MEMBRANE BIOCHEMISTRY (BCH 320)

CELL MEMBRANES

The plasma membrane/ cell membrane is the edge of life, the boundary that separates the living cell from its surroundings. Cell membrane = Plasma membrane = Plasmalemma.
Membranes are barriers that give cells their outer boundaries (plasma membranes) and their inner compartments (organelles).

The functions of membranes can be broadly categorized as highlighted below:

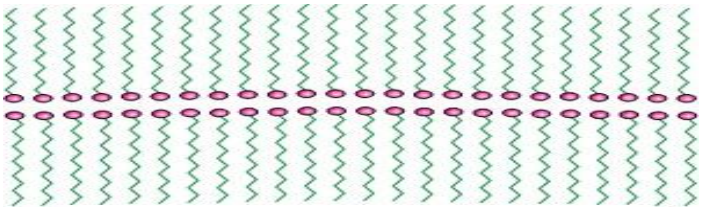
1. Membranes because they are selectively permeable control the movement of substances into and out of the cells thereby regulating the composition of the fluid within an individual cell
2. Membranes control the flow of information between cells either by recognizing signals received from other cells or by sending chemical or electrical signals to other cells.
3. Membranes are involved in the capture and release of energy-photosynthesis and oxidative phosphorylation takes place on membranes.

The boundaries of cells are formed by *biological membranes*, the barriers that *define the inside and the outside of a cell.* These barriers prevent molecules generated inside the cell from leaking out and unwanted molecules from diffusing in; yet they also contain transport systems that allow specific molecules to be taken up and unwanted compounds to be removed from the cell. Such transport systems confer on membranes the important property of *selective permeability.*
Membranes are dynamic structures in which proteins float in a sea of lipids. The lipid components of the membrane form the permeability barrier, and protein components act as a transport system of p u m p s and channels that endow (give) the membrane its selective permeability.
In addition to an external cell membrane (called the plasma membrane), eukaryotic cells also contain internal membranes that form the boundaries of organelles such as mitochondria, chloroplasts, peroxisomes, and lysosomes. Functional specialization in the course of evolution has been closely linked to the formation of such compartments. Specific systems have evolved to

allow targeting of selected proteins into or through particular internal membranes and, hence, into specific organelles. External and internal membranes have essential features in common.

Biological membranes serve several additional important functions indispensable for life, such as energy storage and information transduction, that are dictated by the proteins associated with them.

Below is the representative of a bilayer formed by detergent molecules.



Note - The polar heads (ball) pack together leaving the hydrophobic groups (tail) in contact with air on the inside and outside of the bubble.

COMMON FEATURES OF BIOLOGICAL MEMBRANES

Membranes are as diverse in structure as they are in function. However, they do have in common a number of important attributes:

1. Membranes are *sheet-like structures*, only two molecules thick, that form *closed boundaries* between different compartments. The thickness of most membranes is between 60 Å (6 nm) and 100 Å (10 nm).

2. Membranes consist mainly of *lipids* and *proteins*. Their mass ratio ranges from 1:4 to 4:1. Membranes also contain *carbohydrates* that are linked to lipids and proteins.

3. Membrane lipids are relatively small molecules that have both *hydrophilic* and *hydrophobic* moieties (each of two parts into which a thing is or can be divided). These lipids spontaneously form *closed bimolecular sheets* in aqueous media. These *lipid bilayers* are barriers to the flow of polar molecules.

4. *Specific proteins mediate distinctive functions of membranes*. Proteins serve as pumps, channels, receptors, energy transducers, and enzymes. Membrane proteins are embedded in lipid bilayers, which create suitable environments for their action.

5. Membranes are *noncovalent assemblies*. The constituent protein and lipid molecules are held together by many noncovalent interactions, which are cooperative.

6. Membranes are *asymmetric*. The two faces of biological membranes always differ from each other.

7. Membranes are *fluid structures*. Lipid molecules diffuse rapidly in the plane of the membrane, as do proteins unless they are anchored by specific interactions. In contrast, lipid molecules and proteins do not readily rotate across the membrane. Membranes can be regarded as *two-dimensional solutions of oriented proteins and lipids*.

8. Most cell membranes are *electrically polarized*, such that the inside is negative [typically - 60 millivolts (mV)].

9. Membrane potential plays a key role in transport, energy conversion, and excitability (that occur in retinal rod cells i.e. visual excitation).

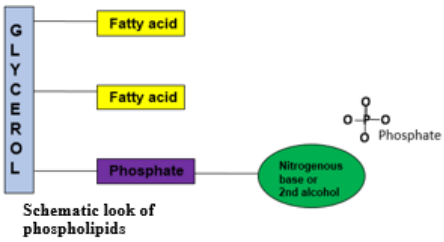
MEMBRANE LIPIDS

Among the most biologically significant properties of lipids are their hydrophobic properties. These properties are mainly due to a particular component of lipids: fatty acids, or simply fats. Fatty acids also play important roles in signal transduction pathways.

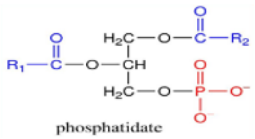
The properties of fatty acids and lipids derived from them are markedly dependent on chain length and degree of saturation. Unsaturated fatty acids have lower melting points than saturated fatty acids of the same length. For example, the melting point of stearic acid is 69.6°C, whereas that of oleic acid (which contains one cis double bond) is 13.4°C. The melting points of polyunsaturated fatty acids of the C18 series are even lower. Chain length also affects the melting point, as illustrated by the fact that the melting temperature of palmitic acid (C16) is 6.5 degrees lower than that of stearic acid (C18). Thus, *short chain length and unsaturation enhance the fluidity of fatty acids and water-insoluble of their derivatives.*

Lipids differ markedly from the other groups of biomolecules. By definition, *lipids are water insoluble biomolecules that are highly soluble in organic solvents such as chloroform.* Lipids have a variety of biological roles: they serve as fuel molecules, highly concentrated energy stores, signal molecules, and components of membranes. The three major kinds of membrane lipids are *phospholipids*, *glycolipids*, and *cholesterol.*

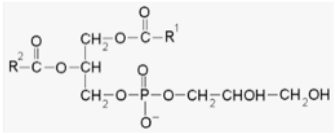
1. **Phospholipids -** *Phospholipids* are abundant in all biological membranes. A phospholipid molecule is constructed from four components: fatty acids, a platform to which the fatty acids are attached, a phosphate, and an alcohol attached to the phosphate. The fatty acid components provide a hydrophobic barrier, whereas the remainder of the molecule has hydrophilic properties to enable interaction with the environment.



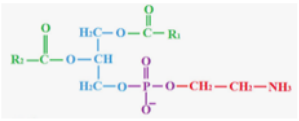
Schematic look of phospholipids



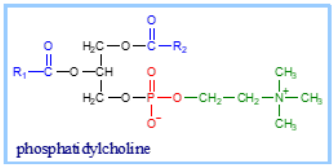
phosphatidate



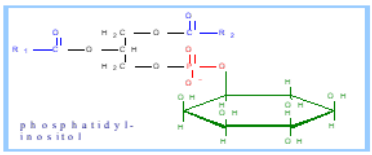
Phosphatidylglycerol



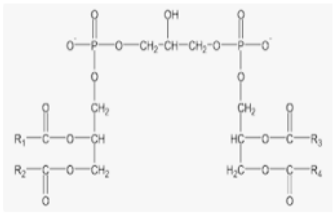
Phosphatidylethanolamine



phosphatidylcholine



phosphatidyl-inositol

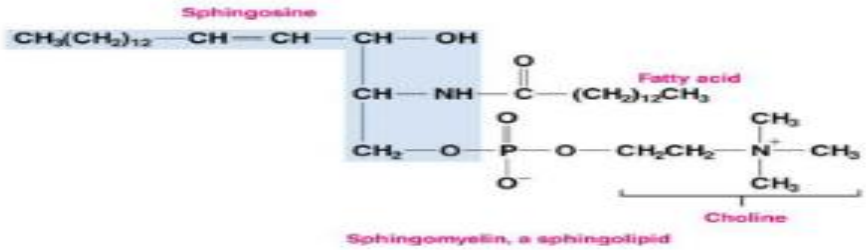


Diphosphatidylglycerol

The platform on which phospholipids are built may be *glycerol*, a 3- carbon alcohol, or *sphingosine*, a more complex alcohol. Phospholipids derived from glycerol are called *phosphoglycerides.* A phosphoglyceride consists of a glycerol backbone to which two fatty acid chains and a phosphorylated alcohol are attached.

In phosphoglycerides, the hydroxyl groups at C-1 and C-2 of glycerol are esterified to the carboxyl groups of the two fatty acid chains. The C-3 hydroxyl group of the glycerol backbone is esterified to phosphoric acid. When no further additions are made, the resulting compound is *phosphatidate (diacylglycerol 3-phosphate)*, the simplest phosphoglyceride. Only small amounts of phosphatidate are present in membranes. The major phosphoglycerides are derived from phosphatidate by the formation of an ester bond between the phosphate group of phosphatidate and the hydroxyl group of one of several alcohols. The common alcohol moieties of phosphoglycerides are the amino alchohol serine, ethanolamine, choline, glycerol, and inositol. ***Sphingomyelin*** is a phospholipid or sphingolipid found in membranes that is not derived from glycerol. Instead, the backbone in sphingomyelin is *sphingosine*, an amino alcohol that contains a long, unsaturated hydrocarbon chain. In sphingomyelin, the amino group of the sphingosine backbone is linked to a fatty acid by an amide bond. In addition, the primary hydroxyl group of sphingosine is esterified to phosphorylcholine.

- **Sphingolipids are phospholipids that have an amide bond between a fatty acid and sphingosine, an 18-carbon alcohol.**

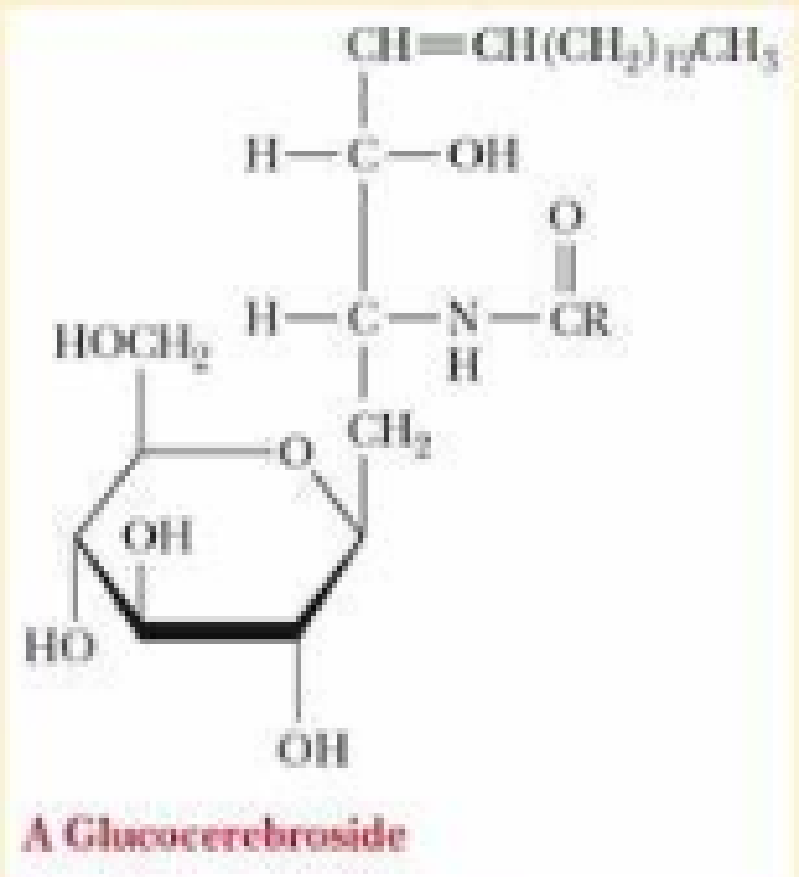


Note- The membranes of archaea differ in composition from those of eukaryotes or bacteria in three important ways. Two of these differences relate to the hostile living conditions of many archaea. First, the nonpolar chains are joined to a glycerol backbone by *ether* rather than ester linkages. The ether linkage is more resistant to hydrolysis. Second, the alkyl chains are *branched* rather than linear. They are built up from repeats of a fully saturated five-carbon

fragment. These branched, saturated hydrocarbons are more resistant to oxidation. The ability of archaeal lipids to resist hydrolysis and oxidation may help these organisms to withstand the extreme conditions, such as high temperature, low pH, or high salt concentration, under which some of these archaea grow. Finally, the stereochemistry of the central glycerol is inverted compared with that of eukaryotes or bacteria.

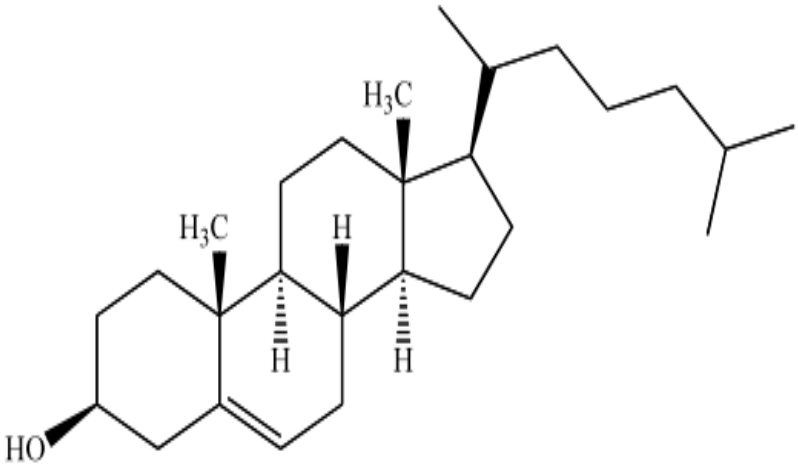
2. **Glycolipids**

Glycolipids, as their name implies, are *sugar-containing lipids.* Like sphingomyelin, the glycolipids in animal cells are derived from sphingosine. The amino group of the sphingosine backbone is acylated by a fatty acid, as in sphingomyelin. Glycolipids differ from sphingomyelin in the identity of the unit that is linked to the primary hydroxyl group of the sphingosine backbone. In glycolipids, one or more sugars (rather than phosphoryl choline) are attached to this group. The simplest glycolipid, called a *cerebroside,* contains a single sugar residue, either glucose or galactose More complex glycolipids, such as *gangliosides*, contain a branched chain of as many as seven sugar residues. Glycolipids are oriented in a completely asymmetric fashion with the *sugar residues always on the extracellular side of the membrane as shown below:*



A Glucocerebroside

3. **Cholesterol -** *Cholesterol* is a lipid with a structure quite different from that of phospholipids. It is a steroid, built from four linked hydrocarbon rings.



Cholesterol

A hydrocarbon tail is linked to the steroid at one end, and a hydroxyl group is attached at the other end. In membranes, the molecule is oriented parallel to the fatty acid chains of the phospholipids, and the hydroxyl group interacts with the nearby phospholipid head groups. Cholesterol is absent from prokaryotes but is found to varying degrees in virtually all animal

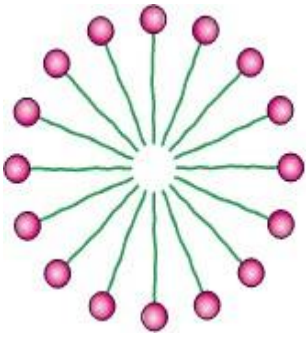
membranes. It constitutes almost 25% of the membrane lipids in certain nerve cells but is essentially absent from some intracellular membranes.

Properties that enable phospholipids to form membranes - *Membrane formation is a consequence of the amphipathic nature of the molecules.* Their polar head groups favor contact with water, whereas their hydrocarbon tails interact with one another, in preference to water. How can molecules with these preferences arrange themselves in aqueous solutions?

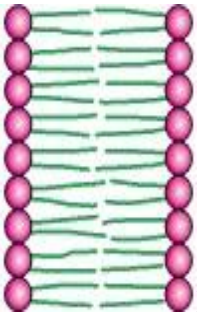
One way is to form a micelle, a globular structure in which polar head groups are surrounded by water and hydrocarbon tails are sequestered inside, interacting with one another, ionized fatty acids readily form such structure, but most phospholipids do not. Alternatively, the strongly opposed preferences of the hydrophilic and hydrophobic moieties of membrane lipids can be satisfied by forming a *lipid bilayer*, composed of two lipid sheets. A lipid bilayer is also called a *bimolecular sheet*. The hydrophobic tails of each sheet interact with one another, forming a hydrophobic interior that acts as a permeability barrier. The hydrophilic head groups interact with the aqueous medium on each side of the bilayer. The two opposing sheets are called leaflets. *The favored structure for most phospholipids and glycolipids in aqueous media is a bimolecular sheet rather than a micelle.* The reason is that the two fatty acyl chains of a phospholipid or a glycolipid are too bulky to fit into the interior of a micelle. In contrast, salts of fatty acids (such as sodium palmitate, a constituent of soap), which contain only one chain, readily form micelles. *The formation of bilayers instead of micelles by phospholipids is of critical biological importance.*

A micelle is a limited structure, usually less than 20 nm (200 Å) in diameter. In contrast, a bimolecular sheet can have macroscopic dimensions, such as a millimeter (106 nm, or 107 Å). Phospholipids and related molecules are important membrane constituents because they readily form extensive bimolecular sheets. The formation of lipid bilayers is a *self-assembly process.* In other words, the structure of a bimolecular sheet is inherent in the structure of the constituent lipid molecules. The growth of lipid bilayers from phospholipids is a rapid and spontaneous process in water. *Hydrophobic interactions are the major driving force for the formation of lipid bilayers*. Water molecules are released from the hydrocarbon tails of membrane lipids as these tails become sequestered in the nonpolar interior of the bilayer. Furthermore, *van der Waals attractive forces between the hydrocarbon tails favor close packing of the tails*. Finally, there are *electrostatic and hydrogen-bonding attractions between the polar head groups and water molecules.* Thus, lipid bilayers are stabilized by the

full array of forces that mediate molecular interactions in biological systems. These hydrophobic interactions have three significant biological consequences: (1) lipid bilayers have an inherent tendency to be *extensive (large in size, containing or dealing with a lot of information and details)*; (2) lipid bilayers will tend to *close on themselves* so that there are no edges with exposed hydrocarbon chains, and so they form compartments; and (3) lipid bilayers are *self- sealing* because a hole in a bilayer is energetically unfavorable.



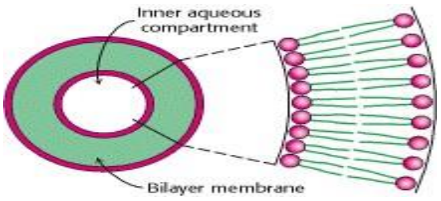
1 Diagram of a Micelle



2 Diagram of a bilayer Membrane

[1]

SYNTHETIC MEMBRANES - The propensity (tendency to behave in a particular way) of phospholipids to form membranes has been used to create an important experimental and clinical tool. *Lipid vesicles*, or *liposomes*, aqueous compartments enclosed by a lipid bilayer can be used to study membrane permeability or to deliver chemicals to cells. Liposome- is a membrane bound vesicle, frequently found by dispersion of phospholipid in aqueous salt solutions (i.e. a small aqueous compartment surrounded by a lipid bilayer). Another well-defined synthetic membrane is a *planar bilayer membrane.*



Liposome

Membrane permeability coefficients (P) - The permeability coefficient is a measure of the ability of an ion or small molecule to diffuse across a lipid bilayer. That is the net number of molecules of solute that can cross each square centimeter of a membrane in a unit time. *Lipid bilayer membranes have a very low permeability for ions and most polar molecules.* Water is a conspicuous exception to this generalization; it readily traverses such membranes because of its small size, high concentration, and lack of a complete charge.

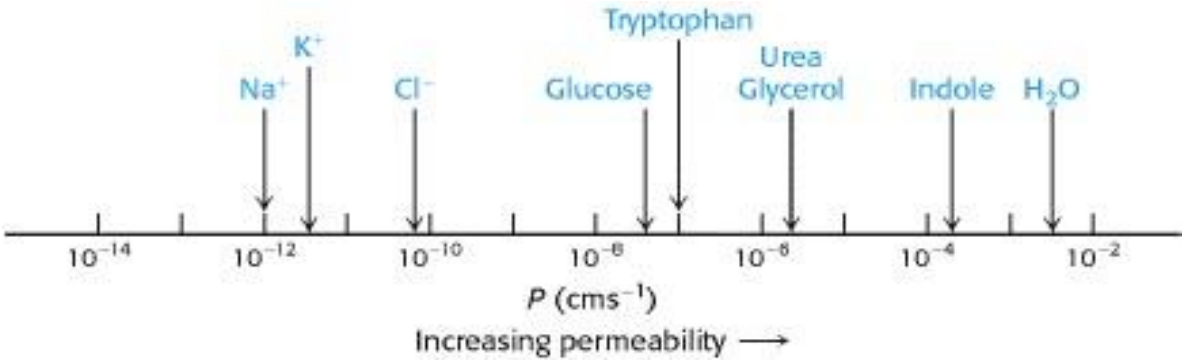


Diagram showing Permeability Coefficients (*P*) of Ions and Molecules in a Lipid Bilayer

The ability of molecules to cross a lipid bilayer spans a wide range of values.

MEMBRANE PROTEINS

Membrane proteins are responsible for most of the dynamic processes carried out by membranes. Membrane lipids form a permeability barrier and thereby establish compartments, whereas *specific proteins mediate nearly all other membrane functions.* In particular, proteins transport chemicals and information across a membrane. Membrane lipids create the appropriate environment for the action of such proteins.

Membranes differ in their protein content. Myelin, a membrane that serves as an insulator around certain nerve fibers, has a low content of protein (18%). Relatively pure lipids are well-suited for insulation. In contrast, the plasma membranes or exterior membranes of most other cells are much more active. They contain many pumps, channels, receptors, and enzymes. The protein content of these plasma membranes is typically 50%. Energy-transduction membranes, such as the internal membranes of mitochondria and chloroplasts, have the highest content of protein, typically 75%.

The protein components of a membrane can be readily visualized by *SDS-polyacrylamide gel electrophoresis.* The electrophoretic mobility of many proteins in SDS-containing gels depends on the mass rather than on the net charge of the protein. The gel-electrophoresis patterns the plasma membrane of erythrocytes, the photoreceptor membrane of retinal rod cells, and the sarcoplasmic reticulum membrane of muscle showed that each of these three membranes contains many proteins but has a distinct protein composition. In general, *membranes performing different functions contain different types of proteins.*

Six major functions performed by proteins of the plasma membrane are;

a) **Transport -** A protein that spans the membrane may provide a hydrophilic channel across the membrane that is selective for a particular solute. Other transport proteins shuttle a substance from one side to the other by changing shape. Some of these proteins hydrolyze ATP as an energy source to actively pump substances across the membrane.

b) **Enzymatic activity -** A protein built into the membrane may be an enzyme with its active site exposed to substances in the adjacent solution. In some cases, several enzymes in a membrane are organized as a team that carries out sequential steps of a metabolic pathway.

c) **Signal transduction -** A membrane protein (receptor) may have a binding site with a specific shape that fits the shape of a chemical messenger, such as a hormone. The external messenger (signaling molecule) may cause the protein to change shape, allowing it to relay the message to the inside of the cell, usually by binding to a cytoplasmic protein.

d) **Cell-cell recognition -** Some glycoproteins serve as identification tags that are specifically recognized by membrane proteins of other cells. This type of cell-cell binding is usually short-lived compared to that shown in (e).

e) **Intercellular joining -** Membrane proteins of adjacent cells may hook together in various kinds of junctions, such as gap junctions or tight junctions. This type of binding is more long-lasting than that shown in (d).

f) Attachment to the cytoskeleton and extracellular matrix (ECM) - Microfilaments or other elements of the cytoskeleton may be non-covalently bound to membrane proteins, a function that helps maintain cell shape and stabilizes the location of certain membrane proteins. Proteins that can bind to cell membrane molecules can coordinate extracellular and intracellular changes.

The ease with which a protein can be dissociated from a membrane indicates how intimately it is associated with the membrane. Some membrane proteins can be solubilized by relatively mild means, such as extraction by a solution of high ionic strength (e.g., 1 M NaCl). Other membrane proteins are bound much more tenaciously (determine to do something and unwilling to stop even when the situation becomes difficult); they can be solubilized only by using a detergent or an organic solvent. Membrane proteins can be classified as being either *peripheral* or *integral* on the basis of this difference in dissociability. *Integral membrane proteins* interact extensively with

the hydrocarbon chains of membrane lipids, and so only agents that compete for these nonpolar interactions can release them. In fact, most integral membrane proteins span the lipid bilayer. In contrast, *peripheral membrane proteins* are bound to membranes primarily by electrostatic and hydrogen-bond interactions with the head groups of lipids. These polar interactions can be disrupted by adding salts or by changing the pH. Many peripheral membrane proteins are bound to the surfaces of integral proteins, on either the cytosolic or the extracellular side of the membrane. Others are anchored to the lipid bilayer by a covalently attached hydrophobic chain, such as a fatty acid. The structures of membrane proteins differ from those of soluble proteins with regard to the distribution of hydrophobic and hydrophilic groups which make them more difficult to purify and crystalline than water soluble proteins.

The two important features of membrane protein structure are first, the parts of the protein that interact with the hydrophobic parts of the membrane are coated with nonpolar amino acid side chains, whereas those parts that interact with the aqueous environment are much more hydrophilic. Second, the structures positioned within the membrane are quite regular and, in particular, all backbone hydrogen-bond donors and acceptors participate in hydrogen bonds. *Breaking a hydrogen bond within a membrane is quite unfavorable, because little or no water is present to compete for the polar groups.*

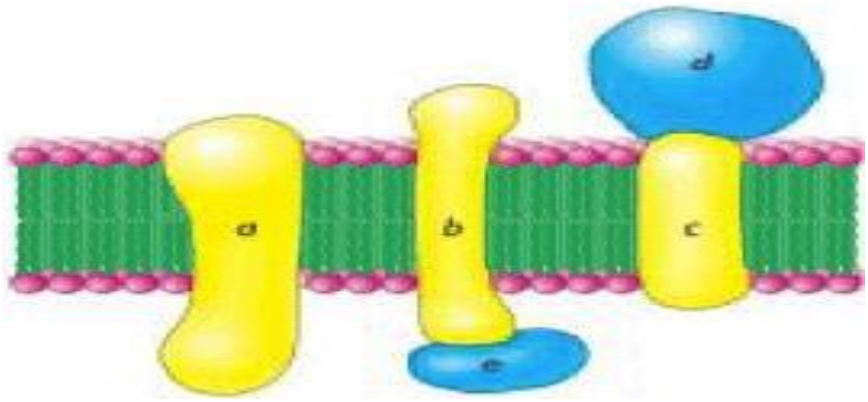


Diagram showing Integral and Peripheral Membrane Proteins. Integral membrane proteins (*a*, *b*, and *c*) interact extensively with the hydrocarbon region of the bilayer. Nearly all known integral membrane proteins traverse the lipid bilayer. Peripheral membrane proteins (*d* and *e*) bind to the surfaces of integral proteins. Some peripheral membrane proteins interact with the polar head groups of the lipids (not shown).

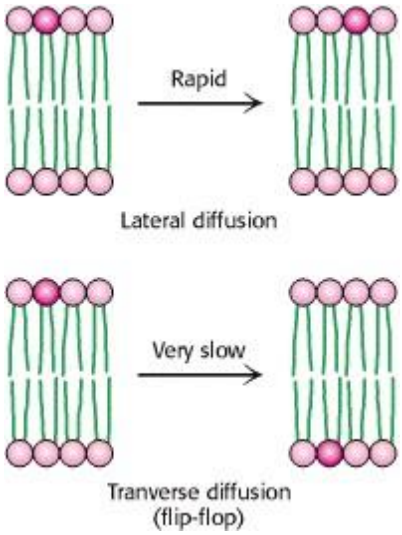
MEMBRANE ANCHORS **-** Membrane anchors are hydrophobic groups that are covalently attached to proteins and aid the binding of proteins to the membrane.

Sometime soluble proteins can associate with membranes if the association is mediated by hydrophobic groups attached to the proteins. Three such groups are (1) a palmitoyl group attached to a specific cysteine residue by a thioester bond, (2) a farnesyl group attached to a cysteine residue at the carboxyl terminus, and (3) a glycolipid structure termed a glycosyl phosphatidyl inositol (GPI) anchor attached to the carboxyl terminus of the hydrophobic amino acid side chain of the protein. These modifications are attached by enzyme systems that recognize specific signal sequences near the site of attachment.

FLUID MOSAIC MODEL

On the basis of the dynamic properties of proteins in membranes, the *fluid mosaic model* for the overall organization of biological membranes was proposed in 1972. The essence of this model is that *membranes are two-dimensional solutions of oriented lipids and globular proteins.* The lipid bilayer has a dual role: (1) it is both a *solvent* for integral membrane proteins and (2) a *permeability barrier.* Membrane proteins are free to diffuse laterally in the lipid matrix unless restricted by special interactions. Biological membranes are not rigid, static structures. On the contrary, lipids and many membrane proteins are constantly in lateral motion, a process called *lateral diffusion* (The rapid motion of lipid or protein molecules within the plane of one leaflet of a lipid bilayer). Although the lateral diffusion of membrane components can be rapid, the spontaneous rotation of lipids from one face of a membrane to the other is a very slow process. The transition of a molecule from one membrane surface to the other is called *transverse diffusion* or *flip-flop (*This is the passage of liquid molecules from one leaflet of a lipid bilayer to other leaflet). The flip-flop of a protein molecule has not been observed because some proteins are often held in place by cytoskeletal filaments. Hence, *membrane asymmetry can be preserved for long periods.*

Experiment carried out to confirm lateral movement of membrane protein- Larry Frye and Michael Edidin, at Johns Hopkins University, labeled the plasma membrane proteins of a mouse cell and a human cell with two different markers and fused the cells. Using a microscope, they observed the markers on the hybrid cell. The mixing of the mouse and human membrane proteins indicates that at least some membrane proteins move sideways within the plane of the plasma membrane. **SOURCE-** L. D. Frye and M. Edidin, The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons, *Journal of Cell Science* 7:319 (1970).



Lipid Movement in Membranes. Lateral diffusion of lipids is much more rapid than transverse diffusion (flip-flop).

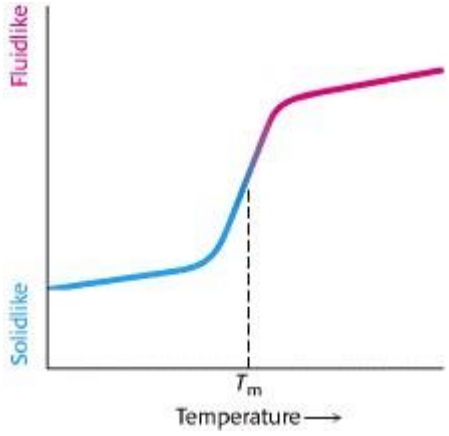
MEMBRANE FLUIDITY

Many membrane processes, such as transport or signal transduction, depend on the fluidity of the membrane lipids, which in turn depends on the properties of fatty acid chains, which can exist in an ordered, rigid state or in a relatively disordered, fluid state. The transition from the rigid to the fluid state occurs rather abruptly as the temperature is raised above T_m, the melting temperature. *This transition temperature depends on the length of the fatty acyl chains and on their degree of unsaturation*. The presence of saturated fatty acyl residues favors the rigid state because their straight hydrocarbon chains interact very favorably with each other. On the other hand, *a cis double bond produces a bend in the hydrocarbon chain. This bend interferes with a highly ordered packing of fatty acyl chains, and so* T_m *is lowered*. The length of the fatty acyl chain also affects the transition temperature. Long hydrocarbon chains interact more strongly than do short ones. That is the longer the chain the higher the melting temperature.

Bacteria regulate the fluidity of their membranes by varying the number of double bonds and the length of their fatty acyl chains. For example, the ratio of saturated to unsaturated fatty acyl chains in the *E. coli* membrane decreases from 1.6 to 1.0 as the growth temperature is lowered from 42°C to 27°C. This decrease in the proportion of saturated residues prevents the membrane from becoming too rigid at the lower temperature.

In animals, cholesterol is the key regulator of membrane fluidity. Cholesterol contains a bulky steroid nucleus with a hydroxyl group at one end and a flexible hydrocarbon tail at the other end. Cholesterol inserts into bilayers with its long axis perpendicular to the plane of the membrane.

The hydroxyl group of cholesterol forms a hydrogen bond with a carbonyl oxygen atom of a phospholipid head group, whereas the hydrocarbon tail of cholesterol is located in the nonpolar core of the bilayer. The different shape of cholesterol compared with phospholipids disrupts the regular interactions between fatty acyl chains. In addition, cholesterol appears to form specific complexes with some phospholipids. Such complexes may concentrate in specific regions within membranes. One result of these interactions is the *moderation of membrane fluidity*, making membranes less fluid but at the same time less subject to phase transitions.



The Phase-Transition, or Melting, Temperature (Tm) for a Phospholipid Membrane. As the temperature is raised, the phospholipid membrane changes from a packed, ordered state to a more random one.

Table showing the melting temperature of phosphatidyl choline containing different pairs of identical fatty acid chains

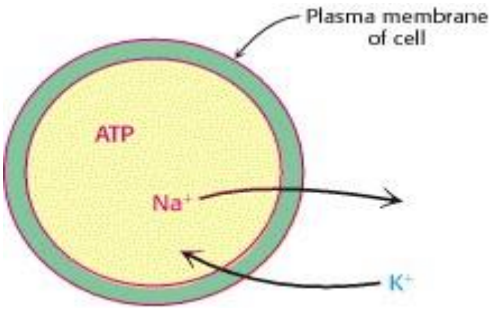
Number of carbons	Number of double bond	Common name of fatty acid	Systematic name	Tm (C)
22	0	Behenate	*n*-Docosanote	75
18	0	Stearate	*n*-Octadecanoate	58
16	0	Palmitate	*n*-Hexadecanoate	41
14	0	Myristate	*n*-Tetradecanoate	24
18	1	Oleate	*Cis*-Δ^9-Octadecenoate	22

MEMBRANES ARE ASYMMETRIC

Membranes are structurally and functionally asymmetric. The outer and inner surfaces of *all known biological membranes have different components and different enzymatic activities.* A

clear-cut example is the pump that regulates the concentration of Na^+ and K^+ ions in cells. This transport protein is located in the plasma membrane of nearly all cells in higher organisms. The Na^+-K^+ pump is oriented so that it pumps Na^+ out of the cell and K^+ into it. Furthermore, ATP must be on the inside of the cell to drive the pump. Ouabain, a specific inhibitor of the pump, is effective only if it is located outside.

Membrane proteins have a unique orientation because they are synthesized and inserted into the membrane in an asymmetric manner. This absolute asymmetry is preserved because membrane proteins do not rotate from one side of the membrane to the other and because *membranes are always synthesized by the growth of preexisting membranes.* Lipids, too, are asymmetrically distributed as a consequence of their mode of biosynthesis, but this asymmetry is usually not absolute, except for glycolipids. For instance, in the red-blood-cell membrane, sphingomyelin and phosphatidyl choline are preferentially located in the outer leaflet of the bilayer, whereas phosphatidyl ethanolamine and phosphatidyl serine are located mainly in the inner leaflet. Large amounts of cholesterol are present in both leaflets.



Asymmetry of the Na^+ - K^+ transport system in plasma membranes. The Na^+-K^+ transport system pumps Na^+ out of the cell and K^+ into the cell.

ASSIGNMENT – Given RBC, explain how to isolate and characterizatize it membrane.

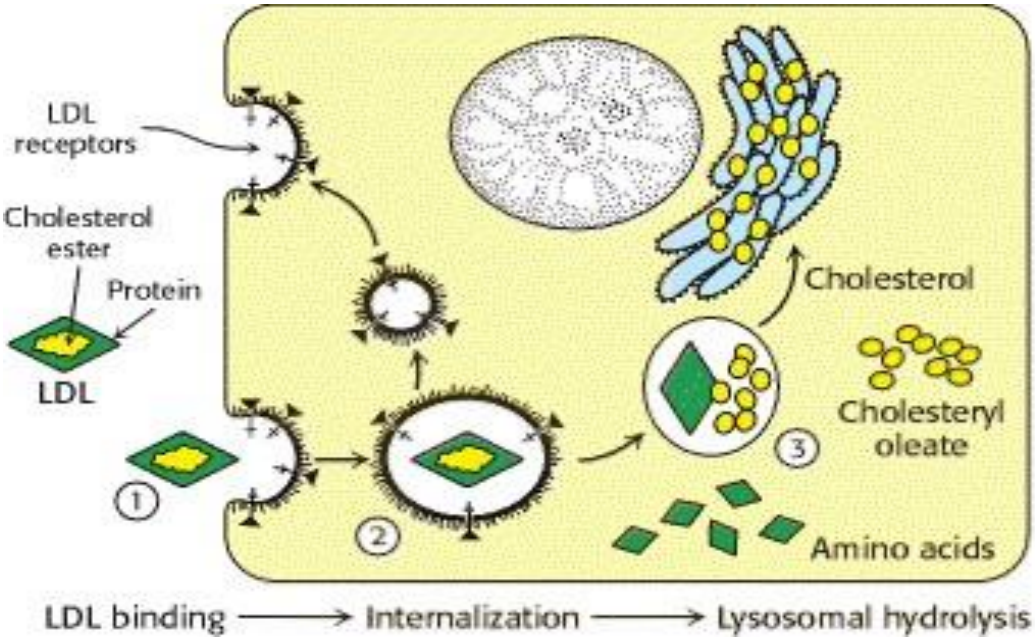
MEMBRANE BUDDING AND FUSION: Membranes must be able to separate or join together to take up, transport, and release molecules. Many take up molecules through the process of *receptor- mediated endocytosis*. Here, a protein or larger complex initially binds to a receptor on the cell surface. After the protein is bound, specialized proteins act to cause the membrane in the vicinity of the bound protein to invaginate. The invaginated membrane eventually breaks off and fuses to form a *vesicle.*

Receptor-mediated endocytosis plays a key role in cholesterol metabolism. Some cholesterol in the blood is in the form of a lipid-protein complex called *low-density lipoprotein* (LDL). Low

density lipoprotein binds to an LDL receptor, an integral membrane protein. The segment of the plasma membrane containing the LDL-LDL receptor complex then invaginates and buds off from the membrane. The LDL separates from the receptor, which is recycled back to the membrane in a separate vesicle. The vesicle containing the LDL fuses with a *lysosome*, an organelle containing an array of digestive enzymes. The cholesterol is released into the cell for storage or use in membrane biosynthesis, and the remaining protein components are degraded. Various hormones, transport proteins, and antibodies employ receptor-mediated endocytosis to gain entry into a cell. A less advantageous consequence is that this pathway is available to viruses and toxins as a means of entry into cells. The reverse process the fusion of a vesicle to a membrane is a key step in the release of neurotransmitters from a neuron into the synaptic cleft.

Synapse: the minute gap across which nerve impulse pass from one neuron to the next, at the end of a nerve fibre. Reaching a synapse, an impulse causes the release of a neurotransmitter, which diffuses across the gap and triggers an electrical impulse in the next neuron; some brain cells have more than 15,000 synapses.

Diagram below showing the Receptor-Mediated Endocytosis -The process of receptor-mediated endocytosis is illustrated for the cholesterol-carrying complex, low-density lipoprotein (LDL): (1) LDL binds to a specific receptor, the LDL receptor; (2) this complex invaginates to form an internal vesicle; (3) after separation from its receptor, the LDL-containing vesicle fuses with a lysosome, leading to degradation of the LDL and release of the cholesterol.



TRANSPORT ACROSS MEMBRANE

No cell exists as a closed system. In order to survive, materials must enter and leave the cell through the plasma membrane. Because different processes take place in different parts of the cell, materials must be transported from one part of the cell to another.

Passive transport: During passive transport, substances move according to their own natural tendency without an input of energy from the cell. No ATP is required.

Diffusion: Diffusion is the net movement of a substance from an area where it has a higher concentration to an area where it has a lower concentration i.e. down its concentration gradient. It is caused by the constant random motion of all atoms and molecules. In addition to simple diffusion, there are 3 special types of diffusion that involve movement of materials across a semipermeable membrane: dialysis, osmosis and facilitated diffusion. Dialysis refers to the diffusion of solutes across a semipermeable membrane (i.e. a membrane where some substances can pass through while others cannot). The ability of solutes to pass through cell membranes depends mainly on size and electrical charge. Osmosis refers to the diffusion of the solvent across a semipermeable membrane. In living systems the solvent is always water, so biologists generally define osmosis as the diffusion of water across a semipermeable membrane:

The osmotic pressure of a solution is the pressure needed to keep it in equilibrium with pure H_20. The higher the [solutes] in a solution, the higher its osmotic pressure. If 2 solutions have the same [solutes], they are called isotonic. If 2 solutions have different [solutes]: The one with the higher [solutes], and lower [solvent], is hypertonic. The one with the lower [solutes], and higher [solvent], is hypotonic.

Cells have developed several ways to survive in a hypotonic environment:

-Pump water out using a contractile vacuole.

-Adjust [solutes] so it is isotonic relative to the environment.

-Develop a thick cell wall that can withstand high turgor pressure.

Even though water molecules are polar, they can pass through the hydrophobic interior of the lipid bilayer because they are so small. However, the flow is fairly limited.

Recent studies have shown that movement of water molecules across cell membranes is facilitated by special protein channels called aquaporins.

Facilitated diffusion refers to the diffusion of solutes through a semipermeable membrane with the help of special transport proteins.

-Non-polar molecules and small polar molecules can diffuse directly through the lipid bilayer of a membrane.

-Ions and large polar molecules cannot, they need help from transport proteins.

Two types of transport proteins can help ions and large polar molecules diffuse through cell membranes:

1) Channel proteins – have a hydrophilic interior for ions or polar molecules to pass through. Some channel proteins can be opened or closed in response to a stimulus. These are called gated channels.
2) Carrier proteins – physically bind to the substance being transported on one side of membrane and release it on the other side.

Characteristics of Facilitated diffusion:

- Is specific – each channel or carrier transports certain ions or molecules only
- Is passive – direction of net movement is always down the concentration gradient
- Saturates – once all transport proteins are in use, rate of diffusion cannot be increased further

Active transport: a cell expends some of its own energy (from ATP) to move a substance against its natural tendency e.g. up a concentration gradient. It requires the use of carrier proteins (transport proteins that physically bind to the substance being transported). 2 types of active transport: membrane pumps (proteinmediated active transport) and coupled transport (cotransport).

1. With membrane pumps, a carrier protein uses energy from ATP to move a substance across a membrane, up its concentration gradient.
2. Coupled transport occurs in 2 stages: First, a carrier protein uses energy from ATP to move a substance across the membrane, up its concentration gradient. This gradient stores energy. Second, a coupled transport protein allows the substance to move back down its concentration gradient. As this happens, the stored energy is released and used to move a second substance up its concentration gradient.

Bulk transport allows small particles, or groups of molecules to enter or leave a cell without actually passing through the membrane. 2 types of bulk transport: endocytosis and exocytosis.

In endocytosis, part of the plasma membrane envelops small particles or fluid, then seals on itself to form a vesicle or vacuole which enters the cell:

- Phagocytosis – the substance engulfed is a solid particle

- Pinocytosis - the substance engulfed is a liquid
- A third type of endocytosis is called receptor-mediated endocytosis. In this process, the molecules to be transported join to specific receptors on the membrane. This causes the membrane to indent, and the molecules are engulfed in a coated vesicle which enters the cell.

The reverse of endocytosis is called exocytosis. During this process, the membrane of a vesicle fuses with the plasma membrane and its contents are released outside the cell.

MEMBRANE CHANNELS AND PUMPS

Permeability of biological membranes is conferred by two classes of membrane proteins, *pumps* and *channels*. Pumps use a source of free energy such as ATP or light to drive the thermodynamically uphill transport of ions or molecules**. *Pump action is an example of active transport***. Channels, in contrast, enable ions to flow rapidly through membranes in a downhill direction. ***Channel action illustrates passive transport***, or *facilitated diffusion*.

Pumps are energy transducers in that they convert one form of free energy into another. Two types of ATP-driven pumps, P-type ATPases and the ATP-binding cassette pumps, undergo conformational changes on ATP binding and hydrolysis that cause a bound ion to be transported across the membrane. Phosphorylation and dephosphorylation of both the Ca^{2+}-ATPase and the Na^{+}-K^{+}-ATPase pumps, which are representative of P-type ATPase, are coupled to changes in orientation and affinity of their ion-binding sites.

One example of channels is the *acetylcholine receptor,* a channel that mediates the transmission of nerve signals across synapses, the functional junctions between neurons. The acetylcholine receptor is a *ligand-gated* channel in that the channel opens in response to the binding of acetylcholine. Another one is the sodium and potassium channels, which mediate action potentials in neuron axon membranes and are opened by membrane depolarization rather than by the binding of an allosteric effector. These channels are *voltage-gated*.

Two factors determine whether a molecule will cross a membrane: (1) the permeability of the molecule in a lipid bilayer and (2) the availability of an energy source.

Some molecules can pass through cell membranes because they dissolve in the lipid bilayer. Such molecules are called *lipophilic molecules*. The steroid hormones provide a physiological example. The transport of some molecules become more complicated for example, sodium ions are present at 143 mM outside the cell and 14 mM inside the cell, yet sodium does not freely

enter the cell because the positively charged ion cannot pass through the hydrophobic membrane interior. Sodium ions pass through specific channels in the hydrophobic barrier formed by membrane proteins.

Examples of pumps:

Na^+ - K^+ pump or the *Na^+ - K^+ ATPase* - The pump is called the Na^+-K^+ ATPase because the hydrolysis of ATP occurs only when Na^+ and K^+ are bound to the pump. Moreover, this ATPase, like all such enzymes, requires Mg^{2+}. The active transport of Na^+ and K^+ is of great physiological significance. Indeed, more than a third of the ATP consumed by a resting animal is used to pump these ions. The hydrolysis of ATP by the pump provides the energy needed for the active transport of Na^+ out of the cell and K^+ into the cell, generating the gradient. The Na+-K+ gradient in animal cells controls cell volume, renders neurons and muscle cells electrically excitable, and drives the active transport of sugars and amino acids.

Ca^{2+}ATPase - the enzyme that transports Ca^{2+} out of the cytoplasm and into the sarcoplasmic reticulum of muscle cells.

The gastric H+-K+ ATPase - the enzyme responsible for pumping sufficient protons into the stomach to lower the pH below 1.0.

Flippases - are enzymes that maintain membrane asymmetry by “flipping” phospholipids from the outer to the inner layer of the membrane.

All these enzymes are referred to as *P-type ATPases* 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.

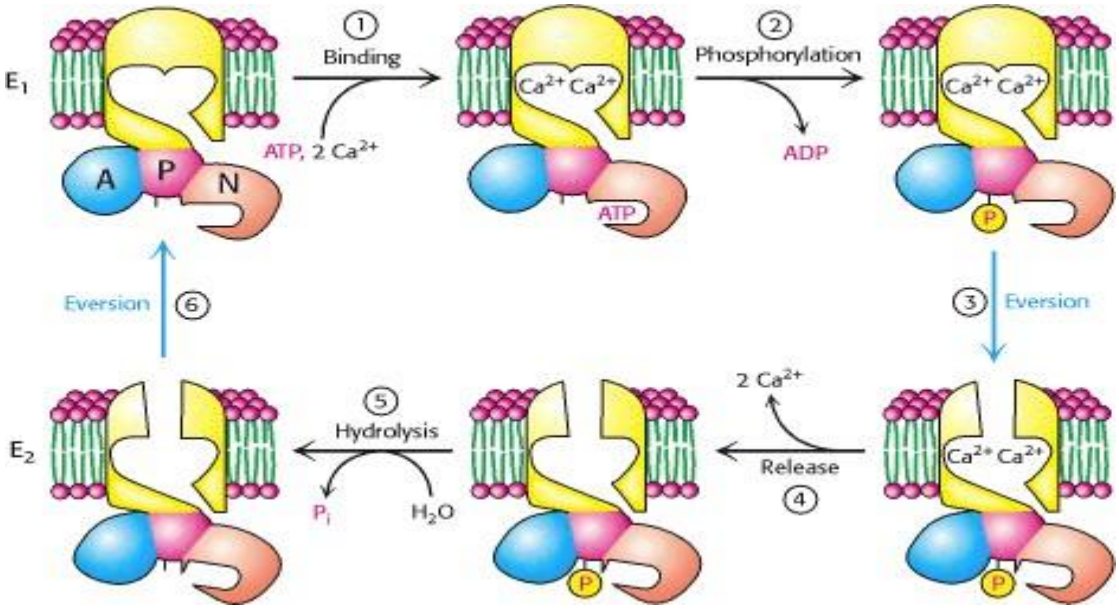
MECHANISM OF P-TYPE ATPase ACTION

Considering the structural and mechanistic features of the Ca^{2+} ATPase found in the sarcoplasmic reticulum (SR Ca^{2+} ATPase) of muscle cells. This enzyme, which constitutes 80% of the sarcoplasmic reticulum membrane protein, plays an important role in muscle contraction, which is triggered by an abrupt rise in the cytosolic calcium level. Muscle relaxation depends on the rapid removal of Ca^{2+} from the cytosol into the sarcoplasmic reticulum, a specialized compartment for calcium storage, by the SR Ca^{2+} ATPase. This pump maintains a Ca^{2+} concentration of approximately 0.1 μM in the cytosol compared with 1.5 mM in the sarcoplasmic reticulum.

Six steps involved in postulated reaction cycle are;

1. **Binding :-** binding of ATP and two Ca^{2+} ions to the E_1 state.
2. **Phosphorylation :-** 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 toward E_2.
3. **Eversion :-** The transition from the E_1 to the E_2 state causes the ion-binding sites to "evert" so that the ions can dissociate only to the luminal side of the membrane.
4. **Release :-** In the E_2-P state, the enzyme has low affinity for the Ca^{2+} ions, so they are released.
5. **Hydrolysis:-** With the release of Ca^{2+}, the phosphor-aspartate residue is hydrolyzed, and the phosphate group is released. (hydrolysis of phosphor-aspartate)
6. **Eversion :-** The enzyme, devoid of a covalently attached phosphoryl group, is not stable in the E_2 form. It everts back to the E_1 form, completing the cycle

Note step *5 and 6 reset the enzyme to its initial state*.



Mechanism of P-Type ATPase Action- The binding of Ca^{2+} and the phosphorylation of the ATPase (steps 1 and 2), illustrated here for the Ca^{2+} ATPase, lead to the eversion of the binding sites (step 3) and the release of Ca2+ to the luminal side of the membrane (step 4). Hydrolysis of phosphoaspartate (step 5) and eversion (step 6) reset the enzyme to its initial state.

Essentially the same mechanism is employed by the Na^+-K^+ ATPase. The E_1 state binds three Na^+ ions and transports them across the membrane and out of the cell as a result of the protein's phosphorylation and transition to the E_2 state. The three Na+ ions are released into the extracellular medium. The E_2 state of this enzyme also binds ions namely, two K^+ ions. These K^+ ions are carried across the membrane in the opposite direction by eversion driven by the hydrolysis of the phosphor-aspartate residue and are released into the cytosol.

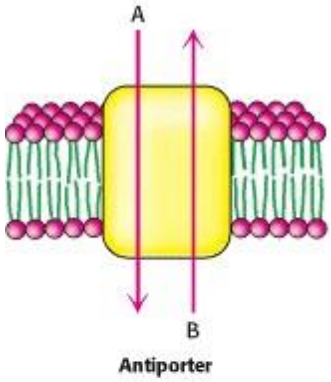
The results of mechanistic studies of the SR Ca^{2+} ATPase and other P-type ATPases have revealed two common features. First, each protein can be phosphorylated on a specific aspartate residue. For the SR Ca^{2+} ATPase, this reaction takes place at Asp 351 only in the presence of relatively high cytosolic concentrations of Ca^{2+}. Second, each pump can interconvert between at least two different conformations, denoted by E_1 and E_2. Thus, at least four conformational states E_1, E_1-P, E_2-P, and E_2 participate in the transport process.

INHIBITORS- Certain steroids derived from plants are potent inhibitors of the Na^+-K^+ pump. Digitoxigenin and ouabain are members of this class of inhibitors, which are known as *cardiotonic steroids* because of their strong effects on the heart. These compounds inhibit the dephosphorylation of the E_2-P form of the ATPase when applied on the *extracellular* face of the membrane. *Digitalis*, a mixture of cardiotonic steroids derived from the dried leaf of the foxglove plant (*Digitalis purpurea*), is of great clinical significance. Digitalis increases the force of contraction of heart muscle, which makes it a choice drug in the treatment of congestive heart failure. Inhibition of the Na^+-K^+ pump by digitalis leads to a higher level of Na^+ inside the cell. The diminished Na^+ gradient results in slower extrusion of $Ca2^+$ by the sodium calcium exchanger. The subsequent increase in the intracellular level of Ca2+ enhances the contractility of cardiac muscle.

CO-TRANSPORTERS (Secondary transporter)

Many active-transport processes are not directly driven by the hydrolysis of ATP. Instead, the thermodynamically uphill flow of one species of ion or molecule is coupled to the downhill flow of a different species. Membrane proteins that pump ions or molecules uphill by this means are termed *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; symporters use the flow of one species

to drive the flow of a different species in the *same direction* across the membrane (see below diagram).



Secondary Transporters. These transporters employ the downhill flow of one gradient to power the formation of another gradient. In antiporters, the chemical species move in opposite directions. In symporters, the two species move in the same direction.

Examples of co-transporters;

1. The *sodium calcium exchanger* in the plasma membrane of an animal cell is an antiporter that uses the electrochemical gradient of Na^+ to pump $Ca2^+$ out of the cell. Three Na^+ ions enter the cell for each Ca^{2+} ion that is extruded. The cost of transport by this exchanger is paid by the Na^+-K^+- ATPase pump, which generates the required sodium gradient.
2. Glucose is pumped into some animal cells by a symporter powered by the simultaneous entry of Na^+. The entry of Na^+ provides a free-energy which is sufficient to generate a 66-fold concentration gradient of an uncharged molecule such as glucose.
3. The lactose permease of *E. coli* is a symporter that uses the H^+ gradient across the *E. coli* membrane generated by the oxidation of fuel molecules to drive the uptake of lactose and other sugars against a concentration gradient.

ION CHANNELS

Pumps and secondary transporters can transport ions at rates approaching several thousand ions per second. Other membrane proteins, *ion channels*, which are passive transport systems, are capable of ion-transport rates that are more than 1000 times as high. These rates of transport through ion channels are close to rates expected for ions diffusing freely through aqueous solution. Yet, ion channels are not simply tubes that span membranes through which ions can rapidly flow. Instead, they are highly sophisticated molecular machines that respond to chemical and physical changes in their environments and undergo precisely timed conformational changes that facilitate their roles as essential components of the nervous and other systems.

Key properties that characterize ion channels:

1. *Ion channels can be highly selective for particular ions*. For example, some channels allow the flow of K^+ very effectively but do not allow appreciable levels of Na^+ to cross the membrane. Other channels transport positively charged ions (cations), but block the flow of negatively charged ions (anions).

2. *Ion channels exist in open and closed states*. These channels undergo transitions from the closed state, incapable of supporting ion transport, to the open state, through which ions can flow.

3. *Transitions between the open and the closed states are regulated*. Ion channels are divided into two classes: *ligand-gated channels* and *voltage-gated channels*. Ligand-gated channels open and close in response to the binding of specific chemicals, whereas voltage-gated channels open and close in response to the electrical potential across the membrane in which they are found.

4. *Open states of channels often spontaneously convert into inactivated states*. Most ion channels do not remain in an open state indefinitely but, instead, spontaneously transform into inactivated states that do not conduct ions. The spontaneous transitions of ion channels from open to inactivated states act as built-in timers that determine the duration of ion flow.

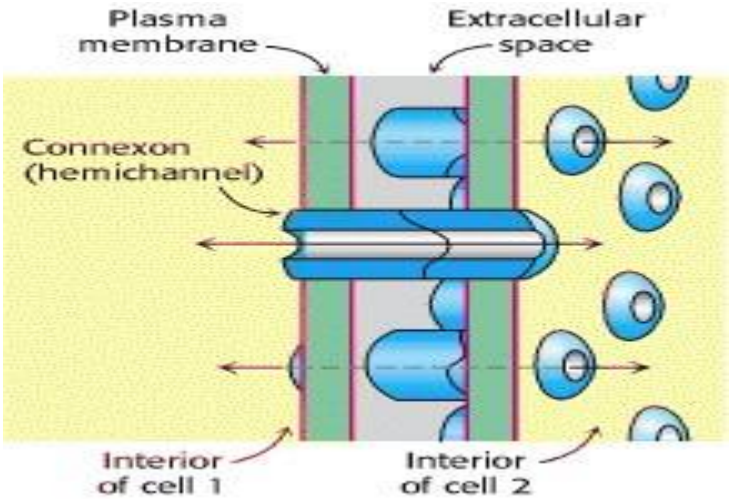
GAP JUNCTIONS (cell to cell channels)

Gap junction is an intercellular network of protein channels that facilitates the cell to cell passage of ions, hormones and neurotransmitters. That is a structure consisting of a series of channels extending between two cells, allowing the passage of ions, small molecules etc.

It is a type of channel with a very different role that serves as passageways between the interiors of contiguous cells. Gap junctions are clustered in discrete regions of the plasma membranes of apposed cells. Small hydrophilic molecules as well as ions can pass through gap junctions.

All polar molecules with a mass of less than about 1 kd can readily pass through these cell-to-cell channels. Thus, *inorganic ions and most metabolites (e.g., sugars, amino acids, and nucleotides) can flow between the interiors of cells joined by gap junctions*. In contrast, proteins, nucleic acids, and polysaccharides are too large to traverse these channels. *Gap junctions are important for intercellular communication*.

Note-Cell-to-cell channels differ from other membrane channels in three respects: (1) they traverse *two* membranes rather that one; (2) they connect cytosol to cytosol, rather than to the extracellular space or the lumen of an organelle; and (3) the hemi-channels forming a channel are synthesized by different cells.



Schematic Representation of a Gap Junction. Connexon proteins are proteins that form a tunnel across gap junctions enabling small molecules of glucose to pass from one cell to another.