

Week 4 & 5 - Plasma Membrane I structure and transport

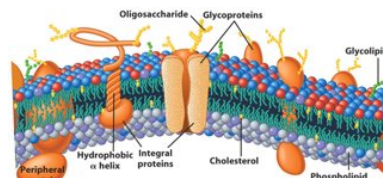
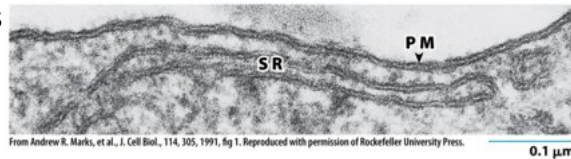
Suggested readings: Karp 9th ed. Chapter 4

4.1 Overview of membrane functions

4.2 Chemical composition of membranes

4.3 & 4.6 Membrane proteins

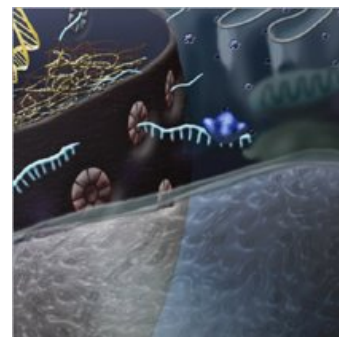
4.4 & 4.5 Membrane lipids



<http://www.dnatube.com/video/551/The-Inner-Life-Of-A-Cell-HQ>

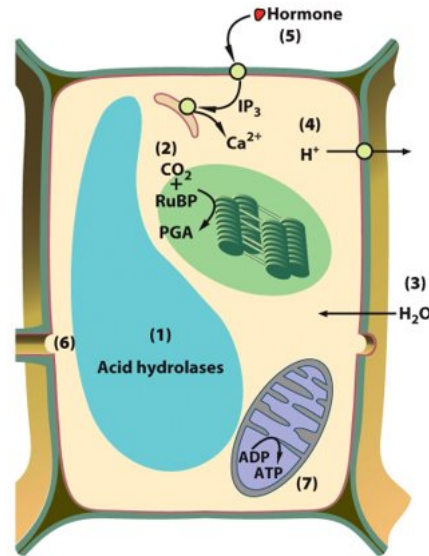
Membranes – An Overview

- Continuous, unbroken sheets, enclosing compartments
- Dynamic structures capable of fusing without losing continuity
- **External membrane:**
 - plasma membrane: encloses contents of entire cells
- **Internal membranes:**
 - nuclear envelope
 - mitochondrial membranes
 - chloroplast membranes
 - lysosomal membrane
 - endoplasmic reticulum



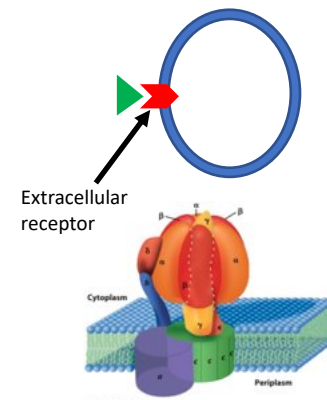
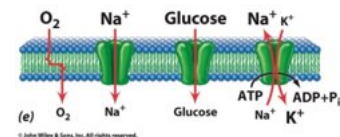
Membranes several structural functions

- **Compartmentalization** Membranes form continuous sheets that enclose intracellular compartments.
- **Scaffold for biochemical activities**
Membranes provide a framework that organizes enzymes for effective interaction.
- **Selectively permeable barrier** Membranes allow regulated exchange of substances between compartments.



Membranes mediate signals and transport

- ***Transporting solutes*** – Membrane proteins facilitate the movement of substances between compartments.
- ***Responding to external signals*** – Membrane receptors transduce signals from outside the cell in response to specific ligands.
- ***Intercellular interaction*** – Membranes mediate recognition and interaction between adjacent cells.
- ***Energy transduction*** – Membranes transduce photosynthetic energy, convert chemical energy to ATP, and store energy.

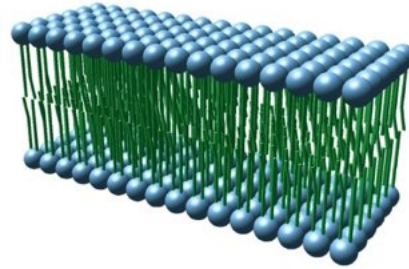


Figures are just examples... we'll go over them later!

Overview of plasma membrane structure

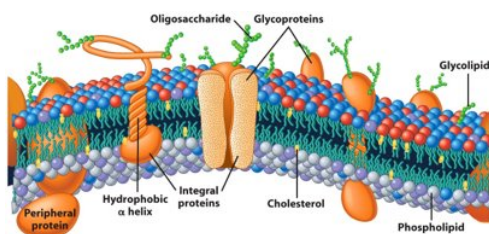
• The lipid bilayer:

- The most energetically favored orientation for the polar head groups is facing the aqueous compartments outside of the bilayer
- The lipid and protein components are bound together by non-covalent bonds.
- Membranes also contain carbohydrates.
- Protein/lipid ratios vary among membrane types



Fluid mosaic model

- lipid bilayer is core of membrane
- lipid molecules are present in a fluid state capable of rotating and moving laterally within membrane.
- proteins occur as "mosaic" of discontinuous particles.
- some proteins penetrate deeply into, and even through lipid bilayer.
- membranes are dynamic structures in which components are mobile.
- components come together to engage in various transient interactions.



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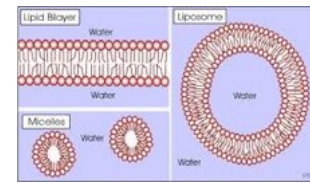
https://www.youtube.com/watch?v=jM_xePC70Yo



Fusing cells: evidence of membrane fluidity

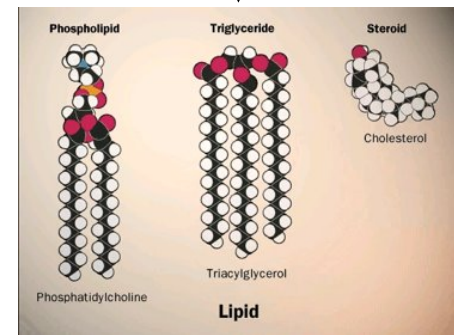
Characteristics of membrane lipids

- All are amphipathic
 - except for glycolipids and cholesterol, all contain phosphate group – hence, they are called phospholipids
 - phospholipids have a glycerol backbone
 - therefore, phospholipids are also phosphoglycerides
- Different lipids in cell membrane:
 - Phosphoglycerides
 - Sphingolipids & Glycolipids
 - Cholesterol



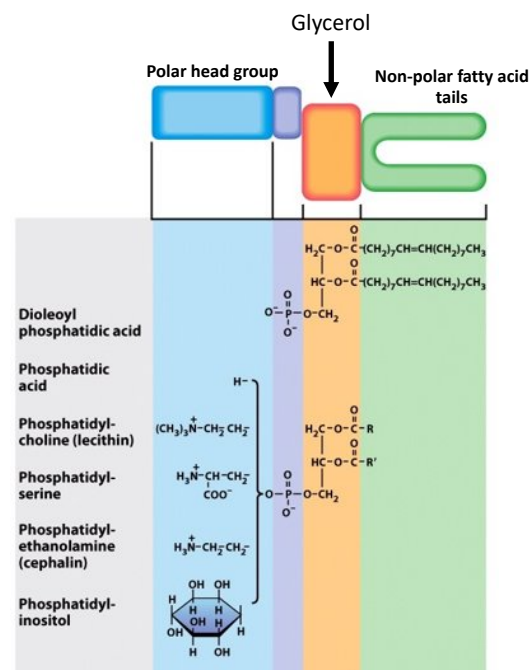
<https://encrypted-tbn2.gstatic.com/>

**Fat...
not amphipathic and
not in membranes!**



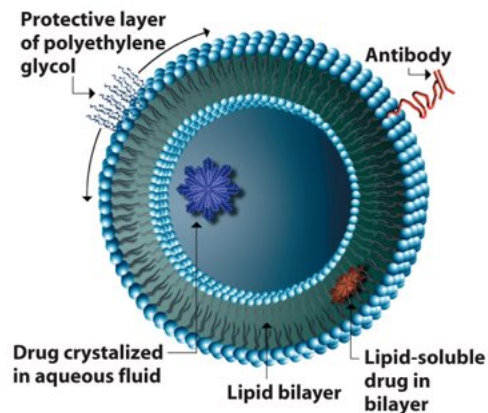
Phosphoglycerides

- Negatively charged phosphate group (purple) and a water soluble domain (blue).
- *Diglycerides*
 - often have one saturated and one unsaturated fatty acid (green) bound to a glycerol backbone (orange).
- Third OH group is coupled to a phosphate plus either:
 - Phosphatidyl choline (PC)
 - Phosphatidyl ethanolamine (PE)
 - Phosphatidyl serine (PS)
 - Phosphatidyl inositol (PI)



Membrane molecules can self assemble and deliver drugs to specific target cells

- phospholipid + water in a test tube -> liposomes
- drugs or DNA can be transported inside the liposome bilayer.
- antibody proteins help target to specific tissues.
- PEG provides protection of the body's immune system (stealth).



What is the difference between a micelle and a liposome?

2. Sphingolipids

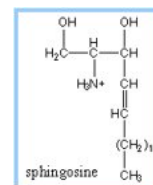
- sphingosine-based lipids.
- sphingosine is an amino alcohol with a long hydrocarbon chain.
- Ceramide = sphingosine + fatty acid (attached to N)

Sphingolipids play roles in

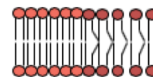
- membrane structure: decreasing membrane fluidity, becoming more gel-like rather than fluid (and therefore resistance to some stresses)
- signaling to other cells – usually because of the sugar groups attached to them, allow other cell receptors to bind
 - a subclass of sphingolipids have sugars attached at the polar head – these sphingolipids are called **glycolipids**

Glycolipids play key roles in:

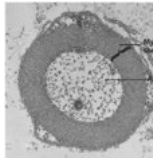
- cell membrane recognition;
- electrical conduction (e.g. myelin around nerve cells)
- inter-cell communication
- binding bacterial toxins (eg. botulism) and some viruses – permitting their entry into the cell



sphingolipid



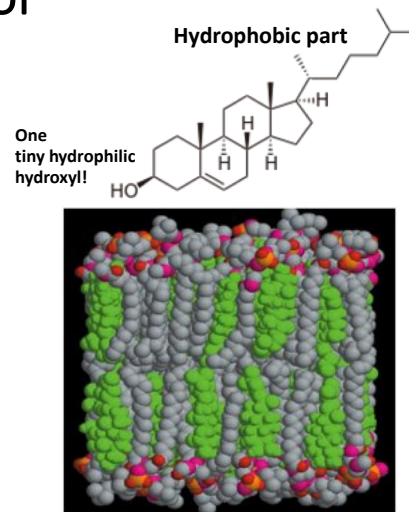
phospholipid



Myelin sheath cells around nerve cells have membranes rich in glycolipids

Cholesterol

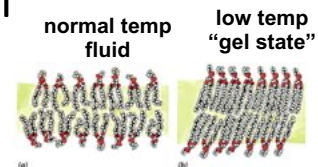
- Our cells need cholesterol!
- Animal cells have more cholesterol – but plants have low levels of cholesterol
- **What's the point to cholesterol of the cell?**
 - Can account for 50% of the PM of certain animal cells
 - Gives membranes stability especially during changes in temperature
- **Does two things:**
- **High temps**
 - Makes membrane too fluid... cholesterol reduces this fluidity
 - Cells will adapt to high temp to produce more cholesterol
- **Low temps**
 - Makes membrane more gel like
 - Prevents phospholipid tails from crystallizing, thus (counterintuitively) maintaining fluidity!!



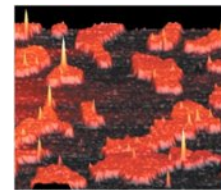
Hydrophilic ends face external surface
Inside – packed phospholipid tails.
Cholesterol = green molecules.

Membrane fluidity is a measure of freedom of movement within the membranes.

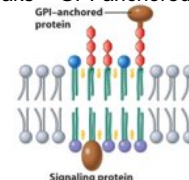
- effect of the fatty acid $(CH_2)_n$ chain on fluidity:
 - longer chains have less fluidity
 - presence of double bonds (unsaturated FA) increases “kinks” and hence fluidity
 - Sphingolipids are more rigid than phosphoglycerides (decrease fluidity)
- fluidity is important to balance mobility and organization
 - cell growth,
 - cell division,
 - inter-cellular junctions,
 - endocytosis and vesicle traffic
- heterogeneity, local patches of membrane may have unique fluidity, e.g. sphingolipid-rich patches or “rafts” – often find cholesterol in lipid rafts
 - Certain proteins (esp. those involved in cell signaling) are preferentially found in lipid rafts



Fluidity is dependent on type of lipid and temperature – high T, more fluid



Lipid rafts – red;
Peaks – GPI-anchored proteins



Cells are able to modify membrane chemistry to maintain correct fluidity

a) remodeling enzymes for short-term response

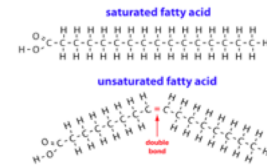
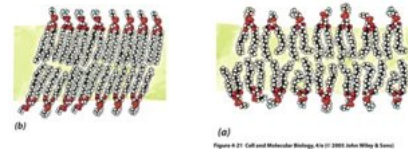
- increased numbers of double-bonds by **desaturase**, alters the fatty acid chains (e.g. exposure of bacteria to cold can induce desaturase activity)

- shuffling of the fatty acid chains on phospholipids

- this is accomplished by **phospholipase** (splits fatty acids from glycerol backbone), and **acyltransferase** (moves fatty acids between glycerol backbones)

b) over longer term, different phosphoglycerides are made by the cell

c) remodeling occurs in prokaryotes and eukaryotes, including animals, plants depending on the temperature they live at; most mammals regulate internal temperature with a few exceptions: extremities in hibernating animals often show changes in lipids



Membrane Carbohydrates

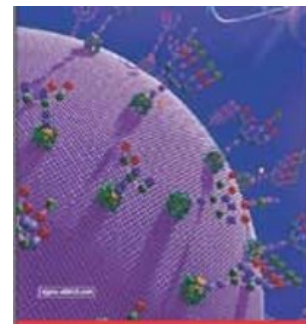
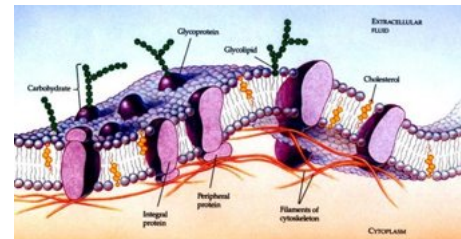
- all of the membrane carbohydrates face outward into extracellular space.
- - covalently linked to either protein or lipid.

- **1. Glycoproteins**

- carbohydrate is present in short, branched oligosaccharides (<15 sugar monomers per chain)
- may be attached to R group of:
 1. asparagine (N-linked)
 2. serine or threonine (O-linked)
- play roles in cell interactions.

2. Glycolipids

- may play role in certain infectious diseases.
- carbohydrates of glycolipids determine blood type (A, B, AB or O)



Membrane proteins have a variety of 'associations' with the membrane

- hundreds of different proteins associated with interior or plasma membrane - each cell type is different.
- highly “sided”: each protein has specific internal/external orientation. E.g.:
 - hormone receptors on outside
 - signal proteins on the cytoplasmic side (G-proteins, protein kinases)

There are **three classes of membrane proteins**

1. *Integral proteins*, (trans-membrane proteins), lipid soluble portion, cytosolic portion and exterior portion.
2. *peripheral proteins*, inside or outside the membrane, associated by non-covalent bonds
3. *lipid-anchored proteins*, inside or outside, covalently linked to a lipid.

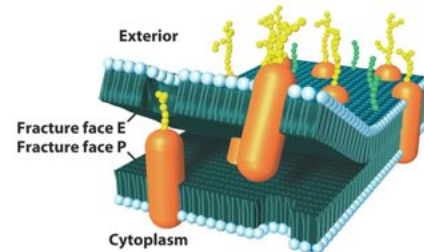
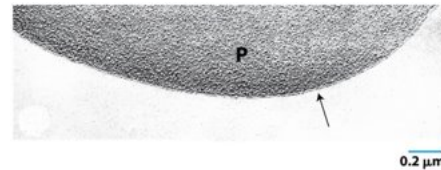


Figure 4-13a Cell and Molecular Biology, 4th (© 2003 John Wiley & Sons)



From Thomas W. Tillack and Vincent T. Marchesi, J. Cell Biol. 45:651, fig. 1 (top), 1970. Reproduced with permission of Rockefeller University Press

1. Integral membrane proteins

- penetrate into lipid bilayer
- usually pass right through membrane
- have segments protruding into extracellular space.
- have segments protruding into cytoplasm.
- transmembrane segments
 - pass through lipid bilayer
 - usually consist mostly of hydrophobic (or neutral) amino acids (red dots on figure) organized in an α -helical conformation
 - hydrophobicity plot – show which amino acids are likely to be found within membrane (above red line)
- some integral membrane proteins can form aqueous channel through lipid bilayer (more than 1 α -helices)

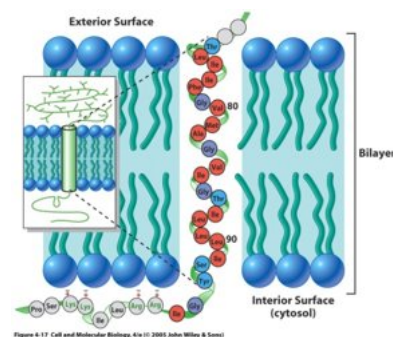
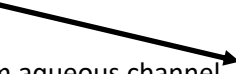
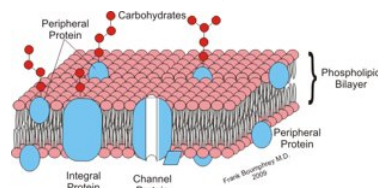


Figure 4-17 Cell and Molecular Biology, 4th (© 2003 John Wiley & Sons)

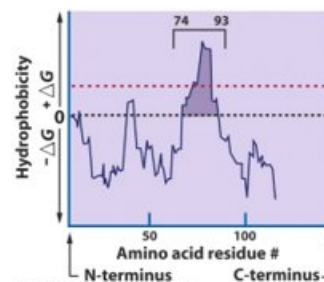
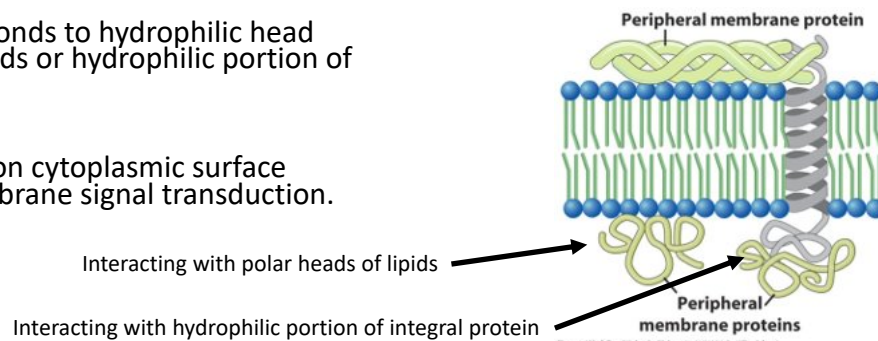


Figure 4-18 Cell and Molecular Biology, 4th (© 2003 John Wiley & Sons)

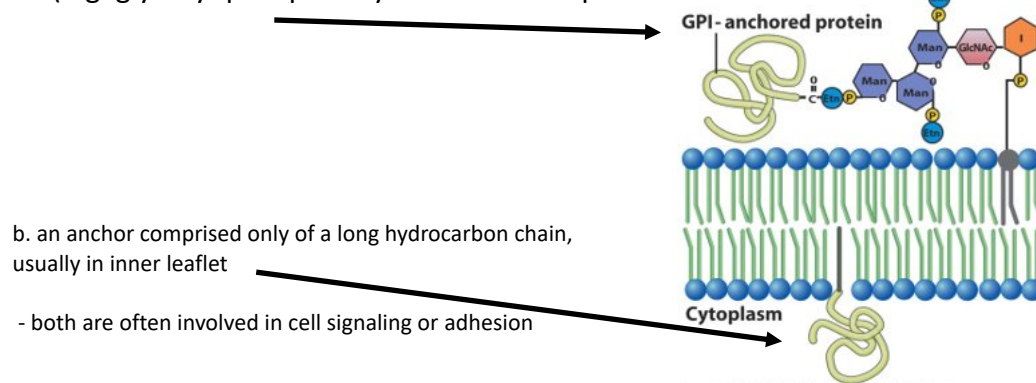
2. Peripheral membrane proteins

- located entirely outside of lipid bilayer.
- either on extracellular or cytoplasmic surface.
- associated with membrane surface by noncovalent bonds.
- weak electrostatic bonds to hydrophilic head groups of phospholipids or hydrophilic portion of integral proteins.
- peripheral proteins on cytoplasmic surface function in transmembrane signal transduction.



3. Lipid-anchored membrane proteins

- Protein is covalently linked to lipid molecules within bilayer.
- two types of lipid anchors (examples will follow in later lectures)
 - a. an anchor comprised of a lipid + carbohydrate chain, usually in outer leaflet (e.g. glycosyl-phosphatidylinositol linked protein)



- both are often involved in cell signaling or adhesion

What you should know after this lecture

- Various functions of membranes
- Fluid mosaic model of the membrane
- Types of lipids within membranes
- How is membrane fluidity altered?
- What changes occur to lipids as temperature changes?
- How are carbohydrates associated with membranes?
- Define the three types of proteins associated with membranes

Membrane transport I - Passive transport

- Structure of lecture:
 1. Overview of 4 ways solutes get across a membrane
 2. Discussion of **diffusion** and **osmosis**
 3. Going over ways passive transport of solutes across a membrane work

Suggested Readings: Karp 9th edition:

4.6 Movement of substances across cell membranes

Intro: Transport across cell membranes

Two functions of membranes (of those we looked at last class):

1. Must retain dissolved material and prevent leakage.
2. Must allow for material to be exchanged into and out of cell.

Why is this exchange of material needed?

1. Cells acquire the molecules and ions they need from their surrounding extra-cellular fluid (ECF) – an unceasing traffic of molecules and ions!!
 - *Within* the cell, molecules and ions must be transported in and out of compartments too!
2. Cells make waste products, which need to be dumped either outside the cell

Four basic mechanisms by which molecules move across membranes

A) Simple diffusion

Movement from higher to lower concentrations straight through the lipid bilayer.

B) Simple diffusion through an channel

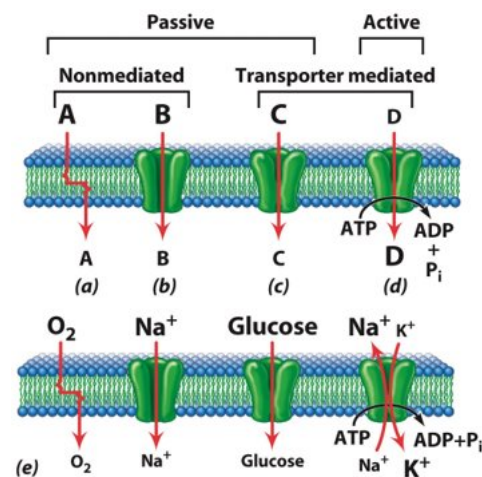
Movement from higher to lower concentrations through the pore of an membrane channel protein

C) Facilitated diffusion

Movement from higher to lower concentrations via a membrane protein carrier (aka 'facilitative transporter')

D) Active transport

Movement from low to high concentrations via a protein transporter. Requires energy, which often comes from ATP hydrolysis.

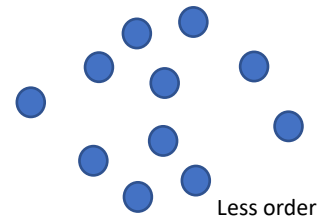


Note that channels, facilitative transporters and active transporters are ALL integral membrane proteins

Mechanism A: Simple diffusion

Molecules and ions move spontaneously down their concentration gradient (i.e., from a region of higher to a region of lower concentration) by **diffusion**.

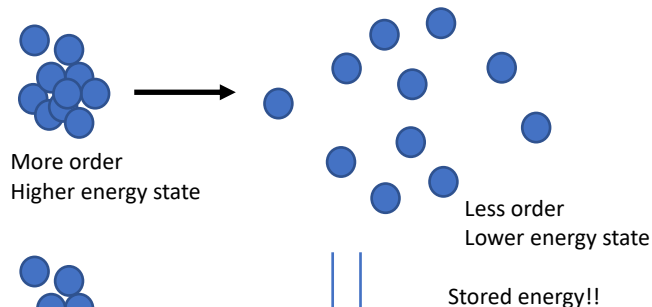
diffusion: the spontaneous movement of solute from regions of high concentration to low concentration - follows the 2nd law of thermodynamics



Molecules and ions can be moved **against** their concentration gradient, but this process, called **active transport**, requires the expenditure of energy (usually from ATP).

In case you forgot (I know I did...), the 2nd law of thermodynamics states: in a natural thermodynamic process, there is an increase in the sum of the entropies of the participating systems.

Maintaining differences in concentration stores energy



When molecules diffuse, the entropy of the system increases.

(remember, entropy = disorder)

Going from higher to lower order dissipates free-energy (ΔG).

Preventing this dissipation of energy with a semipermeable membrane thus *stores* this energy.

This energy can be dissipated later to do stuff.

How much energy?
Assume 10x
difference on each side
of the membrane

$$\Delta G = RT \ln([Cl]/[Co])$$

$$= 1.4 \text{ kcal mole}^{-1} \text{ stored energy}$$

Do not memorize
This stuff

Mechanism A: Simple diffusion continued...

The cell membrane is **permeable** to water molecules and a few other small, uncharged, molecules like oxygen (O_2), carbon dioxide (CO_2), and small non-polar molecules. These diffuse freely in and out of the cell.

Lipid membranes are **not** permeable to:

- ions such as:

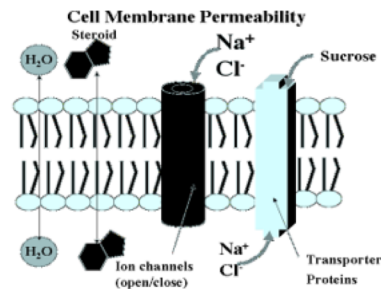
K^+ , Na^+ , Ca^{2+} (called **cations**)

Cl^- , HCO_3^- (called **anions**)

- small hydrophilic molecules like glucose

- macromolecules like proteins and RNA

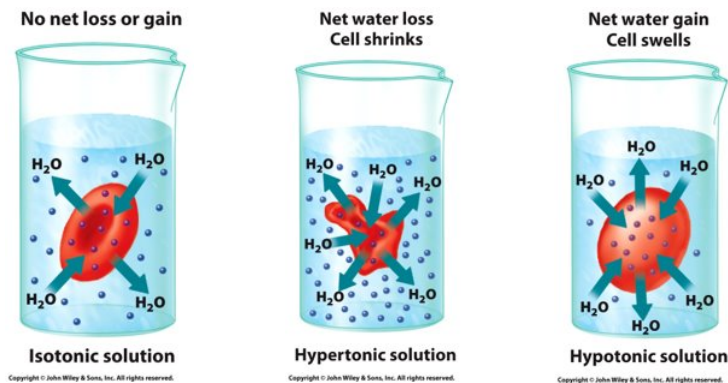
All of these molecules need 'help' to get across the membrane, and are assisted by proteins



Diffusion of water across membranes

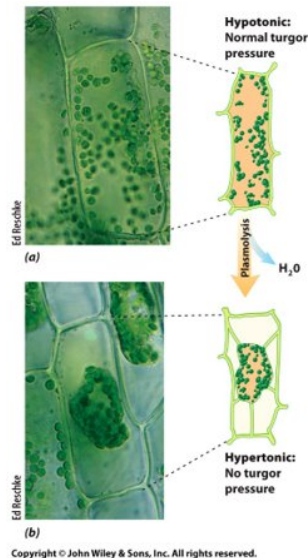
Water can freely move across membranes, solutes (salts, etc.) cannot.

Water will move from an area of low solute concentration to high solute concentration. This is called **osmosis**.



Memory device: *tonic* water is thick with sugar (solute). I *hypertonic* solution has *lots* of sugar.

Why do plants wilt when you forget to water them?

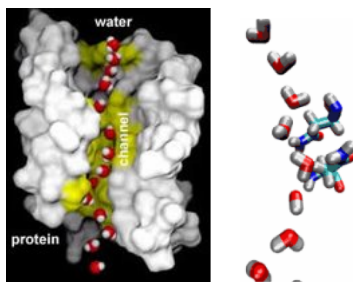


- The fluids outside of plant cells is hypotonic. (inside is hypertonic)
- Thus water flows into the plant cell.
- This causes the cells to swell, but there's a rigid plant cell wall in the way.
- So the cell pushes against the cell wall, creating 'turgor pressure'
- I forgot to water my plants. Again...
- The loss of water in the whole plant causes the fluid outside of the cells to become hypertonic.
- Water flows out of the cells, the cells shrink in a process called **plasmolysis**. The turgor pressure goes to zero, and my plant wilts and looks sad.

Aquaporins mediate the flow of water through some membranes

- in some cell types the movement of water is much greater than it would be if it flowed through the lipid bilayer itself.
- due to the presence of trans-membrane **aquaporin** proteins (movement is still passive, no energy needed, down the conc. gradient).

e.g. 1. In the gut, several liters of water per day are moved into the lumen of stomach, intestine, then re-absorbed in the lower gut.



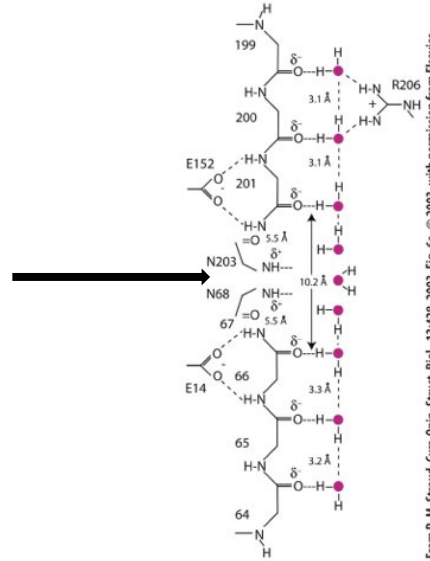
Aquaporin pore/channel allows water molecules to move through in single file

This is an example of B on slide 3!

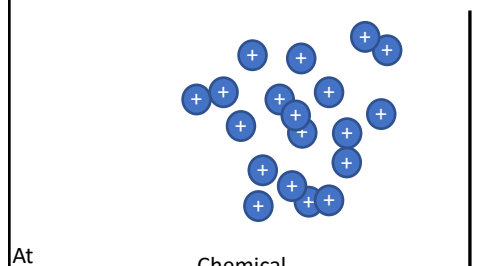
<http://www.ks.uiuc.edu/Research/aquaporins/>

X-ray crystal structures and molecular dynamics of aquaporins have shed light onto how they work

- Water flows single file through the pore... one billion per second!
- Hydrogen bonding from the amino acids and peptide backbone coordinates the water molecules as they flow through.
- The amino acid residues N203 and N68 occur at the narrowest point in the channel. The positive dipole on these residues interrupts the intra-water H-bonds and flip the water around.
- This prevents H⁺ ions that can 'hitch a ride' on the water column from getting through the pore.
- Thus, aquaporins are **selective** for transporting water across a membrane.



Maintaining differences in the concentration of ions stores even more energy



At

10x difference in concentration

Chemical
Concentration
terms

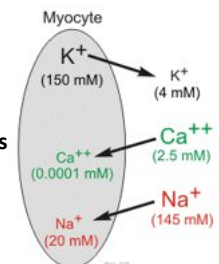
Electrical
terms

$$\Delta G = RT \ln([C_i]/[C_o]) + zFDE_m$$

$$= 3.1 \text{ kcal mole}^{-1} \text{ stored energy}$$

- Ions kept across a membrane still store energy due to the concentration effect
- They ALSO repel each other due to their like charge...
- This adds an extra term to the energy stored when ions kept on one side of a membrane... if the overall charge on that side is their charge state (+ or -)
- This is called an **electrochemical gradient**

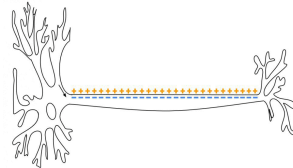
There are some large differences in ion concentration across membranes... this can really add up!



Electrochemical gradients are dispersed by diffusion of ions across membranes

The rapid movement of ions across a membrane (called **conductance**) is important in processes such as:

- Nerve impulses
- Secretion of substances to external space
- Muscle contraction
- Many others!



These rapid movements are from high to low concentration

The movement is done through **ion channels** (another example of B from slide 3)

- these are integral membrane proteins
- the movement is *selective*. Each ion channel will only pass one type of ion
- the movement is *bidirectional* dependant on concentration

Ion channels can be gated

These channels can be maintained in either open or closed states (gated). This allows the to be closed until they exactly need to be opened.

Voltage gated channels

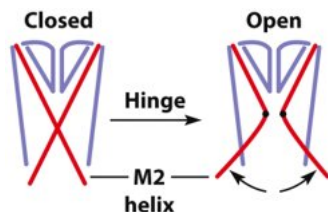
- Monitor the voltage around them and open/close if there's a change

Ligand-gated channels

- Open/close if a specific ligand binds to them... usually *not* what goes through them

• **Mechano-gated channels**

- Monitor mechanical forces and open/close with the appropriate signal



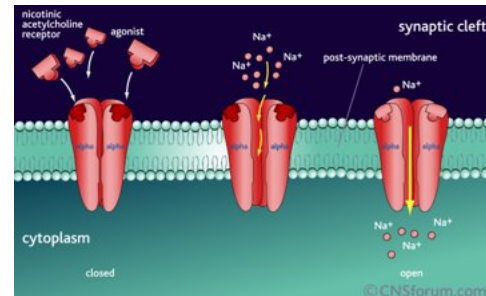
An example of a gate... movements of alpha-helices (red) in the ion channel open and close the pore through which ions can flow.

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Examples of a gated ion channels

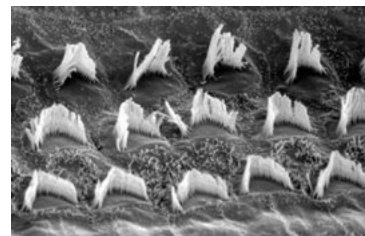
Ligand-gated ion channel. The binding of the neurotransmitter acetylcholine at certain synapses opens the Na^+ channel.

This initiates a nerve impulse or muscle contraction.



Mechanically-gated ion channels

Sound waves bend cilia-like projections on the hair cells of the inner ear; the bending opens up ion channels, leading to the creation of nerve impulses to the brain.



TMC1 is the ion channel that regulates hearing in mammals

Long-Sought Hearing Channel Protein Found

After a decades-long pursuit, researchers have confirmed the identity of the pore of the mechanotransduction channel in vertebrate inner ear hair cells.

Aug. 22, 2018

NIH/NIH

Scientists have been looking for years for the proteins that convert the mechanical movement of inner ear hair cells into an electrical signal that the brain interprets as sound. In a study published today (August 22) in *Neuron*, researchers have confirmed that transmembrane channel-like protein 1 (TMC1) contributes to the pore of the so-called mechanotransduction channel in the cell's membrane.

"The identification of the channel has been missing for a long time," says Anthony Freg, a neuroscientist at the University of Colorado Denver who did not participate in the study. This work "settles the debate as to whether or not [TMC1] is a pore-forming component of the mechanotransduction channel."

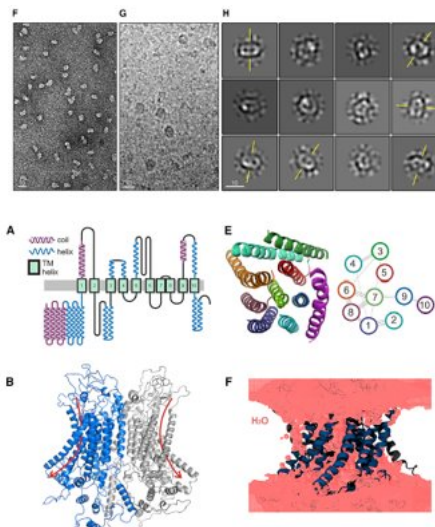
When a sound wave enters the cochlea, it wiggles protrusions called stereocilia on both inner hair cells, which amplify the signals, and outer hair cells, which convert the mechanical signals to electrical ones and send them to the brain. It's been tricky to figure out what protein the inner hair cells use for this conversion, because their delicate environment is difficult to recreate in vitro in order to use candidate channel proteins. In 2000, researchers reported on a promising candidate in flies, but it turned out not to be conserved in mammals.

In a study published in 2013, Jeffrey Holt of Harvard Medical School and Boston Children's Hospital and colleagues showed that genes for TMC proteins were necessary for mechanotransduction in mice. This evidence—combined with *no-fewer* from another group showing that mutations in these genes could cause deafness in humans—pointed to the idea that TMC1 formed the ion channel in inner ear hair cells.

To investigate the protein's role, Holt and colleagues created 17 different TMC1 genetic mutants, based on the best-known site in deafness or their location in the structure of TMC1 that they propose in the study. Each sequence alteration changed the endogenous amino acid to cysteine. The authors next injected an adenoviral vector carrying either the wildtype gene or one of the 17 mutant versions into the ears of mouse pups lacking the gene entirely. Four or five days later, they removed the hearing organs from the ears of the mice and cultured them for another three to 10 days.

While in culture, the researchers made electrophysiological measurements of currents in the hair cells to see the transduction potential of the mutated TMC1 proteins. They also measured currents in the presence of a drug that causes cysteine to form a disulfide bridge, likely affecting the shape of the TMC1 protein. In 16 of the 17 mutant proteins, the drug changed the amplitude of the current they recorded.

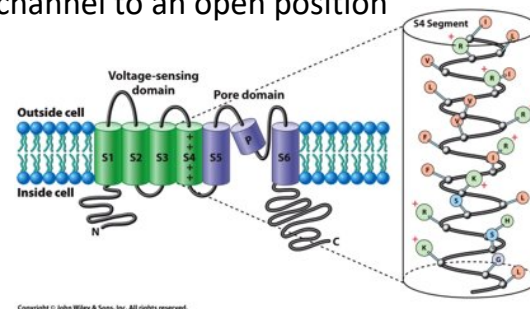
The Scientist, 22 August 2018



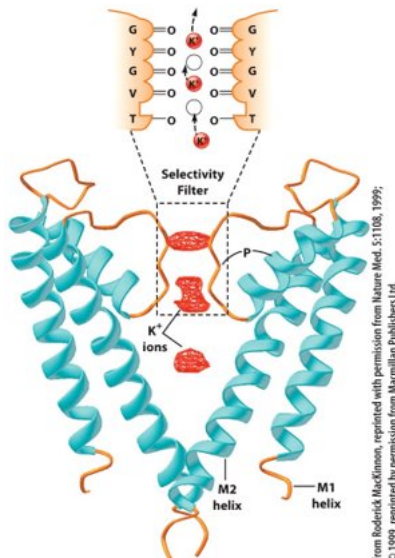
Pan et al 2018 <https://doi.org/10.1016/j.neuron.2018.07.033>

Voltage gated ion channel example : The K^+ channel

- involved in action potential transduction in nerves, muscles, etc.
- Opens *after* Na^+ channels first open
- 2 homodimers, 6 membrane spanning domains each
- The S4 helix is + charged and senses the charge state across the membrane (keeps channel closed)
- When the membrane charge state changes from the Na^+ channels open, S4 moves in a way that reconfigures the channel to an open position



K^+ channel are *selective* for only K^+ ions



How does the K^+ channel not mistakenly pass Na^+ ions?

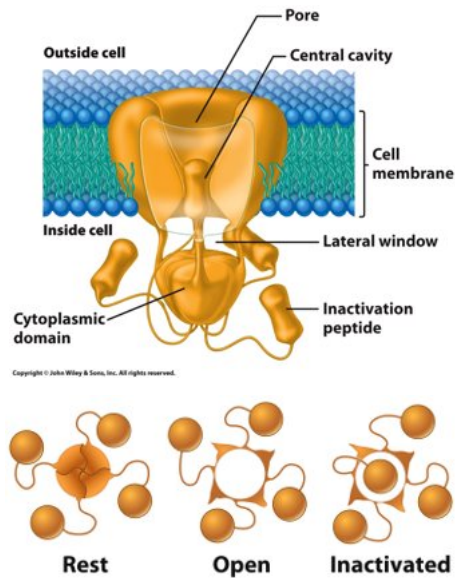
An X-ray crystallography structure of a bacterial K^+ channel shows its selectivity.

Carbonyl oxygen atoms held *just right* inside the pore bind K^+ ions as they go through. This the pore shape and allows passage.

Pore = 3 Å K^+ ion = 2.7 Å

Na^+ ions are smaller... cannot stabilize shape and are not passed.

The K⁺ ion channel has an 'auto-shutoff' feature



Reprinted from Neuron, Vol. 20, C. M. Armstrong and B. Hille, Voltage-Gated Ion Channels and Electrical Excitability, P. 377; Copyright 1998, with permission from Elsevier Science.

After a few milliseconds, the cytoplasmic inactivation peptide domain of the channel slides into the pore area, blocking the ion flow

So the K⁺ ion channel has three 'states':

1. Rest (closed)... S4 helix keeps the pore closed
2. An open state... after the signal has been received and the S4 helix has moved
3. An inactivated state where the inactivation peptide is blocking the pore

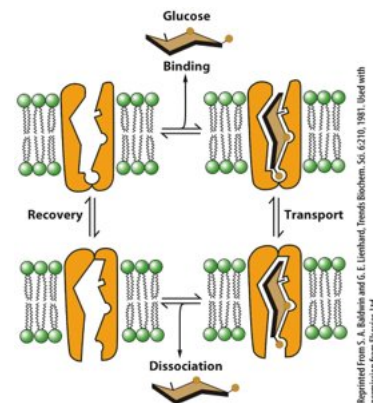
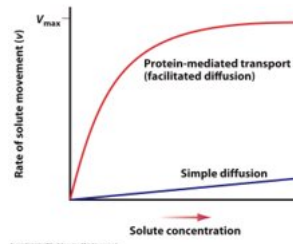
Facilitative transporters transport 'larger' molecules

Aquaporins and ion transporters allow passage of little molecules

Larger molecules can be transported across membranes by facilitative transporters

- We're still talking about passive diffusion from high to low concentration!

1. The glucose molecule binds to the transporter
2. This causes a conformational change in the transporter shape
3. This change exposes the glucose to the other side

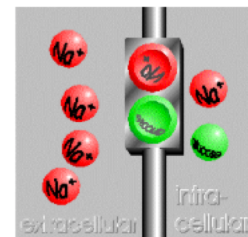


Because there is actual *binding* of the solute to the transporter, these behave a bit like an enzyme. Thus, the binding can *saturate* at high solute concentration!

Membrane transport II: Active transport

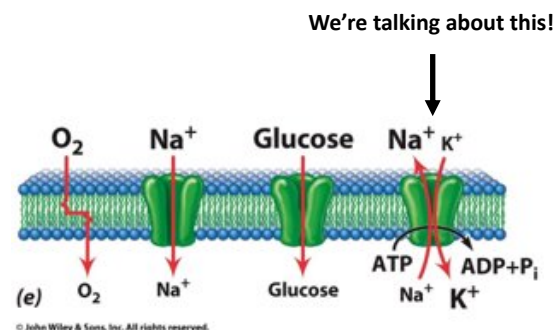
Suggested reading: Karp 9th edition
Active transport 4.6

www.liv.ac.uk



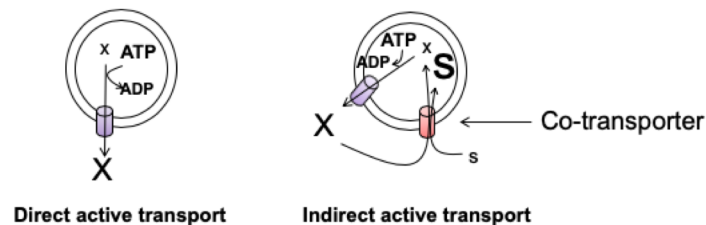
Active transport is the pumping of ions or molecules through a membrane against their concentration gradient

- it ***always*** requires a trans-membrane (integral) protein – usually called a transporter
- - the active transport proteins are
- specific for a given chemical
- their presence in the cell membrane is regulated by the cell
- transport is coupled to the consumption of energy



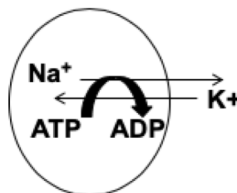
The source of the energy for active transport

- The source of the energy used in active transport is **ATP**.
- The energy of ATP may be used directly or indirectly.
- **Direct Active Transport** - the transporter binds ATP directly and uses the energy of its hydrolysis to drive active transport.
- **Indirect Active Transport** - the transporter uses the energy already stored in the gradient of a directly-pumped **ion**. Direct active transport of the ion establishes a concentration gradient. When this is relieved by facilitated diffusion, the energy released can be harnessed to the pumping of some other ion or molecule.



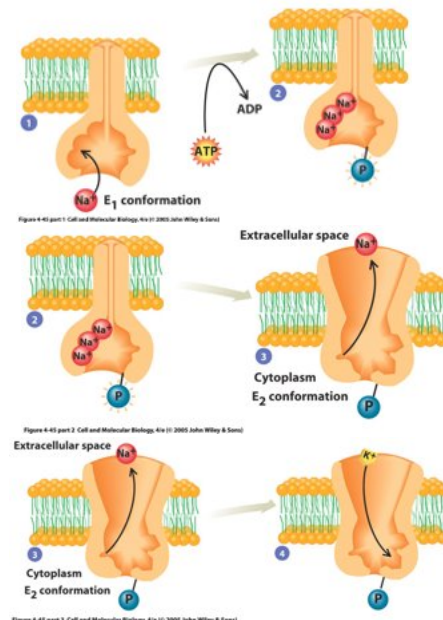
$\text{Na}^+/\text{K}^+\text{ATPase}$ (sodium-potassium pump)

- cells maintain high internal K^+ (100 mM inside, 5 mM outside)
- cells simultaneously maintain high external Na^+ (10-20 mM inside, 150 mM outside)
- sodium-potassium ATPase: an enzyme and a trans-membrane protein that establishes this gradient of ions
- this transporter uses ATP to pump out 3 Na^+ and pump in 2 K^+ , against their concentration gradients
- **electrogenic** pump, unequal transport of charged particles (3 Na^+ for every 2 K^+), important in maintaining normal trans-membrane electrical potential



Steps in transport: Na⁺/K⁺ATPase

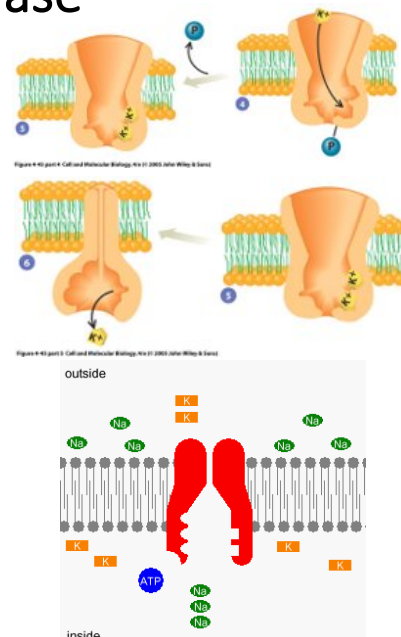
1. To begin with, the transporter has a high affinity for sodium and low affinity for potassium
 - sodium ions bind on the inside of the membrane (at a low conc. on the inside of the cell)
2. ATP is hydrolyzed, transfers phosphate on to protein
3. The protein changes its conformation, changes ion affinity
 - lower affinity for sodium
 - sodium ions diffuse away
4. Change in conformation increases affinity for potassium ions on outside of membrane (where the conc. of the ion is low).



Steps in transport: Na⁺/K⁺ATPase

5. phosphate removed from protein
 - energy from this causes conformational change...
6. protein returns to original conformation
 - lower affinity for potassium
 - higher affinity of sodium

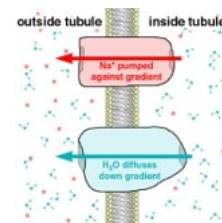
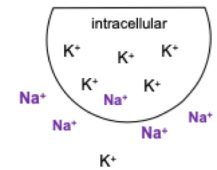
Ready for step 1 again



http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation_how_the_sodium_potassium_pump_works.html

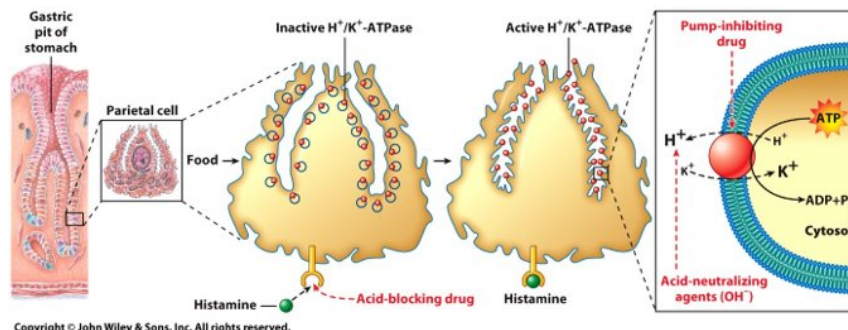
The importance of the Na^+/K^+ ATPase

- It creates a net charge across the plasma membrane with the interior of the cell being negatively charged with respect to the exterior. This **resting potential** prepares nerve and muscle cells for nerve impulses and muscle contraction.
- The accumulation of sodium ions outside of the cell draws water out of the cell and thus enables it to maintain **osmotic balance** (otherwise it would swell and burst from the inward diffusion of water).
- The gradient of sodium ions is harnessed to provide the **energy to run several types of indirect pumps**.
- The crucial roles of the Na^+/K^+ ATPase are reflected in the fact that almost **one-third of all the energy generated in animal cells is used just to run this pump**.
- Note: this pump is known as a P-type ion pump because it phosphorylates the transporter protein



The H^+/K^+ ATPase

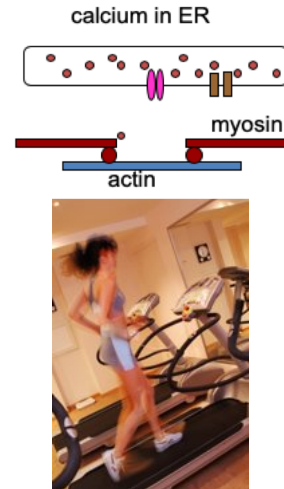
- The parietal cells of your stomach use this pump to secrete gastric juice. These cells transport protons (H^+) from a concentration of about 4×10^{-8} M within the cell to a concentration of about 0.15 M in the gastric juice (giving it a pH close to 1).
- Food in the stomach triggers histamine to bind to cells
- H^+/K^+ ATPase is translocated from vesicles to cell's membrane to initiate pumping of protons into stomach
- three-million fold concentration of protons needs huge amounts of ATP energy



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Ca²⁺ ATPase example in striated muscle

- In resting striated muscle, there is a much higher concentration of calcium ions (Ca²⁺) in the sarcoplasmic reticulum (ER) than in the cytosol.
- Activation of the muscle fiber allows some of this Ca²⁺ to pass by facilitated diffusion into the cytosol where it triggers contraction.
- After contraction, this Ca²⁺ is pumped back into the sarcoplasmic reticulum (= SER of muscle cells).
- This is done by a Ca²⁺ ATPase that uses the energy from each molecule of ATP to pump 2 Ca²⁺ ions.
- A Ca²⁺ ATPase is also located in the plasma membrane of all eukaryotic cells. It pumps Ca²⁺ out of the cell helping to maintain the ~10,000-fold concentration gradient of Ca²⁺ between the cytosol and the ECF.

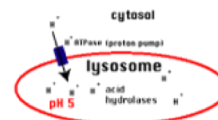


Microsoft Clip Art

ATPase pump types: V, P, ABC

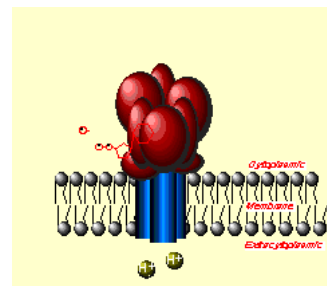
1. "P-type", transporter gets phosphorylated

- Four-subunit structure
- Na⁺/K⁺ ATPase
- Includes Ca²⁺ pump on cell membrane and SER (maintains low cytoplasmic [Ca²⁺])
- Includes stomach acid (H⁺) secretion pump



2. V-type, (also uses ATP, but transporter itself not phosphorylated) – pump found on vacuoles

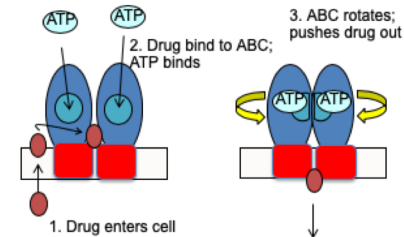
- H⁺ pump only
- Complex 7-subunit structure
- Plant cells use V-ATPase pumps to fill vacuoles with H⁺
- Animal cells have V-type pumps on lysosomes, vesicles, in the kidney (regulating pH in blood)



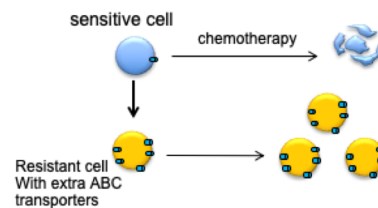
Animation found at:
<http://biologyofcells.blogspot.com/2007/12/pumps.h>

ABC type (ATP-Binding Cassette) family of ion transporters

- Share a unique phosphorylated region: “ATP-binding cassette”
- Human genome has 48 ABC transporters
- ABC transporters are transmembrane proteins that have: a substrate-binding domain at one surface of protein; and a ATP-binding domain at another surface (or domain).
- The substrate-binding domain can be restricted to a single type of molecule.
- The ATP bound to its domain provides the energy to pump the substrate across the membrane. ATP binding causes conformational change, which helps push substrate through

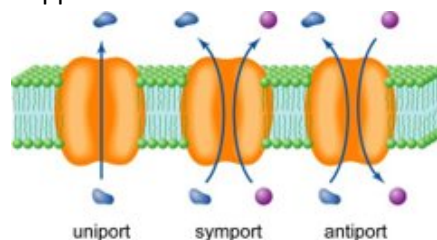


Cancer cells can become resistant to drugs by over-producing ABC transporters that expel the drug from the cell



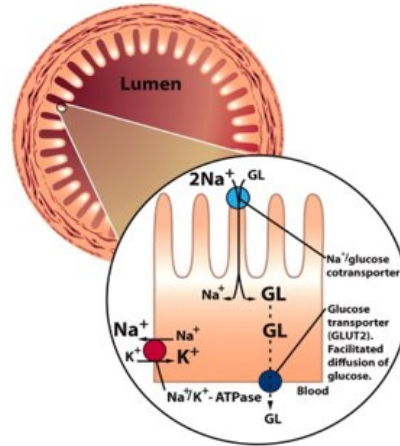
Indirect active transport = co-transport

- Indirect active transport uses the downhill flow of an ion to pump some other molecule or ion against its gradient. The driving ion is usually sodium (Na^+) with its gradient established by the Na^+/K^+ ATPase.
- **Symport Pumps**
 - the driving ion (Na^+) and the pumped molecule pass through the membrane pump in the **same** direction.
- **Antiport Pumps**
 - In antiport pumps, the driving ion (again, usually sodium) diffuses through the pump in one direction providing the energy for the active transport of some other molecule or ion in the opposite direction.



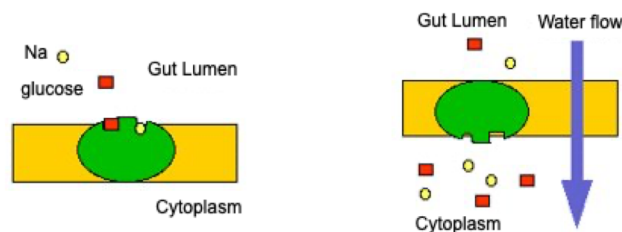
Symport example: Na^+ /glucose co-transporter

- glucose transport from the gut into bloodstream:
 - utilizes a sodium gradient to transport glucose against its concentration gradient
 - two Na^+ and one glucose are transported together
 - Glucose moves from low conc. of glucose in gut to a higher conc. within the cells lining the gut
- the energy source: Na^+ gradient is created by a separate pump (the Na^+/K^+ ATPase) elsewhere in the cell
- next: a passive channel then allows glucose to move into the blood, down its concentration gradient (facilitated diffusion)



Na^+ /glucose co-transporter can be used to absorb water into intestinal cells

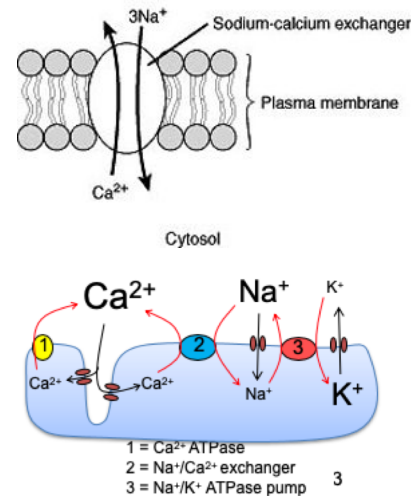
- With cholera or other diseases that cause diarrhea, water loss is serious
- Treatment to prevent water loss can be as simple as feeding sugar-salt solutions



The cotransport protein rotates and releases Na and glucose in the intestinal cells. This raises the osmotic pressure within the cell and sucks water back into the body by osmosis.

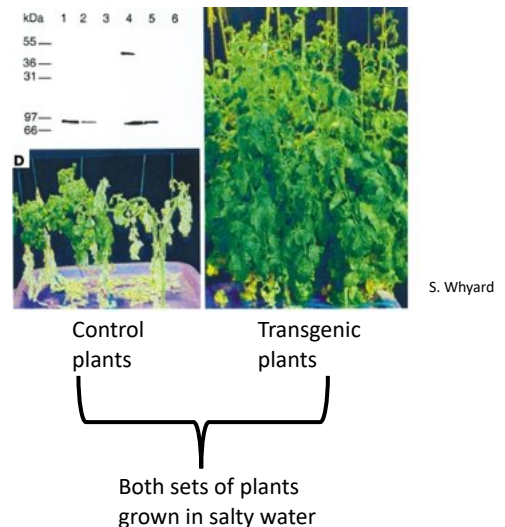
Antiport example – $\text{Na}^+/\text{Ca}^{2+}$ exchanger

- Ca^{2+} ions are returned to the ECF by active transport using
 - an ATP-driven pump like the Ca^{2+} ATPase of skeletal muscle and
 - a $\text{Na}^+/\text{Ca}^{2+}$ exchanger. This antiport pump harnesses the energy of 3 Na^+ ions flowing DOWN their concentration gradient to pump one Ca^{2+} against its gradient.
- Note that calcium is pumped out of the cell by 2 main active transport mechanisms - the $\text{Na}^+/\text{Ca}^{2+}$ exchanger and the Ca^{2+} ATPase pump (described in earlier slide)



Antiport example 2: vacuolar Na^+/H^+ antiport in transgenic tomato plants

- The Na^+/H^+ antiport pump in the vacuoles of some plant cells couples:
 - facilitated diffusion of protons (H^+) out from vacuoles with
 - the active inward transport of sodium ions.
- This sodium/proton antiport pump enables the plant to sequester sodium ions in its vacuole.
- Transgenic tomato plants that over-express this sodium/proton antiport pump are able to thrive in saline soils too salty for conventional tomatoes.

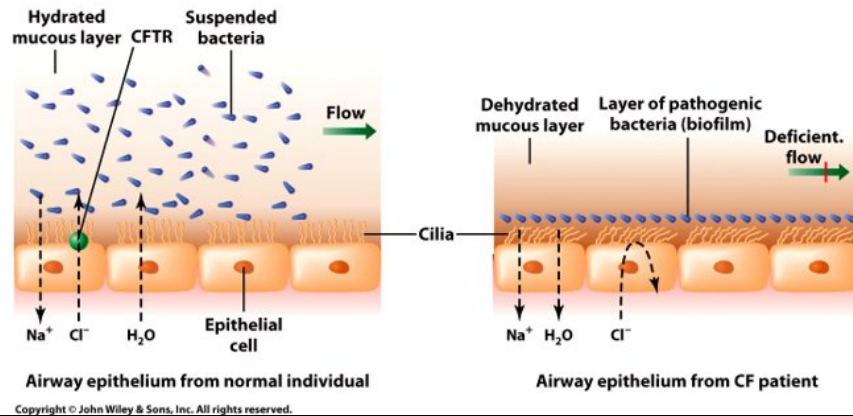


Inherited ion-channel diseases

Some human diseases are caused by inherited mutations in genes encoding channels.

Cystic fibrosis is caused by a defective chloride channel encoded by the *CFTR*

– many tissues affected, but the lungs show the most severe effects, with thick sticky mucous causing impaired breathing



What you should know after this lecture

- The difference between indirect and direct active transport
- The importance of the Na^+/K^+ pump and how it works
- The relevance and functioning of the H^+/K^+ ATPase
- The relevance and functioning of the Ca^{2+} ATPase
- The differences across the P-, V-, and ABC ATPases
- The difference between a symport and an antiport pump
- How the sodium/glucose co-transporter functions
- Two examples of an antiport pump
- How a defective chloride channel is associated with cystic fibrosis