Chapter

6

Solute Transport

PLANT CELLS ARE SEPARATED from their environment by a plasma membrane that is only two lipid molecules thick. This thin layer separates a relatively constant internal environment from highly variable external surroundings. In addition to forming a hydrophobic barrier to diffusion, the membrane must facilitate and continuously regulate the inward and outward traffic of selected molecules and ions as the cell takes up nutrients, exports wastes, and regulates its turgor pressure. The same is true of the internal membranes that separate the various compartments within each cell.

As the cell's only contact with its surroundings, the plasma membrane must also relay information about its physical environment, about molecular signals from other cells, and about the presence of invading pathogens. Often these signal transduction processes are mediated by changes in ion fluxes across the membrane.

Molecular and ionic movement from one location to another is known as **transport**. Local transport of solutes into or within cells is regulated mainly by membranes. Larger-scale transport between plant and environment, or between leaves and roots, is also controlled by membrane transport at the cellular level. For example, the transport of sucrose from leaf to root through the phloem, referred to as *translocation,* is driven and regulated by membrane transport into the phloem cells of the leaf, and from the phloem to the storage cells of the root (see Chapter 10).

In this chapter we will consider first the physical and chemical principles that govern the movements of molecules in solution. Then we will show how these principles apply to membranes and to biological systems. We will also discuss the molecular mechanisms of transport in living cells and the great variety of membrane transport proteins that are responsible for the particular transport properties of plant cells. Finally, we will examine the pathway that ions take when they enter the root, as well as the mechanism of xylem loading, the process whereby ions are released into the vessel elements and tracheids of the stele.

PASSIVE AND ACTIVE TRANSPORT

According to Fick's first law (see Equation 3.1), the movement of molecules by diffusion always proceeds spontaneously, down a gradient of concentration or chemical potential (see Chapter 2 on the web site), until equilibrium is reached. The spontaneous "downhill" movement of molecules is termed **passive transport**. At equilibrium, no further net movements of solute can occur without the application of a driving force.

The movement of substances against or up a gradient of chemical potential (e.g., to a higher concentration) is termed **active transport**. It is not spontaneous, and it requires that work be done on the system by the application of cellular energy. One way (but not the only way) of accomplishing this task is to couple transport to the hydrolysis of ATP.

Recall from Chapter 3 that we can calculate the driving force for diffusion, or, conversely, the energy input necessary to move substances against a gradient, by measuring the potential-energy gradient, which is often a simple function of the difference in concentration. Biological transport can be driven by four major forces: concentration, hydrostatic pressure, gravity, and electric fields. (However, recall from Chapter 3 that in biological systems, gravity seldom contributes substantially to the force that drives transport.)

The **chemical potential** for any solute is defined as the sum of the concentration, electric, and hydrostatic potentials (and the chemical potential under standard conditions):

$$\tilde{\mu}_j = \mu_j^* + RT \ln C_j + z_j FE + \bar{V}_j P \qquad (6.1)$$

$\tilde{\mu}_j$	μ_j^*	$RT \ln C_j$	$z_j FE$	$\bar{V}_j P$
Chemical potential for a given solute, *j*	Chemical potential of *j* under standard conditions	Concentration (activity) component	Electric-potential component	Hydrostatic-pressure component

Here $\tilde{\mu}_j$ is the chemical potential of the solute species *j* in joules per mole (J mol^{-1}), μ_j^* is its chemical potential under standard conditions (a correction factor that will cancel out in future equations and so can be ignored), *R* is the universal gas constant, *T* is the absolute temperature, and C_j is the concentration (more accurately the activity) of *j*.

The electrical term, $z_j FE$, applies only to ions; *z* is the electrostatic charge of the ion (+1 for monovalent cations, –1 for monovalent anions, +2 for divalent cations, and so on), *F* is Faraday's constant (equivalent to the electric charge on 1 mol of protons), and *E* is the overall electric potential of the solution (with respect to ground). The final term, $\bar{V}_j P$, expresses the contribution of the partial molal volume of *j* ($\bar{V}_j$) and pressure (*P*) to the chemical potential of *j*. (The partial molal volume of *j* is the change in volume per mole of substance *j* added to the system, for an infinitesimal addition.)

This final term, $\bar{V}_j P$, makes a much smaller contribution to $\tilde{\mu}_j$ than do the concentration and electrical terms, except in the very important case of osmotic water movements. As discussed in Chapter 3, the chemical potential of water (i.e., the water potential) depends on the concentration of dissolved solutes and the hydrostatic pressure on the system.

The importance of the concept of chemical potential is that it sums all the forces that may act on a molecule to drive net transport (Nobel 1991).

In general, diffusion (or passive transport) always moves molecules from areas of higher chemical potential downhill to areas of lower chemical potential. Movement against a chemical-potential gradient is indicative of active transport (Figure 6.1).

If we take the diffusion of sucrose across a permeable membrane as an example, we can accurately approximate the chemical potential of sucrose in any compartment by the concentration term alone (unless a solution is very concentrated, causing hydrostatic pressure to build up). From Equation 6.1, the chemical potential of sucrose inside a cell can be described as follows (in the next three equations, the subscript *s* stands for sucrose, and the superscripts *i* and *o* stand for inside and outside, respectively):

$$\tilde{\mu}_s^{i} = \mu_s^* + RT \ln C_s^{i} \qquad (6.2)$$

$\tilde{\mu}_s^{i}$	μ_s^*	$RT \ln C_s^{i}$
Chemical potential of sucrose solution inside the cell	Chemical potential of sucrose solution under standard conditions	Concentration component

The chemical potential of sucrose outside the cell is calculated as follows:

$$\tilde{\mu}_s^{o} = \mu_s^* + RT \ln C_s^{o} \qquad (6.3)$$

We can calculate the difference in the chemical potential of sucrose between the solutions inside and outside the cell, $\Delta\tilde{\mu}_s$, regardless of the mechanism of transport. To get the signs right, remember that for inward transport, sucrose is being removed (–) from outside the cell and added (+) to the inside, so the change in free energy in joules per mole of sucrose transported will be as follows:

$$\Delta\tilde{\mu}_\sigma = \tilde{\mu}_\sigma^{\iota} - \tilde{\mu}_\sigma^{o} \qquad (6.4)$$

Substituting the terms from Equations 6.2 and 6.3 into Equation 6.4, we get the following:

$$\begin{aligned} \Delta\tilde{\mu}_s &= \left(\mu_s^* + RT \ln C_s^{i}\right) - \left(\mu_s^* + RT \ln C_s^{o}\right) \\ &= RT\left(\ln C_s^{i} - \ln C_s^{o}\right) \\ &= RT \ln \frac{C_s^{i}}{C_s^{o}} \end{aligned} \qquad (6.5)$$

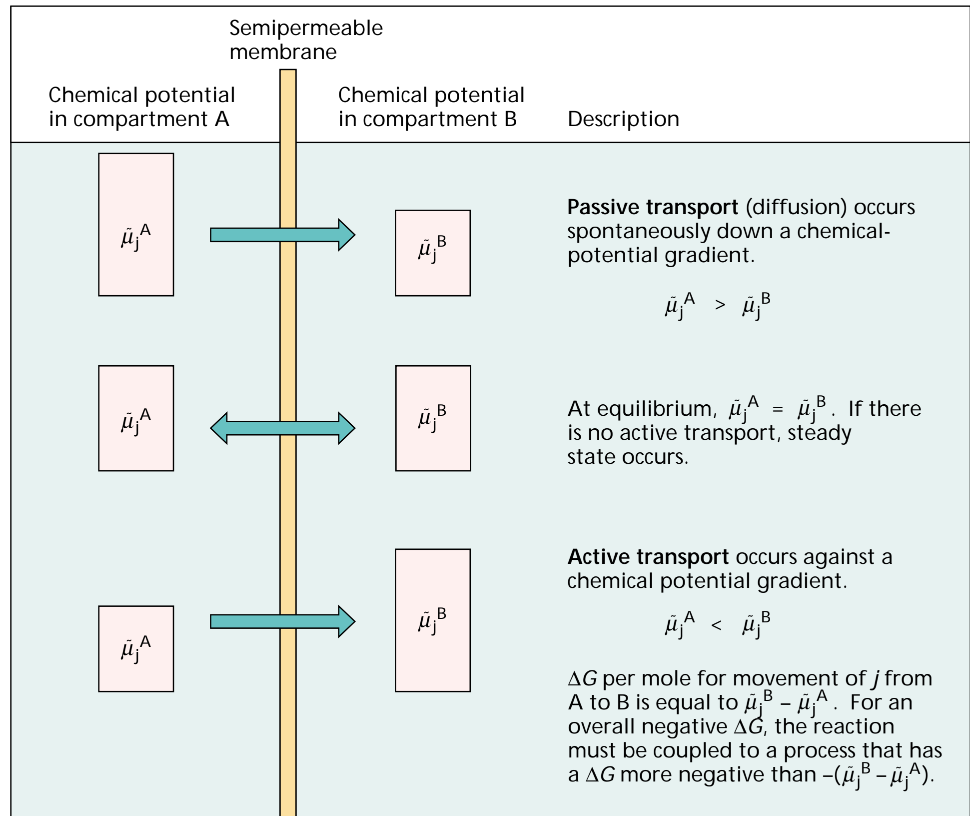


FIGURE 6.1 Relationship between the chemical potential, $\tilde{\mu}$, and the transport of molecules across a permeability barrier. The net movement of molecular species *j* between compartments A and B depends on the relative magnitude of the chemical potential of *j* in each compartment, represented here by the size of the boxes. Movement down a chemical gradient occurs spontaneously and is called passive transport; movement against or up a gradient requires energy and is called active transport.

If this difference in chemical potential is negative, sucrose could diffuse inward spontaneously (provided the membrane had a finite permeability to sucrose; see the next section). In other words, the driving force ($\Delta\tilde{\mu}_s$) for solute diffusion is related to the magnitude of the concentration gradient (C_s^i/C_s^o).

If the solute carries an electric charge (as does the potassium ion), the electrical component of the chemical potential must also be considered. Suppose the membrane is permeable to K^+ and Cl^- rather than to sucrose. Because the ionic species (K^+ and Cl^-) diffuse independently, each has its own chemical potential. Thus for inward K^+ diffusion,

$$\Delta\tilde{\mu}_K = \tilde{\mu}_K^i - \tilde{\mu}_K^o \tag{6.6}$$

Substituting the appropriate terms from Equation 6.1 into Equation 6.6, we get

$$\Delta\tilde{\mu}_s = (RT \ln [K^+]^i + zFE^i) - (RT \ln [K^+]^o + zFE^o) \tag{6.7}$$

and because the electrostatic charge of K^+ is +1, $z = +1$ and

$$\Delta\tilde{\mu}_K = RT \ln\frac{[K^+]^i}{[K^+]^o} + F(E^i - E^o) \tag{6.8}$$

The magnitude and sign of this expression will indicate the driving force for K^+ diffusion across the membrane, and its direction. A similar expression can be written for Cl^- (but remember that for Cl^-, $z = -1$).

Equation 6.8 shows that ions, such as K^+, diffuse in response to both their concentration gradients ($[K^+]^i/[K^+]^o$) and any electric-potential difference between the two compartments ($E^i - E^o$). One very important implication of this equation is that ions can be driven passively against their concentration gradients if an appropriate voltage (electric field) is applied between the two compartments. Because of the importance of electric fields in biological transport, $\tilde{\mu}$ is often called the **electrochemical potential**, and $\Delta\tilde{\mu}$ is the difference in electrochemical potential between two compartments.

TRANSPORT OF IONS ACROSS A MEMBRANE BARRIER

If the two KCl solutions in the previous example are separated by a biological membrane, diffusion is complicated by the fact that the ions must move through the membrane as well as across the open solutions. The extent to which a membrane permits the movement of a substance is called **membrane permeability**. As will be discussed later, permeability depends on the composition of the membrane, as well as on the chemical nature of the solute. In a loose sense, permeability can be expressed in terms of a diffusion coefficient for the solute in the membrane. However, permeability is influenced by several additional factors, such

as the ability of a substance to enter the membrane, that are difficult to measure.

Despite its theoretical complexity, we can readily measure permeability by determining the rate at which a solute passes through a membrane under a specific set of conditions. Generally the membrane will hinder diffusion and thus reduce the speed with which equilibrium is reached. The permeability or resistance of the membrane itself, however, cannot alter the final equilibrium conditions. Equilibrium occurs when $\Delta\tilde{\mu}_j = 0$.

In the sections that follow we will discuss the factors that influence the passive distribution of ions across a membrane. These parameters can be used to predict the relationship between the electrical gradient and the concentration gradient of an ion.

Diffusion Potentials Develop When Oppositely Charged Ions Move across a Membrane at Different Rates

When salts diffuse across a membrane, an electric membrane potential (voltage) can develop. Consider the two KCl solutions separated by a membrane in Figure 6.2. The K^+ and Cl^- ions will permeate the membrane independently as they diffuse down their respective gradients of electrochemical potential. And unless the membrane is very porous, its permeability for the two ions will differ.

As a consequence of these different permeabilities, K^+ and Cl^- initially will diffuse across the membrane at different rates. The result will be a slight separation of charge, which instantly creates an electric potential across the membrane. In biological systems, membranes are usually more permeable to K^+ than to Cl^-. Therefore, K^+ will diffuse out of the cell (compartment A in Figure 6.2) faster than Cl^-, causing the cell to develop a negative electric charge with respect to the medium. A potential that develops as a result of diffusion is called a **diffusion potential**.

An important principle that must always be kept in mind when the movement of ions across membranes is considered is the principle of electrical neutrality. Bulk solutions always contain equal numbers of anions and cations. The existence of a membrane potential implies that the distribution of charges across the membrane is uneven; however, the actual number of unbalanced ions is negligible in chemical terms. For example, a membrane potential of –100 mV (millivolts), like that found across the plasma membranes of many plant cells, results from the presence of only one extra anion out of every 100,000 within the cell—a concentration difference of only 0.001%!

As Figure 6.2 shows, all of these extra anions are found immediately adjacent to the surface of the membrane; there is no charge imbalance throughout the bulk of the cell. In our example of KCl diffusion across a membrane, electrical neutrality is preserved because as K^+ moves ahead of Cl^- in the membrane, the resulting diffusion potential retards the movement of K^+ and speeds that of Cl^-. Ultimately, both ions diffuse at the same rate, but the diffusion potential persists and can be measured. As the system moves toward equilibrium and the concentration gradient collapses, the diffusion potential also collapses.

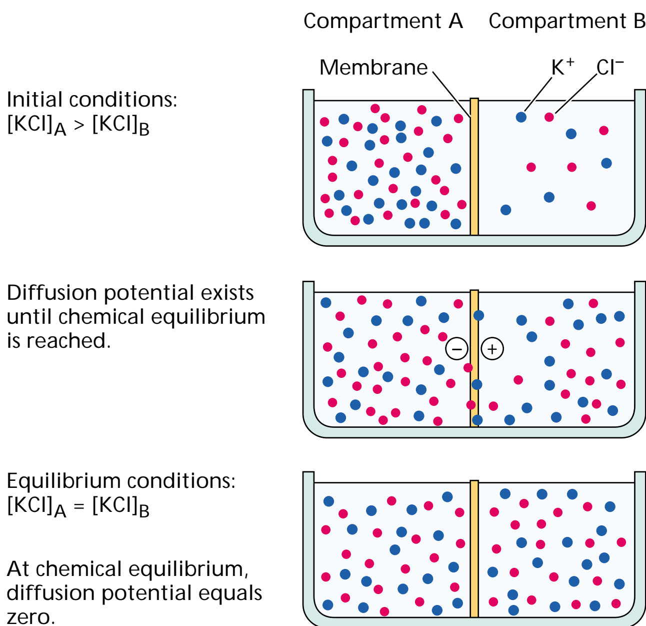


FIGURE 6.2 Development of a diffusion potential and a charge separation between two compartments separated by a membrane that is preferentially permeable to potassium. If the concentration of potassium chloride is higher in compartment A ($[KCl]_A > [KCl]_B$), potassium and chloride ions will diffuse 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.

The Nernst Equation Relates the Membrane Potential to the Distribution of an Ion at Equilibrium

Because the membrane is permeable to both K^+ and Cl^- ions, equilibrium in the preceding example will not be reached for either ion until the concentration gradients decrease to zero. However, if the membrane were permeable to only K^+, diffusion of K^+ would carry charges across the membrane until the membrane potential balanced the concentration gradient. Because a change in potential requires very few ions, this balance would be reached instantly. Transport would then be at equilibrium, even though the concentration gradients were unchanged.

When the distribution of any solute across a membrane reaches equilibrium, the passive flux, J (i.e., the amount of solute crossing a unit area of membrane per unit time), is the same in the two directions—outside to inside and inside to outside:

$$J_{o \to i} = J_{i \to o}$$

Fluxes are related to $\Delta\tilde{\mu}$ (for a discussion on fluxes and $\Delta\tilde{\mu}$, see Chapter 2 on the web site); thus at equilibrium, the electrochemical potentials will be the same:

$$\tilde{\mu}_j^{o} = \tilde{\mu}_j^{i}$$

and for any given ion (the ion is symbolized here by the subscript *j*):

$$\mu_j^* + RT \ln C_j^{o} + z_j F E^{o} = \mu_j^* + RT \ln C_j^{i} + z_j F E^{i} \quad (6.9)$$

By rearranging Equation 6.9, we can obtain the difference in electric potential between the two compartments at equilibrium ($E^i - E^o$):

$$E^i - E^o = \frac{RT}{z_j F}\left(\ln \frac{C_j^{o}}{C_j^{i}}\right)$$

This electric-potential difference is known as the **Nernst potential** (ΔE_j) for that ion:

$$\Delta E_j = E^i - E^o$$

and

$$\Delta E_{\varphi} = \frac{RT}{z_{\varphi} F}\left(\ln \frac{C_{\varphi}^{o}}{C_{\varphi}^{\iota}}\right)$$

or

$$\Delta E_{\varphi} = \frac{2.3RT}{z_{\varphi} F}\left(\log \frac{C_{\varphi}^{o}}{C_{\varphi}^{\iota}}\right)$$

This relationship, known as the **Nernst equation**, states that at equilibrium the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. The Nernst equation can be further simplified for a univalent cation at 25°C:

$$\Delta E_{\varphi} = 59\mu\varsigma \log \frac{C_{\varphi}^{o}}{C_{\varphi}^{\iota}} \quad (6.11)$$

Note that a tenfold difference in concentration corresponds to a Nernst potential of 59 mV ($C_o/C_i = 10/1$; log 10 = 1). That is, a membrane potential of 59 mV would maintain a tenfold concentration gradient of an ion that is transported by passive diffusion. Similarly, if a tenfold concentration gradient of an ion existed across the membrane, passive diffusion of that ion down its concentration gradient (if it were allowed to come to equilibrium) would result in a difference of 59 mV across the membrane.

All living cells exhibit a membrane potential that is due to the asymmetric ion distribution between the inside and outside of the cell. We can readily determine these membrane potentials by inserting a microelectrode into the cell and measuring the voltage difference between the inside of the cell and the external bathing medium (Figure 6.3).

The Nernst equation can be used at any time to determine whether a given ion is at equilibrium across a membrane. However, a distinction must be made between equilibrium and steady state. **Steady state** is the condition in which influx and efflux of a given solute are equal and therefore the ion concentrations are constant with respect to time. Steady state is not the same as equilibrium (see Figure 6.1); in steady state, the existence of active transport across the membrane prevents many diffusive fluxes from ever reaching equilibrium.

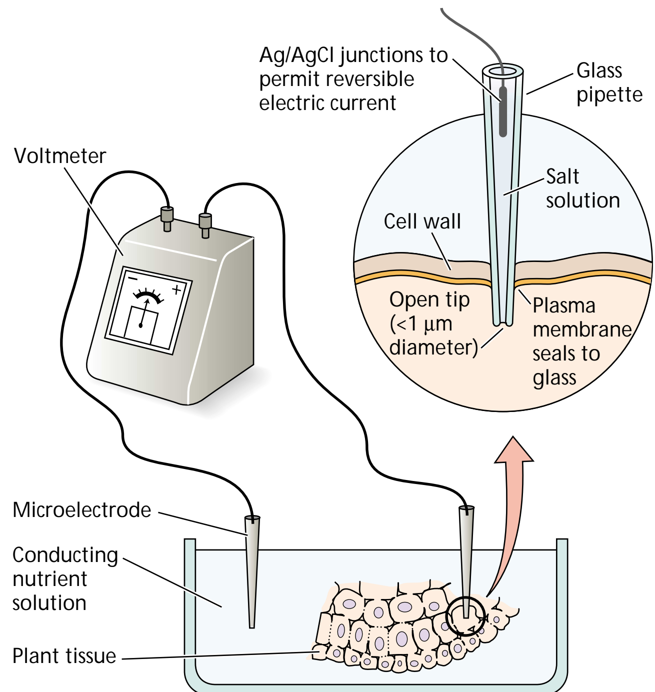


FIGURE 6.3 Diagram of a pair of microelectrodes used to measure membrane potentials across cell membranes. One of the glass micropipette electrodes is inserted into the cell compartment under study (usually the vacuole or the cytoplasm), while the other is kept in an electrolytic solution that serves 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.

The Nernst Equation Can Be Used to Distinguish between Active and Passive Transport

Table 6.1 shows how the experimentally measured ion concentrations at steady state for pea root cells compare with predicted values calculated from the Nernst equation (Higinbotham et al. 1967). In this example, the external concentration of each ion in the solution bathing the tissue, and the measured membrane potential, were substituted into the Nernst equation, and a predicted internal concentration was calculated for that ion.

Notice that, of all the ions shown in Table 6.1, only K^+ is at or near equilibrium. The anions NO_3^-, Cl^-, $H_2PO_4^-$, and SO_4^{2-} all have higher internal concentrations than predicted, indicating that their uptake is active. The cations

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

Ion	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
Ca^{2+}	1	5360	2
NO_3^-	2	0.0272	28
Cl^-	1	0.0136	7
$H_2PO_4^-$	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.

Na^+, Mg^{2+}, and Ca^{2+} have lower internal concentrations than predicted; therefore, these ions enter the cell by diffusion down their electrochemical-potential gradients and then are actively exported.

The example shown in Table 6.1 is an oversimplification: Plant cells have several internal compartments, each of which can differ in its ionic composition. The cytosol and the vacuole are the most important intracellular compartments that determine the ionic relations of plant cells. In mature plant cells, the central vacuole often occupies 90% or more of the cell's volume, and the cytosol is restricted to a thin layer around the periphery of the cell.

Because of its small volume, the cytosol of most angiosperm cells is difficult to assay chemically. For this reason, much of the early work on the ionic relations of plants focused on certain green algae, such as *Chara* and *Nitella*, whose cells are several inches long and can contain an appreciable volume of cytosol. Figure 6.4 diagrams the conclusions from these studies and from related work with higher plants.

- Potassium is accumulated passively by both the cytosol and the vacuole, except when extracellular K^+ concentrations are very low, in which case it is taken up actively.
- Sodium is pumped actively out of the cytosol into the extracellular spaces and vacuole.
- Excess protons, generated by intermediary metabolism, are also actively extruded from the cytosol. This process helps maintain the cytosolic pH near neutrality, while the vacuole and the extracellular medium are generally more acidic by one or two pH units.
- All the anions are taken up actively into the cytosol.
- Calcium is actively transported out of the cytosol at both the cell membrane and the vacuolar membrane, which is called the *tonoplast* (see Figure 6.4).

Many different ions permeate the membranes of living cells simultaneously, but K^+, Na^+, and Cl^- have the highest concentrations and largest permeabilities in plant cells. A modified version of the Nernst equation, the **Goldman equation**, includes all three of these ions and therefore gives a more accurate value for the diffusion potential in these cells. The diffusion potential calculated from the Goldman equation is termed the *Goldman diffusion potential* (for a detailed discussion of the Goldman equation, see Web Topic 6.1).

Proton Transport Is a Major Determinant of the Membrane Potential

When permeabilities and ion gradients are known, it is possible to calculate a diffusion potential for the membrane from the Goldman equation. In most cells, K^+ has both the greatest internal concentration and the highest membrane permeability, so the diffusion potential may approach E_K, the Nernst potential for K^+.

In some organisms, or in tissues such as nerves, the normal resting potential of the cell may be close to E_K. This is not

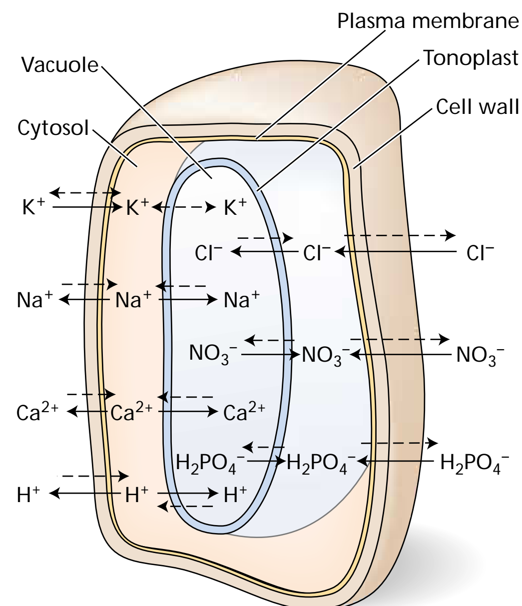
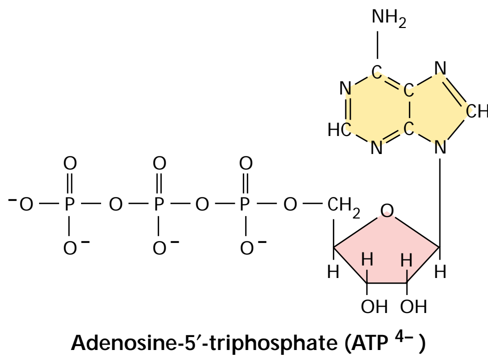


FIGURE 6.4 Ion concentrations in the cytosol and the vacuole are controlled by passive (dashed arrows) and active (solid arrows) transport processes. In most plant cells the vacuole occupies up to 90% of the cell's volume and contains the bulk of the cell solutes. Control of the ion concentrations in 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.

the case with plants and fungi, which may show experimentally measured membrane potentials (often –200 to –100 mV) that are much more negative than those calculated from the Goldman equation, which are usually only –80 to –50 mV. Thus, in addition to the diffusion potential, the membrane potential has a second component. The excess voltage is provided by the plasma membrane electrogenic H^+-ATPase.

Whenever an ion moves into or out of a cell without being balanced by countermovement of an ion of opposite charge, a voltage is created across the membrane. Any active transport mechanism that results in the movement of a net electric charge will tend to move the membrane potential away from the value predicted by the Goldman equation. Such a transport mechanism is called an *electrogenic pump* and is common in living cells.



The energy required for active transport is often provided by the hydrolysis of ATP. In plants we can study the dependence of the membrane potential on ATP by observing the effect of cyanide (CN^-) on the membrane potential (Figure 6.5). Cyanide rapidly poisons the mitochondria, and the cell's ATP consequently becomes depleted. As ATP synthesis is inhibited, the membrane potential falls to the level of the Goldman diffusion potential, which, as discussed in the previous section, is due primarily to the passive movements of K^+, Cl^-, and Na^+ (see Web Topic 6.1).

Thus the membrane potentials of plant cells have two components: a diffusion potential and a component resulting from electrogenic ion transport (transport that results in the generation of a membrane potential) (Spanswick 1981). When cyanide inhibits electrogenic ion transport, the pH of the external medium increases while the cytosol becomes acidic because H^+ remains inside the cell. This is one piece of evidence that it is the active transport of H^+ out of the cell that is electrogenic.

As discussed earlier, a change in the membrane potential caused by an electrogenic pump will change the driving forces for diffusion of all ions that cross the membrane. For example, the outward transport of H^+ can create a driving force for the passive diffusion of K^+ into the cell. H^+ is transported electrogenically across the plasma membrane not only in plants but also in bacteria, algae, fungi, and some animal cells, such as those of the kidney epithelia.

ATP synthesis in mitochondria and chloroplasts also depends on a H^+-ATPase. In these organelles, this transport protein is sometimes called *ATP synthase* because it forms ATP rather than hydrolyzing it (see Chapter 11). The structure and function of membrane proteins involved in active and passive transport in plant cells will be discussed later.

MEMBRANE TRANSPORT PROCESSES

Artificial membranes made of pure phospholipids have been used extensively to study membrane permeability. When the permeability of artificial phospholipid bilayers for ions and molecules is compared with that of biological membranes, important similarities and differences become evident (Figure 6.6).

Both biological and artificial membranes have similar permeabilities for nonpolar molecules and many small polar molecules. On the other hand, biological membranes are much more permeable to ions and some large polar molecules, such as sugars, than artificial bilayers are. The reason is that, unlike artificial bilayers, biological membranes contain **transport proteins** that facilitate the passage of selected ions and other polar molecules.

Transport proteins exhibit specificity for the solutes they transport, hence their great diversity in cells. The simple prokaryote *Haemophilus influenzae*, the first organism for which the complete genome was sequenced, has only 1743 genes, yet more than 200 of these genes (greater than 10% of the genome) encode various proteins involved in mem-

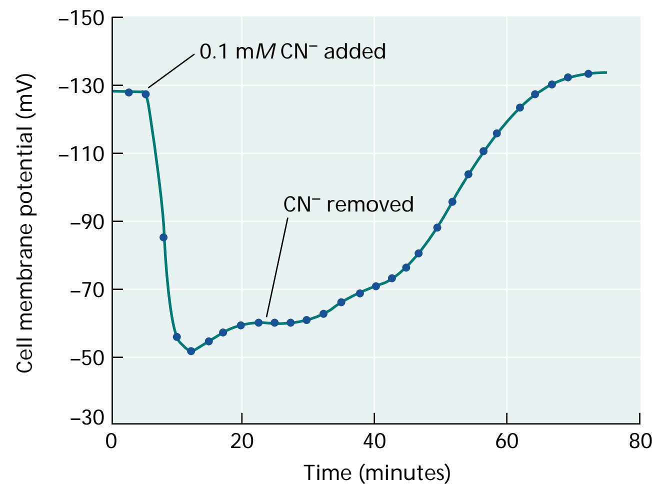


FIGURE 6.5 The membrane potential of a pea cell collapses when cyanide (CN^-) is added to the bathing solution. Cyanide blocks ATP production in the cells by poisoning the mitochondria. The collapse of the membrane potential upon addition of cyanide indicates that an ATP supply is necessary for maintenance 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.)

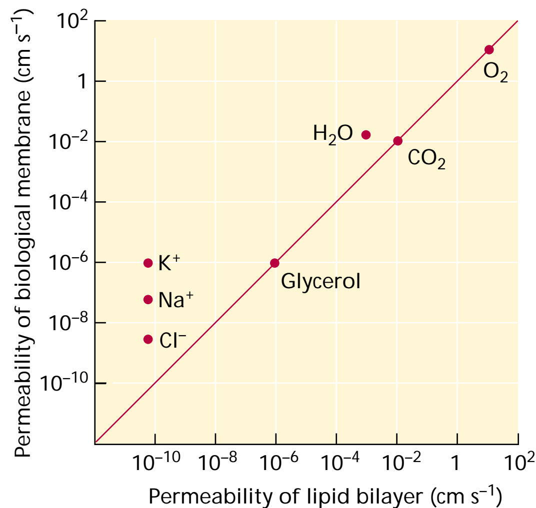


FIGURE 6.6 Typical values for the permeability, *P*, of a biological membrane to various substances, compared with those for an artificial phospholipid bilayer. For nonpolar molecules such as O_2 and CO_2, and for some small uncharged molecules such as glycerol, *P* values are similar in both systems. For ions and selected polar molecules, including water, the permeability of biological membranes is increased by one or more orders of magnitude, because of the presence of transport proteins. Note the logarithmic scale.

brane transport. In *Arabidopsis*, 849 genes, or 4.8% of all genes, code for proteins involved in membrane transport.

Although a particular transport protein is usually highly specific for the kinds of substances it will transport, its specificity is not absolute: It generally also transports a small family of related substances. For example, in plants a K^+ transporter on the plasma membrane may transport Rb^+ and Na^+ in addition to K^+, but K^+ is usually preferred. On the other hand, the K^+ transporter is completely ineffective in transporting anions such as Cl^- or uncharged solutes such as sucrose. Similarly, a protein involved in the transport of neutral amino acids may move glycine, alanine, and valine with equal ease but not accept aspartic acid or lysine.

In the next several pages we will consider the structures, functions, and physiological roles of the various membrane transporters found in plant cells, especially on the plasma membrane and tonoplast. We begin with a discussion of the role of certain transporters (channels and carriers) in promoting the diffusion of solutes across membranes. We then distinguish between primary and secondary active transport, and we discuss the roles of the electrogenic H^+-ATPase and various symporters (proteins that transport two substances in the same direction simultaneously) in driving proton-coupled secondary active transport.

Channel Transporters Enhance Ion and Water Diffusion across Membranes

Three types of membrane transporters enhance the movement of solutes across membranes: *channels*, *carriers*, and *pumps* (Figure 6.7). **Channels** are transmembrane proteins

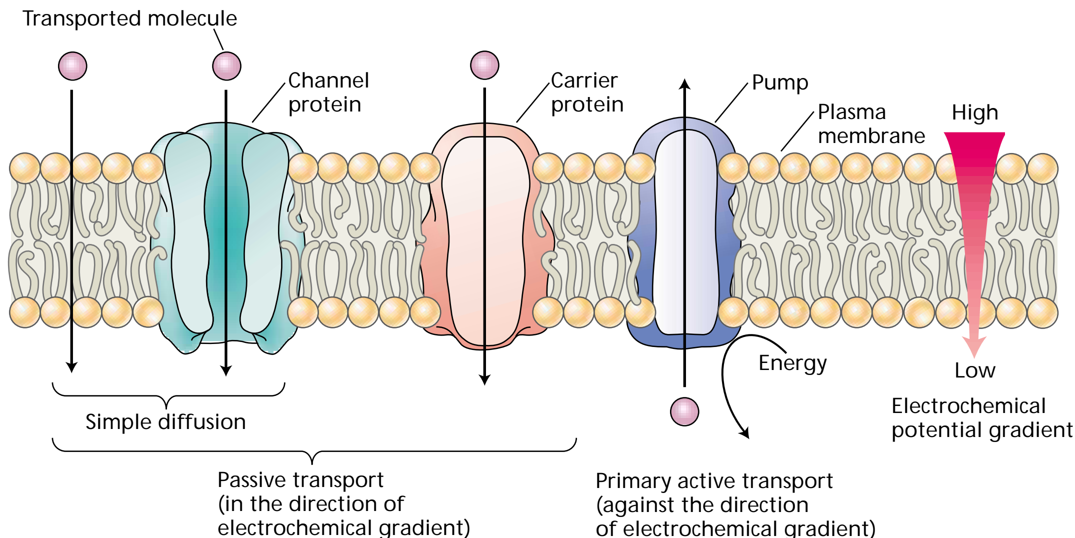


FIGURE 6.7 Three classes of membrane transport proteins: channels, carriers, and pumps. Channels and carriers can mediate the passive transport of solutes across membranes (by simple diffusion or facilitated diffusion), down the solute's gradient of electrochemical potential. Channel proteins act as 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.

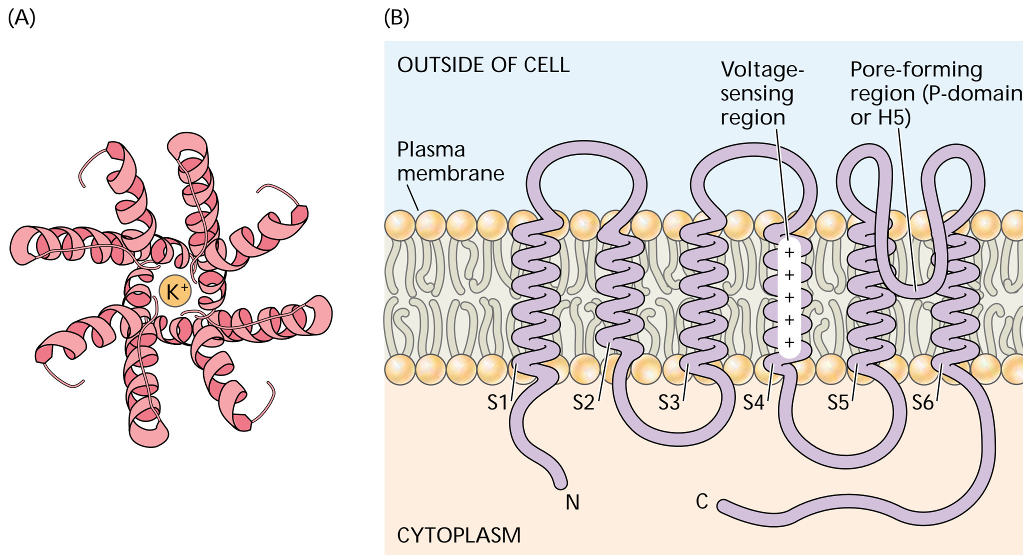


FIGURE 6.8 Models of K^+ channels in plants. (A) Top view of channel, looking through the pore of the protein. Membrane-spanning helices of four subunits come together in an inverted teepee with the pore at the center. The pore-forming regions of the four subunits dip into the membrane, with a K^+ selectivity 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.)

that function as selective pores, through which molecules or ions can diffuse across the membrane. The size of a pore and the density of surface charges on its interior lining determine its transport specificity. Transport through channels is always passive, and because the specificity of transport depends on pore size and electric charge more than on selective binding, channel transport is limited mainly to ions or water (Figure 6.8).

Transport through a channel may or may not involve transient binding of the solute to the channel protein. In any case, as long as the channel pore is open, solutes that can penetrate the pore diffuse through it extremely rapidly: about 10^8 ions per second through each channel protein. Channels are not open all the time: Channel proteins have structures called **gates** that open and close the pore in response to external signals (see Figure 6.8B). Signals that can open or close gates include voltage changes, hormone binding, or light. For example, voltage-gated channels open or close in response to changes in the membrane potential.

Individual ion channels can be studied in detail by the technique of patch clamp electrophysiology (see Web Topic 6.2), which can detect the electric current carried by ions diffusing through a single channel. Patch clamp studies reveal that, for a given ion, such as potassium, a given membrane has a variety of different channels. These channels may open in different voltage ranges, or in response to different signals, which may include K^+ or Ca^{2+} concentrations, pH, protein kinases and phosphatases, and so on. This specificity enables the transport of each ion to be fine-tuned to the prevailing conditions. Thus the ion permeability of a membrane is a variable that depends on the mix of ion channels that are open at a particular time.

As we saw in the experiment of Table 6.1, the distribution of most ions is not close to equilibrium across the membrane. Anion channels will always function to allow anions to diffuse out of the cell, and other mechanisms are needed for anion uptake. Similarly, calcium channels can function only in the direction of calcium release into the cytosol, and calcium must be expelled by active transport. The exception is potassium, which can diffuse either inward or outward, depending on whether the membrane potential is more negative or more positive than E_K, the potassium equilibrium potential.

K^+ channels that open only at more negative potentials are specialized for inward diffusion of K^+ and are known as **inward-rectifying,** or simply **inward,** K^+ channels. Conversely, K^+ channels that open only at more positive potentials are **outward-rectifying,** or **outward,** K^+ channels (see Web Essay 6.1). Whereas inward K^+ channels function in the accumulation of K^+ from the environment, or in the opening of stomata, various outward K^+ channels function in the closing of stomata, in the release of K^+ into the xylem or in regulation of the membrane potential.

Carriers Bind and Transport Specific Substances

Unlike channels, **carrier** proteins do not have pores that extend completely across the membrane. In transport mediated by a carrier, the substance being transported is

initially bound to a specific site on the carrier protein. This requirement for binding allows carriers to be highly selective for a particular substrate to be transported. Carriers therefore specialize in the transport of specific organic metabolites. Binding causes a conformational change in the protein, which exposes the substance to the solution on the other side of the membrane. Transport is complete when the substance dissociates from the carrier's binding site.

Because a conformational change in the protein is required to transport individual molecules or ions, the rate of transport by a carrier is many orders of magnitude slower than through a channel. Typically, carriers may transport 100 to 1000 ions or molecules per second, which is about 10^6 times slower than transport through a channel. The binding and release of a molecule at a specific site on a protein that occur in carrier-mediated transport are similar to the binding and release of molecules from an enzyme in an enzyme-catalyzed reaction. As will be discussed later in the chapter, enzyme kinetics has been used to characterize transport carrier proteins (for a detailed discussion on kinetics, see Chapter 2 on the web site).

Carrier-mediated transport (unlike transport through channels) can be either passive or active, and it can transport a much wider range of possible substrates. Passive transport on a carrier is sometimes called **facilitated diffusion**, although it resembles diffusion only in that it transports substances down their gradient of electrochemical potential, without an additional input of energy. (This term might seem more appropriately applied to transport through channels, but historically it has not been used in this way.)

Primary Active Transport Is Directly Coupled to Metabolic or Light Energy

To carry out active transport, a carrier must couple the uphill transport of the solute with another, energy-releasing, event so that the overall free-energy change is negative. **Primary active transport** is coupled directly to a source of energy other than $\Delta\tilde{\mu}_j$, such as ATP hydrolysis, an oxidation–reduction reaction (the electron transport chain of mitochondria and chloroplasts), or the absorption of light by the carrier protein (in halobacteria, bacteriorhodopsin).

The membrane proteins that carry out primary active transport are called **pumps** (see Figure 6.7). Most pumps transport ions, such as H^+ or Ca^{2+}. However, as we will see later in the chapter, pumps belonging to the "ATP-binding cassette" family of transporters can carry large organic molecules.

Ion pumps can be further characterized as either electrogenic or electroneutral. In general, **electrogenic transport** refers to ion transport involving the net movement of charge across the membrane. In contrast, **electroneutral transport**, as the name implies, involves no net movement of charge. For example, the Na^+/K^+-ATPase of animal cells pumps three Na^+ ions out for every two K^+ ions in, resulting in a net outward movement of one positive charge. The Na^+/K^+-ATPase is therefore an electrogenic ion pump. In contrast, the H^+/K^+-ATPase of the animal gastric mucosa pumps one H^+ out of the cell for every one K^+ in, so there is no net movement of charge across the membrane. Therefore, the H^+/K^+-ATPase is an electroneutral pump.

In the plasma membranes of plants, fungi, and bacteria, as well as in plant tonoplasts and other plant and animal endomembranes, H^+ is the principal ion that is electrogenically pumped across the membrane. The **plasma membrane H^+-ATPase** generates the gradient of electrochemical potentials of H^+ across the plasma membranes, while the **vacuolar H^+-ATPase** and the **H^+-pyrophosphatase** (**H^+-PPase**) electrogenically pump protons into the lumen of the vacuole and the Golgi cisternae.

In plant plasma membranes, the most prominent pumps are for H^+ and Ca^{2+}, and the direction of pumping is outward. Therefore another mechanism is needed to drive the active uptake of most mineral nutrients. The other important way that solutes can be actively transported across a membrane against their gradient of electrochemical potential is by coupling of the uphill transport of one solute to the downhill transport of another. This type of carrier-mediated cotransport is termed **secondary active transport**, and it is driven indirectly by pumps.

Secondary Active Transport Uses the Energy Stored in Electrochemical-Potential Gradients

Protons are extruded from the cytosol by electrogenic H^+-ATPases operating in the plasma membrane and at the vacuole membrane. Consequently, a membrane potential and a pH gradient are created at the expense of ATP hydrolysis. This gradient of electrochemical potential for H^+, $\Delta\tilde{\mu}_{H^+}$, or (when expressed in other units) the **proton motive force** (**PMF**), or Δp, represents stored free energy in the form of the H^+ gradient (see Web Topic 6.3).

The proton motive force generated by electrogenic H^+ transport is used in secondary active transport to drive the transport of many other substances against their gradient of electrochemical potentials. Figure 6.9 shows how secondary transport may involve the binding of a substrate (S) and an ion (usually H^+) to a carrier protein, and a conformational change in that protein.

There are two types of secondary transport: symport and antiport. The example shown in Figure 6.9 is called **symport** (and the protein involved is called a *symporter*) because the two substances are moving in the same direction through the membrane (see also Figure 6.10A). **Antiport** (facilitated by a protein called an *antiporter*) refers to coupled transport in which the downhill movement of protons drives the active (uphill) transport of a solute in the opposite direction (Figure 6.10B).

In both types of secondary transport, the ion or solute being transported simultaneously with the protons is moving against its gradient of electrochemical potential, so its transport is active. However, the energy driving this transport is provided by the proton motive force rather than directly by ATP hydrolysis.

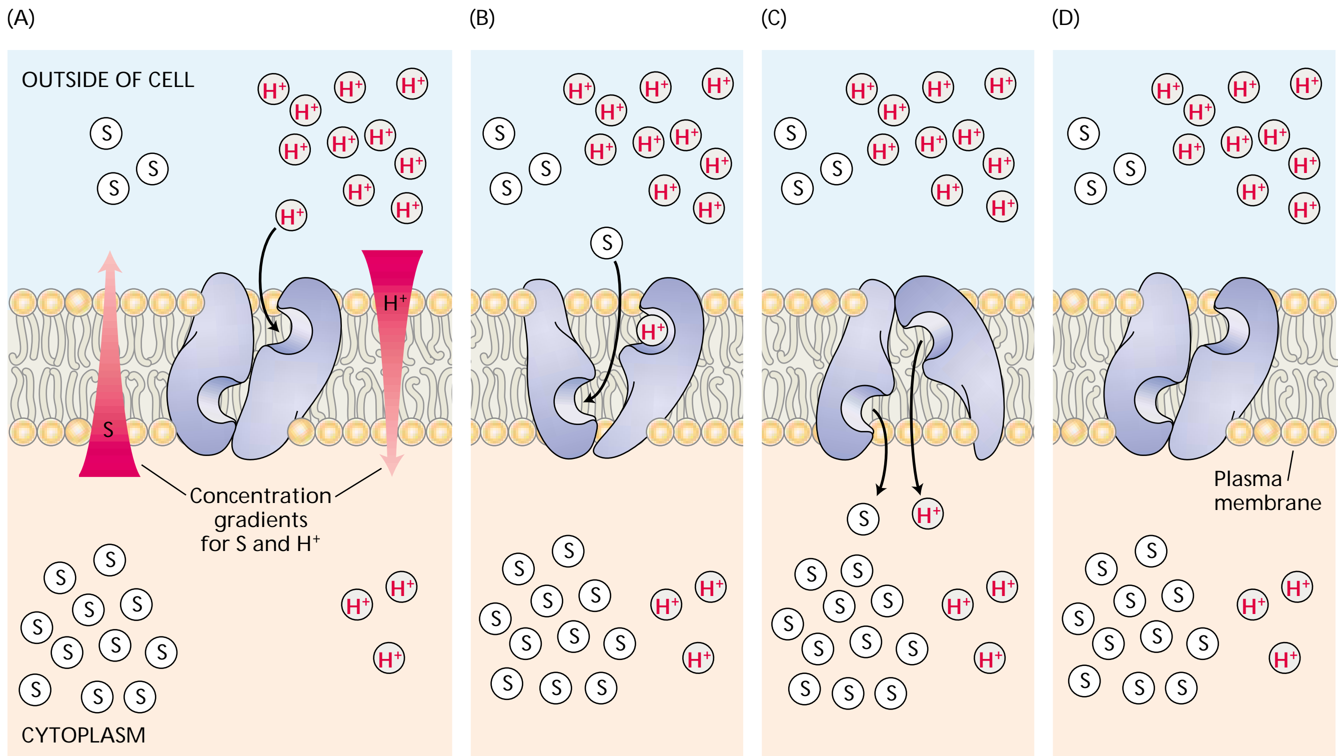


FIGURE 6.9 Hypothetical model for secondary active transport. The energy that drives the process has been stored in a $\Delta\tilde{\mu}_{H^+}$ (symbolized by the red arrow on the right in A) and is being used to take up a substrate (S) against its concentration gradient (left-hand red arrow). (A) In the initial conformation, the binding sites on the protein are exposed to the outside environment and can bind a proton. (B) This binding results in a conformational change that permits a molecule of S to be bound. (C) The binding of S causes another conformational change that exposes the binding sites and their substrates to the inside of the cell. (D) Release of a proton and a molecule of S to the cell's interior restores the original conformation of the carrier and allows a new pumping cycle to begin.

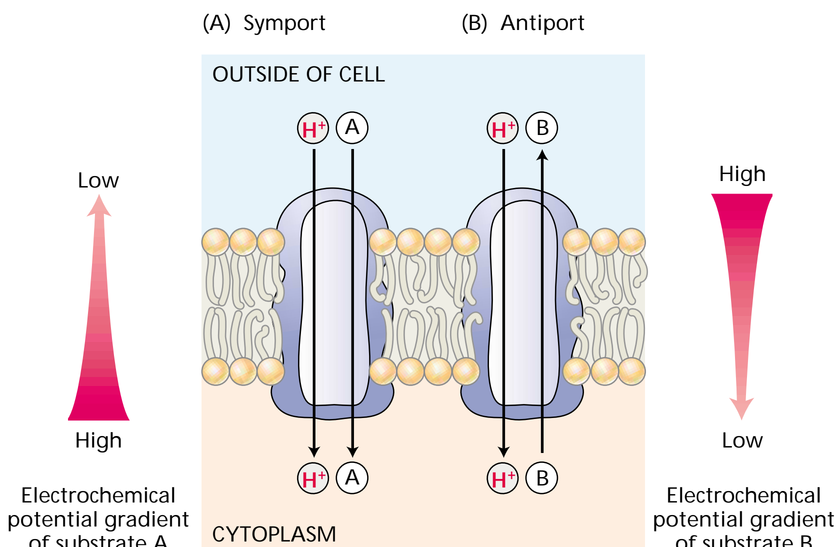


FIGURE 6.10 Two examples of secondary active transport coupled to a primary proton gradient. (A) In a symport, the energy dissipated by a proton moving back into the cell is coupled to the uptake of one molecule of a substrate (e.g., a sugar) into the cell. (B) In an antiport, the energy dissipated by a proton moving back into the cell is coupled to the active transport of a substrate (for example, a sodium ion) out of the cell. In both cases, the substrate under consideration is moving against its gradient of electrochemical potential. Both neutral and charged substrates can be transported by such secondary active transport processes.

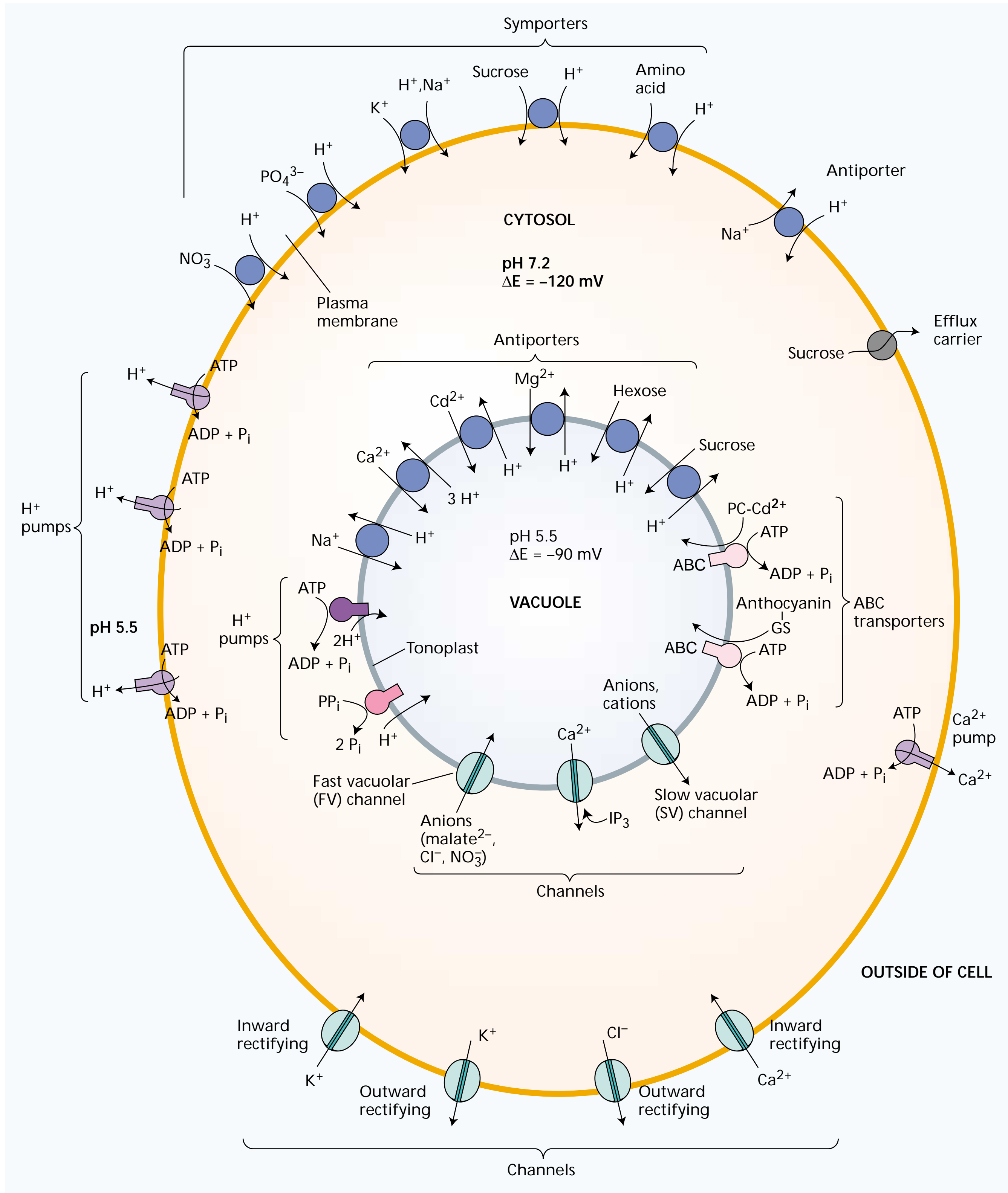


FIGURE 6.11 Overview of the various transport processes on the plasma membrane and tonoplast of plant cells.

Typically, transport across a biological membrane is energized by one primary active transport system coupled to ATP hydrolysis. The transport of that ion—for example, H^+—generates an ion gradient and an electrochemical potential. Many other ions or organic substrates can then be transported by a variety of secondary active-transport proteins, which energize the transport of their respective substrates by simultaneously carrying one or two H^+ ions down their energy gradient. Thus H^+ ions circulate across the membrane, outward through the primary active transport proteins, and back into the cell through the secondary transport proteins. In plants and fungi, sugars and amino acids are taken up by symport with protons.

Most of the ionic gradients across membranes of higher plants are generated and maintained by electrochemical-potential gradients of H^+ (Tazawa et al. 1987). In turn, these H^+ gradients are generated by the electrogenic proton pumps. Evidence suggests that in plants, Na^+ is transported out of the cell by a Na^+–H^+ antiporter and that Cl^-, NO_3^-, $H_2PO_4^-$, sucrose, amino acids, and other substances enter the cell via specific proton symporters.

What about K^+? At very low external concentrations, K^+ can be taken up by active symport proteins, but at higher concentrations it can enter the cell by diffusion through specific K^+ channels. However, even influx through channels is driven by the H^+-ATPase, in the sense that K^+ diffusion is driven by the membrane potential, which is maintained at a value more negative than the K^+ equilibrium potential by the action of the electrogenic H^+ pump. Conversely, K^+ efflux requires the membrane potential to be maintained at a value more positive than E_K, which can be achieved if efflux of Cl^- through Cl^- channels is allowed. Several representative transport processes located on the plasma membrane and the tonoplast are illustrated in Figure 6.11.

MEMBRANE TRANSPORT PROTEINS

We have seen in preceding sections that some transmembrane proteins operate as channels for the controlled diffusion of ions. Other membrane proteins act as carriers for other substances (mostly molecules and ions). Active transport utilizes carrier-type proteins that are energized directly by ATP hydrolysis or indirectly as symporters and antiporters. The latter systems use the energy of ion gradients (often a H^+ gradient) to drive the uphill transport of another ion or molecule. In the pages that follow we will examine in more detail the molecular properties, cellular locations, and genetic manipulations of some of these transport proteins.

Kinetic Analyses Can Elucidate Transport Mechanisms

Thus far, we have described cellular transport in terms of its energetics. However, cellular transport can also be studied by use of enzyme kinetics because transport involves the binding and dissociation of molecules at active sites on transport proteins. One advantage of the kinetic approach is that it gives new insights into the regulation of transport.

In kinetic experiments the effects of external ion (or other solute) concentrations on transport rates are measured. The kinetic characteristics of the transport rates can then be used to distinguish between different transporters. The maximum rate (V_{max}) of carrier-mediated transport, and often channel transport as well, cannot be exceeded, regardless of the concentration of substrate (Figure 6.12). V_{max} is approached when the substrate-binding site on the carrier is always occupied. The concentration of carrier, not the concentration of solute, becomes rate limiting. Thus V_{max} is a measure of the number of molecules of the specific carrier protein that are functioning in the membrane.

The constant K_m (which is numerically equal to the solute concentration that yields half the maximal rate of transport) tends to reflect the properties of the particular binding site (for a detailed discussion on K_m and V_{max} see Chapter 2 on the web site). Low K_m values indicate high affinity of the transport site for the transported substance. Such values usually imply the operation of a carrier system. Higher values of K_m indicate a lower affinity of the transport site for the solute. The affinity is often so low that in practice V_{max} is never reached. In such cases, kinetics alone cannot distinguish between carriers and channels.

Usually transport displays both high-affinity and low-affinity components when a wide range of solute concentrations are studied. Figure 6.13 shows sucrose uptake by soybean cotyledon protoplasts as a function of the external

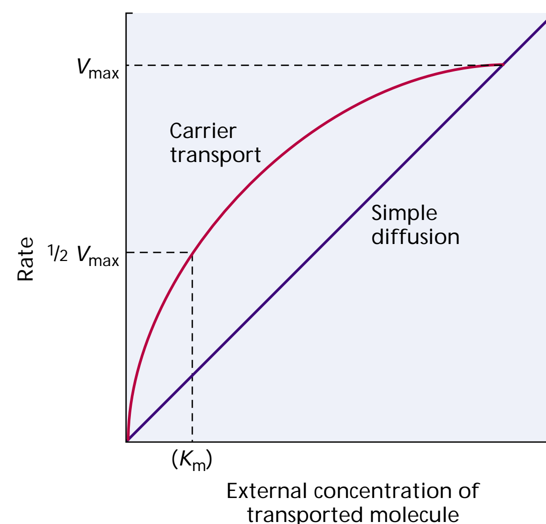


FIGURE 6.12 Carrier transport often shows saturation kinetics (V_{max}) (see Chapter 2 on the web site), because of saturation of a binding site. Ideally, diffusion through channels is directly proportional to the concentration of the transported solute, or for an ion, to the difference in electrochemical potential across the membrane.

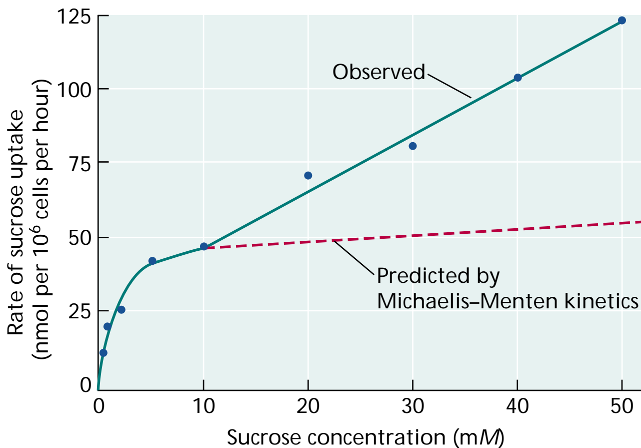


FIGURE 6.13 The transport properties of a solute can change at different solute concentrations. For example, at low concentrations (1 to 10 m*M*), the rate of uptake of sucrose by soybean cells shows saturation kinetics typical of carriers. A curve fit-ted to these data is predicted to approach a maximal rate (V_{max}) of 57 nmol per 10^6 cells per hour. Instead, at higher sucrose concentrations the uptake rate continues to increase linearly over a broad range of concentrations, suggesting the existence of other sucrose transporters, which might be carriers with very low affinity for the substrate. (From Lin et al. 1984.)

sucrose concentration (Lin et al. 1984). Uptake increases sharply with concentration and begins to saturate at about 10 m*M*. At concentrations above 10 m*M*, uptake becomes linear and nonsaturable. Inhibition of ATP synthesis with metabolic poisons blocks the saturable component but not the linear one. The interpretation is that sucrose uptake at low concentrations is an active carrier-mediated process (sucrose–H^+ symport). At higher concentrations, sucrose enters the cells by diffusion down its concentration gradient and is therefore insensitive to metabolic poisons. However, additional information is needed to investigate whether the nonsaturating component represents uptake by a carrier with very low affinity, or by a channel. (Transport by a carrier is more likely in the case of a molecular solute such as sucrose.)

The Genes for Many Transporters Have Been Cloned

Transporter gene identification, isolation, and cloning have greatly aided in the elucidation of the molecular properties of transporter proteins. Nitrate transport is an example that is of interest not only because of its nutritional importance, but also because of its complexity. Kinetic analysis shows that nitrate transport, like the sucrose transport shown in Figure 6.13, has both high-affinity (low K_m) and low-affinity (high K_m) components. In contrast with sucrose, nitrate is negatively charged, and such an electric charge imposes an energy requirement for the transport of the nitrate ion at all concentrations. The energy is provided by symport with H^+.

Nitrate transport is also strongly regulated according to nitrate availability: The enzymes required for nitrate transport, as well as nitrate assimilation (see Chapter 12), are induced in the presence of nitrate in the environment, and uptake can also be repressed if nitrate accumulates in the cells.

Mutants in nitrate transport or nitrate reduction can be selected by growth in the presence of chlorate (ClO_3^-). Chlorate is a nitrate analog that is taken up and reduced in wild-type plants to the toxic product chlorite. If plants resistant to chlorate are selected, they are likely to show mutations that block nitrate transport or reduction.

Several such mutations have been identified in *Arabidopsis,* a small crucifer that is ideal for genetic studies. The first transport gene identified in this way encodes a low-affinity inducible nitrate–proton symporter. As more genes for nitrate transport have been identified and characterized, the picture has become more complex. Each component of transport may involve more than one gene product, and at least one gene encodes a dual-affinity carrier that contributes to both high-affinity and low-affinity transport (Chrispeels et al. 1999).

The emerging picture of plant transporter genes shows that a family of genes, rather than an individual gene, exists in the plant genome for each transport function. Within a gene family, variations in transport characteristics such as K_m, in mode of regulation, and in differential tissue expression give plants a remarkable plasticity to acclimate to a broad range of environmental conditions.

The identification of regions of sequence similarity between plant transport genes and the transport genes of other organisms, such as yeast, has enabled the cloning of plant transport genes (Kochian 2000). In some cases, it has been possible to identify the gene after purifying the transport protein, but often sequence similarity is limited, and individual transport proteins represent too small a fraction of total protein. Another way to identify transport genes is to screen plant cDNA (complementary DNA) libraries for genes that complement (i.e., compensate for) transport deficiencies in yeast. Many yeast transport mutants are known and have been used to identify corresponding plant genes by complementation.

In the case of genes for ion channels, researchers have studied the behavior of the channel proteins by expressing the genes in oocytes of the toad *Xenopus*, which, because of their large size, are convenient for electrophysiological studies. Genes for both inward- and outward-rectifying K^+ channels have been cloned and studied in this way. Of the inward K^+ channel genes identified so far, one is expressed strongly in stomatal guard cells, another in roots, and a third in leaves. These channels are considered to be responsible for low-affinity K^+ uptake into plant cells.

An outward K^+ channel responsible for K^+ flux from root stelar cells into the dead xylem vessels has been

cloned, and several genes for high-affinity K^+ carriers have been identified. Further research is needed to determine to what extent they each contribute to K^+ uptake, and how they obtain their energy (see Web Topic 6.4). Genes for plant vacuolar H^+–Ca^{2+} antiporters and genes for the proton symport of several amino acids and sugars have also been identified through various genetic techniques (Hirshi et al. 1996; Tanner and Caspari 1996; Kuehn et al. 1999).

Genes for Specific Water Channels Have Been Identified

Aquaporins are a class of proteins that is relatively abundant in plant membranes (see Chapter 3). Aquaporins reveal no ion currents when expressed in oocytes, but when the osmolarity of the external medium is reduced, expression of these proteins results in swelling and bursting of the oocytes. The bursting results from rapid influx of water across the oocyte plasma membrane, which normally has a very low water permeability. These results show that aquaporins form water channels in membranes (see Figure 3.6).

The existence of aquaporins was a surprise at first because it was thought that the lipid bilayer is itself sufficiently permeable to water. Nevertheless, aquaporins are common in plant and animal membranes, and their expression and activity appear to be regulated, possibly by protein phosphorylation, in response to water availability (Tyerman et al. 2002).

The Plasma Membrane H^+-ATPase Has Several Functional Domains

The outward, active transport of H^+ across the plasma membrane creates gradients of pH and electric potential that drive the transport of many other substances (ions and molecules) through the various secondary active-transport proteins. Figure 6.14 illustrates how a membrane H^+-ATPase might work.

Plant and fungal plasma membrane H^+-ATPases and Ca^{2+}-ATPases are members of a class known as P-type ATPases, which are phosphorylated as part of the catalytic cycle that hydrolyzes ATP. Because of this phosphorylation step, the plasma membrane ATPases are strongly inhibited by orthovanadate (HVO_4^{2-}), a phosphate (HPO_4^{2-}) analog that competes with phosphate from ATP for the aspartic acid phosphorylation site on the enzyme. The high affinity of the enzyme for vanadate is attributed to the fact that vanadate can mimic the transitional structure of phosphate during hydrolysis.

Plasma membrane H^+-ATPases are encoded by a family of about ten genes. Each gene encodes an isoform of the enzyme (Sussman 1994). The isoforms are tissue specific, and they are preferentially expressed in the root, the seed, the phloem, and so on. The functional specificity of each isoform is not yet understood; it may alter the pH optimum of some isoforms and allow transport to be regulated in different ways for each tissue.

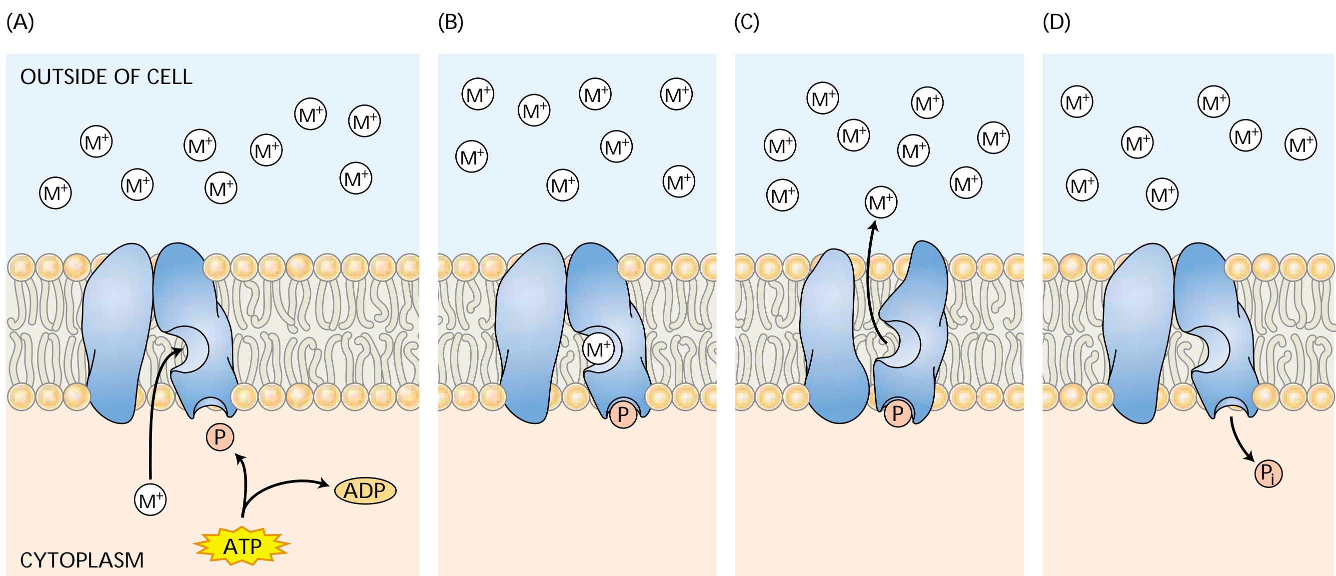


FIGURE 6.14 Hypothetical steps in the transport of a cation (the hypothetical M^+) against its chemical gradient by an electrogenic pump. The protein, embedded in the membrane, binds the cation on the inside of the cell (A) and is phosphorylated by ATP (B). This phosphorylation leads to a conformational 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.

FIGURE 6.15 Two-dimensional representation of the plasma membrane H^+-ATPase. The H^+-ATPase has 10 transmembrane segments. The regulatory domain is the autoinhibitory domain. (From Palmgren 2001.)

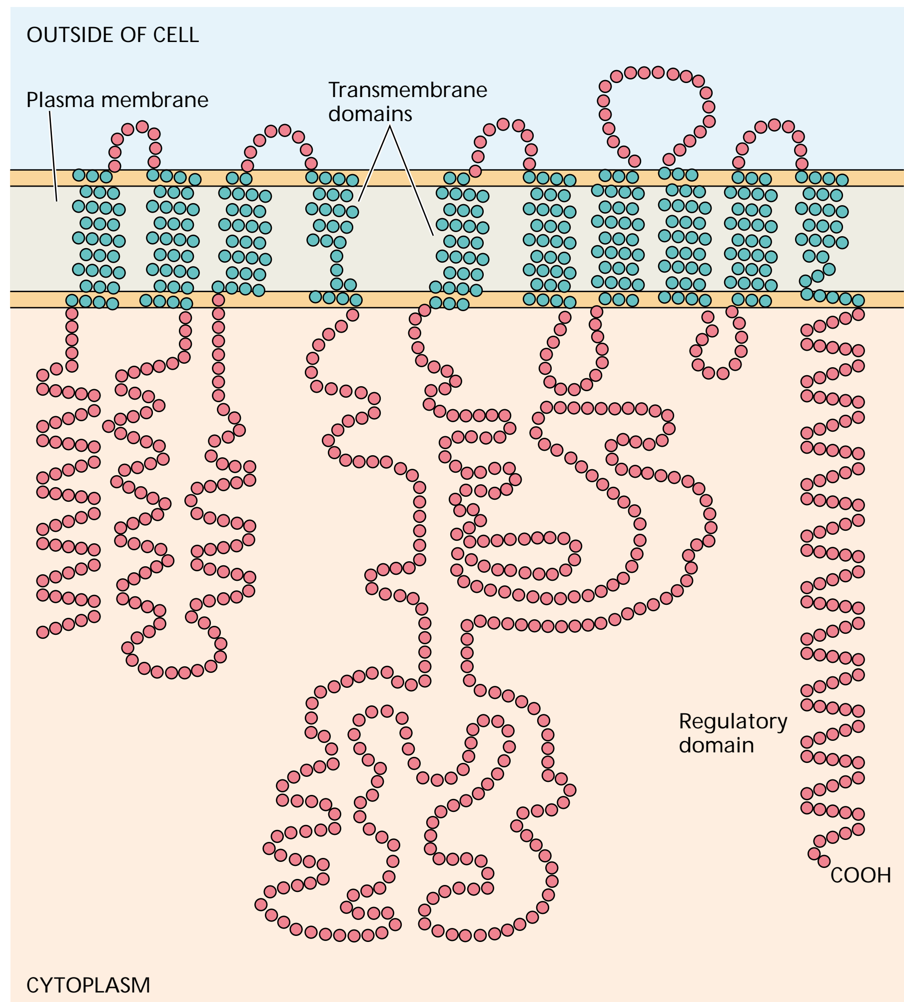


Figure 6.15 shows a model of the functional domains of the plasma membrane H^+-ATPase of yeast, which is similar to that of plants. The protein has ten membrane-spanning domains that cause it to loop back and forth across the membrane. Some of the membrane-spanning domains make up the pathway through which protons are pumped. The catalytic domain, including the aspartic acid residue that becomes phosphorylated during the catalytic cycle, is on the cytosolic face of the membrane.

Like other enzymes, the plasma membrane ATPase is regulated by the concentration of substrate (ATP), pH, temperature, and other factors. In addition, H^+-ATPase molecules can be reversibly activated or deactivated by specific signals, such as light, hormones, pathogen attack, and the like. This type of regulation is mediated by a specialized autoinhibitory domain at the C-terminal end of the polypeptide chain, which acts to regulate the activity of the proton pump (see Figure 6.15). If the autoinhibitory domain is removed through the action of a protease, the enzyme becomes irreversibly activated (Palmgren 2001).

The autoinhibitory effect of the C-terminal domain can also be regulated through the action of protein kinases and phosphatases that add or remove phosphate groups to serine or threonine residues on the autoinhibitory domain of the enzyme. For example, one mechanism of response to pathogens in tomato involves the activation of protein phosphatases that dephosphorylate residues on the plasma membrane H^+-ATPase, thereby activating it (Vera-Estrella et al. 1994). This is one step in a cascade of responses that activate plant defenses.

The Vacuolar H^+-ATPase Drives Solute Accumulation into Vacuoles

Because plant cells increase their size primarily by taking up water into large, central vacuoles, the osmotic pressure of the vacuole must be maintained sufficiently high for water to enter from the cytoplasm. The tonoplast regulates the traffic of ions and metabolites between the cytosol and the vacuole, just as the plasma membrane regulates uptake into the cell. Tonoplast transport became a vigorous area of research following the development of methods for the isolation of intact vacuoles and tonoplast vesicles (see Web Topic 6.5). These studies led to the discovery of a new type of proton-pumping ATPase, which transports protons into the vacuole (see Figure 6.11).

The vacuolar H^+-ATPase (also called **V-ATPase**) differs both structurally and functionally from the plasma membrane H^+-ATPase. The vacuolar ATPase is more closely related to the F-ATPases of mitochondria and chloroplasts (see Chapter 11). Because the hydrolysis of ATP by the vacuolar ATPase does not involve the formation of a phosphorylated intermediate, vacuolar ATPases are insensitive to vanadate, the inhibitor of plasma membrane ATPases discussed earlier. Vacuolar ATPases are specifically inhibited by the antibiotic bafilomycin, as well as by high concentrations of nitrate, neither of which inhibit plasma membrane ATPases. Use of these selective inhibitors makes it possible to identify different types of ATPases, and to assay their activity.

Vacuolar ATPases belong to a general class of ATPases that are present on the endomembrane systems of all

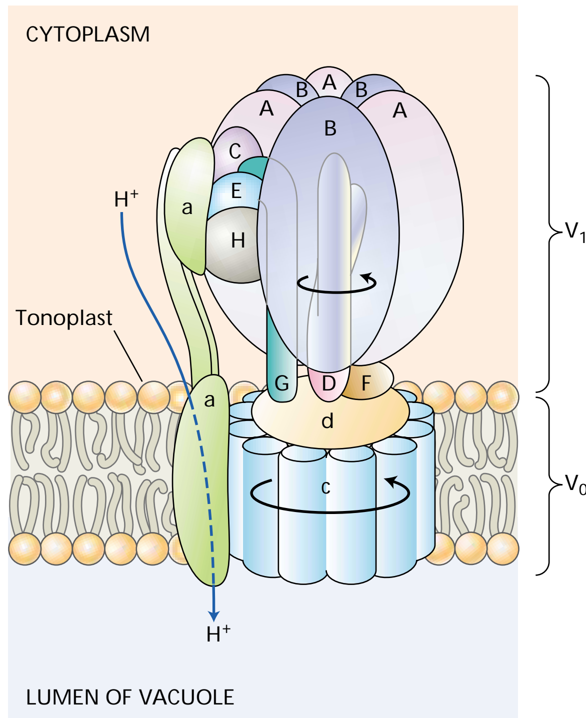


FIGURE 6.16 Model of the V-ATPase rotary motor. Many polypeptide subunits come together to make this complex enzyme. The V_1 catalytic complex is easily dissociated from the membrane, and contains the nucleotide-binding and catalytic sites. Components of V_1 are designated by uppercase letters. The intrinsic 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.)

eukaryotes. They are large enzyme complexes, about 750 kDa, composed of at least ten different subunits (Lüttge and Ratajczak 1997). These subunits are organized into a peripheral catalytic complex, V_1, and an integral membrane channel complex, V_0 (Figure 6.16). Because of their similarities to F-ATPases, vacuolar ATPases are assumed to operate like tiny rotary motors (see Chapter 11).

Vacuolar ATPases are electrogenic proton pumps that transport protons from the cytoplasm to the vacuole and generate a proton motive force across the tonoplast. The electrogenic proton pumping accounts for the fact that the vacuole is typically 20 to 30 mV more positive than the cytoplasm, although it is still negative relative to the external medium. To maintain bulk electrical neutrality, anions such as Cl^- or malate^{2-} are transported from the cytoplasm into the vacuole through channels in the membrane (Barkla and Pantoja 1996). Without the simultaneous movement of anions along with the pumped protons, the charge buildup across the tonoplast would make the pumping of additional protons energetically impossible.

The conservation of bulk electrical neutrality by anion transport makes it possible for the vacuolar H^+-ATPase to generate a large concentration (pH) gradient of protons across the tonoplast. This gradient accounts for the fact that the pH of the vacuolar sap is typically about 5.5, while the cytoplasmic pH is 7.0 to 7.5. Whereas the electrical component of the proton motive force drives the uptake of anions into the vacuole, the electrochemical-potential gradient for H^+ ($\Delta\tilde{\mu}_{H^+}$) is harnessed to drive the uptake of cations and sugars into the vacuole via secondary transport (antiporter) systems (see Figure 6.11).

Although the pH of most plant vacuoles is mildly acidic (about 5.5), the pH of the vacuoles of some species is much lower—a phenomenon termed ***hyperacidification***. Vacuolar hyperacidification is the cause of the sour taste of certain fruits (lemons) and vegetables (rhubarb). Some extreme examples are listed in Table 6.2. Biochemical studies with lemon fruits have suggested that the low pH of the lemon fruit vacuoles (specifically, those of the juice sac cells) is due to a combination of factors:

- The low permeability of the vacuolar membrane to protons permits a steeper pH gradient to build up.
- A specialized vacuolar ATPase is able to pump protons more efficiently (with less wasted energy) than normal vacuolar ATPases can (Müller et al. 1997).

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 (*Opuntia phaeacantha*)[b]	1.4 (6:45 A.M.) 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.

- The accumulation of organic acids such as citric, malic, and oxalic acids helps maintain the low pH of the vacuole by acting as buffers.

Plant Vacuoles Are Energized by a Second Proton Pump, the H^+-Pyrophosphatase

Another type of proton pump, an H^+-pyrophosphatase (H^+-PPase) (Rea et al. 1998), appears to work in parallel with the vacuolar ATPase to create a proton gradient across the tonoplast (see Figure 6.11). This enzyme consists of a single polypeptide that has a molecular mass of 80 kDa. The H^+-PPase harnesses its energy from the hydrolysis of inorganic pyrophosphate (PP_i).

The free energy released by PP_i hydrolysis is less than that from ATP hydrolysis. However, the vacuolar H^+-PPase transports only one H^+ ion per PP_i molecule hydrolyzed, whereas the vacuolar ATPase appears to transport two H^+ ions per ATP hydrolyzed. Thus the energy available per H^+ ion transported appears to be the same, and the two enzymes appear to be able to generate comparable H^+ gradients.

In some plants the synthesis of the vacuolar H^+-PPase is induced by low O_2 levels (hypoxia) or by chilling. This indicates that the vacuolar H^+-PPase might function as a backup system to maintain essential cell metabolism under conditions in which ATP supply is depleted because of the inhibition of respiration by hypoxia or chilling. It is of interest that the plant vacuolar H^+-PPase is not found in animals or yeast, although a similar enzyme is present in some bacteria and protists.

Large metabolites such as flavonoids, anthocyanins and secondary products of metabolism are sequestered in the vacuole. These large molecules are transported into vacuoles by **ATP-binding cassette (ABC) transporters**. Transport processes by the ABC transporters consume ATP and do not depend on a primary electrochemical gradient (see Web Topic 6.6). Recent studies have shown that ABC transporters can also be found at the plasma membrane and in mitochondria (Theodoulou 2000).

Calcium Pumps, Antiports, and Channels Regulate Intracellular Calcium

Calcium is another important ion whose concentration is strongly regulated. Calcium concentrations in the cell wall and the apoplastic (extracellular) spaces are usually in the millimolar range; free cytosolic Ca^{2+} concentrations are maintained at the micromolar (10^{-6} *M*) range, against the large electrochemical-potential gradient that drives Ca^{2+} diffusion into the cell.

Small fluctuations in cytosolic Ca^{2+} concentration drastically alter the activities of many enzymes, making calcium an important second messenger in signal transduction. Most of the calcium in the cell is stored in the central vacuole, where it is taken up via Ca^{2+}–H^+ antiporters, which use the electrochemical potential of the proton gradient to energize the accumulation of calcium into the vacuole (Bush 1995). Mitochondria and the endoplasmic reticulum also store calcium within the cells.

Calcium efflux from the vacuole into the cytosol may in some cells be triggered by inositol trisphosphate (IP_3). IP_3, which appears to act as a "second messenger" in certain signal transduction pathways, induces the opening of IP_3-gated calcium channels on the tonoplast and endoplasmic reticulum (ER). (For a more detailed description of these sensory transduction pathways see Chapter 14 on the web site.)

Calcium ATPases are found at the plasma membrane (Chung et al. 2000) and in some endomembranes of plant cells (see Figure 6.11). Plant cells regulate cytosolic Ca^{2+} concentrations by controlling the opening of Ca^{2+} channels that allow calcium to diffuse in, as well as by modulating the activity of pumps that drive Ca^{2+} out of the cytoplasm back into the extracellular spaces. Whereas the plasma membrane calcium pumps move calcium out of the cell, the calcium pumps on the ER transport calcium into the ER lumen.

ION TRANSPORT IN ROOTS

Mineral nutrients absorbed by the root are carried to the shoot by the transpiration stream moving through the xylem (see Chapter 4). Both the initial uptake of nutrients and the subsequent movement of mineral ions from the root surface across the cortex and into the xylem are highly specific, well-regulated processes.

Ion transport across the root obeys the same biophysical laws that govern cellular transport. However, as we have seen in the case of water movement (see Chapter 4), the anatomy of roots imposes some special constraints on the pathway of ion movement. In this section we will discuss the pathways and mechanisms involved in the radial movement of ions from the root surface to the tracheary elements of the xylem.

Solutes Move through Both Apoplast and Symplast

Thus far, our discussion of cellular ion transport has not included the cell wall. In terms of the transport of small molecules, the cell wall is an open lattice of polysaccharides through which mineral nutrients diffuse readily. Because all plant cells are separated by cell walls, ions can diffuse across a tissue (or be carried passively by water flow) entirely through the cell wall space without ever entering a living cell. This continuum of cell walls is called the *extracellular space*, or *apoplast* (see Figure 4.3).

We can determine the apoplastic volume of a slice of plant tissue by comparing the uptake of ^{3}H-labeled water and ^{14}C-labeled mannitol. Mannitol is a nonpermeating sugar alcohol that diffuses within the extracellular space but cannot enter the cells. Water, on the other hand, freely penetrates both the cells and the cell walls. Measurements of this type usually show that 5 to 20% of the plant tissue volume is occupied by cell walls.

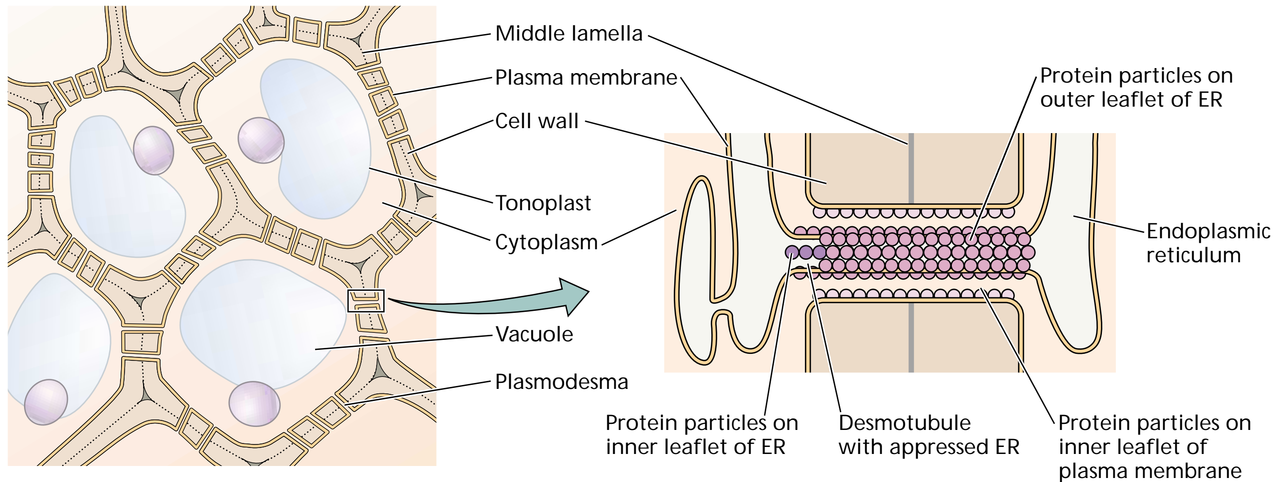


FIGURE 6.17 Diagram illustrating how plasmodesmata connect the cytoplasms of neighboring cells. Plasmodesmata are about 40 nm in diameter and allow diffusion of water and small molecules from one cell to the next. In addition, the size of the opening can be regulated by rearrangements of the internal proteins to allow the passage of larger molecules.

Just as the cell walls form a continuous phase, so do the cytoplasms of neighboring cells, collectively referred to as the *symplast*. Plant cells are interconnected by cytoplasmic bridges called plasmodesmata (see Chapter 1), cylindrical pores 20 to 60 nm in diameter (see Figure 1.27). Each plasmodesma is lined with a plasma membrane and contains a narrow tubule, the desmotubule, that is a continuation of the endoplasmic reticulum.

In tissues where significant amounts of intercellular transport occur, neighboring cells contain large numbers of plasmodesmata, up to 15 per square micrometer of cell surface (Figure 6.17). Specialized secretory cells, such as floral nectaries and leaf salt glands, appear to have high densities of plasmodesmata; so do the cells near root tips, where most nutrient absorption occurs.

By injecting dyes or by making electrical-resistance measurements on cells containing large numbers of plasmodesmata, investigators have shown that ions, water, and small solutes can move from cell to cell through these pores. Because each plasmodesma is partly occluded by the desmotubule and associated proteins (see Chapter 1), the movement of large molecules such as proteins through the plasmodesmata requires special mechanisms (Ghoshroy et al. 1997). Ions, on the other hand, appear to move from cell to cell through the entire plant by simple diffusion through the symplast (see Chapter 4).

Ions Moving through the Root Cross Both Symplastic and Apoplastic Spaces

Ion absorption by the roots (see Chapter 5) is more pronounced in the root hair zone than in the meristem and elongation zones. Cells in the root hair zone have completed their elongation but have not yet begun secondary growth. The root hairs are simply extensions of specific epidermal cells that greatly increase the surface area available for ion absorption.

An ion that enters a root may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may enter the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortex, an ion may either cross the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially all the way to the endodermis via the apoplast. In all cases, ions must enter the symplast before they can enter the stele, because of the presence of the Casparian strip.

The apoplast forms a continuous phase from the root surface through the cortex. At the boundary between the vascular cylinder (the stele) and the cortex is a layer of specialized cells, the endodermis. As discussed in Chapters 4 and 5, a suberized cell layer in the endodermis, known as the Casparian strip, effectively blocks the entry of water and mineral ions into the stele via the apoplast.

Once an ion has entered the stele through the symplastic connections across the endodermis, it continues to diffuse from cell to cell into the xylem. Finally, the ion reenters the apoplast as it diffuses into a xylem tracheid or vessel element. Again, the Casparian strip prevents the ion from diffusing back out of the root through the apoplast. The presence of the Casparian strip allows the plant to maintain a higher ionic concentration in the xylem than exists in the soil water surrounding the roots.

Xylem Parenchyma Cells Participate in Xylem Loading

Once ions have been taken up into the symplast of the root at the epidermis or cortex, they must be loaded into the tracheids or vessel elements of the stele to be translocated to the shoot. The stele consists of dead tracheary elements and

the living xylem parenchyma. Because the xylem tracheary elements are dead cells, they lack cytoplasmic continuity with surrounding xylem parenchyma. To enter the tracheary elements, the ions must exit the symplast by crossing a plasma membrane a second time.

The process whereby ions exit the symplast and enter the conducting cells of the xylem is called **xylem loading**. The mechanism of xylem loading has long baffled scientists. Ions could enter the tracheids and vessel elements of the xylem by simple passive diffusion. In this case, the movement of ions from the root surface to the xylem would take only a single step requiring metabolic energy. The site of this single-step, energy-dependent uptake would be the plasma membrane surfaces of the root epidermal, cortical, or endodermal cells. According to the passive-diffusion model, ions move passively into the stele via the symplast down a gradient of electrochemical potential, and then leak out of the living cells of the stele (possibly because of lower oxygen availability in the interior of the root) into the nonliving conducting cells of the xylem.

Support for the passive-diffusion model was provided by use of ion-specific microelectrodes to measure the electrochemical potentials of various ions across maize roots (Figure 6.18) (Dunlop and Bowling 1971). Data from this and other studies indicate that K^+, Cl^-, Na^+, SO_4^{2-}, and NO_3^- are all taken up actively by the epidermal and cortical cells and are maintained in the xylem against a gradient of electrochemical potential when compared with the external medium (Lüttge and Higinbotham 1979). However, none of these ions is at a higher electrochemical potential in the xylem than in the cortex or living portions of the stele. Therefore, the final movement of ions into the xylem could be due to passive diffusion.

However, other observations have led to the view that this final step of xylem loading may also involve active processes within the stele (Lüttge and Higinbotham 1979). With the type of apparatus shown in Figure 6.19, it is possible to make simultaneous measurements of ion uptake into the epidermal or cortical cytoplasm and of ion loading into the xylem.

By using treatments with inhibitors and plant hormones, investigators have shown that ion uptake by the cortex and ion loading into the xylem operate independently. For example, treatment with the protein synthesis inhibitor cycloheximide or with the cytokinin benzyladenine inhibits xylem loading without affecting uptake by the cortex. This result indicates that efflux from the stelar cells is regulated independently from uptake by the cortical cells.

Recent biochemical studies have supported a role for the xylem parenchyma cells in xylem loading. The plasma

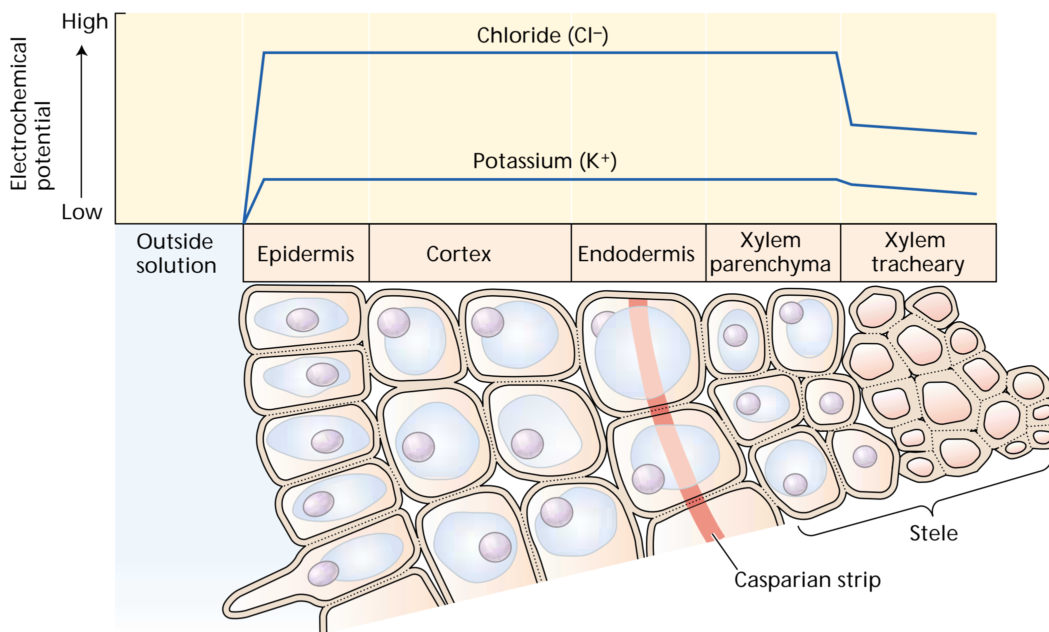


FIGURE 6.18 Diagram showing electrochemical potentials of K^+ and Cl^- across a maize root. To determine the electrochemical potentials, the root was bathed in a solution containing 1 m*M* KCl and 0.1 m*M* $CaCl_2$. A reference electrode was positioned in the bathing solution, and an ion-sensitive measuring electrode was inserted in different cells of the root. The horizontal axis shows the different tissues found in a root cross section. The substantial increase in electrochemical potential for both K^+ and Cl^- between the bathing medium and the epidermis indicates that ions are taken up into the root by an active transport process. In contrast, the potentials decrease at the xylem vessels, suggesting that ions are transported into the xylem by passive diffusion down the gradient of electrochemical potential. (After Dunlop and Bowling 1971.)

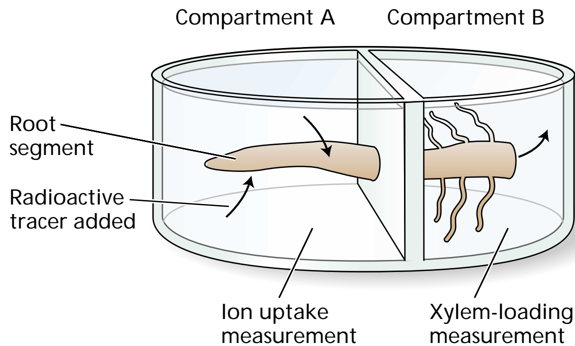


FIGURE 6.19 We can measure the relationship between ion uptake into the root and xylem loading by placing a root segment across two compartments and adding a radioactive tracer to one of them (in this case compartment A). The rate of disappearance of the tracer from compartment A gives a measure of ion uptake, and the rate of appearance in compartment B provides a measurement of xylem loading. (From Lüttge and Higinbotham 1979.)

membranes of xylem parenchyma cells contain proton pumps, water channels, and a variety of ion channels specialized for influx or efflux (Maathuis et al. 1997). In barley xylem parenchyma, two types of cation efflux channels have been identified: K^+-specific efflux channels and nonselective cation efflux channels. These channels are regulated by both the membrane potential and the cytosolic calcium concentration (De Boer and Wegner 1997). This finding suggests that the flux of ions from the xylem parenchyma cells into the xylem tracheary elements, rather than being due to simple leakage, is under tight metabolic control through regulation of the plasma membrane H^+-ATPase and ion efflux channels.

SUMMARY

The movement of molecules and ions from one location to another is known as transport. Plants exchange solutes and water with their environment and among their tissues and organs. Both local and long-distance transport processes in plants are controlled largely by cellular membranes.

Forces that drive biological transport, which include concentration gradients, electric-potential gradients, and hydrostatic pressures, are integrated by an expression called the electrochemical potential. Transport of solutes down a chemical gradient (e.g., by diffusion) is known as passive transport. Movement of solutes against a chemical-potential gradient is known as active transport and requires energy input.

The extent to which a membrane permits or restricts the movement of a substance is called membrane permeability. The permeability depends on the chemical properties of the particular solute and on the lipid composition of the membrane, as well as on the membrane proteins that facilitate the transport of specific substances.

When cations and anions move passively across a membrane at different rates, the electric potential that develops is called the diffusion potential. For each ion, the relationship between the voltage difference across the membrane and the distribution of the ion at equilibrium is described by the Nernst equation. The Nernst equation shows that at equilibrium the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. That voltage difference, or membrane potential, is seen in all living cells because of the asymmetric ion distributions between the inside and outside of the cells.

The electrical effects of different ions diffusing simultaneously across a cell membrane are summed by the Goldman equation. Electrogenic pumps, which carry out active transport and carry a net charge, change the membrane potential from the value created by diffusion.

Membranes contain specialized proteins—channels, carriers, and pumps—that facilitate solute transport. Channels are transport proteins that span the membrane, forming pores through which solutes diffuse down their gradient of electrochemical potentials. Carriers bind a solute on one side of the membrane and release it on the other side. Transport specificity is determined largely by the properties of channels and carriers.

A family of H^+-pumping ATPases provides the primary driving force for transport across the plasma membrane of plant cells. Two other kinds of electrogenic proton pumps serve this purpose at the tonoplast. Plant cells also have calcium-pumping ATPases that participate in the regulation of intracellular calcium concentrations, as well as ATP-binding cassette transporters that use the energy of ATP to transport large anionic molecules. The gradient of electrochemical potential generated by H^+ pumping is used to drive the transport of other substances in a process called secondary transport.

Genetic studies have revealed many genes, and their corresponding transport proteins, that account for the versatility of plant transport. Patch clamp electrophysiology provides unique information on ion channels, and it enables measurement of the permeability and gating of individual channel proteins.

Solutes move between cells either through the extracellular spaces (the apoplast) or from cytoplasm to cytoplasm (via the symplast). Cytoplasms of neighboring cells are connected by plasmodesmata, which facilitate symplastic transport. When an ion enters the root, it may be taken up into the cytoplasm of an epidermal cell, or it may diffuse through the apoplast into the root cortex and enter the symplast through a cortical cell. From the symplast, the ion is loaded into the xylem and transported to the shoot.

Web Material

Web Topics

6.1 Relating the Membrane Potential to the Distribution of Several Ions across the Membrane: The Goldman Equation

A brief explanation of the use of the Goldman equation to calculate the membrane permeability of more than one ion.

6.2 Patch Clamp Studies in Plant Cells

The electrophysiological method of patch clamping as applied to plant cells is described, with some specific examples.

6.3 Chemiosmosis in Action

The chemiosmotic theory explains how electrical and concentration gradients are used to perform cellular work.

6.4 Kinetic Analysis of Multiple Transporter Systems

Application of principles on enzyme kinetics to transport systems provides an effective way to characterize different carriers.

6.5 Transport Studies with Isolated Vacuoles and Membrane Vesicles

Certain experimental techniques enable the isolation of tonoplasts and plasma membranes for study.

6.6 ABC Transporters in Plants

ATP-binding cassette (ABC) transporters are a large family of active transport proteins energized directly by ATP.

Web Essay

6.1 Potassium Channels

Several plant K^+ channels have been characterized.

Chapter References

Barkla, B. J., and Pantoja, O. (1996) Physiology of ion transport across the tonoplast of higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 159–184.

Buchanan, B. B., Gruissem, W., and Jones, R. L., eds. (2000) *Biochemistry and Molecular Biology of Plants.* Amer. Soc. Plant Physiologists, Rockville, MD.

Bush, D. S. (1995) Calcium regulation in plant cells and its role in signaling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 46: 95–122.

Chrispeels, M. J., Crawford, N. M., and Schroeder, J. I. (1999) Proteins for transport of water and mineral nutrients across the membranes of plant cells. *Plant Cell* 11: 661–675.

Chung, W. S., Lee, S. H., Kim, J. C., Heo, W. D., Kim, M. C., Park, C. Y., Park, H. C., Lim, C. O., Kim, W. B., Harper, J. F., and Cho, M. J. (2000) Identification of a calmodulin-regulated soybean Ca^{2+}-ATPase (SCA1) that is located in the plasma membrane. *Plant Cell* 12: 1393–1407.

De Boer, A. H., and Wegner, L. H. (1997) Regulatory mechanisms of ion channels in xylem parenchyma cells. *J. Exp. Bot.* 48: 441–449.

Dunlop, J., and Bowling, D. J. F. (1971) The movement of ions to the xylem exudate of maize roots. *J. Exp. Bot.* 22: 453–464.

Ghoshroy, S., Lartey, R., Sheng, J., and Citovsky, V. (1997) Transport of proteins and nucleic acids through plasmodesmata. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 27–50.

Higinbotham, N., Etherton, B., and Foster, R. J. (1967) Mineral ion contents and cell transmembrane electropotentials of pea and oat seedling tissue. *Plant Physiol.* 42: 37–46.

Higinbotham, N., Graves, J. S., and Davis, R. F. (1970) Evidence for an electrogenic ion transport pump in cells of higher plants. *J. Membr. Biol.* 3: 210–222.

Hirshi, K. D., Zhen, R.-G., Rea, P. A., and Fink, G. R. (1996) CAX1, an H^+/Ca^{2+} antiporter from *Arabidopsis. Proc. Natl Acad. Sci. USA* 93: 8782–8786.

Kochian, L. V. (2000) Molecular physiology of mineral nutrient acquisition, transport and utilization. In *Biochemistry and Molecular Biology of Plants*, B. Buchanan, W. Gruissem, and R. Jones, eds., American Society of Plant Physiologists, Rockville, MD, pp. 1204–1249.

Kuehn, C., Barker, L., Buerkle, L., and Frommer, W. B. (1999) Update on sucrose transport in higher plants. *J. Exp. Bot.* 50: 935–953.

Leng, Q., Mercier, R. W., Hua, B-G., Fromm, H., and Berkowitz, G. A. (2002) Electrophysical analysis of cloned cyclic nucleotide-gated ion channels. *Plant Physiol.* 128: 400–410.

Lin, W., Schmitt, M. R., Hitz, W. D., and Giaquinta, R. T. (1984) Sugar transport into protoplasts isolated from developing soybean cotyledons. *Plant Physiol.* 75: 936–940.

Lüttge, U., and Higinbotham, N. (1979) *Transport in Plants.* Springer-Verlag, New York.

Lüttge, U., and Ratajczak, R. (1997) The physiology, biochemistry and molecular biology of the plant vacuolar ATPase. *Adv. Bot. Res.* 25: 253–296.

Maathuis, F. J. M., Ichida, A. M., Sanders, D., and Schroeder, J. I. (1997) Roles of higher plant K^+ channels. *Plant Physiol.* 114: 1141–1149.

Müller, M., Irkens-Kiesecker, U., Kramer, D., and Taiz, L. (1997) Purification and reconstitution of the vacuolar H^+-ATPases from lemon fruits and epicotyls. *J. Biol. Chem.* 272: 12762–12770.

Nobel, P. (1991) *Physicochemical and Environmental Plant Physiology*. Academic Press, San Diego, CA.

Palmgren, M. G. (2001) Plant plasma membrane H^+-ATPases: Powerhouses for nutrient uptake. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 817–845.

Rea, P. A., Li, Z-S., Lu, Y-P., and Drozdowicz, Y. M.(1998) From vacuolar Gs-X pumps to multispecific ABC transporters. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49: 727–760.

Small, J. (1946) *pH and Plants, an Introduction to Beginners.* D. Van Nostrand, New York.

Spanswick, R. M. (1981) Electrogenic ion pumps. *Annu. Rev. Plant Physiol.* 32: 267–289.

Sussman, M. R. (1994) Molecular analysis of proteins in the plant plasma membrane. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45: 211–234.

Tanner, W., and Caspari, T. (1996) Membrane transport carriers. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 595–626.

Tazawa, M., Shimmen, T., and Mimura, T. (1987) Membrane control in the *Characeae. Annu. Rev. Plant Phsyiol.* 38: 95–117.

Theodoulou, F. L. (2000) Plant ABC transporters. *Biochim. Biophys. Acta* 1465: 79–103.

Tyerman, S. D., Niemietz, C. M., and Bramley, H. (2002) Plant aquaporins: Multifunctional water and solute channels with expanding roles. *Plant Cell Envir.* 25: 173–194.

Vera-Estrella, R., Barkla, B. J., Higgins, V. J., and Blumwald, E. (1994) Plant defense response to fungal pathogens. Activation of host-plasma-membrane H^+-ATPase by elicitor-induced enzyme dephosphorylation. *Plant Physiol.* 104: 209–215.