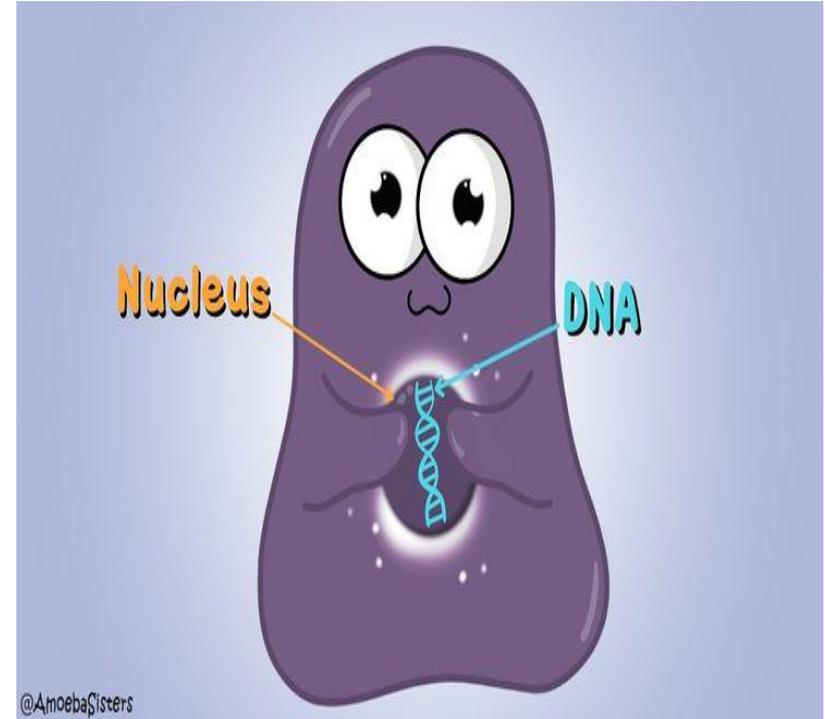


INTRODUCTION TO CYTOLOGY



By

Dr. Heba Sharaf Eldin

Assistant Professor of Histology & Cell Biology

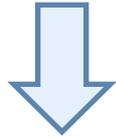
OBJECTIVES OF THE LECTURE

1. Define cytology and methods for studying by using microscopes.
2. Know the parts of the human cell.
3. Describe the molecular composition of the cell membrane.
4. Enumerate the functions of cell membrane.
5. Describe the transport across the cell membrane.

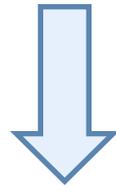
What *is* Cytology



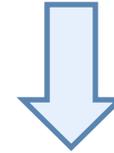
cytology *is*



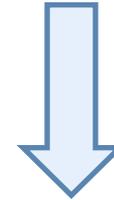
Cyto..



cell



ology



science

- The study of normal cells structure, mainly By using microscopes.
- *Why ?* The small size of **cells** and **matrix** components makes the study of cell dependents on the use of microscopes.

Organization of the human body

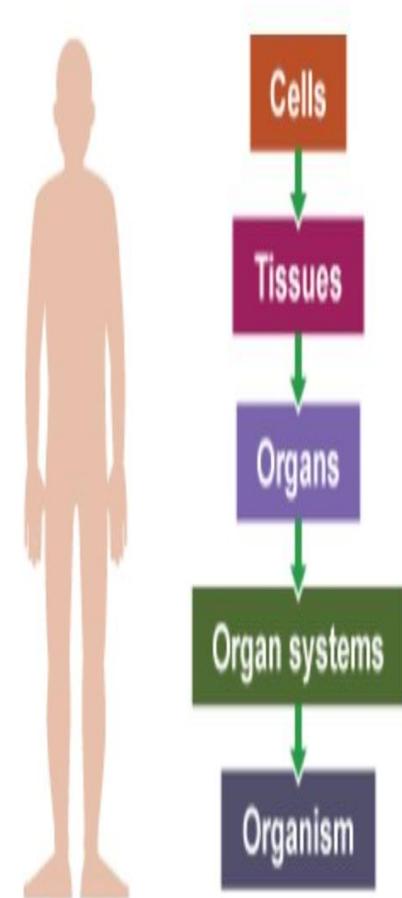
1-CELL: smallest structural & functional unit in the body.

2-TISSUE: 4 basic:

- Epithelial.
- Connective.
- Muscular.
- Nervous.

3-ORGAN : different tissues together perform special function.

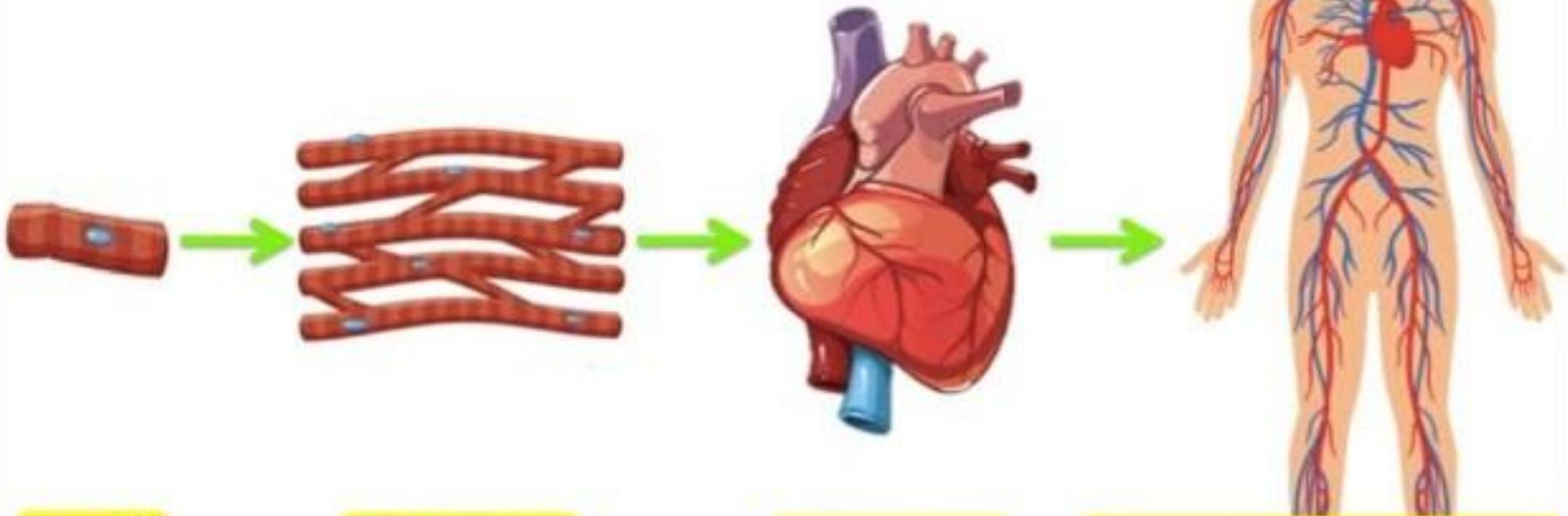
4-SYSTEMS : different organs together perform complex function.



Example

Cardiovascular system

Levels of organisation



Cell

Tissue

Organ

Organ system

How *to* study cytology



How to study cytology

```
graph TD; A[How to study cytology] --> B[Histological sections preparations]; A --> C[Staining]; A --> D[Examination]; B --> E[Microtechniques  
As paraffin sections]; C --> F[Haematoxylin and eosin  
(H & E): Routine stain]; D --> G[Microscopes];
```

Histological sections preparations

Microtechniques
As *paraffin sections*

Staining

Haematoxylin and eosin
(H & E): Routine stain

Examination

Microscopes

Types of microscopes

Magnification

Light microscope

X Up to 1000

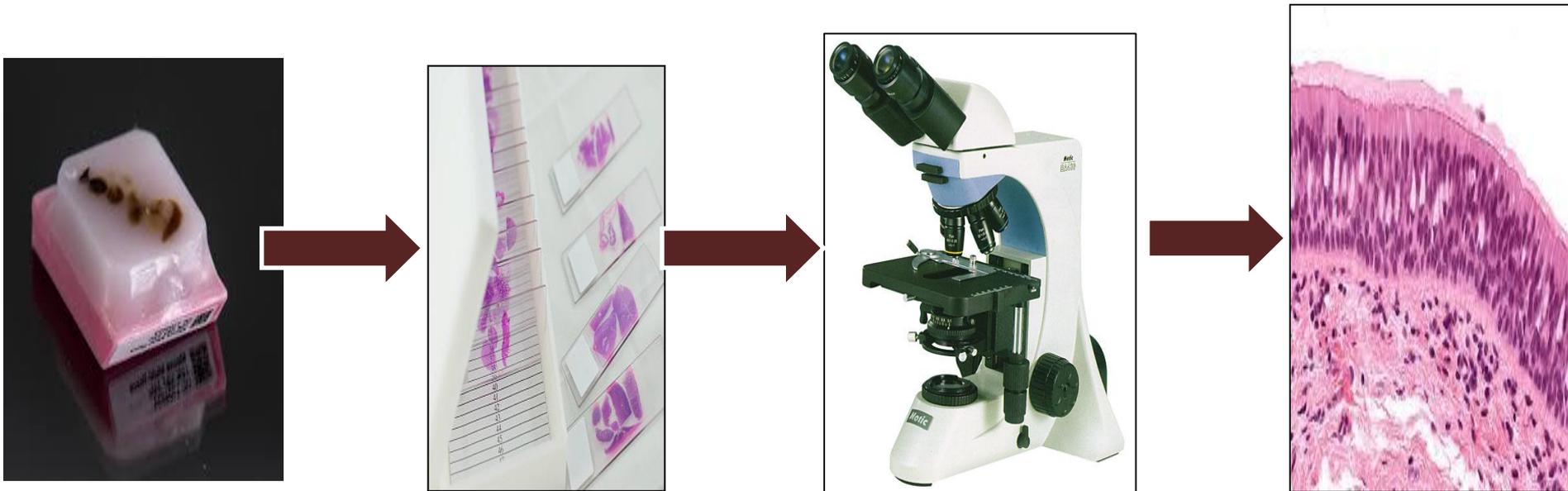


Electron microscope

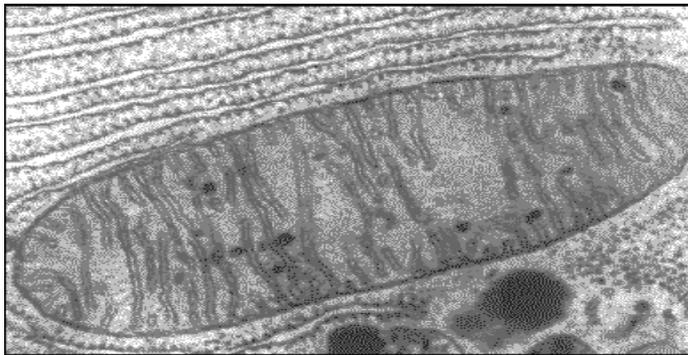
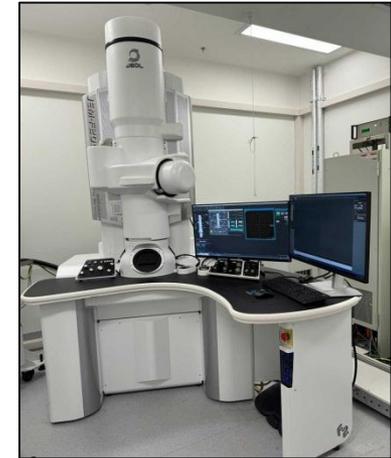
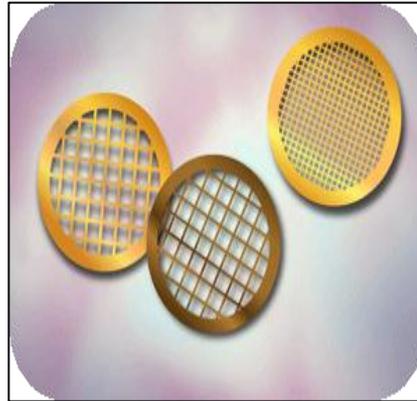
X up to 400,000



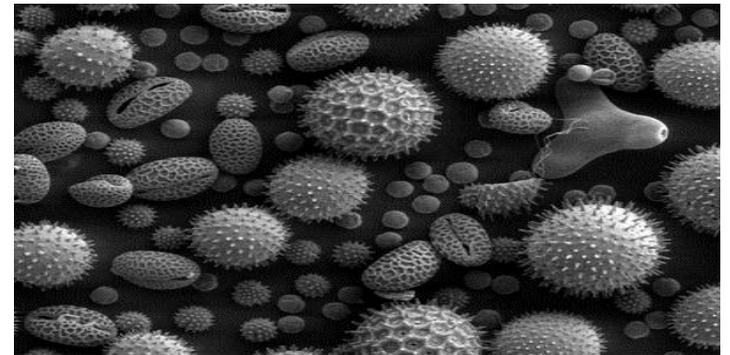
Light microscope



Electron microscope



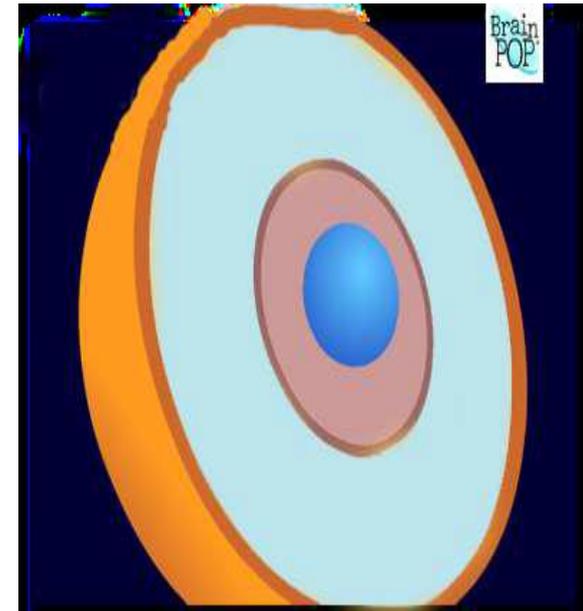
Transmission (TEM)



Scanning (SEM)

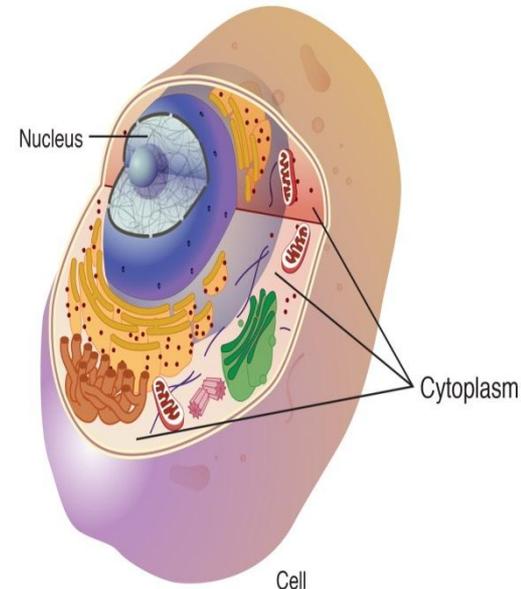
CYTOLOGY (CELL BIOLOGY)

- ❑ *Cytology* : means the study of cells.
- ❑ *The cell*: is the structural and functional unit of the organism.
- ❑ The cell is limited by a **cell membrane**
- ❑ *Protoplasm*, the living substance of the cell, is subdivided into two compartments, the **cytoplasm** and **the nucleus**.



The cytoplasm is formed of:

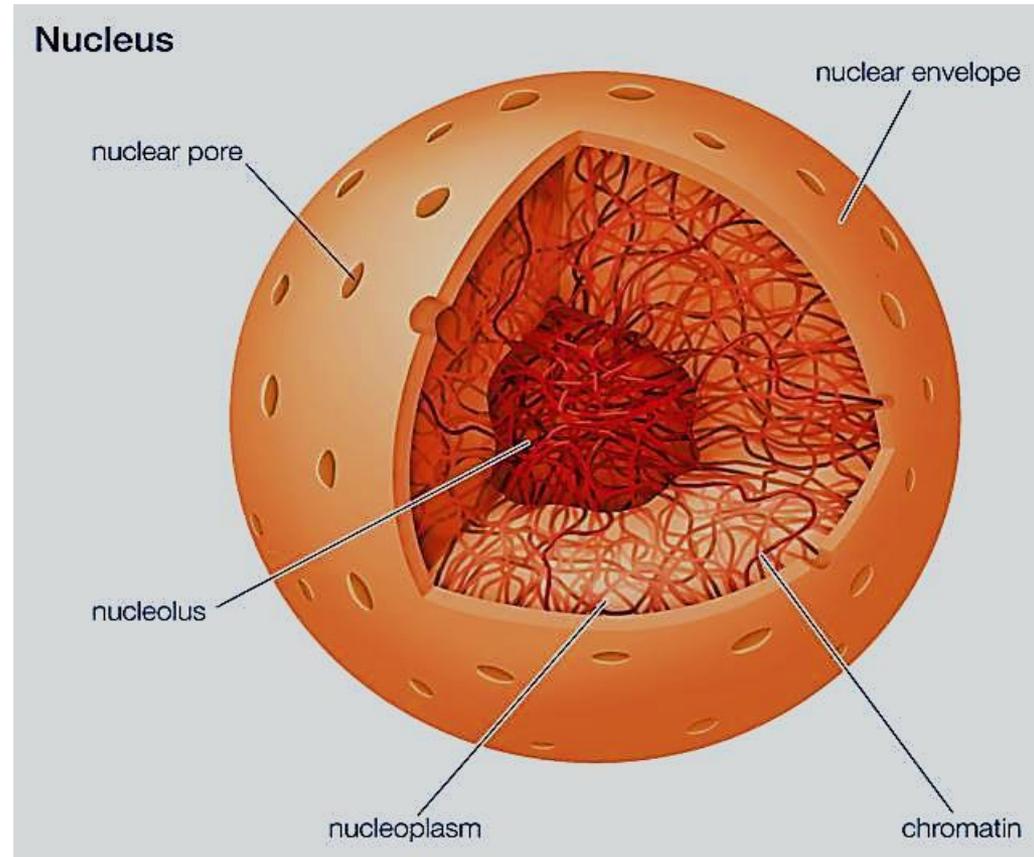
1. **Cytosol**: It is **semifluid** matrix. In which organelles, cytoskeleton and inclusions are embedded
2. **Cytoplasmic organelles**: active **permanent** structures that are **essential for the life** and the function of the cell.
3. **Cytoskeleton**: It is formed of tubules and filaments that maintain the shape of the cells, help in the cell movement.
4. **Cytoplasmic inclusions**: They are **non living temporary** structure, consist of metabolic products, stored nutrients.
They are not essential for the life of the cell.



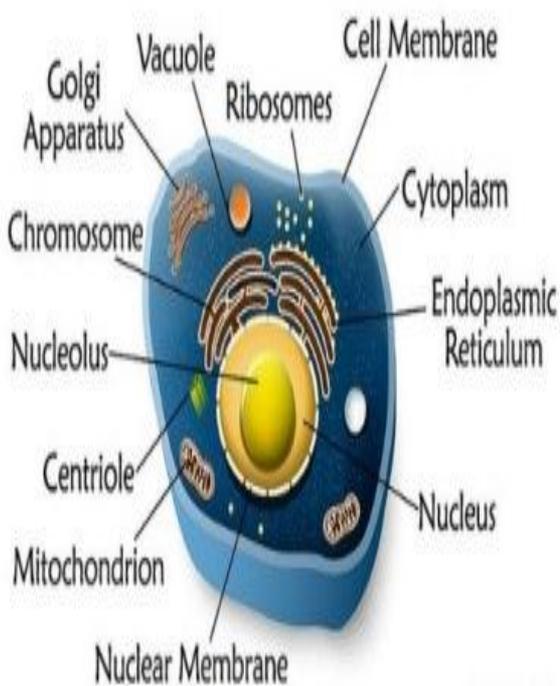
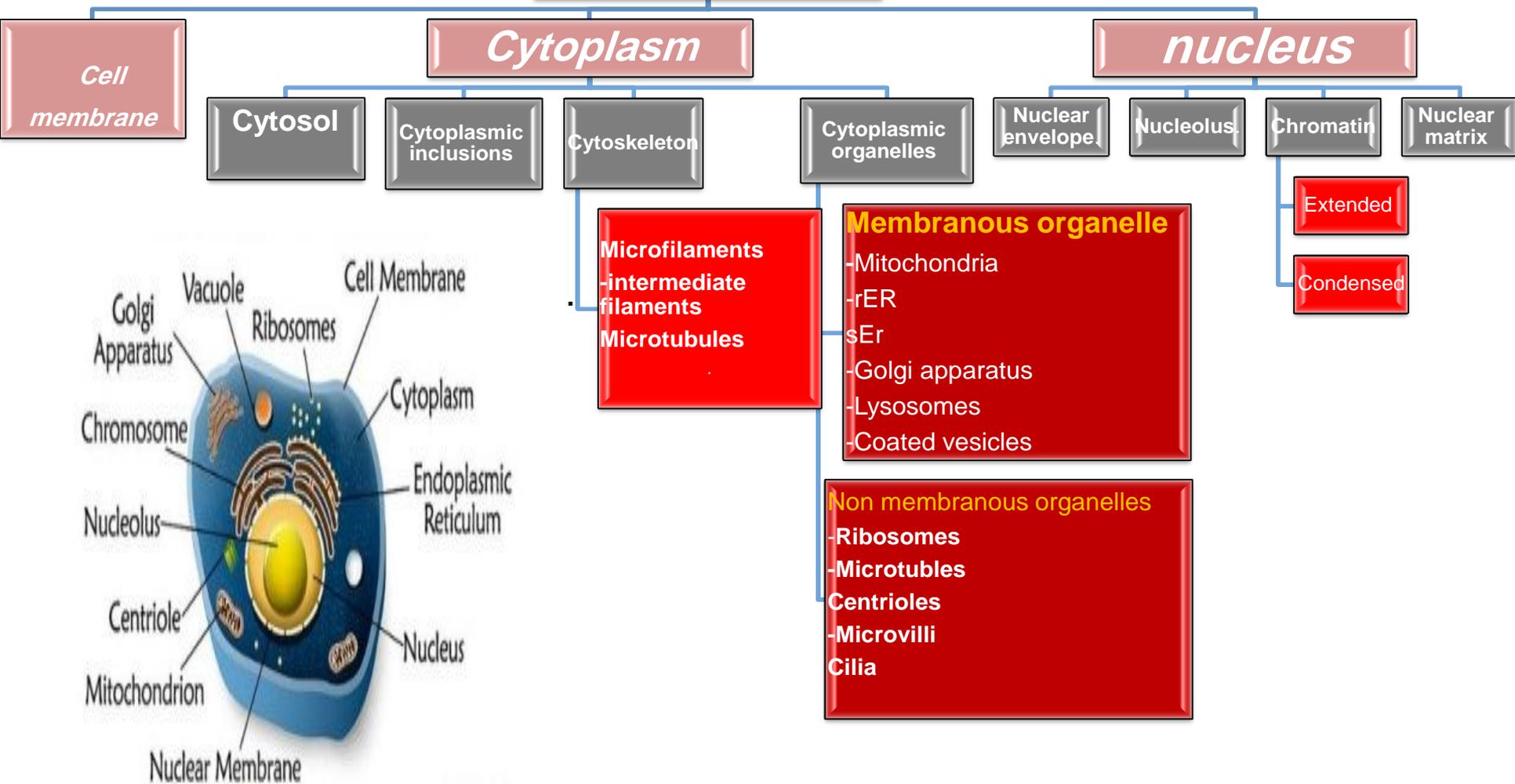
The **nucleus** is formed of:

In **non-dividing cells**; *It consists of the followings:*

1. Nuclear envelope.
2. Nucleolus.
3. Chromatin.
4. Nucleoplasm.



The cell



CELL MEMBRANE

All cells are bounded by a cell membrane (plasma-membrane or plasmalemma).

□ Structure of cell membrane:

Light microscope (L/M): invisible as it is very thin (8-10 nm).

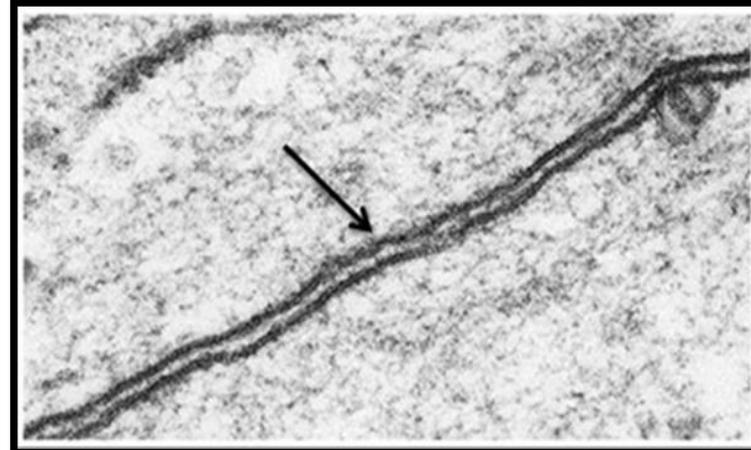
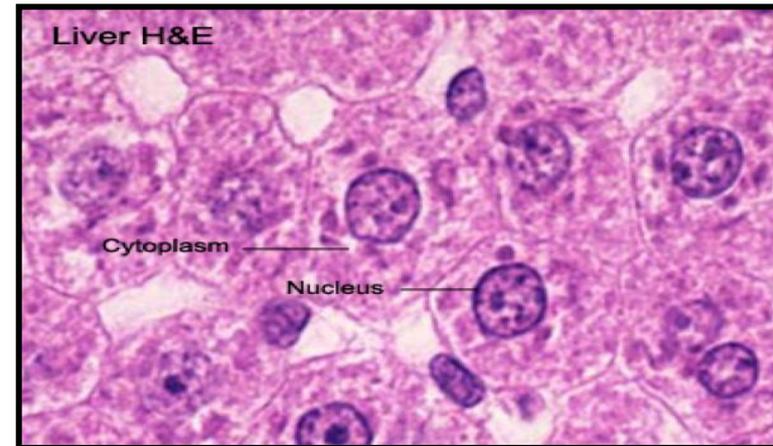
Electron microscope (E/M):

1- Low magnification: it appears as a **dense thin line**.

2- High magnification:

it appears as two **electron-dense layers** (2.5 - 3 nm each) separated by an **electron-lucent (light) intermediate zone** (3.5-4 nm).

it has a characteristic trilaminar appearance that has been also called the unit membrane.

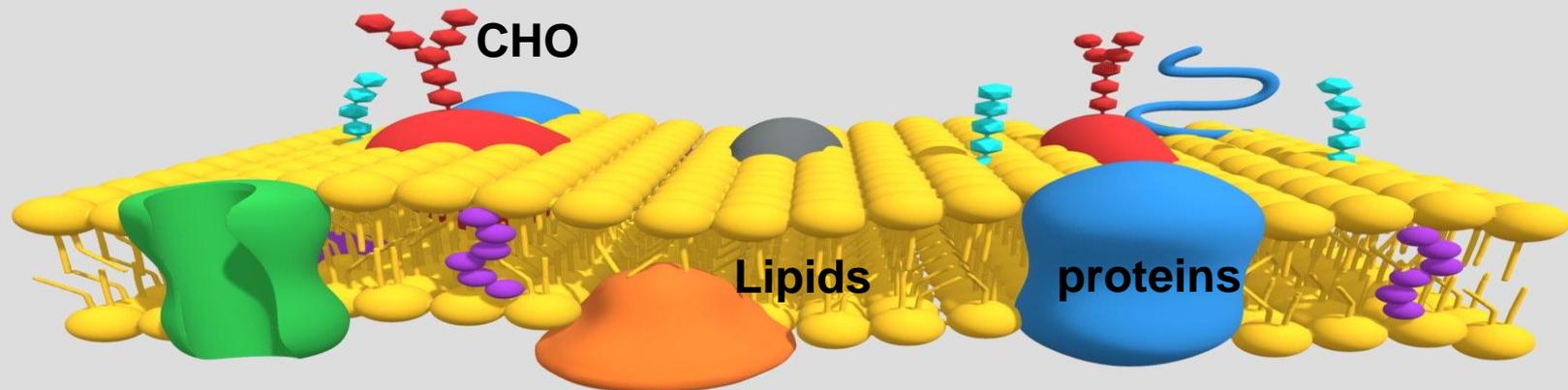


Molecular structure of the cell membrane

According to **The fluid-mosaic model** of Singer and Nicholson

The cell membrane consists mainly of:

1. **Lipids** (Phospholipids bilayer & Cholesterol).
2. **Proteins** (integral & peripheral).
3. **Carbohydrates** (glycoproteins & glycolipids)



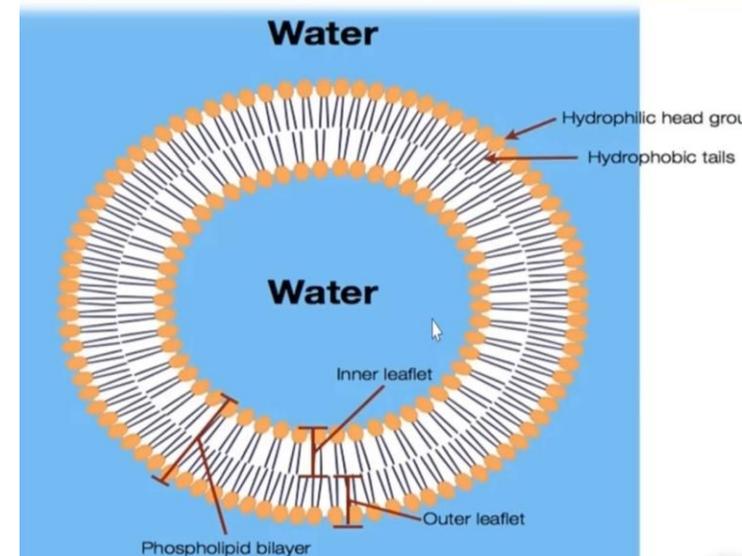
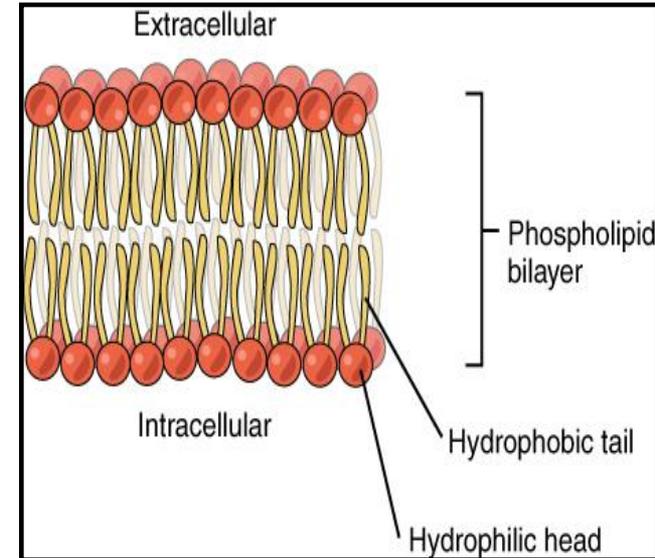
1) Lipids components

- Allow the passage of fat soluble substance through the cell membrane.
- Responsible for the semipermeability of the membrane

Two types of lipids are present

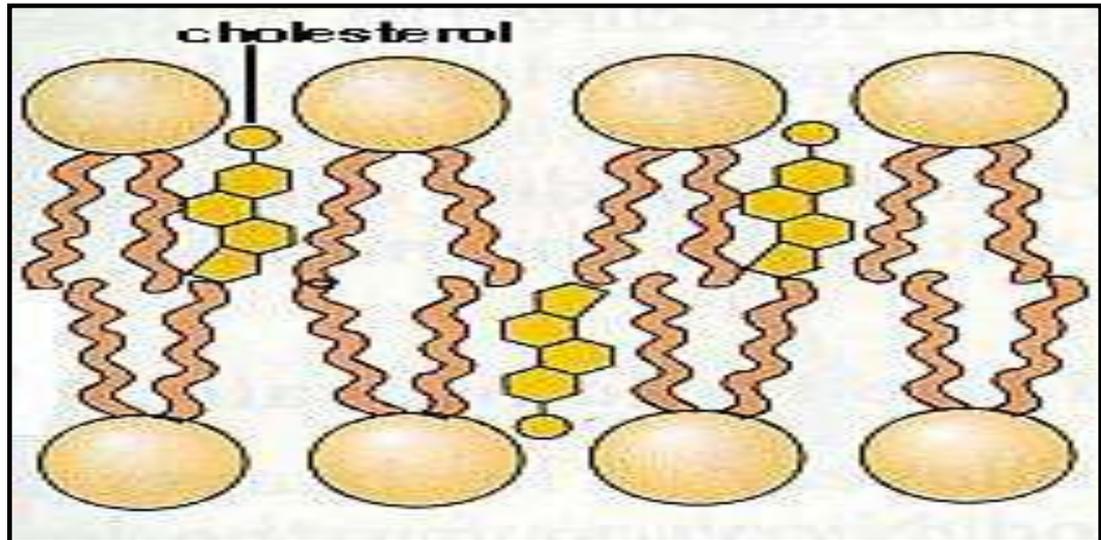
I) Phospholipids bilayer:

- It is the **backbone** of the plasma-membrane.
- It is formed of **double layer** of phospholipids molecules.
- **The phospholipids molecules** have:
 - ❑ Outer phosphate heads which are polar and hydrophilic (water attracting)
 - ❑ Inner two tails of long fatty acid chains which are non-polar hydrophobic (water repelling)

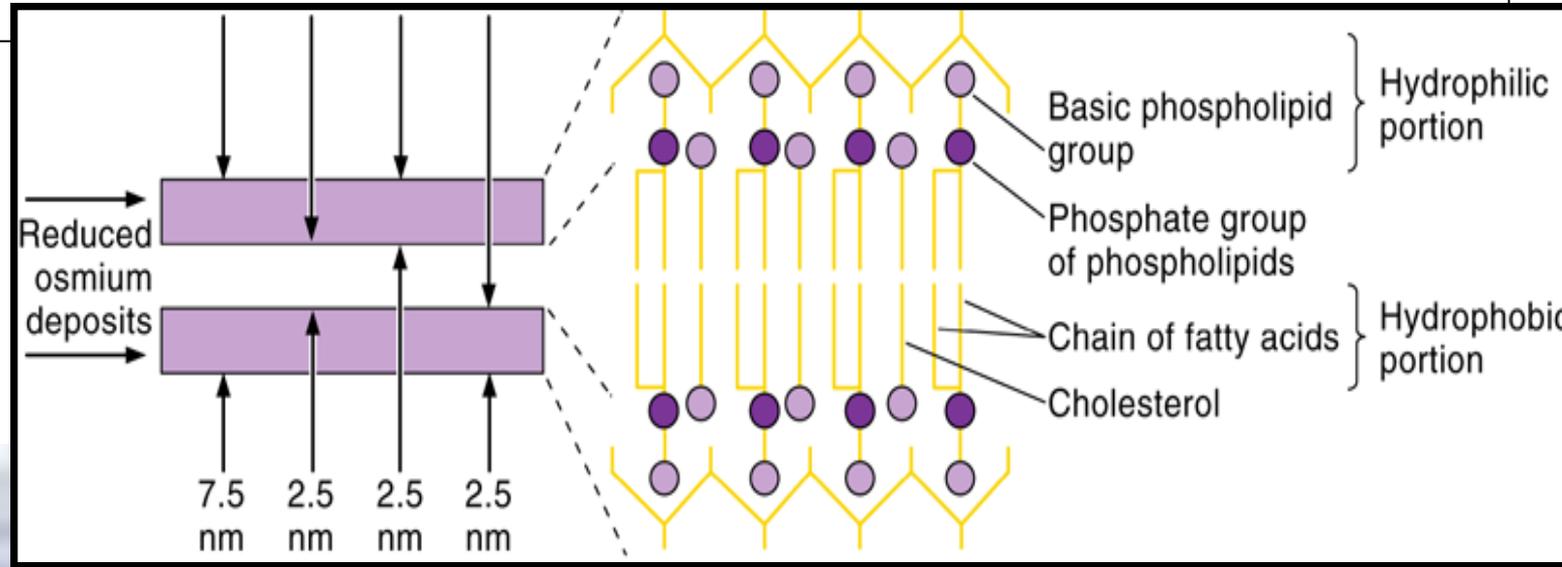


II) Cholesterol molecules:

- They are present in lipid bilayer.
- They **fill the gaps** between the fatty acid tails and prevent their close packing.
- Maintains the **stability of the membrane** by modulating the **fluidity** and movement of all membrane compartments.



E/M of cell membrane by high magnification



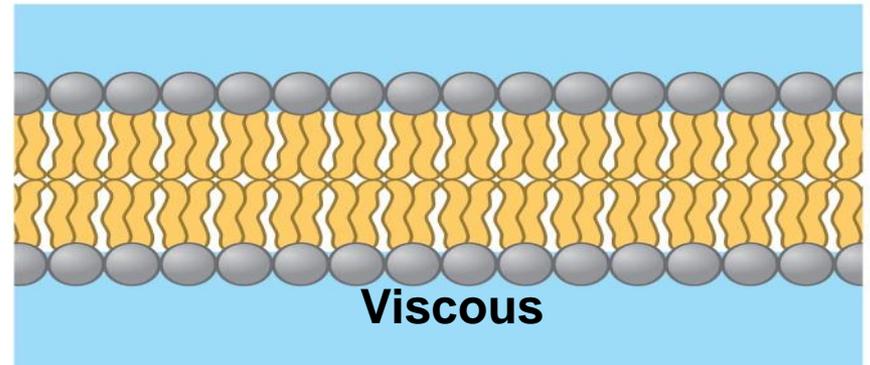
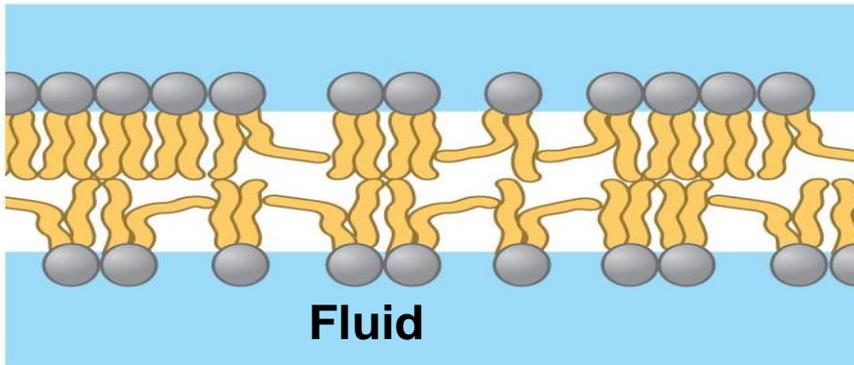
trilaminar appearance:

- two electron dense (dark) layers
- with a electron lucent (light) band in-between

Due to deposition of osmium tetroxide fixative on the outer and inner hydrophilic heads of the lipid bilayer

The Fluidity of cell Membrane

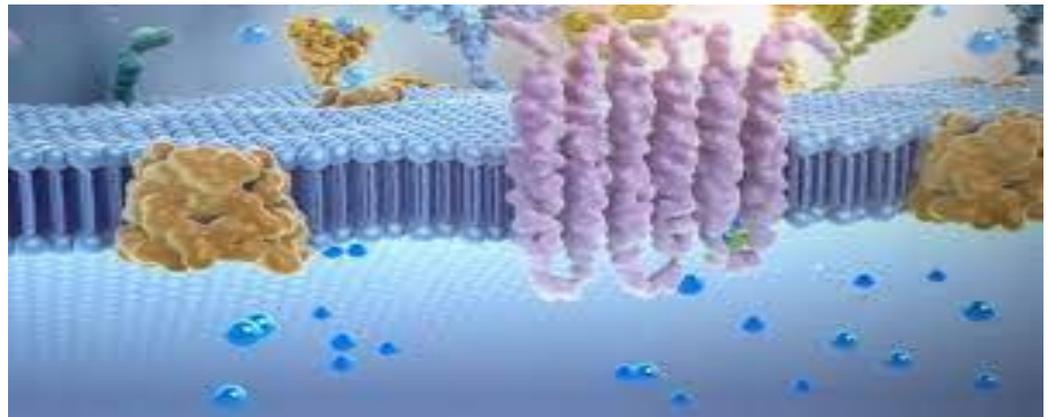
- Phospholipids in the plasma membrane can move within the bilayer
- Most of the lipids, and some proteins, drift laterally.
- Membranes rich in unsaturated fatty acids are more fluid than those rich in saturated fatty acids.
- At warm temperatures (such as 37°C), cholesterol restrains movement of phospholipids
- Membranes must be fluid to work properly; fluidity of cell membrane is essential for endocytosis, exocytosis and membrane biogenesis.



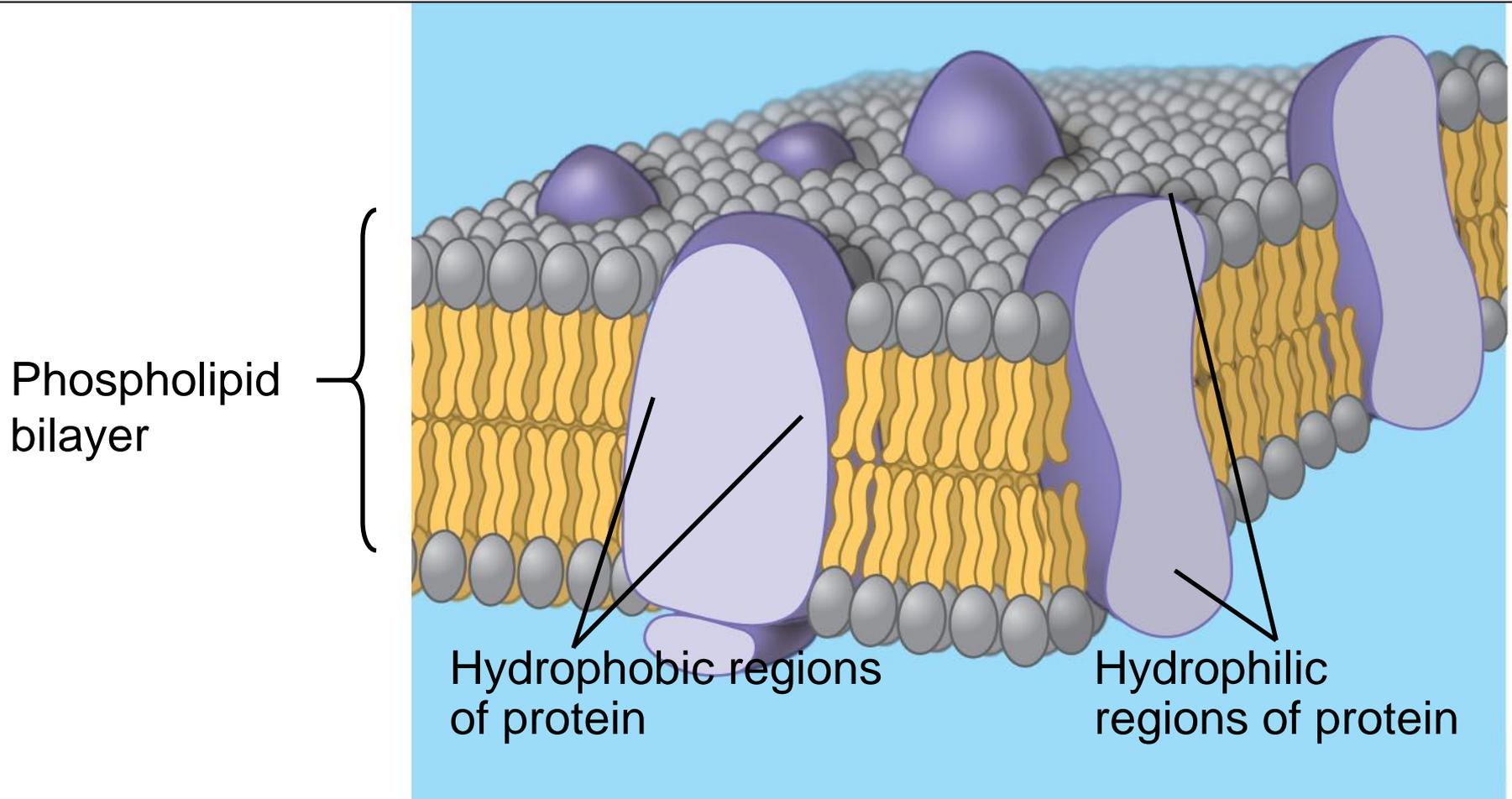
2) Proteins components : They are two types:

A) Integral (intrinsic) proteins:

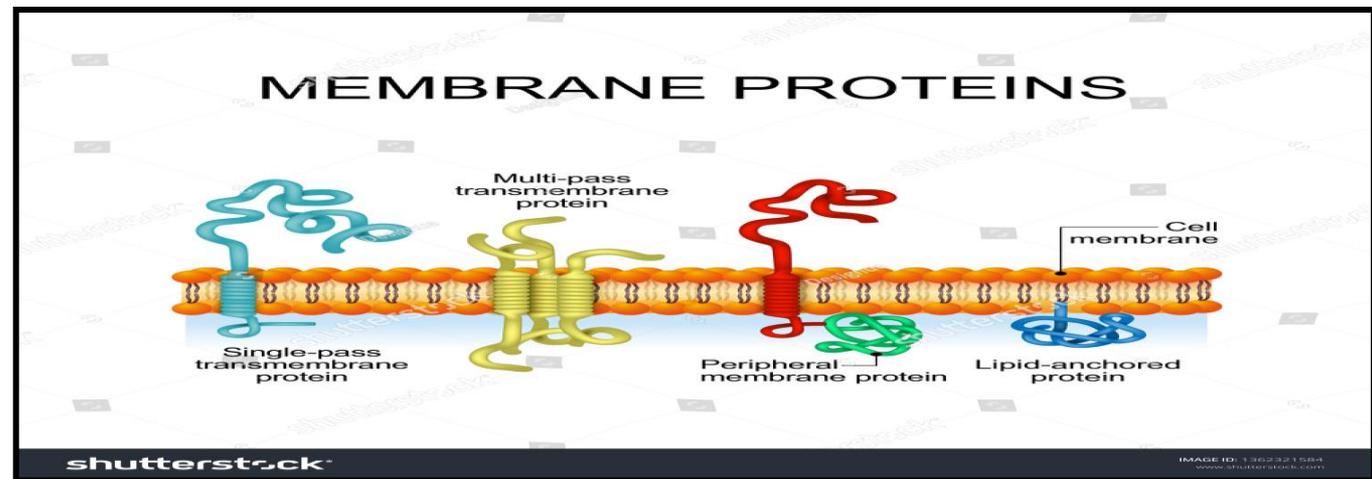
- ❑ They are **present among** lipid molecules.
- ❑ **Some** are **partially** embedded in lipid bilayer.
- ❑ **Other** proteins extend across lipid bilayer and protrude from both membrane surfaces (**transmembrane protein**) that have channels through which water-soluble molecules (e.g. ions)
- ❑ Integral membrane proteins can move within the lipid bilayer if they are not bound to filaments in the underlying cytoplasm.



□ Transmembrane proteins have hydrophobic and hydrophilic regions ; Within membrane :**hydrophobic** amino acids and On outer surfaces of membrane: **hydrophilic** amino acids that extend into extracellular fluid & into cytosol.



- When proteins inserted once through the membrane are called "**single-pass transmembrane proteins.**" and if pass through several times are called "**multipass transmembrane proteins**", they form loops outside the membrane



B) Peripheral Proteins:

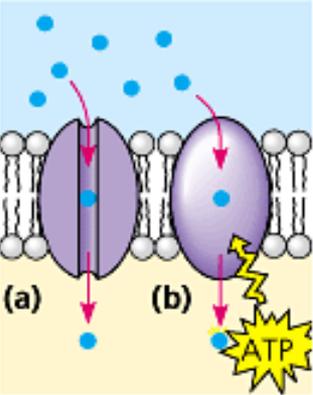
- They are associated with **the surfaces** of the bilayer.
- They are **loosely attached** to the membrane surfaces and can easily **separable** from the lipid bilayer

Functions of Membrane proteins :

- ❑ Act as **channels** allow **transport** materials across the membrane passively.
- ❑ Acts as **pump** to transport ions as Na^+ actively across the membrane.
- ❑ Act as **enzymes** to speed up chemical reactions.
- ❑ Act as **receptors** allow binding of specific molecules as hormones.
- ❑ Act as **linker** to anchor cytoskeleton to extracellular matrix.
- ❑ Act as **structural proteins** to form intercellular junctions.

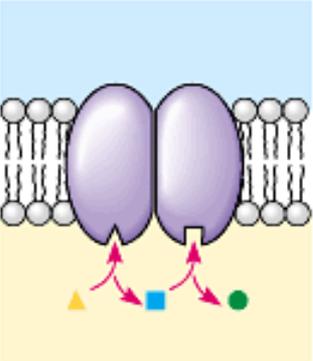
Protein Functions

Transport



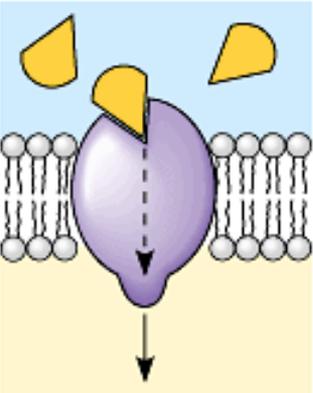
- Passive // Channel Proteins
- Active // Protein Pumps

Enzymatic activity



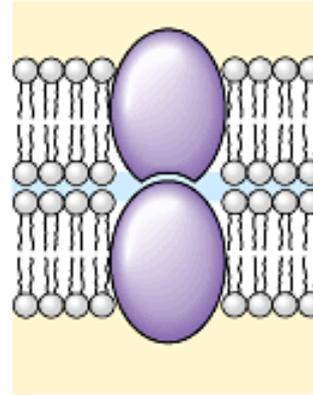
Membrane enzymes produce a variety of substances essential for cell function

Signal transduction (Cell surface Receptor)



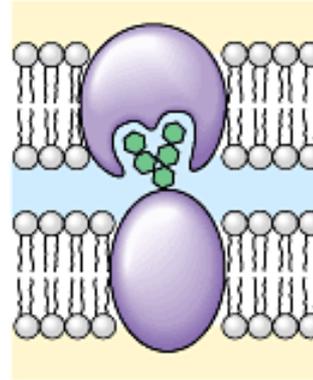
Extracellular signaling molecule activates a membrane receptor creating intracellular response

Intercellular joining



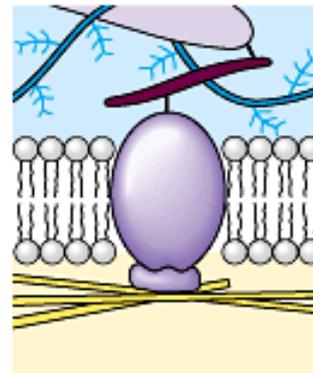
Intercellular junctions

Cell-cell recognition (Cell surface identity Marker)



Some glycoproteins serve as identification tags that are specifically recognized by other cells

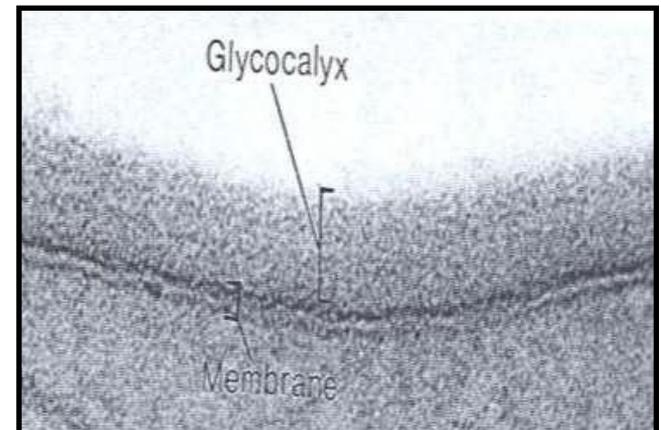
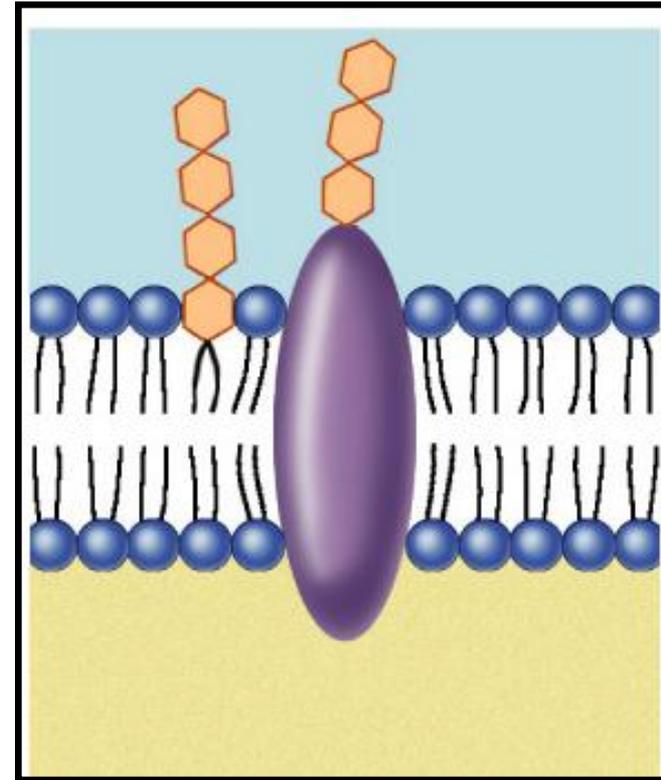
Attachment to the cytoskeleton and extracellular matrix



Microfilaments or other elements bonded to membrane proteins, maintain cell shape and stabilizes the location of certain membrane proteins

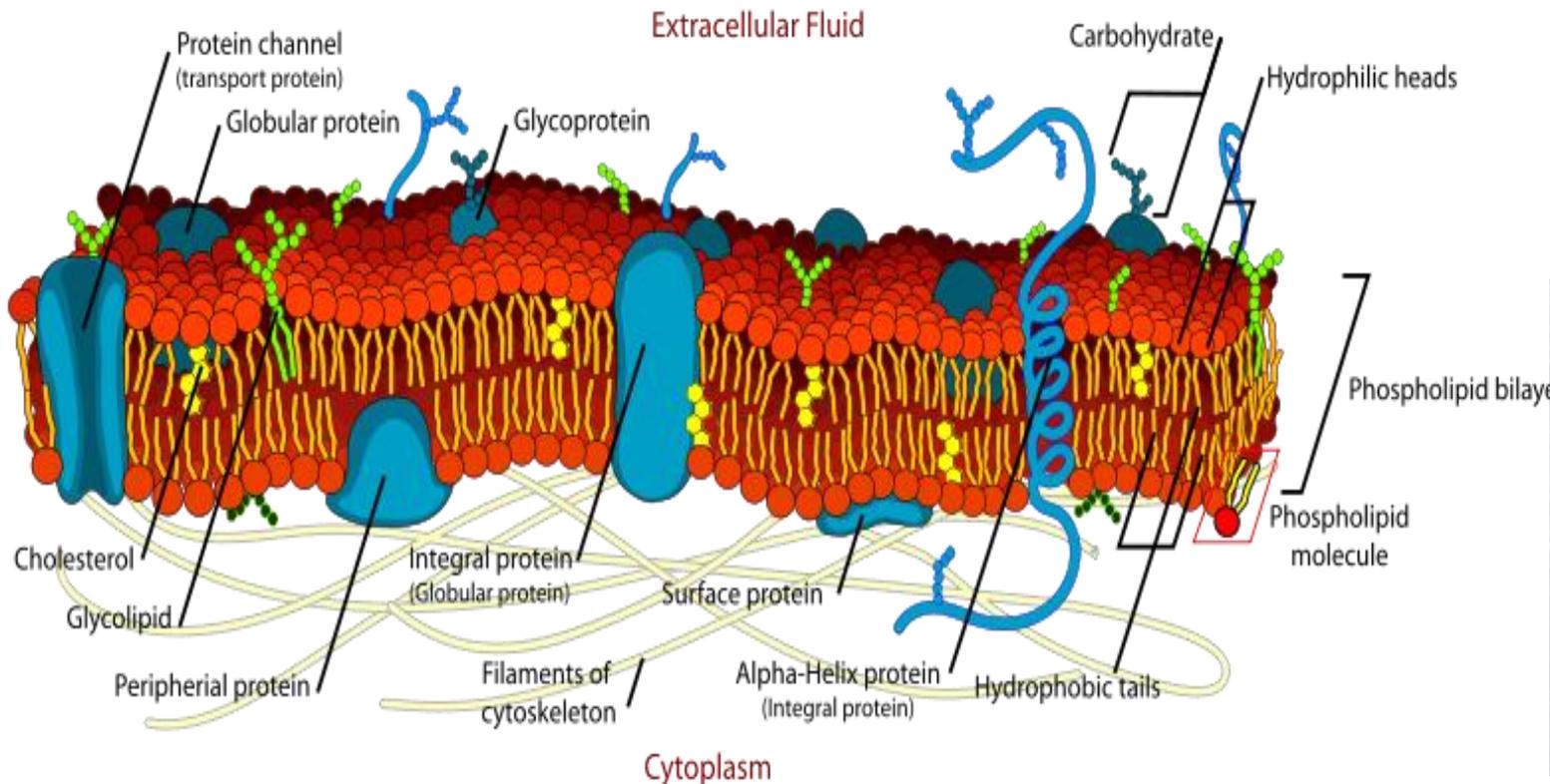
3) Carbohydrates components : Cell coat (Glycocalyx)

- ❑ It is associated with the **outer** surface of cell membrane.
- ❑ It consists of **short chains of polysaccharides** which are conjugated mainly with membrane proteins to form **glycoprotein** and to some of the membrane lipids to form **glycolipids**.
- ❑ **By E/M**, it appears as a fine filamentous material.
- ❑ **Function:**
 - cell **recognition**
 - protection
 - intercellular adhesions
 - **stabilise** membrane structure
 - some act as **receptor molecules**



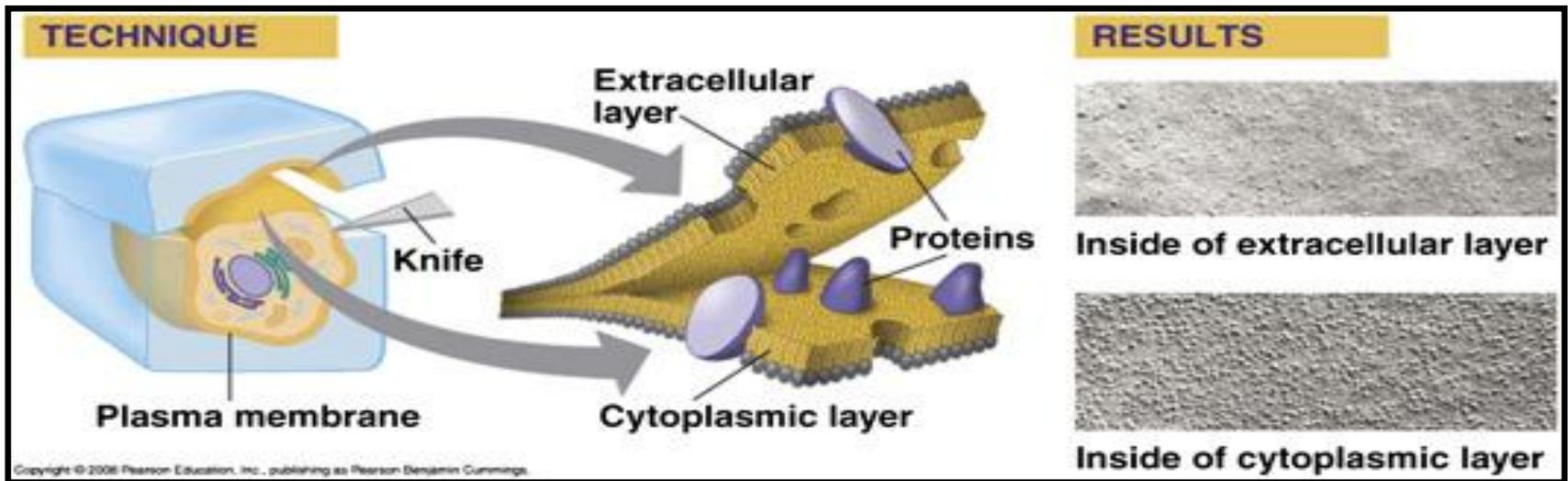
The fluid-mosaic model of Singer and Nicholson

At *body temperature*, the lipid bilayer is **fluid**. So, the mosaic disposition of membrane proteins with the fluid nature of the lipid bilayer constitutes the fluid-mosaic model for membrane structure.



When cells are frozen and fractured (cryofracture), the lipid bilayer of membranes is often cleaved along the **hydrophobic center**. **Splitting** occurs along the line of weakness formed by the fatty acid tails of phospholipids.

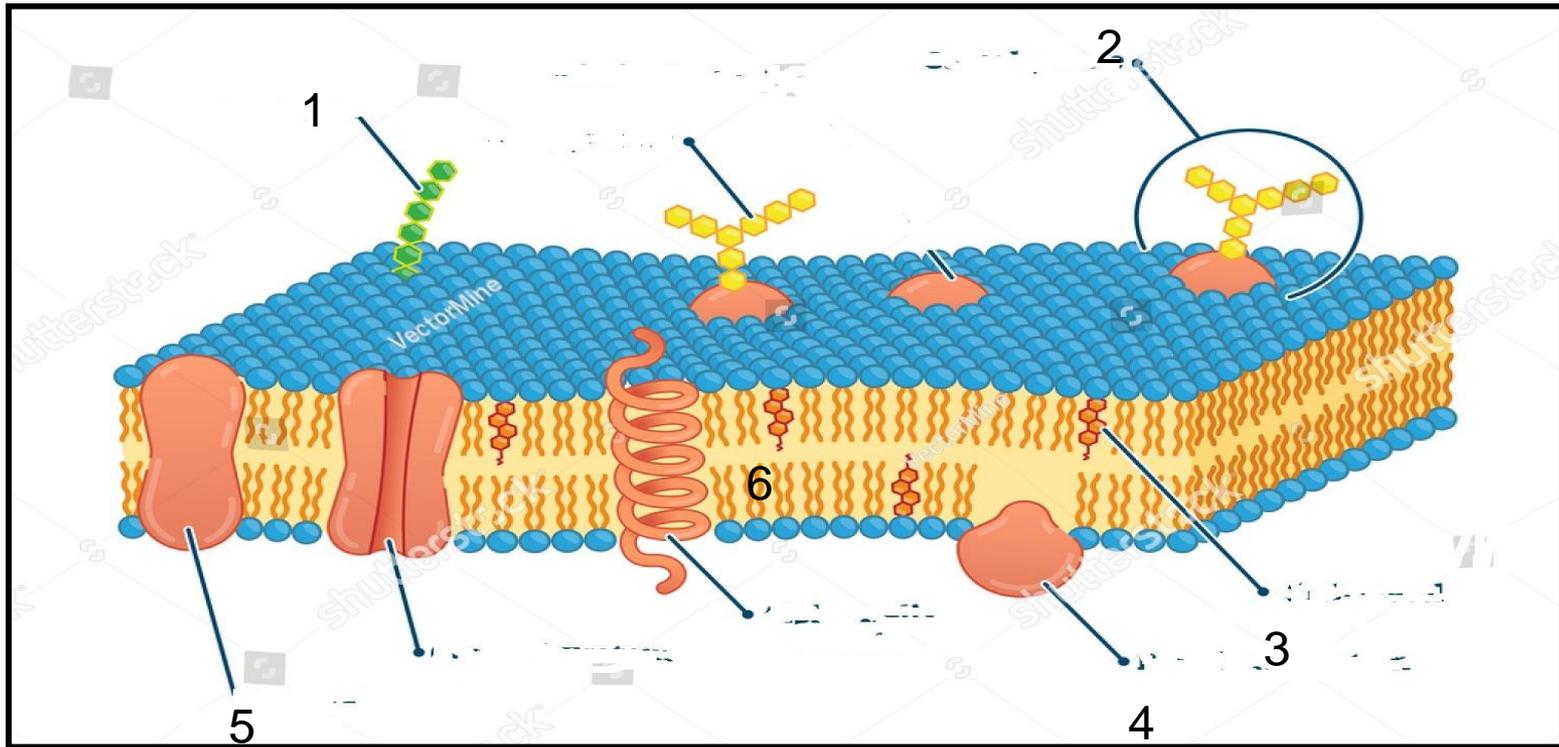
Electron microscopy of such cryofracture preparation replicas provides a useful method for studying membrane structures.



Functions of CELL MEMBRANE

- 1- It maintains the **structural integrity** of the cell.
- 2- **Selective permeability:** It controls **movements of substances** in and out the cell.
- 3- **Cell coat functions:** cell **recognition**, protection, intercellular adhesions, some act as **receptor molecules**
- 4- It acts as an **interface** between the cytoplasm and the external environment
- 5- It can establish **transport systems** for specific molecules.

Lable



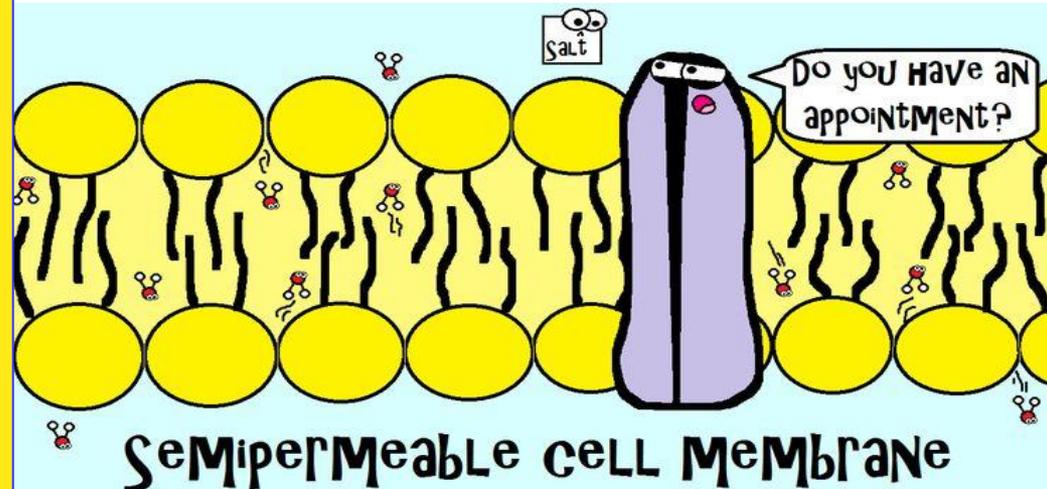
- 1-glycolipid
- 2-glycoprotein
- 3-cholesterol
- 4-peripheral protein
- 5-transmembrane protein
- 6-phospholipid bilayer

Membrane Transport

- The plasma membrane is the site where materials are exchanged between the cell and its environment.
- **Small, lipophilic (fat-soluble) molecules can pass through lipid bilayers by simple diffusion.**

• What molecules can **NOT** get through lipid bilayer directly?

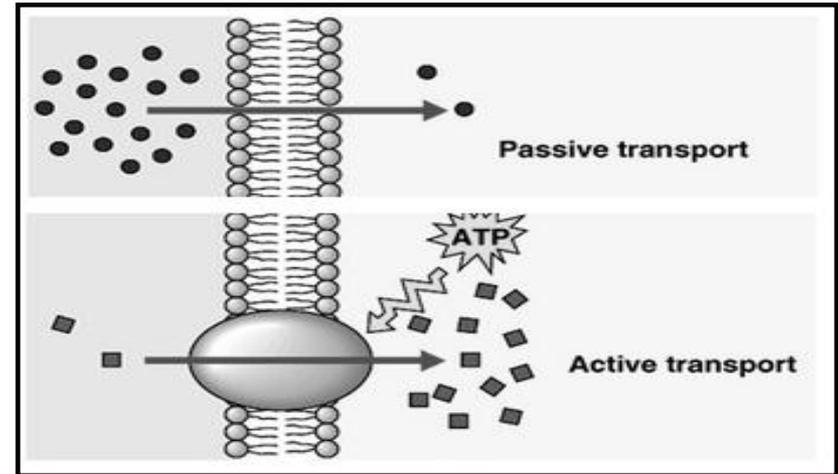
- (H₂O) or water soluble substances
- Polar ions
- Large molecules



Passage of materials across cell membrane

□ Ions and small molecules

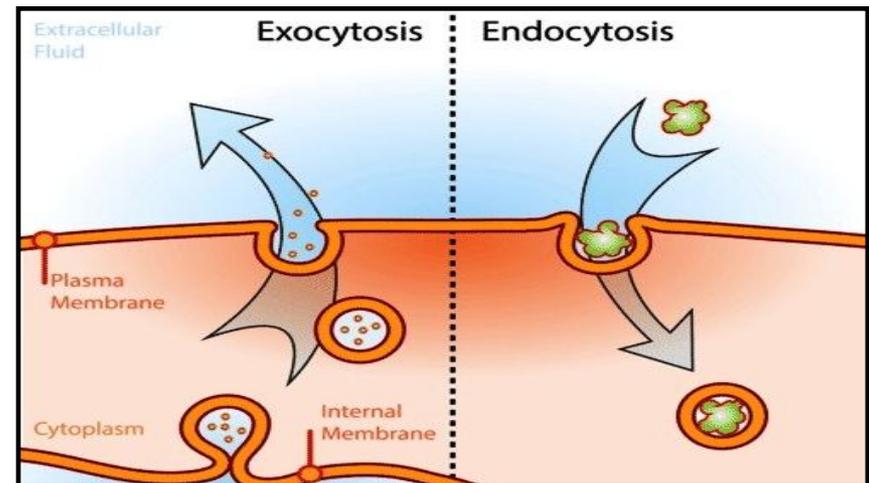
- Passive transport.
- Active transport.



□ Large molecules: Vesicle-mediated transport

(Mass transfer)

- Endocytosis
- Exocytosis



Passage of materials across cell membrane

Ions and small molecules

Passive transport:

- The passage of molecules depends on their concentration gradients.
- No energy is needed during this process.

1- Simple diffusion: Dissolved gases (e.g. O₂&N₂), substances of low molecular weight (e.g. H₂O).

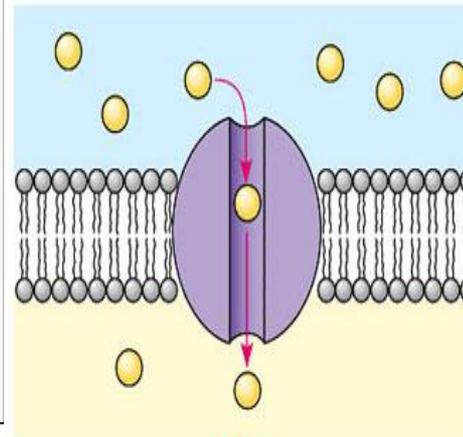
2- Facilitated diffusion: ions and small molecule **not soluble in lipids** need:

- **Channel proteins** do not form a bond with the molecules.
- **Carrier proteins** form a bond with the molecules so that they can move across the membrane.

Active transport:

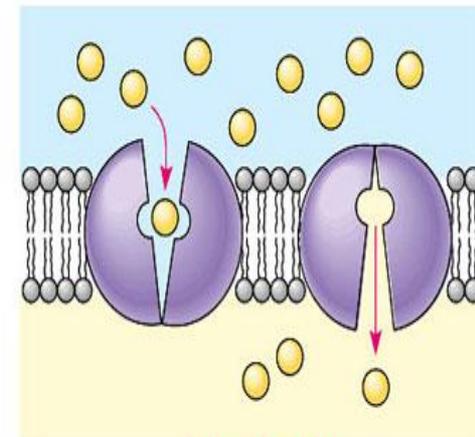
- Certain ions and molecules are transported against **their** concentration gradient from low concentration to higher one (e.g. **sodium-potassium pump**).
- Energy is required in this process.

The process is carried out by **carrier proteins**.



(a)

pore



(b)

carrier protein

II) Vesicle-mediated transport (Mass transfer)

- ❑ It is the process by which mass transport of materials (large molecules) occurs through cell membrane.
- ❑ This type of transport involves **vesicles or vacuoles** that are formed from or fuse with the cell membrane.
- ❑ The process includes either getting substances into the cell (endocytosis) or getting them out of the cell (exocytosis).

1- Endocytosis:

A-Phagocytosis.

B-Fluid phase pinocytosis.

C-Receptor mediated
endocytosis.

2-Exocytosis:

A-Constitutive pathway.

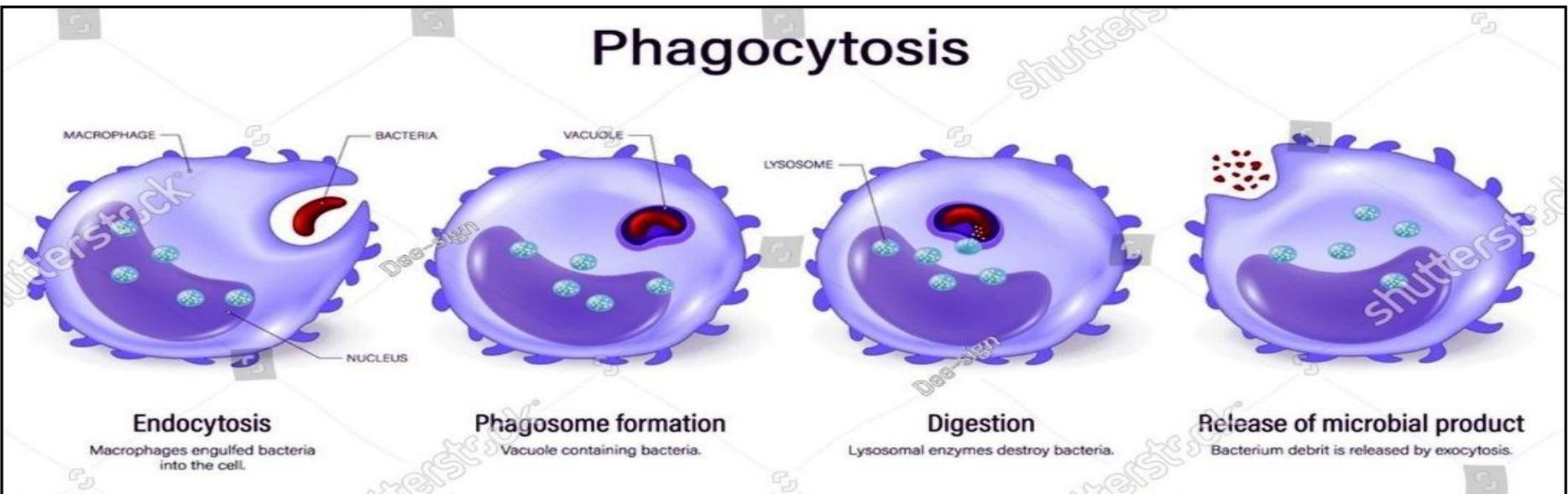
B-Regulated secretory pathway.

A) Phagocytosis (cell eating)

- It is the process of engulfing large solid particles by the cell e.g. bacteria, microorganisms and dead cells.
- It is performed by specialized cells known as **phagocytes** as neutrophils and macrophages.

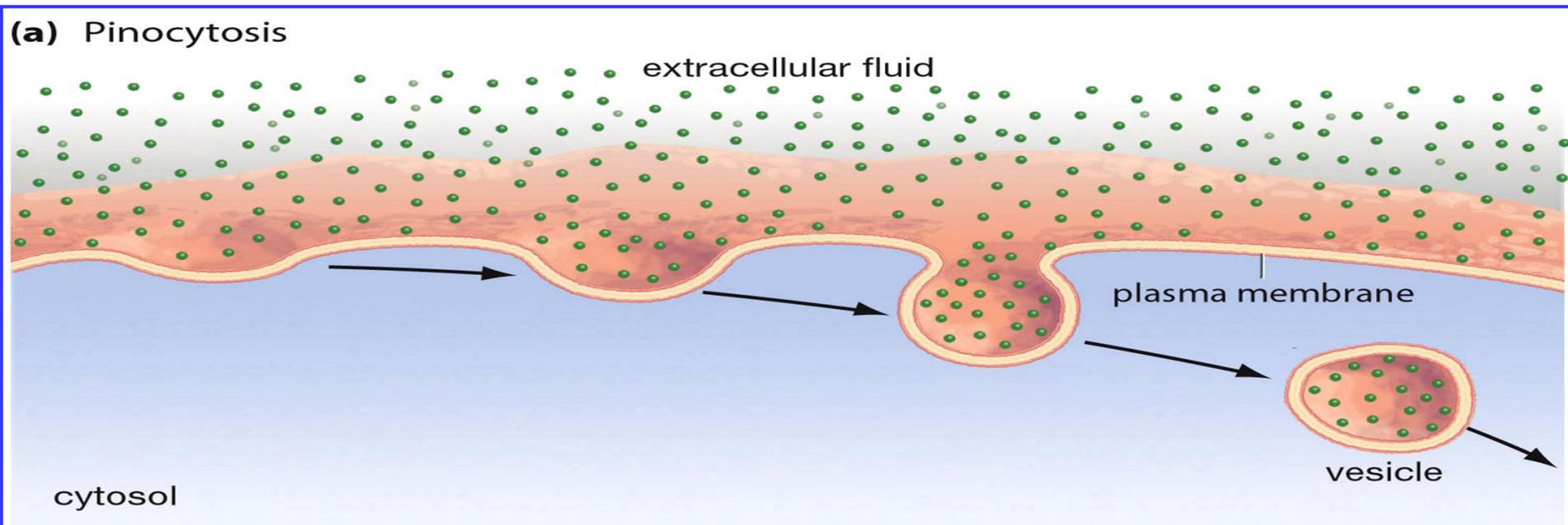
•**Process of phagocytosis:**

- 1.Binding of a bacterium to the cell surface.
- 2.Formation of cell processes (**pseudopodia**) by mobilization of actin and myosin in the cytoplasm.
- 3.Fusion of the cell processes to engulf the bacterium in an intracellular **phagocytic vacuole**.
- 4.Digestion of this vacuole **by lysosomes** to destroy the bacterium.



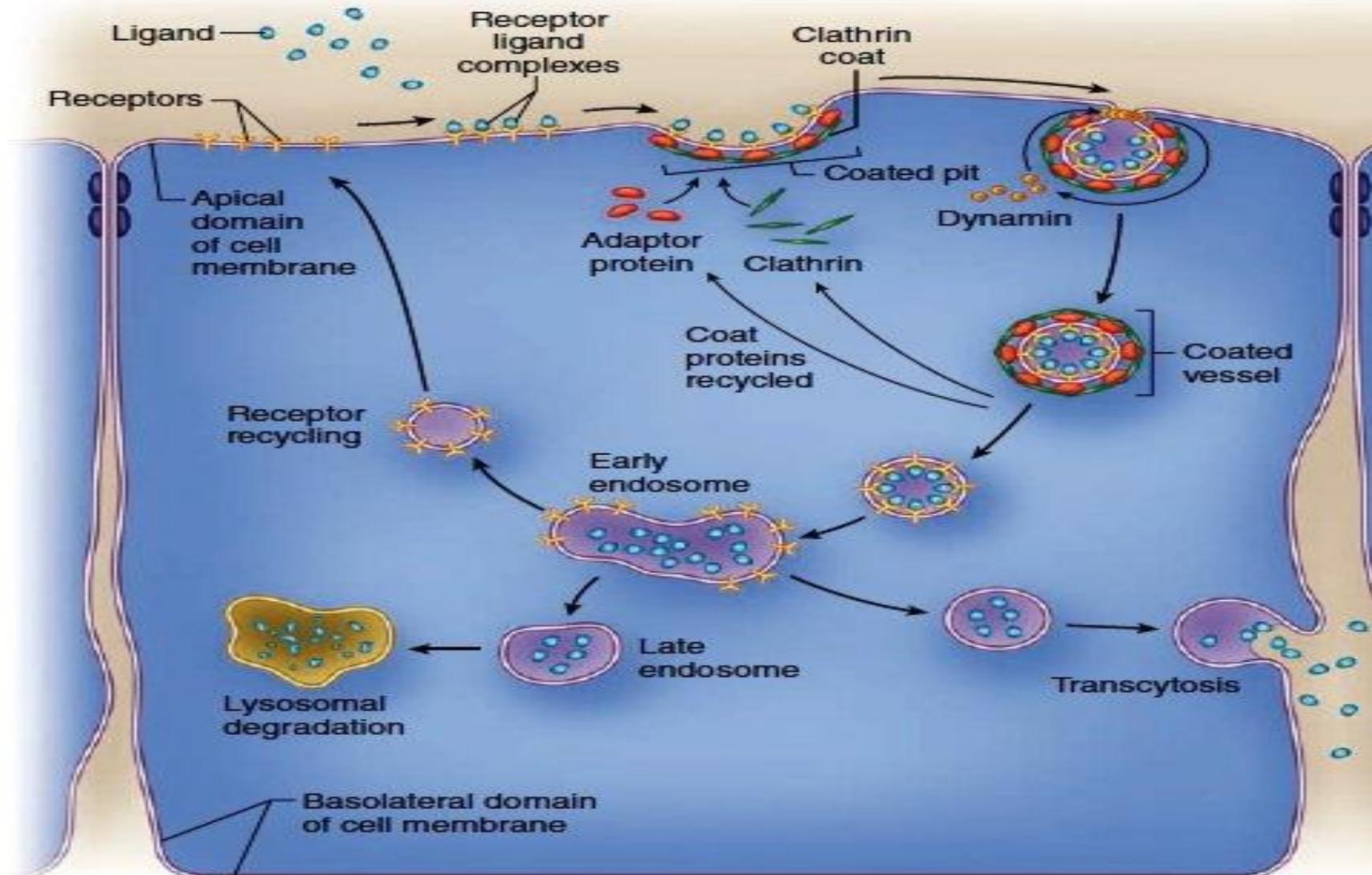
B) Fluid-phase pinocytosis (cell drinking):

- ❑ Non selective process
- ❑ Small invagination of cell membrane is formed and entraps extracellular fluid or any substance in solution forming the **pinocytotic vesicle**.
- ❑ Pinocytotic vesicles pinch off from the cell membrane and ***either*** fuse with lysosomes or move to the opposite surface of the cell and fuse with cell membrane releasing their contents in a process known as **trans-cellular transport (as in endothelial cells)**.



C) Receptor mediated endocytosis:

It is a **highly selective process** requiring the presence of **receptors and their ligands**.



B) Receptor mediated endocytosis:

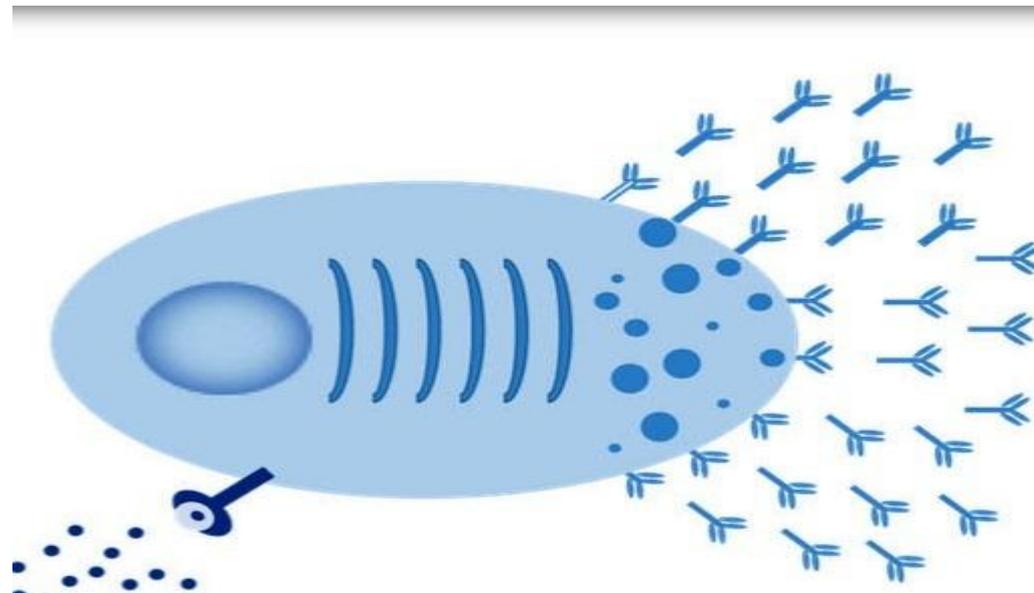
- Specific receptors for substances (*ligands*), e.g. proteins or hormones, are located at the cell surface.
- Binding of a ligand to its receptor forms **ligand-receptor complex** which aggregates in shallow invagination of cell membrane called *coated pit*.
- The cytoplasmic surface of coated pit is coated with spiny fibrous cytoplasmic protein called *clathrin*.
- Coated pits become deep and pinch off the cell membrane to be free in the cytoplasm and are known as *coated vesicles*, which carry ligand-receptor complex into the cell.
 - Coated vesicles soon lose their clathrin coat.
 - Then, they **fuse with endosomes** leading to **dissociation of ligands from their receptors**.
 - Receptors move back to cell membrane to be used again (recycling).

Exocytosis

- ❑ Release of cell products into the extracellular space.
- ❑ A *membrane-limited cytoplasmic vesicle* fuses with the plasma membrane, resulting in the **release of its contents** into the extracellular space.
- ❑ Exocytosis is triggered in many cells by transient increase in **cytosolic Ca^{2+}** .
- ❑ There are two general pathways of exocytosis:
 - A- Constitutive pathway.
 - B- Regulated secretory pathway (stimulus-dependent)

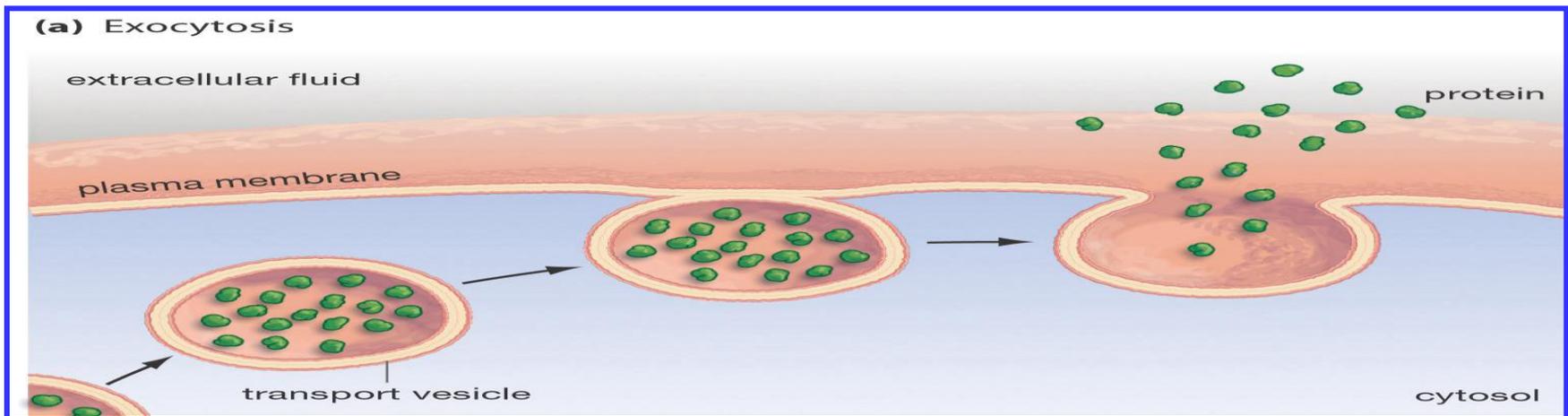
A) Constitutive pathway (Continuous process):

- Proteins that leave the cell by this process are secreted **immediately after their synthesis.**
- An example is the secretion of immunoglobulins by **plasma cells**



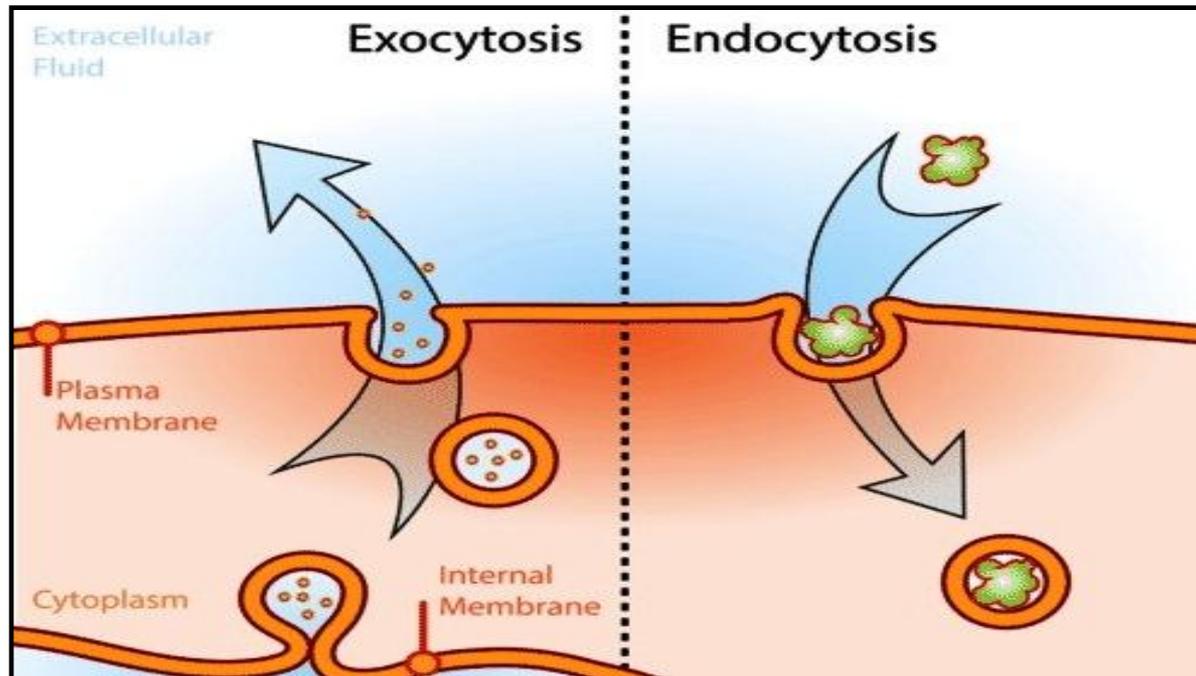
B) Regulated secretory pathway (Discontinuous process):

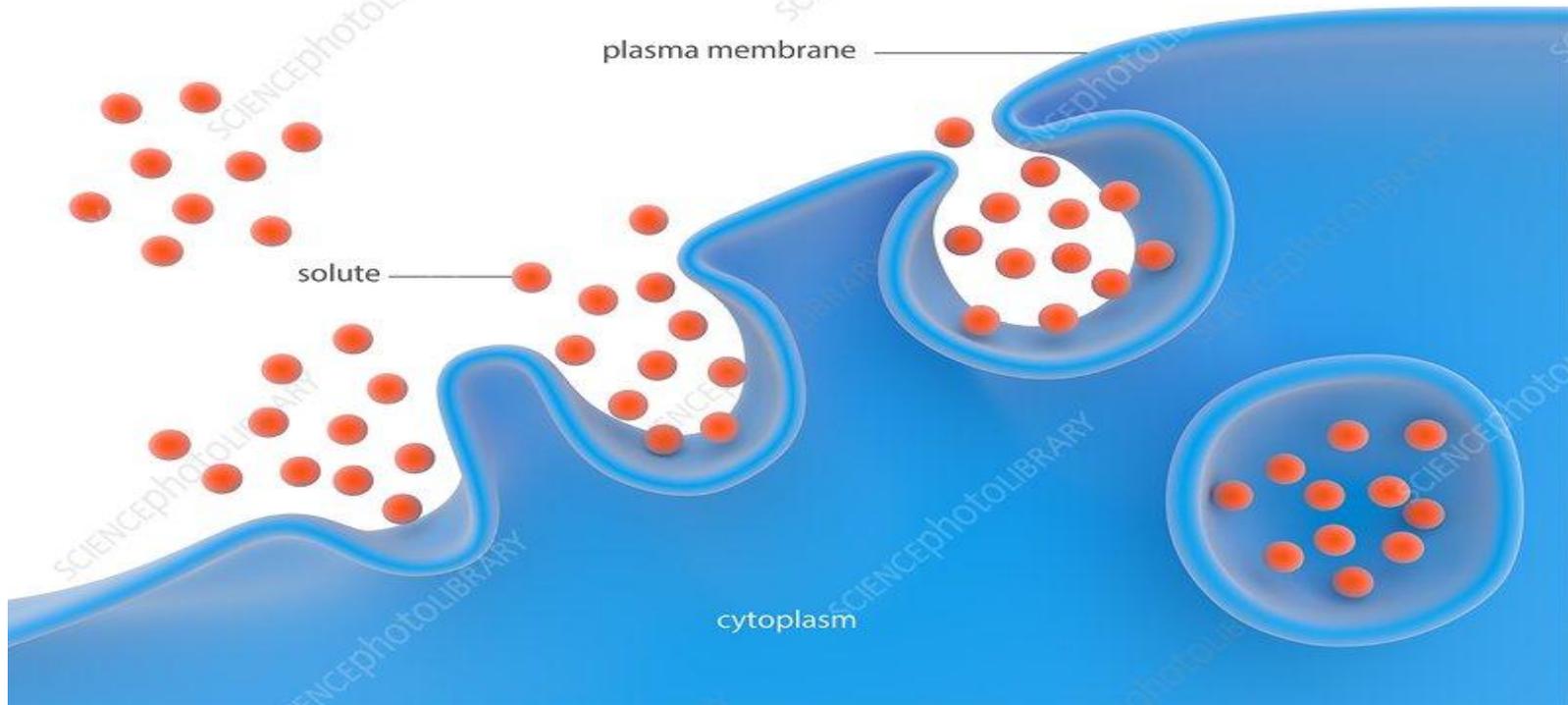
- ❑ the proteins are concentrated and stored in **secretory granules**.
- ❑ These secretory granules, in response to stimulation **fuse with the cell membrane** to release their contents outside the cell.
- ❑ An example of this pathway is the release of contents of granules of pancreatic acinar cells.



Membrane trafficking

- ❑ During endocytosis, portions of the cell membrane are used as endocytotic vesicles.
- ❑ These lost portions are **compensated** by other membranes during the process of exocytosis.
- ❑ This repeated recycling of the cell membrane is called membrane trafficking.





What is this process?

1- phagocytosis

2- pinocytosis

3- Receptor mediated endocytosis

Animation videos

- <https://www.youtube.com/watch?v=ufCiGz75DAk>
- <https://www.youtube.com/watch?v=vSkPEPQ-SpU>



THANK
YOU