Laboratoř růstových regulátorů

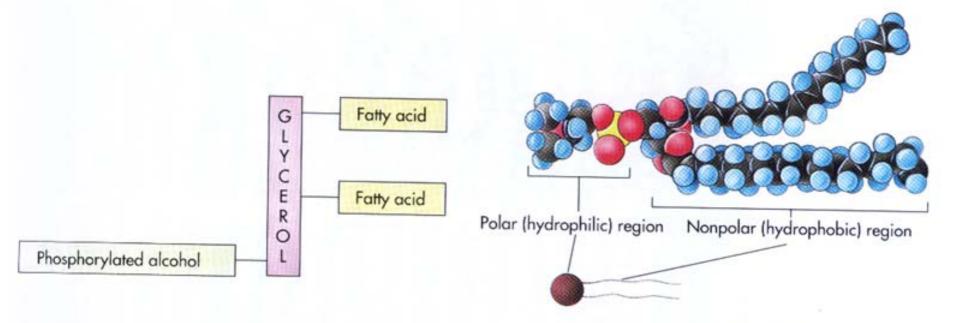
Miroslav Strnad

Membrány a transport látek přes membrány [kap 2]

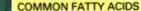


Univerzita Palackého & Ústav experimentální botaniky AV CR

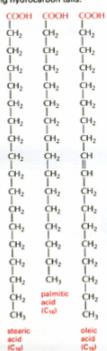




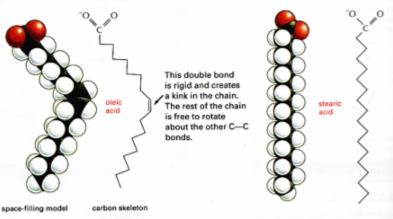
Phospholipid structure. A phospholipid is a composite molecule similar to a triglyceride, except in this case only two fatty acids are bound to the glycerol backbone, the third position being occupied by another kind of molecule called a phosphorylated alcohol. Because the phosphorylated alcohol usually extends from one end of the molecule, and the two fatty acid chains from the other, phospholipids are often diagrammed as a polar head with two nonpolar hydrophobic tails.

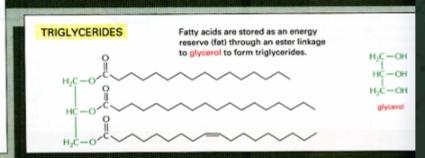


These are carboxylic acids with long hydrocarbon tails.



Hundreds of different kinds of fatty acids exist. Some have one or more double bonds and are said to be unsaturated.





hydrophobic

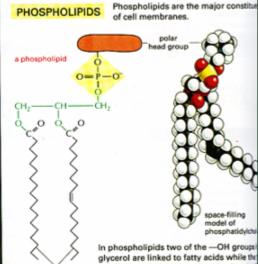
fatty acid

CARBOXYL GROUP

If free, the carboxyl group of a fatty acid will be ionized.

But more usually it is linked to other groups to form either esters

or amides.



In phospholipids two of the —OH groups glycerol are linked to fatty acids while the —OH group is linked to phosphoric acid.1 phosphate is further linked to one of a wal of small polar head groups (alcohols).

LIPID AGGREGATES

Fatty acids have a hydrophilic head and a hydrophobic tail.



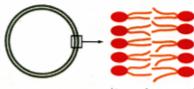
In water they can form a surface film or form small micelles.

Their derivatives can form larger aggregates held together by hydrophobic forces:

Triglycerides form large spherical fat droplets in the cell cytoplasm.

Phospholipids and glycolipids form self-sealing lipid bilayers that are the basis for all cellular membranes.





OTHER LIPIDS

Lipids are defined as the water-insoluble molecules in cells that are soluble in organic solvents. Two other common types of lipids are steroids and polyisoprenoids. Both are made from isoprene units.

STEROIDS

Steroids have a common multiple-ring structure.

cholesterol-found in many membranes

testosterone-male steroid hormone

GLYCOLIPIDS

Lite phospholipids, these compounds are composed of a hydrophobic region, containing two long hydrocarbon tails, and a polar region, which now contains one or more sugar residues and no phosphate.

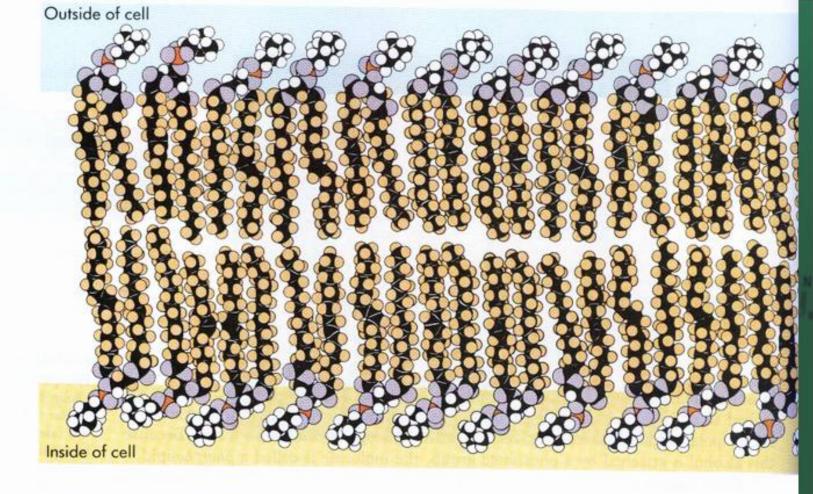
POLYISOPRENOIDS

long chain polymers of isoprene



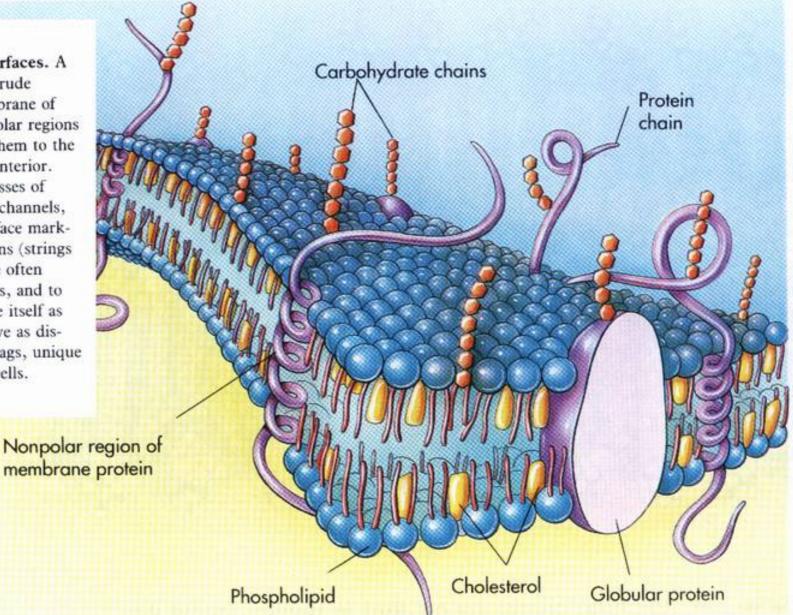
dolichol phosphate—used to carry activated sugars in the membrane-associated synthesis of glycoproteins

and some polysaccharides

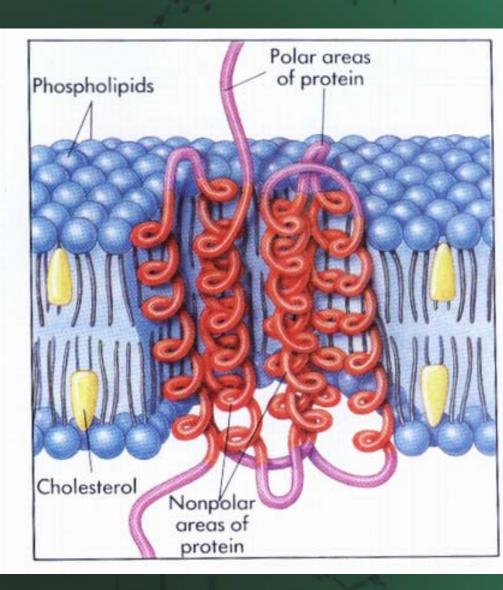


A phospholipid bilayer. The diagram above illustrates how the long nonpolar tails of the phospholipids orient toward one another. Because some of the tails contain double bonds, which introduce kinks in their shape, the tails do not align perfectly and the membrane is "fluid"—individual phospholipid molecules can move from one place to another in the membrane.

Cells have complex surfaces. A variety of proteins protrude through the lipid membrane of animal cells, and nonpolar regions of the proteins tether them to the membrane's nonpolar interior. The three principal classes of membrane protein are channels, receptors, and cell surface markers. Carbohydrate chains (strings of sugar molecules) are often bound to these proteins, and to lipids in the membrane itself as well. These chains serve as distinctive identification tags, unique to particular types of cells.



How proteins are anchored to membranes. Many membrane proteins are anchored within the lipid bilayer by nonpolar segments. In all cases studied to date, these segments have proved to be helical in secondary structure. Two general classes of membrane protein occur: proteins that traverse the membrane only once (receptors and some channels are of this sort), and proteins that traverse the membrane many times, creating a hollow "pipe" through the bilayer, as illustrated here. Many channels are of this sort.



Proteins in membranes move around. Protein movement within membranes can be easily demonstrated by labeling the proteins of a mouse cell with fluorescent antibodies, and then fusing that cell with a human cell. Within 1 hour, the labeled and unlabeled proteins are intermixed throughout the hybrid cell's membranes.

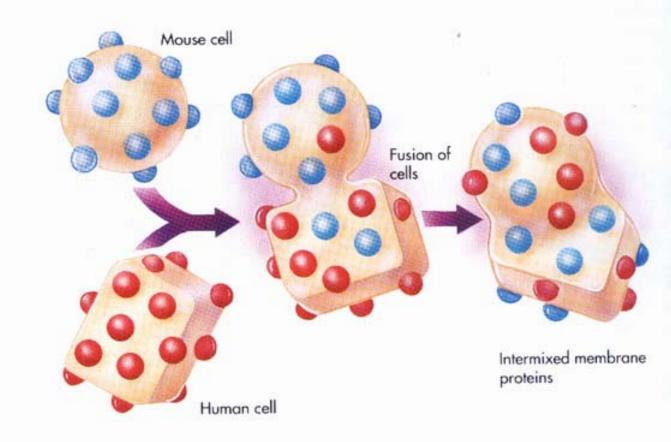
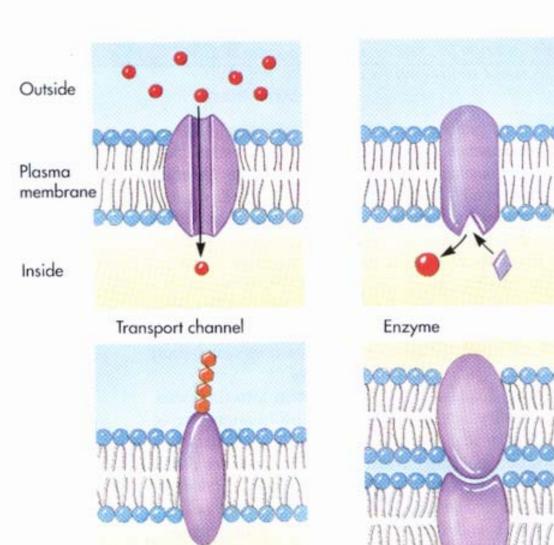
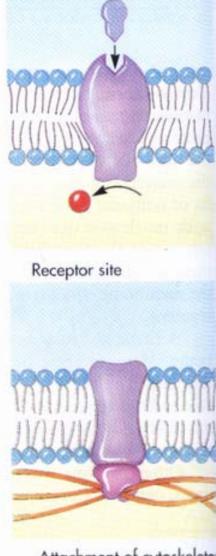


FIGURE 6-8 Functions of plasma membrane proteins.



Cell identity marker



Cell adhesion

Attachment of cytoskeleton

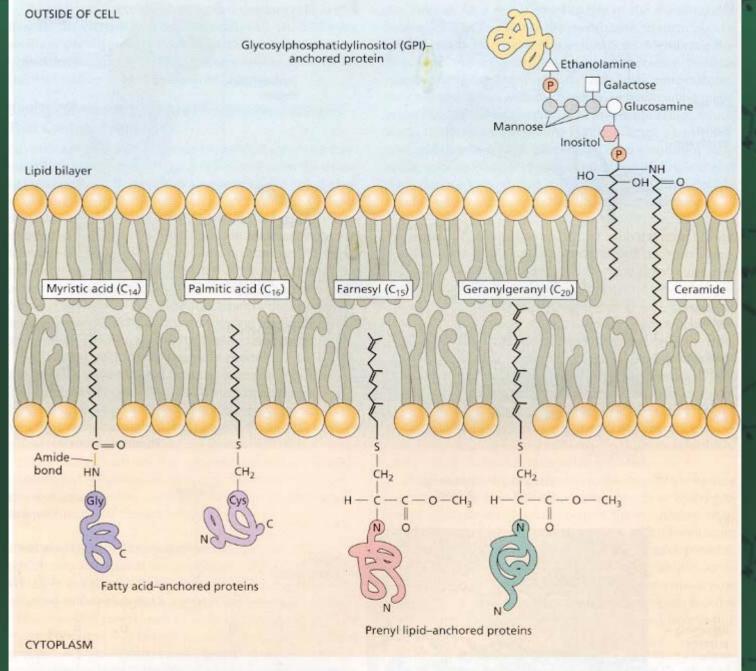
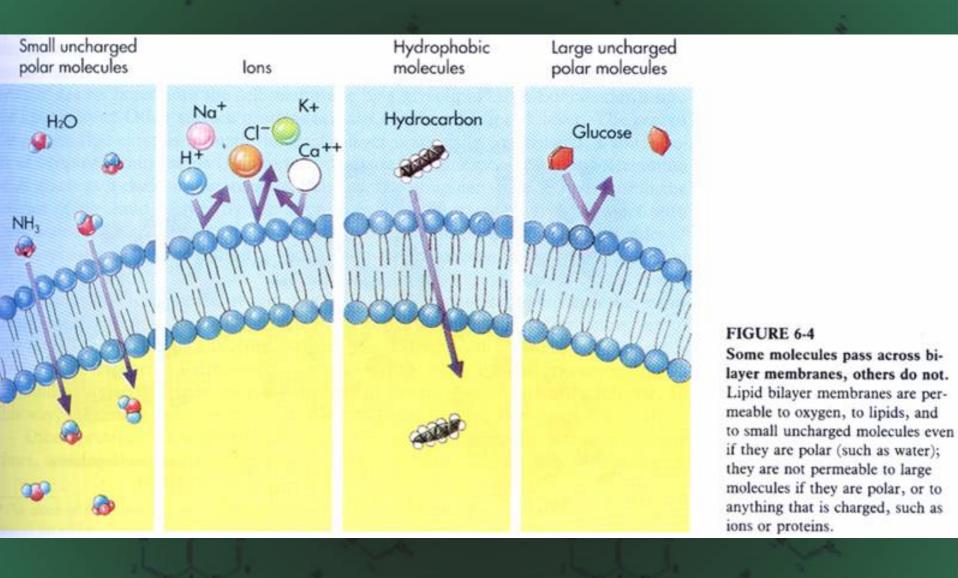


FIGURE 1.6 Different types of anchored membrane proteins that are attached to the membrane via fatty acids, prenyl groups, or phosphatidylinositol. (From Buchanan et al. 2000.)



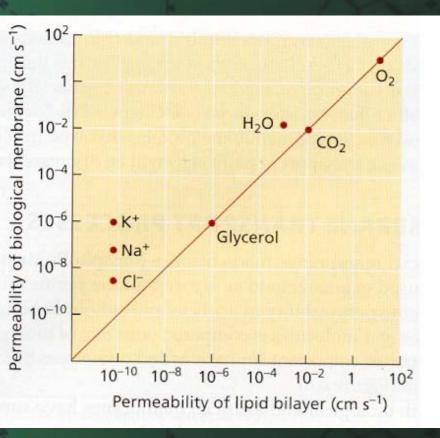


FIGURE 6.6 Typical values for the permeability, *P*, of a logical membrane to various substances, compared with those for an artificial phospholipid bilayer. For nonpola molecules such as O₂ and CO₂, and for some small uncharged molecules such as glycerol, *P* values are sim in both systems. For ions and selected polar molecules, including water, the permeability of biological membra is increased by one or more orders of magnitude, becau of the presence of transport proteins. Note the logarithm scale.

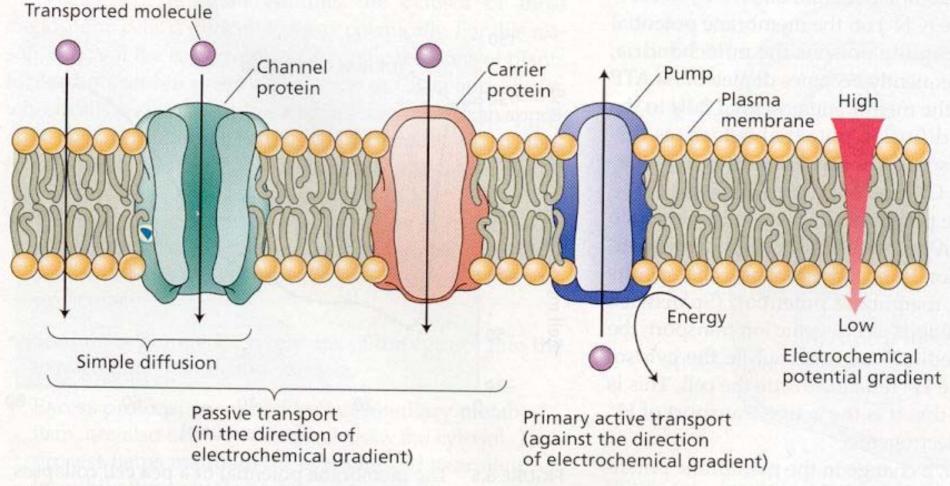
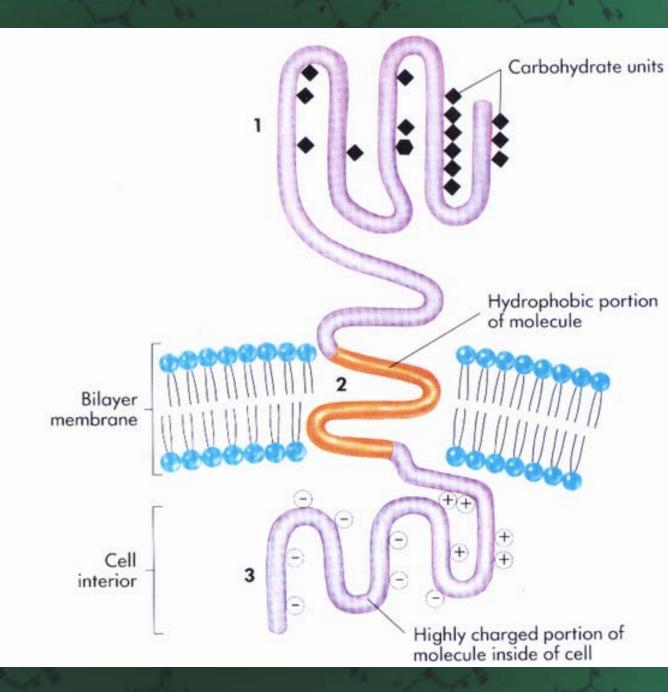


FIGURE 6.7 Three classes of membrane transport proteins: channels, carriers, and pumps. Channels and carriers can mediate the passive transport of solutes across membranes (by simple diffusion or facilitated diffusion), down the solute's gradient of electrochemical potential. Channel proteins act as membrane pores, and their specificity is determined primarily by the biophysical properties of the channel. Carrier proteins bind the transported molecule on one side of the membrane and release it on the other side. Primary active transport is carried out by pumps and uses energy directly, usually from ATP hydrolysis, to pump solutes against their gradient of electrochemical potential.

The molecular structure of the glycophorin A sugar transport channel is known in considerable detail. This channel has three distinctly different domains, or zones.

- 1 The end of the protein protruding from the outside surface has carbohydrates bound to it.
- 2 The central zone possesses a sequence of hydrophobic (nonpolar) amino acids, facilitating the burial of this portion of the protein within the lipid bilayer membrane.
- 3 The end of the protein protruding into the cell possesses many amino acids that are polar and ionized, giving the interior of the channel a negative charge.



Initial conditions:
[KCI]_A > [KCI]_B

Compartment A

Diffusion potential exists until chemical equilibrium is reached.

Equilibrium conditions: $[KCI]_A = [KCI]_B$

At chemical equilibrium, diffusion potential equals zero.

FIGURE 6.2 Development of a diffusion potential and a charge separation between two compartments separated by a membrane that is preferentially permeable to potassium. If the concentration of potassium chloride is higher in compartment A ($[KCl]_A > [KCl]_B$), potassium and chloride ions will diffuse at a higher rate into compartment B, and a diffusion potential will be established. When membranes are more permeable to potassium than to chloride, potassium ions will diffuse faster than chloride ions, and charge separation (+ and –) will develop.

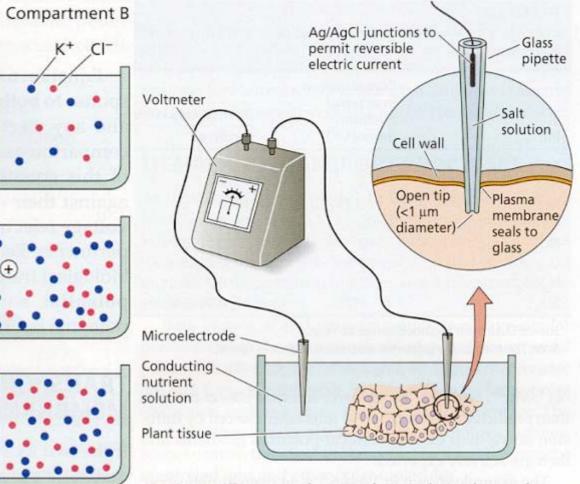


FIGURE 6.3 Diagram of a pair of microelectrodes used to measure membrane potentials across cell membranes. One of the glass micropipette electrodes is inserted into the cell compartment under study (usually the vacuole or the cytoplasm), while the other is kept in an electrolytic solution that serves as a reference. The microelectrodes are connected to a voltmeter, which records the electric-potential difference between the cell compartment and the solution. Typical membrane potentials across plant cell membranes range from –60 to –240 mV. The insert shows how electrical contact with the interior of the cell is made through the open tip of the glass micropipette, which contains an electrically conducting salt solution.

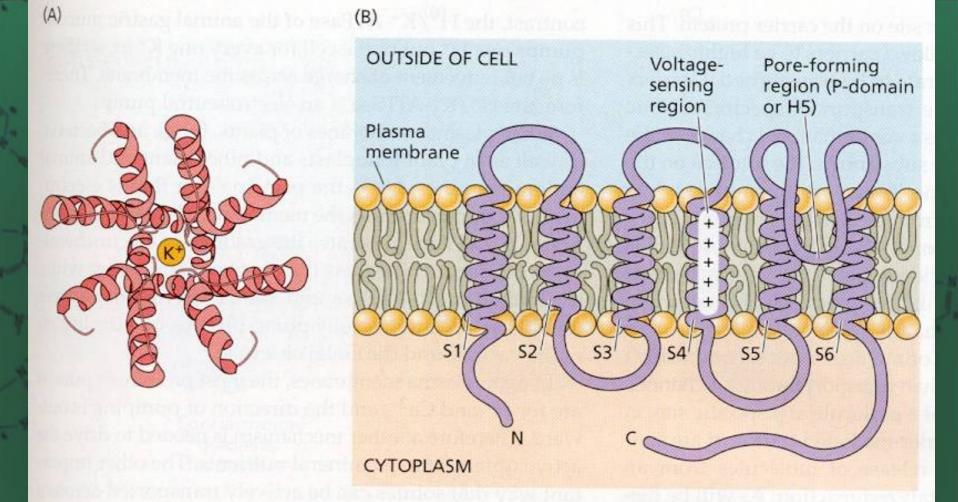


FIGURE 6.8 Models of K⁺ channels in plants. (A) Top view of channel, looking through the pore of the protein. Membrane-spanning helices of four subunits come together in an inverted teepee with the pore at the center. The pore-forming regions of the four subunits dip into the membrane, with a K⁺ selectivity finger region formed at the outer (near) part of the pore (more details on the structure of this channel can be found in Web Essay 6.1). (B) Side view of the inward rectifying K⁺ channel, showing a polypeptide chain of one subunit, with six membrane-spanning helices. The fourth helix contains positively-charged amino acids and acts as a voltage-sensor. The pore-forming region is a loop between helices 5 and 6. (A after Leng et al. 2002; B after Buchanan et al. 2000.)

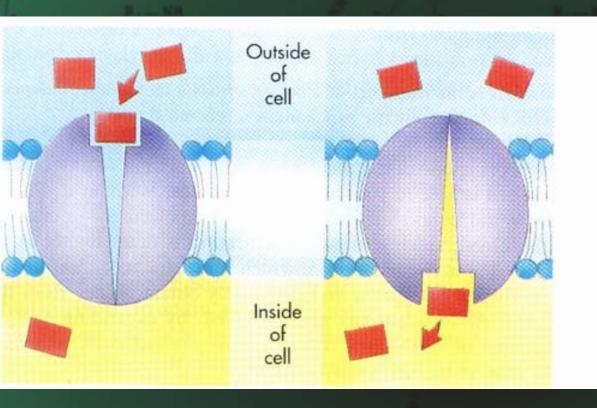


FIGURE 6-17 Facilitated diffusion is a carriermediated transport process.

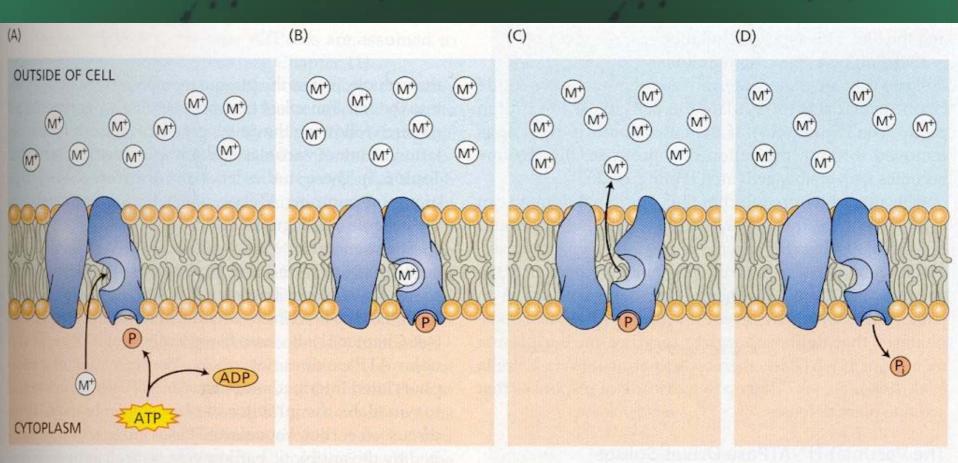
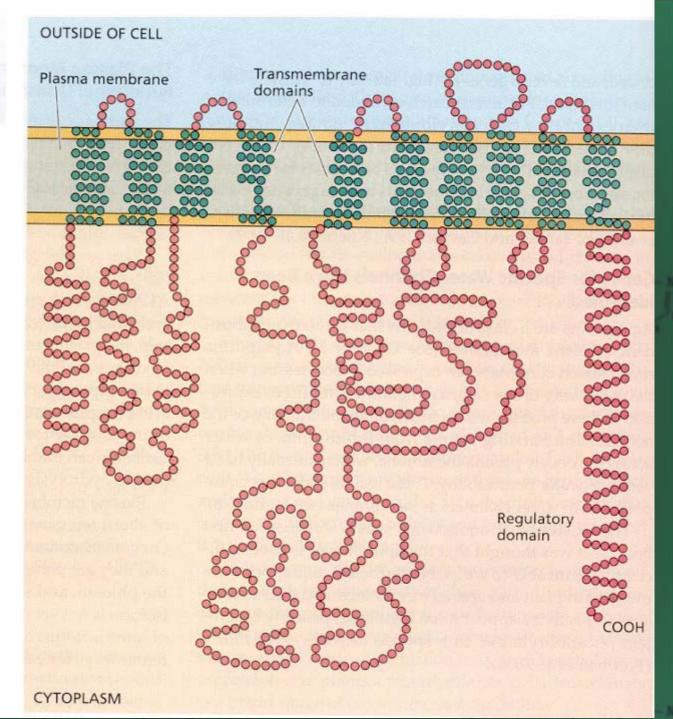


FIGURE 6.14 Hypothetical steps in the transport of a cation (the hypothetical M⁺) against its chemical gradient by an electrogenic pump. The protein, embedded in the membrane, binds the cation on the inside of the cell (A) and is phosphorylated by ATP (B). This phosphorylation leads to a conformational change that exposes the cation to the outside of the cell and makes it possible for the cation to diffuse away (C). Release of the phosphate ion (P) from the protein into the cytosol (D) restores the initial configuration of the membrane protein and allows a new pumping cycle to begin.

resentation of the plasma membrane H+-ATPase. The H+-ATPase has 10 transmembrane segments. The regulatory domain is the autoinhibitory domain. (From Palmgren 2001.)



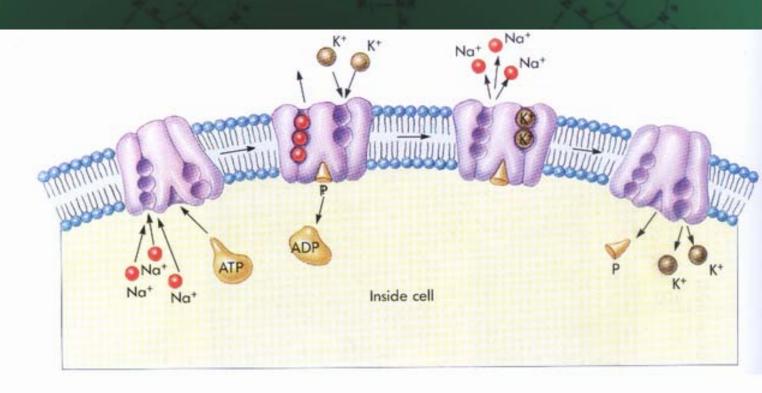
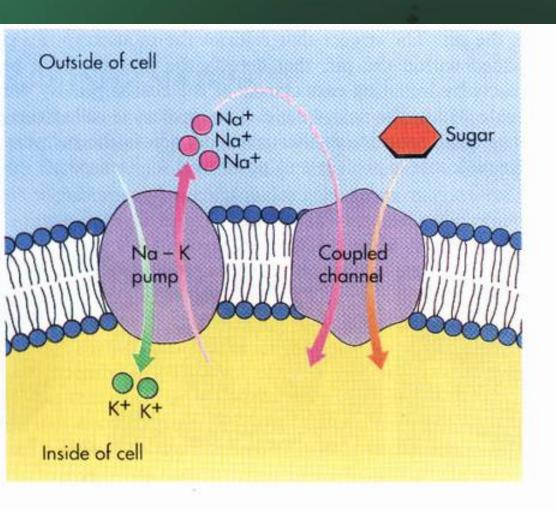


FIGURE 6-20 The sodium-potassium pump.



A coupled channel. The sodiumpotassium pump keeps the Na⁺
ion concentration higher outside
the cell than inside. There is thus
a strong tendency for Na⁺ ions to
diffuse back in through the coupled
channel, but their passage requires
the simultaneous transport of a
sugar molecule as well. The diffusion gradient driving Na⁺ entry is
so great that sugar molecules are
pulled in two, even against a sugar
concentration gradient.

TABLE 6.1
Comparison of observed and predicted ion concentrations in pea root tissue

lon	Concentration in external medium (mmol L ⁻¹)	Internal concentration (mmol L ⁻¹)	
		Predicted	Observed
K ⁺	1	74	75
Na ⁺	1	74	8
Mg ²⁺	0.25	1340	3
Mg ²⁺ Ca ²⁺	1	5360	2
NO ₃ -	2	0.0272	28
CI-	1	0.0136	7
H ₂ PO ₄ -	1	0.0136	21
SO ₄ 2-	0.25	0.00005	19

Source: Data from Higinbotham et al. 1967.

Note: The membrane potential was measured as -110 mV.

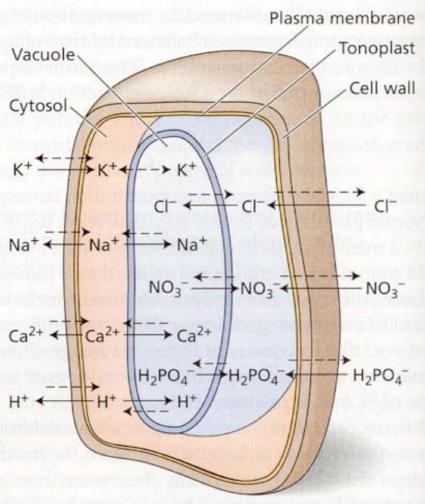


FIGURE 6.4 Ion concentrations in the cytosol and the vacuole are controlled by passive (dashed arrows) and active (solid arrows) transport processes. In most plant cells the vacuole occupies up to 90% of the cell's volume and contains the bulk of the cell solutes. Control of the ion concentrations in the cytosol is important for the regulation of metabolic enzymes. The cell wall surrounding the plasma membrane does not represent a permeability barrier and hence is not a factor in solute transport.

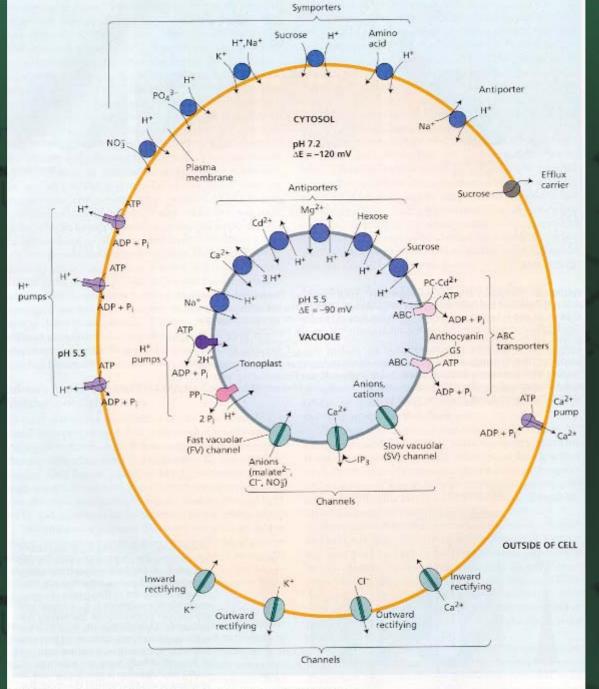


FIGURE 6.11 Overview of the various transport processes on the plasma membrane and tonoplast of plant cells.

TABLE 6.2
The vacuolar pH of some hyperacidifying plant species

Tissue	Species	pH ^a
Fruits		
	Lime (Citrus aurantifolia)	1.7
	Lemon (Citrus limonia)	2.5
	Cherry (Prunus cerasus)	2.5
	Grapefruit (Citrus paradisi)	3.0
Leaves		
	Rosette oxalis (Oxalis deppei)	1.3
	Wax begonia (Begonia semperflorens)	1.5
	Begonia 'Lucerna'	0.9 - 1.4
	Oxalis sp.	1.9 - 2.6
	Sorrel (Rumex sp.)	2.6
	Prickly Pear	1.4 (6:45 A.M.)
	(Opuntia phaeacantha) ^b	5.5 (4:00 P.M.)

Source: Data from Small 1946.

^a The values represent the pH of the juice or expressed sap of each tissue, usually a good indicator of vacuolar pH.

^b The vacuolar pH of the cactus *Opuntia phaeacantha* varies with the time of day. As will be discussed in Chapter 8, many desert succulents have a specialized type of photosynthesis, called crassulacean acid metabolism (CAM), that causes the pH of the vacuoles to decrease during the night.

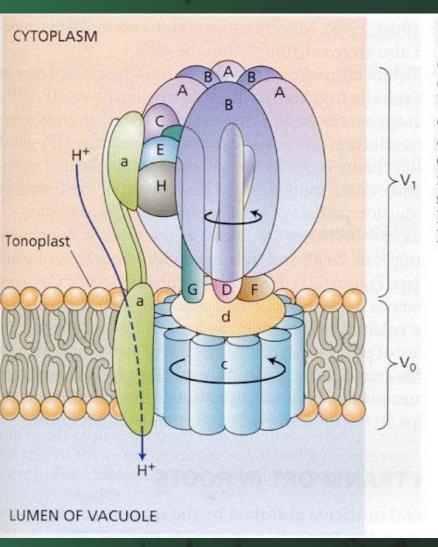


FIGURE 6.16 Model of the V-ATPase rotary motor. Many polypeptide subunits come together to make this complex enzyme. The V_1 catalytic complex is easily dissociated from the membrane, and contains the nucleotide-binding and catalytic sites. Components of V_1 are designated by uppercase letters. The intrinsic membrane complex mediating H^+ transport is designated V_0 , and its subunits are given lowercase letters. It is proposed that ATPase reactions catalyzed by each of the A subunits, acting in sequence, drive the rotation of the shaft D and the six c subunits. The rotation of the c subunits relative to subunit a is thought to drive the transport of H^+ across the membrane. (Based on an illustration courtesy of M. F. Manolson.)

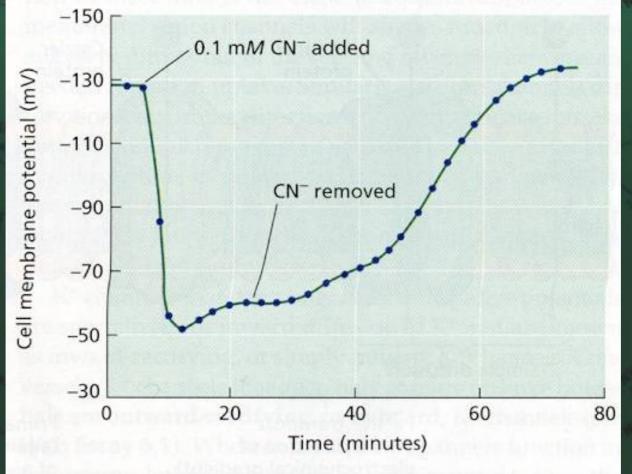


FIGURE 6.5 The membrane potential of a pea cell collapses when cyanide (CN⁻) is added to the bathing solution. Cyanide blocks ATP production in the cells by poisoning the mitochondria. The collapse of the membrane potential upon addition of cyanide indicates that an ATP supply is necessary for maintenance of the potential. Washing the cyanide out of the tissue results in a slow recovery of ATP production and restoration of the membrane potential. (From Higinbotham et al. 1970.)

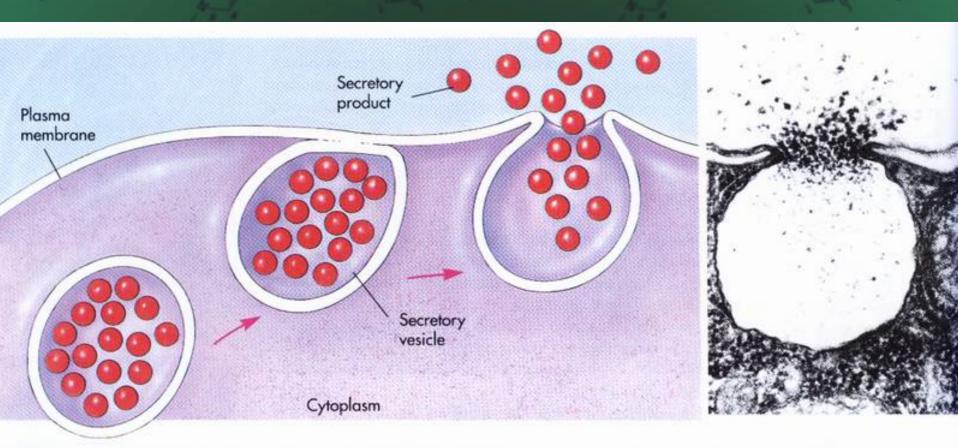
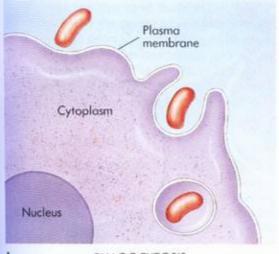
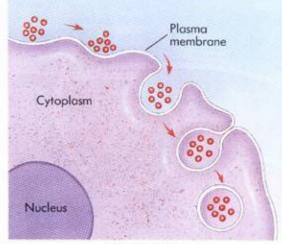


FIGURE 6-16

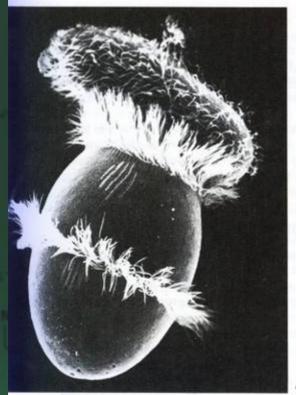
Exocytosis. Proteins and other molecules are secreted from cells in small pockets called vesicles, whose membranes fuse with the cell membrane, releasing their contents to the cell surface.







PINOCYTOSIS



D

FIGURE 6-15
Endocytosis. Both phagocytosis
(A) and pinocytosis (B) are form:
of endocytosis. The large eggshaped protist *Didinium nasutum*(C) has just begun eating the
smaller protist *Paramecium*; (D)
its meal is practically over.