Lecture 2: Golgi apparatus and vesicular transport

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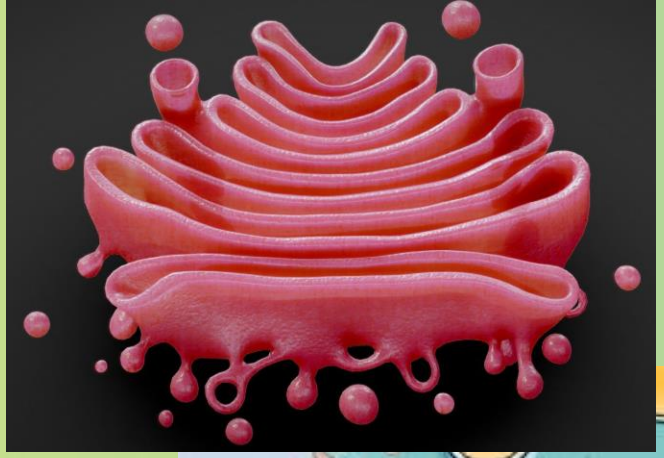
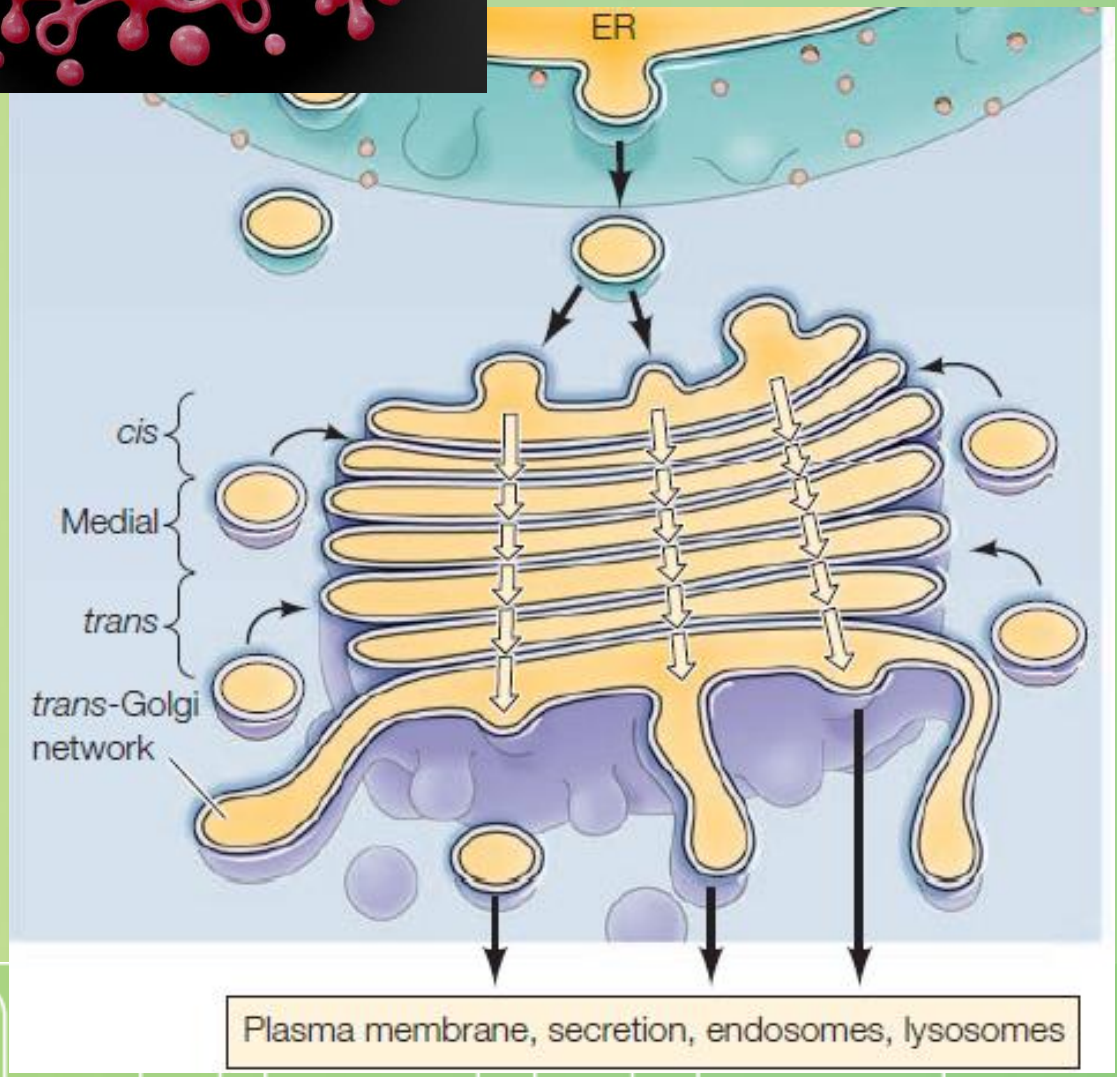
Functions of the Golgi apparatus

- Further protein processing and modification
- Further protein sorting
- Synthesis of glycolipids and sphingomyelin

Structure of the Golgi

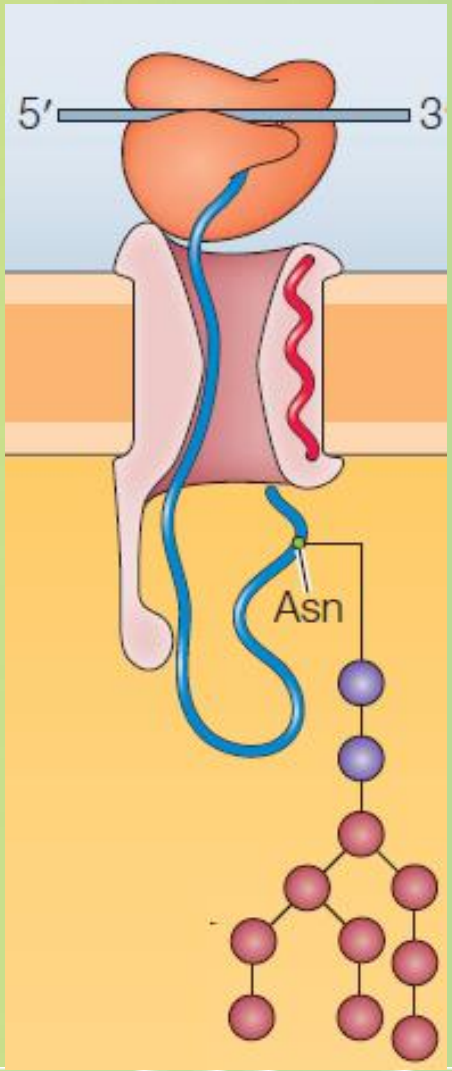


- The Golgi apparatus consists of a stack of flattened sacs (cisternae) of four regions: *cis*, medial, and *trans* compartments and the *trans*-Golgi network.
- Proteins are carried through the Golgi apparatus in the *cis-to-trans* direction.
- Transport vesicles carry the Golgi proteins back to earlier compartments for reuse.

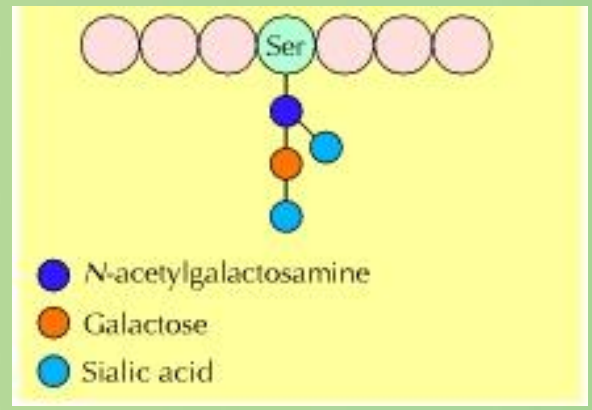


Processing of *N*-linked oligosaccharides in Golgi

The *N*-linked oligosaccharides, which are added to asparagine residues of glycoproteins and transported from the ER, are further modified enzymatically in different compartments of the Golgi.



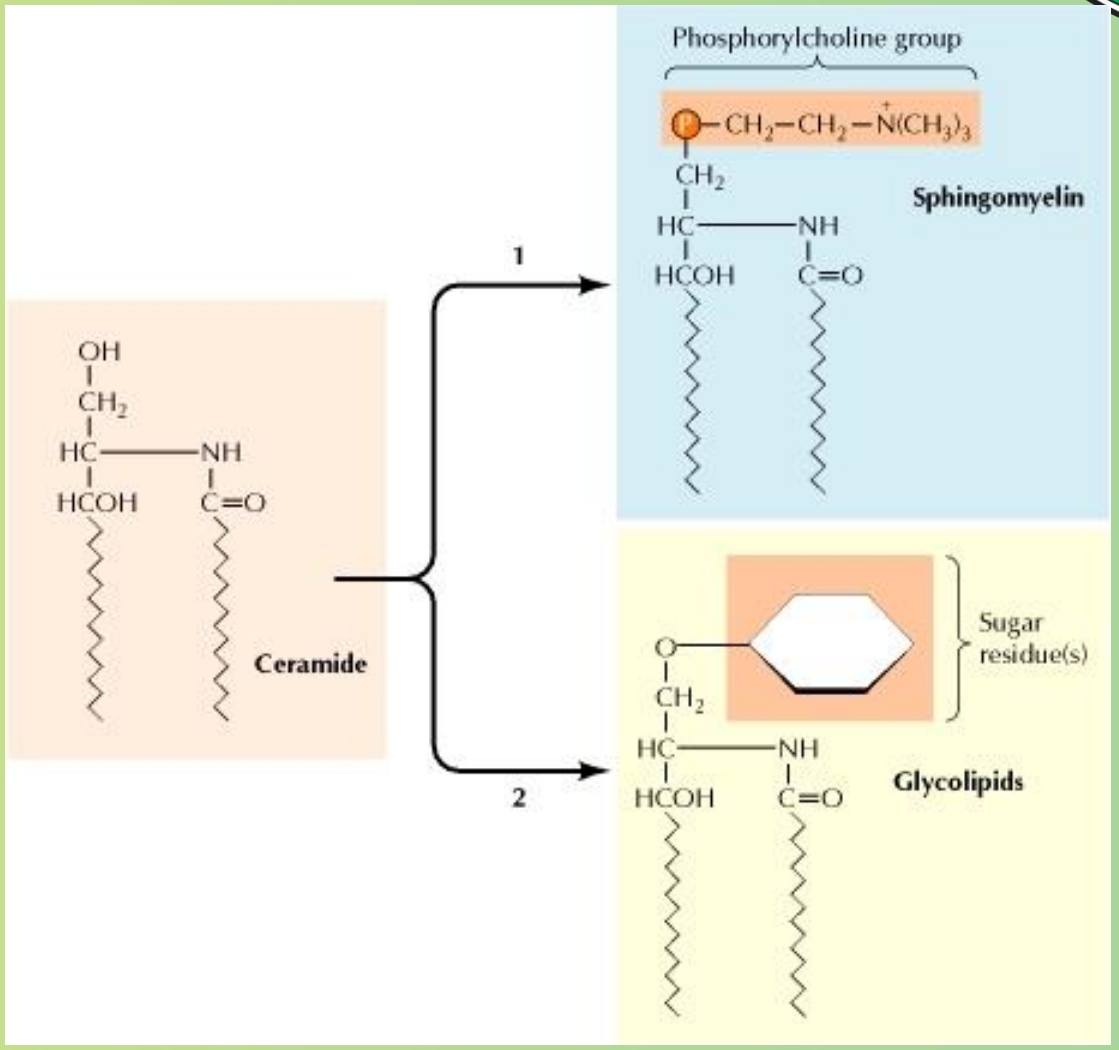
Proteins can also be modified by the addition of carbohydrates to the hydroxyl side chains of serine and threonine residues, hence called O-linked sugars.



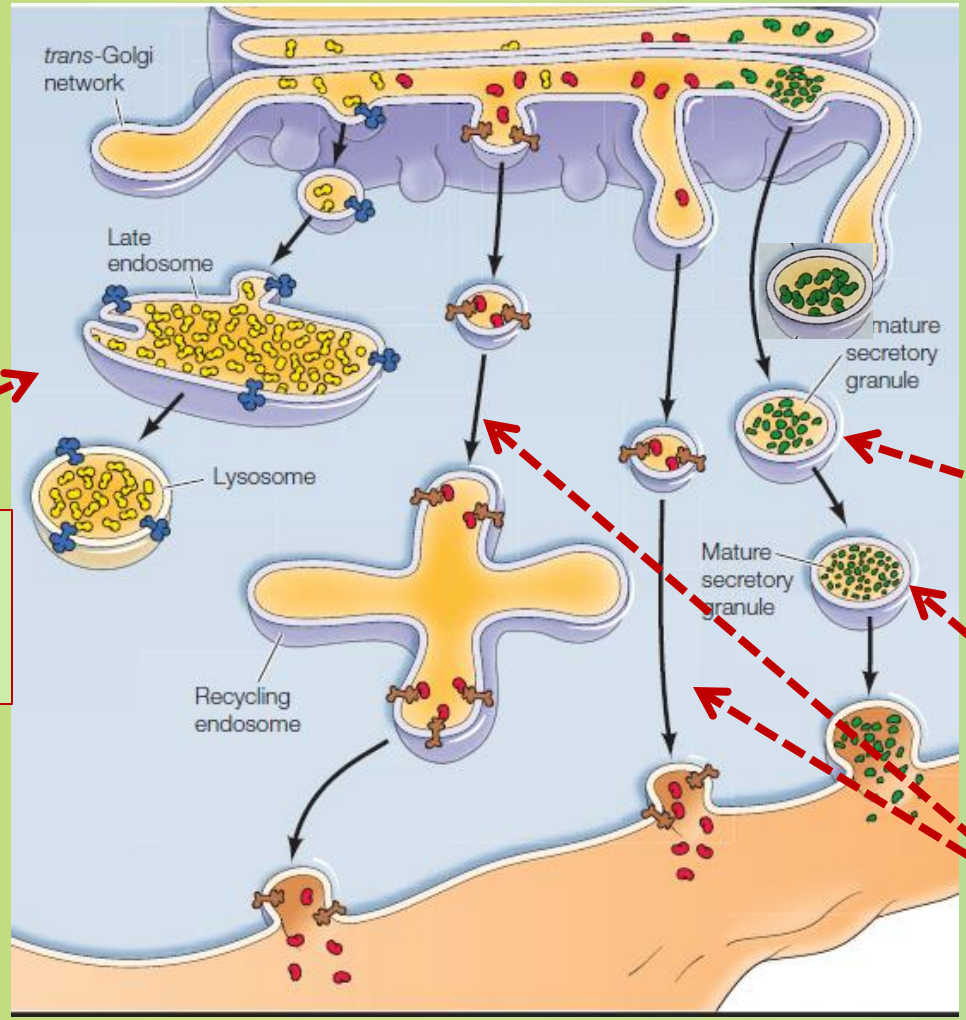
Lipid and Polysaccharide Metabolism in the Golgi

- Ceramide is converted either to sphingomyelin (a phospholipid) or to glycolipids in the Golgi apparatus.

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

Proteins can be targeted to late endosomes, which develop into lysosomes

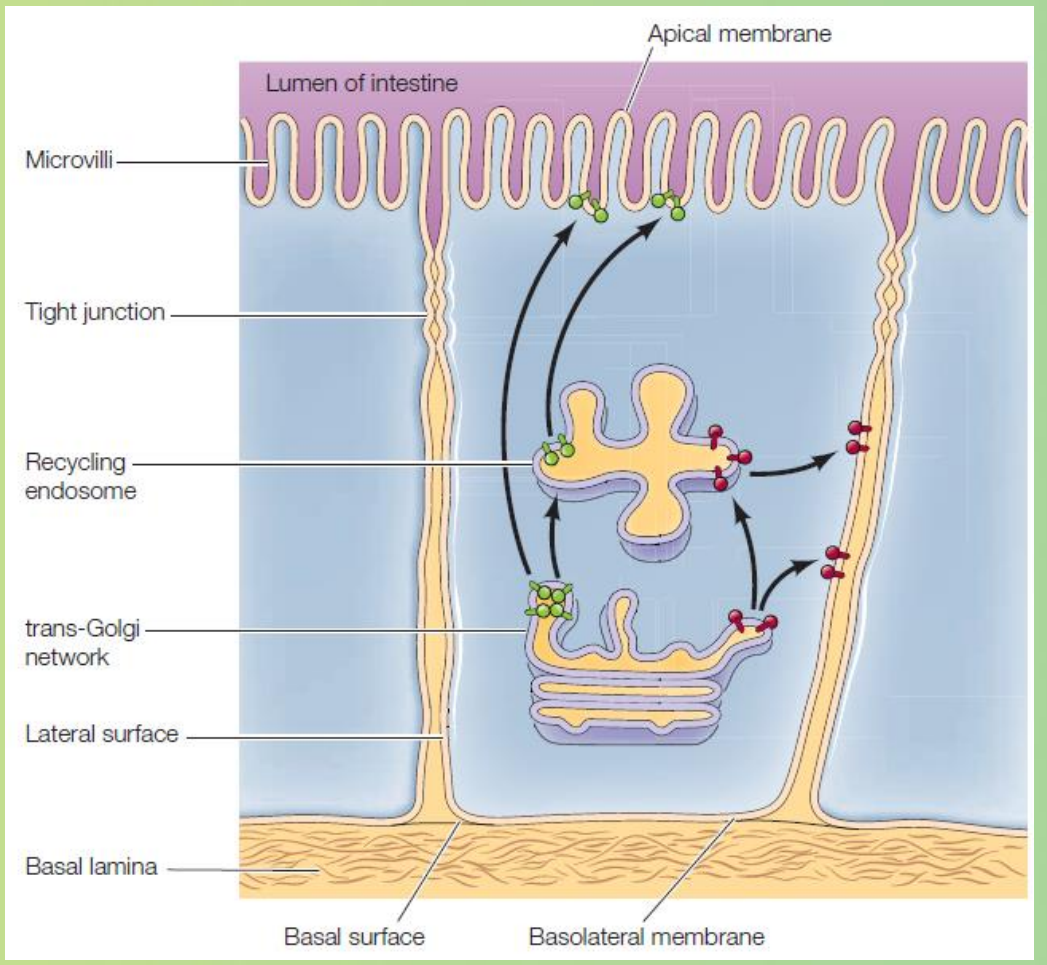
Protein processing in Immature secretory vesicles

Regulated secretion after signaling from specialized vesicles

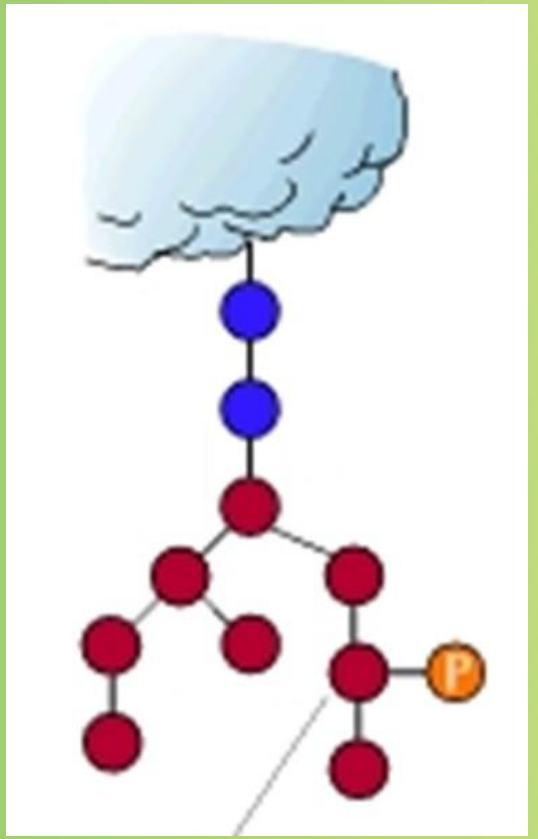
Continuous, unregulated secretion

Transport to the plasma membrane of polarized cells

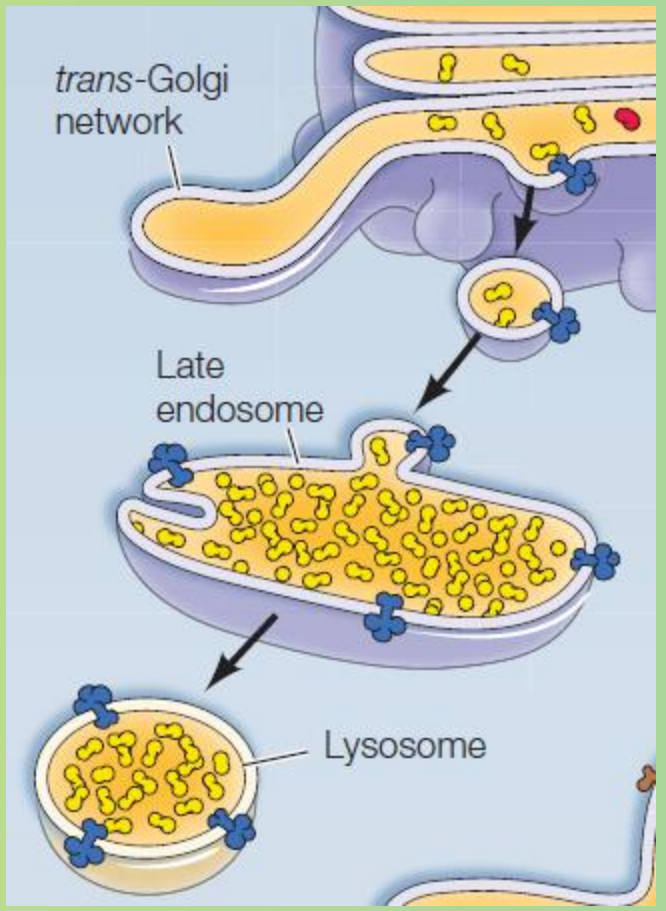
- Proteins are selectively packaged into transport vesicles from the trans-Golgi or recycling endosomes.
- Targeting is determined by special sequences (basolateral) or GPI sugar modification (apical).



Processing of lumenal lysosomal proteins



Protein destined to lysosomes have a signal patch (a three-dimensional structural determinant), which is recognized by modifying enzymes that add mannose-6-phosphate to the proteins.

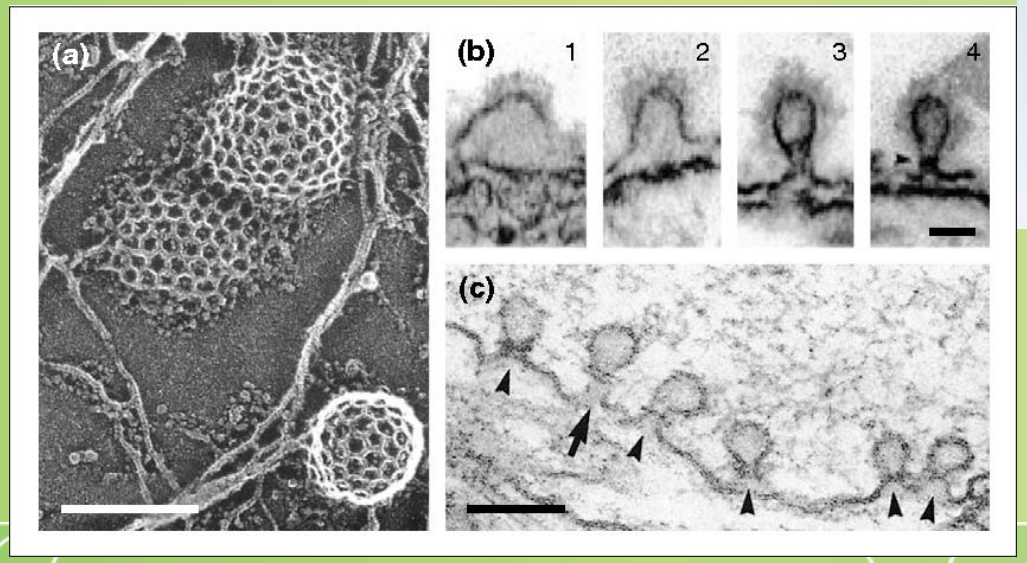


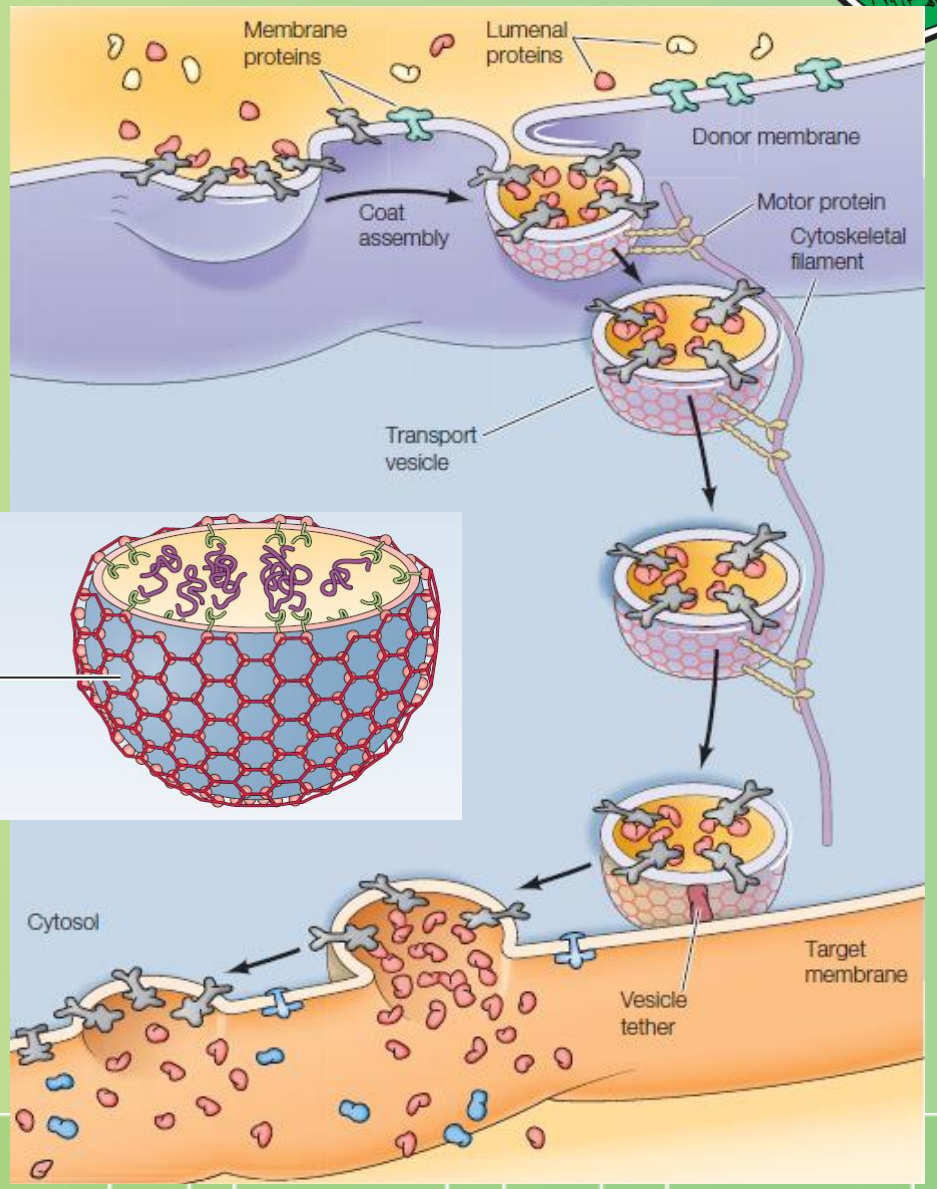
Lumenal lysosomal proteins bind to a mannose-6-phospahte receptor and are transported to late endosome, which mature into lysosomes.

The mechanism of vesicular transport

Formation and fusion of a transport vesicle

- Membrane proteins and lumenal secretory proteins with their receptors are grouped on the Golgi membrane before budding of a transport vesicle coated by a protein called clathrin.
- The clathrin-coated vesicle is then docks at its target membrane, gets uncoated, and fuses with the membrane.



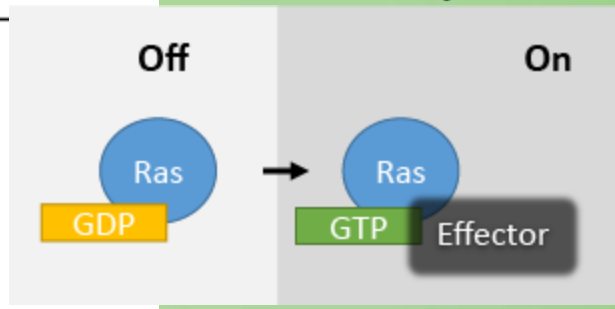


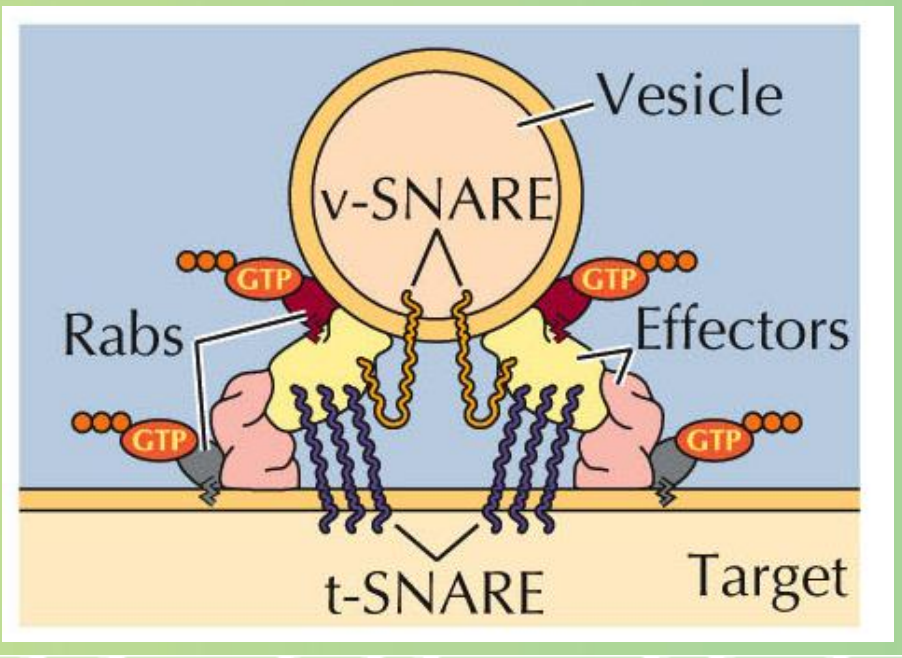
Delivery of vesicles: targeting and fusion

- Small G proteins called Rab determine the membrane targets of vesicles.
  - There are over 60 Rab proteins where different combinations of these proteins mark different transport vesicles.
- v-SNAREs-t-SNAREs proteins are responsible for vesicular fusion with the target membranes.

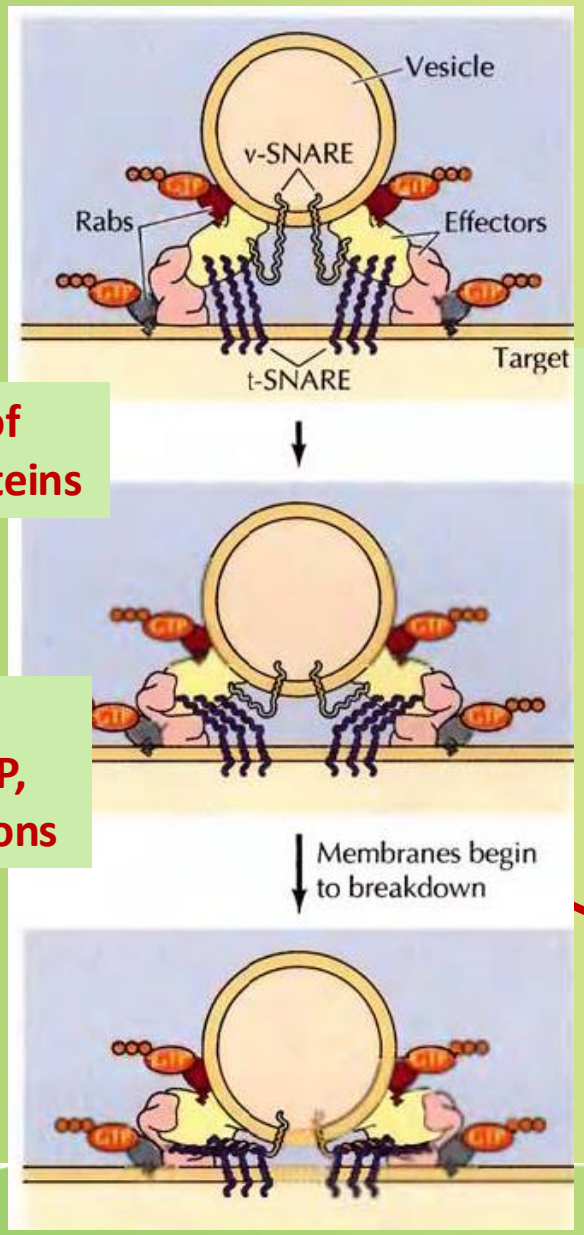
Rab GTPase	Site(s) of action[a]
Rab1	ER to Golgi, intra-Golgi
Rab4	EE to PM
Rab5	PM to EE
Rab6	Golgi to ER, intra-Golgi, EE to TGN
Rab7	EE to LE, LE to lysosome
Rab9	LE to TGN
Rab10	Golgi-associated
Rab11	RE to PM, EE to TGN, TGN to PM

[a] Abbreviations: EE, early endosome; PM, plasma membrane; LE, late endosome; RE, recycling endosome.





The mechanism of fusion

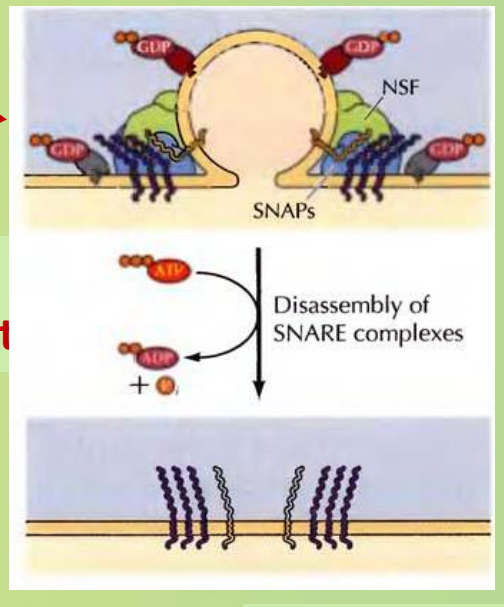


Interaction of effector proteins

Tethering, hydrolysis of GTP, SNARE interactions

Closer vesicle-target

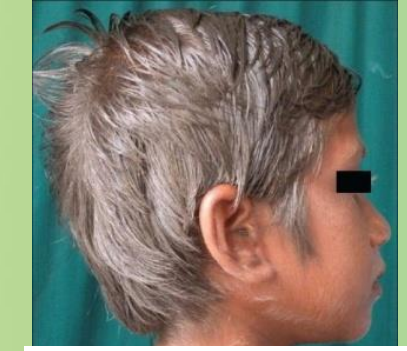
Fusion



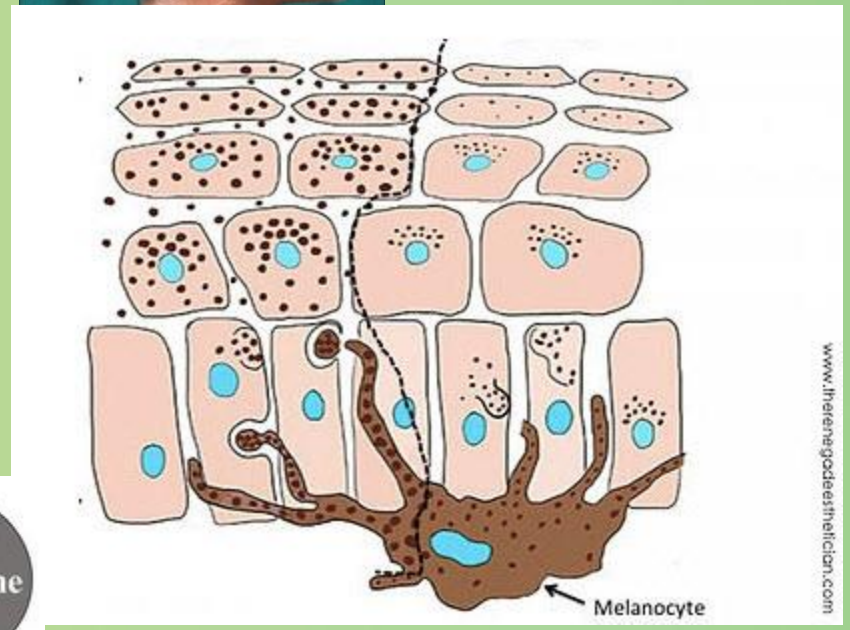
Disassembly of SNARE complex

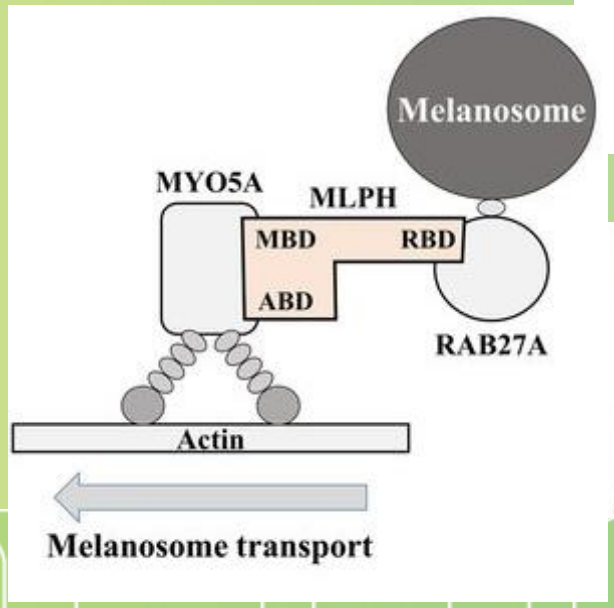
Rab protein binds to a tethering factor associated with the target membrane. SNAREs on the vesicle and target membranes complex together. The SNAREs zip together, bringing the vesicle and target membranes into close proximity, and the membranes fuse

Griscelli syndrome (GS)



- A rare genetic condition
- Mutations in MYO5A (a motor protein), RAB27A and MLPH (a Rab effector protein) 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

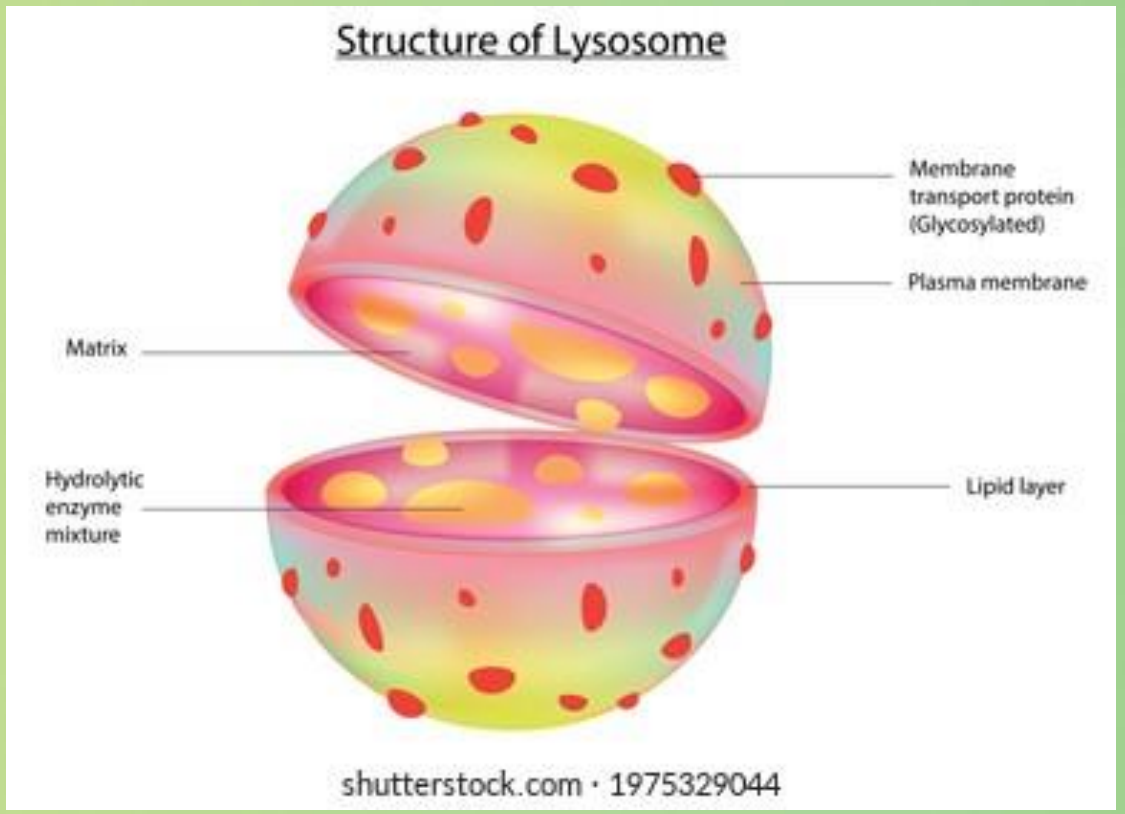




Lysosomes

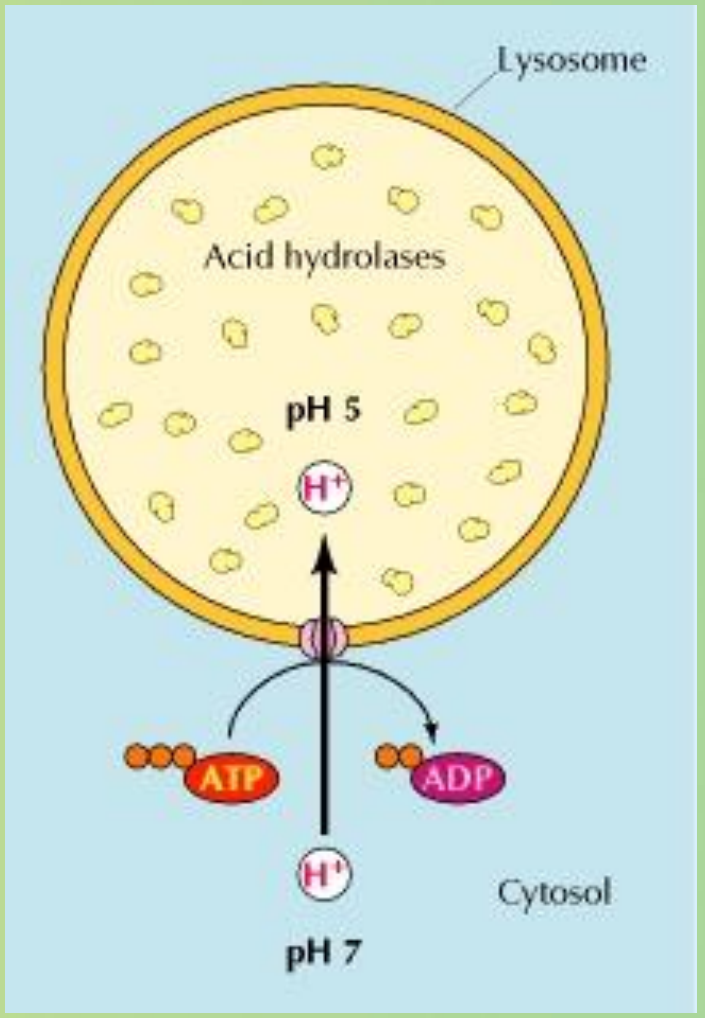
Structure

- Lysosomes are membrane-enclosed organelles that contain various enzymes that break down all types of biological macromolecules.
- Lysosomes degrade material taken up from outside and inside the cell.



Lysosomal enzymes

- Lysosomes contain ~60 different acid hydrolases.
- The enzymes are active at the acidic pH (about 5) that is maintained within lysosomes.
- Levels of cell protection from these hydrolases:
  - Containment
  - Inactive if released
- A proton pump maintains the lysosomal pH.

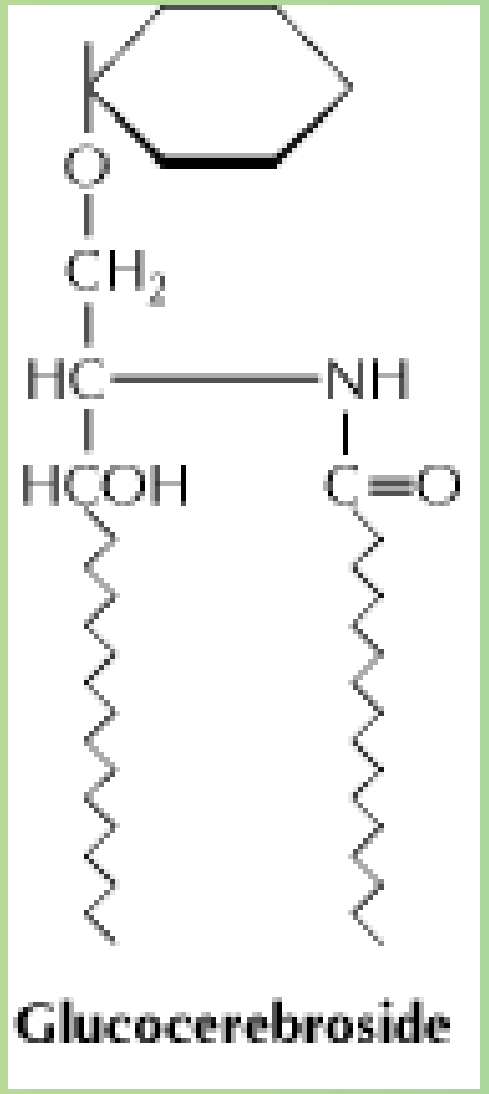


Lysosomal storage diseases

- Glycolipidoses (sphingolipidoses)
- Oligosaccharidoses
- Mucopolysaccharidoses: deficiencies in lysosomal hydrolases of glycosaminoglycans (heparan, keratan and dermatan sulfates, chondroitin sulfates.
  - They are chronic progressively debilitating disorders that lead to severe psychomotor retardation and premature death.

Glucocerebroside

- Glucocerebroside is a glycosphingolipids (a monosaccharide attached directly to a ceramide unit (a lipid)
- It is a byproduct of the normal recycling of red blood cells during, which are phagocytosed by macrophages, degraded and their contents recycled to make new cells.

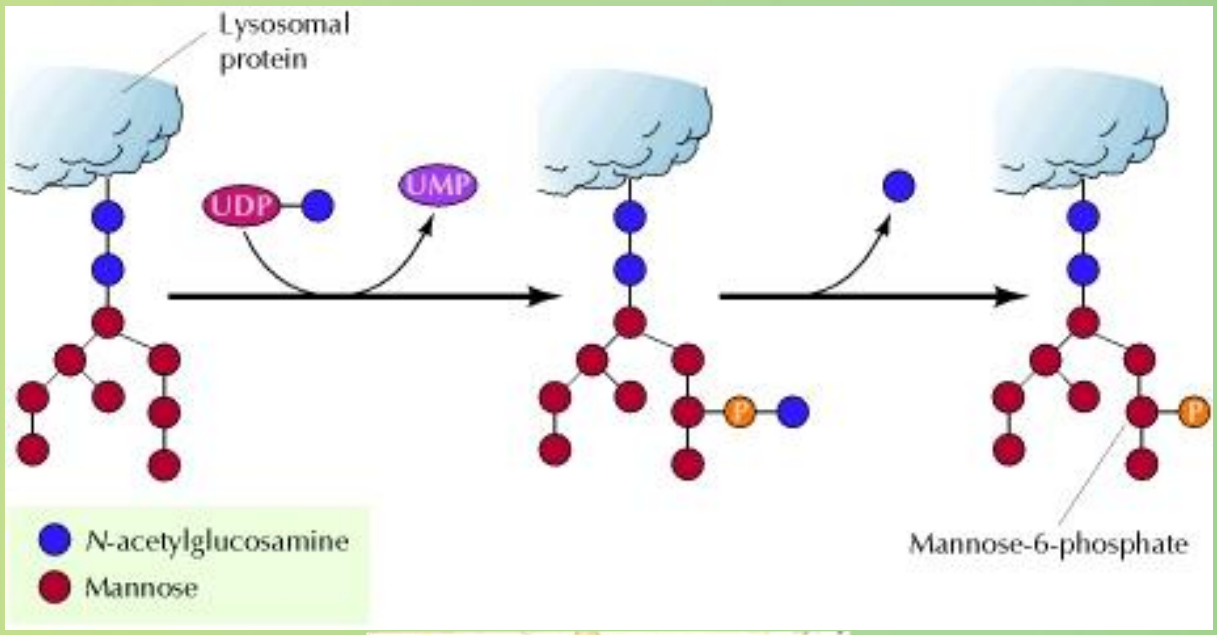


Glucocerebroside

I-cell disease

also called mucolipidosis IIA, or mucolipidosis II alpha/beta: ML-IIα/β

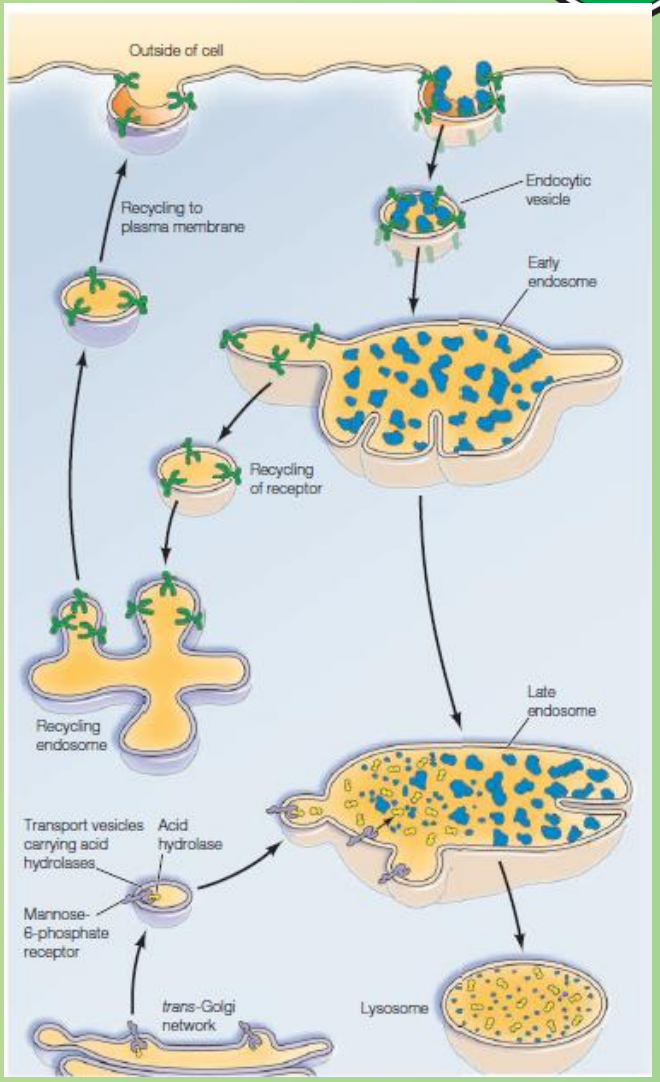
- Defective targeting of lysosomal enzymes from Golgi to the lysosomes
- A deficiency in tagging enzyme that phosphorylates mannose
- Features: severe psychomotor retardation that rapidly progresses leading to death between 5 and 8 years of age.



Endocytosis

- Molecules are taken up from outside the cell in endocytic vesicles, which fuse with early endosomes.
- Early endosomes mature into late endosomes.
- Transport vesicles carrying acid hydrolases from the Golgi fuse with late endosomes, which mature into lysosomes.

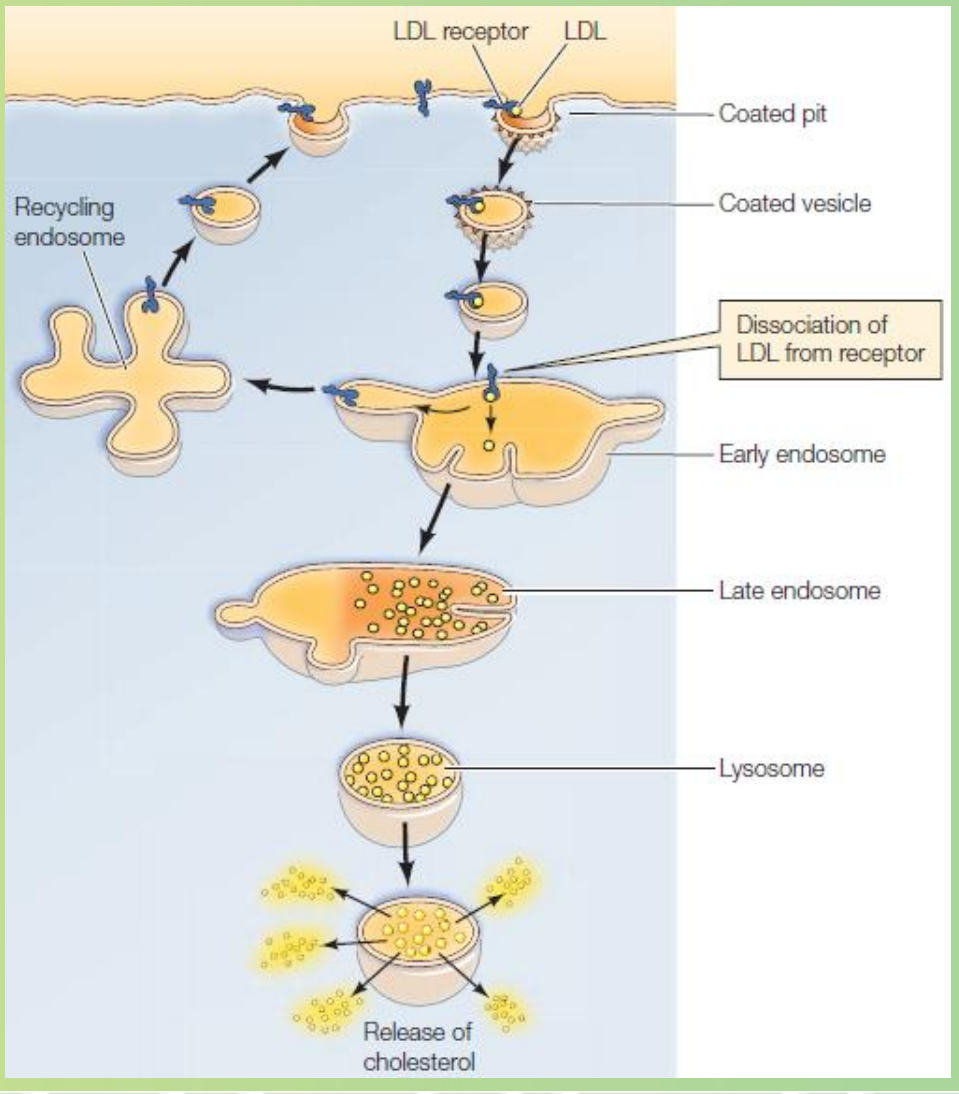
- *Note: the pH in endosomes is 6.0-6.5.*



Clathrin-dependent endocytosis

Receptor-mediated endocytosis

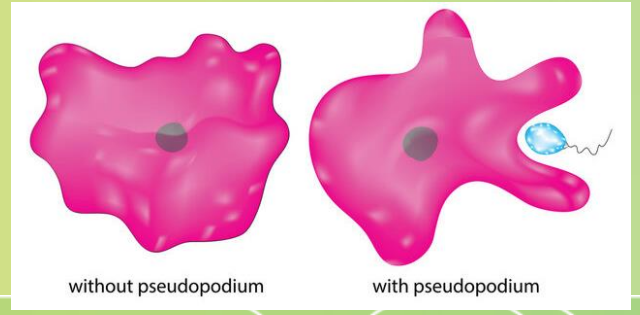
- Ligands bind to their receptors stimulating endocytosis.
- In early endosomes, the acidic pH causes the release of ligands from their receptors.
- Membrane receptors are recycled via recycling endosomes and early endosomes mature into late endosomes.
- Transport vesicles carrying acid hydrolases from the Golgi fuse with late endosomes, which mature into lysosomes.
- Example: removal of plasma cholesterol by low-density lipoprotein (LDL) receptor

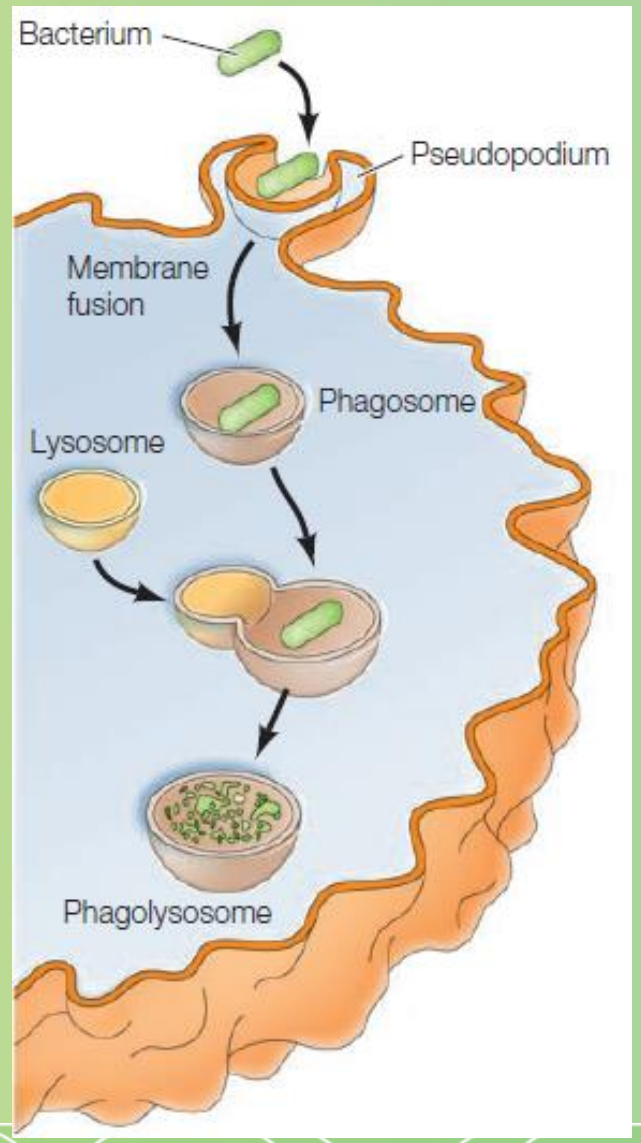


Phagocytosis

- Binding of a bacterium to the cell surface stimulates the extension of a pseudopodium, which eventually engulfs the bacterium.
- Fusion of the pseudopodium membranes then results in formation of a large intracellular vesicle (a phagosome). The phagosome fuses with lysosomes to form a phagolysosome within which the ingested bacterium is digested.
- *Macropinocytosis (clathrin-independent) is cell drinking via the formation of small vesicles.*

A pseudopodium is a temporary arm-like projection of a eukaryotic cell membrane





Autophagy (self-eating)

- Regions of the cytoplasm or internal organelles (such as mitochondria) are enclosed by membranes derived from the endoplasmic reticulum, forming autophagosomes.
- Autophagosomes fuse with lysosomes to form large phagolysosomes in which their contents are digested.
- Purpose: removal of damaged organelles; survival during starvation; tissue remodeling during development