Lecture 3:
Protein Sorting (Golgi Apparatus and Vesicular Transport)

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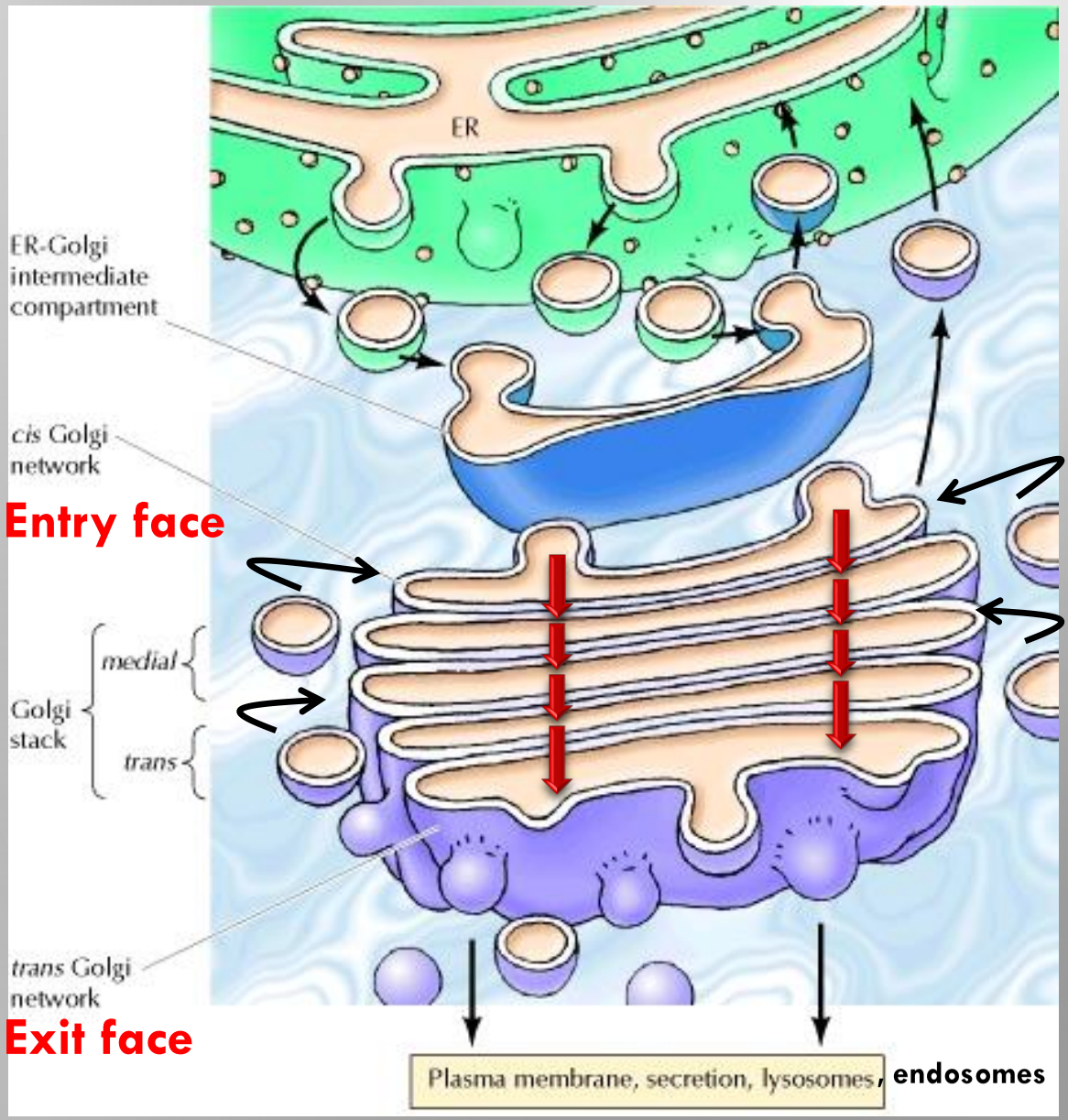
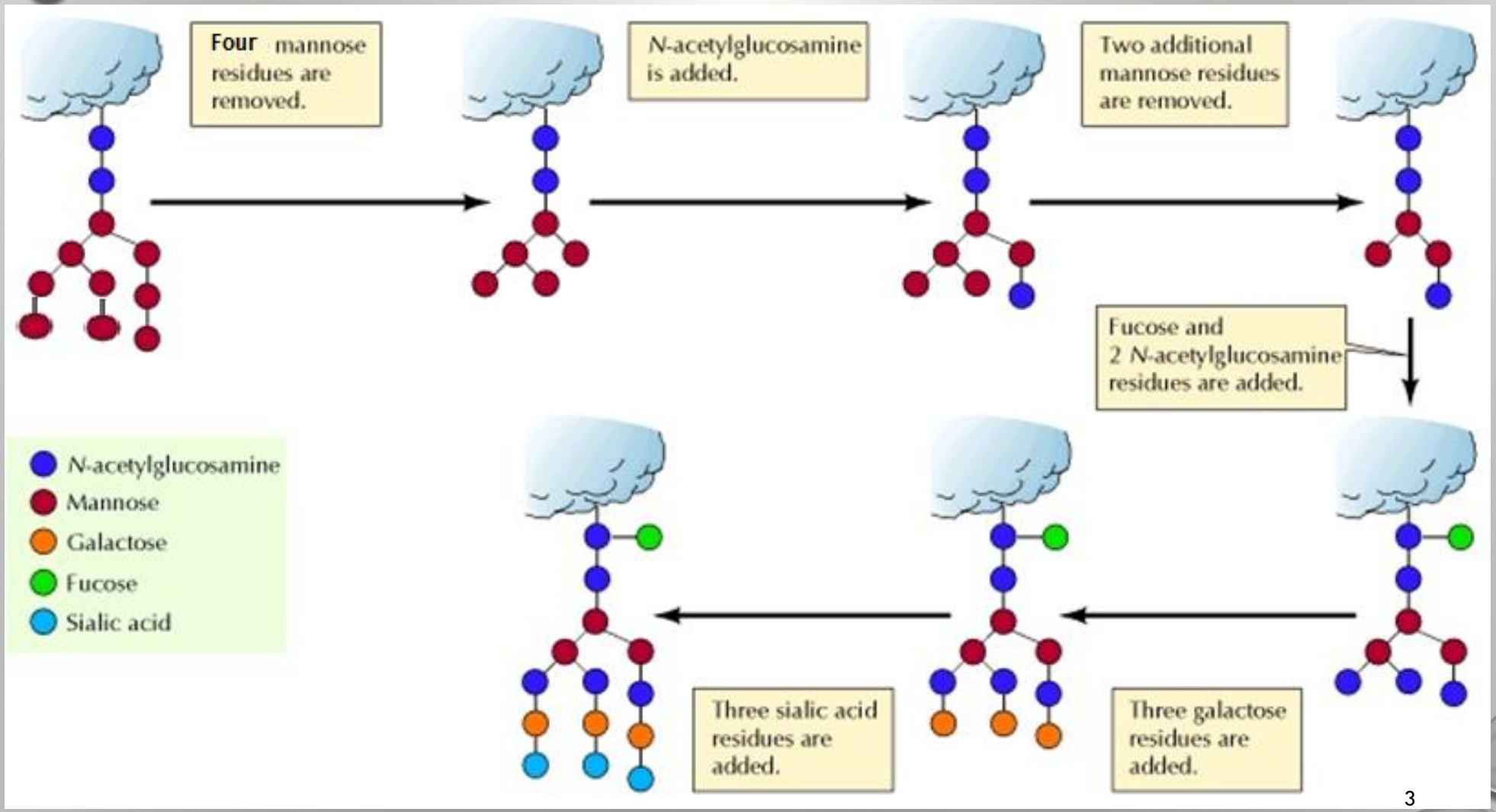
Principles of Genetics and Molecular Biology

Structure and Functions of Golgi

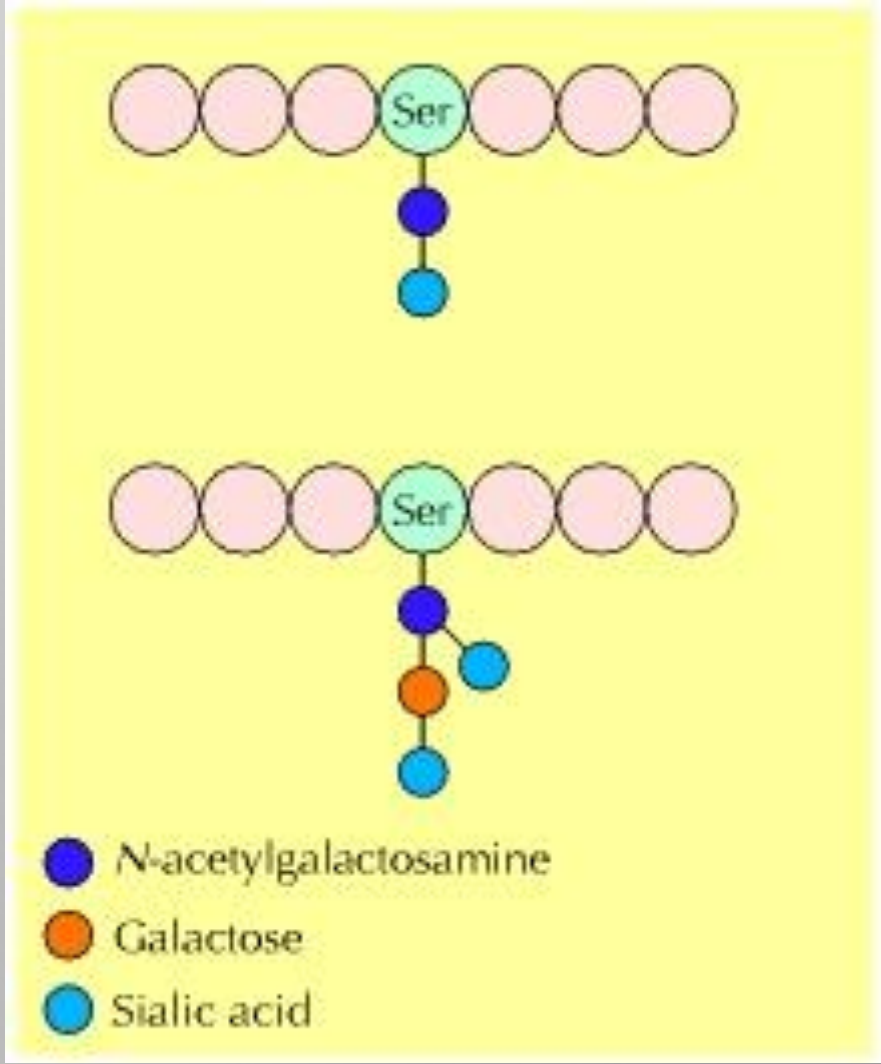


- Composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles.
- Structural and functional polarity.
- Further protein processing and modification
- Protein sorting and distribution
- Synthesis of glycolipids and sphingomyelin

Protein glycosylation within Golgi
Processing of *N*-linked Oligosaccharides in Golgi



O-linked Glycosylation

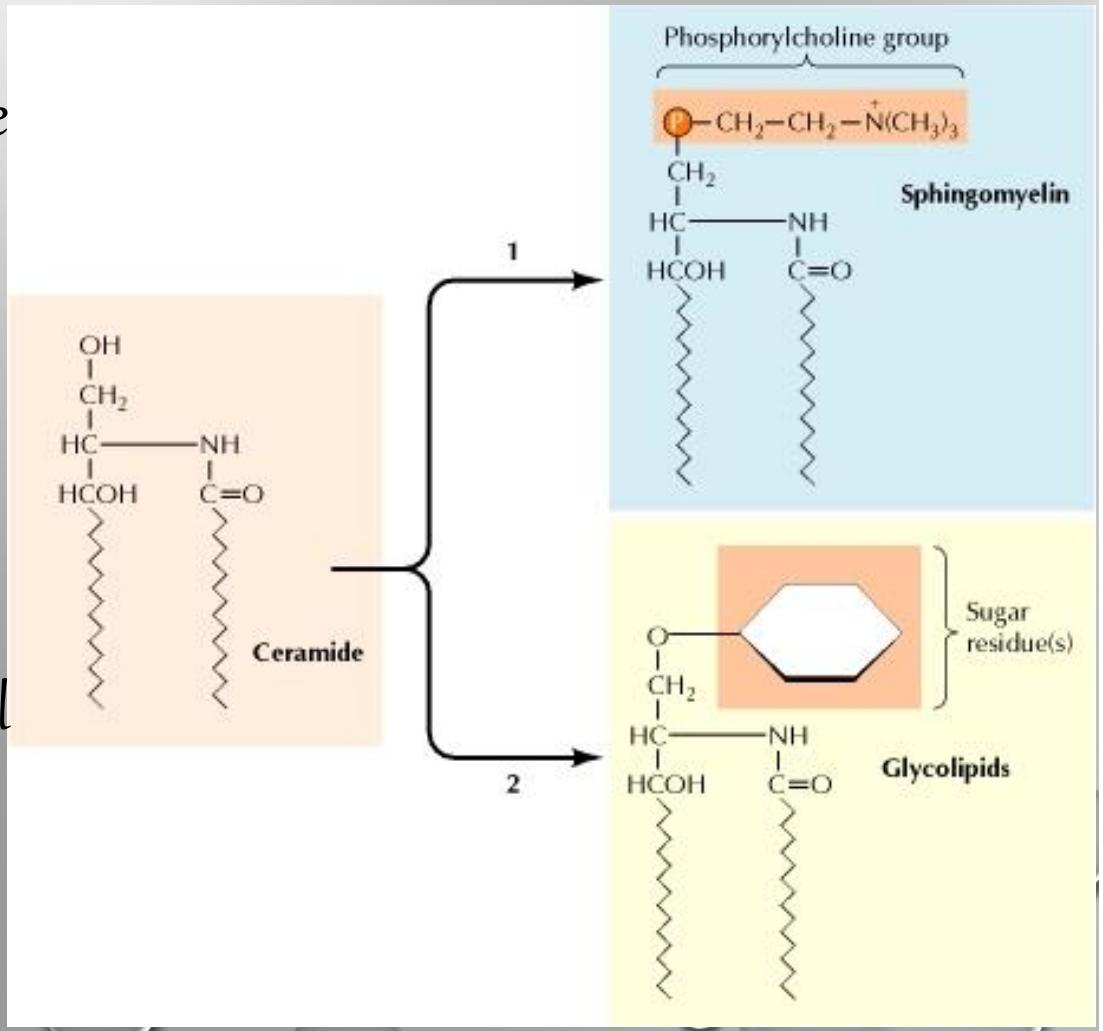


- Carbohydrates are added to the side chains of acceptor serine and threonine residues.
- The serine or threonine is usually linked directly to *N*-acetylgalactosamine, to which other sugars can then be added.
- Some of the added sugars are further modified by the addition of sulfate groups.

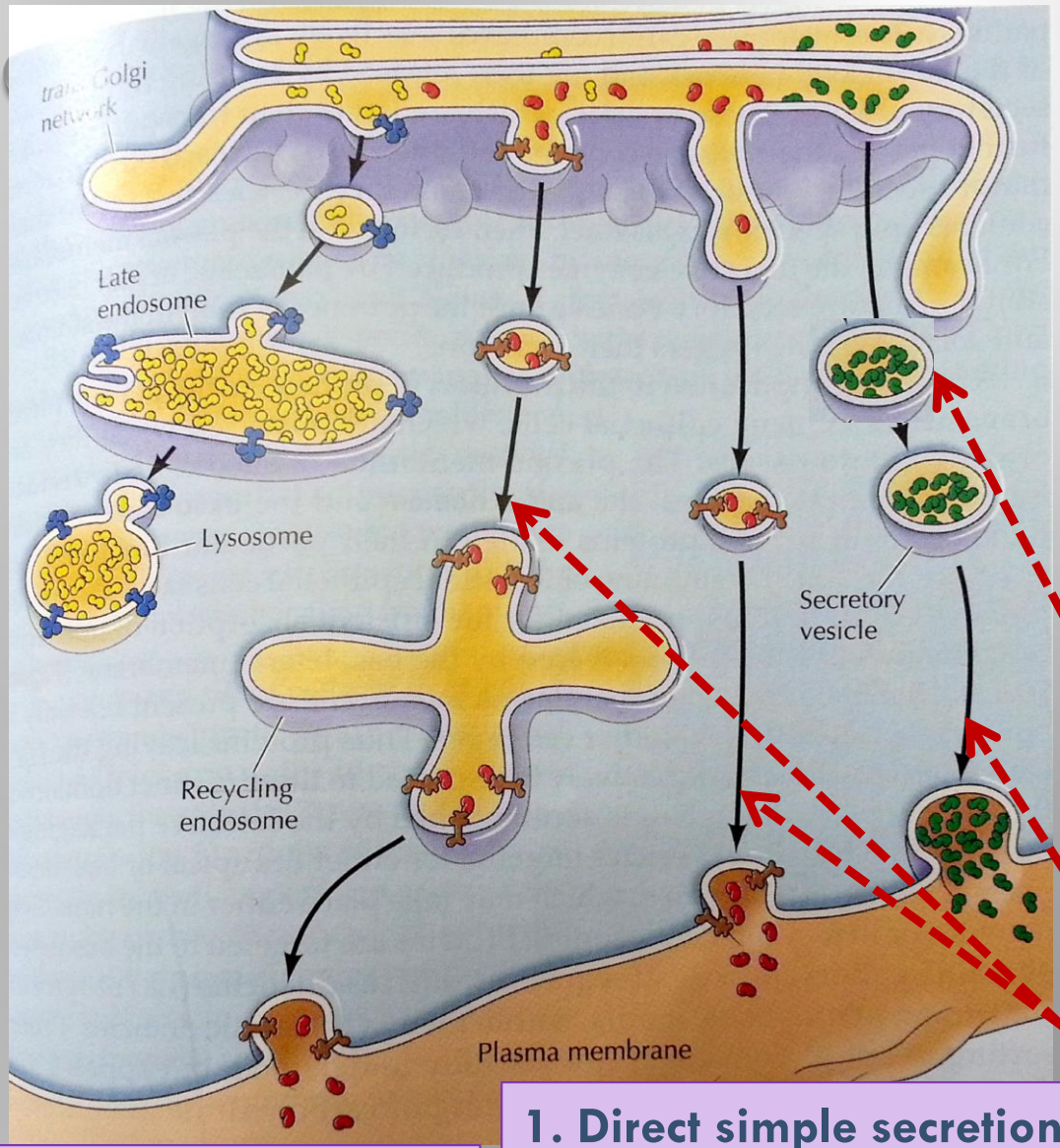
Lipid and Polysaccharide Metabolism in the Golgi

- Transfer of phosphorylcholine group from phosphatidylcholine to ceramide.
  - Sphingomyelin is synthesized on the lumenal surface.
- Addition of sugar residues (glycolipids).
  - Glucose is added to ceramide on the cytosolic side and glucosylceramide then apparently flips and additional carbohydrates are added on the lumenal side of the membrane

Ceramide is synthesized in the ER



Protein Sorting and Export



In contrast to the ER, all of the proteins retained within the Golgi complex are associated with the Golgi membrane rather than being soluble proteins within the lumen. Retention signal is the length of their transmembrane domains

- Protein packaging mediated by cargo receptor
- Processing in immature secretory vesicles

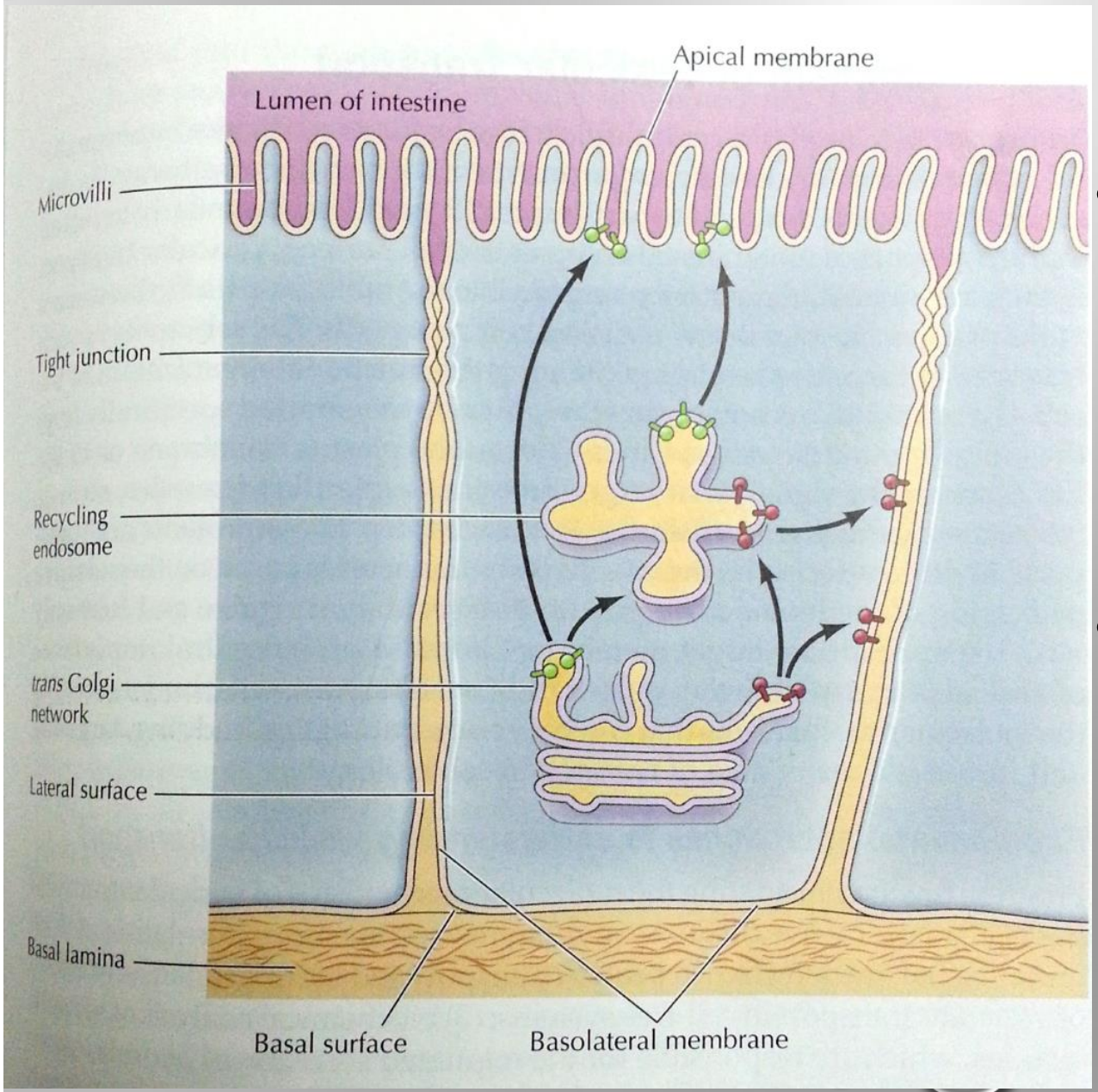
3. Regulated secretion after signaling (e.g. hormones) from specialized vesicles

Continuous, unregulated secretion

1. Direct simple secretion

2. Transport via recycling endosomes

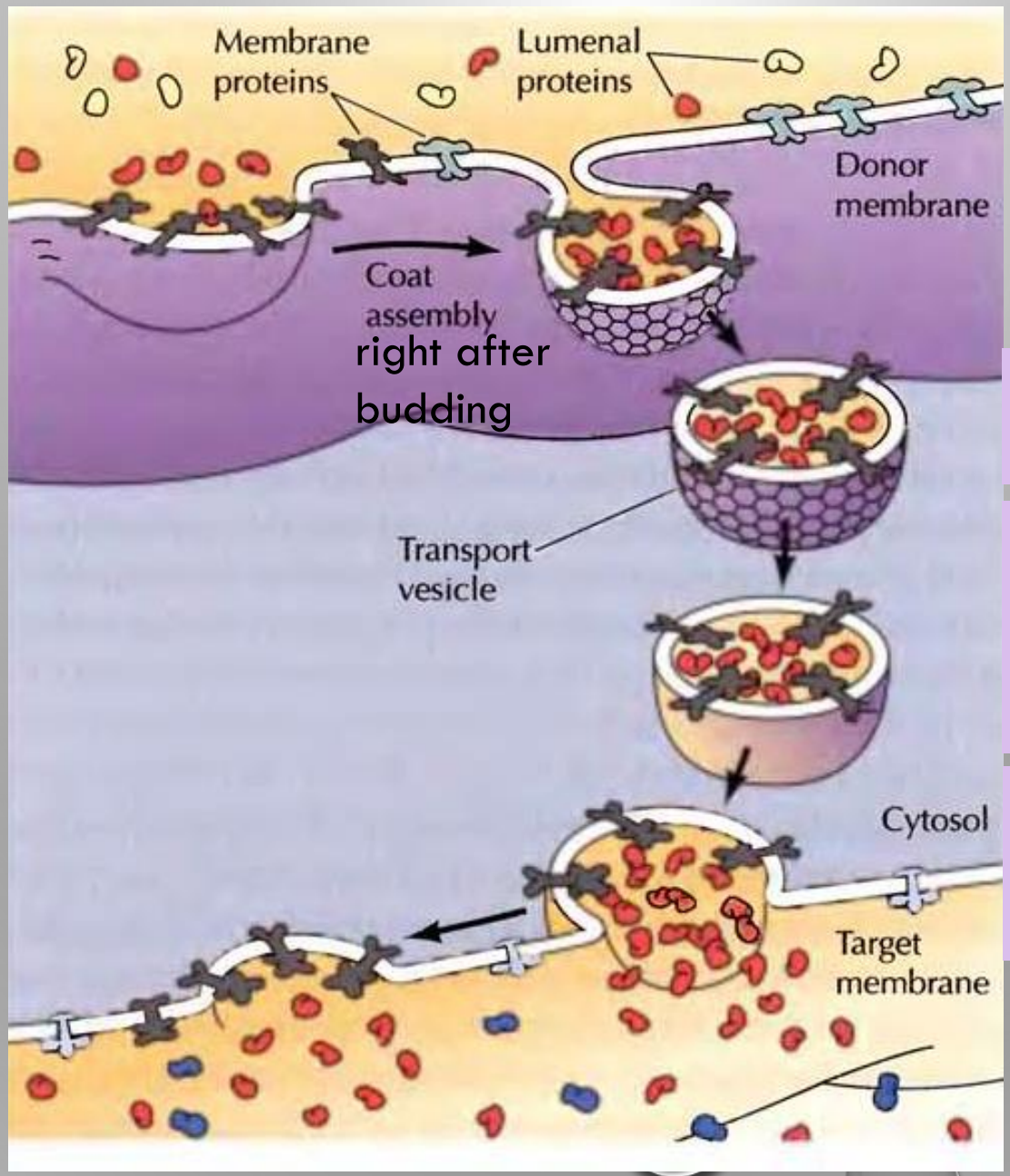
Transport to the plasma membrane of polarized cells



- Selective packaging of proteins into transport vesicles from the *trans* Golgi or recycling endosomes.
- Targeting is determined by special sequences (basolateral) or sugar modification (apical)

The mechanism of vesicular transport

Formation and Fusion of a Transport Vesicle



Vesicular transport

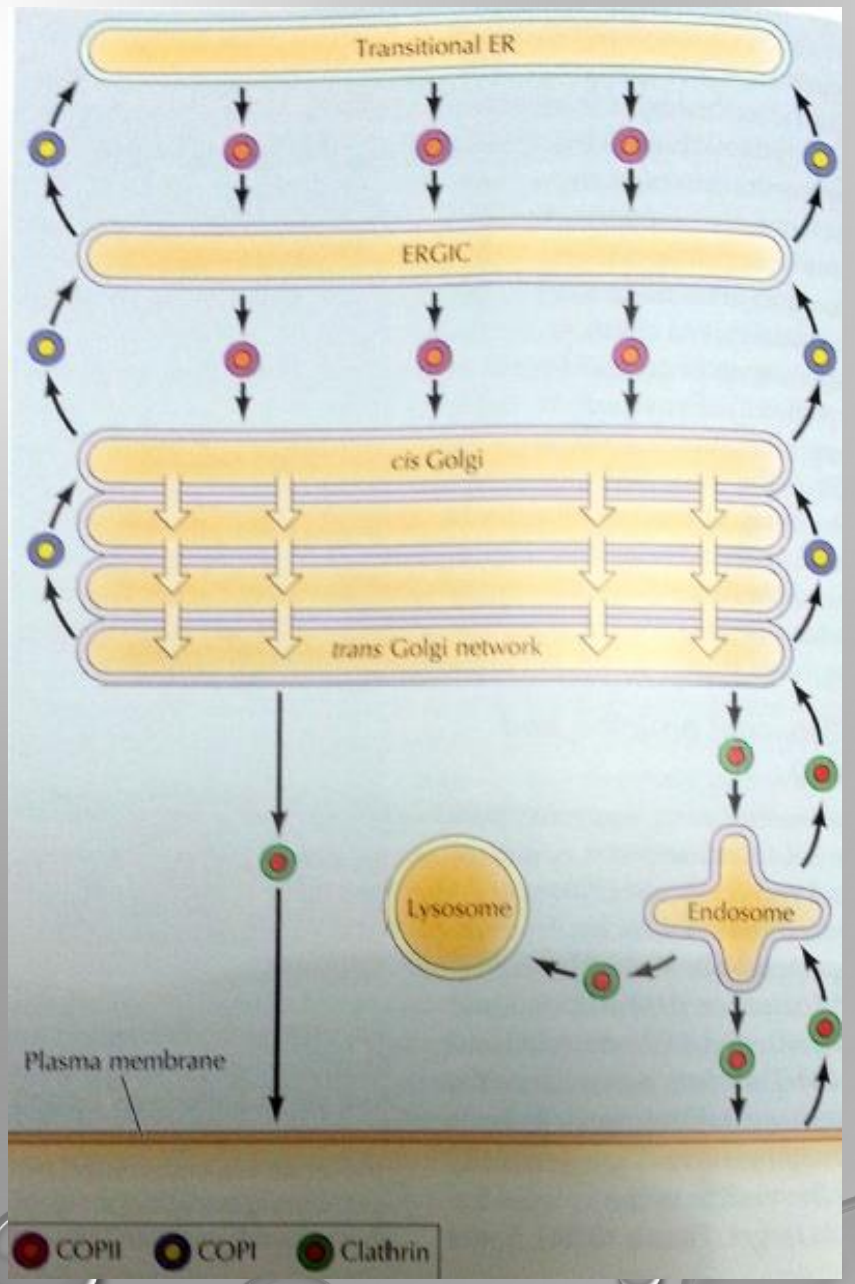
Coat disassembly in cytosol before reaching target membrane

Vesicular docking & fusion

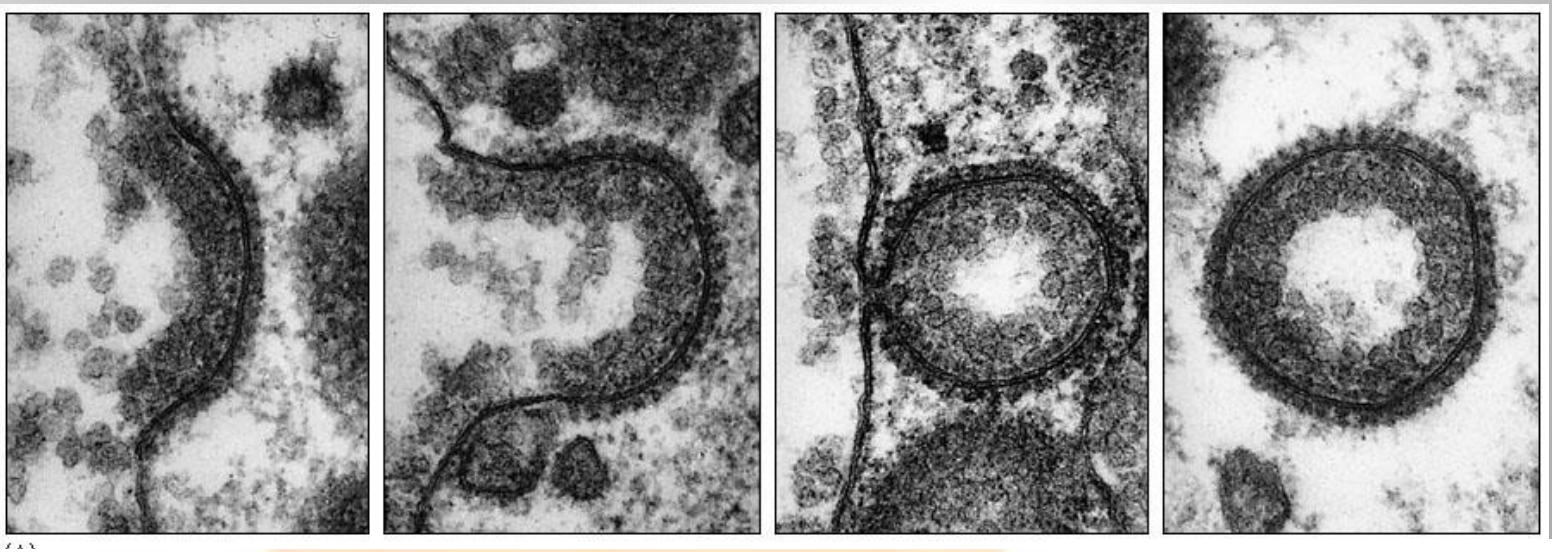
Coat Proteins

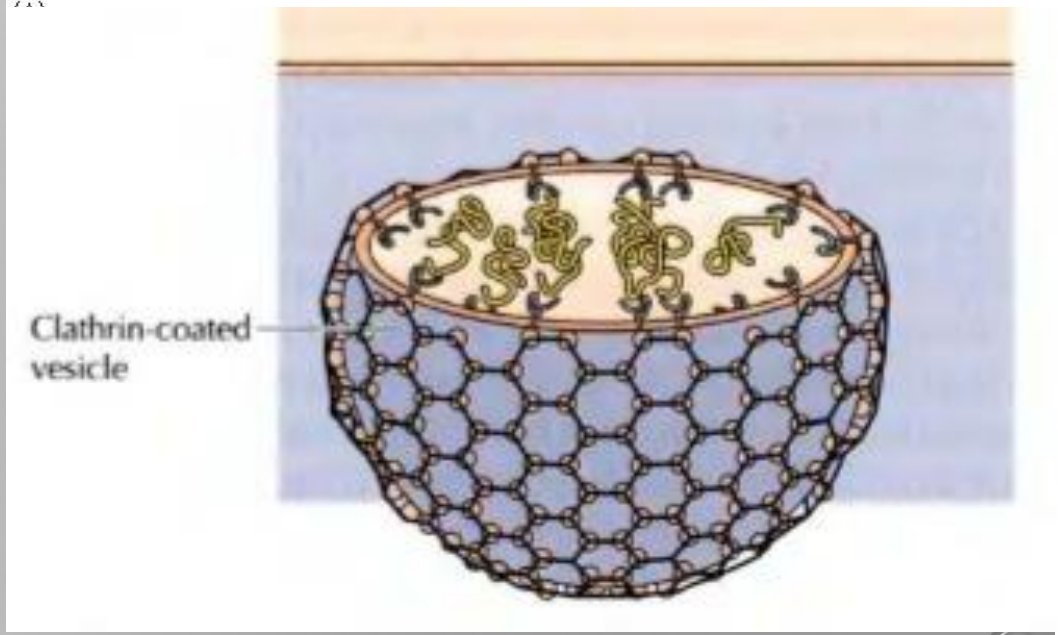
Different coating proteins (clathrin, COPI and COPII) depending on:

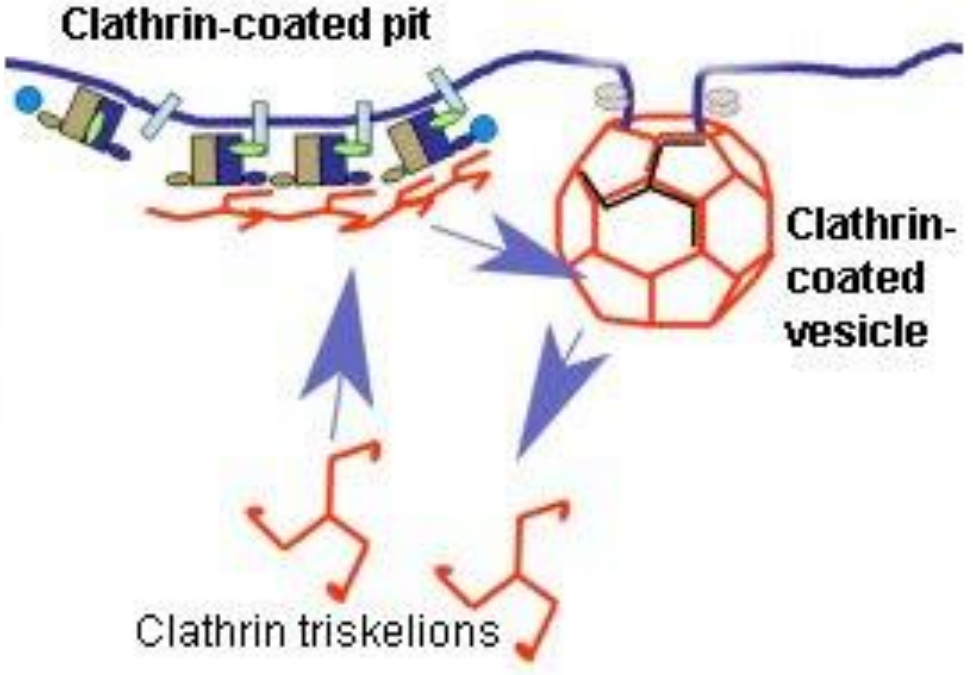
- ✓ The direction of movement
- ✓ The budding location
- ✓ The final destination



Formation of clathrin-coated vesicles

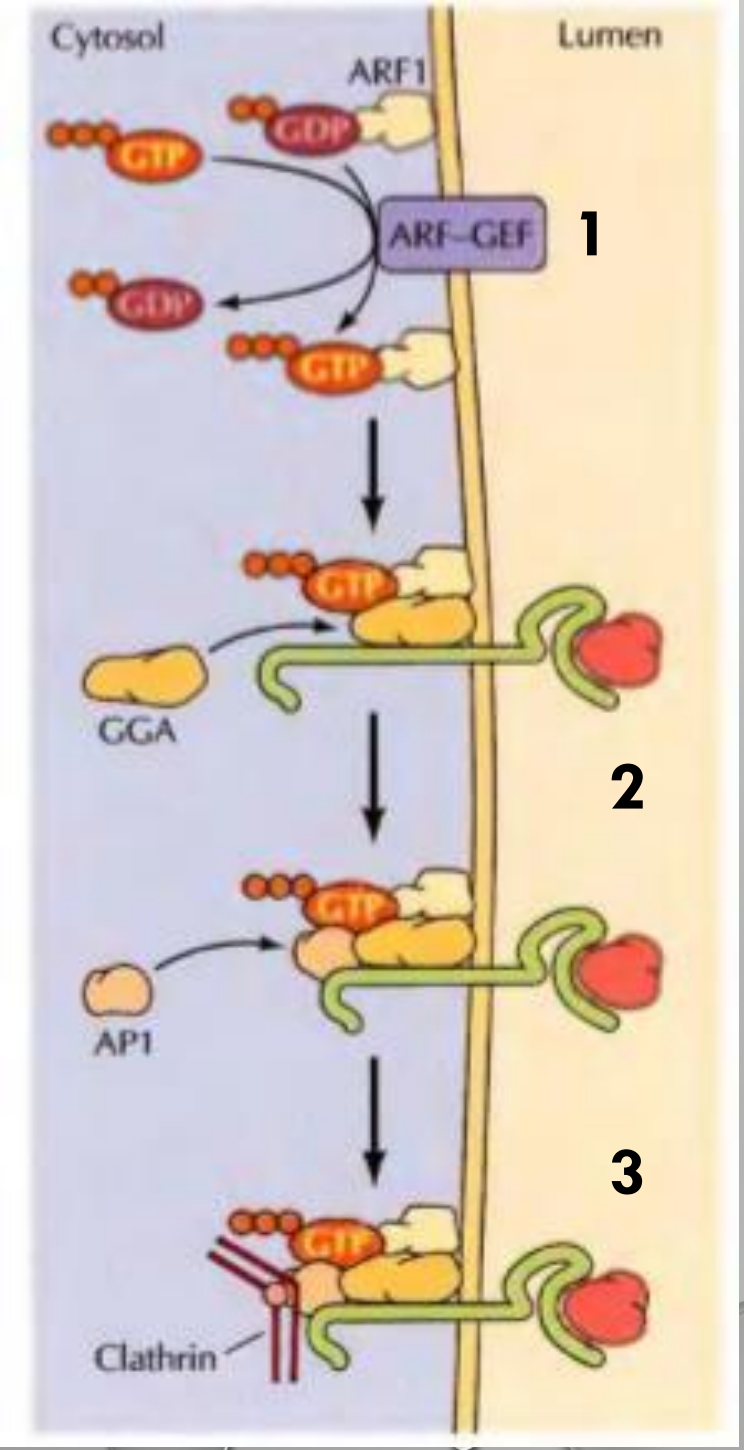






The role of ARF1 in COP1- and clathrin- coated vesicle formation

1. Activation of ARF1 by GEF
2. Recruitment of adaptor protein AP1 and then clathrin
3. Formation of ARF1-clathrin-receptor-cargo complex
4. Formation of vesicle
5. Budding and transport of vesicle
6. Inactivation of ARF1 by GTP hydrolysis and disassembly of coat
7. Vesicle fusion



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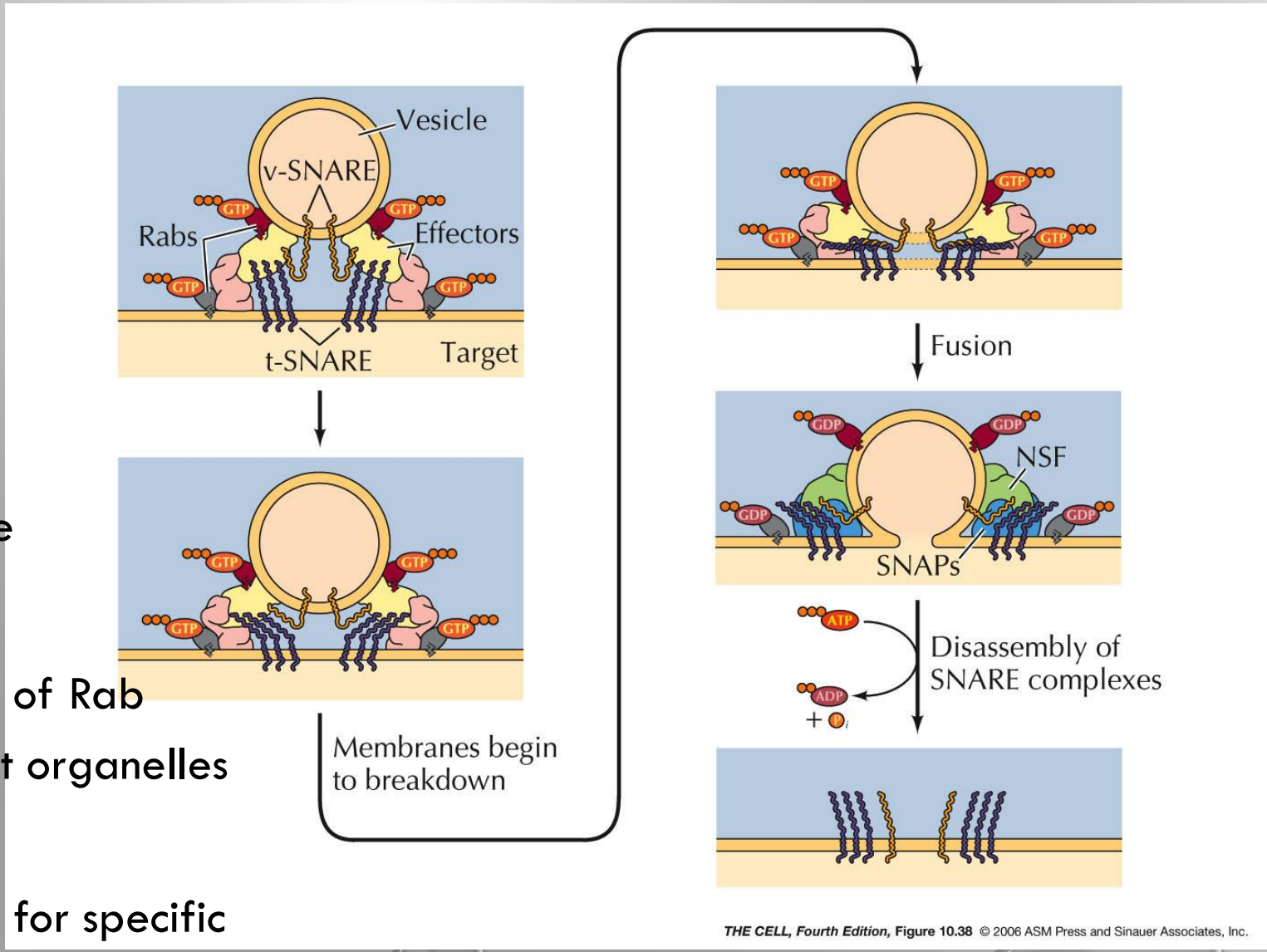
Vesicular fusion

- The formation of v-SNAREs-t-SNAREs complexes leads to membrane fusion.
- GTP-binding Rab proteins function in several steps of vesicle trafficking.
- Different combinations of Rab proteins mark different organelles and transport vesicles.
- Effector proteins allow for specific interaction



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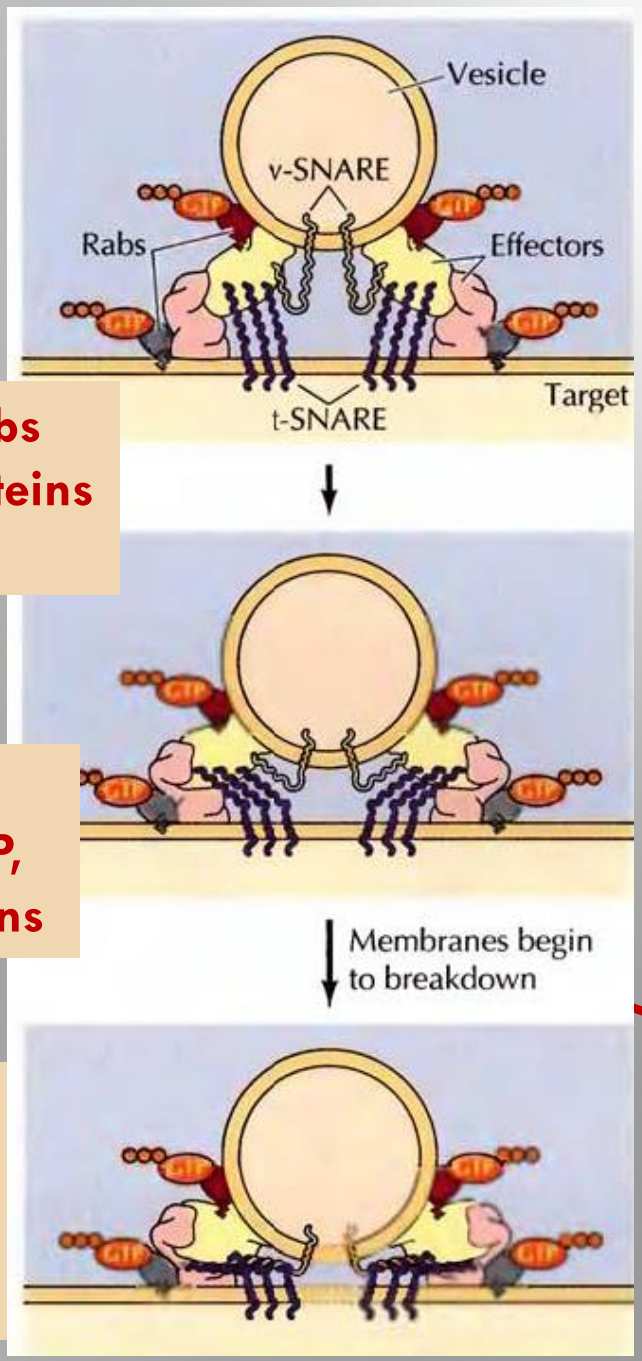
The mechanism of fusion

Docking

Interaction of Rabs with effector proteins and SNAREs

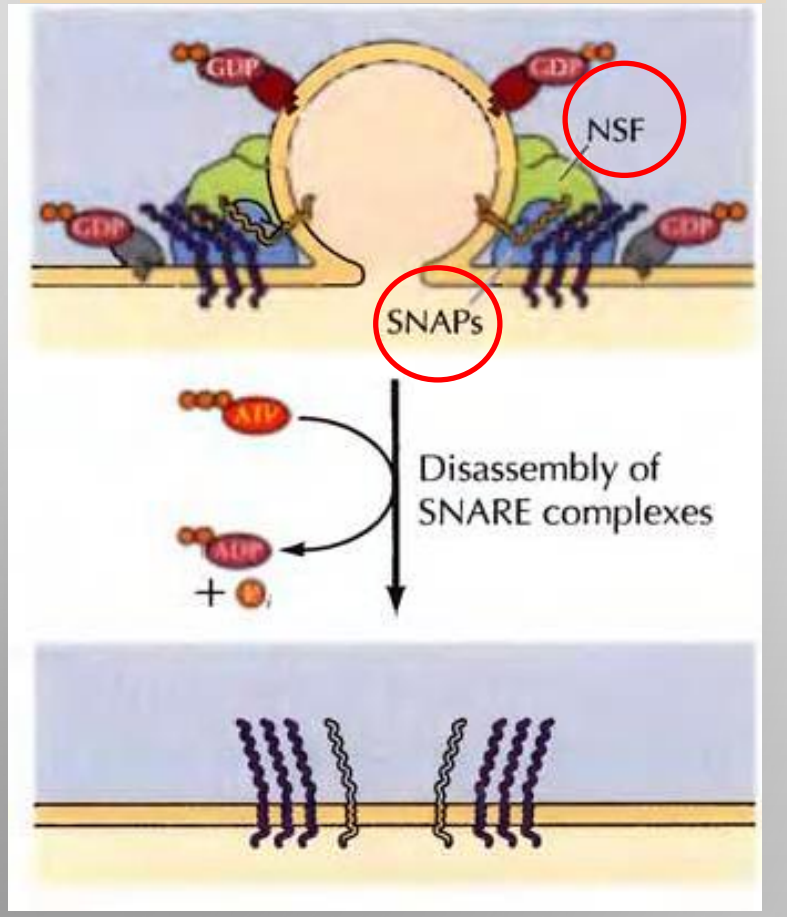
Tethering, hydrolysis of GTP, SNARE interactions

Closer vesicle-target induces membrane instability



Fusion

Disassembly of SNARE complex by NSF-SNAP complex



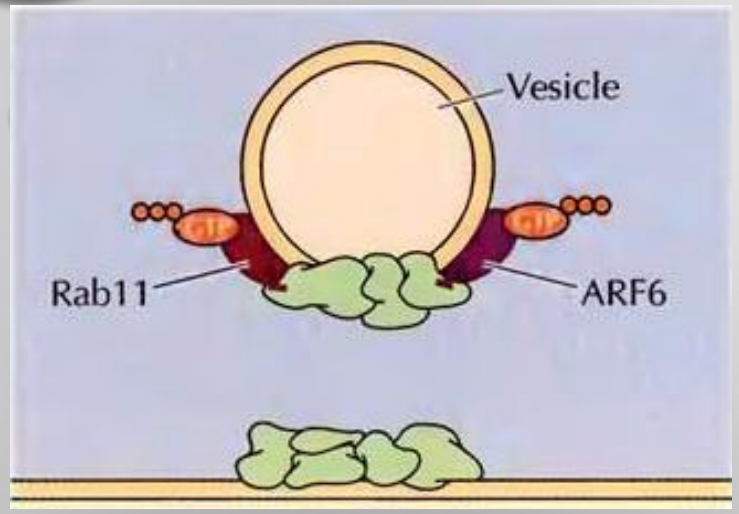
Disassembly of SNARE complex needs energy (ATP)

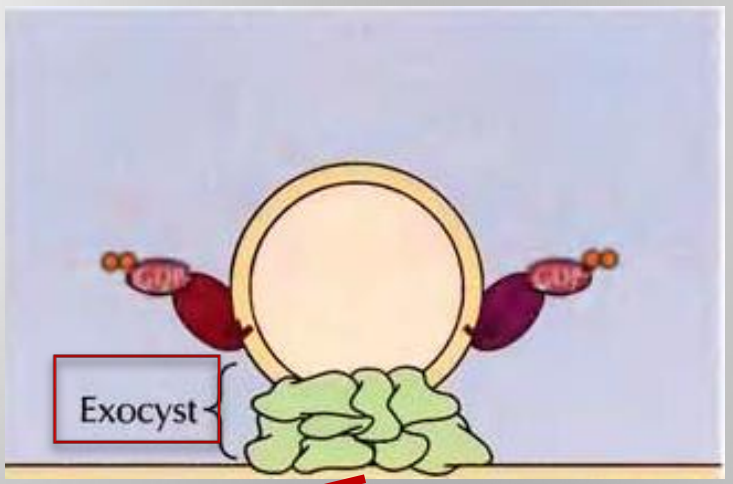
TABLE 10.1 Rab GTP-Binding Proteins and Their Sites of Action

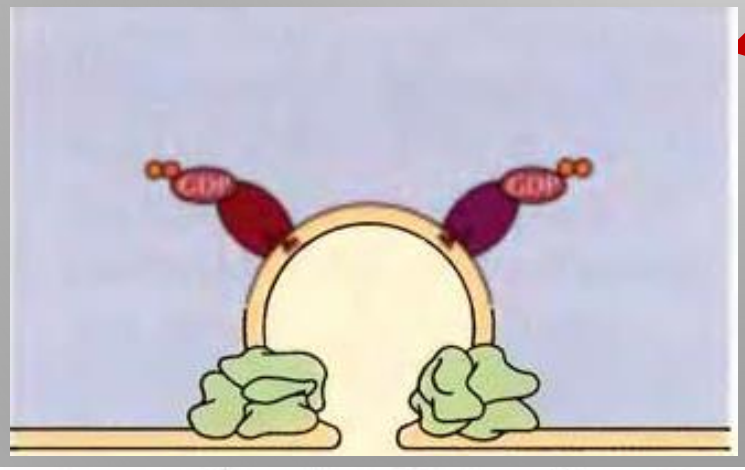
Transport step	Rab proteins involved
Exocytosis	
Transitional ER to Golgi	Rab1, Rab1b, Rab2
Golgi back to ER	Rab6, Rab6b
Intra-Golgi	Rab1, Rab6, Rab6b
trans Golgi network to plasma membrane	Rab11a, Rab11b
Endocytosis	
Plasma membrane to early endosome	Rab5a, Rab5b, Rab5c
Early endosome to plasma membrane	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







Exocysts are specific protein complexes (8 proteins) at which exocytosis occurs

Exocysts protein interaction results in efficient targeting of the vesicle to a specific location on plasma membrane.

FIGURE 10.39 Exocyst assembly and vesicle targeting Exocysts are complexes of eight different proteins formed during exocytosis from proteins present on both transport vesicles and specific regions of the plasma membrane. Tethering and docking at exocysts results in normal SNARE-mediated vesicle fusion. Small GTP-binding proteins including Rab11 and ARF6 regulate assembly of the exocyst complex on the transport vesicle and coordinate its movement to the target site.

Clinical Application: Griscelli syndrome (GS)

- A rare genetic condition
- Type: GS1, GS2, GS3
- Mutations in MYO5A, RAB27A and MLPH genes that encode the MyoVA-Rab27a-Mlph protein complex that function in melanosome transport and fusion.
- Pigmentary dilution of the skin, silver-grey hair, melanin clumps within hair shafts
- Mature melanosomes accumulate in the center of melanocytes.