General Biology

Course No: BNG2003
Credits: 3.00

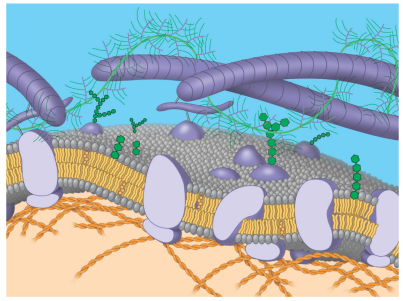
4. Cell Membrane Structure and Function

Prof. Dr. Klaus Heese

1

- Life at the Edge
- ***The plasma membrane*** is the boundary that separates the living cell from its 'nonliving' surroundings

- The plasma membrane exhibits selective permeability - it allows some substances to cross it more easily than others



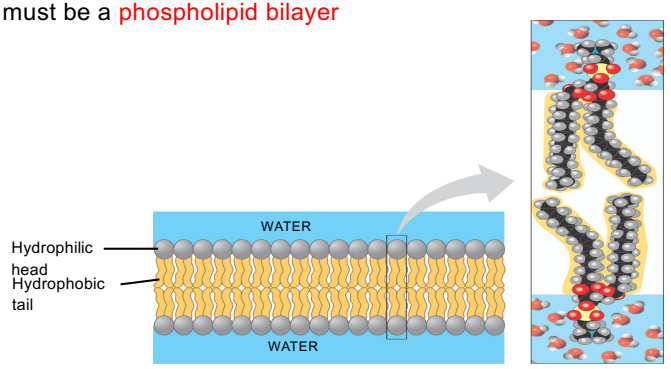
2

- **Cellular membranes** are fluid mosaics of **lipids** and **proteins**
- Phospholipids
  - are the most abundant lipid(s) in the plasma membrane
  - are amphipathic, containing both hydrophobic and hydrophilic regions
- The ***fluid mosaic model of membrane structure***
  - states that a membrane is a fluid structure with a "mosaic" of various proteins embedded in it

3

Membrane Models: Scientific Inquiry

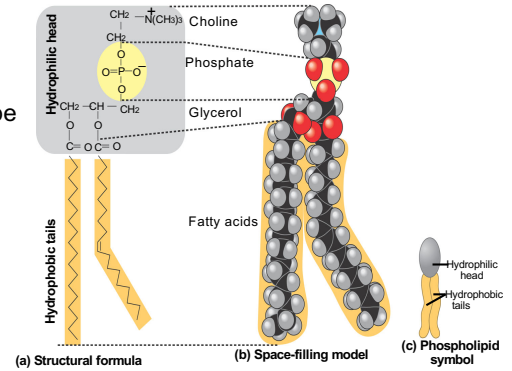
- Membranes have been chemically analyzed and found to be composed of proteins and lipids
- Scientists studying the plasma membrane reasoned that it must be a phospholipid bilayer



4

- The Davson-Danielli sandwich model of membrane structure
  - stated that the membrane was made up of a phospholipid bilayer sandwiched between two protein layers, and this

was supported by electron microscope pictures of membranes



5

Triacylglycerol

Phosphatidylcholine, a typical phosphoglyceride

Lipids

HO--C-H$_2$
HO--C-H
H$_2$--C-OH
<--- glycerol backbone

Fatty acid chains

Hydrophilic head

Phosphate

Hydrophobic tail

Glycerol

Choline

PHOSPHATIDYLCHOLINE

All phosphoglycerides are amphipathic – having a hydrophobic tail (yellow) and a hydrophilic head (blue) in which glycerol is linked via a phosphate group to an alcohol. Either of or both the fatty acyl side chains in a phosphoglyceride may be saturated or unsaturated. In phosphatidic acid (red), the simplest phospholipid, the phosphate is not linked to an alcohol.

6

Phosphoglycerides

Fatty Acids & Lipids

Molecules of a Biomembrane

Head group

PE

PC

PS

PI

Hydrophobic tail

Plasmalogen

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

7

Molecules of a Biomembrane

Fatty Acids & Lipids

Sphingolipids

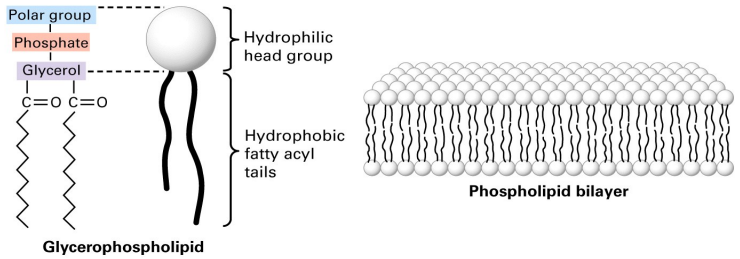
SM

GlcCer

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

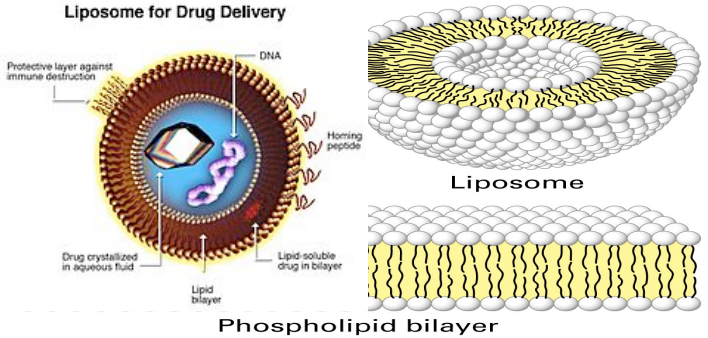
8

Essential Cell Membrane Molecules



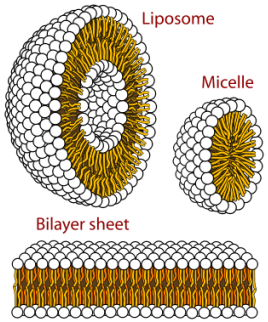
9

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.

10

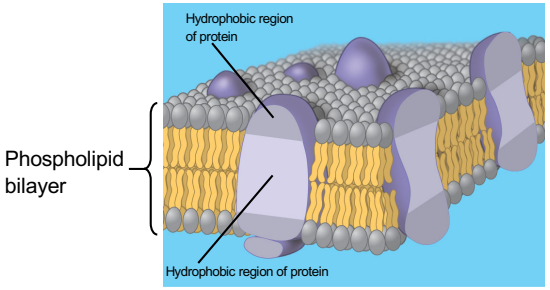


A micelle is an aggregate of surfactant molecules dispersed in a liquid colloid

Micelles are approximately spherical in shape.

11

- In 1972, Singer and Nicolson
  - proposed that membrane proteins are dispersed and individually inserted into the phospholipid bilayer

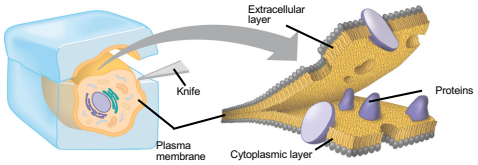


12

- Freeze-fracture studies of the plasma membrane
  - supported the fluid mosaic model of membrane structure

APPLICATION A cell membrane can be split into its two layers, revealing the ultrastructure of the membrane's interior.

TECHNIQUE A cell is frozen and fractured with a knife. The fracture plane often follows the hydrophobic interior of a membrane, splitting the phospholipid bilayer into two separated layers. The membrane proteins go wholly with one of the layers.

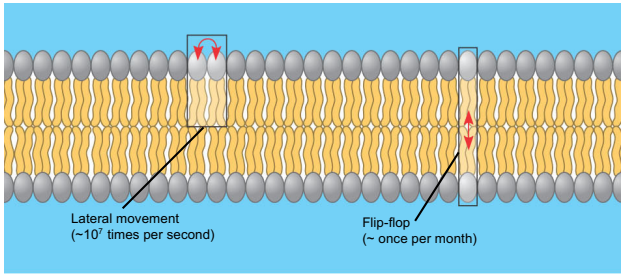


RESULTS These SEMs show membrane proteins (the "bumps") in the two layers, demonstrating that proteins are embedded in the phospholipid bilayer.

13

The Fluidity of Membranes

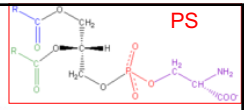
- Phospholipids in the plasma membrane
  - can move within the bilayer

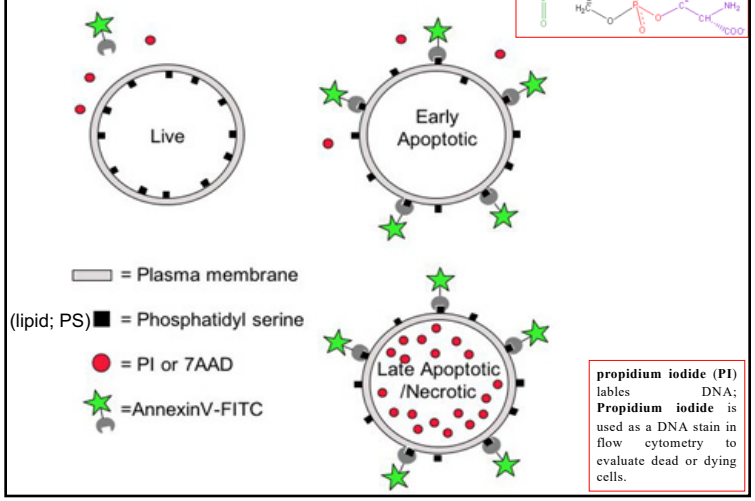


Movement of phospholipids

14

Apoptosis Assay





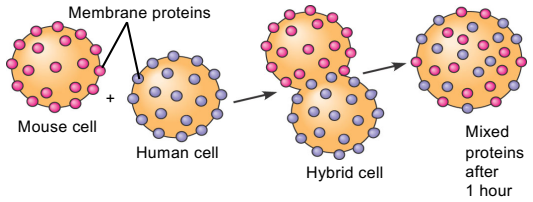
propidium iodide (PI) lables DNA; **Propidium iodide** is used as a DNA stain in flow cytometry to evaluate dead or dying cells.

15

- Proteins in the plasma membrane can drift within the bilayer

EXPERIMENT Researchers labeled the plasma mambrane proteins of a mouse cell and a human cell with two different markers and fused the cells. Using a microscope, they observed the markers on the hybrid cell.

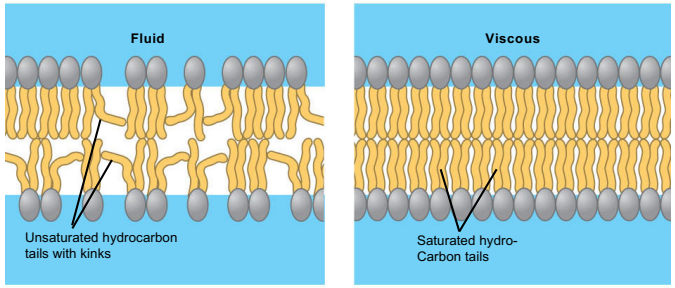
RESULTS



CONCLUSION The mixing of the mouse and human membrane proteins indicates that at least some membrane proteins move sideways within the plane of the plasma membrane.

16

- The type of hydrocarbon tails in phospholipids
  - affects the fluidity of the plasma membrane

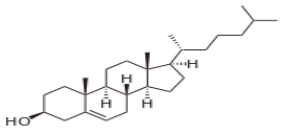


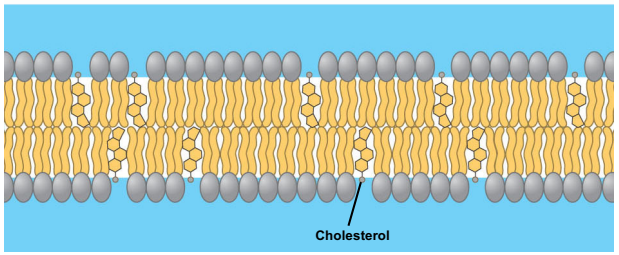
Membrane fluidity

17

- The steroid **cholesterol**

has different effects on membrane fluidity at different temperatures





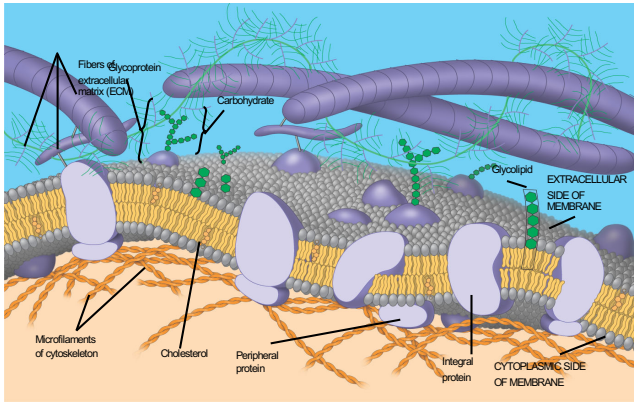
Cholesterol within the animal cell membrane

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.

18

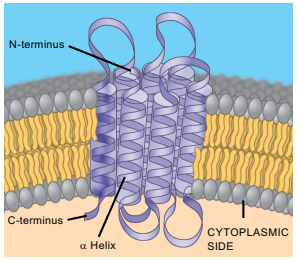
Membrane Proteins and Their Functions

- A membrane is a collage of different proteins embedded in the fluid matrix of the lipid bilayer



19

- *Integral proteins*
  - penetrate the hydrophobic core of the lipid bilayer
  - are often transmembrane proteins, completely spanning the membrane

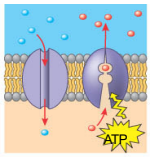


- peripheral proteins are appendages, loosely bound to the surface of the membrane

20

An overview of six major functions of membrane proteins

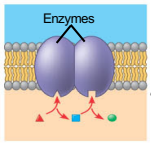
(a) **Transport. (left)** A protein that spans the membrane may provide a hydrophilic channel across the membrane that is selective for a particular solute. **(right)** Other transport proteins shuttle a substance from one side to the other by changing shape. Some of these proteins hydrolyze ATP as an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



e.g. ABC-transporter

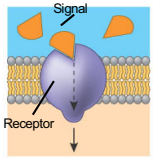
Na/K-ATPase, NMDAR

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



e.g. PLC
γ-secretase

(c) **Signal transduction.** A membrane protein may have a binding site with a specific shape that fits the shape of a chemical messenger, such as a hormone. The external messenger (signal) may cause a conformational change in the protein (receptor) that relays the message to the inside of the cell.



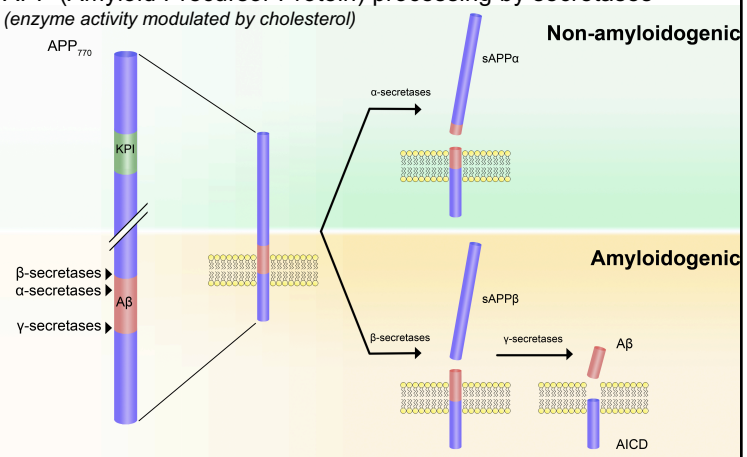
Receptors:
e.g. TRKA
p75NTR
NMDR

21

Regulated Intramembrane Proteolysis / RIP-tide mechanism

APP (Amyloid Precursor Protein) processing by secretases

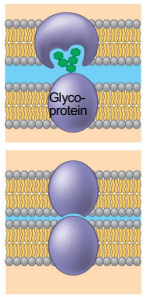
(enzyme activity modulated by cholesterol)



22

(d) **Cell-cell recognition.** Some glyco-proteins serve as identification tags that are specifically recognized by other cells.

(e) **Intercellular joining.** Membrane proteins of adjacent cells may hook together in various kinds of junctions, such as gap junctions or tight junctions.



inter-cell-signalling: e.g.

Notch-Delta

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

23

The Role of Membrane Carbohydrates in Cell-Cell Recognition

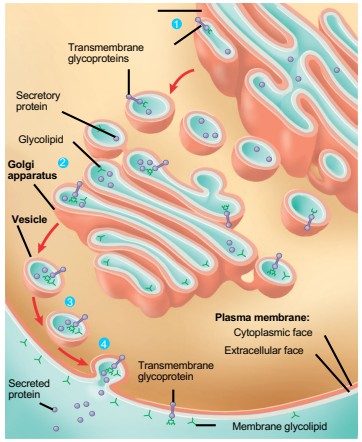
- Cell-cell recognition is a cell's ability to distinguish one type of neighboring cell from another
- Membrane carbohydrates interact with the surface molecules of other cells, facilitating cell-cell recognition

Synthesis and Sidedness of Membranes

- Membranes have distinct inside and outside faces
- This affects the movement of proteins synthesized in the endomembrane system

24

- Membrane proteins and lipids are synthesized in the ER and Golgi apparatus



25

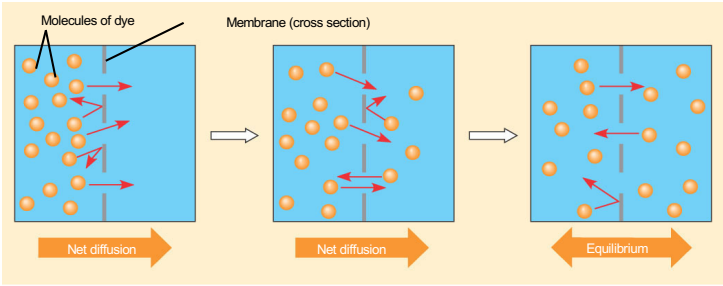
- Membrane structure results in selective permeability
- A cell must exchange materials with its surroundings, a process controlled by the plasma membrane

The Permeability of the Lipid Bilayer

- Hydrophobic molecules are lipid soluble and can pass through the membrane rapidly
- Polar molecules do not cross the membrane rapidly
- *Transport proteins* allow passage of hydrophilic substances across the membrane
- Passive transport is diffusion of a substance across a membrane with no energy investment

26

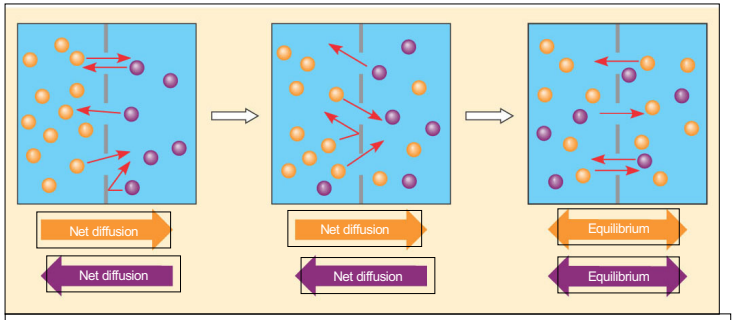
Diffusion is the tendency for molecules of any substance to spread out evenly into the available space



Diffusion of one solute. The membrane has pores large enough for molecules of dye to pass through. Random movement of dye molecules will cause some to pass through the pores; this will happen more often on the side with more molecules. The dye diffuses from where it is more concentrated to where it is less concentrated (called diffusing down a concentration gradient). This leads to a dynamic equilibrium: The solute molecules continue to cross the membrane, but at equal rates in both directions.

27

Substances diffuse down their concentration gradient, the difference in concentration of a substance from one area to another

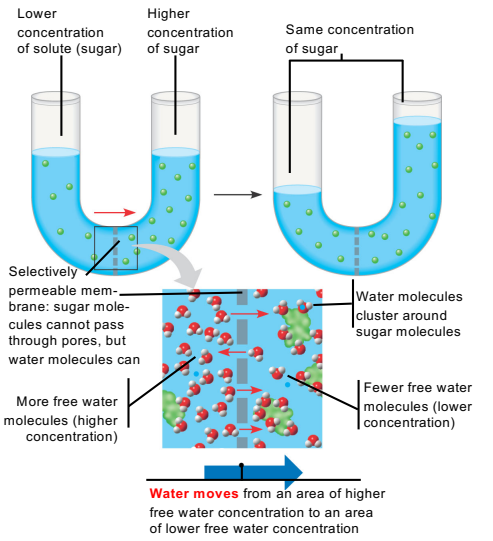


Diffusion of two solutes. Solutions of two different dyes are separated by a membrane that is permeable to both. Each dye diffuses down its own concentration gradient. There will be a net diffusion of the purple dye toward the left, even though the *total* solute concentration was initially greater on the left side.

28

Effects of Osmosis on Water Balance

Osmosis is the movement of water across a semipermeable membrane; it is affected by the concentration gradient of dissolved substances



29

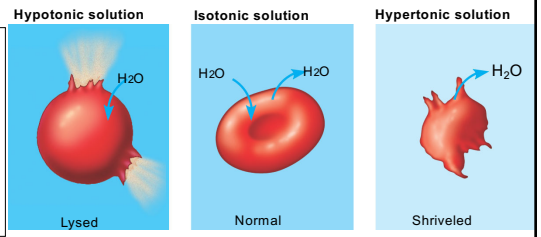
Water Balance of Cells Without Walls

- Tonicity is the ability of a solution to cause a cell to gain or lose water; it has a great impact on cells without walls
- If a solution is isotonic
  - the concentration of solutes is the same as it is inside the cell
  - there will be no net movement of water
- If a solution is hypertonic
  - the concentration of solutes is greater than it is inside the cell
  - the cell will lose water
- If a solution is hypotonic
  - the concentration of solutes is less than it is inside the cell
  - the cell will gain water

30

- Water balance in cells without walls
- Animals and other organisms without rigid cell walls living in hypertonic or hypotonic environments
  - must have special adaptations for **osmoregulation**

Animal cell. An animal cell fares best in an isotonic environment unless it has special adaptations to offset the osmotic uptake or loss of water.



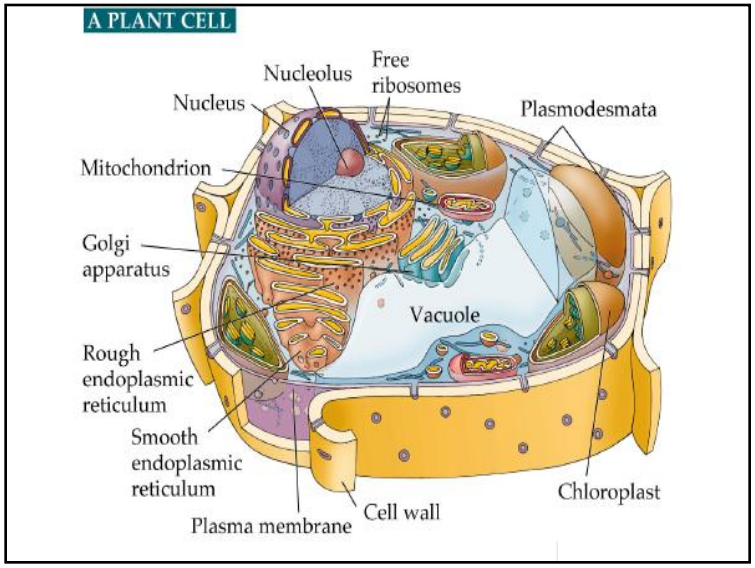
An increase in the salinity (saltiness; salt concentration) of a lake can kill animals there; if the lake water becomes hypertonic to the animal's cells, the cells might shrivel and die. Hypotonic environment is hazardous as well.

31

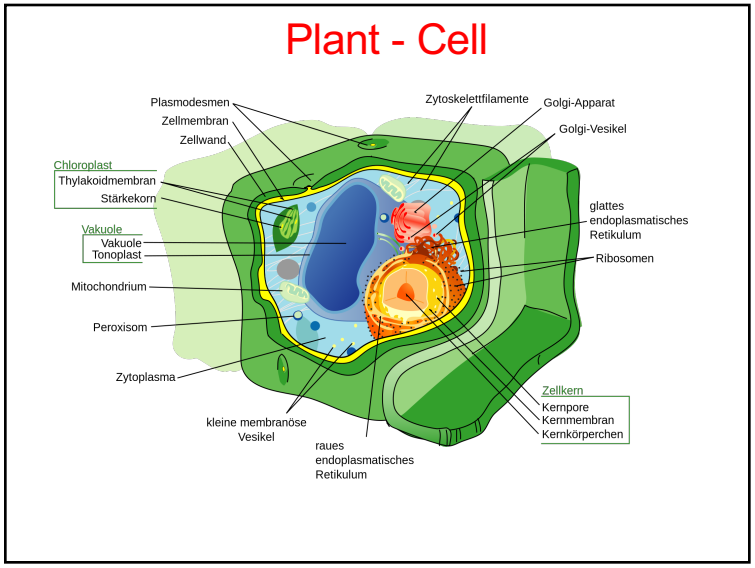
Water Balance of Cells with Walls

- Cell walls help maintain water balance
- If a plant cell is turgid
  - it is in a hypotonic environment
  - it is very firm, a healthy state in most plants
- If a plant cell is flaccid
  - it is in an isotonic or hypertonic environment

32

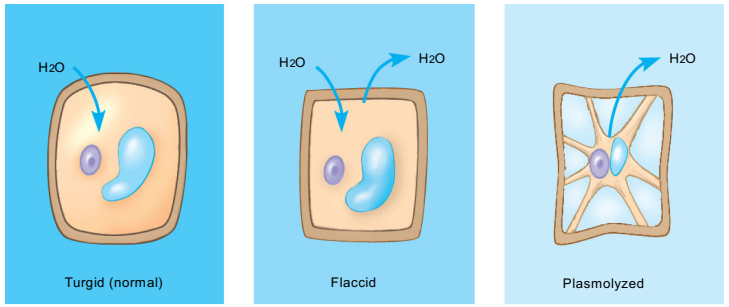


33



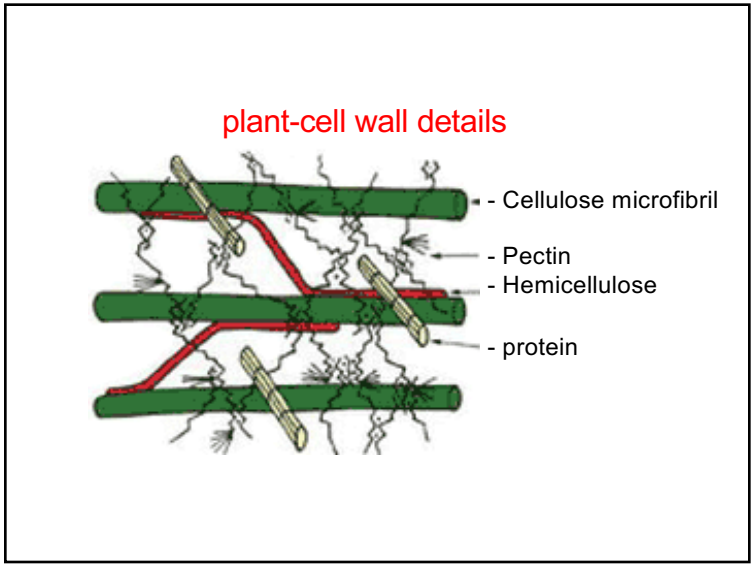
34

- **Water balance in cells with walls**



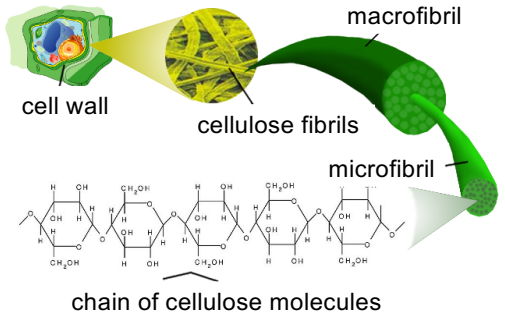
Plant cell. Plant cells are turgid (firm) and generally healthiest in a hypotonic environment, where the uptake of water is eventually balanced by the elastic wall pushing back on the cell.

35



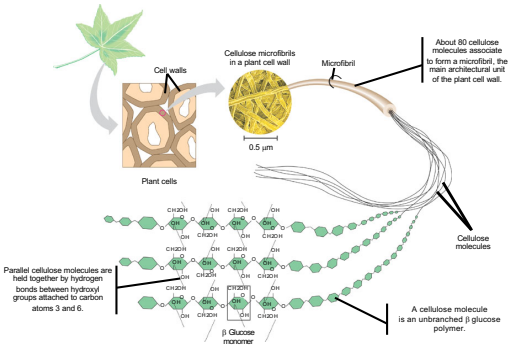
36

plant-cell wall details



37

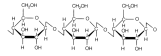
- Cellulose is a major component of the tough walls that enclose plant cells

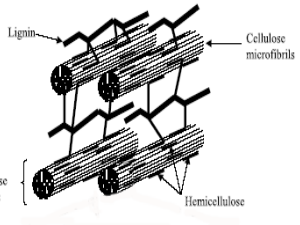


38

- Cellulose is a major component of the tough walls that enclose plant cells

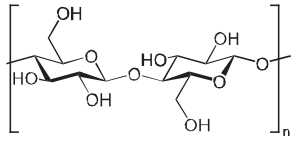
Cellulose is an organic compound with the formula $(C_6H_{10}O_5)n$, a polysaccharide consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units. Cellulose is an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms.

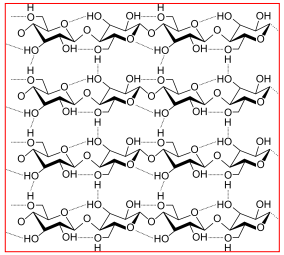


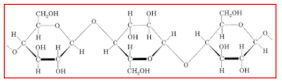


39

Cellulose

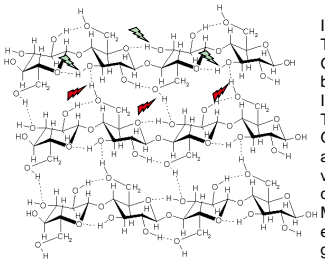






40

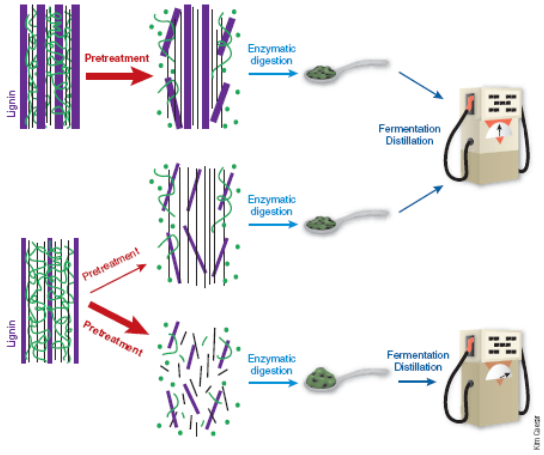
The crystalline regions of cellulose have intramolecular and intermolecular hydrogen bonds, allowing the linear glucan chains to form crystalline structures that exclude water and enzymes.



Intramolecular:
The H of the OH on C3 to the O that makes the glycosidic bonds.
Intermolecular:
The H of the OH on C6 to the O of the OH on C3. These are the bonds that make the very tight structure of cellulose microfibrils. Microfibrils have 30-40 chains each with 2000 to 10,000 glucose units.

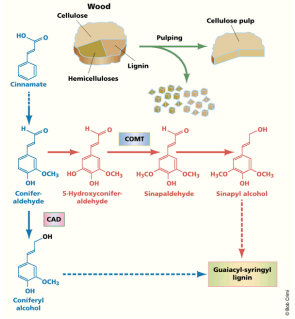
41

Lignin modification may decrease the need for pretreatment

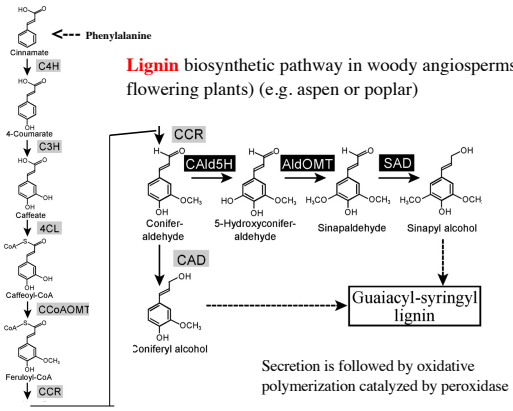


42

Lignin biosynthetic pathway in woody angiosperms (e.g. aspen or poplar)

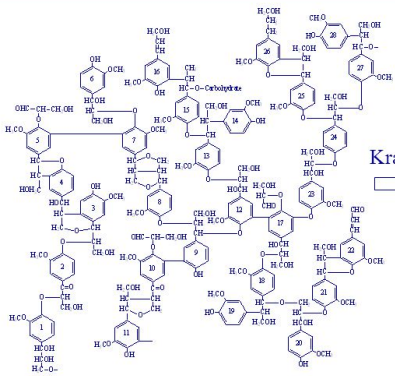


43



44

The Goal of Lignin Reactions in Kraft Pulping

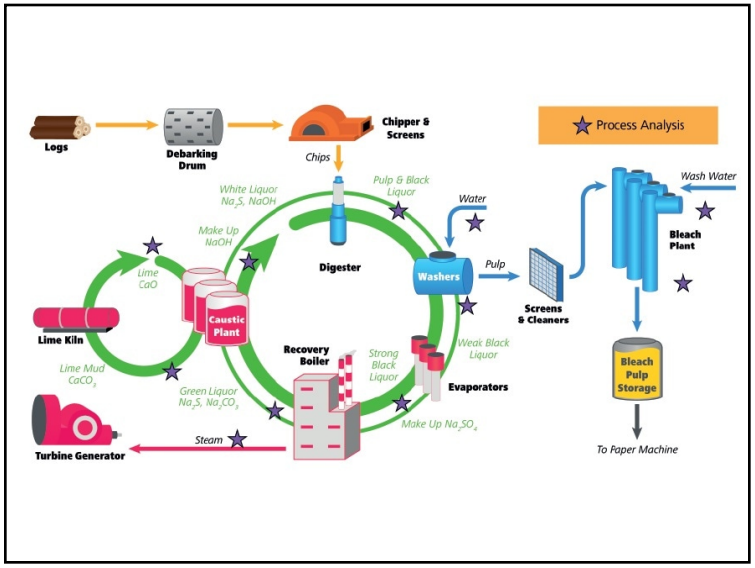


Kraft Pulping → Soluble Fragments

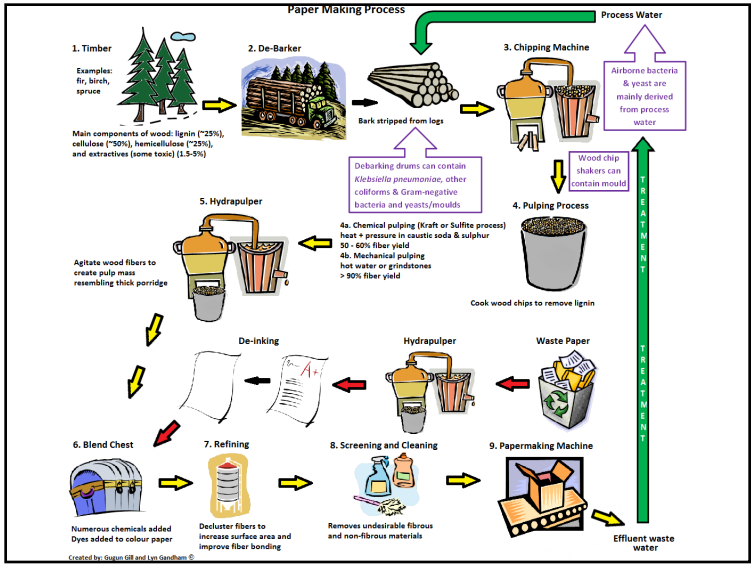
The goal in kraft pulping is to take large insoluble lignin and turn it into small alkali soluble fragments.

2

45

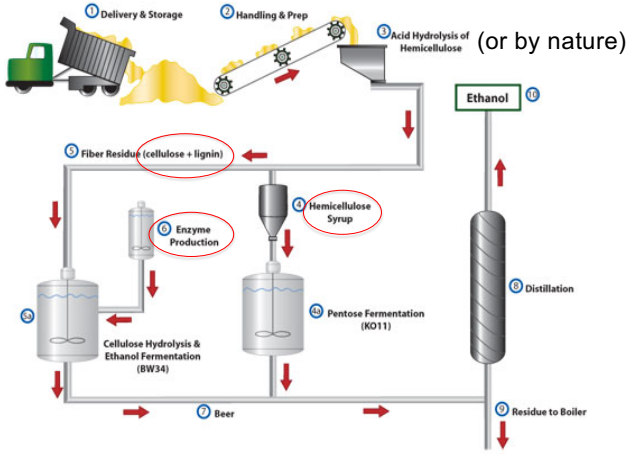


46



47

A simplified scheme for the processing of biomass into ethanol



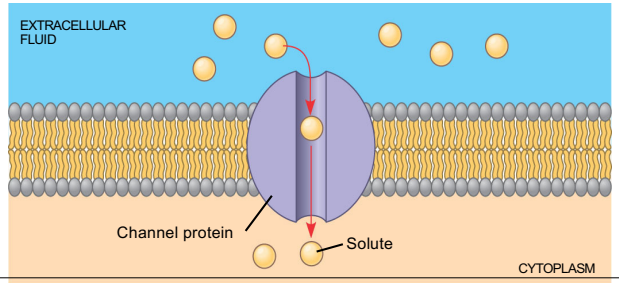
48

Facilitated Diffusion: Passive Transport Aided by Proteins

- In facilitated diffusion
  - *transport proteins* speed the movement of molecules across the plasma membrane

49

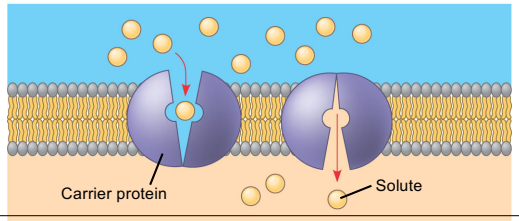
- Channel proteins (e.g. ion channels (various types such as voltage-gated or neurotransmitter receptors) in neurons)
  - provide corridors that allow a specific molecule or ion to cross the membrane



A channel protein (purple) has a channel through which water molecules or a specific solute can pass.

50

- Carrier proteins (various types)
  - undergo a subtle change in shape that translocates the solute-binding site across the membrane (also as 'Co-transporters')



A carrier protein alternates between two conformations, moving a solute across the membrane as the shape of the protein changes. The protein can transport the solute in either direction, with the net movement being down the concentration gradient of the solute.

51

- Active transport uses energy to move solutes against their gradients

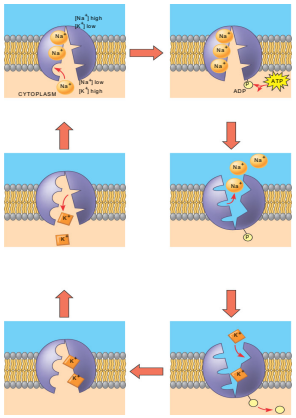
The Need for Energy in Active Transport

- Active transport
  - moves substances against their concentration gradient
  - requires energy, usually in the form of ATP

52

The sodium-potassium pump (Na/K-ATPase is one type of **active** transport system

1. Cytoplasmic Na^+ binds to the sodium-potassium pump.
2. Na+ binding stimulates phosphorylation by ATP.
3. Phosphorylation causes the protein to change its conformation, expelling Na^+ to the outside.
4. Extracellular K^+ binds to the protein, triggering release of the Phosphate group.
5. Loss of the phosphate restores the protein's original conformation.
6. K^+ is released and Na^+ sites are receptive again; the cycle repeats.



53

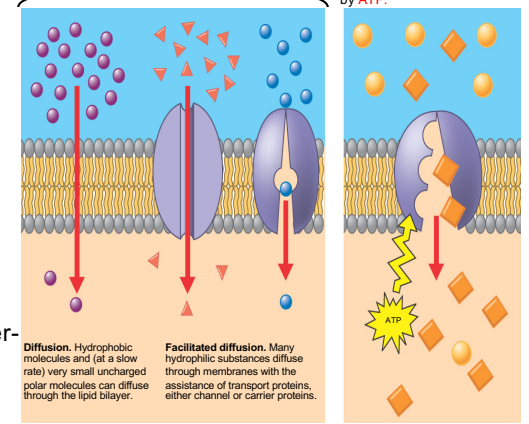
Review passive and active transport compared

Passive transport. Substances diffuse spontaneously down their concentration gradients, crossing a membrane with no expenditure of energy by the cell. The rate of diffusion can be greatly increased by transport proteins in the membrane.

Active transport. Some transport proteins act as pumps, moving substances across a membrane against their concentration gradients. Energy for this work is usually supplied by ATP.

Ion-channels

Neurotransmitter-Receptors



54

Maintenance of Membrane Potential by Ion Pumps (as the Na/K ATPase; proton pump)

Membrane potential is the voltage difference across a membrane

An electrochemical gradient

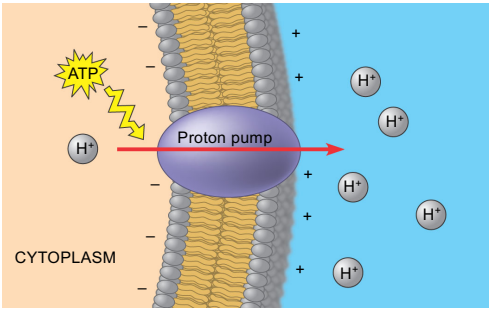
is caused by the concentration electrical gradient of ions across a membrane

Co-transport: Coupled Transport by a Membrane Protein

Cotransport occurs when active transport of a specific solute indirectly drives the active transport of another solute

55

- An electrogenic pump
  - is a transport protein that generates the voltage across a membrane



H^+ gradient is either created by ATP or H^+ gradient is used to make ATP

56

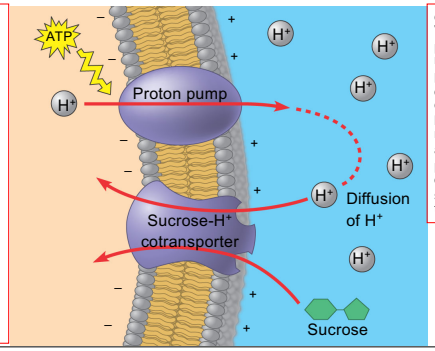
- An electrogenic pump is a transport protein that generates the voltage across a membrane
- Cotransport: active transport driven by a concentration gradient

See also
F-class H^+-pump:

Mitochondria:
ATP generation

Chloroplasts:
Photosynthesis

compare with:
Na/K ATPase

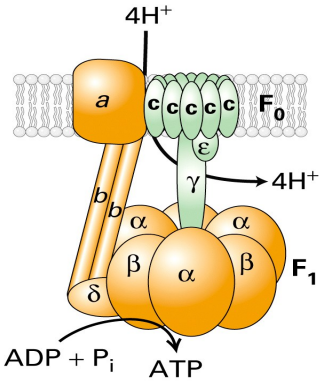


e.g.:
V-class proton pumps at vacuolar membranes in plants, yeast, other fungi; at endosomal and lysomal membranes in animal cells; at plasma membrane of osteoclasts and some kidney tubule cells.

H^+ gradient is either created by ATP, or H^+ gradient is used to make/synthesize ATP (as in choroplasts or mitochondria)

57

ATP-synthesis: F-class H^+-pump



F-class proton pumps

Bacterial plasma membrane

Inner mitochondrial membrane

Thylakoid membrane of chloroplast

electromotive force (emf)

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.

58

- 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 (neurotransmitter release)

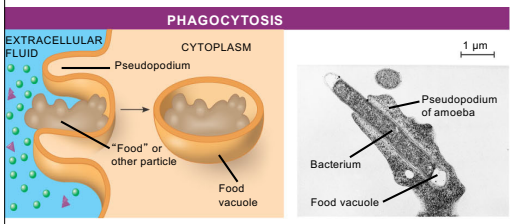
Endocytosis

- In endocytosis the cell takes in macromolecules by forming new vesicles from the plasma membrane

59

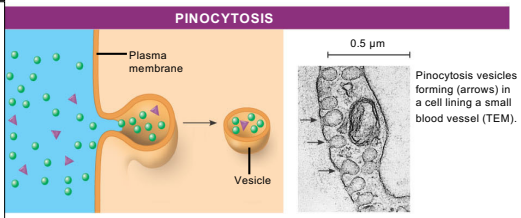
- Three types of endocytosis

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.



An amoeba engulfing a bacterium via phagocytosis (TEM).

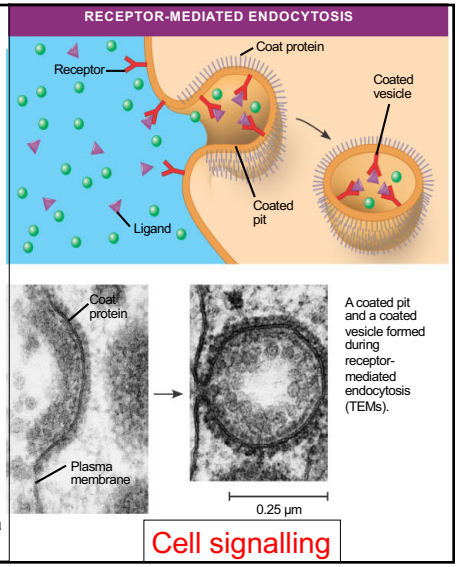
In **pinocytosis,** the cell "gulps" droplets of extracellular fluid into tiny vesicles. It is not the fluid itself that is needed by the cell, but the molecules dissolved in the droplet. Because any and all included solutes are taken into the cell, pinocytosis is nonspecific in the substances it transports.



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

60

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.

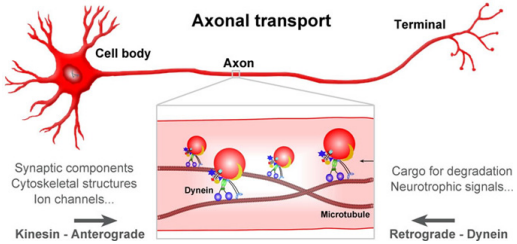


Cell signalling

61

Axonal transport

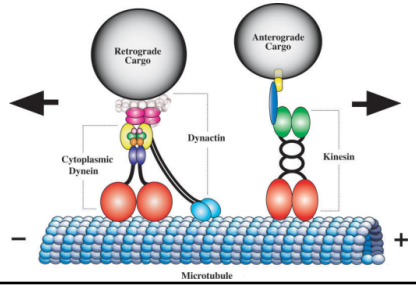
- Anterograde: From cell body to terminal (e.g: neurotransmitter, neurotransmitter enzyme, BDNF)
- Retrograde: From terminal to cell body (e.g: NGF, GPCR internalization)



62

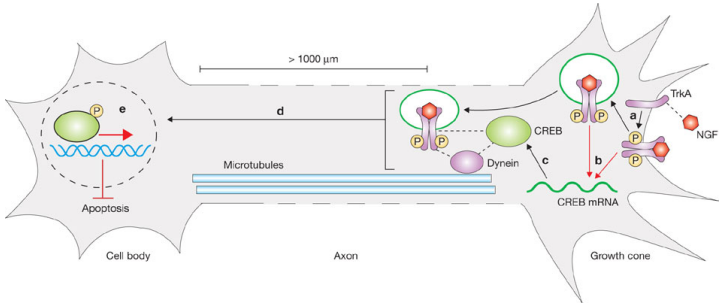
Kinesin & Dynein motor protein

- Retrograde transport requires kinesin and dynein/dynactin motor proteins
- Anterograde transport requires kinesin motor protein



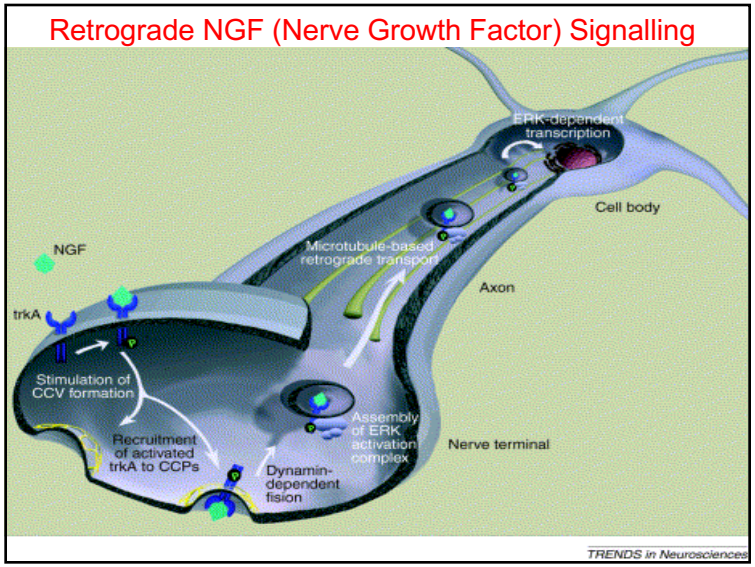
63

NGF is transported retrogradely



64

Retrograde NGF (Nerve Growth Factor) Signalling



65

BNG2003

4. – Biochemistry - Bio-Energetics

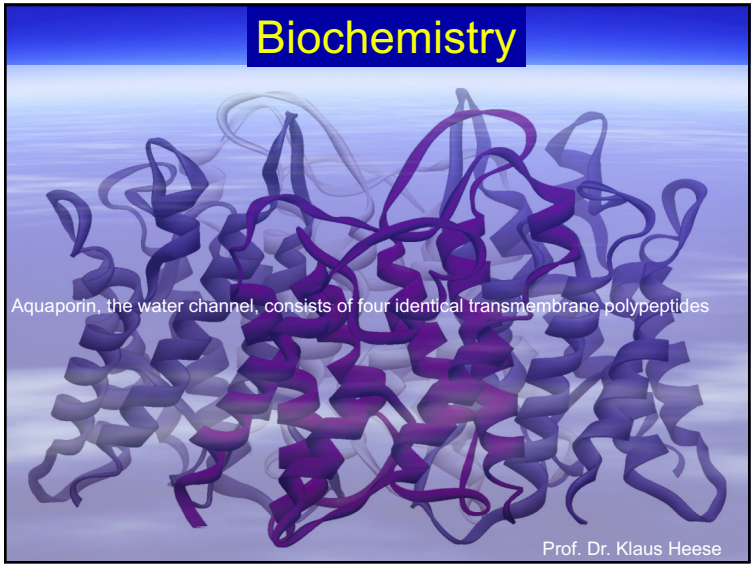
4.2) Transport of ions and small molecules across cell membranes

Key Energy (ATP)-dependent Membrane Proteins (Membrane Transporters)

Prof. Dr. Klaus Heese

1

Biochemistry

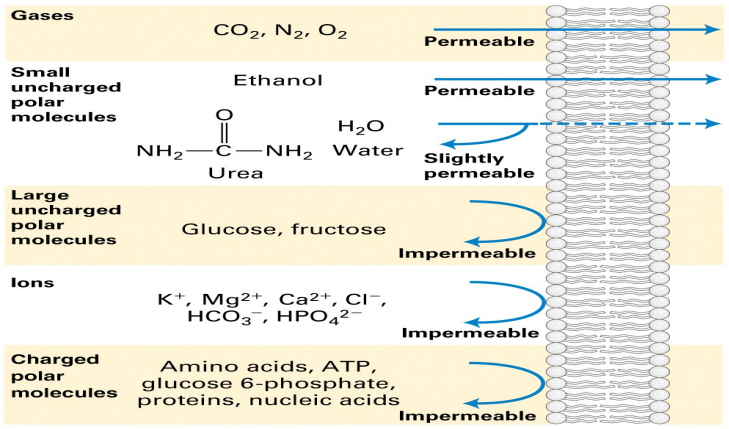


Aquaporin, the water channel, consists of four identical transmembrane polypeptides

Prof. Dr. Klaus Heese

2

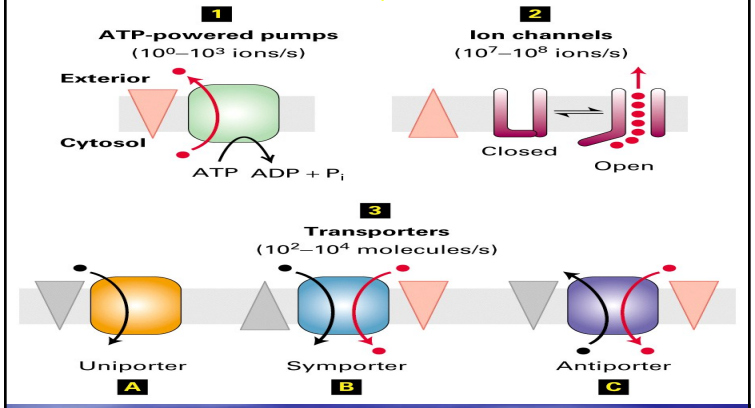
Relative permeability of a pure phospholipid bilayer to various molecules



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.

3

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.

4

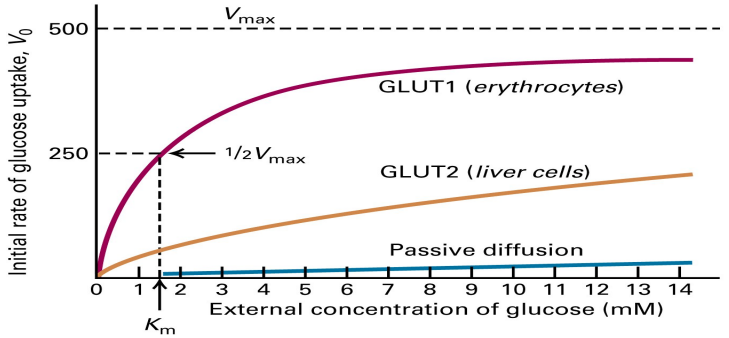
Mechanisms for Transporting Ions and Small Molecules Across Cell Membranes

	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*.

5

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

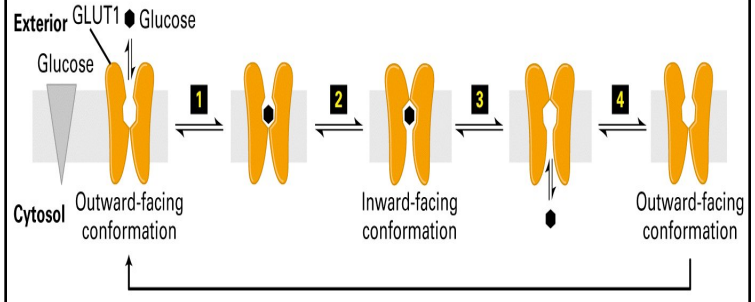


The initial transport rate for the substrate S into the cell catalyzed by e.g. GLUT1: v=Vmax/(1+Km/[S])

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.

6

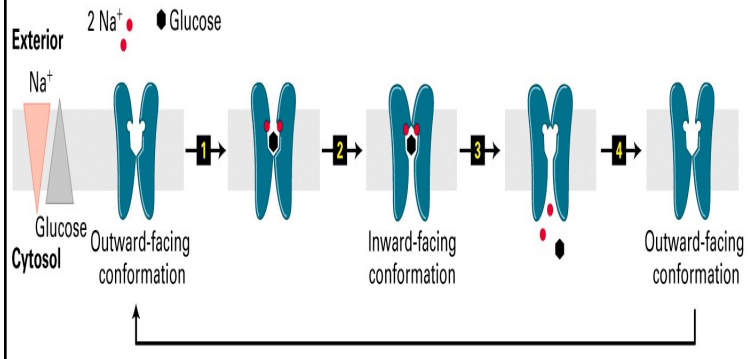
Model of Uniport Transport by GLUT1



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.

7

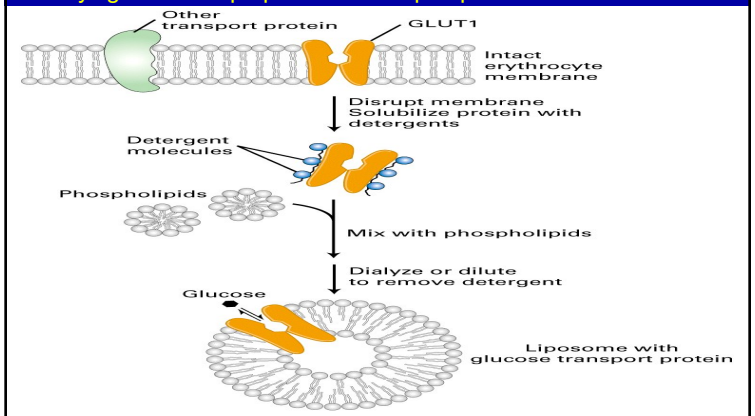
Operational model for the 2-Na^+/1-glucose symporter



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.

8

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



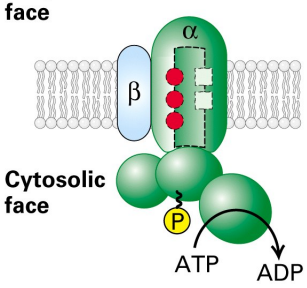
Here, all the integral proteins of the erythrocyte membrane are solubilized by a nonionic detergent, such as octylglucoside. The glucose uniporter GLUT1 can be purified by chromatography on a column containing a specific antibody and then incorporated into liposomes made of pure phospholipids.

9

ATP-Powered Pumps and the Intracellular Ionic Environment

10

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)

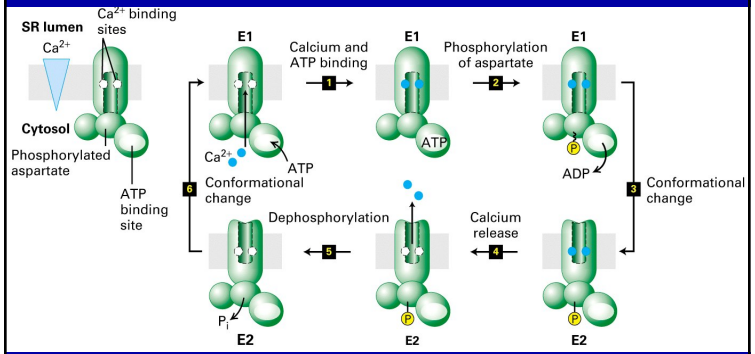
Plasma membrane of all eukaryotic cells (Ca^{2+} pump)

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.

11

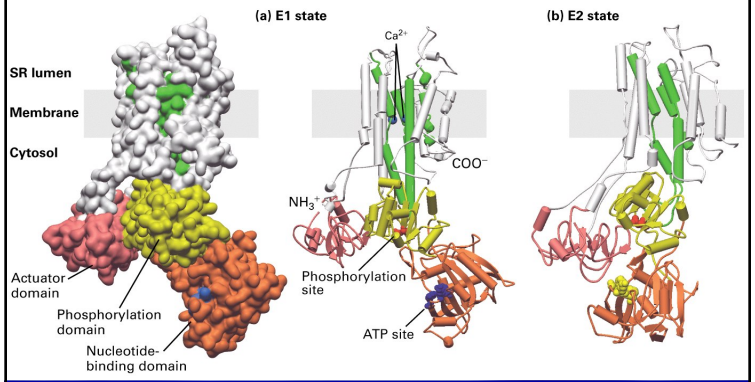
Operational model of the Ca^{2+} ATPase in the SR membrane of skeletal muscle cells



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.

12

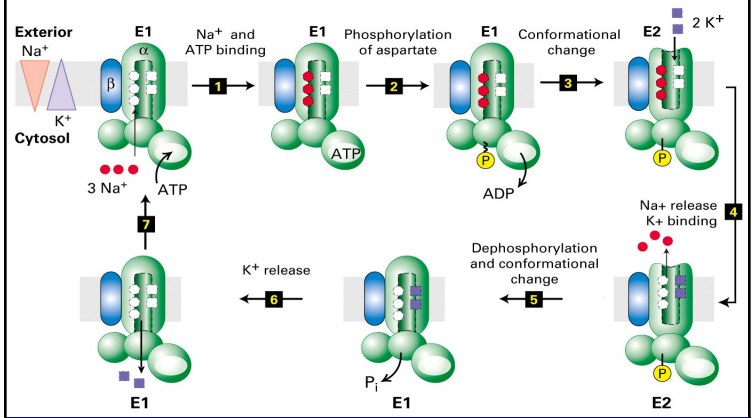
Structure of the catalytic alpha subunit of the muscle Ca^{2+} ATPase



(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).

13

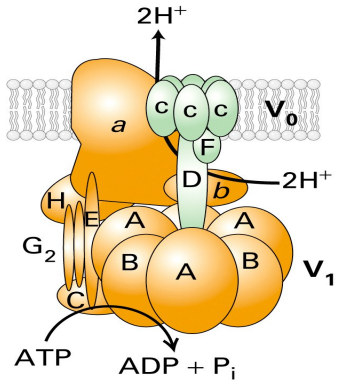
Operational model of the Na^+/K^+ ATPase in the plasma membrane



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 imporant 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.

14

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



V-class proton pumps

Vacuolar membranes in plants, yeast, other fungi

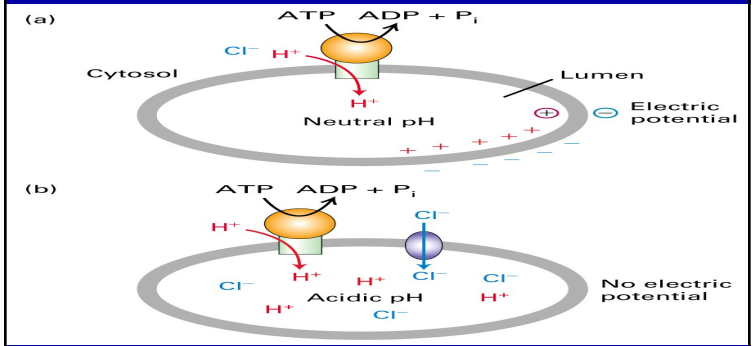
Endosomal and lysosmal membranes in animal cells

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.

15

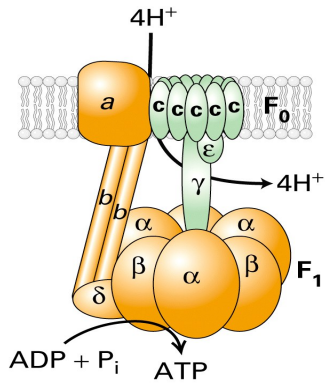
Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes.



(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.

16

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



F-class proton pumps

Bacterial plasma membrane

Inner mitochondrial membrane

Thylakoid membrane of chloroplast

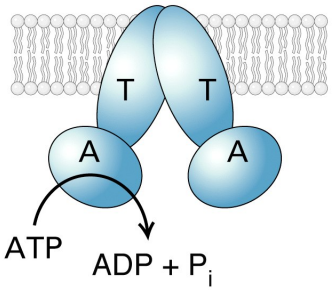
change of ?
pH
[ion]
e^--potential

electromotive force (emf)

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.

17

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



ABC superfamily

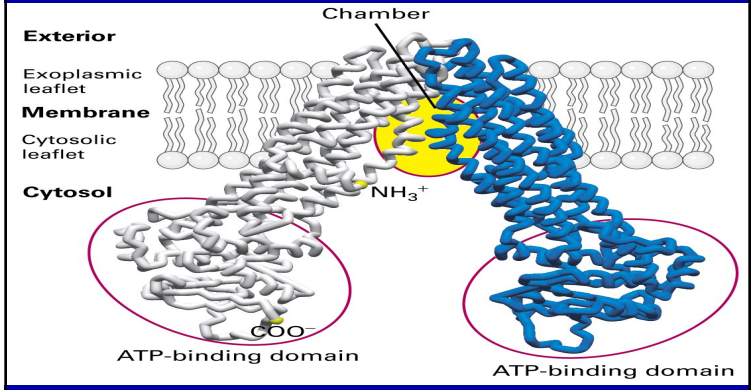
Bacterial plasma membranes (amino acid, sugar, and peptide transporters)

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.

18

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

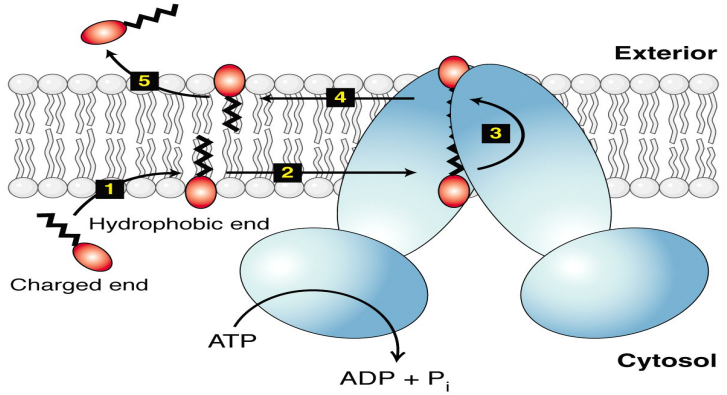


The V-shaped protein encloses a "chamber" within the bilayer where it is hypothesized that bound substrates are flipped across the membrane, as shown in the next slide.

Each identical subunit in this homodimeric protein has one transmembrane domain, comprising six alpha helices, and one cytosolic domain where ATP binding occurs.

19

Flippase model of transport by MDR1 and similar ABC proteins

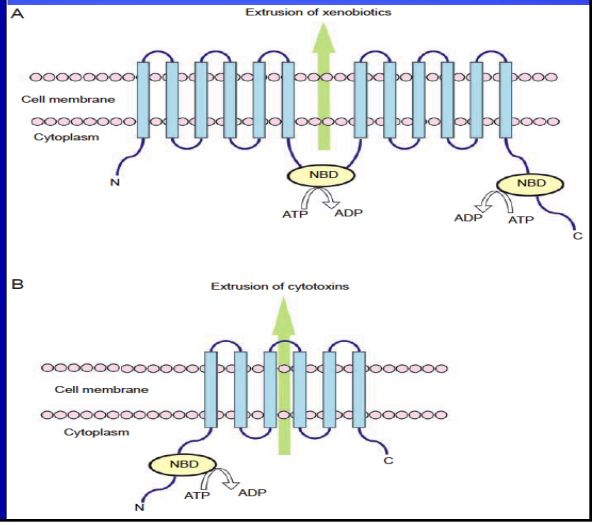


1: the hydrophobic portion (black) of a substrate molecule moves spontaneously from the cytosol into the cytosol-facing leaflet lipid bilayer, while the charged end (red) remains in the cytosol. 2: the substrate diffuses laterally until encountering and binding to a site on the MDR1 protein within the 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.

20

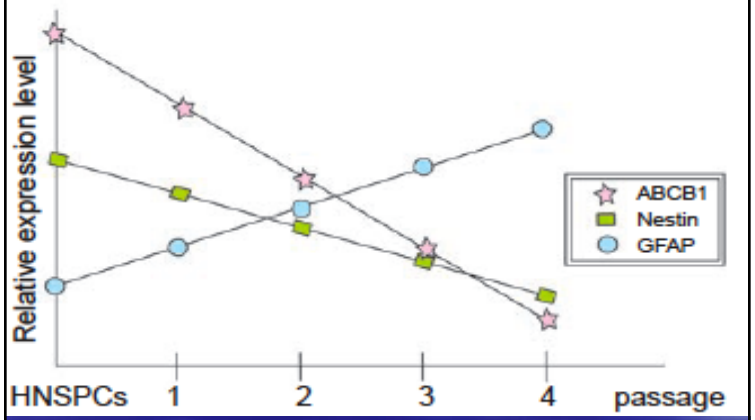
ABC Transporters - and Bio-Tissue Engineering (1)

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.



21

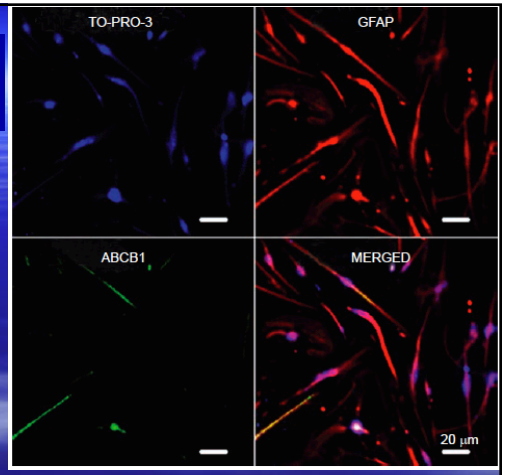
ABC Transporters - and Bio-Tissue Engineering (2)



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.

22

ABC Transporters - and Bio-Tissue Engineering (3)

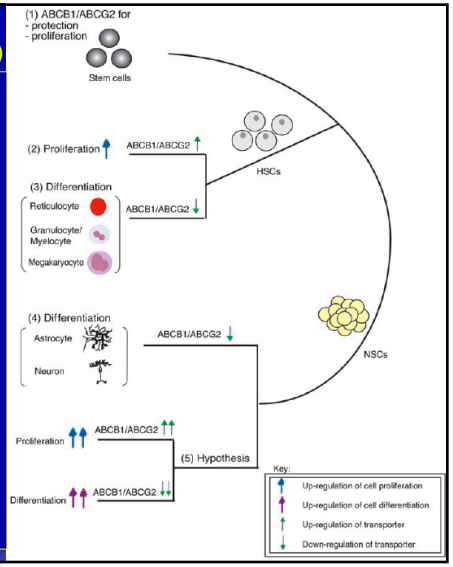


Immunocytochemical analysis of the ABCB1 and GFAP expression pattern in hNSPCs differentiated into glia cells. Nuclei were stained with TO-PRO-3. Scale-bar = 20 μm.

23

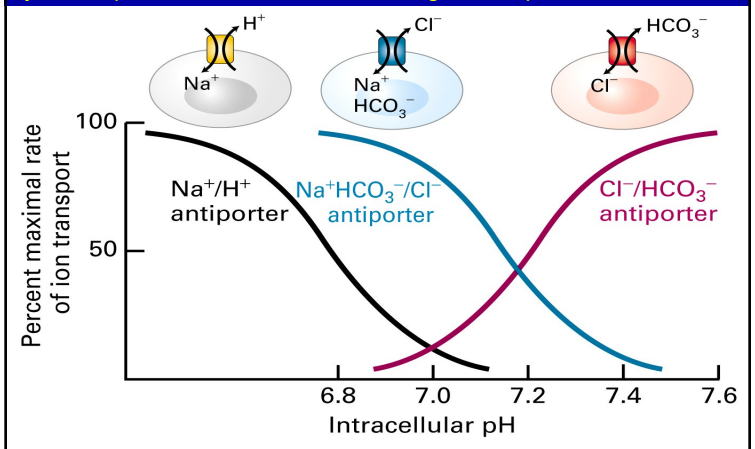
ABC Transporters - and Bio-Tissue Engineering (4)

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.



24

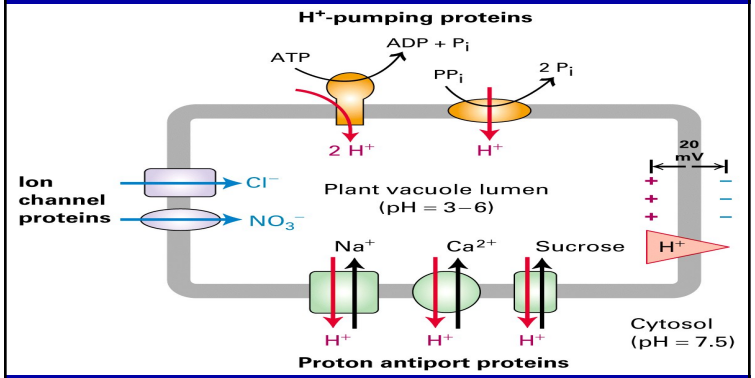
The activity of membrane transport proteins that regulate the cytosolic pH of mammalian cells changes with pH



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

25

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.

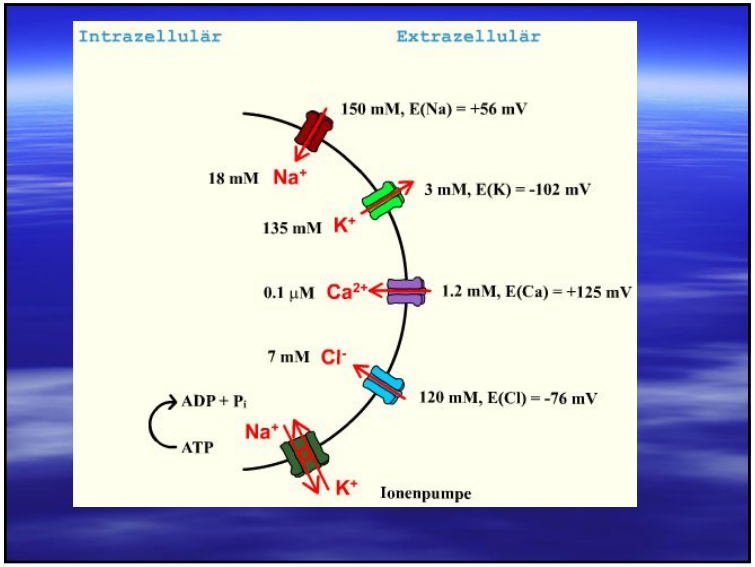
26

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

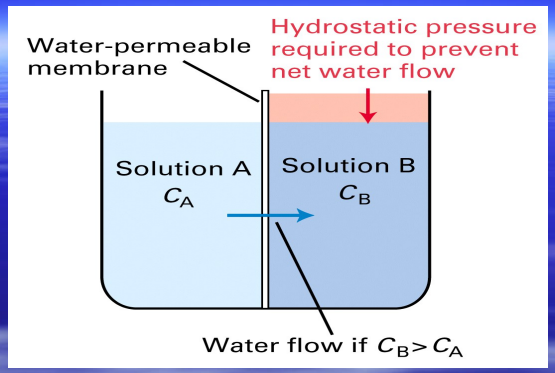
*The large nerve axon of the squid has been widely used in studies of the mechanism of conduction of electric impulses.
†X^- represents proteins, which have a net negative charge at the neutral pH of blood and cells.

27



28

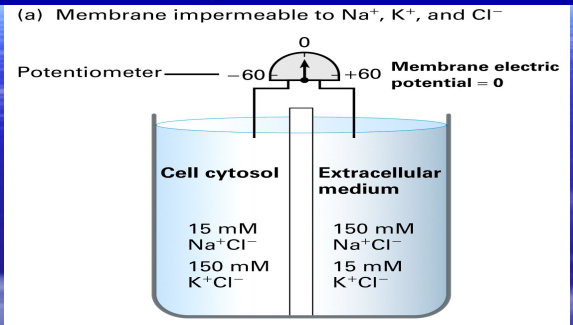
Movement of water - Osmotic Pressure



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.

29

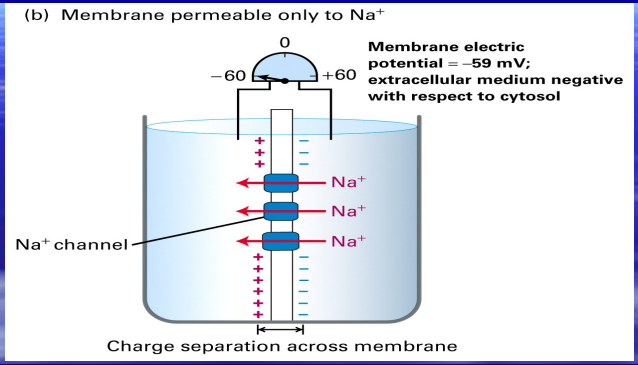
Generation of a transmembrane electric potential (voltage) depends on the selective movement of ions across a semi-permeable membrane (a)



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.

30

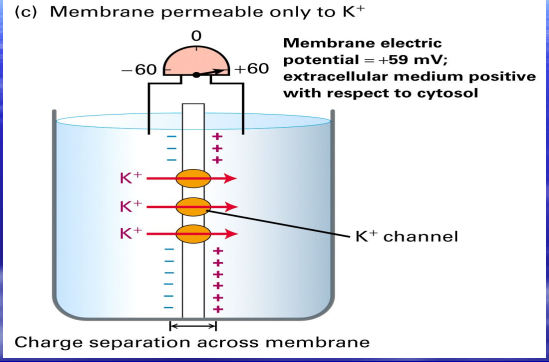
Generation of a transmembrane electric potential (voltage) depends on the selective movement of ions across a semi-permeable membrane (b)



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.

31

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

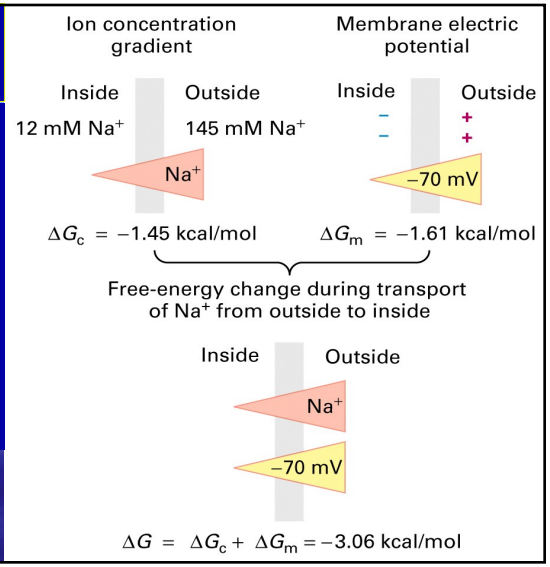


If the membrane is selectively permeable only to K^+, then diffusion of ion through their respective channels leads to a separation of charge across the membrane. At equilibrium, the membrane potential caused by the charge separation becomes equal to the Nernst potential E_K registered on the photometer.

32

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.



33

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

34

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

$2\ Na^+_{out} + glucose_{out} <----> 2\ Na^+_{in} + glucose_{in}$

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.

35

END

36