

Endocytosis and Exocytosis

Endocytosis: Endocytosis is the process by which cells absorb larger molecules and particles from the surrounding by engulfing them. It is used by most of the cells because large and polar molecules cannot cross the plasma membrane. The material to be internalized is surrounded by plasma membrane, which then buds off inside the cell to form vesicles containing ingested material.

The endocytosis pathway is divided into 4 categories:

Endocytosis

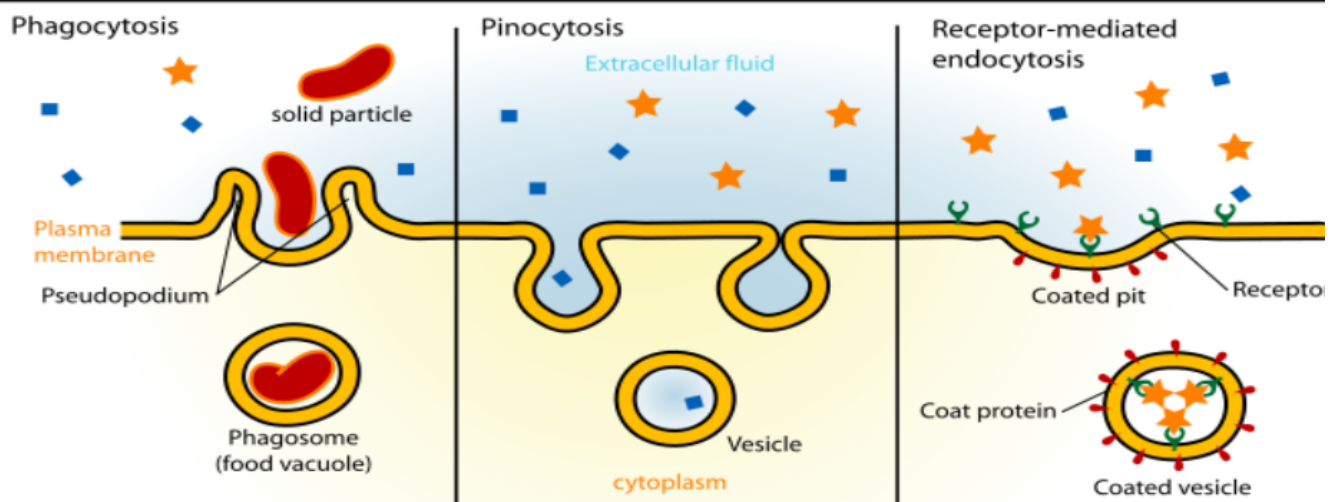


Figure 1: Types of endocytosis process (4th pathway is not shown in above figure)

1. **Phagocytosis:** Phagocytosis is the process by which certain living cells called phagocytes engulf larger solid particles such as bacteria, debris or intact cells. Certain unicellular organisms, such as the protists, use this particular process as means of feeding. It provides them part or all of their nourishment. This mode of nutrition is known as phagotrophic nutrition. In amoeba, phagocytosis takes place by engulfing the nutrient with the help of pseudopods, that are present all over the cell, whereas, in ciliates, a specialized groove or chamber, known as the cytostome, is present, where the process takes place.

When the solid particle binds to the receptor on the surface of the phagocytic cell such as amoeba, then the pseudopodia extends and later surrounds the particle as shown in figure

2. Then their membrane fuses to form a large intracellular vesicle called phagosome. These phagosomes fuse with the lysosome, forming phagolysosomes in which ingested material is digested by the action of lysosomal enzymes. During its maturation, some of the internalized membrane is recycled to plasma membrane by receptor mediated endocytosis.

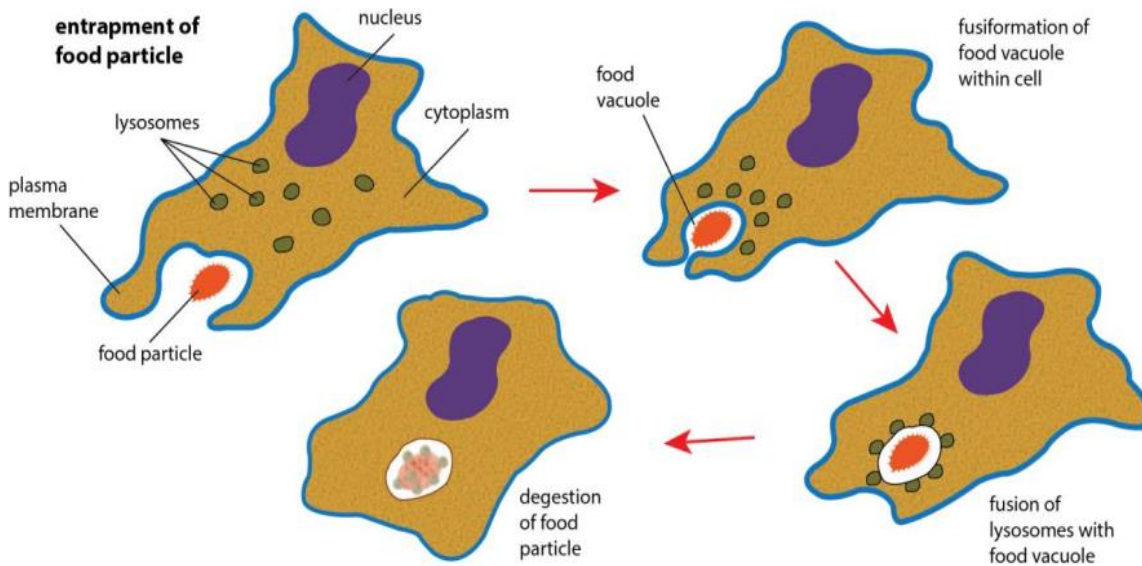


Figure 2: Example of phagocytic process for entrapment of food particle

Draw the above figure

The various phases of phagocytosis in amoeba for food capturing are:

- Adherence of the macromolecules to the receptor on the phagocytic cell
- Extension of pseudopodia and ingestion of microbe by phagocytic cell
- Formation of phagosome by the fusion of surrounding membrane
- Fusion of phagosome and lysosome to form phagolysosome
- Digestion of the ingested macromolecules by the acid hydrolytic enzymes in the lysosome
- Formation of residual body coating indigestible material
- Discharge of waste materials

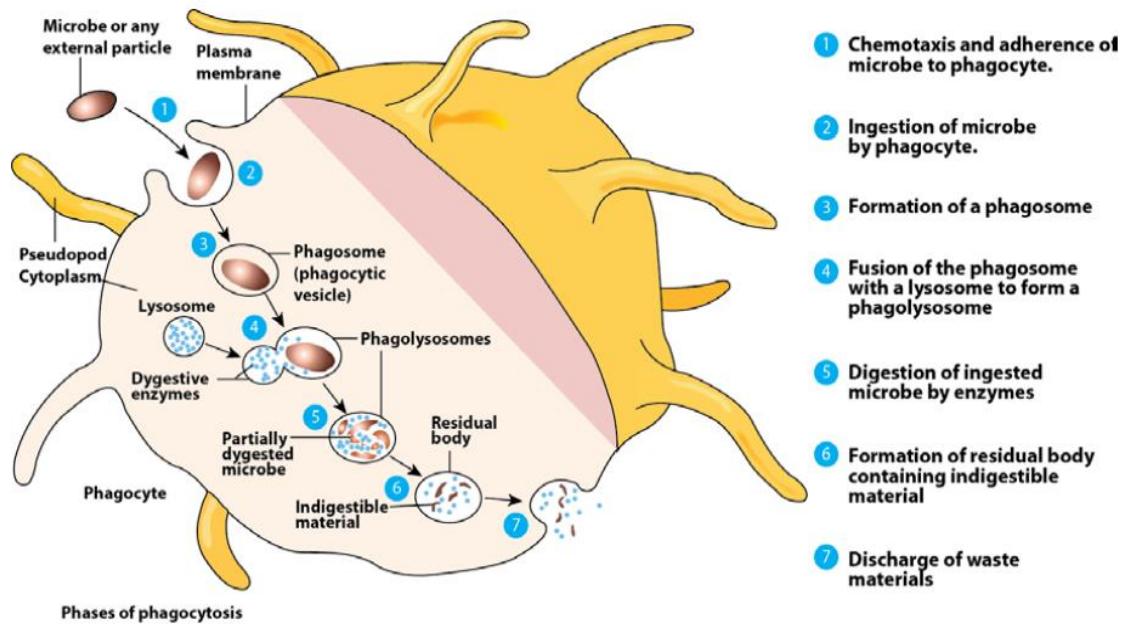


Figure 3: Phases of phagocytosis process Draw this figure

Other examples of phagocytosis include some immune system cells, that engulf and kill certain harmful, infectious micro-organisms and other unwanted foreign materials which in turn provides defence against invading micro-organism and eliminate damaged cells from the body. There are two types of phagocytes (WBC) in mammals: Macrophages and Neutrophils. These WBC acts as defence system by eliminating micro-organisms from infected tissues. In these cells, the engulfment of foreign material is facilitated by actin-myosin contractile system. It allows the cell membrane to expand in order to engulf the particle and then contract immediately, ingesting it. Macrophages also remove dead cells.

Steps of phagocytosis in the immune system:

The WBC cells are activated in the presence of certain bacterial cells, inflammatory cells or other foreign bodies. It includes the following steps:

- Phagocytes get activated by the presence of certain particles around them. As soon as they detect a foreign particle, the phagocytes produce surface glycoprotein receptors which increase their ability to adhere to the surface of the particle.
- The phagocyte slowly attaches to the surface of the foreign particle. The cell membrane of the phagocyte begins to expand and forms a cone around the foreign particle.

- The cell membrane surrounds the foreign particle to create a vacuole, known as phagosome or food vacuole. The phagosome is then passed into the cell for absorption.
- The lysosomes break the food vacuole or phagosome, into its component materials. The essential nutrients, if any, are absorbed in the cell, and the rest is expelled as waste matter. In case of the immune system, the cell creates a peroxisome, a special structure that helps the body to get rid of the toxins.

2. **Clathrin-mediated endocytosis:** Clathrin-mediated endocytosis is also known as receptor mediated endocytosis. It is the process of internalizing molecules into the cell by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being internalized (**Jackson et al.**).

Phases of clathrin-mediated endocytosis:

- Macromolecules (as ligands) bind to the specific cell surface receptors
- Then the receptors are concentrated in specialized regions of plasma membrane and clathrin and adaptor protein binds to these receptor forming clathrin-coated pits
- These pits bud from the membrane and form clathrin-coated vesicles containing receptors, proteins and ligands
- Then these vesicles fuse with early endosomes, in which the contents are sorted for the transport to lysosomes and receptors and proteins are recycled to plasma membrane

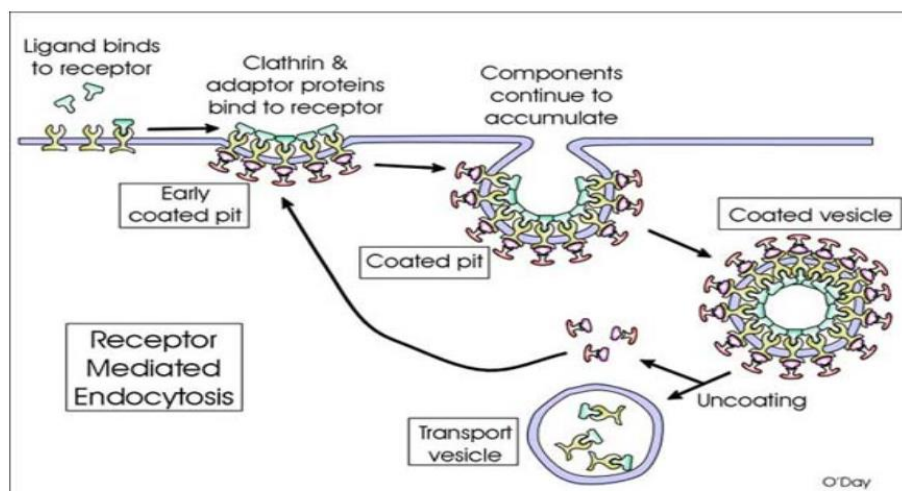


Figure 4: Phases of clathrin-mediated endocytosis

The example for clathrin mediated endocytosis is uptake of cholesterol by the mammalian cells. Here cholesterol is transported through the blood stream in the form of lipoprotein or LDL. The LDL particle consists of phospholipid bilayer, esterified and non-esterified cholesterol and Apo B protein as shown in figure 4. It was first demonstrated by Michael Brown and Joseph Goldstein in which uptake of LDL requires the binding of LDL particle to the specific cell receptor. Later it was found that it is concentrated in clathrin coated pits and internalized by endocytosis. Then receptor is recycled to plasma membrane and LDL is transported to lysosome and cholesterol is released for use by the cell.

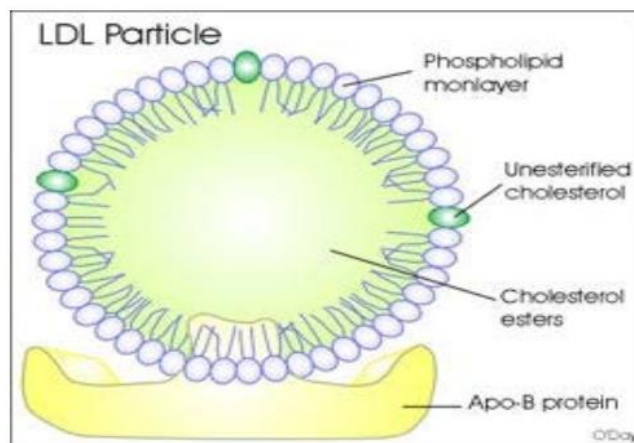


Figure 5: LDL particle or low density lipoprotein

Phases for receptor mediated endocytosis for cholesterol uptake involves:

- Receptor Binding & its activation: Here LDL receptor binds to Apo-B protein on the LDL particle
- Coated Pit Formation: Clathrin forms cage around forming endosome
- Clathrin-Coated Vesicle Budding
- Uncoating of the Vesicle
- Early Endosome associates with other vesicles
- Formation of CURL (Compartment for Uncoupling of Ligand and Receptor) or Late Endosome
- Recycling of the Receptor to the cell surface
- Fusion of Transport Vesicle with Lysosome
- Digestion of the LDL to Release Cholesterol

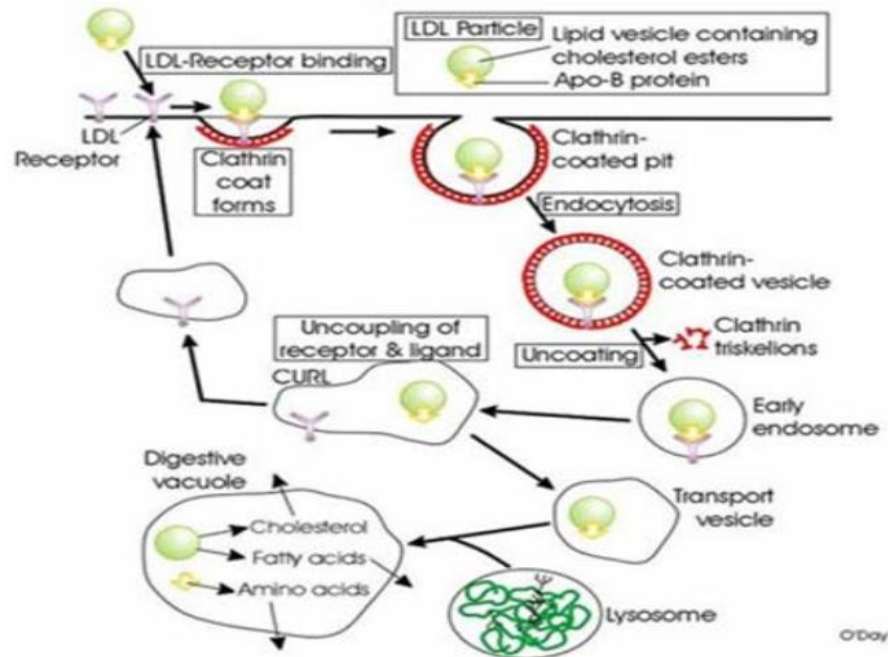


Figure 6: Receptor mediated endocytosis: Cholesterol uptake

In patients suffering from familial hypercholesterolemia, having high levels of cholesterol in serum and hence suffer from heart attacks early in life. Because these patients are unable to internalize LDL from the extracellular fluids, result in high accumulation of cholesterol. Normal individual possess LDL for transport of cholesterol but familial hypercholesterolemia results from inherited mutation in LDL receptor. These mutations can happen in two ways.

Either the patients simply fail to bind with LDL, demonstrating that a specific cell surface receptor is required for uptake of cholesterol. Or the patients are able to bind with LDL but are unable to internalize it. Because they are unable to concentrate in coated pits, demonstrating that coated pits in receptor plays an important role for cholesterol uptake. This mutation lies in the cytoplasmic tail of the receptor and can be subtle as the change of a single tyrosine to cysteine.

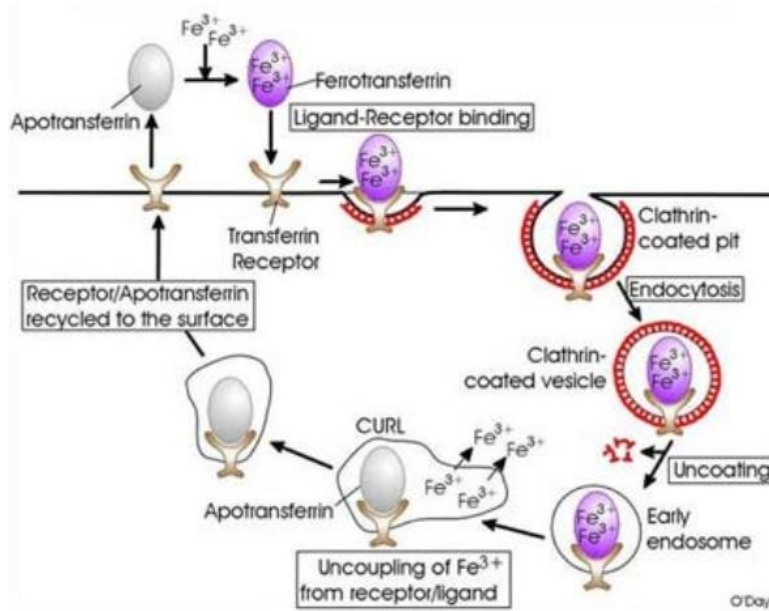


Figure 7: Example for receptor mediated endocytosis for ion uptake

3. Caveolae:

Caveolae is a pathway which is independent of clathrin- endocytosis process and involves in the uptake of molecules in small invaginations of the plasma membrane (50 to 80 nm diameter). These are enriched in lipid rafts of cholesterol, phospholipid and sphingolipids and possess a distinct coat formed by a protein called caveolin (cholesterol binding protein). It is abundant in smooth muscle, type I pneumocytes, fibroblasts, adipocytes, and endothelial cells (Parkar et al., 2009).

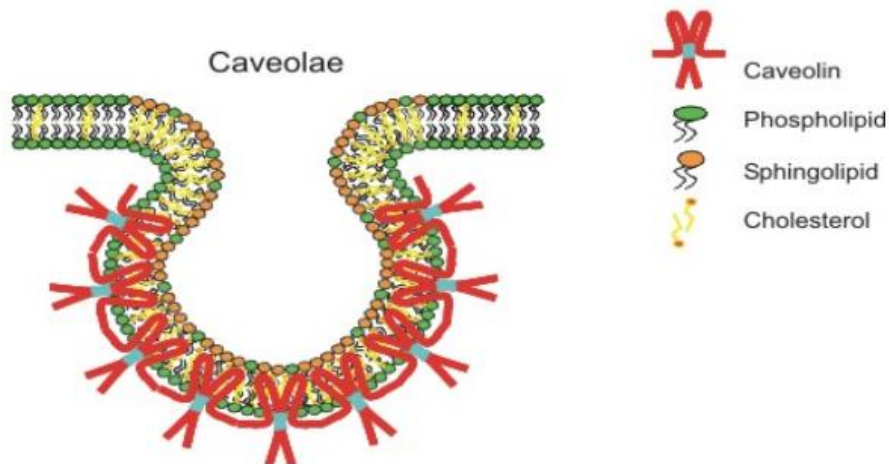


Figure 8: Structure of caveolae

Cells mostly use caveolae for the selective uptake of molecules as small as folate to full size proteins such as albumin and alkaline phosphatase. Many studies have shown that caveolae-mediated uptake of materials is not limited to macromolecules. In certain cell-types, viruses as simian virus 40 and even entire bacteria as some specific strains of *E. Coli* are engulfed and transferred to intracellular compartments in a caveolae-dependant fashion.

4. Macropinocytosis: The process of uptake of fluids in large vesicles (0.15 to 5 μm in diameter) is called macropinocytosis.

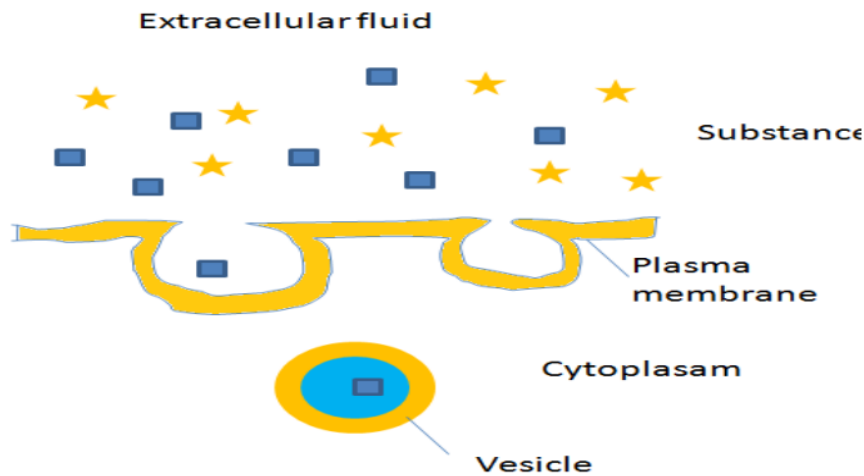


Figure 9: Diagram depicting Pinocytosis

Macropinocytosis is a different phenomenon from phagocytosis.

Macropinocytosis can uphold for particle uptake and involves the uptake of large amounts of fluid and solutes which is non-specific in nature. The receptors that trigger macropinocytosis have other physiological roles and are present on many cell types. These include growth factor receptors that activate common signalling pathways and involve global activation of the actin cytoskeleton resulting in plasma membrane ruffling and the formation of lamellipodia or blebs over the entire surface of the cell. In contrast, phagocytosis is particle-driven, and it depends on receptor interactions over the entire surface of the ingested particle. The receptors that trigger phagocytosis are usually specialized for interaction with the surface components of relevant cargo particles and actin modifications are localized to the phagocytic cup that forms around the particle.

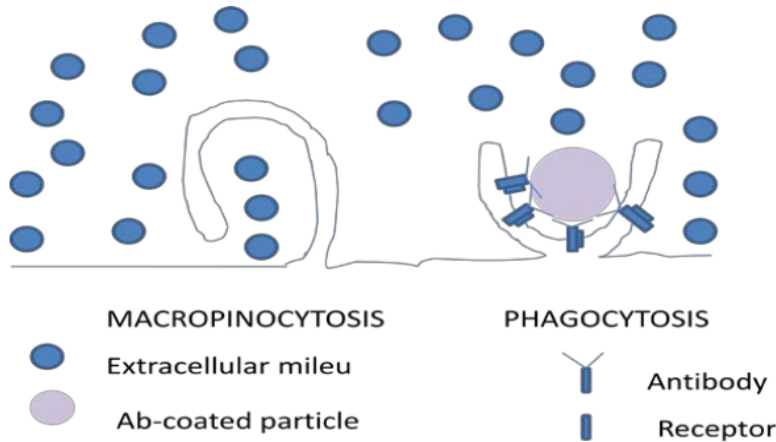


Figure 10: Differences between macropinocytosis and phagocytosis

Protein Trafficking in Endocytosis:

After internalization, clathrin-coated vesicles rapidly shed their coats and fuse with early endosomes which maintain an acidic internal pH and are located in periphery of the cell. This acidic pH leads to the dissociation of many ligands from receptors within early endosome compartment and hence serves as sorting compartment, from which molecules taken up by endocytosis are either recycled to the plasma membrane or transported to lysosomes for degradation. Later, ligands and membrane proteins for degradation are transported to late endosomes which are mediated by movement of large endocytic carrier vesicles along microtubules. The late endosomes are more acidic than early endosomes and fuse with transport vesicles carrying hydrolases from Golgi apparatus. Late endosomes mature into lysosomes when they acquire a full complement of lysosomal enzymes and become acidic. Hence the endocytosed materials are degraded by action of acid hydrolases (Cooper et al., 2000).

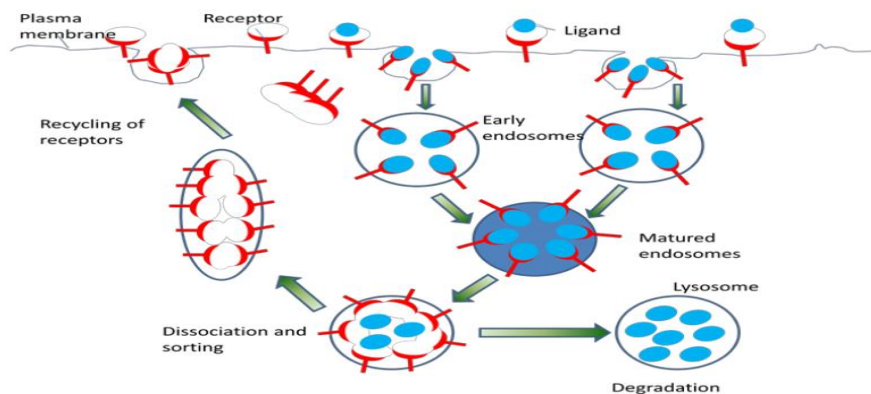


Figure 11: Protein trafficking during endocytosis. The figure shows the recycling of the plasma membrane and degradation of ligands and other membrane proteins in lysosome

The most common example for protein trafficking is recycling of synaptic vesicles. As when an action potential arrives at the terminal of most neuron signals, the fusion of synaptic vesicles with the plasma membrane releases neurotransmitter that carry signal to post synaptic cells. The empty synaptic vesicles are then recovered by plasma membrane in clathrin-coated vesicles, which fuse with early endosomes. The synaptic vesicles are then regenerated directly by budding from endosomes. They accumulate a new supply of neurotransmitter and recycle to the plasma membrane and ready for next cycle of synaptic transmission.

Transcytosis:

The process of transfer of internalized receptor across the cell to opposite domain of the plasma membrane is called transcytosis. It occurs in polarized cells or epithelial cells mostly. It is used for protein sorting and also a mean for transport of macromolecules across the epithelial cell sheets.

Example: Transport of Abs from blood to other secreted fluids. The Abs bind to the receptor on basolateral surface and then transcytosed along with their receptors to apical surface. The receptors are then cleaved and release Abs into extracellular secretions.

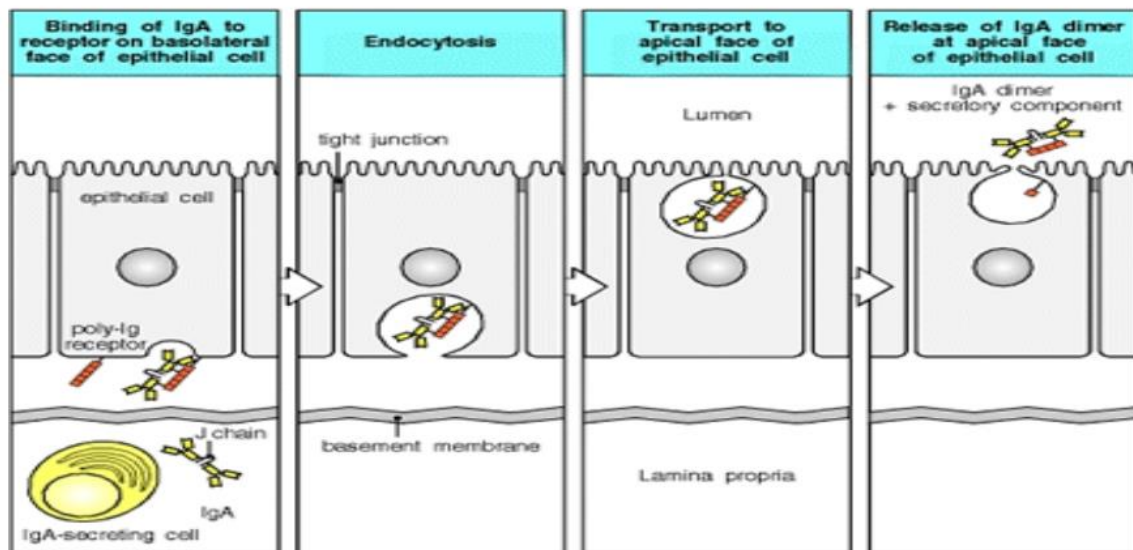


Figure 12: Process of transcytosis of IgA, an immunoglobulin, within an epithelial cell.

Exocytosis:

The process by which the cells direct the contents of secretory vesicles out of the cell membrane is known as exocytosis. These vesicles contain soluble proteins to be secreted to the extracellular environment, as well as membrane proteins and lipids that are sent to become components of the cell membrane. It is the final step in the secretory pathway that typically begins in the endoplasmic reticulum (ER), passes through the Golgi apparatus, and ends at the outside of the cell. Some of the examples include secretion of proteins like enzymes, peptide hormones and antibodies from cells and release of neurotransmitter from presynaptic neurons.

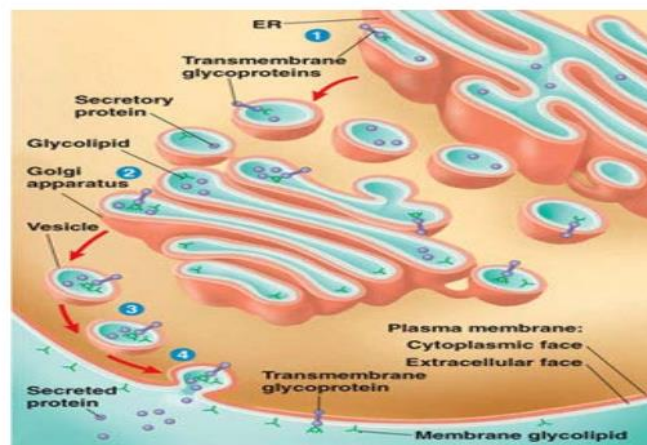


Figure 13: Diagram depicting Exocytosis process

Types of exocytosis: Exocytosis are of two types. Constitutive exocytosis and Regulated exocytosis

- 1. Constitutive exocytosis:** Secretory materials are continuously released without requirement of any specific kind of signal.
- 2. Regulated exocytosis:** Regulated exocytosis requires an external signal, a specific sorting signal on the vesicles for release of components. It contains a class of secretory vesicles that fuse with plasma membrane following cell activation in presence of signal. Examples of regulated exocytosis are secretion of neurotransmitter, hormones and many other molecules

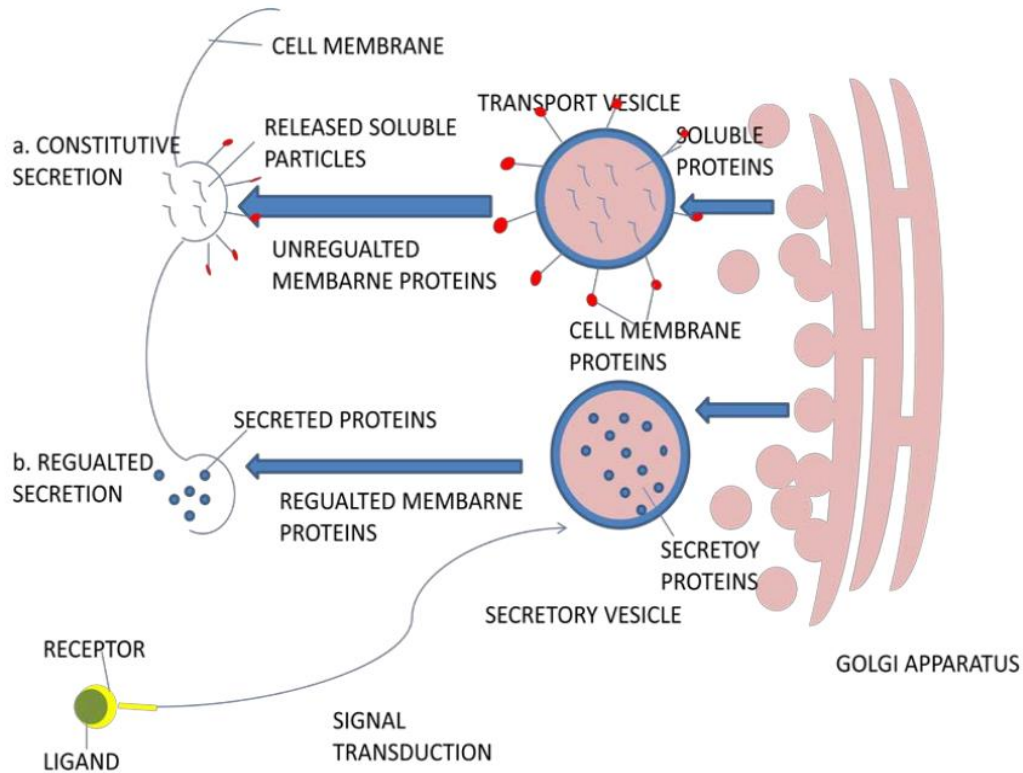


Figure 14: Types of exocytosis. (a) Constitutive secretion and (b) Regulated secretion

Steps in exocytosis:

Vesicles are used to transport the proteins from the Golgi apparatus to the cell surface area using motor proteins and a cytoskeletal track to get closer to cell membrane. Once these vesicles reach their targets, they come into contact with tethering factors that can restrain them. Then the process of **vesicle tethering** distinguishes between the initial, loose tethering of vesicles from the more stable, packing interactions. Tethering involves links over distances of more than about half the diameter of a vesicle from a given membrane surface (>25 nm). The process of holding two membranes within a bilayer's distance of one another (<5-10 nm) is called **vesicle docking**. Stable docking indicates the molecular interactions underlying the close and tight association of a vesicle with its target may include the molecular rearrangements and ATP-dependent protein and lipid modifications, needed to trigger bilayer fusion called **vesicle priming**. It is mostly takes place before exocytosis and used in regulated secretion type of exocytosis but not used in constitutive secretion. It is followed by **vesicle fusion** which includes merging of the vesicle membrane with the target and hence there is release of large biomolecules in the extracellular space with the help of some protein complex.

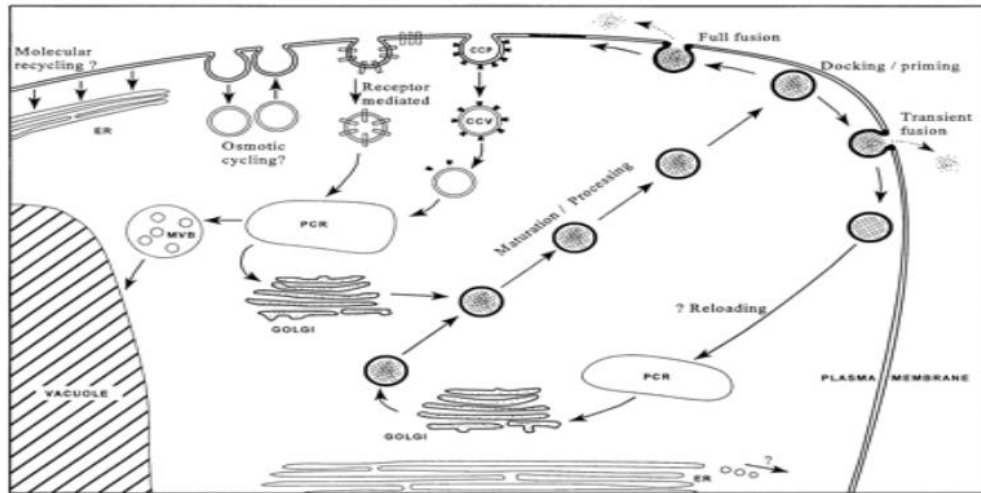


Figure 15: Sequence of Exocytosis and Endocytosis: Transport, Docking, Fusion, Content Release, and Recycling.

Interesting facts:

- Many cells in the body use exocytosis to release enzymes or other proteins that act in other areas of the body like secretion of the hormones glucagon and insulin, or to release molecules that help cells communicate with one another more directly through the products that they secrete like neurotransmitters.
- The immune system also uses exocytosis to communicate information between cells.
- Both endocytosis and exocytosis involve active transport, that is, energy must be expended to move particles against the concentration gradient.
- Both endocytosis and exocytosis involve the formation of vesicles: endocytosis forms them in order to take particles into the cell via the cell membrane and involves a reduction in cell membrane area, as part of the membrane is pinched off to form a vesicle; exocytosis forms them in order to expel things from the cell via the cell membrane and results in an increase in cell membrane, as the vesicle wall joins that of the cell membrane and is incorporated into it. Thus, the two processes also serve to balance each other.

Intracellular analysis

- The vesicle fusion is driven by SNARE proteins process of merging the vesicle membrane with the target one resulting in release of large biomolecules in the extracellular space.
- Ca^{2+} might control the exocytotic/endocytotic balance in plants.
- Synaptotagmin I (syt1) is required for normal rates of synaptic vesicle endo- and exocytosis.

Questions:

1. The transport method of neurotransmitters between nerve cells is:
 - a. Exocytosis
 - b. Passive transport
 - c. Receptor-mediated endocytosis
 - d. Active transport
2. Which of the following membrane activities does NOT require the expenditure of energy by the cell?
 - a. Active transport
 - b. Osmosis
 - c. Endocytosis
 - d. Exocytosis
 - e. Synthesis of more membrane
3. The membrane transport mechanism used when an amoeba engulfs a bacterial cell is called:
 - a. Carrier-mediated facilitated diffusion
 - b. Exocytosis
 - c. Phagocytosis
 - d. Pinocytosis
 - e. Sodium-potassium pump

Intracellular analysis

4. A person has a genetic disease that prevents the phospholipids in the plasma membrane of the white blood cells from freely fusing with the other membranes within the cell. How would this disease affect phagocytosis?
 - a. Lysosomes would not be formed
 - b. Facilitated diffusion would not occur
 - c. Lysosomes would be formed lacking hydrolytic enzymes
 - d. The phagocytic vacuole would not fuse with the lysosome
 - e. Endocytosis would not occur

5. Pinocytosis:
 - a. Is engulfment of large particles by the cell
 - b. Occurs in protozoans and algae but not in more complex organisms
 - c. Involves the specific binding of molecules to receptors on the cell surface
 - d. Is the nonspecific uptake of fluids by pinching inward of the plasma membrane
 - e. Is movement of molecules against the concentration gradient through a permeable membrane

6. Receptor-mediated endocytosis:
 - a. Is a passive process
 - b. Involves only membrane transport proteins
 - c. Brings about the selective uptake of materials by enclosing them in membranous vesicles
 - d. Does not require energy
 - e. Is most likely to be found in cells that release large amounts of hormones

7. Exocytosis is a process by which cells
 - a. Pass substances out of the cell in vesicle
 - b. Pass substances out of the cell through the membrane by osmosis
 - c. release substances directly into the extracellular fluid through a pore
 - d. Release substances directly into the extracellular fluid through a pit
 - e. Identify substances in the environment

8. A cell engaged in phagocytosis must be
 - a. Engulfing a live organism
 - b. Acquiring a liquid
 - c. Engulfing a dead organism
 - d. Transporting bulk dissolved nutrients
 - e. Transporting bulk solid material

9. Coated pits are required for which type of membrane transport?
 - a. Receptor-mediated endocytosis
 - b. Phagocytosis
 - c. Pinocytosis
 - d. Exocytosis

10. Certain white blood cells are designed to engulf and destroy microbes such as bacteria. The process used to achieve this would be:
 - a. Phagocytosis
 - b. Receptor-mediated endocytosis
 - c. Pinocytosis
 - d. Exocytosis

11. What is the primary factor that determines if endocytosis or exocytosis would be needed to move a substance across the cell membrane as opposed to diffusion or osmosis?
 - a. the chemical properties of the molecules being moved
 - b. the size of the molecules being moved
 - c. the differences in concentration of the substance inside and outside the cell
 - d. whether water is being moved across the membrane

12. In endocytosis, a transport vesicle is derived from _____.
 - a. plasma membrane
 - b. ribosomes
 - c. Golgi complex
 - d. Lysosomes

Intracellular analysis

13. Low density lipoproteins (LDL's or bad cholesterol) are taken up "in-bulk" into the cytoplasm of a cell. This process is an example of
- Endocytosis
 - Exocytosis
 - Molecular transport
 - Osmosis
 - Diffusion
14. How the phagocytosis takes place in the immune system of cells?
15. Describe the uptake of cholesterol by the mammalian cells using receptor-mediated endocytosis.
16. What is protein trafficking? Explain with an example.