Diffusion and Transport Across Cell Membranes

(Lecture)

OBJECTIVES

- List the general mechanisms by which molecules cross membranes. For each, give a specific example of a that would cross membranes by that mechanism.
- List the two properties that determine how well a substance can diffuse across a membrane.
- Calculate the fractions of a weak acid or weak base that are charged and uncharged in a given medium, given the drug's pKa and the pH of the medium. If the drug partitions across multiple compartments with different pHs, calculate the amount of drug in each compartment.
- Explain how a drug can become trapped in a body fluid that has a pH different than the blood.
- Compare and contrast transport mediated by channel proteins versus carrier proteins.
- List the forms of energy used by carrier proteins to mediate active transport.
- Describe the differences in the concentrations of Na^+, K^+, Ca^{2+} and Cl^- on the outside of typical plasma membranes of eukaryotic cells versus the inside.
- Explain how the gradients of Na^+, K^+ and Ca^{2+} are maintained.
- Explain the influence of the membrane potential on the electrochemical gradients of Na^+, K^+ and Ca^{2+} and Cl^- across membranes.
- Explain how the Na^+/K^+-ATPase operates. Describe the consequences of inhibition of the Na^+/K^+-ATPase.
- Compare and contrast symport and antiport. Name a symporter and an antiporter and explain how each of them functions.
- Explain the contribution of P-glycoprotein to the blood-brain barrier.
- Name three ways in which an ion channel can be gated.

KEY WORDS

ABC transporter
active transport
antiport
ATP-driven pump
blood-brain barrier
carrier proteins
channel proteins
concentration gradient
coupled transporter
diffusion
electrochemical gradient
electrochemical gradient
Henderson-Hasselbach equation
membrane transport protein
Na^+/ K^+ -ATPase
nicotinic acetylcholine receptor (nAchR) channel
passive transport
P-glycoprotein
pKa
symport
weak acid/weak base

SUPPLEMENTAL READING

Boron and Boulpaep, *Medical Physiology*, Saunders, 2003. pp. 56-71. (This section is detailed, but contains useful descriptions of nearly every type of transport protein you will encounter in other blocks.)

Alberts et al., *Essential Cell Biology*, Garland Publishing, 1998, pp. 371-406.

INTRODUCTION

An important function of a biological membrane is to serve as a barrier to the outside world. However, membranes are not impenetrable walls. Obviously, nutrients must enter the cell and waste products have to leave in order for the cell to survive. For this and many other reasons, it is crucial that membranes be *selectively permeable*. For example, the movement of ions across membranes is important in regulating vital cell characteristics such as cellular pH and osmotic pressure. Membrane permeability is also a key determinant in the effectiveness of drug absorption, distribution, and elimination. For example, a drug taken orally that targets cells in the central nervous system must cross several membranes: first the barrier presented by the intestinal epithelium, then the walls of the capillaries that perfuse the gut, then the blood-brain barrier. Some endogenous substances and many drugs easily diffuse across the lipid bilayer. However, the lipid bilayer presents a formidable barrier to larger and more hydrophilic molecules (such as ions). These substances must be transported across the membrane by special proteins. We will first look briefly at the three major ways that both endogenous substances and drugs cross the barriers presented by cell membranes. We will then discuss in more detail two of these mechanisms, which are the primary ways that drugs cross membranes.

I. MECHANISMS FOR GETTING IN AND OUT OF CELLS

A. Diffusion across the lipid bilayer. Since membranes are held together by weak forces, certain molecules can slip between the lipids in the bilayer and cross from one side to the other. This spontaneous process is termed **diffusion** (you may also see the term *lipid diffusion* used) (Fig. 1). This process allows molecules that are small and lipophilic (lipid-soluble), including most drugs, to easily enter and exit cells. More on this later.

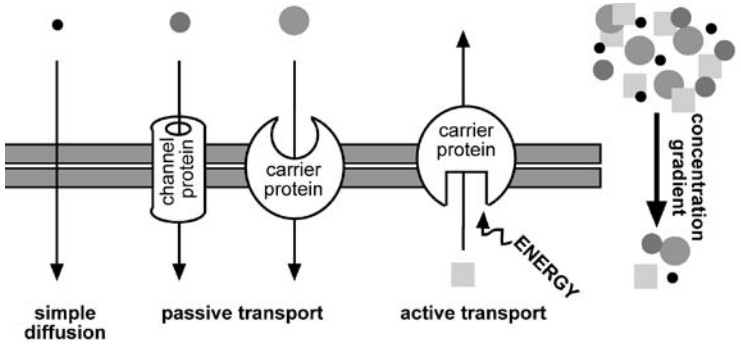

Figure 1. *Types of movement across membranes. Molecules can diffuse across a membrane down a concentration gradient without the aid of a protein or the input of energy. Passive and active transport require membrane transport proteins. Channel proteins carry out passive transport, but carrier proteins can carry out passive or active transport. Active transport, or movement against a concentration gradient requires*

A note on gradients - molecules are driven down their concentration gradients: The difference in the concentration of a molecule on one side of a membrane versus the other is called a gradient. A molecule's **concentration gradient** (also called the **chemical gradient**) drives movement across the membrane until the molecule is at equilibrium. Movement from a high concentration to a low concentration is also referred to as movement "with" or "in the direction of" the concentration gradient or "downhill." Movement from a low concentration to a high concentration is also referred to as "against" the concentration gradient or "uphill."

B. Protein-mediated transport. In order to cross the hydrophobic interior of the bilayer, water-soluble molecules (those that are either charged or have polar groups) and large molecules require the action of **membrane transport proteins.** These integral membrane proteins provide a continuous protein-lined pathway through the bilayer. There are two classes of membrane transport proteins that we will discuss: **carrier proteins**, which literally carry specific molecules across, and **channel proteins**, which form a narrow pore through which ions can pass (Fig. 1). Channel proteins carry out **passive transport,** in which ions travel spontaneously down their gradients. Some carrier proteins mediate passive transport (also called *facilitated diffusion*), while others can be coupled to a source of energy to carry out **active transport**, in which a molecule is transported *against* its concentration gradient (Fig. 1). Membrane transport proteins are important pharmacologically for two reasons. First, some drugs exploit endogenous membrane transport processes to enter or exit cells. Second, membrane transport proteins are major drug targets (discussed in the section on Signaling).

C. Endocytosis/exocytosis. Large macromolecules (e.g., proteins, viruses, lipoprotein particles) require more complex mechanisms to traverse membranes, and are transported into and out of cells selectively via *endocytosis* and *exocytosis* (secretion). Interestingly, endocytosis and exocytosis are not only important for the import/export of large molecules. Often, essential small molecules that are hydrophobic or toxic (e.g., iron) travel through the bloodstream bound to proteins, which enter and exit cells via these mechanisms. Receptor-mediated endocytosis will be discussed in more detail in the Organs block.

We will now focus on lipid diffusion and protein-mediated transport. These mechanisms are the key means by which drugs travel into and out of cells. These processes are critical for movement of endogenous molecules (ions, sugars, amino acids, etc.) across membranes as well.

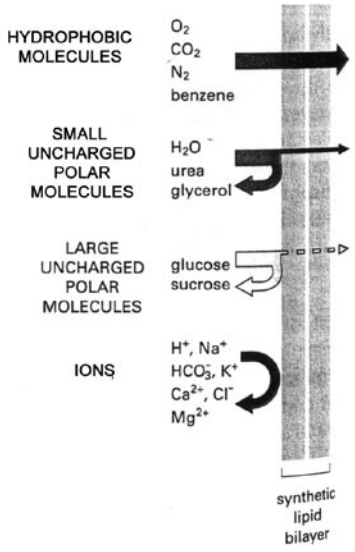

Figure 2. *The relative permeability of a lipid bilayer to different classes of molecules. The smaller the molecule and, more importantly, the less strongly it associates with water, the more rapidly the molecule diffuses across the bilayer. (From Alberts et al. Molecular Biology of the Cell, 4th ed, Garland Publ, 2002, Fig 11-1, p.616.)*

II. LIPID DIFFUSION: THE MAJOR MEANS OF DRUG ABSORPTION AND PERMEATION

The rate at which a molecule diffuses across a membrane depends on its size and its degree of hydrophobicity (Fig. 2). Hydrophobic substances such as gases and steroid hormones diffuse across membranes easily. Due to the fact that they are repelled by the hydrophobic interior of the bilayer, polar molecules do not diffuse across the bilayer as easily, unless they are very small and uncharged (e.g., water and EtOH). Lipid bilayers are much less permeable to larger polar molecules, and are virtually impermeable to ions, which are surrounded by a cage of water. (Note that the reference is to experimentally generated lipid bilayers here--not membranes. Biological membranes contain proteins that effectively render them

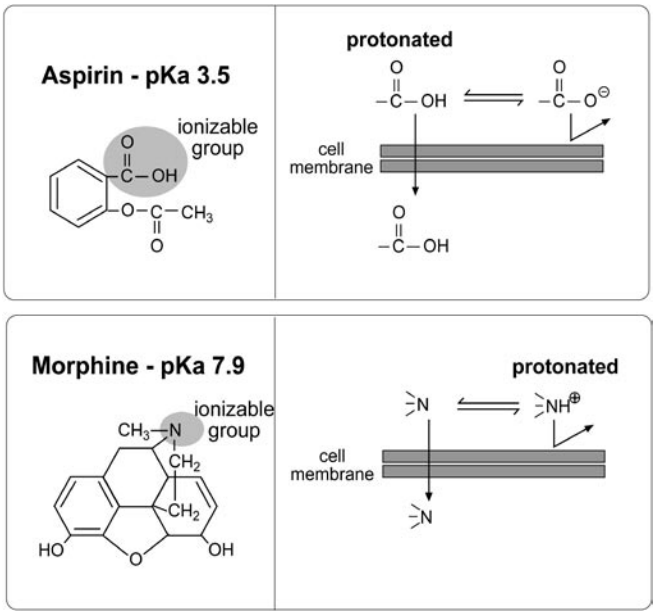

Figure 3. *Ionization of weak acids and bases. Weak acids and bases have groups that can donate or accept protons. Weak acids, such as aspirin, donate a proton to form anions. Weak bases, such as morphine, accept a proton to form cations. Only the fraction of the drug that is uncharged can diffuse across membranes (see right of each panel--only the ionizable portion of each drug is shown). The extent of ionization at a given pH is determined by the drug's pKa. For example, at a pH of less than 3.5, the uncharged form of aspirin predominates and therefore the majority of the drug can diffuse across membranes.*

permeable to polar molecules and ions, which is discussed below.) All in all, relatively few kinds of endogenous molecules cross membranes by lipid diffusion. However, most drugs are small amphipathic compounds, and therefore rely heavily on lipid diffusion to move through different compartments in the body. The ability of drugs to diffuse across membranes is heavily influenced by the ionizability of the drug in the surrounding medium.

The pH of body compartments influences lipid diffusion of drugs. A large fraction of drugs are **weak acids** or **weak bases**. Drugs that are weak acids or weak bases exist in either charged (ionized) or uncharged (nonionized) forms (Fig. 3). The ratio of the charged to uncharged form depends on the drug's **pKa**, and the pH of the environment. (Recall that the pKa is the pH at which 50% of a given substance is protonated.) Weak acids are neutral molecules that can dissociate from a proton to form an anion (e.g., aspirin). Weak bases are defined as neutral molecules that can form a cation when protonated (e.g., morphine). Since diffusion across a lipid bilayer requires that a drug be lipid-soluble, the ionized form of a drug cannot cross membranes. Thus, weak acids that are nonprotonated and weak bases that are protonated cannot diffuse across membranes. At a pH that is equal to a drug's pKa, equal amounts of the protonated and nonprotonated forms are present. Assuming the pH is the same on both sides of a given membrane, the drug will be at equilibrium across the membrane. If the pH is less than the pKa (such that there are excess protons available), the protonated form of a drug predominates. Thus weak acids exposed to a low pH environment are favored to diffuse across membranes, while weak bases are not. The opposite is true at a higher pH.

The Henderson-Hasselbalch equation reveals the amount of drug that is lipid-soluble in a given medium. For a drug that is a weak acid or base, the ratio of the protonated form to the nonprotonated form in a particular bodily fluid can be calculated using the Henderson-Hasselbalch equation if the pKa of the drug and the pH of the fluid are known. The equation is:

$$\log \frac{[\text{protonated form}]}{[\text{nonprotonated form}]} = \text{pKa} - \text{pH}$$

For a weak acid, the protonated form is abbreviated as 'HA,' and the nonprotonated form is 'A-'. For a weak base, the protonated form is abbreviated as 'HB+', and the nonprotonated form is 'B'. Morphine is a weak base with a pKa of 7.9. Thus in the plasma, which has a pH of 7.4, log [HB+]/[B] = 0.5. Therefore, [HB+]/[B] = 3.2/1. Approximately 25% of the total amount of morphine present in the blood can diffuse across membranes to enter or exit cells.

Body fluids can "trap" drugs. Drugs travel through the body primarily in the blood. Other body fluids, however, have significant pH differences from the blood (e.g., urine pH is around 6.0, breast milk pH is around 7.0, stomach lumen pH is around 2.0). The extent of ionization when exposed to a new pH determines how a drug partitions between the two compartments. Take aspirin as an example (Fig. 4). In the blood, [HA]/[A-] of aspirin = 1/7692. In the urine, which typically has a pH of around 6.0, [HA]/[A-] of aspirin = 1/333. The uncharged form of aspirin can freely diffuse across the membrane, but the majority of the drug in both the blood and the urine is ionized. In this example, the total

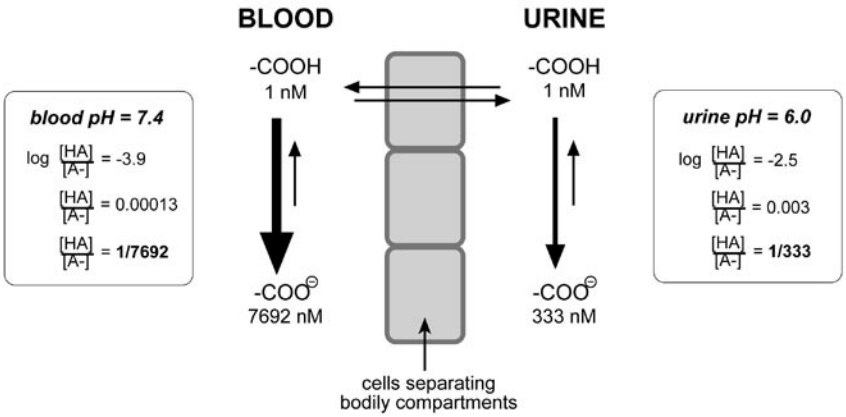

Figure 4. *Partitioning of aspirin into different body fluids. The diffusible uncharged form (only the ionizable group is shown) of aspirin has equilibrated across the cells separating the blood from the urine. But the total concentration of aspirin in the blood is 23 fold higher than in the urine due to more extensive ionization at the more basic pH.*

concentration of aspirin in the blood is 23 times higher than in the urine. Differences in pH across membranes can result in "trapping" of a drug in a body fluid, a phenomenon that can be used therapeutically. For example, in the case of an overdose with a weak acid like aspirin, alkalinization of the urine (by administering sodium bicarbonate intravenously) increases the drug's excretion by trapping it in the more basic environment. If you do the calculation, you will see that increasing the pH of the urine to just 6.3 doubles the extent of ionization in the urine, thereby trapping much more of the drug there. Note, however, that alkalinization or acidification of the urine is not without risk to the patient, and the benefit of these procedures has only been established for a few drugs.

III. MEMBRANE TRANSPORT PROTEINS: CARRIERS AND CHANNELS

As stated above, there are two major classes of membrane transport proteins: carrier proteins and channels. Membrane transport proteins can be classified further by whether they mediate active or passive transport (Fig. 5). Channel proteins, most of which transport ions, open to make a hole in the membrane,

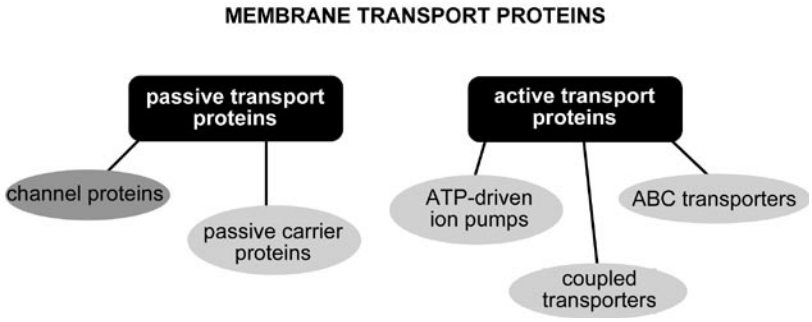

Figure 5. *Classification of the types of membrane transport proteins. Membrane transport proteins can be classified by whether they mediate active or passive transport. Membrane transport proteins are either channel proteins or carrier proteins. Here, carrier proteins are shown in light gray.*

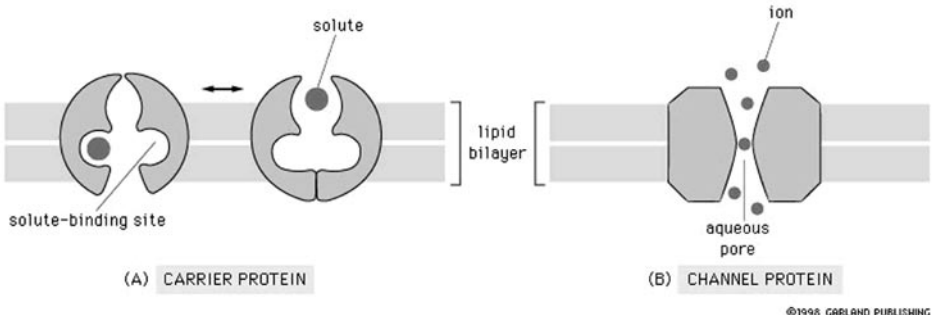

Figure 6. *Carrier proteins and channel proteins. (A) A carrier protein alternates between two conformations, so that the solute-binding site is sequentially accessible on one side of the bilayer and then on the other. (B) In contrast, a channel protein forms a water-filled pore across the bilayer through which specific solutes can diffuse. (From Alberts et al. Essential Cell Biology, Garland Publ, Fig 12-2, p.372, 1998.)*

through which ions can diffuse down their gradients. Channels serve simply as a gateway through a membrane—there are no highly specific interactions that take place between the protein and ions passing through it. In contrast, each carrier protein (also called *permeases* or simply *carriers*) actually binds to a specific molecule and physically carries it across the membrane via a conformational change (Fig. 6). Consequently, carriers are considerably slower than channels. Some drugs resemble endogenous ligands for carriers, and are at least partly absorbed across membranes by these systems. Carriers can be linked to a source of energy to drive active transport, which we will look at first. We will then turn our attention to carrier proteins that mediate passive transport, and channels.

A. Active carriers

Active transport of solutes against their gradients is important for, among other things, maintaining the balance of ions across membranes (discussed below), concentrating metabolites in certain organs or cellular compartments, and exporting foreign substances from cells. We will discuss three types of active transporters: 1) *ATP-driven ion pumps*, 2) *coupled transporters*, and 3) *ABC transporters*. Transport of a molecule against its gradient is energetically unfavorable, and therefore requires that the transporter harness an energy source (Fig. 7). ATP-driven ion pumps and ABC transporters utilize ATP hydrolysis to power uphill transport. Coupled transporters are driven by the energy stored in ion gradients.

1. ATP-driven ion pumps

ATP-driven ion pumps utilize the energy liberated by ATP hydrolysis to move ions across membranes, against their gradients. These proteins maintain ion gradients across both the plasma membrane and intracellular membranes.

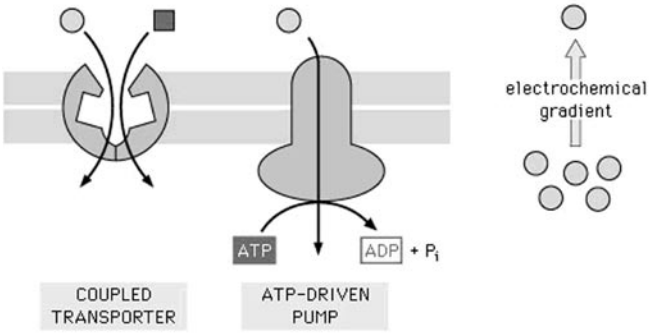

Figure 7. *Energy sources for active transport. Two sources of energy are used by eukaryotic active carrier proteins to transport molecules against the electrochemical gradient. Coupled transporters use the free energy released by the flow of a molecule down its gradient (depicted here as a dark square) to drive uphill transport. ATP-driven pumps use ATP hydrolysis to power uphill transport. (From Alberts et al. Essential Cell Biology, Garland Publ, Fig 12-8, p.377, 1998.)*

Another note on gradients – ion gradients must be actively maintained: Before we examine how ion pumps work, it is useful to understand the special nature of ion gradients, and why their maintenance is critically important to the cell. The internal ion composition of a cell is very different from that of the extracellular fluid, and the maintenance of these gradients is crucial for the viability of a cell. There are four ion gradients that we need to be concerned with: those of sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), and chloride (Cl^-) (Fig. 8). The concentration of Na^+ is higher outside of a cell than on the inside. The constant active transport of Na^+ out of the cell maintains this concentration gradient, which helps to balance osmotic pressure on either side of the membrane. If Na^+ were not actively transported out of the cell, water would rush in to dilute the intracellular contents, and the cell would eventually burst. Ca^{2+}, and Cl^- concentrations are also higher outside the cell than inside. The K^+ gradient is the opposite—the intracellular concentration of K^+ is higher than the extracellular concentration.

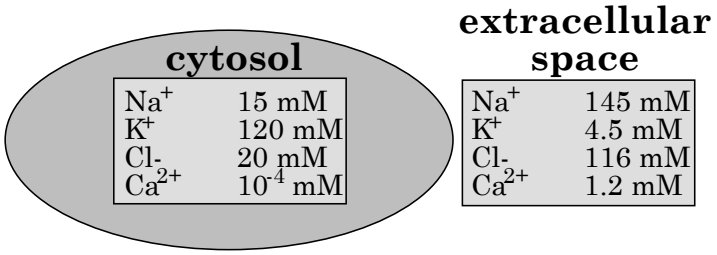

Figure 8. *Typical concentration gradients of cellular ions across the plasma membrane. The physiological concentrations of Na^+, Ca^{2+} and Cl^- are greater outside the cell while the concentration of K^+ is greater inside the cell.*

More on gradients-– ion gradients are influenced by membrane potential: In the case of charged molecules, movement across a membrane is influenced by the molecule's concentration gradient, and by the fact that the interior of the plasma membrane is negatively charged relative to the extracellular side of the membrane. This difference in charge across the membrane is referred to as *membrane potential.* The inside-negative membrane potential exerts a force on any molecule carrying an electrical charge, which determines an **electrical gradient** (or electrical energy difference) for that molecule. The effects of the electrical and concentration gradients of a molecule are combined in what is called the **electrochemical gradient** (Fig. 9). In the case of K^+ and Cl^-, the electrical and concentration gradients of molecules work against each other. K^+ is attracted toward the inside of a cell because of the inside-negative membrane potential, but the molecule's concentration gradient works in the other direction. Thus, K^+ comes almost to equilibrium across the plasma membrane though it tends to move out of cells when given the opportunity. Cl^- also tends move out of cells, despite its higher extracellular concentration, because of the inside-negative membrane potential. With Na^+ (and to a lesser degree Ca^{2+}), the electrical and chemical gradients work in the same direction: molecules are driven into the cell down the chemical gradient, and the membrane potential also pulls Na^+ into the cell. Simply stated, Na^+ enters cells whenever it can, because there is a large electrochemical force driving it to do so. This driving force can be harnessed by coupled transporters to power active transport of other molecules (discussed below).

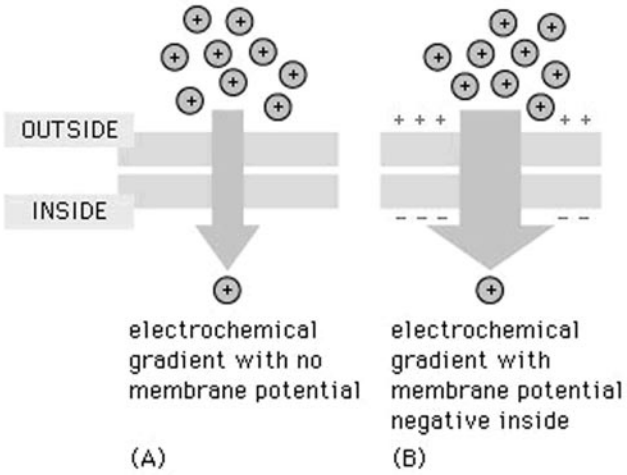

Figure 9. *Two forces at work in an electrochemical gradient. An electrochemical gradient combines the membrane potential and the concentration gradient, which can work additively to increase the driving force on an ion across the membrane (right), as is the case with Na^+, or can work against each other, as is the case with K^+ (not shown). (From Alberts et al. Molecular Biology of the Cell, 4th ed, Garland Publ, 2002, Fig 11-4, p.618.)*

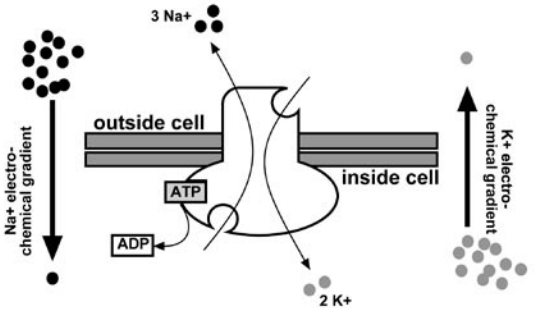

Figure 10. *The Na^+/K^+-ATPase. ATP hydrolysis is coupled to the transport of Na^+ and K^+ uphill against their electrochemical gradients. For every ATP hydrolyzed, three molecules of Na^+ are exported and two molecules of K^+ are imported.*

The best-understood and perhaps the most important example of an ion pump is the **Na^+/ K^+-ATPase**, which is also called the **Na^+/ K^+ pump** (Fig. 10). This pump is an enzyme embedded in the plasma membrane that hydrolyzes ATP so that Na^+ and K^+ can be transported against their concentration gradients. As stated above, if Na^+ were not continually pumped out, the gradient would rapidly be lost, and cells would swell and burst. For this reason, the Na^+/ K^+ ATPase is in continual operation: more than a third of the ATP consumed by a resting animal cell is used by this pump. The Na^+/K^+ pump brings two K^+ ions in and three Na^+ ions out for every molecule of ATP hydrolyzed. An important feature of this mechanism is that ATP hydrolysis and ion transport are tightly coupled—ATP is not hydrolyzed unless the ions are transported. This

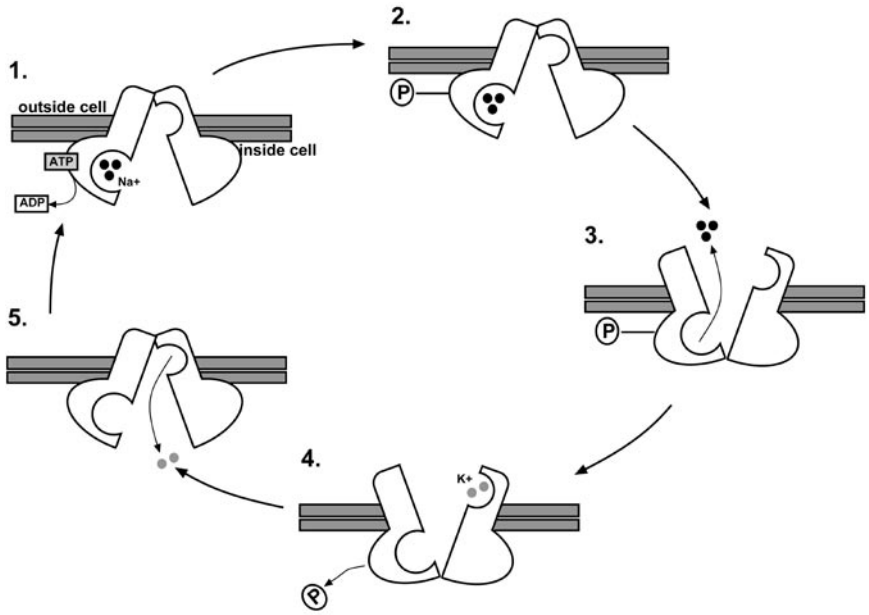

Figure 11. *A schematic model of the Na^+/K^+ ATPase pumping cycle. (1) First, three molecules of Na^+ bind at the binding sites on the intracellular side of the cell. ATP is hydrolyzed subsequently, and (2) the carrier is phosphorylated. (3) A conformational change results in the release of Na^+ to the outside of the cell. (2) Two molecules of K^+ bind to the carrier, and the phosphate linkage is cleaved. (5) The carrier undergoes a conformational change and K^+ is released to the inside of the cell. The cycle can then begin again.*

way, ATP will not be hydrolyzed unnecessarily and wasted. The action of the Na^+/K^+ pump is thought to follow a series of steps (Fig. 11). First, Na^+ and ATP bind the carrier protein. There are three binding sites for Na^+ on the intracellular side of the carrier. Next, ATP is hydrolyzed by the ATPase part of the carrier. As a result, ADP is released and the internal side of the carrier is phosphorylated. Subsequently, a conformational change causes the carrier to transfer Na^+ across the membrane and release it. Two molecules of K^+ then bind to the extracellular surface. Then, the carrier is dephosphorylated and returns to its original conformation, which transfers the K^+ across the membrane. When K^+ is released into the cytosol, the carrier is ready to start the cycle again. The widely used digitalis family of drugs (e.g. digoxin) inhibits this pump by binding to the K^+ binding sites on the outside surface.

There are other examples of ion pumps that operate in much the same way as the Na^+/K^+-ATPase. A *Ca^{2+}-ATPase* is located in the membrane of the sarcoplasmic reticulum (SR), a specialized version of the ER located in muscle cells. In this case, for every ATP hydrolyzed, two Ca^{2+} ions are pumped out of the cytosol and into the SR. This sequesters Ca^{2+} in the SR and maintains the Ca^{2+} gradient, which is important for muscle contraction (discussed below) and other cellular functions. *Proton pumps* are another important example of ATP-driven carrier proteins. These are important for many cellular events, including acidification of intracellular compartments. You will learn about the action of the *H^+/K^+-ATPase*, a pump present on cells in the stomach, in your first Biochemistry/ Pharmacology small group discussion.

2. **Coupled Transporters**

Coupled transporters use the energy stored in ion gradients to actively transport molecules across membranes (Fig. 12). Because they rely on the gradients generated by ion pumps, coupled transporters are also known as *secondary active transporters*. If the carrier protein transports two solutes in the same direction, this is called **symport**. **Antiport** occurs when an ion traverses the membrane in one direction and another metabolite is transported in the other direction. Most often, the source of energy for coupled transporters is the flow of Na^+ down its electrochemical gradient (which, you recall, is generated by the Na^+/K^+ pump). There are also some coupled transporters that use proton gradients to drive uphill transport, for example the uptake of neurotransmitter into synaptic vesicles. There are many well-understood and physiologically important coupled transporters. A few are discussed below.

Symport. The *Na^+-driven glucose pump* on the apical side of intestinal epithelial cells is an example of a symporter. This protein assures that glucose can be transported into gut epithelial cells after a meal even when the concentration of sugar is higher inside the cells than out. This cotransporter relies on energy from the Na^+ gradient, which flows into the cell down its gradient, to drive the transport of glucose into the cell. You will learn more about this protein in the lecture on Epithelia.

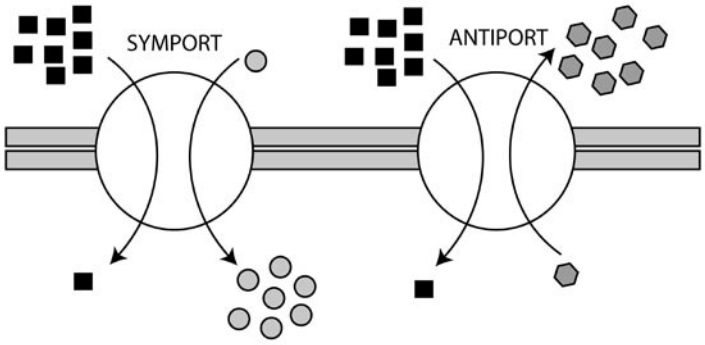

Figure 12. The act*ion of coupled transporters. Coupled transporters transport two molecules across membranes simultaneously. A symporter (shown at the left) transports two molecules in the same direction, and an antiporter (shown at the right) transports two molecules in opposite directions. Both symporters and antiporters use the energy stored in an ion gradient (represented here by black squares) to power uphill transport of another molecule.*

The *K^+/Cl^- coupled transporter*, present on some cells, plays a role in keeping intracellular concentrations of Cl^- low. Movement of K^+ down its concentration gradient promotes Cl^- efflux through this transporter, against its outwardly directed gradient.

Antiport. The *Na^+/Ca^{2+} exchanger* also uses the electrochemical gradient of Na^+ to drive transport of another molecule. In this case, Na^+ flows down its gradient into the cell, which allows Ca^{2+} to be exported from the cell against its gradient. Three Na^+ molecules enter the cell for every Ca^{2+} that exits. Of course, the Na^+/K^+ pump works to pump the Na^+ back out of the cell, maintaining the electrochemical gradient. As mentioned earlier, digoxin is a drug that inhibits the Na^+/K^+-ATPase. But the effects of digoxin are attributed to an inability of the Na^+/Ca^{2+} exchanger to effectively remove Ca^{2+} from cardiac cells. Can you suggest why?

3. **ABC transporters**.

The final type of active carrier we will look at is the **ABC transporter** (ATP-binding cassette) superfamily. ABC transporters all have a similar structure, consisting of two ATP binding domains facing the cytosol and two transmembrane domains (Fig. 13). Similar to the situation seen with ATP-driven ion pumps, the binding and hydrolysis of ATP by ABC transporters is thought to drive conformational changes that transport molecules across the membrane. But while ion pumps transport ions in or out of cells, most ABC transporters in eukaryotes are specialized for pumping small compounds <u>out</u> of cells (these proteins are sometimes referred to as *efflux pumps*). In general, ABC transporters seem to be crucial for getting foreign substances (drugs and other toxins) out of cells, making them extremely important clinically.

Multidrug resistance in cancers. One of the first eukaryotic ABC transporters identified, called **P-glycoprotein,** is responsible for pumping hydrophobic drugs out of the cytoplasm (recall that most drugs

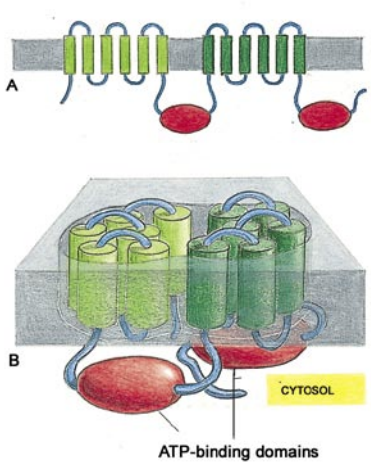

Figure 13. *A typical ABC transporter. (A) A diagram of the topology of the protein. (B) A hypothetical arrangement of the polypeptide chain in the membrane. Six putative membrane-spanning segments form the pathway through which substances are transported. Two ATP-binding cassettes are located within the cytosol. (From Alberts et al,. Molecular Biology of the Cell, 4th ed., Garland Publ, 2002, Fig 11-19, p.630.)*

are small and lipophilic, and can easily diffuse into cells). Often, P-glycoprotein (P-gp) is found to be overexpressed in human cancer cells. The presence of large amounts of this protein makes the cells resistant to a variety of drugs used in cancer chemotherapy, a phenomenon known as multi-drug resistance. (P-gp is also commonly known as the *multidrug resistance (MDR) protein*.) This demonstrates that P-gp can transport a variety of compounds with very different chemical structures, which makes it very unusual compared to other carrier proteins. Clinical trials are underway using drugs (e.g. verapamil) that inhibit P-gp for treatment of certain types of highly drug-resistant cancers.

The blood-brain barrier. Another reason that ABC transporters are important medically is for their contribution to the **blood-brain barrier**. It has been observed that many drugs are not efficiently delivered to the brain, despite the fact that the drugs are hydrophobic enough to diffuse through the membranes. It is thought that ABC transporters present in the plasma membranes of brain capillary endothelial cells pump a wide range of drugs back out into the blood. ABC transporters are also expressed in other tissues (e.g. reproductive tissues of intestinal epithelial cells), and are thought to protect them from foreign organic compounds by a similar mechanism. Recall that carriers bind to molecules in a specific manner. It is unclear at this point how P-gp specifically binds to drugs that are structurally unrelated, although it has been postulated that P-gp contains at least two distinct recognition sites. One goal in this field is to better understand P-gp's binding and transport properties in order to alter the permeability of the blood-brain barrier to drugs.

B. Passive carriers

Passive carrier proteins facilitate the downhill transport of substances across membranes. An example of a carrier protein that carries out passive transport is the glucose transporter, which is located in the plasma membrane of all mammalian cell types. On liver cells the carrier can be open toward the outside of a cell or toward the inside (Fig. 14). The glucose transporter can carry glucose in either direction, depending on the direction of the concentration gradient. For example, just after a meal, glucose levels

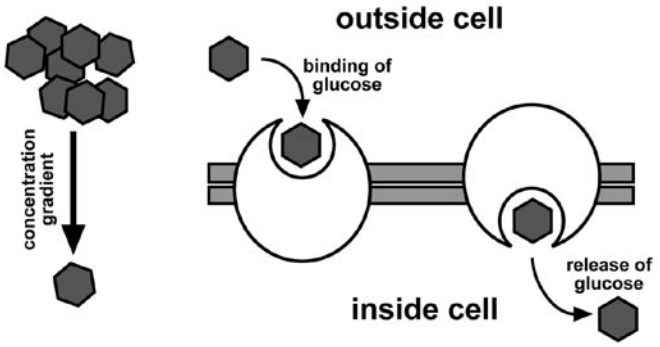

Figure 14. *Passive transport by the glucose carrier. This schematic represents the concentration gradient of glucose just after a meal. Glucose is abundant in the blood, outside of the cells. The glucose carrier can bind to glucose by opening toward the outside of the cell. By undergoing a conformational change, glucose is transported across the membrane and can be released inside the cell. If glucose levels are higher inside the cell, transport can take place in the other direction.*

are high in the blood. The glucose transporter opens toward the outside of the cell and shuttles glucose across the membrane by undergoing a conformational change that opens the carrier toward the inside of the cell. Glucose is then released into the cells, where the concentration is lower. During fasting, blood sugar is low, and the transporter moves glucose from the liver out to the blood. This transporter is also found on the basolateral side of mammalian epithelial cells, which contributes to *transcellular transport* of absorbed solutes. (You will learn more about transcellular transport in the lecture on Epithelia.)

C. Ion channels

Lipid bilayers on their own are virtually impermeable to charged molecules. However, membranes contain channel proteins, which form protein-lined passageways through the membrane and facilitate the trafficking of hydrophilic and charged molecules. Transport through channels does not require an additional input of energy. Channels have an advantage over carrier proteins in terms of the speed of transport—up to a hundred million ions can pass through an ion channel per second (which is 100,000 times greater than any measured rate of transport via a carrier protein). Regulation of this transport is key for all cells. Specifically, ion channels contribute to the electrical excitability of muscle cells and signaling in the nervous system, which will be described in detail in the Signaling and Muscle/Nerve lectures. While drugs do not typically rely on ion channels for transport (the channel opening is too small—see below), many drugs use ion channels as receptors, and therefore regulate ion channel-mediated processes. The **nicotinic acetylcholine receptor (nAchR) channel** is a well-understood example that demonstrates the principles of ion channels. One of the places where the nAchR channel is found is at the neuromuscular junction--the specialized chemical synapse between a motor neuron and a skeletal muscle cell (Fig. 15). When the nAchR channel binds to acetylcholine, a neurotransmitter, the channel opens. The opening of the nAchR channel results in a large net influx of Na^+ down its electrochemical

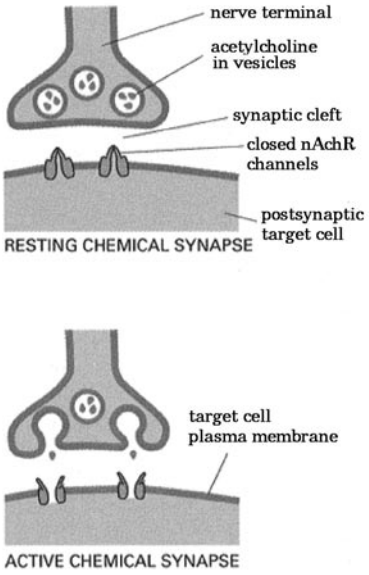

Figure 15. *Schematic of nAchR channel at the neuromuscular junction. At a synapse such as the neuromuscular junction, the presynaptic cell (or nerve terminal) is separated from the postsynaptic cell (in this case a muscle cell) by a narrow synaptic cleft. When the synapse is at rest, the nAchR channel is closed. A change in electrical potential causes release of acetylcholine from synaptic vesicles in the presynaptic cell. Acetylcholine binds the nAchR channel and causes it to open, thereby transmitting a signal from the excited nerve which ultimately results in muscle contraction. (Adapted from Alberts et al., Molecular Biology of the Cell, 4th ed, Garland Publ, 2002, Fig 11-33, p. 645).*

gradient. This influx causes a membrane depolarization that signals the muscle to contract. (You will learn more about membrane depolarization in the Muscle/Nerve lectures.)

Ion channels: general structural characteristics. Ion channels are composed of multiple protein subunits or protein domains that traverse the lipid bilayer (Fig. 16). Most ion channels are formed from four to six subunits, each comprised of membrane-spanning alpha-helices. The subunits associate in a circular formation, resulting in a central hydrophilic pore perpendicular to the membrane. The nAchR channel consists of five similar protein subunits.

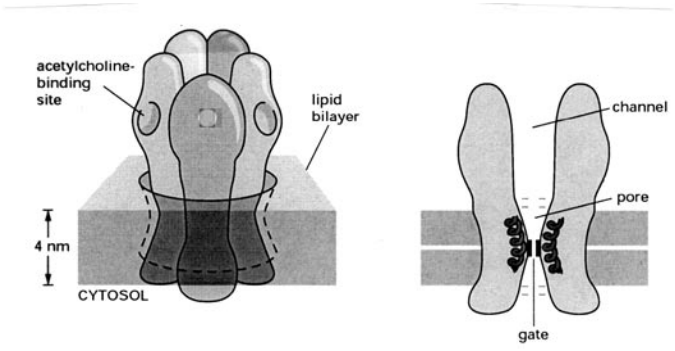

Figure 16. *A model for the structure of the nAchR channel. Five subunits combine to form a transmembrane aqueous pore. The pore is lined by a ring of five alpha-helices, one contributed by each subunit. The gate is thought to be formed by the hydrophobic side chains of five leucines, one from each helix. Acetylcholine binding sites are located on each of two subunits. When acetylcholine binds to both sites, the channel undergoes a conformational change that opens the gate. The negatively charged side chains at either end of the pore ensure that only positively charged ions pass through the channel. (From Alberts et. al., Molecular Biology of the Cell, 4th ed, Garland Publ, 2002., Fig 11-36, p.648.)*

Selectivity of ion channels. Ion channels must allow the passage of ions but exclude other hydrophilic molecules. Clearly, the size of the central pore plays a role in selectivity. Ion channel pores are extremely narrow—Na^+ and K^+ channels have the smallest pores, from 3-5 Å. Ion channels also discriminate between cations and anions. The distribution of charged amino acids along the outside of a channel's opening contributes to charge selectivity. nAchR is characterized by negatively charged amino acids on either side of the pore, serving to guide in positively charged ions (Fig. 16). When the channel is open, any cation with a diameter of less than 65 nm (which includes Ca^{2+}, K^+ and Na^+) has the potential to pass through it.

In contrast to the nAchR, which allows the passage of any cation through its pore, some channels are exquisitely selective for particular ions. For example, K^+ leak channels discriminate between K^+ and Na^+ by a factor of 10,000, despite both cations being of similar size (Na^+ diameter = 0.95A, K^+ diameter = 1.33A). The channel discriminates against the *smaller* ion, a mystery that was solved when the crystal structure of a bacterial K^+ channel was determined. The channel contains what is termed a *selectivity filter* (Fig 17). The filter is an area of the channel that is lined by carbonyl oxygen atoms from the polypeptide backbone. In order to pass through the selectivity filter, an ion must shed its coat of water (all ions are surrounded by a shell of water molecules) and interact with the carbonyl atoms. The filter is large enough to accommodate a dehydrated K^+ ion or a dehydrated Na^+ ion. However, only K^+ ions are large enough to be able to contact the carbonyl oxygens. This energetically favorable interaction balances the energy lost in removal of the waters from the ion. In contrast, when Na^+ loses its surrounding waters, it is too small to intimately contact the wall of the channel. It is energetically more favorable for Na^+ to retain its shell of water and not pass through the filter.

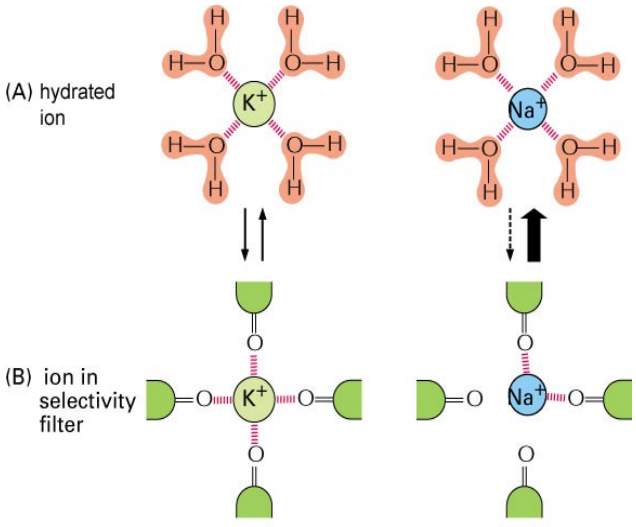

Figure 17. *K^+ specificity of the selectivity filter of a K^+ channel. (A) Na^+ and K^+ ions hydrated with an outer shell of water. (B) The selectivity filter of the pore, viewed in cross section. In the selectivity filter, the carbonyl oxygens are placed precisely to accommodate a dehydrated K^+ ion. The energy required to dehydrate the ion is balanced by the energy regained by the interaction of the ion with the carbonyl oxygens that serve as surrogate water molecules. Because the Na^+ ion is too small to interact with the oxygens, it can enter the selectivity filter only at a great energetic expense. (From Alberts et. al., Molecular Biology of the Cell, 4th ed, Garland Publ, 2002, Fig 11-24, p. 637.)*

Gating of ion channels. In most cases, the central pore of an ion channel is not continuously open, and the regulation of the open versus closed state is crucial to maintaining the balance of ions in a cell. Thus, ion channels are gated: they can switch between an open and closed state by a change in conformation. The open state has an extremely short lifetime, typically a millisecond. The conformational change can occur in response to several types of stimuli: voltage-gated channels respond to changes in voltage across the membrane, ligand-gated channels respond to binding of another molecule to the channel itself, and mechanically-gated channels respond to physical stimuli (Fig. 18).

The nAchR channel is ligand-gated. The endogenous ligand for nAchR is acetylcholine, a neurotransmitter. When acetylcholine is released into the *nerve terminal*, the space between a nerve and muscle, it binds to the channel and induces a conformational change that opens the channel. With ligand bound, the channel flickers between the open and closed states, but is much more often in the open state. A specific enzyme (acetylcholinesterase) located in the neuromuscular junction hydrolyzes acetylcholine, and the channel returns to its resting state. Succinylcholine is a drug that is a functional antagonist of the nAchR receptor. It is used as a paralytic agent—in the case of Mr. Danovic, it is used to aid in intubation. The mechanism of action of succinylcholine will be discussed in more detail in the chapter on Signaling.

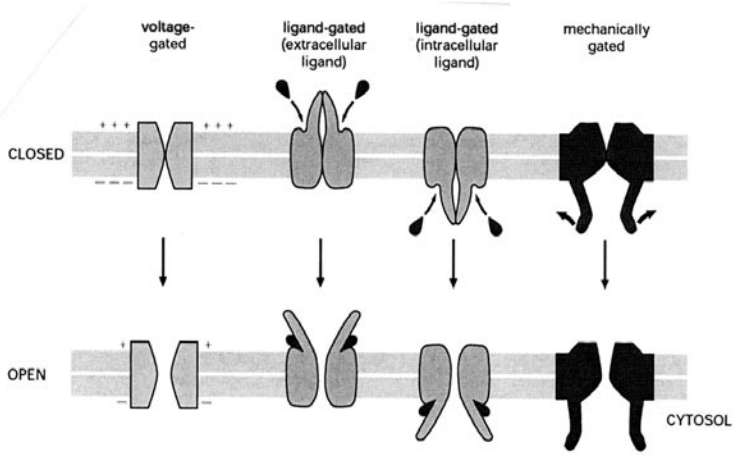

Figure 18. *The gating of ion channels. There are three different kinds of ion channels, each opened by a different kind of stimuli. (From Alberts et al., Essential Cell Biology, Garland Publ, 1998, Fig 12-22, p. 390.)*

PUTTING IT ALL TOGETHER:
The relationship between several transport mechanisms can be observed at the neuromuscular junction when a nerve impulse stimulates a muscle cell to contract (Fig. 19). The response requires the action of at least five sets of ion channels, sequentially activated as follows.

1. The nerve impulse reaches the nerve terminal and depolarizes the plasma membrane of the terminal. This opens voltage-gated Ca^{2+} channels in this membrane. Ca^{2+} flows into the nerve terminal, which triggers the localized release of acetylcholine into the synaptic cleft.

2. The acetylcholine binds to nAchR channels, allowing Na^{+} to flow into the muscle cell, causing a localized membrane depolarization.

3. The local depolarization of the muscle cell plasma membrane opens voltage-gated Na^{+} channels in this membrane, allowing more Na^{+} to enter. This opens neighboring voltage-gated Na^{+} channels, creating a large depolarization (or *action potential*) that spreads to involve the entire plasma membrane.

4. The depolarization of the entire plasma membrane results in the opening of Ca^{2+} channels in the sarcoplasmic reticulum. (You will hear about how the signal is transmitted to the SR from the plasma membrane in the Muscle lecture.) Ca^{2+} then rushes into the cytosol, and its sudden increase in concentration triggers a series of events that cause the muscle cell to contract.

To end the process of contraction, Ca^{2+} must be removed from the cytosol of both the nerve terminal and the muscle cell. In both cells, the Na^{+}/Ca^{2+} exchanger pumps Ca^{2+} out of the cell using the energy of the Na^{+} gradient. The Na^{+}/K^{+}-ATPase pumps Na^{+} back out of the cell, maintaining the Na^{+} gradient. In the muscle cells, the Ca^{2+}-ATPase, located in the membrane of the SR, pumps Ca^{2+} from the cytosol back into the SR using the energy of ATP hydrolysis.

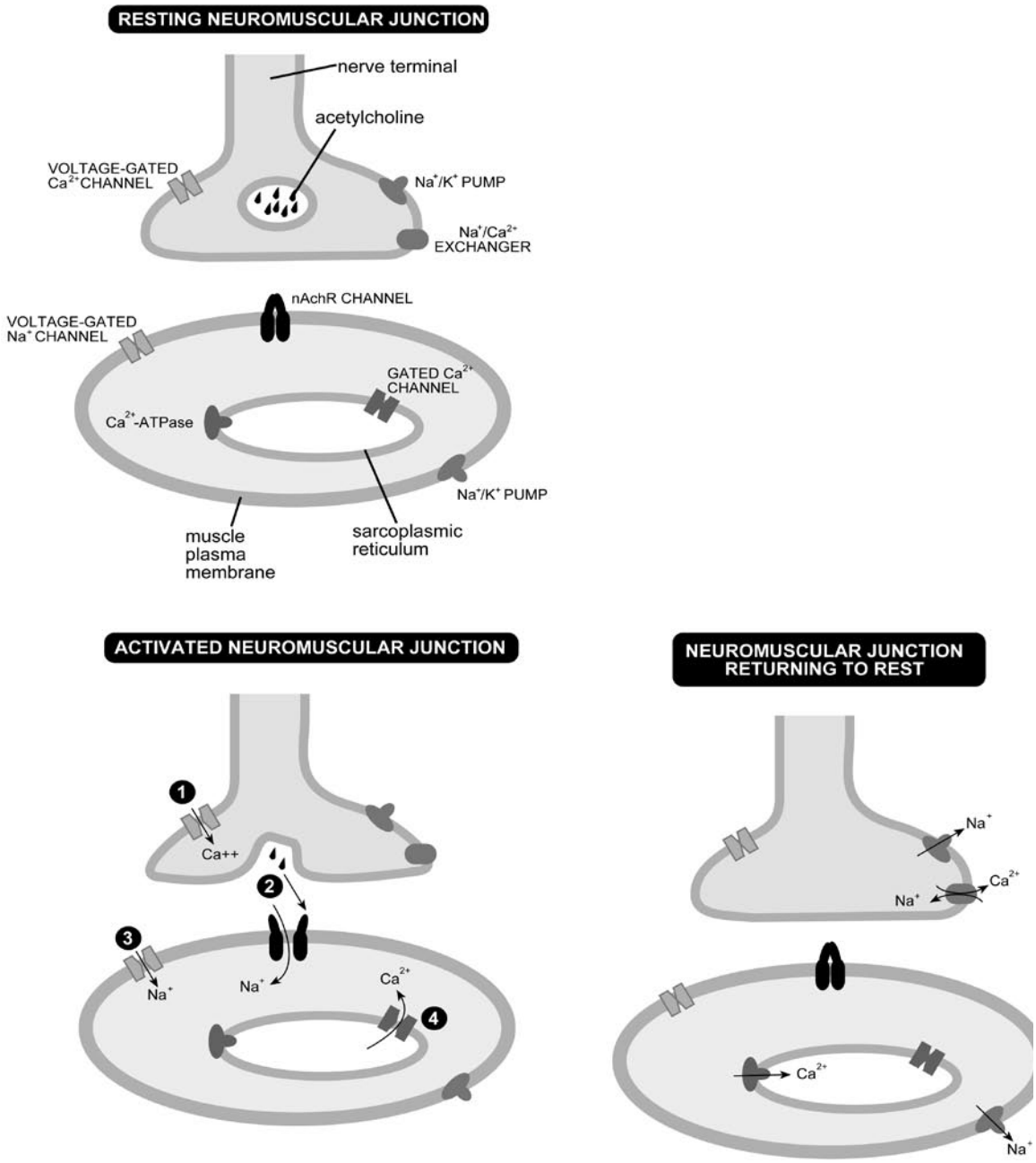

Figure 19. *Relationship between various membrane transport proteins at the neuromuscular junction. The various channels are numbered in the sequence in which they are activated, as described in the text. Note that the Na^+/K^+ pump, the Na^+/Ca^{2+} exchanger and the Ca^+-ATPase are active almost continually, whether the junction is active or at rest. Their activities are shown in the last panel, separate from the activities of the channels for simplicity. (Adapted from Alberts et al., Molecular Biology of the Cell, 4th ed, Garland Publ., 2002, Fig 11-37, p.650.)*