11

TRANSMEMBRANE TRANSPORT OF IONS AND SMALL MOLECULES

REVIEW THE CONCEPTS

1. Like O_2 and CO_2, NO passively diffuses through membranes. As it is produced by an enzyme and accumulates in the endothelial cell cytosol, NO passively diffuses down its concentration gradient though the endothelial cell plasma membrane out of the cell and then passively diffuses through the plasma membrane into the cytoplasm of the smooth muscle cell, where it acts to decrease contraction.

2. Of the two at neutral pH, ethanol is the much more membrane permeant. It has no acidic or basic group and is uncharged at a pH of 7.0. The carboxyl group of acetic acid is predominantly dissociated at this pH and hence acetic acid exists predominantly as the negatively charged acetate anion. It is nonpermeant. At a pH of 1.0, ethanol remains uncharged and membrane permeant. The carboxyl group of acetic acid is now predominantly nondissociated and uncharged. Hence, acetic acid is now membrane permeant. Any difference in permeability is very small.

3. Uniporters are slower than channels because they mediate a more complicated process. The transported substrate both binds to the uniporter and elicits a conformational change in the transporter. A uniporter transports one substrate molecule at a time. In contrast, channel proteins form a protein-lined passageway through which multiple water molecules or ions move simultaneously, single file, at a rapid rate. The major contributor to the free-energy-driving transport through a uniporter is the entropy (ΔS) increase as a molecule moves from a high concentration to a low concentration.

4. The three classes of transporters are uniporters, symporters, and antiporters. Both symporters and antiporters are capable of moving organic molecules against an electrochemical gradient by coupling an energetically unfavorable movement to the energetically favorable movement of a small inorganic ion. The ΔG for bicarbonate has two terms, a concentration term and an electrical term, because bicarbonate is an anion. Glucose is neutrally charged and hence its ΔG for transport has only a concentration term. Unlike pumps, neither symporters nor antiporters hydrolyze ATP or any other molecule during transport. Hence, these cotransporters are better referred to as examples of secondary active transporters rather than as actual active transporters. The term active transporter is restricted to the ATP pumps where ATP is hydrolyzed in the transport process.

5. To be transported, a molecule must fit into the aquaporin channel and form hydrogen bonds with N-H groups of amino acids lining the channel. Although H^+ is smaller than H_2O, it cannot form the required hydrogen bonds. Glycerol is much larger than H_2O, but the three-carbon chain is flexible and the three OH groups can form the required hydrogen bonds.

6. Uniporters mediate substrate-specific facilitated diffusion. Uptake of glucose by GLUT1 exhibits Michaelis-Menten kinetics and approaches saturation as the concentration of glucose is increased. For any given substrate, GLUT1 displays a characteristic K_m at which concentration GLUT1 is transporting the substrate at 50% of V_{max}.
   a. Determination of the rate of erythrocyte (GLUT1) transport of substrate versus concentration allows the determination of K_m for glucose versus galactose versus mannose. The K_m for glucose will be lowest, indicating that GLUT1 is glucose-specific, not galactose- or mannose-specific.
   b. Despite its smaller size, ribose cannot bind to GLUT1 as glucose does because it cannot form the same noncovalent bonds—and thus cannot be transported.
   c. Using Equation 11-1, we find that at 5 mM, GLUT1-expressing cells transport glucose at 77% maximal rate, whereas at 2.8 mM, the cells transport at 65% maximal rate.
   d. Liver cells convert glucose to glycogen, which maximizes the glucose gradient across the plasma membrane.
   e. Tumor cells often express a higher number of glucose transporters than normal cells.
   f. When insulin is low, GLUT4 is stored in intracellular vesicles. Insulin induces a rapid increase in the V_{max} for glucose uptake by stimulating fusion of these vesicles with the plasma membrane, thereby increasing the number of plasma membrane glucose transporters.

7. The four classes of ATP-powered pumps are: P-class, V-class, F-class, and ABC superfamily. Only the ABC superfamily members transport small organic molecules. All other classes pump cations or protons. The initial discovery of ABC superfamily pumps came from the discovery of multidrug resistance to chemotherapy and the realization that ultimately this was due to transport proteins (i.e., ABC superfamily pumps). Today, the natural substrates of ABC superfamily pumps are thought to be small phospholipids, cholesterol, and other small molecules.

8. Direct hydrolysis of the phosphoanhydride bond would result in release of the bond energy as heat, which would thus be "lost." By firsttransferringthephosphate bond to an aspartate (D) residue, the P-class ATPase uses the released bond energy to drive a conformational change in the protein from the E1 to the E2 state.

9. A rise in cytosolic Ca^{2+} concentration causes activation of calmodulin. Some Ca^{2+}-ATPase pumps are activated by Ca^{2+}-calmodulin, which lowers the cyto-solic Ca^{2+} concentration by pumping Ca^{2+} either into the sarcoplasmic reticu-lum/endoplasmic reticulum or out of the cell. An anti-calmodulin drug would inhibit this negative feedback mechanism, leaving higher Ca^{2+} concentration in the cytosol for a longer period of time. In skeletal muscle cells, the result would be to prolong the length and/or strength of muscle contraction.

10. These drugs irreversibly inhibit the H^+/K^+ ATPase in the apical membrane of stomach parietal cells. Although the inhibition of a given H^+/K^+ ATPase is irreversible, the cells eventually make more of the pump.

11. Membrane potential refers to the voltage gradient across a biological membrane. The generation of this voltage gradient involves three fundamental elements: a membrane to separate charge, a Na^+/K^+ ATPase to achieve charge separation across the membrane, and nongated K^+ channels to selectively conduct current. The major ionic movement across the plasma membrane is that of K^+ from inside to outside the cell. Movement of K^+ outward, powered by the K^+ concentration gradient generated by Na^+/K^+ ATPase, leaves an excess of negative charges on the inside and creates an excess of positive charges on the outside of the membrane. Thus, an inside-negative membrane potential is generated. These potassium channels are referred to as resting K^+ channels. This is because these channels, although they alternate between an open and closed state, are not affected by membrane potential or by small signaling molecules. Their opening and closing are nonregulated; hence, the channels are called nongated. K^+ channels achieve selectivity for K^+, versus, say, Na^+, through coordination of the nonhydrated ion with carbonyl groups carried by amino acids within the channel protein. The ion enters the channel as a hydrated ion, the water of hydration is exchanged for interaction with carbonyl residues within the channel, and then as the ion exits the channel it is rehydrated. Within the confinesofthechannelproteinstructure, Na^+, unlike K^+, is too small to replace fully the interactions of water with those with amino acid–carried carbonyl groups. Because of this, the energetic situation is highly unfavorable for Na^+ versus K^+.

12. Expression of a channel protein in a normally nonexpressing cell permits the patch clamp assessment of channel properties. Typically, the cell used is a frog oocyte. Frog oocytes do not normally express plasma membrane channel proteins. Channel protein expression may be induced by microinjection of in vitro–transcribed mRNA encoding the protein. Frog oocytes are large and hence technically easier to inject and to patch clamp than other cells. To determine if the gene coding for putative K+ channel actually codes for a K+ or an Na+ channel, one can vary the composition of the medium and determine whether the ionic movement through the channel occurs in the presence of K+ (then the channel is a K+ channel) or Na+ (Na+ channel).

13. Plant cells, unlike animal cells, are surrounded by a cell wall. This cell wall is relatively stiff and rigid. The hyperosmotic situation within the plant vacuole that typically constitutes most of the volume of the plant cell is resisted by the rigid cell wall and the cell does not burst. Overall, a plant cell is considered to have a turgor pressure because of the hyperosmotic vacuole. The Na^+/K^+ ATPase is key to animal cells avoiding osmotic lysis. Animal cells have a slow inward leakage of ions. In the absence of a countervailing export, this would result in osmotic lysis of the cells even under isotonic conditions. The main countervailing export is the net transport of cations by Na^+/K^+ ATPase (3 Na^+ ions out for 2 K^+ in).

14. The six oxygens in the main-chain carbonyl or side-chain carboxyl groups that bind each of the two Na^+ ions in the symporter are exquisitely positioned with a geometry similar to that of the water molecules with which Na^+ associates in solution. At one site, the carboxyl group of the bound leucine provides one of the coordinating oxygens. When Na^+ ions bind to the oxygens, they lose their water of hydration. The increase in entropy that occurs when hydration water molecules are freed promotes Na^+ ions binding at both sites. K^+ ions (and water molecules themselves) are too big to bind the six oxygens in the proper geometry and so do not compete with Na^+.

15. Glucose uptake from the intestinal lumen into the epithelial cells is driven by symport with 2 Na^+ ions by a 2 Na^+/glucose symporter. Binding of two Na^+ ions and one glucose molecule to high-affinity , outward-facing sites in the protein causes a series of conformational changes in the symporter that eventually allows Na^+ and glucose to be released from low-affinitysitesfacingthecytosol. Transport by this symporter is energetically favorable because movement of Na^+ ions into the cell is driven by both its concentration gradient and the transmembrane voltage gradient. Transport of two Na^+ ions into the cell provides ~6 kcal of energy—enough to generate an intracellular glucose concentration that is 30,000 times higher than in the intestinal lumen.

16. The Na^+/K^+ ATPase located on the basolateral surface of intestinal epithelial cells uses energy from ATP to establish Na^+ and K^+ ion gradients across the intestinal epithelial cell plasma membrane. Cotransporters couple the energetically unfavorable movement of glucose and amino acids into epithelial cells to the energetically favorable movement of Na^+ into these cells. The accumulation of glucose and amino acids here is an important example of secondary active transport. Tight junctions are essential for the process because they seal the interstitial space between cells and hence allow the transport proteins in the apical and basolateral membranes of the epithelial cell to be effective. Effec-tive transport could not be achieved through a leaky cell layer. The coordinated transport of glucose and Na^+ ions across the intestinal epithelium creates a transepithelial osmotic gradient. This forces the movement of water from the intestinal lumen across the cell layer and hence promotes water absorption from sport drinks.