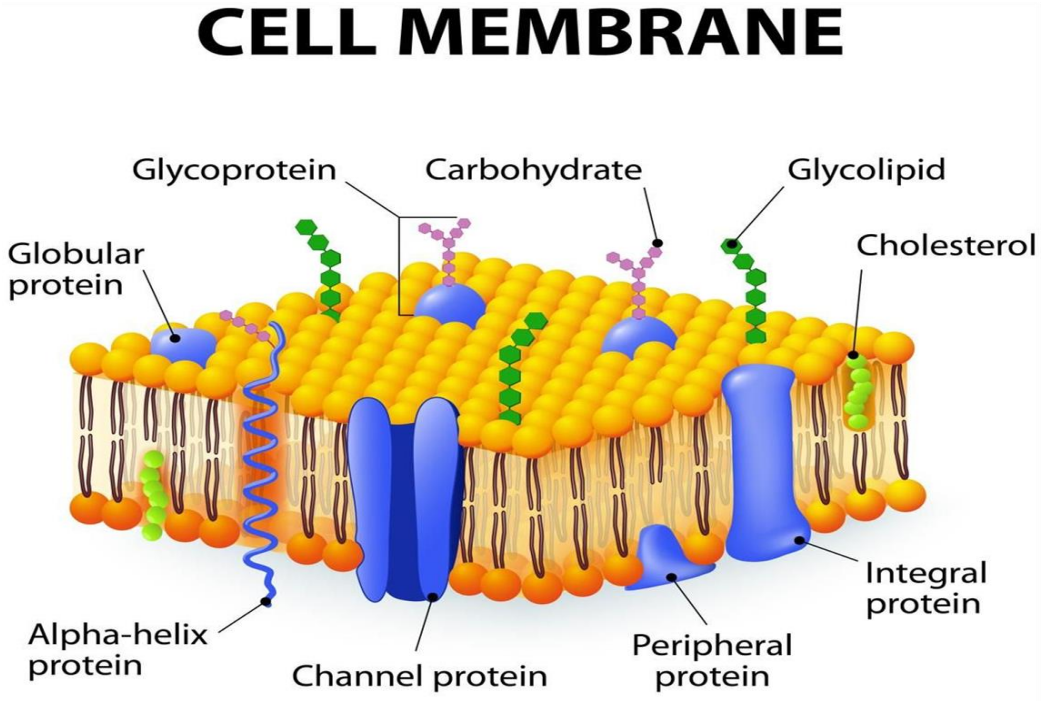
Cell Membrane and Biosignalling

The protoplast of plant cell is separated from their environment by the plasma membrane or plasma lemma. It is presumed that the structure and function of the plasma membrane I the plant is fundamentally similar to that in animals, fungi and bacteria. The plasma membrane of plant cell is primarily composed of lipids and proteins.

The first important hypothesis regarding the structure of biological membrane was proposed by H. Davson and J. Danielli (1935) according to which the membrane is composed of a continuous hydrocarbon phase contributed by the lipid components. This hypothesis was later modified by J.D. Robertson into the unit-membrane hypothesis. This hypothesis states that the membrane is composed ofa bilayer of mixed polar lipids with their hydrophobic tails oriented inward to form a continuous hydrocarbon phase and their hydrophilic heads oriented outwards. Both the surfaces were supposed to be coated with a monomolecular layer of protein molecules.

The most satisfactory model of membrane structure at present is the dynamic fluid mosaic model put forward by Singer and Nicholson (1972). This model states that the amphipathic lipids (phospholipids, glycolipids, sterols) are arranged in a bilayer to form a fluid, liquid-crystalline matrix, in which individual molecules can move laterally. This lateral movement gives the bilayer structure fluidity, flexibility and characteristically high electrical resistance and relative impermeability to highly polar molecules. The globular proteins either remain partially embedded in either side or may completely penetrate the matrix forming a mosaic pattern. As the proteins are free to diffuse laterally in two dimensions, the mosaic pattern is not fixed or static. This fluid-mosaic m0odel satisfactorily accounts for many features and properties of biological membrane- such as-

1. It explains the widely different protein content per unit area,
2. It explains the variation in thickness of the different types of membranes,
3. It accounts for the electrical properties and permeability of membranes, and
4. It also accounts for the movement of some proteins in the cell membrane.



Chemical composition of cell membrane

I. **Lipids-** Lipids are water insoluble biomolecules that are highly soluble in organic solvents such as chloroform. Three major kinds of membrane lipids are phospholipids, glycolipids, and cholesterol.

Phospholipids are abundant in all biological membranes. A phospholipid molecule is constructed from four components- fatty acids, a platform to which the fatty acids are attached which may be either a glycerol or sphingosine, a phosphate group and an alcohol attached to the phosphate. Phospholipids derived from glycerol are called phosphoglycerides. Sphingomyelin is a phospholipid found in membrane that has sphingesine backbone.

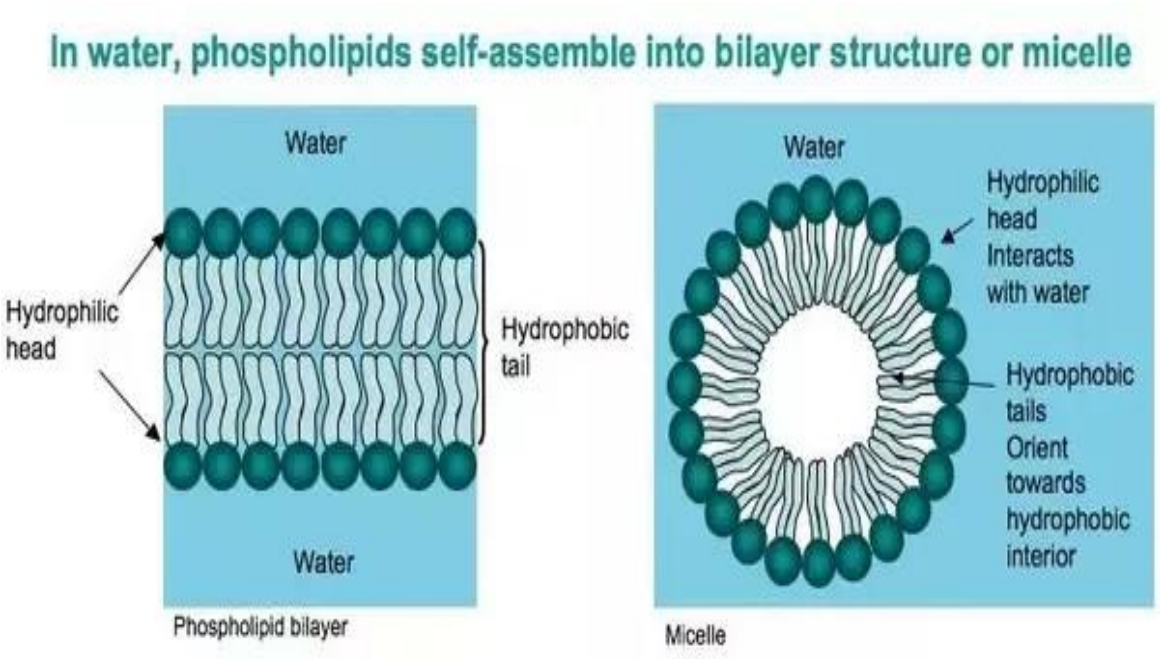
Glycolipids are sugar containing lipids. They too contain a sphingosine backbone. Glycolipids differ from sphingomyelins in having one or more sugar residues, instead of phosphoryl choline, attached to sphingosine. Glycolipds are oriented in a completely asymmetric fashion with the sugar residues always on the extracellular side of the membrane.

Cholesterol is a lipid with a structure quite different from that of phospholipid. It is a steroid built from four linked hydrocarbon rings. A hydrocarbon tail is linked to the steroid at one end and a hydroxyl group is attached at the other end. In membrane, the molecule is oriented parallel to the fatty acid chains of the phospholipids and the hydroxyl group interacts with the nearby phospholipid head group.

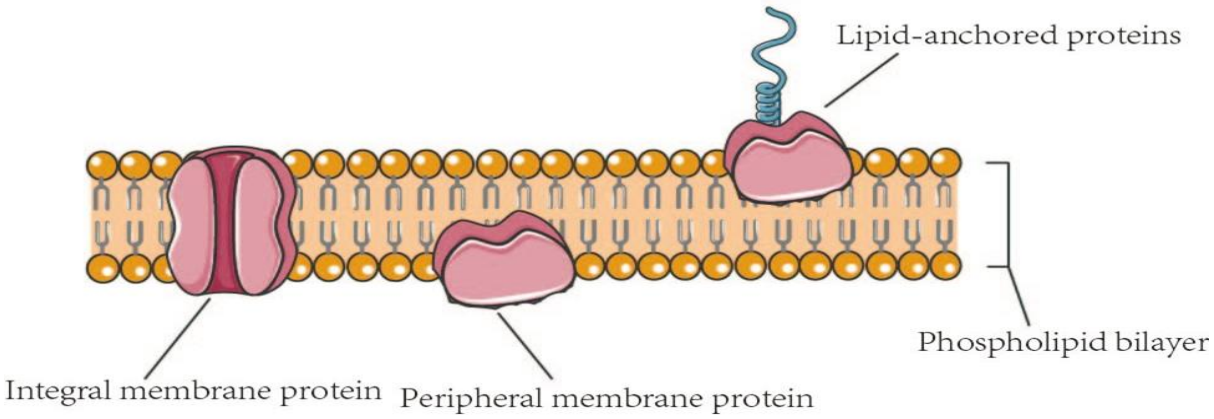
Membrane lipids are amphipathic molecules as they contain both hydrophilic and hydrophobic moiety. Membrane formation is actually a consequence of this amphipathic nature of the lipid molecules. Their polar head groups favour contact with water, whereas their hydrophobic tails interact with each other. With these preferences a lipid molecule can arrange itself in two ways in aqueous solution- either forming a globular micelle or a lipid bilayer. A lipid bilayer, also known as the bimolecular sheet possess the hydrophobic tails of its members interacting with one another forming a hydrophobic interior that acts as a permeability barrier. The hydrophilic head groups interacts with the aqueous medium on each side of the bilayer.

A bimolecular sheet is preferred over a micelle always because of both structural reason as well as biological reason. The formation of lipid bilayer is a self-assembly process, where hydrophobic interactions take part as the major driving force. While van der Waals attractive forces between the hydrocarbon tails favour the close packing of the tails, the electrostatic and hydrogen bonding attraction between the polar head groups and water molecules form the compact hydrophilic side. The presence of several hydrophobic interactions impart three biological significances to the lipid bilayer-

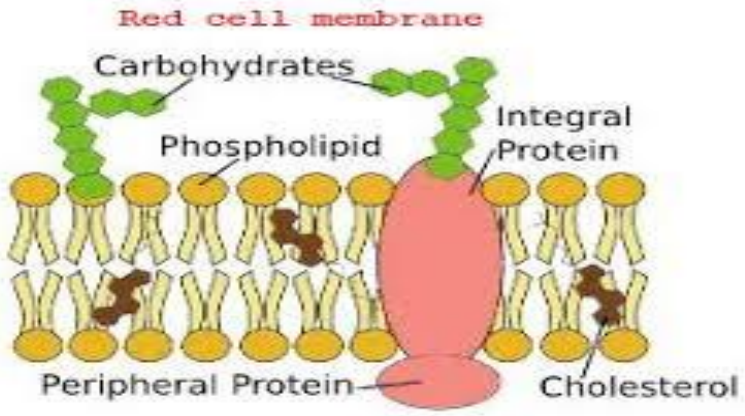
- They have an inherent tendency to be extensive,
- They tend to close on themselves so that there are no edges with open hydrocarbon chains and compartments can be formed,
- Lipid bilayers re self-sealing as a hole in the bilayer is energetically unfavourable.



II. **Proteins-** About 35-40% of the weight of the plasma membrane is accounted for by proteins. As membrane lipids form a permeability barrier, proteins transport chemicals and information across the membrane. Membrane proteins can be classified as being either peripheral or integral on the basis of how easily they can be dissociated from the membrane. Integral membrane proteins interact extensively with the hydrocarbon chains of membrane lipids. In contrast, peripheral membrane proteins are bound to membranes primarily by electrostatic and hydrogen bond interactions with the head groups of lipids.



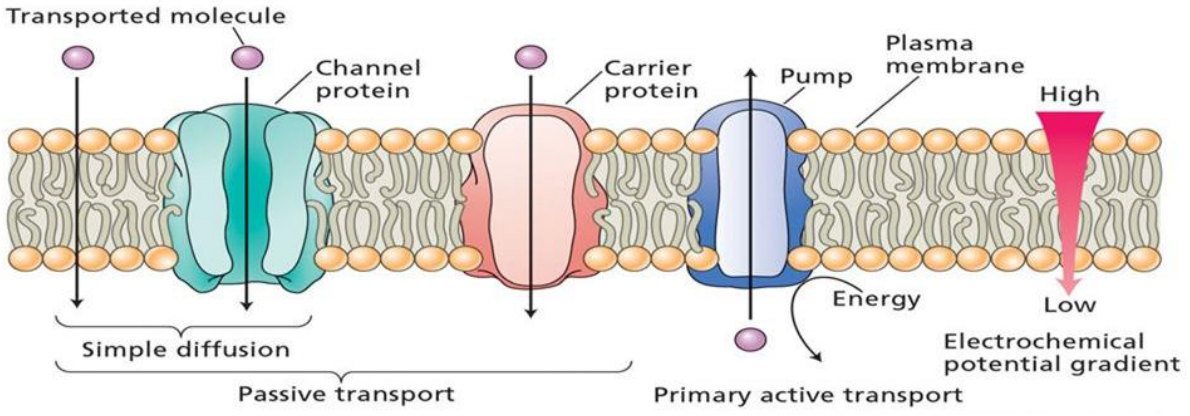
III. **Carbohydrates-** The third major component of the plant plasma membrane is carbohydrate in the form of glycolipids, glycoproteins and various other cell wall polysaccharides. The carbohydrate chains may consist of 2-60 monosaccharide units and can be either straight or branched. Carbohydrates are added to the lipid or protein through a process called glycosylation. Glycoproteins and glycolipids are covalently bound to the plasma membrane protein or lipid respectively and exist at the exoplasmic face of the membrane.



Other than these three major components various enzymes are also found in the plant plasma membrane. K^+-ATPase enzyme acts as a primary energy transducer for the coupling of metabolic energy of ion transport across the plasma membrane. The preferred substrate for the enzyme is the Mg-ATP, while Ca2+ strongly inhibits enzyme activity. Another enzyme glucosyl transferase catalyses the transfer of the sugar moiety of a nucleotide to various components associated with the membranes. Plasma membrane associated cellulase is actually transported from the cytoplasm to the cell wall when necessary to function in the dissolution of cell wall cellulose. Plasma membrane also contain specific binding proteins that function variously, like in auxin transport, transport of α-galactosides, harbouring photoreceptors etc.

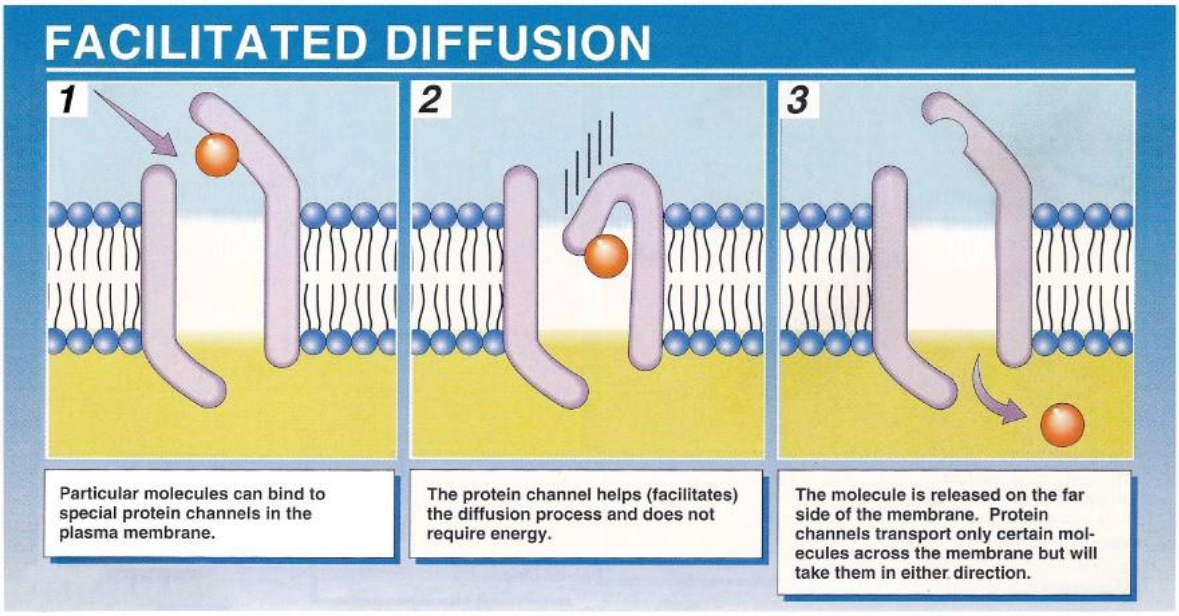
Transport through Membrane

Because of non-polar nature of membranes interior, pure phospholipid bilayers are highly impermeable to ions or polar molecules. Biological membranes contain transport proteins that facilitate the passage of ions and other polar molecules. These transport proteins exhibit specificity in the solutes they transport, which accounts for their great diversity I the cells. Transport proteins can be grouped into three main categories- channels, carriers and pumps.



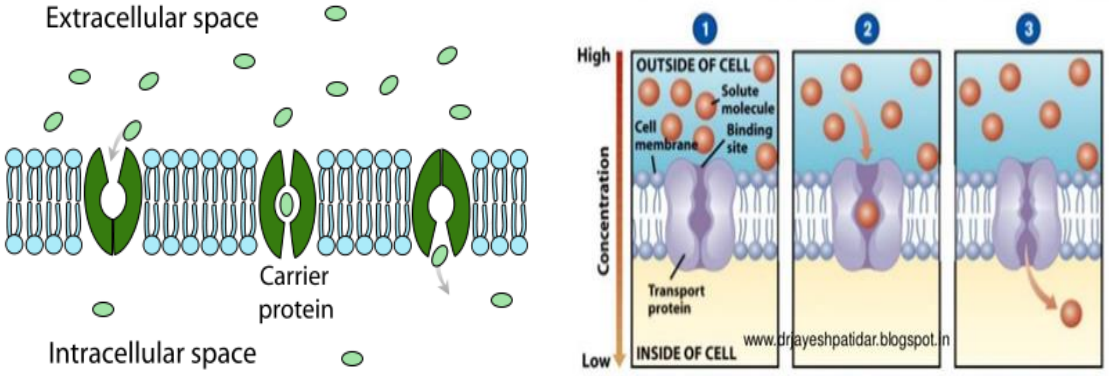
Channels are trans membrane proteins that function as selective pores in the membrane. The size of a pore and the density of surface charges on its interior lining determine its transport specificity. Transport through a channel may or may not involve transient binding of the solute to the channel protein. As long as the channel pore is open, solutes that can penetrate the pore diffuse through it extremely rapidly. However, the channel do not usually stay open for long periods. They have gates that open and close the pore in response to external signals. Transport

through channels is always passive, and because the specificity of transport depends on pore size and electric charge more than on selective binding, channel transport is limited to ions or water. The region of channels that determine specificity is called the selectivity filter.



Patch clamp studies have revealed that gated channels can also be distinguished on the basis of how long the gates remain open in response to a prolonged stimulus. On this basis they can be classified as rapid and slow channels. Such channels have been observed in the guard cell plasma membrane and named as R-type (gates open and close rapidly in response to a voltage stimulus) and S-type (channels that remain open for the total duration of the stimulus).

In case of transport mediated by a carrier, the substance being transported is initially bound to a specific site o the carrier proteinbinding causes a conformationl change in the protein, which exposes the substance to the solution on the other side of the membrane. Transport is complete whenthe substance dissociates from thr carriers binding site. Because a conformational change in the protein is required to transport molecules orions, the rate of transport by carriers is much slower than that of the channels.



Carrier mediated transport can either be active or pasive, and it can transport a wider range of possible substrates. Passive transport on a carrier is sometimes also called as facilotated diffusion.

Primary Active Transport- To carry out active transport, a carrier must couple with the uphill transport of the solute with another energy releasing event, so that the overall free energy change is negative. Primary active transport is coupled directly to a metabolic source of energy, such as ATP hydrolysis, an oxidation-reduction reaction like the electron transport chain in mitochondria or the absorption of light by the carrier protein. The membrane protein that carry out primary active transport are called the pumps. Most pumps transport ions, such as H^+ or Ca^+. However, pumps belonging to the ATP-binding cassette family of transporters can carry large organic molecules into the vacuole.

Ion pumps can further be characterised as either electrogenic or electroneutral. Electrogenic transfer refers to ion transport involving the net movement of charge across the membrane. In contrast, electroneutral transport, involves no net movement of charge. For example, the Na^+/K^+-ATPase pumps three Na+ ions out for every two K^+ ion in, resulting in a net outward movement of one positive charge, and thus it is an electrogenic ion pump. But the H^+/K^+-ATPase pumps one H^+ ion out of the cell for every one K^+ in.so there is no net movement of charge across the membrane and hence it is an electroneutral pump.

In the plasma membrane of plants, fungi and bacteria, as well as in plant tonoplast and other plant endomembrane, H^+ is the principal ion that is electrogenically pumped across the membrane. The plasma membrane H^+-ATPase creates the gradient of electrochemical potentials of H^+ in the plasma membrane, while the vacuolar H^+-ATPase and the H^+-pyrophosphatase electrogenically pump protons into the lumen of vacuole and Golgi cisternae.

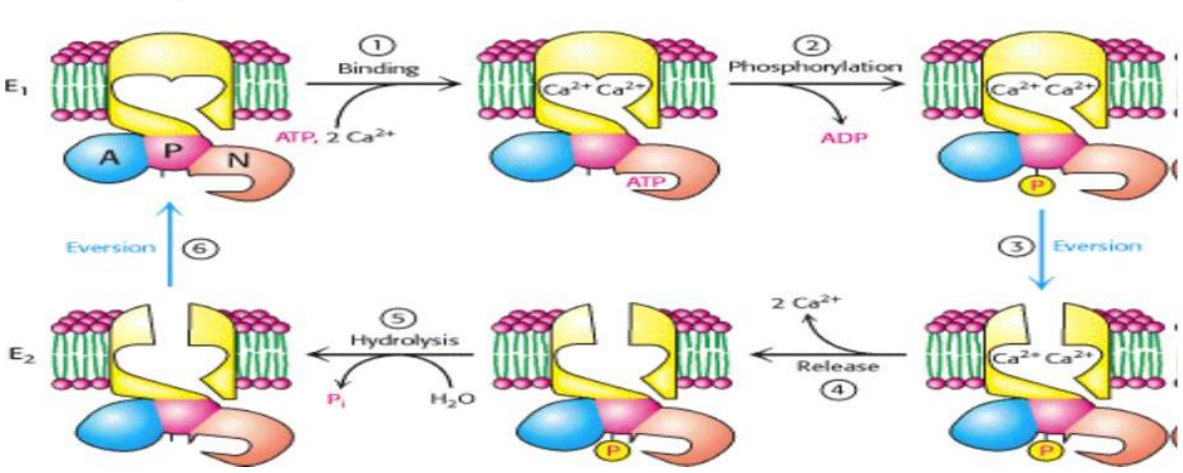
Mechanism of transport- Purification of Na^+/K^+ ATPase, Ca^{2+} ATPase, H^+/K^+ ATPase and other ion pumps has revealed a large family of evolutionarily related ion pumps. These enzymes, along with hundreds of known homologs, are referred to as P-type ATPase because they form a key phosphorylated intermediate. In the formation of this intermediate, a phosphoryl group obtained from the hydrolysis of ATP is linked to the side chain of a specific conserved aspartate residue in the ATPase.

The structural and mechanistic function of these enzymes can be examined via Ca^{2+} -ATPase. It is a single 110 kd polypeptide with a trans membrane domain consisting of 10α helices. A large cytoplasmic head piece constitute nearly half the molecular weight of the protein and consists of three distinct cytoplasmic domains with three distinct functions. One domain (N) binds the ATP nucleotide, another (P) domain accepts the phosphoryl group on its conserved aspartate residue, and the third (A) domain may serve as an actuator for the N domain. The relation between these three domains changes significantly on ATP hydrolysis.

The results of mechanistic studies of P-type ATPase have revealed two common features, first, each protein can be phosphorylated to a specific aspartate residue. Second, each pump can interconvert between at least two different conformations, denoted by E_1 and E_2. Thus, at least four conformational status, E_1, E_1-P, E_2-P and E_2 participate in the transport process. From these four states, it is possible to construct a plausible mechanism of action for these enzymes which is provided below-

1. The postulated reaction cycle begins with the binding of ATP and two Ca^{2+} ions to the E1 state.

2. The enzyme cleaves ATP, transferring the γ- phosphoryl group to the key aspartate residue. Calcium must be bound to the enzyme for the phosphorylation to take place. Phosphorylation shifts the conformational equilibrium of the ATPase towards E_2.
3. The transition from the E_1 to E_2 state cause the ion binding sites to evert, so that the ions can dissociate only to the luminal side of the membrane.
4. In the E_2-P state, the enzyme has low affinity for the Ca^{2+} ions, so they are released.
5. With the release of Ca^{2+}, the phosphoaspertate residue is hydrolysed and the phosphate group is released.
6. The enzyme, devoid of a covalently attached phosphoryl group is not stable in the E_2 form. It evert back to the E_1 form, completing the cycle.



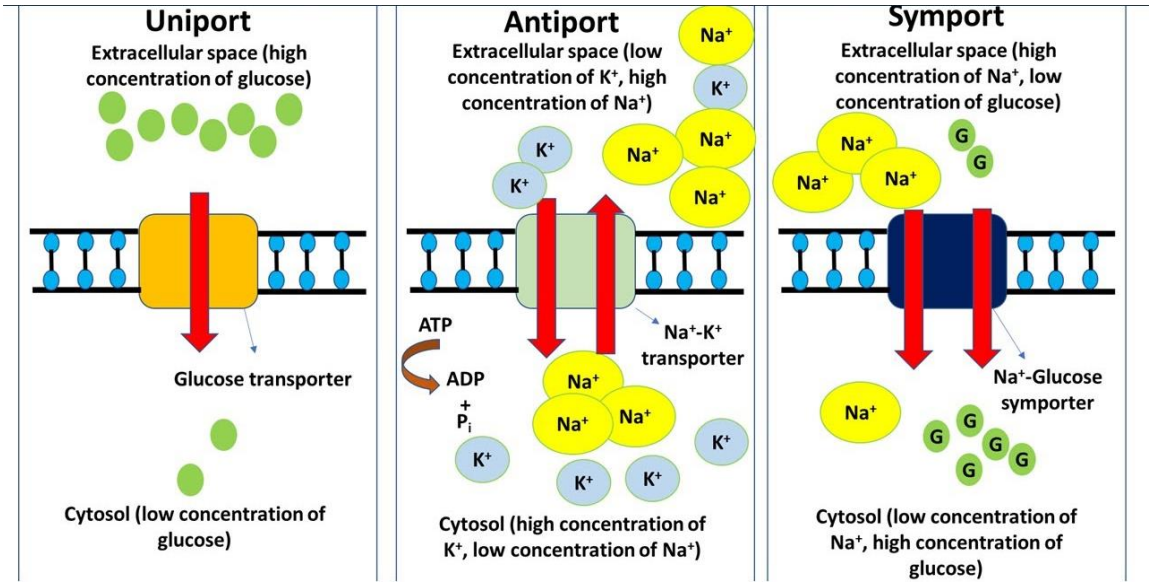
Essentially the same mechanism is employed by the Na^+/K^+ ATPase. The E_1 state binds the three Na^+ ions and transports them across the membrane as a result of the proteins phosphorylation and transition to the E_2 state. The three Na^+ ions are released into the extracellular medium. The E_2 state of the enzyme also binds two K^+ ions, which are carried across the membrane in the opposite direction by eversion driven by the hydrolysis of the phosphoaspertate residue and are released into the cytosol.

Analysis of the complete Yeast genome revealed the presence of 16 proteins that clearly belongs to the P-type ATPase family. All members of this protein family employ the same fundamental mechanism. The free energy of the ATP hydrolysis drives membrane transport by effecting conformational changes associated with the addition and removal of a phosphoryl group at an analogous aspartate site in each protein.

Secondary Active Transport- In plant plasma membrane, only H^+ and Ca^+ appear to be transported by pumps, and the direction of pumping is outward, not inward. Therefore, it is necessary to possess another mechanism to drive the active uptake of most mineral nutrients. The way through which solutes can be actively transported across a membrane against their gradient of electrochemical potential is by coupling the uphill transport of one solute to the downhill transport of another. This type of carrier mediated transport is termed secondary active transport, and it is indirectly driven by pumps.

Membrane proteins that pumps ions or molecules uphill by this means are termed as secondary transporters or cotransporters. These proteins can be classified as either antiporters or symporters. Antiporters couple the downhill flow of one species to the uphill flow of another

in the opposite direction across the membrane; while symporters use the flow of one species to drive the flow of a different species in the same direction across the membrane.



UNIPORT VS SYMPORT VS ANTIPORT

UNIPORT	SYMPORT	ANTIPORT
An integral membrane protein, which transports a single type of substrate species across the cell membrane	Another integral membrane protein involved in the transport of two different molecules in the same direction through the cell membrane	The third type of integral membrane protein involved in the secondary active transport of two different molecules in opposite directions
Types of molecules: One	Types of Molecules: Two	Types of Molecules: Two
Direction of Transport: Single	Direction of Transport: Single	Direction of Transport: Both
Transporter Proteins: Carrier proteins	Transporter Proteins: Cotransporters	Transporter Proteins: Cotransporters
Uses primary active transport	Uses secondary active transport	Uses secondary active transport
Driving Force: ATP	Driving Force: Electrochemical gradient	Driving Force: Electrochemical gradient
Examples: Channel proteins	Examples: Na/glucose symporter	Examples: Na/H antiporter

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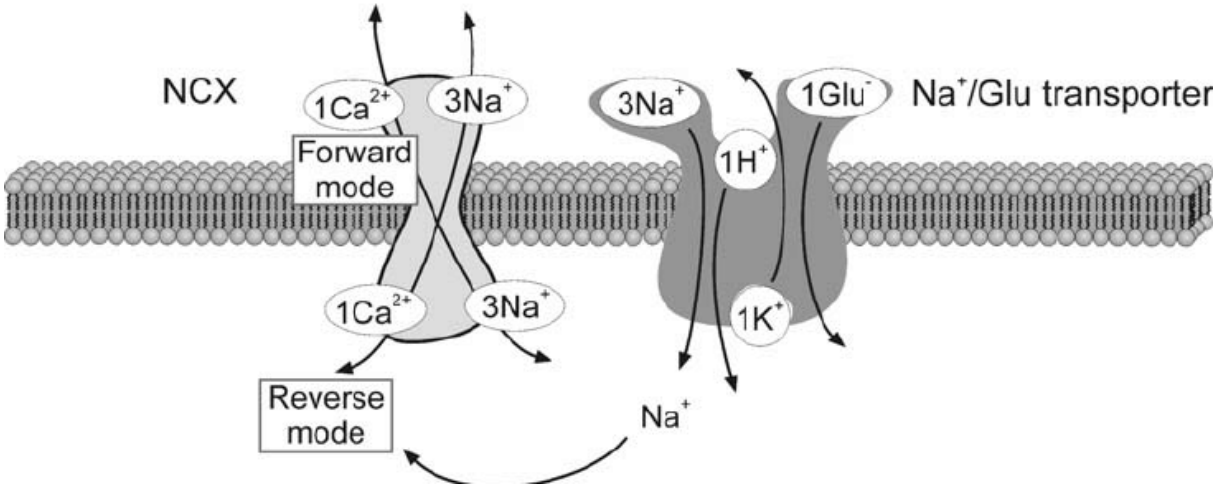
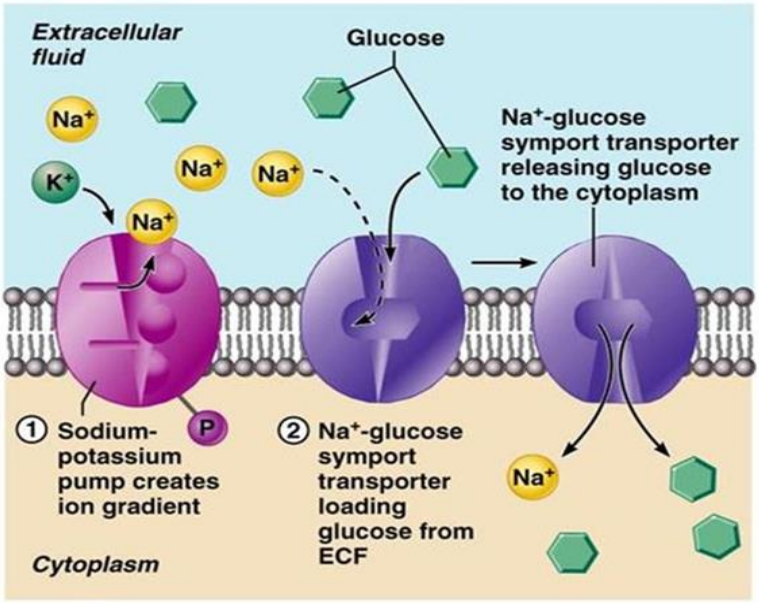
The sodium calcium exchanger in the plasma membrane is an antiporter that uses the electrochemical gradient of Na^+ to pump Ca^{2+} out of the cell. Three sodium ions enter the cell for each Ca^{2+} ion that is extruded. The cost of transport by this exchange is paid by the Na^+/K^+ ATPase pump, which generates the requisite sodium gradient.



NCX= sodium calcium exchanger

Similarly glucose is pumped into the cell by a symporter powered by the simultaneous entry of Na^+. The Na^+/K^+ ATPase pump convers the free energy of phosphoryl transfer into the free energy of a Na^+ ion gradient. The ion gradient can then be used to pump glucose into the cell through the action of a secondary transporter.



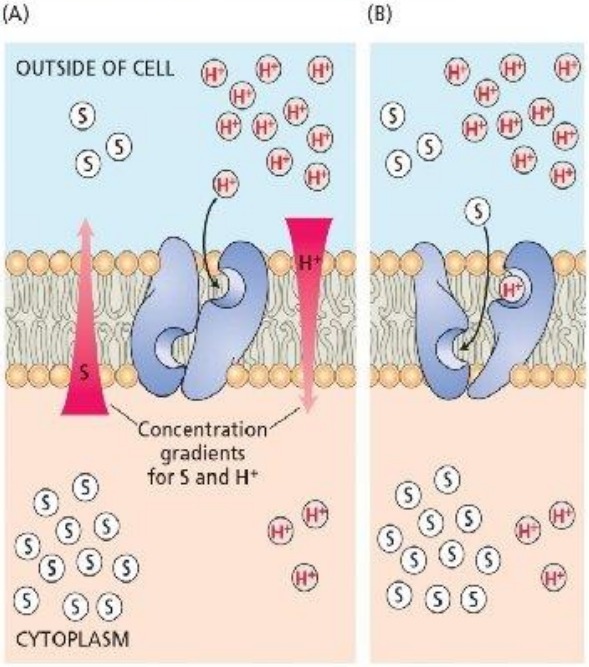
A hypothetical model for the operational mechanism of secondary active transport can be depicted as follows-

1. The energy that drives the process has been stored in a protein gradient and is being used to take up a substrate(s) against its concentration gradient.

2. In the initial conformation, the binding sites of the protein are exposed to the outside environment and can bind a protein.
3. This binding results in a conformational change that permits a molecule of substrate(s) to be bound.
4. The binding of substrate causes another conformational change that exposes the binding sites and their substrates to the inside of the cell.
5. Release of a proton and a molecule of the substrate to the cells interior restores the original conformation of the carrier and allows a new pumping cycle to begin.

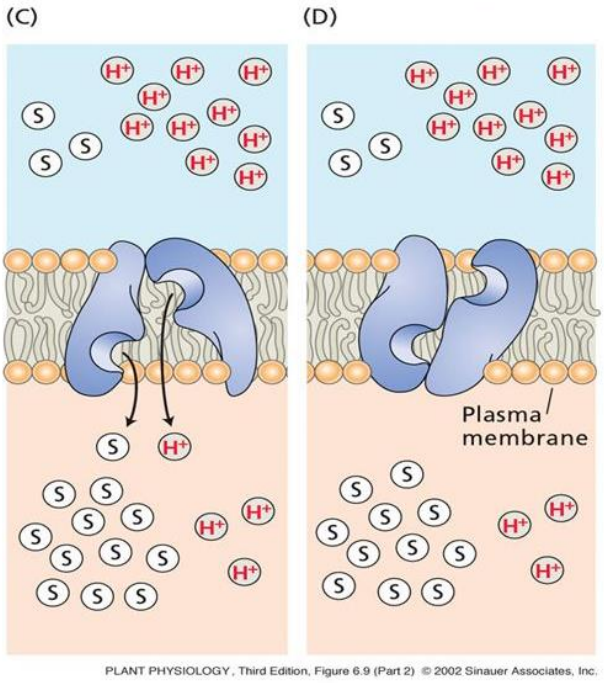
pump-mediated transport against the gradient (secondary active transport)

- ***Involves the coupling of the uphill transport of a molecule with the downhill transport of another***
- (A) the initial conformation allows a proton from outside to bind to pump protein
- (B) Proton binding alters the shape of the protein to allow the molecule [S] to bind



- (C) The binding of the molecule [S] again alters the shape of the pump protein. This exposes the both binding sites, and the proton and molecule [S] to the inside of the cell

- (D) This release restores both pump proteins to their original conformation and the cycle begins again



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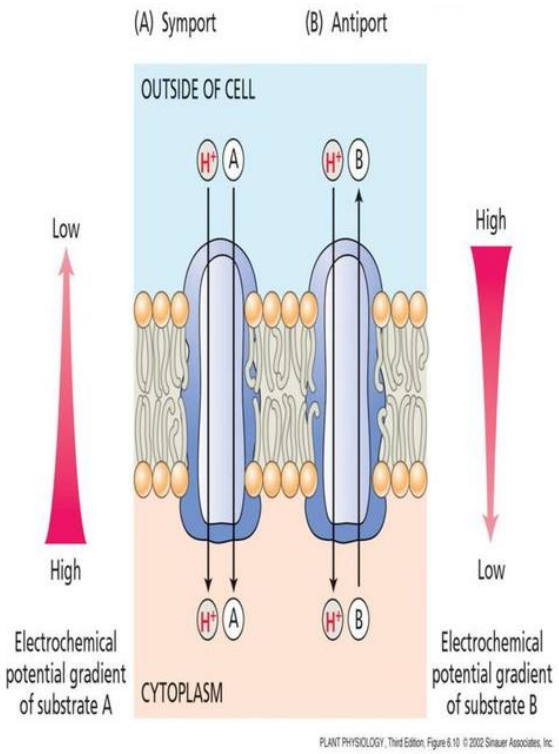
- The proton gradient required for secondary active transport is provided by the activity of the electrogenic pumps
- Membrane potential contributes to secondary active transport
- Passive transport with respect to H+ (proton)



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Uniport- Uniporter is an integral membrane protein which is involved in facilitated diffusion. They can be either a channel or a carrier protein. Uniporter carrier proteins work by binding to one molecule of solute at a time and transporting it with the solute gradient. Uniporter channels open in response to a stimulus and allow the free flow of specific molecules. Uniporters are slower than channels because they mediate a more complicated process. The transported substrate both binds to the uniporter and elicit a conformational change in the transporter. A uniporter transports one substrate molecule at a time, while channel proteins form a protein

linked passageway through which multiple water molecules or ions can move simultaneously at a rapid rate. Thus uniporters mediate substrate specific facilitated diffusion.

Uniport can be distinguished from the primary transport in following three points-

1. The rate of facilitated transport by uniporter is far slower than passive diffusion
2. Uniport transport is specific while passive diffusion is non-specific. Each uniporter transports only single species of molecules or a single group of closely related molecules.
3. Uniport transport occurs via a limited number of uniporter molecules, rather than throughout the phospholipid bilayer.