Molecular Cell Biology

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Module 4

Membrane Organization and Transport Across Membranes

Lecture 1

Cell Membrane and Transport Mechanisms Across The Cell Membrane

Cell membrane

- Cell membrane or plasma membrane mainly regulates the cellular entry and exit of molecules and ions.
- This function of cell membrane is called cell permeability.
- Plant cells have a thick cell wall that covers plasma membrane and protects it.
- Animal cells have a cell coat or external laminae.
- Isolation of membranes from erythrocytes is relatively easy.
- Hypotonic solutions cause swelling of membranes forcing out hemoglobin from red blood cells forming a red cell ghost and hemolysis.
- With mild hemolysis one can get resealed ghost that can be used for permeability studies but with severe hemolysis white ghosts are formed that can be used for biochemical studies.

Components of cell membrane

- The membrane is made up of a lipid bilayer embedded with proteins some of them protruding out from the membrane.
- Red blood cell membrane has 52% protein, 40% lipids and 8% carbohydrates.
- There is a wide variation in this composition among various membranes.
- Phospholipids, cholesterol and galactolipids generally constitute the major lipid portion of membranes but their composition varies among different membranes.
- Major phospholipids include the neutral phospholipids (no net charge at neutral pH) such as phosphatidyl choline, phophatidyl ethanolamine and sphingomyelin.
- Acidic phospholipids (5-20%) are negatively charged that include phosphatidyl inositol, phosphatidyl serine, phosphatidyl glycerol, cardiolipin and sulfolipids.

Asymmetric distribution of membrane components

- The various chemical components are not distributed uniformly between the inner protoplasmic surface and outer surface of the plasma membrane.
- For instance the outer layer of red blood cell membrane consists of lecithin and sphingomeyelin whereas the inner layer is made up of phosphatidyl ethanolamine and phosphatidyl serine. Glycolipids are present in the outer half of the bilayer membrane.

- This kind of stable asymmetry is noticed in many membranes and not much of exchange occurs among the lipids across the bilayer.
- The asymmetric distribution is also noticed among the oligosaccharide components of the membrane. Outer membrane surface mainly contains glycolipids and glycoproteins.
- In erythrocytes all the proteins from the outer surface are glycosylated.
- Hexose, hexosamine, fucose and sialic acid are bound to proteins.
- The negative charge is contributed by sialic acid, phosphate and carboxylic groups.

Membrane proteins

- Proteins are not only structural components but also serve as carriers or channels.
- They also serve as receptors for various signaling ligands in addition being enzymes and antigens.
- Membrane proteins are generally classified into extrinsic (peripheral) or intrinsic (integral) proteins.
- Peripheral proteins are soluble in water/aqueous solutions and do not contain much of lipids. Eg: Spectrin, cytochrome c.
- The majority of membrane proteins are integral proteins that are insoluble in water.
- They are in strong association with lipids and carbohydrates. Membrane bound enzymes, histocompatibility antigens and various drug and hormone receptors belong to this category.
- Hydrophilic or polar /hydrophilic amino acids are mainly present near the surface in the peripheral proteins whereas the nonpolar/hydrophobic amino acids are buried inside
- In the case of integral proteins the nonpolar amino acids are more exposed to the surface.
- Outer surface of some membranes contain acetyl choline esterase, nicotinamide dinunucleotide-adenine dinucleotidase and the ouabain binding site of $Na^+ K^+$ ATPase.

- Inner surface contains NADH-diaphorase, adenylatecyclase, protein kinase and Mg^{++} ATPase.

Theories on membrane structures

- In 1902 it was thought that the membranes had only lipids (Overton).
- In 1926 *Gorter and Grendell* proposed that lipids are capable of forming a double layer.
- In 1935 *Danielli* and *Davson* proposed the lipid bilayer model that includes proteins adhering to both lipid-aqueous interfaces
- Artificial model systems such as the liposomes supported the idea of *Danielli* and *Devson.*
- A droplet of lipid made soluble in an organic solvent can be spread over a small hole on a septum that divides two chambers containing water.
- This set up is useful to study biophysical properties of a bilayer such as permeability and electrical resistance.
- Channels for ions can be formed by adding certain proteins or polypeptides.
- Liposomes act as excellent carriers for different molecules such as chemotherapeutic compounds, insulin and antibodies.

Unit membrane model

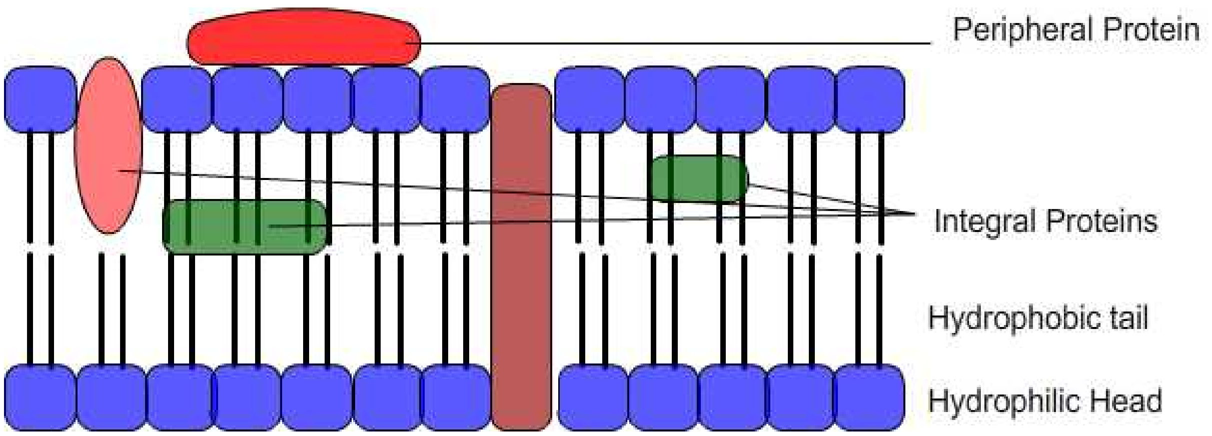
- Robertson in 1959 postulated the unit membrane model.
- This model stated that the central layer of plasma membranes is made up of the hydrocarbon chains of lipids and the proteins constitute the dense surrounding layers on both sides when viewed through an electron microscope.
- Unit membrane model turned out to be an oversimplification model as it cannot account for the no. of protein molecules present across the membranes.

Fluid mosaic model

- Fluid mosaic model proposed by S.J. Singer and G.L. Nicolson (1972) was finally acceptable to most biologists
- This model recognizes that lipids and proteins are in a mosaic arrangement.

- It also recognizes that there is translational movement of lipids and proteins within the lipid bilayer.
- Non covalent interactions ensure a fluid like state for the membranes.
- Integral proteins are intercalated into the continuous lipid bilayer.
- Polar/hydrophilic regions of proteins protrude from the surface while the nonpolar/hydrophobic regions are embedded inside.
- The concept of fluidity is attractive as it explains the considerable freedom of lateral movement for proteins and lipids observed within the bilayer.

Fluid mosaic model



Permeability

- Permeability is an important property of the plasma membrane and other membranes in a living cell.
- This is important for maintaining the required intracellular conditions.
- Basically this determines as to what substances should enter or leave the cell and in turn this is essential to maintain life.
- The composition of important body fluids depends on the permeability.
- Osmotic pressure of intra and extra cellular fluids depends upon the permeability.

Passive permeability

- Membrane acts as a barrier to the passage of water soluble molecules.
- Lipid soluble substances more easily pass through the membrane.
- Size and solubility of molecules are important factors affecting their permeability.
- P=KD/t where P is permeability, K is partition coefficient, D is diffusion coefficient, and t is thickness of membrane.
- If two molecules have the same size, the one with higher solubility in lipids will penetrate the membrane faster.
- If two molecules have equal solubility in lipids, the smaller molecule will penetrate the membrane faster.

Electrical and ionic gradients

- Between the extracellular and intracellular compartments ionic and electrical gradients exist.
- Their interdependence is known since the distribution of ions on both sides of the membrane contributes to the electrical potential.
- Intracellular fluid contains more of K^+ ions and organic anions
- Interstitial fluid contains more of Na^+ and Cl^- ions.
- The resting/steady potential is usually negative inside a cell and varies between -20 and -100 mV.
- The diffusion of ions depends both on the concentration and electrical gradients across the membrane.

Active Transport

- If only passive diffusion operates in cells any increase or decrease in membrane potential would result in asymmetric ionic distribution.
- Many experiments have shown that indeed there are active transport mechanisms in living cells that require energy.
- ATP provides energy for such active transport processes.
- Thus oxygen consumption is required when an ion is transported against the electrochemical gradient.

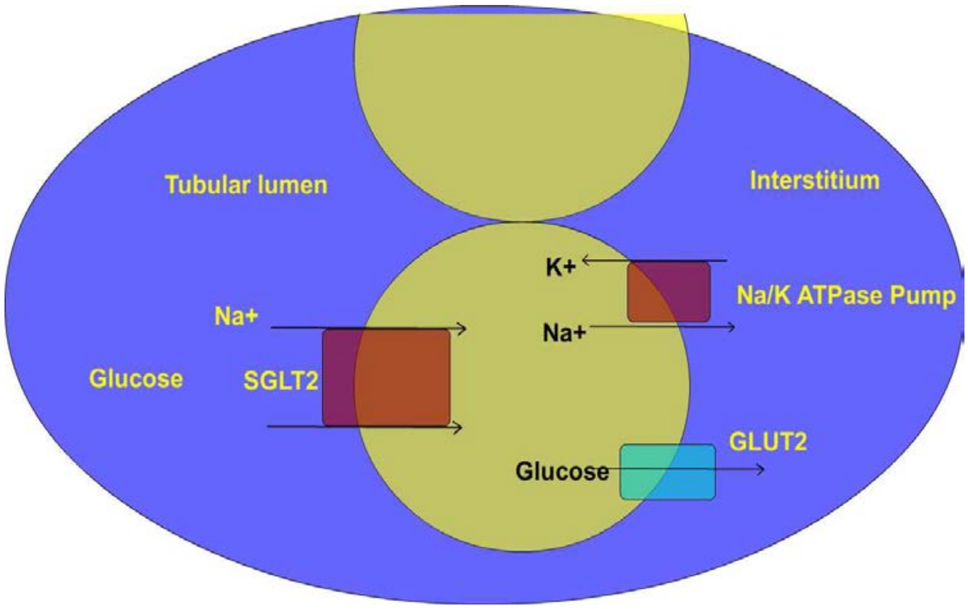
- Active transport is also needed to maintain the resting potential
- The cells are able to keep a constant osmotic pressure by regulating the ionic transport across the cell membrane.
- Potassium ions are present in higher concentrations inside the cells through a pumping mechanism that requires energy to work against the concentration gradient.

Sodium Pump

Although Na^+ does not have a higher mol. wt. in comparison to K^+ and Cl^- ions its ionic radius in the hydrated conditions is higher and thus it cannot enter the cell easily.

- Sodium pump throws out Na^+ ions together water from inside to the outside of the cell by an active transport mechanism.
- Na^+ K^+ ATPase is an enzyme that couples the hydrolysis of ATP with the elimination of Na^+ ions from the cytoplasm against electrochemical gradient.
- Hydrolysis of one ATP can give energy to transport two K^+ ions towards inside and three Na^+ ions towards outside.
- Thus both these ions can activate ATPase and ouabain can inhibit it by binding to it on the extracellular surface of the enzyme.
- Vanadate also inhibits this enzyme but this acts from the cytoplasmic side of the enzyme.
- The first step for this enzyme reaction is the formation of a covalent phosphoenzyme intermediate.
- This happens on the inner side of the membrane in the presence of Na^+ ions but Ca^{++} ions inhibit this reaction
- In the second step the intermediate complex is hydrolyzed forming the free enzyme and phosphate ions and this requires K^+ but is inhibited by ouabain.
- Other substances such as glucose and amino acids may use the sodium pump for their transport.

Sodium Pump



Transport proteins

- Selective transport of molecules across the membranes is also achieved by means of carriers/permeases/transport proteins.
- High degree of specificity by this mechanism is related to the chemical structures of molecules being transported.
- For example the structures of glucose and galactose are very similar except for the position of OH group at carbon 4, but these two molecules cross the membranes by using different carriers.
- Permeases help in achieving this specificity related to the structure and in the process the permeases do not change and get recycled for another round.
- Some permeases work under a favorable concentration gradient in a mode of passive diffusion called facilitated diffusion.
- Some others work against the concentration gradient employing an active transport mechanism.

Mechanisms

- The **carrier mechanism** works by first binding of the molecule with the carrier protein at the outer surface of the cell.
- Then this complex translocates into the cytoplasm by a rotatory movement.
- However, this kind of a mechanism is not thermodynamically favorable as rotation and translocation across the bilayer may not be easy.
- **Fixed pore mechanism** suggests that the carriers are actually integral proteins and they undergo conformational change once a molecule to be transported gets attached with the carrier.
- In the above mechanism, the carrier proteins are suggested to be oligomers forming a channel or pore that has a hydrophilic lining in the middle.
- This mechanism can account for the sodium pump action and also for the transport of glucose and amino acids.

Study Questions

1. What are the functions of plasma membrane?
2. What are the mechanisms by which molecules and ions are transported across membranes?
3. Energy requirement of active transport is met by
   a) GTP b) ATP c) GMP d) cAMP
4. Match the following

Fluid mosaic model	ATPase inhibitor
Danielli and Devson	Negative inside
Ouabain	Lipid bilayer model
Resting potential	Singer and Nicolson

5. Hypotonic solutions cause--------------- of membranes