CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

10

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Vesicular transport

The mechanism of vesicular transport:

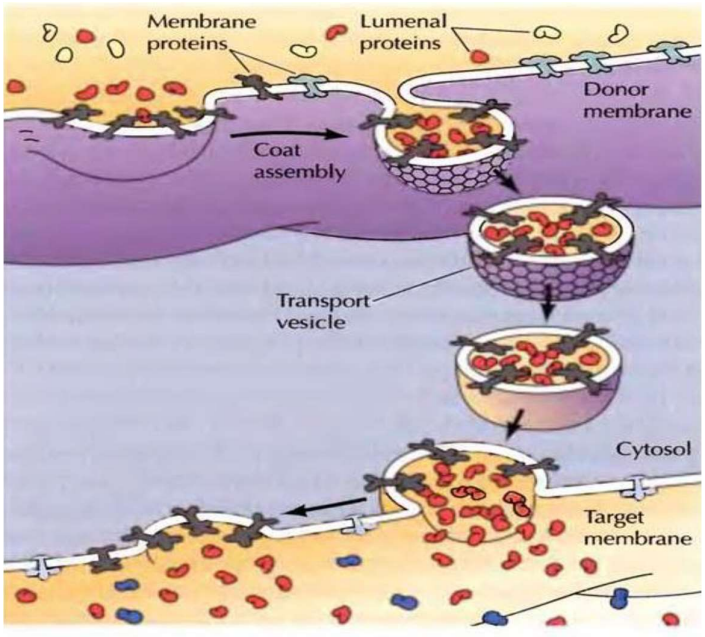
*Cargo proteins are collected into selected regions of a donor membrane.

*The formation of a cytosolic coat results in the budding of a transport vesicle.

(Vesicle budding stimulates coat assembly)

*The vesicle is transported by motor proteins along cytoskeletal filaments to its target.

*The transport vesicle then docks at its target membrane, the coat is removed, and the vesicle fuses with its target.



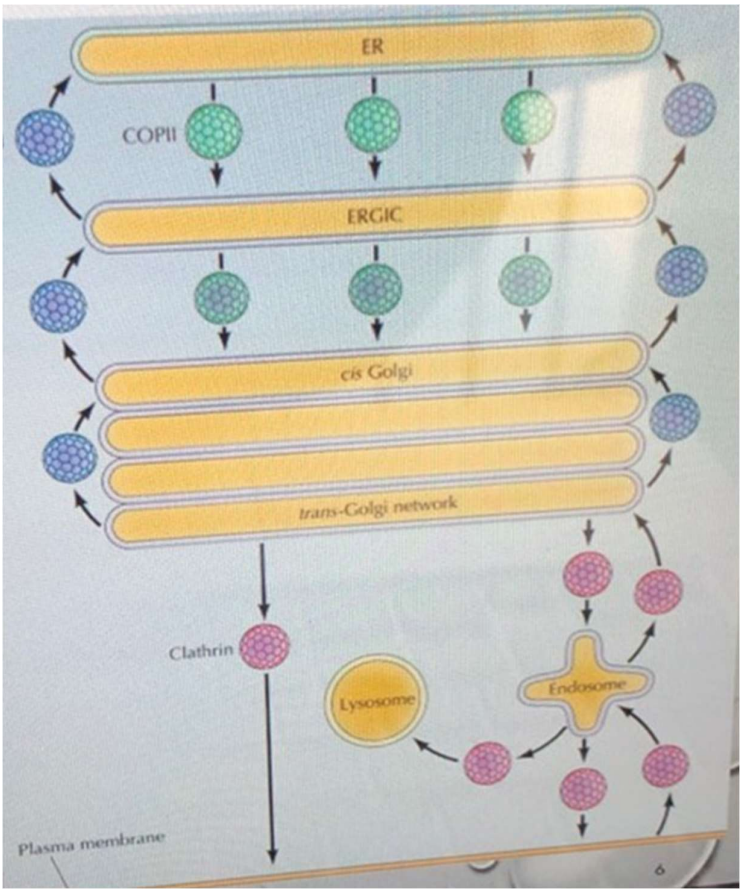
➢ Note that the "coat assembly" takes place right after budding, and the "coat disassembly" takes place before reaching target membrane.

Types of coat proteins:

*Coat proteins are classified according to the direction of vesicular movement, budding location, and final destination.

Coat protein	Pathway
COPI	From: Golgi or ERGIC (ER-Glogi-intermediate-compartment). To: Rough endoplasmic reticulum.
COPII	From: Rough endoplasmic reticulum. To: Cis-Golgi
Clathrin	Between Trans-Golgi, endosomes, lysosomes, and the plasma membrane.

*Note that this variations in coat proteins assists in the direction of vesicles to their final destination.



➢ Notice that proteins moving from Cis-Golgi to Trans-Golgi do not need vesicles to move, while moving from Trans-Golgi to Cis-Golgi needs vesicles coated with COPI.

➢ COPII-Coated-Vesicles move from ER > ERGIC > Cis-Golgi.

➢ COPI-Coated-Vesicles move from Trans-Golgi > Cis-Golgi > ERGIC > ER.

➢ Clathrin-Coated-Vesicles move IN BOTH DIRECTIONS between the Trans-Golgi, endosomes, lysosomes, and the plasma membrane.

Formation of clathrin-coated vesicles

*The formation of coated vesicles is regulated by many proteins, the most important one is a small GTP-binding protein (exchanges between active (GTP-bound) and inactive (GDP-bound) states{not hydrolysis for p-group }) called **Arf protein**.

*Clathrin proteins gather on the vesicle surface forming a “triskelion” structure, this structure maintains the vesicle until it reaches its target where clathrin coat is removed.

*GEF protein: Guanine nucleotide exchange factor, it activates Arf protein by converting GDP to GTP.

*GGA and AP1 are adaptor proteins.

*Receptors are needed to hold soluble proteins in certain regions in the membrane.

*The donor membrane starts to curve inward to induce its cut. (not shown).

1. Activation of ARF1 by GEF.

2. Recruitment of adaptor protein AP1 and then clathrin.

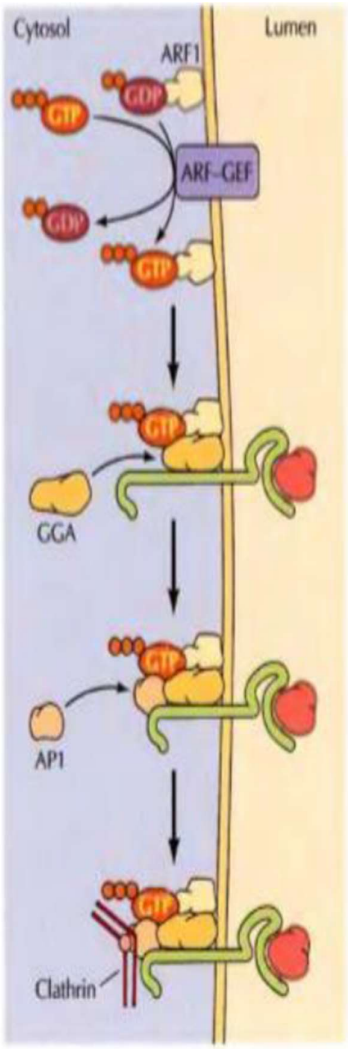
3. Formation of ARF1-clathrinreceptor-cargo complex.

4. Formation of vesicle.

5. Budding and transport of vesicle.

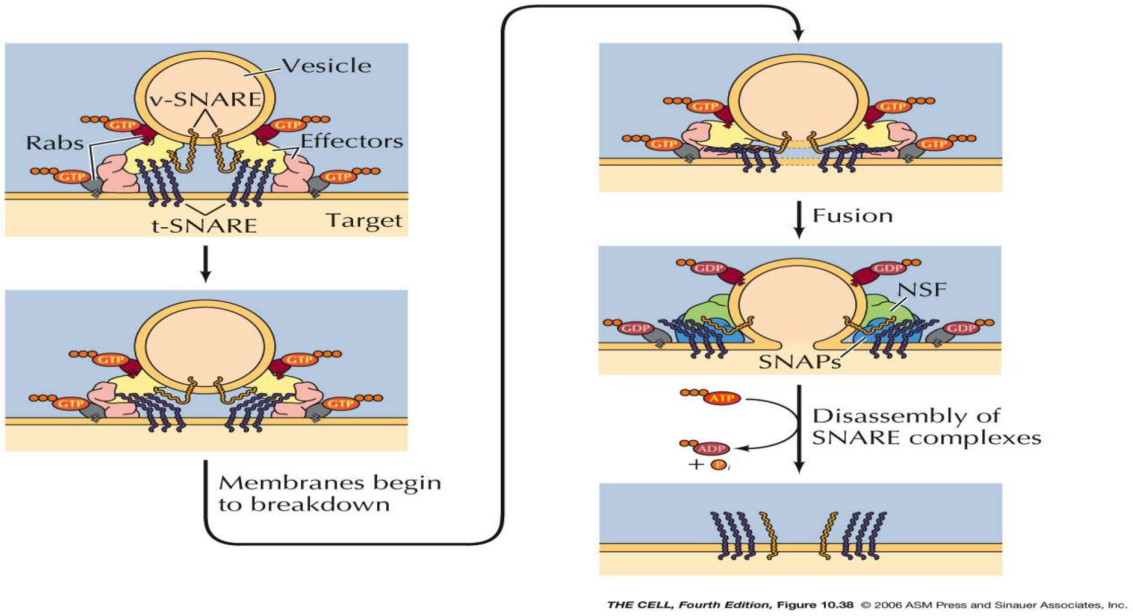
6. Inactivation of ARF1 by GTP hydrolysis and disassembly of coat.

7. Vesicle fusion.



★ *Vesicular Fusion :*

- In the membrane of the vesicle there is a protein called **[v-SNARE],** in the opposite side of the target membrane there is also protein called **[t-SNARE]**.



THE CELL, Fourth Edition, **Figure 10.38** © 2006 ASM Press and Sinauer Associates, Inc.

➔ The binding between v-SNARE and t-SNARE is going to activate the assembly and diffusion protein complex, including RAB'S (GTP binding protein, same idea as ARF).

➔ RAB'S have to be in a GTP binding state, their binding is going to activate the other protein effectors (yellow and pink ones).

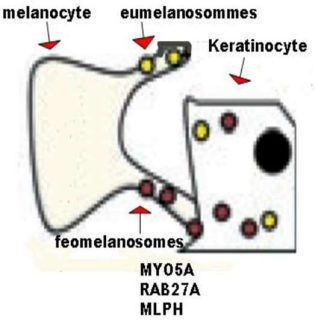
➔ This is going to induce informational shape of SNARS, SO now they pulling each other -the membrane of vesicle is closer to the target membrane- but <u>still is Not close enough to be blended</u>

➔ More pulling between P or RABs, we also need ATP (as a source of energy) to disassembly of SNAR complexes. the target membrane. SNAR is going to make the membranes more close to each other, it reaches the point with instability and induces fusion of the vesicular membrane with with GD

➔ The fusion is done, SO we need now to disassemble the complexes by exchanging GTP

➔ SNAR complexes after that will be part of the target membrane

Clinical Application: Griscellisyndrome (GS)

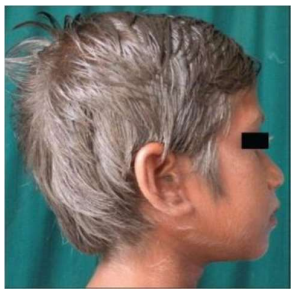


- Melocytes are melanin synthesizing cells, that give the color of skin, eyes, hair, …etc.

Melanin synthesized in melanocyte within malanosome

Melanosome like vesicular structure, not an organelle, it's found in a special of cells

-The melanosomes are going to move from melanocyte to ketatinocytes(skin cells), for example to give the colour for the skin cell, these vesicles in its movement is going to need the vesicular protein for vesicular fusion. these are mutated they NOT eligible to move.



• Lysosome

- *It's rounded shape organelle and It's act as a digesting system .*

Structure:

- Lysosomes can digest any polymerase molecules, such as protein, and types of lipids.
- Lysosomes are membrane-enclosed organelles that contain various enzymes that break down all types of biological polymers.
- Lysosomes degrade material taken up from outside and inside the cell.
- Variable in size and shape.

Lysosomal enzymes:

- PH in cytosole around 6.3, almost 7, on the other hand the PH in lysosomes around 5.
- Approximatly, we have 102 more protons inside the lysosome than cytosole

From this equation

$$PH = -10g[H^+]$$

➔ What makes this different in protons concentration happened?

= proton pump.

➔ We need pumps and ATP to pump the protons inside the lysosomes *against concentration gradient*.

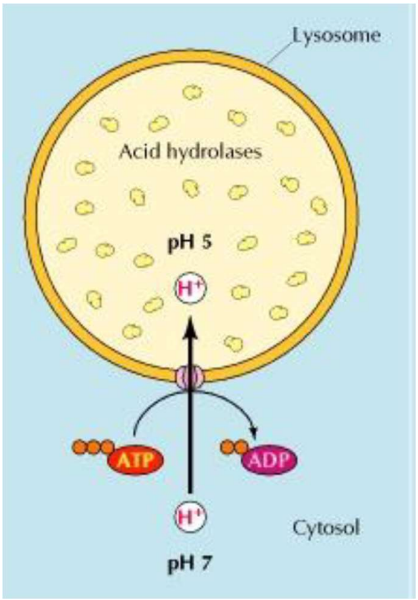
- Why we need this eternal environment inside the lysosomes?
  1- Acidic environment denature the protein (we need to open the protein, so we can allow to the enzymes to do its work)
  2- Some enzymes only work in this acidic environment .why?

(because its release inactive form)

- How do these lysosomes form?
  ⇨ It turns of exporting the lysosomal proteins,from ER to golgi and from golgi network to lysosomes.

➔ Lysosomal protein if they are lumenal proteins (soluble) -> they are labeled, and this lable is on the glycosylation ~we add: { 2 N-Acetylglucosamine, 9 mannose, and 3 glucose in ER => Then we remove 3 glucose and one mannose, in golgi. There is a hole series in changes by adding and removing some types of sugers }~

- Lysosomes contain ~50 different acid hydrolases.
- Enzymes hydrolyze proteins, DNA, RNA, polysaccharides and lipids.
- 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.



- **Processing of lumenallysosomalproteins:**

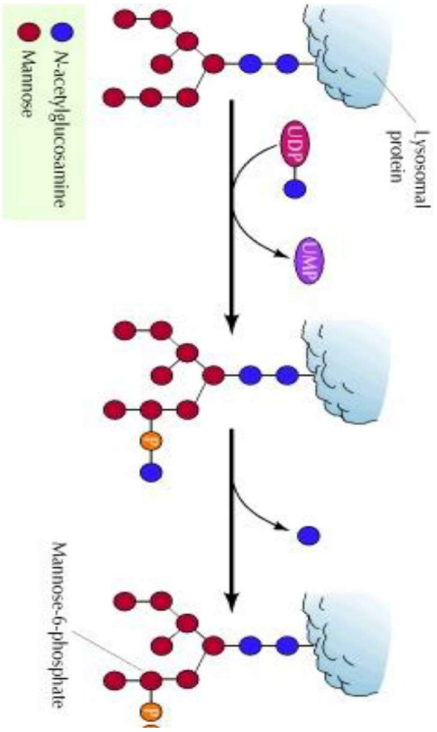
For lysosomal lumenal proteins, will going to get bake to this form when we have

❖ 2 N-acetylglucosamine, and 8 mannoses and we are going to lable them by adding a phosphate group on mannose no.6,

this phosphate group attaches to the **N-acetylglucosamine** (**N-acetylglucosaminephosphates**).

➔ When we have **N-acetylglucosamine**, there is another one from it carried on the nucleotide UDP, we add from it a phosephate group and **N-acetylglucosamine**, to the mannose no.6 -> then we removed **N-acetylglucosamine**-> then the mannose-6-phosphate is attached on lysosomal proteins.

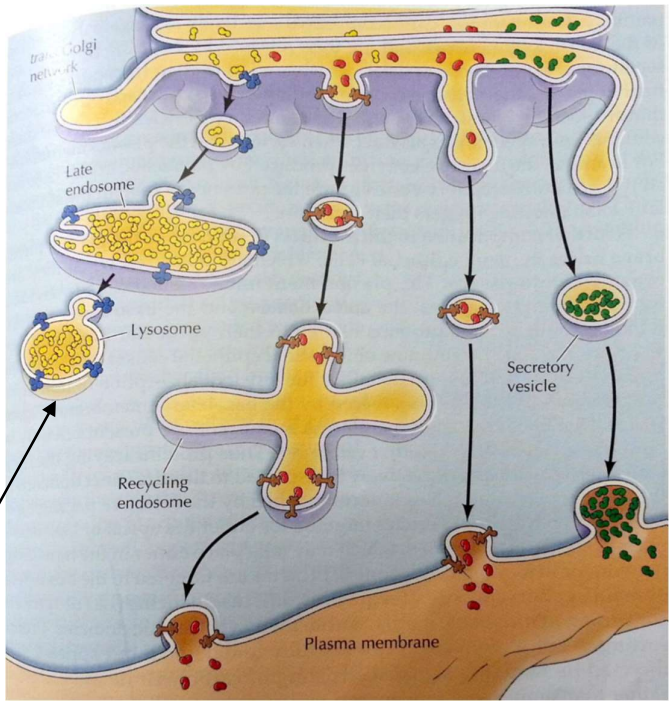
➔ This enzyme recognise acertain region/certain structural region, on the surface of the lysosomal lumenal protein, it's NOT recognize just a certain one protein.



➢ Lumenallysosomalproteins marked by mannose-6-phosphatesbind to a mannose-6-phospahte receptor.

➢ The complexes are packaged into transport vesicles destined for late endosomes, which mature into lysosomes.

➢ Lysosomal membraneproteins are targeted by sequences in their cytoplasmic tails, rather than by mannose-6-phosphates.



The blue protein is a receptor for mannose-6-phosphate

• Lysosomalstorage diseases

- Glycolipidoses(sphingolipidoses)
- Oligosaccharidoses
- Mucopolysaccharidoses: deficiencies in lysosomal hydrolases of GAGs (heparan, keratan and dermatan sulfates, chondroitin sulfates).

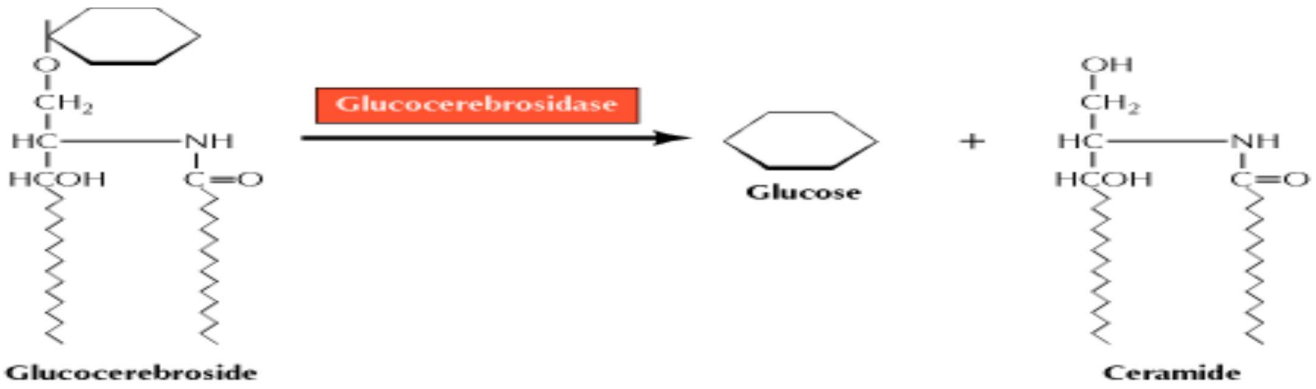
▪ They are chronic progressively debilitating disorders that lead to severe psychomotor retardation and premature death.

➔ These diseases are the resulting of the problem in one or more lysosomal enzymes
➔ These diseases frequently a genetic diseases .
➔ Resulting the accumulation of the substrate on that enzymes.

"Suppose that the enzyme that digest proteins is missing,-> protein starts to accumulate at the lysosome,-> the lysosome be bigger, and it appear under the microscope like bubbles".

1. Glucocerebroside:

✓ In this disease the enzyme "Glucocerebrosidase" is missing, SO the reaction stops, -> so the " Glucocerebroside is going to accumulate inside the lysosomes, that's why we call it lysosomal storage disease.
✓ And this disease called "Glucocerebrosidase deficiency"



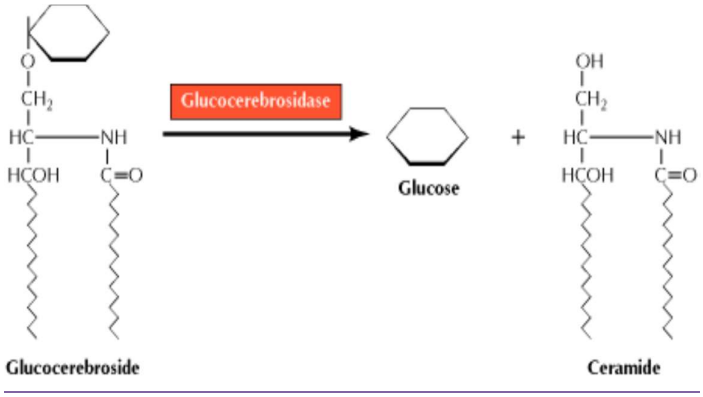
➔ Glucocerebrosideis a glycolipids (a monosaccharide attached directly to a ceramide unit

➔ It is a byproduct of the normal recycling of red blood cells, which are phagocytosed by macrophages, degraded and their contents recycled to make new cells.

2.Gaucherdisease(glucocerebrosidasedeficiency)

- The most common lysosomal storage disease.
- Caused by mutation in the gene encoding acid-beta glucosidase or glucocerebrosidase.

- Failure of lysosomes to degrade substances that they normally break down.
- The accumulation of non-degraded compounds leads to an increase in the size and number of lysosomes within the cell.

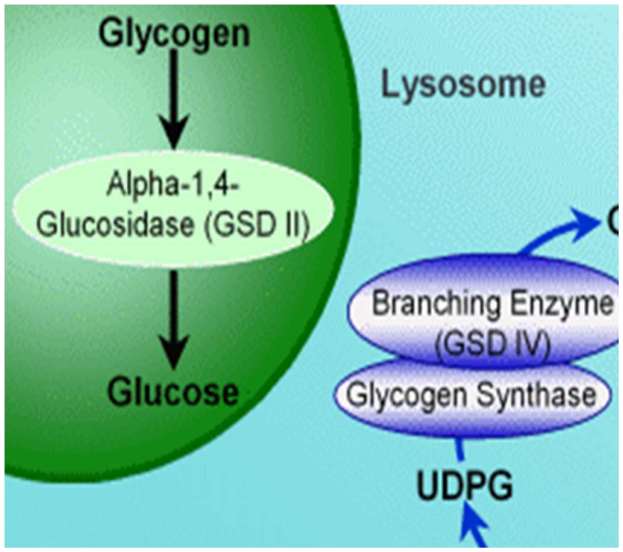


3.Oligosaccharidoses-Pompedisease (type II)

•This disease related to suger

•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



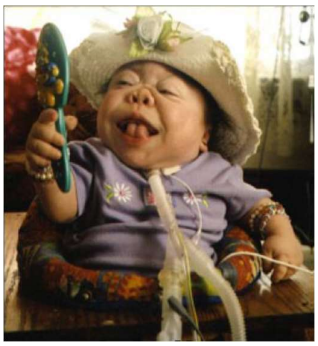
القناعه لا تعارض الطموح القناعه هي حدود الممكن للطموح

4. *I-cell disease:*

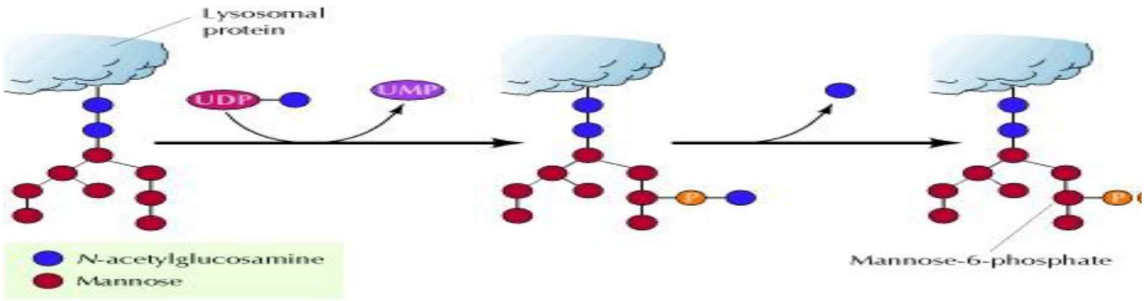
"I-cell disease" is relating to the deficiency of enzyme that does the mannose-6-phosphate tagging -> so that all lysosomal lumenal protein will NOT be targeting to the lysosomes.

*** explanations:-

⇨ The enzyme that add the phosphate group to mannose no.6 is missing.



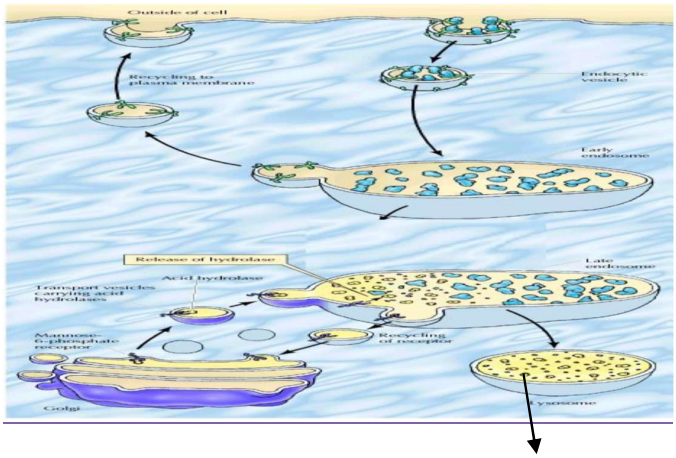
- Lack of targeting of lysosomalenzymes from Golgi
- A deficiency in tagging enzyme
- Features: severe psychomotor retardation that rapidly progresses leading to death between 5 and 8 years of age.



❖ Endocytosis

- What is the difference between endocytosis and processing vesicles?
  - ⇨ I n endocytosis the molecules are taken up from outside to inside the cell in endocytic cells
  - ⇨ On the slides, the legand binds to the green receptors, on the surface, and the vesicle which was formed after the fusion with the cell membrane -> it is going to fuse with the early endosomes (larg , it is the fusion of several vesicles).

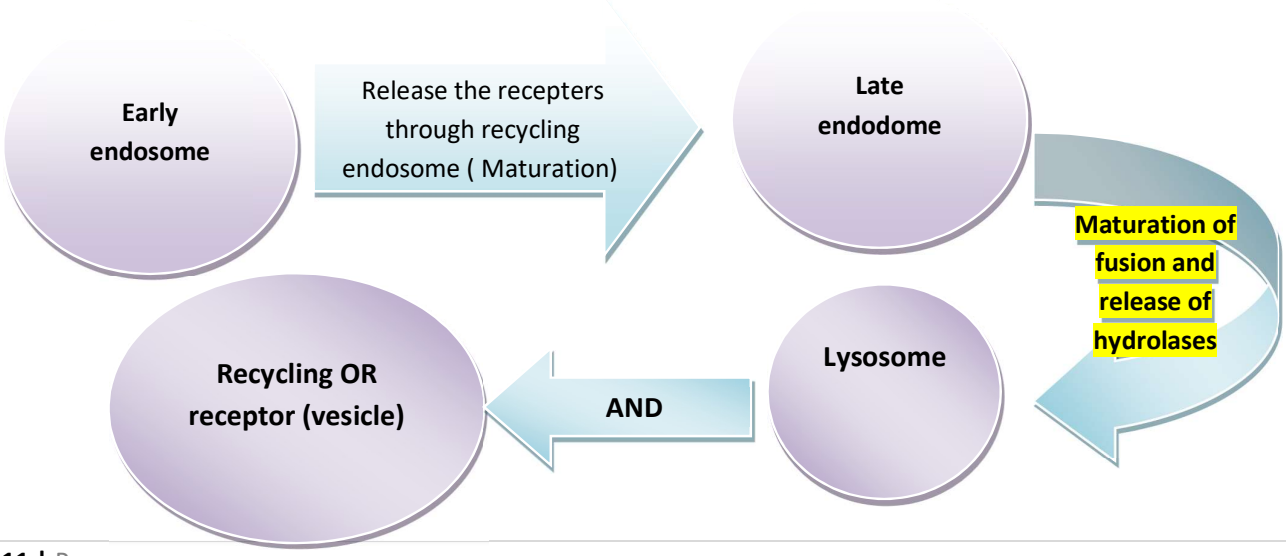
*Notice that inside the endosome the legand is released from the receptor.



- Molecules are taken up from outside the cell in endocytic vesicles, which fuse with early endosomes.
- Early endosomes separate molecules targeted for recycling from those targeted for degradation.
- Membrane receptors are recycled via recycling endosomes.
- Early endosomesmature into late endosomes.
- Transport vesicles carrying acid hydrolasesfrom the Golgi fuse with late endosomes, which mature into lysosomes.
- The acid hydrolasesdissociate from the mannose-6-phosphate receptor and the receptors are recycled to the golgi.

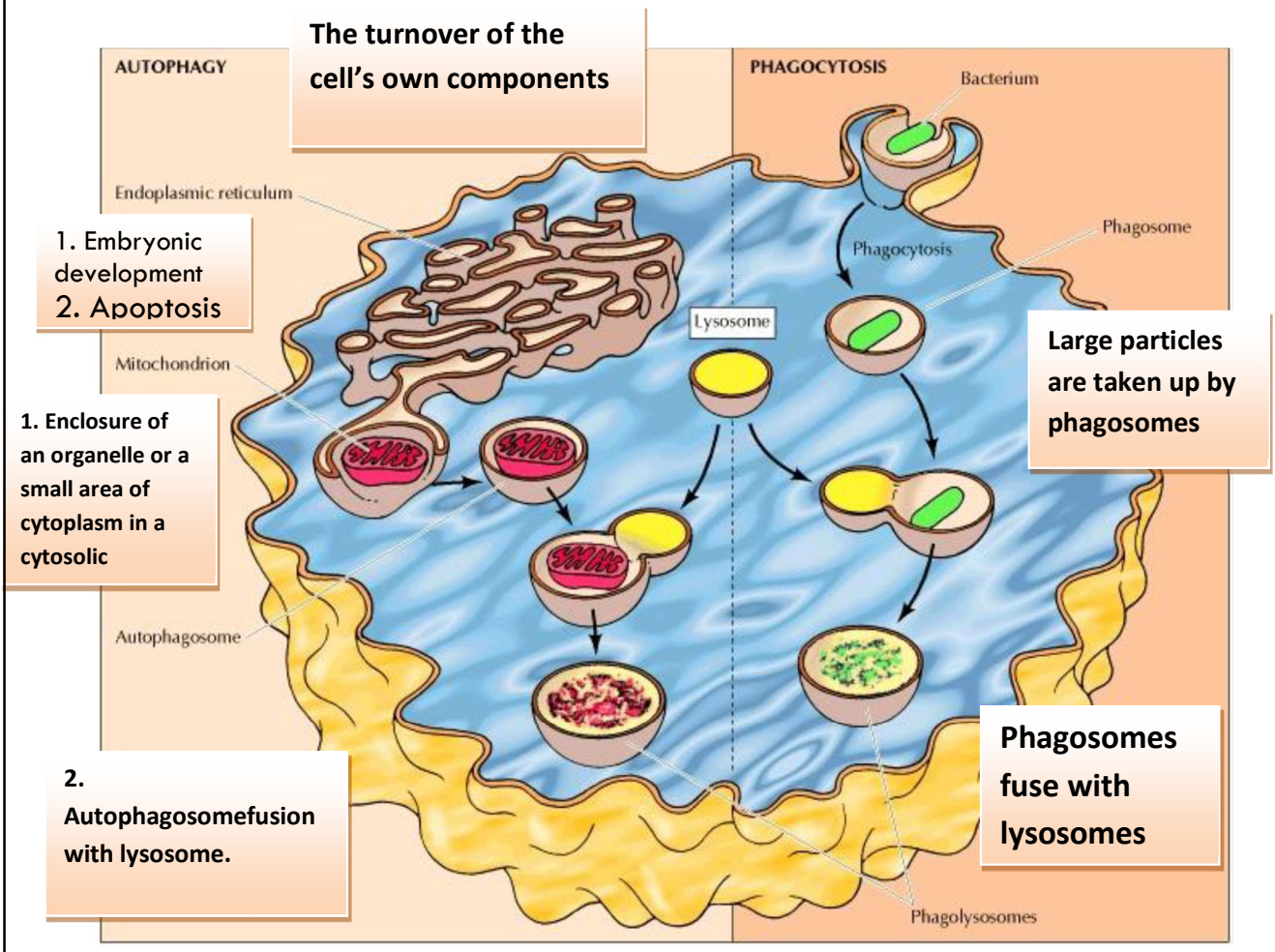
- Now, we need to recycle the receptors and bake it to the membrane. Once they release from the early endosome as a vesicle, then we call it "**Recycling endosome"**

** Once the recycling endosome appears the "Early endosome" became "late endosome".

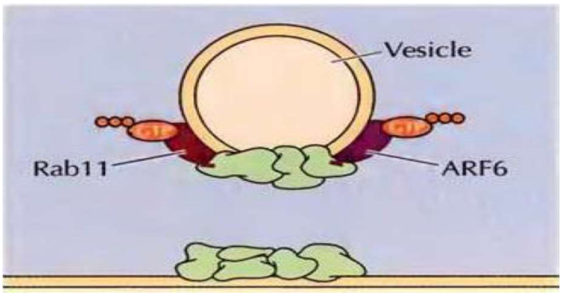


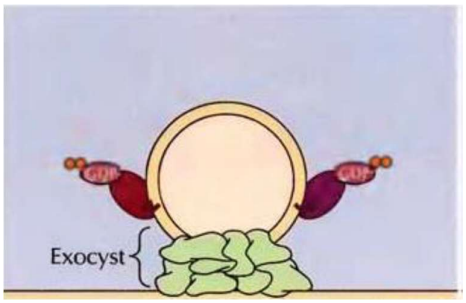
- **Transport vesicles**
  - ➔ Carrying acid hydrolasis from golgi to fuse it with the late lysosome to make maturation after that, then the lysosome is ready .

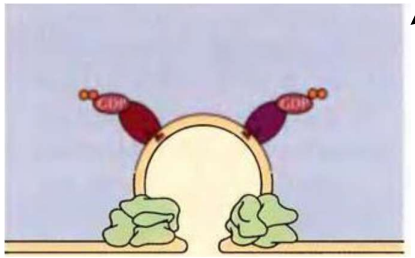
Phagocytosis and autophagy



• Exocytosis







Exocystsare specific protein complexes (8 proteins) at which exocytosis occurs

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

❤ *Never give up on a dream just Because of the time it will take to accomplish it .the time will pass anyway.* ❤