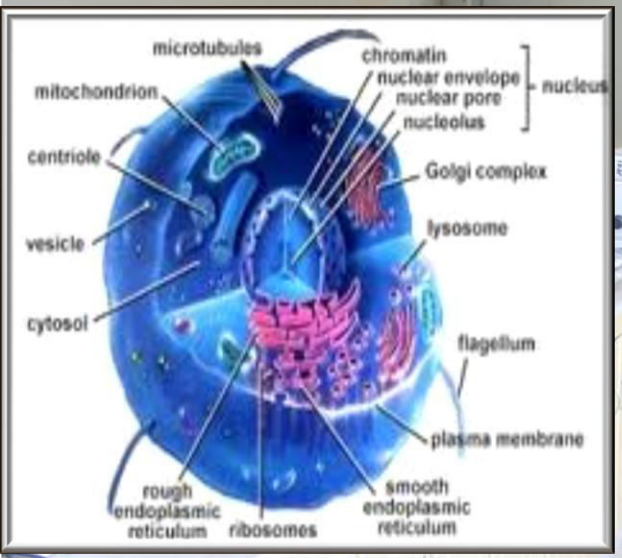
Department of Biochemistry

CELL & BIOLOGICAL MEMBRANES



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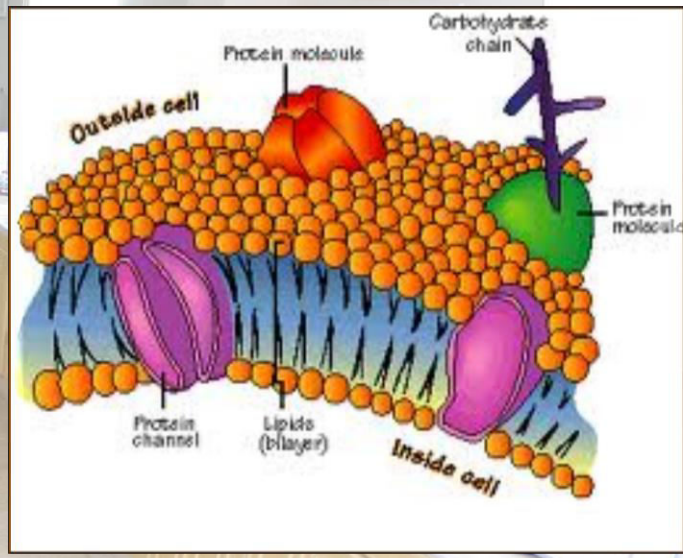
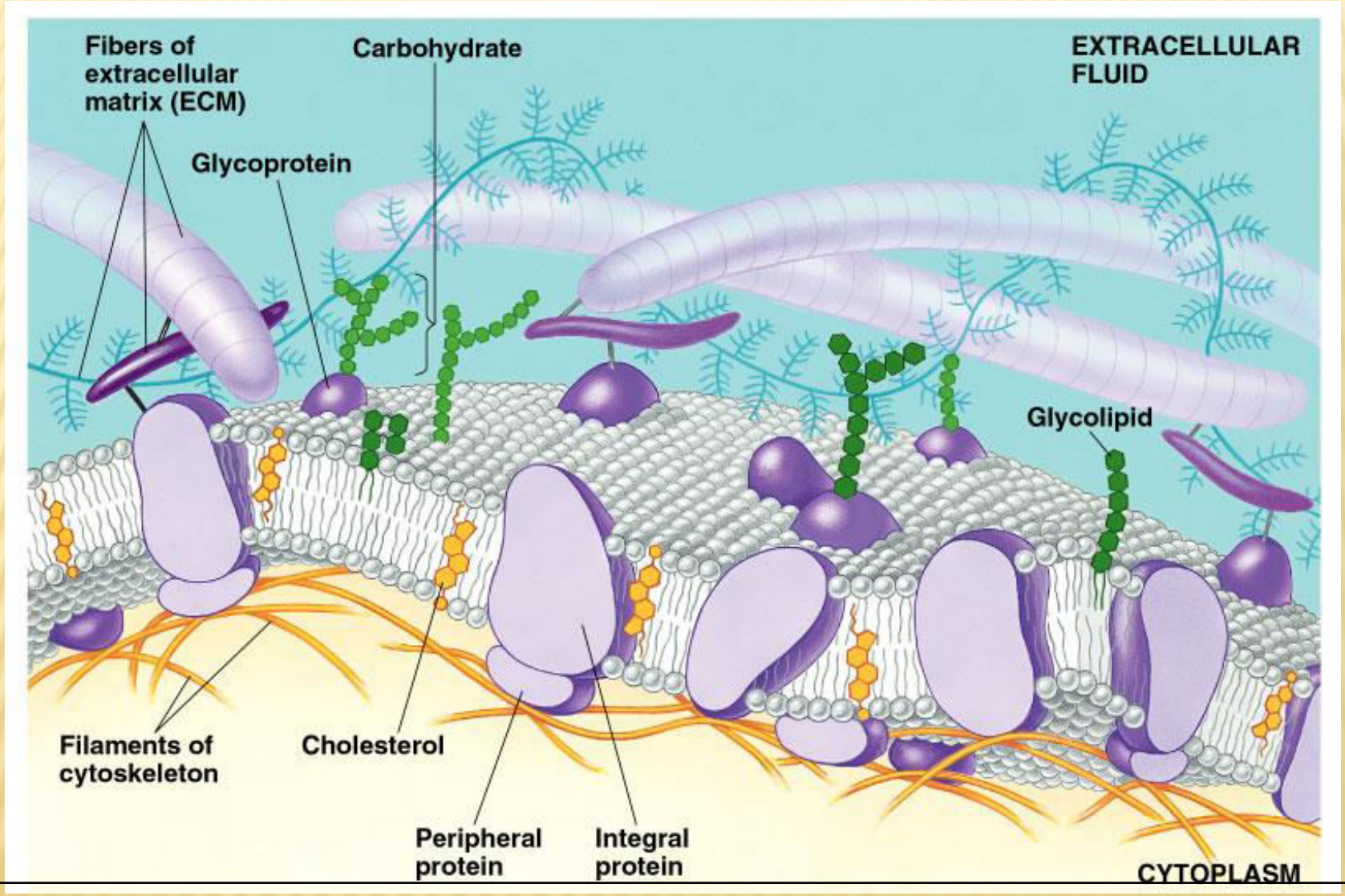


First Year, MBBS

Learning Objectives

By the end of this Lecture, students shall be able to:

1. Describe the fluid **Mosaic Model** of cell membrane
2. Describe the various **types of transport across the cell membrane** with examples
3. Differentiate between the **passive and active transport across cell membrane**



FUNCTIONS OF MEMBRANE LIPIDS

1. Phospholipids form bilayer.

2. Certain lipids determine fluidity of plasma membrane:

- Cholesterol
- Saturated and unsaturated fatty acids (components of phospholipids)

3. Provide permeability barrier for water soluble molecules.

FUNCTIONS OF MEMBRANE LIPIDS

4. Provide a hydrophobic region in which part or major part of membrane proteins are embedded.

5. Provide site for attachment of :

- Peripheral proteins (electrostatic interactions)
- Oligosaccharide chains

FUNCTIONS OF MEMBRANE CARBOHYDRATES

1. Many of them have negative electrical charge which gives most cells an overall negative surface charge that repels other negative objects.
2. The glycocalyx of some cells attaches to the glycocalyx of other cells, thus attaching cells to one another.
3. Carbohydrates play an important role in cell-cell recognition.
4. Many of these act as receptor substance for binding hormones.

MEMBRANE CARBOHYDRATES ARE IMPORTANT FOR CELL-CELL RECOGNITION

- Cell-cell recognition: The ability of a cell to distinguish one type of neighboring cell from another.
- Cell-cell recognition is crucial in the functioning of an organism. It is the basis for:
  - **Sorting** of cells into tissues and organs in an animal embryo's cell.
  - **Rejection** of foreign cells by the immune system.

- The way cells recognize other cells is probably by keying on surface molecules (**markers**)

Markers: Surface molecules found on the external surface of the plasma membrane that distinguish one cell from another.

FUNCTIONS OF MEMBRANE PROTEINS: INTEGRAL MEMBRANE PROTEINS

1. Many of the integral proteins provide structural channels (or pores) through which water molecules & water soluble substances, especially ions, can diffuse b/w the ECF & ICF. These protein channel also have selective properties that allow preferential diffusion of some substances over others e.g. Aquaporins.
2. Other integral proteins act as carrier proteins for transporting substances that otherwise could not penetrate the lipid bilayer.

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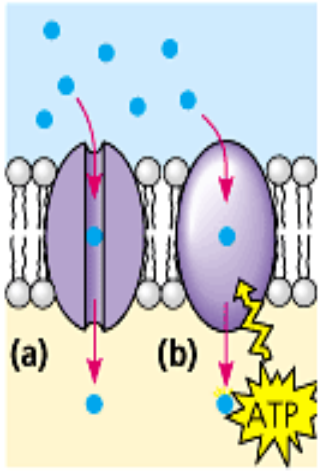
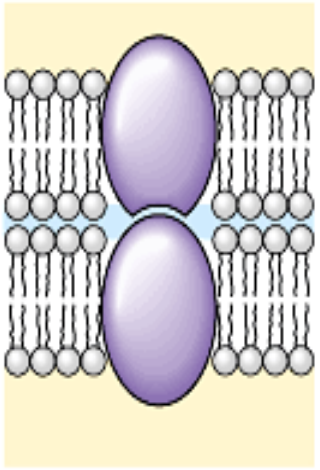
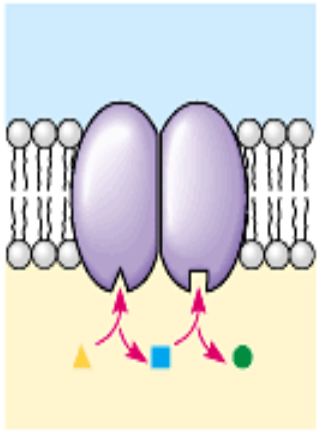


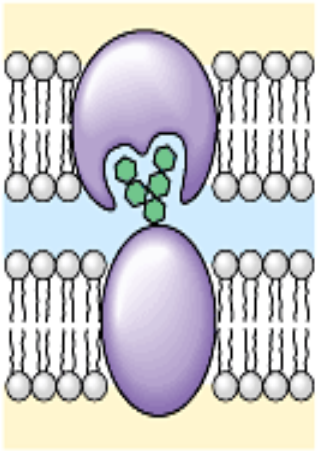
Transport



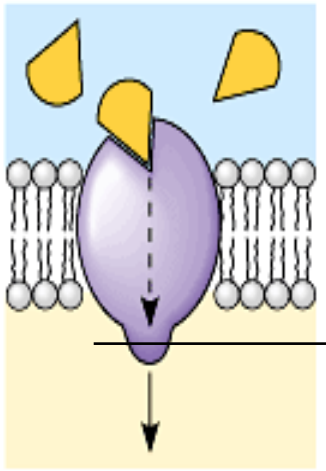
Intercellular joining



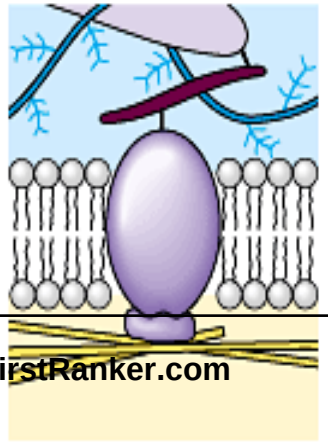
Enzymatic activity



Cell-cell recognition



Signal transduction



Attachment to the cytoskeleton and extracellular matrix (ECM)

TRANSITION TEMPERATURE (TM)

The temperature above which the paracrystalline solid changes to fluid is called Tm. Transition temperature is characteristic for each membrane and depends upon membrane lipid composition.

THE FLUID MOSAIC MODEL

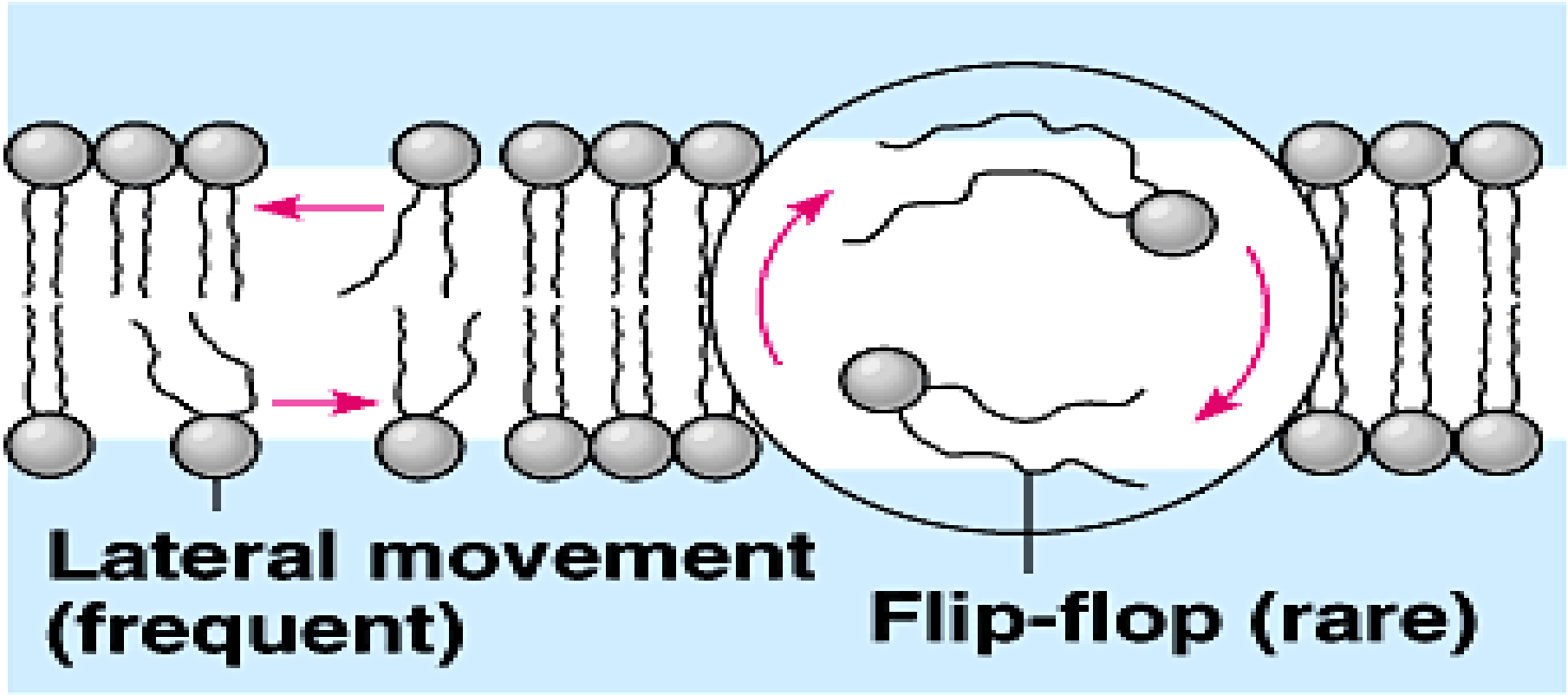
- *Fluid = always moving and changing*
- *Mosaic = made up of many different parts*

Fluid mosaic model was proposed in 1972 by Singer and Nicolson.

According to this model, membrane proteins are like icebergs floating in a sea of phospholipid molecules.

- Later on, it was demonstrated that phospholipids also undergo rapid redistribution in the plane of membrane.
- In FREEZE-FRACTURE TECHNIQUE, cells are frozen to very cold temp and then fractured with a very fine diamond knife. Some cells are fractured between two layers of membrane lipid bilayer. When viewed with Electron microscope, the membrane appeared to be a mosaic, studded with proteins. Due to fluidity of membranes and the appearance of proteins, the concept of membrane structure is called **"the fluid mosaic structure".**

- Fatty acyl chains in the in the interior of membrane form a fluid, hydrophobic region. Integral proteins float in this sea of lipids held by hydrophobic interactions with their non-polar amino acid side chains.
- Both lipids and proteins are free to move laterally in the plane of bilayer, but movement of either from one face of the bilayer to the other (flip-flop movement) is restricted.
- Carbohydrate moieties attached to some proteins and lipids of the plasma membrane are exposed on the extracellular side.



(a) Movement of phospholipids

Molecules **rarely flip transversely** (flip-flop) across the membrane, because hydrophilic parts would have to cross the membrane's hydrophobic core.

2. TEMPERATURE:

Individual hydrocarbon chains of fatty acids are in constant motion.

a) At low temperature relatively little lipid motion occurs & bilayer exists as a nearly crystalline array.

b) Above a certain temperature lipid can under go a rapid motion.

Fluidity of memb effects its functions:

As fluidity increases ⟶ permeability of membrane to water & other hydrophilic substances increases.

STUDY OF CELLS FROM A BIOCHEMICAL VIEWPOINT

Rat hepatocyte is one of the most extensively studied of all cells from a biochemical viewpoint. This is due to following reasons:

1. Available in relatively large amounts
2. Diverse functions
3. Suitable for fractionation studies; contains major organelles (nucleus, mitochondria, ER, free ribosomes, Golgi-apparatus, lysosomes, peroxisomes, plasma membrane, cytoskeletal elements) found in eukaryotic cells.

SUBCELLULAR FRACTIONATION

In order to study the function of any organelle, it is necessary to isolate it in relatively pure form. The usual process by which this is achieved is called subcellular fractionation.

Subcellular fractionation generally entails three procedures:

1. Extraction
2. Homogenization
3. Centrifugation

EXTRACTION

As a first step toward isolating a specific organelle (or molecule); it is necessary to extract it from the cells in which it is located.

Most organelles & many biomolecules are labile & subject to loss of biologic activities; they must be extracted using mild conditions (i.e. employment of aqueous solution, avoidance of extreme of pH, osmotic pressure, high temperature).

Most procedures for isolating organelles are performed at 0-4 ˚C (e.g. In a cold room or using material kept on ice).

Significant losses of activity occur at room temperature, partly owing to the action of various digestive enzymes (protease, nuclease etc) liberated when cells are disrupted.

A common solution for extraction of organelles consist of sucrose, 0.25 mol/l (isoosmotic), adjusted to pH 7.4 by TRIS HCL buffer, 0.05 mol/l, containing K^+ & Mg^{++} ions at near physiologic concentrations; this solution is conveniently called STKM.

Organic solvents are used for extraction of lipids & nucleic acids.

HOMOGENIZATION

Organs (e.g. liver) & their contained cells may be conveniently disrupted by the process of homogenization, in which a manually operated or a motor driven pestle is rotated within a glass tube of suitable dimensions containing minced fragments of the organs under study, & a suitable homogenizing medium, such as STKM. The controlled rotation of the pestle exerts mechanical shearing forces on cells & disrupts them, liberating their constituents in sucrose. The resulting suspension, containing many organelles, is known as HOMOGENATE.

CENTRIFUGATION

Subfractionation of the contents of homogenate is done by differential centrifugation. In the classic method, a series of 03 different centrifugation steps at successively greater speed yield a pellet & supernatant. The supernatant from each step is subjected to centrifugation in the next step. This procedure provides three pellets, named the nuclear fraction, mitochondrial fraction, & microsomal fraction.

NOTE: None of these fractions are composed of absolutely pure organelles. However, it has been established by use of electron microscope & suitable markers (enzymes or chemical components) that the major constituents of each of these 03 fractions are nuclei, mitochondria & microsomes respectively.

Nuclear fraction → contain nuclei & unruptured cells

Mitochondrial fraction → contain mitochondria, lysosomes & peroxisomes

Microsomal fraction → contain mixture of free ribosomes, smooth ER rough ER

MARKER ENZYME OR CHEMICAL

- A marker enzyme or chemical is one that is almost exclusively confined to one particular organelle.
- The marker thus can serve to indicate the presence or absence of the organelle in any particular fraction in which it is contained.

MARKERS

ORGANELLE OR FRACTION	MARKER	MAJOR FUNCTIONS
Mitochondria	Glutamic dehydrogenase	• Citric acid cycle • Oxidative phosphorylation
Endoplasmic reticulum (ER)	Glucose-6- phosphatase	• Membrane bound ribosomes are a major site of protein synthesis • Synthesis of various lipids • Metabolism of drugs
Nucleus	DNA	• Site of chromosome • Transcription
Ribosomes	RNA (high content)	•Site of protein synthesis
Lysosomes	Acid phosphatase	• Site of many hydrolases (enzymes catalyzing degradative reactions)

ORGANELLE OR FRACTION	MARKER	MAJOR FUNCTIONS
Plasma membrane	• Na^+-K^+ ATPase • 5, - Nucleotidase	• Transport of molecules in and out of cells • Intercellular adhesion and communication
Golgi-apparatus	• Galactosyl transferase	• Intra cellular sorting of proteins • Glycosylation reactions • Sulfation reactions
Peroxisomes	• Catalase • Uric acid oxidase	• Degradation of certain fatty acids & amino acids • Production & degradation of H_2O_2
cytosol	• Lactate dehydrogenase	• Enzymes of glycolysis • Fatty acid synthesis

TRANSPORTATION THROUGH CELL MEMBRANE

TRANSPORTATION THROUGH MEMBRANE:

Can occur by following mechanisms:

1. Cross membrane movement of small molecules.
   - Diffusion
   - Active transport
2. Cross membrane movement of large molecules.
   - Endocytosis
   - Exocytosis
3. Signal transmission across membrane.
   - Cell surface receptors
4. Inter cellular contact and communication.

TRANSPORT SYSYTEM

Transport system can be described in a functional sense according to the no. of molecules moved & direction of movement or according to whether movement is towards or away from equilibrium.

Uniport system:

Moves one type of molecules bidirectionally.

Co-Transport system:

The transfer of one solute depends upon the stoichiometric simultaneous transfer of another solute.

- Symport:

  Move these solute in the same direction

  Examples:

  Na^+ - sugar transporters (glucose & certain other sugars) and Na^+- amino acid

  transporters in mammalian cells

- Antiport:

  Movement of two molecules in opposite direction

  Examples:

  Na^+ in & Ca^{2+} out

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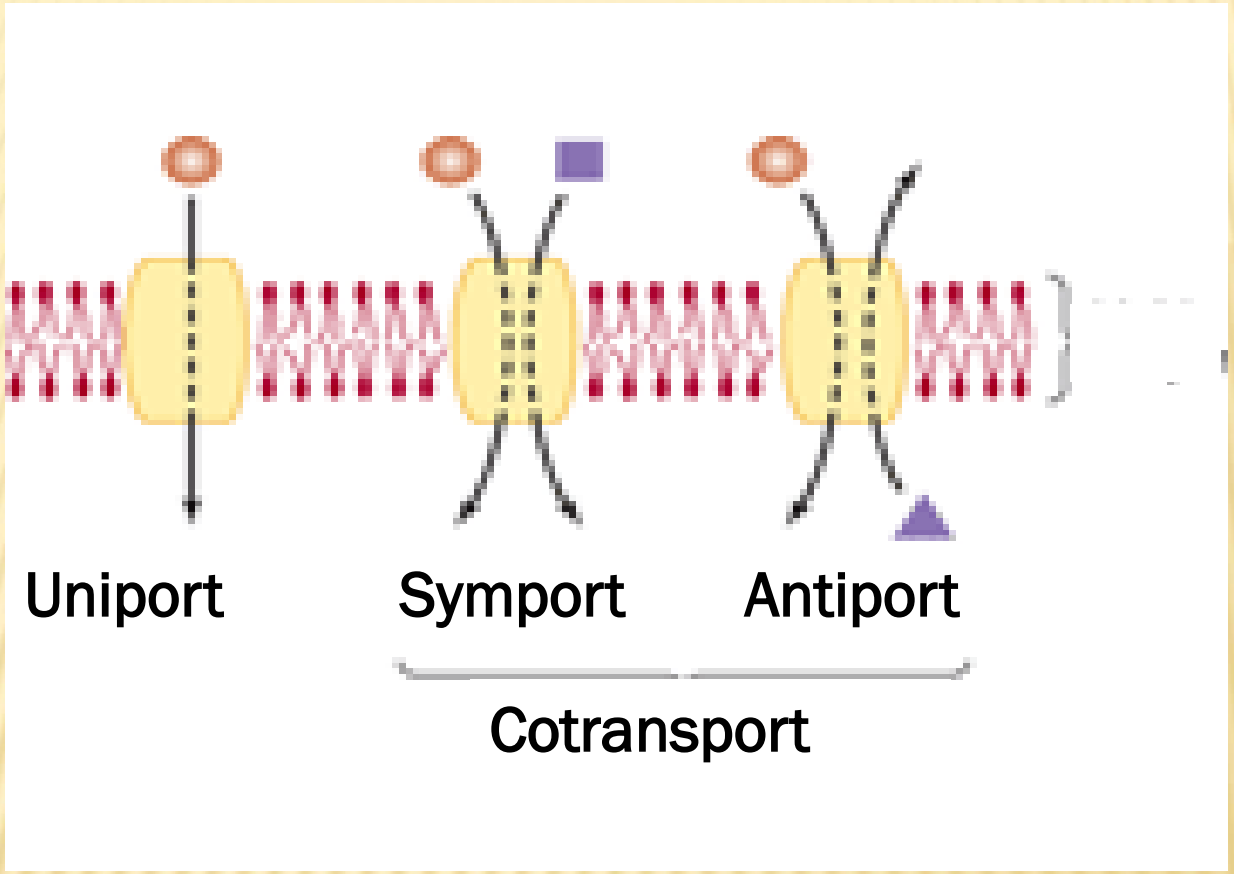

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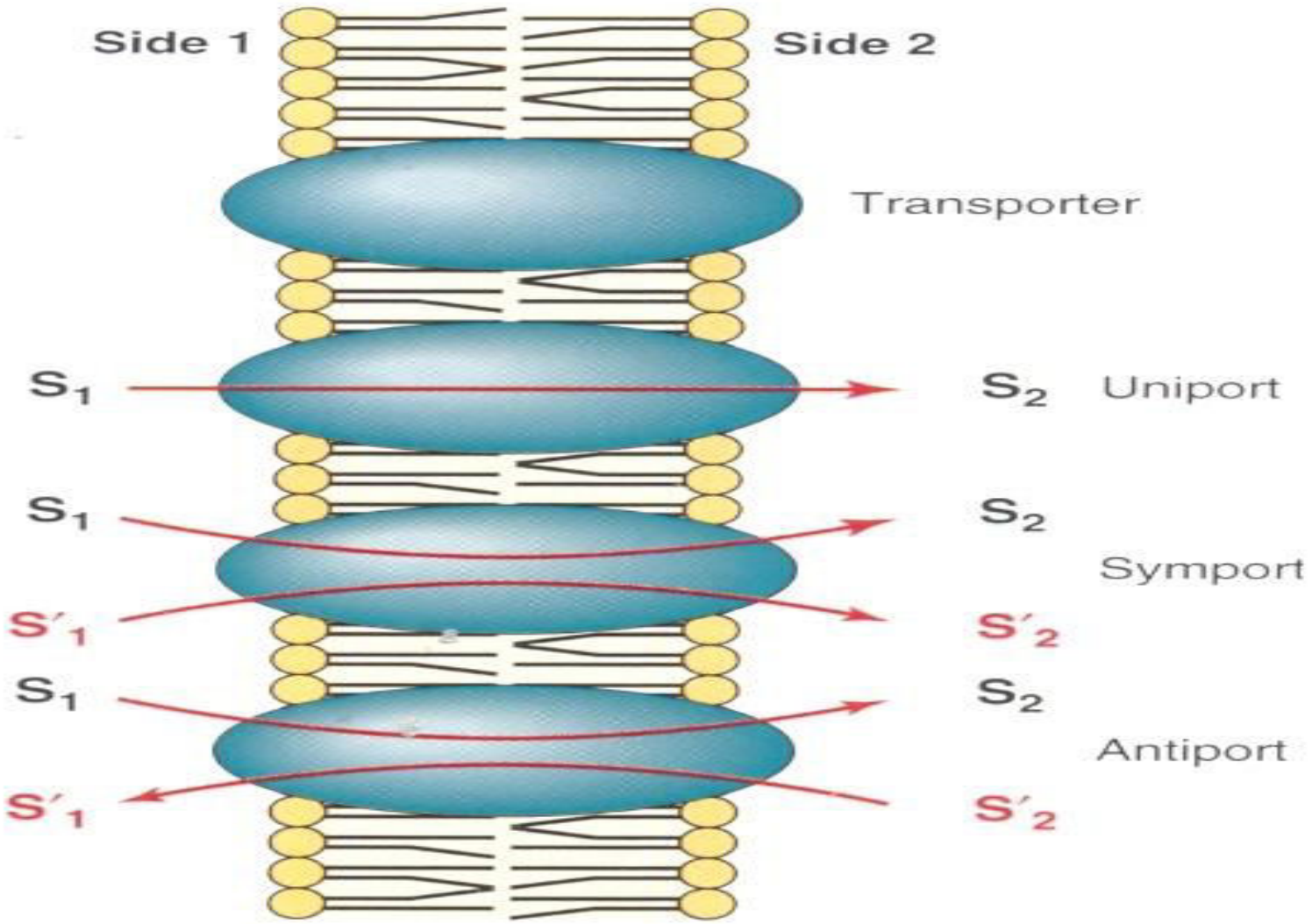


FIGURE 12.45

Uniport, symport, and antiport mechanisms for translocation of substances. S and S′ represent different molecules.

1. Cross membrane movement of small molecules

DIFFUSION:

- Simple
- Facilitated

Molecules can passively traverse the bilayer down electrochemical gradients by simple diffusion or by facilitated diffusion.

FACTORS AFFECTING NET DIFFUSION OF A SUBSTANCE THROUGH MEMBRANE

1. Concentration gradient across the membrane.
2. The electrical potential across the membrane.
3. The permeability coefficient of the substance for the membrane.
4. The hydrostatic pressure gradient across the membrane.
5. Temperature.

The permeability coefficient of the substance is the solubility of that substance in the hydrophobic core of the membrane bilayer.

SIMPLE DIFFUSION

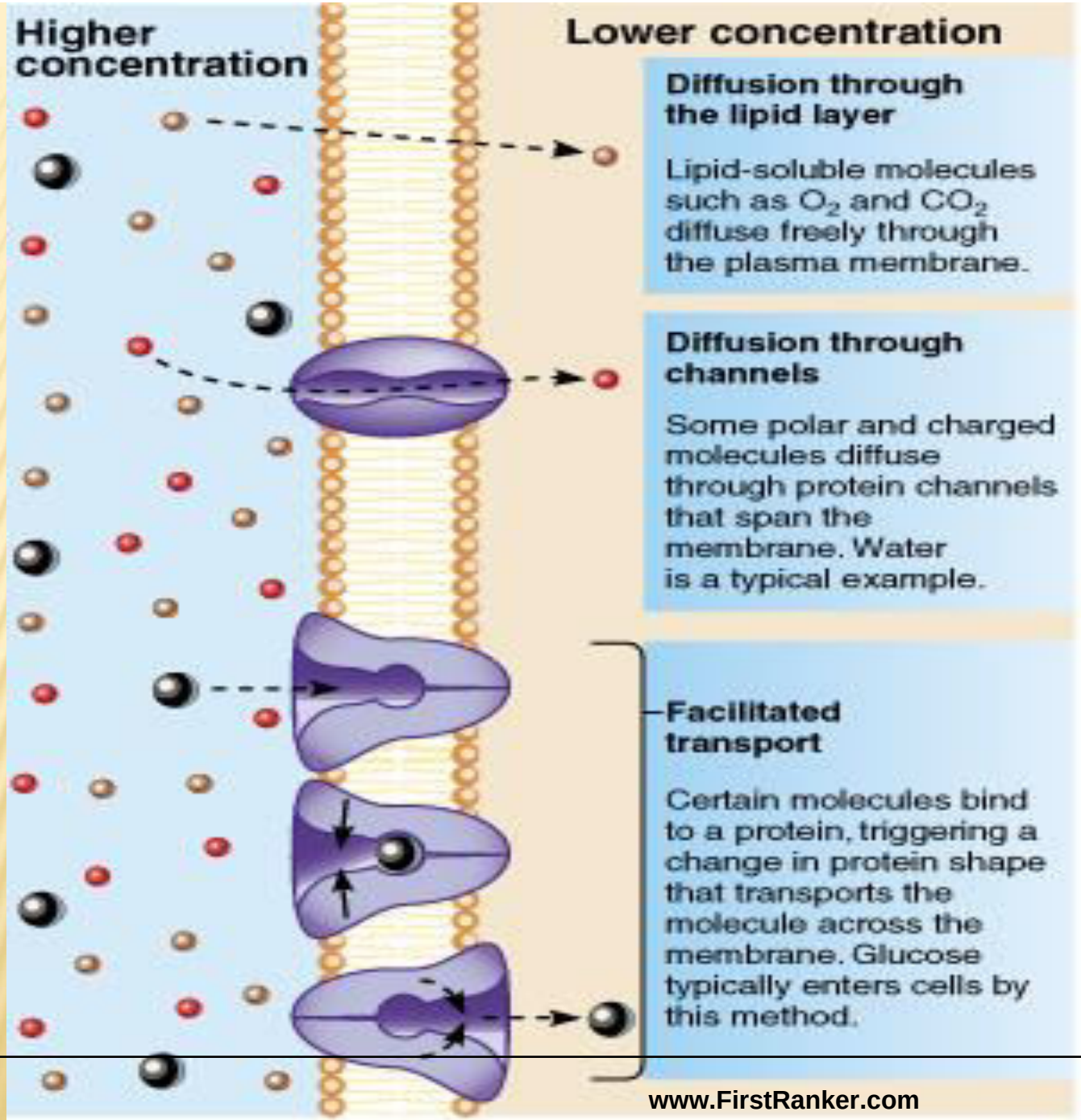
Definition: Movement of molecules/solutes from the region of higher solute concentration to the region of lower solute concentration through the membrane.

Examples:

Non polar gases such as CO_2, O_2 , nitrogen, methane & alcohol.

It can occur by two path ways

1. Through the interstices of the lipid bilayer, if the diffusing substances is lipid soluble.
2. Through watery channels that penetrate all the way through some of large transport proteins.



Solubility is inversely proportionate to the number of hydrogen bonds that must be broken in order for a solute in external aqueous phase to become incorporated in the hydrophobic bilayer.

Electrolytes, poorly soluble in lipids, do not form H- bonds with water, but they do acquire a shell of water from hydration by electrostatic interactions.

Size of shell is directly proportional to the charge density of the electrolyte. Electrolytes with a Large charge density have a larger shell of hydration and thus a slower diffusion rate.

Example: Na^+ has a higher charge density than K^+ so K^+ move easily through the membrane.

CHANNELS AND PORES

Channels and pores facilitate translocation of molecules or ions across cell membrane by creating a central aqueous channel in the protein that permits diffusion of substrate in both directions.

Channel proteins do not bind or sequester the molecule or ions in transit.

Their specificity is based on the size and charge of the substance.

Cation conductive channels are negatively charged within the channel.

The specific channels for Na^{+}, k^{+} Ca^{2+} and Cl^{-} have been identified.

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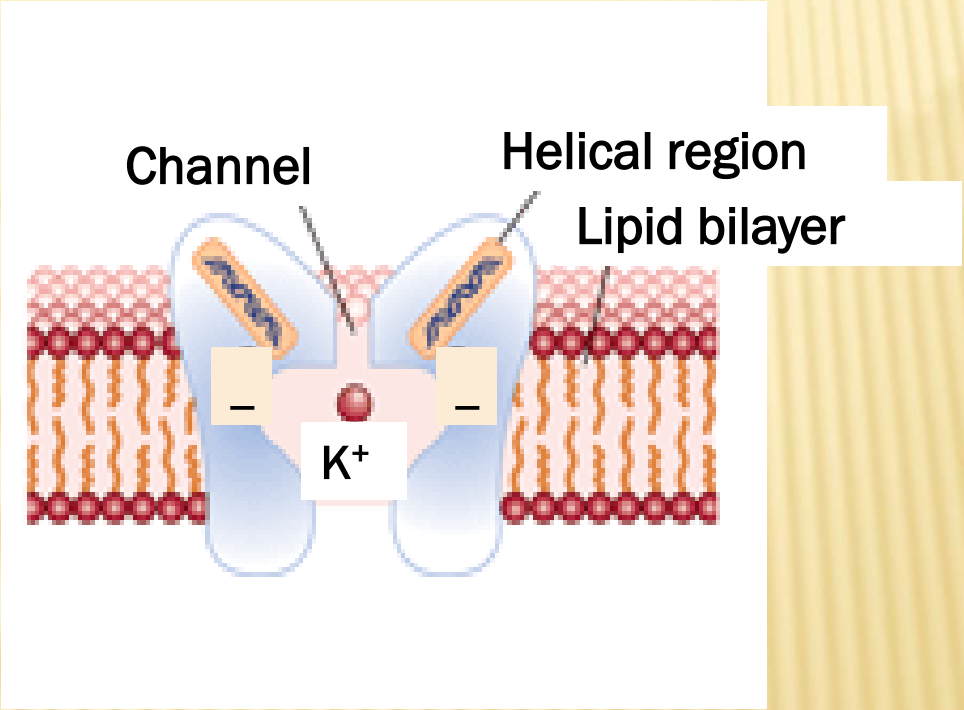

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These are regulated by various mechanisms that open or close the passageway. Channels are open transiently and thus are gated.

- Ligand-gated channels:

  Binding of ligand to receptor opens the channel.

- Voltage-gated channel:

  Open in response to a change in membrane potential.

DIFFUSION OF WATER

Despite polarity, water crosses some membranes slowly by simple diffusion due to high concentration gradient. However, for tissues in which rapid transmembrane water movement is essential e.g. Kidneys, water diffuses through channels formed by specific integral proteins – the aquaporins.

AQUA PORINS (AQP)

Aquaporins are small hydrophobic integral membrane proteins that contain water pore. The aqueous pathway is lined with few hydrophilic residues that attract water.

In addition to water, aquaporins permit translocation of CO_2 , glycerol, urea, purines, pyrimidines and nucleosides.

Eleven (11) mammalian AQPs have been identified.

These are subdivided by amino acid sequence and functional characteristics into

- Aquaporins: channel selective only for water
- Aqua glyceroporins: permit translocation of water and small solutes

Aquaporins are present in different tissues. Kidneys (proximal tubules and collecting ducts) contain AQP1, AQP2, AQP3, AQP4, and AQP6.

CLINICAL CORRELATION

- AQP2 is under hormonal control. Low levels of AQP2 and polyuria are found in acquired nephrogenic diabetes insipidus, acquired hypokalemia and hypercalcemia.

- High levels of AQP2 are found in congestive heart failure, liver cirrhosis and pregnancy, leading to an expansion of the ECF volume

FACILITATED DIFFUSION:

Transmembrane passage of polar compounds & ions is made possible by membrane proteins that lower the activation energy for transport by providing the alternative path for specific solute through the lipid bilayer. These protein are not enzymes but are called transporters.

Specific solutes diffuse down electrochemical gradient across membrane more rapidly than might be expected from their size, change or partition coefficients through facilitated diffusion.

PING PONG MECHANISM

In this model the carrier protein exist in two principal conformations.

In the "pong" state it is exposed to high concentration of solute. Solute molecules bind to specific sites on the carrier protein.

Conformational change occur that exposes the carrier protein to a lower concentration of solute "ping" state and helps transporting solute molecules.

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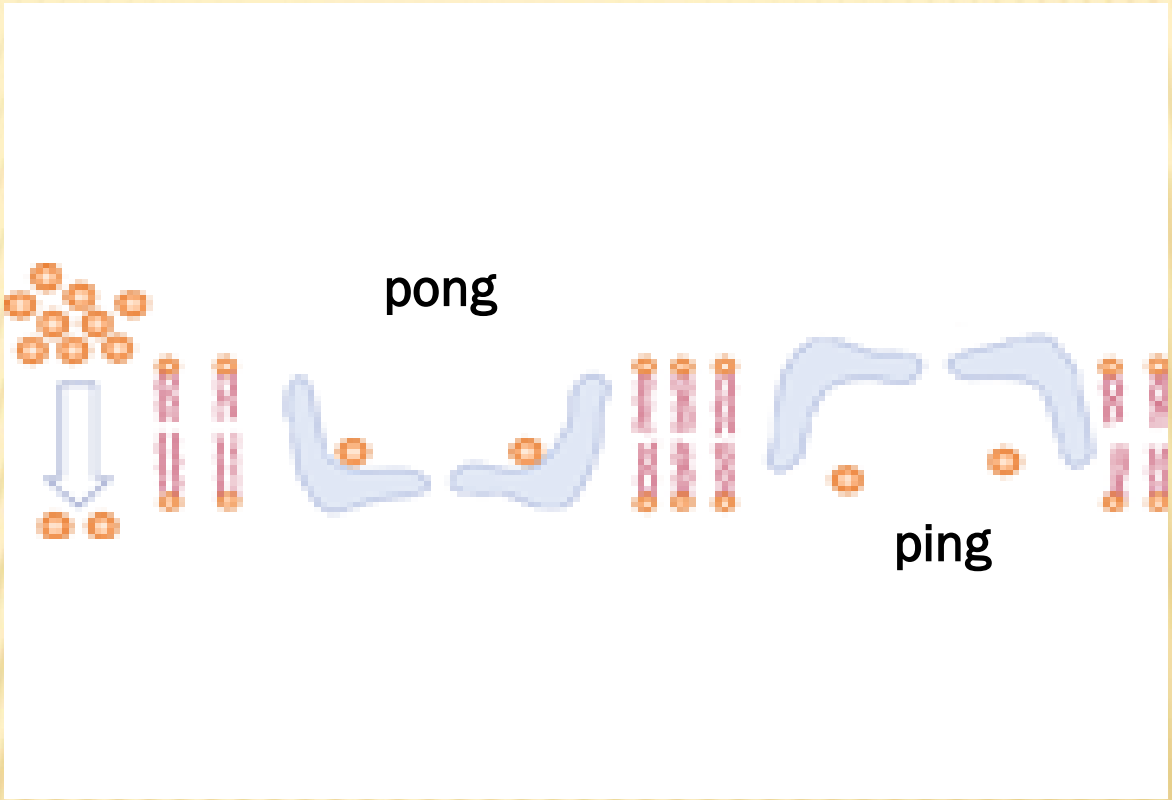

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The rate of solute entry depends on

- The concentration gradient across membrane
- The amount of carrier available
- The rapidity of solute carrier interaction
- The rapidity of conformational change

TRANSPORTORS

- Transporters catalyze movement of a molecule or ion across the membrane by binding and physically moving it across.
- Transporters have the specificity for the substance to be transported --referred to as the substrate, have defined kinetics and can be affected by competitive and non competitive inhibitors.

- Some transporters move their substrate only down the concentration gradient– passive transport, facilitated diffusion or protein mediated diffusion
- Other transporter can move the substrate against concentration gradient– active transport or pump and require input of energy.

ACTIVE TRANSPORT

Active transport is the movement of molecules against concentration gradient and energy is needed for this transport. The energy is obtained from hydrolysis of ATP.

Active transport involves carriers or transport proteins which are named as translocases. These specific carrier proteins are integral membrane proteins

MAJOR TYPES OF ATP-DRIVEN ACTIVE TRANSPORTERS

P- type: Transport of Na^+, K^+ & Ca^{2+}

V-type (V for vacuoles): pumps Proton into lysosomes, & synaptic vesicles.

F-type: Present in mitochondria & transport protons by using ATP but synthesize ATP when function in reverse direction.

ABC Transporter: CFTR protein, a chloride channel involved in the causation of cystic fibrosis.

TYPES OF ACTIVE TRANSPORT

According to the source of energy used to cause the transport:

1. Primary active transport
2. Secondary active transport

1. Primary active transport:

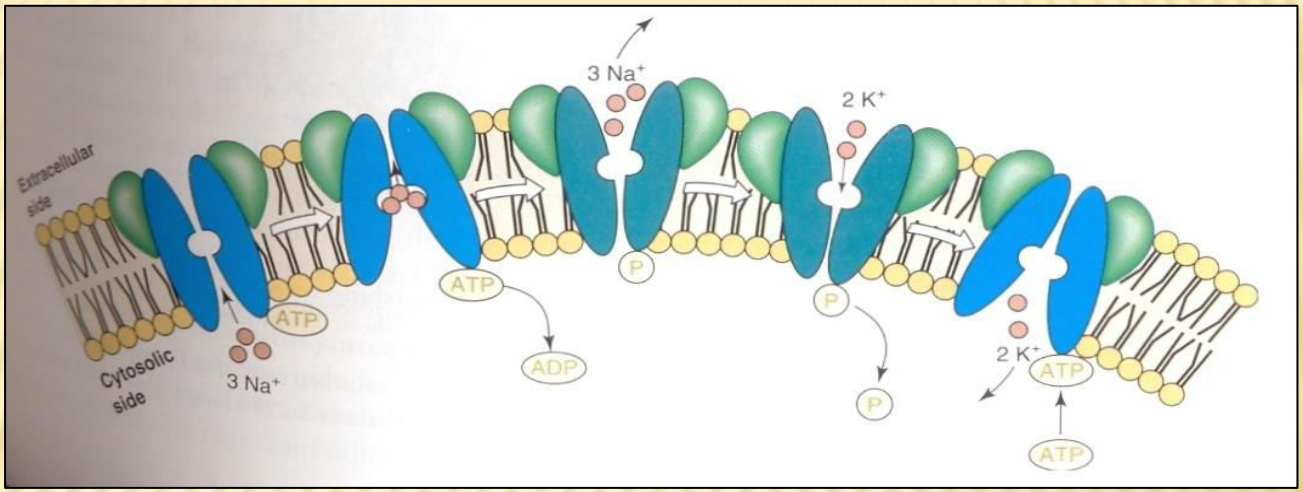
In Primary active transport the energy is derived directly from breakdown of ATP or of some other high energy phosphate compound.

EXAMPLES:

1. Secretion of HCL in the lumen of stomach: The pH of plasma is 7.4 and the pH of stomach lumen is 0.8, this difference in the pH is provided by proton pumps.

   Such proton pumps are also present in endosomes, lysosomes and plasma membrane of some epithelial cells

2. Na^+ -K^+ ATPase system: Na^+ -K^+ ATPase enzyme is a glycoprotein composed of 2α & 2β chains. The enzymes hydrolyzes ATP and released energy is used to transport $3Na^+$ outside and simultaneously $2K^+$ inside across the cell membrane.



3. Active uptake of iodide by the cells of thyroid gland:

   Thyroid gland concentrate iodide from blood against concentration gradient. Iodine is used for synthesis of thyroid hormones

4. Ca^{2+} ATPase:

   This pump produces a Ca^{2+} gradient of upto 1:10000 across the plasma membrane

Active transport and facilitated diffusion share many features e.g.

- Both appear to involve carrier proteins
- Both show specificity for ions, sugars and amino acids

Major differences:

- Facilitated diffusion can operate bidirectionally whereas active transport is usually unidirectional.
- Active transport always occurs against an electrical or chemical gradient and so it requires energy.

2. Secondary active transport:

In Secondary active transport, the energy is derived secondarily from the energy that has been stored in the form of ionic concentration differences of secondary molecular or ionic substances between the two sides of a cell membrane, created originally by primary active transport.

Secondary active transport can be either

co transport or counter transport

Co Transport of glucose and amino acids along with sodium ions:

Glucose and many amino acids are transported into most cells against large concentration gradients; the mechanism of this is entirely by co transport. The transport carrier protein has two binding sites on its external side, one for Na^+ and one for glucose. Concentration of Na^+ is very high on out side and very low inside which provides energy for the transport

A special property of the transport protein is that a conformational change to allow the sodium movement to the interior will not occur until a glucose molecule also attaches. When the both become attached the conformational change takes place automatically, and the sodium and glucose molecule are transported to the interior of the cell at the same time. Hence this is sodium-glucose co-transport.

Sodium co transport of the amino acids occur in the same manner as for glucose, except that it uses a different set of transport proteins. Na^+ co transport of glucose and amino acids occur especially through the epithelial cells of the intestinal tract and renal tubules of kidneys to promote absorption of these substances into the blood.

Sodium counter transport of Calcium and H^+ ions

Counter transport mean transport in a direction opposite to the primary ion.

EXAMPLES:

1. Na^+-Ca^{2+} counter transport

It occurs through almost all cells, Na^+ ions moving to interior and calcium ions moving to exterior, both bound to same transport protein in a counter transport mode.

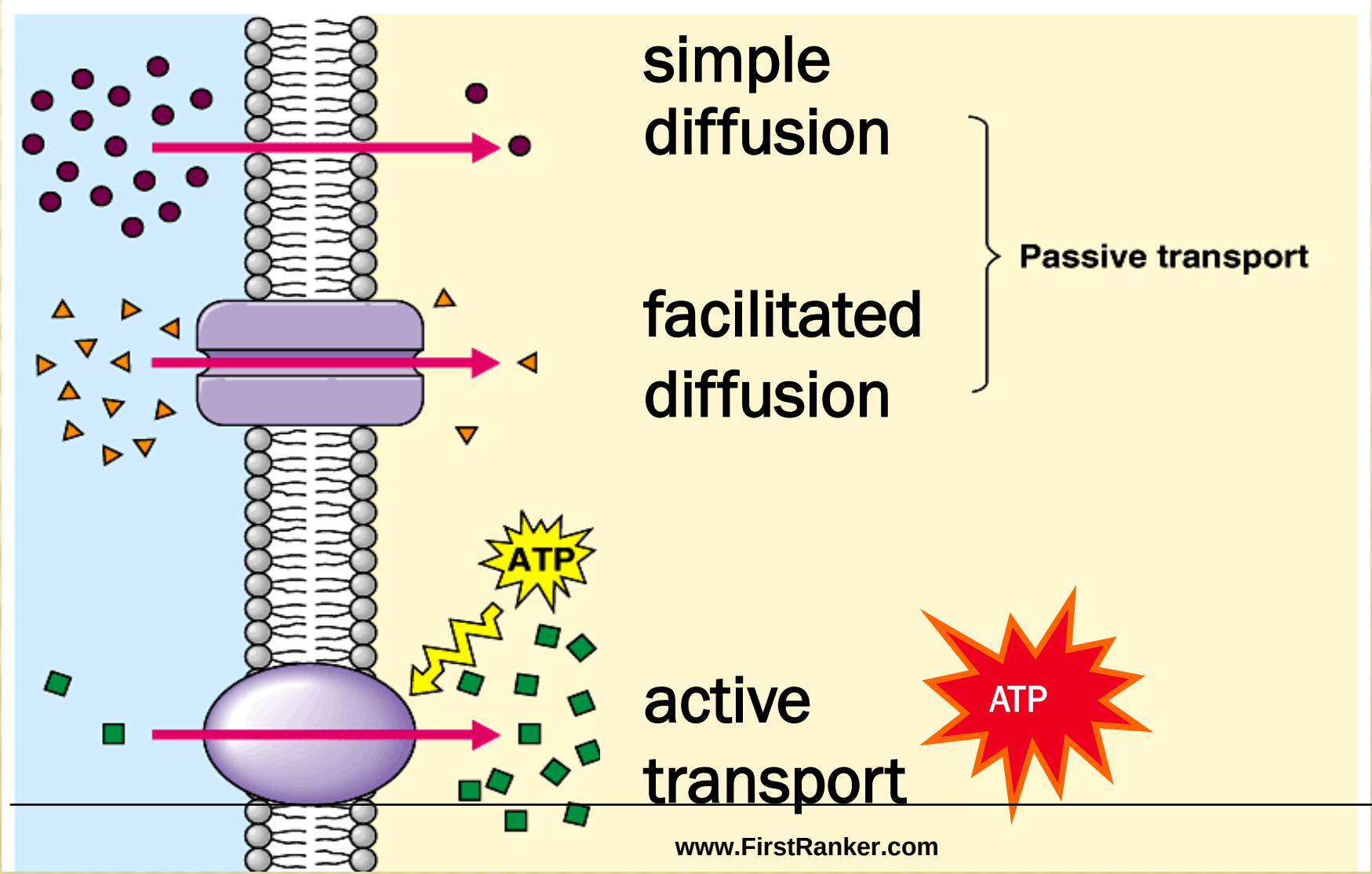
2. Na^+-H^+ counter transport:

It occurs in several tissues especially proximal tubules of kidneys, where Na^+ ions move from the lumen of the tubule to the interior of the tubular cells, while H^+ ions are counter transported in the tubule lumen.

GETTING THROUGH CELL MEMBRANE

- Passive Transport
  - Simple diffusion
    - diffusion of nonpolar, hydrophobic molecules
      - lipids
      - high → low concentration gradient
  - Facilitated transport
    - diffusion of polar, hydrophilic molecules
    - through a protein channel
      - high → low concentration gradient
- Active transport
  - diffusion *against* concentration gradient
    - low → high
  - uses a protein pump
  - requires ATP

TRANSPORT SUMMARY



2. Cross membrane movement of large molecules:

Endocytosis:

"The process by which cells take up large molecules is called endocytosis".

Endocytosis provide a mechanism for regulating content of membrane components - hormone receptors being a case in point.

Endocytosis requires:

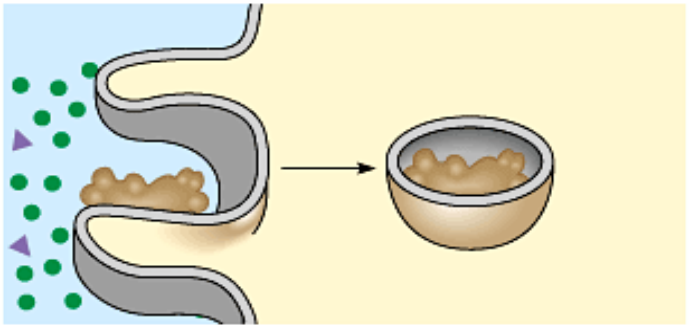
- Energy – usually from hydrolysis of ATP
- Ca^{2+} in ECF
- Contractile element in the cell– the microfilament system

MECHNISM:

Endocytotic vesicles are generated when segments of plasma membrane invaginate, enclosing a minute volume of ECF and its contents. The vesicle then pinches off as the fusion of plasma membrane seals the neck of the vesicle at the original site of invagination. The vesicle Fuses with membrane structures and achieve the transport of its contents to other cellular compartments, usually lysosomes, or back to the cell exterior.

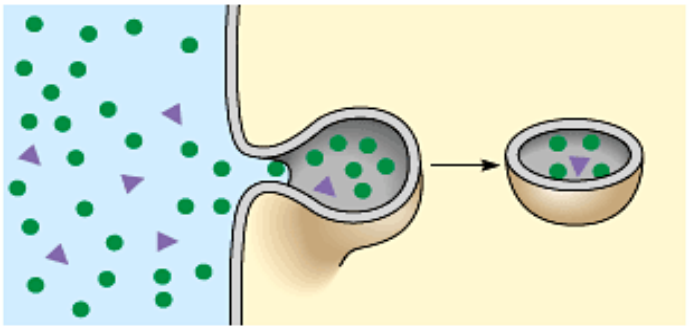
Examples: Polysaccharides, Proteins, polynucleotides,etc.

phagocytosis



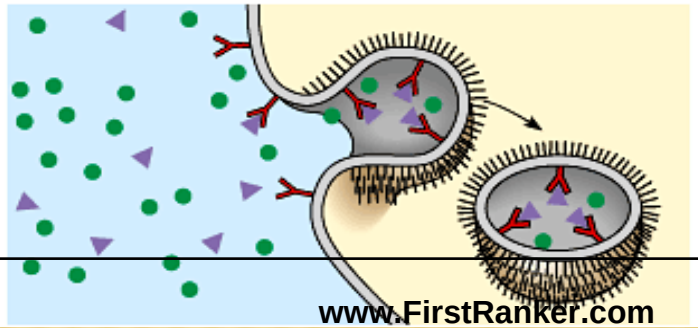
fuse with lysosome for digestion

pinocytosis



non-specific process

receptor-mediated endocytosis



triggered by molecular signal

TYPES:

- Phagocytosis
- Pinocytosis

Phagocytosis:

It occurs only in specialized cells e.g. Macrophages and granulocytes

Involves the ingestion of large particles such as viruses, bacteria, cells or debris.

Pinocytosis:

Property of all cells, leads to the cellular uptake of fluid and fluid contents.

Types of pinocytosis:

1. Fluid phase Pinocytosis
2. Absorptive Pinocytosis

Fluid phase Pinocytosis:

- Non-selctive
- Uptake of solute by formation of small vesicles is simply proportionate to its concentration in the ECF
- Active process

Absorptive Pinocytosis:

- Receptor mediated selective process
- Uptake of macromolecules for which there are a finite number of binding sites on plasma membrane.
- High affinity receptors
- Invaginations (pits) coated on the cytoplasmic site with a filamentous material e.g. Protein clathrin

EXAMPLE:

Uptake of LDL and its receptor

Dark side to receptor mediated endocytosis

- Viruses can cause diseases e.g. Hepatitis, poliomyelitis, and AIDS initiate their damage by this mechanism .
- Iron toxicity also begins with excessive uptake due to endocytosis.

EXOCYTOSIS

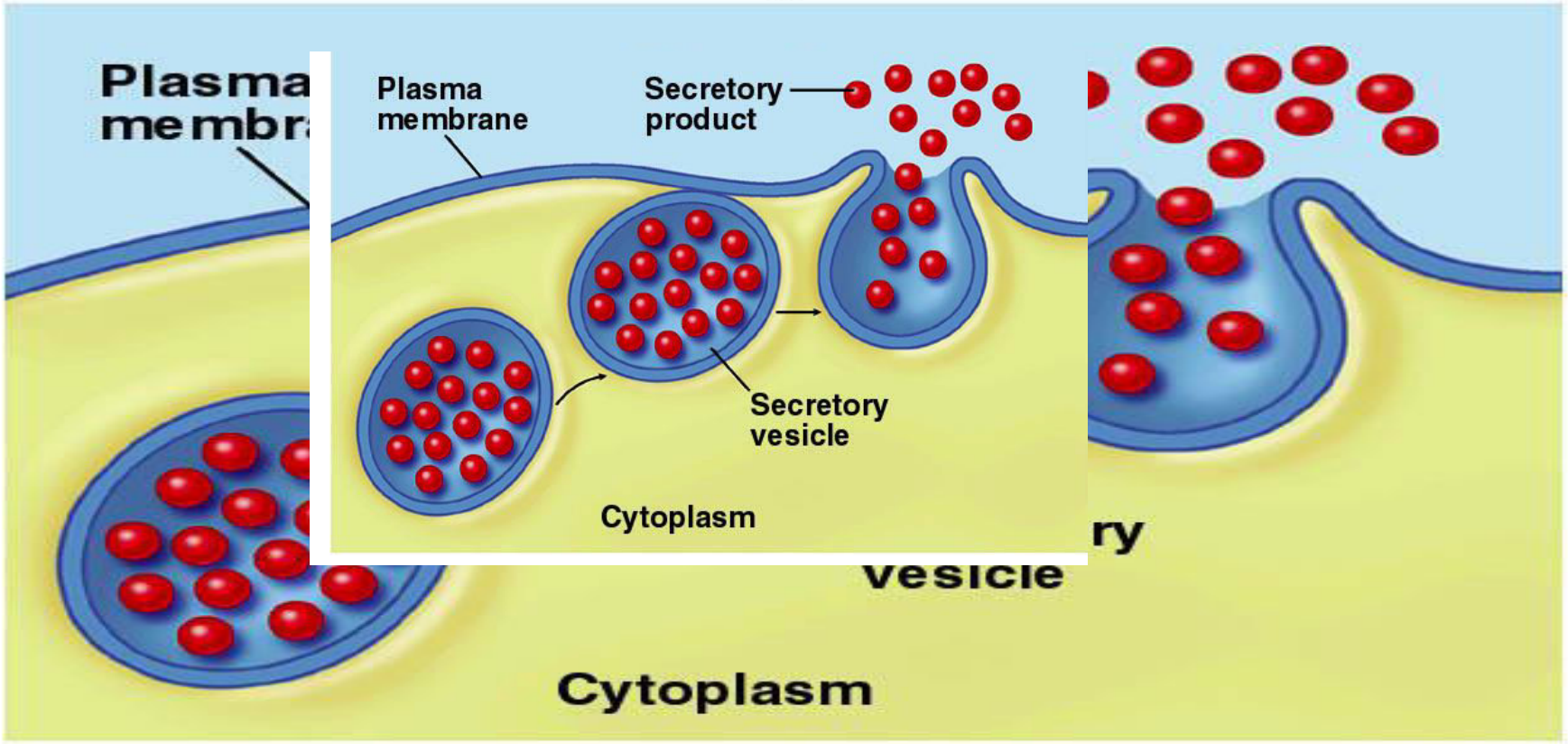
- Cells release macromolecules to the exterior by exocytosis.
- The signal for exocytosis is often a hormone when it binds to cell surface receptors.
- Induces a local and transient change in calcium concentration, calcium triggers exocytosis.

Categories:

Molecules released by exocytosis fall into three categories

- Can attach to the cell surface and become peripheral protein e.g. Antigens
- Can become part of extra cellular matrix e.g. Glycosaminoglycans,
- Can enter ECF and signal other cells e.g. Insulin, catecholamines and parathyroid hormones

Exocytosis

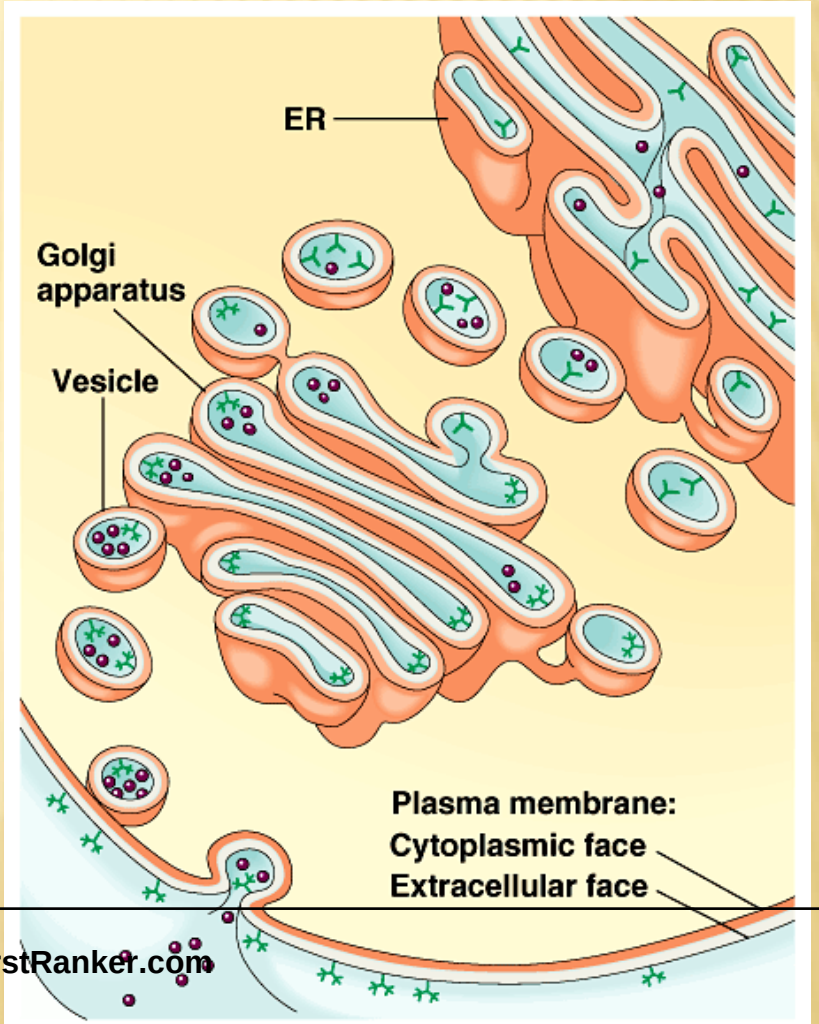


MEMBRANE ASYMMETRY

MEMBRANES HAVE ASYMMETRIC STRUCTURE

1. Proteins are inserted in an asymmetric fashion.
2. Oligosaccharide chains always project towards exterior.
3. Lipid components are also distributed in an asymmetric fashion. e.g., in membrane of RBCs, outer leaflet of bilayer contains mostly phosphatidylcholine and sphingolipids, whereas inner leaflet contains phosphatidylethanolamine and phosphatidylserine.
4. Moreover, cholesterol is generally present in larger amounts on the outside than on the inside.

- Membranes have **asymmetric** inside and outside faces. The membrane's synthesis and modification by the ER determines this asymmetric distribution of lipids, proteins and carbohydrates.



INTEGRINS

Integrins are integral membrane proteins consist of α & β transmembrane polypeptides, their extracellular domains combine to form binding sites for metal ions, proteins of extracellular matrix such as collagen & fibronectin or specific surface proteins of other cells.

FUNCTIONS:

a. Adhesive

b. Receptors

c. Signal transducers

d. Regulate many processes including platelet aggregation at site of wound.

PERIPHERAL MEMBRANE PROTEINS

1. Most of the peripheral membrane proteins function as enzymes e.g.

- Alkaline Phosphatase
- Acetyl –Cholinesterase
- Lipoprotein Lipase

2. Also act as controllers of transport of substances through the cell membrane pores.

3. Certain peripheral membrane proteins interact with the cytoskeleton. These interactions are essential for integrity of cell e.g.

- Ankyrin
- Spectrin

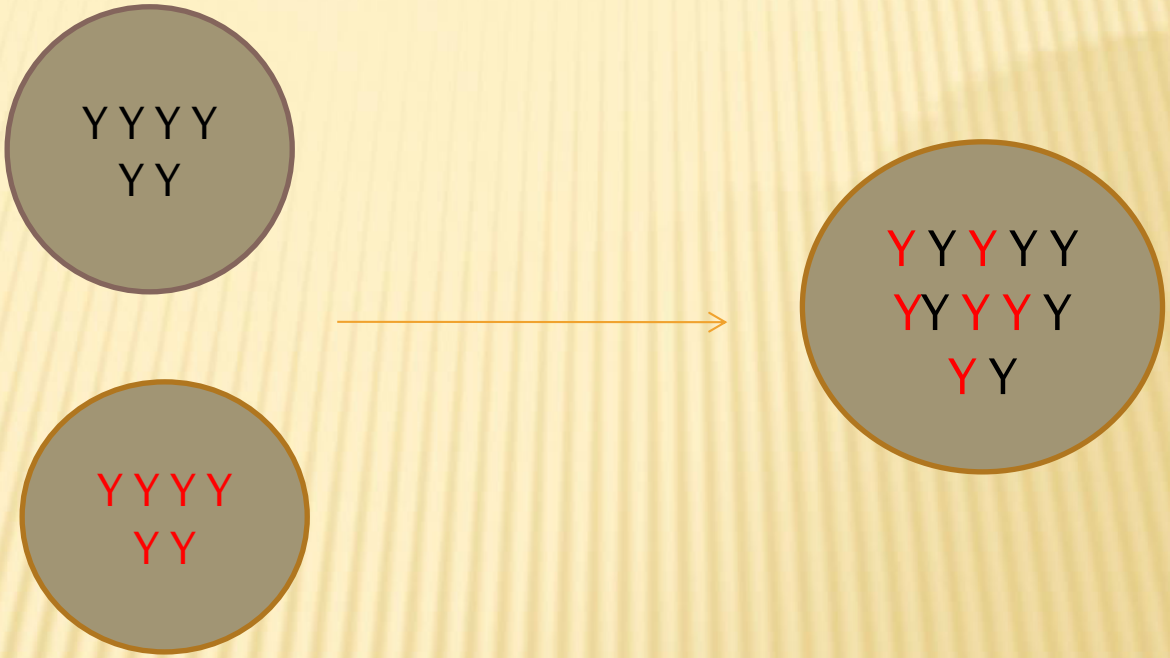
4. Specific proteins are involved in signal transduction pathway e.g.

- Gs (G-stimulatory)
- Gi (G-inhibitory)

FLUID MOSAIC MODEL OF MEMBRANE

FLUID MOSAIC MODEL

EVIDENCE:



*Y Y are species specific integral proteins

- The phase changes and the fluidity of membrane is largely dependent upon two factors:

1. Lipid composition
2. Temperature

1. LIPID COMPOSITION:

- Cholesterol: Has two important effects

1. Below the membrane transition temperature, the cholesterol insertion b/w hydrocarbon fatty acyl chains interferes with the highly ordered packing of these chains and thus increase fluidity.

2. At temperature above the transition temperature when hydrocarbon fatty acyl chains tend to move more freely, cholesterol, due to its rigid ring structure reduces the freedom of neighbouring fatty acyl chains to move, thus limiting fluidity.

- Saturated and unstructured fatty acids of phospholipids:

  When there are more of S.F.A in membrane phospholipids, they tend to pack closely ⟶ hydrocarbons interactions ⟶ more energy (thermal E) to move them farther ⟶ so, transition temperature is high.

Sometimes these even transport substances in the direction opposite to their natural direction of diffusion, which is called 'active transport'.

Glucose transporters (GLUT 1-5) are integral membrane proteins that transport glucose.

3. Some integral membrane proteins act as enzymes e.g.

- Na^+ - K^+ ATPase
- Adenylyl cyclase
- Guanylyl cyclase

4. Integral membrane proteins also serve as receptors for water soluble chemicals, such as peptide hormones, they do not easily penetrate the cell membrane e.g.

- Insulin receptor
- Nicotinic acetylcholine receptor

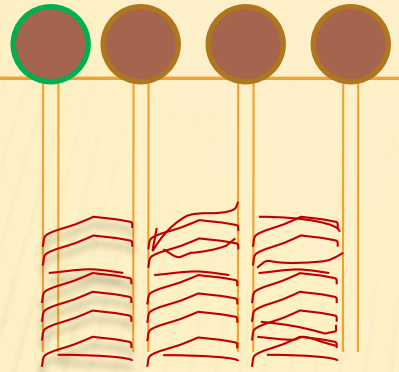
5. Some integral membrane proteins mediate cell-cell interactions & adhesions e.g.

- Integrins

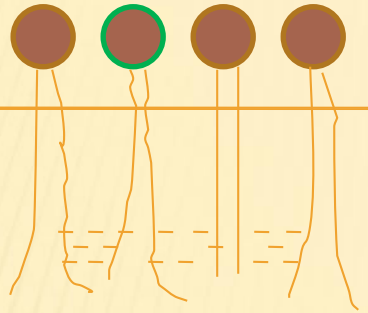
2. At temperature above the transition temperature when hydrocarbon fatty acyl chains tend to move more freely, cholesterol, due to its rigid ring structure reduces the freedom of neighbouring fatty acyl chains to move, thus limiting fluidity.

- Saturated and unstructured fatty acids of phospholipids:

  When there are more of S.F.A in membrane phospholipids, they tend to pack closely ⟶ hydrocarbons interactions ⟶ more energy (thermal E) to move them farther ⟶ so, transition temperature is high.



On the other hand, membrane phospholipids with unsaturated fatty acids (having kinks) cannot be very closely packed → consequently weak interactions with neighbouring → molecules little thermal energy required to move them apart have low transition temperature.



so we conclude:

presence of more S.F.A in membrane → more is transition temp.

presence of more U.F.A in membrane → lower is transition temp.

CELL TECHNOLOGY

During the past 60 years, there has been a revolution in our understanding of the functioning of living organisms. In the years immediately after world war II three important developments were made.

1. Development & increasing availability of electron microscope.

2. Introduction of methodology permitting disruption of cells under relatively mild conditions that preserved function.

3. Increasing availability of high speed, refrigerated ultra centrifuge, capable of generating centrifugal forces sufficient to separate constituents of disrupted cells from one another without overheating them.

STUDY OF CELLS FROM A BIOCHEMICAL VIEWPOINT

Rat hepatocyte is one of the most extensively studied of all cells from a biochemical viewpoint. This is due to following reasons:

1. Available in relatively large amounts
2. Diverse functions
3. Suitable for fractionation studies; contains major organelles (nucleus, mitochondria, ER, free ribosomes, Golgi-apparatus, lysosomes, peroxisomes, plasma membrane, cytoskeletal elements) found in eukaryotic cells.

The development of Electron microscope as a biological instrument by Keith Porter & his colleagues in 1940s was responsible for the resolution of the fine structure of organelles.

- Structures such as mitochondria & lysosomes were not discovered until this time; with use of light microscope they appeared as mere granules.
- Moreover, E/M revealed that membranes of the Golgi-apparatus are often continuous with those of the Endoplasmic reticulum. This discovery is especially significant because of the role both organelles play in protein synthesis.