C4 T – CELL BIOLOGY

UNIT 3: Cytoplasmic organelles I

Protein sorting & Mechanism of vesicular transport (Part – II)

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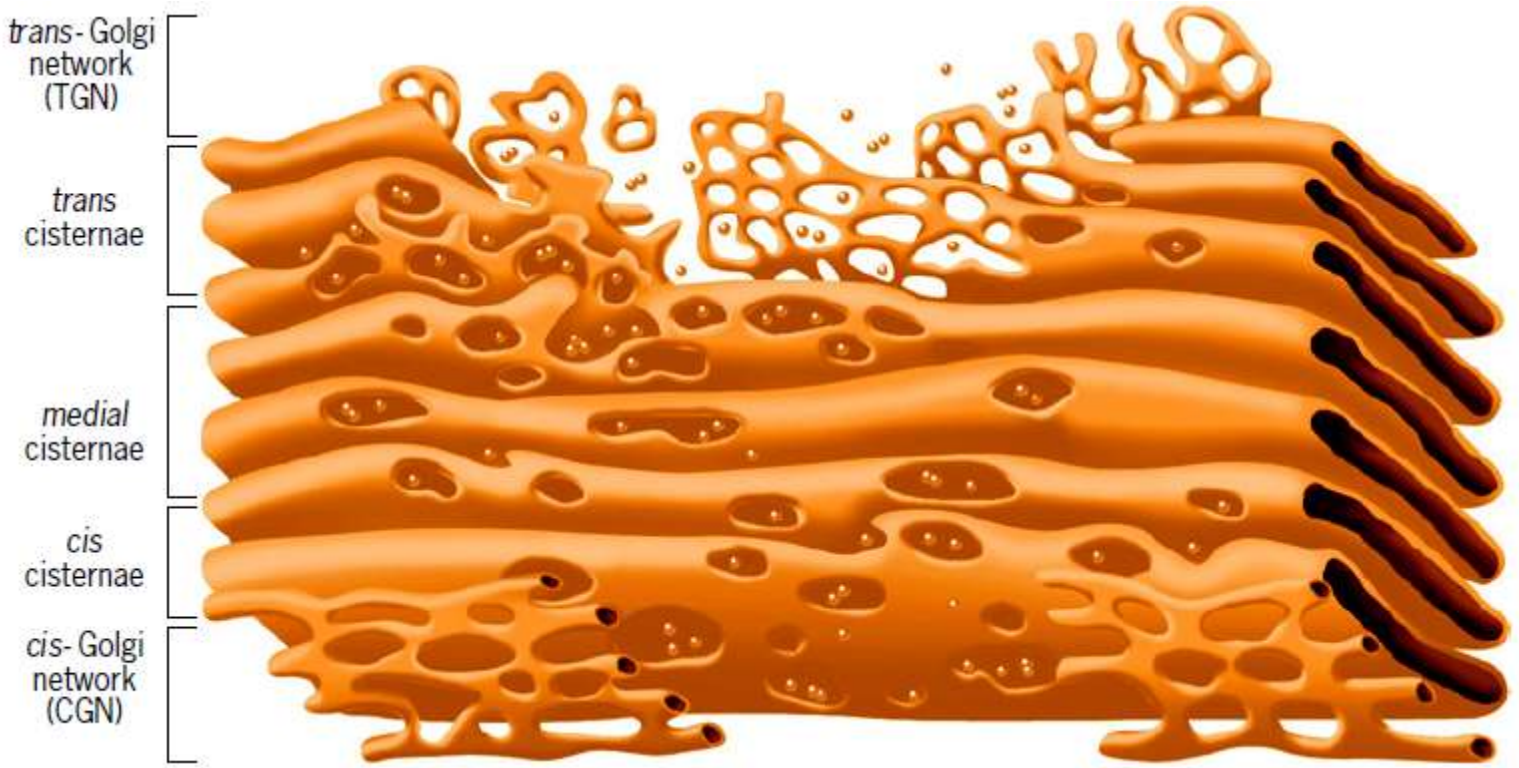
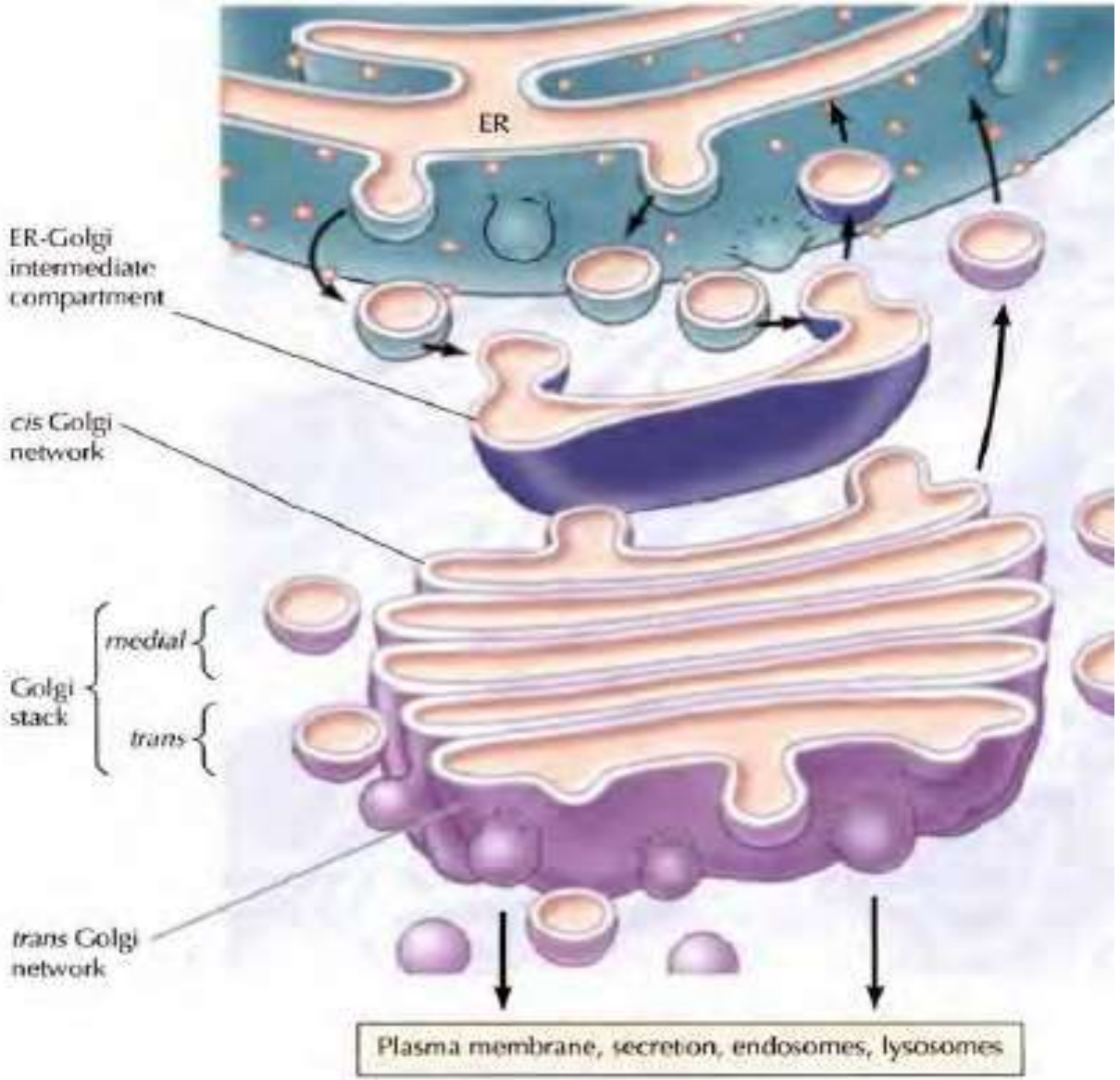
The Golgi Complex



Regions of the Golgi apparatus



Function:

- The Golgi apparatus, or Golgi complex, functions as a factory in which proteins received from the ER are further processed and sorted for transport to their eventual destinations: endosomes, lysosomes, the plasma membrane, or secretion.
- Also, most glycolipids and sphingomyelin are synthesized within the Golgi.
- In plant cells, the Golgi apparatus further serves as the site at which the complex polysaccharides of the cell wall are synthesized.
- The Golgi apparatus is thus involved in processing the broad range of cellular constituents that travel along the secretory pathway.

- Protein processing within the Golgi involves the **modification** and **synthesis** **of the carbohydrate portions** of **glycoproteins**

- N-linked oligosaccharides are processed within the Golgi apparatus in an ordered sequence of reactions.

Protein Glycosylation in the Golgi Complex

- As newly synthesized soluble and membrane glycoproteins pass through the ***cis*** and ***medial cisternae*** of the Golgi stack, most of the mannose residues are also removed from the core oligosaccharides, and other **sugars are added sequentially** by various **glycosyltransferases.**
- The enzyme sialyltransferase, for example, which places a sialic acid at the terminal position of the chain in animal cells, is localized in the *trans face of the* Golgi stack, as would be expected if newly synthesized glycoproteins were continually moving toward this part of the organelle.
- In contrast to the glycosylation events that occur in the ER, which assemble a single core oligosaccharide, the glycosylation steps in the Golgi complex can be quite varied, producing carbohydrate domains of remarkable sequence diversity.
- One of many possible glycosylation pathways is shown in Figure 8.22. Unlike the *N-linked oligosaccharides, whose synthesis begins in the* ER, those attached to proteins by *O-linkages (Figure 4.11) are* assembled entirely within the Golgi complex.

Modification of proteins destined for secretion or for the plasma membrane

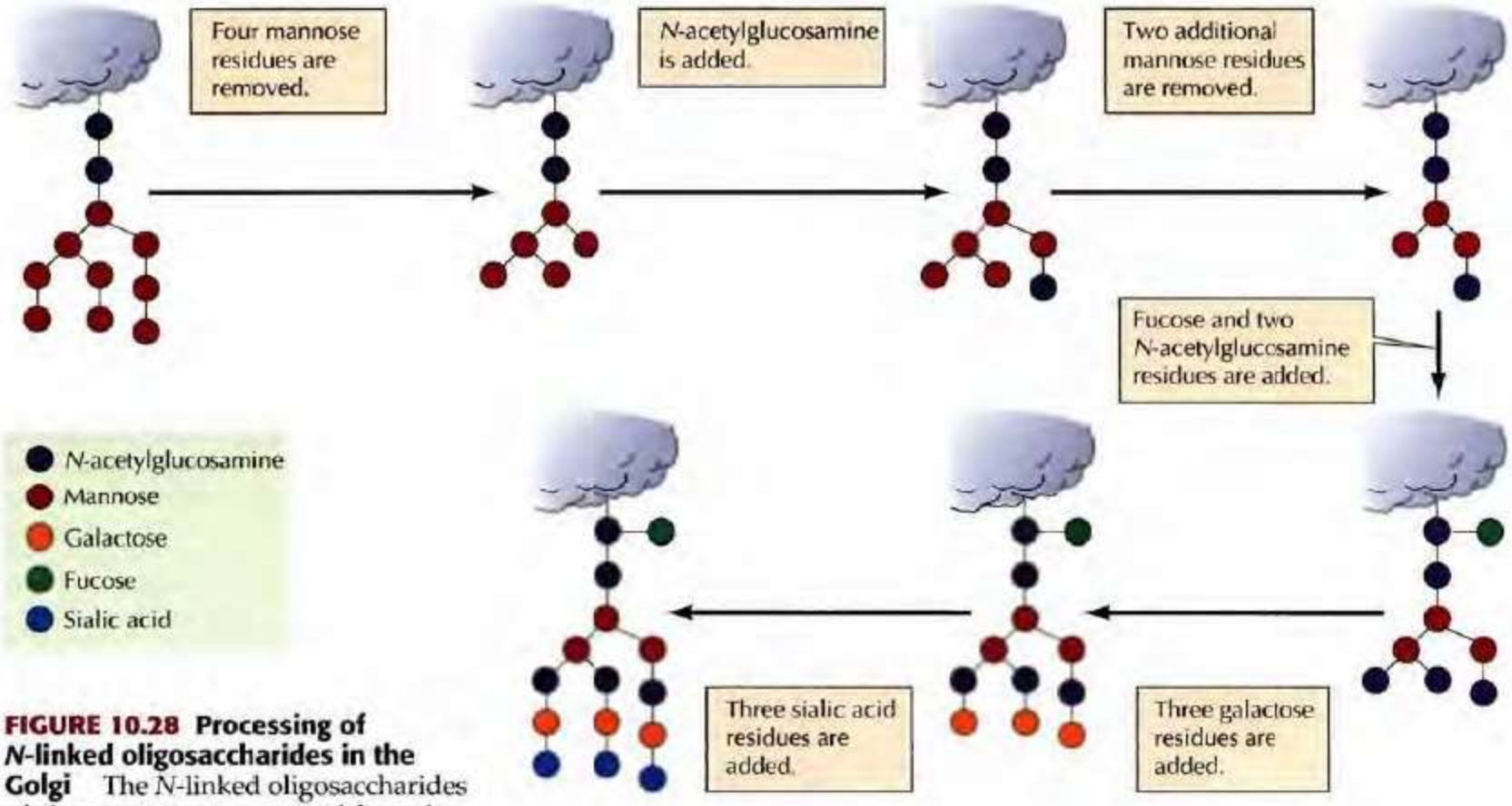
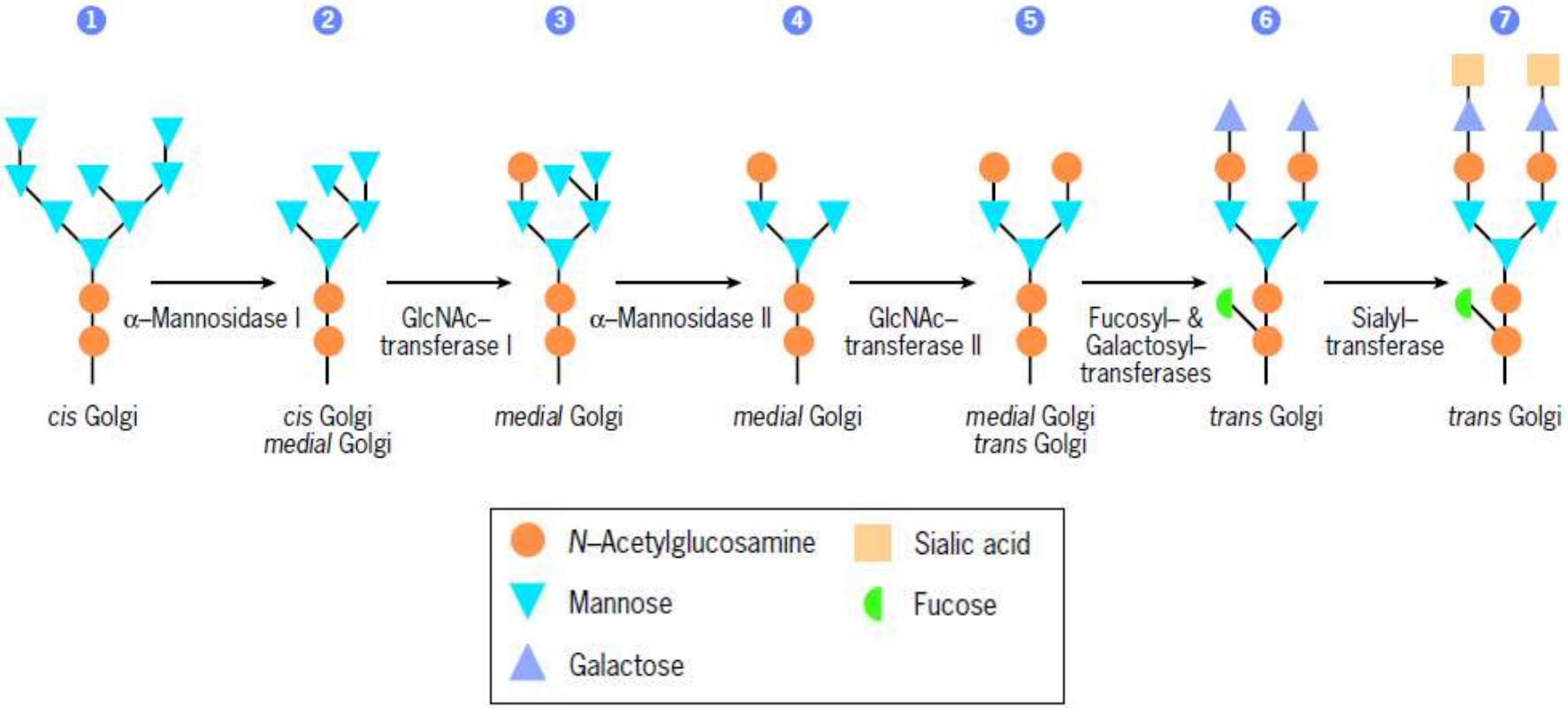


FIGURE 10.28 Processing of *N*-linked oligosaccharides in the Golgi The *N*-linked oligosaccharides of glycoproteins transported from the ER are further modified by an ordered sequence of reactions in the Golgi.

Steps in the glycosylation of a typical mammalian *N-linked oligosaccharide in the* Golgi complex.



The Movement of Materials through the Golgi Complex

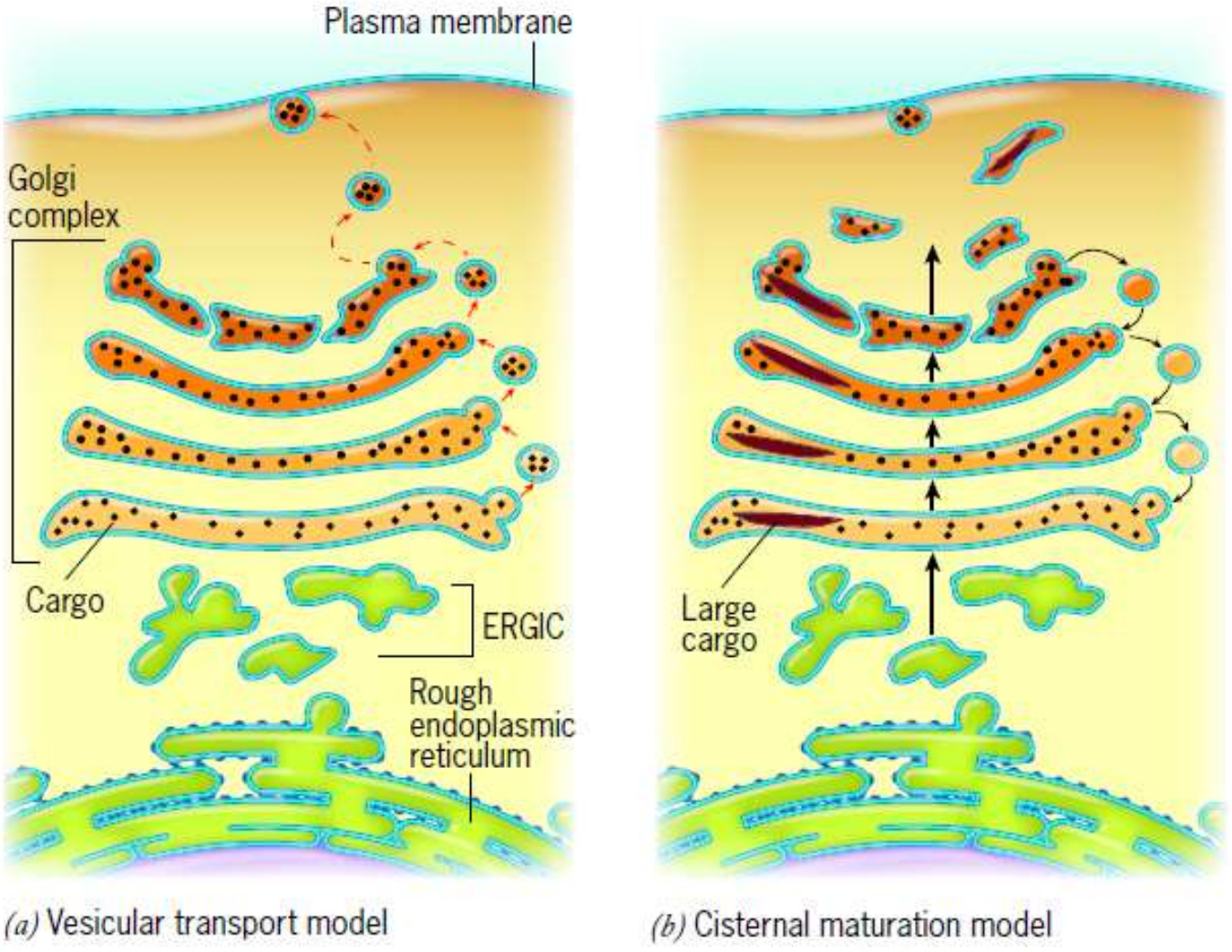
- **Cisternal maturation model** - according to this model, each cisterna "matures" into the next cisterna along the stack

  It was generally believed that **Golgi cisternae were transient structures**, up until the mid-1980s. It was supposed that golgi cisternae formed at the ***cis face of the* stack** by fusion of membranous carriers from the ER and ERGIC and that each cisterna physically moved from the *cis* to the ***trans end of the stack,*** changing in composition as it progressed.

The Movement of Materials through the Golgi Complex

- **Vesicular transport model** - From the mid-1980s to the mid-1990s an alternative model was established, which proposed that the **cisternae of a Golgi stack remain in place as stable compartments**. **Cargo** (i.e., secretory, lysosomal, and membrane proteins) **is shuttled through the Golgi stack**, from the CGN to the TGN, in vesicles that bud from one membrane compartment and fuse with a neighboring compartment farther along the stack

The dynamics of transport through the Golgi complex



Cargo Selection, Coat Proteins and Vesicle Budding

Types of Vesicle Transport and Their Functions

- Materials are carried between compartments by vesicles (or other types of membrane-bound carriers) that bud from donor membranes and fuse with acceptor membranes.
- Electron micrographs for vesicles caught in the act of budding, shows that most of these membranous buds are covered on their cytosolic surface by a “fuzzy,” electron-dense layer.
- Further analysis reveals that the dark-staining layer consists of a **protein coat** formed from soluble proteins that assemble on the cytosolic surface of the donor membrane at sites where budding takes place.
- Each coated bud pinches off to form a **coated vesicle**

Protein coats have at least two distinct functions

(1) they act as a mechanical device that causes the membrane to curve and form a budding vesicle.

(2) they provide a mechanism for selecting the components to be carried by the vesicle.

- Selected components include

(*a)* cargo consisting of secretory, lysosomal, and membrane proteins to be transported

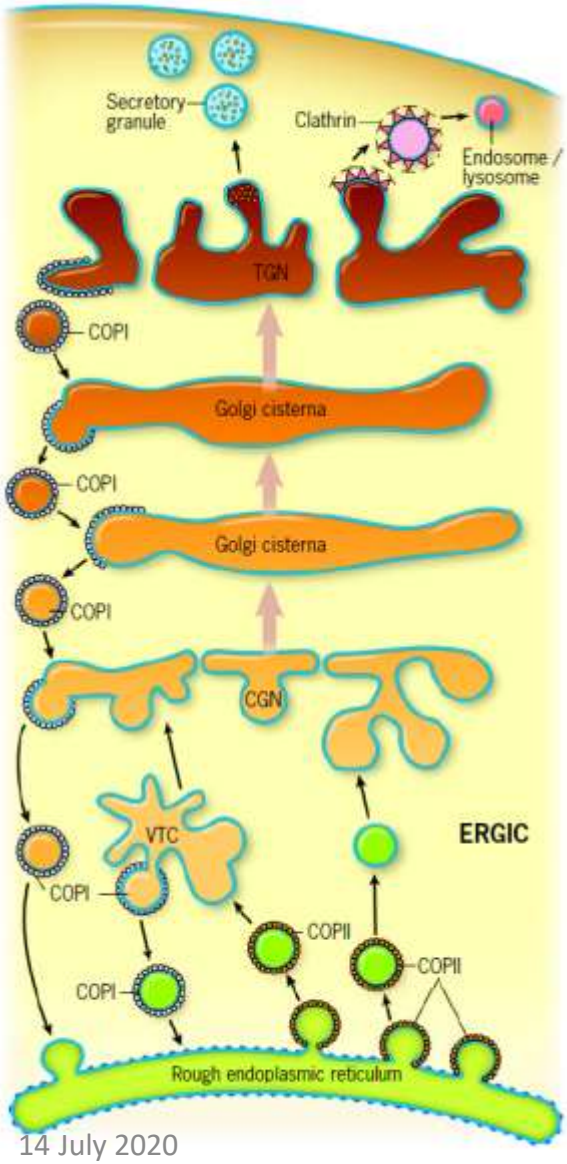
(*b)*the machinery required to target and dock the vesicle to the correct acceptor membrane.

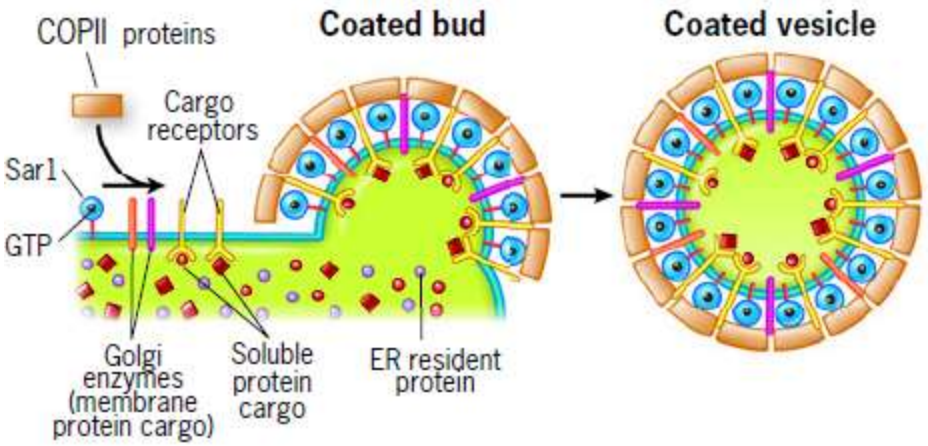
- The formation of coated vesicles is regulated by small GTP-binding proteins related to Ras and Ran. Two families of GTP-binding proteins play roles in **transport vesicle budding**: ADP-ribosylation factors (ARFs 1-3 & Sarl) and a large family of Rab proteins.
- These regulate adaptor proteins that interact directly with a vesicle coat protein. The binding of GTP-binding proteins and adaptor proteins establishes a "platform" on the membrane for a specific process, such as **assembly** and **budding** of a transport vesicle directed from the transitional ER to the Golgi or from the *trans Golgi* network to endosomes and lysosomes.
- Individual proteins in the complex (coat proteins, adaptor proteins, and GTP-binding proteins) may participate in assembly of transport vesicles directed elsewhere, or in vesicle fusion but each protein complex is apparently unique to a particular budding, transport, or fusion pathway.

The three best studied coated vesicles are the following:

- **COPII-coated vesicles** move materials from the ER "forward" to the ERGIC and Golgi complex (COP is an acronym for coat proteins).
- **COPI-coated vesicles** move materials in a **retrograde direction** (1) from the ERGIC and Golgi stack "backward" toward the ER and (2) from trans Golgi cisternae "backward" to cis Golgi cisternae.
- **Clathrin-coated vesicles** move materials from the TGN to endosomes, lysosomes, and plant vacuoles. They also move materials from the plasma membrane to cytoplasmic compartments along the endocytic pathway. They have also been implicated in trafficking from endosomes and lysosomes.

Proposed movement of materials by vesicular transport between membranous compartments of the biosynthetic/secretory pathway.





COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex

- Proteins selected by COPII-coated vesicles include

  (1) enzymes that act at later stages in the biosynthetic pathway, such as the glycosyltransferases of the Golgi complex

  (2) Membrane proteins involved in the docking and fusion of the vesicle with the target compartment

  (3) membrane proteins that are able to bind soluble cargo (such as the secretory proteins)

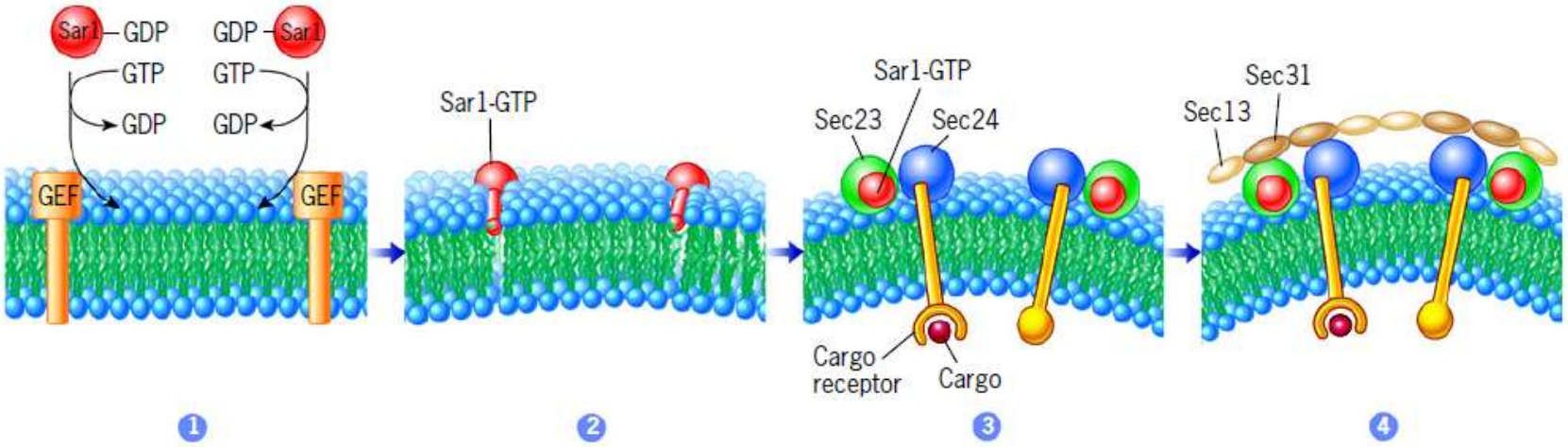
Cells lacking a specific cargo receptor typically fail to transport a specific subset of proteins from the ER to the Golgi complex

Role of **Sar1** in generating membrane curvature, assembling the protein coat, and capturing cargo

One of the COPII coat proteins is a small **G protein** called **Sar1**, which is recruited specifically to the ER membrane. Sar1 initiates vesicle formation and regulates the assembly of the vesicle coat.

- **step 1** - Sar1 is recruited to the ER membrane in the GDP-bound form by a protein called a GEF (guanine-exchange factor) that catalyzes the exchange of the bound GDP with a bound GTP.
- **step 2** - Upon binding of GTP, Sar1 undergoes a conformational change (extended a finger-like helix) that causes its N-terminal helix to insert itself into the cytosolic leaflet of the ER bilayer. This event expands the leaflet and induces the curvature (conversion of a flattened membrane into a spherical vesicle) of the lipid bilayer at that site. Membrane bending is aided by a change in packing of the lipids that make up the two leaflets of the bilayer.

Proposed roles of the COPII coat proteins in generating membrane curvature, assembling the protein coat, and capturing cargo



- **step 3** - a “banana-shaped” dimer composed of two COPII polypeptides (Sec23 and Sec24) has been recruited by the bound Sar1-GTP. Because of its curved shape, the Sec23-Sec24 heterodimer provides additional pressure on the membrane surface to help it further bend into a curved bud in the formation of a vesicle.

  Sec24 also functions as the primary adaptor protein of the COPII coat that interacts specifically with the ER export signals in the cytosolic tails of membrane proteins that are destined to traffic on to the Golgi complex, thus help in cargo accumulation.
- **step 4**, the remaining COPII polypeptides (Sec13 and Sec31) have joined the complex to form an outer structural scaffold of the coat. A certain degree of flexibility is built into the hinge between the Sec13- Sec31 subunits that allow them to form cages of varying diameter, thus accommodating vesicles of varying size.

Fate of COP II coated vesicle:

- Once the entire COPII coat has assembled, the bud is separated from the ER membrane in the form of a COPII coated vesicle.
- Before the coated vesicle can fuse with a target membrane, the protein coat must be disassembled and its components released into the cytosol.
- Disassembly is triggered by hydrolysis of the bound GTP to produce a Sar1-GDP subunit, which has decreased affinity for the vesicle membrane.
- Dissociation of Sar1-GDP from the membrane is followed by the release of the other COPII subunits.

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

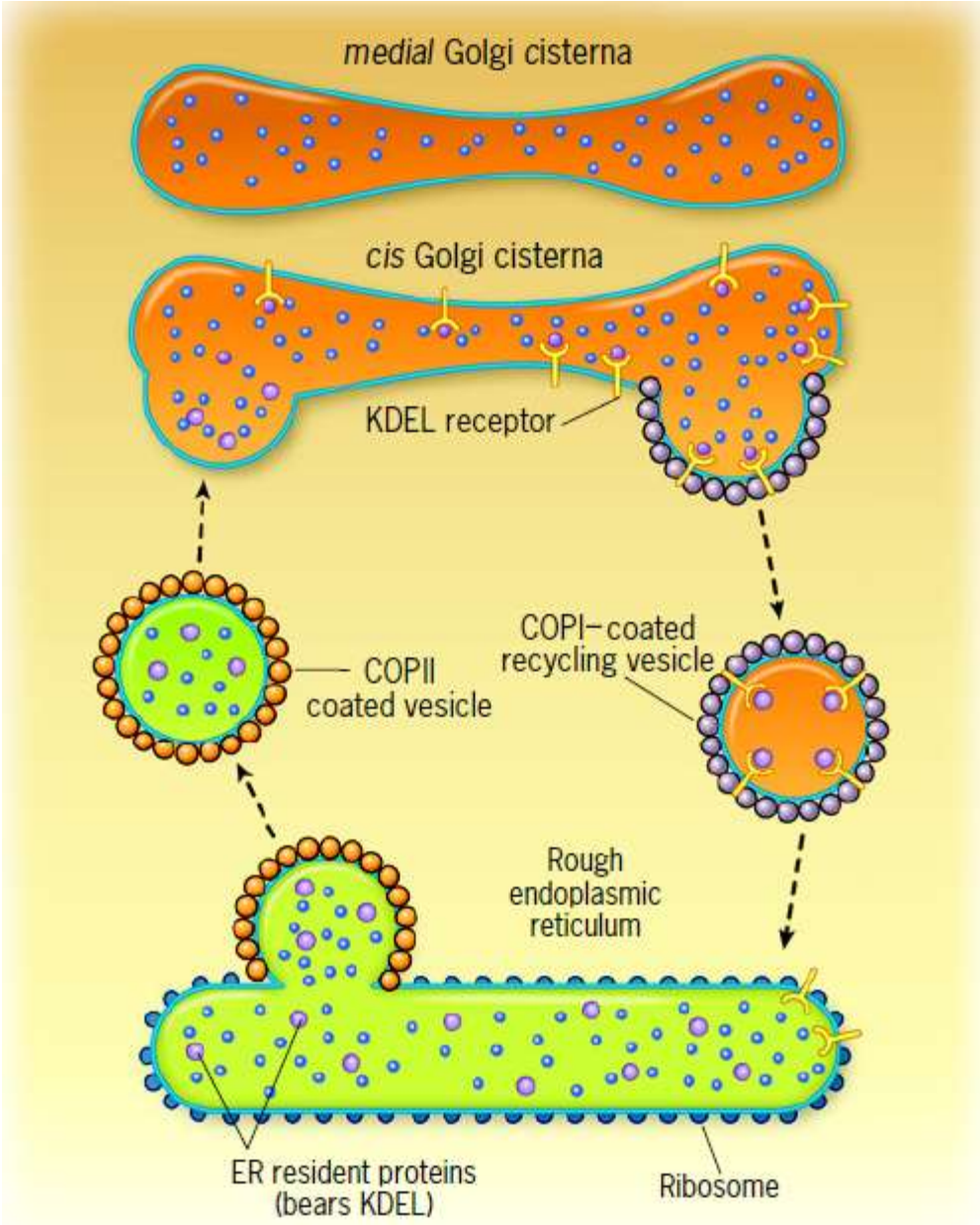
- COPI coated vesicles are engaged in the retrograde transport of proteins.

That includes the movement of

(1) Golgi resident enzymes in a *trans*-to-*cis* direction.

(2) ER resident enzymes from the ERGIC and the Golgi complex back to the ER

Retrieving ER proteins



Retaining and Retrieving Resident ER Proteins

- If vesicles continually bud from membrane compartments, how does each compartment retain its unique composition?

- What determines, whether a particular protein in the membrane of the ER remains in the ER or proceeds on to the Golgi complex?

Two way solution to the problem:

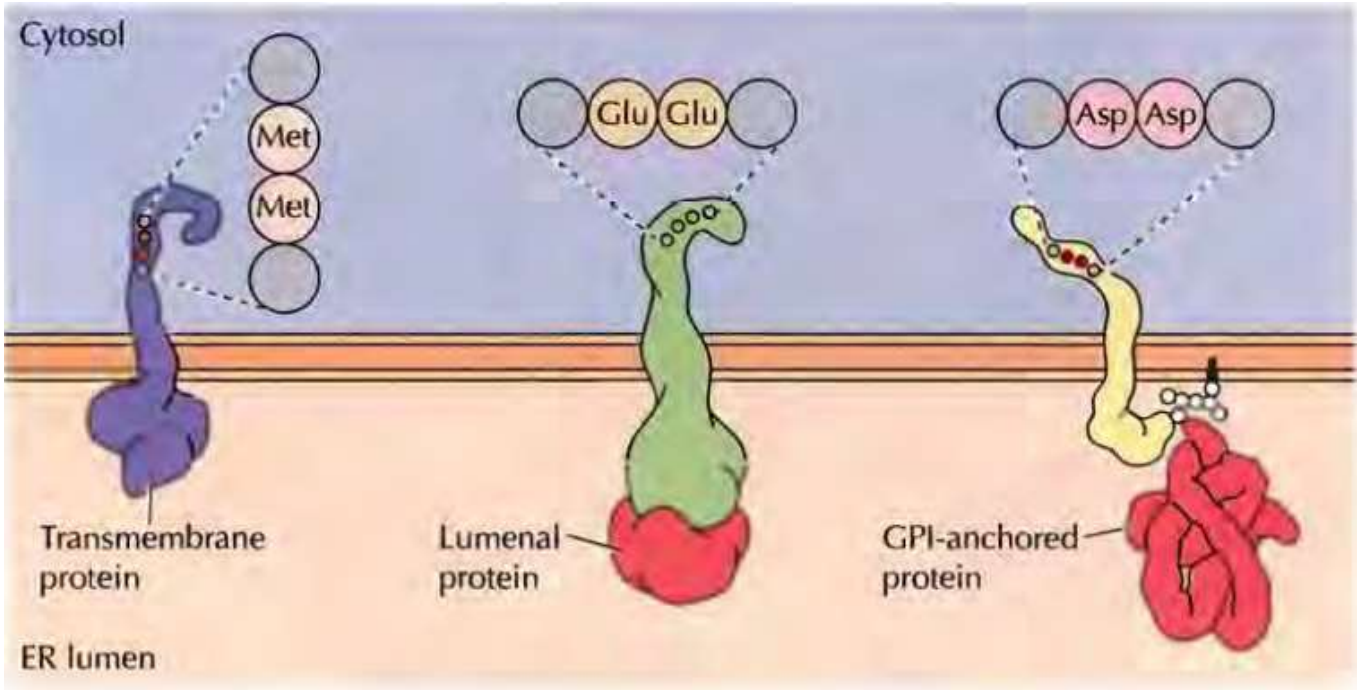
1. Retention of resident molecules that are excluded from transport vesicles. Retention may be based primarily on the physical properties of the protein.

For example, soluble proteins that are part of large complexes or membrane proteins with short transmembrane domains are not likely to enter a transport vesicle.

2. Retrieval of "escaped" molecules back to the compartment in which they normally reside**.**

ER export signals :

- Many transmembrane proteins possess **di-acidic** or **dihydrophobic** amino acid sequences in their cytosolic domains that function as ER export signals.
- Both GPI-anchored proteins (which are marked for export by their GPI-anchors) and lumenal secretory proteins appear to be recognized and sequestered by these transmembrane receptor proteins.



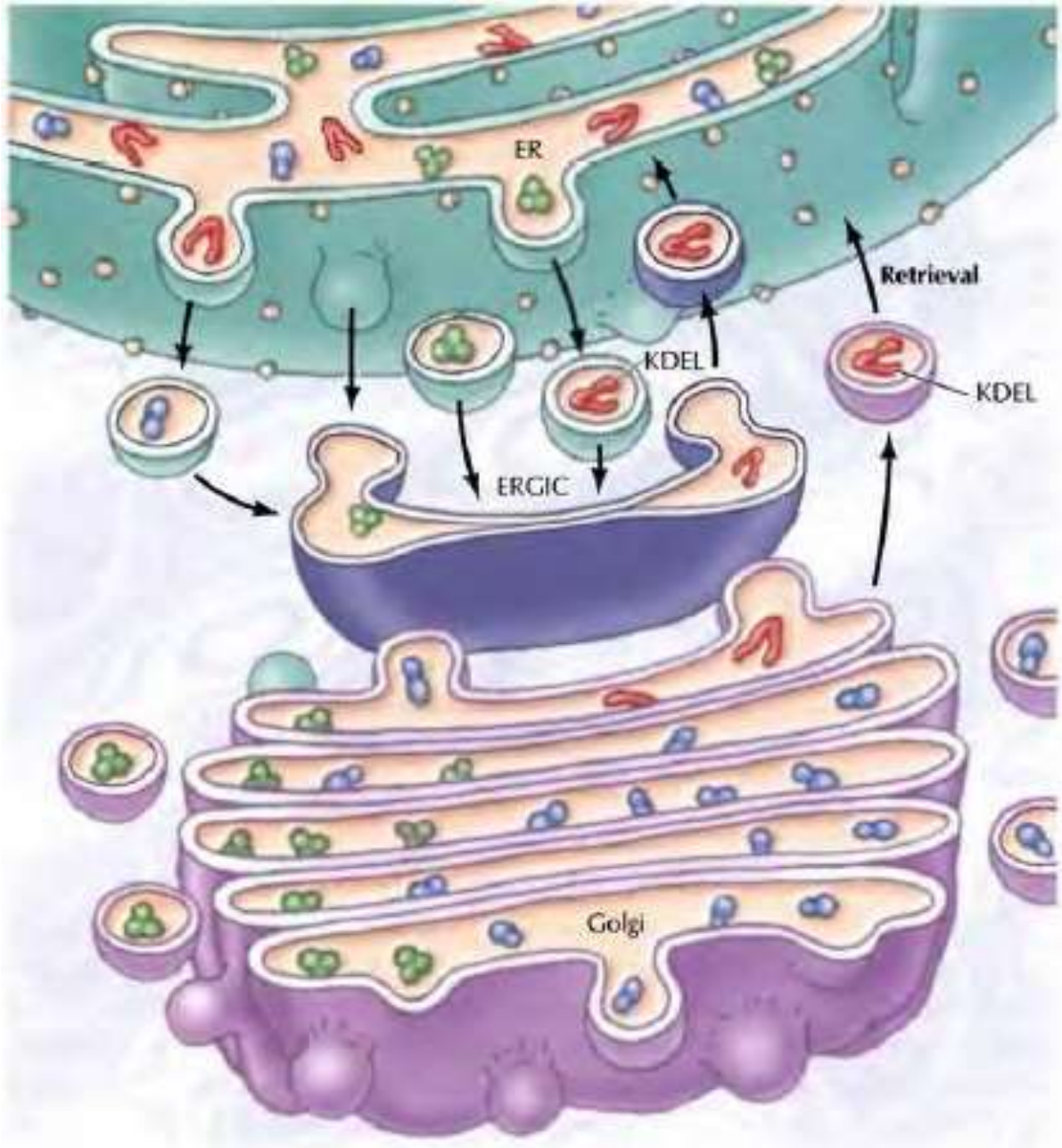
Retrieval signals :

- Proteins that normally reside in the ER (those both in the lumen and in the membrane), contain **short amino acid sequences** at their **C-terminus** that serve as ***retrieval signals,*** ensuring their return to the ER if they should be accidentally carried forward to the ERGIC or Golgi complex.
- **Soluble resident proteins** of the ER lumen (such as protein disulfide isomerase and the molecular chaperones that facilitate folding) typically possess the retrieval signal "lys-asp-glu-leu" (or KDEL in single-letter nomenclature). These proteins are recognized and returned to the ER by the ***KDEL receptor,*** an integral membrane protein that shuttles between the *cis Golgi and the* ER compartments.
- **Membrane proteins** that reside in the ER also have a retrieval signal that binds to the COPI coat, facilitating their return to the ER. The most common retrieval sequences for ER membrane proteins involve two closely linked basic residues, most commonly ***KKXX*** (where K is lysine and X is any residue).

- The KDEL and KKXX signals do not prevent ER proteins from being packaged into vesicles and carried to the Golgi. Instead, these signals cause these ER resident proteins to be selectively retrieved from the ER-Golgi intermediate compartment or the Golgi complex and returned to the ER via a recycling pathway.

- Proteins bearing the KDEL and KKXX sequences bind to specific recycling receptors in the membranes of these compartments and are then selectively transported back to the ER.

Retrieval of resident ER proteins



Beyond the Golgi Complex: Sorting Proteins at the TGN

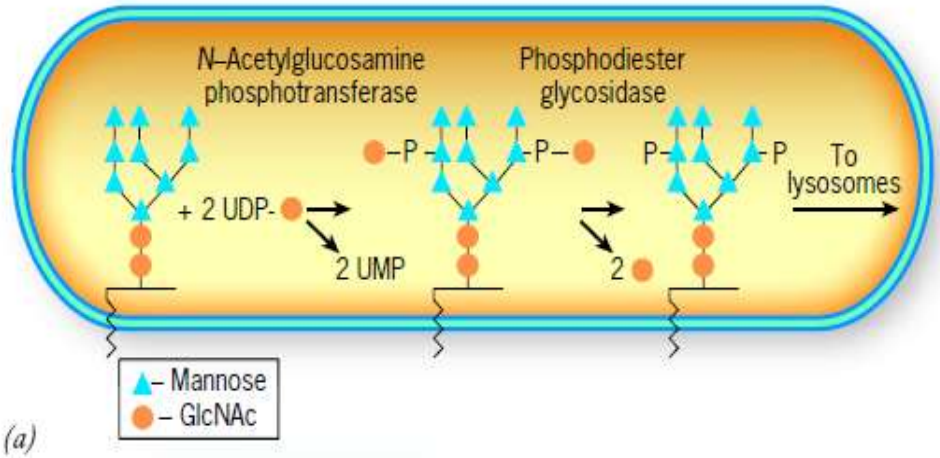
- How a particular protein that has been synthesized in the ER is targeted toward a particular cellular destination ?
- The ***trans* Golgi network (TGN),** which is the last stop in the Golgi complex, functions as a major **sorting station**, directing proteins to various destinations.

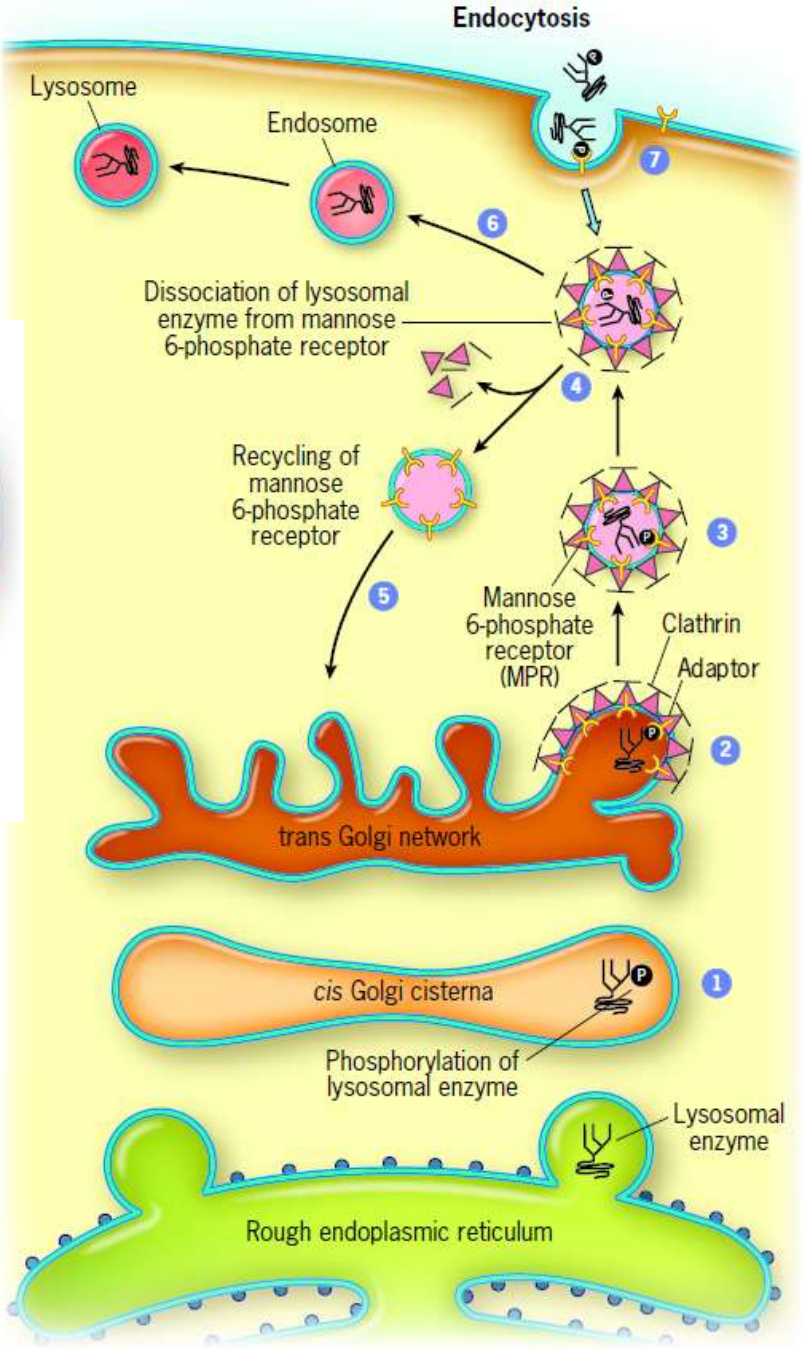
  The best understood of the post-Golgi pathways is one that carries lysosomal enzymes.

Sorting and Transport of Lysosomal Enzymes

- Lysosomal proteins are synthesized on membrane-bound ribosomes of the ER and carried to the Golgi complex along with other types of proteins.
- Once in the Golgi cisternae, soluble lysosomal enzymes are specifically recognized by enzymes that catalyze the two-step addition of a phosphate group to certain mannose sugars of the *N-linked* carbohydrate chains.
- Lysosomal enzymes possess phosphorylated mannose residues, which act as **sorting signals**.
- Lysosomal enzymes carrying a **mannose 6-phosphate signal** are recognized and captured by ***mannose 6-phosphate receptors (MPRs)*****,** which are integral membrane proteins that span the TGN membranes

Targeting lysosomal enzymes to lysosomes

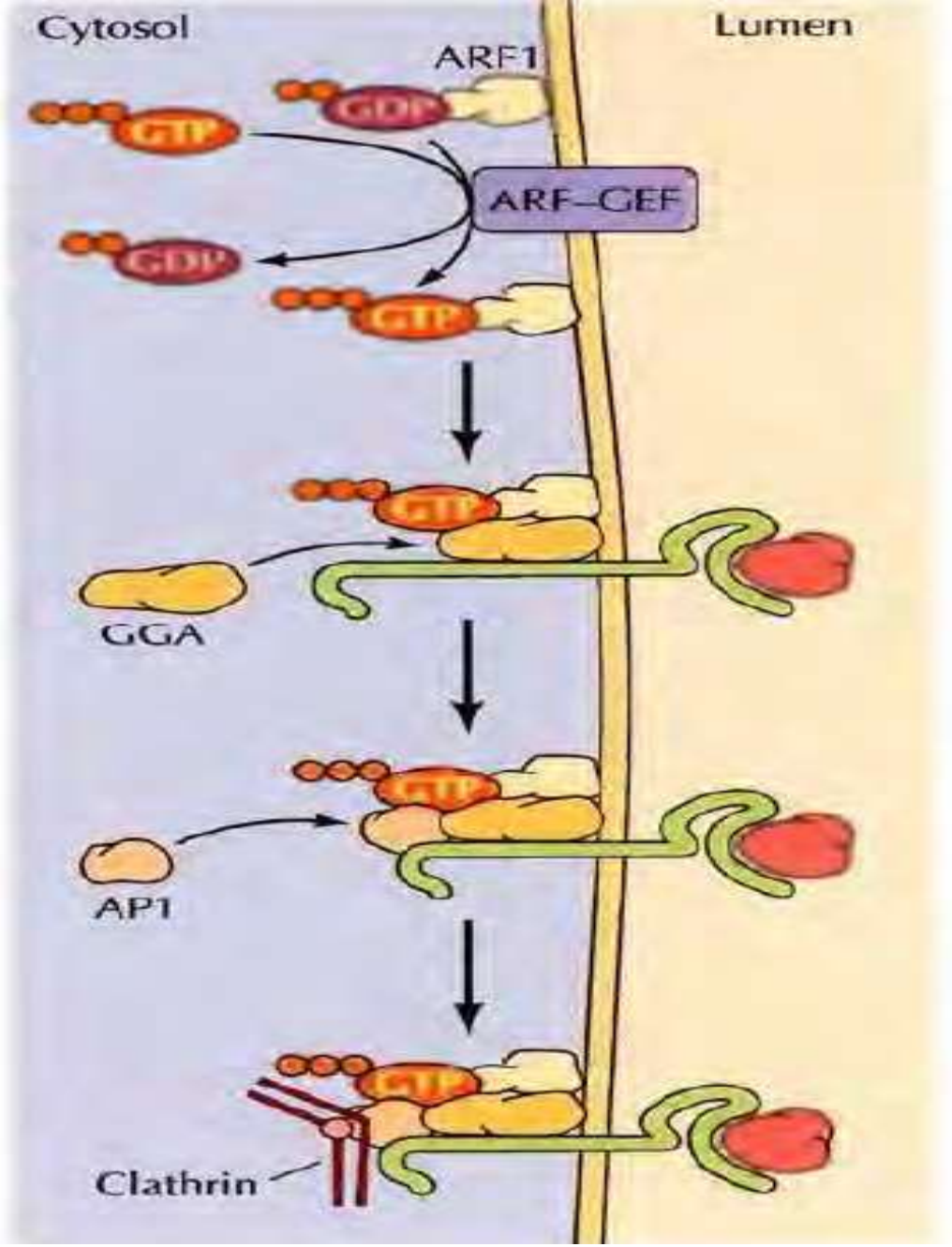




Clathrin-Coated Vesicles

- Responsible for the uptake of extracellular molecules from the plasma membrane by endocytosis as well as the transport of molecules from the *trans* golgi network to endosomes, lysosomes, or the plasma membrane.
- The formation of clathrin-coated vesicles on the ***trans* Golgi network** requires **clathrin**, the GTP-binding protein, **ARF1**, and at least two types of adaptor proteins.
- **At first ARF /GDP binds to proteins in the Golgi membrane. After being delivered to the membrane, ARF/GDP is activated to ARF/GTP by a specific ARF-guanine nucleotide exchange factor (ARF-GEF). ARF /GTP recruits a GGA adaptor protein to the membrane and this protein recruits a transmembrane receptor with its bound lumenal cargo by interacting with the cytosolic tail of the receptor. GGA then recruits a second adaptor protein, APl, which serves as a binding site for assembly of a clathrin coat.**
- Clathrin actually plays a structural role in vesicle budding by assembling into a basketlike lattice structure that distorts the membrane and initiates the bud. During transport, the GTP bound to ARFl is hydrolyzed to GDP and the ARF /GDP is released from the membrane to recycle.

Initiation of a clathrin-coated vesicle by ARFl



Lysosomal enzymes are transported from the TGN in **clathrin-coated vesicles**

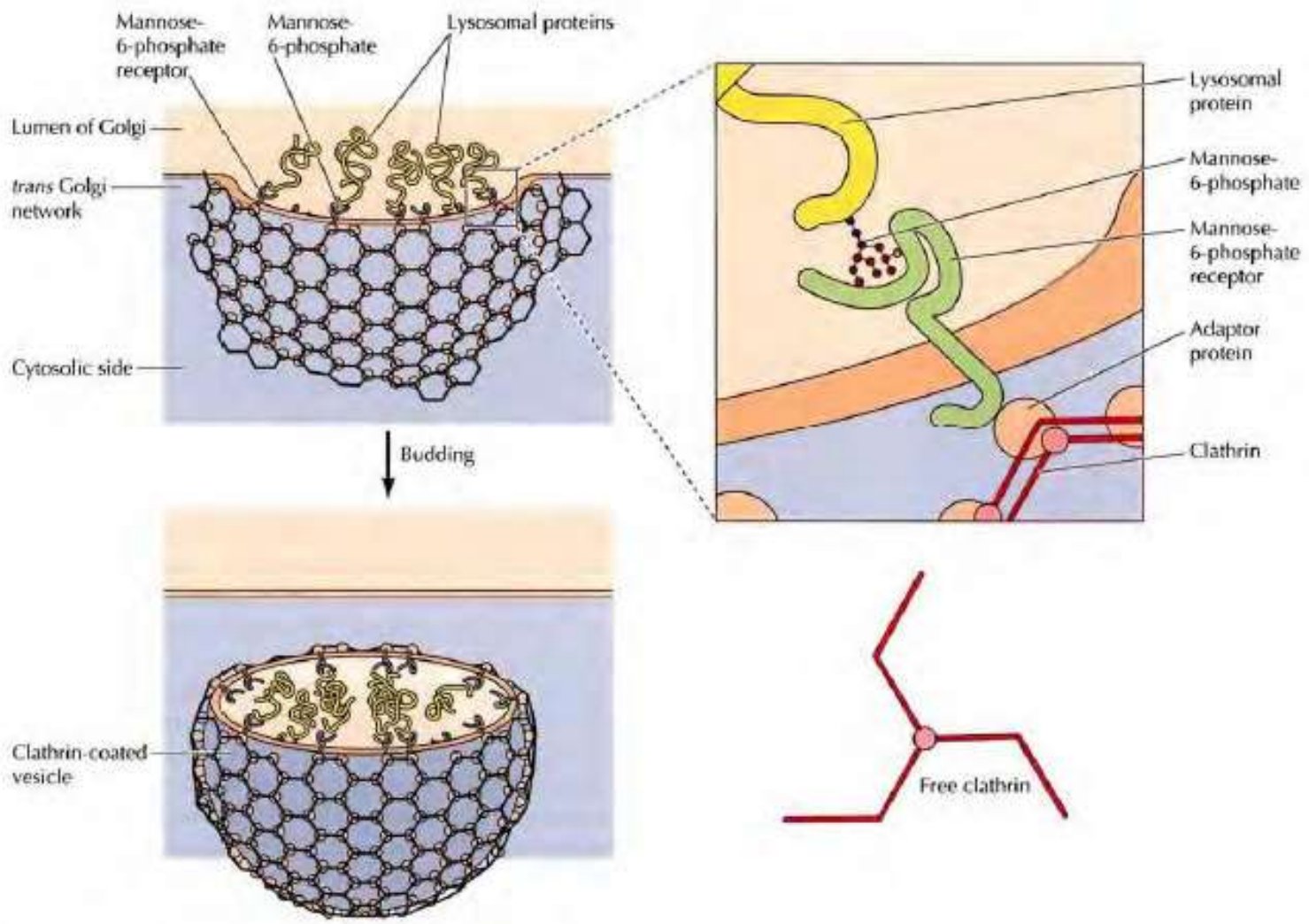
- These vesicles contain

  (1) an outer honeycomb like lattice composed of the protein clathrin, which forms a structural scaffold

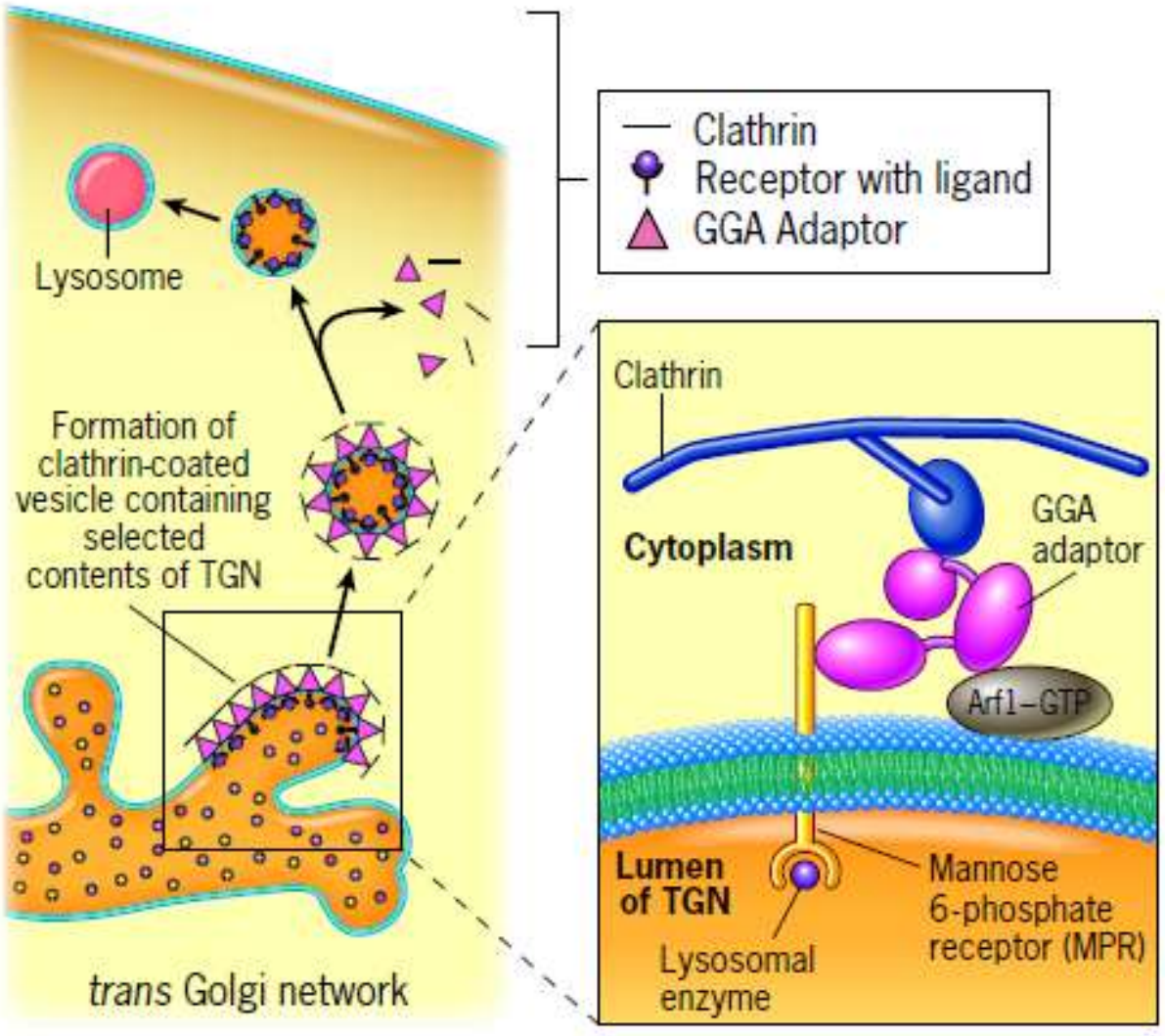
  (2) an inner shell composed of protein adaptors, (a molecule that physically links two or more components) which covers the surface of the vesicle membrane that faces the cytosol.

- Lysosomal enzymes are escorted from the TGN by a family of adaptor proteins called **GGAs.**

Incorporation of lysosomal proteins into clathrin-coated vesicles



The formation of clathrin-coated vesicles at the TGN.



Sorting and Transport of Nonlysosomal Proteins

- Though the mechanisms are poorly understood, still it is believed that membranous carriers are produced as the TGN fragments into vesicles and tubules of various size and shape for transportation.
- Proteins that are discharged from the cell by a process of regulated secretion, are thought to form selective aggregates that eventually become contained in large, densely packed secretory granules. These aggregates are apparently trapped as immature secretory granules bud from the rims of the *trans Golgi* cisternae and TGN.

Sorting and Transport of Nonlysosomal Proteins

- In some cells, long tubules are seen to be pulled out of the TGN by motor proteins that operate along microtubular tracks. These tubules are then split into a number of vesicles or granules by membrane fission. Once they have departed from the TGN, the contents of the secretory granules become more concentrated. Eventually, the mature granules are stored in the cytoplasm until their contents are released following stimulation of the cell by a hormone or nerve impulse.
- The targeted delivery of integral proteins to the plasma membrane appears to be based largely on sorting signals in the cytoplasmic domains of the membrane proteins. membrane proteins destined to reside in the apical portion of the plasma membrane contain different sorting signals from those destined for the lateral or basal portion. These two groups of plasma membrane proteins are clustered into different TGN membrane domains and transported to the cell surface in separate carriers.

- Vesicle fusion requires specific interactions between different membranes. Vesicles from the ER, for example, fuse with the ERGIC or *cis* Golgi network and not with a *trans* cisterna. Selective fusion is one of the factors that ensures a directed flow through the membranous compartments of the cell

SNARE hypothesis : (James Rothman)

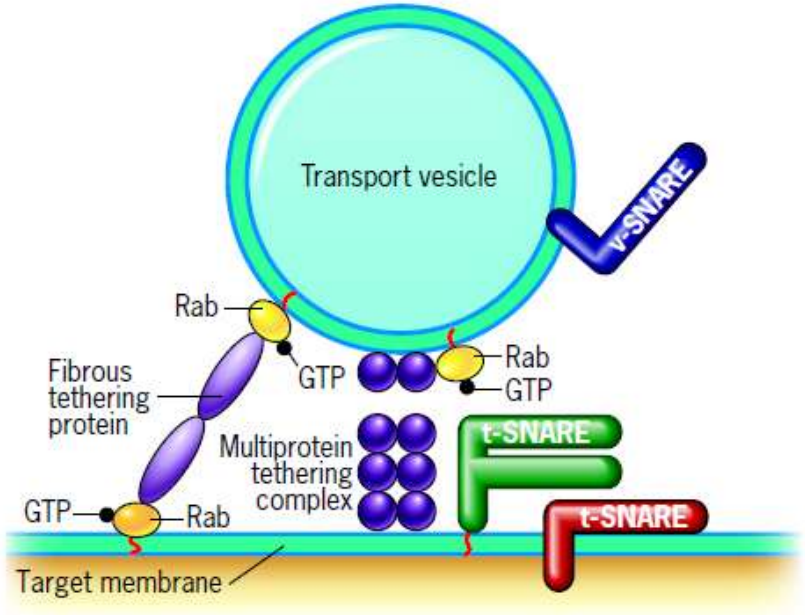
- Vesicle fusion is mediated by interactions between specific pairs of transmembrane proteins, called SNAREs, on the vesicle and target membranes (v-SNAREs and t-SNAREs, respectively).
- According to the hypothesis, the formation of complexes between v-SNAREs on the vesicle and t-SNAREs on the target membranes leads to membrane fusion.

Targeting Vesicles to a Particular Compartment

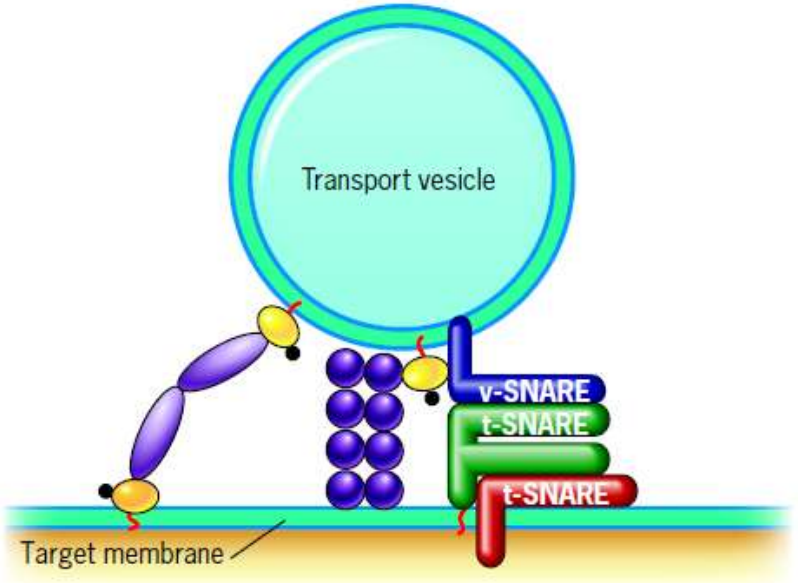
- **Steps:** (between the stages of vesicle budding and vesicle fusion)

1. **Movement of the vesicle toward the specific target compartment-** mediated largely by microtubules, which act like railroad tracks carrying cargo containers along a defined pathway to a predetermined destination.
2. **Tethering vesicles to the target compartment –** The initial contacts between a transport vesicle and its target membrane, are thought to be mediated by a diverse collection of "tethering" proteins.

Two groups of tethering proteins have been identified- rod-shaped, **fibrous proteins** (**golgin**, acts in and around the Golgi complex) that are capable of forming a molecular bridge between the two membranes over a considerable distance (50–200 nm) and large **multiprotein complexes** that appear to hold the two membranes in closer proximity.



(a) Tethering



(c) Docking

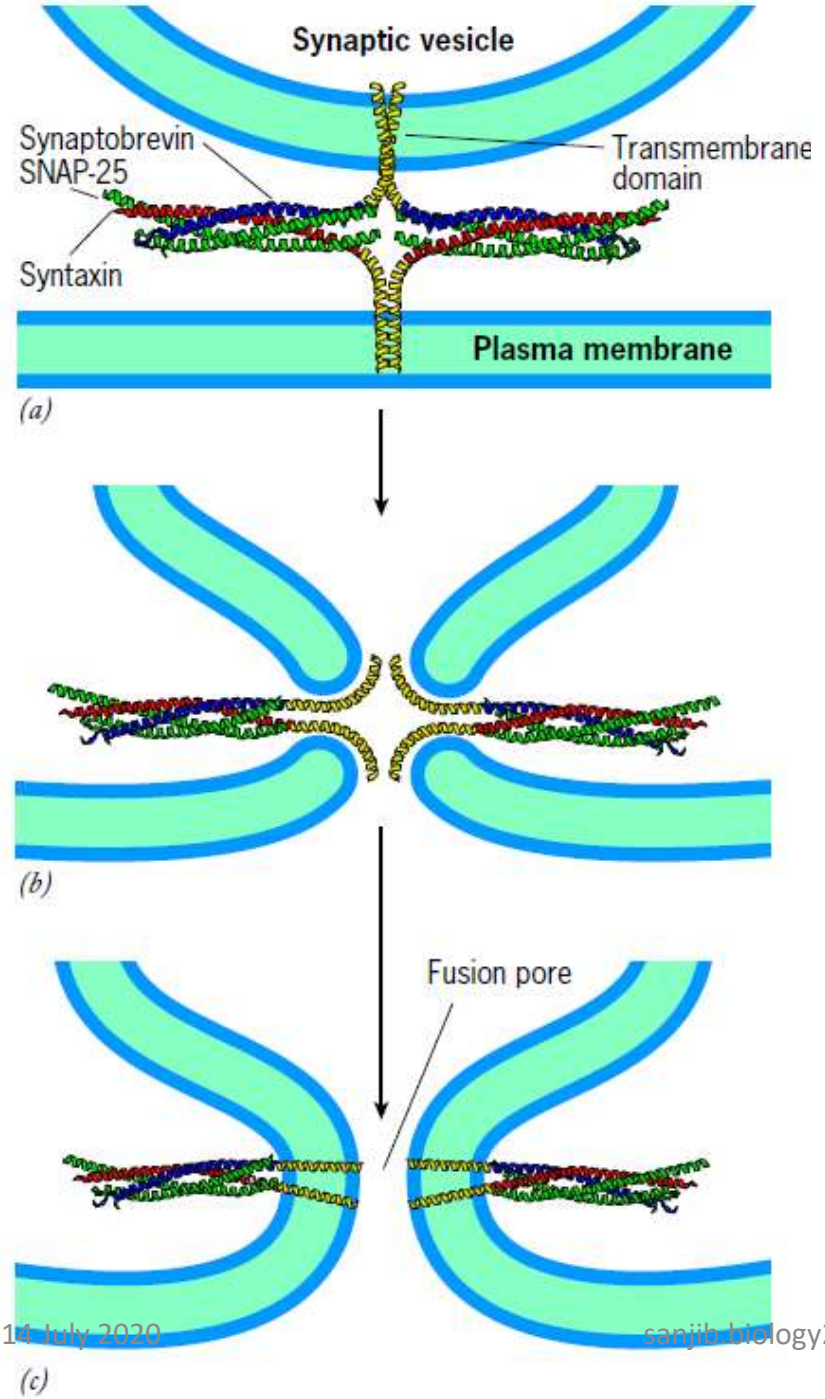
3. **Docking vesicles to the target compartment-** the membranes of the vesicle and target compartment come into close contact with one another as the result of an interaction between the cytosolic regions of integral proteins (**SNAREs**) of the two membranes.

   [Although SNAREs vary considerably in structure and size, all contain a segment in their cytosolic domain called a ***SNARE motif*** that consists of 60–70 amino acids capable of forming a complex with another SNARE motif. SNAREs can be divided functionally into two categories, **v-SNAREs,** which become incorporated into the membranes of transport vesicles during budding, and **t-SNAREs,** which are located in the membranes of target compartments]

4. **Fusion between vesicle and target membranes -** interactions between t- and v-SNAREs are capable of pulling two lipid bilayers together with sufficient force to cause them to fuse. once the lipid bilayers of the two membranes merge, the SNAREs that previously projected from separate membranes now reside in the same membrane

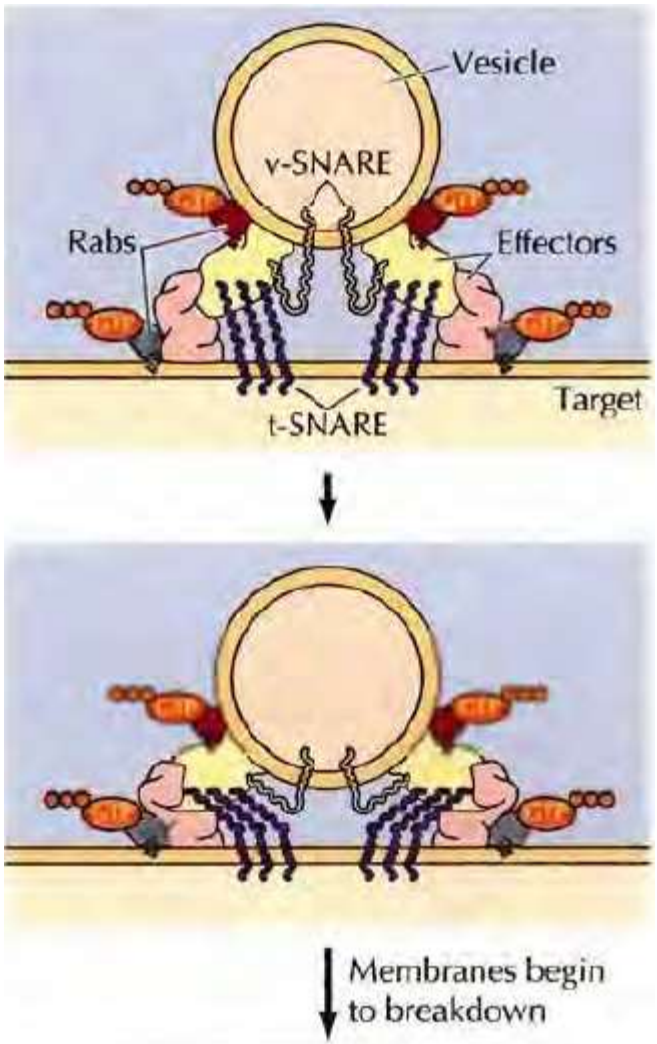
Eg. docking of synaptic vesicles with the presynaptic membrane during the regulated release of neurotransmitters.

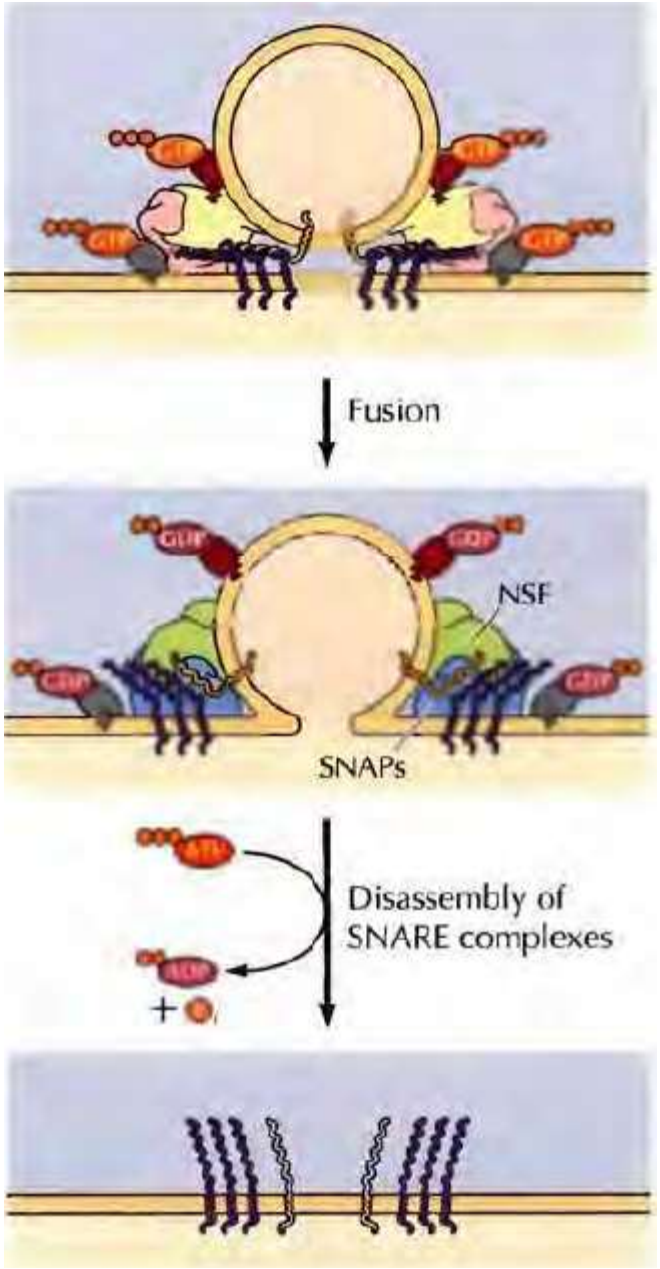
- Here, the plasma membrane of the nerve cell contains two t-SNAREs, syntaxin and SNAP-25, whereas the synaptic vesicle membrane contains a single v-SNARE, synaptobrevin.
- As the synaptic vesicle and presynaptic membrane approach one another, the SNARE motifs of t- and v-SNARE molecules from apposing membranes interact to form four-stranded bundles. Each bundle consists of four helices, two donated by SNAP-25 and one each donated by syntaxin and synaptobrevin. These parallel helices zip together to form a tightly interwoven complex that pulls the two apposing lipid bilayers into very close association for fusion.
- Following membrane fusion, the NSF /SNAP complex disassembles the SNARE complex, allowing the SNAREs to be reused for subsequent rounds of vesicle transport.
- As the energy of SNARESNARE interaction drives the fusion of the membranes, energy from hydrolysis of ATP is required to separate the SNAREs.



A model of the interactions between v- and t-SNAREs leading to membrane fusion and exocytosis

Vesicle fusion





Role of **Rab proteins** in tethering & docking :

- Members of the Rab family of small GTP-binding proteins play key roles in the docking of transport vesicles.
- More than 60 different Rab proteins have been identified and shown to function in specific vesicle transport processes.
- The Rab proteins are carried through the cytosol in their GDP-bound form by GDP-dissociation inhibitors (GDIs). At a membrane, they are removed from GDIs by GDI-displacement factors . Specific guanine nucleotide exchange factors then convert Rab/GDP to the active Rab/GTP state.
- To initiate transport vesicle fusion, Rab/GTP on the transport vesicle interacts with effector proteins and v-SNAREs to assemble a pre-fusion complex.
- A different Rab protein on the target membrane similarly organizes other effector proteins and t-SNAREs.
- When the transport vesicle encounters this target membrane, the effector proteins link the membranes by protein-protein interactions.
- This tethering of the vesicle to the target membrane stimulates Rab/GTP hydrolysis and allows the vSNAREs to contact the t-SNAREs

Delivery of **Rab** t o a membrane

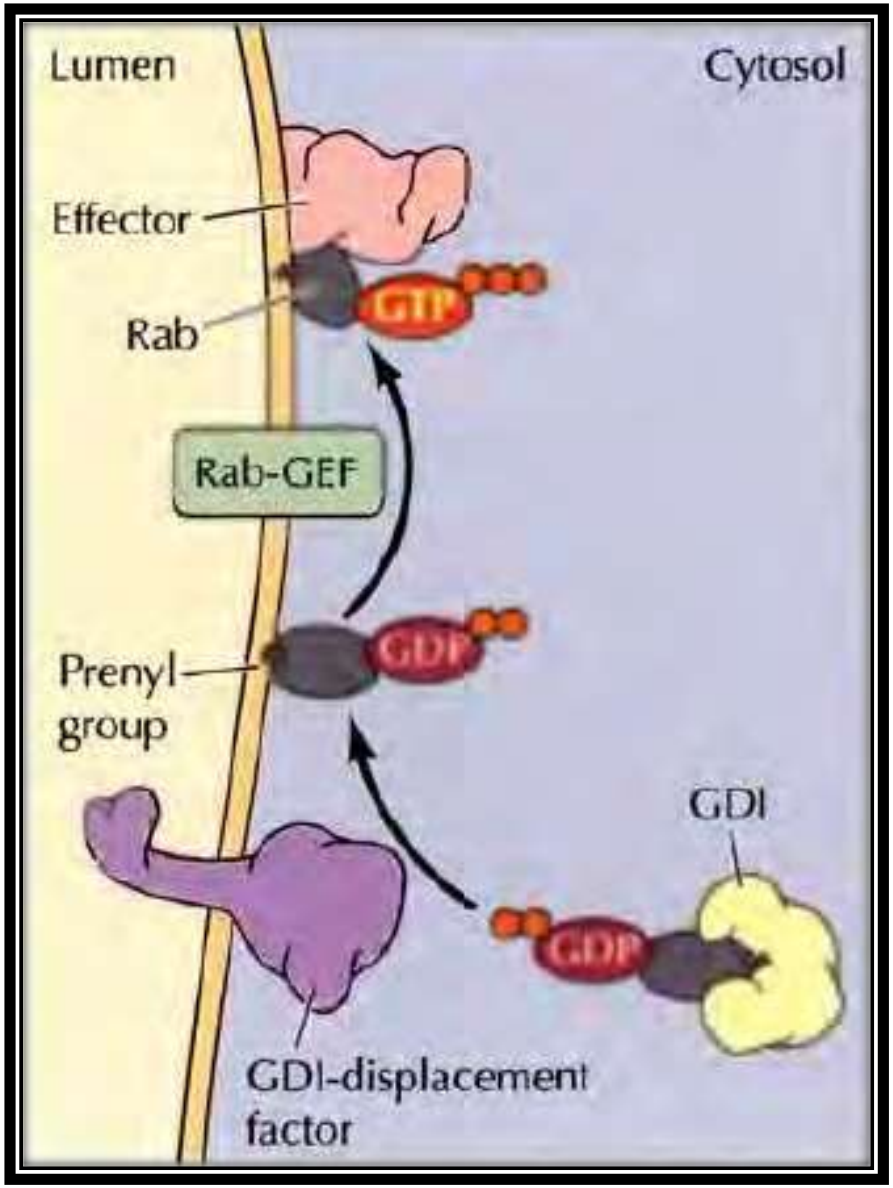


TABLE 10.1 Rab GTP-Binding Proteins and Their Sites of Action

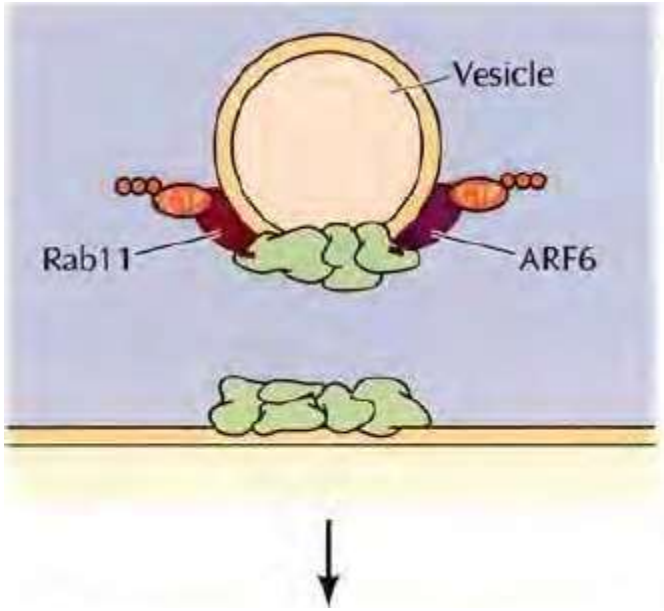
Transport step	Rab proteins involved
Exocytosis	
Transitional ER to Golgi	Rab1, Rab1b, Rab2
Golgi back to ER	Rab6, Rab6b
Intra-Golgi	Rab1, Rab6, Rab6b
trans Golgi network to plasma membrane	Rab11a, Rab11b
Endocytosis	
Plasma membrane to early endosome	Rab5a, Rab5b, Rab5c
Early endosome to plasma membrane	Rab4, Rab15, Rab18
Early endosome to late endosome	Rab7
Special roles	
Exocytosis of secretory granules	Rab8b
Late endosome to *trans* Golgi network	Rab9, Rab11a, Rab11b
trans Golgi network to basolateral membrane	Rab8a
trans Golgi network to apical membrane	Rab21

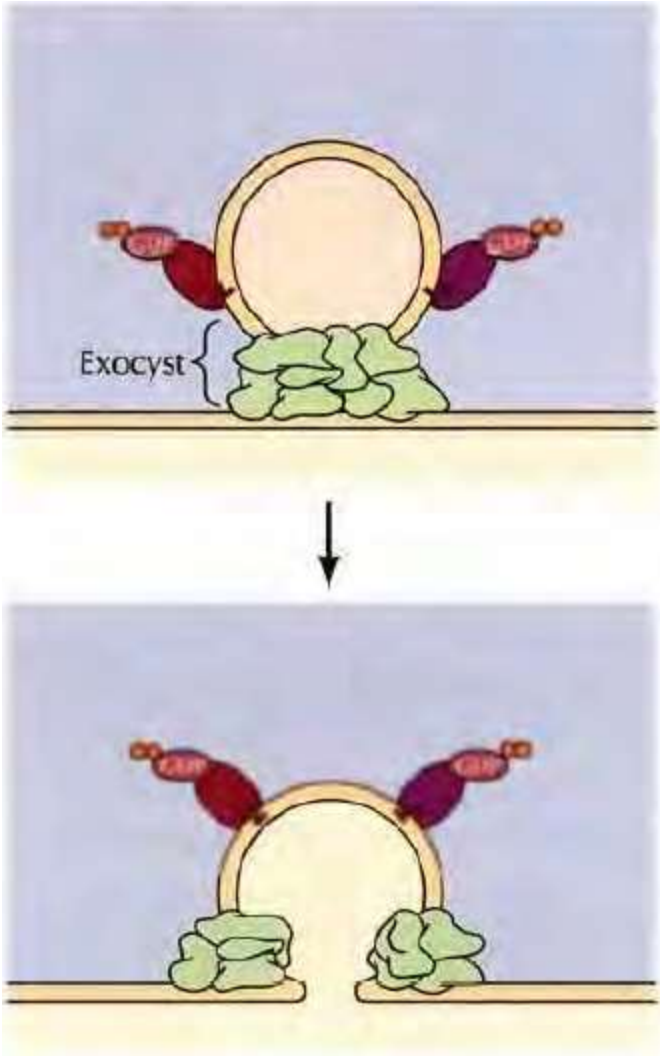
Examples of the more than 60 mammalian Rab proteins whose locations and presumptive functions are known.

Exocytosis:

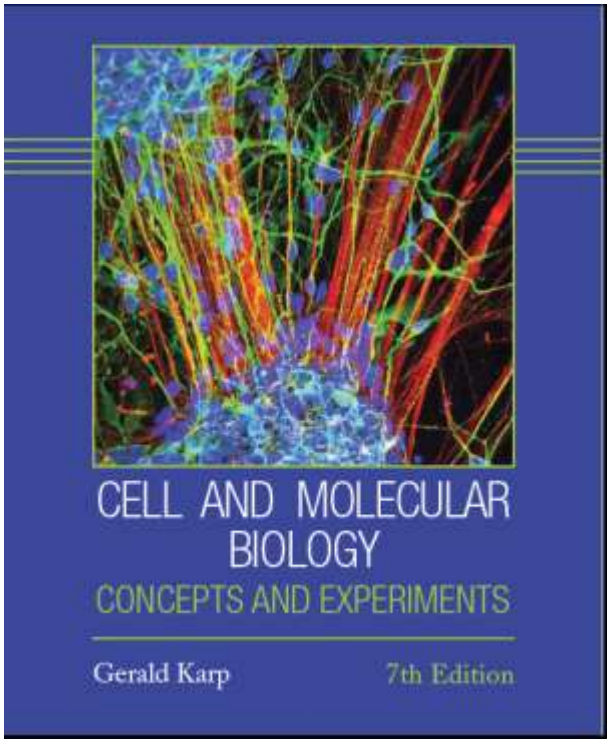
- Exocytosis is the fusion of a transport vesicle with the plasma membrane, resulting in secretion of the vesicle contents.
- Many types of exocytosis occur at specific protein complexes, called exocysts, on the plasma membrane.
- The structure of exocysts is not well understood but their assembly appears to require sequential interactions among **eight exocyst proteins** localized on both the transport vesicle and the target membrane site.
- Interaction of these proteins results in efficient targeting of the transport vesicle to a specific location on the plasma membrane.

Exocyst assembly and vesicle targeting





REFERENCES: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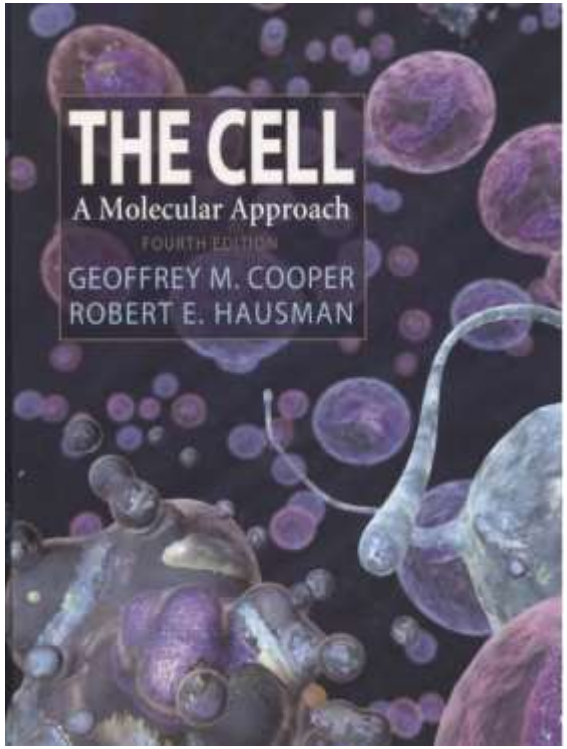
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