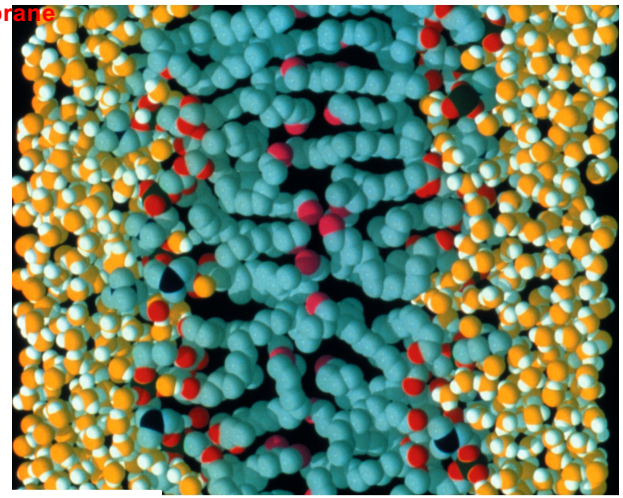
Molecular and Cellular Biology

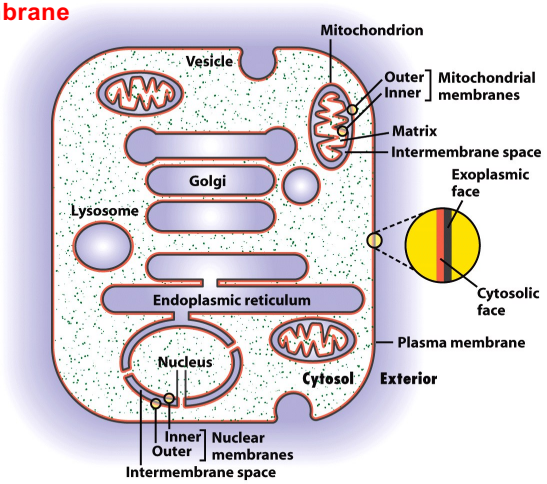
6. Biomembrane: Structure & Function

Prof. Dr. Klaus Heese

Biomembrane

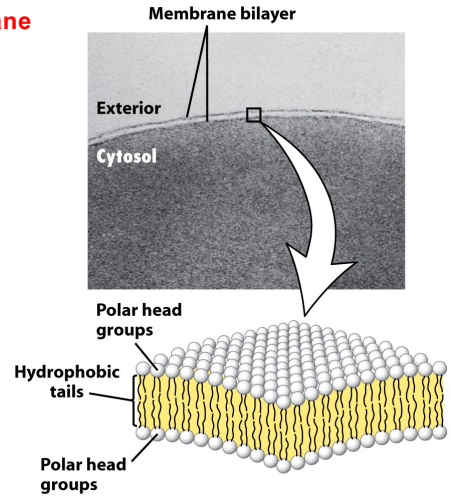


BioMembrane

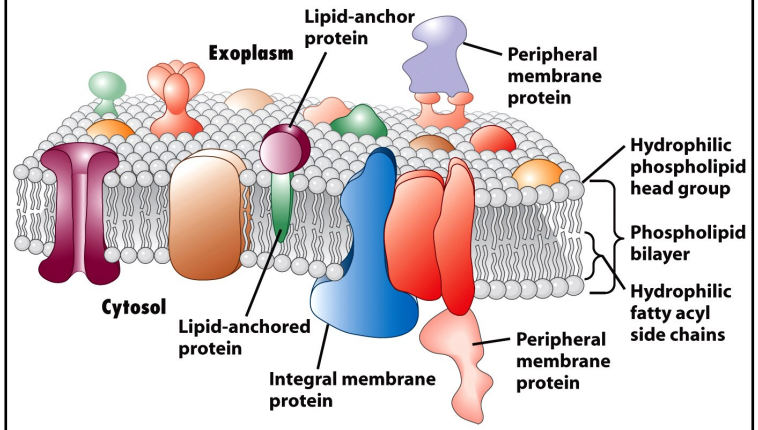


A Cell and its Sub-Compartments with BioMembrane Systems

Biomembrane



Biomembrane



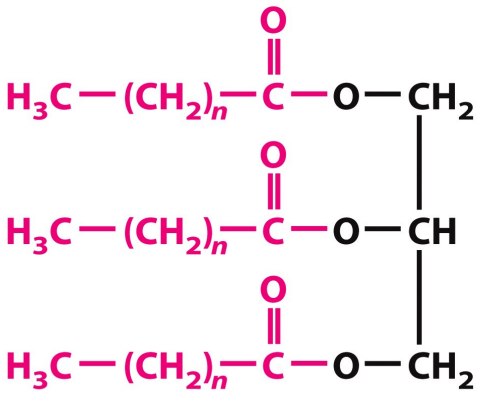
Biomembrane Molecules

Major Lipid Components of Selected Biomembranes

SOURCE/LOCATION	COMPOSITION (MOL %) PC	PE + PS	SM	CHOLESTEROL
Plasma membrane (human erythrocytes)	21	29	21	26
Myelin membrane (human neurons)	16	37	13	34
Plasma membrane (*E. coli*)	0	85	0	0
Endoplasmic reticulum membrane (rat)	54	26	5	7
Golgi membrane (rat)	45	20	13	13
Inner mitochondrial membrane (rat)	45	45	2	7
Outer mitochondrial membrane (rat)	34	46	2	11
Primary leaflet location	Exoplasmic	Cytosolic	Exoplasmic	Both

PC = phosphatidylcholine; PE = phosphatidylethanolamine; PS = phosphatidylserine; SM = sphingomyelin.
SOURCE: W. Dowhan and M. Bogdanov, 2002, in D. E. Vance and J. E. Vance, eds., *Biochemistry of Lipids, Lipoproteins, and Membranes*, Elsevier.

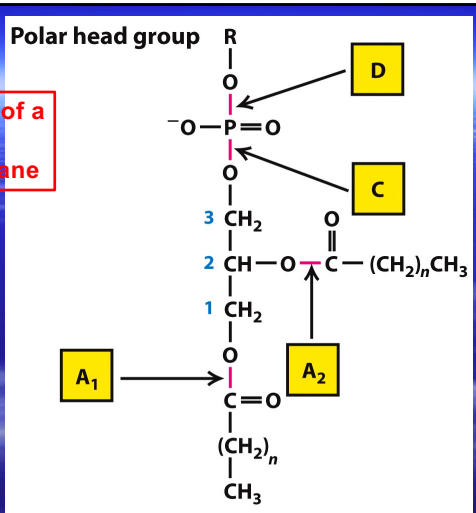
Molecules of a Biomembrane



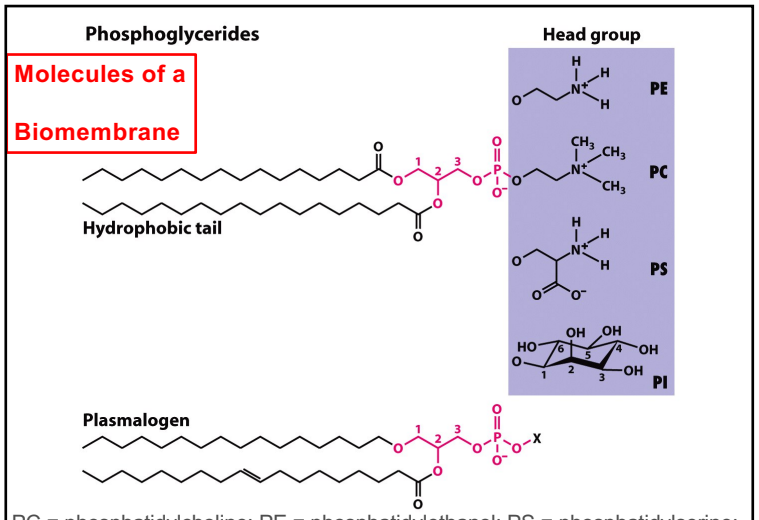
Triacylglycerol

---> fatty acid metabolism

Molecules of a Biomembrane

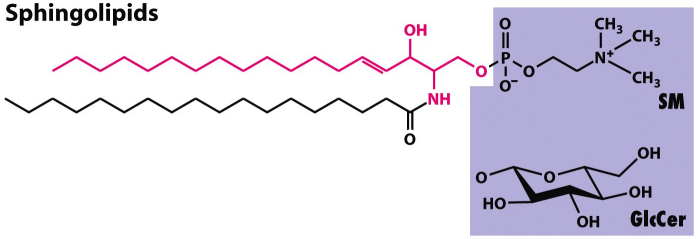


Molecules of a Biomembrane



PC = phosphatidylcholine; PE = phosphatidylethanol; PS = phosphatidylserine; SM = sphingomyelin; PI = phosphoinositol

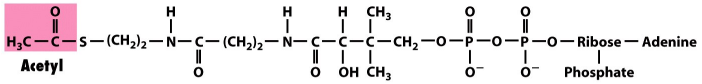
Molecules of a Biomembrane



PC = phosphatidylcholine; PE = phosphatidylethanol; PS = phosphatidylserine; SM = sphingomyelin; PI = phosphoinositol

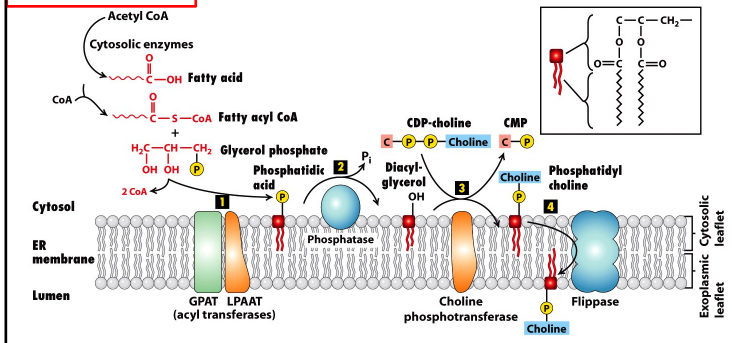
Molecules of a Biomembrane

Acetyl-CoA

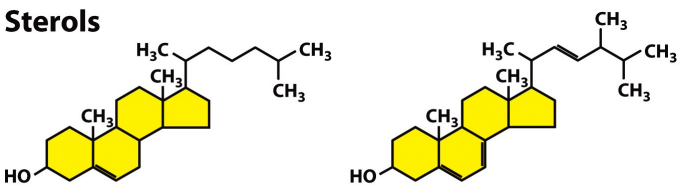


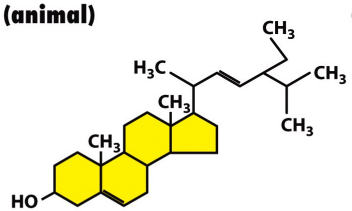
Coenzyme A (CoA)

Molecules of a Biomembrane



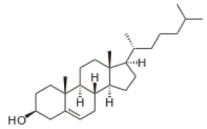
Sterols



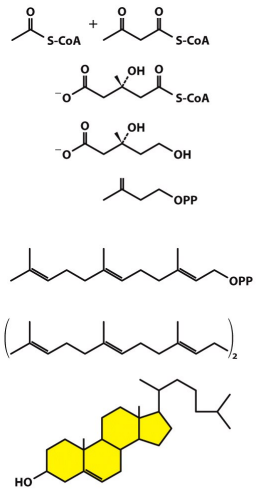


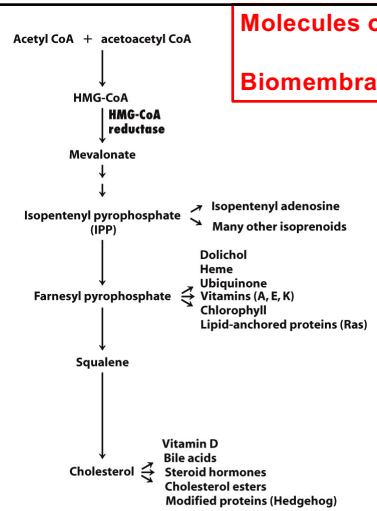
Molecules of a Biomembrane

Cholesterol

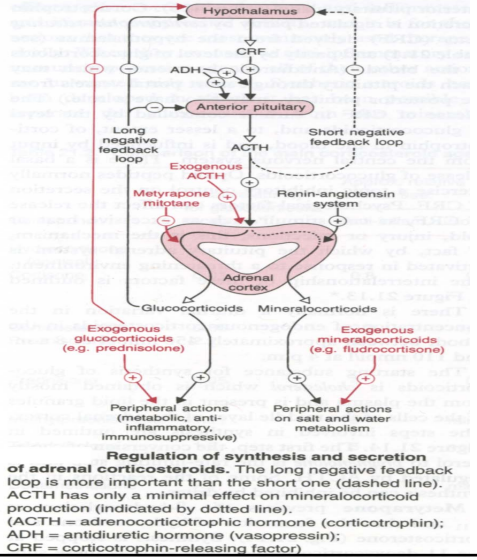


- Plant and animal food contain sterols but only animal food contains cholesterol
- Why? Cholesterol is made in the liver and plants do not have a liver
- Cholesterol is needed to make bile, sex hormones, steroids and vitamin D.
- It is the constituent of cell membrane structure
- Dietary recommendation - <300 mg/d
- Sources – egg yolks, liver, shellfish, organ foods

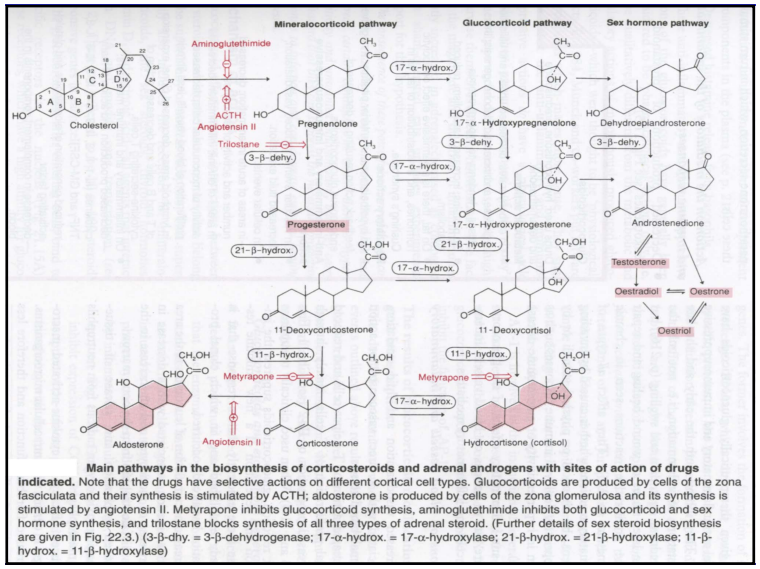




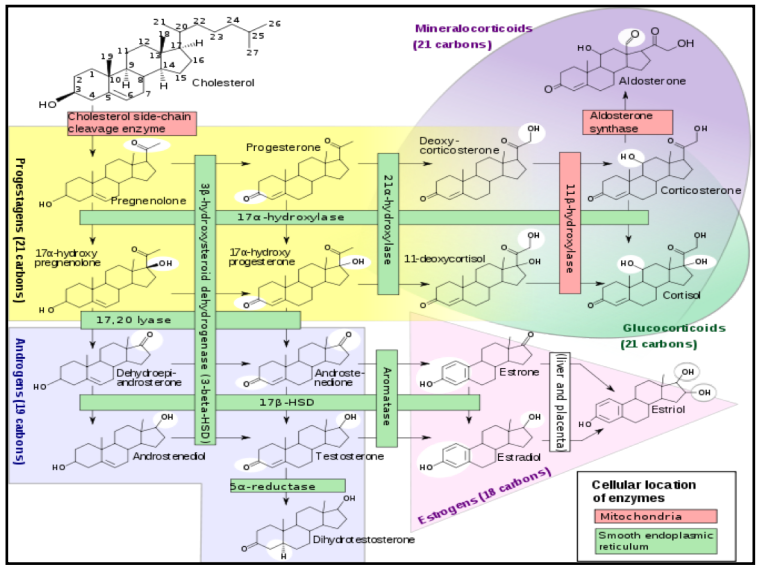
Molecules of a Biomembrane

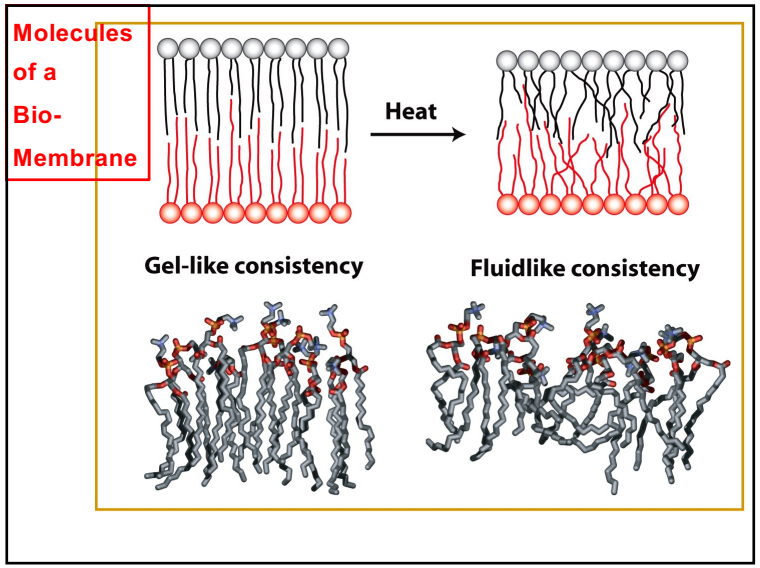


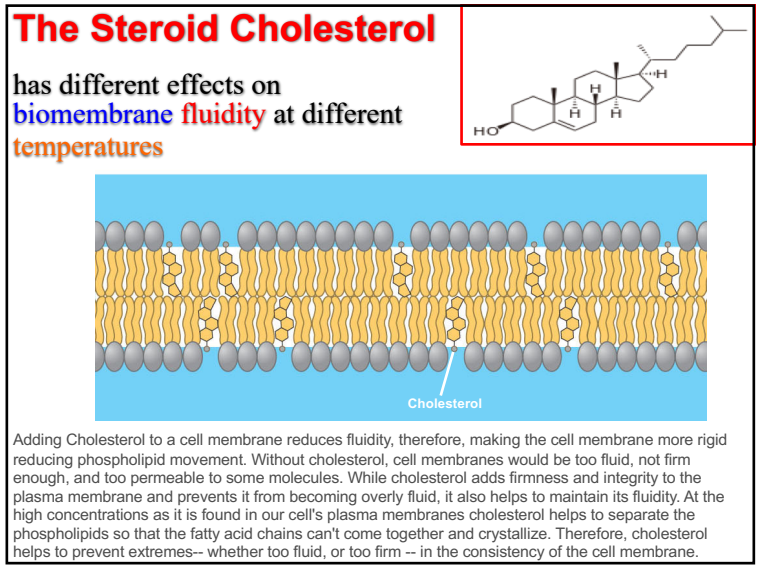
Regulation of synthesis and secretion of adrenal corticosteroids. The long negative feedback loop is more important than the short one (dashed line). ACTH has only a minimal effect on mineralocorticoid production (indicated by dotted line). (ACTH = adrenocorticotrophic hormone (corticotrophin); ADH = antidiuretic hormone (vasopressin); CRF = corticotrophin-releasing factor)



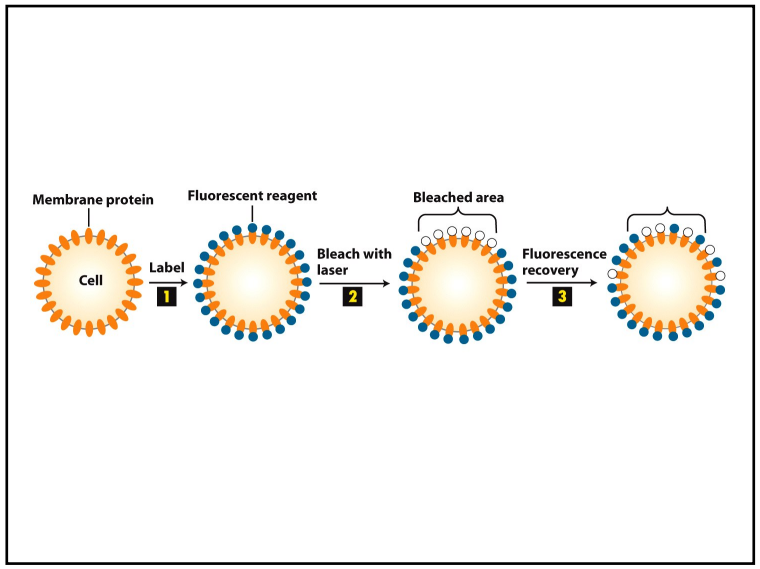
Main pathways in the biosynthesis of corticosteroids and adrenal androgens with sites of action of drugs indicated. Note that the drugs have selective actions on different cortical cell types. Glucocorticoids are produced by cells of the zona fasciculata and their synthesis is stimulated by ACTH; aldosterone is produced by cells of the zona glomerulosa and its synthesis is stimulated by angiotensin II. Metyrapone inhibits glucocorticoid synthesis, aminoglutethimide inhibits both glucocorticoid and sex hormone synthesis, and trilostane blocks synthesis of all three types of adrenal steroid. (Further details of sex steroid biosynthesis are given in Fig. 22.3.) (3-β-dhy. = 3-β-dehydrogenase; 17-α-hydrox. = 17-α-hydroxylase; 21-β-hydrox. = 21-β-hydroxylase; 11-β-hydrox. = 11-β-hydroxylase)

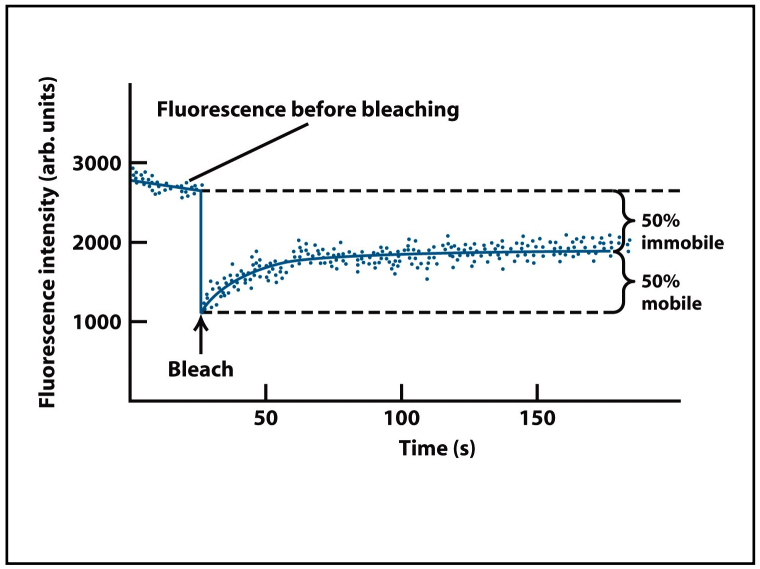


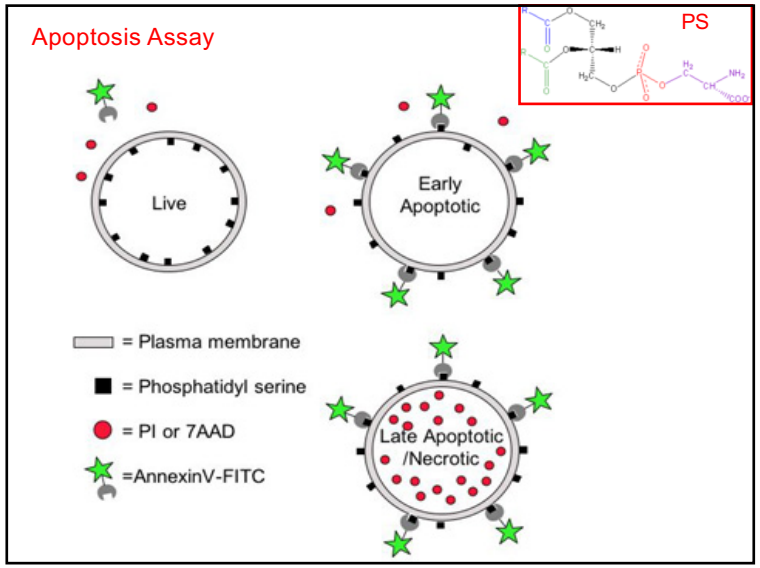


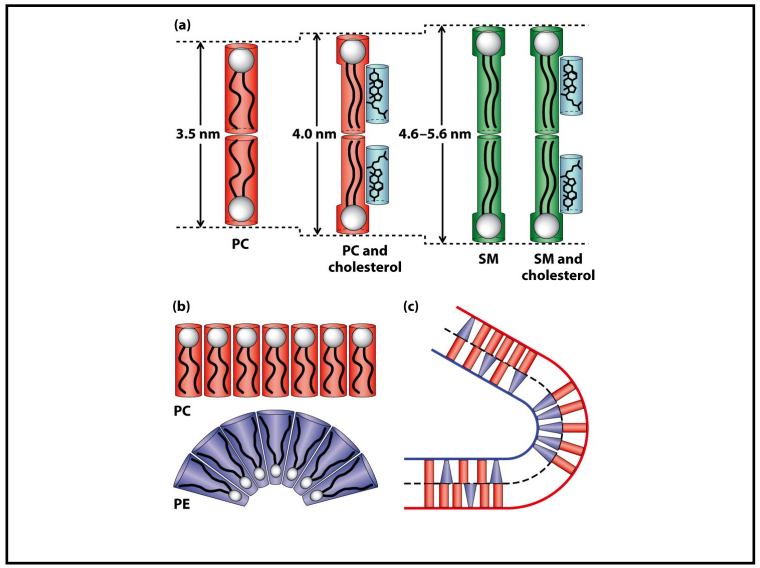


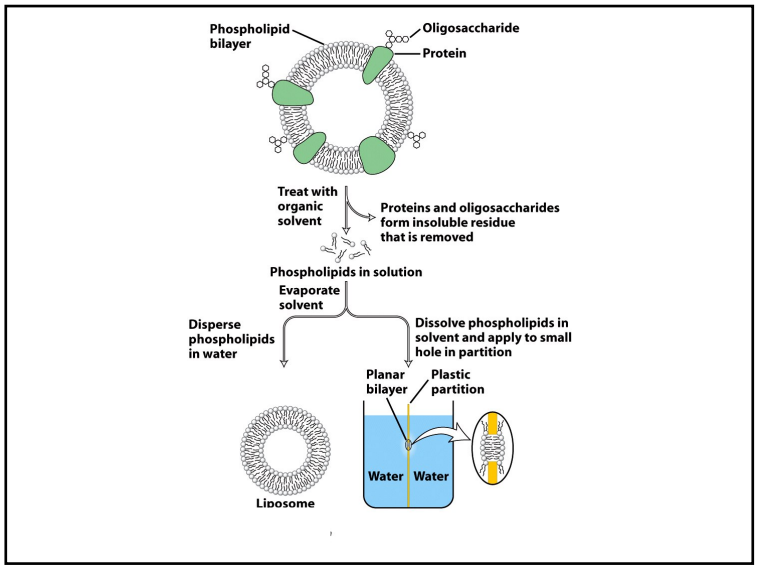
Adding Cholesterol to a cell membrane reduces fluidity, therefore, making the cell membrane more rigid reducing phospholipid movement. Without cholesterol, cell membranes would be too fluid, not firm enough, and too permeable to some molecules. While cholesterol adds firmness and integrity to the plasma membrane and prevents it from becoming overly fluid, it also helps to maintain its fluidity. At the high concentrations as it is found in our cell's plasma membranes cholesterol helps to separate the phospholipids so that the fatty acid chains can't come together and crystallize. Therefore, cholesterol helps to prevent extremes-- whether too fluid, or too firm -- in the consistency of the cell membrane.


Membrane protein
Fluorescent reagent
Bleached area
Cell
Label
1
Bleach with laser
2
Fluorescence recovery
3

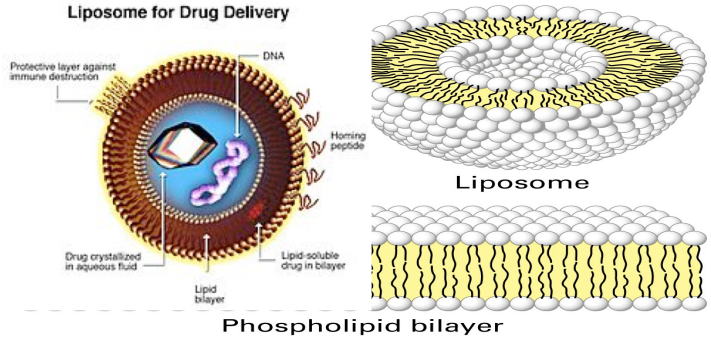

Fluorescence intensity (arb. units)
Fluorescence before bleaching
3000
2000
1000
50% immobile
50% mobile
Bleach
50
100
150
Time (s)


Apoptosis Assay
PS
Live
Early Apoptotic
= Plasma membrane
= Phosphatidyl serine
= PI or 7AAD
=AnnexinV-FITC
Late Apoptotic /Necrotic


(a)
3.5 nm
4.0 nm
4.6–5.6 nm
PC
PC and cholesterol
SM
SM and cholesterol
(b)
PC
PE
(c)

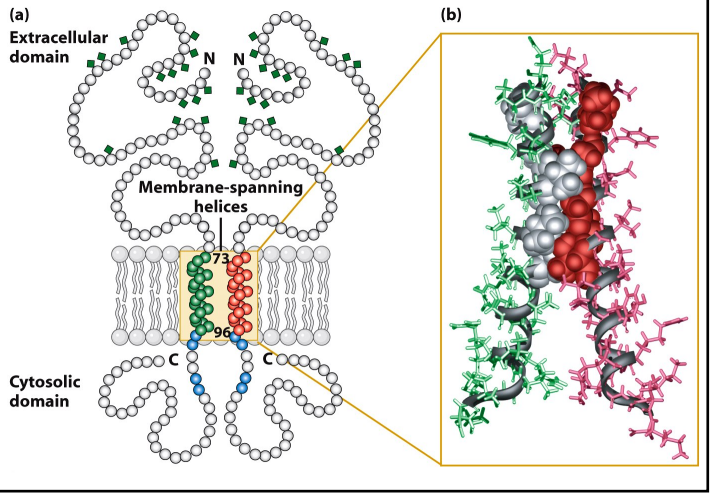


Cross-sectional views of the three structures formed by phospholipids in aqueous solutions

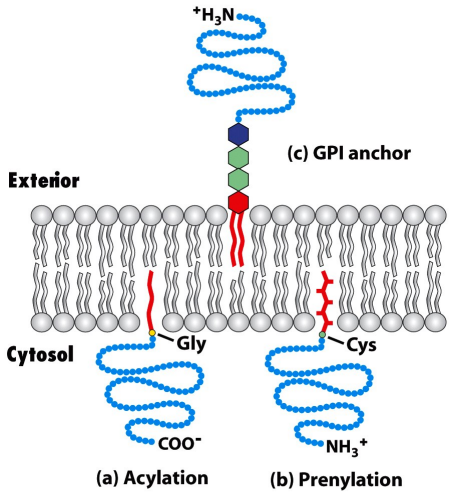


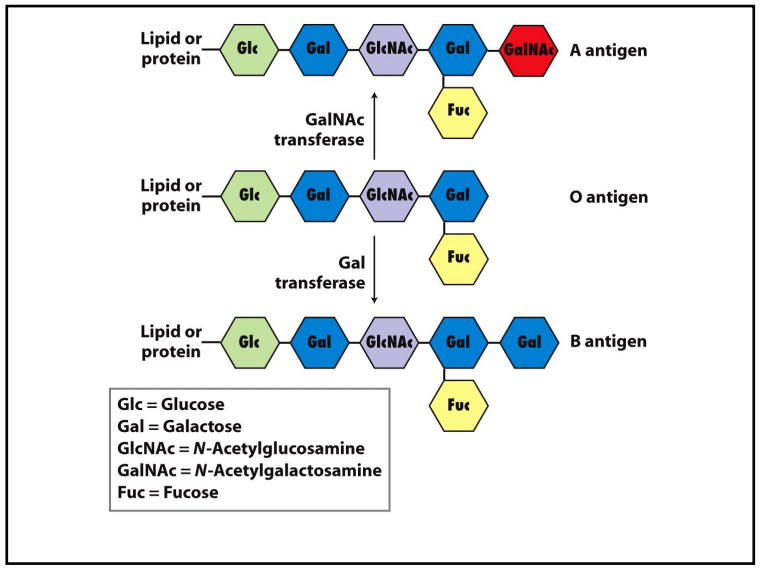
The white spheres depict the hydrophilic heads of the phospholipids, and the squiggly black lines (in the yellow regions) represent the hydrophobic tails. Shown are a spherical micelle with a hydrophobic interior composed entirely of fatty acyl chains; a spherical liposome, which has two phospholipid layers and an aqueous center; and a two-molecule-thick sheet of phospholipids, or bilayer, the basic structural unit of bio-membranes.

Molecules of a Biomembrane – Membrane Proteins



Molecules of a Biomembrane – Membrane Proteins

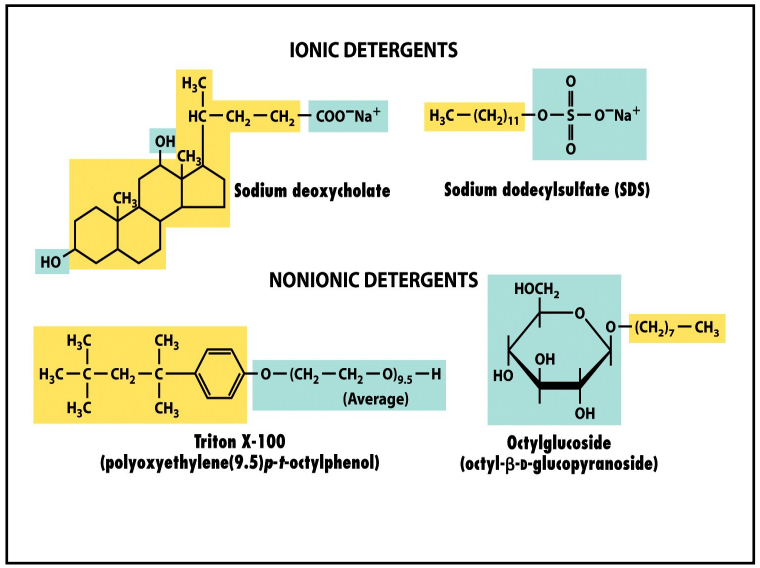


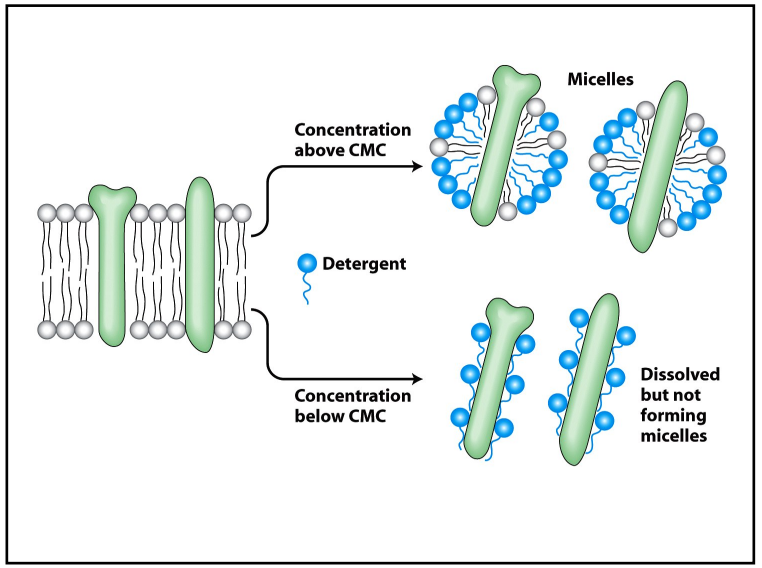


Molecules of a Biomembrane – Membrane Proteins

TABLE 10-2 ABO Blood Groups

BLOOD GROUP	ANTIGENS ON RBCS*	SERUM ANTIBODIES	CAN RECEIVE BLOOD TYPES
A	A	Anti-B	A and O
B	B	Anti-A	B and O
AB	A and B	None	All
O	O	Anti-A and anti-B	O





Biomembranes – Endo- / Exo-cytosis

- Bulk transport across the plasma membrane occurs by exocytosis and endocytosis
- Large proteins cross the membrane by different mechanisms

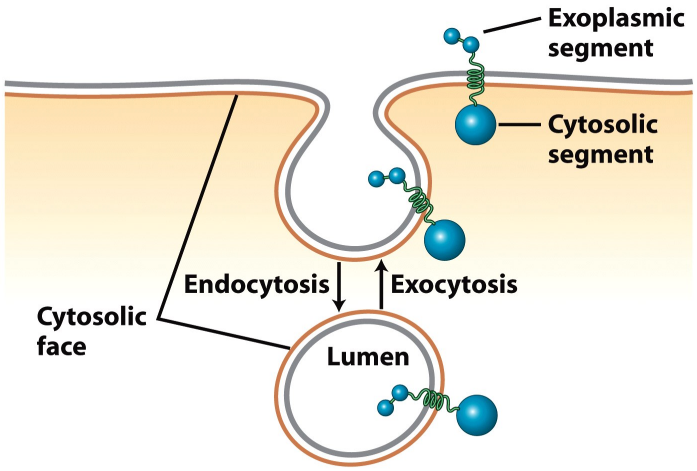
Exocytosis

- In exocytosis transport vesicles migrate to the plasma membrane, fuse with it, and release their contents (compare with synaptic (vesicle) neurotransmitter release)

Endocytosis

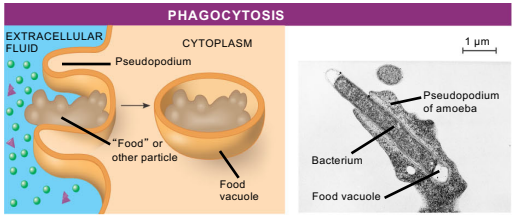
- In endocytosis the cell takes in macromolecules by forming new vesicles from the plasma membrane
- (NGF uptake)

Biomembranes - three types of endocytosis

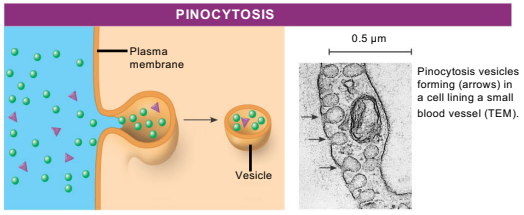


- Three types of endocytosis

In **phagocytosis,** a cell engulfs a particle by Wrapping pseudopodia around it and packaging it within a membrane-enclosed sac large enough to be classified as a vacuole. The particle is digested after the vacuole fuses with a lysosome containing hydrolytic enzymes.

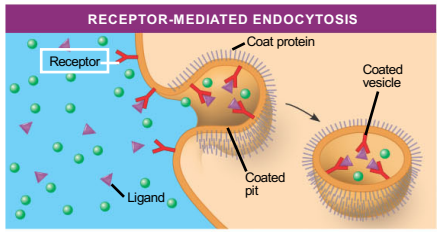


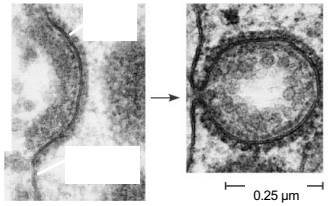
In **pinocytosis,** the cell "gulps" droplets of extracellular fluid into tiny vesicles. It is not the fluid itself that is needed by the cell, but the molecules dissolved in the droplet. Because any and all included solutes are taken into the cell, pinocytosis is nonspecific in the substances it transports.



Pinocytosis vesicles forming (arrows) in a cell lining a small blood vessel (TEM).

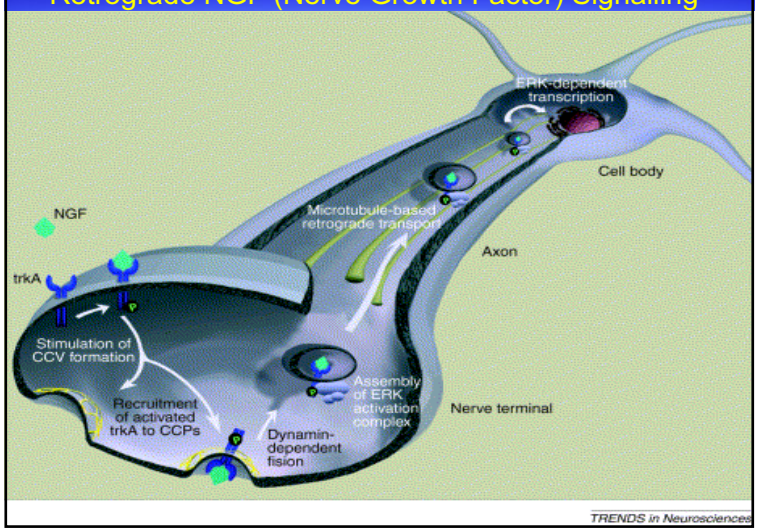
Receptor-mediated endocytosis enables the cell to acquire bulk quantities of specific substances, even though those substances may not be very concentrated in the extracellular fluid. Embedded in the membrane are proteins with specific receptor sites exposed to the extracellular fluid. The receptor proteins are usually already clustered in regions of the membrane called coated pits, which are lined on their cytoplasmic side by a fuzzy layer of coat proteins. Extracellular substances (ligands) bind to these receptors. When binding occurs, the coated pit forms a vesicle containing the ligand molecules. Notice that there are relatively more bound molecules (purple) inside the vesicle, other molecules (green) are also present. After this ingested material is liberated from the vesicle, the receptors are recycled to the plasma membrane by the same vesicle.



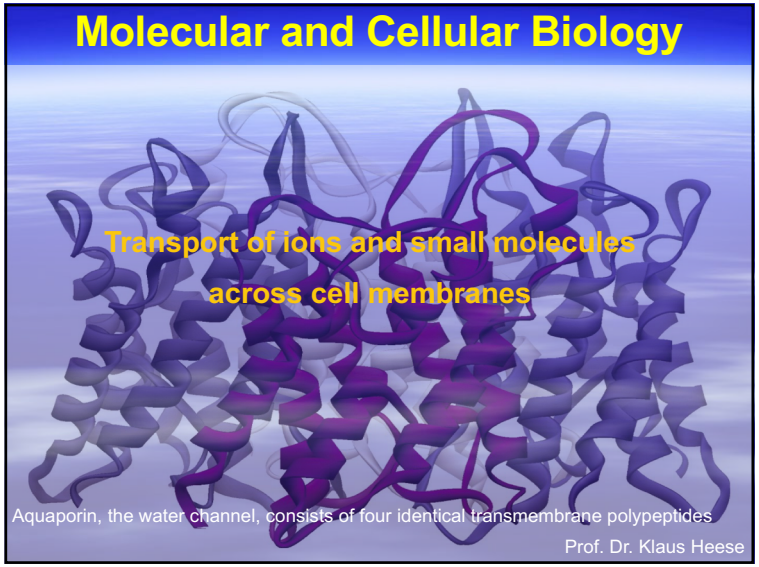


Cell signalling

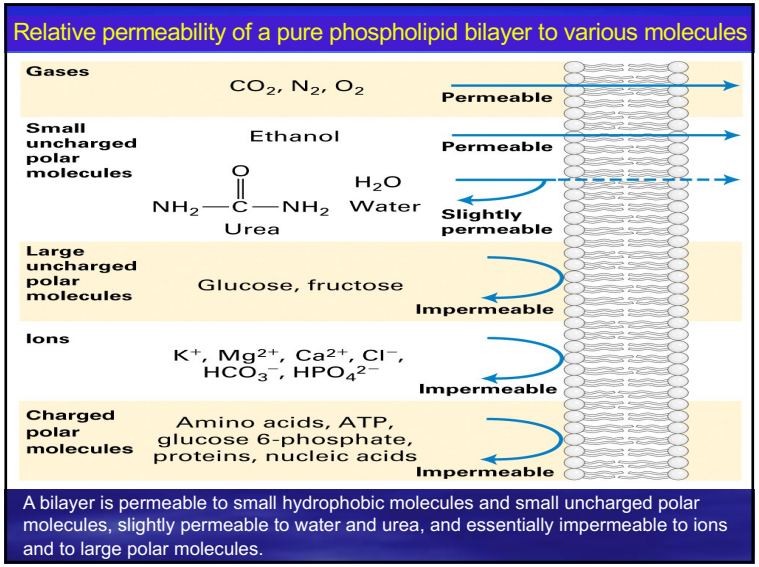
Retrograde NGF (Nerve Growth Factor) Signalling



Molecular and Cellular Biology

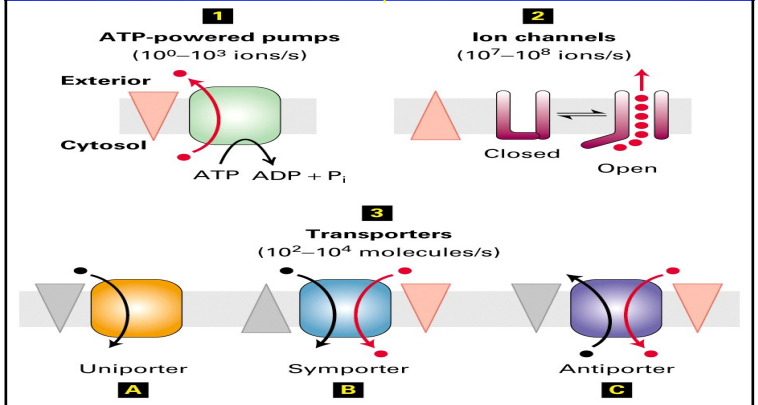


Relative permeability of a pure phospholipid bilayer to various molecules



A bilayer is permeable to small hydrophobic molecules and small uncharged polar molecules, slightly permeable to water and urea, and essentially impermeable to ions and to large polar molecules.

Membrane Transport Proteins



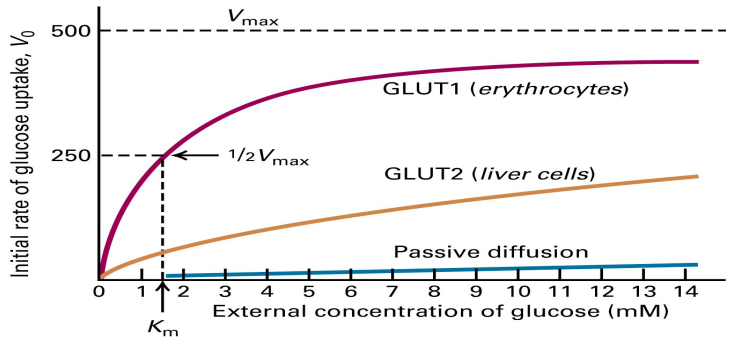
Gradients are indicated by triangles with the tip pointing toward lower concentration, electrical potential, or both. 1: pumps utilize the energy released by ATP hydrolysis to power movement of specific ions (red circles) or small molecules against their electrochemical gradient. 2: Channels permit movement of specific ions (or water) down their electrochemical gradient; they can also be controlled by e.g. ligand binding or phosphorylations etc : Transporters, which fall into three groups, facilitate movement of specific small molecules or ions. Uniporters transport a single type of molecule down its concentration gradient (3A). Cotransport proteins (symporters (3B) and antiporters (3C) catalyze the movement of one molecule against its concentration gradient (black circle), driven by movement of one or more ions down an electrochemical gradient (red circles). Differences in the mechanisms of transport by these three major classes of proteins account for their varying rates of solute movement. Transporters can also depend on ATP.

Mechanisms for Transporting Ions and Small Molecules Across Cell Membranes

	Transport Mechanism			
Property	Passive Diffusion	Facilitated Diffusion	Active Transport	Cotransport*
Requires specific protein	–	+	+	+
Solute transported against its gradient	–	–	+	+
Coupled to ATP hydrolysis	–	–	+	–
Driven by movement of a cotransported ion down its gradient	–	–	–	+
Examples of molecules transported	O_2, CO_2, steroid hormones, many drugs	Glucose and amino acids (uniporters); ions and water (channels)	Ions, small hydrophilic molecules, lipids (ATP-powered pumps)	Glucose and amino acids (symporters); various ions and sucrose (antiporters)

*Also called *secondary active transport*.

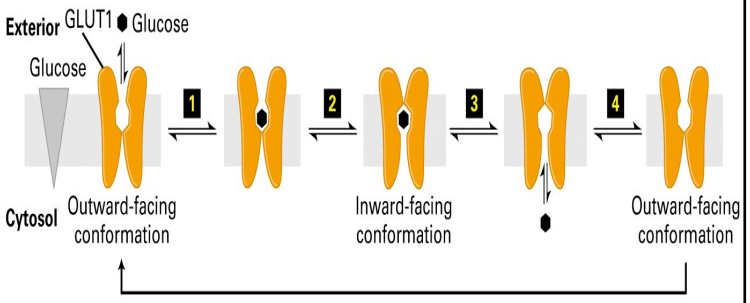
Cellular uptake of glucose mediated by GLUT proteins exhibit simple enzyme kinetics and greatly exceeds the calculated rate of glucose entry solely by passive diffusion



The initial transport rate for the substrate S into the cell catalyzed by e.g. GLUT1: v=Vmax/(1+Km/[S])

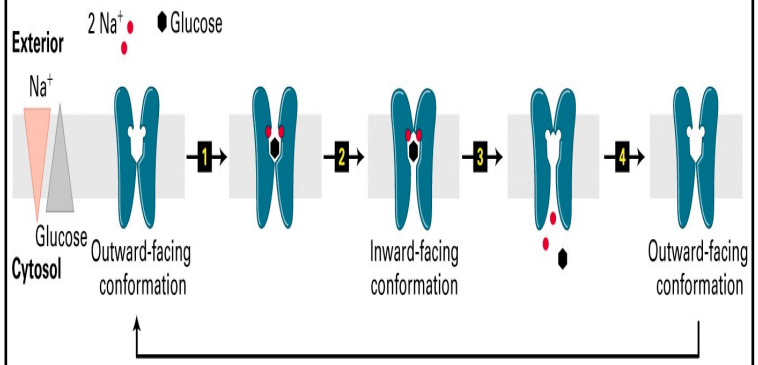
The initial rate of glucose uptake (measured as micromoles per milliliter of cells per hour) in the first few seconds is plotted against increasing glucose concentration in the extracellular medium. In this experiment, the initial concentration of glucose in the cells is always zero. Both, GLUT1, expressed by erythrocytes, and GLUT2, expressed by liver cells, greatly increase the rate of glucose uptake (red and orange curves) at all external concentrations. Like enzyme-catalyzed reactions, GLUT-facilitated uptake of glucose exhibits a maximum rate (Vmax). The Km is the concentration at which the rate of glucose uptake is half maximal. GLUT2, with a Km of about 20 mM, has a much lower affinity for glucose than GLUT1, with a Km of about 1.5 mM.

Model of Uniport Transport by GLUT1



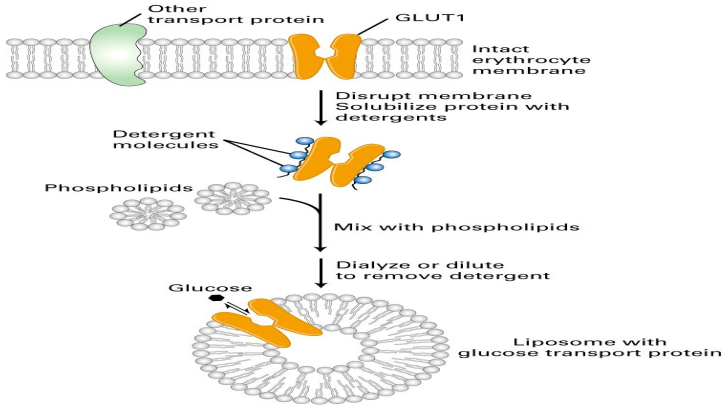
In one conformation, the glucose-binding site faces outward; in the other, the binding site faces inward. Binding of glucose to the outward-facing site (step-1) triggers a conformational change in the transporter that results in the binding site facing inward toward the cytosol (step-2). Glucose then is released to the inside of the cell (step 3). Finally, the transporter undergoes the reverse conformational change, regenerating the outward-facing binding site (step 4). If the concentration of glucose is higher inside the cell than outside, the cycle will work in reverse (step-4 ---> step-1), resulting in net movement of glucose form inside to out. The actual confomrational changes are probably smaller than those depicted here.

Operational model for the 2-Na^+/1-glucose symporter



Simultaneous binding of Na^+ and glucose to the conformation with outward-facing binding sites (step-1) generates a second conformation with inward-facing site (step-2). Dissociation of the bound Na^+ and glucose into the cytosol (step-3) allows the protein to revert to its original outward-facing conformation (step-4), ready to transport additional substrate.

Liposomes containing a single type of transport protein are very useful in studying functional properties of transport proteins



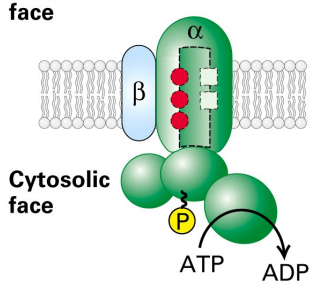
Here, all the integral proteins of the erythrocyte membrane are solubilized by a nonionic detergent, such as octylglucoside. The glucose uniporter GLUT1 can be purified by chromatography on a column containing a specific antibody and then incorporated into liposomes made of pure phospholipids.

ATP-Powered Pumps

And

the Intracellular Ionic Environment

The 4 classes of ATP-powered transport proteins - (1)



P-class pumps

Plasma membrane of plants, fungi, bacteria (H^+ pump)

Plasma membrane of higher eukaryotes (Na^+/K^+ pump)

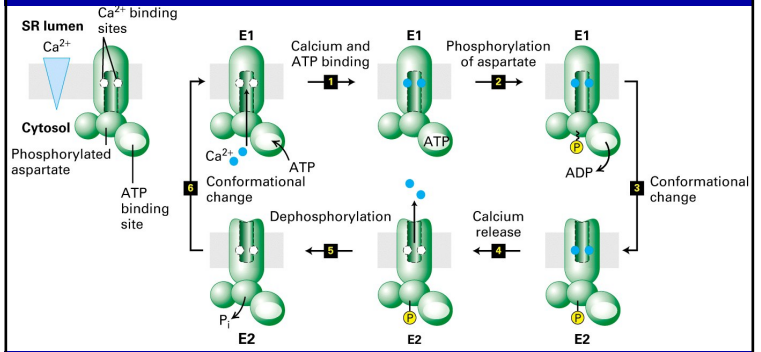
Apical plasma membrane of mammalian stomach (H^+/K^+ pump)

Plasma membrane of all eukaryotic cells (Ca^{2+} pump)

Sarcoplasmic reticulum membrane in muscle cells (Ca^{2+} pump)

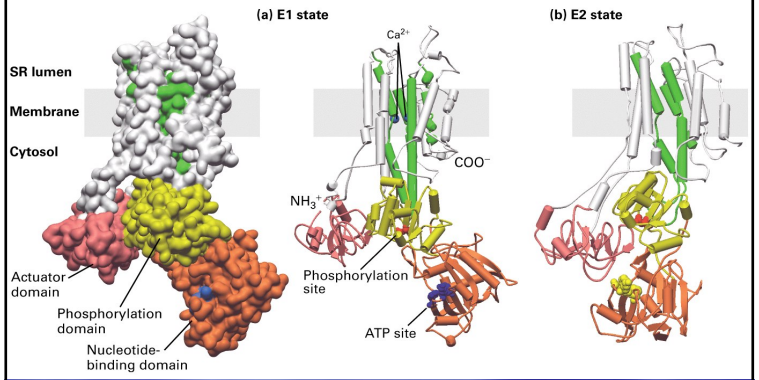
P-class pumps are composed of a catalytic alpha subunit which becomes phosphorylated as part of the transport cycle. A beta subunit, present in some of these pumps, may regulate (regulatory subunit) transport.

Operational model of the Ca^{2+} ATPase in the SR membrane of skeletal muscle cells



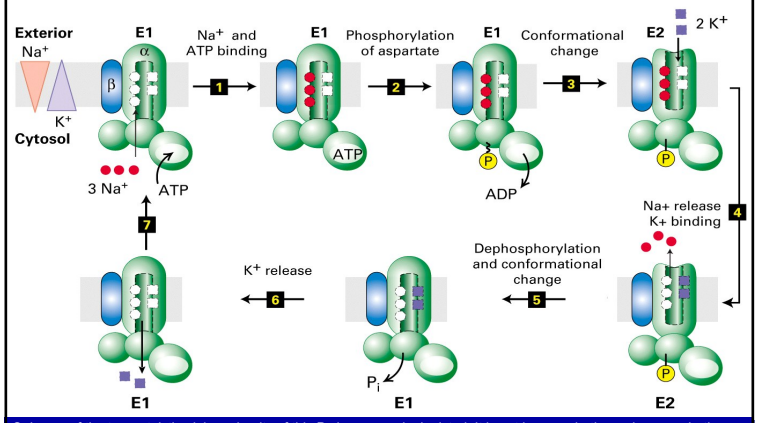
Only one of the two catalytic alpha subunits of this P-class pump is depicted. E1 and E2 are alternative conformations of the protein in which the Ca^{2+}-binding sites are accessible to the cytosolic and exoplasmic faces, respectively. An ordered sequence of steps (1-6) is essential for coupling ATP hydrolysis and the transport of Ca^{2+} ions across the membrane. In this figure ~P indicates a high-energy acyl phosphate bond; -P indicates a low-energy phosphoester bond. Because the affinity of Ca^{2+} for the exoplasmic-facing sites in E2, this pump transports Ca^{2+} uni-directionally from the cytosol to the SR lumen.

Structure of the catalytic alpha subunit of the muscle Ca^{2+} ATPase



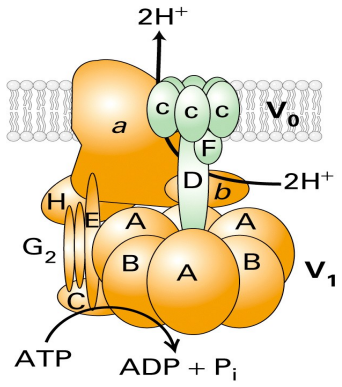
(a) 3-D models of the protein in the E1 state based on the structure determined by X-ray crystallography. There are 10 transmembrane alpha helices, 4 of which (green) contain residues that site-specific mutagenesis studies have identifies as participating in Ca^{2+} binding. The cytosolic segment forms 3 domains: the nucleotide binding domain (orange) the phosphorylation domain (yellow) and the actuator domain (pink) that connects 2 of the membrane-spanning helices.
(b) Hypothetical model of the pump in E2 state, based on a lower resolution structure determined by electron microscopy of frozen crystals of the pure protein. Note the differences between the E1 and E2 states in the confirmations of the nucleotide-binding and actuator domains; these changes probably power the conformational changes of the membrane-spanning alpha helices (green) that constitute the Ca^{2+}-binding site, converting them form one in which the Ca^{2+}-binding sites are accessible to the cytosolic face (E1 state) to one in which they are accessible to the exoplasmic face (E2 state).

Operational model of the Na^+/K^+ ATPase in the plasma membrane



Only one of the two catalytic alpha subunits of this P-class pump is depicted. It is not known whether only one or both subunits in a single ATPase molecule transport ions. Ion pumping by the Na^+/K^+ ATPase (very important in neurons) involves phosphorylation, dephosphorylation and conformational changes similar to those in the muscle Ca^{2+} ATPase, in this case, hydrolysis of the E2-P intermediate powers the E2--->E1 conformational change and concomitant transport of two ions (K^+) inward. Na^+ ions are indicated by red circles; K^+ ions by purple squares; high energy acyl phosphate bond by ~P; low-energy phosphoester bon by -P.

The 4 classes of ATP-powered transport proteins - (2)



V-class proton pumps

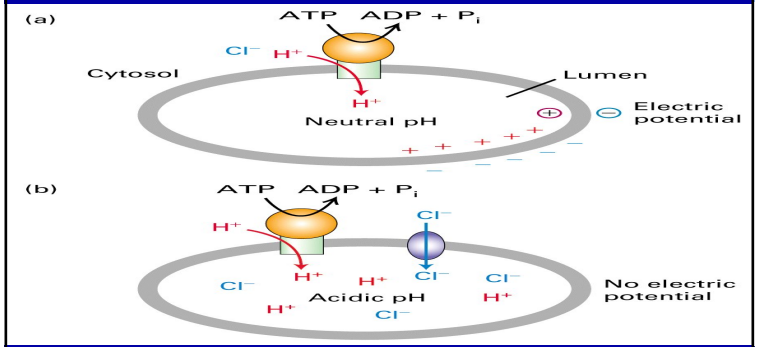
Vacuolar membranes in plants, yeast, other fungi

Endosomal and lysosmal membranes in animal cells

Plasma membrane of osteoclasts and some kidney tubule cells

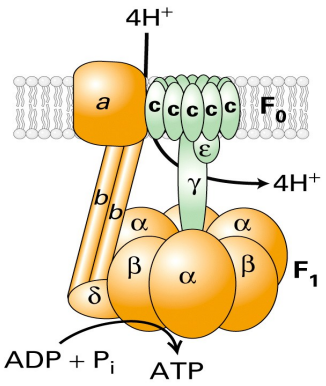
V-class pumps do not form phosphoprotein intermediates and transport only protons. V/F-class structures are similar and contain similar proteins, but none of their subunits are related to the P-class pumps. V-class pumps couple ATP hydrolysis to transport of protons against a concentration gradient.

Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes.



(a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change in the intraluminal pH. (b) if the organelle also contains Cl^- channels, anions passively follow the pumped protons, resulting in an accumulation of H^+ ions (low luminal pH) but no electric potential across the membrane.

The 4 classes of ATP-powered transport proteins - (3)



F-class proton pumps

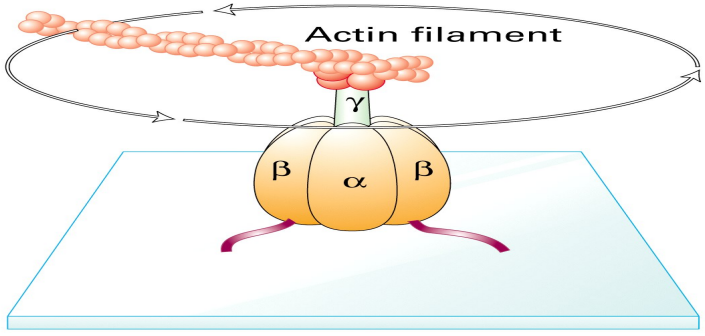
Bacterial plasma membrane

Inner mitochondrial membrane

Thylakoid membrane of chloroplast

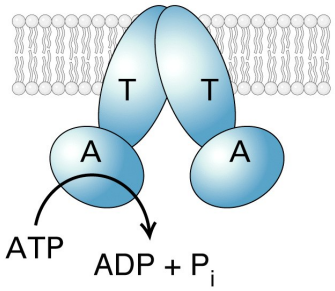
F-class pumps do not form phosphoprotein intermediates and transport only protons. V/F-class structures are similar and contain similar proteins, but none of their subunits are related to the P-class pumps. F-class pumps *operate in the reverse directions* (compared to V-class) to utilize energy in a proton concentration or electrochemical gradient to synthesize ATP.

Rotation of the γ subunit of the F_1 complex relative to the $(\alpha\beta)_3$ hexamer can be observed microscopically.



F_1 complexes were engineered that contained β subunits with an additional His6 sequence, which causes them to adhere to a glass plate coated with a metal reagent that binds histidine. The γ subunit in the engineered F_1 complexes was linked covalently to a fluorescently labeled actin filament. When viewed in a fluorescence microscope, the actin filaments were seen to rotate counterclockwise in discrete 120° steps in the presence of ATP, powered by ATP hydrolysis by the β subunits.

The 4 classes of ATP-powered transport proteins - (4)



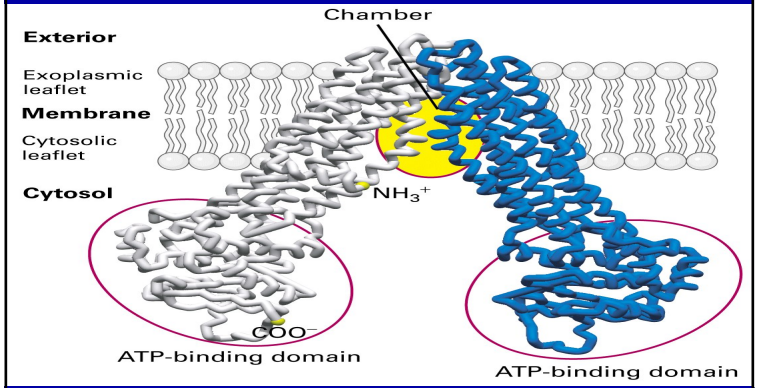
ABC superfamily

Bacterial plasma membranes (amino acid, sugar, and peptide transporters)

Mammalian plasma membranes (transporters of phospholipids, small lipophilic drugs, cholesterol, other small molecules)

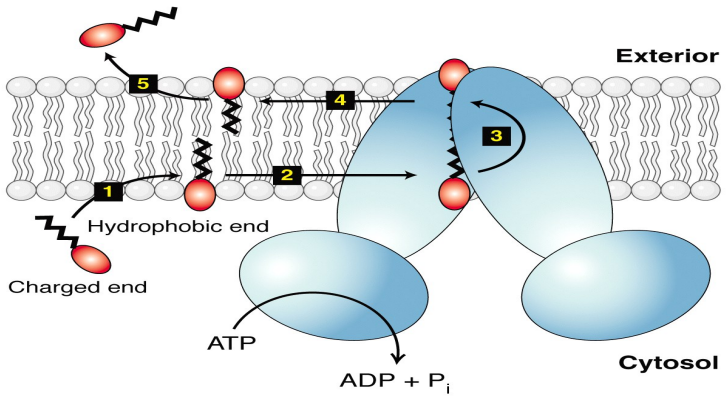
All members of the large ABC superfamily of proteins contain 2 transmembrane (T) domains and 2 cytosolic ATP-binding (A) domains, which couple ATP hydrolysis to solute movement. These core domains are present as separate subunits in some ABC proteins, but are eventually fused to a single polypeptide in other ABC proteins.

Structural model of E. coli lipid flippase, an ABC protein homologous to mammalian MDR1



The V-shaped protein encloses a "chamber" within the bilayer where it is hypothesized that bound substrates are flipped across the membrane, as shown in the next slide. Each identical subunit in this homodimeric protein has one transmembrane domain, comprising six alpha helices, and one cytosolic domain where ATP binding occurs.

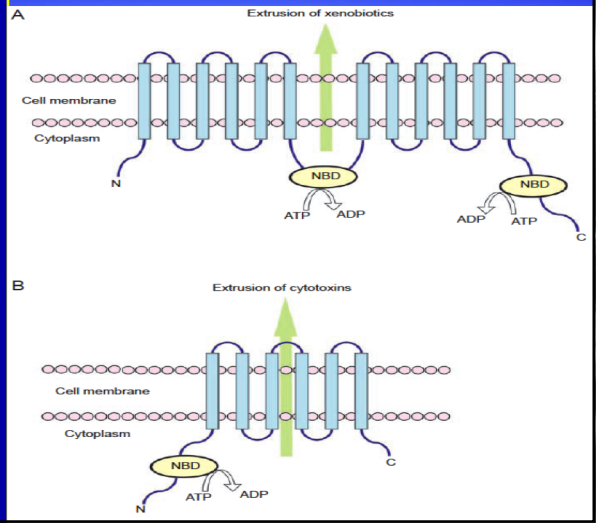
Flippase model of transport by MDR1 and similar ABC proteins



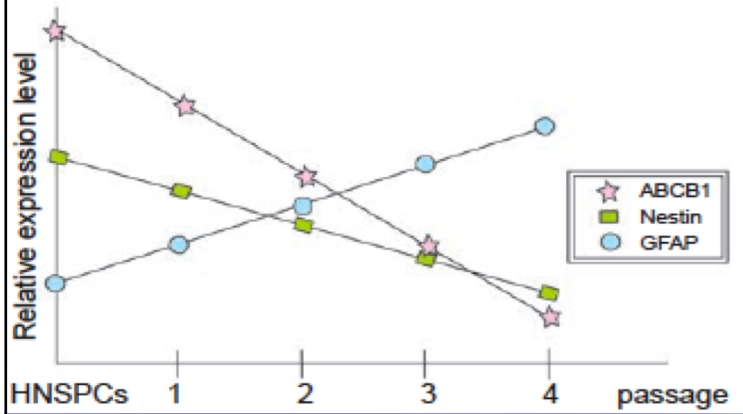
1: the hydrophobic portion (black) of a substrate molecule moves spontaneously from the cytosol into the cytosol-facing leaflet lipid bilayer, while the charged end (red) remains in the cytosol. 2: the substrate diffuses laterally until encountering and binding to a site on the MDR1 protein within the bilayer. 3: the protein then flips the charged substrate molecule into the exoplasmic leaflet, an energetically unfavorable reaction powered by the coupled hydrolysis of ATP by the cytosolic domain. 4+5: Once in the exoplasmic face, the substrate again can diffuse laterally in the membrane and ultimately moves into the aqueous phase on the outside of the cell.

ABC Transporters - and Bio-Tissue Engineering (1)

Structural features of ABC transporters. Most of the transporters are membrane-associated with six membrane-spanning regions, and are characterized by the presence of the ABC region. (A) ABCB1 typically is a full-length transporter of two identical halves as shown, with two NBDs that contain conserved sequences of the ABC. (B) ABCG2, on the other hand, is a half-transporter consisting of one NBD containing an ABC, followed by a six membrane-spanning domain.

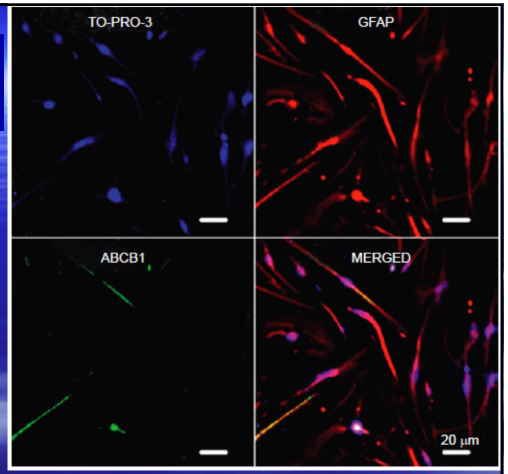


ABC Transporters - and Bio-Tissue Engineering (2)



Expression of ABCB1 during hNSPCs differentiation. hNSPCs (neurospheres) were induced to differentiate into glia and were then passaged four times. The graph shows the relative time course expression of ABCB1, nestin and GFAP in these hNSPCs over the four passages of differentiation.

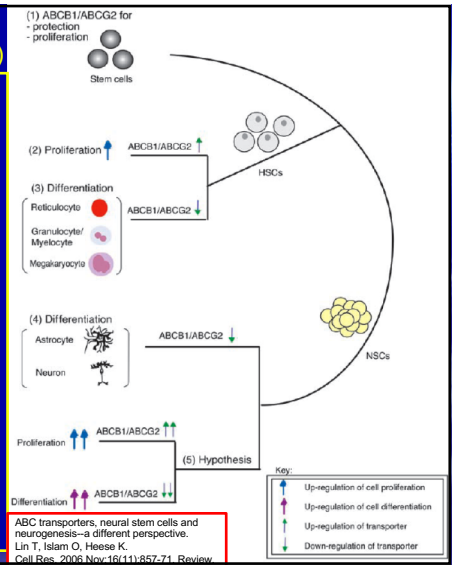
ABC Transporters - and Bio-Tissue Engineering (3)



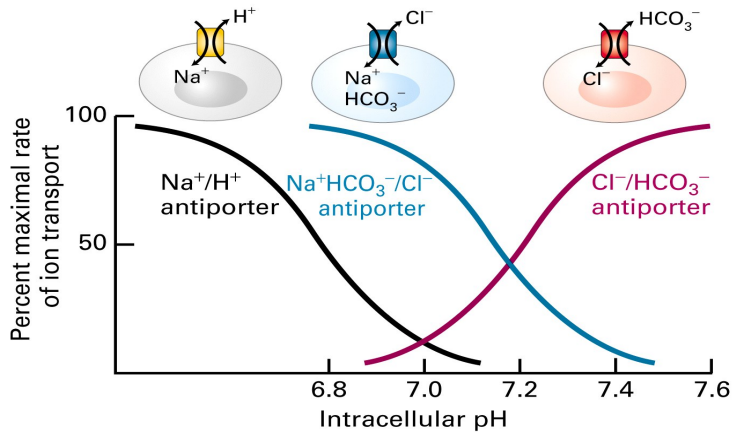
Immunocytochemical analysis of the ABCB1 and GFAP expression pattern in hNSPCs differentiated into glia cells. Nuclei were stained with TO-PRO-3. Scale-bar = 20 µm.

ABC Transporters - and Bio-Tissue Engineering (4)

Schematic illustration of the effect of ABC transporters on stem cell proliferation and differentiation.
(1) Expression of ABC transporters (especially ABCB1 and ABCG2) in stem cells like the NSCs, HSCs or pancreatic stem cells is thought to be essential for their in vivo proliferation and probably their self-renewal activity.
(2) Enforced expression of ABCB1 or ABCG2 leads to enhanced proliferation in HSCs.
(3) Downregulation of ABCB1 or ABCG2 in HSCs is observed with the differentiation of HSCs.
(4) Downregulation of the ABC transporters in NSCs is observed with the differentiation into astrocytes or neurons. (5) The hypothesis suggests that expression of the ABC transporters in NSCs may have an effect on NSC differentiation or proliferation, such that significant upregulation of ABCB1 or ABCG2 expression may lead to an increase in self-renewal of NSCs, and correspondingly, a decrease in ABCB1 or ABCG2 expression may lead to increased differentiation of NSCs.

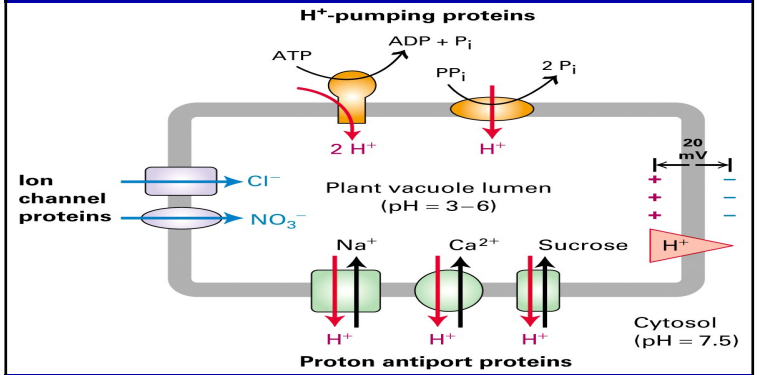


The activity of membrane transport proteins that regulate the cytosolic pH of mammalian cells changes with pH



Direction of ion transport is indicated above the curve for each protein.

Concentration of ions and sucrose by the plant vacuole

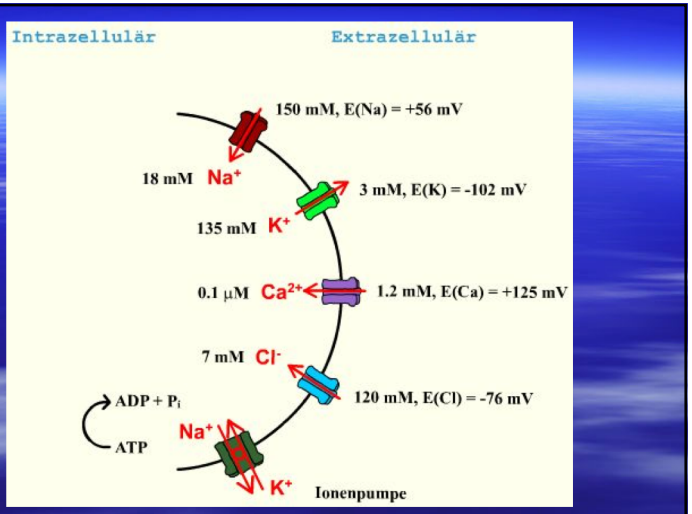


The vacuolar membrane contains two types of proton pumps (orange): a V-class H^+ ATPase (left) and a pyrophosphate-hydrolyzing proton pump (right) that differ from all other ion-transport proteins and probably is unique to plants. These pumps generate a low luminal pH as well as an inside-positive electric potential across the vacuolar membrane owing to the inward pumping of H^+ ions. The inside-positive potential powers the movement of Cl^- and NO_3^- from the cytosol through separate channel proteins (purple). Proton (H^+) antiporters (green), powered by the H^+ gradient, accumulate Na^+ and Ca^{2+} and sucrose inside the vacuole.

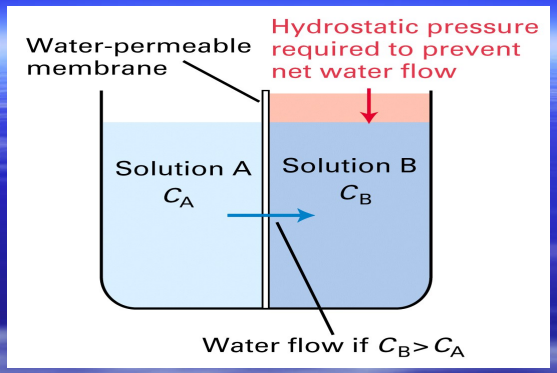
Typical Intracellular and Extracellular Ion Concentrations

Ion	Cell (mM)	Blood (mM)
Squid Axon (Invertebrate)*		
K^+	400	20
Na^+	50	440
Cl^-	40–150	560
Ca^{2+}	0.0003	10
X^{-}†	300–400	5–10
Mammalian Cell (Vertebrate)		
K^+	139	4
Na^+	12	145
Cl^-	4	116
HCO_3^-	12	29
X^-	138	9
Mg^{2+}	0.8	1.5
Ca^{2+}	<0.0002	1.8

*The large nerve axon of the squid has been widely used in studies of the mechanism of conduction of electric impulses.
†X^- represents proteins, which have a net negative charge at the neutral pH of blood and cells.

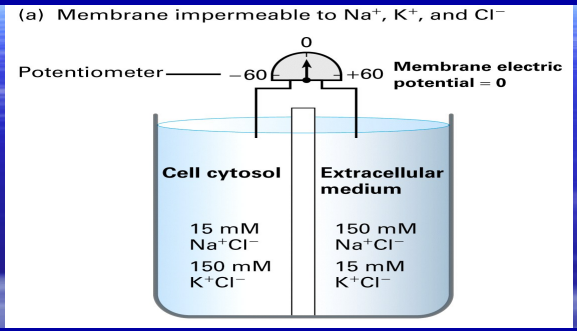


Movement of water - Osmotic Pressure



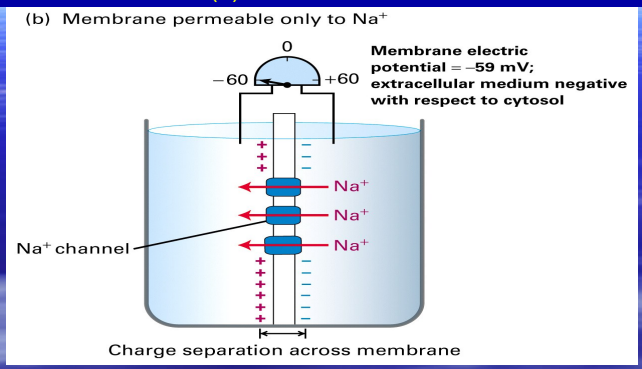
Solution A and B are separated by a membrane that is permeable to water but impermeable to all solutes. If [B] (the total concentration of solutes in solution B) is greater than [A], water will tend to flow across the mebrane form solution A to solution B. the osmotic pressure π between the solutions is the hydrostatic pressure that would have to be applied to solution B to prevent this water flow. From the van't Hoff equation, osmotic pressure is given by $\pi = RT([B]-[A])$, where R is the gas constant and T is the absolute temperature.

Generation of a transmembrane electric potential (voltage) depends on the selective movement of ions across a semi-permeable membrane (a)



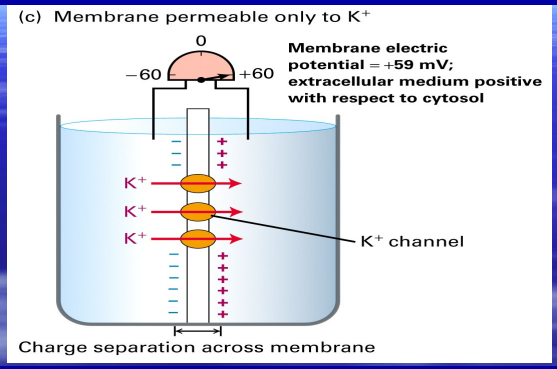
In this experimental system a membrane separates a 15 mM NaCl/150 mM KCl solution (left) from a 150 mM NaCl/15mM KCl solution (right); these ion concentrations are similar to those in cytosol and blood, respectively. If the membrane separating the two solutions is impermeable to all ions (a), no ions can move across the membrane and no difference in electric potential is registered on the potentiometer connecting the two solutions.

Generation of a transmembrane electric potential (voltage) depends on the selective movement of ions across a semi-permeable membrane (b)



If the membrane is selectively permeable only to Na^+, then diffusion of ion through their respective channels leads to a separation of charge across the membrane. At equilibrium, the membrane potential caused by the charge separation becomes equal to the Nernst potential E_{Na} registered on the photometer.

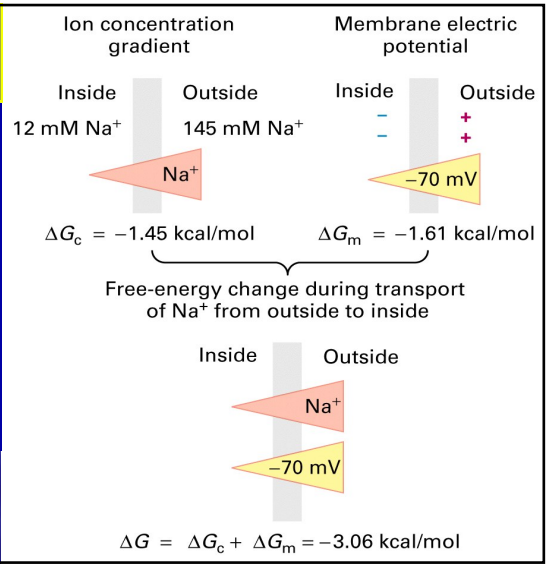
Generation of a transmembrane electric potential (voltage) depends on the selective movement of ions across a semi-permeable membrane (c)



If the membrane is selectively permeable only to K^+, then diffusion of ion through their respective channels leads to a separation of charge across the membrane. At equilibrium, the membrane potential caused by the charge separation becomes equal to the Nernst potential E_K registered on the photometer.

Transmembrane forces acting on Na^+

As with all ions, the movement of Na^+ ions across the plasma membrane is governed by the sum of two separate forces: the ion concentration gradient and the membrane electric potential. At the internal and external Na^+ concentrations typical of mammalian cells, these forces usually act in the same direction, making the inward movement of Na^+ ions energetically favorable.



Na^+ Entry into mammalian cells has a Negative Change in Free Energy (ΔG)

Two forces govern the movement of ions across selectively permeable membranes: the voltage and the ion concentration gradient across the membrane. The sum of the two forces, which may act in the same or in opposite directions, constitute the electrochemical gradient. To calculate the **free-energy change ΔG** corresponding to the transport of any ion across a membrane, we need to consider the independent contributions from each of the forces to the electrochemical gradient. E.g. when Na^+ moves from outside to inside the cell, the free-energy change generated by Na^+ concentration gradient is given by:

$\Delta G_C = RT \ln [Na_{in}]/[Na_{out}]$; at the concentration of $[Na_{in}]$ and $[Na_{out}]$ = 12 mM and 145 mM (typical for many mammalian cells), respectively, ΔG_C , the change in free energy due to the concentration gradient, is -1.45 kcal for transport of 1 mol Na^+ ions from outside to inside the cell, assuming there is no electric potential.

The free-energy change generated from the membrane electric potential is given by:

$\Delta G_m = FE$

(F = Faraday constant, E = membrane electric potential. If E = - 70 mV, then ΔG_m , the free-energy change due to the membrane potential, is -1.61 kcal for transport of 1 mol Na^+ ions from outside to inside the cell, assuming there is no Na^+ concentration gradient. Since both forces in fact act on Na^+ ions, the total **ΔG is the sum of the two partial values:**

$\Delta G = \Delta G_C + \Delta G_m$ = (-1.45) + (-1.61) = -3.06 kcal/mol

Na^+-linked symporters import amino acids and glucose into animal cells against high concentration gradients

$2\ Na^+_{out} + glucose_{out} \leftrightarrow 2\ Na^+_{in} + glucose_{in}$

ΔG is the sum of the free-energy changes generated by glucose concentration gradient, the Na+ concentration gradient and the membrane potential.

$\Delta G = RT \ln [glucose_{in}]/[glucose_{out}] + 2\ RT \ln [Na^+_{in}]/[Na^+_{out}] + 2\ FE$

At equilibrium $\Delta G = 0$.

From previous figure we know that ΔG is about -3 kcal per mole Na^+ transported --->

$0 = RT \ln [glucose_{in}]/[glucose_{out}]$ - 6 kcal --->

$[glucose_{in}]/[glucose_{out}] \sim 30{,}000$

Thus, inward flow of 2 moles of Na^+ can generate an intracellular glucose concentration that is 30,000 times greater than the exterior concentration. For 1 mole Na^+ it would be only 170-fold.