11 Transmembrane Transport of Ions and Small Molecules

Review the Concepts

1. 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.

2. 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.

3. 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.

4. 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}. 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. 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.

5. 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.

6. Total genome information allows the identification of the complete set of open reading frames within an organism. These may then be compared by sequence homology to known example transporters — for example, GLUT1 was characterized from erythrocyte plasma membranes. By homology, there are 12 proteins in the human genome that are highly similar in sequence. As the complete mouse genome is known, a similar homology grouping of proteins can be made. Several of the human GLUT family members remain "orphan" transporters as their natural substrate and physiology remain

unknown. In other words, they are members by homology, but that alone is not sufficient to establish their actual physiological roles. For example, GLUT5 transports fructose. Establishment of actual roles requires "hunt and peck" biochemical and physiological experiments. For example, Northern blotting of a DNA probe to mRNA from various tissues will establish in which tissue(s) a given GLUT family member is present, but nevertheless does not establish actual substrate or physiological importance. A series of different kinds of experiments is needed.

7. The P-class Ca^{2+} ATPase of the sarcoplasmic reticulum (SR) pumps Ca^{2+} ions from the cytosol into the SR. Hence it lowers the cytosolic Ca^{2+} concentration and induces muscle relaxation. Selective inhibition of this Ca^{2+} ATPase will prolong the period of elevated cytosolic Ca^{2+} associated with muscle contraction and hence prolong the length and/or strength of muscle contraction.

8. *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 confines of the channel protein structure, 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^+.

9. 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. One can then vary the ionic composition of the medium and determine whether the presence of Na^+ or of K^+ supports ionic movement through the channel.

10. 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).

11. The key feature that prevents other ions from binding the sodium-leucine transporter is that no water molecules surround either sodium ion. As Na^+ ions lose their water of hydration, when they bind the transporter, instead to bind oxygen atoms. This reduces the activation energy for binding Na ions, and thereby prevents K^+ ions from binding. Na^+ ions are responsible for transporting glucose from the intestinal lumen into the cell. Transport by this symporter is

possible because Na^+ ions are driven by its concentration gradient and by the transmembrane voltage gradient.

12. *Melanosome* is the name given to a melanin-containing vesicle. Immunofluorescence can be used to identify the expression and localization of a protein in cells and/or tissues. Basically, using the zebrafish as our model, skin samples would be chemically fixed or frozen and prepared for histology. Slices, called sections, are made through the tissue and affixed to glass microscope slides. The tissue is washed with physiological buffer and then incubated with an antibody prepared in mouse against SLC24A5, the protein encoded by the zebrafish *golden* gene. After a series of washes to remove unbound antibody, a goat or rabbit secondary antibody that recognizes mouse antibodies would be applied. This secondary antibody is labeled with a fluorochrome that fluoresces, emitting visible light when excited by ultraviolet light. Using a fluorescence microscope the investigator is able to see this visible light, which represents the signal where the original or primary antibody had detected SLC24A5 in the cell. In this case, the signal would be seen highlighting melanosomes. Using modifications and a slightly different approach with the electron microscope, the investigator could extend these results and examine the expression of SLC24A5 at the ultrastructural level. *Complementation* is the term used to describe how a phenotype arising from the expression of a mutant gene can be rescued by expressing an orthologous wild-type gene.

13. 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. Effective 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.

Analyze the Data

a. Transepithelial transport of glucose requires the cotransport of Na^+ down its concentration gradient to drive glucose against its concentration gradient from the apical medium into the cells. This coupling cannot occur when the concentration of Na^+ in the apical medium is low, as is the case for curve 2. Under these conditions, there would be no inward gradient of Na^+ across the apical membrane to drive glucose uptake. Subsequently, for the glucose to move from the cells into the basolateral medium, the glucose is transported down its concentration gradient and thus does not depend on Na^+ and its concentration in the basolateral medium. Accordingly, the concentration of Na^+ in the basolateral medium is not a factor.

b. In order for the epithelial cells to maintain a low Na^+ concentration, the Na^+ must be continually pumped out of the cells by the Na^+/K^+ ATPase. Ouabain inhibits this pump and thus would prevent the cells from keeping their cytosolic $[Na^+]$ low. If Na^+ cannot be pumped out, then the Na^+ gradient driving glucose transport into the cells would dissipate, and glucose transport into the cells would cease. The observation that glucose transport ceases only when ouabain is added to the basolateral medium suggests that the Na^+/K^+ ATPase is localized at and/or only functions in this membrane domain.

c. The affinity of Glut1 for glucose is much higher than is that of Glut2. Accordingly, Glut1 will facilitate glucose transport out of the cells at lower internal glucose concentrations which, in turn, will result in a lower steady state glucose concentration in cells expressing Glut 1 versus Glut2. That concentration may be too low to

meet the energy demands of these epithelial cells, thereby affecting their survival.