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METHODICAL POINTING
from the course
"BIOLOGICAL MEMBRANES AND REGULATION OF METABOLISM"
(part 1. Biological membranes structure and membrane transport)

for the master's degrees of a 1 course of department of biochemistry with English of educating

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1. Membrane structure.

1.1. Phospholipid Bilayer and membranes lipids

Scientists began building molecular models of the membrane decades before membranes were first seen with the electron microscope (in the 1950s). In 1915, membranes isolated from red blood cells were chemically analyzed and found to be composed of lipids and proteins. Ten years later, two Dutch scientists reasoned that cell membranes must be phospholipid bilayers in which hydrophobic tails of the phospholipids are directed from water and hydrophilic heads - to water. In 1935 Hugh Davson and James Danielli suggested that the membrane might be coated on both sides with hydrophilic proteins. They proposed ***a sandwich model***: a phospholipid bilayer between two layers of proteins. When researchers first used electron microscope to study cells (in the 1950s), the images seemed to support the Davson-Danielli model. By the late 1960s, however, many cell biologists recognized two problems with the model. First, inspection of a variety of membranes revealed that membranes with different functions differ in structure and chemical composition. A second, more serious problem became apparent once membrane proteins were better characterized. Unlike proteins dissolved in the cytosol, membrane proteins are not very soluble in water because they are amphipathic. If such proteins were layered on the surface of the membrane, their hydrophobic parts would be in aqueous surroundings. Taking these observations into account, S. J. Singer and G. Nicolson proposed in 1972 that membrane proteins reside in the phospholipid bilayer with their hydrophilic regions protruding. This molecular arrangement would maximize contact of hydrophilic regions of proteins and phospholipids with water in the cytosol and extracellular fluid, while providing their hydrophobic parts with a non aqueous environment. In this ***fluid mosaic model***, the membrane is a mosaic of protein molecules bobbing in a fluid bilayer of phospholipids.

So, today is known that the cell membrane has mosaic structure. Mosaic is the art of creating images with an assemblage of small pieces of colored glass, stone, or other materials. The cell membrane is similar in that it is composed of many different, small units (many different proteins and phospholipids along with cholesterol).

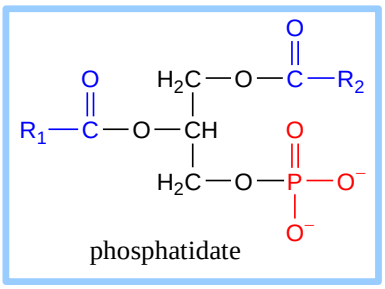
Membranes lipids are:

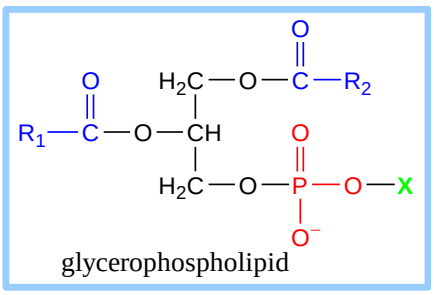
- Phospholipids:
  - Glycerophospholipids;
  - Sphingophospholipids
- Glycolipids:
  - Glyceroglycolipids (mitochondrial, bacterial membranes);
  - Sphingoglycolipids
- Cholesterol

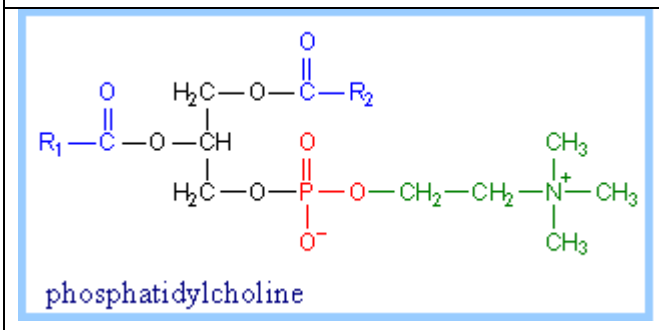
Outer leaflet of membrane has phosphatidylcholine, sphingomyelin and inner leaflet has phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, so plasma membrane is asymmetric. Glycolipids localized only in outer leaflet, carbohydrate on surface. Cholesterol localized in both leaflets.

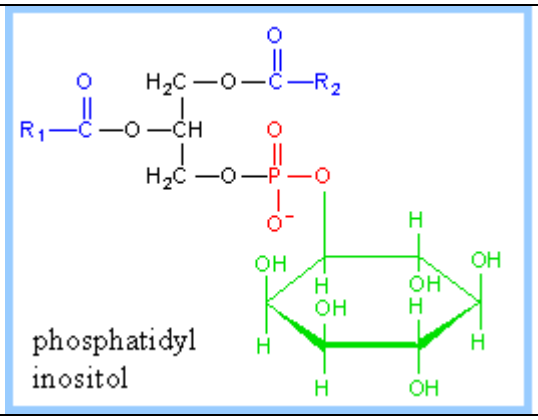
Glycerophospholipids are common constituents of cellular membranes. They have a glycerol backbone and hydroxyls at C1 and C2 are esterified to fatty acids.

In phosphatidate fatty acids are esterified to hydroxyls on C1 and C2 and the C3 hydroxyl is esterified to Pi.







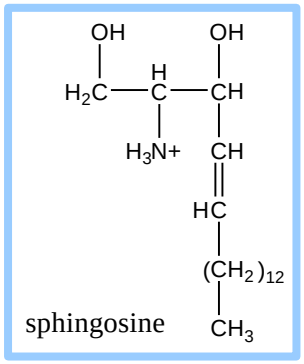


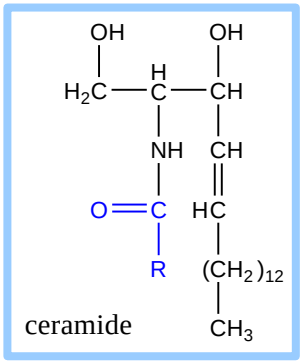
In most glycerophospholipids Pi is in turn esterified to OH of a polar head group (X): e.g., serine, choline, ethanolamine, glycerol, or inositol.

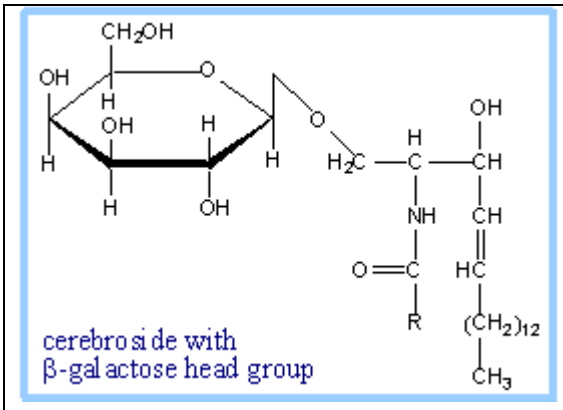
Each glycerophospholipid includes a polar region (glycerol, carbonyl O of fatty acids, Pi, and the polar head group (X)) and non-polar hydrocarbon tails of fatty acids (R1, R2).

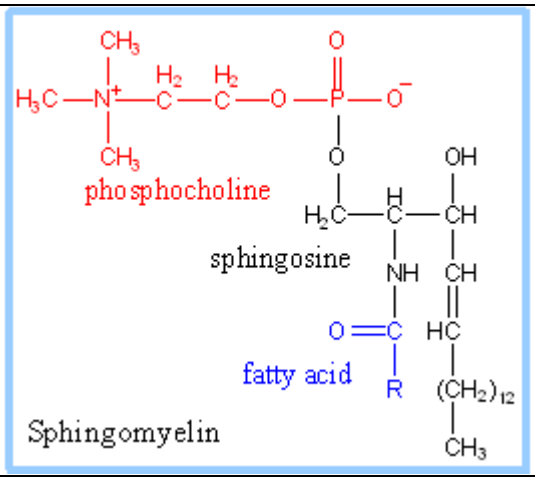
Sphingolipids are derivatives of the lipid sphingosine, which has a long hydrocarbon tail, and a polar domain that includes an amino group. This amino group can form an amide bond with a fatty acid carboxyl, to yield a Ceramide.

In the more complex sphingolipids, a polar “head group" is esterified to the terminal hydroxyl of the sphingosine moiety of the ceramide. ***Sphingomyelin*** has a phosphocholine or phosphoethanolamine head group. ***Cerebroside*** is a sphingolipid (ceramide) with a monosaccharide such as glucose or galactose as polar head group. ***Ganglioside*** is a ceramide with a polar head group that is a complex oligosaccharide, including the acidic sugar derivative sialic acid. Cerebrosides and gangliosides, collectively called glycosphingolipids, are commonly found in the outer leaflet of the plasma membrane bilayer, with their sugar chains extending out from the cell surface.



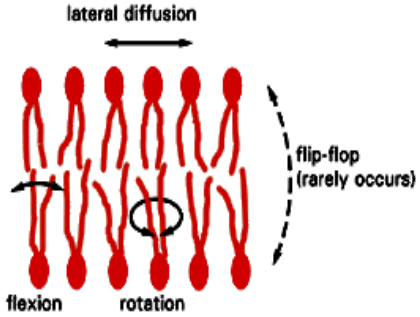






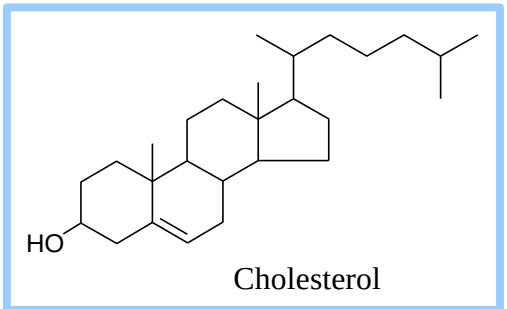
Amphipathic lipids in association with water form complexes in which polar regions are in contact with water and hydrophobic regions away from water. Depending on the lipid there are two possible molecular arrangements - ***various micelle structures*** (e.g., a spherical micelle is a stable configuration for amphipathic lipids with a conical shape, such as fatty acids) and ***bilayer*** (which is the most stable configuration for amphipathic lipids with a cylindrical shape, such as phospholipids).

All the molecules in the cell membrane (proteins, phospholipids, cholesterol) are a fluid and move around independent of each other, unless they are attached to the cytoskeleton or other proteins in oligomeric complexes. Fluidity enhanced by cholesterol and unsaturated lipids.



The speed of lipids lateral movement is about 10^7 times per second, the speed of rotation is faster. Flip-flop of lipids (from one half of a bilayer to the other) is normally very slow – about once per month, as flip-flop would require the polar head-group of a lipid to traverse the hydrophobic core of the membrane. So the two leaflets of a bilayer membrane tend to differ in their lipid composition. Some membranes contain enzymes - ***flippases*** - which actively transport particular lipids from one monolayer to the other and in this case the speed of flip-flop enhances to about once per 1-2 minutes.

Cholesterol, an important constituent of cell membranes, has a rigid ring system and a short branched hydrocarbon tail. Cholesterol is largely hydrophobic. But it has one polar group, a hydroxyl, making it amphipathic. Cholesterol inserts into bilayer membranes with its hydroxyl group oriented toward the aqueous phase and its hydrophobic ring system adjacent to fatty acid chains of phospholipids. The OH group of cholesterol forms hydrogen bonds with polar phospholipid head groups. Interaction with the relatively rigid cholesterol decreases the mobility of hydrocarbon tails of phospholipids. Phospholipid membranes with a high concentration of cholesterol have a fluidity intermediate between the liquid crystal and crystal states.



Cholesterol and sphingolipids (sphingomyelin and glycolipids) in membranes form clusters - ***lipid rafts***. Rafts are highly-ordered versus phospholipid bilayer. Visualize lipid rafts with fluorescent probe Laurdan sensitive to rigidity of phospholipid bilayer (false color).

Lipid rafts are complex sphingolipids tend to separate out from glycerophospholipids and co-localize with cholesterol in membrane microdomains called lipid rafts. Membrane fragments assumed to be lipid rafts are found to be resistant to detergent solubilization, which has facilitated their isolation and characterization. Proteins involved in cell signaling often associate with lipid raft domains. Otherwise soluble signal proteins often assemble in complexes at the cytosolic surface of the plasma membrane in part via insertion of attached fatty acyl or isoprenoid lipid anchors into raft domains. Integral proteins may concentrate in raft domains via interactions with raft lipids or with other raft proteins. Some raft domains contain derivatives of phosphatidylinositol that bind signal proteins with pleckstrin homology domains.

Caveolaes are invaginated lipid raft domains of the plasma membrane that have roles in cell signaling and membrane internalization. Caveolin is a protein associated with the cytosolic leaflet of the plasma membrane in caveolae. Caveolin interacts with cholesterol and self-associates as oligomers that may contribute to deforming the membrane to create the unique morphology of caveolae.

1.2. Membrane proteins

Membrane Proteins differ by their functions. There are:

- Channel Proteins:
  - Tubular
  - Allow passage of molecules through membrane
- Carrier Proteins:
  - Combine with a substance to be transported
  - Assist passage of molecules through membrane
- Cell Recognition Proteins:
  - Provide cells with unique chemical compounds
  - Help body to recognize foreign substances
- Receptor Proteins:
  - Bind to a ligand
  - Cause cell to respond to a ligand
- Enzymatic Proteins:
  - Carry out metabolic reactions

Membrane proteins also differ by their localization in membrane. There are integral proteins, proteins which link to the cytosolic surface of the plasma membrane via a covalently attached hydrophobic anchor and peripheral proteins.

Integral proteins have domains that extend into the hydrocarbon core of the membrane. Intramembrane domains have largely hydrophobic surfaces, which interact with membrane lipids. Residues with aliphatic side-chains (leucine, isoleucine, alanine, and valine) predominate in the middle of the bilayer.

A ***membrane-spanning α-helix*** is the most common structural motif found in integral proteins. If a hydropathy plot has ***1 transmembrane α-helix",*** topology studies are expected to confirm location of N and C termini on opposite sides of membrane. If ***two transmembrane α-helices*** are predicted, N and C termini should be on the same side. The segment between the α-helices should be on the other side.

Integral membrane proteins insert into lipid bilayer and can dissociate with reagents that disrupt hydrophobic interactions – detergents (e.g. octylglucoside, sodium dodecylsulfate (SDS)). ***Detergents*** are amphipathic (both hydrophobic and hydrophilic) compounds that solubilize integral transmembrane proteins.

Some proteins bind to membranes ***via a covalently attached hydrophobic anchor*** that inserts into the bilayer. So, some proteins may link to the cytosolic surface of the plasma membrane ***via a covalently attached fatty acid*** (e.g., palmitate or myristate) or an ***isoprenoid group***.

Palmitate is usually attached via an ester linkage to the thiol of a cysteine residue. Palmitated protein may be released from plasma membrane to cytosol via ***depalmitoylation***, hydrolysis of the ester link.

An ***isoprenoid*** such as a ***farnesyl*** residue is attached to some proteins via a thioether linkage to a cysteine thiol.

Some cytosolic proteins have ***domains that bind to polar head groups of lipids that transiently exist in a membrane.*** E.g., ***pleckstrin homology (PH) domains*** bind to phosphorylated derivatives of phosphatidylinositol. Some PH domains bind *phosphatidyl-inositol-bis-phosphate* (PIP2, = PI-4,5-P2). Other PH domains recognize and bind phosphatidylinositol derivatives with Pi esterified at the 3' OH of inositol, e.g., *phosphatidyl-inositol-3,4,5-triphosphate* (PI-3,4,5-P3), *phosphatidyl-inositol-3,4-bisphosphate* (PI-3,4-P2).

Proteins are much larger than lipids and move more slowly, but some membrane proteins do drift. And some membrane proteins seem to move in a highly directed manner, perhaps driven along cytoskeletal fibers by motor proteins connected to the membrane proteins' cytoplasmic regions. However, many other membrane proteins seem to be held immobile by their attachment to the cytoskeleton or to the extracellular matrix.

1.3. Membrane carbohydrates

Membrane carbohydrates are usually short, branched chains of fewer than 15 sugar units. Some are covalently bonded to lipids, forming molecules called glycolipids. However, most are covalently bonded to proteins, which are thereby glycoproteins. The carbohydrates on the extracellular side of the plasma membrane vary from species to species, among individuals of the same species, and even from one cell type to another in a single individual. The diversity of the molecules and their location on the cell's surface enable membrane carbohydrates to function as markers that distinguish one cell from another. For example, the four human blood types designated A, B, AB, and O reflect variation in the carbohydrate part of glycoproteins on the surface of red blood cells.

1.4. Test quiescence

1. The fluidity of most membranes is reduced by the presence of _____ in the membrane.

A) nucleotides
B) proteins
C) cholesterol
D) glycolipids

2. Which of the following phospholipids is localized to a greater extent in the outer leaflet of the membrane lipid bilayer?

A)Choline phosphoglycerides
B)Ethanolamine phosphoglycerides
C)Inositol phosphoglycerides
D)Serine phosphoglyceride

3. Glycosphingolipids are a combination of

A)Ceramide with one or more sugar residues

B)Glycerol with galactose
C)Sphingosine with galactose
D)Sphingosine with phosphoric acid

4. Plane membrane microdomains, that contain many glycerophospholipids and cholesterol, are called:

A) Lipid rafts;
B) Endosome;
C) caveole;
D) caveosome;
E) peroxysome

5. Amphipatic compounds that can solubilize integral (transmembrane) proteins (for example, octylglucoside, sodium dodecylsulfate (SDS), are called _____.

2. Membrane transport.

There are two main types of membrane transport: passive (diffusion) and active.

2.1. Passive transport: passive diffusion

Diffusion is the random, passive (happens all by itself without transferring energy to the molecules) movement of molecules from a location where they are higher in to a location where they are lower in concentration.

Small hydrophobic molecules like steroids, oxygen molecules, and carbon dioxide have no charge and therefore do not stick to anything, and are small enough to squeeze between phospholipids of the membranes. So they can go through the phospholipid bilayer ***by simple diffusion*** by which molecules move across membrane from high to low concentration without any help from proteins.

2.2. Passive transport: facilitated diffusion

Small hydrophilic molecules (ions, monomers and other small organic molecules) will cross the membrane by ***facilitated diffusion***, by which molecules have net movement from high to low concentration with assistance from a membrane proteins. ***These proteins can form channels (a), can be carrier proteins (b), which by definition change conformation (shape) up binding of solute or can be ionofores (c).***

2.2.1. Mobile transporters

Molecules which cross the membrane by facilitated diffusion ***can go through the membrane faster than normal diffusion process***. The ***velocity of transport is saturable*** in facilitated diffusion**.** The uptake of glucose into erythrocyte is a good example. It is rapidly moved across the membrane down the concentration gradient with the help of a membrane protein that belong to ***permeases family*****.** Permeases are multipass transmembrane proteins used to facilitate the diffusion of specific molecules across biological membrane. Permeases for glucose are proteins GLUTs.

2.2.2. Ion channels

Some ions can pass through membrane by ***ion channels*****.** These structures have two discrete states: open (conducting) and closed (nonconducting). Some channels have also inactivated state (open but nonconducting).

Ligand-gated channels are protein channels that open when a ligand – atom or molecule - binds to a specific site on a protein. *Examples of these channels* are glutamate receptors, nicotinic acetylcholine receptor, vanilloid receptor family. *Examples of extracellular ligands* are acetylcholine, glycine, γ-amino butyric acid, serotonin, ATP and *examples of intracellular ligands* are cADP-ribose, inosytol-1,4,5-triphosphate, Ca^{2+}.

Acetylcholine Receptor (AchR) consists of a pentamer of protein subunits, with two binding sites for acetylcholine, which, when bound, alter the receptor's configuration and cause an

internal pore to open. This pore allows Na^+ ions to flow down their electrochemical gradient into the cell. The AchR also responds to nicotine, and so is called the "nicotinic" acetylcholine receptor –nAChR. *CORBA* snake toxins inhibit AchR.

Mechanic-gated channels can open upon mechanical (physical) stress. In this case it is caused by vibrations from sound entering your ear as the receptor in blue gets pulled open when the cilia vibrate.

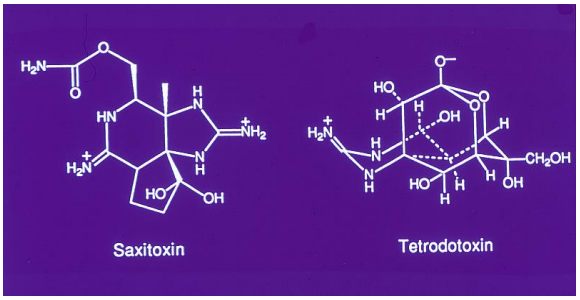
Light-gated channels can open and close in response to light. Channelrhodopsins are a subfamily of retinylidene proteins (rhodopsins) that function as light-gated ion channels. They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis: movement in response to light. Expressed in cells of other organisms, they allow the light-induced depolarization of cells and enable light to control electrical excitability, intracellular acidity, calcium influx, and other cellular processes.

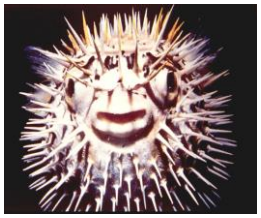
Voltage-gated channels can open in response to changes in membrane potential, subsequently open and inactivate, are specific for a particular ion, have common domain structure and are regulated by external signals.

Voltage-gated sodium channel has α, β, and γ subunits. α subunit is responsible for pore and β, γ subunits modify channel function but are not essential to create the pore. α-subunit (260kda) contains 4 internal repeats (similar amino acid sequence) - domains 1-4. Each repeat contains of 6 transmembrane α helical structures (S1-S6): 5 hydrophobic segments (S1, S2, S3, S5, S6) which form selectivity filter and pore and 1 highly positive charged segment (S4) which is voltage sensors of the channel.

Voltage-gated Na^+ channel binds *specific neurotoxins*: ***Tetrodotoxin*** from puffer fish (10ng lethal dose), ***Saxitoxin*** from Marine Protozoa which are *Na^+ channel blocking toxin* and ***batrachotoxin*** from Colombian frog, ***Veratridine*** from lilies, *that blocks inactivation* so causes channels to open at more negative potentials and to stay open much longer than usual.

Tetrodotoxin from puffer fish is found mainly in the liver and gonads. It binds to the outside of the sodium channel on a one-to-one basis.

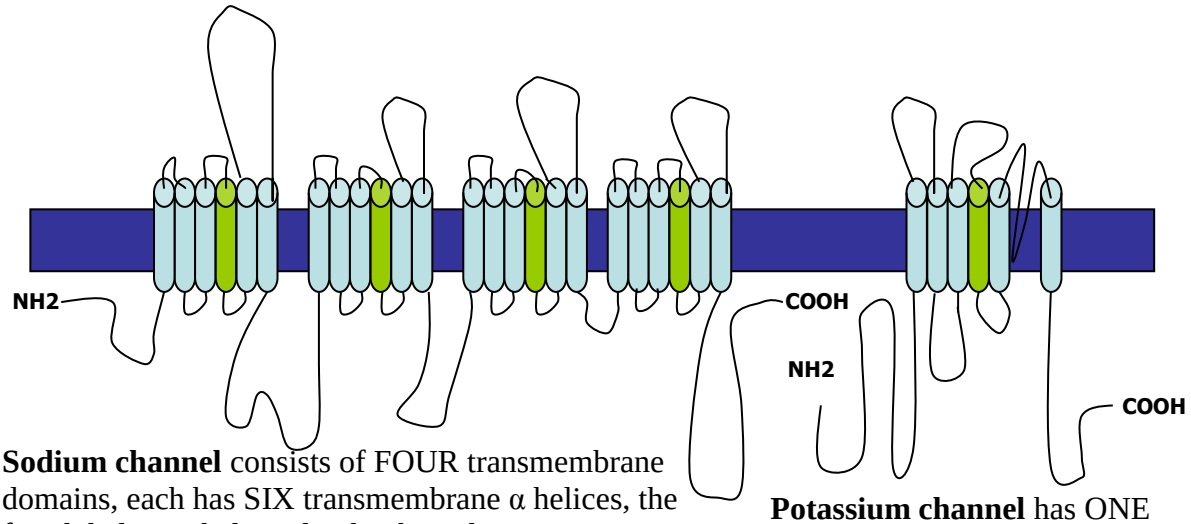




Saxitoxin from algae bio-accumulates to bivalves such as oyster and mussels. Red-tide (algal bloom) could be dangerous. They have sodium channel blockers too. When saxitoxin accumulated in mussels or oysters and consumed by human, paralytic shell-fish poisoning resulted.

Sodium channel blockers can cause suffocation when the nervous system controlling respiration is blocked. At low dose, paralytic effects have been observed in patients intoxicated with these toxins.

Voltage-gated potassium channel is 70 kDa protein that has 4 subunits (is tetramer). Each subunit has S1-S6 membrane-spanning α helices which are homologous to one of the repeated units (domens 1-4) of Voltage-gated Sodium channel. S5 and S6 form the actual pore of the K^+ channel, selective filter and S4 is voltage sensors of the channel.



Sodium channel consists of FOUR transmembrane domains, each has SIX transmembrane α helices, the fourth helice is believed to be the voltage sensor.

Potassium channel has ONE domain of only SIX transmembrane α helices

K^+-ion channel blockers are tityustoxin and Charybdotoxin from scorpions (Tityus serrulatus and Leiurus quinquestriatus), dendrotoxins from Dendroaspis angusticeps, tarantula toxins (hanatoxins) and alkaloid toxins from plants.

Voltage-gated calcium channels in neurons are mostly responsible for the entry of calcium into the presynaptic ending following depolarization (and subsequent exocytosis of neurotransmitter), in heart are coupling to excitation contraction and in all excitable secretory cells (adrenal medulla, pancreas) cause entry of calcium which induces secretion.

They were initially divided into 2 classes – HVA (high voltage activated) and LVA (low voltage activated) Ca^{2+}-channels. HVA Ca^{2+}-channels are further divided into L, N, P/Q and R-types channels, while LVA Ca^{2+}-channels consist of only T-type channels. R-type is occasionally classified as IVA (intermediate-voltage-activated) channels.

There are a lot of ***Chloride Ion Channels*** which have different structure, gating mechanisms and functions. *Extracellular ligand-gated (ELG) Cl^--channels* are pentamers (each subunit has 4 transmembrane domains) and function as receptors (post synaptic GABA and Glycine receptors). *CFTR (cystic fibrosis transmembrane conductance regulator)-channel* belong to ABC (ATP-binding cassette) transporters, has 2 transmembrane domains (each of them consist of 6 α-helical segments) and its gating controlled by ATP and/or phosphorylation by cAMP- , cGMP- dependant kinases. There are also *voltage gated chloride channels (CLC), nucleotide/volume sensitive chloride channels (CLNS1A), chloride intracellular channels (CLIC*) – they have no membrane spanning domains and regulate electrolyte composition and acidification of intravascular spaces) and *calcium activated (CLCA) chloride channels*.

Often chloride ion channels are permeable to other anions, but chloride happens to be the most abundant anion. Chloride channels regulate cell volume, membrane potential, resting potential, depolarization, signal propagation, transport processes.

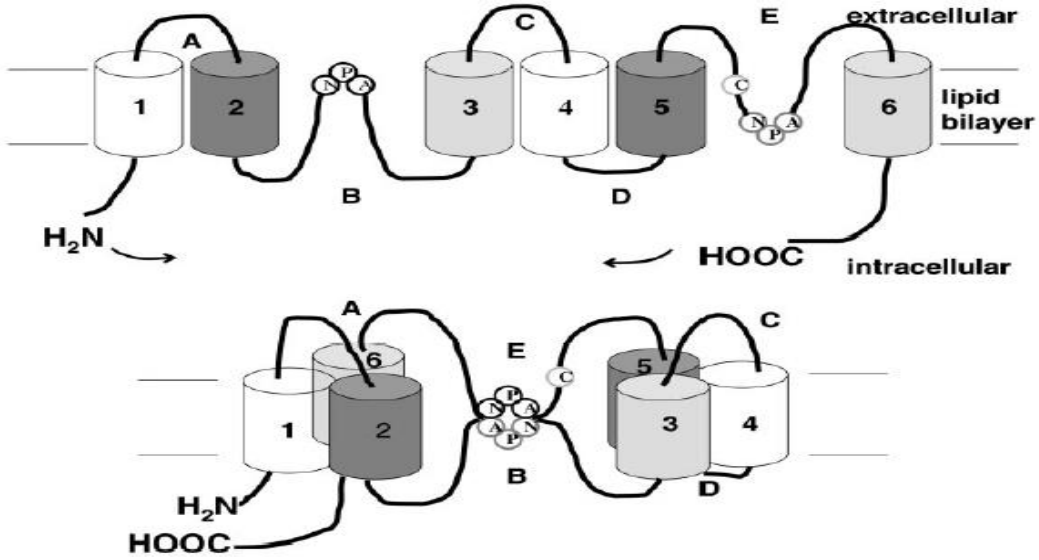
Some diseases associated with chloride ion channels - Cystic Fibrosis (genetic disorder in which gland secretions are abnormally thick), Myotonia Congenita (genetic neuromuscular disorder with mutations in the chloride ion channels of muscle cell plasma membranes) etc.

Cystic Fibrosis has many affects: abnormally thick and sticky gland secretions, many organs in the body are affected by clogging mucous (the lungs are most affected). Air passages are blocked and the mucous also serves as a growth environment for bacteria giving rise to respiratory infections. The decrease in oxygen levels also causes pulmonary arteries to constrict leading to high blood pressure and increases the strain on the heart. 98% of all patients die from cardiopulmonary complications. 1 out of 1500 Caucasian children are affected.

Aquaporines (AQPs***),*** also known as ***water channels,*** were found by Peter Agre (1992; 2003 Nobel Prize in Chemistry was awarded to him for the discovery of aquaporines). For many years, scientists assumed that water leaked through the cell membrane, and some water does. The

presence of water channels increases membrane permeability to water. Many human cell types express them, as do certain bacteria and many other organisms, such as plants for which it is essential for the water transport system. Aquaporines selectively conduct water molecules in and out of the cell, while preventing the passage of ions and other solutes. Some of them, known as ***aquaglyceroporines***, also transport other small uncharged solutes, such as glycerol, CO_2, ammonia and urea across the membrane, depending on the size of the pore. However, the water pores are completely impermeable to charged species, such as protons, a property critical for the conservation of the membrane's electrochemical potential.

Aquaporines are integral membrane pore proteins. Aquaporines form tetramers in the cell membrane, with each subunit acting as a water channel. The different aquaporines contain differences in their peptide sequence, which allows for the size of the pore in the protein to differ between aquaporines. Each monomer consists of *six membrane-spanning segments (1-6)* arranged in two hemi-pores, which fold together to form the hourglass-shaped channel. There are also *five interhelical loop regions (A – E)* that form the extracellular and cytoplasmic vestibules. Loops B and E are hydrophobic loops that contain the highly, although not completely conserved, asparagine–proline–alanine (NPA) motif, which overlap the middle of the lipid bilayer of the membrane forming a 3-D 'hourglass' structure where the water flows through. This overlap forms one of the two well-known channel constriction sites in the peptide, the NPA motif and a second and usually narrower constriction known as 'selectivity filter' or ar/R selectivity filter.



There are 11 different variants of these channels in the human body and more may still be discovered. They are responsible for many reactions in the body and one major function is done in the kidneys.

AQP1 is a widely expressed water channel, whose physiological function has been most thoroughly characterized in the kidney. It is found in the basolateral and apical plasma membranes of the proximal tubules, the descending limb of the loop of Henle. ***AQP2*** is found in the apical cell membranes of the kidney's duct principal cells and in intracellular vesicles located throughout the cell. 70% of water is reabsorbed by AQP1 from primary urine into blood and 10% of water is reabsorbed by AQP2.

AQP2 is the only aquaporine regulated by ***vasopressin*** (the same as ADH, antidiuretic hormone). This regulation can occur in two ways: short-term regulation (minutes) through trafficking of AQP2 vesicles to the apical region where they fuse with the apical plasma

membrane and long-term regulation (days) through an increase in AQP2 gene expression. A deficiency in the vasopressin may be affected by *diabetes insipidus* and show an increase of urine excretion to 10 – 15 liters a day.

2.2.3. Ionophores

Ionophores are common growth enhancers in livestock feed, and are used in veterinary medicine as a coccidiostat in poultry. They are lipid-soluble molecules usually synthesized by microorganisms to transport ions across the lipid bilayer of the cell membrane. There are two main kinds of ionophores: ***mobile ion carriers*** that bind to a particular ion, shielding its charge from the surrounding environment, and thus facilitate its crossing the hydrophobic interior of the lipid membrane (ex. Valinomycin) and ***channel formers*** that introduce a hydrophilic pore into the membrane, allowing ions to pass through while avoiding contact with the membrane's hydrophobic interior (ex. Gramicidin). Ionophores disrupt the transmembrane ion concentration gradients required for the proper functioning and survival of microorganisms, and thus have antibiotic properties. They are produced naturally by a variety of microbes and act as a defense against competing microbes. Many antibiotics, particularly the macrolide antibiotics, are ionophores that exhibit high affinities to Na^+ or K^+.

Gramicidin is a polypeptide with alternating L- and D-amino acids, sharing the general formula: formyl-L-**X**-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-**Y**-D-Leu-L-Trp-D-Leu-L-Trp-ethanolamine (X and Y depend upon the type of gramicidin molecule). It is obtained from the soil bacteria species *Bacillus brevis.* Gramicidin's bactericidal activity is a result of increasing the permeability of the bacterial cell membrane, allowing Na^+ ions to travel through unrestricted and thereby destroying the ion gradient between the cytoplasm and the extracellular environment.

Valinomycin is obtained from the cells of several *Streptomyces*. It consists of enantiomers D- and L-valine, D-hydroxyvaleric acid and L-lactic acid. It functions as a potassium-specific transporter and facilitates the movement of K^+ ions through lipid membranes "down" an electrochemical potential gradient.[1]

2.3. Active Transport

Active transport is the movement of a substance across a cell membrane against its concentration gradient (from low to high concentration) with assistance from a membrane channel protein and an energy source (ATP). Small, hydrophilic molecules will cross the membrane by active transport. Active transport uses ATP hydrolysis directly (***primary active transport***) or indirectly (***secondary active transport***).

2.3.1. Primary active transport

Active transporters are membrane proteins specifically bind and move the molecules across the membrane to a unique direction using ATP hydrolysis as an energy source. Most of the enzymes that perform this type of transport are transmembrane ATPases, which classified according to their protein sequence homology and structures. ***P-type*** of ATPases (from ***p***hosphorylation) examples are *Na^+-K^+-ATPase,* proton-potassium pump (*H^+,K^+-ATPase*) and *Ca^{2+}-ATPase,* they all are sensitive to vanadate inhibition. ***V-type*** (from ***v***acuole type) ATPases regulate H^+ gradients and evoke acidification of lysosomes, endosomes, Golgi, and secretory vesicles. ***F-type*** (from energy coupling ***f***actor) ATPases example is *H^+-ATPase/ATP-synthase* which generate ATP energy from moving the proton across inner mitochondrial membrane. There are F_1 and F_0 subcomplexes in this enzyme: F_1 generates ATP, F_0 lets H^+ go through the membrane and may bind olygomycin that is ATP synthase blocker. There are also **ABC transporters** (from *A*TP-***b***inding ***c***assette) - proteins for active transport of hydrophobic organic chemicals.

The P-type ATPases, also known as ***E1-E2 ATPases,*** are a large group of evolutionarily related ion pumps that are found in bacteria, archaea and eukaryotes. They named P-

type ATPases ***because they catalyze auto- (or self-) phosphorylation of a key conserved aspartate residue within the pump molecule. So they form a high-energy aspartyl-phosphoranhydride intermediate in the reaction cycle, and they interconvert between at least two different conformations, denoted by E1 and E2.*** The E1-E2 notation stems from the initial studies on this family of enzymes made on the Na^+,K^+-ATPase, where the sodium form and the potassium form are referred to as E1 and E2, respectively.

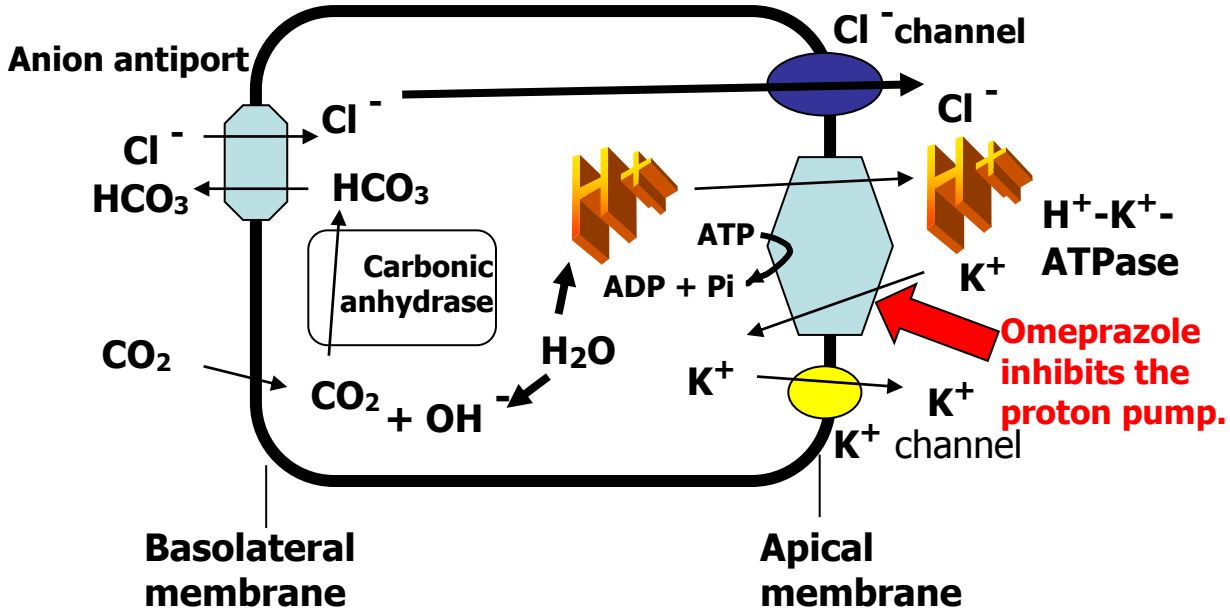
Na^+,K^+-pump is α2β2 tetramer. The α-subunit has 12 transmembrane domains (TMDs). The β-subunit is glycosylated and has one TMD. It actively transports 3 Na^+ ions out and 2 K^+ ions into the cell using a single ATP molecule. This causes a higher concentration of sodium outside and a higher concentration of potassium inside the cell. Such an unequal transport of charge generates a voltage across the membrane or a membrane potential. The outside will become relatively positive and the inside relatively negative since $3Na^+$ (+3 charges) go out for every $2K^+$ (+2 charges) coming in. The result is an electrochemical gradient - a combination of chemical concentration gradient and electrical gradient.

Certain steroids derived from plants are potent inhibitors of Na^+,K^+-pump. So, cardiotonic steroid digitalis from the dried leaf of the foxglove plant inhibits the dephosphorylation of the E2-P form of the ATPase and increases the force of heart muscle contraction because inhibition of Na^+,K^+-pump causes higher level of Na^+ inside the cell, slower extrusion of Ca^{2+} by the Na^+,Ca^{2+}-exchanger and increase in the intracellular level of Ca^{2+} that enhances the ability of cardiac muscle to contract.

The ***plasma membrane Ca^{2+}-ATPase*** (***PMCA***) is a transport protein in the plasma membrane of cells that serves to remove calcium from the cell with a stoichiometry of one Ca^{2+} ion removed for each molecule of hydrolyzed ATP. It is vital for regulating the amount of Ca^{2+} within cells.

The ***sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)*** is a calcium ATPase-type P-ATPase. It is a Ca^{2+}-ATPase that transfers two Ca^{2+} ions from the cytosol of the cell to the lumen of the sarcoplasmic reticulum at the expense of ATP hydrolysis during muscle relaxation.

The ***gastric hydrogen potassium ATPase*** or ***H^+,K^+-ATPase*** is the proton pump of the stomach and, as such, is the enzyme primarily responsible for the acidification of the stomach contents. The H^+,K^+-ATPase is found in parietal cells, which are highly specialized epithelial cells located in the inner cell lining of the stomach called the gastric mucosa. Parietal cells possess an extensive secretory membrane system and the H^+,K^+-ATPase is the major protein constituent of these membranes. The H^+,K^+-ATPase transports one hydrogen ion (H^+) from the cytoplasm of the parietal cell in exchange for one potassium ion (K^+) retrieved from the gastric lumen.



As ion pump the H^+,K^+-ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transferred from adenosine triphosphate to the H^+,K^+-ATPase during the transport cycle. This phosphate transfer powers a conformational change in the enzyme that helps drive ion transport. H^+,K^+-ATPase is an electroneutral antiport: K^+ is removed by K^+-channel and concurrently Cl^- channel removes Cl^- to the same direction.

Two drug categories are commonly used to inhibit H^+,K^+-ATPase activity. Histamine receptor H_2 antagonists like *cimetidine* (Tagamet) inhibit the signaling pathway that leads to histamine-dependent activation of the ATPase. Proton pump inhibitors like *omeprazole* (Prilosec) directly bind to and inactivate the H^+,K^+-ATPase.

ABC (ATP-binding cassette) transporters pass wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids, sterols and drugs**.** Their molecules have 6 or 12 trans-membrane helices with 2 ATP binding sites, drug or ligand- binding sites yet to be clearly identified. Examples are multidrug-resistance protein (MRPs), p-glycoproteins (P-gp - bile salt export pump, multidrug-resistance glycoproteins (MDRs) etc.) that cause active transport and one transporter which accomplishes facilitate diffusion - chloride channel CFTR

Multidrug resistance proteins and glycoproteins are chemical pumps which use ATP energy to actively remove the hydrophobic drugs out of the cells and cause development of resistance to one drug also makes the cell less sensitive to a range of other compounds. After ATP hydrolysis, changes in their structures facilitate the movement of hydrophobic drugs.

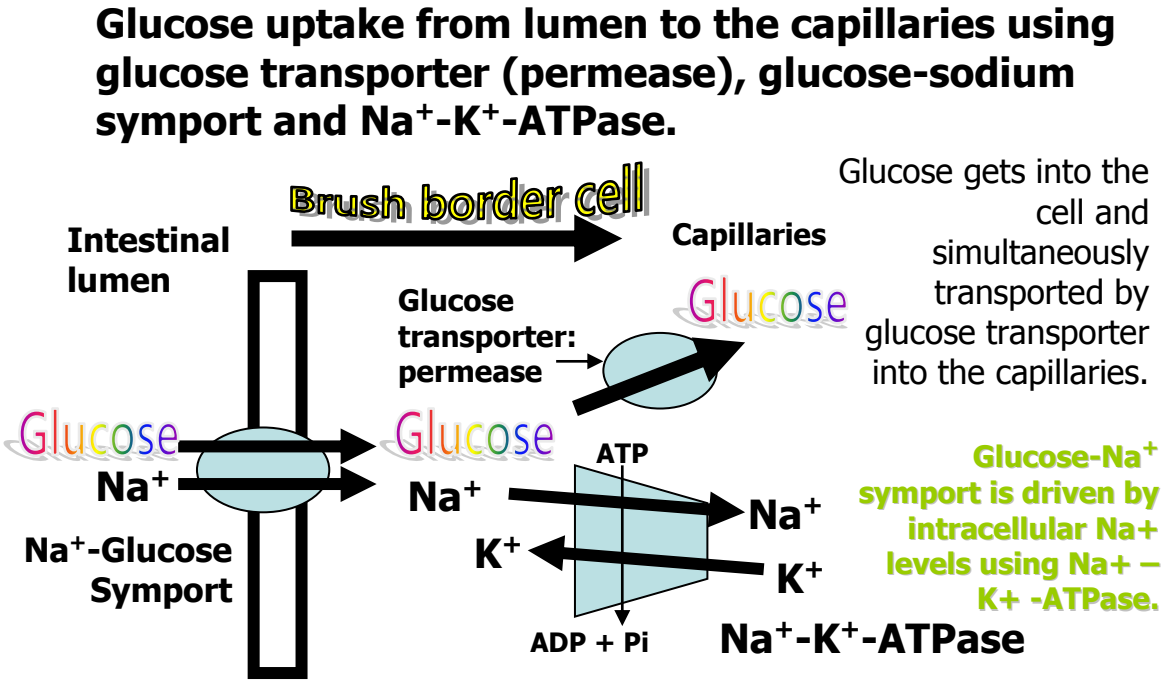
2.3.2. Secondary active transport (cotransport, coupled transport)

A single ATP-powered pump that transports a specific solute can indirectly drive the active transport of several other solutes in a mechanism called cotransport. A substance that has been pumped across a membrane can do work as it moves back across the membrane by diffusion, analogous to water that has been pumped uphill and performs work as it flows back down. Another transport protein, a ***cotransporter,*** separate from the pump, ***can couple the "downhill" diffusion of this substance to the "uphill" transport of a second substance against its own concentration (or electrochemical) gradient***. So secondary active transport needs in special carrier proteins which can use passive flow of one ion (e.g., Na^+) to pull in another ion against its concentration gradient.

The two main forms of secondary active transport are antiport and symport. In ***antiport*** two species of ion or other solutes are pumped in opposite directions across a membrane. An example is the *sodium-calcium exchanger* or antiporter, which allows three sodium ions into the

cell to transport one calcium ion out. By ***symport*** the two species move in the same direction across the membrane. An example is the *glucose symporter SGLT1*, which co-transports one glucose (or galactose) molecule into the cell for every two sodium ions it imports into the cell. This symporter is located in the small intestines, trachea, heart, brain, testis, and prostate.

Primary and secondary active transporters work coordinately in animal cells. They generate membrane potential, generate proton gradient, maintain acidity, etc.



2.3.3. Cytosis as active transport

Large molecules, such as proteins and polysaccharides, as well as larger particles, generally cross the membrane in bulk by mechanisms that involve packaging in vesicles. Like active transport, these processes require energy.

The cell secretes certain biological molecules by the fusion of vesicles with the plasma membrane; this process is called ***exocytosis***. A transport vesicle that has budded from the Golgi apparatus moves along microtubules of the cytoskeleton to the plasma membrane. When the vesicle membrane and plasma membrane come into contact, specific proteins rearrange the lipid molecules of the two bilayers so that the two membranes fuse. The contents of the vesicle then spill to the outside of the cell, and the vesicle membrane becomes part of the plasma membrane.

Many secretory cells use exocytosis to export products. For example, the cells in the pancreas that make insulin secrete it into the extracellular fluid by exocytosis. Other example, neurons (nerve cells) use exocytosis to release neurotransmitters that signal other neurons or muscle cells.

In ***endocytosis***, the cell takes in biological molecules and particulate matter by forming new vesicles from the plasma membrane. Although the proteins involved in the process are different, the events of endocytosis look like the reverse of exocytosis. A small area of the plasma membrane sinks inward to form a pocket. As the pocket deepens, it pinches in, forming a vesicle containing material that had been outside the cell.

There are three types of endocytosis: phagocytosis (“cellular eating”), pinocytosis (“cellular drinking”), and receptor-mediated endocytosis.

In *phagocytosis*, a cell engulfs a particle by wrapping pseudopodia around it and packaging it within a food vacuole. The particle is digested after the food vacuole fuses with a lysosome containing hydrolytic enzymes.

In *pinocytosis*, the cell "gulps" droplets of extracellular fluid into tiny vesicles. It is not the fluid itself that is needed by the cell, but the molecules (salts, monomers, small molecules) dissolved in the droplets. These small molecules are transported by proteins out of the vesicles and into the cytosol. Because any and all included solutes are taken into the cell, pinocytosis is nonspecific in the substances it transports.

Receptor-mediated endocytosis enables the cell to acquire bulk quantities of specific substances, even though those substances may not be very concentrated in the extracellular fluid. This type of endocytosis needs in membrane receptors to which specific ligands bind.

Then the receptor proteins cluster in regions of the membrane called *coated pits*, which are lined on their cytoplasmic side by a fuzzy layer of *coat protein clathrin*. *Adaptin* (4 types) binds clathrin and receptors, acting as a bridge. Next, each coated pit forms a coated vesicle containing the ligand molecules. There are relatively more bound molecules inside the vesicle, but other molecules are also present. *Dynamin* forms a ring around the bud (GTPase). After the ingested material is liberated from the vesicle, the emptied receptors are recycled to the plasma membrane by the same vesicle. *Hsp70 chaperone* and *auxillin* uncoat the vesicle.

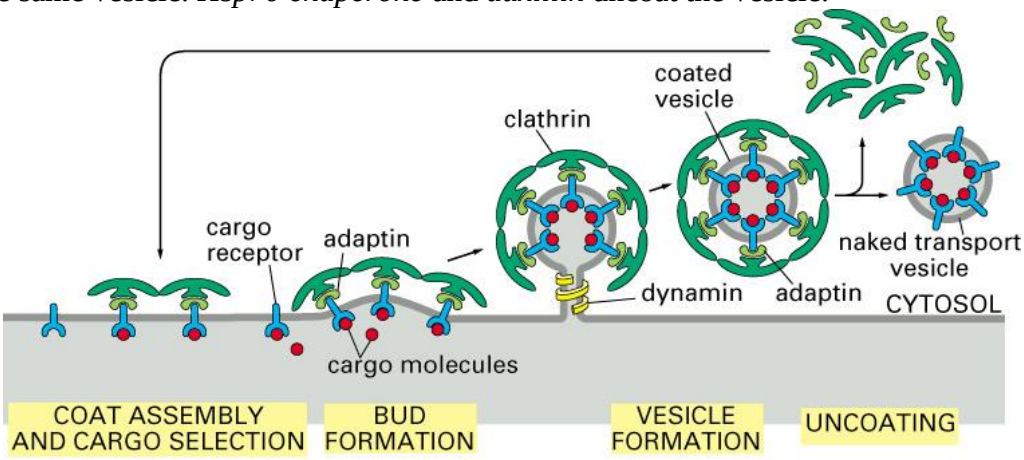


Figure 13–8. Molecular Biology of the Cell, 4th Edition.

Human cells use receptor-mediated endocytosis *to take in cholesterol* for membrane synthesis and the synthesis of other steroids. Cholesterol travels in blood in particles called low-density lipoproteins (LDLs), a complex of lipids and a protein. LDLs bind to LDL receptors on plasma membranes and then enter the cells by endocytosis (LDLs thus act as ligands, a term for any molecule that binds specifically to a receptor site on another molecule). In humans with *familial hypercholesterolemia*, an inherited disease characterized by a very high level of cholesterol in the blood, LDLs cannot enter cells because the LDL receptor proteins are defective or missing. Consequently, cholesterol accumulates in the blood, where it contributes to early atherosclerosis, the buildup of lipid deposits within the walls of blood vessels. This buildup causes the walls to bulge inward, thereby narrowing the vessels and impeding blood flow.

Vesicles not only transport substances between the cell and its surroundings but also provide a mechanism for rejuvenating or remodeling the plasma membrane.

2.4. Test quiescence

1. *What ion channel has such characters: blocked by a specific neurotoxin (Tetrodotoxin from puffer fish); has an α-subunit, which contains 4 internal repeats - each repeat contains 5 hydrophobic segments (S1, S2, S3, S5, S6). Segment S4 is highly positive charged and forms voltage sensor of the channel.*

A) The Voltage-Gated Sodium Channel;
B) The Voltage-Gated Potassium channel;
C) CFTR;
D) aquaporine;
E) The Voltage-Gated Calcium Channel;

F) Acetylcholine-gated cation channel

2. Glucose is a water soluble molecule. By what route does it pass through the cell membrane?

A) Transferred by the carrier proteins
B) Diffuses across the membrane
C) Passes between the phospholipid molecules
D) Enters in a vesicle

3. Which of the following is the difference between active transport and facilitated diffusion?

A) Active transport involves membrane proteins, but facilitated does not.
B) Active transport requires energy from the cell, but facilitated diffusion does not.
C) Facilitated diffusion involves membrane proteins, but active transport uses active proteins.
D) Facilitated diffusion requires energy from the cell and active transport does not.

4. What is the function of a P-glycoprotein?

A) They are involved in the anchoring of cells to an extracellular matrix.
B) They break down the basement membranes round blood vessels prior to angiogenesis.
C) They are responsible for the resistance shown by some cancer cells to anticancer drugs.
D) They induce metastasis.

5. ***Which structures realize facilitated diffusion?***

A) Na+, glucose-cotransport protein;
B) P-glycoproteins;
C) ionophores;
D) GLUT-1;
E) CFTR;
F) H+-ATPase;
G) The Voltage-Gated Sodium Channels activators

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