later to earlier Golgi compartments maintains sufficient levels of these carbohydrate-modifying enzymes in their functional compartments.

Later Stages of the Secretory Pathway



Properly processed cargo proteins reach the *trans-Golgi network, the* most-distal Golgi compartment where they are sorted into vesicles for delivery to their final destination.

Vesicles Coated with Clathrin and/or Adapter Proteins Mediate Several Transport Steps

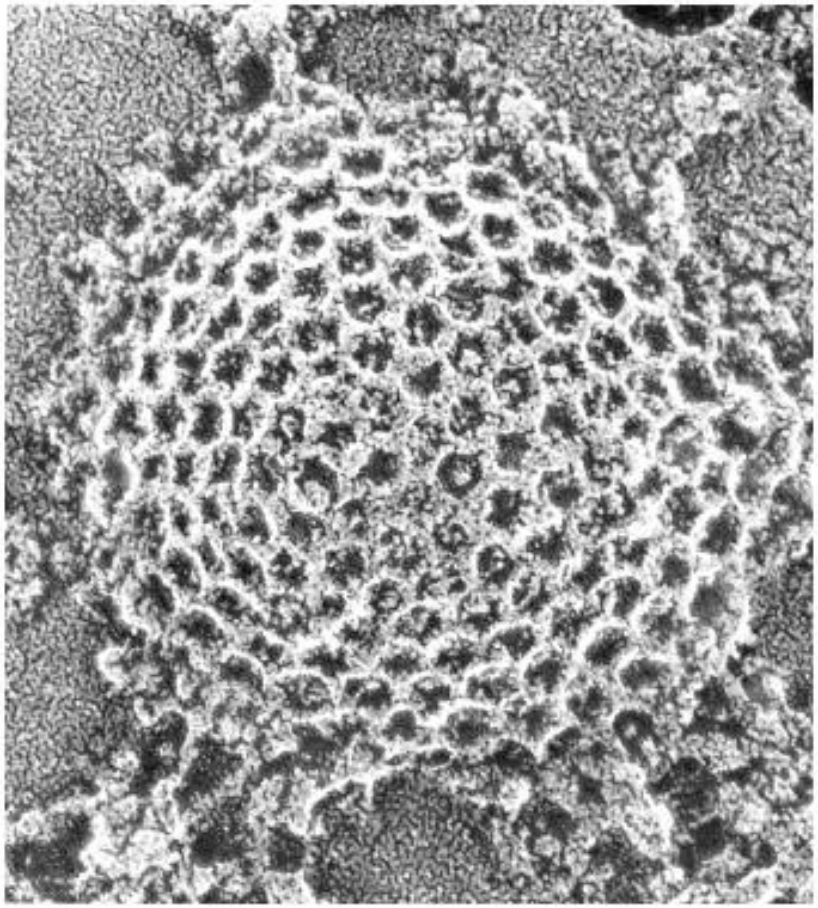


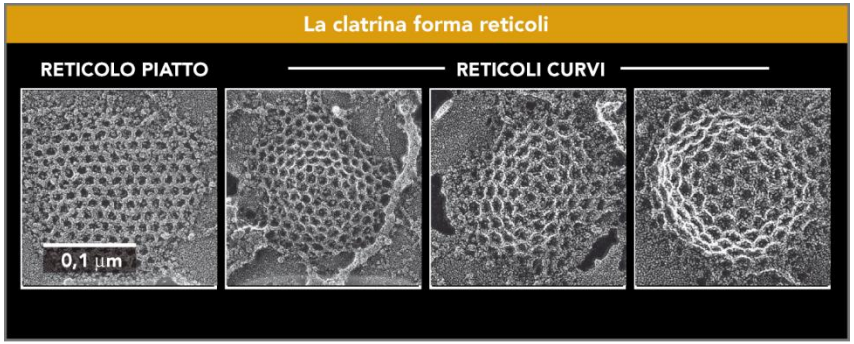
Structure of clathrin coats

The best-characterized vesicles that bud from the ***trans*-Golgi network** (TGN) have a two-layered coat:
- an outer layer composed of the **fibrous protein clathrin** and
- an inner layer composed of **adapter protein (AP)** complexes.

Clathrin: three branched shape, called **triskelion.** Each branch: 1 heavy chain of 180 kDa forming legs from α-helical zigzags and 1 light chain of 35-40 kDa;
Triskelions polymerize to form a polyhedral cage **with intrinsic curvature**. The clathrin triskelions determine the geometry of the clathrin vescicles.

A clathrin-coated pit on the cytosolic face of the plasma membrane





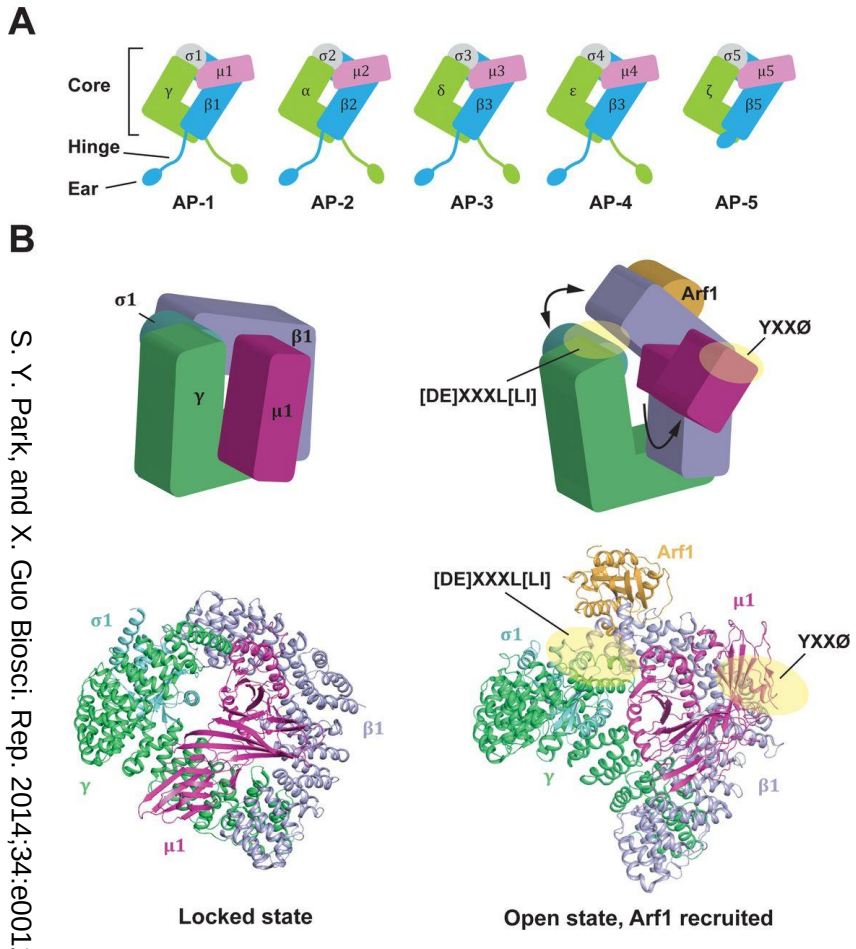
Triskelions assemble in vitro to form empty lattice cages with open hexagonal and pentagonal faces.

They are very similar to those observed in vivo.

Assembly does not require ATP and direct binding to membranes

Figure 17-35

The adapter complexes (AP)



Locked and open structure of AP-1 core complexes

S. Y. Park, and X. Guo Biosci. Rep. 2014;34:e00123

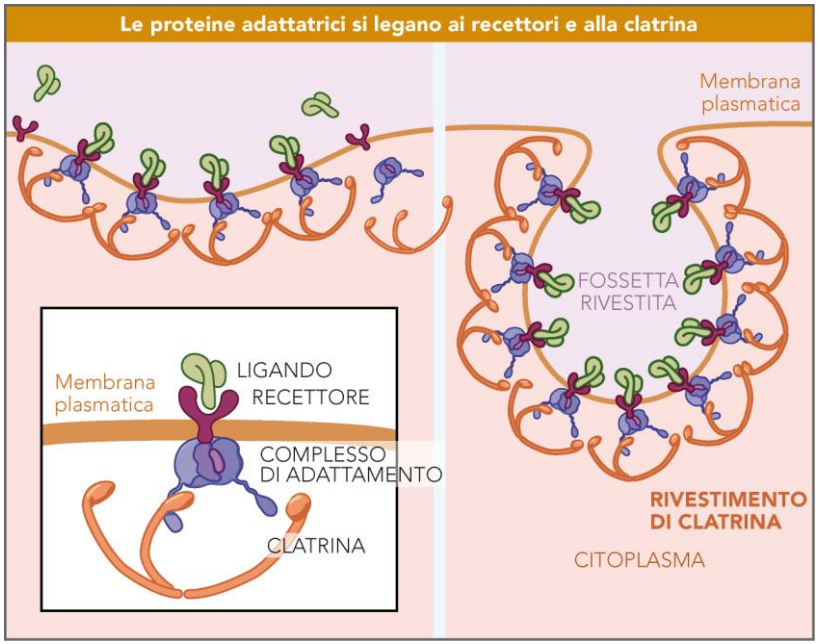
Clathrin polymerization occurs on a donor membrane in association with **AP complexes** (340,000 MW), which assemble between the clathrin lattice and the membrane.

AP complexes are heterotetramers containing one copy each of 4 different adapter subunits.

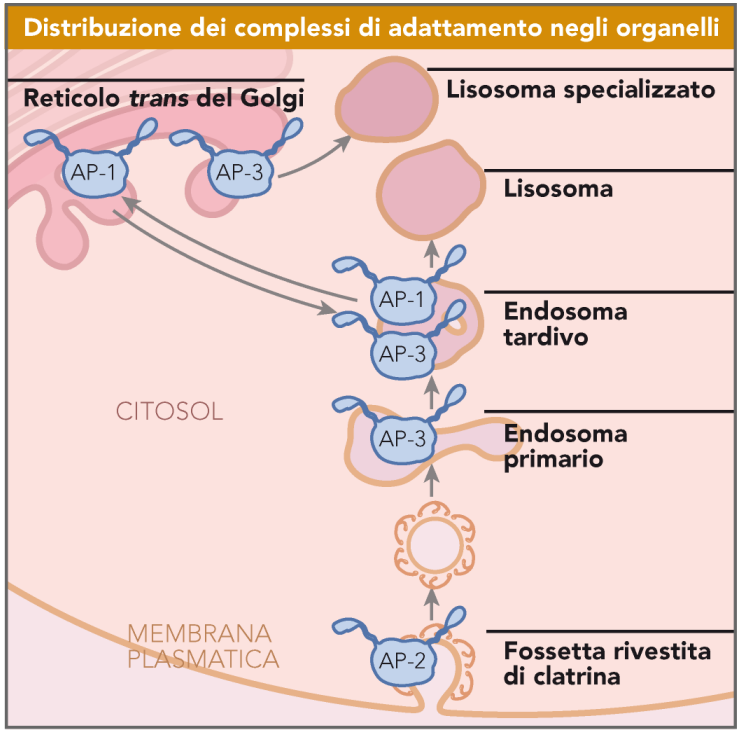
In the presence of **Arf1** binding, AP-1 undergoes a large conformational change to the open state exposing the binding sites for cargo proteins

clathrin/AP1: Proteins containing a Tyr-XX-Φ sequence, (where Φ is a bulky hydrophobic amino acid), or a D/EXXXL/I sequence are recruited into clathrin/AP1 vesicles

Adapter proteins determine which cargo proteins are specifically included in clathrin vesicle



Adapter proteins determine which cargo proteins are specifically included in (or excluded from) a budding transport vesicle.



Vesicles containing different adapter complexes have been found to mediate specific transport steps:

AP1 complex: cargo selection from the TGN and endosomes.

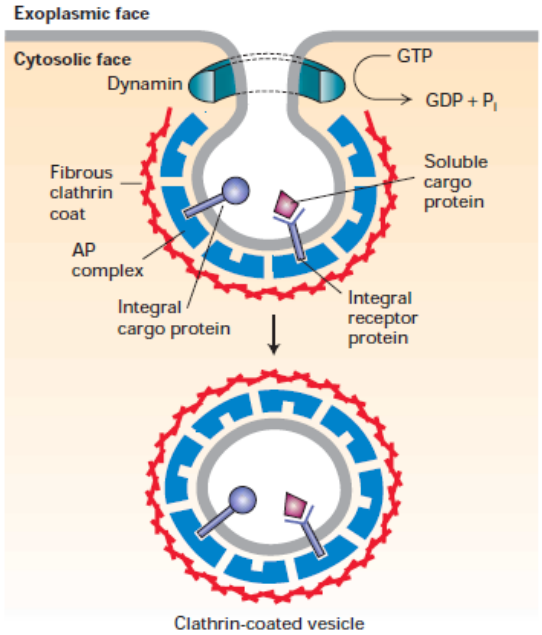
AP2 complex: cargo selection from plasma membrane

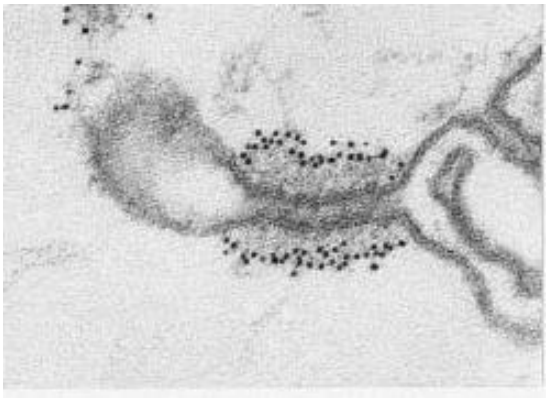
AP3 complex: cargo selection to lysosomes (some types of cells).

Dynamin Is Required for Pinching Off of Clathrin Vesicles

Dynamin is a cytosolic protein that polymerizes around the neck portion and then hydrolyzes GTP.
The energy derived from GTP hydrolysis is thought to drive "contraction" of dynamin around the vesicle neck until the vesicle pinches off.

As with COPI and COPII vesicles, clathrin/AP vesicles normally lose their coat soon after their formation

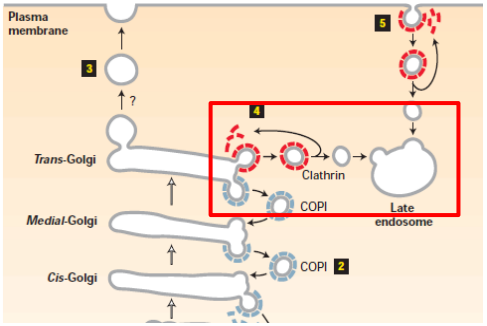




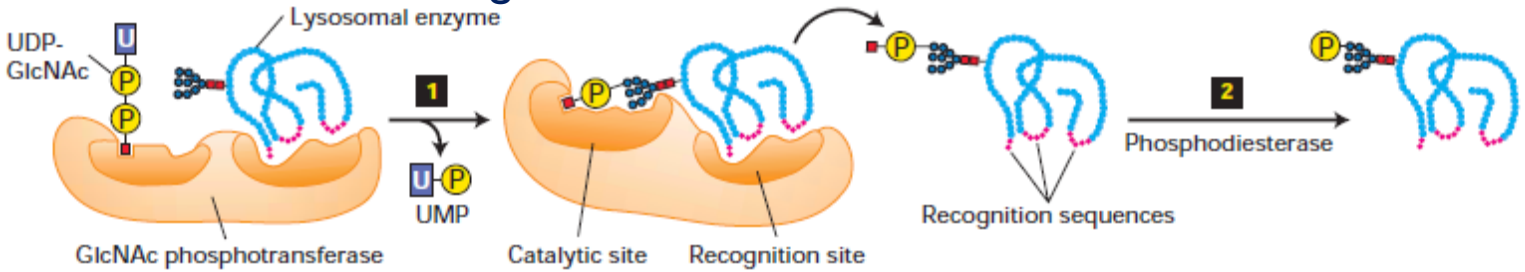
Incubation of cell extracts with a nonhydrolyzable derivative of GTP provides leads to accumulation of clathrin coated vesicle buds with excessively long necks surrounded by polymeric dynamin but do not pinch off

COPI and COPII vesicles appear to pinch off from donor membranes without the aid of a GTPase.

Mannose 6-phosphate (M6P) residues targets soluble enzymes to lysosomes



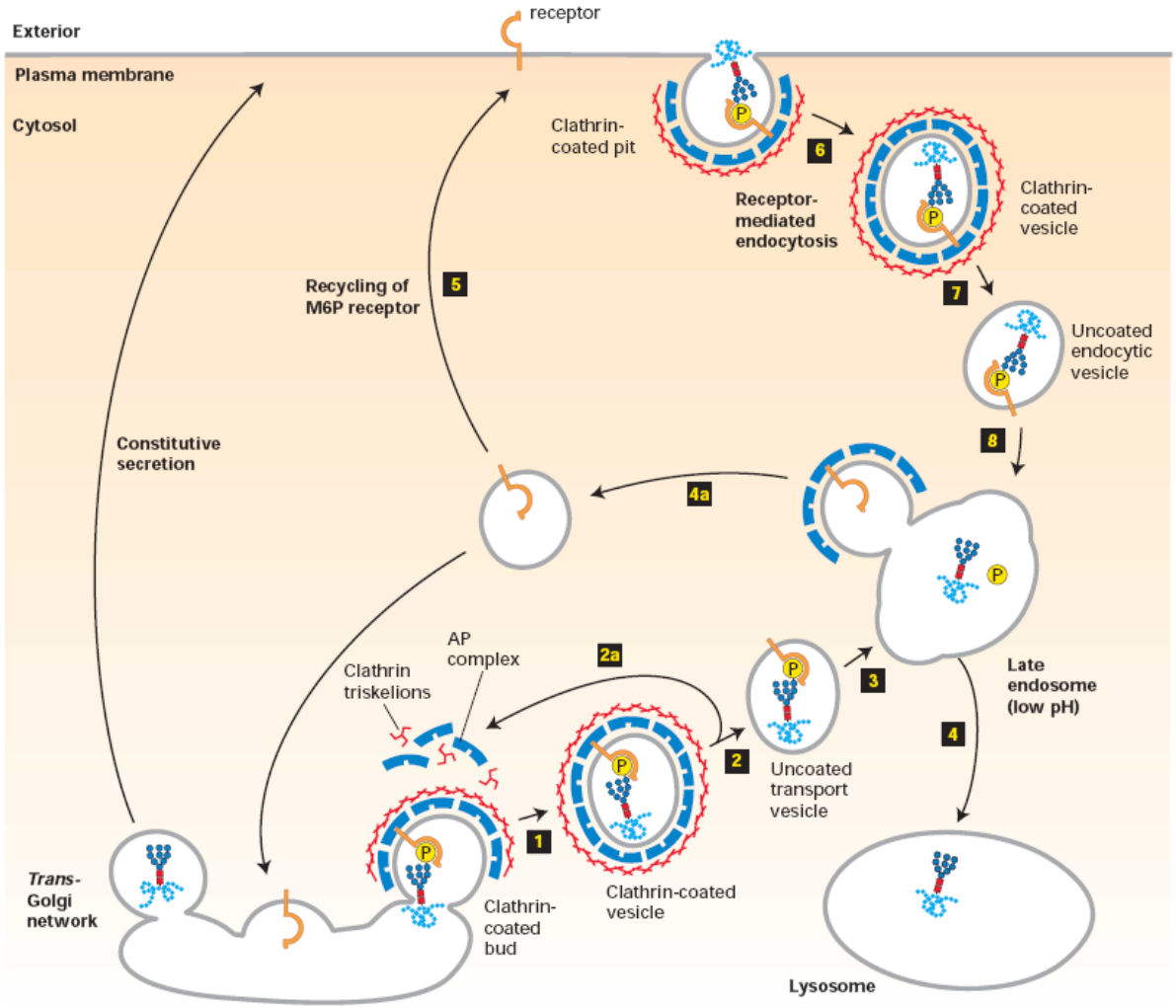
The sorting signal that directs soluble lysosomal enzymes from the trans-Golgi network to the late endosome is a carbohydrate residue, **mannose 6-phosphate (M6P),** which is formed in the cis-Golgi. The N-linked Man8(GlcNAc)2 oligosaccharide present on most lysosomal enzymes undergo a two-step reaction sequence that generates M6P residues.



1) A *GlcNAc phosphotransferase* transfers a phosphorylated GlcNAc group to C6 of one or more mannose residues.
2) A *phosphodiesterase* removes the GlcNAc group, leaving a phosphorylated mannose residue on the lysosomal enzyme.

The addition of M6P prevents lysosomal enzymes from undergoing the further processing reactions as other secreted and membrane proteins: clathrin/AP1 vesicles contain the **M6P receptor** which bounds lysosomal enzymes then bud from the *trans-Golgi network*

Mannose 6-Phosphate Residues Target Soluble Proteins to Lysosomes



M6P receptor and bound lysosomal enzymes then bud from the *trans-Golgi network*, lose their coats, and subsequently fuse with the late endosome.

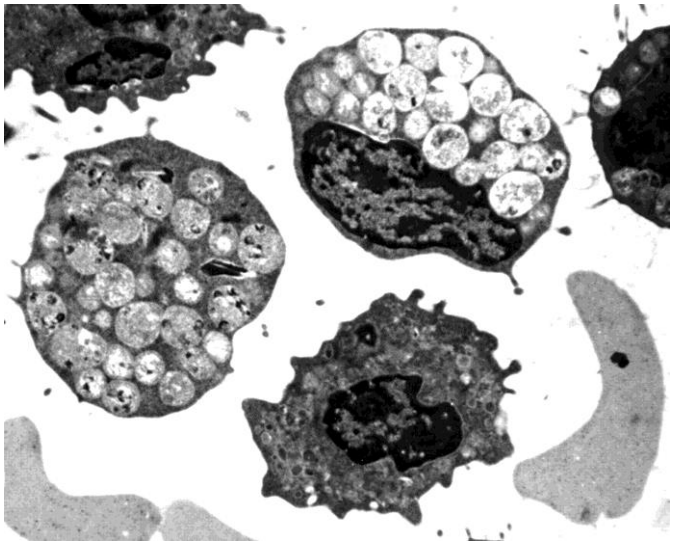
Because **M6P receptors** can bind **M6P** at the slightly acidic pH (≈6.5) of the trans-Golgi network but not at a pH less than 6, the bound lysosomal enzymes are released within **late endosomes** (pH of 5.0–5.5).

A phosphatase within late endosomes usually removes the phosphate from M6P residues on lysosomal enzymes.

Vesicles budding from late endosomes recycle the M6P receptor back to the *trans*-Golgi network.

Study of Lysosomal Storage Diseases Revealed Key Components of the Lysosomal Sorting Pathway

Lacking the M6P sorting signal, the lysosomal enzymes are secreted rather than being sorted to and sequestered in lysosomes.



van der Meer W et al. J Clin Pathol 2001;54:724-726

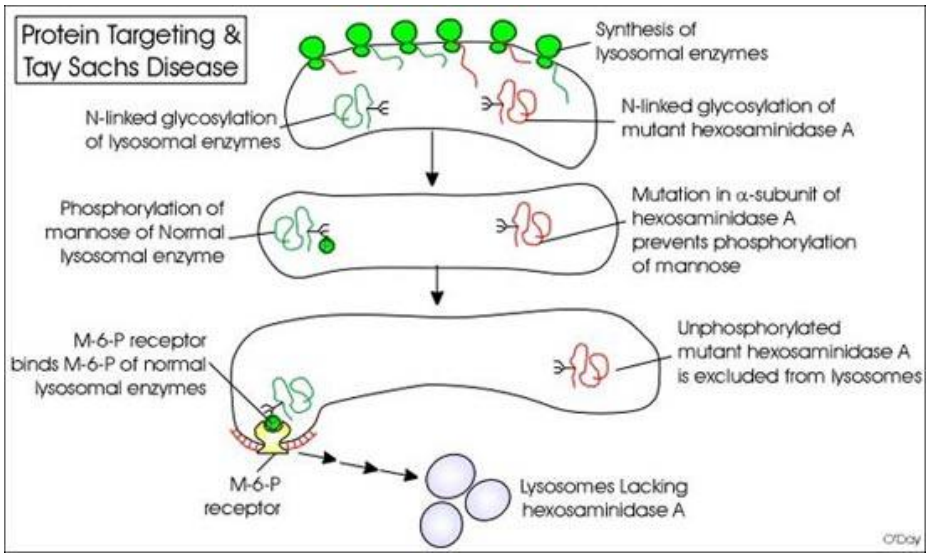
JCP

I-cell disease: lymphocytic vacuoles containing round osmiophilic structures

Lysosomal storage diseases, are caused by the absence of one or more lysosomal enzymes. As a result, undigested glycolipids and extracellular components that would normally be degraded by lysosomal enzymes **accumulate in lysosomes** as large inclusions.

I-cell disease: a severe lysosomal storage disease, in which cells from affected individuals lack the **N-acetylglucosamine phosphotransferase**

Lysosomal storage diseases are characterized by abnormal lisosomes.



http://drustapbio.wikia.com/wiki/Tay-_Sachs

Tay-Sachs GM2 gangliosidosis results in cell accumulation of harmful amounts of lipids (gangliosides) in the brain.

Tay-Sachs GM2 gangliosidosis is an inherited metabolic disorder that results from defects in lysosomal function (Lysosomal storage diseases) due to a **Hexosaminidase A** deficiency in lysosomes.