بسم الله الرحمن الرحيم

CYTOLOGY

MID – Lecture 4

Vesicular Transport & Lysosomes

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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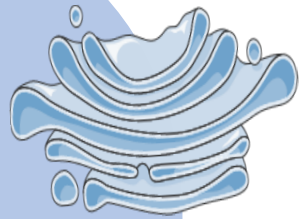
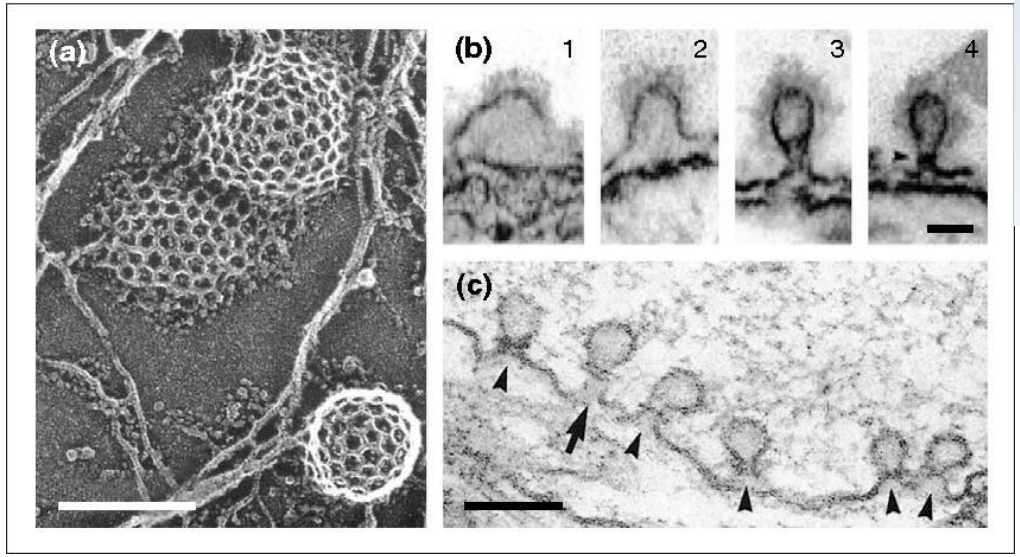
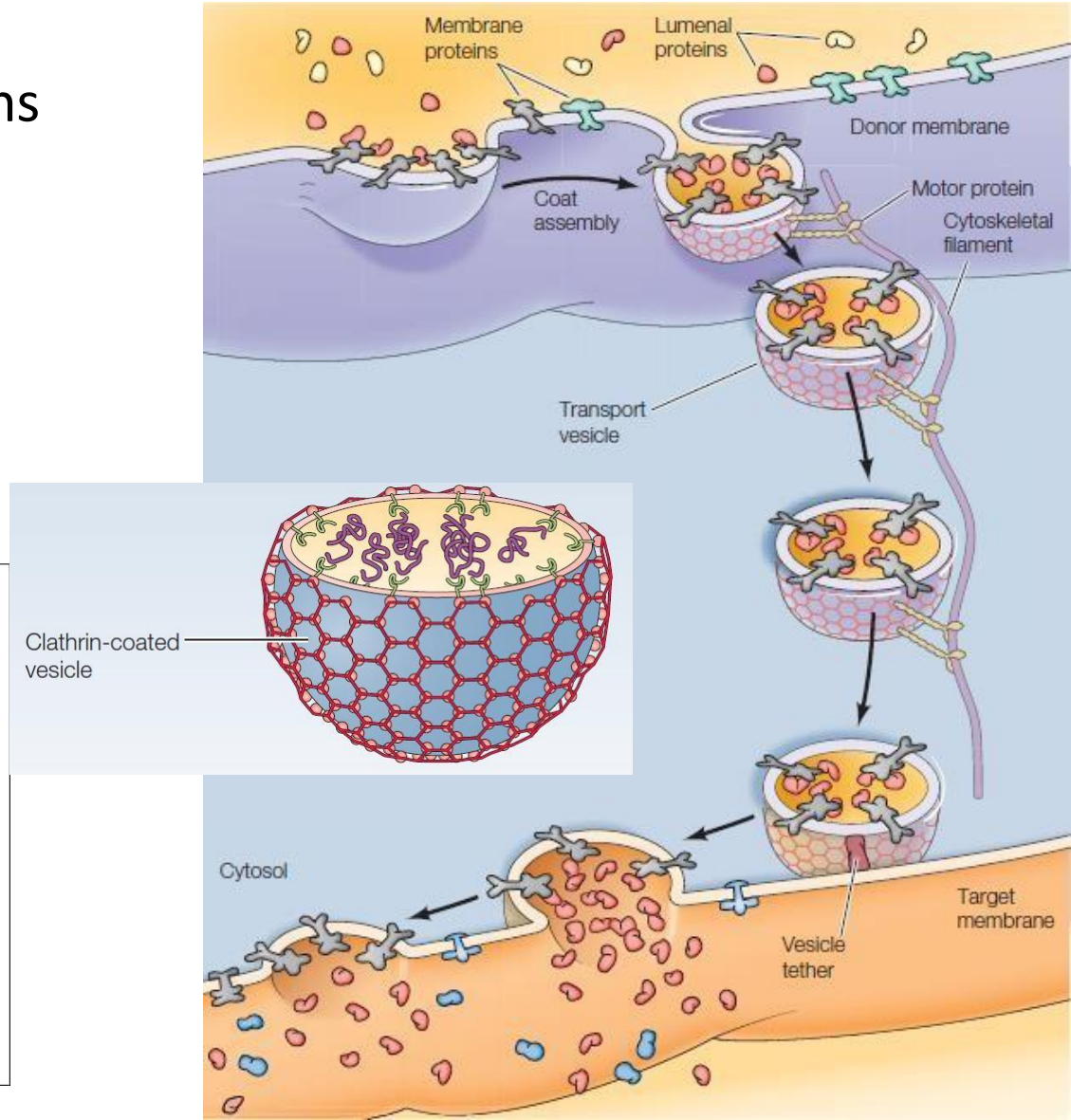


The mechanism of vesicular transport

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 then docks at its target membrane, gets uncoated, and fuses with the membrane. **"clathrin protein network dissociates".**





Formation and fusion of a transport vesicle

Some Vesicles that budded out of Golgi to be transported to the Plasma membrane and vice versa (Vesicles made in the membrane transported to Golgi) are coated with structural proteins called clathrin.

A network surrounding the vesicle is called “clathrin lattice” is formed to serve certain purposes:

1- Preserves the structure and the integrity of the vesicle.

2- Ensures the fusion of the vesicle specifically and properly with the targeted membranes.

Delivery of vesicles: targeting and fusion

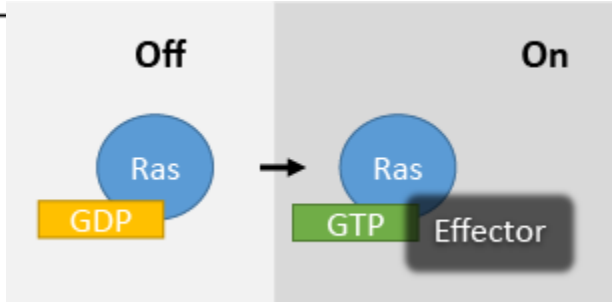
- Small G proteins called Rab determine the membrane targets of vesicles.
  - There are over 60 Rab proteins where different combinations of these proteins mark different transport vesicles.
- v-SNAREs-t-SNAREs proteins are responsible for vesicular fusion with the target membranes.

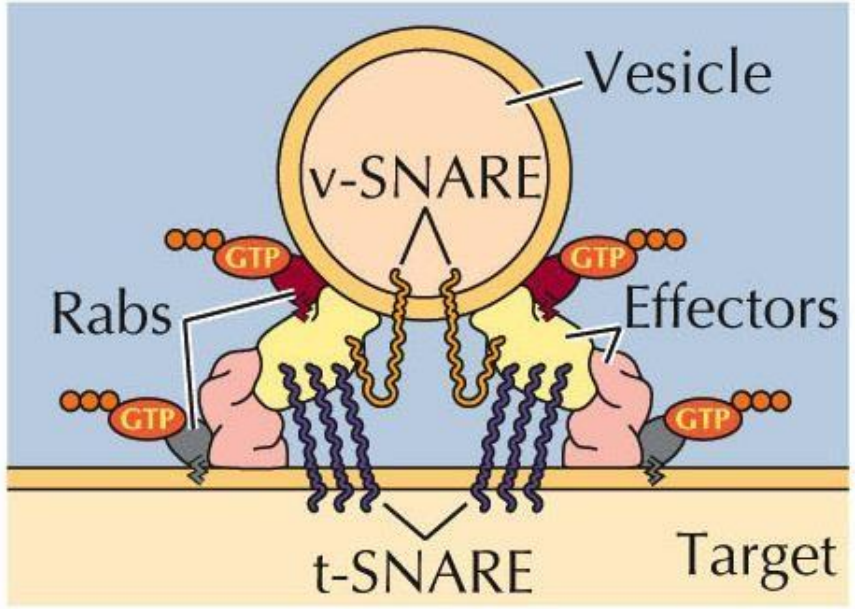
Each type is specific for a certain destination

Don't memorize

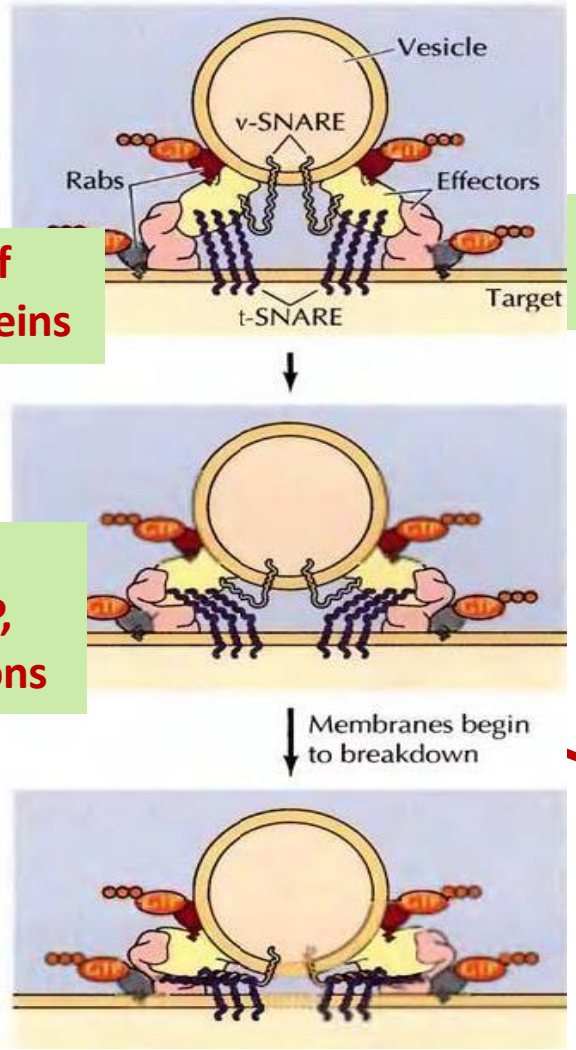
Rab GTPase	Site(s) of action[a]
Rab1	ER to Golgi, intra-Golgi
Rab4	EE to PM
Rab5	PM to EE
Rab6	Golgi to ER, intra-Golgi, EE to TGN
Rab7	EE to LE, LE to lysosome
Rab9	LE to TGN
Rab10	Golgi-associated
Rab11	RE to PM, EE to TGN, TGN to PM

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





The mechanism of fusion

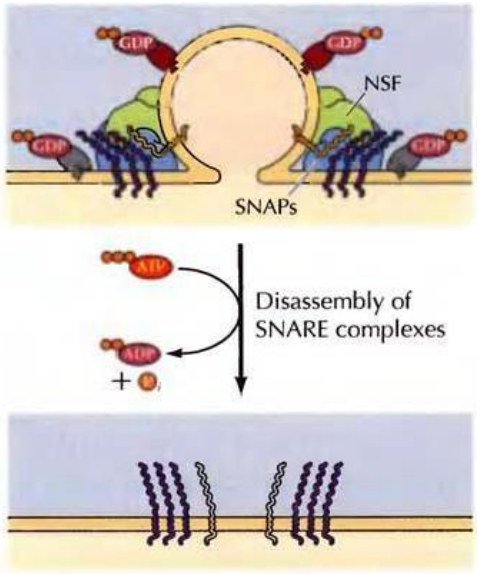


Interaction of effector proteins

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

Delivery of vesicles: targeting and fusion

How vesicles know that they reached their target membrane?

There is a small monomeric G protein called Rab, which is regulated by GTP. When bound to GTP, Rab is active, but when bound to GDP, it becomes inactive. GTP is hydrolyzed to GDP, and GDP is subsequently exchanged for GTP to reactivate the protein.

Those Rab proteins are attached to vesicles from a membrane and transported to the target membrane then interact with a Rab effector protein.

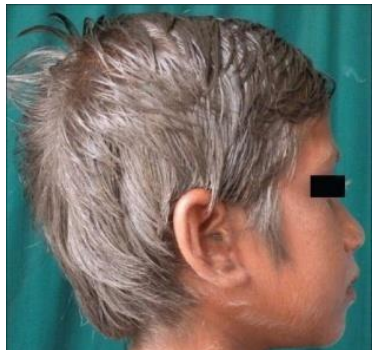
Another structural proteins

v-SNARE found on the vesicle

t-SNARE found on the target membrane.

When Rab protein bind with Rab effector protein the SNARE proteins interact with each other to promote tethering (binding). When this happens the clathrin dissociates, so the vesicle become closer to the membrane and fuse together(The lipid bilayer of both the vesicle and the membrane). After fusion, content is released, if the content is secretory, it either leaves the cell or enters the Golgi, however if it is a membrane protein, the vesicle itself with its contents becomes part of the target membrane and the protein stays in the membrane's surface.

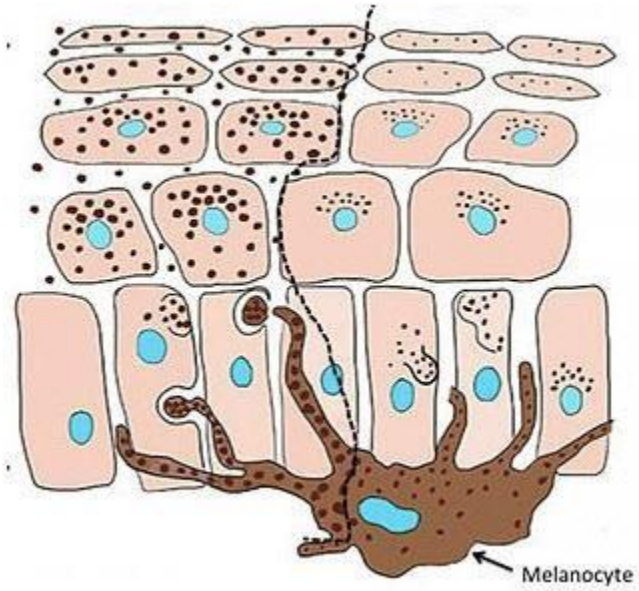
Griscelli syndrome (GS)



- A rare genetic condition

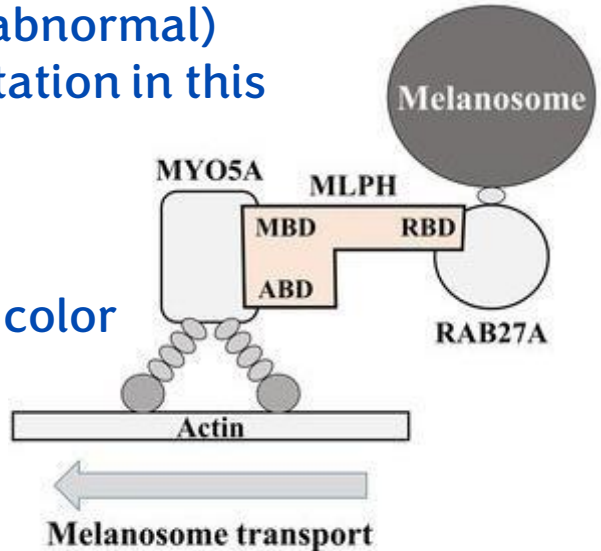
Don't memorize numbers

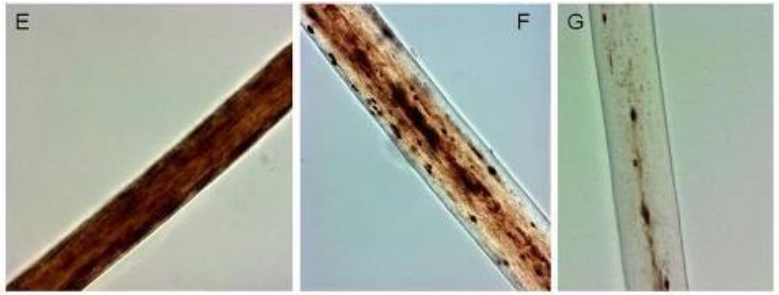
- Mutations in MYO5A (a motor protein), RAB27A and MLPH (a Rab effector protein) genes that encode the MyoVA-Rab27a-Mlph protein complex that function in melanosome transport and fusion.
- Pigmentary dilution of the skin, silver-grey hair, melanin clumps within hair shafts



Grey color (abnormal) (Rab27a mutation in this case)

Normal hair color





Griscelli syndrome (GS)

Melanocytes produce melanin and transport it via vesicles called melanosomes which fuses with the membrane and travel melanin to keratinocytes. Melanin dies the hair black and cause the dark skin color.

People with GS have a disorder in the transportation of vesicles from melanocytes to keratinocytes so they are kept inside melanocytes.

Transportation of melanosomes depends on three proteins any mutation in any of these proteins will affect the skin and hair color melanosomes will clumps in melanocytes.

The three proteins

Myosin: motor protein (moves the protein via using ATP)

Rab27A: functions in targeting the vesicle to a certain direction

MLPH (Rab effector protein): facilitates the reaction between melanosomes and the target membrane

Lysosomes

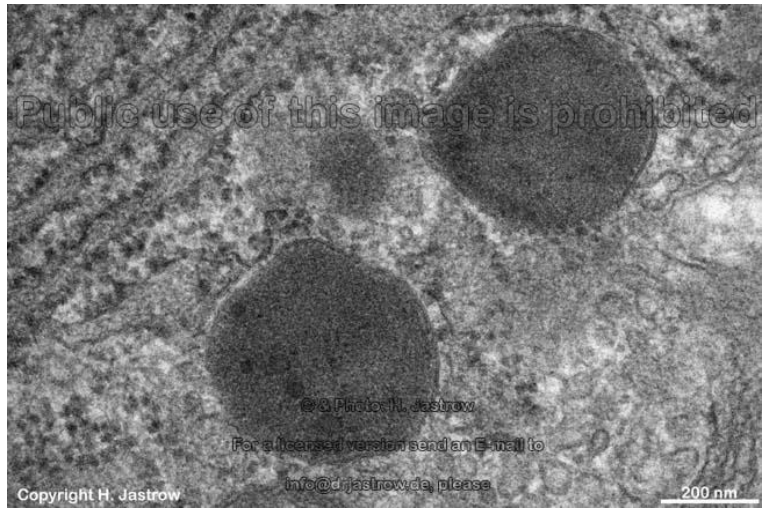
Structure

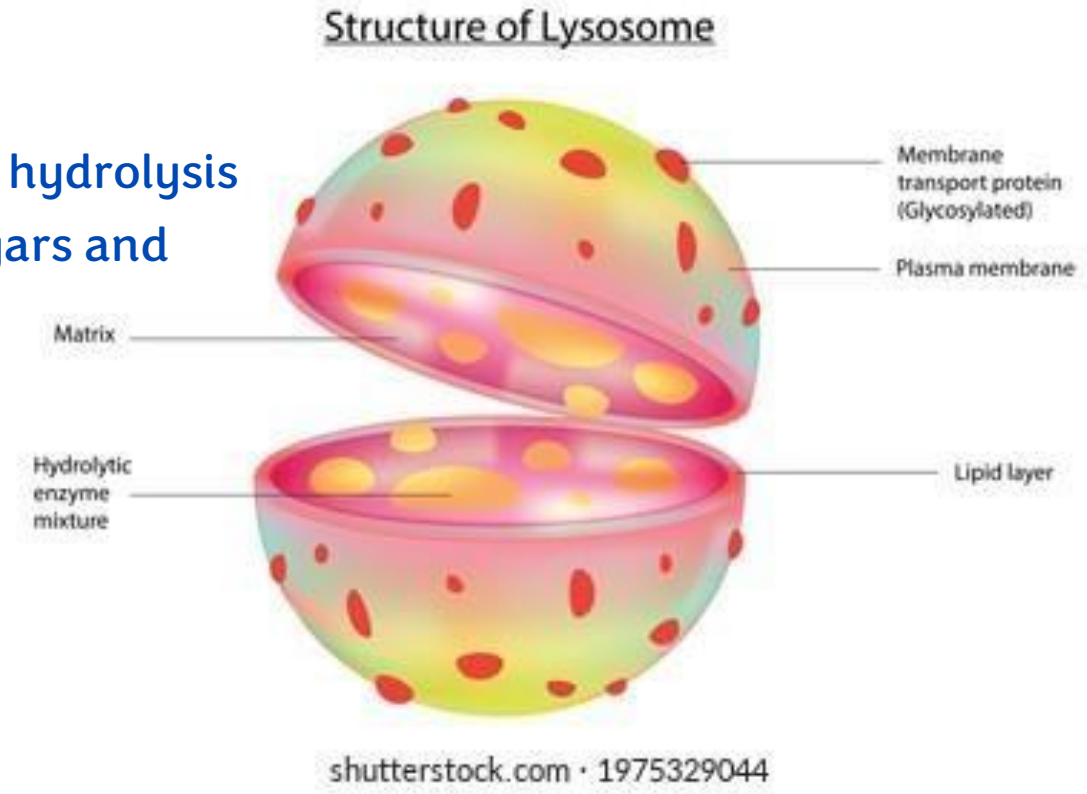
- Lysosomes are membrane-enclosed organelles that contain various enzymes that break down all types of biological macromolecules.
- Lysosomes degrade material taken up from outside and inside the cell.

Single bilayer membrane

Via hydrolysis

Such as lipids, sugars and proteins

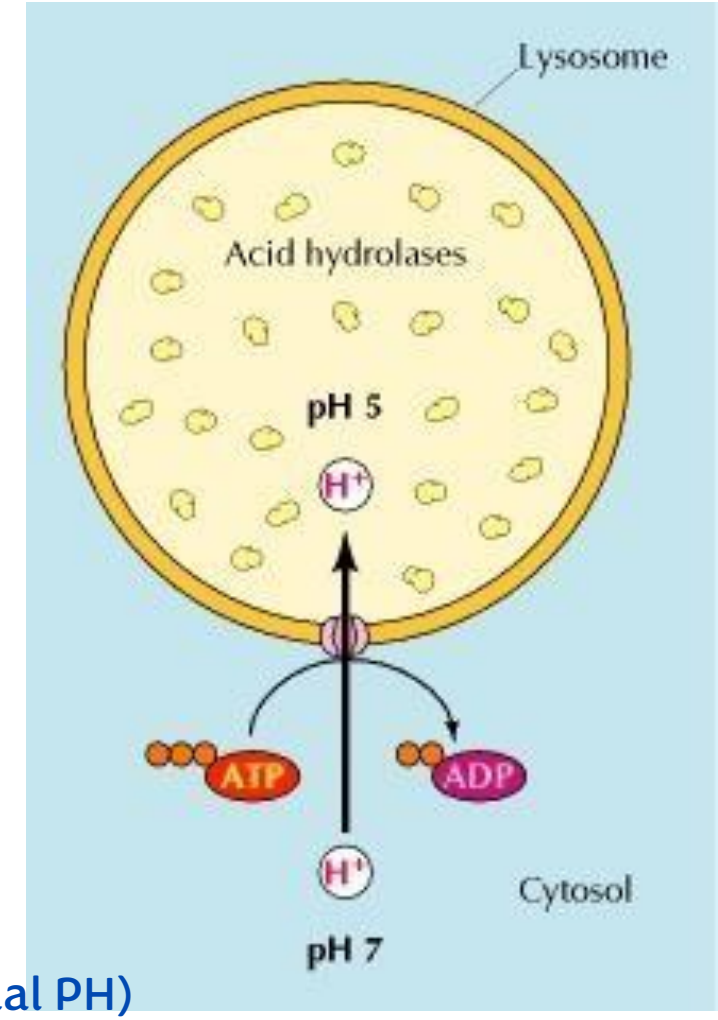




Lysosomal enzymes

- Lysosomes contain ~60 different acid hydrolases. Enzymes that catalyze hydrolysis (the process of adding water to break down molecules)
- The enzymes are active at the acidic pH (about 5) that is maintained within lysosomes.
- Levels of cell protection from these hydrolases:
  - Containment Contain those hydrolases in lysosomes
  - Inactive if released Since the cytoplasmic PH is 7.2~7.4 (not its optimal PH)
- A proton pump maintains the lysosomal pH. ATP dependent pump, pumps H+ into lysosomes

Another benefit for the acidic PH other than providing a suitable environment for the enzymes is to inactivate molecules like proteins that enter the lysosomes and denature them, aiding hydrolytic enzymes break these molecules down.



Lysosomal storage diseases

This condition is caused by defects in lysosomal enzymes, leading to issues in the degradation of macromolecules.

- 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.

Since there are 60 different lysosomal enzymes there are 60 different lysosomal storage diseases
Some are
Protein-related
Sugar-related
Lipid-related

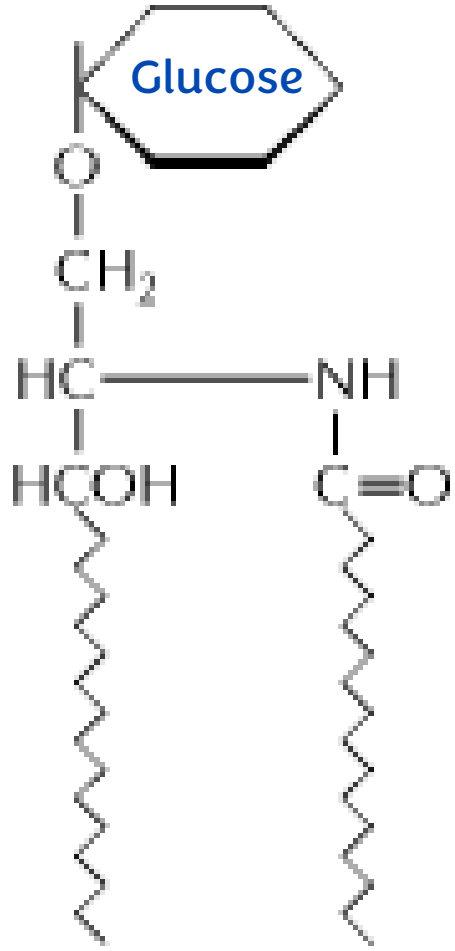
"The severity of these diseases is highly variable and depends on whether the mutation causes the protein to be partially or totally defective."

Obvious but nice to mention: People with the same disease differ in severity (Severe, Mild, etc.)
All related to mutation (the protein is defective partially or totally, and may lead to mental retardation (most likely)or to 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.

For example: If the enzyme glucocerebrosidase (metabolizing glucocerebroside to ceramide and glucose) is defective it can lead to lysosomal storage disease.

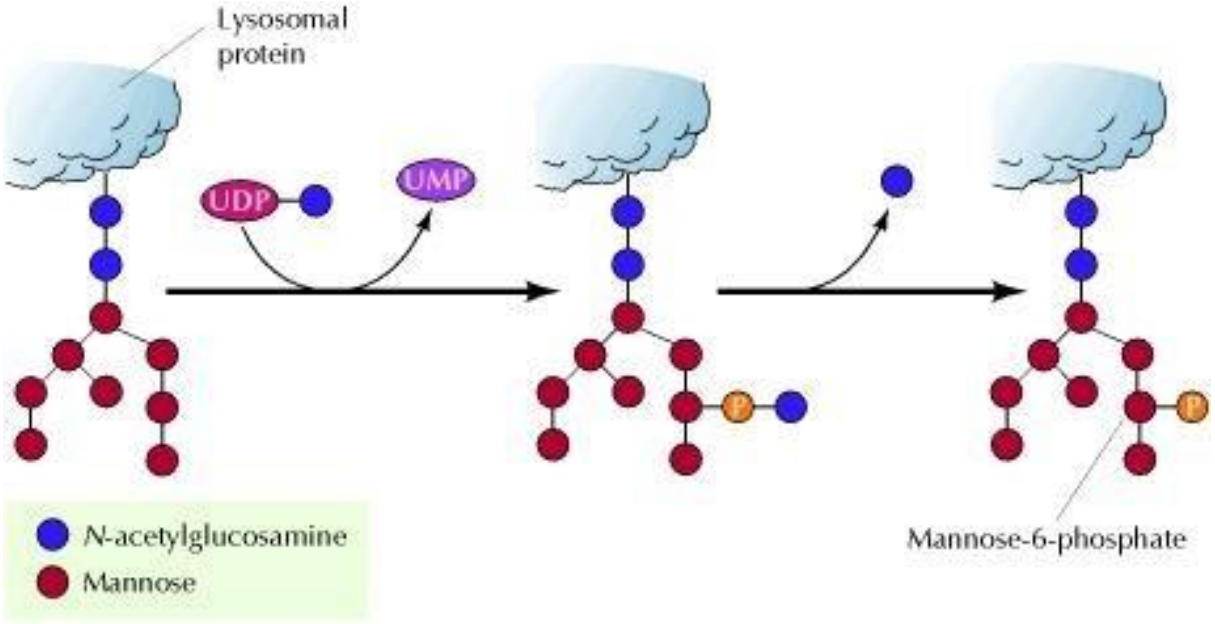


Glucocerebroside

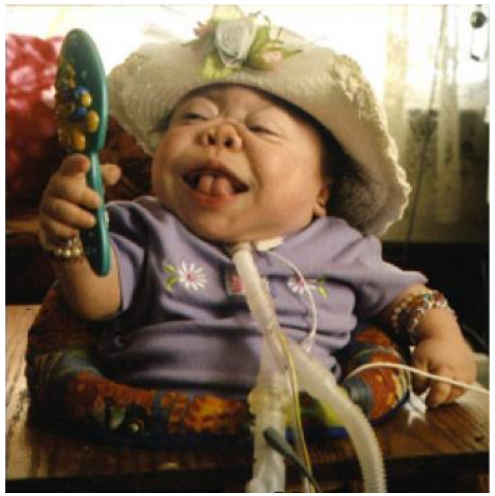
I-cell disease

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.



It is a lysosomal storage disease, but it is not specific to a certain target or substrate, it affects many enzymes and proteins, because the enzyme that phosphorylates mannose is defective so all lysosomal proteins aren't phosphorylated at mannose and they cannot bind to the mannose-6-phosphate receptor, leading to them not being targeted to lysosomes



Endocytosis

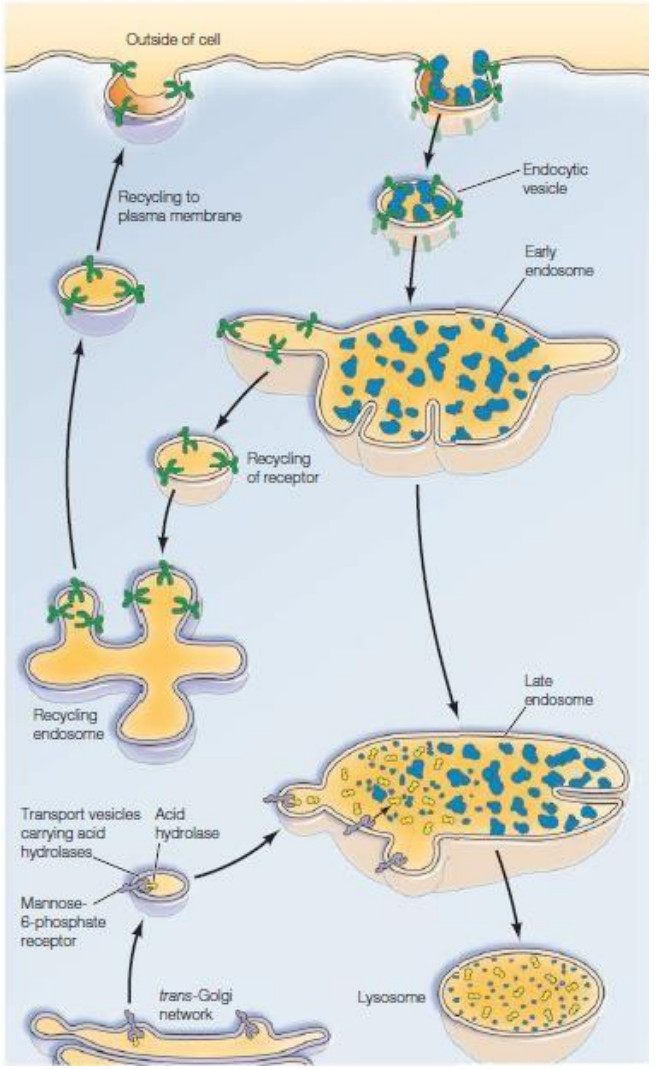
Remember: Exocytosis is when vesicles exit golgi to plasma membrane and release their content outside of the cell

- Molecules are taken up from outside the cell in endocytic vesicles, which fuse with early endosomes.
- Early endosomes mature into late endosomes.

Late endosomes fuse with the lysosomes and then the substances that enters the cell by endocytosis will be degraded inside lysosomes.

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

Acidic



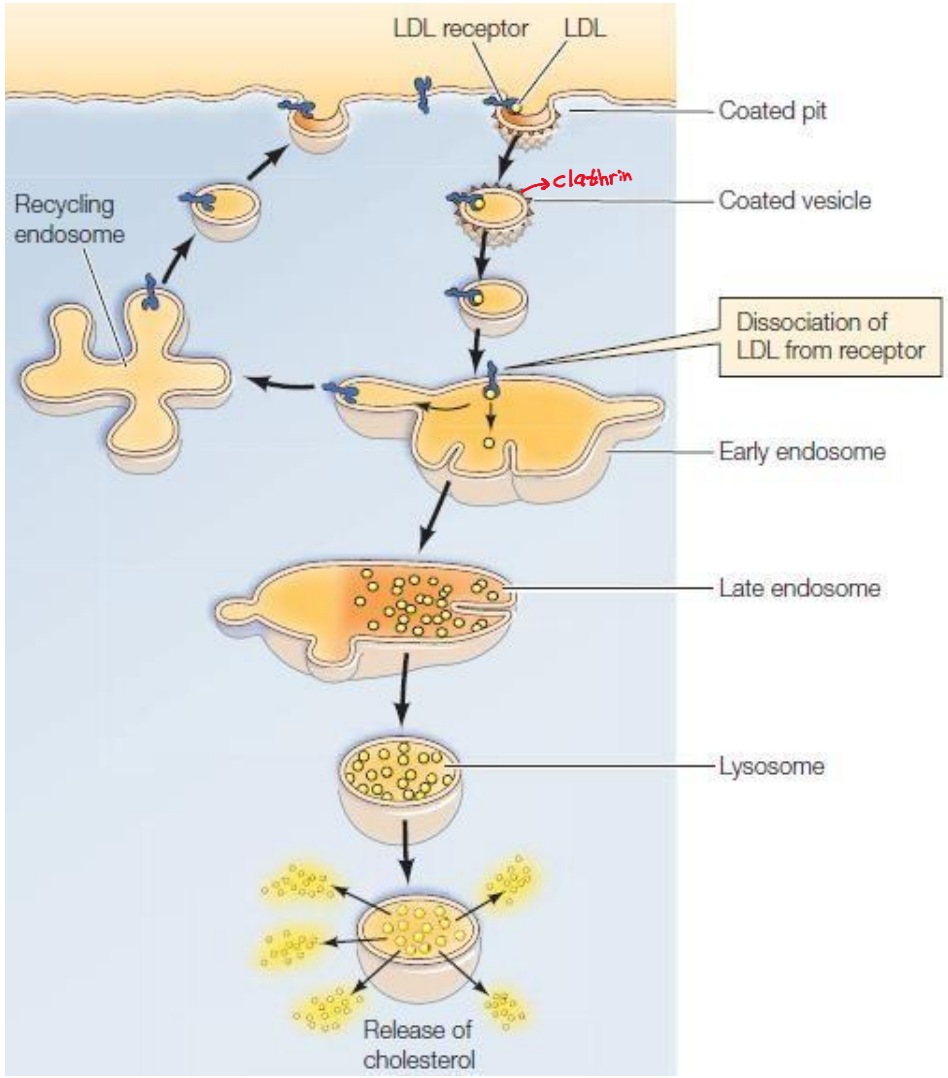
The vesicle is pinched of the plasma membrane into a vesicle that may or may not be coated with clathrin.

Remember: vesicles produced by Golgi fuses with late endosomes and then matures to lysosomes

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



Hydrolysis will degrade apolipoproteins and cholesterol esterase will degrade cholesterol in lysosomes

Steps for simplifying the previous slide (the steps are in order)

- Ligands bind to their receptors stimulating endocytosis.
- Examples: Insulin with its receptors (insulin receptors). LDL (containing cholesterol) binds to its own receptors (LDL receptor) stimulating the formation of a vesicle, stimulating endocytosis
- After the formation of the vesicle(which contains the ligand and its receptor) the vesicle fuses with early endosomes
- In early endosomes, the acidic pH (*6.0-6.5*) causes the release of ligands from their receptors.
- Membrane receptors are recycled via recycling endosomes and early endosomes mature into late endosomes (The receptors return back to the membrane)
- Transport vesicles carrying acid hydrolases from the Golgi fuse with late endosomes, which mature into lysosomes.

Cell eating

Phagocytosis

In Phagocytosis the vesicle is coated with clathrin unlike in pinocytosis

It's endocytosis but since the vesicle(called phagosome) is large it is called phagocytosis

Macrophages(immune cell) use this technique

- 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.

Pinocytosis

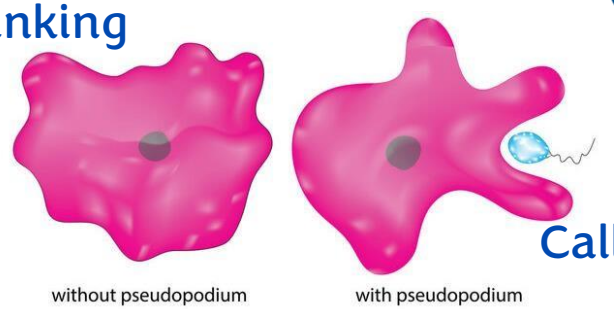
- *Macropinocytosis (clathrin-independent) is cell drinking via the formation of small vesicles.*

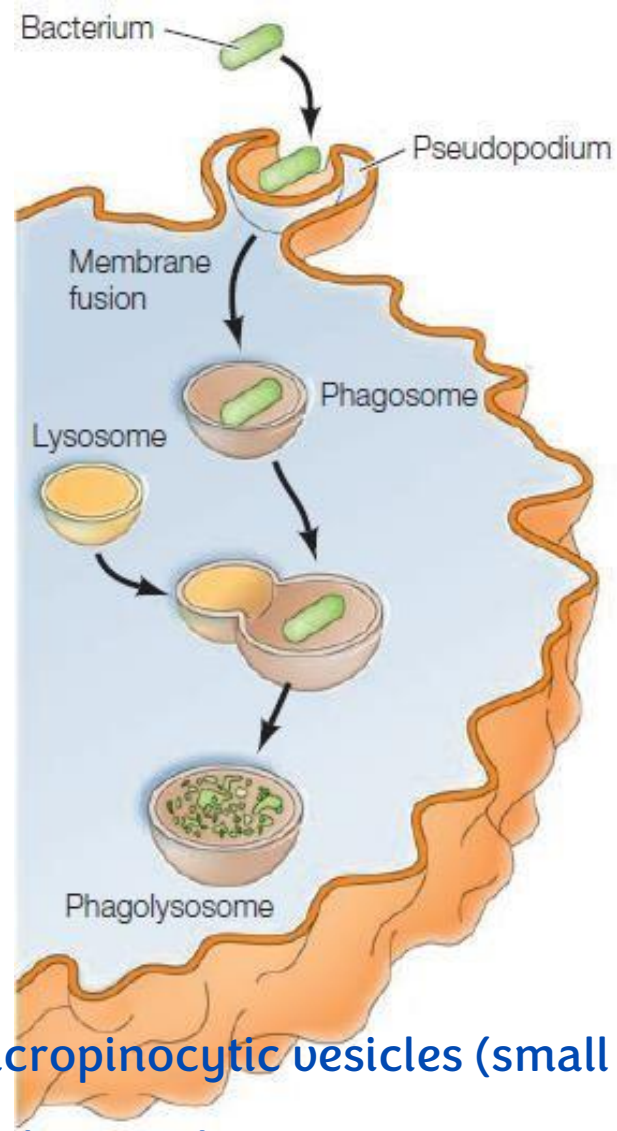
Fake arms

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

These arms surrounds bacterium

Pino=drinking



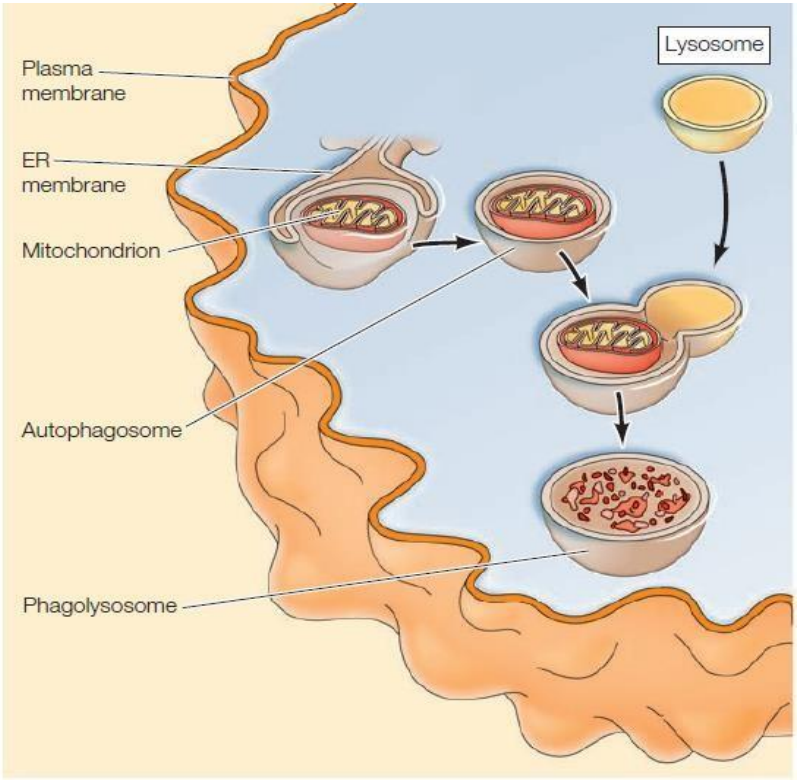


Called macropinocytic vesicles (small vesicles)

Mostly fluids enter however small molecules may enter the cell as well

Autophagy (self-eating)

No endocytosis, the vesicles are formed inside the cell and called autophagosomes



- Regions of the cytoplasm or internal organelles (such as mitochondria) are enclosed by membranes derived from the endoplasmic reticulum, forming autophagosomes. Large vesicles
- 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

Degradation

For renewal of the organelles

Like mitochondria and peroxisomes

A research conducted that fasting induces autophagy which is beneficial since the cell renews itself

Purposes of Autophagy;
1-protects itself (removal of damaged organelles)
2-when the cell is starving it stars eating itself to provide some nutrients for survival and generation of (energy, amino acids, sugars, lipids etc..) to renew itself

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

عنْ عبدالله بن عمرو بن العاص، رضي الله عنْهُمَا أنَّهُ سمِع رسُول الله ﷺ

يقُولُ: مَنْ صلَّى عليَّ صلاَةً، صلَّى الله علَيّهِ بِهَا عشْرًا

صلّوا على الحبيب