بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture #3

Vesicular Transport

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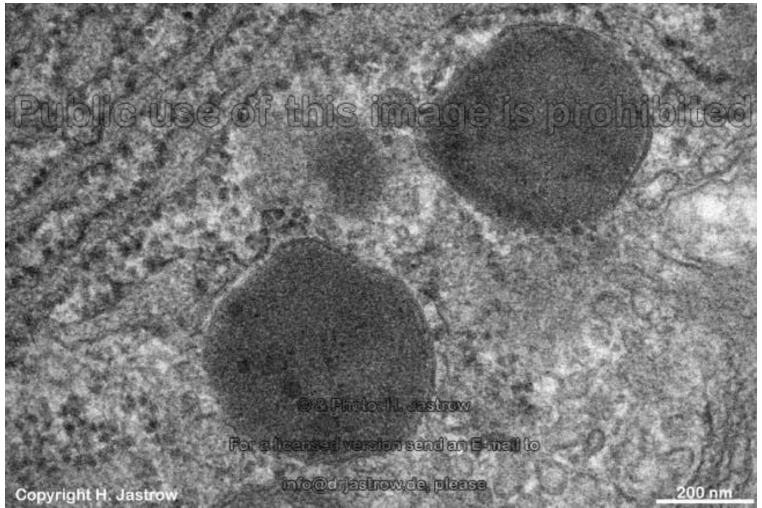
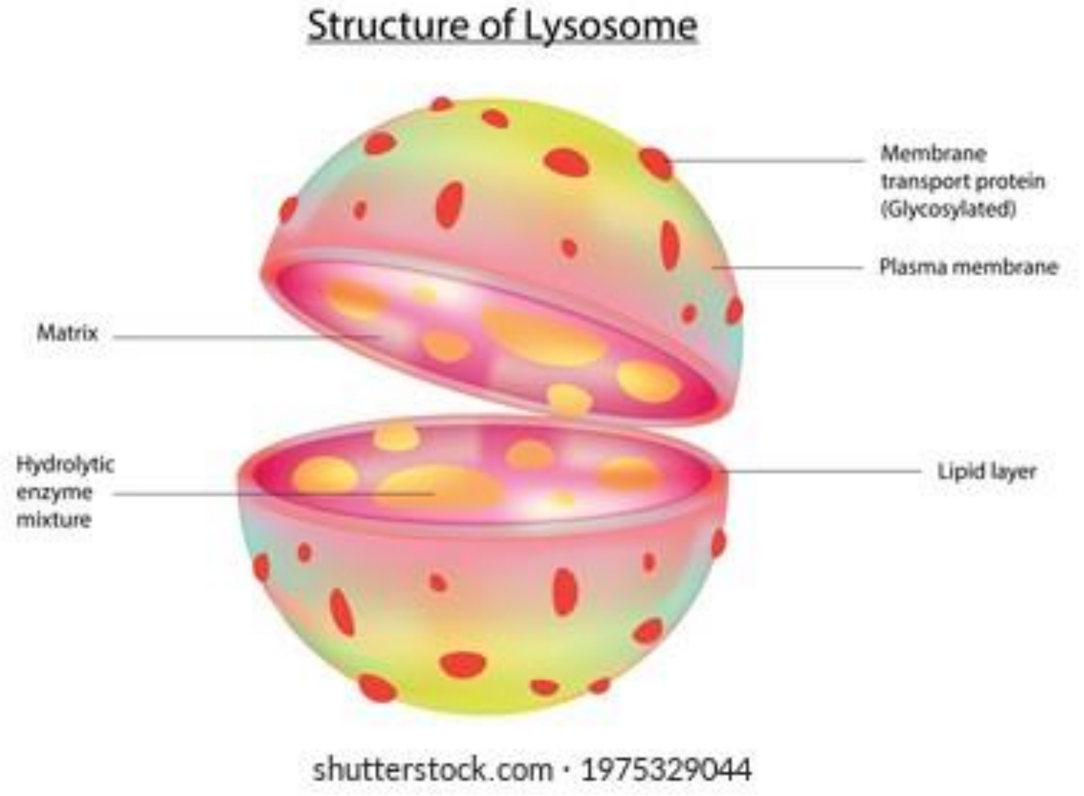
Lysosomes

Structure

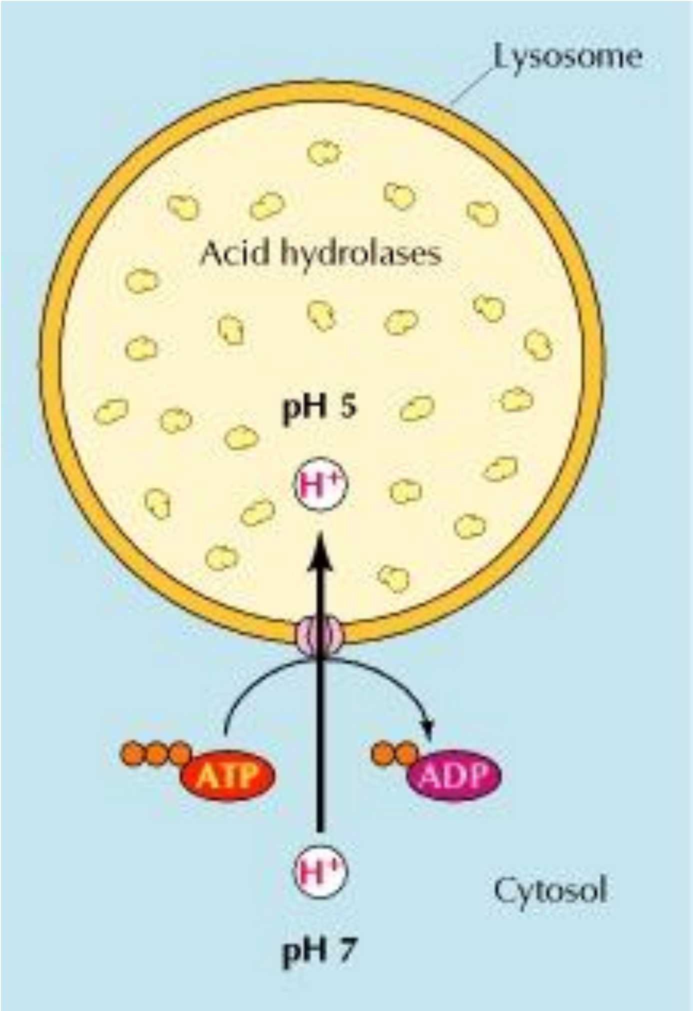
- Lysosomes are vesicles that have a single phospholipid bilayer membrane
- Lysosomes are membrane-enclosed organelles that contain various enzymes that break down all types of biological macromolecules (lipids, proteins, carbohydrates, etc.).
- Lysosomes degrade material taken up from outside and inside the cell.





Lysosomal enzymes

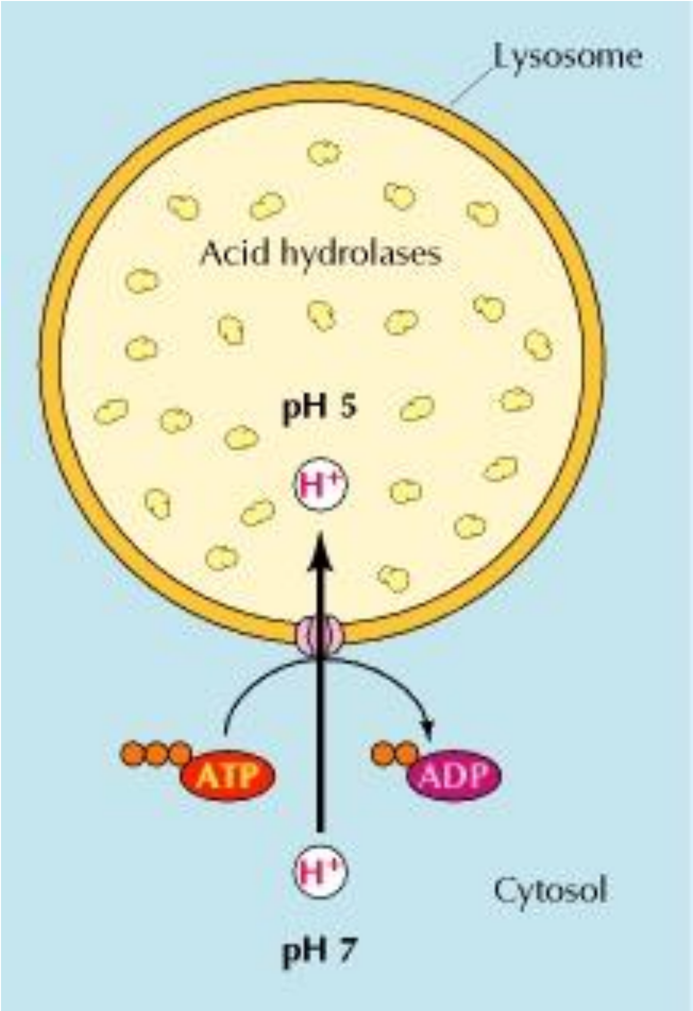
- Lysosomes contain ~60 different acid hydrolases (which uses water to break down other macromolecules).
- The enzymes are active at the acidic pH (about 5) that is maintained within lysosomes.
- Levels of cell protection from these hydrolases:
  - Containment
  - Inactive if released
- An ATP-dependent proton pump maintains the lysosomal pH by pumping protons from the cytosol into the lysosome.



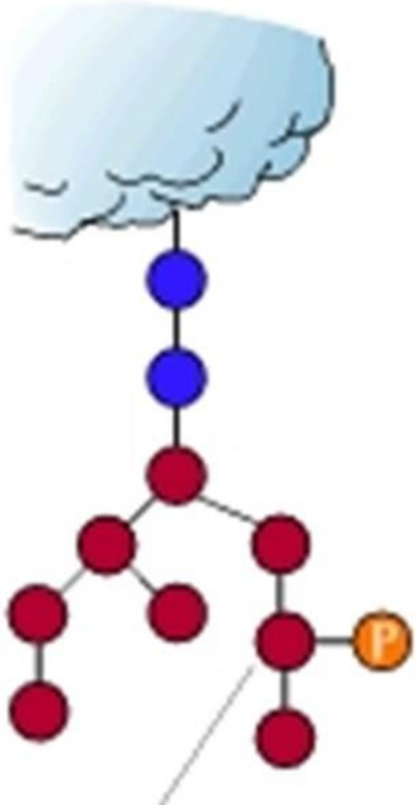
Lysosomal enzymes

The low pH inside lysosomes (4.5-5) is present for 2 reasons:
1- The denaturation (loss of 3D shape) of molecules, (especially proteins). This gives the enzymes of lysosomes access to the covalent bonds of these molecules which helps in the process of degradation.

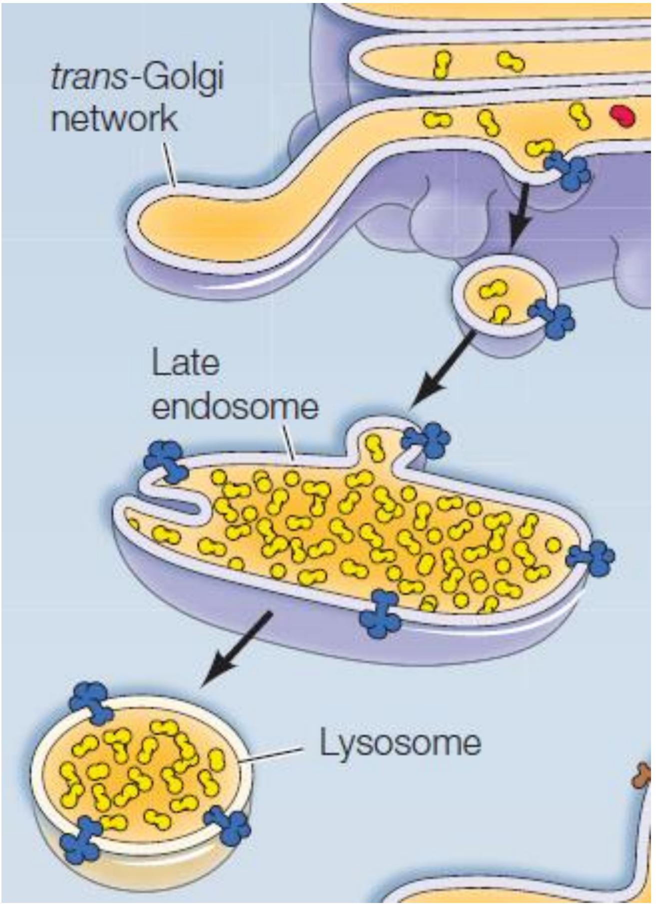
2- If the hydrolases of the lysosomes are released to the cytosol, they won't be active since the pH of the cytosol is around (7.3-7.4), this works as a protective mechanism for the cell, so the lysosomes contain them inside in a low PH , and protect the cell from these hydrolases



Targeting of lysosomal proteins to the lysosomes

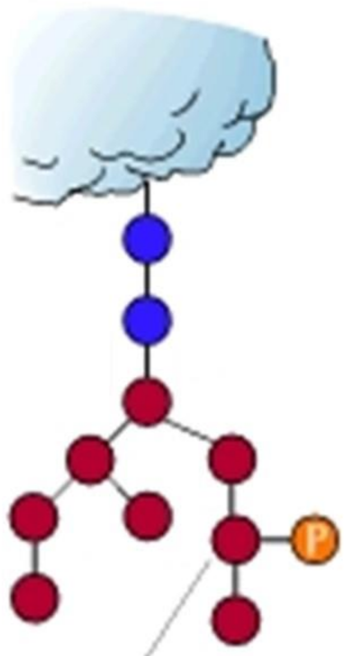


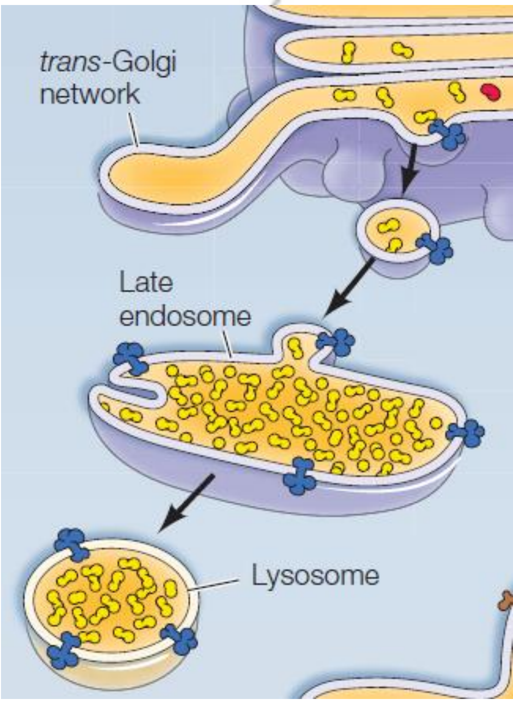
Proteins destined to lysosomes have <u>a signal patch</u> (a three-dimensional structural determinant), which is recognized by modifying enzymes that add mannose-6-phosphate to the proteins.



Lumenal lysosomal proteins bind to a mannose-6-phospahte receptor in the Golgi and are transported to vesicles known as the late endosome, which mature into lysosomes.

Targeting of lysosomal proteins to the lysosomes





- Lysosomal proteins (hydrolases and lysosomal membrane proteins) are synthesized in the surface of ER and modified in the ER and Golgi apparatus, where they undergo glycosylation (adding of sugar molecules).

- All lysosomal proteins are usually tagged with a **mannose** (sugar) in the Golgi due a special signal patch (3D structure), which enable the phosphorylation of mannose to be **mannsose-6-phosphate**.

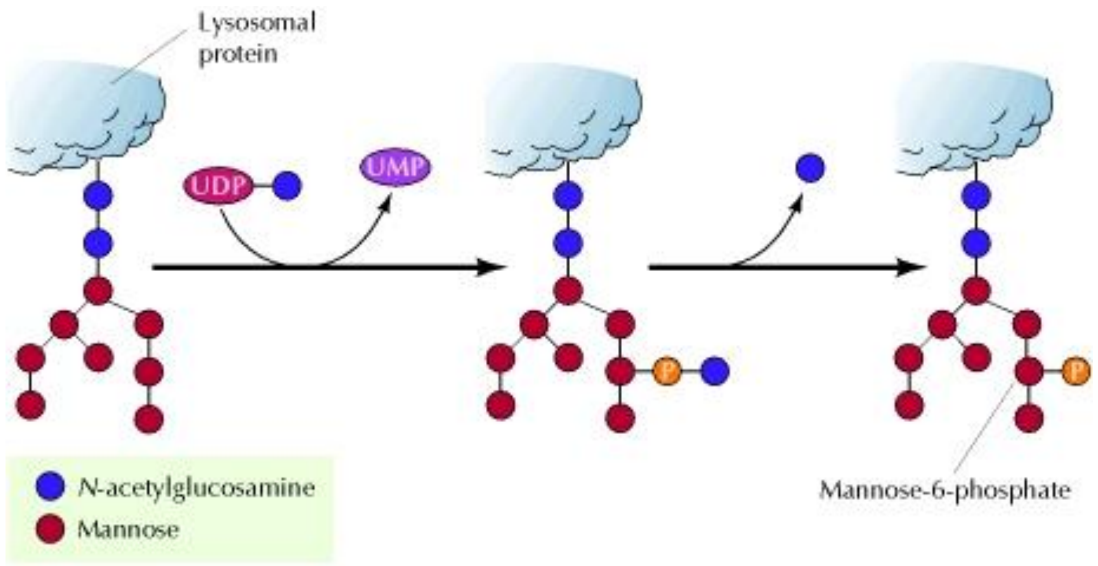
- This phosphorylated mannose allows the lysosomal proteins to bind with a special receptor called (**mannose-6-phosphate receptors**) located in the trans-Golgi network.

- In the trans-Golgi network, vesicles will start forming there containing the lysosomal proteins and they will fuse with the late endosome which will mature to form the lysosome.

I-cell disease

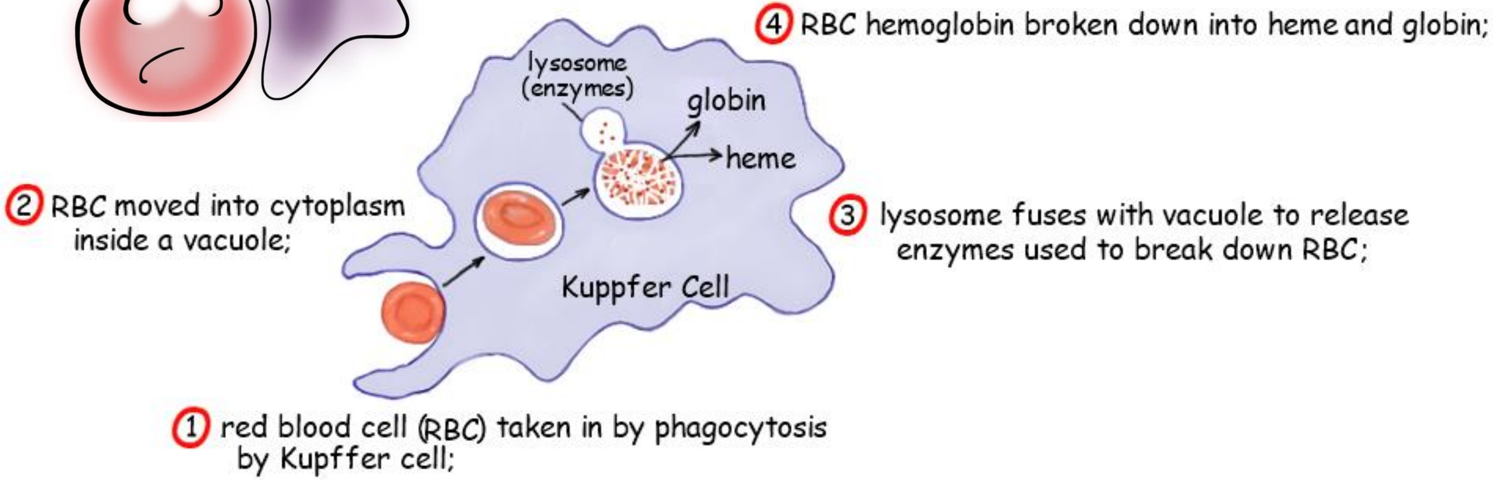
also called mucolipidosis IIA, or mucolipidosis II alpha/beta: ML-IIα/β

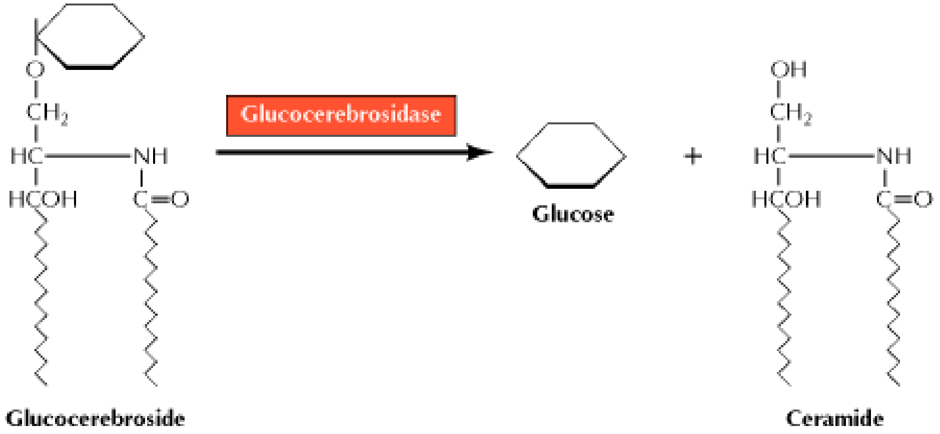
- Defective targeting of lysosomal enzymes from Golgi to the lysosomes
- A deficiency in the tagging enzyme that phosphorylates mannose.
- **So, proteins can't move from Golgi to lysosomes leading to I-cell disease severely affecting the CNS.**
- Features: severe psychomotor retardation that rapidly progresses leading to death between 5 and 8 years of age.



Glucocerebroside

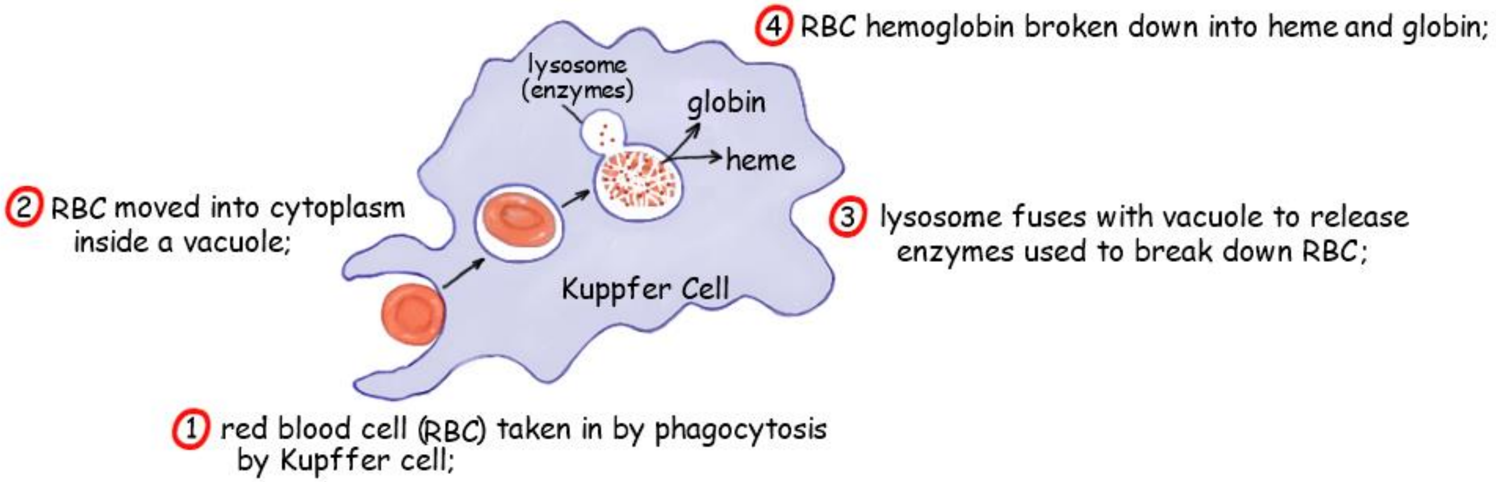
- Glucocerebroside is a glycosphingolipid(a monosaccharide attached directly to a ceramide unit (a lipid)
- It is a byproduct of the normal recycling of red blood cells, which are phagocytosed (eaten up) by macrophages, degraded, and their contents recycled to make new cells.

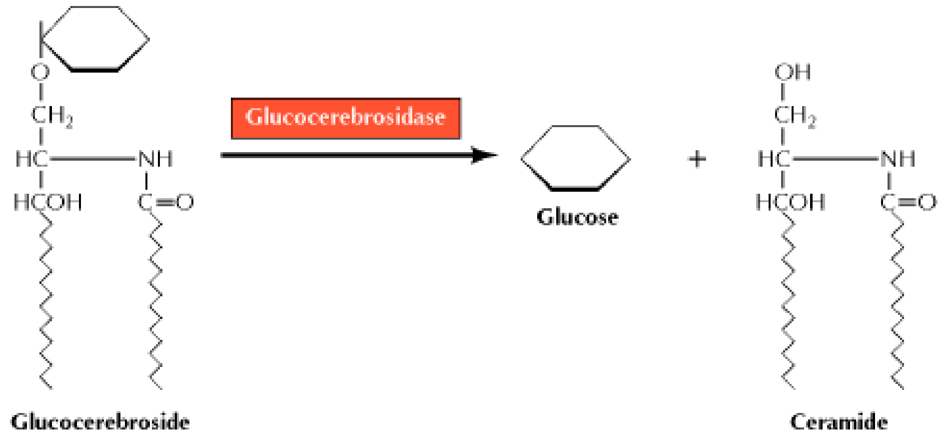




Glucocerebroside

- Glucocerebroside is a molecule found in the plasma membrane. It is a sphingolipid (a lipid that has sphingosine as a backbone instead of glycerol). **Cerebroside** is made of ceramide attached to 1 monosaccharide (glucose or galactose). **Globoside** is made of ceramide attached to 2 monosaccharides or more. Addition of sialic acid gives **ganglioside**. In lysosomes, there is an enzyme called **glucocerebrosidase** which converts Glucocerebroside into free glucose and ceramide.
- Macrophages during phagocytosis of RBCs (that underwent hemolysis) encounter high amounts of glucocerebrosides. So defective glucocerebrosidase leads to the accumulation of glucocerebrosides leading to several symptoms including mental retardation. This process also occurs in the neural cells in the CNS





Lysosomal storage diseases

- Gaucher disease (glucocerebrosidase deficiency) **(most common metabolic syndrome)**
- Glycolipidoses (sphingolipidoses) **(disease where enzymes that degrade sphingolipids are defective)**
- Oligosaccharidoses **(disease where enzymes that degrade sugars are defective)**
- 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.
  - All of these disease affect CNS leading to mental retardation

The general mechanism of vesicular transport

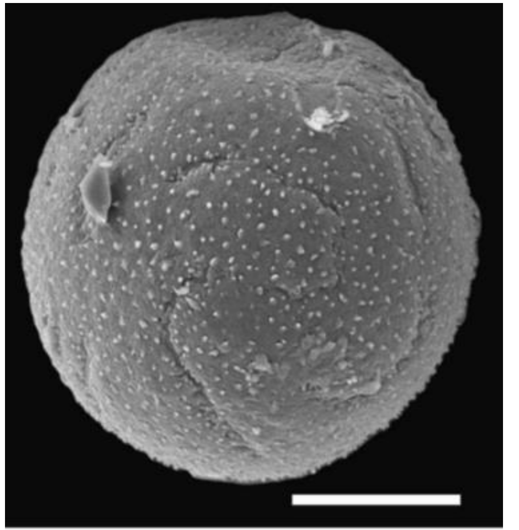
Proteins go into the ER with the help of signal peptides then these proteins go into either nuclear membrane, peroxisomal membrane, or the golgi apparatus.. From the golgi it goes into either the plasma membrane, extracellular milieu (specific region outside the cell), endosomes and lysosomes ,or secretory vesicles.

The general mechanisms for transport of proteins is through *vesicles*

What are transport vesicles?

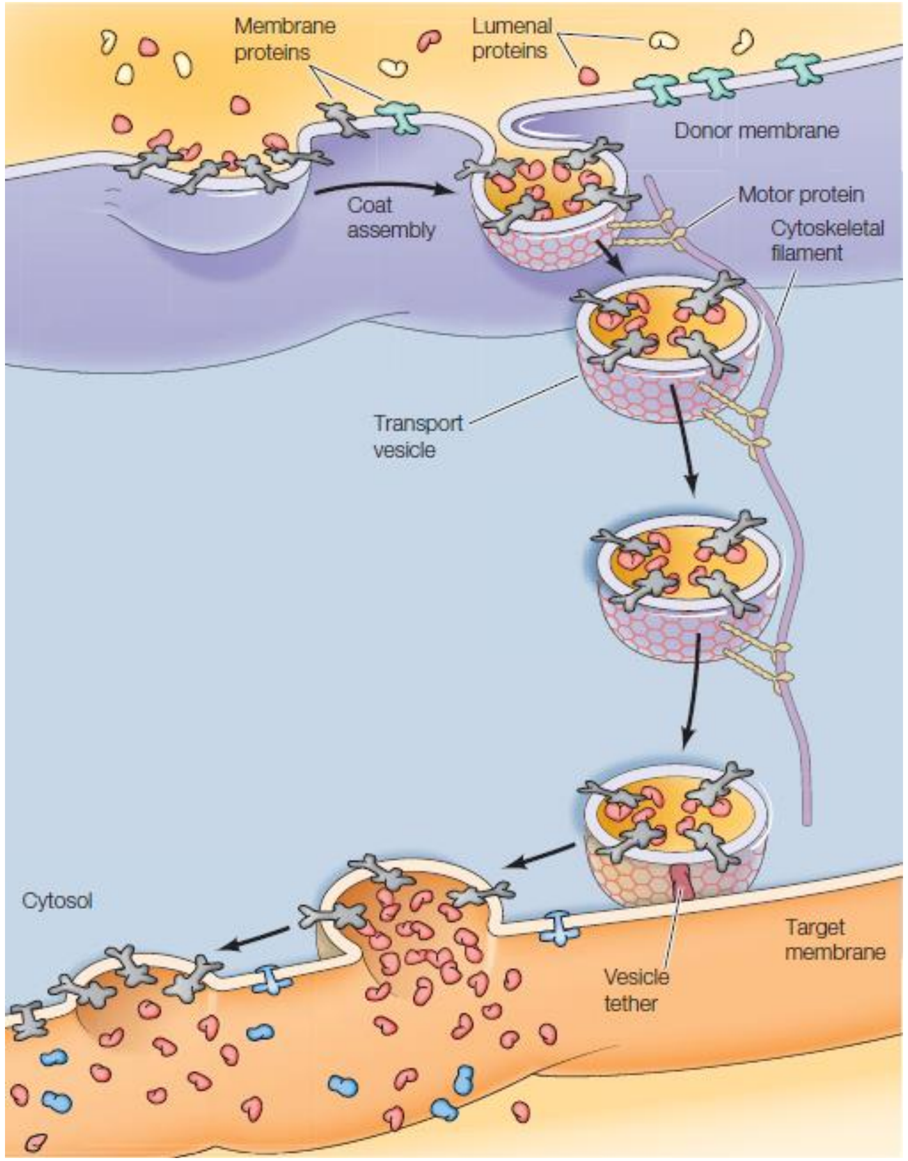
- Vesicles are formed by when an organelle (e.g ER) pinches off to form a ball-like structure.
- Vesicular transport is the movement of molecules in bags (or sacs) called transport vesicles in and out of cells or within cells.
  - Examples: (1) the transport of materials taken up from outside the cells at the cell surface into them, or (2) the transport of lysosomal enzymes from the Golgi apparatus to lysosomes.
- Vesicular transport is selective in terms of destination and packaging (it allows for intracellular communication inside the cell and precise transfer of proteins).
- When molecules enter the cell, they get enclosed by a vesicle. When molecules are released outside the cell the vesicle fuses into the target membrane.

SEM image of vesicle



The general steps of vesicular transport

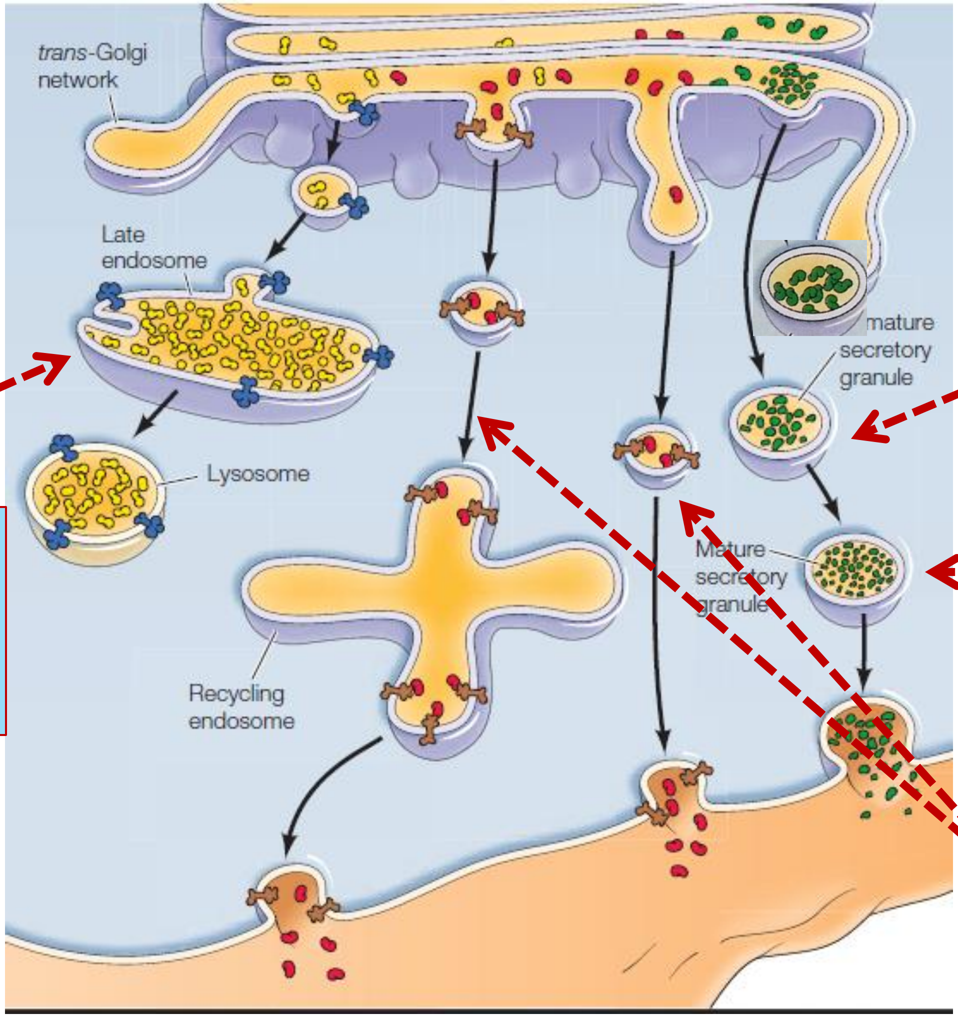
1. The molecules to be transported are aligned along or clustered in the membrane of the donor compartment (e.g ER). Proteins are concentrated in the region that will form the vesicle
2. A vesicle buds from the donor membrane.
3. The bud grows and ultimately pinches off the donor membrane, yielding a transport vesicle.
4. The vesicle is transported along cytoskeletal filaments (microtubules) to a target membrane. This movement is regulated.
5. The vesicle fuses with the target membrane, whereupon the transmembrane proteins are incorporated into that membrane, and the soluble proteins are released (e.g golgi or extracellular milieu). Membrane proteins become part of the target membrane.



The processes of vesicular transport

The secretory pathway (exocytosis)

Cytosis: a cellular process of moving material across membranes



Protein processing in Immature secretory vesicles

2. Regulated, signal-stimulated secretion of processed proteins from specialized vesicles (e.g., neurotransmitters and digestive enzymes)

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

1. Continuous, unregulated secretion directly or via recycling endosomes, which also act to recycle endocytosed proteins to the plasma membrane

SEE NEXT SLIDE

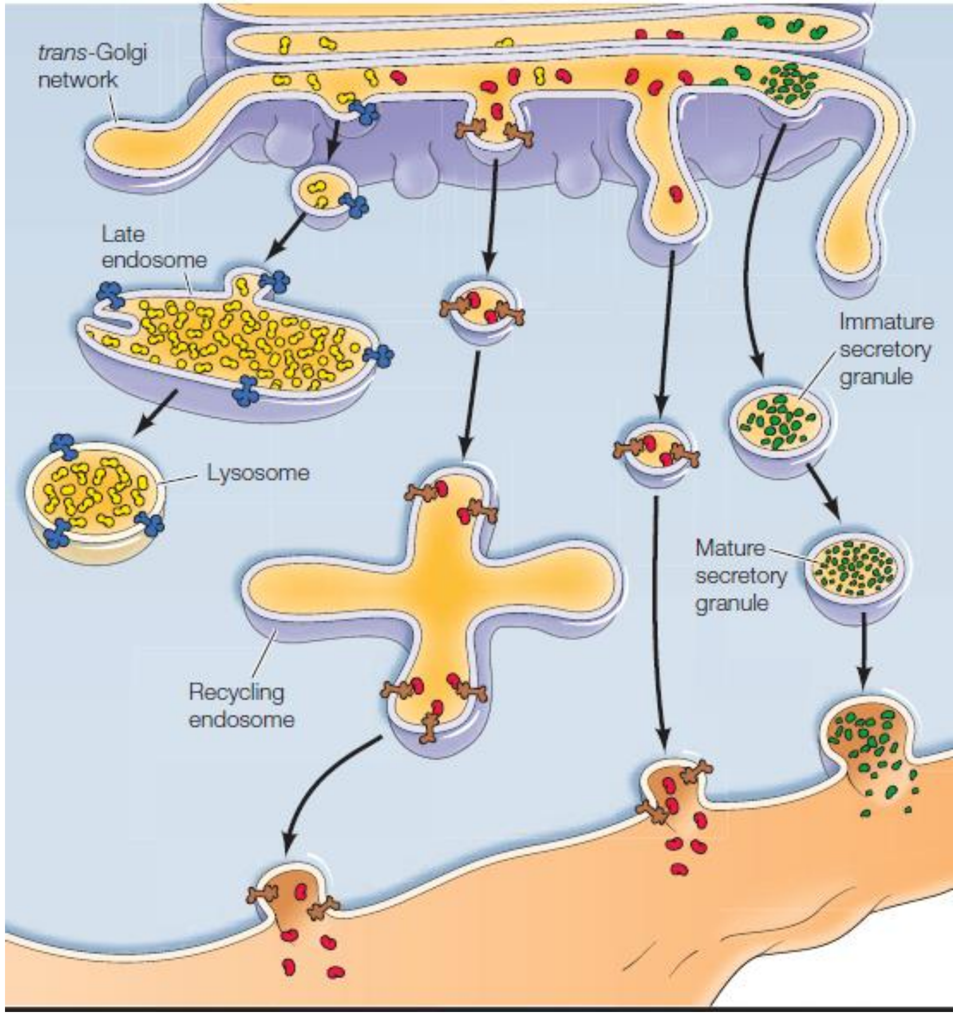
The secretory pathway (exocytosis)

Vesicles that pinch off Golgi could fuse with late endosome, where proteins will get released from the mannose-6-phosphate receptor due to low pH found in endosomes. Then endosome then matures into lysosome which has a lower PH.

Cytosis: a cellular process of moving material across membranes
Exocytosis: the movement of vesicles from inside the cell to outside.

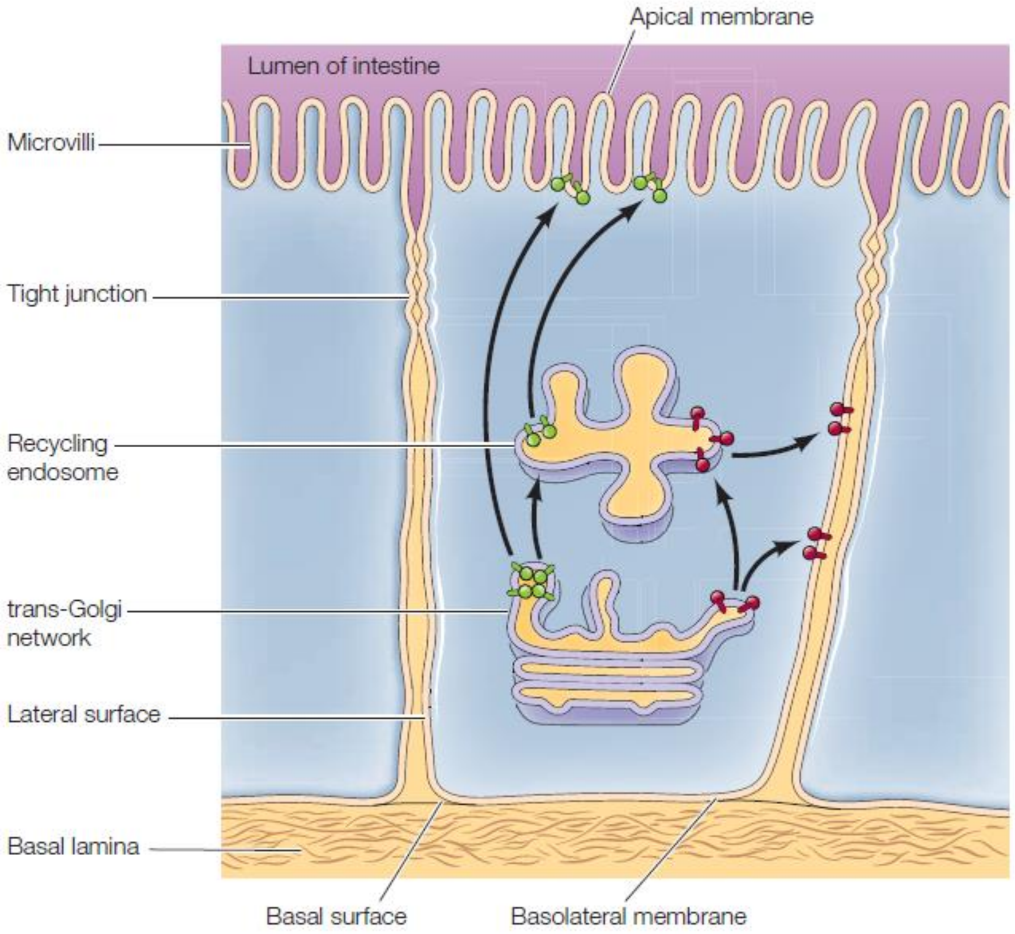
Exocytosis could be :
Continuous exocytosis, which has 2 types:
1- The vesicle moves directly from the Golgi to the Plasma membrane
2- The vesicle moves from the Golgi into a recycling endosomes and fuses with it. Then the recycling endosome fuses with the plasma membrane to release its contents outside the cell.

Regulated exocytosis,
means that after pinching off the Golgi, it remains in the cytosol until a signal appears which allows the vesicle to fuse the plasma membrane and leave the cell.
Regulatory vesicles contain neurotransmitters or digestive enzyme (in pancreatic cells). During this regulated exocytosis, there might be intermediate vesicles where protein processing occurs



Transport to the plasma membrane of polarized epithelial 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).



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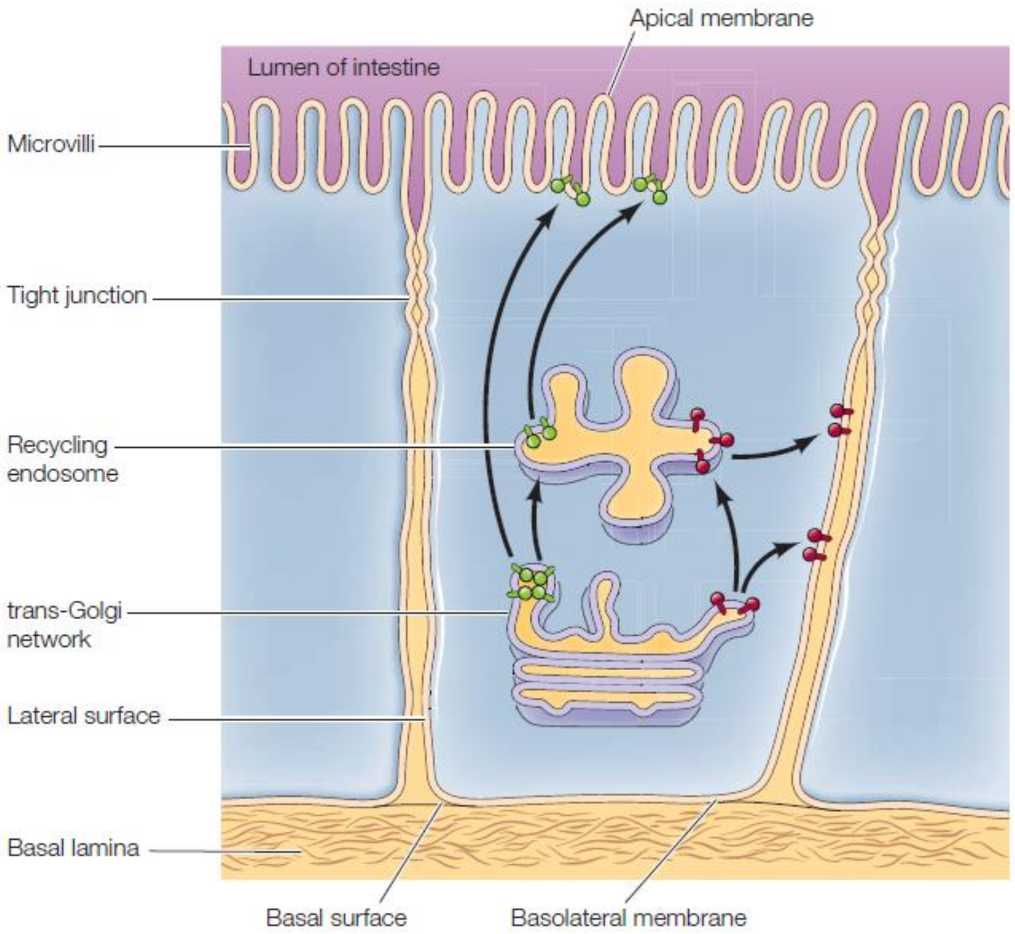
Transport to the plasma membrane of polarized epithelial cells

Targeting of vesicles in polarized cells such as epithelial cells is **not random**.

In intestinal epithelial cells, during absorption, molecules enter the cell from the apical surface and are released into the basolateral surface. This means that distribution of proteins differs between apical and basolateral surfaces which is determined by specific targeting codes.

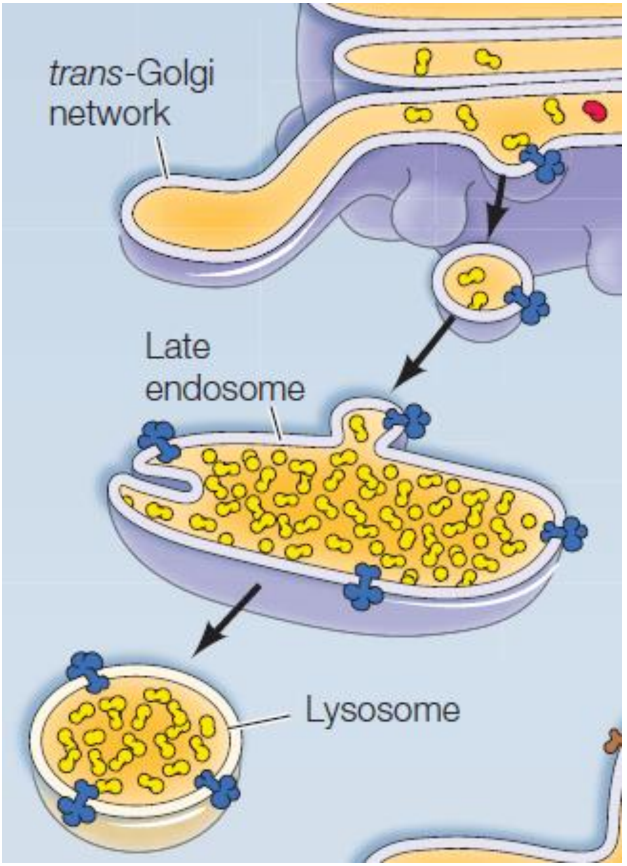


For **apical** surface proteins, these codes are modified by GPI (glycosyl phosphatidyl inositol) sugars.

Whereas for **basolateral** surface proteins, we have special sequences found in the primary structure of proteins

From the Golgi to the lysosomes

- Transport vesicles carrying acid hydrolases from the trans-Golgi network fuse with late endosomes, which mature into lysosomes.

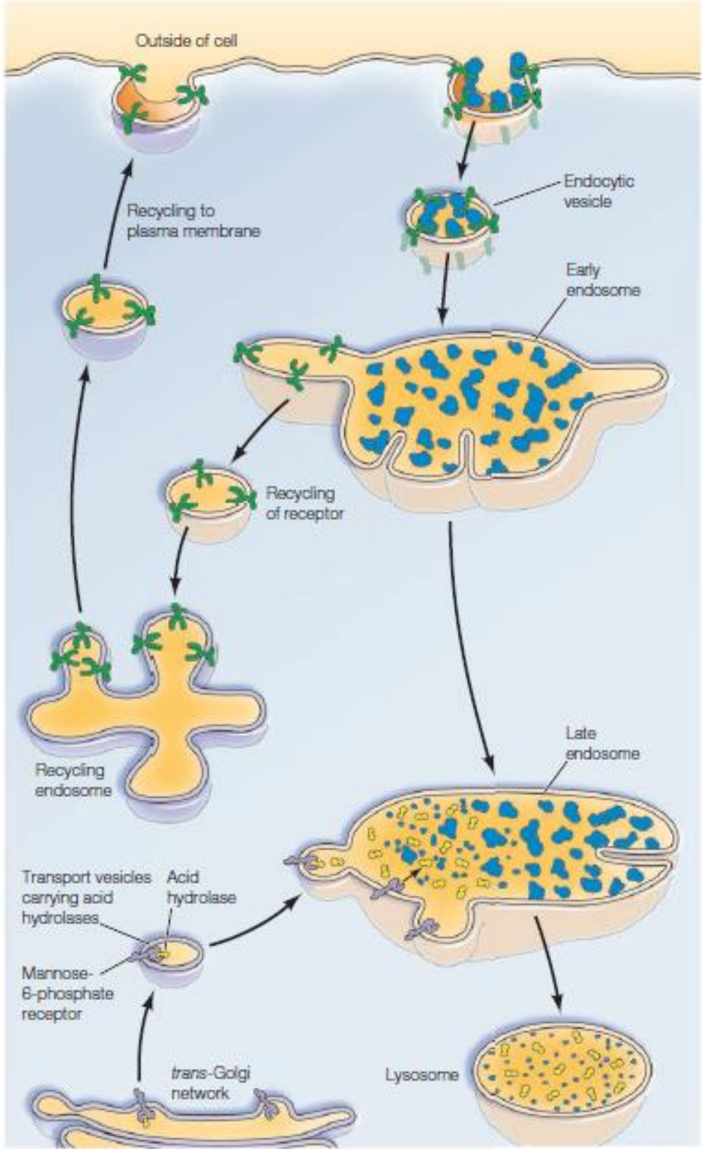


Endocytosis (from outside the cells to inside)

- 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: early endosomes have a pH of around 6.5, late endosomes around 5.5, and lysosomes a very acidic pH of about 4.5 to 5.0.*

Try to memorize pH values



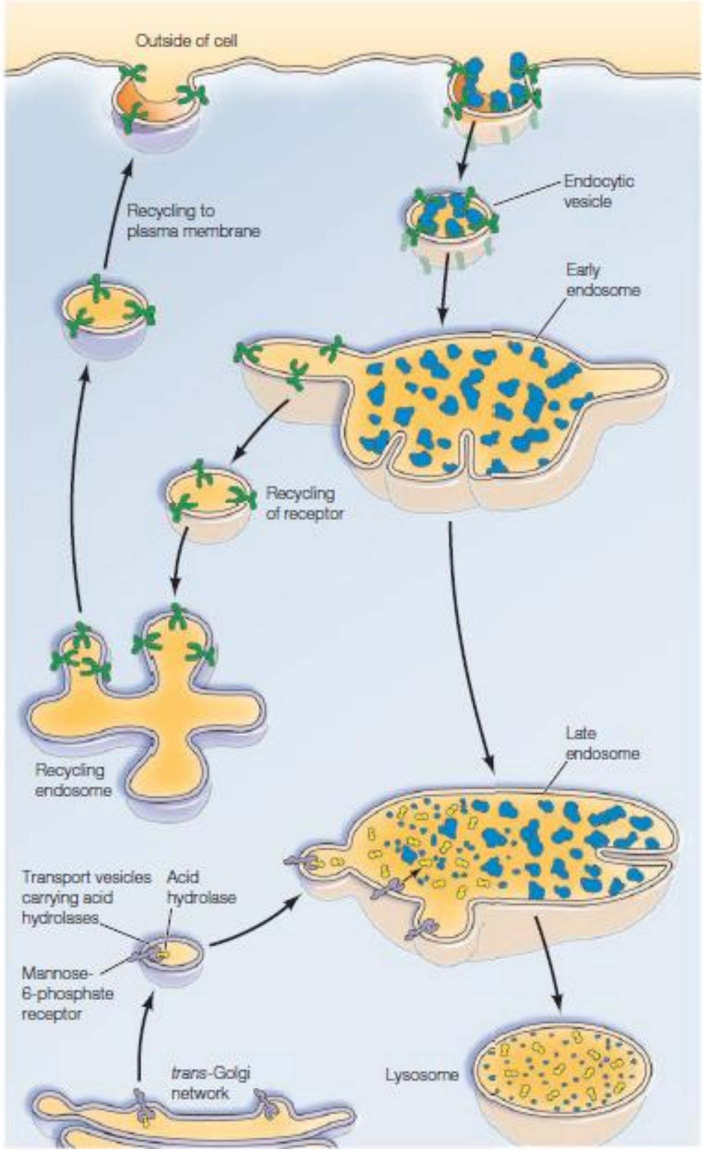
Endocytosis (from outside the cells to inside)

- Endocytosis works by the formation of a vesicle which pinches of the plasma membrane. Then, the newly formed vesicle fuses with an **early endosome**. This early endosome then matures into a late endosome by the addition of some proteins. The low pH (6.5) found inside early endosome is present so that there will be dissociation between the ligand and the receptor. Therefore, the ligands remain in the early endosome while the receptors has 3 possible pathways:

1- Transfer directly into the plasma membrane

2- Transfer to a recycling endosome where it will go back to the plasma membrane

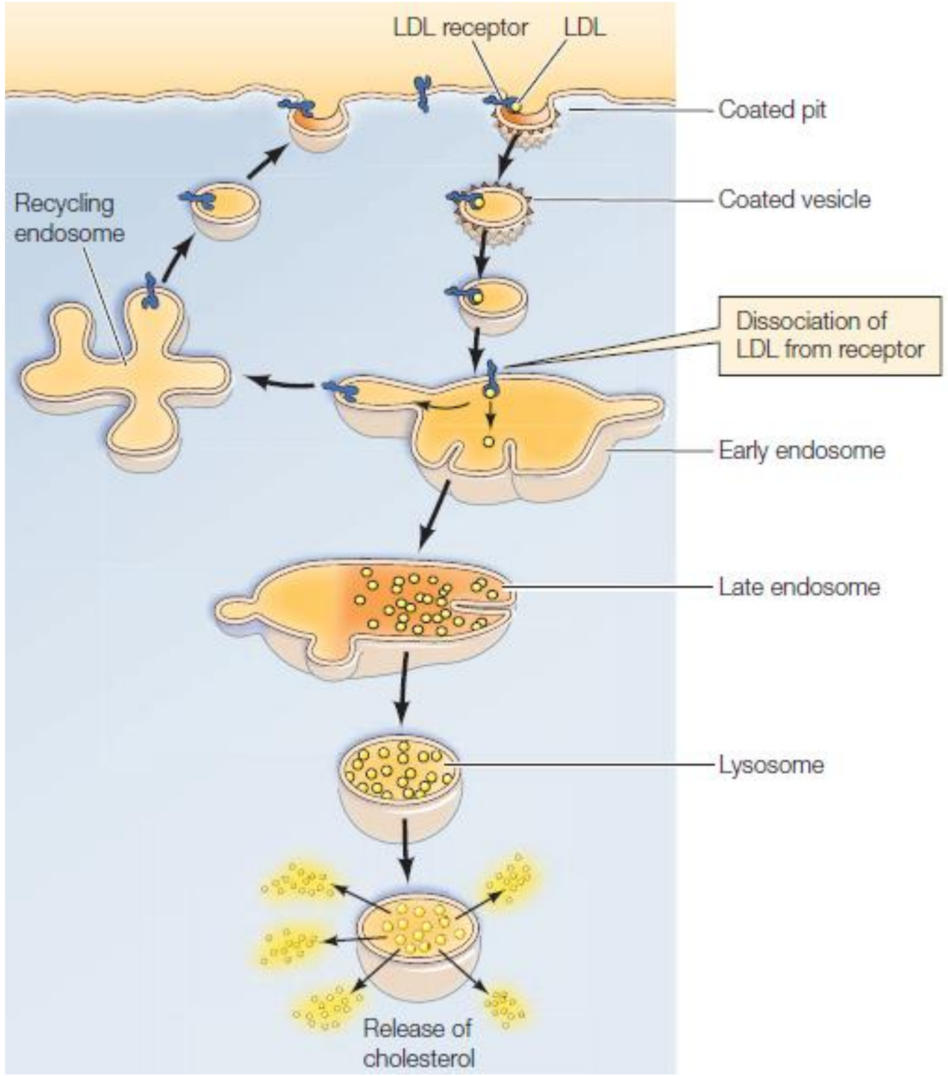
3- Remain in the early endosome until it matures to a late endosome then lysosome where it will be degraded



Clathrin-dependent endocytosis

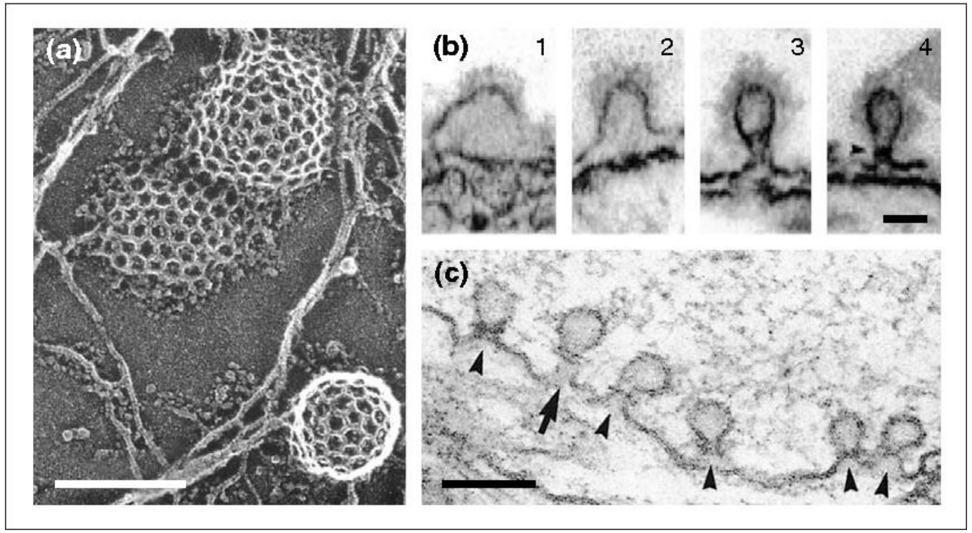
Receptor-mediated endocytosis

- There are different types of endocytosis, one of which is the receptor-mediated endocytosis, which requires the binding of a ligand to the receptor to stimulate endocytosis
- Ligands bind to their receptors stimulating endocytosis by the formation of a vesicle from the plasma membrane which fuses with early endosome.
- 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 and later into a lysosome.
- The ligands in the lysosomes are degraded and are released outside the lysosome via certain channels. (They could get modifications before they degrade). Their products are released into the cytosol.
- Example: removal of plasma cholesterol by low-density lipoprotein (LDL) receptor
- LDL (lipoprotein made of phospholipids and proteins) is rich with cholesterol (since its function is to transfer cholesterol into the cell). Inside the lysosomes, the LDL molecules degrade into cholesterol molecules which undergo modification, simplification, and then release into the cytosol.

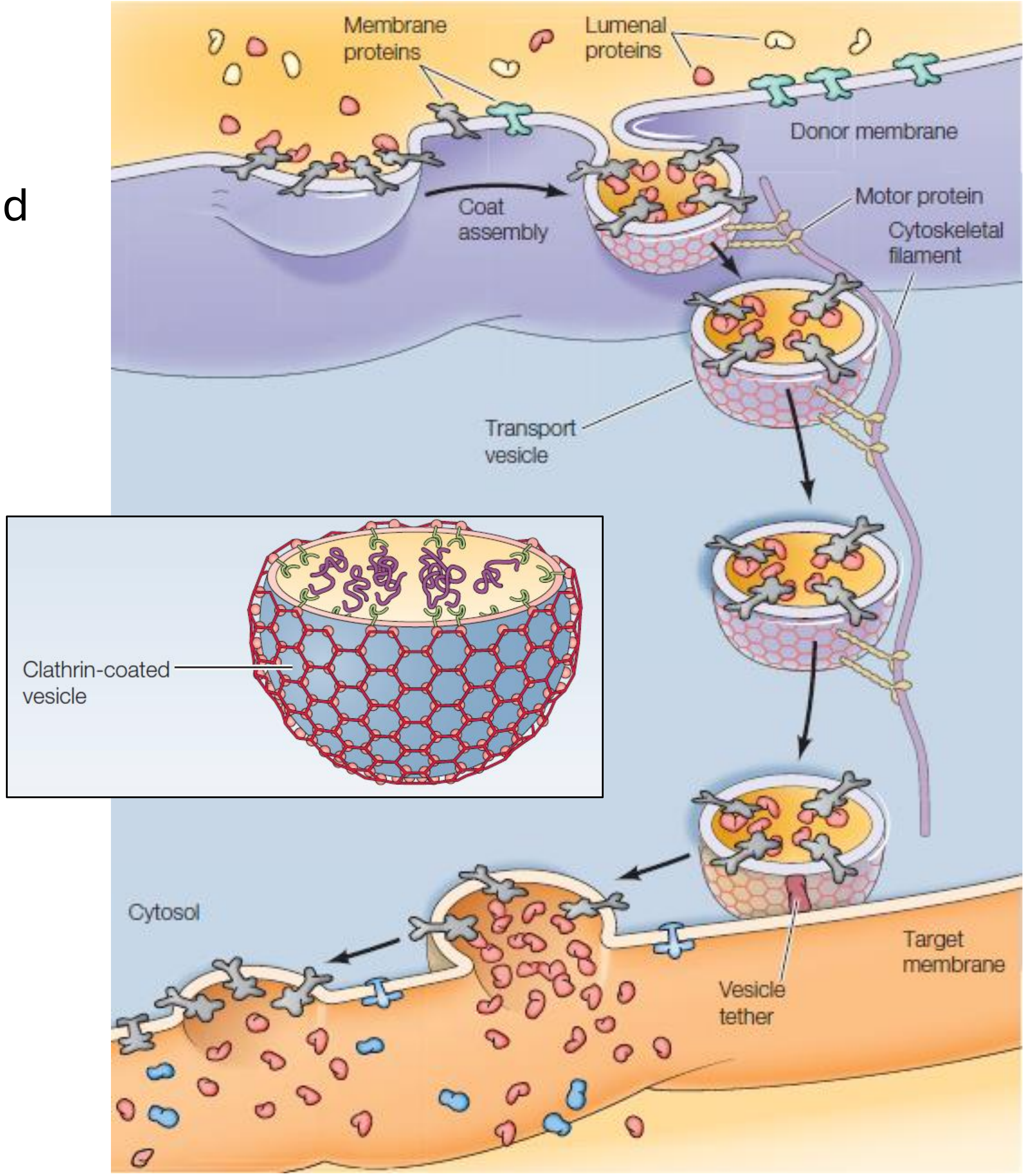


What is clathrin?

- Before budding of a transport vesicle, they get coated by a protein called clathrin.
- When the clathrin-coated vesicle docks at its target membrane, it gets uncoated, and fuses with the membrane.
- Why clathrin? It shapes the budding vesicle, selectively sorts cargo for packaging, facilitates the budding process, and regulates the movement of vesicles between the membranes.



SEM image of clathrin



What is clathrin?

- Receptor-mediated endocytosis is a type of clathrin dependent endocytosis. Clathrin is a protein that makes a network on the outer surface of the vesicle. It is made up of 3 arms, when these arms connect together, they form a coating around the vesicle which forms when the ligand binds the receptor. Before the fusion of the vesicle with the target membrane, this clathrin is dissociated and released.
- Clathrin has several functions:

-It preserves the shape of the vesicle (ball-like structure)

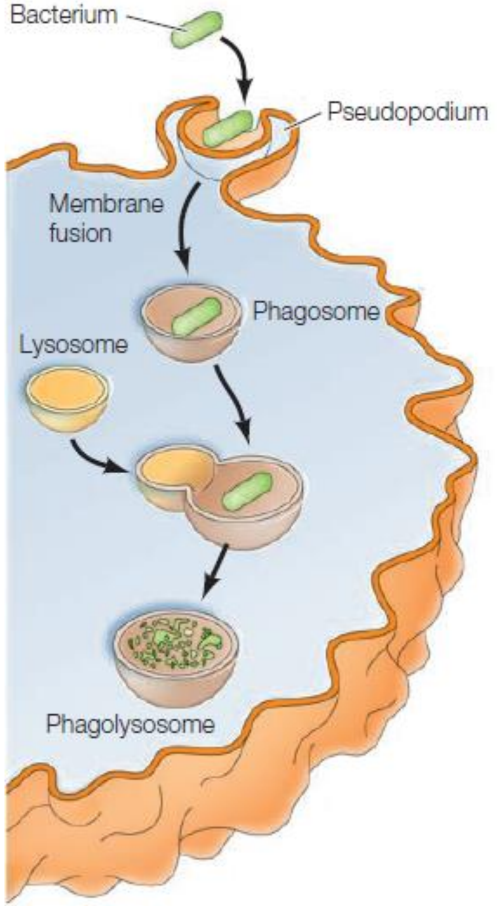
-Sorting of materials that are found inside the vesicle

-Helps in the movement of the vesicle along the microtubules

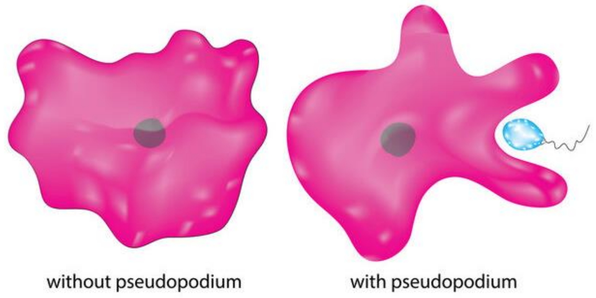
-Helps in the process of budding from the plasma membrane

Phagocytosis (another type of endocytosis) (usually occurs in immune cells such as macrophages) (*Clathrin-independent)*

- Phagocytosis is the process of cell eating through vesicles (phago=eating, cytosis=cell)
- The binding of a bacterium to the cell surface stimulates the extension of a pseudopodium (extending arms from the membrane), which eventually engulfs the bacterium.
- Fusion of the pseudopodium/pseudopods membranes then results in the formation of a large intracellular vesicle (a phagosome).
- The phagosome fuses with lysosomes (containig hydrolytic enzymes) to form a phagolysosome within which the ingested bacterium is digested. This is the process is by which our immune cells remove bacterial cells.
- *Macropinocytosis (also clathrin-independent) is cell drinking via the formation of small vesicles.* (endocytosis of water and nutrients inside)

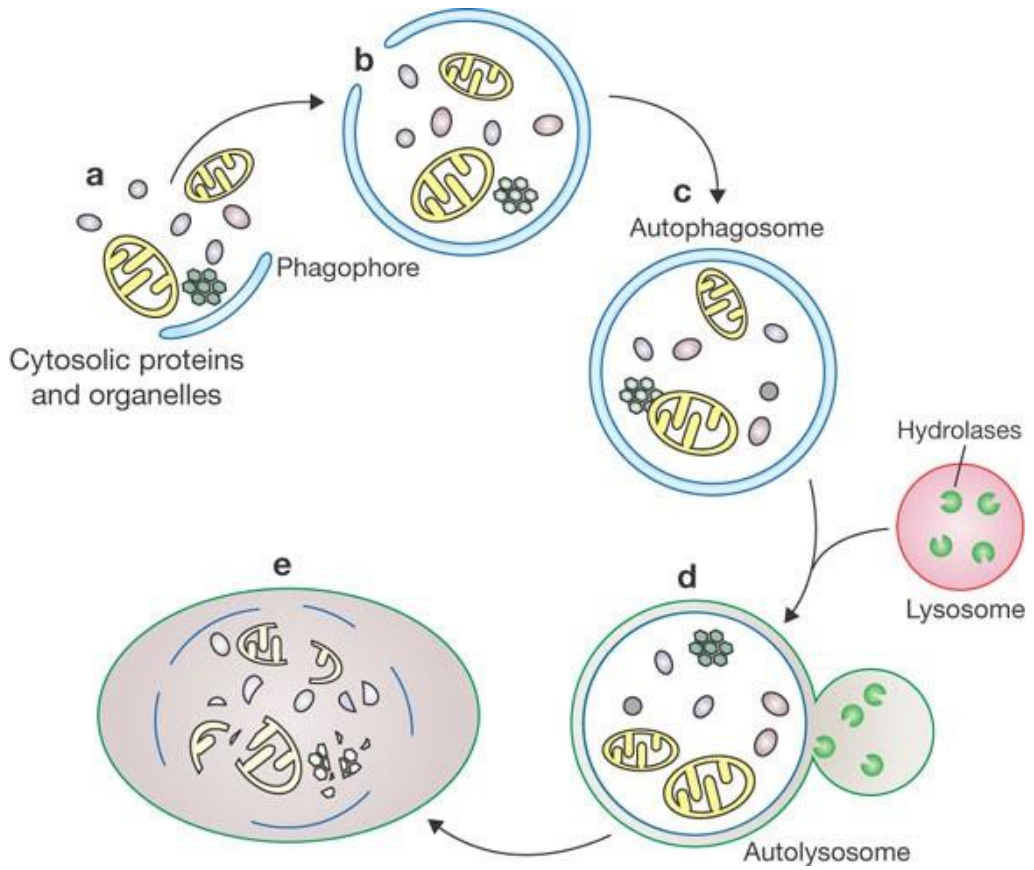


A pseudopodium is a temporary arm-like projection of a eukaryotic cell membrane



Autophagy (self-eating)

- Autophagy involves the formation of a large vesicle called autophagosome **(from an immature structure called phagophore)** at the surface of ER with the with the help of a protein called Atg9 **and other proteins**.
- **These vesicles are formed de novo (they form from scratch).**
- The formation of autophagosome is concomitant with the encapsulation of dysfunctional and harmful **intracellular** substances and proteins, as well as damaged organelles (such as mitochondria).
- Autophagosomes fuse with lysosomes to form large **auto lysosomes** in which their contents are digested.



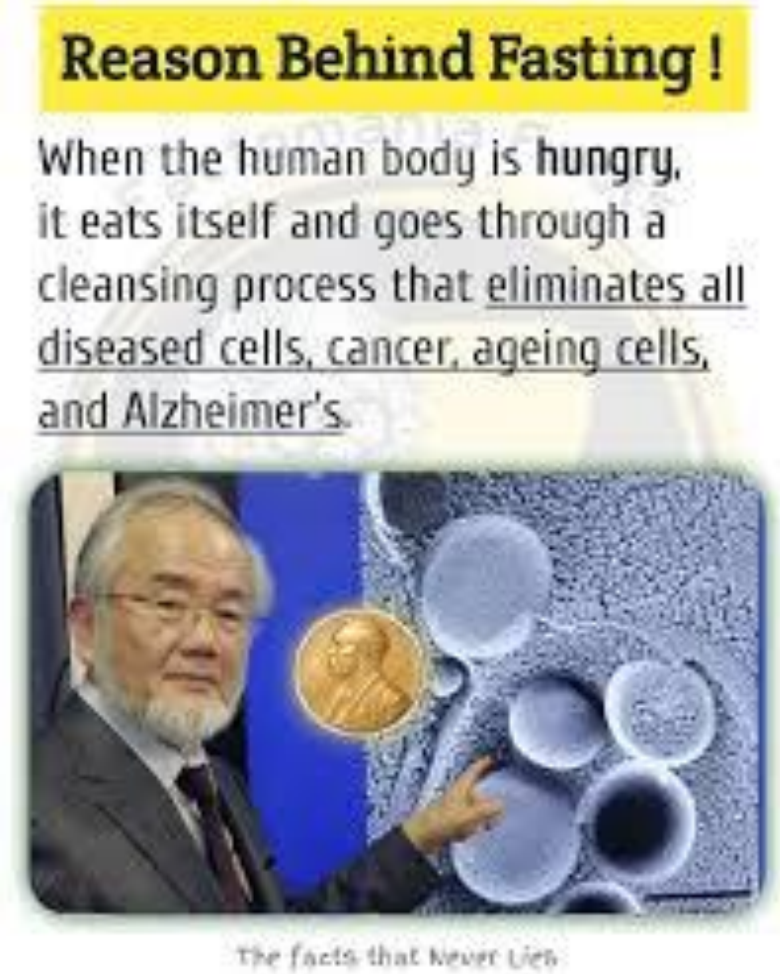
Autophagy in health and disease

The good

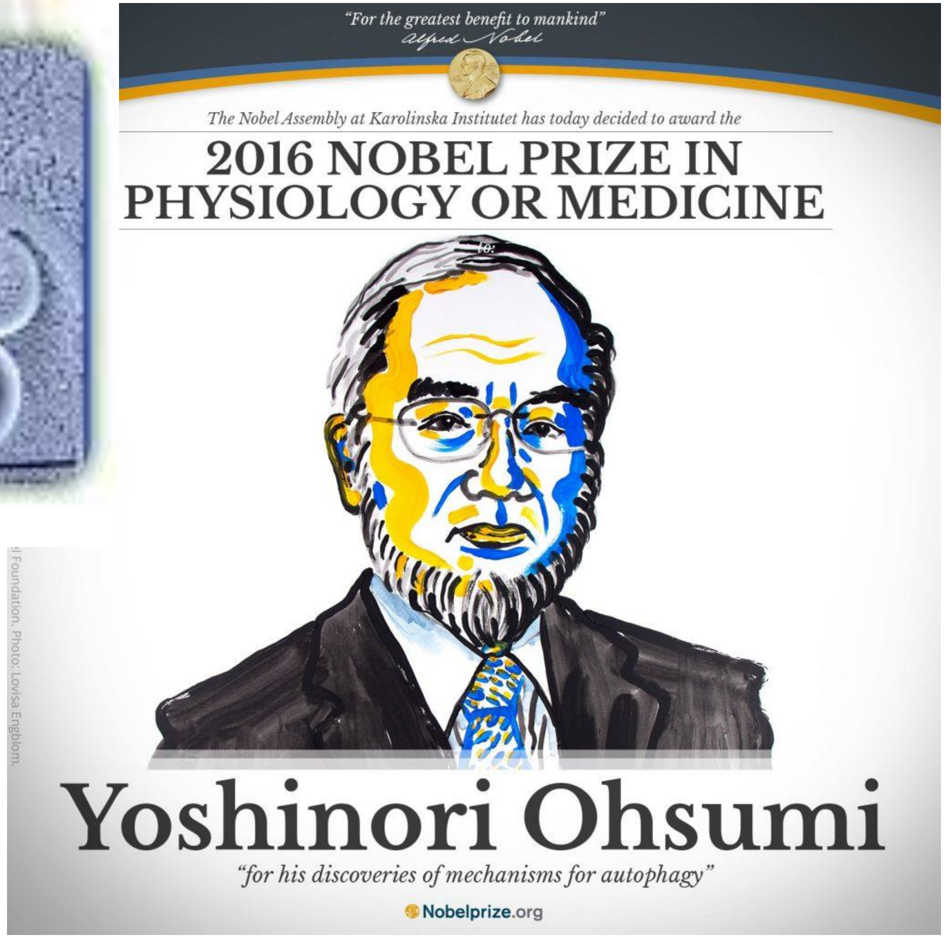
- Removing dysfunctional and harmful substances and proteins, as well as damaged organelles (such as mitochondria).
- Allowing survival during starvation to eliminate nonessential macromolecules (such as extra mitochondria) and replenish ATP.
- Remodeling tissues during development.
- Preventing cancer

The bad and the ugly

- Autophagy can help cancer cells survive and proliferate under suboptimal conditions.
- Autophagy is also implicated in several neurodegenerative diseases.
  - Genes responsible for the autophagic degradation of dysfunctional mitochondria in degenerating cells are found to be mutated in early-onset Parkinson's disease.



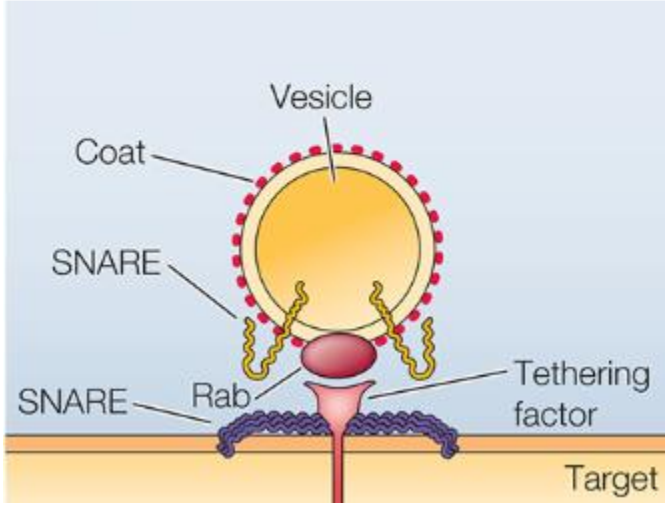
Fasting helps in autophagy, as a result, damaged organelles (mitochondria), and harmful substances are degraded.
So, fasting keeps your health
الصيام حكمة من ربنا، نحافظ بها على صحتنا ونكسب بها أجرًا عظيمًا.



The mechanism of vesicular transport

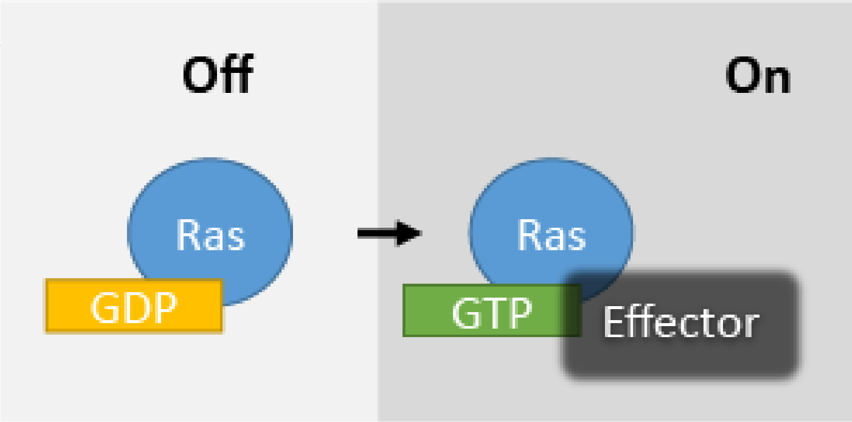
Delivery of vesicles: targeting and fusion

- Vesicular small G proteins called Rab determine the membrane targets of vesicles.
    - There are over 60 Rab proteins that mark different transport vesicles and determine their destinations. (These Rab molecules determine the origin and destination of vesicles. These Rabs also interact with SNARE proteins)
- v-SNAREs (Vesicular SNAREs) -t-SNAREs (Target SNAREs which are found on the target membrane) proteins are responsible for vesicular fusion with the target membranes.

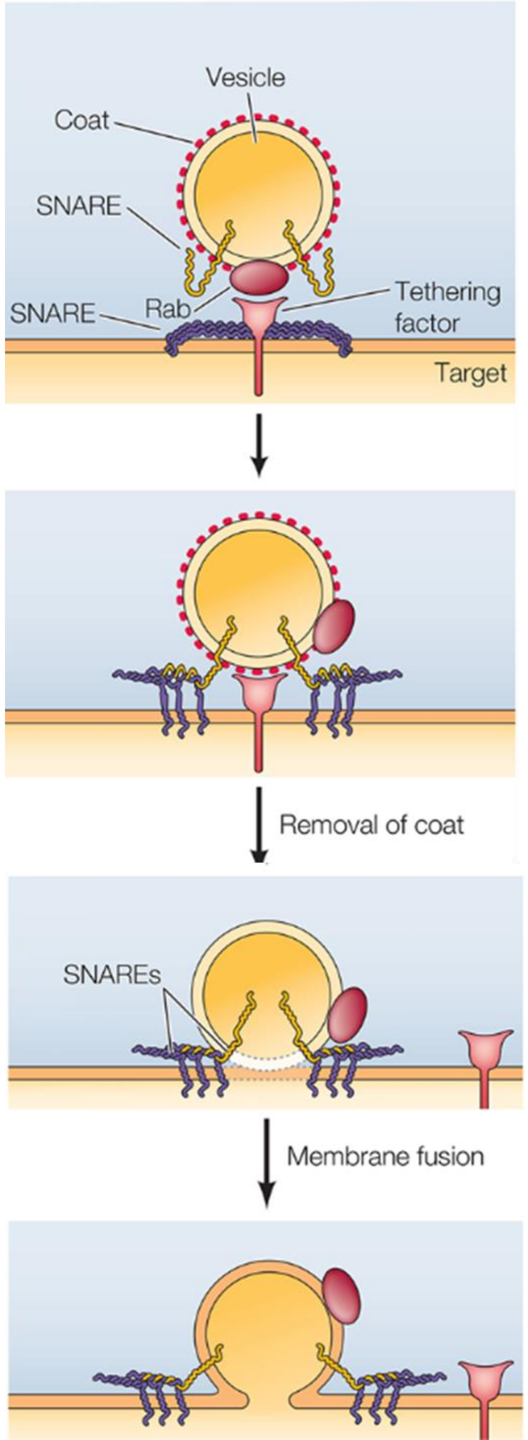


Rab GTPase	Site(s) of action[a]
Rab1	ER to Golgi, intra-Golgi
Rab4	EE to PM
Rab5	PM to EE
Rab6	Golgi to ER, intra-Golgi, EE to TGN
Rab7	EE to LE, LE to lysosome
Rab9	LE to TGN
Rab10	Golgi-associated
Rab11	RE to PM, EE to TGN, TGN to PM

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



G-proteins are inactive when bound to GDP and active when bound to GTP which allows them to bind to their specific receptors.

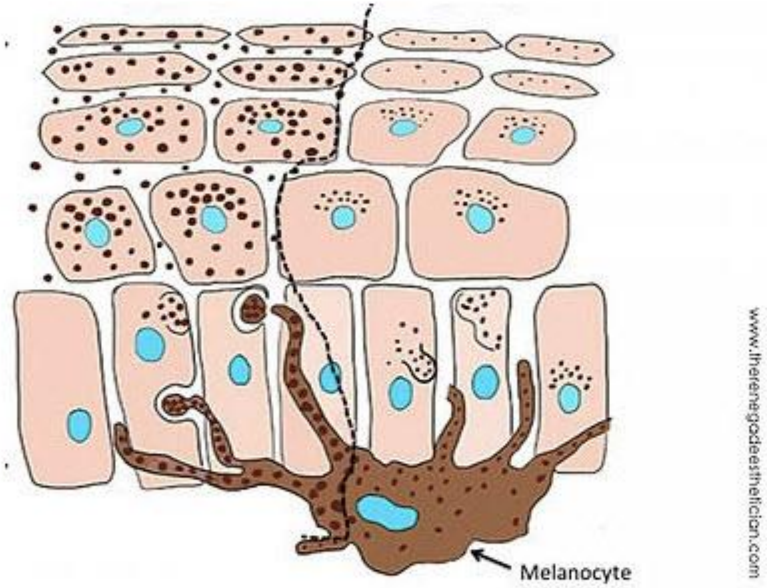


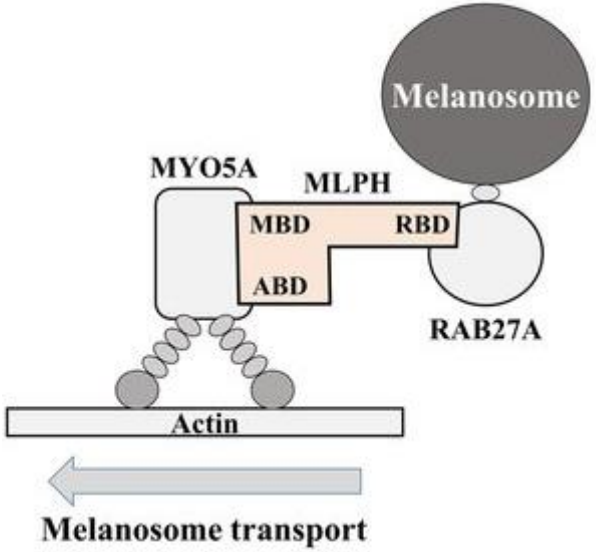
The mechanism of fusion

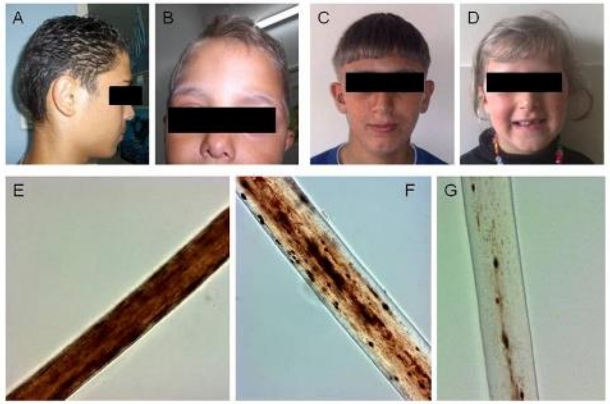
1. A Rab protein binds to a tethering factor(**protein**) associated with the target membrane.
2. SNAREs on the vesicle and target membranes complex together (**interaction forms between them**).
3. The SNAREs zip together (بلفوا حولين بعض), bringing the vesicle and target membranes into close proximity
4. The membranes fuse (vesicle fuses with the target membrane and becomes a part of it).

Griscelli syndrome (GS)

- A rare genetic condition
- Mutations in MYO5A (a motor protein), RAB27A, and MLPH (a Rab effector protein) genes that function as a protein complex in melanosome transport and fusion during the transfer of melanin-containing melanosomes from melanocytes to keratinocytes..
- Pigmentary dilution of the skin, silver-grey hair, melanin clumps within hair shafts

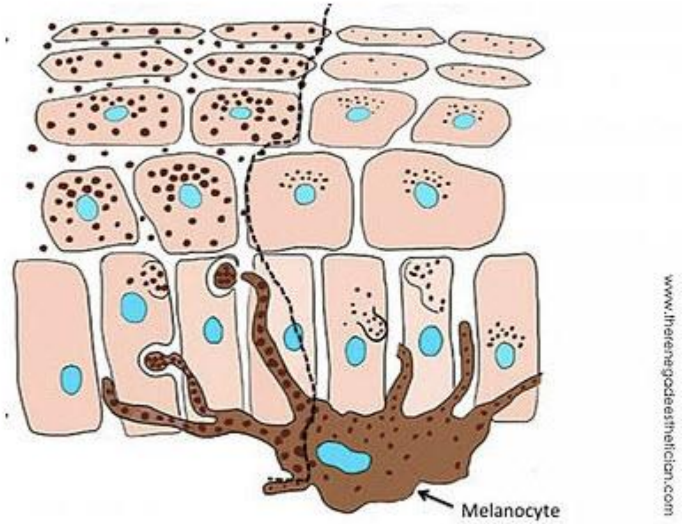






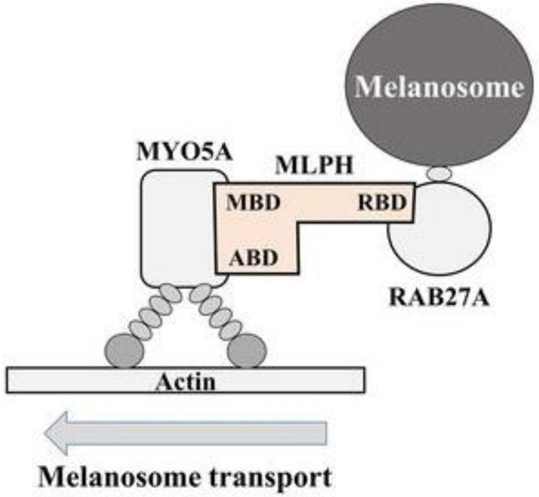
Griscelli syndrome (GS)

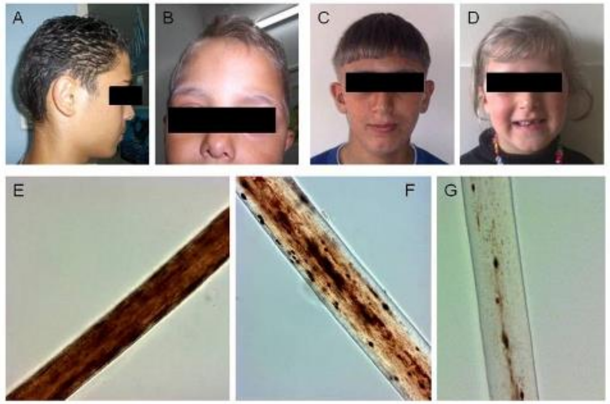
- Griscelli syndrome is a disease where the hair becomes gray instead of the typical brown or black. Melanin(which gives the brown/black color of hair) is produced by melanocytes and are stored in vesicles called melanosomes. These vesicles transfer from melanocytes into keratinocytes which will nutrition hair strands. This transfer occurs via the actin filament. There is a complex of 3 proteins which are responsible for the movement of melanosomes which are: -MYO5A, -MLPH, -RAB27A. MYO5A(Myosin) is the motor protein which provide the energy for movement. RAB27A is the targeting molecule which specifies the destination of melanosomes. MLPH is a mediator that binds MYO5A to RAB27A. If a mutation occurs in any of these proteins, melanosomes can't move from melanocytes to keratinocytes so melanin will remain clustered in a specific region which makes hair greyish



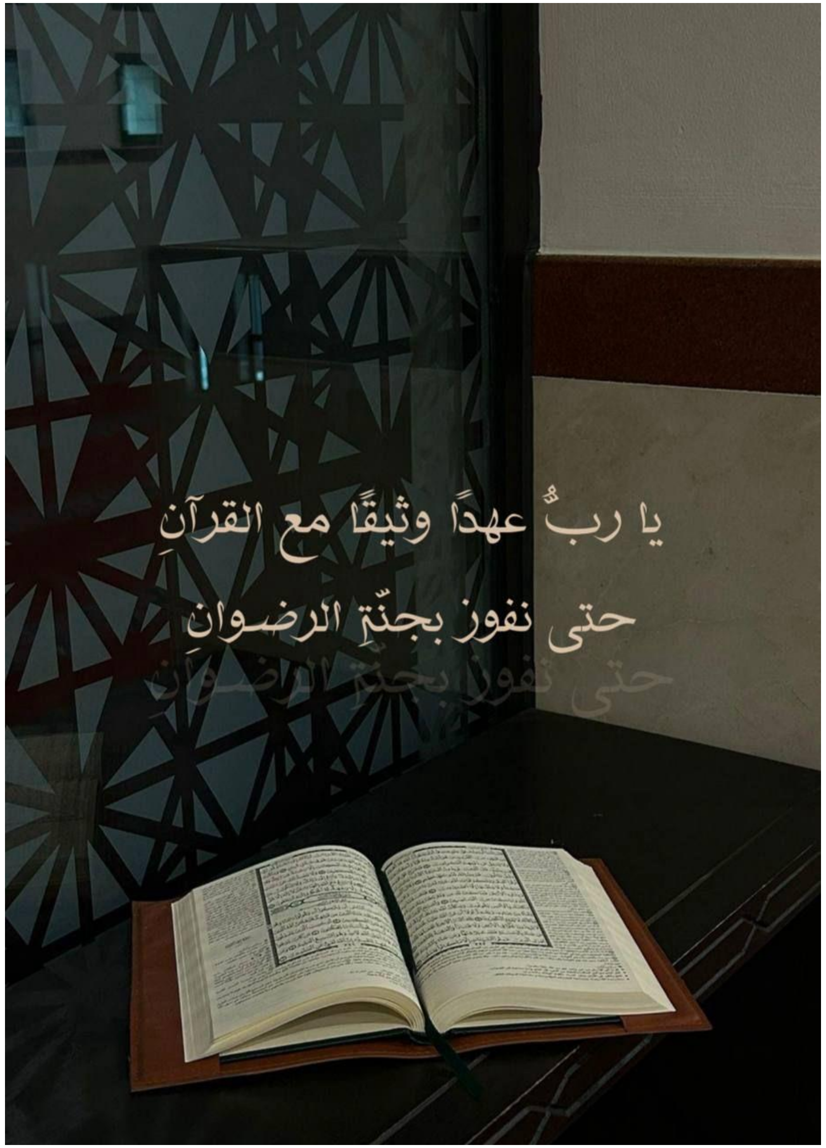
Griscelli syndrome since hair is greyish

WT(Wild type)Normal Phenotype





Additional Resources: رسالة من الفريق العلمي:



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	12 10	Proteins in the ER has signal peptides which lead these proteins into either nuclear membrane, peroxisomal membrane, or the golgi apparatus. (a lipid that has sphingosine as a backbone instead of g)	Proteins go into the ER with the help of signal peptides then these proteins go into either nuclear membrane, peroxisomal membrane, or the golgi apparatus. (a lipid that has sphingosine as a backbone instead of glycerol)
V1 → V2			