

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ  
الَّذِي عَلَّمَ بِالْقَلَمِ ۖ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ۝

## CYTOLOGY

◆ MID | Lecture 4

# Golgi apparatus & vesicular transport pt.2

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# ***Color coding used in the modified:***



*Black: the original slides*



*Orange: the doctor's explanation/words*



*Gray: additional information and explanation*



*Red: important information*

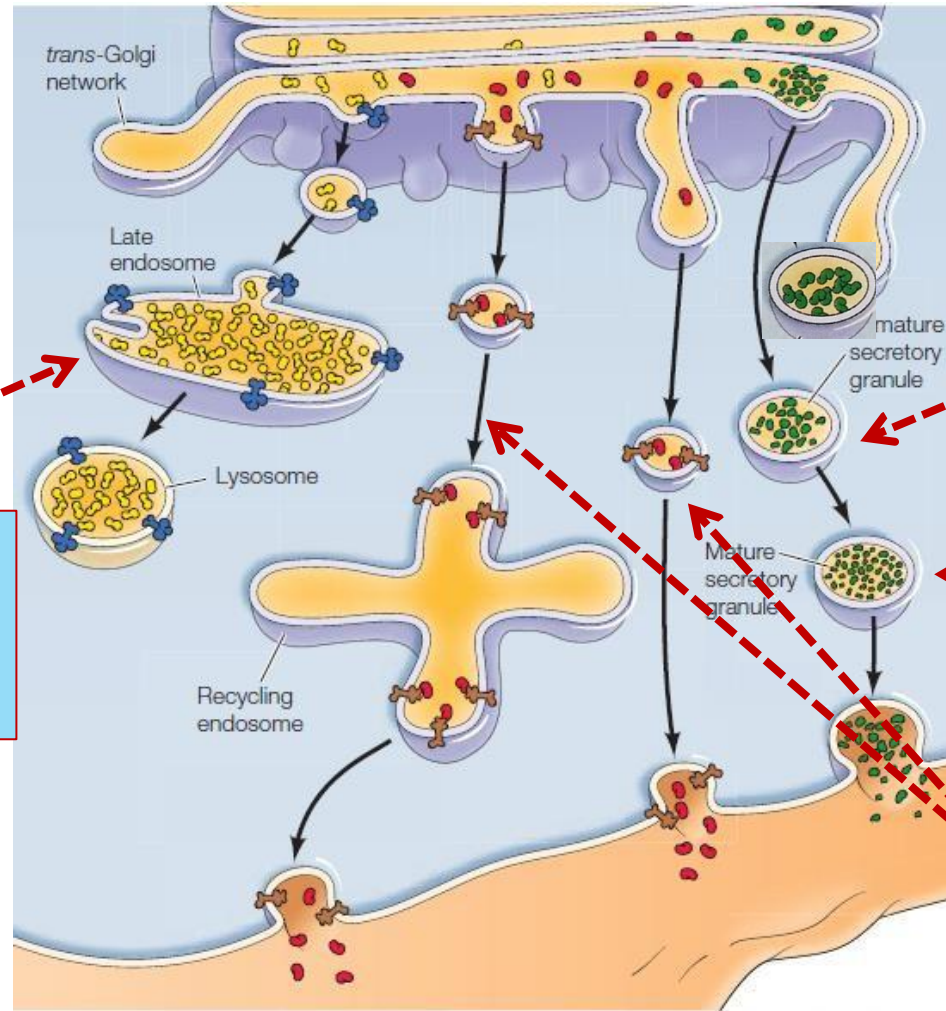
# The processes of vesicular transport

**In the last lecture we talked about:**

- **Secretion:** The release of molecules outside the cell ( exo : out ).
- We also talked about the movement of vesicles from the Golgi apparatus to the lysosomes.
- The signal on the proteins that directs them to the lysosomes is : **Mannose-6-phosphate (M6P)**. In the trans-Golgi, proteins tagged with Mannose-6-phosphate bind to a specific receptor called the **Mannose-6-phosphate receptor (M6P receptor)**.

If the Mannose-6-phosphate receptor is defective, I-cell disease can occur.

# The secretory pathway (exocytosis)



Cytosis: a cellular process of moving material across membranes

Protein processing in Immature secretory vesicles

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

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

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)

**Exocytosis can be regulated or unregulated**

## **1. Unregulated exocytosis :**

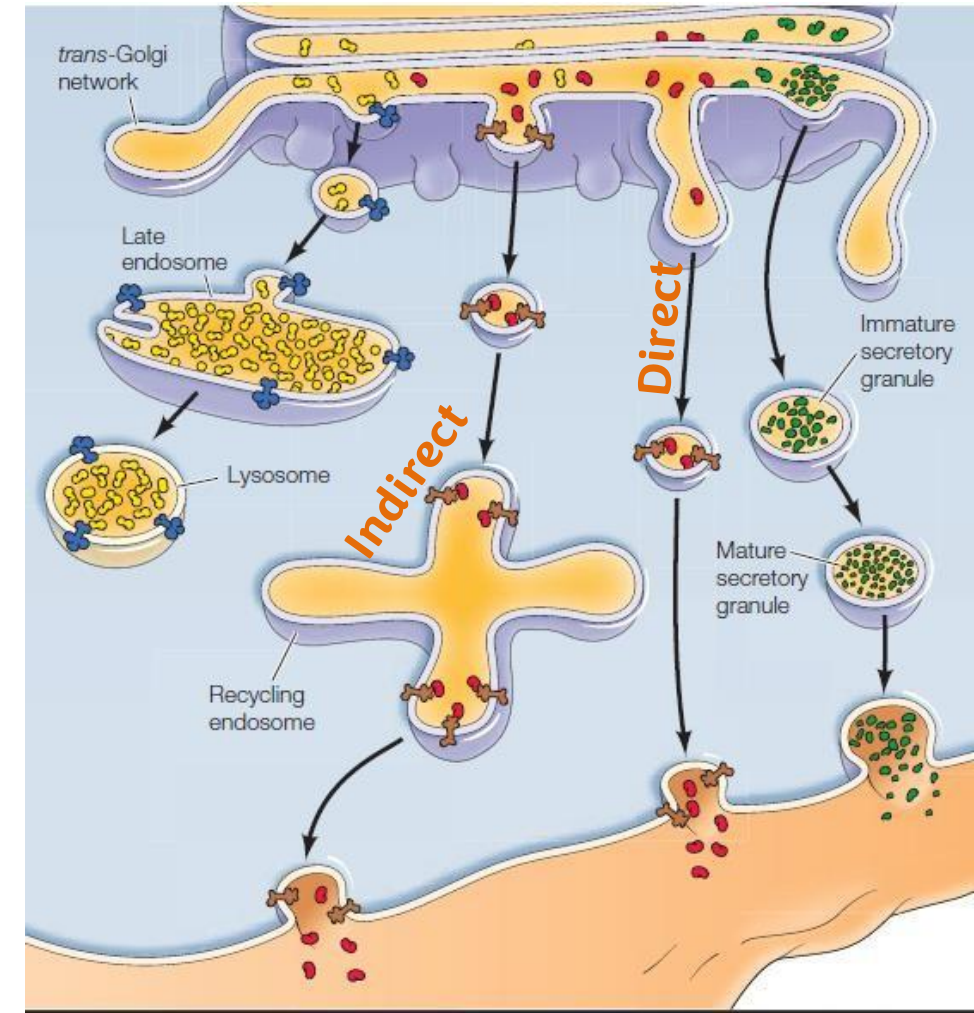
It is the continuous release of molecules from the cell to the outside.

It can occur in two ways:

- **Direct pathway:** Vesicles are formed from the trans-Golgi and continuously move toward the plasma membrane. The vesicles then fuse with the plasma membrane and release their contents outside the cell.
- **Indirect pathway:** Vesicles pass through what is known as **recycling endosomes**, which act as a mediator compartment. They mediate the transport and release of vesicular proteins to the outside of the cell.

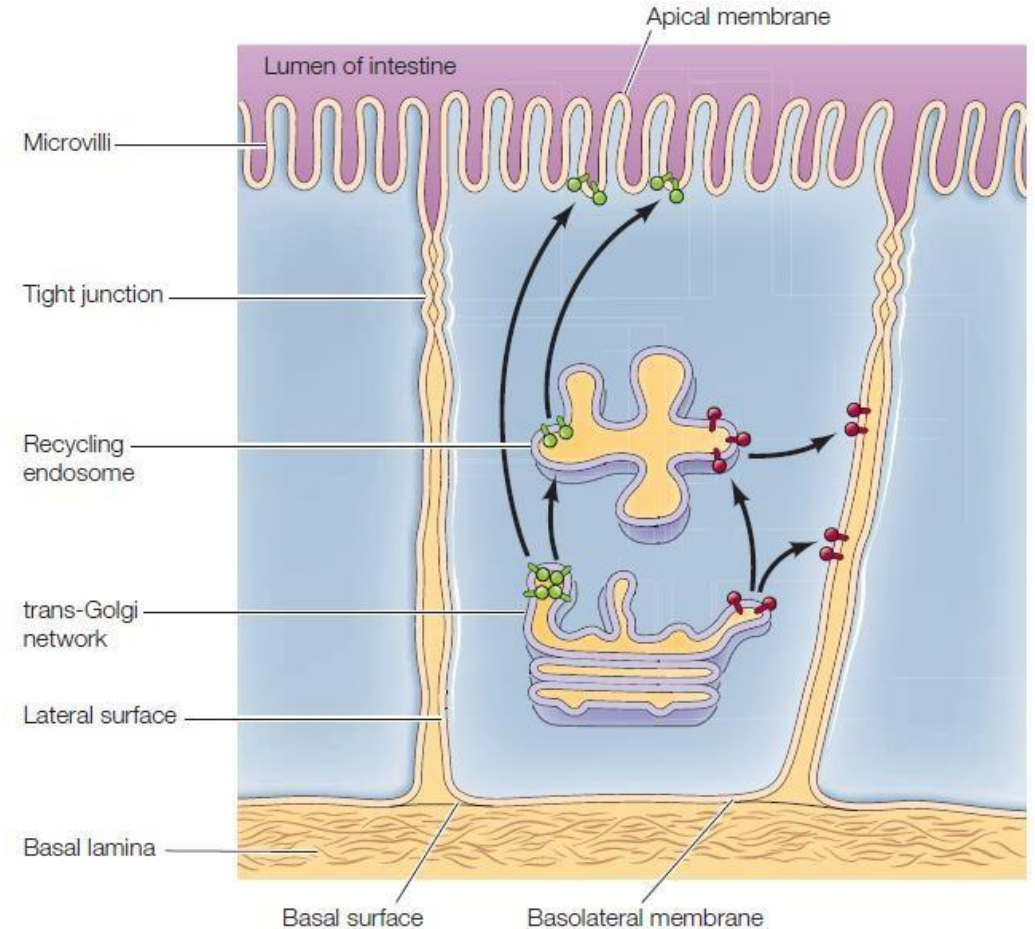
## **2. Regulated exocytosis:**

For example, the release of neurotransmitters. Neurotransmitters are stored in vesicles that are lined up near the plasma membrane. When a signal arrives, the vesicles fuse with the plasma membrane, releasing their contents outside the cell.



# 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).



**SEE NEXT SLIDE**

The movement of proteins specifically to one side of the membrane, rather than the other, occurs in **polarized cells**. **Polarized cells** are cells that have two poles, such as intestinal cells and cells lining the lungs. These cells have two main membrane domains:

- **Apical surface:** The top surface of the cell, which faces the lumen in intestinal cells.
- **Basolateral membrane:** The bottom surface of the cell, which face the underlying tissues and blood vessels.

In intestinal cells, nutrients are absorbed from the lumen through the apical surface and then transported across the cell and released from the basolateral side into the blood vessels to be distributed throughout the body. Therefore, the proteins located on the apical surface are different from those present on the basolateral surface.

**How are these proteins targeted to the correct side?**

**1. Basolateral proteins:**

These proteins contain specific amino acid sequences that act as sorting signals. These signals allow the cell to recognize that the proteins must be transported to the basolateral membrane ( at this level, we do not need to know the exact sequences.)

**2. Apical proteins:**

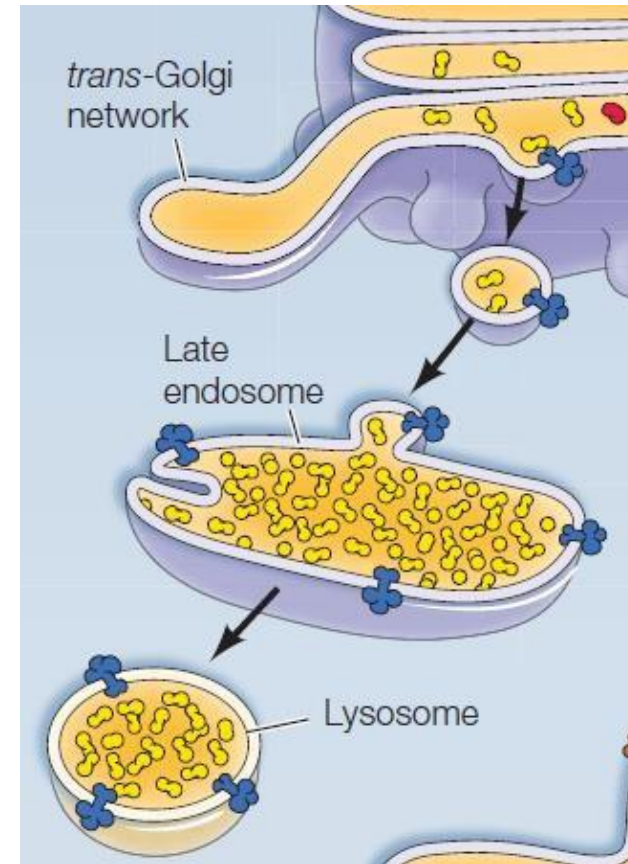
Some apical proteins are modified by the addition of a **GPI (glycosylphosphatidylinositol)** which acts as a signal directing them toward the apical surface.

**Role of tight junctions:**

Tight junctions are protein structures that seal the space between adjacent cells. They prevent proteins from moving freely between the apical and basolateral membrane domains, helping maintain the different protein compositions of these two surfaces.

# From the Golgi to the lysosomes

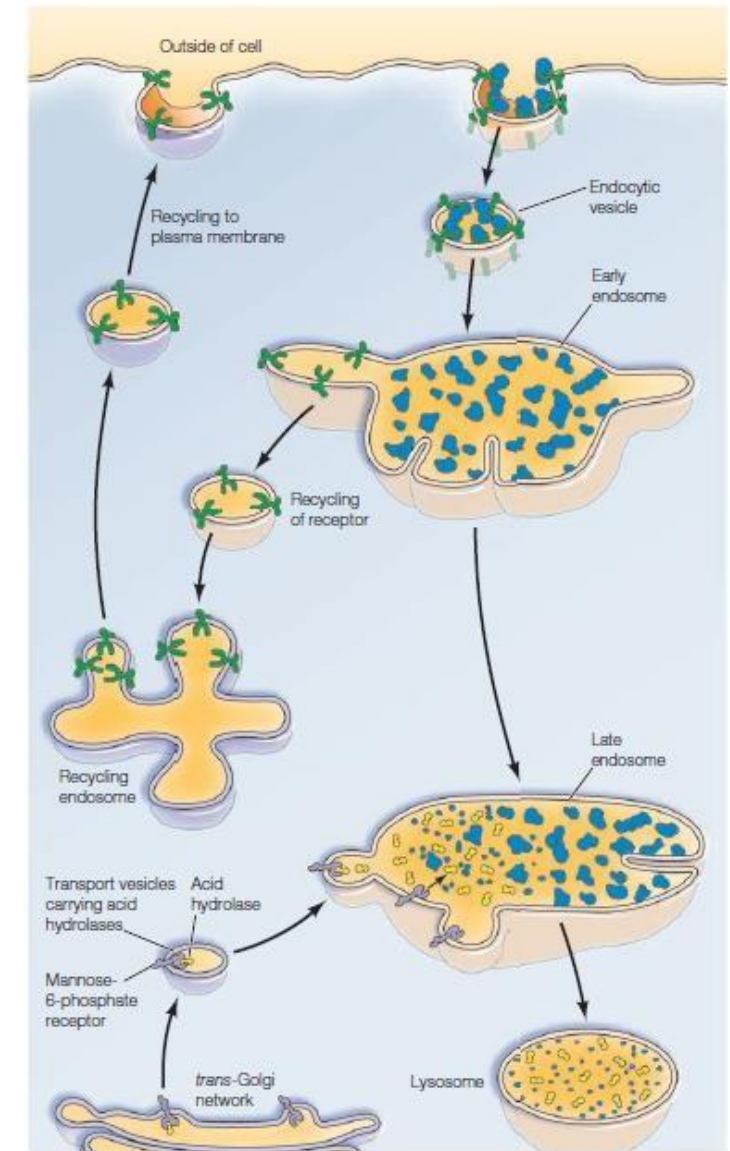
- Transport vesicles carrying acid hydrolases from the trans-Golgi network fuse with late endosomes, which mature into lysosomes.



# 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

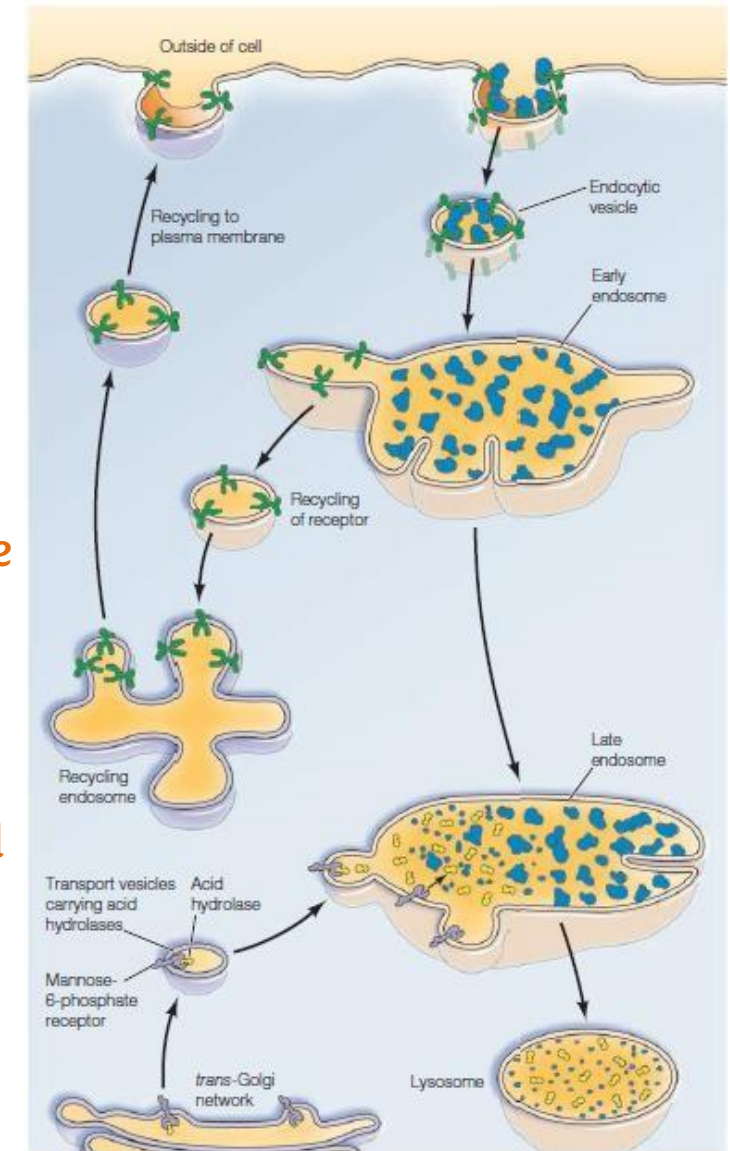


More illustrations in the next slide

# Endocytosis (from outside the cells to inside)

**Endocytosis:** The movement of molecules from outside the cell to the inside. There are different types and mechanisms of endocytosis, but the general process is as follows:

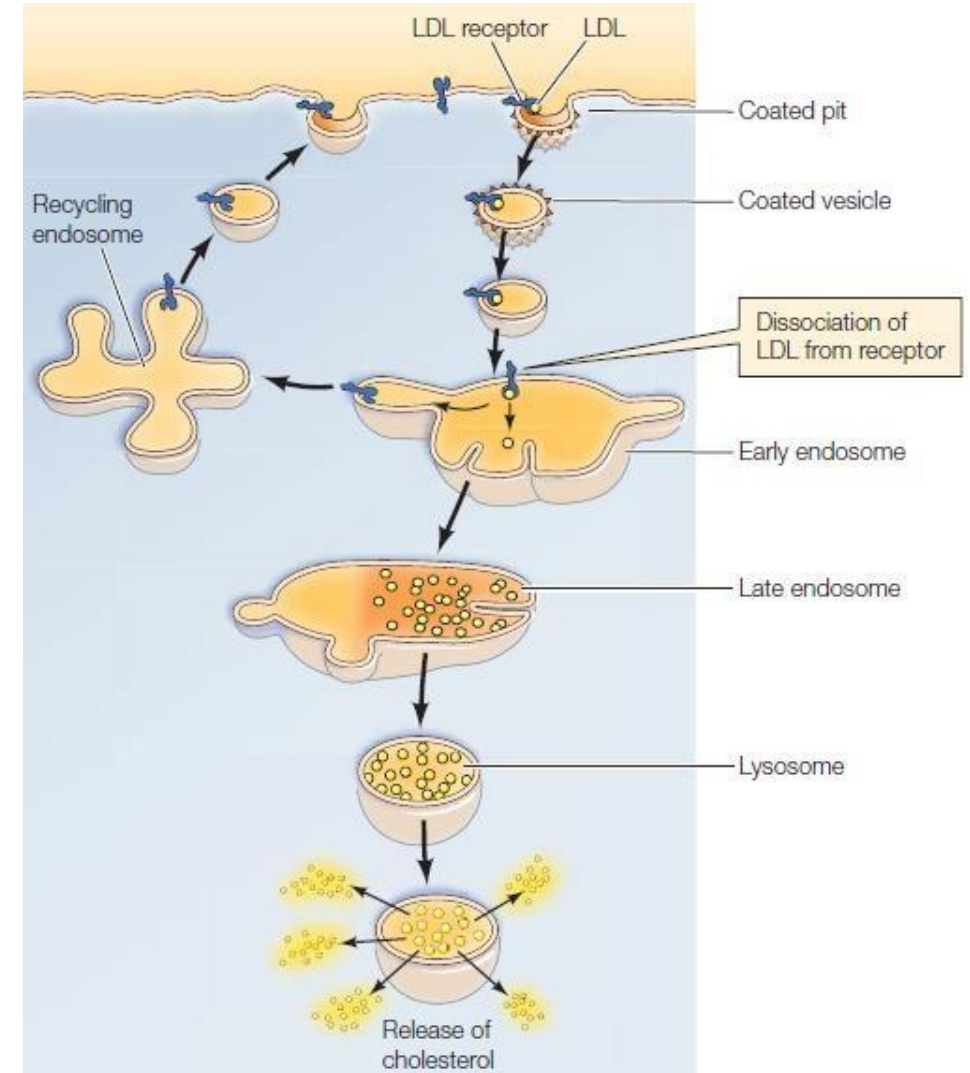
1. **Budding:** A portion of the plasma membrane bends inward and forms a bud (برعم) containing molecules from outside the cell.
2. **Pinching off:** The bud separates from the plasma membrane, forming a vesicle inside the cell.
3. **Early endosomes:** These vesicles fuse with a larger compartment called the **early endosome**, which has an acidic pH of approximately 6.5 (less than 7).
4. **Late endosomes:** Early endosomes mature into late endosomes, which have a lower pH of approximately 5.5.
5. **Lysosomes:** Late endosomes mature into lysosomes, which have an even lower pH of approximately 5. Lysosomes contain enzymes that break down molecules and cellular materials.
6. **Recycling:** Some molecules are sorted in the early endosomes and returned to the plasma membrane. This recycling can occur through **recycling endosomes**.



# 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 (moving back to the plasma membrane) and early endosomes mature into late endosomes
- Example: removal of plasma cholesterol by low-density lipoprotein (LDL) receptor



- We are going to talk about different types and mechanisms of endocytosis. One type is called **receptor mediated endocytosis** or **clathrin-dependent endocytosis**.

- An example of clathrin-mediated endocytosis is **receptor-mediated endocytosis**, which is a very important mechanism of endocytosis.

• **Receptor-mediated endocytosis :**

What happens is that we have a receptor at the cell surface, and the ligand binds to the receptor. Sometimes, when the ligand binds to the receptor, it sends a signal inside the cell. However, in this case, **the receptor and the ligand** are endocytosed, resulting in formation of a vesicle that contains the receptor and the ligand bound to it

- This vesicle then fuses with the early endosome, which has a pH of approximately 6.5.

Because the pH is considered low , the ligand is released from the receptor. The receptor is then recycled back to the plasma membrane, where it can bind to another ligand and repeat the process.

- The ligand is released inside the early endosome, which then turn into late endosome and finally to lysosome, where it is degraded.

- **Receptor-mediated endocytosis is a regulated process.** It occurs when a specific ligand binds to its receptor. However, the receptor is not always recycled back to the plasma membrane. Sometimes, both the receptor and the ligand reach the lysosome, where they are degraded. Therefore, the receptor may either be recycled or degraded, depending on the type of receptor and the mechanism involved.

• **Example: LDL receptor-mediated endocytosis :**

- LDL stands for **low-density lipoprotein**. High levels of LDL cholesterol are associated with atherosclerosis, which can increase the risk of a heart attack.

- LDL carries cholesterol from the liver to peripheral tissues. LDL is formed in the blood from VLDL (very-low-density lipoprotein) as VLDL is metabolized.

What happens is that LDL travels to peripheral tissues, where it binds to the LDL receptor on the surface of peripheral cells.

Once LDL binds to its receptor, both are internalized through endocytosis inside vesicles. These vesicles fuse with early endosomes, where LDL separates from the LDL receptor.

The receptor then returns to the plasma membrane so it can bind to another LDL particle and repeat the process.

- LDL continues toward the lysosome, where it is degraded and cholesterol is released. The cholesterol then moves from the lysosome into the cytosol, where the cell can use it for different purposes, such as making cell membranes, synthesizing steroid hormones in cells capable of producing them, or storing it.

**Regulation of LDL receptor-mediated endocytosis**

This process is regulated according to the amount of cholesterol inside the cell.

When the cell has enough cholesterol, it sends signals that decrease the production of LDL receptors, reducing the amount of LDL cholesterol that enters the cell.

In this way, the cell prevents excessive cholesterol accumulation.

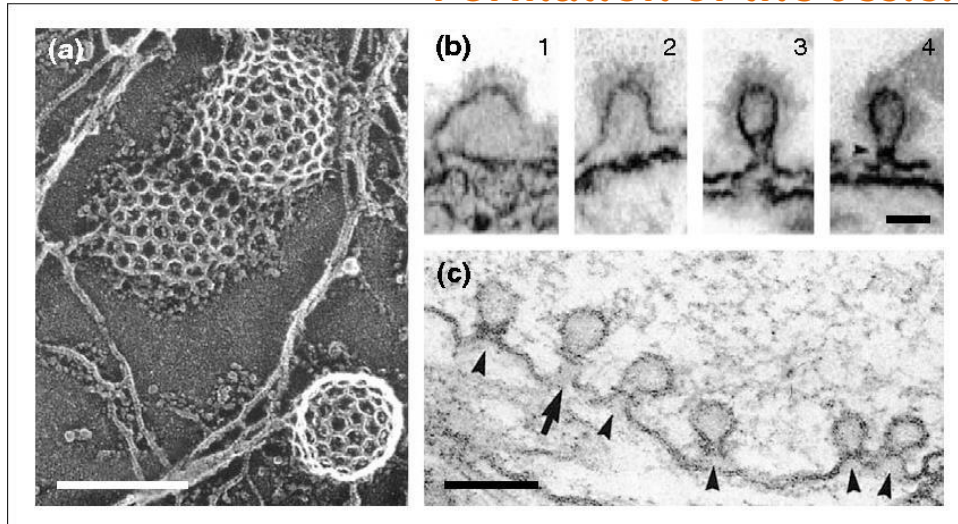
This process is called **receptor-mediated endocytosis**.

Understanding this mechanism helps scientists develop drugs that reduce cholesterol levels in the blood by affecting LDL metabolism and the availability of LDL receptors.

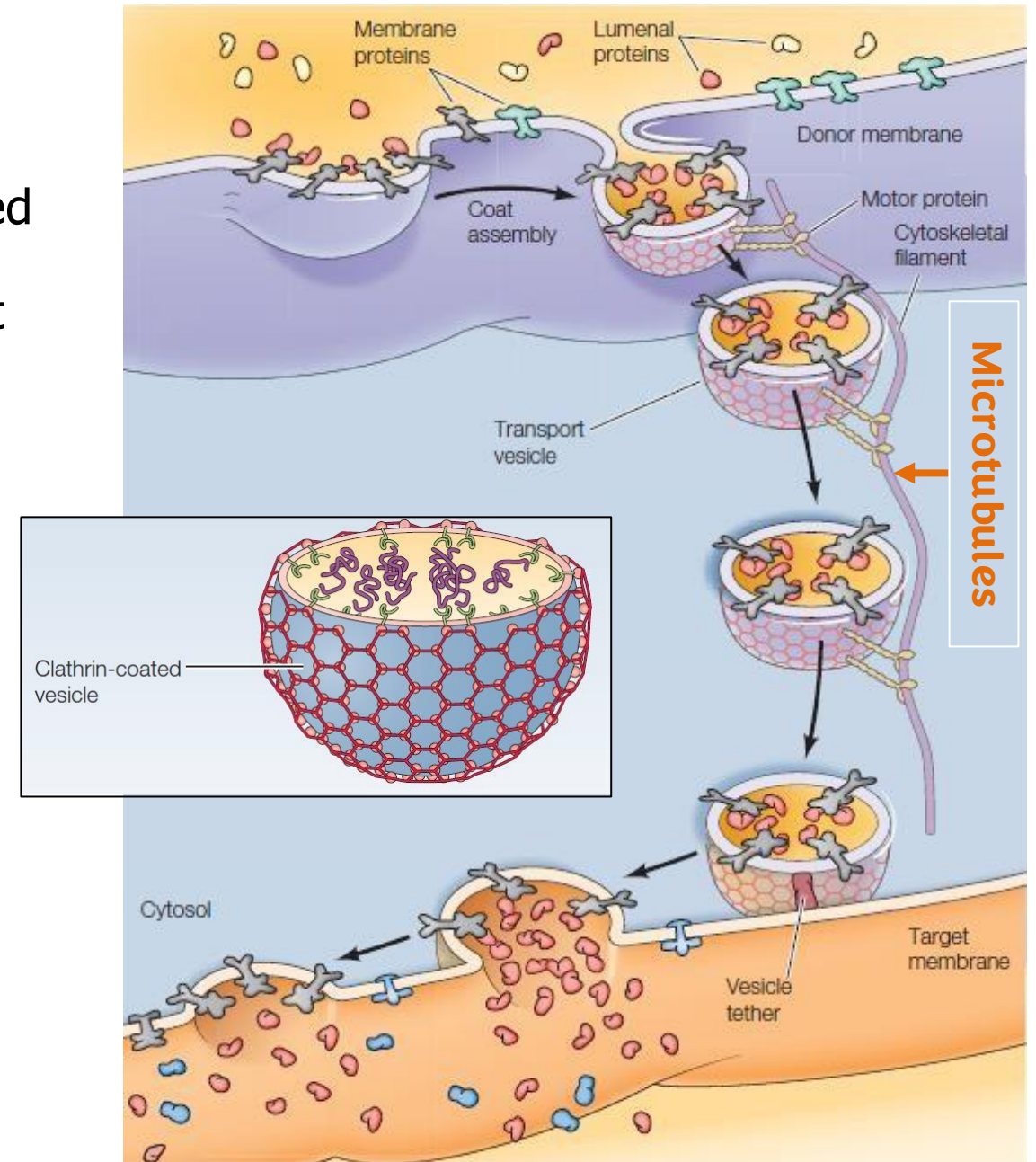
# What is clathrin?

- Before budding of a transport vesicle, they get coated by a **protein** called clathrin. **That works as a scaffold**
- 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.

## Formation of the vesicle



SEM image of clathrin



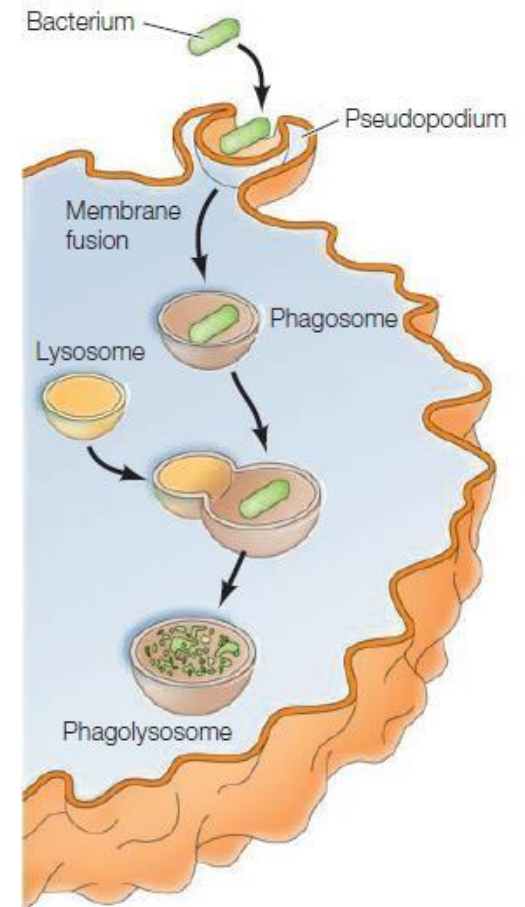
# What is clathrin?

- 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 purposes :
  - It forms the vesicle , it allows the formation of vesicle on the plasma membrane
  - it sorts the package ( cargo )
  - regulate the movement on the cytoskeletal filaments ( microtubules ), vesicles don't diffuse, it actually walks on the cytoskeleton and once it stands on endosomes or golgi ,shedding happens and clathrin coat is removed which allow the fusion of vesicle with the target membrane .
- \*receptor mediated endocytosis depends on the formation of clathrin around the vesicle

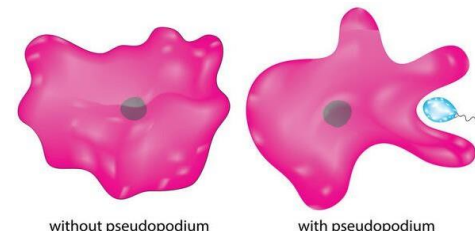
# Phagocytosis (another type of endocytosis) (usually occurs in immune cells such as macrophages)

## *(Clathrin-independent)*

- Cytosis: movement of vesicles inside the cell
- Phago : means eating
- The binding of a bacterium to the cell surface of a **macrophage** stimulates the extension of a pseudopodium (extending arms from the membrane ( fake arms )), which eventually engulfs the bacterium.
- Fusion of the pseudopodium/pseudopods membranes then results in the formation of a large intracellular vesicle (a **phagosome**). **No clathrin covering**
- The phagosome fuses with lysosomes (containing hydrolytic enzymes) to form a phagolysosome within which the ingested bacterium is digested. In phagolysosomes have a low ph so degradation happens to the bacteria.
- **Micropinocytosis** (also clathrin-independent) is cell drinking via the formation of small vesicles( micropinocytic vesicles ) . (endocytosis of water and nutrients inside)
- Clathrin dependent endocytosis depends on the formation of scaffold and needs receptor but phagocytosis and micropinocytosis doesn't need scaffold or a receptor

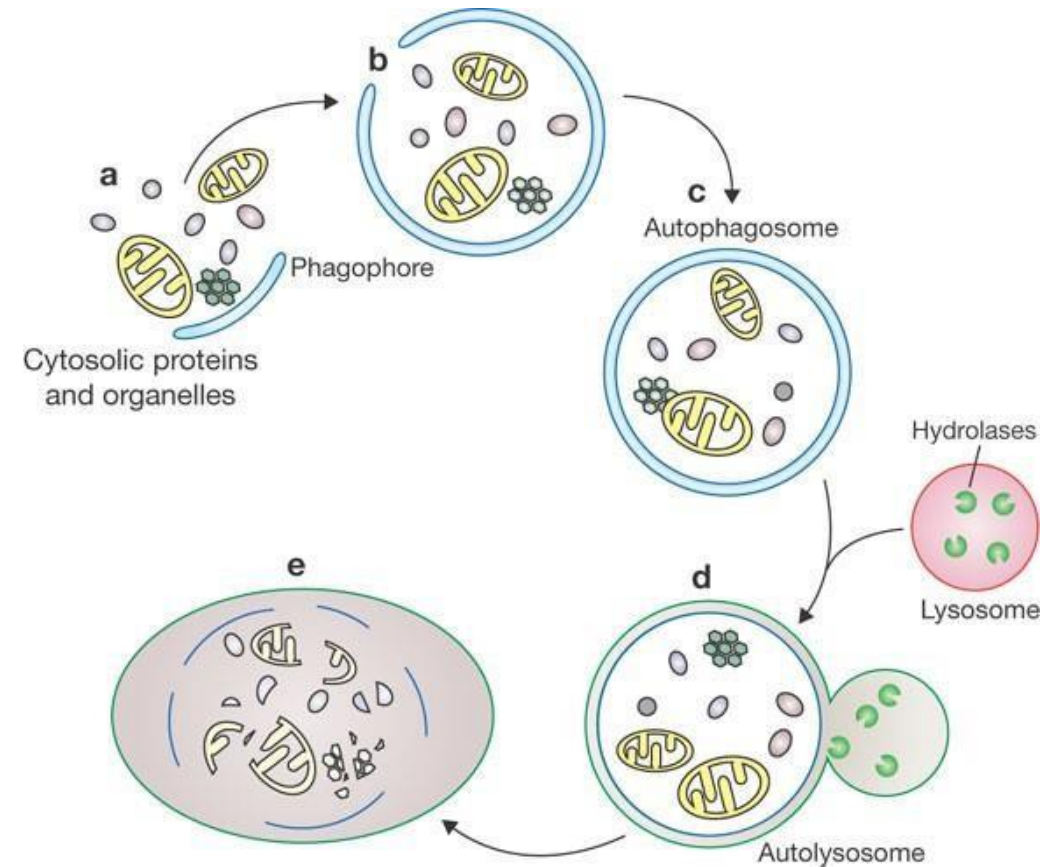


*A pseudopodium is a temporary arm-like projection of a eukaryotic cell membrane*



# Autophagy (self-eating)

- Autophagy involves the formation of a large vesicle called **autophagosome** (depends on a multiple proteins exist in the cell like Atg9) at the surface of ER with the help of a protein called **Atg9**.
- These autophagosomes encircle large damaged molecules, organelles like mitochondria and proteins inside the cell.
- 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** or **autophagolysosomes** 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 or peroxisomes).

### Like in Alzheimer's disease

- Allowing survival during starvation to eliminate **damaged** or nonessential macromolecules (such as extra mitochondria) and replenish ATP.
- Remodeling tissues during Development ( **embryogenesis : differentiation of cells** ) **Formation of tissues** Or Like Forming fingers
- Preventing cancer : cell eats herself to protect my body from cancer
- When I have a misfolded proteins or damaged organelles inside the body they cluster for example in neurons ( amyloid clusters that leads to Alzheimer's disease )

## The bad and the ugly

- Autophagy can help cancer cells survive and proliferate under suboptimal conditions : inside the tumor I have low level of oxygen ( hypoxia ) and nutrients can't reach the core of tumor but it can survive starvation by inducing autophagy but eventually they will die by necrosis which is very bad for the surrounding tissue ( recall Pathology lectures )
- Autophagy is also implicated in several neurodegenerative diseases : like Alzheimer's and Parkinson's disease that prevent the stimulation of autophagy so the damaged and misfolded proteins in nerve cells can survive .
  - Genes responsible for the autophagic degeneration of dysfunctional mitochondria in degenerating cells are found to be mutated in early-onset Parkinson's disease so the damaged cell will survive  
No autophagy = No cell death



**Fasting triggers a biological mechanism called autophagy, which breaks down old cells and literally regenerates the immune system**

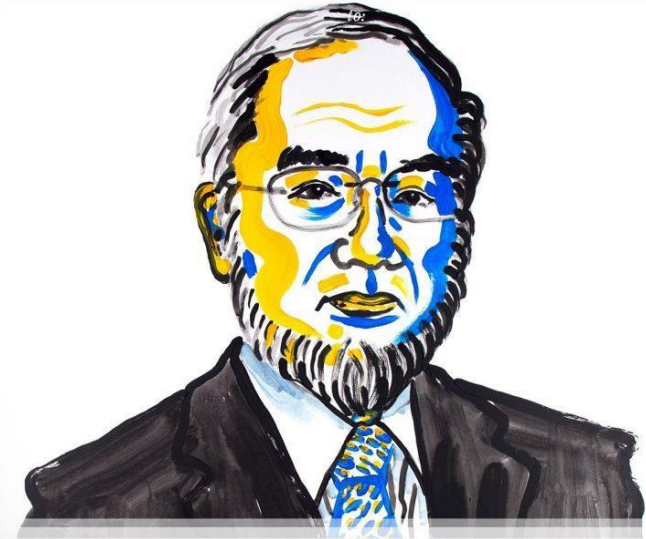
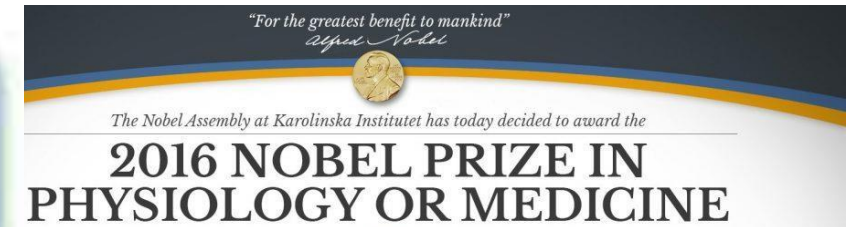
## Reason Behind Fasting !

When the human body is hungry, it eats itself and goes through a cleansing process that eliminates all diseased cells, cancer, ageing cells, and Alzheimer's.



The facts that Never Lie

Fasting helps in autophagy, as a result, damaged organelles (mitochondria, peroxisomes, protein clusters), and harmful substances are degraded. To survive, so fasting is a good protection from cancer.



**Yoshinori Ohsumi**

*"for his discoveries of mechanisms for autophagy"*

Nobelprize.org

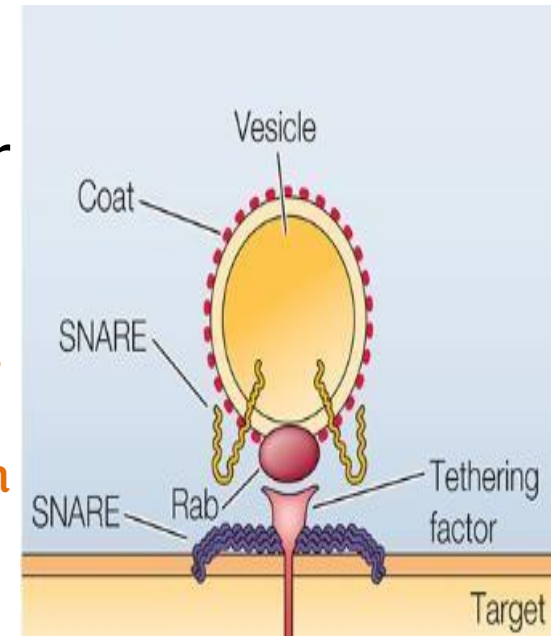
Molecular aspect

# *The mechanism of vesicular transport*

# Delivery of vesicles: targeting and fusion

- Vesicular small **monomeric** G proteins ( **their activity depends on GTP** ) 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 where vehicles should bud off from and where they should move to. and the destination of vesicles.** ( for **example** : Rab-1 are on vesicles that bud off the ER and determine that these vesicles should go to Golgi and if they go to a wrong destination there won't be a diffusion)
  - The other type of protein is SNARE and there is two types of it:
- **v-SNAREs** ( **Vesicular SNAREs are found on the vesicles** ) - **t-SNAREs** ( **target SNAREs which are found on the target membrane** ) proteins are responsible for vesicular fusion with the target membranes.
- Rab proteins decide the from where to where should the vesicle move and the SNARE proteins help in the fusion of the vesicle with membrane. The vesicles move from the donor membrane to the target membrane. Rab proteins that are found on the vesicles bind with receptors on the target membrane then the SNARE protein on both the vesicle & membrane zip together. ( imagine twisting two wires together to tighten the distance)

See the coming second slide

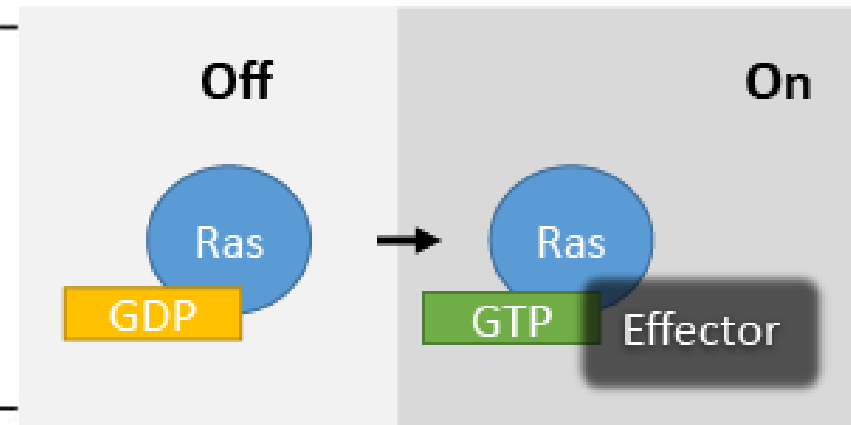


G-proteins are inactive when bound to GDP and active when bound to GTP which allows them to bind to their specific receptors.

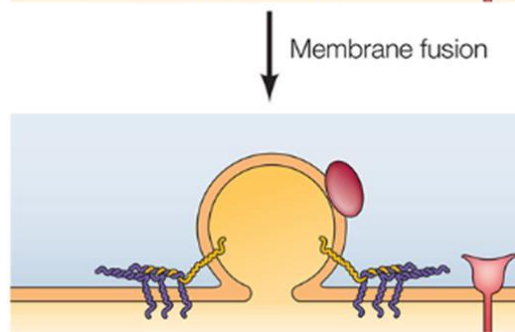
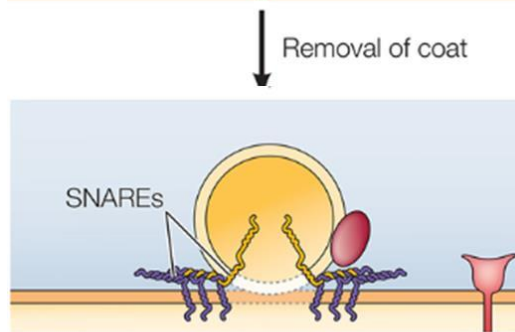
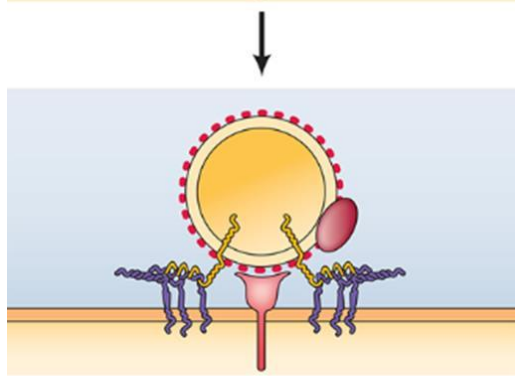
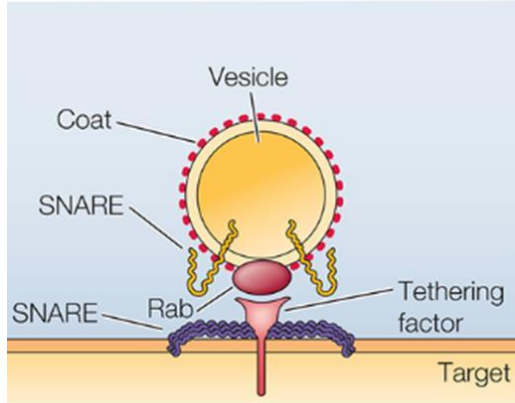
This stable is not for  
memorization

| Rab GTPase | Site(s) of action <sup>a</sup>      |
|------------|-------------------------------------|
| 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      |

<sup>a</sup> Abbreviations: EE, early endosome; PM, plasma membrane; LE, late endosome; RE, recycling endosome.



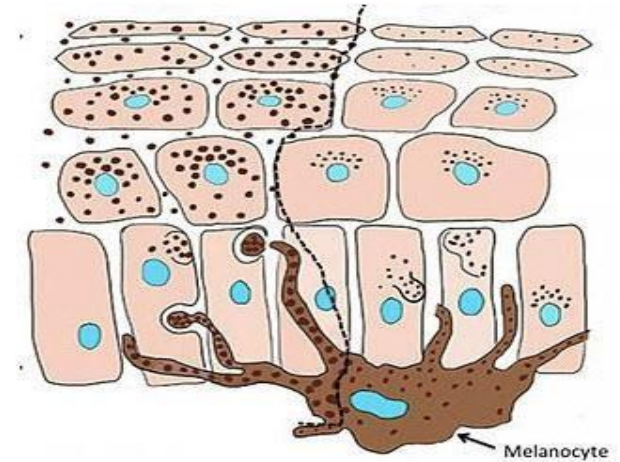
# The mechanism of fusion



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 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).

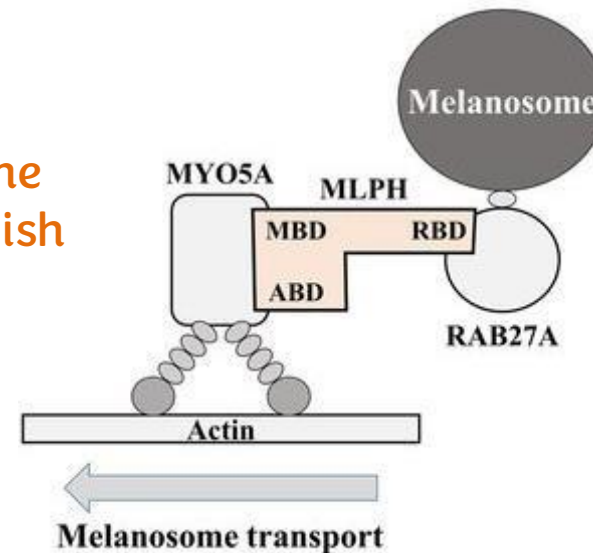
# Griscelli syndrome (GS)

- 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  
since hair is greyish

WT(Wild  
type)Normal  
Phenotype



# 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 and comes from tyrosine ) is produced by melanocytes and are packaged in vesicles called melanosomes . These vesicles transfer from melanocytes into **keratinocytes** on the surface of the skin so the color of the skin and hair comes from these melanocytes that transfer melanin . This transfer occurs via the **actin microfilament**. There is a complex of 3 proteins which are found on the vesicles on the melanosomes which are:
  - -MYO5A ( **myosin** ), this is the motor protein that moves the vesicle like it have legs moving on the actin because this movement needs energy (ATP).
  - MLPH, this is a Rab effector protein which regulates the Rab.
  - RAB27A, this is responsible for targeting so it reaches the membrane and then moves to the Keratinocytes.
- 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 without color, but it doesn't affect the skin color .

Press to Test yourself



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



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

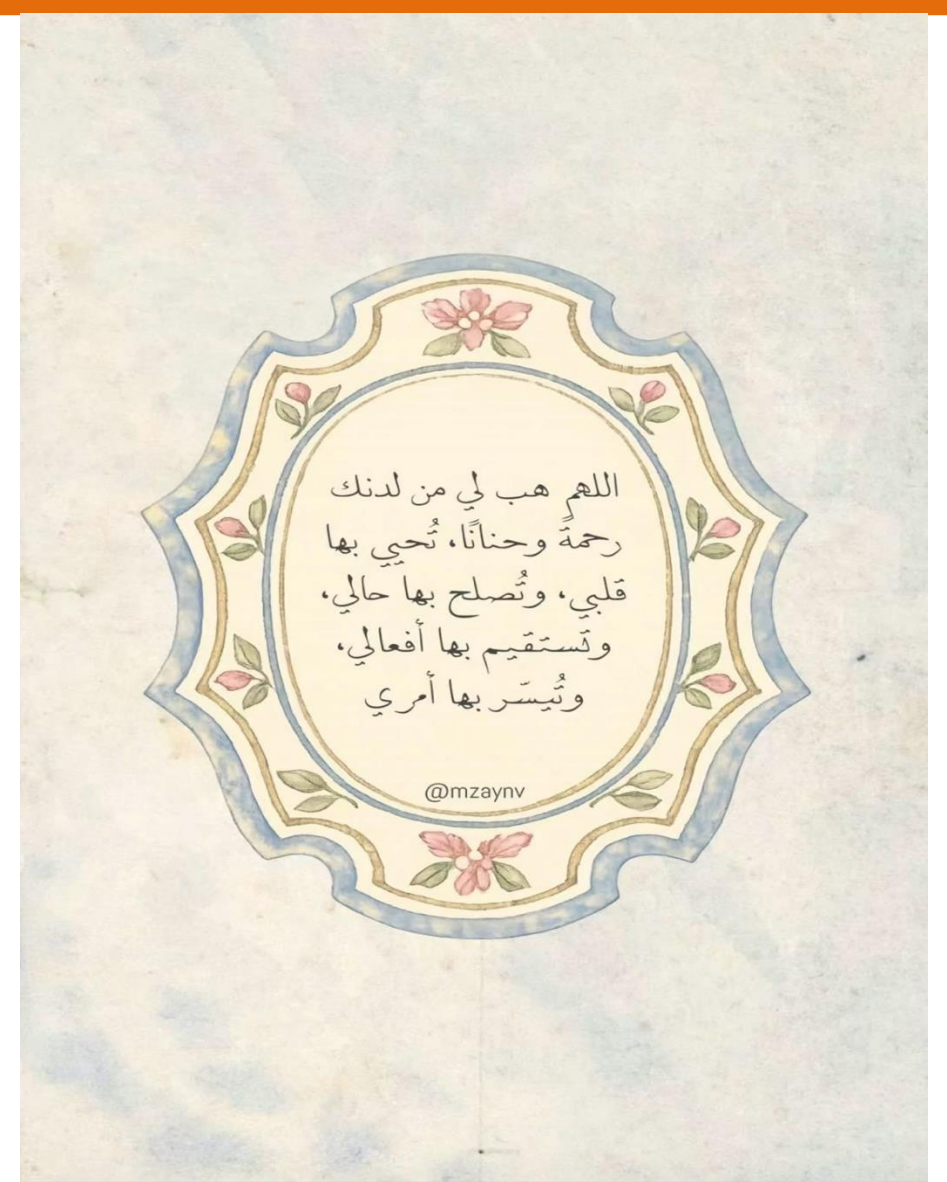
## Additional resources:

### References Used:

Prof. Mamoun Ahram recorded lecture

### Extra References for The Reader to Use:

□ لا تنسونا من صالح دعائكم



رسالة من الفريق العلمي: