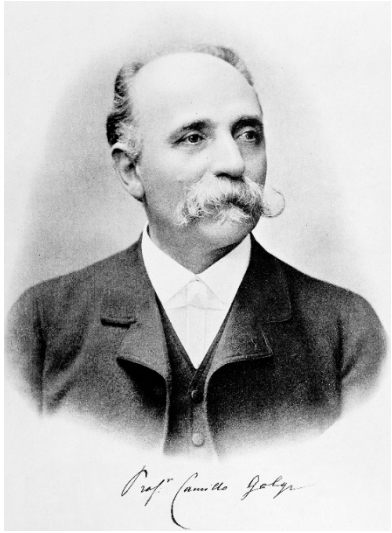
Membranous Organelles:

Golgi Complex, Endosomes, Coated Vesicles

"Cytology, Histology and Embryology"
Stefan Trifonov MD, PhD
24.03.2020

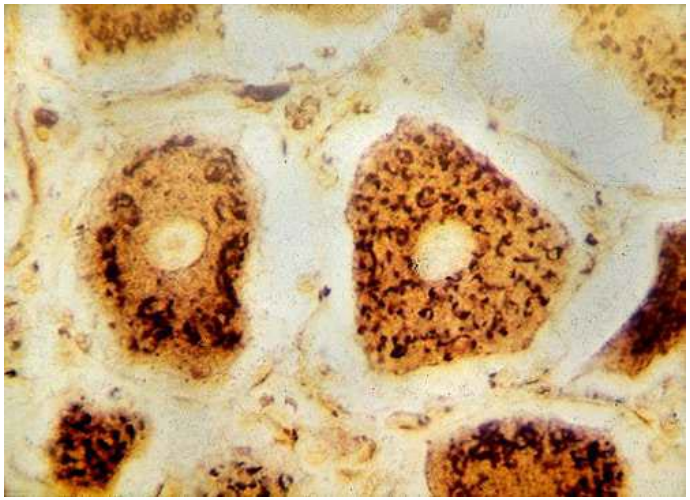
1. Golgi complex – structure and functions.
2. Endosomes – intracellular transport processes:
    a. Early endosomes.
    b. Late endosomes.
3. Coated and secretory vesicles.

Golgi Complex

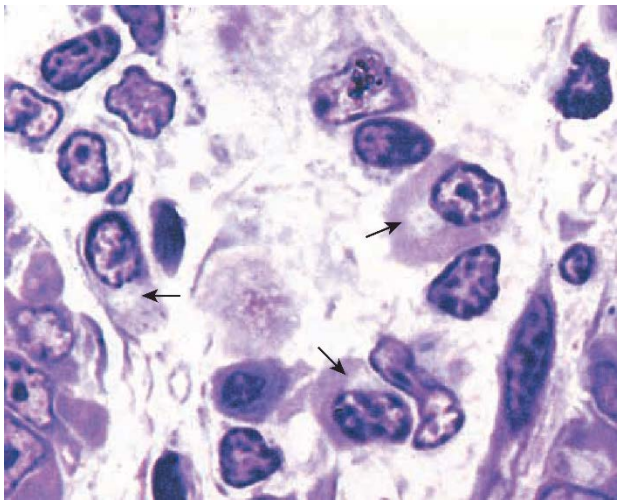


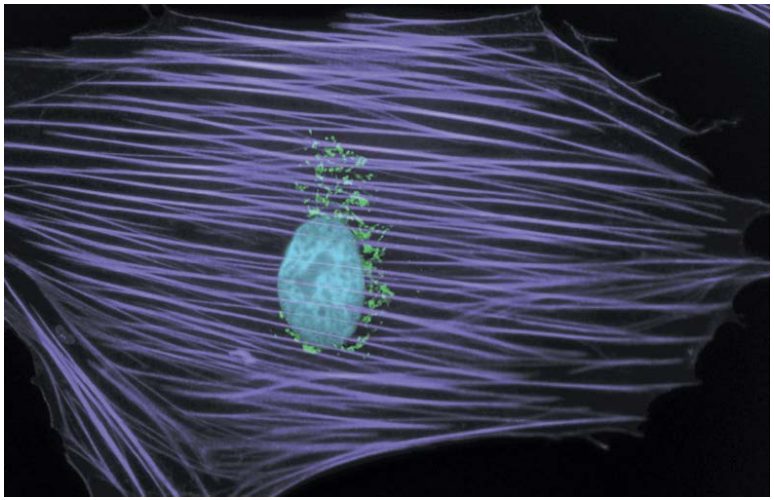
Camillo Golgi
(1843 – 1926)

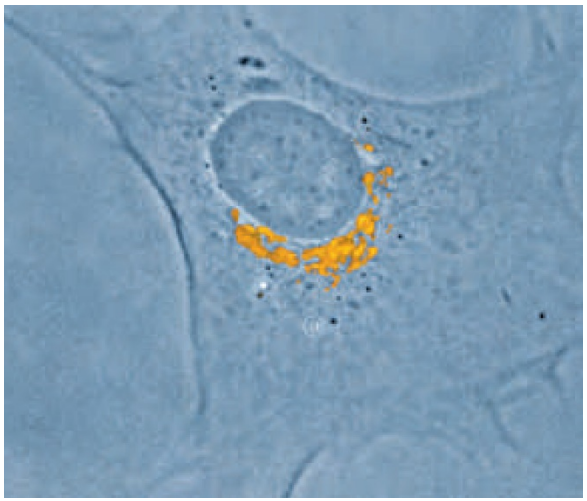
- **Cammillo Golgi** first described it at **1897** as “apparato reticolare interno”. In 1898 it was named after him. He received Nobel prize in physiology or medicine in 1906 for his studies of the structure of the nervous system.



- Synonyms:
  - ✓ **Golgi apparatus**.
  - ✓ **Golgi zone**.

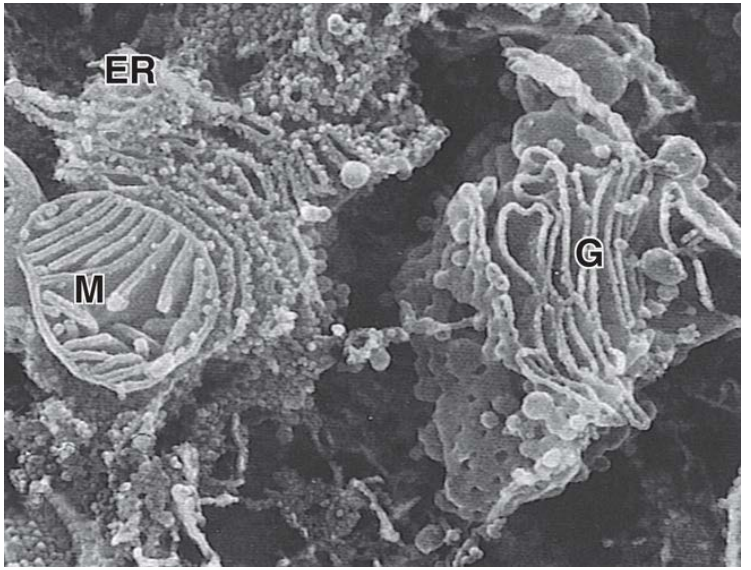


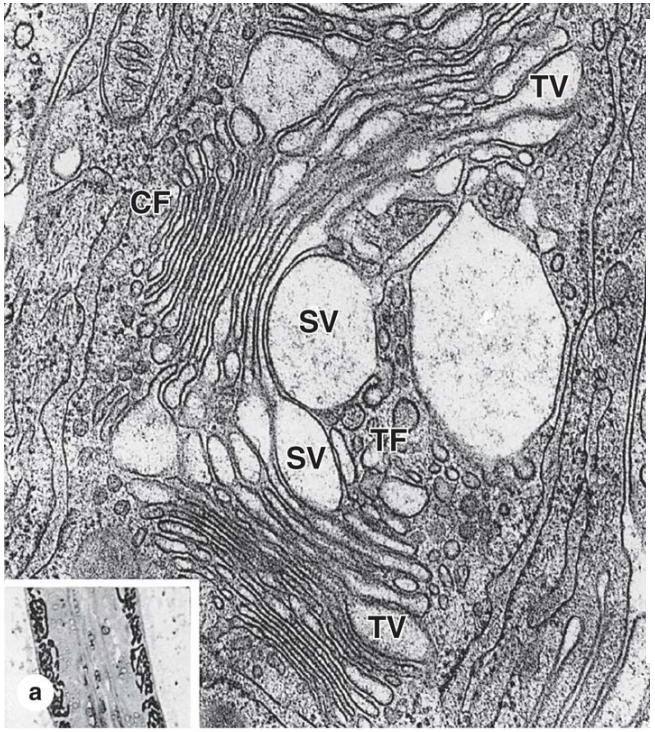


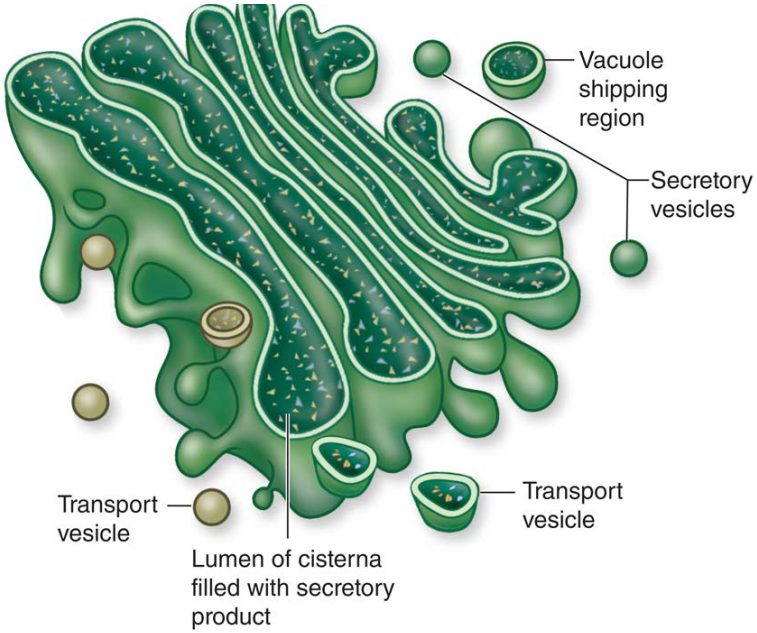


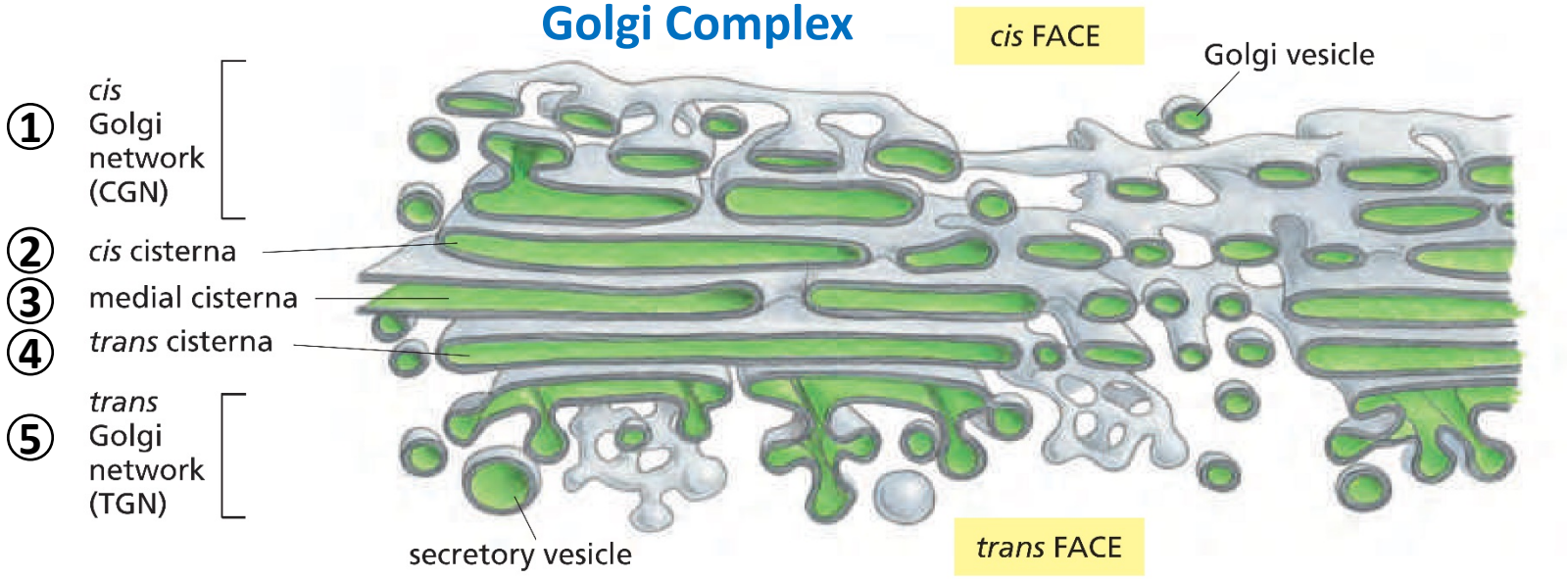
Golgi Complex

- The ultrastructure of the Golgi complex was described by **A. Dalton** and **M. Felix** in **1953**.
- It consists of a collection of flattened, membrane-enclosed compartments called **dictyosomes**:
  - ✓ **4 – 6 flattened cisternae** (50 – 200 nm).
  - ✓ **Vesicles** (30 – 50 nm).
  - ✓ **Large, clear vacuoles** (200 – 300 nm).

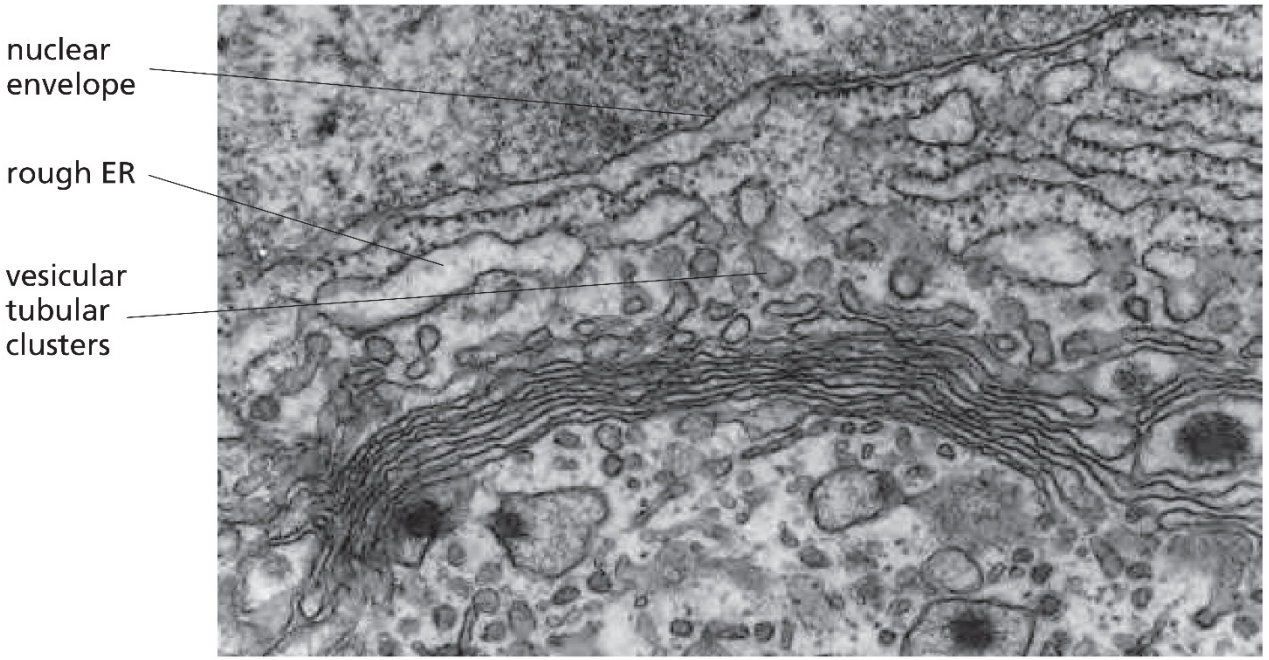







Golgi Complex
cis FACE
Golgi vesicle
1
cis Golgi network (CGN)
2
cis cisterna
3
medial cisterna
4
trans cisterna
5
trans Golgi network (TGN)
secretory vesicle
trans FACE

(A)


nuclear envelope
rough ER
vesicular tubular clusters
1 μm

(B)

Golgi Complex

- The Golgi complex consists of an ordered series of compartments.

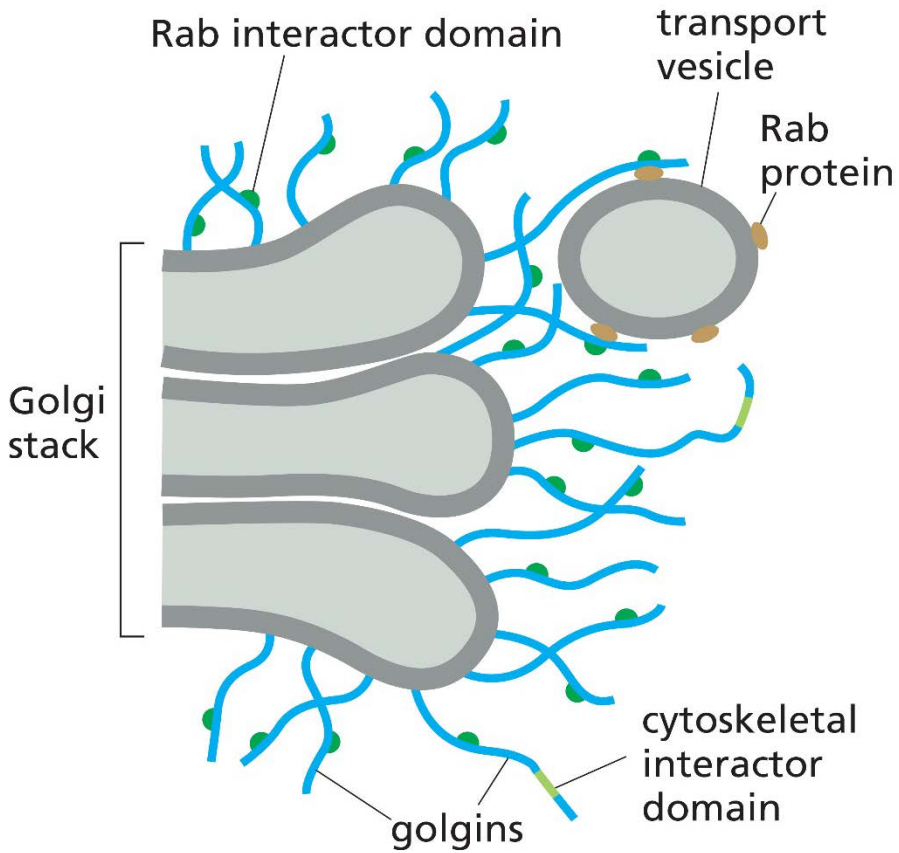
- Each Golgi stack has **five distinct parts**:
  1. ***cis* Golgi network (CGN, entry place).**
  2. ***cis* cisternae.**
  3. **Medial cisternae.**
  4. ***trans* cisternae.**
  5. ***trans* Golgi network (TGN, exit place).**

- Proteins and lipids enter the ***cis* Golgi network** and exit from the ***trans* Golgi network**, bound for the cell surface or another compartment.

Golgi Matrix Proteins Help Organize the Stack

- The unique architecture of the Golgi complex depends on both the **microtubule cytoskeleton** and cytoplasmic **Golgi matrix proteins** (***golgins***).

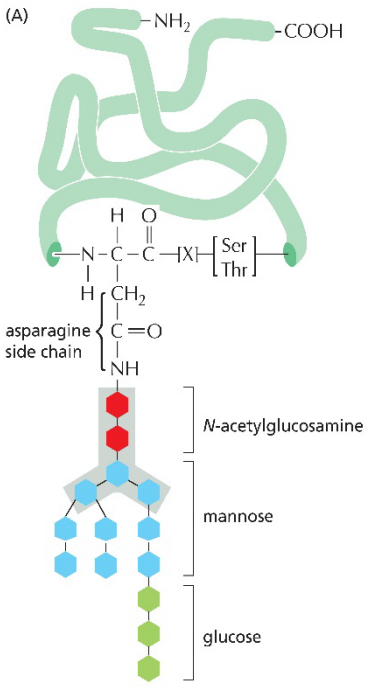
- They form long forest-like tentacles that can extend 100–400 nm from the surface of the Golgi stack.

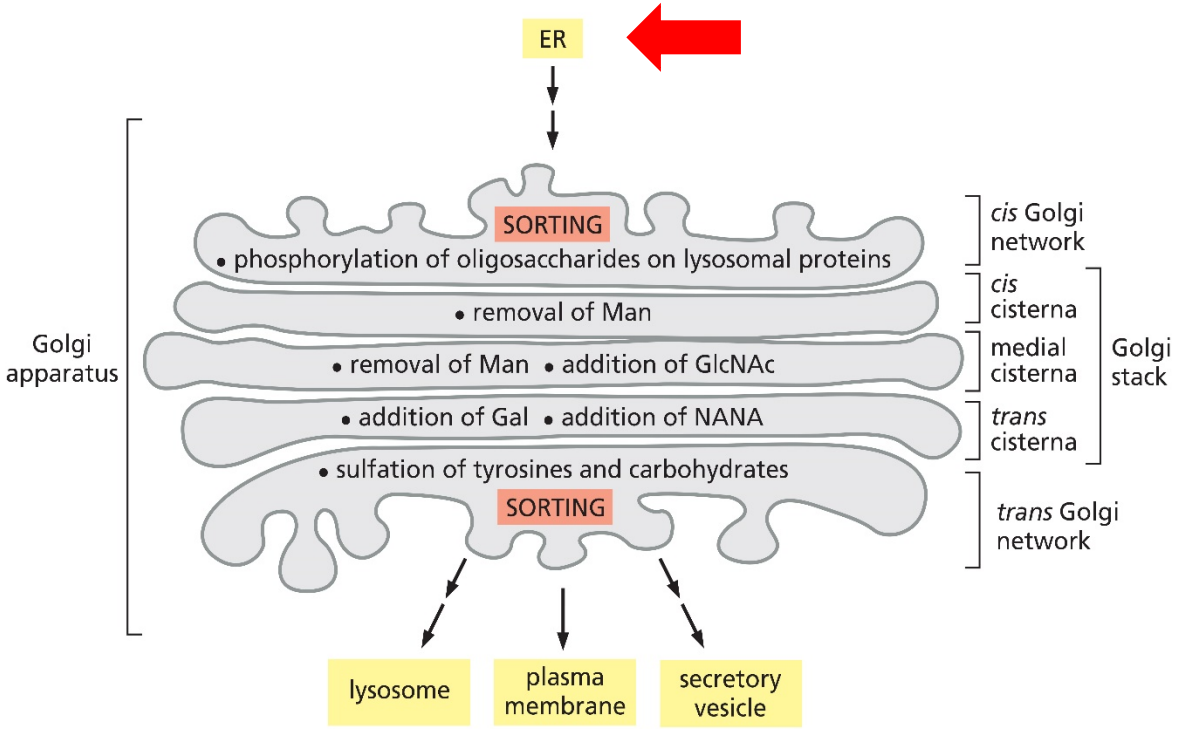


Functions of Golgi Complex

In the rER

- Newly synthesized proteins are translocated into ER cisternae.
- Preassembled **mannose-rich oligosaccharide precursors** are added to specific asparagine residues (*N*-linked).
- Proteins are folded, guided by **chaperones**, with strict quality control.
- **Disulfide bonds** are formed between specific cysteine residues.



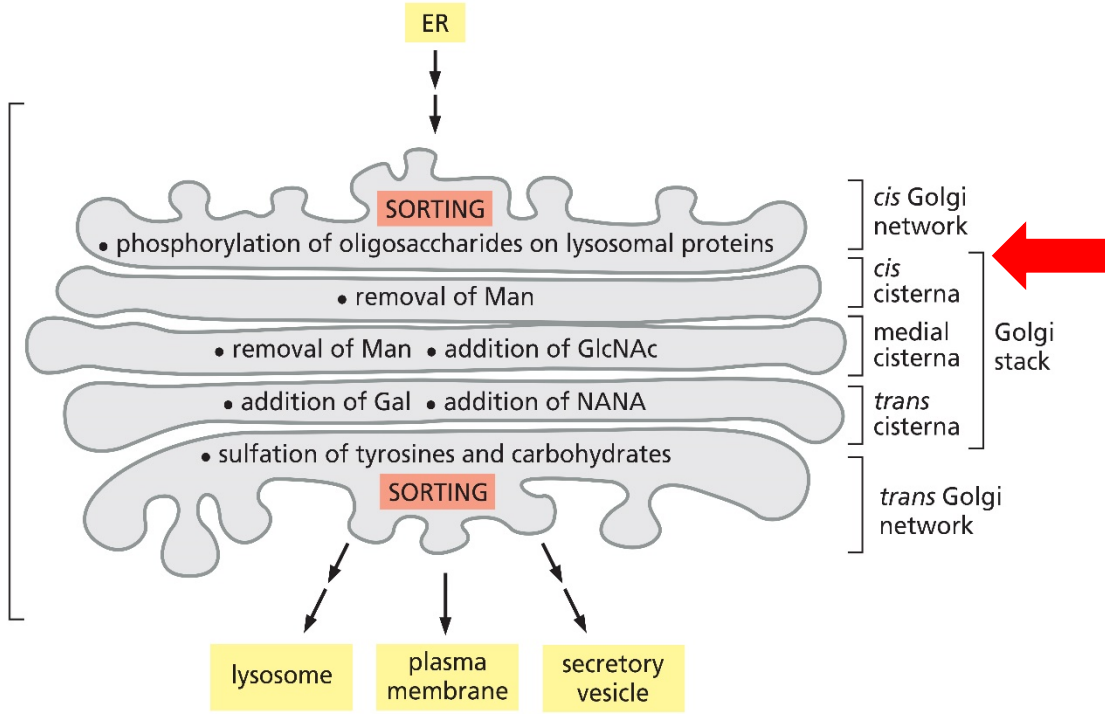


Functions of Golgi Complex

In the CGN and *cis* cisternae

- Vesicle movement from rER and forward through the CGN is promoted by coat protein **COP-II (sorting)**.
- **COP-I** controls retrograde vesicle movements (**sorting**).
- **Mannose-6-phosphate** is added to future lysosomal enzymes.
- *N*-linked **oligosaccharides** are trimmed, and other sugars added.

  - ✓ All of the Golgi **glycosidases** and **glycosyl transferases** are single-pass transmembrane proteins, many of which are organized in multienzyme complexes.

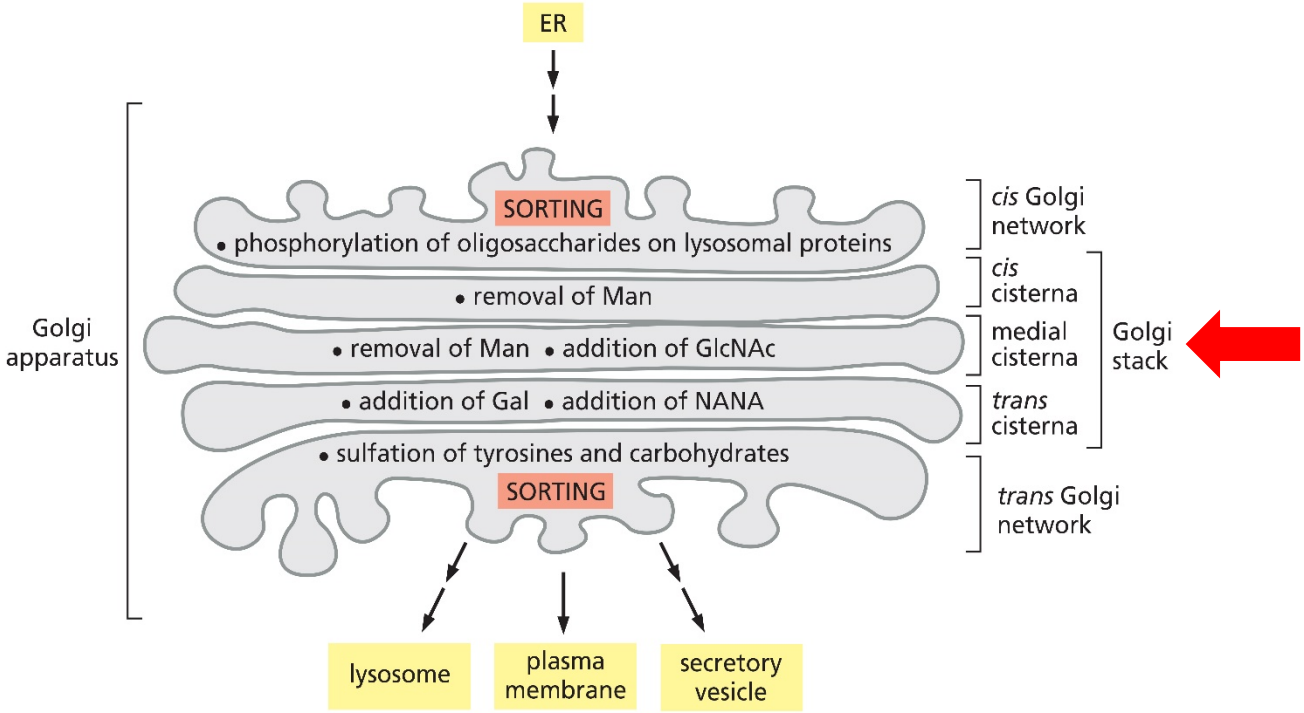


Functions of Golgi Complex

In the medial cisterna

- New **glycosylation** occurs on –OH groups of some lipids and serine and threonine residues (*O*-linked).

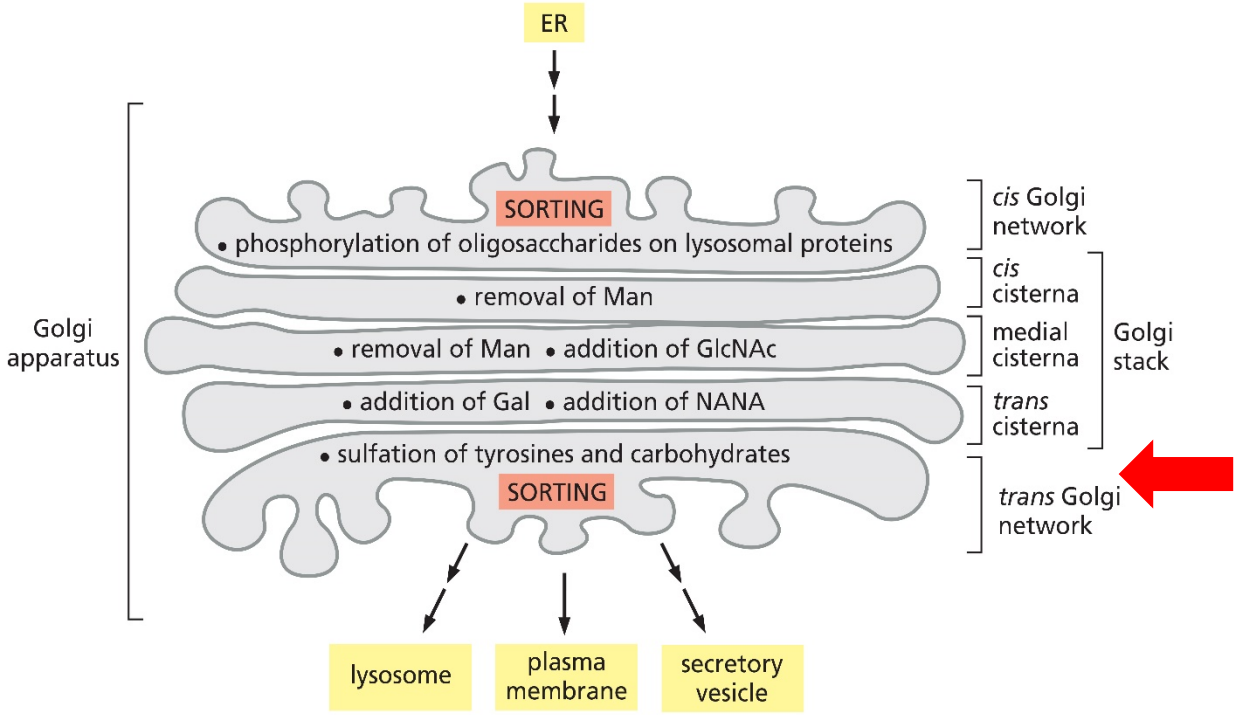
- *N*-linked **oligosaccharides** on proteins are modified further.



Functions of Golgi Complex

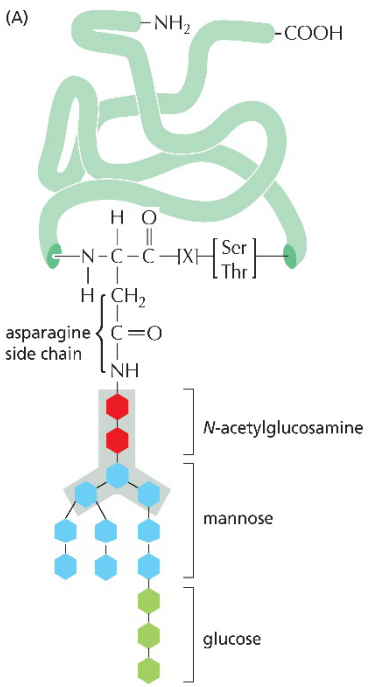
In the *trans* cisterna and TGN

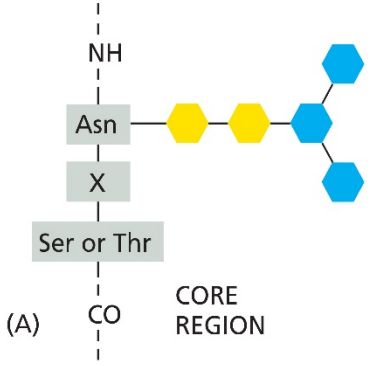
- **Sialic acid** is added as the terminal sugar to certain oligosaccharides.
- **Sulfation** of tyrosine residues and some sugars occurs.
- Glycoproteins and glycolipids are **sorted** into specific vesicles.
- Specific **vesicles** with different destinations are separated from the TGN.

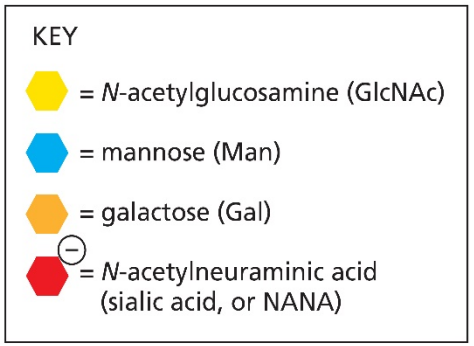


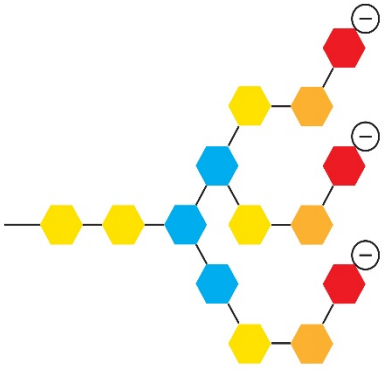
Processing of Oligosaccharide Chains in the Golgi Complex

- Two broad classes of *N*-linked oligosaccharides, the **complex oligosaccharides** and the **high-mannose oligosaccharides**, are attached to mammalian glycoproteins in GC.
- Sometimes, both types are attached in different places to the same polypeptide chain.
- Complex oligosaccharides are generated when the original *N*-linked oligosaccharide added in the ER is trimmed and further sugars are added.

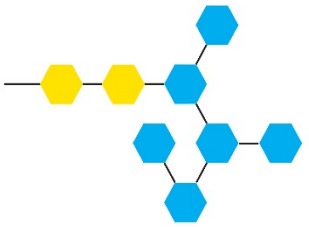






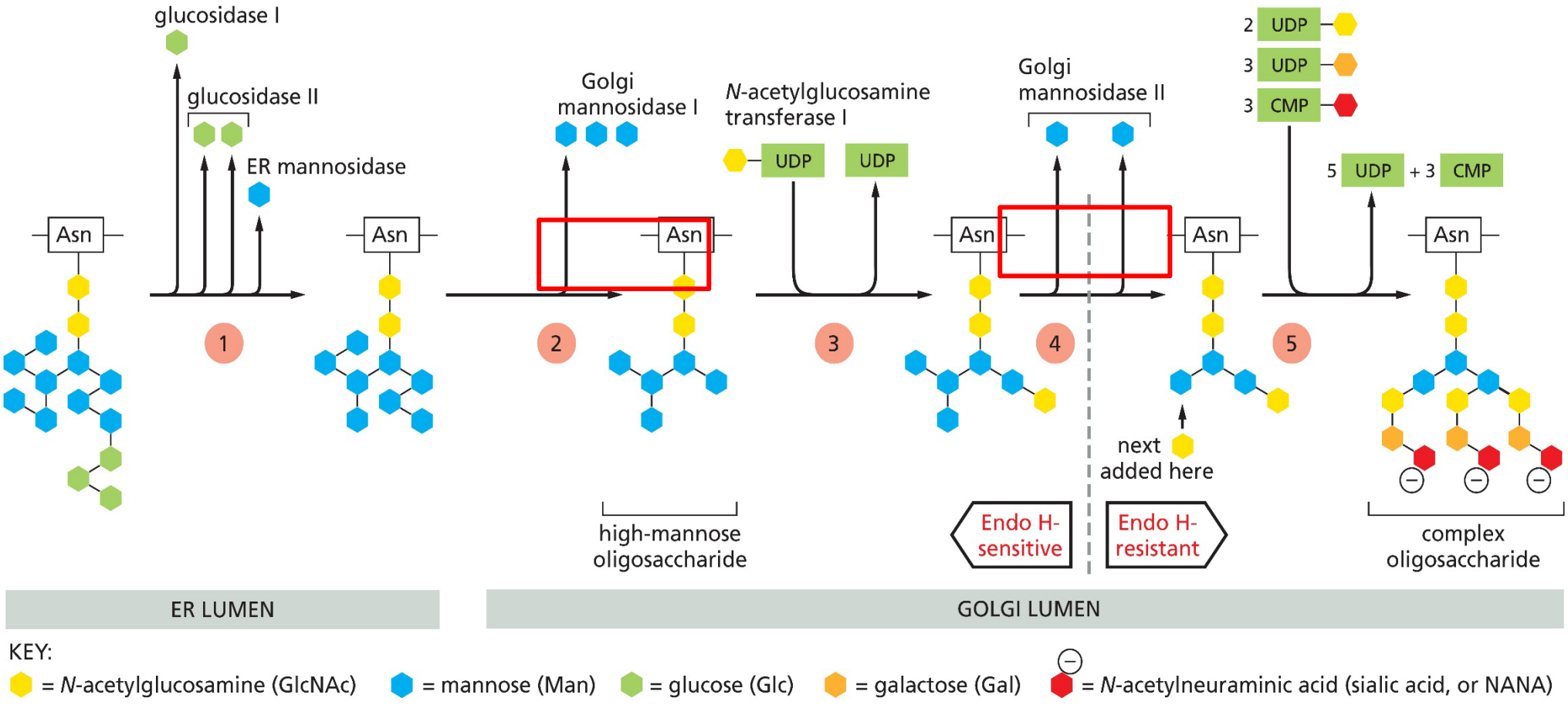


(B) COMPLEX OLIGOSACCHARIDE



(C) HIGH-MANNOSE OLIGOSACCHARIDE

Processing of Oligosaccharide Chains in the Golgi Complex

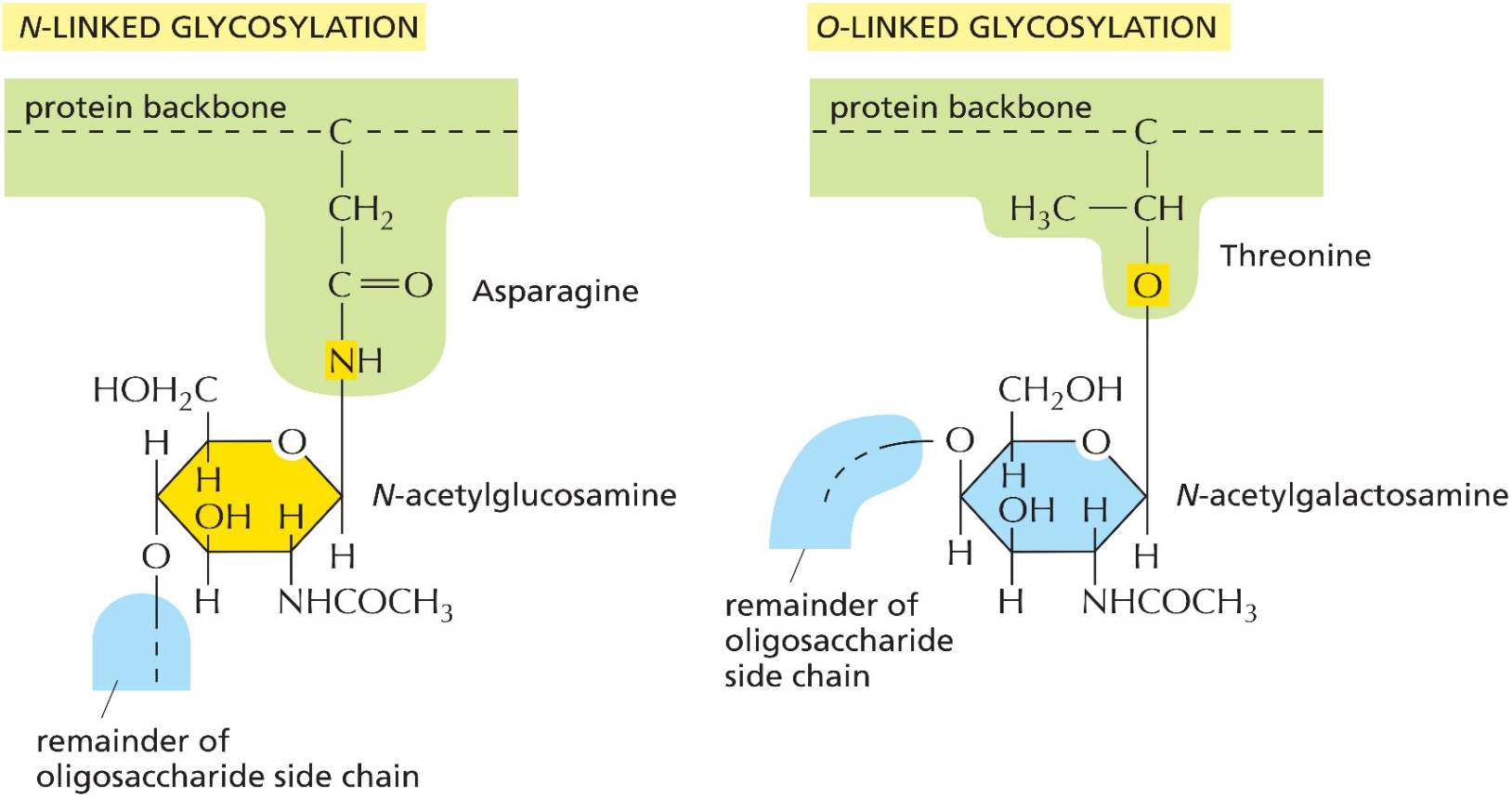


KEY:

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

Processing of Oligosaccharide Chains in the Golgi Complex

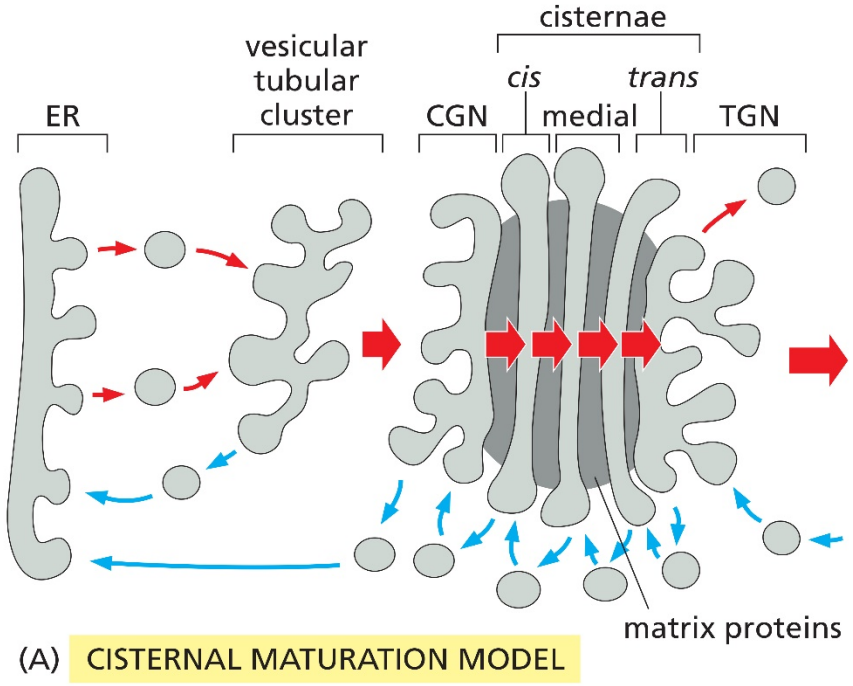
- In addition to the *N*-linked oligosaccharide alterations some proteins have sugars added to the –OH of selected **serines** or **threonines**, or, in some cases (**such as collagens**) to **hydroxylated proline** and **lysine** side chains.



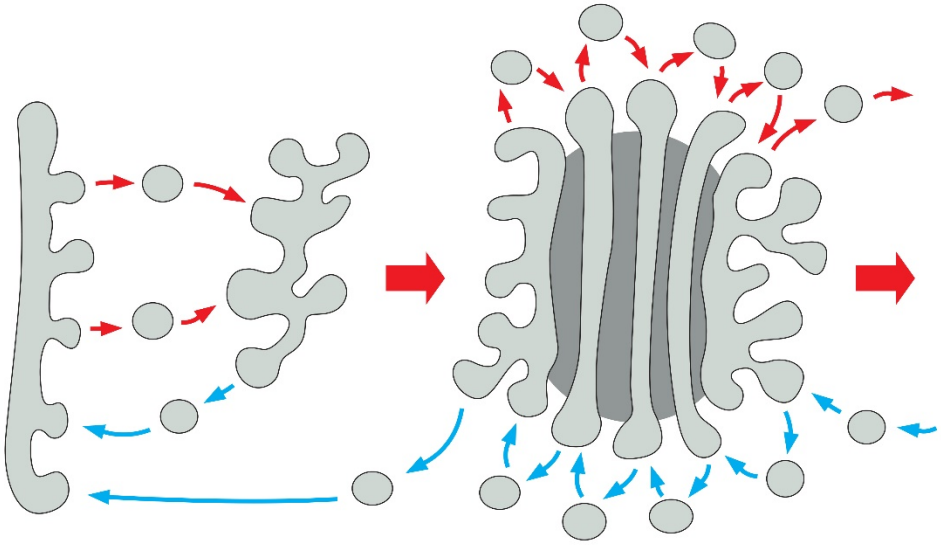
What is the Purpose of Glycosylation?

- *N*-linked glycosylation promotes **protein folding** in two ways:
  - ✓ It has a direct role in making folding intermediates more **soluble**, thereby preventing their aggregation.
  - ✓ It establishes a "glyco-code" that marks the progression of protein folding and mediates the binding of the protein to **chaperones**.
- The presence of oligosaccharides tends to make a glycoprotein more resistant to digestion by proteolytic enzymes (**protection**).
- Glycosylation can also have important **regulatory roles**.
- The recognition of sugar chains by ***selectins*** in the extracellular space is important in many developmental processes and in **cell-cell recognition and communication**.

Two Possible Models Explaining the Organization of the Golgi Apparatus and How Proteins Move Through It



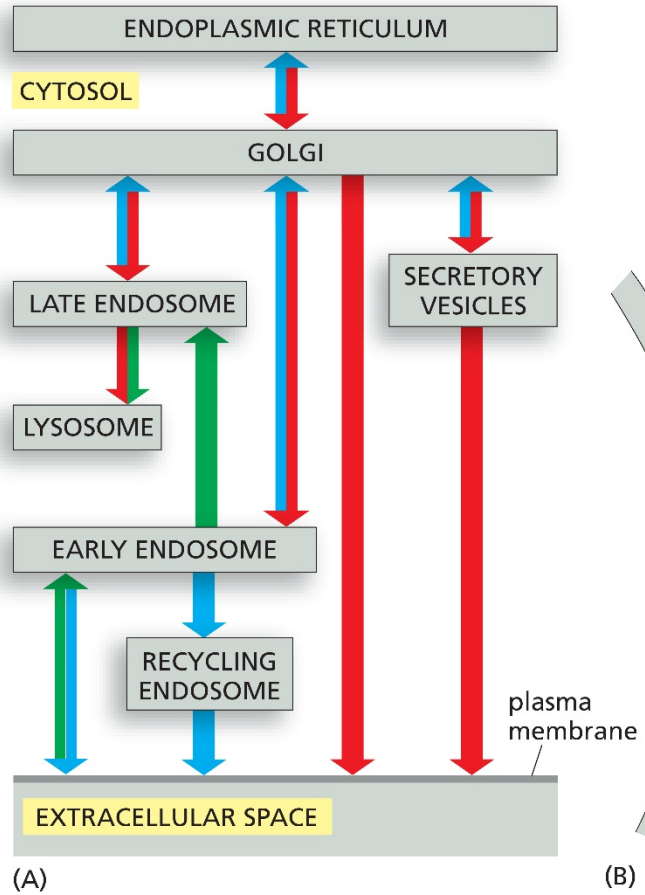
(A) CISTERNAL MATURATION MODEL



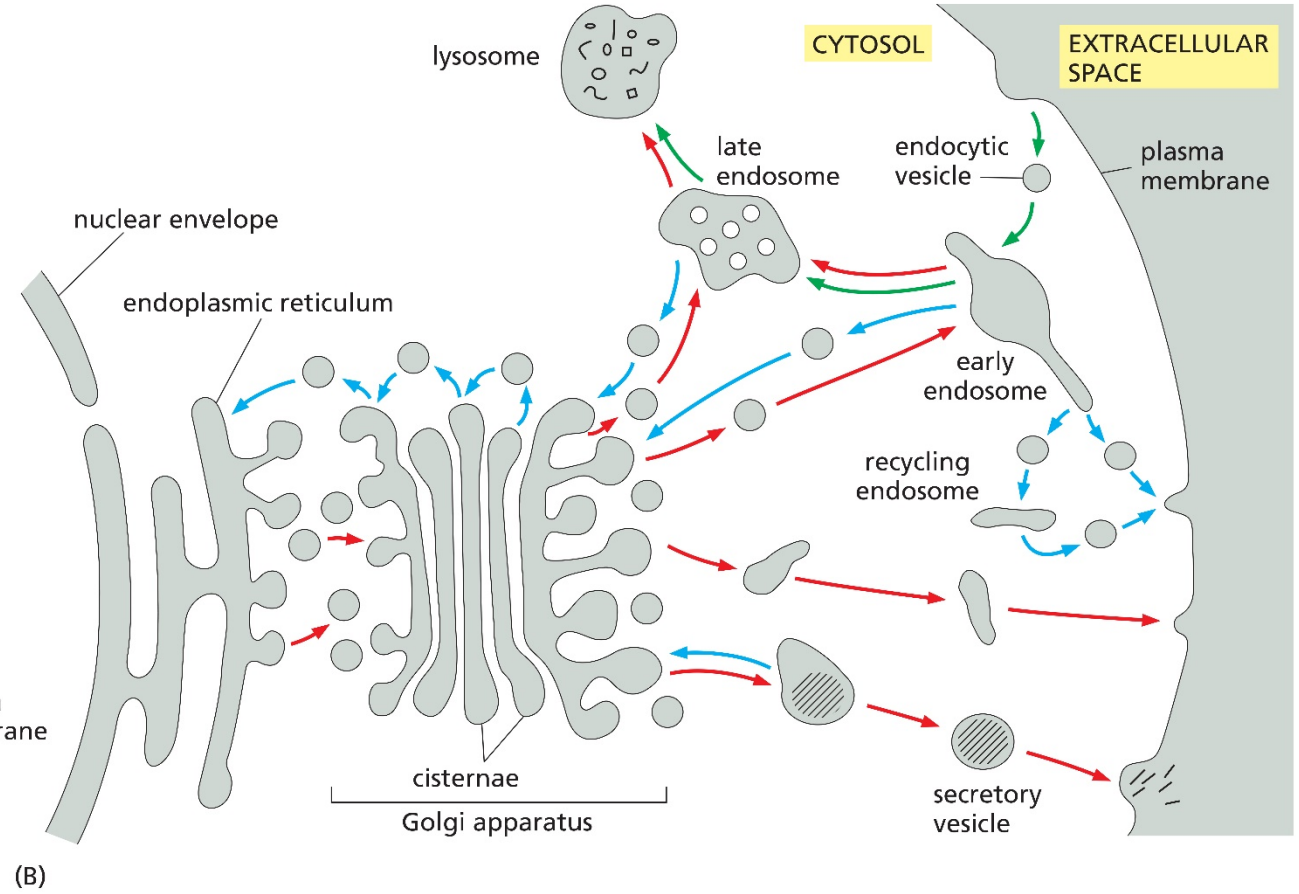
(B) VESICLE TRANSPORT MODEL

Endosomes

- **Endosomes** are a membrane-bound compartments inside all eukaryotic cells.
- Molecules from the **extracellular space** are transported to the endosomes and eventually to lysosomes for degradation, or they can be recycled back to the plasma membrane.
- Molecules are also transported to endosomes from the **TGN** and either continue to lysosomes or recycle back to the Golgi complex.



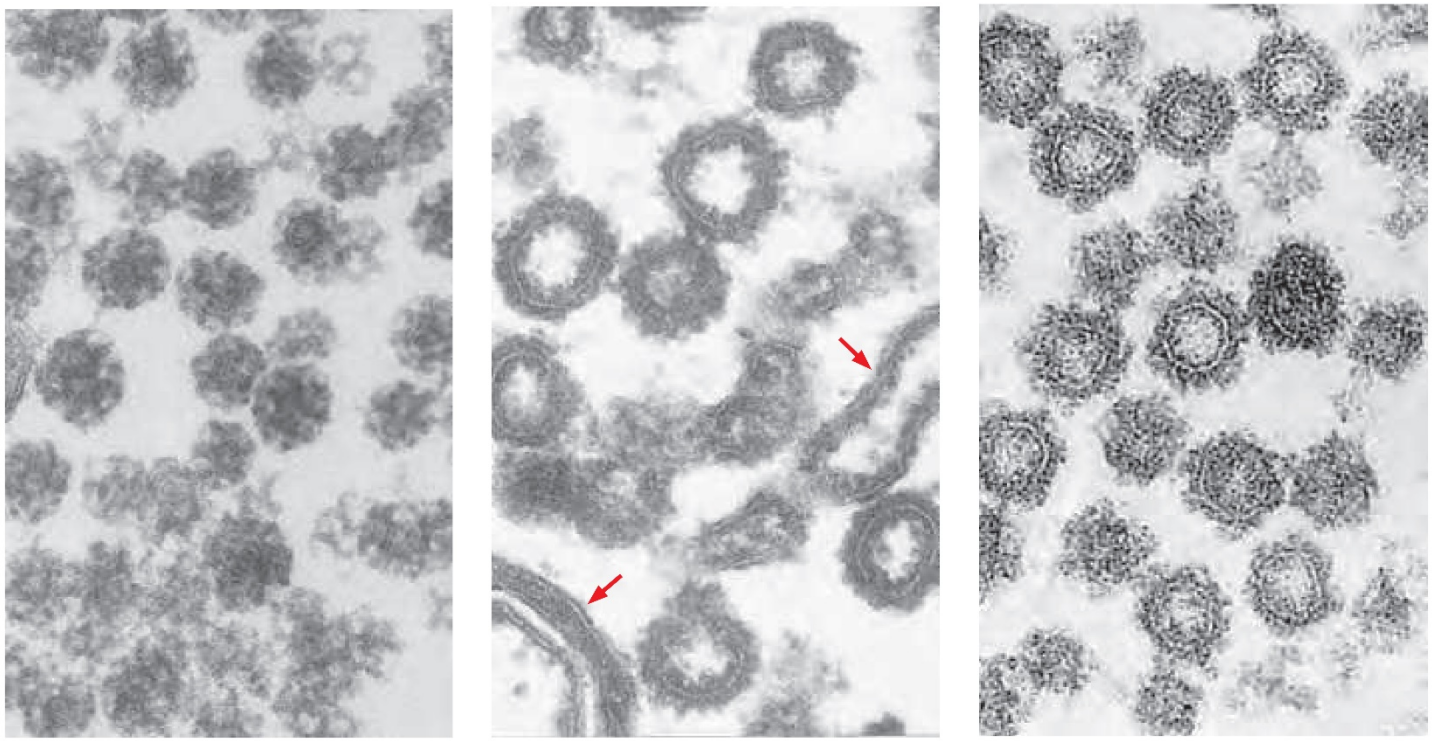
(A)



(B)

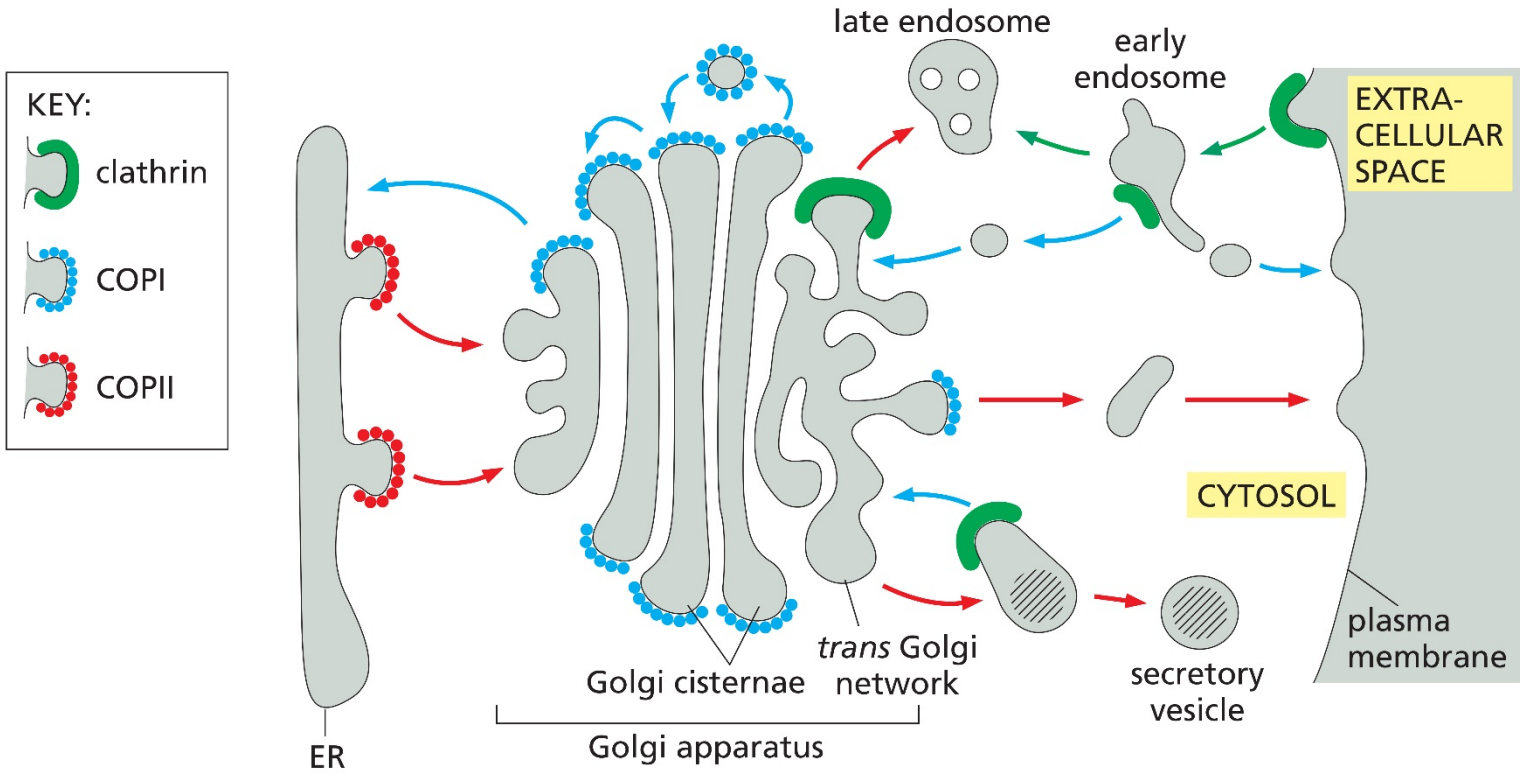
Coated Vesicles

- Most transport vesicles form from specialized, coated regions of membranes.
- They bud off as **coated vesicles**, which have a distinctive cage of proteins covering their cytosolic surface.
- Before the vesicles fuse with a target membrane, they discard their coat.
- There are three well-characterized types of coated vesicles, distinguished by their major coat proteins:
  - ✓ ***Clathrin-coated***.
  - ✓ ***COP-I coated***.
  - ✓ ***COP-II coated***.



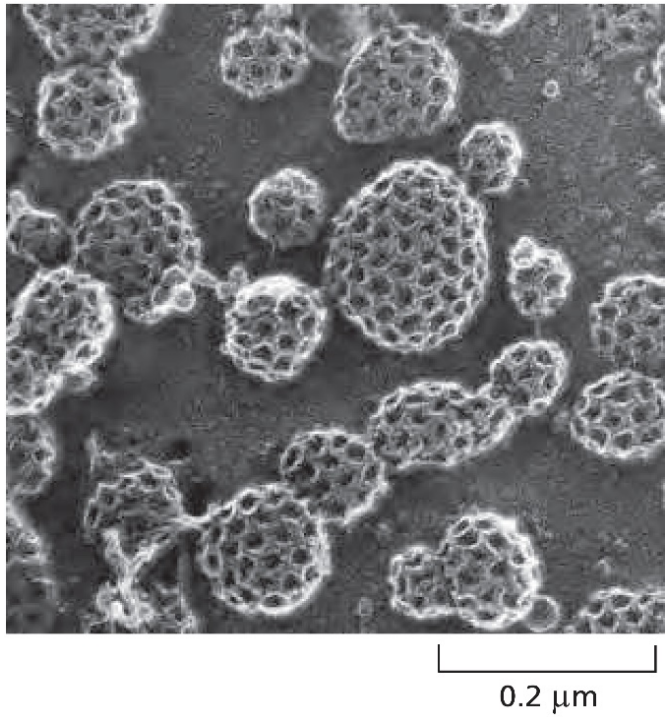
Coated Vesicles

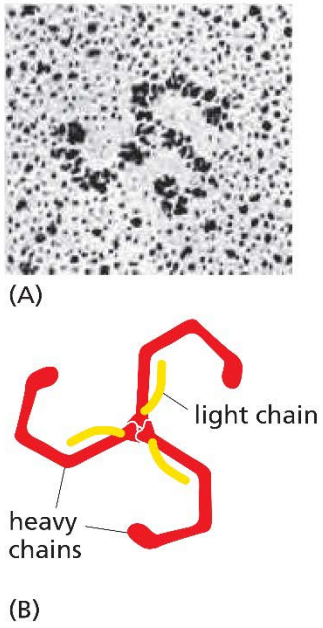
- Each type is used for different transport steps. ***Clathrin-coated* vesicles** mediate transport from the Golgi complex and from the plasma membrane, whereas ***COP-I coated* vesicles** bud from Golgi compartments, and ***COP-II coated* vesicles** bud from the ER.

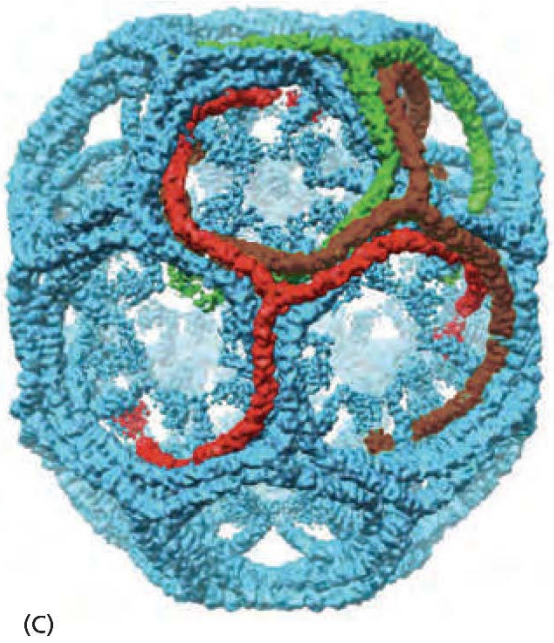


Clathrin-coated Vesicles

- The major protein component of ***clathrin-coated* vesicles** is **clathrin** itself, which forms the outer layer of the coat.
- Each **clathrin** subunit consists of **three large** and **three small polypeptide chains** that together form a three-legged structure called a ***triskelion***.
- Clathrin **triskelions** assemble into a basketlike framework of hexagons and pentagons.

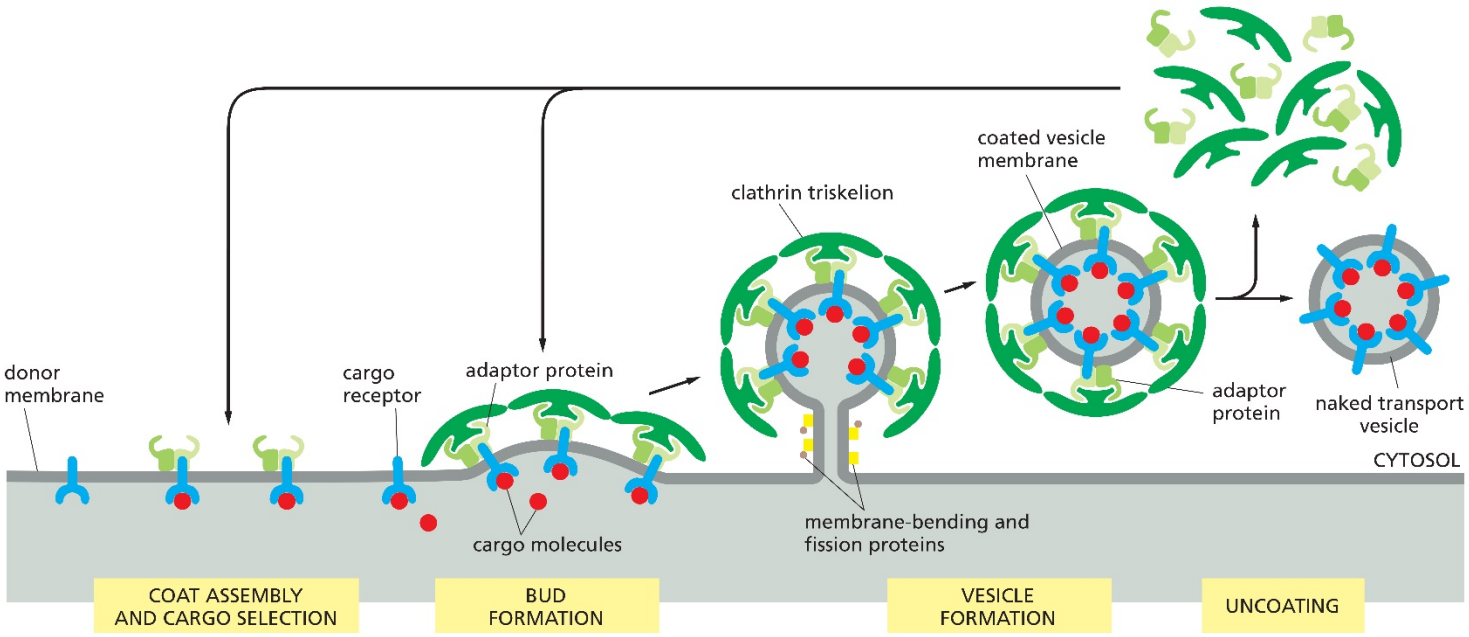


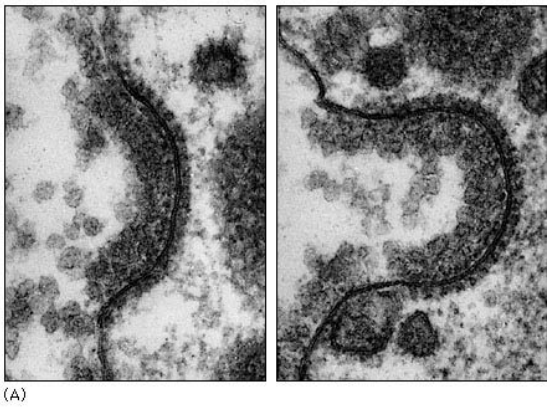


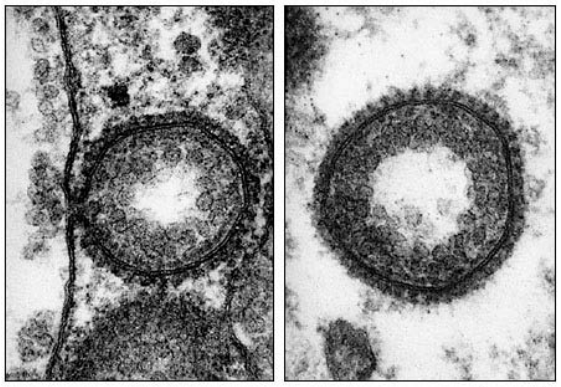


Clathrin-coated Vesicles

- **Adaptor proteins** form a discrete inner layer of the coat, positioned between the clathrin cage and the membrane.
  - ✓ They bind the clathrin coat to the membrane and trap various transmembrane proteins, including **transmembrane receptors (*cargo receptors*)**.

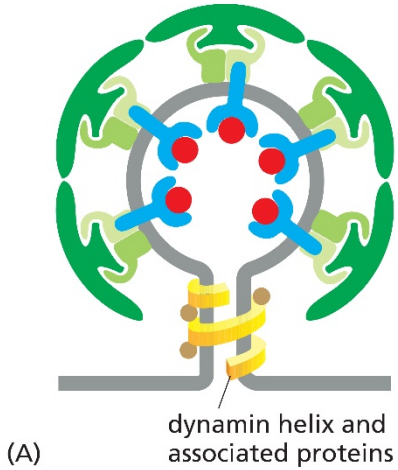


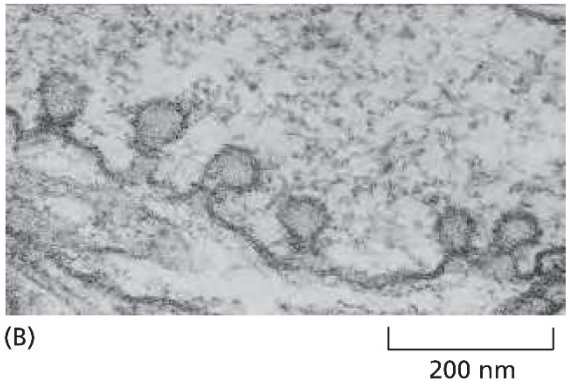


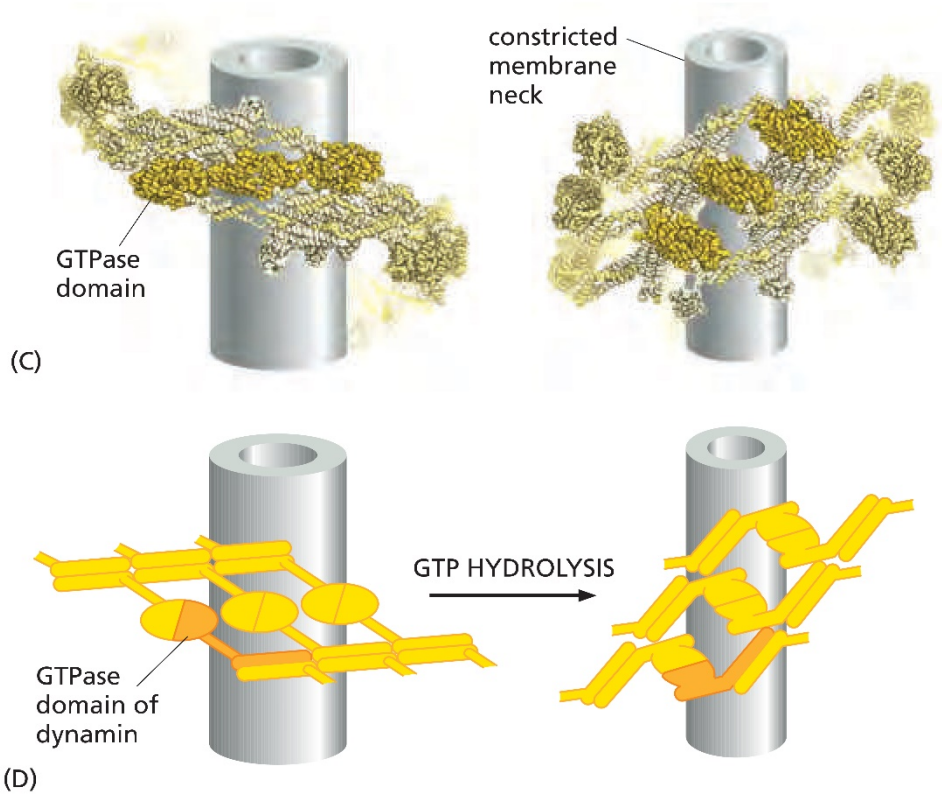


Clathrin-coated Vesicles

- Cytoplasmic proteins regulate the pinching-off of the vesicle from the membrane.
  - ✓ As a *clathrin*-coated bud grows, soluble cytoplasmic protein called **dynamin** assembles at the neck of each bud.
- *Clathrin-* and *COP-I coated* vesicles shed their coat soon after they pinch off.







Rab Proteins Guide Transport Vesicles to Their Target Membrane

- To ensure an orderly flow of vesicle traffic, transport vesicles must be highly accurate in recognizing the correct target membrane with which to fuse.

- Specificity in targeting is ensured because all transport vesicles display **surface markers** that identify them according to their origin and type of cargo, and target membranes display complementary **receptors** that recognize the appropriate markers.

- This crucial process occurs in two steps:
  - ✓ **Rab proteins** and **Rab effectors** direct the vesicle to specific spots on the correct target membrane.
  - ✓ **SNARE proteins** and **SNARE regulators** mediate the fusion of the lipid bilayers.

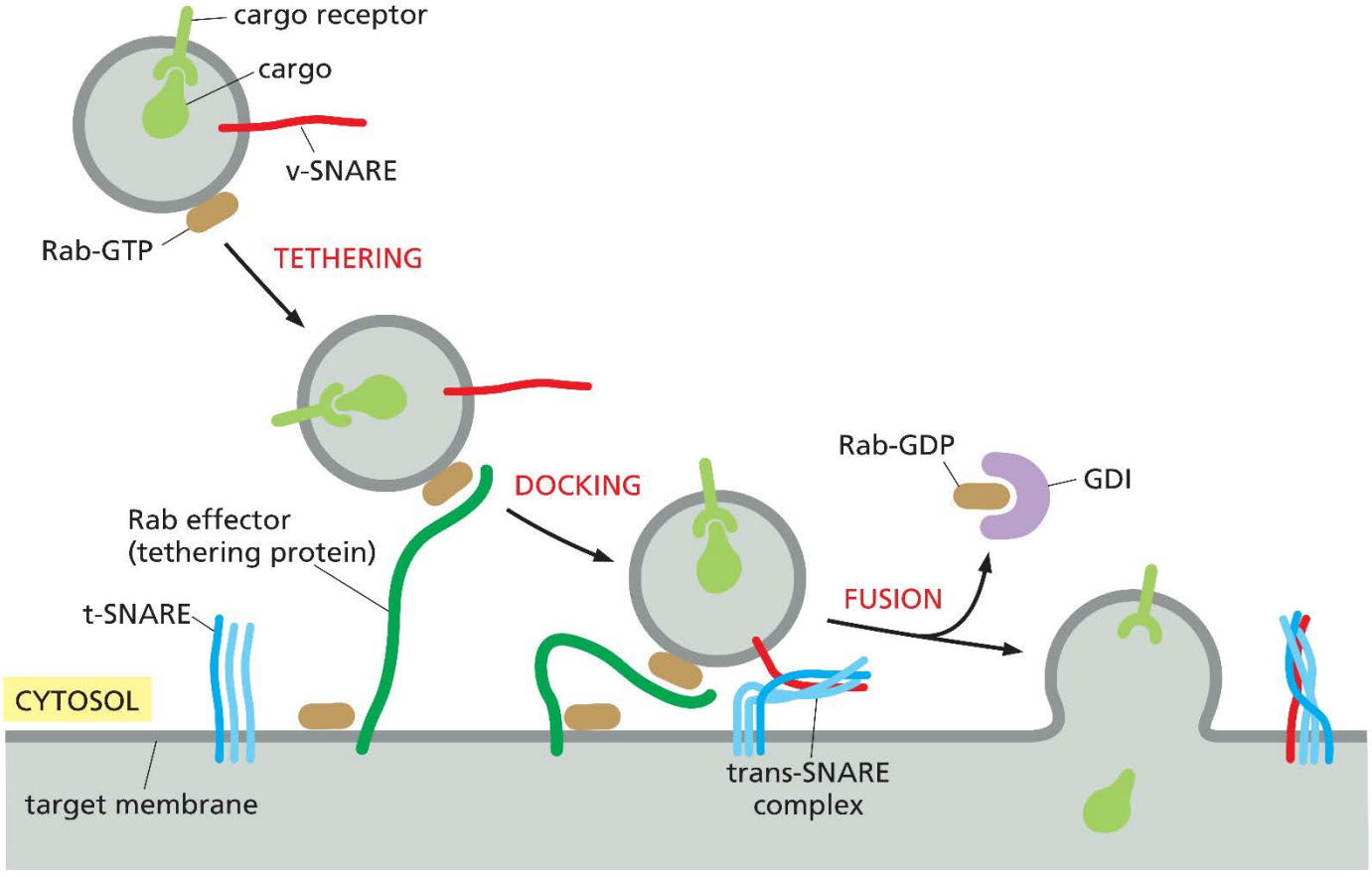
Rab Proteins Guide Transport Vesicles to Their Target Membrane

- **Rab proteins** are monomeric GTPases.
- There are over 60 known members of Rab family.
- Highly selective distribution of Rab proteins turns them in ideal molecular markers for identifying each membrane type and guiding vesicle traffic between them.

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
Rab7	Late endosomes
Rab8	Cilia
Rab9	Late endosomes, *trans* Golgi

Tethering of a Transport Vesicle to a Target Membrane

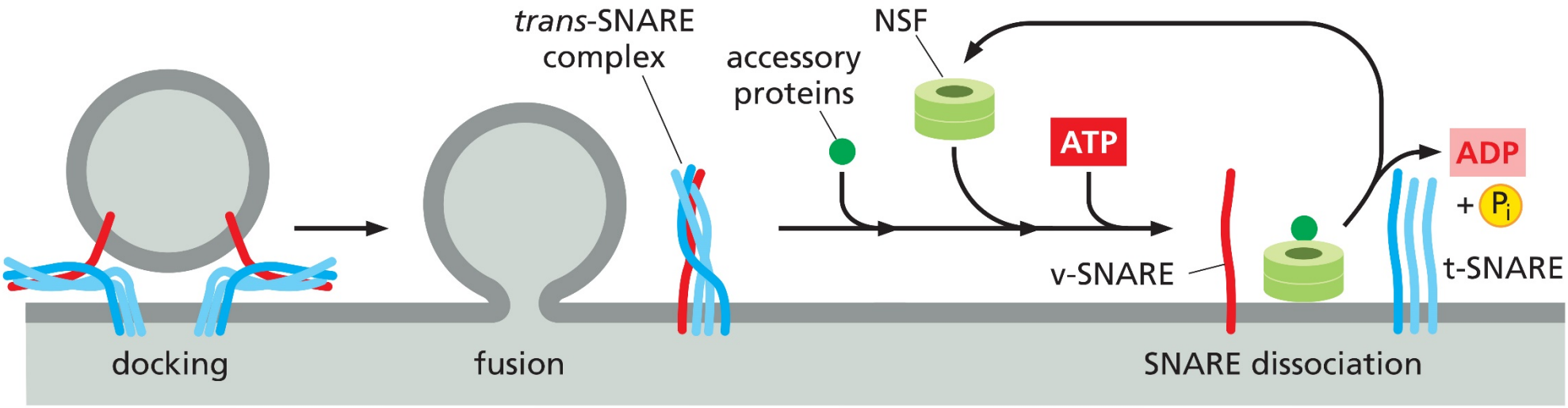
- **Rab effector** proteins interact with active **Rab proteins** to establish the first connection between the two membranes that are going to fuse.
- Next, **SNARE proteins** on the two membranes pair, docking the vesicle to the target membrane and catalyzing the fusion of the two apposed lipid bilayers.



Interacting SNAREs Need to be Pried Apart Before They Can Function Again

- A crucial protein called **NSF** cycles between membranes and the cytosol and catalyzes the **disassembly process**.

- **NSF** is a hexameric ATPase that uses the energy of ATP hydrolysis to unravel the intimate interactions between the helical domains of **paired SNARE proteins**.



Secretory Vesicles

- Secretory vesicle is a vesicle that mediates the transport of cargo (hormones or neurotransmitters) to specific sites at the **cell membrane**, where it docks and fuses to release its content.

- Shape – spherical.

- Diameter – from 0.15 µm to more than 1 µm.

- *Clathrin – coated vesicles.*