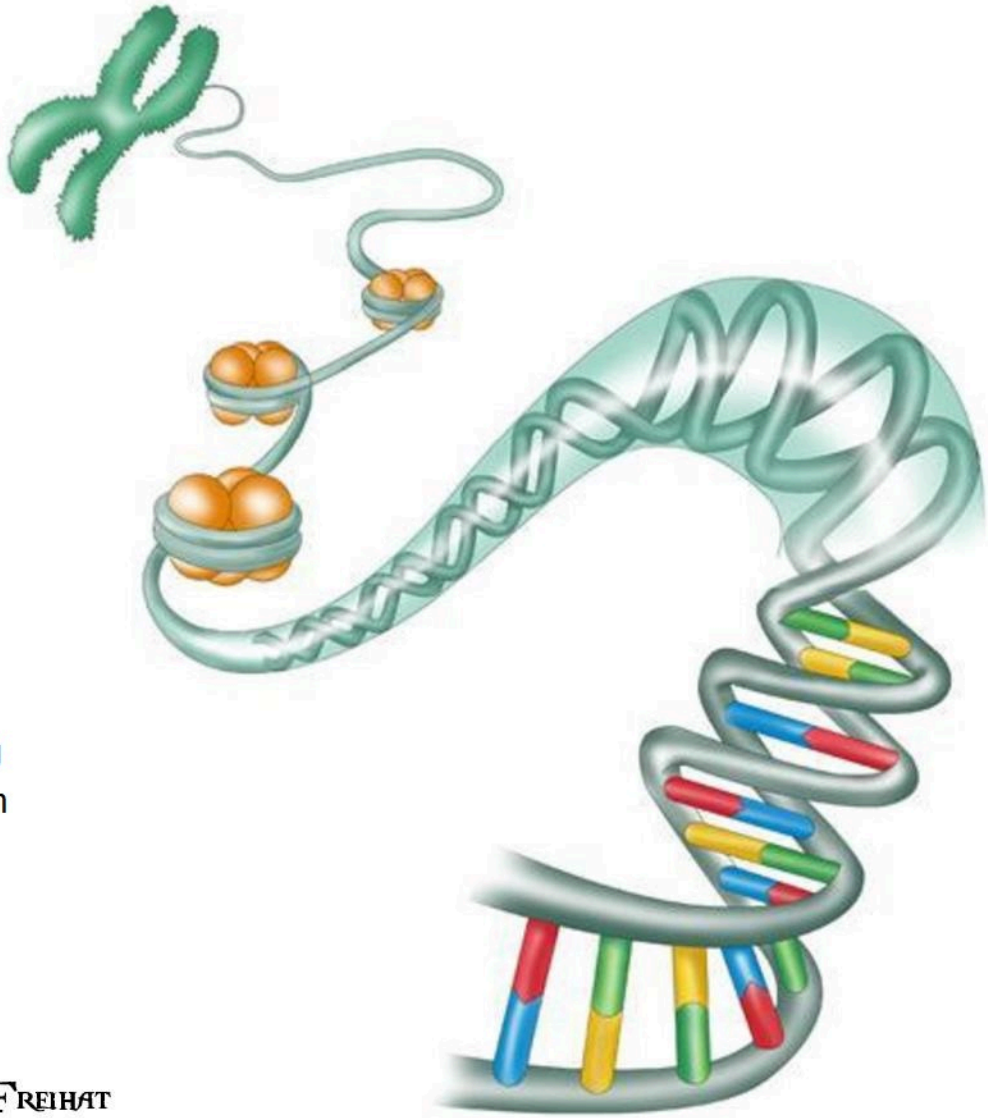
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ○ Sheet ○ Handout ○ other.....



SLIDE #: 3- protein sorting
DR.NAME: Mamoun Ahram

Designed by Nadeen Al-Freihat

Lecture 3: Protein sorting
(Golgi apparatus and vesicular transport)

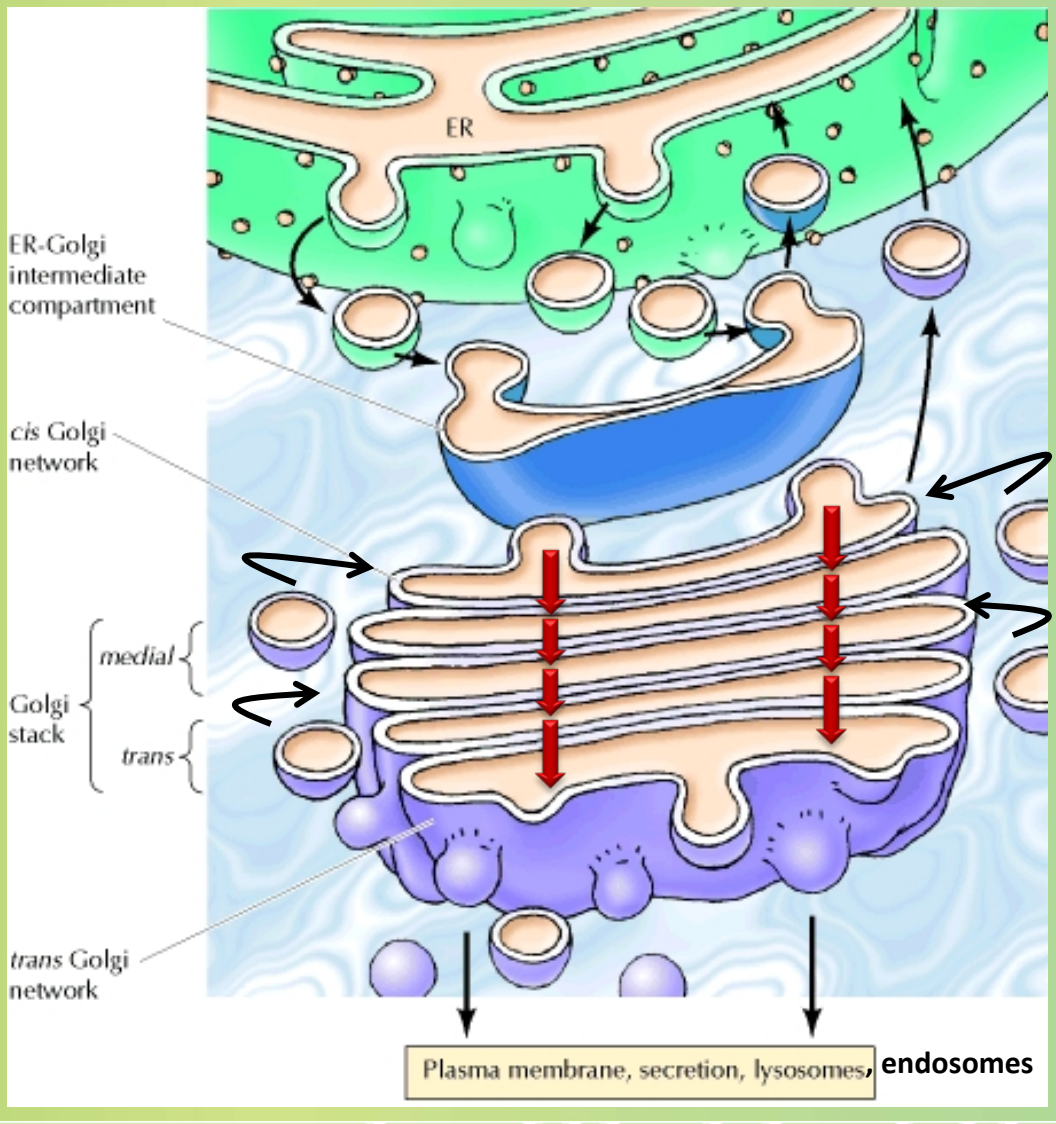
Dr. Mamoun Ahram
Faculty of Medicine
Second year, Second semester, 2014-2014

Principles of Genetics and Molecular Biology

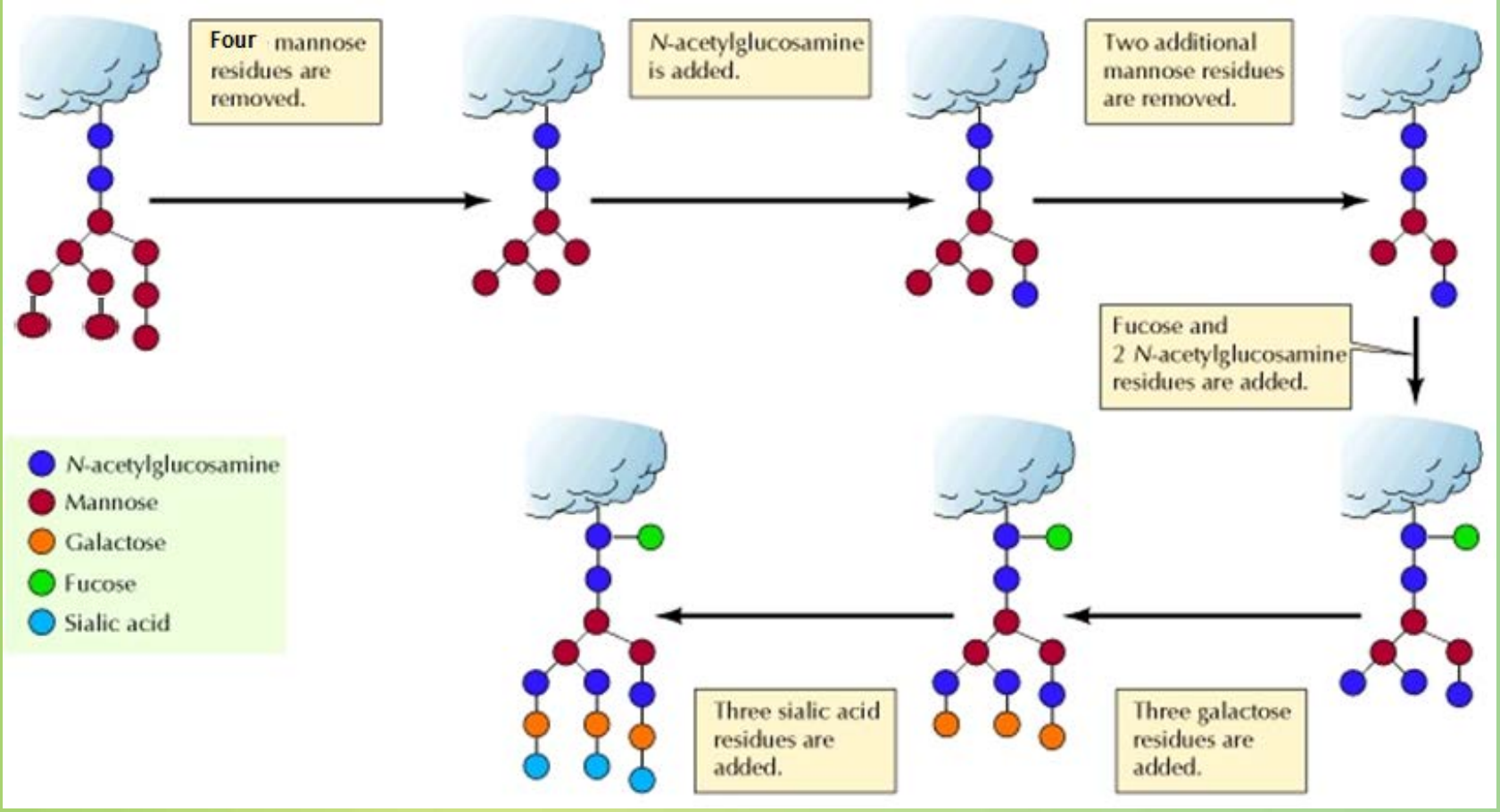
Functions of Golgi

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

Structure of the Golgi

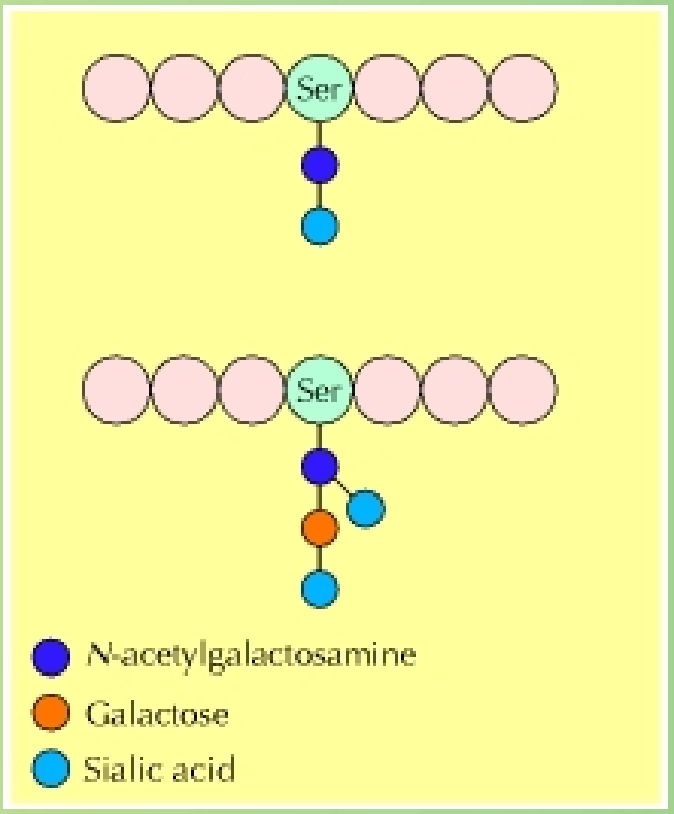


Processing of *N*-linked oligosaccharides in Golgi



O-linked glycosylation

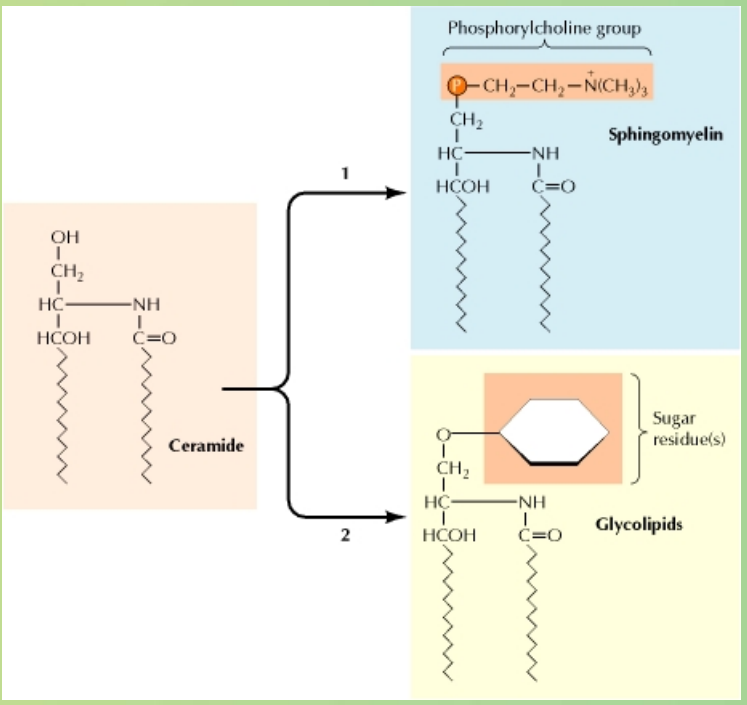
- Proteins can also be modified by the addition of carbohydrates to the side chains of acceptor serine and threonine residues.
- The serine or threonine is usually linked directly to *N*-acetylgalactosamine, to which other sugars can then be added.
- In some cases, these sugars are further modified by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



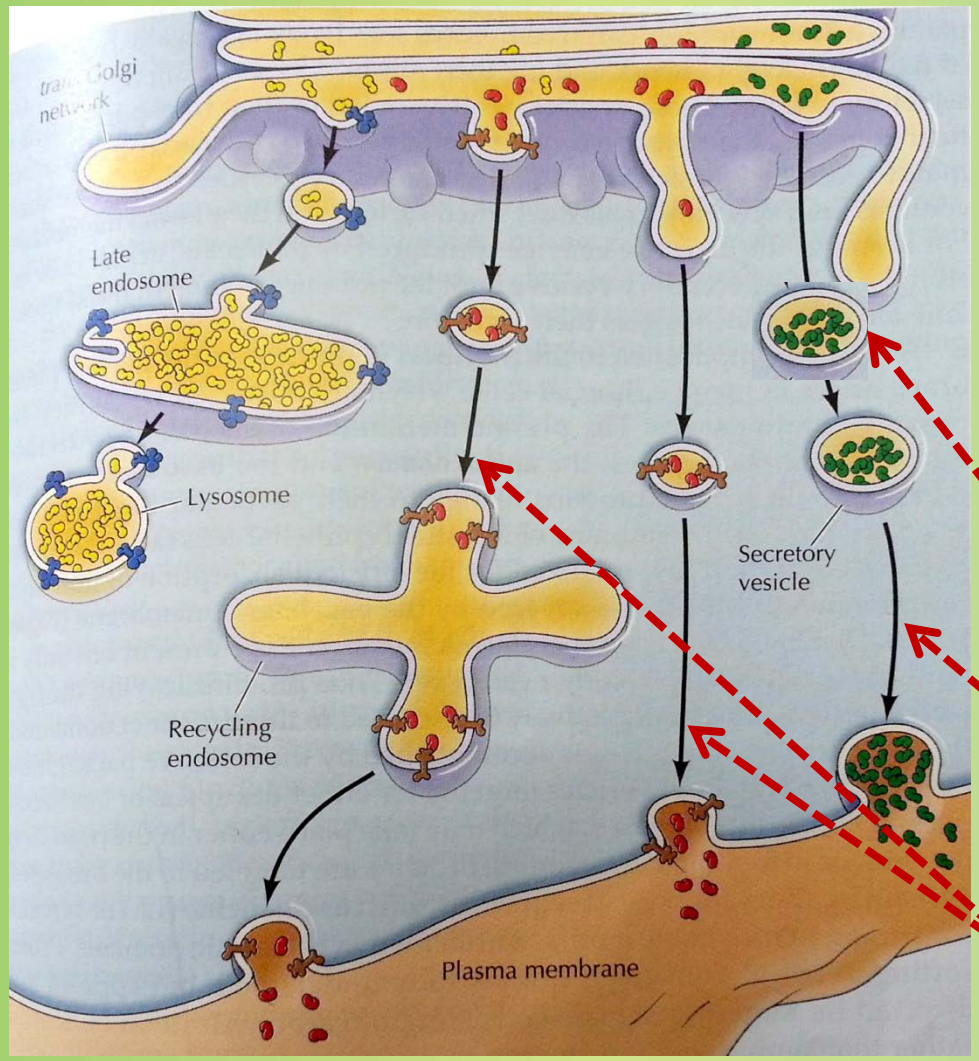
Lipid and Polysaccharide Metabolism in the Golgi

- **Transfer of phosphorylcholine group is from phosphatidylcholine to ceramide.**
  - **Sphingomyelin is synthesized on the lumenal surface.**
- **Addition of sugar residues.**
  - **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

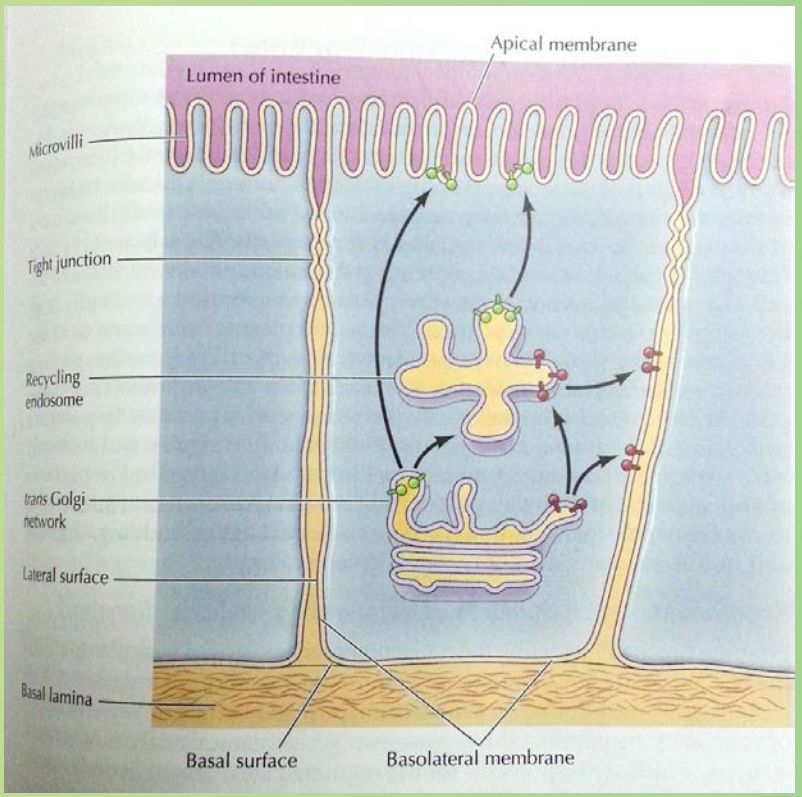
- Protein packaging mediated by cargo receptor
- processing in Immature secretory vesicles

- Regulated secretion after siganling from specialized vesicles

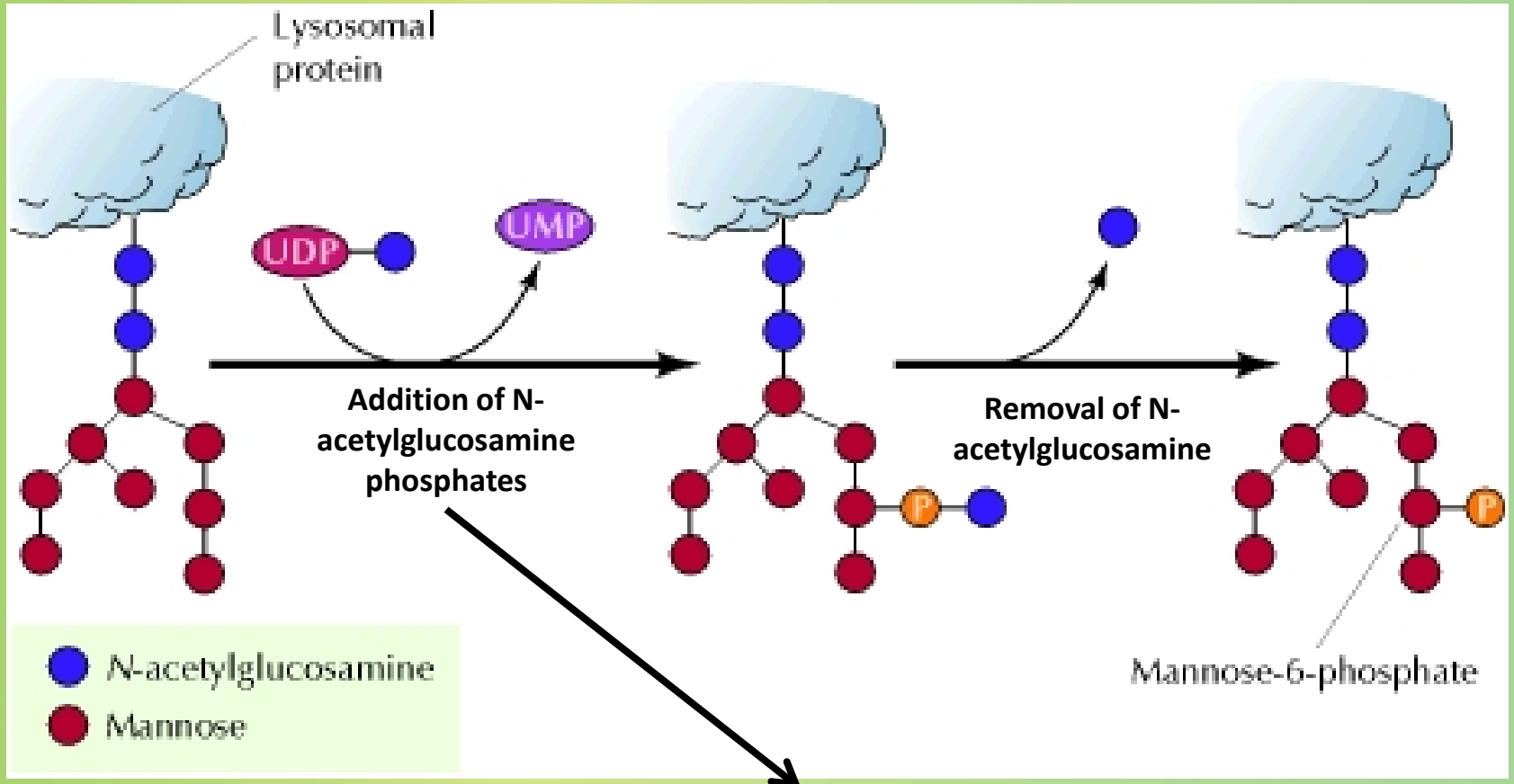
Continuous, unregulated secretion

Transport to the plasma membrane of polarized cells

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



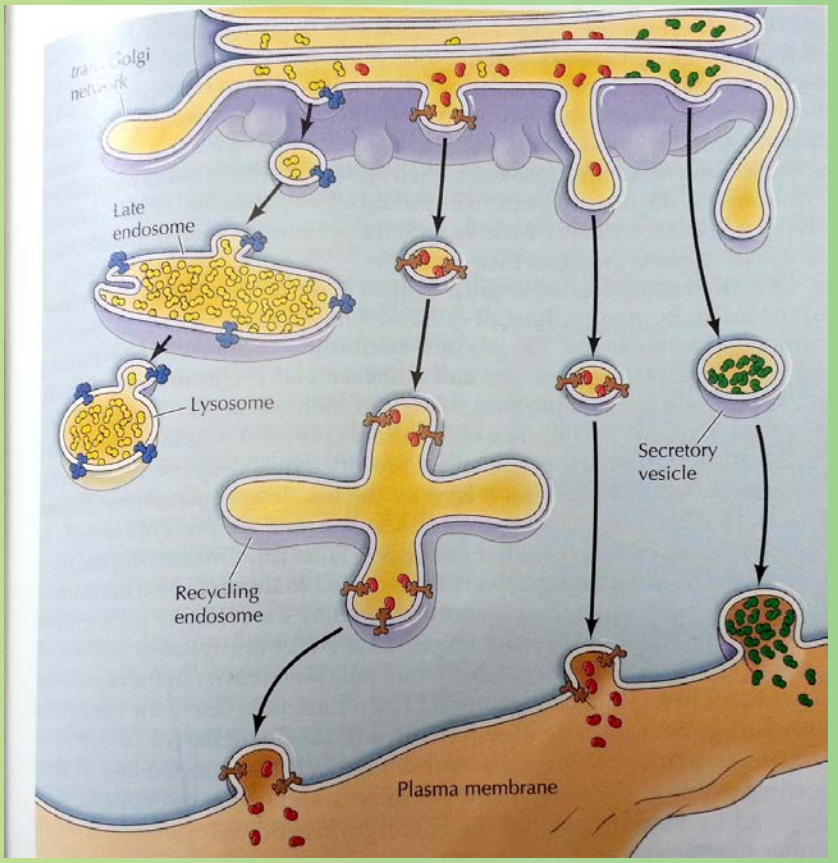
Processing of lumenal lysosomal proteins



The enzyme recognizes a signal patch (a three-dimensional structural determinant) not a sequence.

Transport of lysosomal proteins

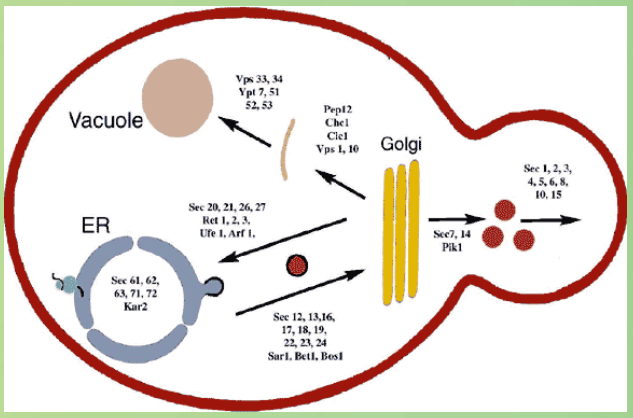
- Lumenal lysosomal proteins marked by mannose-6-phosphates bind to a mannose-6-phospahte receptor.
- The complexes are packaged into transport vesicles destined for late endosome, which mature into lysosomes.
- lysosomal membrane proteins are targeted by sequences in their cytoplasmic tails, rather than by mannose-6-phosphates.



The mechanism of vesicular transport

How have we understood the mechanism?

- Isolation of yeast mutants that are defective in protein transport and sorting (*sec* mutants)
  - The role of Sec61 as translocation channel in the ER
- Reconstitution of vesicular transport in cell-free systems
- Biochemical analysis of synaptic vesicles
- Tracing the path of GFP fusion proteins
- Proteomics analysis

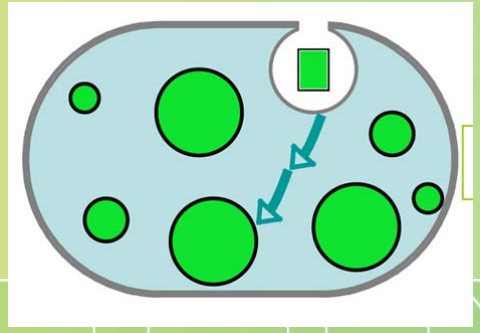


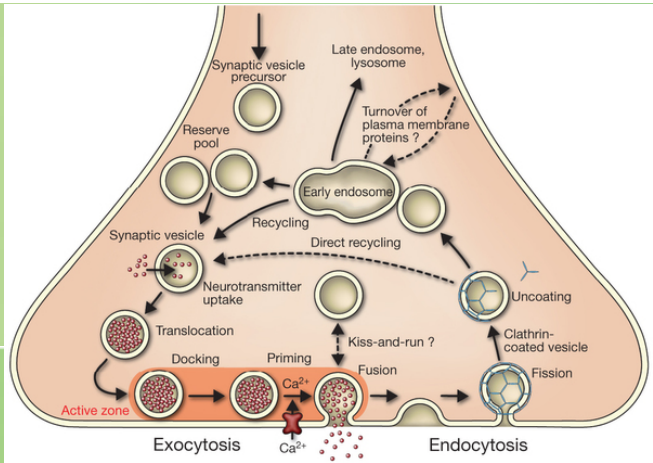
Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 3870–3874, July 1980
Biochemistry

Transport of vesicular stomatitis virus glycoprotein in a cell-free extract

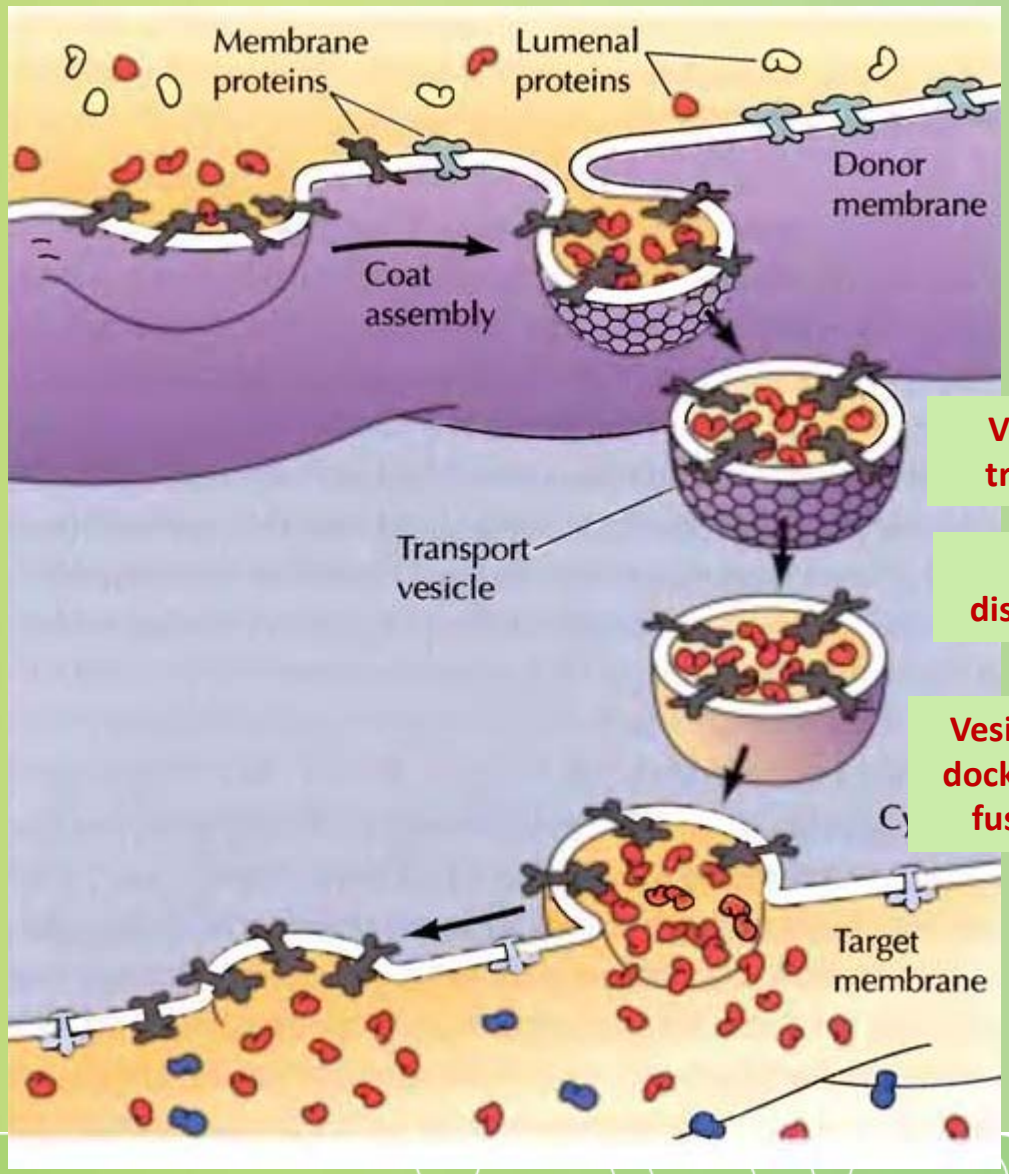
(endoplasmic reticulum/Golgi complex/oligosaccharide processing/membrane assembly)

ERIK FRIES AND JAMES E. ROTHMAN





Formation and fusion of a transport vesicle

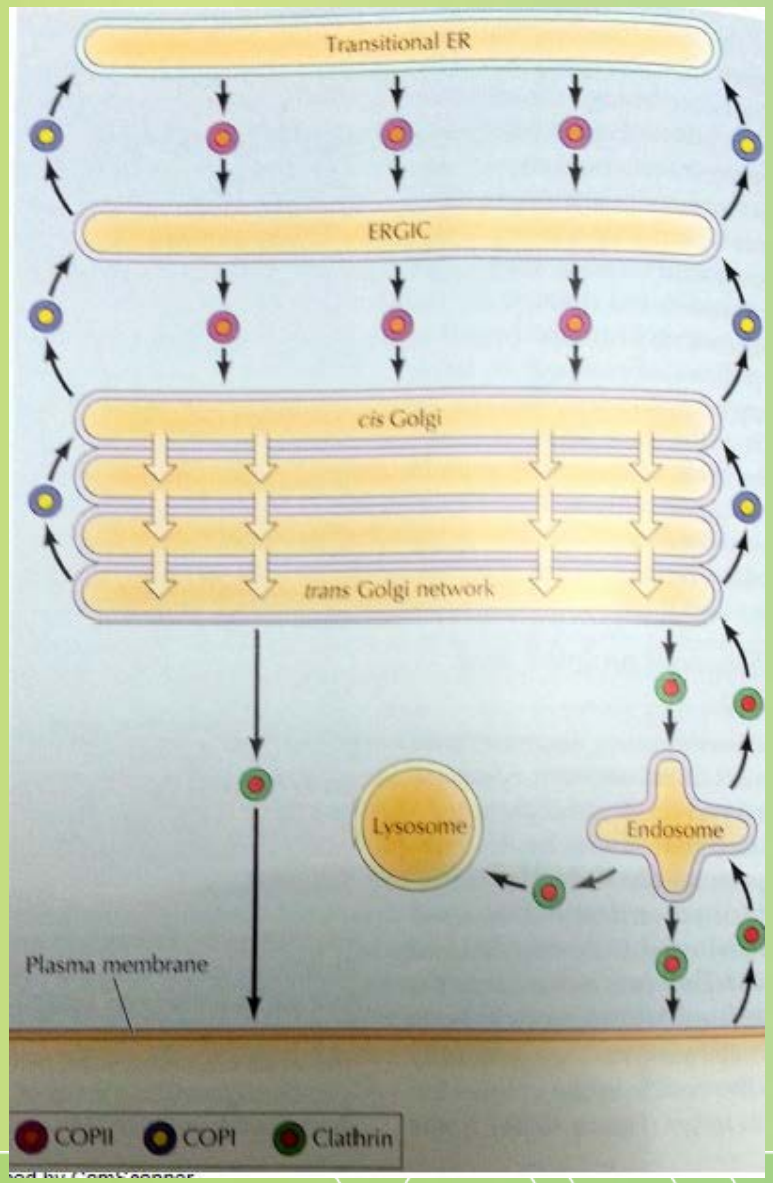


Vesicular transport

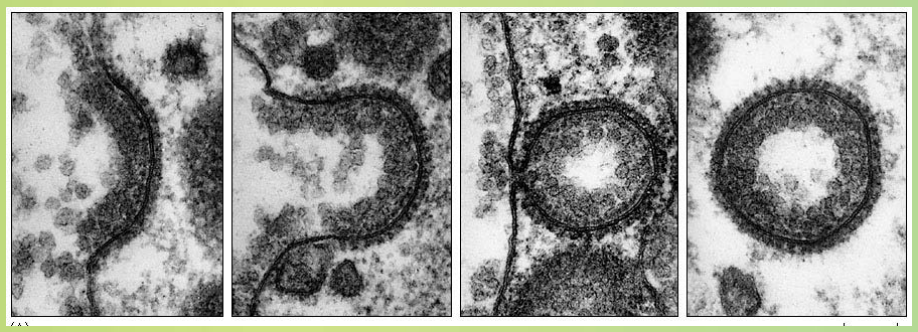
Coat disassembly

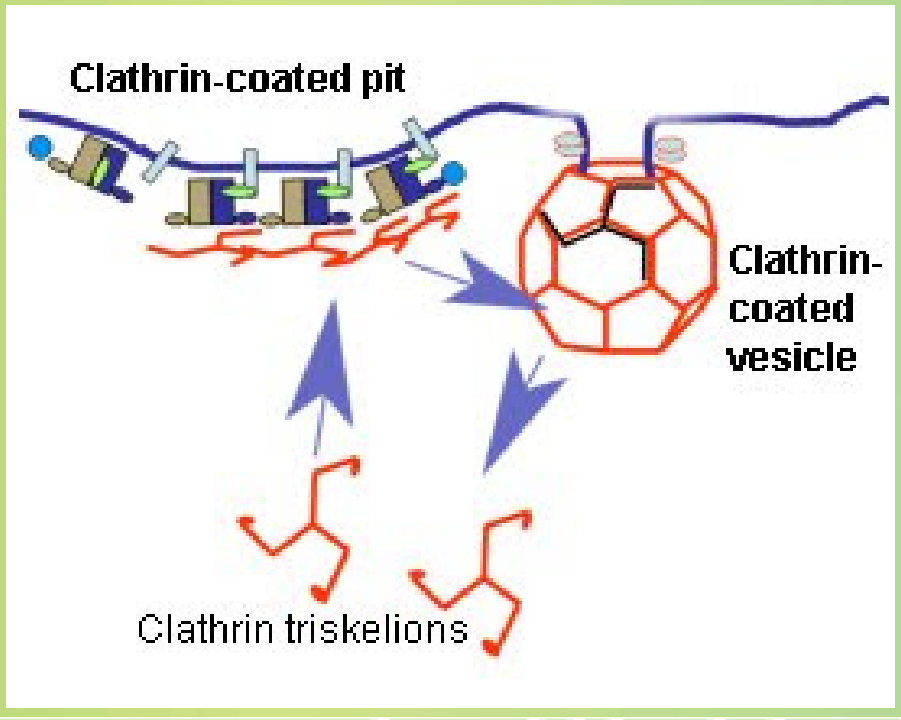
Vesicular docking & fusion

Coat proteins

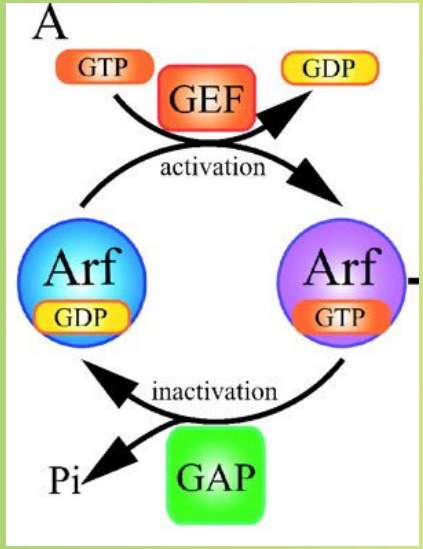


Formation of clathrin-coated vesicles

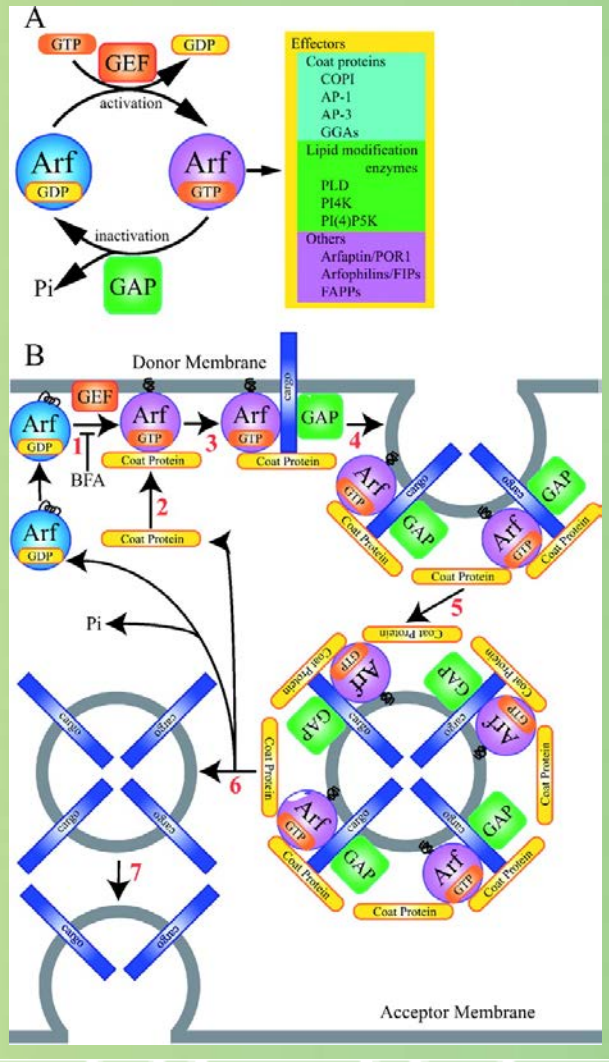




Role of ARF1

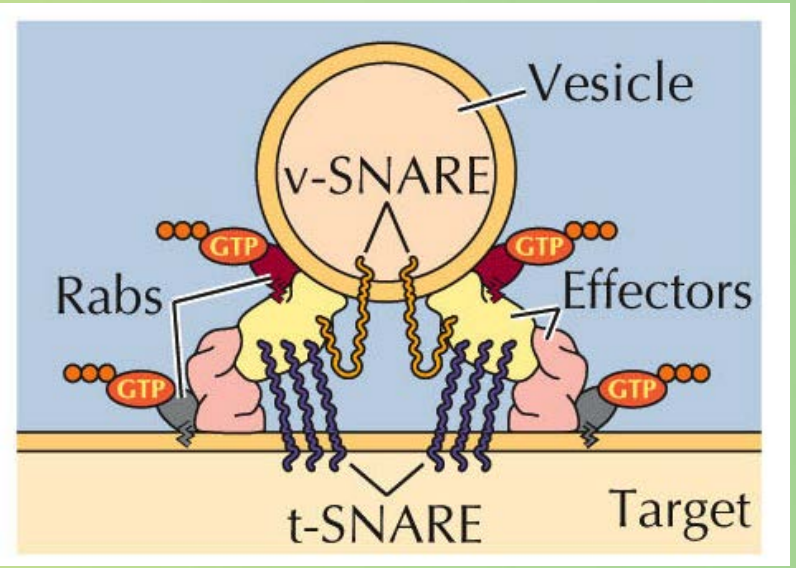


1. Activation of Arf1 by GEF
2. Recruitment of AP1 (not shown) and clathrin
3. Formation of Arf1-clathrin-receptor-cargo complex
4. Formation of vesicle
5. Budding and transport of vesicle
6. Inactivation and of Arf1 and disassembly of coat
7. Vesicle fusion

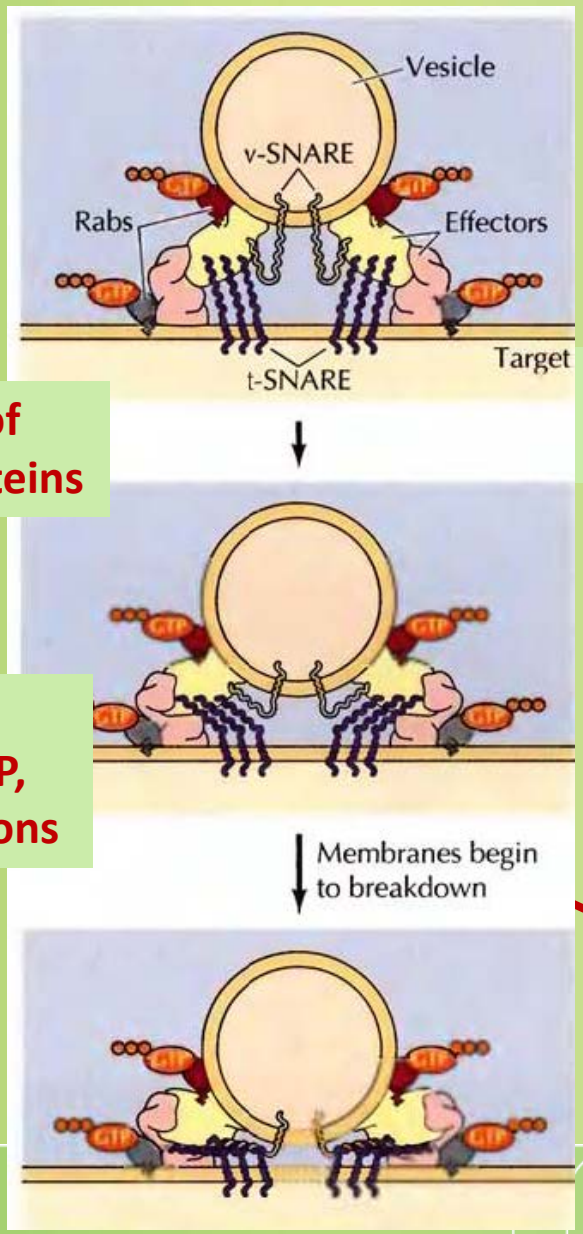


Players of vesicle fusion

- The formation v-SNAREs-t-SNAREs complexes on the 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



The mechanism of fusion

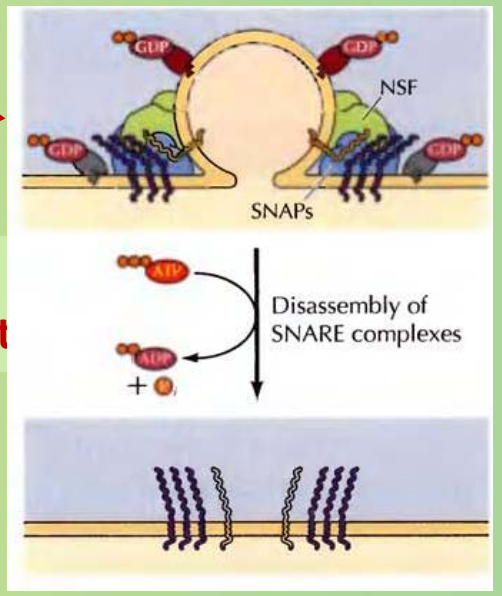


Interaction of effector proteins

Tethering, hydrolysis of GTP, SNARE interactions

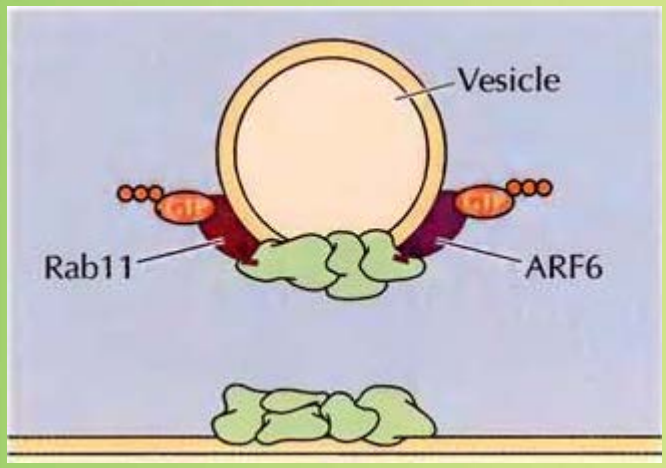
Closer vesicle-target

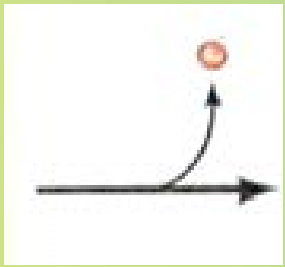
Fusion

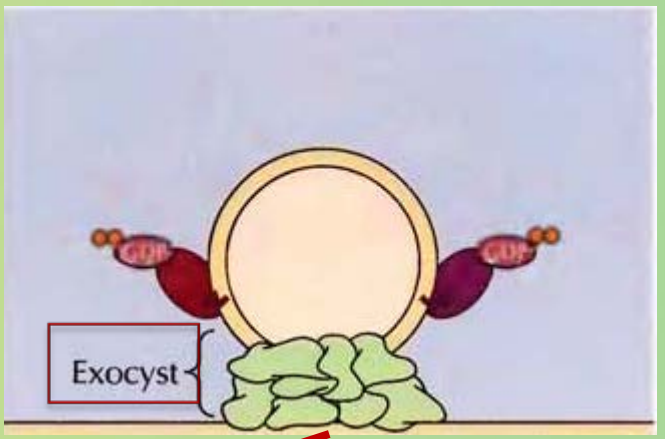


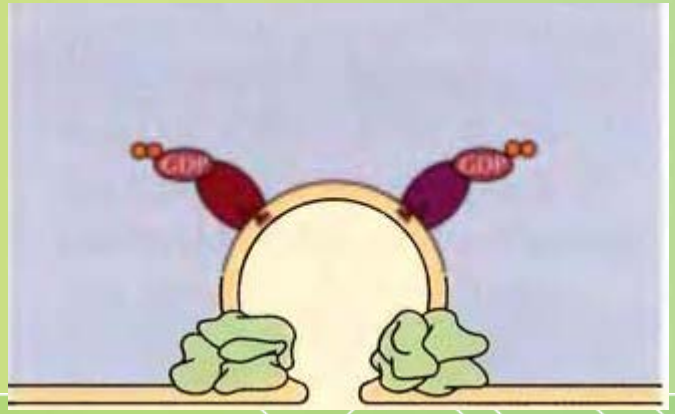
Disassembly of SNARE complex

Exocytosis

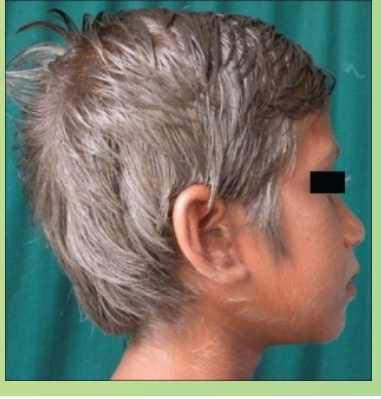




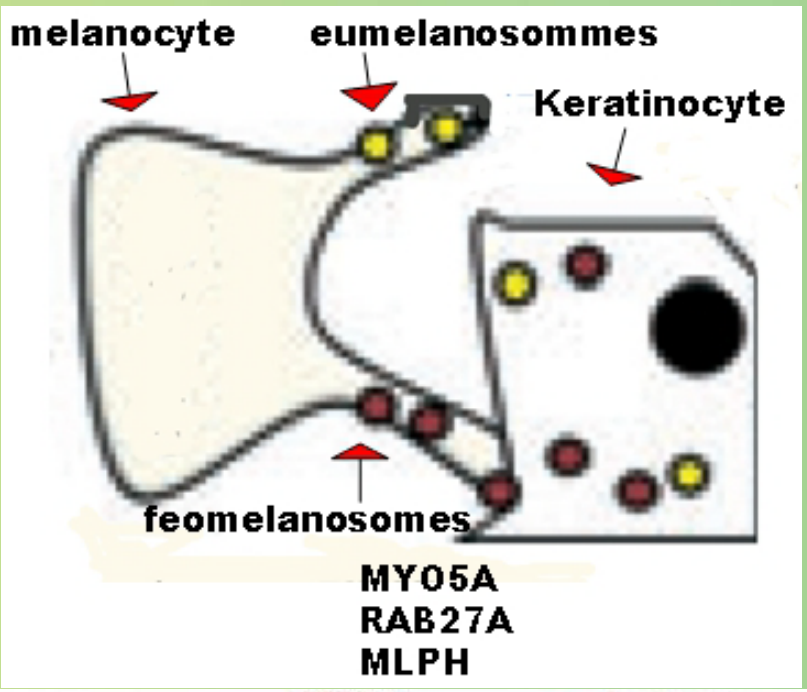


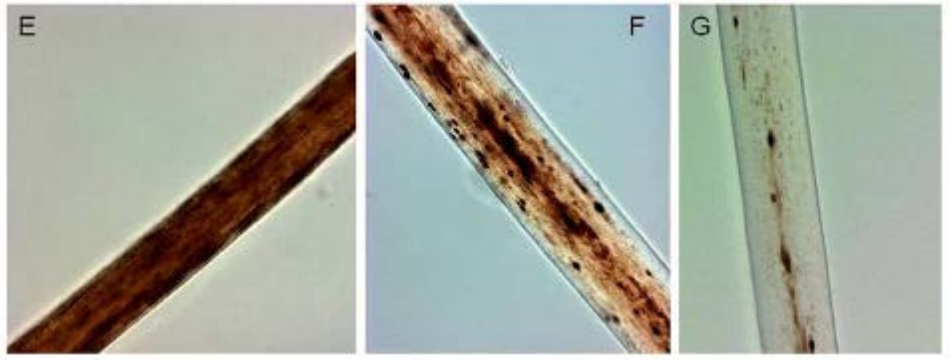


Griscelli syndrome (GS)



- A rare genetic condition
- Type GS: 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 accumulatte in the centre of melanocytes





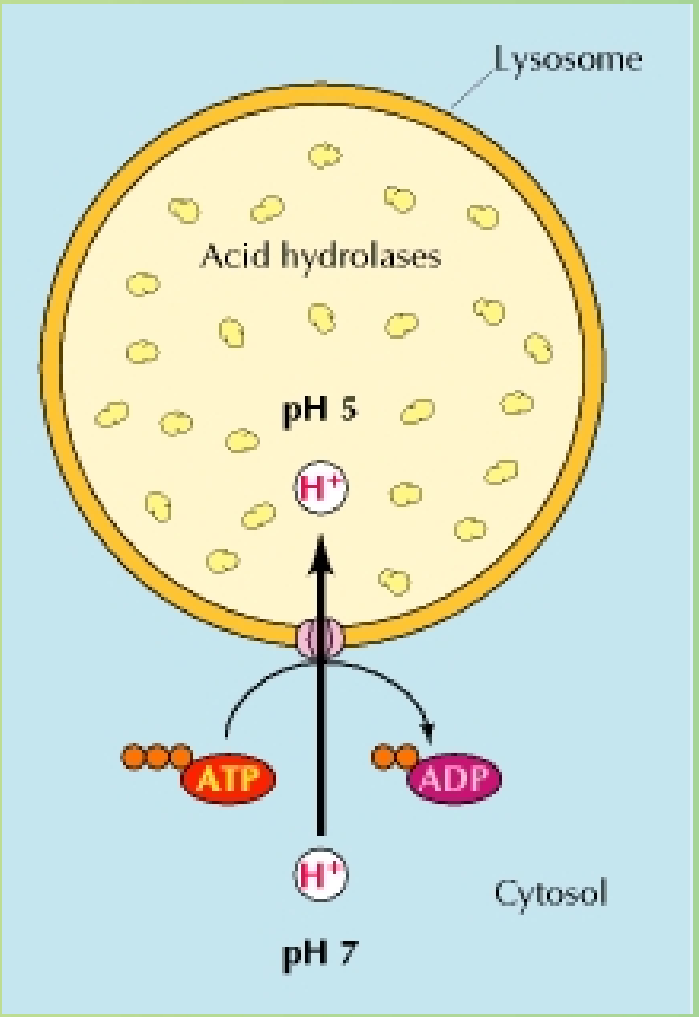
Lysosomes

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 ~50 different acid hydrolases.
- The enzymes are active at the acidic pH (about 5) that is maintained within lysosomes.
- Levels of Protection:
  - Containment
  - Inactive if released
- A proton pump maintains lysosomal pH.

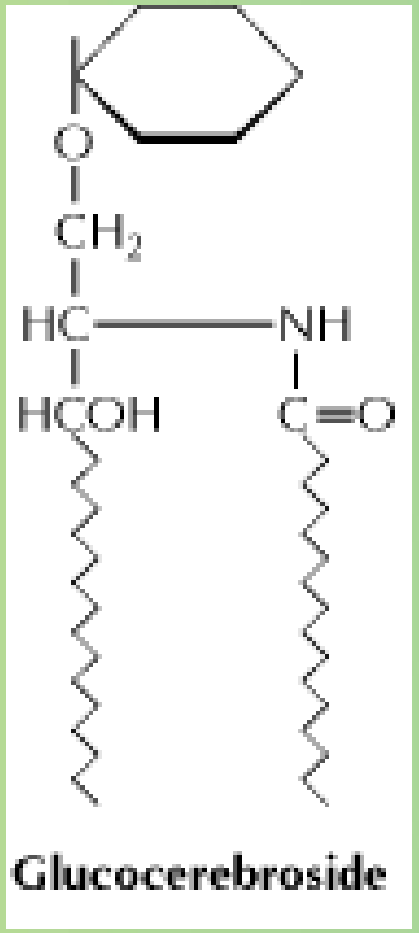


Lysosomal storage diseases

- 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.



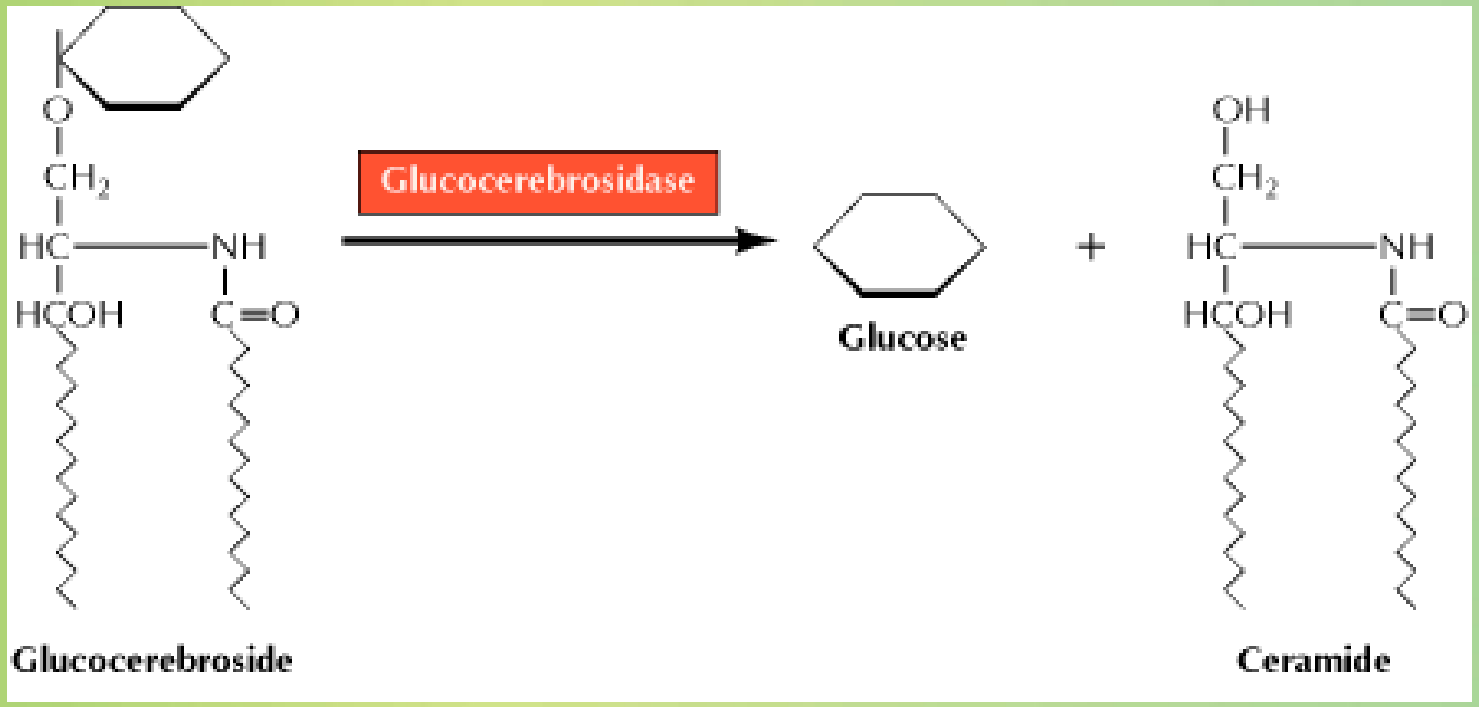
Glucocerebroside

Types

- **Three types according to severity and nervous system involvement**
  - **Type I: (least severe, most common) the nervous system is not involved; spleen and liver enlargement, development of bone lesions**
  - **Types II and III (more severe, much rarer): the only cells affected in Gaucher's disease are macrophages**
    - **Because macrophages function is to eliminate aged and damaged cells by phagocytosis by continually ingesting large amounts of lipids to be degraded in lysosomes**

Gaucher disease, type I (acid glucocerebrosidase deficiency)

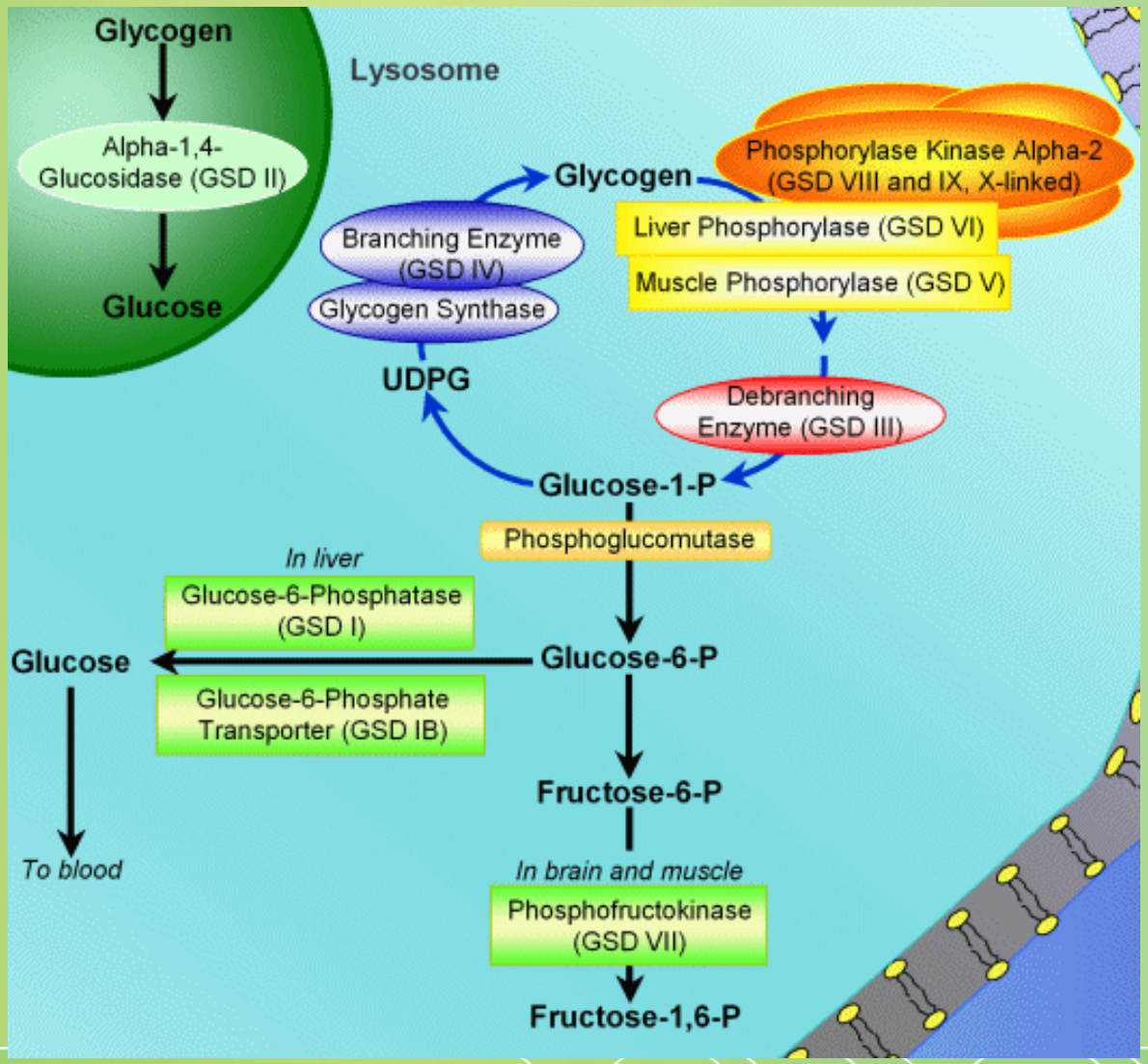
- Caused by mutation in the gene encoding acid-beta glucosidase, or glucocerebrosidase, which catalyzes the hydrolysis of glucocerebroside to glucose and ceramide.



Gaucher disease, type I (glucocerebrosidase deficiency-acid)

- Gaucher's disease is the most common of the lysosomal storage diseases, which are caused by a failure of lysosomes to degrade substances that they normally break down
- The resulting accumulation of nondegraded compounds leads to an increase in the size and number of lysosomes within the cell

Carbohydrate metabolism

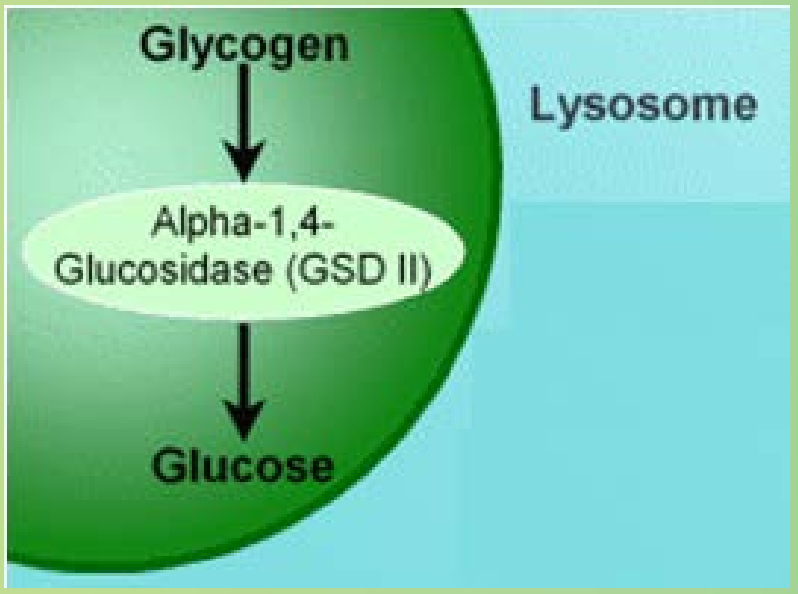


Oligosaccharidoses

No.	Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
0		glycogen synthase-2	Liver		hypoglycemia, early death, hyperketonia
I	Von Gierke disease	Glucose 6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure.	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.
II	**Pompe disease**	**α-1,4-Glucosidase (lysosomal)**	**All organs**	**Massive increase in amount; normal structure.**	**Cardiorespiratory failure causes death, usually before age 2.**
III	Cori disease	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches.	Like type I, but milder course.
IV	Andersen disease	Branching enzyme	Liver and spleen	Normal amount; very long outer branches.	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.
V	McArdle disease	Phosphorylase	Muscle	Moderately increased amount; normal structure.	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.
VI	Hers disease	Phosphorylase	Liver	Increased amount.	Like type I, but milder course.
VII	Tarui Disease	Phosphofructokinase	Muscle	Increased amount; normal structure.	Like type V.
VIII		Phosphorylase kinase	Liver	Increased amount; normal structure.	Mild liver enlargement. Mild hypoglycemia.
IX		phosphorylase kinase, β-subunit	liver, leukocytes, muscle		like VI
	Fanconi-Bickel, hepatorenal glycogenosis	glucose transporter-2 (GLUT-2)	liver	failure to thrive, hepatomegaly, rickets, proximal renal tubular dysfunction	

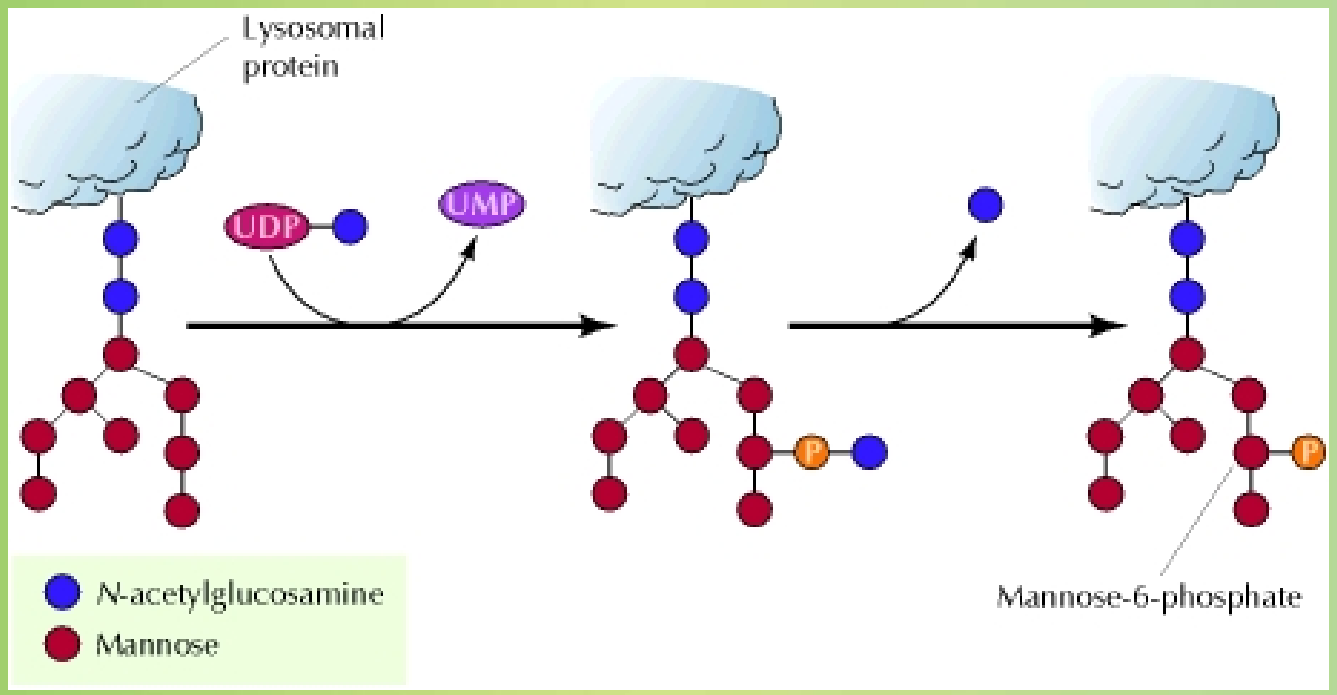
Pompe disease (type II)

- Lysosomes become engorged with glycogen because they lack α-1,4-glucosidase, a hydrolytic enzyme confined to these organelles
- Glycogen structure is normal, but its amount is excessive

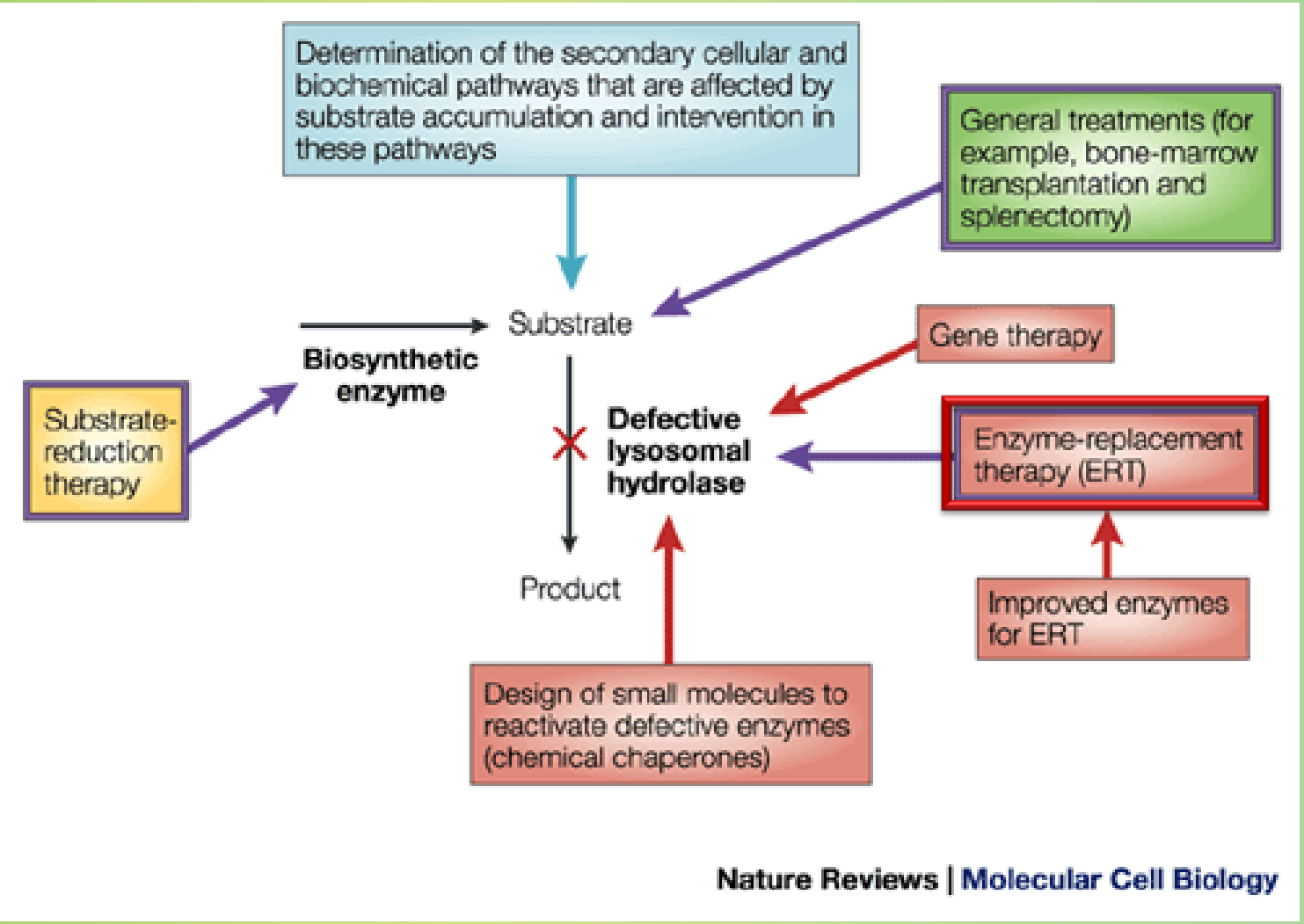


I-cell disease

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

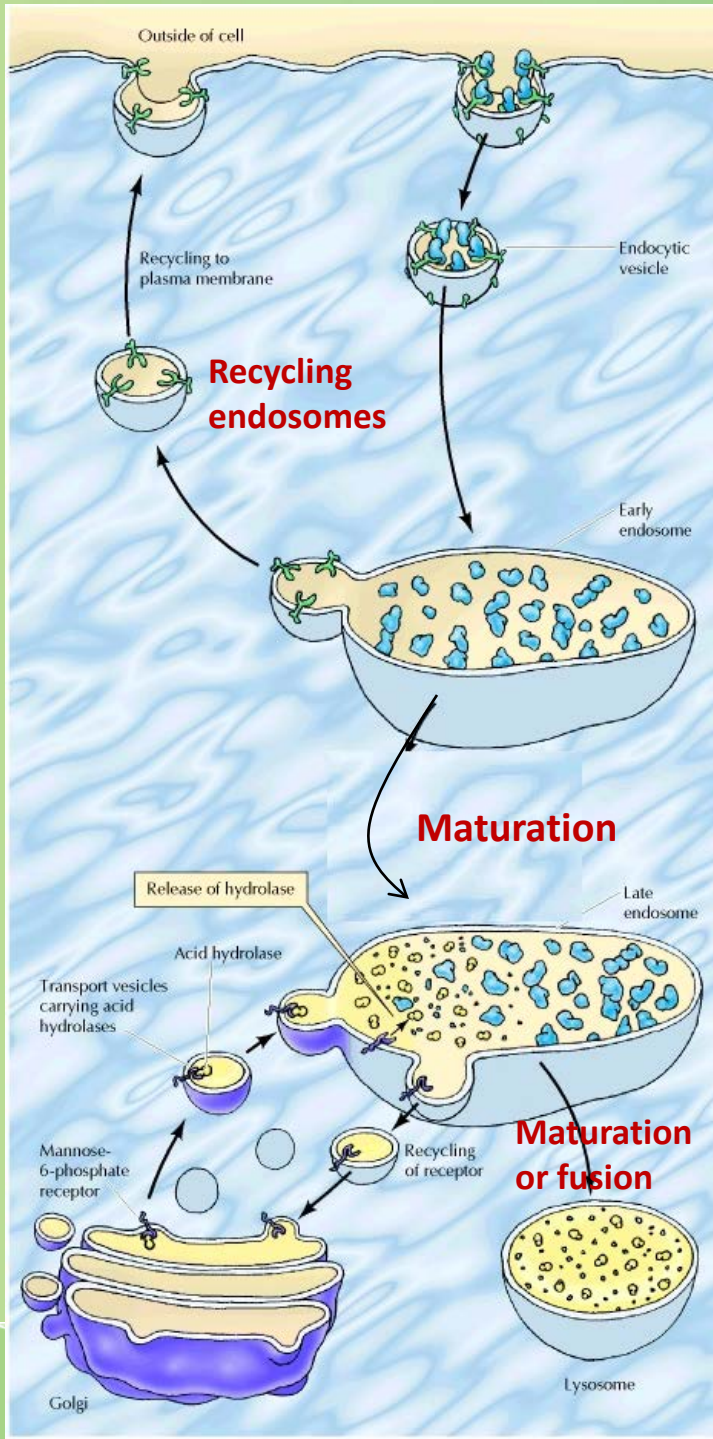


Treatment


Determination of the secondary cellular and biochemical pathways that are affected by substrate accumulation and intervention in these pathways
General treatments (for example, bone-marrow transplantation and splenectomy)
Substrate
Biosynthetic enzyme
Substrate-reduction therapy
Gene therapy
Defective lysosomal hydrolase
Enzyme-replacement therapy (ERT)
Product
Improved enzymes for ERT
Design of small molecules to reactivate defective enzymes (chemical chaperones)
Nature Reviews | Molecular Cell Biology

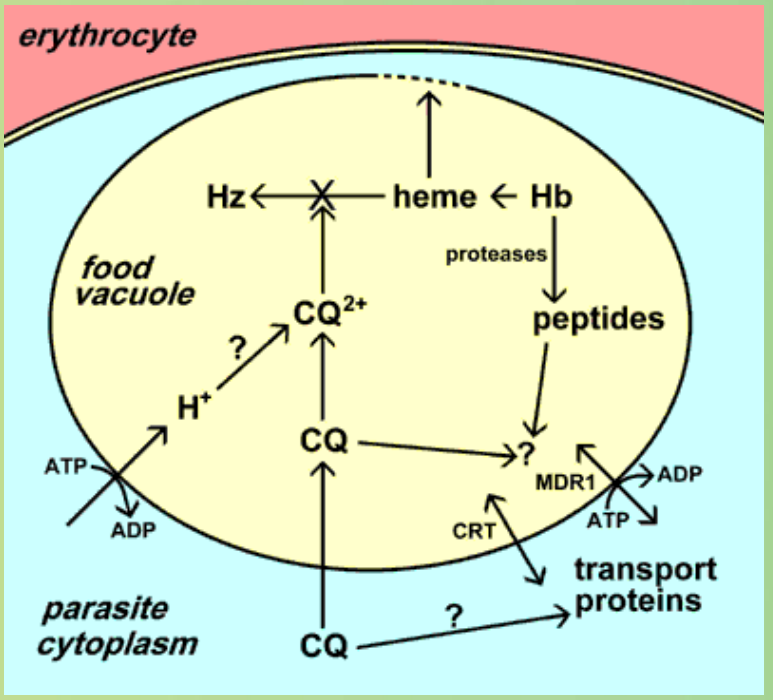
Endocytosis

- Molecules are taken up from outside the cell in endocytic vesicles, which fuse with early endosomes.
- Membrane receptors are recycled via recyling endosomes.
- Early endosomes mature into late endosomes.
- Transport vesicles carrying acid hydrolases from the Golgi fuse with late endosomes, which mature into lysosomes.
- The acid hydrolases dissociate from the mannose-6-phosphate receptor and the receptors are recycled to the Golgi.



Chloroquine

- Anti-malarial agent
- In the parasite's vaculoe, hemoglobin is digested and heme is modified by heme polymerase.
- If heme is not modified, it is toxic to the parasite.
- Chloroquine inhibits the enzyme.
- It is a weak base that becomes charged at acidic pH
- It crosses membranes into the malarial digestive vacuole.



Phagocytosis and autophagy