Semester3/ Membranes and Receptors

Session 6 / Lecture 2

Principles of Receptor Mediated Endocytosis

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AIMS & Learning Outcomes

Objectives:

- How large, hydrophilic molecules can enter cells by associating with a cell surface receptor. This process is called **receptor-mediated endocytosis (RME)**

- How RME process can contribute to the uptake of metabolites.

- The passage of large molecules across cells.

- The control of receptor number at the cell surface and the entry of membrane-enveloped viruses.

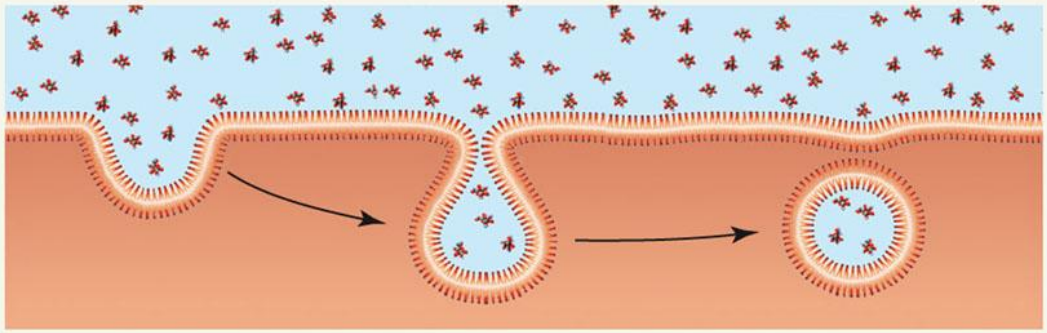
Objective Number 1:

- How large, hydrophilic molecules can enter cells by associating with a cell surface receptor. This process is called receptor-mediated endocytosis (RME)

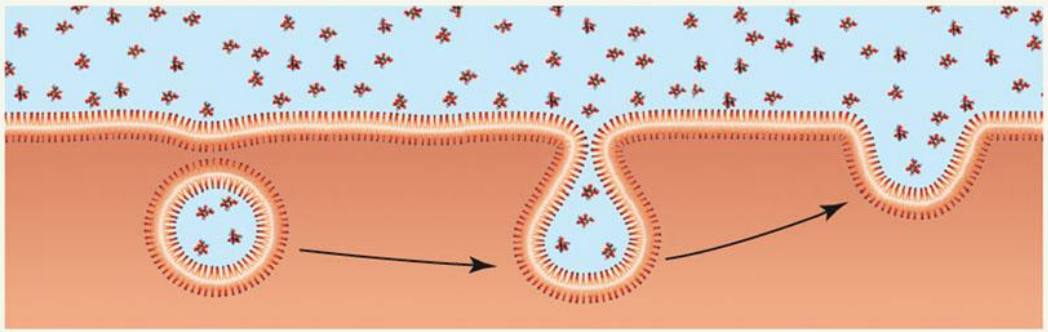
Endocytosis

Is a process by which cells internalize substances (**molecules, bacteria or viruses**) by forming new **vesicles** from the plasma membrane. Vesicles movement brings substances in bulk **into** the cell

Endocytosis and **Exocytosis** continually replace and withdraw patches of the plasma membrane



D **Endocytosis** Vesicle movement brings substances in bulk into cell.



E **Exocytosis** Vesicle movement ejects substances in bulk from cell.

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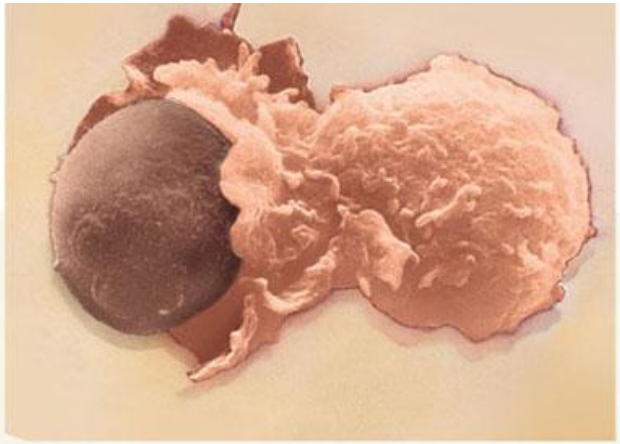
Endocytosis is include the following:

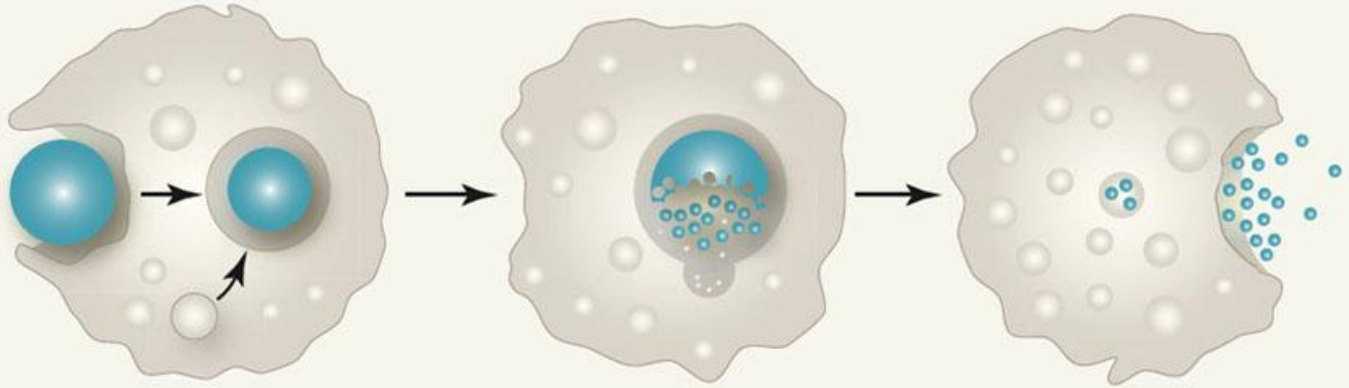
A) Phagocytosis : Called "**cellular eating**".

The cell engulfs a particles (**molecules, bacteria or viruses**) by extending pseudopodia around it and packaging it in a large vacuole.
The contents of the vacuole are digested when the vacuole fuses with a **lysosome** to form **phagolysosomes** in which the particulate material is degraded.

Phagocytosis



A Pseudopods surround a pathogen (*brown*).



B Endocytic vesicle forms.

C Lysosome fuses with vesicle; enzymes digest pathogen.

D Cell uses the digested material or expels it.

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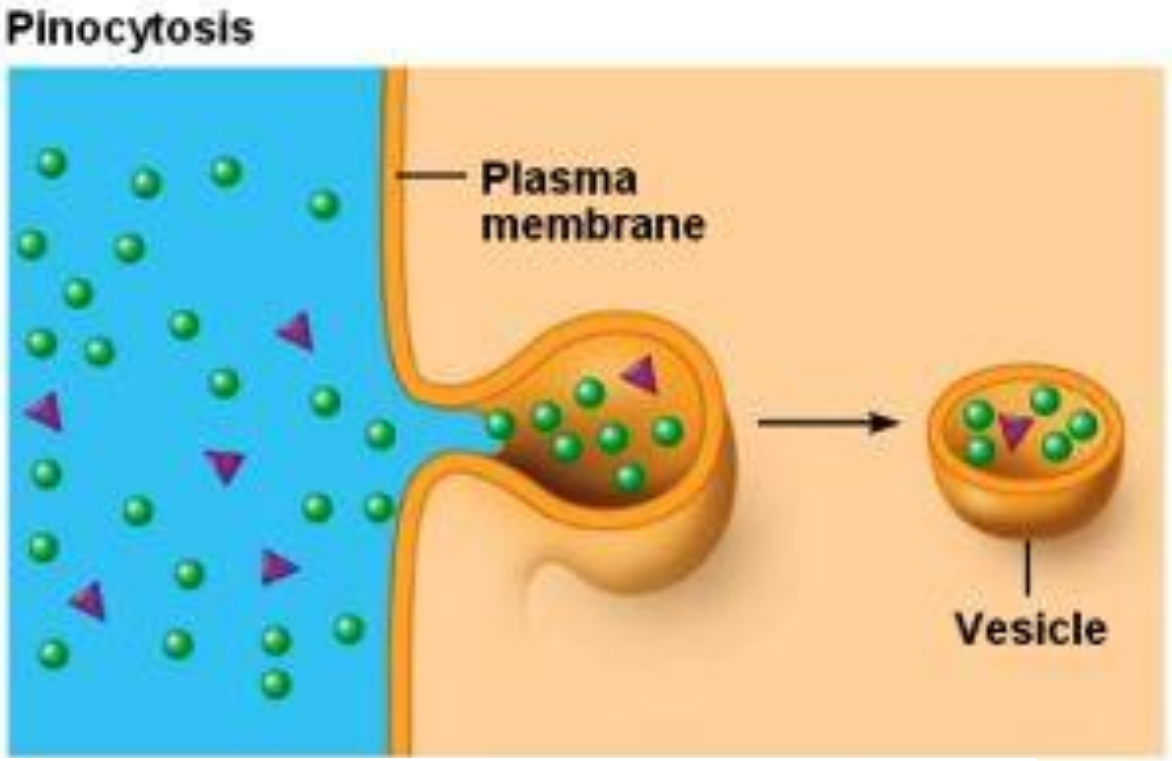
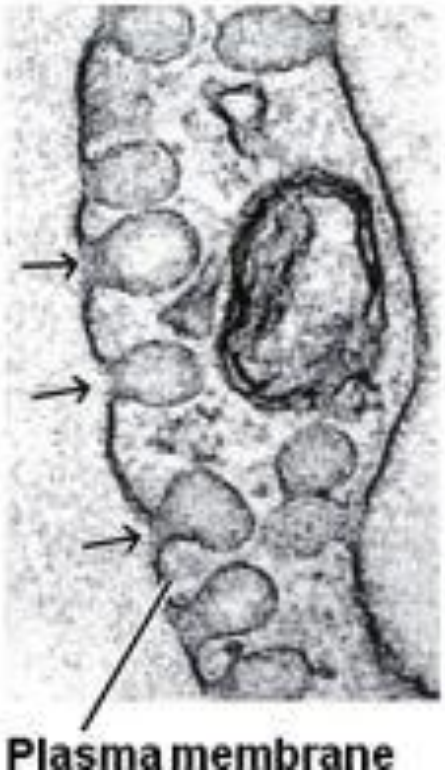
B) Pinocytosis : Called "**cellular drinking**".

The invagination of the plasma membrane to form a lipid vesicle. This permits the uptake of impermeable extracellular solutes and retrieval of plasma membrane. Pinocytosis can be sub divided into two forms, **fluid-phase** and **receptor mediated endocytosis**.





***This is a non-specific process**

C) Receptor-Mediated Endocytosis (RME):

Called "**selective drinking**" called also **Clathrin-dependent endocytosis**, which is very **specific** in what substances are being transported.

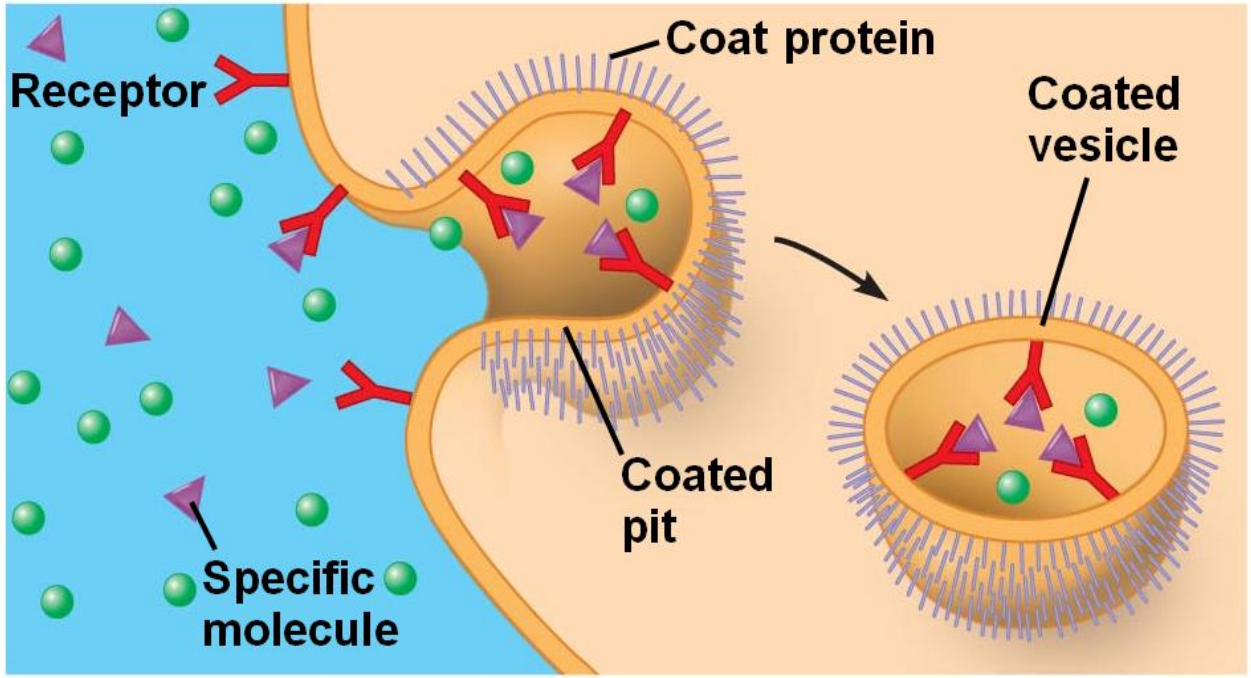
It is triggered when extracellular substances bind to special receptors on the membrane surface. This triggers the formation of a vesicle containing proteins with receptor sites specific to the molecules being internalized.

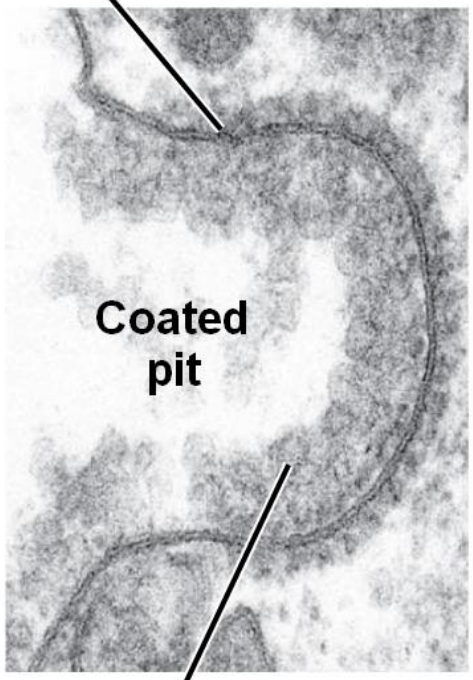
It enables a cell to take large quantities of specific materials that may be in low concentrations in the environment.

As its name implies, it depends on the interaction of that molecule with a specific binding protein in the cell membrane called **a receptor**.

***This is a specific process**

Receptor-mediated endocytosis





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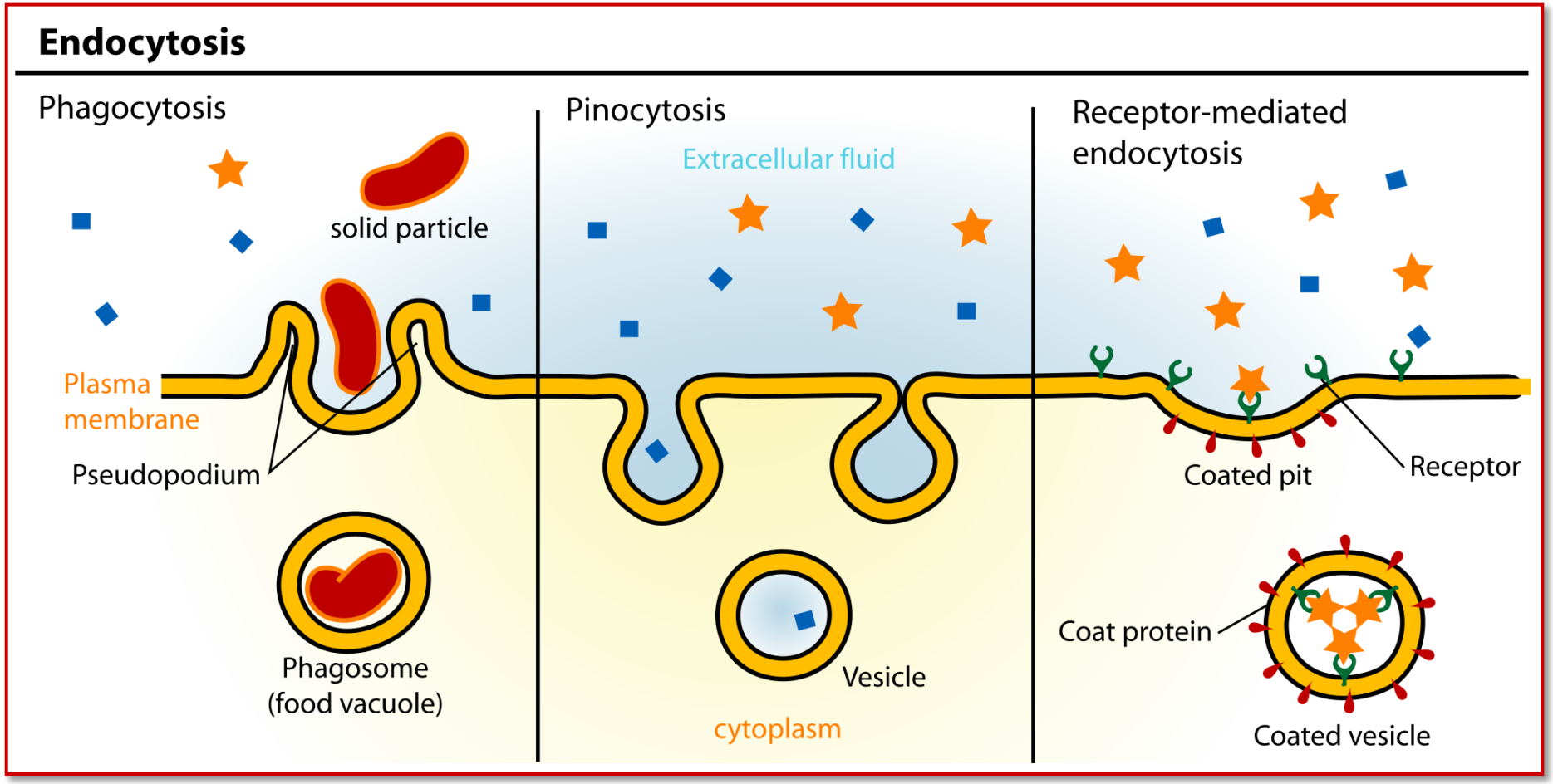
How do cells internalize molecules and other cells?



Objectives Number 2 & 3 :

- How RME process can contribute to the uptake of metabolites.
- The passage of large molecules across cells.

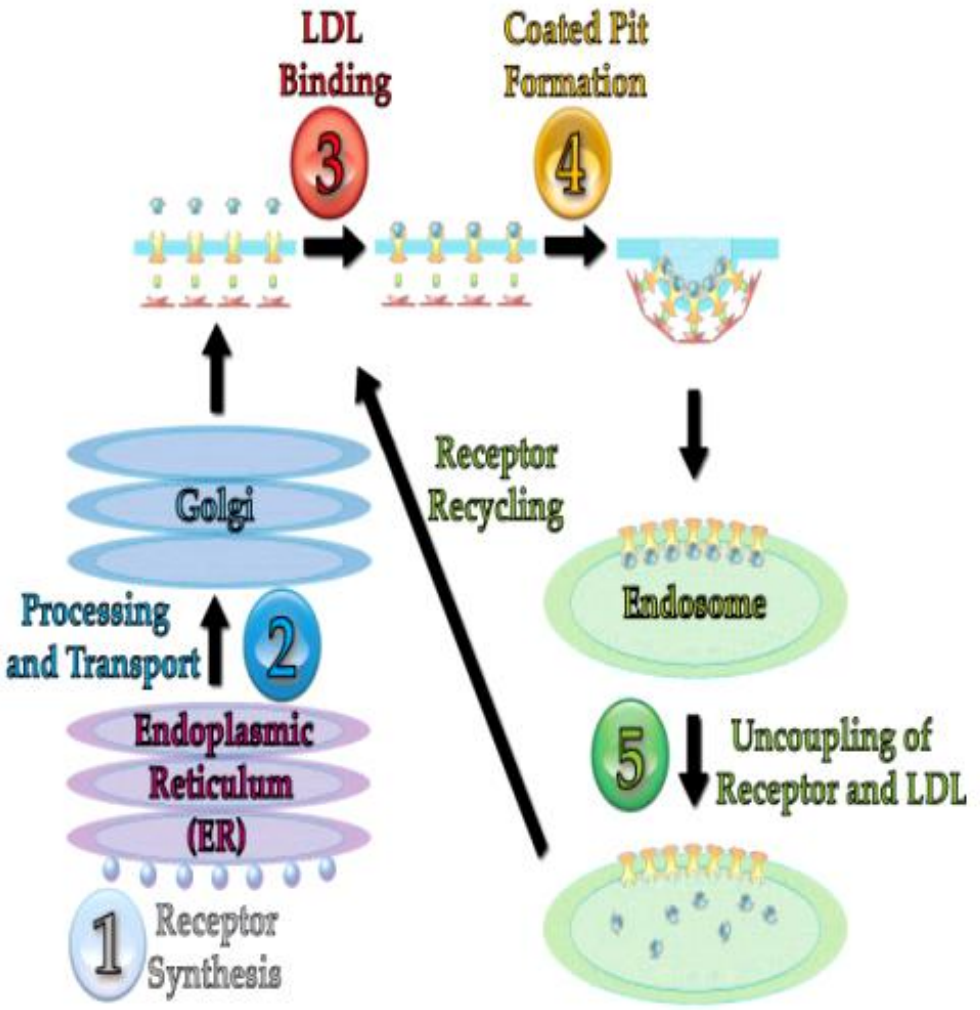
The Four modes of Receptor-Mediated Endocytosis

1-Cholesterol Uptake

- Cholesterol, a hormone precursor and component of plasma membranes, is synthesized in the liver.

- Lipoprotein complexes such as **LDL (low density lipoprotein)** transport the insoluble cholesterol molecules to the body's cells

- The LDL receptor binds to LDL complexes and mediates their endocytosis

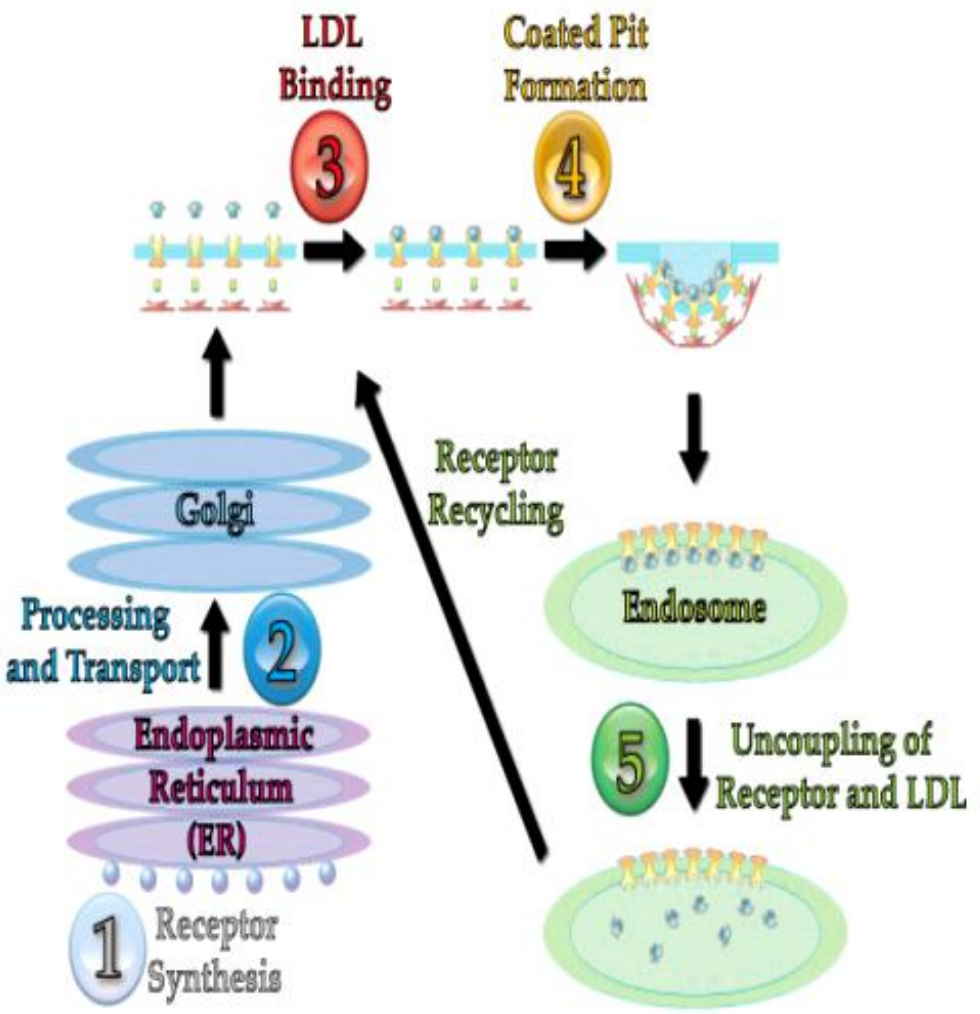


- Loss-of-function **mutations** in the gene encoding the LDL receptor cause **FH (Familial Hypercholesterolemia)** over 1000 different receptor mutations are associated with **FH**.

- FH patients have high plasma levels of LDL-cholesterol.

- High levels of LDL-cholesterol in the blood due to conditions such as FH lead to plaque formation and atherosclerosis.

- which results in coronary heart disease



The events of LDL uptake follow the general steps of receptor-mediated endocytosis:

-The LDL particles binds to the LDL-receptors are localized in clusters over **coated-pits** (2% of cell surface).

- The coated pits invaginate and pinch off from the plasma - membrane to form **coated vesicles** .

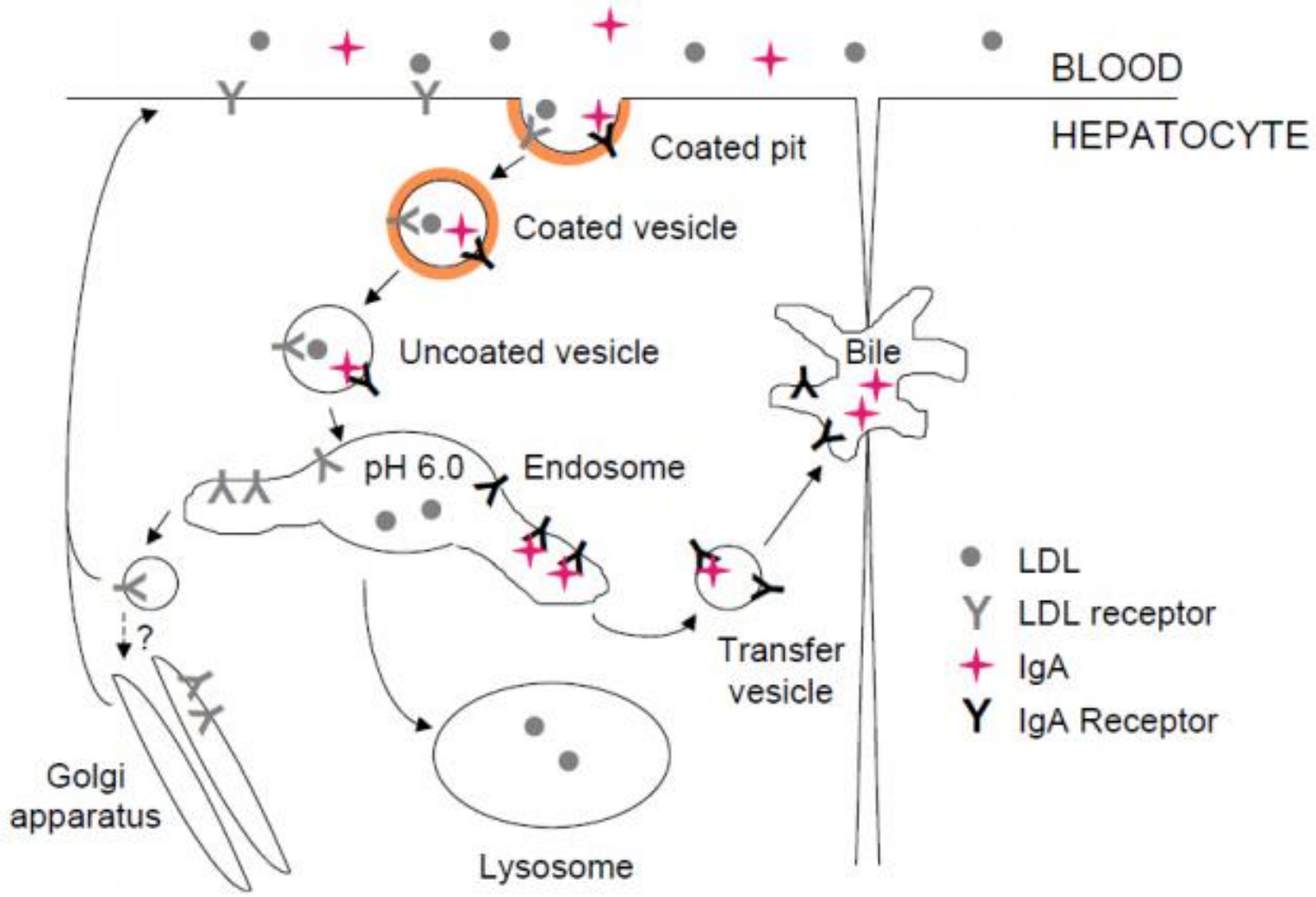
- Coated vesicles are quickly **uncoated** .

- The uncoated vesicles then fuse with larger smooth vesicles called **endosomes**, where the low pH (5.5-6.0) causes a conformational change in the receptors that results in the release of LDL particles

- The receptors recycle to the cell surface.

- The LDL particles are delivered to a lysosome and degraded, free cholestrol released into the cell.

Receptor-mediated endocytosis



Mutations Affecting The LDL-Receptor

Naturally occurring mutations of the LDL-Receptor have been identified in some patients with **Hypercholesterolemia** **Three types of mutations, when found in a homozygous individual lead to three phenotypes:**

- Receptor Deficiency : Mutations that prevent expression of LDL receptor.

– Non-Functional Receptor : No binding of LDL. Normal coated pits and internalization.

– Receptor Binding Normal : No internalization due to a deletion in the C-terminal of the receptor that makes the interaction with the coated pits. LDL- Receptors are found distributed over whole cell surface in these patients.

2- Iron Uptake

- Iron is a cofactor for various enzymes necessary for many metabolic processes, including DNA synthesis, electron transfer, and oxygen transport and storage.

• Cells take up iron from the extracellular environment via receptor mediated endocytosis, but iron does not interact with the receptor directly.

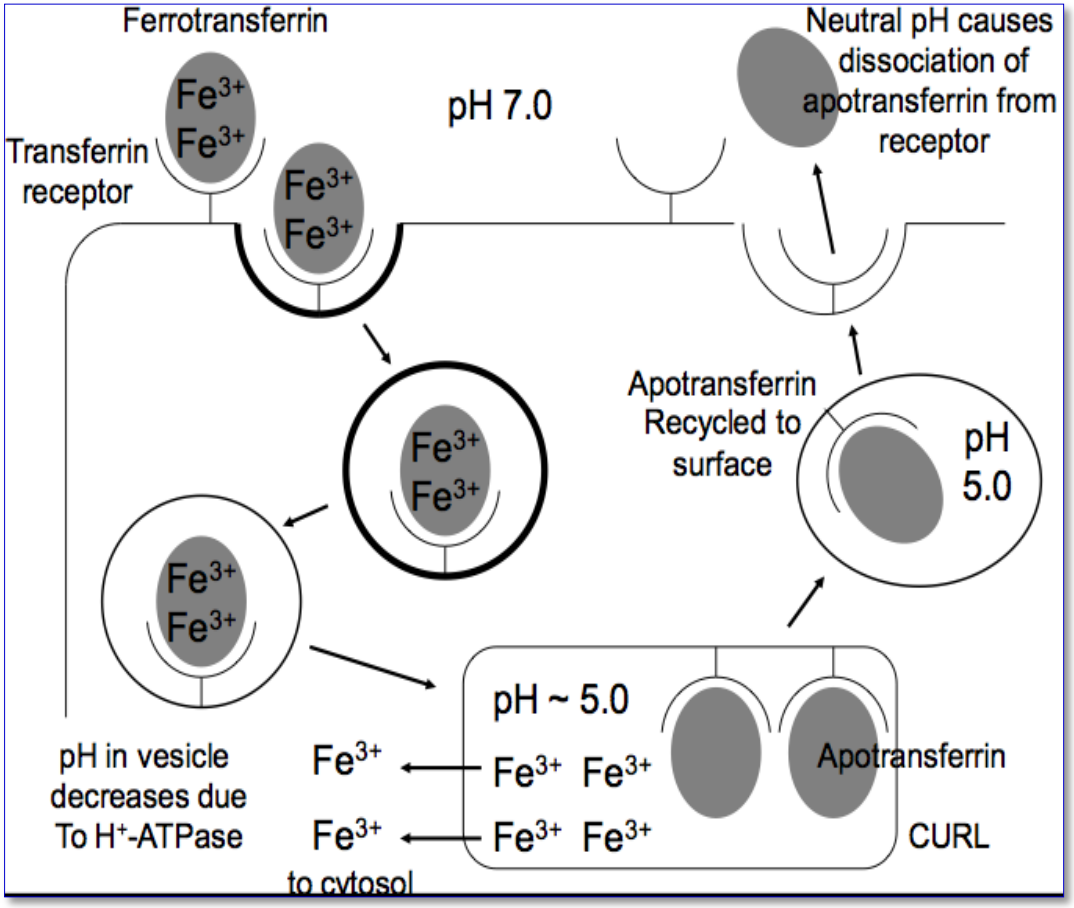
• Instead, iron associates with the protein transferrin, which then binds to the transferrin receptor, and the complex undergoes endocytosis.

• Transferrin contains two iron binding domains, each capable of binding one iron ion.

• In the extracellular environment, which has a pH of 7.4, the affinity of transferrin for ferric iron (Fe^{3+}) is extremely high

1-Two Fe^{3+} ions bind to Apotransferrin to form Transferrin in the circulation.

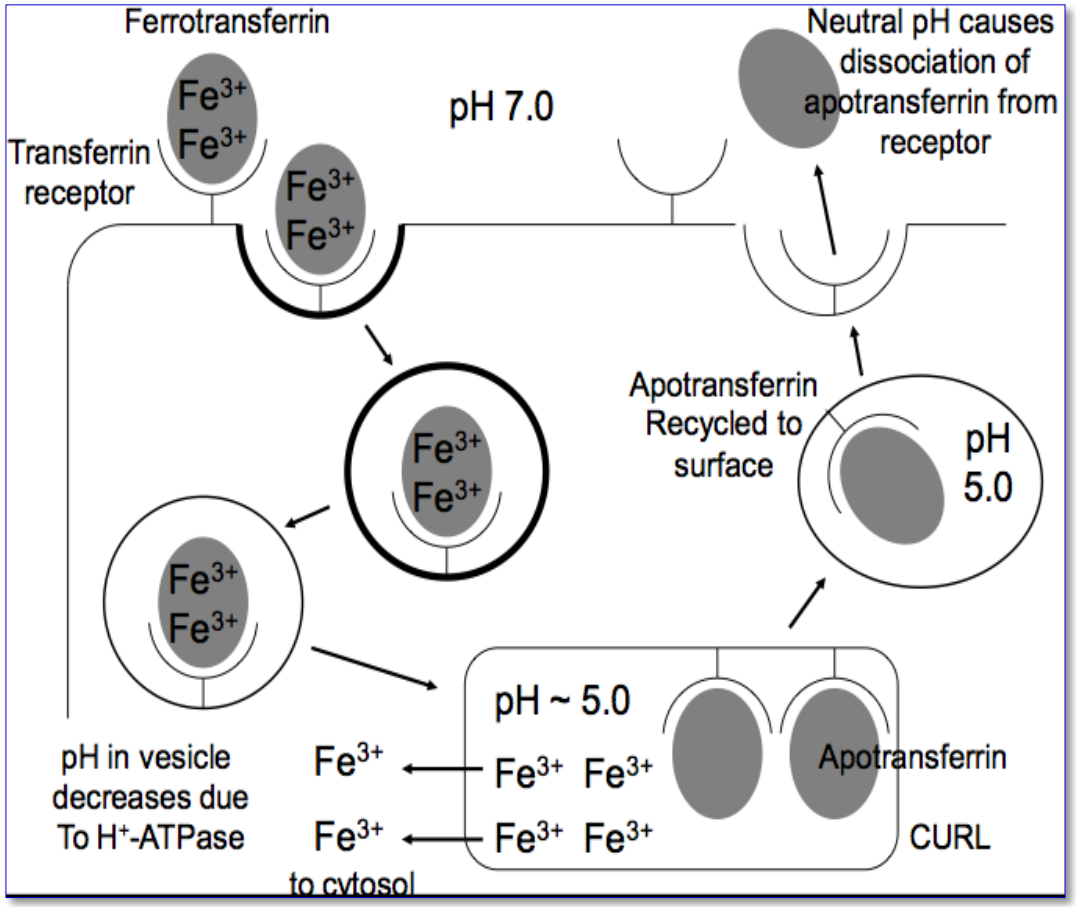
2- Transferrin, but not Apotransferrin, binds to the **Transferrin Receptor** at neutral pH and is internalised in a similar way to LDL as described above.



The endosome is also known as the Compartment for the Uncoupling of Receptor and Ligand (CURL)

3 - Upon reaching the **acidic endosome, the Fe^{3+} ions are released** from the transferrin, but **at this pH the Apoptransferrin remains associated** with the transferrin receptor.

4- The complex is sorted in the CURL for recycling back to the plasma membrane where **at pH 7.4 the Apoptransferrin dissociates** from the receptor again.



3- uptake of Occupied Insulin Receptors

Unlike the previous two examples, insulin receptors **only congregate over clathrin coated pits when their agonist is bound**.

Insulin binding induces a conformational change in the receptor that allows it to be recognised by the pit.

In the endosome Insulin **remains bound** to the receptor and the complex is targeted to the lysosomes for degradation.

This mechanism allows for the reduction in the number of insulin receptors on the membrane surface, desensitising the cell to a continued presence of high circulating insulin concentration.

4- Transcytosis

Some ligands that remain bound to their receptors may be **transported across the cell**. This is Transcytosis. Examples include maternal immunoglobulins to the foetus via the placenta and the **transfer of immunoglobulin A (IgA) from the circulation to bile in the liver**.

During transport of IgA the receptor is cleaved, resulting in the release of immunoglobulin with a bound '**secretary component**' derived from the receptor.

Compare and Contrast the Four modes of Receptor-Mediated Endocytosis

Mode	Fate of Receptor	Fate of Ligand	Examples	Function
1	Recycled	Degraded	LDL	Metabolite Uptake
2	Recycled	Recycled	Transferrin	Metabolite Uptake
3	Degraded	Degraded	Insulin, Epidermal Growth Factor Immune Complexes	Receptor down-regulation Removal from circulation of foreign antigen
4	Transported	Transported	Maternal IgG,IgA	Transfer of large molecules across cell **E.g. Maternal immunity to foetus via placenta** **E.g. Circulation to Bile**

Q/ What types of ligands enter by receptor mediated endocytosis?

❖**Toxins and lectins**
- Diptheria Toxin
- Pseudomonas toxin
- Cholera toxin
- Ricin

❖**Viruses**
- Rous sarcoma virus
- Semliki forest virus
- Vesicular stomatitis virus
- Adenovirus

❖**Hormones and Growth Factors**
- insulin
- Epidermal Growth Factor
- Growth Hormone
- Thyroid stimulating hormone
- Nerve Growth Factor
- Calcitonin
- Prolactin
- Luteinizing Hormone
- Thyroid hormone
- Platelet Derived Growth Factor
- Interferon

❖**Serum transport proteins and antibodies**
- Transferrin
- Low density lipoprotein
- Yolk proteins
- IgE
- Polymeric IgA
- Maternal IgG
- IgG, via Fc receptors

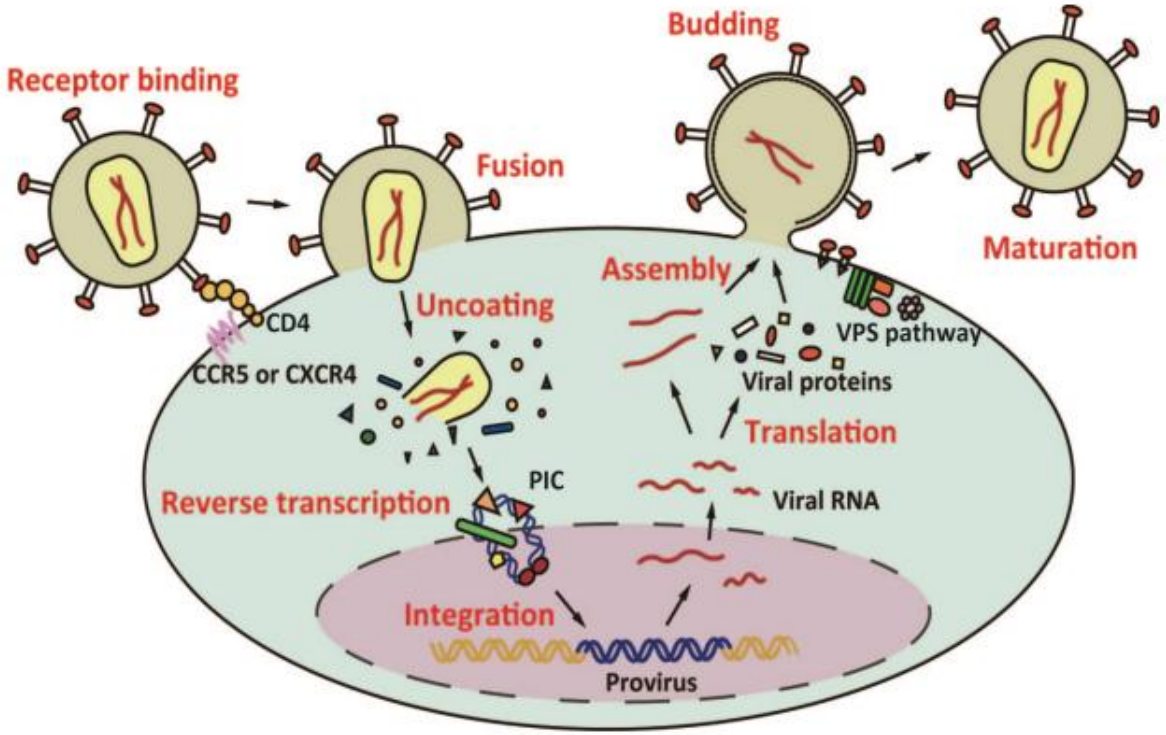
Objective Number 4:

- The control of receptor number at the cell surface and the entry of membrane-enveloped viruses

Viral Entry

- Is the earliest stage of infection in the **viral life cycle**, as the virus comes into contact with the host cell and introduces viral material into the cell.

-The major steps involved in **viral entry** are shown below:-

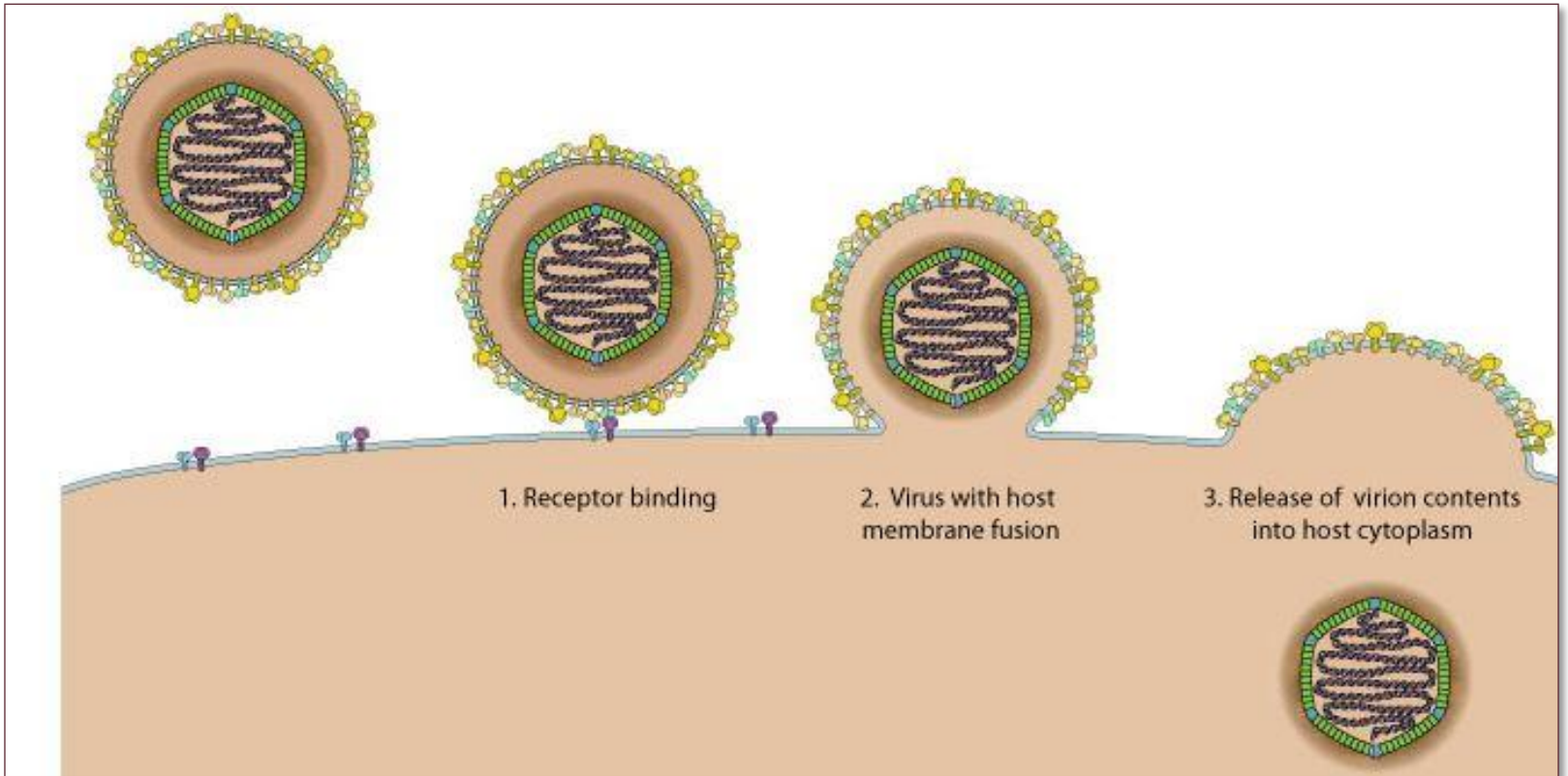


1- Entry via Membrane Fusion

Steps of viral-cell membrane fusion:

1- Receptor Binding : specific association with the virion and cell surface receptors

2- Virus with host membrane fusion:

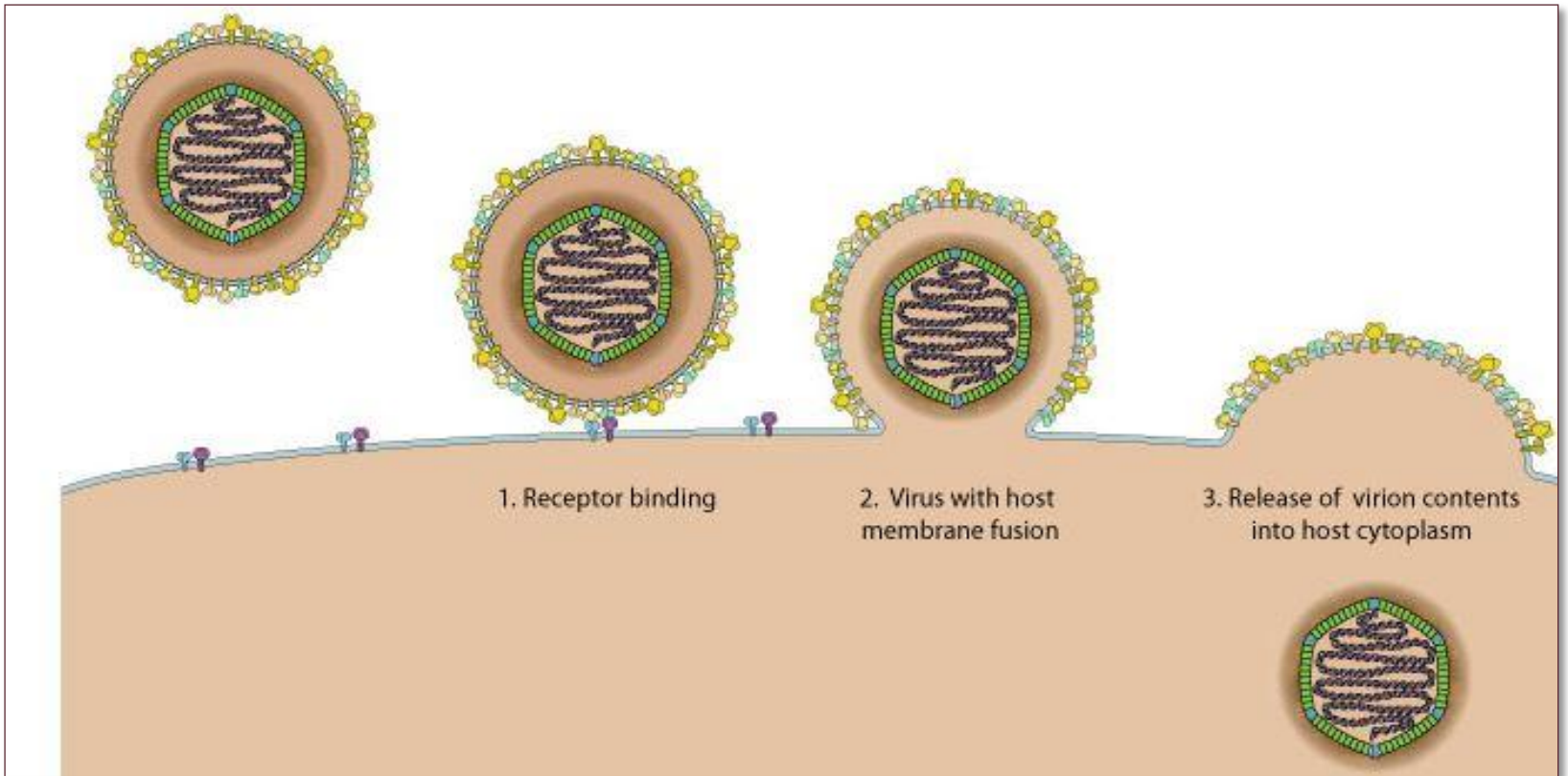


1- Entry via Membrane Fusion

3- unfolding of the viral envelope: The virus's envelope blends with the cell membrane.

4- Release of virion contents into host cytoplasm.

This can only be done with viruses that an envelope viruses, Examples (**HIV** and **Herpes simplex** virus).



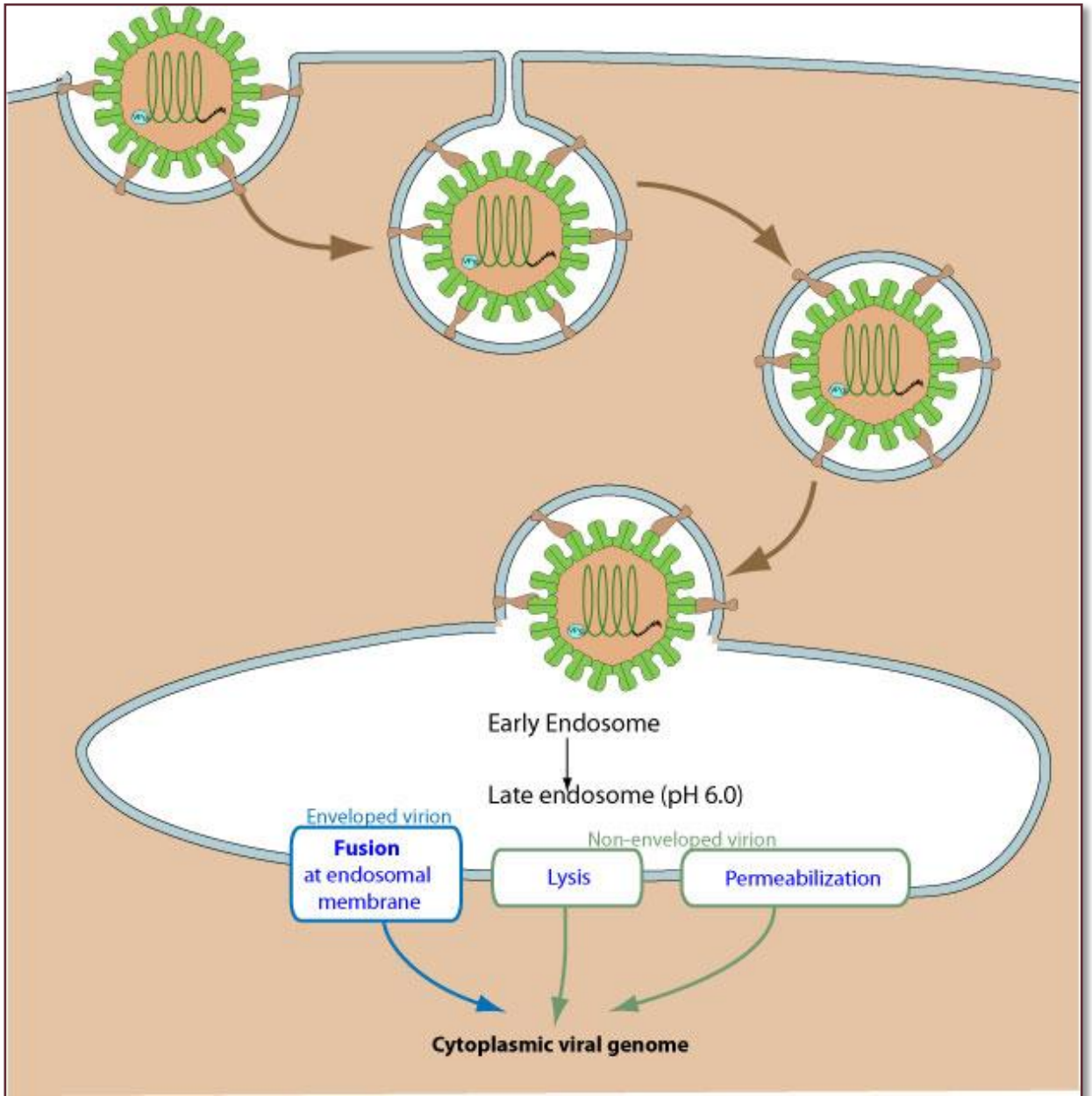
2- Entry via Endocytosis

- Membrane-enveloped viruses and some toxins exploit endocytic pathways to enter cells after binding to receptors in the plasma membrane.

- In the endosome, where the acid pH is favourable, the viral membrane is able to fuse with the endosomal membrane.

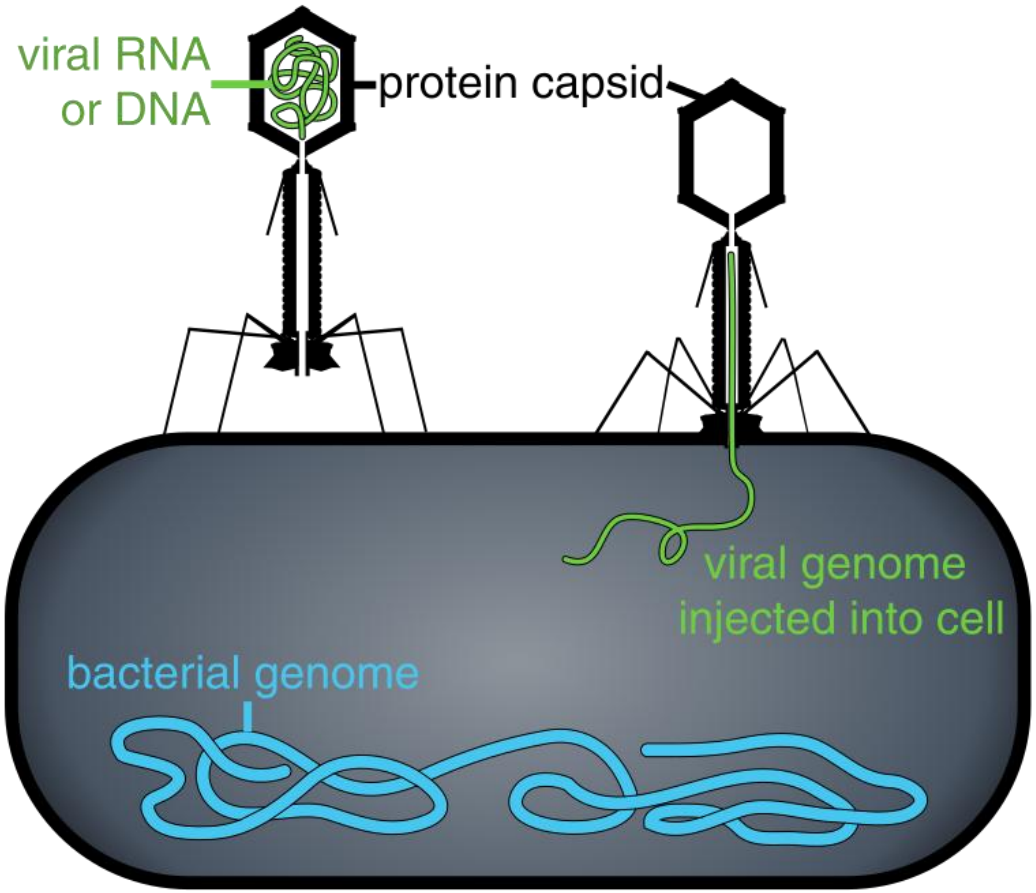
-the virus must now break out of the vacuole to gain access to the cytoplasm, thereby, releasing the viral RNA into the cell where it can be translated and replicated to form new viral particles,
Examples include the (**poliovirus**, **Hepatitis C virus, Foot and mouth disease** virus)

2- Entry via Endocytosis



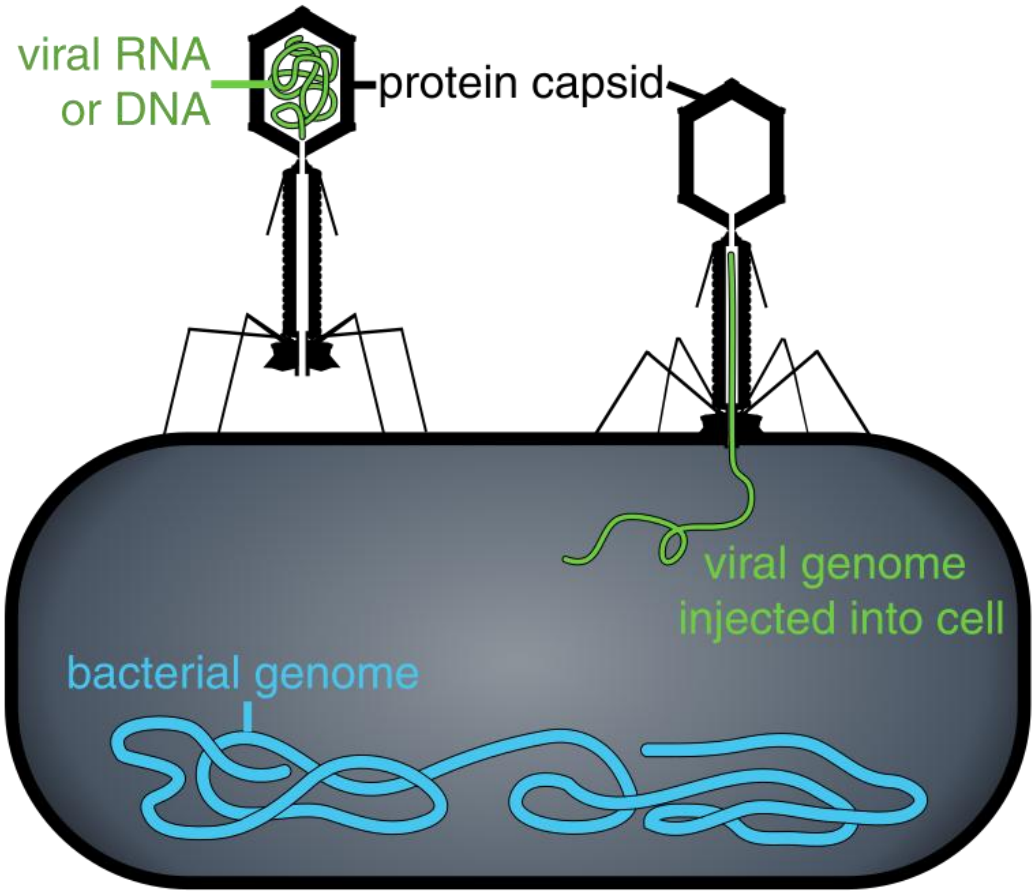
3- Entry via Genetic Injection

• Simply attaching to the surface of the cell via receptors on the cell, and injecting only its gene into the cell, leaving the rest of the virus on the surface.



3- Entry via Genetic Injection

- This is restricted to viruses in which only the gene is required for infection of a cell (most positive-sense, single-stranded RNA viruses) The best studied example includes the **bactriophages (phages)**



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